Review

THE EMBO JOURNAL

"Cancer Reviews 2021" series

ATP and cancer immunosurveillance

Oliver Kepp[1,2], Lucillia Bezu[1,2], Takahiro Yamazaki[3], Francesco Di Virgilio[4], Mark J Smyth[5], Guido Kroemer[1,2,6,7,8,*,†] & Lorenzo Galluzzi[3,9,10,11,12,**,†]

Abstract

While intracellular adenosine triphosphate (ATP) occupies a key position in the bioenergetic metabolism of all the cellular compartments that form the tumor microenvironment (TME), extracellular ATP operates as a potent signal transducer. The net effects of purinergic signaling on the biology of the TME depend not only on the specific receptors and cell types involved, but also on the activation status of *cis*- and *trans*-regulatory circuitries. As an additional layer of complexity, extracellular ATP is rapidly catabolized by ectonucleotidases, culminating in the accumulation of metabolites that mediate distinct biological effects. Here, we discuss the molecular and cellular mechanisms through which ATP and its degradation products influence cancer immunosurveillance, with a focus on therapeutically targetable circuitries.

Keywords ADORA2A; autophagy; CD39; CD73; immune checkpoint inhibitors; immunogenic cell death

Subject Categories Cancer; Immunology; Metabolism

DOI 10.15252/embj.2021108130 | Received 25 February 2021 | Revised 24 March 2021 | Accepted 5 April 2021 | Published online 14 June 2021

The EMBO Journal (2021) 40: e108130

Introduction

According to a widely accepted model, malignant transformation is initiated by relatively common genetic or epigenetic alterations that incapacitate tumor-suppressing mechanisms (for the most part, mechanisms that ensure the preservation of cellular homeostasis) as they activate oncogenic drivers (generally, processes that enable accrued anabolism in support of hyperproliferation) (Hanahan & Weinberg, 2011; Timp & Feinberg, 2013). The vast majority of newly formed malignant cells, however, appears to be controlled by the host immune system prior to forming symptomatic tumors (Vesely & Schreiber, 2013; Lopez-Otin & Kroemer, 2021). While in most cases such control involves the definitive "eradication" of malignant cell precursors, in some instances newly formed cancer cells can resist immune attacks and generate a rudimentary tumor microenvironment that enables some degree of proliferation, a dynamic battle between emerging tumors and their host commonly referred to as "equilibrium" (Vesely & Schreiber, 2013). In this context, malignant cells can acquire additional genetic and epigenetic alterations that either (i) impair their ability to initiate anticancer immune responses, such as the loss or downregulation of genes coding for endogenous danger signals, (ii) limit their visibility to immune effector cells, such as the loss of MHC class I-coding genes or beta-2-microglobulin (*B2M*), (iii) increase their resistance to immune effector molecules, such as the loss of caspase 8 (*CASP8*), or (iv) establish a state of local immunosuppression, such as the upregulation of CD274 (best known as PD-L1) (Galluzzi *et al*, 2018). In this context, the equilibrium between cancer cells and the host immune system ceases to exist in favor of an "escape" phase culminating in uncontrolled tumor growth and metastatic dissemination (Dunn *et al*, 2002; Dersh *et al*, 2021).

Importantly, the TME of malignancies that escaped immunosurveillance (Rao *et al*, 2019) undergoes a considerable reconfiguration, generally involving the accumulation of immunosuppressive myeloid and lymphoid cells including M2-like tumor-associated macrophages (TAMs) and $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells at the expense of M1-like TAMs, type I conventional dendritic cells (cDC1s), T_H1 $CD4^+$ T cells, $CD8^+$ cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells, all of which promote tumor-targeting immunity (Talmadge & Gabrilovich, 2013; Mantovani *et al*, 2017; Lee & Radford, 2019; Sprooten *et al*, 2019; Togashi *et al*, 2019). Beyond such a general trend, however, the precise immune

1 Equipe labellisée par la Ligue contre le cancer, Centre de Recherche des Cordeliers, INSERM U1138, Sorbonne Université, Université de Paris, Paris, France
2 Metabolomics and Cell Biology Platforms, Gustave Roussy Comprehensive Cancer Institute, Villejuif, France
3 Department of Radiation Oncology, Weill Cornell Medical College, New York, NY, USA
4 Department of Medical Sciences, University of Ferrara, Ferrara, Italy
5 Immunology in Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, Brisbane, Qld, Australia
6 Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France
7 Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China
8 Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden
9 Sandra and Edward Meyer Cancer Center, New York, NY, USA
10 Caryl and Israel Englander Institute for Precision Medicine, New York, NY, USA
11 Department of Dermatology, Yale School of Medicine, New Haven, CT, USA
12 Université de Paris, Paris, France
*Corresponding author. Tel: +33 1 4427 7667; E-mail: kroemer@orange.fr
**Corresponding author. Tel: +1 646 962 2095; E-mail: deadoc80@gmail.com
†These authors contributed equally to this work as senior authors
This article is part of the Cancer Reviews 2021 series.

contexture of each neoplasm exhibits considerable heterogeneity (De Sousa *et al*, 2013; Vitale *et al*, 2021) and has a major impact on disease course and response to therapy (Fridman *et al*, 2017). Indeed, it has now become clear that the efficacy of most anticancer agents commonly employed in the clinic, encompassing cytotoxic chemotherapeutics, radiation therapy (RT), and targeted anticancer agents, relies at least partially on the (re)activation of immunosurveillance (Galluzzi *et al*, 2020; Rodriguez-Ruiz *et al*, 2020; Petroni *et al*, 2021). In line with this notion, considerable efforts are being dedicated to the identification of clinically relevant approaches to alter the TME in favor of treatment efficacy, especially for tumors that exhibit rather scarce infiltration by immune effector cells, such as luminal breast cancer and pancreatic carcinoma (Kroemer *et al*, 2015; Ho *et al*, 2020).

All cellular components of the TME including malignant and immune cells engage in a dynamic competition for nutrients, oxygen, and trophic signals (all of which are generally scarce as a consequence of relatively poor vascularization) (Martinez-Outschoorn *et al*, 2017; O'Sullivan *et al*, 2019; Garner & de Visser, 2020). Moreover, the availability of nutrients, oxygen, and trophic signals is not equal across all tumor regions and is not constant over time (e.g., before and after therapy), hence constituting a major driver of intratumoral heterogeneity (ITH) (De Sousa *et al*, 2013; Vitale *et al*, 2021). Indeed, such restrictions *de facto* operate as Darwinian pressures, fostering the selection of cells with an accrued capacity to harness alternative carbon sources (*e.g.*, lactate, extracellular amino acids) for catabolic and anabolic reactions in support of proliferation and tolerance to hypoxia (Chang *et al*, 2015; Xiao *et al*, 2019).

Adenosine triphosphate (ATP) occupies a key position in the overall configuration of the TME. On one hand, intracellular ATP is crucial for each cellular TME component to survive and mediate its functions (including proliferation, for malignant and non-terminally differentiated immune cells) (Leone & Powell, 2020; Bergers & Fendt, 2021). On the other hand, the pool of ATP that accesses the TME upon active secretion by living or dying cells into the extracellular space constitutes a major signal transducer (Di Virgilio *et al*, 2018). The net effect of ATP signaling on the TME, however, depends on multiple factors, including the presence of extracellular ATP-degrading enzymes as well as the expression pattern of purinergic receptors. Here, we will critically discuss the molecular and cellular mechanisms through which extracellular ATP and its degradation products influence the crosstalk between malignant and immune cells and present recent advances on the purinergic system as a potential target for the development of novel anticancer interventions.

Extracellular ATP homeostasis in the TME

Since ATP cannot be synthesized in the extracellular milieu, the microenvironmental levels of ATP are entirely controlled by the balance between its secretion/release and degradation (Fig 1).

