Anti-PD-1/PD-L1 antibodies are emerging as promising anticancer therapeutics. Interestingly, elevated response rates to these agents are mostly documented among patients with tumors that bear high level of somatic mutations, like melanoma or non-small cell lung carcinoma. We herein formulate the hypothesis that high levels of mutational heterogeneity in the tumor could be the key for the success of immune checkpoint-targeting therapies.

Introduction

Recently, promising results have been obtained in patients with advanced cancers like melanoma, renal cell carcinoma (RCC), or non-small cell lung carcinoma (NSCLC) with immune checkpoint blockers, including monoclonal antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PDCD1, best known as PD-1), or its main ligand CD174 (best known as PD-L1), resulting in remarkable and long-lasting clinical responses. Interestingly, Phase I efficacy data on anti-PD-1/PD-L1 agents point to a higher response rate among patients with melanoma and NSCLC, 2 tumor types associated with the highest levels of somatic mutations that develop upon exposure to UV light and carcinogens (tobacco smoke), respectively. Also, preliminary Phase IA data on the anti-PD-L1 antibody MPDL3280A suggest an association between smoking status and response in NSCLC patients.1 These observations may suggest that the mutational heterogeneity of the tumor could be the key of the success of immune checkpoint-targeting therapies. To evaluate this hypothesis, we looked at the overall response rate (ORR) to PD-1/PD-L1-targeting agents among patients affected by solid tumors and compared it to mutational load reported in the literature for neoplasms of the same type. Herein, we report the results of this analysis, provide a rationale to explain how the accumulation of somatic mutations in tumors could improve the response to immune checkpoint blockers and propose future directions to improve the development of these new immunotherapies.

Response Rate to PD-1/PD-L1-Targeting Agents Across Solid Tumors

The PD-1/PD-L1 pathway mediates one major immune checkpoint.7 PD-1/PD-L1 are a receptor/ligand pair that delivers inhibitory signals to activated T cells. Malignant cells are able to avoid immune destruction by diverting such an immune checkpoint. Thus, anti-PD-1/PD-L1 monoclonal antibodies often restore effective antitumor immune responses.

Although anti-PD-1/PD-L1 monoclonal antibodies have been tested across multiple solid tumors, they were first investigated in melanoma and RCC patients, mostly because of renown sensitivity of these tumors to these therapies. Moreover, melanoma and RCC patients are often treated with immune checkpoint blockers, as they benefit from exceptional response rates. The efficacy of anti-PD-1/PD-L1 agents in melanoma and RCC is likely to be due to the high somatic mutation load in these tumors, as mutations may drive novel antigens recognized by the immune system.

Keywords: immune checkpoints, immunotherapy, mutational heterogeneity, PD-1, PD-L1

Abbreviations: BRCA1, breast cancer 1, early onset; BRCA2 breast cancer 1, early onset; CTLA4, cytotoxic T lymphocyte-associated protein 4; IDO1, indoleamine 2,3-dioxygenase 1; IFN, interferon; IL, interleukin; MMR, mismatch repair; MSI, microsatellite instability; NSCLC, non-small cell lung carcinoma; ORR, overall response rate; PDCD1, programmed cell death 1; PD-L1, PD-1 ligand 1; RCC, renal cell carcinoma; TGFβ1, transforming growth factor β1; TKI, tyrosine kinase inhibitor

