

Advances in local targeted gene therapy for arthritis: towards clinical reality



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'[There is] a strong rationale for IFN- β gene therapy as a novel therapeutic approach for arthritis.'

In Western countries, the population is aging and showing an increasing incidence of chronic diseases. Among these, diseases of bones and joints – for example, osteoporosis, rheumatoid arthritis (RA) and osteoarthritis – reduce the quality of life, and impose a huge drain on healthcare funds. For RA, new biologicals such as anti-TNF therapies have improved treatment to a certain extent, with reduced toxic side effects, and have provided proof of principle for targeted therapy. It is now possible to reach 20% improvement in approximately 70% of the RA patients using this approach. However, most of these ACR 20% responders will still have some actively inflamed joints that are probably not responsive to the TNF-blocker. A drawback is the requirement of frequent injections in order to manage the disease. In addition, long-term systemic treatment with anti-TNF agents can result in serious side effects, and not all patients will qualify for treatment.

Persistent inflammation of a (single) joint can be treated with a local corticosteroid injection. Although intra-articular corticosteroid injections are widespread and effective in reducing symptoms of the inflammation, some studies show less favorable results. The application of more than four injections into the same joint per year is advised against because of the risk for procedure- and steroid-associated side effects. Gene therapy could provide a novel approach for local treatment of arthritis, and holds promise as it can result in more prolonged expression of therapeutic proteins after only a single injection. Local delivery of the gene therapy vector can also result in a reduction of the side effects sometimes observed with systemic treatment. In addition, it may also reduce the current high costs, and make these new therapies available to a larger number of patients. Recombinant adeno-associated virus (rAAV) currently has the most potential for the treatment of immune-mediated inflammatory diseases, including RA, over other vectors [1,2]. rAAV gene therapy has become more feasible as a consequence of the

improvement of the production of clinical grade rAAV vectors, resulting in production of larger amounts of vector. In addition, efforts to produce clinical-grade empty-capsid free vector batches have met with success [3], and should significantly reduce the antigen load as many early trials, and even current clinical vector batches, maintain a full:empty capsid ratio of 1:3 to 1:100 (only the full capsids contain the transgene).

Over the past 20 years, remarkable progress has been made in the development of effective gene therapy for the treatment of various diseases. However, only a few clinical trials have been conducted in the field of rheumatological diseases. In order to boost gene therapy in this field, more experience in patients is obviously required. The importance of well-designed clinical trials is underscored by recent observations showing the development of T-cell responses to rAAV capsid in patients, which was not anticipated based on animal studies. A clinical trial in which an rAAV vector was introduced into the liver of hemophilia B subjects indicated that an immune response directed to the rAAV capsid could be a serious obstacle in translating gene therapy to patients [4]. Similar T-cell responses have been reported in a Phase I trial in patients with familial lipoprotein lipase deficiency in which the rAAV vector was injected intramuscularly [5]. On the other hand, injection of small doses of rAAV into immuno-privileged sites suggested that the vector was well tolerated without inducing such immune responses, leading to long-term gene expression of the transgene [6]. Whether this would be the case after injection of the therapeutic vector into the joints of RA patients who are on systemic immunosuppressive drugs can only be answered by performing clinical trials addressing this issue.

Numerous preclinical studies have documented the excellent safety profile of rAAV vectors, along with their remarkable capacity to confer long-term gene transfer and expression in a variety of tissues. For example, in muscle tissue in dogs expression is seen for more than 7 years. In total, at least 46 clinical trials have been conducted or are in progress with rAAV vectors carrying different transgenes, all showing a good safety profile. One subject enrolled in an RA trial

receiving systemic anti-TNF therapy in combination with a rAAV2 vector expressing a TNF-blocking agent locally in the joint developed fatal disseminated histoplasmosis. However, this tragic event was ultimately considered unrelated to the study agent [7].

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Most of the current trials are performed using AAV serotype 2 as the vector. Recently, we have compared the efficacy of five different serotypes of AAV (1–5) vectors in transducing synovial tissue after intra-articular injection. AAV5 was the most efficient in transducing synovial tissue, especially fibroblast-like synoviocytes, after intra-articular injection in two different animal models of arthritis [8,9]. Importantly, although 25–60% of humans have neutralizing antibodies against different AAV serotypes, RA patients exhibit only low titers of neutralizing antibodies against AAV5 compared with AAV2 (Vervoordeldonk MJ *et al.*, University of Amsterdam, The Netherlands. Unpublished Data) and the low titers observed are not anticipated to interfere with local transduction in the joint.

