Highlights

-Adenosine is an endogenous and ubiquitous nucleoside, deriving from dephosphorylation of both intracellular and extracellular ATP, rising under stressed conditions present in several pathologies and acting through interaction with four GPCR receptors, named A_1, A_{2A}, A_{2B} and A_3.

-Adenosine plays a crucial role in the regulation of cancer cell biology by regulating both tumor and immune cells, through interaction with its cognate A_3 and A_{2A} receptors, respectively. Specifically, a vicious cycle of tumor hypoxia, increased adenosine concentrations, immune suppression and cancer growth implies the use of adenosine receptor ligands in tumors.

-Highly selective A_3 agonists with potential as anticancer drugs have been developed, exploiting their anti-proliferative and proapoptotic effects. These ligands represent a new and original anticancer approach, addressing for the first time both cancer and healthy immune cells.

- A_3 receptors are overexpressed in almost all cancer types, indicating that A_3 agonists could offer the opportunity of an innovative personalized cancer therapy. The A_3 receptor represents an effective tumor biomarker, being its overexpression at baseline in correlation with a positive response in patients.

-Potent and selective A_{2A} receptor antagonists have been developed based on the awareness that hypoxic generation of adenosine in tumors is crucial for their immuno-escape A_{2A} receptors-mediated. Based on the ability of these compounds to activate the immune system, clinical trials have been designed to evaluate their antitumor efficacy in combination with immunological drugs.

- The A_3 agonist Namodenoson, as well as A_{2A} antagonists Preladenant, PBF-509, CPI-444 and AZD4635, in clinical development, demonstrate a good safety profile, no side effects, and an acceptable pharmacokinetic behavior, thereby giving hope for the future for a new generation of drugs capable of changing the fate of numerous types of tumor.

This box summarizes key points contained in the article.

Targeting A_3 and A_{2A} adenosine receptors in the fight against cancer

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Key words: Adenosine receptors, cancer, cell proliferation, immunoescaping, immunoprotection, clinical trials

Abstract

Introduction: there exists a vicious cycle of tumor hypoxia, high adenosine levels, immune suppression and cancer growth that involves the use of adenosine receptor ligands in tumors. After several years of research the candidates emerging as promising new anticancer drugs are A_3 adenosine receptor agonists and A_{2A} receptor antagonists.

Areas covered: in this article, the authors give an updated overview of the field related to A_3 receptor agonists and A_{2A} receptor antagonists in cancer and propose their perspectives on the status of the art for these compounds in oncology. In particular, the rationale for the modulation of adenosine receptors in cancer will be addressed, starting from the first in vitro evidences of their efficacy up to the animal and clinical studies.

Expert opinion: A_3 and A_{2A} receptors are attractive targets in oncologic therapy due to their involvement in cancer progression and immune-resistance, respectively. Of relevance the A_3 subtype is also a tumor marker to be used in a personalized drug treatment program while the A_{2A} receptor, playing a non-redundant role in immunomodulation, may be blocked in combination with checkpoint inhibitors to improve their efficacy. Overall, the next years will be determinant to see the real winners, hopefully both, in the fight against cancer.

1 Introduction

Adenosine is an endogenous and ubiquitous nucleoside affecting a wide range of physiological functions in tissues and organs through interaction with four G protein coupled receptors named A_1, A_{2A}, A_{2B} and A_3 [1]. Cancer biology is an important field regulated by adenosine that exerts its control on both neoplastic and immune cells, modulating the main hallmarks of tumors such as proliferation, metastasis, angiogenesis, and immunoescape [2-5]. The production of adenosine occurs in the nanomolar range in normal healthy conditions, but it significantly increases up to the micromolar range, in the hypoxic environment characterizing solid tumors. Indeed hypoxia increases CD73 while decreases AK enzymes, involved in the adenosine accumulation [5]. The starting point of the research connecting adenosine to cancer is represented by the observation that the extracellular milieu of lung and colon adenocarcinomas was characterized by low oxygen concentration and high amount of adenosine able to counteract anticancer activity of immune cells [6]. Importantly, it was then reported that muscles do not develop tumor metastases due to the presence of both adenosine and endogenous A_3 receptor agonists, playing an inhibitory effect on tumor cell growth [7-10]. Subsequently, another milestone relating adenosine to cancer was the evidence that the A_{2A} receptor is an essential non pleonastic inhibitor of inflammation with consequences for tumor survival [11-12]. Specifically, it has been demonstrated that hypoxic cancer cells are protected against immune attack through the inhibition of the host T cell response mediated by adenosine activating A_{2A} receptors [13]. This effect was synergic with the immunosuppression exerted by the hypoxia-inducible factor 1 (HIF-1α) overexpressed in tumors. Since that time several works have been performed by eminent scientists operating in the adenosine receptors cancer area.

Therefore, this perspective is devoted to offer a comprehensive and updated picture about the preclinical and clinical research activity concerning the development of A_3 agonists and/or A_{2A} antagonists, finalized to obtain anti-proliferative and immunosuppressive drugs.

