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Review

A_{2B} adenosine receptor and cancer

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Abstract: There are four subtypes of adenosine receptors (ARs), named A_1, A_{2A}, A_{2B} and A_3, all of which are G protein-coupled receptors (GPCRs). Locally produced adenosine suppresses in anti-tumor immune surveillance. The A_{2B}AR, coupled to both Gαs and Gαq G proteins, is one of the several GPCRs that are expressed in a significantly higher level in some cancer tissues in comparison to adjacent normal tissues. There is growing evidence that the A_{2B}AR plays an important role in tumor cell proliferation, angiogenesis, metastasis, and immune suppression. Thus, A_{2B}AR antagonists are potentially novel attractive anticancer agents. Several antagonists targeting at A_{2B}AR are currently in clinical trials for various types of cancers. In this review, we first describe the signaling, agonists, and antagonists of the A_{2B}AR. We further discuss the role of the A_{2B}AR in the progression of various cancers, and the rationale of using A_{2B}AR antagonists in cancer therapy.

Keywords: adenosine receptor; immune system; cancer therapy; tumor microenvironment; cell proliferation; metastasis

1. Introduction

Adenosine in the extracellular milieu is generated, mainly via the degradation of ATP released under stress conditions, to protect cells and tissues locally. Adenosine and ATP acting at different classes of receptors often have opposite effects in cell proliferation or cell death. ATP and other adenine nucleotides have antitumor effects via the activation of $P2Y_1$ receptor ($P2Y_1R$) subtype (Wei et al., 2011; Burnstock and Di Virgilio, 2013), whereas adenosine induces cancer cell proliferation and growth of many types of tumors via the activation of the A_{2B} adenosine receptor (AR) (Seitz et al., 2019; Wei et al., 2013; Cekic et al., 2012; Kasama et al., 2016; Mittal et al., 2016; Sepulveda et al., 2016; Ryzhov et al., 2008; Iannone et al., 2016). The generation and degradation/removal of adenosine is a multi-step and balanced process in cells involving enzymes (CD39, CD73, CD26, adenosine deaminase, adenosine kinase, S-adenosyl homocysteine hydrolase) and nucleoside transporters (Fredholm et al., 2011), which are not the main topic of this review. Although extracellular adenosine exerts its action via four G protein-coupled receptors (GPCRs), A_1, A_{2A}, A_{2B} and A_3 (Jacobson and Gao, 2006), in this review we will only focus on the importance of A_{2B}AR signaling (Figure 1) in cancer progression and the rationale to use A_{2B}AR antagonists as anticancer agents.

The importance of the A_{2B}AR in cancer progression has only recently been revealed, despite the physiological role of adenosine in cardiac function being realized almost a century ago (Drury & Szent-Györgyi, 1929). Although A_{2B}AR effects in brain slices were characterized in the early 1980s (Daly et al., 1983), until recently the A_{2B}AR has been poorly characterized in comparison to the other three ARs, which is at least in part due to the fact that A_{2B}AR has low affinity for the endogenous agonist adenosine **1** (EC_{50} ~24 μM, Figure 2, Table 1). Thus, it was assumed that A_{2B}AR must have a minor physiological significance. However, increasing evidence has shown that there is a dramatic increase in extracellular adenosine concentration and a significant upregulation of A_{2B}AR expression under many pathological conditions (Borea et al., 2016; 2018; Cekic and Linden, 2016), such as hypoxia, inflammation and cancer, which may indicate the critical role of A_{2B}AR in many diseases. For example, adenosine concentration has been reported to increase 10-fold in patients with septic shock (Ramakers et al., 2011). Hypoxia-inducible factor 1 (HIF-1α) has been reported to up-regulate

A_{2B}AR expression on activated macrophages (Philip et al., 2017). Lan et al. (2018) found that hypoxia increased expression of A_{2B}AR in human breast cancer cells through the transcriptional activity of HIF-1α. The discovery that A_{2B}AR expression is significantly increased by HIF-1α strongly suggested its involvement in cancer promotion (Lan et al., 2018; Philip et al., 2017; Ma et al., 2010; Kong et al., 2006; Feoktistov et al., 2002). In addition to its role in tumor growth, inhibition of A_{2B}AR genetically or pharmacologically dramatically decreased lung metastasis after implantation of breast cancer cells into the mammary fat pad of immunodeficient mice (Lan et al., 2018). It has also been recently shown that bladder urothelial carcinoma expresses high levels of A_{2B}AR, which is suggested to be associated with a poor patient prognosis (Zhou et al., 2017). A tissue microarray of 232 breast cancer samples, that included 66 triple negative breast cancer cases suggest that A_{2B}AR could serve as a prognostic biomarker and a potential therapeutic target (Horigome et al., 2018). Kasama et al. (2015) showed that A_{2B}AR controls cellular proliferation via HIF-1α activation, indicating that A_{2B}AR may be a key regulator of tumoral progression in oral squamous cell carcinoma. Thus, the A_{2B}AR is consistently and convincingly demonstrated to be involved in tumor cell proliferation, metastasis, angiogenesis, and immune suppression. Furthermore, the A_{2B}AR and A_3AR seem to be the only AR subtypes that are expressed in significantly higher levels in cancer tissues in comparison to normal adjacent tissues, similar to several other GPCRs (Li et al., 2005; Xiang et al., 2006; Kasama et al., 2015; Sepulveda et al., 2016; Cohen and Fishman, 2019).

Although all four ARs are reported to be involved in cancer progression (Borea et al., 2018; Koszałka et al., 2016; Marwein et al., 2019; Gorain et al., 2019), the other three ARs have been shown both to be pro- and anti-tumoral (Borea et al., 2018). For example, both pro- and antitumoral effects have been reported for the A_1AR (Borea et al., 2018). Targeting A_{2A}AR has been considered as a double-edged sword (Allard et al., 2016; Borea et al., 2018). It has been suggested that adenosine accumulation in the tumor microenvironment facilitates tumor growth through inhibition of effector T cells and natural killer (NK) cells (Cekic and Linden, 2014), and inhibition of A_{2A}AR alone was found to be sufficient to establish anti-tumor immunity and protect against metastasis in various mouse models of cancer (Cekic and Linden, 2014). However, A_{2A}AR deletion does not inhibit the growth of all tumor types and might have the opposite effect. For example, an increased tumor growth rate of both B16F10 melanoma and MB49 bladder carcinomas has been observed in A_{2A}AR knockout (KO) mice (Cekic et al., 2012). Blocking A_{2B}AR action might have advantages over the A_{2A}AR as a cancer therapeutic target. Cekic et al. (2012) showed that AR antagonist theophylline slowed the growth of MB49 bladder and 4T1 breast tumors in mice and reduced breast cancer cell metastasis from mammary fat to lung via the A_{2B}AR, but not the A_{2A}AR, based on experiments using A_{2A}AR or A_{2B}AR KO mice. The role of A_3AR has been investigated in various cancer cell types with contrasting results, i.e. both pro- and antiproliferative, as well as pro-apoptotic and anti-apoptotic effects (Borea et al., 2016). Both A_3AR agonists and antagonists have been considered for anti-cancer agents, although only A_3AR agonists have progressed in clinical trials (Cohen and Fishman, 2019).

