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Targeting the CD73-adenosine axis in immuno-oncology

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ABSTRACT

The ectonucleotidases CD39 and CD73 are cell surface enzymes that catabolize the breakdown of extracellular ATP into adenosine. As such, they constitute critical components of the extracellular purinergic pathway and play important roles in maintaining tissue and immune homeostasis. With the coming of age of cancer immunotherapy, ectonucleotidases and adenosine receptors have emerged as novel therapeutic targets to enhance antitumor immune responses. With early-phase clinical trials showing promising results, it is becoming increasingly important to decipher the distinct mechanisms-of-action of adenosine-targeting agents, identify patients that will benefit from these agents and rationally develop novel synergistic combinations. Given the broad expression of ectonucleotidases and adenosine receptors, a better understanding of cell-specific roles will also be key for successful implementation of this new generation of immuno-oncology therapeutics. We here review the latest studies on the roles of CD73 and adenosine in cancer with a focus on cell-specific function. We also discuss ongoing clinical trials and future avenues for adenosine-targeting agents.

1. Biology of the CD73-extracellular adenosine pathway

The adenosinergic pathway is a complex system of enzymes, transporters and receptors regulating the conversion of pro-inflammatory and immuno-stimulatory extracellular ATP into immunosuppressive adenosine [1]. In this system, CD73 has a unique function in that it regulates production of immunosuppressive adenosine. CD73, also called ecto-5′-nucleotidase (encoded by the *NT5E* gene), is a 70-kD glycosylphosphatidylinositol (GPI) anchored cell-surface homodimer. CD73 can also be cleaved from the cell surface and an enzymatically active soluble CD73 can be found in extracellular fluids and blood [2]. CD73 is the principal enzyme responsible for the breakdown of extracellular AMP into adenosine [3]. Extracellular AMP is generally generated from phospho-hydrolysis of ATP by ecto-ATP-Dases such as CD39. Extracellular AMP can further be generated by an alternative, CD39-independent pathway, involving the degradation of extracellular NAD^+ through the sequential enzymatic activity of CD38 and CD203a/PC-1 [4].

CD73-derived adenosine exerts its biological function by binding to one of the four G protein-coupled adenosine receptors (A1, A2a, A2b, and A3), via cellular uptake through equilibrative or concentrative nucleoside transporters (ENTs and CNTs, respectively) or via catabolism into inosine by adenosine deaminase. Because of its short half-life, extracellular adenosine essentially mediates its biologic functions in the proximal cellular environment. Activation of cyclic AMP (cAMP) elevating A2a (expressed on lymphocytes and myeloid cells) and A2b receptors (expressed on myeloid cells) is immunosuppressive, and as such serve to maintain immune homeostasis. Notably, A2a adenosine receptors are significantly upregulated following T cell activation [5]. Activation of A2a receptor potently suppresses T cell receptor signaling through activation of type I protein kinase A (PKA) and its phosphorylation of C-terminal Src kinase (Csk), inhibiting the Src family tyrosine kinases Lck and Fyn [6]. The cAMP-elevating and immunosuppressive effects of adenosine on T cells were first described by Wolberg et al. in 1975 [7].

During inflammatory and hypoxic conditions, activation of the CD73-adenosinergic pathways provides a negative feedback in order to limit excessive tissue damage caused by sustained immune cell activation. In 1997, Jonathan Blay and colleagues reported that solid tumors contained higher levels of extracellular adenosine than surrounding normal tissue (10–20-fold higher), suggesting a role for adenosine in tumor progression [8]. In 1998, Linda F. Thompson and colleagues reported that targeting CD73 with a neutralizing mAb promoted human lymphocyte proliferation [9]. In 2006, Sitkovsky and colleagues demonstrated that the local accumulation of adenosine suppressed anti-tumor immunity via the A2a receptor on effector T cells [10]. Building on this work, we demonstrated in 2009 that CD73-deficient mice have increased anti-tumor immunity and that targeting CD73 could delay

