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Arthritis Gene Therapy and its Tortuous Path into the Clinic

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Abstract

Arthritis is a disease of joints. The biology of joints makes them very difficult targets for drug delivery in a manner that is specific and selective. This is especially true for proteinaceous drugs ("biologics"). Gene transfer is the only technology that can solve the delivery problem in a clinically reasonable fashion. There is an abundance of pre-clinical data confirming that genes can be efficiently transferred to tissues within joints by intra-articular injection using a variety of different vectors in conjunction with *ex vivo* and *in vivo* strategies. Using the appropriate gene transfer technologies, long-term, intra-articular expression of anti-arthritic transgenes at therapeutic concentrations can be achieved. Numerous studies confirm that gene therapy is effective in treating experimental models of rheumatoid arthritis (RA) and osteoarthritis (OA) in the laboratory. A limited number of clinical trials have been completed, which confirm safety and feasibility but only three protocols have reached Phase II; as yet, there is no unambiguous evidence of efficacy in human disease. Only two clinical trials are presently underway, both Phase II studies using allogeneic chondrocytes expressing TGF-β_1 for the treatment of OA. Phase I studies using adeno-associated virus to deliver IL-1Ra in OA and IFN-β in RA are going through the regulatory process. It is to be hoped that the recent successes in treating rare, Mendelian diseases by gene therapy will lead to accelerated development of genetic treatments for common, non-Medelian diseases, such as arthritis.

Introduction

Diseases of joints are common, incurable and often difficult to treat. Of the 100 or so forms of arthritis, osteoarthritis (OA) is the most prevalent, affecting 27 million Americans [1]; this number will rise as the population ages and gains weight. Although OA is considered a non-lethal disease, it is associated with an elevated risk of death [2]. Because OA resists effective therapy, many patients progress to the need for prosthetic joint replacements. In 2004, over 650,000 artificial hips and knees were implanted at a cost of $26 billion. It is predicted that around 2 million hip and knee replacement surgeries will be performed in the year 2015 [3]. Overall, OA generates medical care expenditures exceeding $185 billion per year [4]. OA is also a major clinical problem in veterinary medicine, particularly for horses and dogs.

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Rheumatoid arthritis (RA), the next most prevalent form of arthritis, is a systemic autoimmune disease that affects approximately 1.3 million adult Americans [5]. A further 294,000 individuals have the juvenile form of the disease. RA is associated with increased mortality [6], but treatment has improved dramatically during the past decade thanks to the introduction of proteinaceous antagonists of tumor necrosis factor (TNF) and other so-called biologics. However, less than 30% of patients show robust responses (ACR 70) to these drugs [7] which, as well as being very expensive, are associated with a number of side-effects related to their systemic mode of delivery. Moreover, even in an otherwise responsive patient, there may remain a number of individual joints that do not respond to the therapy.

The TNF antagonists in clinical use constitute the first wave of new, biologic therapies using proteins as drugs to combat arthritis [8]. Although such drugs are delivered systemically by intravenous infusion or subcutaneous injection in RA, their application in OA and other localized forms of arthritis is constrained by the need to deliver the protein locally and specifically to a small number of target joints, and to sustain a therapeutic, intra-articular concentration of the protein for a considerable period [9]. As described in more detail in the following sections, gene transfer is the only clinically reasonable technology that can do this [10].

Thus, although the common forms of arthritis are not genetic diseases in the Mendelian sense, they are amenable to gene therapy, using intra-articular gene transfer as a means of delivering therapeutic gene products to joints in a sustained fashion. When we suggested a gene therapy approach to treating arthritis over 20 years ago [11], it was the first non-Mendelian, non-lethal disease to be considered in this fashion. Other such indications have subsequently followed suit.

The essential biology of the arthritic joint

Arthritis is a disease of diarthrodial (moveable) joints. The anatomy of most such joints conforms to a basic plan where the ends of the long bones articulate within a discrete cavity lined by synovium (figure 1). The articulating surfaces of the bones are covered with articular cartilage, which, in conjunction with lubricating functions provided by the synovial fluid, ensures almost frictionless motion. Two fundamental pathological changes commonly occur during arthritis: inflammation and destruction of the articular cartilage.

Inflammation in joints is seen as a synovitis and hypertrophy of the synovium, with increased volume and leukocytosis of the synovial fluid. In RA, inflammation is driven by autoimmune mechanisms [12,13]. The importance of inflammation in OA, a degenerative condition, is increasingly, but not uniformly, appreciated [14]. Where present, it may be driven by wear particles [15], soluble products released from the extracellular matrix of cartilage [16], crystals [17], cytokines [14] or mechanical forces [18].

Loss of articular cartilage is mediated by proteolytic enzymes directed against the major macromolecules of its extracellular matrix [19]. In OA, these enzymes are synthesized and secreted by the chondrocytes within the cartilage in response to certain cytokines and mechanical forces. In RA this mechanism is supplemented by direct invasion of the cartilage and adjacent bone, by an enlarged, hyperplastic, destructive synovium. Moreover, inflammatory mediators inhibit the compensatory synthesis of matrix macromolecules by chondrocytes, thereby exacerbating the problem [20]. These changes are often accompanied by chondrocyte cell death.

The pharmacokinetics of the joint

The obstacles to delivering drugs to joints in the sustained fashion required of chronic conditions like arthritis are not trivial. When drugs are delivered systemically, they enter the joint via fenestrated synovial capillaries [21]. These exert a sieving effect that restricts the entry of larger molecules. Although this effect is reduced when synovitis is present, it still presents a barrier to entry for large proteins [22]. Thus, to deliver therapeutic amounts of protein to joints in a sustained fashion via the circulation requires repeated, systemic administrations. With RA, which has important systemic, extra-articular involvement, this may be an advantage, but for everything else it is a disadvantage because such methods do not specifically target drugs to joints. Thus non-target organs are exposed to high concentrations of the drug, increasing the potential for unwanted side-effects as well as greatly increasing the amount of drug that needs to be administered and hence costs.

