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Pharmacological and therapeutic effects of A_3 adenosine receptor agonists

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The A_3 adenosine receptor (A_3AR) coupled to G_i (inhibitory regulative guanine nucleotide-binding protein) mediates anti-inflammatory, anticancer and anti-ischemic protective effects. The receptor is overexpressed in inflammatory and cancer cells, while low expression is found in normal cells, rendering the A_3AR as a potential therapeutic target. Highly selective A_3AR agonists have been synthesized and molecular recognition in the binding site has been characterized. In this article, we summarize preclinical and clinical human studies that demonstrate that A_3AR agonists induce specific anti-inflammatory and anticancer effects through a molecular mechanism that entails modulation of the Wnt and the NF-κB signal transduction pathways. At present, A_3AR agonists are being developed for the treatment of inflammatory diseases, including rheumatoid arthritis (RA) and psoriasis; ophthalmic diseases such as dry eye syndrome and glaucoma; liver diseases such as hepatocellular carcinoma and hepatitis.

Introduction

The A_3 adenosine receptor (A_3AR) is a subtype of the adenosine receptor (AR) family, which additionally includes A_1, A_{2A} and A_{2B} receptors [1,2]; each receptor is encoded by a separate gene and has different physiological roles. The G_i-coupled A_3AR is less widely distributed than other AR subtypes with expression in human lung, liver, brain, aorta, testis and heart. The utilization of the A_3AR as a therapeutic target and a biological predictive marker is based on two major findings: (i) the A_3AR is overexpressed in cancer and inflammatory cells, while low expression is found in normal cells [3–5]. The high receptor expression is also found in peripheral blood mononuclear cells (PBMCs) of patients with cancer or inflammatory diseases [5,6]. (ii) Highly selective A_3AR agonists have been synthesized and induce specific anti-inflammatory and anticancer effects through a molecular mechanism that entails modulation of the Wnt and the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signal transduction pathways [6–8] (Fig. 1). A protective effect of the agonists on normal cells was recorded as well, suggesting that this unique differential effect of the agonists will contribute to a safety profile of these drug candidates in both preclinical and clinical studies. At present, A_3AR agonists are being developed for the treatment of inflammatory, ophthalmic and liver diseases and demonstrate excellent safety and efficacy in Phase II clinical studies.

A_3AR agonists

The human A_3AR was cloned in 1993 [1] and soon thereafter found to have cerebroprotective and cardioprotective properties [9,10]. Like other G protein-coupled receptors (GPCRs), it is also known to affect G protein-independent signaling, such as translocation of arrestins, leading to rapid desensitization of the A_3AR *in vitro* (typically within ~20 min in the presence of agonist) [11,12]. Highly selective A_3AR agonists have been synthesized, and molecular recognition in the binding site has been characterized using site-directed mutagenesis and molecular modeling. Typical A_3AR agonists are adenosine derivatives that contain 5′-uronamide and N^6-benzyl modifications leading to nanomolar receptor

