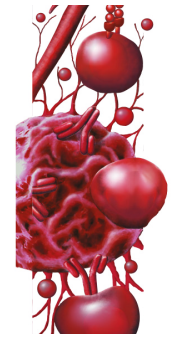
Review

For reprint orders, please contact: reprints@futuremedicine.com

Immunotherapy



CD73: an emerging checkpoint for cancer immunotherapy

Siqi Chen[1], Derek A Wainwright[2], Jennifer D Wu[3], Yong Wan[4], Daniela E Matei[4], Yi Zhang[5] & Bin Zhang*,[1]

[1]Robert H Lurie Comprehensive Cancer Center, Department of Medicine-Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

[2]Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

[3]Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

[4]Department of Obstetrics & Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

[5]Biotherapy Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, Henan, China

*Author for correspondence: Tel.: +1 312 503 2435; Fax: +1 312 503 0189; bin.zhang@northwestern.edu

CD73 is a novel immune checkpoint associated with adenosine metabolism that promotes tumor progression by suppressing antitumor immune response and promoting angiogenesis. The inhibition of CD73, in combination with immune checkpoint blockade, targeted therapy or conventional therapy, improves antitumor effects in numerous preclinical mouse models of cancer. Emerging evidence suggests that the combination of anti-CD73 and immune checkpoint blockade has promising clinical activity in patients with advanced solid tumors. In this review, we will discuss the specific role of CD73 on both tumor cells and non-tumor cells in regulating tumor immunity and tumorigenesis and provide an update on the current view of the antitumor activity of targeting CD73 by mAb or small molecule selective inhibitors in preclinical and clinical settings.

First draft submitted: 9 December 2018; Accepted for publication: 7 June 2019; Published online: 21 June 2019

Keywords: adenosine receptor • CD73 • immune checkpoint blockade • immunotherapy • tumor evasion

CD73, known as ecto-5′-nucleotidase, is a cell surface glycosylphosphatidlinositol-anchored glycoprotein. CD73 is essential for the generation of extracellular adenosine from 5′-adenosine monophosphate (5′-AMP) through the coordinated action of CD39 (ecto-nucleoside triphosphate diphosphohydrolase-1; ecto-NTPDase1) that catalyzes the phosphohydrolysis of ATP and ADP to AMP) [1,2]. Additionally, extracellular adenosine could be generated from the noncanonical pathway of CD38 (NAD^+ nucleosidase)-CD203a (ecto-nucleotide pyrophosphatase/phosphodiesterase 1)-CD73 that is independent of CD39 [3]. CD73 is found on the surface of a variety of cell types, including endothelial cells, subtypes of lymphocytes, stromal cells and select types of tumor cells. Adenosine induces immunosuppression [4], angiogenesis [5], mucosal hydration [6] and ischemic preconditioning [7]. During pathophysiological conditions, CD73-generated adenosine protects from tissue destruction through inflammation, ischemia and hypoxia [2]. Despite the lack of extracellular adenosine signaling in $CD73^{-/-}$ mice, extracellular ATP and ADP levels remain almost completely unchanged. Strikingly, $CD73^{-/-}$ mice are viable, indicating that CD73 is not vital under normal physiologic conditions. However, recent work found that, CD73 insufficiency contributes to arterial calcification in humans with an autosomal recessive disease caused by loss-of-function mutations of *Cd73*. CD73 knockout ($CD73^{-/-}$) mice also recapitulated some of the characteristics associated with arterial calcification cue to deficiency of CD73 (ACDC) [8].

Previous studies have underscored a negative role for extracellular adenosine within the tumor microenvironment by activating four distinct adenosine receptors (G protein-coupled receptors): A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R. Adenosine activation of $A_{2A}R$ triggers cyclic AMP and protein kinase A signaling, which inhibits T cell receptor signaling [9] and promotes the induction of $Foxp3^+$ regulatory T cells (Tregs) [10]. In contrast, adenosine activation of $A_{2B}R$ decreases the barrier function of vascular endothelium [11] and promotes myeloid cells [12–15] to acquire an anti-inflammatory (M2) phenotype that suppresses immune-mediated tumor cell eradication. Consistent with the results from $A2AR^{-/-}$ [16] and $A2BR^{-/-}$ mice [17], we and others have shown that $CD73^{-/-}$ mice more readily

Future Medicine

reject tumors as compared with wild-type (WT) mice due to enhanced antitumor immunity [18] and decreased carcinogenesis [19]. Unexpectedly, one recent report indicated a decrease of CD73 expression in poorly differentiated advanced-stage endometrial carcinoma and ovarian high-grade serous carcinoma [20]. Strikingly, CD73-generated adenosine inhibits disease progression in these tumors [20]. CD73-generated adenosine seems to be required to sustain a physiological balance that maintains epithelial integrity in endometrial carcinomas with stable cell–cell adhesions mediated by A_1R. As the epithelial barrier function is essential for the inhibition of endometrial carcinomas with fewer intervening stromal or inflammatory cells in interconnected malignant glands [21], the loss of CD73 promotes adenosine deaminase endometrial tumor progression. This phenomenon is completely contrary to that observed in other tumor types, and results likely from context-dependent functions of the receptor in different tumor types. These findings provide a distinct perspective on the role of CD73-generated adenosine in tumor development. The roles of CD73 on tumor and host cells as well as its prognosis and therapeutic value in human cancers have been recently discussed [22,23]. In this review, we aim at providing a broader view of how CD73 is involved in the immune evasion and tumor progression, thereby highlighting the importance of combination treatment arising from CD73 targeting currently under investigation in early phase clinical trials. The limitations, challenges and the possible future research directions of anti-CD73 immunotherapy are also reviewed.

Effects of CD73 activity by tumor cells

CD73 is expressed by many different types of cancer and promotes tumor outgrowth, metastasis [18,24–31] and drug resistance [32,33] among leukemia, malignant glioma, melanoma, ovarian-, colon-, breast- and bladder-cancer [34–37].

A number of factors contribute to the modulation of CD73 expression on tumor cells. Estrogen receptors (ERs) have been shown to dominantly and negatively regulate CD73 expression, and its ability to generate adenosine in breast cancer [37]. As a result, the increased expression of CD73 may relate to breast cancer progression in a subset of ER-negative tumor cells. CD73 expression is also often induced under hypoxic conditions in the tumor microenvironment, as the *cd73* gene transcription is directly mediated by HIF-1α [38–41]. In addition, many proinflammatory factors promote induction of CD73 expression, including TGF-β, IFNs, TNF, IL-1β and prostaglandin E2 [42,43]. Furthermore, tumor cell CD73 expression is regulated through the Wnt and cAMP pathways [44,45]. CD73 expression is also induced epigenetically, as CD73 expression is downregulated via methylation-dependent transcriptional silencing in human melanoma cell lines [46]. Particularly, melanomas lacking CD73 methylation are more likely to relapse. In addition, activated MAPK pathway in cooperation with the proinflammatory cytokines such as TNFα, promotes CD73 expression on melanoma cells [47,48]. Emerging evidence also suggests aberrant CD73 regulation at the transcriptional and post-transcriptional (e.g., miRNA) level in a variety of different cancer subtypes [49]. Together, these observations collectively support the potential for therapeutically targeting CD73 in melanoma and beyond.

The extracellular adenosine generated by CD73-expressing tumor cells [24,25] negatively regulates the activation and effector phases of the antitumor T cell response, while also promoting T cell apoptosis. CD73 is also required for cancer cell proliferation independent of immune regulation. For example, silencing CD73 expression with specific shRNAs inhibits the proliferation of breast cancer cells (MB-MDA-231), leading to increased cell-cycle arrest and apoptosis [31]. Likewise, treatment with APCP (α, β-methylene adenosine-5-disphosphate), a selective CD73 enzyme inhibitor, inhibits cancer cell proliferation in a dose-dependent manner [31,50,51]. Conversely, CD73 overexpression in breast cancer cells (MCF-7) increases cell viability and promotes cell-cycle progression. Similarly, CD73 overexpressing MCF-7 cells grow more rapidly than parental MCF-7 cells, while suppressing CD73 mRNA with siRNA suppresses tumor growth in mouse xenograft models [28,31]. In glioma cells, APCP treatment causes a 30% reduction of cell proliferation, while the addition of adenosine increases cell proliferation by 35%. Taken together, CD73-generated adenosine may promote cancer cell growth via its enzyme activity [29]. However, this effect is not universal, as adenosine induces apoptosis in gastric carcinoma cells [52], and ovarian cancer cells through the pro-apoptotic molecules Bax and caspase-3 [53]. Tumor cell CD73 expression also promotes tumor metastasis in mouse models, likely depending on the autocrine activation of A_{2B}R [24]. Tumor cell CD73 expression [28–30], or the activation of other adenosine receptors [54,55], promotes chemotaxis and invasiveness. Strikingly, CD73 activity by tumor cells also involves tumor angiogenesis by facilitating VEGF production in a mouse breast cancer model [56]. CD73 is also overexpressed on cancer stem cells [57,58] or cancer-initiating cells [59], and CD73 inhibition attenuates sphere formation and tumor initiation [57,59] highlighting the druggability of CD73 in the context of cancer stem cell/cancer-initiating cell-directed therapies. These results indicate a complex and contextual role for CD73 in regulating cancer cell viability, stemness and immune suppression, warranting further investigation *in vivo*.

