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Immunomodulatory effect of ADO on liver tumor microenvironment

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ABSTRACT

Liver cancer is affected by adenosine expression and metabolic byproducts. Cancer patients treated with different adenosine antagonists had their pathological alterations rectified by influencing proliferation, apoptosis, and angiogenesis. Our review focuses on new carcinogenic mechanisms driving adenosine activity, especially changes in immunological mediators and immune archetypes. In this study, we look at how adenosine affects the reprogramming of tumor immune cells like dendritic cells, T cells, Tregs, and neutrophils. This study covers current knowledge of the molecular mechanisms underlying adenosine's unique carcinogenic mechanisms via modification of the expression pattern of immune mediators in the tumor immune microenvironment, such as PD-1, CTLA-4, TGF-β, and IL-12, IL-23, and FOXP3. Adenosine reprograms tumor immunological architecture and mediators, making it a viable therapeutic target for tumor eradication through its multiple mechanisms of carcinogenicity.

ABBREVIATIONS: CTLA-4: cytotoxic T lymphocyte antigen-4; **FOXP3:** forkhead box protein P3; **IDO:** indoleamine 2,3 dioxygenase; **IL-12:** Interleukin 12; **PD-1:** programmed cell death 1; **TGF-β:** transforming growth factor-beta.

Keywords: *Adenosine; Immune microenvironment; TGF-β; Treg.*

1. Introduction

Adenosine (ADO) is primarily produced in the extracellular environment by the breakdown of adenosine 5′-triphosphate (ATP), which is released under stressful conditions to provide localized protection for cells and tissues. When acting on distinct kinds of receptors, ADO and ATP frequently have contradictory effects on either cell proliferation or cell death **(Robert D. Leone & Leisha A. Emens, 2018)**. ADO stimulates the growth of many different types of cancers by activating the A2B adenosine receptor (A2BAR); whereas ATP and other adenine nucleotides have anticancer effects by activating the P2Y1 receptor (P2Y1R) subtype (AR). Enzymes, Cluster of Differentiation 39 (CD39), CD73, CD26, ADO deaminase, ADO kinase, and S-adenosyl homocysteine hydrolase) and nucleoside transporters, which are not the focus of this review, are involved in the multi-step and balanced process in which ADO is produced and removed from cells **(Franco & Rivas-Santisteban, 2021)**.

ADO effect on cancer proliferation

The initial stages of carcinogenesis involve modifications to tissue architecture and the development of preneoplastic nodules **(Nahla E. El-Ashmawy, Khedr, El-Bahrawy, & Abd El-Fattah, 2016, 2017)**. These changes lead to localized hypoxia that promotes tissue stem cell survival, proliferation, and angiogenesis. They are associated with cell phenotypic changes such as epithelial-mesenchymal transition and cell migration **(Feitelson et al., 2015)**.

Most known targeted therapies now aim to prevent signal transduction pathways from becoming permanently engaged breakpoint cluster region and Abelson murine leukaemia viral oncogene homologue (Bcr-Abl), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), mesenchymal-epithelial transition factor (c-Met), and rapidly accelerated fibrosarcoma (Raf). Although these drugs originally worked to halt tumor growth, the development of resistant clones is a common clinical occurrence, underscoring the need for additional therapeutic choices **(E. E. Abd El-Fattah et al., 2021; Abdelhamid et al., 2022; Granada et al., 2020)**.

Polydeoxyribonucleotide (PDRN), A2AR antagonist, therapy in diabetic mice increased cyclin D1/CDK6 and cyclin E/CDK2 expression and lowered p15 and p27 inhibitors at day 6 following damage in an incisional skin wound model **(Altavilla et al., 2011)**. The WNT/β-catenin signaling pathway is inhibited by pharmacological blockage of A2AR, which contributes to its ability to prevent cutaneous fibrosis in conditions like scleroderma **(J. Zhang et al., 2017)**. Rapid cellular β-catenin level increases caused by the A2AR-selective agonist CGS21680 boosted Col3 production in cutaneous fibrosis and scarring **(Shaikh, Zhang, Perez-Aso, Mediero, & Cronstein, 2016)**. While adenosine A2 receptor antagonist, DMPX treatment blocked PDRN effects on Wnt/-catenin signaling, PDRN stimulation of the Wnt/ β-catenin pathway lowered apoptosis 24 hours after spinal cord injury **(Irrera et al., 2018)**.

ADO effect on cancer apoptosis

A hallmark and goal of clinical oncology have been the development of medications that accelerate the effective elimination of cancer cells via apoptosis. Many factors, including cellular stress, DNA damage, and immune surveillance, cause programmed cell death and involve numerous signaling pathways **(Carneiro & El-Deiry, 2020)**.

At reperfusion, ADO and CGS21680, a specific adenosine A2A subtype receptor agonist, greatly reduced the level of upregulated Bax and dramatically enhanced the expression of downregulated Bcl-2 (percent of normal myocardium) **(Zhao et al., 2001)**. Rats with intestinal ischemia and reperfusion (I/R) saw an increase in Bcl2L1 gene expression that was aided by ATP **(Fagundes, Carrara, Teixeira, Simões, & Taha, 2018)**. Western blot examination revealed significant ERK1/2 activation, elevated Bcl-2 expression, and reduced Bax expression following I/R in lungs pretreated with the selective A2AR agonists ATL 313 **(Rivo et al., 2007)**. After spinal cord injury, ATL 313 and CGS21680 reduced tissue damage, TUNEL staining, tumor necrosis factor α (TNF-α) expression, Bax, Fas-L, and Caspase-3 expression, Annexin-V staining, and increased Bcl-2 expression in an animal model of spinal cord trauma **(Eslam E. Abd El-Fattah et al., 2022; Genovese et al., 2010)**.