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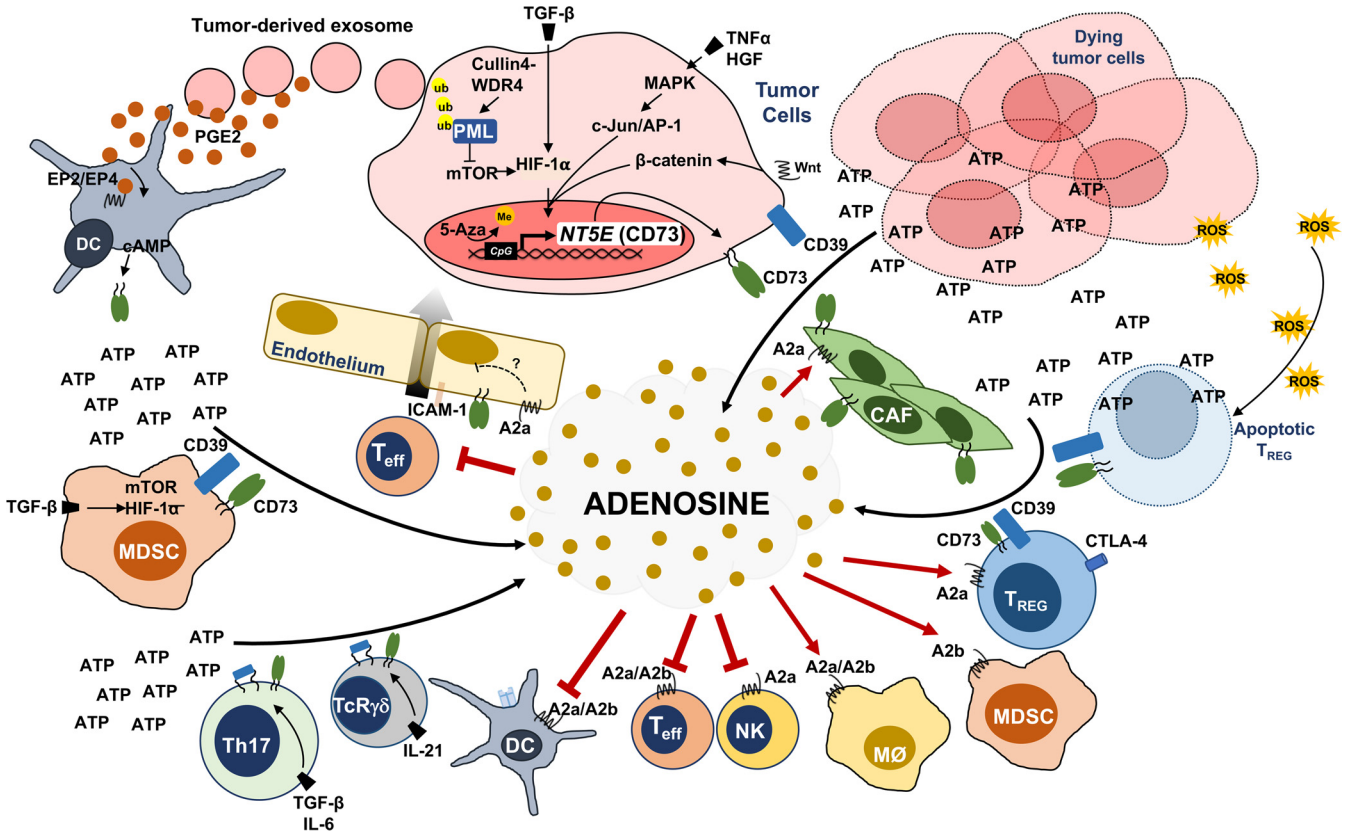

Fig 1. The cellular compartmentalization of CD73 in the regulation of the immunosuppressive tumor microenvironment. CD73 is expressed widely in the TME and generated adenosine contributes extensively to the creation of an immunosuppressive tumor growth-permissive microenvironment. CD73 is expressed on a variety of cells found within the tumor microenvironment, and HIF-1α is a major regulator of its expression. In tumor cells, TGF-β, chemotherapeutic agents and PML degradation-mediated activation of mTOR were shown to induce CD73 expression through HIF-1α stabilization. Alternatively, Wnt signaling can induce CD73 promoter activity through β-catenin and pro-inflammatory cytokines such as TNFα and HGF cooperates with MAPK signaling through the c-Jun/AP-1 transcription factor complex to activate CD73 transcription. CpG islands within CD73 promoter are also subjected to methylation and the DNA-methyltransferase inhibitor 5-azacitidine induces CD73. Tumor-derived exosomes were shown to induce CD73 expression on dendritic cells in a cAMP-dependent manner through the secretion of PGE2. CD73 and CD39 are induced on MDSCs upon TGF-β exposure through mTOR/HIF-1α signaling. TGF-β and IL-6 upregulates CD73 on Th-17 cells, and CD73 expression may be induced on TcR-γδ T cells by IL-21. CD73 levels may also be upregulated on CAFs, Tregs and tumor endothelial cells. Upon tumor immunogenic cells death, tumor cells release ATP, and they also release ROS into the TME. ROS triggers apoptosis in Tregs, a process which contributes to the extensive ATP release and its subsequent conversion to adenosine. Abundant adenosine exerts immunosuppressive activity on a variety of immune cells. It hinders antigen presentation by DC, favors development and accumulation of Treg and MDSCs, inhibits proliferation and cytotoxicity of T_{eff} and NK cells through the A2a and A2b receptors, skews macrophages polarization toward pro-tumorigenic M2 type through the A2a receptor and induces proliferation and expression of inhibitory molecules on Tregs. Adenosine also drives proliferation of CAFs through the A2a receptor, and impairs T_{eff} homing to tumor sites via an incompletely understood mechanism involving A2a signaling and ICAM-1 reduction on endothelial cells.

LEGEND: TME = tumor microenvironment; EP2/EP4 = PGE_2 receptors; ROS = reactive oxygen species; HGF = Hepatocyte Growth Factor; CAF = cancer-associated fibroblast; MDSC = myeloid-derived suppressor cell; MØ = macrophage; DC = dendritic cell; NK = natural killer cell; Teff = effector T cells. Red arrows indicate immunosuppressive activities.

tumor growth in mice [11], observations that were subsequently validated by independent groups [12].

