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CD73 in glioblastoma: Where are we now and what are the future directions?

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ABSTRACT

Glioblastoma (GB) is the most aggressive type of brain tumor with heterogeneity, strong invasive ability, and high resistance to therapy due to immunosuppressive mechanisms. CD73 is an overexpressed enzyme in GB that acts via two main mechanisms: (1) CD73 acts as an adhesion protein independent of the enzymatic activity or (2) via the catalyses of AMP to adenosine (ADO) generating a strong modulatory molecule that induces alterations in the tumor cells and in the tumor microenvironment cells (TME). Taken together, CD73 is receiving attention during the last years and studies demonstrated its dual potential benefit as a target to GB therapy. Here, we review the roles of CD73 and P1 receptors (ADO receptors) in GB, the impact of CD73 in the immune interactions between tumor and other immune cells, the proposed therapeutic strategies based on CD73 regulation, and discuss the gap in knowledge and further directions to bring this approach from preclinical to clinical use.

1. Introduction

Glioblastomas (GB; grade 4 glioma) are among the most challenging malignancies to treat, largely owing to (a) the often inoperable nature of the tumors and its infiltrative behavior, (b) the intrinsic or acquired chemoresistance, (c) the difficulties of drug delivery imposed by blood-brain barrier (BBB), (d) tumor heterogeneity, and (e) the pro-tumorigenic influence of the tumor microenvironment (TME) [1,2]. Currently, there are only limited treatment options beyond maximal safe tumor resection followed by radio- and/or chemotherapy with temozolomide, GB usually follows an aggressive course which results in morbidity and mortality, and a median survival of 12–15 months for patients after diagnosis [3,4]. There is a clear need for the development of innovative treatment strategies. However, decades of clinical trials failed in improve this devasting outcome [5,6].

To determine the success or failure of a drug candidate, the selection of an appropriate target proves to be a crucial step. The ideal target mechanism should play a key role in the vital molecular processes involved in cancer progression [7]. It could both directly impairs the tumor cell proliferation or targets the tumor-associated non-transformed cells that support tumor growth. In the last 10 years, the purinergic signaling emerged as an important pathway to be target in GB, specially the adenosinergic pathway [8–10].

Adenosine (ADO) levels are mutable and can be affected in the TME by CD73 overexpression, hypoxia, or stress, raising from physiological (1–50 nM) to pathological (1000 nM) levels [11]. GB is characterized by extensive hypoxia areas, which exhibit increased ADO levels [12]. ADO, in a physiological context, has been recognized to mediate immunomodulatory/immunosuppressive responses to protect adjacent tissues from inflammation [13]. However, in a pathological context, this nucleoside has been reported as a mediator of tumor cell proliferation, angiogenesis, and immunosuppression placing the adenosinergic pathway as a key tumor-promoter and a interesting candidate for immunotherapy [14].

Ecto-5′-nucleotidase/CD73 (CD73) is a protein bounded to the outer surface of plasma membrane by a glycosylphosphatidyl inositol (GPI) anchor and it is co-localized within lipid rafts. It hydrolyzes monophosphate nucleosides released by a variety of cell types in response to stress signals, such as injury, hypoxia and inflammatory conditions present in the TME. Moreover, it plays a crucial role in tumor

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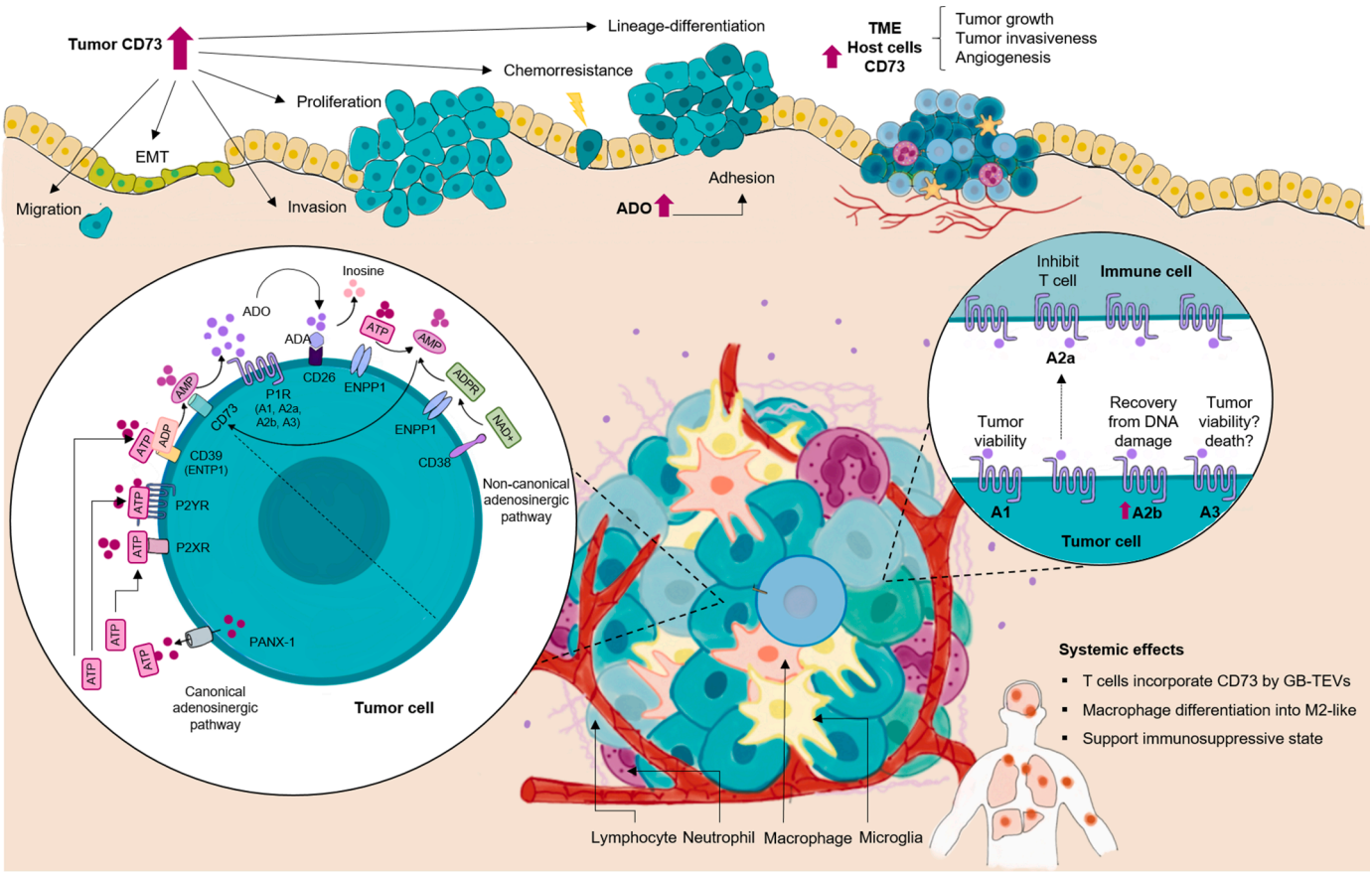

