1.0 SCIENTIFIC ABSTRACT OF THE PROTOCOL

Inflammatory arthritides, such as rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS), are chronic systemic autoimmune inflammatory diseases of unknown etiology, characterized by chronic joint swelling, pain, and stiffness leading to progressive damage and destruction of the joints. They constitute the most prevalent chronic inflammatory diseases in the US. More than two million Americans are affected with RA, of whom over 75% are women, with the peak onset occurring between the ages of 20 and 45. PsA and AS are less common, but still affect 180,000 and 500,000 Americans, respectively, with similar incidence rates between men and women.

TNF-α is strongly implicated as a major participant in the inflammatory cascade that leads to joint damage and destruction in inflammatory arthritis patients. Although there is no cure, treatment has been revolutionized by the advent of anti-TNF-α therapies. These include etanercept (Enbrel®), infliximab (Remicade®) and adalimumab (Humira®), which consist of soluble TNF receptors, chimeric human-mouse anti-TNF-α monoclonal antibodies and fully human anti-TNF-α monoclonal antibodies, respectively. Clinical studies have shown these products improve the signs and symptoms, inhibit the structural damage, and impact functional outcomes in patients with inflammatory arthritis. However, some inflammatory arthritis patients have one or two persistently symptomatic joints despite systemic TNF-α blockade, or only a few problematic joints that do not warrant the use of systemic TNF-α antagonists, but may be at risk for progressive joint destruction. These patients might benefit from intra-articular anti-TNF-α therapy using gene transfer, which provides a mechanism to achieve expression of therapeutically levels of TNFR:Fc protein in the joint. In the latter group of patients, local TNFR:Fc expression may also reduce the incidence of the serious, but uncommon, adverse events associated with systemic TNF-a blockade that have been reported through the FDA’s post-marketing passive surveillance program. These adverse events include serious bacterial infections, reactivation of latent tuberculosis, demyelinating syndromes, malignancy and lupus-like reactions (FDA Briefing Document, 2003).
tgAAC94 is a recombinant adeno-associated virus (rAAV) serotype 2 vector which contains the cDNA for the human tumor necrosis factor receptor (hTNFR)-immunoglobulin (IgG1) Fc fusion (hTNFR:Fc) gene. The DNA sequence of hTNFR:Fc in tgAAC94 codes for a protein sequence identical to that of etanercept (Enbrel®). Intra-articular delivery of the hTNFR:Fc gene should result in expression of the secreted protein in the joint space and provide local concentrations of soluble hTNFR:Fc at the site of disease. A Phase 1 safety study of a single injection of tgAAC94 is currently underway in a dose escalation study of 24 human subjects with inflammatory arthritis who are not taking TNF-α antagonists (Protocol 13E04). In the 12 subjects dosed to date, no safety concerns have arisen. The proposed initial dose (1x10^11 DRP/mL joint volume) level in this proposed new protocol is the same as that administered in the second cohort of the ongoing trial (13E04). The safety data from the second cohort in the current study will be available prior to initiation of the corresponding cohort in this proposed clinical study (13G01).

Extensive non-clinical studies were conducted in normal rats, rats with experimentally induced arthritis, and non-human primates to provide data on the pharmacologic effects, as well as the toxicity profile, of tgAAC94. Data were previously submitted and reviewed in support of the first Phase 1 study (RAC protocol 0307-588). The safety of tgAAC94 over a range of doses (~1x10^11 to ~1x10^13 DRP/mL of joint volume) was demonstrated in normal Lewis rats. No toxicity attributable to the test article was noted at the maximal dose that could be administered in a single injection based on vector concentration and joint volume constraints. Similarly, no toxicity was noted in studies of tgAAV2-rat TNFR:Fc in normal rats and rats with experimentally induced arthritis. In both the rat and non-human primate models after a single intra-articular injection TNFR:Fc mRNA expression was detected at day 90, the last time-point tested.

The safety of repeat intra-articular doses of tgAAC94 (6x10^{12} DRP/mL of joint volume) and tgAAV2-ratTNFR:Fc (~1x10^{13} DRP/mL of joint volume) was evaluated in normal rats on an accelerated schedule, by administrating the vector once monthly for three consecutive months at doses that were 60-100 times higher than the proposed starting dose in the 13G01 protocol (1x10^{11} DRP/mL joint volume). This study was designed in collaboration with the FDA. The final data audit is ongoing. Once monthly intra-articular injections of tgAAC94 or tgAAV2-ratTNFR:Fc caused acute, transient mild to moderate swelling at the injection
site after repeat dosing. Most of these observations were recorded within a few days of the second dose and were observed in only 20% of the animals. There were no changes in body weight, hematology, or clinical chemistry that were attributable to the test articles.

