Editorial

Liquid biopsies come of age in lung cancer

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Comment on: Low SK, Ariyasu R, Uchibori K, *et al.* Rapid genomic profiling of circulating tumor DNA in non-small cell lung cancer using Oncomine Precision Assay with Genexus™ integrated sequencer. Transl Lung Cancer Res 2022;11:711-21.

Submitted Apr 06, 2022. Accepted for publication May 04, 2022.
doi: 10.21037/tlcr-22-268
View this article at: https://dx.doi.org/10.21037/tlcr-22-268

The catalytic impact of next-generation sequencing (NGS) on modern oncology cannot be overstated: high-throughput screening for actionable genetic alterations has advanced understanding of cancer biology, accelerated development of targeted drugs, and guided their use with unprecedented survival gains during the last decade. Yet, the additional impact from full integration of liquid biopsies (LB) into the clinical routine remains hard to appreciate. For all we can say at present, LB represent a second revolution, poised to become as important as NGS itself, which they gradually relieve from several important limitations.

In the current issue of *Translational Lung Cancer Research*, Low *et al.* offer us another glimpse of how wonderful a LB-dominated world of non-small-cell lung cancer (NSCLC) management could become in the near future (1). "Rapid genomic profiling of circulating tumor DNA" would ensure molecular results before treatment start for every single lung cancer patient, including those 25% with insufficient tissue samples (2), as well as many others hampered by high procedural risk, hardly accessible tumors, and lack of local expertise. Surprisingly, a shocking 30–50% of patients will not receive adequate biomarker profiling at initial diagnosis today, even in the most advanced health care systems of the USA and western Europe, according to recent real-world data (3,4). On the other hand, LB combine the ease of blood collection with the ability to preserve and ship samples for several days in special tubes, which is sufficient to overcome any barriers. In fact, accumulating evidence already argues for the feasibility and expediency of a "blood-first" approach in the molecular workup of NSCLC: besides the present work by Low *et al.*, another important example is the prospective head-to-head comparison of the NILE study, in which upfront LB could detect guideline-recommended treatable mutations in more NSCLC patients, i.e., 87% (77/89) *vs.* 67% (60/89), than standard-of-care tissue testing (5). An alternative promising strategy are "reflex" or "rescue" LB for newly diagnosed NSCLC with negative tissue NGS results, which will detect established therapeutic targets, including *EGFR*, *ALK*, *ROS1*, *RET*, *BRAF*, and *MET* mutations in almost one-third (29%) of such cases, with full benefit from subsequent use of the respective approved drugs (6). This ability of LB to capture clinically relevant mutations missed by conventional tissue sampling demonstrates how liquid assays can overcome the obstacle of spatial tumor heterogeneity and was also highlighted in the study by Low *et al.* with several premium examples: *EGFR* L858R, *KRAS* G12C, *MET* D1028N (1). Of note, technical failures were infrequent in this study, i.e., <10% (11/119) at first sequencing attempt, and could all be "salvaged" by resequencing on Ion 540 chips, which have equivalent reads with GX5 chips on the Ion S5 Prime system (1). However, an important problem of most current LB platforms, including that employed by Low *et al.*, is limited sensitivity, with a minimum detectable variant allelic fraction (VAF) of 0.1–0.01%, which will miss up to approximately 1/3 of mutations found in tissue

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NGS (1). Therefore, an optimal strategy to maximize diagnostic yield today would be the complementary use of both tissue and LB (7). With that being said, technical progress is rapid and will probably overcome such "teething troubles" soon. For example, the limit-of-detection for LB could be brought down to 0.004% in a pivotal study using larger, tumor-informed DNA panels with the bioinformatics pipeline from CAPP-Seq and digital error suppression (8).