In the past 10 years, the role of the CD73-adenosinergic pathway in suppression of anti-tumor immunity has increasingly been recognized [13,14]. In this review, we discuss the latest findings dealing with the regulation of anti-tumor immunity by CD73 and adenosine. We also discuss ongoing clinical trials and future avenues of targeting the CD73-adenosine axis in immuno-oncology.

1.1. CD73 in antitumor immunity

CD73 expression is upregulated in the tumor microenvironment (TME) as a result of tissue hypoxia [15], epithelial-to-mesenchymal transition [16], inflammation (e.g. TNF-α) [17] and/or cytotoxic stress (e.g. chemotherapy) [18]. CD73 can be found expressed on cancer cells, endothelial cells, fibroblasts, lymphocytes and myeloid cells. Depending on cell type, CD73 expression has specific impacts on anti-tumor immunity (Fig. 1). Deciphering the contribution of cell-specific CD73 expression will be important for effective translation of adenosine-targeting agents to the clinic.

1.2. Regulation of CD73 expression in cancer

CD73 transcription is directly regulated by HIF-1α, which explains why hypoxic tumors often display elevated levels of CD73 [19] and HIF-1α gene signature positively correlates with CD73 gene expression [20]. Moreover, activation of PKA by adenosine receptors further enhances HIF-1 activity and expression of CD39 and CD73 [15]. TGF-β is another important inducer of CD73 expression, partly through stabilization of HIF-1α. TGF-β-mediated EMT further contributes to promote CD73 expression by tumor cells [21]. In breast cancer and melanoma, high CD73 expression has also been associated with demethylation of the NT5E promoter [22,23]. Activation of Wnt and downstream β-catenin [24], MAPK [17], EGFR [25], and AKT signaling have further been shown to promote CD73 expression on tumor cells [26]. Overexpression of CD73 was shown in turn to induce increased nuclear

localization of β-catenin [27].

Invasive EMT-like melanoma phenotype has been associated with expression of CD73, which is induced by MAPK signaling and inflammatory cytokines, such as TNF-α and HGF. Upregulation of CD73 has also been shown following adoptive T cell immunotherapy, and in some melanoma patients receiving anti-PD-1 immunotherapy [17]. In another interesting study, Wang et al. identified CD73 upregulation downstream of WDR4-mediated PML degradation [28]. They described a mechanism by which tumors downregulate the tumor suppressor PML through the overexpression of the WDR4 adaptor-containing Cullin4 ubiquitin ligase [28]. By transcriptomic analysis, they identified CD73 to be induced following PML degradation in lung cancer, due to the lack of PML-mediated inhibition of HIF-1α [28]. Notably, expression of WD4R4 promoted tumor cell migration, invasion, and metastasis, and this was abrogated by targeting CD73 expression. In a genetically engineered mouse model of lung cancer, WDR4 ablation suppressed tumor development by increasing antitumor immunity, and importantly this could be reproduced using a CD73 inhibitor [28].

1.3. CD73 on tumor cells

In addition to hypoxia, inflammatory cytokine and specific oncogenic pathways (described above), cytotoxic stress can also induce CD73 expression on tumor cells. In human triple negative breast cancer (TNBC) cells, we previously demonstrated that a wide-range of cytotoxic drugs induced both CD39 and CD73 expression on the tumor cell surface [29]. While the underlying mechanism remains to be defined, it is observed across cancer types [29]. Recently, it was shown that multiple chemotherapeutic agents can induce HIFs, thus inducing CD73 expression. Interestingly, this was shown to be accompanied by HIF-driven upregulation of PD-L1 and CD47 as well. It was proposed that chemotherapy-induced upregulation of CD73, PD-L1 and CD47 contribute to immune evasion in TNBC [18].

Tumor-derived CD73 can potently inhibit anti-tumor T cell functions [11,30]. In murine models, tumor-derived CD73 was shown to significantly contribute to the suppression of T cell and NK cell-mediated anti-tumor immunity, and to contribute to the efficacy of CD73 and A2a receptor targeting therapies. In addition to its enzymatic functions, a growing body of evidence indicates that tumor-derived CD73 also promotes tumor growth and metastasis independently of extracellular adenosine. While adenosine-independent function of CD73 is still poorly understood, it could have important implications in the activity of CD73 therapeutic agents (discussed below).

1.4. CD73 on host cells – regulatory t cells

In addition to tumor-derived CD73, expression of CD73 by non-transformed 'host' cells also significantly contributes to tumor immune evasion and progression. Using CD73 deficient mice and bone marrow transplantation experiments, we and others demonstrated that deletion of CD73 in either hematopoietic or non-hematopoietic cells was sufficient to enhance anti-tumor immunity [12,31]. In mice, a critical role for Treg-derived CD73 was demonstrated by in vivo Treg reconstitution studies. Accordingly, reconstitution of Treg-deficient mice with CD73-deficient Treg was unable to fully rescue tumor growth compared to reconstitution with CD73-proficient Tregs [12,31]. These results support a role for CD73-derived adenosine in the immunosuppressive function of Tregs [32,33].