Because they are discrete, accessible cavities, joints lend themselves to direct, intra-articular injection [9]. Although direct injection of proteins overcomes physiological barriers to entry, it is a futile therapeutic strategy because macromolecules are rapidly cleared from joints via the lymphatics [22]. The intra-articular half-life of a soluble protein is typically a few hours, regardless of its size. Repeated intra-articular injections are not reasonable and the insertion of an infusion pump is not clinically feasible. Barriers to delivery are overcome, however, by the genetic transduction of cells within the joint, whereby the transgene product is synthesized and secreted locally for an extended period, accumulating in the synovial fluid and articular tissues to provide sustained, therapeutic concentrations lacking the peaks and troughs of intermittent application. This is the core logic that underpins arthritis gene therapy [10]. The synovium has traditionally been the target tissue for gene transfer within the joint (figure 2) [11] but, as described later in this review, other articular tissues may also be important.

Pre-clinical research

Several detailed reviews summarize comprehensively the pre-clinical, experimental findings concerning arthritis gene therapy [10,23-25]. In essence the data confirm that genes may be transferred to the joints of experimental animals using *ex vivo* or *in vivo* strategies in conjunction with a variety of viral and non-viral vectors. If the host immune response is not activated, intra-articular transgene expression can persist for months [26], and possibly years, when using viral vectors. Intra-articular transgene expression using non-viral vectors tends to be low and transient [27].

A variety of different transgenes, reviewed in reference 25 [25] have shown convincing efficacy in animal models of OA and RA, providing undeniable proof of principle. The majority of these transgenes encode immunomodulatory and anti-inflammatory cytokines, as well as proteinase inhibitors and growth factors for the protection and repair of cartilage. Those that have advanced into clinical trials, or are at an advanced stage of pre-clinical development, encode the interleukin-1 receptor antagonist (IL-1Ra), etanercept (a fusion protein of TNF soluble receptors and the Fc domain of IgG), transforming growth factor-β_1 (TGF-β_1) and interferon-β (IFN-β). Progress in their clinical translation will now be described.

Clinical trials – ex vivo delivery

MFG-IRAP

When we first suggested the use of gene therapy to treat arthritis, retrovirus vectors based upon the Moloney murine leukemia virus were the most advanced vectors available for

human gene therapy, having been used in the first human clinical trials. Because retrovirus vectors require host cell division for effective transduction, it was necessary to develop an *ex vivo* strategy. It is a relatively straightforward matter to harvest and establish monolayer cultures of synovial fibroblasts, so these were an obvious choice of cells, especially as surgical synovectomy was a common clinical procedure.

Of the pathophysiological mediators present in arthritic joints, IL-1 seems a promising target. Because of its involvement in inflammation, immune function and cartilage destruction, it has potential involvement in both RA and OA. At the time these studies were starting IL-1Ra, a naturally occurring antagonist of IL-1, had been identified and cloned [28]. This molecule has many advantages as a therapeutic molecule: it has no agonist activity, even at very high concentration; its dose-response is an uncomplicated rectangular hyperbola; it is safe; it is a small protein whose full coding sequence is easily cloned into a retrovirus without modification. Using a cDNA encoding the full length, native IL-1Ra molecule reduces the potential for complications with immunogenicity or other emergent properties than can occur with fusion proteins or other novelties.

Human IL-1Ra cDNA was cloned into a derivative of the Moloney murine leukemia virus known as MFG to produce the vector MFG-IRAP. Used in conjunction with autologous synovial fibroblasts, MFG-IRAP successfully transferred IL-1Ra cDNA into the knee joints of rabbits [29], mice [30] and dogs [31], with high levels of intra-articular transgene expression. A similar retrovirus constructed by Makarov's group achieved this in rats [32]. Safety of MFG-IRAP was confirmed in a number of studies, the most compelling of which involved the stable transduction of marrow cells in mice, leading to life-long, high levels of IL-1Ra expression without obvious harm [33].

Efficacy of gene transfer of IL-1Ra was confirmed in animal models of RA including antigen-induced arthritis in rabbits [34], zymosan- and collagen-induced arthritis in mice [30] and streptococcal cell wall-induced arthritis in rats [32]. Promise of a therapeutic effect in humans was suggested by experiments in which human cartilage was co-implanted with human, rheumatoid synovium under the kidney capsule of SCID mice. When synovium was first transduced with MFG-IRAP, destruction of the cartilage matrix by chondrocytes was inhibited [35].

Translating these efficacious animal model results into a clinical trial for RA was constrained by the fact that this was the first protocol to come before the authorities proposing to perform human gene therapy for arthritis or any other non-lethal, non-Mendelian disease. Although RA is associated with reduced life-expectancy [6] it is not considered lethal in the conventional sense, a circumstance that considerably skewed the risk:benefit ratio so that safety became of predominant concern. The protocol thus included two important safety features: the recruitment of post-menopausal females to eliminate the possibility of germ-line transgene transmission, and gene delivery to joints that were scheduled for joint replacement surgery [36]. We knew from pre-clinical studies that the transduced cells did not migrate from the joints into which they were injected, so joint replacement surgery should remove the genetically modified cells and eliminate the possibility of unpredictable, late responses.