Recent advances in the signaling and function of the A_{2B}AR (Vecchio et al., 2019; Cekic and Linden, 2016; Gao et al., 2018; Allard et al., 2016) and the availability of selective ligands (Müller et al., 2018; Gao et al., 2014; Alnouri et al., 2015), have greatly facilitated understanding of the role of A_{2B}AR in cancer progression and the rationale for development of A_{2B}AR antagonists as anti-tumor drugs. In this review, we first describe the distribution, signaling, agonists and antagonists of the A_{2B}AR. We then discuss the role of the A_{2B}AR in the progression of various types cancers, and the rationale of using A_{2B}AR antagonists in cancer therapy.

2. A_{2B}AR distribution and expression

In rat, A_{2B}AR mRNA was detected at various levels in all tissues studied (Dixon et al.,1996). In mouse, by replacing exon 1 of the A_{2B} gene with a reporter construct containing β-gal, mouse tissue-specific activation of the A_{2B} gene promoter was conveniently determined in various organs and specific cells within those organs, with the primary site of expression being the vasculature (Yang et

al., 2006). Yang et al. (2006) found that mouse smooth muscle cells, endothelial cells and macrophages exhibit high A_{2B}AR expression levels. The high level of A_{2B}AR expression in endothelial cells suggests a potential role in angiogenesis. In human primary cells, A_{2B}AR has been found in endothelial cells, mast cells, dendritic cells, macrophages, and neutrophils (Cekic and Linden, 2016; Hasko et al., 2009). High expression levels in dendritic cells and macrophages indicate a possible role in modulation of immunity. In human cancer tissues, A_{2B}AR expression levels were found to be higher than in adjacent normal tissues (Li et al., 2005; Xiang et al., 2006; Kasama et al., 2015; Sepulveda et al., 2016; Zhou et al., 2017). High levels of A_{2B}AR have been suggested to be associated with worse prognosis in bladder urothelial carcinoma (Zhou et al., 2017). Mittal et al. (2016) suggested that high A_{2B}AR expression is associated with worse prognosis in triple-negative breast cancer (TNBC). In a TNBC mouse model, A_{2B}AR activation increased metastasis (Neumann et al., 2018), while A_{2B}AR antagonism in mouse models reduced tumor burden by immune mechanisms and action on tumor cells. The high A_{2B}AR expression has also been found in many human tumor cell lines, such as PC3 prostate, T24 bladder, 1321N1 astrocytoma (Gao et al.,2018), U373MG astrocytoma (Fiebich et al., 1996), MDA-MB-231 breast (Lan et al., 2018), Jurkat T cells (Nonaka et al., 1994), BON-1 pancreatic and KRJ-I intestinal (Kalhan et al., 2012), A375 melanoma (Merighi et al., 2009), and THP-1 human monocytes (Zhong et al., 2006). The high expression of the A_{2B}AR in those cancer cells indicates its potential role in cancer progression. In a glioblastoma cell line derived from a mouse line containing spatially expressed A_{2B}AR, this receptor is highly upregulated leading to proliferation, angiogenesis and invasiveness (Yan et al., 2019). Mouse KO of CD73, which forms adenosine locally from AMP, reduced A_{2B}AR signaling in the glioblastoma, to decrease pathogenesis and increase sensitivity to chemotherapy. A_{2B}AR expression was also demonstrated in human lung epithelial cells (Giacomelli et al., 2018). Consistent with the high A_{2B}AR expression in bladder cancer and breast cancer cells, Cekic et al. (2012) showed that A_{2B}AR antagonists delayed the growth of bladder and breast tumors and reduced lung metastasis. Lan et al. (2018) found that genetic or pharmacological inhibition of A_{2B}AR expression or activity dramatically impaired tumor initiation and lung metastasis in mice. Thus, high A_{2B}AR expression is related to tumor growth and metastasis, and therefore A_{2B}AR antagonists are potential therapeutic agents for various types of cancers including lung cancer.

3. A_{2B}AR signaling

Classical A_{2B}AR signaling has been initially and primarily demonstrated in CHO cells expressing the recombinant human A_{2B}AR (Rivkees and Reppert, 1992; Pierce et al., 1992; Schulte and Fredholm, 2003). A_{2B}AR activation leads to dissociation of the Gαs and Gβγ subunits and subsequent activation of the adenylyl cyclases, which in turn hydrolyze intracellular ATP into cyclic AMP (cAMP), which activates protein kinase A (PKA) and many downstream signaling molecules. The Gs-cAMP-PKA axis is an important A_{2B}AR-mediated signaling pathway. For example, Xu et al. (2008) found that A_{2B}AR-mediated cAMP is both necessary and sufficient to suppress interferon-γ (IFN-γ)-mediated immune responses. Jing et al. (2015) showed that A_{2B}AR activation in hematopoietic stem cells induced chemokine CXCL8 production via cAMP-PKA signaling and mediated hematopoiesis. In addition to PKA, cAMP also activates 'exchange protein directly activated by cAMP' (EPAC), another important signaling molecule related to cell migration and angiogenesis (Fang and Olah, 2007). In CHO cells expressing the recombinant human A_{2B}AR, the nonselective AR agonist NECA **3** activated cAMP response element-binding protein (CREB) and P38 (a mitogen-activated protein kinase, MAPK) but not Akt (protein kinase B). Extracellular signal-regulated kinase 1/2 (ERK1/2) and GTPase Rap1 were blocked by PKA inhibitor H89 (Schulte and Fredholm, 2003). Phosphorylation of Akt and ERK1/2 was blocked by a phosphoinositide 3-kinase (PI3K) inhibitor, wortmannin. Thus, A_{2B}AR activating various downstream MAPKs may be via different signaling pathways. Although PKA-independent in CHO cells, the Rap1 activation seems PKA-dependent in HEK293 cells (Ntantie et al., 2013). The coupling of A_{2B}AR to β-arrestin signaling has also been reported (Mundell et al., 2001; Gao et al., 2014).