Adenosine triphosphate secretion is an active, regulated process that can occur via multiple mechanisms and involve different cellular sources. Molecular mechanisms for ATP secretion encompass: (i) exocytosis of ATP-containing vesicles, a process that may (but does not necessarily) involve cell death (Imura *et al*, 2013; Martins *et al*, 2014) and mechanistically relies on vesicular loading by solute carrier family 17 member 9 (SLC17A9) (Imura *et al*, 2013; Cao *et al*, 2014) and the SNAP receptor (SNARE)- and Rho-associated, coiled-coil containing protein kinase 1 (ROCK1)-dependent fusion of exocytosis-competent ATP-rich vesicles with the plasma membrane (Imura *et al*, 2013; Martins *et al*, 2014); (ii) liberation of cytosolic ATP molecules via gap junction protein alpha 1 (GJA1, best known as CX43) hemichannels at gap junctions (Stout *et al*, 2002; Eltzschig *et al*, 2006; Kang *et al*, 2008); and (iii) gradient-driven efflux via oligomeric pannexin 1 (PANX1) channels (also known as "pannexons") (Dahl, 2015), which are sensitive to activation by mechanical forces (Bao *et al*, 2004), by the pro-inflammatory CASP1 (Narahari *et al*, 2021), and by apoptotic caspases such as CASP3 and CASP7 (Chekeni *et al*, 2010; Medina *et al*, 2020). That said, while both vesicular ATP secretion and PANX1-dependent release have been documented in living and dying malignant cells (Martins *et al*, 2014; Martin *et al*, 2017), CX43 hemichannels appear to be mostly operational in non-malignant cells of the TME, including astrocytes (Stout *et al*, 2002), as well as (at least potentially) neutrophils (Dosch *et al*, 2018) and macrophages (Dosch *et al*, 2019). Indeed, while both neutrophils and macrophages have been shown to release ATP via CX43 hemichannels in non-oncological settings, whether such function is preserved in TAMs and tumor-infiltrating neutrophils (TINs) remains to be elucidated. Along similar lines, platelets are known as major sources of extracellular ATP upon degranulation (Yeaman, 2014), but their contribution to extracellular ATP availability in the TME has just begun to emerge (Schumacher *et al*, 2013; Gaertner & Massberg, 2019). Additional cellular compartments that may secrete ATP in the TME encompass (at least in some settings) endothelial cells (Sáez *et al*, 2018; Yang *et al*, 2020d), fibroblasts (Pinheiro *et al*, 2013; Murata *et al*, 2014), dendritic cells (DCs) (Tappe *et al*, 2018; Martinek *et al*, 2019), and activated CTLs (Tokunaga *et al*, 2010). Importantly, while some cells spontaneously secrete at least some ATP in their physiological status, for the most part, ATP is actively released in the context of adaptive responses to microenvironmental perturbations, including mechanical cues (Bao *et al*, 2004), inflammatory signals (Beckel *et al*, 2018), hypoxia (Lim To *et al*, 2015), and exposure to a variety of cancer therapeutics (Michaud *et al*, 2011; Tatsuno *et al*, 2019; Rodriguez-Ruiz *et al*, 2020). In most such instances, abundant ATP secretion by stressed cells (which is key for extracellular ATP to mediate immunostimulatory effects, see below) involves functional autophagic responses (Michaud *et al*, 2011), potentially linked to the ability of autophagy to preserve intracellular ATP pools during stress (Rybstein *et al*, 2018; Anderson & Macleod, 2019). Consistent with this notion, genetic and pharmacological interventions aimed at blocking or boosting autophagic responses in cancer cells have been consistently associated with reduced and increased ATP secretion, respectively, in response to immunogenic chemotherapy (Michaud *et al*, 2011; Pietrocola *et al*, 2016; Chen *et al*, 2019; Kepp & Kroemer, 2020; Wang *et al*, 2020). Obviously, all dying cells abruptly release their cytosolic ATP pool when they undergo plasma membrane permeabilization (PMP) as the final step of cellular demise. However, while PMP itself has now been shown to be an active (rather than an osmosis-driven) process even in the context of post-apoptotic, secondary necrosis (Kayagaki *et al*, 2021), the consequent spillage of cytosolic content into the extracellular milieu remains a largely unregulated phenomenon.

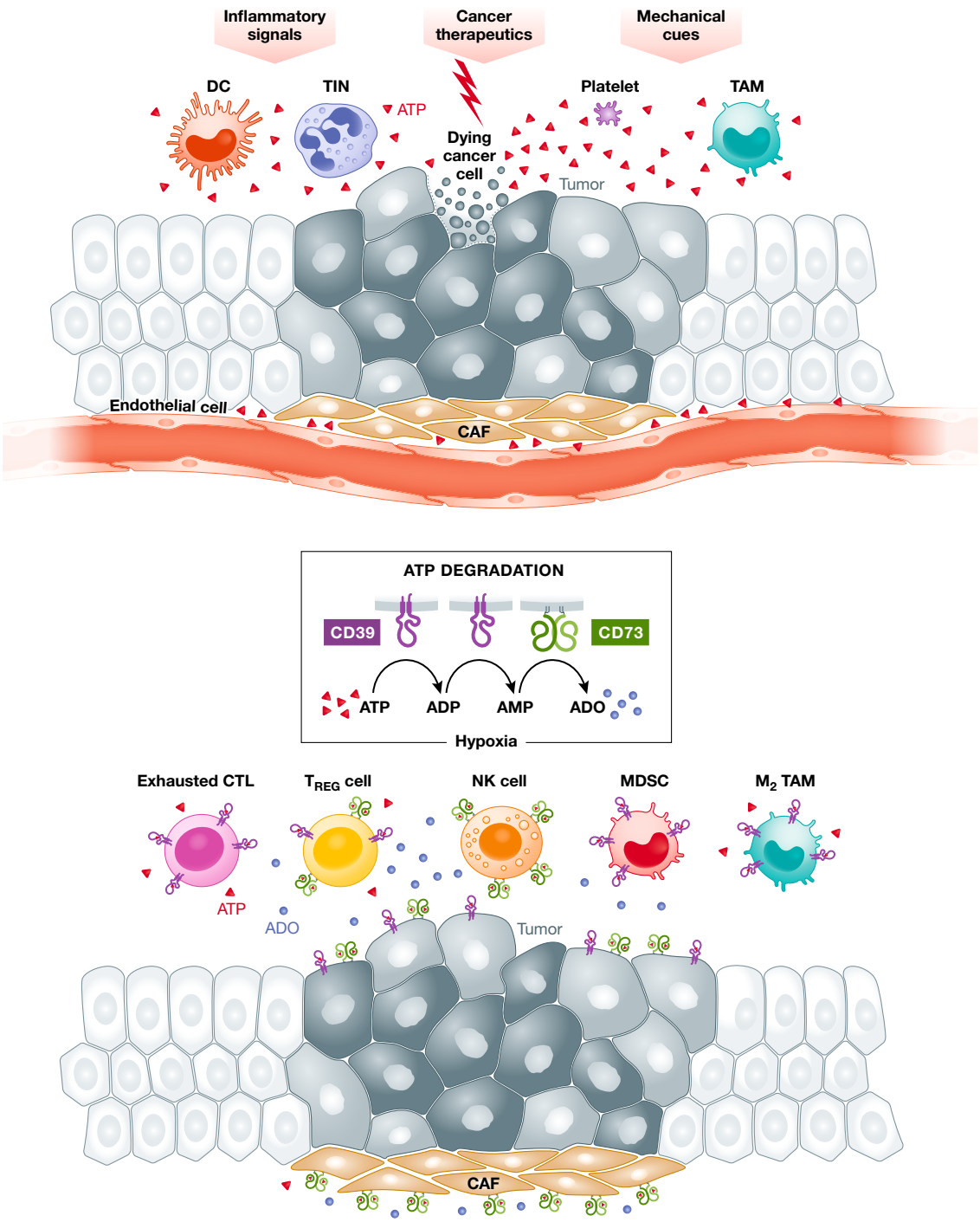

Figure 1. Extracellular ATP homeostasis in the tumor microenvironment.

The concentration of extracellular ATP in the tumor microenvironment is determined by the balance between ATP release and degradation. A variety of cells release ATP either as part of their physiological state or as they respond to stress and potentially die, including cancer cells, dendritic cells (DCs), tumor-infiltrating neutrophils (TINs), tumor-associated macrophages (TAMs), and platelets. Extracellular ATP is catabolized by the sequential activity of ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, best known as CD39), which converts ATP into ADP and AMP, and 5'-nucleotidase ecto (NT5E, best known as CD73), which converts AMP into adenosine (ADO). CD39 and CD73 are expressed by multiple cell type that populate the tumor microenvironment, including some malignant cells, cancer-associated fibroblasts (CAFs), exhausted cytotoxic T lymphocytes (CTLs), regulatory T (T_{REG}) cells, an immunosuppressive subset of natural killer (NK) cells, M2-like TAMs, and myeloid-derived suppressor cells (MDSCs). Hypoxia is a major driver of ATP degradation in the tumor microenvironment.