The study that was eventually approved [36] involved the injection of autologous synovial fibroblasts into the metacarpophalangeal (MCP; knuckle) joints 2-5 on one hand of 9 post-menopausal female subjects with RA (figure 3). In a double-blind fashion, two of the 4 MCP joints were injected with unmodified cells and two with transduced cells. The MCP joints are common sites of RA, and before the advent of TNF antagonists they were commonly replaced with sialistic prostheses. Thus it was possible to inject these joints one week before

all 4 MCP joints were removed and replaced. This provided tissue for subsequent analysis of gene expression and other functions (figure 3).

This study was completed without incident and confirmed that it was possible to transfer genes into human, arthritic joints in a manner that is safe and acceptable to patients [37]. The intraarticular expression of an active transgene product was confirmed. Because of the end stage of the disease, the small number of subjects, and the short time between injection and removal of the transgene, the study was not designed to study efficacy. However, several subjects reported symptomatic improvement.

A follow-up study by Wehling et al [38] included outcome measures based on a visual analog pain scale and the diameter of the MCP joint. Their study followed the one described above, but allowed 4 weeks between the injection of the cells and their removal, which was accomplished by synovectomy instead of joint replacement. Although the local ethics committee allowed 6 subjects to be included in the study, adverse events in an unrelated clinical trial of X-linked SCID which also used a derivative of MFG as the vector [39], caused the study to be closed after only two subjects had completed the protocol. One of these two responded in a dramatic fashion [38]. The other also reported improvement. Synovial expression of IL-1Ra was confirmed and the expression of matrix metalloproteinase-3 (stromelysin-1) was strongly inhibited. No adverse events were noted.

Collectively, these are very encouraging data but, for a variety of reasons, no further development of this approach has occurred. One issue brought home by these studies was the enormous cost and complexity of *ex vivo* gene therapy using expanded, autologous cells. Moreover, during the course of these studies the first cases of insertional mutagenesis caused by a retroviral vector were reported [39]. This threw the risk:benefit ratio back into high relief and made it even more difficult to apply to non-lethal diseases. Furthermore, the FDA tightened the regulations to require a 12-year follow up for gene therapy trials using an integrating vector. On top of this, the anti-TNFs and other biological drugs made an increasing impact on the treatment of RA [8], reducing (but not eliminating) the scope for a gene therapy. In view of these developments we decided to turn our attention to OA using *in vivo* gene delivery, as described later.

TissueGene-C

One way to make *ex vivo* gene delivery less cumbersome and expensive is to use an universal donor cell line. This has been accomplished for joints using a line of human chondrocytes established from the cartilage of a new born with polydactyly. These cells have been stably transduced with a retrovirus carrying cDNA encoding TGF-β_1 [40,41], which is thought to promote the healing of cartilage. The therapy based upon genetically modified chondrocytes is known as “TissueGene-C” or “TG-C”.

In many ways OA is a better target than RA for intra-articular gene therapy because, unlike RA, OA affects a limited number of joints and has no important systemic or other extra-articular components. Moreover, unlike the case with RA, there are no reliably effective treatments for OA.

Because the transduced chondrocytes are aneuploid it is necessary to irradiate the cells prior to injection to eliminate their ability to divide and generate tumors. The irradiated, transduced cells are mixed with untransduced, unirradiated cells prior to injection (figure 4). Two Phase I studies using this protocol have been completed in the USA and Korea. The cells were injected into the knee joints of subjects with OA prior to undergoing surgery to insert an artificial knee. No serious adverse events were reported [42]. Phase II studies are now underway and encouraging preliminary data were recently presented [43].

In vivo delivery – emergence of AAV as the vector of choice

The encumbrances of *ex vivo* gene delivery to joints were recognized early, and considerable research evaluated vectors derived from adenovirus, herpes virus, high-titer retrovirus, lentivirus and a wide range of non-viral vectors as vehicles for *in vivo* gene delivery to joints [44-48]. However, all of these vectors have shortcomings, and adeno-associated virus (AAV) has emerged as the vector of choice.

The general advantages of recombinant AAV as a vector are well known: wild-type virus causes no known disease; it transduces non-dividing cells; it is thought to have low immunogenicity etc. [49,50] However, only Gouze et al [26] have specifically identified the requirements of a successful vector for achieving long-term expression in joints and demonstrated how AAV uniquely satisfies these requirements.

As part of a study into the factors that limit transgene expression in joints, Gouze et al [26] compared an integrating virus (lentivirus) with a non-integrating virus (adenovirus) in immunocompetent and athymic rats. Green fluorescent protein (GFP) and human IL-1Ra were used as the transgenes, with expression driven by the cytomegalovirus (CMV) or elongation factor 1α promoter. Long-term expression was not achieved in immunocompetent animals. However, GFP and IL-1Ra expressed for the life of the athymic animals, regardless of the vector or promoter. This suggests that long-term transgene expression can be achieved if neither the vector nor the transgene triggers an immune reaction in the host; furthermore, this does not require viral integration. The latter conclusion suggests that enduring populations of quiescent cells exist within joints and sustain transgene expression from a constitutive promoter for extended periods of time.

Examination of joints by fluorescence microscopy (figure 5) helped identify the location of such cells. Early after the injection of virus, most of the GFP-fluorescence occurred in the synovium. This was lost with time, possibly due to turnover of the synovial cells. Persistent GFP expression occurred in fibroblasts present in ligaments and capsule, as well as in the region where the synovium meets the cartilage. The persistence of transgene expression in ligaments agrees with older data of Oligino et al [46] using herpes virus vectors.

Consideration of these findings leads to the conclusion that AAV is about the only clinically reasonable option, at present, for obtaining persistent, therapeutic levels of transgene expression after *in vivo* delivery to joints. Lentivirus is very powerful [48,51] but, as an integrating retrovirus, raises too many safety issues. Non-integrating lentiviruses have been developed but are not widely available. Adenovirally-transduced cells are cleared by the immune system. Although “gutted” adenovirus vectors lack this limitation, they are difficult to produce and are not readily available. Vectors based on herpes simplex virus are cytotoxic to most of the mesenchymal cells of the joint. Non-viral vectors provide only transient, low levels of transgene expression.