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Most of the early studies on A_{2B}AR signaling utilized CHO or HEK293 cells transfected with recombinant human A_{2B}AR (Pierce et al., 1992, Schulte and Fredholm, 2003, Gao et al., 1999). However, in various types of cells endogenously expressing the A_{2B}AR, the receptor was able to couple to either Gi or Gs, depending on the cell type and downstream signaling pathway measured (Gao et al., 2018). For example, A_{2B}AR agonist NECA stimulates ERK1/2 phosphorylation via Gαi in T24 bladder cancer cells (Gao et al., 2018), but via Gαs in CHO cells (Schulte and Fredholm, 2003). The Gαi inhibitor pertussis toxin, but not Gαq KO, diminished NECA-stimulated ERK1/2 activity suggesting the involvement of Gαi rather than Gαq (Gao et al., 2018). A_{2B}AR downregulates ERK1/2 activity via Gαs in 1321N1 astrocytoma cells (Gao et al., 2018) and in MDA-MB-231 breast cancer cells (Koussémou et al., 2018). ERK1/2 reduction in MDA-MB-231 cells was triggered by an A_{2B}AR agonist and forskolin, but abolished by the PKA inhibitor H89, suggesting an important role for the cAMP-PKA pathway in controlling ERK1/2 activity in MDA-MB-231 cells. A_{2B}AR-mediated intracellular calcium mobilization in T24 cells was mainly via Gi, although Gs may also play a minor role, but Gq is not involved (Gao et al., 2018). Thus, it is conceivable that in many cases the predominant A_{2B}AR coupling is through Gαi rather than Gαs. Many important A_{2B}AR functions from primary cells or tissues have recently been related to the PI3K-Akt and RAP1B-EPAC pathways (Ou et al., 2016; Ntantie et al., 2013; Phosri et al., 2017; Lim et al., 2019; Ni et al., 2018; Shen et al., 2018). However, it has not been extensively explored whether those signaling molecules are actually downstream of A_{2B}AR-mediated Gαi or Gαs proteins. The A_{2B}AR-mediated major signaling pathways are illustrated in Figure 1.

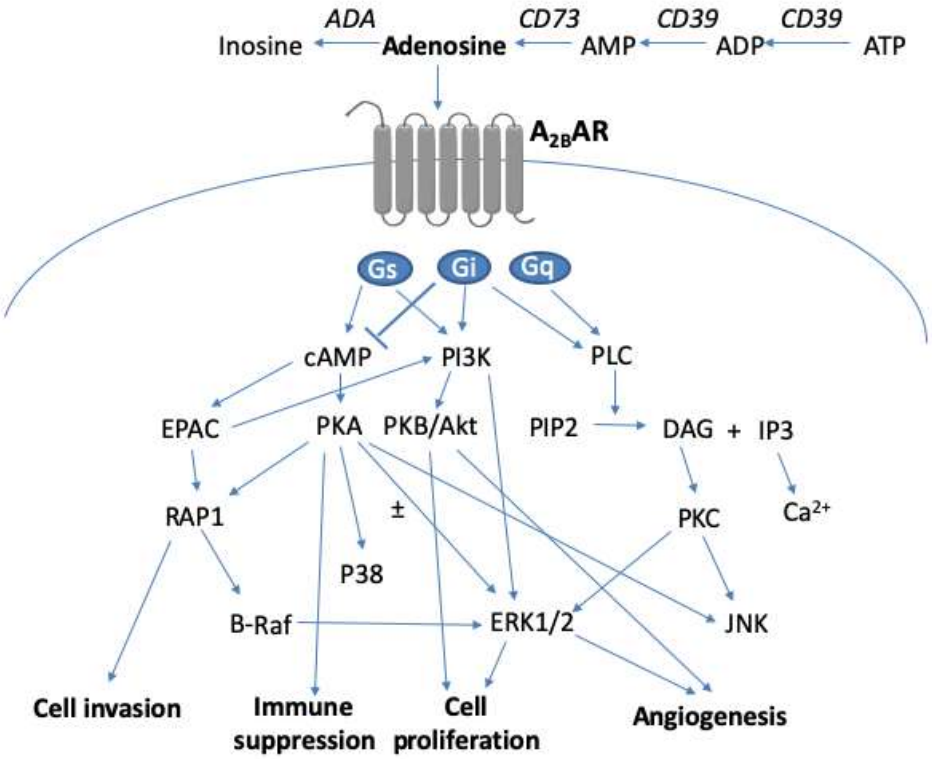

Figure 1. A_{2B}AR signaling in mammalian cells and in the tumor microenvironment, as explained in the text. The three G proteins shown act through either Gα, i.e. on cAMP, or Gβ,γ subunits, i.e. on PI3K. PKA has either a stimulatory or inhibitory effect on ERK1/2. For more detail see: Gao et al., 2018; Giacomelli et al., 2018; Borea et al., 2016; Ntantie et al., 2013; Koussémou et al., 2018. For effects on specific immune cells, see: Cekic and Linden, 2016; Allard et al., 2016.

4. A_{2B}AR agonists and antagonists as pharmacological tools

Although numerous antagonists and a few agonists for the A_{2B}AR have been reported, in this section we focus on the agonists and antagonists that are commercially available as pharmacological tools and those in clinical trials for cancer patients (Table 1). In addition to selective antagonists and agonists, various specialized pharmacological tools can be used to characterize A_{2B}AR and its activity. Radiolabelled compounds are used to investigate A_{2B}AR binding activity including both tritiated ligands and ^{18}F-labeled compounds for positron emission tomography (Hinz et al., 2018; Lindemann et al., 2018). Ligands that have been tritiated for A_{2B}AR binding experiments are: agonists **3** and **8**; antagonists **13**, **21**, **22a**, and ZM241,385 (structure not shown). Fluorescent antagonists of high affinity at the A_{2B}AR were recently reported (Baressi et al., 2018; Köse et al., 2018). A_{2B}AR allosteric modulators have been reported but not extensively characterized (Trincavelli et al., 2014).

