Molecular residual disease and adjuvant trials design in solid tumors

Maria Coakley[1], Isaac Garcia-Murillas[1], Nicholas C. Turner[1,2]

1 Breast Cancer Now Research Centre, Institute of Cancer Research, London, SW3 6JB, UK.

2 Breast Unit, Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK

Running Title: Molecular Residual Disease and Adjuvant Trial Design

Keywords: Molecular, Residual, Relapse, Adjuvant, Trial.

Additional Information:

Financial support has been received from the following organizations for the following authors:

- **Maria Coakley** Cancer Research UK, The Wellcome Trust
- **Isaac Garcia-Murillas** Breast Cancer Now
- **Nicholas Turner** Breast Cancer Now, NHS funding to the NIHR Royal Marsden Biomedical Research Centre, Cancer Research UK, The Wellcome Trust.

Corresponding Author: Nicholas Turner, Breast Unit, Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK. Phone number: +44 20 7352 8171. Fax number: +44 20 8643 0373. Email address: Nick.Turner@icr.ac.uk

Conflict of Interest Disclosure Statement: Nicholas Turner has received advisory board honoraria from Astra Zeneca, Bristol-Myers Squibb, Lilly, Merck Sharpe and Dohme, Novartis, Pfizer, Roche/Genentech, Tesaro and research funding from Astra Zeneca, BioRad, Pfizer, Roche/Genentech.

Word Count: 4183

Figures: 3

Tables: 2

Abstract

Advances in diagnosis and treatment have resulted in a high rate of survival for many patients with early stage cancers. However, identifying who is at ongoing risk of relapse remains of high priority to direct subsequent adjuvant therapy.

Multiple recent retrospective studies have shown that detection of tumor derived materials in blood, in particular with circulating tumor DNA (ctDNA) analysis, can identify patients with residual disease prior to clinical or radiological evidence of metastatic disease, anticipating relapse with relatively high sensitivity and high specificity.

We discuss how these emerging technologies are defining new subgroups of patients with "***Molecular Residual Disease"*** and "***Molecular Relapse"***. We outline how novel clinical trials in the adjuvant setting designed for these new subgroups of patients may improve selection for adjuvant therapies, and provide new surrogate endpoints that may allow for early registration of adjuvant therapies and novel clinical trial designs in the adjuvant setting.

We discuss the current limitations of these techniques and the routes to clinical implementation.

Introduction

Adjuvant therapy, delivered after definitive treatment of the primary tumor (via surgery or with radiotherapy) may improve patient survival by treating undetectable micro-metastatic disease (1). Sequential improvements in adjuvant treatment strategies with clinical trials designed to intensify treatments have resulted in improved patient survival. Yet many patients are cured by definitive treatment of the primary tumor alone and the absolute gains offered by adjuvant therapies remain low, leading to toxicity in patients who may not have required therapy, and increasing financial costs of multi-modality therapies (2). For example, the absolute overall survival (OS) benefit from adjuvant chemotherapy in Non-Small Cell Lung Cancer (NSCLC) post resection is a 4% improvement in OS at 5 years (3). Similarly, adjuvant clinical trials often require very large numbers of patients to display a clinical benefit. For example, the APHINITY clinical trial (NCT/01358877) randomized a total of 4805 patients with early HER2-positive breast cancer to receive chemotherapy and trastuzumab plus either pertuzumab or placebo, with an estimated 3-year invasive disease free survival (DFS) benefit of only 0.9% (4).

Some tumor derived surrogates are useful in assessing the effects of treatment or directing therapy, including pathological complete response (pCR) to neo-adjuvant chemotherapy (5-7), and gene expression analysis of the primary cancer to predict risk of micro-metastatic dissemination (8). Yet for many patients these offer only a modest improvement in establishing risk of relapse. Similarly, following definitive therapy, traditional protein-based biomarkers may play a role in identifying early relapse in certain disease states, for example carcinoembryonic antigen (CEA) in colorectal carcinoma (9). However in many disease settings, for example following treatment for early breast cancer, intensive follow up, including the monitoring of protein-based biomarkers, is not recommended (10). This recommendation is based on data which shows that intensive follow up fails to improve patient outcomes (11). Therefore, better biomarkers are required which identify patients at risk of relapse, or are developing an early subclinical relapse, at a timepoint amenable to therapy, potentially through the detection of micro-metastatic disease, ideally combined with predictive markers of who will benefit from future treatment.

Technological advances have resulted in a transformation in our ability to detect and analyze tumor derived material in blood through minimally invasive or non-invasive methods *(12)*. The oldest report of tumor material in blood can be traced to 1869, when Thomas Ashworth

reported circulating tumor cells (CTCs) in a post mortem (13). Subsequently, in the 1970s S.A. Leon *et al.* reported high levels of circulating free DNA (free DNA) in the serum of cancer patients (14), with tumor origin circulating tumor DNA (ctDNA) confirmed in 1994, with the identification of mutated *KRAS* in plasma of patients with pancreatic adenocarcinoma (15). Now two decades later, recent advances in detecting tumor derived material in the plasma and other body fluids, and circulating tumor DNA (ctDNA) analysis in plasma in particular, have identified alternative strategies that identify patients at very high risk of relapse, and therefore in need of adjuvant therapy. In this review we discuss how the analysis of tumor derived material from the blood or other body fluids, and in particular ctDNA can be incorporated into adjuvant clinical trials to treat newly defined subgroups of patients, and to provide new surrogate endpoints to allow for accelerated approval of adjuvant therapies and novel clinical trial design in the adjuvant setting.

Analysis of tumor derived material from the blood

Circulating tumor ctDNA:

ctDNA detection offers a high level of accuracy for detection of residual disease, and offers great potential for translation into clinical trials (16). ctDNA is stable when collected appropriately, and in preservative tubes may be shipped to central laboratories for delayed processing, facilitating centralized testing for multi-center trials and routine clinical use (17, 18).

Translation of ctDNA analysis to the early cancer setting requires detection of very low levels of ctDNA in plasma (19-21). Detection of such low levels of ctDNA in the plasma is performed by digital PCR or by error corrected sequencing, which typically includes molecular barcoding for efficient recovery of circulating free DNA molecules and bioinformatic *in silico* techniques to eliminate background artifacts (22). These methodologies are reviewed in depth elsewhere (23). A broad range of assays are currently available, and with only a limited number of studies of cross-platform comparison of these technologies (24, 25), it is in general unknown whether evidence from one assay can be safely applied to other assays. A recent American Society of Clinical Oncology (ASCO) position paper has highlighted the need for standardization in techniques and reporting, along with more studies on cross-assay comparisons (26).

The choice of ctDNA assays in future prospective studies will need to balance considerations in sensitivity, specificity, availability, cost and practicalities for implementation. For example, patient specific multi-gene or mutation panels may be highly sensitive, but have high cost; whereas tracking one clonal mutation may offer less sensitivity, but be practical and cost effective to implement in large studies. Future prospective studies discussed below, where ctDNA is suggested as a method of selecting patients for further therapies, should aim to concurrently validate assays which can be readily translated into clinical practice by demonstrating that they are capable of positively impacting patient outcomes in a cost-effective manner in a clinical setting.

Studies to date detecting ctDNA in the adjuvant setting may be split into two general types. Studies that seek to identify ***"molecular residual disease (MRD)"*** shortly after finishing definitive treatment, and those with sequential ctDNA testing during follow-up seeking to

detect residual disease as it starts to expand and identify ***"molecular relapse (MR)"*** with a lead time prior to clinical relapse.

