REVIEW

The inhibitory effect of adenosine on tumor adaptive immunity and intervention strategies

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Abstract   Adenosine (Ado) is significantly elevated in the tumor microenvironment (TME) compared to normal tissues. It binds to adenosine receptors (AdoRs), suppressing tumor antigen presentation and immune cell activation, thereby inhibiting tumor adaptive immunity. Ado downregulates major histocompatibility complex II (MHC II) and co-stimulatory factors on dendritic cells (DCs) and macrophages, inhibiting antigen presentation. It suppresses anti-tumor cytokine secretion and T cell activation by disrupting T cell receptor (TCR) binding and signal transduction. Ado also inhibits chemokine secretion and KCa3.1 channel activity, impeding effector T cell trafficking and infiltration into the tumor site. Furthermore, Ado diminishes T cell cytotoxicity against tumor cells by promoting immune-suppressive cytokine secretion, upregulating immune checkpoint proteins, and enhancing immune-suppressive cell activity. Reducing Ado production in the TME can significantly enhance anti-tumor immune responses and improve the efficacy of other immunotherapies. Preclinical and clinical development of inhibitors targeting Ado generation or AdoRs is underway. Therefore, this article will summarize and analyze the inhibitory effects and molecular mechanisms of Ado on tumor adaptive immunity, as well as provide an overview of the latest advancements in targeting Ado pathways in anti-tumor immune responses.

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1. Introduction

Ado is primarily generated through the sequential action of extracellular hydrolysis of adenosine triphosphate (ATP) hydrolysis by ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase1/CD39) and extracellular 5′-nucleotidase (5′-NT/CD73)[1,2]. In the canonical pathway, CD39 enzymatically converts ATP into adenosine diphosphate (ADP) and adenosine monophosphate (AMP). In the non-canonical pathway, CD38 utilizes nicotinamide adenine dinucleotide (NAD) as a substrate to generate ADP-ribosylation (ADPR). Subsequently, ADPR is converted to AMP through the action of nucleotide pyrophosphatases/phosphodiesterase 1. Both pathways lead to the production of AMP, which is further converted to Ado by the action of CD73. This process is considered the rate-limiting step in the Ado generation[3–5]. Additionally, Ado can also be released from the cytoplasm through nucleoside transport proteins[6]. Ado can be metabolized into inosine by membrane-associated Ado deaminase, or it can be transported into the cell through specific nucleoside transporters (Fig. 1)[7,8].

Under normal physiological conditions, Ado serves as a constituent of ATP and ADP, regulating the levels of ATP and ADP, and playing a crucial role in maintaining energy balance and metabolism[3,6]. Additionally, it functions as a vital neurotransmitter in the nervous system. Ado is widely expressed in tissues throughout the body, but its concentration is relatively low, ranging from 0.05 to 0.2 μmol/L[9]. However, in solid tumors, due to factors such as cell necrosis and other secretory mechanisms, conditions like hypoxia, inflammation, nutrient scarcity, and cytotoxic drug treatments have a dual impact on Ado levels. On one hand, tumor cells release a substantial amount of ATP, directly leading to an increase in its hydrolysis product, Ado[10,11]. On the other hand, factors including hypoxia-inducible factor 1 (HIF1) and cytokines like transforming growth factor beta (TGF-β) promote the transcription and expression of CD39 and CD73 enzymes, enhancing the breakdown of ATP and further elevating Ado concentrations in the TME[12–15]. Through these two mechanisms, Ado levels can rise to as high as 1–10 mmol/L (Fig. 1). Furthermore, CD39 and CD73 are pivotal extracellular enzymes responsible for Ado production, and they are highly expressed in various cell types within the TME, including tumor cells, stromal cells, immune cells, endothelial cells, and fibroblasts[16,17].

Ado exerts its immunosuppressive effects by binding to AdoRs and triggering signaling cascades. Currently, four types of G protein-coupled receptors (GPCRs) are known to be AdoRs, including A1R, A2aR, A2bR, and A3R[17]. Ado mainly exerts their immunosuppressive effects by activating A2aR and A2bR. A2aR is expressed in most immune cells, while A2bR is mainly expressed by macrophages and DCs[18]. The A2aR which is a GPCR forms a complex with intracellular Gαs and Gβ-Gγ subunits on the surface of immune cells. When Ado binds to A2aR, Gαs dissociates from Gβ-Gγ, activating adenylyl cyclase (AC). AC then breaks down intracellular ATP into ADP and cyclic adenosine monophosphate (cAMP), with cAMP serving as a second messenger to activate protein kinase A (PKA) within the cell[19]. PKA phosphorylates cyclic-AMP response binding protein (CREB), leading to the upregulation of immunosuppressive cytokines, including interleukin 10 (IL-10) and forkhead box P3 (Foxp3). At the same time, phosphorylated CREB suppresses the expression of anti-tumor cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6[20]. In addition, the binding of Ado to A2bR triggers immunosuppressive effects by activating the AC/cAMP/PKA signaling pathway. However, activating A1R and A3R on immune cells inhibits AC and cAMP (Fig. 1)[21]. Ado binds to A2aR/A2bR on DCs and macrophages, inhibiting their antigen-presenting function. It also binds to A2aR on T cells, activating the cAMP/PKA/CSK signaling pathway and suppressing the secretion of chemokines, thereby inhibiting T cell activation and infiltration. Additionally, Ado can induce functional exhaustion of effector T cells through both external and internal regulatory mechanisms. Moreover, in solid tumors such as lung cancer, hepatocellular carcinoma, bladder urothelial carcinoma, pheochromocytoma, cervical squamous cell carcinoma, and gastric adenocarcinoma, components of the Ado pathway, including A2A and A2B, are significantly upregulated. This results in a stronger immunosuppressive effect of Ado in these solid tumors and suggests that inhibitors targeting the Ado pathway may have more pronounced therapeutic effects in these cancers[17,22].

Based on the inhibition of Ado's suppressive effects on tumor adaptive immunity, strategies involving the inhibition of Ado generation or the blockade of AdoRs interactions hold the potential to improve the TME and alleviate Ado's immunosuppressive effects. To date, several inhibitors targeting Ado generation or antagonists blocking Ado receptors are in preclinical or clinical development stages. Therefore, this article will delve into the inhibitory actions and mechanisms of Ado at various stages of regulating tumor adaptive immunity. Additionally, it will discuss research on Ado antagonists aimed at enhancing the efficacy of immune checkpoint inhibitors (ICIs) and clinical treatment approaches.

