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Title: A Phase II Trial of Nilotinib in KIT-driven Advanced Melanoma (NICAM)

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Summary (current 150, max 150 words)

Mucosal (MM) and acral melanomas (AM) are rare melanoma subtypes of unmet clinical need; 15-20% harbour *KIT* mutations potentially targeted by small molecule inhibitors, but none yet approved in melanoma. This multicentre, single-arm phase 2 trial (NICAM) investigates nilotinib safety and activity in *KIT* mutated metastatic MM and AM. *KIT* mutations are identified in 39/219 screened patients (18%); of 29/39 treated, 26 are evaluable for primary analysis. Six patients were alive and progression-free at 6 months (local radiology review, 25%); 5/26 (19%) had objective response at 12 weeks; median OS was 7.7 months. ddPCR assay correctly identifies *KIT* alterations in circulating tumour DNA (ctDNA) in 16/17 patients.

Nilotinib is active in *KIT*-mutant AM and MM, comparable to other KIT inhibitors, with demonstrable activity in non-hotspot *KIT* mutations, supporting broadening of *KIT* evaluation in AM and MM. Our results endorse further investigations of nilotinib for the treatment of *KIT* mutated melanoma.

Keywords (max 10): *KIT* mutation, tyrosine kinase inhibitor, melanoma, mucosal, acral, liquid biopsy

Clinical Trial Registration: ISRCTN39058880, EudraCT 2009-012945-49

Introduction

Acral melanomas (AM) and mucosal melanomas (MM) are rare subtypes of melanomas (comprising around 5%), and arise from non-glabrous skin including mucosa (MM), soles, palms, and the nail bed (AM)[1,2]. MM and AM are clinically and genetically distinct from the common cutaneous melanomas. MM exhibit aggressive clinical behaviour, commonly recur after surgical removal, resulting in five-year survival rates of just 14%, compared to 90% five-year survival of patients with cutaneous melanomas[3,4]. AM have inferior outcomes compared to UV-associated cutaneous melanomas[2,5-7], due to frequently delayed diagnosis and inherently more aggressive disease course[8] .

UV-driven mutagenesis is limited in AM and only found in a small proportion of MM from sun-exposed mucosa, including the conjunctiva and lips[3,9-16]. MM and AM have low tumour mutational burden, and instead are characterised by higher levels of chromosomal complexity[16]. *BRAF* mutations, present in ~40-50% of common cutaneous melanomas[17], are detected in ~20% AM[18-20] and are largely absent in MM[21,22]; thus, only a minority of these patients are suitable for treatment with BRAF&MEK targeting agents. Immune checkpoint blockade (ICB) has transformed the outcomes of patients with cutaneous metastatic melanoma with 5-year survival rates of ~50% [23]; however, the proportion of patients with AM and MM who benefit from ICB is significantly lower by comparison: programmed death-1 (PD1) blockade response rate of 15-40% vs 40-50% and overall survival of 11.5 vs 25.8 months[12,24-27]. Thus, AM and MM have relatively limited treatment options, further aggravated by the disease rarity, frequent exclusion from phase III clinical trials, and lack of evidence-base for clinical decision making.

The aim of this study was to evaluate the efficacy of nilotinib in advanced *KIT* mutated melanoma, to explore the particularities of *KIT* mutation and copy number amplification and benefit from treatment, and to assess the value of droplet digital PCR (ddPCR) for the liquid biopsy of melanomas with uncommon *KIT* mutations and complex aberrations.

Results

Patients

Between December,15 2009 and August,4 2014, 219 patients with the diagnosis of advanced acral or mucosal melanoma meeting eligibility criteria were screened for the presence of *KIT* mutations. *KIT* mutations were detected in 39 (18%) of patients, of which 29 (13%) were considered eligible to enter the treatment part of the trial (Figure 1). One of the 10 ineligible patients was excluded due to the finding of exon 17 *KIT* mutation that likely conferred resistance to nilotinib based on prior reports[28]. Baseline characteristics of enrolled patients are shown in Table 1 (see supplementary Table S1 for baseline features of all screened patients). Six patients presented with AM (20.7%), and 23 with MM (79.3%). *KIT* mutations were found in exon 11 (n=20, 69%), exon 13 (n=4, 14%), exon 17 (n=4,14%) and exon 9 (n=1, 3%). Twenty-one (72%) mutations were single nucleotide variants, while eight (28%) were insertions or deletions (indels). The most common mutation was L576, which we observed in nine patients (31%) (Figure 2, supplementary Table S2).

Amongst patients who received at least one dose of nilotinib (n=28), median time on treatment was 3.7 months (Q1-Q3 2.2 – 11.7 months) (Figure 3). One patient remained on treatment for more than 50 months. Overall, 22 patients (79%) had at least one dose reduction, delay or missed treatment (supplementary Figure S1); of these, 8 patients (29%) had at least one nilotinib dose reduction (4/8 due to abnormal liver function, 2/8 due to other toxicities, 2/8 due to omitting doses in error). At data cut-off the median follow-up for patients on trial was 7.1 months (Q1-Q3 3.0, 19.1 months).

Overall, 26 patients were evaluable for the primary endpoint. Three unevaluable participants (all MM) included one who discontinued due to toxicity prior to the first scan, but deemed unevaluable as was taking a prohibited concomitant medication; one patient who withdrew consent for all trial procedures after 1.4 months of treatment; and one who progressed prior to receiving any trial treatment.

Safety

All patients who received at least one dose of nilotinib (n=28) were assessed for safety. NCI-CTC grade 3 adverse events (AE) or higher were reported in 18 patients (64%) while on treatment (Table 2). The most frequent AEs of any grade were fatigue (N=21 75%), nausea (n=17, 61%) and constipation (n=14, 50%). Sixteen serious AE (SAEs) in 10 patients were reported, of which only two events in one patient were deemed to be related to study drug (SAR). This patient experienced both SAR within two months

of commencing treatment (raised ALT grade 4, AST grade 3 and bilirubin grade 2) and permanently discontinued nilotinib. We note that this patient had been taking concomitant prohibited herbal medication which may have contributed to the liver dysfunction. A further patient experienced a treatment-related toxicity (deranged liver function) leading to 50% dose reduction and then treatment discontinuation. There were no treatment-related deaths.

Antitumour activity

Of the first 24 evaluable patients as pre-specified in the two-stage design, six patients were progression-free at six months as reported locally (25% 90%CI 12-44, p=0.11), thus not fulfilling the pre-specified success criteria. However, central review of the primary endpoint indicated that there were seven patients who were progression-free at six months (29%, 90%CI 15-47, p=0.05). Accounting for the two-stage design, the local and central estimate of 6-month PFS were, respectively, 30% and 33%. Over all 26 evaluable patients, the estimates for 6-month PFS rate accounting for the two-stage design were 29% (90%CI: 11-44, p=0.14) as per local review and 31% (90%CI 14-45) as per central review. Of note, all acral-subtype patients progressed by six months.

Objective RECIST 1.1 OR at 12 weeks was 5/26 patients (19%, [95%CI 7-39]) based on local reporting. Median PFS was 3.7 months (95%CI 2.7-5.9), and PFS at six months as estimated by Kaplan-Meier (supplementary Figure S2) was 23% (95% CI 9-40). Median OS was 7.7 months (95%CI 5.3-17.3); OS at 12 months was 44% (95%CI 25-62) (supplementary Figure S2). Disease burden at baseline (measured by the sum of target lesion diameters, in cm) was not statistically associated with PFS (HR=1.04 [95%CI 0.96-1.11] p=0.34) but was associated with worse overall survival (HR=1.08 [95%CI 1.00-1.16] p=0.043). Acral tumours had worse median PFS (2.3 months) and OS (5.1 months) than mucosal tumours (PFS 5.4, OS 7.7 months), although differences were not significant.

The presence of indolent disease at baseline could be centrally reviewed in 19 patients where pre-baseline scans were available. Of these, 4/19 (21%) presented indolent disease at baseline (see Methods), but only one patient with indolent disease was alive and progression-free at 6 months. It does not seem therefore that indolent disease is driving the observed response to nilotinib.

Association of KIT mutation and gene amplification with antitumour activity

Central assessment of antitumour activity was used for the following association analyses. No significant differences according to the exon in which the *KIT* mutation were observed in OR at 12 weeks (exon 11: 3/19 (16%); exon: 13 1/4 (25%); exon 17: 2/3 (67%), p=0.15) or median PFS (exon 11: 2.9 months; exon 13: 2.3 months; exon 17: 5.4 months; p=0.75) (Figure 4A). Median OS was 13.8 months for patients with mutations in exon 11, 5.1 months in exon 13 and 6.5 months in exon 17, although the differences were not significant (Figure 4B, p=0.26). Note that 3 out of 4 mutations found in exon 13 corresponded to acral tumours (supplementary Table S2). We observed an outlier patient with D820V *KIT* mutation (exon 17) who remained on treatment for 54 months. In terms of mutational class, OR rate at 12 weeks was 14.3% (1/7) in patients with complex indels and 26.3% (5/19) in patients with single nucleotide variants (Figure 4C) with no significant difference found in median PFS (2.7 months vs 5.4, p=0.38) nor OS (20.8 vs 6.5, p=0.34, Figure 4D).

mK-CN tumour values (see Methods), reflecting copy number status of *KIT* gene, could be inferred in 22 evaluable patients in baseline tumour samples. Median mK-CN was 3.5 (first-third quartiles Q1-Q3: 1.3-7.1, supplementary Figure S3A), consistent with presence of high-level *KIT* amplification in a subset of patients (see type of mutation by mk-CN amplification in supplementary Table S3). We did not find a significant correlation between tumour mK-CN and overall disease burden at baseline (supplementary Figure S3B).

There was no significant difference in the distribution of mK-CN between patients with OR at 12 weeks compared to non-responders (supplementary Figure S3C, p=0.56). mK-CN (considered continuous variable, centred to its mean and scaled by its standard deviation) was not significantly associated with PFS (HR=0.98 [95%CI 0.63-1.53] p=0.93) nor OS (HR=1.08 [95%CI 0.68-1.73] p=0.73). Median PFS was 3.7 months in patients with mK-CN at or above the median (amplified) compared to 5.3 months in patients with mK-CN below the median (non-amplified, p=0.73). Median OS was 7.1 and 7.7 months, respectively (supplementary Figure S3D, p=0.64). Best tumour shrinkage at 12 weeks by type of mutation and amplification is presented in supplementary Figure S4.

To explore the intratumour heterogeneity of *KIT* amplification we performed FISH in six evaluable samples (supplementary Table S4) observing some degree of heterogeneity in at least one case

(supplementary Figure S5) with mean *KIT* copies = 5.9. Supplementary Table S4 also refers to whole genome and exome sequencing performed for 2 and 4 patients in the trial.

Mutation analysis in plasma

Finally, we explored the feasibility of ddPCR testing to identify *KIT* alterations in plasma. For this purpose, baseline blood samples were available for 18 evaluable patients. The design of specific primer/probes for mutation analysis in ctDNA and matched FFPE tumour was successful for all but one patient, where ddPCR could not satisfactorily differentiate the wild type and the complex *in-del* mutated sequence. Concordance of mutations detected in ctDNA and FFPE tumour was 100%.

KIT VAF_{adj}, which is the frequency of the variant allele in plasma, adjusted for mK-CN, could be inferred in all 17 blood samples. We did not find a significant correlation between VAF_{adj} and overall disease burden at baseline (supplementary Figure S6A). There was no significant difference in baseline plasma VAF_{adj} between responders and non-responders (supplementary Figure S6B). Baseline plasma VAF_{adj} (as a continuous variable, centred to its mean and scale to its SD) was not significantly associated with PFS (HR=0.70 [95%CI 0.37-1.31], p=0.27) nor OS (HR=0.94 [95%CI: 0.58-1.53], p=0.82).

Discussion

Rare cancers pose a unique challenge for clinical development of new therapies as the scarcity of appropriate patient population makes it difficult to perform sufficiently powered studies to gain evidence[29,30]. The advent of molecular stratification and personalised medicine such as the current approaches for BRAF mutant cutaneous melanoma and *KIT*-mutant gastrointestinal tumours offer hope to these patients. However, additional challenges exist in the setting of a rare cancer with infrequent targetable alterations[31]. This is evident in our study, where 219 patients were screened, with only 29 entering the trial.