Extracellular ATP is rapidly catalyzed by the sequential activity of two ectonucleotidases, that is, ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, best known as CD39), which converts ATP into ADP and AMP, and 5'-nucleotidase ecto (NT5E, best known as CD73), which converts AMP into adenosine as the rate-limiting step of this enzymatic cascade (Allard *et al*, 2020; Moesta *et al*, 2020). CD39 is mostly expressed by T_{REG} cells (Borsellino *et al*, 2007), M2-like TAMs (d'Almeida *et al*, 2016), and myeloid-derived suppressor cells (MDSCs, an immature population of myeloid cells with potent immunosuppressive activity) (Li *et al*, 2017), as well as by specific cancer cell types, such as adult T-cell leukemia/lymphoma cells (Nagate *et al*, 2021), type 16 human papillomavirus (HPV-16)-associated cervical carcinoma cells (de Lourdes Mora-García *et al*, 2019), and ovarian carcinoma cells (Häusler *et al*, 2011). Moreover, $CD8^+$ CTLs undergoing terminal exhaustion as a consequence of chronic antigen stimulation generally exhibit a $CD39^+$ phenotype (Canale *et al*, 2018). Conversely, CD73 is expressed by a wide variety of malignant cells as well as by cancer-associated fibroblasts (CAFs) (Yu *et al*, 2020), T_{REG} cells (Stagg *et al*, 2011), and a regulatory subset of NK cells (Neo *et al*, 2020). Interestingly, the whole-body deletion of purinergic receptor P2X 7 (*P2RX7*), which codes for one of the main receptors of extracellular ATP, has a major impact on extracellular ATP levels in the TME of experimental P2RX7-competent melanomas (De Marchi *et al*, 2019), at least in part as a consequence of altered tumor infiltration by $CD39^+$ and $CD73^+$ T_{REG} cells and decreased ATP release by TAMs (De Marchi *et al*, 2019). Such an effect, however, cannot be recapitulated by the pharmacological P2RX7 antagonist A740003 as a result of its mixed activity on immune cells (it fails to alter tumor infiltration by T_{REG} cells, decreases the abundance of intratumoral $CD39^+$ and $CD73^+$ effector T (T_{EFF}) cells, and inhibits ATP secretion by TAMs) and malignant cells (it favors ATP secretion by malignant cells) (De Marchi *et al*, 2019).

Of note, extracellular ATP degradation does not necessarily require the expression of CD39 and CD73 on the same cell (*in cis*), but can also occur efficiently when these ectonucleotidases are expressed by different cellular compartments that are in proximity to each other within the TME (Schuler *et al*, 2014). CD73 is abundant in T_{REG} cell-derived exosomes (Smyth *et al*, 2013), which are highly mobile and hence further promote the overall catalytic efficiency of ATP degradation within the TME. Moreover, CD38 (also known as cyclic ADP-ribose hydrolase) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), which are expressed by some cancer cells and exhausted T cells, can compensate for limited CD39 activity as they catalyze the conversion of extracellular NAD^+ into ADP ribose and AMP (Morandi *et al*, 2015). Finally, the expression of both CD39 and CD73 can be upregulated by hypoxia, which is relatively common in the TME of solid neoplasms, via a transcriptional mechanism that involves hypoxia-inducible factor 1 subunit alpha (HIF1A, best known as HIF-1α) (Giatromanolaki *et al*, 2020; Synnestvedt *et al*, 2002).

In summary, the levels of extracellular ATP in the TME are dynamically determined by the mutually opposed inputs of secretion/release *vs*. degradation. As the factors governing these aspects of the ATP biology exhibit a considerable degree of ITH, regional and temporal fluctuations in extracellular ATP levels are likely to play a major role in the outcome of purinergic signaling in the TME, as discussed further below.

Immunostimulation by extracellular ATP

Extracellular ATP mediates two main functions: (i) It operates as a chemotactic cue for myeloid cells, upon binding to the purinergic receptor P2Y2 (P2RY2), a metabotropic receptor (Elliott *et al*, 2009; Chekeni *et al*, 2010), and (ii) it promotes activation of the inflammasome and hence CASP1-dependent secretion of interleukin 1 beta (IL1B) and IL18 upon binding to P2RX7, an ionotropic receptor (Perregaux *et al*, 2000). Importantly, both these effects are required for the optimal activation of tumor-specific immune responses by (and hence the complete efficacy of) immunogenic chemotherapeutics such as anthracyclines and oxaliplatin, as demonstrated in $P2ry2^{-/-}$, $P2rx7^{-/-}$, $Casp1^{-/-}$ and $Il18^{-/-}$ mice, as well as mice lacking a core component of the inflammasome ($Nlrp3^{-/-}$ mice), the main IL1B receptor ($Il1r1^{-/-}$ mice) or treated with a purinergic receptor antagonist (suramin) or an IL1B-blocking antibody (Ghiringhelli *et al*, 2009; Aymeric *et al*, 2010; Ma *et al*, 2013).

In this setting, DC precursors newly recruited to the TME via ATP released by cancer cells succumbing to immunogenic cell death (ICD) not only mature upon ATP-driven inflammasome activation and migrate to tumor-draining lymph nodes or tertiary lymphoid structures to prime adaptive anticancer immunity, but also recruit a population of IL17-producing γδ T cells that is critical for tumor infiltration by primed CTLs (Ma *et al*, 2011). In accordance with this notion, optimal anticancer immune responses (and consequent superior therapeutic efficacy) driven by immunogenic chemotherapeutics are compromised in $Il17a^{-/-}$ and $Il17ra^{-/-}$ mice (Ma *et al*, 2011). Intriguingly, it has recently shown that the chemotactic activity of ATP on DCs also involves P2RX7 and PANX1 (Saez *et al*, 2017), suggesting the existence of a feed-forward loop whereby intracellular ATP stores may contribute to DC migratory capacity (Saez *et al*, 2017). Moreover, elevated levels of extracellular ATP appear to induce pyroptosis in $P2RX7^+$ M2-like TAMs, hence supporting T cell-mediated antitumor immunity upon the depletion of immunosuppressive cells from the TME (Bidula *et al*, 2019).

Importantly, the net immunomodulatory effect of extracellular ATP depends on the activation of additional signaling pathways. Indeed, the PANX1-dependent co-release of ATP and a wide panel of metabolites including ADP, AMP, GMP, creatine, spermidine, and glycerol-3-phosphate (G3P) by dying cells reportedly promote the removal of cell corpses while preventing the initiation of inflammatory reactions (Medina *et al*, 2020; Narahari *et al*, 2021). Moreover, extracellular ATP can have direct tumorigenic functions. Specifically, the cancer cell-driven release of ATP from platelets initiates a P2RY2-dependent signaling cascade that promotes tumor extravasation and metastatic dissemination upon the opening of endothelial barriers (Schumacher *et al*, 2013; Chen *et al*, 2019; Wang *et al*, 2020). The autophagy-dependent secretion of ATP by melanoma cells has been shown to promote invasiveness and resistance to the BRAF inhibitor vemurafenib, a process that requires P2RX7 expression in the malignant cell compartment (Martin *et al*, 2017). Similar findings have been obtained with human triple-negative breast cancer (TNBC) MDA-MB-231 cells upon the ATP-dependent activation of the transcription factor SRY-box transcription factor 9 (SOX9) (Yang *et al*, 2020a). Finally, NME/NM23 nucleoside diphosphate kinase 1 (NME1, best known as NDPK-A) and NME2 (best known as NDPK-B) expression on extracellular vesicles from MDA-

MB-231 cells reportedly support the formation of pulmonary metastatic niches as a consequence of extracellular ATP generation *in situ* and consequent activation of purinergic receptor P2Y1 (P2RY1) (Duan *et al*, 2021).

Consistent with the multipronged effects of extracellular ATP on the TME, a large body of clinical literature suggests that genetic or epigenetic defects affecting ATP signaling influence disease outcome in cancer patients in a context-dependent manner (Table 1). For instance, while loss-of-function polymorphisms in *P2RX7* (rs3751143; rs208294) have been associated with advanced stage or poor disease outcome in cohorts of patients with breast carcinoma (Ghiringhelli *et al*, 2009), chronic lymphocytic leukemia (CLL) (Thunberg *et al*, 2002; Wiley *et al*, 2002; Zhang *et al*, 2003), and papillary thyroid carcinoma (PTC) (Dardano *et al*, 2009), no impact on

Table 1. Pathophysiological relevance of extracellular ATP signaling in human cancer.

