DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES IN RELAPSED ADVANCED/METASTATIC NON-SMALL CELL LUNG CANCER

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Declaration of Authorship

I declare that this thesis and the work contained herein is my own except where explicitly stated otherwise, and that this work has not been submitted for any other degree or professional qualification.

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Signed   Dated

Abstract

Recent advances in the treatment of advanced NSCLC include introduction of targeted therapies and immune-checkpoint inhibitors, leading to improvements in survival outcomes. However, significant new challenges and questions have arisen, including availability of adequate material for tissue molecular testing, overcoming resistance to first-line TKIs and immunotherapy agents, and the emerging role of ctDNA genotyping.

This thesis describes the conduct of two national real-world studies of outcomes in patients with advanced adenocarcinoma NSCLC treated with pembrolizumab in the treatment-naïve setting and combination docetaxel/nintedanib in the relapsed setting, demonstrating their safety and effectiveness during the early days of uptake in UK patients, while also benchmarking outcomes in a target population for a phase Ib/II clinical trial to determine the safety and efficacy of a novel therapeutic combination of nab-paclitaxel with nintedanib, the set-up of which is also described.

This thesis also describes a single-centre retrospective study validating the adequacy of rebiopsy tissue for genotyping in relapsed NSCLC, followed by a study of optimal methods of tissue acquisition in the context of CRUK SMP2 programme, both providing valuable data to guide clinicians at a time when evidence was building for the need for repeated molecular genotyping, and subsequently also for the benefits of broader tissue NGS profiling.

Finally, results of a prospective feasibility study of implementation of a national clinical EGFR ctDNA testing service in the NHS are presented, followed by a study evaluating the real-world clinical utility of ctDNA-based NGS demonstrating it's complementary role when used with current standard-of-care molecular profiling technologies, by increasing the proportion of patients with actionable genomic variants in a rapid and minimally-invasive manner.

The work performed towards this thesis has enabled me to develop knowledge, skills and confidence to continue to contribute to the implementation and advancement of personalised cancer medicine and towards improving patient outcomes.

Acknowledgements

Attribution of work

Chapter 2

I conceived and developed the proposals for the two real-world national evaluation studies together with my primary supervisor. I independently wrote and submitted the proposals to the RMH R&D Committee and created the validated data collection tool with statistical support from Ranga Gunapala, RM RDSU statistician. Together with my primary supervisor, we sent invitations to participate to UK oncologists and I coordinated subsequent collaboration. I collated the data received from all participating centres and conducted data cleaning. Following statistical analysis in collaboration with the statistician, I conducted the interpretation of statistical results and comparison with trial data. I was the first author of the abstract and poster publications.

The N3 study was conceived and idea developed by Prof Sanjay Popat as Chief Investigator of the trial, with support from IMP providers and part-funders Boehringer Ingelheim and Celgene. Following submission of application for sponsorship to the RM CCR and approval-in-principle, I drafted and submitted the responses to scientific and statistical comments, leading to successful approval of full sponsorship by RM CCR. I wrote the N3 Trial Protocol (Appendix 3), with statistical input from Ann Petruckevitch, RM RDSU Lead Statistician and input on governance sections from Aude Espinasse, RM CTU Senior Trial Coordinator, and oversight from Prof Popat. I wrote the REC and MHRA submissions, and together with Prof Popat presented the study to the Research & Ethics Committee, subsequently gaining full regulatory approval for the study. I wrote additional trial documents including Patient Information Sheets (Appendices 6 & 7) and Informed Consent Forms (Appendix 8). I presented the trial protocol and the patient facing documents to an RM Patient Focus Group, obtaining highly positive opinion and incorporated their feedback into the documents. I worked with the CTU Trial Coordinators to write the Trial Steering Committee Charter (Appendix 4) and with the Lead Statistician to write the Independent Data Monitoring Committee Charter (Appendix 5). I wrote the N3 Trial Laboratory Manual (Appendix 10) and worked with the Lead Statistician and CTU Trial Coordinators to create the CRF and the trial database (Appendix 9), and prepared the SIV slides. I worked with the Sponsor legal department and co-ordinated during contract negotiations between the Sponsor and the two trial funders.

Chapter 3

I conceived and developed the idea for the retrospective single-centre study of image-guided rebiopsies together with my primary supervisor. I independently wrote the study proposal and developed the data collection tool, with statistical support from Ranga Gunapala, RM RDSU statistician. I obtained approvals from the RM Audit Committee. I performed data collection together with Dr Sarah Barth, Core Medical Trainee at the Royal Marsden Hospital. I conducted all statistical analyses and data interpretation. I presented the oral poster presentation at BTOG 2017 international meeting and was first author of the paper publication in Journal of Thoracic Oncology.

CRUK SMP2 is a collaborative programme between Cancer Research UK, the Experimental Cancer Medicine Centres Network, NHS, University of Birmingham Cancer Research Clinical Trials Unit (CRCTU), Illumina, Astra Zeneca and Pfizer. The programme is managed and coordinated by the CRUK Precision Medicine Team, which I joined as a volunteer collaborator in September 2017. Work on collating, converting and summarising the pre-analytical and analytical SMP2 data was performed by Tara Mills, Precision Medicine Data Analyst, and Sahar Rehman, Senior Business Analyst and Project Manager. The rationale for the study of optimal methods of tissue acquisition in the context of SMP2 programme was developed together with the Precision Medicine Team and Prof Sanjay Popat. I devised the study objectives and methods and performed all data statistical analysis and result interpretation presented here.

Chapter 4

The national feasibility study of EGFR ctDNA testing was conceived and designed by Prof David Gonzalez de Castro and Prof Sanjay Popat in collaboration with Dr Philippe Taniere, Dr Rachel Butler, Dr Andrew Wallace and Dr Lisa Thompson. The study proposal was written by Dr Alexandra Pender, Research Fellow at the Royal Marsden Hospital Lung Unit, with statistical input from Ranga Gunapala, RM RSDU statistician. I conducted collation of data from participating centres and performed all data cleaning, statistical analyses and interpretation with oversight from Prof Gonzalez de Castro.

I conceived and designed the study of ctDNA NGS in real-world UK patients together with Prof Sanjay Popat. I wrote the study proposal and obtained study approvals from the RM

Research and Development Committee. I independently conducted data collection, analyses and interpretation with oversight from Prof Popat.

List of Publications

Molecular Adequacy of Image-guided Rebiopsies for Molecular Retesting in Advanced NSCLC: A Single Centre Experience. Tokaca N, Barth S, O'Brien M, Bhosle J, Fotiadis N, Wotherspoon A, Thompson L, Popat S. Journal of Thoracic Oncology 2017, Vol. 13(1): 63-72. Winner of first prize for poster and oral presentation at BTOG 2017 international meeting, Dublin, Ireland.

Outcomes with nintedanib and docetaxel in patients with relapsed NSCLC adenocarcinoma treated within the UK Nintedanib Individual Patient Supply programme. N. Tokaca, M.S. Crawford, A. Greystoke, W. Appel, R. Lal, N. Steele, C. Ali, P. Bezecny, S. Fernando, E. Karapanagiotou, G. Skailes, N. Dorey, S. Harrow, J. Bhosle, O. Khan, T. Newsom-Davis, J. Spicer, L. Toy, M. O'Brien, R. Gunapala, S.K. Lu, S. Popat. Lung Cancer 2017; Vol. 103:S32-S33.

A phase I/II trial of combination nab-paclitaxel and nintedanib or nab-paclitaxel and placebo in relapsed NSCLC adenocarcinoma (N3). N. Tokaca, A. Espinasse, A. Petruckevitch, S. Ellis, N. Yousaf, J. Bhosle, M. O'Brien, S. Popat. Lung Cancer 2017; Vol. 103: S77-S78.

Real-world outcomes with pembrolizumab in patients with treatment-naive advanced/metastatic NSCLC in the UK: multicentre retrospective observational study. N. Tokaca, F. Gomes, S. Lau, et al. Lung Cancer 2019; Vol. 127, Supplement 1, S33–S34.

Clinical utility of circulating tumour (ct) DNA next generation sequencing (NGS) for target identification in diagnostic and acquired resistance settings in metastatic NSCLC (mNSCLC): Single centre experience. Tokaca N, Cui W, Faull I, et al. Annals of Oncol 2020, Vol. 31, Suppl 4, S867-S868.

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List of Abbreviations

ADC	Adenocarcinoma
AE	Adverse event
ALK	Anaplastic lymphoma kinase
ALK+	ALK fusion positive
ALT	Alanine aminotransferase
AMP	The Association for Molecular Pathology
ANC	Absolute neutrophil count
ANG-2	Agiopoietin-2
ARMS	Amplification refractory mutation system
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
ATM	A-T mutated
ATP	Adenosine triphosphate
AUC	Area under the curve
BID	''bis in die'', twice daily
bp	Base pairs
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BRCA1	BReast CAncer-1 gene
BRCA2	BReast CAncer-2 gene
BSC	Best supportive care
bTMB	Blood-based TMB
CAP	The College of American Pathologists
CAPP-seq	Cancer personalized profiling by deep sequencing
CCL	C-C-motif chemokine ligand
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A
CE-SSCA	Capillary electrophoresis single-strand conformation analysis
cfDNA	Circulating free DNA
CH	Clinical Hub
CHIP	Clonal haematopoiesis of indeterminate potential

ChT	Chemotherapy
CI	Confidence interval
c-KIT	Tyrosine-protein kinase Kit
CLIA	The Clinical Laboratory Improvement Amendments
CNS	Central nervous system
CR	Complete response
CRUK	Cancer Research UK
CSF1	Macrophage colony-stimulating factor 1
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumour DNA
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCL12	C-X-C-motif chemokine ligand 12
CYP	Cytochrome P450
DCR	Disease control rate
ddPCR	Digital droplet PCR
DDR2	Discoidin Domain Receptor Tyrosine Kinase 2
DLT	Dose limiting toxicity
dMMR	Mismatch repair deficient
dNTP	Deoxynucleoside triphosphate
EAMS	Early access to medicines scheme
EBUS	Endobronchial ultrasound
ECMC	Experimental Cancer Medicine Centre
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
EGFR+	EGFR mutation positive
EMA	European Medicines Agency
ErbB	Avian erythroblastosis oncogene B
ESCAT	The ESMO Scale for Clinical Actionability of Molecular Targets
ESMO	European Society of Medical Oncology

EUS	Endoscopic ultrasound
FASL	FAS antigen ligand
FDA	U.S. Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FGFR	Fibroblast growth factor receptor
FISH	Fluorescence in-situ hybridisation
FNA	Fine needle aspiration
G360	Guardant®360 CDx assay
GCSF	Granulocyte colony-stimulating factor
GCP	Good Clinical Practice
GLH	Genomic Laboratory Hub
GM-CSF	Granulocyte–macrophage colony-stimulating factor
H&E	Hematoxylin and eosin
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IC_{50}	Half-maximal inhibitory concentration
ICI	Immune checkpoint inhibitor
IDMC	Independent Data Monitoring Committee
IDO-1	Indoleamine 2,3-Dioxygenase 1
IHC	Immunohistochemistry
IMP	Investigational medicinal product
IQR	Interquartile range
IR AE	Immune related adverse event
ITT	Intention-to-treat
JAK	Janus kinase
KRAS	Kirsten rat sarcoma viral oncogene homolog
LAG3	Lymphocyte Activating Gene 3
MAF	Mutant allele fraction
MAMA	Multi-arm multi-agent
MEK	Mitogen activated protein kinase
MET	Mesenchymal to epithelial transition

MHRA	Medicines and Health products Regulatory Agency
mNSCLC	Metastatic non-small cell lung cancer
mOS	Median overall survival
mPFS	Median progression free survival
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MSI-H	Microsatellite instability-high
MTD	Maximum tolerated dose
Nab-P	Albumin-bound paclitaxel
NF1	Neurofibromin-1
NGIS	National Genomic Information System
NGS	Next-generation sequencing
NHS	U.K. National Health Service
NHS GMS	NHS Genomic Medicine Service
NICE	The National Institute for Health and Care Excellence
NIPS	Named individual patient supply
NLCA	National Lung Cancer Audit
NLMT	National Lung MATRIX Trial
NR	Not reached
NRAS	Neuroblastoma RAS
NSCLC	Non-small cell lung cancer
NSCLC NOS	NSCLC not otherwise specified
NTRK	Neurotrophic tropomyosin receptor kinase
ORR	Objective response rate
OS	Overall survival
PARP	Poly (ADP-ribose) polymerase
PCR	Polymerase chain reaction
PD	Progressive disease
PD-1	Programmed cell death-1
PDGFR	Platelet-derived growth factor receptor
PD-L1	Programmed death-ligand 1

PFS	Progression free survival
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PR	Partial response
PS	Performance status
PTEN	Phosphatase and tensin homolog
QC	Quality control
RB1	Retinoblastoma 1
RCT	Randomised controlled trial
RDSU	Research Data and Statistics Unit
RECIST	Response Evaluation Criteria In Solid Tumours
RET	Rearranged during transfection
RM-CTU	Royal Marsden Clinical Trials Unit
RMH	Royal Marsden Hospital
ROS1	c-ros oncogene 1
ROS1+	ROS1 rearrangement positive
ROSE	Rapid on-site evaluation
RP2D	Recommended phase 2 dose
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcriptase PCR
SAE	Serious adverse event
SCC	Squamous cell carcinoma
SCLC	Small cell lung cancer
SCNA	Single copy number alterations
SD	Stable disease
SE	Service evaluation
SMP2	Stratified Medicine Programme 2
SNP	Single nucleotide polymorphism
SOC	Standard of care
SOP	Standard operating procedure
STK11	Serine/threonine kinase 11
TAT	Turn-around time

TAM	Tyro3, Axl, Mer receptors
TDM-1	Trastuzumab emtansine
TGFβ	Transforming growth factor β
TH	Technical Hub
TIGIT	T Cell Immunoreceptor With Ig And ITIM Domains
TIL	Tumour infiltrating lymphocyte
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
TKI	Tyrosine kinase inhibitor
TMB	Tumour mutational burden
TME	Tumour microenvironment
TMG	Trial Management Group
TP53	Tumour protein p53
TPS	Tumour proportion score
TRK	Tropomyosin receptor kinase
TSC	Trial Steering Committee
TTF	Time to treatment failure
TTP	Time to progression
UICC TNM	The Union for International Cancer Control TNM staging
VAF	Variant allele frequency
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organisation
wt	Wild type

Chapter 1 Introduction and literature review

1.1 Non-small cell lung cancer incidence

Lung cancer remains the commonest cause of cancer mortality worldwide. In 2020, the most recent year for which global World Health Organisation data are available, there were an estimated 2.2 million new cases of lung cancer every year (comprising 11% of total new global cancer cases), resulting in around 1.8 million deaths.[1] In the UK, lung cancer is the 3rd most common cancer with around 47,000 new cases each year (2014-2016), accounting for 13% of all new cancer cases, but the most common cause of cancer deaths with around three quarter of cases diagnosed at a late stage.[2]

Non-small cell lung cancer (NSCLC) accounts for 80%–90% of lung cancers, while small cell lung cancer (SCLC) has been decreasing in frequency.[3] During the last 25 years, the distribution of histological types of NSCLC has changed: in the United States, squamous cell carcinoma (SCC), formerly the predominant histotype, decreased, while adenocarcinoma has increased in both genders. In Europe, similar trends have occurred in men, while in women both SCC and adenocarcinoma rates are still increasing. These trends likely reflect the changes in cigarette smoking patterns. However, and despite an overall reduction in lung cancer mortality rates by more than a quarter (28%) since the early 1970, widespread continued cigarette smoking means that lung cancer will remain a significant public health problem for the foreseeable future, with an estimated 71% of global lung cancer deaths caused by smoking.

Conversely, about 500,000 deaths annually are attributed to lung cancer in lifetime never-smokers and an increasing trend in the proportion of NSCLC in never-smokers has been observed, especially in Asian countries.[4] Non-smoking-associated lung cancer is now considered a distinct disease entity with specific molecular and genetic tumour characteristics.[5] Identification of specific oncogenic driver aberrations such a *EGFR* activating mutations and *ALK* gene rearrangements, predominantly in non-squamous NSCLC, and development of corresponding targeted therapeutic agents has resulted in significant improvements in outcomes for these patients.

Treatment of advanced/metastatic non-small cell lung cancer consists of use of sequential systemic therapies, with aim of prolonging overall survival and improving quality of life. The choice of systemic therapy is guided by the histological diagnosis, presence or absence of

targetable driver mutations, performance status and patient preferences in individual patients. There have been significant recent advances in treatment of advanced NSCLC, with introduction of targeted therapies and immune-checkpoint inhibitors (ICIs), with 5 year overall survival in patients treated with immunotherapy reaching around 20% compared with historical 5-year rates for stage IV NSCLC of 1% to 8%.[6] However, outcomes for most patients, particularly those with relapsed disease after first-line therapy, remain poor. Therefore, development of novel combination therapies and identification of new predictive and prognostic biomarkers in relapsed NSCLC patients with no currently identifiable driver mutations or with resistance to existing targeted therapies is of paramount importance in NSCLC research.

1.2 Current treatment options in advanced/metastatic NSCLC with no known oncogenic drivers

Platinum-doublet chemotherapy was for many decades the mainstay of systemic therapy for advanced NSCLC. A study by Schiller et al of 1,155 patients treated with 4 different platinum-doublet regimen showed a median survival of 8 months, 1-year survival rate of 33% and 2-year survival of 11%, with no difference in survival between different platinum doublet regimens.[7]

The identification of *EGFR* mutations as the driver of benefit in patients with *EGFR* mutant lung cancer treated with gefitinib in the 2009 IPASS study heralded the era of targeted treatment with tyrosine kinase inhibitors and the implementation of routine molecular testing.[8] Since that time, several other NSCLC tumour gene alterations have been identified as oncogenic drivers (gene alterations responsible for the initiation and maintenance of the cancer, often found in genes that encode for signaling proteins that are critical for maintaining normal cellular proliferation and survival) and associated targeted therapeutics developed and in some cases licenced as systemic therapies for advanced NSCLC.

A further major development was the identification of benefit of immune checkpoint inhibitors, which have demonstrated improved outcomes and tolerability over chemotherapy in NSCLC, with differential responsiveness according to programmed death-ligand 1 (PD-L1) expression levels, leading to introduction of routine PD-L1 testing, but with limited activity in oncogene-addicted NSCLC.

Where no oncogenic driver alterations are present, treatment choice depends on histology and expression levels of PD-L1 on the surface of lung tumour cells, and includes a choice of immune checkpoint inhibitor therapy alone or in combination with platinum-doublet chemotherapy (ChT), or platinum-doublet chemotherapy alone.

1.2.1 No oncogenic drivers, PD-L1 ≥50%

In untreated advanced NSCLC with PD-L1 expression levels of ≥50%, there is data supporting multiple treatment options including ICI monotherapy,[9–11] ICI-chemotherapy combinations[12–18] and dual immune checkpoint inhibition with PD-L1 and CTLA-4 inhibitors[19]. However there are no direct comparisons of efficacy of single-agent ICI versus ICI-ChT or ICI-ICI combinations in NSCLC with PD-L1 expression of ≥50%, while cross-trial

comparison suggests similar OS outcomes but greater toxicities with the combination regimens, therefore current international guidelines recommend use of single-agent ICI where PD-L1 is ≥50%, and combination regimens elsewhere, in absence of contraindications to immune checkpoint inhibitor therapy.[20]

Data for the anti-PD-1 agent pembrolizumab in this setting comes from results of two phase III trials KEYNOTE-024 and KEYNOTE-042.[9, 10] In KEYNOTE-024, 305 patients with NSCLC, no driver mutations and PD-L1 tumour proportion score (TPS)[*] ≥50% were randomised in a 1:1 fashion to receive 200mg pembrolizumab every 3 weeks for up to 2 years or 4–6 cycles of standard platinum-doublet chemotherapy (ChT). All efficacy measures were improved with pembrolizumab, including progression-free survival (PFS, hazard ratio (HR) 0.5, 95% confidence interval (CI): 0.37–0.68, p<0.001) and overall survival (OS, HR 0.6, 95% CI: 0.41–0.89, P=0.005), as well as the safety profile.[9] After additional follow-up, magnitude of OS benefit was further demonstrated with median OS of 30 months for pembrolizumab versus 14 months for ChT.

Subsequent KEYNOTE-042 phase III trial examined the PD-L1 threshold for benefit with pembrolizumab, randomising patients with PD-L1 ≥1% to either pembrolizumab or ChT.[10] This trial demonstrated that while improved OS was seen at threshold level of ≥1%, the preponderance of OS benefit was driven by patients with PD-L1 ≥50%, with no significant increase seen in patients with PD-L1 1%–49% (HR 0.92, 95% CI: 0.77–1.11). Another anti-PD-1 inhibitor nivolumab failed to demonstrated survival benefit over ChT in the same patient population in the phase III CheckMate-026 trial when a PD-L1 cut off of 5% was used.[21]

Atezolizumab, an anti-PD-L1 inhibitor, is another promising single-agent option in the setting of high PD-L1 expression, based on the interim survival analysis results of IMPower110 study which reported increased median overall survival by 7.1 months for atezolizumab compared with chemotherapy (20.2 months vs. 13.1 months; HR for death, 0.59; p=0.01) in the subgroup of patients with PD-L1 expresion on ≥50% of tumor cells or ≥10% of tumor-infiltrating immune cells (as assessed by the SP142 immunohistochemical

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[*] Tumour proportion score (TPS) is a PD-L1 measurement which is applied in lung cancer, head and neck cancer and melanomas. Within this approach, only membranous staining of tumour cells by immunohistochemistry is regarded as significant staining.

assay).[11] Atezolizumab is FDA approved, but not EMA licenced as a single-agent in the first-line setting.

1.2.2 No oncogenic drivers, any PD-L1

Platinum-doublet chemotherapy remains an important treatment option in this group of patients, particularly where there are contraindications to the use of ICIs, however several new treatment options have emerged with the publication of large phase III trials supporting the role of ChT-ICI combinations, ICI-ICI combinations as well as single-agent ICI therapy for both non-squamous and squamous NSCLC.

In KEYNOTE-189[12] patients with non-squamous advanced NSCLC without driver mutations were randomised between platinum/pemetrexed ChT plus pembrolizumab or placebo every 3 weeks for 4 cycles, followed by pembrolizumab or placebo for up to a total of 35 cycles plus pemetrexed maintenance therapy. Median OS in the pembrolizumab/ChT arm was 22.0 months versus 10.3 months in the ChT arm (HR 0.56, 95% CI: 0.45–0.70, p<0.00001), and the benefit was observed in all PD-L1 subgroups, including in those with PD-L1 TPS <1%. IMpower130[13] was another multicentre, randomised, phase 3 study randomising stage IV non-squamous NSCLC patients to receive anti-PD-L1 ICI atezolizumab (1,200 mg every 3 weeks) and carboplatin/albumin-bound paclitaxel (nab-P) for 4–6 cycles, followed by maintenance atezolizumab, to ChT alone. This trial showed a significant improvement in OS (18.6 vs. 13.9 months; HR 0.79, 95% CI: 0.64–0.98, P=0.033) and PFS (7.0 vs. 5.5 months, HR 0.64, 95% CI: 0.54–0.77, p<0.0001) with atezolizumab plus ChT versus ChT as first-line treatment, and represents another treatment option in this group of patients. More recently, final results of IMPower132 trial have reported, of atezolizumab in combination with platinum-pemetrexed ChT versus ChT alone (followed by maintenance atezolizumab and pemetrexed versus pemetrexed alone).[16] In this study, co-primary endpoint of PFS was met, with significant improvement for the atezolizumab-ChT group (mPFS 7.6 vs. 5.2 months, HR 0.60, p<0.001), while there was a trend towards improvement for the co-primary endpoint of OS albeit not reaching significance (mOS 7.5 vs. 13.6 months, HR 0.86, p=0.1546). The authors note that nearly half of all patients in ChT-alone group went on to receive immunotherapy as 2nd or subsequent line therapy, which may have impacted survival outcomes.

In IMpower150[15], addition of atezolizumab to bevacizumab plus ChT significantly improved PFS and OS among patients with metastatic non-squamous NSCLC, regardless of PD-L1 expression. Of note, this trial also included patients with sensitising *EGFR* or *ALK* gene alterations and showed a trend towards survival benefit of ICI/ChT combinations in this subgroup (OS HR 0.54, 95% CI: 0.29–1.03). While this was a post hoc unplanned analysis in a small group of patients (58 patients with *EGFR* sensitising mutations and fewer than 20 ALK+ patients), this combination could be considered as a treatment option in these patients after targeted therapies have been exploited and is EMA licenced in this setting.

In metastatic squamous NSCLC, benefit of ICI+ChT combination therapy was demonstrated by KEYNOTE-407,[14] a phase III trial which randomised SCC patients to receive carboplatin and paclitaxel (every 3 weeks) or nab-paclitaxel (weekly) plus pembrolizumab or placebo for 4 cycles, followed by pembrolizumab or placebo for a total of 35 cycles, with OS benefit observed across all PD-L1 expression levels (overall OS HR 0.64, mOS 15.9 vs. 11.3 months, p=0.0008). Atezolizumab in combination with ChT in patients with metastatic squamous NSCLC was investigated in the IMPower131 study.[17] Patients were randomised to atezolizumab plus ChT with carboplatin plus paclitaxel or nab-paclitaxel or ChT alone. Atezolizumab + ChT resulted in PFS improvement compared with ChT alone (HR 0.71, p=0.0001), but no improvement in OS was seen in the ITT population (mOS 14.2 vs. 13.5 months). Patients with high PD-L1 expression (≥50% of tumour cells or ≥10% tumour-infiltrating immune cells) appeared to derive an OS benefit with the addition of atezolizumab (HR 0.48, 95% CI: 0.29–0.81) however the study was not powered for this analysis.

Efficacy of combined PD-1 and CTLA-4 checkpoint inhibition in first-line NSCLC was investigated in CheckMate-227 phase III study which randomly assigned patients with treatment naïve advanced NSCLC and a PD-L1 expression level of ≥1% in a 1:1:1 ratio to receive nivolumab plus ipilimumab, nivolumab alone, or chemotherapy.[19] The patients who had a PD-L1 expression level of <1% were randomly assigned to receive nivolumab plus ipilimumab, nivolumab plus chemotherapy, or chemotherapy alone. Overall survival benefit was observed for nivolumab plus ipilimumab versus chemotherapy in the PD-L1 ≥1% subgroup (mOS 17.1 vs. 14.9 months, HR 0.79, p=0.007), an effect predominantly driven by PD-L1 ≥50% patients. OS benefit for nivolumab plus ipilimumab was also reported in PD-L1

negative patients, where OS was a pre-specified exploratory endpoint. Of note, this trial reported a 32.8% rate of severe adverse events for nivolumab plus ipilimumab combination, significantly higher than rates observed with single agent ICI therapy. Earlier reports of PFS analyses in this trial suggested tumour mutational burden (TMB) as a predictive biomarker of response to nivolumab plus ipilimumab combination therapy, with significantly longer PFS compared with chemotherapy regardless of PD-L1 status (HR 0.58, 97.5% CI: 0.41–0.81, p<0.001), however this effect did not translate into overall survival benefit.

Treatment with nivolumab and ipilimumab in combination with 2 cycles of histology-based platinum-doublet chemotherapy was studied in the CheckMate9LA trial.[18] In the pre-planned interim OS analysis, median OS was significantly longer in the experimental group than in control group: 14.1 vs. 10.7 months (HR 0.69; p=0.00065), with the effect seen for both squamous and non-squamous histology, however with grade 3-4 treatment-related adverse events occurring in nearly half of patients in the experimental group compared with a third in the control group.

Evidence for the role of single agent immunotherapy in this setting, as studied in KEYNOTE-042 and CheckMate-026 trials, was discussed in the previous section.[10, 21]

Based on the results of trials outlined above, introduction of immune checkpoint inhibitors is likely to be indicated for most patients with treatment-naïve NSCLC and no known oncogenic driver alterations.

Summary of key ICI trials in first-line advanced NSCLC is presented in Table 1.1.

1.2.3 No oncogenic drivers with contraindications to use of immunotherapy

Platinum-doublet chemotherapy is standard of care in all patients with advanced NSCLC and performance status of 0-2 where immunotherapy is contraindicated, based on the results of two large meta-analyses showing improvements in survival and quality of life with chemotherapy over best supportive care.[22, 23] Survival benefit of doublet over single-agent ChT regimens was reported in a 2004 meta-analysis, while no survival benefit was observed for triplet over doublet regimens.[24] Platinum-doublet regimens are recommended based on a 2006 meta-analysis, which showed a 22% reduction in the risk of death at 1 year for platinum over non-platinum combinations.[25] No OS benefit has been observed for 6 versus 4 cycles of platinum-doublet ChT, albeit with longer PFS at a cost of higher toxicities with 6 cycle regimens.[26] Therefore, 4 cycles of platinum-based doublet chemotherapy followed by

maintenance monotherapy (where indicated) or up to a maximum of 6 cycles are currently the standard approach.

Evidence for pemetrexed "switch-maintenance" or "continuation-maintenance" therapy after first-line platinum-doublet ChT comes from several phase III trials showing PFS and OS benefits in patients with non-squamous NSCLC and good performance status.[27–29]

Treatment options for advanced NSCLC without oncogenic driver mutations are summarised in Figure 1.1. Included are all evidence-based regimens which are recommended by the current European guidelines,[20] with the NICE approved regimens and those currently NHS funded via the Cancer Drugs Fund (as well as the current Covid-19 emergency funded regimens) are outlined as a subset.

Trial Year of publication	Histology	Therapeutic regimen	Chemotherapy drugs	No. of patients	PD-L1 strata	PFS (m)	HR for PFS (CI)	OS (m)	HR for OS (CI)	HR for OS by PD-L1 strata	Median follow-up time (m)
KEYNOTE-024 2016	NSCC SCC	Pembro vs. ChT	AP or AC CPa or GP or GC	305	≥50%	10.3 vs. 6.0	0.50 (0.37 - 0.68)	30.0 vs. 14.2	0.63 (0.47 - 0.86)	-	25.2
KEYNOTE-042 2019	NSCC SCC	Pembro vs. ChT	AC CPa	599	≥50% ≥20% ≥1%	7.1 vs. 6.4	0.81 (0.67 - 0.99)	20.0 vs. 12.2	0.69 (0.56 - 0.85)	0.69 (0.56, 0.89) 0.77 (0.64, 0.92) 0.81 (0.71, 0.93)	12.8
CheckMate-026 2016	NSCC SCC	Nivo vs. ChT	AP or AC CPa or GP or GC	541	>5% >50%	4.2 vs. 5.9	1.15 (0.91 - 1.45)	14.4 vs. 13.2	1.02 (0.80 - 1.30)	1.02 (0.8, 1.3) 0.90 (0.63, 0.29)	13.5
MYSTIC† 2018	NSCC SCC	Durva vs. Durva/Treme vs. ChT	AP or AC CPa or GP or GC	1118	≥25%	4.7 vs. 5.4	0.87 (0.67 - 1.14)	16.3 vs. 12.9	0.76 (0.56 – 1.02)	-	NK
KEYNOTE-189 2018, 2019	NSCC	Pembro + ChT vs. ChT	AP or AC	202	<1% 1-49% ≥50%	9.0 vs. 4.9	0.48 (0.40 - 0.58)	22 vs. 10.7	0.56 (0.45 - 0.70)	0.52 (0.36, 0.74) 0.62 (0.42, 0.92) 0.59 (0.39, 0.88)	18.7
KEYNOTE-407 2018, 2019	SCC	Pembro + ChT vs. ChT	CPa or CNPa	559	<1% 1-49% ≥50%	8.0 vs. 5.1	0.57 (0.47 - 0.69)	17.1 vs. 11.6	0.71 (0.58 - 0.88)	0.79 (0.56, 1.11) 0.59 (0.42, 0.84) 0.79 (0.52, 1.21)	14.3
IMPower-130 2019	NSCC	Atezo + ChT vs. ChT	CNPa	723	High Low Negative	7.0 vs. 5.5	0.64 (0.54 - 0.77)	18.6 vs. 13.9	0.79 (0.64–0.98)	0.84 (0.51, 1.39) 0.70 (0.45, 1.08) 0.81 (0.61, 1.08)	18.5
IMPower-132 2018	NSCC	Atezo + ChT vs. ChT	AP or AC	578	High Low Negative	7.6 vs. 5.2	0.6 (0.49 – 0.72)	18.1 vs. 13.6	0.81 (0.64–1.03)	0.41 (0.13, 1.25) 1.14 (0.73, 1.80) 0.71 (0.47, 1.07)	14.8
IMPower-150 2018	NSCC	Atezo + Bev/ChT vs. Bev/ChT	CPa	692	N/A*	8.3 vs. 6.8	0.62 (0.52 - 0.74)	19.2 vs. 14.7	0.78 (0.64 - 0.96)	-	20.0
IMPower-131 2018	SCC	Atezo + ChT vs. ChT	CPa or CNPa	683	High Low Negative	6.5 vs. 5.6	0.74 (0.62 - 0.87)	14.6 vs. 14.3	0.92 (0.76 - 1.12)	0.48 (0.29, 0.81) 0.86 (0.67, 1.11) 0.87 (0.67, 1.13)	12.8
CheckMate-227‡ 2019	NSCC SCC	Ipi + Nivo vs. ChT	AP or AC GP or GC	793	<1% ≥1%	5.1 vs. 5.6	0.82 (0.69 - 0.97)	17.1 vs. 14.9	0.79 (0.65 - 0.96)	0.62 (0.48, 0.78) 0.79 (0.65, 0.96)	29.3

Table 1.1. Summary of key phase III trials of immune checkpoint inhibitors alone or in combination with chemotherapy for treatment naïve NSCLC. A, pemetrexed; Atezo, atezolizumab; Bev, bevacizumab; C, carboplatin; ChT, chemotherapy; CI, confidence interval; Durva, durvalumab; G, gemcitabine; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab; NK, not known; NPa, nab-paclitaxel; NSCC, non-squamous cell carcinoma; OS, overall survival; P, cisplatin; Pa, paclitaxel; Pembro, pembrolizumab; PFS, progression-free survival; Treme, tremelimumab. † Survival data presented for Durva. vs. ChT arm. ‡ Survival data presented for PDL≥1 subgroup. *Teff-gene signature used for stratification.
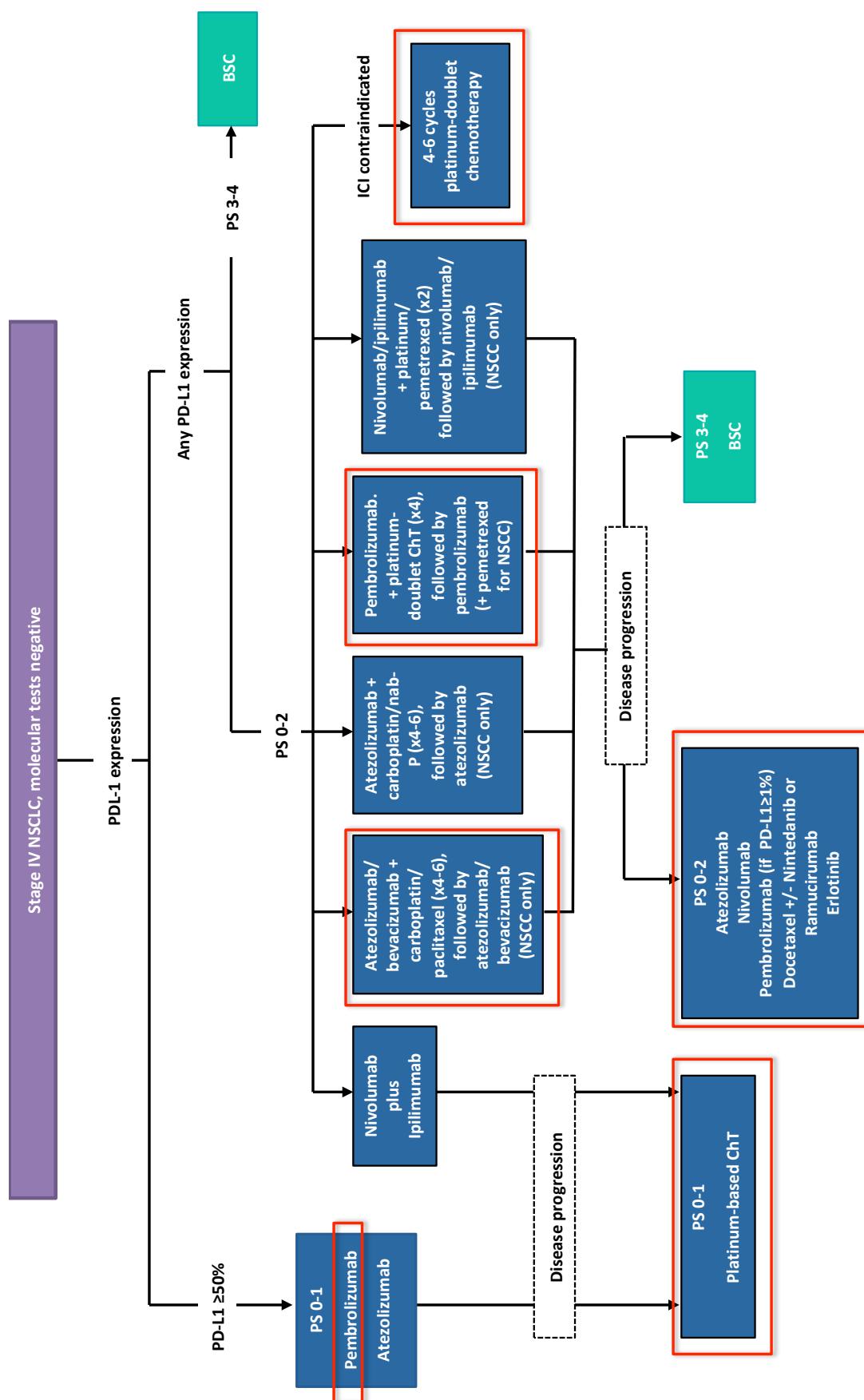
Figure 1.1 Treatment algorithm for advanced/metastatic NSCLC with no driver mutations. BSC, best supportive care; ChT, chemotherapy; ICI, immune-checkpoint inhibitor; NSCC, non-squamous cell carcinoma; PS, performance status.

ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann Oncol (2018) 29 (suppl 4): iv192–iv237; https://www.esmo.org/Guidelines/Lung-and-Chest-Tumours/Metastatic-Non-Small-Cell-Lung-Cancer.)

1.2.4 Treatment options for metastatic NSCLC with no oncogenic drivers after relapse following first-line systemic therapy

Current standard of care after relapse following first-line therapy, is a choice of platinum-doublet chemotherapy (if not previously received), single-agent immune checkpoint inhibitor (if not previously received), single agent chemotherapy (e.g. docetaxel, pemetrexed) with or without angiogenesis inhibitors, or EGFR tyrosine kinase inhibitors.

1.2.4.1 Immune checkpoint inhibitors in second-line advanced NSCLC

ICIs atezolizumab, nivolumab and pembrolizumab are all licenced in the second-line immunotherapy-naïve setting, with the former two licenced at any PD-L1 expression level and pembrolizumab in patients with PD-L1≥1%. All have demonstrated OS benefit in phase III studies when compared with single-agent docetaxel.[30–34] However, differential responsiveness is observed according to PD-L1 status.

Nivolumab has been tested in phase III trials CheckMate-017 and CheckMate-057 in squamous and non-squamous NSCLC, respectively, and any PD-L1 expression, with modest if any benefit in PD-L1 negative patients (Figure 1.2.).[30, 31]

Atezolizumab was tested in OAK trial, a phase III trial that recruited patients with both squamous and non-squamous histology and any level PD-L1 expression.[33] Modest benefit was observed in the PD-L1 low or negative group (TC0/IC0 by SP142 assay; mOS 12.6 vs. 8.9 months; HR 0.75, 95% CI: 0.59–0.96), however the SP142 assay has since demonstrated significantly lower sensitivity than SP263 or 22C3 assays in the Blueprint study,[35] with those included in the PD-L1 low or negative groups more likely to be PDL1 positive by SP263 or 22C3.

In the phase III KEYNOTE-010 study of pembrolizumab versus docetaxel, PD-L1 negative patients were excluded due to modest benefit reported in the phase I KEYNOTE-001 trial in these patients (Figure 1.3).[34, 36]

None of the trials have compared ICIs with docetaxel in combination with nintedanib or ramucirumab, both of which have demonstrated improved efficacy over docetaxel alone and are licenced in this setting.[37, 38] While the survival benefit demonstrated in phase III trials of these combination regimens is significant, it is modest and mostly limited to adenocarcinomas, with nintedanib EMA approved but not FDA approved, so uptake of these combination regimes globally has been low.

Key data from second-line phase III ICI trials are summarised in Table 1.2.

1.2.4.2 Single-agent chemotherapy in second-line advanced NSCLC

Three-weekly docetaxel 75mg/m^2 and pemetrexed 500mg/m^2 are licensed for second line treatment of NSCLC. Improvement in OS and disease-related symptoms for these agents was demonstrated in phase III trials.[39–41] However, responses to these agents are in the range of 7-10% and benefits in terms of prolongation of PFS and OS are small.[42] Furthermore toxicities from three-weekly docetaxel are prohibitive for many patients. Febrile neutropaenia rates vary from 4-25% and around a 40% admission rate is observed.[42, 43] Efficacy of these agents after first-line immunotherapy has not been formally assessed, however there is emerging evidence of increased responsiveness to chemotherapy after exposure to immune-checkpoint inhibition.[44–46]

Trial Year of publication	Histology	Therapeutic regimen	PD-L1 strata	No. of patients	PFS (m)	HR for PFS (CI)	OS (m)	HR for OS (CI)	HR for OS by PD-L1 (CI)	Median follow-up (m)
CheckMate-017 2015	SCC	Nivolumab vs. Docetaxel	<1% ≥1% <5% ≥5%	272	3.5 vs. 2.8	0.62 (0.47 - 0.81)	9.2 vs. 6.0	0.59 (0.44 - 0.79)	0.58 (0.37, 0.92) 0.69 (0.45, 1.1) 0.70 (0.47, 1.0) 0.53 (0.31, 0.89)	NK
CheckMate-057 2015	NSCC	Nivolumab vs. Docetaxel	≥1% <1% ≥5% <5% ≥5% <5%	582	2.3 vs. 4.2	0.92 (0.77 – 1.11)	12.2 vs. 9.4	0.72 (0.60 - 0.88)	0.59 (0.43, 0.82) 0.90 (0.66, 1.24) 0.43 (0.30, 0.63) 1.01 (0.77, 1.34) 0.40 (0.26, 0.59) 1.00 (0.76, 1.31)	17.2
Keynote-010† 2016	SCC + NSCC	Pembrolizumab vs. Docetaxel	1-49% ≥50%	678	3.9 vs. 4.0	0.88 (0.74 -1.05)	10.4 vs. 8.5	0.71 (0.58 - 0.88)	0.53 (0.40, 0.70) 0.76 (0.60, 0.96)	13.1
OAK 2017	SCC + NSCC	Atezolizumab vs. Docetaxel	<1%* ≥1%	850	2.8 vs. 4.0	0.95 (0.82 - 1.10)	13.8 vs. 9.6	0.73 (0.62 – 0.87)	0.74 (0.58, 0.93) 0.75 (0.59, 0.960)	21

Table 1.2. Summary of key phase III trials of immune-checkpoint inhibitors in second-line advanced NSCLC. CI, confidence interval; HR, hazard ratio; m, months; NK, not reported; NSCC, non-squamous cell carcinoma; OS, overall survival; SCC, squamous cell carcinoma; PFS, progression-free survival; † Data presented for Pembrolizumab 2mg/kg arm vs. docetaxel. *PD-L1 strata by SP142 assay: TC1/2/3 or IC1/2/3 (PD-L1 ≥1%) vs TC/IC 0 (PD-L1 <1%).

PD-L1 expression level	Nivolumab n	Docetaxel n	Unstratified HR (95% CI)	Interaction P-value
OS				
≥1%	123	123	0.59 (0.43, 0.82)	0.06
<1%	108	101	0.90 (0.66, 1.24)	
≥5%	95	86	0.43 (0.30, 0.63)	<0.001
<5%	136	138	1.01 (0.77, 1.34)	
≥10%	86	79	0.40 (0.26, 0.59)	<0.001
<10%	145	145	1.00 (0.76, 1.31)	
Not quantifiable at baseline	61	66	0.91 (0.61, 1.35)	
PFS				
≥1%	123	123	0.70 (0.53, 0.94)	0.02
<1%	108	101	1.19 (0.88, 1.61)	
≥5%	95	86	0.54 (0.39, 0.76)	<0.001
<5%	136	138	1.31 (1.01, 1.71)	
≥10%	86	79	0.52 (0.37, 0.75)	<0.001
<10%	145	145	1.24 (0.96, 1.61)	
Not quantifiable at baseline	61	66	1.06 (0.73, 1.56)	

Figure 1.2. Top: Overall Survival and Progression-free Survival Hazard Ratios by PD-L1 Expression at Baseline in Non-Squamous NSCLC (CheckMate-057 trial, Borghaei et al, NEJM 2015). Bottom: Kaplan-Meier Curve of Overall Survival by PD-L1 Expression Level in Squamous NSCLC (CheckMate-017 trial, Brahma et al, NEJM 2015).

	Events, n/N	Median OS, mo (95% CI)	5-Year OS Rate, % (95% CI)
TPS ≥ 50%	104/138	15.4 (10.6 to 18.8)	25.0 (18.0 to 32.5)
TPS 1–49%	146/168	8.5 (6.0 to 12.6)	12.6 (7.9 to 18.5)
TPS < 1%	83/90	8.6 (5.5 to 10.6)	3.5 (0.7 to 10.0)

No. at risk

TPS ≥ 50%	138	101	83	70	56	52	45	42	40	39	37	32	16	6	3	1
TPS 1–49%	168	112	78	61	51	41	37	28	26	26	23	21	6	3	1	0
TPS < 1%	90	58	36	29	25	21	16	10	7	7	5	4	1	0	0	0
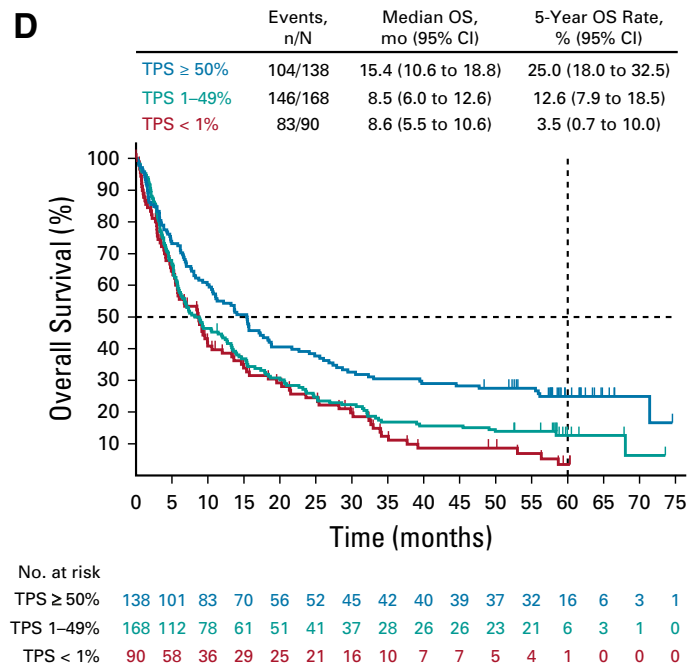
Figure 1.3. Five-year overall survival rates for pembrolizumab by PD-L1 TPS in KEYNOTE-001 trial (Garon et al, J Clin Oncol 2019).

1.2.4.3 Role of anti-angiogenesis inhibitors in relapsed NSCLC

Angiogenesis is involved in tumour growth and metastasis. Vascular endothelial growth factor (VEGF) and its receptor are crucial for the formation of new tumour vessels and are proven drug targets. Role of anti-angiogenic agents in advanced NSCLC has been investigated in multiple phase III trials and several drugs, such as bevacizumab, ramucirumab and nintedanib, have been found to improve outcomes when added to standard chemotherapy regimens, however others have failed to demonstrate a survival advantage. Most of these trials were done in the 1990s and early 2000s, prior to the molecular stratification era and before development of immune checkpoint inhibitors. More recently anti-angiogenic agents have been added to immunotherapy/chemotherapy combinations with some success, as discussed earlier. The key trials of anti-angiogenics in combination with chemotherapy in NSCLC are reviewed below, with landmark phase III trials summarised in Table 1.3.

Phase III trials of multi-targeted oral tyrosine kinase inhibitors cediranib, sorafenib and vandetanib were all negative for survival benefit when added to chemotherapy. Cediranib, oral inhibitor of VEGFR1–3, PDGFR-α/β, FGFR1 and c-kit, did not confer a survival advantage when added to carboplatin/paclitaxel chemotherapy versus chemotherapy alone in first-line advanced NSCLC, while toxicity was significantly increased and the trial was halted early.[47] Two large phase III trials of sorafenib, inhibitor of VEGFR2–3, PDGFR-β, c-kit, RAF and flt-3, failed to demonstrate survival benefit, with one trial of sorafenib with carboplatin/paclitaxel reporting a potential detriment with excess deaths in squamous patient group,[48] and the other showing improvement in PFS but not OS, when combined with gemcitabine/cisplatin in the first-line setting.[49] Sorafenib monotherapy was assessed versus placebo in a phase III trial in patients with non-squamous NSCLC, with significant improvement in median PFS (HR 0.61, p<0.0001), but not overall survival.[50] Results of a phase II trial combining sorafenib with pemetrexed in the second-line were disappointing with no improvement in PFS or OS, and the combination was not taken to phase III.[51] After promising phase II results, vandetanib, an inhibitor of VEGFR2, VEGFR3, RET and EGFR, was extensively investigated in several phase III trials in the second-line setting: in combination with docetaxel versus docetaxel alone in the ZODIAC trial,[52] with pemetrexed versus pemetrexed alone in the ZEAL trial,[53] and also as a single agent versus erlotinib alone in the ZEST trial,[54] with none

demonstrating improvement in overall survival, and with only ZODIAC trial showing modest PFS improvement.

On the background of these many negative trials, bevacizumab stands out as the first anti-angiogenic agent to confer survival benefit in NSCLC, but only when combined with paclitaxel. A humanized monoclonal antibody to vascular endothelial growth factor, it has been found to prolong OS and PFS when added to first-line platinum-based chemotherapy in patients with advanced non-squamous NSCLC and is licensed for use in this setting. A systematic review and meta-analysis of all randomised phase II/III trials of bevacizumab in the treatment of 1st line advanced or metastatic NSCLC has found that compared with chemotherapy alone, bevacizumab significantly prolonged OS (HR 0.90; 95% CI: 0.81–0.99; p=0.03), and PFS (0.72; 95% CI: 0.66–0.79; p<0.001).[55] Bevacizumab has been combined with 3-weekly nab-paclitaxel and carboplatin in first-line non-squamous advanced or metastatic NSCLC in an open-label single arm phase II trial.[56] This combination was well tolerated, with mild neutropaenia, manageable side effects and median overall survival of 16.8 months (95% CI: 10.4–21.6 months). In the phase III ULTIMATE trial,[57] bevacizumab in combination with weekly paclitaxel was compared to docetaxel in the second and third line setting in non-squamous NSLCL. 166 patients with non-squamous NSCLC progressing after 1 or 2 previous lines of treatment, were randomised in a 2:1 fashion to receive weekly paclitaxel plus bevacizumab (paclitaxel 90 mg/m² D1, 8, 15 and bevacizumab 10 mg/kg D1, 15, q28d) or docetaxel (75mg/m2 q21d). The trial met its primary end-point of progression free survival, with an adjusted hazard ratio for PFS of 0.62 in favour of bevacizumab and weekly paclitaxel over docetaxel (95% CI: 0.44–0.86, p=0.005). Median PFS was 5.4 months for weekly paclitaxel/bevacizumab vs. 3.9 months for docetaxel, while ORR was 22.5% and 5.5% respectively (p=0.006). Crossover was allowed, with 38% of patients crossing over from docetaxel to weekly paclitaxel/bevacizumab arm, and no difference in OS was observed.

Ramucirumab, a recombinant human monoclonal antibody of the immunoglobulin G1 class that specifically binds to and blocks the activation of vascular endothelial growth factor receptor-2 (VEGFR-2), is FDA and EMA approved for use in combination with docetaxel for the treatment of patients with relapsed metastatic NSCLC regardless of histology, based on improvement in OS with an acceptable toxicity profile in a randomized, multicenter, double-blinded, placebo-controlled trial of 1,253 patients with relapsed metastatic NSCLC.[38] In the REVEL trial, patients who received ramucirumab in combination with docetaxel had

improved OS versus docetaxel alone (mOS 10.5 vs. 9.1 months, HR 0.86; 95% CI: 0.75–0.98), but unlike bevacizumab and nintedanib (see following section) where benefit was largely seen in non-squamous NSCLC, the OS benefit for ramucirumab was observed in both squamous and non-squamous NSCLC patients, hence ramucirumab is licenced in this setting in all histologies. Ramucirumab has also shown activity in the 1st line setting in combination with platinum/pemetrexed chemotherapy in non-squamous NSCLC[58] and in combination with erlotinib in patients with advanced NSCLC and activating EGFR mutations.[59]

Clinical Trial/ Year of publication	Setting/ Histology	Treatment regime	No. of patients	Median PFS (m)	HR (95% CI)	Median OS (m)	HR (95% CI)
ECOG4599 2006	First-line NSCC	CPa+Bev vs. CPa	434 444	6.2 vs. 4.5	0.66 (0.57–0.77)	12.3 vs. 10.3	0.79 (0.67–0.92)
PointBreak 2013	First-line NSCC	PemCBev vs. PacCBev	472 467	5 vs. 5.6	0.83 (0.71–0.96)	12.6 vs. 13.4	1.0 (0.86–1.16)
BEYOND 2015	First-line NSCC	CPa+Bev vs. CPa+Placebo	138 138	9.2 vs. 6.5	0.40 (0.29–0.54)	24.3 vs. 17.7	0.68 (0.50–0.93)
AVAPERL1 2016	First-line NSCC	PemCisBev→PemBev vs. PemCisBev→Bev	128 125	7.4 vs. 3.7	0.5 (0.44–0.75)	17.1 vs. 13.3	0.87 (0.63–1.21)
REVEL 2014	Second-line NSCC + SCC	Doc+Ram vs. Doc+Placebo	628 625	4.5 vs. 3.0	0.76 (0.68–0.86)	10.5 vs. 9.1	0.86 (0.75–0.98)
LUME-Lung1† 2014	Second-line NSCC + SCC	Doc+Nintedanib vs. Doc	655 659	4.2 vs. 2.8	0.77 (0.62–0.96)	12.6 vs. 10.3	0.83 (0.70–0.99)
LUME-Lung2 2016	Second-line NSCC + SCC	Pem+Nintedanib vs. Pem+Placebo	353 360	4.4 vs. 3.6	0.83 (0.70–0.99)	12.0 vs. 12.7	1.01 (0.85–1.21)
ULTIMATE 2016	Second/Third-line NSCC	wPa+Bev vs. Doc	111 55	5.4 vs. 3.9	0.62 (0.44–0.86)	9.9 vs. 10.8	1.15 NK
ALTER 2018	Third-line NSCC + SCC	Anlotinib vs. Placebo	296 143	5.4 vs. 1.4	0.25 (0.19–0.31)	9.6 vs. 6.3	0.68 (0.54–0.87)

Table 1.3. Key phase III trials of combination chemotherapy with anti-angiogenic agents in advanced NSCLC. Bev, bevacizumab; C, carboplatin; Cis, cisplatin; Doc, docetaxel; HR, hazard ratio; m, months; NK, not reported; Pa, paclitaxel; Pem, pemetrexed; Ram, ramucirumab; wPa, weekly paclitaxel. †survival results given for adenocarcinoma patients.

1.2.4.4 Development of nintedanib

Nintedanib (BIBF 1120) is an orally available potent small molecule triple kinase inhibitor targeting VEGFR 1-3, fibroblast growth factor receptors (FGFR) 1-3 as well as platelet-derived growth factor receptor (PDGF) α and ß in the low nanomolar range. The specific and simultaneous abrogation of these pathways may be more effective than inhibition of endothelial cell growth via VEGF pathway alone. Furthermore preclinical models have shown that nintedanib may have a direct anti-tumour effect on those malignant cells which overexpress PDGFR and/or FGFR.

The efficacy of nintedanib in combination with chemotherapy in relapsed advanced or metastatic NSCLC was investigated in LUME-Lung 1, a randomised phase III trial, in which nintedanib plus docetaxel was compared to placebo plus docetaxel in relapsed NSCLC patients.[37] 1,314 patients with stage IIIb/IV recurrent NSCLC who had received one prior chemotherapy treatment were randomised in a 1:1 fashion to receive docetaxel with nintedanib or docetaxel with placebo; primary end-point was PFS by central independent review, and a key secondary endpoint was OS. PFS was significantly improved in the docetaxel plus nintedanib group compared with the docetaxel plus placebo group (median 3.4 months vs. 2.7 months; HR 0.79, p=0.0019) in the overall patient population. PFS for patients with adenocarcinoma was statistically significantly longer with docetaxel/nintedanib compared with docetaxel/placebo both at the time of primary PFS analysis (median PFS 4.0 vs. 2.8 months, HR 0.77, p=0.0193) and at the time of final PFS analysis (median PFS 4.2 vs. 2.8 months, HR 0.84; p=0.0485). After a median follow-up of 31.7 months, overall survival was significantly improved for patients with adenocarcinoma histology (322 patients in the docetaxel plus nintedanib group and 336 in the docetaxel plus placebo group; median OS 12.6 vs. 10.3 months; HR 0.83, p=0.0359), but not in the total study population (median OS 10.1 vs. 9.1 months; HR 0.94, 95% CI: 0.83–1.05, p=0.2720). In a predefined subgroup of patients with adenocarcinoma who had progressed within 9 months after start of first-line treatment, overall survival was significantly longer in the docetaxel plus nintedanib group (206 patients) compared with those in the docetaxel plus placebo group (199 patients; median 10.9 months vs. 7.9 months; HR 0.75, p=0.0073).

In this phase III study, adverse events that were more common in the docetaxel plus nintedanib group than in the docetaxel plus placebo group were diarrhoea, reversible increases in ALT and AST, neutropaenia grade ≥3, nausea, decreased appetite and vomiting.

The rate of grade ≥3 febrile neutropaenia in adenocarcinoma patients was 7.2% in the nintedanib arm (compared to 4.5% in the placebo arm). In the adenocarcinoma population, 17.2% of patients required at least one dose reduction to nintedanib while 16.9% of patients required a docetaxel dose reduction. The number of patients in the adenocarcinoma population who experienced a fatal AE unrelated to PD was 20 vs. 8 (6.2% vs. 2.4%) in the nintedanib and placebo arms respectively. The most common fatal AEs were sepsis (3 vs. 0), respiratory failure (2 vs. 0), and dyspnoea (0 vs. 2).

This trial led to EMA license for nintedanib at a dose of 200mg BID d2-21, q21, in combination with docetaxel 75mg/m^2 d1, q21 in adenocarcinoma non-small cell lung cancer after first line chemotherapy.

1.2.4.5 Docetaxel and nintedanib in relapsed NSCLC after ICIs

Since the introduction of ICIs as standard first-line systemic therapy in advanced NSCLC, there is emerging evidence for the role of anti-angiogenic agents in the immunotherapy era. Pre-clinical and early clinical studies have demonstrated that VEGF is an important factor in the immunosuppressive tumour micro-environment (TME), enabling the tumour to evade immune surveillance, promoting and inducing inhibitory immune-cell growth, including regulatory T-cells and myeloid derived suppressor cells (MDSCs), as well as inhibiting T-cell development and lymphocyte adhesion.[60–62] Abnormal tumour vasculature results in hypoxia and acidosis, which in turn leads to immunosuppressive TME via several different mechanisms including increased activation and expansion of immunosuppressive immune cells (regulatory T-cells, inflammatory monocytes and tumour-associated macrophages), suppression of dendritic cell maturation, causing impaired antigen presentation and activation of tumour-specific cytotoxic T lymphocytes, and up-regulation of PD-1 and PD-L1 expression on the immune cells as well as on tumour cells.[63] The interplay between immunosuppression and angiogenesis in the tumour micro-environment is illustrated in Figure 1.4. VEGF inhibitors have been shown to normalise the tumour vasculature and improve delivery of therapeutic agents to the tumour,[64, 65] as well as increasing infiltration and accumulation of immune effector cells in the tumour.[66] Therefore, by converting the immunosuppressive TME into an immunosupportive one, combining anti-angiogenic agents and immunotherapies may lead to increased effectiveness of immunotherapy and a favourable therapeutic outcome.

The synergistic effect of immunotherapy and anti-angiogenesis was investigated in several clinical trials combining ICIs with anti-angiogenic agents including the previously discussed phase III IMPower150 trial which established atezolizumab/bevacizumab plus ChT as a potential treatment option in first-line non-squamous advanced NSCLC.[67] In this trial, when compared to the control arm of chemotherapy alone (carboplatin/paclitaxel), survival benefit was only observed after addition of bevacizumab to atezolizumab/ChT combination (4 drug combination) and not for the atezolizumab/ChT arm (3 drug combination). This effect was particularly evident in the *EGFR* mutant NSCLC subgroup. Furthermore, evidence is emerging for the role of anti-angiogenesis agents in the setting of ICI resistance, following progression on or after ICI therapy. In one retrospective study of US patients treated with VEGF2 inhibitor ramucirumab-containing regimens and/or ICIs, patients receiving ramucirumab after previous ICI-based therapy had an unadjusted median OS of 26.5 months (95% CI: 23.7–33.0) after diagnosis, compared with 23.1 months for those treated with ICIs who never received ramucirumab containing regimens.[68] In another retrospective study of 11 European patients treated with nintedanib/docetaxel after platinum-based chemotherapy and immunotherapy reported an ORR of 36% and DCR of 82%.[69] Results from an initial analysis of a German prospective non-interventional study of nintedanib-docetaxel after prior platinum-based chemotherapy and treatment with an ICI reported an ORR of 58%, and median PFS of 5.8 months (ORR 4.7% and PFS 4.2 months in the LUME-Lung1 trial) for docetaxel/nintedanib after ICIs, with the overall survival data not yet mature.[70] While these were small non-randomised studies and direct comparisons with phase III trial data is not appropriate, taken together these data show a consistent signal of increased effectiveness of chemotherapy/anti-angiogenic agent combinations after exposure to ICIs that need to be further explored in larger randomised placebo-controlled trials, with several phase I/II trials currently ongoing (NCT01454102, NCT02574078, NCT02681549, NCT02039674).
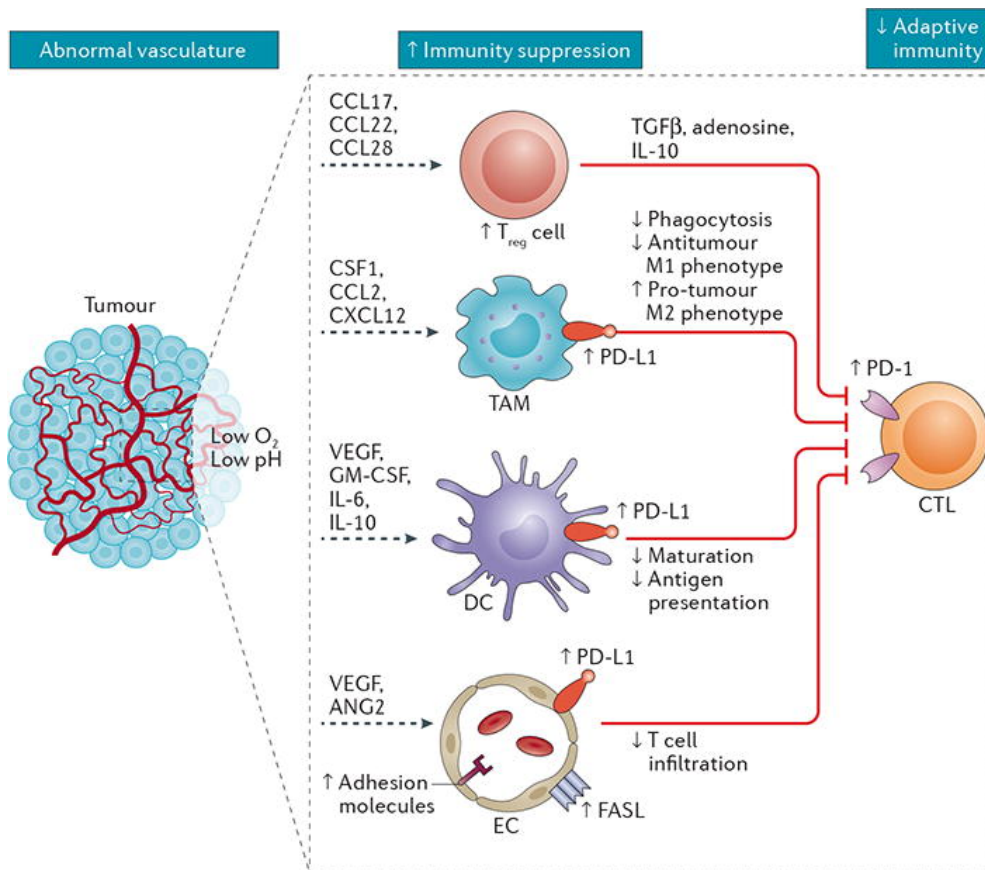
Figure 1.4. The role of tumour angiogenesis in inducing immunosuppression in the tumour microenvironment. ANG2, angiopoietin 2; CCL, C-C-motif chemokine ligand; CXCL12, C-X-C-motif chemokine ligand 12; CSF1, macrophage colony-stimulating factor 1; FASL, FAS antigen ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; TGFβ, transforming growth factor β. (Reproduced with permission from: Fukumura et al, Nat Rev Clin Oncol. 2018 May; 15(5): 325–340.)

1.3 Genotype-directed therapy for advanced/metastatic NSCLC

1.3.1 Development of currently licenced targeted therapies in advanced NSCLC

Identification of driver somatic aberrations in advanced NSCLC has led to rational implementation of genotype-directed therapy, with international guidelines recommending molecular testing.[71, 72] EGFR and ALK kinase inhibitors have demonstrated marked improvement in progression free survival over chemotherapy in those harbouring activating *EGFR* mutations and *ALK* rearrangements, respectively, and are licensed alongside ROS1 kinase inhibitors.[73–79]

1.3.1.1 Treatment of EGFR-mutated NSCLC

Oncogenic driver alterations in the *EGFR* gene are found in around 10-20% patients with NSCLC (with much higher prevalence in Asian countries), representing the commonest targetable oncogenic driver in NSCLC.[80, 81] Several first-, second- and third-generation EGFR-TKIs are licenced in EGFR-positive (EGFR+) NSCLC having demonstrated significant improvements in PFS, tolerability and safety over chemotherapy in this patient subgroup.[74, 75, 82, 83] None had successfully demonstrated an overall survival benefit, likely due to high levels of crossover, until publication in 2014 of a pooled analysis of LUX-Lung 3 and LUX-Lung 6, two large phase III trials of afatinib versus chemotherapy, which reported a 3 month overall survival benefit of afatinib in the subgroup of treatment-naïve patients with *EGFR* exon 19 deletion.[84] Subsequently, ARCHER 1050 phase III trial comparing second-generation EGFR-TKI dacomitinib with first-generation TKI gefitinib,[85, 86] reported significant improvement in PFS (mPFS 14.7 vs. 9.2 months; HR 0.59, 95% CI: 0.47–0.74, p<0.0001) and OS (mOS 34.1 months vs. 26.8 months; HR 0.76, 95% CI: 0.58–0.993, p=0.04) for dacomitinib over gefitinib. This trial excluded patients with CNS metastases, and while the overall impact of this is unknown, this could have had an impact on several of the efficacy analyses.

In the FLAURA trial, third-generation wild-type-sparing irreversible EGFR inhibitor osimertinib, which was originally developed to target the resistance *EGFR* mutation *T790M*, was compared against first-generation EGFR-TKIs (erlotinib or gefitinib) in treatment-naïve EGFR+ NSCLC,[87, 88] with PFS as the primary endpoint and OS a key secondary endpoint. This showed significant improvement in PFS (mPFS 18.9 vs. 10.2 months, HR 0.46, 95% CI: 0.37–0.57, p<0.001) and OS (mOS 38.6 vs. 31.8 months, HR 0.80, 95% CI: 0.64–1.00, p=0.046). Cross-over to osimertinib was permitted upon confirmation of presence of T790M

resistance mutation, but only 31% of patients in the erlotinib/gefitinib group crossed over to osimertinib. This may have contributed to the observed OS benefit, which only just reached statistical significance. In this trial, osimertinib also had improved CNS activity, with CNS progression events observed in 6% of patients in the osimertinib group versus 15% in the 1st generation TKI group.[87]

Toxicity profiles vary between EGFR-TKIs, with irreversible inhibitors dacomitinib and afatinib associated with higher incidence of grade 3 skin and gastrointestinal toxicity. In LUX-Lung 7 trial, a phase IIb trial of afatinib vs. gefitinib, treatment-related grade 3 or 4 adverse events of diarrhoea occurred in 13% of patients treated with afatinib vs. 1% on gefitinib, and rash or acne in 9% on afatinib vs. 3% on gefitinib.[89] In the ARCHER 1050 trial, the rate of grade 3-4 treatment-related acneiform dermatitis was 14% with dacomitinib vs. 0% for gefitinib, and 8% vs. 1% for grade 3-4 diarrhoea.[85] Third-generation inhibitors are associated with a more favourable tolerability, with a rate of grade 3 or higher adverse events for osimertinib of 34% versus 45% for 1st generations TKIs.[87]

EGFR-TKIs have been combined with angiogenesis inhibitors in several phase II and phase III trials, with some evidence of improvement in PFS with addition of bevacizumab or ramucirumab to erlotinib, however so far no overall survival benefit has been observed, with final OS results from two phase III trials pending.[59, 90]

Landmark phase III trials of first-generation reversible EGFR-TKIs gefitinib and erlotinib, second-generation irreversible ErbB family inhibitors afatinib and dacomitinib, and third-generation inhibitor osimertinib are summarised in Table 1.4.

Trial/ Year of publication	EGFR TKI vs. comparator	Patient population	No. of patients	PFS (m)	HR for PFS (CI)	OS (m)	HR for OS (CI)	HR for OS (CI) del19 v. L858R	Median follow-up (m)
IPASS 2009, 2011	Gefitinib vs. Carboplatin + Paclitaxel	Adenocarcinoma Any EGFR status (EGFR+ subgroup analysis) Asian	261 EGFR+ (total 1217)	9.5 vs. 6.3	0.48 (0.36 - 0.64)	21.6 vs. 21.9	1.00 (0.76 - 1.33)	-	17.0
EURTAC 2012	Erlotinib vs. Carbo/cisplatin + Docetaxel or Carbo/cisplatin + Gemcitabine	EGFR+ (European)	173	9.7 vs. 5.2	0.37 (0.25 – 0.54)	19.3 vs. 19.5	1.04* (0.65 - 1.68)	-	18.9
LUX-LUNG 3 2013	Afatinib vs. Cisplatin + Pemetrexed	EGFR+ (Global)	345	11.1 vs. 6.9	0.58 (0.43 – 0.78)	28.2 vs. 28.2	0.88 (0.66 - 1.17)	0.54 (0.36, 0.79) 1.30 (0.80, 2.11)	41
LUX-LUNG 6 2014	Afatinib vs. Cisplatin + Gemcitabine	EGFR+ (Asian)	364	11.0 vs. 5.6	0.28 (0.20 – 0.39)	23.1 vs. 23.5	0.93 (0.72-1.22)	0.64 (0.44, 0.94) 1.22 (0.81, 1.83)	33
LUX-LUNG 7† 2016, 2017	Afatinib vs. Gefitinib	EGFR+	319	11.0 vs. 10.9	0.73 (0.57 – 0.95)	27.9 vs. 24.5	0.86 (0.66 - 1.12)	0.83 (0.58, 1.17) 0.91 (0.62, 1.36)	42.6
ARCHER 1050 2017, 2018	Dacomitinib vs. Gefitinib	EGFR+	452	14.7 vs. 19.2	0.59 (0.47 – 0.74)	34.1 vs. 26.8	0.76 (0.58 - 0.99)	0.88 (0.61, 1.26) 0.71 (0.48, 1.04)	31.3
FLAURA 2018, 2020	Osimertinib vs. Gefitinib or Erlotinib	EGFR+	556	18.9 vs. 10.2	0.46 (0.37 – 0.57)	38.6 vs. 31.8	0.80 (0.64 - 1.00)	0.68 (0.51, 0.90) 1.00 (0.71, 1.40)	35.8

Table 1.4. Summary of selected phase III trials of EGFR TKIs in first-line EGFR+ advanced/metastatic NSCLC. †Randomised Phase IIb trial; *exploratory analysis. CI, confidence interval; EGFR, erythrocyte growth factor receptor; HR, hazard ratio; m, months; mt, mutation; ORR, objective response rate; OS, overall survival; PFS, progression free survival; TKI, tyrosine kinase inhibitor.

1.3.1.2 Treatment of ALK-rearranged NSCLC

Anaplastic lymphoma kinase (ALK) gene rearrangements are found in around 4%–5% of all NSCLC patients. First-generation ALK-TKI crizotinib was the first to demonstrate improved efficacy over chemotherapy in both recurrent and treatment-naïve *ALK*-positive (ALK+) NSCLC in PROFILE 1007 and PROFILE 1014 trials.[76, 77] Subsequently, second-generation ALK inhibitors alectinib and brigatinib have shown improved intracranial efficacy over crizotinib.[91, 92] In a direct head-to-head with crizotinib in treatment-naïve ALK+ NSCLC in the global phase III ALEX trial, treatment with alectinib resulted in doubling of the PFS (mPFS 25.7 vs. 10.4 months; HR 0.50; 95% CI: 0.36–0.70; P<0.001) and near quadrupling of the intracranial control rates (12% vs. 45% rate of CNS progression).[91] Crizotinib is licenced for treatment-naïve ALK+ NSCLC, while alectinib, brigatinib and ceritinib are licenced in treatment-naïve and crizotinib-resistant ALK+ NSCLC. Third-generation ALK-TKI lorlatinib, developed to have greater intracranial penetration, is currently licenced after progression on alectinib or ceritinib as first ALK-TKI therapy; or after crizotinib and at least one other ALK-TKI. Interim results of the global phase III CROWN study of lorlatinib versus crizotinib in treatment-naïve ALK+ NSCLC were recently published.[93] Median PFS by blinded independent central review was significantly longer with lorlatinib (mPFS 18.3 vs. 14.8 months, HR 0.28, 95% CI: 0.19–0.41, p<0.001). The rate of intracranial response among those with measurable brain metastases was 82% with lorlatinib versus 23% with crizotinib, with 71% of the patients who received lorlatinib having an intracranial complete response. There was an increased rate of grade 3-4 adverse events with lorlatinib (72.5% vs. 56), these were mainly laboratory lipid abnormalities, and did not lead to an increased rate of treatment discontinuation for lorlatinib (7% vs. 9% for crizotinib). Optimal sequencing of ALK-TKIs in ALK+ NSCLC remains a subject of ongoing research.[94]

1.3.1.3 Treatment of ROS1-rearranged NSCLC

ROS1 gene rearrangements are found in around 2% of NSCLC patients.[95] Crizotinib is licenced in the first-line setting or as second line in patients with ROS1+ metastatic NSCLC. Due to small patients numbers, evidence for efficacy of crizotinib in this patients population comes largely from small prospective phase II and retrospective studies with mPFS ranging between 9 to 13.4 months and response rates of up to 80%.[78, 96] Entrectinib, a multikinase inhibitor with activity against ROS1, pan-TRK and ALK, is licenced in treatment-naïve ROS1+

NSCLC, having demonstrated activity in a prespecified integrated sub-groups analysis of 53 ROS1-TKI naïve patients from three phase I/II trials.[97] Response rate was 77%, median PFS was 19 months and intracranial response rate 55% (11 out of 20 patients with baseline CNS metastases). Within the limitations of cross-trial comparisons, rates of ORR and PFS for entrectinib were similar to those reported for crizotinib in PROFILE 1001 trial, however entrectinib appears to have much better brain penetration than crizotinib, with no data on CNS efficacy in ROS1+ patients reported for crizotinib. While the data is limited, entrectinib appears to have minimal or no efficacy when used after crizotinib.[98] Ceritinib, brigatinib, lorlatinib and repotrectinib have all also shown promising anti-ROS1 activity in small phase I/II studies.[99–101]

1.3.1.4 Treatment of KRAS-mutated NSCLC

KRAS is one of the most commonly mutated oncogenes in cancer. *KRAS* mutations are found in around a third of NSCLC adenocarcinomas, with majority being a single point glycine-to-cysteine substitution at codon 12 (*KRAS* G12C) resulting in constitutive activation of the KRAS oncoprotein.[102] Until very recently, KRAS was considered "undruggable" after years of repeated efforts to directly target mutant KRAS yielded minimal success. A breakthrough arrived with development of novel covalent inhibitors targeting the mutant cysteine residue, with the first-in-class agent sotorasib being granted FDA and EMA approval in 2021 for advanced *KRAS* G12C-mutated NSCLC after progression on at least one prior therapy, based on the primary analysis results of the CodeBreaK100 trial, an ongoing phase I/II registrational trial, which demonstrated ORR of 37% and median PFS of 6.7 months with sotorasib in this patient population.[103] Several other inhibitors targeting *KRAS* G12C are currently being tested in clinical trials (NCT03785249, NCT04006301), along with a number of other mutation-specific inhibitors and pan-KRAS strategies, both as monotherapy and in combination with ICIs (NCT04111458, NCT03948763, NCT044117087). *KRAS* G12C mutation testing should be undertaken routinely at time of first diagnosis preferably as part of a reflex testing approach in parallel with other driver targets and is included in The National Genomic Test Directory for cancer multi-target NGS panel for NSCLC. A number of questions remain regarding *KRAS* testing after prior systemic therapy, clonal versus sub-clonal *KRAS* mutations and impact of co-occurring mutational landscape in targeting *KRAS*-mutant NSCLC, further strengthening the case for broader molecular profiling where possible.

1.3.1.5 Treatment of NSCLC with other oncogenic drivers

Alterations in *BRAF*, *RET*, *MET*, *HER2* and *NTRK* have all been identified as rare oncogenic drivers and potential therapeutic targets in advanced NSCLC.

BRAF mutations are found in 1-2% of lung adenocarcinoma NSCLC. BRAF inhibitor dabrafenib is licenced in combination with MEK inhibitor trametinib for treatment of advanced NSCLC with *BRAF V600* mutations, based on the results of a prospective phase II trial which reported the ORR of 66% and mPFS 10.2 months with combination dabrafenib and trametinib in pretreated patients, and the ORR of 64% and mPFS 10.9 months in treatment-naïve patients.[104, 105] Updated survival analyses have reported median OS of 18.2 and 24.6 months in pretreated and treatment-naïve patients, respectively.[105, 106]

RET fusions (found in 1-2% of NSCLC), *MET* aberrations (3-4%) and *HER2* dysregulation (1-5%) are all promising targets with inhibitors in clinical trial development. Specific *RET*-fusion inhibitors pralsetinib and selpercatinib have both demonstrated promising safety and efficacy in *RET*-rearranged NSCLC in ongoing phase I/II trials, and are FDA-approved but not yet EMA licenced. For pralsetinib, ORR was 61% and 73% among patients with previously treated and treatment-naive RET fusion-positive NSCLC, respectively.[107] Selpercatinib had an ORR of 64% in patients previously heavily pre-treated patients and 85% in previously untreated patients, with objective intracranial response observed in 10 out of 11 patients with measurable intracranial disease at baseline.[108]

MET exon 14 variants are the commonest oncogenic *MET* aberration and are sensitive to inhibition by crizotinib, with ORR of 32% and mPFS of 7.3 months reported for 69 patients with *MET*-exon-14-altered NSCLCs enrolled in an expansion cohort of the phase I trial PROFILE 1001.[109] Targeting *HER2* mutations (primarily exon 20 insertions) and *HER2* overexpression with antibody-drug conjugate adotrastuzumab emtansine (TDM-1) in advanced NSCLC has shown promising results in phase II basket.[110, 111] Fusions in *NTRK* genes are rare oncogenic drivers in multiple cancers including NSCLC (<1%). Basket trials of inhibitors of NTRK fusion proteins have demonstrated durable responses in solid tumours including NSCLC,[98, 112, 113] with entractinib and larotrectinib both EMA and FDA licenced for *NTRK* fusions-positive solid tumours regardless of histological subtype.

1.3.2 Acquired TKI resistance

Most patients with oncogene-addicted NSCLC will experience good initial responses to oncogene-directed therapies, however resistance inevitably develops leading to disease progression. Multiple mechanisms of acquired resistance to molecular-directed therapy have been identified including on-target mechanisms (emergence of acquired somatic changes to the drug binding site leading to reduced affinity for drug, for instance the *EGFR T790M* gatekeeper mutation; gene copy number increase), off-target effects (histological transformation, bypass track activation), lack of CNS penetration and pharmacokinetic issues (CYP up and down-regulation, interactions with other agents such as tobacco and proton pump inhibitors), as well as other as yet unidentified mechanisms.[114–118]

The most common mechanism of acquired resistance to 1st and 2nd generation EGFR TKIs, occurring in around 50-60% cases, is the T790M mutation in the EGFR tyrosine kinase domain.[117, 119] It was first identified in 2005 as a secondary mutation in patients treated with gefitinib after initial response, by two independent groups of researchers.[120, 121] Threonine 790 is the "gatekeeper" residue involved in determining inhibitor specificity in the ATP binding pocket. T790M mutation increases the ATP affinity of mutant EGFR, thereby reducing the potency of ATP-competitive inhibitors, an effect which can be overcome by irreversible inhibitors through covalent binding.[114] A handful of other secondary *EGFR* mutations have been identified including L747S, D761Y and T854A.[122–124] Mutations in *ALK* tyrosine kinase domain are also an important on-target mechanism of resistance in ALK+ patients, occurring in around 20-30% of patients, which can co-occur with *ALK* copy number gain.[125]

The commonest off-target effect is the activation of so called bypass mechanisms, which use alternative cellular pathways to activate the same downstream effectors of tumour cell survival and growth. Amplification of *HER2* occurs in 10-15% of patients and *MET* amplification in around 5% patients with EGFR TKI resistance, and can co-occur with T790M, while in ALK+ tumours important bypass track mechanisms include *EGFR* mutations and amplification, *KRAS* mutations, *HER2* phosphorylation and *cKIT* amplification.[119, 126] Phenotypic transformation to small-cell lung cancer is another well described off-target mechanism of resistance in up to 10% case, while epithelial-to-mesenchymal transition occurs in around 1-2% cases.[116, 119, 127–129]

Currently described molecular mechanisms of acquired resistance in EGFR+ to 1st and 2nd generation EGFR TKIs and ALK+ NSCLC are illustrated in Figure 1.5.

Therapeutic strategies to overcome mechanisms of acquired resistance are being developed, and in some cases licensed. For example, the *EGFR* mutation-specific irreversible kinase inhibitor osimertinib has high potency and inhibitory activity both against classical activating *EGFR* mutations (e.g. apparent IC_{50} of 9nm and 12nm for L858R and exon 19 deletion, respectively, in vitro) and the resistance mutation T790M (apparent IC_{50} 3nm to 13nm), leading to inhibition in cell growth, while showing significantly less activity in wild type cell lines.[130] Results of a randomised phase III trial of osimertinib versus platinum-pemetrexed in *EGFR* T790M-positive lung cancer on or after first-line EGFR-TKI demonstrated significantly longer median PFS for osimertinib,[131] resulting in FDA and EMA licenses for osimertinib in this indication. Second and third generation ALK-TKIs targeting acquired resistance mutations in the ALK tyrosine kinase domain have also been identified and licenced as described above, however the licences for these agents are not conditional on the resistance genotype.
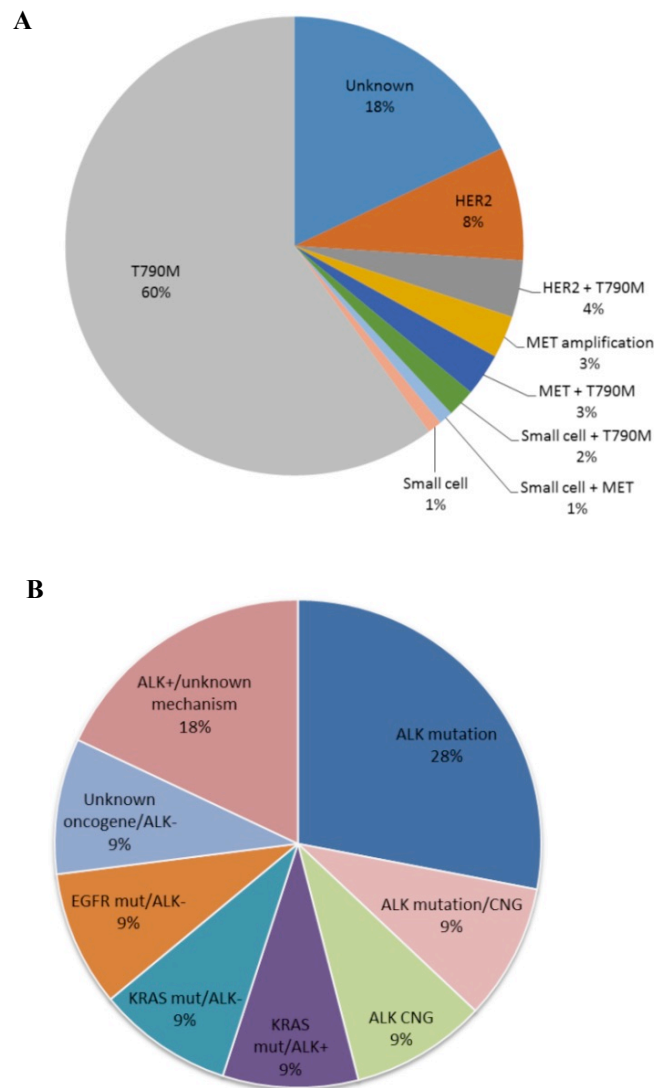
Figure 1.5. Relative frequencies of TKI resistance mechanisms in EGFR+ (A) and ALK+ (B) NSCLC (adapted from Yu et al, Clin Cancer Res. 2013, 19(8): 2240–2247 and Doebele et al, Clin Cancer Res. 2012, 18(5): 1472–1482)

1.3.3 Current directions

With increasing understanding of NSCLC as a heterogenous group of diseases driven by multiple somatic aberrations occurring at varying frequencies, a variety of global efforts are underway to identify and validate the efficacy of genotype-directed therapy in NSCLC through the design of multi-arm multi-agent (MAMA) trials, such as the NCI-MATCH trial (NCT02465060) and the UK National Lung MATRIX Trial (NCT02664935). When identifying novel drug-target combinations for future development, MAMA or Platform trials are able to investigate multiple hypotheses through concurrent sub-studies, resulting in greater efficiency in terms of cost, time and patient numbers, as well as providing enhanced

adaptivity (using pre-specified adaptation rules) and improved standardisation through use of a master protocol, compared to multiple separate small phase Ib/II trials. Such trials commonly employ molecular pre-screening protocols, involving extensive next-generations sequencing-based molecular genotyping panels, to molecularly characterise and sub-classify tumours and match them to rationally selected targeted agents. Such molecular panels are also designed to include known tumour suppressor genes and other molecular alterations that are associated with therapeutic resistance. The global NCI-MATCH trial which enrolled over 6000 patients of varying histologies after relapse on standard therapies, including over 400 patients with NSCLC, recently reported an interim analysis of feasibility of such trial design, with successful molecular profiling achieved in 87% of patients, potentially actionable alterations identified in over one third of patients and nearly 18% assigned to a matched therapy.[132] The causes for the high attrition rate between proportion of actionable alterations identified and the rate of matching to a treatment included identification of co-occurring alteration known to confer resistance leading to exclusion from treatment in over 37% of patients with an actionable alteration and lack of sub-protocol availability due to accrual being reached. For those sub-protocols whose targeted alteration had a prevalence of <1.5%, none reached the accrual goal, highlighting the challenge of developing novel therapies in less common molecular subgroups, where much larger screening population will be needed to enrol sufficient patient numbers.

1.4 Molecular genotyping in NSCLC: current technologies and challenges

Target identification at diagnosis, identification of T790M and other resistance mechanisms, choice of optimal therapy and development of novel treatment strategies in NSCLC are all predicated by tumour histological and molecular characterisation. Repeated molecular profiling is likely to be required at multiple time points during the treatment pathway, as is already the case for *EGFR* T790M mutation detection, given molecular heterogeneity identified from sequencing studies, and evolutionary pressures of molecular selection from targeted therapy in oncogene-addicted NSCLCs. [133, 134]

Current international molecular testing guidelines recommend testing for alterations in *EGFR*, *ALK*, *ROS1, KRAS* and *BRAF* genes as a minimum in all patients with non-squamous non-small cell lung cancer (NSCLC), and should be considered for patients with squamous cell NSCLC with atypical clinical features (younger, never-smokers).[72] Additional repeated molecular testing performed upon development of resistance to initial targeted therapies may help to identify acquired molecular resistance mechanisms and any drug susceptibility. Increasingly, it may be beneficial to extend molecular testing beyond those molecular alterations for which targeted therapies are approved by regulatory agencies to include molecular alterations for which there is compelling evidence of effective investigational targeted therapies from published clinical trials, with guidelines recommending *RET*, *MET* and *ERBB2 (HER2* alterations be included in any extended panel testing. Furthermore, the role of broader genotyping outside the scope of therapy selection is gaining in traction, with accumulating data, for instance, that *RB1* and *TP53* inactivating mutations predict a high risk of SCLC transdifferentiation in *EGFR* mutant NSCLC,[135] and similarly that *STK11* mutations may predict poor response to PD-1 inhibitors regardless of PDL1 expression in *KRAS*-mutant NSCLC.[136]

Several different technologies have been developed in the arena of molecular diagnostics, from single-gene assays to next-generation sequencing (NGS) platforms which allow simultaneous testing of a broad panel of genes. Furthermore, molecular testing using plasma circulating tumour DNA (ctDNA) obtained from peripheral blood samples has been developed as a potentially less invasive and faster alternative to a biopsy sample, or at least where a biopsy is not possible.[137–139] Additionally, in the relapsed metastatic setting, ctDNA may be representative of tumour DNA shedding from multiple disease sites, and therefore may enable broad sampling of different tumour sub-clones.[138] However, much is still

unknown about the relative benefits of ctDNA versus tissue-based testing, while wide implementation of NGS-based testing is often limited by resources and tissue quality resulting in variable tumour DNA and RNA quality and quantity.

1.4.1 Tissue molecular genotyping in NSCLC

Whilst ctDNA genotyping is an effective and validated technology for some alleles (e.g. *EGFR* T790M), contingent on clinical setting, the low specificity of some genotyping technologies coupled with the low ctDNA shedding rate for M1a disease (defined by the TNM 8th edition as metastases in contralateral lung or pleural/pericardial nodule/malignant effusion) may limit clinical interpretation. Tissue-based molecular genotyping therefore used to be the gold standard in the majority of clinical settings, but limitations of tissue genotyping are increasingly recognised including an appreciable false negative rate.[140] Increasing requirements for repeated molecular testing represent a challenge in terms of tissue availability and adequacy as well as potential for patient morbidity related to repeated invasive procedures. Safety and tissue diagnostic yields of biopsies at first diagnosis of lung cancer are well established,[141–143] however data remain limited on the adequacy of tumour material obtained by repeat biopsies in order to molecularly characterise tumours for clinical decision making.

Optimal tissue sampling technique, adequacy of formalin-fixed paraffin-embedded (FFPE) tissue versus cytology samples, optimal handling of samples in histopathology laboratories and minimum thresholds for sample cellularity required for molecular analyses are some of the questions that continue to pose challenges to tissue molecular genotyping in NSCLC. General consensus guidelines, developed to define pre-analytic measures and procedures which would optimise tissue-based molecular testing in the diagnostic setting, recommend involvement of a multidisciplinary team to provide individualised diagnostic and therapeutic plan, development of local standard operating procedures (SOPs) for handling of tissue samples and provide guidance on tissue conserving techniques.[144, 145]

1.4.1.1 Tissue molecular genotyping techniques

Methods for molecular genotyping in NSCLC are continually evolving, with conventional methods such as direct single-gene sequencing and FISH now largely replaced by DNA allele-specific PCR methods, NGS DNA and RNA sequencing and RNA fusion panels, due to

improved speed, sensitivity, cost-efficency and semi-automation achieved with the newer technologies.

