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Targeting CD73 to augment cancer immunotherapy

Meejeon Roh[1], **Derek A Wainwright**[2], **Jennifer D Wu**[3], **Yong Wan**[4], **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 and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

Abstract

CD73 (ecto-5′-nucleotidase) is a novel immunoinhibitory protein that plays a key role for tumor growth and metastasis. Its main function is to convert extracellular ATP to immunosuppressive adenosine in concert with CD39 in normal tissues to limit excessive immune response. However, tumors take advantage of the CD73-mediated adenosinergic mechanism to protect them from immune attack. In particular, inducible expression of CD73 along with other adenosinergic molecules on both cancer cells and host cells sustains immunosuppressive tumor microenvironment by affecting multiple aspects of the immune response. Owing to its multifaceted capacity to tumor promotion as an emerging immune checkpoint, CD73 is an ideal therapeutic target for cancer treatment especially in combination with conventional therapy and/or other immune checkpoint inhibitors. In this review, we will discuss the roles of CD73 on tumor and immune cells and will highlight the therapeutic value of CD73 for combination therapy.

*Address correspondence to: Bin Zhang, Robert H. Lurie Comprehensive Cancer Center; Department of Medicine- Division of Hematology/Oncology; Northwestern University Feinberg School of Medicine; 300 E Superior Street-Tarry 4-726; Chicago, Illinois 60611, bin.zhang@northwestern.edu, Fax: 312-503-0189; Ph: 312-503-2435.

CRediT authorship contribution statement

Meejeon Roh: Writing - original draft, Writing - review & editing, Artwork-figures. **Derek A Wainwright:** Writing-review & editing. **Jennifer D Wu:** Writing - review & editing. **Yong Wan**: Writing-review & editing. **Bin Zhang**: Writing - original draft, Writing - review & editing, Supervision.

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Keywords

CD73; Immunotherapy

Introduction

CD73, also known as ecto-5′-nucleotidase (ecto-5′-NT, EC 3.1.3.5) is a glycosylphosphatidyl inositol (GPI)-anchored cell surface protein that is encoded by *NT5E* gene. CD73 is widely expressed on different tissues [1,2] and cell types including, but not limited to the subsets of T cells and B cells [1,3–5], endothelial cells [4,6] and epithelial cells [7].

The balance between ATP and adenosine is crucial to prevent uncontrolled tissue damage due to excessive inflammatory responses. CD73, as a rate-limiting enzyme for adenosine production, plays a critical role to maintain tissue homeostasis by converting/switching ATP-triggered immune activation to adenosine-mediated immunosuppression, although the relative contribution of non-canonical adenosinergic pathways led by alkalike phosphatases and/or NAD^+ ectohydrolase CD38 may need consideration. The extracellular ATP level is elevated in stressful situations such as inflammation, malignancy, and ischemia [8,9]. While ATP mediates inflammatory responses through their P2 purinergic receptors, i.e. P2XRs and P2YRs, it is rapidly hydrolyzed by the enzymatic cascade via CD39 (NTPDases) and CD73 (ecto-5'-nucleotidase) to generate adenosine that acts as an anti-inflammatory mediator to downregulate the immune cell function through its four receptors (A1, A2A, A2B, and A3). As such, CD73, by degrading extracellular AMP to adenosine, is a key player for the establishment of an immunosuppressive tumor microenvironment (TME). In this review, the roles of CD73 on tumor and immune cells, as well as its therapeutic potential will be discussed.

