Editorial Commentary

Can liquid biopsy dynamics stratify patients with small cell lung cancer?

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Lung cancer remains the deadliest type of cancer in the world (1). The histological subtype small cell lung cancer (SCLC), which represents about 13% of lung cancer cases (2), has a very poor prognosis with a 2-year survival rate of only 16% and 19% in men and women, respectively (2).

Stage IV SCLC is an advanced and aggressive form of lung cancer characterized by rapid growth, early metastasis, treatment resistance and disease recurrence, resulting in a poor prognosis for patients (3). Platinum-etoposide chemotherapy was the standard first-line treatment for extensive-stage SCLC (ES-SCLC) for several decades; however, the need for additional treatment strategies remained (3,4). After decades of little progress, immune checkpoint inhibitors (ICI) revolutionized the treatment landscape, leading to a new standard of care in the first-line setting (3-6). Still, there is an unmet need to gain deeper insight into the biology of SCLC.

In a recent study, published in *Clinical Cancer Research* (7), Sivapalan and colleagues evaluate circulating tumor DNA (ctDNA) dynamics in ES-SCLC patients treated with ICI and/or chemotherapy. In total 33 patients participated in the retrospective study (median follow-up of 11 months; range, 1–63 months), where blood was collected at minimum three timepoints: baseline (BL), during treatment and at clinical progression, resulting in 139 serial plasma samples (*Figure 1*). The authors perform hybrid-capture based next-generation sequencing (NGS) of cell-free DNA (cfDNA) obtained from each plasma sample using targeted error-correction sequencing (TEC-seq), which has previously been described by the authors (8). In addition, they perform TEC-seq on matched peripheral blood mononuclear cells (PBMC) in 32/33 (97%) cases. TEC-seq covers 58 genes and they identify single-nucleotide variants (SNV) and small insertions/deletions (indel) using VariantDx. They classify variants as: (I) germline [variant allele fraction (VAF) >25% and non-hotspot]; (II) clonal hematopoiesis (CH) (variant present in PBMC sample or in *DNMT3A*); or (III) tumor-derived. The authors also perform genome-wide copy number alteration (CNA) analysis of cfDNA based on the TEC-seq data. Unique for this study, they combine the information gained from the tumor-derived SNV/indel analysis and the CNA analysis to a single biomarker which they call cell-free tumor load (cfTL).

Based on the cfTL dynamics in serial blood samples they group the SCLC patients accordingly (*Figure 2*). They define groups as: (I) molecular responders (n=9, cfTL at BL, but undetectable in subsequent blood samples); (II)

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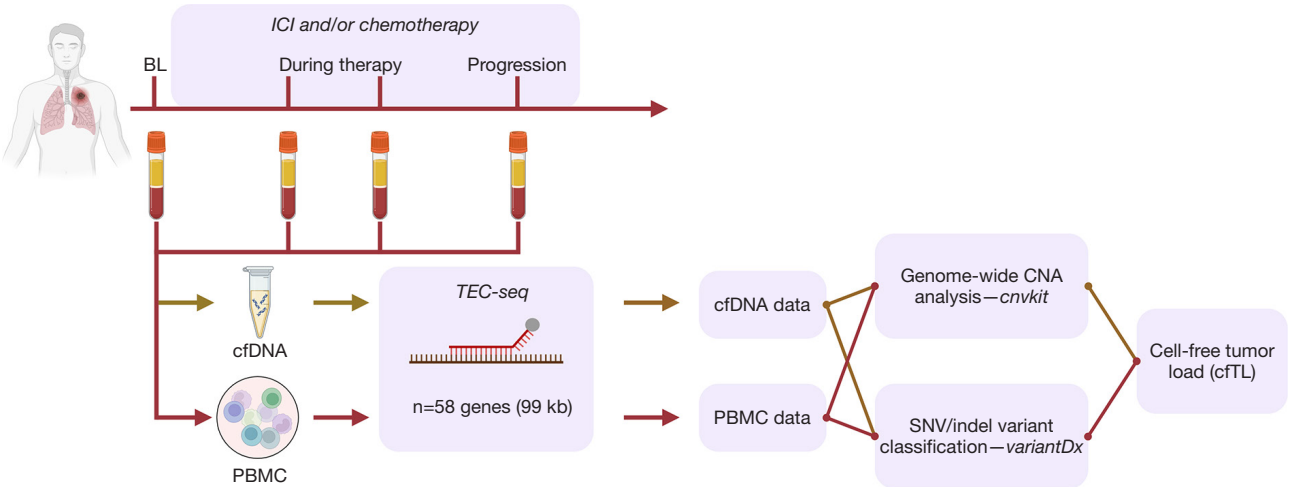

Figure 1 Experimental setup made by Sivapalan *et al.* (created with BioRender.com). BL, baseline; ICI, immune checkpoint inhibitor; cfDNA, cell-free DNA; PBMC, peripheral blood mononuclear cells; TEC-seq, targeted error-correction sequencing; cfTL, cell-free tumor load; CNA, copy-number alteration; indel, insertion/deletion; SNV, single-nucleotide variant.