2. Adenosine inhibits tumor antigen presentation

DCs capture TAAs and use them to generate homologous peptide−MHC complexes. These complexes are accompanied by co-stimulatory molecules like CD80 and CD86. Subsequently, DCs present these complexes to T cells, initiating their activation and immune response. Numerous studies have now clearly demonstrated the inhibitory effects of Ado on DCs. Mature DCs predominantly express A2aR and A2bR, and Ado activates A2aR and A2bR on DCs, thereby increasing intracellular cAMP levels. cAMP inhibits CD86 co-stimulatory signal expression and decreases the expression of MHC II on DCs through the PKA/EPAC signaling pathway[23]. Simultaneously, EPAC activation inhibits the nuclear factor κB (NF-κB) signaling pathway, resulting in a decrease in the secretion of pro-inflammatory cytokines like IL-12 and TNF-α[24]. Additionally, it leads to an increase in the production of factors that promote tumor growth and progression, including IL-10, IL-6, TGF-β, and vascular endothelial growth factor (VEGF). As a result, the antigen-presenting capacity of DCs is diminished[25,26]. The Ado/cAMP signaling pathway can selectively target the PKA/EPAC pathway, leading to the polarization of activated mouse DCs towards an inhibitory phenotype. This phenotypic change promotes tumor suppression and hinders pro-inflammatory cytokine production, ultimately suppressing T-cell activation[27]. In addition, Ado also exerts inhibitory effects on macrophages with antigen-presenting functions. Ado binds to A2bR receptors on macrophages, inhibiting the expression of CD86 co-stimulatory signals and MCH II, thereby suppressing the antigen-presenting capacity of macrophages[3]. In Lewis lung cancer mouse models, A2bR blockade significantly enhances antigen presentation by macrophages, increases CD8$^+$ T cell responses, and inhibits tumor growth[18].
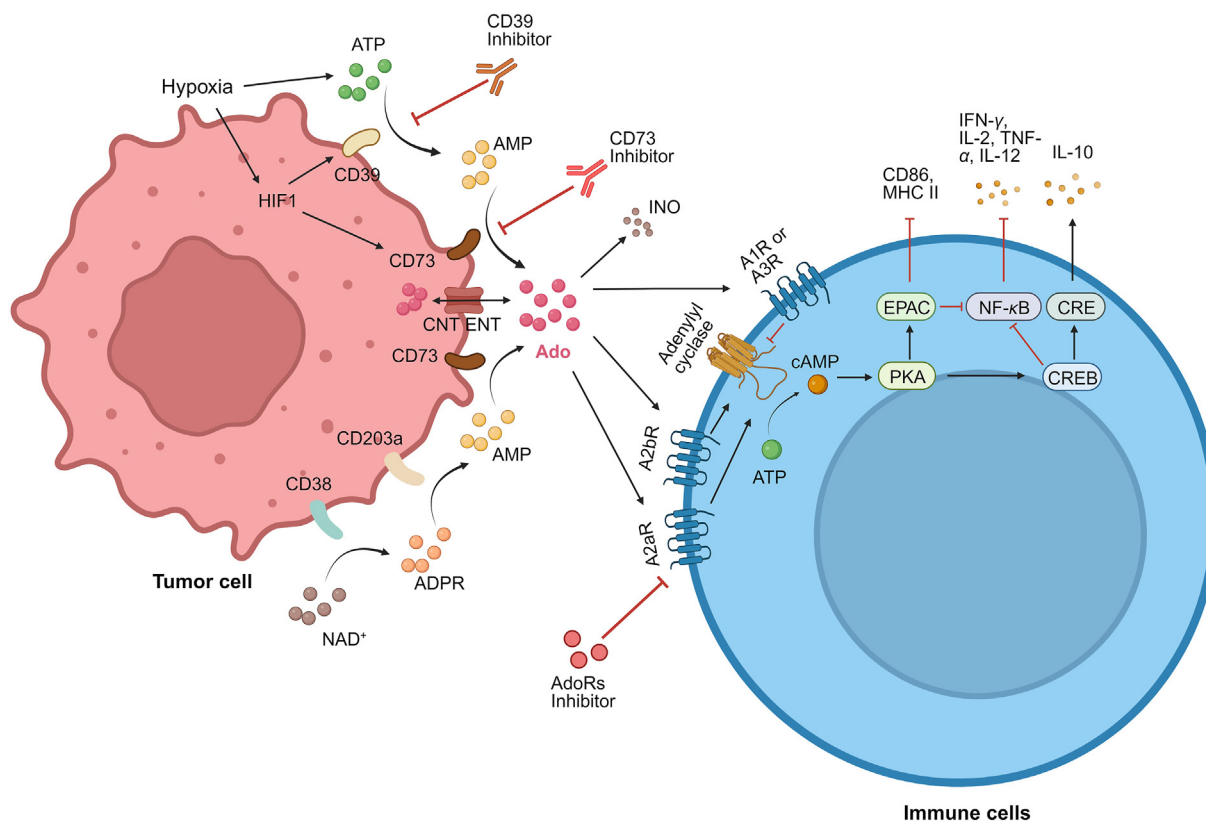
Figure 1 Adenosine metabolism pathway and signaling. The generation of Ado involves both canonical and non-canonical pathways. In the canonical pathway, ATP is converted into ADP and AMP by CD39. In the non-canonical pathway, CD38 utilizes NAD as a substrate to generate ADPR, which is further converted into AMP by ENPP1. Both pathways result in the production of AMP, which is then cleaved by CD73 to generate Ado. Ado can also be released from the cytoplasm through nucleoside transport proteins. Generated Ado can be metabolized into INO by ecto-ADA or transported into cells *via* ENTs and CNTs. Upon binding to A2aR, Ado dissociates Gαs from Gβ-Gγ, activating AC. AC then breaks down intracellular ATP into ADP and cAMP. cAMP serves as a second messenger to activate PKA, which phosphorylates CREB, promoting the expression of immunosuppressive cytokines such as IL-10. Phosphorylated CREB also inhibits the expression of TNF-α, IL-1β, and other anti-tumor cytokines. Additionally, activation of EPAC by Ado binding to A2bR inhibits the expression of CD86 and MHC II. Furthermore, activation of A1R and A3R on immune cells exerts inhibitory effects on AC and cAMP. Inhibitors targeting the Ado pathway act by selectively inhibiting CD39, CD73, and AdoRs, thereby suppressing Ado generation and signaling, ultimately aiming to alleviate the immunosuppressive function of adenosine. Abbreviations: Ado, Adenosine; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CD38, the cluster of differentiation 38; NAD, Nicotinamide adenine dinucleotide; ADPR, ADP-ribosylation; ENPP1, nucleotide pyrophosphatases/phosphodiesterase 1; ecto-ADA, membrane-associated adenosine deaminase; INO, Inosine; ENTs, equilibrative nucleoside transporters; CNTs, concentrative nucleoside transporters; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, Protein kinase A; CREB, cyclic-AMP response binding protein; IL-10, interleukin 10; TNF-α, tumor necrosis factor-α; EPAC, exchange protein directly activated by cAMP; MHC II, major histocompatibility complex II; AdoRs, adenosine receptors.

