Human Immunology xxx (xxxx) xxx

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Review

Targeting adenosine and regulatory T cells in cancer immunotherapy

Alexey Churov *, Galina Zhulai

Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russian Federation

ARTICLE INFO

Article history:
Received 30 October 2020
Revised 7 December 2020
Accepted 10 December 2020
Available online xxxx

Keywords:
Adenosine
Treg
CD39
CD73
FOXP3
Immunosuppression
Tumor immunity

ABSTRACT

Immunosuppressive activity of regulatory T cells (Tregs) is one of the mechanisms promoting carcinogenesis. Intratumoral Tregs have some phenotypic and functional traits that lower the efficiency of antitumor immune response, which makes them a good target for immunotherapy. Several approaches to cancer immunotherapy are being developed along this vector: deletion of tumor-infiltrating Tregs, inhibition of their homing to the tumor microenvironment, and functional downregulation of Tregs.

Studies of the past decade have demonstrated the role of Tregs and ectonucleotidases CD39 and CD73 in the generation of immunosuppressive extracellular adenosine. Pharmacological targeting of CD39 and CD73 can restrain the activity of suppressor cells and promote the efficiency of cancer therapy.

Here we review the latest data on issues regarding the role of extracellular adenosine and its receptors in antitumor immune response, adenosine generation mechanisms involving Tregs and the membrane proteins CD39 and CD73. Innovative approaches to antitumor immunotherapy and clinical studies of Treg targeting and application of anti-CD39/CD73 antibodies, adenosine receptor antagonists, and small-molecule inhibitors of ectonucleotidase activity are explored.

Contents

1. Introduction . . . 00
2. Extracellular adenosine and its receptors in carcinogenesis . . . 00
3. Role of Tregs in adenosine production . . . 00
4. Clinical trials of therapies targeting adenosinergic pathway . . . 00
   4.1. Agents blocking CD73 activity . . . 00
   4.2. CD39 blocking agents . . . 00
   4.3. Selective A2AR/A2BR inhibitors . . . 00
   4.4. CD38/CD157-based therapies . . . 00
5. Conclusions . . . 00
   Funding . . . 00
   Declaration of Competing Interest . . . 00
   References . . . 00

Abbreviations: Tregs, regulatory T cells; FOXP3, transcription factor forkhead box P3; IL, Interleukin; CTLA-4, cytotoxic T-lymphocyte associated antigen 4; GITR, glucocorticoid-induced TNFR-related protein; PD1, programmed death 1; LAG3, lymphocyte-activation gene 3; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin mucin 3; APC, antigen-presenting cell; DCs, dendritic cells; TME, tumor microenvironment; Th2, T helper 2; Th17, T helper 17; NK, natural killer; ATP, adenosine triphosphate; MDSC, myeloid-derived suppressor cells; NAD^+, nicotinamide adenine dinucleotide; ADPR, adenosine diphosphatase ribose; TGF-β, transforming growth factor-β; CT, clinical trial.

* Corresponding author at: Department of Immunology, Institute of Biology of Karelian Research Centre, Russian Academy of Sciences, 185910 Petrozavodsk, Russian Federation.

E-mail address: achurou@yandex.ru (A. Churov).

https://doi.org/10.1016/j.humimm.2020.12.005
0198-8859/

Please cite this article as: A. Churov and G. Zhulai, Targeting adenosine and regulatory T cells in cancer immunotherapy, Human Immunology, https://doi.org/10.1016/j.humimm.2020.12.005

1. Introduction

In the process of carcinogenesis, tumor cells induce immunosuppression in their microenvironment. A critical role in this process belongs to regulatory T cells (Tregs). Tregs are involved in maintaining immune homeostasis, prevent autoimmune reactions, and participate in the pathogenesis of cancer and other diseases [1–3]. Tregs are strongly involved in antitumor immune response and an elevated ratio of tumor-infiltrating FOXP3$^+$ Tregs to T cells is a poor prognostic factor in various types of cancer [4,5]. Cancer cells build an immunosuppressive environment which favors a rise in the tumor-infiltrating Treg cell population [6].

The phenotype of tumor Tregs is characterized by enhanced expression of CD45RO, costimulatory (GITR, ICOS, OX40) and inhibitory (CTLA-4, PD-1, LAG3, TIGIT, Tim-3) molecules, as well as chemokine receptors (CCR4 and CCR8) [6,7]. This makes them capable of effectively suppressing antitumor immune response. The main known targets for Treg-driven immunosuppression are CD4$^+$ and CD8$^+$ T cells, B cells, NK, NKT, and antigen-presenting cells (APCs) (i.e. dendritic cells (DCs), monocytes, macrophages) [8].

Currently, the development of immunotherapy based on Tregs is actively underway [9]. Experiments have demonstrated that Treg inhibition reduces the tumor burden and improves the efficiency of antitumor immunity [10–12], corroborating that the Treg-targeting strategy has a high therapeutic potential. There are now several vectors for developing Treg-targeting immunotherapy: elimination of tumor-infiltrating Tregs, inhibition of their recruiting into the tumor microenvironment (TME), their functional downregulation.

For instance, drugs based on humanized anti-CD25 antibodies – daclizumab and basiliximab, coupled with a dendritic cell vaccine, reduce Treg cell numbers in melanoma and glioblastoma therapies, and in the case of basiliximab the drug potentiates the vaccine [13]. Another drug targeting IL-2 and capable of selectively eliminating Tregs – Denileukin diftitox (Ontak), is a recombinant fusion protein product of IL-2 and diphtheria toxin, which was approved for treating cutaneous T-cell lymphoma. Its variants are now going through clinical trials (CTs) in combination with other antitumor therapy approaches [13].

Membrane proteins CTLA-4 and PD-1/PD-L1 also act as targets for Treg-cell depletion, since intratumoral Tregs feature a high constitutive expression of these inhibitor molecules. Immunotherapy targeting CTLA-4 and PD-1/PD-L1 proved to be quite effective in achieving long-term remission in therapy-responsive patients [13].

Treg recruitment to tumor localizations can be inhibited by therapy with chemokine receptor blocking antibodies. FDA has recently endorsed mogamulizumab, humanized anti-CCR4 antibody, for treating rare forms of cutaneous T-cell lymphoma. In CTs, mogamulizumab therapy substantially depleted intratumoral Tregs [14]. However, the CCR4 receptor is expressed also by effector T cells and, as demonstrated by phase 1A trial [15], mogamulizumab depleted not only Tregs, but also Th2 and Th17 cells.

One of the suppression mechanisms for Tregs in the TME is their involvement in extracellular adenosine production through expression of the membrane proteins CD39 and CD73, which possess ectonucleotidase activity [16,17]. Adenosine is present in all cells, mostly in the form of adenine nucleotides, which are involved in energy metabolism. At the same time, adenosine can exist in the free form, regulating some biochemical processes. In particular, interacting via cellular receptors, adenosine inhibits the activity of DCs and macrophages, suppresses the proliferation and effector functions of cytotoxic CD8$^+$ T cells and NK cells [18]. Adenosine level in tumors is higher than in normal tissue. Extracellular adenosine generation in the TME is now regarded as a potential target for tumor immunotherapy.

This review aimed to explore the role of adenosine and Tregs in generation of the immunosuppressive TME, as well as to discuss advances and future perspectives for targeting Tregs, and CD39/CD73/adenosine pathway for cancer immunotherapy.

2. Extracellular adenosine and its receptors in carcinogenesis

The main pathway for intracellular adenosine production is through hydrolysis of S-adenosylhomocysteine. Nucleoside transporter proteins carry adenosine from the cell to the intercellular space [18]. In cases of inflammation, hypoxia or ischemia, intercellular adenosine accumulation is mostly a result of extracellular ATP dephosphorylation mediated by CD39/CD73 activity (Fig. 1 A,D). Tissue hypoxia, such that develops during tumor growth, is the key factor that alters adenosine metabolism. As the metabolic activity of rapidly growing tumor cells increases, they begin to consume more oxygen, wherefore free-radical oxidation is stimulated and local pH is altered. Tumor causes hypoxia to become chronic [19]. It has been demonstrated that hypoxia can induce CD39 and CD73 expression, as well as downregulate adenosine kinase, thus inhibiting adenosine conversion to final metabolites [20]. Hypoxia has a powerful immunosuppressive effect both on effector cells and on APCs [21]. Mice exposed to respiratory hyperoxia (60% oxygen) exhibited a decline in adenosine level in the TME [22].

