**IN THE SPOTLIGHT**

Lessons from the A2A Adenosine Receptor Antagonist-Enabled Tumor Regression and Survival in Patients with Treatment-Refractory Renal Cell Cancer

Michail V. Sitkovsky

Summary: In this issue of Cancer Discovery, Fong and colleagues describe the encouraging observations of tumor regression, disease control, and survival of patients with otherwise refractory renal cell cancer with progressive disease after treatment with the conceptually novel oral antagonist of the A2A adenosine receptor (A2AR), ciprofloxacin. A2AR antagonists may represent the until now missing but critically important part of more effective immunotherapies of cancer, because they prevent the inhibition of tumor-reactive T and natural killer cells by blocking the immunosuppressive hypoxia–A2A-adenosinergic signaling, which represents an emerging immunosuppressive hallmark of tumors that are the most resistant to therapies.

See related article by Fong et al., p. 40 (1).

Currently, the majority of patients with cancer are still eventually refractory to any cancer therapy despite a massive and decades-long effort. The hope for the solution to this acute medical problem may come from taking a different and novel therapeutic path, as did Fong and colleagues (1), who, in an “out-of-the-box” approach, treated patients with refractory renal cell cancer (RCC) with a drug that inactivates the biochemical, hypoxia-A2-adenosinergic, immunosuppressive tumor protection (2–8). This powerful mechanism of tumor protection inhibits the antitumor T and natural killer (NK) cells near and within tumors, thereby making them the most resistant to cancer therapies (3, 4, 7), even after the blockade of immunologic negative regulators (4, 6).

THE A2A ADENOSINE RECEPTOR IS A LIFESAVER IN INFECTIOUS DISEASES AND THE LIFE-TAKER IN CANCER

The A2A adenosine receptor (A2AR) has a high affinity for extracellular adenosine, and it was shown to prevent the excessive collateral tissue damage to noninfected but inflamed normal tissues of vital organs by overactive antipathogen T cells and myeloid cells (2). Having the “molecules of life” such as oxygen, or the lack of oxygen, and adenosine as the triggering stimuli, hypoxia-A2-adenosinergic signaling is likely to be the most fundamental anti-inflammatory and tissue-protecting mechanism (4). Unfortunately, A2AR-mediated signaling also misguidedly protects the hypoxic and extracellular adenosine-rich cancerous tissues (3, 4, 7). This is why A2AR blockade with synthetic A2AR antagonists has been proposed for a long time (2, 3) as a therapeutic tool to unleash tumor-reactive T and NK cells to enable immunotherapy-mediated tumor regression (3–7). The synthetic A2AR antagonists can also be termed “super-caffeine,” because the research and development of these highly selective for A2AR and long-lived in vivo drugs was in part prompted by observations of favorable effects of caffeine consumption in patients with Parkinson disease. Originally, not only the A2AR but also the low-affinity A2B adenosine receptor (A2BR) were considered to be targets to antagonize to improve immunotherapies of cancer (3). However, the subsequent biochemical considerations of the differences between the Gs-coupled A2AR and Gs/Gq-coupled A2BR, as well as the more detailed preclinical tumor immunology comparisons of A2AR versus A2BR, led to the prioritization of targeting the upstream hypoxia-inducible factor-1α (hypoxia-HIF1α) stage by supplemental oxygenation or oxygenation agents (Fig. 1). Nevertheless, it would be interesting to see the clinical outcomes of testing the dual antagonists of A2AR and A2BR in immunotherapies of cancer. The two most important of several anti-hypoxia-A2-adenosinergic drugs that are expected to be the most effective in clinically eliminating the immunosuppression in hypoxic and adenosine-rich tumors (Fig. 1; refs. 4, 7, 8) are: (i) an A2AR antagonist (2, 3) and (ii) the hypoxia-HIF1α-mediated immunosuppression-weakening oxygenation of tumors.

TUMOR REGRESSION AND SURVIVAL OF PATIENTS WITH REFRACTORY RCC

Fong and colleagues are among the first clinical development teams that aimed to block not only the immunologic negative regulators (9), but also the powerful A2A-adenosinergic negative regulators of antitumor immunity (4, 8). It is reported (1) that the antagonist of A2AR does enable durable...