Effects of CD73 activity by nontumor cells

Endothelial cells

A subset of endothelial cells expresses CD73 [60] and may contribute to angiogenesis within the tumor microenvironment. Indeed, WT mice formed more capillary-like structures in pulmonary microvascular endothelial cells than $CD73^{-/-}$ hosts, and this difference was more evident when pulmonary microvascular endothelial cells were *in vitro* cultured with cancer cell-conditioned medium. The extent and density of tumor angiogenesis was greater in WT mice as compared with $CD73^{-/-}$ deficient mice *in vivo* [61]. Additionally, the treatment of anti-CD73 monoclonal antibody (mAb) or APCP led to impaired angiogenesis and decreased tumor growth in several murine tumor models [56,62]. There was also evidence showing that the *in vitro* formation of capillary-like tubes by human umbilical vein endothelial cells is affected by CD73 expression but independent of its associated enzyme activity (i.e., extracellular adenosine) [56]. Furthermore, tumor cell CD73 promotes metastasis through adenosine-independent attachment to endothelium [63]. Taken together, current studies demonstrate that both tumor and endothelial cell CD73 synergistically contribute to tumor angiogenesis. However, the exact role of adenosine-independent function of CD73 demands additional investigation.

In an experimental lung metastasis model, $CD73^{-/-}$ mice were found to be resistant to tumor metastasis after the intravenous injection of B16F10 melanoma cells or TRAMP-C1 prostate cancer cells [19,26]. Notably, the pro-metastatic effects of host CD73 were dependent on its expression by nonhematopoietic cells; most likely attributable to endothelial cells. On the other hand, we found that endothelial cell CD73 expression was associated with limited T cell infiltration of tumors [18] and an enhancement of tumor growth. Despite remaining not fully understood, the bulk of the existing evidence points to CD73-expressing endothelium as a contributor to tumor growth and metastasis.

T cells

Regulatory T cells (Tregs; $CD4^+CD25^+FoxP3^+$) mediate immunotolerance and help tumor cells evade immunosurveillance by suppressing the immune response. One of the main mechanisms for Treg-mediated tumor immunosuppression is dependent on the extracellular adenosine generated by CD73 [18]. CD73 is abundantly expressed by Tregs and is frequently coexpressed with CD39. CD73, in combination with CD39, renders an enzymatically driven accumulation of immunosuppressive adenosine by Tregs. Accordingly, Tregs derived from either $CD73^{-/-}$ or $CD39^{-/-}$ mice have impaired suppressive functions [64,65]. Unlike WT murine Tregs, $CD73^{-/-}$ Tregs fail to promote tumor growth [18,26]. Similarly, human Treg CD73 inhibition decreases their immunosuppressive capability, *in vitro* [66]. Coincidently, CD73 expression is observed on circulating Tregs isolated from head and neck cancer patients as compared with healthy donors [67]. In contrast to murine Tregs, natural human Tregs are often CD39-positive, but express little or no cell surface CD73. Inducible $CD39^+CD73^+$ Tregs significantly increase in peripheral blood, sentinel lymph node and tumor infiltrating lymphocytes of melanoma patients [68]; especially in response to high-dose IL-2 therapy [69]. Although the full role of CD73 on human Tregs is still being characterized, it is generally accepted that they generate adenosine through paracrine interaction(s) with local CD73-expressing cells and/or tumor-derived exosomes [70,71].

Aside from Tregs, murine $CD4^+Foxp3^-$ conventional T and $CD8^+$ T cells express CD73 when exposed to TGF-β. In healthy donors, peripheral human $CD8^+$ T cells express CD73 [72] and the activation of $CD8^+$ T cells downregulate CD73 expression [73]. Within tumors, CD73 is highly expressed by memory $CD8^+$ T cells and negligible on terminally differentiated effector $CD8^+$ T cells [74]. CD73-mediated adenosine signaling through $A_{2A}R$ is associated with the transition from naive/memory $CD8^+$ T cells into effector cells by the inhibition of Wnt-signaling [74,75]. CD73 expression is also associated with a subset of $CD4^+$ effector T cells enriched in polyfunctional Th1/17 cells [76]. In resected human breast and ovarian tumors, multicolor immunofluorescence confirms the codetection of $CD73^+CD4^+$ effector T cells and $CD39^+$ Tregs within the immune infiltrates [76]. Increased CD73 levels are associated with increased adenosine generation and immunosuppressive Th17 functions, and increased tumor growth in mouse tumor models [77]. In contrast, genetic ablation of CD73 or treating with IL-1β rather than TGF-β to program Th17 cells, *ex vivo*, induces stemness and improves the antitumor effects of adoptive Th17 cell therapy [78].

Natural killer cells

Within the tumor microenvironment, natural killer (NK) cells highly express CD73, which is found at negligible levels in healthy individuals [79]. NK cell CD73 expression endows them with suppressive functions via adenosine

generation. $A_{2A}R$ is primarily expressed by NK cells and upregulated further under pathological conditions, such as lung injury and inflammation [80]. $A_{2A}R$ activation suppresses the activation and cytotoxic function of NK cells [81–84]. Accordingly, CD73 adenosine and $A_{2A}R$ activation causes NK cell suppression of antitumor functions while simultaneously promoting tumor metastasis [81,85,86]. Therefore, blockade of the CD73-$A_{2A}R$ axis may potentiate the antitumor effects of NK cell-based immunotherapy. However, different from the effect of $A_{2A}R$ activation, $A_{3A}R$ agonists can stimulate NK cytotoxicity and promote the antitumor function [87]. Furthermore, the presence of adenosine renders NK cells hyporesponsive to IL-12 and IL-15 stimulation *ex vivo*, exhibiting enhanced IFN-γ expression from $CD56^+$ subsets [84]. These results argue against the adenosine-mediated general NK cell inhibition and suggest instead the cellular contexture dependent effect of adenosine on NK cells.

Macrophages

In healthy individuals, circulating $CD14^+$ monocytes express low levels of CD73, while tissue-resident macrophages express both CD39 and CD73 [88]. Activation of macrophages through TLR activation induces the release of ATP, which is rapidly hydrolyzed by cell surface CD39 [89]. The expression levels of CD39 and CD73 regulates adenosine generation [90]. Macrophages are divided into two primary subsets, the pro-inflammatory M1 population and the anti-inflammatory M2 population. M1 macrophages express lower levels of CD39 and CD73 as compared with the M2 subset [90]. Changes in the ectoenzyme activities of CD39 and CD73 may fine-tune their functions in the inflammatory setting. In a murine model of myocardial infection, CD73 blockade by APCP augmented the predominance of the M1 subset [91]. Similarly, ablating CD73 activity in tumor-bearing mice decreases M2 macrophages and increases the M1 subset [27]. However, LPS and TNF-α induced CD73 on human macrophages, and the addition of exogenous AMP or CD73 inhibitor has no effect on their polarization [92]. To better identify the effects of CD73 on macrophages during tumor outgrowth, additional studies are required to evaluate the importance of CD73 for differentiation and function of tumor-associated macrophages isolated, especially from human tumors.

Myeloid-derived suppressor cells

MDSCs (myeloid-derived suppressor cells) are a heterogeneous population consisting of different immature myeloid cell subsets that accumulate during tumor development. MDSCs infiltrate human tumors and their presence is associated with decreased efficacy of immune checkpoint blockade and other immunotherapies [93]. In tumor-bearing mice, CD39 and CD73 are coexpressed on MDSCs [14,94,95] and subsequent $A_{2B}R$ activation promote their expansion [14]. Furthermore, $A_{2B}R$ blockade reduces tumor-infiltrating MDSCs in a mouse melanoma model [96]. Conversely, treatment with an $A_{2B}R$ agonist enhances MDSC tumor infiltration [97]. These results are supported by human studies showing higher CD39 and CD73 levels on MDSCs isolated from cancer patients as compared with healthy donors [98–100]. CD39 and CD73 coexpression also identifies a distinct inflammatory subpopulation among activated MDSCs with enriched suppressive molecular signatures in non-small-cell lung cancer patients [99]. Specifically, tumor-derived TGF-β induces CD39/CD73 coexpression on MDSCs through activation of the mTOR-HIF-1α pathway. Moreover, MDSC CD39/CD73 contributes to their immunosuppressive function and predicts the response to chemotherapy [99]. Consistently, metformin treatment decreases the expression and ectoenzyme activity of CD73 on monocytic and polymononuclear MDSC subsets in ovarian cancer (OC) patients, which is associated with improved T cell-mediated antitumor immunity [100]. OC patients treated with metformin have a downregulation of CD39/CD73 expression on MDSCs through activation of AMP-activated protein kinase α and subsequent suppression of HIF-1α. Metformin treatment induces a simultaneous increase in the antitumor activities of circulating $CD8^+$ T cells and correlates with a longer overall survival in diabetic patients with OC. Collectively, these data suggest that the inhibition of CD39/CD73-dependent MDSCs may provide for new opportunities to improve future cancer immunotherapeutic strategies.

Other immune cells & stromal cells

CD73 has long been recognized to be a marker for B cell differentiation [101]. Several studies demonstrated that, CD73 is expressed on a subset of memory B cells [102,103]. *In vitro* blockade of B cell CD73 activity from healthy controls results in an impairment of IgG class switching. Although there is a paucity of data for B cell CD73 expression and its function in cancer, a recent study reported a critical role for B cells in providing antitumor activity while using APCP in a mouse melanoma model [104]. We have shown that CD73 is expressed on type 2 innate lymphoid cells (ILC2s) and can be significantly elevated on tumoral $ST2^+$ ILC2s in the IL-33-rich tumor

microenvironment [90]. Importantly, the ILC2-derived CD73 is likely involved in suppressing NK cell-mediated antitumor activities [90].

