

1 *Review*

2 **A_{2B} adenosine receptor and cancer**

3 **Zhan-Guo Gao***, **Kenneth A. Jacobson***

4 Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and
5 Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States.

6 *Correspondence: *Zhan-Guo Gao* (zg21o@nih.gov); *Kenneth A. Jacobson* (kennethj@niddk.nih.gov).

7 **Abstract:** There are four subtypes of adenosine receptors (ARs), named A₁, A_{2A}, A_{2B} and A₃, all of
8 which are G protein-coupled receptors (GPCRs). Locally produced adenosine suppresses in anti-
9 tumor immune surveillance. The A_{2B}AR, coupled to both G_{as} and G_{aq} G proteins, is one of the
10 several GPCRs that are expressed in a significantly higher level in some cancer tissues in comparison
11 to adjacent normal tissues. There is growing evidence that the A_{2B}AR plays an important role in
12 tumor cell proliferation, angiogenesis, metastasis, and immune suppression. Thus, A_{2B}AR
13 antagonists are potentially novel attractive anticancer agents. Several antagonists targeting at A_{2B}AR
14 are currently in clinical trials for various types of cancers. In this review, we first describe the
15 signaling, agonists, and antagonists of the A_{2B}AR. We further discuss the role of the A_{2B}AR in the
16 progression of various cancers, and the rationale of using A_{2B}AR antagonists in cancer therapy.

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

19

20 **1. Introduction**

21 Adenosine in the extracellular milieu is generated, mainly via the degradation of ATP released
22 under stress conditions, to protect cells and tissues locally. Adenosine and ATP acting at different
23 classes of receptors often have opposite effects in cell proliferation or cell death. ATP and other
24 adenine nucleotides have antitumor effects via the activation of P2Y₁ receptor (P2Y₁R) subtype (Wei
25 et al., 2011; Burnstock and Di Virgilio, 2013), whereas adenosine induces cancer cell proliferation and
26 growth of many types of tumors via the activation of the A_{2B} adenosine receptor (AR) (Seitz et al.,
27 2019; Wei et al., 2013; Cekic et al., 2012; Kasama et al., 2016; Mittal et al., 2016; Sepulveda et al., 2016;
28 Ryzhov et al., 2008; Iannone et al., 2016). The generation and degradation/removal of adenosine is a
29 multi-step and balanced process in cells involving enzymes (CD39, CD73, CD26, adenosine
30 deaminase, adenosine kinase, S-adenosyl homocysteine hydrolase) and nucleoside transporters
31 (Fredholm et al., 2011), which are not the main topic of this review. Although extracellular adenosine
32 exerts its action via four G protein-coupled receptors (GPCRs), A₁, A_{2A}, A_{2B} and A₃ (Jacobson and Gao,
33 2006), in this review we will only focus on the importance of A_{2B}AR signaling (Figure 1) in cancer
34 progression and the rationale to use A_{2B}AR antagonists as anticancer agents.

35 The importance of the A_{2B}AR in cancer progression has only recently been revealed, despite the
36 physiological role of adenosine in cardiac function being realized almost a century ago (Drury &
37 Szent-Györgyi, 1929). Although A_{2B}AR effects in brain slices were characterized in the early 1980s
38 (Daly et al., 1983), until recently the A_{2B}AR has been poorly characterized in comparison to the other
39 three ARs, which is at least in part due to the fact that A_{2B}AR has low affinity for the endogenous
40 agonist adenosine 1 (EC₅₀ ~24 μM, Figure 2, Table 1). Thus, it was assumed that A_{2B}AR must have a
41 minor physiological significance. However, increasing evidence has shown that there is a dramatic
42 increase in extracellular adenosine concentration and a significant upregulation of A_{2B}AR expression
43 under many pathological conditions (Borea et al., 2016; 2018; Cekic and Linden, 2016), such as
44 hypoxia, inflammation and cancer, which may indicate the critical role of A_{2B}AR in many diseases.
45 For example, adenosine concentration has been reported to increase 10-fold in patients with septic
46 shock (Ramakers et al., 2011). Hypoxia-inducible factor 1 (HIF-1α) has been reported to up-regulate

47 A_{2B}AR expression on activated macrophages (Philip et al., 2017). Lan et al. (2018) found that hypoxia
48 increased expression of A_{2B}AR in human breast cancer cells through the transcriptional activity of
49 HIF-1 α . The discovery that A_{2B}AR expression is significantly increased by HIF-1 α strongly suggested
50 its involvement in cancer promotion (Lan et al., 2018; Philip et al., 2017; Ma et al., 2010; Kong et al.,
51 2006; Feoktistov et al., 2002). In addition to its role in tumor growth, inhibition of A_{2B}AR genetically
52 or pharmacologically dramatically decreased lung metastasis after implantation of breast cancer cells
53 into the mammary fat pad of immunodeficient mice (Lan et al., 2018). It has also been recently shown
54 that bladder urothelial carcinoma expresses high levels of A_{2B}AR, which is suggested to be associated
55 with a poor patient prognosis (Zhou et al., 2017). A tissue microarray of 232 breast cancer samples,
56 that included 66 triple negative breast cancer cases suggest that A_{2B}AR could serve as a prognostic
57 biomarker and a potential therapeutic target (Horigome et al., 2018). Kasama et al. (2015) showed that
58 A_{2B}AR controls cellular proliferation via HIF-1 α activation, indicating that A_{2B}AR may be a key
59 regulator of tumoral progression in oral squamous cell carcinoma. Thus, the A_{2B}AR is consistently
60 and convincingly demonstrated to be involved in tumor cell proliferation, metastasis, angiogenesis,
61 and immune suppression. Furthermore, the A_{2B}AR and A₃AR seem to be the only AR subtypes that
62 are expressed in significantly higher levels in cancer tissues in comparison to normal adjacent tissues,
63 similar to several other GPCRs (Li et al., 2005; Xiang et al., 2006; Kasama et al., 2015; Sepulveda et al.,
64 2016; Cohen and Fishman, 2019).

65 Although all four ARs are reported to be involved in cancer progression (Borea et al., 2018;
66 Koszałka et al., 2016; Marwein et al., 2019; Gorain et al., 2019), the other three ARs have been shown
67 both to be pro- and anti-tumoral (Borea et al., 2018). For example, both pro- and antitumoral effects
68 have been reported for the A₁AR (Borea et al., 2018). Targeting A_{2A}AR has been considered as a
69 double-edged sword (Allard et al., 2016; Borea et al., 2018). It has been suggested that adenosine
70 accumulation in the tumor microenvironment facilitates tumor growth through inhibition of effector
71 T cells and natural killer (NK) cells (Cekic and Linden, 2014), and inhibition of A_{2A}AR alone was
72 found to be sufficient to establish anti-tumor immunity and protect against metastasis in various
73 mouse models of cancer (Cekic and Linden, 2014). However, A_{2A}AR deletion does not inhibit the
74 growth of all tumor types and might have the opposite effect. For example, an increased tumor
75 growth rate of both B16F10 melanoma and MB49 bladder carcinomas has been observed in A_{2A}AR
76 knockout (KO) mice (Cekic et al., 2012). Blocking A_{2B}AR action might have advantages over the
77 A_{2A}AR as a cancer therapeutic target. Cekic et al. (2012) showed that AR antagonist theophylline
78 slowed the growth of MB49 bladder and 4T1 breast tumors in mice and reduced breast cancer cell
79 metastasis from mammary fat to lung via the A_{2B}AR, but not the A_{2A}AR, based on experiments using
80 A_{2A}AR or A_{2B}AR KO mice. The role of A₃AR has been investigated in various cancer cell types with
81 contrasting results, i.e. both pro- and antiproliferative, as well as pro-apoptotic and anti-apoptotic
82 effects (Borea et al., 2016). Both A₃AR agonists and antagonists have been considered for anti-cancer
83 agents, although only A₃AR agonists have progressed in clinical trials (Cohen and Fishman, 2019).

84 Recent advances in the signaling and function of the A_{2B}AR (Vecchio et al., 2019; Cekic and Linden,
85 2016; Gao et al., 2018; Allard et al., 2016) and the availability of selective ligands (Müller et al., 2018;
86 Gao et al., 2014; Alnouri et al., 2015), have greatly facilitated understanding of the role of A_{2B}AR in
87 cancer progression and the rationale for development of A_{2B}AR antagonists as anti-tumor drugs. In
88 this review, we first describe the distribution, signaling, agonists and antagonists of the A_{2B}AR. We
89 then discuss the role of the A_{2B}AR in the progression of various types cancers, and the rationale of
90 using A_{2B}AR antagonists in cancer therapy.

91

92 **2. A_{2B}AR distribution and expression**

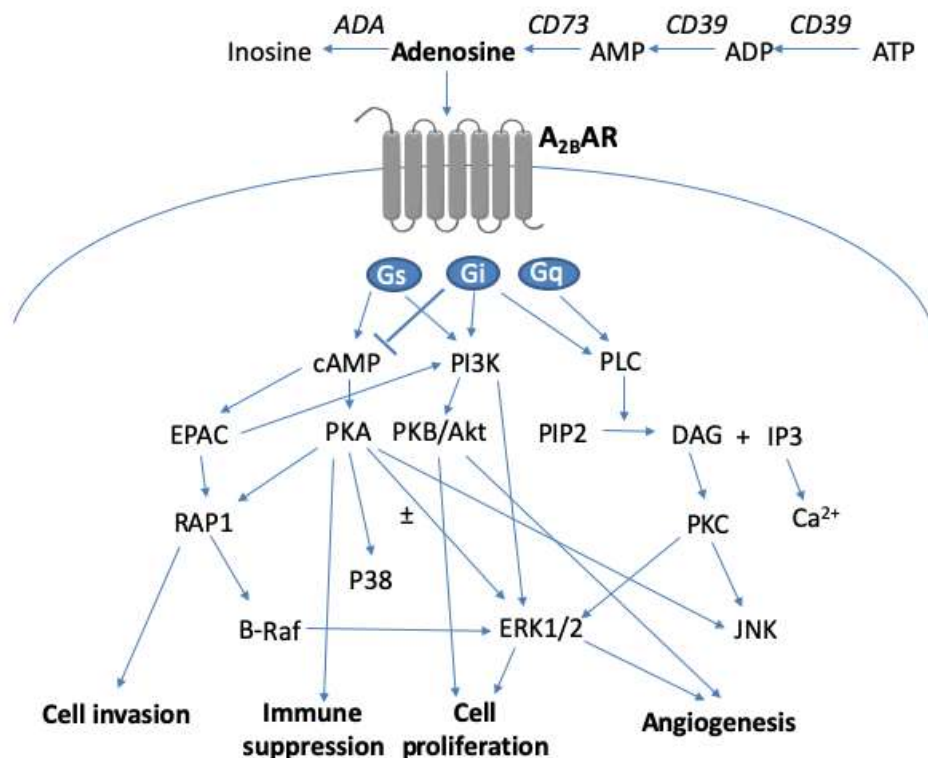
93 In rat, A_{2B}AR mRNA was detected at various levels in all tissues studied (Dixon et al., 1996). In
94 mouse, by replacing exon 1 of the A_{2B} gene with a reporter construct containing β -gal, mouse tissue-
95 specific activation of the A_{2B} gene promoter was conveniently determined in various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

