

## Meeting report

# The 3rd International Meeting on Gene Therapy in Rheumatology and Orthopaedics

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## Abstract

The 3rd International Meeting on Gene Therapy in Rheumatology and Orthopaedics was held in Boston, Massachusetts, USA in May 2004. Keystone lectures delivered by Drs Joseph Glorioso and Inder Verma provided comprehensive, up-to-date information on all major virus vectors. Other invited speakers covered the application of gene therapy to treatment of arthritis, including the latest clinical trial in rheumatoid arthritis, as well as lupus and Sjögren's syndrome. Applications in mesenchymal stem cell biology, tissue repair, and regenerative medicine were also addressed. The field has advanced considerably since the previous meeting in this series, and further clinical trials seem likely.

## Introduction

Every 3 years, a loosely affiliated network of investigators holds an informal, 2-day meeting to discuss progress in the general area of arthritis gene therapy. The name of the meeting varies slightly on each occasion to reflect the inclinations of the local organizers. The First International Meeting on Gene Therapy of Arthritis and Related Disorders (held in Bethesda, MA, USA by the National Institutes of Health in 1998) [1] and the Second International Meeting on Gene and Cell Therapies of Arthritis (held in Montpellier, France in 2001) [2] attracted a predominately rheumatologic audience. The latest meeting (held in Boston, MA, USA in May 2004) included substantial coverage of bone, cartilage and ligament healing, as well as osteoporosis, disc degeneration and osteogenesis imperfecta (OI). The Third International Meeting on Gene Therapy in Rheumatology and Orthopaedics thus attracted participants from both the rheumatologic and orthopaedic communities. Approximately 85 individuals attended.

## Keynote lectures

Two tremendous Keynote Lectures by former Presidents of the American Society of Gene Therapy ensured a successful start. Joseph Glorioso (University of Pittsburgh, Pittsburgh, PA, USA), American Editor of the journal *Gene Therapy*, lifted the mood by discussing 'Why gene therapy will work'. His lecture focused on the major nonintegrating viral vectors, adenovirus and, especially, herpes simplex virus, whose development as a vector for gene therapy was pioneered by Glorioso and colleagues [3]. This vector is particularly well suited to applications in the nervous system, and impressive preclinical data were shown in animal models of pain [4]. This is clearly of relevance to rheumatology and orthopaedics, in which intransigent pain is a frequent dominant symptom.

Inder Verma (the Salk Institute, La Jolla, CA, USA), Editor-in-Chief of the journal *Molecular Therapy*, discussed 'Gene delivery: novel vectors'. By focusing largely on retroviral and lentiviral vectors, it formed a fitting complement to Glorioso's lecture. Verma's laboratory pioneered the development of lentiviral vectors [5], and his presentation emphasized both the remarkable efficiency of these vectors and the problems associated with insertional mutagenesis as a result of integration into genomic DNA. A major effort is underway to develop vectors with predictable, safe integration sites. Without such assurances, gaining regulatory approval for the use of such vectors for nonlethal indications such as arthritis and tissue repair may be difficult.

## Arthritis and autoimmune diseases

Lentiviral vectors were the subject of the first talk in the following session on 'Gene transfer to synovium'. The

AAV = adeno-associated virus; ACL = anterior cruciate ligament; APC = antigen-presenting cell; BMP = bone morphogenetic protein; LMP = lim mineralization protein; MSC = mesenchymal stem cell; OI = osteogenesis imperfecta; RA = rheumatoid arthritis.

synovium is an obvious site for gene transfer when treating joint diseases by local gene therapy [6], and this shaped the strategy of the first clinical trials. Although a number of vectors can transfer genes to synovium quite effectively, it has been difficult to achieve long-term transgene expression in the joints of experimental animals. Elvire Gouze (University of Florida, Gainesville, FL, USA) described the remarkable efficiency of HIV-derived vectors in transferring genes to the synovial linings of rat joints by *in vivo* intra-articular injection [7]. Moreover, experiments conducted with athymic rats clearly identified the immune system as a major barrier to prolonged intrasynovial transgene expression [8]. Her experiments also identified a subpopulation of synovial cells with a very low turnover and the ability to support long-term transgene expression.

Another vector for *in vivo* transfer of genes to joints, namely adeno-associated virus (AAV), was described by Florence Apparailly (Hopital Lapeyronie, Montpellier, France). AAV is becoming the vector of choice for many human applications, including arthritis, because it is perceived to be very safe [9]. This consideration overrides its relatively modest transduction of synovium and the cost and complexities of its manufacture. Later in the meeting, Haim Burstein (Targeted Genetics Inc., Seattle, WA, USA) described the first human clinical trial using AAV in arthritis gene therapy.