A number of studies confirm the ability of AAV to deliver genes to the joints of small animals in an appropriate fashion [49,52], especially when using self-complementing viruses that avoid the need for second strand DNA synthesis [53-55]. Several laboratories have compared different serotypes of AAV for their ability to transduce articular tissues *in vitro* and *in vivo,* identifying serotypes 1, 2, 2.5, 5, 8 and 9 as interesting candidates [56-59].

Only one study has investigated gene transfer in large animals with joints that are similar in size to human joints, and thus provide a more reliable indicator of what might occur in human clinical use. Watson *et al* [59] injected recombinant AAV.GFP into the midcarpal and metacarpophalangeal joints of horses. Fluorescence microscopy revealed impressive transduction of the synovial lining cells (figure 6) and chondrocytes, especially in areas of

cartilage damaged by OA (figure 7). Transduction of chondrocytes by AAV was surprising as this had not been observed in the joints of small animals (e.g. figure 5), although the in vitro experiments of Madry et al. [60] suggested that this might be possible. It is of particular relevance to OA where the enhanced transduction of cells within cartilagenous lesions would bring many advantages.

Because equine joints, unlike the joints of small animals, can be aspirated it was possible to gain an accurate measure of IL-1Ra in the synovial fluid; human IL-1Ra was used as the transgene, so it was possible to distinguish this product from endogenous equine IL-1Ra. Synovial fluid concentrations of 1-2 ng/ml were maintained for 5 weeks, after which an immune reaction to the human IL-1Ra eliminated expression [59]. Based upon our accumulated experience with these types of experiments, a concentration in this range is predicted to be therapeutic. When the equine IL-1Ra cDNA was used, intraarticular expression was higher and persisted for many months (Ghivizzani *et al.* unpublished data).

Experience from human clinical trials suggests that immune reaction to AAV can be problematic [61]. In agreement with such observations, intra-articular injection of AAV2 into the MCP joints of horses generated a persistent neutralizing antibody response to the vector [62] that would presumably interfere with re-dosing.

As well as providing confidence that these strategies will work in human joints, the equine studies form the basis for veterinary application in using AAV-based, *in vivo* gene therapy to treat equine OA and, by extension, OA in other domestic species.

Completed and pending clinical trials using AAV

*rAAV2-TNFR:Fc (*tgAAC94)

Etanercept is a TNF antagonist created by the fusion of two soluble TNF receptors to the Fc domain of immunoglobulin [63]. As a recombinant protein, it is widely used to treat patients with RA and certain other inflammatory arthritides, such as psoriatic arthritis. It is delivered by sub-cutaneous self-injection twice per week. A cDNA encoding etanercept has been incorporated into AAV2 to form the vector rAAV2-TNFR:Fc (tgAAC94). Because of the size of etanercept cDNA, this is a single-stranded vector. It is intended to be used in individual symptomatic joints of patients receiving systemic treatment for RA, as well as individual joints suffering from other inflammatory conditions.

The AAV-etanercept vector showed efficacy in rat streptococcal cell wall-induced arthritis [64] and entered a Phase I study involving 14 subjects with RA and one with ankylosing spondylitis [65]. Subjects were given a single injection of 10^{10} or 10^{11} virions/ml, with the volume depending on the joint; this ranged from 0.5ml for MCP joints to 5ml for knee joints. No adverse events were noted, leading to a Phase II study [66] involving over 100 patients with RA, as well as patients with ankylosing spondylitis and psoriatic arthritis, whose disease was not adequately controlled by standard therapy [67].

In the Phase II study the dose range was expanded to include 10^{12} and 10^{13} virions/ml, repeat dosing was allowed and, unlike the case in the Phase I study, concomitant treatment with conventional TNF blockers was also allowed. This study attracted considerable notoriety when a subject died shortly after receiving a second injection of the highest dose of the vector [68,69]. The subject died with disseminated histoplasmosis accompanied by a massive retroperitoneal hematoma weighing at least 3.5 kg. Histoplasmosis is a known risk factor associated with the application of anti-TNFs and, controversially, the subject was taking adalimumab, an anti-TNF antibody, during the gene therapy trial. The FDA

suspended the trial while these circumstances were investigated, but allowed the study to proceed to completion with minor changes to the consent form and protocol.

Apart from this fatality, which was concluded by the FDA not to be the result of AAV-mediated gene therapy, the trial did not encounter any severe adverse events directly related to the study. However, as with many such studies, there were minor injection site reactions. Viral genomes were not detected in the peripheral blood of subjects receiving the lowest dose of vector, but were detected in 46% of those receiving the middle dose and 61% of those receiving the highest dose. However, viral DNA was no longer detectable in peripheral blood cells after 12-18 weeks. No viral genomes were detected in a range of solid organs, including liver, brain, and heart, obtained at autopsy. Neutralizing antibodies to AAV2 were generated, but there was no evidence of a cell-mediated response. However, clinical responses were modest [67] and it is not clear if further development of this product will occur.

Sc-rAAV2.5IL-1Ra

As noted earlier in this review, our group is now focusing its attention on developing an *in vivo* gene therapy using recombinant, self-complementing (sc) AAV to deliver IL-1Ra cDNA to joints, with OA as the initial target disease.