Ectonucleotidases are expressed by many cell types (Fig. 1B), e.g. CD39 is found on monocytes, and CD73 on myeloid cells, bone marrow stromal cells, thymic epithelial cells. Some cell types simultaneously carry both proteins (CD39 and CD73) on their membrane: B cells, Tregs, T helper 17 cells, NK cells, and myeloid-derived suppressor cells (MDSC). Tumor cells can also express CD39 and CD73 [23]. Exosomes of some solid human tumors (colorectal cancer, breast cancer, prostate cancer) also express CD39 and CD73, and stimulate the formation of adenosine to suppress T cells [24]. Many authors have noted an overexpression of these ectonucleotidases in relation to many types of tumors. Changes in CD39 and CD73 expression can be of prognostic value (Table 1).

There is an alternative pathway for adenosine production from extracellular nicotinamide adenine dinucleotide (NAD$^+$) (Fig. 1 A, D). Glycoproteins CD38 and CD157, expressed on tumor and immune cells, function both as receptors and as NAD$^+$ converting ectoenzymes [25]. NAD$^+$ glycohydrolase CD38 converts extracellular NAD$^+$ into adenosine diphosphatase ribose (ADPR) directly or through the intermediate cyclic ADPR (cADPR). ADPR is then converted by CD203a (ecto-nucleotidepyrophosphatase / phosphodiesterase 1 or ENPP1) to produce AMP. AMP, in turn, is metabolized into adenosine by CD73. This adenosine generation pathway has been described in less detail than the classical one, and was originally demonstrated for the Jurkat cell line [25]. An alternative pathway for adenosine-mediated suppression has been shown to exist also in human melanoma cells [26]. It was also demonstrated that suppression of CD4$^+$ and CD8$^+$ T cell proliferation was reverted by adding CD38 and CD73 specific inhibitors. Similar to CD38, CD157 is a NAD$^+$ metabolizing ectoenzyme with glycohydrolase and cyclase activities and is capable of ADPR and cADPR production from NAD$^+$, respectively. CD157, although less efficient in ADPR/cADPR formation than CD38, is probably also involved in the CD38/CD157/CD203a/CD73 adenosinergic pathway [25].

There are four known subtypes of receptors to adenosine - A1, A2A, A2B, and A3, which differ in localization, affinity for adenosine and signaling pathways. Adenosine receptors on cell surface are associated with G proteins, which can either induce (A2A and

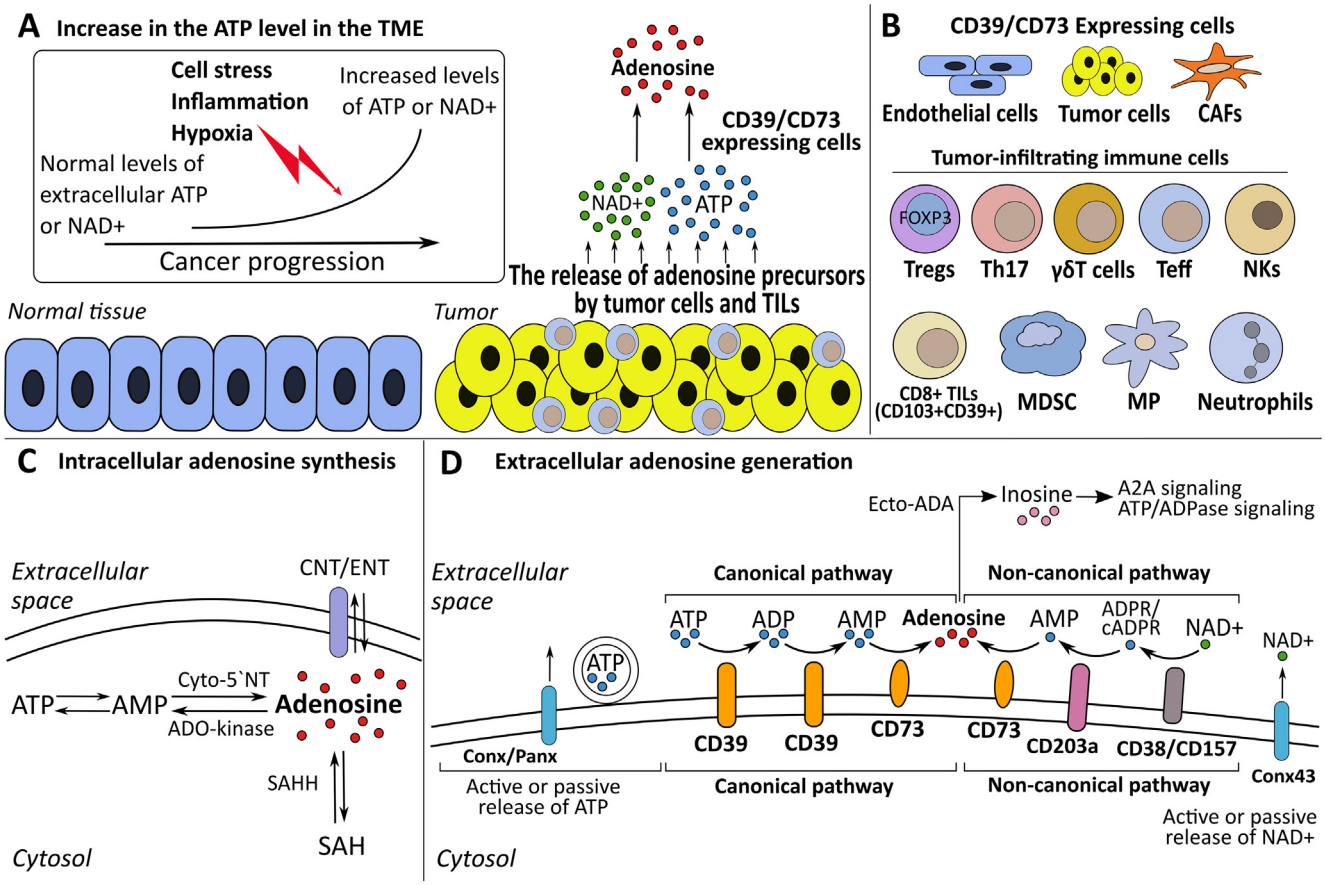

Fig. 1. Adenosine production and signaling. A) During oncogenesis, hypoxia, chronic inflammation, and cellular stress induce active release of adenosine precursors (ATP and NAD^+) from tumor cells and tumor-infiltrating lymphocytes within the TME. As a result, the concentration of adenosine precursors in the TME becomes much higher than in normal tissues. In the extracellular tumor milieu, adenosine precursors are enzymatically processed by a set of ectonucleotidases (CD39 and CD73) expressed by various cell types (B). C) Intracellular adenosine synthesis is controlled by the balance of the activity of adenosine kinase (ADO-kinase) and cytoplasmic 5'nucleotidase (Cyto-5'NT) or through the direct metabolism of S- adenosylhomocysteine (SAH) by the S-adenosyl-homocysteine hydrolase (SAHH). Adenosine is transported into and out of the cell by concentrative or equilibrative nucleoside transporters (ENTs and CNTs, respectively). D) During the extracellular generation of adenosine, ATP or NAD^+ are released into the extracellular space through passive mechanisms (cell lysis and cell death) or active pathways. Active transport of ATP may occur under the control of transport proteins connexin and pannexin (Conx/Panx). ATP can also be released in ATP-containing vesicles. NAD^+ active transport is controlled by connexin 43 (Conx43). The canonical pathway of adenosine synthesis involves the hydrolysis of ATP to AMP by CD39 and the hydrolysis of AMP by CD73. The non-canonical pathway involves the use of NAD^+ as a substrate by CD38 or CD157 to generate ADP-ribose (ADPR) directly or through its cyclic form (cADPR). ADPR is then processed to AMP by CD203a (ENPP1). Once generated, extracellular adenosine can bind to its receptors or be metabolized to inosine by adenosine deaminase (ecto-ADA). Inosine can also induce signals via A2A receptors or ATP/ADPase enzymes that metabolize ATP or ADP to AMP.

Table 1
Prognostic implications of CD39 and CD73 expression in carcinogenesis.

Marker	Favorable prognostic factor for overall survival	Unfavorable prognostic factor for overall survival
CD39	Pancreatic cancer, n = 28 [27] (mRNA; OS, P = 0.0466).	Hepatocellular carcinoma, n = 324 [28] (Protein; OS, P = 0.002). Gastric cancer, n = 101 [29] (mRNA; OS, P = 0.0315). Renal cancer, n = 243 [30] ($CD39^+CD8^+$ T cells; OS, P < 0.0001).
CD73	Bladder cancer, n = 207 [31] (protein; OS, P = 0.0022).	Breast cancer (triple negative), n = 661 [32] (mRNA; OS, P = 0.029). Head and neck carcinoma, n = 162 [33] (mRNA; OS, P = 0.002). Glioblastoma, n = 163 [34] (mRNA; OS, P = 0.048). Colorectal cancer, n = 135 [35] (protein; OS, P = 0.04).

