Identification of BRAF Kinase Domain Duplications Across Multiple Tumor Types and Response to RAF Inhibitor Therapy

The Raf family (ARAF, BRAF, CRAF) of serine/threonine kinases are activators of downstream MEK kinases in the Ras-Raf-MEK-ERK signaling pathway. BRAF is the most potent activator of MEK kinases, and alterations in the Ras-Raf-MEK-ERK pathway are observed in nearly 30% of human malignant neoplasms. More than 300 BRAF mutations are described, mostly within exons 11 through 15 of the catalytic kinase domain (CR3), and BRAF V600E accounts for 90% of observed BRAF mutations.1 Alternate mechanisms of BRAF activation including amplification, non-V600 mutations, and chromosomal rearrangements are less well studied. BRAF fusions have been observed infrequently, and BRAF amplification arises more commonly as a resistance mechanism to RAF-directed tyrosine kinase inhibitor therapy.2 BRAF kinase domain duplication (BRAF-KD) has only been observed in gliomas.

Report of a Case | A woman in her 30s with an advanced acinic cell tumor of the right parotid gland was initially treated with systemic chemotherapy, followed by empirical erlotinib hydrochloride therapy. Her disease ultimately progressed with neck, liver, and lung lesions (Figure 1A). Progression biopsy of the right parotid gland mass was subjected to comprehensive genomic profiling revealing an intrachromosomal duplication event at 7q34 with a breakpoint at intron 9 resulting in duplication of the entire BRAF kinase domain (Figure 2A-D), and an additional breakpoint in the intergenic space downstream of the BRAF gene (Figure 2E). No other oncogenic driver alterations were observed across the 315 genes assayed (data not shown). She was treated with regorafenib monohydrate and achieved a considerable partial response in all disease sites (Figure 1). As this article went to press, the patient continued to maintain a partial response lasting more than 12 months with regorafenib therapy.

Results | To identify additional BRAF-KD events, we interrogated sequencing data from 50,000 clinical samples in the Foundation Medicine Inc database. Nine samples harbored 3′ duplication of the BRAF gene, with 8 of 9 cases (89%) having a genomic breakpoint at intron 9. In a single sample the BRAF breakpoint occurred at intron 3, resulting in duplication of exons 3 through 18 (Figure 2E). BRAF-KD was mutually exclusive from other tyrosine kinase fusions and established oncogenic alterations. BRAF-KD represented 0.5% of BRAF alterations and was not identified in available Catalogue of Somatic Mutations in Cancer or Cancer Genome Atlas data.

Discussion | Using comprehensive genomic profiling, we identified BRAF-KD across multiple tumor types and demo-
strate response to RAF-directed therapy. The tandem duplications observed here lack the N-terminal autoinhibitory domain, and deletion of the N-terminal RAS-binding domain is known to cause constitutive kinase activation.\(^3\) BRAF-KD is predicted to generate a protein with 2 functional kinase domains, one of which cannot be regulated by the CR1 regulatory domain. Nearly all BRAF-KDs occurred at breakpoints in intron 9 of the BRAF gene, and intron 9 insertions generate a truncated transcript analogous to the BRAF alternate splice form that is an observed resistance mechanism in melanoma.\(^4\) BRAF duplication results in increased BRAF mRNA and expression of CCND1, a well-established downstream mitogen-activated protein kinase (MAPK) target gene.\(^5\) Thus, the BRAF-KD observed here would be expected to activate downstream MAPK signaling. The salivary acinic cell carcinoma contained a BRAF-KD with no other putative driver alterations, suggesting that the response is attributable to BRAF inhibition, and regorafenib has demonstrated efficacy in BRAF fusions retaining the entire BRAF kinase domain in preclinical models.\(^6\) However, regorafenib is a multitargeted tyrosine kinase inhibitor and in the absence of functional validation we cannot definitively conclude that BRAF-KD inhibition is the sole mechanism of efficacy.

Increasing clinical incorporation of tumor-profiling technologies is likely to further refine the clinicopathologic features of BRAF-KDs. Overall, this is the first report and largest series examining BRAF-KDs, providing evidence that BRAF-KDs are a clinically important genomic alteration and therapeutic target.