Fig. 1. CD73-associated roles in GB. CD73 is widely expressed in tumors and its presence is associated with migration, EMT, invasion, proliferation, chemoresistance, lineage-differentiation, tumor growth, angiogenesis, and invasiveness. CD73 is present in tumor microenvironment, mainly in GB cells, and connects canonical, from CD39-CD73 (left side), and non-canonical adenosinergic pathway, composed by CD38-NPP1-CD73 (right side). ADO, the resultant product of CD73 catalysis, increases cell adhesion and interacts with P1 receptors. The expression of P1 receptors is also associated with distinct functions: A1 increases cell viability, A2A was associated with inhibitory effects on T cells and A2B was associated with recovery of DNA damage. A3 has controversial effects while some studies point to tumor viability while others associated the augmented expression with cell death. Regarding systemic effect, GB can liberate TEVs, containing CD73 that can be incorporated by T cells and ADO that helps to support the immunosuppressive status, with markedly induction of macrophages to M2-*like* polarization.
EMT: epithelial-mesenchymal transition; ADO: adenosine; GB: glioblastoma; TEVs: tumor extracellular vesicles; TME: tumor microenvironment, ENPP-1: Ecto-nucleotide pyrophosphatase/phosphodiesterase 1; ATP: adenosine triphosphate, ADP: adenosine diphosphate; AMP: adenosine monophosphate; CD73: ecto-5′-nucleotidase; CD39: NTPDase 1; PANX-1: pannexin 1; P2X/P2Y: Purinoceptors; P1: ADO receptors.

progression and recurrence by controlling extracellular nucleotide/nucleoside levels and the further sensitization of purinergic receptors located at cancer as well as non-transformed cells that compose TME, for instance tumor associated macrophages/microglia and lymphocytes [15–17].

ADO production from the substrate AMP is processed primarily by CD73, the most important mechanism of extracellular ADO source. In this instance, CD73 has demonstrated *in vitro* and *in vivo* enzymatic and non-enzymatic functions in GB progression [18]. Notably, extracellular ADO production by CD73 enzyme activity plays an important role in cancer immunosuppression, angiogenesis, migration/invasion, cell proliferation and chemoresistance via P1 purinoceptor sensitization [12,16,19,20]. Moreover, it is involved in tumor cell-extracellular matrix interactions which may favor tumor invasion and metastasis [21,22].

This review aims to discuss the relationship between CD73 and GB malignity, showing what is already known for CD73 in GB, and discussing future perspectives.

2. General aspects of purinergic signaling

The extracellular metabolism of ATP to ADO is performed by ecto-nucleotidases. The hydrolysis of ATP to AMP could be done by a family of enzymes called ecto-nucleoside triphosphate diphosphatehydrolase (NTPDase) *e.g.*, NTPDase1/CD39, while CD73 catalyzes the conversion of AMP to ADO. Different mechanisms including passive diffusion and active intracellular transport contribute to extracellular ADO levels. However, one mechanism that has been drawing attention is through enzymatic hydrolysis of extracellular ATP via CD39-CD73 [17]. On the other hand, ADO can be formed by non-canonical pathway: from NAD+ conversion through CD38, followed by the conversion of ADPR by Ecto-nucleotide pyrophosphatase/phosphodiesterase 1(NPP-1) generating AMP that will be converted to ADO by CD73, connecting both pathways [16,23].

