OncoTargets and Therapy

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REVIEW

Minimal Residual Disease Monitoring in Radically Treated Non-Small Cell Lung Cancer: Challenges and Future Directions

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Abstract: Circulating tumor DNA (ctDNA) analysis can identify patients with residual disease before it is clinically or radiologically evident. Minimal residual disease (MRD) is an advancing area in the management of radically treated solid tumors. Which MRD assay is optimum and when it should be used is still not defined. Whilst promising, the clinical utility of this technology to guide patient care is still investigational in non-small cell lung cancer (NSCLC) and has not entered routine care. Once technically and clinically optimized, MRD may be utilized to personalize adjuvant therapy, detect disease relapse earlier and improve cure rates. In this review, we discuss the current status of MRD monitoring in NSCLC by summarizing frequently used MRD assays and their associated evidence in NSCLC. We discuss the potential applications of these technologies and the challenge of demonstrating MRD clinical utility in trials.

Keywords: MRD, ctDNA, NSCLC, adjuvant therapy, minimal residual disease, non-small cell lung cancer, circulating tumor DNA

Background

Cell-free DNA (cfDNA) refers to fragments of DNA released into the circulation by endothelial cells and white blood cells by active release or passively through apoptosis and necrosis.[1,2] The fraction of cfDNA that originates from tumor is known as circulating tumor DNA (ctDNA). In 2016 the first commercial ctDNA test was approved for use in non-small cell lung cancer (NSCLC); the cobas EGFR mutation test, a real-time polymerase chain reaction (PCR) companion diagnostic test, was designed to identify advanced stage NSCLC patients eligible for treatment with erlotinib.[3] Since then, ctDNA use has exponentially increased in the management of advanced NSCLC.

In the advanced setting, evidence supports the use of multigene next generation sequencing (NGS) ctDNA assays upfront, concurrently, or sequentially with tissue biopsies to genotype newly diagnosed patients. ctDNA analysis is also advantageous throughout treatment. In multiple solid tumors, including NSCLC, a "molecular response" has been shown to correlate with superior clinical outcomes. This refers to a reduction in ctDNA variant allele frequency (VAF) following the initiation of treatment and may identify a response to therapy earlier than imaging.[4–13] In oncogene-addicted NSCLC, ctDNA analysis is frequently used at the point of disease progression to potentially identify any resistance mechanisms to aid next-line treatment selection.[14,15]

Furthermore, ctDNA VAF is prognostic in early NSCLC, stage I patients with preoperatively detectable ctDNA have an increased risk of recurrence compared to those with stage I disease with no ctDNA identified prior to resection.[16] This suggests the presence of detectable ctDNA in such patients may represent the existence of occult micro metastatic disease at the time of surgery. ctDNA can also be used in the surveillance of radically treated patients to detect minimal residual disease (MRD), i.e., molecular evidence of cancer soon after curative treatment. In contrast to the management of a metastatic patient, where ctDNA assessment is necessary to identify specific targetable alterations to guide therapy, in

Received: 1 August 2022
Accepted: 11 March 2023
Published: 7 April 2023

the radical setting it is the presence of ctDNA in any quantity, not quality, which is important. However, MRD identification is challenging. Assays need to be sufficiently sensitive to detect the small fraction of tumor-derived ctDNA among the abundant cfDNA from normal cells, risking false negative and false positive results. Moreover, even if MRD can be reliably assessed, it's assessment is not currently recommended by international guidelines as the clinical utility is yet to be sufficiently established, randomized trials are ongoing to define this. Once optimized, MRD status may be used to personalize adjuvant therapy, detect disease relapse earlier, and improve cure rates. In this review, we summarize frequently used MRD assays and their associated observational evidence in NSCLC. We discuss the potential applications of these technologies and the challenge of demonstrating MRD clinical utility in trials.

Minimal Residual Disease Assays

Tumor Genotype Informed and Tumor Genotype Uninformed Assays

Assays used in the detection of MRD can be divided into the tumor genotype informed and genotype uninformed (Figure 1). The tumor informed approach involves prior knowledge of a tumor genotype, via analysis of a tumor specimen and/or preoperative cfDNA, to develop and customize a panel of mutations for each individual patient, the unique panel is subsequently used to track the tumor-specific mutations longitudinally. This personalized approach of tracking tumor specific variants lowers the incidence of false positives from background noise of non-tumor variants. The requirement of sufficient tissue for assay development limits the use of these assays to patients with large tumor biopsy samples or resection specimens with adequate tumor DNA content and quality. These tumor informed assays are also constrained to the mutations detected in the utilized biopsy or resection sample and may not represent the genomic heterogeneity of the primary cancer. Consequently, if a tumor relapses with an alternative set of clonal genotypes to that under analysis, this approach may result in a false negative result due to failure to identify the new sub-clones. Another disadvantage of this approach is the long turnaround time and resources required to develop the personalized assays.