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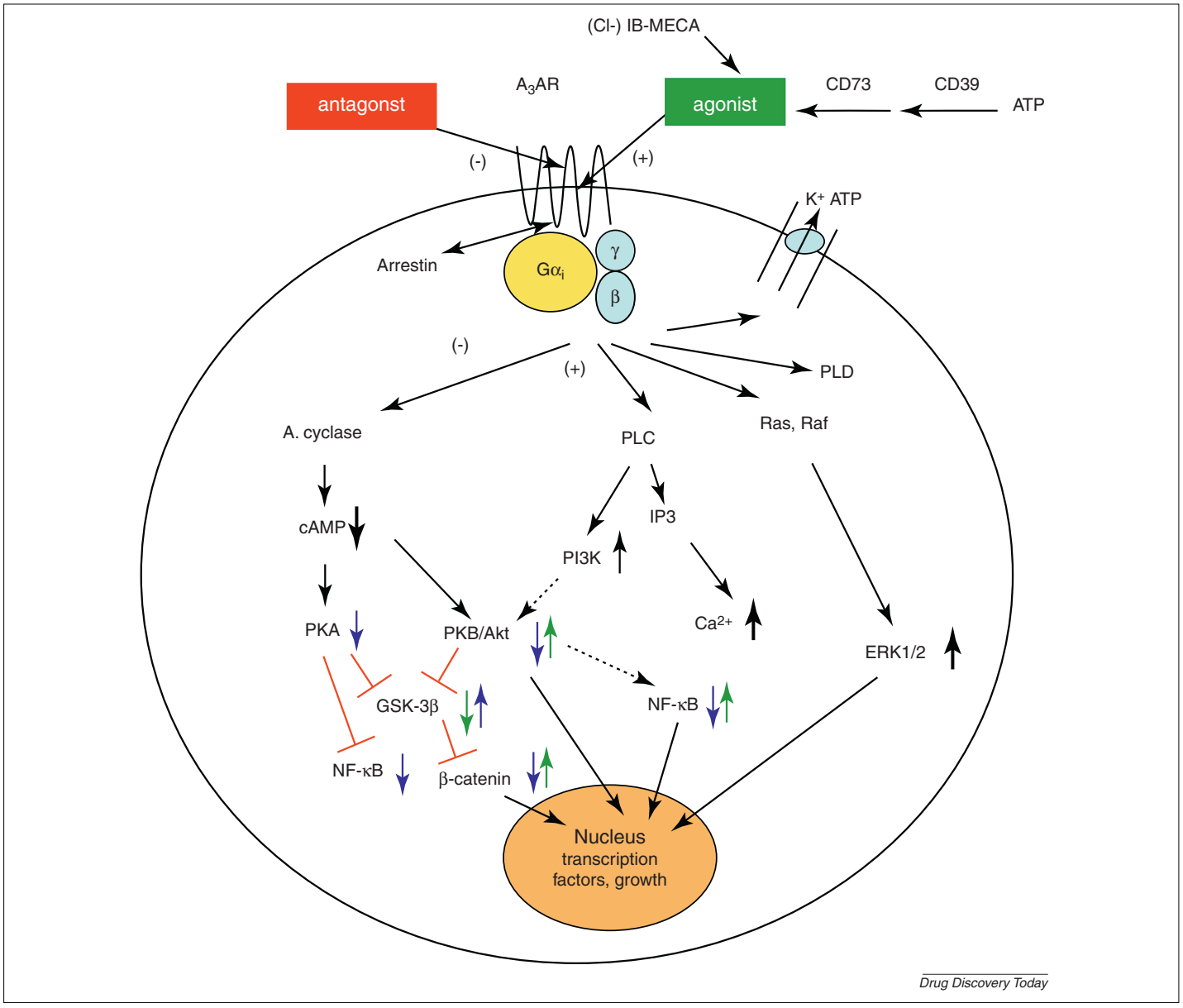

FIGURE 1

Signaling pathways involved in the action of A_3AR agonists. Pathways proposed to be involved in anticancer, anti-inflammatory and cardioprotective effects of A_3AR agonists. A_3AR signals through both G protein-dependent (through G_i or β, γ subunits) and independent pathways. Translocation of arrestin to the A_3AR would be associated with receptor downregulation [22] and G protein-independent signaling. Not all pathways are present in all circumstances, and other pathways not shown can also affect cell survival, proliferation and differentiation. In cancer (blue arrows), activation of the A_3AR corrects an imbalance in the downstream Wnt signaling pathway [6,34]. Administration of an A_3 agonist to activate its cell surface receptor inhibits the formation of cAMP and indirectly decreases phosphorylation (and therefore decreased inactivation) of the serine/threonine kinase GSK-3β. The resulting increased phosphorylation of β-catenin causes it to be removed from the cytoplasm by ubiquitination and therefore preventing its nuclear import. This results in a net suppression of cyclin D1 and c-myc, which leads to cell growth inhibition. With respect to cancer, NF-κB is a potent antiapoptotic agent in malignant cells and its activation is strongly associated with tumors [76]. Additionally, as a major cell survival signal, NF-κB is involved in multiple steps in carcinogenesis and in cancer cells' resistance to chemo- and radiotherapy. Thus, drugs aimed to decrease expression or activity of NF-κB could abrogate its antiapoptotic effect. In inflammatory models [7–9,20,29,71], the reduced activation of NF-κB (in synoviocytes, neutrophils and other immune cells) has an anti-inflammatory effect, in part by reducing the expression of TNF-α. Opposite effects of A_3AR activation on some of these pathways (green arrows) are associated with myeloprotective (through increased NF-κB in splenocytes [39]) and cardioprotective (GSK-3β inhibition [47]) responses to A_3AR agonists. In the heart, there are opposing effects of GSK-3β at different stages of prolonged ischemia (GSK-3β protects) and reperfusion (GSK-3β inhibition protects) [77].