Artificial chromosomes provide an alternative means of presenting and sustaining transgenes within target cells [10]; Margriet Vervoordeldonk (University of Amsterdam, Amsterdam, The Netherlands) described their first use in synovium. Because the artificial chromosomes are duplicated, segregated, and distributed to daughter cells upon cell division in the manner of endogenous chromosomes, there is the potential for long-term carriage of transgenes. The large size of the chromosome circumvents the packaging restrictions of viral vectors. At the moment, the strategy is technologically demanding. The artificial chromosomes must be transfected into synovial cells *in vitro* before implantation, and the efficiency of successful transfection is low. Although this can to some degree be compensated for by including selectable markers, many of the proteins encoded by the selectable marker genes, such as neomycin phosphotransferase, are of nonhuman origin. They are therefore antigenic and thus likely to provoke immune destruction of the transfected cells.

After systemic injection into experimental animals, certain types of cells home to arthritic joints and other sites of disease, such as lymphoid organs. This permits transgenes to be delivered locally to multiple affected sites by a single, systemic injection – a strategy known as ‘facilitated local delivery’ [11]. By combining the specificity and safety of local delivery with the ease of systemic application, this approach offers many advantages. Moreover, the types of cells that are most useful in this regard, namely antigen-presenting cells

(APCs) and lymphocytes, are not passive suppliers of transgene products but are active participants in suppressing disease.

In the session on ‘Antigen presenting cells and lymphocytes’, Paul Robbins (University of Pittsburgh, Pittsburgh, PA, USA) discussed data confirming the remarkable potency of genetically modified dendritic cells as antiarthritic agents in murine, collagen-induced arthritis. A variety of transgenes, including interleukin-4 and Fas ligand, as well as nuclear factor- $\kappa$ B decoys, are active in this regard [12,13]. Robbins also described microvesicles, termed exosomes, which are produced by the genetically modified APCs and are as potent as the parental immunosuppressive APCs in blocking, or even reversing, established murine arthritis [14].

John Mountz (University of Alabama, Birmingham, AL, USA) has pioneered the use of APCs expressing a death gene. He described the latest iteration of this approach in which an adenovirus is used to deliver TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) cDNA, under the control of a tet-inducible promoter, to dendritic cells. Impressive suppression of murine collagen-induced arthritis was achieved when the genetically modified dendritic cells were first pulsed with type II collagen [15].

Gary Fathman (Stanford University, Palo Alto, CA, USA) covered the use of genetically modified lymphocytes to treat autoimmune diseases – an approach he has named ‘adoptive cellular gene therapy’ [16]. Like dendritic cells, the lymphocytes home to sites of disease involvement where they confer a therapeutic effect [17]. One advantage of lymphocytes is their ability to divide in response to antigen, thereby amplifying the therapeutic response. A disadvantage for clinical application is the difficulty of isolating autoreactive T cells in sufficient numbers.

A severe combined immunodeficient mouse model developed by Gay and colleagues [18] has proved very useful in evaluating various elements of gene transfer to synovium in human tissues *in vivo*. Ulf Muller (University of Regensburg, Regensburg, Germany) described this model and the accumulated data obtained with it. The model involves the coimplantation of human synovium or isolated synoviocytes with small pieces of human cartilage into severe combined immunodeficient mice. Both synovial invasion of the cartilage and chondrocytic chondrolysis occur, and are susceptible to various genes transferred to the human synovial tissue before implantation.

Other talks in the session on ‘Autoimmune disease’ were represented by lectures on lupus and Sjögren’s syndrome. Rizgar Mageed (University College, London, UK) described various genetic approaches to treating lupus, many of which are based upon the delivery of immunosuppressive cytokines or cytokine antagonists [19]. Sjögren’s syndrome is an

autoimmune disease that affects the salivary glands. Direct gene transfer to the salivary glands can be accomplished in a relatively noninvasive manner. Bruce Baum (National Institutes of Health, Bethesda, MA, USA) described the suppression of disease in a murine model of Sjögren's syndrome using recombinant AAV to deliver interleukin-10 cDNA [20]. Because the salivary glands have important secretory functions, they can also be targeted for the systemic delivery of secreted gene products [21].