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malignancies to immunotherapy. Indeed, PD-1/PD-L1-targeting agents are associated with significant response rates in these clinical settings\(^1,2,3,4,5,6\), as shown in Table 1: ORRs up to 38% (44/117) or 27% (9/33) have been reported in melanoma patients treated with the anti-PD-1 antibody lambrolizumab or RCC patients treated with nivolumab (another PD-1-targeting antibody), respectively.\(^2,3\). Nonetheless, comparable response rates were later on observed in subjects with neoplasms expected to be poorly immunogenic. For instance, the ORR observed in NSCLC patients treated with MDP3280A and nivolumab is 23% (12/53) and 18% (7/46), respectively.\(^6\). Individuals bearing both the squamous and non-squamous NSCLC subtype seems to equally benefit from PD-1/PD-L1-targeting agents: ORRs of 8–33% and 11–27% were observed among squamous and non-squamous NSCLC patients, respectively.\(^6\) (Table 1). This suggests that the immunogenicity of tumors, as evaluated by their sensitivity to interleukin (IL)-2 or interferon (IFN) α is not a good surrogate marker for the efficacy of anti-PD-1/PD-L1 antibodies. Interestingly, patients affected by other solid tumors exhibit null or very low ORRs to anti-PD-1/PD-L1 agents: 0% in colorectal cancer patients (0/19 with nivolumab, 0/18 with lambrolizumab), 0% in prostate cancer (0/13 with nivolumab) and 6% in ovarian carcinoma (1/17 with BMS-936559).\(^6\) The expression of PD-L1 by malignant cells has been suggested to constitute a predictive marker of clinical efficacy for anti-PD-1/PD-L1 agents.\(^1,2,8\) Nevertheless, the method to quantify PD-L1 positivity is still debated and available data show a significant amount of responses among PD-L1-negative tumors. The expression of PD-L1 by malignant cells may therefore not constitute such an exclusive and critical predictive factor and other tumor characteristics may be as important for its sensitivity to anti-PD-1/PD-L1 agents. The remarkable ORRs observed upon the administration of anti-PD-1/PD-L1 antibodies to NSCLC patients raise the question on how NSCLC, melanoma and RCC differ from other solid tumors, which are associated with limited responses to these immunotherapeutics. Recent Phase I data on the treatment of NSCLC patients with MPD3280A revealed a higher ORR of 26% (11/43) among former/current smokers than among never smokers (10%, 1/10).\(^1\) These preliminary results in the setting of NSCLC and the ORRs documented among melanoma patients suggest that carcinogens (tobacco smoke or UV light) may play a key role in the susceptibility of tumors to PD-1/PD-L1 blockers, perhaps through their ability to promote mutagenesis. We thus formulated the hypothesis that the specific mutational profile of NSCLC, melanoma and RCC could underlie, at least in part, their ability to respond to checkpoints blockers.

### Mutational Heterogeneity Across Solid Tumors

To support this contention we looked at mutations rates that had been previously reported in the literature.
for various solid tumors. Somatic mutations may be the consequence of defective DNA repair, the infidelity of the DNA replication machinery or the exposure to mutagens. Recent advances in the sequencing technology nowadays allow for the identification of a large panel of somatic mutations across different types of cancer and offer the possibility to validate predictive models in humans. The frequency of somatic mutations has been shown to be highly variable across cancer types, ranging from 0.001 to more than 400 per megabase (Mb). The highest mutational rate is observed in melanoma (median of 13.2 mutations per Mb) and in NSCLC (8.17 and 6.43 mutations per Mb for the squamous and non-squamous subtype, respectively), in which mutations are known to be secondary to UV light and tobacco smoke exposure, respectively. As expected, the mutation rate is radically different between NSCLC patients that are former/current smoker and their counterparts who never smoked. The median of somatic mutations per Mb is indeed 10.5 for the former and 0.6 for the latter. On the contrary, relatively low median rates of somatic mutations are observed in tumor types exhibiting limited responses to anti-PD-1/PD-L1 agents: 3.2 mutations per Mb for colorectal carcinoma, 1.53 mutations per Mb for RCC, 1.65 mutations per Mb for ovarian carcinoma and 0.73 mutations per Mb for prostate cancer. As a hypothesis-generating case, we observed a significant association between the mutational load of tumors and their susceptibility to PD-1/PD-L1-targeting agents (Fig. 1).