Mutations in the stem cell factor receptor gene *KIT* are reported in ~5-20% of AM and MM[11,12,15,16,32,33] and is the sole currently targetable molecular alteration in these patients. Mutant KIT targeting has been trialled with varied success with response rates ranging from 0 to 26% (supplementary Table S5) [34-44]. Critically, the impact of the *KIT* mutation type, especially outside exon 11, and the additional presence of *KIT* amplification, on the treatment response has not been investigated prospectively. Moreover, the utility of ctDNA analysis, which is established for the more common melanoma genotypes[45,46] is only explored to a limited degree in *KIT* mutated melanomas[47].

Our data show that nilotinib has activity in the setting of *KIT* mutant melanoma, comparable to other KIT inhibitors with toxicity profile consistent with previous reports[42]. Despite the time lapse since the study conception and the advancements in the analytical technologies that have become available, there have been no breakthrough advances in terms of targeted therapy for AM and MM, no dedicated randomised phase III trials and KIT inhibitors remain unlicenced in most countries. Our results will, therefore, add to the body of evidence to plan future trials in these cancers of unmet need.

We also show that ddPCR in the plasma can accurately pinpoint[40] the tumour mutational profile. Additionally, we showed that tumour-informed ddPCR is a feasible and reliable tool for evaluating *KIT* aberrations, including complex insertion-deletions, hence we propose that it could be implemented in future personalised oncology strategies, such as disease response monitoring and minimal residual disease assessment in the adjuvant setting of AM and MM. The findings regarding the prognostic value of plasma mk-CN require validation but nonetheless warrant further investigations. Similar to our findings, the concomitant *KRAS* mutation and amplification has a predictive effect for bigger benefit from treatment in *KRAS* mutated lung cancers[48], and high allele fraction for *BRAF* mutation, which is an adverse prognostic factor in colorectal cancers, is associated with a higher benefit from triplet therapy with EGFR-BRAF-MEK inhibitors (OS HR=0.17) compared to the cancers with low *BRAF* mutation allele frequency cancers (OS HR=0.90)[49]. Concomitant mutation and amplification could indicate oncogene addiction, but since targeted therapy for *KIT* mutated AM and MM is generally not licenced and not available for broad use it is challenging to obtain samples to validate our study. However, these considerations could be taken into account for future clinical trials design.

The variety of *KIT* alterations including complex mutations across multiple exons with or without gene amplification creates a complicated scenario for successful targeting of KIT protein in melanoma[50,51]. Also, similarly to previous observations with imatinib[40], the same mutations were associated with variable responses in different patients, which might suggest a complex interaction between multiple oncogenic pathways. In contrast, *KIT* alterations in gastrointestinal stromal tumours are more homogenous, with 70-90% being exon 11 deletions, and potentially relatedly KIT inhibitors are an effective standard of care across most patients with *KIT*-mutated GIST. *KIT* aberrations in acral and mucosal melanoma include hotspot point mutations at the juxta membrane and tyrosine kinase domain, respectively (L576P (Ex 11) and K642E (Ex 13)) as well as complex in or out of frame indels or duplications involving exons 11,13 and 17 (kinase domain).

Our approach facilitated the detection of these complex variants, which would not be discovered by hotspot assays. Consistent with literature reports most mutations were localised in exon 11 (n=20, 69%) and the most common mutation was L576, observed in 9 patients (31%), and we showed that tumour responses are not restricted to exon 11 mutations. Our findings have relevant ramifications for *KIT* testing strategies, because despite the availability of tests with broader capture of *KIT* alterations, most *KIT* tests still currently in use in clinic for economic reasons fail to detect non-L576 or non-exon 11 mutations, thus missing patients who could benefit from KIT-targeted treatment. We suggest that an extended assessment of *KIT* to detect indels and complex aberrations across exons 11, 13 and 17 would provide a useful therapeutic option for patients who have no therapeutic alternatives and whose tumour harbour *KIT* mutations currently undetected. This could pose concerns about the high cost of genetic sequencing[52] and the availability of tissue could be an additional limit. This is particularly important given the high number of patients that would need to be screened for *KIT* variants, and also the possible limited quality outputs when using archival FFPE samples to test *KIT* amplifications with alternative methods like gene sequencing or FISH. However, these limitations should be considered in the context of the scarce alternative therapeutic options and limited benefit from ICB that these patients have, and based on our results we recommend the use of technologies that, albeit more expensive, enable a more complete detection of *KIT* alterations in a clinical setting.

Limitations of the Study

Based oun our results, suggesting a prognostic value of plasma mk-CN, we hypothesise that concomitant mutation and amplification could indicate oncogene addiction. However, we could not verify this hypothesis *in vitro* and could not obtain additional patient samples to validate our study because targeted therapy for KIT mutated AM and MM is generally not licenced and not available for broad use.

Conclusion

Nilotinib has an activity comparable to what has been reported for other *KIT* inhibitors and is a viable therapeutic option, including for *KIT* mutations not captured in current standard protocols. ddPCR-based *KIT* analysis appears feasible and accurate for *KIT* testing in patients with metastatic MM and AM and could be proposed for liquid biopsies testing.

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Author's contributions

Conceptualization: JL, RM, JB, ST; Methodology: JL, RM, NP, JB, SV, SMT; Formal Analysis: NP, DGdC, LT, SV; Investigation & Resources: JL, RM, DGdC, LPm CM, GS, LT, KE, SS, PL, TRJE, PC, EM, MRM, PN, SN, CO, RP, SV, ST; Data Curation: NP, DGdC, GS, LT, KE, SS, SV; Writing original draft: JL, RM, NMP, LP, JB, SV, ST; Writing editing/reviewing: all authors; Visualization: NP, SV, ST; Supervision: JL, RM, JB, ST; Project Admin: JBa, JB, Funding acquisition: JL, RM, JB, ST;

Declaration of Interests

JL declares the following: Honorariums: Eisai, Novartis, Incyte, Merck, touchIME, touchEXPERTS, Pfizer, Royal College of Physicians, Cambridge Healthcare Research, Royal College of General Practitioners, VJOncology, Agence Unik, BMS, Immatics, Insighter, GCO. Consultancy: iOnctura, Apple Tree, Merck, BMS, Eisai, Debipharm, Incyte, Pfizer, Novartis. Speaker fee: Pierre Fabre, BMS, Ipsen, Roche, EUSA Pharma, Novartis, Aptitude, AstraZeneca, GSK, Eisai, Calithera, Ultimovacs, Seagen, Merck, eCancer, Inselgruppe, Pfizer, Goldman Sachs, MSD, Regional British Society of Gastroenterology, Agence Unik. Institutional research support: BMS, MSD, Novartis, Pfizer, Achilles Therapeutics, Roche, Nektar Therapeutics, Covance, Immunocore, Pharmacyclics, Aveo. Grants: Achilles, BMS, MSD, Nektar, Novartis, Pfizer, Roche, Immunocore, Aveo, Pharmacyclics.

RM is an expert witness for Pfizer and may benefit financially from commercialised programmes.

PL declares the following: Honorariums: Novartis, PierreFabre, Merck, BMS, MSD, NeraCare GmbH, Amgen, Roche, OncologyEducation Canada, Nektar; Consultancy: Merck Sharp & Dohme, Bristol-Myers Squibb, Amgen, Pierre Fabre, Novartis, Nektar, NeraCare GmbH; Speakers' Bureau: Merck Sharp & Dohme, Novartis, Bristol-Myers Squibb, Pierre Fabre; Institutional Research Funding: BMS, Pierre Fabre; Travel, accommodations, expenses: Merck Sharp & Dohme, Bristol-Myers Squibb.

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PN reported having received funding for advisory boards and/or speakers bureau from the following sources: AZ, BMS, Esai, Ideaya, Immunocore, Ipsen, Medicenna, MSD, Merck, Novartis, Pfizer.

RP reported, in the last 4 years, having received Honoria for attending advisory boards from Pierre Faber, Bayer, Novartis, BMS, Cybrexa, Ellipses, CV6 Therapeutics, Immunocore, Genmab, Astex Therapeutics, Medivir, and Sanofi Aventis. RP also reported to have received honoraria as an IDMC member for Alligator Biosciences, GSK, Onxeo and SOTIO Biotech AG, AstraZeneca, and have been paid for delivery of educational talks or chairing educational meetings by AstraZeneca, Novartis, Bayer, MSD and BMS. She has received funds to support attendance at conferences from MSD and BMS.

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NP, DFdC, LP, CM, GS, LT, KE, SS, JBa, PC, EM, SN, CO have no conflicts of interest.

Main Figure titles and legends

Figure 1: Patient flow-chart in the NICAM trial

Figure 2: c-*KIT* molecular characterisation in the NICAM trial
The chart shows the individual c-*KIT* mutation characterization in the 29 trial patients who were enrolled in the molecular profiling. The gene fragment affected by mutations spanned from exon 9 to 17, comprising the Ig-like-C2 type 5 domain (green), a junction domain (pink) and the protein kinase domain (light blue). Each lollipop anchor corresponds to individual mutation sites (complex mutations are in purple and missense single nucleotide mutations are in blue) and the height of the lollipop is indicative of the mutation frequency in the trial population.

Figure 3: Time on treatment for all entered NICAM patients, by cKIT mutation exon.
Bar length indicate months on treatment; objective disease progression and death are indicated in the figure. Patients were allowed to continue treatment as long as clinically indicated by the treating physician.

Figure 4: Association of mutation with outcome data (A) Percentage change from baseline at 12 weeks in sum of target lesions as per RECIST 1.1 by exon where *KIT* mutation was detected; (B) overall survival by exon where *KIT* mutation was detected; (C) percentage change from baseline at 12 weeks in sum of target lesions as per RECIST 1.1 by type of *KIT* mutation; (D) overall survival by type of *KIT* mutation
For the waterfall plots (A) and (C), only evaluable patients with data at the 12-week scan since start of nilotinib are included.

Main tables and corresponding titles and legends

Table 1: Baseline characteristics of patients entered into the NICAM trial

	Patients entered N=29	
	N	**%**
Patient demographics		
Sex		
Female	20	69
Male	9	31
Age at registration/entry (yr), mean(SD)	67.1 (9.1)	
Ethnicity		
Caucasian	22	75.9
Asian	2	6.9
Other	4	13.8
Unknown	1	3.4
Skin type (Fitzpatrick classification)		
I	3	10.3
II	1	3.4
III	17	58.6
IV	3	10.3
V	1	3.4
VI	2	6.9
Unknown	2	6.9
Disease at presentation & past treatments		
Melanoma subtype		
Acral	6	20.7
Location		
Hand	*1*	*3.4*
Foot	*5*	*17.3*
Stage at presentation		
Localised	*3*	*10.3*
Regional lymph node metastasis	*2*	*6.9*
Unknown	*1*	*3.4*
Mucosal	23	79.3
Location		
Head and neck	*5*	*17.2*
Upper gastrointestinal tract	*2*	*6.9*
Anorectal	*5*	*17.2*
Urogenital	*11*	*37.9*
Other[a]	*1*	*3.4*
Stage at presentation		
Localised I	*6*	*20.7*
Localised II	*7*	*24.1*
Localised III	*1*	*3.4*
Unknown	*9*	*31.0*
Prior treatments		
Radiotherapy	9	31
Systemic treatment (palliative)[b,c]	4	13.8
Disease at trial entry		
Time from diagnosis (yr) to trial entry, median (Q1-Q3)	1.3 (0.7-3.3)	
ECOG performance status		
0	16	55.2
1	12	41.4
2	1	3.4
Location of disease[c]		
Local	5	17.2
Lymph nodes	20	69.0
Liver	11	37.9

	Patients entered N=29	
	N	**%**
Lung	21	72.4
Brain	0	0
Other	8	27.6
Disease burden at trial entry (sum of target lesions in cm as per RECIST 1.1), median (Q1-Q3)	7.2 (4.8-10.5)	
LDH at trial entry (U/L), median (Q1-Q3), N=25	259 (199-358)	

yr: year; SD: standard deviation, Q1: first quartile, Q3: third quartile [a]*One patient specified 2 primary sites (urogenital and other –unknown)* [b]*Includes immunotherapy (n=3): interferon & interleukin 2 (n=1), ipilimumab (n=1), other (n=1); chemotherapy (n=3)* [c]*More than one option per patient could be specified*

Table 2: Treatment-emergent adverse events in the NICAM trial (N=28, safety population)