2. A_3 adenosine receptor agonists

2.1 A_3 receptor as a biomarker for cancer

A huge amount of literature data report that A_3 receptors appear highly expressed in both cancer cells and solid tumors [7,14-18]. Specifically, A_3 receptors have been detected in blood tumors such as leukemia and lymphoma, as well as in different cancer cells including melanoma, glioblastoma, prostate, colon and mesothelioma [14,19-26]. Furthermore, they have been found upregulated in

tumor tissues isolated from patients affected by colorectal, breast and thyroid cancer [27-29]. Subsequently, they are overexpressed also in the highly aggressive neoplasm mesothelioma, when compared to the healthy pleura [26]. Importantly, polymorphonuclear and T cells obtained through peripheral venous sampling from patients affected by colon and hepatocellular carcinoma (HCC), reported the same upregulation found in the related cancer tissues suggesting that, A_3 receptors present in neutrophils and lymphocytes, reflect the status of the receptor in solid tumors [28,30].

2.2 In vitro proofs of concept

Ligands activating the A_3 adenosine receptors are characterized by the capability to strongly reduce cancer cells proliferation [26,31-32]. A cytostatic activity has been demonstrated for A_3 adenosine receptor agonists, at the beginning of the research, in leukemia cells, where telomerase function was contrasted [14,33]. Furthermore, other crucial behaviors of cancer cells such as growth, migration, and apoptosis were strongly blocked by A_3 receptor agonists in prostate, malignant mesothelioma and glioblastoma cells [25,26,34-36]. The relevant antitumoral effects displayed by this class of compounds led researcher to deeply investigate the molecular mechanism and the intracellular pathways responsible for tumor growth arrest [15,37-39]. Interestingly, the main process activated by A_3 adenosine receptor involves the Wnt machinery, responsible for cell cycle activation and cell growth occurring in both embryogenesis and tumorigenesis. Specifically, signaling triggered by A_3 receptor, through Gi protein coupling, inhibits adenylyl cyclase and thus PKA and PKB/Akt phosphorylation, which in turn rise glycogen synthase kinase 3β (GSK-3β) function. As a consequence β-catenin is phosphorylated and ubiquitinated, while cyclin D1 and c-myc cell cycle regulators are decreased. Another actor in the apoptotic cell death-A_3 receptor dependent is NF-kB, that being inhibited, reduces melanoma and hepatocellular carcinoma growth [15] .

Interestingly, the effects induced by A_3 adenosine receptor agonists in immune system were different and opposite from those exerted in cancer cells [10]. The first studies indeed, demonstrated that these molecules increased the number of murine bone marrow cells, through the granulocyte colony-stimulating factor (G-CSF) production. This effect resulted in an increased white cell number in mice treated with adenosine and a cytotoxic antitumoral drug [33,40-41]. The signal transduction mechanism of this response involved enrollement of PI3K, PKB/Akt, IKK and NF-kB factors [39,42]. Other studies showed that A_3 receptor recruited other cell types for the immune fight against cancer cells. Indeed it rose the ability of natural killer (NK) cells to destroy cancer cells and stimulated, following ex vivo exposure, the release of TNF-α from CD8+ T cells, resulting in an anticancer therapeutic effect, after animal injection [24,43-44].

2.3 Animal studies

Following the positive results obtained in *in vitro* studies the A_3 receptor agonists, IB-MECA and Cl-IB-MECA, named CF101 and CF102, respectively, were tested in different *in vivo* animal models of cancer, spanning from syngeneic, xenograft, orthotopic and metastatic models of numerous different type of tumors such as melanoma, colorectal, prostate and liver carcinomas [4].

The first evidence of a role for adenosine in the reduction of tumor growth come back to studies performed in mice reporting that oncologic animals, affected by melanoma or sarcoma, exposed to medium obtained from skeletal muscle cells, presented a reduction of lung metastasis [8]. Few years later, by studying mice affected by melanoma, it was discovered that the antiproliferative action of adenosine was instead due to endogenous A_3 receptor agonists [9].

Then, it was confirmed that Cl-IB-MECA, administered to animals with melanoma, was effective not only to inhibit the development of melanoma lung metastases, but also to potentiate the therapeutic anticancer efficacy of cyclophosphamide, without myelotoxic drawbacks [33,39,45]. Importantly, the agonist effect was antagonized by the A_3 receptor blocker, MRS1523 and involved the pathway triggered by A_3 receptor recruiting c-Myc, cyclin D1 as well as GSK-3β [46]. In addition, IB-MECA showed the same antitumor effect when given to mice transplanted with human prostate carcinoma [47]. Moreover, CF101 orally administered, blocked the growth and liver metastasis of primary colon carcinoma in syngeneic animal models, through stimulation of interleukin-12 production and NK cell function. Similar to CF102, CF101, given in combination with 5-fluorouracyl (5-FU), to mice bearing human colon cancer, produced a greater antitumoral effect, preventing immune depression, usually associated with 5-FU therapy [24,48]. Again the signaling machinery triggered by the A_3 receptor agonist involved the increase of GSK-3β as well as the inhibition of NF-kB, as previously demonstrated [38,49]. Other studies, performed in xenograft animals treated with CF102, showed a reduction of hepatocellular and breast cancer, as well as a decrease of liver inflammation and pain due to bone breast metastasis, respectively [30,50-51].