Cancer associated fibroblasts (CAFs) are an important stromal component in a majority of tumor microenvironments. High levels of CAF CD73 expression is associated with poor prognosis in patients with OC [105] and nonmuscle invasive bladder cancer [106]. CD73-expressing CAFs are also detected in human triple negative breast cancer [107] and OC [108]. In these two studies, CAFs enhanced the survival, as well as the content of Tregs; partly dependent on CD73 activity. In a mouse model of OC, CAF CD73 expression inhibits T cell-mediated antitumor immunity [105], similar to the immunosuppressive effect of tumor cell CD73 [25]. Furthermore, CD73 expression in prostate epithelium is associated with immune suppression and progression of prostate cancer, while its expression in the stroma is shown to be associated with a good disease prognosis [109].

CD73 blockade in cancer therapy

In 2006, the Sitkovsky group presented seminal findings on how A_2AR protect tumors from antitumor-armed effector T cells [16]. Based on the first report, accumulating evidence has supported the therapeutic potential of targeting the CD73-A_2AR axis in numerous types of cancer over the past decade. Stagg *et al.* originally showed that, CD73 mAb treatment inhibited breast tumor growth and metastasis [24], while we reported that CD73 small molecule inhibitor treatment with APCP decreased the progression of OC and increased animal subject survival [25]. These two independent groups both subsequently demonstrated that host and tumor cell CD73 contribute to tumor growth and metastasis using multiple mouse models, highlighting the requirement for optimal antitumor activity to target both tissue compartments. The different experimental methods for inhibiting CD73 expression and its activity in the preclinical setting is summarized in Table 1.

While single agent CD73 blockade is not curative, CD73 inhibition improves the antitumor activity of blockade immune checkpoint treatments including CTLA-4- and PD-1-mAb [116–119]. Strikingly, tumor cell CD73 expression attenuates the immune response evoked by anti-PD-1 mAb treatment, while anti-PD-1 mAb treatment augments the expression level of $A_{2A}R$, especially on $CD8^+$ tumor-infiltrating lymphocytes [117]. As expected, the combination of anti-PD-1 and anti-CD73 mAbs, or an $A_{2A}R$ antagonist, results in more effective antitumor T cell-mediated immune responses [117]. Interestingly, the activation of $A_{2A}R$ enhances PD-1 expression on tumor-specific $CD8^+$ T cells and $CD4^+$ $Foxp3^+$ Tregs [116], indicating a reciprocal loop of the CD73-$A_{2A}R$ axis and how PD-1 contributes to the synergy of combination immunotherapy. Indeed, the removal of hypoxic environment, a primary driver of the CD73-$A_{2A}R$ axis, promotes antitumor activity of dual PD-1 and CTLA-4 blockade in an $A_{2A}R$ dependent manner [85]. In addition, blockade of $A_{2A}R$ profoundly increases the antitumor activity of CAR T cell, particularly when combined with PD-1 blockade [120]. Similarly, anti-CD73 improves the intratumoral homing of CAR-NK *in vivo*. Furthermore, the combination of anti-CD73 with NKG2D-engineered CAR-NK cells achieves synergistic antitumor effect in $CD73^+$ human lung cancer xenograft model [121].

Aside from the immune checkpoint-targeted treatments, combination of agonistic anti-4-1BB therapy and CD73 blockade can achieve tumor regression in several mouse models of cancer [122]. Interestingly, anti-4-1BB treatment preferentially induces CD73-negative effector T cell response for tumor inhibition. However, the efficacy of agonistic anti-4-1BB therapy is diminished in TGF-β-rich tumors that sustain the expression of CD73 on intratumoral $CD8^+$ T cells. Mechanistically, the efficacy of anti-4-1BB therapy is determined by the activation of STAT3 and mutual regulation between TGF-β and 4-1BB ligation in concomitant $CD8^+$ T cell activity. Similar to anti-4-1BB treatment, either anti-GITR or anti-OX40 treatment preferentially drives CD73-negative $CD8^+$ T cell immunity. Accordingly, tumor regression has been achieved by combination therapy of CD73 blockade and anti-GITR [122]. Nevertheless, the contribution of CD73-mediated adenosinergic effect to the resistant mechanism of immunostimulatory agonistic cancer therapy remains to be further defined.

NAMPT inhibitors has been combined with anti-CD73 mAbs to improve immune-mediated antitumor effector function(s). NAMPT is a key enzyme in the regulation of intracellular NAD^+ pool. NAMPT inhibitors suppress tumor cell growth by reducing NAD^+ and ATP levels [123,124], and have entered the clinical trials. In human cells, CD73 is capable of converting extracellular NAD^+/nicotinamide mononucleotide to nicotinamide riboside as a precursor for intracellular NAD^+ biosynthesis [125,126]. Therefore, antitumor functions of combined NAMPT (FK866) and CD73 (APCP) inhibition leads to a significant increase in survival of tumor-bearing mice compared as compared with single treatment in a human OC model [127].

In immunocompetent mouse models of HER2/ErbB2-driven breast cancer, anti-CD73 mAb treatment improves the efficacy of anti-ErbB2 mAb for treating engrafted or spontaneous primary tumors and lung metastases [128].

Table 1. The strategies to block CD73 in tumor cells and tumor-bearing animals.

Tumor cell	Cancer type	Treatment	Efficiency
MDA-MB-231	Human breast cancer	APCP	Xenograft of MDA-MB-231 cells in nude mice with APCP treatment have a lower volume and weight than those of the control group [50]
		Anti-CD73 (AD2)	Anti-CD73 monoclonal antibody inhibits metastasis formation by a mechanism independent of CD73 catalytic activity but fails to inhibit primary tumor growth [110]
		SiRNA-CD73	CD73 siRNA effectively causes MB-MDA-231 tumor growth suppression *in vitro* and *in vitro*, prevention of adhesion to extracellular matrix and inhibition of invasion and migration [28,31]
U138MG	Human brain glioblastoma	APCP	*In vitro* APCP treatment of glioma cells results in a significant reduction of glioma cell proliferation [111]
T24	Human bladder carcinoma	APCP	*In vitro* APCP treatment results in a significant decrease of T24 cell number as compared with control cells [51]
GBC-SD	Human gallbladder carcinoma	SiRNA-CD73	CD73 knockdown inhibits tumor cell proliferation, migration and motility [112]
Glioblastoma Multiforme	Human brain cancer	SiRNA-CD73	CD73 knockdown reverses multiple-drug resistance of GBM cells [33]
E0771	Murine breast cancer	Anti-CD73 (TY/23)	Anti-CD73 monoclonal antibody treatment significantly delays primary E0771 tumor growth in immune-competent mice [24]
ID8	Murine ovarian cancer	APCP	A survival advantage is observed in ID8-bearing mice with APCP treatment *in vivo* [25]
		Anti-CD73 (TY/23)	Peritoneal ID8 tumor model showed a median 146-day survival of mice treated with TY/23 as superior to that of mice without treatment [18]
ID8-OVA	Murine ovarian cancer	SiRNA-CD73	CD73 knockdown renders tumor cells more susceptible to T-cell killing [25]
B16-SIY	Murine melanoma	APCP	*In vivo* APCP treatment effectively suppresses B16-SIY tumor growth [18]
		Anti-CD73 (TY/23)	Delayed tumor growth was observed in B16-SIY bearing mice treated with the anti-CD73 mAb TY/23 as compared with a control group [18]
B16F10	Murine melanoma	APCP	*In vivo* APCP treatment induces significant tumor regression by promoting the release of Th1- and Th17-associated cytokines in the tumor microenvironment [104]
		CD73 ablation	CD73 ablation significantly suppresses the growth and metastasis of B16F10 [18]
4T1.2	Murine breast cancer	Anti-CD73 (TY/23)	Anti-CD73 monoclonal antibody treatment significantly delays primary 4T1.2 and E0771 tumor growth in immune-competent mice and significantly inhibits the development of spontaneous 4T1.2 lung metastases [24]
4T1	Murine breast cancer	siRNA-CD73 loaded nanoparticle	Decreased expression of tumor cell CD73 contributes to decreased tumor growth and metastasis and improves animal subject survival [113,114]
MCA-induced fibrosarcoma	Murine	Anti-CD73 (TY/23)	Anti-CD73 monoclonal antibody treatment effectively suppresses growth of established 3-methylcholanthrene (MCA)-induced tumors [19]
		CD73 ablation	CD73 deficiency suppresses the development of MCA-induced fibrosarcomas [19]
TRAMP-C1	Murine prostate cancer	Anti-CD73 (TY/23)	Anti-CD73 monoclonal antibody treatment suppresses the growth of TRAMP-C1 prostate tumors and inhibits the development of TRAMP-C1 lung metastases [19]
Prostate cancer	Murine	CD73 ablation	CD73 deficiency also suppresses prostate tumorigenesis in TRAMP transgenic mice [19]
LWT1	Murine melanoma	Anti-CD73 (TY/23)	Anti-CD73 monoclonal antibody treatment significantly decreases metastatic burden as compared with control treatment [115]
		CD73 ablation	CD73 ablation improves metastatic control as compared with wild-type mice [115]
MC38-OVA	Murine colon cancer	CD73 ablation	CD73 ablation significantly suppresses the growth of MC38-OVA [26]
EL4/EG7	Murine lymphoma	CD73 ablation	CD73 ablation significantly suppresses the growth of EL4/EG7 [18,26]
AT-3	Murine mammary tumor	CD73 ablation	CD73 ablation significantly suppresses the growth of AT-3 [26]

A recent study reported the increased CD73 expression in melanoma patients with advanced BRAF mutation$^+$ disease [129]. Administration of BRAF inhibitor, with or without MEK inhibitor, downregulates CD73 expression, and combination anti-BRAF, MEK and A2A receptor inhibitor treatment results in a significant suppression of tumor initiation and metastasis in a mouse melanoma model [129]. It is becoming increasingly appreciated that a synergy occurs during concurrent treatment with CD73 blockade and conventional antineoplastic agents against cancer [130]. CD73 inhibition enhances doxorubicin-mediated antitumor effects in mice with established metastatic breast tumors [131]. In contrast, CD73 blockade triggers a beneficial effect in the tumor microenvironment of

Table 2. CD73 blockade in the clinic.