	Grade 1+		Grade 3+	
	N	**%**	**N**	**%**
Fatigue	21	75.0%	3	10.7%
Nausea	17	60.7%	2	7.1%
Constipation	14	50.0%	1	3.6%
Rash	12	42.9%	0	0.0%
Anorexia	12	42.9%	0	0.0%
Anaemia	10	35.7%	1	3.6%
Vomiting	8	28.6%	2	7.1%
Alopecia	8	28.6%	0	0.0%
Abdominal pain	7	25.0%	3	10.7%
Diarrhoea	6	21.4%	1	3.6%
Arthralgia	6	21.4%	1	3.6%
Bone pain	6	21.4%	0	0.0%
Peripheral oedema	6	21.4%	0	0.0%
Pruritus	6	21.4%	0	0.0%
Headache	4	14.3%	1	3.6%

The above toxicities were pre-specified in the Case Report Form (CRF) at each cycle; additional toxicities graded 3+ not pre-specified in the CRF were observed in 12 patients: Alanine aminotransferase increased (1, 4%), Aspartate aminotransferase increased (1,4%), Back pain (1 pt, 4%), Blood lactate dehydrogenase increased (1, 4%), Breast cancer female (1, 4%), Cellulitis (2, 8%), Chest pain (1, 4%), Convulsion (1, 4%), Deep vein thrombosis (1, 4%), Dehydration (1, 4%), Dyspnoea (1, 4%), Embolism (1, 4%), Hypertension (1, 4%), Lower respiratory tract infection (2, 8%), Muscular weakness (1, 4%), Oesophageal pain (1, 4%), Pain (1, 4%), Pleural effusion (1, 4%), Pneumonia (1, 4%), Urogenital haemorrhage (1, 4%)

STAR Methods

Resource availability

Lead Contact

Further information and requests for resources should be directed to the Lead Contact, Prof Samra Turajlic, Skin and Renal Units, The Royal Marsden Hospital NHS Foundation Trust, London, UK (samra.turajlic@crick.ac.uk).

Materials Availability

There is no availability of biological material because we utilised unique patient samples that were utilised in their entirelty. This study did not generate new unique reagents and the ddPCR primer sequences are available from BioRad Assay Design Tool by inputing the *KIT* alteration sequences.

Data and code availability

- The ddPCR primer sequences are available from BioRad Assay Design Tool. De-identified data reported in this paper will be shared upon request; applicants can contact the Lead applicant of the Clinical Trials and Statistics Unit at the Institute of Cancer Research (ICR-CTU), who coordinated this study. Trial data are collected, managed, stored, shared, and archived according to ICR-CTSU Standard Operating Procedures to ensure the enduring quality, integrity, and utility of the data. Formal requests for data sharing are considered in line with ICR-CTSU procedures with due regard given to funder and sponsor guidelines. Requests are via a standard proforma describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a formal data sharing agreement that describes the conditions for release and requirements for data transfer, storage, archiving, publication, and intellectual property. Restrictions relating to patient confidentiality and consent will be limited by aggregating and anonymising identifiable patient data. Additionally, all indirect identifiers that could lead to deductive disclosures will be removed in line with Cancer Research UK Data Sharing Guidelines. Further information can be found here: https://www.icr.ac.uk/our-research/centres-and-collaborations/centres-at-the-icr/clinical-trials-and-statistics-unit/working-with-us/data-sharing
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

Experimental model and and study participant details

NICAM is a multicentre, open-label, investigator-initiated, single-arm two-stage phase 2 study conducted across 16 UK sites (supplementary Table S6). Eligible patients were 18 years or older, with *KIT* mutated histologically proven advanced (unresectable locally advanced or metastatic) mucosal or acral melanoma. Patients whose tumours harboured *KIT* mutation previously characterised as conferring resistance to nilotinib were excluded. Patients were required to have one or more clinically or radiologically measurable lesions (≥10mm), Eastern Cooperative Oncology Group (ECOG) performance status 0-2, and adequate organ function. Patients with intracranial disease were excluded (unless present and stable for >6 months). Prior exposure to tyrosine kinase inhibitors was excluded. The full list of inclusion and exclusion criteria are provided in supplementary Table S7.

Patients provided written informed consent before enrolment; initially for *KIT* mutation screening and, once eligibility was confirmed, for entry into the treatment stage of the trial.

Method details

Pre-Screening

KIT mutation status was ascertained from the genomic DNA extracted from formalin fixed paraffin embedded tumour tissue (either archived or obtained for the purpose of trial screening). Exons 9, 11, 13 and 17 were evaluated by PCR amplification, followed by Capillary Electrophoresis Single-Strand Conformation Analysis (CE-SSCA) and direct Sanger sequencing for identification of the exact mutation. CE-SSCA for *KIT* detects >95% of mutations with a limit of detection of 5-10%, while direct sequencing has a limit of detection of 20-30%. Most analyses were conducted by a central accredited laboratory at The Royal Marsden NHS Foundation Trust. Sites with a laboratory accredited to perform *KIT* mutational analysis also performed *KIT* gene sequencing and analyses, but all reports were centrally reviewed. The suitability of the patient to enter the study based on the mutational profile were determined by the chief investigator. Patients whose tumours were found to harbour *KIT* mutation were eligible for the trial. Patients whose tumours were wild type for *KIT* or did not enter the trial for any reason were treated according to local protocols.

Trial procedures

All patients who were included in the NICAM study received oral nilotinib (two 200 mg capsules) twice a day (800 mg per day in total) in 4-week cycles for as long as there was evidence of clinical benefit; treatment beyond radiological progression was allowed. Patients attended for visits on days 1, 15, 29,

57 and then every 4 weeks in year 1; and 8 weekly thereafter for as long as they were receiving trial treatment and were able to attend. Patients underwent CT scans of the thorax, abdomen and pelvis for tumour assessment at screening and after 12 and 26 weeks following initiation of treatment. Further CT scans were performed 3-monthly until 3 years, and 4-monthly thereafter, until progression of disease. Adverse events were recorded according to the National Cancer Institute Common Terminology Criteria (NCI-CTC) version 3. Guidance on drug interruptions or dose reductions for relevant haematological and non-haematological toxicities were implemented as outlined in the protocol. After treatment discontinuation, patients were followed for survival status.

Translational analyses

Whole EDTA blood samples were collected pre-treatment (baseline), 2 weeks after start of nilotinib and at disease progression. Formalin fixed paraffin embedded (FFPE) tumour blocks were also available for exploratory analyses where patients provided additional consent.

Genomic DNA isolation:

Genomic DNA was isolated as described previously[47,53]; in brief, DNA was extracted from plasma using QIAamp Circulating Nucleic Acid Kits (Qiagen) and quantified with Qubit Assay (Thermofisher Scientific). Based on the *KIT* mutation determined during screening custom primers and probe sets were designed using BioRad Assay Design Tool; BioRad ddPCR assays utilised ddPCR Supermix for probes (cat 1863024) and FAM/HEX kits (cat 10031276, 10031279, 10049550, 10049047). Wild type and mutant alleles in the tumour and circulating tumour DNA (ctDNA) were quantified by ddPCR; custom drop-off probes were designed to detect complex mutations that would not be detected by standard ddPCR assays[54]. The specificity of the primer/probes was tested using healthy donor peripheral blood mononuclear cells' DNA as negative control, and patient-matched tumour DNA was used as positive control.

Allele quantification:

The amount of mutant and wild type DNA in each sample was quantified using Bio-Rad QX200 platform and expressed as variant allele frequency (VAF, the fraction of mutant droplets in the total number of mutant and wild-type droplets). Mutated *KIT* copy number (mK-CN, the fraction of mutant *KIT* droplets over the number of droplets positive for the reference gene *hTERT*), was calculated utilising the median values of three technical replicates as previously described[47].

Fluorescent in situ hybridisation (FISH):

KIT gene amplification confirmation was exploratorily tested in FFPE archival tumour samples by means of FISH, that was performed with dapi staining for nuclei and Pishes Empire fluorescent probes for chromosome 4 centromer (5-fluoreshein (FITC), and *KIT* (5-tamra) using the producer's protocols; the stained slides were evaluated on a Zeiss Imager.M1, AX10 or Zeiss M200 FL microscope.

Whole genome/exome sequencing (WGS/WES):

Exploratory WGS/WES was pursued in a small subset of NICAM patients co-enrolled in tissue biobanking study [3]. For WGS, DNA was sequenced using Illumina Hiseq2000 sequencers, the FASTQ files of the paired-end reads were aligned to the human reference genome (GRCh37) and processed using default settings BWA[55], Samtools[56] and Picard (https://broadinstitute.github.io/picard/). We used SomaticSniper (score threshold ≥ 40, a mapping threshold ≥ 40, and depth in tumour and normal ≥ 10) to call the somatic single nucleotide variants (SNVs)[57], applying pre-determined filters to remove likely false-positive SNVs 20[58]. Somatic indels were called using Strelka[59] removing low-confidence indels. All SNVs and indels were annotated[60], and SNVs and indels present in dbSNP 135 were excluded. We used Illumina's cancer pipeline to identify copy number alterations (CNAs) and assessed the somatic structural variations with CREST[61] (default settings for comparison between normal and tumour).

Whole human exome capture and sequencing was performed using Agilent SureSelect sample preparation protocol V2 (37 Mb) with Illumina GAIIX sequencer (76 bp paired-end reads) or Agilent SureSelect sample preparation protocol V4 (50 Mb) with HiSeq 2000 sequencer (100 bp paired-end reads). Sequences were aligned to the NCBI build 37 reference genome using BWA[55] and processed with Picard and GATK[62]. Somatic SNVs were called using Varscan with predetermined filters to remove false positives[58] and SomaticSniper[57]. We used SomaticIndelDetector to identify somatic indels (https://gatk.broadinstitute.org/hc/en-us) and Ensembl Variant Effect Predictor to annotate somatic variants[60].

Outcomes

The primary endpoint was the proportion of patients who were alive and progression free at six months according to RECIST 1.1[63]. Progression free survival (PFS) was measured from the date of enrolment into the treatment phase until the first date (following start of treatment) of either death or confirmed progressive disease according to RECIST 1.1. The secondary endpoints of the study included objective response (OR) rate (complete or partial response as per RECIST 1.1) at 12 weeks, overall survival (OS,

measured from the date of enrolment until the date of death due to any cause) and the safety and tolerability profile of nilotinib. Post-hoc exploratory endpoints included assessment of the primary endpoint as reviewed centrally, and proportion of patients presenting indolent disease at trial entry as ascertained by central assessment of pre-baseline (within three months of trial entry) and baseline scans. The presence of indolent disease can impact interpretation of drug effectivenessparticularly in this non-randomised trial. Indolent disease was defined as stable disease or lesion growth <20% between pre-baseline and baseline scans. Translational secondary endpoints were the association of particular *KIT* mutations and *KIT* gene amplification with response to treatment and survival.

Quantification and Statistical analyses

Efficacy endpoints were reported in the subgroup of patients considered evaluable for the primary endpoint assessment. Safety was reported on all patients who received at least one dose of study drug. A cohort of 24 evaluable patients was targeted under a two-stage design (nine in stage one, 15 in stage two), where there would be an 86% power for nilotinib to show sufficient activity (≥15%) to pursue further investigation (one-sided alpha=5%) if the true proportion of patients progression-free at six months was 40%. At least 2/9 and 7/24 patients to be progression-free at 6 months were required as success criteria at stage one and two, respectively. To account for the two-stage design, the 2-sided 90% confidence interval for PFS at six months and p-value for decision making were obtained as per Koyama and Chen (2008)[64]. The PFS at six months was also estimated by the uniformly minimum variance unbiased estimator (UMVUE) to account for the two-stage design[65]. The R library OneArmPhaseTwoStudy was used to obtain these adjusted parameters (R version 4.1.3)[66]. Given that the trial over-recruited to account for non-evaluable patients, these estimates were also obtained for the whole evaluable cohort. Kaplan-Meier estimates for PFS and OS were graphically summarised in survival curves. Response rates were summarised with 95% exact binomial confidence intervals. Most common (by NCI-CTC grade), dose-limiting and serious adverse events and reactions were summarised by frequencies and percentages. As exploratory analysis, we analysed the association between disease burden at baseline (as measured by sum of target lesions) and PFS and OS with Cox Proportional Hazards models. Association of mutations and amplification with OR and best change from baseline in tumour size at 12 weeks were summarised descriptively, and groups compared by appropriate non-parametric tests (i.e., Kruskal-Wallis or Mann-Whitney, respectively). Cox proportional hazard models were used to quantify association of continuous biomarkers with PFS and OS. Exploratory cut-offs based on the median of

the biomarkers were used to categorise them, as no clear clusters of data were observed. Kaplan-Meier estimates of the survival function for each biomarker category (amplified vs non-amplified as per the median value) were graphically presented and compared by log-rank tests. Correlations between tumour and plasma DNA, and with baseline disease burden were measured by Spearman correlation coefficient. Due to the small number of patients, the p-values presented are considered hypothesis-generating.