2.4 Human clinical trials

The encouraging results coming from the first human study involving CF101 and CF102, orally administered in healthy young men, were that these drugs presented a safe profile with good tolerability, and an acceptable pharmacokinetic behavior [52]. Therefore, CF102 (Namodenoson) has been introduced in clinical trials for treatment of advanced hepatocellular carcinoma (HCC). The

results of the Phase I/II (NCT00790218) study showed that Namodenoson was devoid of severe drug-dependent side effects. The median overall survival (OS) of 18 subjects included in the study was 7.8 months, and it is important to underline that for the 67% of them CF102 drug treatment was the second-line therapy [53]. CF102 preserved liver function over a 6-month period. A correlation between receptor upregulation and patients' outcome was observed. A Phase II trial in this patient population affected also by Child-Pugh Class B (CPB) cirrhosis has been completed (Phase 2, NCT02128958). The study, where 78 patients were separated into the three degree of pathology severity CPB7, CPB8, and CPB9, did not achieve its primary end point of median OS in the whole groups. However, the CPB7 subpopulation of 56 patients, demonstrated a median OS of 6.8 months compared to placebo (n=22) with 4.3 months. In addition, for this group, progression free survival was 3.5 months in the namodenoson-treated group versus 1.9 months in the placebo group. Finally, for the all group, 9% of patients cured with namodenoson presented a reduction of tumors versus none in the placebo group (Table 1). Considering the lack of treatment for this pathology, the drug has already been granted fast-track status by the FDA. On these basis the biotechnology company Canfite biopharma Ltd is currently designing and planning the protocol for a Phase III study.

3 A_{2A} adenosine receptor antagonists

3.1 A_{2A} receptor as a non-redundant actor of immunosuppression

Another area of research exploited to develop novel anticancer drugs inside adenosine receptors system take advantage of the important and non-redundant role of the A_{2A} receptor as inhibitor of the immune system, when activated by adenosine generated at high levels by CD73, overexpressed in cancer [11]. Indeed, A_{2A} adenosine subtype is present on the lymphocytes cell membrane and through enrollement of Gs proteins stimulates cAMP, thus reducing the activation of the machinery triggered by TCR in immune cells. This effect induces a series of inhibitory events, concerning T cell responses, resulting in a decrease of inflammation, thanks to which adenosine has been suggested to be like a body guardian angel [4]. However, in the case of solid tumors, where cancer and T cells cohexist, this virtuous immunosuppressive behavior becomes a sort of dangerous boomerang, that should be suppressed [54]. In more detail, considering the hypoxic milieu characterizing solid tumors, the activation of the master regulator of cell functions during low oxygen concentrations named hypoxia inducible-factor 1 (HIF-1) occurs. This event leads to the reduction of T cell functions, stimulates genesis of new blood vessels as well as induces an increase in adenosine levels CD73-dependent, overall allowing the phenomenon called “tumor immune escape”, thus resulting in a worse life

expectation [55]. Interestingly, genetic data obtained from more than 6,000 breast tumor cases, demonstrated that high levels of CD73 were related to a worse prognosis in triple-negative breast cancers [56-57]. Accordingly, in ovarian tumor-bearing mice adoptive T-cell immunotherapy was efficacious, only following tumor CD73 knockdown and in animal characterized by CD73 overexpression chemoresistance to anthracycline drugs occurred, via engagement of A_{2A} receptors, allowing the introduction of anti-CD73 antibodies and A_{2A} receptor antagonists in human clinical studies [56,58].

3.2 In vitro proofs of concept

The inhibitory anti-inflammatory signaling induced by the A_{2A} adenosine receptor, involves the recruitment of cAMP/PKA molecules in CD8+ T, CD4+ T, B, natural killer (NK) and dendritic cells, as well as monocytes and macrophages, thus promoting tumor evasion [59-68].
Specifically, adenosine through A_{2A} activation inhibits NK cell differentiation and proliferation thus causing a reduction of perforin- and Fas-ligand-dependent cytotoxicity, and decreases TNF-α, IFN-γ, GM-CSF, MIP-1α cytokines production [68-70]. In addition, A_{2A} enrollement in CD8+ lymphocytes reduces IL-2, IFN-γ, TNF-α, GM-CSF, MIP-1α, MIP-1β, IL-13 and RANTES production, provoking inhibition of proliferation and cytotoxicity. Also, A_{2A} stimulation inhibits macrophages activation while increases VEGF production; reduces IL-4 and IFN-γ secretion by CD4+T, Th1 and Th2 cells and decreases pro-inflammatory cytokines without change in the anti-inflammatory ones on T lymphocytes [12,71-74]. Another component of the immune system relevant for the contribution of adenosine to the immunoescaping is represented by regulatory T (Treg) cells, that repress autoreactive T cells, thus preventing autoimmune diseases development [75-76]. Indeed, inside cancer environment Treg cells, following apoptotic cell death, produce ATP generating adenosine, responsible for blunting of the immune system A_{2A} adenosine receptor-dependent [77-78]. Specifically, activation of A_{2A} adenosine receptor in these cells increases CD39 and CD73 enzymes, through E2F-1 and CREB transcription factors, thus accelerating adenosine formation [79].