ADO effect on angiogenesis

Tumors are fed and oxygenated via a sophisticated tumor microvasculature network, allowing adequate metabolite drainage. Tumor angiogenesis results from a series of processes that culminate in cancer progression. The angiogenic process begins when a tumor reaches a specific size, and the cells become hypoxic which initiates tumor angiogenesis **(Prager & Poettler, 2012)**.

As a result, hypoxic cancer cells produce angiogenic chemicals like growth factors, cytokines, bioactive lipids, and matrix-degrading enzymes, which bind to receptors on vascular endothelial cells in surrounding blood vessels and stimulate the formation of new capillaries. The vascular endothelial growth factor (VEGF) is the most crucial proangiogenic agent that promotes tumor angiogenesis and, as a result, metastasis **(Lugano, Ramachandran, & Dimberg, 2020)**. Endothelial tip cell motility is stimulated by VEGF-binding A's to VEGFR2. Once activated, VEGFRs affect cellular survival and proliferation, cytoskeleton remodeling, and vascular permeability through a variety of downstream pathways **(Dimova, Popivanov, & Djonov, 2014)**.

In human macrophages, CGS21680 increased VEGF, but the antagonist SCH58261 attenuated it **(Ernens et al., 2010)**. A2BAR induces VEGF overproduction in ex vivo glomeruli exposed to high glucose concentrations and necessitates PKC and Erk1/2 activation **(Cárdenas et al., 2013)**. Antagonizing AR with coffee reversed the VEGF up-regulation caused by local 5'-N-ethylcarboxyamidoadenosine (NECA; an A1 and A2 receptor agonist) injection into the mouse hind limb, and this resulted in a 46 percent reduction of neovascularization in a mouse ischemia hind limb model **(Ryzhov, McCaleb, Goldstein, Biaggioni, & Feoktistov, 2007)**. **Gu et al. (1999)** found that A2R antagonist 8-(3-chlorostyryl)-caffeine completely blocked ADO-induced VEGF protein and mRNA expression and significantly reduced baseline VEGF protein levels by up to around 60%.

According to the research above, proliferation, oxidative stress, apoptosis, and metastasis were all involved in ADO-induced carcinogenesis. We investigated the immunomodulatory effect of ADO on the liver tumor

microenvironment (Immune mediators and immune cells) in addition to the pro-tumorigenic effect of ADO on liver tissue, which adds to the multiple carcinogenic mechanisms of ADO and thus immune fitness modulation.

A- Immunomodulatory mediators

Transforming growth factor-beta (TGF-β)

Transforming growth factor-beta is an immunosuppressive cytokine that promotes cell proliferation, suppresses the immune system, and contributes to cancer development. In HCC, TGF-β is linked to immune cell exhaustion **(Eslam E. Abd El-Fattah & Zakaria, 2022; Saber et al., 2021)**. TGF-β regulates immune cells in the liver, balancing immunological tolerance and activation **(Youssef et al., 2021)**. TGF-β is a growth factor that controls immune cells **(Fig. 1)**. TGF-β inhibits Th1 and Th2 cells by downregulating SRY-box (Sox4) expression and possibly boosting Th1 cell differentiation into Th2 cells **(Chen, Gingold, & Su, 2019)**.

Roa et al. (2009) mentioned that when ADO's bioavailability is increased, activation of the low-affinity A2BAR results in the release of TGF-β1 from diabetic rats' glomeruli, a pathogenic event that may promote the development of glomerulopathy. Utilizing pharmacological methods and animals genetically modified, **Vasiukov et al. (2021)** determined that the effects of NECA, an ADO analog, on the TGF-β pathway happen in a fashion that depends on the A2A/A2B AR, AC, and PKA. **Qu et al. (2009)** results suggest that In mesangial cells, a high glucose content quickly raises the extracellular levels of ATP. TGF-β1 is upregulated as a result of ATP activating ERK1/2, an effect that is at least partially dependent on ROS. ZM241385 and MRS1754, respectively, which are selective antagonists of A2AR and A2BR, effectively inhibited the production of TGF-β1 in cervical cancer cells **(García-Rocha et al., 2019)**.

Programmed cell death 1 (PD-1)

The transmembrane receptor PD-1 is expressed by activated T cells, natural killer cells, B cells, and antigen-presenting cells. It has been discovered that PD-1 has a negative regulatory effect on autoimmune diseases. PD-L1 is a PD-1 ligand on the surface of hematopoietic and nonhematopoietic cells. Chronic inflammation increases the expression of PD-L1, and its binding to the PD-1 receptor causes T-cell exhaustion, which prevents autoimmunity from developing. The role of the PD-L1/PD1 axis in tumor microenvironment formation and immune escape is now well understood. PD-L1 has been shown to cause activated tumor-reactive T-cell death, which results in local T-cell immune suppression, promoting tumor growth and spread **(Macek Jilkova, Aspord, & Decaens, 2019) (Fig. 1)**.

