Co-blockade of immune checkpoints and adenosine A2A receptor suppresses metastasis

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Immunosuppressive pathways active within the tumor microenvironment must be targeted in combination to sufficiently bolster antitumor immune defenses. Inhibition of A2A adenosine receptor signaling in combination with immune checkpoint blockade enhances CD8+ T and NK cell anti-metastatic activity. This results in reduced metastatic burden and improved survival in pre-clinical models.

Antibody-directed blockade of immune checkpoint molecules, such as PD-1 and CTLA-4, is now established as a significant therapeutic modality for the treatment of cancer.1,2 Yet for some patients where an immune reaction exists, single immune checkpoint blockade may fail due to the multiple non-redundant immunosuppressive mechanisms that exist to facilitate tumor growth. Targeting multiple immune checkpoint molecules in tandem heightens objective response rates, however, the occurrence of immune-related adverse events (irAEs) is also increased.3 It is therefore imperative to identify alternate immunosuppressive pathways active within the tumor that may be targeted in combination to strengthen antitumor immune defenses with minimal associated irAEs.

The immunosuppressive adenosinergic pathway has risen to prominence as an anticancer target due to its association to hypoxic conditions often evident within the tumor microenvironment. Generation of adenosine is mediated by the ectoenzyme CD73, expressed on both hematopoietic and non-hematopoietic cell types. In addition, tumor-derived CD73 is associated with increased migratory and invasive capacity and holds potential as a tumor biomarker for poor prognosis and metastatic progression.4,5 Adenosine acts through four adenosine receptors, in particular signaling via the A2A and A2B adenosine receptors both dampens immune effector functions and increases the prevalence and activity of immunosuppressive cell subsets.6,7 Previous pre-clinical investigations identified A2A and A2B adenosine receptor antagonism by small molecular inhibitors to be protective against metastatic progression of CD73+ tumors.7 Monotherapeutic activity of A2A, but not A2B, adenosine receptor inhibitors appeared mediated by lymphocytic populations as Rag2−/−γc−/− mice, lacking natural killer (NK), T, and B lymphocytes, rendered this therapy inactive.7 Due to this dependence on effector immune cell populations, particularly NK cells and CD8+ T lymphocytes, we hypothesized that targeting multiple immunosuppressive mechanisms in combination may increase anti-metastatic activity. In our recent study, we investigated whether immune checkpoint blockade in combination with targeted inhibition of A2A adenosine receptor signaling could further increase immune effector functions to reduce metastatic progression.8

We assessed the validity of this therapeutic combination in both an experimental lung and spontaneous metastasis mouse model, using B16F10-CD73hi melanoma and 4T1.2 mammary carcinoma, respectively. In both models, combining A2A adenosine receptor inhibition with mAbs targeting immune checkpoint molecules, in particular anti-PD-1, significantly improved the antitumor immune response comparative to single agent activity alone.8 This was observed as significantly prolonged survival in the spontaneously metastasizing 4T1.2 model. Similarly, pulmonary metastatic burden of B16F10-CD73hi was significantly reduced in an apparent synergistic manner by this therapeutic combination.

Therapeutic efficacy for this combinatorial approach, targeting A2A adenosine receptor inhibition alongside anti-PD-1, was mediated by both CD8+ T and NK cells. In addition, a critical dependency on IFNy and to a lesser extent perforin was also established. These molecules modulate the effector functions and cytotoxic capabilities of both CD8+ T and NK cells. In addition, a critical dependency on IFNy and to a lesser extent perforin was also established. These molecules modulate the effector functions and cytotoxic capabilities of both CD8+ T and NK cells. In addition, a critical dependency on IFNy and to a lesser extent perforin was also established. These molecules modulate the effector functions and cytotoxic capabilities of both CD8+ T and NK cells.
causing reduction in tumor growth, alongside increased infiltration of CD8+ T cells heightening the T effector: T regulatory ratio.9

When targeting immunosuppressive adenosine, two main strategies have been employed with success in pre-clinical models. These approaches can be separated into those targeting generation of adenosine vs. adenosine signaling.4,7 While adenosine signaling has been solely targeted by small molecular inhibitors, both antibody blockade and pharmacological intervention have proven efficacy in preventing adenosine generation. The anticipated benefits and concerns surrounding these therapeutic approaches have been summarized in Fig. 1.

Diversity in function of CD73 (producing immunosuppressive adenosine, enhancing angiogenesis and mediating tumor and immune cell migration) suggests that this molecule may prove to be an excellent target for enhancing antitumor immunity. Antibody-blockade of CD73 may also enable additional immune-dependent benefits through Fc receptor-mediated responses. However, lack of humanized CD73 therapies provides an obstacle in proceeding to the clinic (Fig. 1). Nonetheless, pre-clinical studies have supported the combinatorial administration of anti-CD73 or targeted inhibition, by APCP, in tandem with immune checkpoint blockade.9,10 Tumor regression and in some cases complete rejection was observed via IFNγ-dependent expansion of tumor-specific CD8+ T cells.10 This further emphasizes the importance and possible cohesive nature of targeting immunosuppressive adenosine in combination with immune checkpoint blockade.

Understanding the tumor microenvironment most likely to benefit from adenosine-related therapies remains an important consideration for its clinical success. Presence of CD73 on the tumor may provide a biomarker to stratify patients for which these therapeutic options are likely to elicit an optimal antitumor immune response.7,8 While early, concurrent treatment appears most effective in mouse models, this dosing schedule may require optimization moving forward to clinical utility. Importantly, the presence of adenosine, or adenosine analog NECA, was shown to enhance PD-1, but not CTLA-4, expression on infiltrating Tregs and tumor-specific CD8+ T cells.10

**Figure 1.** Targeting adenosine generation and signaling in combination with immune checkpoint blockade enhances antitumor response. Adenosine receptor signaling and interaction of the immune checkpoint receptor, PD-1, with its ligand, PDL-1, can decrease immune effector functions leading to tumor progression. Combinatorial approaches, which inhibit multiple immunosuppressive pathways in tandem, can improve antitumor immunity. Therapies directed toward both adenosine signaling and generation may be able to overcome adenosine-generated immunosuppression.
Antagonism of A\textsubscript{2A} adenosine receptor signaling, as well as CD73-deficiency, was able to reduce PD-1 expression. This apparent association suggests that targeting immunosuppressive adenosine preceding anti-PD-1 may potentiate therapeutic efficacy by increasing the ratio of antibody to target.

Advantageously, A\textsubscript{2A} adenosine receptor antagonists are currently undergoing clinical testing for Parkinson’s disease and display excellent safety profiles, indicating a possible transition to clinical utility in an oncology setting. Targeting non-redundant immunosuppressive pathways in combination may improve overall response rates to cancer. Our study, with others, provides evidence for the clinical utility of targeting A\textsubscript{2A} adenosine receptor inhibition and immune checkpoint blockade to improve antitumor immunity.8-10

Disclosure of Potential Conflicts of Interest

Professor Mark Smyth and Dr. John Stagg declare financial research support from Medimmune LLC.

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