97 al., 2006). Yang et al. (2006) found that mouse smooth muscle cells, endothelial cells and macrophages
98 exhibit high A_{2B}AR expression levels. The high level of A_{2B}AR expression in endothelial cells suggests
99 a potential role in angiogenesis. In human primary cells, A_{2B}AR has been found in endothelial cells,
100 mast cells, dendritic cells, macrophages, and neutrophils (Cekic and Linden, 2016; Hasko et al., 2009).
101 High expression levels in dendritic cells and macrophages indicate a possible role in modulation of
102 immunity. In human cancer tissues, A_{2B}AR expression levels were found to be higher than in adjacent
103 normal tissues (Li et al., 2005; Xiang et al., 2006; Kasama et al., 2015; Sepulveda et al., 2016; Zhou et
104 al., 2017). High levels of A_{2B}AR have been suggested to be associated with worse prognosis
105 in bladder urothelial carcinoma (Zhou et al., 2017). Mittal et al. (2016) suggested that high A_{2B}AR
106 expression is associated with worse prognosis in triple-negative breast cancer (TNBC). In a TNBC
107 mouse model, A_{2B}AR activation increased metastasis (Neumann et al., 2018), while A_{2B}AR
108 antagonism in mouse models reduced tumor burden by immune mechanisms and action on tumor
109 cells. The high A_{2B}AR expression has also been found in many human tumor cell lines, such as PC3
110 prostate, T24 bladder, 1321N1 astrocytoma (Gao et al., 2018), U373MG astrocytoma (Fiebich et al.,
111 1996), MDA-MB-231 breast (Lan et al., 2018), Jurkat T cells (Nonaka et al., 1994), BON-1 pancreatic
112 and KRJ-I intestinal (Kalhan et al., 2012), A375 melanoma (Merighi et al., 2009), and THP-1 human
113 monocytes (Zhong et al., 2006). The high expression of the A_{2B}AR in those cancer cells indicates its
114 potential role in cancer progression. In a glioblastoma cell line derived from a mouse line containing
115 spatially expressed A_{2B}AR, this receptor is highly upregulated leading to proliferation, angiogenesis
116 and invasiveness (Yan et al., 2019). Mouse KO of CD73, which forms adenosine locally from AMP,
117 reduced A_{2B}AR signaling in the glioblastoma, to decrease pathogenesis and increase sensitivity to
118 chemotherapy. A_{2B}AR expression was also demonstrated in human lung epithelial cells (Giacomelli
119 et al., 2018). Consistent with the high A_{2B}AR expression in bladder cancer and breast cancer cells,
120 Cekic et al. (2012) showed that A_{2B}AR antagonists delayed the growth of bladder and breast tumors
121 and reduced lung metastasis. Lan et al. (2018) found that genetic or pharmacological inhibition of
122 A_{2B}AR expression or activity dramatically impaired tumor initiation and lung metastasis in mice.
123 Thus, high A_{2B}AR expression is related to tumor growth and metastasis, and therefore A_{2B}AR
124 antagonists are potential therapeutic agents for various types of cancers including lung cancer.
125

126 3. A_{2B}AR signaling

127 Classical A_{2B}AR signaling has been initially and primarily demonstrated in CHO cells expressing
128 the recombinant human A_{2B}AR (Rivkees and Reppert, 1992; Pierce et al., 1992; Schulte and Fredholm,
129 2003). A_{2B}AR activation leads to dissociation of the G_{αs} and G_{βγ} subunits and subsequent activation
130 of the adenylyl cyclases, which in turn hydrolyze intracellular ATP into cyclic AMP (cAMP), which
131 activates protein kinase A (PKA) and many downstream signaling molecules. The G_s-cAMP-PKA
132 axis is an important A_{2B}AR-mediated signaling pathway. For example, Xu et al. (2008) found that
133 A_{2B}AR-mediated cAMP is both necessary and sufficient to suppress interferon- γ (IFN- γ)-mediated
134 immune responses. Jing et al. (2015) showed that A_{2B}AR activation in hematopoietic stem cells
135 induced chemokine CXCL8 production via cAMP-PKA signaling and mediated hematopoiesis. In
136 addition to PKA, cAMP also activates 'exchange protein directly activated by cAMP' (EPAC), another
137 important signaling molecule related to cell migration and angiogenesis (Fang and Olah, 2007). In
138 CHO cells expressing the recombinant human A_{2B}AR, the nonselective AR agonist NECA 3 activated
139 cAMP response element-binding protein (CREB) and P38 (a mitogen-activated protein kinase,
140 MAPK) but not Akt (protein kinase B). Extracellular signal-regulated kinase 1/2 (ERK1/2) and GTPase
141 Rap1 were blocked by PKA inhibitor H89 (Schulte and Fredholm, 2003). Phosphorylation of Akt and
142 ERK1/2 was blocked by a phosphoinositide 3-kinase (PI3K) inhibitor, wortmannin. Thus, A_{2B}AR
143 activating various downstream MAPKs may be via different signaling pathways. Although PKA-
144 independent in CHO cells, the Rap1 activation seems PKA-dependent in HEK293 cells (Ntantie et al.,
145 2013). The coupling of A_{2B}AR to β -arrestin signaling has also been reported (Mundell et al., 2001; Gao
146 et al., 2014).

147 Most of the early studies on A_{2B}AR signaling utilized CHO or HEK293 cells transfected with
 148 recombinant human A_{2B}AR (Pierce et al., 1992, Schulte and Fredholm, 2003, Gao et al., 1999).
 149 However, in various types of cells endogenously expressing the A_{2B}AR, the receptor was able to
 150 couple to either Gi or Gs, depending on the cell type and downstream signaling pathway measured
 151 (Gao et al., 2018). For example, A_{2B}AR agonist NECA stimulates ERK1/2 phosphorylation via G_{ai} in
 152 T24 bladder cancer cells (Gao et al., 2018), but via G_s in CHO cells (Schulte and Fredholm, 2003).
 153 The G_{ai} inhibitor pertussis toxin, but not G_{αq} KO, diminished NECA-stimulated ERK1/2 activity
 154 suggesting the involvement of G_{ai} rather than G_{αq} (Gao et al., 2018). A_{2B}AR downregulates ERK1/2
 155 activity via G_s in 1321N1 astrocytoma cells (Gao et al., 2018) and in MDA-MB-231 breast cancer cells
 156 (Koussémou et al., 2018). ERK1/2 reduction in MDA-MB-231 cells was triggered by an A_{2B}AR agonist
 157 and forskolin, but abolished by the PKA inhibitor H89, suggesting an important role for the cAMP-
 158 PKA pathway in controlling ERK1/2 activity in MDA-MB-231 cells. A_{2B}AR-mediated intracellular
 159 calcium mobilization in T24 cells was mainly via Gi, although G_s may also play a minor role, but G_q
 160 is not involved (Gao et al., 2018). Thus, it is conceivable that in many cases the predominant A_{2B}AR
 161 coupling is through G_{ai} rather than G_s. Many important A_{2B}AR functions from primary cells or
 162 tissues have recently been related to the PI3K-Akt and RAP1B-EPAC pathways (Ou et al., 2016;
 163 Ntantie et al., 2013; Phosri et al., 2017; Lim et al., 2019; Ni et al., 2018; Shen et al., 2018). However, it
 164 has not been extensively explored whether those signaling molecules are actually downstream of
 165 A_{2B}AR-mediated G_{ai} or G_s proteins. The A_{2B}AR-mediated major signaling pathways are illustrated
 166 in Figure 1.
 167



168

169

170

171

172

173

174

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 G_α, i.e. on cAMP, or G_{β,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.

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

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

186 There are two major classes of A_{2B}AR agonists that are commercially available (Figure 2). The
187 adenosine derivatives include adenosine, NECA and CPCA **4**, which are considered as full and
188 balanced agonists and often used as standard A_{2B}AR agonists albeit nonselective (Gao et al., 2014).
189 The non-adenosine 3,5-dicyanopyridine class of A_{2B}AR agonists that are commercially available
190 include BAY60-6583 **8**, LUF5834 **7** and BAY68-4986 (A₁AR agonist Capadenoson **6**). BAY60-6583 is an
191 A_{2B}AR-selective agonist but shows variable agonist E_{max} and potencies in different types of cells and
192 tissues (Gao et al., 2014; Gao et al., 2018). Partial and biased agonists for the A_{2B}AR have been reported
193 (Gao et al., 2014; Gao et al., 2018; Hinz et al., 2014; Baltos et al., 2017; Vecchio et al., 2019). In cAMP
194 accumulation assays, 5'-substituted nucleosides NECA and CPCA, and non-adenosine agonists
195 BAY60-6583 and BAY68-4986 are all full agonists in cells overexpressing the recombinant human
196 A_{2B}AR. In calcium mobilization, ERK1/2 phosphorylation and β-arrestin translocation, only 5'-
197 substituted adenosine analogs CPCA and NECA are full agonists. A quantitative operational model
198 characterized BAY60-6583 as an ERK1/2-biased agonist and N⁶-substituted agonists as biased against
199 calcium and β-arrestin pathways. Interestingly, a partial A_{2B}AR agonist BAY60-6583 behaved as an
200 A_{2B}AR antagonist in MIN-6 mouse pancreatic β cells expressing low A_{2B}AR levels, to induce insulin
201 release (Gao et al., 2014). It remains to be determined whether BAY60-6583 behaves as a partial
202 agonist or an antagonist in other cell types endogenously expressing low levels of the A_{2B}AR.

