Clinical Cancer Research

Clonal Hematopoiesis: A New Layer in the Liquid Biopsy Story in Lung Cancer

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Cell-free DNA (cfDNA) is a unique biospecimen that contains multiple sources of DNA, including tumor, germline, fetal, and others. Clonal hematopoiesis, a process that leads to expansion of mutations in peripheral blood cells, is an additional source of DNA that adds a layer of complexity when interpreting results. *Clin Cancer Res; 24(18); 4352–4. ©2018 AACR.*

See related article by Hu et al., p. 4437

In this issue of *Clinical Cancer Research*, Hu and colleagues report on recurrent mutations in KRAS, p53, and JAK2 identified in peripheral blood cells (PBC) from patients with non–small cell lung cancer (NSCLC; ref. 1). The authors compared the DNA isolated from PBCs with next-generation sequencing (NGS) results from plasma cell-free DNA (cfDNA) and tumor tissue. By demonstrating that these alterations exist in PBCs, but not in paired tumor tissue, the authors conclude that these are a consequence of clonal hematopoiesis, a process that can lead to expansion of mutations in PBCs. The authors state that this represents a false-positive finding in cfDNA testing, introducing an additional layer of complexity when interpreting cfDNA reports.

The recent introduction of cfDNA platforms has altered the diagnostic paradigm for the targeted treatment of advanced NSCLC, allowing physicians to overcome many common barriers in biopsy tissue stewardship. cfDNA has a number of advantages: It is minimally invasive, can be performed serially, and molecular alterations identified in cfDNA seem to be as predictive of response to targeted therapies as those identified in tissue (2). The College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology (CAP-IASLC-AMP) have recently updated their guidelines to recommend the use of cfDNA to "rule in" the presence of a targetable aberration among patients with advanced NSCLC with limited biopsy samples (3). Despite the promise of cfDNA, there are certain limitations. cfDNA has a lower sensitivity for the detection of mutations than tumor tissue, and so it is not an effective tool to "rule out" the presence of a target (2). The rapid development of multiple commercial-based plasma platforms has introduced additional challenges including lack of harmonization of preanalytic workflows as well analytic validations that have yet to be standardized across assays. Furthermore, a test that is analytically validated with reproducible results is not necessarily clinically valid or useful (4). Each assay sets sensitivity cut-off points to amplify signal and minimize noise, but the very nature of the sample means DNA will be heterogeneous. DNA captured in a plasma cfDNA specimen may be from multiple sources other than tumor, including germline, fetal, post-organ transplant, and others (Fig. 1). These mixed sources have been routinely detected in commercial-based assays and can impact the interpretability of results.

Clonal hematopoiesis, or the development of somatic mutations in hematopoietic cells, adds a further wrinkle to this discussion. As evidenced by a recent retrospective analysis evaluating paired tumor and blood samples from 5,649 nonhematologic patients, these mutations occur in more than 25% of patients with NSCLC ($n = 818$) and are strongly associated with both smoking and prior radiotherapy (5). The majority of these mutations are from leukemia-associated genes; however, less frequent clonal hematopoiesis alterations with known relevance in NSCLC have been identified, including JAK2, KRAS, and TP53. In an unpublished subset analysis of the aforementioned series, TP53 and JAK2 related to clonal hematopoiesis were each noted in only 1% of patients with NSCLC (11/818 and 9/818, respectively; M. Zehira; personal correspondence). Interestingly, in the article by Hu and colleagues, TP53 mutations were identified at a much higher rate (108/143 specimens), and a majority of patients with paired tissue samples did not have TP53 mutations in tumor (1). In all, 15% (5/33) of patients with paired tumor tissue and 3% (5/143) of the total cohort had TP53 mutations isolated from PBCs alone. This higher rate observed compared with the work by Coombs and colleagues may be due to assay sensitivity but underscores the importance for further research that may characterize distinct clonal hematopoiesis profiles in patients with NSCLC.

A disparate clonal hematopoiesis profile of NSCLC could have important clinical implications. We would argue that one must view the term "false positive" through the same prism in which we consider the validity of the test. If a test reliably and accurately identifies a mutation with potential clinical implications (e.g., JAK2 mutations in PBCs or BRCA1 mutations in nontumor cells), it would be hard to call that a clinically false-positive result. Although we agree that these findings make the interpretation of cfDNA more complex, we remain uncertain whether the term "false positive" is the most appropriate and believe that more data

