

Targeting immunosuppressive adenosine in cancer

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Abstract | Despite the success of anti-programmed cell death protein 1 (PD1), anti-PD1 ligand 1 (PDL1) and anti-cytotoxic T lymphocyte antigen 4 (CTLA4) therapies in advanced cancer, a considerable proportion of patients remain unresponsive to these treatments (known as innate resistance). In addition, one-third of patients relapse after initial response (known as adaptive resistance), which suggests that multiple non-redundant immunosuppressive mechanisms coexist within the tumour microenvironment. A major immunosuppressive mechanism is the adenosinergic pathway, which now represents an attractive new therapeutic target for cancer therapy. Activation of this pathway occurs within hypoxic tumours, where extracellular adenosine exerts local suppression through tumour-intrinsic and host-mediated mechanisms. Preclinical studies in mice with adenosine receptor antagonists and antibodies have reported favourable antitumour immune responses with some definition of the mechanism of action. Currently, agents targeting the adenosinergic pathway are undergoing first-in-human clinical trials as single agents and in combination with anti-PD1 or anti-PDL1 therapies. In this Review, we describe the complex interplay of adenosine and adenosine receptors in the development of primary tumours and metastases and discuss the merits of targeting one or more components that compose the adenosinergic pathway. We also review the early clinical data relating to therapeutic agents inhibiting the adenosinergic pathway.

The deconvolution of the tumour microenvironment (TME) has led to the identification of metabolic pathways that are fundamental for tumour cell survival. Tumour hypoxia induces a wide network of metabolic and immunological changes that favour tumour growth and progression^{1–3}. That solid tumours display regions of irregular oxygen tension has been known since 1955 (REF. 4), but only in the past two decades has there been a renaissance in studying tumour hypoxia and its downstream effects on the TME. Oxygen deprivation limits the availability of energy sources and induces the accumulation of extracellular ATP and subsequently adenosine in tumours^{5,6}. Following its release into the TME, ATP signals via the P2 purinergic receptors (P2XRs and P2YRs) primarily to promote antitumour immunity. Alternatively, extracellular ATP is rapidly degraded to adenosine, and adenosine binds to the P1 purinergic receptors (also called the adenosinergic receptors) to hamper immune cell infiltration and activation^{7,8} (FIG. 1).

The ectonucleotidases CD39 (also known as NTPDase 1) and CD73 (also known as 5'-NT) are critical mediators of adenosine accumulation in the TME. Specifically, ATP conversion to ADP and/or AMP occurs in the presence of CD39, while CD73 dephosphorylates

AMP to adenosine (FIG. 1). The accumulated extracellular adenosine then mediates its regulatory functions by binding to one of four adenosine receptors, A1R, A2AR, A2BR and A3R. Each receptor exhibits different affinities for adenosine. A1R, A2AR and A3R respond to low levels of adenosine (250–700 nM) and are thus categorized as high-affinity adenosine receptors. By contrast, activation of the low-affinity receptor A2BR occurs only under pathological conditions such as in the TME where adenosine accumulates at high concentrations (25 µM)⁹. Adenosine receptors are also subdivided on the basis of their ability to induce the downstream signalling molecule intracellular cyclic AMP (cAMP). Signalling via cAMP (as seen with A2AR and A2BR) is typically associated with profound immunosuppression^{10–12}, while activation of A1R and A3R inhibits cAMP generation, and therefore these receptors are generally viewed as immune-promoting adenosine receptors^{13,14}.

Inhibiting hypoxia and adenosine represents a potential approach for anticancer therapy. Accordingly, groundbreaking research by Sitkovsky and colleagues^{1,2} demonstrated that supplementing mice with 60% oxygen reversed tumour hypoxia and resulted in a significant reduction in solid tumour growth and metastases

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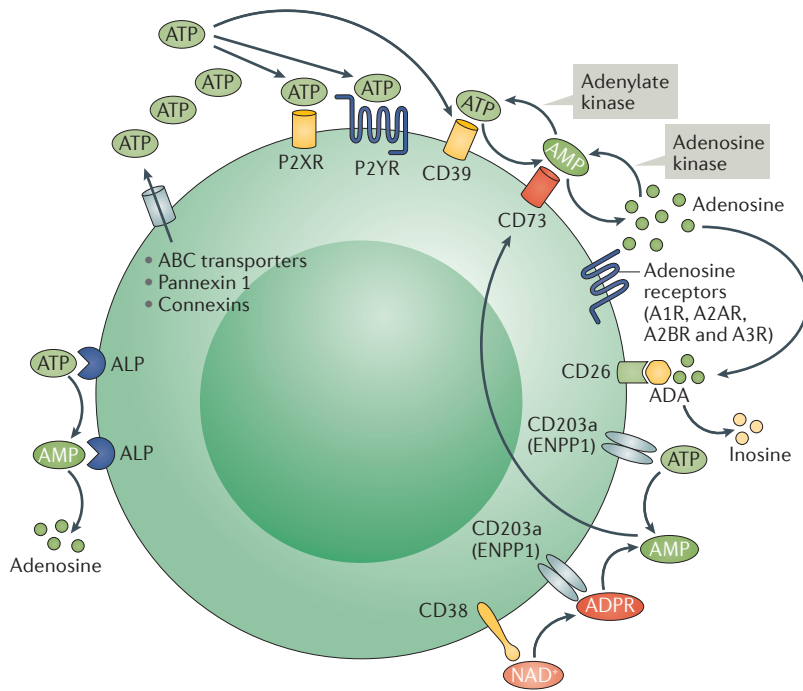


Figure 1 | Adenosine generation and signalling. Hypoxia induces the release of ATP through ATP-binding cassette (ABC) transporters, pannexin 1 or connexins. The accumulated ATP can either stimulate P2 purinergic receptors (P2XR and P2YR) or be further degraded to adenosine by the sequential action of the ectonucleotidases CD39 and CD73. This degradation pathway could be reversed in the presence of adenosine kinase. In addition to ectonucleotidases, alkaline phosphatase (ALP) can also contribute to the production of extracellular adenosine. In some cancers, NAD⁺ released by the salvage pathway can be hydrolysed to adenosine through the CD38 (or its paralogue CD157)–CD203a (also known as ENPP1)–CD73 pathway as demonstrated. Accumulated adenosine can be further degraded to inosine in the presence of adenosine deaminase (ADA) through its association with CD26, although this pathway may be inhibited in some cancers by adenosine itself¹³⁸. Overall, this purinergic pathway can be observed on tumour, stromal and immune cells within the tumour microenvironment (TME). ADPR, ADP-ribose.

Hypoxia
The disorganized arrangement of blood vessels around a tissue such as cancer, which often results in irregular distribution of oxygen within that tissue; low oxygen levels are often seen in regions of tissues further away from blood vessels.

Ectonucleotidases
Families of nucleotide-metabolizing enzymes that possess an active catalytic site and are expressed on the plasma membrane. These enzymes are associated with the catalysis of nucleotides to their corresponding nucleosides.

via an A2AR-mediated mechanism¹. Similarly, antagonism of adenosine generation and signalling has also resulted in durable antitumour responses in mice^{15–17}. In particular, A2AR inhibitors potentiate antitumour effects largely through the modulation of immune cell functions, such as enhancing the effector functions of cytotoxic lymphocytes and preventing the recruitment and polarization of immunosuppressive cell types in the TME^{11,18–20}. By contrast, the pro-tumour effects of A2BR and CD73 occur through both tumour-intrinsic and host-mediated pathways^{16,18,20–23}. CD39 is broadly expressed across a diverse range of cancer cell types²⁴ and immune cell types^{25–30}. Although the role of CD39 in altering tumour immunity is only now emerging, current evidence indicates that CD39 may modulate both tumour²⁴ and immune cells^{30,31} to promote tumour growth. Within the immune cell compartment, A2AR and CD73 primarily impair the function of natural killer (NK) cells and CD8⁺ T cells^{11,18,20,23,32–36}. In addition to acting directly on NK cells and CD8⁺ T cells, A2AR also acts through myeloid cells to hamper cytotoxic lymphocyte functions¹⁹. The immune inhibitory functions of

CD39, CD73 and A2AR also occur through the modulation of CD4⁺CD25⁺ forkhead box P3 (FOXP3)⁺ regulatory T cells (T_{reg} cells)^{23,30,37}, while A2BR elicits immunosuppression mainly via myeloid cells^{27,38,39}.

Such differences in the mechanism of action by adenosinergic molecules have thus allowed testing of combination strategies that might target potential non-redundant adenosinergic pathways within the TME. To this end, dual targeting of A2AR and CD73 showed a significant combination benefit in controlling primary tumour growth and lung metastases in mice²⁰. Furthermore, combination therapies targeting the adenosinergic pathway alongside immune checkpoint blockade, chemotherapies, targeted therapies and adoptive cellular therapies (ACTs) have displayed improved antitumour efficacy^{8,15,34,36,40}. These encouraging results were instrumental in the initiation of clinical trials in 2016 of some small molecule inhibitors targeting A2AR (NCT02403193 (REF. 41), NCT02655822 (REF. 42) and NCT02740985 (REF. 43)) and another examining the efficacy of a CD73 monoclonal antibody (mAb) (NCT02503774)⁴⁴ in patients with solid cancers. This Review details the role of adenosine in cancer progression and highlights the potential of using adenosinergic targets in the treatment of patients with cancer. In particular, we present the results from the first phase I clinical trial data and discuss strategies that could improve adenosine receptor antagonist or mAb development in the treatment of solid cancers.

Adenosinergic molecules in cancer

A hypoxic TME provides a strong selection pressure for tumour cells, which consequently increases their aggressiveness⁴⁵. The lack of oxygen results in nutrient deprivation, forcing both tumour and immune cells to compete for essential nutrients. During this process, cancer cells may hamper the proliferation and effector functions of lymphocytes by competing for glucose⁴⁵ and thereby evade immune surveillance, resulting in their survival and escape to distant organs. Notably, hypoxia-inducible factor 1α (HIF1α), the master transcriptional regulator of hypoxia, is a strong inducer of CD39, CD73 and A2BR expression^{46–48}. Hence, it is not surprising that CD39, CD73 and A2BR are overexpressed in various cancers and often correlate with poor prognosis in patients^{16,21}. Indeed, high expression of ectonucleotidases has consistently correlated with poor prognosis in patients with gastric, ovarian and breast cancers, hepatocellular carcinoma (HCC) and non-small-cell lung cancers (NSCLCs) (TABLE 1), thus justifying the rationale to target CD73 and CD39 in the clinic.