affinity (compounds numbered in bold as shown in Fig. 2) [13]. The prototypical agonists IB-MECA **1**, N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine, CF101, and its 2-chloro analog Cl-IB-MECA **2**, 2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine, CF102, are 9-ribosides with A_3AR selectivity. Other selective A_3 agonists include the 4′-thio derivative **3**, 3′-amino-3′-deoxy derivatives CP-608,039 **5**, (2S,3S,4R,5R)-3-amino-5-{6-[5-chloro-2-(3-methylisoxazol-5-ylmethoxy)benzylamino]-purin-9-yl}-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide, and its dichlorobenzyl analog CP-532,903 **4**, which were originally developed for cardioprotection, and the N^6-methyl-2-ethynyl derivative **6** [9,14]. Introduction of a fused

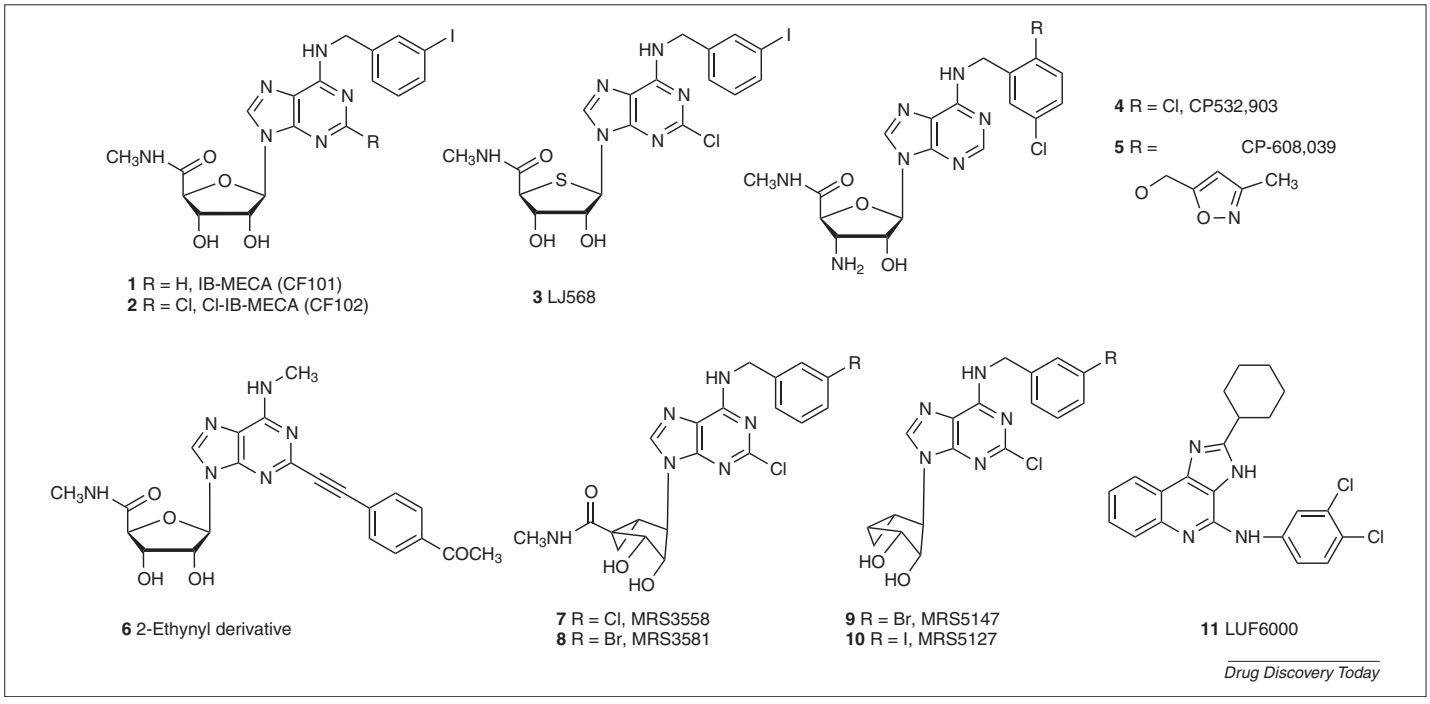

FIGURE 2

Representative agonists (**1**–**8**) and partial agonists (**9**, **10**) of nanomolar affinity at the A_3AR and a positive allosteric modulator **11**. Nucleosides **7**–**10** contain the (North) methanocarba substitution of the ribose ring, which maintains an A_3AR-preferred conformation.

