

## IN THE SPOTLIGHT

## Whole-Genome Sequencing and Cancer Therapy: Is Too Much Ever Enough?

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**Summary:** This issue of *Cancer Discovery* features an article that describes the use of whole-genome sequencing to discover an actionable genetic alteration that was not detected using a lower resolution diagnostic approach. This finding highlights the growing debate surrounding the optimal deployment of powerful new genomics technologies in the clinical oncology arena. *Cancer Discov*; 2(9); 766-8. ©2012 AACR.

Commentary on Dahlman et al., p. 791 (8).

Cancer genome discovery continues to unveil new insights into “driver” mechanisms that govern the genesis, persistence, and progression of many malignancies. However, clinical indications for tumor genetic testing and therapeutic choice remain narrow compared with the possibilities brought forth by genome discovery. For example, malignant melanoma contains numerous genetic alterations that may be “druggable” in principle (1)—but clinical genetic tests in this malignancy focus primarily on codon 600 of the *BRAF* oncogene because mutations therein predict clinical response to RAF inhibitors such as vemurafenib (2, 3) and dabrafenib (4). Several academic cancer centers and commercial vendors are pursuing a more categorical approach to tumor genomic profiling by leveraging so-called “allele-based” technologies that additionally interrogate approximately 40 to 500 individual mutations affecting approximately 10 to 40 known cancer genes (5-7). Although these efforts eclipse the scope of many clinical tests, they vastly underrepresent the spectrum of known cancer gene mutations that are “actionable” in principle. This “actionability gap” highlights a fundamental question pertaining to implementation of “precision” cancer medicine: Should we continue on a validation path that queries a relatively small number of genes/mutations at a time? Or is it preferable to adopt a more aggressive framework that leverages state-of-the-art technologies and analytics to produce comprehensive genomic information and thereby identify a fuller spectrum of plausibly “actionable” events in individual tumors?

In this issue of *Cancer Discovery*, Dahlman and colleagues (8) address these questions in a case study of aggressive, meta-

static melanoma. In this patient, “allele-specific” tumor genetic studies had come up empty: the melanoma was “wild-type” for *BRAF*<sup>V600E/K</sup> mutations (which occur in 40%–50% of cutaneous melanoma) and several common *KIT* mutations (which are typically observed in acral and mucosal melanomas). To determine what may have been missed by the allele-based assays in this aggressive tumor, the investigators conducted post-mortem whole-genome sequencing (WGS) of the tumor and paired germline DNA. This effort identified a *BRAF* mutation only 3 codons away from the site queried by existing genetic tests: encoding a leucine-to-arginine substitution at position 597. From a biologic standpoint, this observation was not unexpected: approximately 10% of *BRAF* mutations involve sites other than codon 600, and the *BRAF*<sup>L597R</sup> mutation has previously been reported in melanoma and other cancers (Fig. 1; ref. 9). Nonetheless, one cannot help but wonder whether knowledge of this mutation might have opened additional clinical trial avenues for this patient had it been identified while the patient was still alive.

Before delving further into this possibility, it is fair to ponder whether WGS might have been “overkill” in this particular instance. After all, most information that emerged from WGS seemed irrelevant to the “index” observation of a “non-V600” *BRAF* mutation that drove the subsequent focus of this study. Although the investigators invested a considerable and laudable effort into identifying the myriad alterations present in this tumor genome (>60% of which consist of cytosine-to-thymidine transitions characteristic of UV light-induced DNA damage), most of this information would likely be uninterpretable to all but the most genomically rarefied oncologists. Moreover, it remains costly to sequence and analyze an entire cancer genome in an environment of Clinical Laboratory Improvement Amendments (CLIA). Finally, few if any groups have yet achieved routine WGS of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue. This limitation poses an additional technical constraint on the practical clinical use of this technology.

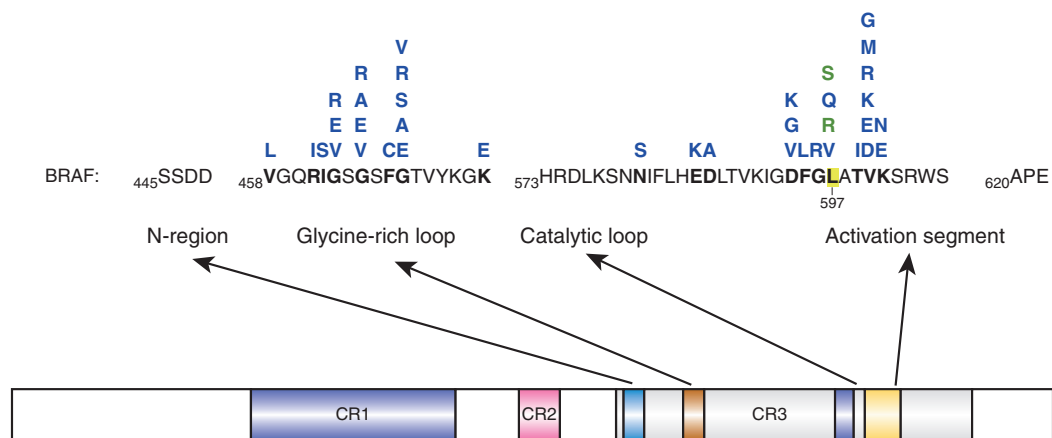
At the same time, many translational oncologists recognize that genomic profiling platforms focused solely on individual mutations are becoming outdated. The study by Dahlman and colleagues (8) exemplifies the singular limitation of such approaches: They miss many plausibly “actionable” alterations. Indeed, neither mass spectrometric nor PCR-based approaches are able to detect most chromosomal