3.3 Animal studies

The important anticancer evidences obtained from the huge amount of *in vitro* studies on A_{2A} receptor inhibition in immune system were soon confirmed on animal cancer models. Indeed, the use of engineered mice knocked down for the A_{2A} receptors generated in them protection versus spread of

cancer cells from the primary tumor to distant organs, suggesting a crucial role of A_{2A} receptors in the metastasis development. At the same time treatment of wild type animals with A_{2A} blockers, such as caffeine, or use of siRNA technology, to blunt down A_{2A} receptors expression in T lymphocytes, arrested cancer and metastases development [12]. Recently, it has been reported that *ex vivo* treatment of T cells, derived from breast tumor mice, with polyethylene glycol (PEG)-chitosan-lactate (PCL) nanoparticles (NPs) containing A_{2A} siRNA, inhibited differentiation of T cells toward Treg, through a pathway involving protein kinase A/cAMP-response element binding protein (PKA/CREB) and nuclear factor-κB (NF-κB) [80]. Accordingly, the efficacy of the A_{2A} receptor block was evaluated in a xenograft model of lung adenocarcinoma, where this procedure reduced tumor development, thus further supporting the use of A_{2A} receptor antagonists as anticancer drugs [81]. Furthermore, antagonism of A_{2A} receptor induced maturation of NK cells and stimulated the presence of NK cell granzyme B, thus reducing breast and melanoma metastasis [82]. Interestingly, the great potential immunosuppressive behavior of A_{2A} receptor antagonist has been exploited in cancer immunotherapy. Indeed anti-CTLA-4 and anti-PD-1/PD-L1 antibodies affected immunosuppressive mechanisms thus improving patient survival, but their activity was limited by the expression of CD73 on tumor cells responsible for an increase in adenosine. In this context, A_{2A} receptors inhibition enhanced anti-PD-1 antibody effect, by rising T cells secretion of IFN-γ and Granzyme B, resulting in cancer arrest and animal survival [83]. Recently, the immunosuppressive propriety of A_{2A} antagonists has been used to improve efficacy of chimeric antigen receptor T cells versus hematological tumors [84]. Accordingly, a new A_{2A} receptor antagonist stimulated T cells activity versus tumor cells causing lung metastasis inhibition with a potentiated ex vivo immunosuppressive effect by coadministration with anti-PD-1 or anti-PD-L1 antibodies [85]. New evidence of the arresting effect induced by A_{2A} antagonists on head and neck squamous cell cancer provides a preclinical basis for the use of A_{2A} blockers on prophylactic experimental therapy of these tumors [86]. Interestingly, new derivatives of A_{2A} receptor antagonists, not successfull as anti-Parkinson drugs, like preladenant and tozadenant, proved their ability to modulate immune system, with fluorinated tozadenant being the best molecule [87]. Importantly, it has been recently confirmed that the immunomodulatory activity of A_{2A} antagonists was independent on the tissue localization of cancer and that was fundamental to counteract immunosuppression exerted by tumor microenvironment on CD8+ cells function [88]. In addition, the novel A_{2A} antagonist, CPI-444, reduced different checkpoint receptors, PD-1 and LAG-3, on both CD8+ and Treg lymphocytes in tumor bearing mice, primarily at tumor-draining lymph nodes. Importantly, this molecule improved the effect of anti-PD-1 therapy and increase antitumor response [89-90].

3.4 Human clinical trials

The efficacy of A_{2A} receptor antagonists to avoid immunoescaping of tumor cells observed in preclinical studies has been translated in clinical trials, evaluating essentially four different A_{2A} blockers including Preladenant, PBF-509, CPI-444, AZD4635 (Table 1).

A phase Ib/II trial evaluating Preladenant safety alone and in combination with the anti-PD-1 drug Pembrolizumab in patients affected by advanced cancers (NCT03099161), has been recently terminated, but the results are not available yet.

PBF-509 was investigated as single agent or in combination with PDR001 (programmed cell death 1 receptor antibody) to determine its safety, tolerability, feasibility and preliminary efficacy in phase I/II study, involving patients with non-small cell lung cancer (NSCLC) (NCT02403193). In addition this A_{2A} antagonist will be evaluated in other two planned trials. One is a phase 2, multicentre, open label study, recruiting patients with advanced solid tumors and non Hodgkin lymphoma (NCT03207867), the other is a Phase I/Ib, open-label, multi-center, study in patients with advanced malignancies, where its efficacy will be tested in combination with the anti-CD73 compound NZV930 (NCT03549000).