Tumor	Drug	Phase	Period	Sponsor	Identifier
Advanced solid tumors	MEDI9447 as monotherapy and in combination with MEDI4736	Phase I	2015–2021	MedImmune LLC	NCT02503774
Advanced solid tumors	BMS-986179 as monotherapy and in combination with nivolumab (BMS-936558)	Phase I/IIa	2016–2020	Bristol-Myers Squibb	NCT02754141
NSCLC, RCC, PDAC, Ovarian cancer, MSS and TNBC	NZV930 as monotherapy and in combination with PDR001 and/or NIR178	Phase I	2018–2022	Novartis Pharmaceuticals	NCT03549000
NSCLC	MEDI9447 in combination with osimertinib or AZD4635	Phase I Phase II	2018–2021	MedImmune LLC	NCT03381274
Ovarian cancer	MEDI9447 in combination with durvalumab (anti-PD-L1), tremelilumab (anti-CTLA-4), and MEDI0562 (anti-OX40)	Phase II	2018–2023	Nordic Society for Gynaecologic Oncology	NCT03267589
TNBC	MEDI9447 in combination with paclitaxel plus carboplatin and durvalumab	Phase I Phase II	2018–2022	Jules Bordet Institute	NCT03616886
NSCLC, RCC, CRC, TNBC, cervical cancer, ovarian cancer, pancreatic cancer, endometrial cancer, sarcoma, HNSCC, bladder cancer and mCRPC	CPI-006 as monotherapy and in combination with CPI-444 or pembrolizumab	Phase I	2018–2023	Corvus Pharmaceuticals, Inc.	NCT03454451

CRC: Colorectal cancer; HNSCC: Squamous cell carcinoma of the head and neck; mCRPC: Metastatic castration-resistant prostate cancer; MSS: Colorectal cancer microsatellite stable; NSCLC: Non-small-cell lung cancer; PDAC: Pancreatic ductal adenocarcinoma; RCC: Renal cell cancer; TNBC: Triple-negative breast cancer.

irradiated tumors, reflected by an increased infiltration and activity of $CD8^+$ T cells and dendritic cells, with an accompanying decrease of Treg accumulation [132], associated with complete tumor regression during dual CD73 and CTLA-4 blockade in combination with irradiation.

CD73 in clinic

In different types of human cancer, including melanoma, breast cancer, acute lymphocytic leukemia, CLL, glioblastoma, head and neck cancers, high-grade serous OC, endometrial cancer, colorectal, prostate, bladder cancer, gastric cancer, kidney cancer, and pancreatic carcinomas [133,134], the high expression level of CD73 within tumor microenvironment is often associated with worse clinical outcomes. Recently, 122 samples from the BIG 02-98 adjuvant Phase III clinical trial further supports the association of a worse prognosis with higher CD73 expression in the resected tumor. A reduced anti-tumor immune response also correlates with CD73 expression on those samples [135]. CD73 upregulation is also detected in melanoma patients progressing under adoptive T-cell transfer or immune checkpoint blockade [136], indicating a potential adaptive resistance mechanism.

Several inhibitors targeting CD73 or $A_{2A}R$ are currently being developed and tested in early clinical trials. CD73-targeted mAbs include MEDI9447, BMS-986179, NZV930 and CPI-006 (Table 2). MEDI9447 (Astrazeneca-Medimmune) is a human IgG1λ mAb selectively binding to and inhibiting the ectonucleotidase activity of CD73, which crossreacts with both mouse and human CD73 [119,137]. MEDI9447 removes CD73 from the cell surface by internalization, resulting in the inhibition of AMP conversion into adenosine, relieving AMP-mediated inhibition on T cell proliferation [119]. In immunocompetent mouse tumor models, MEDI9447 decreases immunosuppression and promotes antitumor function(s) [119]. Moreover, the combination of MEDI9447 with an anti-PD-1 antibody, induces greater antitumor effects as a single agent, supporting the initiation of multiple MEDI9447-based Phase I/II trials. Preliminary Phase I data of MEDI9447 (NCT02503774) were recently reported [138]. Treatment with MEDI9447 and durvalumab (anti-PD-L1) had a manageable safety profile and PD consistent with its mechanism of action. There is encouraging clinical activity in pancreatic cancer, and potentially, in colorectal cancer patients following combination therapy. Similar to MEDI9447, BMS-986179 (BMS) is a high-affinity antibody that inhibits CD73 enzyme activity and mediates the internalization of CD73. BMS-986179 treatment enhances antitumor activities of anti-PD-1 mAb in preclinical models [139]. Importantly, preliminary results of the first-in-human Phase I/IIa study of BMS-986179 + nivolumab (anti-PD-1 mAb) in patients with advanced solid tumors (NCT02754141), showed a safety profile similar to that of nivolumab monotherapy and with measurable immunological responsiveness [140]. Aside from anti-CD73 mAbs, small molecule inhibitors targeting CD73 have generated considerable interest. Novel substituted benzothiadiazine derivatives are being developed by GSK

to inhibit CD73 activity [141]. Arcus Biosciences have discovered AB680, a highly potent, reversible and selective inhibitor of CD73, with a favorable projected human PK profile suitable for parental administration [142]. Moreover, coadministration of AB680 with an anti-PD-1 mAb, results in a significant reduction of tumor growth associated with increased tumor-infiltrating immune cells [143]. According to a recent global immuno-oncology landscape survey in September 2018 [144], there are 16 active reagents targeting CD73 currently under development. These data collectively support the further evaluation of this approach with other immune checkpoint inhibitors for patients with advanced solid tumors in large cohort clinical studies.

Interestingly, CD73 and $A_{2A}R$ functions have recently been shown to be nonredundant in the tumor microenvironment. Antitumor activity of combined inhibition with CD73 and $A_{2A}R$ coinhibition is more potent than monotherapy with either agent. Thus, targeting these interdependent pathways combinatorially, significantly suppresses tumor initiation, growth and metastasis [115]. Notably, combination AZD4635 (A2AR inhibitor) with MEDI9447 treatment in NSCLC patients is currently undergoing clinical trial (NCT03381274) evaluation.

Importantly, the soluble CD73 (sCD73) enzyme activity is observed in the plasma [145–147]. In patients with metastatic melanoma stage IV, increased basal levels of sCD73 enzyme activity in the blood, before starting nivolumab treatment, are associated with lower response rates to therapy [148], suggesting a potential value of measuring sCD73 activity in the serum from cancer patients to predict the benefit of immune checkpoint therapy [148]. Interestingly, sCD73 and alkaline phosphatase catabolizes AMP to adenosine, whereas soluble ADA (sADA) mediates conversion of adenosine to inosine [145]. There are also significantly higher activities of serum total sADA in patients with breast cancer than healthy subjects [149,150]. However, the expression patterns of these plasma purine-metabolizing enzymes that result in elevated plasma concentrations of anti-inflammatory adenosine in cancer patients remain elusive. Still, more researches are needed to confirm if the sCD73 and/or sADA can be used as serologic prognostic biomarkers in the future.

Conclusion

It has become clear that, CD73 generates adenosine that suppresses antitumor immunity and contributes to tumor outgrowth and/or metastasis. Apart from the immune-dependent effect(s) of CD73, it also promotes tumorigenesis independent of the immune effects. Strikingly, CD73 blockade also suppresses angiogenesis in the tumor [56,61], and both CD73-mediated enzyme and nonenzyme functions contribute to tumor angiogenesis [56]. CD73 also promotes tumor cell adhesion to extracellular matrix proteins [151,152]. These results have led to continued interest in the rapid development of anti-CD73 cancer therapy using mAb and/or small molecule inhibitors in early stage clinical trials. In addition, high levels of CD73 are associated with lymph node metastases and poor prognoses in various types of cancer [153]. CD73 is found to be an independent prognostic factor in cancer patients, including prostate cancer [154] and triple-negative breast cancer (TNBC) [155]. Furthermore, a recent study suggests that sCD73 is a reliable biomarker in patients with metastatic melanoma patients treated with nivolumab [148]. Whether surface CD73 and/or sCD73 serves as a viable biomarker requires further validation.