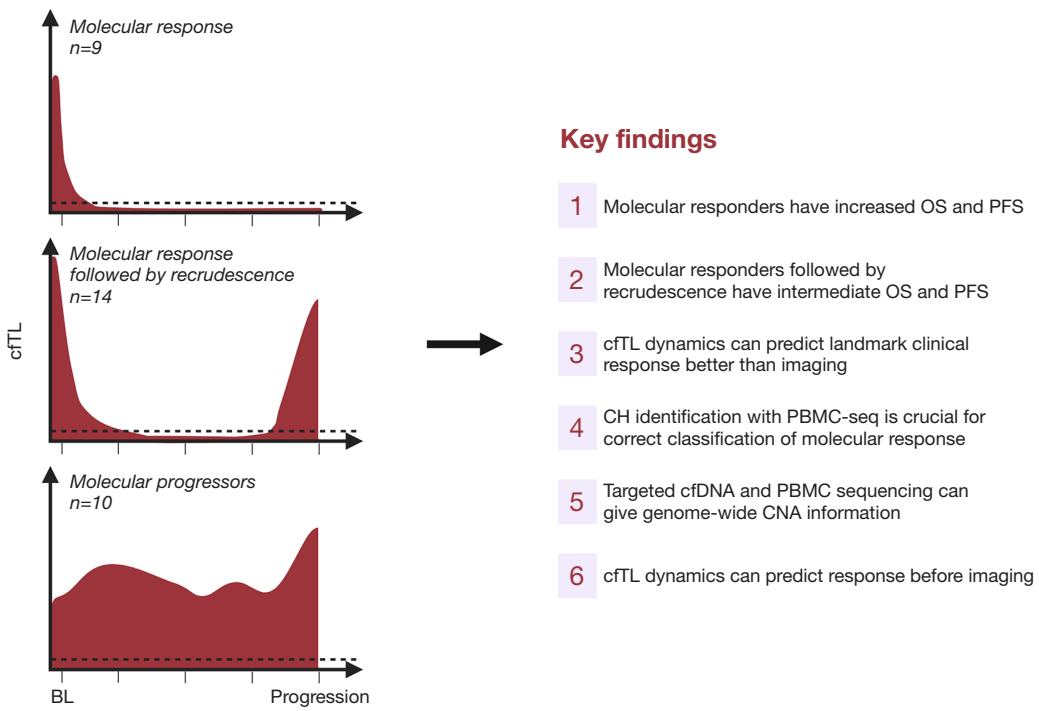

Figure 2 Molecular analysis and key findings by Sivapalan *et al.* (created with BioRender.com). Three illustrative examples of different cfTL dynamics observed with liquid biopsies. The dashed line indicates the detection limit for cfTL and vertical ticks indicate different blood samples. cfTL, cell-free tumor load; BL, baseline; OS, overall survival; PFS, progression-free survival; CH, clonal hematopoiesis; cfDNA, cell-free DNA; PBMC, peripheral blood mononuclear cell; CNA, copy-number alteration.

molecular response, followed by recrudescence (n=14, cfTL at BL followed by cfTL elimination but with subsequent re-emergence); or (III) molecular progressors (n=10, persistent cfTL across all timepoints). They find that the three groups have different overall survival (OS), progression-free survival (PFS), durable clinical benefit (DCB), and radiographic response. Interestingly, the novel classification of molecular responders followed by recrudescence, which demonstrate an intermediate molecular response, also fall between molecular responders and progressors in terms of PFS and OS.

Previous studies on ctDNA dynamics in SCLC have shown similar results (9-13). Similar to the study by Sivapalan *et al.*, these studies are also based on targeted NGS and SNV/indel detection. However, the molecular classification of patients based on ctDNA dynamics vary between individual studies. In a study by Iams *et al.* from 2020 SCLC patients treated with chemoradiation or surgical resection were classified as "ctDNA never detected" or "ctDNA ever detected" (11). The ctDNA is detected using a targeted NGS panel designed by the authors to specifically identify mutations frequently observed in SCLC (13). Patients with ctDNA detected at any timepoint after therapy had reduced PFS and OS. This broad dichotomization differs from the analysis by Sivapalan *et al.* where the SCLC patients are separated into three groups based on cfTL dynamics, and importantly demonstrate the difference between molecular progressors and molecular response followed by recrudescence. While cfTL clearance during therapy is an important measure of response, the reemergence of cfTL through serial monitoring is a clear indication of tumor relapse and possess great clinical value. Feng *et al.* from 2022 also separated SCLC patients into three groups based on ctDNA dynamics, although this was based on two serial blood samples (BL and on treatment) and only considering mutations in *TP53* or *RB1* (9). Patients which were ctDNA positive in both samples had the worst PFS and OS, whereas patients clearing the ctDNA following therapy had the best PFS and OS. Patients with ctDNA negative samples at both timepoints had intermediary PFS and OS. They also demonstrated that ctDNA responses correlated with radiographic responses and in some cases could detect disease progression earlier than CT scans. It is important to note that the patients in the study by Feng *et al.* primarily have limited disease (69%) in contrast to the solely ES-SCLC patients in the study by Sivapalan *et al.*