Immunosuppression is a classical hallmark of cancer, and identifying strategies that can overcome this suppressive barrier is essential⁴⁹. cAMP potently dampens the immune response⁵⁰; thus, this Review focuses on the role of cAMP-inducing A2AR and A2BR in the TME. Overexpression of A2BR was associated with poor survival in patients with triple negative breast cancer (TNBC), multiple myeloma, acute myeloid leukaemia (AML) and liposarcoma²¹. Wang *et al.*⁵¹ showed that the microRNA miR-128b (also known as miR-128-2),

Regulatory T cells

(T_{reg} cells). A subpopulation of CD4⁺ T cells that are involved in modulating inflammation and preventing autoimmunity. However, in the tumour microenvironment, the accumulated presence of these suppressor populations has an important role in impairing antitumour immunity.

Adoptive cellular therapies

(ACTs). Treatments used to help the immune system fight diseases, such as cancer and infections with certain viruses. T cells are collected from a patient and grown *ex vivo* to increase the number of T cells that are able to kill cancer cells or fight infections. These T cells are then infused back into the patient. Also called cellular adoptive immunotherapy.

Recurrence-free survival

Relating to cancer therapy, refers to the time after a treatment when patients show no signs of disease re-appearance (that is, these patients are cancer-free). This is also called disease-free survival or relapse-free survival.

a repressor of A2BR expression, is downregulated in patients with gastric cancer. This downregulation invariably increased A2BR expression and tumour cell proliferation, migration and survival. Unlike CD39, CD73 and A2BR, A2AR is regulated by the transcription factor nuclear factor-κB (NF-κB)⁵², but there is a limited understanding of how A2AR influences tumour cell function. A recent study observed that high expression of A2AR in patients with adenocarcinoma correlated favourably with recurrence-free survival⁵³. While this observation appears paradoxical to our current understanding of A2AR in cancers, it is important to highlight that the tumour biopsy samples in this study were also used to

investigate levels of CD73. Patients with CD73^{hi}A2AR^{hi} tumours displayed significantly poorer prognosis compared with patients with CD73^{lo}A2AR^{hi} tumours⁵³, thus implying that these effects could be attributed in part to tumour CD73 expression rather than A2AR expression. Additionally, CD73^{hi}A2AR^{lo} tumours exhibited high immune cell infiltration compared with CD73^{lo}A2AR^{hi} tumours, although the nature of these infiltrates is unknown⁵³. It is conceivable that adenosine produced within a CD73^{hi}A2AR^{lo} tumour, but not a CD73^{lo}A2AR^{hi} tumour, may activate A2BR, which subsequently induces the accumulation of suppressive myeloid cells in the tumour.

Table 1 | Involvement of adenosinergic molecules in human cancers

Enzyme or receptor	Cancer type	Major finding	Refs
CD39	High-grade serous ovarian cancer	In patients displaying high CD39 expression, a trend (<i>P</i> = 0.0507) towards poor OS was identified (meta-analysis)	58
	HCC	Poor OS and RFS were seen in patients with high CD39 expression	140
	Gastric cancer	Elevated CD39 expression in the tumour correlated with poor prognosis (<i>n</i> = 42 out of 84 patients)	141
	CLL	Increased expression of CD39 on T cells, but not B cells, was linked to poor survival (<i>n</i> = 15 out of 68 patients)	142
CD73	Gastric cancer	High CD73 expression was associated with poor prognosis (<i>n</i> = 31 out of 68 patients)	143
	TNBC	A relationship between high CD73 expression and poor prognosis was seen only in patients with TNBC and not in patients with HER2 ⁺ or luminal breast cancer	94
	NSCLC	High CD73 expression was associated with poor prognosis (<i>n</i> = 66 out of 653 patients)	53
	Rectal adenocarcinoma	High tumour-derived CD73 expression was associated with worse outcomes (<i>n</i> = 47 out of 90 patients)	144
	Colorectal cancer	Increased CD73 expression negatively correlated with prognosis in 16 patients tested	102
	Renal cell carcinoma	High CD73 expression was associated with poor prognosis (<i>n</i> = 136 out of 235 patients) and increased tumour grade	145
	Ovarian cancer	High CD73 expression was a poor prognostic marker in these patients, which was also associated with a high number of CD73 ⁺ CD8 ⁺ T cells in the tumour (meta-analysis)	58
	Prostate cancer	High CD73 expression in the tumour stroma and tumour epithelium was associated with shorter RFS and shorter bone-metastasis-free survival in these patients (<i>n</i> = 137 out of 285 patients)	146
	Oral SCC	Poor RFS and OS were seen in patients who showed elevated CD73 expression (<i>n</i> = 66 out of 113 patients)	95
	HNSCC	Patients exhibiting increased CD73 expression showed poorer OS (<i>n</i> = 100 out of 162 patients). In this study, high CD73 expression was also seen in metastatic lymph nodes	95
	Urothelial bladder cancer	High CD73 expression reduced the rate of cancer progression in these patients (<i>n</i> = 46 out of 174 patients)	147
Endometrial cancers	High CD73 expression was associated with better prognosis in these patients	148	
A2AR and A2BR	NSCLC	High A2AR expression was indicative of better OS and RFS (<i>n</i> = 316 out of 642 patients)	53
	TNBC	Patients with TNBC and high A2BR expression showed poor survival, while no correlation was seen in patients with HER2 ⁺ or luminal breast cancer (meta-analysis)	21

CLL, chronic lymphocytic leukaemia; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small-cell lung cancer; OS, overall survival; RFS, recurrence-free survival; SCC, squamous cell carcinoma; TNBC, triple negative breast cancer.

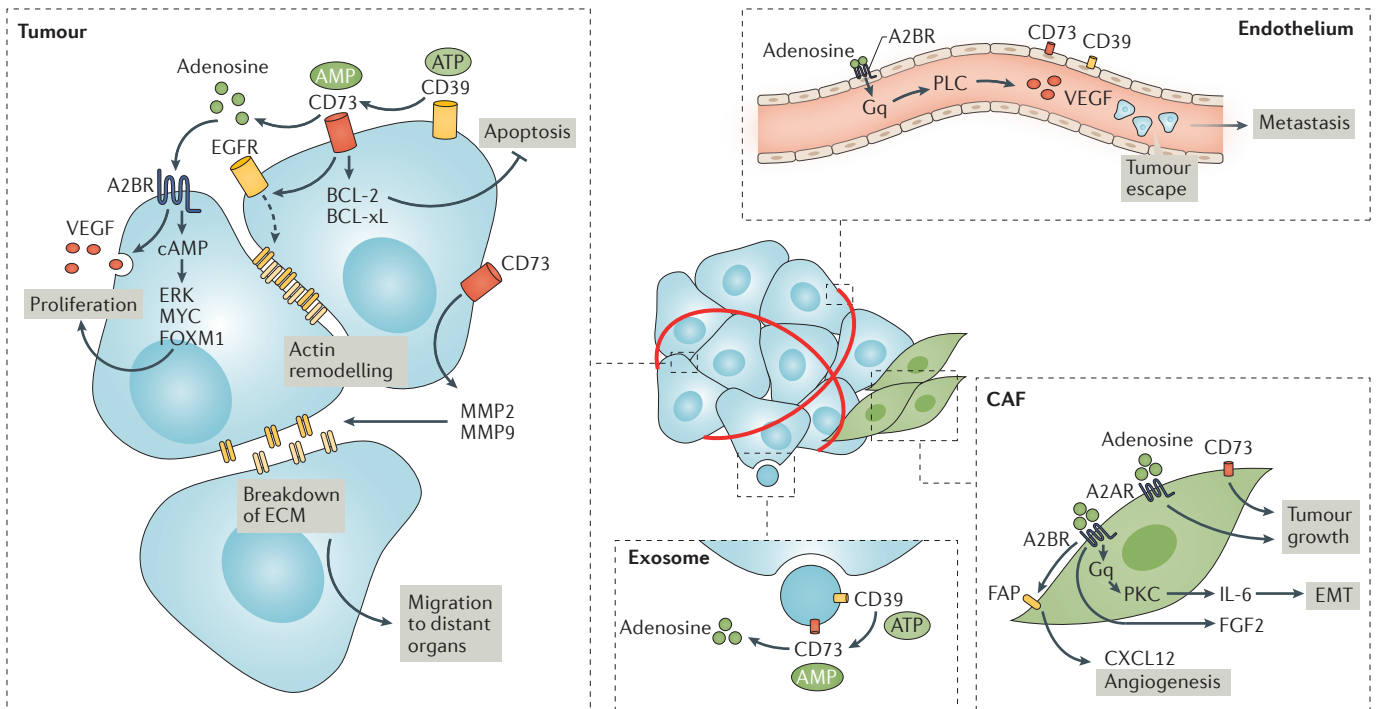


Figure 2 | Effect of adenosinergic molecules on the tumour and surrounding stroma. Adenosine accumulation favours tumour growth and metastasis through effects on tumour cells and stroma. For example, activation of CD73 on tumour cells favours cell adhesion potentially through epidermal growth factor receptor (EGFR) signalling (indicated by dashed arrow) and inhibits tumour apoptosis. CD73 activation also releases matrix metalloproteinases (MMPs) that facilitate breakdown of extracellular matrix (ECM), thus enabling tumour cells to invade and migrate to distant organs. In addition, A2BR activation on tumour cells promotes proliferation and angiogenesis through secretion of vascular endothelial growth factor (VEGF). In cancer-associated fibroblasts (CAFs), A2BR induces fibroblast growth factor 2 (FGF2) expression and increases the number of fibroblast activation protein (FAP)-positive fibroblasts. A2BR activation leads to elevated release of CXCL12 by these FAP⁺ fibroblasts, thus increasing the number of CD31⁺ endothelial cells within the tumour¹³⁹. A2BR can engage the G protein-coupled receptor Gq–protein kinase C (PKC) signalling pathway to activate interleukin-6 (IL-6), which in turn mediates epithelial-to-mesenchymal transition (EMT). Additionally, some tumour exosomes co-express CD39 and CD73, which might be associated with tumour dissemination to distant organs. cAMP, cyclic AMP; FOXM1, forkhead box M1; PLC, phospholipase C.