CD73 on tumor cells

CD73 expression level is higher in the majority of human solid tumors. Its expression and activity are closely associated with tumor invasiveness and metastasis [10]. We [11] and others [12] have demonstrated that extracellular adenosine generated by CD73 on tumor cells is sufficient to mediate immune evasion, facilitating tumor growth and metastasis. The importance of CD73 on tumor cells versus host cells in tumorigenesis has been further documented using multiple CD73-deficient tumor models [11,13–16]. Besides the immune regulation of CD73 by tumor cells [11,12], CD73 affects multiple aspects of tumorigenesis such as proliferation, adhesion/migration, angiogenesis and metastasis. It promotes proliferation of tumor cells by regulating cell cycle, apoptosis, and signaling pathways such as EGFR, β-catenin/cyclin D1, VEGF, and AKT/ERK[17–21]. Independent of its enzymatic function, CD73 can also promote cell-to-cell adhesion, migration, invasion of cancer cells as well as stemness [17,20,22–24]. Interestingly, CD73 on both tumor cells and host cells is required for tumor angiogenesis [25,26]. It has been also demonstrated the importance of CD73-A2AR signaling for tumor-associated lymphangiogenesis [27,28], further supporting the potential use of adenosine blocking agents to inhibit pathological lymphangiogenesis in

cancers and prevent tumor dissemination. In addition, two recent studies reported that cancer cell-intrinsic CD73 expedited metastasis by driving epithelial-to-mesenchymal transition (EMT) through PI3K/AKT signaling pathway [29] and RICS/Rho GTPase signaling pathway [30], respectively. In support, CD73 expression is often associated with worse prognosis [18,21,31–33] and poor response to therapeutic agents [34,35]. However, CD73 is not always upregulated in cancers and its expression has been reported to be correlated with a positive prognosis [36,37]. In fact, aberrantly glycosylated CD73 [38], as well as a human specific CD73 isoform (CD73s) [39] have been identified in human hepatocellular carcinoma (HCC), leading to the functional suppression of tumor CD73. CD73 was also downregulated in advance stage prostate [40], laryngeal [41] and high grade colon carcinomas [42]. Lower expression levels of CD73 were observed in poorly-differentiated and advanced stage of endometrial carcinomas compared to normal and well-differentiated, early state tumors, and higher CD73 expression was associated with better overall survival [43]. CD73-generated adenosine was further shown to protect epithelial integrity via actin polymerization in early-stage endometrial tumors [43]. Thus, the role of CD73 in cancers seems to be complex possibly due to the non-tumor promoting effects mediated by CD73.

In addition, evidence supports the existence of a soluble form of CD73 (sCD73) [44] and its increased levels in the plasma of cancer patients compared to healthy individuals (Q Huang *et al.*, abstract 1538, 106th American Association for Cancer Research, Philadelphia, April 2015). Although the role of sCD73 is less explored, high levels of sCD73 enzyme activity in serum, before nivolumab (anti-PD-1 Ab) treatment, was found to be associated with poor survival of metastatic melanoma patients [45], indicating sCD73 as a potential prognostic marker for cancer immunotherapy.

Interestingly, CD73 together with CD39 were found on exosomes isolated from mesothelioma patients [46] and CD73^{+} exosome suppressed immune cell function [46,47]. Furthermore, prostate cancer cell–derived exosome was able to induce CD73 expression on dendritic cells (DC), thereby inhibiting T cell function [48]. Thus, CD73 by tumor cells or their derived exosomes exerts its immunosuppressive function in an adenosine-dependent manner.

CD73 on immune cells

CD73 along with other adenosinergic molecules play critical roles in the establishment of an immunosuppressive TME by affecting multiple types of immune cells [11,13–16] (see Figure1). Thereafter we will summarize the roles of CD73 on the following major immune cell populations.

Regulatory T (Treg) cell:

In mice, CD73 is expressed on different subsets of T lymphocytes, but it is particularly abundant in Foxp3^{+} Tregs [49,50]. CD73 is crucial for Treg-mediated inhibition of effector T cell function as shown by impaired immunosuppressive capability of Treg cells in CD73–deficient tumor-bearing mice [14,15]. These effects are mediated mainly through A2AR on T effector cells. A2AR activation in naive CD4^{+} T cells promotes their differentiation towards Foxp3^{+} and LAG-3^{+} Treg cells and induce a long-term anergy [51,52]. In human,

the CD73 expression level in Tregs is low, but increased in certain cancer patients [53,54]. especially after high-dose IL-2 therapy in melanoma patients [55]. Similar to mouse cell system, CD73 inhibition decreases Treg-mediated immunosuppressive function [56].

