THERAPEUTIC FOCUS

Adenosine Receptors – The Promise of A3AR Research

By: Pnina Fishman, PhD

INTRODUCTION

Adenosine is a neuromodulator that suppresses synaptic transmission. Adenosine receptors, part of the superfamily of G-protein-coupled receptors (GPCRs), come in four sub-varieties: A1, A1A, A2B, and A3. A separate gene encodes each of these four, and each has different physiological roles. The A3AR is less widely distributed that the A1, A1A, and A2B adenosine receptors, and in humans, the A3AR finds expression in the lungs, liver, brain, aorta, testis, and heart. Recent research has demonstrated that the A3AR holds promise as both a therapeutic target and as a biological predictive marker.

THE PROMISE OF A3AR RESEARCH

The reasons for such optimism are twofold. First, researchers have shown that the A3AR is overexpressed in cancer and inflammatory cells, while low expression is found in normal cells. They have also demonstrated that peripheral blood mononuclear cells (PBMCs) of patients with cancer or inflammatory disease show high receptor expression.

Specifically, A3AR is overexpressed in various neoplastic cells, including leukemia, lymphoma, astrocytoma, melanoma, and pineal tumor cells. Research captured similar data in other studies that showed the receptor expression levels in tumor tissues derived from patients with breast, colon, hepatocellular, pancreatic, small cell lung carcinomas, and melanoma in direct comparison with adjacent normal tissues. Furthermore, studies have described a direct correlation between A3AR tissue expression levels and disease progression in breast and colon cancer.

A similar pattern of receptor overexpression was described in inflammatory cells both in experimental animal models and humans. Rat studies of rheumatoid arthritis (RA) detected A3AR overexpression in paw tissue. When mice inhaled ipopolysaccharides (LPS), findings showed similar data in colon tissues, as well as in colon tissues derived from rats with colitis.

Similarly, over-expression of A3AR has been described in PBMCs of patients with psoriasis and patients with Crohn's Disease, suggesting that this is a general phenomenon in immuno-inflammatory diseases.

The A3AR is also highly expressed in anterior segment tissues derived from eyes with pseudoexfoliation syndrome when compared to the eyes of healthy subjects.

Second, scientistS have synthesized highly selective A3AR agonists that clearly induce specific anti-inflammatory and anticancer effects.

Additionally, experiments have illustrated a protective effect of the agonists on normal cells. This has caused many to believe that this unique differential effect of the agonists will contribute to a safety profile of these drug candidates in both preclinical and clinical studies.

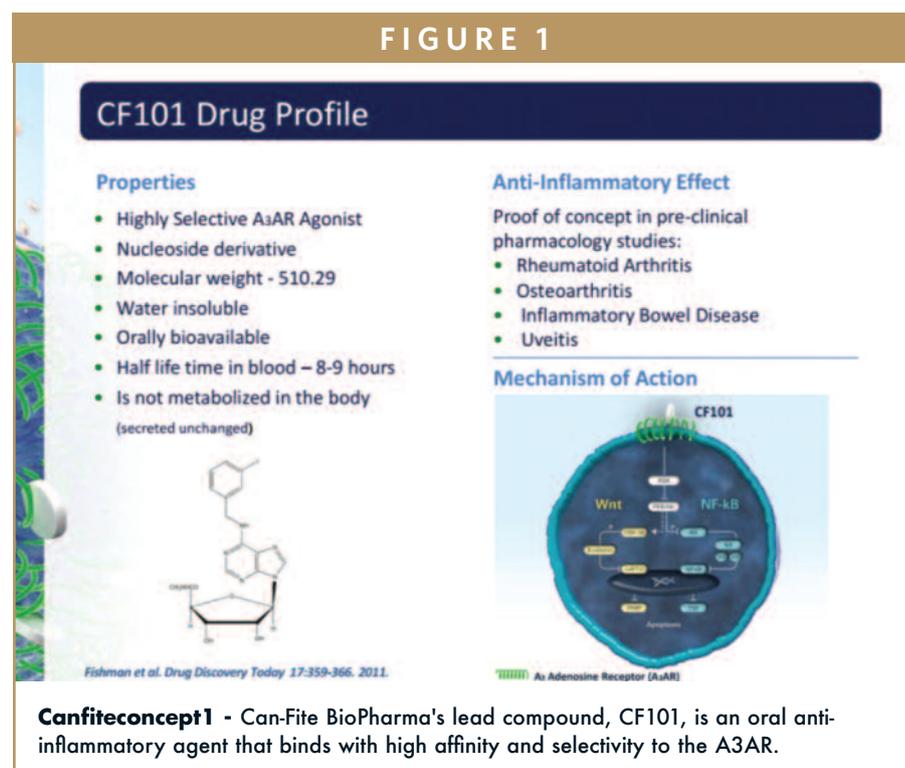At present, A3AR agonists are being developed for the