Adenosine was found to increase PD-L1 level. According to **H. Xu, Liang, Liu, and Chen (2021)**, patients who tested positive for PD-L1 had a higher likelihood of having aggressive clinicopathologic features. Significantly, the immunohistochemical expression of PD-L1 was linked with the response rates to PD-L1/PD1 targeting medications in most clinical studies, indicating that PD-L1 expression could be used as a predictor of immunotherapy sensitivity. Because of their role in CD8+ T cell depletion, therapeutic monoclonal antibodies targeting PD1 or PDL1 have been shown to treat a variety of advanced cancers effectively. **(S. Zhang, Li, & Cheng, 2020)**.

High AFP levels, satellite nodules, macrovascular invasion, poor differentiation, and CK19 expression were all substantially linked with PD-L1 expression. Overexpression of PD-Ls in HCC patients is linked to tumor recurrence and a poor prognosis **(Calderaro et al., 2016; Jung et al., 2017)**. TGF-β1 activation stimulates HCC cell proliferation by increasing PD-L1 gene expression. By demethylating PD-1/PD-L1 promoters, TNF-α activates TGF-β1 and IKK/NF-κB signaling pathways **(Arrese et al., 2018; Asgarova et al., 2018; Sow, Ren, Camps, Ossendorp, & Ten Dijke, 2019).** PD-L1 gene upregulation is caused by overexpression of NF-κB p50 **(Asgarova et al., 2018)**.

Patients who received anti-PD-1 monotherapy, anti-PD-1 plus anti-CTLA4 antibody therapy, or anti-VEGF medications had a stronger response and longer overall survival if their initial tumors expressed more PD-L1 and less A2AR **(Kamai et al., 2021)**. **L. Li et al. (2012)** found that Dendritic cells (DCs) that have been stimulated by A2AR signaling express more B7-DC (also known as PDL2), a ligand for the inhibitory receptor PD1. A2AR increases the surface expression of PD1 and CTLA-4 and suppresses T cell proliferation and cytokine production **(Vijayan, Young, Teng, & Smyth, 2017)**. **Turiello, Capone, Morretta, Monti, and Madonna (2022)** found that patients with melanoma who did not react to therapy may respond differently to anti-PD-1 drugs if they had early-on high expression levels of exosomal CD73. **Beavis et al. (2015)** found that A2AR antagonists can greatly increase the efficacy of anti-PD-1 mAb, and CD73 expression may serve as a possible biomarker for anti-PD-1 mAb effectiveness in cancer patients. Because ADO signaling may favorably control TGF-β levels, DO also

causes a rise in PD-1 levels. TGF-β is primarily responsible for inhibiting effector T cell activation and promoting the activity of PD-1-expressing antigen-presenting cells **(Scheffel et al., 2021)**.

Indolemine 1,2 dioxygenase

Tryptophan (Trp), an important amino acid, is broken down by the 403-amino-acid enzyme in the cytosol known as indoleamine 2,3-dioxygenase (IDO) via the kynurenine (Kyn) route (KP). Trp must be destroyed for proper Kyn concentrations and other crucial cellular functions, and IDO causes this to happen. Trp catabolites can kill CD4+ T cells. Kyn, 3-HK, and 3-HAA all suppress T cell proliferation while also inducing apoptosis **(Eslam E. Abd El-Fattah, 2022) (Fig. 1)**.

Different allostimulatory behaviors and increased quantities of pro-inflammatory, angiogenic, and immune suppressor/tolerogenic effector molecules (such as VEGF, IL-8, IL-6, IL-10, COX-2, TGF-β, and IDO) are produced by ADO-differentiated DCs **(Kumar, 2013)**. ADO also causes DCs to exhibit suppressive traits by secreting IL-10, TGF-β, arginase, and IDO **(Sergey V Novitskiy et al., 2008)**.

Interleukin 12 (IL-12)

Interleukin 12 is a cytokine that activates NK and CD8+ T cells to help fight cancer. IL-12, IL-23, and IL-27, all members of the IL-12 family of cytokines, are essential mediators of innate and adaptive immunity. IL-12 enhances T helper 1 (Th1) development and cell-mediated immune responses by promoting IFN-γ production by NK cells and naive T cells. IL-12 therapy prompted NK cells to secrete IFN-γ, which reduced tumor angiogenesis in the HCC-Hu-PBL-NOD/SCID mouse model **(Lo et al., 2010; Yue et al., 2016) (Fig. 1).**

Through both adenosine A2AR-dependent and independent pathways, ADO reduces the synthesis of IL-12 and TNF-α **(Haskó et al., 2000)**. ADO signaling increases the expression of vascular endothelial growth factor (VEGF) and IL-10 while suppressing the TLR-dependent expression of TNF-α, IL-12, IFN-γ, and several other inflammatory cytokines by macrophages **(Ferrante et al., 2013)**. **Takahashi et al. (2007)** found that ADO prevented IL-18 from increasing the expression of ICAM-1 on human monocytes and stopped the production of IL-12, IFN-γ, and TNF-α. **Schnurr et al. (2004)** found that mature ADO secreted by plasmacytoid dendritic cells inhibits the synthesis of interleukin-6 (IL-6), IL-12, and IFN-α in response to CpG oligodeoxynucleotides (ODN). **Wilson et al. (2009)** found that TNF-α and IL-12 were suppressed by NECA (5'-N-ethylcarboxamidoadenosine) in a concentration-dependent manner, although IL-10 production was elevated. **Koscsó et al. (2012)** found that ADO increases IL-10 synthesis while decreases IL-6, TNF-α, and IL-12 release by PGN- or LPS-activated BV-2 microglia.

