Translating Pembrolizumab to Clinical Practice: Speak Immunology and Learn Fast!

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Running Title: Pembrolizumab: Learning to Speak Immunology

Disclosure of Potential Conflicts of Interest

A. van Elsas is a founder of and has ownership interest in BioNovion, and has provided expert testimony on the history of checkpoint inhibitors to the European Patent Office (EPO). H. van Eenennaam is a founder of and has ownership interest in BioNovion. J.B. Haanen reports receiving commercial research grants from Bristol-Myers Squibb, GlaxoSmithKline, and Merck, and is a consultant/advisory board member for Bristol-Myers Squibb and Merck. No other potential conflicts of interest were disclosed.
Summary

T-cell checkpoint inhibitors treat the cancer patient’s immune system potentially inducing significant long-term survival. Pembrolizumab demonstrates clinical activity in patients diagnosed with melanoma and other cancers. Its mode of action suggests a rationale for combination with other treatment modalities, urging oncologists to brush up their knowledge of immunology.
In this issue of *Clinical Cancer Research*, Patnaik and colleagues (1) report on the first clinical application of pembrolizumab (MK-3475) to 30 patients diagnosed with advanced solid cancers of varying histology, including melanoma and non-small-cell lung carcinoma. Over all dose levels and schedules tested, evidence of clinical activity was obtained with 2 complete, 3 partial responses and 15 patients with stable disease. Adverse events reported were consistent with immunological activity of pembrolizumab and allowed the authors to conclude that up to the highest exposure tested (10 mg/kg infused every two weeks) no dose limiting toxicities were observed. Intrapatient dose escalation studies were used to gain sufficient understanding of pembrolizumab pharmacokinetic (PK) properties and build a predictive model. Importantly, the authors used an *ex vivo* read-out of peripheral blood T cell activation to establish a PK-PD (pharmacodynamic response) relationship, focused on identifying an optimal pembrolizumab dose level and schedule achieving maximal immunological activity. The assay deployed involved the use of *Staphylococcus* enterotoxin B (SEB) to stimulate a fraction of blood-derived T cells to produce IL-2. In healthy donor and cancer patient whole blood this response was known to be modulated in a dose-dependent fashion by antibodies targeting PD-1, PD-L1 and CTLA-4. Patnaik et al. (1) further expanded on these observations by testing the *ex vivo* SEB response in patients dosed with pembrolizumab essentially establishing a longitudinal data set explaining optimal PD-1 engagement in relation to dose. In addition, the authors report on mouse studies relating blood antibody levels to intratumoral concentrations, leading to a rather precise prediction that a schedule of 2 mg/kg q3w achieves maximal engagement in the tumor compartment.

Instead of targeting tumor cells directly, cancer immunotherapy aims to treat a patient’s immune cells to reject their own cancer with the promise of potential long-term response due to T cell memory. In 2011, the first drug in new a class of cancer
immunotherapies termed 'checkpoint inhibitors', ipilimumab, was approved by the FDA for treatment of patient with advanced melanoma based on improved overall survival (2). Ipilimumab blocks CTLA-4, an inhibitory receptor or ‘checkpoint’ expressed on T cells, and induced long-term survival in approximately 20-22% of the metastatic melanoma patients treated. Continuing survival analysis demonstrated that the vast majority of responders remained alive up to 10 years post treatment initiation (3), igniting speculation about the curative potential of checkpoint inhibitors in melanoma. Following ipilimumab, unprecedented successes were reported using checkpoint inhibitors targeting the PD-1 pathway inducing objective clinical responses in melanoma, advanced lung cancer (non-small cell and small cell), renal, bladder and other cancers (4-7). In 2014, first pembrolizumab and subsequently nivolumab received FDA approval for the treatment of advanced melanoma, followed earlier this year by nivolumab approval in NSCLC. For both agents, long-term survival data are maturing and eagerly awaited.

Medical oncology professionals are quickly brushing up their knowledge of immunology and checkpoint inhibition, as ipilimumab, pembrolizumab, nivolumab are becoming part of their armamentarium, and their activity is not be mistaken for chemo- or targeted therapy. In addition to unusual response patterns, such as pseudo-progression caused by massive infiltration of tumor lesions by immune cells, immunological adverse events are reported, some severe and rarely observed in clinical practice.

Enormous attention is devoted to further pinpointing the precise mechanism-of-action of these checkpoints inhibitors. That knowledge seems key to enable selection of responder patients, optimal combination with other drugs, and to identify alternative means to enhance productive tumor immunity. Thus far, we have learned that successful tumor rejection involves effector T cells (both CD8 and CD4) and IFNγ, and is inhibited by the
intratumoral presence of suppressive regulatory T cells, myeloid cells and other factors. Enhancing the effector versus regulatory T cell ratio appears to be critical to a productive anti-tumor response.

