Pharmacological and therapeutic effects of A3 adenosine receptor agonists

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The A3 adenosine receptor (A3AR) coupled to G1 (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 A3AR as a potential therapeutic target. Highly selective A3AR 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 A3AR 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, A3AR 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 A3 adenosine receptor (A3AR) is a subtype of the adenosine receptor (AR) family, which additionally includes A1, A2A and A2B receptors [1,2]; each receptor is encoded by a separate gene and has different physiological roles. The G1-coupled A3AR is less widely distributed than other AR subtypes with expression in human lung, liver, brain, aorta, testis and heart. The utilization of the A3AR as a therapeutic target and a biological predictive marker is based on two major findings: (i) the A3AR 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 A3AR 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, A3AR agonists are being developed for the treatment of inflammatory, ophthalmic and liver diseases and demonstrate excellent safety and efficacy in Phase II clinical studies.

A3AR agonists
The human A3AR 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 A3AR in vitro (typically within ~20 min in the presence of agonist) [11,12]. Highly selective A3AR agonists have been synthesized, and molecular recognition in the binding site has been characterized using site-directed mutagenesis and molecular modeling. Typical A3AR agonists are adenosine derivatives that contain 5′-uronamide and N6-benzyl modifications leading to nanomolar receptor...
affinity (compounds numbered in bold as shown in Fig. 2) [13]. The prototypical agonists IB-MECA 1, N°-(3-iodobenzyl)-5'-N-methylcarboxamidoadenosine, CF101, and its 2-chloro analog CI-IB-MECA 2, 2-chloro-N°-(3-iodobenzyl)-5'-N-methylcarboxamidoadenosine, CF102, are 9-ribosides with A3AR selectivity. Other selective A3 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°-methyl-2-ethynyl derivative 6 [9,14]. Introduction of a fused
bicyclic ring in the rigid analog MRS3558 7, (1′R,2′R,3′S,4′R,5′S)-4-[2-chloro-6-[(3-chlorophenyl)methylamino]-purin-9-yl]-1-
(methylaminocarbonyl)-bicyclo[3.1.0]hexane-2,3-diol, increased A3AR 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 comparison with arrestin signaling [12]. Truncation of nucleosides at the 4′-position reduces efficacy while
retaining affinity of binding to the A3AR. Thus, the methanocarba
analog 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]hex-
ane, and its 3-iodo analog MRS5127 10 are low efficacy partial
A3AR agonists, that are selective in both human and rat [15].
Recently, macromolecular conjugates (e.g. polyamidoamine dendir-
mers) of chemically functionalized AR agonists were intro-
duced as potent polycation activators of the receptors that are
qualitatively different in pharmacological characteristics in com-
parison with the monomeric agonists [16]. Several A3AR positron
emission tomography (PET) ligands have been introduced for in
vivo imaging: the antagonist [18F]FE@SUPPY 5-(2-fluoroethyl)2,4-
diethyl-3-(ethoxysulfanylcarbonyl)-6-phenylpyridine-5-carboxy-
late [16], and a pair of nucleosides (e.g. low efficacy agonist
[76Br]MRS5147 9 and full agonist [76Br]MRS581 8).

The selectivity of A3AR 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 characteriza-
tion 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-1H-im-
dazo[4,5-c]quinolin-4-amine, is a selective positive allosteric mod-
ulator of the human A3AR [18], increasing the maximal effect of
inhibition of adenylate cyclase. Species-dependence of the affini-
ty and selectivity of A3AR antagonists should be carefully considered
in preclinical studies. Functional polymorphism of A3AR is known;
a high-transcript haplotype of the A3AR gene was associated with
the development of cutaneous hyper-reactivity to aspirin [19].

**Differential expression of A3AR in pathological and normal cells**

A3AR 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 com-
parison with adjacent normal tissues [3,4,6]. A direct correlation
between A3AR 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 A3AR 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 lipopolysacchar-
dides (LPS) by mice [26,27]. The receptor was also highly expressed
in anterior segment tissues derived from eyes with pseudoxofilia-
tion syndrome in comparison with healthy subjects’ eyes [28].

The high expression levels of A3AR seen in tumor and inflam-
matory cells were also found in PBMCs derived from tumor-bear-
ing animals and cancer patients [3,6]. Similarly, high receptor
expression levels were found in PBMCs and from patients with autoimmune inflammatory diseases, such as RA, psoriasis and Crohn’s disease [5,7,29].

These data suggest that A3AR expression levels in PBMCs mirror the receptor expression levels in the remote tumor or inflammatory tissue, rendering the receptor a biological marker. A3AR 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 (IkB), IkB kinase (IKK) and the transcription factor NF-κB [24,28]. The latter is known to act as an A3AR 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 A3AR promoter region. It is well established that proinflammatory cytokines regulate the cell expression levels of each of these transcription factors, hence regulating A3AR 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 A3AR 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, A3AR 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 A3AR agonists

Anticancer effect

In experimental animal models, A3AR 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 A3AR agonists entails modulation of the NF-κB and the Wnt signaling pathways. In tumor lesions of A3AR 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 expression 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 protein (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

A3AR 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-κB 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.