203 A_{2B}AR expression levels often determine the potency and E_{max} of a given A_{2B}AR agonist. BAY60-
204 6583 was found to be a partial agonist in stimulating cAMP accumulation in several cell types
205 endogenously expressing the A_{2B}AR (Gao et al., 2014). For example, in an assay of cAMP
206 accumulation in HEK293 cells endogenously expressing the A_{2B}AR, the EC₅₀ and agonist E_{max} values
207 of BAY60-6583 are 242 nM and 73%, respectively. However, in HEK293 cells overexpressing the
208 recombinant A_{2B}AR, the EC₅₀ and E_{max} of BAY60-6583 are 6.1 nM and 102%, respectively (Gao et al.,
209 2014). BAY60-6583 did not show any agonist activity in stimulating calcium mobilization or ERK1/2
210 phosphorylation in T24 bladder cancer cells. BAY60-6583 also did not show any agonist activity in
211 stimulating calcium transients in HEK293 cells, although it showed a robust effect in stimulating
212 cAMP accumulation and ERK1/2 activity. LUF5834 has been reported as a nonselective A_{2B}AR agonist
213 showing an EC₅₀ of 12 nM in cAMP accumulation and an agonist E_{max} of 74% in comparison with
214 NECA (E_{max}=100%) (Beukers et al., 2004). Using CHO cells overexpressing the human A_{2B}AR, Baltos
215 et al. (2017) found that the A₁AR agonist BAY68-4986 shows potent A_{2B}AR agonist activity in
216 stimulating cAMP accumulation, with an EC₅₀ of 1.1 nM. However, when tested in cAMP
217 accumulation in HEK293 cells endogenously expressing the A_{2B}AR, BAY68-4986 showed an EC₅₀ of
218 500 nM and E_{max} of 95% (Gao and Jacobson, unpublished data). Thus, for all nucleoside and non-
219 nucleoside A_{2B}AR agonists commercially available, only the partial agonist BAY60-6583 is A_{2B}AR
220 selective, which may show agonist activity in some signaling pathways, and antagonist properties in
221 other signaling events (Gao et al., 2014). Full agonists selective for A_{2B}AR are not yet available. Future
222 efforts could be the development of selective and full agonists for A_{2B}AR, in order to have a full range
223 of A_{2B}AR efficacies for studying cell proliferation, angiogenesis, metastasis and immune suppression.

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

227 heterocyclic selective A_{2B}AR antagonists, such as recently reported LAS101053 **25**, AB928 **26** and
228 ISAM140 **27** (Müller et al., 2018; Seitz et al., 2019). Commercially available A_{2B}AR antagonists as
229 pharmacological tools include 8-arylloxanthine derivatives MRS1754 **13**, MRS1706 **14**, GS6201 **18**, PSB-
230 1115 **21**, PSB-603 **22a** and PSB-0788 **23**. Recently, an alkylloxanthine with a picomolar affinity at the
231 human A_{2B}AR, PSB-1901 **22b**, was reported (Jiang et al., 2019). Antagonists that are in clinical trials
232 (AB928 **26**, PBF-1129 and theophylline **11**) will be discussed in Section 9.

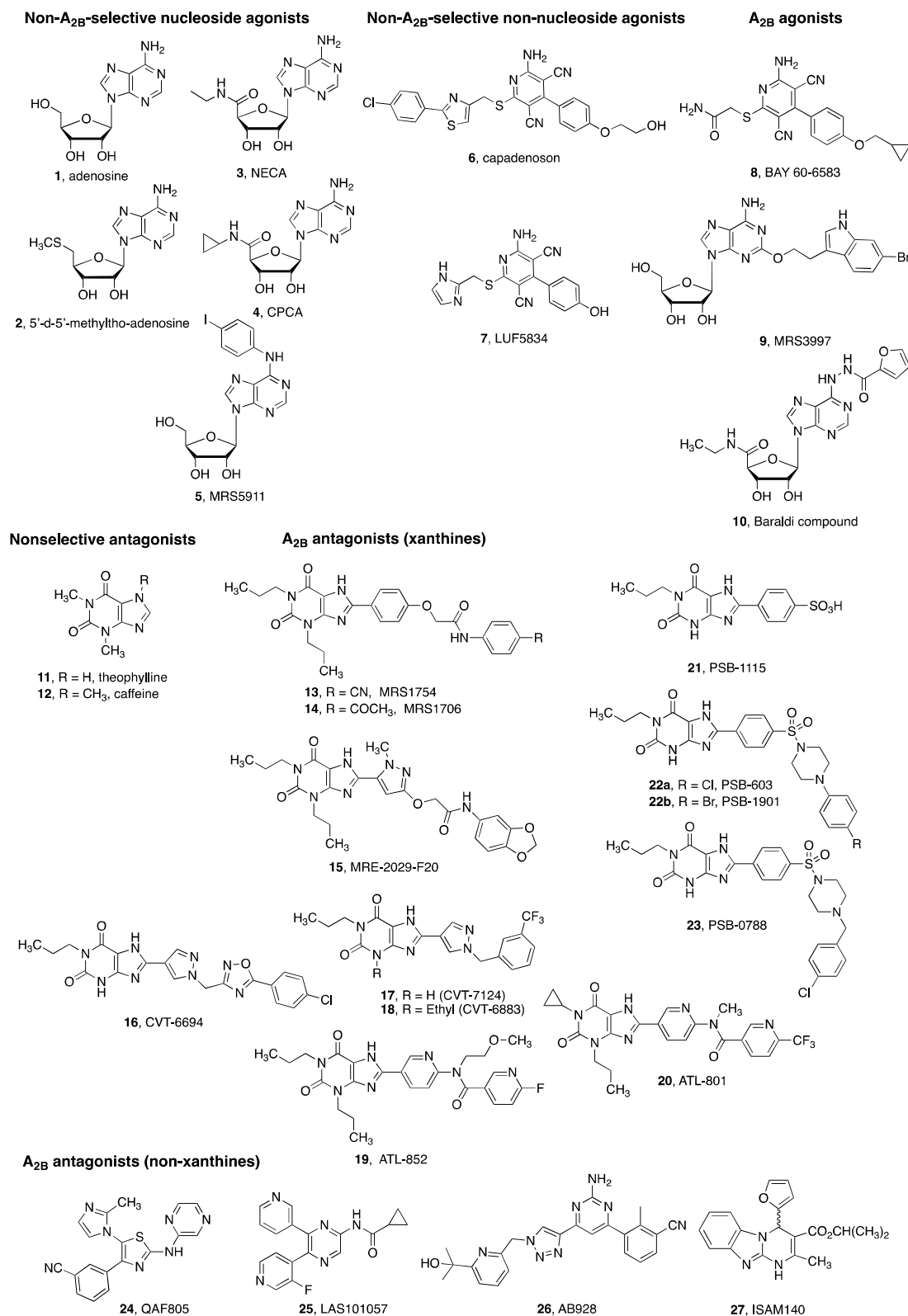
233

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

237 K_i (nM) or EC_{50} (E_{max} , %)

238 Compound	A_1	A_{2A}	A_{2B}	A_3	Reference
239 <i>Agonists</i>					
240 1, Adenosine ^a	310	700	24,000	290	Fredholm et al., 2001
241			4620 ^c (97%)		Gao et al., 2014
242 3, NECA ^b	14	20	1900	25	Alnouri et al., 2015
243 3, NECA ^a	12	60	104 (100%)	11	Beukers et al., 2014
244 4, CPCA	1.9 ^b	50 ^b	267 ^c (102%)	108 ^b	Gao et al., 2014
245 6, BAY68-4986 ^a	0.66	1400	1.1 (93%)	2400	Baltos et al., 2017
246 (Capadenoson)			522 ^{c,d} (95%)		
247 7, LUF5834	2.6 ^b	28 ^b	12 ^a (74%)	538 ^b	Beukers et al., 2004
248 8, BAY60-6583 ^b	390	>10,000	110	220	Alnouri et al., 2015
249			242 ^c (73%)		Gao et al., 2014
250			6.1 ^e (102%)		Gao et al., 2014
251 <i>Antagonists</i>					
252 11, Theophylline ^b	6200	1710	7850	22,300	Alnouri et al., 2015
253 12, Caffeine ^b	44,900	9560	33,800	13,300	Alnouri et al., 2015
254 13, MRS1754 ^b	403	503	1.7	570	Alnouri et al., 2015
255 14, MRS1706 ^b	157	112	1.4	230	Alnouri et al., 2015
256 18, GS6201 ^b (CVT-6833)	1940	3280	22	1070	Elzein et al., 2008
257 21, PSB-1115 ^b	>10,000	3790	53.4	>10,000	Alnouri et al., 2015
258 22a, PSB-603 ^b	>10,000	>10,000	0.55	>1000	Alnouri et al., 2015
259 22b, PSB-1901 ^b	>1000	>1000	0.060	>1000	Jiang et al., 2019
260 23, PSB-0788 ^b	2240	333	0.39	>1000	Alnouri et al., 2015
261 27, LAS101057 ^b	>10,000	2500	24	>10,000	Eastwood et al., 2011
262 26, AB928 ^b	64	1.5	2.0	489	Walters et al., 2017
263 27, ISAM140 ^b	>10,000	>10,000	0.55	>1000	El Maatougui et al., 2016
264 PBF-1129	nd	nd	nd	nd	

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



270

271

272

273

274

275

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

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

292 The results from A_{2B}AR blockade with antagonists were consistent with those from genetic
293 knockdown and KO of the A_{2B}AR in various animal models of solid tumors (Cekic et al.,
294 2012; Kasama et al., 2015; Ryzhov et al., 2008), further confirming the critical role of this receptor in
295 cancer cell proliferation and growth.