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**Figure 1.** Oncogenic *BRAF* mutations have been described in approximately 8% of human cancers, including at least 50% of melanoma. The most common mutations (V600E/K) occur at codon 600 in the activation segment, but other *BRAF* mutations can also drive carcinogenesis by activating MEK/ERK signaling. Some *BRAF* loss of function mutations may still activate MEK/ERK signaling through heterodimerization with CRAF (19). Amino acid changes in cancer (blue) are shown above the *BRAF* sequence. L597 is highlighted and the S and R substitutions are shown (green).

copy number alterations and rearrangements at the DNA level; they are also highly insensitive for inactivating tumor suppressor gene mutations. Toward this end, the *BRAF*<sup>L597R</sup> mutation described in this study occurred in the context of *BRAF* gene amplification, thus drawing additional attention to its likely importance as a driver in this tumor. More generally, the report by Dahlman and colleagues (8) illustrates that allele-specific technologies have proven inadequate even for detection of lower frequency mutations in known, “actionable” oncogenes. This vexing limitation is readily overcome by massively parallel sequencing-based platforms that interrogate larger cancer gene panels (e.g., hundreds of known cancer genes; refs. 10, 11). Thus, although global approaches such as WGS or whole-exome sequencing may arguably overshoot the present clinical demand, it seems likely that some expansion in the scope of tumor genomic profiling may prove beneficial for emerging clinical studies of “precision” cancer medicine—particularly if such approaches can be implemented using archival (e.g., FFPE) materials and at a cost and turn-around time that is acceptable to clinicians.

Having identified *BRAF*<sup>L597R</sup> in this sample and confirmed its recurrence in 49 additional melanoma tumors that lacked *BRAF*<sup>V600E/K</sup> mutations, the investigators postulated that this or similar “non-V600” *BRAF* mutations might confer sensitivity to mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitors. Indeed, biochemical studies of cells expressing the mutant *BRAF* constructs suggested that pharmacologic MEK inhibition might be more effective than RAF inhibition in suppressing MAP kinase (ERK) activation in these settings. Most importantly, the investigators genetically characterized a *BRAF* “wild-type” melanoma from a patient who experienced a partial response to MEK inhibition and found that this tumor harbored a very similar *BRAF*<sup>L597S</sup> mutation. Together, these results suggested that mutations involving codon 597 or other “non-V600” changes might predict sensitivity to MEK inhibitors.

When these provocative mechanistic and clinical findings are considered in light of the WGS data from the “index”

melanoma tumor, several more subtle aspects emerge that collectively augment the potential benefits of comprehensive tumor genomic profiling. Here, it is important to note that other developmental therapeutic avenues aside from MEK inhibitors might in principle have been entertained for this patient, had the investigators known about the *BRAF*<sup>V597R</sup> mutation. For example, the investigators may conceivably have considered a trial of a RAF inhibitor with properties distinct from that of vemurafenib (e.g., a small molecule with enhanced potency or measurable activity against other RAF isoforms), or perhaps a clinical trial combining RAF and MEK inhibitors. This combination results in a high response rate and doubles the time to disease progression when compared with single-agent *BRAF* or MEK inhibitors in *BRAF*-mutant melanoma (12). Alternatively, they might have considered an ERK inhibitor (such agents have recently entered phase I trials). RAF inhibitors, however, are contraindicated in tumors that contain upstream RAS activation—these drugs produce a paradoxical activation of MEK/ERK signaling when administered to tumor cells that exhibit dysregulated RAS signaling (13–15). In melanoma, the most common mechanism of RAS activation involves oncogenic *NRAS* mutation, which occurs in 15% to 20% of patients. Importantly, *NRAS* mutations can co-occur with “non-V600” *BRAF* mutations in melanoma (5). Although the “index” tumor did not contain *NRAS* mutations, it did harbor an unusual *KRAS* mutation known to occur as a pathogenic germline event in patients with cardiofacial-cutaneous syndromes (characterized by aberrant RAS/MAP kinase signaling). In addition, this tumor contained a mutation in *NFI*, a tumor suppressor gene whose inactivation causes dysregulated RAS signaling. Neither the *KRAS* nor *NFI* mutations would have been detected by allele-based tumor genomic profiling platforms; however, their presence effectively excludes RAF inhibition as a viable therapeutic option in this patient.

