

AUTHOR'S VIEW

## Genomic profile, smoking, and response to anti-PD-1 therapy in non-small cell lung carcinoma

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### ABSTRACT

The recent successes of immune checkpoint therapies have established a new era for the treatment of patients with cancer, yet the predictors of response remain largely undetermined. We recently demonstrated that the genomic landscape of lung cancers substantially influences the response to programmed cell death 1 receptor (PD-1) blockade, providing new insights into the molecular determinants of the response to immunotherapy.

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### Introduction

Immune checkpoint inhibitors are revolutionizing treatment for patients with cancer. The far reaching potential of programmed cell death 1 receptor (PD-1) blockade therapy has been extended beyond the tumors classically thought to be immunogenic, such as melanoma and renal cell carcinoma. The list of tumor types in which responses have been observed following anti-PD-1 therapy now includes bladder cancers, gastric cancers, head and neck cancers, lung cancers, breast cancers, and lymphomas, and continues to grow. This broad activity has validated the concept that the host immune system can be harnessed for the treatment of cancers. However, a unifying mechanistic basis of response across tumor types has not been identified.

Based on initial observations that some of the cancer types with the best response to PD-1 blockade are those characterized by a relatively high mutation burden (such as melanoma and lung cancers<sup>1-3</sup>), we hypothesized that the mutational landscape of a given tumor might be an important predictor of response to PD-1 blockade. DNA damage may cause mutations that can cause tumor cells to appear foreign, thus activating the immune response.

### Mutation burden and response to PD-1 blockade

To examine this hypothesis, we characterized the mutational landscape of patients with non-small cell lung cancer (NSCLC) who were treated with pembrolizumab as part of a phase I study.<sup>4</sup> Similar to previous series of sequenced NSCLC tumors,<sup>5,6</sup> we found a substantial range in the mutation burden of samples in our study, with some tumors harboring as few as 11 somatic nonsynonymous mutations and others having as many as 1,192 mutations. Mutation burden substantially correlated with clinical benefit of treatment with pembrolizumab.

Patients with a durable benefit lasting >6 months (termed “durable clinical benefit” or DCB) had a significantly higher mutation burden than those with no durable benefit (NDB) (median 299 *versus* 127 mutations,  $p = 0.0008$ ). Additionally, the rate of objective response, DCB, and progression-free survival were all significantly greater in patients with an elevated mutation burden (59% *versus* 12%,  $p = 0.01$ ; 79% *versus* 18%,  $p = 0.001$ ; hazard ratio [HR] 0.19, 95% confidence interval [CI] 0.08–0.47, respectively).

As rates of DCB were similar in the 51–75th percentile (86%) and the 76–100th percentile (71%) of mutation burden in our cohort, the clinical correlation between mutation burden and response to PD-1 blockade appears to have a threshold effect. Thus, in the context of considering mutation burden as a potential biomarker, it may be feasible to identify a binary cut-point that efficiently identifies those patients most likely to benefit from PD-1 blockade. In our cohort, only one patient with a mutation burden <178 had DCB (8%) compared to 72% for patients with mutation burden  $\geq 178$ . We are currently performing additional larger prospective trials in patients with NSCLC who were treated with PD-1 blockade to determine exact mutational thresholds that associate with clinical benefit to immunotherapy. We are also working to enhance the speed of bioinformatics processing of whole-exome sequencing and determination of mutational burden so that we can apply these genomic biomarkers as real-time predictive tools.

### Molecular smoking signature and benefit from immunotherapy

When considering the correlation between mutation burden and clinical benefit from PD-1 blockade, a fundamental question arises: what biologic processes are responsible for the variation in somatic mutations in NSCLC? It is known that the

carcinogens in tobacco smoke are responsible for much of the mutagenesis in NSCLC<sup>7</sup> and that smoking-related lung cancers are characterized by a greater mutation burden than lung cancers that occur in never smokers.<sup>8</sup> To assess the effects of smoking on the mutational landscape and pembrolizumab response, we applied a classifier designed to identify the molecular signature of smoking in lung cancer exomes.<sup>6</sup> Based on the frequency of C>A transversions (which is characteristic of smoking-related genotoxicity), samples were defined as “transversion high” (TH, smoking signature) or “transversion low” (TL, never-smoking signature). We found that the presence of the TH molecular signature highly correlated with both elevated mutation burden and clinical benefit with pembrolizumab. Notably, the mutational smoking signature was a far more robust predictor of clinical benefit than smoking history. This molecular signature provides a more objective and quantitative determination of tobacco carcinogen-induced DNA damage. We believe that the mutational smoking signature may have broad application as a biomarker of response to PD-1 pathway blockade; not just for lung cancer, but also for tobacco carcinogen-related tumors in general. Going forward, we will expand our denominator of NSCLC patients treated with PD-1 pathway blockade and will extend this analysis to patients with head and neck, esophageal, and bladder cancers. With the increasing rapidity and decreased cost of exome-based analyses, this approach could provide a more granular predictor of response to PD-1 blockade than immunohistochemistry-based analyses alone.