296 The specific mechanisms related to A_{2B}AR-mediated proliferation of various cancer cells and
297 growth of different types of tumors have not been extensively and systematically explored. As it has
298 been suggested that different agonists may bind in different modes and induce different A_{2B}AR
299 conformational changes (Thimm et al., 2013), together with the recent finding that A_{2B}AR may couple
300 variably to at least three G proteins in different cell types, it is possible that each agonist may activate
301 a particular mix of signaling cascades in a specific cell type, or the same agonist may activate different
302 signaling pathways in other cell types (Gao et al., 2018). Thus, the signaling mechanisms related to
303 A_{2B}AR-mediated cell proliferation may be diverse in different types of cancers. Nevertheless, multiple
304 studies have shown the importance of several signaling pathways related to A_{2B}AR activation and
305 the subsequent release of various cytokines and growth factors, which eventually led to cancer cell
306 proliferation. MAPK signaling is involved in multiple cellular processes and is often active in cancer
307 cells, promoting proliferation and metastasis (Loi et al., 2016). A_{2B}AR was demonstrated to couple to
308 all three types of MAPKs (Schulte and Fredholm, 2003), the extracellular signal-regulated kinases
309 (ERK1/2), the stress-activated protein kinases P38 and the c-jun N-terminal kinase (JNK). The cAMP-
310 EPAC pathway and ERK1/2 phosphorylation are known to be involved in A_{2B}AR-mediated
311 proliferation of some endothelial cells (Fang & Olah, 2007; Grant et al., 2001). Limm et al., (2014)
312 showed that PKC, but not cAMP or Ca²⁺, is involved in 5'-methylthioadenosine (2)-induced and
313 A_{2B}AR-mediated melanoma cell proliferation. Forskolin can mimic adenosine-induced proliferation
314 of MDA-MB-231 breast cancer cells, suggesting that Gs-cAMP signaling is involved, although it is
315 not clear whether PKA or EPAC is the downstream mediator. Recent evidence correlates the A_{2B}AR-
316 mediated cAMP/PKA and MAPK/ERK pathway activation with the epithelial-mesenchymal
317 transition in lung cancer cells (Giacomelli et al., 2018). A_{2B}AR has been shown to activate the PI3K-
318 Akt pathway (Schulte and Fredholm, 2010), which is known to induce cell proliferation and protects
319 against apoptosis in many cancer cell types. The A_{2B}AR-mediated PI3K-Akt pathway has been shown
320 to be critical for proliferation of glioblastoma stem cells (Liu et al., 2014). The importance of Akt
321 signaling in cell survival has been demonstrated in many cell types. However, it remains to be
322 investigated whether the A_{2B}AR-mediated PI3K-Akt pathway is downstream of G_{ai}, G_{as} or both.

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

324 A_{2B}AR activation plays a critical role in cell motility and migration, which are part of the multi-
325 step process of metastasis (Ntantie et al., 2013; Sepúlveda et al., 2016; Sun & Huang, 2016). Adenosine
326 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 G_s-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; Adisheshaiah 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.

352 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 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

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

382 8. A_{2B}AR and immunity

383 It has been well documented that cancer cells can escape from anti-tumor immune surveillance
384 especially under conditions with impaired immunity. Adenosine has demonstrated its role as an
385 important modulator of immune cell functions at least in part via its action at the A_{2B}AR (Cekic and
386 Linden, 2016; Hasko et al., 2009; Allard et al., 2016). A_{2B}AR activation is known to suppress IFN- γ -
387 enhanced expression of major histocompatibility complex class II (MHC-II) transactivator (Xaus et
388 al., 1999; Xu et al., 2008). In addition to the well-described roles of CD73 and CD39, adenosine
389 deaminase is known to control the local adenosine concentration, and this enzyme also binds to the
390 A_{2B}AR (Herrela et al., 2001). Adenosine deaminase deficiency is one of the serious immune diseases
391 which is due to the increased adenosine concentration and subsequently suppressed immune
392 responses. Thus, in addition to its direct effects on metastasis, proliferation and angiogenesis, the
393 A_{2B}AR can have a direct or an indirect role on cancer progression via modulation of the immune
394 system. The role of the A_{2B}AR in cell immunity was mostly neglected until recently partly due to
395 adenosine having a low A_{2B}AR affinity (Jacobson and Gao, 2006; Fredholm et al., 2011), although
396 early findings indicated that A_{2B}AR was the AR subtype responsible for the immune suppressive
397 function of T cells, macrophages and dendritic cells (Cekic and Linden 2016; Fredholm et al., 2011;
398 Hasko et al., 2009). Also, early work on CD26/DPP4 (dipeptidyl peptidase 4), a T cell surface antigen
399 that cleaves various bioactive peptides, mainly focused on its role in T cells (Dong and Morimoto,
400 1996; Morimoto and Schlossman, 1998) that highly express the A_{2A}AR (Hoskin et al., 2008; Kjaergaard
401 et al., 2018; Erdmann et al., 2005). More recently, in addition to CD39 and CD73, the importance of
402 A_{2B}AR and DPP4 in dendritic cells and macrophages also gained appreciation (Zhong et al., 2013).
403 DPP4 has been identified as one of the macrophage-related gene signatures predicative of increased
404 risk in gliomas (Sun et al. 2019). DPP4 inhibitor vildagliptin has been reported to suppress lung cancer
405 growth via a macrophage-mediated mechanism (Jang et al., 2019). Considering the increased
406 adenosine concentration and increased A_{2B}AR expression in the tumor microenvironment (Allard,
407 2016; Cekic and Linden, 2016, Sorrentino and Morello, 2017) together with the high expression levels
408 of both A_{2B}AR and DPP4 in macrophages and dendritic cells, growing evidence suggests a critical
409 role of A_{2B}AR together with CD39 and CD73 in modulating cancer progression at least in part via
410 immune suppression. Furthermore, DPP4 physically associates with adenosine deaminase, which
411 controls adenosine concentration and binds to the A_{2B}AR. Thus, A_{2B}AR blockade may enhance the
412 function of immune cells (Hasko et al., 2009; Cekic and Linden 2016; Allard et al., 2016).

413 A_{2A}AR has been shown to be critical in regulating TLR-induced cytokine production. However,
414 a recent study utilizing macrophages isolated from A_{2B}AR KO mice showed that adenosine elicits IL-
415 6 production from macrophages via the A_{2B}AR (Philip et al., 2017). IFN- γ upregulates A_{2B}AR
416 expression on macrophages resulting in an increased responsiveness of macrophages to the
417 stimulatory effects of NECA (Cohen et al., 2015). The pharmacologic inhibition or the genetic deletion
418 of the A_{2B}AR results in a hyperinflammatory response to TLR ligation, similar to IFN- γ treatment
419 of macrophages, suggesting the NECA-mediated effect is via A_{2B}AR, but not A_{2A}AR (Cohen et al.,
420 2015). The role of A_{2B}AR in regulating dendritic cell function has been defined using A_{2B}AR KO mice
421 and selective agonists and antagonists for A_{2B}AR (Cekic and Linden 2016; Hasko et al., 2009). In mice
422 bearing MB49 and/or 4T1 tumors, Cekic et al. (2012) demonstrated that selective blockade of A_{2B}AR
423 resulted in a CXCR3-dependent reduction of tumor growth and lung metastases from breast tumors
424 through enhancement of dendritic cell activation. Inhibition of A_{2B}AR activation by PSB-603 was
425 shown to suppress regulatory T cell (Treg) differentiation and IL-10 production, without affecting
426 effector T cell activation measured by IL-2 production and CD25 expression (Nakatsukasa and
427 Tsukimoto, 2011). A_{2B}AR was also suggested to modulate the phenotype of bone marrow-derived
428 dendritic cells. A_{2B}AR activation impairs MHC-II transcription in IFN- γ -stimulated cells (Fang et al.,

429 2013; Xia et al., 2015). MHC-II expression is required for CD4⁺ T cell anti-tumor responses, and loss
430 of MHC-II is associated with aggressiveness of colorectal cancer and decreased levels of tumor-
431 infiltrating lymphocytes (Warabi et al., 2000). Shi et al. (2006) also reported that both major MHC-II
432 transactivator (CIITA) and MHC-II are decreased in highly metastatic cancer cells. Thus, A_{2B}AR
433 blockade has a potential to enhance anti-tumor immunity in cancers where tumor-infiltrating
434 lymphocytes and MHC-II levels are decreased.

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

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

454 As described above, A_{2B}AR activation induces tumor proliferation, growth of solid tumor, tumor
455 angiogenesis, tumor cell invasion and metastasis, and immune suppression. Thus, A_{2B}AR blockade
456 holds great promise as an anti-cancer therapy. For example, A_{2B}AR inhibition by the antagonist PSB-
457 1115 was shown to decrease tumor metastasis of CD73⁺ melanoma cells and mammary carcinoma
458 cells (Mittal et al., 2016). Iannone et al. (2013) observed that PSB-1115 delayed tumor growth and
459 enhanced the anti-tumor activity of dacarbazine, a drug currently used in metastatic melanoma.

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

465 Antagonists in clinical trials for cancer patients (ClinicalTrials.gov NCT Identifier) include the
466 mixed A_{2A}AR/A_{2B}AR antagonist AB928 26 (Phase 1, lung cancer, 03846310; Phase 1, breast and
467 ovarian cancer, 03719326; Phase 1, gastrointestinal cancer, 03720678; Phase 1, advanced cancer,
468 03629756), PBF-1129 (structure not disclosed; Phase 1, non-small cell lung cancer, 03274479) and
469 theophylline 11 (see below). The first dual-acting A_{2A}AR/A_{2B}AR antagonist AB928 is being tested
470 clinically in multiple arms in combination with pegylated liposomal doxorubicin, nanoparticle
471 albumin-bound paclitaxel, or a PI3K- γ inhibitor. AB928 has exhibited excellent safety, PK, and PD
472 profiles in a Phase 1 clinical trial in healthy volunteers and is currently being evaluated in patients
473 with non-small cell lung cancer, breast cancer, ovarian cancer, colorectal and six other types of cancers
474 (clinicaltrials.gov). One of the cancer immunotherapy drugs, AB122, a fully human immunoglobulin
475 G4 monoclonal antibody targeting human programmed cell death protein 1 (PD-1), will be tried in
476 combination with AB928. AB928 was able to produce maximal AR blockade assessed as a function of
477 NECA-stimulated pCREB induction in peripheral blood CD8⁺ T cells (Seitz et al., 2019). AB928 was
478 shown to relieve adenosine-mediated immune suppression (Waters et al., 2018). Combining AR
479 inhibition with AB928 and chemotherapy results in greater immune activation and tumor control.