Functions of adenosinergic molecules

Adenosinergic molecules cause strong immunosuppression in the TME through multiple non-overlapping functions, including enzymatic and non-enzymatic pathways as well as through the activation of tumour-intrinsic and host-mediated pro-tumour mechanisms^{8,17}. The therapeutic inhibition of the adenosinergic pathway may not only reverse immunosuppression but, via CD39 blockade, can also consequently lead to the accumulation of ATP in the TME. Remarkably, by associating with its cognate P2 purinergic receptors, ATP can trigger a form of cancer cell death, termed immunogenic cell death (ICD). During ICD, ATP acts as a potent immunogenic signal, allowing dying cancer cells to induce an anticancer vaccine effect^{54,55}. In addition to activating ICD, adenosine inhibition may also directly impact tumour cells, stromal cells (FIG. 2) and immune cell subsets (FIG. 3).

Adenosinergic molecules influence tumour growth, survival, adhesion and migration. Dual inhibition of A2AR and CD73 resulted in synergistic antitumour responses in preclinical models²⁰, and therefore more attention should

be paid to the interrelationships between the adenosinergic molecules. The functions of CD39⁺ tumour cells and CD73⁺ tumour cells appear to be overlapping, such that inhibition of CD39 activity in human SK-MEL-5 melanoma cells²⁴ or knockdown of CD73 on mouse ID8 ovarian cancer cells⁵⁶ or human MB-MDA-231 TNBC cells⁵⁷ elevates the proliferation and effector functions of CD8⁺ T cells *in vitro*. Furthermore, CD73 expression on tumour cells can directly influence their survival and proliferation⁵⁸. In addition to the well-understood enzymatic function of CD73, this ecto-enzyme also serves as an adhesion molecule, participating in the migration of melanoma⁵⁹ and breast cancer cell lines^{57,60,61}.

A2BR activation in TNBC cells increased their survival via the ERK signalling pathway. Knockdown of A2BR in these same cells reduced lung metastases and directly hampered cell proliferation, survival and invasion²¹. Intriguingly, Vecchio *et al.*¹² showed that A2BR is constitutively activated in prostate cancer cells, and this is critical to the proliferation of these cancer cells *in vitro* (FIG. 2). This constitutive activation was observed to be independent of its ligand availability, indicating an adenosine-independent function for A2BR in these

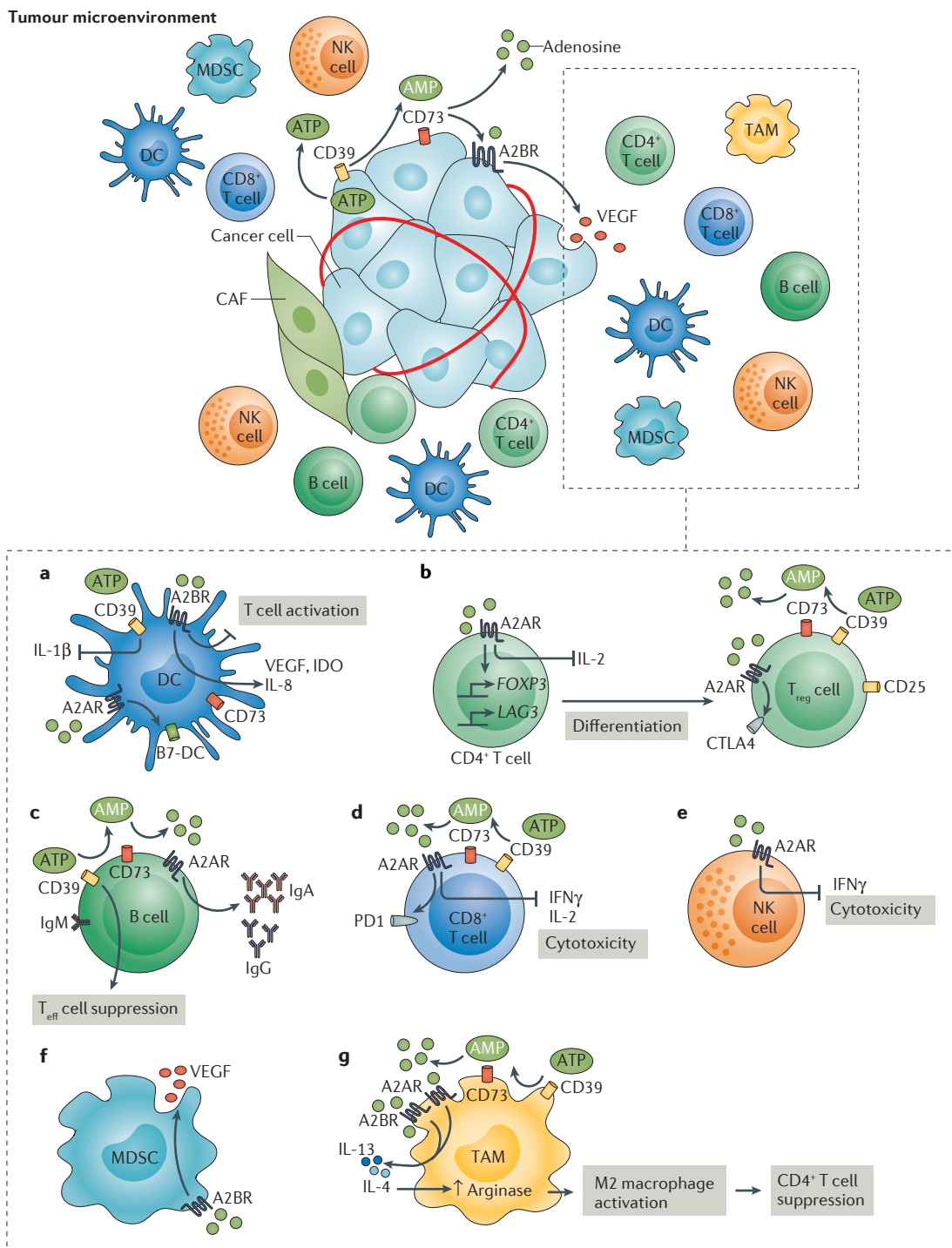


Figure 3 | Adenosine-mediated immunosuppression in the tumour microenvironment. Expression and activation of cyclic AMP (cAMP)-inducing adenosinergic molecules impairs the proliferation and effector functions of cytotoxic lymphocytes while simultaneously promoting the generation and infiltration of immunosuppressive cells, including regulatory T (T_{reg}) cells, myeloid-derived suppressor cells (MDSCs), B cells and tumour-associated macrophages (TAMs). **a** | On dendritic cells (DCs), activation of CD39, A2BR or A2AR impairs DC antigen presentation and subsequent T cell activation. **b** | Stimulation of A2AR on naive $CD4^+$ T cells promotes the development of T_{reg} cells through activation of forkhead box P3 (FOXP3) and lymphocyte activation gene 3 (LAG3). T_{reg} cells also express high levels of CD39 and CD73, and these cells have increased immunosuppressive potential. **c** | Similarly, CD39, CD73 and A2AR expression on B cells suppresses T effector (T_{eff}) cell functions and induces the secretion of immunoglobulin A (IgA) and IgG type antibodies. **d,e** | On $CD8^+$ T cells and natural killer (NK) cells, A2AR activation impairs the cytotoxic potential of these cells. **f** | A2BR activation on MDSCs induces vascular endothelial growth factor (VEGF) secretion and angiogenesis. **g** | A2BR stimulation in TAMs favours M2 macrophage polarization. B7-DC, also known as PDL2; CTLA4, cytotoxic T lymphocyte antigen 4; IDO, indoleamine 2,3-dioxygenase; IFN γ , interferon- γ ; IL, interleukin; PD1, programmed cell death protein 1.

cancer cells. Similarly, A2AR expression in human melanoma and breast cancer cell lines was associated with increased cell survival and proliferation^{62,63}; however, these studies were only conducted *in vitro*. Thus, it remains likely that the major site of action for A2AR in the TME is on host immune cells.

Adenosinergic molecules in the endothelium, lymph nodes and exosomes. Adenosine was initially identified as a mediator of cardiovascular and angiogenic functions⁶⁴, yet our understanding of how adenosinergic molecules directly influence endothelial cells in tumours is limited (FIG. 2). Global loss of CD39, CD73, A2AR or A2BR resulted in reduced vascular endothelial growth factor (VEGF) and CD31 (also known as PECAM1) staining on tumour vessels in mouse cancer models^{22,31,65,66}. By use of bone marrow chimeric mice, it was shown that optimal antitumour effects relied on blocking CD73 expression on both bone-marrow-derived host cells and host cells not derived from the bone marrow^{23,67}. However, it remains unknown if any of these effects are intrinsic to endothelial cells. We recently developed an endothelial-cell-specific conditional vascular endothelial cadherin (VE cadherin)-Cre × A2BR floxed strain of mice and found no apparent role for A2BR on these cells in the control of metastasis (M.J.S., unpublished observations). Morello and colleagues³⁹ also showed that CD11b⁺Gr1⁺ myeloid-derived suppressor cells (MDSCs) were the major producers of VEGF in a B16F10 mouse melanoma model and that the inhibition of A2BR reduced MDSC infiltration and VEGF levels and resulted in significantly reduced angiogenesis in these mice (FIG. 3).

Lymph nodes (LNs) might serve as reservoirs for the dissemination of tumour cells to trigger distant metastasis. Yang *et al.*⁶⁸ reported higher CD73 expression on prostate cancers that had metastasized to LNs than on non-metastasizing tumours. Furthermore, patients with melanoma with more advanced clinical staging, as assessed by their level of nodal metastases, express higher levels of CD73 (REF. 40). Systemic administration of the adenosine analogue 5'-N-ethylcarboxamidoadenosine (NECA) increased metastatic formation in the LNs, but not primary tumour growth, in a tamoxifen-inducible melanoma mouse model⁴⁰.

Exosomes isolated from patients with mesothelioma co-express CD39 and CD73 (REF. 69). As exosomes rapidly disseminate from the primary tumour through the lymphatics and into the LNs⁷⁰, the presence of CD39 and CD73 on these exosomes could potentially suppress systemic immunity and help facilitate the generation of pre-metastatic niches. Loss of host CD73 in mice increased lymphocyte influx into the LNs, and this occurred via an L-selectin-dependent mechanism⁷¹. By contrast, CD73 expression on lymphocytes facilitates binding of these cells to the endothelium, resulting in lymphocyte migration and extravasation into tissues⁷². Therefore, while a CD73 mAb could inhibit tumour metastasis to LNs, it may either increase or decrease endothelial entry of tumour-reactive T cells into the tumour.