In contrast to murine Tregs, human Tregs do not constitutively express cell-surface CD73, but are positive for CD39 [34]. However, it was reported that human Tregs rather predominantly expressed CD73 intracellularly [34]. Nevertheless, human $CD39^+$ Tregs can rapidly hydrolyze ATP to adenosine when in the presence of other $CD73^+$ cells, such as cancer cells or other immune cells, or in the presence of soluble CD73 or CD73-expressing microvesicles [35]. Human Tregs co-expressing CD73 and CD39 have been reported under certain conditions [36], including high-dose IL-2 [37]. Recently, it was also shown that apoptotic Tregs were enriched in the TME and that apoptotic Tregs were more suppressive than live Tregs due to increased production of adenosine. Accordingly, suppressive activity of tumor-associated apoptotic Tregs was shown to rely on the conversion of released ATP into immunosuppressive adenosine through CD39 and CD73. Notably, in vivo blockade of ATP catabolism by a specific CD39 inhibitor reduced the immunosuppressive function of apoptotic Tregs and enhanced anti-PD-1 therapy. Importantly, similar results were obtained in vitro using human tumor associated apoptotic Tregs. These results indicate that blocking ATP catabolism is a promising approach to dampen Treg-mediated immunosuppression in the TME.

1.5. CD73 on effector T cells

In humans, CD73 is expressed on a small proportion of peripheral effector T cells. On circulating $CD8^+$ T cells, CD73 is essentially expressed by naïve cells and small fraction of memory cells. In contrast, in the TME, high levels of CD73 can be observed on memory $CD8^+$ T cells [38]. The exact function of CD73 expression by memory $CD8^+$ T cells still remains poorly understood but may favor maintenance of long-lived memory T cells [38]. Similarly, circulating $CD4^+$ T cells expressing CD73 are mostly memory cells [39]. Interestingly, CD73 was identified as a marker of human effector $CD4^+$ T cells enriched in polyfunctional Th1.17 cells [35]. In breast and ovarian tumors, $CD73^+$ $CD4^+$ effector T cells express lower levels of immune checkpoint receptors compared to $CD73^-$ $CD4^+$ cells. Notably, Th17 cells expressing CD39 and CD73 infiltrate human breast tumors and correlate with poor outcome [40]. In murine models of cancer, Th17 cells generated in the presence of TGF-β expressed high levels of CD39 and CD73, suppressed T cell effector function and promoted tumor growth in an adenosine-dependent manner [41]. Remarkably Th17 cells generated in the absence of TGF-β did not express CD73 [40,41] and rather mediated anti-tumor effects upon adoptive transfer in tumor-bearing mice [40].

1.6. CD73 on myeloid cells

Myeloid-derived suppressors cells (MDSCs) are now recognized as important regulators of immune responses within the TME. MDSCs are distinct from mature differentiated myeloid cells and accumulate in tumors as a consequence of aberrant myelopoiesis. While MDSCs use multiple immunosuppressive factors to suppress anti-tumor immunity (arginases, nitric oxide, reactive oxygen species, indoleamine 2,3-dioxigenase, TGF-β and PGE2), production of adenosine is emerging as an additional mechanism of MDSC-mediated immunosuppression. MDSCs are indeed found to express high levels of CD73 and CD39 in various cancer models, possibly driven by TGF-β production in the tumor microenvironment [42].

In lung cancer patients, an enrichment of MDSCs co-expressing CD73 and CD39 was observed. Notably, MDSCs expressing CD39 and CD73 were shown to be associated with increased metastasis and resistance to chemotherapy [43]. Data suggest that TGF-β serves to activate mTOR-HIF1α pathways in infiltrating MDSCs, leading to upregulation of CD73 and CD39 [43]. Adenosine-producing MDSCs were also reported in colorectal cancer (CRC) patients. Notably, the immunosuppressive activity of CRC-associated MDSCs could be reversed by blocking CD39 or CD73 activity [44].

Tumor-associated macrophages (TAM) are another critical component of the TME. In some cancers, TAMs can constitute up to 50% of the tumor tissue and severely impair anti-tumor immunity. In human ovarian ascites, $CD14^+$ TAMs were shown to selectively co-express CD73 and CD39. Co-culture of healthy donor monocytes with ovarian cancer cells induced the differentiation of monocytes into anti-inflammatory M2 macrophages expressing high level of CD73 and CD39 [45]. These adenosine-producing TAMs were further demonstrated to inhibit $CD4^+$ T cells activation in vitro [45]. Interestingly, similar

findings were recently reported in patients with mesothelioma. In these patients, expression of CD73 was selectively induced in $CD14^+$ cells extracted from pleural effusions and not from blood. In pleural effusions, increased levels of PGE2 were shown to induce CD73 expression on $CD14^+$ cells in a cAMP/PKA/p38 dependent manner. Together, these results suggest that upon tumor infiltration, monocytes/macrophages can acquire CD73 expression and exert immunosuppressive functions by producing adenosine.

