

Targeting the A₃ adenosine receptor to treat cytokine release syndrome in cancer immunotherapy

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Abstract: Cancer patients undergoing immunotherapy may develop cytokine release syndrome (CRS), an inflammatory cytokine storm condition, followed by neurotoxic manifestations and may be life-threatening. The current treatments for CRS successfully reduce the inflammatory response but may limit the anticancer effect of the given immunotherapy and fail to overcome the neurotoxic adverse events. Adenosine, a ubiquitous purine nucleoside, induces a plethora of effects in the body via its binding to four adenosine receptors A₁, A_{2a}, A_{2b}, and the A₃. Highly selective agonists to the A₃ adenosine receptor act as inhibitors of proinflammatory cytokines, possess robust anti-inflammatory and anticancer activity, and concomitantly, induce neuroprotective effects. Piclidenoson and namodenoson belong to this group of compounds, are effective upon oral administration, show an excellent safety profile in human clinical studies, and therefore, may be considered as drug candidates to treat CRS. In this article, the detailed anti-inflammatory characteristics of these compounds and the rationale to use them as drugs to combat CRS are described.

Keywords: A₃, adenosine receptor, cytokine release syndrome, treatment, immunotherapy

Introduction

Cancer immunotherapy includes checkpoint inhibitors, bispecific antibodies, and chimeric antigen receptor (CAR) T cells, altogether utilizing the patient's own immune system to fight cancer, having a high potential to reach complete remission.^{1,2} This approach is especially beneficial in patients presenting an advanced stage of disease at the time of diagnosis where traditional cancer treatments have very limited efficacy and in patients with refractory/relapsed diseases.²⁻⁷ However, along with the high beneficial effects, patients treated with immunotherapy drugs may experience cytokine release syndrome (CRS) as an adverse or severe adverse event (AE or SAE, respectively).

CRS is defined as an inflammatory condition occurring when a large number of lymphocytes and/or myeloid cells are being activated, releasing high levels of inflammatory cytokines. CRS usually occurs within minutes or hours following treatment; however, it can also take place days or weeks later. A recent meta-analysis looking at the efficacy and safety of bispecific T-cell engager (BiTE) antibody blinatumomab for the treatment of relapsed/refractory acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL) found that the pooled occurrence rate of grade ≥ 3 CRS was 0.04 (95% CI: 0.01–0.06) and the pooled occurrence of grade ≥ 3 neurological events was 0.12 (95% CI: 0.08–0.12).^{6,2}

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The timing and symptoms can vary depending on the type of immunotherapy and the magnitude of immune cell activation. CRS is manifested by high fever, nausea, headache, tachycardia/hypotension, cardiac dysfunction, rash, and shortness of breath with some patients experiencing severe inflammatory syndrome resulting in multiorgan failure which can lead to a life-threatening event.^{8–10} Neurotoxicity is an additional manifestation, mostly appears after the CRS has been resolved and is characterized by signs of neurological dysfunction which may be lethal. It has been speculated that this form of neurotoxicity is linked to immunotherapy which targets the CD19 antigen.^{11–13}

The main cytokines involved with CRS include tumor necrosis factor- α (TNF- α), interferon γ (IFN- γ), interleukin 1 β (IL-1 β), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 8 (IL-8), and interleukin 10 (IL-10), all known to be involved in the regulation of the innate and cellular immunity.¹⁰ Following immunotherapy, the inflammatory cytokines are released, enhancing the immune response and activating the proliferation of immune cells to further secrete more inflammatory cytokines. This chain of events leads to a loop between the inflammatory cytokines and the immune cells, which may result in a cytokine storm.¹⁴

Although many cytokines contribute to CRS, previous work indicates that CRS is at least partially IL-6 mediated.^{1,8,15,16} IL-6 is involved in promoting neutrophil trafficking, B-cell differentiation, and autoantibody production.¹⁷ In patients with CRS, following CAR-T therapy, IL-6 levels reach a peak during maximal T cell proliferation, suggesting that IL-6 blockade will reduce CRS toxicity.¹⁰ However, new findings suggest that circulating monocytes secreting IL-1 are the primary cells responsible for the initiation of CRS. It was found that IL-1 is secreted hours before IL-6 and is capable of inducing both IL-6 secretion and soluble IL-6 receptor, and that the IL-1 α receptor antagonist, anakinra, reduces both CRS and neurotoxicity.¹⁸ Similarly, the anti-IL-6 monoclonal antibody, tocilizumab, is US FDA approved and has also been shown to reduce the incidence of CAR-T-induced CRS.⁶³