There are two major classes of A_{2B}AR agonists that are commercially available (Figure 2). The adenosine derivatives include adenosine, NECA and CPCA **4**, which are considered as full and balanced agonists and often used as standard A_{2B}AR agonists albeit nonselective (Gao et al., 2014). The non-adenosine 3,5-dicyanopyridine class of A_{2B}AR agonists that are commercially available include BAY60-6583 **8**, LUF5834 **7** and BAY68-4986 (A_1AR agonist Capadenoson **6**). BAY60-6583 is an A_{2B}AR-selective agonist but shows variable agonist E_{max} and potencies in different types of cells and tissues (Gao et al., 2014; Gao et al., 2018). Partial and biased agonists for the A_{2B}AR have been reported (Gao et al., 2014; Gao et al., 2018; Hinz et al., 2014; Baltos et al., 2017; Vecchio et al., 2019). In cAMP accumulation assays, 5′-substituted nucleosides NECA and CPCA, and non-adenosine agonists BAY60-6583 and BAY68-4986 are all full agonists in cells overexpressing the recombinant human A_{2B}AR. In calcium mobilization, ERK1/2 phosphorylation and β-arrestin translocation, only 5′-substituted adenosine analogs CPCA and NECA are full agonists. A quantitative operational model characterized BAY60-6583 as an ERK1/2-biased agonist and N^6-substituted agonists as biased against calcium and β-arrestin pathways. Interestingly, a partial A_{2B}AR agonist BAY60-6583 behaved as an A_{2B}AR antagonist in MIN-6 mouse pancreatic β cells expressing low A_{2B}AR levels, to induce insulin release (Gao et al., 2014). It remains to be determined whether BAY60-6583 behaves as a partial agonist or an antagonist in other cell types endogenously expressing low levels of the A_{2B}AR.

A_{2B}AR expression levels often determine the potency and E_{max} of a given A_{2B}AR agonist. BAY60-6583 was found to be a partial agonist in stimulating cAMP accumulation in several cell types endogenously expressing the A_{2B}AR (Gao et al., 2014). For example, in an assay of cAMP accumulation in HEK293 cells endogenously expressing the A_{2B}AR, the EC_{50} and agonist E_{max} values of BAY60-6583 are 242 nM and 73%, respectively. However, in HEK293 cells overexpressing the recombinant A_{2B}AR, the EC_{50} and E_{max} of BAY60-6583 are 6.1 nM and 102%, respectively (Gao et al., 2014). BAY60-6583 did not show any agonist activity in stimulating calcium mobilization or ERK1/2 phosphorylation in T24 bladder cancer cells. BAY60-6583 also did not show any agonist activity in stimulating calcium transients in HEK293 cells, although it showed a robust effect in stimulating cAMP accumulation and ERK1/2 activity. LUF5834 has been reported as a nonselective A_{2B}AR agonist showing an EC_{50} of 12 nM in cAMP accumulation and an agonist E_{max} of 74% in comparison with NECA (E_{max}=100%) (Beukers et al., 2004). Using CHO cells overexpressing the human A_{2B}AR, Baltos et al. (2017) found that the A_1AR agonist BAY68-4986 shows potent A_{2B}AR agonist activity in stimulating cAMP accumulation, with an EC_{50} of 1.1 nM. However, when tested in cAMP accumulation in HEK293 cells endogenously expressing the A_{2B}AR, BAY68-4986 showed an EC_{50} of 500 nM and E_{max} of 95% (Gao and Jacobson, unpublished data). Thus, for all nucleoside and non-nucleoside A_{2B}AR agonists commercially available, only the partial agonist BAY60-6583 is A_{2B}AR selective, which may show agonist activity in some signaling pathways, and antagonist properties in other signaling events (Gao et al., 2014). Full agonists selective for A_{2B}AR are not yet available. Future efforts could be the development of selective and full agonists for A_{2B}AR, in order to have a full range of A_{2B}AR efficacies for studying cell proliferation, angiogenesis, metastasis and immune suppression.

The structures and potencies of the commercially available antagonists as pharmacological tools are listed in Figure 2 and Table 1, respectively. The first selective A_{2B}AR antagonists were reported by Kim et al. (2000), which were xanthine derivatives, and currently there are chemically diverse

heterocyclic selective A_{2B}AR antagonists, such as recently reported LAS101053 **25**, AB928 **26** and ISAM140 **27** (Müller et al., 2018; Seitz et al., 2019). Commercially available A_{2B}AR antagonists as pharmacological tools include 8-arylxanthine derivatives MRS1754 **13**, MRS1706 **14**, GS6201 **18**, PSB-1115 **21**, PSB-603 **22a** and PSB-0788 **23**. Recently, an alkylxanthine with a picomolar affinity at the human A_{2B}AR, PSB-1901 **22b**, was reported (Jiang et al., 2019). Antagonists that are in clinical trials (AB928 **26**, PBF-1129 and theophylline **11**) will be discussed in Section 9.

Table 1. Binding affinity (K_i, nM) or functional potency (EC_{50}, nM; E_{max} as %) of commercially available A_{2B}AR agonists and antagonists as pharmacological tools and A_{2B}AR antagonists in clinical trials for cancer patients. Refer to Figure 2 for structures.

	K_i (nM) or EC_{50} (E_{max}, %)				
Compound	A_1	A_{2A}	A_{2B}	A_3	Reference
Agonists					
1, Adenosine[a]	310	700	24,000	290	Fredholm et al., 2001
			4620[c] (97%)		Gao et al., 2014
3, NECA[b]	14	20	1900	25	Alnouri et al., 2015
3, NECA[a]	12	60	104 (100%)	11	Beukers et al., 2014
4, CPCA	1.9[b]	50[b]	267[c] (102%)	108[b]	Gao et al., 2014
6, BAY68-4986[a]	0.66	1400	1.1 (93%)	2400	Baltos et al., 2017
(Capadenoson)			522[c,d] (95%)		
7, LUF5834	2.6[b]	28[b]	12[a] (74%)	538[b]	Beukers et al., 2004
8, BAY60-6583[b]	390	>10,000	110	220	Alnouri et al., 2015
			242[c] (73%)		Gao et al., 2014
			6.1[e] (102%)		Gao et al., 2014
Antagonists					
11, Theophylline[b]	6200	1710	7850	22,300	Alnouri et al., 2015
12, Caffeine[b]	44,900	9560	33,800	13,300	Alnouri et al., 2015
13, MRS1754[b]	403	503	1.7	570	Alnouri et al., 2015
14, MRS1706[b]	157	112	1.4	230	Alnouri et al., 2015
18, GS6201[b] (CVT-6833)	1940	3280	22	1070	Elzein et al., 2008
21, PSB-1115[b]	>10,000	3790	53.4	>10,000	Alnouri et al., 2015
22a, PSB-603[b]	>10,000	>10,000	0.55	>1000	Alnouri et al., 2015
22b, PSB-1901[b]	>1000	>1000	0.060	>1000	Jiang et al., 2019
23, PSB-0788[b]	2240	333	0.39	>1000	Alnouri et al., 2015
27, LAS101057[b]	>10,000	2500	24	>10,000	Eastwood et al., 2011
26, AB928[b]	64	1.5	2.0	489	Walters et al., 2017
27, ISAM140[b]	>10,000	>10,000	0.55	>1000	El Maatougui et al., 2016
PBF-1129	nd	nd	nd	nd	

[a]EC_{50} values (nM) from cAMP assays. [b]K_i values (nM) from radioligand binding. [c]EC_{50} values (nM) from cAMP assays in HEK293 endogenously expressing the A_{2B}AR. [d]unpublished data. [e]The EC_{50} and E_{max} values of Bay60-6583 stimulated cAMP accumulation in HEK293 cells expressing the recombinant human A_{2B}AR (Gao et al., 2014); Percentages shown in the A_{2B} column represent the agonist E_{max} in comparison to NECA as 100%. nd, not disclosed.