Cancer	No. patients	Variable	Technology	Impact	References
Breast cancer	225	*P2RX7* rs3751143	SNP analysis	Metastatic dissemination Decreased OS	Ghiringhelli *et al* (2009)
	1,067 1,992	*BECN1*	Gene expression profiling	Improved disease outcome	Tang *et al* (2015)
	152	MAP1LC3B	IHC	Improved MFS Improved OS	Ladoire *et al* (2016)
	152 1,646	MAP1LC3B	IHC	Improved PFS	Ladoire *et al* (2015)
CLL	36	*P2RX7* rs3751143	SNP analysis	Disease stage	Wiley *et al* (2002)
	144	*P2RX7* rs3751143	SNP analysis	Marginally decreased OS	Zhang *et al* (2003)
	170	*P2RX7* rs3751143	SNP analysis	Decreased OS	Thunberg *et al* (2002)
	111	*P2RX7* rs3751143	SNP analysis	No correlation with clinical outcome	Nückel *et al* (2004)
	121	*P2RX7* rs3751143	SNP analysis	No correlation with clinical outcome	Starczynski *et al* (2003)
	21	P2RX7	Immunoblotting	Disease progression	Adinolfi *et al* (2002)
Colorectal cancer	2,297	*MAP1LC3B*	Gene expression profiling	Increased OS	Li *et al* (2020)
Gastric cancer	14	*P2RY2*	Gene expression profiling	Disease	Aquea *et al* (2014)
	354	*MAP1LC3C*	Gene expression profiling	Improved OS	Wang *et al* (2021)
	354	*ATG4D*	Gene expression profiling	Decreased OS	Wang *et al* (2021)
	402	MAP1LC3B and SQSTM1	IHC, immunoblotting a,nd RT–PCR	Decreased OS	Kim *et al* (2019)
Head and neck cancer	79	MAP1LC3B	IHC	Disease stage	Jiang *et al* (2012)
Hepatocellular carcinoma	190	MAP1LC3B	IHC	Improved OS	Lee *et al* (2013)
	1,086	*BECN1*	Gene expression profiling	Improved OS	Qin *et al* (2018)
Multiple myeloma	136	*P2RX7* rs3751143	SNP analysis	No correlation with clinical outcome	Paneesha *et al* (2006)
Ovarian cancer	1,497	*BECN1*	Gene expression profiling	Improved OS and PFS	Chen *et al* (2020c)
	1,497	*MAP1LC3B*	Gene expression profiling	No correlation with clinical outcome	Chen *et al* (2020c)
Pancreatic cancer	73	BECN1	IHC	Disease progression	Ko *et al* (2013)
	86	BECN1 and MAP1LC3B	IHC and RT–PCR	Metastatic dissemination Disease stage Decreased OS	Cui *et al* (2019)
Papillary thyroid cancer	121	*P2RX7* rs3751143	SNP analysis	Disease type	Dardano *et al* (2009)
	121	*P2RX7* rs208294	SNP analysis	No effect on disease stage	Dardano *et al* (2009)
Salivary gland carcinoma	48	BECN1 and MAP1LC3B	IHC	Low disease stage	Li *et al* (2019a)

CLL, chronic lymphocytic leukemia; IHC, immunohistochemistry; MFS, metastasis-free survival; N/A, not available; OS, overall survival; PFS, progression-free survival; SNP, single nucleotide polymorphism.

clinicopathological variables could be attributed to rs3751143 in other cohorts of subjects with CLL (Starczynski *et al*, 2003; Nückel *et al*, 2004), multiple myeloma (Paneesha *et al*, 2006), and PTC (Dardano *et al*, 2009), while increased expression levels of P2RX7 have been linked to disease progression in an independent cohort of CLL patients (Adinolfi *et al*, 2002). Likewise, elevated P2RY2 levels have been associated with gastric malignant transformation (Aquea *et al*, 2014). A variety of immunohistochemical and transcriptional signatures of proficient autophagic responses have been linked to worsened disease outcome in cohorts of breast (Yamazaki *et al*, 2020), gastric (Kim *et al*, 2019; Wang *et al*, 2021), pancreatic (Ko *et al*, 2013; Cui *et al*, 2019), and head and neck (Jiang *et al*, 2012) cancer patients, while the contrary held true (or there was no impact on clinicopathological variables) in independent series of patients with breast (Ladoire *et al*, 2015; Tang *et al*, 2015; Ladoire *et al*, 2016), ovarian (Chen *et al*, 2020c), hepatocellular (Lee *et al*, 2013; Qin *et al*, 2018), gastric (Wang *et al*, 2021), colorectal (Li *et al*, 2020), and salivary gland (Li *et al*, 2019a) carcinoma.

In summary, while an abundant preclinical literature mechanistically implicates ATP secretion by stressed and dying cells in the initiation of anticancer immune responses (Fig 2), additional, hitherto poorly characterized factors and mechanisms appear to influence the net effect of ATP signaling in the TME.

Immunosuppression by extracellular ATP metabolites

Extracellular ATP degradation by the sequential activity of CD39 and CD73 mediates immunosuppressive effects not only as a consequence of limited ATP-dependent immunostimulation (see above), but also due to the generation of adenosine, which per se promotes tumor progression via immunological and non-immunological mechanisms (Allard *et al*, 2020). Consistent with this notion, transgene-driven overexpression of CD39 by malignant cells has been associated with impaired anticancer immunity driven by immunogenic chemotherapy (and hence poor disease outcome) in syngeneic immunocompetent mouse models of fibrosarcoma (Michaud *et al*, 2011; Pietrocola *et al*, 2016). Similarly, experimental CD73 overexpression in human cervical carcinoma as well as human and mouse breast carcinoma cells has been shown to promote invasiveness and metastatic potential (Zhou *et al*, 2007; Gao *et al*, 2017), at least in part as a consequence of autocrine–paracrine adenosine signaling via adenosine A2b receptor (ADORA2B) and ADORA2B-driven neovascularization (Stagg *et al*, 2010; Mittal *et al*, 2016; Ludwig *et al*, 2020). Together with ADORA2A, ADORA2B is indeed the main receptor for extracellular adenosine signaling in the TME (Allard *et al*, 2020). However, while ADORA2B is expressed by various malignant cell types including breast cancer (Stagg *et al*, 2010; Lan *et al*, 2018), cervical cancer (Torres-Pineda *et al*, 2020), and melanoma (Mittal *et al*, 2016) cells, as well as by endothelial cells (Ludwig *et al*, 2020), DCs (Chen *et al*, 2020b), and M2-like TAMs (Cohen *et al*, 2015), ADORA2A expression is mostly restricted to myeloid cells (Nakamura *et al*, 2020), NK cells (Young *et al*, 2018), CTLs (Kjaergaard *et al*, 2018; Shi *et al*, 2019), and lymphatic endothelial cell precursors (Allard *et al*, 2019).

From a mechanistic standpoint, ADORA2A- and ADORAB-driven immunosuppression results from the activation of intracellular cyclic AMP (cAMP) signaling, the ultimate functional outcome of which depends on the specific immune cell type expressing these receptors. Thus, cAMP signaling directly inhibits TCR activation and cytokine production in CTLs (Lappas *et al*, 2005), promotes forkhead box P3 (FOXP3) synthesis and the upregulation of the co-inhibitory receptor cytotoxic T lymphocyte-associated protein 4 (CTLA4) in T_{REG} cells (Klein & Bopp, 2016), impairs NK cell effector and secretory functions (Raskovalova *et al*, 2005), inhibits NF-κB-dependent inflammatory responses (Minguet *et al*, 2005) and drives the secretion of immunosuppressive cytokines such as IL10 from TAMs and MDSCs (Németh *et al*, 2005; Cekic *et al*, 2014), and perturbs the maturation of DCs (Kayhan *et al*, 2019). Of note, both ADORA2A and ADORA2B can be upregulated by hypoxia (Sitkovsky *et al*, 2014; Lan *et al*, 2018), in thus far resembling CD39 and CD73. Thus, particularly hypoxic regions of the TME are expected to exhibit robust adenosinergic signaling and hence (i) potent immunosuppression, (ii) accrued neovascularization, (iii) increased vascular permeability, and (iv) enhanced cancer cell motility, *de facto* representing ideal niches for metastatic dissemination. This is further aggravated by immunometabolic circuitries driven by hypoxia. Specifically, hypoxic tumor regions are rich in glycolytic lactate, which mediates multipronged immunosuppressive effects involving CTLs (Brand *et al*, 2016), NK cells (Husain *et al*, 2013), T_{REG} cells (Watson *et al*, 2021), and MDSCs (Yang *et al*, 2020c).

