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Next-generation ctDNA-driven clinical trials in precision immuno-oncology

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Based on an improvement in our understanding of the biology and underpinnings of the biology of cancer, we have witnessed a sea change in our ability to deliver novel therapies, such as immunotherapy and targeted therapy for the appropriate subset of patients. The study of tumor clonal evolution has been largely aided by access to tumor tissue using repeat biopsies. While access to sequential tumor biopsies provides a clear insight into the tumor genomic landscape, the real-time applicability of this approach is often fraught by issues such as the invasive nature of the biopsies, and the potential of associated complications. With the availability of new technologies, such as cell-free circulating tumor DNA (ctDNA), there is now a compelling advantage of a minimally invasive approach that can be repeated sequentially and may provide a more comprehensive view of tumor clonal evolution over time. A variety of ctDNA-based technologies have been developed, including next-generation sequencing (NGS) of a broad panel of mutations, evaluation of copy number changes and amplification patterns. Other technologies such as methylation signatures and fragmentomics are being explored for early detection of cancer. Genotyping using NGS of ctDNA is already an integral component of routine clinical practice for a variety of tumor types. In the setting of certain advanced malignancies, such as non-small cell lung cancer (NSCLC), ctDNA is predominantly used as a complement to tissue genotyping for selection of biomarker-directed first-line therapy. However, its use is not limited to merely detection of genomic alterations—by extension to evaluation of tumor mutational burden (TMB), and microsatellite instability (MSI) status, ctDNA evaluation in patients receiving immune checkpoint inhibitors have shown both predictive and prognostic value. In the clinic, ctDNA-based genotyping is also being used as a real-time tool for monitoring of emergent resistance mutations in patients receiving targeted therapy—with direct clinical impact and ability to alter therapy and make treatment decisions based on evolving tumor biology. In addition to these applications, the ability to repeat ctDNA assessment in a minimally invasive fashion offers a unique opportunity to use early on-treatment changes in ctDNA for real-time assessment of therapeutic response and outcome. In the current issue of this journal, Sivapalan and colleagues outline the use of ctDNA-based liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy.[1]

Despite promising data supporting the prognostic impact of baseline ctDNA levels and early clearance or molecular response associated with improved outcomes with checkpoint inhibitors, use of liquid biopsy to direct immunotherapy in lung cancer is not quite ready for prime time. To evaluate how ctDNA-based approaches may fit into our clinical care paradigm, we must first examine the current scope of validated predictive biomarkers for immunotherapy. Biomarkers such as programmed death ligand-1 (PD-L1) tumor cell expression and tumor agnostic MSI remain limited to tissue-based assays with assessment performed at the time of initial diagnostic biopsy. Tissue testing for MSI is widely available, and while ctDNA-based approaches can report MSI status, use has been limited due to lack of an immediate clinical need. PD-L1 assessment by liquid biopsy using exosomes and other plasma markers remains under investigation.

Assessment of other biomarkers using ctDNA is, however, of interest. Evaluation of TMB is an area of particular relevance, where ctDNA may play a key role. Concordance of plasma TMB and tissue TMB has already been established, and aside from serving as a non-invasive biomarker when tissue is lacking, plasma TMB may have other advantages. High spatial and intratumoral heterogeneity of the immune microenvironment

may make it challenging to rely on a single tissue biopsy to predict immune signatures. Plasma TMB may overcome this by more comprehensively capturing tumor genomic heterogeneity, including assessment of both the primary and metastatic sites. Several studies have demonstrated an association between baseline plasma TMB (≥10–20 mutations per megabase, mut/Mb) and immunotherapy outcomes in NSCLC. However, correlation of plasma TMB with outcomes on prospective clinical trials has not been perfect. This may be related in part to the lack of algorithmic standardization and harmonization with various thresholds tested in clinical trials. In cohort C of the B-FAST trial, patients with treatment-naïve advanced NSCLC with high-plasma TMB (≥10 mut/Mb using a clinical trial assay) received either atezolizumab or chemotherapy irrespective of tumor PD-L1 expression. No differences were seen in progression-free survival (PFS) or overall survival (OS). In a prespecified analysis of patients with TMB ≥16 mut/Mb, there was a statistically non-significant trend to better PFS with immunotherapy, (stratified PFS HR 0.77; p=0.053). In the NEPTUNE trial, PFS and OS in the 25% of the patients with high plasma TMB (≥20 mut/Mb) was numerically but not statistically improved in those receiving durvalumab plus tremelimumab versus chemotherapy (OS HR 0.71, PFS HR 0.77, p=NS). As highlighted by Sivapalan *et al*, pretreatment liquid biopsy predictors of immunotherapy benefit will likely require multimodal assays, human leukocyte antigen (HLA)-correction of TMB measurement and more before clinical use can be achieved.[1]