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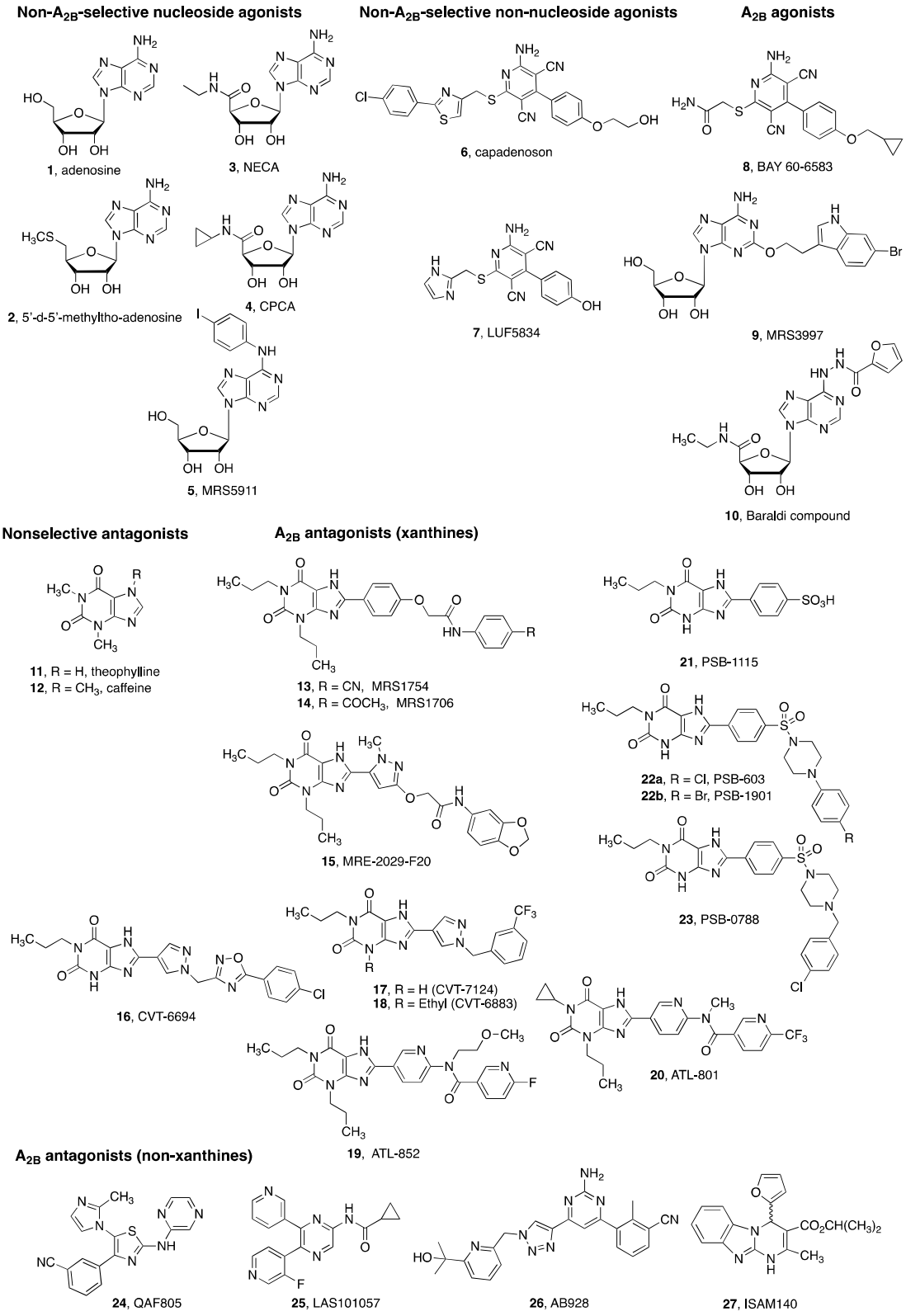

Figure 2. Chemical structures of both commercially available and literature-reported A_{2B}AR agonists (**1** – **10**) and antagonists (**11** – **27**) as pharmacological tools and an A_{2A}AR/A_{2B}AR mixed antagonist (**26**) in a clinical trial for cancer treatment. For more detail, see Gao et al., 2014; Müller et al., 2018.

5. A_{2B}AR receptors in cell proliferation and tumor growth

A_{2B}AR activation can promote proliferation of multiple types of cancer cells and growth of solid tumors. Activation of the A_{2B}AR by BAY 60-6583 was shown to stimulate both proliferation and migration of MDA-MB-231 cells (Fernandez-Gallardo, 2016). The A_{2B}AR-mediated effects were blocked by an A_{2B}AR antagonist GS-6201. Wei et al. (2013) found the A_{2B}AR to be the most highly expressed AR in several human prostate cancer cell lines, including PC-3, and A_{2B}AR activation promotes cell proliferation and decreases cell apoptosis. An A_{2B}AR-selective antagonist PSB-603 decreased proliferation of prostate cancer cell lines (Vecchio et al., 2016; Wei et al., 2013), and colon cancer cells (Ma et al., 2010). Activation of the A_{2B}AR with agonist BAY 60-6583 increased tumor growth in a mouse model of melanoma (Iannone et al., 2013). In a model of bladder cancer, inhibition of tumor growth by the non-selective antagonist theophylline was demonstrated to be mediated by A_{2B}AR but not A_{2A}AR blockade (Cekic et al., 2012). A_{2B}AR selective antagonist ATL801 also inhibited growth of MB49 bladder and 4T1 breast tumor volume (Cekic et al., 2012) and melanoma in mice (Iannone et al., 2013). Stagg et al. (2010) showed that A_{2B}AR activation promoted 4T1.2 tumor-cell chemotaxis in vitro and metastasis in vivo. High A_{2B}AR expression levels have also been found in hepatocellular carcinoma (Xiang et al., 2006). It is suggested that high A_{2B}AR levels are generally associated with worse prognosis or poor survival (Mittal et al., 2016; Cekic and Linden, 2016).