Molecular Residual Disease (MRD):

ctDNA has been shown in multiple tumor types to detect MRD including nasopharyngeal carcinoma (27), colorectal carcinoma (28-32), locally advanced rectal carcinoma (33), breast carcinoma (34) , pancreatic carcinoma (35, 36) and lung carcinoma (37) ***(Table 1)***. ctDNA in the plasma has a short half-life (<2 hours), however, it has been found that ctDNA may be elevated 24 hours following intervention in early post-operative samples, most likely due to release of ctDNA from damaged tissue (28). Allowing for this early elevation to resolve, these studies show in patients with ctDNA detected at a later single timepoint (e.g. 10 days - 16 weeks) following definitive treatment (via surgery or with radiotherapy) Disease Free Survival (DFS) is significantly reduced and the Hazard Ratio (HR) for recurrence is significantly elevated (HR 3.1- 43.4 across studies), compared to patients where ctDNA was not detected. Some studies have also shown ctDNA detection in the post-treatment period to be associated with a reduced OS (HR 3.4, 6.7) (31, 32).

Some of these studies, along with additional studies have examined the effect of serial sampling of ctDNA from the plasma to detect MR at a later timepoint, which may be missed by the first post treatment sample.

Molecular Relapse (MR):

Serial sampling for ctDNA from plasma following definitive therapy or adjuvant treatment, performed at various intervals ranging from monthly (31) to 6 monthly (34) has been shown to detect MR with lead times from detection of ctDNA to clinical or radiological relapse reported as up to 6 months in nasopharyngeal carcinoma (38), or at a median of 7.9 - 11 months in breast carcinoma (34, 39), 6.5 months in pancreatic carcinoma (40), 70 days – 5.2 months in lung carcinoma (37, 41), and 167 days - 10 months in colorectal carcinoma (29, 30, 42, 43) **(Table 2)**.

ctDNA has been shown to have superior sensitivity in detecting MR compared to the protein-based tumor marker CEA (29). Serial sampling during or after adjuvant therapy has also been shown to correspond with clinical events, which may provide early information on the effects of adjuvant therapy, with a number of patients displaying a decrease in ctDNA during

adjuvant chemotherapy (28, 29, 44). This property may allow for ctDNA to be employed as a novel surrogate endpoint in adjuvant clinical trials as discussed below.

Whether detection of MRD or MR has clinical utility unknown, and later in this review we discuss how well-designed clinical trials may establish the clinical utility of detection of disease at these new timepoints.

Furthermore, due to differences in blood sampling schedules in studies to date, and with no studies directly comparing between chosen timepoints, the optimal blood sampling schedule to test for MRD and MR is yet unknown. More frequent testing may allow for earlier detection of micro-metastatic disease, however at a higher financial cost and increased stress of an intensive follow up schedule for patients. Studies designed to directly compare various sampling schedules would be helpful to design optimal schedules in this setting.

Sensitivity and Specificity of ctDNA in Detection of Molecular Residual Disease (MRD) and Molecular Relapse (MR):

There is marked heterogeneity in these studies with investigators analyzing various tumor sites, sample sizes, investigative points, assays and follow up time-points; along with numerous different outcomes measured and reported (**Table 1, 2**). Many studies are also limited as they are retrospective. Yet despite heterogeneity in studies, clear themes are emerging.

ctDNA analysis has a ***high level of specificity*** as detection of ctDNA after definitive treatment is associated with a high risk of future relapse. Although in many studies a small number of patients did not relapse after ctDNA being detected in the post-treatment setting, it is anticipated that this may be due to short follow-up, and that the patients were likely to clinically relapse outside of the follow up period of the study (34). Reporting of longer follow-up is required to address this problem. A further confounding factor is that the effects of further treatments (i.e. efficacy of adjuvant chemotherapy) may also have affected specificity results, as for example in some studies patients had adjuvant therapy following MRD testing, which would potentially impact specificity (28, 29, 44). As larger studies are conducted in this setting, specificity is likely to be affected by additional factors such as new primary tumors, clonal hematopoiesis, and possibly somatic mutations leading to “field defects” with mutations that accumulate in healthy tissue with age (45).

The ***sensitivity across studies is variable***. For example, in testing for MRD in colorectal carcinoma, sensitivities reported across various studies ranges between 48-100% (28, 31). The variability in sensitivity across studies may be due to a number of factors. In some patients the level of residual disease may be low, and there may be insufficient ctDNA present in the plasma. This may partially account for the low sensitivity reported in early stage tumors, i.e. stage II colorectal carcinoma (29). In other patients, who have sampling after completing adjuvant chemotherapy, tumor cells may be quiescent and not release sufficient ctDNA for detection. Likely only proliferating cancers can be detected by ctDNA analysis, as quiescent cancer cells also have low rates of cell death. Variability in assays is a concern, and as discussed above there is a growing need for cross-assay comparisons and standardization in methodologies (26). Many differences in outcomes across studies may be attributed to differences in study design and patient selection with a broad range of tumor sites and stages studied, a broad range in chosen timepoints for the first post-treatment sample (10 days - 16 weeks), differences in the follow up time period chosen, and variations in timing of sampling in assaying for MR (ranging from 1 - 6 monthly).

Sensitivity of serial sampling for MR is higher than with sampling at a single timepoint for MRD, and approaches 100% in many studies (31, 36, 42). However, patients relapse without evidence of ctDNA occur in many series, and may be described as having "***dark metastases***". In certain anatomical sites, e.g. Central Nervous System (CNS) ctDNA in the plasma may be undetectable. In future, for patients who are particularly high-risk, consideration may need to be given to analysis of other body fluids such as Cerebrospinal Fluid (CSF) to detect such occult recurrences (20, 34) if clinical outcomes could be improved by early detection of metastases in these sites.

Ultimately, for implementation of ctDNA monitoring into clinical practice in the adjuvant setting, investigators will need to demonstrate that ctDNA detection is sufficiently sensitive and specific to allow potential changes to management.

Circulating Tumor Cells (CTCs):

Multiple techniques have been developed for assaying CTCs, however the most widely utilized method in clinical research is the FDA approved CellSearch system which positively selects for cells expressing EpCAM (46). There are some studies analyzing the ability of CTCs to detect MRD following definitive therapy. In early breast cancer CTCs have been detected after surgery, prior to adjuvant chemotherapy in 21.5% of patients. However, detection of CTCs in this patient group only modestly associated with a reduced DFS (HR 2.1). Although a higher level of CTCs (>5 CTCs) had a stronger association with a reduced DFS (HR 4.5), this was only found in approximately 3% patients (47).

In contrast, the presence of CTCs in patients with a history of lymph node-positive and high-risk lymph node-negative hormone receptor-positive early stage breast cancer approximately 5 years after diagnosis, was found to be associated with a higher risk of recurrence (HR 13.1). Again, a higher CTC burden was associated with a higher risk of recurrence. However, in hormone receptor-negative patients detection of CTCs, mostly a single cell, was not predictive of relapse (48). It is unknown how useful ctDNA would be in this context to predict late recurrences.

Although these results are promising that detection of CTCs can detect residual disease, they also demonstrate limitations of current CTC assays. Detection of high levels of CTCs is associated with a high probability of risk of future relapse, but with low sensitivity. Conversely, detection of one CTC is likely subject to false positive results, which lowers specificity and raises concerns over the cut off where a result may be considered clinically significant.