A2B) or inhibit (A1 and A3) intracellular cAMP production by regulating adenylyl cyclase activity. All adenosine receptors are linked to the MAPK signaling pathway system (including ERK, JNK, and p38 MAPK pathways), which orchestrate gene transcription, metabolism, proliferation, mobility of cells, apoptosis, and other processes that are important for tumor progression [18,36,37]. Stimulation of adenosine receptors also results in CREB, PKA, and EPAC signaling. PKA and EPAC are associated with many immunosuppressive effects [37]. Adenosine receptors are widely represented on cells of different types. Activation of adenosine receptors on tumor-infiltrating cells and directly on tumor cells triggers various reactions modulating the immune response (Fig. 2).

A majority of immune cells express the A2A-type adenosine receptor (A2AR), whose interplay with adenosine mainly leads to an immunosuppressive effect [38,39]. Blocking of A2AR on T cells suppresses tumor progression, inhibits angiogenesis and metastatic spread. This effect has been demonstrated for some human

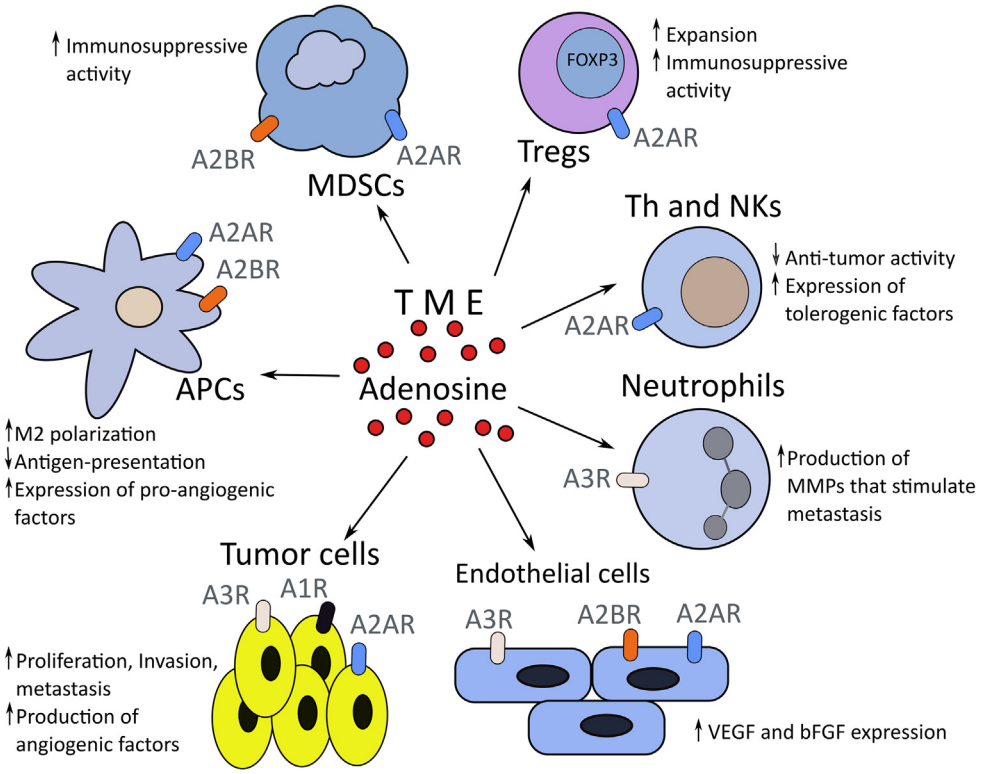

Fig. 2. Adenosine effects on various types of cells in the tumor microenvironment. TME, tumor microenvironment; APCs, antigen-presenting cells; MMPs, matrix metalloproteinases; MDSCs, myeloid-derived suppressor cells.

cancers [40–42]. Tumor-infiltrating $CD4^+$ T helper cells, cytotoxic $CD8^+$ T cells, and NK cells are sensitive to high extracellular adenosine concentrations, which stimulates A2AR on these cells, weakens their effector functions, and induces the expression of the membrane proteins CTLA-4 and PD-1 [18,20]. Activation of adenosine receptors of the same type on Tregs and on MDSCs triggers an expansion of these cells and weakens anti-tumor immunity [43–45]. In view of the above, A2AR are regarded as a potential target in cancer immunotherapy (Fig. 2).

Fast-growing cancer cells are in need of nutrition, derived through the blood vessel network. The formation of new vessels in the TME is promoted by endothelial and tumor cells producing proangiogenic factors: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angioprotein, and IL-8. Secretion of these biomolecules can be stimulated by the activation of A2A and A2B receptors [46]. Also, angiogenesis can be supported by adenosine-differentiated DCs featuring a high expression of angiogenic, anti-inflammatory, and immunosuppressive factors [47,48].

APCs, first of all DCs and macrophages, effectively recognize tumor antigens and launch the adaptive immune response. High adenosine concentration in tumors can modulate DC activity via A2AR and A2BR receptors, discouraging APC-mediated T cell activation. Macrophages impacted by adenosine via A2B receptors can undergo polarization from the pro-inflammatory M1 type to the M2 type. Such macrophages secrete indoleamine 2,3-dioxygenase, arginase, and TGF-β, which possess immunosuppressive activity [20,46] and induce tumor progression by promoting metastasis. It was also demonstrated that when A2BR and A1R receptors are activated, tumor-infiltrating neutrophils upregulate the production of metalloproteases, which accelerate metastasis [46].

Extracellular adenosine also produces versatile direct effects on tumor cells: receptors A1R, A2AR, A2BR, and A3R promote the proliferation of tumor cells, their survival, migration and invasiveness [46,49–51].

3. Role of Tregs in adenosine production

Adenosine production appears to be one of the mechanisms for the suppressive activity of Tregs. Deaglio et al. (2007) were among the first to demonstrate that Tregs can participate in adenosine generation from extracellular nucleotides [52]. Tregs in mice constitutively express CD39 and CD73 as much as other key markers for Tregs: FOXP3 and CTLA-4 [52]. Later studies revealed that Tregs in the blood of $CD39^{-/-}$ mice have a weakened suppressive activity *in vitro* and cannot inhibit the graft-versus-host desease [53]. Furthermore, application of CD73/CD39 inhibitors significantly reduces the ability of Tregs to suppress the proliferation of $CD4^+$-$CD25^-$ T cells. The catalytic capacity of CD39 and CD73 is synchronized with Treg activation. Thus, murine Tregs showed increased CD39 activity after activation of their T-cell receptor [54]. In addition to exploiting adenosine as an immunosuppressive agent, Tregs use it to augment their functional activity. Moreover, stimulation of the adenosine receptor A2A has been shown to promote Treg proliferation, CTLA-4 and PD-1 expression, and functional activity [44].

Surface expression of CD73 has been clearly detected in $CD4^+$-$CD39^+$ Tregs of mice. Human Tregs differ from other immune cells in an higher expression of CD39, but CD73 expression, on the other hand, can be very low if at all detectable [55]. Schuler et al. (2014) showed that only around 1% of circulating Tregs have the $CD39^+$-$CD73^+$ phenotype. In order to produce adenosine $CD4^+CD39^+$ Tregs need to engage membrane-bound CD73, which is possible in a TME containing $CD4^+CD73^+$ T cells, B cells, or $CD39^+CD73^+$ exosomes [56]. In cancer patients (head and neck carcinoma, melanoma, pancreatic cancer), Tregs express both the CD39 and CD73 markers,

Table 2
Clinical trials of antitumor drugs targeting the adenosine signaling pathway.