Adenosinergic molecules and immunosuppression.

A successful immunotherapy is effective by engaging effector CD8⁺ T cells and NK cells within the TME^{73,74}. Adenosine suppresses tumour immunity, largely by restricting immune cell infiltration, cytotoxicity and the production of cytokines, such as interferon-γ (IFNγ)^{1,2,18,75} (FIG. 3). Thus, genetic ablation or therapeutic inhibition of CD73 or A2AR improves the effector functions of cytotoxic lymphocytes and significantly reduces tumour growth^{11,20,23,33,36,76}. These findings correlate well with clinical observations reported in patients with high-grade serous ovarian cancer, where the prognostic value of infiltrating CD8⁺ T cells was improved when tumours had low CD73 expression⁵⁸.

Re-invigoration of exhausted or dysfunctional T cells in the TME is another area where therapeutic targeting of adenosine might have a direct or indirect role. Elevated levels of CD39⁺ T_{reg} cells were identified in patients with follicular lymphoma, which resulted in T cell hyporesponsiveness⁷⁷, and tumour-infiltrating CD4⁺ and CD8⁺ T cells in the AT-3 mammary carcinoma model also expressed high levels of CD39 (REF. 78). Recently, human CD39⁺CD8⁺ T cells isolated from patients with chronic viral infections displayed gene signatures consistent with an exhausted or dysfunctional T cell phenotype, including high expression of the inhibitory receptors programmed cell death protein 1 (PD1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA4)²⁹. Anergy is postulated to be a peripheral tolerance mechanism whereby T cells lose the ability to produce autocrine growth factors and proliferate in response to antigen. A newly defined population of anergic CD4⁺FOXP3⁻ T cells present in mouse secondary lymphoid organs was shown to express high levels of CD73 (REF. 79). These observations imply that CD39 and CD73 expression on tumour-infiltrating T cells could influence local and peripheral tolerance, further emphasizing the therapeutic potential of targeting these molecules in cancer therapy.

Signalling via A2AR in dendritic cells (DCs) increases the expression of B7-DC (also known as PDL2)⁸⁰, a ligand for the inhibitory receptor PD1. Expression analysis of A2AR in T cells from wild-type (WT) and heterozygous *Adora2a*^{+/-} mice demonstrated that there is no A2AR reserve in T cells and that A2AR-driven cAMP accumulation was significantly reduced in heterozygous mice compared with WT mice⁸¹. This study indicated that the number of A2AR molecules per T cell directly correlates with the level of immunosuppression in the TME. Indeed, A2AR inhibits T cell proliferation and cytokine production and elevates surface expression of PD1 and CTLA4 (REFS 76,82,83). Moreover, A2AR activation in T cells inhibited T cell receptor (TCR)-mediated transcription factor AP-1 signalling and promoted T cell tolerance⁸⁴. Thus, genetic ablation of *Adora2a* in CD8⁺ T cells or treatment of mice with an A2AR antagonist improved ACT and resulted in significant tumour suppression¹¹. Antagonism of A2AR has also been shown to improve memory T cell responses in tumour-bearing mice³⁶. Sitkovsky *et al.*^{18,85} proposed that tumour suppression induced by A2AR antagonism was mediated by CD8⁺ T cells via the release of cytotoxic granules and/or

Myeloid-derived suppressor cells

(MDSCs). A heterogeneous population of myeloid immune cells that originate from the bone marrow and exhibit potent suppressive functions.

Exosomes

Microvesicles of endocytic origin that are secreted by several cells, including tumour cells.

Mesothelioma

An aggressive form of cancer originating around the lining (mesothelium) of organs such as the lungs, abdomen or heart.

Exhausted or dysfunctional T cells

A state of T cells generally associated with progressive loss of T cell effector functions, resulting in exhaustion or dysfunction. Exhausted T cells are commonly observed during many chronic infections and cancer.

FAS ligand (FASL) ligation of the death receptor FAS (also known as CD95). Alternatively, in A2AR-deficient mice, tumour angiogenesis was reduced and starvation of tumour cells ultimately led to their death^{11,85}.

Adenosinergic molecules can also indirectly hamper the proliferative and cytotoxic potential of lymphocytes^{24,86,87}. For example, conditional deletion of A2AR on myeloid cells resulted in improved antitumour immunity *in vivo* by reducing immunosuppressive interleukin-10 (IL-10) cytokine production, which subsequently improved infiltration, activation and effector functions of CD8⁺ T cells and NK cells in the tumour¹⁹. Likewise, inhibition of A2BR improved DC activation and subsequent adaptive responses in mice, resulting in reduced growth of MB49 bladder and 4T1 mammary carcinomas⁸⁸. Adenosine also promotes the development of suppressive T_{reg} cells and MDSCs, which correlates with poor prognosis in patients with cancer^{89,90} (FIG. 3). Adoptive transfer of T_{reg} cells from CD39-deficient mice, but not WT mice, into immunodeficient mice improved NK cell functions, signifying a fundamental tumour-promoting function for CD39⁺ T_{reg} cells³⁰. Similarly, CD73⁻ T_{reg} cells fail to suppress effector T cell functions^{23,65}. Activation of A2AR in naive CD4⁺ T cells drives their differentiation towards CD4⁺FOXP3⁺ T cells. Furthermore, A2AR expression on T_{reg} cells augments the suppressive phenotype of these cells⁹¹. While the expression and role of A2BR on lymphocytes is not well defined, there is compelling evidence in support of antagonism of A2BR suppressing tumour growth mainly via reducing the accumulation of MDSCs in the TME^{38,39}. CD39 expression has also been identified on MDSCs isolated from the peripheral blood of patients with colorectal cancers, although the functional relevance of this finding needs further investigation⁹². Similarly, adenosine-differentiated DCs displayed high levels of tolerogenic molecules such as VEGF and indoleamine 2,3-dioxygenase (IDO), which together impaired DC allostimulatory functions and resulted in accelerated tumour growth in mice⁹³. In addition to MDSCs, tumour-associated macrophages (TAMs) also express CD39 and CD73, which suppress CD4⁺ T cell proliferation through the production of adenosine⁸⁶.

Regulation of adenosinergic molecules

Adenosine-generating enzymes and adenosine receptors may not be ubiquitously expressed throughout cancer development. Identification of factors or drivers that modulate their expression in tumour development and progression will provide essential clues to understanding when adenosine-based therapies may be most clinically relevant. For example, high expression of the genes encoding CD73 or A2BR correlated with poor survival in patients with advanced TNBC^{21,94}. Similarly, high CD73 expression is seen in patients with advanced-stage melanoma⁴⁰ and with head and neck squamous cell carcinoma (HNSCC) exhibiting LN metastasis⁹⁵. By contrast, high CD73 expression was associated with poor prognosis only in patients with stage I or II, but not stage III or IV, oral SCC⁹⁵. Likewise, expression of *NT5E*, the gene encoding CD73, was highest in the C1 molecular subtype

of high-grade serous ovarian cancer, a tumour subtype characterized by increased stromal infiltration and angiogenesis⁵⁸. Importantly, increased tumour aggressiveness in CD73^{hi} tumours may not necessarily correlate with increased adenosine but could also indicate activation of alternative CD73 pathways (see below) that could favour tumour development and spread.

Several immunological and non-immunological events might drive the activation of the adenosinergic pathway in the TME (FIG. 4). For example, adenosine accumulation and signalling occurs as a consequence of an overactive immune response¹⁰, and a negative feedback mechanism such as this may well dampen inflammation in the TME. Indeed, pro-inflammatory factors such as tumour necrosis factor (TNF) have been reported to upregulate the expression of adenosinergic receptors A2AR and A2BR on host cells, most likely through the activation of NF- κ B^{52,96}. Activation with an agonistic CD40 mAb moderately increased CD73 and CD39 expression in an *in vivo* mouse tumour model⁷⁸. In the same study, PD1 expression was also reduced in the TME⁷⁸. Therefore, it will be essential to delineate whether upregulation of CD39 and CD73 occurred in response to inflammation or whether this is a potential compensatory immunoregulatory mechanism arising from reduced PD1 expression. Indeed, increased tumour-derived CD73 is observed in a subset of patients with melanoma after receiving anti-PD1 immunotherapy⁹⁷. However, this finding requires further validation with larger patient cohorts and long-term survival outcomes.

In addition to hypoxia, transforming growth factor- β (TGF β) is another critical regulator of tumour growth and angiogenesis in the TME⁹⁸ and can drive the expression of both CD39 and CD73 on myeloid cells and innate lymphoid cells^{27,99}. The expansion of these cells directly correlated with angiogenesis and favoured tumour growth and metastasis²⁷. Furthermore, conditioning both CD8⁺ and CD4⁺ T cells with TGF β also elevated their CD73 expression¹⁰⁰. While TGF β induces FOXP3 expression in CD4⁺ T cells, elevated CD73 expression was generated independently of T_{reg} cell polarization¹⁰⁰ (FIG. 4). Therefore, co-targeting hypoxia and TGF β represents an alternative combination strategy that could be investigated.

Many human cancers, including TNBC, ovarian cancer and colorectal cancer, carry mutations in *TP53* (REF. 101). These cancer types also exhibit high levels of CD73 expression, which are associated with poor prognosis^{58,94,95,102}. In addition, patients with melanoma harbouring *TP53* mutations showed significantly increased tumour-derived CD73 expression⁴⁰. This observation indicates that *TP53*-mutant tumours, particularly those displaying high CD73 expression, may present a more suitable tumour type for targeting the adenosine pathway. By contrast, A2BR is a direct transcriptional target of p53 (FIG. 4), and in cancers expressing WT p53, adenosine signalling by A2BR favours tumour cell apoptosis through negative regulation of the anti-apoptotic molecules BCL-2 and BCL-xL¹⁰³.