Defining which Antitumor Immune Responses are Stimulated by Immune Checkpoint Blockers

In the past decades, cancer vaccine specialists have been focusing on the identification of antigens selectively expressed by various solid tumors to developed efficient anticancer vaccines. Generally, tumor-specific T cells can recognize antigens resulting from oncogenic proteins, differentiation-associated proteins, overexpressed or aberrantly expressed proteins, proteins coded by oncogenic viruses, and
cancer-testis proteins. All these tumor-associated antigens are usually shared by different patients affected by the same neoplasm and sometimes even by distinct tumor types. Unfortunately, the results of clinical trials that have tested anticancer vaccines so far are deceiving, with null or very low ORRs. Now that immune checkpoint blockers are showing encouraging results, the future of anticancer immunotherapy looks more promising. Indeed, checkpoint-targeting agents represent, together with the adoptive transfer of tumor-infiltrating lymphocytes, the only immunotherapeutics that have been associated with high ORR and long-term responses. Compared with peptide-based vaccines or the adoptive transfer of engineered T cells with a narrow specificity, immune checkpoint blockers activate immune responses targeting a broad spectrum of antigens. Interestingly, patients responding to checkpoint-targeting agents show modest reactivity to known tumor-associated antigens, suggesting they are not strongly implicated in the immune response elicited by these agents. In fact, the tumor antigens that drive efficient T-cell responses in patients who obtain clinical benefits from immune checkpoint blockers have not been clearly identified so far. The exome-guided immunomonitoring of patients treated with checkpoint-targeting agents has recently revealed that tumors are producing a large panel of neo-antigens that can drive immune responses. These neo-antigens originate from the so-called tumor “mutanome,” the ensemble of tumor-specific (and hence most often patient-specific) mutations accumulated in the course of oncogenesis and tumor progression, which can involve both oncogenes and passenger genes. Moreover, mutations can result in neo-epitopes that are characterized by an improved MHC-binding profile, resulting in superior presentation to T cells. New technologies based on next-generation sequencing or peptide/MHC multimers are being developed to identify the numerous potential T-cell epitopes resulting from neo-antigens and to create optimal polytropic vaccines. However, since neo-antigens most often are tumor-specific, it seems particularly complex nowadays to employ these technologies to develop personalized treatments at a large scale.

**Mutational Heterogeneity and Immune Response**

Elevated mutation frequencies are sometimes attributable to the exposure to well known carcinogens, such as UV radiation in the case of melanoma and tobacco smoke in the case of lung cancer. As UV rays form pyrimidine dimers, melanoma-associated mutations show a high prevalence of C- > T transitions on the untranscribed, as compared with the transcribed, strand. The mutational signature linked to NSCLC is dominated by C- > A transitions, which are associated with the exposure to the polycyclic hydrocarbons found in tobacco smoke. Indeed, whole genome and transcriptome sequencing revealed a much higher number of mutations per Mb in NSCLC patients who were or had been tobacco smokers (mutations per Mb: median 10.5, range 4.9–17.6) than in never smokers (mutations per Mb: median 0.6, range 0.6–0.9), with a possible dose-response relationship between the amount and duration of smoking and the frequency of mutations. Such an extreme mutational heterogeneity (be it intrinsic or induced by mutagens) represents a fundamental problem for targeted therapies, as it is responsible for the evolution of cancer cells exhibiting multiple molecular subtypes that exhibit variable susceptibility to treatment, hence facilitating resistance. As any mutation can potentially produce neo-antigens, the levels of mutagenesis may correlate with the degree of immunogenicity. Also, contrarily to targeted therapies, the immune system is highly adaptable, implying that a high mutational heterogeneity could play a key role in the tumor susceptibility to checkpoint blockers by eliciting a wide immune response to neo-antigens and therefore improving efficacy in particular against individual subclonal heterogeneity. Unlike anticancer vaccines, which may allow for the outgrowth of antigen-loss tumor variants, these neo-antigens could help to shape the antitumor immune response toward an improved fitness. High amounts of neo-antigens may also induce a beneficial effect by broadening the repertoire of responding T cells. Moreover, high levels of mutational heterogeneity may also promote immunogenicity by facilitating the interaction between tumor-derived epitopes and MHC class I and II molecules, resulting in improved presentation to T cells. The immune system is known to shape the tumor immunogenicity through immunoediting, reflecting the constant selection pressure exerted by the immune system on malignant cells. On the other side, tumor’s mutational heterogeneity could reciprocally improve anticancer immune responses by widening the T-cell repertoire. In this setting, checkpoint blockers could reinstate therapeutically relevant immune responses against a hidden repertoire of neo-antigens.