3. Adenosine inhibits T cell activation and infiltration

3.1. Adenosine inhibits T cell activation

After TCR recognizes a homologous peptide—MHC complex, the TCR—CD3 complex undergoes clustering, and its intracellular region is phosphorylated by the tyrosine kinase Lck. This process triggers signal transduction by phosphorylating the immunoreceptor tyrosine-based activation motif, which leads to the interaction between ZAP-70 and CD3ζ, subsequently activating T cells[28,29]. T cells express abundant A2aR, and Ado binding to A2aR on T cells activates PKA in a cAMP-dependent manner. Activated PKA then phosphorylates and inactivates Lck through activating tyrosine-protein kinase (CSK). This reduces the tyrosine phosphorylation of the TCR/CD3ζ chain during T cell activation, disrupting the transmembrane signaling of TCR in T cells.

Blocking A2aR can restore T cell function[30−33]. Furthermore, CSK activation hinders the IL-2R mediated signal transduction in T cells and suppresses the expression of the co-stimulatory molecule CD28 consequently. This inhibition ultimately impedes effector T cell activation and proliferation[34]. Additionally, A2aR activation triggers cAMP/PKA/CREB signaling and leads to upregulation of CREB. By inhibiting NF-κB and the nuclear factor of activated T cells, CREB suppresses the production of anti-tumor cytokines including IL-6, IL-2, IL-4, TNF-α, IL-5, and interferon-gamma (IFN-γ). Consequently, this inhibits the activation of T cells and leads to a reduction in the secretion of these cytokines with anti-tumor properties[20]. The interaction between the TCR on CD4+ T cells and TAAs stimulates the production of IFN-γ, which in turn promotes T cell differentiation and activation. However, A2aR activation on T cell surfaces by Ado inhibits transmembrane signaling triggered by TCR, leading to a 98%

reduction in TCR-mediated production of IFN-γ and subsequently impairing T cell function[35].

3.2.   Adenosine inhibits T cell trafficking and infiltration

The trafficking and infiltration of effector T cell into the TME are critical steps in cancer immune responses, which is primarily achieved through the interaction of chemokines and their receptors[36]. The expression and production of chemokines C−X−C motif ligand 9 (CXCL9) and CXCL10 are mainly induced by IFN-γ[37−39]. In the solid tumor TME, Ado accumulates and inhibits the chemotactic response and tumor infiltration level of tumor-specific T cells by activating A2aR[40]. The activation of the cAMP/PKA signaling pathway occurs when Ado binds to A2aR on effector T cells. This activation causes a considerable decrease in IFN-γ secretion. Consequently, there is a significant reduction in the expression of chemokines, including chemokine CXCL9 and CXCL10, within tumor cells. As a result, the recruitment and infiltration of effector T cells into the tumor site are inhibited[41,42].

Research findings indicated that inhibition of AdoRs in mouse breast cancer models enhanced IFN-γ-induced CXCL10 production, which facilitated T cell recruitment and hindered tumor growth[43]. In a melanoma lung metastasis mouse model, the Ado signaling pathway in the TME inhibited the production of chemokines CXCL9 and CXCL10 in late-stage metastatic lesions, resulting in reduced infiltration of effector T cells[38]. In tumor mouse models (B16F10 and SM1WT1) lacking A2aR, increased CD8$^+$ T cells infiltration into the tumor and significant tumor growth inhibition were observed[44]. Similarly, in a head and neck squamous cell carcinoma (HNSCC) mouse model, A2aR blockade using the small molecule inhibitor SCH58261 enhanced CD8$^+$ T cells' presence within the tumor and promoted the expression of IFN-γ and TNF-α, thereby improving the tumor-killing capabilities[45]. However, the presence of Ado reduced the chemotaxis (movement towards a chemical signal) of CD8$^+$ T cells in HNSCC and had a more profound effect on HNSCC CD8$^+$ T cells compared to CD8$^+$ T cells from healthy donors[40]. Ion channels play a regulatory role in various functions of T cells, such as cytokine production and chemotactic ability[46−48]. The KCa3.1 channel in T cells regulates cell chemotaxis and is involved in Ado's inhibitory effects[46]. Ado promotes cAMP production and triggers PKA activation *via* A2aR. Consequently, PKA inhibits the KCa3.1 channel, resulting in the decrease of T cell chemotaxis and T cell migration[49,50]. HNSCC CD8$^+$ T cells exhibit the decreased activity of the KCa3.1 channel. However, the chemotactic capability of HNSCC CD8$^+$ T cells can be restored in the presence of Ado by activating the KCa3.1 channel using 1-EBIO[40,50].

4.   Adenosine inhibits T cell killing of tumor cells

Within the TME, cytotoxic T lymphocytes (CTLs) may experience exhaustion, characterized by a decline in effector cytokine production, upregulation of inhibitory receptors, and decreased cytotoxicity, ultimately enabling tumor immune evasion. The regulation of T cell exhaustion in TME can be divided into external and internal pathways[51]. In terms of external regulatory mechanisms, several cell types play a role in mediating T cell exhaustion, including Tregs, tumor-associated macrophages (TAMs), DCs, MDSCs, and tumor cells[52−55]. Key extrinsic cytokines involved in the phenomenon of T cell exhaustion include IL-10 and TGF-β[56,57]. Tregs, MDSCs, and tumor cells inhibit the function of CTLs by releasing TGF-β, while M2 macrophages promote CTLs exhaustion by expressing signal transducer and activator of transcription 3 (STAT3)[58,59]. Additionally, tumor cells secrete immune-suppressive mediators such as indoleamine 2,3-dioxygenase 1, programmed cell death ligand-1 (PD-L1), cyclooxygenase-2, and STAT3 within the TAMs to suppress CTLs activity. Tregs also express immune checkpoint receptor proteins to inhibit the activity of CD8$^+$ T cells[60,61]. Concerning the internal mechanisms, the binding of Ado to AdoRs increases the expression of immune checkpoint proteins, including programmed cell death 1 (PD-1), cytotoxic T lymphocyte antigen 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3, and the binding of immune checkpoint proteins bind to their cognate ligands inhibits the tumor-killing capability of T cells and leads to the exhaustion of T cells. Consequently, tumor cells can evade immune surveillance by the host cells[62,63]. Numerous studies have indicated that Ado contributes to T cell functional exhaustion by mediating both external and internal regulatory pathways, thereby suppressing their antitumor response.

