Review

Clinical Cancer Research

Molecular Residual Disease and Adjuvant Trial Design in Solid Tumors

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

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 before 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 4,805 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 (CTC) in a post-mortem (13). Subsequently, in the 1970s Leon and colleagues (14) reported high levels of circulating-free DNA (free DNA) in the serum of patients with cancer, 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.

[1]Breast Cancer Now Research Centre, Institute of Cancer Research, London, United Kingdom. [2]Breast Unit, Royal Marsden Hospital, Fulham Road, London, United Kingdom.

Corresponding Author: Nicholas C. Turner, Institute of Cancer Research and The Royal Marsden Hospital, London, SW3 6JB, UK. Phone: 207-153-5574; E-mail: Nick.Turner@icr.ac.uk

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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 multicenter 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 article 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 affecting 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 before 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) DFS is significantly reduced and the hazard ratio (HR) for recurrence is significantly elevated (HR, 3.1–43.4 across studies), compared with 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; refs. 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

Table 1. Summary of studies reporting ctDNA detection of Molecular Residual Disease (MRD) with corresponding clinical outcomes and accuracy data reported by the study authors

Tumor type	Study, number of patients (*n*)	Clinical outcomes in patients with ctDNA detected post treatment compared to patients with no ctDNA detected	Reported data on accuracy of ctDNA for detecting MRD
Nasopharyngeal carcinoma	Chan ATC, et al. (2002) (27), $n = 170$	High versus low EBV DNA; 1-year PFS 48% versus 93%; RR for recurrence 11.9	Positive predictive value 87% Negative predictive value 83%
Colorectal carcinoma	Diehl F, et al. (2008) (28), $n = 18$	Recurrence rate with or without ctDNA detected statistically significant (P=0.006)	Sensitivity 100% 15/16 patients with ctDNA detected recurred
	Tie J, et al. (2016) (29), $n = 230$	*No adjuvant chemotherapy group:* 3-year RFS 0% versus 90%; HR for recurrence 18 *Adjuvant chemotherapy group:* HR for recurrence 11	Sensitivity 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; HR for OS 3.4	92% of patients with ctDNA detected recurred 7% of patients without ctDNA detected recurred
	Overman MR, 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), $n = 27$	3-year RFS 0% versus 73%; HR for recurrence 37.7; HR for 5-year OS 6.7	Sensitivity 100% Specificity 100%
Rectal carcinoma	Tie J, et al. (2018) (33), $n = 159$	3-year RFS 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 Not Reached; HR 25.1	6/12 (50%) patients who relapsed had ctDNA detected 96% of patients who did not relapse did not have ctDNA detected
Pancreatic carcinoma	Peitrasz D, et al. (2016) (35), $n = 31$	DFS 4.6 versus 17.6 months OS 19.3 versus 32.3 months	4/6 patients with ctDNA detected relapsed
	Lee B, et al. (2017) (36), $n = 42$	DFS 5.4 versus 17.1 months; HR 5.4 OS 10.6 months versus Not Reached; HR 4.5	Recurrence occurred in 13/13 patients with ctDNA detected despite over half receiving chemotherapy.
Lung carcinoma	Chaudhuri AA, et al. (2017) (37), $n = 40$	3-year DFS 0% versus 93%; HR 43.4	1 patient who recurred did not have ctDNA detected 100% specificity

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Table 2. Summary of studies reporting ctDNA detection of molecular relapse (MR) with lead times and accuracy data reported by the study authors

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%) of relapses had 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 ctDNA detected 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 before recurrence 1/12 patients persistent ctDNA detected who did not recur.

Abbreviations: ctDNA, circulating tumor DNA; DFS, disease-free survival; EBV, Epstein Barr Virus; HR, hazard ratio; NA = Not Applicable; n = number of patients; OS, Overall Survival; PFS, progression-free survival; RFS, Recurrence Free Survival; RR, risk ratio. For full data, including confidence intervals of HR provided and assays used the reader is advised to refer to the original texts referenced.