Preclinical data in rabbits [53] and horses [59] confirm the ability of scAAV.IL-1Ra to generate therapeutic amounts of IL-1Ra intra-articularly. In horses, intra-articular IL-1Ra expression persists at an undiminished rate for at least several months when the cDNA encodes the equine product (Ghivizzani *et al.*, unpublished), with high transgene expression in synovium and areas of damaged cartilage (figures 6 and 7). A Pre-IND meeting was held with the FDA in December, 2011 and a large pharmacology-toxicology-efficacy study is now underway in rats.

ART-I02 (AAV.IFN-β)

The Dutch company, Arthrogen BV, is developing AAV5 carrying cDNA encoding IFN-β, under the control of an inflammatioñ inducible promoter, as an intra-articular gene therapy for RA. Interest in IFN-β as a treatment for autoimmune diseases such as RA is generated by its immunomodulatory, anti-inflammatory and anti-angiogenic properties [70]. When delivered systemically as a recombinant protein, it has shown efficacy in clinical trials for multiple sclerosis [71] but not RA [72]. However, repeated application of IFN-β in mice [73] and monkeys [74] with collagen-induced arthritis was efficacious, suggesting that sustained delivery of this cytokine was necessary to produce a therapeutic effect in RA. Evidence in favor of this conclusion was obtained by the i.p. injection of fibroblasts that constitutively secreted IFN-β in rats with adjuvant arthritis, a model of human RA [75]. The intra-articular injection of AAV5.IFN-β has also shown efficacy in rats with adjuvant arthritis [76].

Perspectives

Tables 1 and 2 list the arthritis clinical trials to date. For a field that is over 20 years old, the number of trials is modest. Moreover, as described in the preceding text, it is unlikely that the IND-approved products listed in Table 1 will be developed further; as far as we know, only 2 new human studies are in the pipeline.

Several factors account for this glacial rate of progress [10,77]. Funding, of course, is an issue and the clinical translation of arthritis gene therapy is hampered by its lack of appeal to pharmaceutical companies who see long time-lines, questionable return on investment, and risk. Nevertheless, as noted in the acknowledgements to this review article, federal funding agencies have supported our research. But the sums of money now needed to perform the

pre-clinical testing required to satisfy the demands of a successful IND application are enormous. Moreover, the regulatory barriers have become much more restrictive, as evidenced by our own experience. Our first gene therapy trial, using the retrovirus MFG-IRAP, took approximately 7 years from concept to injecting the first subject. We have been working on the next one, using AAV.IL-1Ra, for 9 years, and it is still at the Pre-IND stage.

Some optimism can be gained from recent progress in treating a number of genetic diseases by gene therapy. These include adrenal leukodystrophy, β-thalassemia, adenosine deaminase (ADA) deficiency, X-linked SCID, chronic granulomatous disease, Leber congential amaurosis, and lipoprotein lipase deficiency [78]. Indeed, an AAV-based gene therapeutic for lipoprotein lipase deficiency has just received marketing approval by the European Medicines Agency as the drug Glybera. According to one newspaper report [79], this could cost as much as $1.6 million for the single injection necessary to confer lifetime therapy. Clearly, this pricing would not be appropriate for a disease as common as arthritis.

All of the above examples of success concern rare, Mendelian diseases. It is to be hoped that success in this domain will generate enthusiasm for using genes to treat common, non-genetic diseases like arthritis.

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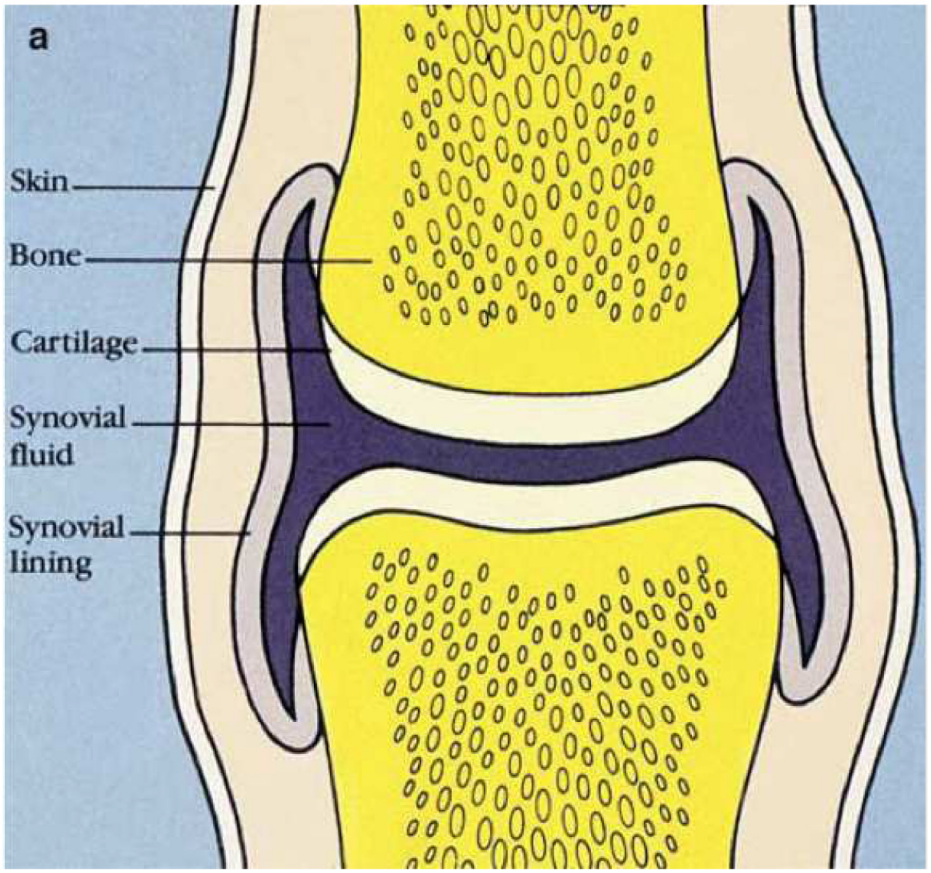

Figure 1. Basic anatomy of the normal diarthrodial joint

The articulating surfaces of the long bones are covered in articular cartilage that permits almost frictionless motion within a synovial cavity (joint space) containing a small volume of synovial fluid. The inner surface of the joint space is lined by synovium (synovial lining).