In addition, TNF- α is a key regulatory cytokine of the inflammatory response known to mediate inflammation in rheumatoid arthritis (RA), inflammatory bowel disease, ankylosing spondylitis, psoriasis, inflammatory diseases of the central nerve system, cardiovascular, renal, and respiratory diseases.^{8,19} The release of TNF- α has been shown to play a role in the pathogenesis of CRS.⁶⁴

CRS: current treatment

CRS therapy needs to meet two criteria, ie, to overcome the severity of the symptoms, aiming at the prevention of a

life-threatening toxicity without having any negative anti-tumor effect of the immunotherapy.

Corticosteroids are immunosuppressive agents widely used in the treatment of CRS-related diseases and were proven to be efficacious in combating CRS.^{20,21} However, corticosteroids have widespread adverse effects on the immune system that can limit or damage the antitumor treatment.²²

The current preferred treatment for CRS is tocilizumab, a recombinant humanized monoclonal antibody against IL-6 receptor, which blocks IL-6 from binding to its receptor. Currently, tocilizumab is used for the treatment of RA,²³ juvenile idiopathic arthritis,²⁴ and poly-articular juvenile RA,²⁵ known to bind and reduce IL-6 levels, thereby acting as an anti-inflammatory agent. The most common AEs of tocilizumab include elevation in liver enzymes, neutropenia, and thrombocytopenia.¹⁰ Preliminary clinical results demonstrate that although tocilizumab is effective in resolving CRS condition, it may fail preventing the neurotoxicity outcome, which may follow CRS.^{11,12}

Although in most cases, the patients overcome the CRS symptoms, in some situations, CRS remains unresolved even after using a combined treatment of corticosteroids and tocilizumab. In those patients, the mortality outcome can be fatal.^{11,26} In patients with severe CRS, a delayed recovery of the hematopoietic system was observed, increasing the chances of infections particularly while using tocilizumab, which can worsen neutropenia.²⁶

Overall, it looks like the current treatments for CRS are not satisfactory, and there is a need for a drug that will concomitantly act as a robust anti-inflammatory and prevent as well the neurotoxic manifestations, while supporting the antitumor effect of the immunotherapy.

Adenosine inhibits inflammatory cytokine production via the A₃ adenosine receptor

Adenosine is a ubiquitous purine nucleoside produced during inflammation, hypoxia, ischemia, or trauma and is released into the extracellular environment from metabolically active or stressed cells.²⁷ Adenosine is known to regulate proliferation, differentiation, and cell death by binding to one of its four G protein-associated cell surface receptors A₁, A_{2a}, A_{2b}, and A₃.^{27,28} Adenosine induces an inhibition of cyclic adenosine monophosphate (cAMP) upon binding to the A_{2a} and A_{2b} adenosine receptors (ARs), whereas A₁AR and A₃AR activation inhibits adenylate cyclase and cAMP. A₃AR activation also results in the inhibition of PI3K/Akt and a subsequent deregulation of nuclear factor κ B (NF- κ B) and MAPK signaling pathways resulting in anti-inflammatory