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

483 Theophylline is a nonselective AR antagonist, which was tested for anticancer efficacy in two
484 previous clinical trials (incidentally, as an inhibitor of intracellular cAMP in chronic lymphocytic
485 leukemia, Phase 2, 00003808; a withdrawn trial in combination with an allogeneic tumor cell-vaccine
486 (gp96-Ig vaccine) and oxygen therapy, which lowers adenosine levels (Hatfield and Sitkovsky, 2016),
487 in non-small cell lung cancer, Phase 1, 01799161). The first theophylline trial description did not even
488 reference AR antagonism, but there was a correlation found between in vitro apoptosis in leukemia
489 cells and clinical response in a subset of patients (Wiernik et al., 2004). Theophylline in combination
490 prednisone and dextromethorphan has also been in a clinical trial (Phase 1, 01017939) for patients
491 with metastatic castration-resistant prostate cancer. Aminophylline, a salt of theophylline, in
492 combination with Bacillus Calmette-Guerin has been in a trial (early Phase 1, 01240824) for patients
493 with bladder cancer. However, it should be noted that theophylline is nonselective and may block all
494 four ARs.
495

496 10. Summary

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

508 **Acknowledgments:** We thank the NIDDK Intramural Research Program for support (ZIADK031117).

509 **Author Contributions:** ZGG, wrote manuscript; KAJ, wrote manuscript.

510 **Conflicts of Interest:** none.

511 References

- 512 1. Adair TH. Growth regulation of the vascular system: an emerging role for adenosine. *Am J Physiol*
513 *Regul Integr Comp Physiol.* **2005**, *289*, R283-R296.
- 514 2. Adisheshaiah P, Lindner DJ, Kalvakolanu DV, Reddy SP. FRA-1 proto-oncogene induces lung
515 epithelial cell invasion and anchorage-independent growth in vitro, but is insufficient to promote
516 tumor growth in vivo. *Cancer Res.* **2007**, *67*, 6204-11.
- 517 3. Allard B, Beavis PA, Darcy PK, Stagg J. Immunosuppressive activities of adenosine in cancer. *Curr*
518 *Opin Pharmacol.* **2016**, *29*, 7-16.
- 519 4. Alnouri MW, Jepards S, Casari A, Schiedel AC, Hinz S, Müller CE. Selectivity is species-dependent:
520 Characterization of standard agonists and antagonists at human, rat, and mouse adenosine receptors.
521 *Purinergic Signal.* **2015**, *11*, 389-407.
- 522
523
524

525

- 526 5. Baltos JA, Vecchio EA, Harris MA, Qin CX, Ritchie RH, Christopoulos A, White PJ, May LT.
527 Capadenoson, a clinically trialed partial adenosine A₁ receptor agonist, can stimulate adenosine
528 A_{2B} receptor biased agonism. *Biochem Pharmacol.* **2017**, *135*, 79-89.
529
- 530 6. Barresi E, Giacomelli C, Daniele S, Tonazzini I, Robello M, Salerno S, Piano I, Cosimelli B, Greco G, Da
531 Settimo F, Martini C, Trincavelli ML, Taliani S. Novel fluorescent triazinobenzimidazole derivatives
532 as probes for labelling human A₁ and A_{2B} adenosine receptor subtypes. *Bioorg Med Chem.* **2018**, *26*,
533 5885-95.
- 534 7. Beukers MW, Chang LC, von Frijtag Drabbe Künzel JK, Mulder-Krieger T, Spanjersberg RF, Brussee J,
535 IJzerman AP. New, non-adenosine, high-potency agonists for the human adenosine A_{2B} receptor with
536 an improved selectivity profile compared to the reference agonist N-ethylcarboxamidoadenosine. *J*
537 *Med Chem.* **2004**, *47*, 3707-9.
538
- 539 8. Beavis PA, Divisekera U, Paget C, Chow MT, John LB, Devaud C, Dwyer K, Stagg J, Smyth MJ, Darcy
540 PK. Blockade of A_{2A} receptors potently suppresses the metastasis of CD73+ tumors. *Proc Natl Acad*
541 *Sci U S A.* **2013**, *110*, 14711-6.
542
- 543 9. Belguise K, Kersual N, Galtier F, Chalbos D. FRA-1 expression level regulates proliferation and
544 invasiveness of breast cancer cells. *Oncogene* **2005**, *24*, 1434-44.
545
- 546 10. Borea PA, Gessi S, Merighi S, Varani K. Adenosine as a Multi-Signalling Guardian Angel in Human
547 Diseases: When, Where and How Does it Exert its Protective Effects? *Trends Pharmacol Sci.* **2016**, *37*,
548 419-434.
549
- 550 11. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of Adenosine Receptors: The State
551 of the Art. *Physiol Rev.* **2018**, *98*, 1591-1625.
552
- 553 12. Burnstock G, Di Virgilio F. Purinergic signalling and cancer. *Purinergic Signal.* **2013**, *9*, 491-540.
554
- 555 13. Cekic C, Sag D, Li Y, Theodorescu D, Strieter RM, Linden J. Adenosine A_{2B} receptor blockade slows
556 growth of bladder and breast tumors. *J Immunol.* **2012**, *188*, 198-205.
557
- 558 14. Cekic C, Linden J. Adenosine A_{2A} receptors intrinsically regulate CD8+ T cells in the tumor
559 microenvironment. *Cancer Res.* **2014**, *74*, 7239-49.
560
- 561 15. Cekic C, Linden J. Purinergic regulation of the immune system. *Nat Rev Immunol.* **2016**, *16*, 177-92.
562
- 563 16. Clark AN, Youkey R, Liu X, Jia L, Blatt R, Day YJ, Sullivan GW, Linden J, Tucker AL. A₁ adenosine
564 receptor activation promotes angiogenesis and release of VEGF from monocytes. *Circ Res.* **2007**, *101*,
565 1130-8.
566
- 567 17. Cohen HB, Ward A, Hamidzadeh K, Ravid K, Mosser DM. IFN- γ Prevents Adenosine Receptor
568 (A_{2B}R) Upregulation to Sustain the Macrophage Activation Response. *J Immunol.* **2015**, *195*, 3828-37.
569
- 570
- 571

- 572 18. Cohen S, Fishman P. Targeting the A₃ adenosine receptor to treat cytokine release syndrome in cancer
573 immunotherapy. *Drug Des Devel Ther.* **2019**, *13*, 491-97.
- 574
575 19. Daly, J. W., Butts-Lamb, P., Padgett, W. Subclasses of adenosine receptors in the central nervous
576 system: interaction with caffeine and related methylxanthines. *Cell Mol Neurobiol*, **1983**, *3*, 69-80.
- 577
578 20. Desmet CJ, Gallenne T, Prieur A, Reyal F, Visser NL, Wittner BS, Smit MA, Geiger TR, Laoukili J, Iskit
579 S, Rodenko B, Zwart W, Evers B, Horlings H, Ajouaou A, Zevenhoven J, van Vliet M, Ramaswamy
580 S, Wessels LF, Peeper DS. Identification of a pharmacologically tractable Fra-1/ADORA2B axis
581 promoting breast cancer metastasis. *Proc Natl Acad Sci U S A.* **2013**, *110*, 5139-44.
- 582
583 21. Dixon AK, Gubitz AK, Sirinathsinghi DJ, Richardson PJ, Freeman TC. Tissue distribution of
584 adenosine receptor mRNAs in the rat. *Br J Pharmacol.* 1996, *118*, 1461-8.
- 585
586 22. Dong RP, Kameoka J, Hegen M, Tanaka T, Xu Y, Schlossman SF, Morimoto C. Characterization of
587 adenosine deaminase binding to human CD26 on T cells and its biologic role in immune response. *J*
588 *Immunol* **1996**, *156*, 1349-55.
- 589
590 23. Du X, Ou X, Song T, Zhang W, Cong F, Zhang S, Xiong Y. Adenosine A_{2B} receptor stimulates
591 angiogenesis by inducing VEGF and eNOS in human microvascular endothelial cells. *Exp Biol Med*
592 (Maywood). **2015**, *240*, 1472-9.
- 593
594 24. Drury AN, Szent-Györgyi A. The physiological activity of adenine compounds with especial
595 reference to their action upon the mammalian heart. *J Physiol.* **1929**, *68*, 213-37.
- 596
597 25. Eastwood P, Esteve C, González J, Fonquerna S, Aiguadé J, Carranco I, Doménech T, Aparici M,
598 Miralpeix M, Albertí J, Córdoba M, Fernández R, Pont M, Godessart N, Prats N, Loza MI, Cadavid
599 MI, Nueda A, Vidal B. Discovery of LAS101057: A potent, selective, and orally efficacious A_{2B}
600 adenosine receptor antagonist. *ACS Med Chem Lett.* **2011**, *2*, 213-8.
- 601
602 26. El Maatougui A, Azuaje J, González-Gómez M, Miguez G, Crespo A, Carbajales C, Escalante
603 L, García-Mera X, Gutiérrez-de-Terán H, Sotelo E. Discovery of potent and highly selective
604 A_{2B} adenosine receptor antagonist chemotypes. *J Med Chem.* **2016**, *59*, 1967- 83.
- 605
606 27. Erdmann AA, Gao ZG, Jung U, Foley J, Borenstein T, Jacobson KA, Fowler DH. Activation of Th1 and
607 Tc1 cell adenosine A_{2A} receptors directly inhibits IL-2 secretion in vitro and IL-2-driven expansion in
608 vivo. *Blood.* **2005**, *105*, 4707-14.
- 609
610 28. Elzein E, Kalla RV, Li X, Perry T, Gimbel A, Zeng D, Lustig D, Leung K, Zablocki J. Discovery of a
611 novel A_{2B} adenosine receptor antagonist as a clinical candidate for chronic inflammatory airway
612 diseases. *J Med Chem.* **2008**, *51*, 2267-78.
- 613
614 29. Fredholm BB, Irenius E, Kull B, Schulte G. Comparison of the potency of adenosine as an agonist at
615 human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem Pharmacol.* **2001**, *61*,
616 443-8.
- 617
618 30. Fang Y, Olah ME. Cyclic AMP-dependent, protein kinase A-independent activation of extracellular
619 signal-regulated kinase 1/2 following adenosine receptor stimulation in human umbilical vein