Forkhead box protein P3 (FOXP3)

Forkhead box protein transcription factor P3 is a crucial biochemical marker for Treg cells, suppressing the immune system **(Eslam E. Abd El-Fattah & Abdelhamid, 2021; N. E. El-Ashmawy, Salem, Abd El-Fattah, & Khedr, 2021)**. Treg cells prevent other leukocytes from activating and fulfilling their duties, inhibiting the immune system. FOXP3 overexpression promotes cell metastasis and invasion in HCC cell lines by modulating MMP-1 **(H. Zhang et al., 2020)**.

The average tumor volume, Treg/CD4+ T cell ratio, and levels of IL10, TGF-β, and VEGF decreased after FOXP3shRNA treatment compared to untreated HCC mice; however, levels of IFN-γ and IL2 rose. By lowering the immunosuppressive role of Tregs, FOXP3 knockdown may decrease the progression of HCC in mice **(Shi et al., 2018) (Fig. 1)**.

Overall survival was predicted by the ratio of FOXP3+ Tregs to CD4+ T cells, with higher Treg levels in tumor tissues indicating a poor prognosis. As a result, FOXP3+ Tregs play a role in HCC's immunosuppressive microenvironment. HCC is linked to a high number of tumor-infiltrating Tregs **(Tu et al., 2016)**. CD4+CD25+FOXP3+ levels in the peripheral blood are elevated. Treg levels are an independent predictor of poor prognosis in stage B HCC patients **(F. Li et al., 2014)**.

Bao et al. (2016) found that in a sepsis model, ADO induces FOXP3 expression in Treg cells via activating the JNK/AP-1 pathway. FOXP3-expressing CD4+CD25+ cells that also express CD39, CD73, and CTLA-4 were increased by A2AR activation **(A. Ohta et al., 2012)**. **Bynoe and Viret (2008)** found that ectoenzymes, catalytic membrane proteins with their active sites outside the cell, CD39 and CD73 are significant contributions to the regulatory function of FOXP3(+)CD4(+) T cells by producing the immunosuppressive factor ADO. In allergic asthma, FOXP3 mRNA, TGF-β, and FEV1 percent pred were all positively linked with A2AR mRNA **(L. Wang**

et al., 2018). The CD4+ FOXP3+ population was greatly enhanced by the A2AR agonist **(Akio Ohta et al., 2012)**.

Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4)

cytotoxic T-lymphocyte-associated antigen 4 is an inhibitory protein expressed on activated T cells that helps maintain the balance between pro-and anti-immune responses by regulating T cell communication **(Salem, El-Ashmawy, Abd El-Fattah, & Khedr, 2021)**. CTLA-4 is a critical susceptibility locus for autoimmune diseases and cancer, according to numerous genetic association studies. On the other hand, the mechanism through which the CTLA-4 polymorphism prevents cancer is unknown. The link between CTLA-4 single nucleotide polymorphisms (SNPs) and cancer susceptibility has been studied in recent research, including gastric cancer, cervical cancer, colorectal cancer, and lung cancer **(J. J. Wang, Wang, & Tan, 2019) (Fig. 1)**.

The inhibitory signals provided by CTLA-4 binding to CD80 and CD86 cancel out the stimulatory signals produced by CD28: TCR: MHC binding and CD80 and CD86 binding **(Sobhani et al., 2021)**. Inhibitory signaling techniques include direct inhibition at the TCR immunological synapse, suppression of CD28 or its signaling pathway, and increased T cell mobility resulting in a diminished ability to engage with APCs **(Perez-Ruiz et al., 2019)**. CTLA-4 reduces cytotoxic T cell activation and the CD8+ cell population by inhibiting T cell receptor attachment to its stimuli CD80 and CD86. CTLA-4 binds more strongly to CD80 and CD86 than CD28 **(Perez-Ruiz et al., 2019)**. **Sobhani et al. (2021)** concluded that antibodies or tiny chemicals that block CTLA-4 but do not influence CTLA-4 levels in Treg cells could be innovative and ultimately more effective in combating cancer cells while maintaining the ability of Treg cells to avoid autoimmunity.