Target	CT ID (sponsor)	CT phase, status	Condition	Drug	Type	Ref.
CD73	NCT02503774 MedImmune LLC	I	Solid tumors (advanced)	Oleclumab in combination with other agents	anti-CD73 mAb	[59,61]
	NCT03736473 AstraZeneca	I	Solid tumors (advanced)	Oleclumab in combination with other agents	anti-CD73 mAb	[62]
	NCT03742102 AstraZeneca	IB/II	Triple negative breast cancer	Oleclumab in combination with other agents	anti-CD73 mAb	[63]
	NCT02754141 Bristol-Myers Squibb	I/IIA	Solid tumors (advanced)	BMS-986179 in combination with other agents	IgG2-IgG1 mAb	[64]
	NCT03822351 MedImmune LLC	II	Stage III non-small cell lung cancer (unresectable)	Oleclumab in combination with other agents	anti-CD73 mAb	[65]
	NCT03454451 Corvus Pharmaceuticals, Inc.	I/IB	13 types of cancer including solid tumors and NHL	CPI-006 in combination with other agents	anti-CD73 mAb	[66]
	NCT03549000 Novartis Pharmaceuticals	I/IB	Solid tumors (advanced)	NZV930 (SRF373) in combination with other agents	IgG4 mAb	[67]
	NCT03954704 Gilead Sciences	IA/IB	Solid tumors (advanced)	GS-1423 (AGEN1423)	Chimeric CD73- and TGF-β-specific mAb	[68]
	NCT04322006 I-Mab Biopharma Co. Ltd.	I/II	Solid tumors (advanced)	TJ004309 monotherapy and in combination with anti-PDL-1 therapy	anti-CD73 mAb	[69]
	NCT03835949 Tracon Pharmaceuticals Inc.	I	Solid tumors (advanced or metastatic cancer)	TJ004309 monotherapy and in combination with anti-PDL-1 therapy	anti-CD73 mAb	[70]
	NCT03677973 Arcus Biosciences, Inc.	I	Healthy volunteers	AB680	CD73 small molecule inhibitor	[71]
CD39	NCT03884556 Trishula Therapeutics, Inc.	I/IB	Solid tumors (advanced)	TTX-30 monotherapy and combination therapy	anti-CD39 mAb IgG4	[72]
	NCT04306900 Trishula Therapeutics, Inc.	I/IB	Solid tumors (advanced)	TTX-30 combination therapy	anti-CD39 mAb IgG4	[73]
	NCT04261075 MedImmune LLC	I	Solid tumors (advanced)	IPH5201 monotherapy and combination therapy	anti-CD39 mAb	[74]
	NCT04336098 Surface Oncology	I	Solid tumors (advanced)	SRF617 monotherapy	anti-CD39 mAb	[75]
A2AR	NCT02655822 Corvus Pharmaceuticals, Inc.	I/IB	Some therapy-resistant cancers (renal cancer, prostate cancer)	Ciforadenant (CPI-444) monotherapy and combination therapy	Small molecule	[76]
	NCT04280328 Corvus Pharmaceuticals, Inc.	I	Some therapy-resistant cancers (multiple myeloma)	Ciforadenant (CPI-444) combination therapy	Small molecule	[77]
	NCT03337698 Corvus Pharmaceuticals, Inc.	IB/II	Metastatic non-small cell lung cancer	Ciforadenant (CPI-444) combination therapy	Small molecule	[78]
	NCT04089553 AstraZeneca	II	Prostate cancer	AZD4635 combination therapy	Small molecule	[79]
	NCT03099161 Merck Sharp & Dohme Corp.	I (trial terminated)	Solid tumors (advanced)	Preladenant (MK-3814)	Small molecule	[80]
	NCT02403193	I/II	Advanced non-small cell lung cancer	PBF-509	Small molecule	[81]
A2BR	NCT03274479 Palobiofarma SL	I	Non-small cell lung cancer	PBF-1129	Small molecule	[82]
A2AR and A2BR	NCT03720678 Arcus Biosciences, Inc.	I/IB	Gastrointestinal tumors	Etrumadenant (AB928) combination therapy	Small molecule, selective A2AR/A2BR inhibitor	[83]
	NCT04262856 Arcus Biosciences, Inc.	II	PDL-1 positive non-small cell lung cancer	Etrumadenant (AB928) monotherapy and combination therapy	Small molecule, selective A2AR/A2BR inhibitor	[84]
CD38	NCT00574288 Janssen Research & Development, LLC	II	Multiple Myeloma	Daratumumab monotherapy	anti-CD38 mAb	[85]
	NCT01084252 Sanofi	I/II	Selected $CD38^+$ Hematological Malignancies	Isatuximab monotherapy	anti-CD38 mAb	[86]
CD157	NCT02353143	I	Relapsed/Refractory Acute Myeloid Leukemia.	MEN1112 monotherapy	anti-CD157 mAb	[87]

and efficiently contribute to adenosine generation from ATP [55,56]. Tumor-infiltrating Tregs have higher CD39 expression compared to Tregs in healthy tissue or circulating Tregs [29,57]. It was demonstrated in a murine model of metastatic liver cancer that CD39 expression on $CD4^+Foxp3^+$ Tregs suppresses NK-cell-mediated antitumor immunity and promotes metastasis [58].

4. Clinical trials of therapies targeting adenosinergic pathway

4.1. Agents blocking CD73 activity

Among agents that directly block ectonucleotidase activity, monoclonal antibodies to the CD73 membrane protein are most commonly tested in CTs. Some drugs in this group are going through their first phase I/II CTs (Table 2).

The most widely tested in CTs is Oleclumab (MEDI9447) – a drug based on anti-CD73 monoclonal antibodies, developed by Medimmune and AstraZeneca. The drug's active ingredient is a modified non-Fc-binding IgG1. Oleclumab (MEDI9447) has a dual mode of action and is capable of inhibiting the formation of the CD73 catalytic site as well as generating CD73 dimers [59,60].

Another drug of interest is BMS-986179, which is also based on anti-CD73 mAbs. BMS-986179 is a chimeric (IgG2-IgG1) protein constructed so that it exhibits no Fc-binding activity. The drug is currently in phase I/II CTs (NCT02754141) [64,88].

Both drugs intensify membrane CD73 internalization, which gives rise to other anti-CD73 antibody properties which may potentially be useful in therapy. For instance, it was found that CD73 can move from membrane-bound to soluble form through shedding from the cell membrane while retaining its enzymatic activity. Geoghegan et al. [60] showed that MEDI9447 can inhibit sCD73 generation, mainly by blocking immobilized receptors on the membrane and internalizing them [60].

Another development to be highlighted is the drug by Gilead Sciences, called GS-1423 (AGEN1423), which is a hybrid antibody capable of simultaneously blocking the activity of the CD73 and the pleiotropic cytokine TGF-β [68,89]. There are also developments of small-molecule inhibitors of CD73 ectonucleotidase activity. An example is AB680 (Arcus Biosciences, Inc.), which is a reversible, slow-onset competitive inhibitor of human CD73 [90]. This drug was designed to treat patients with solid tumors.

4.2. CD39 blocking agents

Agents targeting CD39 activity have so far been less actively studied. CTs are currently underway for three agents suppressing CD39 ectonucleotidase activity – TTX-30 (Trishula Therapeutics, Inc.), IPH5201 (MedImmune LLC), and SRF617 (Surface Oncology). All agents in this group are mAbs to the CD39 membrane protein, which are now in phase I CTs for advanced solid tumor therapy (Table 2).

There are several drugs not yet in CTs that can be highlighted as promising pharmacological agents inhibiting CD39 activity. Such is ES002 (developed by Elpisciences), based on ATP-blocking anti-CD39 mAb. ES002 has so far been tested only within preclinical trials – in an animal model of multiple myeloma. This drug exhibits robust efficacy in inhibiting tumor growth *in vivo*. It can downregulate the suppressive activity of Tregs towards effector T cells, and engage APCs and NK cells to facilitate its antitumor activity *in vivo* [91,92].

Another potential agent for tumor immunotherapy is a locked nucleic acid (LNA)-based antisense oligonucleotide. The anti-nucleotidase activity of this antisense oligonucleotide (ASO), which specifically blocks CD39 mRNA (CD39-specific ASO), has so far been tested only in human tumor cell lines and *in vivo* in tumor-bearing mice. The first results of the studies look very promising. CD39-specific ASOs suppressed the expression of CD39 mRNA and protein in various murine and human cancer cell lines and in primary human T cells. The application of CD39-specific ASO lowered extracellular ATP catabolism, and augmented $CD8^+$ T-cell proliferation. Besides, the treatment of tumor-bearing mice with CD39-specific ASOs induced dose-dependent decrease in the CD39 protein level in Tregs and in intratumoral macrophages. The number of tumor-infiltrating Tregs was notably lower in mice treated with CD39-specific ASO. As a result, intra-tumoral $CD8^+$ T cells to Tregs ratio in the mice increased, and PD-1 expression was induced in tumoral $CD8^+$ T cells. Tumor therapy in mice using CD39-specific ASOs in combination with anti-PD-1 mAb effectively slowed down tumor progression [93].

4.3. Selective A2AR/A2BR inhibitors

Another emerging class of compounds for antitumor therapy is pharmacological substances selectively targeting adenosine receptors. This class of agents is of special interest since some of them have already been tested in advanced CTs (phase III), particularly Parkinson's disease and other neurological conditions. In these CTs, the drugs proved to be highly tolerable and safe for the patients [94–96].