Table 1 Adenosine anti-inflammatory effect is A₃AR mediated

	Study	Findings
Vincenzi et al 2013 ⁴²	Pulsed electromagnetic fields increased the anti-inflammatory effect of A _{2a} and A ₃ adenosine receptors in human T/C-28a2 chondrocytes and hFOB 1.19 osteoblasts.	An in vitro model of inflammatory bone and joint disorder – A ₃ AR activation by adenosine was efficacious in decrease of some of the most relevant pro-inflammatory cytokines release such as IL-6 and IL-8.
Antonioli et al 2010 ⁴³	The blockade of adenosine deaminase ameliorates chronic experimental colitis through the recruitment of adenosine A _{2a} and A ₃ receptors.	Inflammatory model of colitis – ADA inhibitors and A ₃ AR antagonists induced a decrease in TNF- α and IL-6. By blocking ADA, adenosine accumulates in the inflamed environment and binds to its receptors. Cytokine inhibition was counteracted by antagonizing the A ₃ AR or A _{2a} AR (but not A ₁ AR and A _{2b} AR), demonstrating that at least part of the adenosine effect was A ₃ AR mediated.
Baharav et al 2002 ⁴⁴	Suppression of experimental zymosan-induced arthritis by intraperitoneal administration of adenosine.	The administration of adenosine significantly reduced serum TNF- α levels and resulted in remarkable clinical benefit in an animal model of zymosan-induced arthritis. Since adenosine is a small molecule with a half-life time of <20 seconds, rapidly metabolizing to inosine, it cannot be utilized as a drug candidate. ²⁸
Madi et al 2007 ⁶¹	Overexpression of A ₃ adenosine receptor in peripheral blood mononuclear cells in rheumatoid arthritis: involvement of nuclear factor-kappa B in mediating receptor level.	Overexpression of A ₃ AR was found in PBMC of RA patients. Receptor upregulation was induced by inflammatory cytokines controlling the expression of the A ₃ AR transcription factor NF-kappa B.
Gessi et al 2004 ⁶⁵	Expression of A ₃ adenosine receptors in human lymphocytes: upregulation in T cell activation.	An in-depth investigation of A ₃ receptors in human lymphocytes demonstrates that, under activating conditions, they are upregulated and may contribute to the effects triggered by adenosine.

Abbreviations: A₃AR, A₃ adenosine receptor; ADA, adenosine deaminase; IL, interleukin; NF-kappaB, nuclear factor κ B; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α .

and anticancer effects.^{29,30} Interestingly, at the same time, adenosine induces cardio-, neuro- and chemo-protective effects manifested by regulation of electrophysiological properties, suppressing neurotransmitter release, modulating dopaminergic motor activity, inhibiting cytokine release and platelet aggregation, inducing erythropoietin production, and modulating lymphocyte function.^{28,31–33} This differential effect of adenosine depends on its extracellular concentration, receptor density on the cell surface, and the physiological state of the target cell, leading to apoptosis of pathological cells and the protective effects toward normal body cells.²⁸

A₃AR is expressed on all types of the immune cells with a broad distribution in inflammatory cells compared with very low expression on normal cells.^{34,35} In addition, a direct correlation has been found between A₃AR expression level and disease progression in inflammatory and cancer diseases in both experimental animal models and humans.^{35–38}

The involvement of adenosine in mediating the anti-inflammatory effects of methotrexate (MTX) and aspirin has been documented.

MTX increases the extracellular adenosine concentration as part of its metabolism, subsequently binding to the A_{2a}AR and A₃AR, inhibiting the release of TNF- α , IL-6, and IL-1.^{39,40} Adenosine was also found to be part of the anti-inflammatory effect of aspirin via inhibition of adenosine deaminase (ADA), an enzyme responsible for the conversion

of adenosine into inosine, resulting in adenosine accumulation and induction of an anti-inflammatory effect.⁴¹

Several additional studies support the notion that the anti-inflammatory effects of adenosine are A₃AR mediated (Table 1).

1. An in vitro model of inflammatory bone and joint disorder – A₃AR activation by adenosine was efficacious in inhibiting IL-6 and IL-8.⁴²
2. Inflammatory model of colitis – ADA inhibitors and A₃AR antagonists induced a decrease in TNF- α and IL-6. By blocking ADA, adenosine accumulates in the inflamed environment and binds to its receptors. Cytokine inhibition was counteracted by antagonizing the A₃AR or A_{2a}AR (but not A₁AR and A_{2b}AR), demonstrating that at least part of the adenosine effect was A₃AR mediated.⁴³ In the zymosan-induced arthritis model, adenosine reduced TNF- α secretion.⁴⁴

Since adenosine is a small molecule with a half-life time of <20 seconds, rapidly metabolizing to inosine, it cannot be utilized as a drug candidate.²⁸

A₃AR agonists inhibit inflammatory cytokine production

Highly selective A₃AR agonists inhibit the production of inflammatory cytokines via downregulation of NF- κ B.⁴⁵ The NF- κ B pathway has long been considered a proto-

typical proinflammatory signaling pathway. NF- κ B is a transcription factor that induces the production of a panel of pro-inflammatory cytokines including TNF- α , IL-1, IL-6, all of which have well-defined roles in the pathogenesis of RA, inflammatory bowel disease, asthma, and COPD, and affects leukocyte recruitment and mediation of cell survival.⁴⁶ At the same time, NF- κ B is present in the A₃AR gene promoter, thereby controlling the expression level of the receptor. Ochaion et al showed a direct correlation between A₃AR over-expression and NF- κ B levels in peripheral blood mononuclear cells derived from patients with RA, psoriasis, and Crohn's disease.⁴⁷ A₃AR agonists induce an inhibition of PI3K-PKB/Akt, upon binding to the receptor, which is over-expressed in inflammatory cells. As a result, a decrease in NF- κ B expression level takes place followed by inhibition of TNF- α .⁴⁸⁻⁵⁰