By enhancing the tumor immune response in a mouse melanoma model, administration of APCP, a specific inhibitor of the ADO-generating nucleotidase CD73, increased the efficacy of anti-CTLA4 mAb **(Iannone, Miele, Maiolino, Pinto, & Morello, 2014)**. CD152 is increased by cAMP in human CD4(+) T cells **(Vendetti et al., 2002)**. **Kamai et al. (2021)** concluded that adenosine 2A receptor expression is elevated in metastatic renal cell carcinoma, which is linked to a shorter survival time and a worse response to anti-vascular endothelial growth factor drugs and anti-PD-1/Anti-CTLA4 antibodies. **B. Allard, S. Pommey, M. J. Smyth, and J. Stagg (2013)** concluded that targeted inhibition of CD73 may amplify therapeutic approaches that target immune checkpoint inhibitors more broadly by increasing the therapeutic activity of anti-PD-1 and anti-CTLA4 mAbs. A2AR agonists not only induce FOXP3 expression, which skews T cells toward the Treg lineage, but they also improve the ability of Treg cells to suppress responder T cells, at least in part through increasing CTLA-4 levels **(Vigano et al., 2019)**. A2AR signaling induces the enhanced expression of other immune checkpoint pathways, such as PD-1, CTLA-4, and LAG-3, on both effector and regulatory T cells **(R. D. Leone & L. A. Emens, 2018)**. In mice, **Bertrand Allard, Sandra Pommey, Mark J Smyth, and John Stagg (2013)** found that treatment with CD73 mAb markedly improved the efficacy of anti-CTLA4 and anti-PD1 treatments in tumor-bearing animals. Preclinical studies targeting CD73-derived ADO with small molecules or monoclonal antibodies, particularly in combination with immune checkpoint inhibitors like PD-1 and CTLA-4, have produced favorable outcomes for the management of melanoma, and several clinical trials have recently been initiated to assess the therapeutic potential of targeting CD73-derived ADO in solid tumors **(Soleimani et al., 2020)**.

IL-23

IL-23 was discovered to be overexpressed in many human malignancies, consistent with its involvement in encouraging tumor growth in mice. Similarly, two functional IL-23R genetic variations (IL-23R rs1884444 T>G and IL-23R rs6682925 T>C) have contributed to susceptibility to solid and blood cancers.

Activated DC and macrophages create IL-23, a member of the IL-12 cytokine family. IL-23 works by attaching to its receptor, which comprises two subunits: IL-12R1 and IL-23R. This causes janus kinase (JAK) activation and phosphorylation of signal transducer and activator of transcription (STAT) subtypes 3 and 4 **(Yan, Smyth, & Teng, 2018) (Fig. 1)**.

Infiltration of M2 macrophages, neutrophils, and their increased secretions of immunosuppressive cytokines TGF-β, IL-10, and VEGF into tumor tissues is promoted by IL-23, as is the rise of the matrix metalloprotease MMP9. IL-23 also raises the expression of the endothelial and proliferative markers CD31 and Ki67 in malignancies. Furthermore, IL23 suppresses the immune system by lowering the invasion of CD4+ and CD8+ T lymphocytes into tumor tissues **(Nie, Yu, Sang, & Gao, 2017)**.

Mice lacking IL-23p19 were resistant to the development of skin papillomas brought on by DMBA/TPA, and this resistance was correlated with an increase in CD8+ T cells penetrating the skin as well as a decrease in IL-17A,

MMP9, CD31, granulocytes (Gr-1+), and macrophages (CD11b+, F4/80+) **(Yan et al., 2018)**. IL-17A did not enhance MCA-induced fibrosarcomas, proving that IL-23p19 has tumor-promoting properties apart from IL-17A. A2AR activation can boost the release of IL-23 by ankylosing spondylitis patients' monocyte-derived macrophages **(Akhtari et al., 2020)**.

When compared to 5′-N-ethylcarboxamidoadenosine (NECA) stimulation alone, the stable ADO analog NECA considerably reduces the amount of TNF-α-induced by LPS and potently increases the amount of IL-23p19 mRNA by LPS **(Crean et al., 2015)**. **Wilson et al. (2011)** found that in the presence of ADO or the stable ADO mimic 5'-(N-ethylcarboximado) adenosine, mouse naive CD4(+) T cells cocultured with DCs led to the differentiation of IL-17 and IL-22 secreting cells and an increase in mRNA that encodes signature Th17-associated molecules, such as IL-23R and RORγt. **Liu et al. (2018)** found that in addition to reducing the synthesis of chemokines including CCL2, CXCL8, and IP-10, activation of ARA1 signaling also reduced the production of IL-12 and IL-23. The net effect on immune mediators were gathered in **Table 1**

Table 1: Effect of ADO on tumor immune mediators.

	Type Of immune mediator	Net effect on activity
ADO	CTLA-4	↓
	PD-L1	↓
	IL-12	↑
	FOXP3	↓
	TGF-β	↓
	IL-23	↓