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 (refs. 29, 30, 42, 43; Table 2).

ctDNA has been shown to have superior sensitivity in detecting MR compared with 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 used 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 and molecular relapse. There is marked heterogeneity in these studies with investigators analyzing various tumor sites, sample sizes, investigative points, assays and follow-up timepoints; along with numerous different outcomes measured and reported (Tables 1 and 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% and 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, that is, 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 to 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, for example, Central Nervous System (CNS) ctDNA in the plasma may be undetectable. In future, for patients who are particularly high-risk,

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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. Multiple techniques have been developed for assaying CTCs; however, the most widely used method in clinical research is the FDA approved CellSearch system that 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, before 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 with 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 (TEP; ref. 58), and ctDNA methylation status (59–61) that 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:

(i) 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, whereas patients without ctDNA detection derive clinically insignificant benefit.

(ii) 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 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 (Fig. 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 (Fig. 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 Tables 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 before consideration of a clinical trials designed to de-escalation therapy in high-risk disease.

Prospective trials of molecular residual disease or molecular relapse 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 (Fig. 2A). Treatments with characteristic adverse effects that unblind the randomization, may require all patients to undergo standard

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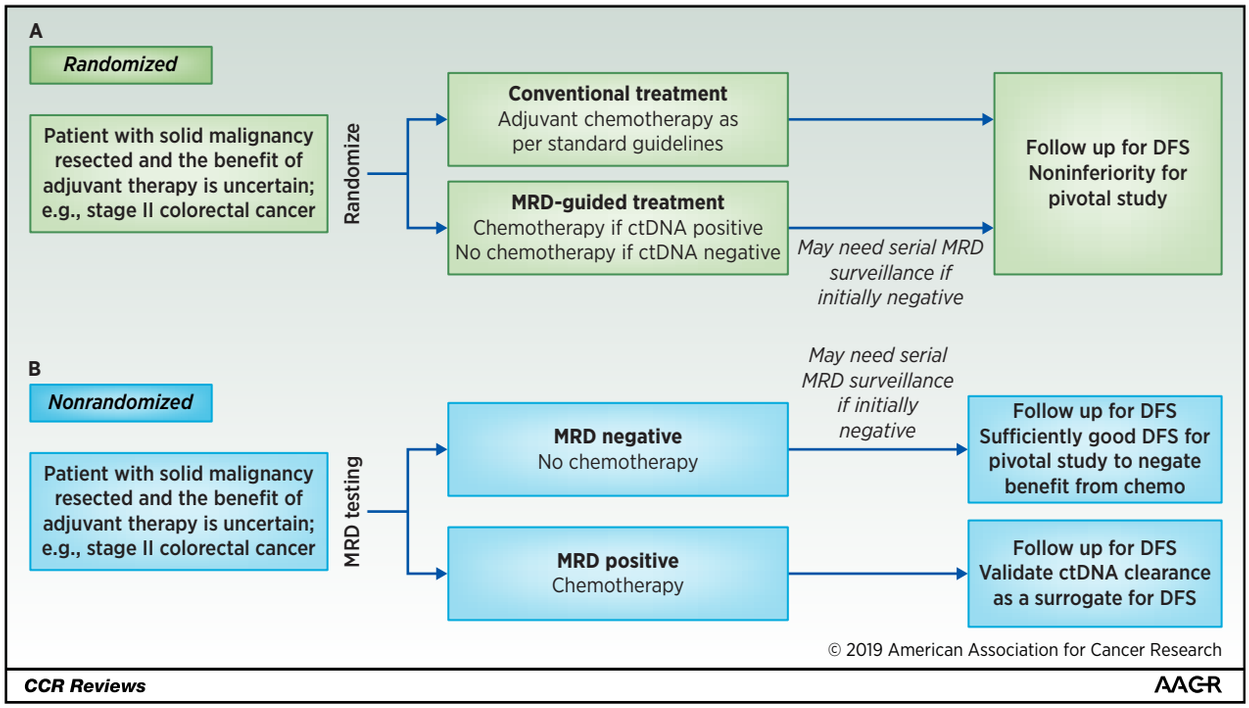

Figure 1.