Massively-parallel sequencing or next-generation sequencing (NGS) is a high-throughput method which allows rapid simultaneous sequencing of large numbers of DNA fragments, utilising bioinformatics analyses to map the individual reads to a human reference genome. Several different NGS approaches have been developed, including pyrosequencing-based techniques (Roche 454 pyrosequencing), sequencing by synthesis (Illumina, Qiagen GeneReader) and sequencing by ligation (SOLiD, CompleteGenomics), but all have several steps in common including library preparation (DNA fragmentation, ligation of adapter), clonal amplification on a solid surface (PCR or non-PCR based), sequencing and analysis against a reference genome. Illumina has become a dominant platform, due to it's maturity as a technology, depth of coverage, accuracy and availability of a range of instruments from the low-throughput benchtop units to the ultra-high-throughput instruments capable of population-level whole genome sequencing, epigenetic applications and transcriptome sequencing (RNA-seq). Following fragmentation and addition of adapters, Illumina technology utilises solid-phase bridge amplification creating many millions of clonal clusters directly on a patterned flow cell. After template enrichment, a mixture of primers, DNA polymerase and modified dNTPs, containing a terminator (allowing addition of only one nucleotide at a time) and labelled with a base-specific fluorophore, are added. After addition of each nucleotide, the clusters are imaged detecting the characteristic fluorescent signal of each base. The dye is then cleaved and the cycle of nucleotide addition, image capture and cleavage begins again, with the number of cycles determining the length of the read. For any given cluster, identical strands are read simultaneously with hundreds of millions of clusters sequenced in parallel producing billions of reads. Similar reads are clustered and aligned to the reference genome for analysis. The overview of Illumina NGS workflow is illustrated in Figure 1.6.
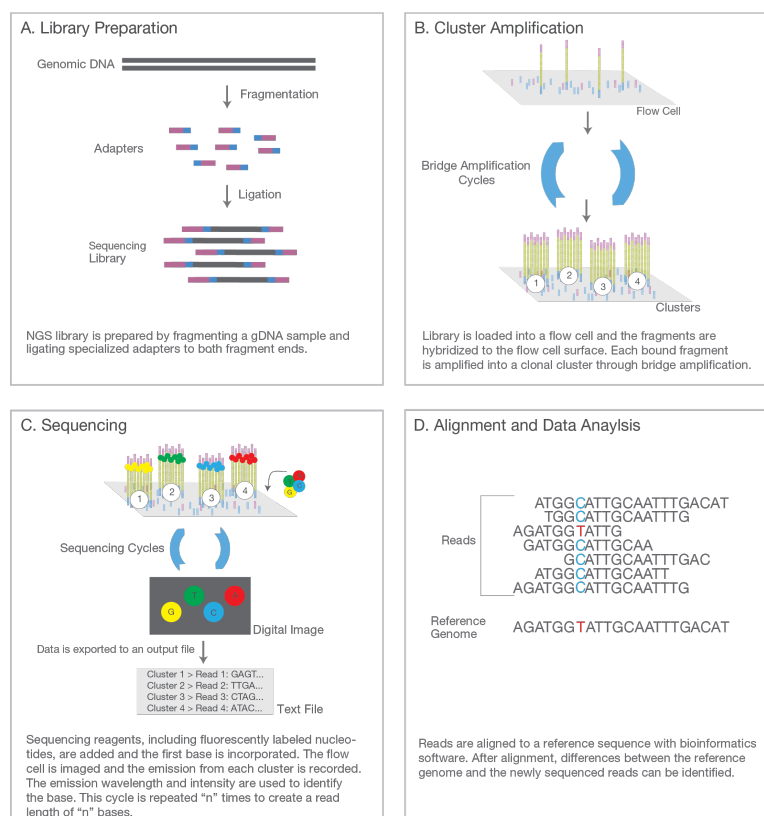
Figure 1.6. The four key steps of Illumina NGS: (A) library preparation, (B) cluster generation,(C) sequencing, and (D) alignment and data analysis. Reproduced with permission from illumina.com

The above described so-called "second-generation" NGS methods are designed to produce vast numbers of short reads in parallel, usually between 50-300bp in length depending on the platform, however novel "third-generation" methods aim to sequence much longer DNA and RNA reads in excess of several kilobases, allowing improved resolution of more complex areas of the genome such as copy number alterations and structural variants.

Complex NGS assays have been validated as highly sensitive and specific,[146] have become increasingly integrated into routine clinical practice. In 2019, NHS England launched the NHS Genomic Medicine Service with aim of providing equitable access and standardisation of a range of clinically appropriate genomic tests to patients in England, with establishment of a network of Genomic Laboratory Hubs and a National Genomic Test Directory setting out which genomic and genetic tests are commissioned, the technology by which they are available and patient eligibility. This includes multi-target NGS panels in NSCLC for detection of small variants, structural variants and copy-number variants (Figure 1.7.) and is to be reviewed annually, following a structured evidence-based process.

Multi-target NGS panel - small variant (EGFR, ALK, BRAF, KRAS)	EGFR, ALK, BRAF, KRAS	Small variant detection	Panel	Usually non-squamous NSCLC although there may be scenarios where clinicians wish to test other subtypes of NSCLC e.g. unusual phenotype, eligible for tyrosine kinase inhibitor therapy
Multi-target NGS panel - structural variant (ROS1, RET, ELM4-ALK, NTRK1, NTRK1, NTRK3)	ROS1, RET, ELM4-ALK, NTRK1, NTRK2, NTRK3	Structural variant detection	Panel	Molecular assessment will aid diagnosis or management NB Usually used in cases of non-squamous NSCLC although there may be scenarios where clinicians wish to test other subtypes of NSCLC e.g. unusual phenotype, eligible for tyrosine kinase inhibitor therapy / Patient's clinical status means they are eligible for an NTRK inhibitor in the event an NTRK rearrangement is detected
Multi-target NGS panel - copy number variant (MET)	MET	Copy number variant detection to exon level resolution	Panel	Molecular assessment will aid diagnosis or management
EGFR hotspot tumour	EGFR	Small variant detection	Simple targeted mutation testing	Usually non-squamous NSCLC although there may be scenarios where clinicians wish to test other subtypes of NSCLC e.g. unusual phenotype, eligible for tyrosine kinase inhibitor therapy, in rare cases where this cannot be delivered by panel testing. NB. Will be subject to close audit
EGFR hotspot ctDNA	EGFR	Small variant detection	Simple targeted mutation testing	To be used for detection of activating EGFR mutations in ctDNA when biopsy unavailable and patient otherwise eligible for tyrosine kinase inhibitor therapy
ROS1 rearrangement FISH/RT-PCR	ROS1	Structural variant detection	FISH/Simple targeted mutation testing	Molecular assessment will aid diagnosis or management
RET rearrangement FISH/RT-PC	RET	Structural variant detection	FISH	Molecular assessment will aid diagnosis or management
MET copy number FISH	MET	Copy number variant detection to genomewide resolution	FISH	Molecular assessment will aid diagnosis or management
EML4-ALK FISH/RT-PCR	ELM4-ALK	Structural variant detection	FISH/Simple targeted mutation testing	Usually non-squamous NSCLC although there may be scenarios where clinicians wish to test other subtypes of NSCLC e.g. unusual phenotype, eligible for tyrosine kinase inhibitor therapy
ALK hotspot cDNA	ALK	Small variant detection	Simple targeted mutation testing	Usually non-squamous NSCLC although there may be scenarios where clinicians wish to test other subtypes of NSCLC e.g. unusual phenotype, where knowledge of ALK mutations would alter management

Figure 1.7. Currently commissioned genomic tests, test methods and patient eligibility in NSCLC, The National Genomic Test Directory for Cancer 2020-2021. Adapted from https://www.england.nhs.uk/publication/national-genomic-test-directories/

1.4.2 Circulating tumour DNA genotyping in NSCLC

Tumour cells release fragments of DNA into the circulation, which can be isolated in the cell-free fraction of blood, together with DNA fragments from normal cells. These tumour-derived DNA fragments are known as circulating tumour DNA or ctDNA (to be distinguished from cell-free DNA or cfDNA, a broader term that describes DNA that is freely circulating in the blood stream but is not necessarily of tumour cell origin). The mechanism of tumour DNA shedding into the bloodstream is mostly via passive release from apoptotic and necrotic tumour cells.[147] ctDNA is highly fragmented with most fragments measuring between 160 and 200 base pairs.[148] Isolation of ctDNA typically requires 5–10 mL of plasma, collected in tubes containing EDTA, with plasma preferred to serum for ctDNA extraction.[149]

1.4.2.1 ctDNA for detection of EGFR mutations

Isolation and molecular analysis of ctDNA in non-small cell lung cancer was first investigated in the context of *EGFR*-mutated NSCLC in the diagnostic setting for detection of sensitising mutations and for prediction of response to first-line TKI therapy. In 2007, Kimura et al analysed the *EGFR* mutation status in tumour and plasma of 42 patients treated with gefitinib and demonstrated concordance with tumour *EGFR* mutation status of 92.9%,

sensitivity of 78.9% and a specificity of 97.0% using Scorpion-ARMS.[150] There was a correlation between the presence of *EGFR* mutations in plasma DNA and the objective responses to gefitinib as well as a trend towards increased overall survival in patients treated with gefitinib. Subsequently, several meta-analyses that collectively included more than 30 studies in 3000 patients reported pooled specificities of *EGFR* testing ranging from 88% to 97% and sensitivities from 62% to 67% for ctDNA with tissue as reference.[151–153] The studies were highly heterogenous in terms of patient populations, blood sampling protocols and DNA extraction and analysis methods, resulting in significant inter- and intra-method variability, albeit consistently reporting higher levels of specificity than sensitivity for ctDNA and best sensitivity for ARMS-based methods and the Roche cobas *EGFR* mutation test, an allele-specific PCR method. In terms of clinical utility of ctDNA *EGFR* testing in the setting of first-line EGFR-TKI therapy, results of pre-planned exploratory analyses from several large randomised trials inferred the same clinical utility for ctDNA positive *EGFR* as for tissue positive *EGFR* mutation, and are summarised in Table 1.5.[82, 139, 154–156] However, a substantial number of patients who were plasma-negative still benefited from EGFR-TKI therapy, reinforcing that tissue testing remains a gold standard for establishing a primary diagnosis of lung adenocarcinoma, due to lower sensitivity of ctDNA, except in those clinical settings in which tissue is limited and/or insufficient for molecular testing.

| Trial/ Year of publication | EGFR TKI | No. of patients | ctDNA Genotyping Method | ctDNA performance | | | Clinical outcome for TKI vs. chemotherapy | | | |
| | | | | Concordance | Sensitivity | Specificity | Tissue + | | ctDNA + | |
							RR	PFS HR	RR	PFS HR
IPASS 2009, 2011	Gefitinib	233	DxS ARMS	66.3%	43.1%	100%	69%	0.70	75%	0.29
EURTAC 2012	Erlotinib	76	Taqman	72.7%	78%	100%	65%	0.34	60%	0.36
FASTACT-2 2015	Erlotinib	238	Cobas	88%	75%	96%	NR	0.25	66%	0.22
ENSURE 2015	Erlotinib	180	Cobas v2	77%	81.4%	96.4%	62.7	0.34	NR	0.29
LUX-LUNG 3 2013	Afatinib	258	Therascreen 29*	28.6%	NR	NR	56%	0.58	62%	0.35
LUX-LUNG 6 2014	Afatinib	298	Therascreen 29	60.5%	NR	NR	67%	0.28	67%	0.25

Table 1.5. Clinical outcomes of patients according to plasma EGFR mutation status in randomised trials of first-line EGFR-TKIs versus chemotherapy. ctDNA, circulating tumour DNA; EGFR, erythrocyte growth factor receptor; HR, hazard ratio; NR, not reported; PFS, progression free survival; RR, response rate; TKI, tyrosine kinase inhibitor. *serum in LUX-LUNG 3, plasma in LUX-LUNG 6.

In the setting of EGFR-TKI resistance, sensitivity, specificity and clinical utility of ctDNA testing for presence of *EGFR* T790M resistance mutation were investigated in the AURA series of trials. Oxnard et al reported sensitivity of 70% and specificity of 69% for ctDNA using digital droplet PCR using tissue as a reference test of *EGFR* T790M, in a cohort of 216 patients enrolled in the phase I cohort of AURA trial, a first-in-man study of third-generation TKI osimertinib.[138] Patients positive for T790M by ctDNA had clinical outcomes with osimertinib that were equivalent to those positive for T790M by tissue-based methods. Subsequently, pooled exploratory analyses from phase II AURA studies (AURA extension cohort and AURA2) in pre-treated T790M tissue-positive patients were reported with similar results, reporting sensitivity of ctDNA *EGFR* T790M testing of 61% and specificity of 79%, using cobas *EGFR* ctDNA method.[157]

These studies also reported a correlation between burden of disease and detectability of T790M in plasma, with patients who had extrathoracic metastatic disease (TNM seventh edition category M1b) more likely to have detectable T790M in plasma than those with M0/M1a disease (M0 - no distant metastases; M1a - metastases in contralateral lung or pleural/pericardial nodule/malignant effusion).[157]

ctDNA specificity reported in above studies was somewhat lower than that observed for *EGFR*-sensitising mutations, due to a proportion of patients who were T790M positive by ctDNA testing (with the presence of T790M in plasma confirmed by next generation sequencing methods) but tissue-negative. These patients had favourable clinical outcomes, similar to T790M-tissue positive patients, suggesting that the reduced specificity and tissue "false negatives" could in part be due to intra-tumour heterogeneity and that ctDNA analysis may be more representative of overall tumour mutation status.

In 2014, data from a phase IV open-label single-arm study of gefitinib in Caucasian EGFR+ patients was published, including data for a preplanned exploratory biomarker objective showing concordance of 94.3%, specificity of 99.8% and sensitivity of 65.7% for *EGFR* ctDNA testing in plasma of 652 patients with matched tumour *EGFR* testing.[158] This also included 12 out of 201 patients with unknown tissue *EGFR* status where a mutation was identified on a matched plasma sample. On the basis of this data, the licence for EGFR-TKI gefitinib was updated to allow the use of plasma for detection of *EGFR* sensitising mutations where tissue was not available, and in 2016, the United States Food and Drug Administration (FDA) approved cobas *EGFR* as the first companion diagnostic plasma *EGFR* test. In 2017,

osimertinib marketing authorisation was updated to allow detection of T790M from ctDNA obtained from plasma, at the time of development of acquired resistance. In view of the above data, showing that plasma ctDNA *EGFR* testing is less sensitive using the tissue test as the reference, the 2018 IASLC Consensus Statement on Liquid Biopsy recommended ctDNA testing for treatment-naïve patients when tumour tissue is scarce, unavailable, or a significant delay is expected in obtaining tissue, and that a tissue biopsy should be considered whenever possible when the plasma result is negative at time of progression.[149] Since then, and with publication of data validating ctDNA NGS in the treatment-naïve setting, as well as it's role in real-time treatment monitoring and in gene-fusion positive NSCLC, the statement is shortly due to be updated to allow "blood first" strategy.[159–162]

1.4.2.2 ctDNA and next generation sequencing methods

Several retrospective and prospective studies have been conducted to evaluate the utility of ctDNA NGS when compared with standard-of-care tissue genotyping,[160, 163] tissue NGS,[164, 165] or where tissue genotyping was not available or possible.[166] In the NILE study, a multicentre prospective cohort study of 283 patients, investigators set out to demonstrate non-inferiority of ctDNA NGS to SOC tissue genotyping in the setting of newly diagnosed non-squamous metastatic NSCLC (mNSCLC).[159] They reported a 48% increase in identification of guideline-recommended biomarkers when ctDNA NGS was used in addition to tissue, a benefit that was seen primarily in those patients in whom tissue was insufficient for testing or where biomarker identified by ctDNA was not assessed in tissue. They also reported a significantly lower median turnaround time for ctDNA compared with tissue genotyping of 9 versus 15 days. In a study by Aggarwal et al, 323 patients with mNSCLC were prospectively enrolled at a single centre to receive plasma NGS testing, including 166 at initial diagnosis and 157 at time of disease progression.[164] Contemporaneous tissue NGS was requested in 207 and performed successfully in 128 patients. Adding plasma NGS to tissue NGS increased detection of therapeutically targetable mutations by 17%, this figure rising to 74% when including patients in whom tissue NGS was not possible. In this study, plasma NGS utility was lower for patients with M1a (intrathoracic only) disease. In a study by Zugaziagotia et al, a cohort of 93 advanced adenocarcinoma NSCLC patients with no available tissue for genotyping were enrolled to receive ctDNA NGS reporting a 57% rate of detection of potentially actionable variants.[166]

An ongoing Phase II/III blood-first assay screening trial (BFAST) in treatment-naïve NSCLC has recently reported initial results from the ALK+ cohort.[160] Of the 2,219 patients screened, blood-based NGS identified 119 patients (5.4%) with ALK+ disease, out of which 87 were treated with first-line alectinib. ORR was 92% by independent review with 12-month PFS rate of 78%. Other ongoing arms of the trial are looking to evaluate and prospectively validate blood-based assays of tumour mutational burden (TMB) and alterations in *RET* as predictors of efficacy and safety of first-line atezolizumab and alectinib, respectively. [167]

These studies demonstrate a clear role for ctDNA NGS in the settings where tissue is unavailable or inadequate, or indeed as a "blood-first" strategy, for monitoring and early detection of minimal residual disease.[168]

1.4.3 Toward routine delivery of predictive genotyping in NSCLC: CRUK Stratified Medicine Programme and UK National MATRIX trial

Cancer Research UK's Stratified Medicine Programme (SMP) is a national observational pre-screening study, which is investigating the feasibility of delivery of precision medicine via a "national screening to national trials" approach. Following on from the pilot Phase 1 of the programme (SMP1) conducted in multiple tumour types between 2011 and 2013,[169] Phase 2 of the programme (SMP2) focuses on the recruitment of late stage metastatic non-small cell lung carcinoma (NSCLC) patients. Patient are enrolled across a number of UK-based clinical hubs and their tumour samples are collected and sent for analysis at one of three national genetics laboratories (technology hubs) with the aim to molecularly profile approximately 2,000 NSCLC patients per year. Launched in January 2015, more than 3,000 NSCLC patients have been enrolled with data on around 1,500 tissue samples molecularly profiled using a 28 gene Illumina NGS panel.[170]

SMP2 provides a national molecular pre-screening service for the UK National Lung MATRIX trial (NCT02664935). This is a multi-arm open-label non-comparative phase II umbrella trial in which patients are allocated to the appropriate targeted therapy according to the molecular genotype of their tumour. The trial includes a common set of outcome measures for all molecularly defined cohorts with flexibility to select a cohort-specific primary end point, looking for robust signals of activity such as would be expected from a bona fide targeted therapy.

1.5 Thesis aims and overview of chapters

In this thesis I will present the work I conducted in the course of my research degree, the primary aim of which was development of novel therapies in relapsed advanced/metastatic non-small cell lung cancer based on two hypotheses:

- Hypothesis 1: In relapsed advanced/metastatic non-small cell lung cancer (NSCLC) adenocarcinoma, oral anti-angiogenic TKI nintedanib in combination with taxane-chemotherapy improves outcomes.

- Hypothesis 2: In relapsed advanced/metastatic non-small cell lung cancer, broader molecular genotyping is feasible in the NHS and leads to identification of novel prognostic and predictive biomarkers.

In Chapter 2, I will represent the findings of two real-world studies of outcomes, in terms of overall response rate, progression free survival and overall survival, in patients with advanced/metastatic NSCLC adenocarcinoma treated with pembrolizumab monotherapy in the treatment naïve setting and combination of docetaxel plus nintedanib in the relapsed setting, followed by the work on set up of a phase Ib/II clinical trial to determine the safety and efficacy of a novel therapeutic drug combination of chemotherapy drug nab-paclitaxel with nintedanib.

In Chapter 3, I will present the work conducted with aim of optimising tissue molecular genotyping in relapsed NSCLC, including a study validating the pathological and molecular adequacy of rebiopsy tissue for genotyping in relapsed NSCLC and work on identifying the optimal methods for tissue acquisition for molecular genotyping in the context of CRUK SMP2 programme.

In Chapter 4, I will present the work investigating the role of ctDNA molecular genotyping in advanced/metastatic NSCLC including results of a feasibility study of implementation of a clinical EGFR ctDNA testing service in the NHS and an evaluation of clinical utility of ctDNA-based next-generation sequencing for target identification in the diagnostic and acquired resistance settings in advanced NSCLC.

Chapter 2 Improving outcomes in advanced/metastatic NSCLC with no driver mutations

In this chapter I will present the work done towards investigating novel systemic therapies in non-oncogene addicted NSCLC, including 2 real-world studies of, at the time, newly licenced agents and combinations in advanced NSCLC, including UK national real-world data on the effectiveness and safety of single agent pembrolizumab in previously untreated advanced NSCLC, and docetaxel-nintedanib combination in second-line adenocarcinoma NSCLC. These studies benchmarked the use and performance of these agents in the UK NSCLC patients, also providing comparator data and the rationale for development of a phase Ib/II trial, named N3, of a novel combination of nab-paclitaxel with nintedanib in second and third-line advanced NSCLC including patients previously treated with immune-checkpoint inhibitors, the set-up of which will also be presented here.

Clinical trial data alone may be insufficient for optimal decision-making when assessing the real-world health-economic value of a novel drug or technology. The reasons for this include highly selected populations, idealised environments for treatment and restrictions on data collection and reporting. Real-world data is a term used to describe healthcare related data collected outside of randomized clinical trials.[171] Value of real world data is increasingly recognised particularly in the post-authorisation setting and to support decision making for drug reimbursement purposes.[172, 173] Post-authorisation, real-world data can provide an understanding of how efficacy and safety established within the clinical trial environment translates in the real world and an oversight of how a drug is used in practice. Evidence obtained from real-world data is already in routine use in UK and the EU, and a number of initiatives to increase the utility of such evidence are ongoing as outlined in the European Commission Expert Group on Safe and Timely Access to Medicines for Patients ("STAMP") documents, including establishment of a specialist "Big Data" task force to explore how regulators can use real world data to support research, innovation and robust medicines development, and the IMPACT HTA programme which is investigating statistical methods and tools to combine randomised clinical trial and real world data in the economic evaluation of medicines. The strengths and limitations of the use of real-world data and

real-world evidence in oncology will be discussed further in the discussion and conclusions section at the end of the chapter.

2.1 Real-world efficacy of pembrolizumab in treatment-naïve advanced or metastatic NSCLC

2.1.1 Background

As discussed in Chapter 1.2, while there are a number of therapy choices, anti-PD1 inhibitor pembrolizumab has become standard therapy for previously untreated advanced or metastatic NSCLC with PD-L1 ≥50% following publication of results from KEYNOTE-001, KEYNOTE-024 and KEYNOTE-042 trials, having demonstrated improved overall survival and tolerability over chemotherapy.[10, 36, 174–176]

Pembrolizumab is a humanised monoclonal antibody which binds to the programmed cell death-1 (PD-1) receptor and blocks its interaction with ligands PD-L1 and PD-L2. The PD-1 receptor is a negative regulator of T-cell activity that has been shown to be involved in the control of T-cell immune responses. Pembrolizumab potentiates T-cell responses, including anti-tumour responses, through blockade of PD-1 binding to PD-L1 and PD-L2, which are expressed in antigen presenting cells, tumour cells or other cells in the tumour microenvironment.

Early evidence of efficacy of pembrolizumab in untreated advanced/metastatic NSCLC came from KEYNOTE-001, a very large phase 1 study designed to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and antitumor activity of pembrolizumab in patients with melanoma or NSCLC (Figure 2.1).[174] 550 patients with NSCLC received treatment with pembrolizumab within KEYNOTE-001, including 101 patients who had not received prior systemic treatment for advanced/metastatic disease. Overall response rate in the 101 previously untreated patients was 24.8% with median duration of response of 23.3 months. Median progression-free survival was 6.0 months for these patients.
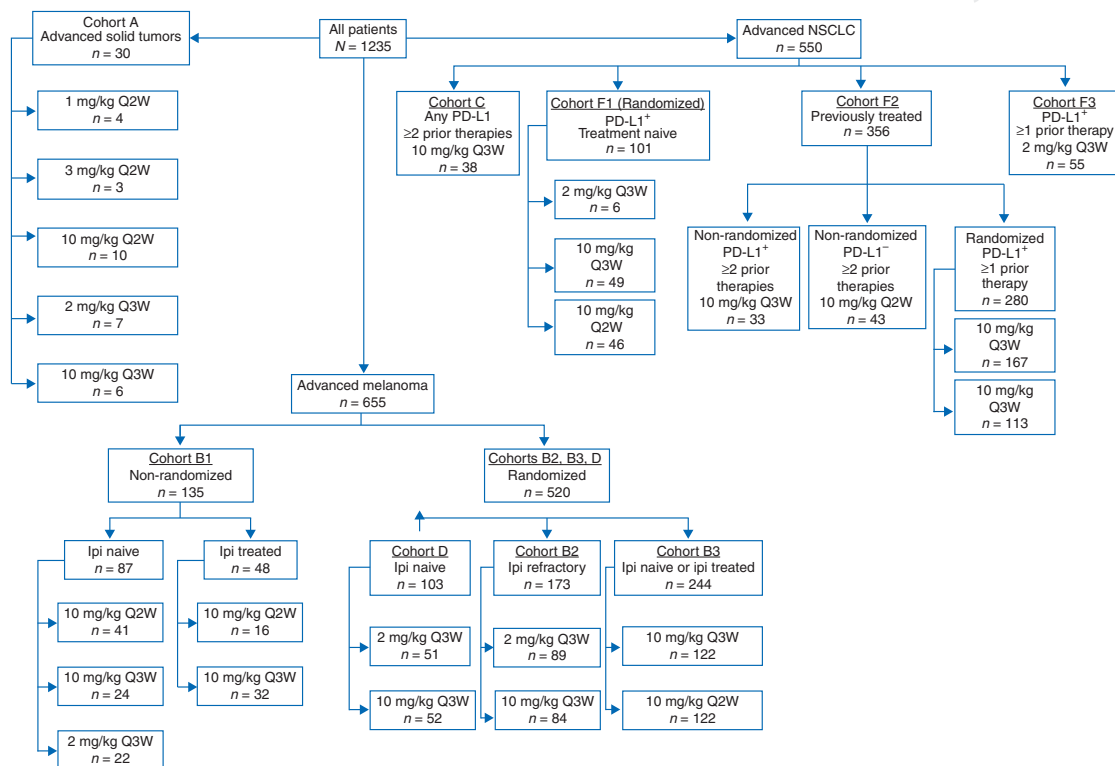
Figure 2.1. KEYNOTE-001 treatment cohorts. Ipi, ipilimumab; PD-L1, programmed death ligand 1; Q2W, every 2 weeks; Q3W, every 3 weeks. Copyright © Kang et al, 2017. Published by Oxford University Press on behalf of the European Society for Medical Oncology.

Analysis of PD-L1 expression showed that PD-L1 tumour proportion score (TPS) of at least 50% was associated with a higher response rate and longer progression-free and overall survival in both previously treated and untreated patients (ORR 50%, median PFS 12.5 months, median OS not reached in untreated patients). Updated overall survival results from KEYNOTE-001 were presented at ASCO 2017 Annual Meeting, with median OS of 35 months, 2-year OS rate 67% and 3-year OS rate 25% in treatment naïve patients with TPS ≥50.[36] Data was also presented for the subgroup of previously treated patients with *EGFR* mutations (n=74), who had a significantly lower median OS (6.0 months, 95% CI 4.4-8.8), which was similar across all levels of PD-L1 expression. Minimal activity in EGFR mutant patients was also observed in KEYNOTE-010 trial, a randomised open-label trial of pembrolizumab at two dose levels (2mg/kg and 10mg/kg) versus docetaxel in previously treated NSCLC with PD-L1 of at least 1%, which reported an OS benefit for pembrolizumab in the overall population and at both dose levels.[34]

Following the early encouraging results from KEYNOTE-001 in treatment-naïve patients, KEYNOTE-024 was a multicentre, randomised, controlled small confirmatory phase 3 trial of pembrolizumab in previously untreated metastatic NSCLC with high PD-L1 expression (≥50% TPS) versus investigator's choice platinum-containing chemotherapy.[9] *EGFR* mutant and *ALK* positive patients were excluded. The primary endpoint was overall survival. Patients were randomised (1:1) to receive pembrolizumab at a dose of 200 mg every 3 weeks (n=154) or investigator's choice platinum-containing chemotherapy (n=151). The flat dose was chosen based on pharmacokinetic modelling which suggested that the 200mg fixed dose would provide similar exposures to the weight-based dosing regimens used in early KEYNOTE studies.[177] Crossover on progression was allowed. Median progression-free survival was 10.3 months in the pembrolizumab group versus 6.0 months in the chemotherapy group (HR 0.50; 95% CI 0.37-0.68; p<0.001). At the time of second interim analysis, despite cross-over, OS was significantly longer in the pembrolizumab group (6-month OS rate 80.2% vs. 72.4%, median OS not reached in either group, HR 0.60; 95% CI 0.41-0.89; p=0.005) and the trial was stopped early. In the updated OS analysis, after 25.2 months median follow-up and 169 events, median OS was 30 months with pembrolizumab and 14.2 months with chemotherapy (HR 0.63; 95% CI 0.47-0.86; p=0.002).[178]

The response rate was higher in the pembrolizumab group than in the chemotherapy group (44.8% vs. 27.8%), and there were fewer treatment-related grade ≥3 adverse events (26.6% vs. 53.3%). In a subgroup analysis, reduced survival benefit of pembrolizumab compared to chemotherapy was observed in the small number of patients who were never-smokers, although no definitive conclusions could be drawn due to small numbers of patients.

Pembrolizumab is most commonly associated with immune-related adverse reactions, defined as a unique constellation of inflammatory toxicities caused by immune-checkpoint inhibitors via promoting the activity of the immune system. Common treatment-related IR AEs of pembrolizumab include fatigue, rash, diarrhoea, pruritus, decreased appetite, and nausea. Drug-related pneumonitis has been reported in around 3% of patients, with grade ≥3 pneumonitis in around 1%. The commonest immune-mediated AE is hypothyroidism (all grade 8.5%).

Following the presentation of results of KEYNOTE-024 at the 2016 ESMO Congress on 9 October 2016, on 15 December 2016 the existing EMA licence (for pembrolizumab as second-line treatment of patients with previously treated locally advanced or metastatic

NSCLC with PD-L1 expressing tumours) was further expanded to include first-line treatment in adults whose tumours express PD-L1 with a TPS ≥50% and with no EGFR or ALK tumour alterations. Pembrolizumab received NICE approval for the first-line indication on 31 May 2017.

Prior to this, and based on the results from the KEYNOTE-001 trial showing that PD-L1 TPS of at least 50% was associated with a higher response rate and longer PFS and OS in both previously treated and untreated patients, an Early Access to Medicines Scheme (EAMS) was opened by the UK Government on 15 March 2016, allowing access to pembrolizumab for patients with PD-L1 TPS ≥50% to all lines of therapy. At that time, UK patients were also able to access pembrolizumab privately and within clinical trial protocols, but the real-world efficacy and safety of pembrolizumab was unknown.

2.1.2 Aims and objectives

I set out to benchmark the outcomes of patients with treatment-naive advanced/metastatic NSCLC treated with pembrolizumab at the Royal Marsden Hospital (RMH) and other participating hospitals in the UK and compare with the KEYNOTE-024 trial data.

Primary objective was to evaluate the progression-free survival after first-line pembrolizumab in the UK patient population.

Secondary objectives were to evaluate, in the same patient population: overall response rate, duration of pembrolizumab treatment, duration and efficacy of pembrolizumab treatment beyond first documented progression, overall survival, safety of pembrolizumab, prognostic factors associated with PFS, OS and ORR (age, gender, smoking status, PDL1 expression level, PS, mutational status, previous systemic therapy), and time from PD-L1 report to start of pembrolizumab treatment.

2.1.3 Methods

I designed and conducted a multicentre retrospective observational study as a National Service Evaluation, with 27 participating centres in the UK.

Approvals were obtained from the RMH Research and Development Committee as the initiating centre, with local permissions at each participating centre. Participating clinicians were approached to securely provide anonymised patient data. Patients were identified at each participating centre from pharmacy and clinic lists of patients with diagnosis of advanced NSCLC who received pembrolizumab in the treatment-naïve setting. Patients were

enrolled regardless of PD-L1 expression status and method of PD-L1 evaluation (22C3 antibody or other), with data on PD-L1 expression level included in data collection and analysis.

Data was collected on patient demographics (age, gender, ethnicity, smoking status, ECOG PS); baseline disease characteristics (date of NSCLC diagnosis, clinical stage at diagnosis, date of diagnosis of advanced disease, histological subtype, mutational status); prior treatment (type of treatment, treatment intent); PD-L1 testing (date of PD-L1 test report, PD-L1 expression level); pembrolizumab treatment (start and end date of pembrolizumab, best response as determined by treating clinician, date of PD, treatment continuation beyond PD); toxicities (name and grade of any CTCAE grade 3 and above toxicities, name and grade of any immune-related toxicities, use of corticosteroids or other immune suppressants) and clinical outcomes (date of last follow-up, disease status at last follow-up). List of data items and possible responses can be found in Appendix 1. Data was retrospectively collected through evaluation of case notes, anonymised and captured on a predefined excel spreadsheet tool developed and validated in conjunction with the RMH Research Data and Statistics Unit (RDSU). The same spreadsheet served as a standard data entry form, with an empty spreadsheet forwarded to participating physicians from external centres. Completed anonymised data were returned for pooling, cleaning and analysis, via a dedicated secure NHS email with use of encryption, and stored in a secure password-protected drive. Each patient was allocated a unique study number in order of enrolment. Data was reviewed to confirm eligibility criteria were satisfied and to check for any inconsistencies. Data queries were sent to participating centres and returned via the same dedicated secure NHS email. The study opened for data collection on 30 August 2017 and closed on 6 March 2018.

2.1.3.1 Eligibility criteria and data collection

Included were all patients who received pembrolizumab as first-line systemic therapy as part of their routine care for advanced/metastatic NSCLC in the UK between 15 March 2016 and January 2018, regardless of route of access to pembrolizumab (e.g. private healthcare, expanded access programme) or PD-L1 status. Patients receiving pembrolizumab via the EAMS programme would have needed to satisfy specific EAMS eligibility at the time of EAMS enrolment, but there were no such specified limitations, for instance to the patient's

PD-L1 expression level and ECOG performance status, for enrolment into this study. Frequency of CT imaging for on-treatment monitoring was performed according to local standard-of-care treatment protocols. Treatment beyond first documented progression was allowed.

Exclusion criteria were:

- Patients with no confirmed histological diagnosis of NSCLC;

- Patients receiving pembrolizumab in second-line or other setting;

- Patients with incomplete or no follow-up data available.

2.1.3.2 Endpoints

Primary endpoint was PFS, defined as date of commencing pembrolizumab treatment to date of first documented progression or death. Patients not progressed/died were censored at their last follow-up visit.

Secondary endpoints were:

- Overall response rate, investigator defined and reported in routine care as complete response or partial response (CR + PR).

- Time to treatment failure (TTF), defined as median time in months from first pembrolizumab dose to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient preference, or death.

- Time to further progression, defined as median time in months from date of first documented progression to further progression on pembrolizumab or death; only patients continuing on pembrolizumab beyond first described progression were included; patients still alive without further progression were censored at their last follow-up visit.

- OS, defined as median time from date of first dose of pembrolizumab to date of death from any cause, where surviving patients were censored at their last follow-up visit.

- Rate of all grade ≥3 treatment-related adverse events (AEs), investigator defined and graded as per Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

- Rate of any grade immune-related adverse events (IR AEs), investigator defined and graded as per CTCAE version 4.0.

- Proportion of patients experiencing dose delays.

- Proportion of patients requiring immunosuppressant therapy (e.g. steroids) and type of immune suppressant used (steroids, mycophenolate, etc.).

- Prognostic factors (age, gender, smoking status, PD-L1 expression level, ECOG performance status (PS), mutational status, previous systemic therapy) associated with ORR, PFS and OS.

- Median number of days from date of PD-L1 report to start of pembrolizumab treatment.

2.1.3.3 Statistical considerations and analysis methods

PFS and OS were calculated using Kaplan-Meier methods using SPSS software, where the median survival and 6-months survival rates were given with 95% confidence intervals. The response rates were calculated as the proportion of patients with CR or PR from the total treated and reported with 95% CIs. Cox regression was used to assess the influence of prognostics variables (age, gender, smoking status, PD-L1 expression level, PS, mutational status, previous systemic therapy) on OS and PFS. Binary logistic regression was used to assess the influence of the above prognostics variables on ORR. Univariate analysis was performed with each factor first, and only those with a p-value <0.2 tested in the multivariate model. Factors with a p-value <0.05 in the multivariate analysis were considered significant. Hazard ratios (HR) for OS and PFS and odds ratio for ORR were reported together with 95% confidence intervals.

Rates of treatment-related toxicities were expressed in the form of total number and percentages for each grade. Maximum toxicity grade experienced by each patient was evaluated and number and percentage of patients experiencing treatment-related toxicity grade ≥3 and any grade immune-related toxicity reported. Proportion of dose delays and immunosuppressant requirements were expressed in the form of percentages.

Total estimated number of patients to be evaluated was estimated at 200 based on the median PFS of 10.3 months as reported in the KEYNOTE-024 trial and assuming minimum 12 months of follow-up for patients in this study from commencing treatment, with 80% power to determine a similar median survival rate to within a width of +/- 6.9% (margin of error assuming 95% level of confidence).

2.1.4. Results

2.1.4.1 Patients

Complete data for 219 patients who received first-line pembrolizumab between 14 July 2016 and 24 January 2018 at 27 centres was included in the analysis. Data was received for additional 13 patients, however on review they were found not to meet eligibility criteria having received pembrolizumab in second or subsequent line of treatment for advanced NSCLC, and were excluded from final analysis.

59.8% were male, 92.7% were former or current smokers and 88.2% had clinical stage 3B or stage 4 disease at diagnosis. Nearly 80% had non-squamous histology with only 20% squamous cell carcinomas, which may reflect more aggressive presentation of SCC with clinicians opting for first line chemotherapy aiming to achieve more rapid disease control, at a time when ICI/ChT combinations were not licenced. 88.1% had an ECOG PS of 0 or 1 at start of treatment and 11.4% had ECOG PS of ≥2. Data on demographics and baseline characteristics is summarised in Table 2.1. 80.8% of patients had no detected oncogenic driver mutations, while in 10.5% mutational status was unknown (not tested or not recorded). 19 patients (8.7%) had a confirmed oncogenic driver mutation including 10 patients with a *KRAS* mutation, 2 patients with *BRAF* and 2 patients with *EGFR* exon 20 mutations (Table 2.2). There were no patients with sensitising *EGFR* mutations or *ALK* rearrangements. Distribution of patients across the UK centres is shown in Table 2.3.

2.1.4.2. PD-L1 reporting

All but one patient had PD-L1 TPS of ≥50%. 1 patient with PD-L1 TPS of 0% received pembrolizumab in the context of a clinical trial protocol. Distribution of PD-L1 TPS is illustrated in Figure 2.2.

Date of PD-L1 report was available for 218 patients (Figure 2.3.). Median time from diagnosis of advanced/metastatic NSCLC to PD-L1 report was 18 days (range 0-748; 95% CI 16-21) while median time from PD-L1 report to start of pembrolizumab treatment was 23 days (range 2-545; 95% CI 21-26).

BASELINE AND DEMOGRAPHIC DATA (n=219)		n	% (out of 219)
Age (median, range)	70 (42-87)		
Gender			
Female		88	40.2
Male		131	59.8
Ethnicity			
Caucasian		204	93.2
Asian		5	2.3
Black		1	0.5
Other		6	2.7
Unknown		3	1.4
Smoking status			
Ex-smoker		160	73.1
Current smoker		43	19.6
Never smoker		16	7.3
Clinical stage at diagnosis*			
IA		2	0.9
IB		6	2.7
IIA		4	1.8
IIB		4	1.8
IIIA		10	4.6
IIIB		17	7.8
IV		176	80.4
ECOG PS at enrolment			
0		48	21.9
1		145	66.2
2		20	9.1
3		4	1.8
4		1	0.5
NE		1	0.5
Histological subtype			
Squamous		45	20.5
Non-squamous		174	79.5

Table 2.1. Demographics and baseline characteristics of 219 patients included in the analysis of UK real-world first-line pembrolizumab in advanced NSCLC. *Staging according to UICC TNM 8th edition. CRT, chemoradiotherapy; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Mutational status at enrolment (n=219)		n (%)
No known variants		177 (80.8)
Unknown		23 (10.5)
Mutation present		19 (8.7)
	KRAS	10 (4.6)
	EGFR exon 20 insertion	2 (0.9)
	BRAF	2 (0.9)
	TP53	4 (1.8)
	NF1	2 (0.9)
	NRAS	1 (0.5)
	CDKN2A	1 (0.5)
	FGFR	1 (0.5)
	PTEN	1 (0.5)
	PIK3CA	1 (0.5)

Table 2.2. Mutational status at baseline for all 219 patients included in the analysis. 19 patients had a known tumour genomic alteration identified prior to commencing pembrolizumab.

INSTITUTION	Freq.	Percent
Brighton and Sussex University Hospital	6	2.7
Blackpool Victoria Hospital	26	11.9
The Christie Hospital	26	11.9
Croydon University Hospital	4	1.8
Chelsea and Westminster Hospital	7	3.2
Dorset County Hospital	3	1.4
East Sussex Healthcare NHS Trust	1	0.5
Newcastle Freeman Hospital	3	1.4
Cumberland Infirmary Carlisle	1	0.5
Queen Elizabeth Hospital Gateshead	1	0.5
University Hospital of North Durham	2	0.9
Great Western Hospital	7	3.2
Heart of England NHS Foundation Trust	9	4.1
Ipswich Hospital	10	4.6
NHS Lanarskhire	6	2.7
Peterborough City Hospital	2	0.9
The Princess Grace Hospital	4	1.8
Poole Hospital NHS Foundation Trust	1	0.5
Queen Alexandra Hospital	13	5.9
Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust	11	5.0
Royal Devon and Exeter Hospital	10	4.6
Royal Marsden Hospital NHS Foundation Trust	27	12.3
St James' University Hospital	11	5.0
Southampton Hospital	15	6.9
St Richard's Hospital	3	1.4
William Harvey Hospital	2	0.9
Western Sussex Hospitals NHS Trust	8	3.7
Total	219	100.0

Table 2.3. Distribution of patients across 27 UK centres.
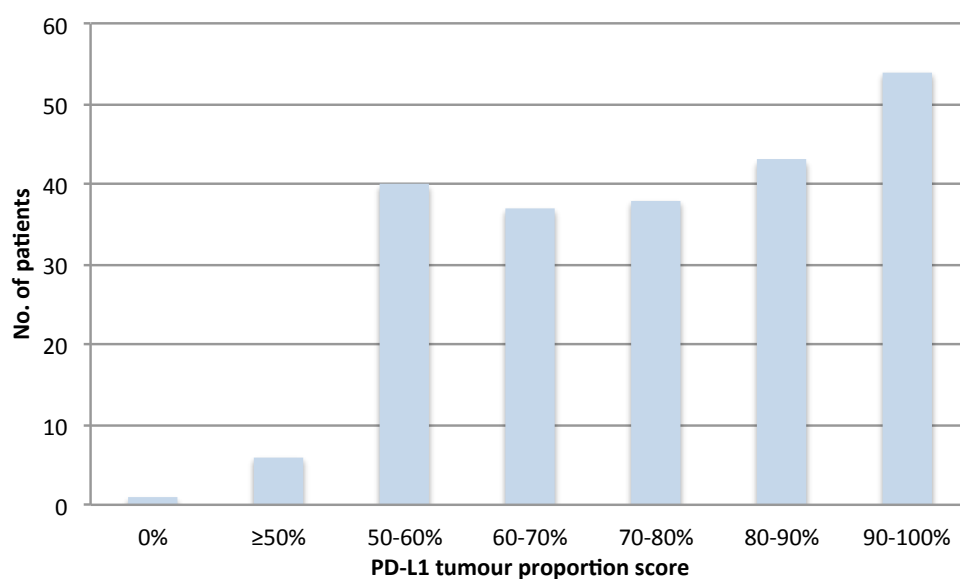
Figure 2.2. Distribution of PD-L1 TPS. One patient had a PD-L1 TPS of 0 and received pembrolizumab in the context of a clinical trial protocol. 6 patients had PD-L1 TPS recorded as being ≥50%, with exact percentage not specified/recorded.

Time from diagnosis to PD-L1 report and start of pembrolizumab (Tukey plot)
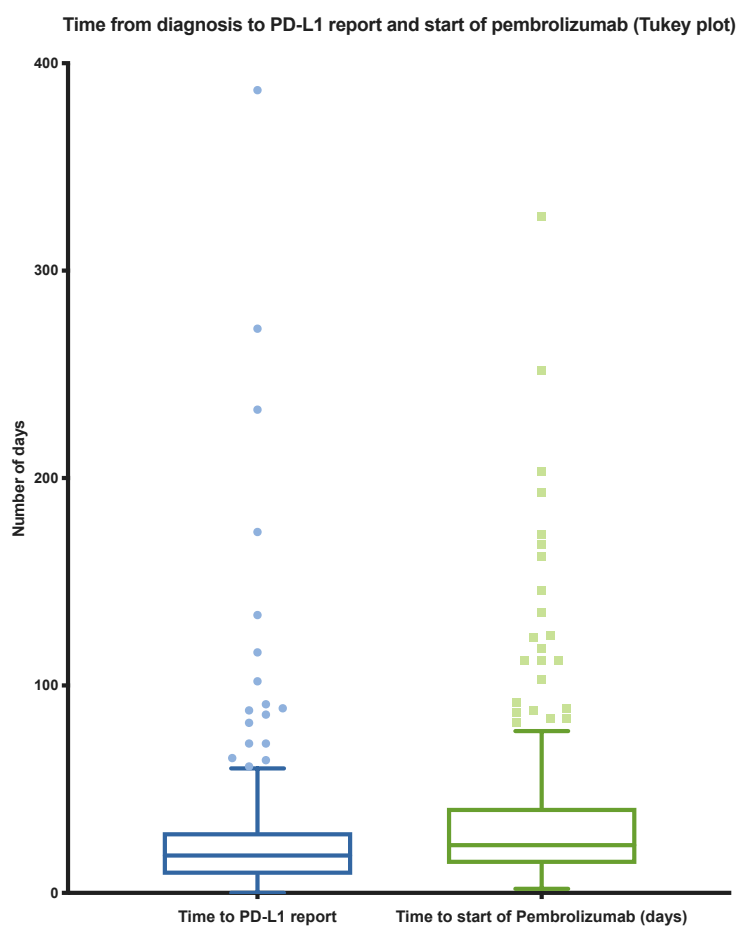
Figure 2.3. Time from diagnosis of advanced NSCLC to PD-L1 report (left) and to start of treatment with pembrolizumab.

2.1.4.3. Efficacy outcomes

After median follow-up of 5.7 months (95% CI 4.6-6.9), there were 90 events of progression or death. Median PFS was 8.2 months (95% CI 5.5-NR). 6-month PFS rate was 57.4% (95% CI 49.6-64.4) and 1-year PFS 44.2% (95% CI 34.8-53.1).

203 out of 219 patients received more than one dose of pembrolizumab and were included in the Kaplan-Meier analysis of time to treatment failure. Median TTF was 8.2 months (95% CI 6.2-15.2), which is almost identical to median PFS, likely due to data being relatively immature with an overall short median follow-up. Out of 57 patients with sufficient follow-up, 43 were still receiving pembrolizumab at 6 months and 19 patients at 1 year after starting treatment, respectively.

69 out of 219 patients (31.5%) had described progression on pembrolizumab at time of data capture. 20 patients (9% of the overall population or 29% of patients with PD) continued treatment with pembrolizumab beyond first progression, with 9 events of PD or death at time of data capture and median time to further progression of 2.8 months (95% CI: 1.9-NR) in these patients. While median time to further progression is low at under 3 months, 11 out of 20 patients continuing on pembrolizumab after initial progression had not experienced further PD and remained on pembrolizumab at time of data capture including 2 patients continuing beyond 6 months from first documented PD (Figure 2.4.).
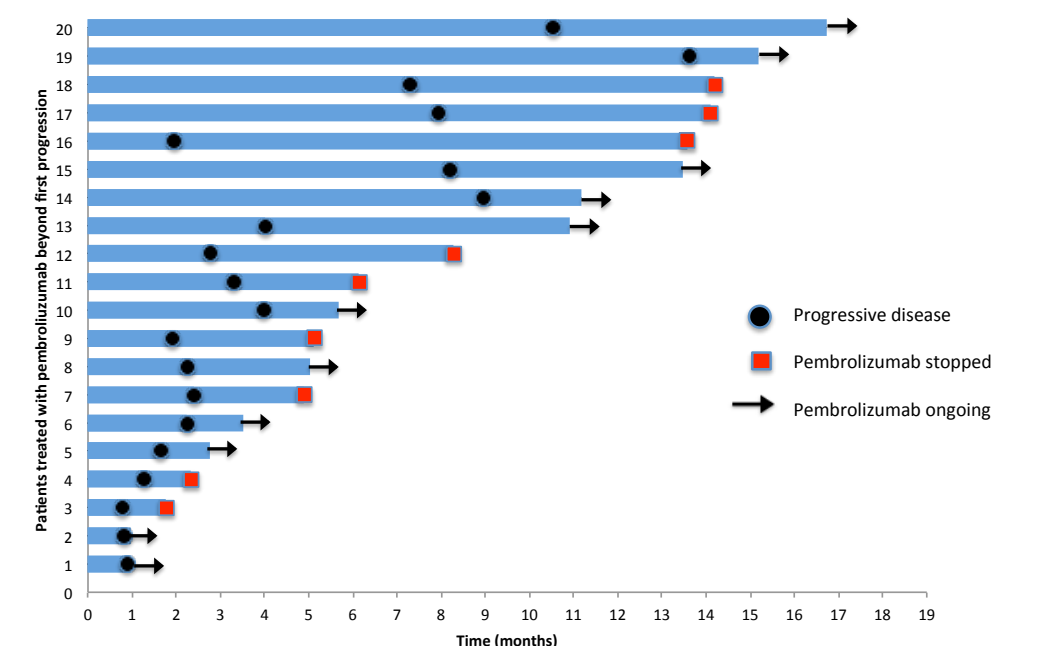
Figure 2.4. Swimmers plot for 20 patients who continued treatment with pembrolizumab beyond first documented progression.

After median follow-up of 5.6 months, there were 53 deaths from any cause. 166 patients (76%) were still alive and were censored at last follow-up date. Median OS was not reached (Figure 2.5.). 6-month and 1-year OS rates were 73.8% and 68.2%, respectively.

Following the univariate Cox regression analysis of overall survival, the variables included in the multivariate analysis were ECOG PS, mutational status, prior radical treatment and PD-L1 expression level. Poor performance status (defined as ECOG PS ≥2; HR 2.1; p=0.044) and presence of a confirmed mutation or unknown mutational status (HR 2.7; p=0.024) were associated with increased risk of death (Tables 2.4. and 2.5.). ECOG PS ≥2 was also strongly associated with shorter PFS (HR 2.23, p=0.006). There was a trend towards reduction in risk of death with increasing PD-L1 expression level, but this was not found to be significant in the multivariate analysis. Higher PD-L1 expression level was found to be significantly associated with improved PFS in the univariate and multivariate analyses (p=0.028). In an exploratory subgroup analysis of PFS according to PD-L1 expression level, patients with PD-L1 50-79% (n=114) had a median PFS of 7.3 months, while those with PD-L1 80-100% (n=97) had a median PFS of 13.6 months, although the difference did not reach statistical significance (HR 0.76; 95% CI 0.49-1.17, p=0.22). Median OS was not reached in either PD-L1 subgroup with no significant difference between the survival curves (HR 0.95; 95% CI 0.55-1.64; p=0.84). Baseline variables of age, smoking status, histology and gender were not significantly associated with survival outcomes.

Out of 182 evaluable patients, 103 had a partial response or complete response, resulting in ORR of 56.6% (95% CI 49-64; Figure 2.6.). Additional 26 patients had stable disease as best response, with disease-control rate of 76.4% (95% CI 70-82). 37 patients (17.8%) were not evaluable for response, with 19 patients awaiting first response assessment at time of data collection; 17 clinically deteriorated and died before first response assessment; 1 patient had clinically determined progressive disease without imaging confirmation. No clinical variable was found to be significantly associated with ORR in the univariate logistic regression analysis.
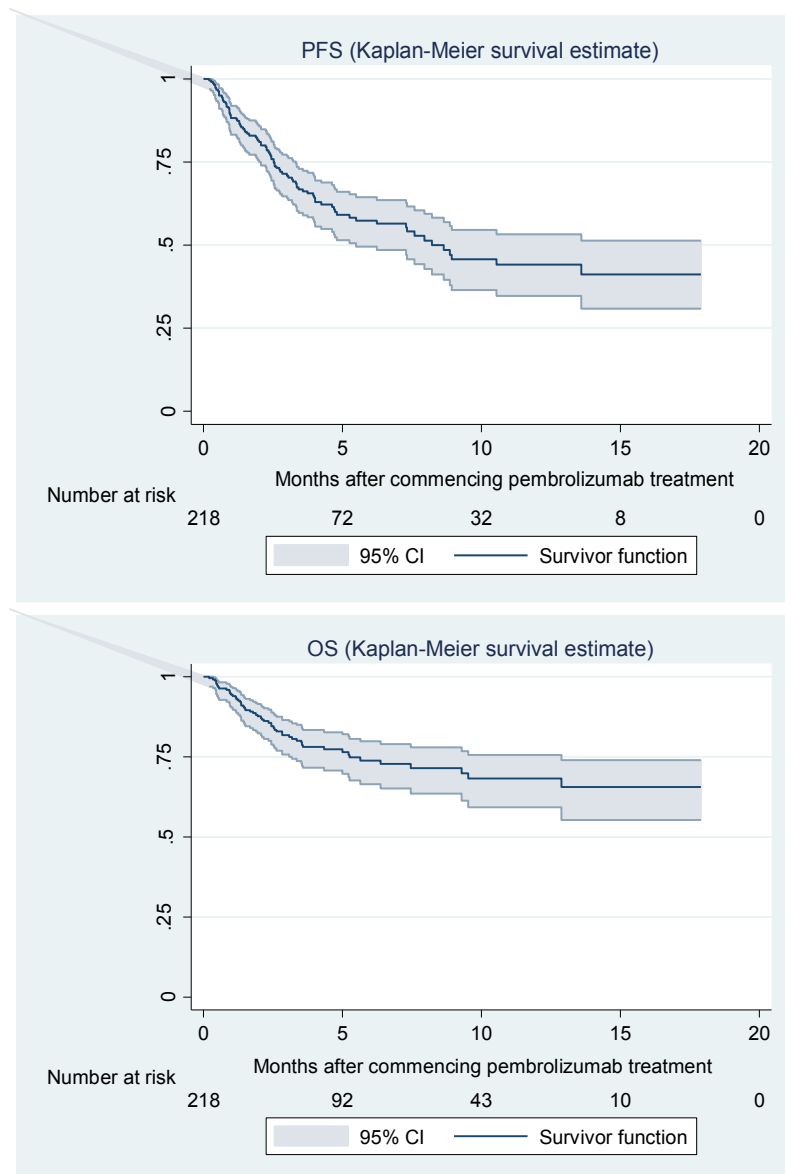
Figure 2.5. Kaplan-Meier survival estimate curves for progression free survival (top) and overall survival (bottom), in all patients who received first-line pembrolizumab (n=219)
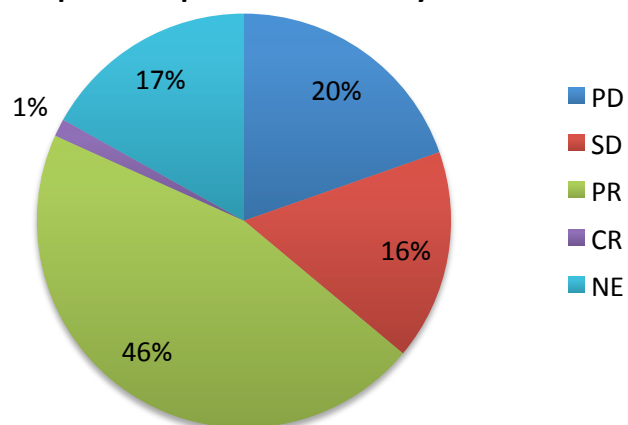
Figure 2.6. Best response according to RECIST 1.1 criteria (investigator assessed). CR, complete response; NE, not evaluable; SD, stable disease; PD, progressive disease; PR, partial response.

Univariate analysis of OS	N	Hazard Ratio (95% CI)	P-value
Age at diagnosis of NSCLC	219	0.99 (0.96-1.02)	0.593
Gender			
Female	88	1.0	
Male	131	1.25 (0.71-2.20)	0.447
Smoking Status			
Never smoker	16	1.0	
Ex-smoker	160	1.53 (0.47-4.93)	0.479
Current	43	0.95 (0.25, 3.68)	0.941
Overall			0.391
ECOG PS at Diagnosis			
PS 0/1	193	1.0	
PS 2/3/4	25	1.73 (0.84, 3.55)	0.134
Mutation Present			
No	177	1.0	
Yes	19	1.20 (0.47, 3.05)	0.706
Unknown	23	2.49 (1.27, 4.89)	0.008
Overall			0.052
Previous Radical Treatment			
No	188	1.0	
Yes	31	0.42 (0.15, 1.18)	0.100
PDL1 Expression Level			
50-60%	30	1.0	
60-70%	31	0.71 (0.28, 1.79)	0.466
70-80%	36	0.73 (0.31, 1.69)	0.458
80-90%	36	0.14 (0.03, 0.62)	0.010
90-100%	84	0.70 (0.33, 1.49)	0.350
Overall			**0.031**

Table 2.4. Univariate Cox regression analysis of OS adjusted for prognostic factors (age, gender, smoking status, PDL1 expression level, PS, mutational status, previous systemic therapy).

Multivariate analysis of OS	Hazard Ratio	95% CI	P-value
Mutation Present			
No	1.0	-	
Yes	1.19	(0.47, 3.03)	0.718
Unknown	2.70	(1.37, 5.35)	0.004
Overall			**0.024**
ECOG PS at Diagnosis			
PS 0/1	1.0	-	
PS 2/3/4	2.11	(1.02, 4.36)	**0.044**

Table 2.5. Multivariate Cox regression analysis of OS, using forward stepwise procedure, including all variables from univariate analysis where p value was <0.2. Any variable with p-value <0.05 was considered significant in the final model, as presented above.

2.1.4.4. Safety outcomes

50 out of 219 patients (22.8%) experienced at least one CTCAE version 4 grade ≥3 treatment-related adverse event during pembrolizumab therapy. There were 8 grade 5 treatment-related adverse events including: sepsis (n=3), lung infection (n=2), cardiac arrest (n=1), thromboembolic event (n=1) and non-specific interstitial pneumonitis (n=1).

83 patients (37.9%) experienced any grade immune-related adverse events (IR AEs). 17 patients (7.8%) experienced grade ≥3 IR AEs, including one grade 4 event, while there were no grade 5 IR AEs.

Commonest grade 1 and 2 IR AEs were hypothyroidism (7.3%), rash (5.9%) and hyperthyroidism (3.7%). Commonest grade 3 IR AEs were hepatitis (2.3%) and pneumonitis (1.4%). 38% experienced any-grade immune-related toxicities the most common being hypothyroidism (7.3%), rash (5.9%) and hyperthyroidism (3.7%). All IR AEs are shown in Table 2.6.

77 patients (35.2%) required at least one dose delay and 58 (26.5%) required immunosuppressant therapy (Table 2.7.). 21 patients (9.6%) permanently discontinued pembrolizumab due to adverse events.

Immune-related adverse events	n (%)	n (%)
Adverse Event*	Any Grade	Grade 3,4 or 5
Any	83 (37.9)	17 (7.8)
Hypothyroidism	16 (7.3)	0 (0.0)
Rash/dry skin	14 (6.4)	1 (0.5)
Pneumonitis	9 (4.1)	3 (1.4)
Hepatitis	8 (3.7)	5 (2.3)
Colitis	8 (3.7)	1 (0.5)
Fatigue	8 (3.7)	1 (0.5)
Hyperthyroidism	8 (3.7)	0 (0.0)
Diarrhoea	7 (3.2)	1 (0.5)
Arthralgia/myalgia	5 (2.3)	1 (0.5)
Nausea	5 (2.3)	0 (0.0)
Adrenal insufficiency	3 (1.4)	0 (0.0)
ALT elevation	2 (0.9)	1 (0.5)
Creatinine elevation	2 (0.9)	1 (0.5)
Pruritus	2 (0.9)	0 (0.0)
Vomiting	2 (0.9)	0 (0.0)
Anaemia	1 (0.5)	1 (0.5)
Myasthenia gravis	1 (0.5)	1 (0.5)
Peripheral sensory neuropathy	1 (0.5)	1 (0.5)
Alopecia	1 (0.5)	0 (0.0)
Hypophisitis	1 (0.5)	0 (0.0)
Mucositis	1 (0.5)	0 (0.0)

Table 2.6. All immune-related adverse events, investigator reported and graded according to CTCAE version 4. Listed in order of frequency.

Management of immune-related adverse events

Dose delays	n (%)
No	142 (64.8)
Yes	77 (35.2)
Immunosuppressant use	
No	161 (73.5)
Yes	58 (26.5)
Prednisolone use	49 (22.4)
Other immunosuppressant use	11 (5.0)
Dexamethasone	4 (1.8)
Hydrocortisone	4 (1.8)
Dexamethasone + infliximab	1 (0.5)
Methylprednisolone	1 (0.5)
Mycophenolate mofetil	1 (0.5)

Table 2.7. Management of immune-related adverse events in UK patients treated with first-line pembrolizumab.

2.1.5 Discussion and conclusions

This national retrospective data demonstrated that efficacy and safety of first-line pembrolizumab in the real-world UK population were comparable to published trial data, with median PFS of 8.2 months (95% CI: 5.5, NR) compared with 10.3 months in KEYNOTE-024, 6-month OS rate of 73.8% (KN-024: 80.2%) and 1-year OS rate of 68.2% (KN-024: 70.3%). Median OS was not reached. In KN-024, median OS was also not reached at interim analysis, with updated analyses showing median OS of 30 months after 25 months of follow-up.[178] Comparison of efficacy and safety outcomes between UK real-world data and trial data for first-line pembrolizumab from KEYNOTE-001, KEYNOTE-024, KEYNOTE-042 trial, as well as first-line atezolizumab from IMPower110 trial is shown in Table 2.8.

Outcomes	UK real-world data (n=219)	KEYNOTE-001 (n=20)	KEYNOTE-024 (n=154)	KEYNOTE-042 (n=299)	IMPower110 (n=205)
ORR	56.6%	50%	44.8%	39%	38.3%
Median PFS	8.2 months	12.5 months	10.3 months	7.1 months	8.1 months
6-month PFS	57.4%	-	62.1%	-	59.8%
Median OS	NR	35 months	30 months	20 months	20.2 months
6-month OS	73.8%	-	80.2%	-	76.3%
1-year OS	68.2%	-	70.3%	-	64.9%
2-year OS	-	67%	-	45%	-
Grade ≥3 AEs	22.8%	12%	26.6%	18%	30.1%
IR AEs	38%	-	29.2%	28%	40.2%
Discontinuation rate	9.6%	6%	7.1%	9%	6.3%

Table 2.8. Efficacy and safety outcomes in NSCLC patients with PD-L1 TPS ≥50% from UK real-world data and KEYNOTE trials for first-line pembrolizumab and IMPower110 trial for first-line atezolizumab (data for subgroup of TC3/IC3 patients; TC3/IC3 indicates patients with PD-L1 expression on ≥50% tumour cells or ≥10% of tumour-infiltrating immune cells). AEs, adverse events; IR AEs, immune-related adverse events; ORR, objective response rate; OS, overall survival; PFS, progression free survival.

These similarities in outcome were observed despite considerable differences in the baseline patient characteristics between the UK cohort and trial population, including over 10% of our real-world patients having ECOG performance status of ≥2 and 1 in 12 having a confirmed molecular oncogenic driver in their tumour. Both of these groups of patients were excluded from KEYNOTE-024 trial, while earlier

KEYNOTE-001 and KEYNOTE-010 trials reported minimal activity of pembrolizumab in the *EGFR* mutant/*ALK* + subgroups.[34, 175] A large retrospective study of immune-checkpoint inhibitors (ICIs) in NSCLC harbouring oncogenic driver alterations including *KRAS*, *EGFR*, *BRAF*, *MET*, *HER2*, *ALK*, *RET* and *ROS1* reported reduced efficacy in this population with median PFS of 2.8 months, OS 13.3 months and response rate of 19% with single agent ICIs, although there was significant variability between different alterations, with no activity seen in ALK+ patients and higher responses observed in patients with *KRAS* mutations.[179] This is borne out in this cohort of UK patients, with known positive or unknown mutation status associated with poorer OS outcomes in the multivariate OS analysis (p=0.024) although the patient numbers were small.

ECOG PS of ≥2 was also associated with a higher risk of death (HR 2.1; p=0.044) in the multivariate analyses, providing cautionary data to clinicians when deciding whether to utilise pembrolizumab in this patient population in absence of proven benefit from randomised trial data. Subsequent retrospective real-world studies in other European countries and the US have corroborated these findings.[180–182] For example, real-world data from 234 US patients with PD-L1 ≥50% treated with first-line pembrolizumab, including 39 (17%) with ECOG PS 2, showed that patients with ECOG PS 2 had a significantly lower response rates (ORR 43.1% vs. 25.6%; p=0.04) and shorter overall survival (median OS 20.3 vs. 7.4 months; HR 0.42; p<0.001) than those with PS 0-1.[180] The estimated OS at 1 year was 73% vs. 41% in patients with ECOG PS 0–1 vs. 2, respectively. Real-world data from a pan-European patient cohort of 302 patients including 52 with ECOG PS 2 also reported significantly worse survival for PS 2 patients compared with those with PS 0-1 (median OS 7.2 months vs. NR, HR 3.80, 95% CI 2.49-5.78).[182] Prospective trial data in patients with poor performance status (ECOG PS ≥2) was limited at that time, but a prospective single-arm phase 2 trial of pembrolizumab in 60 NSCLC patients with ECOG PS 2 has recently reported evidence of durable clinical benefit (primary endpoint defined as the occurrence of complete response, partial response, or stable disease that continues until at least the second CT scan scheduled at 18 weeks) in around a third of patients, with an overall response rate of 28.3%, median PFS of 5.4 and OS of 11.7 months.[183] In this study fewer than half of patients received pembrolizumab in the first-line setting and

45% showed no PD-L1 expression, representing a somewhat different patient population. Data for safety and activity of other ICIs in PS 2 NSCLC patients comes from subgroup analyses of CheckMate-817 (nivolumab plus ipilimumab in treatment-naïve patients), CheckMate-171 (pre-treated squamous NSCLC) and CheckMate-153 (pre-treated squamous and non-squamous NSCLC).[184–186] In CheckMate-817, a multi-cohort single-arm study of combination nivolumab/ipilimumab in treatment-naïve advanced NSCLC unselected for PD-L1, the cohort of patients with ECOG PS 2 or co-morbidities (untreated brain metastases, hepatic or renal impairment, HIV) had lower survival than the PS 0-1 cohort with median OS of 9.9 vs. 17 months, respectively, and with no difference in safety between the cohorts.[184] Similar findings of reduced efficacy and lower overall survival in PS 2 patients were reported with single agent nivolumab in previously treated NSCLC.[185, 186] Currently, several trials are ongoing to specifically evaluate different ICIs in PS ≥2 patients with NSCLC, such as the phase III IPSOS trial of atezolizumab in treatment-naïve patients with PS 2-3 (NCT03191786), the phase III eNERGY trial (NCT03351361) of nivolumab plus ipilimumab, and a phase II trial of pembrolizumab with or without chemotherapy in previously treated patients with ECOG PS 2 (NCT02581943).

Conversely, a trend towards improved clinical outcomes according to level of PD-L1 expression was observed which was not statistically significant for OS but was significant for PFS (p=0.028). Median PFS for patients in the PD-L1 subgroup of 80-100% expression was nearly double that for patients with PD-L1 50-79% (13.6 vs. 7.4 months), albeit the difference did not reach statistical significance in this exploratory analysis. A recent retrospective analysis of outcomes in 172 US patients treated with first-line pembrolizumab reported improved ORR (45.2% vs. 20.6%), PFS (5.3 vs. 2.4 months) and a trend to improved OS (33.6 vs. 20.6 months, HR=0.60; p=0.056) for patients with PD-L1 75-100% versus those with PD-L1 50-74%.[187] However, when compared with patients with PD-L1 expression of 50%-89%, patients with an expression level of 90%-100% did have a significantly longer median OS (NR vs. 15.9 months, HR 0.39, p = 0.002).[188] In our UK cohort, patients with PD-L1 90-100% had a similar PFS and OS compared to those with PD-L1 50-89% (9 vs 8.5 months) in an exploratory analysis. It is important to note that data on the methods of PD-L1

testing was not collected as part of the UK study and that immunohistochemistry for PD-L1 was not at the time implemented into routine diagnostic protocols for treatment-naïve patients, therefore lack of standardisation and variability in PD-L1 reporting methodology may have had an impact on the findings. This may also be reflected in the relatively prolonged timelines from diagnosis of advanced NSCLC to PD-L1 report of 18 days (95% CI 16-21), although data was not available for the timelines between dates of PD-L1 test request (or clinical decision to perform PD-L1 testing) to date of PD-L1 report. Median time of 23 days from PD-L1 report to start of pembrolizumab treatment likely reflects the complex access routes to pembrolizumab for many patients prior to UK NICE approval, including EAMS application and approval, or clinical trial screening and enrolment.

Safety data from the real-world UK patients were comparable to trial data with no new safety concerns identified. 22.8% patients experienced at least one CTCAE grade ≥3 treatment-related adverse event compared with 26.6% in KN-024, and 38% experienced any grade immune-related adverse events during pembrolizumab therapy, only 8% being grade 3 or above (29.2% and 9.7% in KN-024, respectively). Over a quarter of patients (26.5%) required use of immune suppressant therapy at some time, predominantly corticosteroids (prednisolone, dexamethasone and hydrocortisone) with use of mycophenolate and infliximab reported in only 2 patients. This is in line with other available data in NSCLC and other tumour types treated with anti-PD-1 therapy, with reported use of corticosteroids for IR AEs ranging from 24% to 43% [189–191]

These results were presented at the British Thoracic Oncology Group Meeting in 2019 and provided valuable real-world data demonstrating safety and efficacy of first-line pembrolizumab in UK treatment-naïve NSCLC patients, as well as much needed data in poor PS patients and oncogenic driver-associated NSCLC. The main limitations of this study are that this was a retrospective review and that the data are relatively immature, with an overall short median follow-up of 5.6 months at the time of data analysis, and relatively small numbers of patients in the subgroups with poor PS and mutations. Results of ongoing prospective phase II and III trials will be required to elucidate further the efficacy in these subgroups.

2.2 Retrospective review of outcomes with docetaxel and nintedanib within the UK nintedanib named-patient supply programme

2.2.1 Background

As discussed in Chapter 1.2, immune-checkpoint inhibitors are approved in relapsed NSCLC and have rapidly become established as standard-of-care, having demonstrated superior overall survival and fewer grade ≥3 toxicities in this setting over docetaxel monotherapy,[30, 31, 192] albeit not against docetaxel-nintedanib combination, an alternative licensed therapy choice for relapsed NSCLC-adenocarcinoma subtype.

Nintedanib is an oral triple-angiokinase inhibitor with activity against vascular endothelial growth factor receptors (VEGFR) 1-3, fibroblast growth factor receptors (FGFR) 1-3 and platelet-derived growth factor receptors (PDGFR) α and β.[193] In 2014, results of LUME-Lung 1 randomized phase 3 trial were reported, demonstrating that in relapsed advanced NSCLC patients following first-line platinum-based chemotherapy, addition of nintedanib led to a PFS benefit in the intention-to-treat population and an OS survival benefit (a pre-specific key secondary endpoint) over docetaxel alone, in patients with adenocarcinoma-subtype NSCLC, with greatest benefit observed in patients progressing within 9 months of starting first-line systemic therapy.[37] Based on this data, nintedanib in combination with docetaxel was licenced by the European Medicines Agency (EMA) and other geographical territories for the treatment of relapsed NSCLC adenocarcinoma.[194] The National Institute for Health and Care Excellence (NICE) approved nintedanib in England as per the licenced indication in July 2015.[195]

Between presentation of the LUME-Lung 1 trial data and EMA license (January 2015), Boehringer-Ingelheim allowed access to nintedanib in combination with docetaxel in the UK and Ireland as per the LUME-Lung 1 indication, through a named individual patient supply (NIPS) programme. This NIPS was approved by the RMH Drugs and Therapeutics Committee and required patients to meet the LUME-Lung 1 trial eligibility criteria and to be treated as per protocol paradigm. The nintedanib NIPS programme closed in January 2015 with a total of 62 patients treated nationally.

I conducted a retrospective review of outcomes in these patients with the aim to benchmark real-world use and clinical outcomes of docetaxel-nintedanib combination, at a time when immune checkpoint inhibitor therapy was not established as standard.

2.2.2 Methods

This was a national retrospective multi-centre review of outcomes for all patients who received treatment with docetaxel-nintedanib within the nintedanib NIPS programme at participating centres in the UK (and IE).

Patient were identified from anonymised lists of all patients and investigators who received approval for treatment within the programme, obtained from Boehringer-Ingelheim Ltd. Investigator consent for contact details to be shared for purposes of participation in the study was sought at time of NIPS request. Investigators were contacted to invite them to contribute anonymised data on their patients participating in the programme.

Overall regulatory approvals were granted by the Royal Marsden Research and Development Committee as the initiating centre, with local governance approvals sought by each participating centre for patient data collection.

A validated excel spreadsheet data capture tool was created in collaboration with RMH RDSU and used for data capture. Data was collected on: patient demographics (age, gender, ethnicity, smoking status); baseline disease characteristics (histology, clinical stage at diagnosis, mutational status, ECOG PS at time of NIPS request; brain metastases at time of NIPS request); prior therapies (prior radical surgery or radiotherapy, first-line systemic therapy for advanced/metastatic disease, first-line maintenance therapy, best-response to first-line chemotherapy investigator-defined as partial response, stable disease, complete response or progressive disease, date of PD on or after first-line therapy, any prior anti-angiogenic therapies); data on nintendanib NIPS (start and end dates of docetaxel and nintedanib; number of cycles of docetaxel-nintedanib combination, docetaxel alone and nintedanib alone; best response; date of PD; dose reductions and dose delays; use of granulocyte-colony stimulating factor; all toxicities; grade ≥3 treatment-related toxicities); and follow-up data (subsequent systemic therapy; date of last follow-up; disease status at last

follow-up). All data items collected, with possible responses, can be found in Appendix 2. All patients who received approval within nintedanib NIPS were eligible for inclusion in the study, regardless of whether they commenced treatment with docetaxel-nintedanib, duration of treatment or baseline characteristics such as histology or performance status. On-treatment assessments were performed according to standard local clinical guidelines and protocols, with no pre-specified response assessment imaging criteria. According to nintedanib NIPS access criteria, patients were permitted to continue with nintedanib monotherapy after discontinuation of docetaxel following completion of planned number of docetaxel cycles or due to intolerance of docetaxel, provided they received at least one cycle of combination treatment.

Completed spreadsheets of anonymised data were returned by secure NHS email and collated at initiating centre (RMH) for pooling, data cleaning, and analysis. Each patient was allocated a unique study ID on receipt of data at the pooling centre. Data cleaning was performed to confirm eligibility criteria were satisfied, to identify any missing data and inconsistencies, and for correct formatting of data in preparation for statistical analyses. Any data queries were sent to participating investigators via secure NHS e-mail.

Data collection commenced in July 2015 and closed in May 2016.

2.2.2.1 Objectives, endpoints and definitions

The primary objective was to evaluate the progression free survival of all patients treated with docetaxel-nintedanib within the UK nintedanib NIPS programme. Secondary objectives were: to evaluate the overall survival and objective response rate in the same patient population; to identify any prognostic factors associated with OS and PFS; and to assess the safety and tolerability of the combination.

The primary endpoint was median PFS, defined as the median time from commencement of docetaxel-nintedanib to disease progression or death.

Secondary endpoints were:
- Median OS, defined as the median time from start of docetaxel-nintedanib to death from any cause, and 1-year overall survival rate, defined as proportion

of patients still alive at 1 year following commencement of docetaxel-nintedanib;

- Objective response rate (ORR, investigator-defined as best response of partial response or complete response);

- Prognostic factors associated with OS and PFS, including age, gender, smoking status, performance status, presence/absence of brain metastases, previous treatment modalities;

- Median OS and PFS for the subgroup of patients progressing within 9 months of commencing first line therapy;

- All CTCAE v 4.0 grade ≥3 treatment-related toxicities;

- Number of treatment cycles delivered of docetaxel-nintedanib doublet (combination therapy), of docetaxel alone (docetaxel monotherapy) and nintedanib alone (nintedanib monotherapy);

- Treatment delays;

- Rates of hospitalization;

- Use of granulocyte-colony stimulating factor (GCSF) prophylaxis, primary and secondary;

- Proportion of patients receiving approval for use of nintedanib within nintedanib NIPS programme but not commencing treatment.

2.2.2.2 Statistical considerations

Median PFS and OS, in the overall population and for the subgroups of patients progressing within 9 months of first-line therapy, were calculated using Kaplan-Meier methods, using SPSS software, with median survival and 1-year OS rates given with 95% confidence intervals. Surviving patients were censored at the date of last follow-up.

The objective response rate was calculated as the proportion of patients with investigator-assessed CR and PR, from the total treated, with 95% confidence intervals given.

Cox regression was used to assess influence of prognostics variables (age, gender, smoking status, PS, presence of brain metastases, previous treatment modalities) on OS and PFS, with hazard ratios given with 95% confidence intervals. Univariate

analysis was performed with each factor first, and only those with a p-value <0.2 were tested in the multivariate model. Factors with a p-value <0.05 in the multivariate analysis were considered significant.

Proportion of patients experiencing any treatment-related toxicity of grade ≥3, dose reductions, dose delays, hospitalisation and GCSF use will be reported in the form of percentages with 95% CIs. Number of treatment cycles administered will be given as median number with range.

2.2.3 Results

2.2.3.1 Patients

Complete data was collected on all 62 patients from 19 clinicians and 13 centres who received approval for use of nintedanib with docetaxel within the NIPS programme between December 2013 and March 2015. 1 patient died shortly after a request for NIPS access was made and prior to approval being received, and is excluded from analysis after baseline characteristics.

Median age of patients was 62 (range 33-82) and female to male ratio was 54% to 46%. 82% of patients were current or former smokers and all patients had adenocarcinoma-subtype NSCLC. Two thirds of patients had stage IIIB or stage IV disease at diagnosis (all had advanced or metastatic disease at time of NIPS application), while 21% and 15% had previously undergone radical surgery and radical radiotherapy, respectively. 18% of patients had an ECOG PS of >1 at time of nintedanib NIPS application. 94% of patients received platinum-doublet chemotherapy in the first-line of systemic treatment for advanced NSCLC and a third of patients received pemetrexed maintenance. 2 patients had a known *EGFR* sensitising mutation and had progressed after first-line EGFR TKI therapy. 5 patients had a known *KRAS* variant. None of the patients had received prior immune-checkpoint inhibitor therapy. 71% of patients had developed progressive disease within 9 months of start of first-line therapy. Patient demographics and baseline characteristics are summarised in Table 2.9.

Patient Characteristics (n = 62)	n	% (out of 62)
Median Age (range)	62 (33-82)	
GENDER		
Female	34	54.8
Male	28	45.2
SMOKING		
Missing	1	1.6
Current	12	19.4
Ex-smoker	39	62.9
Never smoker	10	16.1
CLINICAL STAGE AT DIAGNOSIS		
Stage <IIIB	15	24.2
Stage IIIB	4	6.5
Stage IV	43	69.3
NSCLC HISTOLOGICAL SUBTYPE		
Adenocarcinoma	62	100.0
ECOG PS AT TIME OF NINTEDANIB NIPS‡ REQUEST		
0	10	16.1
1	40	64.5
2	11	17.8
Missing	1	1.6
PREVIOUS RADICAL SURGERY		
No	49	79.0
Yes	13	21.0
PREVIOUS RADICAL RADIOTHERAPY		
No	53	85.5
Yes	9	14.5
FIRST-LINE SYSTEMIC THERAPY FOR ADVANCED OR METASTATIC DISEASE		
Non-platinum based	3	4.8
Platinum-based	58	93.6
Unknown	1	1.6
PREVIOUS BEVACIZUMAB		
No	61	98.4
Yes	1	1.6
FIRST-LINE MAINTENANCE THERAPY		
None	38	61.3
Pemetrexed	21	33.9
Erlotinib	1	1.6
Other*	2	3.2
BEST RESPONSE TO 1ST LINE THERAPY†		
Partial response	33	53.2
Stable disease	18	29.0
Progressive disease	7	11.3
Complete response	2	3.2
Not known/not available	2	3.2
PD <9 MONTHS FROM START OF 1ST LINE THERAPY		
Yes	44	71.0
No	17	27.4
Unknown	1	1.6
METASTASES AT TIME OF NINTEDANIB NIPS REQUEST		
No	1	1.6
Yes	61	98.4
BRAIN METASTASES AT TIME OF NINTEDANIB NIPS REQUEST		
No	37	59.7
Not known	21	33.9
Yes	4	6.5

Table 2.9. Demographics and baseline characteristics for all 62 patients enrolled in the UK Nintedanib NIPS programme. *2 patients received pazopanib/placebo within the EORTC 08092 Phase III trial. †Best response to first line therapy as clinically assessed and reported by treating clinician.

2.2.3.2 Delivery of docetaxel/nintedanib

52 out of 62 patients (84%) went on to receive at least one cycle of docetaxel with nintedanib. Reasons why patients did not receive treatment with docetaxel-nintedanib despite obtaining NIPS approval include: clinical deterioration or death due to progressive disease (6 patients), start of other anti-cancer therapy (2 patients), patient decision not to proceed (1 patient) and loss to follow-up (1 patient).

For the 52 patients who received at least one cycle of docetaxel-nintedanib combination, median number of all treatment cycles was 5 (range 1-18). 22 out of 52 patients (42.3%) received nintedanib monotherapy maintenance, with 16 (30.8%) continuing nintedanib after completion of the total planned number of cycles of docetaxel-nintedanib combination therapy (median 4; range 1-12). 4 (7.7%) patients received nintedanib monotherapy after discontinuing docetaxel early due to toxicity, and 2 (3.8%) after treating physician's decision to discontinue docetaxel. Median number of nintedanib monotherapy cycles was 3 (range 1-12). 8 out of 52 patients (15.4%) received at least 1 cycle of docetaxel monotherapy, including 3 (5.8%) who discontinuing nintedanib due to toxicity, and 5 (9.6%) who received docetaxel monotherapy while awaiting NIPS approval for nintedanib. Median number of docetaxel monotherapy cycles was 1 (range 1-5).

Starting dose of docetaxel was $75mg/m^2$ in 42 out of 52 (80.8%) patients. 16 out of 42 patients (38.1%) who commenced treatment at $75mg/m^2$ required at least one docetaxel dose reduction. 10 out of 52 (19.2%) patients received $60mg/m^2$ as the initial docetaxel dose, with 3 (30%) of these patients requiring at least one further dose reduction. Overall dose reductions of docetaxel were required for 19 patients or 36.5%. Nintedanib dose reductions were required for 5 out of 52 patients (9.6%). Primary GCSF prophylaxis was given to 10 out of 52 (19.2%) patients while 5 patients (9.6%) received secondary GCSF prophylaxis. 33 out of 52 patients (63.5%) did not receive any GCSF. Data on GCSF use was missing for 4 patients (7.7%).

2.2.3.3 Efficacy

At the time of data capture, after a median follow-up of 21.2 months (95% CI 18.6-26.3), 41 out of 52 patients (79%) had died, 9 (17.3%) had developed progressive disease but remained alive and 2 (3.7%) were alive with no evidence of disease progression.

Median PFS for all 52 patients treated with docetaxel-nintedanib was 4.2 months (95% CI 2.7-5.2) with 6-month and 1-year PFS rates of 28.2% and 6.8%, respectively (Figure 2.7(a)). Median OS was 9.2 months (95% CI 6.5-10.8), with 1-year OS rate of 31.4% (95% CI 19.3%-44.2%) and 2-year OS rate of 17.7% (95% CI 8.1%-30.5%) (Figure 2.7(b)).

None of the prognostic covariates demonstrated a significant effect during univariate analysis, and no covariates were analysed within the multivariate model.

ORR was 28.9% with partial response to docetaxel-nintedanib observed in 15 out of 52 patients, and no patients achieved a complete radiological response. 22 out of 52 patients (42.3%) patients had stable disease as best response. Disease control rate (defined as combined rate of PR, CR and SD) was 71.2% (95% CI 56.9%-82.9%).

Median PFS and OS were also assessed for the subgroup of patients who progressed within 9 months of commencing first-line systemic therapy for advanced/metastatic disease. Out of 52 patients who received docetaxel-nintedanib, 36 or 69.2% had developed progressive disease within 9 months from start date of first-line therapy. For these patients, median PFS was 2.8 months (95% CI 1.6-4.8, Figure 2.7(c)) and OS was 8.8 months (95% CI 4.9-12.6, Figure 2.7(d)).
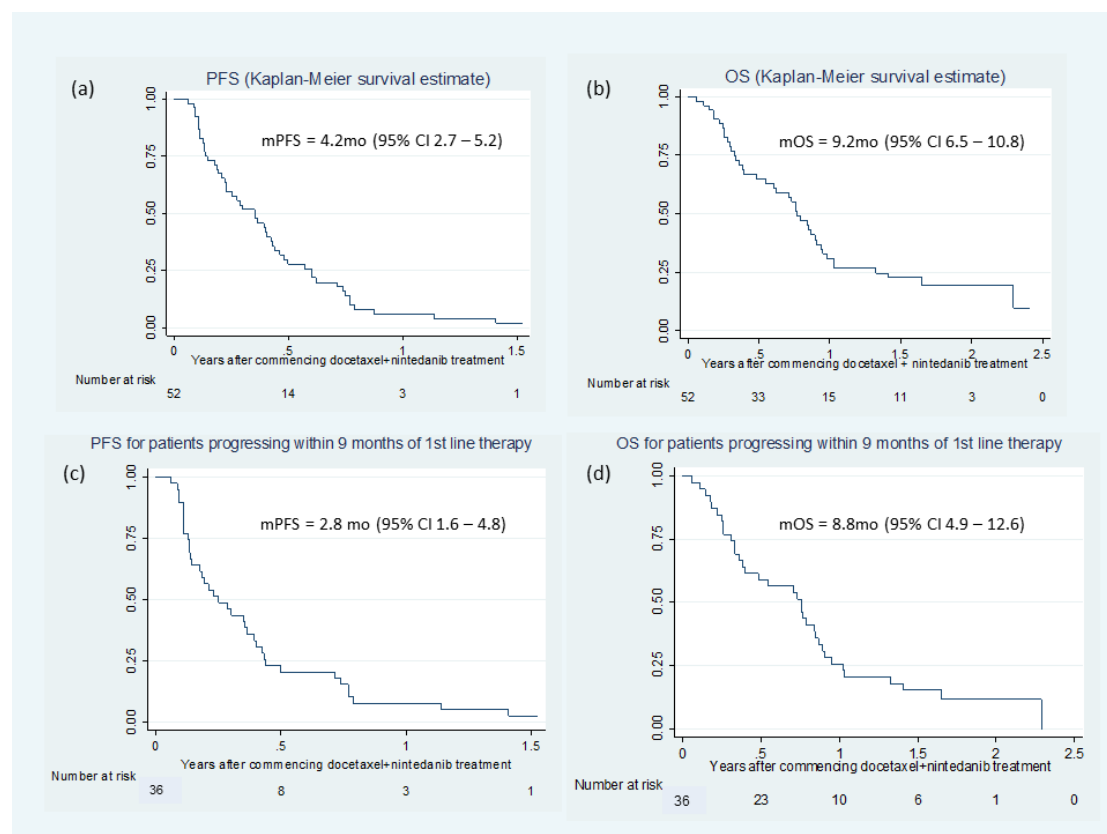
Figure 2.7. Kaplan-Meier survival estimates for: (a) PFS patients who received docetaxel-nintedanib within nintedanib NIPS (n=52); (b) OS for patients who received docetaxel-nintedanib within nintedanib NIPS (n=52); (c) PFS for patients who received docetaxel-nintedanib having progressed within 9 months of first-line therapy (n=36); (d) OS for patients who received docetaxel-nintedanib having progressed within 9 months of first-line therapy (n=36).

2.2.3.4 Safety

15 out of 52 patients (28.8%) experienced treatment-related CTCAE grade ≥3 adverse events. The commonest grade ≥3 events were febrile neutropaenia (13.5%), decreased neutrophils (5.8%) and dyspnoea (3.8%). There was one grade ≥3 diarrhoea event (1.9%) and no grade ≥3 transaminitis events. There were no grade 4 or 5 events. All reported adverse events are summarised in Table 2.10.

22 out of 52 patients (42.3%) required hospitalisation for any reason during treatment. In 12 out of 52 patients (23.1%), hospitalisation was treatment-related, in 6 patients (11.5%) related to disease progression and in 4 patients (7.7%) attributable to other causes.

Adverse events	All grades (%)	Grade ≥3 (%)
Any AE leading to dose reduction of nintedanib	7 (13.5)	2 (3.8)
Any AE leading to dose reduction of docetaxel	18 (34.6)	10 (19.2)
Any AE	31 (59.6)	15 (28.8)
Fatigue	10 (19.2)	1 (1.9)
Febrile neutropaenia	7 (13.5)	7 (13.5)
Decreased neutrophils	4 (7.7)	3 (5.8)
Diarrhoea	4 (7.7)	1 (1.9)
Increased hepatic enzyme	4 (7.7)	0 (0)
Dyspnoea	3 (5.8)	2 (3.8)
Peripheral sensory neuropathy	3 (5.8)	0 (0)
Pain	3 (5.8)	1 (1.9)
Lung infection	2 (3.8)	1 (1.9)
ALT increased	2 (3.8)	0 (0)
Nausea	2 (3.8)	0 (0)
Stomatitis	2 (3.8)	0 (0)
AST increased	1 (1)	0 (0)
Oedema	1 (1)	0 (0)
Decreased appetite	1 (1)	0 (0)
Infection – other*	1 (1)	0 (0)
Radiation recall	1 (1)	0 (0)
Pericardial effusion	0 (0)	1 (1.9)
Oesophageal fistula	0 (0)	1 (1.9)

Table 2.10. Overview of all reported adverse events, classified according to CTCAE version 4.0, for all patients who received docetaxel-nintedanib. AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events. *Not specified.

2.2.3.4 Post-docetaxel-nintedanib therapy

26 out of 52 patients (50%) received third-line therapy after docetaxel-nintedanib. 17 patients received third-line erlotinib, 5 patients received experimental trial treatment (3 within phase I clinical trial protocols, 2 within phase II), 3 patients received platinum-doublet chemotherapy and 1 patient received vinorelbine monotherapy.

2.2.4 Discussion and conclusions

In the era before immune-checkpoint inhibitors for advanced NSCLC, chemotherapy remained the mainstay of systemic therapies in relapsed NSCLC, but options after progression on first-line platinum-doublet chemotherapy were limited and an area of significant unmet need. Publication of LUME-Lung 1 trial was welcomed as one of the first phase III trials to demonstrate survival benefit of addition of oral anti-angiogenic agents to standard docetaxel chemotherapy in adenocarcinoma NSCLC, but the real-world efficacy and safety of docetaxel-nintedanib combination in patients with relapsed advanced or metastatic NSCLC were unknown.

LUME-Lung 1 was an international, multi-center, phase III, double-blind trial which randomised 1314 patients with stage IIIB or IV NSCLC after relapse after one prior systemic chemotherapy to receive docetaxel with nintedanib or docetaxel with placebo.[196] Progression free survival was significantly improved in the docetaxel-nintedanib group compared with the docetaxel-placebo group (median PFS 3.4 vs. 2.7 months; HR 0.79; p=0.0019) in the intention to treat population. Overall survival benefit did not reach significance in the overall study population, but in a pre-planned subgroup analysis, median OS was significantly longer for patients with adenocarcinoma histology (mOS 12.6 vs. 10.3 months; HR 0.83; p=0.0359), with the greatest benefit observed in a predefined subgroup of patients with adenocarcinoma who had progressed within 9 months after start of first-line treatment (HR 0.75, p=0.0073).

Findings from real-world data in UK patients are comparable to that from LUME-Lung 1 trial, with median PFS (mPFS) of 4.2 months, mOS of 9.2 months and 1-year OS rate of 31.4% for 52 patients who received at least one cycle of combination docetaxel-nintedanib within the UK nintedanib NIPS programme, compared to mPFS

of 4.2 months, mOS of 12.6 months and 1-year OS of 52.7% for adenocarcinoma subgroup in LUME-Lung 1. In the subgroup of patients progressing <9 months from start of first-line therapy, mPFS was 2.8 months and mOS 8.8 months (3.6 and 10.9 months in LUME-Lung 1, respectively). The comparison of efficacy outcomes between UK real-world data and LUME-Lung 1 trial data is outlined in Table 2.11.

Endpoint	UK Nintedanib NIPS n=52	LUME-Lung 1 (Adenocarcinoma) n=320	LUME-Lung 1 (ITT population) n=655
mPFS	4.2 months	4.2 months	3.5 months
mOS	9.2 months	12.6 months	10.1 months
1-year OS rate	31.4%	52.7%	-
ORR	28.9%	4.7%	10.4%*
DCR	71%	60%	63.4%*
mPFS if PD <9mo from 1L treatment	2.8 months	4.2 months	-
mOS if PD <9mo from 1L treatment	8.8 months	10.9 months	-

Table 2.11. Comparison of efficacy outcomes between UK nintedanib NIPS patients and LUME-Lung 1 trial population. *At time of final OS analysis. DCR, disease control rate; ITT, Intention-to-treat; mOS, median overall survival; mPFS, median progression free survival; ORR, objective response rate; PD, progressive disease.

While mPFS (overall and for the subgroup of early progressors after first-line therapy) is similar to that reported in LUME-Lung 1 trial, mOS and 1-year OS rates were lower in the NIPS cohort compared with the LUME-Lung 1 trial population, likely reflecting the unselected nature of our patient population. For instance, 11 out of 52 (17.8%) of patients had an ECOG performance status of 2 and 6.5% patients had confirmed brain metastases at the time of NIPS request, while these patient groups were excluded from enrolment into LUME-Lung 1.

10 patients out of 62 who received approval for use of docetaxel-nintedanib through the NIPS scheme did not go ahead to receive treatment with at least one cycle of combination therapy, including 6 patients (9.7%) who developed clinical deterioration or death due to progressive disease in the interval between nintedanib NIPS request and approval, which is reflective of the natural history of metastatic NSCLC adenocarcinoma, while screen failure rate in LUME-Lung 1 due to clinical

deterioration or death during screening is not reported. Patients who went on to receive docetaxel-nintedanib may also have experienced deterioration in their symptoms and performance status during the time interval between nintedanib NIPS request and start of docetaxel-nintedanib, which could have had a bearing on subsequent outcomes. Furthermore, median time from first diagnosis to randomisation within LUME-Lung 1 trial was reported as 8.8 months, while in the NIPS cohort median time from start of first-line systemic therapy for advanced/metastatic disease to start of docetaxel-nintedanib was over 12 months. Hence, we cannot exclude that this population was prognostically superior to that of LUME-Lung 1. The higher rate of reported ORR in the nintedanib NIPS patients (30% vs 10% in trial patients) is due to the fact that responses were clinician reported without mandated use of RECIST criteria or independent radiological review.