PD-1 inhibits T cell function after engagement with one of its ligands, PD-L1 or PD-L2. In healthy tissues PD-L1 regulates peripheral immunological tolerance limiting exaggerated inflammatory and autoimmune disease. PD-1 ligands (mostly PD-L1) are aberrantly expressed on cancer cells as well as cells in the tumor microenvironment (7). In tumor samples of different histology PD-L1 overexpression was found to be a poor prognostic indicator, and nivolumab clinical activity was associated with detection of PD-L1 protein in tumor biopsies (4). However, also patients without evidence of PD-L1 expression may respond to PD-1 or PD-L1 targeting agents, explained either by assuming their site of action is elsewhere, or, more likely, that PD-L1 expression is dynamic and cannot be assessed reliably in a single isolated pre-treatment biopsy. PD-L1 expression is upregulated by IFNγ, a product of tumor-specific T effector cells. This implies the existence of a tumor-immune dialogue promoting immune evasion. Human melanoma biopsies sampled in the course of pembrolizumab treatment demonstrated that pre-existing CD8+, PD-1+ T cells in close proximity to PD-L1 expressing cells at the margin or inside the tumor correlated with subsequent response to treatment (8), confirming that pembrolizumab acts by overcoming PD-1 down-regulation of T cell effector function in the tumor.

The T cells associated with clinical activity of checkpoint inhibitors are directed towards tumor antigens derived from somatic mutations. Similar to melanoma patients responding to ipilimumab, in NSCLC patients treated with pembrolizumab clinical response was found to be associated with the mutational landscape and the appearance of novel T cell epitopes (9). Such neoantigen-specific T cells could be detected in patients following...
initiation of treatment and preceding assessment of clinical response. These reports set the stage for rational combination of checkpoint inhibitors with treatment modalities that induce tumor cell killing and release of tumor antigens (‘endo-vaccination’) (Fig. 1). Several standard-of-care chemotherapeutic agents, such as cisplatin, cyclophosphamide and temozolomide, targeted therapeutics and radiotherapy may support a productive anti-tumor immune response. Indeed, long-term efficacy of tumor-targeting agents may require involvement of a functional immune system as was suggested from mouse studies of inhibition of oncogenic driver mutations (10). Also, various combinations of immune modulating agents are being explored. Clinical testing of ipilimumab plus nivolumab as a treatment of metastatic melanoma demonstrated fast and deep clinical responses in up to 53% of patients (11), albeit with significant tolerability issues.

Until tumor-directed T cells were isolated from patients and their cognate tumor-associated antigens cloned in the early 90’s, the historically prevailing opinion had been that cancer patients displayed insufficient pre-existing adaptive immunity targeting their cancer cells and that chemotherapy further crippled their immune capabilities. Checkpoint inhibitors demonstrate that simply releasing the brakes on T lymphocytes is sufficient to trigger profound and durable clinical responses, confirming that there is potent pre-existing immunity and/or that sufficient immune capabilities are preserved in many patients despite pre-treatment.

This publication of first-in-human study of pembrolizumab in advanced cancer patients (1) follows after the publication of pivotal data in ipilimumab-refractory melanoma (6,12). In an interesting approach, after completing the dose-escalation phase pembrolizumab was tested in a variety of expansion cohorts constituting an unusually large Phase 1 study as a spring board for pivotal studies in several indications. Mid-May 2015,
clinicaltrials.gov listed 97 studies with pembrolizumab and 82 for nivolumab being tested for activity in a broad range of cancer indications. This is a clear indication that cancer immunology and immune checkpoint inhibition have come of age and may serve a huge medical need in advanced cancer patients in the near future.

References


Figure 1. Rationale for combination of immune checkpoint blockade with other treatment modalities.

A. A tumor lesion consists of multiple supporting cells collectively addressed as ‘tumor microenvironment’. These include tumor-associated macrophages and regulatory T cells limiting effector T cells and potentially pushing these towards a deep state of tolerance or exhaustion. B. Various established and novel therapeutic modalities induce tumor cell death and disrupt the immune suppressive tumor microenvironment. C. Pre-existing T cell immunity can be re-activated and novel responses induced in draining lymph nodes by neoantigens released from a dying tumor. These mechanisms may be invoked by other treatment modalities, but probably not to the extent unveiled using checkpoint inhibitors. D. As a consequence of successful immune checkpoint blockade, alone or in combination with other modalities, not just the tumor lesion in sight (‘index’ lesion) is rejected but also distant tumor sites (referred to as abscopal effect in the context of radiotherapy).
Figure 1:

A. Immunosuppressive tumor microenvironment

B. Surgery, radiotherapy, chemotherapy, targeted therapy

C. Combination with immune checkpoint inhibitors

D. Rejection of local and distant tumors

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