Consistent with the potent immunosuppressive effects of adenosine signaling in the TME, an abundant clinical literature links elevated expression levels of adenosine-generating enzymes or adenosine receptors to poor disease progression in cohorts of patients with various tumors (Table 2). Thus, high levels of CD39 have been associated with advanced grade or poor disease outcome in multiple cohorts of patients with CLL (Pulte *et al*, 2011), renal cell carcinoma (Wu *et al*, 2020a), endometrial tumors (Aliagas *et al*, 2014), pancreatic carcinoma (Künzli *et al*, 2007), and non-small cell lung cancer (NSCLC; Li *et al*, 2017). Similar clinical findings have been correlated with various single nucleotide polymorphisms affecting *Entpd1* (i.e., rs10748643, rs11188513, rs2226163) in patients with colorectal carcinoma (Tokunaga *et al*, 2019; Gallerano *et al*, 2020), but the functional impact of these variants on CD39 functions remains unclear. Moreover, abundant tumor-infiltrating or circulating levels $CD4^+$ or $CD8^+$ cells expressing CD39 have been linked to disease progression or resistance to therapy in various cohorts of individuals with CLL (Perry *et al*, 2012), colorectal carcinoma (Gallerano *et al*, 2020), head and neck squamous cell carcinoma (Gallerano *et al*, 2020), pancreatic cancer (Gallerano *et al*, 2020), NSCLC (Koh *et al*, 2020), and renal cell carcinoma (Qi *et al*, 2020). Along these lines, intratumoral or circulating biomarkers of CD73 proficiency (including expression levels and enzymatic activity) have been correlated with poor disease outcome in patients with diffuse large B-cell lymphoma (Wang *et al*, 2019), ovarian cancer (Turcotte *et al*, 2015), pancreatic carcinoma (Chen *et al*, 2020a; Tahkola *et al*, 2021), NSCLC (Li *et al*, 2017), renal cell carcinoma (Tripathi *et al*, 2020), breast cancer (Loi *et al*, 2013; Buisseret *et al*, 2018), glioma (Xu *et al*, 2013), colorectal carcinoma (Messaoudi *et al*, 2020), and metastatic melanoma (Turiello *et al*, 2020). The *Nt5e* polymorphism rs2229523 (of hitherto unclear functional significance) correlated with limited overall survival in patients with colorectal carcinoma (Tokunaga *et al*, 2019), as did tumor infiltration by $CD8^+CD73^+$ cells in prostate cancer patients (Leclerc *et al*, 2016). Finally, ADORA2A expression or signaling was linked to poor

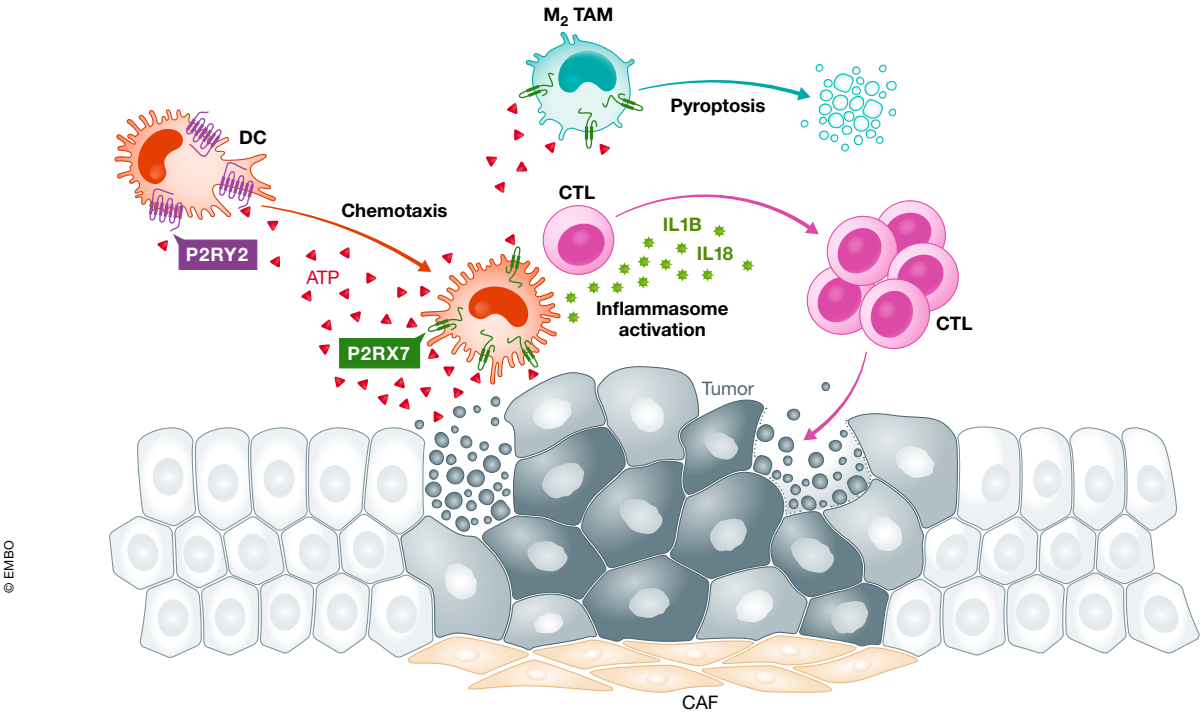

Figure 2. Immunostimulation by extracellular ATP.

Extracellular ATP mediates chemotactic effects on myeloid cells, upon binding to purinergic receptor P2Y2 (P2RY2), and promotes the inflammasome-dependent secretion of interleukin 1 beta (IL1B) and IL18 upon binding to purinergic receptor P2X 7 (P2RX7). These effects are critical for mature dendritic cells (DCs) to prime cytotoxic T lymphocytes (CTLs) against tumor-derived antigens and hence initiate adaptive anticancer immunity. Alongside, extracellular ATP triggers pyroptosis in tumor-associated macrophages (TAMs), hence depleting the tumor microenvironment of generally immunosuppressive cells along with the emission of pyroptosis-dependent immunostimulatory signals.

disease outcome in individuals with diffuse large B-cell lymphoma (Wang *et al*, 2019), renal carcinoma (Kamai *et al*, 2021), and a variety of tumors from The Cancer Genome Atlas (Sidders *et al*, 2020), as was the *ADORA2B* polymorphism rs2015353 in patients with colorectal cancer (Tokunaga *et al*, 2019), although the functional implications of rs2015353 on ADORA2B activity remain to be elucidated.

Of note, the discovery that the TME contains unusually high levels of extracellular ATP has prompted innovative therapeutic approaches that specifically harness this biochemical feature. For instance, a monoclonal antibody specific for the immunostimulatory receptor TNF receptor superfamily member 9 (TNFRSF9, best known as CD137) has been engineered to drive CD137 signaling only in the presence of nearly millimolar ATP levels, thus inducing a potent anticancer immune response in the absence of adverse effects due to extratumoral activity (Kamata-Sakurai *et al*, 2020). This agent is currently being tested in combination with the immune checkpoint inhibitor (ICI) atezolizumab (a PD-L1-blocking antibody) for safety and preliminary efficacy in a phase I clinical trial enrolling patients with solid tumors (JapicCTI-205153). Along similar lines, it has recently been shown that a novel positive allosteric modulator of P2RX7 (which only acts in the presence of high extracellular ATP levels) potentiates the therapeutic effects of an ICI specific for programmed cell death 1 (PDCD1, best known as PD-1) by stimulating DCs to release IL18 in support of the effector functions of tumor-infiltrating NK cells and CTLs (Douguet *et al*, 2021). Incidentally, these therapeutic applications provide an independent and convincing demonstration (mutatis mutandis, almost an "ex adiuvantibus" proof) of the accuracy of early measurements of extracellular ATP concentration in the TME (Pellegatti *et al*, 2008).

Taken together, these observations point to CD39/CD73-dependent adenosine generation and consequent adenosinergic signaling via ADORA2A and ADORA2B as a key immunosuppressive mechanism supporting the progression and metastatic dissemination multiple tumors (Fig 3). Of note, while additional adenosine receptors including ADORA1 and ADORA3 (which inhibit cAMP signaling) are expressed by malignant cells and some tumor-infiltrating immune cells (Stagg & Smyth, 2010), their role in immunosurveillance remains poorly investigated.