Trial designs in Clinical scenarios where the benefit of adjuvant therapy is uncertain. **A,** Randomized design ctDNA guided therapy versus standard therapy. Such a design for a pivotal phase III study potentially requires a non-inferiority design. **B,** 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.

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 (Fig. 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; ref. 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

Because of 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 (Fig. 2C). Targeted therapy may subsequently be directed to the genomics of the residual disease, that may differ from the original primary due to intratumoral 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 before disease progression (Fig. 3).

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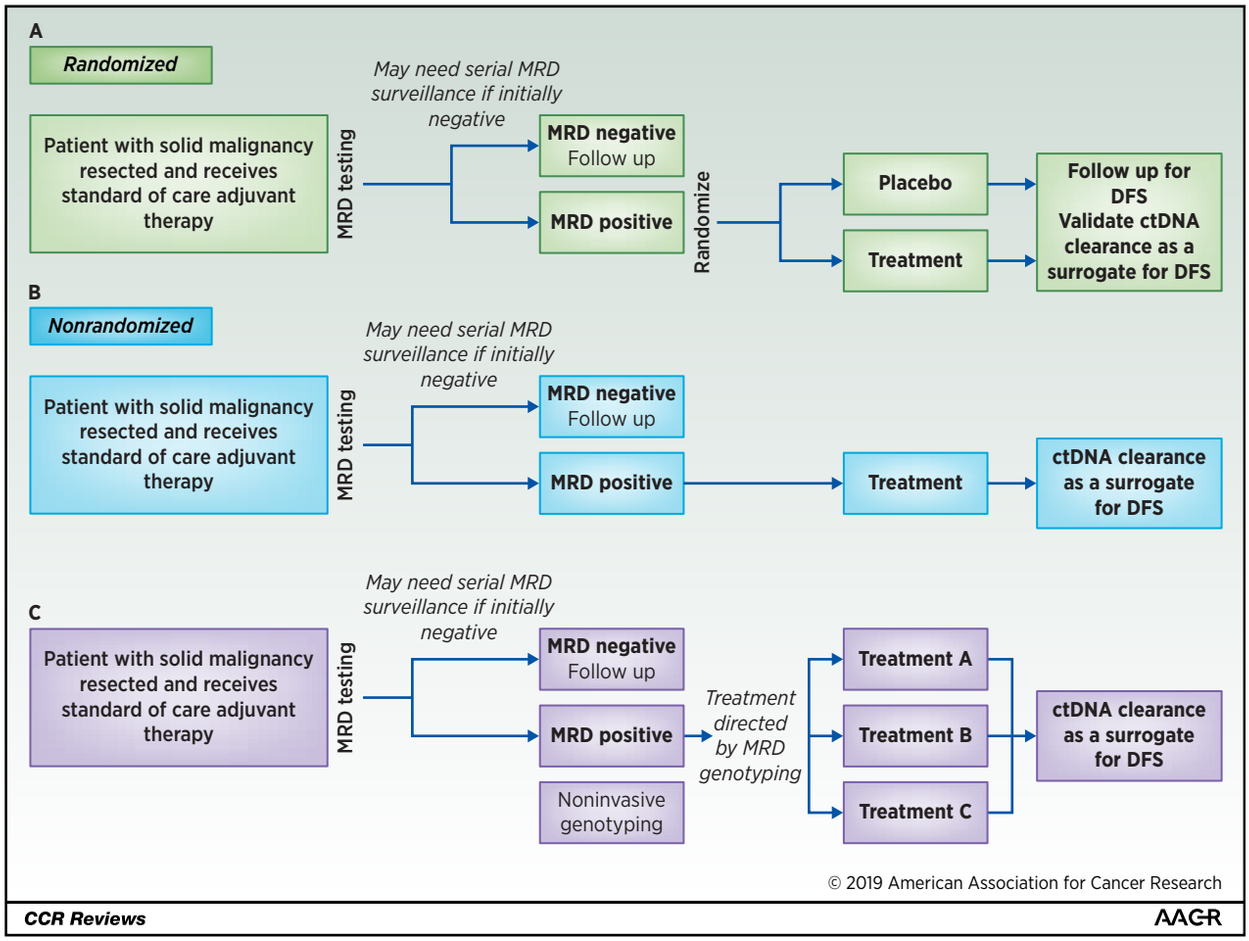

Figure 2.