Effector T cell:

High level of CD73 is associated with the exhausted or anergic T cell phenotype [57,58]. Th17 cells express CD73 and CD39, and suppress effector T cell function dependent on the enzymatic activity of CD39/CD73 [59]. Furthermore, genetic ablation of CD73 or reducing CD73 by reprogramming Th17 cells improves antitumor effects by increasing their effector function [60]. As expected, A2AR agonist treatment inhibits T cell activation and proliferation, and induces T cell anergy [61–63]. Despite the role of CD73 by effector $CD8^+$ T cells remains elusive, a recent study supported a prognostic value of $CD8^+$ T cells expressing CD73 particularly after immunotherapy [64].

Natural killer (NK) cell:

The CD73 expression level in NK cells is low, but increased under specific conditions. For example, CD73 was induced on NK when co-cultured with human mesenchymal stem cells [65]. In gastrointestinal stromal tumors, tumor-infiltrating NK cells express higher levels of CD73 than those in PBMCs [66]. CD73 was also found on NK cells isolated from mouse melanoma [67], suggesting that tumor-infiltrating NK cells might acquire CD73 expression. CD73-produced adenosine suppresses NK cell functions primarily through A2AR [68,69]. A2AR activation hinders NK cell maturation, activation and cytotoxic function [67,68,70–72]. In contrast, loss of A2AR signaling in NK cells ameliorates $CD73^+$ tumor metastasis and enhances anti-tumor immune response [66,73]. A recent study demonstrated that NK cells underwent phenotypic and functional switch to immunosuppressive population through acquiring CD73 in the TME [74], suggesting the importance of targeting CD73 for NK-based immunotherapy.

Myeloid derived suppressor cell (MDSC):

CD39 and CD73 levels on MDSC are higher in cancer patients [75–77]. CD73-mediated adenosine promotes MDSC function mainly through A2BR. A2BR antagonist inhibited the accumulation of tumor-infiltrating MDSCs in TME and this led to the delayed tumor growth in a mouse model [78]. In contrast, mice treated with a A2BR agonist accelerated tumor growth through enhanced MDSC infiltration to tumor and angiogenesis [79]. Tumor-derived TGF-β induced CD39 and CD73 on MDSCs through mTOR/HIF-1α pathway and $CD39^+CD73^+$ MDSCs represented a distinct inflammatory subpopulation associated with immunosuppressive signatures and chemotherapeutic response in the NSCLC patients [76]. On the other hand, metformin was found to reduce CD39 and CD73 via activation of AMP-activated protein kinase α, thereby blocking MDSC activity in patients with ovarian cancer [77]. These data suggest the targeting CD39/CD73 improves antitumor immunity in part through inhibition of MDSCs.

Macrophages:

In mice, CD39 and CD73 are expressed on resident macrophages [80,81], and their expression level changes depending on the activation state of the macrophage [82]. Modulation of CD73 activity determines macrophage function by switching M1 and M2 phenotype [13,82,83]. Notably, tumor-associated macrophages (TAMs) express CD39/CD73 that suppress $CD4^+$ T cell proliferation through adenosine generation [84]. Furthermore, fasting-mediated tumor inhibition was related to reduced M2 polarization of TAMs with less CD73 and lower adenosine level in the TME [85]. Together, these data support the idea that activity of CD73 together with CD39 is required for fine-tuning of TAM function during tumor progression. However, more studies are needed for further clarification due to the conflicting report [86].