treatment of inflammatory, ophthalmic, and liver diseases and demonstrate excellent safety and efficacy in Phase II clinical studies.

THE EXAMPLE OF PLAQUE PSORIASIS

Plaque psoriasis affects 2% to 3% of the population, and it is the most common of the five varieties of psoriasis (guttate, inverse, pustular, and erythrodemic being the others). The plaque variety appears in itchy red patches covered in dead skin or scale that looks like a white, silvery build up. Often, these patches will bleed.

Traditional dermatology has focused on topical treatments, such as corticosteroids or salicylic acid and coal tar-based substances, although Hippocrates himself recommended arsenic. In general, these topical treatments have enjoyed enough success that they remain the first line of defense in dealing with plaque psoriasis.

Alternatively or in addition, phototherapy has shown some positive effect on the condition as well. There are two types of UVB treatment, broad band and narrow band. The major difference between them is that narrow band UVB light bulbs release a smaller range of ultraviolet light. Narrow-band UVB is similar to broad-band UVB in many ways. Several studies indicate that narrow-band UVB clears psoriasis faster and produces longer remissions than broad-band UVB. It also may be effective with fewer treatments per week than broad-band UVB. However, during UVB treatment, psoriasis may worsen temporarily before improving. The skin

FIGURE 1

Canfiteconcept1 - Can-Fite BioPharma's lead compound, CF101, is an oral anti-inflammatory agent that binds with high affinity and selectivity to the A3AR.

may redden and itch from exposure to the UVB light which can discourage the patient from further treatment, and consistency in phototherapy is vital to success.

In the past decade or so, however, data have demonstrated that the disease is much more systemic than previously known. Therefore, systemic treatments show great promise in treating plaque psoriasis – this is all the more the case when topical treatment and phototherapies have failed. Such systemic treatments include Acitretin (Soriatane), Cyclosporine, and Methotrexate, as well as off-label systemic treatments. Each has shown some efficacy, but each also has side effects and may not be tolerated well by certain individuals.

Can-Fite BioPharma's lead compound, CF101, is an oral anti-inflammatory agent that binds with high affinity and selectivity to the A3AR. CF101 has been successfully tested in animal models and is currently in advanced stages of clinical development

for rheumatoid arthritis (RA) and psoriasis. All human clinical studies have demonstrated this drug to have an excellent safety profile.

In two Phase II studies in which CF101 has been given as a stand-alone drug, data indicated that it acted as a disease-modifying anti-inflammatory drug in RA patients. The Phase II and the interim analysis of the Phase II/III clinical studies in psoriasis were successfully concluded showing efficacy and safety of CF101. Can-Fite has conducted a Phase II clinical study of the safety and efficacy of CF101 for the treatment of patients with moderate-to-severe plaque psoriasis. In previous Phase II studies conducted in patients with rheumatoid arthritis, CF101 demonstrated a marked anti-inflammatory effect.