Statistical analyses were done with Stata software (version 13 & later), on a snapshot of the clinical data taken on 9 January 2017, when all patients have completed trial follow-up. Biological and biomarker data for translational analyses presented in this report were generated after trial completion.

Additional resources

The study was approved by the Oxfordshire Research Ethics Committee (REC reference 09/H0606/103), and co-sponsored by The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research (ICR), London, UK. The trial was conducted in accordance with the principles of good clinical practice and overseen by an Independent Data Monitoring and Steering Committee. A Trial Management Group (TMG) was responsible for the day-to-day running of the trial. The Clinical Trials and Statistics Unit at ICR (ICR-CTSU) had overall responsibility for trial coordination, monitoring, and data analysis.

Trial registration: ISRCTN39058880, EudraCT 2009-012945-49.

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KEY RESOURCES TABLE

TABLE FOR AUTHOR TO COMPLETE

Please do not add custom subheadings. *If you wish to make an entry that does not fall into one of the subheadings below, please contact your handling editor or add it under the "other"* subheading. ***Any subheadings not relevant to your study can be skipped.*** *(**NOTE:** references should be in numbered style, e.g., Smith et al.[1])*

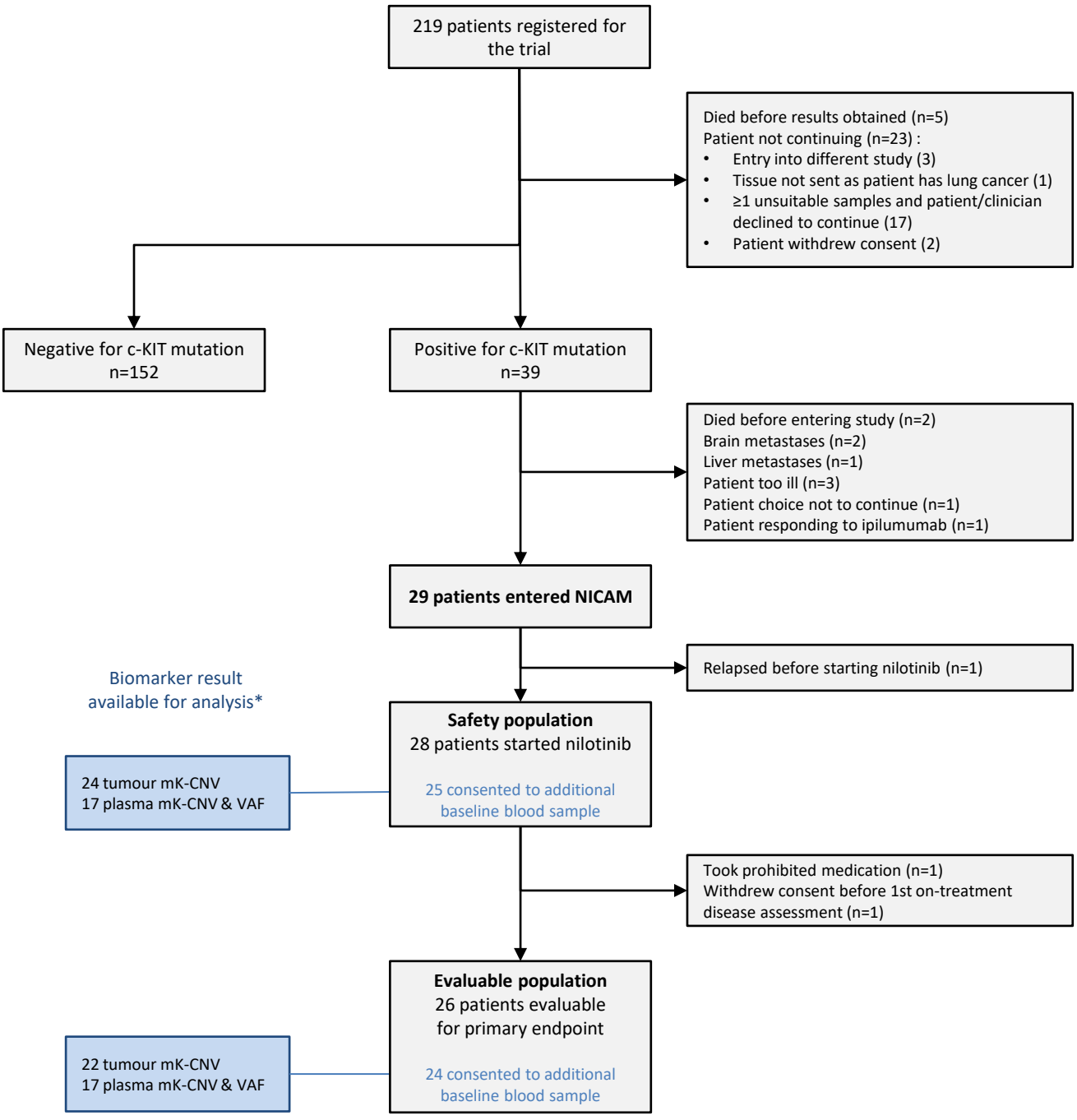
Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
FFPE tumour samples	Patients	N/A
Plasma samples	Patients	N/A
Critical commercial assays		
Droplet digital Polymerase Chain Reaction SuperMix for probes	BioRad	Cat #1863024
Droplet digital Polymerase Chain Reaction primers and FAM/HEX probes	BioRad	cat #10031276, #10031279, #10049550, #10049047
Fluorescent probes for chromosome 4 centromer (5-fluoreshein (FITC), and *KIT* (5-tamra)	Pishes Empire	Cat # KIT-CHR04-20- ORGR
QIAamp Circulating Nucleic Acid Kits	Qiagen	Cat #55114
Agilent SureSelect sample preparation protocol V2	Agilent	https://www.agilent.com/cs/library/brochures/SureSelect%20CREV2%20Brochure%205991-7572EN%204.9%20(Single%20Page).pdf
Agilent SureSelect sample preparation protocol V4	Agilent	https://www.agilent.com/cs/library/flyers/Public/5990-9857en_lo.pdf
Software and algorithms		
STATA v13 & later	StataCorp	https://www.stata.com/
R package OneArmPhaseTwoStudy (run in R version 4.1.3)	Kieser et al.[59]	N/A
BWA	Li et al[55]	https://github.com/lh3/bwa
Samtools	Li et al[56]	http://www.htslib.org
Picard	http://picard.sourceforge.net/index.shtml	
SomaticSniper	Larson et al[57]	https://gmt.genome.wustl.edu/packages/somatic-sniper/documentation.html

Strelka	Saunders et al[59]	https://github.com/Illumina/strelka
CREST	Wang J et al[61]	
GATK	McKenna et al[62]	https://gatk.broadinstitute.org/hc
Varscan	Koboldt et al[58]	https://varscan.sourceforge.net
SomaticIndelDetector	McKenna et al[62]	http://www.broadinstitute.org/gatk/gatkdocs/org_broadinstitute_sting_gatk_walkers_indels_SomaticIndelDetector.html
Ensembl Variant Effect Predictor	McLaren et al[60]	https://www.ensembl.org/vep
Other		

Figure 1

Click here to access/download;Figure;Figure 1 - CONSORT.pdf



mK-CNV: Mutated KIT copy number variation; VAF: Variant allele frequency

*1 patient who consented to additional blood sample for translational analysis was excluded, as the assay led to false positive results due to the failure of primer/probe design.