Another important innovation introduced by Low *et al.* is the parallel sequencing of both circulating tumor-derived RNA and DNA (ctDNA) within the same workflow (1). Meanwhile, there is ample evidence that additional RNA-alongside DNA-NGS can enhance sensitivity for the detection of oncogenic fusions, e.g., *ALK*, *ROS1*, *RET*, *NTRK*, as well as *MET* exon 14 skipping (9,10). Based on these insights, combined DNA/RNA-NGS is already the standard of care for tissue testing and has demonstrated feasibility and clinical utility in the real-world setting (11). With the pivotal study by Low *et al.*, a similar standard is now set for LB as well, but with one crucial difference: while at least 5–10 working days are necessary for combined tissue DNA/RNA-NGS pipelines, Low *et al.* could complete their entire sample-to-result workflow in less than 24 hours, using fully automated procedures with minimal hands-on time (1). This turnaround time is astonishingly short and much faster than other LB platforms, for example Guardant360, FoundationOne Liquid CDx, or AVENIO, which all need several days to deliver results. The accelerated procedure reported by Low *et al.* represents an important step forward with major implications for all key players in the field: treating oncologists could provide accelerated patient care owing to the immediate availability of results; small diagnostic facilities in the periphery could easily adopt the faster and user-friendlier workflow in order to provide rapid, fully automated services with the highest standards for medically underserved areas; and major high-throughput centers with heavy workloads could also employ the method in order to free-up many workhours that could be reinvested in assay development, biobanking and translational research activities. Thus, the study by Low *et al.* demonstrates how superior logistics, already a key advantage of liquid over tissue assays, can be improved even more to further boost dissemination of LB use within the oncological community.

Quantity has a quality of its own, and the ability to test more can open several exciting new prospects. Repeat LB at the time of disease progression can identify resistance mechanisms and guide the choice of next-line therapy, as several proof-of-concept series have recently demonstrated (12). Of note, tissue rebiopsies become increasingly difficult along the disease course, with a feasibility ≤50% beyond the first line, which further highlights the better practicability of LB as a crucial advantage over tissue sampling in the pretreated setting (13). Another bonus from the use of LB for tumor retesting is their prognostic relevance: ctDNA detectability at the time of disease progression is associated with shorter time-to-next-treatment and shorter overall survival, which could provide additional support for decision-making in subsequent management, for example inform the choice of whether to continue the current drug or switch systemic therapy in case of oligoprogression (14). Moreover, serially performed LB at regular intervals have demonstrated clinical utility for the close monitoring of advanced NSCLC, for example they can trace clonal dynamics and reveal treatment failure several months earlier than radiologic restaging (15). This is especially important for patients with high-risk oncogene-driven NSCLC, for example "double-positive" *EML4-ALK*v3^{+}*TP53*^{mut} or *EGFR*non-del19^{+}*TP53*^{mut} tumors, which have a very aggressive course (16,17). Nonetheless, better surveillance is also essential for every advanced NSCLC, because 30–50% of these patients will not be able to receive available and effective subsequent therapies after each treatment failure due to rapid clinical deterioration (18,19). For immunotherapy-treated tumors, serial LB have recently also demonstrated great clinical importance, as they can identify a very favorable patient subset with "ctDNA clearance" (aka "complete molecular response") and excellent long-term outcome under immune checkpoint inhibitors (20). Last but not least, accumulating data further extend the clinical applicability of LB to early-stage lung cancer: detection of ctDNA can, for example, identify patients with inoperable stage 3 tumors after chemoradiation who will benefit from subsequent durvalumab consolidation (21), patients with a higher chance of pathologic complete response after neoadjuvant treatment (22), and operable tumors carrying a higher risk of relapse (23). For the non-invasive monitoring of disease without detectable mutations in the blood, alternative ctDNA parameters are currently in development, such as the trimmed median absolute deviation from copy number neutrality (t-MAD) score as a measure of copy number alterations (24), or fragmentomic and epigenetic markers (25), with very promising results. Even the most challenging tasks dependent on minute tumor volumes in the human body, such as assessment of minimal residual