ADO-mediated effects occur via P1 purinoceptors sensitization. There are four distinct G protein-coupled receptors: A1, A2A, A2B, and A3. A1 and A3 are Gi-coupled described as inhibitory receptors since they reduce the intracellular cyclic AMP (cAMP) levels by inhibiting the activity of adenylyl cyclase. On the other hand, A2A and A2B are usually considered as excitatory receptors due to its ability to increase intracellular cAMP levels by activating adenylyl cyclase. Alternatively, A2B also can be Gq-coupled, increasing intracellular calcium via PLC-β cascade [24].

The P1 receptors are differentially sensitized by ADO depending on its extracellular levels. For instance, at lowest levels, ADO interacts preferentially with A1 receptor, while at higher levels, it favorably binds to A2A and A3. A2B usually is activated in pathological situations due to

its lowest affinity for ADO when compared with the other receptors [25].

P1 receptors are widely expressed in the body, especially in the central nervous system, cardiovascular, immune, and renal systems. Regarding immune activation, A1 receptors are expressed in neutrophils and immature dendritic cells (DCs) and are involved with neutrophil chemotaxis [26,27]. A2A is expressed in most immune cells and platelets promoting immunosuppressive/immunomodulatory responses in immune cells and preventing platelet aggregation [27]. A2B is expressed in macrophages, DCs and mast cells promoting IL-6 and VEGF release by macrophages and DCs and driving mast cell degranulation [28]. Finally, A3 is also expressed in neutrophils and mast cells, reducing the neutrophil chemotaxis and stimulating mast cells degranulation [27].

3. CD73 roles in GB

CD73 is an important factor in GB pathogenesis, due to its high expression in tumor cells, acting as the main enzyme source of ADO in GB TME [19]. On the other hand, CD39 is less expressed in tumor cells, therefore non-tumor cells in TME are the main source, especially microglia [18,29,30]. Multiple roles are associated with the CD73 expression as a membrane protein and its catalytic activity including effects in adhesion, cell growth, epithelial-mesenchymal transition (EMT) process, invasion, and others [12,21,31] (Fig. 1).

In vitro experiments have elucidated the roles of CD73 in GB. In terms of adhesion, ADO is suggested as a key player [21]. CD73 mediates adhesion in lymphocytes and is important to efficient entry of lymphocytes into the CNS during experimental autoimmune encephalomyelitis [32,33]. In GB context, Cappellari et al. [21] evaluated the roles of ADO in the U138MG human GB cell line and found a great importance in tumor cell adhesion. The study reveals that ADO increased 40% the tumor cell adhesion, which was impaired by the pharmacological enzyme CD73 inhibitor (APCP). Moreover, tumor aggressiveness is also described to be associated with CD73 expression [34]. Indeed, Azambuja et al. [18] demonstrated that ADO promoted an increase in GB cell viability, which was mediated by A1R sensitization. In line with the participation of CD73 as extracellular source of ADO, CD73 knockdown or its pharmacological inhibition decreased GB cell proliferation, migration, and invasion by reducing metalloproteinase-2 and vimentin expression. CD73 blockage also potentiated temozolomide (TMZ) cytotoxic effect on GB. Interestingly, CD73 silencing upregulated p-Akt and NF-kB-p65, which are two well known pathways related with GB progression. This effect was ADO-independent and further experiments are needed to better understand the effect of CD73 expression on Akt/NFkB signaling in GB. Furthermore, CD73 inhibition abrogated *in vivo* tumor growth in a preclinical GB model. The reduction of GB progression was parallel to the decrease of ADO levels in the cerebrospinal fluid [12].

Additionally, the role of CD73 activity in tumor cell migration was described by Kitabatake et al. [35] in CD73-overexpressing A172 GB human cell line. CD73 also regulates the expression of other genes, such as SNAIL-1, an activator of EMT [31], and MRP-1 - a multidrug-resistant protein that acts as a pump for the removal of different metabolites and xenobiotics, including antitumor drugs [36]. Taken together, all these studies have demonstrated the essential role of CD73 expression in GB progression, since its overexpression in cancer cells is deeply related to tumor adhesion, migration, invasion, and proliferation. As aforementioned discussed, GB cells widely overexpress CD73 when compared to normal brain parenchyma; however, other sources of this enzyme are available in the TME. Indeed, GB TME host cells, particularly the immunosuppressive-associated tumor cells, also express CD73 and play a role in cancer pathogenesis, supporting tumor growth, angiogenesis and invasiveness [37,38]. In addition, mesenchymal stem cells (MSCs) were investigated by Shahar et al. [39] as a prediction factor to overall survival, indicating worse prognosis when present in GB patients.

