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CASE BASED REVIEW

The A_3 adenosine receptor (A_3AR): therapeutic target and predictive biological marker in rheumatoid arthritis

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Abstract The Gi protein-associated A_3 adenosine receptor (A_3AR) is over-expressed in inflammatory cells, and this high expression is also reflected in the peripheral blood mononuclear cells of patients with autoimmune inflammatory diseases such as rheumatoid arthritis, psoriasis, and Crohn's disease. CF101, a selective agonist with high affinity to the A_3AR, is known to induce robust anti-inflammatory effect in experimental animal models of adjuvant-, collagen-, and tropomyosin-induced arthritis. The effect is mediated via a definitive molecular mechanism entailing deregulation of the nuclear factor-κB (NF-κB) and the Wnt signal transduction pathways resulting in apoptosis of inflammatory cells. CF101 was found to be safe and well tolerated in all preclinical, phase I, and phase II human clinical studies. In two phase II clinical studies where CF101 was administered to rheumatoid arthritis (RA) patients as a stand-alone drug, a significant anti-rheumatic effect and a direct significant correlation were found between receptor expression at baseline and patients' response to the drug, suggesting that A_3AR may be utilized as a predictive biomarker. The A_3AR is a promising therapeutic target in rheumatoid arthritis and can be used also as a biological marker to predict patients' response to CF101. This is a unique type of a personalized medicine approach which may pave the way for a safe and efficacious treatment for this patient population.

Keywords A_3 adenosine receptor · Predictive marker · Rheumatoid arthritis · Therapeutic

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Abbreviations

A_3AR	A_3 adenosine receptor
cAMP	Cyclic adenosine monophosphate
MTX	Methotrexate
PBMCs	Peripheral blood mononuclear cells
PKB/Akt	Protein kinase B/Akt
NF-κB	Nuclear Factor-κB
RA	Rheumatoid arthritis
TNF-α	Tumor necrosis α

Introduction

Adenosine, a ubiquitous purine nucleoside, is released into the extracellular environment by metabolically active and stressed cells. It acts as an important regulatory molecule through its binding to G protein-associated cell surface receptors identified as A_1, A_{2A}, A_{2B}, and A_3 receptors [1–3]. A3 receptors for adenosine are found in almost all organs and cells of the body and are over-expressed in tumor and inflammatory cells [4]. Activation of the A_3 adenosine receptor (A_3AR) by highly selective agonists such as CF101 chemically known as 1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-*N*-methyl-β-D-ribofuranuronamide (IB-MECA) activates G protein inhibitory pathways that subsequently decrease cyclic adenosine monophosphate (cAMP) and the kinases PKB/Akt and PKA. This leads to the deregulation of the Wnt and the nuclear factor-κB (NF-κB) pathways resulting in apoptosis of inflammatory cells [5–8].

A_3AR is highly expressed in inflammatory tissues derived from patients with active rheumatoid arthritis (RA). Interestingly, the high receptor expression is also found in peripheral blood mononuclear cells (PBMCs), reflecting receptor status in the remote inflammatory organs [8–10].

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CF101 is an A_3AR agonist currently developed for the treatment of RA. It has a molecular weight of 510.29 Da and substitutions at the 2, N6, and 5′ positions creating metabolic stability and substantial A_3AR selectivity over the A_1, A_{2A}, and A_{2B} adenosine receptors. CF101, as the free base, exists as a non-hygroscopic stable white powder. The compound is insoluble in organic solvents and water [11].

Anti-arthritic effect of CF101 in experimental animal models

CF101 has been found to exert a marked anti-rheumatic effect in three different experimental animal models of poly-articular inflammatory arthritis. The anti-inflammatory effect of CF101 was examined in rat adjuvant-induced arthritis, in mouse collagen-induced arthritis, and in rat tropomyosin-induced arthritis [12]. The animals were treated orally twice daily with CF101, and the effect on arthritis severity was assessed clinically and histologically in all models. Additionally, the effect of CF101 on tumor necrosis factor α (TNF-α) secretion from the synovial tissue, draining lymph nodes, and spleen-derived cells was determined in mice. CF101 at 10 or 100 μg/kg/day markedly ameliorated the clinical and histological features of arthritis in the various autoimmune models. CF101 also inhibited TNF-α secretion in cells derived from mouse synovial tissue, lymph nodes, and spleen.

CF101 acts differentially on pathological and normal cells, due to the fact that the A_3AR target is over-expressed in inflammatory cells. CF101 induces apoptosis of inflammatory cells, while normal cells are refractory to the effect of the drug. Furthermore, in distinction from TNF-α blockers, CF101 induces only partial inhibition of TNF-α. Thus, CF101 is not considered as an immunosuppressive agent and is not expected to present the infection-related risks of therapy with anti-TNF biological agents [8, 9, 13].