Improved treatment using gene therapy directed at novel targets is needed to further increase efficacy. We and others have performed extensive research on the pleiotropic cytokine IFN- β belonging to a family of naturally secreted proteins with immunomodulatory functions [10]. IFN- β has anti-inflammatory properties and plays a role in bone homeostasis. The ability of IFN- β to reduce the secretion of pro-inflammatory cytokines, which are key players in the pathogenesis of RA, is of special interest. We have shown that IFN- β treatment is very effective in animal models of RA, including collagen-induced arthritis in both mice and Rhesus monkeys. However, clinical improvement could not be induced using systemic treatment with recombinant IFN- β in RA patients when administered only three-times weekly, most likely due to pharmacokinetic issues. It appears likely that continuous levels of IFN- β at the site of inflammation are required to induce clinical efficacy, which might be achieved by intra-articular gene therapy. After successful proof-of-concept studies, we have investigated the potential of intra-articular IFN- β gene therapy using an adenoviral vector (Ad) and in follow-up studies rAAV5 expressing rat IFN- β , using different animal models of arthritis. Local delivery of Ad or rAAV5 vectors expressing rat IFN- β after the onset of disease

reduced paw swelling impressively in both the treated and untreated contralateral joints [11,12]. Strikingly, IFN- β treatment protected against joint destruction, which is a hallmark of RA. Together, the results provide a strong rationale for IFN- β gene therapy as a novel therapeutic approach for arthritis. The advantage of using IFN- β , which is already approved as therapy for multiple sclerosis, is that it will help to bring local gene therapy for RA, on a relatively short term, into the clinic.

The Academic Medical Center/University of Amsterdam (The Netherlands) has extensive experience with the conduct of small proof of principle trials providing a high density of data that can provide information on the therapeutic response in an early stage of drug development [13]. Phase I clinical trials for RA gene therapy will mainly focus on assessing safety and establishing dosing for future trials. However, especially important in an RA gene therapy trial where only one inflamed joint is treated will be the collection of synovial tissue biopsies, the target tissue in RA, from the treated joint. The systematic evaluation of changes in synovial tissue after commencing treatment enables identification of an early therapeutic effect, using relatively small numbers of patients and the expression of the therapeutic gene locally at the site of inflammation [13]. Several studies from our group, which have been confirmed by others, have consistently shown that the number of synovial sublining macrophages may serve as a sensitive biomarker of clinical response to therapeutic intervention in RA, whereas after ineffective treatment no change in the number of synovial sublining macrophages was observed. The biomarker approach has recently been endorsed by the international Outcome Measures in Rheumatology (OMERACT) Special Interest Group on Synovial Analysis in Clinical Trials. With respect to trial design, it will also be important for a study focused on proof of concept for gene therapy to include only RA patients fulfilling the ACR criteria who are on stable treatment with one single (conventional) DMARD to minimize variability. In addition, the patients should be selected on the basis of having active arthritis in the same joint, for instance the knee joint, to facilitate standardized evaluation of disease activity.

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A recent clinical trial performed by Targeted Genetics Inc. (WA, USA) was one of the first trials for a nongenetic, nonlethal disease, and as such important in the translation of gene therapy for arthritic diseases from animal models to the patient. The recently reported results [14] on the Phase II clinical trial by Targeted Genetics Inc. do not provide statistical evidence of efficacy, but have improved knowledge regarding the safety profile of direct *in vivo* delivery of a viral vector into the joint. The next step in clinical trials evaluating gene therapy for arthritis should involve: the use of optimized vectors; inclusion of better-defined patient groups; use of validated outcome measures for single joint assessment; synovial tissue analysis to evaluate transduction efficiency, the expression of the transgene and therapeutic protein, and the effects on inflammation; and imaging modalities

to visualize the effects on inflammation and destruction. Such studies should obviously also provide data on the influence of pre-existing neutralizing antibodies on transduction efficacy and the effects of the immune response to the vector to facilitate the design of larger clinical trials aimed at the assessment of clinical effectiveness.

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