CPI-444 will be assayed in a phase 1/1b (NCT02655822) open-label, multicenter, dose-selection study as oral drug acting on T-lymphocytes and other immune cells. This trial will evaluate its safety, tolerability, and efficacy when administered alone and in co-treatment with atezolizumab, a PD-L1 blocker against NSCLC, Malignant Melanoma, Renal Cell Cancer, Triple Negative Breast Cancer, Colorectal Cancer, Bladder Cancer and Metastatic Castration Resistant Prostate Cancer. Furthermore this compound will be assessed in a phase Ib/II, open-label, multicenter, randomized umbrella study, measuring the efficacy and safety of multiple immunotherapy-based treatment combinations in patients with metastatic NSCLC (NCT03337698). Finally, CPI-444 will enter a phase 1/1b open label, multicenter, study in cotreatment with the anti-CD73 CPI-006, in patients affected by various solid tumors and non-Hodgkin lymphoma (NCT03454451).

AZD4635 will be studied, as continuous oral administration, in a Phase 1, open-label, multicenter trial in patients with advanced solid cancers. The primary endpoint of the study will be to calculate the maximum safe dose of AZD4635 in combination with the anti-PD-L1 drug, Durvalumab (NCT02740985). Finally, this A_{2A} antagonist will be also evaluated for safety, tolerability and antitumor activity in combination with the anti-CD73 drug MEDI9497 and with the EGFR inhibitor Osimertinib, in subjects with NSCLC (NCT03381274).

4 Conclusion

This prospective review briefly summarizes the state of the art concerning adenosine A_{2A} and A_3 receptors as therapeutic targets in cancer therapy. It highlights the message that drugs stimulating A_3 and blocking A_{2A} subtypes are emerging as a novel promising approach for fighting oncologic diseases (Figures 1 and 2). Indeed, over the last two decades, many in vitro and animal studies published by scientists working in the purinergic field have demonstrated their efficacy in the reduction of cancer growth and metastasis development, suggesting their great therapeutic potential. In addition, human studies have shown that these drugs have a good safety profile, no side effects, and an acceptable pharmacokinetic behavior, thereby giving hope for the future for a new generation of drugs capable of changing the fate of numerous types of tumor.

5 Expert opinion

In the past, conventional antitumor therapy consisted of surgery, chemotherapy, and radiotherapy [91]. Even though these methods have proved to be essential for removing primary tumors, patient survival is still compromised by remaining tumor cells or cancer metastases causing cancer recurrence, and different approaches are required to eradicate the resistant tumor cells [92].

In this context, increasing understanding of the link between cancer and immune cells has led to a revolution in cancer therapy, namely the development of cancer immunotherapy based on monoclonal antibodies (mAbs), as well as vaccines, adoptive cell therapy and immune checkpoint therapy [93]. However, the efficacy of this type of treatment is limited by the phenomenon of resistance to immunotherapy. Individuals will display a range of responses, as tumors employ different strategies to evade the immune system. Such mechanisms include reduced antigen expression, affecting the MHC complex, for instance, and altered levels of cell receptors involved in immune recognition, such as PD-1/PD-L1 and CTLA-4 proteins, as well as alterations in enzymes and metabolic pathways. An example of the former is CD39/CD73 overexpression, which modifies adenosine levels in the tumor microenvironment, and thereby results in a lack of response to immunotherapy.

Issues such as these highlight a need for personalized medicine, a goal whose achievement will depend on our discovery of biomarkers able to identify which patients will benefit. We also need to find new regulatory pathways that can enhance the immune response to tumors, and in this context adenosine is a viable target, as it regulates both tumor and immune cells through interaction with its cognate A_3 and A_{2A} receptors, respectively [94-95]. This makes these receptors appealing therapeutic targets that may potentially turn the tide in the fight against cancer by exploiting a dual approach

involving A_3 receptor stimulation to support adenosine's anti-proliferative effects and A_{2A} receptor antagonism against immunosuppression.

Indeed, highly selective A_3 agonists with potential as anticancer drugs have been developed following in-depth investigation, spanning 20 years, of the intracellular molecular signalling behind adenosine's anti-proliferative and proapoptotic effects. These molecules offer a unique and original anticancer strategy, targeting for the first time both cancer and healthy immune cells, with anti- and pro-proliferative effects, respectively. Evidence suggests that A_3 receptors are up-regulated in almost all cancer types, indicating that this class of drugs could be widely applicable, and may be the lynchpin of an innovative personalized therapeutic strategy for cancer. Indeed, the A_3 receptor may provide an effective tumor biomarker, as there is a correlation between its overexpression at baseline and a positive response in patients.

Despite the wide range of beneficial properties of A_3 agonists, however, only two clinical trials have been completed to date, both on Namodenoson in patients affected by hepatocellular carcinoma (HCC). This apparent lack of interest in such an exciting field of research may be explained by the fact that, to our knowledge, Namodenoson is the only A_3 receptor agonist with such high affinity and selectivity found to date. Canfite, which owns the drug, is currently recruiting patients with autoimmune diseases like rheumatoid arthritis and psoriasis, but such clinical trials are likely to be expensive and protracted.