Analysis of patient data confirmed the preclinical findings, showing a huge effect of CD73 on patients survival rate [15,40]. *In silico* analysis performed by Wang and Matosevic [40] have shown an increased CD73 expression in all GB molecular subtypes and a closer relationship between NT5E overexpression with shorter disease-free survival. Xu et al. [41] study endorses these findings. CD73 downregulation has a positive prognostic factor related to the extended disease-free survival in GB patients. Recent studies have been investigating the crosstalk among non-transformed cells that compose TME [42]. Indeed, extracellular ADO levels increase proportionally with the interaction of non-transformed cells, driving to poor clinical outcomes. Furthermore, CD73 expression was associated with genotype, lineage-differentiation, and dynamic functional states such as hypoxia in high-grade gliomas [30]. In this regard, Alarcón et al. [43] evaluated the most aggressive types of GB stem cells, identifying that mesenchymal (MES GSC) exhibits higher extracellular ADO levels when compared to proneural (PN GSC), which was related to the decreased expression and activity of ENT1 transporter. All together, these studies indicate a strong direct association between CD73 expression with poor prognosis in GB patients. Besides, the CD73 expression/ activity is present not only in tumor, but also in non-transformed immunosuppressive cells from the TME, which proves to be an interesting target for immunotherapies.

4. ADO receptors and nucleoside transporters as protumorigenesis players

CD73 expression is closely related to the amount of ADO in TME [18,30,44]. In this regard, ADO receptors and nucleoside transporters contribute to tumor outcome. The accumulation of ADO in the GB TME led to activation of P1 receptors: A1, A2A, A2B and A3. A1 receptor is the most sensitive receptor and it was related to cell viability *in vitro* experiments [18].

A2A receptor seems important in the modulation of immune cells. Wang et al. [45] studied the effects of CD73-positive tumor-derived extracellular vesicles *in vitro* experiments. They demonstrated that these vesicles have the capacity to inhibit T-cell aerobic glycolysis, cell cycle entry and clonal proliferation systemically in an interaction-dependent process with A2A, sustaining an immunosuppressive TME and contributing to tumor progression. In addition to tumor expansion, the activation of A2B was associated with recovery from DNA damage γ-radiation-induced, enhanced cell migration and actin remodeling [35]. Yan et al. [46] found in their experiments a 20-fold increase in A2B expression on GB compared to control group. Therefore, inhibiting A2B could be an interesting approach for GB therapy. Preliminary studies have demonstrated that the inhibition of A2B suppressed γ-radiation-induced DDR and promoted γ-radiation-induced cell death [35] and increased GB chemosensitivity to TMZ [46].

Although P1 receptors commonly are associated to protumorigenesis functions, A3 effects are controversial. While Ceruti et al. [47] found that A3 antagonism induces *in vitro* apoptosis and impaired the clonogenicity of GB stem cell, Azambuja et al. reported that A3 agonism mediates GB cell death [18] and Mendes et al. [48] demonstrated *in vitro* toxicity effect of α-bisabolol with the increase of A3 expression.

Another way to control extracellular ADO concentrations are concentrative nucleoside transporters (CNT) and equilibrative nucleoside transporters (ENT). Alarcón et al. [43] studied these transporters and found that CNT-mediated ADO transport corresponds to a smaller fraction of ADO transport in glioma stem cell subtypes. To the best of our knowledge, there are no studies comparing the ADO signaling from different receptors and transporters in GB (P1 receptors and CNT/ENT).

This section shows how P1 receptors and ADO transporters contribute to cancer advancement. Furthermore, these receptors are proposed as a potential target for GB management.

5. GB-immune cell interaction mediated by CD73/ADO signaling: new strategies

Immune infiltration is considered a hallmark of cancer, especially in

GB [49]. Therefore, the immune composition in TME can support the immunosuppressive state [50]. GB tumors can be described as a cold tumor since their configuration involves a great number of myeloid and regulatory cells, and do not respond to classical immunotherapy [1,38]. In this regard, Xu et al. [41] investigated the ectonucleotidase characteristics of GBs and infiltrating $CD4^+$T lymphocytes isolated from newly diagnosed malignant glioma patients. They identify that ADO formation is dependent on CD39 derived from T lymphocytes and CD73 from GB cells that act synergistically, resulting in suppression of responder $CD4^+$T-cell proliferation.

Moreover, recent studies have demonstrated that therapies affect not only GB cells but also associated-immune populations. Gonçalves et al. [51] performed *in vitro* experiments using cisplatin on U87-MG and LN-18 cell lines. The results indicate a loss of $CD146^+/CD73^+$ MSC cell subpopulations after treatment of U87MG cultures, while LN-18 exhibited $CD73^+/CD90^+$ MSC subpopulations increase. Figueiró et al. [44] evaluated the treatment of Methotrexate (MTX) in C6 cell line and found that MTX treatment enhances CD73 expression/activity with consequent ADO accumulation and establishment of an immunosuppressive state. This condition was further confirmed by an increased CD39 expression on $CD8^+$ T lymphocytes, followed by a rise in CD73 expression on both $CD4^+$ T and $CD8^+$T lymphocytes. Besides, the MTX treatment decreased the frequencies of $CD4^+$T, $CD8^+$T, and Treg lymphocytes in the TME.

In an attempt to use CD73 as a therapeutic target, Azambuja et al. [20] used a nanoemulsion containing siRNA to block CD73 activity/expression in a GB preclinical model. The treatment was performed using nasal route and impaired the *in vivo* GB growth via apoptosis. Furthermore, the treatment promoted a reduction of Tregs, microglia, and macrophages population in TME, followed by increased expression of IL-6, CCL17, and CCL22 in the tumor tissue. Therefore, CD73 expression decreased in the GB cells as well as in the tumor-associated macrophages/microglia, re-establishing an immune inflammatory TME.