Lastly, investigations into the spatial heterogeneity of immune cell density within the tumour core and invasive margin and how this is altered in response to adenosine

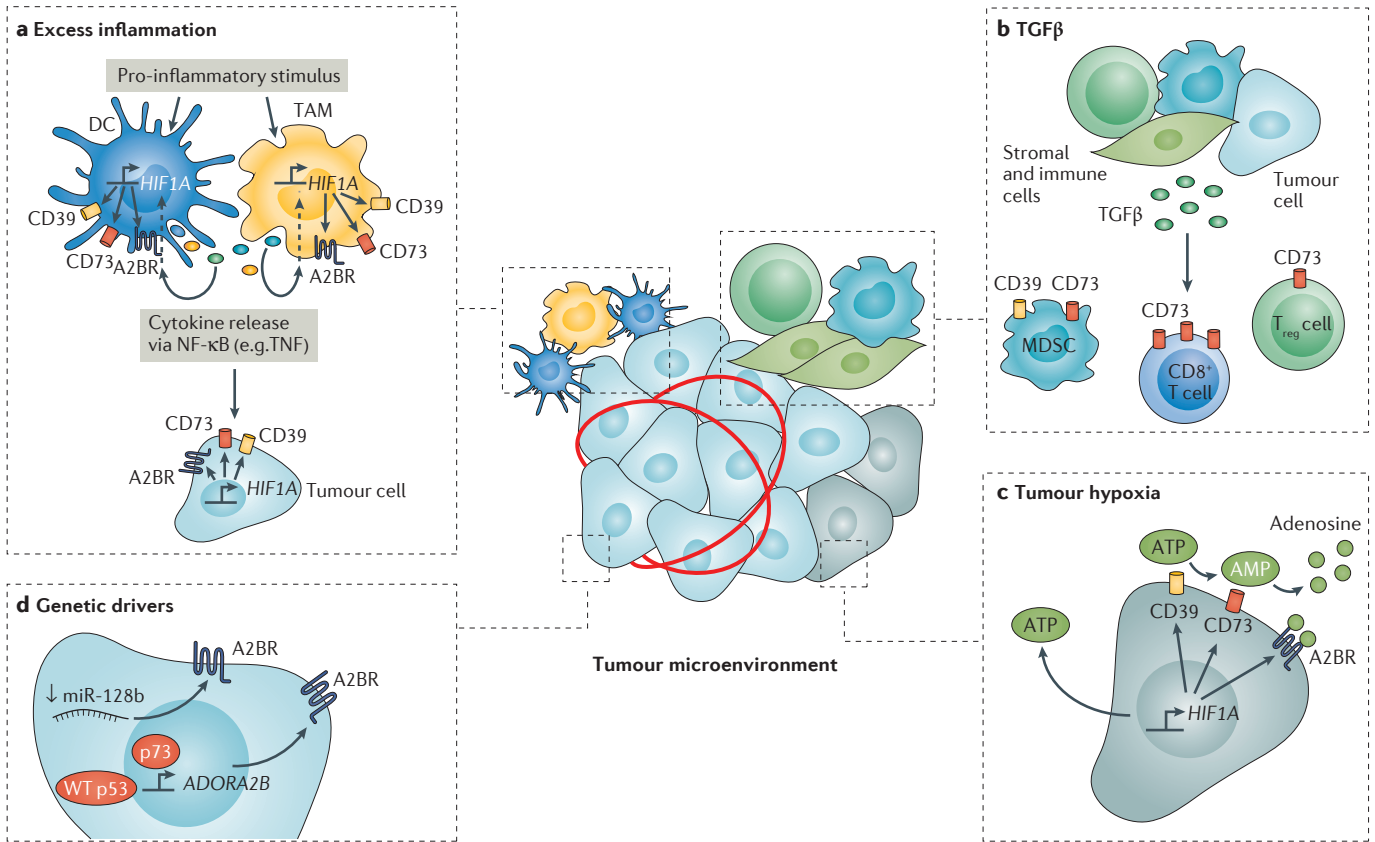


Figure 4 | Regulation of adenosinergic molecules in the tumour microenvironment. **a** | Pro-inflammatory processes such as cellular stress or chronic infection result in accumulation of cytokines such as tumour necrosis factor (TNF) through nuclear factor- κ B (NF- κ B) activation. The excess cytokines trigger a negative feedback loop in either an autocrine or paracrine manner to induce the expression of adenosine molecules, most likely via hypoxia-inducible factor 1 α (HIF1 α). **b** | The secretion of the immunoregulatory cytokine transforming growth factor- β (TGF β) by tumour cells and immune suppressive cells in the tumour microenvironment (TME) can induce expression and activation of CD39 and CD73 on these cells. **c** | Hypoxic tumours, through the activation of HIF1 α , can activate the adenosinergic pathway. **d** | In addition to immunological drivers, the transcriptional regulators p73 and wild-type (WT) p53 can induce expression of the gene *ADORA2B* encoding A2BR on tumour cells. In addition, tumours downregulate microRNA miR-128b, a transcriptional repressor of A2BR, which subsequently leads to elevated expression of A2BR on tumours. DC, dendritic cell; MDSC, myeloid-derived suppressor cell; TAM, tumour-associated macrophage; T_{reg} cell, regulatory T cell.

could provide valuable insight into how a hypoxic TME regulates localization of immune cell infiltrates. For example, Hatfield *et al.*^{1,2} observed that CD8⁺ and CD4⁺ T cells were excluded from hypoxic areas of tumours but resided in adjacent normoxic regions of the tumour. Furthermore, hyperoxic conditions reduced the density of blood vessels encapsulating the tumours through marked reduction in VEGF levels². Importantly, reducing tumour hypoxia by supplemental oxygenation reduced HIF1 α , extracellular adenosine and hence A2AR signalling within these tumours^{1,2}. In tumour-bearing mice deficient in A2AR, a marked upregulation of tumour and host CD73 expression was observed compared with WT control tumours²⁰. On the basis of these observations, future experiments are required to identify whether CD73 expression (i) is hypoxia-dependent, (ii) modulates adenosine levels and signalling within the TME and/or (iii) is transient or constitutive through tumour development. Importantly, examination of the

temporal kinetics of adenosine induction within the tumour core may further our understanding of cancer growth and development.

Targeting the adenosinergic pathway

Much anticipation and excitement awaits the first clinical trial results in patients with cancer who are receiving new agents that reduce extracellular adenosine generation or activity in tumours. Vernalis (licensed by Corvus Pharmaceuticals), Palobiofarma (licensed by Novartis) and Heptares (licensed by AstraZeneca) have developed A2AR inhibitors, namely, CPI-444 (NCT02655822)⁴², PBF-509 (NCT02403193)⁴¹ and AZD4635 (NCT02740985)⁴³, for the treatment of cancers. All three compounds were initially developed for central nervous system (CNS) indications (that is, good penetration into the brain, where they compete with adenosine receptors). This touches on a key question — will these types of compounds ever be able to

Hyperoxic conditions
A condition where cells or tissues are exposed to an elevated concentration of oxygen.

Stable disease

A term commonly used in cancer to describe the condition where tumours neither progress to distant organs nor regress.

G protein-coupled receptors

(GPCRs). Transmembrane receptors that detect extracellular molecules to initiate signalling pathways essential for cellular processes and maintenance of homeostasis.

effectively block adenosine signalling in the TME, where adenosine concentrations could be orders of magnitude higher than in the brain?

CPI-444 is an orally available selective A2AR inhibitor (and has an inhibitory constant K_i in the low nM range and >50-fold selectivity over A1R and >400-fold selectivity over A2BR and A3R) with a reported plasma half-life in the range of hours in humans. In the first evaluation of safety and clinical activity of an A2AR antagonist presented at the Annual Meeting of the American Association of Cancer Research in 2017, CPI-444 was administered alone or with atezolizumab (anti-PD1 ligand 1 (PDL1)) in patients with a spectrum of advanced tumours (TABLE 2). These included patients with NSCLC, melanoma, renal cell carcinoma, TNBC and other cancers who had received one to five lines of previous therapy, with about half of these patients being refractory to anti-PD1 and anti-PDL1 therapy¹⁰⁴. A two-step trial design included a dose-selection accrual of CPI-444 alone followed by a cohort expansion by disease. The dosing ranged from 50–200 mg with daily dosing for 14 or 28 days. Some patients at the 50 and 100 mg CPI-444 doses received concomitant 840 mg atezolizumab every two weeks. There were no reported grade 3 or 4 adverse events with single-agent CPI-444. A small number of immune-related adverse events were only seen with the atezolizumab and CPI-444 combination, so CPI-444 appears to be well tolerated in combination at doses up to 100 mg daily. The clinical data presented thus far are quite preliminary in terms of follow-up (4–44 weeks), but overall, 96 patients have been evaluated (52 with CPI-444 and 44 with CPI-444 and atezolizumab combination). Interestingly, 38% of patients treated with CPI-444 only (including those who were naive and those who were refractory to anti-PD1 and anti-PDL1 therapy) had partial responses or stable disease. This result compared favourably against patients receiving the CPI-444 and atezolizumab combination (39%), taking into consideration the heavy pretreatment and advanced state of these patients. Responses appeared to occur in both patients who were naive and in those who were refractory to anti-PD1 and anti-PDL1 therapy, but disappointingly, complete responses have not yet been recorded in any tumour type. Given that a much smaller proportion of all patients with cancer strictly evaluated by

computed tomography (CT) scan ($n=70$) have recorded tumour regressions ($n=14$), it will be important to evaluate more mature data on these patients in the coming 6–12 months. Examples of increased CD8⁺ T cell infiltrates in some lesions of patients after CPI-444 treatment have been observed. Overall, it is too early to make broad conclusions. The drug appears to provide good inhibition of the target at 100 mg, but less so at 50 mg, and it remains possible the target is not fully inhibited and dosing higher for a full 28 days or longer may be necessary to optimally test CPI-444 (REF. 104).

Palobiofarma is testing the combinatorial blockade of the A2AR antagonist PBF-509 with anti-PD1 treatment in patients with NSCLC (NCT02403193)⁴¹, while AstraZeneca is investigating the efficacies of co-targeting A2AR (AZD4635) with durvalumab (MEDI4736, anti-PDL1) in patients with solid malignancies⁴³ (TABLE 2). MedImmune has developed a mouse and human cross-reacting CD73 mAb, MEDI9447, that increased anti-tumour immunity in preclinical tumour models¹⁰⁵. A phase I trial using MEDI9447 in combination with MEDI4736 is currently underway (NCT02503774)⁴⁴. Other CD73 mAbs are also in development¹⁰⁶. The results of these trials are eagerly awaited (TABLE 2).