4.1.   Adenosine promotes T cell exhaustion through extrinsic regulatory mechanisms

There are various immunosuppressive cells in the TME, including Tregs, TAMs, DCs, MDSCs, and tumor cells, which negatively regulate the function of cytotoxic T cells. Ado amplifies the inhibitory effect of immunosuppressive cells and further inhibits the activity of cytotoxic T cells consequently[64]. By binding to A2aR, Ado promotes CD4$^+$ T cells to differentiate into Tregs through Foxp3 and LAG3 activation. This increase in CD4$^+$ Foxp3$^+$ Tregs inhibits the tumor-killing effect of CTLs by increasing immunosuppressive cytokines secretion[33,65,66]. Additionally, Tregs promote CD39 and CD73s′ expression, leading to Ado accumulation in the TME, which further stimulates A2aR and A2bR signaling pathways through paracrine or autocrine mechanisms, forming a positive feedback loop that enhances the suppressive function of Tregs[67−70]. In a mouse model of HNSCC, an increased number of Tregs expressing A2aR was observed. Inhibition of A2aR with the antagonist SCH58261 suppressed tumor growth, decreased Treg cells, and enhanced CTLs' anti-tumor response[45]. And in a mouse model of chronic lymphocytic leukemia, the use of an A2aR inhibitor reactivated T cells while limiting the expansion of Tregs[71]. Administration of high oxygen levels to tumor-bearing mice, reducing tumor hypoxia and inhibiting extracellular Ado accumulation driven by hypoxia in the TME, weakened the immunosuppressive effects of Tregs[72]. Furthermore, Hu et al.[73] discovered that tumor-infiltrating CD73$^+$ $\gamma\delta$Tregs were the predominant Tregs in human breast cancer. They subsequently demonstrated that IL-6 secreted by cancer-associated fibroblasts (CAFs) induced the differentiation of normal breast tissue into CD73$^+$ $\gamma\delta$Tregs through the IL-6/STAT3 pathway, leading to the increased production of Ado. Subsequently, Ado activated the A2bR/p38/MAPK signaling pathway to promote CAFs in secreting IL-6, thereby establishing an IL6-Ado positive feedback loop.

In the TME, macrophages can polarize into M1-type macrophages, exerting an anti-tumor effect, in response to factors such as TNF-α, IFN-γ, Toll-like receptors (TLRs), IL-12, and others[74,75]. Conversely, stimulation by cell factors like VEGF, TGF-β, IL-10, IL-13, and prostaglandin E2 leads to the polarization of

macrophages into M2-type macrophages, which suppresses T cell functions and promotes T cell exhaustion, thereby facilitating tumor proliferation and metastasis[76]. Ado activates A2aR on macrophages, upregulates cAMP levels, and triggers the cAMP/PKA signaling and PI3K/PKC/HIF1 signaling pathways, thereby promoting the secretion of VEGF. Additionally, activation of A2bR by Ado triggers the MAPK/AP-1 signaling pathway, further promoting VEGF secretion, and creating a microenvironment conducive to M2 polarization of macrophages[3,77]. Moreover, Ado binding to A2aR activates the orphan nuclear receptor 4A transcription factor *via* the cAMP/PKA pathway and also triggers the cAMP/EMAC/P38/CREB signaling pathway. Both pathways interfere with the activation of NF-κB, which leads to decreased expression of IL-12 and TNF-α. As a result, macrophages undergo polarization towards the M2 phenotype[78−81]. Furthermore, upregulation of cAMP levels triggers the EPAC/p38/C/EBP signaling pathway, leading to increased secretion of IL-10 and the polarization of macrophages into M2-type macrophages[82].

MDSCs inhibit the tumor-killing ability of CTLs in the TME, and Ado activates A2bR on MDSCs to promote further expansion[83]. Blocking the Ado−A2bR signaling pathway in a melanoma mouse model reduced MDSC accumulation, restored CTL function, and decreased MDSC-mediated tumor growth and immune suppression[84]. Additionally, in a Lewis lung cancer mouse model, Ado promoted the expansion of CD11b$^+$ Gr1$^+$ MDSCs and enhanced their suppressive effects on T cells[85].

4.2. Adenosine promotes T cell exhaustion through intrinsic regulatory mechanisms

Activation of A2aR on T cells also enhances co-inhibitory receptors' expression, including PD-L1, CTLA-4, LAG3, and TIM3[86]. The selective A2aR agonist ATL313 promotes Ado−A2aR signaling, leading to the increased expression of PD-1 and CTLA-4 on T cells, thereby inhibiting T cell function and weakening immune activation[87]. In the MC38-OVA tumor-bearing mouse model, treatment with Ado receptor agonist NECA significantly increased the expression levels of PD-1 on antigen-specific CD8$^+$ tumor-infiltrating lymphocytes (TILs) and CD4$^+$ Foxp3$^+$ TILs. However, when an A2aR antagonist (SCH58261) was administered to tumor-bearing mice, NECA-mediated upregulation of PD-1 on TILs was inhibited[88]. Moreover, stable expression of Notch1 can increase the resistance of CD8$^+$ T cells to tumor-induced immune suppression and is associated with enhanced maintenance of T cell function and increased IFN-γ production. In activated CD8$^+$ T cells, A2aR activation inhibits phosphorylation of ZAP70, an upstream component of the TCR, thereby reducing Notch1 protein expression, suppressing the generation of active Notch1 intracellular domain, and ultimately inhibiting CD8$^+$ T cells' function[33].

In summary, Ado exerts immune inhibitory effects in the tumor immune cycle by binding to Ado receptors on various cells involved in tumor immune response, including tumor cells. Ado activates different signaling pathways through AdoRs activation, contributing to immune suppression (Fig. 2).

5. Clinical treatment options

5.1. Targeting adenosine-generating enzymes (CD39 and CD73) and A2aR

Ado exerts inhibitory effects on tumor adaptive immunity within the TME. However, its half-life is less than 10 s, making direct targeting of Ado unrealistic. Therefore, extensive preclinical and clinical studies have explored the disruption of immune suppression by targeting Ado-generating enzymes (CD39 and CD73) to inhibit Ado production or block A2aR.

In various mouse models of cancer, Ado pathway inhibitors had demonstrated significant anti-tumor effects. In a mouse model of melanoma, the administration of anti-CD39 monoclonal antibody (mAb) stimulated the release of IFN-γ, resulting in the eradication of cancer cells[89]. The dual-specific mAb ES014 targeting CD39/TGF-β can simultaneously inhibited CD39 and autocrine/paracrine TGF-β, thereby suppressing Ado and TGF-β accumulation in the TME to restore anti-tumor immunity[90]. Furthermore, in a mouse model of breast cancer with tumor formation, the mAb 3F7 targeting CD73 exhibited notable inhibition of tumor growth and metastasis[91]. Similarly, in a mouse xenograft model utilizing human epithelial ovarian cancer, the CD73 inhibitor APCP effectively impeded CD73 activity, leading to tumor regression, heightened T cell-mediated anti-tumor responses, and improved survival rates in mice[92,93]. Moreover, an increasing body of preclinical research indicates that antagonizing A2aR can significantly enhance anti-tumor immunity. Among drugs targeting Ado signaling, A2aR inhibitors are currently the most extensively studied and well-supported agents. A2aR inhibitors have been demonstrated to augment the effector function of CTLs and disrupt the recruitment and polarization of immunosuppressive immune cells within the TME, thereby increasing their anti-tumor effects[94,95].