Regarding human samples, Goswami et al. [34] have identified a population of macrophages with high expression of CD73 that persists after anti-PD1 treatment. Robert et al. [52] found that GSCs with ZEB1 and CD73 inhibition could influence the phenotype of T-cells and monocytes, becoming a mixed-population of pro-inflammatory and anti-inflammatory macrophages and DCs, while only CD73 inhibition decreased CD209 expression in monocyte-derived macrophages. Besides, results have described a decrease in the percentage of $CD64^+$ monocyte-derived macrophages cells and an increase of $CD11c^+$ DC cells. On the other hand, there was an increased expression of $CD64^+CD209^+$ protumorigenic macrophages after treatment with tumor conditioned media in GSCs, indicating an increase of the tumor-associated macrophages. All together, these results suggest that CD73 participates in macrophages and DCs protumor activation, modulating treatment responses.

Recently, strategies using immune cells have emerged as a therapeutic option. In this regard, Wang et al. [53] presented genetically engineered human NK (CD73.mCAR-pNK) cells as an option to anti-GB therapy. The study proposes the autophagy functions from NK cells as target mechanism to reprogram TME and sensitize tumor cells to combined treatments. More studies should be performed at this point to clarify the tumor-response using these new strategies.

In summary, regulating CD73 not only changes GB cells, but also modifies other immune cell populations in diverse ways. At this point, understanding how this modulation affects tumor development and progression and which patterns would offer better patient prognosis would be useful to the development of new strategies to GB treatment.

6. CD73/ADO signaling modulation as therapeutic promises for GB management

Since the essential functions associated with CD73 and ADO in GB pathogenesis and growth, investigating blockade mechanisms for these molecules looks insightful. At this point, many researchers have contributed using different models and strategies (Table 1).

Table 1
New therapeutical approaches in CD73/ADO signaling modulation to GB management.

Target strategy	Type of experiment	Main findings	Refs.
Anti-CTLA-4, Anti-PDL-1	*In vivo* ($CD73^{-/-}$ mice)	Lack of CD73 improved survival rates	[34]
CD73-siRNA-loaded nanoemulsion	*In vitro* (C6, primary astrocytes	Reduction of CD73 expression, AMPase activity and cell viability in C6 GB cell line No astrocytes toxicity	[57]
CD73 knockdown (siRNA-CD73 Nanoemulsion containing siRNA-CD73 (NE-siRNA-CD73) Combination of temozolomide (TMZ) and NE-siRNA-CD73	*In vitro* (C6, U87MG) *In vivo* (Preclinical GB model in Wistar rats)	Treatment reduced AMPase activity and cell viability (C6, U87MG) Treatment via nasal route reduced tumor growth, CD73 expression in GB cells and ADO levels in liquor CD73 blockade induced tumor cell apoptosis *In vitro* CD73 knockdown promoted a reduction of IC50 for TMZ No synergistic or additive effect combining TMZ and NE-siRNA-CD73 *in vivo* GB model	[18–20, 56]
α-bisabolol	*In vitro* (U138MG)	Cytotoxic effect against glioma cells Increase of A3 expression in glioma cells Increase of CD73 activity	[48]
LaSOM63 (monastrol derivative	*In vitro* (C6)	Cytotoxic activity *in vitro* Reduction of CD73 activity	[54]
Bozepinib (BZP	*In vitro* (C6 and U138MG)	Reduction of AMP levels at high concentration (250 μM) in U138MG BZP did not alter the expression of CD73 Decrease of AMPase activity	[55]
Pentoxifylline (phosphodiesterase inhibitor	*In vitro* (JHH520, 407 and SF188)	Inhibition of ZEB1 and CD73 expression Decreased viability, clonogenicity, and invasion of GSC	[31]

CTLA-4: Cytotoxic T-lymphocyte-Associated Protein 4; PDL-1: Programmed cell death ligand protein 1; CD73: ecto-5′-nucleotidase; TMZ: temozolomide; AMP: Adenosine Monophosphate; ADO: adenosine; GB: glioblastoma.

In vitro studies that modulated CD73 activity have shown promising results. Figueiró et al. [54] have tested the effects of LaSOM63 in C6 cell line, showing that this compound had exerted cytotoxic activity and has reduced CD73 activity. Running alongside, Tsiampali et al. [31] have demonstrated the participation of CD73 in the enhancement of the EMT process. Thus, they have evaluated pentoxifylline, a phosphodiesterase inhibitor, and their findings included inhibition of ZEB1 and CD73 expression, decreased viability, clonogenicity, and invasion of GSC cultures. Interestingly, Dias et al. [55] have investigated the effect of