Figure 2

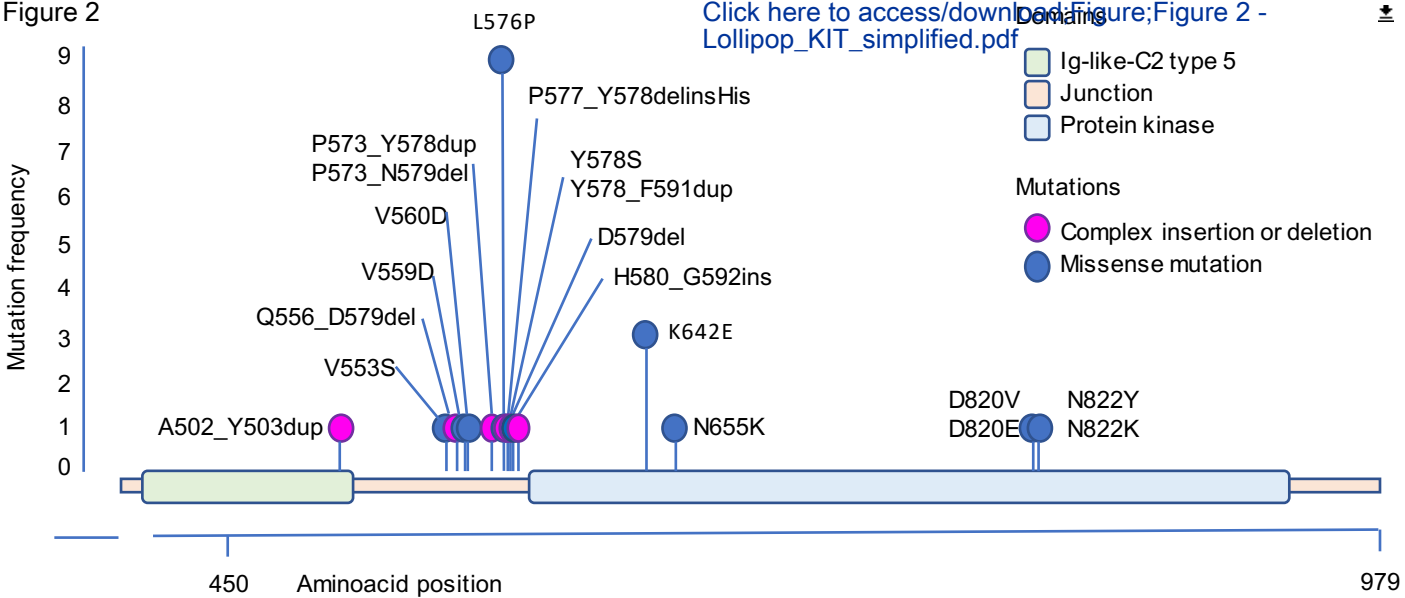


Figure 3

Click here to access/download;Figure;Figure 3

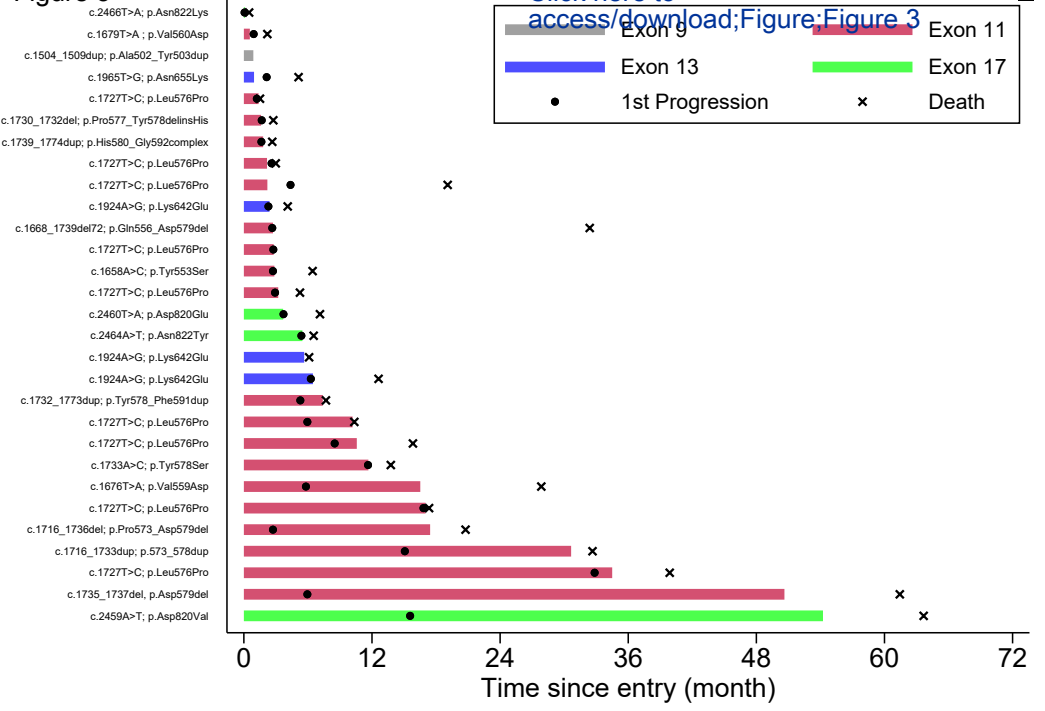
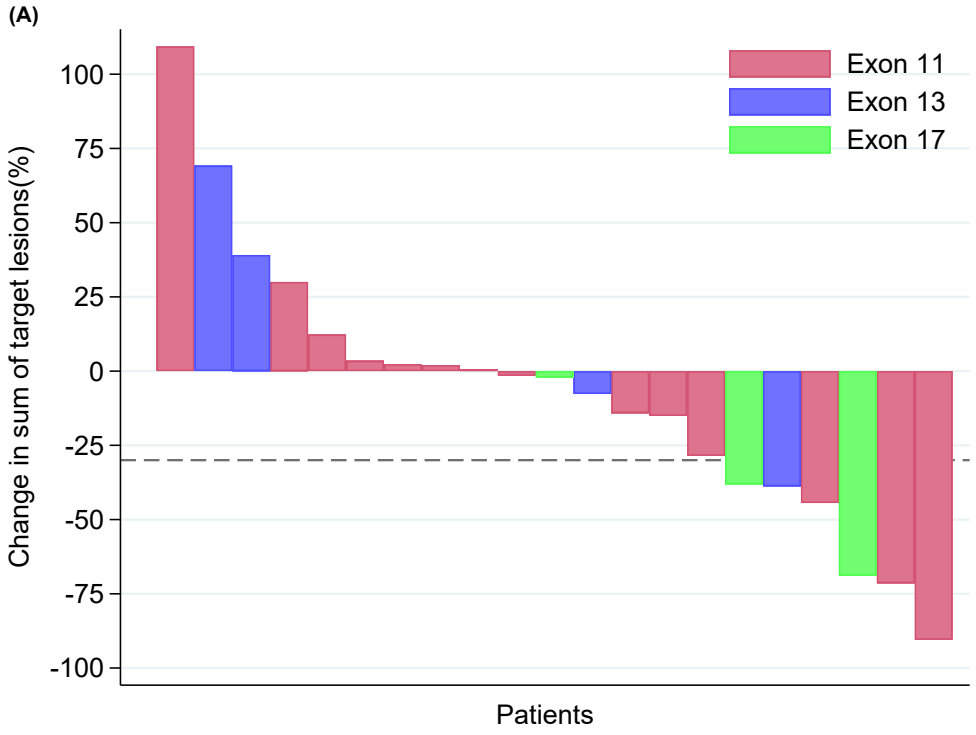
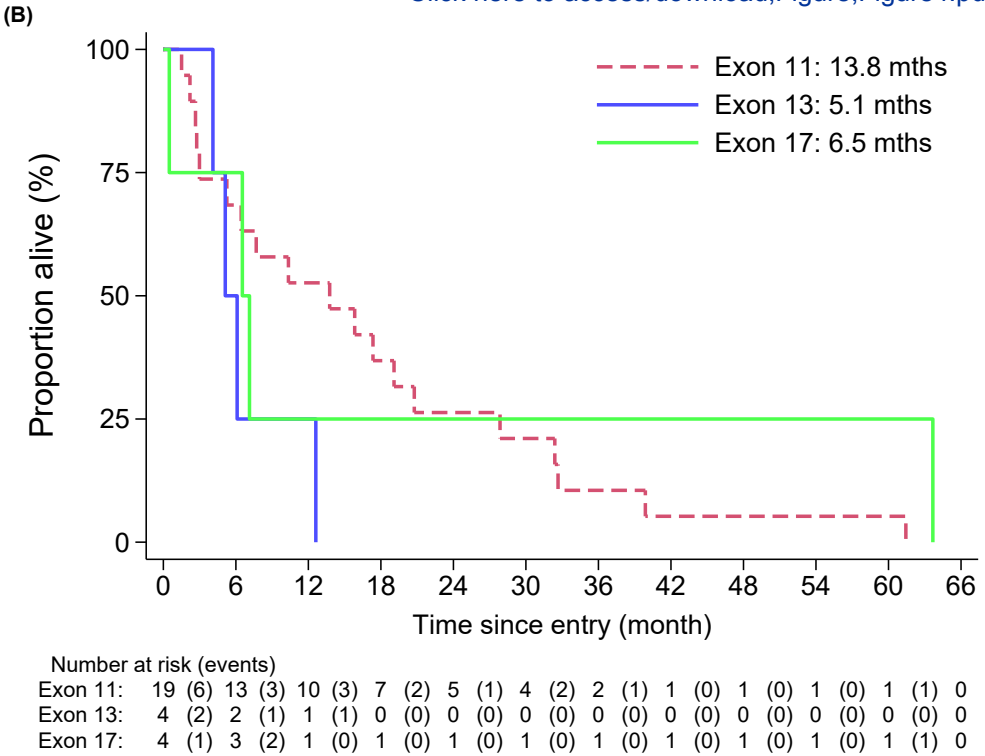
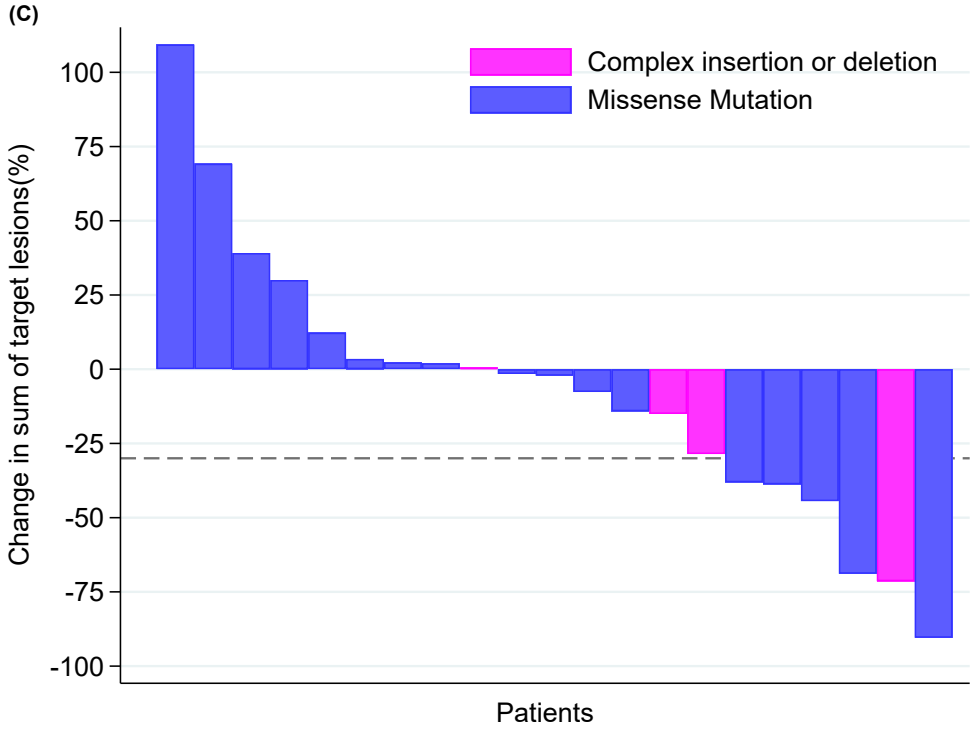
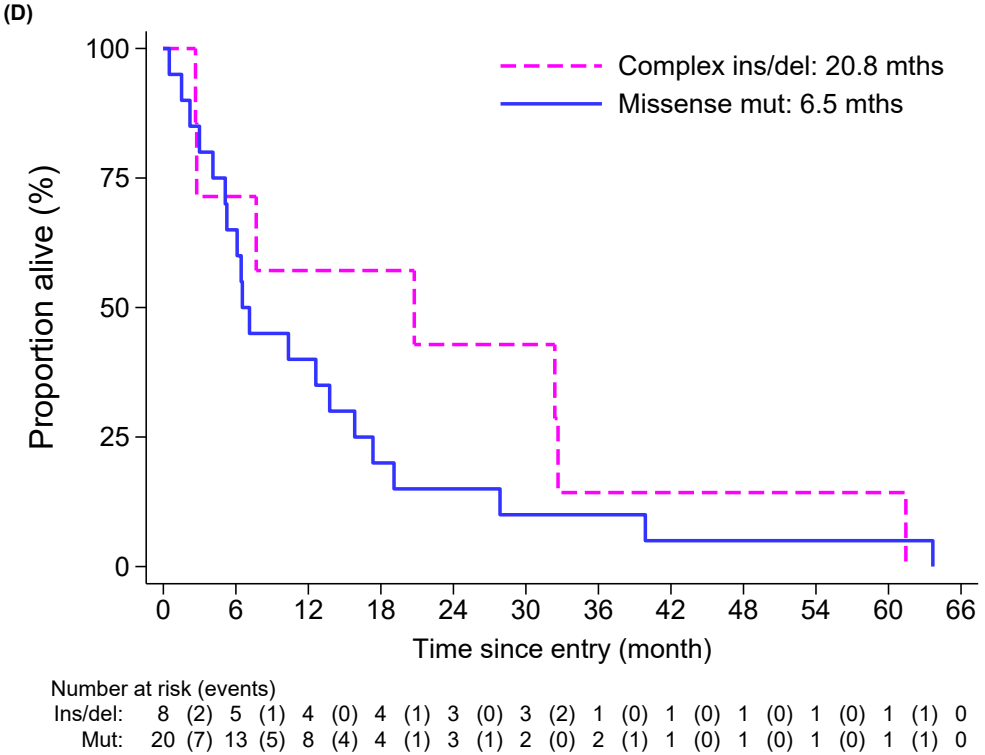


Figure 4









Supplementary Tables

Table S1: Baseline characteristics of all patients screened in NICAM
Related to Figure 1 and Table 1

	Patients screened (N=218[a]) N	%
Patient demographics		
Sex		
Female	135	61.9
Male	80	36.7
Unknown	3	1.4
Age at registration/entry (yr), mean(SD)	65.6 (12.1)	
Ethnicity		
Caucasian	185	84.9
Asian	6	2.8
Other	10	4.6
Unknown	17	7.8
Skin type (Fitzpatrick classification)		
I	14	6.4
II	21	9.6
III	96	44
IV	10	4.6
V	4	1.8
VI	5	2.3
Unknown	68	31.2
Melanoma subtype		
Acral	67	30.7
Location		
Finger	*5*	*2.3*
Heel	*8*	*3.7*
Instep	*1*	*0.5*
Sole (non specific type)	*21*	*9.6*
Subungual (Foot)	*4*	*1.8*
Subungual (Hand)	*3*	*1.4*
Toe	*24*	*11*
Stage at presentation		
Localised	*44*	*20.2*
Regional lymph node metastasis	*7*	*3.2*
Distant metastasis	*12*	*5.5*
Unknown	*4*	*1.8*
Mucosal	151	69.3
Location		
Head and neck	*48*	*22*
Upper gastrointestinal tract	*10*	*4.6*
Anorectal	*30*	*13.8*
Urogenital	*56*	*25.7*
Upper respiratory tract	*2*	*0.9*
Other[b]	*6*	*2.8*
Stage at presentation		
Localised I	*41*	*18.8*
Localised II	*42*	*19.3*
Localised III	*22*	*10.1*
Unknown	*46*	*21.1*

[a]No baseline features available for one patient screened for c-kit mutation (but not entered) [b]Includes lower gastrointestinal tract (n=3), lower respiratory tract (n=1), eyes (n=1) and unknown (n=1) Yr: year; SD: standard deviation.