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A, BRAF is located on chromosome 7q34. B, The kinase domain spans exons 11 through 18. C, The BRAF kinase domain is duplicated owing to genomic rearrangement. D and E, Graphical representation of the 3' portion of the BRAF gene from 9 patients with BRAF kinase domain duplication. D, Eight patients harbored a breakpoint in intron 9. E, One patient sample contained a breakpoint in exon 3. All observed events completely encompass the kinase domain spanning exons 11 through 18. Dotted lines show intergenic space, solid lines represent noncoding sequence, black boxes are coding sequences, and shaded blue boxes show duplicated sequences. Arrowheads represent breakpoints.
Associations Between Industry Sponsorship and Results of Cost-effectiveness Analyses of Drugs Used in Breast Cancer Treatment

A 1999 investigation found that industry sponsorship of cost-effectiveness analyses (CEAs) for oncology drugs was associated with lower likelihood of reporting unfavorable conclusions relative to CEAs with other sponsorship. Over the past 15 years, the CEA literature for oncology drugs has expanded dramatically, and oncology now accounts for the largest single pharmaceutical sales area worldwide. We sought to determine whether the association between industry sponsorship and CEA results has persisted.

Methods | We examined CEAs for breast cancer, the most common target diagnosis among oncology CEAs (36% of studies). We obtained data on all such CEAs published between 1991 and 2012 from the Tufts Cost-Effectiveness Analysis Registry, which was created by searching MEDLINE for all English language CEAs using the key words “QALYs” (quality-adjusted life-years), “cost-effectiveness analysis,” and “breast cancer.” From the registry we extracted study characteristics, results (cost per QALY), and registry-assigned quality ratings (which ranged from 2 to 6). We considered a study industry-sponsored if a pharmaceutical company provided funding or if 1 or more study authors was a company employee. Study authors provided sponsorship information for 13 studies with unclear funding information in the publication.

We converted each study’s results to 2013 US dollars using purchasing power parity conversion factors, categorized study results based on 3 thresholds ($500 000, $100 000, and $150 000/QALY), and classified studies as “cost-effective” if all results were equal to or more favorable than the chosen threshold, “not cost-effective” if none were, or “mixed” otherwise (note: each study could contain multiple analyses, with varying assumptions). Using JMP Pro statistical software (version 11.0.0, SAS Institute), we tested bivariate associations between industry sponsorship and study characteristics. We then fitted logistic regressions to estimate independent associations between industry sponsorship and study results, adjusting for drug class, cancer stage targeted, and study quality score.

Results | Of 105 CEA studies, 65 were industry funded (Table 1). Study quality ratings were nonsignificantly higher among industry-sponsored studies (mean rating, 4.8 vs 4.4 among studies with other sponsorship; P = .09).

Industry-sponsored studies were statistically significantly more likely than other-sponsored studies to report favorable cost-effectiveness results: 75.4% vs 40.0% at $500 000/QALY (P = .004), 80.0% vs 57.5% at $100 000/QALY (P = .03), and 87.7% vs 67.5% at $150 000/QALY (P = .04) (Table 2).

Among the subset of CEAs with high quality ratings (≥4.5), industry-sponsored studies were more likely to report favorable findings (75.5% vs 45.5%, P = .04, at the $500 000/QALY threshold).

Discussion | Our analysis of breast cancer CEAs suggests that pharmaceutical industry-sponsored studies continue to be more likely to report favorable estimates than studies with other sponsorship. These findings have multiple possible explanations.

First, most CEAs have retrospective designs, which can allow investigators to identify and then conduct, based on early looks at clinical and resource profiles, those trials most likely to yield positive outcomes. Second, potential conflicts of interest exist. Pharmaceutical companies can exert influence through grants, educational funds, or manuscript review requirements. Investigators set the values assigned to quality of life, determine the price and duration of interventions, and make other methodological choices that can affect study findings. Making these choices transparently and before results are known could enhance the credibility of CEAs.

Our study has limitations. We examined drugs for breast cancer only. Financial relationships between pharmaceutical companies and researchers were considered, but other less readily detectable factors influencing study findings may exist.

Additional studies are needed to determine whether similar associations between industry sponsorship and results ex-