Safety data is consistent with previously reported with rate of all grade ≥3 events of 28.8% (LUME-Lung 1: 31.3%), dose reductions to docetaxel and/or nintedanib in 36.5% and toxicity-related hospitalizations of 23.1%. Of note, use of primary GCSF prophylaxis was observed in nearly 20% of patients, while prophylactic or primary GCSF was at the time not approved by NICE in the UK for palliative regimens.

The main weaknesses of this study are that this is a small cohort of patients, where data was collected retrospectively, with clinician assessed and reported objective responses and toxicities. Results of future studies designed to prospectively collect post-marketing data in patients commencing newly licenced drugs or combination therapies are required to obtain timely and accurate information on the real-world efficacy, safety and cost-effectiveness of novel drug regimes. Nevertheless, efficacy and safety of docetaxel-nintedanib combination used in real life in the UK seems comparable to that published in LUME-Lung 1, benchmarking real-world clinical utility and providing independent data for efficacy, safety and health technology appraisals when the comparator arm in relapsed adenocarcinoma-subtype advanced NSCLC is docetaxel-nintedanib. Studies such as this one, mapping real world experiences and patient outcomes of novel regimes to clinical trial data, can help corroborate clinical efficacy, financial feasibility, assist in clinical decision-making, as well as decision making regarding drug reimbursement.

2.3 Set-up of a phase I/II trial of combination nab-paclitaxel and nintedanib or nab-paclitaxel and placebo in relapsed NSCLC adenocarcinoma: N3 trial

2.3.1 Trial rationale

As discussed in Chapters 1.2.4 and 2.2, combination of docetaxel and nintedanib is now a standard choice of second-line therapy for advanced NSCLC adenocarcinoma-subtype in the UK, however with low response rates, modest OS benefit and, for many patients, prohibitive toxicity rates. Furthermore, efficacy of these agents after first-line immunotherapy has not been formally assessed, with emerging evidence of increased responsiveness to chemotherapy after exposure to immune-checkpoint inhibition, as well as evidence of a synergistic effect of immunotherapy and anti-angiogenesis. Evidence of increased objective response rates and prolonged PFS with ramucirumab-containing regimens and nintedanib plus docetaxel after ICIs is currently derived mainly from retrospective data, but there is also some early prospective data to corroborate this, with an overall strong signal of increased effectiveness that requires further investigation in larger prospective randomised trials.[68–70]

Nab-paclitaxel, nanoparticle-albumin-bound paclitaxel, has been developed to improve the therapeutic index of paclitaxel and has been shown to have an improved toxicity profile compared to solvent-bound paclitaxel and docetaxel in breast and lung cancer patients.[197, 198] Weekly nab-paclitaxel, alone or in combination with other cytotoxic chemotherapy, has been investigated in treatment-naive and relapsed advanced NSCLC with evidence of activity and a good safety profile.[199–204]

Maximum tolerated dose (MTD) and activity of single agent weekly nab-paclitaxel in previously untreated NSCLC were investigated in an open-label single arm phase I/II study, with the dose of 125 mg/m^2 on days 1, 8, and 15 of a 28-day cycle determined to be the MTD.[202] In the expanded cohort of 40 patients treated at the MTD, the objective response rate was 30%, median PFS 5 months, median OS was 11 months and 1-year OS was 41%. Subsequent phase II dose finding study of weekly and three-weekly nab-paclitaxel at several different dose levels in combination with q3w

carboplatin in previously untreated NSCLC found that weekly dosing at 100mg/m^2 resulted in improved safety and efficacy profile compared with three-weekly dosing (ORR 47% vs. 30%).[198] This dosing schedule was subsequently taken forward into a large phase III study of weekly nab-paclitaxel with carboplatin versus solvent-bound paclitaxel with carboplatin in treatment-naïve patients.[199] Nab-paclitaxel demonstrated a significant improvement in the primary endpoint of ORR (33% vs. 25%; 95% CI 1.08-1.59; p=0.005) compared with paclitaxel, with a non-significant trend towards improvement in PFS and OS, and a significantly lower rate of grade ≥3 peripheral neuropathy and neutropenia. OS was significantly longer with nab-paclitaxel in the subgroup of elderly patients (age ≥70; median OS 19.9 vs. 10.4 months; HR 0.583; p=0.009).[200] These findings led to weekly nab-paclitaxel being FDA approved and EMA licensed for the treatment of 1st line advanced NSCLC in combination with carboplatin, at a dosage of 100 mg/m^2 on Days 1, 8, and 15 in combination with carboplatin (AUC = 6) on Day 1, every 21 days. Nab-paclitaxel has also demonstrated single agent activity in the setting of previously treated NSCLC. In a phase II single-arm study of weekly nab-paclitaxel at 100mg/m2 on days 1, 8 and 15 of a 4-weekly cycle in patients with ECOG PS 0-2 relapsed after at least one prior line of treatment, demonstrated ORR of 19%, median PFS of 4.5 months, median OS of 15.7 months, and 1-year OS rate of 54.8%.[205] In a randomised phase II study, weekly nab-paclitaxel at a dose of 150mg/m^2 on day 1 and 8 of a 3-weekly cycle demonstrated equivalent efficacy and safety to pemetrexed in the second-line setting in NSCLC unselected for histology, with median OS of 9.9 months vs. 9.4 months, and median PFS of 5.1 vs. 4.6 months for nab-paclitaxel and pemetrexed, respectively.[203] More recently, a phase III study comparing weekly nab-paclitaxel (100 mg/m^2 on days 1, 8, and 15 q3w) with docetaxel (60mg/m^2 on day 1 q3w) in previously treated NSCLC reported non-inferiority of nab-paclitaxel with ORR 29.9% vs. 15.4%, median PFS 4.2 vs. 3.4 months, and median OS of 16.2 vs. 13.6 months for nab-paclitaxel vs. docetaxel respectively, while the trend towards superiority of nab-paclitaxel was not statistically confirmed.[206]

At the time the study was developed, the recommended phase 2 dose (RP2D) of combination nab-paclitaxel and nintedanib was unknown and the efficacy of the combination has not been investigated to date. We designed a phase Ib/II trial to

explore the safety, tolerability and efficacy of combination nab-paclitaxel and nintedanib in relapsed adenocarcinoma NSCLC (N3 trial).

Part 1 of the trial (Phase Ib) was designed to evaluate the incidence of dose limiting toxicities when nab-paclitaxel is given in combination with nintedanib and to determine the recommended phase II dose.

Hypothesis to be explored in Part 2 of the trial (Phase II) is that addition of nintedanib to nab-paclitaxel is safe, tolerable and active in patients with relapsed advanced or metastatic adenocarcinoma NSCLC.

The participating patient population will be patients with relapsed advanced or metastatic non-small cell lung cancer of adenocarcinoma histology, in whom second and subsequent line treatment options are extremely limited and there is evidence of promising activity of nintedanib in combination with chemotherapy agents, as well as evidence of enhanced activity after prior immune checkpoint inhibitors, and where tolerability is a significant limitation to delivery of currently available second line treatments. Patients with adenocarcinoma and known driver mutations in the EGFR and ALK genes would also be included provided they have received prior treatment with an appropriate tyrosine kinase inhibitor in the first or second advanced or metastatic treatment line setting.

The chosen nab-paclitaxel dose and schedule is $100mg/m^2$ on day 1 and day 8 of every 3-weekly cycle. As noted above, the dose of weekly nab-paclitaxel licenced in first-line setting in combination with carboplatin was $100 mg/m^2$ on days 1, 8, and 15 every 3 weeks. The decision to allow one week rest period in this study, with no treatment on day 15, was made taking into account that patients with previously treated NSCLC are unlikely to tolerate the same dose-intensity compared to treatment-naïve patients, with previous trial experience with taxanes showing higher rate of dose reductions in previously treated patients, and therefore the proposed schedule would be more likely to allow consistent dose-intensity for NSCLC patients in this setting. The same schedule was also taken forward in other ongoing trials of nab-paclitaxel in second-line NSCLC in combination with targeted agents and immunotherapy (NCT02250326; NCT02289456).

The exploratory phase II part of the trial is to directly explore activity and efficacy of combination nab-paclitaxel and nintedanib in this patient population. If the

combination is felt to be safe and efficacious, phase 3 trial would be considered and powered to detect improvements in PFS and OS over current standard treatments.

2.3.2 Trial design

This is a phase Ib/II multi-centre dose-finding study to explore the safety, tolerability and efficacy of combination nab-paclitaxel and nintedanib in patients with relapsed advanced or metastatic NSCLC of adenocarcinoma histology. The study will consist of two parts, as show in the trial schema Figure 2.8.
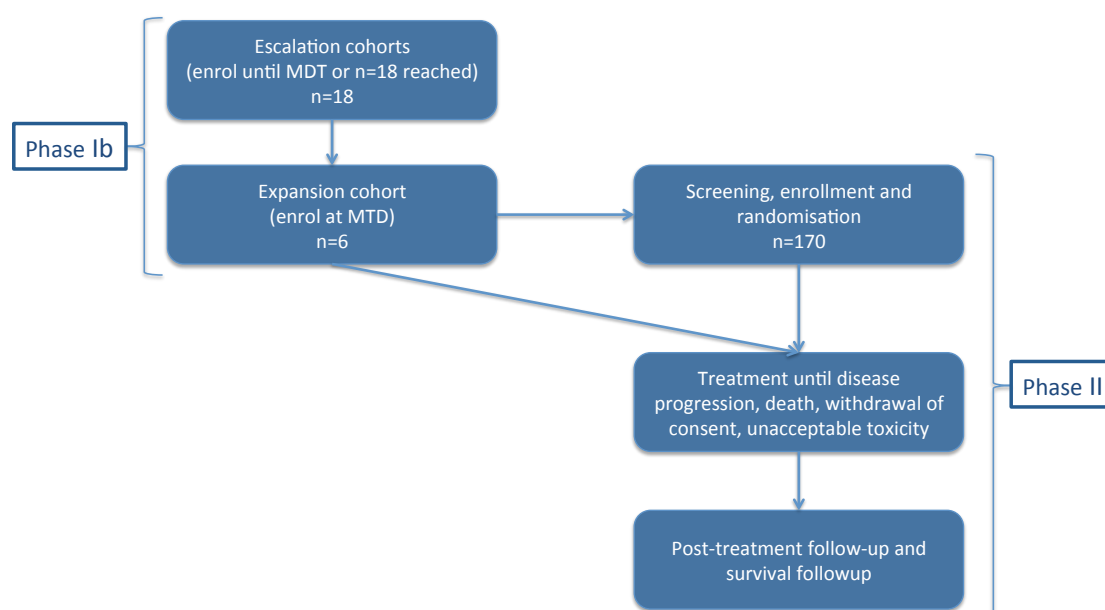
Figure 2.8. N3 overall trial schema

2.3.2.1 Part 1 (Phase Ib) trial design

Part 1 of the trial is designed as a Phase Ib multicentre dose-finding study, with a standard 3+3 design, to determine the maximum tolerated dose/recommended phase 2 dose (MTD/RP2D, as defined in section 2.3.2.1.2), safety and tolerability of nintedanib in combination with nab-paclitaxel. All patients will receive weekly nab-paclitaxel 100mg/m^2 IV on d1, d8, every 21 days. There will be three nintedanib dose cohorts, each with 3 or 6 patients. Nintedanib will not be given on day of nab-paclitaxel dosing (due to potential effects on nintedanib pharmacokinetics). Nintedanib dose levels will be:

- Dose level -1: 100mg po BID d2-7, 9-21, q21

- Dose level 1: 150mg po BID d2-7, 9-21, q21
- Dose level 2: 200mg po BID d2-7, 9-21, q21

Dose limiting toxicities (DLTs) used to determine dose-escalation or cohort expansion will be based on Cycle 1 (DLT window cycle 1 d1-21). DLTs are defined in section 2.3.2.1.1.

Nintedanib will commence at dose level 1 (150mg po BID) and escalate to level 2. In case dose level 1 is not tolerable, nintedanib dose will de-escalate to level -1 (100mg po BID).

Within each cohort, every patient will need to undergo a safety review on day 22 before next dose level cohort can commence. In each cohort, if there are no DLTs among the first 3 patients, then the next dose level cohort will commence. If there is 1 DLT, another 3 patients will be added to the current cohort. If there are no further DLTs (i.e. 1 DLT in 6 patients only), the next dose level cohort can commence. If at least 2 out of the 6 patients have a DLT, the trial dose escalation stops and no higher dose will be used. If DLT is identified in dose level 1, then dose level -1 will be explored. The decision to dose-escalate to the next dose level or to declare an MTD/RP2D will be determined by the Trial Steering Committee (TSC) based on results from clinical and laboratory safety data for a given cohort. The TSC will also determine the dose appropriate for the Phase II portion of the study (or the RP2D). The RP2D will be the highest dose in the Phase I where there is an acceptable safety profile and where no more than 1 DLT was experienced out of the 6 patients in the cohort. Six additional patients will be recruited and given the same dose to confirm tolerability and safety before proceeding to Part 2. If there are more than 1 in 6 DLTs at dose level -1, the TSC will determine whether to stop the dose expansion phase and Part 2 of the trial.

Any withdrawals or dropouts prior to the end of the first cycle for any other reason than DLT, or any patients that do not meet the eligibility criteria will be replaced. Patients who do not complete the first cycle due to a DLT will not be replaced. Patients will be issued with screening number before enrolment onto the study over the 28 day screening period.

Part 1/Phase Ib trial schema is shown in Figure 2.9.
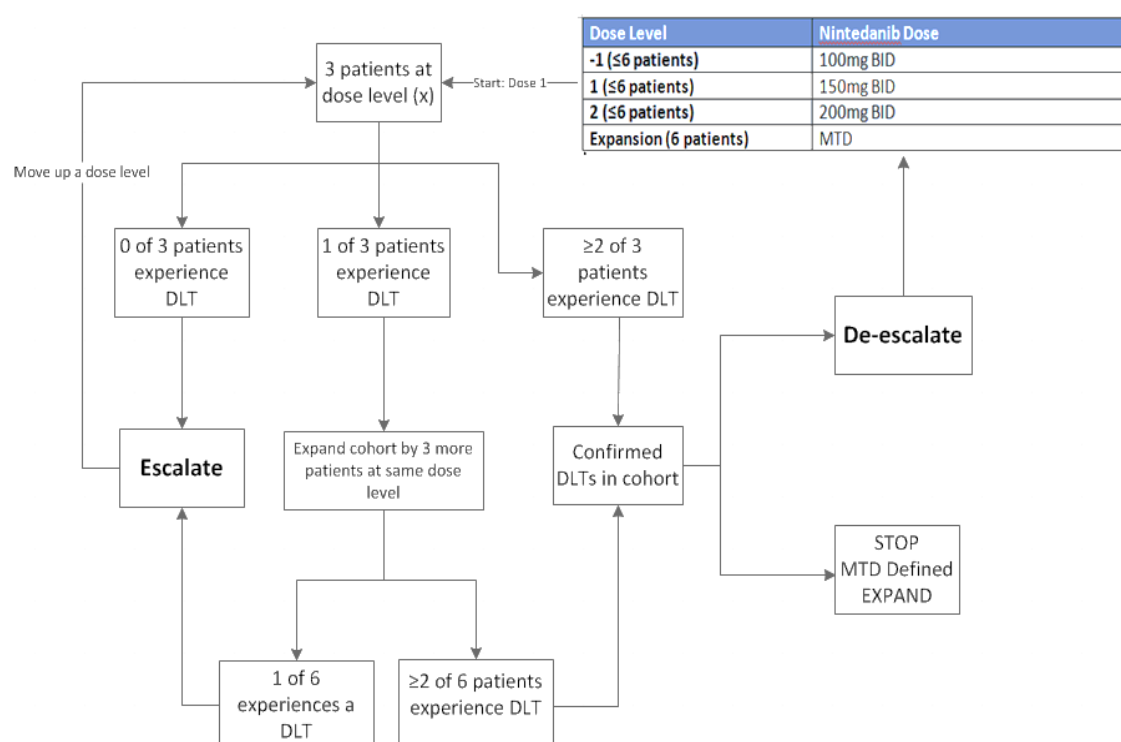
Figure 2.9. N3 trial: Part 1 (Phase Ib) trial schema.

2.3.2.1.1 Definition of DLT

The following adverse events occurring within first 3 weeks of treatment will qualify as DLT if considered drug-related: non-haematological toxicity ≥ CTCAE grade 3, in particular gastrointestinal toxicity (e.g. nausea, vomiting, diarrhoea, abdominal pain) or hypertension despite optimal supportive care/ intervention; nintedanib-related liver toxicity except isolated GGT[*] including AST/ALT >5x ULN[†] independent of bilirubin and AST/ALT >2.5x ULN[‡] together with total bilirubin >1.5 ULN[§]; haematological toxicity including CTCAE Grade 4 neutropenia that is uncomplicated (not associated with fever ≥38.5°C) only if continuing for >7 Days, CTCAE grade 4 febrile neutropenia of any duration if associated with fever ≥38.5ºC, and CTCAE grade 4 platelet decrease or CTCAE grade 3 platelet decrease if associated with bleeding or requiring transfusions.

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[*] isolated GGT elevation with no corresponding ALT/AST/ increase will not be considered

[†] corresponding to grade 3 toxicity according CTCAE

[‡] corresponding to grade 2 toxicity according CTCAE

[§] corresponding to grade 2 toxicity according CTCAE

Inability to resume nintedanib dosing within 14 days of stopping due to treatment related toxicity will also be considered a DLT.

In case adverse events with CTCAE grade 3 or 4 were not judged as DLT from a clinical point of view, the sponsor will obtain a confirmation from the investigator regarding the appropriateness of the judgment.

All DLT events will have to be reported immediately to the sponsor. All DLT events that occur in individual patients at any time during repeated treatment courses or the follow-up period must also be reported as Significant Adverse Events (SAE).

2.3.2.1.2 Definition of MTD

The MTD is defined as the highest dose of nintedanib associated with the occurrence of DLTs in fewer than 2/6 patients. The MTD estimated will be the dose level at which 0/3 or 1/6 patients will experience a DLT during the first cycle of treatment and will be below the maximum administered dose if the next higher dose has at least 1/3 or 2/6 patents experiencing DLTs.

2.3.2.2 Part 2 (Phase II) trial design

Part 2 is designed as a randomised double-blind placebo-controlled Phase II trial of nab-paclitaxel and nintedanib at recommended phase 2 dose as determined during Part 1, versus nab-paclitaxel and placebo. All patients will receive weekly nab-paclitaxel 100mg/m^2 IV d1, d8, q21 and will be randomised into 2 arms at a 1:1 ratio to receive nintedanib RP2D or placebo. In Arm A, patients will receive nab-paclitaxel 100mg/m^2 IV d1, d8, q21 with placebo. In Arm B, patients will receive nab-paclitaxel 100mg/m^2 IV d1, d8, q21 with nintedanib at RP2D. Part 2 (Phase II) trial schema is shown in Figure 2.10.

Schedule of dosing of nab-paclitaxel and ninitedanib will be the same in Phase II as in Phase Ib. Patients discontinuing nab-paclitaxel due to toxicity or patient/physician decision are allowed to continue nintedanib/placebo monotherapy. Patients discontinuing nintedanib/placebo due to toxicity or patient/physician decision are allowed to continue nab-paclitaxel monotherapy until disease progression. There will be no restriction on the maximum number of nab-paclitaxel cycles that can be administered.
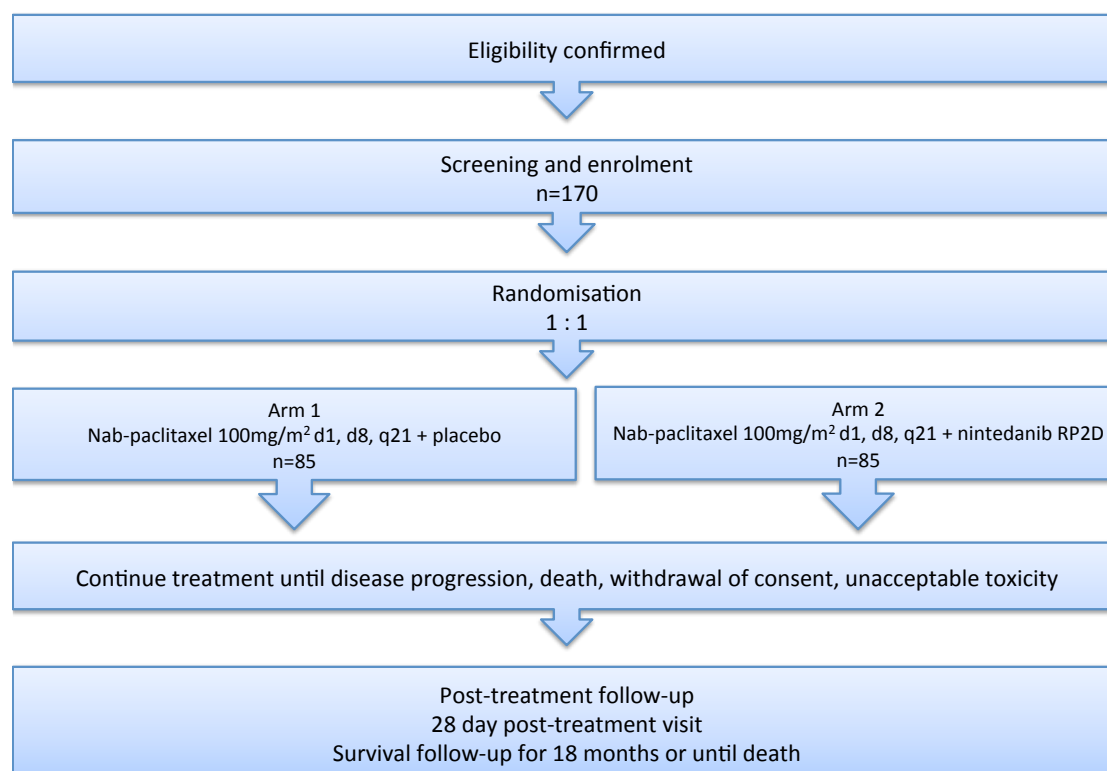
Figure 2.10. N3 trial: Part 2 (Phase II) trial schema

2.3.3 Objectives and outcome measures/endpoints

2.3.3.1 Primary objective

Primary objectives of Part 1 (Phase Ib) of the trial are: to evaluate the safety and tolerability of combination nab-paclitaxel and nintedanib in patients with relapsed stage III and IV adenocarcinoma of the lung in second and third treatment line setting; to determine the MTD/ RP2D of nintedanib when given with nab-paclitaxel at 100mg/m2 d1, d8 q21; and the incidence of DLTs during cycle 1 of treatment at different dose levels of nintedanib.

Primary objective of Part 2 (Phase II) of the trial is to explore the efficacy of combination nab-paclitaxel and nintedanib versus nab-paclitaxel and placebo in the same patient population, with nintedanib/placebo given at the recommended phase 2 dose (RP2D) as defined by phase I of the study.

2.3.3.2 Secondary objectives

Phase Ib secondary objectives are: to examine the frequency of all adverse events graded by NCI-CTCAE version 4.0.; to examine the objective tumour response according to RECIST 1.1 criteria (investigator reported), and the overall response rate; and to explore the number of cycles of nab-paclitaxel with nintedanib given.

Phase II secondary objectives are: to examine the frequency of all adverse events graded by NCI-CTCAE version 4.0.; to examine the objective tumour response according to RECIST 1.1 criteria (investigator reported), and the overall response rate; and to examine overall survival in the intention to treat population and in the predefined subgroups according to progressive disease before or after 9 months from start of first-line chemotherapy, and according to prior or no prior immunotherapy.

2.3.3.3 Primary and secondary endpoints

Phase Ib primary endpoint is the incidence of dose limiting toxicities during cycle 1 and the RP2D of nintedanib in combination with nab-paclitaxel.

Phase II primary endpoint is the PFS rate at 12 weeks after the first dose of nab-paclitaxel with nintedanib/placebo. Timing of the phase II primary endpoint was chosen in order to detect a strong early efficacy signal for the combination.

Phase Ib secondary endpoints are:

1. The incidence of all adverse events (AEs) graded by NCI-CTCAE version 4.0.

2. The objective tumour response according to RECIST (investigator reported) and the overall response rate (ORR; definitions according to RECIST 1.1 can be found in the N3 Trial Protocol, Appendix 3).

3. The number of cycles of nab-paclitaxel with nintedanib given.

Phase II secondary endpoints are:

1. The frequency of all adverse events (AEs) graded by NCI-CTCAE version 4.0.

2. The objective tumour response according to RECIST (investigator reported) and the ORR.

3. The overall survival in the ITT population and the predefined subgroups as defined above.

Primary Objectives	Primary Endpoints	Timelines
Phase I: To evaluate the safety and tolerability of combination nab-paclitaxel and nintedanib.	Phase I: Maximum tolerated dose (MTD) of nintedanib, defined as the highest dose of nintedanib at which only 1 DLT occurs for 6 patients treated.	Phase I: After cycle 1 of treatment.
Phase II: To explore the efficacy of combination nab-paclitaxel and nintedanib versus nab-paclitaxel and placebo.	Phase II: Progression free survival rate at 12 weeks after first dose of study treatment by each treatment arm with their respective 95% CIs.	Phase II: At 12 weeks after first dose of phase II study treatment.
Secondary Objectives	**Secondary Endpoints**	**Timelines**
1. To evaluate the frequency of all adverse events graded by NCI-CTCAE version 4.0 (phase I and II)	**1.** Expressed as frequencies, percentages and descriptive summary measures for all AEs	End of trial
2. To evaluate the objective tumour response and overall response rates by RECIST (phase I and II)	**2.** Expressed as a proportion for each treatment arm and with their respective 95% CIs	
3. To define number of cycles of nintedanib and nab-paclitaxel given (phase I)	**3.** Expressed as median and range	
4. To explore the overall survival rates by treatment arm in ITT and predefined subgroups by time to progression after start of 1_{st} line treatment and by previous immunotherapy (phase II)	**4.** Expressed as median OS estimates with 95% confidence intervals calculated using Kaplan Meier methods, by treatment arm in ITT and respective subgroups	

Table 2.12. N3 trial: Objectives and endpoints.

2.3.4 Study duration

In both Part 1 and Part 2 of the study, patients will enter a 28-day screening period, and if eligible (and the cohort/group is open for recruitment) will proceed to the treatment phase. Patients may remain on treatment at the discretion of the investigator until disease progression, unacceptable toxicity, until beginning of a new anticancer therapy, withdrawal of consent, refusal, physician decision, or death.

All patients in both parts of the study will be followed for 28 days after discontinuing treatment for safety and monitoring of adverse events, until progression (if applicable) for response, and for 18 months after the last dose of nab-paclitaxel and/or nintedanib/placebo for survival and new anticancer therapies.

The Phase 2 part of the study will begin when the RP2D has been declared in the Phase 1 part.

2.3.5 Study population and eligibility criteria

In Part 1 of the study, at least 12 to a maximum of 24 patients will be enrolled, within 3 dose escalation cohorts and 1 dose expansion cohort. In Part 2 of the study, up to 170 patients will be enrolled and randomised into 2 arms, with 85 patients per arm. The same inclusion and exclusion criteria will apply for both parts of the trial. Key inclusion and exclusion criteria are outlined below (full list of criteria can be found in the N3 Trial Protocol in Appendix 3).

2.3.5.1 Inclusion Criteria

Patients must meet all of the following criteria to be enrolled in the study:

1. Male or female patients aged 18 or over.

2. Patients with a pathologically confirmed diagnosis of stage IIIb or stage IV adenocarcinoma of the lung; patients with locally recurrent disease (stage IIIa) and no radical treatment options are also eligible.

3. Patients who have previously received no more than 2 lines of systemic therapy for NSCLC with palliative intent:

i. Chemotherapy as first or second line with palliative intent

ii. Relapsing within 6 months of adjuvant chemotherapy after surgery or as part of radical chemo-radiotherapy, which count as one line of therapy

iii. Licenced or experimental maintenance therapy is allowed (e.g. pemetrexed)

iv. Immunotherapy at prior line of treatment (first or second line) is allowed.

4. Patients with ECOG performance status 0-1.

5. Patients with estimated life expectancy of ≥12 weeks.

6. Patients with at least one radiologically measurable tumour lesion as defined by RECIST 1.1 criteria.

7. Patients with adequate haematopoietic, hepatic and renal function.

8. Signed informed consent in accordance with local legislation.

2.3.5.2 Exclusion criteria

The presence of any of the following will exclude a patient from enrolment:

1. Patients with a known *EGFR* sensitising mutation or *ALK* gene fusion prior to enrolment who have not received prior TKI (patients enrolled and subsequently found to be positive will remain on protocol). Patients with known *EGFR* activating

mutation or *ALK* fusion who have received appropriate TKI treatment will be allowed.

2. Any concurrent anticancer systemic therapy.

3. Prior treatment with nintedanib or any other VEGFR inhibitor; prior treatment with bevacizumab is allowed.

4. Patients refractory to prior taxane therapy for advanced disease. Prior taxane used in the adjuvant setting does not exclude eligibility provided there is no disease recurrence within 12 months upon completion of chemotherapy in that setting.

5. Inadequate laboratory parameters

6. Proteinuria CTCAE grade 2 or greater.

7. Pre-existing peripheral sensory neuropathy CTCAE grade 2 or greater.

8. Use of any investigational drug within 4 weeks of randomisation.

9. Radiotherapy within 4 weeks prior to randomisation.

10. Major surgery (other than biopsy) within 4 weeks prior to randomisation.

11. Active brain metastases or leptomeningeal disease.

12. Any other active current malignancy.

13. Active or uncontrolled infections or serious illnesses or medical conditions that in the opinion of the investigator could interfere with the patient's participation in the study.

14. Therapeutic anticoagulation.

15. Radiographic evidence (CT or MRI) of cavitary or necrotic tumours or local invasion of major blood vessels by tumour.

16. Pregnancy or breast feeding.

2.3.6 Statistics and data analysis

2.3.6.1 Sample size calculation

For Part 1, the sample size, using the 3+3 design, has been arbitrarily determined to gain confidence in tolerability recruiting up to a maximum of 24 patients (up to 18 patients in the 3 dose cohorts and 6 additional patients at the MTD).

For Part 2, the sample size is based on the 12 week expected PFS rates for the control and experimental arms being 45% and 65%, respectively (on the basis of the LUME-Lung 1 trial PFS data), with a two-sided alpha of 0.1 and power of 80%. Using a

chi-squared test without correction, this gives an intended recruitment in each arm of 85 patients, allowing for a 10% dropout, the total number of patients needed to show a 20% difference between arms will be 170, respectively. Nquery software was used to calculate the sample size required.

2.3.6.2 Planned recruitment rate and subject population

For Part 1, a 3+3 design will be used to assess DLTs in potentially 3 dose cohorts with an additional 6 patients treated at the derived RP2D. All evaluable patients recruited to Part 1 of the study will be analysed based on dose-escalation and expansion decisions, respectively. For Part 2, patients will be randomized in a 1:1 ratio between the two study arms with competitive enrolment between study sites. Those patients randomised to the study will be analysed using an ITT approach.

2.3.6.3 Statistical analysis plan summary

Part 1 of the study will define the MTD and evaluate the incidence of DLTs during Cycle 1. The incidence of DLTs will be presented using percentages and frequencies with 95% confidence intervals assigned respectively. Secondary endpoints as defined in Section 2.3.3 will be presented using appropriate descriptive summary measures such as means, medians, standard deviations and ranges for interval data, and proportions/percentages with frequencies for categorical data.

In Part 2, all randomised patients will be included in the primary endpoint analysis. The 12-week PFS rates will be calculated using Kaplan-Meier methods and compared using the log rank test, respectively. Progression is defined as per RECIST 1.1 criteria. Objective tumour response and ORRs will be reported by treatment arm with their 95% confidence intervals, respectively. Toxicity will be examined using frequency tabulation reports for each treatment arm. Overall survival will be calculated using Kaplan-Meier methods and the median survival estimates with 95% confidence intervals will be presented for each treatment arm; and for any predefined subgroups. Patients will be followed until death, loss to follow-up or 18 months after EOT, whichever occurs first with those patient alive censored at this point, respectively.

2.3.6.3.1 Adjusted and subgroup analyses

Survival analyses may need to be adjusted for common clinical factors (e.g. age, stage, previous treatment lines). Any adjusted survival analyses will be carried out using Cox proportional hazards modelling taking account of patient variables thought to impact on outcome. Assumptions of proportionality will be assessed and tested for any constructed survival models, respectively. Multiple logistic regression modelling will be used for binary outcomes in the same way.

Exploratory analyses of overall survival differences in subgroups according to time to progression as defined above after first line systemic therapy and according to prior or no prior immunotherapy are planned.

2.3.6.3.2 Interim analyses and criteria for the premature termination of the trial

In Part 1, the decision to dose-escalate to the next dose level or to declare an MTD/RP2D will be determined by the TSC based on results from clinical and laboratory safety data for a given cohort. An Independent Data Monitoring Committee (IDMC) will be established to meet half-yearly to review the data from Part 2/Phase II of the trial. At the end of the trial, if the combination is felt to be safe and efficacious, but statistical significance is not definitively achieved, statistical inference, using the point estimate and its 95% confidence interval, will be used to measure the experimental arm's potential effect. On the basis of this, the next stage, if suggestive of a benefit from the experimental treatment arm, would be a larger confirmatory trial in the form of a phase 3 randomised trial powered for OS, between nab-paclitaxel/placebo and nab-paclitaxel/nintedanib combination.

2.3.6.3.3 Procedures to account for missing or spurious data

Missing data will be reported using patient listings and percentage frequencies for baseline and outcome variables, respectively. For the progression-free survival rate analysis, patients who are alive with no recorded progression at the time of analysis will be censored at the date of the CT scan when they were last recorded with an evaluable measure that was not progression. For the overall survival time analysis, patients who are alive at the time of analysis will be censored at the date last seen alive.

2.3.7 Study setting and trial governance

This is a multi-centre study. Phase I of the study will be run at a limited number sites in the UK in the first instance and then open to up to a larger number of sites during the Phase II part of the study.

This trial is sponsored by the Royal Marsden NHS Trust and will be conducted in accordance with the professional regulatory standards required for non-commercial research in the NHS under the Research Governance Framework for Health and Social Care and Good Clinical Practice. Boerhinger Ingelheim and Celgene will provide funding support and provision of study drug/placebo.

Royal Marsden Clinical Trials Unit (RM-CTU) has the overall responsibility for facilitating and coordinating the conduct of the trial, for the day-today management of the trial including safety reporting, as well as for collating data obtained, and undertaking and reporting all analyses. Duties and responsibilities of RM-CTU and participating sites are listed in full in N3 Trial Protocol (Appendix 3).

A Trial Management Group (TMG) will be set up with membership to include Chief Investigator (Prof Sanjay Popat), Chief Co-Investigator, Trial Statistician and Trial Manager. Principal Investigators and other key study personnel will be invited to join the TMG as appropriate. The TMG have operational responsibility for the conduct of the trial.

Extended TMG or the Trial Steering Committee (TSC) will consist of the TMG plus the site PI or representative from sites participating in Phase 1 and an independent chair and clinician. TSC will meet at the end of each cohort to regularly review toxicity data, define DLTs, and make decision to proceed to the next cohort (or not) cohort dosing and expansion cohort, and define the RP2D. The role of the TSC is to monitor trial progress and to ensure the protocol and GCP principles are adhered to. The TSC's terms of reference, roles and responsibilities are defined in the TSC charter (Appendix 4). Further internal or external experts may be consulted by the TSC as necessary.

The Data Monitoring Committee (DMC) will consist of Chair from the Trial Steering Committee, independent clinician and statistician. The DMC will meet approximately 6 monthly to perform a monitoring role to review the data of the Phase II trial. They

will be provided all relevant results as necessary to perform this role. This will be conducted according to the IDMC charter (Appendix 5).

During the trial RM-CTU is responsible for monitoring data quality in accordance with relevant standard operating procedures. Incoming data will be monitored for protocol compliance and if any inconsistent or missing data is identified queries will be sent to the site for resolution, while any systematic inconsistencies may trigger an onsite monitoring visit.

2.3.8 Role in trial set-up

As the trial physician, I participated in establishing the trial design with the chief investigator and wrote the entire trial protocol. I wrote and presented the submissions to the RMH Committee for Clinical Research, Ethics Committee, MHRA and other regulatory authorities, successfully obtaining full regulatory approval for the trial. I lead and conducted discussions and negotiations on trial design and legal contracts between Trial Sponsor and IMP providers. I presented the trial design to the RMH Patient Review Panel and incorporated their feedback into the trial design. I worked with the RMH Trials Unit team to produce all trial documentation including patient information sheets, informed consent forms, TSC and IDMC charters, data collection tools, site initiation materials, as well as working with the trial IT unit on designing and validating the trial database.

2.3.9 Current status of trial

On 21 November 2020 the part-funder Celgene-BMS unilaterally decided to withdraw funding for the trial after an internal review of funding priorities of legacy investigator-initiated trial protocols after the merger of Celgene with BMS. BI were unable to increase the funding requirement and so the trial was formally closed without opening for enrolment.

2.4 Discussion and Conclusions

The U.S. FDA guidance defines the real-world data as the "data relating to patient health status and/or delivery of health care routinely collected from a variety of sources", while real-world evidence is "the clinical evidence regarding the usage, and potential benefits or risks, of a medical product derived from the analysis of RWD".[207] While randomized controlled trials (RCTs) are designed to test a therapeutic hypothesis under optimal conditions, with highly selected patients, optimal management conditions, and ideal settings, such conditions are very different from those encountered in real life. Thus, RCTs provide information on the "efficacy" or the extent to which medical interventions achieve health improvements under ideal circumstances, where the priority is to obtain unbiased estimates of treatment effects. Such unbiased estimates have high internal validity, are considered high-quality data and are used by regulatory agencies to make drug approval decisions, however the limitations are that strict inclusion and exclusion criteria limit generalizability to broader patient populations and to less ideal conditions, while a typically short follow-up duration is not designed to detect rare or long-term outcomes. Conversely, real-world evidence provides estimates of "effectiveness" or the extent to which medical interventions achieve health improvements in real practice settings. Real-world study populations reflect a broader, more heterogeneous distribution of patients observed in clinical practice, which can include different subgroups. Real-world studies have the potential to estimate true therapy use and to answer questions which cannot be answered by RCTs, such as dosing, compliance, adherence and off-label us. They can also be used to assess a broad range of outcomes, including economic, patient reported outcomes and quality of life measure, and can include larger sample sizes and longer follow-up, allowing to assess rarer/long-term outcomes. Due to diversity of patient populations, more diverse drug comparators can also be considered within the same study. However, with no guarantee of comparable patient groups, real-world data has the potential for biases and confounding, with larger populations, sophisticated designs and complex analyses (such as propensity score-weighted analyses) required to provide sufficient power and to control for bias/confounding. Data quality can

also be an issue where data on key variables may be missing or incomplete (e.g. follow-up and survival outcomes, baseline patient data, disease characteristics such as biomarkers and mutational status), while feasibility and sample size are dependent upon market uptake of the medication under study. Furthermore, while assessments and outcomes are normally strictly defined in clinical trials, the definition of some outcomes can be less defined and controlled in real-world studies, and assessments not carried out at protocol pre-specified fixed intervals and are not independently assessed. Real-world evidence cannot be directly compared with RCT data, however the relationship between RCT efficacy and real-world effectiveness for oncology treatments, as well as how this relationship varies depending on the use of different endpoints, has been studied with the finding that real-world effectiveness was similar to treatment efficacy when trials used overall survival as endpoints, but it was somewhat lower than trial efficacy when trials used surrogate endpoints such as PFS or time to progression.[208]

While many of the limitations of real-world evidence can be identified in the studies presented in this chapter, they nevertheless provided important additional safety and effectiveness data during the early days of uptake of first-line pembrolizumab in NSCLC patients in the UK, particularly those with reduced performance status and known oncogenic driver mutations, while real-world data on docetaxel-nintedanib was used during considerations to support reimbursement in territories where the combination was not yet approved. Also, together these real-world studies provided a benchmark for outcomes in patients who were the target population for the N3 trial. While this trial did not go on to open and recruit patients, similar questions are being interrogated in several trials that are ongoing or in some cases already reported. The ULTIMATE trial has reported on the efficacy and safety of weekly paclitaxel with bevacizumab versus docetaxel as second or third-line treatment in patients with advanced non-squamous NSCLC, with significantly improved PFS (5.4 vs. 3.9 months) and ORR (22.5% vs. 5.5%) with weekly paclitaxel-bevacizumab over docetaxel, although this trial did not report on the efficacy in the subgroup of patients previously treated with immune-checkpoint inhibitors.[209] The question of post-ICI salvage therapies is being asked in the context of several ongoing trials, with strategies including switch to chemotherapy, immunotherapy continuation with

addition of chemotherapy and immunotherapy sequencing. For instance, the phase III INSIGNA trial (NCT03793179) is recruiting and randomizing patients with non-squamous NSCLC patients with PD-L1 ≥1% where patients will receive upfront pembrolizumab, switching to pemetrexed/carboplatin chemotherapy on progression, in one of the trial arms, and with continuation of pembrolizumab but addition of chemotherapy in another, while another phase II trial is looking at continuation of pembrolizumab therapy with addition of single agent chemotherapy (docetaxel, pemetrexed or gemcitabine) at time of first progression (NCT03083808).

Chapter 3 Optimising tissue molecular genotyping in advanced NSCLC

As discussed in Chapters 1.3 and 1.4, identification of driver somatic aberrations in advanced NSCLC has led to rational implementation of genotype-directed therapy and repeated biopsies for molecular characterisation purposes may be required for a variety of indications and with aim of guiding optimal management of patients with relapsed advanced NSCLC, including target identification, resistance mechanism identification, as well as novel therapeutic target development in the context of clinical trial protocols. These increasing requirements for repeated and more extensive molecular testing represent a challenge in terms of availability, adequate quantity and quality of material for testing, as well as questions over safety of repeated invasive procedures.

In the first part of this chapter I will present the work to benchmark the adequacy for molecular testing and safety of repeated image-guided biopsies in NSCLC, followed by the work to evaluate optimal methods of sample acquisition for molecular genotyping in the context of a national observational pre-screening study, the CRUK Stratified Medicine part 2 (SMP2) programme.

3.1 Validating the pathological and molecular adequacy of rebiopsy tissue for molecular genotyping in relapsed NSCLC

3.1.1 Introduction

Image guided percutaneous transthoracic core needle biopsies are a standard diagnostic tool used to obtain tumour tissue at point of diagnosis or relapse. Safety and tissue diagnostic yields of biopsies at first diagnosis of lung cancer are well established.[141, 143, 210] However, at this time data remained limited on the adequacy of tumour material obtained by repeat image-guided percutaneous biopsies in order to molecularly characterise tumours. I designed and conducted a retrospective evaluation of safety and adequacy for molecular testing of tumour material obtained from image-guided transthoracic rebiopsies in NSCLC patients at the RMH.

3.1.2 Methods

This is a retrospective analysis of patients undergoing image-guided lung rebiopsies at a single cancer centre between 2011 and 2014. Rebiopsy was defined as biopsy after cancer progression following anti-cancer therapy (any line) or repeated biopsy where initial histological or molecular analysis was inadequate or incomplete for clinical decision-making. Approvals were obtained from the RMH audit committee.

3.1.2.1 Patients

Patients were identified through search of electronic patient records for those attending RMH Lung Unit with diagnosis of NSCLC undergoing image-guided lung biopsies between November 2011 and April 2014. These dates were chosen to allow sufficient follow-up time for evaluation of post-biopsy clinical outcomes, with data collection commencing in December 2015 and closing in April 2016. Patients with any stage disease were allowed, however only those attending for a rebiopsy and whose biopsy material was considered for molecular analysis were included in the final analysis. Patients with other primary thoracic malignancies (e.g. small cell lung cancer, mesothelioma, thymic malignancies, carcinoid tumours) and patients undergoing first diagnostic biopsies were excluded from further analysis.

Individual case notes were hand-searched for pre-defined data items including fields on demography (age, gender, smoking history, pulmonary comorbidities, history of other malignancies), lung cancer (diagnosis, disease stage, number of previous lines of systemic anti-cancer treatment, somatic mutational status at biopsy time), rebiopsy data (biopsy indication, image guidance mode, number of passes, needle gauge, number of cores obtained), post-procedure complications (pneumothorax, haemoptysis, hospitalization), rebiopsy tissue sample (presence/absence of malignancy, histological subtype, molecular analysis performed, mutations identified, molecular success, molecular failure reasons). A validated data capture spreadsheet was created and populated by two independent investigators who reviewed case-notes, identified and entered data.

3.1.2.2 Objectives

Primary objective was to determine the pathological success rate, defined as proportion of rebiopsy cases confirmed to contain malignant cells (as documented in the pathology reports).

Secondary and exploratory objectives included: technical success rate; adequacy of rebiopsy material for molecular analysis; concordance of pre-and post-biopsy histological subtype; number and nature of new mutations identified; association between number of cores obtained and adequacy for molecular analysis; and incidence of complications.

3.1.2.3 Definitions

Technical success was defined as successful insertion of biopsy needle into target lesion and cells or lung tissues were present in specimen, as documented in the pathology reports. Histological concordance was determined by comparison of original histological diagnoses, as documented in case-notes, with histological diagnoses on rebiopsy specimens, which were reviewed and classified by a dedicated lung pathologist using the 2015 WHO classification. Diagnostic biopsies were re-reviewed by a dedicated thoracic pathologist where possible. Molecular analysis of rebiopsy material was performed as clinically indicated for individual cases.

"Pre-test molecular adequacy" of rebiopsy material was defined as presence of a minimum of 30% viable tumour cells in the rebiopsy sample, as assessed by a dedicated thoracic pathologist as per routine practice. Reasons for inadequacy as reported by the pathologist were identified by case notes review and grouped into consistent themes.

"Post-test molecular adequacy" was defined as the proportion of successfully informative individual gene analyses (regardless of whether a variant was identified or not) out of the total number of genes analysed.

3.1.2.4 Statistical analysis

Differences in inter-gene failure rates were tested using the chi-square test for comparing multiple proportions with a significance level of α=0.05, with Bonferroni correction for multiple pairwise comparisons. The relationship between number of

cores (<3 versus ≥3 cores) and molecular adequacy was tested using the Fisher's exact test.

3.1.3 Results

One hundred and three patients were identified from case-notes searching with a diagnosis of thoracic malignancy undergoing image-guided percutaneous transthoracic procedures between November 2011 and April 2014. 7 patients had pleural drain insertion or pleural fluid aspiration and were excluded from analysis. 16 out of 103 patients underwent an initial diagnostic biopsy for suspected lung cancer (14 to obtain a histological diagnosis and 2 for completion of staging at diagnosis), and were excluded from further analysis, as this was an initial biopsy as opposed to a rebiopsy. 14 patients with a diagnosis of other thoracic malignancy including 10 mesotheliomas, 2 SCLC, and 2 thymic malignancies, were excluded from further analysis.

66 patients with NSCLC rebiopsy were included in final analysis. Median age was 67 (range 33-84), 71% of patients were former or current smokers and 68% were adenocarcinomas. 59 out of 66 patients (89%) had stage IIIB or stage IV disease at diagnosis, while 7 patients (11%) had stage II or IIIA disease at diagnosis previously treated on a radical treatment paradigm and were being investigated for relapsed disease at the time of rebiopsy. 76% had received at least one prior systemic therapy for advanced NSCLC. 10 patients (16%) had a known oncogenic driver alteration at the time of rebiopsy (9 patients with *EGFR* sensitising mutations and 1 with an *ALK* rearrangement). Patient characteristics are summarised in Table 3.1.

Demographic variable	No. out of 66 (%)
Median Age	67 (IQR 60-71)
Sex	
Male	35 (53)
Female	31 (47)
Smoking (at time of diagnosis)	
Ex-smoker	35 (53)
Never smoker	18 (27)
Active smoker	12 (18)
Unknown	1 (2)
Pulmonary Comorbidities	
None	57 (86)
COPD	5 (7)
Previous pulmonary TB	2 (3)
Asthma	1 (2)
Emphysema	1 (2)
Other malignancy	
Yes*	4 (6)
No	62 (94)
Histological subtype at primary diagnosis	
Adenocarcinoma	45 (68)
Squamous cell carcinoma	14 (21)
Adenosquamous	1 (2)
NSCLC NOS	6 (9)
Stage at diagnosis	
II	6 (9)
III	7 (11)
IV	53 (80)
Previous lines of systemic treatment for advanced disease	
0	16 (24)
1	24 (36)
2	14 (21)
3	7 (11)
4	5 (8)
Mutational status at time of biopsy	
EGFR	
Unknown	37 (56)
EGFR WT	20 (30)
EGFR mutation present	9 (14)
ALK	
Unknown	51 (77)
No rearrangement	14 (21)
Rearrangement present	1 (2)

Table 3.1. Demographics and baseline characteristics for all patients included in the analysis (n=66). *Other malignancies: 3 patients had past history of endometrial cancer (1), breast cancer (1) and basal cell carcinoma lip (1). 1 patient had a concurrent diagnosis of thymoma. COPD, chronic obstructive pulmonary disease; NOS, not otherwise specified; TB, tuberculosis; WT, wild type.

3.1.3.1 Procedures

Mode of image guidance was computed tomography (CT) in 60 out of 66 cases (91%) and ultrasound (US) in 6 cases (9%). Four patients had a CT-guided chest wall biopsy. All procedures were performed by an experienced interventional radiologist using dedicated CT-guided biopsy software (i-sequence and i-spiral) on a Somatom Definition Edge CT scanner (Siemens, Erlangen, Germany). Rapid on-site evaluation (ROSE) was not used for any of the procedures.

Although all rebiopsies were considered for molecular analysis, primary indications for rebiopsy varied. Majority of patients underwent rebiopsy primarily for molecular testing (41/66, 62.1%), including 11 patients for first-time molecular analysis, 13 patients for repeat analysis due to previous failure, 11 for expanded molecular profiling and 6 for *EGFR* T790M mutation detection. In 12 patients documented primary indication for repeat biopsy was histological confirmation of disease relapse, in 4 patients primary indication was to exclude clinical suspicion of high grade neuroendocrine transformation, while in 2 patients it was disease restaging. Seven out of 66 patients had a rebiopsy in the context of a research protocol.

Technical success was achieved in all 66 patients (100% rate). Mean target lesion size was 40.7mm (95% CI: 35.9–45.5), with mean distance to pleura of 15mm (95% CI: 11.35–18.55). A range of needle gauge sizes was used, from 14G to 18G, with majority procedures performed using an 18G needle (86% or 45/52 cases where needle gauge size was documented). Median number of cores obtained was 3 (range: 1 to 6), in one case reported as "multiple", and not documented in 3 cases. Target lesion locations were evenly distributed between all lobes of the lung (53% in upper and 45% in lower lobes), with one lesion located in the right middle lobe.

3.1.3.2 Pathological findings

Pathological success was achieved in 54 out of all 66 patients (81.8%). In 8 patients no malignant cells were found in the sample, with 7 cases containing benign lung tissue and 1 case containing necrotic material only. 3 out of the 8 cases were patients with known metastatic NSCLC on systemic therapy where rebiopsy was performed on an enlarging metastatic nodule, while 5 out of 8 were new nodules

suspicious for relapse in patients following radical treatment for limited disease. Presence or absence of malignant cells was non-evaluable in 4 cases, when rebiopsy was performed as part of a research protocol. These 4 cases were not evaluated for histopathology and were therefore excluded from further analyses. Therefore the pathological success rate for evaluable cases was 54/62 (87.1%).

Histological concordance was evaluable in 52 cases (in 2 out of 54 cases containing malignant cells histological subtype was not reported on rebiopsy tissue). Concordance of pre- and post-rebiopsy histological subtype was observed in 40/52 (76.9%). Discordance was observed in 12 (23.1%) cases as detailed in Table 3.2. In one case, rebiopsy sample histopathology was consistent with thymoma, in a patient with known synchronous diagnoses of NSCLC adenocarcinoma and thymoma.

Original histology	n	Rebiopsy histology	Number (%)
Adenocarcinoma	38	Adenocarcinoma	36 (94.8)
		NSCLC NOS	2 (5.2)
Squamous cell carcinoma	9	Squamous cell carcinoma	3 (33.3)
		Adenocarcinoma	4 (44.5)
		NSCLC NOS	1 (11.1)
		Pleomorphic ca. rhabdoid subtype	1 (11.1)
NSCLC NOS	4	NSCLC NOS	1 (25.0)
		Squamous cell carcinoma	3 (75.0)
Adenosquamous carcinoma	1	Adenocarcinoma	1 (100)
Total*	**52**	**Concordant**	**40 (76.9)**
		Discordant	**12 (23.1)**

Table 3.2. Histological discordance rates. *Total of 52 cases were evaluable for histological concordance. 14 cases were non-evaluable including: 8 cases with no malignant cells in sample (pathological fail), 4 cases sent to research laboratory, 2 cases histological subtype not reported. NOS, not otherwise specified.

3.1.3.3 Molecular analysis

We analysed the pre-test and post-test molecular adequacy of the 66 rebiopsy cases. Pre-test molecular adequacy (defined as presence of a minimum of 30% viable tumour cells in the sample), was determined and routinely reported by the lung pathologist, and confirmed on review of rebiopsy pathology reports.

52 out of 66 cases were found to meet the definition, resulting in pre-test molecular adequacy of 78.8% of all rebiopsy cases, and 52 out of 54 (96.3%) of cases where malignant cells were identified in the sample. 2 cases containing malignant cells (pathologically successful) were inadequate for molecular analysis due to "poor sample quality".

Molecular analyses were performed in 51 out of 66 patients, with a total number of 209 genes analysed. In one patient whose rebiopsy sample showed NSCLC with rhabdoid differentiation, tissue was subjectively adequate for molecular analysis, but molecular testing was not requested as not clinically indicated.

Genes analysed on at least one occasion were *EGFR*, *ALK*, *KRAS*, *NRAS*, *BRAF*, *DDR2*, *ROS1* and *RET*. Individual PCR-based gene assays were performed including: cobas 480®(Roche) for *EGFR* and *KRAS* mutations, capillary electrophoresis single-strand conformation analysis (CE-SSCA) for *EGFR*, *BRAF* exon 15 mutation and *NRAS* mutations, and direct sequencing for *BRAF* exon 11 and *DDR2* as next generation sequencing (NGS) was not routinely implemented during this period. Fluorescence in situ hybridisation (FISH) was used to detect *ALK* and *ROS1* rearrangements.

Post-test molecular adequacy, defined as the number of informative gene tests out of all tests performed, was 87.1% or 182 out of 209 genes analysed. 27 gene tests (22.9%) failed molecular analysis and were uninformative, as illustrated in Figure 3.1., a consort diagram showing the study flow from patient selection, to assessment of technical success rate, pathological success rate, pre-test and post-test molecular adequacy.

Next, we examined the inter-gene variation in molecular failure rates, by comparing proportions of tests that failed molecular analysis between different genes, including differences between *EGFR* and *ALK*, *EGFR* and *KRAS*, and *ALK* and *KRAS*. There was

significant overall inter-gene variation in molecular failure rates (p=0.005). For instance, *EGFR* analysis was performed in 50 and *ALK* analysis in 40 patients, with molecular failure rates of 4% and 2.5% respectively, while *KRAS* was analysed 41 times with a failure rate of 24.4% (p=0.04 and p=0.04, respectively). Rates of molecular success and failure by gene are shown in Table 3.3. The observed inter-gene variation in failure rates is likely due to sequential nature of individual gene tests performed, with less material available for each subsequent analysis. Reason for molecular analysis failure, where recorded, was in each case poor sample quality. Finally we explored whether the number of cores obtained contributed to post-test molecular adequacy, by comparing rates of molecular test failure between cases where fewer than 3 cores versus 3 or more cores were obtained. This cut off was chosen because of some guidance at the time recommending 3-6 cores to maximise volume of tissue.[145] We found no significant difference in molecular failure rates between these two groups (p=0.185). There did not appear to be any clear links between incidence of molecular test failure and patient characteristics or other technical aspects of rebiopsy.
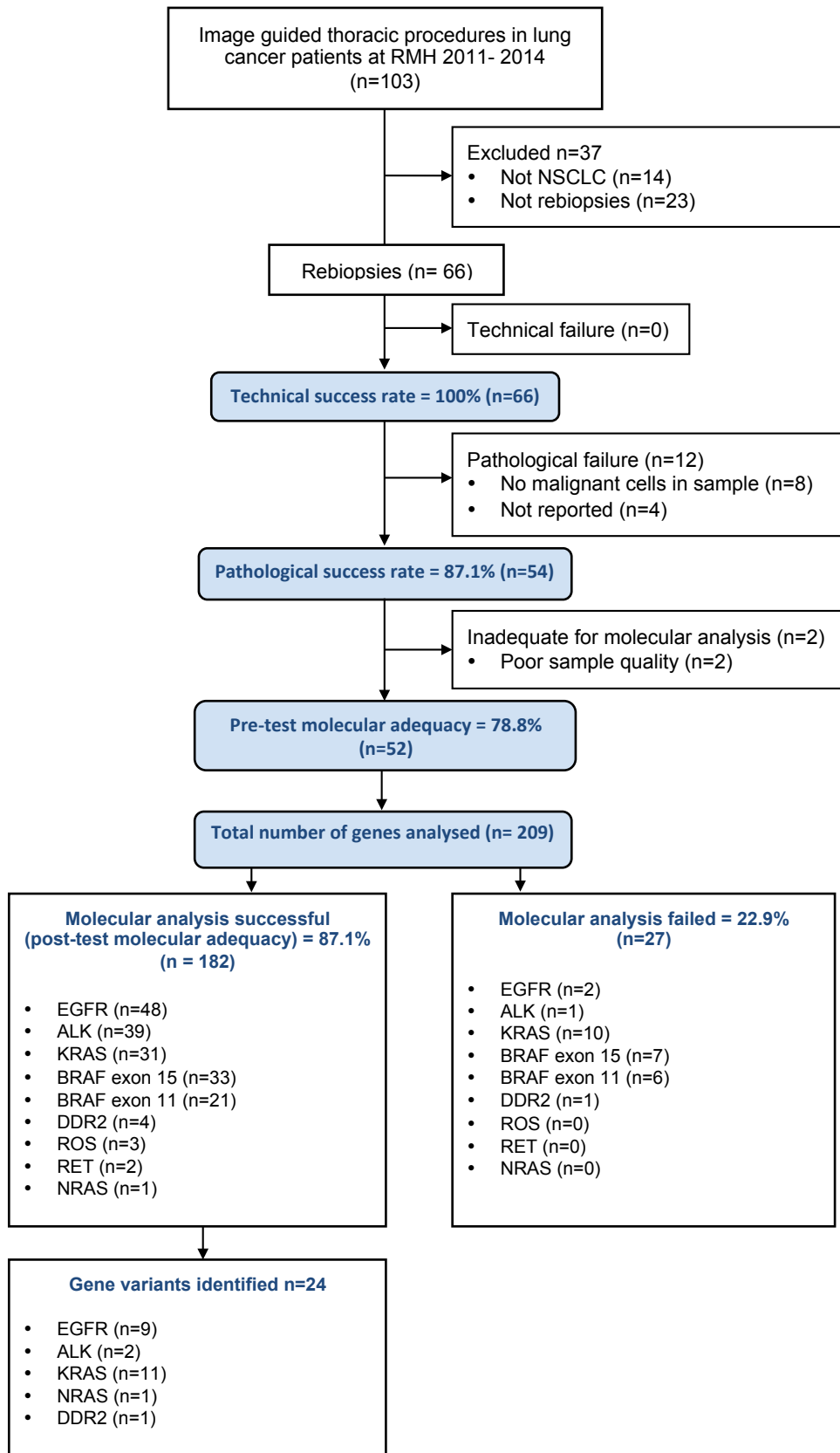
Figure 3.1. Consort diagram

In total, twenty four genetic aberrations were identified, including 20 new previously unknown potentially targetable mutations including: activating mutations in *EGFR* in two patients in whom molecular testing had previously failed (one *EGFR* exon 19 deletion and one S768I point mutation); two *EGFR* T790M acquired resistance mutations; one *EGFR* primary resistance mutation (exon 20 deletion). *ALK* rearrangements were identified in 2 patients. 11 patients were found to have a *KRAS* mutation, 1 patient had a *NRAS* Q61L mutation and 1 had a *DDR2* mutation.

Gene	No. analysed	No. failed	Wild type	Variant present	Failure rate
EGFR	50	2	39	9	4%
ALK	40	1	37	2	2.5%
KRAS	41	10	20	11	24.4%
BRAF Exon 11	27	6	21	0	22.2%
BRAF Exon 15	40	7	33	0	17.5%
DDR2	5	1	3	1	20%
ROS1	3	0	3	0	0%
RET	2	0	2	0	0%
NRAS	1	0	0	1	0%
TOTAL	209	27	158	24	12.9%

Table 3.3. Rates of molecular success and failure by gene analysed.

3.1.3.4 Safety

Rate of all complications was 25.7% (17 out of 66 patients). Presence of pneumothorax was assessed in all patients by post-procedure plain chest radiograph or limited post-procedure chest computed tomography (CT) and confirmed in 12/66 cases (18.2%). However, only 2 out of 12 cases required intervention with chest drain insertion (3.0%). Median age of patients suffering a pneumothorax was similar to that of overall study population (63 (range 37-76) versus 67 (37-84)). Rate of ex or current smoking was slightly higher in the pneumothorax group than in the overall population (83.3% vs. 71.2%), but none had a history of significant pulmonary comorbidities compared with 13% in the overall group.

Haemoptysis was reported in 5 out of 66 cases (7.6%), and not recorded in 2 patients. All cases were categorised as mild haemoptysis (<30ml over 24hrs) not requiring further intervention. 2 patients (3.0%) required prolonged hospitalisation post-procedure (>48 hours) for management of pneumothorax requiring chest drain insertion. Three patients required a prolonged admission for unrelated reasons.

3.1.3.5 Post-rebiopsy clinical outcomes

We extracted data on post-rebiopsy clinical treatment pathways, to explore the ways in which rebiopsy affected clinical decision-making. This data is summarised in Table 3.4. In 42 out of 66 patients (63.6%), rebiopsy had a direct impact on the choice of subsequent treatment, including 13 (19.7%) who commenced licensed targeted therapies for newly identified somatic mutations (7, 54% in clinical trial setting) or histology-specific chemotherapy. Four patients (6%) were too unwell for further systemic therapy following rebiopsy.

Post-Rebiopsy Clinical Outcomes	Number of patients
Potentially actionable genetic mutation identified	**20**
Patients started licenced TKI*	6
Patients entered clinical trial of targeted therapy*	7
Patients started chemotherapy but potentially eligible for future clinical trial*	4
Patients too unwell for further systemic therapy	3
Activating mutation confirmed/no acquired resistance mutation	**4**
Patients switched to chemotherapy	2
Patients switched to second generation TKI	1
Patients too unwell for systemic therapy	1
Mandatory biopsy within research protocol – patients entered clinical trial*	**6**
Histological discordance identified – new treatment paradigm*	**4**
Histological confirmation of NSCLC recurrence*	**12**
Patients started palliative treatment*	10
Patients started radical treatment*	2
NSCLC recurrence ruled out – patients continued surveillance*	**3**
Pathological or molecular failure	**13**
No actionable mutations identified	**4**
Total	66

Table 3.4. Rebiopsy outcomes and post-biopsy patient pathways. *indicates patients in whom rebiopsy informed subsequent choice of treatment.

3.1.4 Discussion and Conclusions

This was a retrospective study of adequacy of image-guided transthoracic rebiopsies in 66 patients in terms of safety, technical success rates, and adequacy for pathological and molecular analysis.

With 100% technical success rate, 87.1% pathological adequacy and 78.8% molecular adequacy as subjectively assessed by a lung pathologist, image guided lung rebiopsies are feasible and can yield tissue adequate for analysis of multiple biomarkers in the setting of standard clinical practice. The rates of pneumothorax (18%), chest drain insertion (3%) and mild haemoptysis (8%) are similar to those previously reported in large series of percutaneous transthoracic biopsies in primary diagnostic setting,[211–214] and therefore do not appear to pose an increased risk compared to primary biopsies.

There was a relatively high rate of histological discordance of 23% between rebiopsy material and prior diagnostic biopsies. In cases where histological discrepancy was observed, initial diagnostic biopsies were re-reviewed where available to explore possible causes for the differences. In two cases where squamous cell carcinoma at initial biopsy was reclassified as adenocarcinoma on rebiopsy, and where diagnostic biopsy material was available for review, rebiopsy tumour material showed some features of overlap between adenocarcinoma and squamous cell carcinoma. The discordance between biopsies may therefore reflect sampling of different components of the same tumour with both adenocarcinoma and squamous cell carcinoma features. Another possible explanation for the observed differences may be sampling bias, with patients whose initial samples were inadequate for optimal histological assessment and diagnosis selected for rebiopsy, leading to higher rates of histological discordance observed in our cohort (e.g. 3 instances of NSCLC NOS were reclassified as squamous cell carcinoma).

Recognising the difficulties that can arise in making a diagnosis on small biopsies in NSCLC, in 2011 the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society jointly proposed new terminology to be used for NSCLC classification based on small biopsies and cytology, subsequently adopted in the 2015 WHO classification.[215, 216] Recommendations included minimising the use of the terms NSCLC or NSCLC NOS, providing as specific

a histologic classification as possible, and emphasised the use of immunohistochemical studies (over those based on hematoxylin and eosin (H&E) examination), and integration with histochemical and molecular studies.

Overall 182 of 209 (87.1%) individual gene tests were performed successfully in 51 patients. Molecular success rates varied significantly between individual gene assays. *EGFR* testing was completed successfully in 48 out of 50 cases (96%), in line with rates reported in several previous studies of adequacy of rebiopsy tissue for *EGFR* testing.[134, 217–220] Two prospective studies of rebiopsies in 121[220] and 162[217] patients with acquired resistance to EGFR-TKIs reported rates of 86% and 95.6% respectively. Another recent prospective study enrolled 24 *EGFR* mutant patients commencing afatinib therapy with a view to rebiopsy for *EGFR T790M* analysis at progression. Out of 23 patients who developed progressive disease, only 14 completed a rebiopsy, with 11 samples (78.6%) sufficient for molecular analysis.[134]

At this time, most studies of rebiopsies have focused on mechanisms of acquired resistance to EGFR-TKI and in particular detection of T790M mutation, and few studies evaluated adequacy for multiple biomarker testing on rebiopsy tissue outside of this context.[114, 221, 222] Tam et al have reported a retrospective analysis of adequacy of percutaneous transthoracic core needle biopsies for the evaluation of multiple molecular biomarkers within the context of the genotype-directed BATTLE trial.[221] 170 biopsies were performed in 151 NSCLC patients screened for the trial. Specimens of 82.9% of patients were found to have adequate tumour tissue for analysis of 11 different biomarkers within *EGFR*, *KRAS*, *BRAF*, *VEGFR*, *RXR* and *Cyclin D* genes. Pneumothorax and chest tube insertion rates were 15.3% and 9.4%, respectively. In this study, rates of pre-test (87.1%) and post-test molecular adequacy (78.8%) are similar to those reported in the BATTLE trial despite our relatively unselected patient cohort in the setting of standard clinical practice.

The main limitation of these data is that this is a retrospective observational study based on clinical experience of a single oncology centre which, as a tertiary referral centre and an institution with well-established infrastructure and experience in this area, may not be representative of the patient profile and resources available in other community-based centres. Secondly, the discrepancy between subjective pathologist assessed pre-test molecular adequacy and post-test molecular success

rate has been difficult to explore in absence of complete data on reasons for test failure. Thirdly, incomplete data on technical aspects of each procedure precluded analysis of potential relationship between incidence of molecular analysis failure and the way procedures were performed, which would help define optimal conditions to obtain adequate tissue samples. Finally, instead of single-gene tests performed in parallel or sequentially, many centres have now moved to implementing NGS-based molecular genotyping,[223–226] and so the individual molecular success rate at individual genes may not reflect changes in gene-testing methodologies.

Nevertheless, in a real world setting, this data identifies the clinical utility and limitations of rebiopsies in advanced NSCLCs, demonstrating a clinically important utility in decision-making and for molecular characterization. Improvements in the histological yield and molecular adequacy of rebiopsies may be achieved by implementation of standardised protocols and algorithms in radiology departments and laboratories to ensure optimal handling of samples for molecular analyses as highlighted in the 2013 CAP/IASCL/AMP Guidelines,[227] which recommended *EGFR* and *ALK* testing for all patients with advanced adenocarcinomas regardless of sex, race, smoking history or other clinical risk factors, as well as setting out recommendations for prioritisation of tissue for *EGFR* and *ALK* testing, minimum test turnaround times, treatment of tissue destined for molecular analyses, specimen requirements such as cancer cell content, DNA quality and quantity, and molecular testing methods for *EGFR* and *ALK*. Use of rapid on-site evaluation (ROSE) of specimens at the time of procedure has been shown to improve diagnostic yield, decrease the need for repeat procedures and facilitate collection of sufficient material for molecular testing,[228] although resource considerations are likely to affect wide-spread use of this technique.

Validation of circulating tumour DNA (ctDNA), initially for detection of *EGFR T790M* and more recently of plasma-based next generation sequencing methods in the diagnostic setting, is facilitating a less invasive molecular genotyping approach in advanced NSCLC. However, due to issues of lower sensitivity, particularly in the context of limited metastatic disease and for detection of gene fusions and copy number alterations, tissue based genotyping remains an important tool both at diagnosis and for identification of well-recognised resistance mechanisms to TKIs,

including those that may result in histological non-concordance, such as histological trans-differentiation into small cell carcinoma after EGFR TKI therapy,[217, 229, 230] or neuroendocrine change on lorlatinib.[231, 232] In this study, tissue rebiopsies provided clinically relevant information, helping to guide the choice of treatment in nearly two thirds of patients, through identification of new actionable driver and resistance mutations, change in histological classification, and confirmation or exclusion of recurrent disease, and provided valuable data on the role and utility of rebiopsy for molecular analysis of multiple molecular markers in a heterogeneous group of NSCLC patients, thereby validating the pathological and molecular adequacy rates of rebiopsies in the setting of standard clinical practice. The findings were presented at the oral poster session of the annual British Thoracic Oncology Group Meeting 2017, followed by paper publication in the Journal of Thoracic Oncology (Appendix 11).

3.2 Optimal methods for tissue acquisition for molecular genotyping in the context of CRUK SMP2 programme

3.2.1 CRUK SMP2 programme design

The Stratified Medicine Programme 2 (SMP2) is a national observational pre-screening study for advanced lung cancer that was launched in 2014, led, sponsored, and funded by Cancer Research UK (CRUK), in collaboration with the NHS Experimental Cancer Medicine Centres (ECMC) Network, University of Birmingham Cancer Research Clinical Trials Unit (CRCTU), and pharmaceutical industry partners Illumina, Astra Zeneca and Pfizer. It is recruiting patients with locally advanced or late-stage metastatic NSCLC to undergo molecular characterisation of their tumours using a custom NGS panel to allow molecular pre-screening to the genotype-directed multi-arm platform trial, the National Lung MATRIX trial (NCT02664935). The custom NGS panel initially comprised 28 genes, with a larger NGS gene panel implemented in early 2020.

CRUK SMP2 is run through a national network of participating cancer medicine centres termed clinical hubs (CHs), each operating a hub-and-spoke model with patients being referred in from nearby hospitals (feeder sites), and each clinical hub linked to one of 3 technical hubs (THs). The CRUK SMP2 network is illustrated in Figure 3.2. Patients are eligible for CRUK SMP2 if they have stage III or IV disease, that is not amenable to radical treatment with radiotherapy or surgery, and if they are of performance status 0-2. Eligible patients are identified and recruited through a network of 23 CHs (including 18 ECMCs and 5 non-ECMC centres), with patients from local feeder hospitals also referred via the CHs for enrolment in the study. Once patients are identified, clinical sites obtain patients' consent for screening on SMP2 at either primary diagnosis or at relapse using a local consent form or the specific CRUK SMP2 consent form. After consenting, a sample from a diagnostic biopsy together with matched blood sample is sent to one of the three technology hubs (THs) for molecular sequencing. The THs are either ISO 15189 or CPA-accredited NHS Molecular Genetics Laboratories located at Birmingham (BMH; West Midlands Regional Genetics Service), Cardiff (All Wales Medical Genetics Service) and at the

Royal Marsden Hospital London (RMH; The Centre for Molecular Pathology). The CRUK SMP2 patient and sample pathway is shown in Figure 3.3.
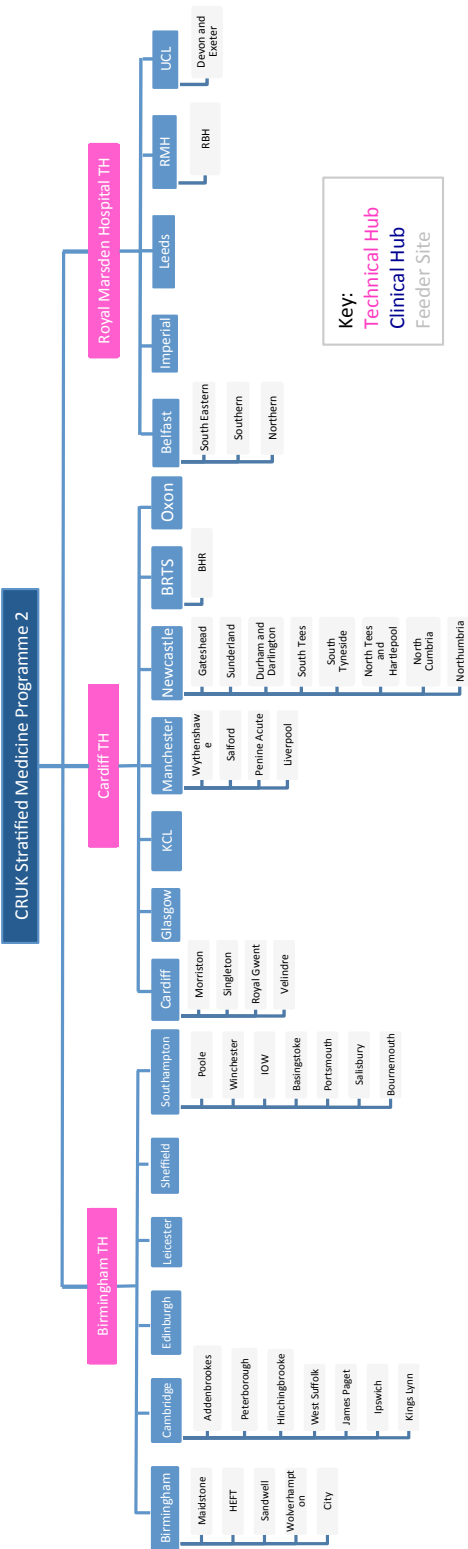
Figure 3.2. CRUK SMP2 network diagram. TH, Technical hub.
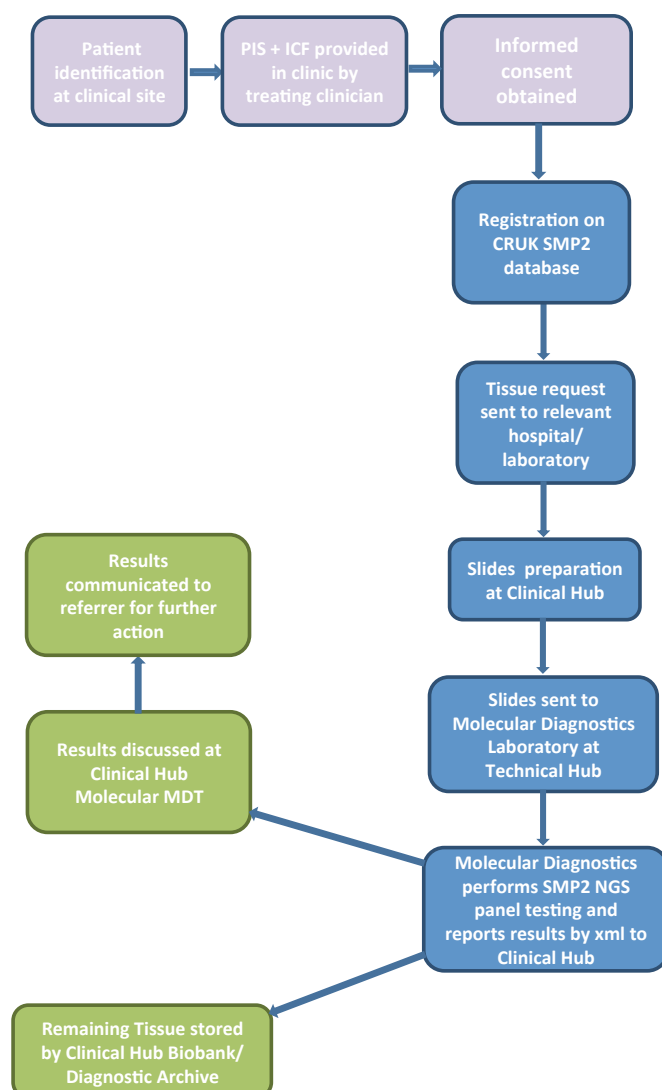
Figure 3.3. CRUK CMP2 patient and sample pathway. PIS, patient information sheet; ICF, informed consent form; MDT, multidisciplinary team.

3.2.2 The National Lung Matrix Trial Design

Based on the results of the SMP2 panel, patients are assessed by the clinical hubs for eligibility for entry into the National Lung Matrix trial (NLMT), a multi-centre, multi-arm, umbrella phase II platform trial (NCT02664935, ISRCTN38344105, EudraCT 2014-000814-73). Aberrations identified on the panel are classified as Tier 1, Tier 2 and Tier 3, according to a comprehensive tiering system based on extensive examination of genetic databases, pre-clinical and clinical data to define "actionability", where Tiers 1 and 2 denote aberrations that confer eligibility for one or more National Lung MATRIX Trial cohorts and Tier 3 for non-eligible variants. Each arm was designed to test an experimental targeted drug intervention in a population stratified by multiple pre-specified target biomarkers, with a Bayesian adaptive

design to screen for signals of efficacy in each selected molecularly-defined cohort. The drugs were chosen on the basis of preclinical evidence of mechanism of action in genotype-directed models and after ratification from the MATRIX trial management group, funding agreement from the CRUK and approval from the trial steering committee. In addition, in order to offer a therapeutic option for patients with successful screening in the trial but without specific eligibility for one of the targeted interventional arms, patients with no actionable genetic changes could be included in the NA arm and treated with a drug from a planned sequential pipeline of experimental agents (e.g. the first drug chosen for the NA arm was durvalumab). The trial initially opened with 20 cohorts using 7 different drugs, subsequently increasing to 22 cohorts and 8 drugs, with the protocol continuously being developed to add further arms and cohorts. The NLMT trial schema is shown in Figure 3.4., with trial cohorts and associated molecular eligibility criteria in Table 3.5. The trial opened to recruitment in 2015, and is ongoing. Interim results from 19 cohorts were recently reported.[233]
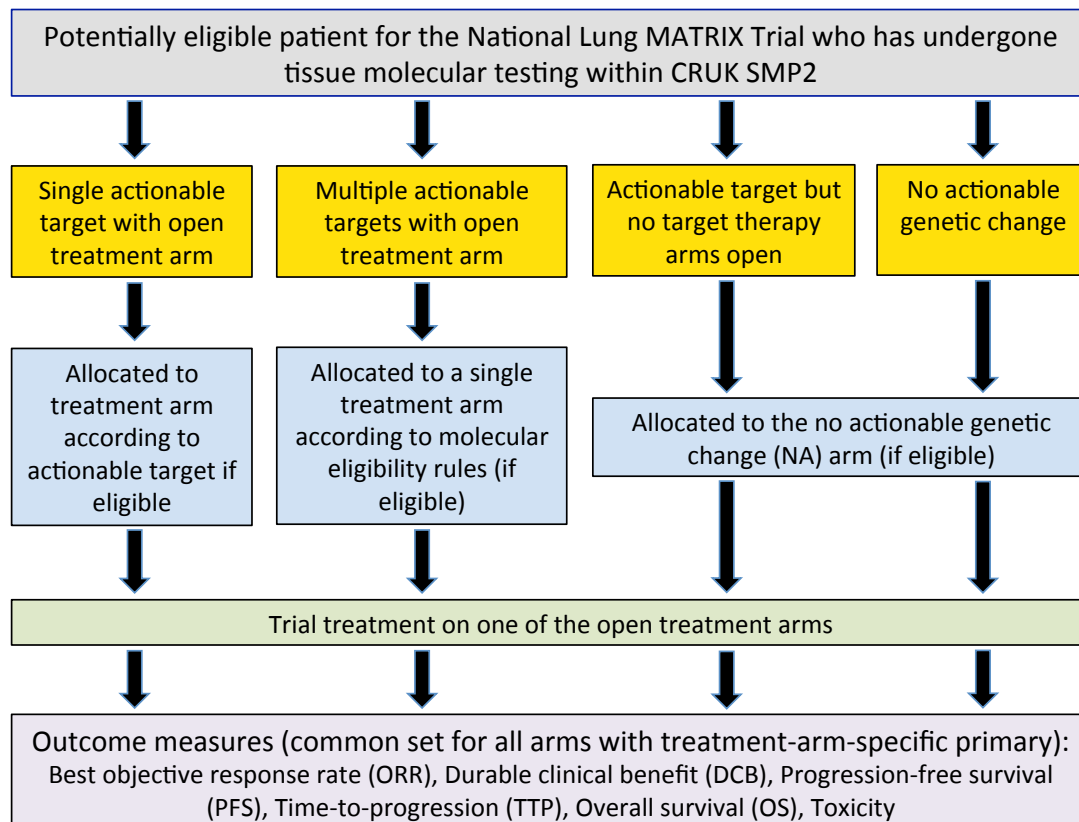
Figure 3.4. The National Lung MATRIX trial schema.

Currently open arms and cohorts

Arm	Investigational Medicinal Products	Cohort Number	NSCLC Histology	Molecular Cohort
C	Palbociclib – CDK-4/6 Inhibitor	C1	SCC	p16 (CDKN2A) loss of function with proficient Rb
		C3	NSCLC	CDK4 amplification with proficient Rb
		C4	NSCLC	CCND1 amplification with proficient Rb
		C5	NSCLC	STK11/LKB1 mutation or homozygous deletion, or TSC1/2 mutation AND Activated KRAS/MAPK pathway i.e. concomitant KRAS, NRAS or NF1 mutation AND Proficient Rb (no loss of Rb function either by mutation or deletion)
D	Crizotinib – ALK Inhibitor	D1	NSCLC	MET amplification
		D3	NSCLC	MET exon 14 skipping (splice mutation or deletion)
E	Selumetinib – MEK Inhibitor & Docetaxel	E1	SCC	NF1 mutation
		E2	ADC or NOS NSCLC	NF1 mutation
		E3	NSCLC	NRAS mutation
J	AZD6738 – ATR inhibitor & Durvalumab – PD-L1 Inhibitor	J1	NSCLC	KRAS mutation STK11/LKB1 successful test result
		NAJ	NSCLC	No actionable genetic change for other trial arms

Closed arms and cohorts

Arm	Investigational Medicinal Products	Cohort Number	NSCLC Histology	Molecular Cohort
A	AZD4547 – FGFR Inhibitor	A1	NSCLC	FGFR2/3 mutation
B	Vistusertib – MTORC-1/2 Inhibitor	B1	NSCLC	TSC1/2 mutation
B	Vistusertib – MTORC-1/2 Inhibitor	B2	NSCLC	STK11/LKB1 mutation or homozygous deletion
C	Palbociclib – CDK-4/6 Inhibitor	C2	ADC or NOS NSCLC	p16 (CDKN2A) loss of function with proficient Rb
		C6	NSCLC	KRAS mutation with proficient Rb (No concomitant STK11/LKB1 mutation or deletion, no PIK3CA mutation or amplification, no PTEN mutation or homozygous deletion, no AKT mutation, no EGFR mutation, no FGFR2/3 mutation, no TSC1/2 mutation, and no HER2 mutation)
D	Crizotinib – ALK Inhibitor	D2	NSCLC	ROS1 gene fusions
F	AZD5363 - AKT Inhibitor	F1	SCC	PIK3CA mutation & no KRAS, NF1, NRAS, HRAS or BRAF aberrations
		F2	SCC	PIK3CA amplification & no KRAS, NF1, NRAS, HRAS or BRAF aberrations
		F3	NSCLC	PIK3CA mutation or PIK3CA amplification & no KRAS, NF1, NRAS, HRAS or BRAF aberrations (ADC or NOS NSCLC) PTEN mutation or PTEN loss & no KRAS, NF1, NRAS, HRAS or BRAF aberrations (ADC or NOS NSCLC)

Table 3.5. The National Lung MATRIX trial arms, investigational medicinal products and molecular cohorts. Source https://www.birmingham.ac.uk/research/crctu/trials/lung-matrix/professionals/design-treatments.aspx

3.2.3 Sample requirements for CRUK SMP2

Samples required for molecular testing within CRUK SMP2 include sections from formalin-fixed paraffin-embedded (FFPE) samples from various sample types with ≥20% tumour content and medium–high cellularity (defined as >4,000 cells, assessed by a senior pathologist as part of routine NHS care), or locally extracted DNA from tumour biopsies (containing 70ng of tumour DNA at a minimum concentration of 2ng/µl) together with a marked hematoxylin and eosin (H&E) slide. Matched blood samples for germline comparison (minimum 4ml EDTA) were initially mandated, although this requirement was subsequently discontinued. Tumour DNA is extracted at the THs using the Maxwell 16 FFPE Plus LEV DNA Purification Kit (Promega; Birmingham TH and Cardiff TH) or the Qiagen DNA extraction kit (Qiagen; RMH TH) according to the manufacturer's instructions. The extracted DNA was quantified using Qubit high-sensitivity FFPE assay (Thermo Fisher Scientific) according to the manufacturer's instructions. Samples with DNA concentrations lower than 50ng were deemed to have failed the quality control step (QC fail) and were not sent on for SMP2 panel molecular testing.

3.2.4 CRUK SMP2 panel design

The SMP2 v.01 custom panel was designed using Illumina DesignStudio, a web-based design tool that converts target regions to capture probes. 28 target genes were identified and selected by CRUK and partners (Figure 3.5). Genetic variants were identified through a custom Nextera Rapid Capture sequencing assay (Illumina).

AKT1	ALK	BRAF	CCND1
CCND2	CCND3	CCNE1	CDK2
CDK4	CDKN2A	EGFR	FGFR2
FGFR3	Her2*	HRAS	KRAS
MET	NF1	NRAS	NTRK1
PIK3CA	PTEN	RB1	RET
ROS1	STK11	TSC1	TSC2

Figure 3.5. 28 genes included in the CRUK SMP2 v0.1 panel.

The SMP2 v.01 panel was updated to SMP2 v.02 in March 2017, with changes made to improve coverage of genes with high failure rates in panel v.01 (RB1 and FGFR3) and to improve detection of fusions and alternative splicing events. Additionally, targets for single-nucleotide polymorphisms (SNPs) common in the population were added, to confirm that tumour and normal samples were derived from the same patient.

The panel underwent comprehensive validation confirming that the panel can detect single-nucleotide variants and indels at >5% frequency (10/200 reads).[234] Somatic copy number alterations (SCNA) could be confidently detected by NGS in samples with a high tumour percentage (>60% tumour content) and if the SCNA was large. Low-level or suspected SCNAs are confirmed by fluorescence in-situ hybridization (FISH) before reporting to the CHs. Similarly, FISH was used to confirm deletions identified by NGS and determine whether the deletion was homozygous or heterozygous. FISH analyses were performed for the following genes to confirm SCNAs and deletions identified by NGS: *MET, ROS1, PIK3CA, PTEN, CCND1, CDK4*, and *CDKN2A*.

3.2.5 Variant reporting and SMP2-specific data collection

Variants identified are gathered into an Excel spreadsheet to assist with summarizing and reporting the data. SMP2 panel results are sent to the clinical hubs in the form

of a report, identifying the aberrations and tier classification, in XML format in order to allow ease of electronic transfer of data between THs, CHs and local electronic patient medical records. For any individual gene where testing is unsuccessful and result is uninformative, this is designated a test fail on the panel report and reason for test failure specified, if known. Example CRUK SMP2 NGS report can be found in Appendix 12).

SMP2-specific analytical data items (including patient demographics, disease characteristics, sample type, methods of sample collection; see Methods and Appendix 13 for full list of data items) were collected at clinical sites by dedicated personnel (research nurses, histopathology and molecular laboratory staff), captured in XML outputs and sent to CRUK SMP2 database for XML collation. Patient consent for data collection, storage and analysis was obtained at the time of informed consent for enrolment into SMP2 programme.

3.2.6 Methods of tissue acquisition within CRUK SMP2 trial

The samples used for molecular analysis within the CRUK SMP2 programme were obtained at a variety of sites and via many different acquisition methods. Early on in the implementation of SMP2, quality control failure rates were observed to be high and a potential correlation with type of sample and method of acquisition was postulated. In cooperation with the CRUK Precision Medicine team, I was granted access to the CRUK central SMP2 database, with aim of investigating the optimal methods of tissue acquisition for molecular genotyping in the context of CRUK SMP2 programme. Here, I present the results of this work.

3.2.6.1 Methods

I conducted a retrospective analysis of data collected for patients enrolled into CRUK SMP2 programme, through an established collaboration with the CRUK Precision Medicine team.

Data was analysed for samples received at the three technology hubs (THs) between August 2014 and December 2017. Anonymised clinical, technical, pathological and genotyping data for more than 3,500 samples were collected from participating Clinical and Technical Hubs and were available for analysis. XML data outputs received at the CRUK SMP2 database from CHs and THs were collated and used to

populate a master Excel spreadsheet by a dedicated CRUK data analyst, which was updated on a quarterly basis. Full list of data items collected and XML definitions are found in Appendix 13.

In my analysis, I included the following data items: sample source (clinical hub, technical hub), sample type (FFPE tissue, cytology cell block, extracted DNA), method of sample acquisition (CT-guided biopsy, surgical resection, bronchoscopic biopsy, endonronchial ultrasound and transbronchial needle aspirate, other), patient baseline demographics (age, gender, ethnicity, smoking status, ECOG performance status at time of SMP2 enrolment), disease baseline characteristics (histological subtype, clinical stage at time of SMP2 enrolment, mutational status at time of SMP2 enrolment, number of prior lines of therapy); QC status of each sample (fail or pass); molecular testing outcome (number of genes that failed or passed NGS panel testing for each sample); and data on turnaround timelines (date sample sent from CH to TH; date sample received in TH; date molecular report released).

Additional data was collected at each TH for purposes of sample tracking including the date each patient was approached and provided consent for entry into CRUK SMP2 study. I was able to obtain access to this data for 360 patients enrolled at the Royal Marsden Hospital TH, in order to evaluate the timelines between patient consent, sample receipt at TH and molecular testing report release.

This project was included within the remit of the research governance approvals for the overall CRUK SMP2 study.

3.2.6.1.1 Objectives and Endpoints

The primary objective was to identify the optimal method of sample acquisition in terms of adequacy for molecular genotyping.

The secondary objectives were: to determine CRUK SMP2 panel success and failure rates according to type of material genotyped; to determine SMP2 panel success and failure rates according to method of sampling; to determine SMP2 panel success and failure rates according to source of material genotyped; to explore the timelines between patient consent, tissue acquisition and SMP2 panel report.

The primary endpoint was the rate of quality control (QC) step failure according to method of tissue acquisition. Samples were deemed to have failed QC if they contained less than 50ng of tumour DNA at a minimum concentration of 2ng/μl.

Secondary endpoints were:

- Rate of QC failure according to type of sample received (e.g. FFPE, cytology cell block, extracted DNA);

- Proportion of SMP2 panel gene fails according to method of sampling;

- Rate of QC failure and number of SMP2 panel gene failures according to source of material genotyped;

- Median time in days between:

    - Sample receipt in TH hub and QC result;

    - Sample receipt in TH hub and molecular report release;

    - Sample receipt in TH hub and molecular report release according type of sample;

    - Patient consent and sample receipt at the TH.

3.2.6.1.2 Statistical methods

Rates and proportions are expressed as percentages, with 95% confidence intervals. Comparisons were made using the chi-square test or Fishers Exact Test as appropriate.

3.2.6.2 Results

3,525 samples from 3,146 patients were included. The distribution of samples across the three THs is shown in Figure 3.6. Patient demographics were generally representative of the UK NSCLC population (Appendix 14).
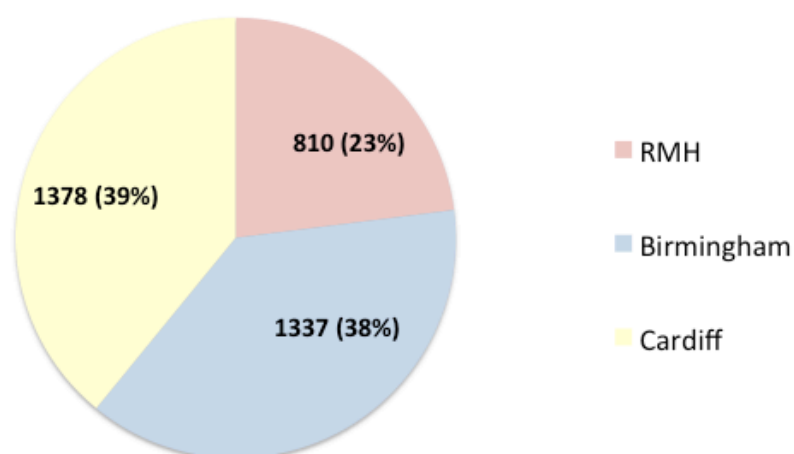
Figure 3.6. Number of samples (and proportion of total) received at each Technical Hub between August 2014 and December 2017.

There were three distinct sample types received at THs: FFPE tissue blocks (48.1% of all samples), cytology cell blocks (19.1%) and extracted DNA (32.3%). For 19 out of 3,525 (0.6%) samples, sample type was unknown due to missing or incomplete data. Table 3.6. shows types of sample received by each TH.