Given the extensive immunosuppressive effects of Ado observed in preclinical studies and the significant anti-tumor effects of inhibiting the Ado pathway, an increasing number of Ado pathway inhibitors have entered clinical trials. However, most of these trials are still in the early stages of development, and no drugs have been approved for the market yet. The most advanced drug in development, CPI-444, is in Phase II clinical trials (Table 1). The CD39 inhibitor ES002023 restored anti-tumor immunity by stabilizing extracellular ATP and inhibiting Ado synthesis within the TME (NCT05075564). The CD73 selective inhibitor AB680 has favorable pharmacokinetic properties and has entered Phase I clinical trials[96]. In a Phase Ib-2 clinical study (NCT03381274), the anti-CD73 mAb MEDI9447 in combination with the EGFR tyrosine kinase inhibitor osimertinib demonstrated moderate activity and acceptable tolerability in previously treated advanced EGFR-mutated non-small cell lung cancer (NSCLC) patients[97]. A2aR inhibitors currently in clinical trials include CPI-444, NIR178, AZD4635, and others. In a Phase I/II clinical study (NCT02403193), 24 advanced NSCLC patients who had undergone treatment were evaluated for NIR178. It exhibited good tolerance, manageable adverse effects, and no Grade 4 drug-related adverse events[98].

5.2. Combination with immune checkpoint inhibitors

ICIs can alleviate T cell exhaustion and restore immune responses against tumor cells. Due to the tremendous success observed in clinical trials, ICIs are currently widely used in the treatment of various types of cancers[99,100]. However, several challenges such as low response rates, lack of known biomarkers, immune-related toxicities, and innate and acquired resistance, among others, still need to be addressed, which restrict their clinical application[101]. Moreover, Ado generated within the TME exerts immunosuppressive effects. As a result, many studies and clinical investigations have attempted to combine inhibitors of the Ado pathway with ICIs to disrupt immune suppression and enhance anti-tumor efficacy
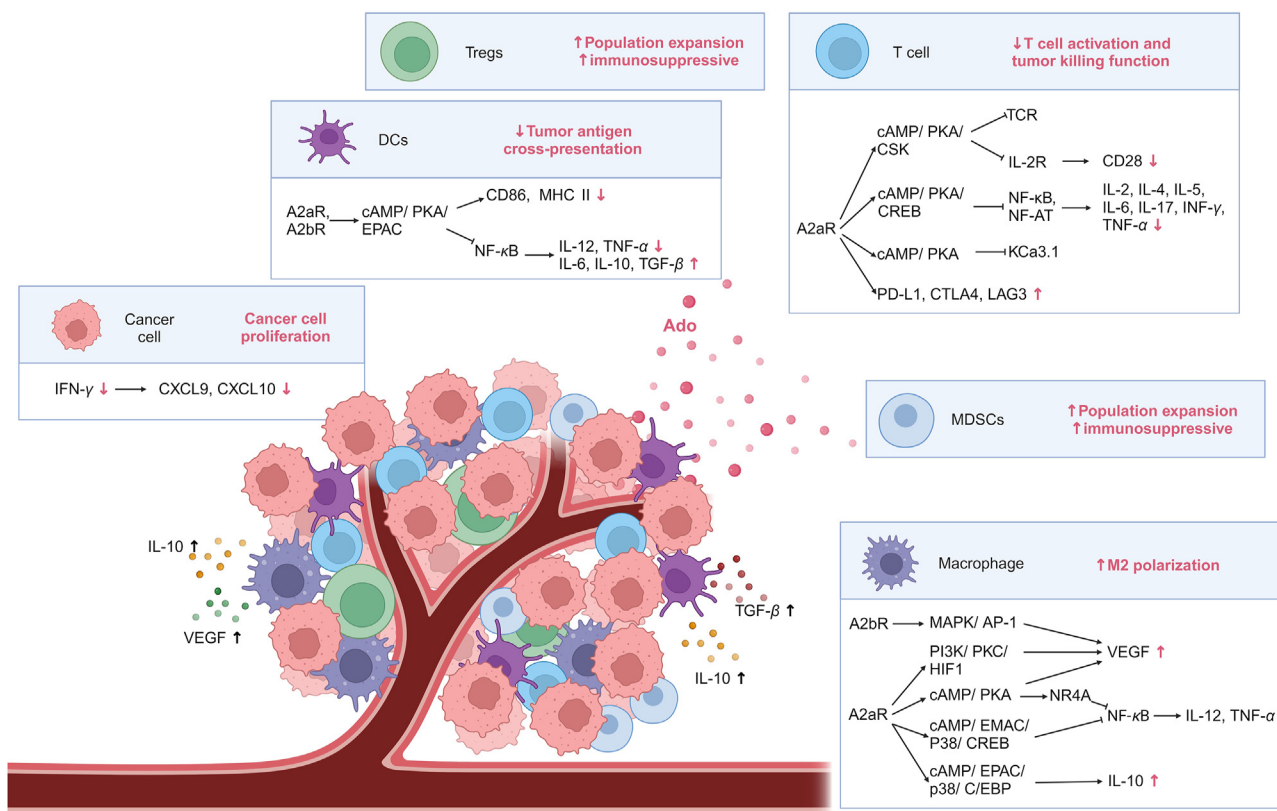
Figure 2 Adenosine suppresses tumor immune cells in the tumor microenvironment. Ado downregulates the expression of MHC II and co-stimulatory molecules on DCs and macrophages, inhibiting antigen presentation to T cells. Ado inhibits the binding of the TCR to its ligand and transmembrane signaling on T cells, while also suppressing the secretion of anti-tumor cytokines, thereby dampening T cell activation. Ado also inhibits the secretion of chemokines and the function of KCa3.1 channels, thereby impeding the trafficking and infiltration of effector T cells to the tumor site. Ado restrains the cytotoxicity of T cells against tumor cells through several mechanisms, including promoting the secretion of immunosuppressive cytokines, increasing the expression of immune checkpoint proteins, and enhancing the activity of immunosuppressive cells. Abbreviations: DCs, dendritic cells; TCR, T cell receptor; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells.

(Table 1). Additionally, the A2aR antagonist CPI-444 reinstated T cell signaling, as well as the production of IL-2 and IFN-γ, which were inhibited by Ado. Furthermore, when combined with anti-PD-1 or anti-CTLA-4 therapies, CPI-444 demonstrated remarkable efficacy in eliminating tumors in 90% of treated mice. This effect was observed even in models that had shown incomplete responses to anti-PD-1 or anti-CTLA-4 therapy, indicating that CPI-444 can restore immune responses and enhance the effectiveness of these immunotherapies[65]. In various mouse models with tumor growth, the addition of anti-CD73 monoclonal antibodies to treatment regimens augmented the immunotherapeutic effects of PD-1 antibodies[88,102−104]. While tumor growth was significantly reduced in the combination therapy group, this effect disappeared in mice with IFN-γ deficiency, highlighting the critical role of an inflamed TME in the success of immune-based therapy[105].

In a Phase I/Ib clinical trial registered under NCT02655822, the antagonistic role of the Ado pathway in cancer immunotherapy was demonstrated. The trial involved 68 patients diagnosed with renal cell carcinoma (RCC) who were treated with either CPI-444 alone or CPI-444 in combination with atezolizumab (an anti-PD-L1 antibody). The study showcased the anti-tumor activity of both monotherapy with anti-PD-L1 and combination therapy in patients with refractory RCC. The objective response rate (ORR) in the monotherapy group was 3% ($n = 33$), with a median progression-free survival of 4.1 months.

