

Effective Cancer Genotyping—Many Means to One End

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Precision cancer medicine requires effective genotyping of every patient's tumor to optimally design treatment plans. Despite its imperfect sensitivity, the rapidity and convenience of cell-free DNA sequencing makes it an essential

complement to tumor genotyping, which, when used appropriately, can aid the pursuit of effective genotyping for all patients.

See related article by Leigh *et al.*, p. 4691

In this issue of *Clinical Cancer Research*, Leigh and colleagues describe a prospective study demonstrating the clinical utility of cell-free DNA (cfDNA) analysis for first-line genotyping of metastatic non-small cell lung cancer (NSCLC) as compared with tissue genotyping (1). Their data raise important and timely questions about the best approaches to achieve effective cancer genotyping in patients with metastatic NSCLC.

Our evolving diagnostic capabilities are moving us toward an inflection point in precision cancer medicine. Since the inception of medical oncology, we have attempted to characterize tumors by their various subsets—beginning with tissue type and morphology, progressing to include key genetic changes (driver mutations, resistance mutations) and broad genomic features (tumor mutational burden, other signatures), and now capturing immunologic factors (tumor microenvironment, expression of immune checkpoint receptors/ligands). Our collective understanding of the molecular makeup of NSCLC and other solid tumors across these categories at the time of initial diagnosis, progression, and therapy resistance is growing rapidly. We have more molecular targets than ever before, and we also have more pharmaceuticals available than we could have dreamed just a few decades ago.

This explosion in development of targeted therapies has been accompanied by a similarly exponential growth in the availability of sophisticated diagnostic tools for genotyping. Nonetheless, more than a decade after the discovery that NSCLCs harboring *EGFR* driver mutations are responsive to tyrosine kinase inhibitors, *EGFR* genotyping is still not performed for every newly diagnosed NSCLC. Indeed, Leigh and colleagues reference multiple studies in which up to 20%–30% of patients with newly diagnosed NSCLC did not have *EGFR* or *ALK* genotyping results prior to initiating therapy. The reasons for this lack of genotyping are myriad, and can include inadequate tumor tissue, testing fatigue, the increasing complexity of care, and perhaps diagnostic nihilism. In some cases, clinicians send PD-L1 IHC to guide

immunotherapy treatment without any genetic sequencing for known drivers—this is still clearly inadequate, as the response rate to pembrolizumab in PD-L1-high (>50%) NSCLC was only 39% in the recent KEYNOTE-042 study (2). Genotyping must inform this decision point, as outcomes on immunotherapy are reliably poor in patients with targetable genotypes and are reliably better in those with a high tumor mutational burden (3). Finally, in addition to these cases in which no genotyping is done, another common scenario is "under-genotyping," or incomplete genotyping (Fig. 1). This can also happen for a variety of reasons, but often results from single-gene testing, which can exhaust tissue and preclude more comprehensive next-generation sequencing (NGS).

In this study, Leigh and colleagues attempt to address the problem of "under-genotyping" by studying the potential for cfDNA sequencing to provide a breadth of genotyping information with a shorter turnaround time (TAT) than tumor tissue genotyping. In their study, 282 patients underwent cfDNA sequencing with Guardant360, an NGS platform assessing for alterations (point mutations, indels, fusions, and amplifications) in 73 genes of interest. They find that cfDNA analysis via the Guardant360 NGS assay detected clinically relevant NSCLC-associated biomarkers at a similar rate as standard-of-care (SOC) testing, noting that during this study the SOC was not tumor NGS for all patients. They further demonstrate that the combination of tissue-based genotyping and cfDNA analysis resulted in a meaningfully higher frequency of identification of NSCLC driver mutations than either method alone. Tissue-based genotyping identified 67% (60/89) of the guideline-recommended biomarkers in first pass, with reflex cfDNA testing identifying the final 33%. In cases where cfDNA genotyping was utilized first, 87% (77/89) of the biomarkers were identified initially, with reflex tumor genotyping identifying the remaining 13%. Notably, the median TAT for cfDNA analysis was significantly lower than that for SOC tumor genotyping (9 days vs. 15 days), with progressive shortening of TAT over the course of the trial as technologies improved.

We share the authors' enthusiasm for cfDNA sequencing as a convenient option for routine NSCLC genotyping. There is now a body of data demonstrating high positive predictive value (PPV) of cfDNA analysis in solid tumor genotyping (4)—a necessary performance characteristic of this diagnostic platform, given the unacceptability of false positives. However, we and others have shown that sensitivity of cfDNA genotyping can be low in patients with lower metastatic burden, likely due to reduced shed of tumor DNA into the plasma (4). This insensitivity of cfDNA sequencing