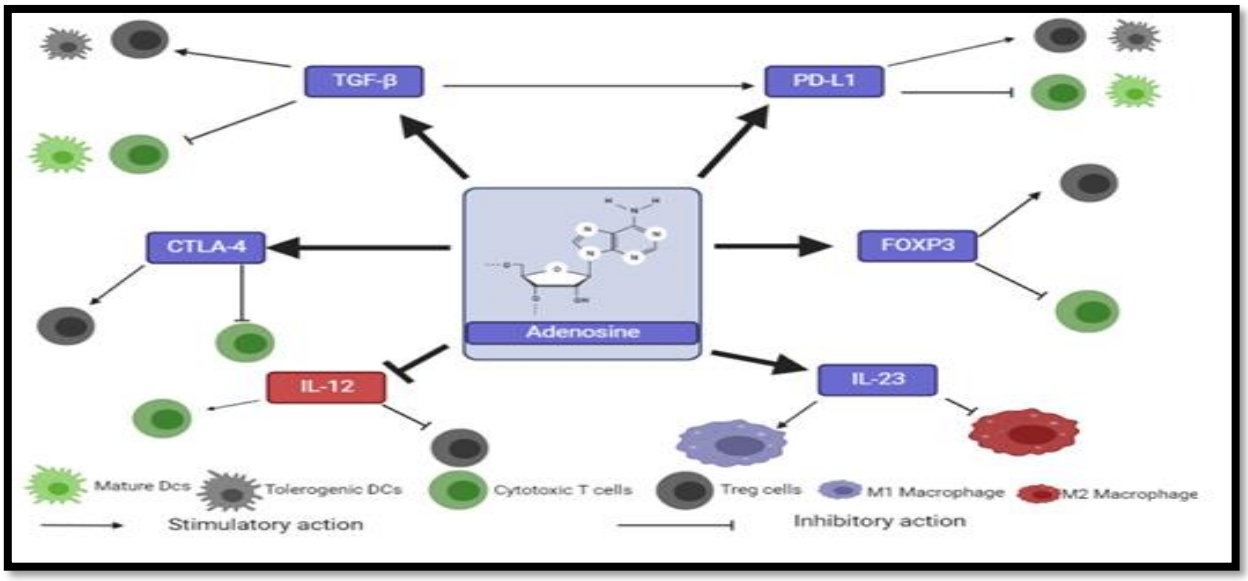

Figure 1: Effect of ADO on tumor immune mediators. ADO alters the tumor microenvironment in favor of immunosuppressive activity via a variety of mechanisms, including (1) Stimulation of both PDL1 and TGF-β1, which stimulates both Treg cells and tolerogenic DCs while inhibiting cytotoxic T cells and mature DCs, (2) Stimulation of FOXP3, and CTLA-4, which stimulates Treg cells and inhibits cytotoxic T cells, (3) Stimulation of IL-23 which stimulates M2 macrophage and inhibits M1 macrophage, and (4) Inhibition of IL-12 which stimulates Treg cells and M2 macrophages and inhibits cytotoxic T cells and M1 macrophages. CTLA-4: Cytotoxic T lymphocyte antigen-4; Il-12: interleukin 12; PD-L1: Programmed death-ligand 1; TGF-β: Transforming growth factor-β; DCS: Dendritic cells; Foxp3: Forkhead box protein P3; Treg: Regulatory T cells.

B- Immunosuppressive cells

Neutrophils

Inflamed tissues emit chemotactic molecules such as IL-8, which aid neutrophil recruitment. Tumor-associated neutrophils (TANs) infiltrate the tumor with neutrophils, promoting cancer growth. The neutrophil/lymphocyte ratio has been demonstrated to have a deleterious impact on prognosis in various cancers, including HCC. Infiltrating TANs have antitumorigenic (N1) or protumorigenic (N2) characteristics. Neutrophils and platelets are attracted to wounded areas quickly. Platelets, neutrophils, and endothelial cells are all involved in this trafficking, which is mediated by coordinated ligand-receptor interactions **(Giese, Hind, & Huttenlocher, 2019)**.

ADO inhibits neutrophil trafficking and effector processes like the synthesis of inflammatory mediators, oxidative burst, and granule release by acting at the lower-affinity A(2A) and A2BAR **(Barletta, Ley, & Mehrad, 2012)**. CSG21680, an A2AR agonist, inhibited neutrophil extracellular traps (NETs) in a manner comparable to that of ADO, whereas ZM241385 prevented the NET-inhibitory effects of ADO **(K. Xu et al., 2019)**. **Säve, Mohlin, Vumma, and Persson (2011)** found that The selective A(2A) receptor agonist CGS21680 dramatically reduced neutrophil transuroepithelial migration after UPEC stimulation. **Lovászi et al. (2022)** found that A2AR activation prevents cell death, slows down neutrophil aging, and helps neutrophils change from an N1 to an N2 phenotype. **Sullivan, Linden, Buster, and Scheld (1999)** found that with or without rolipram (0-0.01 microgram/kg/h), the selective A2AR agonist WRC-0470 (0-0.9 microgram/kg/h) suppressed pleocytosis and decreased the lipopolysaccharide-induced rise in blood-brain barrier permeability, indicating lessened neutrophil-induced damage.

Regulatory T Cells (Tregs)

Regulatory T cells control the immune system, preventing autoimmune illness, and infiltrate the tumor microenvironment, dispersing it. CD4+CD25+ cells that express FOXP3 are known as Tregs **(Eslam E. Abd El-Fattah & Abdelhamid, 2021; Y. Wang et al., 2016a)**. TGF-β1 generated by hepatocellular carcinoma may increase Tregs, but TGF-β1 deficiency may reduce Tregs and decrease immunosuppression in the tumor microenvironment **(Y. Wang et al., 2016b)**. In HCC patients, Tregs were detected in large quantities, and having a large number of Tregs was linked to a bad prognosis. TGF-β1 was released by liver cancer cells, which aided in Treg development. TGF-β1 knockdown lowered the number of Tregs and metastatic nodules in mice in vivo experiments **(Y. Wang et al., 2016a) (Fig. 2)**.