bicyclic ring in the rigid analog MRS3558 **7**, (1′R,2′R,3′S,4′R,5′S)-4-{2-chloro-6-[(3-chlorophenylmethyl)amino]-purin-9-yl}-1-(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, increased A_3AR potency and selectivity, and identified the North conformation of the ribose ring as the preferred conformation in receptor binding. **7** also shows a preference in potency for the cAMP pathway, in comparsion with arrestin signaling [12]. Truncation of nucleosides at the 4′-position reduces efficacy while retaining affinity of binding to the A_3AR. Thus, the methanocarba analogs MRS5147 **9**, (1′R,2′R,3′S,4′R,5′S)-4′-[2-chloro-6-(3-bromobenzylamino)-purine]-2′,3′-O-dihydroxybicyclo-[3.1.0]hexane, and its 3-iodo analog MRS5127 **10** are low efficacy partial A_3AR agonists, that are selective in both human and rat [15]. Recently, macromolecular conjugates (e.g. polyamidoamine dendrimers) of chemically functionalized AR agonists were introduced as potent polyvalent activators of the receptors that are qualitatively different in pharmacological characteristics in comparison with the monomeric agonists [16]. Several A_3AR positron emission tomography (PET) ligands have been introduced for *in vivo* imaging: the antagonist [^{18}F]FE@SUPPY 5-(2-fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate [16], and a pair of nucleosides (e.g. low efficacy agonist [^{76}Br]MRS5147 **9** and full agonist [^{76}Br]MRS3581 **8**).

The selectivity of A_3AR agonists differs between *in vitro* and *in vivo* models and between species, although the sequence identity is high (84.4%) within the transmembrane region. The characterization of a given nucleoside derivative as full or partial agonist is highly dependent on the pharmacological system, such that **2** ranges from full agonist to low efficacy partial agonist [17]. LUF6000 **11**, *N*-(3,4-dichloro-phenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine, is a selective positive allosteric modulator of the human A_3AR [18], increasing the maximal effect of inhibition of adenylate cyclase. Species-dependence of the affinity and selectivity of A_3AR antagonists should be carefully considered in preclinical studies. Functional polymorphism of A_3AR is known; a high-transcript haplotype of the A_3AR gene was associated with the development of cutaneous hyper-reactivity to aspirin [19].

Differential expression of A_3AR in pathological and normal cells

A_3AR was found to be overexpressed in various neoplastic cells, including leukemia, lymphoma, astrocytoma, melanoma and pineal tumor cells, whereas low or almost no receptor expression was found in normal cells [20–25]. Similar data were reported in other studies, the receptor expression levels in tumor tissues derived from patients with colon, breast, small cell lung, pancreatic and hepatocellular carcinomas, and melanoma in direct comparison with adjacent normal tissues [3,4,6]. A direct correlation between A_3AR tissue expression levels and disease progression was described in breast and colon cancer [3,4].

A similar pattern of receptor overexpression was described in inflammatory cells both in experimental animal models and humans. The most studied inflammatory disease was rheumatoid arthritis (RA) in which A_3AR overexpression was detected in paw tissue, draining lymph nodes and synovial cells of rats with adjuvant-induced arthritis and in synovial cells from patients with RA [7]. Similar data were observed in colon tissues derived from rats with colitis and in lungs upon inhalation of lipopolysaccharides (LPS) by mice [26,27]. The receptor was also highly expressed in anterior segment tissues derived from eyes with pseudoexfoliation syndrome in comparison with healthy subjects' eyes [28].

The high expression levels of A_3AR seen in tumor and inflammatory cells were also found in PBMCs derived from tumor-bearing animals and cancer patients [3,6]. Similarly, high receptor

expression levels were found in PBMCs derived from experimental animal models of inflammation and from patients with autoimmune inflammatory diseases, such RA, psoriasis and Crohn's disease [5,7,29].