Future perspective

The field of cancer immunotherapy is evolving rapidly, and CD73-targeted therapies serve as a valuable example of new immune target derived from fundamental new therapeutic approaches. Despite the effective preclinical antitumor activity by CD73 inhibition, in combination with blockade of other immune checkpoints or agonist immunotherapies, the clear therapeutic potential of CD73-targeted therapy remains minimally defined to date in patients with cancer. In addition, manipulating the metabolism of adenosine from CD73 as therapeutic modalities targeting the downstream cascade (such as adenosine deaminase) appears compelling. Deep characterization of CD73 expression, and its function as well as the downstream cascade in the tumor microenvironment during ongoing clinical trials, holds the key to addressing the potential for targeting CD73-mediated adenosinergic axis to substantially benefit human subjects with aggressive malignancies.

Acknowledgments

We thank S Khare for her critical reading of the manuscript.

Financial & competing interests disclosure

This research was in part supported by National Institutes of Health (grant numbers CA149669, CA208354 and CA222963) P50CA221747 SPORE Pilot, Melanoma Research Alliance Pilot Award, the Marsha Rivkin Center for Ovarian Cancer Research Pilot

Award, CBC HTS Supplemental Grant to B Zhang, Northwestern University RHLCCC Flow Cytometry Facility, a Cancer Center Support Grant (grant number NCI CA060553), the RH Lurie Comprehensive Cancer Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- CD73 is essential for generation of extracellular adenosine through the coordinated action of CD39. Alternatively, extracellular adenosine could be generated from the noncanonical pathway of CD38-CD203a-CD73 that is independent of CD39.
- We aim at providing a broader view of how CD73 is involved in the immune evasion and tumor progression, thereby highlighting the importance of combination treatment arising from CD73 targeting currently under investigation in early phase clinical trials.

Effects of CD73 activity by tumor cells

- CD73 is expressed by many different types of cancer and promotes tumor outgrowth, metastasis and drug resistance.
- There is a complex and contextual role for CD73 in regulating cancer cell viability, stemness and immune suppression.

Effects of CD73 activity by nontumor cells

- CD73-mediated adenosinergic effect has been extensively investigated on a variety of immune cell populations including CD4, CD8, NK, MDSC, macrophage, B cell, ILC2 in the regulation of tumor immunity.
- Both tumor and endothelial cell CD73 synergistically contribute to tumor angiogenesis.
- CAF CD73 expression inhibits T cell-mediated antitumor immunity, similar to the immunosuppressive effect of tumor cell CD73.

CD73 blockade in cancer therapy

- Host and tumor cell CD73 contribute to tumor growth and metastasis in multiple mouse models, highlighting the requirement for optimal antitumor activity to target both tissue compartments.
- While single agent CD73 blockade is not curative, CD73 inhibition improves the antitumor activity of blockade of CTLA-4/PD-1 or agonistic anti-4-1BB/GITR therapy.
- Targeting the CD73-$A_{2A}R$ axis augments the antitumor activity of CAR-T cells or CAR-NK cells.
- A synergy occurs during concurrent treatment with CD73 blockade and conventional antineoplastic agents, or targeted cancer therapies such as BRAF, MEK, NAMPT and $A_{2A}R$ inhibitors.

CD73 in clinic

- High expression level of CD73 within tumor microenvironment is often associated with worse clinical outcomes.
- Several inhibitors targeting CD73 or $A_{2A}R$ with other immune checkpoint inhibitors are currently being developed and tested in early clinical trials.
- Early evidence suggests a potential value of measuring sCD73 activity in the serum/plasma from cancer patients to predict the benefit of immune checkpoint therapy.

Conclusion & future perspective

- Apart from the immune-dependent effect(s) of CD73, it also promotes tumorigenesis independent of the immune effects.
- CD73 blockade is expected to act additively or synergistically with other immune checkpoint inhibitors and agonist immunotherapies.
- Whether surface CD73 and/or soluble CD73 serves as a viable biomarker requires further validation.

References

Papers of special note have been highlighted as: • of interest

1. Resta R, Yamashita Y, Thompson LF. Ecto-enzyme and signaling functions of lymphocyte CD73. *Immunol. Rev.* 161, 95–109 (1998).
2. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5′-nucleotidase (CD73). *Purinergic Signal.* 2, 351–360 (2006).
3. Horenstein AL, Chillemi A, Zaccarello G *et al.* A CD38/CD203a/CD73 ectoenzymatic pathway independent of CD39 drives a novel adenosinergic loop in human T lymphocytes. *Oncoimmunology* 2, e26246 (2013).
4. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 414, 916–920 (2001).
5. Linden J. Adenosine in tissue protection and tissue regeneration. *Mol. Pharmacol.* 67, 1385–1387 (2005).

6. Madara JL, Patapoff TW, Gillececastro B *et al.* 5′-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial-cell monolayers. *J. Clin. Invest.* 91, 2320–2325 (1993).
7. Eckle T, Krahn T, Grenz A *et al.* Cardioprotection by ecto-5′-nucleotidase (CD73) and A2B adenosine receptors. *Circulation* 115, 1581–1590 (2007).
8. Li QL, Price TP, Sundberg JP, Uitto J. Juxta-articular joint-capsule mineralization in CD73 deficient mice: similarities to patients with NT5E mutations. *Cell Cycle* 13, 2609–2615 (2014).
9. Linden J, Cekic C. Regulation of Lymphocyte Function by Adenosine. *Arterioscl. Throm. Vas.* 32, 2097–2103 (2012).
10. Zarek PE, Huang CT, Lutz ER *et al.* A(2A) receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood* 111, 251–259 (2008).
11. Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK. A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* 111, 2024–2035 (2008).
12. Novitskiy SV, Ryzhov S, Zaynagetdinov R *et al.* Adenosine receptors in regulation of dendritic cell differentiation and function. *Blood* 112, 1822–1831 (2008).
13. Wilson JM, Ross WG, Agbai ON *et al.* The A(2B) adenosine receptor impairs the maturation and immunogenicity of dendritic cells. *J. Immunol.* 182, 4616–4623 (2009).
14. Ryzhov S, Novitskiy SV, Goldstein AE *et al.* Adenosinergic regulation of the expansion and immunosuppressive activity of CD11b(+)Gr1(+) cells. *J.Immunol.* 187, 6120–6129 (2011).
15. Koscso B, Csoka B, Kokai E *et al.* Adenosine augments IL-10-induced STAT3 signaling in M2c macrophages. *J. Leukocyte Biol.* 94, 1309–1315 (2013).
16. Ohta A, Gorelik E, Prasad SJ *et al.* A2A adenosine receptor protects tumors from antitumor T cells. *Proc. Natl Acad. Sci.USA* 103, 13132–13137 (2006).

- **Paper showing the role of A2AR pathway in antitumor T cells, highlighting the therapeutic potential of A2AR inhibitors.**

17. Ryzhov S, Novitskiy SV, Zaynagetdinov R *et al.* Host A(2B) adenosine receptors promote carcinoma growth. *Neoplasia* 10, 987–995 (2008).
18. Wang L, Fan J, Thompson LF *et al.* CD73 has distinct roles in nonhematopoietic and hematopoietic cells to promote tumor growth in mice. *J. Clin.Invest.* 121, 2371–2382 (2011).
19. Stagg J, Beavis PA, Divisekera U *et al.* CD73-deficient mice are resistant to carcinogenesis. *Cancer Res.* 72, 2190–2196 (2012).
20. Bowser JL, Blackburn MR, Shipley GL, Molina JG, Dunner K, Broaddus RR. Loss of CD73-mediated actin polymerization promotes endometrial tumor progression. *J. Clin. Invest.* 126, 220–238 (2016).
21. Silverberg SG. Problems in the differential diagnosis of endometrial hyperplasia and carcinoma. *Modern Pathol.* 13, 309–327 (2000).
22. Allard D, Chrobak P, Allard B, Messaoudi N, Stagg J. Targeting the CD73-adenosine axis in immuno-oncology. *Immunol. Lett.* 205, 31–39 (2019).
23. Ghalamfarsa G, Kazemi MH, Raoofi Mohseni S *et al.* CD73 as a potential opportunity for cancer immunotherapy. *Expert Opin. Ther. Targets* 23, 127–142 (2019).
24. Stagg J, Divisekera U, McLaughlin N *et al.* Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc. Natl Acad. Sci. USA* 107, 1547–1552 (2010).

- **The proof-of-concept study showing that anti-CD73 therapy improves adaptive antitumor immunity and inhibits metastasis of breast cancer.**

25. Jin D, Fan J, Wang L *et al.* CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression. *Cancer Res.* 70, 2245–2255 (2010).

- **Data demonstrating a potential mechanism for tumor CD73-mediated immune evasion and developing an immunotherapy strategy by targeting the enzymatic activity of CD73.**

26. Stagg J, Divisekera U, Duret H *et al.* CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. *Cancer Res.* 71, 2892–2900 (2011).
27. Yegutkin GG, Marttila-Ichihara F, Karikoski M *et al.* Altered purinergic signaling in CD73-deficient mice inhibits tumor progression. *Eur. J. Immunol.* 41, 1231–1241 (2011).
28. Zhi XL, Chen SF, Zhou P *et al.* RNA interference of ecto-5′-nucleotidase (CD73) inhibits human breast cancer cell growth and invasion. *Clin. Exp. Metastas.* 24, 439–448 (2007).
29. Zhou P, Zhi XL, Zhou TT *et al.* Overexpression of Ecto-5′-nucleotidase (CD73) promotes T-47D human breast cancer cells invasion and adhesion to extracellular matrix. *Cancer Biol. Ther.* 6, 426–431 (2007).
30. Wang L, Zhou XR, Zhou TT *et al.* Ecto-5′-nucleotidase promotes invasion, migration and adhesion of human breast cancer cells. *J. Cancer Res. Clin.* 134, 365–372 (2008).
31. Zhi XL, Wang YJ, Zhou XR *et al.* RNAi-mediated CD73 suppression induces apoptosis and cell-cycle arrest in human breast cancer cells. *Cancer Sci.* 101, 2561–2569 (2010).