An additional benefit of comprehensive tumor genomic profiling may involve consideration of so-called pertinent negatives. (This term refers to diagnostic abnormalities whose absence

carries clinical importance.) For example, WGS found no evidence of *MAP2K1* or *MAP2K2* mutations (these genes encode MEK kinases, which signal directly downstream of RAF in the MAP kinase cascade). *MEK1/2* mutations occur in approximately 5% to 10% of melanomas (16), and some may confer resistance to MEK inhibitors (17). Furthermore, this tumor contained a *PTEN* tumor suppressor gene mutation but apparently lacked concomitant mutations in the *RB1* gene [encodes the retinoblastoma (RB) tumor suppressor]—this absence is important in light of preclinical studies showing that combined *PTEN*/*RB* loss can confer MEK independence (and therefore resistance to MEK inhibitors) in melanoma (18). Of course, certifying the pertinent negative status of such genes requires that all exons be sequenced to a depth of coverage that ensures robust mutation detection. Nonetheless, to a first approximation, the WGS data enabled identification of multiple pertinent positive and negative genetic observations that might have supported enrollment of this patient into a clinical trial of a MEK inhibitor.

Even if robust tumor genetic profiling (e.g., hundreds of cancer genes queried simultaneously) ultimately proves necessary, this intervention will not likely be sufficient for high-impact “precision” cancer medicine. Innovative approaches will also be needed to discern therapeutic combinations that might be tested in individual patients based on tumor genetic/molecular profiling information. The availability of WGS data in the study by Dahlman and colleagues (8) again offers a tantalizing view into such prospects. The aforementioned *PTEN* mutation might have endorsed a clinical trial of combined MEK and phosphoinositide 3-kinase inhibition. Moreover, the sequenced melanoma tumor harbored a *BRCA2* mutation, which raises the intriguing notion of adding a PARP inhibitor to a MEK inhibitor in such settings. Expanded efforts are needed to design clinical trials that test both genomics-driven hypotheses and dosing/scheduling of novel anti-cancer drug combinations. In parallel, advances in pharmacodynamic and imaging modalities will be needed to determine whether the relevant therapeutic targets are being adequately intercepted at the tumor site that was subjected to genomic profiling. Overall, the study by Dahlman and colleagues (8) elaborates one of many components that will be required to “move the needle” of precision cancer medicine in a manner that provides durable benefit to many patients with cancer. And for the time being, it may also suggest that a comprehensive analysis of *BRAF*-activating mutations beyond V600 should be incorporated into the emerging framework for melanoma-targeted therapy.

### Disclosure of Potential Conflicts of Interest

L.A. Garraway has a commercial research grant from Novartis, has ownership interest in Foundation Medicine, and is a consultant/advisory board member for Novartis, Foundation Medicine, and Millennium. No potential conflicts of interest were disclosed by the other author.

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### REFERENCES

- Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. *Cell* 2012;150:251–63.
- Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010;363:809–19.
- Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012;366:707–14.
- Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012;380:358–65.
- Thomas RK, Baker AC, Debiassi RM, Winckler W, Laframboise T, Lin WM, et al. High-throughput oncogene mutation profiling in human cancer. *Nat Genet* 2007;39:347–51.
- MacConaill LE, Campbell CD, Kehoe SM, Bass AJ, Hatton C, Niu L, et al. Profiling critical cancer gene mutations in clinical tumor samples. *PLoS One* 2009;4:e7887.
- Dias-Santagata D, Akhavanfard S, David SS, Vernovsky K, Kuhlmann G, Boisvert SL, et al. Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol Med* 2010;2:146–58.
- Dahlman KB, Xia J, Hutchinson K, Ng C, Hucks D, Jia P, et al. *BRAF*<sup>L597</sup> mutations in melanoma are associated with sensitivity to MEK inhibitors. *Cancer Discov* 2012;2:791–7.
- Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. *Cancer Cell* 2004;6:313–9.
- Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov* 2012;2:82–93.
- Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat Med* 2012;18:382–4.
- Weber JS, Flaherty KT, Infante JR, Falchook GS, Kefford R, Daud A, et al. Updated safety and efficacy results from a phase I/II study of the oral BRAF inhibitor dabrafenib (GSK2118436) combined with the oral MEK 1/2 inhibitor trametinib (GSK1120212) in patients with BRAF-naïve metastatic melanoma. *J Clin Oncol* 30, 2012 (suppl; abstr 8510).
- Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 2010;140:209–21.
- Poulidakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 2010;464:427–30.
- Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* 2010;464:431–5.
- Nikolaev SI, Rimoldi D, Iseli C, Valsesia A, Robyr D, Gehrig C, et al. Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. *Nat Genet* 2012;44:133–9.
- Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci U S A* 2009;106:20411–6.
- Xing F, Persaud Y, Pratilas CA, Taylor BS, Janakiraman M, She QB, et al. Concurrent loss of the PTEN and RB1 tumor suppressors attenuates RAF dependence in melanomas harboring (V600E)BRAF. *Oncogene* 2012;31:446–57.
- Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004;116:855–67.

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