Considerations for the clinic

The adenosinergic receptors A2AR and A2BR are G protein-coupled-receptors (GPCRs), and the preparation of mAbs against conformationally active GPCRs is notoriously difficult to achieve. Thus, no antibodies currently exist to target these receptors. Small molecule inhibitors are currently the only available option in the clinic, and therefore it is essential to design molecules that do not lose potency in a high adenosine environment. Jaakola *et al.*¹⁰⁷ showed that binding of the A2AR antagonist ZM241385 to A2AR hinders the activation of A2AR through competitive inhibition and improves the K_i value of the compound. Likewise, the A2AR inhibitor AZD4635 developed by AstraZeneca has >30-fold selectivity over other adenosine receptors and a K_i of 1.7 nM (REF. 108). More potent A2AR and CD73 antagonists may also reach trials in the future. Similarly, small molecule CD73 inhibitors (such as A001421), with potencies (30 pM) that exceed that of any reported mAb, have been generated¹⁰⁶.

Table 2 | Ongoing clinical trials of inhibitors of the adenosinergic pathway in patients with malignancies

Target	Drug	Company	Clinical trial number	Study phase	Cancer type	Combination partner
CD73	MEDI9447	MedImmune	NCT02503774	I	Solid tumours	Anti-PDL1 (MEDI4736; durvalumab)
A2AR	CPI-444	Corvus Pharmaceuticals	NCT02655822	I and Ib	<ul style="list-style-type: none"> • NSCLC • Malignant melanoma • Renal cell carcinoma • TNBC • Colorectal cancer • Bladder cancer 	Anti-PDL1 (MPDL3280A; atezolizumab)
	PBF-509	Palobiofarma	NCT02403193	I and Ib	NSCLC	Anti-PD1 (PDR001)
	AZD4635	AstraZeneca	NCT02740985	I	Advanced cancers	Anti-PDL1 (MEDI4736; durvalumab)

NSCLC, non-small-cell lung cancer; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; TNBC, triple negative breast cancer.

A major drawback of the use of small molecule inhibitors is their fairly short half-life. Thus, they require regular administration to obtain maximal therapeutic efficacy. Since small molecules can readily diffuse across physiological barriers, it is essential to identify the optimal pharmacokinetics and pharmacodynamics as well as the associated risk of developing cardiovascular and brain-related complications. Remarkably, Arcus Biosciences Inc. has recently designed a next generation dual A2AR and A2BR antagonist (AB928), which unlike other available A2AR inhibitors does not cross the blood–brain barrier. Pharmacologically, the inhibitor has a longer half-life than other A2AR inhibitors and has a potency of less than 10 nM. This compound is expected to enter into clinical trials in late 2017 (REF. 109). As A2BR could potentially compensate for the absence of A2AR through direct modulation of tumour cell survival or through activation of MDSCs, a dual-purpose inhibitor with increased sensitivity towards both A2AR and A2BR is an attractive option. In addition to developing a single-agent antagonist, scientists are also actively testing a bi-specific inhibitory molecule against A2AR and CD73 (REF. 110; an abstract presented at the 2017 Annual Meeting of the American Association for Cancer Research). Currently, the precise mechanism of action for this bi-specific inhibitor is unknown. Given the therapeutic potential this combination may hold²⁰, screening of such bi-specific inhibitors with dual pharmacological properties would be extremely beneficial.

CD73 and CD39 mAbs are currently in development. With the emerging role of enzymatic and non-enzymatic functions of CD73 in cancer, the design of appropriate mAbs is extremely important. The recognition of these functions, coupled with the identification of unique CD73 epitopes, is a critical consideration in the design of CD39 and CD73 mAbs. To this end, MedImmune designed MEDI9447, an antibody that cross-reacts with human and mouse CD73. MEDI9447 binds the CD73 epitope distal from the substrate-binding site and sterically abolishes the conversion of both membrane-associated and soluble CD73 to a catalytically active conformation¹¹¹. Likewise, Corvus Pharmaceuticals has developed two versions of a CD73 mAb, CPX-006 and CPX-016, each with different abilities to interact with the CD73 active site. CPX-006 is a competitive inhibitor of AMP, while CPX-016 inhibits AMP hydrolysis in an allosteric fashion¹¹². The therapeutic effectiveness of these different CD73 mAbs in patients with cancer is eagerly awaited.

Our recent findings using CD73 mAbs also indicate that CD73 enzyme inhibition is helpful but may not always be sufficient, as our study describes an alternative mechanism of action for CD73 mAbs in control of lung metastases in mice. Specifically, the optimal anti-metastatic effect of anti-CD73 required tumour CD73 expression as well as engagement of activating Fcγ receptor IV (FcγRIV) on myeloid cells in mice^{20,32}. In addition, human anti-CD73 required intact FcR binding for optimal cytokine production²⁰. Therefore, anti-CD73 therapies designed for clinical use may benefit from exploiting these enlisted mechanistic approaches.

Combination therapies

Each adenosinergic molecule acts selectively to engage both immune cells and tumour cells in the TME; identifying non-redundant pathways is therefore essential to the development of suitable combination strategies (FIG. 5). Additionally, targeting adenosinergic molecules combines effectively with conventional chemotherapies or immune checkpoint molecules, such as anti-PD1 or anti-PDL1 and anti-CTLA4 therapies (FIG. 6).

Combinations with other members of the adenosinergic pathway

CD39 acts upstream of CD73 in the generation of adenosine; however, recent evidence suggests that both CD39 and CD73 have multiple and dynamic roles in tumour progression. These roles are not restricted to enzyme activity but could be extended to other cellular functions, including tumour cell adhesion, migration, receptor internalization and recycling^{15,17}, thus presenting opportunities to co-target these molecules in the clinic. For example, Terp *et al.*⁶⁰ demonstrated that targeting CD73 on MDA-MB-231 cells using a human CD73 mAb did not inhibit enzyme activity but induced clustering and internalization of CD73. This loss of surface CD73 was essential for protection against metastasis formation. Similarly, upregulation of CD39 on IL-2-primed human Vγ9Vδ2 T cells, a major innate-like peripheral T cell subset, directly induced dephosphorylation of isoprenoid diphosphates, a metabolic intermediate derived from the known oncogenic mevalonate pathway. Through this pathway, CD39 reduced Vγ9Vδ2 T cell activation and IFNγ production¹¹³. Thus, co-blocking CD39 and CD73 could potentially inhibit tumour growth through effects on the mevalonate pathway and inhibition of ATP degradation by CD39, thus activating ICD as well as inducing CD73-mediated internalization of surface CD73 resulting in loss of tumour adhesion and escape.

Additionally, Xu *et al.*¹¹⁴ demonstrated that in the TME of patients with glioma, infiltrating CD4⁺ T cells expressed CD39, while CD73 levels were extremely low. By contrast, CD73 expression was largely restricted to glioma cells. Functionally, this dichotomy appeared to confer optimal immunosuppression, as inhibition of CD4⁺CD39⁺ T cells or CD73⁺ glioma cells alone did not block immunosuppression. Similarly, in patients with chronic lymphocytic leukaemia (CLL), CD39 expression in LNs was observed on tumour cells and surrounding stroma, while CD73 expression was more confined to the proliferative centres of CLL¹¹⁵. In a mouse model of bleomycin chemotherapy-induced scleroderma, limited synergism or additive effects were seen in double-knockout mice deficient in both CD39 and CD73 compared with mice deficient in CD39 or CD73 alone¹¹⁶. Nonetheless, the antitumour efficacy of the double-knockout mice has not been tested in a cancer setting. CD39 and CD73 thus elicit a multitude of adenosine-dependent and adenosine-independent functions that, depending on the cell type, could have different effects. Therefore, an understanding of how these ectoenzymes are anatomically oriented in a TME could be extremely beneficial.

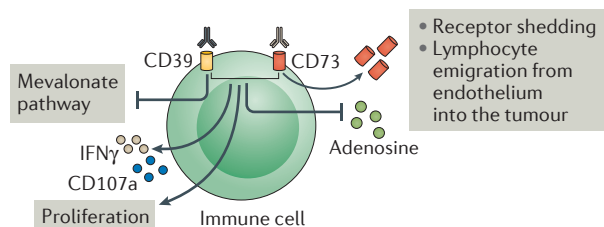
Proliferative centres

Regions within a tumour microenvironment that are characterized by increased tumour proliferation and are commonly identified by elevated Ki-67 staining.

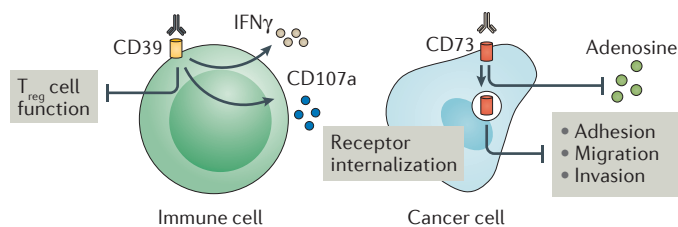
Scleroderma

An autoimmune condition that affects the connective tissue in the body. Scleroderma commonly results in thickening and hardening of skin in areas such as the hands and face.

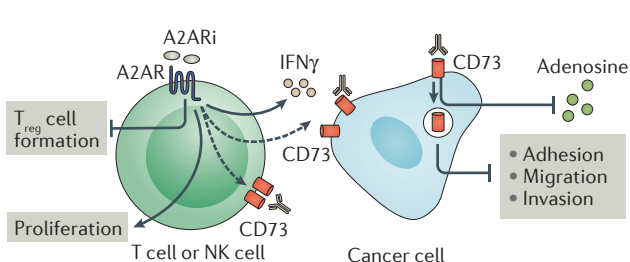
a Anti-CD39 and anti-CD73 co-targeted on immune cells



b Anti-CD39 and anti-CD73 targeted on different cells



c A2ARi and anti-CD73 co-targeting



d A2ARi and A2BRi co-targeting

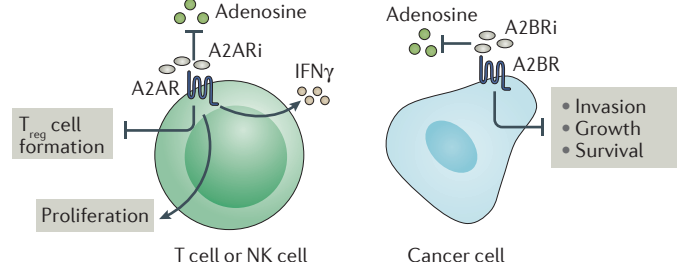


Figure 5 | Co-targeting members of the adenosinergic pathway to facilitate lymphocyte-mediated cytotoxicity.