32. Ujhazy P, Berleth ES, Pietkiewicz JM *et al.* Evidence for the involvement of ecto-5′-nucleotidase (CD73) in drug resistance. *Int. J. Cancer* 68, 493–500 (1996).
33. Quezada C, Garrido W, Oyarzun C *et al.* 5′-ectonucleotidase mediates multiple-drug resistance in glioblastoma multiforme cells. *J. Cell. Physiol.* 228, 602–608 (2013).
34. Serra S, Horenstein AL, Vaisitti T *et al.* CD73-generated extracellular adenosine in chronic lymphocytic leukemia creates local conditions counteracting drug-induced cell death. *Blood* 118, 6141–6152 (2011).
35. Bavaresco L, Bernardi A, Braganhol E *et al.* The role of ecto-5′-nucleotidase/CD73 in glioma cell line proliferation. *Mol. Cell. Biochem.* 319, 61–68 (2008).
36. Sadej R, Spychala J, Skladanowski AC. Expression of ecto-5′-nucleotidase (eN, CD73) in cell lines from various stages of human melanoma. *Melanoma Res.* 16, 213–222 (2006).
37. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5′-nucleotidase and adenosine in breast cancer. *Clin. Cancer Res.* 10, 708–717 (2004).
38. Synnestvedt K, Furuta GT, Comerford KM *et al.* Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J. Clin. Invest.* 110, 993–1002 (2002).
39. Hart ML, Grenz A, Gorzolla IC, Schittenhelm J, Dalton JH, Eltzschig HK. Hypoxia-Inducible factor-1 alpha-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5′-nucleotidase (CD73) and the A2B adenosine receptor. *J. Immunol.* 186, 4367–4374 (2011).
40. Robinson A, Keely S, Karhausen J, Gerich ME, Furuta GT, Colgan SP. Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* 134, 145–155 (2008).
41. Eltzschig HK, Bratton DL, Colgan SP. Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. *Nat. Rev. Drug Discov.* 13, 852–869 (2014).
42. Antonioli L, Pacher P, Vizi ES, Hasko G. CD39 and CD73 in immunity and inflammation. *Trends Mol. Med.* 19, 355–367 (2013).
43. Kiss J, Yegutkin GG, Koskinen K, Savunen T, Jalkanen S, Salmi M. IFN-beta protects from vascular leakage via up-regulation of CD73. *Eur. J. Immunol.* 37, 3334–3338 (2007).
44. Spychala J, Zimmermann AG, Mitchell BS. Tissue-specific regulation of the ecto-5′-nucleotidase promoter – role of the cAMP response element site in mediating repression by the upstream regulatory region. *J. Biol. Chem.* 274, 22705–22712 (1999).
45. Spychala J, Kitajewski J. Wnt and beta-catenin signaling target the expression of ecto-5′-nucleotidase and increase extracellular adenosine generation. *Exp. Cell Res.* 296, 99–108 (2004).
46. Wang H, Lee S, Lo Nigro C *et al.* NT5E (CD73) is epigenetically regulated in malignant melanoma and associated with metastatic site specificity. *Brit. J. Cancer* 106, 1446–1452 (2012).
47. Reinhardt J, Landsberg J, Schmid-Burgk JL *et al.* MAPK signaling and inflammation link melanoma phenotype switching to induction of CD73 during immunotherapy. *Cancer Res.* 77, 4697–4709 (2017).
48. Liu L, Mayes PA, Eastman S *et al.* The BRAF and MEK inhibitors dabrafenib and trametinib: effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. *Clin. Cancer Res.* 21, 1639–1651 (2015).
49. Kordass T, Osen W, Eichmuller SB. Controlling the immune suppressor: transcription factors and microRNAs regulating CD73/NT5E. *Front. Immunol.* 9, 813 (2018).
50. Zhou XR, Zhi XL, Zhou P *et al.* Effects of ecto-5′-nucleotidase on human breast cancer cell growth *in vitro* and *in vivo*. *Oncol. Rep.* 17, 1341–1346 (2007).
51. Rockenbach L, Bavaresco L, Farias PF *et al.* Alterations in the extracellular catabolism of nucleotides are involved in the antiproliferative effect of quercetin in human bladder cancer T24 cells. *Urol. Oncol.* 31, 1204–1211 (2013).
52. Wang MX, Ren LM. Growth inhibitory effect and apoptosis induced by extracellular ATP and adenosine on human gastric carcinoma cells: involvement of intracellular uptake of adenosine. *Acta Pharmacol. Sin.* 27, 1085–1092 (2006).
53. Shirali S, Aghaei M, Shabani M, Fathi M, Sohrabi M, Moeinifard M. Adenosine induces cell cycle arrest and apoptosis via cyclinD1/Cdk4 and Bcl-2/Bax pathways in human ovarian cancer cell line OVCAR-3. *Tumor Biol.* 34, 1085–1095 (2013).
54. Richard CL, Tan EY, Blay J. Adenosine upregulates CXCR4 and enhances the proliferative and migratory responses of human carcinoma cells to CXCL12/SDF-1 alpha. *Int. J. Cancer* 119, 2044–2053 (2006).
55. Woodhouse EC, Amanatullah DF, Schetz JA, Liotta LA, Stracke ML, Clair T. Adenosine receptor mediates motility in human melanoma cells. *Biochem. Bioph. Res. Co.* 246, 888–894 (1998).
56. Allard B, Turcotte M, Spring K, Pommey S, Royal I, Stagg J. Anti-CD73 therapy impairs tumor angiogenesis. *Int. J. Cancer* 134, 1466–1473 (2014).
57. Kaplan HB. Methodological problems in the study of psychosocial influences on the AIDS process. *Soc. Sci. Med.* 29, 277–292 (1989).
58. Song L, Ye W, Cui Y *et al.* Ecto-5′-nucleotidase (CD73) is a biomarker for clear cell renal carcinoma stem-like cells. *Oncotarget* 8, 31977–31992 (2017).

59. Lupia M, Angiolini F, Bertalot G *et al.* CD73 regulates stemness and epithelial-mesenchymal transition in ovarian cancer-initiating cells. *Stem Cell Rep.* 10, 1412–1425 (2018).
60. Zukowska P, Kutryb-Zajac B, Toczek M, Smolenski RT, Slominska EM. The role of ecto-5′-nucleotidase in endothelial dysfunction and vascular pathologies. *Pharmacol. Rep.* 67, 675–681 (2015).
61. Wang L, Tang SX, Wang YJ *et al.* Ecto-5′-nucleotidase (CD73) promotes tumor angiogenesis. *Clin. Exp. Metastas.* 30, 671–680 (2013).
62. Koszalka P, Pryszlak A, Golunska M *et al.* Inhibition of CD73 stimulates the migration and invasion of B16F10 melanoma cells *in vitro*, but results in impaired angiogenesis and reduced melanoma growth *in vivo*. *Oncol. Rep.* 31, 819–827 (2014).
63. Koszalka P, Golunska M, Stanislawowski M *et al.* CD73 on B16F10 melanoma cells in CD73-deficient mice promotes tumor growth, angiogenesis, neovascularization, macrophage infiltration and metastasis. *Int. J. Biochem. Cell B.* 69, 1–10 (2015).
64. Deaglio S, Dwyer KM, Gao W *et al.* Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204, 1257–1265 (2007).
65. Romio M, Reinbeck B, Bongardt S, Huls S, Burghoff S, Schrader J. Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells. *Am. J. Physiol.Cell Physiol.* 301, C530–C539 (2011).
66. Mandapathil M, Hilldorfer B, Szczepanski MJ *et al.* Generation and accumulation of immunosuppressive adenosine by human CD4(+)CD25(high)FOXP3(+) regulatory T cells. *J. Biol. Chem.* 285, 7176–7186 (2010).
67. Mandapathil M, Szczepanski MJ, Szajnik M *et al.* Increased ectonucleotidase expression and activity in regulatory T cells of patients with head and neck cancer. *Clin. Cancer Res.* 15, 6348–6357 (2009).
68. Di Gennaro P, Gerlini G, Caporale R *et al.* T regulatory cells mediate immunosuppresion by adenosine in peripheral blood, sentinel lymph node and TILs from melanoma patients. *Cancer Lett.* 417, 124–130 (2018).
69. Sim GC, Martin-Orozco N, Jin L *et al.* IL-2 therapy promotes suppressive ICOS+ Treg expansion in melanoma patients. *J. Clin. Invest.* 124, 99–110 (2014).
70. Schuler PJ, Saze Z, Hong CS *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, 531–543 (2014).
71. Clayton A, Al-Taei S, Webber J, Mason MD, Tabi Z. Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production. *J. Immunol.* 187, 676–683 (2011).
72. Murata K, Tsukahara T, Emori M *et al.* Identification of a novel human memory T-cell population with the characteristics of stem-like chemo-resistance. *Oncoimmunology* 5, e1165376 (2016).
73. Toth I, Le AQ, Hartjen P *et al.* Decreased frequency of CD73(+) CD8(+) T cells of HIV-infected patients correlates with immune activation and T cell exhaustion. *J. Leukocyte Biol.* 94, 551–561 (2013).
74. Flores-Santibanez F, Fernandez D, Meza D *et al.* CD73-mediated adenosine production promotes stem cell-like properties in mouse Tc17 cells. *Immunology* 146, 582–594 (2015).
75. Bono MR, Fernandez D, Flores-Santibanez F, Rosemblatt M, Sauma D. CD73 and CD39 ectonucleotidases in T cell differentiation: beyond immunosuppression. *Febs. Lett.* 589, 3454–3460 (2015).
76. Gourdin N, Bossennec M, Rodriguez C *et al.* Autocrine adenosine regulates tumor polyfunctional CD73(+)CD4(+) effector T cells devoid of immune checkpoints. *Cancer Res.* 78, 3604–3618 (2018).
77. Chalmin F, Mignot G, Bruchard M *et al.* Stat3 and Gfi-1 transcription factors control Th17 cell immunosuppressive activity via the regulation of ectonucleotidase expression. *Immunity* 36, 362–373 (2012).
78. Chatterjee S, Thyagarajan K, Kesarwani P *et al.* Reducing CD73 expression by IL1 beta-programmed Th17 cells improves immunotherapeutic control of tumors. *Cancer Res.* 74, 6048–6059 (2014).
79. Buisseret L, Garaud S, Allard B *et al.* CD73 expression on tumor-infiltrating breast cancer leukocytes. *Cancer Res.* 75, Abstract nr 3361 (2015).
80. Wallace KL, Linden J. Adenosine A(2A) receptors induced on iNKT and NK cells reduce pulmonary inflammation and injury in mice with sickle cell disease. *Blood* 116, 5010–5020 (2010).
81. Beavis PA, Divisekera U, Paget C *et al.* Blockade of A(2A) receptors potently suppresses the metastasis of CD73(+) tumors. *Proc. Natl Acad. Sci. USA* 110, 14711–14716 (2013).
82. Hausler SFM, del Barrio IM, Strohschein J *et al.* Ectonucleotidases CD39 and CD73 on OvCA cells are potent adenosine-generating enzymes responsible for adenosine receptor 2A-dependent suppression of T cell function and NK cell cytotoxicity. *Cancer Immunol. Immun.* 60, 1405–1418 (2011).
83. Raskovalova T, Lokshin A, Huang XJ, Jackson EK, Gorelik E. Adenosine-mediated inhibition of cytotoxic activity and cytokine production by IL-2/NKp46-activated NK cells – involvement of protein kinase A isozyme I (PKA I). *Immunol. Res.* 36, 91–99 (2006).
84. Chambers AM, Wang J, Lupo KB, Yu H, Atallah Lanman NM, Matosevic S. Adenosinergic signaling alters natural killer cell functional responses. *Front. Immunol.* 9, 2533 (2018).
85. Hatfield SM, Kjaergaard J, Lukashev D *et al.* Immunological mechanisms of the antitumor effects of supplemental oxygenation. *Sci. Transl. Med.* 7, 277ra30 (2015).