Therapeutic strategies involving A_{2A} antagonism, on the other hand, are being intensively pursued. This is largely because several big pharmaceutical companies can boast different potent A_{2A} antagonists. The safety of these drugs has previously been demonstrated in clinical trials on Parkinson's disease patients, meaning that one of the major barriers to novel clinical studies in cancer has already been overcome [96-98]. Furthermore, the observation that hypoxic generation of adenosine in tumors is crucial for their immuno-escape has led to the development of potent and selective A_{2A} receptor antagonists [99]. Based on the ability of these compounds to activate the immune system, clinical trials have been designed to evaluate their efficacy in cancer in combination with immunological drugs. Even though the results of these studies are not yet available, this approach seems very promising, because it is designed to act on a crucial pathway—linking hypoxia, adenosine, A_{2A} receptors and immunosuppression—that limits the efficacy of immunotherapy. In order to extend such an approach to a wide range of tumors, however, it would be useful to design novel antagonists unable to cross the blood–brain barrier. This would avoid potential CNS problems in patients affected by cancer located in other body districts.

Other research has detected increased A_{2A} receptor levels in patients treated with PD-1/PD-L1 drugs. As these are potentially responsible for immuno-escape, as well as limiting antibody efficacy,

increased A_{2A} may therefore represent an early biomarker. Moreover, a new drug delivery system has recently been developed using nanoparticles loaded with A_{2A}-receptor siRNA [80]. This resulted in the suppression of Treg differentiation and the loss of the immunosuppressive adenosinergic effect, suggesting that it may one day be useful in other anticancer treatments. Another interesting approach is being investigated further to evidence that hyperoxia reduces tumor growth. This suggests that A_{2A} receptor antagonists could be used synergistically in combination with supplemental oxygenation, making oxygenation of cancers as a new checkpoint inhibitor, in association with anticancer immunostimulation, an appealing therapeutic target [100-101].

However, the applicability of this approach in patients is still undetermined, and even in the wider field, clinical trials of A_{2A} antagonists and A_3 agonists will not allow us to draw conclusions about which strategy looks more promising in the fight against cancer for some time. Nonetheless, we can speculate on which might be the best way forward. As such drugs have different targets, it is possible to foresee that combinations of them may have complementary effects, and therefore added therapeutic value. Indeed, taken together the two receptors have a diverse biological repertoire, with A_3 receptor signalling inhibiting tumor growth but promoting immune function, and A_{2A} receptor signalling blocking the antitumor activity of immune cells. In vitro studies suggest that both A_3 and A_{2A} agonists may promote angiogenesis and metastasis, and the addition of an A_{2A} antagonist could block this tumor-promoting mechanism, thereby potentially enhancing the efficacy of A_3 agonists. Likewise, the immunostimulation induced by A_{2A} antagonists may be responsible for the development of a pathological cytokine ‘storm’, which may, however, be counteracted by the beneficial action of A_3 agonists [102-104] (Figure 3).

That being said, before such innovative strategies may be even considered, we will have to wait for the results of the ongoing clinical trials on each class of drugs alone. Indeed, Namodenoson, as well as Preladenant, PBF-509, CPI-444 and AZD4635, are only at the beginning of clinical development. Nevertheless, this field of research is full of promise, and shows the potential to yield novel anticancer drugs that act against a wide variety of tumors, not to mention biomarkers that may take us one step closer to the overarching goal of personalized medicine.