1.7. CD73 on other immune cells

The role of CD73 on other immune cell subsets is still poorly defined. Intriguingly, we and others have observed that a large fraction of tumor-infiltrating NK cells express CD73, in contrast to peripheral NK cells [46]. CD73 expression on human NK cells has also been reported [47,48]. Further investigations are needed to better understand the function of CD73 on NK cells and its impact on anti-tumor immunity.

Tumor-infiltrating B cells can also express CD73. Tumor-infiltrating B cells are now recognized as important regulator of anti-tumor immunity. In some tumor types, such as high-grade serous ovarian cancer and metastatic melanoma, the presence of B cells in tumors correlates with favorable outcomes [49]. In other cancers, such as lung cancer, B cells have sometimes been associated with suppression of T cell function [50]. Interestingly, human circulating B cells express CD73 as well as CD39. Recently, a subpopulation of adenosine producing, $CD39^{hi}$ $CD73^+$ B cells was shown to have regulatory functions relying at least in part on adenosine production. The exact contribution of adenosine-producing B cells on tumor immunity and the activity of immune checkpoint inhibitors remains to be investigated.

1.8. CD73 on cancer-associated fibroblasts

Cancer associated fibroblasts (CAFs) are an important component of the tumor microenvironment. CAF promote cancer progression by providing growth factors, angiogenic factors, extracellular matrix remodeling and through immunosuppression [51]. Several studies, including our own work, indicate that human CAFs often express high levels of CD73, in different cancer indications. Using mouse models of cancer, we showed that adenosine produced by CAF can promote tumor immune escape [52–54]. In ovarian cancer, we demonstrated that high levels of CD73 expression on CAFs were associated with poor prognosis [52]. CD73-expressing CAFs were also identified in human TNBC [54,55]. In TNBC, $CD73^+$ CAFs exhibit potent immunosuppressive functions and promote Treg recruitment, survival, and differentiation. Interestingly, the immunosuppressive properties of TNBC $CD73^+$ CAFs were shown to be partly dependent on adenosine production. $CD73^+$ CAFs were also recently reported in lung cancer [56]. Notably, targeting A2a with a small-molecule antagonist inhibited lung cancer CAFs proliferation.

1.9. CD73 on endothelial cells

CD73 expression on endothelial cells regulates leukocytes adhesion and transmigration across the endothelial barrier in inflammatory conditions [57]. In the TME, however, the role of endothelial CD73 on anti-tumor immunity is still unclear. One report suggested that CD73 expression on tumor vascular endothelial cells impaired T cell homing to tumors [12]. Other studies, including one from our group, rather suggest a tumor-promoting role of endothelial-derived CD73. Interestingly, our in vitro studies using human umbilical cord endothelial cells (HUVEC) suggest that at least part of the pro-angiogenic effects of CD73 are adenosine-independent [58].

In addition to vascular endothelial cells, lymphatic endothelial cells (LECs) are also recruited to the TME where they can modulate anti-tumor immunity. In mouse and human, LECs exhibit high level of CD73 and express adenosine receptors. Preliminary results generated in our lab (unpublished) indicate that adenosine signaling promotes tumor-associated lymphangiogenesis and favor regional metastasis to sentinel lymph node. The role of CD73 and adenosine signaling in the tumor vasculature and LECs biology remains to be further investigated.

Using a CD73-EGFP reporter mouse, Breitbach et al. [59] recently demonstrated high CD73 expression in perivascular cells in different organs, as well as strong expression on sinusoidal endothelial cells in the bone marrow. Expression of CD73 on sinusoidal bone marrow cells may indicate a potential role for CD73 in regulation of the hematopoietic stem cell niche. In support of this, Jin et al. [60] showed that adenosine promotes hematopoietic stem and progenitor cell (HSPC) development via A2b receptor, which increases CXCL8 production by vascular endothelial cells.

1.10. CD73 on microvesicles

Tumor cell-derived exosomes purified from different types of human cancers have been shown to exhibit ATP/AMP hydrolytic activity relying on the presence of enzymatically-active forms of CD73 and/or CD39 at their surface [61]. Supporting these in vitro observations, exosomes extracted from pleural effusion of mesothelioma patients showed ATP-degrading activity and inhibited T cell activation in an A2a-dependent manner [61]. Recently, similar observations were made in multiple myeloma where microvesicles expressing high levels of CD73, CD39, but also CD38 and CD203 ectoenzymes, were found to be enriched in the plasma of myeloma patients compared to healthy individuals [62]. These microvesicles were able to produce adenosine from ATP, NAD^+, ADPR or AMP and thus to create an immunosuppressive microenvironment in the bone marrow niche where malignant cells proliferate in multiple myeloma. Consistent with these data, exosomes circulating in the blood of patients with head-and-neck cancer were shown to express high levels of CD73 and to suppress effector T cells and NK cells through adenosine production. Other cell types composing the tumor microenvironment, such as Treg and mesenchymal stromal cells, have also been shown to produce $CD73^+$ exosomes [63,64].