Sample type	RMH		Birmingham		Cardiff		All samples	
	n	%	n	%	n	%	n	%
FFPE block	599	74.0	487	36.4	609	44.2	1,695	48.1
Cytology cell block	179	22.1	322	24.1	171	12.4	672	19.1
Extracted DNA	13	1.6	528	39.5	598	43.4	1,139	32.3
Blood	3	0.4	0	0.0	0	0.0	3	0.1
Other	16	2.0	0	0.0	0	0.0	16	0.5
Total	810	100.0	1337	100.0	1,378	100.0	3,525	100.0

Table 3.6. Sample types received by each Technical Hub.

There was considerable variation in sample types between the three THs, reflecting the differences in local clinical and laboratory protocols and nature of predominant cancer activity of the associated Clinical Hubs. For instance, extracted DNA represented 43.4% and 39.5% of all samples at the Cardiff and Birmingham THs respectively, but only 1.6% at RMH TH where 74% of samples were FFPE tissue blocks. Methods of sample acquisition included image-guided lung biopsies, surgical lung resection specimens, bronchoscopic biopsies, endobronchial and endoscopic ultrasound fine needle aspirates (FNA), image-guided biopsies, surgical biopsies and surgical resection specimens from extra-thoracic sites, as well as bronchial washings and pleural effusion sampling for cytology cell block preparation. Methods of sample acquisition according to type of sample and by TH are shown in Table 3.7. For 19 samples, method of acquisition was unknown or not recorded.

Sample type	RMH n	RMH %	Birmingham n	Birmingham %	Cardiff n	Cardiff %	All samples n	All samples %
FFPE tissue block	**599**	**74.0**	**487**	**36.4**	**609**	**44.2**	**1,695**	**48.1**
CT guided lung biopsy	88	10.9	201	15.0	98	7.1	387	11.0
Surgical resection	138	17.0	79	5.9	205	14.9	422	12.0
Bronchoscopic lung biopsy	136	16.8	107	8.0	187	13.6	430	12.2
Other tissue biopsy*	237	29.3	100	7.5	119	8.6	456	12.9
Cytology cell block	**179**	**22.1**	**322**	**24.1**	**171**	**12.4**	**672**	**19.1**
EBUS/EUS FNA	144	17.8	268	20.0	120	8.7	532	15.1
CT guided FNA	7	0.9	6	0.4	5	0.4	18	0.5
Other cytology cell block**	28	3.5	48	3.6	46	3.3	122	3.5
Extracted DNA	**13**	**1.6**	**528**	**39.5**	**598**	**43.4**	**1,139**	**32.3**
CT guided lung biopsy	1	0.1	125	9.3	130	9.4	256	7.3
Surgical resection	12	1.5	99	7.4	49	3.6	160	4.5
Surgical lung biopsy	0	0.0	40	3.0	7	0.5	47	1.3
EBUS/EUS FNA	0	0.0	64	4.8	130	9.4	194	5.5
Other tissue biopsy*	0	0.0	183	13.7	272	19.7	455	12.9
Other FNA cytology	0	0.0	17	1.3	10	0.73	27	0.8
Blood	**3**	**0.4**	**0**	**0.0**	**0**	**0.0**	**3**	**0.1**
Other	**16**	**2.0**	**0**	**0.0**	**0**	**0.0**	**16**	**0.5**
Total	**810**	**100**	**1,337**	**100**	**1,378**	**100**	**3,525**	**100**

Table 3.7. Methods of sample acquisition for all 3525 samples and by TH. *Other tissue biopsy: there was insufficient additional data to further sub-classify the samples in these categories, the methods of sampling are likely to include image-guided biopsies or surgical biopsies from non-thoracic sites (e.g. liver, lymph nodes etc.). ** Other cytology cell block: samples included 65 pleural effusions, 12 bronchial washings, and 45 cell block samples that were not otherwise defined.

3.2.6.2.1 Rate of QC failure according to sample type

723 (20.5%) of all samples failed QC. QC fail rate for FFPE tissue samples was 24.9%, for cytology cell block samples 24% and for extracted DNA samples 11.3% (Figure 3.7.). There was no statistically significant difference in QC failure rate between the tissue and cytology cell block sample types (Chi-square=0.2283, p=0.63), while the extracted DNA had significantly lower QC failure rate compared with tissue and cytology blocks (Chi-square = 84.376. p< 0.00001). There was a significant difference in QC failure rates between the three THs (p<0.0001, Table 3.8), with significantly lower rate of QC failure observed for Birmingham and Cardiff TH samples, likely reflecting the higher proportion of extracted DNA samples submitted to these THs.
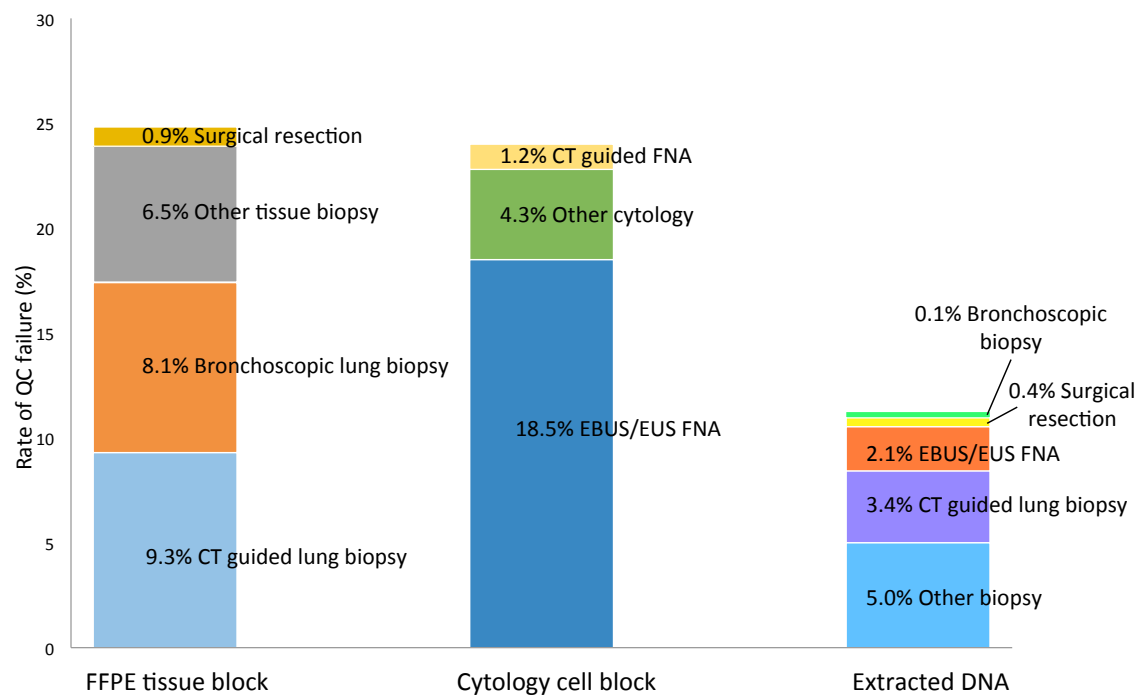
Figure 3.7. Rates of QC failure (%) according to method of sample acquisition (all samples). EBUS, endobronchial ultrasound; EUS, endoscopic ultrasound; FFPE, formalin-fixed paraffin-embeded; FNA, fine needle aspirate.

TH	Total number of samples	No. (%) of QC pass	No. (%) of QC fail	% of all QC failures	P
RMH	810	579 (71.5)	231 (28.5)	32.0	
Birmingham	1,337	1,034 (77.3)	303 (22.7)	41.9	0.0023
Cardiff	1,378	1,189 (86.3)	189 (13.7)	26.1	0.00001
Total	3,525	2,802 (79.5)	723 (20.5)	100.0	

Table 3.8. Rates of QC failure by Technical Hub, and as proportion of all samples. QC, quality control; RMH, Royal Marsden Hospital; TH, Technical Hub.

3.2.6.2.2 Rate of QC failure according to method of sample acquisition

For QC failure rates according to method of sample acquisition, and in view of the significant difference in failure rates between extracted DNA samples and tissue FFPE/cytology samples, tissue and cytology samples were analysed separately (Table 3.9 and Figure 3.8.). The highest rate of QC failures were observed for CT-guided lung biopsies and FNAs: 40% and 44%, respectively (although the total number of CT-guided FNAs was very small), and the lowest for surgical resection specimens (3.8%), while endoscopically acquired FNA cytology samples had a QC failure rate of 23%.

For extracted DNA samples, there was no significant difference in failure rates between different methods of sampling, except for surgical resections which has a significantly lower rate at 3.2%, compared with CT guided lung biopsies with 15% and EBUS cytology with 12.4% (Table 3.10).

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Method of acquisition (tissue and cytology samples only)	Total number	No. failed QC	% failed QC	p
Tissue - CT guided lung biopsy	387	158	40.8	
Tissue - Surgical resection	422	16	3.8	< 0.00001
Tissue - Surgical (bronchoscopic) lung biopsy	430	137	31.9	0.0077
Tissue - Other tissue biopsy	456	111	24.3	<0.00001
Cytology - EBUS/EUS FNA	532	124	23.3	< 0.00001
Cytology- CT guided FNA	18	8	44.4	0.76
Cytology- Other cytology	122	29	23.8	0.0006
TOTAL	**2,367**	**583**	**24.6**	

Table 3.9. Rates of QC failure according to method of sampling for tissue FFPE and cytology cell block samples (n=2,367).

	FFPE - CT guided lung biopsy	FFPE - Surgical resection	FFPE - Surgical (bronchoscopic) lung biopsy	FFPE - Other tissue biopsy	Cytology - EBUS/EUS FNA	Cytology- CT guided FNA	Cytology- Other cytology
n passed QC	229	406	293	345	408	10	93
n failed QC	158	16	137	111	124	8	29
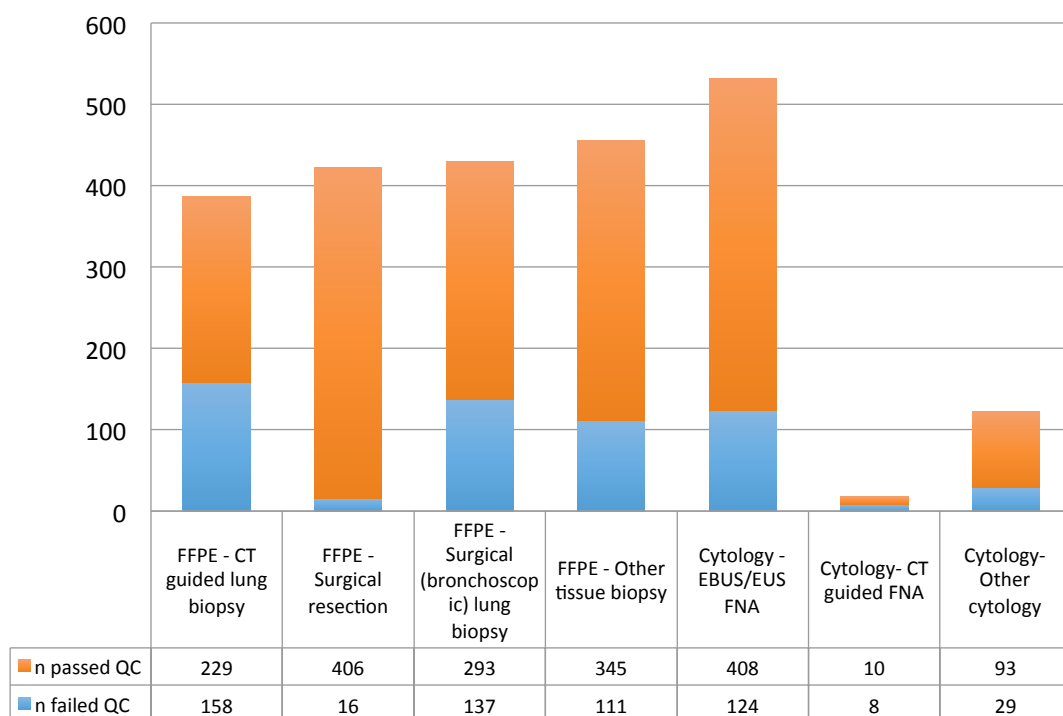
Figure 3.8. Comparison of rates of QC failure according to method of sampling for tissue FFPE and cytology cell block samples (n=2,367).

Method of acquisition (Extracted DNA samples only)	Total number	No. failed QC	% failed QC	p
CT guided lung biopsy	256	39	15.2	
Surgical resection	160	5	3.1	0.0001
Surgical lung biopsy	47	1	2.1	0.1468
EBUS/EUS FNA	194	24	12.4	0.3843
Other tissue biopsy	455	57	12.5	0.3125
Other FNA cytology	27	3	11.1	0.56868
Total	1,139	129	11.3	

Table 3.10. Comparison of rates of QC failure according to method of sample acquisition for extracted DNA samples (n=1,139).

3.2.6.2.3 Number of SMP2 panel gene failures according to method of sampling

2,802 samples passed QC step and were submitted for SMP2 28-gene panel testing. 1,099 samples (39.2%) successfully returned results for all 28 gene analyses, while 289 (10.3%) failed all 28 gene tests. Figure 3.8. shows the distribution of samples according to number of gene fails. This was similar across all three main sample types (FFPE tissue, cytology cell block and extracted DNA, Figure 3.9. middle) and across different methods of sample acquisition, with around 40% samples passing all 28 genes, approximately 10% failing all 28 genes and the remaining 50% distributed in a highly similar pattern.
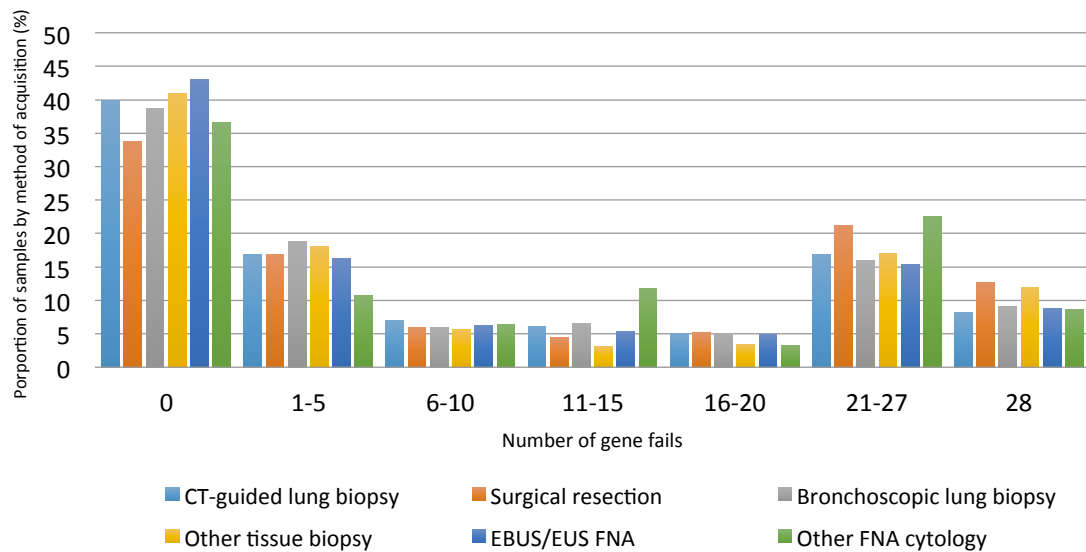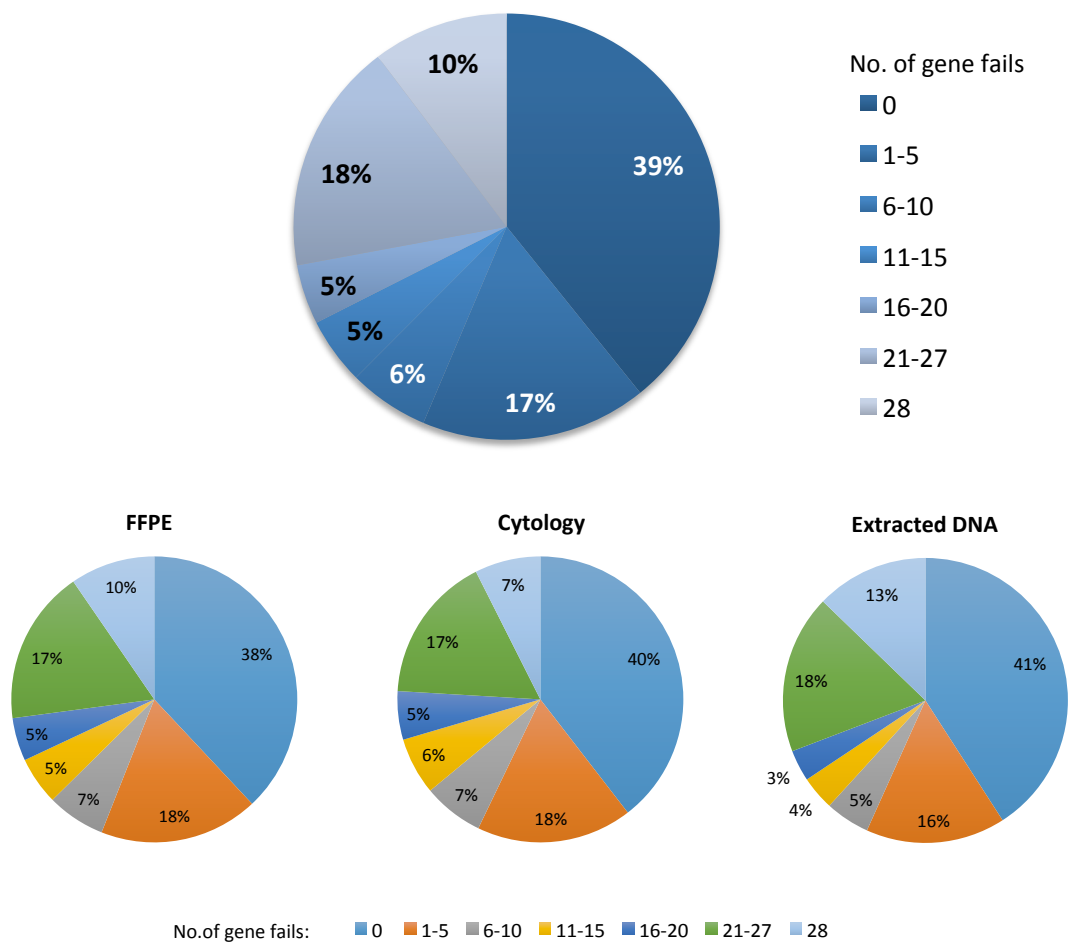
Figure 3.9. Distribution of samples according to number of SMP2 panel gene fails for all 2,802 samples that passed QC step and underwent SMP2 panel testing (top), by sample type (middle) and by method of sample acquisition (bottom).

3.2.6.2.4 Timelines

Timelines between samples being received in the TH laboratory, QC step results availability and sequencing report release were analysed. For the 723 samples that failed QC, this result was available after a median of 14 days (range 1-459, IQR 8-22). For 2,802 samples that passed QC step and went on to NGS, median time from sample receipt to SMP2 panel sequencing report release was 29 days (range 1-993, IQR 23-44). Median time to sequencing report release was 28 days (IQR 22-40) for extracted DNA samples, and 31 days for tissue and cytology samples (IQR 24-49 and 24 to 41 respectively, Figure 3.10.). Evaluation of timelines between patient consent to SMP2 and sample receipt in the laboratory for 360 patients from the RMH TH demonstrated a median time from patient consent to sample being received in molecular lab was 17 days (range 0-380, IQR 6-29).
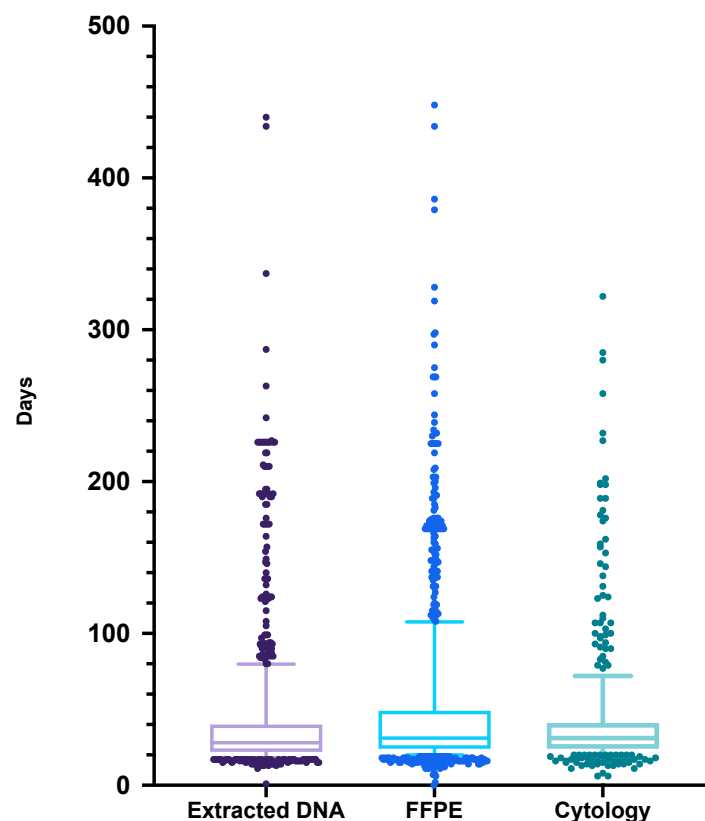
Figure 3.10. Time in days from sample received in laboratory to SMP2 panel sequencing report release according to type of sample analysed.

3.2.7 Discussion and conclusions

These data and CRUK SMP2 experience demonstrate the challenges in implementation of broad panel next generation sequencing in patients with NSCLC due to high variability in quantity and quality of material available for DNA extraction and sequencing.

The overall rate of QC failure was high at 20.5%, but this is consistent with other similar programmes of large-scale genomic testing such as the 2015 study at MD Anderson where 23% of the 2,601 enrolled patients did not undergo testing as a result of inadequate tissue or DNA quantity or quality.[235] There was significant variability in QC failure rates between different types of sample and methods of sampling. Samples of extracted DNA received at the molecular testing hubs would likely already have undergone a quality check process at site of extraction and therefore unsurprisingly have a significantly lower QC failure rate of 11.3% compared with tissue and cytology cell block samples at around 24%. With respect to method of sampling, surgical resection specimens predictably have a vastly lower QC failure rate than other methods of sampling due to quantity of tissue available, but it is perhaps more interesting that cytology samples obtained by EBUS FNA perform significantly better than image-guided biopsies (23.3% vs. 40.8%, p<0.00001). Endobronchial ultrasound transbronchial needle aspiration has a high sensitivity and specificity in confirming intrathoracic lymph node metastasis and is the recommended modality to stage the mediastinum in patients with NSCLC.[236] EBUS FNA samples have been shown to be adequate for identification of *EGFR* and *ALK* mutations by single gene tests in several studies, with a large meta-analysis reporting adequacy for *EGFR* identification of 94.5% and *ALK* in 94.9%.[237] Adequacy for NGS has also been assessed albeit in smaller studies with one study in 54 patients reporting 98% adequacy for a 50 gene panel and another study in 115 patients showing 86.1% adequacy for a larger (>300 gene) panel of EBUS obtained cytology specimens.[238, 239] Formalin fixation can result in significant degradation of DNA[240] and one reason for good performance of EBUS cytology for NGS may be that alcohol-based cytology fixatives may result in better preservation of high-quality nucleic acids and nuclear structure, providing a benefit in molecular testing, however data for methods used for cytology sample preparation were not available here. Another

possibility is that close visualisation and targeting with EBUS may result in samples that have high cellularity, with low numbers of red cells or benign bronchial cells.[239] The difference in QC failure rates between CT guided biopsies and EBUS cytology were not observed for extracted DNA samples, and again this was likely due to the samples passing a local quality control step before being sent to TH.

A National Lung Cancer Audit (NLCA) Spotlight report on molecular testing in advanced lung cancer published it's findings in January 2020 on the efficacy and outcomes of routine testing for *EGFR*, *ALK* and PD-L1 in England.[241] This national audit, commissioned by the Healthcare Quality Improvement Partnership, looked at 1,157 patients with stage IIIB and IV NSCLC from 60 trusts in England between June and December 2017, aiming to determine the adequacy of tumour sampling, proportion of patients undergoing molecular testing, timeliness of testing as well as treatments received and survival. The key findings of the report were that 83% of patients with advanced adenocarcinoma underwent molecular testing for all three molecular biomarkers (*EGFR*, *ALK* and PD-L1), and that 96% of molecular tests were successful in providing adequate results. One of the highlights of the report was the finding in a multivariate analysis of increased likelihood of requiring a second biopsy for molecular testing if the initial sampling attempt was through pleural biopsy or aspiration (adjusted odds ratio 2.37; 95% CI: 1.20-4.70), suggesting that cell blocks from pleural aspirates may be less suitable for molecular testing. There was no correlation between patient characteristics or other sampling techniques and requirement for second biopsy. In our data, there were only 65 cases where cytology cell blocks could be confirmed as being from pleural effusion specimens. Out of these 65 cases, 13 (20%) resulted in a QC fail, closely in line with the overall QC fail rate for all samples (20.5%) and similar to fail rate for all cytology cell block samples (23%). For the 52 cases that went on to SMP2 NGS panel testing, 17 (33%) successfully returned all 28 gene results and 5 failed all 28 genes on the panel (9.6%). While the numbers here are small, they do not appear to bear out the findings of the NLCA report, although a direct comparison is not possible due to significant differences in methodology between the two studies.

For all samples that passed the QC step, 90% returned a valid result for at least one gene, with around 40% successfully tested for all 28 genes, while 10% failed all 28

genes despite passing the QC step. This pattern of panel performance was consistent between different sample types and sample acquisition methods.

Median time from sample receipt in TH to report release was 29 days. When combined with the median time from patient consent to sample receipt in the laboratory, this results in an overall duration of around 46 days or over 6 weeks from patient consent to report being available to clinicians for interpretation and treatment decision making. This timeline reflects the relative complexity of the pathway with time required to identify and locate archival samples, followed processing at local laboratory and transport to centralized THs (17 days), followed by quality control check (14 days) and subsequent panel testing and preparation of NGS report (15 days). This may be one of the factors in the observed high attrition rate between numbers of patients with confirmed molecular eligibility for entry into one of the NLMT arms and trial enrolment (14% of molecularly eligible patients or 5% of all screened patients enrolled into NLMT as of November 2019, with death or deterioration in PS precluding enrolment in 46% of patients).[242]

CRUK SMP2 and the NLMT trial demonstrate the feasibility of delivering a national screening programme of NGS tissue molecular genotyping at scale in advanced NSCLC, utilizing existing NHS infrastructure. The data from this analysis provides additional information about the key challenges to successful implementation, including minimizing the number of QC fails by optimal sample selection wherever possible, with this data suggesting improved performance of surgical specimens and EBUS samples over image-guided biopsy specimens, and performing local DNA extraction where technically feasible to avoid processing of unsuitable samples and therefore a delay to acquisition of additional samples where needed. The main limitations of this analysis, was that, due to data input from variety of sites and by different personnel, this resulted in variability of accuracy and completeness of key data items, for instance large number of cases where method of sample acquisition is stated as "other" and not further specified (1,077 or nearly a third of all samples). One of the optional SMP2-specific data items for collection (data item 23 in Appendix 13) was "type of biopsy" (diagnostic biopsy, repeat biopsy after test fail, repeat biopsy after prior therapy) which was largely unpopulated, possibly due to this information not being available to the individual performing data input or due to

difficulty in extracting this data from patient records where a degree of clinical knowledge may be required, and was therefore not able to be included in this analysis, but may have provided useful information regarding the relationship between rates of QC fail and type of biopsy material. Therefore, and while this is also likely a resource issue, additional instruction or training to teams performing data entry may lead to improvement in data completeness and accuracy to allow further deep data mining and to answer additional questions regarding the relationship between molecular adequacy and sample factors.

3.3 Conclusions

The two studies in this chapter were performed to provide data and guidance to clinicians when considering tissue molecular genotyping in advanced NSCLC in the early years of the molecular era in NSCLC in the UK, where evidence was building for the need for repeated molecular genotyping, and subsequently also for the benefits of broader tissue NGS profiling, both for diagnosis and identification of resistance mechanisms after TKI therapy. Since then, genomic testing has become routinely commissioned in the UK and in January 2019 the national NHS Genomic Medicine Service (GMS) was established with the aim of providing equitable and extended access to molecular diagnostics and routine genomic testing to all patients with cancer. Utilizing the infrastructure developed during the 100,000 Genomes Project,[243] the NHS GMS is delivered via a network of seven Genomic Laboratory Hubs (GLHs), responsible for coordinating the service in their respective regions and performing the molecular testing as outlined in the National Genomic Test Directory. Included in the Test Directory are guidelines on the use of multi-targeted tissue NGS panels to aid diagnosis and management of advanced non-small cell lung cancer.[244]

This new infrastructure has brought with it several challenges to effective implementation including complex pathways for sample collection, registration and tracking; need for standardised delivery and interpretation of results; handling of large volumes of data; and provision of training for clinicians and information for patients. The National Genomic Information System (NGIS) is an informatics platform in development by Genomics England on behalf of the NHS in order to provide a national framework for digital patient registration, consent, data entry, sample labelling and tracking and result delivery for patients undergoing molecular testing within the GMS. Interpretation of the results requires implementation of central molecular tumour boards and local oncology multidisciplinary team meetings, with involvement and input from cancer geneticists, enabling effective and accurate interpretation of results, aiding with assessment of significance and pathogenicity of rare variants, and evidence-based clinical translation. Training of more clinicians to be able to interpret genomic data in the clinical context is required, with integration of cancer genomics training in the medical oncology training curriculum.

Additionally, availability of suitable and adequate material for genomic analyses remains a significant challenge for clinicians and patients with advanced NSCLC. Clinicians should work closely with their pathology and molecular scientist colleagues to assess and select material suitable for testing, with their radiology and interventional radiology teams to identify the sites and methods of sample acquisition most likely to yield adequate samples, and consider acquisition of additional material early in the patient treatment pathway. Meanwhile, ongoing technological advances in the development of minimally invasive ctDNA NGS approaches is likely to lead to their increasing use to complement tissue genotyping where samples are not available or inadequate, as well introduction of "blood-first" strategies in the future.[160, 245]

Chapter 4 Clinical utility of circulating tumour DNA genotyping in advanced NSCLC

As discussed in chapter 1.4.2, development and validation of ctDNA for diagnosis, target identification and resistance mutation identification has heralded a new era of minimally invasive technologies for tumour molecular characterisation and clinical decision making in NSCLC. However, at this time, feasibility of routine implementation of ctDNA testing was unknown and the data for the role and relative utility of ctDNA NGS were limited.

In this chapter, I will present the work to evaluate the feasibility of implementation of routine EGFR ctDNA in the NHS and in the second part, the work in gathering real-world data for the clinical utility of ctDNA NGS in advanced NSCLC.

4.1 Feasibility of implementation of a clinical EGFR ctDNA testing service in the NHS

4.1.1 Introduction

Detection of *EGFR* mutations in ctDNA from patients with advanced NSCLC is now considered a surrogate for tissue genotyping and a licenced tool to guide EGFR-TKI treatment both in the treatment-naïve setting, via detection of activating/sensitising *EGFR* mutations and, at the time of progression after TKI treatment, via detection of the *EGFR T790M* resistance mutation. Publication of the AURA series of trials,[131, 157, 246, 247] which demonstrated activity of osimertinib in T790M mutated NSCLC after progression on first-line EGFR TKIs, followed by wider availability of osimertinib within the NHS in the same indication (initially via the Cancer Drugs Fund from October 2016, followed by NICE recommendation for routine NHS use from October 2020), created a growing demand for *EGFR* ctDNA testing in the UK. The molecular diagnostic laboratories started to implement ctDNA *EGFR* genotyping principally for identification of *EGFR* T790M mutation, although the ctDNA assays were also able to identify the primary sensitising *EGFR* mutations, thereby introducing their potential use in the diagnostic setting. Several clinical trials had evaluated the diagnostic test performance of plasma-based compared with FFPE tissue *EGFR* testing with

relatively good concordance, sensitivity and specificity, as outlined in section 1.4.2.1, however, at this time, data from multicentre real-life studies was limited, particularly in the UK where no multi-centre data on the feasibility of routine ctDNA *EGFR* testing within the NHS was available. With hospitals and laboratories yet to establish routine clinical pathways for *EGFR* ctDNA testing, including sample collection and shipping to centralised molecular laboratories, the likely turn-around-times (TATs) that could be achieved were unknown and whether these would be relevant for routine clinical decision making. We performed a national service evaluation of ctDNA *EGFR* testing across several NHS molecular hubs to evaluate the results generated and validate their suitability for timely clinical decision making.

4.1.2 Methods

This was a prospective multi-centre observational study with the aim to establish the feasibility of delivering a timely and accurate routine clinical service for *EGFR* ctDNA testing across the UK, using NHS reference laboratories at 4 regional participating centres: Birmingham, Cardiff, Manchester and the Royal Marsden Hospital. These centres had previously demonstrated the required capacity and expertise to deliver molecular diagnostics in lung cancer on a regional scale and as part of the Cancer Research UK Stratified Medicine Programme.

Relevant regulatory and ethical approvals were sought and obtained from the RMH R&D committee, with local approvals obtained at participating centres.

4.1.2.1 Objectives and endpoints

The primary objective was to assess the feasibility of implementation of a clinical *EGFR* ctDNA testing service in the NHS in patients with advanced NSCLC.

The primary endpoint was the proportion of *EGFR* ctDNA tests providing a valid test result (regardless of whether a mutation was detected or not) ≤14 calendar days from test request in >80% of blood specimens sent for testing.

The secondary objectives were: to assess the rate of detection of *EGFR* T790M mutation at the time of progression in patients with *EGFR+* NSCLC treated with tyrosine kinase inhibitors; to compare the concordance of *EGFR* mutational status between FFPE tissue testing and ctDNA testing; to assess sensitivity of *EGFR* ctDNA tests; to assess specificity of *EGFR* ctDNA tests; to describe the turn-around times

from date of sample request and date of sample receipt in the laboratory to date of result.

The secondary endpoints were:

- Proportion of valid tests out of all *EGFR* ctDNA tests performed;
- Proportion of tests detecting a *EGFR* T790M mutation in patients with confirmed clinical progression after EGFR TKI therapy;
- Rate of concordance of *EGFR* ctDNA with tissue *EGFR* testing;
- Sensitivity of *EGFR* ctDNA testing compared with tissue;
- Specificity of *EGFR* ctDNA testing compared to tissue;
- Turn-around time (TAT) in days from date of sample request to date of result published for *EGFR* ctDNA tests.

4.1.2.2 Inclusion and exclusion criteria

The patient inclusion criteria for the study were:

- Patients with a histologically confirmed diagnosis of non-squamous advanced or metastatic NSCLC;
- Patients with treatment-naïve NSCLC for whom *EGFR* tissue testing has been requested, is in process or has been completed;
- Patients with pre-treated *EGFR* mutant NSCLC at time of clinical progression where *EGFR* T790M mutation testing would be considered appropriate by the treating physician;

The exclusion criteria were:

- Patients without definitive histological diagnosis of NSCLC;
- Patients with squamous NSCLC;
- Patients where *EGFR* mutation testing is not appropriate according to national or local guidelines.

4.1.2.3 Patient identification and testing

Patients satisfying the inclusion/exclusion criteria were identified by clinicians at each participating centre between June 2016 to October 2017. Tissue-based *EGFR* testing was requested as routine standard of care by clinicians and performed in the local molecular diagnostics laboratory. For plasma sampling, a blood sample was

taken in pre-specified tubes according to the chosen reference centre, to be sent alongside the appropriate request form to the local molecular testing centre. Sample preparation, DNA extraction and *EGFR* mutation testing was performed by molecular laboratories on receipt of a blood sample and completed request form, using their validated diagnostic protocol at the time of testing, with different ctDNA *EGFR* assays used by different laboratories.

The results were communicated to the referring clinician electronically or by post, in line with the laboratory's standard operating procedures. The *EGFR* ctDNA results were made available for clinical use as per local practice and in line with licencing guidelines at the time.

4.1.2.4 Data collection

The anonymised data was captured at the participating molecular diagnostic laboratories and entered into a validated data collection tool, then transferred via secure NHS e-mail for central pooling, cleaning and analysis. Data items collected included: anonymised data on patient demographics and baseline characteristics (age, gender, TNM stage, histological diagnosis, prior systemic anti-cancer therapy); disease characteristics at time of ctDNA collection (presence/absence of extrathoracic metastases, presence/absence of brain metastases); data on tissue *EGFR* testing (date/time/result of tissue *EGFR* test, tissue testing method); data on *EGFR* ctDNA testing methods (date/time of blood draw, blood tube used for blood draw, date/time of DNA extraction, *EGFR* testing method, date/time of *EGFR* ctDNA result, whether mutation was detected, type of EGFR mutation identified). Full list of data items and possible responses can be found in Appendix 15.

4.1.2.5 Statistical analysis

Over the 12-month time frame, an expected sample size of 600 patients undergoing ctDNA analyses was chosen to reflect national activity of the molecular diagnostic laboratories involved and to give reasonable chance of reaching the primary endpoint. The primary endpoint was chosen as a pragmatic reflection of whether the test could function as a reliable and accurate test across the UK, i.e. that it is able to provide a valid result in 80% of cases and that this result is available within 2 weeks from request, as an acceptable time frame to allow timely clinical decision-making.

With an expected valid result to be detected in 80% (i.e. 480 out of 600) of blood specimens sent for testing would give a 95% confidence interval of 77% to 83% with this number of patients (based on exact binomial confidence interval).

The following analyses were carried out:

- Proportion of *EGFR* ctDNA testing providing a informative or valid result (i.e. mutation detected or mutation not detected) out of all patients tested overall and within 14 days of test request, expressed as a percentages with 95% confidence interval. TATs were expressed as medians with range. Comparisons of TATs between different methods of *EGFR* ctDNA testing were performed using Mann-Whitney U test for two groups or Kruskal-Wallis test for multiple groups as appropriate.

- For patients undergoing *EGFR* ctDNA testing after progression on EGFR TKI therapy, the proportion of patients where the T790M mutation was detected on *EGFR* ctDNA testing, expressed as a percentage with 95% confidence interval.

- Concordance was measured based on *EGFR* mutation identification in tissue compared to blood and calculated as the sum of patients where the methods agree out of the total number of patients expressed as a percentage.

- Sensitivity was measured as the proportion of positive results correctly identified by the test (sensitivity = true positive/(true positive + false negative)).

- Specificity was measured as the proportion of negative results correctly identified by the test (specificity = true negative/(true negative + false positive)).

- Difference in sensitivity and specificity between paired tissue and ctDNA samples for *EGFR* mutation detection was tested using the McNemar's test for paired samples.

4.1.3 Results

Data was collected for 657 cases undergoing ctDNA *EGFR* testing between June 2016 and October 2017 at 4 major regional centres: Birmingham 303, Manchester 122, RMH 181 and Cardiff 51.

Data on ctDNA testing methodology, sampling and reporting timelines, clinical setting of testing (at diagnosis/treatment naïve or at clinical progression) and *EGFR* test results was available for all 657 patients.

4.1.3.1. Patients

Complete data including patient demographics and baseline clinical characteristics was available for 354 patients from 3 centres (Manchester, Cardiff, RMH), but was not available for 303 patients from Birmingham, due to local ethics committee restrictions.

Table 4.1. shows patient demographics, baseline characteristics and technical data for the 354 patients for whom this data was available. The median age was 68 (range 27-90), there was a somewhat greater proportion of female versus male patients (57% vs 43%) and the great majority had a confirmed diagnosis of adenocarcinoma NSCLC with stage IV disease at diagnosis.

A third of patients had disease limited to the thorax, while 15% had confirmed brain metastases at time of blood sampling.

Out of all 657 patients, 287 patients underwent *EGFR* ctDNA testing in the diagnostic/treatment naïve setting and 364 patients had testing after progression on or after prior TKI therapy, as clinically determined by treating clinician. For 6 patients, data on clinical setting for testing was not available.

Patient demographics and baseline characteristics (n=354)	n	%
Age		
Median (range)	68 (27-90)	
Gender		
Male	153	43.2
Female	201	56.8
Histology		
Adenocarcinoma	316	89.3
Adenosquamous carcinoma	4	1.1
NSCLC NOS	10	2.8
Other*	17	4.80
Unknown	7	2.0
UICC TNM Stage		
1B	9	2.5
2A	1	0.3
2B	3	0.9
3A	10	2.8
3B	19	5.4
4A	101	28.5
4B	182	51.4
Unknown	29	8.2
Thoracic disease only at time of ctDNA testing		
Yes	119	33.6
No	218	61.6
Unknown	17	4.8
Brain metastases at time of ctDNA testing		
No	284	80.2
Yes	54	15.3
Unknown	16	4.5

Table 4.1. Patient demographics and baseline characteristics for 354 patients from 3 regional centres. NSCLC NOS: not otherwise specified. UICC TNM: The Union for International Cancer Control lung cancer TNM staging, 7th edition. *17 other: 7 squamous, 1 pleomorphic, 1 large cell, 2 LCNEC/carcinoid, 1 neuroendocrine/adenoid cystic, 1 mesothelioma, 4 not specified.

4.1.3.2 Sample collection and testing methods

Plasma samples for *EGFR* ctDNA testing were collected using 4 different sample collection bloods tubes: Roche cell-free DNA blood collection tube (Roche tube; Ariosa Diagnostics, Inc., San Jose, USA), Streck cell-free DNA blood collection tube (Streck Tube; Streck Inc., Omaha, USA) and K2-EDTA blood collection tube (EDTA tube; BD Vacutainer®). Roche tube and PAXgene tube were used for the majority of samples (44% and 46%, respectively), with Streck tubes used for 8% and EDTA tube for 2% of all samples.

EGFR ctDNA testing was performed using the Roche cobas real-time PCR test (Roche Molecular Diagnostics, Basel, Switzerland) in 92.2% cases, with 7.6% tests performed using digital droplet PCR (ddPCR). Birmingham, Manchester and RMH all used Roche cobas, while ddPCR was used for the 51 samples at Cardiff molecular diagnostic centre.

Table 4.2. summarises type of blood collection tubes used and method of EGFR ctDNA testing according to molecular testing centre.

Molecular laboratory	Blood tube	Method of *EGFR* ctDNA testing	n (%)
Birmingham	PAXgene tube	Roche cobas	303 (100.0)
Cardiff	Streck tube	ddPCR	50 (98.0)
		Other	1 (2.0)
Manchester	Roche tube	Roche cobas	107 (87.7)
	EDTA tube	Roche cobas	15 (12.3)
RMH	Roche tube	Roche cobas	181 (100.0)

Table 4.2. Blood sample collection tubes and methods of EGFR ctDNA testing used at each molecular diagnostic centre.

4.1.3.3 EGFR ctDNA testing results

A valid *EGFR* test result was obtained for 651 out of all 657 samples (99.1%). For 6 invalid results, 3 had failed testing with no reason given, 1 sample was labelled incorrectly, 1 sample had suboptimal plasma volume and 1 sample was discarded due to a laboratory error (possible sample swap).

Data on TAT from test request to test result were available for 600 of 657 cases (in 57 cases data was incomplete for either date of request or date of result, or both). Out of 600 tests for which data was available, 584 or 97.3% returned a valid result within 14 days of test request (95%CI 95.7-98.5), meeting the primary endpoint. For 16 patients where time to result was >14 days, this was secondary to a delay between test request and blood sample draw in 8 cases (50%), while only 8 cases required longer than 14 days (with a range of 15-18 days) for sample processing in the molecular laboratory.

Overall median TAT from request to result was 6 days (range 0-71). There was no significant difference in TATs between Roche cobas and ddPCR testing methods (Mann-Whitney U test p=0.227), or between different blood collection tubes used (Kruskal-Wallis test p=0.06).

Out of all 651 valid tests, an *EGFR* mutation was detected in 244 samples (37.5%). 80 *EGFR* T790M mutations were detected: 78 in 364 (21.4%, 95%CI 17.3-26.0) of patients tested on clinical progression, 1 in a treatment naïve patient and 1 where no data on clinical setting was available. For one patient tested in treatment naïve setting, concurrent *EGFR* L858R and T790M mutations were detected. Data on variant allele frequency (VAF) was not available and not collected, with Roche cobas technology not able to provide VAF quantification, therefore it is not possible to say whether the baseline T790M detected in one patient was a germline or somatic variant.

All *EGFR* variants identified are shown in Table 4.3, while Figure 4.1. shows variants identified according to clinical setting.

EGFR variants detected (n=244)	n	%
Exon 19 deletion	86	35.2
Exon 19 deletion & T790M	51	20.9
Exon 19 deletion & G719X	1	0.4
L858R	60	24.6
L858R & T790M	17	7.0
L858R & T790M & S768I	1	0.4
L858R & S768I	1	0.4
G719X	2	0.8
G719X & L861Q	2	0.8
G719X & S768I & T790M	1	0.4
G719X & T790M & Exon 20 insertion	1	0.4
S768I	1	0.4
S768I & G719X	4	1.6
L861Q	5	2.0
L861Q & T790M	1	0.4
T790M	8	3.3
Exon 20 insertion	2	0.8
Total	**244**	**100.0**

Table 4.3. List of all EGFR variants detected from 651 valid EGFR ctDNA tests.
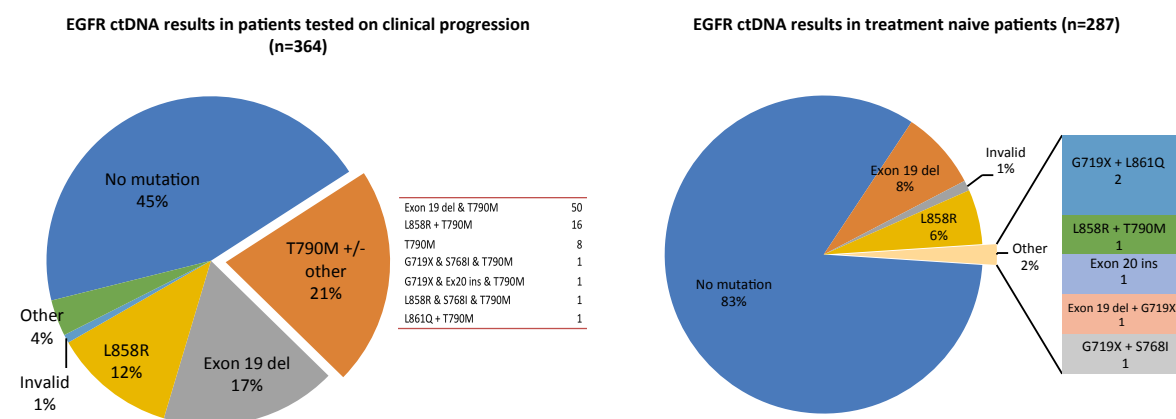
Figure 4.1. *EGFR* ctDNA test results according to clinical setting, with mutations identified in patients tested on clinical progression on the left and those identified in treatment naïve patients on the right.

4.1.3.4 Concordance with tissue EGFR testing

Result of a paired tissue *EGFR* test was available for 228 cases: 203 in treatment naïve setting and 25 in clinical progression setting. In the treatment naïve setting, any tissue *EGFR* test performed in the diagnostic setting was considered a paired tissue test. In the clinical progression setting, a paired tissue test was considered to be paired if tissue sampling was performed within 16 weeks of *EGFR* ctDNA tests without intervening EGFR TKI therapy.

Overall concordance of *EGFR* ctDNA with tissue *EGFR* testing for all 228 paired samples was 87.7% (95% CI 82.8%-91.4%). Sensitivity of plasma *EGFR* testing was 63.1% and specificity 97.6% (Table 4.4.). There was a significant difference between tissue and ctDNA in detecting *EGFR* mutations (Chi-sq = 12.89, p = 0.0003).

All paired samples (n=228)		ctDNA *EGFR*		
		No mutation	Mutation	Total
Tissue *EGFR*	No mutation	159	4	163
	Mutation	24	41	65
	Total	183	45	228
Chi-sq = 12.893 (1 degree of freedom), p = 0.0003				

Statistic	Value	95% CI
Concordance	**87.7%**	**82.82% – 91.37%**
Sensitivity	63.08%	50.15% to 74.45%
Specificity	97.55%	93.54% to 99.21%
Positive Predictive Value	91.11%	77.87% to 97.11%
Negative Predictive Value	86.89%	80.92% to 91.25%

Table 4.4. Concordance, sensitivity, specificity, positive and negative predictive values for all 228 paired *EGFR* ctDNA and tissue tests

There were 4 discordant results where plasma ctDNA testing identified an *EGFR* sensitising mutation (*EGFR* exon 19 deletion) which was not identified on tissue testing, including 3 cases where tissue test was invalid/failed and 1 case in the treatment naïve setting were an *EGFR* exon 19 deletion was not detected on a valid tissue test. There were further 2 cases in the clinical progression setting where ctDNA identified a T790M mutation not identified on tissue testing (Table 4.5.).

Tissue	n	ctDNA	n
EGFR wt	146	EGFR wt	144
		Exon 19 del	1
		Invalid	1
Sensitising EGFR mutation detected	59	Sensitising mutation detected	35
		Sensitising mutation & T790M detected	2
		No mutation detected	21
		Invalid	1
T790M mutation detected	6	T790M mutation detected	2
		Sensitising mutation only	2
		No mutation detected	2
Invalid/fail	17	Invalid	1
		EGFR wt	13
		Exon 19 del	3
Total	228	Total	228

Table 4.5. Concordance of *EGFR* mutation status between plasma and tissue for 228 paired samples. wt, wild type

The sensitivity and specificity were also analysed separately for the two groups according to clinical setting for test: treatment naïve/at diagnosis or on clinical progression on/after TKI. For the 203 paired samples in the treatment naïve/diagnosis group, concordance was 91.1%, sensitivity 66.7% and specificity 98.1% (Table 4.6.).

Treatment naïve cases paired FFPE and ctDNA tests (n=203)		ctDNA result		
		No mutation	Mutation	Total
Tissue result	No mutation	155	3	158
	Mutation	15	30	45
	Total	170	33	203

Statistic	Value	95% CI
Concordance	**91.13%**	86.42% to 94.32%
Sensitivity	**66.67%**	50.95% to 79.56%
Specificity	**98.10%**	94.11% to 99.51%
Positive Predictive Value	**90.9%**	74.53% to 97.62%
Negative Predictive Value	**91.17%**	85.60% to 94.80%

Table 4.6. Concordance, sensitivity, specificity, positive and negative predictive values for 203 paired cases in treatment naïve/diagnostic setting.

In 30 out of 203 cases (14.8%) both tissue and ctDNA detected an *EGFR* sensitising mutation. The type of mutation detected was concordant in 29 out of 30 cases (96.7%). In one case where tissue testing detected a G719X mutation, ctDNA also detected an *EGFR* exon 19 deletion concurrent with a G719X mutation. 3 additional *EGFR* exon 19 deletions were detected by ctDNA that were not identified on tissue testing: in two cases where tissue *EGFR* testing was invalid, and in one case where *EGFR* was reported as wild type on tissue testing. There were 14 false negative ctDNA tests, where an *EGFR* mutation was detected on paired tissue testing. 11 of these 14 patients (78.6%) had intrathoracic disease only (10 patients) or a solitary brain metastasis as the only site of metastatic disease (1 patient).

In the setting of clinical progression on or after TKI therapy, there were only 25 paired samples available for comparison. Concordance was 60%, sensitivity 55% and specificity 80%. In 4 patients, neither tissue nor ctDNA identified any *EGFR* mutation, despite all having a tissue-confirmed *EGFR* sensitising mutation at diagnosis. In 9

cases, ctDNA failed to detect any *EGFR* mutations, including 8 cases where paired tissue testing confirmed a sensitising *EGFR* mutation and 1 case where paired tissue detected both exon 19 deletion and T790M. In one case, ctDNA confirmed a sensitising *EGFR* exon 19 mutation while tissue testing was negative. In 11 patients both ctDNA and paired tissue identified at least one *EGFR* variant (Table 4.7.).

Patient	Tissue *EGFR* result	ctDNA *EGFR* result
1	Exon 20 ins	Exon 20 ins
2	Exon 19 del & T790M	Exon 19 del
3	Exon 19 del	Exon 19 del & T790M
4	L858R & S768I & T790M	L858R & S768I
5	Exon 19 del & T790M	Exon 19 del & T790M
6	Exon 19 del	Exon 19 del
7	L858R	L858R
8	Exon 19 del	Exon 19 del
9	L858R	L858R
10	G719X & S768I	G719X & S768I & T790M
11	Ex19 del	Ex19 del

Table 4.7. Results for 11 patients where *EGFR* variant was identified on paired testing of both tissue and ctDNA in the setting of clinical progression on prior TKI therapy. ctDNA identified two additional T790M mutations not identified on tissue testing (patients 3 and 10), but failed to identify T790M in two cases (patients 2 and 4).

For all 228 paired tissue and plasma samples, plasma testing was performed by Roche cobas in 216 (94.7%) cases using Roche blood tubes (203 cases, 89%), with only 12 cases (5.3%) using ddPCR for ctDNA testing, therefore meaningful comparison of concordance, sensitivity and specificity between different ctDNA technologies was not possible. However, out of 12 cases tested by ddPCR, we observed discordant results in 5 cases (41.7%), including 4 cases where a sensitising *EGFR* mutation was detected on tissue but not plasma (three EGFR exon 19 deletions and 1 L858R mutation) and 1 case where tissue testing failed to produce a valid result while ctDNA testing identified *EGFR* exon 19 deletion. Data on the technologies used for tissue testing were not available.

4.1.4 Discussion

Circulating tumour DNA is the fraction of cell-free DNA circulating freely in the blood stream derived from tumour cells, after being shed primarily through passive processes of apoptosis and necrosis. Isolation and molecular analysis of ctDNA in advanced non-small cell lung cancer was developed as a less invasive method of tumour genotyping for purposes of identifying patients suitable for licenced TKI therapies and, following validation in randomised clinical studies, ctDNA genotyping is now in routine use in the UK and globally both in the diagnostic and resistance settings.

This study was conducted at the very early stages of routine clinical application of ctDNA testing in NSCLC patients in the UK, and provided valuable evidence that ctDNA *EGFR* analysis in plasma from patients with NSCLC is a reliable tool that can be used in a timely and accurate way to aid molecular characterization and complement tissue analysis.

The primary objective of valid result being available within 2 weeks of clinical decision to test was met in 97% of cases, with a median TAT of 6 days. The results also showed that ctDNA *EGFR* analysis in real-life routine clinical care in different UK laboratories is highly specific (>97%), while confirming lower levels of sensitivity (63%) that are comparable to data obtained from published clinical studies, as well as those from the ASSESS study, a large real-world study of *EGFR* ctDNA testing in European and Japanese patients which reported concordance of mutation status in 1162 paired tissue and plasma samples of 89%, sensitivity of 46% and specificity of 97%.[248] The reasons for lower sensitivity of ctDNA *EGFR* consistently observed across multiple studies is likely due to several factors. Very low fraction of ctDNA in the blood stream can be present in some patients with advanced NSCLC and this may correlate with the burden and distribution of disease, with evidence of reduced sensitivity in patients with intra-thoracic only disease (M1a). In our cohort, 10 out of 15 patients (67%) in the treatment naïve setting and 2 out of 9 patients on clinical progression (22%) in whom an *EGFR* mutation was detected on tissue but not on ctDNA had no metastatic disease outside the thorax. Furthermore, there are inherent differences between technologies used for ctDNA analysis, with allele-

specific methods such as Roche cobas *EGFR* ctDNA generally having lower sensitivity and higher mutant allele fraction detection limits (0.1-1%) than digital PCR methods which tends to be highly sensitive (MAF detection limit 0.01-0.1%) at the cost of lower specificity. Problem of low sensitivity of ctDNA is being addressed with advancement of ctDNA NGS technologies and application of deep sequencing, as well as the ability to test simultaneously for a broad panel of variants requiring smaller quantities of DNA for genotyping.

We observed good levels of concordance with tissue *EGFR* testing for the common sensitizing *EGFR* mutations, both overall (88%) and particularly in the treatment naïve group (91%), similar to data available from clinical studies with centralized testing. With paired tissue and ctDNA data derived primarily from treatment naïve patient cohort, these results indicated that ctDNA *EGFR* testing could be incorporated in the diagnostic pathway of advanced NSCLC prior to initiation of first line therapy, particularly for patients where tissue is not available or is inadequate for molecular analysis, at a time when ctDNA *EGFR* was being used primarily in the resistance setting.

The results for "clinical progression" group produced a lower overall concordance of 60% and sensitivity of 55%, although numbers in this particular group were low, with only 25 paired samples available. Furthermore, compared to clinical trial data were all patients require confirmed RECIST progression, in this real-life study samples were submitted for analysis on or after TKI treatment based on local clinical decision-making (e.g. on clinical progression in absence of radiological progression). This may have resulted in an increased rate of ctDNA "false negatives" due to very low mutant allele fraction of both the original sensitising *EGFR* mutation, as well as any resistance mutation such as T790M, being present in the blood at early stages of development of resistance and disease progression, with absence of original sensitising *EGFR* mutation on ctDNA in most cases of discordance in this setting (9 out of 10 cases).

Discordance may also be observed secondary to application of diverse ctDNA and tissue DNA genotyping technologies with potential differences in breadth and scope for identification of different mutant alleles and mutant allele frequencies, however data on technologies used for paired tissue testing was not available. For most cases

where a false negative result was obtained on ctDNA (20 out of 24, or 83%), testing was performed by Roche cobas *EGFR* ctDNA test with coverage of all common *EGFR* variants, including exon 19 deletions, L858R, G719X, S768I, L861Q, exon 20 insertions and T790M.

In 6 cases, ctDNA identified a mutation not detected on paired tissue testing. While in three of these cases tissue testing failed to produce a valid result, there were three cases that produced a valid but "false negative" result. Causes for false negatives to be observed on tissue testing may include tumour heterogeneity, where sampling from one section of the tumour or metastasis may not be representative of the entire tumour, because of presence of different genomic sub-clones within the same primary tumour, or between primary tumour and metastases, particularly in the setting of acquired resistance. However sensitivity of detection of *EGFR* mutations in tissue also varies depending on technology used, with some studies reporting reduced sensitivity of tissue EGFR Roche cobas test particularly in setting of small biopsies with lower tumour content.[140]

In this study, blood sampling for ctDNA was performed using a variety of blood tubes, but molecular testing with the Roche cobas EGFR ctDNA assay was used in the large majority of cases (92.2%). There were no significant differences observed in the proportion of valid results obtained or TATs for the different methods of sampling or testing. While the numbers were insufficient for direct comparison of concordance with tissue tests for the different methods of sampling and testing in this study, prior studies comparing the performance of cell-free DNA collection tubes have shown comparable results between Streck, Roche and PAXgene tubes for ctDNA stabilisation, extraction and testing,[249, 250] while cross-platform comparisons of technologies for ctDNA testing, including Roche cobas and ddPCR, have demonstrated high specificity and sensitivity for detection of sensitising *EGFR* mutations in the clinical trial setting, with digital technologies demonstrating better sensitivity but lower specificity for detection of T790M over non-digital assays.[251]

Overall, this data demonstrated feasibility of routine *EGFR* mutation detection by molecular testing of ctDNA in NHS laboratories across the UK, with clinically meaningful turnaround times and sufficient sensitivity and specificity to be used to direct clinical treatment. While sensitivity observed in this real-world study was

relatively low at 66%, this level of sensitivity was within the established limits of ctDNA *EGFR* assays and consistent with other clinical trial datasets, with international guidance at the time recommending ctDNA *EGFR* testing up-front on progression on first-line TKIs, with tissue biopsy to be considered whenever possible when the plasma result is negative.[72] Recent availability of osimertinib in the first-line for untreated *EGFR*-mutated NSCLC, including in the UK following recent positive recommendation by NICE, brings into questions the relevance and value of *EGFR* T790M testing upon progression on first-line osimertinib therapy, with the focus shifting to identification of diverse mechanisms of resistance to osimertinib and increasing importance of next generation sequencing in relapsed advanced EGFR+ NSCLC.[252, 253]

4.2 Clinical utility of ctDNA-based next-generation sequencing for target identification in the diagnostic and acquired resistance settings in advanced NSCLC

As discussed in section 1.4.2.2, several studies have demonstrated a potential role for ctDNA NGS particularly in the settings where tissue is unavailable or inadequate. Multiple commercial platforms have been developed which, while not routinely available in the UK National Health Service, can be used in the private care setting for NGS genotyping to guide therapy selection, and also as a commercial research trial screening tool and for self-funding patients. However, its role and relative benefits over other technologies, such as the tissue and ctDNA single-gene assays commonly used in routine practice, are not fully known. Guardant360® CDx (Guardant Health, Inc) is a ctDNA NGS-based assay of 74 genes, including all oncogenic drivers in NSCLC with currently licenced targeted therapies, namely *EGFR*, *ALK*, *ROS1* and *BRAF*, as well as emerging targets such as *RET*, *MET*, *HER2* and *KRAS*. Guardant360® CDx uses targeted high throughput hybridization-based capture technology for detection of single-nucleotide variants, insertion-deletion variants, fusion alterations and copy-number amplifications in select genes, on circulating cell-free DNA extracted from plasma of peripheral whole blood. Following collection of whole blood into Streck cell-free DNA BCT tubes, samples are shipped to the CLIA/CAP accredited Guardant Health Clinical Laboratories (Redwood City, CA, USA) for processing. Plasma is isolated by centrifugation and cfDNA extracted. 5-30ng of cfDNA is used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are sequenced using the Illumina NextSeq 550 NGS platform. Sequencing data are analysed using a custom bioinformatics pipeline and results reported in a written report to requester, with quantitative reporting of SNVs mutant allele fraction and gene copy number alterations, separation of germline or somatic alterations, and identification of actionable alterations identified as those for which there is an FDA-approved treatment or that serve as eligibility criteria for later phase clinical trials.

Guardant360 cell free DNA assay has undergone analytical and clinical validation with specificity of >99.9% and sensitivity of >85% for detection of mutated oncogenes in several solid tumour types including NSCLC in patients with stage III/IV disease.[254]

I designed and conducted a study to benchmark the clinical utility of ctDNA NGS using Guardant360® CDx assay compared with other standard of care tissue and ctDNA molecular testing in diagnosis and therapy selection in patients with advanced/metastatic NSCLC.

4.2.1 Methods

This was a retrospective analysis of clinical utility of molecular tumour genotyping by comprehensive ctDNA NGS using Guardant360® CDx assay (G360) in advanced lung cancer patients at the Royal Marsden Hospital.

4.2.1.1 Patients

Case notes for patients with advanced/metastatic NSCLC undergoing ctDNA NGS by G360 as part of routine care between June 2016 and September 2019 were reviewed. Patients were identified through search of electronic patient record for those with a confirmed histological diagnosis of non-small cell lung cancer (NSCLC) and G360 ctDNA NGS molecular genotyping result. The G360 test in April 2016 covered 54 genes, with the next version covering 70 genes and the latest panel, which became commercially available in November 2016, covering 74 genes.

Patients with non-NSCLC diagnosis and those with incomplete demographic, treatment or follow-up data were excluded. Individual case notes were hand-searched for pre-defined data items including fields on demography (age, gender, smoking history), lung cancer diagnosis (histology, disease stage, sites of metastatic disease), data on any prior molecular genotyping (known tumour variants, tissue source, methods and dates of prior testing) as well as ctDNA NGS outcomes data (genomic variants identified, date of sampling and date of report issue). A validated data capture spreadsheet was created and populated by two independent investigators, following approvals from the local research committee.

4.2.1.2 Objectives

Primary objective was to determine the proportion of informative ctDNA NGS tests out of all tests performed, defined as reporting any genomic variant. Secondary and exploratory objectives were: to document indications for ctDNA NGS testing request; to describe genomic variants identified and compare with variants identified by standard-of-care (SOC) methods of tissue and ctDNA molecular genotyping (established and contemporaneous specimens); to evaluate turnaround times between ctDNA NGS request and report, and compare to other methods of molecular genotyping.

4.2.1.3 Definitions

ctDNA NGS testing was classified as informative or not if any genomic variants were reported (or not). Genomic variants identified were classified according to AMP/ASCO/CAP guidelines[255] by their clinical significance and strength of available evidence into four categories: variants of strong clinical significance, including therapeutically targetable variants (Tier I); variants of potential clinical significance (Tier II); variants of unknown clinical significance (Tier III); and benign or likely benign variants (Tier IV, Figure 4.2.).

4.2.1.4 Statistical considerations

Proportions of informative tests were expressed as percentages with 95% confidence intervals where appropriate. Genomic variants were grouped according to clinical significance and presented in tabulated form. Differences in proportions of patients with Tier I mutations identified by ctDNA NGS and standard of care tests were compared using the Chi-square and Fisher's exact test as appropriate. TATs were reported as medians with range. Comparisons of TATs between standard of care tests and ctDNA NGS were performed using a paired t test.

Tier I: Variants of Strong Clinical Significance	Tier II: Variants of Potential Clinical Significance	Tier III: Variants of Unknown Clinical Significance	Tier IV: Benign or Likely Benign Variants
Therapeutic, prognostic & diagnostic	*Therapeutic, prognostic & diagnostic*		
Level A Evidence FDA-approved therapy Included in professional guidelines	**Level C Evidence** FDA-approved therapies for different tumor types or investigational therapies Multiple small published studies with some consensus	Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases No convincing published evidence of cancer association	Observed at significant allele frequency in the general or specific subpopulation databases No existing published evidence of cancer association
Level B Evidence Well-powered studies with consensus from experts in the field	**Level D Evidence** Preclinical trials or a few case reports without consensus		

Figure 4.2. Evidence-based variant categorization. Somatic variants are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics. Variants in tier I are of strongest clinical significance, and variants in tier IV are benign or likely benign variants. Copyright © 2017 American Society for Investigative Pathology and the Association for Molecular Pathology.

4.2.2 Results

Fifty four ctDNA NGS G360 reports from forty seven patients were identified between June 2016 and September 2019 and included in the analysis. Median age at time of testing was 61 years (range 31 to 79). Proportion of female to male patients was 66% to 34% respectively. More than half of patients were never smokers (53.2% versus 46.8% of current or former smokers). The majority of patients had adenocarcinoma NSCLC (44 out of 47 or 93.6%) with two cases of pleomorphic NSCLC and one case of squamous cell NSCLC. All patients had clinical stage IV disease at time of ctDNA NGS test request. 4 out of 47 patients (8.5%) had CNS-only metastases and one quarter (25.5%) had intrathoracic-only metastases (Table 4.8.). ctDNA NGS testing was performed in two distinct indications: first, to identify an oncogenic driver in patients with advanced/metastatic NSCLC as part of diagnosis/target discovery; and second, to identify an acquired resistance mechanism for patients with oncogene-addicted advanced NSCLC. 19 patients (40.4%) had a known oncogenic variant previously identified on tissue genotyping. Known tissue variants at time of ctDNA NGS are shown in Figure 4.3.

Patient characteristics (n=47)	n	%
Median age at diagnosis (range)	61 (31-79)	
Sex		
Male	16	34
Female	31	66
Smoking		
Never	25	53
Ex/current	22	47
Histology		
Adenocarcinoma	44	94
Pleomorphic	2	4
Squamous	1	2
M Stage at time of ctDNA NGS*		
M1A	13	28
M1B	5	11
M1C	29	62
Site of metastases		
CNS only	4	9
Intrathoracic only	12	26

Table 4.8. Patient demographics and baseline characteristics. *8th addition of the International Union for Cancer Control (UICC) TNM staging staging system for lung cancer. CNS, central nervous system.

Known variants in tissue of patients undergoing ctDNA NGS
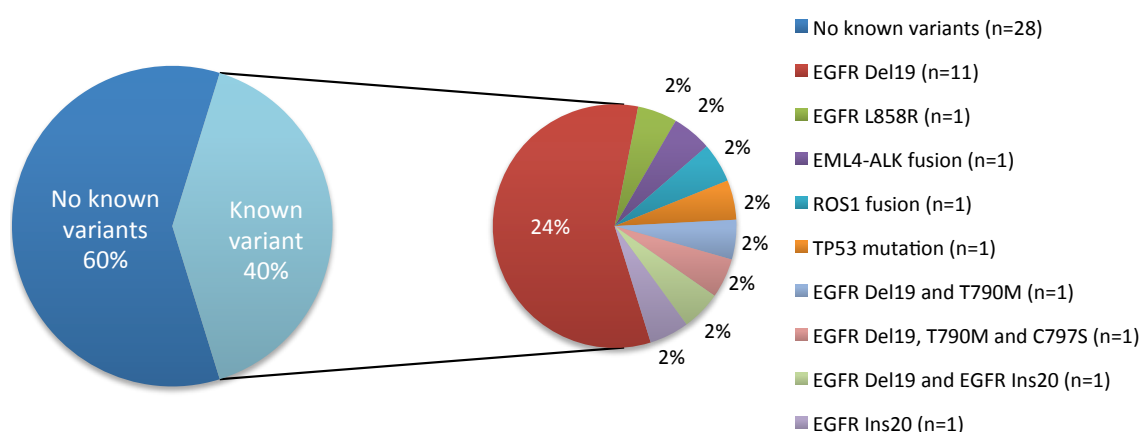
Figure 4.3. Known tissue variants at time of ctDNA NGS request.

4.2.2.1 Utility of ctDNA NGS for diagnosis/target discovery

There were thirty four ctDNA NGS tests in thirty four patients in the diagnosis/target discovery setting. All 34 patients also had tissue genotyping performed as per routine standard of care, including at least *EGFR* and *ALK* testing, and in some cases broader genomic profiling using tissue NGS, either using commercial platforms or in the context of a clinical trial. 31 of the 34 patients had adenocarcinoma histology, two had pleomorphic NSCLC and 1 was squamous cell carcinoma subtype NSCLC.

ctDNA NGS testing was informative in 30 out of 34 patients in the diagnostic setting (88.2%; 95% CI 72.6% to 96.7%). All variants identified by ctDNA NGS in the diagnosis/target discovery setting are shown in Table 4.9. ctDNA NGS identified 17 Tier I variants in 16 patients, 53.3% of informative cases or 47.1% of all 34 cases, including 4 Tier IA variants and 13 Tier IB variants. In one patient, two Tier IB variants were identified, *HER2* exon 20 insertion and *HER2* amplification. The number of patients with a Tier I variant increased by 69% (13 to 22 patients) when ctDNA NGS was performed in addition to tissue testing. 9 Tier IIC variants and 28 Tier IID variants were identified in 19 patients. Median number of variant identified per patient was 2 (range 1-7).

Two patients (5.9%) commenced new treatment paradigms with targeted therapies as a direct result of ctDNA NGS identifying a therapeutically targetable variant not identified on tissue testing: one patient commenced alectinib for *ALK-HIP* fusion to subsequent excellent partial response; the second patient enrolled in a clinical trial of BLU-677 (NCT03037385) targeting *RET* fusion to good partial response. A *RET* fusion was identified on ctDNA NGS in one other patient, who was not eligible for clinical trial entry due to reduced performance status at time of test result. There were five patients in whom ctDNA NGS identified an *EGFR* exon 20 insertion or *HER2* exon 20 insertion, which are currently targetable in context of recruiting clinical trials (NCT03037385, NCT02716116, NCT03318939), but these were not available at the time of testing in the year 2018.

Tier I: Variants of Strong Clinical Significance	Tier II: Variants of Potential Clinical Significance	Tier III: Variants of Unknown Clinical Significance
Level A EGFR Del19 EML4-ALK Fusion ALK-HIP1 fusion EGFR Del19 **Level B** KRAS G12V HER2 G660D HER2 G776_V777delinsAVE EGFR Exon 20 insertion KIF5B-RET Fusion HER2 P780_Y781insGSP (Exon 20 ins) HER2 A775_G776insYVMA (Exon 20 ins) KIF5B-RET Fusion HER2 P780_Y781insGSP (Exon 20 ins) EGFR A767_V769dup (Exon 20 ins) KIF5B-RET fusion KRAS G12V HER2 AMP	**Level C** TP53 R273H NF1 I679fs PIK3CA AMP TP53 Q331 TP53 M246V TP53 R175H TP53 S127F TP53 Y103H BRCA2 N818fs **Level D** PTEN G143fs KIT AMP TP53 A161T APC E1157fs BRAF K601E PTEN P248fs ARID1A Q1573* EGFR AMP TP53 P278L STK11 Q302* EGFR AMP GNAS R201H GNAS R201C TP53 R282W TP53 S90fs ARID1A S1316* CDKN2A Y44* TP53 C238Y TP53 R282W SMAD4 E330K MYC AMP TP53 C277Y AR AMP TP53 H179D CTNNB1 S37F PIK3CA A1066T PIK3CA AMP AR AMP	BRAF R178Q TP53 spl site SNV NF1 H1748R CDK12 S625 APC D971N NF1 N1805D FGFR2 T137P CCND2 Q263* NRAS E132K APC A2608T BRCA2 P1510S MAPK3 T223I MYC G104S FBXW7 *708*† FGFR2 R592H CDH1 F423S ATM Q2442P AR A358P BRCA1 S361P KIT I531I† EGFR D916D PDGFRA V683V† HER2 P780P† TP53 T125T† BRCA1 R866H BRCA2 T2703T† STK11 P324P† BRCA2 V950I EGFR B255Q APC R1105Q SMAD4 S343 PIK3CA L866M BRCA2 A2764G

Table 4.9. All genomic variants identified by ctDNA NGS in the diagnosis/target discovery setting, grouped according to clinical impact category. †Synonymous mutations

ctDNA NGS was non-informative in 4 cases (11.8%), as shown in Table 4.10. All of these cases were patients with limited metastatic disease distribution, including two patients with CNS-only metastases, one patient with small volume intrapulmonary-only disease and one patient with metastatic disease confined to cutaneous scalp lesions. In 3 of these cases, ctDNA NGS failed to identify a known driver variant which had been identified on contemporanous tissue testing, including: a patient with scalp-only metastases and small volume pulmonary disease, identified to

harbour a *KRAS* G12D variant on a tissue NGS panel in the context of a research study (Illumina® Nextera Rapid Capture NGS assay within CRUK SMP2); a patient with small volume pleural disease only, with contemporaneous diagnostic tissue EGFR Roche cobas assay identifying *EGFR* exon 19 deletion and a de novo T790M mutation; and a patient with CNS-only metastases and *EGFR* exon 19 deletion by contemporaneous EGFR Roche cobas on tissue from a diagnostic biopsy of the lung primary tumour.

Case no.	M stage*	Metastatic disease distribution	G360 report date	Paired tissue date**	Paired tissue testing methods	Paired tissue test result	Paired EGFR ctDNA Roche cobas report date	Paired EGFR ctDNA Roche cobas result
7	4B	Intracranial metastasis only	10/06/16	21/07/16	EGFR Roche cobas	No mutation	Not performed	n/a
					ALK, ROS1 and RET FISH	No rearrangement		
					BRAF and KRAS NGS TSCA†	Fail		
8	4B	Cutaneous scalp metastasis only	08/06/16	25/05/16	EGFR Roche cobas	No mutation	08/06/16	No mutation
					ALK and ROS1 FISH	No rearrangement		
					Illumina® Nextera Rapid Capture NGS assay	KRAS G12D mutation		
29	4A	Intrapulmonary metastases only	17/07/17	31/07/17	EGFR Roche cobas	EGFR Del19 + T790M	17/07/17	No mutation
44	4C	Intracranial metastases only	01/03/19	11/02/19	EGFR Roche cobas	EGFR Del19	27/02/19	No mutation
					BRAF Roche cobas	No mutation detected		

Table 4.10. Results of paired tissue genotyping and EGFR ctDNA testing in patients with non-informative ctDNA NGS by G360. *8th addition of the International Union for Cancer Control (UICC) TNM staging staging system for lung cancer. **Date tissue histopathology report issued. † Illumina TruSeq Custom Amplicon. NGS, next generation sequencing.

Case number	M stage*	Metastatic disease distribution	G360 report date	G360 result	Paired tissue date**	Paired tissue test methods	Paired tissue test result	Paired EGFR ctDNA Roche cobas result
12	4B	Intracranial metastasis only	11/02/17	KIT amp	28/06/17	FoundationOne® CDx NGS assay	ROS1-CD74 fusion	Not performed
27	4A	Intrapulmonary metastases only	30/05/17	BRCA2 P1510S	22/06/16	Illumina® Nextera Rapid Capture NGS assay	KRAS G12S mutation BRAF G466V mutation	No mutation
53	4A	Intrapulmonary metastases only	12/08/19	ATM Q2442P BRCA2 T2703T	10/07/19	ALK FISH	ALK rearrangement	Not performed

Table 4.11. Results of paired tissue and EGFR ctDNA Roche cobas testing in patients with informative G360 result but where oncogenic driver mutation was missed by G360. *8th addition of the International Union for Cancer Control (UICC) TNM staging staging system for lung cancer. **Date tissue histopathology report issued. NGS, next generation sequencing.

ctDNA NGS failed to identify an oncogenic driver variant in 3 further cases where this was identified by diagnostic tissue genotyping including: a patient with CNS-only disease shown to harbour a *ROS1* fusion by commercial tissue NGS (FoundationOne® CDx, Foundation Medicine, Inc., Cambridge, MA) from a contemporaneous metastatic resection specimen; a patient with intrapulmonary-only metastases with tissue NGS (Illumina® NGS assay in the context of CRUK SMP2 study) from an archival resection specimen identifying *KRAS* G12S and *BRAF* G466V; and a patient with pleomorphic subtype NSCLC with intrapulmonary-only disease, where contemporanous diagnostic tissue testing confirmed presence of an *ALK* translocation by FISH (Table 4.11.). In each of the three cases, ctDNA NGS was informative, identifying a Tier II variant (*KIT* amplification) in the first case and Tier III variants in the latter two cases (*BRCA2* P1510S and *ATM* Q2442P with *BRCA2* T2703, respectively). Overall, ctDNA NGS failed to identify a known tissue oncogenic driver variant in 6 out of 34 cases (false negative rate of 17.6%; Figure 4.4. left).
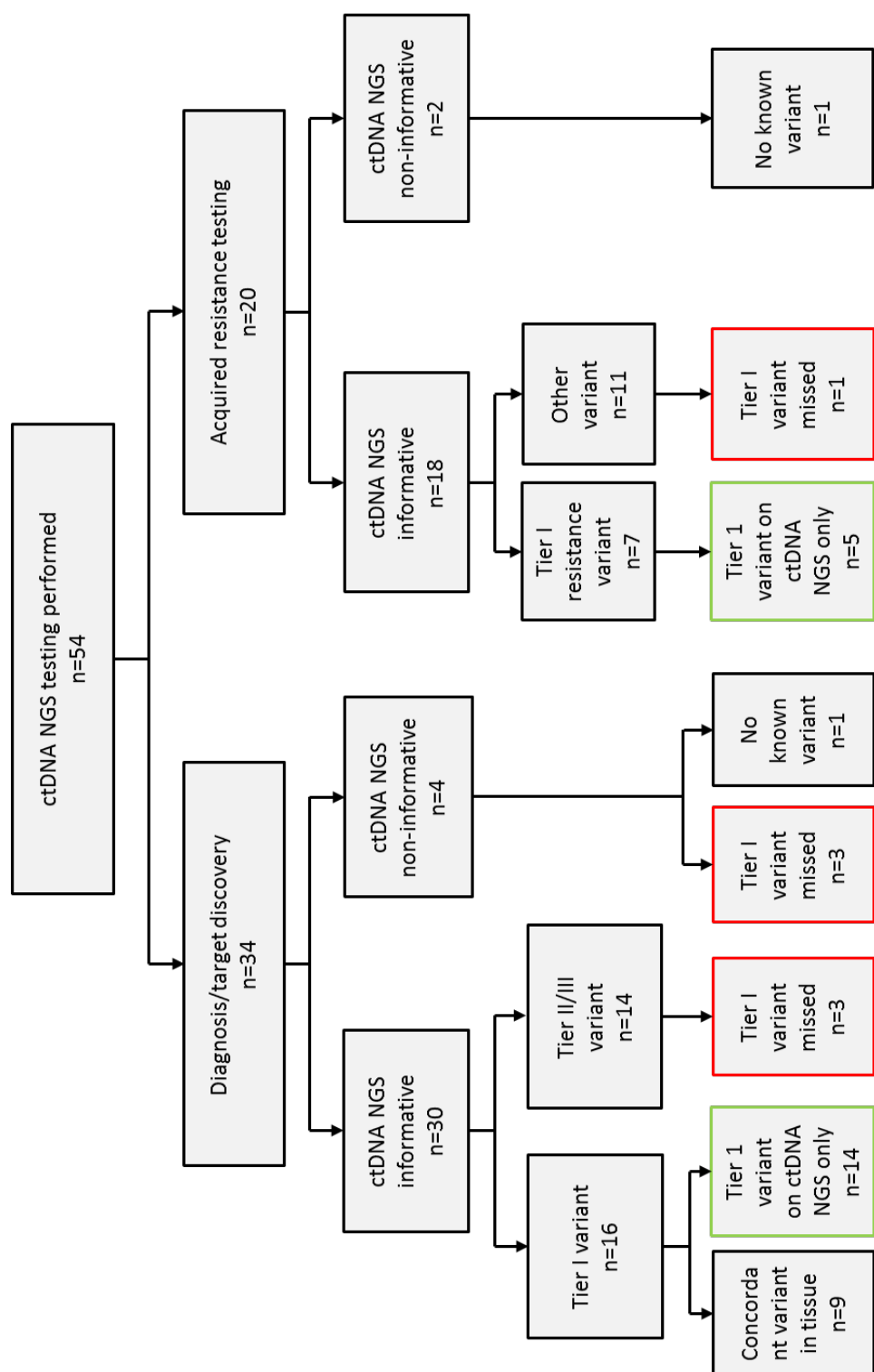
Figure 4.4. Consort diagram. All cases included in analysis.

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4.2.2.2 Utility of ctDNA NGS in acquired resistance setting

Acquired drug resistance mechanism testing by ctDNA NGS was performed on 20 occasions from fourteen patients over the same time period. One patient underwent 4 tests over time, one patient underwent 3 tests, one patient underwent 2 tests and 11 patients had 1 test each.

All patients had adenocarcinoma subtype NSCLC. 11 patients were known to harbour activating *EGFR* mutations, two had a known *ALK* fusion and one had a known *ROS1* fusion. For all but one of the 11 *EGFR* mutant patients, the activating mutation was *EGFR* exon 19 deletion, while the remaining one patient had *EGFR* L858R mutant genotype. All patients had developed progressive disease having received at least one prior line of systemic targeted therapy at point of ctDNA NGS test request.

In the acquired resistance setting, ctDNA NGS testing was informative in 18 out of the 20 cases (90%). All variants identified are shown in Table 4.12.

Tier I: Variants of Strong Clinical Significance	Tier II: Variants of Potential Clinical Significance	Tier III: Variants of Unknown Clinical Significance
Level A	**Level C**	TP53 Splice Site SNV
EGFR Del19	BRAF AMP	PTEN Y240
EGFR Del19 and T790M	PIK3CA E542K	EGFR P1136P
EGFR Del19 and T790M	PIK3CA E545K	RB1 C666
EGFR L858R and T790M	ATM R3008H	BRCA2 N2436N
EGFR Del19 and T790M		MTOR G590C
EGFR Del19 and T790M		TP53 S33S
EGFR Del19 and T790M	**Level D**	NOTCH1 C423C
EGFR Del19	TP53 C238_M243del	CCND2 I287T
EGFR Del19	TP53 G244D	NTRK1 V630M
EGFR Del19	TP53 C238_M243del	FGFR2 R165Q
EGFR Del19	TP53 S127F	KIT L631L
EML4-ALK fusion	EGFR AMP	FGFR2 T333S
EGFR Del19	TP53 C141W	CDK12 I76T
EGFR Del19	EGFR AMP	NOTCH1 N180K
	TP53 R273L	BRAF R271H
Level B	EGFR G724S	
EGFR C797S	CTNNB1 S37F	
	EGFR L792F	
	TP53 C176F	
	CDK4 AMP	
	AR AMP	

Table 4.12. Variants identified by G360 ctDNA NGS in the acquired resistance setting

Of the 20 cases, half had contemporaneous paired tissue molecular genotyping. These were all *EGFR* mutant patients and tissue testing was usually by EGFR Roche cobas assay. In 14 of the 20 cases, contemporaneous ctDNA *EGFR* single gene testing by EGFR Roche cobas was also performed (Figure 4.5). Of the 10 cases with no contemporaneous tissue testing, in 7 cases progressive disease was non-biopsiable, in 2 cases patient was too unwell to undergo rebiopsy and in 1 case G360 result confirmed T790M resistance mutation while patient was awaiting tissue rebiopsy, therefore clinical decision was made not to proceed.
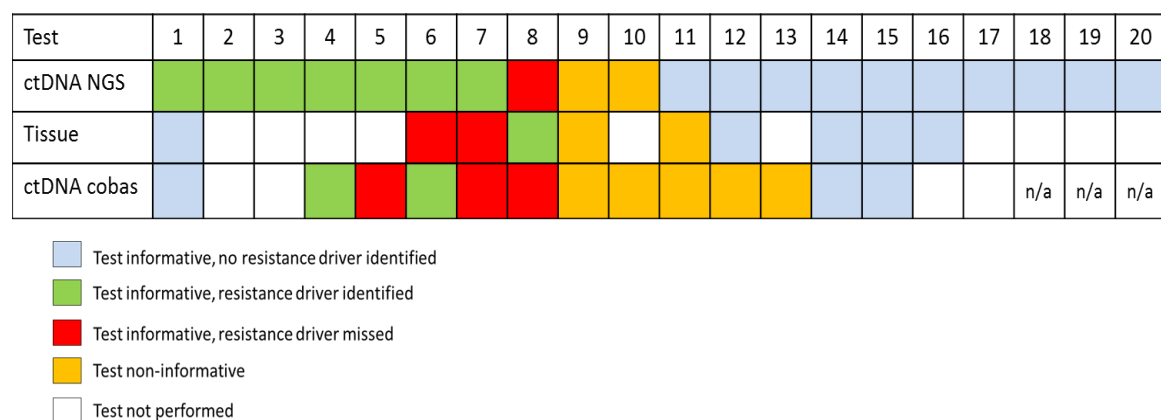
Figure 4.5. Heat map of paired testing by Guardant360® CDx ctDNA NGS, tissue and ctDNA EGFR Roche cobas in the acquired resistance setting. Test 1-17 performed in EGFR+ patients; Test 18,19, 20 performed in ALK+ and ROS1+ patients.

ctDNA NGS identified a Tier I resistance variant in 7 out of 20 cases (35%), compared to 3 out of 20 cases (15%) with standard of care testing alone, although this difference did not reach statistical significance (Chi-square 2.133, p=0.1441). ctDNA NGS identified a *EGFR* T790M acquired resistance mutation in 6 cases. There was one case where *EGFR* exon 19 deletion was confirmed with no T790M (concordant with ctDNA and tissue EGFR Roche cobas) but where ctDNA NGS additionally identified a *BRAF* amplification as a potential bypass track resistance mechanism. In three cases where ctDNA NGS identified a T790M mutation that was not identified on contemporaneous tissue or ctDNA EGFR cobas testing, T790M was present at very low VAF (VAFs of 0.2%, 0.4% and 0.6%).

In further 11 tests from 7 patients, ctDNA NGS was informative and confirmed a known Tier I oncogenic driver mutation in 7 cases (6 cases with *EGFR* exon 19 deletion and 1 case with *ALK* rearrangement). In all but one of these cases paired tissue and ctDNA *EGFR* testing, where performed, also failed to identify an acquired resistance variant. In one case of a patient with small volume intrapulmonary progressive disease, paired tissue testing by EGFR Roche cobas assay from a CT-guided lung rebiopsy identified an acquired T790M mutation, which was not identified on contemporaneous ctDNA NGS testing, representing a false negative result.

ctDNA NGS was non-informative in 2 cases in the acquired resistance setting, both in patients with a known EGFR exon 19 deletion identified at diagnosis. In one EGFR+ patient, contemporaneous tissue and ctDNA EGFR Roche cobas testing both showed wildtype *EGFR*; in the second case, paired tissue testing was not performed, with *EGFR* wild type by contemporaneous ctDNA EGFR cobas. In 2 further cases ctDNA NGS was informative but did not identify the original oncogenic driver mutation identified at diagnosis, an EGFR exon 19 deletion in one case and ALK fusion in another. In the EGFR+ patient, disease progression was evident only in a solitary CNS site and tissue sampling for repeat *EGFR* testing was not possible. Paired ctDNA EGFR testing by Roche cobas identified no *EGFR* mutations. However CSF was obtained, and following DNA extraction and *EGFR* testing using Roche cobas EGFR assay, *EGFR* exon 19 deletion was detected. No contemporaneous tissue testing was performed in the ALK+ patient. The above 4 cases are summarised in Table 4.13.

Overall, in 9 out of 13 cases where ctDNA NGS did not identify an acquired resistance variant, the predominant pattern was one a limited extent of disease progression, including intrapulmonary-only progression, CNS oligoprogression, and one case each of progression in a solitary liver lesion and bone-only progressive disease.

Case number	M stage*	Oncogenic driver at diagnosis	Site of progressive disease	G360 report date	G360 result	Paired tissue date**	Paired tissue testing result	Paired EGFR ctDNA cobas date	Paired EGFR ctDNA cobas result
5	4C	EGFR Del19	Intrapulmonary and abdominal soft tissue	13/07/17	Non-informative	02/08/17	EGFR wild type	17/07/17	EGFR wt
20	4C	EGFR Del19	Intrapulmonary and pleural	21/04/18	Non-informative	n/a	Not performed	27/03/18	EGFR wt
3	4C	EGFR Del19	Intracranial oligoprogression	21/11/17	EGFR P1136P mutation	n/a	Not performed†	12/09/17	EGFR wt
51	4C	ALK fusion	Bone	27/06/19	NTRK1 V630M mutation CDK12 I76T mutation BRAF R271H mutation	n/a	Not performed	n/a	n/a

Table 4.13. Summary of 4 cases where ctDNA NGS did not identify the original driver variant when performed at the time of acquired resistance to first line TKI therapy. †In this patient, contemporaneous testing of CSF fluid by Roche cobas EGFR assay confirmed presence of *EGFR* exon 19 deletion.

4.2.2.3 Timelines

For all 54 cases, median time from date of ctDNA NGS blood sampling to date of report issue was 10 days (range 6-19). For 30 cases where paired ctDNA EGFR Roche cobas test was performed and reported, median time from blood sampling to report was 8 days (range 2-25, Figure 4.6(a)). There was no significant difference between median TATs for ctDNA NGS and ctDNA EGFR Roche cobas (median 10 vs. 8 days, p=0.1945).

For the ten cases in the acquired resistance setting where paired tissue genotyping was performed contemporaneous with ctDNA NGS (defined as tissue sampling within 16 weeks of ctDNA NGS blood sampling, with no intervening change in therapy), median time from tissue sampling request to molecular report issue was 33.5 days (range 8-57), while median time from tissue acquisition to tissue molecular report was 18 days (range 8-47, Figure 4.6(b)). Median TAT for ctDNA NGS was significantly shorter than for tissue (median 8 vs. 18 days, p=0.013).

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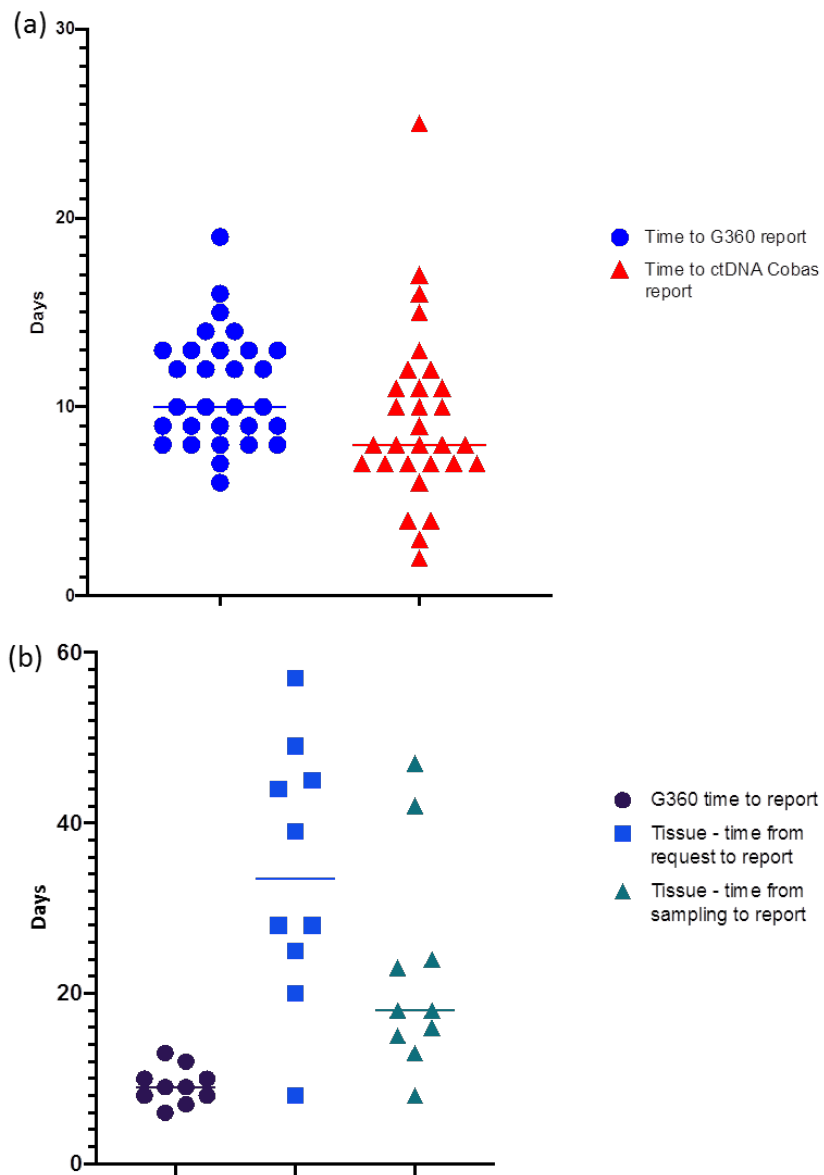
Figure 4.6. Comparison of timelines in days for: (a) blood sampling to molecular report for paired Guardant360®
CDx and ctDNA EGFR cobas (n=30); (b) tissue genotyping request to report and tissue sampling to report for
paired Guardant360® CDx and tissue EGFR Roche cobas in acquired resistance setting (n=10).