B cell:

The CD73 expression level is considered as an indication of B cell maturity [1,5]. In adult human, majority of B cells express CD73 [1], but neonatal B cells are deficient in CD73, and this deficiency seems to be responsible for impaired B cell function in early life [87]. Moreover, CD73 is required for class switch recombination in B cells [88,89]. In a murine melanoma model, CD73 activity in B cells was reported to play an important role in tumor growth [90]. Treatment with adenosine 5'-(α,β-methylene) diphosphate (APCP), a CD73 specific inhibitor, induced IL17A and facilitated the presence of B cells and the production of IgG2b within the melanoma [90].

CD73 as a novel therapeutic target for combination therapy

CD73 has recently emerged as a promising target for novel immunotherapy due to its critical role for anti-tumor immunity. In fact, inhibition of CD73 using either monoclonal antibodies (mAb) or small molecule inhibitor such as APCP have demonstrated antitumor effects in preclinical tumor mouse models [10,91]. Furthermore, a number of anti-CD73 mAbs (MEDI9447, BMS986179, SRF373/NZV930, CPI-006/CPX-006, IPH5301, TJ004309) and selective small molecule inhibitors (LY3475070, AB680, CB-708) are being tested in early phase clinical trials [92–94]. Notably, CD73 expression and activity can be increased upon different therapeutics. We will thus review the evidence below to support feasibility of targeting CD73 in combination with chemotherapy, radiation therapy, and other immunotherapies (see Figure 2).

Inhibition of the adenosinergic pathway:

Stimulated by the seminal work of Sitkovsky group showing the promise of A2AR inhibition for cancer immunotherapy [95], we [10] and others [91] have further demonstrated the therapeutic potential of targeting CD73-A2AR axis in multiple types of cancer. Inhibition of both CD73 and A2AR showed synergy in anti-tumor response in several mouse tumor models [67]. Importantly, a promising clinical response was reported in renal cell cancer (RCC) patients receiving A2AR antagonist (ciforadenant) alone or in combination with an anti-PD-L1 antibody (atezolizumab) [96]. Moreover, ciforadenant-mediated antitumor activity was associated with high levels of adenosine gene signature expression before treatment, suggesting that adenosine gene signature might serve as a

predictive biomarker for adenosine blockade [67]. Although blockade of CD73 and A2AR is likely the most efficient strategy to neutralize tumor-driving adenosine effects, co-inhibition of CD73 and other adenosinergic members (e.g. A2BR, and CD39) is also a viable option, given their distinct expression pattern and nonredundant functionality. For example, blockade of CD73 and CD39 enzymatic activities resulted in greater inhibitory effect on human MDSC-mediated suppressive function [77]. Moreover, the anti-CD39/CD73 mAb combination at suboptimal doses acted in synergy to promote the proliferation of T cells from healthy donors and cancer patients [94]. A recent study also showed a synergistic anti-metastatic effect between anti-CD39 mAb and A2AR antagonists in mouse models of experimental and spontaneous metastases [97]. Different from other agents targeting the adenosinergic pathway, CD39 enzyme blockade-mediated anti-tumor effect was attributed to the critical role of an eATP-P2X7-ASC-NALP3-inflammasome-IL-18 pathway as mechanism of action [97,98]. On the other hand, it was reported that a non-canonical adenosinergic pathway led by CD38 might contribute to the immunosuppressive TME, especially serving as a mechanism of tumor cell escape from PD-1/PD-L1 blockade [99]. In addition, CD73 was upregulated via adenosine-A2BR circuit in cancer-associated fibroblasts (CAF), and inhibition of A2AR and A2BR together with CD73 blockade significantly enhanced antitumor immunity in murine CAF-rich tumors [100].