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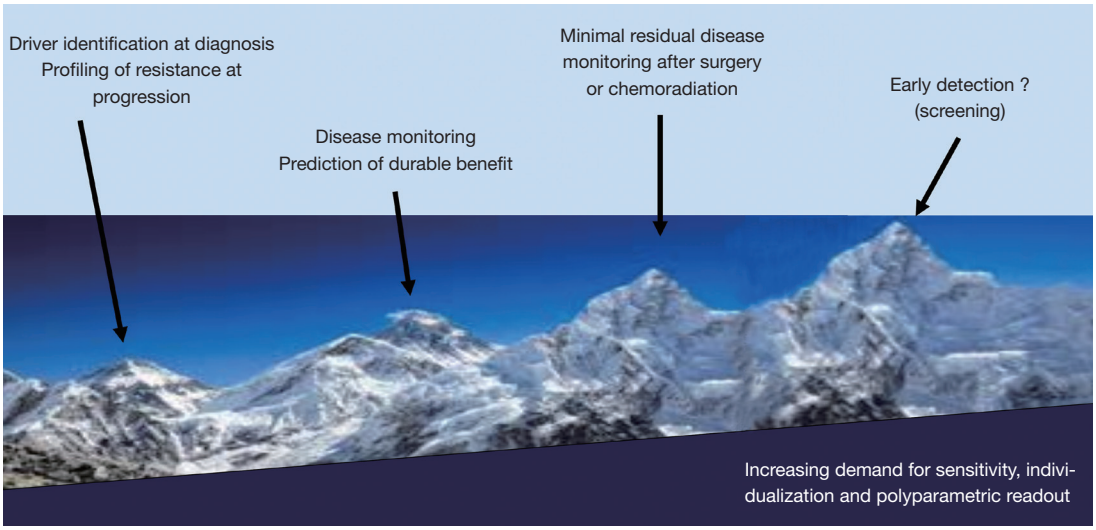

Figure 1 Emerging applications of liquid biopsies in thoracic oncology. From the identification of actionable drivers at initial diagnosis of lung cancer, as described by Low *et al.* and other studies (1,5), to the profiling of acquired resistance at the time of disease progression (12,14), longitudinal monitoring (15,24) with prediction of durable benefit under targeted therapies (14) and immunotherapy (20,22), detection of minimal residual disease (MRD) after surgery (20) or chemoradiation (22), and the ongoing trials of cancer screening in high-risk populations, liquid biopsies master progressively difficult tasks in thoracic oncology, which is becoming a model field for the successful clinical application of novel molecular tools.

disease (MRD) after curative treatments, and screening for lung and other cancers in high-risk populations, are taken on by LB today, since the sensitivity of the method can be increased as needed by the simultaneous use of multiple parameters in personalized assays (*Figure 1*).

The actual obstacle hampering multifaceted routine use of LB in oncology today is financial and ultimately caused by two fundamental issues. First, NGS costs are much higher for ctDNA compared to tissue DNA testing, still well over 1,000 € for one single LB, because allelic frequencies of tumor mutations are much lower in the blood, typically below 1%, and therefore much deeper sequencing coverage is needed to discover the precious "needles" of actionable variants in the "haystack" of abundant circulating germline DNA. Second, despite the promising data no regulatory approval has been granted and no public reimbursement is available for multigene LB panels in Europe and many other parts of the world, yet. In order to overcome the resistance posed by regulatory authorities and financial stakeholders, the clinical utility of LB needs to be demonstrated even more clearly, by exploiting technical innovations in conjunction with well-designed clinical studies. This is one further reason, why the work by Low *et al.* presented in the current issue of *Translational Lung Cancer Research* has particular importance and makes the future of molecular lung cancer diagnostics look even brighter, and closer than ever before.

Acknowledgments

Funding: This work was funded by the German Center for Lung Research (DZL).

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Lung Cancer Research*. The article did not undergo external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-268/coif). The author declares research funding from AstraZeneca, Amgen, Merck, Novartis, Roche, and Takeda; speaker's honoraria from AstraZeneca, Novartis, Roche, Takeda; support for attending meetings from AstraZeneca, Daiichi Sankyo, Eli Lilly, Gilead, Janssen, Novartis, Takeda; and personal fees for participating to advisory boards from Boehringer

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Ingelheim, Chugai, Pfizer and Roche; all outside the submitted work. The author has no other conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Christopoulos P. Liquid biopsies come of age in lung cancer. Transl Lung Cancer Res 2022;11(5):706-710. doi: 10.21037/tlcr-22-268

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