86. Mittal D, Young A, Stannard K *et al.* Antimetastatic effects of blocking PD-1 and the adenosine A2A receptor. *Cancer Res.* 74, 3652–3658 (2014).

87. Harish A, Hohana G, Fishman P, Arnon O, Bar-Yehuda S. A3 adenosine receptor agonist potentiates natural killer cell activity. *Int. J. Oncol.* 23, 1245–1249 (2003).

88. Hamidzadeh K, Mosser DM. Purinergic signaling to terminate TLR responses in macrophages. *Front. Immunol.* 7, 74 (2016).

89. Cohen HB, Briggs KT, Marino JP, Ravid K, Robson SC, Mosser DM. TLR stimulation initiates a CD39-based autoregulatory mechanism that limits macrophage inflammatory responses. *Blood* 122, 1935–1945 (2013).

90. Zanin RF, Braganhol E, Bergamin LS *et al.* Differential macrophage activation alters the expression profile of NTPDase and Ecto-5′-nucleotidase. *PLoS ONE* 7, e31205 (2012).

91. Ponce NE, Sanmarco LM, Eberhardt N *et al.* CD73 inhibition shifts cardiac macrophage polarization toward a microbicidal phenotype and ameliorates the outcome of experimental chagas cardiomyopathy. *J. Immunol.* 197, 814–823 (2016).

92. Eichin D, Laurila JP, Jalkanen S, Salmi M. CD73 activity is dispensable for the polarization of M2 macrophages. *PLoSONE* 10, e0134721 (2015).

93. de Haas N, de Koning C, Spilgies L, de Vries IJM, Hato SV. Improving cancer immunotherapy by targeting the STATe of MDSCs. *Oncoimmunology* 5, e1196312 (2016).

94. Ye C, Geng Z, Dominguez D *et al.* Targeting ornithine decarboxylase by alpha-difluoromethylornithine inhibits tumor growth by impairing myeloid-derived suppressor cells. *J. Immunol.* 196, 915–923 (2016).

95. Ryzhov SV, Pickup MW, Chytil A *et al.* Role of TGF-beta signaling in generation of CD39(+)CD73(+) myeloid cells in tumors. *J. Immunol.* 193, 3155–3164 (2014).

96. Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Blockade of A(2b) adenosine receptor reduces tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse model of melanoma. *Neoplasia* 15, 1400-+ (2013).

97. Sorrentino C, Miele L, Porta A, Pinto A, Morello S. Myeloid-derived suppressor cells contribute to A2B adenosine receptor-induced VEGF production and angiogenesis in a mouse melanoma model. *Oncotarget* 6, 27478–27489 (2015).

98. Limagne E, Euvrard R, Thibaudin M *et al.* Accumulation of MDSC and Th17 cells in patients with metastatic colorectal cancer predicts the efficacy of a FOLFOX-bevacizumab drug treatment regimen. *Cancer Res.* 76, 5241–5252 (2016).

99. Li JY, Wang LP, Chen XF *et al.* CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF–mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncoimmunology* 6, e1320011 (2017).

100. Li LF, Wang LP, Li JY *et al.* Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* 78, 1779–1791 (2018).

101. Yamashita Y, Hooker SW, Jiang H *et al.* CD73 expression and fyn-dependent signaling on murine lymphocytes. *Eur. J. Immunol.* 28, 2981–2990 (1998).

102. Anderson SM, Tomayko MM, Ahuja A, Haberman AM, Shlomchik MJ. New markers for murine memory B cells that define mutated and unmutated subsets. *J. Exp. Med.* 204, 2103–2114 (2007).

103. Tomayko MM, Steinel NC, Anderson SM, Shlomchik MJ. Cutting edge: hierarchy of maturity of murine memory B cell subsets. *J. Immunol.* 185, 7146–7150 (2010).

104. Forte G, Sorrentino R, Montinaro A *et al.* Inhibition of CD73 improves B cell-mediated anti-tumor immunity in a mouse model of melanoma. *J. Immunol.* 189, 2226–2233 (2012).

105. Turcotte M, Spring K, Pommey S *et al.* CD73 is associated with poor prognosis in high-grade serous ovarian cancer. *Cancer Res.* 75, 4494–4503 (2015).

106. Koivisto M, Tervahartiala M, Kenessey I, Jalkanen S, Bostrom PJ, Salmi M. Cell-type specific CD73 expression is an independent prognostic factor in bladder cancer. *Carcinogenesis* 40, 84–92 (2018).

107. Costa A, Kieffer Y, Scholer-Dahirel A *et al.* Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell* 33, 463–479 e410 (2018).

108. Givel AM, Kieffer Y, Scholer-Dahirel A *et al.* miR200-regulated CXCL12 beta promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. *Nat. Commun.* 9, 1056 (2018).

109. Leclerc BG, Charlebois R, Chouinard G *et al.* CD73 expression is an independent prognostic factor in prostate cancer. *Clin. Cancer Res.* 22, 158–166 (2016).

110. Terp MG, Olesen KA, Arnspang EC *et al.* Anti-human CD73 monoclonal antibody inhibits metastasis formation in human breast cancer by inducing clustering and internalization of CD73 expressed on the surface of cancer cells. *J. Immunol.* 191, 4165–4173 (2013).

111. Braganhol E, Tamajusuku AS, Bernardi A, Wink MR, Battastini AM. Ecto-5′-nucleotidase/CD73 inhibition by quercetin in the human U138MG glioma cell line. *Biochim. Biophys. Acta* 1770, 1352–1359 (2007).

112. Xiong L, Wen Y, Miao XY, Yang ZL. NT5E and FcGBP as key regulators of TGF-1-induced epithelial-mesenchymal transition (EMT) are associated with tumor progression and survival of patients with gallbladder cancer. *Cell Tissue Res.* 355, 365–374 (2014).

113. Jadidi-Niaragh F, Atyabi F, Rastegari A *et al.* CD73 specific siRNA loaded chitosan lactate nanoparticles potentiate the antitumor effect of a dendritic cell vaccine in 4T1 breast cancer bearing mice. *J. Control. Rel.* 246, 46–59 (2017).

114. Ghalamfarsa G, Rastegari A, Atyabi F *et al.* Anti-angiogenic effects of CD73-specific siRNA-loaded nanoparticles in breast cancer-bearing mice. *J. Cell Physiol.* 233, 7165–7177 (2018).