The results from A_{2B}AR blockade with antagonists were consistent with those from genetic knockdown and KO of the A_{2B}AR in various animal models of solid tumors (Cekic et al., 2012; Kasama et al., 2015; Ryzhov et al., 2008), further confirming the critical role of this receptor in cancer cell proliferation and growth.

The specific mechanisms related to A_{2B}AR-mediated proliferation of various cancer cells and growth of different types of tumors have not been extensively and systematically explored. As it has been suggested that different agonists may bind in different modes and induce different A_{2B}AR conformational changes (Thimm et al., 2013), together with the recent finding that A_{2B}AR may couple variably to at least three G proteins in different cell types, it is possible that each agonist may activate a particular mix of signaling cascades in a specific cell type, or the same agonist may activate different signaling pathways in other cell types (Gao et al., 2018). Thus, the signaling mechanisms related to A_{2B}AR-mediated cell proliferation may be diverse in different types of cancers. Nevertheless, multiple studies have shown the importance of several signaling pathways related to A_{2B}AR activation and the subsequent release of various cytokines and growth factors, which eventually led to cancer cell proliferation. MAPK signaling is involved in multiple cellular processes and is often active in cancer cells, promoting proliferation and metastasis (Loi et al., 2016). A_{2B}AR was demonstrated to couple to all three types of MAPKs (Schulte and Fredholm, 2003), the extracellular signal-regulated kinases (ERK1/2), the stress-activated protein kinases P38 and the c-jun N-terminal kinase (JNK). The cAMP-EPAC pathway and ERK1/2 phosphorylation are known to be involved in A_{2B}AR-mediated proliferation of some endothelial cells (Fang & Olah, 2007; Grant et al., 2001). Limm et al., (2014) showed that PKC, but not cAMP or Ca^{2+}, is involved in 5′-methylthioadenosine (**2**)-induced and A_{2B}AR-mediated melanoma cell proliferation. Forskolin can mimic adenosine-induced proliferation of MDA-MB-231 breast cancer cells, suggesting that Gs-cAMP signaling is involved, although it is not clear whether PKA or EPAC is the downstream mediator. Recent evidence correlates the A_{2B}AR-mediated cAMP/PKA and MAPK/ERK pathway activation with the epithelial-mesenchymal transition in lung cancer cells (Giacomelli et al., 2018). A_{2B}AR has been shown to activate the PI3K-Akt pathway (Schulte and Fredholm, 2010), which is known to induce cell proliferation and protects against apoptosis in many cancer cell types. The A_{2B}AR-mediated PI3K-Akt pathway has been shown to be critical for proliferation of glioblastoma stem cells (Liu et al., 2014). The importance of Akt signaling in cell survival has been demonstrated in many cell types. However, it remains to be investigated whether the A_{2B}AR-mediated PI3K-Akt pathway is downstream of Gαi, Gαs or both.

6. A_{2B}AR receptors and tumor metastasis

A_{2B}AR activation plays a critical role in cell motility and migration, which are part of the multi-step process of metastasis (Ntantie et al., 2013; Sepúlveda et al., 2016; Sun & Huang, 2016). Adenosine binding to A_{2B}AR on tumor cells was found to enhance their metastatic capability (Rodrigues et al.,

2007; Ntantie et al., 2013). It was reported that the A_{2B}AR has higher expression in metastatic versus non-metastatic derived colorectal cancer cell lines (Ma et al., 2010). A_{2B}AR activation has been shown to enhance tumor cell chemotaxis and lung metastasis in animal models of breast cancer and melanoma (Cekic et al., 2012; Mittal et al., 2016; Stagg et al., 2010). Consistent with A_{2B}AR agonist-induced metastasis, A_{2B}AR-selective antagonists and genetic knockdown with shRNA suppressed lung metastasis (Desmet et al., 2013; Mittal et al., 2016; Cekic et al., 2012).

The mechanisms behind A_{2B}AR-mediated cell migration and metastasis have been explored (Ntantie et al., 2013). A_{2B}AR-mediated cell motility and metastasis is related to the PKA-dependent suppression of Rap1B, a Rho member of the Ras superfamily of small GTPases that activate MAP kinases (Ntantie et al., 2013). It was found that A_{2B}AR activation may delay Rap1B prenylation in breast, lung, and pancreatic cancer cell lines, and suggested that A_{2B}AR inhibition may be an effective method to prevent metastasis. Similarly, Wilson et al. (2015) found that another Gs-coupled GPCR family, the β-adrenergic receptors, suppresses Rap1B prenylation via a PKA-dependent mechanism and promotes the metastatic phenotype in MDA-MB-231 breast cancer cells. Desmet et al. (2013) suggested that the enhanced metastasis may involve A_{2B}AR-increased gene expression of a key metastatic transcription factor, Fos-related antigen-1 (Fra-1), the expression level of which is associated with increased cell motility and invasion (Belguise et al., 2005; Adiseshaiah et al., 2007). Fra-1 is regulated by ERK, and its overexpression is associated with a poor clinical outcome (Zhao et al., 2014). Fra-1 and A_{2B}AR positively correlate at the mRNA level, and it was shown using chromatin immunoprecipitation (ChIP) experiments that Fra-1 binds the promoter of A_{2B}AR gene in human breast cancer cells (Desmet et al., 2013). Ou et al. (2016) discovered that hypoxia as well as extracellular ATP cause a reversible increase in the centrosome-nucleus distance and reduced cell motility through the A_{2B}AR and specifically activate the Epac1/RapGef3 pathway. Epac1 is critically involved, and Rap1B is important in the relative positioning of the centrosome and nucleus, which is related to cell motility and migration.

7. A_{2B}AR receptors and angiogenesis

Tumor growth is enhanced by angiogenesis, the formation of new blood vessels, which involves the migration, differentiation and growth of endothelial cells inside the blood vessels. Adenosine signaling plays an important role in angiogenesis. Adenosine has been reported to promote angiogenic responses via all four AR subtypes (Clark et al., 2007; Feoktistov, et al., 2004; Adair et al., 2005; Koszalka et al., 2016). The endothelial cells express high levels of the A_{2B}AR suggesting its potentially critical role in promoting angiogenesis. A_{2B}AR stimulation promotes the production of angiogenic cytokines by mast cells (Ryzhov et al., 2008) and dendritic cells (Novitskiy et al., 2008). It has been suggested that adenosine increases endothelial cell proliferation, chemotaxis and capillary tube formation (Grant et al., 2001; Acurio, et al., 2014). A_{2B}AR activation has also been shown to stimulate production of vascular endothelial growth factor (VEGF), basic fibroblast growth factor and insulin-like growth factor-1 (IGF1) by human HMEC-1 microvascular endothelial cells (Feoktistov et al., 2002). Adenosine was demonstrated to promote VEGF production in rat myocardial myoblasts (Gu et al., 2000) and in macrophages from C57BL/6 mice (Leibovich et al., 2002). It has been demonstrated that AR stimulation could increase VEGF production five-fold in tumor-associated $CD45^+$ immune cells, an effect that is not observed in $CD45^+$ cells from A_{2B}AR KO mice (Ryzhov et al., 2008). The A_{2B}AR induces production of VEGF (Feoktistov et al., 2002; Ryzhov et al., 2008; 2014) and interleukin (IL)-8 in human melanoma cells (Merighi et al., 2009), which are essential for tumor angiogenesis. Bay60-6583, a selective A_{2B}AR agonist, was demonstrated to induce in tumor expression of VEGF-A (Sorrentino et al., 2015). A_{2B}AR inhibition by a selective antagonist PSB-1115 **21** significantly decreased tumor growth by blocking angiogenesis and increasing T cells numbers within the tumor microenvironment.