Table S2: List of NICAM patients - *cKIT* mutation details, RECIST response and key endpoints
Related to Table 1, Figures 2,3,4

ID	Melanoma subtype	Exon	*cKIT* mutation	mK-CN (biopsy)	RECIST - Baseline sum of target lesions (cm)	RECIST – best % change in sum of target lesions within 12 weeks	Months on treatment	Months to progression	Months to death
NI01	Mucosal	11	c.1658A>C; p.Tyr553Ser	1.2	7.3	12.3	2.9	2.7	6.4
NI02	Mucosal	11	c.1668_1739del72; p.Gln556_Asp579del	3.4	2.8	-28.6	2.7	2.7	32.4
NI03	Mucosal	11	c.1676T>A; p.Val559Asp	10.6	12	-14.2	16.5	5.8	27.9
NI04	Mucosal	11	c.1679T>A , p.Val560Asp	7.2	2.4		0.6	0.9	2.2
NI05	**Mucosal**	**11**	**c.1716_1733dup; p.573_578dup**		**6.5**	**0**	**30.7**	**15.1**	**32.7**
NI06	Acral	11	c.1716_1736del; p.Pro573_Asp579del	3.3	2.1*		17.4	2.7	20.8
NI07	**Mucosal**	**11**	**c.1727T>C; p.Leu576Pro**	**5.4**	**5**	**2**	**34.5**	**32.9**	**39.9**
NI08	**Mucosal**	**11**	**c.1727T>C; p.Leu576Pro**	**13**	**2.1**	**-90.5**	**17.0**	**16.8**	**17.3**
NI09	**Mucosal**	**11**	**c.1727T>C; p.Leu576Pro**	**4.4**	**20.2**	**3.5**	**10.6**	**8.5**	**15.8**
NI10	Mucosal	11	c.1727T>C; p.Leu576Pro	12.3	15.8		1.4	1.2	1.5
NI11	Mucosal	11	c.1727T>C; p.Leu576Pro	2.9	6.2	-1.6	10.2	5.9	10.3
NI12	Mucosal	11	c.1727T>C; p.Leu576Pro	5.9	21.3	30	3.2	2.9	5.3
NI13	Acral	11	c.1727T>C; p.Leu576Pro	0.8	3.2	109.4	2.8	2.8	2.8+
NI14	Mucosal	11	c.1727T>C; p.Leu576Pro		9	-44.4	2.2	2.6	3
NI15	Acral	11	c.1730_1732del; p.Pro577_Tyr578delinsHis	3.6	12.5	1.6¥	1.6	1.7	2.8
NI16	Mucosal	11	c.1732_1773dup; p.Tyr578_Phe591dup	1.2	19.9	-15.1	7.4	5.3	7.7
NI17	**Mucosal**	**11**	**c.1733A>C; p.Tyr578Ser**	**3.5**	**4.3**	**2.3**	**11.7**	**11.6**	**13.8**
NI18	Mucosal	11	c.1735_1737del, p.Asp579del	10.1	1.4	-71.4	50.6	5.9	61.4
NI19	Mucosal	11	c.1739_1774dup; p.His580_Gly592complex	13.2	9.7	40.2¥	1.8	1.6	2.7
NI20	Acral	13	c.1924A>G; p.Lys642Glu		3.9	-7.7	6.5	6.3	12.6
NI21	Acral	13	c.1924A>G; p.Lys642Glu		4.6	39.1	2.4	2.3	4.1
NI22	Mucosal	13	c.1924A>G; p.Lys642Glu	1.4	4.9	-38.8	5.7	6.1	6.1
NI23	Acral	13	c.1965T>G; p.Asn655Lys	1.3	2.6	69.2	1.0	2.1	5.1
NI24	**Mucosal**	**17**	**c.2459A>T; p.Asp820Val**	**0.7**	**6.3**	**-38.1**	**54.2**	**15.6**	**63.7**
NI25	Mucosal	17	c.2460T>A; p.Asp820Glu	5.6	9.1	-2.2	3.7	3.7	7.1
NI26	Mucosal	17	c.2464A>T; p.Asn822Tyr	1	11.9	-68.9	5.5	5.4	6.5
NI27	*Mucosal*	*11*	*c.1727T>C; p.Leu576Pro*	*1.8*	*4.2*		*2.2*	*4.4*	*19.1*
NI28	*Mucosal*	*9*	*c.1504_1509dup; p.Ala502_Tyr503dup*	*13.6*	*22.3**		*0.9*	*1.4§*	*1.4§*
NI29	*Mucosal*	*17*	*c.2466T>A; p.Asn822Lys*				*0*	*0*	*0.5*

mK-CN: mutated *KIT* copy number (measured on baseline biopsy sample). RECIST: Response Criteria for Solid Tumours 1.1 as per central review, except for * where this was not available, and local assessment is reported instead. In bold, patients alive and progression free as per local assessment (primary endpoint). Patient NI11 was considered alive and progression free as per central review. Patients NI27, NI28, NI29 were not evaluable for the primary endpoint. ¥ Reported at progression<12 weeks. +Patient alive at last follow-up (lost to follow-up after progression). §Patient alive and progression free at last follow-up (withdrew from trial assessments). Cases with the same c-KIT mutation are highlighted in grey.

Table S3: *cKIT* mutation detail, by type of mutation and mutated *KIT* copy number amplification
Related to Figures 2,3,4

	Exon	mk-CN<p50 (non-amplified)		mK-CN ≥p50 (amplified)		Total	
		n	%	n	%	n	%
Complex insertion or deletion		3	27.3	3	27.3	6	27.3
c.1668_1739del72; p.Gln556_Asp579del	*11*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1716_1736del; p.Pro573_Asp579del	*11*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1730_1732del; p.Pro577_Tyr578delinsHis	*11*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
c.1732_1773dup; p.Tyr578_Phe591dup	*11*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1735_1737del, p.Asp579del	*11*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
c.1739_1774dup; p.His580_Gly592complex	*11*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
Missense mutation		8	72.7	8	72.7	16	72.7
c.1658A>C; p.Tyr553Ser	*11*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1676T>A; p.Val559Asp	*11*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
c.1679T>A ; p.Val560Asp	*11*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
c.1727T>C; p.Leu576Pro	*11*	*2*	*18.2*	*5*	*45.5*	*7*	*31.8*
c.1733A>C; p.Tyr578Ser	*11*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1924A>G; p.Lys642Glu	*13*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.1965T>G; p.Asn655Lys	*13*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.2459A>T; p.Asp820Val	*17*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
c.2460T>A; p.Asp820Glu	*17*	*0*	*0*	*1*	*9.1*	*1*	*4.5*
c.2464A>T; p.Asn822Tyr	*17*	*1*	*9.1*	*0*	*0*	*1*	*4.5*
Total		**11**	**100**	**11**	**100**	**22**	**100**

mK-CN: mutated *KIT* copy number; highlighted cells represents occurrences with patients with PFS≥6 months (3/5 patients for *c.1727T>C; p.Leu576Pro* and mK-CN amplified):

PFS>=6m
3/5 PFS>=6m

Table S4. Summary of the molecular analyses performed on the tumour biopsy samples and clinical outcomes

Related to Figure 2

ID[1]	*cKIT* mutation	mK-CN (biopsy)	WGS ID[2]	WES ID[2]	FISH[3]	RECIST – % change in sum of target lesions within 12 weeks	Months on treatment	Months to progression
NI02	c.1668_1739del72; p.Gln556_Asp579del	3.4		N10213		-28.6	2.7	2.7
NI03	c.1676T>A; p.Val559Asp	10.6	N05408		CEN4/nucleous~2-4 *KIT*:CEN4>1 *KIT*:nucleus~2-4	-14.2	16.5	5.8
NI10	c.1727T>C; p.Leu576Pro	12.3			CEN4:nucleous~3 *KIT*:CEN4=1 *KIT*:nucleus>2		1.4	1.2
NI11	c.1727T>C; p.Leu576Pro	2.9			Heterogeneous CEN4:nucleous~3 *KIT*:CEN4=1 *KIT*:nucleus>2	-1.6	10.2	5.9
NI12	c.1727T>C; p.Leu576Pro	5.9			Heterogeneous; some areas are euploids and *KIT*:CEN4=1, others appear CEN4:nucleous~2-5 *KIT*:CEN4~1 *KIT*:nucleus~2-5	30	3.2	2.9
NI15	c.1730_1732del; p.Pro577_Tyr578delinsHis	3.6			Very heterogeneous, some areas are euploidy with *KIT*:CEN4=1, others CEN4:nucleous=1 *KIT*:CEN4=~4 *KIT*:nucleus~4	1.6[¥]	1.6	1.7
NI16	c.1732_1773dup; p.Tyr578_Phe591dup	1.2	N01803			-15.1	7.4	5.3
NI18	c.1735_1737del, p.Asp579del	10.1		N06610		-71.4	50.6	5.9
NI19	c.1739_1774dup; p.His580_Gly592complex	13.2		N01502		40.2[¥]	1.8	1.6
NI22	c.1924A>G; p.Lys642Glu	1.4			Euploid and *KIT*:CEN4=1	-38.8	5.7	6.1
NI27	c.1727T>C; p.Leu576Pro	1.8		N00101			2.2	4.4

[1]All Mucosal type except NI15 acral; all Exon 11 except NI22 (exon 13; NI27 considered not evaluable for primary endpoint analysis

[2]Whole genome sequencing (WGS) and whole exome sequencing (WES) identifiers (ID) used in a in a small subset of NICAM patients co-enrolled in a tissue biobanking study (Furney, Turajlic et al., Journal of Clinical Pathology, 2013) . Results are not reproduced here to avoid data duplication

[3]The count of probe signals for KIT and the centromere of chromosome 4 (CEN4) per nucleous in cancer cells for the 6 samples that could be analysed with FISH. Some cells appear to have duplications of both KIT and centromere of chromosome 4, others to have duplications of KIT with normal number of centromere of chromosome 4.

Table S5. Systematic review of studies of targeted therapies in advanced melanoma harbouring KIT alterations
Related to discussion, Table 1, Figure 4

	Patients (n)	Patients with KIT mutation	RR (%)	OS (median)	PFS (median)	TTP (median)	Length of FU (median)	Intervention
Kluger 2011	36	36	5	12 .0	2	--	--	Dasatinib
Kim 2008	22	22	5	7.5		1.4		Imatinib
Kalinsky 2017	73	3/51 stage 1 22/22 stage 2	5.9 KIT- 18.2 KIT+	7.5	2.1		59.5 stage 1 23.2 stage 2	Dasatinib
Hodi 2013	24	24	21.0[a]	12.5	3.5	3.7[b]	10.6	Imatinib
Buchbinder 2015	52	13	9.7	7.5[c]	--	2.6[d]	--	Sunitinib
Guo 2011	43	43	23.3	15	3.5	--	12	Imatinib
Carvajal 2011	25[e]	25[e]	16	10.7	--	2.8	--	Imatinib
Carvajal 2015	19	11 Cohort A[f] 8 Cohort B[f]	18.2 0	14.2 4.3	--	3.4 2.6	16.2 11.7	Nilotinib
Guo 2017	42	42	26.2	18	4.2	--	25.8[f]	Nilotinib
Lee 2015	42	42	16.7	17.5	8.5		12.2	Nilotinib

Length of time reported in months. Abbreviations: OS, overall survival; PFS, progression-free survival; RR, response rate, TTP, time to progression, FU Follow-up; UNK, unknown.

a: RR reported also as 29% but only 21% confirmed response

b: 3.9 months with subset analysis KIT mutations and 3.4 months with amplifications

d: median based on 8.6 KIT-;6.4 KIT+;6.2 KIT UNK

c: median based on 2.8 KIT-;3.2 KIT+;1.8 KIT UNK

e: 28 KIT+ patients overall, only 25 evaluable

f: Cohort A: refractory or intolerant to a prior KIT inhibitor, Cohort B: those with brain metastases

g: reported only for 3 living patients

Table S6. NICAM Inclusion and exclusion criteria (as per Protocol V8)
Related to STAR Methods