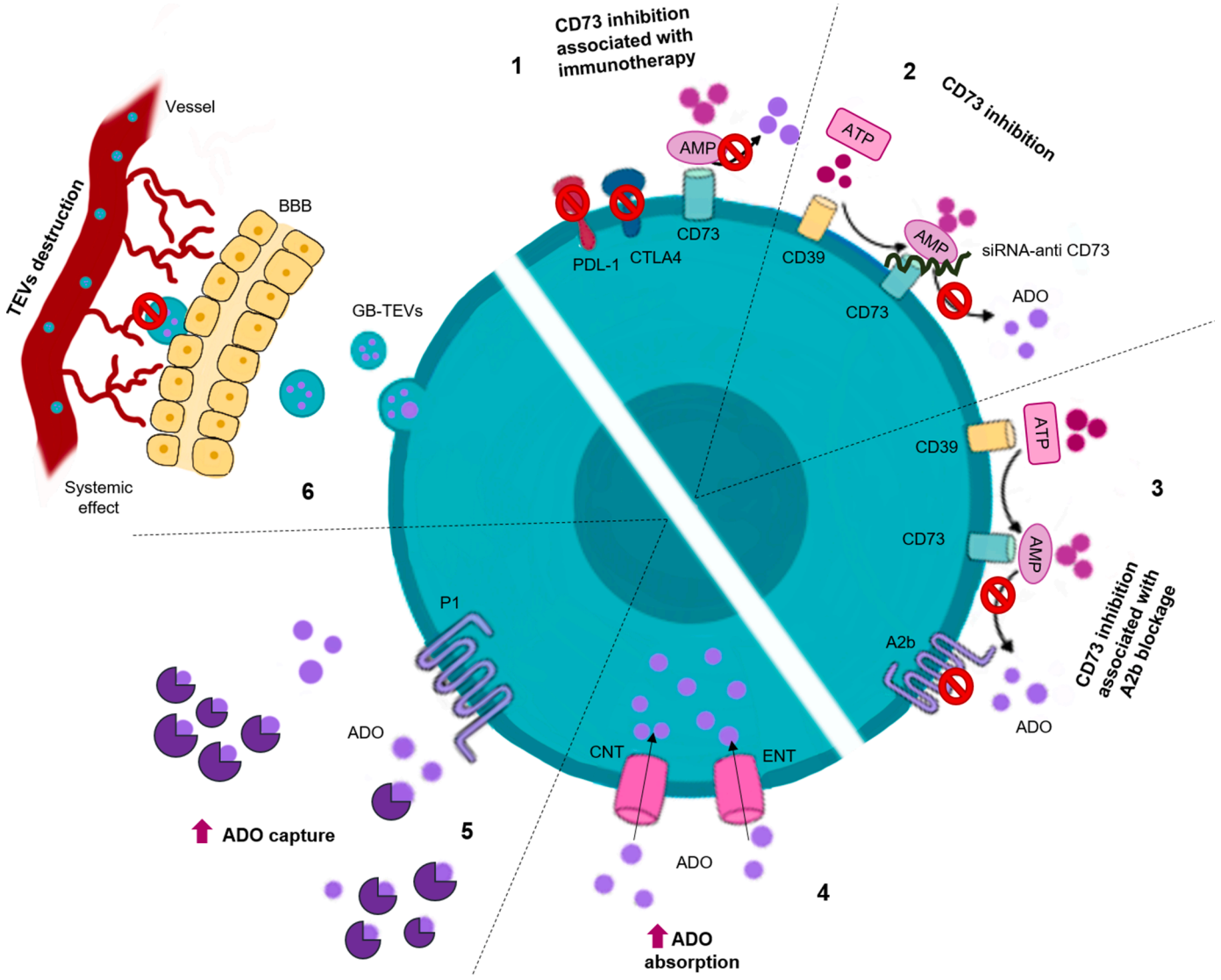

Fig. 2. Adenosinergic-based strategies for GB therapy: promising strategies and new possibilities. Different possibilities are proposed in the regulation of CD39-CD73-P1 receptors axis to trap GB advance. In the right side are presented the strategies most established in literature that includes: [1] the blockade of CD73 in combination with immunotherapy [2], CD73 knockdown using siRNA and [3] the combination between CD73 inhibition and A2b blockage. In the left side are presented some unexplored options that may include [4] the modulation of CNT and ENT channels to ADO uptake to establish normal levels of extracellular ADO [5], the capture/ trapping of accumulated ADO in TME that might be done by the discovering of new drugs [6], the modulation of TEVs that could include its inhibition, blockade of spreading and/or destruction of TEVs. Specially from the ones pointed into the left side, new research is necessary to better understand mechanisms and possibilities.

CNT: concentrative nucleoside transporters; ENT: equilibrative nucleoside transporters; ADO: adenosine; TEV: tumor extracellular vesicles; TME: tumor microenvironment; TEV: tumor extracellular vesicle; BBB: blood brain barrier; ATP: adenosine triphosphate, AMP: adenosine monophosphate; GB: glioblastoma, CTLA-4: Cytotoxic T-lymphocyte-Associated Protein 4; PDL-1: Programmed cell death ligand protein 1; CD73: ecto-5′-nucleotidase; CD39: NTPDase 1.

bozepinib (BZP) in U138MG and found a decreased AMP hydrolysis with no changes in CD73 expression, suggesting that only modulation of activity would have promising effects in GB.