Multiple signaling molecules have been found to be related to A_{2B}AR-mediated angiogenesis. Du et al. (2015) suggested the A_{2B}AR activation-driven angiogenesis is via cAMP-PKA-CREB mediated VEGF production and PI3K/Akt-dependent upregulation of endothelial nitric oxide synthase (eNOS) in HMEC-1 cells. Ryzhov et al. (2014) suggested that VEGF appears to be stimulated

by a mechanism involving the transcription factor JunB downstream of A_{2B}AR-mediated PLC-Rap1-MEK activation. Fang and Olah (2007) showed that cyclic AMP-dependent, protein kinase A-independent activation of ERK1/2 following AR stimulation in human umbilical vein endothelial cells was via Epac1.

8. A_{2B}AR and immunity

It has been well documented that cancer cells can escape from anti-tumor immune surveillance especially under conditions with impaired immunity. Adenosine has demonstrated its role as an important modulator of immune cell functions at least in part via its action at the A_{2B}AR (Cekic and Linden, 2016; Hasko et al., 2009; Allard et al., 2016). A_{2B}AR activation is known to suppress IFN-γ-enhanced expression of major histocompatibility complex class II (MHC-II) transactivator (Xaus et al., 1999; Xu et al., 2008). In addition to the well-described roles of CD73 and CD39, adenosine deaminase is known to control the local adenosine concentration, and this enzyme also binds to the A_{2B}AR (Herrela et al., 2001). Adenosine deaminase deficiency is one of the serious immune diseases which is due to the increased adenosine concentration and subsequently suppressed immune responses. Thus, in addition to its direct effects on metastasis, proliferation and angiogenesis, the A_{2B}AR can have a direct or an indirect role on cancer progression via modulation of the immune system. The role of the A_{2B}AR in cell immunity was mostly neglected until recently partly due to adenosine having a low A_{2B}AR affinity (Jacobson and Gao, 2006; Fredholm et al., 2011), although early findings indicated that A_{2B}AR was the AR subtype responsible for the immune suppressive function of T cells, macrophages and dendritic cells (Cekic and Linden 2016; Fredholm et al., 2011; Hasko et al., 2009). Also, early work on CD26/DPP4 (dipeptidyl peptidase 4), a T cell surface antigen that cleaves various bioactive peptides, mainly focused on its role in T cells (Dong and Morimoto, 1996; Morimoto and Schlossman, 1998) that highly express the A_{2A}AR (Hoskin et al., 2008; Kjaergaard et al., 2018; Erdmann et al., 2005). More recently, in addition to CD39 and CD73, the importance of A_{2B}AR and DPP4 in dendritic cells and macrophages also gained appreciation (Zhong et al., 2013). DPP4 has been identified as one of the macrophage-related gene signatures predicative of increased risk in gliomas (Sun et al. 2019). DPP4 inhibitor vildagliptin has been reported to suppress lung cancer growth via a macrophage-mediated mechanism (Jang et al., 2019). Considering the increased adenosine concentration and increased A_{2B}AR expression in the tumor microenvironment (Allard, 2016; Cekic and Linden, 2016, Sorrentino and Morello, 2017) together with the high expression levels of both A_{2B}AR and DPP4 in macrophages and dendritic cells, growing evidence suggests a critical role of A_{2B}AR together with CD39 and CD73 in modulating cancer progression at least in part via immune suppression. Furthermore, DPP4 physically associates with adenosine deaminase, which controls adenosine concentration and binds to the A_{2B}AR. Thus, A_{2B}AR blockade may enhance the function of immune cells (Hasko et al., 2009; Cekic and Linden 2016; Allard et al., 2016).

A_{2A}AR has been shown to be critical in regulating TLR-induced cytokine production. However, a recent study utilizing macrophages isolated from A_{2B}AR KO mice showed that adenosine elicits IL-6 production from macrophages via the A_{2B}AR (Philip et al., 2017). IFN-γ upregulates A_{2B}AR expression on macrophages resulting in an increased responsiveness of macrophages to the stimulatory effects of NECA (Cohen et al., 2015). The pharmacologic inhibition or the genetic deletion of the A_{2B}AR results in a hyperinflammatory response to TLR ligation, similar to IFN-γ treatment of macrophages, suggesting the NECA-mediated effect is via A_{2B}AR, but not A_{2A}AR (Cohen et al., 2015). The role of A_{2B}AR in regulating dendritic cell function has been defined using A_{2B}AR KO mice and selective agonists and antagonists for A_{2B}AR (Cekic and Linden 2016; Hasko et al., 2009). In mice bearing MB49 and/or 4T1 tumors, Cekic et al. (2012) demonstrated that selective blockade of A_{2B}AR resulted in a CXCR3-dependent reduction of tumor growth and lung metastases from breast tumors through enhancement of dendritic cell activation. Inhibition of A_{2B}AR activation by PSB-603 was shown to suppress regulatory T cell (Treg) differentiation and IL-10 production, without affecting effector T cell activation measured by IL-2 production and CD25 expression (Nakatsukasa and Tsukimoto, 2011). A_{2B}AR was also suggested to modulate the phenotype of bone marrow-derived dendritic cells. A_{2B}AR activation impairs MHC-II transcription in IFN-γ-stimulated cells (Fang et al.,

2013; Xia et al., 2015). MHC-II expression is required for $CD4^+$ T cell anti-tumor responses, and loss of MHC-II is associated with aggressiveness of colorectal cancer and decreased levels of tumor-infiltrating lymphocytes (Warabi et al., 2000). Shi et al. (2006) also reported that both major MHC-II transactivator (CIITA) and MHC-II are decreased in highly metastatic cancer cells. Thus, A_{2B}AR blockade has a potential to enhance anti-tumor immunity in cancers where tumor-infiltrating lymphocytes and MHC-II levels are decreased.