Targeting purinergic signaling for cancer therapy

Consistent with the key role of extracellular ATP and its degradation products in the control of immunosurveillance, a variety of pharmacological and genetic approaches designed to boost ATP-driven immunostimulation or inhibit adenosine-dependent immunosuppression (alone or combined with other treatments) have been shown to mediate prominent antineoplastic effects in immunocompetent mouse models of cancer.

Table 2. Pathophysiological relevance of adenosinergic signaling in human cancer.

Cancer	No. patients	Variable	Technology	Impact	References
Breast cancer	122	CD73	IHC	Decreased OS	Buisseret *et al* (2018)
	>6,000	*NT5E*	Gene expression profiling	Reduced pCR	Loi *et al* (2013)
CLL	34	Circulating $CD4^+CD39^+$ and $CD8^+CD39^+$ cells	Flow cytometry	Disease stage	Pulte *et al* (2011)
	62	Circulating $CD4^+CD39^+$ T cells	Flow cytometry	Disease stage	Perry *et al* (2012)
Colorectal cancer	107	*ADORA2B* rs2015353	SNP analysis	Decreased OS	Tokunaga *et al* (2019)
	107 215	*ENTPD1* rs11188513	SNP analysis	Decreased OS	Tokunaga *et al* (2019)
	215	*ENTPD1* rs2226163	SNP analysis	Decreased OS	Tokunaga *et al* (2019)
	107	*NT5E* rs2229523	SNP analysis	Decreased OS	Tokunaga *et al* (2019)
	129	*ENTPD1*, *NT5E*, and *ADORA2B*	SNP analysis	No correlation	Tokunaga *et al* (2019)
	60	Circulating $CD8^+CD39^+$ T cells	Flow cytometry	Disease progression	Gallerano *et al* (2020)
	60	*ENTPD1* rs10748643	SNP analysis	Disease progression	Gallerano *et al* (2020)
	215	CD73	IHC	Disease progression Decreased OS	Messaoudi *et al* (2020)
	193	Circulating CD73	ELISA	Decreased OS	Messaoudi *et al* (2020)
DLBCL	91	ADORA2A on TILs	IHC	Decreased OS	Wang *et al* (2019)
	91	CD73	IHC	Decreased OS	Wang *et al* (2019)
Endometrial tumors	29	CD39	IHC	Tumor grade	Aliagas *et al* (2014)
Glioma	500	*NT5E*	Gene expression profiling	Limited DFS	Xu *et al* (2013)
HNSCC	19	Circulating $CD8^+CD39^+$ T cells	Flow cytometry	Disease progression	Gallerano *et al* (2020)
Melanoma	546	Circulating CD73	AMPase activity	Disease progression Decreased OS	Turiello *et al* (2020)
NSCLC	132	Circulating $CD8^+CD39^+$ T cells	Flow cytometry	Decreased OS	Koh *et al* (2020)
	24	Circulating $CD8^+CD39^+$ MDSCs	Flow cytometry	Tumor infiltration by MDSCs	Li *et al* (2017)
Ovarian cancer	208	CD73	IHC	Decreased OS	Turcotte *et al* (2015)
Pancreatic cancer	28	*ENTPD1*	RT–PCR	Decreased OS	Künzli *et al* (2007)
	3	Circulating $CD8^+CD39^+$ T cells	Flow cytometry	Disease progression	Gallerano *et al* (2020)
	110	CD73	IHC	Decreased OS	Tahkola *et al* (2021)
	168	*NT5E*	Gene expression profiling	Disease progression Decreased OS	Chen *et al* (2020a)
Prostate cancer	285	Circulating $CD8^+CD73^+$ T cells	IHC	Disease progression	Leclerc *et al* (2016)
Renal cell carcinoma	60	ADORA2A expression	IHC	Metastatic dissemination Decreased OS	Kamai *et al* (2021)
	138	CD73	IHC	Decreased OS	Tripathi *et al* (2020)
	243	$CD8^+CD39^+$ T cells	IHC	Disease progression	Qi *et al* (2020)
	367	CD39	IHC and RT–PCR	Disease stage Disease progression	Wu *et al* (2020a)
Various tumor types	N/A	ADORA2A-regulated gene expression	Gene expression profiling	Decreased OS	Sidders *et al* (2020)

CLL, chronic lymphocytic leukemia; DBLCL, diffuse large B-cell lymphoma; DFS, disease-free survival; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; MDSC, myeloid-derived suppressor cell; N/A, not available; OS, overall survival; pCR, pathological complete response; SNP, single nucleotide polymorphism; TIL, tumor-infiltrating lymphocyte.

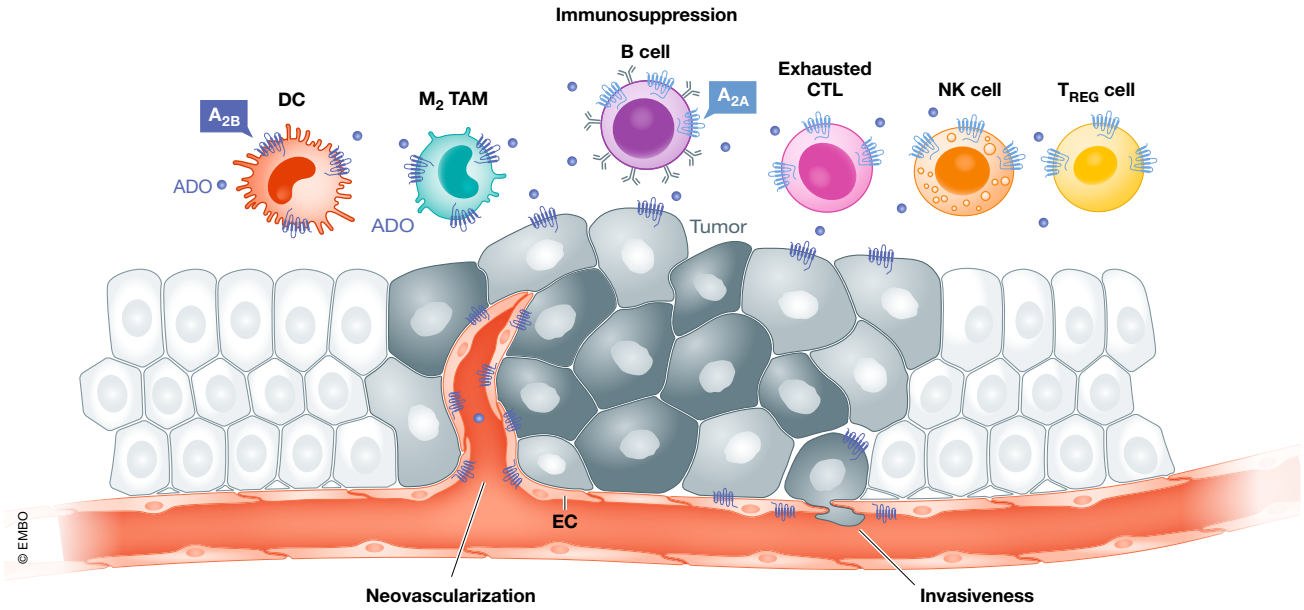

Figure 3. Immunosuppression by extracellular ATP metabolites.

Extracellular adenosine (ADO) not only favors metastatic dissemination by binding adenosine A2b receptor (ADORA2B) on malignant cells, hence promoting invasiveness, and endothelial cells (ECs), thus promoting neovascularization, but also modulates the functions of multiple immune cells upon interaction with ADORA2B or adenosine A2a receptor (ADORA2A). Specifically, adenosine signaling inhibits dendritic cell (DC) maturation and interferes with the effector functions of cytotoxic T lymphocytes (CTLs), B cells, and natural killer (NK) cells, while favoring immunosuppression by M2-like tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T (T_{REG}) cells.