When directly compared in the metastatic disease setting, ctDNA has shown superior sensitivity than CTCs (49-51) and has been shown to capture a broad spectrum of a patient's heterogeneous mutational profile (52, 53). These findings are in contrast to earlier reports of CTCs being more sensitive than ctDNA to detect an activating EGFR mutation (54), however, this predates improved ctDNA techniques in recent years (19, 20). To date, there are no direct comparisons between ctDNA and CTC detection in monitoring for MRD or MR in the adjuvant setting.

Emerging technologies:

There are numerous emerging technologies, such as the analysis of circulating extracellular vesicles (55, 56), circulating free tumor derived RNA (57), Tumor Educated Platelets (TEPs) (58) and ctDNA methylation status (59-61) which hold promise for detection of residual disease in the future, but currently lack strong evidence for translation into clinical practice or clinical trials at this point in time.

Clinical trials guided by the analysis of ctDNA in blood

Treating newly defined subgroups of patients:

The very high rates of relapse associated with detection of ctDNA after definitive treatment, identify entirely new subgroups of patients. Although data of preliminary clinical *validity* is present for many different ctDNA assays, this does not imply clinical *utility* – that use of ctDNA to identify residual disease can improve patient outcome. Two routes to demonstration of clinical utility are potentially available:

1) Prospectively planned collection, with retrospective analysis, of baseline (pre-treatment) samples from an already conducted randomized control trial of treatment versus placebo, to demonstrate that patients with ctDNA detection derive benefit from treatment, whilst patients without ctDNA detection derive clinically insignificant benefit.

2) Large prospective clinical trials of ctDNA assays with treatment guided by ctDNA detection, to test the hypothesis that an intervention at the point of ctDNA detection can lead to an improvement in patient outcomes (26).

Prospective trials of molecular residual disease (MRD) detection to guide standard therapy:

Designing trials to direct standard adjuvant therapy, for example adjuvant chemotherapy, are challenged by the high risk of relapse in patients with ctDNA analysis. Standard trial designs that randomize patients between no treatment and treatment, or between placebo and treatment if side effect profiles unblind the randomization, are likely not practical. Two potential designs are available to direct standard adjuvant chemotherapy, in settings where there is uncertainty over the benefit of adjuvant chemotherapy:

1. Randomize patients between ctDNA guided therapy and standard therapy (**Figure 1a**) This design is currently being explored in stage II colorectal carcinoma, such as the **DYNAMIC clinical trial (ACTRN/12615000381583**), currently recruiting in Australia (62). Such a design for a pivotal phase III study most logically requires a non-inferiority design, and a very large number of patients.

2. Non-randomized studies, with ctDNA negative patients not receiving adjuvant chemotherapy **(Figure 1b),** with the objective being to show that ctDNA negative patients have a sufficiently good outcome to not have been able to receive sufficient benefit from chemotherapy. This design requires substantially less patients, but is predicated on baseline relatively low risk.

As discussed above, and summarized in Table 1 and 2, sensitivity of assays in detecting ctDNA in the adjuvant setting is variable. Therefore, at present we do not recommend clinical trials designed to omit adjuvant therapy on the basis of a negative ctDNA result in clinical settings where there is substantial absolute benefit from adjuvant chemotherapy. In such scenarios, we recommend more sensitive diagnostic development, showing consistently high sensitivity approaching 100% across multiple studies prior to consideration of a clinical trials designed to de-escalation therapy in high risk disease.

Prospective trials of molecular residual disease (MRD) or molecular relapse (MR) detection to guide additional treatment after standard therapy:

Trial designs to escalate therapy after completing standard adjuvant chemotherapy, are potentially more straightforward. Patients with evidence of MRD may be randomized to new treatment or placebo on the background of standard therapy, provided such therapies do not unblind randomization (**Figure 2a**). Treatments with characteristic adverse effects that unblind the randomization, may require all patients to undergo standard therapy to avoid patient dropout. Such designs are being trialed in the **c-TRAK-TN clinical trial (NCT/03145961**) currently recruiting in the United Kingdom (63). Pivotal studies, with endpoints of DFS or OS from the time of randomization at ctDNA detection, would require substantially less patients to be treated than traditional adjuvant trials, although an approximately similar number of patients overall are required to enter MRD surveillance. The importance of routine follow-up for DFS, which in most disease sites consists of interval imaging is highlighted in the figures, to ensure all relapses, particularly those not detectable by ctDNA are not missed.

Potentially, future studies may not require randomization with a placebo arm if it is first proven that ctDNA detection, and then ctDNA clearance by treatment, is a robust surrogate for DFS, particularly in phase II clinical trials **(Figure 2b).** Such studies would offer a benefit over conventional clinical trials as they would require smaller cohorts of patients to display efficacy, and all patients would be offered the opportunity to potentially benefit from the trial treatment. However, these benefits would need to be carefully balanced with risk of bias, and the need for larger confirmatory studies.

It has historically been difficult to alter the disease course detecting progression with protein-based biomarkers (e.g. rising CA125 in ovarian carcinoma), with historical treatment modalities (64). There is uncertainty regarding the best management when a biomarker such as Prostate Specific Antigen (PSA) is detected following definitive treatment for early cancer (65). With new treatment modalities, such as immunotherapy and targeted therapies, efforts to alter the disease course in these settings continue in clinical trials (e.g. NCT02649439) (66). The hope is that as ctDNA detection is opening up additional disease settings, such as MRD and MR at a substantially earlier stage than was previously possible, and with newer treatment modalities, that clinical trials in these settings will lead to improved clinical outcomes for a greater number of patients than has been historically possible.

The ability to select patients with actionable mutations for targeted therapy in the adjuvant setting:

Due to the rich genomic information offered by the analysis of tumor derived material from blood these techniques are uniquely positioned to guide targeted therapies in the adjuvant setting. Similarly to the clinical trial design suggested above, a non-randomized approach may be possible, if ctDNA were first validated as a surrogate endpoint **(Figure 2c).** Targeted therapy may subsequently be directed to the genomics of the residual disease, that may differ from the original primary due to intra-tumoral heterogeneity or tumor evolution (34). During adjuvant targeted therapy, ctDNA analysis could detect the development of resistance mutations to trigger an appropriate change in therapy, as most clearly demonstrated in advanced lung cancer with EGFR T790M mutations in advanced EGFR-mutated NSCLC (67). Furthermore, sequential analysis of ctDNA through adjuvant treatment may therefore provide rich genomic information on mechanisms of response and resistance, allowing tailoring of therapy prior to disease progression **(Figure 3).**

Surrogate Endpoint in Clinical Trials:

Perhaps one of the most exciting developments is that detection of MRD and MR, and subsequent clearance, may act as a surrogate endpoint in clinical trials. The role of surrogate endpoints in clinical trials is hotly debated (68), however, it is generally accepted that well validated surrogate endpoints (e.g. pCR) can be utilized as primary endpoints in clinical trials to demonstrate clinical benefit, and may even provide evidence for accelerated drug approval (69). It is possible to consider potential future uses of ctDNA detection as a surrogate endpoint.

1. ***Detection of ctDNA as an early readout from adjuvant trials***. Detection of ctDNA associates strongly with DFS. Decreased detection rate of ctDNA after completing standard adjuvant therapy with a new therapy, compared to standard adjuvant therapy alone, may present a surrogate end point for DFS to allow accelerated approval. Such concepts are established in hematological malignancy (70, 71).
2. ***Clearance of ctDNA as a surrogate endpoint of efficacy of ctDNA directed treatment***. Analogous to the above, in trials where further treatment is directed by detection of ctDNA, clearance of ctDNA might be a surrogate for treatment efficacy and subsequent improvement of DFS. Here direct evidence is lacking, although in the metastatic setting early suppression of ctDNA is associated with

PFS in multiple settings (72, 73). Issues around clearance of ctDNA, criteria for testing, optimal time-points for testing, and how to deal with stochastic issues of detection near the assay lower limit of detection will all need establishing in trials.