In the HCC tumor microenvironment, Tregs were discovered in substantially higher numbers than in tissue-surround and biopsy tissues from healthy livers. In HCC patients, a relationship between TGF-β1 and Treg cells was discovered, and TGF-β1 and IL-10 expression was connected to the disease's progression **(Roudi, D'Angelo, Sirico, & Sobhani, 2021; Shen et al., 2015)**.

In autologous T cell cocultures with HIV, ADO deaminase dramatically decreased CD4(+)CD25(hi) forkhead box p3(+) cells produced by HIV-1. HIV-1-specific CD4(+) responder T cells are dramatically increased by HIV-1-pulsed dendritic cells **(Naval-Macabuhay et al., 2016)**. The iTreg-mediated suppression of mitogenic T cells is in part caused by cAMP **(Su et al., 2019)**. **Kinsey et al. (2012)** found that lack of both ADO production (CD73-deficient Tregs) and ADO responsiveness (A(2A)R-deficient Tregs) reduced Treg activity.

Cytotoxic $CD8^+$ T Cells

The most prevalent anti-tumor cells are CD8+ T cells, which, when activated, change into CTLs, release perforin and granzyme-containing granules, and have anti-tumor properties **(Abdelhamid et al., 2021; N. E. El-Ashmawy et al., 2021; Salem et al., 2021)**. CD4+ Th1 cells produce IL-2 and IFN-γ, which boost T cell priming, activation, and cytotoxicity, leading to anti-tumor action. To boost the efficacy of the anti-tumor response, T cell-based anti-tumor immunity requires both cytotoxic CD8+ T cells and Th1 cells **(Gao et al., 2007)**. With CD8+ T and Th1 cell infiltrates, TME, MDSCs, and DCs inhibit T cell activity, which has been linked to a better prognosis in cancer patients **(van der Leun, Thommen, & Schumacher, 2020)**. For B cells to develop into plasmablasts that produce IgG, CD8+CXCR5+ T cells had to release the hormone IL-21. This was crucial for HCC humoral immunity **(Ye et al., 2019) (Fig. 2)**.

According to **Raskovalova et al. (2007)** research, intratumorally generated ADO may hinder the capacity of T lymphocytes to infiltrate tumors by inhibiting the effector function of anti-melanoma specific T cells. ADO signaling via the A2AR in human peripheral CD8+ T cells and TILs results in greater vulnerability to ADO-mediated inhibition of T central memory cells with impairment of peripheral and tumor-expanded T cell effector capacities. Adolescence-related immunosuppressive effects are mediated by increased PKA activity, which impacts the mTORC1 pathway **(Mastelic-Gavillet et al., 2019)**. **Cekic and Linden (2014)** found that mice with

tumors developed more ectopic melanomas, had fewer tumor-associated T cells, less effector memory differentiation, and less antiapoptotic IL7R (CD127) expression on cells exposed to antigens. Similar reductions in CD8(+) T-cell density were seen with intratumoral pharmacologic inhibition in wild-type hosts. **Briceño et al. (2021)** mentioned that the differentiation and metabolic fitness of cytotoxic T lymphocytes are restricted by CD73-mediated ADO synthesis.

Dendritic Cells

Dendritic cells (DCs) are a type of leukocyte that may identify and notify the immune system about illnesses. They are required for the initiation of both innate and adaptive immune responses. Before transmitting antigenic peptides from pathogens to naive T cells in lymphoid organs in the presence of major histocompatibility molecules (MHCs), they must first look for pathogens in peripheral tissues, recognize, trap, and process them. DCs play an essential part in the formation of antigen-specific immune responses as a result of these techniques. They also serve as a vital connection between innate and adaptive immunity **(Lu, Yu, & Xu, 2012).**

Because of their high expression of cell surface and vesicular PRRs, immature DCs in peripheral tissues can detect exogenous PAMP-carrying bacteria. Pathogens and phagocytoses are recognized by DCs, which break them down into peptides. Because innate immunity does not always protect against sickness, an adaptive immune response aimed at pathogen-associated antigenic epitopes may be necessary to combat the immunological danger. In the presence of pro-inflammatory cytokines, a pathogen-digesting immature DC develops and migrates to lymphoid regions where it can present the antigen peptide to immature T cells **(Lu et al., 2012) (Fig. 2)**.

S. V. Novitskiy et al. (2008) showed that VEGF, IL-8, IL-6, IL-10, COX-2, TGF-β, and IDO are among the many angiogenic, pro-inflammatory, immunological suppressor, and tolerogenic factors that are highly expressed by ADO-differentiated DCs. They also exhibit decreased allostimulatory activity. Inflammatory ADO deamination maintains dendritic cell activation **(Desrosiers et al., 2007)**. A2AR suppression improves cancer immunotherapy using dendritic cells **(Masjedi et al., 2020)**. Human dendritic cell differentiation is skewed toward a tolerogenic phenotype with reduced CD8(+) T-cell priming ability by ADO and cAMP signaling **(Challier, Bruniquel, Sewell, & Laugel, 2013)**. The net effect on immune cells were gathered in **Table 2**

Table 2: Effect of ADO on tumor immune mediators.

	Type Of cells	Net effect on activity
ADO	Neutrophils	↓
	Cytotoxic T cell	↓
	Treg Cells	↑
	Dendritic cells	↓

Conclusion

According to our findings, our review focused the point on how ADO causes the tumor immune milieu to transition from an immunostimulant to an immunosuppressive state which is considered a new determinant of immune microenvironment and thus an immune target.