Azambuja et al. and Teixeira et al. extensively have demonstrated that a nanoemulsion loaded with siRNA-CD73 (NE-siRNA-CD73) knockdown CD73, reducing AMPase activity and cell growth *in vitro* with no toxicity associated to astrocytes [18,19,56,57]. Further, the *in vivo* potential was assessed with the nasal administration of NE-siRNA-CD73. The formulation was able to reduce tumor size, induce apoptosis, reduce both ADO levels in cerebrospinal fluid and CD73 expression in the tumor. Finally, they have tested the association of NE-siRNA-CD73 and TMZ treatment. The proposed therapy have demonstrated higher response than TMZ, but no synergic effects were observed [19,20,56].

Goswami et al. [34] have performed reverse translational studies using $CD73^{-/-}$ mice and found that the animals with lack of CD73 have improved survival rates after treatment with anti-CTLA-4 and anti-PD-1. In the immunotherapy field, Anzai et al. [58] have identified that the antibody 0614-5-ADC, which is produced from exosomes immunization, targets CD73 as an antigen, suggesting that it might be used to treat cancers with high CD73 expression.

On the other hand, Mendes et al. [48] have shown cytotoxic effects *in vitro* in U138MG using α-bisabolol with an enhanced A3 receptor expression and increased CD73 activity. The opposite mechanisms observed in the studies mentioned above. Lopes et al. [59] evaluated the effects of NSAIDs and found that MTX decreased cell viability with an increase of CD73 in GB cells.

Taken together, these findings suggest that up or downregulation of CD73 produces effects in GB viability *in vitro*. However, more studies are necessary to evaluate the better strategy with regards to CD73 and ADO

Table 2
Clinical trials targeting CD73 to cancer treatment.

Drug/ Molecule	Action mechanism	Number of studies	Disease	NCT
AGEN1423	Target CD73 and TGFβ:-block CD73 activity - intracellular trapping (TGF-β	1	Pancreatic cancer	NCT05632328
AK119	Inhibits CD73 CD73 internalization	4	Solid tumors	NCT04572152; NCT05173792; NCT05689853; NCT05559541
BMS-986,179	Internalization of CD73	1	Malignant Solid Tumor	NCT02754141
CPI-006	Inhibits CD73 enzyme activity	1	Non small cell lung cancer, renal cell cancer, triple negative breast cancer, colorectal cancer, bladder cancer, cervical cancer, uterine cancer, sarcoma, endometrial cancer, and metastatic castration resistant prostate cancer.	NCT03454451
IBI325	Inhibits CD73 enzyme activity	2	Advanced unresectable or metastatic tumors	NCT05119998; NCT05246995
INCA00186	Inhibits CD73 enzyme activity	1	Advanced Solid Tumors, Squamous Cell Carcinoma of the Head and Neck (SCCHN) Gastrointestinal (GI) Malignancies	NCT04989387
IPH5301	Inhibits CD73 enzyme activity	1	Incurable advanced and/or metastatic cancer	NCT05143970
JAB-BX102	Inhibits CD73 enzyme activity	1	Confirmed metastatic or locally advanced solid tumor	NCT05174585
Oleclumab (MEDI 9447)	Internalization of CD73	7	Ovarian cancer, solid tumor, breast cancer, pancreatic cancer, Non-small Cell Lung Cancer, sarcoma	NCT03267589; NCT03616886; NCT02503774; NCT04940286; NCT03381274; NCT04668300; NCT03875573
PT199	Inhibits soluble and membrane-bound CD73	1	Solid tumors	NCT05431270
Sym024	Enzymatic inhibition Inhibits soluble and membrane-bound CD73	1	Squamous cell carcinoma of the head and neck; Non-small-cell lung carcinoma-adenocarcinoma histology subtype; Pancreatic ductal adenocarcinoma; Cholangiocarcinoma; Colorectal cancer; Gastric carcinoma (includes gastroesophageal carcinoma) carcinoma (microsatellite stable [MSS] and microsatellite instability-high [MSI-H] phenotypes); Gastric carcinoma (includes gastroesophageal carcinoma); Esophageal carcinoma (includes squamous cell and adenocarcinoma); Mesothelioma (pleural and peritoneal); Cervical carcinoma (CC) (includes adeno, squamous and mixed adeno-squamous carcinoma histology subtypes)	NCT04672434
TJ004309 Uliledlimab	Block CD73:-non-competitive-intra-dimer binding mode	2	Ovarian cancer, solid tumor, metastatic cancer	NCT05001347; NCT03835949

NCT: Number clinical trial.

signaling.

7. Systemic immunosuppressive effects of CD73/ADO signaling

GB is a tumor characterized by its extended immunosuppression that affects the whole body [60]. However, few studies have explored this circumstance. In this regard, Wang et al. [45] have identified a high concentration of tumor derived extracellular vesicles (TEV) positive for CD73 enriched in exosomes in central and peripheral body fluids of GB patients compared to other brain tumours. In addition, T cells have presented a high expression of CD73 in the surface, indicating that GB-TEV could be taken up by T cells. Besides, *in vitro* experiments have shown that $CD73^{+}$ GB-TEVs could be uptake by T-lymphocytes. Similar results suggesting a major role of EVs/exosomes was demonstrated by Azambuja and colleagues [15]. *Ex vivo* and *in vivo* experiments have demonstrated the functions of exosomes from GB in reprogramming immune cells. Indeed, findings include macrophage differentiation into M2-*like* profile, and alterations in number and functions of all subsets of immune cells to support a strongly immunosuppressive TME.