Therefore, biomarkers such as ctDNA may be chosen as surrogate endpoints in adjuvant trials, which would allow for a more rapid advancement in the field of adjuvant therapies by providing earlier endpoints than DFS or OS to display treatment effect. Careful evaluation and analysis to validate the proposed novel surrogate endpoint will be required, as is the case for pathological complete response in the neo-adjuvant treatment of breast cancer, which continues to undergo careful re-evaluation in meta-analysis as additional long-term outcome data becomes available (74).

As discussed above, variable sensitivity in detecting MRD and MR is a significant problem to be overcome for ctDNA to be utilized to direct therapies in adjuvant clinical trials. However, as assays continue to evolve and studies become more standardized, it is expected this limitation may be overcome. Emerging techniques such as incorporation of fragment size analysis or tracking multiple mutations in plasma may boost ctDNA detection, especially in tumor types with low levels of ctDNA (75). Future approaches may also incorporate commercial or multi-omic approaches, as such generalized mutation panels have even reported to hold promise in identifying malignancy in screening of healthy cohorts of patients (e.g. CancerSEEK) (76). At present, ctDNA analysis requires sequencing of the primary tumor, although it is conceivable that further developments in methylation analysis in ctDNA may remove the requirement for this. It is critical to emphasize the need for prospective, multi-center studies to validate the findings of currently largely retrospective and proof-of-principle studies.

Conclusion

For the analysis of tumor derived material from the blood to be incorporated into clinical practice, the results of adjuvant clinical trials with robust methodology are required. Analysis of tumor derived material from the blood and in particular ctDNA in the plasma, has been shown in the retrospective research setting to be sensitive and specific to detect recurrent malignancy prior to clinical or radiological evidence of metastatic disease. This early detection of minimal residual disease (MRD) or molecular relapse (MR) is redefining new cohorts of patients which may be useful to improve patient selection in the adjuvant setting.

In this review we have discussed various clinical trial designs aimed at limiting adjuvant therapies to a much smaller number of patients than with current clinical practice, where large numbers of patients are treated in order to improve outcomes for a small percentage of patients. Such a change in practice would lead to less treatment toxicities in the short term, improved long-term survivorship outcomes and lower overall costs of early stage cancer care.

Finally, it should be noted that clinical trials incorporating analysis of tumor derived material in the blood face additional challenges as open questions remain regarding assay design, optimal scheduling, sensitivity and specificity. Future clinical trials in this setting should concurrently aim to validate the assay of choice by demonstrating that the assay is robust and sufficiently sensitive and specific to direct changes in clinical management which lead to meaningful improvements in outcomes for patients.

Figure 1. Trials designs in Clinical scenarios where the benefit of adjuvant therapy is uncertain.

1a. Randomized design ctDNA guided therapy versus standard therapy. Such a design for a pivotal phase III study potentially requires a non-inferiority design.

1b. Non-randomized studies, with ctDNA negative patients not receiving adjuvant chemotherapy. The objective is to show that ctDNA negative patients have a sufficiently good outcome to not have been able to receive sufficient benefit from chemotherapy. This design requires substantially less patients, but is predicated on baseline relatively low risk.

MRD = Molecular Residual Disease; ctDNA = circulating tumor DNA; DFS = Disease Free Survival

Figure 2. Prospective trials of molecular residual disease and relapse detection to guide additional treatment after standard therapy.

2a. Randomized studies: Patients may be randomized to new treatment or placebo on the background of standard therapy, provided such therapies do not unblind randomization.

2b + 2c. Non-Randomized studies: Potential future studies may not need randomization if it is proven that MRD detection, and clearance, is a valid surrogate for DFS.

2c. MRD testing may also provide additional data on disease genotyping which may be utilized to select patients for targeted therapies.

MRD = Molecular Residual Disease; ctDNA = circulating tumor DNA; DFS = Disease Free Survival

Figure 3. MRD testing as a surrogate endpoint for accelerated approval

ctDNA analysis in follow-up after completion of a randomized adjuvant trial, may provide an early readout for accelerated approval. Decreased rates of MRD with new treatment, may provide sufficiently robust surrogate of improved DFS to warrant accelerated approval.

MRD = Molecular Residual Disease

References

1. Steeg PS. Targeting metastasis. Nat Rev Cancer. 2016;16(4):201-18.
2. Pondé NF, Zardavas D, Piccart M. Progress in adjuvant systemic therapy for breast cancer. Nature Reviews Clinical Oncology. 2018:1-18.
3. NSCLC Meta-analyses Collaborative Group, Arriagada R, Auperin A, Burdett S, Higgins JP, Johnson DH, *et al.* Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. Lancet 2010 375:1267–77.
4. von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, *et al.* Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. New England Journal of Medicine. 2017;377(2).
5. ClinicalTrials.gov. I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer (I-SPY 2); ClinicalTrials.gov Identifier: NCT01042379 https://clinicaltrials.gov/ct2/show/NCT01042379 2018 [updated October 31, 2018.
6. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, *et al*. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. N Engl J Med. 2018;378(21):1976-86.
7. von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, *et al.* Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. New England Journal of Medicine. 2018.
8. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, *et al.* A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer. New England Journal of Medicine. 2004;351:2817-26.
9. Sørensen CG, Pommergaard HC , Burcharth J, Rosenberg J. The diagnostic accuracy of carcinoembryonic antigen to detectcolorectal cancer recurrencee. A systematic review. International Journal of Surgery. 2016;25:134-44.
10. Senkus E, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, Zackrisson S., *et al.* Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2015; 26(Supplement 5): v8-v30.
11. Rosselli Del Turco M, Palli D, Cariddi A, Ciatto S, Pacini P, Distante V. Intensive Diagnostic Follow-up After Treatment of Primary Breast Cancer. A Randomized Trial. JAMA. 1994;271(20):1593-7.
12. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, *et al.* Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223-38.

13. Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Australian Medical Journal. 1869;14:146–7.
14. Leon SA, Shapiro B, Sklaroff DM, and Yaros MJ. Free DNA in the Serum of Cancer Patients and the Effect of Therapy. Cancer Research. 1977;37:646-50.
15. Sorenson GD, Pribish DM, Valone FH, Memoli VA, Bzik DJ, Yao SL. Soluble Normal and Mutated DNA Sequences from Single-Copy Genes in Human Blood. Cancer Epidemiology, Biomarkers and Prevention. 1994;3:67-71.
16. Corcoran RB, Chabner BA. Application of Cell-free DNA Analysis to Cancer Treatment. New England Journal of Medicine. 2018;379(18):1754-65.
17. Hrebien S, O'Leary B, Beaney M, Schiavon G, Fribbens C, Bhambra A, *et al*. Reproducibility of Digital PCR Assays for Circulating Tumor DNA Analysis in Advanced Breast Cancer. PLoS One. 2016;11(10):e0165023.
18. Kang Q, Henry NL, Paoletti C, Jiang H, Vats P, Chinnaiyan AM, *et al.* Comparative analysis of circulating tumor DNA stability In K3EDTA, Streck, and CellSave blood collection tubes. Clinical Biochemistry. 2016;49:1354-60.
19. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(23):9530-5.
20. Bettegowda C, Sausen M, Leary R, Kinde I, Wang Y, Agrawal N, *et al.* Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. Science Translational Medicine. 2014;6(224).
21. Newman AM, Bratman SV, To J, Wynne JF, Eclov NCW, Modlin LA, *et al.* An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nature Medicine. 2014;20(5):552-8.
22. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, *et al.* Integrated digital error suppression for improved detection of circulating tumor DNA. Nature Biotechnology. 2016;34(5):547-55.
23. Volik S AM, Morin RD, Collins C. Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies. Molecular Cancer Research. 2016;14(10):898-908.
24. Thress KS, Brant R, Carr TH, Dearden S, Jenkins S, Brown H, *et al.* EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. Lung Cancer. 2015;90:509-15.
25. Xu T, Kang X, You X, Dai L, Tian D, Yan W, *et al.* Cross-Platform Comparison of Four Leading Technologies for Detecting EGFR Mutations in Circulating Tumor DNA from Non-Small Cell Lung Carcinoma Patient Plasma. Theranostics. 2016;7(6):1437-46

26. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, *et al.* Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. Journal of Clinical Oncology. 2018;36(16).

27. Chan ATC, Lo YMD, Zee B, Chan LYS, Ma BBY, Leung SF, *et al.* Plasma Epstein-Barr virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. Journal of the National Cancer Institute. 2002;94(21):1614-9.

28. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, *et al.* Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985-90.

29. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, *et al.* Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92.

30. Overman MJ, Vauthey JN, Aloia TA, Conrad C, Chun YS, Pereira AAL, *et al.* Circulating tumor DNA (ctDNA) utilizing a high-sensitivity panel to detect minimal residual disease post liver hepatectomy and predict disease recurrence. Journal of Clinical Oncology. 2017;35(no.15_suppl):3522

31. Schøler LV, Reinert T, Ørntoft MW, Kassentoft CG, Árnadóttir SS, Vang S, *et al.* Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clincal Cancer Research. 2017;23(18):5437-45.

32. Diehn M, Alizadeh AA, Adams HP, Lee JJ, Klassen S, Palma JF, *et al.* Early prediction of clinical outcomes in resected stage II and III colorectal cancer (CRC) through deep sequencing of circulating tumor DNA (ctDNA). Journal of Clinical Oncology. 2017;35(No. 15_suppl):3591

33. Tie J, Cohen J, Wang YX, Li L, Kinde I, Elsaleh H, *et al.* Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut. 2018;0:1-9.

34. Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, *et al.* Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med. 2015;7(302):302ra133.

35. Pietrasz D, Pecuchet N, Garlan F, Didelot A, Dubreuil O, Doat S, *et al.* Plasma Circulating Tumor DNA in Pancreatic Cancer Patients Is a Prognostic Marker. Clinical Cancer Research. 2017;23(1):116-23.

36. Lee B, Lipton LR, Cohen J, Tie J, Javed AA, Li L. Circulating tumor DNA as a prognostic biomarker in early stage pancreatic cancer. Journal of Clinical Oncology. 2018;36(15).

37. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, *et al*. Early Detection of Molecular Residual Disease in Localized Lung Cancer by Circulating Tumor DNA Profiling. Cancer Discovery. 2017;7(12):1394-403.

38. Lo DYM, Chan LY, Chan AT, Leung SF, Lo KW, Zhang J, *et al.* Quantitative and Temporal Correlation between Circulating Cell-Free Epstein-Barr Virus DNA and Tumor Recurrence in Nasopharyngeal Carcinoma1. Cancer Research. 1999;59:5452-5.

39. Olsson E, Winter C, George A, Chen Y, Howlin J, Tang MH, *et al.* Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med. 2015;7(8):1034-47.

40. Sausen M, Phallen J, Adleff V, Jones S, Leary RJ, Barrett MT, *et al.* Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun. 2015;6:7686.

41. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, *et al.* Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017 545(7655):446-51.

42. Reinert T, Scholer LV, Thomsen R, Tobiasen H, Vang SR, Nordentoft I, *et al.* Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. Gut. 2016;65(4):625-34.

43. Ng SB, Chua C, Ng M, Gan A, Poon PS, Teo M, *et al.* Individualised multiplexed circulating tumour DNA assays for monitoring of tumour presence in patients after colorectal cancer surgery. Scientific Reports. 2017;7:40737.

44. Tie J, Cohen J, Wang YX, Lee M, Wong R, Kosmider S. Serial circulating tumor DNA (ctDNA) analysis as a prognostic marker and a real-time indicator of adjuvant chemotherapy (CT) efficacy in stage III colon cancer (CC). Journal of Clinical Oncology. 2018;36(15):3516-3516

45. Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, et al. Somatic mutant clones colonize the human esophagus with age. Science. 2018;362:911-7.

46. Cabel L, Proudhon C, Gortais H, Loirat D, Coussy F, Pierga JY, *et al.* Circulating tumor cells: clinical validity and utility. International Journal of Clinical Oncology; Tokyo 2017;22(3):421-30.

47. Rack B, Schindlbeck C, Jückstock J, Andergassen U, Hepp P, Zwingers T, *et al.* Circulating tumor cells Predict Survival in early Average-to-High risk Breast cancer Patients. Journal of the National Cancer Institute. 2014;106(5).

48. Sparano J, O Neill A, Alpaugh K, Wolff AC, Northfelt DW, Dang CT, *et al.* Association of Circulating Tumor Cells With Late Recurrence of Estrogen Receptor–Positive Breast Cancer. A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncology. 2018;4(12):1700-6.

49. Dawson SJ, Tsui DWY, Murtaza M, Biggs H, Rueda OM, Chin SF, *et al.* Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer. New England Journal of Medicine. 2013; 368:1199-209.

50. Bidard FC, Mariani MJ, Mariani P, Piperno-Neumann S, Rampanou A, Servois V, *et al.* Detection rate and prognostic value of circulating tumor cell sand circulating tumor DNA in metastatic uveal melanoma. Int J Cancer. 2014;134:1207-13.

51. Punnoose EA, Atwal S, Liu WQ, Raja R, Fine BM, Hughes BGM, *et al.* Evaluation of Circulating Tumor Cells and Circulating Tumor DNA in Non-Small Cell Lung Cancer: Association with Clinical Endpoints in a Phase II Clinical Trial of Pertuzumab and Erlotinib. Clinical Cancer Research. 2012;18(8):2391-401.

52. Hazar-Rethinam M, Kleyman M, Han GC, Liu D, Ahronian LG, Shahzade HA, *et al.* Convergent Therapeutic Strategies to Overcome the Heterogeneity of Acquired Resistance in BRAFV600E Colorectal Cancer. Cancer Discovery. 2018;8(4):417-27.

53. Shaw JA, Guttery DS, Hills A, Fernandez-Garcia D, Page K, Rosales BM, *et al.* Mutation Analysis of Cell-Free DNA and Single Circulating Tumor Cells in Metastatic Breast Cancer Patients with High Circulating Tumor Cell Counts. Clincal Cancer Research. 2016;23(1):88-96.

54. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, *et al.* Detection of mutations in EGFR in circulating lung-cancer cells. New England Journal of Medicine. 2008;359(4):366-77.

55. Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, *et al.* Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer. 2009;100(10):1603-7.

56. Mitchell PJ, Welton J, Staffurth J, Court J, Mason MD, Tabi Z, *et al.* Can urinary exosomes act as treatment response markers in prostate cancer? Journal of Translational Medicine. 2009;7(4).

