CD73: A potential biomarker for anti-PD-1 therapy

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In our recent study, we show that tumoral CD73 expression limits the efficacy of anti-PD-1 therapy, and this is rescued by concomitant A2A blockade. Since CD73 is known to be overexpressed in several human cancers and A2A antagonists have undergone clinical trials for Parkinson’s Disease, this combination warrants further investigation.

The success of the checkpoint inhibitors anti-PD-1 and anti-CTLA-4 in clinical trials has greatly intensified interest in the potential applications of immunotherapy in cancer. Although their success to date has been a landmark in the field of immunotherapy, their effectiveness is currently limited to an undefined subset of patients.1 There is a clear need to identify these checkpoint inhibitors and in this regard biomarkers are clearly lacking. In studies analyzing immune correlates for predictive power with regard to efficacy of PD-1 blockade, PDL-1 expression on the tumor cells and/or infiltrating immune cells has emerged as a promising biomarker for response to PD-1 blockade. For example, in the study by Taube et al., the efficacy of anti-PD-1 in patients with PDL-1+ biopsies was 39%; while this figure is greatly improved from the overall rate of 24%. However, further biomarkers are needed to improve prediction of responsiveness in patients with PDL-1+ tumor biopsies.1

In our recent study, we identified CD73 as a potential biomarker for response to anti-PD-1.2 Our finding that CD73 expression on tumor cells reduces the immune response evoked by anti-PD-1 mAb therapy, supports our previous findings that CD73 expression suppresses the immune response induced by anthracyclines3 and the observations by Iannone et al. that inhibition of CD73 enhanced the efficacy of anti-CTLA-4 in a melanoma model.4 In humans, CD73 expression has been observed in several cancer types, driven by multiple factors in the tumor microenvironment including hypoxia,5 and its expression is correlated with poor prognosis in triple negative breast cancer.3 CD73 is an ectoenzyme which suppresses the antitumor immune response due to its conversion of AMP to adenosine which consequently suppresses immune responses due to the activation of A2A and A2B receptors on a wide range of immune cells including T lymphocytes.5 Targeting this pathway by either direct inhibition of CD73 or the downstream A2A/A2B receptors has been shown to induce antitumor immunity3,6,7 (and reviewed by Leone et al5).

Since activation of T cells results in increased expression of A2A receptors, we hypothesized that activation of T cells following anti-PD-1 may increase their expression of A2A receptors and consequently suppress the immune response induced by anti-PD-1. Indeed, we showed that PD-1 blockade resulted in enhanced A2A expression on tumor-infiltrating CD8+ T cells and that combined treatment with anti-PD-1 and an A2A antagonist led to greater antitumor immune responses. Single-agent treatment with anti-PD-1 was associated with transient increases in IFNγ and Granzyme B expression by CD8+ Tumor-infiltrating lymphocytes (TILs) whereas combined PD-1 and A2A blockade led to prolonged expression of IFNγ and Granzyme B. We propose that the transient increases in T cell effector functions following anti-PD-1 monotherapy may be due to enhanced adenosine mediated immunosuppression resulting from the increased expression of A2A receptors (Fig. 1). Notably, our findings that A2A blockade can enhance the activity of anti-PD-1 are supported by the recent study by Sitkovsky and colleagues who showed that the removal of the hypoxic (adenosine promoting) environment could enhance the activity of dual PD-1 and CTLA-4 blockade in an A2A dependent manner.7 Although our data supports a number of studies showing that blockade of the A2A receptor enhances antitumor immunity3,5,7 it has recently been observed that genetic ablation of A2A can be deleterious in some tumor models due to reduced T cell effector memory differentiation/survival.8 Therefore, it may be that the pharmacodynamic and pharmacokinetic properties of A2A antagonists are vital to their therapeutic outcome.

The enhanced expression of A2A on CD8+ TILs following PD-1 blockade correlates well with the findings by Allison and colleagues that blockade of PD-1 results in enhanced expression of CTLA-4 on CD8+ TILs.9 The increased expression of alternative checkpoint inhibitors/immunosuppressive receptors following the blockade of a single pathway may underpin the increased efficacy of combined immunotherapy.9 It
has also been suggested that blockade of PD-1 and CTLA-4 may be synergistic due to the blockade of distinct immunosuppressive signaling pathways in T cells. Since PD-1 signals to dampen TCR signaling through engagement of the SHP-1/SHP-2 pathway, and A2A activation suppresses T cell activity through enhancing the intracellular concentration of cAMP, dual PD-1 and A2A blockade represents an alternative way to enhance T cell activation by blocking multiple immunosuppressive signaling pathways.

Interestingly, another paper recently reported that dual PD-1 and A2A blockade could enhance anti-metastatic NK cell responses. However, we observed no expression of PD-1 on NK cells in tumor bearing mice, and no increase in NK cell number following PD-1 blockade. Although this data strongly suggests that anti-PD-1 has little direct effect on NK cell activity in this setting, it remains possible that NK cell function may be indirectly enhanced through modulation of PD-1 immunosuppressive subsets. Notably however, Mittal et al. did not observe any enhanced antitumor effect following CD4 depletion, seemingly precluding a role for Tregs in this effect. In any case, A2A antagonists are known to enhance NK cell responses and so the ability of A2A antagonists to activate an antitumor immune response through multiple cell types is clearly a therapeutic advantage.

In summary, our results support the notion that targeting multiple immunosuppressive pathways is likely to be required for optimal therapeutic outcomes. Since A2A antagonists, including SYN-115 as used in our study, have undergone clinical trials for Parkinson’s disease and shown to be safe, there is a clear rationale to trial combination immunotherapies therapies including A2A blockade in cancer patients.

**Figure 1.** Combined blockade of PD-1 and A2A leads to enhanced CD8+ antitumor immune responses. (A) In tumors expressing both PDL-1 and CD73, CD8+ T cells are suppressed by both PDL-1 and adenosine: A2A interactions. (B) Following PD-1 blockade with anti-PD-1/anti-PDL-1 the activation of CD8+ T cells results in increased production of effector molecules such as IFNγ and Granzyme B but also an increase in expression of A2A which limits the CD8+ T cell response. (C) Combined treatment with anti-PD-1 and an A2A antagonist eliminates this negative feedback mechanism, resulting in further increases in CD8+ cytotoxicity/cytokine production and greater antitumor efficacy.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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