4.2.3 Discussion

This is a retrospective analysis of real-world clinical utility of comprehensive ctDNA-based NGS molecular profiling using Guardant360® CDx assay, alongside standard-of-care methods of tissue and ctDNA molecular genotyping, for target discovery and acquired resistance mechanism identification in metastatic NSCLC.

ctDNA NGS testing was informative in 88.2% and 90% of cases in diagnostic and acquired resistance settings, respectively. A Tier I variant was identified in 47.1% (16 out of 34) cases in the diagnosis/target discovery setting, and the number of Tier I variants identified increased by 69% (13 to 22 pts) when ctDNA NGS was performed in addition to tissue testing. The primary reason for this in our cohort of patients appears to be the greater breadth of variants included in the G360 ctDNA NGS panel compared with standard of care testing, with several patients identified to harbour Tier 1 *HER2* and *RET* variants, for instance, which were not tested for on tissue in the majority of cases. However, there were also two cases of tissue "false negatives", including where ctDNA NGS identified an *EGFR* exon 20 insertion (VAF 1.4%) where *EGFR* was previously reported as wild type on tissue testing, and one case where an *ALK* fusion was identified on ctDNA NGS in a patient where tissue *ALK* testing failed. In the setting of acquired resistance to prior TKI therapy, ctDNA NGS identified a resistance variant in 35% of cases, compared with 15% by standard of care testing alone, although this difference was not statistically significant (p=0.1441). Overall, 78% of non-informative or false negative ctDNA NGS tests occurred in patients with pulmonary-only or CNS-only disease. Time to ctDNA NGS report was significantly shorter than time to tissue genotyping report for those cases where paired contemporaneous testing was performed (median 8 vs. 18 days, p=0.0013).

These results are in line with trial data and other studies of ctDNA utilization, with reported additional clinical utility of ctDNA NGS over tissue testing alone of between 17% and 74%.[163, 164, 256] In the NILE study,[159] a prospective cohort of 282 previously untreated advanced NSCLC patients underwent ctDNA NGS genotyping by Guardant360® CDx along with standard of care tissue genotyping at physicians discretion in the diagnostic setting. Performing ctDNA NGS in addition to tissue,

increased the detection of 8 biomarkers (*EGFR, ALK, ROS1, BRAF, RET, HER2, MET* amplification and *MET* exon 14 skipping mutations) by 48%, from 60 to 89 patients. This included all patients with negative, not assessed or insufficient tissue results, comprising over three quarters of the study population, with only 64 patients having attempted tissue genotyping of all 8 biomarkers. When analysis was restricted to these 64 patients, the total number of biomarkers identified was the same for tissue and ctDNA NGS (22 biomarkers), however each had false negative results (3 in each group). In our study, false negatives were also observed both in tissue (1 in diagnostic setting and 2 in acquired resistance setting) and plasma NGS (6 patients in diagnostic setting and one in acquired resistance setting). As discussed earlier in the chapter, false negative results in tissue may be due to tumour heterogeneity and assay limit of detection. Causes for false negative results in plasma may include low rate of ctDNA shedding in some patients, with the finding of lower plasma NGS sensitivity in patients with limited metastatic disease distribution also borne out here, with more than two thirds of all non-informative/false negative ctDNA NGS tests occurring in patients with intrapulmonary-only or CNS-only metastatic disease. Others have found that technical factors specific to different ctDNA NGS assays may contribute to false negatives, such as those related to bioinformatics filtering processes which may exclude variants with variant allele fraction (VAF) below bioinformatic calling thresholds, those associated with high background noise or variants considered to be germline based on high VAF.[257] Conversely, false positives in plasma could be attributed to some variants being identified as tumour-derived but which are in fact non-malignant mutations harboured by hematopoietic cells, known as clonal haematopoiesis of indeterminate potential (CHIP).[258, 259] CHIP is an age-related phenomenon of clonal expansion of a distinct subpopulation of haematopoietic stem cells or other early progenitor blood cells with presence of one or more somatic mutations. It has been reported in as many as 25% of patients with non-haematological malignancies, including in lung cancer-associated oncogenes such as *KRAS*, *TP53* and *JAK*.[260] Caution should therefore be exercised in interpreting sequencing results when only ctDNA or only tissue is used without matched samples, due to possible clonal haematopoiesis variants confounding the results, with false positives potentially leading to misguided clinical management. However, this can be

mitigated by examining the VAFs of these variants CHIP variants generally have very low VAFs (<1%).[257, 258]

The main limitation of this data is the relatively small numbers of patients, reflecting the non-standard-of-care use of ctDNA NGS in the UK, with a highly selected patient population included in the study, which is likely enriched for those with therapeutically targetable molecular variants. This real-world data provides important additional evidence for the complementary role of ctDNA NGS when used with current standard-of-care tissue and ctDNA molecular profiling technologies, by increasing the proportion of patients identified with actionable genomic variants in a rapid and minimally invasive manner, while appropriate patient selection is key to ensure maximal clinical benefit, reduce false negatives and optimise use of resources. It also shows that commercial ctDNA NGS assay is quicker that tissue NGS and suggests that if a tier 1 variant is identified on ctDNA NGS additional tissue NGS may not be required due to mutual exclusivity.

Chapter 5 Current updates and future directions

Over the past decade and a half, the pace and scope of advances in the treatment of advanced lung cancer have been extraordinary and have transformed the landscape of systemic therapies for NSCLC, from ever increasing range of molecular targets and licenced targeted therapies, to immune-checkpoint inhibitors becoming standard of care for most patients at some stage during their treatment for advanced NSCLC, and the increasing sophistication, speed and cost effectiveness of technologies for molecular characterization of NSCLC tumours. Since the work presented in this thesis was performed, the field has moved forward significantly in many areas. For instance, next generation sequencing is now used as standard in the UK and globally, immune-checkpoint inhibitors in combination with chemotherapy with and without anti-angiogenic agents have become standard of care in the first-line setting, and the role of ctDNA genotyping for target identification and on acquired resistance development is increasingly promising. Furthermore, experimental strategies for discovery and development of novel therapies have shifted from the traditional sequential approaches where small phase I trials are followed by larger randomized phase II followed by very large randomized phase III trials, looking for small median OS benefits. Instead, molecularly targeted drugs are now being licensed by the FDA on the basis of results from expanded cohorts in phase I trials, particularly for rare subgroups which will never enter randomized phase III testing, as was the case for crizotinib in ROS1-positive NSCLC and larotrectinib and entrectinib for NTRK fusion-positive solid tumours, with researchers and regulators looking for larger magnitude of benefit, for instance ORRs of 60% or greater. For immune-checkpoint inhibitors, trials have moved to very large phase I trials with multiple expansion cohorts of different clinical setting, with findings taken directly to phase III, such as the phase I trials of pembrolizumab (KEYNOTE-001), nivolumab (CheckMate012) and atezolizumab (NCT01375842).

In this chapter I will present the latest updates and discuss the emerging and future directions in the arena of systemic therapies for advanced non-small cell lung cancer.

5.1 Advanced NSCLC and post-ICI landscape

With the integration of ICIs as a standard treatment option for most patients with advanced NSCLC in the first-line setting, either as single agent, in combination with chemotherapy or as ICI-ICI combos, the key future challenges surround the question of ICI resistance and post-ICI therapeutic strategies, including optimal sequencing of ICI/chemotherapy, understanding the mechanisms of primary and secondary resistance to ICIs and developing strategies for overcoming ICI resistance, identifying new and improved predictive biomarkers of ICI response and developing novel post-ICI treatment regimens. Prospective clinical studies are required and in some cases are ongoing in order to answer these questions.

Various mechanisms of ICI resistance have been postulated and described, with the constantly evolving interactions of immune cells and other components of the tumour microenvironment (TME) playing a central role.[261, 262] Tumour infiltrating lymphocytes (TILs) are the key effectors of the anti-tumour immune response in the TME. The levels of TIL activity and extent of tumour infiltration can vary significantly between so called "hot" and "cold" tumours and have been associated with differential ICI efficacy.[263] Combination ICI strategies are being developed to convert cold into hot tumours and potentiate anti-tumour immune responses.[264] New immune checkpoints such as LAG-3, TIM-3 or TIGIT are being identified and investigated as drug targets in pre-clinical and clinical trials, primarily in combination with anti-PD-1/anti-PD-L1 inhibition in NSCLC (ClinicalTrials.gov Identifier: NCT02750514; NCT02817633; NCT04619797) with anti-TIGIT monoclonal antibody tiragolumab now in phase III development (ClinicalTrials.gov Identifier: NCT04294810) having recently been granted FDA breakthrough therapy designation based on the primary analysis of results from the phase II CITYSCAPE study, a randomized double-blind placebo-controlled trial showing improved ORR and median PFS for tiragolumab in combination with atezolizumab versus atezolizumab and placebo, in previously untreated PD-L1-positive metastatic NSCLC.[265] As a consequence multiple tiragolumab trials, as well as trials of other anti-TIGIT monoclonal antibody drugs, have been launched in different indications (e.g. SKYSCRAPER-01 in NSCLC (NCT04294810) and SKYSCRAPER-02 in extensive-stage small cell lung cancer (NCT04256421)).

The number of non-synonymous single nucleotide variants in a tumour, referred to as the tumour mutation burden (TMB), has long been hypothesized as a likely predictor of response to ICIs, based on the observation that some of the best initial ICI responses are observed in carcinogen-driven cancers such as melanoma and NSCLC that typically have a higher burden of mutations, and that primary targets of many human tumour immune responses are tumour-specific neo-antigen peptides which arise from somatic mutations in cancer genomes. However, clinical trials have reported divergent results with some demonstrating a strong link between high TMB and responses particularly to combined anti-PD-L1 and anti-CTLA-4 therapy independent of PD-L1 expression,[266] while others showed no correlation between treatment responses and clinical outcomes and TMB.[19] This could be the result of inconsistencies in the measuring and reporting of TMB, with use of different cut-offs and different methodologies for TMB assessment, but may also result from variable immunogenicity and antigenicity of different neo-antigens, with some evidence suggesting that only clonal TMB predicts for ICI response whereas subclonal TMB does not.[267] Nevertheless, FDA has recently approved the use of pembrolizumab in all advanced solid tumours with high TMB, defined as ≥10 mutations/megabase, after progression on at least one prior therapy, based on the results of the single arm phase II KEYNOTE-158 study which showed that the subgroup of patients with high TMB (102 patients or 13% of the study population) had significantly higher response rates than non-TMB-high patients (29% vs. 6%) regardless of tumour site. Furthermore, blood-based assessment of TMB (bTMB) has been prospectively validated as a predictive biomarker of ICI efficacy in NSCLC. B-F1RST, a phase II trial of atezolizumab in advanced first-line NSCLC patients unselected for PD-L1 expression, found that patients with high bTMB by FoundationOne ctDNA panel (using ≥16 mutations/megabase as cut-off) had a higher ORR (28.6% vs. 4.4%), although no statistically significant differences in PFS and OS were detected.[268] In the exploratory analysis of MYSTIC trial, a phase III trial of durvalumab and tremelimumab in first-line metastatic NSCLC which did not meet its primary efficacy endpoints, high bTMB of ≥20 mutations/Mb was identified to confer improved outcomes including OS benefit for durvalumab and tremelimumab versus chemotherapy in this subgroup (mOS 21.9 vs. 10.0 months; HR, 0.49; 95% CI, 0.32-

0.74). Blood-based TMB is also being used to study dynamic on-treatment TMB changes as a potentially stronger predictor of response over single pre-treatment TMB measurement.[269, 270] Strategies to increase immunogenicity by increasing the TMB and mutation burden using PARP inhibitors together with immunotherapy are also under investigation in phase II studies such as the multi-arm JASPER trial of niraparib in combination with anti-PD-1 inhibitors pembrolizumab or dostarlimab (NCT03308942) and the umbrella HUDSON trial of olaparib with anti-PD-L1 inhibitor durvalumab after progression on prior anti-PD-1/PD-L1 therapy (NCT03334617).

Other strategies to overcome ICI resistance include combining ICIs with metabolic targets, such as arginase inhbitors (NCT02903914) and adenosine signaling pathway inhibitors (HUDSON, NCT03334617; COAST, NCT03822351), while development of initially promising IDO-1 inhibitors in combination with ICIs halted after disappointing results in phase III testing in melanoma.[271] Strategies of combining ICIs with anti-angiogenic agents are also ongoing, in a progressively increasing number of studies. Nintedanib is being investigated in combination with ipilimumab and nivolumab in a phase I/II trial including cohorts of treatment-naïve and previously-ICI exposed patients (ClinicalTrials.gov Identifier: NCT03377023). Following the benefit demonstrated in the phase III IMpower-150 trial in PD-L1 unselected NSCLC, bevacizumab is now being studied in combination with atezolizumab versus atezolizumab alone in first-line advanced NSCLC with PD-L1 ≥1% in the phase II BEAT trial (NCT03896074) and in combination with atezolizumab and chemotherapy in EGFR+ NSCLC after progression on TKI therapy in a phase II trial (NCT03786692). Ramucirumab is being studied in phase II trials in combination with atezolizumab (RamAtezo-1; NCT03689855) and in combination with nivolumab (NCT03527108) after progression on prior ICIs, after demonstrating durable responses in combination with pembrolizumab in a cohort of NSCLC patients in a phase Ia/b JVDF trial (mPFS 9.7 months, mOS 26.2 months).[272] Other multi-targeted agents with anti-angiogenic acitivity, such as anlotinib and lenvatinib are also being combined with ICIs with success. Results from the safety run-in of the phase III LEAP-006 study of lenvatinib, receptor tyrosine kinase inhibitor of VEGFR 1–3, FGFR 1–4, PDFGFR α, c-KIT, and RET, with pembrolizumab and platinum-based chemotherapy in first-line advanced non-squamous NSCLC were presented at the 2020 European Society of

Medical Oncology (ESMO) annual meeting, showing ORR of 69% with the 4 drug combination.[273] LEAP-008 is a phase III study of lenvatinib with pembrolizumab after prior immunotherapy and chemotherapy, with docetaxel as a comparator (NCT03976375). Sitravatinib, a receptor tyrosine kinase inihibitor of VEGFR, PDGFR, c-KIT, MET, and the TAM family of receptors (TYRO3, AXL, and MER), is being combined with nivolumab in the phase III SAPPHIRE trial (NCT03906071), randomized versus docetaxel, in NSCLC patients previously treated with platinum-based chemotherapy and immunotherapy, after the combination showed clinical activity in this setting in a phase II study.[274, 275]

Development of reliable biomarkers of response to post-ICI therapy remains an area of significant research need, with some hypothesis generating studies proposing dynamics of neutrophil-to-lymphocyte ratio and absolute neutrophil counts as possible predictors of response to salvage chemotherapy after ICIs, with others working to develop a clinical "post-ICI" score to identify the patients most likely to benefit, however validation of these approaches in larger prospective trials is required.[276, 277]

5.2 Precision medicine and advanced NSCLC

In 2014, ESMO published a position paper on the delivery of precision medicine in oncology, defined as the "the use of an individual patient's molecular information (including genomics and proteomics) to inform diagnosis, prognosis, treatment and prevention of cancer for that patient".[278] With the era of stratified medicine evolving into personalized or precision medicine, the paper identified the key challenges ahead including tumour heterogeneity, molecular evolution and drug resistance; the need for technical feasibility, validation, standardization and reproducibility of increasing numbers of biomarkers; availability of suitable biological material; the need for effective information technologies to allow integration and interpretation of large volumes of genomic data; and considerations around value and cost-effectiveness. It also recognized that as more biomarkers become identified and clinically actionable, multiple single gene tests would become unfeasible, requiring a move away from the single-diagnostic/single-drug paradigm and instead towards full molecular characterisation of tumours using multi-gene assays. In 2018, the ESMO

Scale for Clinical Actionability of Molecular Targets (ESCAT) was published to provide a systematic framework for ranking of molecular targets based on evidence supporting their utility as clinical targets,[279] and in August 2020, the ESMO Precision Medicine Working Group published a report on the use of next generation sequencing of metastatic cancers which recommended routine use of tumour multi-gene NGS in NSCLC, cholangiocarcinoma, prostate and ovarian cancers.[280] In NSCLC, the recommendations stated that profiling using NGS could be performed on tumour or plasma samples from patients with advanced non-squamous NSCLC in order to detect level I alterations (defined as a target suitable for routine use and alteration-drug match is associated with improved outcome in clinical trials), while centres that run drug development programs and clinical trials should run multi-gene sequencing in the context of molecular screening programmes, to identify level II–IV alterations with aim to accelerate cancer research and drug development through clinical trials, provide access to innovation to patients and to collect data (Table 5.1).

NGS is now routinely commissioned by the NHS in England with the 2020/2021 National Genomic Test Directory for Cancer setting out the genomic tests, the technology by which they are available, and the patients eligible to access the test.[244] The number of commissioned genomic tests is likely to continue to expand with increasing evidence for targeting of rarer alterations in NSCLC such as those involving *MET*, *RET*, *PIK3CA* and *HER2*, common but previously not actionable alterations such as *KRAS G12C*, and development of pan-tumour diagnostics such a *NTRK*.

Gene	Alteration	Prevalence	ESCAT
EGFR	Common mutations (*Del19, L858R*)	15% (50%–60% Asian)	IA
	Acquired *T790M* exon 20	60% of *EGFR* mutant NSCLC	IA
	Uncommon *EGFR* mutations (*G719X* in exon 18, *L861Q* in exon 21, *S768I* in exon 20)	10%	IB
	Exon 20 insertions	2%	IIB
ALK	Fusions (mutations as mechanism of resistance)	5%	IA
	Mutations *ex 14 skipping*	3%	IB
MET	Focal amplifications (acquired resistance on EGFR TKI in *EGFR*-mutant tumours)	3%	IIB
BRAF[V600E]	Mutations	2%	IB
ROS1	Fusions (mutations as mechanism of resistance)	1%–2%	IB
NTRK	Fusions	0.23%–3%	IC
RET	Fusions	1%–2%	IC
KRAS[G12C]	Mutations	12%	IIB
ERBB2	Hotspot mutations Amplifications	2%–5%	IIB
BRCA 1/2	Mutations	1.2%	IIIA
PIK3CA	Hotspot mutations	1.2%–7%	IIIA
NRG1	Fusions	1.7%	IIIB

Table 5.1. List of genomic alterations level I/II/III according to ESCAT in advanced non-squamous non-small-cell lung cancer (NSCLC). Reproduced with permission from Mosele F. et al., Annals of Oncology, Volume 31, Issue 11, 1491 – 1505.

Recently, selective inhibitors of MET capmatinib and tepotinib have been approved in the US and Japan for the treatment of metastatic NSCLC patients whose tumours have a mutation leading to *MET* exon 14 skipping. In the phase II GEOMETRY mono-1 trial, which included cohorts of patients with *MET* exon 14 skipping mutation or *MET* amplification, capmatinib demonstrated an ORR of 68% and median PFS of 12.4 months in previously untreated patients with *MET* exon 14 skipping mutations, while efficacy was more limited in previously treated patients and those with *MET* amplifications.[281] Good intracranial activity was also demonstrated with half of patients who had baseline brain metastases demonstrating a partial response and nearly all achieving disease control. There was a 99% concordance between reverse transcriptase PCR (RT-PCR) analyses and next-generation sequencing using the tissue-based FoundationOne CDx panel. In February 21, the FDA granted accelerated approval for tepotinib for both treatment naïve and previously treated *MET* exon 14 skipping mutation positive NSCLC, based on results of the open-label phase II VISION trial where tepotinib demonstrated an ORR of 43% and durable responses in both patient groups.[282] In this study patients underwent molecular profiling on tissue (using the RNA-NGS Oncomine Focus Assay) or plasma (using Guardant360 NGS panel) or both, as well as molecular response monitoring by cfDNA. No responses were observed in patients with concomitant activating point mutations in *PI3KCA*, *KRAS*, *NRAS* or with inactivating mutations in *PTEN* at baseline, comprising 12% of overall study patient population, suggesting a potential primary resistance mechanism involving the RAS–RAF and PI3K–AKT pathways, which have previously been associated with MET inhibitor resistance.[283, 284] For 51 patients who had matched baseline and on-treatment cfDNA profiling, 27 patients had a complete molecular cfDNA response (defined as 100% depletion of MET exon 14 alterations in cfDNA i.e. no detection) and 7 had a deep molecular response (>75% but <100% depletion). Among the patients with a molecular cfDNA response, 71% also had a radiographic response and 88% had disease control, while 4 patients had disease progression. In those patients where an increase from baseline in the frequency of MET exon 14 variant was observed, only 1 (10%) had a response. Overall, there was

a high level of concordance between molecular and clinical RECIST-based responses. This study illustrates the promising strategies of using broad NGS profiling to identify cohorts of patients who are less likely to benefit due to primary resistance, and use of minimally invasive plasma-based molecular monitoring of disease response, but further understanding of reasons for incomplete concordance between molecular and clinical responses and how these methods could be used in routine clinical practice is required.

Pan-tumour or tumour-agnostic therapeutic and diagnostic strategies have emerged as a radical new approach in oncology over the last few years, whereby tumour genomic profile supersedes tumour histology, ever since pembrolizumab in 2017 became the first anti-cancer agent in history to be approved for treatment of adult and paediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumours that have progressed after prior therapy and who have no suitable alternative treatment options. Since then two further agents have received tumour-agnostic approvals, tropomyosin receptor kinase (TRK) inhibitors larotrectinib and entrectinib, both for the treatment of patients with metastatic *NTRK* gene fusion positive tumours and no other alternative treatment options. All three received approvals based on pooled data from several phase I/II trials (KEYNOTE-164 and KEYNOTE-158 for pembrolizumab; LOXO-TRK-14001, NAVIGATE and SCOUT for larotrectinib; ALKA-372-001, STARTRK-1 and STARTRK-2 for entrectinib) highlighting increasing importance of basket trials in cancer drug development, enabling investigation of therapeutics for rare tumour types and less common genotypes of common cancers. There are several pan-tumour therapies currently in development in basket trials, such as repotrectinib in patients with advanced solid tumours with *ALK*, *ROS1*, or *NTRK1-3* rearrangements (TRIDENT-1 trial, NCT03093116); selpercatinib (LIBRETTO-001 trial, NCT03157128) and pralsetinib (ARROW trial, NCT03037385) for *RET*-fusion positive solid tumours; Debio1347 in solid tumours harbouring *FGFR* gene fusions (FUZE trial, NCT03834220); PLX8394 in advanced *BRAF*-mutated solid tumours (NCT02428712). Such studies will allow identification of early signals of efficacy while recruiting relatively small numbers of patients from each solid tumour, however

future challenge remains validation of these efficacy signals in larger later-phase randomized trials, including identification of suitable controls across disparate patient groups, while post-approval adoption of these agents depends on integration of relevant molecular tests into standard diagnostic protocols to ensure target populations are reached.

5.3 Plasma-based genotyping - future directions in NSCLC

As a result of studies such as AURA, FLAURA, NILE and BFAST, plasma-based molecular genotyping has been established as complementary to tissue genotyping in NSCLC.[157, 160, 162, 163] Disadvantages of plasma-based genotyping, such as higher rates of false negatives previously demonstrated compared with tissue, are being addressed by ongoing technological developments with improved ability to detected lower mutant allele fractions, and together with advantages of being minimally invasive and not relying on availability of suitable and sufficient tissue, applications of plasma NGS are a focus of ongoing intensive research. Application of nationwide plasma-based molecular screening projects for efficient genomic screening and identification of rare or novel molecular targets is being tested in trials such as Japan's LC-SCRUM.[285] Initially established primarily to screen *ALK*, *RET* and *ROS1* fusions using RT-PCR and FISH in advanced non-squamous NSCLC without EGFR mutations, the project was expanded in 2015 to an academic-industrial collaboration with broader eligibility criteria and tissue sample analysis by next-generation sequencing, followed by introduction of plasma-based screening using the Guardant360 panel (LC-SCRUM-Liquid) in December 2017. Overall, over 7,700 patient have been enrolled and a large concordance study between tissue and liquid NGS analysis has been performed in 2,000 patients, with results awaited. Large trials of plasma-based molecular profiling are also ongoing in other tumour types such as the plasmaMATCH trial (NCT03182634), a UK multi-centre multi-arm open-label phase II trial in advanced breast cancer matching designated targeted therapies with targetable mutations identified through ctDNA screening.[286]

As well as target identification in advanced NSCLC, utility of ctDNA is also under investigation for monitoring of response to treatment in oncogene addicted

metastatic NSCLC, for identification of responders to ICI therapy (as discussed earlier), in early NSCLC for detection of minimal residual disease (MRD) after resection and identification of patients at increased risk of disease recurrence, and in lung cancer screening.

In the oncogene-addicted metastatic NSCLC setting, longitudinal ctDNA monitoring and dynamics of ctDNA changes are being evaluated as a predictive biomarker of response and for early detection of progression on TKI therapy. In the AURA3 trial, early clearance of EGFR mutations, as assessed by digital droplet PCR or the Guardant360 ctDNA NGS assay, after three weeks of treatment with osimertinib was associated with a longer median PFS (10.9 vs. 5.7 months) and a higher ORR (81% vs. 50%).[287] Similarly, in the FLAURA trial, early ctDNA clearance was associated with longer PFS for both osimertinib and comparator EGFR TKIs.[162] ctDNA dynamics were also evaluated in ALK positive NSCLC, in a cohort of 92 patients treated with ALK-TKIs including crizotinib, alectinib, ceritinib and brigatinib.[288] ctDNA analysis was performed using Guardant360 NGS assay at baseline, 2 months into treatment and at progression. Absence of detectable ctDNA at baseline was associated with significantly longer progression-free survival (mPFS 36.1 vs. 11.6 months, HR 0.432, p=0.004) and overall survival (mOS NR vs. 27.9 months, HR 0.418, p = 0.034), while patients with clearance of ctDNA at two months had significantly longer PFS and OS than those without clearance (n=29 vs. n=22; mPFS 25.4 vs 13.9 months, HR 0.343, p=0.030; mOS NR vs. 25.7 months, HR 0.173, p = 0.035), suggesting a role for ctDNA as both a prognostic and predictive biomarker.

Based on studies documenting marked differences between pre- and post-surgical levels of ctDNA in resectable NSCLC,[289, 290] several studies have investigated the role of ctDNA for early detection of relapse after surgery, utilizing both "tumour-informed" and "tumour-uninformed" methods. In the TRACERx study, whole exome sequencing of multiple regions of resected tumour was used to identify and select patient-specific clonal and sub-clonal single nucleotide variants (SNVs) and create a bespoke multiplex-PCR assay-panel for each patient, which were then used for post-operative longitudinal ctDNA monitoring.[291] There was strong correlation between post-surgical ctDNA detection (defined as detection of at least 2 pre-defined SNVs)

and radiological relapse, with 13 out of 14 patients having detectable ctDNA before or at the time of relapse, with median interval of 70 days between first ctDNA detection and radiological relapse. In patients who received adjuvant chemotherapy, ctDNA profiling also appeared to reflect resistance to adjuvant treatment, with 3 patients in whom there was a continuing increase in detectable ctDNA SNVs despite adjuvant chemotherapy relapsing within 1 year of surgery, while one patient with detectable ctDNA post-operatively but decreasing to undetectable post-adjuvant chemotherapy remained disease free at 2 years. Simultaneously, Chaudhuri et al performed ctDNA monitoring by a cancer personalized profiling by deep sequencing (CAPP-seq) assay after surgery in 37 patients with stage I to III resectable NSCLC.[292] Detectable ctDNA was present in 20 out of 37 patients all of whom developed relapsed disease. Patients with undetectable ctDNA within 4 months after surgery had a significantly higher relapse-free survival and overall survival than those with detectable ctDNA. While these are promising strategies, questions remain surrounding feasibility and cost-effectiveness of personalised ctDNA assays, clinical utility of earlier treatment of relapsed disease in the routine clinical setting, the number of mutations needed for detection to avoid false negatives, as well as risk of false positives due to processes such as clonal hematopoiesis. Technologies such as LUNAR (**L**iquid biopsy **U**sing **N**GS to **A**ssay high-**R**isk patients), a tumour-uninformed plasma-based assay developed to detect genomic alterations and epigenomic signatures with high clinical sensitivity and specificity down to allele frequencies of 0.01 percent and to filter out biological noise sources, such as mutations caused by clonal hematopoiesis, are under ongoing investigation both for MRD detection post-definitive treatment and in lung cancer screening of at risk populations (NCT03774758).

5.4 Conclusions

Systemic therapy for advanced NSCLC is a rapidly evolving field of oncology, with recent advances leading to dramatic paradigm shifts in our treatment approaches and year-on-year evolution of approved and available novel therapies and treatment combinations, all with the ultimate benefit of improved patient outcomes. Lung

cancer death rates have fallen sharply in the Western world in recent years, while survival rates have improved, driven in large part by reductions in smoking rates, however recent analysis of the SEER database shows that death rates for NSCLC have fallen faster than incidence rates in recent years, an effect most likely driven by advances in treatment, and not observed in other types of lung cancer such as small cell lung cancer where decline in death rates largely parallels the incidence rates.[293] Despite these advances NSCLC remains a challenge to treat, particularly in those patients with resistance to immune-checkpoint inhibitors and no identifiable oncogenic driver mutations. Furthermore, the ongoing Covid-19 pandemic may lead to reversal in some of these positive trends, with evidence emerging that lung cancer patients have been particularly badly affected in the UK, likely as a result of a combination of factors including delays to presentation, diagnosis and treatment, as well as poorer outcomes for patients on treatment who contract Covid-19.[294]

The work done in this thesis includes contributions to understanding of the real-world utility of, at the time, novel therapies and molecular genotyping strategies, helping to guide their implementation in the clinic and to advance their further development. Ongoing research into combination systemic therapies, overcoming primary and acquired resistance to targeted therapies and ICIs, identification of prognostic and predictive biomarkers of response using validated molecular diagnostic tools, effective and accurate interpretation of large-scale genomic profiling data and integration with clinical data, while ensuring feasibility, standardisation and cost-effectiveness of ever more complex genomic technologies, remain some of the key future challenges in developing optimal treatment paradigms in advanced NSCLC and improving patient outcomes.

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Appendices

APPENDIX 1. LIST OF DATA ITEMS COLLECTED FOR STUDY OF PEMBROLIZUMAB IN

TREATMENT-NAÏVE ADVANCED NSCLC

PATIENT INITIALS
INSTITUTION (CENTRE)
GENDER (M/F)
ETHNICITY (ASIAN/BLACK/CAUCASIAN/OTHER)
SMOKING STATUS (CURRENT SMOKER/EX-SMOKER/NEVER SMOKER)
DATE OF DIAGNOSIS OF NSCLC (DD/MM/YY)
AGE AT DIAGNOSIS
ECOG PS AT DIAGNOSIS (0,1,2,3,4)
CLINICAL STAGE AT DIAGNOSIS (IA/IB/IIA/IIB/IIIA/IIIB/IV)
NSCLC HISTOLOGY SUBTYPE (SQUAMOUS/NON-SQUAMOUS)
NSCLC HISTOLOGY SUBTYPE (NAME)
MUTATION PRESENT (Y/N/UNKNOWN, IF YES – NAME)
PREVIOUS RADICAL TREATMENT (Y/N, IF YES NAME - (SURGERY, CHEMORADIOTHERAPY, OTHER))
DATE OF DIAGNOSIS OF RELAPSED/ADVANCED/METASTATIC DISEASE (DD/MM/YY)
DATE OF PD-L1 REPORT (DD/MM/YY)
PD-L1 EXPRESSION LEVEL (0%, 1-49%, 50-100%; ABSOLUTE NUMBER)
START DATE OF PEMBROLIZUMAB (DD/MM/YY)
END DATE OF PEMBROLIZUMAB (DD/MM/YY)
BEST RESPONSE (SD, PR, CR, PD, NE)
DATE OF PD1 (DD/MM/YY)
CONTINUED PEMBROLIZUMAB BEYOND PD1 (Y/N)
IF YES, DATE OF PD2 (DD/MM/YY)
DOSE DELAYS (Y/N)
GRADE >=3 TOXICITIES (Y/N, NAMES)
ANY IMMUNE-RELATED TOXICITIES (Y/N, NAMES, CTCAE V.4 GRADES)
PREDNISOLONE USE (Y/N)
MMF USE (Y/N)
OTHER IMMUNOSUPPRESSANT USE (Y/N, NAME)
DATE OF LAST FOLLOW-UP (DD/MM/YY)
DISEASE STATUS AT LAST FOLLOW-UP (DEATH/PD/SD/ONGOING PR/ONGOING CR)
DATE OF DEATH (DD/MM/YY)

APPENDIX 2. LIST OF DATA ITEMS COLLECTED FOR STUDY OF DOCETAXEL AND

NINTEDANIB IN RELAPSED NSCLC

PATIENT ID
PATIENT INITIALS
INSTITUTION (CENTRE)
GENDER (M/F)
ETHNICITY (ASIAN/BLACK/WHITE/OTHER)
SMOKING STATUS (CURRENT SMOKER/EX-SMOKER/NEVER SMOKER)
CLINICAL STAGE AT DIAGNOSIS (<IIIB/IIIB/IV/UNKNOWN)
NSCLC HISTOLOGY SUBTYPE (ADENOCARCINOMA/ADENOSQUAMOUS/LARGE CELL/OTHER)
KNOWN MUTATION (EGFR/ALK/OTHER (free text))
PREVIOUS RADICAL SURGERY (Y/N)
PREVIOUS RADICAL RT (Y/N)
PREVIOUS FIRST-LINE THERAPY FOR RELAPSE/METASTATIC DISEASE (PLATINUM-BASED/NON-LATINUM BASED/OTHER (free text)/UNKNOWN)
PREVIOUS BEVACIZUMAB (Y/N)
FIRST LINE MAINTENANCE THERAPY (PEMETREXED/ERLOTINIB/BEVACIZUMAB/OTHER (free text)/UNKNOWN)
BEST RESPONSE TO FIRST-LINE THERAPY (CR/PR/SD/PD/UNKNOWN)
DATE OF 1ST CYCLE OF FIRST-LINE THERAPY (DD/MM/YY)
DATE OF PROGRESSION AFTER FIRST-LINE THERAPY (DD/MM/YY)
BI NPP APPROVAL DATE (DD/MM/YY)
NINTEDANIB COMMENCED (Y/N)
REASONS FOR NOT COMMENCING NINTEDANIB DESPITE APPROVAL (free text)
AGE OF PATIENT AT TIME OF NPP REQUEST
ECOG PS AT TIME OF NPP REQUEST (0,1,2,3,4)
PRESENCE OF METASTASES AT TIME OF NPP REQUEST (Y/N)
BRAIN METS AT TIME OF NPP REQUEST (Y/N/UNKNOWN)
DATE OF 1ST CYCLE OF DOCETAXEL+NINTEDANIB (DD/MM/YY)
DATE OF LAST CYCLE OF DOCETAXEL+NINTEDANIB (DD/MM/YY)
DATE OF LAST CYCLE OF NINTEDANIB MONOTHERAPY (DD/MM/YY)
DATE OF LAST CYCLE OF DOCETAXEL MONOTHERAPY (DD/MM/YY)
TOTAL NUMBER OF COMBINED DOCETAXEL AND NINTEDANIB CYCLES
TOTAL NUMBER OF NINTEDANIB MONOTHERAPY CYCLES

TOTAL NUMBER OF DOCETAXEL MONOTHERAPY CYCLES
REASON FOR CHANGING TO MONOTHERAPY (END OF PLANNED NUMBER OF DOCETAXEL CYCLES/DOCETAXEL TOXICITY/NINTEDANIB TOXICITY/PHYSICIAN'S DECISION/OTHER (free text))
REASON FOR STOPPING ALL THERAPIES (DISEASE PROGRESSION/DRUG TOXICITY/PATIENT'S CHOICE/OTHER (free text))
BEST RESPONSE (CR/PR/SD/PD/NE)
DATE OF RADIOLOGICAL PROGRESSION (DD/MM/YY)
DOSE REDUCTION OF NINTEDANIB (YES/NO)
IF YES, NAME OF TOXICITY 1, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 2, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 3, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
STARTING DOSE OF DOCETAXEL mg/m^2 (60/75)
GCSF USE (PRIMARY PROPHYLAXIS/SECONDARY PROPHYLAXIS/NIL/UNKNOWN)
DOSE REDUCTION OF DOCETAXEL (YES/NO)
IF YES, NAME OF TOXICITY 1, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 2, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 3, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
TREATMENT DELAY FOR NINTEDANIB (YES/NO)
IF YES, NAME OF TOXICITY 1, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 2, CTCAE GRADE, CAUSALITY (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 3, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
TREATMENT DELAY FOR DOCETAXEL (YES/NO)
IF YES, NAME OF TOXICITY 1, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 2, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
IF YES, NAME OF TOXICITY 3, CTCAE GRADE (1,2,3,4), CAUSALITY (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
ANY HOSPITAL ADMISSION (Y/N)
REASON (DRUG TOXICITY/DISEASE PROGRESSION/OTHER (free text))
OTHER GRADE ≥3 AE 1 (free text)
CAUSALITY FOR NINTEDANIB (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/DEFINITELY/ UNKNOWN)

CAUSALITY FOR DOCETAXEL (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
OTHER GRADE ≥3 AE 2 (free text)
CAUSALITY FOR NINTEDANIB (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/DEFINITELY/ UNKNOWN)
CAUSALITY FOR DOCETAXEL (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
OTHER GRADE ≥3 AE 3 (free text)
CAUSALITY FOR NINTEDANIB (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/DEFINITELY/ UNKNOWN)
CAUSALITY FOR DOCETAXEL (NOT RELATED/UNLIKELY/ POSSIBLY/PROBABLY/ DEFINITELY/UNKNOWN)
3RD LINE THERAPY (Y/N/UNKNOWN)
REGIME (free text)
BEST RESPONSE (CR/PR/SD/PD/NE)
DATE OF LAST FOLLOW-UP (DD/MM/YY)
DISEASE STATUS AT LAST FOLLOW-UP (PROGRESSIVE/NON-PROGRESSIVE/NOT EVALUABLE/UNKNOWN)
DATE OF DEATH (DD/MM/YY)

Study Title: A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA - N3 -

Sponsor: Royal Marsden

Sponsor Number: CCR4448

EudraCT: 2016-000109-35

Clinical trials.gov Number: TBC

IRAS Number: 199962

Protocol version and date: 1.3 dated 21 April 2017

REC: TBC

IMP/s:
- Nab-paclitaxel
- Placebo
- Nintedanib

This protocol has regard for the HRA guidance and order of content

NHS Health Research Authority   **NHS** Health Research Authority   The ROYAL MARSDEN Life demands excellence

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CHIEF INVESTIGATOR AND SPONSOR SIGNATURE PAGE

STUDY TITLE: A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA (N3)

The under-signed confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator: Sanjay Popat, FCRP PhD

Signature: .... **Date:** ..../............./……..………

STATISTICIAN SIGNATURE PAGE

STUDY TITLE: A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA (N3)

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to EMEA ICH Topic E9 'Statistical Principles for Clinical Trials' and the relevant standard operating procedures and policies used by the Sponsor the Royal Marsden.

Name of Statistician: ………………………………………………………………………………………

Title: ………………………………………………………………………………………………………

Signed: …………………………………………………………………………………………………..

Organisation/Company: …………………………………………………………………………………..

Date: ………………………………………………………………………

STUDY TITLE: A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA (N3)

I have read and agree to the protocol, as detailed in this document. I am aware of my responsibilities as an Investigator under the UK Clinical Trials Regulations[1], the guidelines of Good Clinical Practice (GCP) the Declaration of Helsinki and the applicable regulations of the relevant NHS Trusts and the trial protocol. I agree to conduct the trial according to these regulations and guidelines and to appropriately direct and assist the staff under my control who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Address of Institution:

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

Name of Investigator: ……………………………………………………………………………………………

Title: ……………………………………………………………………………………………

Signed: ……………………………………………………………………………………………

Date: ……………………………………………………………………………………………

---

[1] The Medicines for Human Use (Clinical Trials), Regulations (S.I. 2004/1031) and any subsequent amendments to it

STUDY CONTACTS

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Funder(s)	**BOEHRINGER INGELHEIM:** Natasha Black IIS co-ordinator Boehringer Ingelheim Ellesfield Avenue Bracknell RG12 8YS	**CELGENE:** Celgene Limited

Study Statistician	**Ann Petruckevitch** Lead Statistician Research Data Management and Statistics Unit (RDSU) The Royal Marsden NHS Foundation Trust Tel: 0208 915 6261 Email: ann.petruckevitch@rmh.nhs.uk

FUNDING AND SUPPORT

ROLE OF STUDY SPONSOR AND FUNDER

For this trial some of the duties of the sponsor have been delegated to the Chief Investigator (CI), for example the CI has overall responsibility for the design and development of the protocol. The sponsorship agreement describes the allocation of such responsibilities, and a summary of this can be provided by the sponsor upon request.

FUNDER(S)	NATURE OF SUPPORT PROVIDED
Boehringer Ingelheim:	• Provision of study drug (nintedanib and placebo) • Funding support
Celgene	• Provision of study drug (nab-paclitaxel) • Funding support
The Royal Marsden NHS Foundation Trust Biomedical Research Unit	• Study coordination/management

TRIAL SUMMARY

Title	**A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA**
Study acronym	N3
Sponsor	Royal Marsden
Indication	Second or third line relapsed NSCLC, adenocarcinoma
Design	**Part 1 (Phase Ib):** A dose-finding study of nintedanib with nab-paclitaxel with a standard 3+3 design. In the dose escalation part there will be 3 dose cohorts of nintedanib: Dose level -1: 100mg po BID d2-7, 9-21, q21 Dose level 1: 150mg po BID d2-7, 9-21, q21 Dose level 2: 200mg po BID d2-7, 9-21, q21 In the dose expansion part, 6 additional patients will be enrolled at the maximum tolerated dose (MTD), prior to proceeding to part 2. **Part 2 (Phase II):** A placebo-controlled, randomised, double-blind, 2-arm, phase 2 multi-centre clinical trial of nab-paclitaxel with nintedanib and nab-paclitaxel alone. **Arm A: nab-paclitaxel + placebo** **Arm B: nab-paclitaxel + nintedanib**
Study Endpoints	**Part 1** **Primary:** • To define Maximum tolerated dose (MTD) and evaluate incidence of dose-limiting toxicities (DLTs) during Cycle 1 **Secondary:** • To examine the frequency of all Adverse Events graded by NCI-CTCAE version 4.0 • To examine the objective tumour response according to RECIST 1.1 (investigator reported), and the overall response rate • To define the number of cycles of nab-paclitaxel with nintedanib given **Part 2** **Primary:** • To explore PFS rate at 12 weeks from first dose of nab-paclitaxel with nintedanib/placebo **Secondary*:*** • To examine the frequency of all Adverse Events graded by NCI-CTCAE

	version 4.0 • To examine the objective tumour response according to RECIST 1.1 (investigator reported), and the overall response rate • To examine overall survival in the ITT and predefined subgroups (PD pre/post 9 months from start of first line chemotherapy; prior or no prior immunotherapy).
Sample size	Part 1: Maximum 24 patients (maximum 18 in dose escalation part and 6 in dose expansion part) Part 2: up to 85 patients in each arm (170 in total)

Key Inclusion Criteria	1. Stage III/IV NSCLC adenocarcinoma, without radical treatment options
	
	 2. ECOG 0-1
	
	 3. Previously received no more than 2 lines of systemic therapy for NSCLC with palliative intent:
	
	    • Chemotherapy as first or second line with palliative intent
	
	    • Relapsing within 6 months of adjuvant chemotherapy after surgery or as part of radical chemoradiotherapy, which count as one line of treatment
	
	    • Licensed or experimental maintenance therapy is allowed (e.g. pemetrexed)
	
	    • Immunotherapy in prior line of treatment (first or second line) is allowed
	
	 4. Radiologically measurable disease
	
	 5. Written informed consent (as per ICH-GCP)
	
	 6. Adequate haematopoietic, hepatic and renal function

Key Exclusion Criteria	1. Patients with known EGFR activating mutation or ALK fusion who have not received appropriate prior TKI treatment (patients enrolled and subsequently found to be positive will remain on protocol). Patients who have received appropriate TKI treatment will be allowed 2. Any concurrent anticancer systemic therapy 3. Patients who are refractory to prior taxane therapy for advanced disease. Prior taxane in the adjuvant setting is allowed provided there is no disease recurrence within 12 months. 4. Active or uncontrolled infections or serious illnesses or medical conditions that in the opinion of the investigator could interfere with the patient's ongoing participation in the study. 5. Gastro-intestinal abnormalities, including inability to take oral medication,

	requirement for intravenous feeding, active peptic ulcer, prior surgical procedures affecting absorption, any medical co-morbidity affecting gastrointestinal absorption.
	6. Radiotherapy within 4 weeks prior to randomisation.
	7. Major surgery (other than biopsy) within 4 weeks prior to randomisation.
	8. Active brain metastases (defined as stable for <4 weeks and/or symptomatic and/or leptomeningeal disease).
	9. Any other active current malignancy (other than non-melanomatous skin cancer, *in situ* breast or *in situ* cervical cancer, prostate cancer diagnosed more than 3 years prior, breast cancer diagnosed more than 5 years prior).
	10. Known pre-existing interstitial lung disease.
	11. History or presence of clinically relevant cardiovascular abnormalities such as uncontrolled hypertension, congestive heart failure NYHA classification of 3, unstable angina or poorly controlled arrhythmia. Myocardial infarction within 6 months prior to randomisation.
	12. Patients unwilling to use a medically acceptable method of contraception during trial and for 3 months after.
	13. Use of any investigational drug within 4 weeks of randomisation.
	14. Known allergy to nab-paclitaxel, nintedanib, or other ingredients
	15. Patients unable to comply with the protocol
Statistical methods	Part 1: This part of the study is descriptive. No formal sample size calculation has been carried out but arbitrarily chosen to gain confidence in treatment tolerability. Part 2: Patients will be randomized 1:1 between the two study arms with competitive enrolment between study sites. The primary endpoint will be 12-week PFS rate. The sample size is based on the expected PFS rates for the control and experimental arms being 45% and 65%, respectively (on the basis of the LUME-Lung 1 trial [docetaxel +/- nintedanib] PFS data for adenocarcinoma subgroup), with a two-sided alpha of 0.1 and power of 80%. Using a chi-squared test without correction, this gives an intended recruitment in each arm of 85 patients, allowing for a 10% dropout, the total number of patients needed to show a 20% difference between arms will be 170, respectively (85 in each arm). The secondary endpoints will be AEs frequency, ORRs and overall survival. All randomised patients will be included in the primary endpoint analysis.

	The 12-week PFS rates will be reported by treatment arm using Kaplan Meier methods and compared using the log rank test, respectively. ORRs will be reported by treatment arm with exact binomial 95% confidence intervals. Toxicity will be examined using frequency reports for each treatment arm. Overall survival will be calculated using Kaplan-Meier methods and the median survival estimates with 95% confidence intervals will be presented for each treatment arm.

LIST OF ABBREVIATIONS

AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CI	**Chief Investigator**
CRF	**Case Report Form**
CRO	**Contract Research Organisation**
CTA	**Clinical Trial Authorisation**
CTIMP	**Clinical Trial of Investigational Medicinal Product**
DMC	**Data Monitoring Committee**
DSUR	**Development Safety Update Report**
EC	**European Commission**
eCRF	**Electronic CRF**
EU	**European Union**
EUCTD	**European Clinical Trials Directive**
EudraCT	**European Clinical Trials Database**
EudraVIGILANCE	**European database for Pharmacovigilance**
GCP	**Good Clinical Practice**
GMP	**Good Manufacturing Practice**
IB	**Investigator Brochure**
ITT	**Intention to treat**
ICF	**Informed Consent Form**
ICH	**International Conference on Harmonisation of technical**
requirements for	**registration of pharmaceuticals for human use.**
IDMC	**Independent Data Monitoring Committee**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
ISF	**Investigator Site File**
ISRCTN	**International Standard Randomised Controlled Trials Number**
MA	**Marketing Authorisation**
MHRA	**Medicines and Healthcare products Regulatory Agency**
MS	**Member State**
NHS R&D	**National Health Service Research & Development**
NIMP	**Non-Investigational Medicinal Product**
PI	**Principal Investigator**
PIC	**Participant Identification Centre**
PIS	**Participant Information Sheet**
PP	**Per protocol**
QA	**Quality Assurance**
QC	**Quality Control**
QP	**Qualified Person**
RCT	**Randomised Control Trial**
REC	**Research Ethics Committee**
SAE	**Serious Adverse Event**
SAR	**Serious Adverse Reaction**
SDV	**Source Data Verification**
SOP	**Standard Operating Procedure**
SmPC	**Summary of Product Characteristics**
SSI	**Site Specific Information**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TMG	**Trial Management Group**
TSC	**Trial Steering Committee**
TMF	**Trial Master File**

1. BACKGROUND

Non-small cell lung cancer remains the commonest cause of cancer mortality worldwide. Treatment options for relapsed advanced or metastatic non-small cell lung cancer (NSCLC) are limited and outcomes remain poor, particularly in those patients with no identifiable driver mutations, therefore this remains an area of significant unmet medical need.

Current standard of care in the second line, after relapse following first line platinum-based chemotherapy, is a choice of single agent chemotherapy (e.g. docetaxel, pemetrexed or gemcitabine), EGFR TKI (e.g. Erlotinib) or immune checkpoint inhibition. The PD-1 inhibitor nivolumab has demonstrated improved median survival by around 3 months versus docetaxel in two randomised phase 3 trials, in both squamous and non-squamous relapsed NSCLC[1, 2]. However, differential responsiveness according to PD-L1 status is observed in non-squamous patients, where patients who had low PD-L1 expression levels (<10%) had comparable outcomes with nivolumab and docetaxel[1]. Nivolumab is FDA approved for both squamous and non-squamous relapsed NSCLC and currently EMA approved in relapsed squamous NSCLC.

Three-weekly docetaxel 75mg/m2 Q3W and paclitaxel 175mg/m2 Q3W are licensed for second line treatment of NSCLC. However, responses to these agents are in the range of 7-10% and benefits in terms of prolongation of PFS and OS are small[3,4]. Furthermore toxicities from three-weekly docetaxel are prohibitive for most patients. Febrile neutropaenia rates vary from 4-25%[5] and around a 40% admission rate is observed.

Weekly paclitaxel has activity in advanced NSCLC in combination with carboplatin and as a single agent. In the setting of previously untreated advanced non-small cell lung cancer, weekly paclitaxel with carboplatin has shown equivalent efficacy with improved toxicity profile over 3-weekly administration[6, 7]. The optimal weekly schedule was explored in a multi-arm randomised trial with paclitaxel 100 mg/m^2 weekly for 3 of 4 weeks with carboplatin (AUC = 6) administered on day 1, demonstrating the most favorable therapeutic index in patients with advanced NSCLC[8], with the rate of grade 3 and 4 haematological toxicities at 32%. In the setting of relapsed NSCLC, several phase II studies have shown efficacy of weekly paclitaxel as a single agent[9–13] or in combination with carboplatin or gemcitabine[14–16]. Incidence of grade 3-4 haematological toxicities ranged from 8-45% when used as single agent and 14-60% when used in combination. Non-haematological toxicities, primarily peripheral sensory neuropathy, occurred in 8-40% with single agent and up to 75% with combination treatment.

Nab-paclitaxel, nanoparticle-albumin-bound paclitaxel, has been developed to improve the therapeutic index of paclitaxel. It has been shown to have an improved toxicity profile compared to solvent-bound paclitaxel and docetaxel in breast and lung cancer patients [17, 18].

Weekly nab-paclitaxel has been investigated in 1st line advanced NSCLC with evidence of activity and a predictable safety profile.

A phase III comparison study conducted in patients with advanced NSCLC compared weekly nab-paclitaxel (100 mg/m^2) and once every three weeks carboplatin (area under the curve [AUC] = 6) against once every three weeks conventional solvent-based paclitaxel (200 mg/m^2) and carboplatin (AUC = 6) as first-line therapy. The nab-paclitaxel arm demonstrated significantly greater efficacy by overall response rate (ORR), with a trend toward greater response in PFS and overall survival (OS). There were significantly more ≥ Grade 3 events of sensory neuropathy, neutropaenia, arthralgia, and myalgia in the conventional solvent-based paclitaxel arm, but more events of thrombocytopaenia and anaemia in the nab-paclitaxel arm [18–20]. The anaemia was readily corrected with a single blood transfusion in the majority of cases. The thrombocytopaenia did not lead to increased rates of haemorrhages. These findings have led to weekly nab-paclitaxel being FDA approved and EMA licensed for the treatment of 1st line advanced NSCLC in combination with carboplatin, at a dosage of 100 mg/m^2 on Days 1, 8, and 15 in combination with carboplatin (AUC = 6) on Day 1, every 21 days.

Nab-paclitaxel single agent activity in 1st line advanced NSCLC has been demonstrated. In a phase I/II trial of nab-paclitaxel monotherapy in chemotherapy naïve patients with advanced NSCLC nab-paclitaxel achieved an objective response rate of 30% (95% CI, 16% to 44%), median time to progression of 5 months (95% CI, 3 to 8 months), and median overall survival of 11 months (95% CI, 7 months to not reached)[21]. The 1-year survival was 41%.

Nab-paclitaxel has also shown single agent activity in relapsed NSCLC, comparable to other approved second line chemotherapy agents. In a phase II study in second line advanced NSCLC, at a dose of 100mg/m2 on day 1, 8 and 15 of 21 day cycle, six-month PFS rate was 18 % (95 % CI 7.8–28.7 %), with median PFS of 3.5 months (95 % CI 1.9–5.8 months), median overall survival of 6.8 months (95 % CI 4.7–9.3 months) and overall response rate of 16.1% [22]. A further phase II study in third and later line relapsed NSCLC showed similar results, with objective response rate of 18.75% and disease control rate of 50%, with the median time to progression of 2.15 months, when given at a dose of 130mg/m2 on day 1, 8 and 15 of a 28 day cycle [23].

Nab-paclitaxel is being further commercially developed for use in the second-line setting in patients with advanced NSCLC, in combination with molecularly targeted agents (ClinicalTrials.gov Identifier: NCT02250326), as maintenance therapy after induction chemotherapy in combination with carboplatin (ClinicalTrials.gov Identifier: NCT02027428), in the first line in elderly patients (ClinicalTrials.gov Identifier: NCT02151149) and patients with reduced performance status (ClinicalTrials.gov Identifier: NCT02289456).

Angiogenesis is involved in tumour growth and metastasis. Vascular Endothelial Growth Factor (VEGF) and its receptor are crucial for the formation of new tumour vessels and are proven drug targets. Bevacizumab, a humanized monoclonal antibody to vascular endothelial growth factor, has been found to improve outcomes when added to standard chemotherapy regimens for bevacizumab-naive patients with non-squamous NSCLC in the first-line[24–26] and is licensed for use in this setting. A systematic review and meta-analysis of all randomised phase II/III trials of bevacizumab in the treatment of 1st line advanced or metastatic NSCLC has found that compared with chemotherapy alone, bevacizumab significantly prolonged OS (HR 0.90; 95% confidence interval 0.81, 0.99; P=0.03), and PFS (0.72; 95% CI 0.66, 0.79; P<0.001)[26]. Bevacizumab has been combined with 3-weekly nab-paclitaxel and carboplatin in first line non-squamous advanced or metastatic NSCLC in an open-label single arm phase II trial[27]. This combination was well tolerated, with mild neutropaenia, manageable side effects and median overall survival of 16.8 months (95% CI 10.4-21.6 months). In a recently published phase III ULTIMATE trial[28], bevacizumab in combination with weekly paclitaxel was compared to docetaxel in the second and third line setting in non-squamous NSLCL. 166 patients with non-squamous NSCLC progressing after 1 or 2 previous lines of treatment, were randomised in a 2:1 fashion to received weekly paclitaxel plus bevacizumab (paclitaxel 90 mg/m² D1, 8, 15 and bevacizumab 10 mg/kg D1, 15, q28d) or docetaxel (75mg/m² q21d). The trial met its primary end-point of progression free survival, with an adjusted hazard ratio for PFS of 0.62 in favour of bevacizumab and weekly paclitaxel over docetaxel (95%CI 0.44-0.86, p=0.005). Median PFS was 5.4 months for weekly paclitaxel/bevacizumab vs. 3.9 months for docetaxel, while ORR was 22.5% and 5.5% respectively (p=0.006). Crossover was allowed, with 38% of patients crossing over from docetaxel to weekly paclitaxel/bevacizumab arm, and no difference in OS was observed. Ramucirumab, a recombinant human monoclonal antibody of the immunoglobulin G1 class that specifically binds to and blocks the activation of vascular endothelial growth factor receptor-2 (VEGFR-2), is FDA and EMA approved for use in combination with docetaxel for the treatment of patients with metastatic NSCLC with disease progression on or after platinum-based chemotherapy, based on improvement in overall survival (OS) with an acceptable toxicity profile in a randomized, multicenter, double-blinded, placebo-controlled trial of 1,253 patients with relapsed metastatic NSCLC[29]. Patients who received ramucirumab in combination with docetaxel had improved OS (HR: 0.86; 95% CI: 0.75, 0.98). Median OS was 10.5 months on the ramucirumab plus docetaxel arm versus 9.1 months in the placebo plus docetaxel arm. Ramucirumab has also shown activity in the 1st line setting in combination with platinum/pemetrexed chemotherapy in non-squamous NSCLC[30] and is being investigated in the first line setting in combination with erlotinib in patients with advanced NSCLC and activating EGFR mutations (ClinicalTrials.gov Identifier: NCT02411448).

Nintedanib (BIBF 1120) is a small molecule tyrosine kinase inhibitor targeting VEGFRs, PDGFRs (platelet-derived growth factor receptors) and FGFRs (fibroblast growth factor receptors). The specific and simultaneous abrogation of these pathways may be more effective than inhibition of endothelial cell growth via VEGF pathway alone. Furthermore preclinical models have shown that nintedanib may have a direct anti-tumour effect on those malignant cells which overexpress PDGFR and/or FGFR (e.g. H1703 NSCLC cells).

Two phase III trials have investigated the efficacy of nintedanib in combination with chemotherapy in relapsed advanced or metastatic NSCLC: the LUME-Lung 1 trial in which nintedanib plus docetaxel was investigated as compared to placebo plus docetaxel in relapsed NSCLC patients [31] and the LUME-Lung 2 trial in which nintedanib plus pemetrexed was compared to placebo plus pemetrexed in second line non-squamous NSCLC (ClinicalTrials.gov Identifier NCT00806819). The LUME-Lung 2 trial was stopped early based on a pre-planned DMC futility analysis of investigator-assessed PFS, after randomization of 713 of 1300 planned patients (no safety issues were identified). Subsequent ITT analysis of the 1° endpoint, based on independently reviewed PFS, showed that primary end-point was met (median 4.4 vs 3.6 mo, HR 0.83 [95% CI: 0.7–0.99], p=0.04). There was no difference in OS, however the trial was underpowered to detect a difference in OS due to recruitment being halted early[32].

In the LUME-Lung 1 trial, 1314 patients with stage IIIb/IV recurrent NSCLC who had received one prior chemotherapy treatment were randomised in a 1:1 fashion to receive docetaxel with nintedanib or docetaxel with placebo; primary end-point was PFS by central independent review, and a key secondary endpoint was OS. PFS was significantly improved in the docetaxel plus nintedanib group compared with the docetaxel plus placebo group (median 3·4 months [95% CI 2·9–3·9] vs 2·7 months [2·6–2·8]; HR 0·79 [95% CI 0·68–0·92], p=0·0019) in the overall patient population. PFS for patients with adenocarcinoma was statistically significantly longer with docetaxel/nintedanib compared with docetaxel/placebo both at the time of primary PFS analysis (median PFS 4.0 vs. 2.8 months, HR 0.77 [95% CI 0.62 – 0.96], p=0.0193) and at the time of final PFS analysis (median PFS 4·2 [95% CI 3·6 to 4·4] vs. 2·8 [95% CI 2·6 to 3·2] months, HR 0·84 [95% CI, 0·71 to 1·00]; p=0·0485). After a median follow-up of 31·7 months, overall survival was significantly improved for patients with adenocarcinoma histology (322 patients in the docetaxel plus nintedanib group and 336 in the docetaxel plus placebo group; median overall survival 12·6 months [95% CI 10·6–15·1] vs. 10·3 months [95% CI 8·6–12·2]; HR 0·83 [95% CI 0·70–0·99], p=0·0359), but not in the total study population.

In a predefined subgroup of patients with adenocarcinoma who had progressed within 9 months after start of first-line treatment, overall survival was significantly longer in the docetaxel plus nintedanib group (206 patients) compared with those in the docetaxel plus placebo group (199

patients; median 10·9 months [95% CI 8·5–12·6] vs. 7·9 months [6·7–9·1]; HR 0·75 [95% CI 0·60–0·92], p=0·0073). In this population of patients, median PFS was significantly longer in the docetaxel plus nintedanib group both at time of the primary PFS analysis (3·6 months [95% CI 2·8–4·3] vs 1·5 months [1·4–2·6]; HR 0·63 [95% CI 0·48–0·83], p=0·0008]) and at the time of the final overall survival analysis (4·2 months [95% CI 3·2–4·4] vs 1·5 months [1·4–2·6]; HR 0·68 [95% CI 0·54–0·84], p=0·0005)[31]. This trial led to EMA license for nintedanib at a dose of 200mg BID d2-21, q21, in combination with docetaxel 75mg/m^2 d1, q21 in adenocarcinoma non-small cell lung cancer after first line chemotherapy.

In this phase III study, adverse events that were more common in the docetaxel plus nintedanib group than in the docetaxel plus placebo group were diarrhoea, reversible increases in ALT and AST, neutropaenia grade ≥3, nausea, decreased appetite and vomiting. Most of these adverse events were manageable with supportive treatment or dose reduction. The rate of grade ≥3 febrile neutropaenia in adenocarcinoma patients was 7.2% in the nintedanib arm (compared to 4.5% in the placebo arm). In the adenocarcinoma population, 17.2% of patients required at least one dose reduction to nintedanib while 16.9% of patients required a docetaxel dose reduction. The number of patients in the adenocarcinoma population who experienced a fatal AE unrelated to PD was 20 vs 8 (6.2% vs 2.4%) in the nintedanib and placebo arms respectively. The most common fatal AEs were sepsis (3 vs. 0), respiratory failure (2 vs. 0), and dyspnoea (0 vs 2).

The present trial will explore the safety and efficacy of nintedanib in combination with nab-paclitaxel in patients with advanced relapsed adenocarcinoma non-small cell lung cancer.

2. NAME AND DESCRIPTION OF INVESTIGATIONAL MEDICINAL PRODUCT(S)

2.1. Nab-paclitaxel

Nab-paclitaxel (ABI-007, ABRAXANE® for Injectable Suspension [Celgene Corporation, Summit, New Jersey, United States]) is a human serum albumin bound nanoparticle formulation of paclitaxel with a mean particle size of approximately 130 nanometers. Nab-paclitaxel has been developed to improve the therapeutic index of paclitaxel. The chemotherapeutic effect is enhanced by exploiting endogenous transport pathways to deliver higher doses of paclitaxel to the tumor [33], while at the same time the toxicities associated with conventional solvent-based paclitaxel formulations using a Cremophor® EL (BASF, Ludwigshafen, Germany) and ethanol vehicle are reduced.

Nab-paclitaxel is bound to albumin in amorphous state and, unlike conventional solvent-based paclitaxel formulations where micellar entrapment is observed [34–36], has linear pharmacokinetic (PK) characteristics. Based on these pharmacokinetic properties, the dose and short infusion time, an

increase of the maximum concentration (C_{max}) of free paclitaxel up to 10-fold greater than with conventional solvent-based paclitaxel has been reported in the literature [37]. The transport of paclitaxel across the endothelium is enhanced through albumin receptor mediated transcytosis, and the delivery of paclitaxel to tumors may be enhanced by binding of the albumin-bound paclitaxel to interstitial albumin binding proteins, such as secreted protein acidic and rich in cysteine (SPARC; also known as osteonectin)[38]. Nab-paclitaxel is not known to cross the blood-brain- barrier.

Although it has been hypothesized that SPARC expression may result in an increased concentration of nab-paclitaxel in tumors due to its albumin-binding ability, and may play a role in the enhanced antitumor activity, clinical studies remains conflicting [38–40] and therefore there is not sufficient data supporting the relationship of SPARC expression to clinical outcomes of nab-paclitaxel treatment.

Type of solid tumors had no significant effect on paclitaxel pharmacokinetics in patients who received nab-paclitaxel. Ethnic origin had no discernible effect upon PK parameters according to the studies conducted in Western countries, Japan and China.

The novel nab-paclitaxel nanoparticles conferred the ability to achieve a higher maximum tolerated dose (MTD) based on every 3-weeks dosing: 300 mg/m^2 for nab-paclitaxel versus 175mg/m^2 for conventional solvent-based paclitaxel [41]. The use of albumin also enables nab-paclitaxel to be given in a shorter, more convenient infusion time of 30 minutes compared with 3 hours to 24 hours with conventional solvent-based paclitaxel. Nab-paclitaxel is given without steroid and antihistamine premedication, which is required for conventional solvent-based paclitaxel to prevent solvent-related hypersensitivity reactions. Cremophor EL has been shown to leach plasticizers, specifically di (2-ethylhexyl) phthalate (DEHP), from polyvinyl chloride (PVC) bags and polyethylene-lined tubing [42, 43]. Although no controlled epidemiologic toxicity studies have been conducted in humans exposed to DEHP, severe effects (eg. carcinogenicity, cardiopulmonary toxicity, hepatotoxicity, and nephrotoxicity) have been observed in experimental models. Solvent-based paclitaxel product information instructs users to prepare, store, and administer solutions in glass, polypropylene, or polyolefin containers; non-PVC-containing infusion sets (eg, those with polyethylene lining) should be used. By comparison, standard tubing and intravenous (IV) bags may be used for the IV administration of nab-paclitaxel [34, 41].

Clinical studies of nab-paclitaxel have demonstrated:

1. The ability to achieve a higher MTD of nab-paclitaxel at 300 mg/m^2 vs. 175 mg/m^2 for conventional solvent-based paclitaxel.

2. Elimination of the need for premedication, which is required with conventional solvent-based paclitaxel to prevent solvent-related hypersensitivity reactions.

3. Shortened infusion times (infusion time of 30 minutes with nab-paclitaxel vs. 3 hours for conventional solvent-based paclitaxel).

4. Elimination of the need for specialised infusion set apparatus (standard infusion sets suffice whereas non-DEHP [diethylhexylphthalate] sets are required for conventional solvent-based paclitaxel).

In Phase 1 studies conducted in the adult population with advanced solid tumors designed to determine the MTD of nab-paclitaxel, the following dose-limiting toxicities were observed: keratitis, blurred vision, sensory neuropathy, stomatitis, and Grade 4 neutropaenia. In general, haematologic toxicities were not important dose-limiting events; no life-threatening neutropaenic infections and no Grade 4 anaemia or thrombocytopaenia were reported. The most frequently (> 50%) reported toxicities were all expected for this therapeutic drug class, namely fatigue, myalgia, nausea, alopecia, and stomatitis.

Nab-paclitaxel is approved globally for the treatment of metastatic breast cancer at a dosage of 260 mg/m2 administered IV over 30 minutes once every 3 weeks, in the EU and USA for the first line treatment of locally advanced or metastatic NSCLC at a dosage of 100 mg/m on Days 1, 8, and 15 in combination with carboplatin (AUC = 6) on Day 1, every 21 days, and in the USA and EU for the treatment of first-line metastatic pancreatic adenocarcinoma at a dosage of 125 mg/m2 (followed immediately by gemcitabine) on Days 1, 8, and 15 of each 28-day cycle.

Please refer to the current Investigator Brochure for detailed information concerning the available pharmacology, toxicology, drug metabolism, clinical studies and adverse event (AE) profile of the IP.

2.2. Nintedanib

Nintedanib (VARGATEF®) is an orally available potent small molecule triple kinase inhibitor inhibiting VEGFR 1-3, FGFR 1-3 as well as PDGF receptor α and ß in the low nanomolar range. VEGFR-2 is considered the crucial receptor involved in initiation of the formation as well as the maintenance of tumour vasculature. In vivo experiments demonstrated good anti-tumour efficacy at doses of 50 – 100 mg Nintedanib, leading to a substantial delay of tumour growth or even complete tumour stasis in xenografts of a broad range of differing human tumour types. Furthermore, established xenograft tumours rapidly responded to treatment with nintedanib. Histological examination of treated tumours showed a marked reduction of tumour vessel density by approximately 80%[44]. In vivo, the combination of nintedanib with docetaxel, pemetrexed or vinorelbine in xenografts showed clear

antitumour efficacy with a tumour/control ratio of 30%, 23% and 124% at suboptimal dose levels with the single agents. In a xenograft model of human ovarian cancer using the SKOV-3 tumour line, nintedanib was active (tumour control ratio of 25% at 50 mg/kg daily), and the combination of low doses of nintedanib and cisplatin showed more than additive efficacy (data on file).

Considering its antiangiogenic mechanism of action, it is anticipated that treatment with nintedanib will slow tumour growth in human cancers. Moreover, tumour regression may also be achieved by induction of apoptosis of immature tumour vessels. In addition, a therapeutic effect may also result from inhibition of tumour autocrine and paracrine growth factor loops involving VEGF, PDGF and bFGF. It is likely that long-term treatment may be needed to ensure maximal clinical benefit.

The metabolism of nintedanib is predominantly characterised by the ester cleavage of the methyl ester moiety yielding BIBF 1202, which was further metabolised by conjugation to glucuronic acid yielding the 1-O-acylglucuronide. Nintedanib has a favourable PK and excretion profile with almost no elimination via the urine, as well as metabolic characteristics that are predominantly independent of cytochrome P450-catalysed metabolic pathways[45].

Available pharmacokinetic data indicate that the systemic exposure required for biological activity can be achieved in cancer patients. Maximum plasma concentrations occurred mainly 1 to 4 hours after administration. There was no detectable deviation from dose proportionality in the pharmacokinetics of nintedanib. Steady state was reached within 9 days of treatment at the latest. The gMean terminal half-life was between 7 to 19 hours. The main metabolite of nintedanib was BIBF 1202 which was in vitro further glucuronidated to the BIBF 1202 glucuronide via the udp glucuronosyltransferase (UGT) 1A1 enzyme. In humans, 93.4% of total [14C] radioactivity was excreted in the faeces within 120 hours after oral administration of nintedanib. Only 0.7 % of total [14C] radioactivity was eliminated via the urine.

Based on the Phase I dose escalation trials with nintedanib monotherapy, the maximum tolerated dose was defined to be 250 mg for twice daily dosing in Caucasians and 200 mg twice daily in Japanese patients with a manageable safety profile in advanced cancer patients. The maximum tolerated dose for combination therapy of nintedanib and other anti-cancer drugs (such as docetaxel, paclitaxel, pemetrexed, carboplatin, 5-FU, oxaliplatin) was determined to be 200 mg twice daily. Based on the overall safety profile a dose of 200 mg twice daily of nintedanib is the recommended phase III dose for combination treatments with pemetrexed, docetaxel, paclitaxel/carboplatin and FOLFOX. Available pharmacokinetic data indicate that the systemic

exposure needed for biological activity can be achieved starting with doses of 100 mg nintedanib once daily.

In the phase I trials where nintedanib was combined with chemotherapeutic regimens, there was no change of the pharmacokinetic parameters of nintedanib or of the cytotoxic compounds due to the combined treatment. Combination of nintedanib with other anti-cancer drugs revealed a similar adverse event profile as compared to nintedanib monotherapy except for the chemotherapy related toxicities. Dose limiting toxicity consisted mostly of liver transaminase elevations as in the monotherapy phase I trials with the exception of the combination of nintedanib with pemetrexed, where fatigue was the most relevant dose limiting toxicity. Hypertension or thromboembolic events were rare and did not suggest an increased frequency as a consequence of therapy with nintedanib.

Based upon a non-clinical safety study *in vitro*, nintedanib may have a potential risk of phototoxicity (skin and eyes) *in vivo*. Few cases of photosensitivity reactions (less than 1%) and of CTCAE grade 1 intensity only have been reported from the clinical studies to date. If adequate precautions are taken (avoidance of prolonged ultraviolet (UV) exposure, use of broad spectrum sunscreen and sunglasses), treatment with nintedanib is considered safe.

As of July 10, 2009, a total of 739 cancer patients, 423 patients with idiopathic pulmonary fibrosis and 59 healthy volunteers have been treated in multiple dose studies with nintedanib or blinded nintedanib/placebo. The predominant adverse events were nausea, diarrhoea, vomiting, abdominal pain and fatigue of mostly low to moderate intensity after monotherapy with nintedanib. Dose limiting toxicities were dose dependent hepatic enzyme elevations that were reversible after discontinuation of nintedanib treatment. These liver enzyme elevations were only in a few cases accompanied by a simultaneous increase of bilirubin. In general, CTCAE v.3 grade 3 liver enzyme increases were reported in the dose groups of 250 mg twice daily or higher. They also were reversible and usually occurred within the first two months of treatment.

Nintedanib is EMA approved in combination with docetaxel 75mg/m^2 for the treatment of adult patients with locally advanced, metastatic or locally recurrent NSCLC of adenocarcinoma tumour histology after first-line chemotherapy.

For further details on nintedanib please refer to the current Investigator Brochure.

3. RATIONALE

The recommended phase 2 dose (RP2D) of combination nab-paclitaxel and nintedanib is unknown and the efficacy of the combination has not been investigated to date. We propose to explore the safety, tolerability and efficacy of combination nab-paclitaxel and nintedanib in relapsed adenocarcinoma NSCLC.

Part 1 one of the trial will evaluate the incidence of dose limiting toxicities when nab-paclitaxel is given in combination with nintedanib and a recommended phase 2 dose will be determined. Hypothesis to be explored in Part 2 of the trial is that addition of nintedanib to nab-paclitaxel is safe, tolerable and active in patients with relapsed advanced or metastatic adenocarcinoma NSCLC.

The participating patient population will be patients with relapsed advanced or metastatic non-small cell lung cancer of adenocarcinoma histology, in whom:

- second and subsequent line treatment options are extremely limited;
- there is evidence of promising activity of nintedanib in combination with chemotherapy agents;
- tolerability is a significant limitation to delivery of currently available second line treatments.

Patients with adenocarcinoma and known driver mutations in the EGFR and ALK genes will be included provided they have received prior treatment with an appropriate tyrosine kinase inhibitor in the first or second advanced or metastatic treatment line setting.

This exploratory phase 2 trial is the first to date to directly explore activity and efficacy of combination nab-paclitaxel and nintedanib in this patient population. If the combination is felt to be safe and efficacious, phase 3 trial will be considered and powered to detect improvements in PFS and OS over current standard treatments.

Choice of nab-paclitaxel dose and schedule (100mg/m2 D1, D8, Q3W) has been based on current nab-paclitaxel development trial experience in the second-line setting in patients with advanced NSCLC, in combination with molecularly targeted agents.

3.1. Assessment and management of risk

Although considerable progress has occurred in understanding the biological characteristics of cancer as well as the development of more effective treatment regimens, most patients with locally

advanced or metastatic tumours succumb to their disease. Thus, there is a substantial need for novel therapeutic strategies to improve the outcome for patients with advanced or metastatic NSCLC.

Antiangiogenic treatment with the orally available triple angiokinase inhibitor nintedanib with inhibition of VEGFR, PDGFR and FGFR offers the chance to control both locally recurrent and distant metastatic disease on an outpatient basis. Treatment with nintedanib may have the potential to provide significant benefit to patients with locally advanced and/or metastatic NSCLC by slowing tumour progression and metastasis, since its cellular target is expressed on the tumour vasculature in most malignancies.

The risks of antiangiogenic therapy with nintedanib in adult patients are primarily related to:

- the gastro-intestinal tract (nausea, vomiting, diarrhoea, abdominal pain)
- increases in liver enzymes (AST, ALT, γ-GT)
- fatigue, asthenia and anorexia.

According to the SPC, liver enzymes must be followed closely during treatment with nintedanib. Therapy with the trial drugs must be interrupted in the event of relevant hepatic toxicity and further treatment is to be withheld until recovery of the abnormal laboratory parameters.

The most clinically significant adverse reactions associated with the use of nab-paclitaxel across all studied indications are related to the blood and lymphatic system (e.g., neutropaenia), the nervous system disorder (e.g., peripheral neuropathy), the musculoskeletal system (e.g., arthralgia/myalgia), and the gastrointestinal system (nausea, vomiting, and constipation).

The major clinical side effects observed after therapy with nab-paclitaxel are distinct from nintedanib induced adverse events, yet some overlap may occur e.g. regarding mild gastrointestinal toxicity or liver enzyme increases. In view of the low potential for drug-drug interactions of nab-paclitaxel and nintedanib, it is not likely that enhanced toxicity due to pharmacokinetic interaction between the drug and the cytotoxic chemotherapy will occur. Due to the partially overlapping toxicity profile, e.g. the occurrence of nausea, vomiting and diarrhoea, liver enzyme increases, may be increased.

4. TRIAL DESIGN

This is a phase Ib/II multi-centre dose-finding study to explore the safety, tolerability and efficacy of combination nab-paclitaxel and nintedanib in patients with relapsed advanced or metastatic NSCLC of adenocarcinoma histology. The study will consist of two parts:

4.1.   Part 1 (Phase Ib)

Part 1 of the trial is designed as a Phase Ib multicentre dose-finding study, with a standard 3+3 design, to determine the MTD/RP2D, safety and tolerability of nintedanib in combination with nab-paclitaxel. All patients will receive weekly nab-paclitaxel 100mg/m2 IV d1, 8, q 21. There will be three nintedanib dose cohorts, each with 3 or 6 patients. Nintedanib will not be given on day of nab-paclitaxel dosing. Nintedanib dose levels will be as follows:

Dose level -1: 100mg po BID d2-7, 9-21, q21

Dose level 1: 150mg po BID d2-7, 9-21, q21

Dose level 2: 200mg po BID d2-7, 9-21, q21

Nintedanib will commence at dose level 1 (150mg po BID) and escalate to level 2. In case dose level 1 is not tolerable, nintedanib dose will de-escalate to level -1 (100mg po BID).

Dose limiting toxicities (DLTs) used to determine dose-escalation or cohort expansion will be based on Cycle 1.

Within each cohort, every patient will need to undergo a safety review on day 22 before next dose level cohort can commence. In each cohort:

- if there are no DLTs among the first 3 patients, then the next dose level cohort will commence
- If there is 1 DLT, another 3 patients will be added to the current cohort
- If there are no further DLTs (i.e. 1 DLT in 6 patients only), the next dose level cohort can commence
- If at least 2 out of the 6 patients have a DLT, the trial dose escalation stops and no higher dose will be used
- If DLT is identified in dose level 1, then dose level -1 will be explored

The decision to dose-escalate to the next dose level or to declare an MTD/RP2D will be determined by the extended Trial Management Group (exTMG) based on results from clinical and laboratory safety data for a given cohort. The exTMG will also determine the dose appropriate for the Phase II

portion of the study (or the RP2D). The RP2D will be the highest dose in the Phase I where there is an acceptable safety profile and where no more than 1 DLT was experienced out of the 6 patients in the cohort. Six additional patients will be recruited and given the same dose to confirm tolerability and safety before proceeding to Part 2. If there are more than 1/6 DLTs at dose level -1, the exTMG will determine whether to stop the dose expansion phase and Part 2 of the trial.

Any withdrawals or dropouts prior to the end of the first cycle for any other reason than DLT, or any screen-fails will be replaced. Patients who do not complete the first cycle due to a DLT will not be replaced. Patients will be issued with screening number before enrolment onto the study over the 28 day screening period.

4.1.1 Definition of DLT

The following adverse events occurring within first 3 weeks of treatment will qualify as DLT if considered drug-related:

- Non haematological toxicity ≥ CTCAE Grade 3 (except transient electrolyte abnormality, alopecia, untreated vomiting or diarrhoea, and isolated elevation of gamma glutamyl transpeptidase). In particular:

    - Gastrointestinal toxicity (e.g. nausea, vomiting, diarrhoea, abdominal pain) or hypertension ≥ CTCAE Grade 3 despite optimal supportive care/ intervention

    - Nintedanib related liver toxicity except GGT*** as specified below:

AST/ALT/ > 5x ULN* independent of bilirubin
AST/ALT/ > 2.5x ULN** together with **total bilirubin > 1.5 ULN****

    * corresponding to grade 3 toxicity according CTCAE
    ** corresponding to grade 2 toxicity according CTCAE
    *** isolated GGT elevation with no corresponding ALT/AST/ increase will not be considered as DLT

- Haematological toxicity

    - CTCAE Grade 4 neutropenia that is uncomplicated (not associated with fever ≥38.5°C) only if continuing for > 7 Days

    - CTCAE Grade 4 febrile neutropenia of any duration if associated with fever ≥38.5ºC

    - Platelet decrease to CTCAE Grade 4, or decrease to CTCAE grade 3 associated with bleeding or requiring transfusions.

- Inability to resume nintedanib dosing within 14 days of stopping due to treatment related toxicity

- In case adverse events with CTCAE Grade 3/4 were not judged as DLT from a clinical point of view, the sponsor will obtain a confirmation from the investigator regarding the appropriateness of the judgment.

All DLT events have to be reported immediately to the sponsor.   All DLT events that occur in individual patients at any time during repeated treatment courses or the follow-up period must also be reported as significant Adverse Events (SAE).

4.1.2   Definition of MTD

The MTD is defined as the highest dose of nintedanib associated with the occurrence of DLTs in fewer than 2/6 patients. The MTD estimated will be the dose level at which 0/3 or 1/6 patients will experience a DLT during the first cycle of treatment and will be below the maximum administered dose (MAD) if the next higher dose has at least 1/3 or 2/6 patents experiencing DLTs.

4.2.   Part 2 (Phase II)

Part 2 is designed as a randomised double-blind placebo-controlled Phase II trial of nab-paclitaxel and nintedanib at RP2D (as determined during Part 1), versus nab-paclitaxel and placebo.

All patients will receive weekly nab-paclitaxel 100mg/m2 IV d1, 8, q 21 and will be randomised into 2 arms at a 1:1 ratio to receive nintedanib RP2D or placebo:

- Arm A: nab-paclitaxel 100mg/m2 IV d1, 8, q 21 + placebo
- Arm B: nab-paclitaxel 100mg/m2 IV d1, 8, q 21 + nintedanib RP2D

Schedule of dosing of nab-paclitaxel and ninitedanib will be the same in Phase II as in Phase Ib.

Patients discontinuing nab-paclitaxel due to toxicity or patient/physician decision are allowed to continue nintedanib/placebo monotherapy.

Patients discontinuing nintedanib/placebo due to toxicity/ or patient/physician decision are allowed to continue nab-paclitaxel monotherapy until disease progression. There will be no restriction on the maximum number of nab-paclitaxel cycles that can be administered.

5. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

5.1.   Primary objective

Phase I:

a. To evaluate the safety and tolerability of combination nab-paclitaxel and nintedanib in patients with relapsed stage III and IV adenocarcinoma of the lung in second and third treatment line setting

b. To determine the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of nintedanib when given with nab-paclitaxel at 100mg/m2 d1, d8 q21, and the incidence of

dose limiting toxicities (DLTs) during cycle 1 of treatment at different dose levels of nintedanib.

Phase II:

To explore the efficacy of combination nab-paclitaxel and nintedanib versus nab-paclitaxel and placebo in the same patient population, with nintedanib/placebo given at the recommended phase 2 dose (RP2D) as defined by phase I of the study.

5.2.   Secondary objectives

Phase I:

1. To examine the frequency of all adverse events graded by NCI-CTCAE version 4.0.
2. To examine the objective tumour response according to RECIST 1.1 criteria (investigator reported), and the overall response rate
3. To explore the number of cycles of nab-paclitaxel with nintedanib given

Phase II:

1. To examine the frequency of all adverse events graded by NCI-CTCAE version 4.0.
2. To examine the objective tumour response according to RECIST 1.1 criteria (investigator reported), and the overall response rate.
3. To examine overall survival in the intention to treat population and in the predefined subgroups: according to progressive disease before or after 9 months from start of first-line chemotherapy; and according to prior or no prior immunotherapy.

5.3.   Outcome measures/endpoints

5.3.1.   Primary endpoint

Phase I:

To determine the incidence of dose limiting toxicities during Cycle 1 and define the RP2D of nintedanib in combination with nab-paclitaxel.

Phase II:

To measure the progression free survival (PFS) rate at 12 weeks after the first dose of nab-paclitaxel with nintedanib/placebo.

5.3.2. Secondary endpoints

Phase I:

1. The incidence of all adverse events (AEs) graded by NCI-CTCAE version 4.0

2. To measure the objective tumour response according to RECIST (investigator reported)* and the overall response rate (ORR)*

3. To describe the number of cycles of nab-paclitaxel with nintedanib given.

Phase II:

1. To measure the frequency of all adverse events (AEs) graded by NCI-CTCAE version 4.0

2. To measure the objective tumour response according to RECIST (investigator reported)* and the ORR*

3. To describe the overall survival in the ITT population and the predefined subgroups as defined above

For definitions see RECIST 1.1, Appendix 1, page 86.

5.4. Summary of trial objectives and corresponding endpoints

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective		
Phase I: To evaluate the safety and tolerability of combination nab-paclitaxel and nintedanib.	Phase I: Maximum tolerated dose (MTD) of nintedanib, defined as the highest dose of Nintedanib at which only 1 DLT occurs out of 6 patients treated.	Phase I: After cycle 1 of treatment.
Phase II: To explore the efficacy of combination nab-paclitaxel and nintedanib versus nab-paclitaxel and placebo.	Phase II: Progression free survival rate at 12 weeks after 1st dose of study treatment by each treatment arm with their respective 95% CIs.	Phase II: At 12 weeks after 1st dose of phase II study treatment.
Secondary Objectives **1.** To evaluate the frequency of all adverse events graded by NCI-CTCAE version 4.0 (phase I and II) **2.** To evaluate the objective tumour response and overall response rates by RECIST (phase I and II) **3.** To define number of cycles of nintedanib and nab-paclitaxel given (phase I) **4.** To explore the overall survival rates by treatment arm in ITT and predefined subgroups by time to progression after start of 1st line treatment and by previous immunotherapy (phase II)	**1.** Expressed as frequencies, percentages and descriptive summary measures for all AEs **2.** Expressed as a proportion for each treatment arm and with their respective 95% CIs **3.** Expressed as median and range **4.** Expressed as median OS estimates with 95% confidence intervals calculated using Kaplan Meier methods, by treatment arm in ITT and respective subgroups	End of trial

6. STUDY DURATION

In both Part 1 and Part 2 of the study, patients will enter a 28-day screening period, and if eligible (and the cohort/group is open for recruitment) will proceed to the treatment phase. Patients may remain on treatment at the discretion of the investigator until disease progression, unacceptable toxicity, until he/she begins a new anticancer therapy, withdrawal of parent/guardian/patient consent/assent, parent/guardian/patient refusal, physician decision, or death.

All patients in both parts of the study will be followed for 28 days after discontinuing treatment for safety and monitoring of adverse events, until progression (if applicable) for response, and for 18 months after the last dose of nab-paclitaxel and/or nintedanib/placebo for survival and new anticancer therapies.

The Phase 2 part of the study will begin when the RP2D has been declared in the Phase 1 part.

6.1. Trial Flow Charts

Part 1 trial schema: Determination of MTD and RP2D.
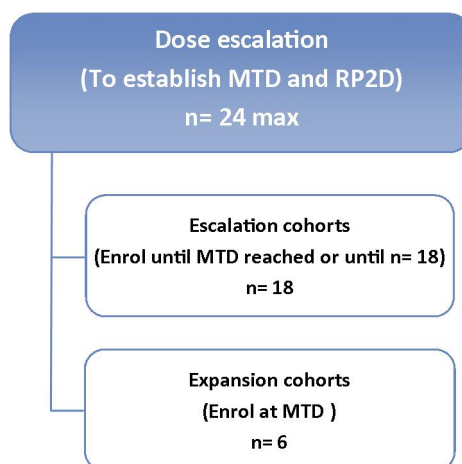
Part 1 - 3+3 dose escalation schema
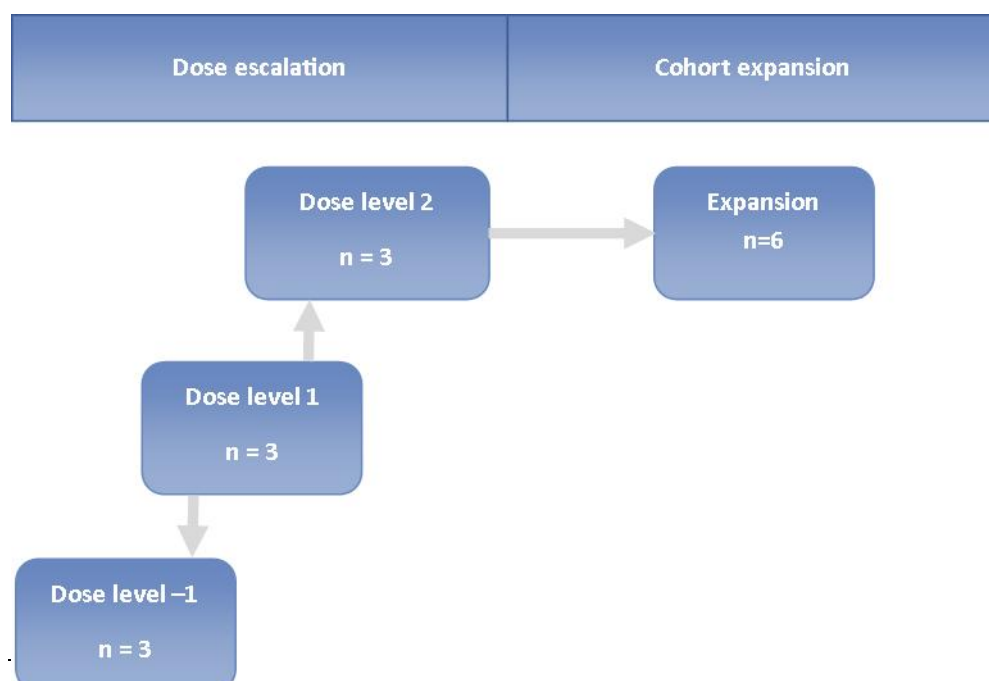
Part 1 - 3+3 Dose Escalation Decision Process

Number of subjects at given dose level with DLT	Action
0 out of 3 subjects	Escalate to next dose level
1 out of 3 subjects	Accrue 3 additional evaluable subjects at current dose level for a total of 6 evaluable subjects
1 out of 6 subjects	Escalate to the next dose level
2 or more subjects in a dosing cohort (up to 6 subjects)	Maximum tolerated dose has been exceeded. Evaluate at dose level below.

Part 2 trial schema
Randomised placebo-controlled 2 arm trial (Phase II)

Eligibility confirmed

Screening and enrolment
n=170

Randomisation
1:1

| Arm 1
Nab-paclitaxel 100mg/m2 d1, d8, q21 + placebo
N=85 | Arm 2
Nab-paclitaxel 100mg/m2 d1, d8, q21 + nintedanib RP2D
N=85 |

Continue treatment until disease progression, death, withdrawal of consent, unacceptable toxicity

Post-treatment follow-up
28 day post-treatment visit
Survival follow-up for 18 months or until death

7. STUDY SETTING

This is a multi-centre study. Phase I of the study will be run at a limited number sites in the UK in the first instance and then open to up to a larger number of sites during the Phase II part of the study.

7.1. Eligibility Criteria

7.1.1. Study population

In the Phase 1 portion of the study, at least 12 to a maximum of 24 patients will be enrolled, within 3 dose escalation cohorts and 1 dose expansion cohort.

In the Phase 2 portion of the study, up to 170 patients will be enrolled and randomised into 2 arms, with 85 patients per arm.

The same inclusion and exclusion criteria will apply for both phase I <u>and</u> phase II parts of the trial.

7.1.2. Inclusion Criteria

Patients must meet all of the following criteria to be enrolled in the study:

1. Male or female patients aged 18 or over.
2. Patients with a pathologically confirmed diagnosis of stage IIIb or stage IV adenocarcinoma of the lung; patients with locally recurrent disease (stage IIIa) and no radical treatment options are also eligible.
3. Patients who have previously received no more than 2 lines of systemic therapy for NSCLC with palliative intent:
   i.   Chemotherapy as first or second line with palliative intent
   ii.   Relapsing within 6 months of adjuvant chemotherapy after surgery or as part of radical chemo-radiotherapy, which count as one line of therapy
   iii. Licenced or experimental maintenance therapy is allowed (e.g. pemetrexed)
   iv.   Immunotherapy at prior line of treatment (first or second line) is allowed.
4. Patients with Eastern Cooperative Oncology Group (ECOG) performance status 0-1.
5. Patients with estimated life expectancy of ≥ 12 weeks.
6. Patients with at least one radiologically measurable tumour lesion as defined by RECIST 1.1 criteria.
7. Patients with adequate haematopoietic, hepatic and renal function.
8. Signed informed consent in accordance with local legislation.

7.1.3. Exclusion criteria

The presence of any of the following will exclude a patient from enrolment:

1. Patients with a known EGFR kinase sensitising mutation or ALK gene fusion prior to enrolment who have not received prior TKI (patients enrolled and subsequently found to be positive will remain on protocol). Patients with known EGFR activating mutation or ALK fusion who have received appropriate TKI treatment will be allowed.

2. Any concurrent anticancer systemic therapy.

3. Prior treatment with nintedanib or any other VEGFR inhibitor; prior treatment with bevacizumab is allowed

4. Patients refractory to prior taxane therapy for advanced disease. Prior taxane used in the adjuvant setting does not exclude eligibility provided there is no disease recurrence within 12 months upon completion of chemotherapy in that setting.

5. Inadequate laboratory parameters defined by:

   i. Absolute neutrophil count (ANC) < 1,500/μl (1.5×10^9/L).

   ii. Platelets < 100,000/μl (100×10^9/L).

   iii. Haemoglobin < 9.0 g/dl or requiring transfusions.

   iv. Creatinine clearance < 45 ml/min (by local institutional methods).

   v. Total bilirubin outside normal limits:

   vi. ALT and/or AST > 1.5 x ULN in patients without liver metastasis.

   vii. ALT and/or AST > 2.5 x ULN in patients with liver metastasis.

   viii. International normalised ratio (INR) > 2, prothrombin time (PT) and partial thromboplastin time (PTT) > 50% of deviation of institutional ULN.