Immune checkpoint blockade (ICB):

In several murine tumor models, combination therapy of anti-CD73 with PD-L1 and/or anti-CTLA-4 enhanced therapeutic activity compared to monotherapy [101–104], Likewise, co-targeting A2AR and ICB showed therapeutic synergy [105]. Although ICB is effective in certain cancer patients, many patients do not respond (innate/primary resistance) or acquire resistance after initial response (acquired resistance). This might be due to the existence of alternative and/or therapy-induced immunosuppressive pathways in the TME. Supporting this notion, CD73 level was found to be upregulated in melanoma patients who received PD-1 immunotherapy [106]. Furthermore, comprehensive immune profiling indicated a unique $CD73^{high}$ macrophage population that persisted in glioblastoma patients after anti-PD-1 therapy [107]. CD73 deficiency enhanced the efficacy of anti-PD-1 and anti-CTLA-4 in a murine model of glioblastoma [107].

Adoptive cell therapy:

CD73 knockdown on tumor cells was sufficient to facilitate T cell effector function following tumor-specific T cell transfer, leading to tumor regression [11]. We further demonstrated that inhibition of CD73 activity by APCP or anti-CD73 mAb improved the efficacy of adoptive T cell therapy (ACT) using B16-SIY melanoma model and peritoneal ID8 tumor models [14]. CD73 upregulation was also observed in melanoma patients during ACT therapy [106]. Furthermore, CD73 was induced in relapsed melanomas in a mouse model of T-cell immunotherapy [106], providing the potential foundation for combining ACT therapy with CD73 blockade. Similarly, inhibition of A2AR pathway in T cells also increased the efficacy of ACT [95] and chimeric antigen receptor (CAR)-T cell therapy [108]. In addition, targeting CD73 activity with anti-CD73 antibody enhanced therapeutic efficacy of engineered CAR-NK cells against $CD73^{+}$ tumors in human lung cancer xenograft

models [109]. Thus, CD73 blockade could inhibit tumor growth *in vivo* dependent of both adaptive and innate immunity of ACT.

Agonistic immunotherapy:

Similar to blocking immune inhibitory molecules, activating immune co-stimulatory molecules on T cells is an open area to explore. Using preclinical models, we recently demonstrated that CD73 expression by T cells conferred tumor resistance to agonistic immunotherapy targeting 4–1BB, an inducible costimulatory molecule in the TNFR superfamily, while anti-4–1BB therapy preferentially mediated CD73-negative effector T cell response for tumor inhibition [110]. In addition, the synergistic antitumor effect was achieved by combination of CD73 blockade with anti-GITR (another potent T cell costimulatory molecule) as well [110]. Based on this exciting result, we infer that immunotherapeutic agonists targeting TNFR costimulatory receptors such as 4–1BB, GITR, OX40, or CD40 in combination with CD73 and/or other adenosinergic signaling molecules may be attractive for clinical development. Indeed, combination of CD73/A2AR blockade and anti-OX40 is being exploited in early phase clinical trials [110].

Chemotherapy:

CD73 has been shown to contribute to multidrug–resistance [34,111]. For instance, CD73 especially in TNBC patients was correlated with resistance to doxorubicin [35]. Doxorubicin treatment increased CD73 expression that led to the suppression of $CD8^+$ T cells [35]. Increased frequency of $CD47^+CD73^+PD\text{-}L1^+$ cell population in TNBC cells was also reported after treatment of other chemotherapeutic reagents such as carboplatin, gemcitabine, and paclitaxel [112]. By analyzing the sensitivity of NCI-60 cell lines to a panel of chemotherapeutic drugs, *CD73* expression was found to be negatively associated with sensitivity to several chemotherapeutic reagents. And *CD73* level was indeed elevated in platinum resistant ovarian cancer cells [113]. It was also reported that mesenchymal stem/stromal cells-derived IL-6 promoted cisplatin resistance by upregulating CD73 in nasopharyngeal carcinoma [114]. CD73 upregulation after chemotherapy seemed to be an attempt to counterbalance excess ATP released from dying tumor cells after therapy [115,116]. Additionally, CD73-mediated adenosine signaling seems to downregulate ABC transporters and P-glycoprotein, a drug efflux transporter, thereby contributing to drug resistance [111,117,118].