As our study sample for CF101 as a treatment for plaque psoriasis, we enrolled males and females, aged 18 to 70 years with moderate-to-severe chronic plaque psoriasis of at least 6 months duration and a Psoriasis Area Sensitivity
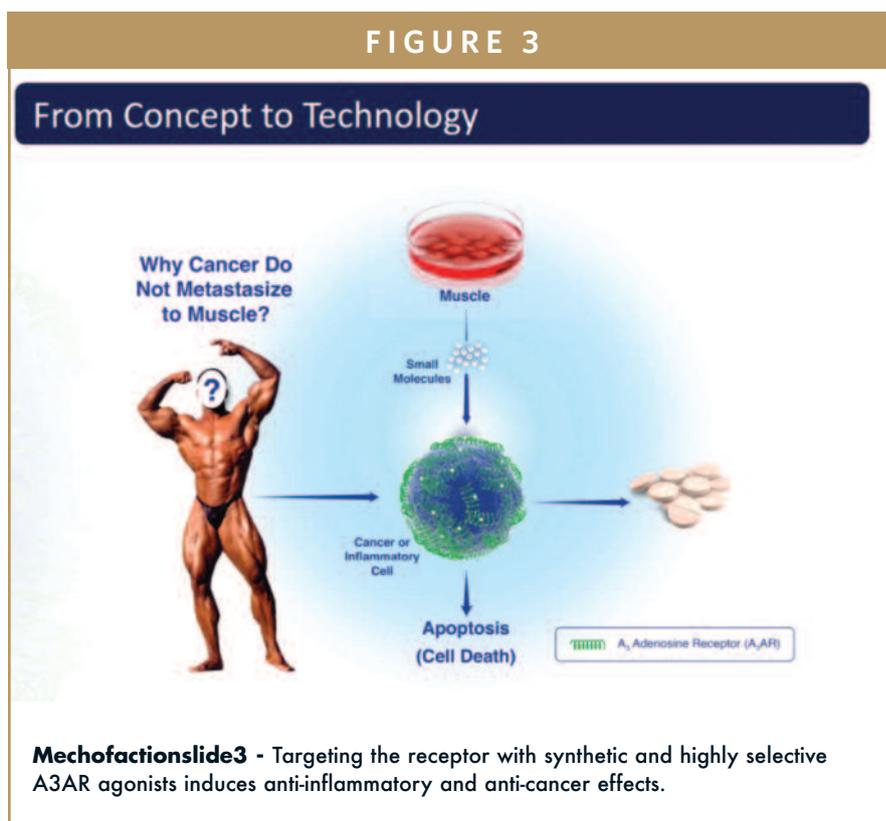
FIGURE 2

CF101Psoraisislide - The Phase II and the interim analysis of the Phase II/III clinical studies in Psoriasis were successfully concluded showing efficacy and safety of CF101.Brains

Index (PASI) score ≥10. The exclusion list was fairly comprehensive, disqualifying patients with: a diagnosed with erythro dermic, guttate, or pustular psoriasis; history of treatment with systemic retinoids, corticosteroids, or immuno-suppressants within 6 weeks of the baseline visit; treatment with moderate-high potency topical corticosteroids (Class I–III), calcification within 2 weeks of the baseline visit; treatment with phototherapy or Dead Sea climato-therapy within 4 weeks of the baseline visit; treatment with a biological agent within a period of time equal to five times its circulating half-life, or 30 days, whichever is longer, prior to the baseline visit; history of poor clinical response to methotrexate after an adequate regimen and duration of treatment; pregnancy, planned pregnancy, lactation, or inadequate contraception as judged by the investigator or other conditions that would confound the study evaluations or endanger patient safety.

This was a Phase II, multicenter, randomized, double-blind, dose-ranging, placebo-controlled study. Eligible patients were assigned to one of three sequential dosing cohorts with planned sample sizes of approximately 15 patients each. A total of 84 patients with moderate-to-severe plaque psoriasis were screened. Patients who met the inclusion criteria (n=75) were randomized to placebo (n=19), CF101 1 mg q12 hours (n=25), CF101 2 mg q12 hours (n=17) or CF101 4 mg q 12 hours (n=15). Patients were permitted to use emollients along the study period. Most of the patients completed the study (n=64, 84.2%).

We set as our exploratory efficacy endpoints: Change from Baseline (CFB) in PASI Scores; proportion of patients achieving 50% and 75% improvement in PASI scores (PASI 50 and PASI 75, respectively); and static Physicians' Global Assessment (PGA), which measures the physician's impression of the disease at a single point (graded on

a 0-5 scale as follows: 0=absent, 1=slight, 2=mild, 3=moderate, 4=marked, 5=severe). These parameters were calculated at weeks 2, 4, 8, 12, and 14. Safety assessments included recording of treatment-emergent adverse events, and changes in vital signs, physical examinations, clinical laboratory tests and electrocardiography findings.

