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Targeting PD-1/PD-L1 interactions for cancer immunotherapy

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Tumors have developed multiple immunosuppressive mechanisms to turn down the innate and the effector arms of the immune system, thus compromising most of the immunotherapeutic strategies that have been proposed during the last decade. Right after the pioneering success of Ipilimumab (anti-CTLA4) in metastatic melanoma, several groups have conducted trials aiming at subverting other immune checkpoints. Two articles that recently appeared in the New England Journal of Medicine.[1,2] highlight the therapeutic potential of agents that target PD-1 or its ligand PD-L1 in patients with advanced cancer, even individuals with lung or brain metastases. If confirmed, this clinical breakthrough will open novel avenues for cancer immunotherapy. In contrast to CTLA4 which regulates the amplitude of early activation of naive and memory T cells following TCR engagement, PD-1 mostly restrains (but not exclusively, see below) the activity of T cells in the periphery during chronic inflammation, infection or cancer, thereby limiting autoimmunity. However, in contrast to CTLA4-deficient mice that exhibit a dramatic lymphoproliferative and autoimmune disorder, PD-1-deficiency results in more subtle autoaggressive manifestations (lupus-like disease, dilated cardiomyopathy, Type 1 diabetes, bilateral hydronephrosis) that mostly manifest in autoimmunity-prone strains after the first year of age.[3]

Programmed cell death- 1 (PD-1), an immunoinhibitory receptor of the CD28 family, plays a major role in tumor immune escape.[4,5] The PD-1/PD-L1 interaction inhibits T lymphocyte proliferation, survival and effector functions (cytotoxicity, cytokine release),[6] induces apoptosis of tumor-specific T cells,[7] promotes the differentiation of $CD4^+$ T cells into $Foxp3^+$ regulatory T cells,[8] as well as the resistance of tumor cells to CTL attack.[9,10] The extent of PD-1 inhibition depends on the strength of the TCR stimulation, with more inhibitory effects at low levels of TCR engagement, preventing the induction of the survival factor Bcl-X_L and the transcription factors GATA-3, EOMES and T-BET. Recruitment of SH2-domain containing protein tyrosine phosphatases (SHP-1 and SHP-2) to the immunoreceptor tyrosine based switch motif within the PD-1 cytoplasmic tail inhibits positive signaling events downstream of the TCR, namely PI3K/Akt activation.

CD28 or IL-2 can override the negative impact of PD-1 on T cells. IL-2 triggers Akt activation through STAT5 and circumvents PD-1-mediated inhibition of Akt activation. In contrast, CTLA4 does not interfere with PI3K activation and rather acts at a more downstream level, by blocking Akt phosphorylation via the PP2A phosphatase. The comparison of the gene expression profiling of T cells exposed to anti-CTLA4 vs. anti-PD-1 Ab revealed that PD-1 has a more pronounced inhibitory activity (90% vs. 67% inhibition of gene products upregulated through the combined addition of anti-CD3 and anti-CD28 antibodies). Moreover, CTLA4 fails to downregulate the survival gene Bcl-X_L, suggesting that only PD-1 engagement has the potential to induce T cell apoptosis.[11]

The PD-1/PD-L1 pathway delivers inhibitory signals that regulate both peripheral and central tolerance. In the thymus, PD-L1 is expressed on the thymic cortex, on thymocytes and in the thymic medulla, participating in positive a well as negative selection.[12] Tolerogenic dendritic cells express PD-L1 and PD-L2, and reduce the initial phase of activation and expansion of self reactive T cells.[13] The PD-1 pathway is also involved in limiting the reactivation, expansion and effector functions of T cells.[14]

Even though the main biological effect of anti-PD-1 Ab consists in restoring the function of exhausted $CD8^+$ T cells in chronic viral infections or cancer, this antibody exerts other potentially interesting functions on additional cell types. Thus, it prevents the depletion of activated memory B cell in SIV-infected macaques, restoring antibody titers.[15] B cells expressing PD-L1 and PD-L1 interact with $PD\text{-}1^+$ follicular T helper cells in germinal centers to regulate the formation of memory B cells. In the absence of PD-1 signaling, the generation of long-lived plasma cells was found to be markedly reduced.[16]