In contrast, the tumor agnostic or uninformed approach utilizes "off the shelf" panels designed to cover genes recurrently mutated in the subtype of cancer under analysis and are not patient specific. This approach has a higher risk of false positives due to technical and biological sources of error and is less reliable at detecting variants at a VAF of ≤0.5%.[17] However, unlike the tumor informed approach, these multigene panels allow for tumor heterogeneity and detection of evolving variants.[17] Table 1 summarizes of some of the available tumor genotype informed and tumor genotype naïve MRD assays, their technology, and limits of detection.

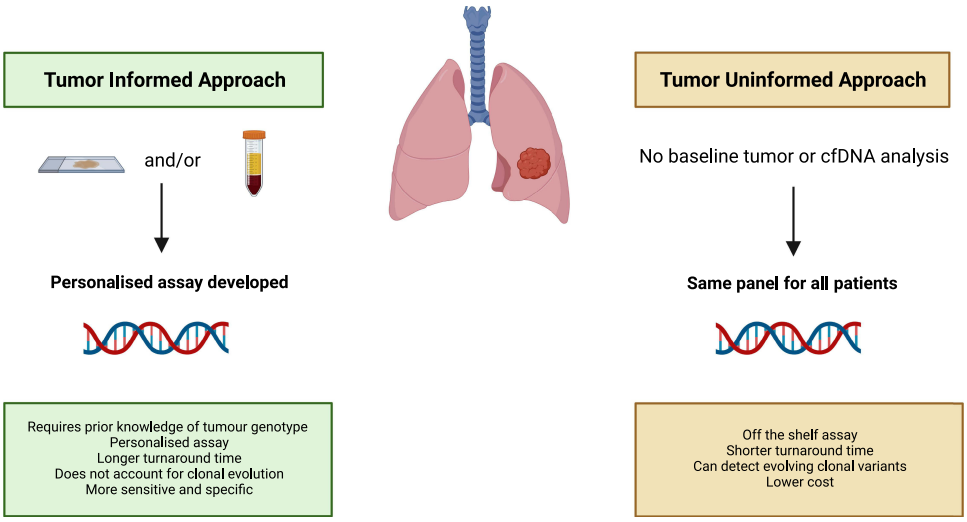

Figure 1 Tumor Informed and Tumor Uninformed Approaches for MRD Assessment. Comparison of tumor informed and tumor uninformed assays. Created with biorender.com.

Table 1 Assays to Detect Minimal Residual Disease

Assay	Method	Tumor Genotype Informed	Variants Assessed	Reported LOD	Published Validation Studies in NSCLC (n)
Signatera[19]	Multiplex PCR based NGS	Yes	Top 16 somatic SNVs and Indels	0.01% VAF	Abbosh et al (n = 100)[18]
RaDaR[20]	Multiplex PCR based NGS	Yes	SNVs, indels and CNAs	0.001% VAF	Gale (n = 88)[21]
CAPP-Seq[22]	Hybridization capture based NGS	Yes	SNVs	0.003% VAF	Chaudhuri et al (n = 37)[22] Moding et al (n = 65)[23] Jun et al (n = 39)[24] [Abstract only]
AVENIO Surveillance kit[27]	Hybridization capture based NGS	Yes	SNVs, indels, fusions and CNAs	0.1% VAF	Nil to date
PhasED-seq[28]	Hybridization capture based NGS	Yes	SNVs and Phased Variants	0.000094% VAF	Kurtz et al (n = 5)[28]
MRDetect[67]	WGS-based	Yes	SNVs and CNAs	0.001% VAF	Zviran et al (n = 22)[30] Tan et al (n = 52)[68] [Abstract only]
Guardant Reveal[33,69]	Hybrid capture based NGS and methylation	No	SNVs, indels and methylation	0.01% VAF	Nil to date
DELFI[36]	Fragmentomics	No	Fragment size	NA	Cristiano et al (n = 12)[36] Mathios et al (n = 46)[37]

Abbreviations: LOD, limit of detection; VAF, variant allele frequency; NGS, next generation sequencing; PCR, polymerase chain reaction; WGS, whole genome sequencing; CNAs, copy-number aberrations; indel, insertion or deletion; SNVs, single-nucleotide variants; CAPP-Seq, cancer personalized profiling by deep sequencing; DELFI, DNA evaluation of fragments for early interception; NA, not applicable.