Moreover, the combination therapy showed more significant results, with an ORR of 11% ($n = 35$) and a median progression-free survival of 5.8 months. The disease control rate at 6 months was 39%, and the overall survival rate at 25 months was >90%[106]. In early-phase trials involving colorectal and pancreatic cancer patients, the combination therapy of CD73 antibody MEDI9447 and durvalumab (anti-PD-1) demonstrated manageable safety and disease progression consistent with its mechanism of action. Clinical responses were observed[107]. In a randomized Phase II COAST trial (NCT03822351) conducted in stage III NSCLC patients, the results indicated that in the group treated with the combination of the anti-CD73 monoclonal antibody oleclumab and durvalumab, dual blockade of CD73/PD-L1 yielded an ORR of nearly 40%. Compared to using anti-PD-L1 alone, there was a significant improvement in progression-free survival at 10 months (39.2% vs. 64.8%)[108,109]. And in a recent clinical trial (NCT02403193), in patients with NSCLC, the combination of the A2aR inhibitor NIRI178 and the anti-PD-1 monoclonal antibody natalizumab exhibited a significantly higher rate of disease stabilization. Out of 25 patients, 14 achieved disease stabilization when treated with combination therapy, whereas only 7 patients achieved disease stabilization when treated with PD-1 antibody monotherapy. This suggests that the combination of NIRI178 and natalizumab provides a more effective approach to managing NSCLC compared to the use of the PD-1 antibody alone[110].

Table 1 Investigation of inhibitors targeting the Ado pathway in clinical trials (httpsclinicaltrials.gov).

Drug	Target	NCT number	Status	Phase	Cancer type	Immunotherapy
Ciforadenant (CPI-444)	A2aR	NCT05501054	Recruiting	Ib/II	RCC	Ipilimumab (anti-CTLA4), Nivolumab (anti-PD-1)
		NCT02655822	Completed	I/Ib	RCC and mCRPC	Atezolizumab (anti-PD-L1)
Taminadenant (NIR178)	A2aR	NCT03207867	Terminated	II	Multiple solid tumors and diffuse large B-cell lymphoma	PDR001 (anti-PD-1)
		NCT03549000	Terminated	I	Advanced cancers	PDR001 (anti-PD-1)
		NCT04237649	Active, not recruiting	I/Ib	Advanced cancers	KAZ954, PDR001, NZV930 (anti-CD73)
Inupadenant (EOS100850)	A2aR	NCT05117177	Recruiting	I	Solid tumor, adult	Monotherapy
PBF-509	A2aR	NCT02403193	Completed	I	NSCLC	PDR001 (anti-PD-1)
PBF-999	A2aR	NCT03786484	Completed	I	Advanced cancers	Monotherapy
AZD4635	A2aR	NCT04089553	Completed	II	Prostate Cancer, mCRPC	Oleclumab (anti-CD73), Durvalumab (anti-PD-L1)
		NCT03381274	Active, not recruiting	Ib/II	Carcinoma, NSCLC	Oleclumab (anti-CD73)
		NCT02740985	Completed	I	mCRPC	Durvalumab (anti-PD-L1)
CS3005	A2aR	NCT04233060	Completed	I	Advanced solid tumor	Monotherapy
INCB106385	A2aR	NCT04580485	Active, not recruiting	I	CD8 T-cell-positive advanced solid tumors and specified GI malignancies	INCMGA00012 (anti-PD-1)
TT-10	A2aR	NCT04969315	Recruiting	II	Renal cell cancer, castrate resistant prostate cancer, NSCLC	Monotherapy
EXS21546	A2aR	NCT04727138	Completed	I	Oncology	Monotherapy
Etrumadenant (AB928)	A2aR/A2bR	NCT03629756	Completed	I	Advanced malignancies	Zimberelimab (AB122) (anti-PD-1)
		NCT04262856	Active, not recruiting	II	NSCLC	Zimberelimab (AB122) (anti-PD-1), Domvanalimab (anti-TIGIT)
		NCT03846310	Active, not recruiting	I	NSCLC	Zimberelimab (AB122) (anti-PD-1), Pembrolizumab (anti-PD-1)
		NCT04381832	Recruiting	Ib/II	mCRPC	Zimberelimab (AB122) (anti-PD-1)
		NCT04660812	Active, not recruiting	Ib/II	Metastatic colorectal cancer	AB680 (anti-CD73), Zimberelimab (anti-PD-1)
		NCT04262856	Active, not recruiting	II	NSCLC	Zimberelimab (anti-PD1), Domvanalimab (anti-TIGIT)
INCB106385	A2aR/A2bR	NCT04580485	Active, not recruiting	I	Advanced solid tumors	INCMGA00012 (anti-PD-1)
JAB-BX102	CD73	NCT05174585	Recruiting	I	Solid tumor	Pembrolizumab (anti-PD-1)
ATG-037	CD73	NCT05205109	Recruiting	I	Locally advanced or metastatic solid tumors	Pembrolizumab (anti-PD1)
AK119	CD73	NCT05173792	Recruiting	I	Solid tumor	Monotherapy
		NCT05559541	Recruiting	I/II	Solid tumor, adult	AK104 (anti-PD-L1/CTLA4)
		NCT05689853	Recruiting	I/II	Solid tumor, adult	AK112 (anti-PD-1/VEGF)
CPI-006	CD73	NCT03454451	Active, not recruiting	I	Advanced cancers	Pembrolizumab (anti-PD1), Ciforadenant (anti-A2aR)
IPH5301	CD73	NCT05143970	Recruiting	I	Advanced and/or metastatic solid tumors	Retifanlimab (anti-PD-1), INCB106385 (anti-A2aR/A2bR)

(continued on next page)

Table 1 (*continued*)