57. Sestini S, Boeri M, Marchiano A, Pelosi G, Galeone C, Verri C, *et al.* Circulating microRNA signature as liquid-biopsy to monitor lung cancer in low-dose computed tomography screening. Oncotarget. 2015;6(32):32868-77.

58. Best MG, Sol N, In ‘t Veld SGJG, Vancura A, Muller M, Niemeijer AN, *et al.* Swarm Intelligence-Enhanced Detection of Non-Small-Cell Lung Cancer Using Tumor-Educated Platelets. Cancer Cell. 2017;32:238-52.

59. Takahashi H, Kagara N, Tanei T, Naoi Y, Shimoda M, Shimomura A, *et al.* Correlation of Methylated Circulating Tumor DNA With Response to Neoadjuvant Chemotherapy in Breast Cancer Patients. Clinical Breast Cancer. 2016;17(1):61-9.

60. Wallner M, Herbst A, Behrens A, Crispin A, Stieber P, Goke B, *et al.* Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clinical Cancer Research. 2006;12(24):7347-52.

61. Haldrup C, Pedersen AL, Ogaard N, Strand SH, Hoyer S, Borre M, *et al.* Biomarker potential of ST6GALNAC3 and ZNF660 promoter hypermethylation in prostate cancer tissue and liquid biopsies. Molecular Oncology. 2018;12(4):545-60.

62. **NHMRC**. Circulating tumour DNA (ctDNA) analysis informing adjuvant chemotherapy in Stage II Colon Cancer; A study to evaluate the use of circulating tumour DNA to guide adjuvant chemotherapy on recurrence-free survival in patients with stage II Colon or rectal cancer; ACTRN12615000381583 2018 updated 18 July 2018. Available from: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12615000381583.

63. Institute of Cancer Research UK. A Trial Using ctDNA Blood Tests to Detect Cancer Cells After Standard Treatment to Trigger Additional Treatment in Early Stage Triple Negative Breast Cancer Patients (c-TRAK-TN); NCT03145961 clinicaltrials.gov2018 updated 30 October 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT03145961.

64. Rustin GJ, van der Burg ME, Griffin CL, Guthrie D, Lamont A, Jayson GC, *et al.* Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. The Lancet 2010;376(9747):1155-63.

65. Parker C, Gillessen S, Heidenreich A, Horwich A. Cancer of the prostate: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2015; 26(Supplement 5):v69-v77.

66. Madan RA, Karzai F, Bilusic M, Strauss J, Slovin SF, Harshman LC, *et al.* Immunotherapy for biochemically recurrent prostate cancer. Journal of Clinical Oncology. 2018;36(no. 6_suppl):215-215.

67. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, *et al*. Osimertinib or Platinum–Pemetrexed in EGFR T790M–Positive Lung Cancer. New England Journal of Medicine. 2017;376(7):629-40.

68. Kemp R, Prasad V. Surrogate endpoints in oncology: when are they acceptable for regulatory and clinical decisions, and are they currently overused? BioMed Central. 2017;15(134).

69. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research. Guidance for Industry. Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval: Food and Drug Administration; 2014 Available from:
https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm305501.pdf

70. Gokbuget N, Dombret H, Bonifacio M, Reichle A, Graux A, Faul C, *et al*. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood. 2018;131(14):1522-31.

71. Jen EY, Xu Q, Schetter A, Przepiorka D, Shen YL, Roscoe D, *et al*. FDA Approval: Blinatumomab for Patients with B-cell Precursor Acute Lymphoblastic Leukemia in Morphologic Remission with Minimal Residual Disease. Clinical Cancer Research. 2018:1-5.

72. O'Leary B, Hrebien S, Morden JP, Beaney M, Fribbens C, Huang X, *et al.* Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nature Communications. 2018;9(896).

73.

Garlan F, Laurent-Puig P, Sefrioui D, Siauve N, Didelot A, Sarafan-Vasseur N, *et al*. Early Evaluation of Circulating Tumor DNA as Marker of Therapeutic Efficacy in Metastatic Colorectal Cancer Patients (PLACOL Study). Clinical Cancer Research. 2017;23(18):5416-25.

74. Cortazar P, hang L, Untch M, Mehta K, Costantino JP, Wolmark N,*et al.* Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. The Lancet. 2014;384(9938):164-72.

75. Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, *et al.* Enhanced detection of circulating tumor DNA by fragment size analysis. Science Translational Medicine. 2018;10(466).

76. Cohen JD, Li L, Wang YX, Thoburn C, Afsari B, Danilova L, *et al.* Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926

Table 1; Molecular Residual Disease (MRD)

Tumor type	***Study, Number of patients (n)***	***Clinical Outcomes in patients with ctDNA detected post treatment compared to ctDNA patients with no ctDNA detected***	***Reported data on accuracy of ctDNA testing for detecting MRD***
Nasopharyngeal Carcinoma	***Chan ATC, et al. 2002.*** (27) n=170(27)	High versus low EBV DNA: PFS at 1yr 48% versus 93%. RR for recurrence 11.9	Negative predictive value 83% Positive Predictive value 87%
Colorectal Carcinoma	***Diehl F, et al. 2008.(28)*** n=18	Recurrence Rate with/without ctDNA statistically significant (p=0.006)	100% sensitive 15/16 patients with ctDNA detected recurred
	Tie J, et al 2016. (29) n=230	***No adjuvant chemotherapy group***: RFS at 3 years 0% versus 90% HR for recurrence 18. ***Adjuvant chemotherapy group following completion of chemotherapy:*** HR for recurrence 11.	Sensitivity for recurrence at 36 months 48% Specificity 100% (in patients not treated with chemotherapy)
	Diehn M, et al. 2017. (32) n=145	2-year DFS 17% versus 88%. HR for recurrence 10.3 OS HR 3.4	92% of ctDNA detected patients recurred 7% of ctDNA not detected patients recurred
	Overman MJ, et al. 2017. (30) n = 54	2-year RFS 0% versus 47%. HR for recurrence 3.1	Sensitivity 58% Specificity 100%
	Schøler LV, et al. 2017. (31) n=27	RFS at 3 years 0% versus 73% HR for recurrence 37.7 5-year OS HR 6.7	Sensitivity 100% Specificity 100%
Rectal Carcinoma	***Tie J, et al. 2018.*** (33) n=159	RFS at 3 years 33% versus 87% HR 13.0	11/19 (58%) with ctDNA detected recurred 12/140 (8.6%) ctDNA not detected recurred
Breast Carcinoma	***Garcia-Murillas I, et al. 2015.*** (34) n=55	DFS 6.5 months versus NR. HR 25.1	6/12 (50%) patients relapsed had ctDNA detected. 96% of patients who did not relapse did not have ctDNA detected.
Pancreatic Carcinoma	***Pietrasz D., et al. 2016.*** (35) n=31	DFS 4.6 versus 17.6 months OS 19.3 versus 32.2 months	4/6 patients with ctDNA detected relapsed
	Lee B., et al. 2018. (36) n = 42	DFS 5.4 versus 17.1 months HR 5.4 OS 10.6 versus NR months HR 4.5	Recurrence occurred in 13/13 patients with detectable ctDNA despite over half receiving chemotherapy
Lung Carcinoma	***Chaudhuri AA, et al. 2017.*** (37) n=40	DFS at 36 months of 0% versus 93%. HR 43.4	One patient who recurred did not have ctDNA detected 100% specificity

Table 2; Molecular Relapse (MR)