Furthermore, trials of individual anti-A2AR agents were not accompanied by immunotoxicity such as happens in therapies with anti-CTLA-4 and anti-PD-1 mAbs [97].

Ongoing CTs concentrate on three groups of adenosine receptor inhibitors for antitumor therapy: A2AR inhibitors, A2BR blockers, and substances co-targeting both receptor types (Table 2). All the adenosine receptor inhibiting agents are selective small molecules. Among A2AR inhibitors, CTs are underway for three drugs: Ciforadenant (CPI-444), AZD4635, and PBF-509 (Table 2).

One more CT (started in 2017) with the drug preladenant (MK-3814) alone and in combination with pembrolizumab in patients with solid tumors was recently terminated as the therapy results did not support the study endpoints. Namely, the preladenant/pembrolizumab combination therapy caused serious adverse events in two of the ten patients in this CT. Moreover, in some patients receiving preladenant monotherapy the disease was progressing even when the dose was raised (from 25 mg to 50 mg) [80]. There occurred fatal cases (all causes) in patients receiving monotherapy, whereas in the group treated with the preladenant/pembrolizumab combination there were no fatal cases.

The only A2BR-targeting drug tested in CTs so far was named PBF-1129, but data on its clinical efficacy are yet missing [82].

Finally, the third group is agents that block both A2AR and A2BR simultaneously. In this group, only one pharmacological substance is being tested in different types of cancer, including some gastrointestinal tumors, non-small cell lung cancer, breast cancer, and some others [83,84,98,99]. Etrumadenant is so far the only dual-action adenosine receptor inhibitor of its kind. Owing to its properties, etrumadenant can prevent adenosine-mediated suppression of not only tumor-infiltrating T cells (express A2AR) and NK cells (express A2BR), but also affects myeloid cells – DCs, macrophages, MDSCs (co-express A2AR and A2BR). These unique properties make etrumadenant one of the most promising agents targeting adenosine receptors.

4.4. CD38/CD157-based therapies

Finally, an important therapy that deserves attention in this review is the treatment based on immunomodulatory molecules CD38 and CD157. As mentioned above, CD38 and CD157 are glycoproteins with NAD^+ ectonucleotidase activity, which are also associated with the pro-oncogenic properties of the TME. The CD38 molecule is present on the surface of cells (including plasma B cells, CD38-expressing Treg subsets and other CD38-expressing cells), in lymphoid tumors, in particular multiple myeloma, post-transplant and AIDS-associated lymphomas [100–102]. Moreover, CD38 and CD157 NAD^+-converting ectonucleotidases play an important immunomodulatory role in solid tumors. The relationship of CD38 with clinical prognosis and/or impact on the therapy

efficacy has been noted in hepatocellular carcinoma, lung cancer, breast cancer and other solid tumors. CD157 functions have also been demonstrated in some types of solid tumors [25]. Together this makes these molecules interesting targets for anticancer therapy. There are some completed as well as undergoing CTs where CD38 and CD157 are the targets for immunotherapy. There are several CTs of daratumumab and isatuximab, which are IgG1-derived mAbs directed against CD38. These drugs are tested for hematological malignancies, [77,85,86]. There's also ongoing CTs using MEN1112, a CD157 targeted mAb, in patients with relapsed or refractory acute myeloid leukemia [87] (Table 2).

5. Conclusions

Cancer cells display multiple immunosuppressive mechanisms to evade antitumor immune responses, including T-cell response. One such mechanism is the suppressive effect of adenosine on tumor-infiltrating immune cells. Tregs are also involved in adenosine generation and regulation of immune cells within TME. CD39 and CD73 are nucleases constitutionally expressed in Tregs. In the TME Tregs can hydrolyse extracellular ATP or ADP into AMP and produce adenosine with immunosuppressive properties. Adenosine transmits inhibitory signals through adenosine receptors on the surface of various types of effector and antigen-presenting intratumoral immune cells. Thus, CD39/CD73 and adenosine receptors are important immune checkpoints whose blockade can reestablish antitumor immune response.

In view of known data on the role of molecular mechanisms regulating the adenosinergic signaling in immune and tumor cells, as well as the results of first preclinical studies regarding inhibition of adenosine-driven suppression, the application of inhibitors of CD39/CD73/adenosine receptor pathway in tumor therapy is of substantial interest.

This approach enables to control both the level of intercelluluar adenosine and to target the functions of immune cells (Tregs, APCs, and effector cells).

Early clinical trials are currently underway for a number of agents based on mAbs and small molecules to test their safety and efficacy for antitumor immunotherapy. Yet, a few questions remain to be answered before this type of immunotherapy can be successfully used in practice.

First, there is still little data on the possible molecular mechanisms of the emergence of tumor resistance to this type of therapy, which does not allow us to fully assess the potential of CD39/CD73 and adenosine receptor inhibitors. Secondly, no phase III CTs have so far been carried out, and so data on effective dosage for therapies with CD39/CD73 and adenosine receptor inhibitors, or on their bioavailability in the TME are insufficient. Thirdly, CT results do not clearly indicate how effective is the therapy using Treg-targeting adenosine signaling inhibitors. Meanwhile, Tregs are the key cells for building a kind of an immunosuppressive "dome" that protects tumor cells against being recognized and killed. Finally, there is so far too little data concerning the efficacy of immune-checkpoint inhibitor combinations with other agents and therapies. A potentially promising approach is to combine CD39/CD73 and adenosine receptor inhibitors with agents targeting intratumoral Tregs.

Thus, it is necessary to further study the role of adenosinergic signaling pathway in the formation of immunosuppression in tumors and conduct further CTs to determine the efficacy of CD39/CD73 and adenosine receptor inhibitors and select the optimal drug dosages. In addition, more research has to be done to confirm the role of other, less well- studied molecules involved in the extracellular adenosine production as promising targets for immunotherapy. For example, CTs of drugs based on mAbs to CD38 and CD157 are already underway.