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survival, tumor regression, and disease control even in patients with RCC who are refractory to all other treatments. Such responses were observed among the 68 patients with RCC with progressive disease in the beginning of the study. These patients with RCC previously were not responsive to either PD-L1 antibody or any other therapy. The clinical responses described included partial responses (PR) in 11% of patients with RCC who were treated with A2AR antagonist in combination with anti–PD-L1 antibody and in 3% of patients treated with A2AR alone. Tumor regression was also observed in an additional 24% of patients, but it was less than required to be defined as PR by RECIST criteria.

These percentages are within the range of clinical responses to PD-1 or CTLA4 blockade in cancers other than melanoma, except that these patients with RCC were, until receiving A2AR antagonist, untreated and refractory to PD-1 blockade. The observed tumor regressions likely happened only in those patients (i) whose tumor was immunogenic; (ii) who did develop tumor-reactive effector T cells; (iii) who did retain at least a functionally meaningful number of effector cells even after previous toxic cancer chemotherapies; and (iv) whose tumors were protected only by immunosuppressive extracellular adenosine→A2AR signaling, so that A2AR antagonist enabled the invasion and tumor-rejecting effector functions of T and NK cells. However, the levels of antitumor immunity in these responsive patients were still not sufficiently high to accomplish a complete response.

The lack of tumor-reactive T and NK cells or their low numbers in a refractory patient due to the poor immunogenicity of tumor or due to toxic past chemotherapies seems to represent the major limitation of both immunologic and biochemical negative regulators. Indeed, according to previous insights (2, 3), an A2AR antagonist was predicted to be therapeutically most effective only in those patients who are known to have a sufficient number of aggressive and multifunctional tumor-reactive T cells. Otherwise, an A2AR antagonist was expected to have antitumor effects only if combined with cancer vaccines or adoptive T-cell transfer (3) that increase the number of tumor-reactive T cells in a patient. Those patients would then also be treated with the blockade of immunologic negative regulators (5, 9).

Fong and colleagues had to deal with this major limitation because the status of refractory patients in terms of quality and numbers of tumor-reactive T cells and NK cells is not possible to know. Therefore, the predictably best clinical outcomes of treatment with A2AR antagonist are to be expected only in patients who are infused with sufficient numbers of the highly aggressive tumor-reactive T and NK cells by adoptive cell transfer (ACT). The future combination of A2AR antagonism with ACT is now even more promising to test with refractory patients in view of clinical observations (1). It seems unavoidable to eventually turn to ACT to partner with A2AR antagonism as the only immunotherapy that can ensure sufficient T-cell and NK-cell presence in a patient.
To support their point that the A2AR antagonist did accomplish encouraging disease control and survival benefit without very high objective response rates, the authors highlight the results of the recent meta-analysis of 87 clinical trials of solid tumors treated with checkpoint inhibitors. The unexpected and potentially paradigm-shifting message was that there is no correlation between response rate and survival. This led the authors to hypothesize that the A2AR antagonist may be enabling the more persistent and durable elevation of tumor-controlling immunity. These considerations should be resolved by longer follow-up and additional studies, thereby clarifying the ongoing discussion of the relative value of evaluating the treatment in terms of the disease control and survival benefit versus the number of patients showing high objective responses.

This is the first step. In view of the much-appreciated problem with the predominance of patients with refractory cancer, it is surprising that it took so long since the first insights (2) and demonstrations of the immunotherapeutic use of A2AR antagonists in cancer (3) to the current publication with supporting clinical data (1). However, it is now much clearer as to what must be done to develop the next generation of the most effective anti-hypoxia-A2A-adenosinergic immunotherapies. The future clinical designs will likely incorporate instructive insights from reported clinical data (1) and earlier preclinical mechanistic studies. In this sense, the testing of A2AR mono-therapy, as well as dual therapy with PD-1 blockade (1), was valuable for future designs because the obtained clinical data allowed evaluation of tolerance to A2AR antagonist alone and evaluation of possible synergies with the blockade of PD-1. The blockade of immunologic negative regulators will continue to be an important part of any therapy, especially to unleash the antitumor T and NK cells in nonhypoxic and adenosine-poor tumors, where there may be less needed for A2AR antagonists.

WHICH OTHER TYPES OF CANCERS COULD BE TARGETED NEXT BY A2AR ANTAGONISTS?

Because hypoxic and adenosine-rich tumors are protected from cytolytic T and NK cells even after the blockade of immunologic-negative regulators, the reported clinical data (1) support the feasibility of extending the use of A2AR antagonists to treat nonresponsive patients with other types of cancer, because the hypoxic and adenosine-rich microenvironments constitute the general tumor biology of all solid tumors, especially the most resistant tumors.