Animals were sacrificed one and 30 days after the third injection. There were no gross lesions at necropsy that were considered test article related. Microscopic findings of inflammation and hemorrhage at the injection site were observed in animals sacrificed one day following the third injection, which could be attributed to the test article since the severity of the findings was higher than in the vehicle control animals. In the recovery group animals sacrificed 30 days after the third injection, decreased average severity and number of microscopic findings at the injection site was observed, indicating the inflammation was resolving. Hyperplasia of the popliteal lymph nodes was also noted in the rAAV vector treated groups. This hyperplasia may be explained by the increased serum neutralizing antibody titer against AAV2 capsids that was observed with each subsequent dose of rAAV2. After the first dose, the median antibody titer was 1:128 which increased to 1:2048 and 1:16384 after the second and third doses respectively. An increase in serum neutralizing antibodies after repeat dosing is expected.

In rodent models, administration of a high dose tgAAV2-ratTNFR:Fc (~1x10^{13} DRP/mL joint volume) showed inhibition of transduction of a second AAV2 vector, rAAV2-Luc, (containing the firefly luciferase cDNA), administered at one or three months after the initial injection. This inhibition is most likely due to the presence of high serum AAV2 neutralizing antibodies (median antibody titers of 1:2560-1:38,420). The study in normal rodents is ongoing and will determine the level of neutralizing antibodies that permits repeat dosing at later time-points. Although neutralizing antibodies can hinder rAAV transduction following re-administration in animal models, the significance in human clinical trials needs further clarification. It is difficult to predict whether humans will elicit the same titers of neutralizing antibodies as seen in the animal studies. In the current ongoing Phase 1 study, tgAAC94 injected at 1x10^{10} DRP/mL of joint volume did not result in an increase in detectable serum neutralizing antibodies, suggesting that there may be potential differences between rodents and humans with respect to anti-AAV2 immune responses. In the above rodent studies, it should be noted that TNFR:Fc mRNA was detected at 49 days after a single intra-articular injection. In other studies in the rat and non-human primate models after a single intra-
articular injection TNFR:Fc mRNA expression was detected at day 90, the last time-point tested. Intra-articular delivery of tgAAV2-ratTNFR:Fc suppresses arthritis in the rat model of induced arthritis at a dose of ~1x10^{12} DRP/mL of joint volume. Thus, frequent repeat administration at such high doses (1 x 10^{13} DRP/mL of joint volume) may not be needed. As indicated above, there were no safety issues related to repeat delivery of rAAV in the presence of anti-AAV2 capsid neutralizing antibodies other than a transient swelling at the injection site and microscopic findings of inflammation and hemorrhage at the injection site. The proposed removal of synovial fluid prior to injection may help remove neutralizing anti-AAV2 antibodies in the joint space that, if present, could have a negative impact on gene transfer after the second injection. In addition, it is expected that most subjects will be taking immunosuppressants, such as methotrexate, because of their inflammatory arthritis, which may blunt the development of AAV2 neutralizing antibodies.

In summary, the proposed protocol is designed to evaluate the safety of tgAAC94 in 40 subjects with and without TNF-\(\alpha\) antagonists, the duration of response after a single injection and safety of repeat administration, where the second dose will be given no earlier than 12 weeks after the initial dose. This new study is based on the safety data from (1) the subjects enrolled thus far in the first two cohorts in the ongoing Phase 1 clinical study (TGC Protocol 13E04, RAC Protocol 0307-588) (2) the rodent toxicology study which indicates no major safety concerns other than a transient mild-to-moderate increase in joint swelling after repeat administration. The study is divided into two segments, a blinded segment followed by an open label extension. In the first segment, two groups of 20 subjects each will be enrolled and randomly assigned to a single injection of either tgAAC94 or placebo in a 3:1 ratio of active study drug to placebo. If no safety concerns arise after dosing the first cohort, the second cohort will receive a higher dose of tgAAC94. In the second segment, each subject will receive an injection of open label tgAAC94 at the dose level of their originally assigned cohort 12 to 36 weeks after the initial injection, depending on when the swelling in the injected joint meets predetermined criteria for re-injection. An independent panel of experts will oversee the study and recommend that the study be stopped if safety concerns arise. The primary endpoint is safety, which will be assessed through clinical examination, laboratory monitoring, and adverse event reporting. Secondary end points include improvement in tenderness and swelling in the injected joint, duration of response as
measured by time to second injection, and improvement in overall disease. The expression of tgAAC94 at the injected site will be assessed by testing for human TNFR:Fc protein in synovial fluid, and the development of anti-AAV2 neutralizing antibodies will be measured. Changes in joint inflammation and damage will be assessed using serial magnetic resonance imaging scans in a subset of subjects.