Boosting ATP secretion with autophagy-activating maneuvers, including weekly cycles of nutrient deprivation and administration of so-called caloric restriction mimetics (CRMs, i.e., molecules that induce autophagy and cause other biochemical correlates of nutrient deprivation in the absence of sizeable weight loss), has been linked to improved therapeutic responses to immunogenic chemotherapies in immunocompetent mouse models of fibrosarcoma, correlating with decreased infiltration by T_{REG} cells, via a mechanism that depends on expression of the essential autophagy gene autophagy-related 5 (*ATG5*) in cancer cells and intact immune responses (Pietrocola *et al*, 2016; Castoldi *et al*, 2020; Wu *et al*, 2020b). Along similar lines, short-term starvation reportedly boosts the responsiveness of mouse breast cancer cells growing in immunocompetent syngeneic hosts to radiation therapy (RT) (Saleh *et al*, 2013; Simone *et al*, 2016). However, proficient autophagic responses in mouse breast cancer cells limit the efficacy of RT *in vivo* as a consequence of an improved disposal of permeabilized mitochondria that would otherwise release mitochondrial DNA in the cytosol and trigger cyclic GMP-AMP synthase (CGAS) signaling coupled to type I interferon (IFN) secretion (Medler *et al*, 2019; Yamazaki *et al*, 2020). This suggests that whole-body autophagy activation by short-term fasting may support the efficacy of RT by mechanisms unrelated to ATP secretion in the TME, potentially linked to improved autophagic responses in immune cells, most of which rely on autophagy for optimal functions (Clarke & Simon, 2019). That said, even though cyclic/short-term nutrient deprivation could be safely implemented in at least some cancer patients (Krstic *et al*, 2020), clinicians remain cautious on implementing clinical trials involving such a nutritional measure. Similarly, while many CRMs with robust anticancer activity in preclinical models are currently available as over-the-counter medications (e.g., aspirin) (Castoldi *et al*, 2020) or vitamin supplementations (e.g., nicotinamide) (Buqué *et al*, 2020), clinical development remains at bay, at least in some cases reflecting potential dosing issues.

Blocking CD39 or CD73 has also been associated with robust antineoplastic effects in immunocompetent mouse models of cancer. For instance, whole-body *Entpd1* deletion as well as reconstitution of radiosensitive hematopoietic cells with *Entpd1*$^{-/-}$ precursors have been shown to inhibit *in vivo* growth and metastatic dissemination of mouse melanoma and colorectal carcinoma cells (Sun *et al*, 2010; Perrot *et al*, 2019). Similar findings have been obtained in *Entpd1*$^{-/-}$ mice xenografted with mouse melanoma or fibrosarcoma cells and treated with immunogenic chemotherapy or ICIs (Perrot *et al*, 2019), as well as with (i) monoclonal antibodies targeting human CD39 in human *ENTPD1* knock-in mice bearing mouse fibrosarcoma cells (Perrot *et al*, 2019), (ii) monoclonal antibodies targeting mouse CD39 in wild-type mice used as hosts for mouse melanoma, fibrosarcoma, or colorectal carcinoma cells (Li *et al*, 2019c; Yan *et al*, 2020), (iii) antisense oligonucleotides targeting *Entpd1* in wild-type mice bearing syngeneic breast cancer cells (Kashyap *et al*, 2019), and (iv) pharmacological CD39 inhibitors in wild-type mice xenografted with mouse colorectal carcinoma cells (Michaud *et al*, 2011). Of note, in the latter models, the anticancer effects of CD39 blockage could be abrogated by *P2rx7* or *Nlrp3* whole-body deletion, as well as by co-administration of monoclonal antibodies targeting IL18 (Li *et al*, 2019c; Yan *et al*, 2020), formally linking therapeutic activity to accrued extracellular ATP signaling (rather than to mere adenosine depletion). Mice lacking *Nt5e* have

Table 3. Ongoing clinical trials targeting purinergic signaling for cancer therapy.[a]

Agent	Target	Indications	Phase	Status	Notes	Ref.
AZD4635 (AstraZeneca)	ADORA2A	CRPC	II	Recruiting	In combination with oleclumab (anti-CD73), durvalumab (anti-PD-L1)	NCT04089553
		NSCLC	I/II	Active	In combination with oleclumab (anti-CD73)	NCT03381274
		Solid tumors	I	Active	Single agent and in combination with durvalumab (anti-PD-L1), oleclumab (anti-CD73), docetaxel, abiraterone acetate, enzalutamide	NCT02740985
		Solid tumors	I	Recruiting	Single agent	NCT03980821
Ciforadenant CPI-444/V81444 (Corvus Pharmaceuticals)	ADORA2A	NSCLC	I/II	Recruiting	Single agent and in combination with atezolizumab (anti-PD-L1)	NCT03337698
		CPRC Renal cell carcinoma	I	Recruiting	Single agent and in combination with atezolizumab (anti-PD-L1)	NCT02655822
EOS100850 (iTeos Therapeutics)	ADORA2A	Solid tumors	I	Recruiting	Single agent	NCT03873883
Etrumadenant AB928 (Arcus Biosciences)	ADORA2A ADORA2B	CRC GEC	I	Active	In combination with mFOLFOX	NCT03720678
		NSCLC	I/I	Recruiting	In combination with carboplatin, pemetrexed and pembrolizumab	NCT03846310
		Ovarian cancer TNBC	I	Recruiting	In combination with IPI-549 (PI3γ inhibitor), doxorubicin, paclitaxel	NCT03719326
		Solid tumors	I	Active	In combination with zimberelimab (AB122, anti-PD1)	NCT03629756
Taminadenant NIR178/PBF-509 (Pablobio/Novartis)	ADORA2A	NSCLC	I	Active	Single agent and in combination with spartalizumab (anti-PD-1)	NCT02403193
		TNBC	I	Recruiting	In combination with spartalizumab (anti-PD-1) and LAG525 (anti-LAG-3)	NCT03742349
		Solid tumors	I	Recruiting	Single agent and in combination with NZV930 (anti-CD73), spartalizumab (anti-PD1)	NCT03549000
		Solid tumors	I	Recruiting	Single agent and in combination with spartalizumab (anti-PD-1); NZV930 (anti-CD73), KAZ954	NCT04237649
		Solid tumors	II	Recruiting	Single agent and in combination with spartalizumab (anti-PD-1)	NCT03207867
SRF617 (Surface Oncology)	CD39	Solid tumors	I	Recruiting	Single agent and in combination with paclitaxel, gemcitabine, pembrolizumab (anti-PD-L1)	NCT04336098
TTX-030 (AbbVie)	CD39	Lymphoma Solid tumors	I/I	Recruiting	Pembrolizumab (anti-PD-L1), docetaxel, gemcitabine	NCT03884556
		Solid tumors	I	Recruiting	In combination with budigalimab (anti-PD-1), mFOLFOX	NCT04306900
BMS986179 (Bristol Myers Squibb)	CD73	Solid tumors	I/II	Active	Single agent and in combination with nivolumab (anti-PD-1)	NCT02754141
CPI-006 (Corvus Pharmaceuticals)	CD73	NHL Solid tumors	I/I	Recruiting	Single agent and in combination with ciforadenant (ADORA2A antagonist), pembrolizumab (anti-PD-L1)	NCT03454451
LY3475070 (Eli-Lilly)	CD73	Solid tumors	I	Recruiting	Single agent or in combination with pembrolizumab (anti-PD-L1)	NCT04148937
NZV930 (Surface Oncology)	CD73	Solid tumors	I	Recruiting	Single agent or in combination with spartalizumab (anti-PD-1), NIR178 (ADORA2A antagonist)	NCT03549000
Oleclumab MEDI9447 (AstraZeneca)	CD73	Bladder cancer	I	Recruiting	In combination with durvalumab (anti-PD-L1)	NCT03773666
		NSCLC	II	Recruiting	In combination with durvalumab (anti-PD-L1)	NCT03334617
		Ovarian cancer	II	Recruiting	Single agent or in combination with durvalumab (anti-PD-L1)	NCT03267589
		Pancreatic cancer	I/II	Recruiting	In combination with gemcitabine, paclitaxel, durvalumab (anti-PD-L1), FOLFOX	NCT03611556
		TNBC	I/II	Recruiting	In combination with durvalumab (anti-PD-L1), paclitaxel	NCT03742102

Table 3 (continued)

Agent	Target	Indications	Phase	Status	Notes	Ref.
TJ004309 TJD5 (Tracon Pharmaceuticals)	CD73	Solid tumors	I	Recruiting	In combination with atezolizumab (anti-PD-L1)	NCT03835949

CRC, colorectal cancer; CRPC, castration-resistant prostate cancer; GEC, gastroesophageal cancer; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer.
[a]Restricted to active and recruiting studies, as per www.clinicaltrials.gov on February 15, 2021.

been shown to be poorly permissive to the growth of syngeneic glioblastoma, lymphoma, melanoma, ovarian cancer, colorectal carcinoma, and breast cancer cells (Stagg *et al*, 2011; Wang *et al*, 2011; Yegutkin *et al*, 2011; Young *et al*, 2016; Yan *et al*, 2019). Moreover, blockage of CD73 with monoclonal antibodies or pharmacological agents mediates standalone therapeutic effects or enhances the efficacy of other treatments (encompassing chemotherapeutics, RT, and ICIs) in immunocompetent mouse models of breast cancer (Loi *et al*, 2013; Allard *et al*, 2014; Young *et al*, 2016; Wennerberg *et al*, 2020), colorectal carcinoma (Allard *et al*, 2013; Hay *et al*, 2016; Tsukui *et al*, 2020), melanoma (Iannone *et al*, 2014; Young *et al*, 2016), ovarian cancer (Häusler *et al*, 2014; Li *et al*, 2019b), head and neck squamous cell carcinoma (Deng *et al*, 2018), and prostate cancer (Allard *et al*, 2013). In line with such an abundant preclinical literature, several monoclonal antibodies targeting CD39 or CD73 are currently being tested for their safety and anticancer efficacy, either as standalone therapeutics or combined with ICIs, in clinical trials (Table 3). Preliminary findings from such studies point to an acceptable safety profile and promising clinical activity encompassing disease stabilization and (at least in some patients) partial or complete responses (Mobasher *et al*, 2019; Bendell *et al*, 2020).