### Mesenchymal stem cells and the repair of bone and cartilage

So-called mesenchymal stem cells (MSCs) attract considerable attention as pluripotent adult cells with the ability to differentiate into most, if not all, musculoskeletal tissues [22]. They thus form a very attractive basis for the repair and regeneration of these tissues, especially when their abilities to do so are enhanced by genetic modification. Many of the technologies being developed for this purpose combine elements from tissue engineering and regenerative medicine with gene therapy. MSCs were originally identified in bone marrow isolates, but similar cells have subsequently been isolated from a number of additional sources.

Considerable research is devoted to comparing the properties of MSCs derived from various different locations and evaluating their suitability for tissue regeneration. In the session on 'Mesenchymal stem cells', talks by Dan Gazit (Hebrew University, Jerusalem, Israel), Hairong Peng (University of Pittsburgh, Pittsburgh, PA, USA), and Ronda Schreiber (Macropore Inc., San Diego, CA, USA) described the properties of MSCs derived from bone marrow [23], muscle [24], and adipose tissue [25], respectively. Collectively, the talks indicated that MSCs can readily be grown from these sources, genetically manipulated in the laboratory, and used successfully to enhance the repair of surgically created lesions in the long bones and crania of experimental animals.

The session on MSCs overlapped with sessions on 'Cartilage repair' and 'Bone healing', as well as certain topics considered in a session on 'Other orthopedic applications of gene therapy'. Steven Ghivizzani (University of Florida, Gainesville, FL, USA) described a novel approach to enhancing cartilage repair, in which bone marrow is aspirated and, as it clots, mixed with vectors carrying chondrogenic genes. The clot containing genetically modified chondroprogenitor cells and, possibly, bound vector is known as a 'gene plug' and can be press-fitted into the defect as an immediate source of reparative cells expressing chondrogenic cDNAs [26]. A key element of this approach is the ability of progenitor cells to undergo chondrogenesis in response to gene transfer, and this was shown for MSCs transduced with cDNAs encoding transforming growth factor- $\beta_1$  [27]. Promising, preliminary *in vivo* data were obtained from experiments in which genetically modified bone

marrow was implanted into osteochondral lesions in the femoral condyles of rabbits.

*Ex vivo* approaches to the repair of cartilage using genetically modified chondrocytes and MSCs were discussed by Alan Nixon (Cornell University, Ithaca, NY, USA) and Klaus Von der Mark (University of Erlangen-Nuernberg, Germany), respectively. The former speaker described experiments in horses in which allogeneic chondrocytes were transduced with equine insulin-like growth factor-1 or human bone morphogenetic protein (BMP)-7, incorporated into a fibrin clot, and implanted into surgically created, partial thickness cartilage lesions in horses [28]. Genetic manipulation of the donor chondrocytes accelerated early healing, but ultimately there was little difference from controls. Several issues remain to be resolved, including the fate of the donor allografted cells. Von der Mark's group established monolayer cultures of MSCs from the rib perichondrium of rats and transduced the cells with recombinant adenoviruses carrying BMP-2 and insulin-like growth factor-1 cDNAs [29]. The modified cells were incorporated into fibrin glue and placed into partial thickness lesions in the patellar groove of the femur. Both transgenes enhanced the repair process, but BMP-2 expression was associated with the formation of osteophytes.

The use of gene transfer to enhance bone healing is a popular field of study, not least because bone responds so well to this type of manipulation and cDNAs encoding a variety of different BMPs are readily available. Several different approaches were discussed. Greg Helm (University of Virginia, Charlottesville, VA, USA) and Axel Baltzer (University of Düsseldorf, Düsseldorf, Germany) reported the use of direct adenovirus delivery of osteogenic cDNAs to ectopic and intraosseous sites [30,31]. Immune reactions to the adenovirus and the transgene product emerge as important factors in the success of these approaches. In general, it appears that intramuscular injection of recombinant first-generation adenovirus carrying an osteogenic BMP cDNA fails to induce bone in immunocompetent rats and mice, although immunodeficient animals respond by producing abundant ectopic bone. When the same adenovirus is injected intraosseously, however, there is a good osteogenic response in immunocompetent animals, with healing of experimental, critical size defects. Baltzer also described preliminary data suggesting that the direct, intralesional injection of adenovirus carrying BMP-2 cDNA promotes the healing of fractures in osteoporotic sheep [32].