6. Proteinuria CTCAE grade 2 or greater.

7. Pre-existing peripheral sensory neuropathy CTCAE grade 2 or greater.

8. Use of any investigational drug within 4 weeks of randomisation.

9. Radiotherapy within 4 weeks prior to randomisation.

10. Major surgery (other than biopsy) within 4 weeks prior to randomisation.

11. Active brain metastases or leptomeningeal disease (defined as stable for <4 weeks, no adequate previous treatment with radiotherapy, symptomatic, requiring treatment with anti-convulsants; dexamethasone therapy will be allowed if administered as stable dose for at least 4 weeks prior to randomisation).

12. Any other active current malignancy (other than non-melanomatous skin cancer, in situ breast or in situ cervical cancer, prostate cancer diagnosed more than 3 years prior, or breast cancer diagnosed more than 5 year prior to randomisation).

13. Active or uncontrolled infections or serious illnesses or medical conditions that in the opinion of the investigator could interfere with the patient's participation in the study, including:

    a. Known active or chronic hepatitis C and/or B infection.

    b. Known pre-existing interstitial lung disease or pneumonitis

    c. Presence of significant cardiovascular diseases (i.e. uncontrolled hypertension, unstable angina, history of infarction within the past 12 months prior to start of study treatment, congestive heart failure > NYHA II, serious cardiac arrhythmia, pericardial effusion).

    d. Gastro-intestinal abnormalities, including inability to take oral medication, requirement for intravenous feeding, active peptic ulcer, prior surgical procedures affecting absorption, any medical co-morbidity affecting gastrointestinal absorption.

    e. History of clinically significant haemorrhagic or thromboembolic event in the past 6 months.

    f. Known inherited predisposition to bleeding or thrombosis.

    g. Major injuries within the past 10 days prior to start of study treatment with incomplete wound healing and/or planned surgery during the on-treatment study period.

    h. Drug or alcohol abuse.

14. Therapeutic anticoagulation (except low-dose heparin and/or heparin flush as needed for maintenance of indwelling intravenous device) or anti-platelet therapy (except low dose therapy with acetylsalicylic acid <325mg her day).

15. Radiographic evidence (CT or MRI) of cavitary or necrotic tumours or local invasion of major blood vessels by tumour.

16. Pregnancy or breast feeding; female patients must have a negative pregnancy test (beta-HCG test in urine or serum) prior to commencing study treatment.

17. Patients who are sexually active and unwilling to use a medically acceptable method of contraception during the trial and for at least three months after ceasing study therapy (medically acceptable methods of contraception include total true abstinence*, permanent sterilisation (see section 7.1.4), combined oral, transdermal or intra-vaginal hormonal contraceptives, methoxyprogesterone injections (e.g. Depo-provera), copper-banded intra-uterine devices, hormone-impregnated intra-uterine systems and vasectomised partners; all methods of contraception, with the exception of total abstinence, should be used in combination with the use of a condom by male sexual partners).

18. Known hypersensitivity or any contraindications to the trial drugs, including nab-paclitaxel/nintedanib, to their excipients or to contrast media or other ingredients including peanuts and soya.

19. Patients unable to comply with the protocol.

* True abstinence, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

7.1.4. Definition of a woman of childbearing potential (WOCBP)

A woman is considered WOCBP i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

8. SCHEDULE OF ASSESSMENTS

8.1. Schedules of Assessments table

PART 1 & 2 Trial Period:	Screening Phase	Treatment Cycles – Every 21 Days to be repeated until patient discontinues trial treatment										End of Treatment[7]	Safety Follow-Up	Survival Follow-Up
Treatment Cycle:	Screening	Cycle 1			Cycle 2			Cycle 3[1]	Cycle 4[1]	Cycle 5[1]	Cycle 6 onwards[2]	At discontinuation of IMP	30 days after last dose.	Every 12 weeks from safety follow up
		Wk 1 Day 1	Wk 2 Day 8	Wk 3 Day 15	Wk 4 Day 1	Wk 5 Day 8	Wk 6 Day 15	Wk 7-9 Day 1	Wk 10 – 12 Day 1	Wk 13 – 15 Day 1				
Visit Window (Days):	-28 to -1				± 3			± 3	± 3	± 3	± 3	± 3	± 3	± 7
Administrative Procedures														
Informed Consent	✕													
Inclusion/Exclusion Criteria	✕													
Demographic & Medical History	✕													
Survival Status														✕
Clinical Procedures / Assessments														
Full Physical Exam	✕													
Blood panel[3]	✕	✕	✕	✕[4]	✕	✕		✕[3]	✕[3]	✕[3]	✕[3]	✕	✕	
PT and aPTT	✕													
Urinalysis for protein	✕													
Pregnancy Test*	✕				✕			✕			✕			
Adverse Events Review		✕	✕	✕[4]	✕	✕		✕	✕	✕	✕	✕	✕	
Con Medication Review	✕	✕	✕	✕[4]	✕	✕		✕	✕	✕	✕	✕	✕	
Vital Signs	✕	✕	✕	✕[4]	✕	✕		✕	✕	✕	✕	✕	✕	
Height	✕													
Weight[5]	✕	✕			✕			✕	✕	✕	✕	✕		
ECOG Performance Status	✕	✕	✕	✕[4]	✕	✕		✕	✕	✕	✕	✕	✕	
Tumour Imaging	✕						✕[6]		✕[6]		✕[6]	✕[8]		
Targeted Physical Exam		✕	✕	✕[4]	✕	✕		✕	✕	✕	✕	✕	✕	
Receive IV nab-paclitaxel[9]		✕	✕		✕	✕		✕[4,9]	✕[4,9]	✕[4,9]	✕[4,9]			
Dispense Nintedanib/Placebo		✕			✕			✕	✕	✕	✕			
Tumour Biopsies/Archival Tissue Collection/Correlative Studies Blood														
Archival Tumour Collection and Blood Sample	✕													

1. Day 1 and Day 8 visit assessments to continue at each cycle as shown for cycle 2.
2. Cycles to be extended past cycle 5 for as long as applicable. MTD will be determined during cycle 1 of part 1.
3. Blood panel to include haematology (FBC and differential white cell count) and biochemistry (U&E, LFTs, Glucose, Calcium) at baseline, prior to initiation of each cycle and prior to each nab-paclitaxel dose. Bloods can be taken within 72 hours in advance of dosing. Results need to be available and reviewed before dosing. Coagulation panel (INR, PT and PTT) to be performed at screening, subsequently as clinically indicated.
4. Day 15 visit mandatory for cycle 1 Part 1 only. Day 1 and Day 8 visits only for each subsequent cycle of Part 1 and all cycles of Part 2.
5. Weight to be recorded at screening and within 72 hours in advance of day 1 dosing of each cycle.
6. Tumour imaging to be done every 2 cycles (every 6 weeks) from the date of the 1st dose irrespective of delays in treatment cycles (can be up to 7 days before visit to ensure results at the visit). Brain imaging (CT or MRI) to be performed at screening and subsequently as clinically indicated for patients with no known diagnosis of brain metastases and any symptoms suspicious for brain metastases.
7. End of treatment visit assessments are only applicable if the patient comes off treatment between cycles.
8. Only if no RECIST assessment has been completed in the last 6 weeks.
9. Nab-paclitaxel will be given on d1 and d8, q21. Nintedanib will be dosed continuously except on day of nab-paclitaxel dosing (d1 and d8 of each cycle). Compliance with oral nintedanib will be reviewed at each visit.
*Serum β-HCG pregnancy test will be performed in women of childbearing potential within 10 days of Cycle 1 Day 1 and prior to day 1 of each cycle. Urine pregnancy test will be performed within 72 hours of Cycle 1 Day 1 if a serum pregnancy test occurred >72 hours of first IMP dose.

8.2. Recruitment

Following signing of the informed consent form, this information will be entered into the study database which will generate a unique trial identification number which will be used to identify the patient throughout the study.   Once all of the screening assessments have been completed and the data entered into the eCRFs, the patient will be assessed for eligibility.

Phase I - During the screening period, patients will be given a Screening number to be used during the screening period only. If eligible, the patient will be enrolled into the Phase I part of the study. However, if the patient is found not to be eligible then the local investigator will make alternative arrangements for the future treatment of that patient. If a patient does not complete at least the first cycle of treatment for any other reason than a DLT (e.g., rapid disease progression or through patient choice) then that patient will be replaced in that cohort.

Phase II - Patients who withdraw from the study after randomisation in the Phase II (Part 2) will not be replaced.

8.2.1. Patient identification

Potentially eligible male or female patients with NSCLC will be identified in clinic by members of patient's direct clinical team or via the local MDTs. Potentially eligible patients will be approached in clinic and offered trial participation by a member of the investigator team trained in the study procedures as per GCP guidelines and provided with the current version of the patient information sheet (PIS). In addition to this, each patient will also be required to consent to the retrieval of archival tissue for future research.

Patients will be recruited into the study by the Principal Investigator or delegate listed on the study delegation log.

8.2.2. Consent

It is the responsibility of the Principal Investigator/designee to provide each patient, prior to inclusion in the trial with full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. At least 24 hours should be allowed for the patient to consider their participation into the trial. Patients must be informed about their right to withdraw from the trial at any time. Written patient information must be given to each patient before enrolment. The written patient information is an approved PIS according to national guidelines.

The Principal Investigator must obtain documented consent from each potential patient or their legally acceptable representative <u>prior</u> to participating in a clinical trial. Consent must be documented by the patient by signing and dating the consent form along with the date and signature of the person delivering the consent discussion. If a translator is required to fully explain the trial they are also required to sign and date the consent form under the witness signature line. Only the Principal Investigator (PI) and those Sub-Investigator(s) delegated responsibility by the PI, having signed the delegation of responsibilities log, are permitted to gain informed consent from patients and sign the consent form. **All signatures must be obtained <u>prior</u> to the occurrence of any medical intervention required by the protocol.**

A copy of the signed and dated consent form should be given to the patient before participation in the trial. The original consent form should be stored in the Investigator Site File (ISF) with a copy also being placed in the patients' medical notes.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the patient must receive the REC approval/favourable opinion in advance of use. The patient or his or her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the patient's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the patient's or the patient's legally acceptable representative's dated signature. The informed consent will adhere to REC requirements, applicable laws and regulations.

8.3. Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies (If applicable)

Patients will be asked to provide consent for an EDTA blood sample and archival tissue samples for biobanking and future research.

8.4. SCREENING

Once informed consent has been obtained (as described in Section 7.2), screening evaluations will be performed for all patients to determine study eligibility.

Any questions regarding patient eligibility should be directed to the study sponsor and waivers to the protocol <u>will not</u> be granted during the conduct of this trial, under any circumstances. Safety laboratory analyses and all assessments will be performed locally. Laboratory normal ranges will be collected at study set-up by the trials team. Screening laboratory values must demonstrate patient eligibility, but may be repeated within the screening window if necessary.

8.4.1. Screening investigations to be completed within 28 days prior to first dose

- Cancer history (including specific information regarding diagnosis, staging, histology and all available biomarker information e.g. PD-L1 status)

- Demographics (initials, date of birth, sex, race and ethnicity, smoking history)

- Prior cancer therapies (includes surgery, radiation, systemic or any other therapy for the patient's cancer) including best documented response to previous therapies.

- Complete medical history (all relevant medical conditions occurring more than 28 days before screening should also be included)

- Prior and concomitant procedures (including all procedures occurring ≤ 28 days before screening) and concomitant medication

- Archival tumor sample collection (optional): If available and consented, the sample should be retrieved and sent after patient eligibility is confirmed. It is recommended that this be sent before the end of Cycle 1

- Physical examination , height and weight

- Vital signs (including blood pressure, temperature and heart rate)

- ECOG Performance status

- Tumour evaluation. Patients with historical tumour scans evaluable per RECIST 1.1 performed ≤ 28 days before the first dose need not repeat scans for the purposes of screening

- Patients with no known diagnosis of brain metastases and any symptoms suspicious for brain metastases must have a brain scan at screening to confirm eligibility (CT or MRI)

- Full blood count (FBC) with differential, including but not limited to red blood cell (RBC) count, haemoglobin, white blood cell (WBC) count, absolute neutrophil count (ANC), and platelet count. ANC should be measured with automated count where available.

- Chemistry panel including, but not limited to, sodium, potassium, urea, creatinine, glucose, albumin, total protein, calcium, alkaline phosphatase, bilirubin, aspartate aminotransferase (AST) or alanine aminotransferase (ALT), and coagulation parameters (INR, PT and PTT).

- Urinalysis for proteins

- A single EDTA sample will be taken from all patients for biobanking.

- Pregnancy test is required for all female patients of childbearing potential. Serum β-hCG pregnancy test will be performed at screening and repeated before beginning each new cycle. Urine pregnancy test will be performed to assess patient eligibility within 72 hours prior to the administration of IP, if the serum pregnancy test did not already occur within 72 hours of dosing.

Adverse event assessment begins when the patient signs the informed consent form

8.5.   Allocation of treatment

In the first part of the study (Phase Ib), patients will be recruited to an open-label, non-randomised dose-finding protocol of nintedanib and nab-paclitaxel combination in a 3+3 design.

Part 2 is designed as a randomised double-blind placebo-controlled trial with all patients receiving nab-paclitaxel plus nintedanib or placebo with a 1:1 random treatment allocation ensuring up to 85 patients in each treatment group.

8.5.1.   Method of implementing the allocation sequence

Phase Ib of the study is not randomised, so treatment will be allocated according to the dose cohort to which the patients are recruited.

The 1:1 random allocation sequence without stratification in phase II will be implemented using a web-based IWRS system, accessed by sites on participant enrollment. The modalities, specifications and specific randomisation procedures are detailed in the trial specific randomisation SOP.

8.6.   Blinding

Phase I is not blinded. In Phase II, the treatment allocation will be blinded to patients, investigators, clinical personnel and the trial team until the point of database lock, where treatment allocation will be divulged to the study statistician. Treatment allocation will not be stored in the trial database, and treatment allocation lists will remain with the randomisation provider. Upon participant randomisation, a notification will be sent by the IWRS system to the central study email address, not including the treatment allocation.

Medication packs will be blinded and Medication ID or label will not reveal study allocation. Placebo will be matched to the active study medication nintedanib.

8.7.   Unblinding

8.7.1.   Routine unblinding

When a participant completes the study, the individual treatment allocation may be revealed by the investigator. In such case, designated investigators at a site will access a dedicated section of the online randomisation system and perform the code break. Upon breaking the blind, a system notification will be sent to the central study email address N3@rmh.nhs.uk, not including the treatment allocation. At the point of database lock, the randomisation service provider will supply upon request treatment allocation list to the study statistician, subject to the internal processes for Royal Marsden sponsored studies.

8.7.2. Emergency unblinding

Patients can be unblinded on an individual basis in exceptional circumstances when knowledge of the treatment allocation is required for appropriate clinical management. Emergency unblinding procedures will be available to the investigator via the IWRS in the event of a medical emergency where a patient's study treatment information must be obtained. The sponsor should be immediately informed of the unblinding, preferably before it takes place and the reason for the unblinding should be documented in the patient's medical record.

In case of unblinding following withdrawal of consent or patient's request to discontinue active treatment, the patient's usual physician will assess status and recommend suitable treatment.

In case of unblinding required for appropriate clinical management, or adverse event, patients may be allowed to continue active treatment only (nab-paclitaxel and/or nintedanib as appropriate) provided they experience clinical benefit and other criteria for treatment continuation are met (see Section 11).

8.8. Trial assessments

8.8.1. Treatment period

The patient will begin treatment at the assigned dose upon confirmation of eligibility and authorisation from the sponsor that there is a slot available in the current cohort/group(s). The patient must start treatment within 28 days of signing the informed consent form (ICF). For all subsequent visits, an administrative window of ± 3 days is permitted.

Each treatment cycle is 21 days in duration.

The following evaluations will be performed at the frequency specified in the Schedule of Assessments. The evaluations should be performed prior to dosing on the visit day, unless otherwise specified:

- Concomitant medications evaluation
- Physical examination
- Vital signs: In general, on-treatment vital sign measurements will be source documented only. However, if an abnormal (out of range) value is reported at any given visit, that parameter should be collected in the case report form (CRF) at every subsequent scheduled visit until it returns to normal
- Weight
- Complete blood count with differential
- Chemistry panel
- Performance status

- Adverse event evaluation (continuously)

- Compliance with oral medication evaluation

- Response assessment/tumour evaluation

8.8.2. End of Treatment

An end of treatment (EOT) evaluation should be performed for patients who are withdrawn from treatment for any reason as soon as possible after the decision to permanently discontinue treatment has been made.

The following evaluations will be performed as specified in the Table of Events:

- Physical examination

- Vital signs and weight

- Concomitant medications evaluation

- Concomitant procedures evaluation

- Performance status

- Adverse event evaluation

- Complete blood count with differential

- Chemistry panel

Response assessment/tumour evaluation will be continued at the schedule defined in the Schedule of Assessments, and does not need to be performed specifically for the EOT visit except if no RECIST assessment has been completed in the last 6 weeks.

8.9. Follow-up assessments

All patients will be monitored for reporting of new or follow-up of existing AEs for 28 days after the last dose of IMP. If the 28-day follow-up visit occurs within 7 days of the end of treatment (EOT) visit and EOT laboratory values are not of clinical significance, laboratory collection is not required at the 28-day follow-up visit. The 28-day follow-up assessments include:

- Physical examination (source documented only)

- Vital signs and weight

- Concomitant medications evaluation

- Concomitant procedures evaluation

- Performance status

- Adverse event evaluation

- Complete blood count with differential

- Chemistry panel

8.10. Efficacy Follow-up

All patients who discontinue treatment for reasons other than disease progression, start of a new anticancer therapy, or withdrawal of consent from the entire study will be followed for response as specified in Section 8.11.

8.10.1. Survival Follow-up

All patients will be followed for survival until end of trial, when the last recruited patients has been followed-up until death, loss to follow-up or for at least 18 months from EOT visit, whichever occurs first. This evaluation should be conducted every 12 weeks for at least 18 months from the last dose of IP. Survival follow-up may be conducted by record review (including public records) and/or telephone contact with the patient, family, or the patient's treating physician. Information about subsequent anti-cancer therapies will be requested and recorded at each survival follow-up review.

8.11. Response Assessments

Response assessments (tumour evaluations) should be performed at screening (up to 28 days before the start of IP) and every 6 weeks (± 7 days) from Cycle 1 Day 1 until disease progression, start of a new anticancer therapy, or withdrawal of consent from the entire study. Evaluation of response should be performed using RECIST 1.1 guidelines.

8.11.1. Assessment of Response According to RECIST 1.1

Response will be assessed using RECIST 1.1. Response assessments will be performed using computed tomography (CT) scan. The regions to be imaged are the chest and abdomen, as well as any other studies required for tumour imaging. The same mode of imaging for lesion evaluation at screening must be used consistently throughout the study. The CT imaging should include contrast unless medically contraindicated. Conventional CT should be performed with contiguous cuts of 5 mm or less in slice thickness. Spiral CT should be performed by use of a 5 mm contiguous reconstruction algorithm.

All patients with evidence of objective tumour response (CR or PR) should have the response confirmed with repeat assessments at the next scheduled scan, but after no less than 4 weeks. Response assessments must have occurred ≥ 4 weeks from Cycle 1 Day 1 to be considered as stable disease (SD) for a best response.

Additional details and definitions of response are found in Appendix A.

8.11.2. Other Assessments

Patients who do not have a diagnosis of brain metastases but who have symptoms suggestive or suspicious for brain metastasis must undergo a brain scan at screening to confirm eligibility. Additional scans, including

further brain scans, MRI of the head, or nuclear medicine bone scan may be performed if clinically indicated (e.g. symptoms of brain metastasis) at the discretion of the investigator.

8.11.3   Assessment of compliance

Compliance with oral study medication will be assessed prior to each treatment cycle by direct questioning and counting of returned tablets. Non-compliance will be defined as <70% or >130% of expected dose taken during one cycle of treatment.

Non-compliance will also be defined as >4 consecutive study visits missed. Compliance information will be recorded at the time of study visit on the source document and non-compliance reported to the sponsor as soon as possible and ideally within 48 hours of investigator becoming aware of non-compliance.

8.12.   Withdrawal criteria and subject replacement

8.12.1. Removal of individual patients

A patient has to be withdrawn from active treatment in case any of the following applies:

- documented progressive disease
- the patient requests discontinuation of active treatment
- the patient withdraws informed consent
- the patient is no longer able to participate in the study (e.g. AE, surgery, pregnancy, concomitant diagnoses, concomitant therapies, or administrative reasons). The investigator may also stop a patient's treatment, if the patient is no longer able to attend study visits e.g. due to worsening of disease
- significant deviation from the protocol or eligibility criteria. The decision to continue or withdraw treatment will be made after discussion between the sponsor and the investigator
- the patient cannot tolerate nintedanib/placebo in combination with nab-paclitaxel, or further dose reductions are necessary but not allowed. Patients discontinuing nintedanib/placebo due to toxicity/patient/physician decision are allowed to continue nab-paclitaxel monotherapy. Patients discontinuing nab-paclitaxel due to toxicity/patient/physician decision are allowed to continue nintedanib/placebo monotherapy.
- the patient receives prohibited concomitant medication (refer to Section 12).

The End of Treatment information has to be obtained. All patients who end active treatment (but not the trial) will be followed up as described in Section 8.10.

All withdrawals will be documented and the reason for withdrawal recorded and discussed, as necessary, in the clinical trial report. Patients who withdraw prior to randomisation will not be included in the analysis but will

be entered into the trial database, the reason for withdrawal documented and reported descriptively and by patient listing in the report of this trial.

Patients who withdraw from the study after randomisation in the Phase II (Part 2) will not be replaced.

8.12.2. Discontinuation of the trial by the sponsor

The sponsor reserves the right to discontinue the trial overall or at a particular trial site at any time for the following reasons:

1. Failure to meet expected enrolment goals overall or at a particular trial site

2. Emergence of any efficacy/safety information that could significantly affect continuation of the

trial

3. Violation of GCP, the Clinical Trial Protocol (CTP), or the contract by a trial site or investigator, disturbing the appropriate conduct of the trial.

In the event of early trial discontinuation, patients remaining on the study who are continuing to benefit from trail drug will continue to receive supply of drug free of charge for as long as they continue to benefit from it and contingent on the recommendation from the DMC.

8.13. Storage and analysis of samples

Archival tissue samples will only be collected for all participants that have consented and have available samples for future research. FFPE tumour blocks will be requested where available. Archival tumour blocks will be returned to the source at the end of the study or, upon request, earlier if required for the participant's clinical management. Cut sections will be retained by the study team. These are archived samples and as such participants will not need to attend extra visits or undergo extra procedures.

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and/or the 2006 Human Tissue (Scotland) Act (as applicable).

8.14. Chain of Custody of Biological Samples

In all cases, patients will be consented for the collection and use of their biological samples and a full chain of custody will be maintained for all samples throughout their lifecycle. The Investigator at each site is responsible for maintaining a record of full traceability of biological samples collected from patients while these are in storage at the site, either until shipment or disposal. Anyone with custody of the samples e.g. sub-contracted service provider will have to keep full traceability of samples from receipt to further shipment or disposal (as appropriate).

RM-CTU will keep overall oversight of the entire lifecycle through internal procedures and monitoring of study sites.

8.15. Samples Confidentiality

All samples collected as part of this study as well as the information associated with the samples will be coded and stored appropriately to ensure confidentiality of the patient's information and to enable destruction of the samples if requested.   Since the evaluations are exploratory and for research purposes only, the results will not be placed in the patient's medical record and will not be made available to members of the family, the personal physician, or other third parties, except as specified in the informed consent.

9. END OF TRIAL

The End of Trial is defined as either the date of the last visit of the last patient to complete the study, or the date of receipt of the last data point from the last patient that is required for primary, secondary and/or exploratory analysis, as pre-specified in the protocol and/or the Statistical Analysis Plan, whichever is the later date.

10. TRIAL MEDICATION

According to the definition of the EU clinical trial directive 2001/20/EC, an investigational medicinal product is a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorisation, but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.

For further guidance on the study medication please refer to the current Pharmacy Manual.

10.1. Legal status of the drugs

10.1.1. Nab-paclitaxel

Nab-paclitaxel is approved globally for the treatment of metastatic breast cancer at a dosage of 260 mg/m2 administered IV over 30 minutes once every 3 weeks, in the EU and USA for the first line treatment of locally advanced or metastatic NSCLC at a dosage of 100 mg/m on Days 1, 8, and 15 in combination with carboplatin (AUC = 6) on Day 1, every 21 days, and in the USA and EU for the treatment of first-line metastatic pancreatic adenocarcinoma at a dosage of 125 mg/m2 (followed immediately by gemcitabine) on Days 1, 8, and 15 of each 28-day cycle.

10.1.2. Nintedanib

Nintedanib is EMA approved in combination with docetaxel 75mg/m^2 for the treatment of adult patients with locally advanced, metastatic or locally recurrent NSCLC of adenocarcinoma tumour histology after first-line chemotherapy. In addition, nintedanib has been approved in the USA by the US FDA for the treatment of idiopathic pulmonary fibrosis.

10.2. Investigator Brochure

For the purpose of this trial, Investigator Brochures will be used for both nab-paclitaxel and nintedanib.

Reference safety information for the purpose of this trail is deemed to be as follows:

Nab-paclitaxel: Section 6.3. 8 – Reference Safety Information of the Investigator brochure

Nintedanib: Section 7 – Summary of Data and Guidance for the Investigator

There are no expected adverse events for matching placebo of nintedanib soft gelatine capsules.

10.3. Drug storage and supply

All supplies must be stored in a secure, limited access storage area. Please refer to the respective Investigator Brochures for detailed information and stability.

10.3.1. Nab-paclitaxel

All study drug supplied by Celgene will be labelled, QP released and distributed via courier. There are no special storage conditions for nab-paclitaxel. Un-reconstituted vials should be stored at ambient temperature.

10.3.2. Nintedanib

All study drug supplied by BI, including placebo, will be QP released by BI, and labelled, packaged and distributed via courier. Nintedanib should not be stored above 30° C and protected from excessive humidity.

For both phases of the study, patients will be supplied with sufficient medication for each cycle. Nintedanib will be dispensed on cycle 1 of each cycle. There will be sufficient tablets in the bottle to cover the visit window.

Please refer to the pharmacy manual for further information.

10.4. Preparation and labelling of Investigational Medicinal Products

10.4.1. Nab-paclitaxel

Nab-paclitaxel will be supplied by Celgene as sterile lyophilized powder for reconstitution before use. Infusion solutions should be prepared using 20mL of **0.9% Sodium Chloride solution for injection** to a vial of nab-paclitaxel. Each mL of the reconstituted solution will contain 5mg/mL nab-paclitaxel. At any given dose of nab-paclitaxel in mg/m^2, the total dose of nab-paclitaxel to be administered should be calculated by the physician using the height/weight conversion chart or other standard method for calculation of the patient's body surface area (BSA). The exact total dosing volume of 5mg/mL suspension required for the patient is calculated using the following formula:

Dosing volume (mL) = Total Dose (mg) / 5 (mg/mL)

Sites are permitted to utilise dose-rounding practices in accordance with the latest guidance issued by NHS England for Anti-Cancer Therapies.

Nab-paclitaxel will be packaged, labelled and delivered to the participating sites via courier. The IMP will be supplied specifically for the trial and should not be used for any other purpose than that stated in this protocol. The drug will be labelled in accordance to Good Manufacturing Practice Annex 13. As a minimum the labels will include the following information:

a. Name, address and telephone number of the Sponsor

b. Name of drug, form, strength, quantity of dose units and route of administration

c. Lot number to identify the contents and packaging operation

d. Blank space for recording the subject number.

e. "Keep vial in outer carton in order to protect from light."

g. Protocol number

h. No special storage conditions

i. Expiry date

j. "For clinical trial use only"

k. "Keep out of reach of children".

Nab-paclitaxel should be administered using an infusion set incorporating a 15µm filter.

10.4.2. Nintedanib

Nintedanib is provided by BI as a soft gelatin capsule containing a suspension of milled active as the salt. For the purpose of this trial, 100mg and 150mg dose strength formulations will be used.

The corresponding placebo capsules are filled with medium chain triglycerides, hard fat and lecithin in addition to titanium dioxide as drug substance substitute.

Nintedanib will be packaged, labelled and delivered to the participating sites via courier. The IMP will be supplied specifically for the trial and should not be used for any other purpose than that stated in this protocol. The drug will be labelled in accordance to Good Manufacturing Practice Annex 13. As a minimum the labels for will include the following information:

a. Name address and telephone number of the Sponsor

b. Name of drug, form, strength, quantity of dose units and route of administration

c. Batch number to identify the contents and packaging operation

d. Trial ID

e. Medication ID

e. Directions for use

f. PI name

g. Protocol number

h. Storage conditions

i. Expiry date

j. "For clinical trial use only"

k. "Keep out of reach of children".

10.5.   Dosage schedules

10.5.1. Nintedanib

Initial Phase I dose will be 150 mg two times per day orally, with dose escalations and de-escalations depending on incidence of DLTs according to Phase I study design (outlined in Section 4). Dose will not be taken on day of administration of nab-paclitaxel i.e. day 1 and 8 of every 21 day cycle.

Initial Phase II dose will be the RP2D established during Phase I part. The capsules of the defined dose should be swallowed un-chewed with a glass of water of about 250 ml. The dose interval should be of around 12 hours at the same times every day, usually in the morning and the evening after food intake. In case of mis-dosing patients should proceed with the intake of medication according to the predefined schedule and take the next scheduled dose when it is due.

10.5.2. Placebo to Nintedanib

Capsules matching nintedanib.

The capsules should be swallowed un-chewed with a glass of water of about 250 ml. The dose interval should be of around 12 hours at the same times every day, usually in the morning and the evening after food intake.

In case of mis-dosing patients should proceed with the intake of medication according to the predefined schedule and take the next scheduled dose when it is due.

For dose modifications and reductions see section 11.

10.5.3. Nab-paclitaxel

In both part 1 and part 2 of the trial nab-paclitaxel will be given as an intravenous infusion at a starting dose of $100mg/m^2$ on day 1 and day 8 of each 21 day cycle. No premedication with corticosteroids or antihistamines will be required. Patients will receive standard antiemetic premedication as per local practice (e.g. Metoclopramide 10mg IV bolus at t -30mins).

Infusion of nab-paclitaxel will be given undiluted over 30 minutes. Limiting the infusion of nab-paclitaxel to 30 minutes will reduce the likelihood of infusion related reactions. An infusion completed in less than 25 minutes may increase Cmax by approximately 20%; therefore a nab-paclitaxel infusion completed in less than 25 minutes will meet the infusion rate criterion for an overdose.

11. DOSAGE MODIFICATIONS

11.1. Criteria for initiation of nab-paclitaxel and nintedanib/placebo treatment:

All of the following criteria must be met:
Nausea CTCAE grade ≤ 1 or baseline Vomiting CTCAE grade ≤ 0 or baseline Diarrhoea CTCAE grade ≤ 1 or baseline AST or ALT ≤ 1.5 x ULN (2.5 x ULN in case of liver metastases) ANC > 1500 /µL (* or WBC > 3000/µL) Platelet count > 100 000/µL Haemoglobin > 9 g/dl Bilirubin values ≤ ULN No uncontrolled infection

Table 1: Criteria for initiation of treatment, provided inclusion & exclusion criteria are met
  *Determination of ANC is recommended. In case ANC cannot be obtained, WBC may be used instead of the ANC, if thenumber of neutrophils at the previous visit (visit number x.1) was >50%

11.2. Criteria to continue nintedanib/placebo treatment on day 1 of a subsequent treatment cycle

The eligibility to continue treatment with nintedanib/placebo has to be assessed at the respective visit. A patient is eligible to continue nintedanib/placebo if all criteria listed in table 2 are met. If a patient has to interrupt intake of nintedanib/placebo due to an adverse event for more than 14 days, the decision to restart treatment with nintedanib needs to be discussed and agreed upon between the investigator and the sponsor.

All criteria must be met in order to continue nintedanib/placebo
Nausea CTCAE grade ≤ 2
Vomiting CTCAE grade ≤ 1
Diarrhoea CTCAE grade ≤ 2
AST or ALT CTCAE grade ≤ 2 and bilirubin ≤ 1.5 ULN corresponding to CTCAE grade 1
No other haematological or non-haematological adverse event grade CTCAE ≥ 3 which is considered drug-related

Table 2: Criteria to assess eligibility to continue nintedanib/placebo treatment on day 1 of a subsequent treatment course without prior interruption of intake due to an adverse event

11.3. Adverse events that require interruption of treatment with nintedanib/placebo

Treatment with nintedanib has to be interrupted in case any of the criteria listed in table 3 is fulfilled.

If one criterion is met, nintedanib/placebo has to be interrupted
Nausea of CTCAE grade ≥ 3 despite supportive care
Vomiting of CTCAE grade ≥ 2 despite supportive care
Diarrhoea of CTCAE grade ≥ 2 for more than seven consecutive days despite supportive care
AST and/or ALT of CTCAE grade ≥ 2 in conjunction with bilirubin of CTCAE grade ≥ 2
AST and/or ALT of CTCAE grade ≥ 3
Other non-haematological adverse event of CTCAE grade ≥ 3 considered drug-related

Table 3: Criteria when to interrupt treatment with nintedanib/placebo due to an adverse event

11.4. Criteria to assess eligibility to restart nintedanib/placebo after prior interruption of intake due to adverse events

If a patient has to interrupt intake of nintedanib /placebo due to an adverse event for more than 14 days, the decision to restart treatment with nintedanib needs to be discussed and agreed upon between the investigator and the sponsor. Patients who have to interrupt nintedanib/placebo during combination therapy shall continue chemotherapy in regular intervals unless re-treatment criteria for chemotherapy are not met.

To restart nintedanib /placebo after prior interruption of intake due to an adverse event, all AEs have to be recovered to or below baseline levels.

If a patient is eligible to restart nintedanib /placebo, please refer to section 11.5. for dose adjustments as well as sections 11.8. for specific cases in order to select the appropriate dose level of nintedanib /placebo. There are 2 possible dose reduction steps described. In case a further dose reduction would be necessary due to adverse events, treatment with nintedanib/placebo has to be permanently discontinued, and no reintroduction of oral therapy is possible.

11.5. Dose adjustments of nintedanib/placebo

The following dose levels will be used in case dose adjustments are required for management of undue toxicity. Dose re-escalation is not permitted.

Dose-level*:	0	-1	-2	-3
Dose:	2 x 200 mg/day	2 x 150 mg/day	2 x 100 mg/day	0

Table 4: Nintedanib/placebo dose levels

*It is anticipated that RP2D will be 2x200mg. Should the RP2D be lower than 2x200mg, the above dose levels will still be used for dose reductions in Part 2, only with fewer steps.

11.6. Criteria to administer nab-paclitaxel on Days 1 and 8 of subsequent cycles

Do not administer nab-paclitaxel on Day 1 of a cycle until ANC > 1500 /µL and platelet count > 100000 /µL.

Do not administer nab-paclitaxel on Day 8 of a cycle unless ANC at least > 500 /µL and platelet count at least 50000 /µL.

Dose-reduce nab-paclitaxel in the event of grade 3 or 4 peripheral neuropathy.

In subjects who develop Grade ≥ 3 haematological toxicity or Grade ≥ 3 peripheral neuropathy, upon resumption of dosing permanently reduce nab-paclitaxel dose as outlined in section 11.8. Re-escalation is not permitted.

For any other Grade 3 or 4 non-haematological toxicity or other investigator defined unacceptable toxicity, interrupt treatment until the toxicity improves to ≤ Grade 2, then restart treatment as per guidelines in section 11.8.

11.7. Dose adjustments of nab-paclitaxel

The following dose levels will be used in case dose adjustments are required for management of undue toxicity:

Dose-level:	0	-1	-2	-3
Dose:	100mg/m^2 d1, d8 Q3W	75mg/m^2 d1, d8 Q3W	50mg/m^2 d1, d8 Q3W	Discontinue treatment

Table 5: Dose reductions of nab-paclitaxel

11.7.1. Dose omissions

If for administrative reasons treatment with nab-paclitaxel cannot be administered on the planned visit date, it may be administered plus or minus 3 days from the scheduled date.

If the dose held or missed was the Day 1 of the next cycle, that cycle will not be considered to start until the first dose is actually administered.

If the dose held or missed was the Day 8 of the next cycle, that dose will be skipped. Next dose will be Day 1 of the next scheduled cycle.

If any doses of nintedanib are missed, skip that day and resume as appropriate, do not double up on subsequent days.

11.8. Management of specific AEs

Dose modifications during treatment			
Adverse drug reaction	Occurrence	Nab-paclitaxel	Nintedanib/Placebo
Haematological			
Platelets < 50000 /μL	1st	Stop until recovery[1], restart at level -1	Stop until recovery[1], restart at dose level -1
	2nd	Discontinue nab-paclitaxel	Stop until recovery, restart at dose level -2
	3rd or with bleeding	N/A	Discontinue nintedanib/placebo
Neutropenia of any grade/ duration accompanied by fever > 38°C	1st	Stop until recovery, restart at level -1	Stop until recovery, restart at level -1
or	2nd	Stop until recovery, restart at level -2	Stop until recovery, restart at level -2
Neutropenia grade 4 >7 days duration without fever	3rd	Discontinue nab-paclitaxel	Discontinue nintedanib/placebo
Non-haematological			
*Diarrhoea Grade 2 >7 days despite optimal medical management	1st	Stop until recovery[1], restart at level -1**	Stop until recovery[1], restart at level -1**
or Grade ≥3		Stop until recovery[1], restart at level -2**	Stop until recovery[1], restart at level -2**
or Any diarrhoea leading to hospitalisation	2nd 3rd	Discontinue nab-paclitaxel	Discontinue nintedanib/placebo
Liver enzyme elevations: ALT and/or AST >5x ULN	1st	Stop until recovery[1], restart at full dose	Stop until recovery[1], restart at level -1
or	2nd		Stop until recovery[1], restart at level -2
ALT and/or AST >2.5x ULN in conjunction with total bilirubin > 1.5 ULN	3rd	Stop until recovery[1], restart at dose level -1	Discontinue nintedanib/placebo
or	4th	Stop until recovery[1], restart at dose level -2 Discontinue nab-paclitaxel	N/A
AST and/or ALT >3x ULN in conjunction with total bilirubin ≥2x ULN and ALKP <2x ULN	Any occurrence	Discontinue nab-paclitaxel, unless an alternative cause is established	Discontinue nintedanib/placebo, unless an alternative cause is established

Dose modifications during treatment (cont'd)			
Adverse Drug Reaction	**Occurrence**	**Nab-paclitaxel**	**Nintedanib/Placebo**
Non-haematological (cont'd)			
Vomiting Grade ≥2*** and/or Nausea Grade ≥3 occurring ≤ day 3 after nab-paclitaxel	1st	Stop until recovery[1], restart at full dose	Stop until recovery[1], restart at full dose
	2nd	Stop until recovery[1], restart at dose level -1	Stop until recovery[1], restart at dose level -1
	3rd	Stop until recovery[1], restart at dose level -2	Stop until recovery[1], restart at dose level -2
	4th	Discontinue nab-paclitaxel	Discontinue nintedanib/placebo
Vomiting Grade ≥2*** and/or Nausea Grade ≥3 occurring ≥ day 4 after nab-paclitaxel	1st	Stop until recovery[1], restart at full dose	Stop until recovery[1], restart at dose level -1
	2nd	Stop until recovery[1], restart at full dose	Stop until recovery[1], restart at dose level -2
	3rd	Stop until recovery[1], restart at full dose	Discontinue nintedanib/placebo
	4th	Discontinue nab-paclitaxel	N/A
Peripheral neuropathy Grade ≥3	1st	Stop until recovery[1], restart at dose level -1	Stop until recovery, restart at dose level -1
	2nd	Stop until recovery[1], restart at dose level -2	Stop until recovery, restart at dose level -2
	3rd	Discontinue nab-paclitaxel	Discontinue nintedanib/placebo
Any other Grade 3 or 4 non-haematological AEs or other investigator defined unacceptable toxicity	1st	Stop until recovery[1], restart at dose level -1	Stop until recovery[1], restart at dose level -1
	2nd	Stop until recovery[1], restart at dose level -2	Stop until recovery[1], restart at dose level -2
	3rd	Discontinue nab-paclitaxel	Discontinue nintedanib/placebo

Until resolution to Grade 1 or baseline

*Diarrhoea Grade 1 or Grade 2 should be managed with anti-diarrhoeal treatment according to the local standard e.g. Loperamide prn, No dose reductions for nab-paclitaxel or nintedanib/placebo are required.

** AND anti-diarrhoeal treatment according to local standard

***Nausea Grade 1 or 2 and/or vomiting Grade 1 require no interruption or dose reduction to nab-paclitaxel or nintedanib/placebo. Anti-emetic treatment according to local standard

11.9. Additional precautions for nintedanib

During treatment with nintedanib /placebo, all study patients will be advised to avoid sun exposure or artificial UVA/UVB radiation in solaria or tanning booths. If exposure to sunlight cannot be avoided, protective clothing and broad spectrum (UVA/UVB) sunscreens should be used. After discontinuation of nintedanib/placebo treatment all protective measures should be continued for at least 2 weeks.

11.9.1. Permanent discontinuation of treatment with nintedanib and/or nab-paclitaxel

Patients should PERMANENTLY discontinue treatment with nintedanib and/or nab-paclitaxel in the event of:

- Intolerable Adverse Events (CTCAE grade 3 or 4) that cannot be managed by dose reduction, as described in section 11.8.

- Withdrawal of informed consent.

11.9.2. Rescue medication and additional treatments

Rescue medication to reverse the actions of nintedanib is not available. Potential side effects of nintedanib have to be treated symptomatically.

11.10. Known drug reactions and interaction with other therapies

Nintedanib is a substrate of P-glycoprotein (P-gp). Co-administration with the potent P-gp inhibitor ketoconazole increased exposure to nintedanib 1.61-fold based on AUC and 1.83-fold based on C_{max} in a dedicated drug-drug interaction study. Potent P-gp inducer rifampicin decreased exposure to nintedanib to 50.3% based on AUC and 60.3% based on C_{max}. Co-administration of potent P-gp inhibitors (eg ketoconazole, erythromycin) should be avoided and patients monitored for tolerability and side-effects. Co-administration of potent P-gp inducers (eg. rifampicin, carbamazepine, phenytoin and St. John's Wort) should be avoided.

The metabolism of paclitaxel is catalysed in part by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Therefore, caution should be exercised when administering nab-paclitaxel concomitantly with medicines known to inhibit (eg, ketoconazole, erythromycin, fluoxetine, imidazole antifungals, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (eg, rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) either CYP2C8 or CYP3A4.

Only a minor extent of the biotransformation of nintedanib consists of CYP pathways. Nintedanib and its metabolites did not inhibit or induce CYP enzymes in preclinical studies. The likelihood of drug-drug interactions with nintedanib based on CYP metabolism is therefore considered to be low.

12. CONCOMITANT MEDICATION

At screening corticosteroids (eg. dexamethasone) for treatment of brain metastases will only be allowed if the patient has been on a stable dose for >4 weeks. Long-term low dose inhaled corticosteroids are permitted. Following enrolment, use of corticosteroids for palliation of symptoms and management of chronic conditions (eg exacerbations of COPD, asthma etc.) will be permitted. Therapeutic anticoagulation e.g. full dose low molecular weight heparin for treatment of venous thromboembolism (except low-dose heparin and/or heparin flush as needed for maintenance of indwelling intravenous device) or anti-platelet therapy (except low dose therapy with acetylsalicylic acid ≤325mg her day) will not be permitted.

Co-administration of nintedanib with potent P-gp inhibitors (e.g. ketoconazole, erythromycin or ciclosporin) may increase exposure to nintedanib. While concomitant use of this medication is not prohibited, the patients should be monitored closely for tolerability of nintedanib.

Metabolism of paclitaxel is catalysed, in part, by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when administering nab-paclitaxel concomitantly with medicines known to inhibit (e.g. ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g. rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) either CYP2C8 or CYP3A4.

13. TRIAL RESTRICTIONS

Additional chemo-, immuno-, hormone- or radiotherapies are not allowed during the active treatment period of this trial. Palliative radiotherapy may be permitted for symptomatic control of pain from bone metastases in extremities after discussion with the Principal Investigator, provided that the radiotherapy does not affect target lesions, and the reason for the radiotherapy does not reflect progressive disease.

14. PHARMACOVIGILANCE

14.1 Definitions

14.1.1 Adverse Event (AE):

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a study drug, whether or not considered related to the study drug.

An AE includes but is not limited to those in the following list:

- A clinically significant worsening of a pre-existing condition. This includes conditions that may resolve completely and then become abnormal again.

- Abuse, withdrawal and overdose (accidental or intentional) of an investigational product. Any sequelae of an accidental or interventional overdose should be reported as an AE or serious adverse event (SAE).

- AEs occurring from lack of efficacy of an IMP, for example if the investigator suspects that a drug batch is not efficacious or if the Investigator suspects the IMP has contributed to disease progression.

Other reportable events that must be treated as AEs are listed below:

- Pregnancy exposure to the IMP. Any pregnancy occurring in a patient or a patient's partner during treatment with an IMP or occurring within six months of the last IMP administration, must be reported to the RM-CTU in the same timelines as an SAE. These should be reported even if the patient is withdrawn from the trial.

- Overdose or inadvertent or accidental exposure to an IMP with or without an AE.

- Any AE that could be related to the protocol procedures, and which could modify the conduct of the trial.

14.1.2 Serious Adverse Event (SAEs):

An SAE is any AE, regardless of dose, causality and expectedness, that:

- Results in death: the patient's death is suspected as being a direct outcome of the AE.

- Is life-threatening: refers to an event in which the subject was at risk of death at the time of the event. It also refers to an event that would result in death with the continued use of the product; it does not refer to an event which hypothetically might have caused death if it were more severe

- Requires hospitalisation, or prolongation of existing inpatient hospitalisation: admission to hospital overnight or prolongation of a stay in hospital was necessary as a result of the AE. Outpatient treatment in an emergency room is not itself an SAE, although the reasons for it may be. Hospital admissions/surgical procedures planned for a pre-existing condition before a patient is randomised to the study are not considered SAEs, unless the illness/disease deteriorates in an unexpected way during the study

- Results in persistent or significant disability or incapacity: the AE results in a significant or persistent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life

- Is a congenital anomaly or birth defect

- Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

14.1.3 Adverse Reaction (AR):

All untoward and unintended responses to the study drug related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions, i.e. an AR is possibly, probably or definitely related to the study drug. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

14.1.4 Serious Adverse Reaction (SAR) and Adverse Event of Special Interest (AESI):

An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
AESIs shall include:

- Any gastrointestinal- and non-gastrointestinal perforation, leakage, fistula formation, abscess
- Hepatic Injury (for patients with normal liver function at baseline) defined by the following alterations of liver parameters:
  - an elevation of AST and/or ALT >5 fold ULN without bilirubin elevation measured in the same blood draw sample.
  - an elevation of AST and/or ALT >3 fold ULN combined with an elevation of bilirubin >1.5 fold ULN measured in the same blood draw sample.
- QT prolongation
- Steven Johnson Syndrome

Protocol-specified AESI are to be reported in an expedited manner similar to Serious Adverse Events, even if they do not meet any of the seriousness criteria

14.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR):

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the investigator's brochure (IB).

14.1.6 Operational definitions for (S)AEs

In this trial, AEs and SAEs will be collected from registration until 30 days after last IMP administration. Adverse events and adverse reactions should be recorded in the medical notes and the appropriate section of the CRF. Serious Adverse Events and Serious Adverse Reactions should be reported to the sponsor as detailed in section 14.6.

14.2   Assessment of Severity

All adverse events should be graded for severity according to the NCI-CTCAE Toxicity Criteria (Version 4.0).

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied in 14.1.2 above.

14.3   Assessment of Seriousness

Criteria for assessment of seriousness are described in section 14.1.2

14.4   Assessment of Causality

All non-serious Adverse Events (including abnormal laboratory values) will be evaluated by the Investigator for potential relationship to IMP(s) according to table 2 below.   All serious adverse events (SAE) will be evaluated by the Investigator for potential relationship to IMP(s) according to table 2 below and reported as described in sections below.

Abnormal clinical laboratory values of clinical significance which were present at baseline and did not change in severity or frequency and/or which can obviously be attributed to the underlying disease will be evaluated by the investigator and recorded in the "unrelated" category.

Adverse Event Relationship to IMP	Description
Definitely related	A causal relationship is clinically/biologically certain. **This is therefore an Adverse Reaction**
Probably related	A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the IMP and there is a reasonable response on withdrawal. **This is therefore an Adverse Reaction**
Possibly related	A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the IMP. **This is therefore an Adverse Reaction.**
Unlikely to be related	A causal relation is improbable and another documented cause of the AE is most plausible. **This is therefore an Adverse Event.**
Unrelated	A causal relationship can be definitely excluded and another documented cause of the AE is most plausible. **This is therefore an Adverse Event.**

Table 2: Relationship of Adverse Event to IMP

The investigator must endeavour to obtain sufficient information to determine the causality or the AE (i.e. IMP(s), other illness, progressive malignancy etc.) and must provide his/her opinion of the causal relationship between each AE and each IMP (s). This may require instituting supplementary investigations of significant AEs based on their clinical judgment of the likely causative factors and/or include seeking a further opinion from a specialist in the field of the AE.

14.5   Assessment of Expectedness

Assessment of causality and expectedness for all SAEs will be made by the PI/designee and Chief Investigator or delegate against the current reference safety information defined in the Investigators Brochure section of this protocol (section 10.2). If updated versions of the Investigator Brochure are released during the course of the trial then they will be assessed for changes to the reference safety information, which will be submitted if relevant for regulatory approval. Assessment of expectedness will be made against the current regulatory approved version.

14.6   Recording and reporting of SAEs and SARs

14.6.1   Reporting of SAEs and SARs

All SAEs / SARs occurring from the time of written informed consent until 30 days post cessation of trial treatment must be reported to RM-CTU within 24hrs of knowledge of the event.- SAEs should be documented on an SAE report form using the completion guidelines provided. This form should be sent to:

Email: n3@rmh.nhs.uk

Fax: 020 8915 6762

Each episode of an SAE must be recorded on a separate SAE report form, graded according for severity according to section 14.2 and the worst grade recorded. If new or amended information on a previously reported SAE becomes available, the investigator must report this to RM-CTU on a new SAE report form. Should the investigator become aware of any drug-related SAEs after the patient go "off study", these must also be reported within the specified timelines above.

For each **SAE / SARs,** the following information will be collected:
- Full details in medical terms and case description
- Event duration (start and end dates, if applicable)
- Action taken
- Outcome
- Seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator

- Whether the event would be considered expected or unexpected.

Any change of condition or other follow-up information should be faxed to RM-CTU within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached. For screening failures, serious adverse events (SAEs) will be reported to the RM-CTU from the date of consent until the date the patient is confirmed as ineligible.

RM_CTU will report all safety events related to either IMP to the relevant manufacturer. Details of the reporting requirements will be specified in the latest version of the contracts.

14.6.2   Recording of SAEs and SARs in the eCRF

All AEs, including SAEs, must be recorded in the eCRF for eligible patients until completion of the safety follow-up. All concomitant medications, including herbal medications and supplements must be recorded. Any therapy used to treat the event must be recorded. AEs will be followed up until resolution, stability or it becomes clinically unfeasible to do so. The final outcome must be recorded in the eCRF as well as in the participants' medical record. Any unresolved AEs at the patient's last visit should be followed up as long as medically indicated, but without further recording in the eCRf. The eCRF will be reconciled with the safety database during and at the end of the trial. Therefore, the sites should ensure the data entered on the SAE report form and the data entered into the eCRF are consistent. The Trial Steering Committee and the Investigator(s) will regularly review the safety data from both the safety and the clinical database.

14.6.3   Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

All SUSARs (as defined in section 9.1.5) are subject to expedited reporting. The Sponsor delegates the responsibility of SUSAR notification to the Chief Investigator, via the RM-CTU (see section 14.6.1) The Chief Investigator must report all the relevant safety information previously described, to the Sponsor, MHRA and REC. The Chief Investigator shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

14.6.4   Minimal criteria for initial expedited reporting of SUSARS

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted within the time limits as soon as the minimum following criteria are met:

  a)   A suspected investigational medicinal product,
  b)   An identifiable subject (e.g. trial subject code number),

c) An adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,

d) An identifiable reporting source,

And, when available and applicable:

a) A unique clinical trial identification (EudraCT number or in case of non-European Community trials the sponsor's trial protocol code number)

b) A unique case identification (i.e. sponsor's case identification number).

14.6.5 Fatal or life-threatening SUSARs

All parties listed in 14.6.3 must be notified as soon as possible but no later than **7 calendar days** after the trial team and Sponsor has first knowledge of the minimum criteria for expedited reporting. In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to all parties within an additional **8 calendar days**.

14.6.6 Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues must be reported to all parties listed in 14.6.3 as soon as possible but no later than **15 calendar days** after first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

14.6.7 Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. Further available relevant information should be reported as follow-up reports. In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

14.6.8 Format of the SUSARs reports

Electronic reporting is the expected method for expedited reporting of SUSARs to the competent authority. The format and content as defined by the competent authority should be adhered to.

15   NOTIFICATION OF DEATHS

All deaths will be reported to the sponsor irrespective of whether the death is related to disease progression, the IMP, or an unrelated event within 24hrs of learning of the event.

15.1   Reporting urgent safety measures

The Sponsor or Investigator may take appropriate urgent safety measures (USMs) in order to protect the patient of a clinical trial against any immediate hazard to their health or safety. This includes procedures taken to protect patients from pandemics or infections that pose serious risk to human health. USMs may be taken without prior authorisation from the competent authority.

Should the site initiate a USM, the Investigator must inform the Sponsor immediately either by:

- E-mail: n3@rmh.nhs.uk

- Telephone: 020 8915 6506; or

- Fax: 020 8915 6762

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures. The initial notification to the MHRA and REC should be by telephone. A notice in writing must be sent within 3 days. The notice should set out the reasons for the USM and the plan for further action.

15.2   The type and duration of the follow-up of subjects after adverse events

All adverse events will be recorded from randomisation until completion of the safety follow-up in the eCRF. AEs will be followed up until resolution, stability or it is clinically feasible to do so. The final outcome must not only be documented in the eCRF but also recorded in the participants' medical records. Serious Adverse Events (SAEs) will also be recorded throughout the study until the safety follow-up visit. The reporting timeframe for adverse events meeting any serious criteria is described in 14.6.1.

Any unresolved AEs at the patient's last visit should be followed up for as long as medically indicated, but without further recording in the eCRF.

If an Investigator learns of any SAEs, including death, at any time after a patient has completed the study and he/she considers there is a reasonable possibility that the event is related to the study IMP, the Investigator should notify the RM-CTU.

The following details will be collected in the eCRF for each AE:

- AE description / diagnosis

- Date of onset and date of resolution

- NCI-CTCAE grade maximum intensity

- Seriousness

- Investigator causality rating against the study medication (yes or no)

- Action taken with regard to study medication

- Outcome

In addition, any adverse events occurring during the screening period that are a result of a protocol-specified intervention should also be recorded according to guidelines for standard AE reporting.

15.2.1 Development safety update reports

It is the responsibility of the sponsor to submit the Development Safety Update Report annually to the MHRA/ REC on the anniversary of the studies MHRA/REC approval. This will facilitate the authorities continuing review of the study. These authorities will also be informed of the end of the study by the sponsor within 90 days of the trial completion.   Copies of these reports will also be held within the main Trial Master File.

16   PREGNANCY REPORTING

The Investigator must make every effort to try and ensure that a clinical trial patient or a partner of a clinical trial patient does not become pregnant during the trial or for 6 months after cessation of study treatment. This should be done as part of the consent process by explaining clearly to the patient the potential dangers of becoming pregnant and also providing each patient with information about appropriate medically approved contraception. Two forms of medically approved contraception should be used, such as:

- oral contraceptives and condom
- intra-uterine device (IUD) and condom

Contraceptives should be used from the time the patient joins the trial, throughout the trial and for 6 months after the last dose of IMP. It should be explained to the patient that if his partner is pregnant or breast-feeding when he enters the trial, the patient should use barrier method contraception (condom plus spermicidal gel) to prevent the unborn baby or the baby being exposed to the IMP(s). The Investigator must ensure that all patients are aware at the start of a clinical trial of the importance of reporting all pregnancies (in themselves and their partners) that occur whilst being treated with the IMP and occurring up to 6 months after the last IMP administration. All pregnancies or suspected pregnancies occurring in either a female subject of childbearing potential or partner of childbearing potential of a male subject are immediately reportable events.

Pregnancies and suspected pregnancies occurring while the subject is on study product or within 6 months of the subject's last dose of study product, must be reported to the Chief Investigator and the Sponsor within 24 hours using the Pregnancy Notification Form. Participants who become pregnant must be discontinued from trial treatment immediately. It is the Investigator's responsibility to obtain consent for follow-up from the patient or patient's partner. All neonatal deaths, congenital anomalies or any other event that satisfies the seriousness criteria (see section 14. 1 for definition) occurring within 28 days of birth should be reported, without regard to causality, as SAEs. The Sponsor will follow-up all pregnancies for the pregnancy outcome via the Investigator and document on the pregnancy form.

The Investigator should offer counselling to the participant and/or the partner, and discuss the risks of continuing with the pregnancy and the possible effects on the foetus. With appropriate consent, monitoring of the participant and/or the partner and the baby should continue until the conclusion of the pregnancy. Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE and reported as such.

17 Cytotoxic chemotherapy can cause temporary or permanent infertility. Male patients will be advised on conservation of sperm prior to treatment.OVERDOSE

On a per dose basis, an overdose is defined as 10% over the protocol-specified dose of IMP assigned to a given patient, regardless of any associated adverse events or sequelae. On a schedule or frequency basis, an overdose is defined as anything more frequent than the protocol required schedule or frequency. On an infusion rate basis, an overdose is defined as any rate faster than the protocol-specified rate.

Complete data about drug administration, including any overdose, regardless of whether the overdose was accidental or intentional, should be reported in the case report form.

18   STATISTICS AND DATA ANALYSIS

18.1   Sample size calculation

Part 1: The sample size, using the 3+3 design, has been arbitrarily determined to gain confidence in tolerability recruiting up to a maximum of 24 patients (up to 18 patients in the 3 dose cohorts and 6 additional patients at the MTD).

Part 2: The sample size is based on the 12 week expected PFS rates for the control and experimental arms being 45% and 65%, respectively (on the basis of the LUME1 trial [docetaxel +/- nintedanib] PFS data), with a two sided alpha of 0.1 and power of 80%. Using a chi-squared test without correction, this gives an intended recruitment in each arm of 85 patients, allowing for a 10% dropout, the total number of patients needed to show a 20% difference between arms will be 170, respectively. Nquery software was used to calculate the sample size required.

18.2   Planned recruitment rate

Phase 1: A 3+3 design will be used to assess DLTs in potentially 3 dose cohorts with an additional 6 patients treated at the derived RP2D.

Phase 2: Patients will be randomized in a 1:1 ratio between the two study arms with competitive enrolment between study sites.

18.3   Subject population

Phase 1: All evaluable patients recruited to the study will be analysed based on dose-escalation and expansion decisions, respectively.

Phase 2: Those patients randomised to the study will be analysed using an ITT approach.

18.4   Statistical analysis plan summary

Phase 1:

This part of the study will define the maximum tolerated dose (MTD) and evaluate the incidence of dose-limiting toxicities (DLTs) during Cycle 1. The incidence of DLTs will be presented using percentages and frequencies with 95% confidence intervals assigned respectively. Secondary endpoints as defined in section 5. 3.2will be presented using appropriate descriptive summary measures such as means, medians, SDs and ranges for interval data; and proportions/percentages with frequencies for categorical data.

Phase 2:

All randomised patients will be included in the primary endpoint analysis. The 3-month/12 week PFS rates will be calculated using Kaplan-Meier methods and compared using the log rank test, respectively. Progression is defined as per RECIST 1.1 criteria (Appendix 1, page 89). Objective tumour response and ORRs will be reported by treatment arm with their 95% confidence intervals, respectively. Toxicity will be examined using frequency tabulation reports for each treatment arm. Overall survival will be calculated using Kaplan-Meier methods and the median survival estimates with 95% confidence intervals will be presented for each treatment arm; and for any predefined subgroups. Patients will be followed until death, loss to follow-up or 18 months after EOT,

whichever occurs first with those patient alive censored at this point, respectively. Any adjusted survival analyses will be carried out using Cox proportional hazards modelling taking account of patient variables thought to impact on outcome. Assumptions of proportionality will be assessed and tested for any constructed survival models, respectively. Multiple logistic regression modelling will be used for binary outcomes in the same way.

18.4.1 Primary outcome / endpoint analysis

Phase 1

- To define the maximum tolerated dose (MTD), RP2D, and evaluate incidence of dose-limiting toxicities (DLTs) during Cycle 1

Phase 2

Primary endpoint:

- To measure the PFS rate at 12 weeks in each treatment group, respectively

18.4.2 Secondary outcome / endpoint analysis

Phase 1

Secondary endpoints:

- To measure the frequency of all Adverse Events graded by NCI-CTCAE version 4

- To measure the objective tumour response according to RECIST (investigator reported), and the overall response rate, at the time of the final analysis of the primary endpoint

- To describe the number of cycles of nab-paclitaxel with nintedanib given

Phase 2

Secondary endpoints:

- To measure the frequency of all Adverse Events graded by NCI-CTCAE version 4, in particular those with ≥ grade 3

- To measure the objective tumour response according to RECIST (investigator reported), and the overall response rate, at the time of the final analysis of the primary endpoint

- To estimate the overall survival at 18 month follow-up in the ITT population and in predefined subgroups:

  - according to progressive disease pre/post 9 months from start of 1st line systemic therapy

  - according to prior or no prior immunotherapy.

18.4.3 Subgroup analyses

Exploratory analyses of overall survival differences in subgroups according to time to progression as defined above after first line systemic therapy and according to prior or no prior immunotherapy are planned.

18.4.4 Adjusted analysis

Survival analyses may need to be adjusted for common clinical factors (eg. age, stage, previous treatment lines). Any need for adjusted survival analyses will be carried out using Cox proportional hazards modelling taking account of patient variables thought to impact on outcome. Assumptions of proportionality will be assessed and tested for any constructed survival models, respectively. Multiple logistic regression modelling will be used for binary outcomes in the same way.

18.5 Interim analysis and criteria for the premature termination of the trial

In Phase I, the decision to dose-escalate to the next dose level or to declare an MTD/RP2D will be determined by the extended Trial Management Group (exTMG) based on results from clinical and laboratory safety data for a given cohort. An Independent Data Monitoring Committee (IDMC) will be established to meet half-yearly to review the data of the phase 2 part. At the end of the trial if the combination is felt to be safe and efficacious, but statistical significance is not definitively achieved, statistical inference, using the point estimate and its 95% confidence interval, will be used to measure the experimental arm's potential effect. On the basis of this, the next stage, if suggestive of a benefit from the experimental treatment arm, would be a larger confirmatory trial in the form of a phase 3 randomised trial powered for OS, between nab-paclitaxel-placebo and nab-paclitaxel-nintedanib combination.

18.6 Procedure(s) to account for missing or spurious data

Missing data will be reported using patient listings and percentage frequencies for baseline and outcome variables, respectively.

For the progression-free survival rate analysis, patients who are alive with no recorded progression at the time of analysis will be censored at the date of the CT scan when they were last recorded with an evaluable measure that was not progression.

For the overall survival time analysis, patients who are alive at the time of analysis will be censored at the date last seen alive.

18.7   Timing and responsibility for analyses

All study analysis will be carried out by the study statistician using the statistical software package STATA version 13. **A more detailed statistical analysis plan will be produced prior to any analyses taking place giving further details of them.**

18.8   Other statistical considerations

Not applicable.

18.9   Economic evaluation

Not applicable

19   DATA MANAGEMENT AND HANDLING

19.1   Source Data

All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial are classified as source data. Source data are contained in source documents; these are defined as original documents, data, and records e.g., hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

19.2   Language

All e-CRFs will be in English. Generic names for concomitant medications should be recorded in the CRF wherever possible. All written material to be used by patients must use vocabulary that is clearly understood, and be in the language appropriate for the study site.

19.3   Data Collection

The medical records/medical notes should be clearly marked and to allow for easy identification of a patient's participation in the clinical trial. The Investigator (or delegated member of the site study team) must record all data relating to protocol procedures, IMP administration, laboratory data, safety data and efficacy data into the e-CRF.

19.4 Data collection and documentation

It is the Investigator's responsibility to ensure that all relevant data is clearly recorded in the medical records. The Investigator must allow the RM-CTU direct access to relevant source documentation for verification of data entered into the e-CRF, taking into account data protection regulations. The clinical data should be recorded in the e-CRF and must be verifiable by the source data.

The patients' medical records, and other relevant data, may also be reviewed by appropriate qualified personnel independent from the sponsor appointed to audit the trial, or by REC. Details will remain confidential and patients' names will not be recorded outside the hospital.

The Principal Investigators at each centre are confirming agreement with his/her local NHS Trust to ensure that:

- sufficient data is recorded for all participating patients to enable accurate linkage between hospital records and e-CRFs
- source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit visits
- original consent forms are dated and signed by both patient and investigator and are kept together in a central log together with a copy of the specific patient information sheet(s) given at the time of consent
- all essential documents must be retained after the trial ends to comply with current legislation

No study document will be destroyed without prior written agreement between the Sponsor and the PI. Should the PI wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

19.5 Electronic Recording of Data

Patients' data will be documented on a trial specific e-CRF designed by RM-CTU. Should the eCRF not be available before the study is ready to begin paper CRFs will be used until the switch can be made. Upon signing the informed consent form, the patient is assigned to the next sequential Patient screening number available. Following confirmation of eligibility patient will be randomized and assigned a randomization number.

The Investigator is responsible for ensuring the accuracy, completeness, clarity and timeliness of the data reported in the e-CRFs. Only the Investigator, and those personnel who have completed the Study Team Responsibilities Signature Log/Delegation Log as authorised by the PI, should enter or change data in the e-CRFs. All protocol required investigations must be reported in the e-CRF. The Investigators must retain all original reports, traces and images from these investigations for future reference. The data will be entered in a clinical trials database (Macro V4). If a patient withdraws from the study, the reason must be noted on the e-CRF.

Authorised site personnel must not enter study-specific data directly into e-CRFs and ensure all results are appropriately documented in the patients' medical records. The e-CRF will be signed electronically by the Investigator or by an authorised staff member. Study specific information will be entered into an e-CRF visit by visit. Data that are derived should be consistent with the source documents or the discrepancies should be explained. All e-CRF data should be anonymous, *i.e.* identified by study patient number only.

19.6 Data Management

Data management will be carried out by RM-CTU using an electronic database and in accordance with the data management plan agreed by the RM-CTU and RDSU. Data entry will be carried out by appropriately trained personnel at participating centres. Queries will be raised centrally by the trial manager / trial monitor and sent to the participating centre for resolution.

19.7 Study Management Structure

19.7.1 Delegations of Responsibilities

This trial is sponsored by the Royal Marsden and will be conducted in accordance with the professional regulatory standards required for non-commercial research in the NHS under the research governance framework for health and social care and good clinical practice. The following responsibilities have been delegated to:

19.7.2 RM-CTU

RM-CTU has overall responsibility for facilitating and coordinating the conduct of the trial and is also responsible for collating data obtained, and undertaking and reporting all analyses.

The responsibilities of RM-CTU for the day-to-day management of the trial will include the following;

- ensuring an appropriate ethics opinion has been sought, and any amendments have been approved
- giving notice of amendments to protocol, make representations about amendments to the Main REC and MHRA as applicable
- notifying sites and Sponsor that the trial has ended
- randomising patients
- raising and resolving queries with local investigators
- keeping records of all serious adverse events (SAEs), overdose incidents, pregnancies and ECI's reported by investigators
- notifying the Main REC, MHRA and Investigators of related Serious Adverse Events

19.7.3 Participating Sites

- Inserting and keeping in place arrangements to adhere to the principles of GCP
- keeping a copy of all 'essential documents' (as defined under the principles of GCP) and ensuring appropriate archiving of documentation once the trial has ended
- taking appropriate urgent safety measures
- sites proposing to recruit to this study will be required to provide evidence that they are able to deliver protocol treatment for the duration of the study
- responsibilities are defined in an agreement between an individual participating centre and RM-CTU, which must be signed and in place before recruitment can commence

19.8 Trial Management

The RM-CTU will be responsible for the day-to-day coordination and management of the trial. This includes all duties relating to safety reporting. A trial agreement will be signed between the site and RM-CTU. Once all relevant trial approvals are in place an initiation (visit or teleconference) will be conducted. In addition, training and ongoing advice will be provided by trial training workshop(s), site initiation and ongoing site support to each participating site by Trial Management Group (TMG).

19.8.1 Trial Management Group

A Trial Management Group (TMG) will be set up and membership will include Chief Investigator, Chief Co Investigator, Trial Statistician and Trial Manager. Principal Investigators and other key study personnel will be invited to join the TMG as appropriate. The TMG have operational responsibility for the conduct of the trial.

19.8.2 Safety Review Meetings

At the beginning of the study the TMG will meet every 2 – 4 weeks to review any safety aspects relating to the trial until the RP2D has been defined and the expansion cohort is completed.

19.8.3 Trial Steering Committee

The Trial Steering Committee (TSC) will consist of TMG plus the site PI or representative from sites participating in Phase 1 and an independent chair and clinician.

The extended TMG (TSC) will meet at the end of each cohort to regularly review toxicity data, define DLTs, and make decision to proceed to the next cohort (or not) cohort dosing and expansion cohort, and define the RP2D.The role of the TSC is to monitor trial progress and to ensure the protocol and GCP principles are adhered to. The TSC's terms of reference, roles and responsibilities will be defined in a charter. Further internal or external experts may be consulted by the TSC as necessary.

19.8.4 Data Monitoring Committee

The Data Monitoring Committee (DMC) will consist of Chair from the Trial Steering Committee, independent clinician and statistician.

The DMC will meet approximately 6 monthly to perform a monitoring role to review the data of the phase 2 trial. They will be provided all relevant results as necessary to perform this role. This will be conducted according to the IDMC charter.

20 MONITORING

During the trial RM-CTU is responsible for monitoring data quality in accordance with relevant standard operating procedures (SOPs). Incoming data will be monitored for protocol compliance and if any inconsistent or missing data is identified queries will be sent to the site for resolution. Any systematic inconsistencies may trigger an onsite monitoring visit.

The trial statistician will periodically examine the data for anomalies and outliers, such as too few or too many events. Queries will be raised by the trial coordinators in such situations and communication with the clinical teams will take place. In addition statistical monitoring of unusual dates and inconsistent data will take place. Again these will raise queries via the trial coordinators.

If an on-site monitoring visit is required, RM-CTU will contact the site to agree convenient date. The site must ensure that relevant site file and patient notes are available for review. RM-CTU staff conducting onsite monitoring will review the investigator site file and carry out source data verification to confirm compliance with the protocol, trial agreement.

20.1 Quality Control and Quality Assurance

Quality Control (QC) will be performed according to RM-CTU internal procedures. The study may be audited by a Quality Assurance (QA) representative of the Sponsor. All necessary data and documents will be made available for inspection.

20.2 Clinical study report

Clinical data will be presented at the end of the trial based on final data listings. The CI/designee together with the trial statistician will prepare a brief clinical study report / publication based on the final data listings. A summary of the report must be provided to the Research Ethics Committee and the MHRA within 1 year from the submission of the end of trial notification.

20.3 Record retention

Essential documents are documents that individually and collectively permit evaluation of the conduct of the trial and substantiate the quality of the data collected. During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical trial and the quality of the data produced to be evaluated and verified in accordance with current legislation.

RM-CTU will maintain essential documents to facilitate the management of the trial, audit and inspection in accordance with RM G-SOPs and in compliance with the clinical trial regulatory requirements.   The medical files of trial subjects shall be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice. All medical records and TMF documentation will be retained for a minimum of 5 years after the study has concluded.

21 REPORTING AND PUBLICATION

The trial results will be submitted for publication in a relevant medical journal with authorship according to the criteria defined by the ICMJE (http://www.icmje.org). These state that: Authorship credit should be based 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Draft publications (manuscripts, abstracts, slides and posters) should be submitted to the RM-CTU for circulation to the relevant parties to allow sufficient time for review prior to submission. There will be a fifteen (15) day period to review abstracts or posters and a thirty (30) day period to review slides and manuscripts and respond to the author with any revisions.

22 ETHICAL CONSIDERATIONS

Before starting the trial, the protocol, patient information sheet and consent form must be approved by the RM/ICR joint Committee for Clinical Research. Once approved, the study may then be submitted to the relevant regulatory authorities.

It is the Chief and Principal Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The Chief and Principal Investigator must ensure this is documented in the patient's medical records and the patient is re-consented, where appropriate.

The Sponsor and Chief and Principal Investigator must ensure that the trial is carried out in accordance with the GCP principles and requirements of the UK Clinical Trials regulations (SI 2004/1031 and SI 2006/1928 as amended) and The Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/).

23 PEER REVIEW

This trial has been peer reviewed as part of the sponsorship approval process by the Royal Marsden and Institute of Cancer Research (ICR) Committee for Clinical Research (CCR). The CCR is comprised of senior staff within Royal Marsden and ICR with significant expertise in clinical research. The CCR approval process consists of expert peer review by a consultant-level clinician and a statistician who are both independent of the study team. The study has also received expert input from various support departments within the Trust as part of this process.

24 PUBLIC AND PATIENT INVOLVEMENT

The patient information sheet for this study will involve patients, service users, and/or their carers, or members of the public in particular to review the design of the research taking place.

25 REGULATORY COMPLIANCE

The study will be performed in compliance with UK regulatory requirements. Clinical Trial Authorisation (CTA) from the Medicines and Healthcare products Regulatory Authority (MHRA) will be obtained prior to the start of the study. In addition, the MHRA must approve amendments (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

25.1 Protocol compliance

The study will be performed in compliance with UK regulatory requirements. Clinical Trial Authorisation (CTA) from the Medicines and Healthcare products Regulatory Authority (MHRA) will be obtained prior to the start of the study. In addition, the MHRA must approve amendments (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

- prospective, planned deviations or waivers to the protocol are **not** allowed under the UK regulations on Clinical Trials and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol

- accidental protocol deviations can happen at any time. Deviations should be recorded and considered for their overall impact on the trial and/or participants. If a protocol deviation is suspected to be a potential serious breach, it must be escalated immediately to the Trials office who

will then inform the GCP compliance team as per sponsor gSOP-10. Regardless of seriousness, all deviations should be recorded, and the complete list should be available to the study statistician.

- deviations from the protocol which are found to frequently recur are not acceptable, will require immediate corrective & preventative action and could potentially be classified as a serious breach.

25.2   Notification of Serious Breaches to GCP and/or the protocol

The Sponsor will notify the MHRA and REC in writing of any serious breaches of:

a. The condition and principles of GCP in connection with the trial

b. The protocol

This will be done within 7 days if becoming aware of that breach, in accordance with the applicable UK regulations as amended from time to time.

For the Purpose of the regulations a "serious breach" is a breach which is likely to affect to a significant degree

a. The safety or physical or mental integrity of the subjects of the trial; or

b. The scientific integrity of the trial.

Systematic or persistent non-compliance by the site with GCP and/or the study protocol, including failure to report SAEs occurring on trial within the specified timeframes, may be deemed a serious breach.

26   DATA PROTECTION AND PATIENT CONFIDENTIALITY

The Principal investigator at each site must ensure that the patient's confidentiality is maintained throughout the study in compliance with the UK Data Protection Act of 1998. On the e-CRFs or other documents submitted to the RM-CTU, patients should be identified by their initials, date of birth and a patient study number only.

In compliance with GCP guidelines, it is required that the investigator and institution permit authorised representatives of the sponsor and of the regulatory agency(s) direct access to review the patient's original medical records for verification of study-related procedures and data. Direct access includes examining, analysing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform and obtain the consent of the patient to permit named representatives to have access to his/her study-related records without violating the confidentiality of the patient.

26.1   Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management

There are no competing interests for this study.

26.2 Indemnity

There are no specific compensation arrangements for harmful events which might arise from participation in this trial. However, the study is covered for negligent claims occurring with the NHS by Crown indemnity. There is no pre-existing arrangement for non-negligent claims arising from the conduct of the study.

26.3 Approval of Amendments

Any protocol amendment should be agreed with the appropriate trial oversight committee and be approved by the sponsor prior to submission and review by the relevant Ethics Committee and Competent Authority (as appropriate). Once Favourable Opinion is REC has been obtained amendments may be disseminated to sites and implemented following local R&D approvals. It is the responsibility of the Principal Investigator to submit amendment to their R&D department for R&D approval Amendments requiring REC approval may be implemented only after a copy of the REC/CA's approval letters has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to patients may be implemented prior to receiving Sponsor or REC/CA approval. However, in this case, approval must be obtained as soon as possible after implementation. Amendments to the protocol or associated documentation will be made when necessary and should be agreed by the trial management group or equivalent committee. The CI has responsibility for preparing the amendment and deciding whether the amendment is substantial. All amendments will be submitted for sponsorship approval prior to making REC and/or MHRA applications, and will not be implemented until NHS permission at the research site has been granted.

Amendments to the protocol will be summarised in the relevant appendix.

26.4 Access to the final trial dataset

Custodian of the data generated in this study will be held by the Chief Investigator.

27 DISSEMINATION POLICY

27.1 Dissemination of results policy

The trial results will be submitted for publication in a relevant peer-reviewed medical journal (see Authorship eligibility guidance in section 27.2). A summary of the trials results will also be uploaded to Clinical Trials.gov, and on the Royal Marsden website to be accessed by patients if desired.

Draft publications (manuscripts, abstracts, slides and posters) should be submitted to the RM-CTU for circulation to the relevant parties to allow sufficient time for review prior to submission. There will be a fifteen

(15) day period to review abstracts or posters and a thirty (30) day period to review slides and manuscripts and respond to the author with any revisions.

27.2   Authorship eligibility guidelines and any intended use of professional writers

The trial results will be submitted for publication in a relevant medical journal with authorship according to the criteria defined by the ICMJE icmje.org). These state that: Authorship credit should be based:

   i.   On substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND

   ii.   Drafting the work or revising it critically for important intellectual content; AND

   iii.   Final approval of the version to be published; AND

   iv.   Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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29 APPENDICES

APPENDIX A – RECIST 1.1

The following information is extracted/summarized from New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1)[46]. Please refer to the primary reference for further information.

1.1. Definitions

At screening, tumor lesions/lymph nodes will be categorized as measurable or nonmeasurable.

1.1.1. Measurable Disease

Tumor Lesions. Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as nonmeasurable)
- 20mm by chest X-ray

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

1.1.2. Nonmeasurable Disease

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥ 10 to < 15mm short axis) as well as truly nonmeasurable lesions. Lesions considered truly nonmeasurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.1.3. Special Considerations for Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local (radiation) therapy should be considered measurable or nonmeasurable.

1.2. Tumor Response Evaluation

1.2.1. Target Lesions

When more than one measurable tumor lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. Note that pathological nodes must meet the measurable criterion of a short axis of ≥ 15 mm by CT scan and only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered nontarget lesions. Nodes that have a short axis < 10 mm are considered nonpathological and should not be recorded or followed. At baseline, the sum of the target lesions (longest diameter of tumor lesions plus short axis of lymph nodes: overall maximum of 5) is to be recorded.

After baseline, a value should be provided on the CRF for all identified target lesions for each assessment, even if very small. If extremely small and faint lesions cannot be accurately measured but are deemed to be present, a default value of 5 mm may be used. If lesions are too small to measure and indeed are believed to be absent, a default value of 0 mm may be used.

1.2.2. Nontarget Lesions

All nonmeasurable lesions (or sites of disease) plus any measurable lesions over and above those listed as target lesions are considered nontarget lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as "present," "absent," or "unequivocal progression."

1.2.3. Response Criteria

Target and nontarget lesions are evaluated for response separately, and then the tumor burden as a whole is evaluated as the Overall response.

1.2.3.1. Target Lesion Response

Target lesions will be assessed as follows:

- Complete Response (CR). Disappearance of all target lesions. Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to <10 mm.
- Partial Response (PR). At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD). At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease (SD). Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

1.2.3.2. Nontarget Lesion Response

Nontarget lesions will be assessed as follows:

- Complete Response (CR). Disappearance of all nontarget lesions and normalisation of tumor marker level. All lymph nodes must be nonpathological in size (<10mm short axis).
- Non-CR/Non-PD. Persistence of one or more nontarget lesion(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease (PD). Unequivocal progression (see comments below) of existing nontarget lesions. (Note: the appearance of one or more new lesions is also considered progression).

When the patient also has measurable disease. In this setting, to achieve "unequivocal progression" on the basis of the nontarget disease, there must be an overall level of substantial worsening in nontarget disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more nontarget lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in nontarget disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only nonmeasurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above; however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in nonmeasurable disease burden. Because worsening in nontarget disease cannot be easily quantified (by definition: if all lesions are truly nonmeasurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in nonmeasurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: ie an increase in tumor burden representing an additional 73% increase in "volume" (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from "trace" to "large," an increase in lymphangitic disease from localised to widespread, or may be described in protocols as "sufficient to require a change in therapy." If "unequivocal progression" is seen, the patient should be considered to have had overall PD at that point. While it would be

ideal to have objective criteria to apply to nonmeasurable disease, the very nature of that disease makes it impossible to do so: therefore, the increase must be substantial.

1.2.3.3. Overall Response

Overall response should be assessed according to Table 10 for patients with target lesions, and Table 11 for patients with only nontarget lesions.

Table 10: Time Point Response: Patients With Target (± Nontarget) Disease

Target Lesions Response	Nontarget Lesion Response	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/ non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NE = inevaluable.

Table 11: Time Point Response: Patients With Nontarget Disease Only

Nontarget Lesions Response	New Lesions	Overall Response
CR	No	CR
Non-CR/ non-PD	No	Non-CR/ non-PDa
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

a "Non-CR/non-PD" is preferred over "stable disease" for nontarget disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NE = inevaluable.

1.2.4. Symptomatic Deterioration

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic

deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease.

APPENDIX B: ECOG PERFORMANCE STATUS

GRADE	DEFINITION
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

Appendix C: PROTOCOL AMENDMENT LOG

Appendix C: PROTOCOL AMENDMENT LOG

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APPENDIX 4. N3 TRIAL TSC CHARTER

N3

A Phase I/II Trial Of Combination Nab-paclitaxel And Nintedanib Or Nab-paclitaxel And Placebo In Relapsed NSCLC Adenocarcinoma

CCR 4448

IRAS: 199962

EUDRACT: 2016-000109-35

ClinicalTrials.gov Number:TBC

CTA: TBC

Trial Steering Committee Charter

Version 1.0, Date April 2017

Authorised by:

Name:	[name]	Role:	[Director]
Signature:		Date:	dd-mmm-yyyy

Prepared by:
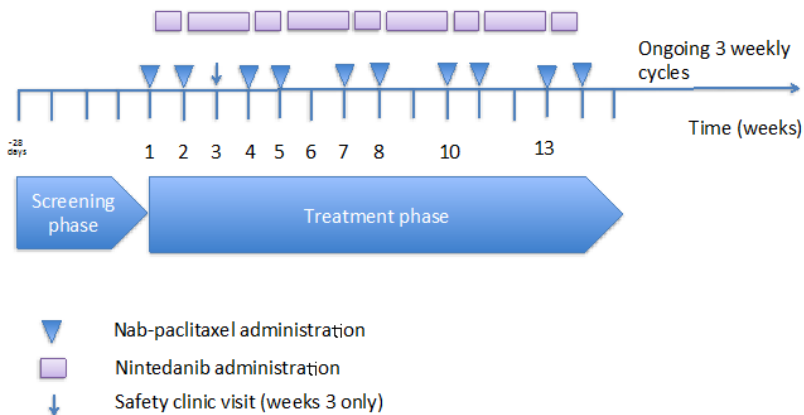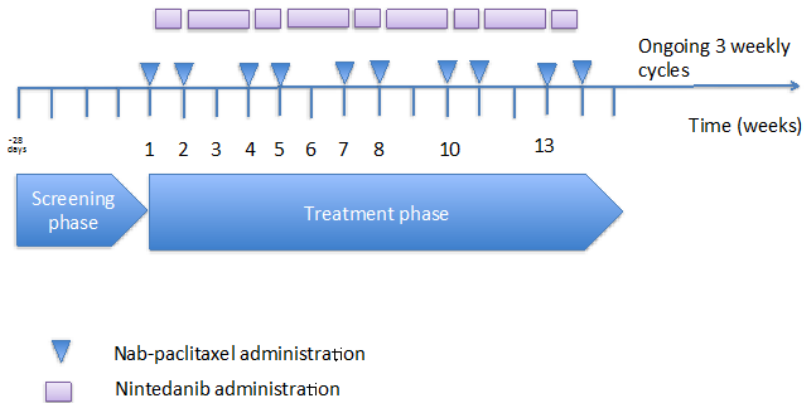
Name:	[name]	Role:	[role]
Signature:		Date:	dd-mmm-yyyy

CONTENT	DETAILS OF T/SSC
1. Introduction	
Name (& Sponsor's ID) of trial	A phase I/II trial of combination Nab-Paclitaxel and Nintedanib or Nab-Paclitaxel and Placebo in Relapsed NSCLC Adenocarcinoma
Objectives of trial, including interventions being investigated	**Study Objectives:** We propose to explore the safety, tolerability and efficacy of combination nab-paclitaxel and nintedanib in relapsed adenocarcinoma NSCLC. Part 1 one of the trial will evaluate the incidence of dose limiting toxicities when nab-paclitaxel is given in combination with nintedanib and a recommended phase 2 dose will be determined. Hypothesis to be explored in Part 2 of the trial is that addition of nintedanib to nab-paclitaxel is safe, tolerable and active in patients with relapsed advanced or metastatic adenocarcinoma NSCLC. **Study Endpoints:** **Part 1** **Primary:** - To define Maximum tolerated dose (MTD) and evaluate incidence of dose-limiting toxicities (DLTs) during Cycle 1 **Secondary:** - To examine the frequency of all Adverse Events graded by NCI-CTCAE version 4.0 - To examine the objective tumour response according to RECIST 1.1 (investigator reported), and the overall response rate - To define the number of cycles of nab-paclitaxel with nintedanib given **Part 2** **Primary:** - To explore PFS rate at 12 weeks from first dose of nab-paclitaxel with nintedanib/placebo **Secondary*:*** - To examine the frequency of all Adverse Events graded by NCI-CTCAE version 4.0 - To examine the objective tumour response according to RECIST 1.1 (investigator reported), and the overall response rate - To examine overall survival in the ITT and predefined subgroups (PD

CONTENT	DETAILS OF T/SSC
	pre/post 9 months from start of first line chemotherapy; prior or no prior immunotherapy). Trial visit schema phase I: ▼ Nab-paclitaxel administration ▢ Nintedanib administration ↓ Safety clinic visit (weeks 3 only) Trial visit schema: ▼ Nab-paclitaxel administration ▢ Nintedanib administration
Outline of scope of Charter	The purpose of this document is to describe the membership, terms of reference, roles, responsibilities, authority, decision-making and relationships of the Trial/Study Steering Committee (T/SSC) for this trial/study, including the timing of meetings, methods of providing information to and from the T/SSC, frequency and format of meetings and

CONTENT	DETAILS OF T/SSC
Facilitation	relationships with other trial/study committees. If the T/SSC covers only one Trial/Study then it will run for the duration of the Trial/Study. A member of the CTU staff will be nominated as a facilitator for the trial/study. The facilitator will be responsible for the organisation of meetings and should be copied into all communications with and between the T/SSC.

2. Roles and responsibilities

A broad statement of the aims of the TSC	To act as the oversight body for Phase I of the N3 trial on behalf of the Sponsor/Funder.
Terms of reference	The role of the TSC is to provide oversight for Phase I of the trial. It should also provide advice through its independent Chairman to the Trial/Study Management Group (T/SMG), Sponsor, Funder and the CTU on all aspects of the trial/study.
Specific roles of T/SSC	• provide expert oversight of the trial/study • maintain confidentiality of all trial information that is not already in the public domain • make decisions as to the future continuation (or otherwise) of the trials/studies • monitor recruitment rates and encourage the T/SMG to develop strategies to deal with any recruitment problems • review regular reports of the trial from the trials unit (sent on behalf of the Trial/Study Management Group (T/SMG)) • receive letters of feedback from the IDMC and consider their recommendations • assess the impact and relevance of any accumulating external evidence • monitor completion of CRFs and comment on strategies from TMG to encourage satisfactory completion in the future • monitor follow-up rates and review strategies from TMG to deal with problems • censure sites that are deviating from the protocol • approve any amendments to the protocol, where appropriate, such as in cases of significant change to the trial design • approve any proposals by the TMG concerning any change to the design

CONTENT	DETAILS OF T/SSC
	of the trial, including additional substudies
	• oversee the timely reporting of trial results
	• be informed of any abstracts and presentations of any results *during* the running of the trial
	• approve external or early internal requests for release of data or subsets of data or samples including clinical data and stored biological samples
	• maintain confidentiality of all trial/study information that is not in the public domain

3. Before or early in the trial/study

The involvement/input of the T/SSC into the protocol	All independent T/SSC members should have sight of the protocol soon after the TSC has been approached. Before recruitment begins the trial/study will have undergone review by the Sponsor, scrutiny by other trial committees and a research ethics committee. Therefore, if a potential independent TSC member has major reservations about the trial/study (e.g. the protocol, the logistics, ethical concerns) they should report these to the CTU/CI and may decide not to accept the invitation to join. TSC members should be constructively critical of the ongoing trial, but also supportive of aims and methods of the trial.
Whether members of the T/SSC will have a contract	T/SSC members will not be asked to formally sign a contract but should formally register their agreement to join the group by confirming (1) that they agree to be a member of the TSC and (2) that they agree with the contents of this Charter. Any potential competing interests should be declared at the same time. Members should complete and return the form in Annexes 2 or 3. Any observers (attendees who are not members) will sign a confidentiality agreement on the first occasion they attend a meeting (Annexe 4). .

4. Composition

Membership and size of the T/SSC	The Trial Steering Committee (TSC) will consist of TMG (Chief Investigator, Chief Co Investigator, Trial Statistician and Trial Manager) plus the site PI or representative from sites participating in Phase 1 and an independent chair. Further internal or external experts may be consulted by the TSC as necessary. Members of the T/SSC are listed in Annexe 1 of the Charter.

CONTENT	DETAILS OF T/SSC
The Chair, how they are chosen and the Chair's role.	The Chair should have previous experience of serving on trial/study committees and experience of Chairing meetings, and should be able to facilitate and summarise discussions; knowledge of the disease area would be beneficial.
Responsibilities of the CTU Trial Team	The Trial team will produce a short report on the trial before each meeting of the T/SSC. A template report will be agreed by the members prior to the first meeting and followed at all subsequent meetings.
Responsibilities of the CI and other members of the T/SMG	The CI (and, if appropriate, other TMG members) should be present at T/SSC meetings in person or by teleconference and no major decisions should be made without consultation with the CI and other appropriate members of the TMG.
The responsibilities of the observers	Additional observers may be in attendance through (parts of) the TSC meetings in order to provide input on behalf of the CTU, the trial/study's Sponsor/Funder or to provide specific relevant expertise.

5. Relationships	
Relationships with Chief Investigators, other trial committees (e.g. TMG and IDMC), Sponsor/Funder and regulatory bodies	The responsibilities and relationships of each trial/study committee are detailed in the protocol and in the respective Charters.
Advisory and executive bodies	The TSC is the oversight body and is delegated the roles in Section 2 by the Sponsor. All substantial issues regarding the trial must go to the T/SSC for consideration.
Payments to TSC members	No payments or rewards will be given to professional members.
The need for TSC members to disclose information about any real or potential competing interests	Any competing interests, both real or potential, should be disclosed. These are not restricted to financial matters, involvement in other trials or intellectual investment. Although members may be able to act objectively despite such connections, complete disclosure enhances credibility. (See Annex 2 and 3)
	T/SSC members should not use any trial data to inform trading in pharmaceutical shares, and careful consideration should be given to trading in stock of companies with competing products. Changes in declarations of real or potential competing interests should be minuted at the start of each

CONTENT	DETAILS OF T/SSC
	meeting.
6. Organisation of meetings	
Expected frequency of T/SSC meetings	The TSC will meet in person or by TC at the end of each cohort to regularly review toxicity data, define DLTs, and make decision to proceed to the next cohort (or not) cohort dosing and expansion cohort, and define the RP2D. At the request of the TSC, interim meetings, in person or by teleconference, will be organised. Some trial issues may need to be dealt with between meetings, by phone or by email. TSC members should be prepared for such instances.
Attendance of TSC members at meetings	Effort will be made to ensure that all members can attend. The Facilitator will work for a date that enables this. Members who cannot attend in person should be encouraged to participate by teleconference. If, at short notice, any TSC members cannot attend then the TSC may still meet if at least two members, including the Chair (unless otherwise agreed), will be present including a member of the trials office team. If the TSC is considering a major action after such a meeting the TSC Chair should communicate with the absent members, including the CI, as soon after the meeting as possible to check they agree. If they do not, a further teleconference should be arranged with the full TSC.
Meeting organisation for TSC	Presence will be usually limited to the TSC members. Other attendees may be invited for all or part of the meeting at the discretion of the TSC and the Facilitator.
TSC members inputting into a meeting they are unable to attend	The TSC report should be circulated before the meeting with sufficient time for members to read. TSC members who will not be able to attend the meeting may pass comments to the TSC Chair or Facilitator for consideration during the meeting.
Independent members who do not attend meetings	If a member does not attend a meeting or provide comments when requested between meetings, it should be ensured that is available for the next meeting. If a member does not attend the next meeting or provide comments when next requested, they should be asked if they wish to remain part of the TSC.