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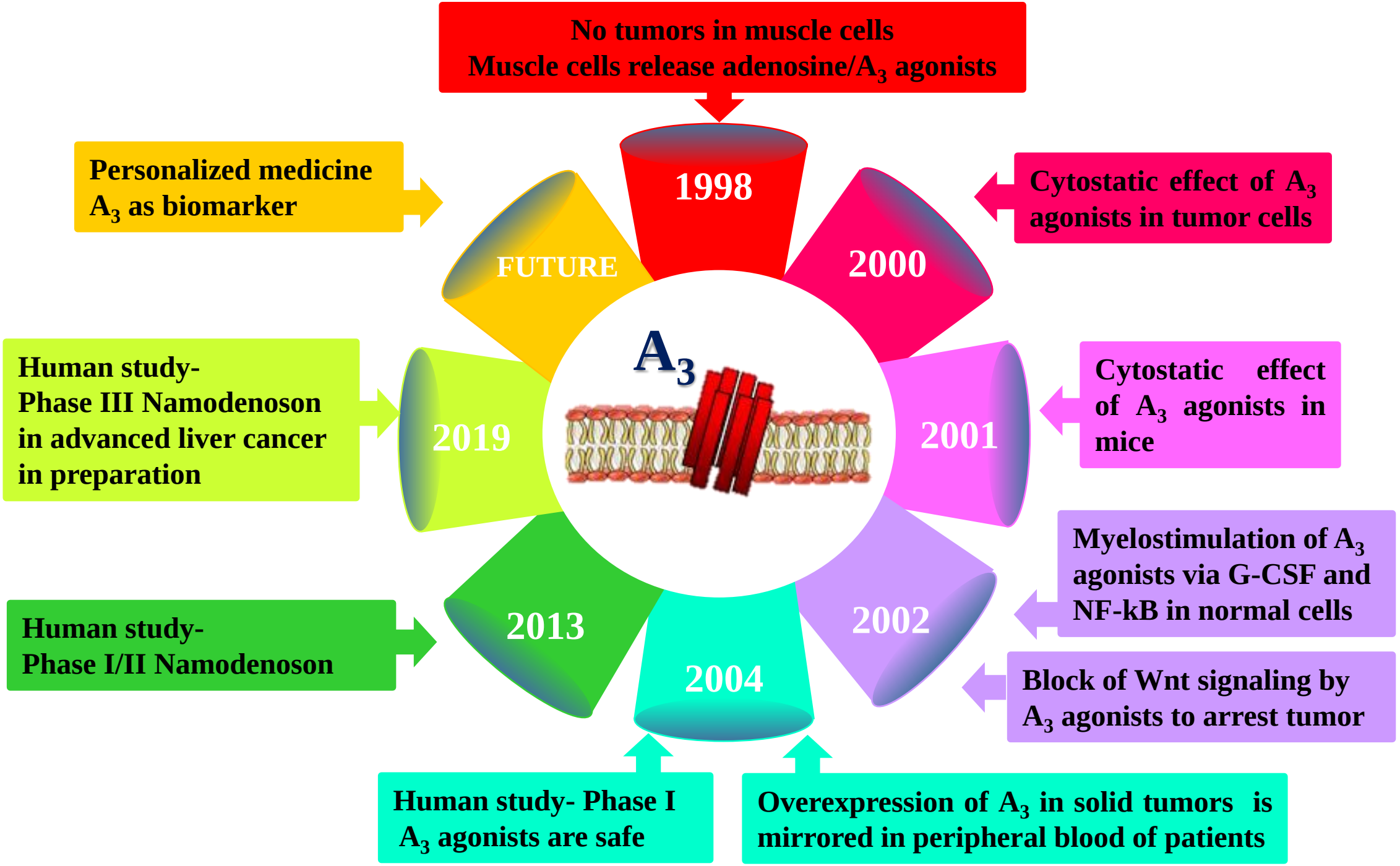
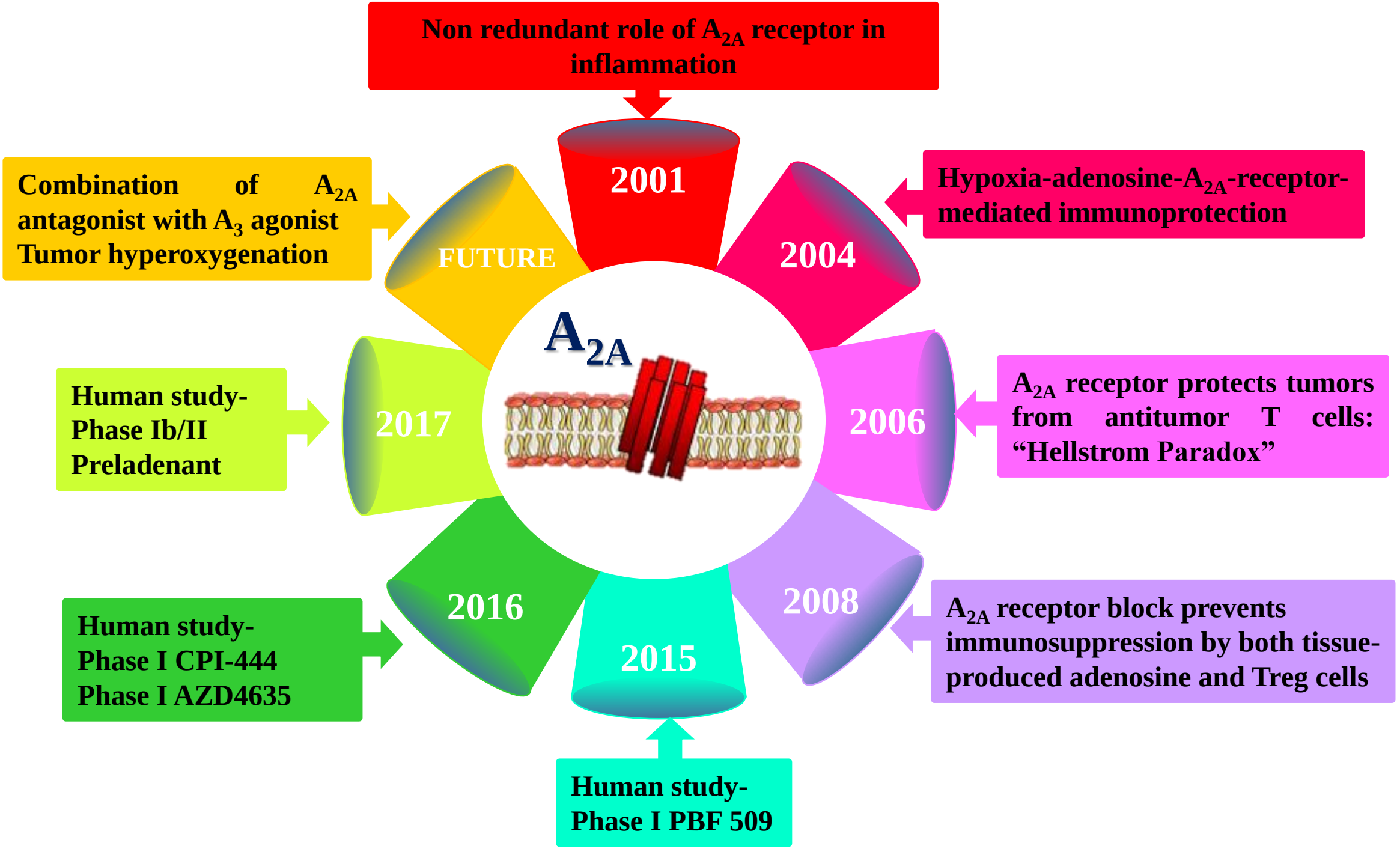
Table 1-Clinical molecules selective for A_3 and A_{2A} adenosine receptors candidates as new anticancer drugs