This mechanism of action and the subsequent anti-inflammatory effect have been recorded in a plethora of in vitro and in vivo studies as is specified herewith.

In vitro studies

1. Cultured lymphocytes obtained from RA patients showed that A₃AR was upregulated over two fold when compared with healthy controls.⁶⁵ A₃AR activation by CI-IB-MECA markedly reduced the secretion of the inflammatory cytokines TNF- α , IL-6, and IL-1 β and reduced NF- κ B activation after phorbol myristate acetate induction, and the inhibitory effect mediated by A₃AR was more prominent in RA patients than in healthy controls.⁴⁵
2. CF502, a novel A₃AR agonist with high affinity and selectivity at the human A₃AR, induced a dose-dependent inhibitory effect on the proliferation of fibroblast-like synoviocytes via deregulation of the NF- κ B signaling pathway. A subsequent decrease in TNF- α and the expression of inflammatory response as measured by glycogen synthase kinase-3 beta (GSK-3 β), β -catenin was also observed via regulation of NF- κ B.⁵⁰
3. The effects of IB-MECA, a selective agonist of A₃AR, were evaluated in a psoriasis model of human keratinocytes (HaCat) cells. IB-MECA induced inhibition of interleukin 17 and interleukin 23 expression levels, mediated via NF- κ B downregulation and inhibition of TNF- α .⁵¹
4. CI-IB-MECA suppressed the expression of pro-inflammatory biomarkers including inducible nitric oxide synthase (iNOS), IL-1 β , and TNF- α in murine macrophages (RAW 264.7) activated with lipopolysaccharide (LPS). CI-IB-MECA also reduced mRNA levels and inhibited LPS-induced PI3 kinase/Akt activation,

NF- κ B binding activity, and β -catenin expression in a dose-dependent manner.⁵²

5. Adenosine and CI-IB-MECA suppressed LPS-induced TNF- α protein and mRNA levels. CI-IB-MECA inhibited LPS-induced NF- κ B DNA binding and luciferase reporter activity. A₃AR activation suppresses TNF- α production by inhibiting PI3 kinase/AKT and NF- κ B activation in LPS-treated BV2 microglial cells.⁵³

In vivo studies

Cytokines storm appears in many inflammatory models including sepsis, which is considered a life-threatening condition, liver inflammation, RA, and more. Through our investigation, we identified several supportive models that demonstrate evidence supporting the anti-inflammatory effect of A₃AR agonists.

1. LPS induced sepsis – CI-IB-MECA treatment inhibited the pro-inflammatory cytokines iNOS, IL-1 β , and TNF- α resulting in a better survival outcome (66.7% for 0.2 mg/kg; 71.4% for 0.5 mg/kg; 0% in vehicle-treated control group).⁵²
2. Endotoxemic mouse model – Pre-treatment with IB-MECA (0.2 and 0.5 mg/kg) decreased interleukin 12 and IFN- γ secretion and protected mice against LPS-induced lethality.⁵⁴
3. Colitis and lung injury – A₃AR agonists significantly reduced IL-1, IL-6, IL-12, and TNF- α levels accompanied by a decrease in immune cell infiltration. This was suggested to be attributed to the presence of the A₃AR on the immune cells and plays a central role in the regulation of cytokine secretion. A₃AR was also found to be critically involved in mediating pulmonary polymorphonuclear cells (PMN) trafficking. Reduced accumulation of PMN was associated with decreased release of relevant cytokines into the alveolar air space.^{43,55,56}
4. Con.A induced liver inflammation – CI-IB-MECA (CF102) 0.1 mg/kg reduced inflammation as measured by markedly reduced serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase in comparison to the vehicle-treated group. CF102 treatment also decreased the expression level of phosphorylated GSK-3 β , NF- κ B, and TNF- α and prevented apoptosis in the liver of Con.A mice.⁴⁸
5. Adjuvant-induced arthritis, collagen-induced arthritis, and tropomyosin-induced arthritis – IB-MECA reduced TNF- α level in the spleen, lymph node, and the synovial tissue manifested also by significant reduction in joint inflammation.^{44,57}