These data suggest that A_3AR expression levels in PBMCs mirror the receptor expression levels in the remote tumor or inflammatory tissue, rendering the receptor a biological marker. A_3AR upregulation is attributed to factors, including elevated adenosine and cytokines, which are characteristic of the microenvironment of cancer and inflammatory cells [29,30]. Under stressed metabolic conditions, extracellular adenosine of intracellular origin accumulates in the surroundings [30,31]. Upon binding to cell surface receptors, adenosine might induce, through an autocrine pathway, the expression of its own receptors. The proinflammatory cytokine tumor necrosis factor-α (TNF-α) initiates downstream signaling by binding to its cell surface receptor, to result in upregulation of protein kinase B (PKB)/Akt, the inhibitor of NF-κB light polypeptide gene enhancer in B cells (IκB), IκB kinase (IKK) and the transcription factor NF-κB [24,28]. The latter is known to act as an A_3AR transcription factor.

Bioinformatic analyses revealed that besides NF-κB, other transcription factors, such as c-Rel, MyoD, c-fos, GR, CREB, AP-1, GATA-1, C/EBP, c-Jun and PU.1 bind to the A_3AR promoter region. It is well established that proinflammatory cytokines regulate the cell expression levels of each of these transcription factors, hence regulating A_3AR expression levels [5]. Taken together, it seems that receptor overexpression in tumors and inflammatory cells is a consequence and manifestation of the disease state, rather than a causative factor.

Interestingly, *in vivo* pharmacological data revealed that chronic treatment with A_3AR agonist in various experimental animal models of cancer and inflammation did not desensitize the receptor. This was evidenced by the downregulation of receptor expression levels shortly after the last drug administration in a chronic mode of treatment [6,7,32]. In addition, 24 hours after the last agonist administration, A_3AR protein expression level was fully recovered to the control level, demonstrating that chronic treatment does not reduce the receptor expression levels [32].

In vivo pharmacological profile of A_3AR agonists

Anticancer effect

In experimental animal models, A_3AR agonists were efficacious in combating growth of solid tumors, including melanoma, prostate, colon and hepatocellular carcinoma (Table 1). The agonists showed efficacy upon chronic oral treatment, which was initiated after the tumor was already established. Overall, the drugs were much more potent in the syngeneic models rather than in the xenograft models, pointing toward an immunological effect on top of the direct anticancer effect. Supporting this notion are the findings showing that treatment with **2** increased interleukin (IL)-12 and potentiated natural killer (NK) activity in an animal model of melanoma [33].

The direct mechanism of the anticancer effect of A_3AR agonists entails modulation of the NF-κB and the Wnt signaling pathways. In tumor lesions of A_3AR agonist-treated animals, the expression levels of PKB/Akt, IKK, NF-κB and TNF-α signaling proteins were downregulated. The expression of glycogen synthase kinase-3β (GSK-3β) was upregulated, whereas the expresssion of its downstream proteins, β-catenin, lymphoid enhancer-binding factor-1 (LEF1) and c-Myc, was decreased, leading to inhibition of tumor cell growth [6,32,34]. Apoptosis, an additional mechanism of action, was demonstrated in hepatocellular carcinoma tumors, and manifested by increased expression of the proapoptotic proteins Bcl-2-associated death promoter (BAD), Bcl-2-associated X protien (BAX) and Caspase-3 upon treatment with **2** [6]. These data prompted the selection of **2** as a drug candidate to be developed as an anticancer agent for the treatment of hepatocellular carcinoma.

Anti-inflammatory effect

A_3AR agonists possess a robust anti-inflammatory effect mediated by the inhibition of proinflammatory cytokines [35–37]. **1**, **2** and **3** exert anti-inflammatory effects in experimental animal models of inflammatory bowel disease, systemic toxemia, pulmonary inflammation, RA, osteoarthritis and liver inflammation (Table 2). The molecular mechanism involved with the anti-inflammatory activity entails deregulation of the NF-κB signaling pathway, leading to inhibition of TNF-α, IL-6, IL-12, macrophage inflammatory proteins (MIPs)-1α, MIP-2 and receptor activator of NF-Kβ ligand (RANKL), resulting in apoptosis of inflammatory cells [7,38].

Protective effects

Chemoprotective effect

Myelotoxicity is a severe and dose-limiting complication of chemotherapy. Drug-induced myelosuppression is a major toxic factor that limits the administration of larger, potentially more effective doses of chemotherapy.