Inclusion Criteria	
1.	Patients with c-KIT mutated histologically proven advanced mucosal or acral melanoma in which the mutation is not known to be associated with nilotinib resistance.
2.	Advanced mucosal and acral melanoma defined as unresectable locally advanced or metastatic disease
3.	The presence of one or more clinically or radiologically measurable lesions at least 10mm in size
4.	Age 18 or greater
5.	ECOG performance status 0, 1 or 2
6.	Life expectancy greater than 12 weeks
7.	At least 14 days since any major surgery
8.	The capacity to understand the patient information sheet and ability to provide written informed consent
9.	Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests and other study procedures
10.	Women must not be pregnant or lactating with no intention of pregnancy during study treatment. Women of child bearing potential must have a negative serum pregnancy test prior to study entry (even if surgically sterilised). Men and women of childbearing potential must use adequate birth control measures (e.g. abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, implantable or injectable contraceptives or surgical sterilisation) for the duration of the study and should continue such precautions for 6 months after receiving the last study treatment
11.	Serum alanine transaminase (ALT) or serum aspartate aminotransferase ≤2.5 x upper limit of normal (ULN) and total serum bilirubin ≤1.5 x ULN
12.	Serum creatinine ≤1.5 x ULN
13.	Serum lipase and amylase <1.5 x ULN
14.	Haemoglobin ≥9.0 g/dL, absolute neutrophil count ≥1.5 x 109/L, platelets ≥100 x 109/L
15.	Prothrombin time (PT) ≤1.5 x ULN
16.	Able to swallow and retain oral medication.
Exclusion Criteria	
1.	Intracranial disease, unless there has been radiological evidence of stable intracranial disease > 6 months. In the case of a solitary brain metastasis, evidence of a disease-free interval of at least 3 months post surgery. All patients previously treated for brain metastases must be stable off corticosteroid therapy for at least 28 days
2.	Women who are pregnant, nursing, or planning to become pregnant during the course of the trial
3.	Men who plan to father a child during the course of the trial
4.	Use of any investigational drug within 30 days prior to screening (both cancer and non cancer treatments)
5.	Use of herbal or chinese medication
6.	Use of therapeutic coumarin derivatives (ie warfarin, acenocoumarol, phenprocoumon)
7.	Significant cardiac disease including patients who have or who are at significant risk of developing prolongation of QTc
8.	Severe and/or uncontrolled medical disease
9.	Known chronic liver disease
10.	Past medical history of chronic pancreatitis
11.	Known HIV infection
12.	Previous radiotherapy to 25% or more of the bone marrow
13.	Radiation therapy in the 4 weeks prior to study entry
14.	Prior exposure to a tyrosine kinase inhibitor
15.	Known lactose intolerance
16.	Any malabsorption syndrome (i.e. partial gastrectomy, small bowel resection, Crohn's disease or ulcerative colitis).

Supplementary Figures

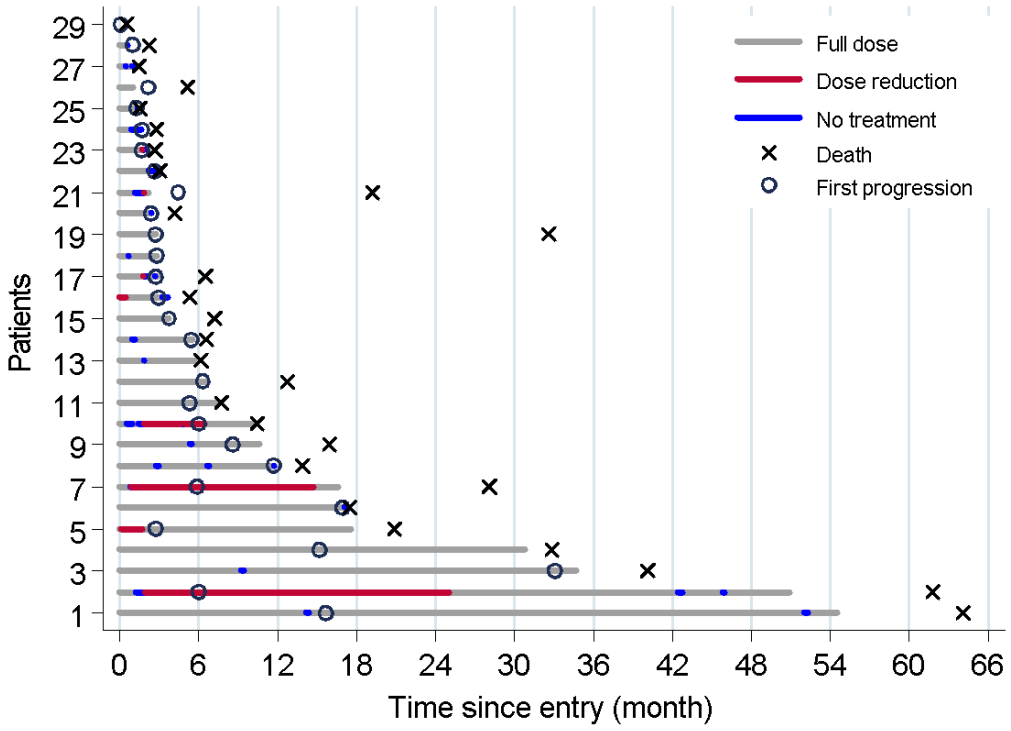


Figure S1: Time on treatment (grey) with periods of dose reduction (red), delay or missing treatment (blue), time of first progression and time of death in all NICAM patients (N = 29)

Bar length indicate months on treatment; objective disease progression and death are indicated in the figure. Patients were allowed to continue treatment as long as clinically indicated by the treating physician.

Related to Figure 3, Table 2

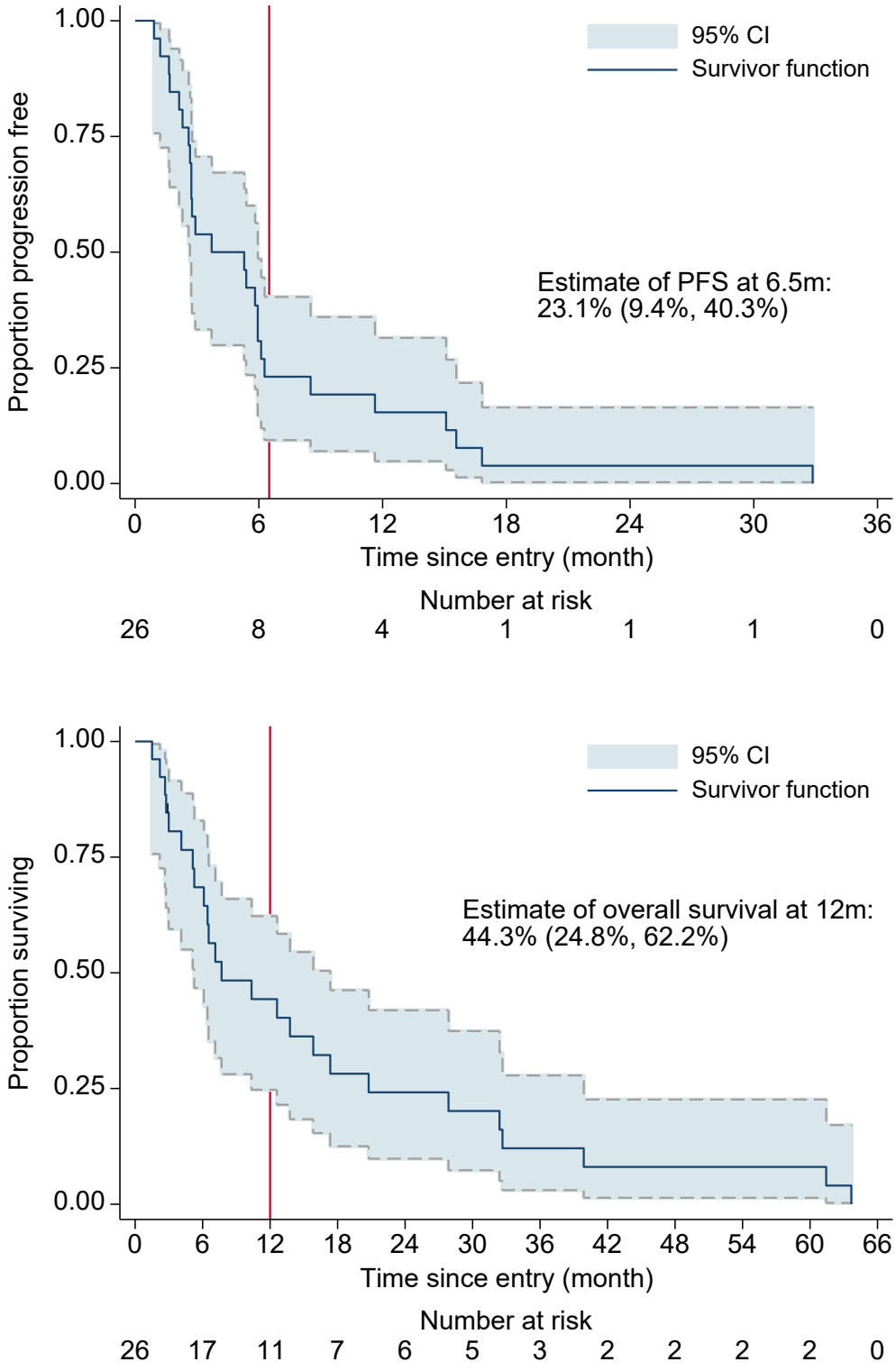


Figure S2: Progression Free Survival (top) and Overall Survival (bottom) Kaplan-Meier estimates on the evaluable population (n=26)

Related to Figures 3, 4

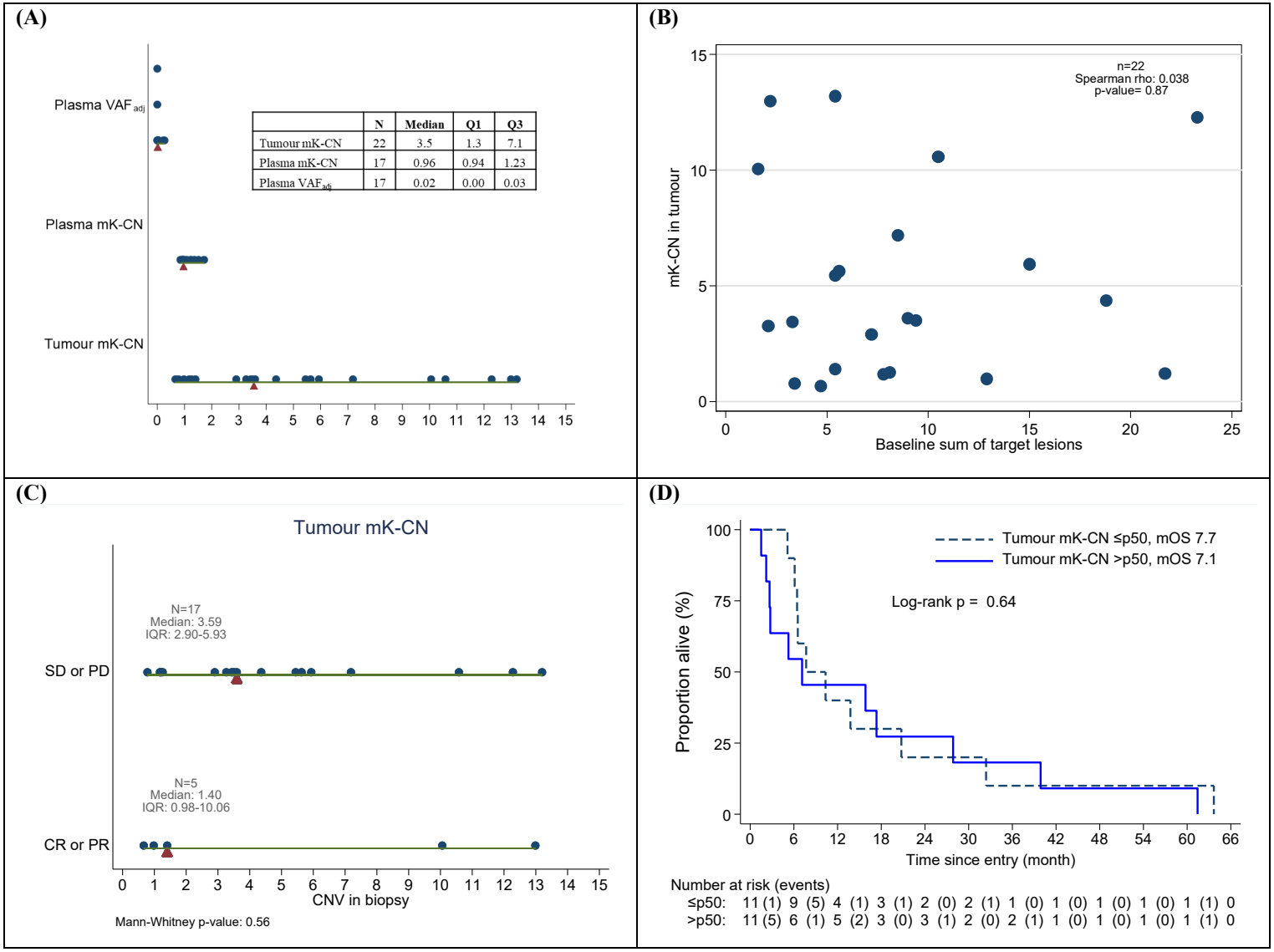


Figure S3 Association of gene amplification in tissue with antitumour activity
(A) Distribution of mK-CN in tumour, mK-CN in plasma and VAF_{adj} in plasma (B) Association of mK-CN in biopsy with disease burden at baseline (represented by sum of target lesions as per RECIST 1.1) (C) Baseline mK-CN in tumour with objective response (RECIST 1.1) at 12 weeks (D) overall survival by baseline mK-CN in tumour, groups defined by its median
mK-CN below median in the analysis set (<p50=3.5); amplified: mK-CN at or above median in the analysis set (≥p50=3.5). All the cfDNA data are the mean of 3 technical replicates for one patient biological sample.
Related to Table 1, Figure 4