CF101 has shown a favorable safety profile in nonclinical toxicology testing and is an orally bioavailable small molecule, features which make it an attractive candidate as a human therapeutic. Thus, if the nonclinical experimental findings of anti-inflammatory efficacy can be extrapolated to human disease, an adenosine A_3 agonist such as CF101 could demonstrate clinically significant beneficial activity in patients with immuno-inflammatory diseases.

CF101—mechanism of action

The A_3AR belongs to the family of Gi protein-associated cell surface receptors, which upon stimulation inhibit the formation of cAMP and its downstream effectors, PKA, and protein kinase B/Akt (PKB/Akt). The latter mediates the expression and activity of NF-κB, which plays a key role in the NF-κB–TNF-α signaling pathway, and the glycogen synthase kinase-3β, a key protein in the Wnt pathway. Upon A_3AR activation, in inflammatory cells and tissues, the expression level and activity of PKB/Akt is reduced, resulting in deregulation of the NF-κB–TNF-α and the Wnt signaling pathways. This chain of events inhibits cell growth regulatory genes, such as c-myc and cyclin D1, resulting in apoptosis of inflammatory cells [8, 14] (Fig. 1).

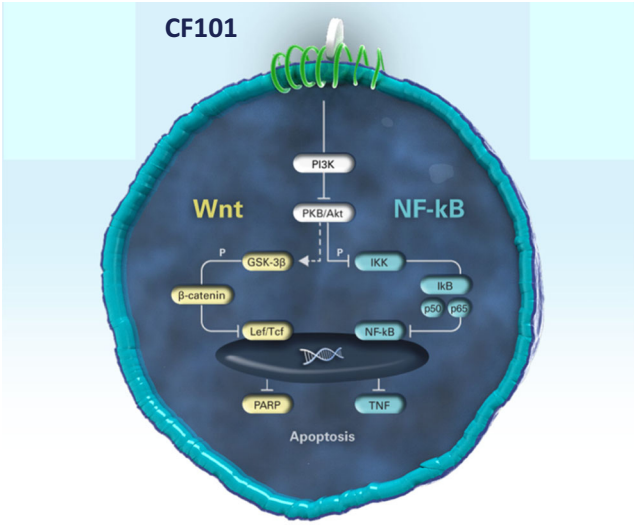

Fig. 1 CF101 molecular mechanism of action

Clinical development of CF101

Following the successful conclusion of couple of phase I clinical studies with CF101 showing that the drug is safe and well tolerated, it has subsequently been tested in phase II trials to establish dose and efficacy (orally administered tablet formulations of 0.1, 1, and 4 mg CF101, given twice daily) in the following clinical settings:

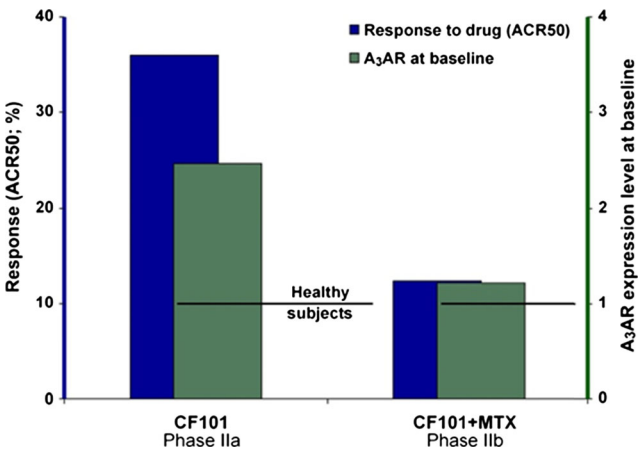

Fig. 2 Direct correlation between A_3AR expression at baseline and patients' response to CF101

Table 1 ACR response rate in the last phase IIb study. The latter reached the primary end point which was ACR20 and safety

	CF101 1 mg ($N=37$)	Placebo ($N=32$)
ACR20	48.6 % **	25 %
ACR50	18.9 %	9.4 %
ACR70	10.8 %	3.1 %

**Statistically significant p value = 0.0352

A phase IIa study utilizing CF101 as a stand-alone drug

This first phase II study was conducted in patients with rheumatoid arthritis, randomized to receive CF101 in one of three dosages—0.1, 1, and 4 mg. It was a double-blind study, and CF101 was given as a monotherapy and was administered twice daily for 12 weeks. The protein expression level of the A_3AR has been tested in the PBMCs, separated from blood samples withdrawn from the patients at the baseline visit. Study data showed that CF101 was safe and well tolerated. Maximal responses were observed with 1.0 mg drug whereas lower at 0.1 and 4.0 mg. At 12 weeks, 55.6, 33.3, and 11.5 % of the patients receiving 1.0 mg CF101 achieved ACR20, 50, and 70 % responses, respectively [15].

In addition, a direct statistically significant correlation between A_3AR expression level at baseline and the patients' response to CF101 has been observed [15]. This led to the conclusion that A_3AR, besides being a therapeutic target, may also be utilized as a predictive biomarker for response and that patients should be selected for CF101 treatment based on A_3AR expression level prior to treatment.

