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Enabling a genetically informed approach to cancer medicine: evaluation of the impact of comprehensive tumor sequencing

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Abstract

“The role of next-generation sequencing to determine appropriateness of anti-PD1/PD-L1 therapy needs further investigation, but has shown promise in predicting response and survival in melanoma and urothelial carcinoma.”

Keywords

immunotherapy; mutations; NGS; PD-1; sequencing; targeted therapy

Molecularly guided therapy has rapidly evolved over the last decade. The initial success of the tyrosine kinase inhibitor, imatinib, in the treatment of chronic myelogenous leukemia and of the monoclonal antibody targeting the HER2 transmembrane receptor, trastuzumab, for HER2-positive breast cancer, spurred investigation of the genomic landscape of tumors and the development of novel agents to target genetic abnormalities. Targeted therapies have demonstrated success in several malignancies including non-small-cell lung cancer (*EGFR* mutations, *ALK* fusions) and melanoma (*BRAF* mutations); however, many other targeted therapy approaches have proven less effective. This is not surprising given that most solid tumors are not driven by a single mutation but are instead characterized by a high degree of genomic complexity. Because of this complexity, immunotherapy has evolved as another treatment strategy and has improved survival in several cancers. Even with these successes, predictive biomarkers are needed to better characterize that patients derive the most benefit from both targeted and immune therapy strategies.

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Identification of targeted therapy options

While the value of identifying highly validated targets (e.g., *BRAF* mutations, *ALK* fusions, *HER2* amplifications) is clear, the clinical utility of more extensive sequencing is more difficult to quantify. In 2014, we evaluated our clinical experience using a hybrid capture-based next-generation sequencing (NGS) platform that sequenced exons from 236 cancer-related genes (FoundationOne®, Foundation Medicine, MA, USA) [1]. Among the first 103 patients sequenced, 83% had at least one potentially actionable genetic alteration. Of these, 21% received genotype-directed therapy, whereas 35% received standard chemotherapy options and 13% had rapid clinical deterioration precluding further therapy. Patients who responded to genotype-directed treatment included BRAF inhibition for a melanoma patient whose *BRAF*V600 mutation had not been detected with previous testing, an *EML4-ALK* fusion detected in a lung mucoepidermoid carcinoma that responded to crizotinib, and a T-cell prolymphocytic leukemia found to have a *JAK1* mutation that responded to ruxolitinib [1].

Another larger observational study more clearly demonstrated clinical benefit from sequencing. Among 339 profiled patients from a single institution, 188 (37.6%) received therapy either matched to identified genomic alterations (n = 122) or unmatched therapy (n = 66) [2]. In multivariate analyses, patients with matched therapy had a trend toward improved clinical outcomes (p = 0.06), and patients with direct therapeutic matches (e.g., BRAF inhibitor for *BRAF* mutation) had improved outcomes compared with those with indirect therapy matches (e.g., PIK3CA inhibitors for *PTEN* mutations). Furthermore, tumors with higher matching scores (a metric that incorporated number of alterations treated vs those not treated) had improved outcomes, with improved progression-free survival (PFS) at 6 months.

“Currently, it is reasonable to use next-generation sequencing to identify well-validated targets that could inform treatment decisions, either with a standard therapy or enrollment in a clinical trial.”

On the other hand, a prospective, Phase II trial (SHIVA) casts some doubt on the clinical utility of routine NGS testing. In this study, 741 patients with advanced cancer were sequenced with an amplicon-based approach evaluating 739 mutations in 45 cancer-associated genes [3]. Of these, 293 patients (40%) matched to one of ten different targeted therapy regimens and eligible patients were randomized 1:1 to molecularly targeted therapy versus physician's choice chemotherapy. No differences in PFS, overall survival (OS) or toxicities were observed between arms. Interestingly, follow-up analysis from this study showed that a sizable minority (37%) of patients who crossed over from standard to targeted therapy had at least a 30% increase in PFS following crossover, suggesting substantial benefit for these patients [4].

In light of these results, we view molecular profiling using NGS panels as a powerful tool to identify the US FDA-approved therapies for patients (e.g., *BRAF* mutations in melanoma). We also posit that this approach can identify key targets for off-label therapy in occasional patients. In our opinion, this approach should primarily be pursued for well-validated targets (e.g., BRAF inhibitors when *BRAF*^{V600} mutations are identified in nonmelanomas), and for clinical trial accrual, rather than more speculative off-label therapies (e.g., mTOR inhibitors

for *PIK3CA* mutations). Large studies like the NCI-MATCH should also help clarify additional high yield targets. Other ‘big data’ efforts such as the American Society of Clinical Oncology's CancerLinq® (American Society of Clinical Oncology, VA, USA), educational resources (My Cancer Genome®, Vanderbilt, TN, USA) and local-level resources (molecular tumor boards) can also provide tools when unclear results are obtained from NGS [5]. The other critical function of NGS is to facilitate clinical trial entry. As the number of so-called ‘basket trials’ increases [6], profiling for numerous mutations simultaneously will potentially identify multiple clinical trial options.