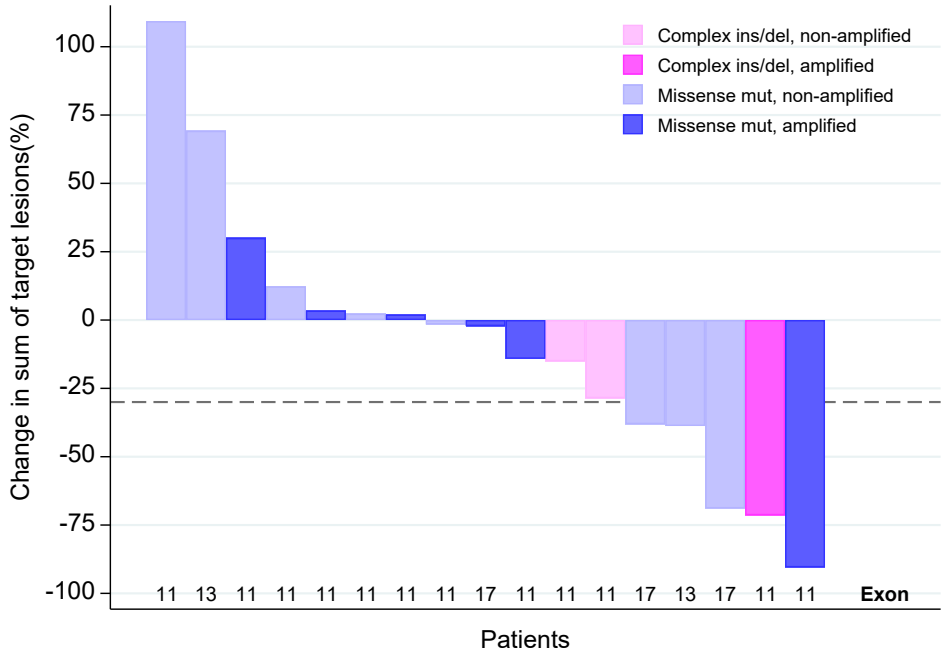


Figure S4. Best percentage change from baseline at 12 weeks in sum of target lesions as per RECIST 1.1 (central review) by type of *KIT* mutation and mutated *KIT* copy number amplification
Complex ins/del= complex insertion or deletion; Missense mut: missense mutation; non-amplified

Related to Figure 4

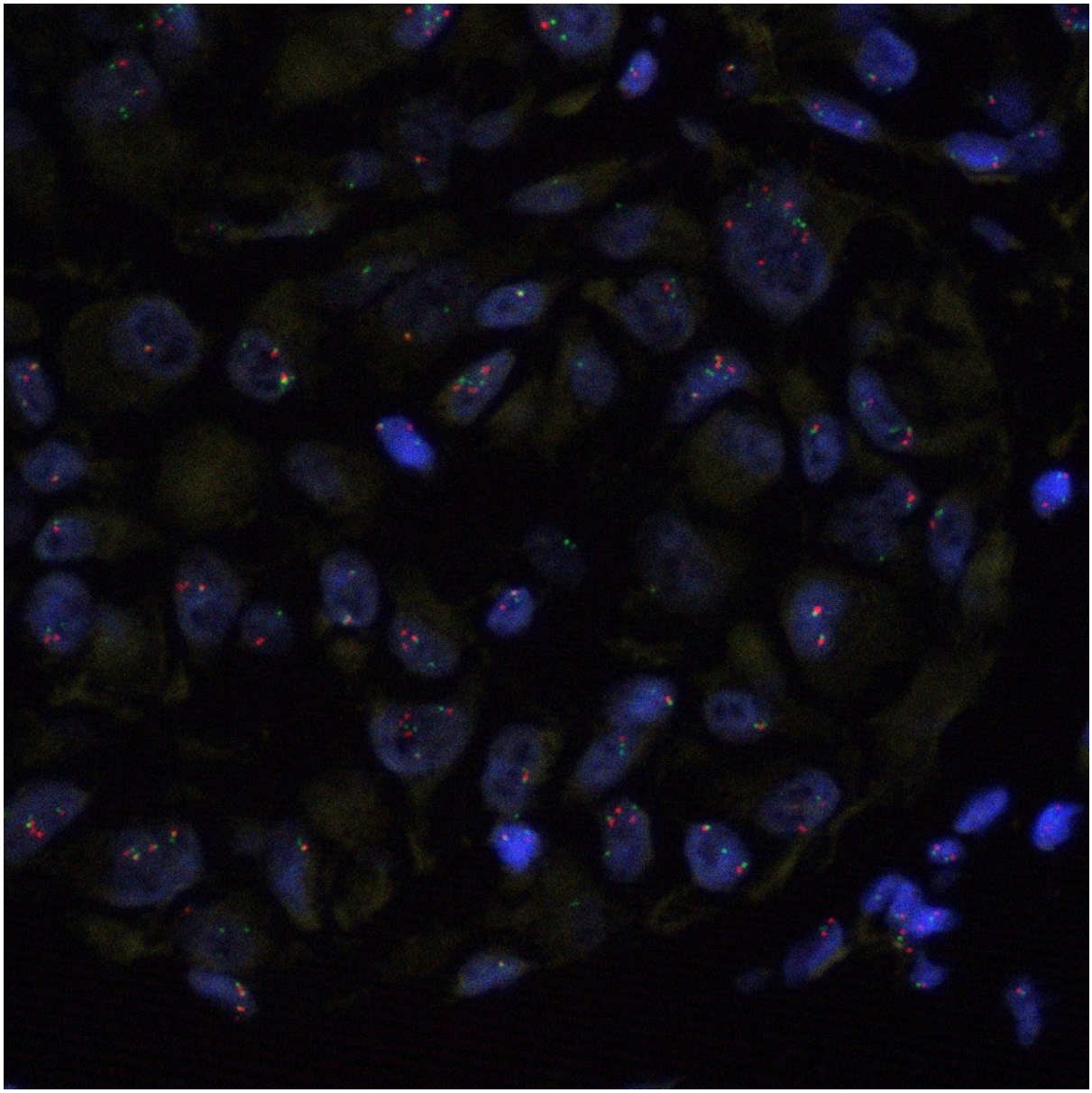
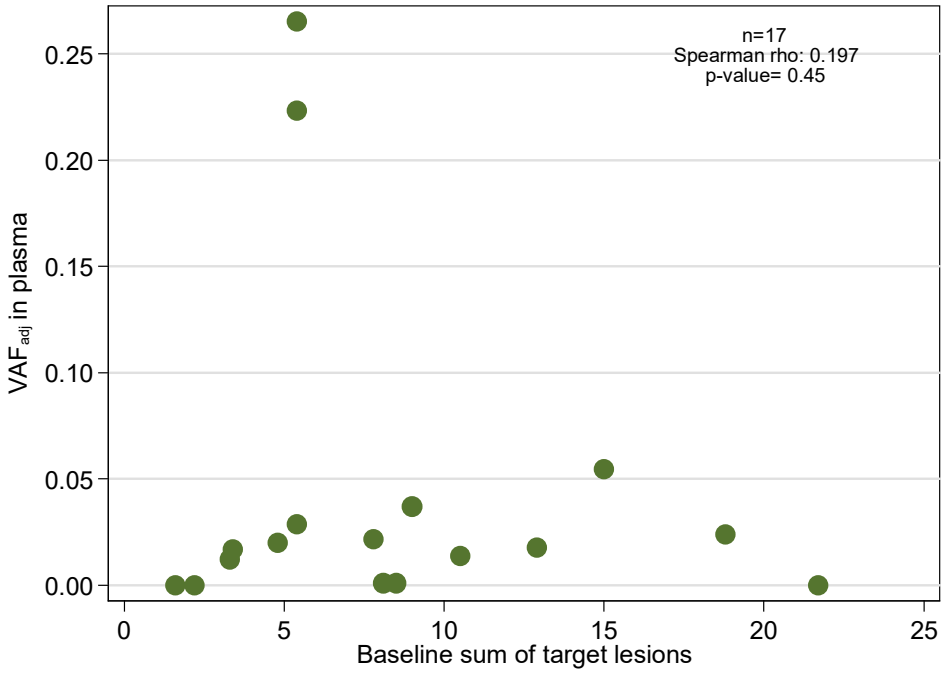


Figure S5. The micrograph shows the fluorescent in situ hybridisation of the paraffin fixed tumour sample of patient NI12 (see Table S4). The nuclei are stained in blue (dapi), the green dots correspond to the chromosome 4 centromere (fluorescein probes) and the orange dots correspond to KIT (temra probes).The white arrows indicate examples of nuclei with two green and two orange dots (diploid for chromosome 4 and KIT), the red arrows highlight examples of cells with more than two copies of chromosome 4 and KIT per nuclei. This patient had mean KIT copies = 5.9 in the tumour and = 1.2 in cfDNA as measured by ddPC

Related to Figure 2, Figure 4

(A)



(B)

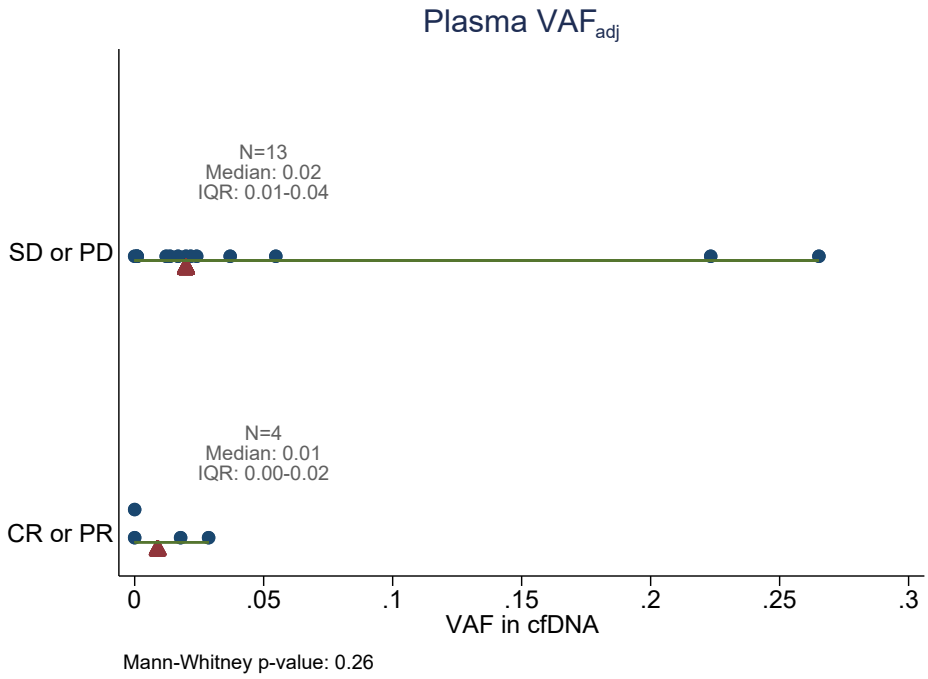


Figure S6: Association of gene amplification in plasma with antitumour activity
(A) Association of VAF_{adj} in blood, with disease burden at baseline (represented by sum of target lesions as per RECIST 1.1) **(B)** Association of Baseline VAF_{adj} in plasma with objective response (RECIST 1.1) at 12 weeks
All the cfDNA data are the mean of 3 technical replicates for one patient biological sample.

Related to Table 1, Figure 4