Multiplex PCR Based NGS Assays: Signatera and RaDaR

TRACERx is a prospective study enrolling early NSCLC patients eligible for primary surgery (NCT01888601). Enrolled patients undergo longitudinal sampling with the objective of defining the relationship between intratumor heterogeneity and clinical outcome. In 2017 Abbosh et al published seminal ctDNA data regarding 100 patients with resectable stage I–IIIB NSCLC analyzed using the Signatera test.[18] Signatera uses a tumor informed PCR amplicon based NGS approach (Table 1). Whole exome sequencing is performed on a sample of tumor tissue, with paired whole blood sequencing to filter out germline variants and clonal hematopoiesis. Sixteen somatic variants are selected based on clonality, detectability and frequency. PCR primers are developed for each of the chosen 16 variants. During surveillance, cfDNA is analyzed using this personalized 16-plex PCR pool. The amplicon products are tagged with sequencing barcodes and pooled for ultra-deep NGS to an average depth of 100,000×, followed by data analysis to detect the presence or absence of ctDNA. The limit of detection (LOD) for Signatera, measured in VAF, is 0.01%.[18,19] Patients are considered ctDNA positive if at least two tumor informed single nucleotide variants (SNVs) are detected in plasma.[18] Abbosh et al found that non-adenocarcinoma histology, high Ki67 and lympho-vascular invasion were predictors of ctDNA positivity preoperatively.[18] Twenty-four patients had plasma ctDNA profiled with the Signatera test pre- and post-operatively every three months for two years, and six monthly thereafter, coinciding with a clinical assessment and chest radiographs for a median follow up of 775 days (range 688–945). Of the 14 patients that experienced disease relapse during follow up, 13/14 (93%) had detectable ctDNA before or at the time of clinical relapse. The lead time between ctDNA detection and confirmed NSCLC relapse was 70 days (10–346 days).[18]

Similar to Signatera, RaDaR is a tumor informed multiplex PCR amplicon based NGS assay, which tracks up to 48 somatic variants with a reported LOD of 0.001%.[20] RaDaR was employed in the LUCID study (LUng cancer CIrculating tumor DNA) which recruited 100 patients with stage I to IIIB NSCLC.[21] Personalized assays were successfully made for 88 patients, plasma samples were collected pre, during, and after radical treatment at three monthly intervals for a median of three years (range 42 days to 5 years). During longitudinal monitoring, 28 patients experienced disease relapse, of whom 18 had ctDNA detected, demonstrating a sensitivity of 64.3% (18/28). For 12 patients ctDNA was detected in samples collected during observation before recurrence was clinically evident, the median lead time was 212.5 days (range 19–687). Gale et al also performed a landmark time analysis using RaDaR, a time point of ≥2 weeks and <4 months from the end of radical treatment was used. During this period, 59 patients had samples collected of which 10/59 (17%) had ctDNA detected. These patients had shorter recurrence free survival (RFS) and overall survival (OS) compared to those who did not have MRD at the landmark timepoint, hazard ratio (HR) 14.8, $p<1\times10^{-5}$ and HR 5.48,

p<0.0003, respectively. Patients with detectable ctDNA pre-treatment also had shorter RFS and OS compared with patients for whom ctDNA was not detected at baseline, HR 3.14, p = 0.01 and HR 2.97, p = 0.003, respectively.

Hybrid-Capture Sequencing: Capp-Seq, Phased-Seq, AVENIO

The CAPP-seq assay (Cancer Personalized Profiling by deep sequencing) uses a hybridization capture based NGS approach. This deep sequencing assay, targeting 128 genes recurrently mutated in lung cancer, was utilized by Chaudhuri et al to longitudinally profile 37 patients with localized NSCLC (64% were stage III). Samples were taken every 2 to 6 months, post treatment with curative intent to coincide with surveillance imaging.[22] They first hypothesized that residual ctDNA at a landmark time, within four months post definitive treatment, would be associated with an increased risk of disease recurrence. ctDNA was detected at the landmark time point in 17/18 patients who ultimately recurred (94% sensitivity). At 36 months the freedom from progression (FFP) rate in patients who were MRD positive at the landmark analysis was 0%, versus 93% in those who were MRD negative (p<0.001, HR 43.4). Detection of ctDNA preceded radiographic progression in 72% of patients and by a median of 5.2 months.

Moding et al used CAPP-seq to interrogate whether ctDNA can act as a biomarker to identify patients who benefit from consolidation therapy.[23] Patients (n = 65) undergoing definitive chemoradiotherapy (CRT) had plasma samples taken pre- and post-CRT and retrospectively analyzed with CAPP-seq. The outcome of patients with detectable ctDNA after CRT who had consolidation immune checkpoint inhibitors (ICI) was compared to patients with detectable ctDNA post CRT who did not receive consolidation ICI. The consolidation cohort had significantly better FFP compared to those who had CRT alone (p = 0.0006), suggesting that additional therapy can improve outcomes in ctDNA positive patients. In contrast, patients with undetectable ctDNA after CRT in the consolidation ICI cohort had similar FFP to patients with undetectable ctDNA in the no consolidation ICI cohort. Suggesting the cost and risk of toxicity associated with consolidation ICI may be omitted in ctDNA negative patients. The CAPP-seq assay was used in a pre-planned analysis of the BTCRC LUN 16–081 Phase 2 study of consolidation nivolumab or nivolumab plus ipilimumab following CRT in patients with unresectable stage III NSCLC. Patients with detectable ctDNA MRD after completion of CRT demonstrated significantly inferior progression free survival (PFS) than patients who were MRD-negative. They also found patients with decreasing or undetectable ctDNA levels after one cycle of ICI had improved outcomes compared to patients with increasing ctDNA levels.[24]