Co-blockade of CD39 and CD73 may provide therapeutic benefit both through disarming suppressive functions on immune effector cells (panel **a**) and/or via cumulative effects on immune cells and tumour cells (panel **b**). On T cells, anti-CD39 could inhibit the mevalonate pathway while also inducing T cell proliferation and effector functions. A CD73 monoclonal antibody (mAb) is thought to increase lymphocyte infiltration within tumours and increase cytotoxic lymphocyte granule exocytosis as defined by exteriorization of CD107a (also known as LAMP1). On the other hand, as CD73 is also expressed on tumour cells, its inhibition on tumours would suppress tumour cell adhesion, migration and dissemination to distant organs. Similarly, A2AR and CD73 could be co-targeted on immune cells and tumours, which could then be targeted using a CD73 mAb (as indicated by the dashed arrows). Similarly, the co-inhibition of the adenosine receptors A2AR and A2BR could be explored (panel **d**), where an A2BR inhibitor (A2BRi) would effectively reduce tumour cell growth, while an A2ARi would improve functions of cytotoxic lymphocytes. IFN γ , interferon- γ ; NK, natural killer; T_{reg} cell, regulatory T cell.

Salvage pathway

A pathway in which nucleosides that have been released during RNA and DNA degradation are synthesized to form nucleotides. The activation of this pathway is usually observed in cells or tissues that are unable to undergo *de novo* synthesis.

While CD39-blocking mAbs represent a potentially useful antitumour therapeutic in the clinic, the biological effects of CD39 inhibition need to be carefully assessed. ATP accumulation in the TME could have a bi-functional role. Specifically, the concentration of ATP in the milieu determines if ATP signalling may have pro-tumour or antitumour roles, as reviewed by Di Virgilio and Adinolfi¹¹⁷. Importantly, although CD39-deficient mice showed significant antitumour immunity in transplantable tumour models^{30,31}, aged CD39-deficient mice were reported to spontaneously develop HCC owing to high accumulation of ATP and mTOR signal activation¹¹⁸.

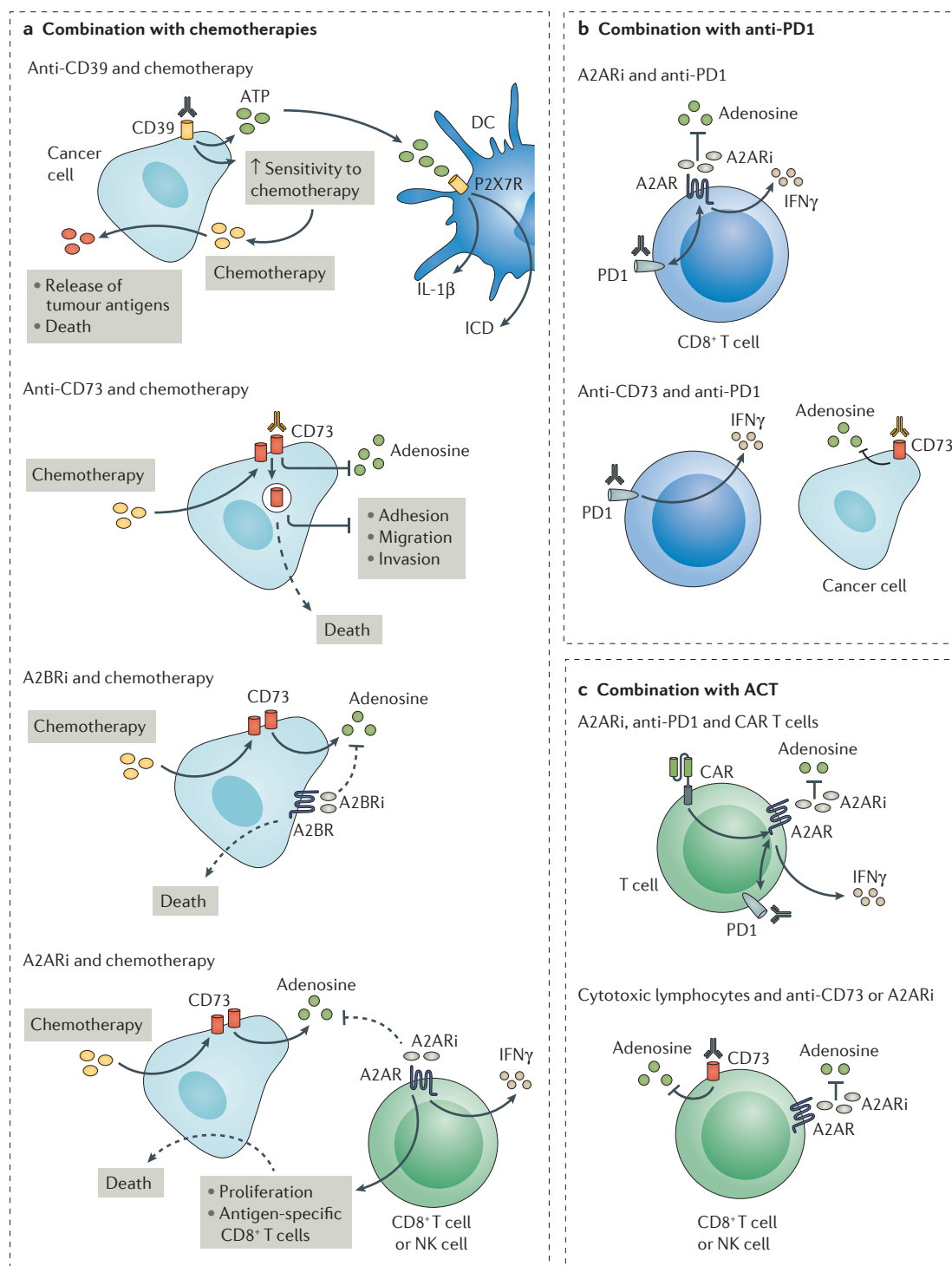
Alongside the CD39–CD73 pathway, additional adenosine-generating pathways are operative in cancers: (i) the CD38 (or its paralogue member CD157)–CD203a (also known as ENPP1 or PC1)–CD73 salvage pathway, (ii) the prostatic acid phosphatase (PAP) pathway, and (iii) the alkaline phosphatase (ALP) pathway. The CD38–CD203a–CD73 adenosine-generating pathway occurs independently of ATP but through the degradation of pyridine metabolites such as NAD⁺. Like ATP, NAD⁺ is also a metabolic intermediate of the glycolytic pathway. Specifically, CD38 hydrolyses NAD⁺ to adenosine diphosphate ribose (ADPR), and ADPR is further degraded to AMP by CD203a. CD73 finally dephosphorylates AMP to adenosine^{119,120} (FIG. 1). This non-classical pathway has been reported in gliomas, melanomas, prostate cancer¹²¹

and multiple myeloma¹¹⁹, and the presence of NAD⁺ has been linked to supporting tumour cell survival¹²². At present, it is unclear if both the CD39–CD73 and CD38–CD203a–CD73 pathways are prominent in the TME and can compensate for each other or if the relative expression of CD38, CD203a and CD39 determines which pathway might be active in the TME. Importantly, CD203a can degrade both ADPR and ATP to AMP¹²⁰, and therefore it is reasonable to assume that in the absence of CD39 in the TME, alternative non-canonical pathways may emerge that could compensate for CD39 activity. Further studies are warranted to understand the relationship between the CD39–CD73 and CD38–CD203a–CD73 pathways in adenosine generation within the TME and whether they reciprocally compensate for each other. Since both the CD39 and CD38–CD203a pathways converge at CD73 for the generation of adenosine, therapeutic efficacies co-blocking CD38 and CD39 could be assessed. Accordingly, two antagonistic CD38 mAbs, namely, daratumumab (NCT02944565)¹²³ and isatuximab (NCT01084252)¹²⁴, are currently being tested in patients with haematological malignancies. This trial could provide insights into the potency of a CD38 mAb as a monotherapeutic agent as well as provide an indication as to whether other adenosine pathways such as the CD39–CD73 axis compensate, due to anti-CD38 therapy-mediated upregulation of CD39 and CD73.

Similarly to CD73, PAP exhibits 5'-ectonucleotidase activity and can dephosphorylate AMP to adenosine. Currently, PAP is widely used as a diagnostic marker in prostate cancer¹²⁵. It is conceivable that a compensatory pathway could be active in patients with prostate cancer, and therefore the possibility of targeting PAP along with CD73 in prostate cancer might be a more effective option. Indeed, double-knockout mice deficient in CD73 and PAP had significantly reduced frequencies of T_{reg} cells

compared with mice deficient in CD73 or PAP alone, thus indicating the lack of redundancy between PAP and CD73 and suggesting potential synergy in co-inhibiting these molecules¹²⁶.

Elevated ALP, which dephosphorylates ATP and ADP to adenosine, has been reported in several cancer types¹²⁷⁻¹²⁹ (FIG. 1). Tumour-derived ALP is significantly increased in metastatic prostate cancer cells, and the inhibition of its phosphatase activity reduced cancer



cell survival and de-differentiated mesenchymal cells to epithelial cells, thus impairing the dissemination of tumour cells¹²⁸. Identification of how these independent adenosine-generating pathways are regulated in relation to each other during the progression of various cancers would be extremely beneficial.

Finally, Young *et al.*²⁰ explored whether CD73 and A2AR played redundant roles within primary tumours and metastatic TMEs, identifying significant additive protection using the combination of a CD73 mAb and an A2AR inhibitor compared with monotherapy alone. Similarly, double-knockout mice deficient in A2AR and CD73 showed significantly reduced metastasis, primary tumour growth and carcinogen-induced tumour initiation.