- 620 endothelial cells: role of exchange protein activated by cAMP 1 (Epac1). *J Pharmacol Exp Ther.* **2007**,
621 322, 1189-200.
- 622
- 623 31. Fang M, Xia J, Wu X, Kong H, Wang H, Xie W, Xu Y. Adenosine signaling inhibits CIITA-mediated
624 MHC class II transactivation in lung fibroblast cells. *Eur J Immunol.* **2013**, *43*, 2162-73.
- 625
- 626 32. Feoktistov I, Goldstein AE, Ryzhov S, Zeng D, Belardinelli L, Voyno-Yasenetskaya T, Biaggioni I.
627 Differential expression of adenosine receptors in human endothelial cells: role of A_{2B} receptors in
628 angiogenic factor regulation. *Circ Res.* **2002**, *90*, 531-8.
- 629
- 630 33. Feoktistov, I. Feoktistov I, Ryzhov S, Zhong H, Goldstein AE, Matafonov A, Zeng D, Biaggioni I.
631 Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A_{2B}
632 angiogenic phenotype. *Hypertension* **2004**, *44*, 649-654
- 633
- 634 34. Fernandez-Gallardo M, González-Ramírez R, Sandoval A, Felix R, Monjaraz E. Adenosine Stimulate
635 Proliferation and Migration in Triple Negative Breast Cancer Cells. *PLoS One.* **2016**, *11*, e0167445. doi:
636 10.1371/journal.pone.0167445.
- 637
- 638 35. Fiebich BL, Biber K, Gyufko K, Berger M, Bauer J, van Calker D. Adenosine A_{2B} receptors mediate an
639 increase in interleukin (IL)-6 mRNA and IL-6 protein synthesis in human astrogloma cells. *J*
640 *Neurochem.* **1996**, *66*, 1426-31.
- 641
- 642 36. Figueiredo AB, Souza-Testasica MC, Mineo TWP, Afonso LCC. *Leishmania amazonensis*-Induced
643 cAMP Triggered by Adenosine A_{2B} Receptor Is Important to Inhibit Dendritic Cell Activation and
644 Evade Immune Response in Infected Mice. *Front Immunol.* **2017**, *8*, 849. doi:
645 10.3389/fimmu.2017.00849.
- 646
- 647 37. Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE. International Union of Basic and
648 Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update.
649 *Pharmacol Rev.* **2011**, *63*, 1-34.
- 650
- 651 38. Gao Z, Chen T, Weber MJ, Linden J. A_{2B} adenosine and P2Y₂ receptors stimulate mitogen-activated
652 protein kinase in human embryonic kidney-293 cells. cross-talk between cyclic AMP and protein
653 kinase c pathways. *J Biol Chem.* **1999**, *274*, 5972-80.
- 654
- 655 39. Gao ZG, Balasubramanian R, Kiselev E, Wei Q, Jacobson KA. Probing biased/partial agonism at the G
656 protein-coupled A_{2B} adenosine receptor. *Biochem Pharmacol.* **2014**, *90*, 297-306.
- 657
- 658 40. Gao ZG, Inoue A, Jacobson KA. On the G protein-coupling selectivity of the native A_{2B} adenosine
659 receptor. *Biochem Pharmacol.* **2018**, *151*, 201-13.
- 660
- 661 41. Giacomelli C, Daniele S, Romei C, Tavanti L, Neri T, Piano I, Celi A, Martini C, Trincavelli ML. The
662 A_{2B} adenosine receptor modulates the epithelial- mesenchymal transition through the balance of
663 cAMP/PKA and MAPK/ERK pathway activation in human epithelial lung cells. *Front Pharmacol.*
664 **2018**, *9*, 54. doi: 10.3389/fphar.2018.00054
- 665

- 666 42. Gorain B, Choudhury H, Yee GS, Bhattamisra SK. Adenosine receptors as novel targets for the
667 treatment of various cancers. *Curr Pharm Des.* **2019** Jul 15. doi: 10.2174/1381612825666190716102037.
668 [Epub ahead of print]
- 669 43. Grant MB, Davis MI, Caballero S, Feoktistov I, Biaggioni I, Belardinelli L. Proliferation, migration, and
670 ERK activation in human retinal endothelial cells through A_{2B} adenosine receptor stimulation. *Invest*
671 *Ophthalmol Vis Sci.* **2001**, *42*, 2068-73.
672
- 673 44. Gu JW, Ito BR, Sartin A, Frascogna N, Moore M, Adair TH. Inhibition of adenosine kinase induces
674 expression of VEGF mRNA and protein in myocardial myoblasts. *Am J Physiol Heart Circ*
675 *Physiol.* **2000**, *279*, H2116-23.
676
- 677 45. Haskó G, Csóka B, Németh ZH, Vizi ES, Pacher P. A_{2B} adenosine receptors in immunity and
678 inflammation. *Trends Immunol.* **2009**, *30*, 263-70.
679
- 680 46. Hatfield SM, Sitkovsky M. A_{2A} adenosine receptor antagonists to weaken the hypoxia-HIF-1 α driven
681 immunosuppression and improve immunotherapies of cancer. *Curr Opin Pharmacol.* **2016**, *29*, 90-6.
682 <https://doi.org/https://doi.org/10.1016/j.coph.2016.06.009>
683
- 684 47. Herrera C, Casadó V, Ciruela F, Schofield P, Mallol J, Lluís C, Franco R. Adenosine A_{2B} receptors
685 behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and
686 cultured cells. *Mol Pharmacol.* **2001**, *59*, 127-34.
687
- 688 48. Hinz S, Lacher SK, Seibt BF, Müller CE. BAY60-6583 acts as a partial agonist at
689 adenosine A_{2B} receptors. *J Pharmacol Exp Ther.* **2014**, *349*, 427-36.
690
- 691 49. Hinz S, Alnouri WM, Pleiss U, Müller CE. Tritium-labeled agonists as tools for studying adenosine
692 A_{2B} receptors. *Purinergic Signal.* **2018**, *14*, 223-33.
693
- 694 50. Horigome E, Fujieda M, Handa T, Katayama A, Ito M, Ichihara A, Tanaka D, Gombodorj
695 N, Yoshiyama S, Yamane A, Yamada K, Horiguchi J, Shinozuka K, Oyama T, Nishiyama M, Rokudai
696 S. Mutant TP53 modulates metastasis of triple negative breast cancer through adenosine A_{2B} receptor
697 signaling. *Oncotarget* **2018**, *9*, 34554-66.
698
- 699 51. Hoskin DW, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and natural killer cell
700 function by adenosine and its contribution to immune evasion by tumor cells (Review). *Int J*
701 *Oncol.* **2008**, *32*, 527-35.
702
- 703 52. Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Blockade of A_{2B} adenosine receptor reduces
704 tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse
705 model of melanoma. *Neoplasia.* **2013**, *15*, 1400-9.
706
- 707 53. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov.* **2006**, *5*, 247-
708 64.
709
- 710 54. Jang JH, Janker F, De Meester I, Arni S, Borgeaud N, Yamada Y, Gil Bazo I, Weder W, Jungraithmayr
711 W. The CD26/DPP4-inhibitor vildagliptin suppresses lung cancer growth via macrophage-mediated
712 NK cell activity. *Carcinogenesis* **2019**, *40*, 324-34.
713

- 714
715 55. Jiang J, Seel CJ, Temirak A, Namasivayam V, Arridu A, Schabikowski J, Baqi Y, Hinz S, Hockemeyer
716 J, Müller CE. A_{2B} adenosine receptor antagonists with picomolar potency. *J Med Chem.* **2019**, *62*,
717 4032–55.
- 718
719 56. Jing L, Tamplin OJ, Chen MJ, Deng Q, Patterson S, Kim PG, Durand EM, McNeil A, Green
720 JM, Matsuura S, Ablain J, Brandt MK, Schlaeger TM, Huttenlocher A, Daley GQ, Ravid K, Zon LI.
721 Adenosine signaling promotes hematopoietic stem and progenitor cell emergence. *J Exp Med.* **2015**,
722 *212*, 649-63.
- 723
724 57. Kalhan A, Gharibi B, Vazquez M, Jasani B, Neal J, Kidd M, Modlin IM, Pfragner R, Rees DA, Ham J.
725 Adenosine A_{2A} and A_{2B} receptor expression in neuroendocrine tumours: potential targets for therapy.
726 *Purinergic Signal.* **2012**, *8*, 265-74.
- 727
728 58. Kasama H, Sakamoto Y, Kasamatsu A, Okamoto A, Koyama T, Minakawa Y, Ogawara K, Yokoe
729 H, Shiiba M, Tanzawa H, Uzawa K. Adenosine A_{2B} receptor promotes progression of human
730 oral cancer. *Clin Cancer Res.* **2016**, *22*, 158-66.
- 731
732 59. Kim YC, Ji XD, Melman N, Linden J, Jacobson KA. Anilide derivatives of an 8-phenylxanthine
733 carboxylic congener are highly potent and selective antagonists at human A_{2B} adenosine receptors. *J*
734 *Med Chem.* **2000**, *43*, 1165-72.
- 735
736 60. Kjaergaard J, Hatfield S, Jones G, Ohta A, Sitkovsky M. A_{2A} Adenosine Receptor Gene Deletion or
737 Synthetic A_{2A} Antagonist Liberate Tumor-Reactive CD8⁺ T Cells from Tumor-Induced
738 Immunosuppression. *J Immunol.* **2018**, *201*, 782-91.
- 739
740 61. Köse M, Gollos S, Karcz T, Fiene A, Heisig F, Behrenswerth A, Kieć-Kononowicz K, Namasivayam V,
741 Müller CE. Fluorescent-Labeled Selective Adenosine A_{2B} Receptor Antagonist Enables Competition
742 Binding Assay by Flow Cytometry. *J Med Chem.* **2018**, *61*, 4301–16.
- 743
744 62. Koszałka P, Gołuńska M, Urban A, Stasiłojć G, Stanisławowski M, Majewski M, Składanowski
745 AC, Bigda J. Specific Activation of A₃, A_{2A} and A₁ Adenosine Receptors in CD73-Knockout Mice
746 Affects B16F10 Melanoma Growth, Neovascularization, Angiogenesis and Macrophage Infiltration.
747 *PLoS One.* **2016**, *11*(3):e0151420. doi: 10.1371/journal.pone.0151420.
- 748
749 63. Koussémou M, Lorenz K, Klotz KN. The A_{2B} adenosine receptor in MDA-MB-231 breast cancer cells
750 diminishes ERK1/2 phosphorylation by activation of MAPK-phosphatase-1. *PLoS One* **2018**,
751 *13*:e0202914. doi: 10.1371/journal.pone.0202914.
- 752
753 64. Lan J, Lu H, Samanta D, Salman S, Lu Y, Semenza GL. Hypoxia-inducible factor 1-dependent
754 expression of adenosine receptor 2B promotes breast cancer stem cell enrichment. *Proc Natl Acad Sci*
755 *U S A.* **2018**, *115*, E9640-8.
- 756
757 65. Leclerc BG, Charlebois R, Chouinard G, Allard B, Pommey S, Saad F, Stagg J. CD73
758 Expression Is an Independent Prognostic Factor in Prostate Cancer. *BMC Cancer* **2015**, *15*, 563. doi:
759 10.1186/s12885-015-1577-2.
- 760