In general, the study showed CF101 to be safe and well tolerated. There were 20 adverse events reported, and 15 of the 20 were adverse events that were possibly related to the study drug; all were mild or moderately severe except for one exacerbation of psoriasis judged to be severe. Within the CF101 1-mg group, the incidence of adverse events was 58.3%, and it is in this group that the severe incident was reported. The adverse event rate was 17.6% in the CF101 2-mg group and 13.3% in the CF101 4-mg group. Within the placebo group, the rate was 21.1%. Within the placebo group, we had a single serious adverse event - moderately severe arrhythmia/atrial fibrillation occurred during this study and was not attributed to study medication.

No clear patterns of difference between the CF101 treatment groups and the placebo group were evident for any body system/organ classes. Only four patients were withdrawn from the study due to adverse events, including arrhythmia patient from the placebo group.

Turning to the efficacy of CF101 shown in the study, there was no therapeutic effect in the 1-mg q12 hour group. However, looking at the mean change in the baseline PASI score at week 12 revealed a statistically significant difference between the 2-mg

CF101-treated group and the placebo group (P<0.001 vs. baseline and P=0.031 vs. placebo). The 4-mg CF101 dose treatment resulted in lesser improvement than that of the 2-mg dose treatment.

Within the 2-mg CF101-treated group, we saw a progressive improvement in the mean change from baseline in the PASI score throughout the study period was observed (week 2: 1.64±0.9; week 4: 3.76±1.9; week 8: 6.22±1.9; week 12: 8.77±2.1), with a statistically significant difference from placebo at weeks 8 and 12 (P=0.047 and P=0.031, respectively).

In the 2-mg CF101-treated group, 35.3% (6 out of 17) of the patients achieved PASI ≥50 response. Among this group, 11.8% (2 out of 17) of the patients achieved a response that was very close to PASI 50 (47.7% and 49.9%) at week 12. Furthermore, of those patients achieving PASI 50, one showed PASI improvement of 73%, and one reached PASI 90%. Because there was no entry requirement for a minimum PGA score, analysis of PGA data was performed on patients who entered the trial with a PGA score >1 to avoid confounding by low baseline scores.

At week 12, 23.5% of the patients treated with the 2-mg CF101 dose achieved a score of 0 or 1, in comparison with 0% in the placebo group (P<0.05).The percentage of patients presenting only slight or no clinical signs (PGA score 0-1) increased throughout the study period in the 2-mg CF101-treated group.

Based on these largely successful results, Can-Fite has finalized enrollment in its Phase II/III trial of CF101 for the treatment of psoriasis with over 300

FIGURE 3

Mechofactionslide3 - Targeting the receptor with synthetic and highly selective A3AR agonists induces anti-inflammatory and anti-cancer effects.

patients through 17 clinical centers in the US, Israel, and Europe. Top line results from the trial are expected in the first quarter of 2015.

The Phase II/III double-blind, placebo-controlled study is designed to test the efficacy of CF101 in 300 patients with moderate-to-severe plaque psoriasis. The first study cohort was composed of three arms with patients receiving: 1 mg of CF101; 2 mg of CF101; and placebo. All patients receiving placebo were switched to either 1 mg or 2 mg of CF101 after 12 weeks. The primary efficacy endpoints are a statistically significant improvement in standard measures used by dermatologists to assess psoriasis, including the PASI score and the PGA score as well as various safety parameters.

Not long ago, we released our interim safety and efficacy results from the first 103 patients who completed 24 weeks of treatment in the trial. The positive clinical effects of CF101 at the 2-

mg dose relative to placebo were observed through PASI and PGA scores, with the responses accumulating steadily over the 24-week treatment period. To allow the trial to meet its full objectives, the study protocol has been amended to enroll patients for the 2-mg dose and placebo administration for an extended study period of 32 weeks.

BEYOND PLAQUE PSORIASIS

Can Fite's scientists believe that CF101 offers a great deal of hope for those with plaque psoriasis because of its anti-inflammatory effect, its well-defined mechanism of action, and the excellent safety profile. Moreover, because CF101 has shown utility in other treatments, further investigation of its uses for other indications makes sound scientific sense. CF101 is also currently developed for the treatment of rheumatoid arthritis (PhaseIIb glaucoma (Phase II).