Funding

The study was funded from the Federal Budget under state order (project No. 0218-2019-0083; Modification of transcription programs of regulatory T cell differentiation in immunoinflammatory diseases and cancer).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.B. Wing, A. Tanaka, S. Sakaguchi, Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer, Immunity 50 (2019) 302–316, https://doi.org/10.1016/j.immuni.2019.01.020.
[2] B. Liu, Y. Shao, X. Liang, D. Lu, L. Yan, A. Churov, R. Fu, CTLA-4 and HLA-DQ are key molecules in the regulation of mDC-mediated cellular immunity by Tregs in severe aplastic anemia, J. Clin. Lab. Anal. (2020) e23443, https://doi.org/10.1002/jcla.23443.
[3] P.N. Kravchenko, G.A. Zhulai, A.V. Churov, E.K. Oleinik, V.M. Oleinik, O.Y. Barysheva, et al., Subpopulations of regulatory T-lymphocytes in the peripheral blood of patients with rheumatoid arthritis, Vestn. Ross. Akad. Med. Nauk. 71 (2016) 148–153, https://doi.org/10.15690/vramn656.
[4] L. Xu, W. Xu, S. Qiu, S. Xiong, Enrichment of CCR6+Foxp3+ regulatory T cells in the tumor mass correlates with impaired CD8+ T cell function and poor prognosis of breast cancer, Clin. Immunol. 3 (2010) 466–475, https://doi.org/10.1016/j.clim.2010.01.014.
[5] M. Watanabe, K. Kanao, S. Suzuki, H. Muramatsu, S. Morinaga, K. Kajikawa, et al., Increased infiltration of CCR4-positive regulatory T cells in prostate cancer tissue is associated with a poor prognosis, Prostate 14 (2019) 1658–1665, https://doi.org/10.1002/pros.23890.
[6] J. Stockis, R. Roychoudhuri, T.Y.F. Halim, Regulation of regulatory T cells in cancer, Immunology 157 (2019) 219–231, https://doi.org/10.1111/imm.13064.
[7] H. Yano, L.P. Andrews, C.J. Workman, D.A.A. Vignali, Intratumoral regulatory T cells: markers, subsets and their impact on anti-tumor immunity, Immunology 157 (2019) 232–247, https://doi.org/10.1111/imm.13067.
[8] C.M. Paluskievicz, X. Cao, R. Abdi, et al., T regulatory cells and priming the suppressive tumor microenvironment, Front. Immunol. 10 (2019) 2453, https://doi.org/10.3389/fimmu.2019.02453.
[9] A.V. Churov, A.I. Siutkina, K.Y. Mamashov, E.K. Oleinik, The potential of CD4+ regulatory T cells for the therapy of autoimmune diseases, Bull. RSMU. 6 (2019) 66–69, https://doi.org/10.24075/brsmu.2019.082.
[10] E. Pastille, K. Bardini, D. Fleissner, et al., Transient ablation of regulatory T cells improves antitumor immunity in colitis-associated colon cancer, Cancer Res. 74 (2014) 4258–4269, https://doi.org/10.1158/0008-5472.CAN-13-3065.
[11] K. Sato, N. Sato, B. Xu, et al., Spatially selective depletion of tumor-associated regulatory T cells with near-infrared photoimmunotherapy, Sci. Transl. Med. 8 (2016) 352ra110, https://doi.org/10.1126/scitranslmed.aaf6843.
[12] F. Arce Vargas, A.J.S. Furness, I. Solomon, Fc-optimized anti-CD25 depletes tumor-infiltrating regulatory T cells and synergizes with PD-1 blockade to eradicate established tumors, Immunity 46 (4) (2017) 577–586, https://doi.org/10.1016/j.immuni.2017.03.013.
[13] A. Sharma, D. Rudra, Regulatory T cells as therapeutic targets and mediators, Int. Rev. Immunol. 38 (5) (2019) 183–203, https://doi.org/10.1080/08830185.2019.1621310.
[14] D. Sugiyama, H. Nishikawa, Y. Maeda, M. Nishioka, A. Tanemura, I. Katayama, S. Ezoe, Y. Kanakura, E. Sato, Y. Fukumori, J. Karbach, E. Jager, S. Sakaguchi, Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking antitumor immune responses in humans, Proc. Natl. Acad. Sci. 110 (44) (2013) 17945–17950, https://doi.org/10.1073/pnas.1316796110.
[15] K. Kurose, Y. Ohue, H. Wada, et al., Phase I a study of FoxP3+ CD4 Treg depletion by infusion of a humanized anti-CCR4 antibody, KW-0761, in cancer patients, Clin. Cancer Res. 21 (2015) 4327–4336, https://doi.org/10.1158/1078-0432.CCR-15-0357.
[16] G.A. Zhulai, E.K. Oleinik, A.V. Churov, A.A. Romanov, P.N. Kravchenko, V.M. Oleinik, Significance of treg cells for adenosine-mediated immune suppression in colorectal cancer, Med. Immunol. (Russia) 19 (2017) 89–94. https://doi.org/10.15789/1563-0625-2017-1-89-94.
[17] G.A. Zhulai, A.V. Churov, E.K. Oleinik, A.A. Romanov, P.N. Semakova, V.M. Oleinik, Activation of CD4+CD39+ T cells in colorectal cancer, Bull. RSMU. 7 (2018) 47–53. https://doi.org/10.24075/brsmu.2018.027.
[18] L. Antonioli, M. Fornai, C. Blandizzi, P. Pacher, G. Haskó, Adenosine signaling and the immune system: when a lot could be too much, Immunol. Lett. 205 (2019) 9–15, https://doi.org/10.1016/j.imlet.2018.04.006.
[19] D. Liao, R.S. Johnson, Hypoxia: a key regulator of angiogenesis in cancer, Cancer Metastasis Rev. 26 (2) (2007) 281–290, https://doi.org/10.1007/s10555-007-9066-y.