AVENIO is a ctDNA assay developed by Roche that consists of three different assays: a "targeted kit" that assesses NCCN recommended biomarkers in ctDNA in patients with advanced cancer,[25] an "expanded kit" that assesses a more expansive list of genomic biomarkers,[26] and a "surveillance kit." The surveillance assay is a hybrid capture based NGS assay that profiles 197 genes, assessing SNV, indels, fusions and CNVs. It is used in a tumor genotype informed fashion with tissue or plasma assessment to establish a genomic baseline. It is reported to have >99% specificity and >99% PPV for all classes of mutations with an LOD of 0.1% VAF.[27] Studies are ongoing using the AVENIO ctDNA surveillance kit in NSCLC (Table 2).

PhasED-seq (Phased Variant Enrichment and Detection Sequencing), a more sensitive hybrid capture-based sequencing approach, tracks multiple variants on a single cfDNA molecule known as phased variants (PVs). Unlike CAPP-seq which uses duplex sequencing, PhasED-seq is not limited by the rate of recovery of DNA duplexes (both stands are often only recovered in 20–25% of all recovered molecules).[28] Tracking PVs reduces the impact of sequencing errors, without the decrease in yield inherent to duplex-sequencing. Using the PhasED-seq technology, Kurtz et al designed tumor informed PV assays for six solid tumor patients (5 lung cancer, 1 breast cancer) to analyze 24 samples. Personalized SNV based assays were also used to analyze the samples and the results were compared. The SNV assay detected ctDNA in 9/24 plasma samples while PhasED-Seq identified ctDNA in six more samples that the SNV assay had deemed negative. These six samples had very low tumor fractions with PhasED-Seq detecting ctDNA at levels as low as 0.000094%.[28]

Whole-Genome Sequencing: MRDetect

MRDetect uses a whole genome sequencing tumor informed approach. The assay combines prior knowledge of thousands of somatic SNVs and copy number alterations to query plasma for MRD. An AI-based error suppression model is used to increase the signal to noise ratio for precise ctDNA detection, and improve the accuracy of readouts especially to detect low

Table 2 Examples of Ongoing Clinical Trials Assessing MRD in NSCLC

Trial	Phase	MRD Trial Type	Assay	Patient Population	Intervention	Primary Endpoint
ORACLE NCT05059444	NA	Surveillance	Guardant Reveal	Stage II–III NSCLC Undergoing curative intent treatment *Other tumor types also enrolled	CtDNA assessment at the end of radical treatment and during follow up.	Distant Recurrence Free Interval
BTCRC-LUN19-396 NCT04367311	II	Surveillance	CAPP-seq	Resected stage IB, II, IIIA NSCLC	All patients have adjuvant platinum-based doublet CT + 13 cycles of atezolizumab. CtDNA testing every 3 months.	Percentage with undetectable ctDNA at defined time points
NCT04585477	II	Treatment Intensification	AVENIO surveillance kit	Stage I–III NSCLC who have completed surgery or definitive SABR and SOC adjuvant CT if required Patients planned for adjuvant ICI will be excluded	MRD positive patients will receive durvalumab for 12 months. MRD negative patients will receive standard of care surveillance.	Change in ctDNA level after 2 cycles of durvalumab
SCION NCT04944173	II	Treatment Intensification	AVENIO surveillance kit	Stage I NSCLC	SABR and 4 cycles of durvalumab, then evaluated for MRD. MRD negative patients will have no further therapy. MRD positive patients will be randomized to no further therapy or 8 further cycles of durvalumab.	Overall Risk of Relapse
MERMAID-1 NCT04385368	III	Treatment Intensification	ArcherDx	Resectable stage II–III NSCLC	MRD-positive patients post operatively are randomized to adjuvant durvalumab plus platinum-based doublet CT or placebo plus platinum-based doublet CT (SOC).	DFS in MRD positive patients
MERMAID-2 NCT04642469	III	Treatment Intensification	ArcherDx	Resectable stage II–III NSCLC	Patients who become MRD positive during a 96-week surveillance period will be randomized to durvalumab or placebo.	DFS in the PD-L1 TC≥1% analysis set
NCT04585490	III	Treatment Intensification	AVENIO surveillance kit	Unresectable stage III NSCLC that have completed definitive CRT	MRD positive patients will receive 4 cycles of platinum-based doublet CT plus durvalumab. MRD negative patients will receive durvalumab (SOC).	Change in ctDNA level following CT
NCT05286957	II	Treatment Intensification	Not specified	Resected stage IIA, IIB, IIIA NSCLC who have completed adjuvant CT	MRD positive patients will receive tislelizumab.	Percentage of patients changed from MRD positive to MRD negative post 8 cycles of Tislelizumab
NCT05457049	NA	Treatment De-escalation	Not specified	Stage IB-IIIA NSCLC patients who have a complete resection and undetectable landmark MRD.	Patients will have MRD assessed at two time points post operatively. If MRD negative, they will not have adjuvant CT and undergo routine MRD assessment instead.	Two-years DFS rates for patients with longitudinal undetectable MRD