- 761 66. Leibovich SJ, Chen JF, Pinhal-Enfield G, Belem PC, Elson G, Rosania A, Ramanathan M, Montesinos
762 C, Jacobson M, Schwarzschild MA, Fink JS, Cronstein B. Synergistic up-regulation of vascular
763 endothelial growth factor expression in murine macrophages by adenosine A_{2A} receptor agonists and
764 endotoxin. *Am J Pathol.* **2002**, *160*, 2231-44.
- 765
766 67. Li S, Huang S, Peng SB. Overexpression of G protein-coupled receptors in cancer cells: involvement in
767 tumor progression. *Int J Oncol.* **2005**, *27*, 1329-39.
- 768
769 68. Lim B, Jung K, Gwon Y, Oh JG, Roh JI, Hong SH, Kho C, Park WJ, Lee HW, Bae JW, Jung YK.
770 Cardioprotective role of A_{2B} in myocardial infarction through ADORA2B. *Cell Death Dis.* **2019**,
771 *10*(7), 511. doi: 10.1038/s41419-019-1746-3.
- 772
773 69. Limm K, Wallner S, Milenkovic VM, Wetzel CH, Bosserhoff AK. The metabolite 5'-
774 methylthioadenosine signals through the adenosine receptor A_{2B} in melanoma. *Eur J Cancer* **2014**, *50*,
775 2714-24.
- 776
777 70. Lindemann M, Hinz S, Deuther-Conrad W, Namasivayam V, Dukic-Stefanovic S, Teodoro R,
778 Toussaint M, Kranz M, Juhl C, Steinbach J, Brust P, Müller CE, Wenzel B. Radiosynthesis and in vivo
779 evaluation of a fluorine-18 labeled pyrazine based radioligand for PET imaging of the adenosine A_{2B}
780 receptor. *Bioorg Med Chem.* **2018**, *26*, 4650-63.
- 781
782 71. Loi S, Dushyanthen S, Beavis PA, Salgado R, Denkert C, Savas P, Combs S, Rimm DL, Giltnane JM,
783 Estrada MV, Sánchez V, Sanders ME, Cook RS, Pilkinton MA, Mallal SA, Wang K, Miller VA,
784 Stephens PJ, Yelensky R, Doimi FD, Gómez H, Ryzhov SV, Darcy PK, Arteaga CL, Balko JM.
785 RAS/MAPK Activation Is Associated with Reduced Tumor-Infiltrating Lymphocytes in Triple-
786 Negative Breast Cancer: Therapeutic Cooperation Between MEK and PD-1/PD-L1 Immune
787 Checkpoint Inhibitors. *Clin Cancer Res.* **2019**, *25*, 1437. doi: 10.1158/1078-0432.CCR-18-4264.
- 788
789 72. Ma DF, Kondo T, Nakazawa T, Niu DF, Mochizuki K, Kawasaki T, Yamane T, Katoh R. Hypoxia-
790 inducible adenosine A_{2B} receptor modulates proliferation of colon carcinoma cells. *Hum Pathol.* **2010**,
791 *41*, 1550-7.
- 792
793 73. Marwein S, Mishra B, Chandra De U, Acharya PC. Recent progress of adenosine receptor modulators
794 in the development of anticancer chemotherapeutic agents. *Curr Pharm Des.* **2019**, Jul 16. doi:
795 10.2174/1381612825666190716141851. [Epub ahead of print]
- 796
797 74. Merighi S, Simioni C, Gessi S, Varani K, Mirandola P, Tabrizi MA, Baraldi PG, Borea PA. A_{2B} and A₃
798 adenosine receptors modulate vascular endothelial growth factor and interleukin-8 expression in
799 human melanoma cells treated with etoposide and doxorubicin. *Neoplasia* **2009**, *11*, 1064-73.
- 800
801 75. Mittal D, Sinha D, Barkauskas D, Young A, Kalimutho M, Stannard K, Caramia F, Haibe-Kains
802 B, Stagg J, Khanna KK, Loi S, Smyth MJ. Adenosine 2B Receptor Expression on Cancer Cells Promotes
803 Metastasis. *Cancer Res.* **2016**, *76*, 4372-82.
- 804
805 76. Morello S, Miele L. Targeting the adenosine A_{2B} receptor in the tumor microenvironment overcomes
806 local immunosuppression by myeloid-derived suppressor cells. *Oncoimmunology* **2014**, *3*:e27989.
- 807

- 808 77. Morimoto C, Schlossman SF. The structure and function of CD26 in the T-cell immune response.
809 *Immunol Rev.* **1998**, *161*, 55-70.
- 810
811 78. Müller CE, Baqi Y, Hinz S, Namasivayam V. Chapter 6, Medicinal chemistry of A_{2B} adenosine
812 receptors, In: *The Adenosine Receptors, The Receptors*, Borea, P.A. et al. (ed.). Springer, **2018**, *34*, 137-
813 168.
- 814
815 79. Nakatsukasa H, Tsukimoto M, Harada H, Kojima S. Adenosine A_{2B} receptor antagonist suppresses
816 differentiation to regulatory T cells without suppressing activation of T cells. *Biochem Biophys Res*
817 *Commun.* **2011**, *409*, 114-9.
- 818
819 80. Neumann CA, Levine K, Oesterreich S. Targeting adenosine receptor 2B in triple negative breast
820 cancer. *J Cancer Metastasis Treat.* **2018**, *4*, 13. <http://dx.doi.org/10.20517/2394-4722.2017.60>
- 821
822 81. Ni Y, Liang D, Tian Y, Kron IL, French BA, Yang Z. Infarct-Sparing Effect of Adenosine A_{2B} Receptor
823 Agonist Is Primarily Due to Its Action on Splenic Leukocytes Via a PI3K/Akt/IL-10 Pathway. *J Surg*
824 *Res.* **2018**, *232*, 442-9.
- 825
826 82. Nonaka H, Ichimura M, Takeda M, Nonaka Y, Shimada J, Suzuki F, Yamaguchi K, Kase H. [KF17837](#)
827 [\(\(E\)-8-\(3,4-dimethoxystyryl\)-1,3-dipropyl-7-methylxanthine\)](#), a potent and selective adenosine A₂
828 [receptor antagonist](#). *Eur J Pharmacol.* **1994**, *267*, 335-41.
- 829
830 83. Novitskiy SV, Ryzhov S, Zaynagetdinov R, Goldstein AE, Huang Y, Tikhomirov OY, Blackburn MR,
831 Biaggioni I, Carbone DP, Feoktistov I, Dikov MM. Adenosine receptors in regulation of dendritic cell
832 differentiation and function. *Blood* **2008**, *112*, 1822-31.
- 833
834 84. Ntantie E, Gonyo P, Lorimer EL, Hauser AD, Schuld N, McAllister D, Kalyanaraman B, Dwinell
835 MB, Auchampach JA, Williams CL. An adenosine-mediated signaling pathway suppresses
836 prenylation of the GTPase Rap1B and promotes cell scattering. *Sci Signal.* **2013**, *6(277)*:ra39. doi:
837 10.1126/scisignal.2003374.
- 838
839 85. Ou Y, Chan G, Zuo J, Rattner JB, van der Hoorn FA. Purinergic A_{2b} Receptor Activation by
840 Extracellular Cues Affects Positioning of the Centrosome and Nucleus and Causes Reduced Cell
841 Migration. *J Biol Chem.* **2016**, *291*, 15388-403.
- 842
843 86. Pierce KD, Furlong TJ, Selbie LA, Shine J. Molecular cloning and expression of an adenosine A_{2b}
844 receptor from human brain. *Biochem Biophys Res Commun.* **1992**, *187*, 86-93.
- 845
846 87. Phosri S, Arieayawong A, Bunrukchai K, Parichatikanond W, Nishimura A, Nishida M, Mangmool S.
847 Stimulation of Adenosine A_{2B} Receptor Inhibits Endothelin-1-Induced Cardiac Fibroblast Proliferation
848 and α -Smooth Muscle Actin Synthesis Through the cAMP/Epac/PI3K/Akt-Signaling Pathway. *Front*
849 *Pharmacol* **2017**, *8*, 428. doi: 10.3389/fphar.2017.00428.
- 850
851 88. Philip K, Mills TW, Davies J, Chen NY, Karmouty-Quintana H, Luo F, Molina JG, Amione-Guerra
852 J, Sinha N, Guha A, Eltzschig HK, Blackburn MR. HIF1A up-regulates the ADORA2B receptor on
853 alternatively activated macrophages and contributes to pulmonary fibrosis. *FASEB J.* **2017**, *31*, 4745-8.
- 854