Several groups demonstrated that PD-L1 also mediated the differentation of regulatory T cells (Tregs), which express both PD-1 and PD-L1. Sharpe and coworkers[17] showed that in the presence of anti-CD3 Ab and TGFβ, PD-L1Ig can induce a profound increase in the de novo generation of $CD4^+Foxp3^+$Tregs (iTreg) from naive $CD4^+$ T cells. Further engagement of $Foxp3^+$ iTregs by PD-L1-Ig resulted in the maintenance of Foxp3 expression and enhanced suppressive activity. The mechanisms underlying this phenomenon have been unraveled by Haxhinasto et al.,[18] Sauer et al.[19] and Francisco et al.[17] Indeed, augmenting PTEN expression and/or blocking the Akt/mTOR pathway resulted in the

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promotion and maintenance of Foxp3$^+$ iTregs. As loss of PTEN augments PD-L1 expression, the PD-1 pathway may activate a negative feedback loop to restrain its otwn function.[20]

The PD-1/PD-L1 axis may also regulate NK cell functions in tumor-bearing mice. IL-18 (either recombinant or tumor-derived) can promote the differentiation and accumulation of a distinct subset of immature NK cells (defined as KIT$^+$CD27$^-$) in the primary and secondary lymphoid organs of tumor bearers. This KIT$^+$ NK cells overexpress B7-H1/PD-L1, CTLA4 and LAG3 and kill DC in lymph nodes in a PD-1/PD-L1-dependent manner. Hence, PD-L1/PD-1 blockade in *nu/nu* mice has a profound anti-metastatic effect.[21,22] These data imply that, at least in mice, the DC/NK cell crosstalk leading to activation of mature (effector) NK cells can be regulated by third-party immature NK cells in a PD-1/PD-L1-dependent manner. Whether this applies to the human system remains to be determined.

PD-1 has two potential ligands, PD-L1 and PD-L2 endowed with a different spectrum of expression and regulation. Reportedly, PD-L1 is expressed constitutively in most hematopoietic cells and some parenchymal cells (such as pancreatic islet cells and vascular endothelial cells) while PD-L2 expression is restricted to macrophages and dendritic cells. Obviously, the question arises which tumor types express which PD-1 ligand.

The expression of PD-L1 in tumors has been described in many histological types such as melanoma, lung cancers, breast and ovarian, pancreatic and esophagus adenocarcinoma, kidney tumors and bladder cancers as well as in hematopoietic malignancies.[23–28] In renal cell carcinoma (RCC), tumor- and/or tumor infiltrating lymphocyte-associated PD-L1 expression was associated with a 4.5 fold increased risk of dying from the RCC,[29,30] as shown in 196 RCC studied on frozen tissue sections using the 5H1 Ab.[7] In primary melanoma, there was a correlation between the level of PD-L1 expression (using the clone 27A2)[24] and the vertical growth of primary melanoma (tumor thickness Breslow index, Clark level) but not ulceration.[27] Constitutive PD-L1 expression has been described to be driven by oncogenes such as loss of function of PTEN.[20]

Somewhat at odds with the aforementioned data, Taube et al. recently unraveled that PD-L1 upregulation by cancer cells may represent a novel "adaptive resistance mechanism of immune escape," in addition to the loss of MHC Class I or tumor antigen.[31] Indeed, there was a highly significant concordance between membranous expression of PD-L1 by naevi and in situ or advanced melanoma (35–39% exhibit a > 5% positivity using the 5H1 Ab) with the presence of CD3$^+$ and CD8$^+$ immune infiltrates (TILs).[31] Interferon Type II was detectable by qRT-PCR assessed after laser capture microdissection of the interface between TILs and PD-L1 expression by tumor cells. Taube et al. detected a positive correlation between PD-L1 expression and overall survival in metastatic disease (but not in localized melanoma that were not treated with prior immunotherapy).[31] In this study, PD-L1 expression was not associated with the natural course of the disease (vertical growth, TNM stage, geographic locations).