Can-Fite is also developing a commercial biomarker blood test kit for the A3AR predictive biomarker. The kit is designed for use at any molecular biology lab prior to treatment to help identify an individual patient's responsiveness to the company's drugs, thus providing personalized medicine. The US Patent and Trademark Office had previously issued Can-Fite a patent for A3AR as a biomarker to predict patient response to CF101 in autoimmune inflammatory indications.

OTHER A3AR AGONISTS

In addition to CF101, Can-Fite is working to develop a different A3AR agonist called CF102, an oral small molecule drug generically known as Cl-IB-MECA (2-chloro-N6-(3-iodobenzyl)-adenosine-5'- N-methyl-uronamide). CF102 has potent anti-cancer effect, particularly against hepatocellular carcinoma, and anti-inflammatory activity demonstrated in preclinical animal models of liver inflammation.

CF102's mechanism of action is mediated via de-regulation of the NF-κB and the Wnt signal transduction pathways, resulting in apoptosis of tumor cells. The protective effect of CF102 is mediated via down-regulation of the NF-κB signal transduction pathway and preventing apoptosis. The safety of CF102 has been demonstrated in preclinical studies, a Phase I clinical study, and in Phase I/II clinical studies demonstrating a favorable safety profile.

Recently, Can-Fite submitted to the US FDA the protocol for its global Phase II trial for the treatment of advanced hepatocellular carcinoma (HCC) with Child-Pugh Class B cirrhosis. The planned Phase II study will be conducted in Israel, Europe, and the US with 78 subjects and will investigate the efficacy and safety of CF102 as compared to placebo. Following Can-Fite's submission, the FDA agreed with the protocol design. The FDA had also previously granted Can-Fite Orphan Drug designation for CF102 in this indication.

Moreover, Israel's Ministry of Health has just approved CF102 for Compassionate Use for a liver cancer patient who has already benefited from the drug during clinical trials.

In addition, the European Union granted Can-Fite a patent for its invention titled, Method for Inducing Hepatocyte Proliferation and Uses Thereof. The patent covers CF102 in the treatment of liver function following liver resection (surgery) by helping the liver to regenerate and repair itself. Preclinical studies have found CF102 offers potential efficacy not only for cancer patients after a tumor has been surgically removed from the liver, it may also offer important benefits for patients with other kinds of liver diseases.

The final drug coming from this technology platform is CF602, a novel A3AR allosteric modulator that enhances the receptor activity in the presence of the native ligand. The molecule is characterized by high selectivity at the A3AR and is capable to avoid receptor desensitization, thus magnifying the agonist activity at low doses. The activity of CF602 was examined in experimental animal models of arthritic diseases. Oral administration of CF602 induced an impressive anti-inflammatory effect, manifested by a decrease in the disease signs and symptoms. CF602 induces down-regulation of the PI3K-PKB/Akt-NF-kB signaling pathway in the inflammatory cells.

CONCLUSION

The finding that the Gi protein coupled A3 adenosinereceptor (A3AR) is highly expressed in inflammatory and cancer cells whereas low expression is found in normal body cells offers a new way to fight such diseases. Targeting the receptor with synthetic and highly selective A3AR agonists induces anti-inflammatory and anti-cancer effects. In addition, the receptor is suggested as a biological marker based on human clinical data showing that high receptor expression at baseline predicts good patient's response to drug treatment. ◆

BIOGRAPHY

Dr. Pnina Fishman is the scientific founder of Can-Fite BioPharma and was previously a professor of Life Sciences and headed the Laboratory of Clinical and Tumor Immunology at the Felsenstein Medical Research Institute, Rabin Medical Center. Prof. Fishman is a very accomplished scientist and has authored or co-authored over 150 publications and presented the findings of her research at many major scientific meetings. Her scientific work was the foundation on which Can-Fite was built. This scientific work has gained recognition as one of the leading approaches for new- generation therapies for cancer and other diseases. Her past managerial experience included 7 years as CEO of Mor Research Application (MRA), a company that was in charge of the commercialization of intellectual property from all hospitals and research centers of Clalit Health Services, the largest healthcare provider in Israel, and was also the first clinical CRO in Israel. She was also involved in the establishment and served on the Board of Directors of several life sciences technology start-ups.