Other tumors also produce exosomes that express CD73 including bladder cancer, head and neck, breast and melanoma [61–63]. $CD73^{+}$ exosomes contribute to immunosuppression through different mechanisms, including adenosine generation, suppression of T cell functions and IL-2 production, changes in mRNA expression profile of recipient cells and promotion of angiogenesis [15,61,62,64].

Further investigation is necessary to understand the role of CD73/ADO signaling in promoting systemic immunosuppressive state, by modulating and reprogramming circulatory immune cells through the release of TEVs in GB.

8. Future perspectives for CD73/ADO signaling in GB studies

From the last 40 years, many efforts have been made to shed light on GB pathogenesis and discovery of new strategies to overcome the challenges imposed by the disease (Fig. 2). However, there are still many points that need to be clarified.

First of all, newer technologies (*e.g.* RNA*seq*) are deeply exploring the gene signature of single cells in TME and also its interactions, but further effort is needed to mapping cell states within native tissue architecture [30]. The full understanding of the cell position/interaction role into disease progression can improve the design of new treatments or even elucidate the causes of current treatment failures. Still in the beginning, there is a vast knowledge about P1 receptors and ADO accumulation, but not much into CNT/ENT transporters. Instead of P1 blockade, modulating these transporters might improve the proinflammatory state in TME, therefore, avoiding ADO accumulation. Moreover, there is still a lack of knowledge about tumor and non-transformed crosstalk inside TME and systemically. The recent studies about EVs and exosomes brought new prospects about tumor communication and reprogramming of surrounding cells – blockade/destruction of EVs would be a strategy to decrease immunomodulatory effects?

Advances in science and technology over the years have shown the impact of CD73/ADO in GB progression. Ceruti et al. [47] observation asserts that well-designed *in vivo* experiments can brighten inconsistencies and highlights the achievable advantages of targeting the adenosinergic signaling for GB therapies.

Studies have targeted CD73/ADO signaling and have demonstrated the potential outcome improvement for instance: blockade, inhibition, or knockdown of CD73; blockade of CD73 in combination with immunotherapy (PD-1, CTL-4), blockade of A2B receptor in combination with CD73 blockade (Table 1). Since the CD73/ADO signaling has recently become a promising target, up to now studies have shown mostly *in vitro*

effects. With regards to the *in vivo* approach, more studies are needed to support these findings and better estimate potential risks of these molecules and the best administration regimen, dosage, and frequency. From the ones that have already been explored *in vivo,* such the one presented by Azambuja and colleagues using NE-siRNA-CD73, phase 1 clinical trials seem reasonable to evaluate the safety in humans of the proposed therapy.

Studies analyzing tumors from GB patients can explore phenotypic characteristics from TME cell composition to find additional knowledge to establish better therapeutic strategies. In parallel, investigation regarding tumor response changes according to patient phenotype is a promising step for current approved therapies and new candidates, specially in modifications in CD39-CD73-P1 receptors axis.

Finally, few researchers have proposed CD73 as a prognostic biomarker in GB [40]. Since CD73 is differentially expressed in tissues/cells in patients and enzyme modulation can alter its expression/activity, more effort in this area should be reasonable to predict treatment response/prognostic with different therapies.

8.1. Clinical trials

Currently, there is no clinical trial proposing anti-CD73 antibodies to GB therapy. There are some trials including 12 different antibodies (Abs) exploring other tumor types such as head and neck, solid tumors, ovarian, breast, lung, colorectal and pancreatic cancer (Table 2). The mechanism includes the blockade/inhibition of CD73 activity. Some of them, such as AK119, BMS-986,179 and Oleclumab (MEDI 9447) promote CD73 internalization [65]. PT199 and Sym024 can block both the membrane and soluble form of CD73. Interestingly, AGEN1423 target CD73 and TGFβ with a unique Ab. Uliledlimab (TJ004309) promotes CD73 blockade through a non-competitive binding [66]. These studies are still ongoing and there is no final results. Besides that, it is difficult to predict responses in GB patients since most of them excludes patients with active brain metastasis and/or central nervous compromising. In this point, it is important to direct studies specifically to GB considering its unique characteristics.

8.2. Concluding remarks

In this review, we aimed to discuss the roles of CD73 in GB and the future perspectives for this area. In summary, CD73 is a key factor into disease progression and GB associated immunosuppression contributing to the alarming clinical scenario of this fatal tumor. Advances occurred in exploring options to modulate CD73 to GB treatment. However, there is a lack of *in vivo* experiments and, especially, clinical trials evaluation of promising therapies to confirm/reject preclinical findings.

Authors contribution

Juliana Hofstätter Azambuja (JHA) and Elizandra Braganhol (EB) contributed to the study conception. E.B. critically revised the manuscript and supervised all stages of manuscript preparation. Nicolly Espindola Gelsleichter (NEG) searched and selected the articles. N.E.G. and J.H.A wrote the first version of the manuscript. Dominique Santos Rubenich (DSR) drew the figures and reviewed the text. All authors commented on previous versions of manuscript. All authors have read and approved the final manuscript.

Consent for publication

All authors agree with the content of the manuscript and approved the submission.

Declaration of Competing Interest

Authors declare no conflict of interest.

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