Tumor type	***Study, number of patients (n)***	***Lead time reported***	***Reported data on accuracy of serial ctDNA testing for detecting MR***
Nasopharyngeal Carcinoma	***Lo DYM, et al. 1999.*** (38) n=17	Up to 6 months	100% sensitivity and specificity
Breast Carcinoma	***Garcia-Murillas I, et al. 2015.*** (34). n=55	Median 7.9 months	12/15 (80%) ctDNA detected and 96% of patients who did not relapse did not have ctDNA detected
	Olsson E, et al. 2015. (39) n=20	Median 11 months	93% sensitivity; 100% specificity
Pancreatic Carcinoma	***Sausen M, et al. 2015.*** (40) n=51	Median 6.5 months	NA
Lung Carcinoma	***Abbosh CBN, et al. 2017.*** (41) n=24	Median 70 days	13/14 (93%) of patients had ctDNA at or before clinical relapse. 1/10 (10%) patients ctDNA detected without evidence of relapse
	Chaudhuri AA et al. 2017. (37) n=40	Median 5.2 months	100% specificity
Colorectal Carcinoma	***Tie J, et al. 2016.*** (29) n=230	Median 167 days	23/27 positive ctDNA at time of recurrence
	Reinert t, et al. 2016. (42) n=11	Median 10 months	100% sensitivity, 100% specificity
	Overman MJ, et al. 2017. (30) n=54	Median 5.1 months	NA
	Ng SB, et al. 2017. (43) n=13	Up to 255 days	11/15 cases with ctDNA detected had ctDNA detected prior to recurrence 1/12 patients persistent ctDNA detected who did not recur

ctDNA = circulating tumor DNA; EBV = Epstein Barr Virus; PFS = Progression Free Survival; RR= Risk ratio; HR = Hazard Ratio; DFS = Disease Free Survival; RFS = Recurrence Free Survival; OS = Overall Survival. For full data, including confidence intervals of HR provided and assays utilized the reader is advised to refer to the original texts referenced.

Fig 1

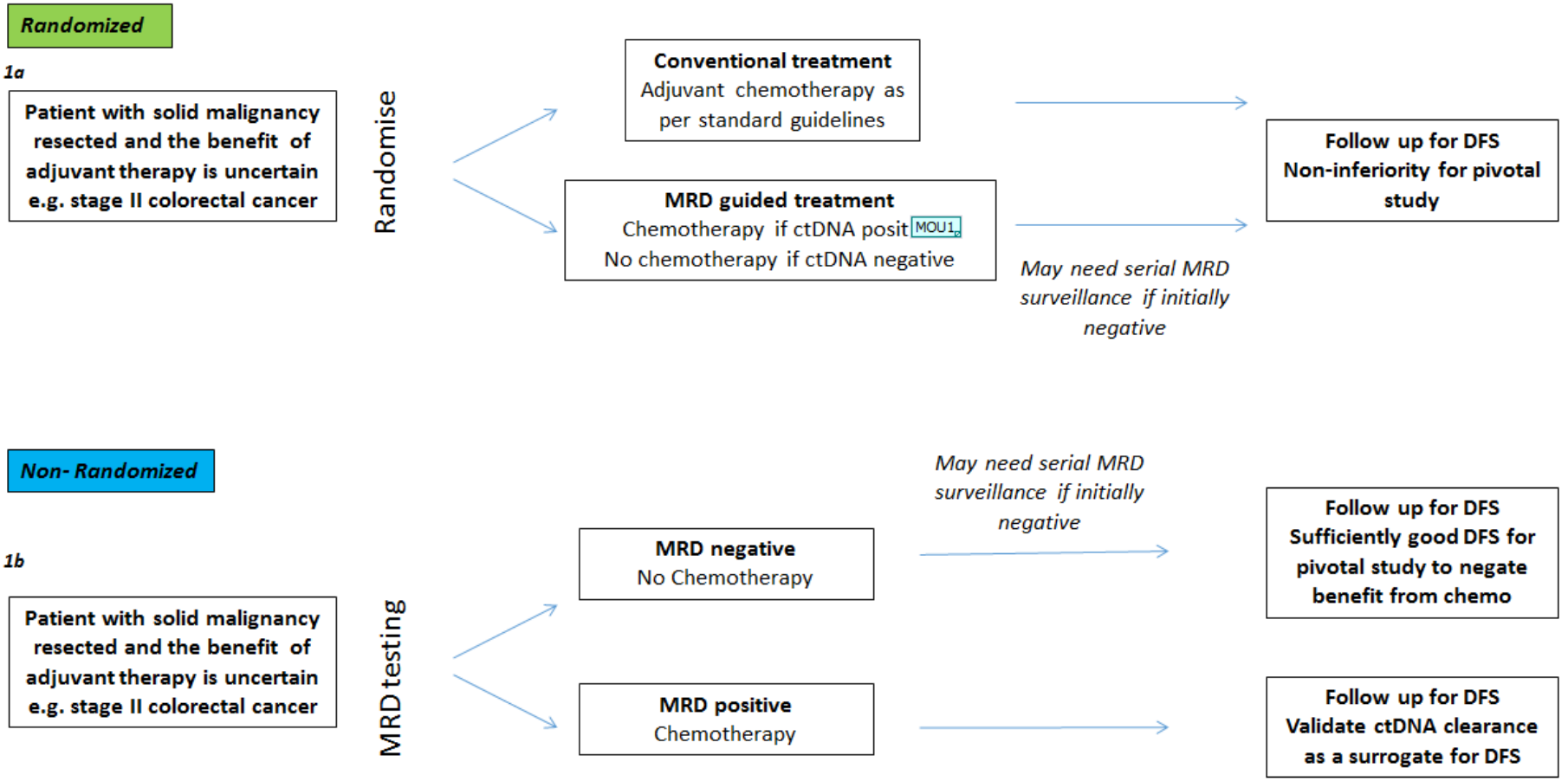

Randomized
1a
Patient with solid malignancy resected and the benefit of adjuvant therapy is uncertain e.g. stage II colorectal cancer
Randomise
Conventional treatment
Adjuvant chemotherapy as per standard guidelines
MRD guided treatment
Chemotherapy if ctDNA posit
No chemotherapy if ctDNA negative
Follow up for DFS
Non-inferiority for pivotal study
May need serial MRD surveillance if initially negative
Non- Randomized
1b
Patient with solid malignancy resected and the benefit of adjuvant therapy is uncertain e.g. stage II colorectal cancer
MRD testing
MRD negative
No Chemotherapy
MRD positive
Chemotherapy
May need serial MRD surveillance if initially negative
Follow up for DFS
Sufficiently good DFS for pivotal study to negate benefit from chemo
Follow up for DFS
Validate ctDNA clearance as a surrogate for DFS