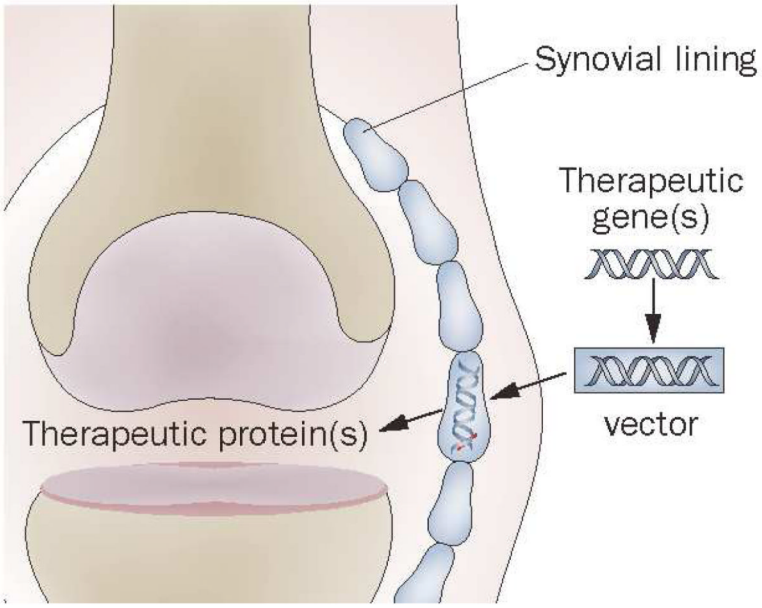

Figure 2. Basic concept behind local, intra-articular gene therapy for arthritis

Complementary DNA encoding an anti-arthritic product, typically a secreted protein, is introduced into the joint. Cells within the synovium, and elsewhere, become transduced and synthesize the encoded transgene endogenously in a sustained fashion.

From reference 10, with permission.

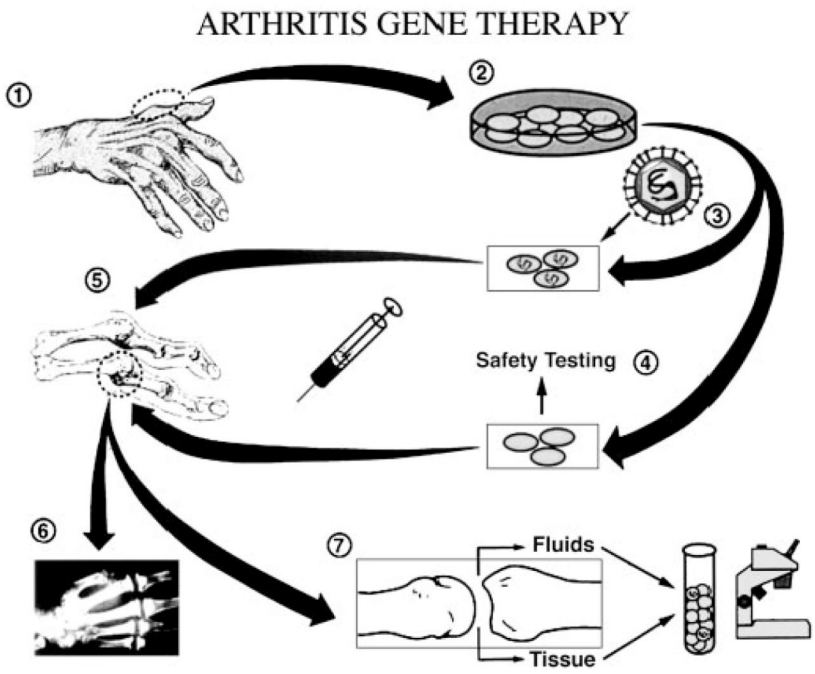

Figure 3. Protocol for the first gene transfer to human joints

Surgery of the joints of the hand or foot (step 1) provided autologous synovium, which was used to establish cultures of synovial fibroblasts (step 2). Half the cells were transduced with the retroviral vector (step 3), and all cells were tested for replication competent retrovirus and adventitious agents (step 4) before injection into MCP joints numbers 2–5 on one hand (step 5). In a double-blinded fashion, two joints received transduced cells, and two received control cells. One week later, the injected joints were surgically removed during total joint replacement surgery (step 6), and the retrieved tissues were analyzed for evidence of successful gene transfer and gene expression (step 7).

From reference 23 with permission.