The specific signaling pathways related to A_{2B}AR-mediated immune suppression have been explored. Xu et al. (2008) found that A_{2B}AR-mediated cAMP is both necessary and sufficient to suppress the IFN-γ-mediated immune response. Figueiredo et al. (2017) showed that cAMP accumulation induced by A_{2B}AR activation is important to inhibit dendritic cell activation and to evade the immune response in infected mice. In human monocytes, it has been suggested that A_{2B}AR-triggered cAMP accumulation inhibits the immune response by lowering the amount of MHC class I and class II molecules (Sciaraffia et al., 2014). A_{2B}AR-induced cAMP accumulation was also found to reduce STAT1 phosphorylation and impair its binding to CIITA promoter while fostering synthesis of TGF-β, known to antagonize MHC-II transactivation (Fang et al., 2013; Xia et al., *2015*). Iannone et al. (2013) showed that melanoma-bearing mice treated with the selective A_{2B}AR agonist BAY60-6583 had increased melanoma growth, which was associated with higher levels of immune regulatory mediators IL-10 and monocyte chemoattractant protein 1 and accumulation of tumor-associated CD11b+ and Gr1+ cells and myeloid-derived suppressor cells. Depletion of CD11b+Gr1+ cells completely reversed the pro-tumor activity of BAY60-6583. Inhibition of A_{2B}AR with PSB-1115 reversed immune suppression in the tumor microenvironment, leading to a significant delay in melanoma growth. The authors suggest that the antitumor activity of PSB-1115 relies on its ability to lower accumulation of tumor-infiltrating myeloid-derived suppressor cells (MDSCs) and restore an efficient antitumor T cell response.

9. A_{2B}AR antagonists as novel anticancer agents

As described above, A_{2B}AR activation induces tumor proliferation, growth of solid tumor, tumor angiogenesis, tumor cell invasion and metastasis, and immune suppression. Thus, A_{2B}AR blockade holds great promise as an anti-cancer therapy. For example, A_{2B}AR inhibition by the antagonist PSB-1115 was shown to decrease tumor metastasis of $CD73^+$ melanoma cells and mammary carcinoma cells (Mittal et al., 2016). Iannone et al. (2013) observed that PSB-1115 delayed tumor growth and enhanced the anti-tumor activity of dacarzabine, a drug currently used in metastatic melanoma.

Cekic et al. (2012) demonstrated that the antitumor effect of theophylline occurs via the A_{2B}AR rather A_{2A}AR, based on a study using A_{2A} and A_{2B}AR KO mice. Nevertheless, simultaneous antagonism of both subtypes has been proposed to be possibly synergistic against some types of tumors (Cekic and Linden, 2016; Allard et al., 2016), although it is not clear whether the blockade of both A_{2A}AR and A_{2B}AR could also produce more adverse effects than either subtype separately.

Antagonists in clinical trials for cancer patients (ClinicalTrials.gov NCT Identifier) include the mixed A_{2A}AR/A_{2B}AR antagonist AB928 **26** (Phase 1, lung cancer, 03846310; Phase 1, breast and ovarian cancer, 03719326; Phase 1, gastrointestinal cancer, 03720678; Phase 1, advanced cancer, 03629756), PBF-1129 (structure not disclosed; Phase 1, non-small cell lung cancer, 03274479) and theophylline **11** (see below). The first dual-acting A_{2A}AR/A_{2B}AR antagonist AB928 is being tested clinically in multiple arms in combination with pegylated liposomal doxorubicin, nanoparticle albumin-bound paclitaxel, or a PI3K-γ inhibitor. AB928 has exhibited excellent safety, PK, and PD profiles in a Phase 1 clinical trial in healthy volunteers and is currently being evaluated in patients with non-small cell lung cancer, breast cancer, ovarian cancer, colorectal and six other types of cancers (clinicaltrials.gov). One of the cancer immunotherapy drugs, AB122, a fully human immunoglobulin G4 monoclonal antibody targeting human programmed cell death protein 1 (PD-1), will be tried in combination with AB928. AB928 was able to produce maximal AR blockade assessed as a function of NECA-stimulated pCREB induction in peripheral blood CD8+ T cells (Seitz et al., 2019). AB928 was shown to relieve adenosine-mediated immune suppression (Waters et al., 2018). Combining AR inhibition with AB928 and chemotherapy results in greater immune activation and tumor control.

Phase I trial of the selective A_{2B}AR antagonist PBF-1129 (structure not disclosed) in patients with advanced non-small cell lung cancer is being conducted. PBF-1129 is being administered in a dose escalation study of tolerability without other therapy.

Theophylline is a nonselective AR antagonist, which was tested for anticancer efficacy in two previous clinical trials (incidentally, as an inhibitor of intracellular cAMP in chronic lymphocytic leukemia, Phase 2, 00003808; a withdrawn trial in combination with an allogeneic tumor cell-vaccine (gp96-Ig vaccine) and oxygen therapy, which lowers adenosine levels (Hatfield and Sitkovsky, 2016), in non-small cell lung cancer, Phase 1, 01799161). The first theophylline trial description did not even reference AR antagonism, but there was a correlation found between in vitro apoptosis in leukemia cells and clinical response in a subset of patients (Wiernik et al., 2004). Theophylline in combination prednisone and dextromethorphan has also been in a clinical trial (Phase 1, 01017939) for patients with metastatic castration-resistant prostate cancer. Aminophylline, a salt of theophylline, in combination with Bacillus Calmette-Guerin has been in a trial (early Phase 1, 01240824) for patients with bladder cancer. However, it should be noted that theophylline is nonselective and may block all four ARs.

10. Summary

A_{2B}AR signaling is a major pathway contributing to cancer cell proliferation and solid tumor growth, angiogenesis and metastasis, and immune suppression. Thus, A_{2B}AR antagonists are potentially a novel anticancer therapy, either in combination with other anticancer drugs or as a mono-therapy. Several A_{2B}AR antagonists are now in clinical trials for patients with various types of cancers. The nonselective A_{2B}AR antagonist, theophylline, in combination with other anticancer drugs has been evaluated in patients with bladder cancer and prostate cancer. Dual acting A_{2A}AR/A_{2B}AR antagonist AB928 has exhibited excellent safety, PK, and PD profiles in a Phase 1 clinical trial in healthy volunteers and is currently being evaluated in patients with non-small cell lung cancer, breast cancer and ovarian cancer. A_{2B}AR selective antagonist PBF-1129 is also in clinical trial for patients with non-small cell lung cancer. Thus, A_{2B}AR antagonism is a promising direction for the development of new cancer therapeutics.

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