Radiation therapy:

Beside direct cancer cell killing by radiation, radiation therapy can also affect immune response [119]. For example, radiation induced apoptosis of NK cells and T cells, and B cells [120], but recruited and activated DCs. This differential effect of radiation on immune regulation is likely dependent on the ratio of ATP to adenosine. Interestingly, enhanced CD73 enzymatic activity was observed in irradiated lung tissue, and involved in pulmonary fibrosis [121], which is a severe side effect of thoracic irradiation. In particular, treatment with anti-CD73 mAb significantly reduced radiation-induced lung fibrosis [121], suggesting that CD73 inhibition might be a promising mean in limiting lung toxicities associated with the treatment of thoracic malignancies. Radiation also increased CD73 expression on human breast cancer cells, and CD73 inhibition combined with radiotherapy showed better

antitumor response due to enhanced antitumor T cell response [122]. However, pharmacological inhibition or knocking out CD73 was found to rescue proliferative capacity of T24 human bladder cancer cells, thereby reducing their sensitivity to radiation [123].

Targeted therapy:

High levels of CD73 gene expression were found to link significantly with poor outcome in a randomized phase III clinical trial evaluating the activity of the anti-HER2/ErbB2 mAb trastuzumab, indicating the potential role of CD73 in conferring tumor resistance to targeted therapies [124]. Indeed, anti-CD73 mAb therapy augmented the efficacy of anti-ErbB2 mAb in immunocompetent mouse models of HER2/ErbB2-driven breast cancer. Furthermore, it is of note that clinical trials are ongoing with anti-CD73 mAb in combination with anti-EGFR therapy or A2AR inhibitors in non-small cell lung cancer (NCT03381274). Similarly, more advanced clinical stage disease was associated with increased CD73 expression despite CD73 expression was not an independent prognostic factor in melanoma [125]. Interestingly, activating MAPK mutations and growth factors drove CD73 expression [106], while BRAF and MEK inhibition potently reduced CD73 expression [125]. Inhibition of adenosine signaling with A2AR antagonist along with BRAF and MEK inhibition enhanced antitumor effects of BRAF-mutated melanoma in mice [125]. These studies together open new avenues for developing targeting CD73-mediated adenosine signaling in combination with targeted therapies and provide insights into how CD73 is regulated in cancer treatment.

Conclusion

CD73 is an ideal therapeutic target of cancer therapy for the following reasons; (i) CD73 expression by cancer cells and host cells including, but limited to, a variety of immune cell populations, creates immunosuppressive adenosine-rich TME. Evolving data support the tumor-promoting role of cancer cell-intrinsic CD73. (ii) CD73 promotes tumor growth and metastasis primarily via its enzymatic activity. The role of CD73 independent of its enzymatic activity in tumorigenesis warrants intensive investigation, providing novel insight into the regulatory function of CD73 in cancer. (iii) As CD73 expression and activity seem to be modulated upon many therapies, co-targeting CD73 with other therapeutic reagents represents a rational strategy. CD73 inhibition in general is expected to boost immune response to keep the tumor cells in control. However, certain concerns on undesirable side effects remain due to ubiquitous expression of CD73 on multiple cell types in various tissues. Notably, there were patients that received BMS-986179, an anti-CD73 mAb that experienced cardiac events while on the clinical study; this led to a change in the inclusion criteria for recruitment of patients. Nevertheless, BMS-986179 in combination with nivolumab appears to have the same toxicity as nivolumab alone (LL Siu *et al.*, abstract CT180, 109th American Association for Cancer Research, Chicago, April 2018). With several clinical trials currently evaluating inhibitors of the adenosine pathway in cancers, the pathophysiological role of adenosine with a focus on effects on antitumor immunity has been comprehensively reviewed [126]. We believe that designing anti-CD73 mAbs that incorporate alternative action mechanism such as Fc receptor engagement to maximize anti-tumor effects and development of more potent and selective small molecule inhibitors with longer half-life would greatly enhance therapeutic efficacy. More attention should be paid

especially to important avenues of clinical studies in the future including evaluation of membrane and soluble CD73 as a potential prognostic or/and predictive biomarker, and clarification of the mechanisms of action for CD73 blockade and in combination therapy together with adverse effects.