A_3AR agonists administered in combination with chemotherapeutic agents to tumor-bearing mice prevented the myelotoxic

TABLE 1

Effects of A_3AR agonists on growth of solid tumors in experimental animal models

Inflammatory condition	Experimental animal model	Refs
RA	Adjuvant and collagen-induced arthritis	[7,38,68–70]
Osteoarthritis	Monosodium iodoacetate-induced osteoarthritis	[71]
Inflammatory bowel diseases	Dextran sodium sulfate or 2,4,6-trinitrobenzene sulfonic acid-induced colitis; spontaneous colitis in IL-10 gene deficient mice	[26,37,72]
Uveitis	IRBP induced experimental autoimmune uveitis	[73]
Sepsis/toxemia	CLP and LPS-induced sepsis	[35,74]
Pulmonary inflammation	LPS inhalation	[27]

Abbreviations: CLP: cecal/ligation and puncture; IRBP: interphotoreceptor retinal binding protein.

TABLE 2

Effects of A_3AR agonists in experimental animal models of inflammatory disease

Tumor type	Animal strain; cell line	Experimental model	Refs
Melanoma	Mice; B16-F10	Syngeneic; metastatic Syngeneic; subcutaneous	[33,62,63]
Prostate cancer	Rat; AT6.1 Mice; PC3	Xenograft; metastatic Xenograft, subcutaneous	[32,64]
Colon carcinoma	Mice; HCT-116 Mice; CT-26	Xenograft, subcutaneous Syngeneic; metastatic	[34,65,66]
Breast cancer	Mice; SK-BR-3	Xenograft, subcutaneous	[75]
Hepatocellular carcinoma	Rat; N1S1 Mice; Hep3B	Syngeneic; orthotopic Xenograft, subcutaneous	[6,67]

effects of chemotherapy [39]. Coadministration of **1** prevented a decline in white blood cell (WBC) and neutrophil counts, resulting in full recovery of myeloid system parameters. The A_3AR agonist induced the production of granulocyte colony-stimulating factor (G-CSF), which stimulates myeloid progenitor cell expansion in the bone marrow and increases the WBC and neutrophil counts in the peripheral blood. The molecular mechanism underlying the events before G-CSF production includes the upregulation of its transcription factor NF-κB and the upstream kinases PI3K, PKB/Akt and IKK [39].

In the cardiovascular system, A_3AR agonists induce cardioprotection against chemotherapy-induced damage. The anthracycline antibiotic doxorubicin (DOX) or adriamycin has been an effective treatment for leukemias, lymphomas and solid tumors, including breast cancer.

Acute cardiotoxicity of DOX develops during and shortly after the initiation of therapy. However, chronic or late DOX-induced cardiotoxicity has a latency lasting for years before the development of overt heart failure. At present, only dexrazoxane, a free-radical scavenger, shows promise as a cardioprotective agent during DOX treatment [40]. Developing new methods to reduce both acute and chronic cardiotoxicity should increase the effectiveness of this anticancer therapy.

In this context, it is interesting to note that the A_3 agonist **2** can protect against mitochondrial damage and helps preserve ATP production in cultured rat cardiomyocytes. Repeated intravenous injection of **2** before DOX administration in rats helped prevent left ventricular wall thinning and dysfunction [41,42]. It is unknown whether continuous treatment with **2** can delay or prevent the late DOX cardiotoxicity.

Cardioprotective anti-ischemic effect

A_3AR agonists protect against myocardial ischemia/reperfusion (I/R) injury, this has been demonstrated using selective agonists and A_3AR-knockout mice, which are otherwise physiologically normal [43]. The cardioprotective effect is evident in role of the A_3AR in ischemic preconditioning and in direct protection during ischemia. The A_3AR might also be involved in mediating the post-conditioning effect given its ability to reduce infarct size when it is administered during reperfusion [9]. The A_3AR has the lowest level of myocardial expression among the ARs, at least for the murine heart. However, evidence has accumulated indicating that stimulation of an endogenous cardiac A_3AR, independent of circulating immune inflammatory cells or resident mast cells, can result in cardioprotection [44]. An anti-inflammatory action of the A_3AR *in vivo* might also contribute importantly to the cardioprotective effect of A_3 agonist. Both a direct cardioprotective mechanism and an anti-inflammatory effect exerted at the immune cell level *in vivo* might be important. Future studies are needed to address this question. A cardioprotective role of the A_3AR was also found in nonrodent mammals, such as rabbits and dogs [45,46].