Another key application of ctDNA as a biomarker is in the association of molecular response assessment using plasma-based changes in ctDNA levels. The ctDNA clearance has been demonstrated as a predictor of immunotherapy response, and conversely, lack of ctDNA decrease has been associated with higher risk of progression (HR 5.4, 95% CI 1.6 to 18.4) and death (HR 6.9, 95% CI 1.4 to 35.0). At the same time, increase in ctDNA has been demonstrated to precede radiographic progression by a median of 8.7 weeks.[2 3] In a pooled analysis of 200 patients with NSCLC in five studies receiving immunotherapy, early on-treatment decreases in ctDNA levels were associated with response, PFS (HR 1.76, 95% CI 1.3 to 2.4) and OS (HR OS 2.28, 95% CI 1.6 to 3.2). Different definitions of ctDNA response were explored, including a −50% change in variant allelic fraction (VAF). The best predictive performance was with a three-level variable separating patients with the greatest increase and decrease in VAF levels and categorizing the rest as intermediate.[4] Collectively, these studies demonstrate that molecular response can be directly correlated to therapeutic response. Although these studies using research-only assays have established proof of principle for use of ctDNA as a dynamic biomarker, we need a readily available assay that could be used readily in the clinic, as the results could rapidly be incorporated into routine clinical practice. Additional questions pertaining to the panel size, type of assay, optimal timing and threshold for ctDNA response remains unclear. Prospective clinical trials are using ctDNA-based molecular response monitoring as a biomarker to intensify therapy with the addition of chemotherapy among patients receiving pembrolizumab alone (Canadian Cancer Trials Group BR.36, NCT04093167) or among those receiving first-line nivolumab plus ipilimumab (NCT04966676).

As immunotherapy moves into the early-stage setting, there has been significant interest in the application of ctDNA as both a prognostic and predictive biomarker in the perioperative setting of NSCLC. In the adjuvant setting, on the IMpower010 trial, patients derived benefit from adjuvant atezolizumab irrespective of whether postoperative ctDNA (minimal residual disease, MRD) was detected using the Signatera tumor-informed assay. Despite the assay's sensitivity with a 95% lower limit of detection of 0.01%, more than 30% of the patients without detectable postoperative ctDNA experienced disease relapse by 36 months.[5] In the setting of neoadjuvant chemo-immunotherapy, ctDNA clearance has been correlated with pathological complete response (pCR). However, not all patients who achieve pCR experience ctDNA clearance and vice versa, with pCR as the better predictor of event-free survival. Likely composite endpoints such as ctDNA clearance plus pCR will identify those at lowest risk of relapse, where de-escalation of further therapy could be tested with more sensitive MRD assays.

In the future, the development of robust integrated ctDNA assays would require standardization, harmonization and cross validation. Longitudinal analyses, including serial monitoring for patients receiving immunotherapy are a promising tool for escalation and de-escalation strategies, especially in the setting of immunotherapy, however several questions remain such as the appropriate timing of response assessment, the type of assay to be used and the calculation, or method of response measurement. Incorporation of ctDNA assays into clinical trials, and integration with other biomarkers such as mutational profiles, TMB will be required to definitively assess whether integrated approaches can enhance the predictive accuracy of these assays. Finally, large scale, national and international collaborative efforts with a multipronged approach to tackle both the analytic, and clinical issues will be instrumental in the clinical implementation of ctDNA analysis for immuno-oncology to improve patient outcomes.

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