Noninvasive cancer biomarkers in solid malignancies: circulating tumor DNA—clinical utility, current limitations and future perspectives

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Since the first publication of human genome, the cancer community witnessed a substantial progress in available technologies to genetically characterize tumor samples (1). Turnaround times and costs are rapidly decreasing, along with the availability of a myriad of tests using a variety of samples other than conventional biopsy tumor tissue. Detection of circulating tumor DNA (ctDNA) in cancer patients has the potential to offer a precise and non-invasive tumor evaluation. Ranging from cancer diagnosis to prognostic stratification, and treatment guidance, applicability of ctDNA may allow its broad incorporation into clinical practice (2-4).

Both tissue and non-invasive blood tests are commercially available from a variety of vendors, and can be order for treatment decisions outside a clinical trial setting. Questions regarding which patients will benefit from a genomic tumor test evaluation, type and extend of panel testing, and the appropriate biological sample to be evaluated are fundamental topics to be considered when discussing genomic test applicability. Also, the appropriate time for tumor genomic testing in a cancer patient journey can be challenging.

Recently, companies dedicated to this segment experienced a substantial expansion, possibly fomented by the relatively low financial cost, in alignment with feasible massively parallel sequencing of both tumors and germline genomic tests. Internet expansion access, which may even include mobile devices to access both somatic and genomic databases, with tools to communicate and interpret genomic data in a medical context, can likewise increase commercial appeal for a broader audience of physicians and health care providers. Nevertheless, pre-analytical considerations, analytical and clinical validity, as well as utility for daily clinical practice adoption are still a matter of debate and uncertainty.

The appealing denomination of liquid biopsy, and the promise of a patient-friendly method, minimally invasive, with implications for cancer detection, monitoring and treatment, unleashed a plethora of excitement both from the scientific community, and from patient advocacy and media. Recently, the American Society of Clinical Oncology (ASCO) published a review on current literature regarding ctDNA assays, providing a framework and insights for future research on this important growing area of research (5).

Determination of which somatic mutations the assay will evaluate is important for its applicability and indications. Some tests will offer only single or a small variety of mutations reported, while others are able to detected a broader number of variants in multiple genes, through next-generation sequencing (NGS) technology (6). Analytical validity is often performed by comparison between variants detected in tumor biopsy and plasma (7). Although agreement between driver mutations in plasma circulating DNA and metastatic tissue biopsy is ultimately desired to guide tumor evaluation and treatment decisions, there are situations in which this concordance is not entirely fulfilled. Factors such as intratumoral heterogeneity may be crucial for this discordance, as the distribution of genetically distinct tumor cell subpopulations across a single lesion or within distinct disease sites can result in discrepancies among ctDNA and focal tumor biopsy.

Accordingly, concordance between tissue and plasma genomic variant detection can greatly vary (7,8). Of note, this aspect may be seen as a strength from plasma collected samples, as tissue biopsies have spatial limitations, and may represent alterations limited to a specific tumor location. Conversely, peripheral blood genomic evaluation has the potential to access tumor heterogeneity from multiple metastatic sites, truly representing the relevant mutations that might be driving tumor progression in a certain moment of disease evolution (9). Insufficient amount of ctDNA being present in the specimen can also be responsible for failure to detect a somatic variant in a ctDNA assay (6). Also, although not definitively proven, the preferred moment to collect plasma for ctDNA analysis might be when tumor is progressing, instead of responding or stable disease to therapy.

Another important challenge is interpretation of ctDNA results. Relative enrichment of leukocyte DNA may vary on the basis of pre-analytical issues. Cell-free DNA (cfDNA) in the blood circulation must be differentiated from ctDNA (10). In fact, most of the mutations in cfDNA are highly correlated to the ones found in white blood cells (WBCs). Detection of ctDNA by removing the background mutations in WBC and cfDNA are important for ctDNA report refinement and accuracy (11). Relative allele fractions can inform physicians about clonal or subclonal variants, with implications to degree of response to targeted response to targeted therapies directed to the mutation.

Reported allele frequency thresholds can greatly vary among the different commercially available tests, with novel technologies capable to identify lower variant allele frequency (12). Quantification of the proportion of variant reads for a given mutation, representing the proportion of tumor cells that display a specific mutation, can improve the understanding of the disease, with the ultimate goal of predicting how these mutations drive tumor behavior, refining prognostic and targeted treatment benefit prediction.

Once clinical validity has been established, clinical utility should be demonstrated in order enable broad assay adoption. In general, use of ctDNA assays for treatment selection in patients with advanced malignancies is limited due to lack of prospective data, and by moderate test sensitivity and specificity (13,14).

The dilemma of finding potential germline DNA mutations is also another issue for these tests. During somatic tumor DNA sequencing, germline genomic mutations can incidentally be discovered (15). Most of the times, pretest counseling is not routinely offered to these patients, and only after a suspicious alteration is identified, the patient is informed that the somatic mutation represents an underlying germline mutation that could potentially has further health implications, both for the patient itself and also for family members. Performing a family history and genetic counseling prior to obtaining next generation sequencing test are important considerations to be taken into account before ordering it. Ultimately, all stakeholders should be involved, as responsible and efficient application of information obtained with the test is warranted.

Ideally, before establishing ctDNA clinical utility, tissue genomic evaluation clinical utility would have to be definitely established, a premise that is not fully accomplished. A previous trial demonstrated that in metastatic cancer patients in which biopsy samples were obtained and sequenced, a targetable genomic alteration was identified in 46%. Personalized treatment was offered for 13% of the study population. Among them, 9% had an objective response, and 21% had stable disease. Although results seem disappointing, it is important to highlight that patient population was heavily pretreated (16). Additionally, progression-free survival comparison for patients who received targeted therapies with those who did was not performed. To date, the few prospective trials evaluating a genetic biomarker treatment selection for a broad cancer population failed to establish a survival benefit (17). This trial sampled tumor tissue to guide treatment. Nonetheless, prior data established that incorporating a biomarker for treatment selection of cancer patients in clinical trials led to more efficacious therapies (18).

The future of cancer treatment undoubtedly depends on the incorporation of a precision oncology strategy in many disease scenarios. Clinicians are used to the conventional strategy of drug development, in which a compound candidate for commercial approval must fulfill rigorous regulatory steps, including a clear demonstration of clinical benefit in well-conducted randomized prospective clinical trials. Conversely, biomarkers tests can be commercially available even before clinical utility has been established. Some of these tests can result in profound patient management modifications; it is reasonably to evaluate and validate them in a similar rigorous development platform. In this context, the ctDNA ASCO review for its use in current clinical practice in patients with cancer, highlights a clear caution warning, and incorporates the recommendation for further development and refinement of these tests. Community is advised about the lack of proven clinical

utility for most assays commercially available. Further research is needed to enable better assessment of the clinical validity and utility of ctDNA assays.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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