115. Young A, Ngiow SF, Barkauskas DS *et al.* Co-inhibition of CD73 and A2AR adenosine signaling improves anti-tumor immune responses. *Cancer Cell.* 30, 391–403 (2016).

- **Paper showing the nonredundant activity of CD73 and $A_{2A}R$ inhibition to promote therapeutic response by coblockade of adenosine signaling in leukocytes.**

116. Allard B, Pommey S, Smyth MJ, Stagg J. Targeting CD73 enhances the antitumor activity of anti-PD-1 and anti-CTLA-4 mAbs. *Clin. Cancer Res.* 19, 5626–5635 (2013).

- **The first preclinical study indicating a synergistic combination of anti-CD73 mAb and blockade of CTLA-4 and PD-1.**

117. Beavis PA, Milenkovski N, Henderson MA *et al.* Adenosine receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses. *Cancer Immunol. Res.* 3, 506–517 (2015).

118. Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Adenosine limits the therapeutic effectiveness of anti-CTLA4 mAb in a mouse melanoma model. *Am. J. Cancer Res.* 4, 172–181 (2014).

119. Hay CM, Sult E, Huang QH *et al.* Targeting CD73 in the tumor microenvironment with MEDI9447. *Oncoimmunology* 5, e1208875 (2016).

120. Beavis PA, Henderson MA, Giuffrida L *et al.* Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. *J. Clin. Invest.* 127, 929–941 (2017).

121. Wang J, Lupo KB, Chambers AM, Matosevic S. Purinergic targeting enhances immunotherapy of CD73(+) solid tumors with piggyBac-engineered chimeric antigen receptor natural killer cells. *J. Immunother. Cancer* 6, 136 (2018).

122. Chen S, Fan J, Zhang M *et al.* CD73 expression on effector T cells sustained by TGF-beta facilitates tumor resistance to anti-4-1BB/CD137 therapy. *Nat. Comm.* 10, 150 (2019).

- **The first preclinical study indicating a synergistic combination of anti-CD73 mAb and agonist anti-4-1BB or anti-GITR therapy.**

123. Galli U, Travelli C, Massarotti A *et al.* Medicinal chemistry of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors. *J. Med. Chem.* 56, 6279–6296 (2013).

124. Garten A, Petzold S, Korner A, Imai S, Kiess W. Nampt: linking NAD biology, metabolism and cancer. *Trends Endocrinol. Metab.* 20, 130–138 (2009).

125. Grozio A, Sociali G, Sturla L *et al.* CD73 protein as a source of extracellular precursors for sustained NAD(+) biosynthesis in FK866-treated tumor cells. *J. Biol.Chem.* 288, 25938–25949 (2013).

126. Garavaglia S, Bruzzone S, Cassani C *et al.* The high-resolution crystal structure of periplasmic Haemophilus influenzae NAD nucleotidase reveals a novel enzymatic function of human CD73 related to NAD metabolism. *Biochem. J.* 441, 131–141 (2012).

127. Sociali G, Raffaghello L, Magnone M *et al.* Antitumor effect of combined NAMPT and CD73 inhibition in an ovarian cancer model. *Oncotarget* 7, 2968–2984 (2016).

128. Turcotte M, Allard D, Mittal D *et al.* CD73 promotes resistance to HER2/ErbB2 antibody therapy. *Cancer Res.* 77, 5652–5663 (2017).

129. Young A, Ngiow SF, Madore J *et al.* Targeting adenosine in BRAF-mutant melanoma reduces tumor growth and metastasis. *Cancer Res.* 77, 4684–4696 (2017).

130. Antonioli L, Novitskiy SV, Sachsenmeier KF, Fornai M, Blandizzi C, Hasko G. Switching off CD73: a way to boost the activity of conventional and targeted antineoplastic therapies. *Drug Discov. Today* 22, 1686–1696 (2017).

131. Loi S, Pommey S, Haibe-Kains B *et al.* CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc.Natl Acad. Sci. USA* 110, 11091–11096 (2013).

132. Wennerberg E, Formenti S, Demaria S. Adenosine regulates radiation therapy-induced anti-tumor immunity. *Int. J. Radiat. Oncol.* 96, S126–S127 (2016).

133. Allard D, Allard B, Gaudreau PO, Chrobak P, Stagg J. CD73-adenosine: a next-generation target in immuno-oncology. *Immunotherapy* 8, 145–163 (2016).

134. Antonioli L, Yegutkin GG, Pacher P, Blandizzi C, Hasko G. Anti-CD73 in cancer immunotherapy: awakening new opportunities. *Trends Cancer* 2, 95–109 (2016).

135. Buisseret L, Pommey S, Allard B *et al.* Clinical significance of CD73 expression in triple-negative breast cancer from the BIG 02-98 adjuvant phase III clinical trial. *Cancer Res.* 78, (2018).

136. Reinhardt J, Landsberg J, Schmid-Burgk JL *et al.* MAPK signaling and inflammation link melanoma phenotype switching to induction of CD73 during immunotherapy. *Cancer Res.* 77, 4697–4709 (2017).

137. Geoghegan JC, Diedrich G, Lu X *et al.* Inhibition of CD73 AMP hydrolysis by a therapeutic antibody with a dual, non-competitive mechanism of action. *mAbs.* 8, 454–467 (2016).

- **Analysis of the properties of the first antihuman CD73 monoclonal antibody that is currently tested in clinical trials.**

138. Overman MJ, LoRusso P, Strickler JH, Patel SP, Clarke SJ, Noonan AM. Safety, efficacy and pharmacodynamics (PD) of MEDI9447 (oleclumab) alone or in combination with durvalumab in advanced colorectal cancer (CRC) or pancreatic cancer (panc). *J. Clin. Oncol.* 36, Abstract no. 4123 (2018).

139. Barnhart BC, Sega E, Yamniuk A *et al.* A therapeutic antibody that inhibits CD73 activity by dual mechanisms. *Cancer Res.* 76, Abstract no. 1476 (2016).

140. Lorenzen S, Siu LL, Burris H *et al.* Preliminary Phase Iprofile of BMS-986179, an anti-CD73 antibody, in combination with nivolumab in patients with advanced solid tumors. *Oncol.Res. Treat.* 41, 263–263 (2018).

141. Gong YP, Wan RZ, Liu ZP. Evaluation of WO2017098421: GSK's benzothiazine compounds as CD73 inhibitor filings. *Expert Opin. Ther. Pat.* 28, 167–171 (2018).

142. Kenneth LJ, Lawson V, Jeffrey JL *et al.* Discovery and characterization of AB680, a potent and selective small-molecule CD73 inhibitor for cancer immunotherapy [abstract]. *Cancer Res.* 78, Abstract no. 1756 (2018).

143. Becker NNA, Yin F, Zhang K *et al.* CD73 inhibitors (CD73i) reverse the AMP/adenosine-mediated impairment of immune effector cell activation by immune checkpoint inhibitors (ICI) [abstract]. *Cancer Res.* 78, Abstract no. 710 (2018).

144. Tang J, Pearce L, O'Donnell-Tormey J, Hubbard-Lucey VM. Trends in the global immuno-oncology landscape. *Nat. Rev. Drug Discov.* 17, 783–784 (2018).

145. Pettengill M, Robson S, Tresenriter M *et al.* Soluble ecto-5′-nucleotidase (5′-NT), alkaline phosphatase, and adenosine deaminase (ADA1) activities in neonatal blood favor elevated extracellular adenosine. *J. Biol. Chem.* 288, 27315–27326 (2013).

146. Maksimow M, Kyhala L, Nieminen A *et al.* Early prediction of persistent organ failure by soluble CD73 in patients with acute pancreatitis*. *Crit. Care Med.* 42, 2556–2564 (2014).

147. Vaara ST, Hollmen M, Korhonen AM *et al.* Soluble CD73 in critically Ill septic patients – data from the prospective FINNAKI study. *PLoSONE* 11, e0164420 (2016).

148. Morello S, Capone M, Sorrentino C *et al.* Soluble CD73 as biomarker in patients with metastatic melanoma patients treated with nivolumab. *J. Transl.Med.* 15, 244 (2017).

149. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Diagnostic value of adenosine deaminase activity in benign and malignant breast tumors. *Arch. Med. Res.* 41, 14–18 (2010).

150. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Adenosine deaminase activity in the serum and malignant tumors of breast cancer: the assessment of isoenzyme ADA1 and ADA2 activities. *Clin. Biochem.* 38, 887–891 (2005).

151. Sadej R, Skladanowski AC. Dual, enzymatic and non-enzymatic, function of ecto-5′-nucleotidase (eN, CD73) in migration and invasion of A375 melanoma cells. *Acta Biochim. Pol.* 59, 647–652 (2012).

152. Sadej R, Inail K, Rajfur Z *et al.* Tenascin C interacts with Ecto-5′-nucleotidase (eN) and regulates adenosine generation in cancer cells. *Biochim. Biophys. Acta* 1782, 35–40 (2008).

153. Jiang T, Xu X, Qiao M *et al.* Comprehensive evaluation of NT5E/CD73 expression and its prognostic significance in distinct types of cancers. *BMC Cancer* 18, 267 (2018).

154. Leclerc BG, Charlebois R, Chouinard G *et al.* CD73 expression is an independent prognostic factor in prostate cancer. *Clin. Cancer Res.* 22, 158–166 (2016).

155. Buisseret L, Pommey S, Allard B *et al.* Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a Phase III clinical trial. *Ann. Oncol.* 29, 1056–1062 (2018).