Drug	Target	NCT number	Status	Phase	Cancer type	Immunotherapy
PT199	CD73	NCT05431270	Recruiting	I	Locally advanced or metastatic solid tumors	Anti-PD-1 monoclonal antibody
		NCT05173792	Recruiting	I	Solid tumor	Monotherapy
		NCT04572152	Active, not recruiting	I	Advanced or metastatic solid tumors	AK104 (anti-PD-L1/CTLA4)
TJ004309	CD73	NCT05001347	Active, not recruiting	II	Advanced or metastatic solid tumors	Atezolizumab (anti-PD-L1)
		NCT04322006	Recruiting	I/II	Advanced solid tumor	Toripalimab (anti-PD-1)
		NCT03835949	Active, not recruiting	I	Solid tumor, metastatic cancer	Atezolizumab (anti-PD-L1)
Sym024	CD73	NCT04672434	Recruiting	I	Metastatic cancer, solid tumor	Sym021 (anti-PD-1)
HLX23	CD73	NCT04797468	Withdrawn	I	Advanced solid tumor	Zimberelimab, (anti-PD-1)
IBI325	CD73	NCT05119998	Active, not recruiting	I	Solid tumor	Sintilimab (anti-PD-1)
AB680	CD73	NCT04104672	Recruiting	I	Advanced pancreatic cancer	Monotherapy
LY3475070	CD73	NCT04148937	Completed	I	Advanced cancer	Pembrolizumab (anti-PD-1)
NZV930	CD73	NCT03549000	Terminated	I	Advanced cancers	PDR001 (anti-PD-1), NIR178 (anti-A2aR)
INCA00186	CD73	NCT04989387	Recruiting	I	Advanced solid tumors	Retifanlimab (anti-PD-1), INCB106385 (anti-A2aR/A2bR)
ORIC-533	CD73	NCT05227144	Recruiting	I	Relapsed or refractory multiple myeloma	Monotherapy
MEDI9447 (oleclumab)	CD73	NCT03736473	Completed	I	Advanced solid malignancies	Monotherapy
		NCT03616886	Active, not recruiting	I/II	Triple negative breast cancer	MEDI4736 (anti-PD-L1)
		NCT04668300	Recruiting	II	Recurrent refractory or metastatic sarcoma	Durvalumab (anti-PD-L1)
		NCT03267589	Completed	II	Ovarian cancer	Durvalumab (anti-PD-L1), Tremelilumab (anti-CTLA4)
		NCT02503774	Active, not recruiting	I	Solid tumors	Durvalumab (anti-PD-L1)
		NCT03822351	Active, not recruiting	II	NSCLC	Durvalumab (anti-PD-L1)
BMS-986179	CD73	NCT02754141	Completed	I/IIa	Malignant solid tumor	Nivolumab (anti-PD-1)
Dalutrafusp (GS-1423)	CD73/TGFβ	NCT03954704	Terminated	I	Advanced solid tumors	Dalutrafusp alfa (anti-CD73/TGFβ)
PUR001	CD39	NCT05234853	Terminated	I	Advanced solid tumors	Monotherapy
JS019	CD39	NCT05508373	Recruiting	I	Advanced solid tumors	Monotherapy
		NCT05374226	Recruiting	I	Advanced solid tumors or lymphomas	Monotherapy
IPH5201	CD39	NCT04261075	Completed	I	Advanced solid tumors	Durvalumab (anti-PD-L1), Oleclumab (anti-CD-73)
TTX-030	CD39	NCT03884556	Active, not recruiting	I	Solid tumor, lymphoma	Pembrolizumab (anti-PD-1)
		NCT04306900	Active, not recruiting	I	Solid tumor, adult	Pembrolizumab (anti-PD-1)
SRF617	CD39	NCT04336098	Active, not recruiting	I	Advanced solid tumor	Pembrolizumab (anti-PD-1)
		NCT05177770	Completed	II	Prostate cancer	AB928 (anti-A2aR/A2bR), Zimberelimab (anti-PD-1)
ES002023	CD39	NCT05075564	Active, not recruiting	I	Advanced solid tumor	Monotherapy
ES014	CD73/TGFβ	NCT05717348	Recruiting	I	Solid tumor, locally advanced solid tumor, metastatic solid tumor	Monotherapy

Moreover, in several other clinical trials, the combination of A2aR inhibitors with ICIs has demonstrated favorable therapeutic effects in various types of cancer patients[111−113]. CD73 antagonists in combination with PD-1/PD-L1 immunotherapy have also demonstrated significant antitumor efficacy in clinical trials. In a Phase Ia/Ib clinical trial (NCT02740985) involving patients with advanced metastatic castration-resistant prostate cancer (mCRPC), the combination therapy of the A2aR inhibitor AZD4635 with durvalumab (anti-PD-L1) achieved an ORR of 16.2% (6/37). This treatment demonstrated a significantly more pronounced therapeutic effect compared to durvalumab alone (2/33)[114]. In a Phase I clinical trial (NCT03835949) of the CD73 inhibitor uliledlimab in combination with Atezolizumab for the treatment of advanced or metastatic cancer patients, uliledlimab demonstrated clinical activity in both PD-1/PD-L1 treatment-naïve and refractory cancer patients, with an ORR of 23.1% in the combination therapy group[22]. In a clinical trial registered as NCT02503774, the administration of the anti-CD73 monoclonal antibody (MEDI9447), either alone or in combination with the PD-L1 antibody durvalumab, resulted in the downregulation of CD73 expression on peripheral T cells and promoted the infiltration of cytotoxic T cells in a cohort of 126 patients diagnosed with colorectal cancer and pancreatic cancer[115]. These data suggest that blocking the Ado pathway can augment the anti-cancer effects of ICIs, and their combination represents an effective treatment strategy in clinical oncology.

5.3. Targeting the adenosine pathway in combination with chemotherapy drugs

In various mouse models of cancer, blocking Ado generation and inhibiting AdoRs can enhance the therapeutic effects of chemotherapy drugs. In a mouse model of breast cancer, overexpression of CD73 in tumor cells suppressed adaptive immunity and increased chemoresistance to anthracycline chemotherapy drug doxorubicin. Blocking CD73 or A2aR significantly enhanced the antitumor effect of doxorubicin and markedly extended the survival of metastatic breast cancer mice[116]. Furthermore, inhibiting CD39 in fibrosarcoma can restore responsiveness to anthracycline drugs through the activation of P2 receptors and immunogenic cell death-mediated mechanisms[117]. Similar synergistic effects have been observed with the use of A2bR antagonists in combination with chemotherapy drugs such as doxorubicin, gemcitabine, and dacarbazine[84,118]. Clinical trials targeting the Ado pathway in combination with chemotherapy have also been conducted in late-stage cancer patients (Table 2), showing that inhibiting Ado can augment the anticancer effects of chemotherapy.

5.4. Multiple inhibitions of adenosine signaling pathway members

To achieve more complete inhibition of Ado production and receptor signaling, many studies have attempted to simultaneously target two members of the Ado pathway to synergistically suppress tumor growth. In various mouse models, the combination therapy targeting both A2aR and CD73 has demonstrated superior efficacy in suppressing tumor growth compared to individual therapies[44,119]. The combined administration of the small-molecule CD73 inhibitor, sodium polyoxotungstate, and the A2aR inhibitor, AZD4635, effectively disrupted the Ado signaling pathway. This dual inhibition led to immune cells' activation, enhanced production of IFN-γ, and a reduction in the abundance of Tregs[120]. In a co-culture system involving both multiple stromal cells and myeloma cells, the synergistic combination of IPH5201(anti-CD39 mAb) and IPH5301 (anti-CD73 mAb) synergistically inhibited Ado generation, thereby reducing T cell suppression[121]. Besides that, several clinical trials are currently testing the combination targeting of A2aR and CD73, including in patients with EGFR-mutated NSCLC (NCT03381274) and prostate cancer (NCT04089553). According to a Phase I trial report, the combined inhibition of A2aR and CD73 using CPI-444 and CPI-006, respectively, demonstrated significantly improved therapeutic efficacy compared to the use of CPI-006 alone[122].