There are currently six agents blocking the PD-1/PD-L1 pathway in clinical evaluation: MDX-1106/BMS-936558/ONO-4538 (fully human IgG4 anti-PD1 mAb from BMS), CT-011 (humanized IgG1 anti-PD1 mAb from CureTech/Teva), MK-3475 (human IgG4 anti-PD1 mAb from Merck), MPDL3280A/RG7446 (anti-PD-L1 from Genentech), BMS-936559 (fully humanized PD-L1 IgG4 mAb inhibiting ligation to both PD-1 and B7.1) and AMP-224 (a B7-DC/IgG1 fusion protein licensed to GSK) (http://www.clinical trials.gov).

The first-in-human Phase I trial of the MDX-1106 (anti-PD-1 mAb) used intermittent dosing over a wide dose range in 39 patients suffering from advanced metastatic solid tumors. The pharmacodynamic effects of PD-1 receptor occupancy by the high affinity MDX-1106 were prolonged beyond its expected half- life, predicting a high biological durability. These data were compatible with the unexpected spectrum of clinical activity observed in melanoma, NSCLC, kidney and colon cancers.[1] Brahmer et al.[1] pursued their investigations in 207 patients using the BMS-936559 (anti-PD-L1 mAb) in a multicenter Phase 1 trial at multiple escalating doses (from 0.3 to 10 mg/kg). The antibody was administered iv, every 14 days in 6 week-cycles for up to 16 cycles or until the patient had a complete response. Grade 3–4 immune- related toxicity occurred in 9% of patients. Long lasting objective responses (OR of 6–17%) were observed in 9/52 melanoma (29% response rates at 3 mg/kg), 2/17 RCC, 5/49 NSCLC (mostly non squamous subtypes) and 1/17 ovarian cancer (no response in 14 pancreatic, gastric, 18 colorectal, 4 breast cancers). Prolonged stabilization of disease was observed for 12–41% lasting at 24 weeks). The median receptor occupancy was higher > 65% in blood PBMC.[1]

A companion paper written by Topalian and coll.[2] reported the efficacy (OR) of the BMS-936558 (anti-PD-1 antibody) in 20–25% among 296 patients treated over a dose range of 0.1 to 10 mg/kg, every two weeks for 8 week-treatment cycles for 12 cycles until progression or complete response. Patients (including those presenting with stabilized brain metastases) were enrolled from Oct 2008 until September 2012. Grade 3–4 immune- related toxicities occurred in 14% of patients. Long lasting objective responses (OR of 20–25%) were observed in 26/94 melanoma, 9/33 RCC, 14/76 NSCLC (but no response in prostate and colorectal cancers). Prolonged stabilization of disease was observed for 20 out of 31 responses lasting for a year at least.[2]

The most surprising findings can be summarized as follows:

- The treatment induced objective responses (according to RECIST criteria) in NSCLC, a poorly immunogenic tumor subtype.
- The duration of the responses across multiple tumor types appeared greater than that observed with most chemotherapies or kinase inhibitors.
- Objective responses were only observed in PD-L1 expressing tumors treated with the anti-PD-L1 antibody (36% vs. 0% in PD-L1$^+$ and PD-L1$^-$ tumors respectively).
- Slight differences appeared in the differential efficacy of both agents, in favor of the anti-PD-1 mAb, which can block the engagement of PD1 by both PD-L1

and PD-L2. At the same time it should be noted that PD-L1 also binds to B7.1 (CD80), in addition to PD-1,[32] meaning that this result was not totally expected.

We anticipate that the FDA and EMEA will approve PD-1 and PD-L1-targeting antibodies soon, if Phase III trials validate their therapeutic potential, especially if suitable biomarkers allowed to predict which fraction of the patient population may profit from these treatments. Moreover, we surmise that combinatorial regimens associating several blockers of the inhibitory pathways (anti-CTLA, anti-PD-1 or PD-L1/L2, anti-LAG3, anti-TIM3, among others) might have synergistic antitumor effects, although possible autoimmune side effects will have to be excluded.[33] The combination of checkpoint blockers and therapeutic cancer vaccines and/or adoptive T cell therapies may yield important results as well.

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