CONTENT	DETAILS OF T/SSC
7. Trial/Study documentation and procedures to ensure confidentiality and communication	
Intended content of material to be considered during meetings	A short report will be prepared by the CTU trial team following a standard template. This will report on accrual, any matters affecting the trial and safety information. Where relevant, accrual, compliance with follow-up and adherence to treatment may be presented by centre.
Report dissemination	The TSC will receive the report at least 1 week before any meetings.
Access to accumulating data and interim analysis.	The TSC will be have access to Phase I accumulating data and interim analyses in order to assess the safety of the Nab-Paclitaxel/Nintedanib combination and declare the recommended phase 2 dose.
Responsibility for identifying and circulating external evidence (e.g. from other trials/studies/ systematic reviews)	Identification and circulation of external evidence (e.g. from other trials/ systematic reviews) is not the responsibility of the TSC members; it is a responsibility of the TMG. However, the TSC should continue to be made aware of other data that may impact on a trial/study.
Communicating decisions made by the T/SSC	(See Section 9)
What will happen to the papers after the meeting	TSC members would be expected to delete, destroy or store securely copies of the reports to and from the TSC, agenda and minutes, as well as copies of communications between meetings. All documentation should be considered confidential. The Facilitator will keep a central record in the CTU of all minutes, reports and correspondence by the TSC.
8. Decision making	
What decisions will be open to the T/SSC	Possible decisions include:- • Dose escalation • Declaring RP2D and opening of phase II • Early stopping due, for example, to clear benefit or harm of a treatment, futility or external evidence • Sanctioning and/or proposing protocol changes Based on other factors, possible decisions include the decisions above and:- • Censuring centres for poor recruitment/poor data quality • Approving proposed protocol amendments or new trial sub-studies • Approving requests for early release of (subsets of) data • Approving external applications for the use of stored samples

CONTENT	DETAILS OF T/SSC
	• Approving presentation of results during the trial or soon after closure
	• Approval of new centres or strategies to improve recruitment or follow-up
The role of formal statistical methods	Formal statistical methods will have been considered by the study statistician/TMG in making recommendations to the TSC. These methods are usually considered guidelines rather than absolute rules, this is because they generally only consider one dimension of the trial. However any decision to disregard a stopping guideline should be noted in the minutes of the meeting..
How decisions or recommendations will be reached within the T/SSC	Every effort should be made to achieve consensus. The role of the Chair is to summarise discussions and encourage consensus; therefore, it is usually best for the Chair to give their own opinion last. It is important that the implications (e.g. ethical, statistical, practical, and financial) for the trial be considered before any decision is made and minuted.
Quoracy in the TSC	Quoracy will be achieved in the presence of TSC Chair, Chief Investigator, CTU representative and one other principal investigator.
Any specific issues relating to the trial design that might influence the proceedings	(See Section 3)
9. Reporting	
To whom will the T/SSC report their recommendations/decisions, and in what form	The TSC will report their decisions (via the Facilitator) to the Sponsor and TMG who will be responsible for implementing any actions resulting. This should be within 3 weeks of the meeting. The TSC may also provide feedback to the Sponsor/Funder where appropriate. Copies of communications will pass through the Facilitator.
Minutes of the meeting	Minutes of the meeting setting out the key points and actions will be taken by the Facilitator. This will include details of whether potential competing interests have changed for any attendees since the previous meeting. The draft minutes will be initially circulated for comment to those TSC members who were present at the meeting. The TSC Chair will sign off the final version of minutes or notes. The minutes of the meeting will be kept confidentially by the Facilitator. A copy of minutes excluding any confidential issues should be stored in the relevant TMF.

CONTENT	DETAILS OF T/SSC

10. After the trial/study	
Publication of results	The TSC will oversee the timely analysis, writing up and publication of the Phase I results. The members of the T/SSC may have the opportunity to read and comment on the proposed main publications of trial data prior to submission and abstracts and presentations during the trial.
T/SSC information included in published trial reports	TSC members will be named and their affiliations listed in the main report, unless they explicitly request otherwise.
Any constraints on T/SSC members divulging information about their deliberations after the trial has been published	TSC members should not divulge sensitive information about the ongoing trial unless specifically authorised by the TSC, depending on the nature of the information.

Annexe 1: The members of the TSC are:

(1) Dr Thomas Newsom-Davis, Consultant Medical Oncologist, Chelsea & Westminster Hospital (Chair and independent member)

(2) Dr Sanjay Popat, Consultant Medical Oncologist, The Royal Marsden Hospital (Chief Investigator)

(3) Dr Nadza Tokaca, Clinical Research Fellow, The Royal Marsden Hospital (Co-investigator)

(4) Ms Ann Petruckevitch, Senior statistician, The Royal Marsden Hospital.

(5) Ms Aude Espinasse. Senior Trial Manager, The Royal Marsden Hospital

(6) Phase I sites PIs/representatives – to be added

Please note: members may change throughout the duration of the trial and will be documented in the relevant Trial Master File.

Annexe 2: Agreement and competing interests form for independent members

N3 Trial Steering Committee name: Agreement to join the Trial/Study Steering Committee as an independent member and disclosure of potential competing interests

Please complete the following document and return to the T/SSC Facilitator.

(please initial box to agree)

I have read and understood the T/SSC Charter version xx.x, dated __/____/____

I agree to join the Trial/Study Steering Committee for this trial/study as an independent member

I agree to treat all sensitive trial/study data and discussions confidentially

The avoidance of any perception that independent members of a T/SSC may be biased in some fashion is important for the credibility of the decisions made by the T/SSC and for the integrity of the trial.

Potential competing interests should be disclosed via the CTR. In many cases simple disclosure up front should be sufficient. Otherwise, the (potential) independent T/SSC member should remove the conflict or stop participating in the T/SSC. **Table 1** lists potential competing interests.

I have no potential competing interests to declare
I have potential competing interests to declare (please detail below)

Please provide details of any potential competing interests:

Name: _____

Signed: _____   Date: _____

Table 1: Potential competing interests for independent members

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the Sponsor/Funder
- Ongoing advisory role to a company providing drugs to the trial
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial/study
- Hands-on participation in the trial/study
- Involvement in the running of the trial/study
- Emotional involvement in the trial/study
- Intellectual conflict e.g. strong prior belief in the trial/study's experimental arm
- Involvement in regulatory issues relevant to the trial/study procedures
- Investment (financial or intellectual) or career tied up in competing products
- Involvement in the writing up of the main trial/study results in the form of authorship

Annexe 3: Agreement and competing interests form for non-independent members

N3 Trial Steering Committee name: Agreement to join the Trial/Study Steering Committee as an non-independent member and disclosure of potential competing interests

Please complete the following document and return to the Facilitator.

(please initial box to agree)

☐ I have read and understood the T/SSC Charter version xx.x, dated __/____/_____

☐ I agree to join the Trial/Study Steering Committee for this trial as an non-independent member

☐ I agree to treat all sensitive trial/study data and discussions confidentially

The avoidance of any perception that members of a T/SSC may be biased in some undisclosed fashion is important for the credibility of the decisions made by the T/SSC and for the integrity of the trial.

Possible competing interests should be disclosed via the CTR. In many cases simple disclosure up front should be sufficient. Otherwise, the (potential) independent T/SSC member should remove the conflict or stop participating in the T/SSC. **Table 1** lists potential competing interests.

☐ I have no competing interests to declare other than involvement in the trial/study

☐ I have competing interests to declare (please detail below)

Please provide details of any competing interests:

_____

_____

_____

Name: _____

Signed: _____   Date: _____

Table 1: Potential competing interests for non-independent members

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the Sponsor/Funder
- Ongoing advisory role to a company providing drugs to the trial
- Frequent speaking engagements on behalf of the intervention
- Intellectual conflict e.g. strong prior belief in the trial/study's experimental arm
- Involvement in regulatory issues relevant to the trial/study procedures
- Investment (financial or intellectual) in competing products

Annexe 4: Agreement and confidentiality agreement for observers

N3 Trial Steering Committee: Agreement to attend the Trial/Study Steering Committee and treat all information confidentially

Please complete the following document and return to the Facilitator.

(please initial box to agree)

☐	I have received a copy of the T/SSC Charter version xx.x
☐	I agree to attend the Trial/Study Steering Committee meeting on ___/____/_____
☐	I agree to treat as confidential any sensitive information gained during this meeting unless explicitly permitted

Name: _____

Signed: _____   Date: _____

APPENDIX 5. N3 TRIAL IDMC CHARTER

N3 Trial – Royal Marsden CTU

A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA

Data Monitoring Committee (DMC) Charter

Version 1.1, 13th April 2017

Sponsor Protocol Number: CCR4448

EudraCT: 2016-000109-35

N3 Trial – Royal Marsden CTU

CONTENTS

1.   Introduction

The purpose of this document is to define the roles and responsibilities of the Data Monitoring Committee (DMC) for the N3 study, identify the individuals who will form the DMC and layout the schedule, format and timing of all meetings, and methods of providing information throughout the duration of the study In addition to providing advice on statistical issues and examine the relationships with other trial oversight committees.

2.   Roles and Responsibilities

2.1.   Responsibilities of DMC

The Data Monitoring Committee will be responsible for Part 2 of the study which will include:

- Review of the primary endpoint and final analysis, trial's progress including updated figures on recruitment, data quality, adherence to protocol treatment and follow-up and main outcomes and safety data

- Monitor evidence for treatment harm (e.g. toxicity, Serious Adverse Events and Serious Adverse Reactions, deaths)

- Assess the impact and relevance of external evidence

- Decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated, either for everyone or for some participant subgroups and/or centres

- Decide whether trial follow-up should be stopped

- Assess data quality including completeness (and by so doing, encourage collection of high quality data)

- Maintain confidentiality of all trial information that is not in the public domain

- Monitor recruitment figures and loss to follow-up

- Monitor compliance with the protocol by participants and investigators

- Consider the ethical implications of their recommendations.

- Monitor planned sample size assumptions

- Suggest additional data analyses if necessary

- Advise on major protocol modifications proposed by investigators or sponsors (e.g. to inclusion criteria, trial endpoints, or sample size)

- Monitor continuing appropriateness of patient information

- Monitor compliance with previous DMC recommendations
- Advice on the likelihood that continuation of the trial will allow detection of an important effect
  If at any stage an extension to the grant is needed

3. Membership

3.1. DMC Members

Name	Name of Institution	Role	Email
Robin Jones	Royal Marsden Hospital	Independent Chair	robin.jones4@nhs.net
Tom Newsom-Davis	Chelsea and Westminster Hospital	Independent Clinician/ TSC Chair	Tom.Newsom-Davis@chelwest.nhs.uk
Andre Lopes	UCL	Independent Statistician	andre.lopes@ucl.ac.uk
Sanjay Popat	Royal Marsden Hospital	Chief Investigator	sanjay.popat@rmh.nhs.uk
Ann Petruckevitch	RDSU, Royal Marsden	Trial Statistician	Ann.Petruckevitch@rmh.nhs.uk
Aude Espinasse	Royal Marsden Hospital	Senior Clinical Trial Manager	aude.espinasse@rmh.nhs.uk

4. Meetings

Data Monitoring Committee (DMC)

The DMC will meet to perform a monitoring role to review the data of the phase 2 trial of this study. They will be provided with all relevant results as necessary to perform this role. The first meeting will take place at the beginning of the study where the protocol will be reviewed and accepted. The second will be a primary endpoint analysis after all patients have completed their 12 weeks of treatment and the final meeting will be at the end of the study to discuss the final analysis

5.   Meeting Documentation

5.1.   Information to be provided to DMC members

Following information will be provided to DMC members prior to the meeting:

- Accumulating information relating to recruitment and data quality
- Toxicity details based on pooled data will be presented and
- Total numbers of events for the primary outcome measure and other outcome measures
- Efficacy and safety data from all patients enrolled into the study (for closed session)

DMC reports will ideally be prepared 2 weeks before the meeting.

6.   Decision making

6.1.   Decision making for DMC

Possible recommendations from the DMC include:

- No action needed, trial continues as planned
- Early stopping due, for example, to clear benefit or harm of a treatment, clear lack of benefit or external evidence.
- Extending recruitment
- Stopping recruitment within a subgroup
- Proposing or commenting on proposed protocol changes
- Commenting on Statistical Analysis Plan
- Other recommendations at the discretion of the DMC

This will be communicated to the Chief Investigator via the Trial Statistician; as soon as possible after the meeting. A copy of the letter will be logged with the N3 Team. If there is any information in the report that it is thought should not be shared with the CI, then the report should be sent only to the study statistician. In this case the report should explicitly state who should have access to the report. Such reports should be filed in the statisticians confidential files along with the DMC report. The study statistician should also be advised what to report to the CI.

The Chair is to summarise discussions and encourage consensus; it is usually best for the Chair to give their own opinion last. Every effort should be made for the DMC to reach a unanimous decision. If the DMC cannot achieve this a vote may be taken, although details of the vote should not be

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routinely included in the report to the TSC as these may inappropriately convey information about the state of the trial data.

It is important that the implications (e.g. ethical, statistical, practical and financial) for the trial be considered be-fore any recommendation is made. Every effort should be made to ensure that all members can attend, and the N3 Team will try to ensure that a date is chosen to enable this. If, at short notice, any DMC members cannot attend in any capacity then the DMC may still meet if at least one statistician and one clinician, including the Chair (unless otherwise agreed), will be present. If the DMC is considering recommending major action after such a meeting, the DMC Chair should communicate with the absent members as soon after the meeting as possible to check they agree; organising a further teleconference within 3-7 days where necessary. If they do not agree, a further meeting should be arranged with the full DMC.

N3 Trial – Royal Marsden CTU

APPENDIX I - Agreement and Potential Competing Interests Form

Agreement to join the N3 Data Monitoring Committee (DMC) and disclosure of potential competing interests*. Please complete the following document and return to the N3 Trial Manager at N3.trial@rmh.nhs.uk

(Please initial box to agree)

☐	I have read and understood the DMC Charter version 1.0, dated 17th March 2017
☐	I agree to join the DMC for this study
☐	I agree to treat all sensitive trial data and discussions confidentially

The avoidance of any perception that members of a DMC may be biased in some fashion is important for the credibility of the decisions made by the DMC and for the integrity of the trial. Possible competing interest should be disclosed via the N3 team. In many cases simple disclosure up front should be sufficient.   Otherwise, the (potential) DMC member should remove the conflict or stop participating in the DMC. Table 1 lists what is considered potential competing interests.

☐	No, I have no competing interests to declare
☐	Yes, I have competing interests to declare (please detail below)

Please provide details of any competing interests:

_____

_____

_____

Name: _____

Signed: _____   Date: _____

Table 1: Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the Sponsor (including CI for other MRC trials)
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict e.g. strong prior belief in the trial's experimental arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) or career tied up in competing products
- Involvement in the publication in the form of authorship

APPENDIX II - Agreement and Confidentiality Agreement for Observers

N3 Data Monitoring Committee Agreement to attend the Data Monitoring Committee and treat all information confidentially

Please complete the following document and return to the PERM Trial Manager/Coordinator at

N3.trial@rmh.nhs.uk

Please initial box to confirm agreement:

☐	I have received a copy of the DMC Charter version 1.0 dated 17th march 2016
☐	I agree to attend the DMC meetings __ / __ / _____
☐	I agree to treat as confidential any sensitive trial information gained during this meeting unless explicitly permitted

Name: _____

Signed: _____   Date: _____

APPENDIX III – Suggested template report from DMC to CI (where no recommendations are being made)

Date: [insert date]

To: CI
Via: Trial Statistician

Dear [CI]

The Data Monitoring Committee (DMC) for the N3 trial met on [meeting date] to review its progress and interim accumulating data. [List members] attended the meeting and reviewed the report.

The DMC would like to congratulate the investigators and trial team on the running of the trial and its recruitment, data quality and follow-up. The trial question remains important and, on the basis of the data reviewed at this stage, we recommend continuation of the trial according to the current version of the protocol [specify protocol version number and date] with no changes.

We shall next review the progress and data [provide approximate timing]

Yours sincerely,

[Name of meeting Chair]
Chair of Data Monitoring Committee

On behalf of the DMC (all members listed below)

DMC members:
(1) [Insert name and role]
(2) [Insert name and role]
(3) [Insert name and role]
(4) [Insert name and role]

APPENDIX IV - Summary of changes

Version Number	Date Effective	Reason for update
1.0	17th March 2017	revisions
1.1	13th April 2017	n/a

APPENDIX IV - Summary of changes

~To be left blank~

APPENDIX 6. N3 TRIAL PART 1/PHASE Ib PATIENT INFORMATION SHEET

Study EudraCT number: 2016-000109-35
Study Protocol number: CCR 4448

Participant Information Sheet – Part 1 (Phase Ib)

Full title: A Phase I/II trial of Combination Nab-paclitaxel and Nintedanib or Nab-paclitaxel and Placebo in Relapsed Non-Small Cell Lung Adenocarcinoma
Short title: N3 study

Introduction

We would like to invite you to take part in a research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. A member of our team will go through the information sheet with you, if needed the team will support you in your understanding and answer any questions you have. You can discuss the study with your family, friends and general practitioner if you wish. **Part 1** tells you the purpose of this study and what will happen to you if you take part. **Part 2** gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

Part 1

1   What is the purpose of this study?

This study will look at the safety and efficacy of using a new combination of drugs called nintedanib and nab-paclitaxel. The purpose of the study is to determine whether combination of these two drugs is safe and effective in patients with lung cancer who have progressed following initial chemotherapy treatment. The study will consist of two parts. The purpose of the first part (Phase I) of the study is to determine the optimal dose of nintedanib when given with nab-paclitaxel. The second part of the study (Phase II) will look at side-effects and effects on the cancer of the combination of nab-paclitaxel and nintedanib as compared with nab-paclitaxel and placebo. Effects on the cancer will be assessed by taking regular images of your tumours and measuring how these treatments can control the growth of your cancer.

2   What treatment is being tested?

The maximum amount of a drug called nintedanib that can be given with nab-paclitaxel is being studied to see how it makes you feel and if it has an effect in treating cancer.

Nab-paclitaxel is a chemotherapy drug that blocks growth of cancer by disrupting the function of structural proteins (called microtubules) inside cancer cells, preventing them from dividing and ultimately leading to cell death. Several thousand men and women have been treated with nab-paclitaxel for breast, pancreatic and lung cancers. In this study patients will receive nab-paclitaxel

at a dose that has been shown to be effective and tolerable in patients with other types of cancer, including older patients.

Patients taking part in the study will all receive nab-paclitaxel chemotherapy intravenously once per week for two consecutive weeks followed by a week break. This 3 week period is called a treatment cycle.

Nintedanib is a tyrosine kinase inhibitor that blocks the effect of blood vessel growth factors which are important for the development of blood vessels. Tumour cells may produce factors that stimulate the formation of new blood vessels (angiogenesis). The new blood vessels may help the tumour grow and possibly spread to other tissues where the tumour cells are then called metastases. Nintedanib is an "angiogenesis inhibitor" which can block this process so that fewer or no new vessels develop.

New blood vessel formation is not only needed for tumour growth, but also for normal wound healing, monthly changes to the uterus associated with the menstrual cycle, and when blood tissue supply is chronically reduced due to "vascular disease". If you have a serious wound that has not yet healed, or if you have serious vascular disease, you will not be eligible to enter this trial.

Nintedanib is included in capsules of two different strengths. If you participate in this trial you will have to swallow 1 or 2 capsules two times a day, depending on your dosage.

The nintedanib/placebo capsules used in this trial contain gelatin derived from pork. If you feel that taking these capsules may conflict with your personal or religious beliefs please discuss this issue with your treating physician prior to signing the consent form.

Nintedanib and nab-paclitaxel are both approved in the EU and the US for treatment of lung cancer, but have never been combined together.

In Part 1 (Phase I) of the study, patients will also receive nintedanib treatment in the form of capsules to be swallowed every day except on the day of their nab-paclitaxel infusion. Different patients will receive different amounts (doses) of nintedanib, as the purpose of this part of the trial is to establish the maximum amount of nintedanib that can be given with nab-paclitaxel without causing excessive side-effects (called dose-limiting toxicities).

In Part 2 (Phase II) of the study, approximately half of patients will receive nintedanib at the maximum tolerated dose determined during Part 1 together with nab-paclitaxel and half of patients will receive a placebo capsule together with nab-paclitaxel. For each patient a computer will randomly allocate whether they receive nintedanib or placebo. Study doctors, nurses, pharmacists and patients will not know whether the capsules contain nintedanib or placebo. The trial is designed in this way to prevent any bias in selecting patients to either group, which might affect the final results of the trial.

The following information is for patients invited to participate in part 1 (Phase I) of the study.

3 Why have I been chosen?

You have been invited to take part in this study because you have lung cancer that is not being controlled by your current treatment. Your doctor thinks you might be suitable to help with this research. Up to 194 patients are expected to take part (maximum of 24 in part 1 and 170 in part 2) from the Royal Marsden NHS Foundation Trust and several other hospitals in the UK.

4 Do I have to take part?

No. It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will give you this information sheet to keep and ask you to sign a consent form. You are free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive or any care in the future.

The study team or the Sponsor (Royal Marsden NHS Foundation Trust) may decide at any time, and for any reason, to stop the study, even though you may want to continue. This may occur if you have bad side effects during the study or if new information about the drug becomes available. Your doctor will explain the reasons why you have to stop and arrange for your medical care to continue as appropriate.

5 What will happen to me if I take part?

The study is divided into several steps:

Step 1: Consent and Screening Period (1-28 days before you start study treatment)

If you are interested in joining the study, you will be asked to sign and date the study consent form. A number of tests (some routine and some extra for the study) will be carried out in an initial screening period to check that you are eligible for the study. During this screening period, we will collect information from you about your condition and any medication you are taking (e.g. any medicines or over the counter treatments, including herbal or dietary supplements). The screening process will involve the following tests:

Routine tests:
- A physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities;
- Review of your medical history and record and medications you are taking;
- Blood samples (approximately 4 teaspoons/14mL in total) will be taken for routine tests. You will be asked to provide a single additional blood sample, which will be stored after the study has ended in a secure laboratory at the Royal Marsden hospital and may be used in future research.

- Perform Radiographic evaluation (such as Computed Tomography (CT) Scan or Magnetic Resonance Imaging (MRI)). These are special scans that take pictures of your tumour.
- A urine test.
- Collection of tissue that has been previously taken from your tumour for storing and use in subsequent research.
- Because of the possible risks to an unborn child, Pregnancy test is required for all female participants of childbearing potential. A serum pregnancy test will be performed at screening. Urine pregnancy test will be performed to assess participant's eligibility within 72 hours prior to the first administration of study drug, if the serum pregnancy test did not already occur within 72 hours of dosing.

If you are **confirmed to be eligible** to take part in the study you will be enrolled and will receive treatment within Phase I of the study.

If you are **not confirmed to be eligible**, you will not be able to take part in the study. However, your doctor will make alternative more suitable arrangements for the treatment of your condition.

Step 2: Treatment and Follow-up

If you are eligible to take part in this part of the study you will begin treatment with nab-paclitaxel together with nintedanib. You will continue receiving nab-paclitaxel and nintedanib for as long as it controls your cancer or until you have side effects that stop you from taking it. The total length of time you stay on the study will depend on how well your cancer responds to the study treatment and whether you have any side effects.

During the treatment phase we will look at 3 different doses of nintedanib: 100mg, 150mg or 200mg twice daily. The dose of nintedanib you will be given will depend on what stage the study is at. The starting dose of nab-paclitaxel will be the same for all patients.
Doses of nab-paclitaxel and nintedanib/placebo can be reduced in a step-wise fashion depending on any side-effects experienced during treatment. The dose cannot be increased again once it has been reduced.

Number of visits

During the treatment phase you will have clinic visits every week for the first 3 weeks.
After this, you will have clinic visits prior to start of every treatment cycle and prior to day 8 of each cycle (2 visits every 3 weeks) for as long as you are having treatment on the study:

Week 1: You will attend a clinic visit and receive your first dose of cycle 1 of nab-paclitaxel. You will be given capsules of nintedanib to take away with you and start taking the following day.
Week 2: You will attend a clinic visit and receive your second dose of cycle 1 of nab-paclitaxel. You will not take your nintedanib capsules on this day, but will restart the following day.

Week 3: You will attend a clinic visit for doctors to check how you are feeling and for blood tests. You will not receive any nab-paclitaxel but will continue taking nintedanib.

Week 4: You will attend a clinic visit and receive your first dose of cycle 2 of nab-paclitaxel. You will be given more nintedanib capsules to take home to start taking the following day.
Week 5: You will attend a clinic visit and receive your second dose of cycle 2 of nab-paclitaxel. You will not take your nintedanib capsules on this day but will restart the following day.

Week 6: You will not need to attend a clinic visit, this is your break week. You will continue to take nintedanib capsules twice daily until your next visit.

From here on, you will continue visits as outlined for week 4 – 6, on a three weekly cycle.

Trial visit schema phase I:
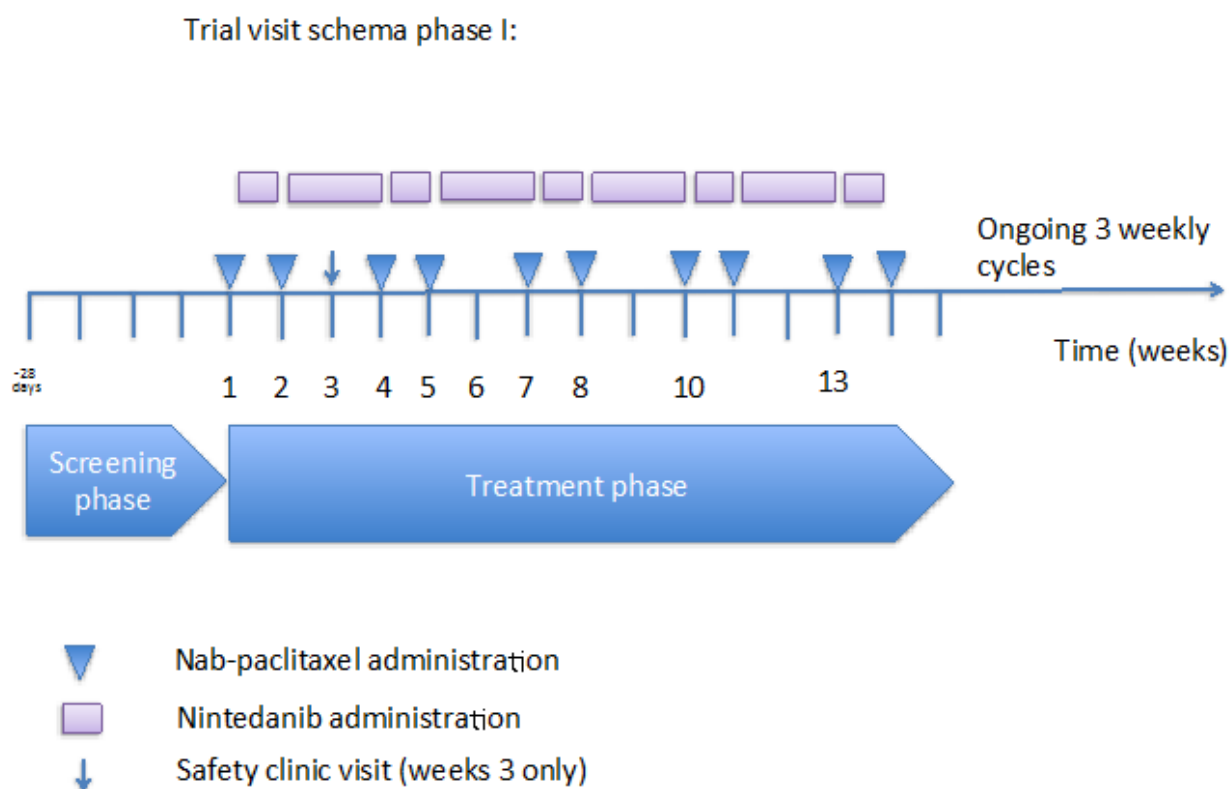
You will continue treatment with nab-paclitaxel and nintedanib as above as long as they are helping to control the cancer and the side-effects are tolerable. If you have to discontinue nab-paclitaxel due to side-effects you will be allowed to continue taking nintedanib, and vice-versa.

Before you are given each new dose of nab-paclitaxel you will need to undergo some tests. These tests are to monitor your progress. At your visit, your doctor will ask you questions to see how you are feeling and if you are having any side effects. Your doctor will also carry out the following:

Routine tests:

- A physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and your disease status;
- Ask if you have taken any new medications;
- Ask about any side effects that you have experienced.
- Take blood samples (approximately 4 teaspoons/14mL) for routine blood tests. Every effort will be made to take all blood samples from a single needle stick.
- Measurements of the size of your tumour using a CT scan (every 6 weeks/after every 2 cycles of treatment as long as you are receiving nab-paclitaxel and/or nintedanib/placebo).

If everything is OK and you wish to continue you will then be given your next dose of nab-paclitaxel and will continue taking nintedanib capsules. This will be repeated at each clinic visit.

Step 3: Discontinuation of study treatment

If your cancer grows, you have unacceptable toxicities or you no longer wish to participate in the study your doctor will ask you to stop taking the study drugs. In addition, Royal Marsden NHS Foundation Trust (Sponsor) may decide to stop the study for reasons other than those listed. When you permanently discontinue study medication (if it is not at a clinic visit), we will immediately see you in clinic for a review visit (end of study treatment visit) and your doctor will carry out the following tests/assessments, with your permission:

- Physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and you disease status;
- Ask about any side effects that you have experienced;
- Blood samples (approximately 4 teaspoons/14mL) for routine blood tests;
- Measurements of the size of your tumour using a CT/MRI scan (if you have not had an measurement taken within the last 6 weeks);

30 Days Safety Follow-up Visit

After the end of study treatment visit, you will be asked to come back to hospital one more time, 30 days later or earlier if you start a new treatment, to follow up on medication you have taken and to check on your general health. The following tests/assessments will be carried out:
- Physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and you disease status;
- Ask about any side effects that you have experienced;
- Blood samples (approximately 4 teaspoons/14mL) for routine blood tests.

Post-Safety Follow-up Visits

We would like to follow you up every 12 weeks. You do not need to attend a visit but the hospital team will review your hospital records and/or contact you or your GP to find out what has been happening with your cancer.

Tissue Samples – Optional

In this study we will also ask for your permission to collect your archived tumour samples that are stored in your local hospital pathology department. These samples were taken at the time of your initial diagnosis or when your cancer came back.

These samples will be stored in a secure laboratory and may be used in subsequent studies to identify specific markers in your cancer cells which may help explain how you cancer responds to treatment.

6   What do I have to do?

If you take part in this study, you will need to follow the treatment plan and hospital appointments outlined in section 5 above. You should consider how these tests and visits will affect your work and family life and decide if you are able to commit to the required visits and tests. We will monitor you closely during the study for any symptoms of side effects, but it is very important that you also tell your doctor about **any** changes in your health, even if you do not think they are related to taking part in the study.

You must inform your doctor of any medications you are currently taking or you intend to use once you have entered the study in case these affect you. Your doctor will provide you with a list of therapies / medications that you must avoid while you are taking part in the study and discuss these with you.

Female participants of child bearing potential and male participants must agree to use effective methods of birth control or complete abstinence from heterosexual contact to prevent pregnancy. Participants with reproductive potential (males and females, including females who have had a tubal ligation) must use reliable means of contraception during the study and for a period of 3 months after the last dose of study drug. Your doctor can discuss suitable methods of birth control with you.
Female participants may not breastfeed while they are in the study

All pregnancies or suspected pregnancies occurring in either a female participant of childbearing potential or partner of childbearing potential of a male participant must be reported to the Chief Investigator and the Sponsor immediately. Participants who become pregnant must be discontinued from trial treatment immediately.

You must not be a blood donor at any time during the study treatment period.

7   Will I be compensated for taking part in this study?

There is unfortunately no payment available for your participation in this study, including lost earnings and your time but a modest bursary is available to help with travelling expenses. The

study treatment, visits to see your research doctor and laboratory tests related to this study will be provided at no cost to you. The doctors and nurses looking after you receive no payment for your participation in this study.

8   What are the possible disadvantages and risks of taking part?

Study medication: You may experience side effects, such as those described in section 9 or others not known yet. During the time you receive treatment you will be examined regularly by your doctor and several tests will be performed to check for side effects.

IV line: for infusion of nab-paclitaxel may cause: discomfort, irritation, mild bruising, bleeding, leakage of drug solution, and rarely infection, nausea, and light-headedness.

Blood Samples: As with all blood tests, there is a possibility of slight redness, inflammation and/or bruising developing at the site where the needle is placed into your arm.

CT (Computerised Tomography) Scan: This is a special type of X-Ray to allow your doctor to see a three dimensional picture of your tumour. This is painless and will take about 10-20 minutes. You may have an injection with a type of dye just before the scan to help make the scan clearer. This may result in a slight burning at the injection site, a metallic taste in your mouth, a sensation of wanting to pass urine or hot flushes. Very rarely an allergic reaction to the contrast dye may occur. Such reactions can involve itching, a rash or in severe cases difficulty in breathing and lowering of blood pressure. If you know of any allergic reaction to imaging contrast dyes you should let your doctor or radiologist know.

Radiation Risks: CT scans and chest x-rays involve exposure to ionising radiation, which carries an associated risk of inducing cancer after a period of time which can be 5-10 years for leukaemia and up to 20-30 years for other tumours. However, the risks are very small compared to the normal lifetime risk of getting cancer, which is 1 in 4, and for patients with your clinical condition these risks may be considered to be negligible when compared to the potential benefits of participation in this study.

MRI (Magnetic Resonance Imaging) Scan: You will have this if appropriate. It is like a CT scan but takes approximately 30 to 45 minutes and is noisy. You will be asked to lie very still on a couch inside a metal tunnel. If you suffer from claustrophobia (fear of enclosed spaces) you will probably find a MRI scan uncomfortable. When needed a special type of contrast dye will be injected into a vein to improve the quality of images. Reactions to this dye are rare and usually no more severe than a headache. MRI scans do not involve the use of radiation.

General: You should be aware that certain insurance covers, such as medical or travel insurance may be affected by participation in a clinical study. Please contact your insurance company to see if participating in the study will affect your insurance.

9   What are the effects of any treatment received when taking part?

You may have side effects while you are in the study, but you will be carefully checked by the study doctor for any problems. As with any drug, an allergic reaction can occur. Allergic reactions can be mild or more serious, and can even result in death. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or breathing difficulties. If you think you are having an allergic reaction, call the trial doctor right away. Patients who have known allergies to the study medications or its ingredients should not take part in this study. This will be the first time that these drugs will be used in combination with one another. Therefore, there may be some risks or side effects that we are not aware of. These side effects may be mild or may be more serious, and, if necessary, your doctor will give you medicine to help lessen the symptoms of the side effects. You should tell the study doctor/ staff about anything that is bothering you or any side effects you have, even if you do not think they are related to the study drug. If you need to contact them outside your schedules visits, then their telephone numbers are at the end of this information sheet.

Nab-paclitaxel

The following is a list of the most medically significant or most common side effects reported in completed studies considered to be related to nab-paclitaxel:

Very common side-effects (may affect more than one in ten participants):

- Loss of hair (the majority of cases of hair loss happened less than one month after starting nab-paclitaxel. When it happens, hair loss is pronounced (over 50%) in the majority of patients)
- Rash
- Abnormal decrease in the number of types of white blood cells (neutrophils, lymphocytes or leukocytes) in the blood
- Deficiency of red blood cells
- Reduction in the number of platelets in the blood
- Effect on peripheral nerves (pain, numbness, tingling, temporary or permanent loss of feeling)
- Pain in a joint or joints
- Pain in the muscles
- Pain in extremities
- Nausea, diarrhoea, constipation,
- Vomiting
- Weakness and tiredness
- Fever
- Dehydration
- Sore mouth, taste disturbance loss of appetite and weight loss
- Low levels of potassium in the blood

- Depression

- Sleep problems

- Headache

- Chills

- Difficulty in breathing

- Dizziness

- Swelling of mucosal and soft tissues

- Increased liver function tests

- Cough

- Abdominal pain

- Nose bleeds

Common side effects (may affect up to 1 in 10 patients):

- Itching, dry skin

- Nail disorders

- Infection, fever with decrease in the number of a type of white blood cell (neutrophils) in the blood, flushing, thrush, severe infection in your blood which may be caused by reduced white blood cells

- Reduction in all blood cell counts

- Chest or throat pain

- Indigestion

- Stuffy nose

- Pain in back, bone pain

- Diminished muscular coordination or difficulty in reading, increased or decreased tears, loss of eyelashes

- Changes in heart rate or rhythm

- Heart failure

- Decreased or increased blood pressure

- Redness or swelling at the site where the needle entered the body

- Anxiety

- Infection in the lungs

- Infection in the urinary tract

- Obstruction in the gut, inflammation of the large bowel, inflammation of the bile duct

- Acute kidney failure

- Increased bilirubin in the blood

- Coughing up blood

- Dry mouth, difficulty in swallowing

- Muscle weakness

- Blurred vision

Uncommon side effects (may affect up to one in a hundred people) :

- Increased weight

- Increased lactate dehydrogenase in the blood increased or decreased blood sugar, increased or decreased phosphorus in the blood

- Decreased kidney function

- Decreased or lack of reflexes, involuntary movements, pain along a nerve, facial nerve paralysis

- Fainting, dizziness when standing up, shaking

- Irritated eyes, painful eyes, red eyes, itchy eyes, double vision, reduced vision, or seeing flashing lights, blurred vision due to swelling of the retina (cystoid macular oedema)

- Ear pain, ringing in your ears

- Coughing with phlegm, shortness of breath when walking or climbing stairs, decreased breath sounds, water on the lung

- Pain and swelling in the nose, runny nose or dry nose

- Loss of voice, dry throat

- Blood clot in the lung

- Painful or sore gums

- Gas, stomach cramps, rectal bleeding

- Painful urination, frequent urination, blood in the urine, inability to hold your urine

- Fingernail pain, fingernail discomfort, loss of fingernails

- Hives, skin pain, skin infections, red skin from sunlight, skin discolouration, increased sweating, night sweats, white areas on the skin, sores, swollen face

- Fluid retention, low albumin in the blood, increased thirst

- Decreased calcium in the blood, decreased sodium in the blood

- Infection due to catheter line

- Bruising

- Pain at site of tumour

- Decreased blood pressure when standing up, coldness in your hands and feet

- Difficulty walking, leg swelling

- Allergic reaction

- Decreased liver function, increased size of liver

- Pain in the breast

- Restlessness

- Small bleedings in your skin due to blood clotsA condition involving destruction of red blood cells and acute kidney failure

Rare side effects (May affect up to one in a thousand people):
- Skin reaction to another agent or lung inflammation following radiation

- Blood clot

- Very slow pulse

- Heart attack

- Leaking of drug outside the vein

- A disorder of the electrical conduction system of the heart (atrioventricular block)

Very rare side effects (May affect up to one in ten thousand people):
- Severe inflammation/eruption of the skin and mucous membranes (Stevens-Johnson syndrome, toxic epidermal necrolysis)

Additional side effects observed during post-marketing surveillance of nab-paclitaxel, not otherwise noted above include:

- lack of movement in the vocal cords with possible voice changes
- skin sensitivity to sunlight
- skin or tissue damage from prior radiation therapy can become damaged again, when a person receives chemotherapy after having had radiation therapy. This is referred to as radiation recall and may involve redness, peeling, pain, and swelling. Skin changes have been noted to range from mild redness to tissue death. Radiation recall may also occur in the lungs and other internal organs.

Elderly

Patients over 65 years old may experience the following side effects more often than younger patients: nose bleed, diarrhoea, dehydration (loss of water and minerals in the body), feeling tired or weak, and swelling caused by fluid held in the tissues, especially of the ankles, feet or fingers.

Nintedanib

Treatment with nintedanib as single agent or in combination with standard doses of other chemotherapy drugs may cause side effects. The possible side effects related to the administration of nintedanib or the combination of nintedanib with standard doses of various chemotherapeutic compounds are listed below and are based on a total of 1932 treated cancer patients.

Very common side effects (may affect more than one in ten people):

- Diarrhoea
- Painful, numb and/or tingling feeling in fingers and toes
- Nausea
- Vomiting
- Pain in the stomach (abdomen)
- Bleeding
- Decrease in the number of white blood cells
- Inflammation of the mucous membranes lining the digestive tract including sores and ulcers in the mouth
- Rash
- Decreased appetite
- Electrolyte imbalance
- Increased liver enzyme values in the blood as seen from blood tests

Common side effects (may affect up to one in ten people):

- Blood poisoning
- Decrease in the number of white blood cells accompanied by fever)
- Decrease in the number of platelets
- Blood clots in the veins
- High blood pressure
- Dehydration
- Abscesses
- Jaundice

Uncommon side effects (may affect up to one in a hundred people):

- Occurrence of holes in the wall of your gut

These treatment-related adverse events were usually reversible and most of these adverse events can be treated or resolving by temporarily or permanently stopping nintedanib. If you experience

any of these side effects or any other side effect which might be related to the intake of the study drug(s), your study doctor may adjust your medication and give you advice on how you can minimise these side effects.

Drugs acting similarly as nintedanib, currently under clinical investigation or already used in the clinical practise, other adverse events than those reported in patients treated with nintedanib alone or in combination with the standard doses of chemotherapy, have been reported. These included renal impairment and blood clots in arteries which may also occur during treatment with nintedanib / nintedanib plus chemotherapy. Please also inform your trial doctor in case you experience such type of adverse event..

10   What are the possible benefits of taking part?

It is hoped that nab-paclitaxel in combination with nintedanib may slow down the growth of your cancer. We cannot promise that the study will help you as an individual directly, however, the information we get might help us understand the behaviour of the drugs, their safety and tolerability. We may gain insights into the treatment of your disease which may lead to better management of patients and you will have helped by taking part.

11   What are the alternatives to treatment?

If you should decide not to participate in, or if you withdraw from this study, the study doctor can recommend other treatments which may include a marketed drug, treatment with a different investigational drug or best supportive care. Please make sure you discuss all the available treatment options with your research doctor before deciding if you want to take part in this study. Your doctor will organise any other treatment or care you may need.

12   What happens when the research study stops?

You will receive nab-paclitaxel and/or nintedanib/placebo for as long as the drugs are controlling your cancer, and the study doctor believes it is in your best interest; you will continue to receive the study drugs until either your disease progresses (becomes worse) or you permanently stop treatment for another reason e.g. you have intolerable side effects or you withdraw your consent. At this point one or the other or both of the drugs will be withdrawn. When the research study stops, your doctor will assess your status and recommend a suitable treatment.

13   What if there is a problem?

Your hospital doctor will be there to answer any questions you might have regarding your disease and your participation in this study. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study there will be several options available to you. The details are included in Part 2 of this information sheet.

14   Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2 of this information sheet

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

15 What if new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to continue. If you decide to continue in the study your research doctor will ask you to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

16 What will happen if I don't want to carry on with the study?

You may decide to stop and withdraw from the study at any time without giving a reason. A decision to withdraw at any time will not affect the standard of care you receive and your legal rights. After discussion with your doctor, you will be offered the treatment felt to be best for you at that time. You must tell your doctor immediately if you no longer wish to take part in the study. If you withdraw, we will not collect any new information about you but will ask you if we can keep previously collected information, blood samples and tissue samples.

Your study doctor can also take you off study treatment at any time if for example your condition becomes worse or another condition develops that may mean you are unable to carry on taking study treatment.

17 What if there is a problem?

If you experience harm

Royal Marsden NHS Foundation Trust holds insurance policies, which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation under the NHS indemnity scheme. This does not affect your legal rights to seek compensation. Please contact your study doctor if you would like further information about the insurance arrangements, which apply to the trial.

If you have any complaints

Regardless of the above, if you wish to complain or have any concerns about the way that you have been approached or treated during the course of this study, you should ask to speak to the PALS team or the researchers who will do their best to answer your questions (contact details at the end of this information sheet). If you remain unhappy and wish to complain formally, the usual National Health Service complaint mechanisms will be available to you. Details can be obtained from your study doctor or nurse. If you are still not satisfied with the response, you may contact the sponsor, Royal Marsden NHS Foundation Trust Research Office.

18 Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be entered onto a database and kept strictly confidential. If you decide to take part in the study, you give us

permission to use information about you and share it with individuals from the Royal Marsden study team, authorised people from UK regulatory bodies and members of the local NHS trust. This permission continues until the study is over, including the length of time that we must keep records about the study. Information will be labelled with a unique code number, and will not include your name or other personal information that directly identifies you. When you sign the consent form, you agree to have your personal and medical information used as described in this section.

19   Will information about this trial be included in a Registry Databank?

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as encouraged for transparency and required by some academic journals. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

20   Involvement of the General Practitioner / Family Doctor

With your permission your GP will be notified of your participation in this study so that they are aware of the treatment you are receiving. We will ask you to sign that you consent to us informing your GP.

21   What will happen to any samples I give?

Blood samples

A single sample of blood taken at the screening visit will be stored at a secure laboratory after the study is completed and kept for the duration of the study. The rest of blood samples taken during the study will be processed and destroyed according to standard practice at the local laboratory.

Tissue samples

Archival tissue samples will be stored at a secure laboratory after the study is completed. Archival tumour samples will be kept for the duration of the study and then returned to your local hospital pathology department.

22   What rights do I have to the results of the research?

Any information derived directly or indirectly from this research, as well as any patents, diagnostic tests, drugs, or biological products developed directly or indirectly as a result of this research, are the sole property of the study sponsor (and its successors and licensees) and may be used for commercial purposes. You will have no right to this property or to any share of the profits that may be earned directly or indirectly as a result of this research. However, in signing the consent form and offering samples for research, you do not give up any rights that you would otherwise have as a participant in research.

23 What will happen to the results of the study?

A group of independent experts will review the progress of the trial and the results will be published in medical journals and/or presented at a national/international meeting as soon as there is enough information to be sure that the results are reliable. The confidentiality of all participants will be kept in all reports and publications that may arise from this study. When the study is completed we aim to make the results available to view on the Royal Marsden website (royalmarsden.nhs.uk, and follow the links to the study results page). If you do not have access to a computer and would like to know the results, please get in touch with the study team who can arrange to send you a copy.

24 Who is organising and funding the research?

This is a study sponsored by Royal Marsden NHS Foundation Trust and coordinated by the Royal Marsden Clinical Trials Unit. Funding is provided by the pharmaceutical companies Celgene and Boehringer Ingelheim who are also providing the study drugs, nab-paclitaxel and nintedanib, free of charge to all participants. Your doctor will not receive any personal financial payment if you take part.

25 Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study was reviewed and given a favourable opinion by XXXX.

26 Contact for further information

If you have any questions or concerns about this study, including study related injury or study medication queries, please contact your study doctor or research nurse. The doctor in charge of this study is*:*

Name:

Title: .

Address:

If at any time you are concerned or require additional information, please contact one of the study team (9 am to 5 pm)

Study Team:
Xxxxxxxxx

PALS Team:
xxxxxxxxx

Cancer Research UK provides general information for patients about cancer and its treatment as well as about clinical trials on their website www.cancerhelp.org.uk. A confidential information

service is provided by specialist nurses on Tel: 0808 8004040. Macmillan Cancer Support (www.macmillansupport.org.uk; Tel: 0808 800 0000) also provides support and counselling to help people living with cancer.

__Thank you for taking time to read this information leaflet. If you think you will take part in the study please read and sign the consent form__

APPENDIX 7. N3 TRIAL PART 2/PHASE II PATIENT INFORMATION SHEET

Study EudraCT number: 2016-000109-35

Study Protocol number: CCR 4448

IRAS 199962

Participant Information Sheet – Part 2 (Phase II)

Full title: A Phase I/II trial of Combination Nab-paclitaxel and Nintedanib or Nab-paclitaxel and Placebo in Relapsed Non-Small Cell Lung Adenocarcinoma

Short title: N3 study

Introduction

We would like to invite you to take part in a research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. A member of our team will go through the information sheet with you, if needed the team will support you in your understanding and answer any questions you have. You can discuss the study with your family, friends and general practitioner if you wish. **Part 1** tells you the purpose of this study and what will happen to you if you take part. **Part 2** gives you more detailed information about the conduct of the study. Please ask us if there is anything that is not clear.

Part 1

1   What is the purpose of this study?

This study will look at the safety and efficacy of using a new combination of drugs called nintedanib and nab-paclitaxel. The purpose of the study is to determine whether combination of these two drugs is safe and effective in patients with lung cancer who have progressed following initial chemotherapy treatment. The study will consist of two parts. The purpose of the first part (Phase I) of the study is to determine the optimal dose of nintedanib when given with nab-paclitaxel. The second part of the study (Phase II) will look at side-effects and effects on the cancer of the combination of nab-paclitaxel and nintedanib as compared with nab-paclitaxel and placebo. Effects on the cancer will be assessed by taking regular images of your tumours and measuring how these treatments can control the growth of your cancer.

2   What treatment is being tested?

The maximum amount of a drug called nintedanib that can be given with nab-paclitaxel is being studied to see how it makes you feel and if it has an effect in treating cancer.

Nab-paclitaxel is a chemotherapy drug that blocks growth of cancer by disrupting the function of structural proteins (called microtubules) inside cancer cells, preventing them from dividing and ultimately leading to cell death. Several thousand men and women have been treated with nab-

paclitaxel for breast, pancreatic and lung cancers. In this study patients will receive nab-paclitaxel at a dose that has been shown to be effective and tolerable in patients with other types of cancer, including older patients.

Patients taking part in the study will all receive nab-paclitaxel chemotherapy intravenously once per week for two consecutive weeks followed by a week break. This 3 week period is called a treatment cycle.

Nintedanib is a tyrosine kinase inhibitor that blocks the effect of blood vessel growth factors which are important for the development of blood vessels. Tumour cells may produce factors that stimulate the formation of new blood vessels (angiogenesis). The new blood vessels may help the tumour grow and possibly spread to other tissues where the tumour cells are then called metastases. Nintedanib is an "angiogenesis inhibitor" which can block this process so that fewer or no new vessels develop.

New blood vessel formation is not only needed for tumour growth, but also for normal wound healing, monthly changes to the uterus associated with the menstrual cycle, and when blood tissue supply is chronically reduced due to "vascular disease". If you have a serious wound that has not yet healed, or if you have serious vascular disease, you will not be eligible to enter this trial.

Nintedanib is included in capsules of two different strengths. If you participate in this trial you will have to swallow 1 or 2 capsules two times a day, depending on your dosage.

The nintedanib/placebo capsules used in this trial contain gelatin derived from pork. If you feel that taking these capsules may conflict with your personal or religious beliefs please discuss this issue with your treating physician prior to signing the consent form.

Nintedanib and nab-paclitaxel are both approved in the EU and the US for treatment of lung cancer, but have never been combined together.

In Part 1 (Phase I) of the study, patients will also receive nintedanib treatment in the form of capsules to be swallowed every day except on the day of their nab-paclitaxel infusion. Different patients will receive different amounts (doses) of nintedanib, as the purpose of this part of the trial is to establish the maximum amount of nintedanib that can be given with nab-paclitaxel without causing excessive side-effects (called dose-limiting toxicities).

In Part 2 (Phase II) of the study, approximately half of patients will receive nintedanib at the maximum tolerated dose determined during Part 1 together with nab-paclitaxel and half of patients will receive a placebo capsule together with nab-paclitaxel. For each patient a computer will randomly allocate whether they receive nintedanib or placebo. Study doctors, nurses, pharmacists and patients will not know whether the capsules contain nintedanib or placebo. The trial is designed in this way to prevent any bias in selecting patients to either group, which might affect the final results of the trial

The following information is for patients invited to participate in part 2 (Phase II) of the study.

3   Why have I been chosen?

You have been invited to take part in this study because you have lung cancer that is not being controlled by your current treatment. Your doctor thinks you might be suitable to help with this research. Up to 194 patients are expected to take part (maximum of 24 in part 1 and 170 in part 2) from the Royal Marsden NHS Foundation Trust and several other hospitals in the UK.

4   Do I have to take part?

No. It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will give you this information sheet to keep and ask you to sign a consent form. You are free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive or any care in the future.

The study team or the Sponsor (Royal Marsden NHS Foundation Trust) may decide at any time, and for any reason, to stop the study, even though you may want to continue. This may occur if you have bad side effects during the study or if new information about the drug becomes available. Your doctor will explain the reasons why you have to stop and arrange for your medical care to continue as appropriate.

5   What will happen to me if I take part?

The study is divided into several steps:

Step 1: Consent and Screening Period (1-28 days before you start study treatment)
If you are interested in joining the study, you will be asked to sign and date the study consent form. A number of tests (some routine and some extra for the study) will be carried out in an initial screening period to check that you are eligible for the study. During this screening period, we will collect information from you about your condition and any medication you are taking (e.g. any medicines or over the counter treatments, including herbal or dietary supplements). The screening process will involve the following tests:

Routine tests:
- A physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities;
- Review of your medical history and record and medications your are taking;

- Blood samples (approximately 4 teaspoons/14mL in total) will be taken for routine tests. You will be asked to provide a single additional blood sample which will be stored after the research has ended in a secure laboratory at the Royal Marsden hospital and may be used in future research.
- Perform Radiographic evaluation (such as Computed Tomography (CT) Scan or Magnetic Resonance Imaging (MRI)). These are special scans that take pictures of your tumour.
- A urine test.
- Collection of tissue that has been previously taken from your tumour for storing and use in subsequent research.
- Because of the possible risks to an unborn child, Pregnancy test is required for all female participants of childbearing potential. A serum pregnancy test will be performed at screening. Urine pregnancy test will be performed to assess participant's eligibility within 72 hours prior to the first administration of study drug, if the serum pregnancy test did not already occur within 72 hours of dosing.

If you are **confirmed to be eligible** to take part in the study you will be enrolled and will receive treatment within Phase II of the study. You will be randomly allocated to one of two treatment groups to receive nab-paclitaxel with nintedanib or nab-paclitaxel with placebo. The treatment will be randomly allocated by a computer, which is like making a choice by tossing a coin. This means that you have an equal chance of being treated with one of the above treatments.

If you are **not confirmed to be eligible**, you will not be able to take part in the study. However, your doctor will make alternative more suitable arrangements for the treatment of your condition.

Step 2: Treatment and Follow-up

If you are eligible to take part in this study you will begin treatment with nab-paclitaxel with nintedanib or placebo. You will continue receiving nab-paclitaxel and nintedanib/placebo for as long as it controls your cancer or until you have side effects that stop you from taking it. The total length of time you stay on the study will depend on how well your cancer responds to the study treatment and whether you have any side effects.

The starting dose of nab-paclitaxel will be the same for all patients. The starting dose of nintedanib/placebo will be the maximum tolerated dose determined during the previously conducted Part 1 of the study.

Doses of nab-paclitaxel and nintedanib/placebo can be reduced in a step-wise fashion depending on any side-effects experienced during treatment. The dose cannot be increased again once it has been reduced.

Number of visits

During the treatment phase you will have visits prior to start of every treatment cycle and a visit prior to day 8 of each cycle (2 visits every 3 weeks) for as long as you are having treatment on the study:

Week 1: You will attend a clinic visit and receive your first dose of cycle 1 of nab-paclitaxel. You will be given capsules of nintedanib or placebo to take away with you and start taking the following day.

Week 2: You will attend a clinic visit and receive your second dose of cycle 1 of nab-paclitaxel. You will not take your nintedanib or placebo capsules on this day.

Week 3: You will not need to attend a clinic visit, this is your break week. You will continue to take your nintedanib or placebo capsules twice daily.

Week 4: You will attend a clinic visit and receive your first dose of cycle 2 of nab-paclitaxel. You will be given more nintedanib/placebo capsules to take home to start taking the following day.

Week 5: You will attend a clinic visit and receive your second dose of cycle 2 of nab-paclitaxel. You will not take your nintedanib/placebo capsules on this day.

Week 6: You will not need to attend a clinic visit, this is your break week. You will continue to take nintedanib/placebo capsules twice daily.

Trial visit schema:
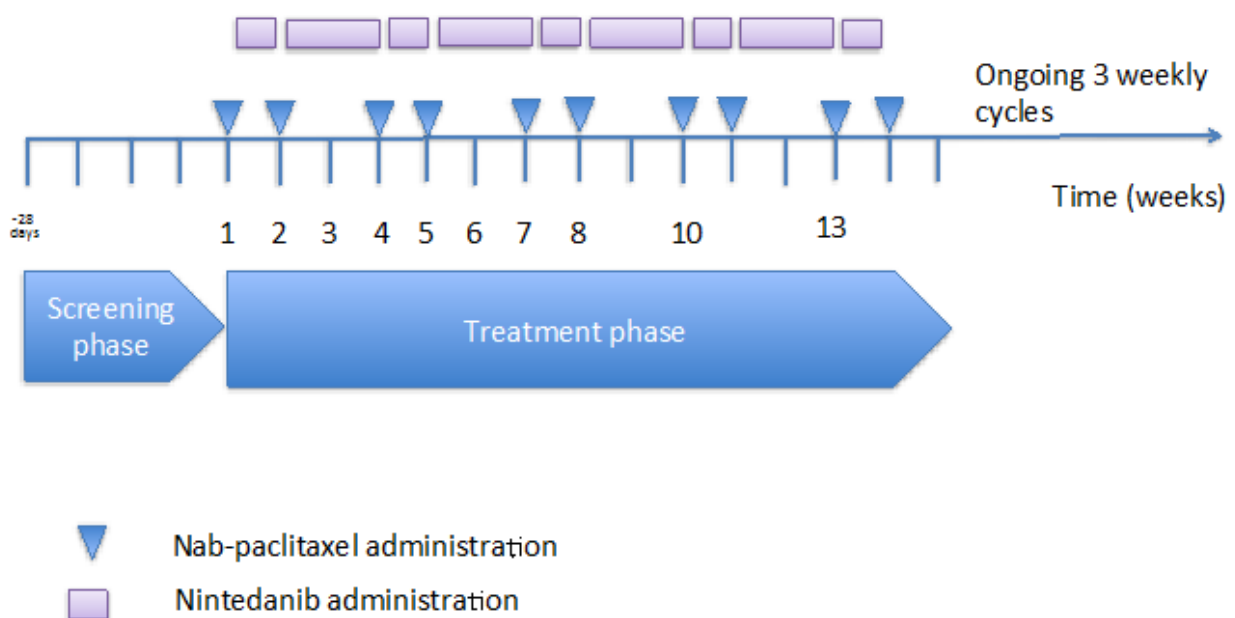
You will continue treatment with nab-paclitaxel and nintedanib every 3 weeks as long as they are helping to control the cancer and the side-effects are tolerable. If you have to discontinue nab-paclitaxel due to side-effects you may be allowed to continue taking nintedanib, and vice-versa; your doctor will discuss this with you.

Before you are given each new dose of nab-paclitaxel you will need to undergo some tests. These tests are to monitor your progress. At your visit, your doctor will ask you questions to see how you are feeling and if you are having any side effects. Your doctor will also carry out the following:

Routine tests:
- A physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and your disease status;
- Ask if you have taken any new medications;
- Ask about any side effects that you have experienced.
- Take blood samples (approximately 4 teaspoons/14mL) for routine blood tests. Every effort will be made to take all blood samples from a single needle stick.
- Measurements of the size of your tumour using a CT scan (every 6 weeks/after every 2 cycles of treatment as long as you are receiving nab-paclitaxel and/or nintedanib/placebo).

If everything is OK and you wish to continue you will then be given your next dose of nab-paclitaxel and will continue taking nintedanib/placebo capsules. This will be repeated at each clinic visit.

Step 3: Discontinuation of study treatment

If your cancer grows, you have unacceptable toxicities or you no longer wish to participate in the study your doctor will ask you to stop taking the study drugs. In addition, Royal Marsden NHS Foundation Trust (Sponsor) may decide to stop the study for reasons other than those listed. When you permanently discontinue study medication (if it is not at a clinic visit), we will immediately see you in clinic for a review visit (end of study treatment visit) and your doctor will carry out the following tests/assessments, with your permission.

- Physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and you disease status;
- Ask about any side effects that you have experienced;
- Blood samples (approximately 4 teaspoons/14mL) for routine blood tests;
- Measurements of the size of your tumour using a CT scan (if you have not had an measurement taken within the last 6 weeks);

30 Days Safety Follow-up Visit

After the end of study treatment visit, you will be asked to come back to hospital one more time, 30 days later or earlier if you start a new treatment, to follow up on medication you have taken and to check on your general health. The following tests/assessments will be carried out:
- Physical examination including measurement of your weight, blood pressure and pulse;
- An assessment of how easily you can carry out daily activities and you disease status;
- Ask about any side effects that you have experienced;
- Blood samples (approximately 4 teaspoons/14mL) for routine blood tests.

Post-Safety Follow-up Visits

We would like to follow you up every 12 weeks. You do not need to attend a visit but the hospital team will review your hospital records and / or contact you or your GP to find out what has been happening with your cancer.

Tissue Samples – Optional

In this study we will also ask for your permission to collect your archived tumour samples that are stored in your local hospital pathology department. These samples were taken at the time of your initial diagnosis or when your cancer came back.

These samples will be stored in a secure laboratory and may be used in subsequent studies to identify specific markers in your cancer cells which may help explain how you cancer responds to treatment.

6   What do I have to do?

If you take part in this study, you will need to follow the treatment plan and hospital appointments outlined in section 5 above. You should consider how these tests and visits will affect your work and family life and decide if you are able to commit to the required visits and tests. We will monitor you closely during the study for any symptoms of side effects, but it is very important that you also tell your doctor about **any** changes in your health, even if you do not think they are related to taking part in the study.

You must inform your doctor of any medications you are currently taking or you intend to use once you have entered the study in case these affect you. Your doctor will provide you with a list of therapies / medications that you must avoid while you are taking part in the study and discuss these with you

Female participants of child bearing potential and male participants must agree to use effective methods of birth control or complete abstinence from heterosexual contact to prevent pregnancy. Participants with reproductive potential (males and females, including females who have had a tubal ligation) must use reliable means of contraception during the study and for a period of 3 months after the last dose of study drug. Your doctor can discuss suitable methods of birth control with you.

Female participants may not breastfeed while they are in the study

All pregnancies or suspected pregnancies occurring in either a female participant of childbearing potential or partner of childbearing potential of a male participant must be reported to the Chief Investigator and the Sponsor immediately. Participants who become pregnant must be discontinued from trial treatment immediately.

You must not be a blood donor at any time during the study treatment period.

7   Will I be compensated for taking part in this study?

There is unfortunately no payment available for your participation in this study, including lost earnings and your time but a modest bursary is available to help with travelling expenses. The study treatment, visits to see your research doctor and laboratory tests related to this study will be provided at no cost to you. The doctors and nurses looking after you receive no payment for your participation in this study.

8   What are the possible disadvantages and risks of taking part?

Study medication: You may experience side effects, such as those described in section 9 or others not known yet. During the time you receive treatment you will be examined regularly by your doctor and several tests will be performed to check for side effects.

IV line: for infusion of nab-paclitaxel may cause: discomfort, irritation, mild bruising, bleeding, leakage of drug solution, and rarely infection, nausea, and light-headedness.

Blood Samples: As with all blood tests, there is a possibility of slight redness, inflammation and/or bruising developing at the site where the needle is placed into your arm.

CT (Computerised Tomography) Scan: This is a special type of X-Ray to allow your doctor to see a three dimensional picture of your tumour. This is painless and will take about 10-20 minutes. You may have an injection with a type of dye just before the scan to help make the scan clearer. This may result in a slight burning at the injection site, a metallic taste in your mouth, a sensation of wanting to pass urine or hot flushes. Very rarely an allergic reaction to the contrast dye may occur. Such reactions can involve itching, a rash or in severe cases difficulty in breathing and lowering of blood pressure. If you know of any allergic reaction to imaging contrast dyes you should let your doctor or radiologist know.

Radiation Risks: CT scans and chest x-rays involve exposure to ionising radiation, which carries an associated risk of inducing cancer after a period of time which can be 5-10 years for leukaemia and up to 20-30 years for other tumours. However, the risks are very small compared to the normal lifetime risk of getting cancer, which is 1 in 4, and for patients with your clinical condition these risks may be considered to be negligible when compared to the potential benefits of participation in this study.

MRI (Magnetic Resonance Imaging) Scan: You will have this if appropriate. It is like a CT scan but takes approximately 30 to 45 minutes and is noisy. You will be asked to lie very still on a couch inside a metal tunnel. If you suffer from claustrophobia (fear of enclosed spaces) you will probably find a MRI scan uncomfortable. When needed a special type of contrast dye will be injected into a vein to improve the quality of images. Reactions to this dye are rare and usually no more severe than a headache. MRI scans do not involve the use of radiation.

General: You should be aware that certain insurance covers, such as medical or travel insurance may be affected by participation in a clinical study. Please contact your insurance company to see if participating in the study will affect your insurance.

9   What are the effects of any treatment received when taking part?

You may have side effects while you are in the study, but you will be carefully checked by the study doctor for any problems.

This will be the first time that these drugs will be used in combination with one another. Therefore, there may be some risks or side effects that we are not aware of. These side effects may be mild or may be more serious, and, if necessary, your doctor will give you medicine to help lessen the symptoms of the side effects. You should tell the study doctor/staff about anything that is bothering you or any side effects you have, even if you do not think they are related to the study drug. If you need to contact them outside your scheduled visits, then their telephone numbers are at the end of this information sheet.

Nab-paclitaxel

The following is a list of the most medically significant or most common side effects reported in completed studies considered to be related to nab-paclitaxel:

Very common side-effects (May affect more than one in ten participants):

- Loss of hair (the majority of cases of hair loss happened less than one month after startingnab-paclitaxel. When it happens, hair loss is pronounced (over 50%) in the majority of patients)
- Rash
- Abnormal decrease in the number of types of white blood cells (neutrophils, lymphocytes or leukocytes) in the blood
- Deficiency of red blood cells
- Reduction in the number of platelets in the blood
- Effect on peripheral nerves (pain, numbness, tingling, temporary or permanent loss of feeling)
- Pain in a joint or joints
- Pain in the muscles
- Pain in the extremities
- Nausea, diarrhoea, constipation
- Vomiting
- Weakness and tiredness
- Fever
- Dehydration
- Sore mouth, taste disturbance loss of appetite and weight loss
- Low levels of potassium in the blood
- Depression
- Sleep problems
- Headache
- Chills

- Difficulty in breathing
- Dizziness
- Swelling of mucosal and soft tissues
- Increased liver function tests
- Pain in extremities
- Cough
- Abdominal pain
- Nose bleeds

Common side effects (May affect up to 1 in 10 patients):

- Itching, dry skin, nail disorder
- Infection, fever with decrease in the number of a type of white blood cell (neutrophils) in the blood, flushing, thrush, severe infection in your blood which may be caused by reduced white blood cells
- Reduction in all blood cell counts
- Chest or throat pain
- Indigestion
- Stuffy nose
- Pain in back, bone pain
- Diminished muscular coordination or difficulty in reading, increased or decreased tears, loss of eyelashes
- Changes in heart rate or rhythm
- Heart failure
- Decreased or increased blood pressure
- Redness or swelling at the site where the needle entered the body
- Anxiety
- Infection in the lungs
- Infection in the urinary tract
- Obstruction in the gut, inflammation of the large bowel, inflammation of the bile duct
- Acute kidney failure
- Increased bilirubin in the blood
- Coughing up blood
- Dry mouth, difficulty in swallowing
- Muscle weakness
- Blurred vision

Uncommon side effects (May affect up to one in a hundred people) :

- Increased weight

- Increased lactate dehydrogenase in the blood, increased or decreased blood sugar, increased or dicreasedphosphorus in the blood

- Decreased kidney function

- Decreased or lack of reflexes, involuntary movements, pain along a nerve, facial nerve paralysis

- Fainting, dizziness when standing up, shaking,

- Irritated eyes, painful eyes, red eyes, itchy eyes, double vision, reduced vision, or seeing flashing lights, blurred vision due to swelling of the retina (cystoid macular oedema)

- Ear pain, ringing in your ears

- Coughing with phlegm, shortness of breath when walking or climbing stairs, decreased breath sounds, water on the lung, loss of voice, dry throat

- Pain and swelling in the nose, runny nose or dry nose

- Loss of voice, dry throat

- Blood clot in the lung

- Painful or sore gums

- Gas, stomach cramps, rectal bleeding

- Painful urination, frequent urination, blood in the urine, inability to hold your urine

- Fingernail pain, fingernail discomfort, loss of fingernails

- Hives, skin pain, skin infections, red skin from sunlight, skin discolouration, increased sweating, night sweats, white areas on the skin, sores, swollen face

- Fluid retention, low albumin in the blood, increased thirst

- Decreased calcium in the blood, decreased sodium in the blood

- Infection due to catheter line

- Bruising

- Pain at site of tumour,

- Decreased blood pressure when standing up, coldness in your hands and feet

- Difficulty walking, leg swelling

- Allergic reaction

- Decreased liver function, increased size of liver

- Pain in the breast

- Restlessness

- Small bleedings in your skin due to blood clots

- A condition involving destruction of red blood cells and acute kidney failure

Rare side effects (May affect up to one in a thousand people):
- Skin reaction to another agent or lung inflammation following radiation

- Blood clot

- Very slow pulse,

- Heart attack

- Leaking of drug outside the vein

- A disorder of the electrical conduction system of the heart (atrioventricular block)

Very rare side effects (May affect up to one in ten thousand people):
- Severe inflammation/eruption of the skin and mucous membranes (Stevens-Johnson syndrome, toxic epidermal necrolysis)

Additional side effects observed during post-marketing surveillance of nab-paclitaxel, not otherwise noted above include:

- lack of movement in the vocal cords with possible voice changes
- skin sensitivity to sunlight
- skin or tissue damage from prior radiation therapy can become damaged again, when a person receives chemotherapy after having had radiation therapy. This is referred to as radiation recall and may involve redness, peeling, pain, and swelling. Skin changes have been noted to range from mild redness to tissue death. Radiation recall may also occur in the lungs and other internal organs.

Elderly

Patients over 65 years old may experience the following side effects more often than younger patients: nose bleed, diarrhoea, dehydration (loss of water and minerals in the body), feeling tired or weak, and swelling caused by fluid held in the tissues, especially of the ankles, feet or fingers.

Nintedanib

Treatment with nintedanib as single agent or in combination with standard doses of other chemotherapy drugs may cause side effects. The possible side effects related to the administration of nintedanib or the combination of nintedanib with standard doses of various chemotherapeutic compounds are listed below and are based on a total of 1932 treated cancer patients.

Very common side effects (may affect more than one in ten people):

- Diarrhoea
- Painful, numb and/or tingling feeling in fingers and toes
- Nausea
- Vomiting
- Pain in the stomach (abdomen)
- Bleeding
- Decrease in the number of white blood cells
- Inflammation of the mucous membranes lining the digestive tract including sores and ulcers in the mouth
- Rash
- Decreased appetite
- Electrolyte imbalance
- Increased liver enzyme values in the blood as seen from blood tests

Common side effects (may affect up to one in ten people):

- Blood poisoning
- Decrease in the number of white blood cells accompanied by fever)
- Decrease in the number of platelets
- Blood clots in the veins
- High blood pressure
- Dehydration
- Abscesses
- Jaundice

Uncommon side effects (may affect up to one in a hundred people)

- Occurrence of holes in the wall of your gut

These treatment-related adverse events were usually reversible and most of these adverse events can be treated or resolving by temporarily or permanently stopping nintedanib. If you experience any of these side effects or any other side effect which might be related to the intake of the study drug(s), your study doctor may adjust your medication and give you advice on how you can minimise these side effects.

Drugs acting similarly as nintedanib, currently under clinical investigation or already used in the clinical practise, other adverse events than those reported in patients treated with nintedanib alone or in combination with the standard doses of chemotherapy, have been reported. These included renal impairment and blood clots in arteries which may also occur during treatment with nintedanib / nintedanib plus chemotherapy. Please also inform your trial doctor in case you experience such type of adverse event.

10   What are the possible benefits of taking part?

It is hoped that nab-paclitaxel in combination with nintedanib may slow down the growth of your cancer. We cannot promise that the study will help you as an individual directly, however, the information we get might help us understand the behaviour of the drugs, their safety and tolerability. We may gain insights into the treatment of your disease which may lead to better management of patients and you will have helped by taking part.

11   What are the alternatives to treatment?

If you should decide not to participate in, or if you withdraw from this study, the study doctor can recommend other treatments which may include a marketed drug, treatment with a different investigational drug or best supportive care. Please make sure you discuss all the available treatment options with your research doctor before deciding if you want to take part in this study. Your doctor will organise any other treatment or care you may need.

12   What happens when the research study stops?

You will receive nab-paclitaxel and/or nintedanib/placebo for as long as the drugs are controlling your cancer, and the study doctor believes it is in your best interest; you will continue to receive the study drugs until either your disease progresses (becomes worse) or you permanently stop treatment for another reason e.g. you have intolerable side effects or you withdraw your consent. At this point one or the other or both of the drugs will be withdrawn. When the research study stops, your doctor will assess your status and recommend a suitable treatment.

13   What if there is a problem?

Your hospital doctor will be there to answer any questions you might have regarding your disease and your participation in this study. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study there will be several options available to you. The details are included in Part 2 of this information sheet.

14   Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2 of this information sheet

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

15 What if new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to continue. If you decide to continue in the study your research doctor will ask you to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

16 What will happen if I don't want to carry on with the study?

You may decide to stop and withdraw from the study at any time without giving a reason. A decision to withdraw at any time will not affect the standard of care you receive and your legal rights. After discussion with your doctor, you will be offered the treatment felt to be best for you at that time. You must tell your doctor immediately if you no longer wish to take part in the study. If you withdraw, we will not collect any new information about you but will ask you if we can keep previously collected information, blood samples and tissue samples.

Your study doctor can also take you off study treatment at any time if for example your condition becomes worse or another condition develops that may mean you are unable to carry on taking study treatment.

17 What if there is a problem?

<u>If you experience harm</u>

Royal Marsden NHS Foundation Trust holds insurance policies, which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation under the NHS indemnity scheme. This does not affect your legal rights to seek compensation. Please contact your study doctor if you would like further information about the insurance arrangements, which apply to the trial.

<u>If you have any complaints</u>

Regardless of the above, if you wish to complain or have any concerns about the way that you have been approached or treated during the course of this study, you should ask to speak to the PALS team or the researchers who will do their best to answer your questions (contact details at the end of this information sheet). If you remain unhappy and wish to complain formally, the usual National Health Service complaint mechanisms will be available to you. Details can be obtained from your study doctor or nurse. If you are still not satisfied with the response, you may contact the sponsor, Royal Marsden NHS Foundation Trust Research Office.

18 Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be entered onto a database and kept strictly confidential. If you decide to take part in the study, you give us permission to use information about you and share it with individuals from the Royal Marsden study team, authorised people from UK regulatory bodies and members of the local NHS trust. This permission continues until the study is over, including the length of time that we must keep records about the study. Information will be labelled with a unique code number, and will not include your name or other personal information that directly identifies you. When you sign the consent form, you agree to have your personal and medical information used as described in this section.

19 Will information about this trial be included in a Registry Databank?

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as encouraged for transparency and required by some academic journals. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

20 Involvement of the General Practitioner / Family Doctor

With your permission your GP will be notified of your participation in this study so that they are aware of the treatment you are receiving. We will ask you to sign that you consent to us informing your GP.

21 What will happen to any samples I give?

Blood samples

A single sample of blood taken at the screening visit will be stored at a secure laboratory after the study is completed and kept for the duration of the study. The rest of blood samples taken during the study will be processed and destroyed according to standard practice at the local laboratory.

Tissue samples

Archival tissue samples will be stored at a secure laboratory after the study is completed. Archival tumour samples will be kept for the duration of the study and then returned to your local hospital pathology department.

22 What rights do I have to the results of the research?

Any information derived directly or indirectly from this research, as well as any patents, diagnostic tests, drugs, or biological products developed directly or indirectly as a result of this research, are the sole property of the study sponsor (and its successors and licensees) and may be used for commercial purposes. You will have no right to this property or to any share of the profits that may be earned directly or indirectly as a result of this research. However, in signing the consent form and offering samples for research, you do not give up any rights that you would otherwise have as a participant in research.

23   What will happen to the results of the study?

A group of independent experts will review the progress of the trial and the results will be published in medical journals and/or presented at a national/international meeting as soon as there is enough information to be sure that the results are reliable. The confidentiality of all participants will be kept in all reports and publications that may arise from this study. When the study is completed we aim to make the results available to view on the Royal Marsden website (royalmarsden.nhs.uk, and follow the links to the study results page). If you do not have access to a computer and would like to know the results, please get in touch with the study team who can arrange to send you a copy.

24   Who is organising and funding the research?

This is a study sponsored by Royal Marsden NHS Foundation Trust and coordinated by the Royal Marsden Clinical Trials Unit. Funding is provided by the pharmaceutical companies Celgene and Boehringer Ingelheim who are also providing the study drugs, nab-paclitaxel and nintedanib, free of charge to all participants. Your doctor will not receive any personal financial payment if you take part.

25   Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study was reviewed and given a favourable opinion by XXXX.

26   Contact for further information

If you have any questions or concerns about this study, including study related injury or study medication queries, please contact your study doctor or research nurse. The doctor in charge of this study is*:*

Name:

Title: .

Address:

If at any time you are concerned or require additional information, please contact one of the study team (9 am to 5 pm)

Study Team:
Xxxxxxxxx

PALS Team:
xxxxxxxxx

Cancer Research UK provides general information for patients about cancer and its treatment as well as about clinical trials on their website www.cancerhelp.org.uk. A confidential information service is provided by specialist nurses on Tel: 0808 8004040. Macmillan Cancer Support (www.macmillansupport.org.uk; Tel: 0808 800 0000) also provides support and counselling to help people living with cancer.

Thank you for taking time to read this information leaflet. If you think you will take part in the study please read and sign the consent form

APPENDIX 8. N3 TRIAL INFORMED CONSENT FORM

Study EudraCT number:
Study Protocol number:
Participant Study Identification Number: < insert participant number >

INFORMED CONSENT FORM

Full title: A Phase I-II trial of Combination Nab-paclitaxel and Nintedanib or Placebo in Relapsed Non-Small Cell Lung Adenocarcinoma

Name of Researcher: ………………………………………………………………………

Please initial each box:

1. I confirm that I have read and understood the participant information sheet *<insert version and date>* for the above study and have been given a copy to keep. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is entirely voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from Royal Marsden NHS Foundation Trust, from UK regulatory authorities or from the local NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers.

5. I understand that the information held and maintained by the Health and Social Care Information Centre (or amend as appropriate) and other central UK NHS bodies may be used to help contact me or provide information about my health status.

6. I agree to my GP being informed of my participation in the study.

7. I give permission for the blood samples described in the participant information sheet to be taken for the purposes of this study.

8. I give permission for the collection of my archival tumour samples for the purposes of this study.

9. I give permission for the collection of archival tissue and blood samples for future research to be stored in a recognised Tissue Bank following completion of the study.

10. I agree that my blood and tissue samples, from which I will not directly be identifiable, may be sent to other approved laboratories, for testing. I understand that I will not receive the results and that they will not affect my clinical care.

11. I will inform the study doctor / team should my contact details change.

12. I agree to take part in the above study.

13.**OPTIONAL** I give permission for any residual blood and tissue samples, as described in the information sheet, to be used for further ethically approved research in the field of cancer research. **If you do not wish to give permission for this, please initial 'no' – however, you may still participate in the study.**	☐ Yes ☐ No

………………………………………………………………. ……………………………………………… ………………………………….............……
Name of participant **(BLOCK CAPITALS)**   Date *(dd/mmm/yyyy)*   Signature

………………………………………………………………. ……………………………………………… ………………………………….............……
Name of witness (if applicable) **(BLOCK CAPITALS)**   Date *(dd/mmm/yyyy)*   Signature

………………………………………………………………. ……………………………………………… ………………………………….............……
Name of person taking consent **(BLOCK CAPITALS)**   Date *(dd/mmm/yyyy)*   Signature
(if different from the researcher)

………………………………………………………………. ……………………………………………… ………………………………….............……
Name of Researcher **(BLOCK CAPITALS)**   Date *(dd/mmm/yyyy*   Signature

(1 copy for participant; 1 copy for researcher (original); 1 copy to be kept in participants medical records)

APPENDIX 9. N3 TRIAL CASE REPORT FORM

Site Code [ ][ ] Code: sitecode
Format: Text - AA

Patient Registration & Demographics

1. Patient Initials Code: patinit
Format: Text - AAA
[ ][ ][ ]

2. Date of Birth Code: dobdat
Format: Date – dd/mm/yyyy
[ ][ ] / [ ][ ] / [ ][ ][ ][ ]   dd / mm / yyyy

3. Sex
Code: patsex
Format: Category – sexcat
Female / Male

4. Race
Code: patrace
Format: Category – racecat
Caucasian / Mixed Race / Asian / African / Caribbean / Oriental / Other

If other, please specify
Code: patraceothsp
Format: Text - 20

5. Smoking status
Code: smoke
Format: Category – smokecat
Never Smoker / Ex-smoker / Current Smoker/Un Known

6. If ever a smoker, number of pack years?
Code: smokeyear
Format: integer - #9

7. PIS Version and Date
[ ][ ] [ ]
Code: pisver
Format: Real Number - #9.9

Code: pisdat
Format: Date – dd/mm/yyyy
[ ][ ] / [ ][ ] / [ ][ ][ ][ ]   dd / mm / yyyy

8. Consent Form Version and Date
[ ][ ] . [ ]
Code: conver
Format: Real Number - #9.9

Code: condat
Format: Date – dd/mm/yyyy
[ ][ ] / [ ][ ] / [ ][ ][ ][ ]   dd / mm / yyyy

9. Date Patient Signed Consent
[ ][ ] / [ ][ ] / [ ][ ][ ][ ]   dd / mm / yyyy
Code: condatsign
Format: Date – dd/mm/yyyy

10. Member of Staff Taking Consent
[ ]
Code: connamesp
Format: Text - 30
[ ]   *Name*

[ ]
Code: conrolesp
Format: Text - 30
[ ]   *Role / Position*

Code: conroledat
Format: Date – dd/mm/yyyy

11. Trial Identification Number
[ ][ ] - [4][2][8] - [ ][ ][ ]
Code: trialid
Format: Text – AA-9999-999
Site Code – CCR Number – Patient Number (Consecutively from 001)

12. Date of Registration
Code: regdat
Format: Date – dd/mm/yyyy
[ ][ ] / [ ][ ] / [ ][ ][ ][ ]   dd / mm / yyyy

13. Cohort
Code:
Format:

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Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Code: d_sitecode Code: d_patinit Code: d_trialid Code: d_visitlabel

The patient must:

Yes / No

1. Be willing and able to provide informed consent for the trial

Code: incq1yn
Format: Category - yesnocat

2. Be ≥ 18 years of age on day of signing informed consent.

Code: incq2yn
Format: Category - yesnocat

3. Have a pathologically confirmed diagnosis of stage IIIb or stage IV adenocarcinoma of the lung or of locally recurrent disease (stage IIIa) with no radical treatment options

Code: incq3yn
Format: Category - yesnocat

4. have previously received no more than 2 lines of systemic therapy for NSCLC with palliative intent:
i.   Chemotherapy as first or second line with palliative intent
ii.   Relapsing within 6 months of adjuvant chemotherapy after surgery or as part of radical chemo-radiotherapy, which count as one line of therapy
iii.   Licenced or experimental maintenance therapy is allowed (eg. pemetrexed)
iv.   Immunotherapy at prior line of treatment (first or second line) is allowed.

Code: incq4yn
Format: Category - yesnocat

5. Have a performance status of 0 -1 on the ECOG performance scale

Code: incq5yn
Format: Category - yesnocat

6. Have an estimated life expectancy of ≥ 12 weeks

Code: incq6yn
Format: Category - yesnocat

7. Have at least one radiologically measurable tumour lesion as defined by RECIST 1.1 criteria

Code: incq7yn
Format: Category - yesnocat

8. Have adequate haematopoietic, hepatic and renal function

Code: incq8yn
Format: Category - yesnocat

All answers should be YES. Add warning message if any of the variables is answered as NO.

Site Code Code: d_sitecode
derived from sitecode

Patient Initials Code: d_patinit
derived from patinit

Trial ID _tr Code:-d_visitlabel
derived from trialid   derived from visits

The patient must not:

Yes / No

1. Have a known EGFR kinase sensitising mutation or ALK gene fusion with no prior TKI treatment.

Code: excq1y
Form t: C tegory - yesnocat

2. Receive any concurrent anticancer systemic therapy.

Code: excq2y
Form t: C tegory - yesnocat

3. Have received prior treatment with nintedanib or any other VEGFR inhibitor (prior Bevacimumab is allowed).

Code: excq3yn
Format: Category - yesnocat

4. Be refractory to prior taxane therapy for avanced disease. Prior taxane used in the adjuvant setting does not exclude eligibility provided there is no disease recurrence within 12 months upon completion of chemotherapy in that setting

Code: excq4yn
Format: Category - yesnocat

5. Demonstrate inadequate laboratory parameters defined by

Code: excq5yn
Format: Category - yesnocat

   i. Absolute neutrophil count (ANC) < 1,500/µl ($1.5x10^9$/L).
   ii. Platelets < 100,000/µl ($100x10^9$/L).
   iii. Haemoglobin < 9.0 g/dl or requiring transfusions.
   iv. Creatinine clearance < 45 ml/min (by local institutional methods).
   v. Total bilirubin outside normal limits:
   vi. ALT and/or AST > 1.5 x ULN in patients without liver metastasis.
   vii. ALT and/or AST > 2.5 x ULN in patients with liver metastasis.
   viii. International normalised ratio (INR) > 2, prothrombin time (PT) and partial thromboplastin time (PTT) > 50% of deviation of institutional ULN.

6. Have Proteinuria CTCAE grade 2 or greater.

Code: excq6yn
Format: Category - yesnocat

7. Have Pre-existing peripheral sensory neuropathy CTCAE grade 2 or greater.

Code: excq7yn
Format: Category - yesnocat

8. Have used any investigational drug within 4 weeks of randomisation

Code: excq8yn
Format: Category - yesnocat

9. Have had radiotherapy within 4 weeks prior to randomisation.

Code: excq9yn
Format: Category - yesnocat

10. Have had major surgery (other than biopsy) within 4 weeks prior to randomisation.

Code: excq10yn
Format: Category - yesnocat

11. Have active brain metastases or leptomeningeal disease (defined as stable for <4 weeks, no adequate previous treatment with radiotherapy, symptomatic, requiring treatment with anti-convulsants; dexamethasone therapy will be allowed if administered as stable dose for at least 4 weeks prior to randomisation).

Code: excq11yn
Format: Category - yesnocat

12. Have any other active current malignancy (other than non-melanomatous skin cancer, in situ breast or in situ cervical cancer, prostate cancer diagnosed more than 3 years prior, or breast cancer diagnosed more than 5 year prior to randomisation).

Code: excq12yn
Format: Category - yesnocat

13. Have active or uncontrolled infections or serious illnesses or medical conditions that in the opinion of the investigator could interfere with the patient's participation in the study, including:

Code: excq13yn
Format: Category - yesnocat

   a. Known active or chronic hepatitis C and/or B infection.
   b. Known pre-existing interstitial lung disease or pneumonitis.
   c. Presence of significant cardiovascular diseases (i.e. uncontrolled hypertension, unstable angina, history of infarction within the past 12 months prior to start of study treatment, congestive heart failure > NYHA II, serious cardiac arrhythmia, pericardial effusion).
   d. Gastro-intestinal abnormalities, including inability to take oral medication, requirement for intravenous feeding, active peptic ulcer, prior surgical procedures affecting absorption, any medical co-morbidity affecting gastrointestinal absorption.
   e. History of clinically significant haemorrhagic or thromboembolic event in the past 6 months.
   f. Known inherited predisposition to bleeding or thrombosis.
   g. Major injuries within the past 10 days prior to start of study treatment with incomplete wound healing and/or planned surgery during the on-treatment study period.
   h. Drug or alcohol abuse.

14. Have taken therapeutic anticoagulation (except low-dose heparin and/or heparin flush as needed for maintenance of indwelling intravenous device) or anti-platelet therapy (except low dose therapy with acetylsalicylic acid <325mg her day).

Code: excq14y Form : Category - yesnocat

15. Have radiographic evidence (CT or MRI) of cavitary or necrotic tumours or local invasion of major blood vessels by tumour.

Code: excq15yn Format: Category - yesnocat

16. Be pregnant or breast feeding; female patients must have a negative pregnancy test (beta-HCG test in urine or serum) prior to commencing study treatment.

Code: excq16yn Format: Category - yesnocat

17. Patients who are sexually active and unwilling to use a medically acceptable method of contraception during the trial and for at least three months after ceasing study therapy (total abstinence, permanent sterilisation (combined oral, transdermal or intra-vaginal hormonal contraceptives, methoxyprogesterone injections (e.g. Depo-provera), copper-banded intra-uterine devices, hormone-impregnated intra-uterine systems and vasectomised partners; all methods of contraception, with the exception of total abstinence, should be used in combination with the use of a condom by male sexual partners).

Code: excq17yn Format: Category - yesnocat

18.Known hypersensitivity or any contraindications to the trial drugs, including nab-paclitaxel/ nintedanib, to their excipients or to contrast media or other ingredients including peanuts and soya.

Code: excq18yn Format: Category - yesnocat

19. Be unable to comply with the protocol.

Code: excq19y Form t: C tegory - yes oc t

All answers should be NO. Add warning message if any of the variables is answered as yes.

Site Code ☐☐   **Patient Initials** ☐☐☐   **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Code: d_sitecode
deri ed from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Cancer History

1. Date of initial diagnosis ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code:pdiagdat
Format: Date – dd/mm/yyyy

2. Type of NSCLC

Code: cantype
Format: Category - cantypecat

Adenosquamous / Adenocarcinoma/other

Any patients with a histological type othe than pure adenocarcinoma should have eligibility checked with sponsor prior to enrollment.

If other, please specify

3. Histology/Cytology at diagnosis

Specify

4. Disease Staging at diagnosis

T   Code: cant
Format: Category - tcat   *T0 / T1 / T2 / T3 / T4 / TX / Tis*

N   Code: cann
Format: Category - ncat   *N0 / N1 / N2 / N3 / NX*
0 / IA / IB / IIA / IIB / IIIA / IIIB /
IVA/IVB

M   Code: canm
Format: Category - mcat   *M0 / M1A / M1B / MX*

Stage   Code: canstage
Format: Category - stagecat

If M ≥ 1 please detail all locations of metastasis

5. Did patient have biopsy on initial diagnosis?

Date of Biopsy   ☐☐☐☐☐ ☐☐☐☐

Was genetic mutation determined?

Check category codes

If yes, specify type

Specify

6. Does the patient have additional locations of metastasis at study entry?

Code: metyn
Format: Category - yesnocat   *Yes / No*

Liver   Code: metliveryn
Format: Category - yesnocat

Bone   Code: metboneyn
Format: Category - yesnocat

Adrenals   Code: metadrnalyn
Format: Category - yesnocat

Brain   Code: metbrainyn
Format: Category - yesnocat

Lung   Code: metalungyn
Format: Category - yesnocat

Other   Code: metotheryn
Format: Category - yesnocat

If yes please specify   Code: metothersp
Format: Category – Text-30

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Site Code [ ][ ]   **Patient Initials** [ ][ ][ ]   **Trial ID** [ ][ ] - [ ][ ][ ][ ] - [ ][ ][ ]

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Systemic Anti-Cancer Treatments

1. Has the patient had any systemic anti-cancer treatments including any novel treatments?

[ ] *Yes / No*

Code: systyn
Format: Category - yesnocat

If yes please provide details of all treatments received below:

Code: systrqg
Repeating Question Group
Number of repeating rows per form = 4

2a. Treatment name   [Code: systnamesp Format: Text - 100]

Start date [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*   **End date** [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*

Code: syststartdat
Format: Date – dd/mm/yyyy

Code: systenddat
Format: Date – dd/mm/yyyy

Number of cycles [Code: systnocyc]   **Best Response** [ ] *CR/PR/SD/PD/NE*   Code: systres Format: Category - respresultcat

Format – integer - #9

Reason stopped [Code: reastop Format: Category - systemiccat] toxicity / progressed / finished course / other / unobtainable

If other specify [Code: reastopothsp Format: Text - 100]

Questions in 2a are repetitive to capture more data if required

2b. Treatment name [ ]

Start date [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*   **End date** [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*

Number of cycles [ ][ ]   **Best Response** [ ] *CR/PR/SD/PD*

Reason stopped [ ] toxicity / progressed / finished course / other

If other specify [ ]

2c. Treatment name [ ]

Start date [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*   **End date** [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*

Number of cycles [ ][ ]   **Best Response** [ ] *CR/PR/SD/PD*

Reason stopped [ ] toxicity / progressed / finished course / other

If other specify [ ]

| Site Code | | Patient Initials | | Trial ID | - | - |

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Surgery

Please enter here any cancer related surgery (excluding biopsies) occurred within the prior 10 years. Please enter non cancer related surgery to Medical History.