Fig 2

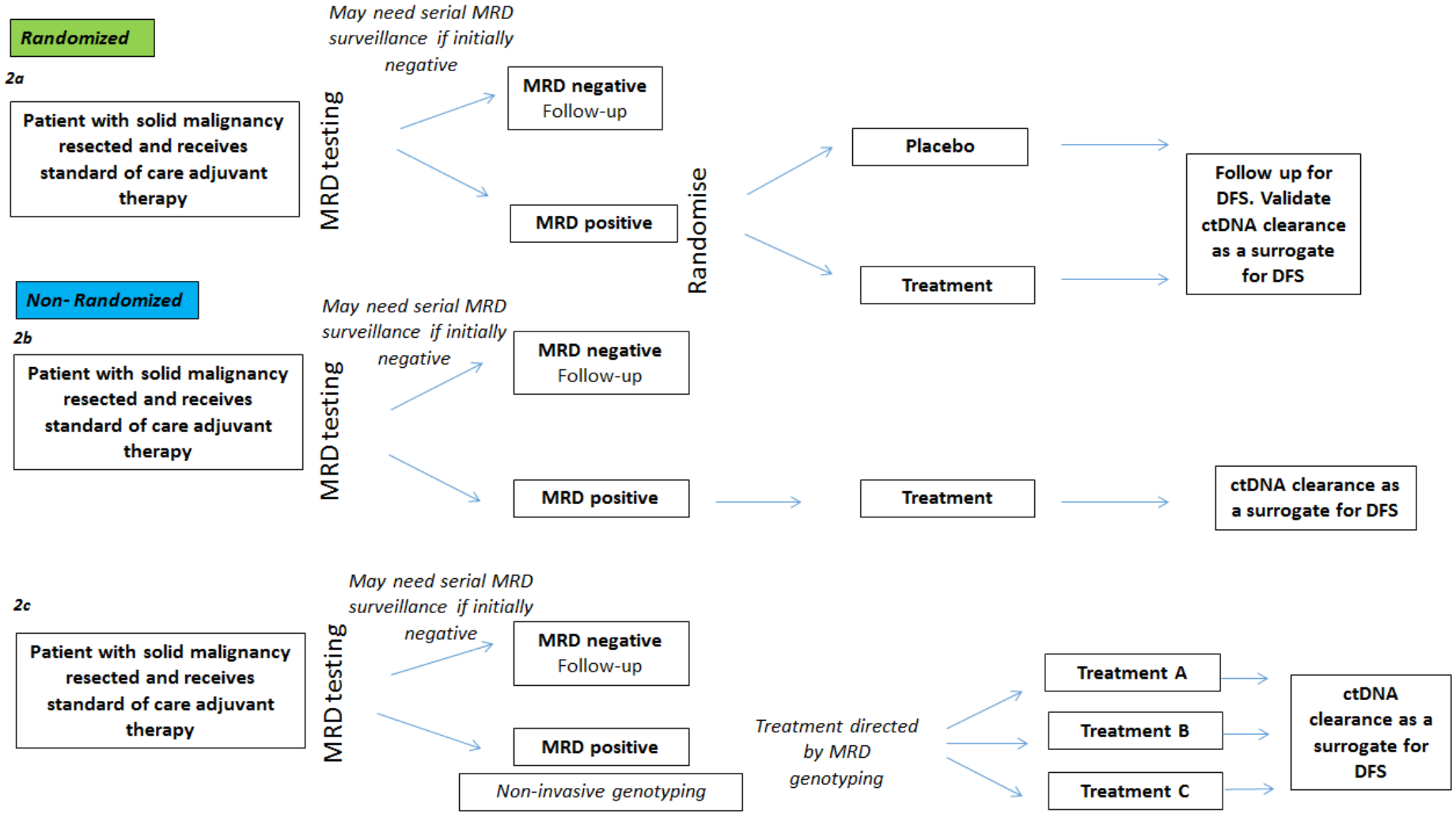
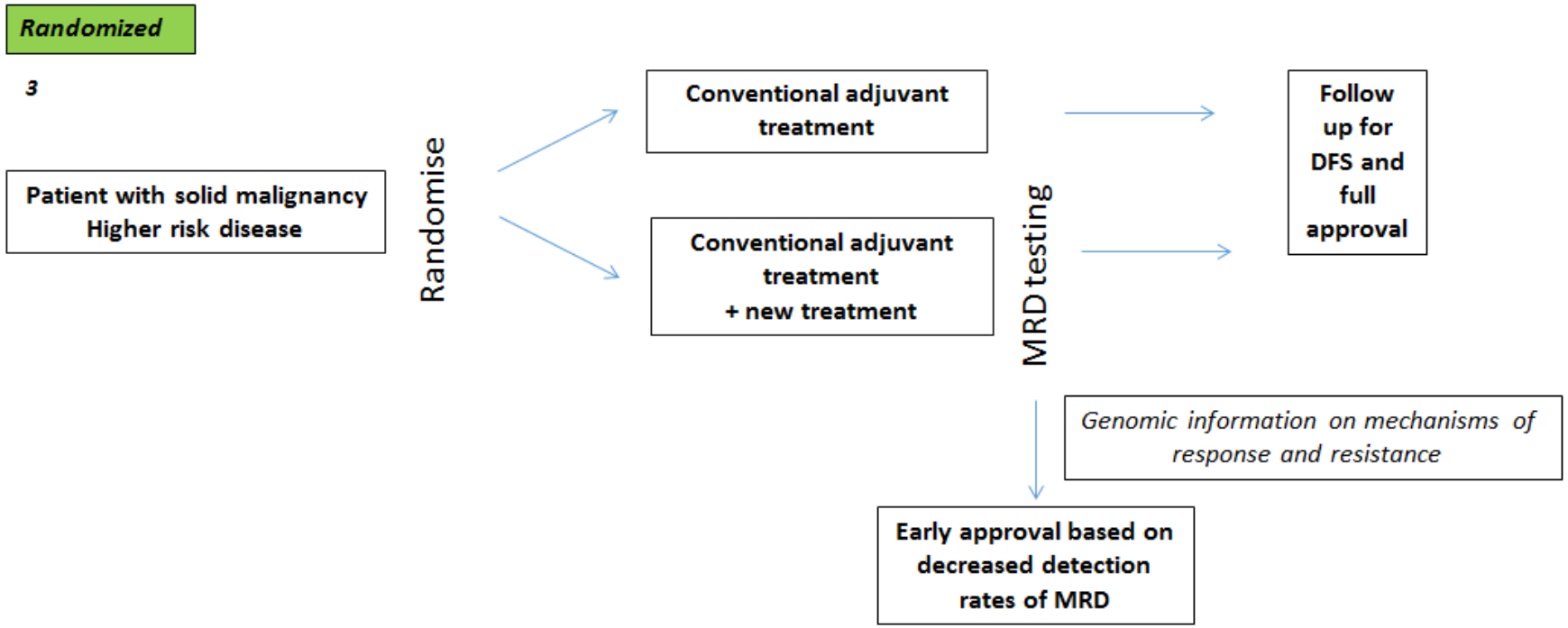

Randomized
2a
Patient with solid malignancy resected and receives standard of care adjuvant therapy
MRD testing
May need serial MRD surveillance if initially negative
MRD negative
Follow-up
MRD positive
Randomise
Placebo
Treatment
Follow up for DFS. Validate ctDNA clearance as a surrogate for DFS
Non- Randomized
2b
Patient with solid malignancy resected and receives standard of care adjuvant therapy
MRD testing
May need serial MRD surveillance if initially negative
MRD negative
Follow-up
MRD positive
Treatment
ctDNA clearance as a surrogate for DFS
2c
Patient with solid malignancy resected and receives standard of care adjuvant therapy
MRD testing
May need serial MRD surveillance if initially negative
MRD negative
Follow-up
MRD positive
Non-invasive genotyping
Treatment directed by MRD genotyping
Treatment A
Treatment B
Treatment C
ctDNA clearance as a surrogate for DFS

Fig 3


Randomized
3
Patient with solid malignancy
Higher risk disease
Randomise
Conventional adjuvant treatment
Conventional adjuvant treatment
+ new treatment
MRD testing
Follow up for DFS and full approval
Genomic information on mechanisms of response and resistance
Early approval based on decreased detection rates of MRD