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Editorials

Plasma-Derived Tumor DNA Analysis at Whole-Genome Resolution

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The last 2 years have witnessed an extraordinary change in our understanding of the somatic mutational landscape of solid tumors, driven by the impressive large-scale sequencing efforts from such groups as the Cancer Genome Atlas and the Cancer Genome Project. As is so often the case with such ambitious scientific endeavors, the complexities these efforts have revealed raise even greater questions for the future. These studies have exposed the scale of diversity in the mutational landscape among tumors of the same histopathologic subtype. For example, between 1 and 3 genes in high-grade serous ovarian cancer or triple-negative breast cancer are subject to somatic mutations in >10% of patients with the same histopathologic subtype of disease *(1, 2)*. The remainder of the somatic mutational landscape is dominated by genes subject to mutations at much lower frequencies. Low-frequency but potentially targetable events challenge conventional approaches to personalized medicine, both in terms of the health-economic costs in developing therapeutics for such small numbers of patients and in terms of tumor screening to identify such low-frequency somatic events.

Adding to this layer of complexity is the increasing evidence for profound heterogeneity in DNA copy number states, somatic mutational and ploidy profiles that may be distinguished within a single tumor sample *(3–5)* or between primary and metastatic sites *(6–9)*. Indeed, such subclonal diversity is thought to contribute to adaptation for tumor growth at distant sites of disease *(10)*. Increasing evidence suggests that the emergence of drug resistance in solid tumors may be predetermined by the presence of low-frequency heterogeneous tumor subclones harboring somatic mutations that confer resistance to the targeting anticancer agent *(11)*. Finally, also emerging is clear evidence that the subclonal dynamics of both hematologic and solid tumors change over time [reviewed in *(10)*] to such an extent that the subclone that ultimately determines disease outcome may barely be detectable at diagnosis *(12)*.

Such findings have begun to raise profound questions regarding how mutational and DNA copy number profiles can be defined rapidly in individual patients and how future personalized cancer therapeutics can be designed to limit disease progression, predict the early emergence of drug resistance, and resolve the changing nature of a tumor's subclonal dynamics over time *(13)*. Given this emerging evidence for intratumor heterogeneity in somatic mutational status between sites of disease and for the risks associated with biopsying multiple sites of disease *(6, 14)*, it is critical to determine how less-invasive techniques can be established to profile a tumor's somatic mutational load more rapidly and to track its evolution over time. Arguably, the future of oncologic care will rely on more accurate and less invasive diagnostic and predictive tools. Progress in our understanding of drug resistance and tumor metastases will undoubtedly depend on our ability to monitor a tumor's subclonal dynamics and the Darwinian selection of tumor subclones, preferably with just a blood sample rather than multiple sequential biopsies of metastatic sites.

The analysis of circulating free tumor DNA (ctDNA)[3] has been primed for some time to overcome the hurdle of invasive biopsies by identifying predetermined somatic mutations via a "liquid biopsy" *(15)*. Until now, however, ctDNA analysis has not been able to resolve distinct copy number events in tumors or to resolve single-nucleotide variants (SNVs) on a genomewide scale. In this cancer-themed issue of *Clinical Chemistry*, Lo and colleagues provide long-awaited evidence that massively parallel sequencing (MPS) analysis of ctDNA can resolve DNA structural aberrations across the entire genome at 1-Mb resolution *(16)*. These authors demonstrate, with 4 patients with hepatocellular carcinoma, the striking representation of copy number events detected in a tumor in events detected in ctDNA, and the almost complete loss of these events from the plasma after tumor resection. In con-

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[3] Nonstandard abbreviations: ctDNA, circulating free tumor DNA; SNV, single-nucleotide variant; MPS, massively parallel sequencing.

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trast, only 1% of the sequencing bins for ctDNA isolated from 4 carriers of the hepatitis B virus with no detectable tumor revealed abnormal copy number aberrations, a finding that emphasizes the potential for ctDNA assessment as a screening tool for this disease. Intriguingly, the authors detected ctDNA aberrations that were not present in the single biopsy obtained from an individual's hepatocellular carcinoma. Although Lo and colleagues reflect that such ctDNA aberrations represent reduced specificity for the use of ctDNA analysis for hepatocellular carcinoma, an alternative explanation is that a single biopsy of a tumor may underrepresent the copy number landscape, as has been shown for renal cancer *(6)*. It would therefore be important to assess an equally plausible explanation—that ctDNA analysis may in fact be superior to a single tumor biopsy for resolving the majority of copy number aberrations present in a single tumor.

Remarkably, Lo and colleagues demonstrated through their study of a patient with synchronous breast and ovarian primaries that the structural DNA copy number aberrations detectable in plasma ctDNA were a hybrid of the ovarian and breast primaries. They elegantly demonstrate that the attenuation of DNA copy number signals in ctDNA after sequential, same-day surgical resection of the breast primary and bilateral ovarian cancers directly mirrors the single-nucleotide polymorphism/comparative genomic hybridization copy number profiles derived from the surgically resected tumors. Although the breast primary was only 3 cm in size and contributed an estimated 2.1% of the total ctDNA, the authors showed that this analytically sensitive technique was still able to detect breast cancer–specific copy number aberrations—such as a deletion in chromosome 6p and amplifications on chromosomes 1q, 7p, and 15q—that were present only in the breast primary and disappeared soon after breast surgery.

These data provide conclusive evidence that the use of plasma-derived ctDNA for unbiased analysis of the structural genomic landscape of tumors over time is now fit for implementation in the research setting. Given the clinical risks of repeat tumor biopsies and the challenges associated with inter- and intratumor heterogeneity, this landmark study will enable rapid progress in our understanding of tumor evolution over time.

Perhaps most importantly, the authors have used ctDNA analysis to explore the phenomenon of intratumoral heterogeneity in ovarian cancer. Although 4 regions of the bilateral ovarian tumors had almost identical copy number profiles, the authors found SNVs specific to a single region, shared SNVs within a single ovarian cancer, and ubiquitous SNVs present bilaterally. Using a mass spectrometry–based iPLEX analytical method, the authors conclusively identified 95% of the 67 SNVs selected for validation in the tumor DNA. The authors then leveraged this analysis to define the presence of these SNVs in the MPS data from ctDNA before and after surgery. In their analysis of the ubiquitous SNVs, the authors estimated that these SNVs accounted for 46% of the ctDNA before surgery and 0.18% after bilateral oophorectomy. When the authors focused their attention on the SNVs shared by one or the other of the ovarian tumors, they found that the degree of a tumor's SNV representation in the ctDNA mirrored the size of the ovarian tumor mass on the side from which these SNVs derived, suggesting that ctDNA may be a useful surrogate of residual disease.

These data have important implications. First, it is apparent that tumor heterogeneity poses a substantial problem to the use of ctDNA focused on tumor-specific mutations as a tool to predict early relapse or to monitor the emergence of drug resistance, if the focus is on somatic events that may not be ubiquitous throughout the tumor mass. Second, these data support the use of unbiased shotgun MPS approaches to ctDNA analysis—particularly in the context of studying the subclonal dynamics of the disease through therapy—for attempting to resolve the emergence of distinct tumor subclones harboring low-frequency somatic events conferring drug resistance. With the future acceleration of DNA sequencing and data analysis, the shotgun MPS approach to ctDNA analysis has the potential to provide a powerful tool for deciphering the Darwinian mechanisms of tumor evolution and to keep pace with tumor subclonal dynamics through the disease course.

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