Abbreviations: SABR, stereotactic radiotherapy; MRD, minimal residual disease; DFS, disease free survival; CT, chemotherapy; vs, versus; SOC, standard of care; ICI, immune check point inhibitors; NA, not applicable; CRT, chemoradiotherapy; PD-L1 TC, programmed death-ligand 1 tumor cells.

ctDNA burden with VAF levels as low as 10^{-5} detected.[29,30] Zviran et al employed this assay to monitor 22 NSCLC stage I–III patients pre- and post-operatively. Patients with detectable ctDNA MRD (n = 10) at the landmark time of 2.5 weeks following surgery had significantly worse RFS than those in whom post-operative ctDNA was not detected (n = 12).[30]

Future Methods of Detecting MRD in NSCLC

Methylation

Aberrantly methylated DNA is more frequent in plasma than mutant DNA, and methylation based assays have been investigated as a cancer screening tool.[31,32] The Guardant Reveal test, formerly called LUNAR-1, assesses epigenomic methylation signatures in addition to somatic mutations.[33] It is a tumor uninformed approach designed to detect the presence of MRD without prior knowledge of the specific molecular alterations present in an individual patient's tumor. Parikh et al demonstrated that the Reveal assay had a sensitivity of 55.6% and a specificity of 100% at a landmark analysis of one month post definitive therapy in a cohort of 103 early colorectal cancer patients. Prospective studies are ongoing evaluating the Reveal test in cancer screening (NCT05117840, NCT03774758) and as an MRD assay in NSCLC and other cancers (NCT05059444, Table 2).

Fragmentomics

As the length of ctDNA is shorter than that of cfDNA of healthy cells, measuring cfDNA fragment length may also be incorporated into MRD assays.[34] This technology has been in use for years in non-invasive prenatal testing due to the fragment length of fetal cfDNA being shorter than that of maternal cfDNA.[35] The DELFI assay (DNA evaluation of fragments for early interception) uses a machine learning model incorporating genome-wide fragmentation. The genome-wide pattern from an individual can be compared to reference populations to determine if the fragmentation pattern is likely healthy or cancer-derived. In a validation study, Cristiano et al performed WGS at 1–2× coverage of cfDNA from 208 patients with cancer, of whom 183 had early disease, including 12 NSCLC patients. Using the DELFI assay, they could classify patients as healthy or having cancer with a sensitivity of 73% (152/208).[36] The DELFI score has also been shown to correlate with cancer stage, with higher scores observed in patients with advanced NSCLC.[37] Furthermore, the DELFI score may be pooled with CEA level, age, smoking status and COPD in a multimodal model ($DELFI_{multi}$) to improve the assay's sensitivity in lung cancer screening.[37] DELFI is currently being assessed as a screening tool in conjunction with CT scanning in the CASCADE-LUNG study for patients at risk of lung cancer (NCT05306288).

Vessie et al combined fragment length score and variant calling (using the AVENIO surveillance kit) to detect post operative MRD and predict recurrence.[38] A fragmentation score was developed by building a reference database of reads that contained tumor informed mutations and their respective lengths. Patients who had a greater fragmentation score, compared to controls with non-malignant disease, were considered positive for MRD. When combined in a validation study of 36 stage II–IIIA NSCLC patients, the fragmentation score and variant calling was more accurate at predicting recurrence in a sample of early NSCLC patients compared to variant calling alone, and fragmentation score alone.[38] Further fragmentomic models are under development.[39,40]