The classification of plasma samples as ctDNA positive or ctDNA negative can also vary between studies. In a study by Nong *et al.* from 2018 they use the mean VAF in the BL sample and group patients as ctDNA high or low based on the median ctDNA level across all samples (12). Patients classified as ctDNA high had worse PFS and OS. In contrast, Sivapalan *et al.* use the VAF of the most abundant tumor mutation and patients are classified as ctDNA negative with cfTL =0. In order for ctDNA to be used in clinical practice to guide treatment strategies we regard standardization of ctDNA dynamics as essential. One approach could be to implement ctDNA-Response Evaluation Criteria in Solid Tumors (ctDNA-RECIST) (14). Using ctDNA-RECIST it would be possible to compare different studies using ctDNA for monitoring patients and thereby enabling wider implementation of liquid biopsies in clinical practice.

One of the strengths in the presented study is the combination of SNV/indel data and genome-wide CNA information gained on targeted NGS. Earlier in 2023 Zhang *et al.* also investigated CNA in plasma from SCLC patients using targeted NGS followed by cnvkit analysis (10). However, in contrast to the presented study, Zhang and colleagues did not combine the CNA with SNV/indel data resulting in limited utility. Sivapalan *et al.* illustrates the importance of CNA analysis because some patients have no ctDNA detected based on mutation analysis, however, structural cancer associated changes are identified. This increases the sensitivity of ctDNA detection which is paramount in order to implement liquid biopsies as a tool for cancer monitoring. However, one of the drawbacks is that cnvkit requires a healthy sample for normalization, which in the case of the paper by Sivapalan *et al.* is the PBMC sample analyzed with TEC-seq (PBMC-seq). This increases sequencing labor and running costs because it requires sequencing of minimum two samples from each patient. However, the PBMC-seq also serves a dual purpose (*Figure 1*). Thus, SNV/indel variants detected in plasma can be classified as germline, CH or tumor-derived based on the variants detected in the PBMC sample. It is important to distinguish between these variants, which is also highlighted in the study by Sivapalan *et al.* If the CH variants are not filtered out, very few patients are classified as molecular responders (n=2) given that CH mutations are not cleared during therapy. As a result, most patients are classified as molecular progressors (n=23) and the molecular subgroups do not have different PFS and OS. These results indicate how PBMC-seq can both increase ctDNA sensitivity and specificity. It is easy to envision how this approach can be

expanded to other solid tumors. Similar results have been observed in non-small cell lung cancer (NSCLC) patients treated with ICI, where ctDNA analysis without CH filtering resulted in weakened stratification as compared to the ctDNA analysis with CH filtering (15).

While the study by Sivapalan *et al.* presents an innovative approach to ctDNA monitoring, further research is still required to better understand how to use liquid biopsies in SCLC. The study is limited by its retrospective approach and a prospective study surveying ctDNA in SCLC patients is required to fully understand the utility of liquid biopsies in an un-biased SCLC cohort. From a methods perspective, the study is also limited by the low number of identified patients with *RB1* mutations (5/33, 15%), where other studies using similar approaches identify between 48% and 80% in plasma from SCLC patients (9-11,16-18). As an example, in the study by Iams *et al.* from 2020 which uses targeted NGS panel specifically designed for SCLC *RB1* mutations is detected in 11/23 (48%) patients (11). In addition, the authors are limited by the variations in timepoints for the on-treatment blood samples between patients as well as the uncoordinated time of radiographic assessment and blood sampling. It will be interesting to see in future studies at what timepoint during treatment ctDNA analysis has the most clinical value.

Moreover, the study focuses mainly on time to response assessment between ctDNA and imaging, and it should be considered how clinically important this information is. The method would be highly relevant if it was possible to detect disease progression earlier with ctDNA, given that molecular progression could be used as a predictive marker for change in treatment strategy (9,19). Although, treatment strategies are limited for ES-SCLC, future studies could delineate whether ctDNA monitoring can be used to determine personalized treatment strategies in SCLC. The study by Sivapalan and colleagues does build upon previous studies and open for the use of ctDNA in SCLC. However, as with many other studies on liquid biopsies, we lack clinical studies comparing ctDNA-guided treatment to standard-of-care in order to solidify ctDNA as a strong predictive biomarker. One very interesting prospective randomized clinical trial for NSCLC (PRELUCA, NCT05889247) is currently ongoing. The study will assess how tumor-informed liquid biopsies can be used to make treatment decisions for NSCLC patients receiving immunotherapy. In the future, similar studies will hopefully also be conducted for SCLC as the field of ctDNA in SCLC is expanding.

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