DRUG	TUMOR TARGET	PHARMACEUTICAL SUPPLIER	PHASE	DESIGN	CLINICAL TRIAL IDENTIFIER CODE
A_3 AGONIST					
Namodenoson CF-102	Hepatocellular Carcinoma	Can-Fite BioPharma Ltd.	I-II	Single agent	NCT00790218
			II	Single agent	NCT02128958
A_{2A} ANTAGONISTS					
Preladenant (MK-3814)	Advanced Solid Tumors	Merck	I	Single agent and + Pembrolizumab (anti-PD-1)	NCT03099161
PBF-509 (NIR 178)	Non-small Cell Lung Cancer	Palobiofarma SL/ Novartis Pharmaceuticals	I/II	Single agent and + PDR001 (anti-PD-1)	NCT02403193
	Non-small Cell Lung Cancer Triple Negative Breast Cancer Pancreatic Ductal Adenocarcinoma Colorectal Cancer Microsatellite Ovarian Cancer Renal Cell Carcinoma	Novartis Pharmaceuticals	I/Ib	+ NZV930 (anti-CD73)	NCT03549000
	Non-Small Cell Lung Cancer Renal Cell Cancer Pancreatic Cancer Urothelial Cancer Head and Neck Cancer Diffused Large B Cell Lymphoma Microsatellite Colon Cancer Triple Negative Breast Cancer Melanoma	Novartis Pharmaceuticals	II	Single agent and + PDR001 (anti-PD-1)	NCT03207867
CPI-444	Non-Small Cell Lung Cancer Malignant Melanoma Renal Cell Cancer	Corvus Pharmaceuticals, Inc.	I/Ib	Single agent and + Atezolizumab (anti-PDL-1)	NCT02655822

	Triple Negative Breast Cancer Colorectal Cancer Bladder Cancer Metastatic Castration Resistant Prostate Cancer				
	Non-Small-Cell Lung Cancer	Hoffmann-La Roche	Ib/II	Immunotherapy-based treatment combinations: Atezolizumab Cobimetinib RO6958688 Docetaxel BL-8040 Tazemetostat CPI-444 Pemetrexed Carboplatin Gemcitabine Linagliptin Tocilizumab	NCT03337698
	Non-Small Cell Lung Cancer Renal Cell Cancer Colorectal Cancer Triple Negative Breast Cancer Cervical Cancer Ovarian Cancer Pancreatic Cancer Endometrial Cancer Sarcoma Head/Neck Squamous Carcinoma Bladder Cancer Metastatic Prostate Cancer Non-hodgkin Lymphoma	Corvus Pharmaceuticals, Inc.	I/Ib	+ CPI-006 (anti-CD73)	NCT03454451
AZD4635	Advanced Solid Malignancies Non-Small Cell Lung Cancer Metastatic Prostate Carcinoma Colorectal Carcinoma	AstraZeneca	I	Single agent and + Durvalumab (anti-PDL-1)	NCT02740985

	Non-Small-Cell Lung	MedImmune LLC	Ib/II	+Oleclumab (MEDI9447) (anti-CD73)	NCT03381274


No tumors in muscle cells
Muscle cells release adenosine/A3 agonists
1998
2000
Cytostatic effect of A3 agonists in tumor cells
2001
Cytostatic effect of A3 agonists in mice
2002
Myelostimulation of A3 agonists via G-CSF and NF-kB in normal cells
Block of Wnt signaling by A3 agonists to arrest tumor
2004
Overexpression of A3 in solid tumors is mirrored in peripheral blood of patients
Human study- Phase I
A3 agonists are safe
2013
Human study-
Phase I/II Namodenoson
2019
Human study-
Phase III Namodenoson
in advanced liver cancer
in preparation
FUTURE
Personalized medicine
A3 as biomarker
A3


Non redundant role of A_{2A} receptor in inflammation
2001
Hypoxia-adenosine-A_{2A}-receptor-mediated immunoprotection
2004
A_{2A} receptor protects tumors from antitumor T cells: "Hellstrom Paradox"
2006
A_{2A} receptor block prevents immunosuppression by both tissue-produced adenosine and Treg cells
2008
Human study- Phase I PBF 509
2015
Human study- Phase I CPI-444 Phase I AZD4635
2016
Human study- Phase Ib/II Preladenant
2017
Combination of A_{2A} antagonist with A_3 agonist Tumor hyperoxygenation
FUTURE
A_{2A}

TO FIGHT CANCER WITH NEW LIGANDS