1.11. CD73 in cancer therapy

Preclinical studies demonstrated the therapeutic potential of targeting CD73 with monoclonal antibodies (mAbs) [11,12], pharmacological inhibitors or siRNA [65,66]. These studies highlight the importance of both hematopoietic and non-hematopoietic expression of CD73 on tumor progression [12,30]. We here review the different mode-of-action of CD73 targeting agents.

1.12. Targeting CD73 with antibodies

Several therapeutic monoclonal antibodies (mAbs) targeting the enzymatic activity of CD73 are being developed (summarized in Table 1) and at least two have entered clinical trials. Anti-CD73 mAb MEDI9447 (Astrazeneca-Medimmune) is a modified IgG1 (with no Fc receptor binding) that inhibits the formation of the catalytically active site of CD73 [67]. In addition, MEDI9447 internalizes CD73 upon binding, which may target ectonucleotidase-independent activities. Anti-CD73 mAb BMS-986179 (Bristol-Myer Squibb) is a human IgG2-IgG1 hybrid also engineered with lack of Fc effector function. Interestingly, the IgG2 sequence of BMS-986179 enhances internalization of CD73. Further work is necessary to clarify the importance of internalization on the activity of anti-CD73 mAbs. Preliminary phase 1 data of BMS-986179 were recently reported [68]. As of Dec 19, 2017, 59 patients had been treated with BMS-986179 alone or with the anti-PD-1 mAb nivolumab. Overall, 7 patients with head and neck, pancreatic, prostate, anal, and renal cancer achieved confirmed partial responses and 10 had stable disease. CD73 target engagement in the tumor and periphery were reported, and safety profile of the combination was

Table 1
Ongoing clinical trials of inhibitors of CD73 in monotherapy or in combination in cancer patients.

INDICATION	PHASE		EXPERIMENTAL GROUPS		TARGET	IDENTIFIER	DEVELOPER	EST. COMPLETION
			DRUG	DRUG TYPE				
Advanced solid cancers	1/2a		BMS-986179	mAb	CD73	NCT02754141	Bristol-Myers Squibb	2020
		+/−	nivolumab	mAb	PD-1			
Advanced solid cancers	1/1b		CPI-006	mAb	CD73	NCT03454451	Corvus Pharmaceuticals, Inc.	2023
		+/−	pembrolizumab	mAb	PD1			
		or	CPI-444	S.M.	A2a			
Solid tumors	1		MEDI9447	mAb	CD73	NCT02503774	MedImmune-AstraZeneca	2022
		+/−	durvalumab	mAb	PD-L1			
Relapsed ovarian	2		MEDI9447	mAb	CD73	NCT03267589	MedImmune-AstraZeneca	2019
		+/−	durvalumab	mAb	PD-L1			
EGFR mut	1b/2		MEDI9447	mAb	CD73			
NSCLC		+/−	AZD4635	S.M.	A2a	NCT03381274	MedImmune-AstraZeneca	2022
		+/−	osimertinib	S.M.	EGFR			

Abbreviations: mAb = monoclonal antibody; S.M. = small molecule; Est. = estimated; mut = mutated

similar to that of nivolumab monotherapy.

1.13. Fc-dependent therapeutic activity of CD73 targeted blockade

Vijayan et al. and Young et al. [69,70] investigated the role of Fc receptor engagement in anti-tumor effects of anti-CD73 mAbs. Young et al. reported that anti-metastatic activities of an anti-mouse CD73 mAb depended on the engagement of FcγRIV on $CD11b^+$ $Gr\text{-}1^+$ myeloid cells [70]. Using distinct human and mouse CD73 cross-reactive mAbs with differences in enzymatic inhibition, Fc binding and CD73 internalization, Vijayan et al. compared anti-tumor effects against mouse and human tumors. In preclinical models, the most efficient inhibition of primary tumor growth required both inhibition of CD73 enzymatic activity and Fc receptor engagement. Notably, control of spontaneous and experimental tumor metastasis was largely dependent on Fc receptor-mediated functions. In addition, engagement of Fc receptors was required for optimal anti-metastatic therapeutic effect of a combination of anti-CD73 mAb with an A2a antagonist or anti-PD-1 mAb.

1.14. Inhibition of CD73 with small molecules

Disclosed small molecule programs targeting CD73 include those of GlaxoSmithKline (GSK) and Arcus Biosciences. GSK filed patent around novel substituted benzothiadiazine derivatives as CD73 inhibitors [71]. Arcus's CD73 inhibitor (AB680) showed low clearance and good tolerability at high doses in rodent and non-rodent models, high potency and high selectivity, and restored IFN-γ production and proliferation of human $CD4^+$ and $CD8^+$ T cells activated in vitro in presence of exogenous AMP [72]. If intracellular CD73 activity is important for tumor growth [34], small molecules might be advantageous over mAbs. Head-to-head comparative studies are still however lacking. For some indications, such as brain cancers, small molecules that cross the blood-brain barrier (BBB) may also be advantageous, although disrupted BBB can permit mAb penetration.