Mediators that have a direct myocardial protective effect include protein kinase C (PKC), K_{ATP} channels, reactive oxygen species, connexin 43, mitochondrial permeability transition pore (MPTP) and GSK-3β. Thus, a signaling cascade might begin with A_3AR stimulation, PKC activation, phosphorylation (and thus inactivation) of GSK-3β, leading to inhibition of MPTP and reduced cardiac myocyte death [47]. The role of sarcolemmal versus that of mitochondrial K_{ATP} channels in mediating the A_3 cardioprotection is not clear. There is extensive evidence for a protective role of mitochondrial K_{ATP} (mito K_{ATP}) channel including the recently elucidated function of connexin 43 in mediating mito K_{ATP} opening by PKC. A recent study, however, showed that sarcolemmal K_{ATP} deletion abrogated the preconditioning effect of A_3AR agonist in murine heart [43]. Given the redundancy of signaling pathways causing cardioprotection, it is possible that species differences exist in the role of such signaling molecules. Genetic background could also modulate cardioprotection not only in mice but also humans.

Protection of skeletal muscle

A_3AR agonists attenuate skeletal muscle injury caused by ischemia and reperfusion or eccentric exercise [48]. Skeletal muscle is susceptible to various forms of injury, including ischemia, trauma and physical exertion. Skeletal muscle is one of the most vulnerable tissues in the extremities. Thus, developing new methods designed to provide cytoprotection to the skeletal muscle is important. Direct infusion of adenosine can mimic the skeletal muscle protective effect of ischemic preconditioning in the extensor digitorum longus muscle before aorta occlusion in the rat in addition to in the pig latissimus dorsi muscle flap model. A_3AR agonist, when administered *in vivo*, signals selectively through phospholipase PLCβ2/β3 to cause a reduction in skeletal muscle injury sustained either during I/R or eccentric exercise [48]. Although A_1 and A_{2A}ARs can also mediate anti-ischemic protection in skeletal muscle, only the A_3AR can induce protection against both I/R and eccentric exercise injuries.

Given that the activation of the A_3AR has a known anti-inflammatory effect; it is possible that skeletal muscle protective effect is

mediated, at least, in part at an immune cell level. The following lines of evidence support this hypothesis. First, activated mast cells and neutrophils are important contributors of skeletal muscle ischemia/reperfusion damage. Second, activation of the A_3AR can block superoxide formation and chemotaxis of murine bone marrow neutrophils [49].

Lung ischemia/reperfusion protection

A_3AR agonist prevents lung injury following ischemia/reperfusion in the cat. Compound **3** produced a sustained protection, which was associated with suppressed p38 protein expression and down-regulation of its phosphorylation [50,51].

Neuroprotection

Evidence from diverse models suggests that neuroprotective effects might be mediated by the A_3AR, but differences between acute and chronic agonist administration have been noted [10]. Ischemic brain injury in a model of forebrain ischemia in gerbils is reduced upon chronic treatment with **1** [52]. A_3AR agonist was found to prevent the loss of retinal ganglion cells following activation of the P2X7 receptor in a rat experimental model [53].

CF101 for the treatment of inflammatory and ophthalmic diseases

Based on the preclinical pharmacology data and encouraging safety data in Phase I studies [54], the anti-inflammatory effect of **1** was tested in a set of three Phase II clinical studies, including RA, psoriasis and dry eye syndrome (Table 3). Overall, the data obtained from these clinical studies showed excellent safety profile and efficacy, positioning **1** as a disease-modifying anti-inflammatory drug.

RA

RA is a chronic, systemic inflammatory disorder attacking joints that results in inflammatory synovitis that could cause destruction of articular cartilage and bone [55]. The mechanisms responsible for causing joint damage and functional impairment in RA are complicated and involve B cell or T-cell products stimulating the release of TNF and other proinflammatory cytokines, including IL-1, IL-6, and TNF-α and degradative enzymes.