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Highlights

- CD73 is a multifunctional ectoenzyme affecting both tumor cells and immune cells.
- CD73 has been hijacked by TME to promote tumor growth and metastasis.
- Targeting CD73 and other adenosinergic molecules orchestrates anti-tumor immunity.
- CD73 blockade achieves synergy in combination with conventional therapy and/or ICB.

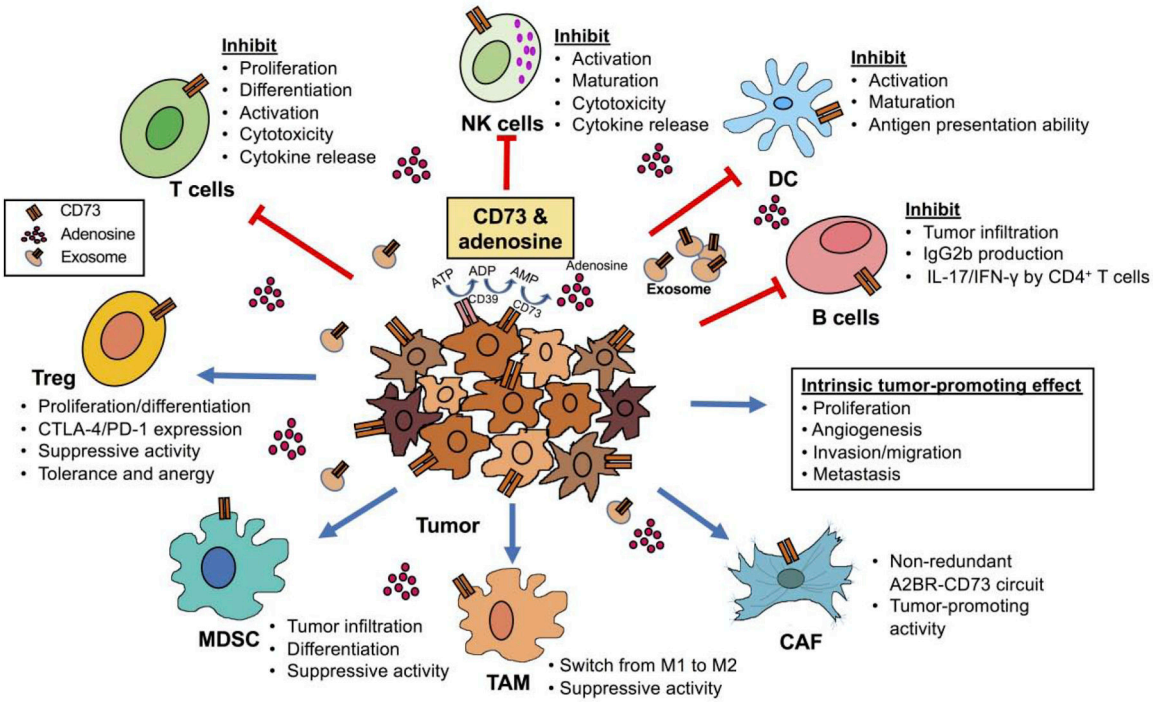

Figure 1. CD73-mediated immunosuppression in the TME

CD73 serve as a major immune suppressive mediator in TME mainly through generation of extracellular adenosine. Besides the effect of cancer cell-intrinsic CD73 on tumor cell proliferation, angiogenesis, invasion/migration and metastasis, CD73 expression by tumor cells and immune cells impairs anti-tumor immunity by suppressing the function of protective immune cells (e.g. effector T cells, NK cells, DC and B cells), while maintaining the function of regulatory immune cells (e.g. Treg, MDSC, TAM and CAF).