A3AR agonists administered in combination with chemotherapeutic agents to tumor-bearing mice prevented the myelotoxic

| TABLE 1 | Effects of A3AR 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.
Effects of $\alpha_3$AR agonists in experimental animal models of inflammatory disease

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Animal strain; cell line</th>
<th>Experimental model</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Mice; B16-F10</td>
<td>Syngeneic; metastatic</td>
<td>[33,62,63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Syngeneic; subcutaneous</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Rat; AT6.1 Mice; PC3</td>
<td>Xenograft; metastatic</td>
<td>[32,64]</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>Mice; HCT-116 Mice; CT-26</td>
<td>Xenograft; subcutaneous</td>
<td>[34,65,66]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Mice; SK-BR-3</td>
<td>Xenograft; subcutaneous</td>
<td>[75]</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Rat; N151 Mice; Hep3B</td>
<td>Syngeneic; orthotopic</td>
<td>[6,67]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xenograft; subcutaneous</td>
<td></td>
</tr>
</tbody>
</table>

The 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 $\alpha_3$AR 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-$\kappa$B and the upstream kinases P13K, PKB/Akt and IKK [39].

In the cardiovascular system, $\alpha_3$AR 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 $\alpha_3$ 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
$\alpha_3$AR agonists protect against myocardial ischemia/reperfusion (I/R) injury, this has been demonstrated using selective agonists and $\alpha_3$AR-knockout mice, which are otherwise physiologically normal [43]. The cardioprotective effect is evident in role of the $\alpha_3$AR in ischemic preconditioning and in direct protection during ischemia. The $\alpha_3$AR might also be involved in mediating the postconditioning effect given its ability to reduce infarct size when it is administered during reperfusion [9]. The $\alpha_3$AR has the lowest level of myocardial expression among the ARs, at least for the murine heart. However, evidence has accumulated indicating that stimulation of endogenous cardiac $\alpha_3$AR, independent of circulating immune inflammatory cells or resident mast cells, can result in cardioprotection [44]. An anti-inflammatory action of the $\alpha_3$AR in vivo might also contribute importantly to the cardioprotective effect of $\alpha_3$ 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 $\alpha_3$AR 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-3$\beta$. Thus, a signaling cascade might begin with $\alpha_3$AR stimulation, PKC activation, phosphorylation (and thus inactivation) of GSK-3$\beta$, leading to inhibition of MPTP and reduced cardiac myocyte death [47]. The role of sarcokemmal versus that of mitochondrial K$_{ATP}$ channels in mediating the $\alpha_3$ 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 sarcokemmal K$_{ATP}$ deletion abrogated the preconditioning effect of $\alpha_3$AR 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
$\alpha_3$AR 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 the pig latissimus dorsi muscle flap model. $\alpha_3$AR agonist, when administered in vivo, signals selectively through phospholipase PLC$\beta$2/3 to cause a reduction in skeletal muscle injury sustained either during I/R or eccentric exercise [48]. Although $\alpha_1$ and $\alpha_2$ARs can also mediate anti-ischemic protection in skeletal muscle, only the $\alpha_3$AR can induce protection against both I/R and eccentric exercise injuries.

Given that the activation of the $\alpha_3$AR 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 A3AR can block superoxide formation and chemotaxis of murine bone marrow neutrophils [49].

**Lung ischemia/reperfusion protection**

A3AR 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 A3AR, 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]. A3AR 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 A3AR expression at baseline and patient response to 1 was observed, rendering the A3AR 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, multi-center, 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>
<thead>
<tr>
<th>Disease</th>
<th>Phase</th>
<th>Primary endpoints</th>
<th>Current status</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>I/II</td>
<td>ACR20 response at week 12</td>
<td>Phase Ib</td>
<td>[56]</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>II</td>
<td>Reduction in PASI score of at least 75% from baseline (PASI 75) to the end of the 12 weeks treatment period</td>
<td>Phase II/III</td>
<td>[57]</td>
</tr>
<tr>
<td>Dry eye syndrome</td>
<td>II</td>
<td>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</td>
<td>Phase III</td>
<td>[60]</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>I/II</td>
<td>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</td>
<td>Ongoing</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C virus infection</td>
<td>I/II</td>
<td>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</td>
<td>Ongoing</td>
<td></td>
</tr>
</tbody>
</table>

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, double-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, A3AR is suggested as a specific and unique therapeutic target to combat proliferative diseases, including inflammation and cancer. The excellent safety profile of A3AR agonists, currently tested in human clinical studies, is attributed to the different protective effects mediated through the receptor. The A3AR has also been identified as a biological marker to predict a patient’s eligibility for treatment with the agonists. Taken together, the utilization of A3AR as both a biological predictive marker and as a therapeutic target encompasses a ‘personalized medicine’ approach and makes the A3AR agonists promising small molecule drug candidates.

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