Clinical Challenges of Assessing MRD in NSCLC

Timing of Landmark Analysis

Several trials are ongoing randomizing patients to different treatments based on their MRD status from a single post operative assessment (Table 2). This raises the question of when to perform the landmark MRD analysis. In plasma samples of patients with resected NSCLC, Gale et al detected ctDNA in 25% (12/48) of patients 1–3 days after surgery and this was not associated with an increased risk of disease recurrence, however ctDNA detected at ≥2 weeks and <4 months post definitive treatment was prognostic.[21] This highlights some logistical challenges; although tumor informed assays can detect ctDNA at lower VAFs than tumor agnostic tests, at least three weeks is required to develop a personalized assay.[19,41] While adjuvant chemotherapy is still efficacious in NSCLC up to four months post-surgery,[42] in the routine oncology clinic there are many steps which may lead to prolonged turnaround times of tumor informed assays, including delays in processing and pathological review of a resection specimen, the necessary shipping time required to transfer the specimen to the relevant assay company, followed by the time needed to perform WES and design a patient specific panel. Consequently, it may be necessary to use diagnostic pre-operative tumor biopsies. Furthermore, if neoadjuvant systemic therapy is utilized the resection specimen may have a high volume of necrosis rendering it unusable.[43] Unfortunately, reliance on tumor biopsies increases failure rates as a biopsy may not be of sufficient quality and quantity, particularly once valuable tissue has been used for standard of care genotyping. This is an issue for both surgical and CRT patients, in the LUCID study 33% (10/30) of non-surgical patients that were recruited could not have tumor informed assays made due to inadequate tissue.[21]

Timing of Surveillance

The optimal schedule and radiological method of follow up in resected NSCLC is unknown. The IFCT-0302[44] trial compared a follow up program of six-monthly clinical examinations and chest radiographs (CXRs) to six monthly physical examinations, CXRs and thoraco-abdominal CT scans in 1775 patients with completely resected stage I-IIIA NSCLC. No difference in OS was found between the two arms post eight years of follow up.[45] The recommended frequency of surveillance imaging also differs amongst international guidelines due to the absence of definitive evidence.[46,47] The lack of consensus regarding how patients with radically treated NSCLC should be surveyed further complicates how MRD surveillance will be incorporated into clinical follow up. Moreover, we have discussed that in

some cases MRD can be detected months prior to clinical evidence of disease relapse.[18,21] As some patients may relapse years after radical therapy, the optimum frequency and duration of MRD surveillance must be defined.

Cumulative Toxicity of Numerous Radical Procedures

The objective of incorporating ctDNA based MRD surveillance into the follow up of radically treated NSCLC is to help identify local disease recurrence, metachronous tumors, and oligo-metastatic disease relapse earlier, thereby enabling clinicians to employ radical locoregional therapy, such as SBRT or surgery. Although SBRT is an effective and tolerated treatment of lung lesions, it is not without toxicity. Treatment of peripheral tumors can lead to chest wall pain and rib fractures, SBRT to apical lesions may damage the brachial plexus, while increased respiratory and gastric complications occur following the treatment of central tumors.[48–50] SBRT planning will need to consider a patient's prior resection location or radiotherapy field, residual lung function and respiratory comorbidities. Image-guided thermal ablation (cryotherapy, microwave, radiofrequency) may be an option for selected patients. In some patients, a segmentectomy, wedge resection or even completion pneumonectomy may be considered. In a population who commonly have baseline chronic obstructive pulmonary disease, the benefits and risks of toxicity of repetitive localized therapies must be considered.

Cost

Another challenge of assessing MRD in NSCLC is the financial implications. Not only must one consider the cost of the assays, but a positive ctDNA result will also lead to further investigations such as a positron emission tomography CT (PET-CT) or bone scintigraphy to assess for occult metastatic disease. This will have knock on effects leading to increased resource utilization and patient outpatient appointments to discuss and action results, in a time scarce public health care system, or increased out of pocket spending for self-funding patients. Indeed, inability to detect any disease on imaging will undoubtedly result in an increased frequency of imaging to confirm relapsed cancer and thereby increase costs and patient anxiety. Moreover, if cancer cannot be detected, the concept of a false positive result may need to be considered resulting in undue patient (and physician) distress.

Combining Precision Medicine and Adjuvant Therapy

Unlike colorectal cancer, where adjuvant chemotherapy can improve survival by 25%,[51] the benefit of adjuvant chemotherapy in resected NSCLC is modest with a 5% improvement in survival at 5 years.[52] Recently, treatment outcomes have improved with the addition of immunotherapy to the adjuvant paradigm[53,54] and targeting common *EGFR* mutations with adjuvant osimertinib.[55] Biomarkers are needed to identify patients that are at high risk of disease recurrence who will derive the most benefit from adjuvant therapy, and those in whom adjuvant treatment can be omitted. An exploratory analysis of the Impower010 study,[53] where ctDNA samples were collected on C1D1 of the enrolment phase (after surgery, prior to chemotherapy) and retrospectively tested using the Signatera assay, found that baseline ctDNA was prognostic. Patients who were ctDNA positive at enrolment had worse DFS than those that were ctDNA negative. Nevertheless, atezolizumab improved DFS in both ctDNA positive and ctDNA negative stage II–IIIA patients, suggesting MRD post operatively may not be used to predict who will benefit from adjuvant immunotherapy post chemotherapy.[56] In the future, it is likely that adjuvant and consolidation systemic anticancer therapy (SACT) choice will be driven by tumor genotype[57,58] and PD-L1 status in addition to pathological staging, similar to the metastatic setting where high PD-L1 expressors (>50%) can be treated with single agent ICI and oncogene addicted NSCLC receive genotype specific targeted therapy.[59] How MRD status will be combined with these clinical and molecular features to further personalize treatment remains to be seen.