### DNA repair and replication mutations

Beyond tobacco carcinogen-induced mutation, defects in DNA repair mechanisms may also be responsible for genetic alterations. We found deleterious mutations in genes such as *polymerase (DNA directed)*, *epsilon*, *catalytic subunit (POLE)*, *polymerase (DNA directed)*, *delta 1*, *catalytic subunit (POLD1)*, and *mutS homolog 2 (MSH2)* in samples with elevated mutation burden and durable benefit to treatment. In a particularly striking example, we identified a *POLD1* E374K mutation in one patient who was a never smoker but nevertheless harbored a substantial mutation burden ( $n = 507$  nonsynonymous mutations) and was one of the few patients with a TL-signature who showed a durable benefit with pembrolizumab. This mutation in *POLD1* occurs in the exonuclease proofreading domain of Pol  $\delta$  and may have contributed to low-fidelity DNA replication and elevated mutation burden, similar to other *POLD1* mutant tumors.<sup>9</sup> These examples illustrate that, in addition to smoking-related genotoxicity, other pathways can contribute to the accumulation of somatic mutations in lung cancers and reveal that multiple factors may be responsible for the variation in mutation burden. As DNA repair pathways are biologically important in a variety of cancers, including microsatellite unstable colon cancers and *POLE*-mutated endometrial cancers, we hypothesize that these tumors may also exhibit sensitivity to immune checkpoint blockade therapy. We are examining a larger series of samples from patients treated with PD-1 blockade to systematically analyze the effects of somatic and germline variants involved in DNA repair and replication to assess which pathways may be particularly associated with elevated mutation burden and response to PD-1 blockade.

### Neoantigen-specific T cells are expanded by PD-1 blockade

What is the biologic connection between elevated mutation burden and response to PD-1 blockade? We hypothesize that neoantigens, cancer-specific mutated peptides formed as a consequence of somatic mutations, underlie this association and that neoantigen-specific T cells may mediate the clinical response seen with PD-1 blockade. Our group has previously developed a computational pipeline for identifying candidate neoantigens from cancer exomes and found recurrent motifs in neoepitopes associated with response to ipilimumab in patients with melanomas.<sup>10</sup> We applied this computational pipeline to examine the neoantigen landscape of one patient with NSCLC who had an excellent response to pembrolizumab. Candidate neoantigens from this patient were synthesized and neoantigen-specific T cell reactivity was identified using autologous peripheral blood lymphocytes. A T-cell response to a neoantigen resulting from a point mutation in *HECT and RLD domain containing E3 ubiquitin protein ligase family member 1 (HERC1 P3278S)* was detected in the peripheral blood and the increase in neoantigen-specific reactivity mirrored the clinical response to pembrolizumab. The T-cell response was only detectable after starting therapy, increased rapidly initially, and then plateaued at levels just above background as tumor regression was maintained over the next year. This is the first neoantigen-specific T-cell response identified in the peripheral blood of a patient treated with PD-1 blockade. These results demonstrate that immunologically relevant neoantigens can be identified using computational methods and suggest that blood-based monitoring of neoantigen-specific T cells may be a feasible strategy for monitoring response to PD-1 blockade.

### Summary

Our work has demonstrated a substantial influence of the genomic landscape of NSCLCs on response to PD-1 blockade, highlighting the potential biologic causes of high mutation burden and demonstrating how neoantigens may mediate the response to PD-1 blockade. As there is a critical need to identify predictive biomarkers associated with benefit from these therapies, we believe that these findings will be important building blocks toward optimizing, and ultimately personalizing, the use of immunotherapies. Importantly, the principles illustrated by our work are applicable to other cancers as the relationship between tumor genetics and response to immunotherapy very likely transcends tumor type. As in our current study, continued collaborations between academic groups and with industry will be absolutely vital to successful integration of our understanding of genomics and immunobiology to ensure greater precision and improved efficacy of immunotherapy in patients with cancer.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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