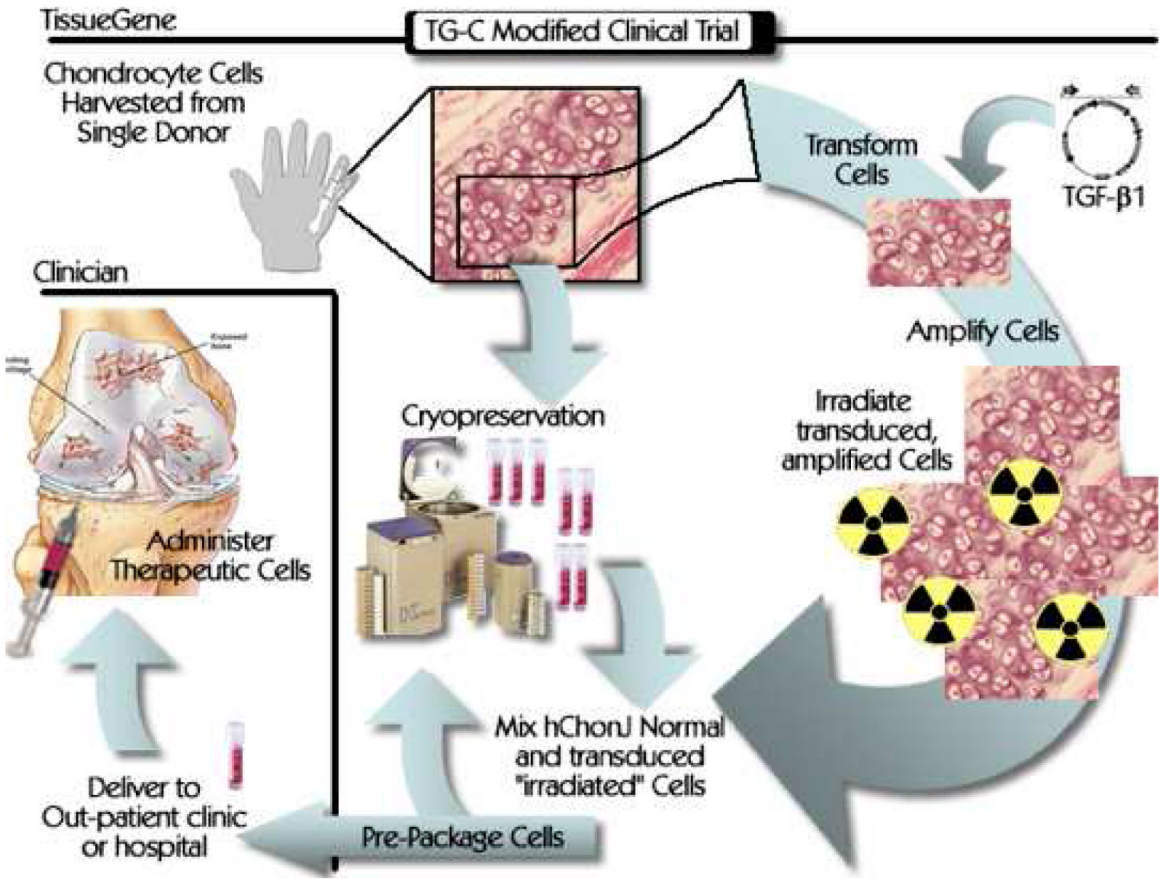

Figure 4. Protocol for use of genetically modified, allogeneic chondrocytes in osteoarthritis

Transduced chondrocytes expressing TGF-β_1 are irradiated and mixed with unmodified cells in a 1:3 ratio. They are delivered to the clinic for intra-articular injection.
From reference 42, with permission.

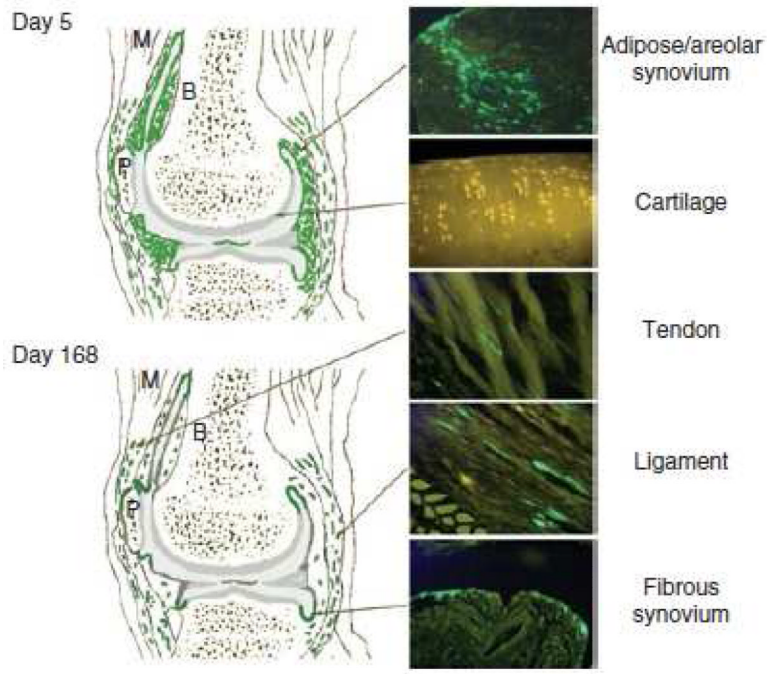

Figure 5. Fibroblasts resident in fibrous articular tissues support stable expression of exogenous transgenes

After intra-articular injection of lentiviral or adenoviral vectors containing the cDNA for GFP into the knees of nude rats, groups of animals were killed at days 5 and 168. The knee joints and surrounding tissues were harvested intact, decalcified, and processed for histology. For each joint, the approximate positions of fluorescent cells identified in serial sagittal whole-knee sections were tabulated in green on knee-joint diagrams similar to that shown on the left. On the right, images are characteristic of the appearance of the GFP+ cells in tissue sections at the different times (×20 magnification). Lines indicate the approximate regions represented by the tissue sections. The numbers of GFP+ cells in the synovium and sub-synovium were reduced dramatically at day 168. The density and distribution of GFP+ cells in the tendon, ligament, and fibrous synovium were largely unchanged over the duration of the experiment. No fluorescent cells were seen in the articular cartilage with either virus at any time point.