Future Directions and Clinical Trial Design

Colorectal cancer has been leading in the field of randomized trials using MRD to guide adjuvant therapy thus far.[60–62] Presented at ASCO Congress 2022 plenary session, the provocative DYNAMIC study randomly assigned post operative stage II colon cancer patients in a 2:1 ratio to ctDNA guided management or standard management. The tumor informed SafeSeq assay was used and MRD was assessed at four and seven weeks post-operatively. In the ctDNA guided management group, those who were ctDNA/MRD positive post-operatively received adjuvant chemotherapy and the ctDNA negative group did not. The trial met its primary end point, no difference in two-year RFS was seen between the two arms.[62]

Compelling data was also generated from an exploratory analysis of the IMvigor010 randomized Phase III trial, which investigated adjuvant atezolizumab versus observation in patients with operable muscle-invasive urothelial

carcinoma.[63] Prospectively collected plasma samples were available for 581 patients and assessed with the tumor informed Signatera assay. Patients who were ctDNA positive at cycle 1 and randomized to the adjuvant atezolizumab arm had improved DFS (HR = 0.58, 95% confidence interval [CI] 0.43–0.79) and OS (HR = 0.59, 95% CI: 0.41–0.86) versus those that were ctDNA positive and randomized to the observation arm. No differences in DFS or OS between treatment arms were found in patients who were negative for ctDNA at baseline. These findings suggest that post-operative ctDNA MRD detection can guide the administration of adjuvant ICIs in operable muscle-invasive urothelial carcinoma. Additionally, patients in the atezolizumab arm that were ctDNA positive at cycle 1 and became ctDNA negative at cycle 3 had superior outcomes compared to those in the same arm who remained ctDNA positive at cycle 3, further demonstrating the prognostic value of ctDNA dynamics.[63]

There are multiple prospective NSCLC trials ongoing (Table 2) aiming to test the hypothesis that ctDNA-based MRD detection identifies patients who benefit from early treatment intensification (Figure 2A). The MERMAID-1 study (NCT04385368) is a Phase III, randomized, double-blind, placebo-controlled study designed to investigate the efficacy of adjuvant durvalumab compared to placebo post platinum-based chemotherapy in patients with resected stage II–III NSCLC