Prospective trials of MRD and relapse detection to guide additional treatment after standard therapy. **A,** Randomized studies: Patients may be randomized to new treatment or placebo on the background of standard therapy, provided such therapies do not unblind randomization. **B** and **C,** 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. **C,** MRD testing may also provide additional data on disease genotyping which may be used to select patients for targeted therapies. MRD, molecular residual disease; ctDNA, circulating tumor DNA; DFS, disease-free survival.

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 used 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 with 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

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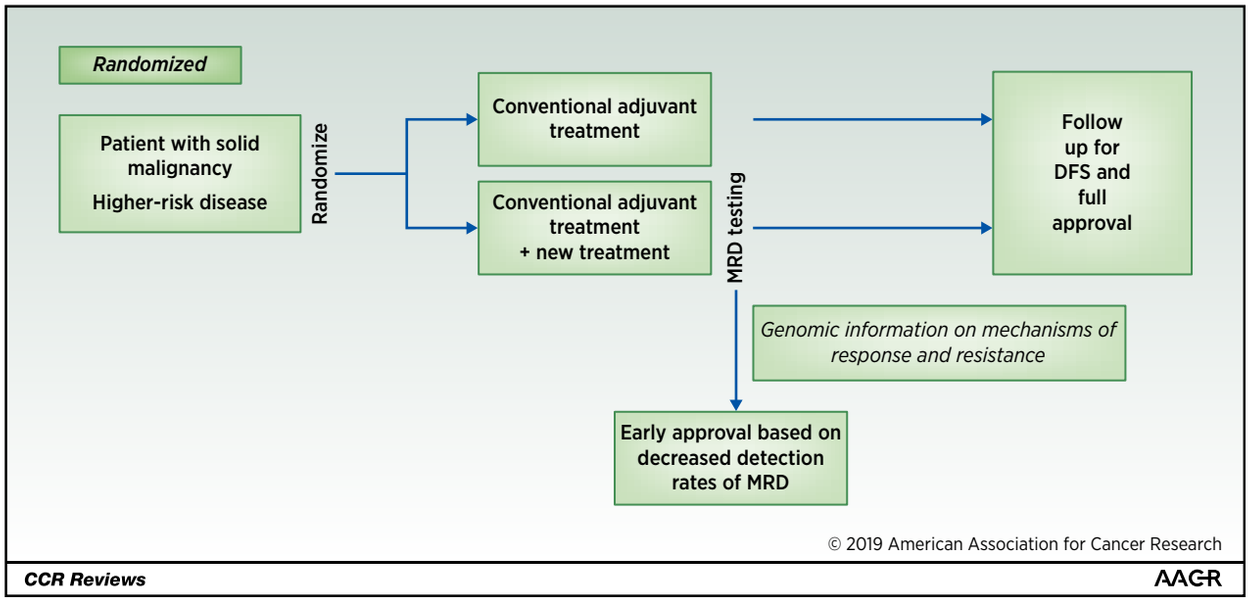

Figure 3.
MRD testing as a surrogate endpoint for accelerated approval. ctDNA analysis in follow-up after completion of a randomized adjuvant trial, may provide an early readout for accelerated approval. Decreased rates of MRD with new treatment, may provide sufficiently robust surrogate of improved DFS to warrant accelerated approval. MRD, molecular residual disease.

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 used 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; ref. 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, multicenter 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 before 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 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.

Disclosure of Potential Conflicts of Interest

N.C. Turner reports receiving commercial research grants from AstraZeneca, Bio-Rad, Pfizer, Roche/Genentech, Clovis, and Guardant Health, and is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Lilly, Merck Sharpe and Dohme, Novartis, Pfizer, Roche/Genentech, Tesaro, and Bicycle Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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