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

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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. *Clin Cancer Res; 21(19); 1–3. ©2015 AACR.*

See related article by Patnaik et al., p. 4286

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 (NSCLC). Over all dose levels and schedules tested, evidence of clinical activity was obtained with 2 complete and 3 partial responses and 15 patients with stable disease. Adverse events reported were consistent with immunologic activity of pembrolizumab and allowed the authors to conclude that up to the highest exposure tested (10 mg/kg infused every 2 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* readout 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 immunologic activity. The assay deployed involved the use of Staphylococcus enterotoxin B (SEB) to stimulate a fraction of blood-derived T cells to produce IL2. In healthy donor and cancer patient’s 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 and colleagues (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 every 3 weeks 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 a new class of cancer immunotherapies termed “checkpoint inhibitors,” ipilimumab, was approved by the FDA for the treatment of patients 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% to 22% of the metastatic melanoma patients treated. Continuing survival analysis demonstrated that the vast majority of responders remained alive up to 10 years after 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, and 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 pseudoprogression caused by massive infiltration of tumor lesions by immune cells, immunologic 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 checkpoint 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 antitumor 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 immunologic 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's clinical activity was associated with detection of PD-L1 protein in tumor biopsies (4). However, patients without evidence of PD-L1 expression may also respond to PD-1– or PD-L1–targeting agents, explained by assuming either that their site of action is elsewhere, or, more likely, that PD-L1 expression is dynamic and cannot be assessed reliably in a single isolated pretreatment 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 preexisting...
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 downregulation of T-cell effector function in the tumor.

The T cells associated with clinical activity of checkpoint inhibitors are directed toward 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 (“endovaccination”; Fig. 1). Several standard-of-care chemotherapeutic agents, such as cisplatin, cyclophosphamide, and temozolomide, targeted therapeutics, and radiotherapy may support a productive antitumor 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 90s, the historically prevailing opinion had been that patients with cancer displayed insufficient preexisting 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 preexisting immunity and/or that sufficient immune capabilities are preserved in many patients despite pretreatment.

This publication of first-in-human study of pembrolizumab in patients with advanced cancer (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 I 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 patients with advanced cancer in the near future.

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.

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