1.15. Targeting multiple targets within the adenosinergic pathway

Since the adenosinergic axis is composed of multiple enzymes and receptors, targeting multiple components of the adenosinergic pathway to achieve enhanced therapeutic benefit has been the subject of recent investigations. Simultaneous administration of an anti-CD73 mAb and an A2a receptor antagonist was shown to be synergistic for control of tumor metastasis [70]. Mechanistically, A2A blockade increases CD73 expression on tumor cells and CD73 blockade results in enhanced therapeutic activity. In addition, tumor growth was also decreased in CD73 and A2a receptor double-KO mice, which showed greater $CD8^+$ T cell infiltration than single KO mice. These results argue for combination therapy targeting both these components simultaneously. Development of bispecific antibodies, targeting multiple epitopes of CD73 or targeting CD73 and other molecules is being pursued [73] as is the development of bispecific inhibitors of CD73 and the A2a receptor [74]. Synergistic effects when targeting other adenosinergic axis molecules, such as the A2b adenosine receptor, CD39 (to increase immunostimulatory ATP levels), or CD38 further need investigation.

1.16. Targeting CD73 in combination therapies

A number of preclinical studies demonstrated synergism between anti-CD73 and anti-PD-1 or anti-CTLA-4 [75–77]. Synergism has also been observed between checkpoint inhibitors and A2a antagonists [14,78,79]. High levels of CD73 gene expression are associated with poor clinical outcome in breast cancer patients treated with trastuzumab [20]. In immunocompetent mouse models of HER2/ErbB2-driven breast cancer, CD73 expression by tumor cells and host cells was found to significantly suppress immune-mediated responses mediated by anti-ErbB2 mAb [20]. Anti-CD73 mAb therapy was further shown to enhance the activity of anti-ErbB2 mAb to treat engrafted or spontaneous tumors as well as lung metastases [20].

The potential benefit of inhibiting the adenosinergic axis in the context of targeted therapies has also recently been investigated. In a recent publication, an increase of CD73 expression in melanoma patients with advanced BRAF mutation positive disease was documented [80]. Interestingly, administration of BRAF inhibitor with or without MEK inhibitor led to a decrease of CD73 expression, and in a mouse melanoma model, a significant therapeutic suppression of tumor initiation and metastasis was observed following anti-BRAF, MEK and A2a receptor inhibitor combination [80]. Emergence of $CD73^+$ melanoma cells was also described during adoptive T cell therapy or anti-PD1 treatment [17], raising a possibility of a therapeutic benefit of targeting CD73 in combination with adoptive T cell therapy. Finally, synergy has been reported between targeting of CD73 and chemotherapy. The biologic basis of this synergy may stem from the prevention of adenosine-induced immunosuppression subsequent to the release of ATP following drug-induced cell death [29]. This may be highly relevant also because, as discussed previously, administration of some chemotherapeutic agents leads to CD73 upregulation [81]. A similar scenario may occur following radiation therapy, which on top of inducing ATP release and ATP to adenosine conversion may also worsen tumor hypoxia [81].

1.17. CD73 as a biomarker to response to immunotherapy

Immune checkpoint inhibition has become a standard of care for

Table 2
Prognostic value of CD73 in human cancers.

		Year	Pts (n)	CD73 Expression Analysis	Reported prognostic findings	References
Gastro-intestinal	Gastric cancer					
	Gastric carcinoma	2013	68	Immunohistochemistry	Overexpressed in tumor tissue Higher expression associated with tumor differentiation, histopathology, depth of invasion, nodal status, metastasis, and stage Poor prognosis (OS)	[86]
	Gallbladder Cancer					
	GBD carcinoma	2014	108	Immunohistochemistry	Overexpressed in tumor tissue Marker of disease progression, clinical biological behaviors and prognosis (OS)	[87]
	Colorectal Cancer					
	Rectal Adenocarcinoma	2015	90	Immunohistochemistry	Overexpressed in both tumor and stromal cells Good prognosis (OS) when expressed in the tumor stroma Poor progrosis (OS) when expressed in tumor cells Better prognostic value when both compartment combined in analysis	[88]
	CRC	2012	16	Western Blot	Overexpressed in tumor tissue	[89]
			223	Immunohistochemistry	Poor prognosis (OS)	
	Neuroendocrine neoplasms					
	Gastro-intestinal	2018	136	Immunohistochemistry	Higher expression in neuroendocrine carcinoma compared with neuro-endocrine tumor (G1/G2) Potential biomarker of anti-PD-1 therapy	[90]
Gynec ological	Breast Cancer					
	TNBC	2013	6.209	Gene-Expression Profiling	Poor prognosis (OS) Association with anthracycline resistance	[29]
		2017	122	Immunofluorescence	Poor prognosis (DFS, OS) and reduced anti-tumor immunity	[91]
	ER+	2012	1.466	Public Microarray data	No prognostic value	[92]
	ER-	2012	476	Public Microarray data	Poor prognosis (RFS)	[92]
	Stage I-III	2012	136	Immunohistochemistry	Good Prognosis (DFS, OS)	[93]
	All Types	2012	256	Methylation-specific PCR; Pyrosequencing	Epigenetically regulated (downregulated when methylated) NT5E CpG island methylation is associated with a good prognosis (DFS, OS, metastatic potential)	[23]
	Ovarian Cancer					
	HGS	2015	285	Immunohistochemistry	Poor prognosis (DFS, OS)	[52]
	Epithelial Ovarian Cancer	2012	167	Immunohistochemistry	Good prognosis (OS)	[94]
Urological	Kidney carcinoma					
	RCC	2015	159	Immunohistochemistry	Associated with tumor type, tumor node metastasis stage and tumor grade Poor prognosis (OS)	[95]
	Bladder Cancer					
	Urothelial Carcinoma	2015	174	Immunohistochemistry	Associated with lower stage, lower grade, less adjacent carcinoma in situ and lower Ki-67 expression Good prognosis (PFS)	[96]
	Prostate Cancer					
		2013	116	Immunohistochemistry; Western blot; qRT-PCR	Marker of lymph node metastasis	[97]
		2016	285	Immunohistochemistry	Poor prognosis in normal adjacent epithelium (BCR-free survival and metastasis-free survival) Good prognosis in tumor stroma (BCR-free survival) High CD73 expression confers a negative prognostic value to intraepithelial CD8+ T infiltration	[98]
Other	Brain Cancer					
	GBM	2013	500	TCGA microarray database	Poor prognosis (DFS)	[99]
	Lung Cancer					
	NSCLC	2017	642	Immunohistochemistry	Poor prognosis (RFS, OS)	[25]
	Head and Neck Cancer					
	HNSCC	2018	65	Immunohistochemistry	Associated with tumor stage Poor prognosis (OS)	[100]
	Hematological Neoplasm					
	B-ALL	2011	270	Genome-wide gene expression comparison	Higher expression in leukemic cells Marker of minimal residual disease	[101]
	B-CLL	2011	299	Immunohistochemistry	Marker of aggressiveness Correlated with CD38 and ZAP-70 expression, two markers of disease progression	[102]
	Melanoma					
	Malignant Melanoma	2012	52	Methylation-specific PCR	Epigenetically regulated (downregulated when methylated) NT5E CpG island methylation is associated with limited metastatic potential	[22]