Co-targeting members of the adenosinergic pathway with other therapies. Stagg and colleagues⁹⁴ observed that CD73 or A2AR blockade could be combined with cytotoxic chemotherapy. Importantly, they found that CD73 expression in patients with TNBC was associated with increased resistance to anthracycline treatment. In addition, CD73 was significantly induced after treatment of mice with doxorubicin and conferred therapeutic resistance⁹⁴. Based on these observations, the combination of CD73 mAb or A2AR antagonism and doxorubicin was tested and showed improved antitumour efficacy in CD73⁺ mouse breast cancer models over monotherapy⁹⁴. Similarly, inhibition of CD39 on fibrosarcomas re-established responsiveness to anthracyclines through the activation of P2 receptors and an ICD-mediated mechanism¹³⁰. A similar synergism using A2BR inhibitors and chemotherapeutic agents such as dacarbazine, gemcitabine or doxorubicin was observed^{21,38} (FIG. 5).

Adenosine inhibits the efficacy of immune checkpoint blockade therapies. A limited study of biopsies from patients treated with anti-PD1 therapy identified that tumours with innate resistance to immunotherapy lacked CD73 expression. By contrast, patients who

developed acquired resistance displayed increased tumour CD73 expression⁹⁷, possibly in response to an inflammation-driven active immune response. In mice, Allard *et al.*⁸³ found that CD73 mAb treatment significantly increased the activity of both anti-CTLA4 and anti-PD1 therapies in tumour-bearing mice. Similarly, Hay *et al.*¹⁰⁵ demonstrated that treatment of a xenograft mouse model with a mouse and human cross-reacting CD73 mAb (MEDI9447) and anti-PD1 therapy retarded mouse CT26 colon carcinoma growth and prolonged survival of these mice (FIG. 6). Iannone *et al.*¹³¹ also reported that co-treatment with the CD73-specific inhibitor APCP and anti-CTLA4 significantly retarded B16F10 melanoma growth *in vivo* compared with either monotherapy alone.

Improved antitumour responses were also seen in mice receiving a combination of anti-PD1 therapy and an A2AR inhibitor^{18,35,36}. In a primary mouse tumour model, anti-PD1 therapy increased A2AR expression on tumour-infiltrating CD8⁺ T cells. Co-blockade with an A2AR inhibitor improved IFN γ and cytotoxic antitumour CD8⁺ T cell responses and resulted in increased tumour suppression¹⁸. Similarly, Mittal *et al.*³⁵ reported that co-treatment with the A2AR inhibitor SCH58261 and anti-PD1 therapy resulted in significantly lower metastatic burden compared with either monotherapy alone. In another study, A2AR-deficient mice bearing EL4 lymphoma, when treated with a soluble B7-DC-Fc fusion protein (which specifically blocks PD1-mediated inhibition), displayed prolonged survival compared with WT mice³⁶.

Adenosinergic therapies may be clinically relevant alongside oncogenic gene-targeted therapies⁴⁰, as evidenced by increased therapeutic benefit in *Braf*-mutant tumour-bearing mice that received an A2AR inhibitor in combination with BRAF and/or MEK inhibitors⁴⁰. Likewise, hypoxic tumours are resistant to radiotherapy¹³² and show increased HIF1 α levels¹³³, thus providing an opportunity to employ anti-adenosine-based therapies with radiotherapy to potentiate therapeutic efficacy. Lastly, combinations with antiangiogenic therapies (for example, anti-VEGF and anti-fibroblast growth factor (FGF)) or therapies that modulate alternative metabolic pathways (for example, IDO) can also be considered but remain to be tested.

Combinations with members of the adenosinergic pathway and ACT. In addition to conventional and targeted therapies and blockade of immune checkpoint molecules, inhibiting the adenosinergic pathway could be potentially combined with adoptive CD8⁺ T cell or NK cell transfer therapies (FIG. 6). CD73 was upregulated in tumour biopsy samples from patients with melanoma that received gene-modified melanoma antigen recognized by T cells 1 (MART1) CD8⁺ T cells, thus emphasizing the potential of inhibiting CD73 for improved ACT⁹⁷. Similarly, adoptive transfer of antigen-specific T cells alone was insufficient to suppress tumour growth in mice; however, upon co-treatment with APCP, a significant inhibition of tumour growth was observed⁶⁷. In addition, treatment with an A2AR selective antagonist or knockdown of A2AR and A2BR specifically in T cells augmented the efficacy of adoptive T cell anticancer therapy in the experimental

◀ **Figure 6 | Potential for targeting adenosinergic molecules to synergize with other cancer therapies.** **a** | Inhibition of CD39 increases the sensitivity of tumours to doxorubicin. This increase in sensitivity occurs mainly due to the inability to hydrolyse ATP, which subsequently activates the P2 purinergic receptor P2X7R on dendritic cells (DCs). This signalling increases inflammasome activity and activation of immunogenic cell death (ICD). Chemotherapy increases expression of CD73 on tumour cells, and therefore co-treatment with the chemotherapeutic agents anthracyclines and a CD73 monoclonal antibody (mAb) reduces tumour growth. Similarly, co-treatment with chemotherapeutic agents and either an A2AR inhibitor (A2ARi) or an A2BRi is known to reduce tumour growth. While the precise mechanism is not entirely understood, it is thought that the action of chemotherapy induces adenosine generation through CD73 upregulation. Furthermore, inhibiting the interaction of adenosine with A2AR or A2BR may potentially contribute to increased antitumour immunity (as indicated by dashed lines). **b** | Targeting CD73 or A2AR with anti-programmed cell death protein 1 (PD1) therapy can be combined effectively preclinically. A PD1 mAb increases the expression of A2AR on CD8⁺ T cells, which upon blockade with A2ARi reduces tumour growth. Similarly, treatment of tumours and/or immune cells with a CD73 mAb inhibits adenosine generation and thus A2AR upregulation on CD8⁺ T cells, and thus a CD73 mAb might be combined with a PD1 mAb. **c** | Engineered chimeric antigen receptor (CAR) T cells activate A2AR, which subsequently can upregulate PD1. Hence, co-inhibiting A2AR and PD1 with adoptively transferred and engineered CAR T cells has substantial antitumour effects. Lastly, therapies targeting adenosinergic molecules might be combined with adoptive T cell and/or natural killer (NK) cell therapies. ACT, adoptive cellular therapy; IFN γ , interferon- γ .

mouse CMS4 sarcoma lung metastasis model and significantly improved survival in the RMA T cell lymphoma syngeneic transplant mouse model¹¹. In two syngeneic HER2⁺ mammary tumour models, Beavis *et al.*³⁴ found that either genetic or pharmacological targeting of A2AR profoundly increased the efficacy of chimeric antigen receptor (CAR) T cells targeting HER2, particularly when combined with PD1 blockade. Mechanistically, this was associated with increased cytokine production by CD8⁺ CAR T cells and increased activation of both CD8⁺ and CD4⁺ CAR T cells³⁴. However, it is important to emphasize that although inhibition of A2AR on CD8⁺ T cells improves the activation and effector functions of these cytotoxic cells, A2AR-deficient T cells show poor survival and memory cell differentiation in certain tumour types¹³⁴. Additionally, A2AR is abundantly expressed on NK cells, and the inhibition of A2AR improves NK cell functions in some tumour settings^{18,35}. It is therefore conceivable that adoptive NK cell transfer therapies could be potentiated by inhibition of A2AR in the TME.

Conclusions

Adenosinergic molecules are dynamically positioned within the cancer-immunity cycle¹³⁵, and targeting this pathway could effectively suppress tumour progression and metastases through multiple redundant and non-redundant mechanisms. Specifically, CD39 inhibition would inhibit hydrolysis of ATP and potentiate ICD, while blocking A2BR would improve characteristics of DC antigen presentation. Targeting A2AR or CD73 is necessary for the activation and infiltration of effector T cells and NK cells into the tumour. Finally, through inhibition of A2BR or CD73, cancer cell survival is significantly hampered.

CD73, CD39 and A2BR affect multiple components of the TME, including tumour cells, stroma and immune cells. Therefore, understanding which cells in the TME are critical for the effectiveness of any given therapy is essential for developing combination strategies. For example, because A2BR and CD73 both exert pro-metastatic effects through direct impact on tumour cells, it is possible that co-blocking these molecules may not provide any substantial combination benefit over monotherapy alone against CD73^{hi} tumours. Furthermore, cAMP-engaging A2BR and A2AR both promote immunosuppression. Hence,

a deeper understanding of their cross-regulation becomes necessary. Specifically, investigating whether the inhibition of A2AR could improve sensitivity to adenosine via A2BR and vice-versa and how this influences the TME is vital. Interestingly, absence of A2AR expression impairs A2BR expression in splenocytes¹³⁶, which illustrates the need to investigate these dynamic interactions within the TME.

As therapies targeting adenosinergic molecules reach clinical utility, it is desirable to appropriately stratify patients most likely to benefit. Notably, CD73 is highly expressed in the tumours of patients with TNBC with poorer prognosis and with resistance to anthracyclines⁹⁴. Similarly, CD73 is expressed in patients with advanced-stage melanoma who exhibit higher nodal metastatic disease⁴⁰. Many patients who exhibited CD73 upregulation were initially responsive to immunotherapies but later developed acquired resistance⁹⁷. These patients are likely to respond to CD73 mAb-based therapies, and hence identification and stratification of patients based on CD73 expression would be useful. By contrast, patients undergoing treatment using a BRAF inhibitor alone or in combination with a MEK inhibitor displayed reduced CD73 expression in tumour biopsy samples. Importantly, in some patients, withdrawal of the BRAF inhibitor resulted in CD73 upregulation, which led to stable or progressive disease in these patients⁴⁰. Thus, due to the dynamic regulation of CD73 in the TME, patients with CD73⁻ tumours before receiving targeted therapies or immunotherapies should not be excluded from receiving anti-CD73 therapies.

Surprisingly, the anti-metastatic effect of an A2AR inhibitor and a CD73 mAb in mice relies on CD73 expression on tumours^{18,20}. Similarly, in some primary tumours, the inhibitory effect of a CD73 mAb is reliant on host A2AR expression¹³⁷. The cross-regulation of these pathways in some tumour settings is exciting and requires further investigation. In addition, blocking inhibitory receptors such as PD1 with anti-PD1 therapy could directly or indirectly modulate the levels of inflammation within the TME and change CD73 and A2AR expression on T cells. An overall understanding of the kinetics of tumour, stroma or immune cell expression of adenosinergic molecules before and after treatment with existing and new immunotherapies and how this relates to response will be of major interest.

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Author contributions

D.V., M.W.L.T. and M.J.S. researched the data for the article. A.Y. provided a substantial contribution to discussions of the content. D.V. wrote the article, and all authors contributed equally to reviewing and/or editing the manuscript before submission.

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