From reference 26, with permission

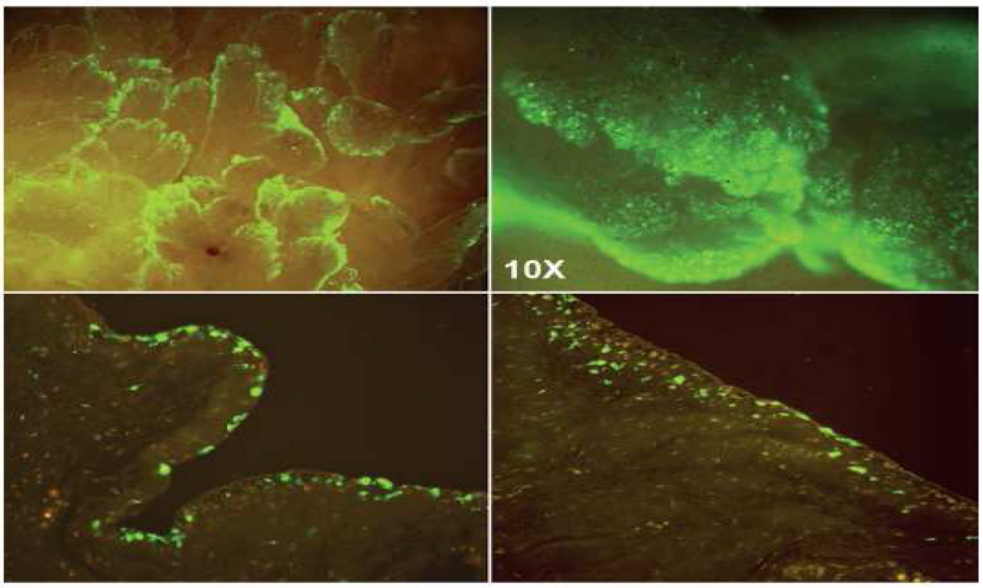

Figure 6. Transgene expression in equine synovium following intraarticular injection of AAV.GFP

Top two panels: Unprocessed tissue

Bottom two panels: Histological sections

From reference 59, with permission

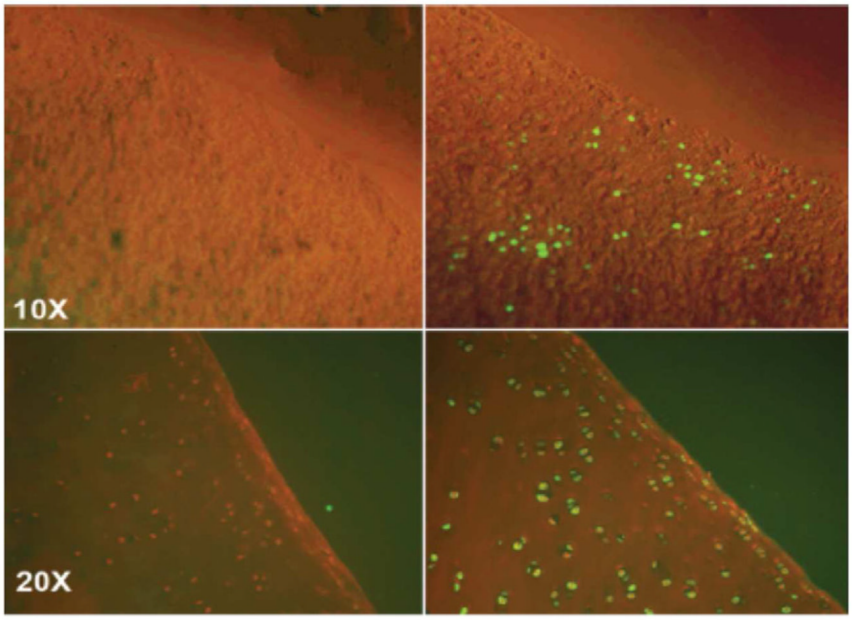

Figure 7. Transgene expression in equine cartilage following intraarticular injection of AAV.GFP

Left two panels: control cartilage

Right two panels: cartilage from joints injected with AAV.GFP

From reference 59, with permission

Table 1

Clinical trials in the gene therapy of rheumatoid arthritis

Transgene	Method of delivery	Phase	Institution or sponsor (principal investigator(s))	NIH OBA protocol number	Number of subject	Reference
IL-1Ra	Retrovirus, Ex Vivo	I	University of Pittsburgh (Evans and Robbins)	9406-074	9	37
IL-1Ra	Retrovirus Ex Vivo	I	University of DŸsseldorf, Germany (Wehling)	NA	2	38
Etanercept	AAV In Vivo	I	Targeted Genetics (Mease)	0307-588 *	15	61
Etanercept	AAV In Vivo	I/II	Targeted Genetics (Mease)	0503-705 **	127	63
INF-β	AAV In Vivo	Pre-Clinical	Arthrogen (Tak)			

OBA: Office of biotechnology activities

NA: Not applicable

Adapted from reference 73

* Included one subject with ankylosing spondylitis

** Included subjects with ankylosing spondylitis and psoriatic arthritis

Table 2

Clinical trials in the gene therapy of osteoarthritis

Transgene	Vector Ex/In Vivo	Phase	PI, Institution or Sponsor	OBA Protocol Number	Status	Number of subjects	Ref.
TGF-β_1	Retrovirus Ex Vivo	I	Ha, Kolon Life Sciences, Korea	NA	Closed	12	42
TGF-β_1	Retrovirus Ex Vivo	I	Mont TissueGene Inc	0307-594	Closed	9	
TGF-β_1	Retrovirus Ex Vivo	IIa	Ha, Kolon Life Sciences, Korea	NA	Closed	28	43
TGF-β_1	Retrovirus Ex Vivo	II	Mont TissueGene Inc	0912-1016	Open	100	
IL-1Ra	AAV In Vivo	I	Evans		Pre-IND		

OBA : Office of Biotechnology Activities

NA = Not Applicable

Adapted from reference 73

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