Phase IIb studies combining CF101 with methotrexate

Two studies combining CF101 with methotrexate (MTX) have been conducted.

The first study entailed 230 patients (NCT00556894) and the second one 252 patients (NCT00280917). Both studies failed and have not demonstrated a significant ACR20 response for patients treated with CF101 + MTX compared to MTX alone (the placebo group) for 12 weeks. Interestingly, cross study analysis revealed low A_3AR expression in baseline blood samples derived from both phase IIb patient populations due to the fact that all patients were pre-treated with MTX. It has been suggested that chronic treatment with MTX most probably led to A_3AR downregulation and thereby diminished the ability of CF101 to engender treatment effects. It is well established that the anti-inflammatory effect of MTX is mediated via the A_{2A} and the A_3 adenosine receptors [16]. Patients, who do not respond to MTX, most likely have low A_{2A} and A_3 adenosine receptor levels. The patients that we were allowed to enroll to the two phase II studies were those who were not good MTX responders, therefore did not respond to CF101 as well (Fig. 2).

A phase IIb study utilizing CF101 as a stand-alone drug

Recent phase IIb study utilizing CF101 as a monotherapy drug has been conducted and has been successfully concluded. The study (NCT01034306) was a 12-week, placebo-controlled study involving 79 patients enrolled into two arms, corresponding to twice-daily monotherapy dose of CF101 (1 mg) or placebo. Enrolled patients had a high-baseline A_3AR biomarker expression (cutoff has been determined at 1.5-fold over a predetermined age-matched standard). This selection criterion was made following the findings during the previous phase IIa and IIb RA studies showing a positive correlation between A_3AR expression at baseline and patients' response to the drug, potentially rendering A_3AR expression as a predictive biomarker. Results demonstrated that CF101 met the primary efficacy endpoint of showing statistically significant improvement vs placebo in reducing signs and symptoms of

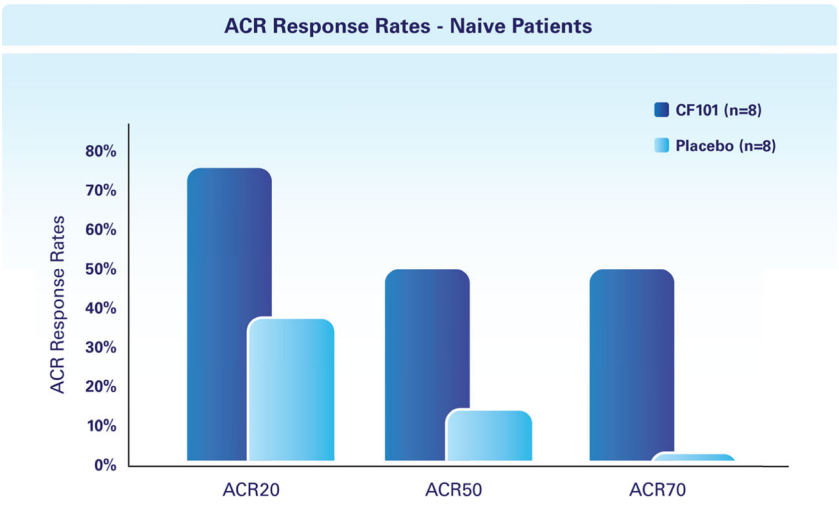

Fig. 3 ACR response rate in the last phase IIb study in naive patients who were treated with CF101 as a first-line therapy

RA, as measured by ACR20 response rates. The treatment arm had an ACR20 response rate of 49 vs 25 % for placebo ($p = 0.035$). The ACR50 (reflecting a >50 % improvement in the ACR composite scale) response rate was 19 % for CF101 vs 9 % for placebo, and the ACR70 response rate was 11 % in the CF101 arm vs 3 % for placebo (Table 1). The study was not designed to show statistical significance in ACR50 and ACR70. CF101 was very well tolerated and showed no evidence of immunosuppression, and there were no severe treatment-emergent adverse events during the study. These safety data are consistent with the positive safety profile reflected in the previously reported phase II CF101 studies comprising over 1200 patients across different systemic inflammatory diseases. In a subpopulation of treatment-naive patients, with no prior systemic therapy, the response to CF101 was higher compared to the response in the whole patient population (Fig. 3).

Furthermore, CF101 showed a linear improvement along the study period, data which are in line with the other phase II studies conducted in RA and psoriasis patients.

Conclusions

CF101 was found to be safe and efficacious when given as a stand-alone therapy for patients with RA. A_3AR is utilized both as a therapeutic target and as a biomarker to predict patients' response to CF101. This novel and unique personalized medicine approach is promising and an oral drug with a safety and efficacy profile as CF101 should be developed to support the care of patients with RA.

Compliance with ethical standards

Disclosures Pnina Fishman is an executive of Can-Fite BioPharma Ltd. and has shares and stock options. Shira Cohen is an employee at Can-Fite BioPharma Ltd. and has stock options.

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