Abbreviations: Pts = patients; TNBC = Triple-Negative Breast Cancer; OS = Overall Survival; ER+ = Estrogen Receptors positive; ER- = Estrogen Receptors negative; RFS = Recurrence-Free Survival; DFS = Disease-Free Survival; NSCLC = Non-Small-Cell Lung Cancer; HNSCC = Head and Neck Squamous Cell Carcinoma; B-ALL = B-cell Acute Lymphoblastic Leukemia; B-CLL = B-cell Chronic Lymphocytic Leukemia; GBM = Glioblastoma Multiform; HGS = High-Grade Serous; BCR = Biochemical relapse; CRC = Colorectal Cancer; PD-1 = programmed death-1; GBD = Gallbladder; AJCC = American Joint Committee on Cancer; RCC = Renal Cell Carcinoma; PFS = Progression-free survival.

patient with metastatic melanoma. Nivolumab is an approved antibody targeting PD-1 for the treatment of advanced melanoma. PD-L1 is well described to be induced on cancer cells upon exposure to IFN-γ [82]. Therefore, tumor status for PD-L1 is not sufficient for predicting what patient will benefit or not from anti-PD-1 therapy. In this regard, Morello et al. showed in advanced melanoma that patients with higher soluble CD73 enzyme activity in their serum prior to nivolumab therapy, had a lower response rate and worse outcome advocating soluble CD73 enzyme activity as a potential prognostic marker to tailor patient treatment [83]. Consistently, TCGA analysis of anti-PD-1 treated melanoma patients identified CD73 expression as a negative prognostic factor (presented at the AACR 2018, [84]). Interestingly, evidence suggest that soluble levels of CD73 are significantly increased in the plasma of cancer patients when compared with healthy controls [85]. Future validations of these results are needed to support the use of soluble CD73 as an effective biomarker.

1.18. Prognostic value of CD73 in cancer

The reported findings in recent literature regarding the prognostic value of CD73 in human cancers are summarized in Table 2. In various malignancies, CD73 is found at higher levels in the tumor when compared to adjacent healthy tissues. In addition, overexpression of CD73 can be found correlated with tumor neovascularization, invasiveness and metastasis. Overall, high CD73 expression is generally associated with poor patient outcomes and correlates with a more aggressive tumor.

2. Conclusion

Immunotherapy has revolutionized cancer treatment management in the past few years. CD73 has gained much appreciation as a novel immune checkpoint target following several conclusive pre-clinical studies and because of the bad prognosis associated with its expression in many human cancers. Several clinical trials involving antibodies targeting CD73 in cancer patients are currently ongoing. In conclusion, while measurement of soluble levels of CD73 in serum of cancer patients may be a promising diagnostic tool that needs further validation, targeting of CD73 has proven efficient in many pre-clinical trials and ongoing clinical trials ought to provide promising hopes to add to the arsenal of immunotherapeutic tools to fight off cancer.

Disclosure of conflict-of-interest

J. Stagg is a permanent member of the Scientific Advisory Board of Surface Oncology, holds stocks of Surface Oncology and has received research grants from Surface Oncology.

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