[20] A.A. Ohta, Metabolic immune checkpoint: adenosine in tumor microenvironment, Front. Immunol. 7 (2016) 109, https://doi.org/10.3389/fimmu.2016.00109.
[21] A. Ohta, M. Sitkovsky, The hypoxia-adenosinergic immunosuppression and redirection of immune response in tumor microenvironmentTumor-Induced Immune Suppression, Springer, New York, 2014, DOI: 10.1007/978-0-387-69118-3.
[22] S.M. Hatfield, J. Kjaergaard, D. Lukashev, T.H. Schreiber, B. Belikoff, R. Abbott, et al., Immunological mechanisms of the antitumor effects of supplemental oxygenation, Sci Transl. Med. 7 (2015) aaa1260, https://doi.org/10.1126/scitranslmed.aaa1260.
[23] L. Antonioli, P. Pacher, E.S. Vizi, G. Haskó, CD39 and CD73 in immunity and inflammation, Trends Mol. Med. 19 (6) (2013) 355–367, https://doi.org/10.1016/j.molmed.2013.03.005.
[24] A. Clayton, S. Al-Taei, J. Webber, M.D. Mason, Z. Tabi, Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production, J.I. 187 (2) (2011) 676–683, https://doi.org/10.4049/jimmunol.1003884.
[25] Y.J. Wo, A.S.P. Gan, X. Lim, I.S.Y. Tay, S. Lim, J.C.T. Lim, J.P.S. Yeong, The roles of CD38 and CD157 in the solid tumor microenvironment and cancer immunotherapy, Cells. 1 (2019) 26, https://doi.org/10.3390/cells9010026.
[26] F. Morandi, B. Morandi, A.L. Horenstein, A. Chillemi, V. Quarona, G. Zaccarello, P. Carrega, G. Ferlazzo, M.C. Mingari, L. Moretta, V. Pistoia, F. Malavasi, A noncanonical adenosinergic pathway led by CD38 in human melanoma cells induces suppression of T cell proliferation, Oncotarget 6 (28) (2015) 25602–25618. https://doi.org/10.18632/oncotarget.4693.
[27] B.M. Künzli, P.O. Berberat, T. Giese, E. Csizmadia, E. Kaczmarek, C. Baker, I. Halaceli, M.W. Büchler, H. Friess, S.C. Robson, Upregulation of CD39/NTPDases and P2 receptors in human pancreatic disease, Am. J. Physiol.-Gastrointest. Liver Physiol. 292 (1) (2007) G223–G230, https://doi.org/10.1152/ajpgi.00259.2006.
[28] X.-Y. Cai, X.-C. Ni, Y. Yi, H.-W. He, J.-X. Wang, Y.-P. Fu, J. Sun, J. Zhou, Y.-F. Cheng, J.-J. Jin, J. Fan, S.-J. Qiu, Overexpression of CD39 in hepatocellular carcinoma is an independent indicator of poor outcome after radical resection, Medicine 95 (40) (2016) e4989, https://doi.org/10.1097/MD.0000000000004989.
[29] X. Cai, X. Wang, J. Li, J. Dong, J. Liu, N. Li, et al., High expression of CD39 in gastric cancer reduces patient outcome following radical resection, Oncol. Lett. 12 (2016) 4080–4086, https://doi.org/10.3892/ol.2016.5189.
[30] Y. Qi, Y. Xia, Z. Lin, Y. Qu, Y. Qi, Y. Chen, Q. Zhou, H. Zeng, J. Wang, Y. Chang, Q. Bai, Y. Wang, Y. Zhu, L. Xu, L. Chen, Y. Kong, W. Zhang, B. Dai, L. Liu, J. Guo, J. Xu, Tumor-infiltrating CD39+CD8+ T cells determine poor prognosis and immune evasion in clear cell renal cell carcinoma patients, Cancer Immunol. Immunother. 69 (8) (2020) 1565–1576, https://doi.org/10.1007/s00262-020-02563-2.
[31] M.K. Koivisto, M. Tervahartiala, I. Kenessey, S. Jalkanen, P.J. Boström, Marko Salmi, Cell-type-specific CD73 expression is an independent prognostic factor in bladder cancer, Carcinogenesis 40 (2019) 84–92.
[32] S. Loi, S. Pommey, B. Haibe-Kains, P.A. Beavis, P.K. Darcy, M.J. Smyth, J. Stagg, CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer, Proc. Natl. Acad. Sci. 110 (27) (2013) 11091–11096, https://doi.org/10.1073/pnas.1222251110.
[33] Z.-H. Ren, C.-Z. Lin, W. Cao, R. Yang, W. Lu, Z.-Q. Liu, Y.-M. Chen, X.I. Yang, Z. Tian, L.-Z. Wang, J. Li, X. Wang, W.-T. Chen, T. Ji, C.-P. Zhang, CD73 is associated with poor prognosis in HNSCC, Oncotarget 7 (38) (2016) 61690–61702.
[34] J. Wang, S. Matosevic, NT5E/CD73 as correlative factor of patient survival and natural killer cell infiltration in glioblastoma, J. Clin. Med. 8 (2019) 1526, https://doi.org/10.3390/jcm8101526.
[35] X.-R. Wu, X.-S. He, Y.-F. Chen, R.-X. Yuan, Y. Zeng, L. Lian, Y.-F. Zou, N. Lan, X.-J. Wu, P. Lan, High expression of CD73 as a poor prognostic biomarker in human colorectal cancer: CD73 and Prognosis of Colorectal Cancer, J. Surg. Oncol. 106 (2) (2012) 130–137, https://doi.org/10.1002/jso.23056.
[36] G. Haskó, L. Antonioli, B.N. Cronstein, Adenosine metabolism, immunity and joint health, Biochem. Pharmacol. 151 (2018) 307–313, https://doi.org/10.1016/j.bcp.2018.02.002.
[37] B. Allard, D. Allard, L. Buisseret, J. Stagg, The adenosine pathway in immuno-oncology, Nat. Rev. Clin. Oncol. 10 (2020) 611–629, https://doi.org/10.1038/s41571-020-0382-2.
[38] A. Ohta, E. Gorelik, S.J. Prasad, F. Ronchese, D. Lukashev, M.K.K. Wong, X. Huang, S. Caldwell, K. Liu, P. Smith, J.-F. Chen, E.K. Jackson, S. Apasov, S. Abrams, M. Sitkovsky, A2A adenosine receptor protects tumors from antitumor T cells, Proc. Natl. Acad. Sci. 103 (35) (2006) 13132–13137, https://doi.org/10.1073/pnas.0605251103.
[39] A. Ohta, A. Ohta, M. Madasu, R. Kini, M. Subramanian, N. Goel, M. Sitkovsky, A2A adenosine receptor may allow expansion of T cells lacking effector functions in extracellular adenosine-rich microenvironments, J. Immunol. 183 (9) (2009) 5487–5493, https://doi.org/10.4049/jimmunol.0901247.
[40] S.-R. Ma, W.-W. Deng, J.-F. Liu, L. Mao, G.-T. Yu, L.-L. Bu, A.B. Kulkarni, W.-F. Zhang, Z.-J. Sun, Blockade of adenosine A2A receptor enhances CD8+ T cells response and decreases regulatory T cells in head and neck squamous cell carcinoma, Mol Cancer 16 (1) (2017), https://doi.org/10.1186/s12943-017-0665-0.
[41] M. Mediavilla-Varela, K. Luddy, D. Noyes, F.K. Khalil, A.M. Neuger, H. Soliman, S.J. Antonia, Antagonism of adenosine A2A receptor expressed by lung adenocarcinoma tumor cells and cancer associated fibroblasts inhibits their growth, Cancer Biol. Ther. 14 (9) (2013) 860–868, https://doi.org/10.4161/cbt.25643.
[42] K. Sek, C. Mølck, G.D. Stewart, L. Kats, P.K. Darcy, P.A. Beavis, Targeting adenosine receptor signaling in cancer immunotherapy, Int. J. Mol. Sci. 19 (2018) 3837, https://doi.org/10.3390/ijms19123837.
[43] C. Sorrentino, L. Miele, A. Porta, A. Pinto, S. Morello, Myeloid-derived suppressor cells contribute to A2B adenosine receptor-induced VEGF production and angiogenesis in a mouse melanoma model, Oncotarget 6 (29) (2015) 27478–27489. https://doi.org/10.18632/oncotarget.4393.
[44] A. Ohta, R. Kini, A. Ohta, M. Subramanian, M. Madasu, M. Sitkovsky, The development and immunosuppressive functions of CD4(+)CD25(+)FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway, Front. Immunol. 3 (2012) 190, https://doi.org/10.3389/fimmu.2012.00190.
[45] C. Cekic, Y.-J. Day, D. Sag, J. Linden, Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment, Cancer Res. 74 (24) (2014) 7250–7259, https://doi.org/10.1158/0008-5472.CAN-13-3583.
[46] P. Vaupel, A. Mayer, Hypoxia-driven adenosine accumulation: a crucial microenvironmental factor promoting tumor progression, in: C.E. Elwell, T.S. Leung, D.K. Harrison (Eds.), Oxygen Transport to Tissue XXXVII. Adv. Exp. Med. Biol., Springer, New York, NY, 2016, p. 876, DOI.org/10.1007/978-1-4939-3023-4_22.
[47] S.V. Novitskiy, S. Ryzhov, R. Zaynagetdinov, A.E. Goldstein, Y. Huang, O.Y. Tikhomirov, et al., Adenosine receptors in regulation of dendritic cell differentiation and function, Blood 112 (2008) 1822–1831.
[48] S. Chen, I. Akdemir, J. Fan, J. Linden, B. Zhang, C. Cekic, The expression of adenosine A2B receptor on antigen-presenting cells suppresses CD8+ T-cell responses and promotes tumor growth, Cancer Immunol. Res. 8 (8) (2020) 1064–1074, https://doi.org/10.1158/2326-6066.CIR-19-0833.
[49] D. Mittal, D. Sinha, D. Barkauskas, A. Young, M. Kalimutho, K. Stannard, F. Caramia, B. Haibe-Kains, J. Stagg, K.K. Khanna, S. Loi, M.J. Smyth, Adenosine 2B receptor expression on cancer cells promotes metastasis, Cancer Res. 76 (15) (2016) 4372–4382, https://doi.org/10.1158/0008-5472.CAN-16-0544.
[50] S. Gessi, S. Bencivenni, E. Battistello, F. Vincenzi, V. Colotta, D. Catarzi, et al., Inhibition of A2A adenosine receptor signaling in cancer cells proliferation by the novel antagonist TP455, Front. Pharmacol. 8 (2017) 1–13, https://doi.org/10.3389/fphar.2017.00888.
[51] Y. Zhou, X. Chu, F. Deng, L. Tong, G. Tong, Y. Yi, J. Liu, J. Tang, Y. Tang, Y. Xia, Y. Dai, The adenosine A2b receptor promotes tumor progression of bladder urothelial carcinoma by enhancing MAPK signaling pathway, Oncotarget 8 (30) (2017) 48755–48768.
[52] S. Deaglio, K.M. Dwyer, W. Gao, et al., Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression, J. Exp. Med. 204 (2007) 1257–1265, https://doi.org/10.1084/jem.20062512.
[53] P.B. Ernst, J.C. Garrison, L.F. Thompson, Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology, J. Immunol. 185 (2010) 1993–1998.
[54] G. Borsellino et al., Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression, Blood 110 (2007) 1225–1232.
[55] I. Shevchenko, A. Mathes, C. Groth, S. Karakhanova, V. Müller, J. Utikal, J. Werner, A.V. Bazhin, V. Umansky, Enhanced expression of CD39 and CD73 on T cells in the regulation of anti-tumor immune responses, OncoImmunology 9 (1) (2020) 1744946, https://doi.org/10.1080/2162402X.2020.1744946.
[56] P.J. Schuler, Z. Saze, C.-S. Hong, L. Muller, D.G. Gillespie, D. Cheng, et al., Human CD4+CD39+ regulatory T cells produce adenosine upon co-expression of surface CD73 or contact with CD73+ exosomes or CD73+ cells, Clin. Exp. Immunol. 177 (2014) 531–543, https://doi.org/10.1111/cei.12354.
[57] P.J. Schuler, B. Schilling, M. Harasymczuk, T.K. Hoffmann, J. Johnson, S. Lang, T. L. Whiteside, Phenotypic and functional characteristics of CD4+CD39+FOXP3+ and CD4+CD39+FOXP3neg T-cell subsets in cancer patients: cellular immune response, Eur. J. Immunol. 42 (7) (2012) 1876–1885, https://doi.org/10.1002/eji.201142347.
[58] X. Sun, Y. Wu, W. Gao, K. Enjyoji, E. Csizmadia, C.E. Müller, T. Murakami, S.C. Robson, CD39/ENTPD1 Expression by CD4+Foxp3+ Regulatory T Cells Promotes Hepatic Metastatic Tumor Growth in Mice, Gastroenterology 139 (3) (2010) 1030–1040, https://doi.org/10.1053/j.gastro.2010.05.007.
[59] C.M. Hay, E. Sult, Q. Huang, K. Mulgrew, S.R. Fuhrmann, K.A. McGlinchey, S.A. Hammond, R. Rothstein, J. Rios-Doria, E. Poon, N. Holoweckyj, N.M. Durham, C.C. Leow, G. Diedrich, M. Damschroder, R. Herbst, R.E. Hollingsworth, K.F. Sachsenmeier, Targeting CD73 in the tumor microenvironment with MEDI9447, OncoImmunology 5 (8) (2016), https://doi.org/10.1080/2162402X.2016.1208875.
[60] J.C. Geoghegan, G. Diedrich, X. Lu, K. Rosenthal, K.F. Sachsenmeier, H. Wu, W. F. Dall'Acqua, M.M. Damschroder, Inhibition of CD73 AMP hydrolysis by atherapeutic antibody with a dual, non-competitive mechanism of action, mAbs 8 (3) (2016) 454–467, https://doi.org/10.1080/19420862.2016.1143182.
[61] MEDI9447 Alone and in Combination With MEDI4736 in Adult Subjects With Select Advanced Solid Tumors. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT02503774.
[62] A Phase I Study of MEDI9447 (Oleclumab) in Japanese Patients. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03736473.