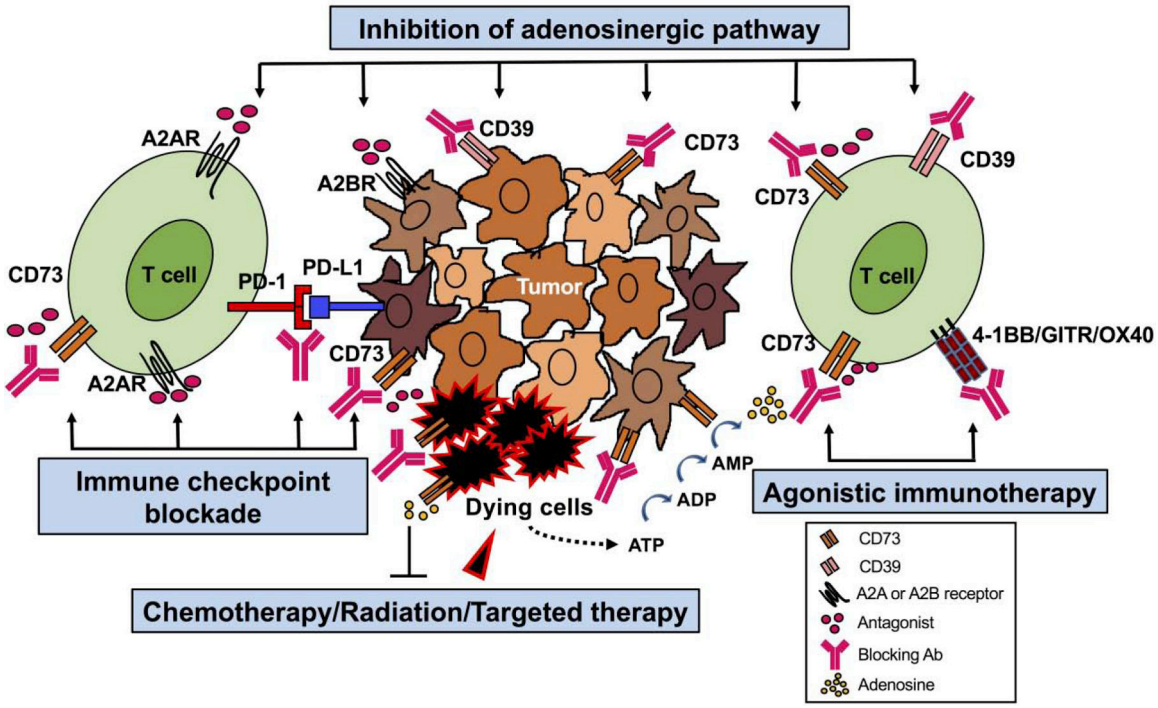

Figure 2. Co-targeting CD73 with other therapies as an attractive therapeutic strategy for cancer treatment

Based on their distinct expression pattern and nonredundant functionality, CD73 along with other molecules of adenosinergic pathway (e.g.CD39, A2AR and A2BR) can be targeted together to achieve synergy in antitumor efficacy by modulating both tumor cells and immune cells (e.g. T cells) in many ways. In addition, CD73 expression and activity seem to be upregulated to confer tumor resistance to therapies. Thus, targeting CD73 with blocking antibodies or small molecule inhibitors in combination with other therapies such as immune checkpoint blockade, adoptive T cell therapy, agonistic immunotherapy, chemotherapy, and radiation therapy is a rational strategy to enhance therapeutic benefit in various cancers. The different combination therapies involving inhibition of CD73 and/or A2AR are already under evaluation in early phase clinical trials.