Indeed, patients with refractory cancer with CD73\textsuperscript{hi} triple-negative breast cancers (TNBC) are likely to be much more susceptible to A2AR antagonists during treatment with doxorubicin, according to Smyth, Stagg, Beavis, Darcy and their colleagues. These authors strongly advanced the field of immuno-oncological use of A2AR antagonists (5–8) by showing that it improves PD-1 blockade (6) and the regression of adenosine-rich and doxorubicin-resistant CD73\textsuperscript{hi} TNBC (7). Their bioinformatics-guided cancer immunology study was influential not only because it identified extracellular adenosine as the main reason for the resistance of these deadly human tumors to current therapies, but also because the authors demonstrated that A2AR antagonists reverse the resistance of CD73\textsuperscript{hi} TNBC to chemotherapy-induced immuno-therapy by enabling doxorubicin to reject these resistant tumors in an appropriate preclinical model.

Metastatic castration-resistant prostate cancer (mCRPC) with high levels of expression of extracellular adenosine-generating prostatic acid phosphatase represents yet another focus for anti-hypoxia-A2-adenosinergic immunotherapies. According to a report at AACR 2019 Annual Meeting (10), treatment with the A2AR antagonist AZD4635 (NCT02740985) was accompanied by examples of durable >99% PSA decrease, immune activation, and disease control in 3 patients plus three confirmed responses of strong tumor regression among 8 A2AR antagonist-treated RECIST-evaluable refractory patients with advanced mCRPC.

These clinical data with patients with refractory mCRPC further support the feasibility of effective treatment of other resistant cancers using an A2AR antagonist, especially if cancers are hypoxic and/or adenosine-rich.

OTHER REQUIREMENTS TO ACCOMPLISH BETTER TUMOR REGRESSION AND SURVIVAL OF REFRACTORY PATIENTS

There is a requirement in predictive biomarkers of adenosine-rich tumors for the best clinical outcomes. The findings with CD73\textsuperscript{hi} TNBC (7) provided the first example of how useful it could be to have predictive biomarkers of response to an A2AR antagonist. Therefore, it is valuable that now there is such diagnostic adenosine signature as “AdenoSig” (1) to select patients most likely to benefit from treatments based on adenosine blockade. This is important because no clinical benefit of A2AR antagonists is expected if the tumor is not extracellular adenosine-rich (2, 3).

But even if the tumor is adenosine-rich and it will be assured that patients with A2AR antagonist–treated refractory cancer have sufficient numbers of antitumor T and NK cells, the immunotherapy will not be fully effective clinically if a tumor is still protected by the HIF1α and hypoxia response element (HRE)–mediated immunosuppressive transcription (Fig. 1). The most attractive therapeutic solution will be to combine A2AR antagonist immunotherapy with the upstream-targeting and strongly anti-immunosuppressive oxygenation of hypoxic and adenosine-rich tumors. The oxygenation of hypoxic tumors is the most obvious, feasible, and justifiable next approach to further improve tumor regression, because both the upstream and the downstream stages of the hypoxia-A2A-adenosinergic pathway need to be targeted to maximally cancel the immunosuppressive transcription by adenosine-A2AR-cAMP-cAMP response element and by the hypoxia–HIF1α–HRE (ref. 4; Fig. 1).

The combinations of an A2AR antagonist with anti-hypoxia-HIF1α drugs such as systemic supplemental oxygen or oxygenation agents will complement the antitumor effects of A2AR antagonists by preventing the hypoxia-driven accumulation of immunosuppressive extracellular adenosine and by blocking the transcription of TGFβ and other immunosuppressive molecules (11). Reassuringly, the provided proof of principle for the clinical efficacy of A2AR antagonism (1) is also predicting the clinical efficacy of those types of anti-hypoxia-A2A-adenosinergic drugs, including oxygenation...
agents, which were shown (11) to act upstream of A2AR in this pathway to reverse immunosuppression in tumors (Fig. 1).

Disclosure of Potential Conflicts of Interest

M.V. Sitkovsky reports receiving commercial research grants from Juno/Therapeutics/Celgene/BMS and is the author of the method-of-use patents issued in the USA, EU, Canada, and Australia that belong to the U.S. National Institutes of Health. These patents cover the combination of A2A adenosine receptor antagonist and/or adenosine deaminase or inhibitors of the extracellular adenosine-generating enzymes and/or oxygen or oxygenation agents with cancer vaccines and T killer cells in immunotherapies of cancer and infectious diseases. No other potential conflicts of interest were disclosed.

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