- 855 89. Ramakers BP, Riksen NP, van der Hoeven JG, Smits P, Pickkers P. Modulation of innate immunity by
856 adenosine receptor stimulation. *Shock* **2011**, *36*, 208-15.
- 857
858 90. Rivkees SA, Reppert SM. RFL9 encodes an A_{2b}-adenosine receptor. *Mol Endocrinol.* **1992**, *6*, 1598-604.
- 859
860 91. Rodrigues S, De Wever O, Bruyneel E, Rooney RJ, Gespach C. Opposing roles of netrin-1 and the
861 dependence receptor DCC in cancer cell invasion, tumor growth and metastasis. *Oncogene* **2007**, *26*,
862 5615-25.
- 863
864 92. Ryzhov S, Novitskiy SV, Zaynagetdinov R, Goldstein AE, Carbone DP, Biaggioni I, Dikov
865 MM, Feoktistov I. Host A_{2B} adenosine receptors promote carcinoma growth. *Neoplasia* **2008**, *10*, 987-
866 95.
- 867
868 93. Ryzhov S, Biktasova A, Goldstein AE, Zhang Q, Biaggioni I, Dikov MM, Feoktistov I. Role of JunB in
869 adenosine A_{2B} receptor-mediated vascular endothelial growth factor production. *Mol*
870 *Pharmacol.* **2014**, *85*, 62-73.
- 871
872 94. Schulte G, Fredholm BB. The G_s-coupled adenosine A_{2B} receptor recruits divergent pathways to
873 regulate ERK1/2 and p38. *Exp Cell Res.* **2003**, *290*, 168-76.
- 874
875 95. Sciaraffia E, Riccomi A, Lindstedt R, Gesa V, Cirelli E, Patrizio M, De Magistris MT, Vendetti S.
876 Human monocytes respond to extracellular cAMP through A_{2A} and A_{2B} adenosine receptors. *J Leukoc*
877 *Biol.* **2014**, *96*, 113-22.
- 878
879 96. Seitz L, Jin L, Leleti M, Ashok D, Jeffrey J, Rieger A, Tiessen RG, Arold G, Tan JBL, Powers JP, Walters
880 MJ, Karakunnel J. Safety, tolerability, and pharmacology of AB928, a novel dual adenosine receptor
881 antagonist, in a randomized, phase 1 study in healthy volunteers. *Invest New Drugs* **2019**, *37*, 711-21.
- 882
883 97. Shen Y, Tang G, Gao P, Zhang B, Xiao H, Si LY. Activation of adenosine A_{2b} receptor attenuates high
884 glucose-induced apoptosis in H9C2 cells via PI3K/Akt signaling. *In Vitro Cell Dev Biol Anim.* **2018**,
885 *54*, 384-91.
- 886
887 98. Sepúlveda C, Palomo I, Fuentes E. Role of adenosine A_{2b} receptor overexpression in tumor
888 progression. *Life Sci.* **2016**, *166*, 92-9.
- 889
890 99. Shi B, Vinyals A, Alia P, Broceño C, Chen F, Adrover M, Gelpi C, Price JE, Fabra A. Differential
891 expression of MHC class II molecules in highly metastatic breast cancer cells is mediated by the
892 regulation of the CIITA transcription Implication of CIITA in tumor and metastasis development. *Int J*
893 *Biochem Cell Biol.* **2006**, *38*, 544-62.
- 894
895 100. Sorrentino C, Miele L, Porta A, Pinto A, Morello S. Myeloid-derived suppressor cells contribute
896 to A_{2B} adenosine receptor-induced VEGF production and angiogenesis in a mouse melanoma model.
897 *Oncotarget.* **2015**, *6*, 27478-89.
- 898
899 101. Sorrentino C, Morello S. Role of adenosine in tumor progression: focus on A_{2B} receptor as potential
900 therapeutic target. *J Cancer Metastasis Treat.* **2017**, *3*, 127-38.
- 901

- 902 102. Stagg J, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, Dwyer KM, Smyth MJ. Anti-
903 CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc Natl Acad Sci U S A.* **2010**,
904 *107*, 1547-52.
- 905
906 103. Sun X, Liu X, Xia M, Shao Y, Zhang XD. Multicellular gene network analysis identifies a macrophage-
907 related gene signature predictive of therapeutic response and prognosis of gliomas. *J Transl*
908 *Med.* **2019**, *17*, 159. <https://doi.org/10.1186/s12967-019-1908-1>
- 909
910 104. Thimm D, Schiedel AC, Sherbiny FF, Hinz S, Hochheiser K, Bertarelli DC, Maass A, Müller CE.
911 Ligand-specific binding and activation of the human adenosine A_{2B} receptor. *Biochemistry* **2013**, *52*,
912 726-40.
- 913
914 105. Trincavelli ML, Marroni M, Tuscano D, Ceruti S, Mazzola A, Mitro N, Abbracchio MP, Martini C.
915 Regulation of A_{2B} adenosine receptor functioning by tumour necrosis factor a in human astroglial
916 cells. *J Neurochem.* **2004**, *91*, 1180-90.
- 917
918 106. Trincavelli ML, Giacomelli C, Daniele S, Taliani S, Cosimelli B, Laneri S, Severi E, Barresi E, Pugliesi I,
919 Greco G, Novellino E, Da Settimo F, Martini, C. Allosteric modulators of human A_{2B} adenosine
920 receptor. *Biochim Biophys Acta (BBA) - General Subjects*, **2014**, *1840*, 1194–203.
- 921
922 107. Vecchio EA, Tan CY, Gregory KJ, Christopoulos A, White PJ, May LT. Ligand-Independent
923 Adenosine A_{2B} receptor constitutive activity as a promoter of prostate cancer cell proliferation. *J*
924 *Pharmacol Exp Ther.* **2016**, *357*, 36-44.
- 925
926 108. Vecchio EA, White PJ, May LT. [The adenosine A_{2B} G protein-coupled receptor: Recent advances and](#)
927 [therapeutic implications.](#) *Pharmacol Ther.* **2019**, *198*, 20-33.
- 928
929 109. Walters MJ, Tan JB, Becker A, Yi F, Park T, Leleti MR, Rosen B, Sharif E, Debieu L, Young S, Lim WH,
930 Garrido-Shaqfeh S, Jaen JC, Powers JP. Characterization of the potent and selective A_{2R} antagonist
931 AB928 for the treatment of cancer. *Cancer Res.* **2017**, *77*(13 Suppl):4572.
- 932
933 110. Walters MJ, Piovesan D, Tan JB, DiRenzo D, Yin F, Miles D, Leleti MR, Park T, Soriano F, Sharif E,
934 Schindler U, Powers JP. Combining adenosine receptor inhibition with AB928 and chemotherapy
935 results in greater immune activation and tumor control. *Cancer Res.* **2018**, *78*(13 Suppl), 5556.
- 936
937 111. Warabi M, Kitagawa M, Hirokawa K. Loss of MHC class II expression is associated with a decrease of
938 tumor-infiltrating T cells and an increase of metastatic potential of colorectal cancer:
939 immunohistological and histopathological analyses as compared with normal colonic mucosa and
940 adenomas. *Pathol Res Pract.* **2000**, *196*, 807-15.
- 941
942 112. Wei Q, Costanzi S, Balasubramanian R, Gao ZG, Jacobson KA. A_{2B} adenosine receptor blockade
943 inhibits growth of prostate cancer cells. *Purinergic Signal.* **2013**, *9*, 271-80.
- 944
945 113. Wei Q, Costanzi S, Liu QZ, Gao ZG, Jacobson KA. Activation of the P_{2Y}₁ receptor induces apoptosis
946 and inhibits proliferation of prostate cancer cells. *Biochem Pharmacol.* **2011**, *82*, 418-25.
- 947
948 114. Wiernik PH, Paietta E, Goloubeva O, Lee SJ, Makower D, Bennett JM, Wade JL, Ghosh C, Kaminer LS,
949 Pizzolo J, Tallman MS; Eastern Cooperative Oncology Group. Phase II study of theophylline in

- 950 chronic lymphocytic leukemia: a study of the Eastern Cooperative Oncology Group (E4998).
951 *Leukemia* **2004**, *18*, 1605-10.
- 952
953 115. Wilson JM, Lorimer E, Tyburski MD, Williams CL. β -Adrenergic receptors suppress Rap1B
954 prenylation and promote the metastatic phenotype in breast cancer cells. *Cancer Biol Ther.* **2015**, *16*,
955 1364-74.
- 956
957 116. Xaus J, Mirabet M, Lloberas J, Soler C, Lluís C, Franco R, Celada A. IFN-gamma up-regulates the A_{2B}
958 adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. *J*
959 *Immunol.* **1999**, *162*, 3607-14.
- 960
961 117. Xu Y, Ravid K, Smith BD. Major histocompatibility class II transactivator expression in smooth
962 muscle cells from A_{2B} adenosine receptor knock-out mice: cross-talk between the adenosine and
963 interferon-gamma signaling. *J Biol Chem.* **2008**, *283*, 14213-20.
- 964
965 118. Xiang HJ, Liu ZC, Wang DS, Chen Y, Yang YL, Dou KF. Adenosine A_{2B} receptor is highly expressed in
966 human hepatocellular carcinoma. *Hepatol Res.* **2006**, *36*, 56-60.
- 967
968 119. Xiang HJ, Chai FL, Wang DS, Dou KF. Downregulation of the adenosine a_{2b} receptor by RNA
969 interference inhibits hepatocellular carcinoma cell growth. *ISRN Oncol.* **2011**, *2011*, 875684. doi:
970 10.5402/2011/875684.
- 971
972 120. Yan A, Joachims ML, Thompson LF, Miller AD, Canoll PD, Bynoe MS. CD73 Promotes Glioblastoma
973 Pathogenesis and Enhances Its Chemoresistance via A_{2B} Adenosine Receptor Signaling. *J Neurosci.*
974 **2019**, *39*, 4387-402.
- 975
976 121. Yang D, Zhang Y, Nguyen HG, Koupenova M, Chauhan AK, Makitalo M, Jones MR, St Hilaire C,
977 Seldin DC, Toselli P, Lamperti E, Schreiber BM, Gavras H, Wagner DD, Ravid K. The A_{2B} adenosine
978 receptor protects against inflammation and excessive vascular adhesion. *J Clin Invest.* **2006**, *116*, 1913-
979 23.
- 980
981 122. Zhao C, Qiao Y, Jonsson P, Wang J, Xu L, Rouhi P, Sinha I, Cao Y, Williams C, Dahlman-Wright K.
982 Genome-wide profiling of AP-1-regulated transcription provides insights into the invasiveness of
983 triple-negative breast cancer. *Cancer Res.* **2014**, *74*, 3983-94.
- 984
985 123. [Zhong H](#), [Wu Y](#), [Belardinelli L](#), [Zeng D](#). A_{2B} adenosine receptors induce IL-19 from bronchial
986 epithelial cells, resulting in TNF-alpha increase. [Am J Respir Cell Mol Biol.](#) **2006**, *35*, 587-92.
- 987
988 124. Zhong J, Rao X, Deiuliis J, Braunstein Z, Narula V, Hazey J, Mikami D, Needleman B, Satoskar AR,
989 Rajagopalan S. A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced
990 visceral inflammation. *Diabetes* **2013**, *62*, 149-57.
- 991
992 125. Zhou Y, Chu X, Deng F, Tong L, Tong G, Yi Y, Liu J, Tang J, Tang Y, Xia Y, Dai Y.
993 The adenosine A_{2B} receptor promotes tumor progression of bladder urothelial carcinoma by
994 enhancing MAPK signaling pathway. *Oncotarget* **2017**, *8*, 48755-48768.