Currently, the most extensively studied cancer types in clinical trials of Ado pathway inhibitors include NSCLC, melanoma, RCC, and mCRPC. Because Ado blockade has shown moderate activity as monotherapy in these tumors. While combining it with ICIs yields more significant therapeutic effects, each cancer type has its unique biological processes determining the responsiveness to Ado blockade. Due to the widespread distribution of Ado throughout the body, including the heart and brain, and its crucial physiological functions, the complexity of the Ado pathway involving multiple components and pathways poses potential limitations and challenges to inhibiting Ado. Ado plays a vital role in maintaining cardiovascular balance, regulating cardiac activity, dilating blood vessels, and reducing cardiac burden within the cardiovascular system. Simultaneously, in the nervous system, Ado functions as both a central excitatory and inhibitory neurotransmitter, highlighting the significance of a balanced AdoRs system in the treatment of brain diseases[9,123]. Therefore, the use of

Table 2 Combination clinical trial targeting the adenosine pathway with chemotherapy (https://clinicaltrials.gov).

Drug	Target	NCT number	Status	Phase	Cancer type	Immunotherapy
SRF617	CD39	NCT04336098	Completed	I	Advanced solid tumor	Albumin-bound paclitaxel (chemotherapy)
IPH5301	CD73	NCT05143970	Recruiting	I	Advanced and/or metastatic solid tumors	Chemotherapy and trastuzumab (anti-HER2)
Oleclumab	CD73	NCT04940286	Recruiting	II	pancreatic cancer	Nab-paclitaxel (chemotherapy)
Dalutrafusp (GS-1423)	CD73/TGFβ	NCT03954704	Terminated	I	Advanced solid tumors	mFOLFOX-6 regimen (chemotherapy)
Inupadenant	A2aR	NCT05403385	Recruiting	II	NSCLC	Carboplatin and pemetrexed (chemotherapy)
Etrumadenant (AB928)	A2aR/A2bR	NCT03720678	Completed	I/Ib	mCRC	SOC (mFOLFOX-6) (chemotherapy)

Ado inhibitors may carry potential side effects, including cardiac and neural toxicity[124]. Additionally, a decrease in Ado concentration in the patient's plasma has been associated with neurogenic syncope[125]. Furthermore, CD73 is expressed in tumor cells, immune cells, and normal cells in various tissues. Therefore, systemic administration of CD73 antagonists may cause adverse reactions, and human *CD73* gene mutations have been associated with arterial and articular calcification, increasing the risk of cardiovascular diseases[126]. Besides that, AdoRs are GPCRs, and constructing mAbs against active GPCRs is extremely challenging. Small molecule inhibitors are currently the only available option in clinical practice, but their main drawback is their short half-life, requiring frequent administration to ensure therapeutic efficacy[127]. Additionally, because small molecules can easily cross physiological barriers, it is crucial to determine the optimal pharmacokinetics and pharmacodynamics, as well as the risk of cardiovascular and brain-related side effects. Therefore, a deeper understanding of the Ado pathway in different tumor TMEs is still needed, and there are ongoing questions to explore during the development of inhibitors targeting Ado pathway members.

6. Conclusions

During tumor development, the concentration of Ado in the TME significantly increases compared to normal physiological levels. Ado exerts inhibitory effects on immune cells involved in tumor adaptive immunity, suppressing tumor antigen presentation, T cell activation and infiltration, as well as cytotoxic T lymphocyte-mediated tumor cell killing, thus promoting immune evasion by tumors. Moreover, Ado activation of A2aR in B cells inhibits NF-κB, thereby disrupting signaling from B cell receptors and TLR4, leading to the suppression of B cell activation[128,129]. Additionally, Ado, through A2aR signaling, reduces the production of Fas ligand, perforin, and IFN-γ in NK cells, promoting the secretion of immunosuppressive cytokines TGF-β and IL-10, thereby impairing the function of NK cells[130,131]. In addition to immune cells, Ado also affects tumor stromal cells in the TME. In solid tumors such as breast cancer, pancreatic cancer, ovarian cancer, and colorectal cancer, CAFs overexpress CD39 and CD73, leading to increased production of Ado[132−134]. Furthermore, in the CRC mouse model, Ado activation of A2bR on CAFs enhanced the expression of CD73, further amplifying the immunosuppressive effects of Ado[133]. Additionally, in NSCLC patients, the activation of A2a receptors promoted the proliferation of CAFs[135]. Moreover, in melanoma, Ado activation of A2b receptors on CAFs enhanced the production of CXCL12 and fibroblast growth factor 2, mediating pro-tumor responses[136]. Besides CAFs, endothelial cells also express CD39 and CD73, and experimental evidence suggests that Ado-mediated signaling promotes tumor cells to secrete VEGF, thereby promoting endothelial cell angiogenesis to facilitate tumor angiogenesis, contributing to tumor hypoxia and further suppressing anti-tumor immune responses[137,138]. Additionally, Ado inhibits T-cell infiltration into tumors by suppressing the expression of intercellular adhesion molecule 1 on endothelial cells[139].

The immunosuppressive TME poses a significant obstacle to the efficacy of cancer immunotherapy and must be surmounted in order to attain robust and long-lasting anti-tumor responses. Novel selective inhibitors that specifically target Ado-generating enzymes, including CD39 and CD73, as well as A2aR, have been successfully developed. These inhibitors have demonstrated remarkable inhibition of tumor growth in preclinical tumor models and have shown synergistic effects when combined with ICIs. Despite extensive evidence from *in vitro* experiments and animal models demonstrating the tremendous potential of targeting CD39, CD73, and A2aR in cancer therapy, there are still some issues to address in clinical applications, which also represent potential research directions for Ado inhibitors. Based on the published results of clinical trials, both single-agent and combination therapy with Ado inhibitors have shown some therapeutic efficacy, but the response rates are relatively low. It is important to delve deeper into characterizing genomic and phenotypic markers associated with treatment success to use Ado inhibitors in tumors that overexpress relevant genes and phenotypes. Furthermore, the overexpression of CD39, CD73, and A2aR in tumors is negatively correlated with immune cell infiltration into tumors and is associated with lower disease-free survival, poorer overall survival, and worse prognosis in cancer patients, CD73 and CD39 also serve as biomarkers for predicting and prognosing various cancers[16,116,121,140,141]. Therefore, incorporating biomarkers into the assessment criteria of clinical trials, summarizing their relevance to treatment success, and predicting the clinical response to Ado inhibitors can be valuable. For A2aR inhibitors, there is still a need to design inhibitors with more pronounced inhibitory effects, longer half-lives, and fewer side effects. Finally, due to the complexity of the Ado pathway and its effects on multiple cell types, further research is needed to determine which cells in the TME are critical for the effectiveness of specific monotherapies. This will facilitate the development of combination therapy strategies with Ado inhibitors.

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Author contributions

Longsheng Wang and Jie Zhang designed and wrote the paper. Wenxin Zhang, Mingming Zheng, Hongjie Guo, Xiaohui Pan, and Wen Li revised the manuscript. Ling Ding and Bo Yang were responsible for the conception and design of the review.

Conflicts of interest

The authors declare no conflicts of interest.

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