As a result of its immunosuppressive activity and its anti-apoptotic, pro-angiogenic, and pro-proliferative, ADO suppressed tumor immune fitness. Immune-stimulatory cells such as cytotoxic T cells, DCs, and neutrophils were blocked by ADO, whereas immunosuppressive cells like Treg and tolerogenic DCs were activated. ADO also decreased immunostimulant mediators like IL-12 while increasing immunosuppressive mediators, including TGF-β, FOXP3, PD-L1, CTLA-4, and IDO. These modifications induced by ADO make it a robust pro-neoplastic agent with multiple mechanisms of action.

Declarations

- **Ethical Approval and Consent to participate:** Not applicable

- **Consent for publication:** Not applicable

- **Availability of supporting data:** Not applicable

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  - Reham M. Goda: Methodology, Investigation, Writing- Original draft preparation.
  - Eslam E. Abd El-Fattah: Conceptualization, Reviewing, and Editing.

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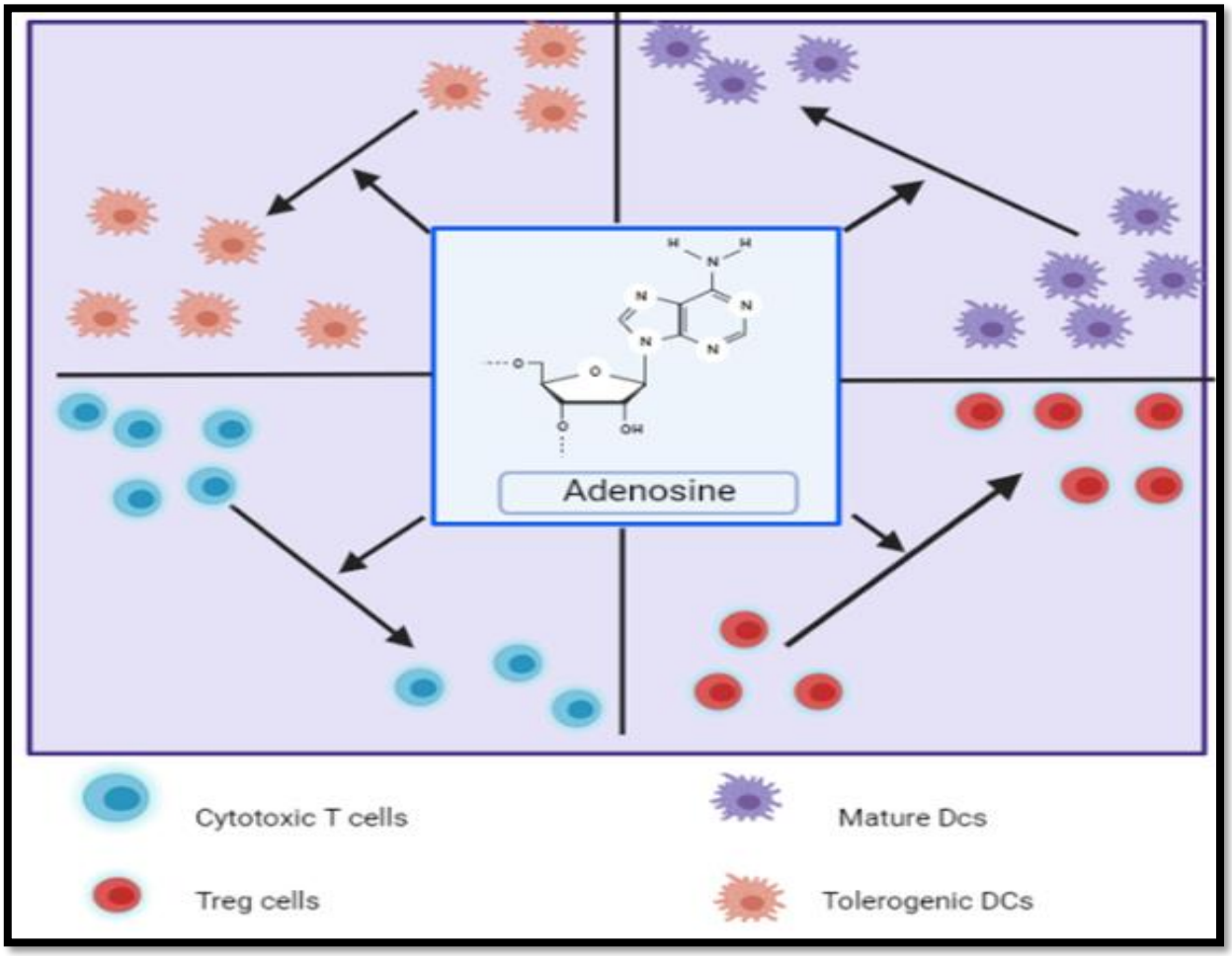

Figure 2: Effect of ADO on tumor immune archetype. ADO alters the tumor microenvironment by various mechanisms, including: **(1)** Shift T cell equilibrium away from cytotoxic type and towards Treg cell type and **(2)** Shift DC equilibrium away from mature type and towards tolerogenic cell type. DCs are dendritic cells, and Tregs are regulatory T cells.

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