1. Has the patient had any cancer related surgeries?   *Yes / No*   Code: surgyn
Format: Category - yesnocat

If yes, please provide details of all treatments received below:

1.a Type of Surgery   Code: surgtype
Format: Category - surgerycat   Pneumonectomy /Lobar resection/ Wedge resection / VATS /Other

If other specify   Code: surother Format: Text - 50

Date of Surgery   Code:surgdat
Format: Date – dd/mm/yyyy

Questions in 1a are repetitive to capture more data if required

1.b Type of Surgery   Pneumonectomy /Lobar resection/ Wedge resection / VATS /Other

If other specify

Date of Surgery

1.c Type of Surgery   Pneumonectomy /Lobar resection/ Wedge resection / VATS /Other

If other specify

Date of Surgery

1.d Type of Surgery   Pneumonectomy /Lobar resection/ Wedge resection / VATS /Other

If other specify

Date of Surgery

The N3 Study	Radiotherapy Treatment History - Screening

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐

Radiotherapy

1. Has the patient had prior radiotherapy treatment? ☐ *Yes / No*

If yes, please provide details of all radiotherapy regimens the patient has received below:

1a. Treatment area ☐ Code: rtsitesp Format: Text - 100

Therapy status ☐ Code: rtstatsp Format: Text - 100 **Technique** ☐ Code: rttechsp Format: Text - 100

Start date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: rtstartdat
Format: Date – dd/mm/yyyy

End date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: rtenddat
Format: Date – dd/mm/yyyy

Total Dose (Gy) ☐☐☐ Code: rtdose
Format: interger - ##9

Number of fractions ☐☐☐ Code: rtfrac
Format: interger - ##9

Questions in 1a are repetitive to capture more data if required

1b. Treatment area ☐

Therapy status ☐ **Technique** ☐

Start date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy* **End date** ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Total Dose (Gy) ☐☐☐ **Number of fractions** ☐☐☐

1c. Treatment area ☐

Therapy status ☐ **Technique** ☐

Start date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy* **End date** ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Total Dose (Gy) ☐☐☐ **Number of fractions** ☐☐☐

1d. Treatment area ☐

Therapy status ☐ **Technique** ☐

Start date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy* **End date** ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Total Dose (Gy) ☐☐☐ **Number of fractions** ☐☐☐

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐

Past Medical History

1. Has the patient had any baseline conditions or significant medical history? ☐ Yes / No

If yes, please provide details of all treatments received below:

Number.	Condition/ diagnosis/sign/symptom	CTCAE Grade *If ongoing*	Start date *dd / mm / yyyy*	Ongoing *yes/no*	End date *If not ongoing dd / mm / yyyy*
2.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
3.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
4.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
5.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
6.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
7.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
8.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
9.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
10.			☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐

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Site Code [ ][ ] **Patient Initials** [ ][ ][ ] **Trial ID** [ ][ ] - [ ][ ][ ][ ] - [ ][ ][ ]

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Archival Tumour Sample

Archival tumour samples can include diagnostic biopsies, surgical excisions and additional research biopsies taken under local hospital consent.

1. Does the patient have any archived tumour samples available for use in this study? [ ] *Yes / No*

Code: arctumyn
Format: Category - yesnocat

If no please provide reason: Code: arctumnosp Format: Text - 100

If yes, please provide details of the samples available below:

2. **Tumour Site** Code: tumsitesp Format: Text - 100

Sample Type

Core Biopsy/Fine needle aspiration/ Surgical specimen
Code: tumtyp1
Format: Category – sample1cat

FFPE Block / Sectioned slides / Both (FFPE and Sectioned slides)
Code: tumtyp2
Format: Category – sample2cat

Sample Date [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
Code: tumdat
Format: Date – dd/mm/yyyy
dd / mm / yyyy

Sample Location
Address
Code: tumlocsp Format: Text - 100

These questions are repetitive to capture more data if required

2a. **Tumour Site** [ ]

Sample Type [ ][ ]

Sample Date [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
dd / mm / yyyy

Sample Location
Address

2b. **Tumour Site** [ ]

Sample Type [ ][ ]

Sample Date [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
dd / mm / yyyy

Sample Location
Address

N3 CRF v0.1 14/03/2017

The N3 Study	Physical Examination		
Site Code ☐☐ Code: d_sitecode derived from sitecode	**Patient Initials** ☐☐☐ Code: d_patinit derived from patinit	**Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐ Code: d_trialid derived from trialid	Code: d_visitlabel derived from visits

Physical Examination

1. Was a physical examination performed? ☐ *yes / no*
Code: physexamyn
Format: Category - yesnocat

If no please provide reason: Code: physexamnosp Format: Text - 100

2. Date of physical examination Code: physexamdat Format: Date – dd/mm/yyyy ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

3. ECOG performance status Code: ecog Format: Category - ecogcat *ECOG0 / ECOG1 / ECOG2 / ECOG3 / ECOG4 / Not Performed*

Normal / Abnormal NOT clinically Significant / Abnormal clinically significant / Not assessed   *If clinically significant please give details?*

4. General Appearance | Code: genappresult Format: Category - resultcat | Code: appsigsp Format: Text - 100

5. Skin | Code: skinresult Format: Category - resultcat | Code: skinsigsp Format: Text - 100

6. Head Eyes Ear Nose Throat | Code: heentresult Format: Category - resultcat | Code: heentsigsp Format: Text - 100

7. Lymphatic | Code: lympresult Format: Category - resultcat | Code: lympsigsp Format: Text - 100

8. Cardiovascular | Code: carresult Format: Category - resultcat | Code: carsigsp Format: Text - 100

9. Respiratory | Code: respresult Format: Category - resultcat | Code: respsigsp Format: Text - 100

10. Abdomen | Code: abdoresult Format: Category - resultcat | Code: abdosigsp Format: Text - 100

11. Genitourinary | Code: geniresult Format: Category - resultcat | Code: genisigsp Format: Text - 100

12. Musculoskeletal | Code: muscresult Format: Category - resultcat | Code: muscsigsp Format: Text - 100

13. Neurologic | Code: neurresult Format: Category - resultcat | Code: neursigsp Format: Text - 100

14. Other physical examinations? Code: ophysexamyn Format: Category - yesnocat *yes / no*

If Other, le se specify   **Physical examination Result** *Normal / Abnormal NOT clinically Significant / Abnormal clinically significant / Not assessed*   *If clinically significa please give details?*

Code: othersp Format: Text - 30 | Code: otheresult Format: Category - resultcat | Code: othersigsp Format: Text - 100

For Screening visit : "Abnormal clinically significant findings at screening should be recorded on the Medical History form"
For all other visits Abnormal clinically significant findings ~~at screening~~ should be recorded on the the Adverse Event form"

N3 CRF v0.1 14/03/2017

The N3 Study		Vital Signs		
Site Code	☐☐	**Patient Initials** ☐☐☐	**Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐	

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Code: vitdoneyn
Format: Category - yesnocat

Vital Signs

1. Were Vital Signs Assessed? ☐ *Yes / No*

If no, please provide reason: | Code: vitdonenosp Format: Text - 100

If yes please complete details below:

2. Date of Assessment Code: vitaldat
Format: Date – dd/mm/yyyy ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

3. Weight (Kg) ☐ Code: weight
Format: real - #99.9

4. Height (cm)
Screening visit only. ☐ Code: height
Format: real - #99.9

5. Temperature (°C) ☐ Code: temp
Format: real - #9.9

6. Pulse (bpm) ☐ Code: pulse
Format: integer - #99

7. Respiration rate (per minute) ☐ Code: resprate
Format: integer - 99

8. Oxygen saturation (%) on room air ☐ Code: oxygensat
Format: real – #99.9

9. Blood Pressure (mmHg)

Systolic blood pressure ☐ Code: standsystolic
Format: integer - #99

Diastolic blood pressure ☐ Code: standdiastolic
Format: integer - #99

See p44 re source documenting vital signs on
treatment

For Screening visit : "Abnormal clinically significant findings at screening should be recorded on the Medical History form"
For all other visits Abnormal clinically significant findings ~~at screening~~ should be recorded on the the Adverse Event form"

The N3 Study	Tumour Imaging - Baseline	
Site Code ☐☐	**Patient Initials** ☐☐☐	**Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐

Tumour Assessment RECIST v1.1

Tumour assessments should be completed at screening and then every 6 weeks from the date of first dose

1. Date of Assessment ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: recistdat
Format: Date – dd/mm/yyyy

2. How many target lesions have been reported? ☐ *1-5*

Code: targetles
Format: Category - targetlescat

Please provide details of target lesions below:

No.	Description of Organ and Sub Sites. *A maximum of 2 lesions per organ*	Method of Assessment *CT / MRI / Clinical*	Length in Longest diameter *short axis diameter for nodal lesions (cm)*
1	Code: tarlessite1sp Format: Text - 50	Code: recistass1 Format: Category - recistasscat	Code: recistsize1 Format: real - #9.9
2	Code: tarlessite2sp Format: Text - 50	Code: recistass2 Format: Category - recistasscat	Code: recistsize2 Format: real - #9.9
3	Code: tarlessite3sp Format: Text - 50	Code: recistass3 Format: Category - recistasscat	Code: recistsize3 Format: real - #9.9
4	Code: tarlessite4sp Format: Text - 50	Code: recistass4 Format: Category - recistasscat	Code: recistsize4 Format: real - #9.9
5	Code: tarlessite5sp Format: Text - 50	Code: recistass5 Format: Category - recistasscat	Code: recistsize5 Format: real - #9.9

3. Sum of longest diameters of target lesions (cm)

Code: targetsum
Calculated sum of longest length
Format: real - #9.9

4. Are there any non target lesions? ☐ *yes / no*

Code: nontarlesyn
Format: Category - yesnocat

4b. Number of non target lesions? ☐ *1-5*

Code: nontargetles
Format: Category - targetlescat

If yes, please provide details of non target lesions below:

Description of Organ and Sub Sites.	*CT / MRI / Clinical*	Visibility
Code: recistnonsite1 Format: Text - 50	Code: recistnonass1 Format: Category - recistasscat	Code: recistnonvis1 Format: Category - recistvisiblecat
Code: recistnonsite2 Format: Text - 50	Code: recistnonass2 Format: Category - recistasscat	Code: recistnonvis2 Format: Category - recistvisiblecat
Code: recistnonsite3 Format: Text - 50	Code: recistnonass3 Format: Category - recistasscat	Code: recistnonvis3 Format: Category - recistvisiblecat
Code: recistnonsite4 Format: Text - 50	Code: recistnonass4 Format: Category - recistasscat	Code: recistnonvis4 Format: Category - recistvisiblecat
Code: recistnonsite5 Format: Text - 50		Code: recistnonvis5 Format: Category - recistvisiblecat

N3 CRF v0.1 14/03/2017

Site Code	☐☐	Patient Initials	☐☐☐	Trial ID	☐☐ - ☐☐☐☐ - ☐☐☐

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Haematology and Coagulation

Was a haematology sample taken ☐   Yes / No

Code: hmyn
Format: Category - yesnocat

Sample Date ☐☐☐☐ ☐☐☐☐   *dd/mmm/yyyy*

Code: hmdat
Format: Date – dd/mmm/yyyy

Sample time (24h) ☐☐ ☐☐   *hh:mm*

Code: hmtim
Date/Time

Haemoglobin (g/dL)
Code: hb
Laboratory Test; ##9.99

Code: hbclinsig
Category

RBC (10^{12}/L)
Code: rbc
Laboratory Test; #9.99

Code: rbcclinsig
Category

Haematocrit (%)
Code: hcrit
Laboratory Test; #9.999

Code: hcritclinsig
Category

Platelet count (10^9/L)
Code: plt
Laboratory Test; ##9.99

Code: pltclinsig
Category

MCV (fl)
Code: mcv
Laboratory Test; #99.9

Code: mcvclinsig
Category

WBC (10^9/L)
Code: wbc
Laboratory Test; ##9.99

Code: wbcclinsig
Category

Neutrophils (10^9/L)
Code: neut
Laboratory Test; #9.99

Code: neutclinsig
Category

Lymphocytes (10^9/L)
Code: lymph
Laboratory Test; 9.99

Code: lymphclinsig
Category

Eosinophils (10^9/L)
Code: eo
Laboratory Test; 9.99

Code: eoclinsig
Category

Monocytes (10^9/L)
Code: mono
Laboratory Test; 9.99

Code: monoclinsig
Category

Basophils (10^9/L)
Code: baso
Laboratory Test; 9.99

Code: basoclinsig
Category

PT (seconds)
Code: pt
Real Number; 9.99

Code: ptclinsig
Category

APPT (seconds)
Code: aptt
Real Number; #9.99

Code: apttclinsig
Category

INR
Code: inr
Real Number; #9.99

Code: inrclinsig
Category

For Screening visit : "Abnormal clinically significant findings at screening should be recorded on the Medical History form"
*For all other visits **Abnormal clinically significant findings at screening should be recorded on the the Adverse Event form"***

Site Code [ ][ ] Code: d_sitecode **Patient Initials** [ ][ ][ ] **Trial ID** [ ][ ] - [ ][ ][ ][ ][ ] - [ ][ ][ ]

Code: d_patinit   Code: d_trialid   Code: d_visitlabel

Biochemistry & thyroid function tests

1. Was a biochemistry sample taken? [ ] Yes / No   Code: bioyn   Format: Category - yesnocat

If no please give reason why. [Code: bionosp Format: Text - 100]

2. Sample Date Code: biodat / Format: Date – dd/mm/yyyy [ ][ ] / [ ][ ] / [ ][ ][ ][ ] dd / mm / yyyy

3. Sample time (24h) Code: biotim / Format: Date – hh:mm [ ][ ] : [ ][ ] hh:mm

	Value	*Value*
4. Sodium *(mmol/L)*	Code:sod / Format: Laboratory Test - ##9	Code: sodresult / Format: Category - sampresultcat
5. Potassium *(mmol/L)*	Code:pot / Format: Laboratory Test - #9.9	Code: potresult / Format: Category - sampresultcat
6. Blood Urea Nitrogen *(mmol/L)*	Code:bun / Format: Laboratory Test - #9.99	Code: bunresult / Format: Category - sampresultcat
7. Creatinine *(µmol/L)*	*Female* Code:creatf Format: Laboratory Test - ##9 *Male* Code:creatm Format: Laboratory Test - ##9	Code: creatresult / Format: Category - sampresultcat
9. Glucose *(mmol/L)*	Code:gluc / Format: Laboratory Test - #9.9	Code: glucresult / Format: Category - sampresultcat
10. Alanine Aminotransferase (ALT) (U/L)	Code: alt / Format: Laboratory Test - ##9	Code: altresult / Format: Category - sampresultcat
11. Alkaine Phosphate (ALP) (U/L)	Code: alp / Format: Laboratory Test - ##9	Code: alpresult / Format: Category - sampresultcat
12. Total bilirubin (µmol/L)	Code:tbi / Format: Laboratory Test - ##9	Code: tbilresult / Format: Category - sampresultcat
14. Total protein *(g/L)*	Code:tprot / Format: Laboratory Test - ##9	Code: tprotresult / Format: Category - sampresultcat
15. Albumin *(g/L)*	Code: alb / Format: Laboratory Test - ##9	Code: albresult / Format: Category - sampresultcat
16. Calcium Corrected *(mmol/L)*	Code: corcalc / Format: Laboratory Test - #9.99	Code: corcalcresult / Format: Category - sampresultcat
17. Phosphate *(mmol/L)*	Code:phos / Format: Laboratory Test - #9.99	Code: phosresult / Format: Category - sampresultcat
18. Magnesium (mmol/L)	Code:mag / Format: Laboratory Test - #9.99	Code: magresult / Format: Category - sampresultcat
19. Uric Acid *(mmol/L)*	*Female* Code: uratef Format: Laboratory Test – 9.99 *Male* Code: uratem Format: Laboratory Test – 9.99	Code: urateresult / Format: Category - sampresultcat
20. Aspartate Aminotransferase (AST) (U/L)	Code: ast / Format: Laboratory Test - ##9	Code: astresult / Format: Category - sampresultcat

For Screening visit : "Abnormal clinically significant findings at screening should be recorded on the Medical History form"
For all other visits Abnormal clinically significant findings at screening should be recorded on the the Adverse Event form"

| **Site Code** ☐☐ | **Patient Initials** ☐☐☐ | **Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐ |

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Urinalysis

1. Was an urinalysis sample taken?   ☐   *yes / no*

Code: urinyn
Format: Category - yesnocat

If no please give reason why.   Code: urinnosp Format: Text - 100

2. Sample Date   Code: urindat
Format: Date – dd/mm/yyyy   ☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

3. Sample time (24h)   Code: urintime
Format: Date – hh:mm   ☐☐ : ☐☐   *hh:mm*

*Nil / Trace / Positive+/ Positive++ /
Positive+++ / Positive++++ /Unobtainable*   *If abnormal please give further details*

4. Glucose   Code: gluresult
Format: Category - urinresultcat   Code: glusigsp Format: Text - 100

5. Protein   Code: proresult
Format: Category - urinresultcat   Code: prosigsp Format: Text - 100

6. Blood   Code: bloresult
Format: Category - urinresultcat   Code: blosigsp Format: Text - 100

*Normal / Abnormal NOT clinically
Significant / Abnormal clinically
significant / Not assessed*

*For Screening visit : "Abnormal clinically significant findings at screening should be recorded on the Medical History form"
For all other visits: "Abnormal clinically significant findings at screening should be recorded on the the Adverse Event
form"*

The N3 Study **Research Blood Sample Collection**

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Research Blood Sample

1. Has the patient consented to research blood collection? ☐ *Yes / No*

Code: rbconyn
Format: Category - yesnocat

2. Was the sample collected? ☐ *Yes / No*

Code: rbyn
Format: Category - yesnocat

If no please provide reason: Code: rbnosp Format: Text - 100

If yes, please provide details of the sample below:

3. Date Sample Taken ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: rbdat
Format: Date – dd/mm/yyyy

The N3 Study	Pregnancy Test		
Site Code ☐☐	Patient Initials ☐☐☐	Trial ID ☐☐ - ☐☐☐☐ - ☐☐☐	

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Serum Pregnancy Test

4. Was a serum pregnancy test performed? ☐ *Yes / No*

Code: spregyn
Format: Category - yesnocat

If no please give reason why.

~~PEAR Study eCRF v23 12.08.2016~~

Code: spregnosp Format: Text - 100

If yes please complete details below:

5. Date sample taken Code: spregdat
Format: Date – dd/mm/yyyy ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

6. Pregnancy test result Code: spregresult
Format: Category - pregresultcat ☐ *Negative / Positive / Inconclusive*

If the serum pregnancy test was performed more than 72hrs from the start of IMP, a urine pregnancy test must be performed

Urine Pregnancy Test

1. Was a urine pregnancy test performed? ☐ *Yes / No*

Code: upregyn
Format: Category - yesnocat

If no please give reason why.

Code: upregnosp Format: Text - 100

If yes please complete details below:

2. Date sample taken Code: upregdat
Format: Date – dd/mm/yyyy ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

3. Pregnancy test result Code: pregresult
Format: Category - pregresultcat ☐ *Negative / Positive / Inconclusive*

If both tests are inconclusive patient cannot begin on the trial as eligibility cannot be confirmed.

Site Code ☐☐ Code: d_sitecode derived from sitecode

Patient Initials ☐☐☐ Code: d_patinit derived from patinit

Trial ID ☐☐ - ☐☐☐☐ - ☐☐☐ Code: d_trialid derived from trialid

Concomitant Medications

1. Has the patient taken any concomitant medications? ☐ *Yes / No*

Code: conmedyn
Format: Category - yesnocat

If yes, please provide details below

Code: conmedrqg
Repeating Question Group
Number of repeating rows per form = 80

Medication	Reason for therapy	Dose	Dose Unit	Route	Dose Frequency	Start Date *dd/mm/yyyy*	Ongoing *yes / no*	End Date *dd/mm/yyyy*
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐
						☐☐ / ☐☐ / ☐☐☐☐		☐☐ / ☐☐ / ☐☐☐☐

Code: conmedsp Format: Text - 50

Code: conmedreasp Format: Text - 50

Code: conmeddose Format: Text - 20

Code: condoseunit Format: Text - 20

Code: conmedroute Format: Category - conroutecat

Code: condosefqy Format: Category - condosefqycat

Code: conmedstdat Format: Date – dd/mm/yyyy

Code: conmedongo Format: Category - yesnocat

Code: conmede dd t Format: D te = dd/mm/yyyy

The N3 Study **Eligibility Assessment**

| Site Code | ☐☐ | Patient Initials | ☐☐☐ | Trial ID | ☐☐ - ☐☐☐☐ - ☐☐☐ |

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Code: d_visitlabel
derived from visits

Eligibility Assessment

1. Have all the patients screening CRFs been completed and are ready for eligibility assessment?   ☐ *Yes*

Code: eligassyn
Format: Category - yescat

2. Date eligibility assessment complete?   ☐☐ / ☐☐ / ☐☐☐☐

Code: eligassdat
Format: Date –
dd/mm/yyyy

If no please complete all CRFs before requesting eligibilty confirmation.

N3 CRF v0.1 14/03/2017

The N3 Study	Eligibility Confirmation

Site Code [ ][ ] **Patient Initials** [ ][ ][ ] **Trial ID** [ ][ ] - [ ][ ][ ][ ] - [ ][ ][ ]

Eligibility Confirmation

> *To be completed by the RM-CTU study team and sent to the site for confirmation of trial entry.*

1. Has the patient been confirmed as eligible   [   ]   *Yes / No*

If yes, please randomise the patient and complete the details below:

2. Date of Eligibility Confirmation   [ ][ ] / [ ][ ] / [ ][ ][ ][ ]   *dd / mm / yyyy*

I confirm that all criteria have been assessed and the patient can be entered into the trial.   [   ] *Yes*

Member of Staff Completing Eligibility Confirmation: [Code: confirmstaff
Format: Text - 15] *Name*

Electronic signature when previous
question answered needs to print
out name / date / time

N3 CRF v0.1 14/03/2017

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐☐ - ☐☐☐

Administration of Nas-Paclitaxel

1. Did the patient receive Nab-Paclitaxel at this visit?

Code: Nabyn
Format: Category - yesnocat

☐ *yes / no*

If no, please provide reason why:

Code: nabnosp Format: Text - 100

2. Date of administration

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: nabdat
Format: Date – dd/mm/yyyy

3. Start Time

☐☐ : ☐☐ *hh:mm*

Code: nabsttim
Format: Date – hh:mm

4. End Time

☐☐ : ☐☐ *hh:mm*

Code: nabendtim
Format: Date – hh:mm

5. Dose Given *(mg)*

☐

Code: nabdose
Format: integer - #99

6. Was this the planned dose?

☐ *yes / no*

Code: nabplanyn
Format: Category - yesnocat

If no, please provide reason why:

Code: nabplannosp Format: Text - 100

7. Did any incident occur during the administration?

☐ *yes / no*

Code: nabincyn
Format: Category - yesnocat

If yes, please provide details:

Code: nbincyessp Format: Text - 250

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Was this cycle delivered as planned ?	If no, specify reason	Other- specify

| Cycle | Code: NINcycle Integer; #9 | Code: NINplanyn Category | Code: NINrea Category | Code: NINreasp Text - 100 |

The cycle should be considered completed as planned if there has been no drug interruption. In case of dose omissions or drug interruptions, use the rows to complete the continuous dosing periods as per the example below:

Batch Number	Start Date	End Date	Dose (mg)	Dose frequency	Tablet/dispersed
1570.1/4	01/10/2016	09/10/2016	150	Twice Daily	Tablet
1570.1/4	10/10/2016	10/10/2016	150	Once Daily	Tablet
1570.1/4	11/10/2016	28/10/2016	160	Twice Daily	Tablet

Batch Number	Start Date	End Date	Dose (mg)	Dose frequency	Tablet/dispersed
Code: NINbatch Text; 15	Code: NINdat Date/Time	Code: NINenddat Date/Time	Code: NINdose Text;20	Code: NINfre Category	Code: NINtab Category
Code: NINbatch Text; 15	Code: NINdat Date/Time	Code: NINenddat Date/Time	Code: NINdose Text;20	Code: NINfre Category	Code: NINtab Category
Code: NINbatch Text; 15	Code: NINdat Date/Time	Code: NINenddat Date/Time	Code: NINdose Text;20	Code: NINfre Category	Code: NINtab Category
Code: NINbatch Text; 15	Code: NINdat Date/Time	Code: NINenddat Date/Time	Code: NINdose Text;20	Code: NINfre Category	Code: NINtab Category
Code: NINbatch Text; 15	Code: NINdat Date/Time	Code: NINenddat Date/Time	Code: NINdose Text;20	Code: NINfre Category	Code: NINtab Category

Patient Survival Status

Site Code		Patient Initials		Trial ID		-		-	

Survival Status

1. Date of survival assessment   ☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

2. Was the survival status of this patient assessed at this visit?   ☐   *yes / no*

If no, please provide reason why: ☐   *Lost to Follow-Up / Patient Withdrawal / Death / Other*

If ~~other~~, please provide details: ☐

If withdrawal, please provide details: ☐   *Patient decision / PI Decision / Lost to Follow-Up / Death*

If the patient has died please complete the death eCRF.

The N3 Study — Adverse Events

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Adverse Events

1. Has the patient had any adverse Events? ☐ *Yes / No*

Code: aeyn
Format: Category - yesnocat

If yes, please provide details below

Code: aergg
Repeating Question Group
Number of repeating rows per form = 80

Code: aectcae
Format: Category - ctcaecat

Code: aeirae
Format: Category - yesnocat

Code: aeeci
Category - yesnocat

Code: aesae
Format: Category - yesnocat

Code: dltae
Category - yesnocat

AE Number	Adverse Event	Start Date	Outcome	End Date	Relationship to study drug	CTCAE Grade	Action taken to study drug	IrAE	ECI	SAE	DLT
Code: aenum Form : integer - ##9	*Description*	*dd/mm/yyyy* Code: aestdat Format: Date – dd/mm/yyyy	*Resolved / Resolved with Sequelae / Ongoing / Fatal.*	*dd/mm/yyyy* Code: aeenddat Format: Date – dd/mm/yyyy	*Definite / Probable / Possible / Unlikely/ Unrelated*	*(1-5)*	*None / Treatment Postponed / Treatment Stopped/ Treatment Interrupted* Code: aeaction	*Yes / No*	*Yes^ / No*	*Yes* / No*	*Yes / No*
☐	Code: aedescripsp Format: Text - 50	☐☐/☐☐/☐☐☐☐	Code: aeoutcome Format: Category - outcomecat	☐☐/☐☐/☐☐☐☐	Code: aerelate Format: Category - relatedcat	☐	Format: Category - actioncat	☐	☐	☐	☐
☐		☐☐/☐☐/☐☐☐☐		☐☐/☐☐/☐☐☐☐		☐		☐	☐	☐	☐
☐		☐☐/☐☐/☐☐☐☐		☐☐/☐☐/☐☐☐☐		☐		☐	☐	☐	☐
☐		☐☐/☐☐/☐☐☐☐		☐☐/☐☐/☐☐☐☐		☐		☐	☐	☐	☐
☐		☐☐/☐☐/☐☐☐☐		☐☐/☐☐/☐☐☐☐		☐		☐	☐	☐	☐
☐		☐☐/☐☐/☐☐☐☐		☐☐/☐☐/☐☐☐☐		☐		☐	☐	☐	☐

These questions are repetitive to capture more data if required.

^ Please refer to ECI guidance in the current protocol.

* Yes should be answered when the adverse event results in death, is life-threatening, requires in-patient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Protocol Deviations / Violations

1. Have there been any protocol deviations / violations for this patient? ☐ yes / no

Code: pdvyn
Format: Category - yesnocat

If yes, please provide details below

Code: pdvrqg
Repeating Question Group
Number of repeating rows per form = 60

Code: d_num
Format: integer

Code: pdvdat
Format: Date – dd/mm/yyyy

SECTION TO BE COMPLETED BY THE SPONSOR STUDY TEAM

No. ##9	Date of Deviation	Description of the Deviation	Deviation / Violation	Category	If other please define	Action to be Taken
		Code: pdvdessp Format: Text - 200	Code: pdvclass Format: Category - pdvclasscat	Code: pdvcat Format: Category - pdvcat	Code: pdvothersp Format: Text - 50	Code: pdvactionsp Format: Text - 50
☐	☐☐/☐☐/☐☐☐☐					
☐	☐☐/☐☐/☐☐☐☐					
☐	☐☐/☐☐/☐☐☐☐					
☐	☐☐/☐☐/☐☐☐☐					
☐	☐☐/☐☐/☐☐☐☐					

These questions are repetitive to capture more data if required.

Deviations Categories:
PD1. Failure to comply to the timeline defined in the visit schedule
PD2. Dose interruptions / modifications not specified in the protocol
PD3. Failure to adhere to the study protocol
PD4. Failure to provide original signed consent forms
PD5. Deviation from recommended IMP handling
PD6. Variation in the management of a participant due to a minor safety concern
PD7. Other…...

Violations Categories:
PV1. The patient received the wrong treatment or incorrect dose i.e IMP dispensing or dosing error
PV2. The patient met withdrawl criteria during the study but was not withdrawn
PV3. The patients received prohibitied medication
PV4. The patient was entered into the study but did not meet the protocols eligibilty criteria
PV5. Failure to perform patients assessments per protocol procedures that specifically relate to primary outcomes.
PV6. Failure to perform patients assessments per protocol procedures that specifically relate to patient safety.
PV7. Inadvertent loss of samples or data

PV8. Failure to obtain informed consent prior to initiation of study related procedures
PV9. Falsifying medical records
PV10. Performing tests and procedures beyond the individuals professional scope or privilage status.
PV11. Repeated protocol deviations
PV12. Working under and expired professional licence or certification
PV13. Other…...

| **Site Code** | | | **Patient Initials** | | | **Trial ID** | | - | | | | | - | | | |

Death Notification Form

Code: deathdat
Format: Date – dd/mm/yyyy

1. **Date of Death**   ☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

2. **Cause of death**   Code: deathcausp Format: Text - 100

3. **Was patient still on trial medication at time of death?**   ☐   *yes / no*
Code: deathmedyn
Format: Category - yesnocat

4. **Has Death been reported as an outcome to an SAE?**   ☐   *yes / no*
Code: deathsaeyn
Format: Category - yesnocat

5. **Was death due to toxicity? Split Nab-Nin**   ☐   *yes / no*
Code: deathpemyn
Format: Category - yesnocat

7. **Please provide any other relevant information**

Code: deathinfosp Format: Text - 200

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Early Discontinuation/Withdrawal

1. Date patient was confirmed to be discontinuing the study treatment.

Code: endtreatdat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

2. Date of last dose of SPLIT

Code: lastpemdat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

3. Reason patient discontinued study treatment.

Code: endtreatreas
Format: Category - discontinuecat

Disease Progression / Toxicity / Intercurrent illness / Non compliance / Administrative reasons / Withdrawal or withdrawal of consent

If withdrawal please select reason

Code: withdraw
Format: Category - withdrawcat

Patient Decision / PI Decision / Lost to Follow-Up / Death

4. Did the patient discontinue at a scheduled cycle visit?

yes / no
Code: cycledisyn
Format: Category - yesnocat

If yes which cycle?

Code: cycledisnum
Format: integer - ##9

If no please complete all end of treatment assessments eCRFs in the end of treatment visit section of the database.

5. Has the patient had a RECIST tumour assessment in the last 6 weeks?

yes / no
Code: recistdisyn
Format: Category - yesnocat

If yes please ensure the details are added to the RECIST eCRF.

If no please ensure the tumour assessment is completed as part of the end of treatment assessments and the details are recorded in the RECIST eCRF. If it is not possible to complete this assessment please give details in the comments box.

Comments:

Code: eotinfosp Format: Text - 200

Site Code ☐☐ *Code: d_sitecode derived from sitecode*

Patient Initials ☐☐☐ *Code: d_patinit derived from patinit*

Trial ID ☐☐ - ☐☐☐☐ - ☐☐☐ *Code: d_trialid derived from trialid*

Study Completion / Follow-Up Completion

1. Did the patient complete the Follow-up?

Code: stcompletyn
Format: Category - yesnocat   *yes / no*

If no, please provide details below

Code: stcompletcat
Format: Category - studycompletcat   *Death / Withdrawal of consent / Unobtainable / Other*

If other, specify

Code: nosttreatsp Format: Text - 100

2. Date of last visit

Code: endstudat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐   *dd / mm / yyyy*

The N3 Study — **Re-consent Form**

Site Code ☐☐ **Patient Initials** ☐☐☐ **Trial ID** ☐☐ - ☐☐☐☐ - ☐☐☐

Code: d_sitecode
derived from sitecode

Code: d_patinit
derived from patinit

Code: d_trialid
derived from trialid

Re-consent

1. Has the patient been asked to re-consent to the trial? ☐ *yes / no*

Code: saetreatrgg
Repeating Question Group
Number of repeating rows per form = 10

Code: reconyn
Format: Category - yesnocat

If yes, please provide details of re-consent below:

2a. PIS Version and Date ☐☐ . ☐

Code: newpisver
Format: Real Number - #9.9

Code: newpisdat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

2b. Consent Form Version and Date ☐☐ . ☐

Code: reconver
Format: Real Number - #9.9

Code: recondat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

2c. Date Consent Signed ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Code: recondatsign
Format: Date – dd/mm/yyyy

Member of Staff Taking Consent

☐ *Name*

Code: reconnamesp
Format: Text - 30

Code: reconrolesp
Format: Text - 30

☐ *Role / Position*

Member of Staff Signature date

Code: reconroledat
Format: Date – dd/mm/yyyy

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

These questions are repetitive to capture more data if required.

3a. Consent Form Version and Date ☐☐ . ☐

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

3b. Date Patient Signed ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

3c. PIS Version and Date ☐☐ . ☐

☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

Member of Staff Taking Consent

☐ *Name*

☐ *Role / Position*

Member of Staff Signature date ☐☐ / ☐☐ / ☐☐☐☐ *dd / mm / yyyy*

The N3 Study — **Tumour Imaging**

Site Code [ ][ ]   **Patient Initials** [ ][ ][ ]   **Trial ID** [ ][ ] - [ ][ ][ ][ ] - [ ][ ][ ]

Code: d_sitecode derived from sitecode Code: d_patinit derived from patinit Code: d_trialid derived from trialid Code: d_visitlabel derived from visits

Tumour Assessment RECIST v1.1

0. Was a Tumour Imaging assessment performed? [ ] *Yes / No*

Code: recistperfyn Format: Category - yesnocat

If no, please provide reason: [Code: recistperfsp Format: Text - 100]

If yes please complete details below:

1. Date of Assessment [ ][ ] / [ ][ ] / [ ][ ][ ][ ] *dd / mm / yyyy*

Code: recistdat Format: Date – dd/mm/yyyy

2. How many target lesions have been reported? [ ] *1-5*

Code: d_targetles Derived from targetles

Please provide details of target lesions below:

No.	Lesion Irradiated? *Yes / No*	Description of Organ and Sub Sites. *A maximum of 2 lesions per organ*	Method of Assessment *CT / MRI / Clinical*	Length in Longest diameter *short axis diameter for nodal lesions (cm)*
1	Code: d_irradles1yn Derived from irradles1yn	Code: d_tarlessite1sp derived from tarlessite1sp	Code: recistass1 Format: Category - recistasscat	Code: recistsize1 Format: real - #9.9
2	Code: d_irradles2yn Derived from irradles2yn	Code: d_tarlessite2sp derived from tarlessite1sp	Code: recistass2 Format: Category - recistasscat	Code: recistsize2 Format: real - #9.9
3	Code: d_irradles3yn Derived from irradles3yn	Code: d_tarlessite3sp derived from tarlessite1sp	Code: recistass3 Format: Category - recistasscat	Code: recistsize3 Format: real - #9.9
4	Code: d_irradles4yn Derived from irradles4yn	Code: d_tarlessite4sp derived from tarlessite1sp	Code: recistass4 Format: Category - recistasscat	Code: recistsize4 Format: real - #9.9
5	Code: d_irradles5yn Derived from irradles5yn	Code: d_tarlessite5sp derived from tarlessite1sp	Code: recistass5 Format: Category - recistasscat	Code: recistsize5 Format: real - #9.9

3. Sum of longest diameters of target lesions (cm) [Code: targetsumv Format: real - #9.9]

3a. Percentage change in target lesions compared with Lowest value (%) [Code: percechange Format: integer - #99]

4. Target Lesion Response [Code: targetres Format: Category - respresultcat] *CR / SD / PR / PD / NE*

5. Are there any non target lesions [ ] *yes / no*

Code: d_nontarlesyn Derived from nontarlesyn

If yes, please provide details of non target lesions below:

5a. Number of non target lesions? [ ] *1-5*

Code: d_nontargetles Format: Category - targetlescat

N3 CRF v0.1 14/03/2017

Description of Organ and Sub Sites.

Method of Assessment
CT / MRI / Clinical

Visibility
present/Absent

Code: d_ntarlessite1 derived from recistnonsite1

Code: d_ntarlessite2 derived from recistnonsite2

Code: d_ntarlessite3 derived from recistnonsite3

Code: d_ntarlessite4 derived from recistnonsite4

Code: d_ntarlessite5 derived from recistnonsite5

Code: recistnontar1
Format: Category - recistasscat

Code: recistnontar2
Format: Category - recistasscat

Code: recistnontar3
Format: Category - recistasscat

Code: recistnontar4
Format: Category - recistasscat

Code: recistnontar5
Format: Category - recistasscat

Code: recistnonvis1
Format: Category - recistvisiblecat

Code: recistnonvis2
Format: Category - recistvisiblecat

Code: recistnonvis3
Format: Category - recistvisiblecat

Code: recistnonvis4
Format: Category - recistvisiblecat

Code: recistnonvis5
Format: Category - recistvisiblecat

5b. Non Target Lesion Response

Code: nontargetres
Format: Category - ntrespresultcat

CR / NCR - NPD / PD/NE

6. Are there any new lesions?

yes / no

Code: newlesionsyn
Format: Category - yesnocat

If yes, please provide details of new lesions below:

Comments:

Code: newlesyessp Format: Text - 100

7. Overall Response

Code: overallres
Format: Category - respresultcat

CR / SD / PR / PD / NE

N3 CRF v0.1 14/03/2017

APPENDIX 10. N3 TRIAL LABORATORY MANUAL

Study Title: A PHASE I/II TRIAL OF COMBINATION NAB-PACLITAXEL AND NINTEDANIB OR NAB-PACLITAXEL AND PLACEBO IN RELAPSED NSCLC ADENOCARCINOMA
- N3 -

Laboratory Manual

Manual Version No:	1.0
Manual Date:	04 Sept 2017
Sponsor:	The Royal Marsden Hospital
CCR No:	4448
REC Ref No:	17/LO/0427
IRAS Project No:	199962
EudractNo	2016-000109-35
Protocol Version No:	1.3
Protocol Date:	21 April 2017

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Royal Marsden Hospital
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Sutton
SM2 5PT

Ext 4222/ 4312

Standard Operating Procedure for Blood Sample and Archival Tissue Sample Collection and Shipping

Introduction

The purpose of this manual is to describe the collection and processing of whole blood and tissue samples for future translational research.

One whole blood sample and one archival tissue sample (preferably FFPE cell blocks where available, otherwise H&E slides) will be collected during the screening phase for each patient, after trial consent has been obtained.

Scope

This manual is intended for participating centres of the **N3** Trial. Further processing and storage of samples after they arrive at the central storage facility is described in their standard operating procedures (SOPs).

Consumables

Sample	Consumables	Provided by
Whole blood	1x 10ml EDTA tube	Sponsor
Whole blood	Venepuncture kit	Site
Whole blood	Tube labels	Sponsor
Whole blood	Sample bag	Sponsor
Whole blood	Royal Mail safe-box	Sponsor
Archival tissue	Label	Sponsor
Archival tissue	Bubble wrap	Site
Archival Tissue	Royal Mail safe-box	Sponsor

WHOLE BLOOD SAMPLES

1. Use 1 x 10ml EDTA tube (purple top) per patient labelled with Unique study number, Patient trial ID, Date and Time.
2. Collect blood via venous puncture directly into the EDTA tube and fill to marked line. Avoid haemolysis.
3. Gently invert the tube 8-10 times.
4. Store tube in an upright position at room temperature.
5. Place blood sample inside the Royal Mail safe box and send to Central Biobank within 24 hours of sample collection. If samples are taken after 15:00, they should be stored locally and sent the next morning.
6. Inform coordinating centre (RMH CTU) by e-mail or telephone that sample has been posted.
7. Record sample collection in eCRF.

TISSUE SAMPLES

1. Optional archival tissue sample (FFPE or slides) to be collected from the local diagnostic pathology laboratory.
2. Samples should be securely wrapped in bubble wrap and inserted into a sample bag.
3. Sample bag should be clearly labelled with the following information: Unique study name and number, Patient trial ID, Pathology number, Hospital of origin, Date sent and Consent status.
4. The sample bags will be placed inside the Royal Mail safe box and sent to the Central Biobank.
5. Inform coordinating centre (RMH CTU) by e-mail or telephone that sample has been posted.
6. Record sample collection in eCRF.

APPENDIX 11. Molecular adequacy of image-guided rebiopsies for molecular retesting in advanced non-small cell lung cancer - Journal of Thoracic Oncology manuscript

Molecular Adequacy of Image-Guided Rebiopsies for Molecular Retesting in Advanced Non–Small Cell Lung Cancer: A Single-Center Experience

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ABSTRACT

Introduction: In the era of biomarker-driven systemic therapy for advanced NSCLC, the role of routine repeated biopsies for decision making outside *EGFR*-mutant disease remains unproven. We report our center's experience of safety and adequacy for molecular retesting of tumor material obtained from image-guided lung rebiopsies in NSCLC.

Methods: We performed a retrospective case note analysis of patients undergoing image-guided lung rebiopsies at a single cancer center between 2011 and 2014. The primary objective was to determine the pathological success rate. Secondary and exploratory objectives were to determine technical success rate, histological concordance, molecular adequacy, genotypes identified, and complication rate.

Results: In all, 103 patients underwent transthoracic image-guided procedures. A total of 66 rebiopsies in NSCLC were identified and analyzed. The pathological success rate was 87.1%. A high histological discordance rate was observed (12 of 52 evaluable cases [23.1%]). Pretest molecular adequacy as determined by the lung pathologist was 78.8% (52 of 66). Of 52 adequate samples 51 were sent for molecular analysis, with a total of 209 genes analyzed (including *EGFR*, ALK receptor tyrosine kinase gene [*ALK*], *KRAS*, *BRAF*, dicoidin domain receptor tyrosine kinase 2 gene [*DDR2*], *NRAS*, *ROS1*, and rearranged during transfection proto-oncogene gene [*RET*]). The rate of postgenotyping molecular adequacy was 87.1% (182 of 209). Overall, 20 new potentially actionable mutations were identified, with 13 of 66 patients (19.7%) starting to receive new targeted treatment as a result. Overall, rebiopsies informed clinical decision making in

63.6% of cases. The rates of complications were 15% for pneumothorax, 3% for pneumothorax requiring chest drain, and 8% for hemoptysis.

Conclusions: We have validated the pathological and molecular adequacy rates of rebiopsies and demonstrated clinical utility in routine decision making.

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Keywords: Lung cancer; Biopsy; Genotype; Non–small cell lung cancer

Introduction

Lung cancer is the most common cause of cancer-related mortality in men and women worldwide,[1,2] with more than 80% classified as NSCLC. Identification of driver somatic aberrations in advanced NSCLC has led to rational implementation of genotype-directed therapy, with international guidelines recommending molecular

testing[3,4] because EGFR and ALK kinase inhibitors have demonstrated markedly superior efficacy over chemotherapy in those harboring activating *EGFR* mutations and ALK receptor tyrosine kinase gene (*ALK*) rearrangements, respectively, and they are licensed for first-line therapy, alongside ROS1 kinase inhibitors.[5–10] However, multiple mechanisms of acquired resistance to molecular-directed therapy have been identified, including emergence of additional somatic mutations with reduced affinity for the drug (for instance, the *EGFR* T790M gatekeeper[11]) but also other less common mechanisms such as histological nonconcordance[12–14] or bypass track activation (e.g., through gene amplification).[15]

Therapeutic strategies to overcome mechanisms of acquired resistance are being developed, and in some cases licensed. For example, the *EGFR* mutation-specific kinase inhibitor osimertinib is active against both classical activating *EGFR* mutations (e.g., L858R or exon 19 deletion) and the resistance mutation T790M, resulting in U.S. Food and Drug Administration and European Medicines Agency licenses for NSCLC progressing during or after a first-line EGFR tyrosine kinase inhibitor (TKI) (afatinib, erlotinib, or gefitinib) and with evidence of T790M mutation.[16,17]

Other potentially targetable somatic aberrations have been identified in up to 70% of patients with the adenocarcinoma subtype of NSCLC[18] and in more than 50% of squamous NSCLC,[19] and a variety of global efforts are under way to identify and validate the efficacy of genotype-directed therapy in relapsed NSCLC through multiarm multiagent designed trials, such as the National Cancer Institute MATCH trial (NCT02465060) and the U.K. National Lung MATRIX Trial (NCT02664935). Although circulating tumor DNA (ctDNA) genotyping is an effective and validated technology for some alleles (e.g., *EGFR* T790M), contingent on clinical setting, the low specificity of some genotyping technologies coupled with the low ctDNA shedding rate for M1a NSCLC may limit clinical interpretation.

Therefore, repeated biopsies for purposes of molecular characterization may be indicated for the optimal management of patients with relapsed advanced NSCLC and are recommended, especially in tumors with oncogene addiction, to identify resistance-associated genotypes and guide therapy choice.[3,20]

Image-guided percutaneous transthoracic core needle biopsies are a standard diagnostic tool used to obtain tumor tissue at the point of diagnosis or relapse. Safety and tissue diagnostic yields of biopsies at first diagnosis of lung cancer are well established.[21–23] However, the data remain limited on the adequacy of tumor material obtained by repeat image-guided percutaneous biopsies to molecularly characterize tumors for clinical decision making. Here, we report our center's experience of safety and adequacy for molecular testing of tumor material obtained from image-guided transthoracic rebiopsies in patients with NSCLC.

Methods

This is a retrospective analysis of patients undergoing image-guided lung rebiopsies at a single cancer center between 2011 and 2014. Rebiopsy was defined as biopsy after cancer progression after anticancer therapy (any line) or repeated biopsy in cases in which initial histological or molecular analysis was inadequate or incomplete for clinical decision making. This study was approved by the local audit committee.

Patients

Patients were identified through a search of electronic patient records for those with a diagnosis of NSCLC who were undergoing image-guided lung biopsies between November 2011 and April 2014. Patients with other primary thoracic malignancies (e.g., SCLC, mesothelioma, thymic malignancies, and carcinoid tumors) were excluded.

Individual case notes were hand-searched for predefined data items, including fields on demography (age, sex, smoking history, pulmonary comorbidities, and history of other malignancies), lung cancer (diagnosis, disease stage, number of previous lines of systemic anticancer treatment, and somatic mutational status at biopsy time), rebiopsy data (biopsy indication, image guidance mode, number of passes, needle gauge, and number of cores obtained), postprocedure complications (pneumothorax, hemoptysis, and hospitalization), and rebiopsy tissue sample (presence/absence of malignancy, histological subtype, molecular analysis performed, mutations identified, molecular success, and molecular failure reasons). A validated data capture spreadsheet was created and populated by two independent investigators (N. T. and S. B.), who reviewed case notes and identified and entered data. Disagreements were reviewed and consensus sought with arbitration by a third reviewer (S. P.).

Objectives

The primary objective was to determine the pathological success rate, defined as the proportion of rebiopsy cases confirmed to contain malignant cells (as documented in the pathology reports).

Secondary and exploratory objectives included determination of the following: technical success rate; concordance of prebiopsy and postbiopsy histological subtype; adequacy of rebiopsy material for molecular analysis; number and nature of new mutations identified; and incidence of complications.

Definitions

Technical success was defined as successful insertion of biopsy needle into the target lesion, with cells or lung tissue present in the specimen, as documented in the pathology reports. Histological concordance was determined by comparison of the original histological diagnoses, as documented in case notes, with histological diagnoses based on rebiopsy specimens, which were reviewed and classified by a dedicated lung pathologist using the 2015 WHO classification. Diagnostic biopsies were re-reviewed by a dedicated thoracic pathologist where possible. Molecular analysis of rebiopsy material was performed as clinically indicated for individual cases. Adequacy of rebiopsy material for molecular analysis was defined as a minimum of 30% viable tumor cells in a sample, as assessed by a dedicated thoracic pathologist per routine practice. Reasons for inadequacy as reported by the pathologist were identified by case note review and grouped into consistent themes. Posttest molecular success rate was defined as the proportion of successfully informative individual gene analyses out of the total number of genes analyzed.

Statistical Analysis

Differences in intergene failure rates were tested by using the chi-square test for comparing multiple proportions with a significance level of α equal to 0.05, with Bonferroni correction for multiple pairwise comparisons. The relationship between number of cores (<3 vs. ≥3 cores) and molecular adequacy was tested by using Fisher's exact test.

Results

Patients

A total of 103 patients were identified from searching case notes for patients with a diagnosis of thoracic malignancy who underwent image-guided percutaneous transthoracic procedures between November 2011 and April 2014. Seven patients had pleural drain insertion or pleural fluid aspiration and were excluded from analysis. Of the 103 patients, 16 underwent an initial diagnostic biopsy for suspected lung cancer (14 to obtain a histological diagnosis and two for completion of staging at diagnosis) and were excluded from further analysis, as this was an initial biopsy as opposed to a rebiopsy. In addition, 14 patients with a diagnosis of other thoracic malignancy, including 10 mesotheliomas, two SCLCs, and two thymic malignancies, were excluded from further analysis.

In all, 66 patients with NSCLC rebiopsy were included in the final analysis. Patient characteristics are summarized in Table 1.

Table 1. Patient Characteristics

Demographic Variable	Value
Median age (IQR), y	67 (60-71)
Sex, (%)	
Male	35 (53)
Female	31 (47)
Smoking (at time of diagnosis), (%)	
Ex-smoker	35 (53)
Never-smoker	18 (27)
Active smoker	12 (18)
Unknown	1 (2)
Pulmonary comorbidities, (%)	
None	57 (86)
COPD	5 (7)
Previous pulmonary TB	2 (3)
Asthma	1 (2)
Emphysema	1 (2)
Other malignancy, (%)	
Yes[a]	4 (6)
No	62 (94)
Histological subtype at time of biopsy, (%)	
Adenocarcinoma	45 (68)
Squamous cell carcinoma	14 (21)
Adenosquamous	1 (2)
NSCLC NOS	6 (9)
Stage at diagnosis, (%)	
II	6 (9)
III	7 (11)
IV	53 (80)
Previous lines of systemic treatment, (%)	
0	16 (24)
1	24 (36)
2	14 (21)
3	7 (11)
4	5 (8)
Mutational status at time of biopsy, (%)	
EGFR	
Unknown	37 (56)
EGFR WT	20 (30)
EGFR mutation present	9 (14)
ALK	
Unknown	51 (77)
No rearrangement	14 (21)
Rearrangement present	1 (2)

[a]Other malignancies: three patients had a history of endometrial cancer (one), breast cancer (one), and basal cell carcinoma lip (one). One patient had a concurrent diagnosis of thymoma.
IQR, interquartile range; COPD, chronic pulmonary obstructive disease; TB, tuberculosis; NOS, not otherwise specified; WT, wild type; *ALK*, ALK receptor tyrosine kinase gene.

Procedures

The mode of image guidance was computed tomography (CT) in 60 of 66 cases (91%) and ultrasonography in six cases (9%). Four patients had a CT-guided chest wall biopsy. All procedures were performed by an experienced interventional radiologist using dedicated CT-guided biopsy software (i-sequence and i-spiral) on a Somatom Definition Edge CT scanner (Siemens,

Table 2. Histological Discordance Rates

Original Histological Subtype	n	Rebiopsy Histological Subtype	n (%)
Adenocarcinoma	38	Adenocarcinoma	36 (94.8)
		NSCLC NOS	1 (2.6)
		Poorly differentiated TTF-1-negative carcinoma	1 (2.6)
Squamous cell carcinoma	9	Squamous cell carcinoma	3 (33.3)
		Adenocarcinoma	4 (44.5)
		NSCLC NOS	1 (11.1)
		Pleomorphic carcinoma rhabdoid subtype	1 (11.1)
NSCLC NOS	4	NSCLC NOS	1 (25.0)
		Squamous cell carcinoma	3 (75.0)
Adenosquamous carcinoma	1	Adenocarcinoma	1 (100)
Total[a]	52	Concordant	40 (76.9)
		Discordant	12 (23.1)

[a]Total of 52 cases were evaluable for histological concordance. Of the 14 cases that were not evaluable, eight had no malignant cells in the sample (pathological fail), four were sent to a research laboratory, and two did not have the histological subtype reported.

NOS, not otherwise specified; TTF-1, thyroid transcription factor 1.

Erlangen, Germany). Rapid on-site evaluation was not used for any of the procedures.

Although all rebiopsies were considered for molecular analysis, the primary indications for rebiopsy varied. Most patients underwent rebiopsy primarily for molecular testing (41 of 66 [62.1%]), including 11 patients for first-time molecular analysis, 13 patients for repeat analysis on account of previous failure, 11 for expanded molecular profiling, and six for *EGFR* T790M mutation detection. In 12 patients, the documented primary indication for repeat biopsy was histological confirmation of disease relapse; in four patients, the primary indication was to exclude clinical suspicion of high-grade neuroendocrine transformation; and in two patients, it was disease restaging. Seven of 66 patients had a rebiopsy in the context of a research protocol.

Technical success was achieved in all 66 patients (a rate of 100%). The mean target lesion size was 40.7 mm (95% confidence interval: 35.9–45.5), with a mean distance to pleura of 15 mm (95% confidence interval: 11.35–18.55). A range of needle gauge sizes was used, from 14G to 18G, with most of the procedures performed using an 18G needle (45 of 52 cases [86%] in which needle gauge size was documented). The median number of cores obtained was 3 (range 1–6), in one case the number was reported as *multiple*, and in 3 cases it was not documented. Target lesion locations were evenly distributed between all lobes of the lung (53% in the upper lobes and 45% in the lower lobes), with one lesion located in the right middle lobe.

Pathological Findings

Pathological success was achieved in 54 of all 66 patients (81.8%). In eight patients no malignant cells were found in the sample. Presence or absence of malignant cells was not evaluable in four cases in which

rebiopsy was performed as part of a research protocol. These four cases were not evaluated for histopathology and were therefore excluded from further analyses. Therefore, the pathological success rate for evaluable cases was 54 of 62 (87.1%).

Histological concordance was evaluable in 52 cases (in two of the 54 cases containing malignant cells histological subtype was not reported on rebiopsy tissue). Concordance of prerebiopsy and postrebiopsy histological subtype was observed in 40 of 52 cases (76.9%). Discordance was observed in 12 cases (23.1%), as detailed in Table 2. In one case, the histopathological features of the rebiopsy sample were consistent with thymoma (in a patient with known synchronous diagnoses of NSCLC adenocarcinoma and thymoma).

Molecular Analysis

A total of 52 cases were adequate for further molecular analysis as subjectively determined by the lung pathologist, resulting in a pretest molecular adequacy of 78.8% of all rebiopsy cases. Two cases containing malignant cells (pathologically successful) were inadequate for molecular analysis owing to *poor sample quality*.

Molecular analysis was performed in 51 of 66 patients, resulting in a total of 209 genes analyzed. In one patient whose rebiopsy sample showed NSCLC with rhabdoid differentiation, the tissue was subjectively adequate for molecular analysis but molecular testing was not requested, as it was not clinically indicated.

The genes analyzed on at least one occasion were *EGFR*, *ALK*, *KRAS*, *NRAS*, *BRAF*, dicoidin domain receptor tyrosine kinase 2 gene (*DDR2*), *ROS1*, and rearranged during transfection proto-oncogene gene (*RET*). Individual polymerase chain reaction–based gene assays were performed; they included the following: cobas 480
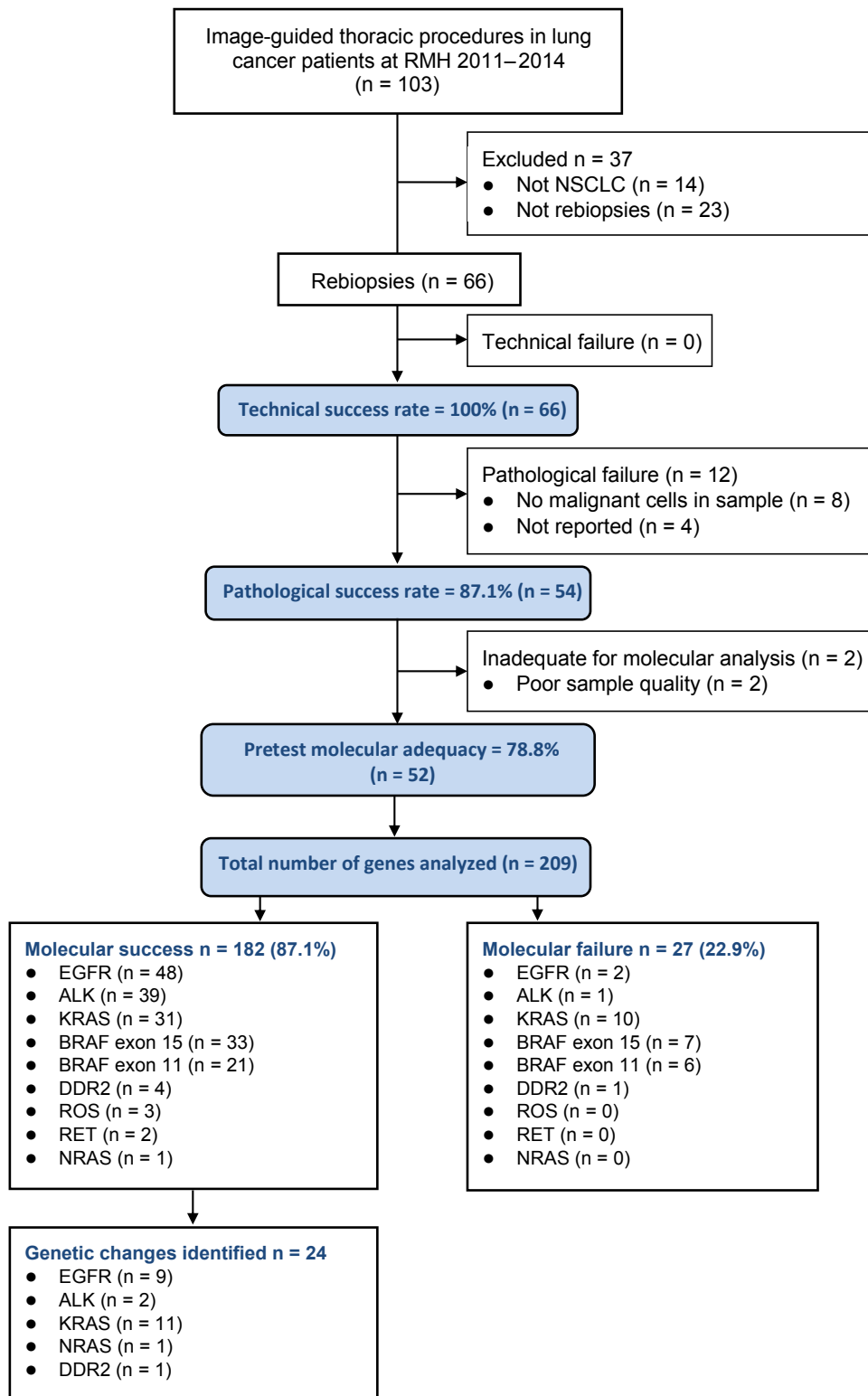
Figure 1. CONSORT diagram. RMS, Royal Marsden Hospital; *ALK*, ALK receptor tyrosine kinase gene; *DDR2*, dicoidin domain receptor tyrosine kinase 2 gene; *RET*, rearranged during transfection proto-oncogene gene.

(Roche, Basel, Switzerland) for *EGFR* and *KRAS* mutations; capillary electrophoresis single-strand conformation analysis for *EGFR*, *BRAF* exon 15 mutation, and *NRAS* mutations; and direct sequencing for *BRAF* exon 11 and *DDR2,* as next-generation sequencing was not routinely implemented during this period. Fluorescence in situ hybridization was used to detect *ALK* and *ROS1* rearrangements.

A total of 182 of 209 genes were analyzed successfully (evaluable), with a posttest molecular success rate of 87.1% (Fig. 1).

There was significant intergene variation in molecular failure rates ($p = 0.005$). For instance, *EGFR* analysis was performed in 50 patients and *ALK* analysis was performed in 40, with molecular failure rates of 4% and 2.5%, respectively, whereas *KRAS* was analyzed 41 times, with a failure rate of 24.4% ($p = 0.04$ and $p = 0.04$, respectively). Rates of molecular success and failure by gene are shown in Table 3. The observed intergene variation in failure rates is likely due to the sequential nature of the individual gene tests performed, with less material available for each subsequent analysis.

The reason for failure of molecular analysis, where recorded, was always poor sample quality. We explored a possible relationship between number of cores obtained and molecular adequacy and found no significant difference in molecular failure rates between cases in which fewer than three cores were obtained and those with three or more cores ($p = 0.185$). There did not appear to be any clear links between incidence of molecular test failure and patient characteristics or technical aspects of rebiopsy.

In all, 24 genetic aberrations were identified, including 20 new (previously unknown) potentially targetable mutations. They included activating mutations in *EGFR* in two patients in whom molecular testing had previously failed (one *EGFR* exon 19 deletion and one S768I point mutation); two *EGFR* T790M acquired resistance mutations; and one *EGFR* primary resistance mutation (exon 20 deletion). *ALK* rearrangements were identified in two patients. Eleven patients were found to have a *KRAS* mutation, one patient had a *NRAS* Q61L mutation, and one had a *DDR2* mutation.

Safety

The rate of all complications was 25.7% (in 17 of 66 patients). Presence of pneumothorax was assessed in all patients by postprocedure plain chest radiograph or limited postprocedure chest CT and confirmed in 12 of 66 cases (18.2%). However, only two of 12 cases required intervention with chest drain insertion (3.0%). The median age of patients experiencing a pneumothorax was similar to that of the overall study population: 63 years (range 37–76) versus 67 years (37–84). The rate of previous or current smoking was slightly higher in the pneumothorax group than in the overall population (83.3% vs. 71.2%), but no one in the pneumothorax group had a history of significant pulmonary comorbidities (compared with a rate of 13% in the overall group).

Hemoptysis was reported in five of 66 cases (7.6%) and not recorded in two patients. All cases were categorized as mild hemoptysis (<30 mL over 24 hours) not requiring further intervention. Two patients (3.0%) required prolonged postprocedure (>48 hours) hospitalization for management of pneumothorax necessitating chest drain insertion. Three patients required a prolonged admission for unrelated reasons.

Postrebiopsy Clinical Outcomes

We extracted data on postrebiopsy clinical treatment pathways to explore the ways in which rebiopsy affected clinical decision making. These data are summarized in Table 4. In 42 of 66 patients (63.6%), rebiopsy had a direct impact on the choice of subsequent treatment, including in 13 (19.7%) who began receiving licensed targeted therapies for newly identified somatic mutations (including seven [54%] in a clinical trial setting) or histological subtype–specific chemotherapy. Four patients (6%) were too unwell for further systemic therapy after rebiopsy.

Discussion

We have reported a retrospective study of the adequacy of image-guided transthoracic rebiopsies in 66

Table 3. Molecular Analysis Results by Gene

Gene	No. Analyzed	No. Failed	Wild Type	Mutation/Rearrangement Present	Failure Rate
EGFR	50	2	39	9	4%
ALK	40	1	37	2	2.5%
KRAS	41	10	20	11	24.4%
BRAF exon 11	27	6	21	0	22.2%
BRAF exon 15	40	7	33	0	17.5%
DDR2	5	1	3	1	20%
ROS1	3	0	3	0	0%
RET	2	0	2	0	0%
NRAS	1	0	0	1	0%
TOTAL	209	27	158	24	12.9%

ALK, ALK receptor tyrosine kinase gene; *DDR2*, dicoidin domain receptor tyrosine kinase 2 gene; *RET*, rearranged during transfection proto-oncogene gene.

Table 4. Rebiopsy Outcomes and Postbiopsy Patient Pathways

Postrebiopsy Clinical Outcomes	No. of Patients
Potentially actionable genetic mutation identified	20
Patients started taking a licensed TKI[a]	6
Patients entered a clinical trial of targeted therapy[a]	7
Patients started undergoing chemotherapy but were potentially eligible for a future clinical trial[a]	4
Patients too unwell for further systemic therapy	3
Activating mutation confirmed/no acquired resistance mutation	4
Patients switched to chemotherapy	2
Patients switched to a second-generation TKI	1
Patients too unwell for systemic therapy	1
Mandatory biopsy within research protocol—patients entered a clinical trial[a]	6
Histological discordance identified—new treatment paradigm[a]	4
Histological confirmation of NSCLC recurrence[a]	12
Patients started receiving palliative treatment[a]	10
Patients started receiving radical treatment[a]	2
NSCLC recurrence ruled out—patients continued surveillance[a]	3
Pathological or molecular failure	13
No actionable mutations identified	4
Total	66

[a]Indicates patients in whom rebiopsy informed the subsequent choice of treatment.
TKI, tyrosine kinase inhibitor.

patients in terms of safety, technical success rates, and adequacy for pathological and molecular analysis.

With a 100% technological success rate, 87.1% pathological adequacy, and 78.8% molecular adequacy as subjectively assessed by a lung pathologist, we have shown that image-guided lung rebiopsies are feasible and can yield tissue adequate for analysis of multiple biomarkers in the setting of standard clinical practice. We report rates of pneumothorax (18%), chest drain insertion (3%), and mild hemoptysis (8%) that are similar to those previously reported in large series of percutaneous transthoracic biopsies in the primary diagnostic setting,[24–27] and we therefore conclude that rebiopsy is not associated with any increased risk compared with primary biopsies.

We observed a relatively high rate of histological discordance (23%) between rebiopsy material and prior diagnostic biopsies. In cases in which histological discrepancy was observed, the initial diagnostic biopsies were re-reviewed where available to explore possible causes for the differences. In two cases in which squamous cell carcinoma at initial biopsy was reclassified as

adenocarcinoma on rebiopsy and where diagnostic biopsy material was available for review, rebiopsy tumor material showed some features of overlap between adenocarcinoma and squamous cell carcinoma. The discordance between biopsy samples may therefore reflect sampling of different components of the same tumor with features of both adenocarcinoma and squamous cell carcinoma. Another possible explanation for the observed differences may be sampling bias, with patients whose initial samples were inadequate for optimal histological assessment and diagnosis selected for rebiopsy, leading to higher rates of histological discordance in our cohort (e.g., three instances of NSCLC not otherwise specified were reclassified as squamous cell carcinoma).

Overall, 182 of 209 individual gene tests (87.1%) were performed successfully in 51 patients. Molecular success rates varied significantly between individual gene assays. *EGFR* testing was completed successfully in 48 of 50 cases (96%), which is in line with the rates reported in several previous studies of adequacy of rebiopsy tissue for *EGFR* testing.[14,28–31] Two prospective studies of rebiopsies in 121 patients[30] and 162 patients[14] with acquired resistance to EGFR TKIs reported rates of 86% and 95.6%, respectively. Another recent prospective study enrolled 24 patients with *EGFR* mutation who began receiving afatinib therapy with a view to rebiopsy for *EGFR* T790M analysis at progression. Of 23 patients in whom progressive disease developed, only 14 completed a rebiopsy, with 11 samples (78.6%) sufficient for molecular analysis.[31]

Most studies of rebiopsies have focused on mechanisms of acquired resistance to EGFR TKIs (in particular, detection of T790M mutation), and few studies have evaluated adequacy for multiple biomarker testing on rebiopsy tissue outside of this context.[32–34] Tam et al. have reported a retrospective analysis of adequacy of percutaneous transthoracic core needle biopsies for the evaluation of multiple molecular biomarkers within the context of the genotype-directed BATTLE trial.[33] A total of 170 biopsies were performed in 151 patients NSCLC who were screened for the trial. Specimens from 82.9% of patients were found to have adequate tumor tissue for analysis of 11 different biomarkers within *EGFR*, *KRAS*, *BRAF*, vascular endothelial growth factor receptor (*VEGFR*), retinoid X receptor gene (*RXR*), and cyclin D genes. The rates of pneumothorax and chest tube insertion were 15.3% and 9.4%, respectively. In our study, the rates of pretest (87.1%) and posttest (78.8%) molecular adequacy were similar to those reported in the BATTLE trial despite our relatively unselected patient cohort in the setting of standard clinical practice.

The main limitation of this study is that it is a retrospective observational study based on the clinical

experience of a single oncology center. As a tertiary referral center and an institution with a well-established infrastructure and experience in this area, our experience may not be representative of the patient profile and resources available in other community-based centers. Second, the discrepancy between subjective pathologist-assessed pretest molecular adequacy and posttest molecular success rate has been difficult to explore in the absence of complete data on the reasons for test failure. Third, incomplete data on the technical aspects of each procedure precluded analysis of the potential relationship between incidence of molecular analysis failure and the way in which procedures were performed, which would help define optimal conditions to obtain adequate tissue samples. Finally, instead of single-gene tests performed in parallel or sequentially, many centers have now moved to implementing next-generation sequencing–based molecular genotyping[35–38]; therefore, the individual molecular success rate at individual genes may not reflect changes in gene testing methodologies.

Choice of optimal treatment and development of treatment strategies in NSCLC are predicated by tumor histological and molecular characterization. Repeated molecular profiling is likely to be required at multiple time points during the treatment pathway, as is already the case for detection of the *EGFR* T790M mutation,[20] given the interpatient and intrapatient molecular heterogeneity identified from sequencing studies[39] and the evolutionary pressures of molecular selection from targeted therapy in oncogene-addicted NSCLCs. Nevertheless, in a real-world setting, our data have identified the clinical utility and limitations of rebiopsies in advanced NSCLCs, demonstrating a clinically important utility in decision making and for molecular characterization. Improvements in the histological yield and molecular adequacy of rebiopsies may be achieved by implementation of standardized protocols and algorithms in radiology departments and laboratories to ensure optimal handling of samples for molecular analyses as highlighted in the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guideline.[4] Use of rapid on-site evaluation of specimens at time of the procedure has been shown to improve diagnostic yield, decrease the need for repeat procedures, and facilitate collection of sufficient material for molecular testing,[40] although resource considerations are likely to affect widespread use of this technique.

Validation of ctDNA for genotyping is facilitating a less invasive approach for detection of *EGFR* T790M at the point of progression,[41] but tissue-based verification remains an important strategy to identify patients

suitable for *EGFR* T790M inhibitors, especially because of the low sensitivity of some ctDNA testing methods. It is also important to verify other resistance mechanisms, such as histological nonconcordance, and to stratify patients for other systemic therapies within clinical trials. In our study rebiopsies produced clinically relevant information, helping to guide the choice of treatment in nearly two-thirds of patients through identification of new actionable driver and resistance mutations, change in histological classification, and confirmation or exclusion of recurrent disease.

Our study provides valuable data on the role and utility of rebiopsy for molecular analysis of multiple molecular markers in a heterogeneous group of patients with NSCLC in the setting of standard clinical practice. We have validated the pathological and molecular adequacy rates of rebiopsies and demonstrated clinical utility in routine decision making.

Acknowledgments

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APPENDIX 12. CRUK SMP2 EXAMPLE NGS REPORT

ROYAL MARSDEN NHS FOUNDATION TRUST - MOLECULAR GENETICS REPORT - CONFIDENTIAL

Date Booked	1 Feb 2019	**Lab No**	19/01191	**Sample Date**	28 Jan 2019
Request Type	Internal	**Request Date**	22 Jan 2019	**Specimen Type**	Other Tissue Sections
Histopathology No	18C00033685	**Pathology No**			

Mutations RESULTS

Studies included: CR-UK National Lung Matrix Trial (NGS CRUK-SMP2 panel v1.0).

Gene	Method	Regions Analysed	Result	Report	Status	Comments
AKT1	Illumina NGS Panel 3	Exon 2	No variant detected	High confidence	Complete	These results are intended for research purposes
ALK	Illumina NGS Panel 3	Exons 18-21 (including fusions), 22, 23 & 25 exon only	No variant detected	Medium confidence	Complete	These results are intended for research purposes
BRAF	Illumina NGS Panel 3	Exons 11, 15	No variant detected	High confidence	Complete	These results are intended for research purposes
CCND1	Illumina NGS Panel 3	Exons 1-5	No variant detected	High confidence	Complete	These results are intended for research purposes
CCND2	Illumina NGS Panel 3	Exons 1-5	No variant detected	High confidence	Complete	These results are intended for research purposes
CCND3	Illumina NGS Panel 3	Exons 1-5	No variant detected	High confidence	Complete	These results are intended for research purposes
CCNE1	Illumina NGS Panel 3	Exons 1-11	No variant detected	Medium confidence	Complete	These results are intended for research purposes
CDK2	Illumina NGS Panel 3	Exons 1-7	No variant detected	High confidence	Complete	These results are intended for research purposes
CDK4	Illumina NGS Panel 3	Exons 2-8	No variant detected	High confidence	Complete	These results are intended for research purposes
CDKN2A	Illumina NGS Panel 3	Exons 1-3	CDKN2A deletion	Tier1 CDKN2A homozygous deletion-confirmed by FISH	Complete	These results are intended for research purposes
EGFR	Illumina NGS Panel 3	Exons 18 - 21	c.2582T>A p. (Leu861Gln); EGFR amplification	Tier1 EGFR variant; Tier3 EGFR amplification	Complete	These results are intended for research purposes
ERBB2	Illumina NGS Panel 3	Exons 1-26	No variant detected	Medium confidence	Complete	These results are intended for research purposes
FGFR2	Illumina NGS Panel 3	Exons 1-17 plus introns 1,17	No variant detected	Medium confidence	Complete	These results are intended for research purposes
FGFR3	Illumina NGS Panel 3	Exons 1-17 plus intron 16	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes

Gene	Method	Region	Result	Confidence	Status	Comment
HRAS	Illumina NGS Panel 3	Exons 1-3	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
KRAS	Illumina NGS Panel 3	Exons 2 - 4	No variant detected	High confidence	Complete	These results are intended for research purposes
MET	Illumina NGS Panel 3	Exons 1-20	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
NF1	Illumina NGS Panel 3	Exons 1-58	No variant detected	High confidence	Complete	These results are intended for research purposes
NRAS	Illumina NGS Panel 3	Exons 2 - 4	No variant detected	High confidence	Complete	These results are intended for research purposes
NTRK1	Illumina NGS Panel 3	Exons 7-14, including introns, plus exon 15	No variant detected	Medium confidence	Complete	These results are intended for research purposes
PIK3CA	Illumina NGS Panel 3	Exons 1-20	No variant detected	High confidence	Complete	These results are intended for research purposes
PTEN	Illumina NGS Panel 3	Exons 1 - 9	No variant detected	High confidence	Complete	These results are intended for research purposes
RB1	Illumina NGS Panel 3	Exons 1-27	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
RET	Illumina NGS Panel 3	Exons 7,8,10-12 plus introns 7,10,11	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
ROS1	Illumina NGS Panel 3	Exons 31-36,38 plus introns 31,33,34,35	No variant detected	High confidence	Complete	These results are intended for research purposes
STK11	Illumina NGS Panel 3	Exons 1 - 9	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
TSC1	Illumina NGS Panel 3	Exons 1-21	No result	Fail. Repeat sample requested if available.	Fail	These results are intended for research purposes
TSC2	Illumina NGS Panel 3	Exons 1-41	No variant detected	Medium confidence	Complete	These results are intended for research purposes

Authorised by DR. SUZANNE MACMAHON **on** 10.6.19

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Please note that some rare mutations in the genes analysed may not be detected with this methodology. Mutations outside the exons or codons analysed cannot be detected. Note the approximate sensitivity limit of the methodologies used (i.e. COBAS 480 5%; CE-SSCA and GeneScan 5-10%; direct sequencing 25%; NGS TSCA 3%). This needs to be taken in consideration together with the level of tumour infiltration and the size of the sample sent, as some mutations may be present below the level of detection of the methodology used.

APPENDIX 13. CRUK SMP2 ALL DATA ITEMS AND XML DEFINITIONS

CH - TH Data Interchange XML Messaging Guidance V3.5					
Location / XML Tag	**XML Description**	**Data Restrictions**	**CH Request Validation**	**TH Results Validation**	**CH Archive Validation**
smClinicalHub (attribute: name)	Name of the clinical hub requesting the tests / sending the samples to the technology hub.	- "1 - Birmingham" - "2 - Cardiff" - "3 - Cambridge" - "4 - Edinburgh" - "5 - Glasgow" - "6 - Leeds" - "7 - Manchester" - "8 - Royal Marsden" - "9 – Barts & Brighton" - "10 – Belfast" - "11 – Imperial" - "12 - KCL" - "13 – Leicester" - "14 – Newcastle" - "15 – Oxford" - "16 – Sheffield" - "17 – Southampton" - "18 - UCL" N.B. XSD type is String and no value list has been enforced	Required	Required	Required
smClinicalHub/patient					

smClinicalHub/patient/organisationCode	NHS Org Code of the hospital where the patient was recruited	String (an5)	Required	Required	Required
smClinicalHub/patient/localPatientIdentifier	Patient Identifier generated at the clinical hub (exact format of this varies per clinical hub)	String (an10)	Required	Required	Required
smClinicalHub/patient/treatingOncologistInitials	Three letter intitials (NHS convention to add Z if no middle initial)	String (an3)	Optional	Optional	Required
smClinicalHub/patient/ageAtAttendance	The number of completed years between the DOB and the Attendance Date or the estimated age of the PATIENT	Number (n3)	Optional	Optional	Required
smClinicalHub/patient/genderCode	Patient's current gender	Value List: 0 - Not Known 1 - Male 2 - Female 9 - Not Specified	Optional	Optional	Required

smClinicalHub/patient/ethnicCategory	The ethnicity of a PERSON, as specified by the PERSON. The 16+1 ethnic data categories defined in the 2001 census is the national mandatory standard for the collection and analysis of ethnicity	Value List: A - White British B - White Irish C - Any other White background Mixed D - White and Black Caribbean E - White and Black African F - White and Asian G - Any other mixed background Asian or Asian British H - Indian J - Pakistani K - Bangladeshi L - Any other Asian background Black or Black British M - Caribbean N - African P - Any other Black background Chinese or Other Ethnic Group R - Chinese S - Any other ethnic group Z - Not stated 99 - Not known	Optional	Optional	Required

smClinicalHub/patient/smokingStatus	Patients current smoking status.	Value List 1 - Current smoker 2 - Ex smoker 3 - Non-smoker - history unknown 4 - Never smoked Z - Not Stated (PERSON asked but declined to provide a response) 9 - Unknown	Optional	Optional	Required
smClinicalHub/patient/noOfPriorLinesTherapy	Number of prior therapy lines (0, 1,2,3,4, N/K, N/A)	String (an5)	Optional	Optional	Required

| smClinicalHub/patient/performanceStatus | Patients Performance status | 0 - Able to carry out all normal activity without restriction
1 - Restricted in physically strenuous activity, but able to walk and do light work
2 - Able to walk and capable of all self care, but unable to carry out any work. Up and about more than 50% of waking hours
3 - Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4 - Completely disabled. Cannot carry on any self care. Totally confined to bed or chair
9 - Not recorded | Optional | Optional | Required |

Sample

sample/clinicalHubElements	**Element containing elements of sample information owned by the clinical hub**			
sample/clinicalHubElements/sourceSampleIdentifier	Sample ID generated by the clinical hub	String an(20)	Required	Required	Required
sample/clinicalHubElements/originOfSample	The Origin of sample is whether the tumour is primary or metastatic	1 - Primary tumour 2 - Metastatic site – lymph node 3 - Metastatic site – other	Optional	Optional	Required

sample/clinicalHubElements/typeOfSample	Format of the sample being sent to the technology hub	Value List: 1 - Blood 3 - Tissue- Resection 8 - Tissue- Bronchoscopic biopsy 9 - Tissue- CT guided biopsy 10 -Tissue- Surgical biopsy 11 - Tissue- Other biopsy 12 - Cytology cell block- EBUS/EUS FNA 13 - Cytology cell block- Bronchoscopic washing 14 - Cytology cell block- CT guided 15 - Cytology cell block- Effusion 16 - Cytology cell block- Other 17 - Extracted DNA	Required	Required	Required
sample/clinicalHubElements/procedureToObtainSample	The type of procedure used to otain the tumour sample	Value List: 1 - CT guided biopsy 2 - US guided biopsy 3 - Surgical lung biopsy 4 - Surgical resection 5 - EBUS 6 - EUS 7 - Other biopsy 8 - Other FNA cytology	Optional	Optional	Required

sample/clinicalHubElements/typeOfBiopsy	Is sample a diagnostic biopsy or repeat biopsy	0 - unknown 1 - Diagnositc biopsy 2 - Repeat biopsy due to sample test failure 3 - Repeat biopsy due to lack of sample after local testing 4 - Mandatory repeat biopsy after targeted first line therapy 5 - Repeat biopsy due to non actionable mutation in diagnostic biopsy 6 - Voluntary repeat biopsy after first line therapy 7 - Voluntary repeat biopsy after targetted therapy	Optional	Optional	Required
sample/clinicalHubElements/dateSampleTaken	The date the Sample to be used for the molecular tests of the SM Programme was taken from the patient. This could be either biopsy or resection, but should be the sample used for molecular profiling.	Date (YYYY-MM-DD)	Optional	Optional	Required

sample/clinicalHubElements/tumourType	Tumour type according to Stratified Medicine categories (defines which tests will be carried out)	Value List: - "1 - Breast" - "2 - Colorectal" - "3 - Lung" - "4 - Melanoma" - "5 - Ovarian" - "6 - Prostate" - "7 - Other"	Required	Required	Required
sample/clinicalHubElements/morphologySnomed	This is the PATIENT DIAGNOSIS for the cell type of the malignant disease recorded as part of a Cancer Care Spell.	String (CDATA wrapped) (Max an18)	Required	Required	Required
sample/clinicalHubElements/pathologyTCategory	T CATEGORY is the Union for International Cancer Control (UICC) code which classifies the size and extent of the primary Tumour based on the evidence from a pathological examination.	Value List: 0 - unknown TX - Primary tumour cannot be assessed T0 - No evidence of primary tumour Tis - Carcinoma in situ T1a - Tumour ≤20 mm diameter T1b - Tumour >20–≤30 mm T2 - Tumour >= 20mm from the carina, invades visceral pleura, partial atelectasis T2a - >30–≤50 mm T2b - >50–≤70 mm T3 - >70 mm; involvement of parietal pleura, mediastinal pleura,	Optional	Optional	Required

		chest wall, pericardium or diaphragm; tumour within 20 mm of the carina; atelectasis/obstructive pneumonitis involving whole lung; separate nodule(s) in the same lobe T4 - Involvement of great vessels, mediastinum, carina, trachea, oesophagus, vertebra, or heart Separate tumour nodule(s) in different ipsilateral lobe 9 - not applicable			

sample/clinicalHubElements/pathologyNCategory	N CATEGORY is the Union for International Cancer Control (UICC) code which classifies the absence or presence and extent of regional lymph node metastases based on the evidence from a pathological examination.	Value List: 0 - unknown NX - Regional lymph nodes cannot be assessed N0 - No regional node involvement N1 - Ipsilateral hilar/intrapulmonary nodes (node stations 10–14) N2 - Ipsilateral mediastinal nodes (node stations 1–9) N3 - Contralateral mediastinal, hilar, ipsilateral or contralateral scalene, supraclavicular nodes 9 - not applicable	Optional	Optional	Required
sample/clinicalHubElements/pathologyMCategory	M CATEGORY is the Union for International Cancer Control (UICC) code which classifies the absence or presence of distant metastases based on the evidence from a pathological examination.	Value List: 0 - unknown M0 - No distant metastasis M1 - Distant metastasis M1a - Separate tumour nodule(s) in a contralateral lobe; pleural nodules or malignant pleural or pericardial effusion. M1b - Distant metastasis 9 - not applicable	Optional	Optional	Required

sample/clinicalHubElements/integratedTNMStageGrouping	Record the overall TNM stage grouping of the tumour, derived from each T, N and M component after treatment. This classification is based on all the evidence available to the clinician(s) with responsibility for assessing the patient. Such evidence arises from physical examination, imaging, endoscopy, biopsy, surgical exploration and other relevant examinations. The overall integrated TNM stage grouping indicates the tumour stage after treatment and/or after all available evidence has been collected. Note: Use UICC coding.	Max an5	Optional	Optional	Required
sample/clinicalHubElements/alkStatus	ALK status of the pathology tumour specimen as assessed by immunohistochemistry.	P-positive N-negative E-equivocal X-not known Z-not performed U-technically unsatisfactory	Optional	Optional	Required
sample/clinicalHubElements/egfrStatus	EGFR mutation status of the pathology specimen assessed in usual referral laboratory before submission of sample to SMP2	M-mutation detected N-no mutation detected X-not known F-test failure Z-not performed Y-other result	Optional	Optional	Required

sample/clinicalHubElements/alkFishStatus	ALK status of the pathology tumour specimen as assessed byfluoresecent in situ hybridisation (FISH) before submission to SMP2.	R-rearrangement detected N-no rearrangement detected X-not known F-test failure Z-not performed Y-other result	Optional	Optional	Required
sample/clinicalHubElements/krasStatus	KRAS mutation status of the pathology specimen assessed in usual referral laboratory before submission of sample to SMP2	M-mutation detected N-no mutation detected X-not known F-test failure Z-not performed Y-other result	Optional	Optional	Required
sample/clinicalHubElements/dateSampleSent	Date the sample was sent from the clinical hub to technology hub	Date (YYYY-MM-DD)	Required	Required	Required
sample/technologyHubElements	**Element containing elements of sample information owned by the technology hub**				
sample/technologyHubElements/dateSampleReceived	Date the physical sample was receipted by the technology hub	Date (YYYY-MM-DD)	N/A	Required	Required
sample/technologyHubElements/labSampleIdentifier	Any lab-based identified for the sample (e.g. barcode).	String Alphanumeric	N/A	Required	Required

sample/technologyHubElements/reportReleaseDate	Date when this patient report was released and the XML message was generated.	Date (YYYY-MM-DD)	N/A	Required	Required
sample/technologyHubElements/volumeBankedNucleicAcid	Volume of nucleic acid which was banked, in microlitres. If no nucleic acid could be banked, this field should be '0' not left blank.	String (CDATA wrapped) (max n3.n2)	N/A	Required	Required
sample/technologyHubElements/concentrationBankedNucleicAcid	Concentration of Nucleic Acid banked from the patient sample in micrograms/microlitre :µg/µl. If no nucleic acid could be banked, this field should be '0' not left blank.	String (Max n3.n2)	N/A	Required	Required
sample/technologyHubElements/bankedNucleicAcidLocation	Location of the nucleic acid bank storage, should be the same of the technology hub name	String (an50)	N/A	Optional	Optional

sample/technologyHubElements/bankedNucleicAcidIdentifier	Identifier used when storing the DNA material. Using this identifier together with the location should allow the sample to be accurately retrieved.	String (an10)	N/A	Optional	Optional
Results					
smTechnologyHub (attribute: name)	Name of the technology hub processing the tests.	Values: - "1 - Birmingham" - "2 - Cardiff" - "3 - Royal Marsden" N.B. XSD type is String and no value list has been inforced	Required	Required	Required
smTechnologyHub/testResults	**Parent element containing repeating groups of 'test' element**				
smTechnologyHub/testResults/test	Group containing all of the fields required for one molecular test result per method of test.				
smTechnologyHub/testResults/test/gene	Name of the gene being tested, should adhere to SM Programme values	Value list: 1 - BRAF 4 - ALK 5 - PIK3CA 6 - PTEN 7 - PTEN LOH 8 - TP53 9 - KIT 10 - NRAS 11 - DDR2 12 - TMPRSS2-ERG 13 - EGFR 14 - KRAS 15 - AKT1 16 - CCND1 17 - CDK4 18 - CDKN2A 19 - CDKN2B	N/A	Required	Required

		20 - FGFR1 21 - FGFR2 22 - FGFR3 23 - HER2 24 - JAK2 25 - KDR 26 - MET 27 - NF1 28 - P16 29 - PDL-1 30 - RB1 31 - RET 32 - ROS1 33 - STAT3 34 - STK11/LKB1 35 - TSC1 36 - TSC2 37 - HRAS 38 - CCND2 39 - CCND3 40 - CCNE1 41 - CDK2 42 - NTRK1			

smTechnologyHub/testResults/test/methodOfTest	Specific method used to detect mutations.	Value list: 1 - FISH 2 - MICROSAT 3 - RQ - PCR 4 - SEQUENCING 5 - DIRECT SEQUENCING 6 - PYROSEQUENCING 7 - HRM-HIGH RESOLUTION MELT 8 - ARMS 9 - CE - SSCA 10 - COBAS 4800 11 - SNAPSHOT 12 - RT - PCR 13 - FRAGMENT LENGTH 14 - Other 15 - Illumina NGS panel 1 16 - Illumina NGS panel 2 17 - Illumina NGS panel 3 18 - Illumina NGS panel 4	N/A	Required	Required
smTechnologyHub/testResults/test/scopeOfTest	Exon or codon scope of the test performed	String (an200) (CDATA wrapped)	N/A	Required	Required
smTechnologyHub/testResults/test/dateTestResultsReleased	Date the individual test results were released. If all test results were released, this date should be set to same as sample/technologyHubElements/reportRelease Date	Date (YYYY-MM-DD)	N/A	Required	Required

smTechnologyHub/testResults/test/testResult	Where mutations have been found, standard HGVS nomenclature should be followed. Where multiple mutations exist, results should be separated using a ";"	String (an100) (CDATA wrapped)	N/A	Required	Required
smTechnologyHub/testResults/test/testReport	Textual description of the above results.	String (an600) (CDATA wrapped)	N/A	Required	Required
smTechnologyHub/testResults/test/testStatus	Status of this gene test at the point of the report	Value List: 1 - Success 2 - Partial Fail 3 - Complete Fail 4 - Not Tested (an1)	N/A	Required	Required

APPENDIX 14. DEMOGRAPHICS AND BASELINE CHARACTERISTICS FOR PATIENTS ENROLLED IN CRUK SMP2 (N=3146)

DEMOGRAPHICS AND BASELINE CHARACTERISTICS (n=3146)	n	%
Number of patients by Technical Hub		
RMH	677	21.52
Birmingham	1214	38.59
Cardiff	1255	39.89
Number of patients by Clinical Hub		
RMH	360	53.18
Leeds	191	28.21
UCL	46	6.79
Imperial	70	10.34
Belfast	10	1.48
Birmingham	432	35.58
Soton	461	37.97
Cambridge	208	17.13
Edinburgh	37	3.05
Leicester	14	1.15
Sheffield	62	5.11
Cardiff	324	25.82
Glasgow	432	34.42
Manchester	217	17.29
Newcastle	237	18.88
KCL	38	3.03
Oxford	4	0.32
Barts	3	0.24
Age at SMP2 enrollment* (median, range)	67, 18-97	
Gender		
Male	1653	52.5
Female	1438	45.7
Missing	55	1.7
Ethnicity		
White British	2174	69.1
White Irish	29	0.9
Any other White background	66	2.1
White and Black Caribbean	3	0.1
White and Black African	1	0.0
White and Asian	2	0.1
Any other mixed background	5	0.2
Indian	19	0.6
Pakistani	6	0.2
Bangladeshi	1	0.0
Any other Asian background	10	0.3
Caribbean	11	0.3

African	8	0.3
Any other Black background	3	0.1
Chinese	14	0.4
Any other ethnic group	21	0.7
Not stated	208	6.6
Not known	565	18.0
Smoking status		
Current smoker	624	19.8
Ex-smoker	1519	48.3
Non/never-smoker	379	12.0
Unknown	584	18.6
Not stated (asked but declined to provide response)	40	1.3
Stage		
0	1	0.0
IA	22	0.7
IB	19	0.6
IIA	29	0.9
IIB	51	1.6
III	20	0.6
IIIA	469	14.9
IIIB	334	10.6
IV	316	10.0
IVA	471	15.0
IVB	745	23.7
NK	669	21.3
Mutational status at enrolment		
EGFR		
Negative	1075	34.2
Positive	170	5.4
Unknown/not performed	1886	59.9
Test failed	15	0.5
ALK IHC		
Negative	731	23.2
Positive	22	0.7
Unknown/not performed	2384	75.8
Test failed	9	0.3
ALK FISH		
Negative	555	17.6
Positive	24	0.8
Unknown/not performed	2544	80.9
Test failed	23	0.7
KRAS		
Negative	197	6.3
Positive	90	2.9
Unknown/not performed	2845	90.4
Test failed	14	0.4

Prior lines of therapy		
0	855	27.2
1	655	20.8
2	376	12.0
3	134	4.3
4	55	1.7
≥5	7	0.2
NK	1064	33.8
ECOG PS at enrolment		
0	522	16.6
1	1594	50.7
2	301	9.6
3	26	0.8
4	4	0.1
NK	699	22.2
Histology		
Adenocarcinoma	1465	46.57
Adenosquamous carcinoma	19	0.60
Anaplastic carcinoma	2	0.06
Atypia suspicious of malignancy	10	0.32
Carcinoma NOS	211	6.71
Large cell carcinoma	37	1.18
Mesothelioma	3	0.10
NSCLC NOS	90	2.86
Small cell carcinoma	18	0.57
Squamous cell carcinoma	680	21.61
Undifferentiated carcinoma	8	0.25
Unknown	481	15.29
Unrecognised snomed code	34	1.08
Other	88	2.80

* not available for 15 patients

APPENDIX 15: LIST OF DATA ITEMS COLLECTED FOR EGFR ctDNA FEASIBILITY STUDY

Metric data point	Response
Patient ID:	123456
Patient Initials:	xx
Institution:	RMH/ Belfast/ Manchester/ Cardiff/Birmingham
Date of birth	dd/mm/yyyy
UICC stage	1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B
T Stage	Tx,0,1,2,3,4
N Stage	Nx,0,1,2,3,
M Stage	Mx,0,1a,1b
Thoracic disease only at time of blood draw?	Yes or No
Brain metastases at time of blood draw?	Yes or No
Date of tissue biopsy	dd/mm/yyyy
Date of EGFR tissue testing request	dd/mm/yyyy
Date and time of first treatment if treatment naive	dd/mm/yyyy hh:mm
Date and time of blood draw	dd/mm/yyyy hh:mm
Blood tube used for blood draw	EDTA/Streck/Ariosa/PaxGene/Other
Date and time of sample receipt in laboratory	dd/mm/yyyy hh:mm
Date and time of plasma processing	dd/mm/yyyy hh:mm
Date and time of DNA extraction	dd/mm/yyyy hh:mm
Plasma Sample Lab No:	XXX
Histopathology Lab No:	XXX
UICC stage M-stage	M0, M1a, M1b
Date of EGFR ctDNA request	dd/mm/yyyy
Date of EGFR ctDNA testing	dd/mm/yyyy
EGFR ctDNA testing method	Roche cobas ®/ Droplet digital PCR, qPCR/ Therascreen/ Other (define)
EGFR ctDNA result	Mutation (define) /No mutation/ Invalid
EGFR T790M ctDNA positive?	Yes or No
Date of EGFR ctDNA result	dd/mm/yyyy
Date of EGFR tissue testing	dd/mm/yyyy
EGFR tissue testing method	Single gene test/NGS/Other (define)
EGFR tissue testing result	Mutation (define)/No mutation/ Invalid
EGFR T790M tissue positive?	Yes or No