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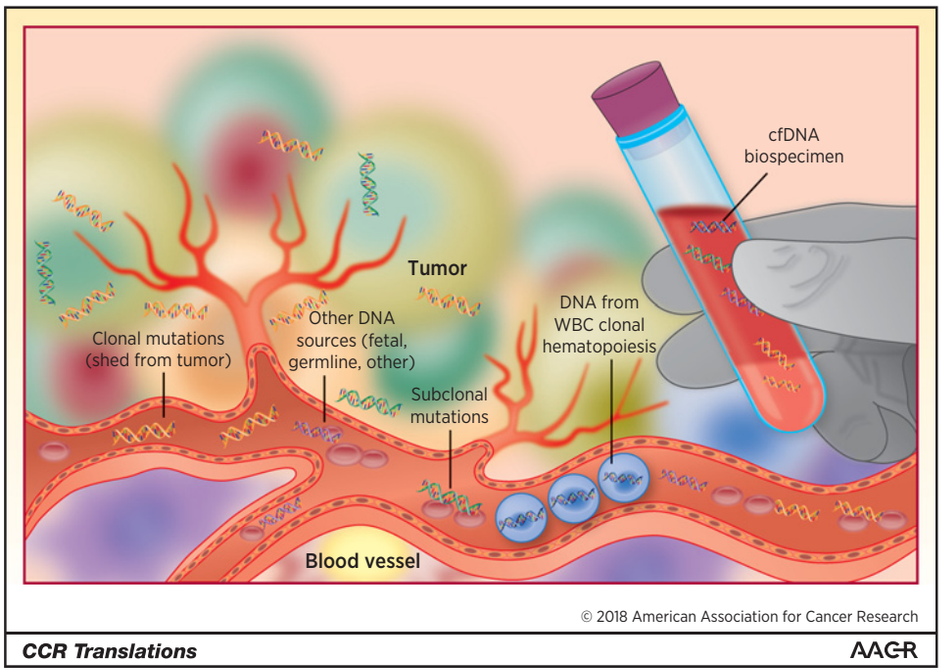

Figure 1.
cfDNA biospecimen contains multiple sources of DNA including clonal alterations, subclonal mutations, clonal hematopoietic aberrations, and other sources of DNA including germline, fetal, post-organ transplant, or concomitant malignancies. WBC, white blood cell.

are needed on the clinical implications of clonal hematopoiesis in NSCLC. Indeed, Coombs and colleagues reported impaired survival among some patients with concerning mutations in clonal hematopoiesis that was not linked to the development of a secondary malignancy but from worse outcomes from their primary cancer diagnosis.

Nevertheless, the authors should be commended for their findings, which have important clinical implications. As the authors noted, a KRAS mutation identified in plasma does not necessarily imply that a patient's tumor harbors KRAS, as this could be a consequence of clonal hematopoiesis. Although KRAS is not currently a targetable aberration, the near mutual exclusivity of KRAS mutation and other targetable molecular alterations may erroneously cause a clinician to stop tissue genotyping efforts whether KRAS was identified in cfDNA. When one considers the exponential increase in the number of targets for NSCLC coupled with highly active therapies that have emerged in the past decade alone, it becomes clear that any result that limits appropriate patient access to these agents is of major concern. These findings amplify our current practices regarding cfDNA testing; they are an excellent adjunct to rule in a targetable aberration but have a limited role in ruling out a target.

Multiple trials are currently underway to utilize cfDNA to identify early recurrences or minimal residual disease after curative intent therapy (6). The authors appropriately raise a concern that the identification of a mutation associated with clonal hematopoiesis after a curative approach could prompt a clinician to inappropriately suspect recurrence. The authors advocate concurrent sequencing of PBCs as done in their study to compare and confirm the presence of tumor DNA. We would suggest an alternative approach would be performing DNA sequencing on the original tumor that informs patient-level assay construction of a more targeted list of mutations to utilize in subsequent cfDNA assessment. The principle remains valid, however. Simply detecting a mutation on cfDNA is insufficient to prove recurrence, and further study on the clinical utility of cfDNA in this setting is warranted.

Despite the appropriate caution these findings provide toward the use of cfDNA, it is important to remember a few key points. The vast majority of mutations reported in clonal hematopoiesis are nonoverlapping with mutations frequently identified in solid tumors. To our knowledge, molecular aberrations that are associated with guideline-recommended therapy for NSCLC have not been reported in clonal hematopoiesis. As such, when patients with NSCLC have a targetable molecular aberration on cfDNA, it remains appropriate to treat this as a clinically actionable result.

As plasma-based NGS platforms continue to increase their breadth and depth of analysis, clinicians will face more challenges in isolating what is clinically actionable in the setting of increased levels of "noise." Although these findings add an appropriate level of caution to the use of cfDNA, we continue to feel that cfDNA testing is an important tool in the diagnosis and management of NSCLC. Indeed, it is possible that some of the noise from clonal hematopoiesis could have clinical import and thus be a separate "signal." It is our hope that future prospective efforts through both industry and cooperative groups will mandate plasma to enable a firmer understanding of how to best interpret and utilize this unique biospecimen.

Disclosure of Potential Conflicts of Interest

J. Bauml reports receiving commercial research grants from Bayer, Carevive Systems, Janssen, Merck, and Novartis, other commercial research support from Incyte, and is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Celgene, Genentech, and Guardant Health. B. Levy reports receiving commercial research grants from Boehringer Ingelheim and Celgene and is a consultant/advisory board member for Admera Health, AstraZeneca, Celgene, Eli Lilly, Genentech, Merck, and Takeda.

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References

1. Hu Y, Ulrich BC, Supplee J, Kuang Y, Lizotte PH, Feeney NB, et al. False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 2018;24:4437–43.
2. Marmarelis M, Thompson JC, Aggarwal C, Evans TL, Carpenter E, Cohen RB, et al. Emerging uses of circulating tumor DNA in advanced stage non-small cell lung cancer. Ann Transl Med 2017;5:380.
3. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018;13:323–58.
4. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. J Clin Oncol 2018;36:1631–41.
5. Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell 2017;21:374–82.
6. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545:446–51.