6. Experimental autoimmune uveitis (EAU) – CF101 treatment reduced the secretion of IL-2, TNF- α , and IFN- γ measured in the condition medium of lymph node cells derived from EAU mice.⁵⁸
7. Cytokine release induced neuropathic pain model of tibia surgery in rats – IB-MECA daily treatment attenuates neuropathic pain by suppressing microglial cells activation in the spinal dorsal horn and results in a decrease in inflammatory cytokine secretion and reduction of pain hypersensitivity.⁵⁹

A₃AR agonists as a potential drug candidate to treat CRS

A₃AR agonists inhibit inflammatory cytokine production and release by binding to the A₃AR receptor, mediating deregulation of the NF- κ B and Wnt/ β -catenin signal transduction pathways.^{36,48} Moreover, NF- κ B was found to directly mediate IL-6 secretion presenting a putative binding site for NF- κ B in the IL-6 promoter region.⁶⁰ TNF- α , IL-6, and IL-1 are known to play an important role in the pathology of inflammation and their regulation became a target to combat this condition. The main goal in CRS treatment is reduction of the inflammatory manifestations through the inhibition of the inflammatory cytokines involved in the pathogenesis of CRS.

Inflammatory cytokines are known to control the A₃AR expression via an autocrine pathway. An increase in cytokine expression results generates downstream signaling pathways leading to upregulation of transcription factors inducing A₃AR upregulation.⁶¹

The capability of A₃AR agonists to inhibit IL-1, IL-6, and TNF- α together with their neuroprotective effects, strongly supports their utilization to combat CRS. Moreover, the A₃AR agonists namodenoson and piclidenoson (generically known as IB-MECA and CI-IB-MECA, respectively) have demonstrated an excellent safety profile in Phase I and Phase II clinical trials. For example, single oral doses of up to 5 mg and repeated oral doses of up to 4 mg of piclidenoson given every 12 hours to healthy men were safe and well tolerated with linear, dose-proportional pharmacokinetics.⁶⁶ Doses of 1 mg BID were generally safe and well tolerated in a 12-week Phase II study in patients with RA.⁶⁷ Results of a Phase I/II study in patients with advanced hepatocellular carcinoma (HCC) demonstrated 1, 5, and 25 mg of namodenoson was safe and well tolerated, showing favorable PK characteristics in Child Pugh A and B HCC patients.⁶⁸ The favorable safety and PK data position these drugs as a promising treatment for CRS.

Summary

The development of CRS in cancer patients undergoing immunotherapy such as CAR T cells and BiTE single-chain antibody constructs is a significant problem and can lead to reduced response or treatment-induced mortality. The incidence of CRS has been steadily decreasing over the years but still ranges between 3% and 48%, with treatment-related mortality averaging around 1%–5%.⁶⁹ A recent meta-analysis looking at the efficacy and safety of blinatumomab for the treatment of relapsed/refractory ALL and NHL found that the pooled occurrence rate of grade ≥ 3 CRS was 0.04 (95% CI: 0.01–0.06) and the pooled occurrence of grade ≥ 3 neurological events was 0.12 (95% CI: 0.08–0.12).⁶²

A viable treatment for CRS must successfully reduce the inflammatory response and subsequent neurotoxic AEs without limiting the anticancer effect of the given immunotherapy. New evidence suggests that circulating monocytes secreting IL-1 are the primary cells responsible for the initiation of CRS. Research shows that highly selective A₃AR agonists inhibit the production of inflammatory cytokines via downregulation of NF- κ B, thus downregulating the production of a panel of pro-inflammatory cytokines including TNF- α , IL-1, and IL-6. Phase I and II clinical data demonstrate that the highly selective A₃AR agonists, namodenoson and piclidenoson, have excellent safety profiles and favorable pharmacokinetics. This suggests that they may be promising drug candidates for the treatment for CRS.

Disclosure

Shira Cohen is a consultant at Can-Fite BioPharma Ltd. Pnina Fishman is an executive at Can-Fite BioPharma Ltd. and has shares and stock options. The authors report no other conflicts of interest in this work.

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