[63] A Study of Novel Anti-cancer Agents in Patients With Metastatic Triple Negative Breast Cancer (BEGONIA) https://clinicaltrials.gov/ct2/show/NCT03742102.
[64] An Investigational Immuno-therapy Study of Experimental Medication BMS-986179 Given Alone and in Combination With Nivolumab. (accessed 19 september 2020) https://clinicaltrials.gov/ct2/show/NCT02754141.
[65] Durvalumab Alone or in Combination With Novel Agents in Subjects With NSCLC (COAST). (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03822351.
[66] CPI-006 Alone and in Combination With Ciforadenant and With Pembrolizumab for Patients With Advanced Cancers. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03454451.
[67] A Phase I/Ib Study of NZV930 Alone and in Combination With PDR001 and /or NIR178 in Patients With Advanced Malignancies. (accessed 16 september 2020) https://clinicaltrials.gov/ct2/show/NCT03549000.
[68] Study of GS-1423 in Participants With Advanced Solid Tumors. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03954704.
[69] A Phase I/II Study of TJ004309 for Advanced Solid Tumor. (accessed 16 september 2020) https://clinicaltrials.gov/ct2/show/NCT04322006.
[70] Study of TJ004309 in Combination With Atezolizumab (Tecentriq®) in Patients With Advanced or Metastatic Cancer. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03835949.
[71] A Study to Investigate the Safety of AB680 in Healthy Volunteers. (accessed 16 september 2020) https://clinicaltrials.gov/ct2/show/NCT03677973.
[72] TTX-030 Single Agent and in Combination With Immunotherapy or Chemotherapy for Patients With Advanced Cancers. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT03884556.
[73] TTX-030 in Combination With Immunotherapy and/or Chemotherapy in Subjects With Advanced Cancers. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT04306900.
[74] IPH5201 as Monotherapy or in Combination With Durvalumab +/- Oleclumab in Subjects With Advanced Solid Tumors. (accessed 19 september 2020) https://clinicaltrials.gov/ct2/show/NCT04261075.
[75] Study of SRF617 in Patients With Advanced Solid Tumors. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT04336098.
[76] Phase 1/1b Study to Evaluate the Safety and Tolerability of Ciforadenant Alone and in Combination With Atezolizumab in Advanced Cancers. (accessed 19 september 2020) https://clinicaltrials.gov/ct2/show/NCT02655822.
[77] Study of Ciforadenant in Combination With Daratumumab in Patients With Relapsed or Refractory Multiple Myeloma. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT04280328.
[78] A Study Of Multiple Immunotherapy-Based Treatment Combinations In Participants With Metastatic Non-Small Cell Lung Cancer (Morpheus-Non-Small Cell Lung Cancer) (Morpheus Lung). (accessed 19 september 2020) https://clinicaltrials.gov/ct2/show/NCT03337698.
[79] An Open-label, Phase II Study of AZD4635 in Patients With Prostate Cancer. (accessed 15 september 2020) https://clinicaltrials.gov/ct2/show/NCT04089553.
[80] Study of Preladenant (MK-3814) Alone and With Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-3814A-062). (accessed 17 september 2020) https://clinicaltrials.gov/ct2/show/study/NCT03099161.
[81] Trial of PBF-509 and PDR001 in Patients With Advanced Non-small Cell Lung Cancer (NSCLC) (AdenONCO). (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT02403193.
[82] PBF-1129 in Patients With NSCLC. (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT03274479.
[83] A Study to Evaluate Immunotherapy Combinations in Participants With Gastrointestinal Malignancies. (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT03720678.
[84] Study to Evaluate Monotherapy and Combination Immunotherapies in Participants With PD-L1 Positive Non-small Cell Lung Cancer (ARC-7). (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT04262856.
[85] Daratumumab (HuMax®-CD38) Safety Study in Multiple Myeloma - Open Label, Dose-escalation Followed by Open Label, Single-arm Study. https://clinicaltrials.gov/ct2/show/NCT00574288 (accessed 7 december 2020).
[86] A Phase I/2 Dose Escalation Safety, Pharmacokinetic and Efficacy Study of Multiple Intravenous Administrations of a Humanized Monoclonal Antibody (SAR650984) Against CD38 in Patients With Selected CD38+ Hematological Malignancies. https://clinicaltrials.gov/ct2/show/NCT01084252 (accessed 7 december 2020).
[87] First in Man Study With MEN1112, a CD157 Targeted Monoclonal Antibody, in Relapsed or Refractory Acute Myeloid Leukemia. (accessed 7 december 2020) https://clinicaltrials.gov/ct2/show/NCT02353143.
[88] L.L. Siu, H. Burris, D.T. Le, A. Hollebecque, N. Steeghs, Jean-Pierre Delord, et al. Abstract CT180: Preliminary phase 1 profile of BMS-986179, an anti-CD73 antibody, in combination with nivolumab in patients with advanced solid tumors, Cancer Res. 78 (2018) CT180; DOI: 10.1158/1538-7445.AM2018-CT180
[89] N.S. Wilson, et al., Anti-CD73 Antibodies and Methods of Use Thereof. U. S. Patent Appl. No. US20190352418A1, Nov 21, 2019.
[90] C.E. Bowman, R.G. da Silva, A. Pham, S.W. Young, An Exceptionally Potent Inhibitor of Human CD73, Biochemistry 58 (31) (2019) 3331–3334, https://doi.org/10.1021/acs.biochem.9b00448.s001.
[91] Elpiscience pipeline, ES002. (accessed 18 september 2020) http://www.elpiscience.com/index.php?c=article&a=type&tid=436.
[92] Y. Qiu et al., The antitumor activity of an anti-CD39 antibody (ES002) in a multiple myeloma model is dependent on NK cells (Poster 790), J. ImmunoTher. Cancer 7 (2019) 283.
[93] A.S. Kashyap, T. Thelemann, R. Klar, S.M. Kallert, J. Festag, M. Buchi, L. Hinterwimmer, M. Schell, S. Michel, F. Jaschinski, A. Zippelius, Antisense oligonucleotide targeting CD39 improves anti-tumor T cell immunity, J. Immunotherapy Cancer 7 (1) (2019), https://doi.org/10.1186/s40425-019-0545-9.
[94] A Study in Healthy Male Volunteers to Investigate a New Drug for the Treatment of Parkinson's Disease. (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT02764892.
[95] Study to Assess the Safety, Tolerability and Pharmacokinetic Profile of PBF-509 (80 mg, 160 mg and 240 mg) "After Multiple Oral Doses" in Healthy Volunteers. (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT02111330.
[96] Study To Assess the Safety and Tolerability of PBF-509 in Male Healthy Volunteers. (accessed 18 september 2020).
[97] R.D. Leone, L.A. Emens, Targeting adenosine for cancer immunotherapy, J. Immunotherapy Cancer 6 (1) (2018), https://doi.org/10.1186/s40425-018-0360-8.
[98] A Study to Evaluate Safety/Tolerability of Immunotherapy Combinations in Participants With Triple-Negative Breast Cancer or Gynecologic Malignancies. (accessed 18 september 2020) https://clinicaltrials.gov/ct2/show/NCT03719326.
[99] Etrumadenant (AB928): the first and only dual adenosine receptor antagonist targeting A2AR and A2BR. (accessed 18 september 2020) https://www.arcusbio.com/etrumadenant/.
[100] A.W. Czura, C.J. Czura, CD38 and CD157: biological observations to clinical therapeutic targets, Mol. Med. 12 (2006) 309–311, https://doi.org/10.2119/2007-00006.Czura.
[101] X. Feng, L. Zhang, C. Acharya, G. An, K. Wen, L. Qiu, et al., Targeting CD38 Suppresses Induction and Function of T Regulatory Cells to Mitigate Immunosuppression in Multiple Myeloma, Clin. Cancer Res. 15 (2017) 4290–4300, https://doi.org/10.1158/1078-0432.CCR-16-3192.
[102] A. Kitadate, H. Kobayashi, Y. Abe, K. Narita, D. Miura, M. Takeuchi, et al., Pre-treatment CD38-positive regulatory T cells affect the durable response to daratumumab in relapsed/refractory multiple myeloma patients, Haematologica 105 (2020) e37–e40, https://doi.org/10.3324/haematol.2019.219683.