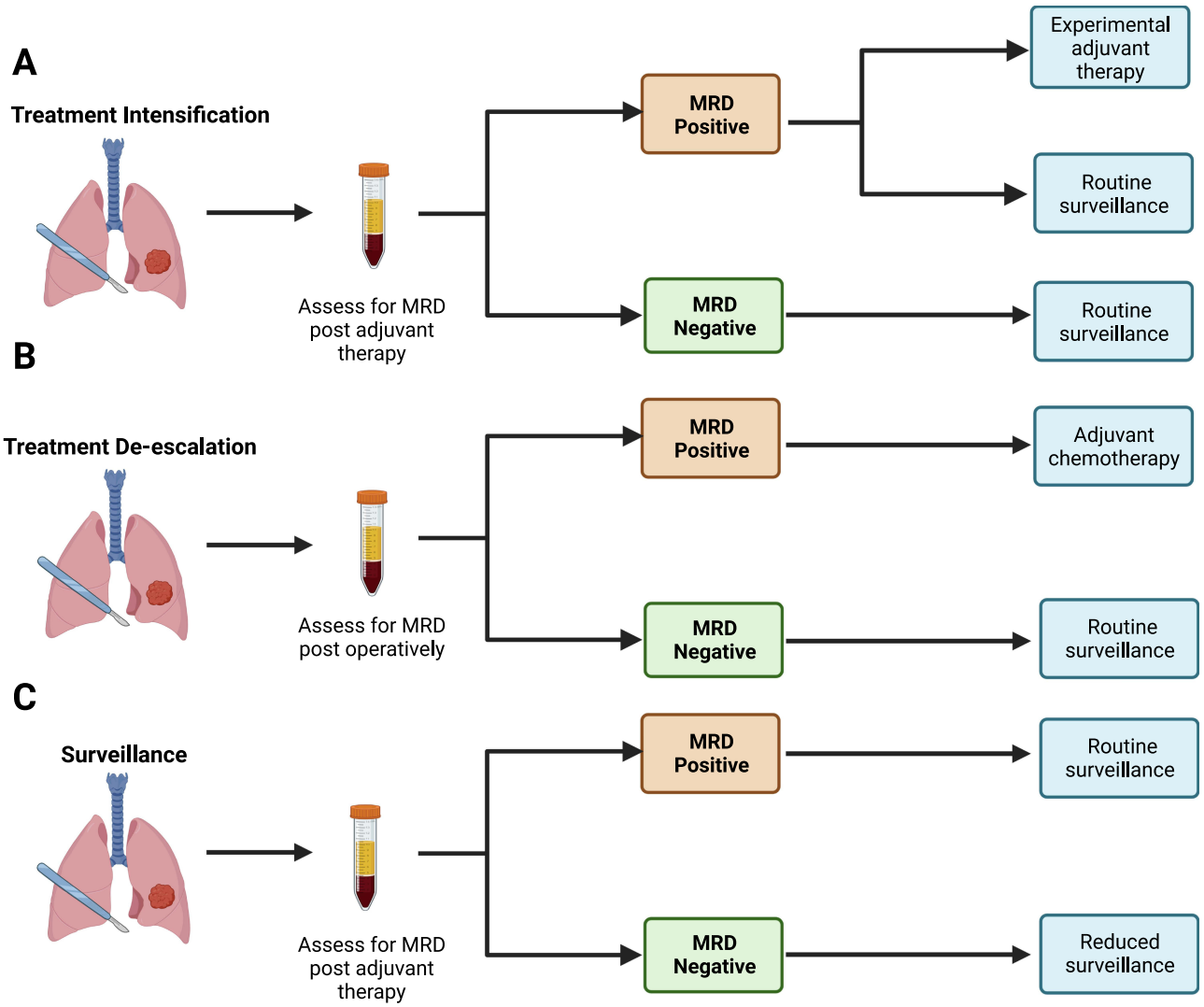

Figure 2 Example clinical trials designs using MRD status to randomize patients. (**A**): MRD status post adjuvant chemotherapy could be used to intensify treatment. In (**A**) MRD positive patients are randomized to an experimental adjuvant therapy or to continue routine surveillance. (**B**): MRD status post operatively could be used de-escalate adjuvant therapy. (**B**) describes a trial where patients that are MRD negative post-operatively have adjuvant therapy omitted. (**C**): MRD status can be used to guide surveillance. (**C**) outlines how MRD negative patients could be assigned a less intensive surveillance follow up that MRD positive patients. Created with biorender.com.
Abbreviation: MRD, minimal residual disease.

who are ctDNA MRD-positive post operatively. DFS in MRD positive patients is the trial primary end point. The NCT04585490 study will investigate stage III NSCLC patients post completion of definitive CRT. MRD positive patients will receive treatment with durvalumab and four further cycles of platinum-based doublet chemotherapy, while MRD negative patients will receive standard of care consolidation durvalumab alone. The study primary end point is change in ctDNA level.

The absence of MRD may also be used to de-escalate adjuvant or consolidative treatment to mitigate potential treatment related toxicity (Figure 2B).[46,47] In NCT05457049, patients with completely resected stage IB-IIIA NSCLC who are MRD negative at two time points post operatively will forego adjuvant chemotherapy and undergo routine MRD assessment instead. The study primary end point is two-years DFS rates for patients with longitudinal undetectable MRD.

Additionally, studies can also be designed to incorporate MRD testing into surveillance. Patients can be stratified to different radiological surveillance protocols according to their MRD status (Figure 2C).[15,64]

Ideally an improvement in OS is the gold standard study end point to demonstrate the clinical utility of MRD assessment in NSCLC.[65,66] Unfortunately the prolonged follow up required to determine an OS benefit is costly leading to the employment of DFS and RFS as surrogate end points instead. Other endpoints, including patient reported quality of life and health economics should also be assessed in prospective MRD studies.

Conclusion

MRD guided treatment has the potential to personalize therapy to improve cure rates and limit unnecessary treatment related toxicity. Nonetheless, the use of ctDNA to assess MRD in NSCLC faces many challenges including assay technology, optimal test timing and robust trial design. Until the clinical utility of escalating or de-escalating NSCLC treatment based on a MRD positive result is proven in a prospective clinical trial design, ctDNA derived MRD will remain a promising academic endeavor.

Disclosure

Dr Andrew Feber is an employee of Nonacus. Professor Sanjay Popat reports personal fees from Amgen, personal fees from AstraZeneca, personal fees from Bayer, personal fees from Beigene, personal fees from Blueprint, personal fees from BMS, personal fees from Boehringer Ingelheim, personal fees from Daiichi Sankyo, personal fees from Guardant Health, personal fees from Incyte, personal fees from Janssen, personal fees from Lilly, personal fees from Merck Serono, personal fees from MSD, personal fees from Novartis, personal fees from Roche, personal fees from Takeda, personal fees from Pfizer, personal fees from Seattle Genetics, personal fees from Turning Point Therapeutics, personal fees from Xcovery, outside the submitted work. The authors report no other conflicts of interest in this work.

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