Applications for immune-oncology

Immune checkpoint inhibitors have had a substantial impact in the treatment of multiple types of cancers. The most widely used and successful of these agents are those targeting the PD-1/PD-L1 axis including pembrolizumab, nivolumab and atezolizumab. These immunotherapy drugs have improved patient outcomes in melanoma, non-small-cell lung cancer, urothelial cancer, renal cell carcinoma, mismatch repair-deficient (MMRD) tumors and others [7]. Despite these advances, only a minority of patients experience durable responses, and the subset of patients that derives the most benefit needs to be more clearly defined.

Interestingly, NGS efforts may provide clinically useful immunotherapy biomarkers. Initial efforts used whole-exome sequencing (WES) and identified total mutational load as a predictor of response to anti-PD-1. Rizvi *et al.* examined 34 non-small-cell lung cancers treated with pembrolizumab. Through WES of two independent cohorts, they found that higher nonsynonymous mutation burden, higher neoantigen burden, certain DNA repair pathway mutations and a molecular smoking signature (transversion high) correlated with improved clinical outcomes [8]. A Phase II study also evaluated the hypothesis that MMRD cancers would have a greater response to anti-PD-1 due to an increased number of somatic mutations. Pembrolizumab was given to 20 patients with MMRD tumors (nine colon, 11 other) and 21 patients with mismatch repair-proficient colon cancer. The response rate and immune-related PFS rate were 40 and 71% for MMRD colorectal cancers, and 71 and 67% for other MMRD tumors, respectively. There were no responses in the mismatch repair-proficient colorectal cancers, and the PFS rate was 11%. Median PFS was not reached in MMRD colon cancer, was 5.4 months in other MMRD tumors and 2.2 months in mismatch repair-proficient colorectal cancers. The mean number of somatic mutations was substantially higher in MMRD tumors versus mismatch repair-proficient tumors (1782 vs 73 mutations per tumor) [9]. These studies suggest that mutation load and perhaps microsatellite stability testing may help guide anti-PD-1 therapy selection independent of histology.

Given the limitations of routine WES to determine mutational load in patient tumors (cost, time, need for matched controls), we assessed whether a more limited NGS panel would predict response to anti-PD-1. We performed NGS (FoundationOne) on archival melanoma tumors to discern whether the number or type of mutation would affect clinical outcomes. In two cohorts, an initial and a validation, response to a PD-1 therapy was associated with higher mutational load (45.6 vs 3.9 mutations/MB – initial; 37.1 vs 12.8 mutations/MB –

validation) compared with nonresponders. We also analyzed response, PFS and OS of three mutational load groups: high (>23.1 mutations/MB), intermediate (3.3–23.1 mutations/MB) and low (<3.3 mutations/MB). Patients with higher mutational load had superior outcomes compared with the intermediate and low mutational load groups. Overall response rate was significantly improved (85 vs 29 vs 14%), as were median PFS and median OS in the high versus intermediate versus low mutational load groups, respectively. We also identified that *NF1* mutations were associated with higher mutational loads and response rates, whereas *BRAF/NRAS/NF1* wild-type melanomas had a lower mutational rate [10]. Our study suggests that NGS can be used to accurately assess mutational load in melanoma patients and may have a role in making treatment decisions.

A recent Phase II study of 310 patients with advanced urothelial cancer receiving atezolizumab also evaluated the effects of mutation load using FoundationOne on response to therapy. In this study, mutation load on pretreatment samples was assessed in 48% of patients. In responders, the mutational load was significantly higher than in nonresponders, (median: 12.4 vs 6.4 mutations/MB) [11]. These findings suggest that targeted NGS panels may serve as an accurate surrogate across multiple cancer types.

Although multiple trials support the notion of a higher mutational burden correlating with response to checkpoint inhibition, another recent melanoma study suggested the importance of other factors including the transcriptome in predicting response. In 38 tumors, mutational load was associated with better OS, but was not predictive of response to anti-PD1 therapy (perhaps due to small sample size). Transcriptome sequencing was also performed, and a set of 26 transcriptomic signatures involving angiogenesis and wound healing were coenriched in patients who failed to respond, referred to as the innate anti-PD-1 resistance signature [12]. Overall, determining response to immunotherapy appears complex, and suggests that transcriptomic (and/or immunohistochemistry assays) may augment the predictive capacity of mutation load evaluation.

In conclusion, the role of sequencing is of increasing importance in the care of cancer patients. Currently, it is reasonable to use NGS to identify well-validated targets that could inform treatment decisions, either with a standard therapy or enrollment in a clinical trial. The role of NGS to determine appropriateness of anti-PD1/PD-L1 therapy needs further investigation, but has shown promise in predicting response and survival in melanoma and urothelial carcinoma. Further prospective studies are needed to confirm these findings and to evaluate their significance in other malignancies.

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Biographies

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