Genetic and pharmacological strategies for ADORA2A and ADORA2B inhibition have also been shown to mediate anticancer effects in preclinical cancer models. For instance, (whole-body or myeloid cell-specific) *Adora2a* or *Adora2b* deletion has been demonstrated to inhibit tumor growth in immunocompetent mice bearing syngeneic melanoma (Ohta *et al*, 2006; Cekic *et al*, 2014; Chen *et al*, 2020b), lymphoma (Waickman *et al*, 2012; Nakamura *et al*, 2020), breast carcinoma (Beavis *et al*, 2013), or lung cancer cells (Chen *et al*, 2020b). Consistent with this notion, pharmacological ADORA2A (e.g., CPI-444, SCH58261, AZD4635) or ADORA2B (e.g., PSB-1115) inhibitors have been attributed robust antineoplastic properties in syngeneic mouse models of melanoma (Willingham *et al*, 2018), fibrosarcoma (Beavis *et al*, 2017), breast cancer (Beavis *et al*, 2013; Mittal *et al*, 2014; Beavis *et al*, 2015; Beavis *et al*, 2017), lymphoma (Nakamura *et al*, 2020), colorectal carcinoma (Beavis *et al*, 2015; Willingham *et al*, 2018), multiple myeloma (Yang *et al*, 2020b), and renal cell carcinoma (Willingham *et al*, 2018), especially when combined with ICIs or other immunotherapies. Importantly, at least in some of these models, co-inhibition of ADORA2A and CD73 mediated superior tumor control as compared to inhibiting ADORA2A or CD73 alone (Young *et al*, 2016), suggesting a non-redundant role for these two factors in the establishment of local immunosuppression. Consistent with these preclinical observations, a number of ADORA2A or dual ADORA2A/ADORA2B inhibitors are currently being investigated for safety and activity in clinical trials (Table 3). Preliminary findings from these studies indicate that many ADORA2A/ADORA2B inhibitors exhibit an acceptable safety profile and at least some degree of clinical activity (Chiappori *et al*, 2018; Fong *et al*, 2020; Lim *et al*, 2020).

Additional approaches that may be harnessed to target adenosine signaling in the TME encompass the use of CD38 or ENPP1 blockers and strategies that revert tumor hypoxia (e.g., respiratory hyperoxygenation) (Hatfield *et al*, 2014; Hatfield & Sitkovsky, 2020). However, CD38-specific agents (including the FDA-approved monoclonal antibody daratumumab) are currently used with the aim of eradicating CD38-expressing myeloma cells (Facon *et al*, 2019), and ENPP1 blockers are still in preclinical development (Carozza *et al*, 2020). Similarly, hyperoxygenation has been employed in the past to improve radiosensitivity (because the ability of radiation therapy to cause DNA damage in cancer cell depends on local oxygen tension) but is no longer used for this purpose.

In summary, although a variety of strategies have been successfully used to target purinergic signaling in preclinical tumor models, CD73, ADORA2A, and (less so) CD39 blockers are the only drugs currently in clinical development as anticancer agents, for the most part in combination with standard of care therapeutics or ICIs (Table 3).

Concluding remarks

In summary, extracellular ATP and its degradation products play a key role in the regulation of the tumor immune contexture, hence have a major influence on the propensity of human neoplasms to respond to therapy. While various agents aimed at boosting extracellular ATP concentrations and/or limiting adenosine signaling are already in clinical development, multiple questions to be addressed and avenues to be explored remain. First, it will be important to determine the contribution of P2RX7 and/or P2RY2 signaling in cancer cells to tumor growth and metastatic dissemination in specific settings. Accumulating evidence indicates that some cancer cells can harness extracellular ATP in support of disease progression and resistance to therapy (Martin *et al*, 2017), suggesting that CD39 and/or CD73 inhibition may not mediate optimal anticancer effects in some settings, and calling for the identification of cancer cell-targeted P2RX7 or P2RY2 inhibitors. Second, it will be crucial to develop combinatorial approaches based on the dual blockage of ATP degradation and adenosine signaling, potentially in the context of ICI-based immunotherapy. Indeed, it appears that the efficacy of ADORA2A and/or ADORA2B blockers can be significantly boosted by CD73 inhibition (Young *et al*, 2016), pointing to a functional non-redundancy that may be harnessed for therapeutic purposes. Also, while inhibiting ATP degradation or adenosine signaling mediates anticancer effects per se (at least in various models), blocking

additional immunosuppressive pathways such as those controlled by co-inhibitory receptors appears to provide improved disease control in most cases (Beavis *et al*, 2017; Leone *et al*, 2018; Goswami *et al*, 2020). Finally, it will be interesting to elucidate whether and how purinergic signaling can be targeted in patients with innate or acquired resistance to ICI-based immunotherapy, reflecting the fact that exhausted T cells generally express high levels of CD39 (Canale *et al*, 2018). Irrespective of these and other incognita, purinergic signaling stands out as a particularly promising target for the development of novel anticancer agents.

Acknowledgements

We are indebted to Giulia Petroni (Weill Cornell Medicine) for help with figure preparation. FDV is supported by the Italian Association for Cancer Research (AIRC) grant # IG13025 and by institutional funds from the University of Ferrara (Italy). MJS is supported by a National Health and Medical Research Council Investigator (1173958) and Program Grant (1132519). GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR)—Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Association Ruban Rose; Cancéropôle Ile-de-France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; Gustave Roussy Odyssea, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; High-end Foreign Expert Program in China (GDW20171100085 and GDW20181100051), Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology; the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM). The LG laboratory is supported by a Breakthrough Level 2 grant from the US Department of Defense (DoD), Breast Cancer Research Program (BRCP) (#BC180476P1), by the 2019 Laura Ziskin Prize in Translational Research (#ZP-6177, PI: Formenti) from the Stand Up to Cancer (SU2C), by a Mantle Cell Lymphoma Research Initiative (MCL-RI, PI: Chen-Kiang) grant from the Leukemia and Lymphoma Society (LLS), by a startup grant from the Dept. of Radiation Oncology at Weill Cornell Medicine (New York, US), by a Rapid Response Grant from the Functional Genomics Initiative (New York, US), by industrial collaborations with Lytix (Oslo, Norway) and Phosplatin (New York, US), and by donations from Phosplatin (New York, US), the Luke Heller TECPR2 Foundation (Boston, US), and Sotio a.s. (Prague, Czech Republic).

Author contributions

LG conceived the article. OK and LG wrote the first version of the manuscript with critical input from LB, TY, FDV, MJS, and GK. OK, TY, and LB prepared display items under supervision from LG. All authors approved the final version of the article.

Conflict of interest

OK reports research funding from Daiichi Sankyo and a co-founder role of Samsara therapeutics. FDV is a member of the Scientific Advisory Board of Biosceptre, Ltd. GK reports research funding from Bayer Healthcare, Eleor, Genentech, Glaxo Smyth Kline, Institut Mérieux, Lytix, PharmaMar, Sotio, and Vasculox (completed), funding from Samsara, consulting/advisory honoraria from The Longevity Labs and Lytix, membership of the Executive Board of Bristol Myers Squibb Foundation France, co-founder role of everImmune, Samsara therapeutics and Therafast-Bio. LG reports research funding from Lytix and Phosplatin (completed), consulting/advisory honoraria from Boehringer Ingelheim, AstraZeneca, OmniSEQ, The Longevity Labs, Inzen, Onxeo, and the Luke Heller TECPR2 Foundation. All other authors declare that they have no conflict to interest.

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