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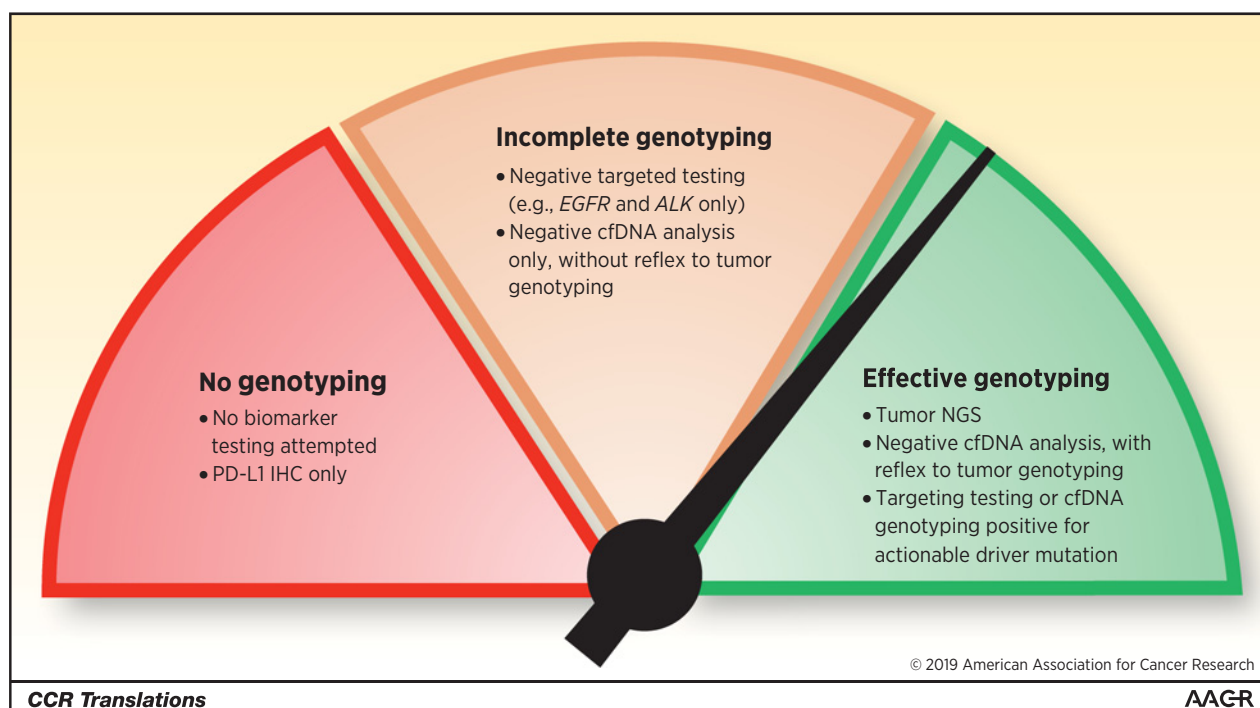


Figure 1.

Achieving effective cancer genotyping: the availability of convenient plasma next-generation sequencing (NGS) assays with a high PPV means that, today, no patients with advanced NSCLC should receive no molecular testing or PD-L1 testing only (red). However, the limitations of our genotyping assays must be recognized if we wish to avoid incomplete genotyping (orange). Targeted assays for common driver mutations (e.g., *EGFR*, *ALK*) can take time and exhaust tissue, making it harder to then test for rarer driver mutations if negative, while cfDNA analysis has imperfect sensitivity such that targetable driver mutations can be missed. These more limited assays can be useful if positive, but further testing must be pursued if they are negative. To offer our patients effective genotyping (green), the most reliable approaches involve either a panel-based assay tested on a high-quality tissue biopsy or cfDNA analysis first, followed by reflex to tumor genotyping if the “liquid biopsy” is negative for a targetable driver mutation.

must be acknowledged as a significant barrier to its application. Although it does not preclude the use of cfDNA sequencing in the appropriate clinical situation, it does require us to be thoughtful about how we apply this technology and the results we conclude from the information gained. Ultimately, negative cfDNA sequencing may be better than no genotyping at all, but it is not sufficient to rule out the presence of targetable driver mutations given the impaired sensitivity of these assays and unknown rate of tumor shed in any given patient. Without careful attention to the sensitivity and specificity of our diagnostic tools, we risk being fooled into thinking we have more information than we actually do.

We must be cautious in thinking, as Leigh and colleagues suggest, that sequencing of cfDNA alone offers “guideline-complete genotyping”—the recent American Society of Clinical Oncology–College of American Pathologists (ASCO–CAP) joint review highlights the need for “reflex to tumor biopsy testing in patients with negative liquid biopsy results” (5). We echo the authors’ suggestion for a role of cfDNA analysis as complementary to tumor analysis, but not a replacement. One could hope that cfDNA sequencing, with its potential for faster TAT and readily available sample in a simple blood draw, could neatly fill some of the gaps that result in incomplete genotyping (Fig. 1). Like our other available diagnostics, it is but one useful addition to our toolkit, and one that we must learn to use well.

In sum, cancer genotyping is not binary. For any given clinical scenario, there are multiple ways to obtain the necessary infor-

mation to make treatment decisions. Our task, as a community of medical oncologists, is to first discern what is the necessary and sufficient genotyping information to fully inform treatment decisions. Second, we must acknowledge and overcome the logistical barriers that prevent our ability to achieve this for every patient. Thanks to a rapidly progressing field in both diagnostics and therapeutics, this will be an iterative process for decades to come. If we can continue to leverage our collective understanding of the biology of solid tumors, the targeted therapies available for clinical use, and the health care delivery systems we work within, we have the ability to make meaningful advances in the field of precision cancer medicine. Through establishing criteria for what qualifies as effective cancer genotyping, we maximize the chance of it being implemented universally for our patients with NSCLC.

Disclosure of Potential Conflicts of Interest

G.R. Oxnard is a consultant/advisory board member for GRAIL, AstraZeneca, Foundation Medicine, DropWorks, Inivata, and Janssen. No potential conflicts of interest were disclosed by the other author.

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