In a multicenter Phase II study, blinded to dose (0.1, 1.0 or 4.0 mg), the drug was administered orally, twice daily for 12 weeks to patients with active RA. The primary efficacy endpoint was an improvement of 20% or more according to the classification of RA responses by the American College of Rheumatology (ACR) [55]. Compound **1** was found to be safe and well tolerated, and the maximal responses were observed in patients treated with a 1.0 mg dose. At 12 weeks, 55.6%, 33.3%, and 11.5% of the patients receiving 1.0 mg **1** achieved ACR 20%, 50%, and 70% responses, respectively. In addition, a statistically significant correlation between A_3AR expression at baseline and patient response to **1** was observed, rendering the A_3AR as a biological predictive marker [56].

Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyper-proliferation and immature differentiation resulting in multisystem pathology and a negative impact on the quality of life of the patients [57]. Proinflammatory cytokines, such as interferon (INF)-γ, TNF-α, IL-23 and T helper (Th)17 are known to have a role in mediating the inflammation and epidermal alterations in psoriasis [58].

The efficacy and safety of **1** were tested in a Phase II, multicenter, randomized, double blind, dose-ranging, placebo-controlled study in patients with moderate to severe chronic plaque-type psoriasis. Compound **1** (1, 2 or 4 mg) or placebo was administered orally twice daily for 12 weeks. Overall, the drug was safe and well tolerated.

The maximal improvement in the mean change from baseline in the psoriasis area and severity index (PASI) score versus placebo and the highest percentage of patients who achieved physician's global assessment (PGA) score of 0 or 1 were observed in the 2 mg **1**-treated group. The improvement was progressive and linear throughout the study period. Thus, **1** was safe and well tolerated.

Dry eye syndrome

Dry eye syndrome is an inflammatory condition of the eye that is caused by decreased tear production or increased tear film evaporation. It is characterized by massive production of proinflammatory cytokines. The dryness and the inflammation could result in eye damage leading to impaired vision [59,60].

TABLE 3

Past and present human clinical studies utilizing CF101 and CF102

Disease	Phase	Primary endpoints	Current status	Refs
RA	IIa	ACR20 response at week 12	Phase IIb	[56]
Psoriasis	II	Reduction in PASI score of at least 75% from baseline (PASI 75) to the end of the 12 weeks treatment period	Phase II/III	[57]
Dry eye syndrome	II	Improvement of 25% or more over baseline at week 12 in tear film BUT or in superficial punctate keratitis as assessed by either FS or ST1 results	Phase III	[60]
Hepatocellular carcinoma	I/II	To determine the safety, tolerability, dose-limiting toxicities, maximum tolerated dose, recommended Phase II dose and to assess the repeat-dose pharmacokinetic behavior of orally administered CF102	Ongoing	
Hepatitis C virus infection	I/II	To determine the safety and tolerability of 15 days of orally administered CF102 in patients with chronic hepatitis C genotype 1, to assess the effects on HCV load during 24 weeks and to assess the repeat-dose pharmacokinetic behavior of CF102	Ongoing	

FS: Fluorescein staining; ST1: Schirmer tear test 1.

Anecdotal findings demonstrating that **1** improved indicators of dry eye syndrome in RA patients led to a separate Phase II clinical study (randomized, multicenter, doubled-masked, placebo-controlled and parallel-group) of the safety and efficacy of **1** (1 mg) administered orally daily for 12 weeks to patients with moderate to severe dry eye syndrome. It was found that compound **1** was safe and well tolerated and no serious adverse events were noted throughout the study.

Treatment with **1** resulted in a statistically significant improvement in the mean change from baseline at week 12 of the clearance of corneal staining, tear break-up time and tear meniscus height in the group treated with **1** versus placebo. Compound **1** was well tolerated and exhibited an excellent safety profile with no serious adverse events. Interestingly, a statistically significant decrease from baseline was observed in the intraocular pressure of the **1**-treated patients in comparison with the placebo-treated group [61]. No serious adverse events in the RA, psoriasis or dry eye clinical studies were observed. The profile of the adverse events was similar between the placebo and **1**-treated groups.

Concluding remarks

Based on the experimental animal data and human clinical study results presented in this article, A_3AR is suggested as a specific and unique therapeutic target to combat proliferative diseases, including inflammation and cancer. The excellent safety profile of A_3AR agonists, currently tested in human clinical studies, is attributed to the different protective effects mediated through the receptor. The A_3AR has also been identified as a biological marker to predict a patient's eligibility for treatment with the agonists. Taken together, the utilization of A_3AR as both a biological predictive marker and as a therapeutic target encompasses a 'personalized medicine' approach and makes the A_3AR agonists promising small molecule drug candidates.

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