**Direct Effects of Carcinogens on the Immune System**

Besides producing neo-antigens, carcinogens like UV rays or tobacco smoke have direct effects on the immune system. In addition to their carcinogenic potential, UV rays exert immunosuppressive effects by impairing the function of antigen-presenting cells, by promoting clonal anergy, by stimulating the accumulation of regulatory T cells or by favoring the secretion of immunosuppressive cytokines. Detrimental mutations induced by tobacco carcinogens are also expected to facilitates the escape of cancer cells from the immune system through different mechanisms: they reduce immune recognition by favoring the loss of MHC class I proteins, by limiting the expression of anti-apoptotic proteins and by promoting the establishing of an immunosuppressive microenvironment characterized by high levels of IL-10, transforming growth factor β1, (TGFβ1) and indoleamine 2–3-dioxygenase 1 (IDO1). Finally, smoking may also help to maintain an inflammatory environment in the lungs, resulting in the IFNγ-driven expression of PD-L1.
Treatment-Induced Mutational Changes

It has been reported that melanoma cell clones becoming resistant to BRAF inhibitors harbor a different mutation profile upon exposure to these therapeutic agents. BRAF inhibitors may therefore contribute to mutational heterogeneity. Since approximately 50% of metastatic melanomas harbor BRAF V600 mutations, many melanoma patients enrolled in Phase I clinical trials have previously received BRAF inhibitors. Likewise, many NSCLC or RCC patients treated with PD-1/PD-L1-targeting agents in the context of Phase I clinical studies had previously been treated with a tyrosine kinase inhibitor (TKI), which may have directly shaped the tumor mutational pattern through drug selective pressure. These drug-induced mutations may play a key role at modeling the clinical response to PD-1/PD-L1-targeting agents. Additionally, these mutations that emerge under drug selection can directly affect the expression of immune checkpoint-related molecules on cancer cells, as it has been reported for melanoma in BRAF inhibitor resistant clones manifesting increased expression levels of PD-L1.

Conclusions and Future Directions

Our analysis is based on a limited amount of data. Moreover, the efficacy observed in Phase I clinical trials is of course very preliminary, and ORR data from Phase II and III studies are extremely expected. Nonetheless, we believe that our analysis has the merit to generate a new hypothesis to explain the responses to checkpoint inhibitors observed so far among patients with solid tumors, notably that mutagen-induced tumors appear to exhibit an improved response to these immunotherapeutic agents. This may indirectly suggest that targeted antitumor agents such as TKIs can affect the immunogenicity of tumors as they impose a selective pressure, a notion that should be taken into consideration for the design of future clinical trials involving immune checkpoint blockers. Moreover, if high levels of mutational heterogeneity increase the tumor immunogenicity, it will be interesting to evaluate the clinical activity of PD-1/PD-L1 agents in DNA mismatch repair (MM)-deficient tumors, such as microsatellite instability (MSI)+ colorectal carcinoma as well as BRCA1 and 2 neoplasms (breast cancer 1and 2, early onset), all of which display severe genomic instability. Other tumors that present foci of localized hypermutations as observed in subsets of breast, pancreatic, lung and hematological cancers, could also be good candidates for immune checkpoint inhibitors, a possibility that should be evaluated in future clinical trials.

Disclosure of Potential Conflicts of Interest

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