Jay Lieberman (University of California at Los Angeles, Los Angeles, CA, USA) described *ex vivo* strategies for healing bone using genetically modified MSCs derived from bone marrow [33] and fat [34]. *Ex vivo* methods are expensive and cumbersome for clinical application, but they are perceived to be safer than *in vivo* methods and, because they provide progenitor cells in addition to genes, they may be more efficient under conditions in which soft tissue support is

compromised. As Lieberman commented, not all bone injuries will need to be treated by gene therapy, and those that do will have available several different strategic options, with different methods suited for different applications [35]. Thus, there is unlikely to be a single preferred gene therapy for all clinical settings in which it is necessary to enhance osteogenesis. Instead, the orthopedic surgeon will have available a variety of options from which to choose, depending on the clinical circumstances.

Large osseous defects are often treated with devitalized, allografted bone. These have a high failure rate because they do not remodel. Edward Schwarz (University of Rochester Medical Center, Rochester, NY, USA) addressed this problem by coating the allograft with AAV carrying cDNAs encoding vascular endothelial growth factor and RANK (receptor activator of nuclear factor- $\kappa$ B) ligand. In a mouse model, the coated allografts underwent remodeling and were eventually replaced with living, host bone – a process known as allograft revitalization [36].

Lim mineralization protein (LMP)-1 is perhaps the most potent osteogenic protein yet identified. When delivered as a cDNA its effects are dramatic, and it has been widely studied in the context of spinal fusion [37]. Because LMP-1 is an intracellular protein, gene transfer has been the technology of choice for enhancing bone healing. However, there are now methods for delivering proteins efficiently into cells by attaching protein transduction domains to their amino-termini. One such protein transduction domain is derived from the TAT protein of HIV; Jeffrey Marx (Medtronic Inc., Minneapolis, MN, USA) described the properties of the protein formed when the TAT protein transduction domain is fused to LMP-1. The potent osteogenic properties of this protein suggest that there are alternatives to gene transfer for certain intracellular proteins that do not require prolonged expression.

### Ligament repair and disc degeneration

The anterior cruciate ligament (ACL) of the knee is frequently ruptured during sporting activities. It does not heal spontaneously. ACL injuries are not only painful and debilitating, but they also predispose to osteoarthritis. In the absence of a repair process, treatment is surgical and of questionable value; the incidence of secondary osteoarthritis, for instance, is not reduced by ACL reconstruction. Martha Murray (Children's Hospital, Boston, MA, USA) described novel approaches to ACL healing based on the migration of cells from a ruptured ACL into suitable, adjacent, collagenous matrices. When these matrices are impregnated with adenovirus vectors, the immigrating cells become transduced and, depending on the transgene, better able to form repair tissue [38].

Intervertebral disc degeneration is a massive and expensive public health problem. It may be possible to prevent disc degeneration through the transfer of protective genes to disc

cells. James Kang (University of Pittsburgh, Pittsburgh, PA, USA) has pioneered research in this area [39]. The disc provides two advantages to gene therapists: the cells within the disc are protected from immune surveillance and they do not divide. Because of these circumstances, it is possible to express foreign genes within the disc for at least a year after the direct injection of first generation adenovirus vectors. A variety of growth factor cDNAs enhance matrix synthesis after *in vivo*, virally mediated gene transfer to the discs of rabbits [40]. Experiments are underway to determine whether they retard disc degeneration in animal models.

### Osteogenesis imperfecta

Until now, applications of gene therapy in rheumatology and orthopedics have focused almost exclusively on the treatment of nongenetic diseases. OI ('brittle bone disease') provides one exception to this engaging paradox. It is caused by mutations in the genes encoding the  $\alpha$ -chains of type I collagen. Gene therapy is complicated by the fact that, in most cases, OI is a dominant negative condition. Christopher Niyibizi (Hershey Medical School, Hershey, PA, USA) studied the gene therapy of OI using the *oim* mouse, which fails to produce the  $\alpha_2$ -chain of type I collagen and has a recessive condition resembling human OI. He has been able to correct the phenotype in cultured fibroblasts *in vitro* and in patches of skin *in vivo*, using an adenovirus to transfer the wild-type cDNA encoding the  $\alpha_2$  chain of type I collagen [41]. This is something of an accomplishment because there was a concern that the high synthesis of the  $\alpha_2$ -chain would disrupt the 2:1 ratio of  $\alpha_1$ : $\alpha_2$  chains in type I collagen. Instead, the inherent editing functions of the cell ensured production of authentic type I collagen molecules. Niyibizi is now exploring the use of genetically modified MSCs as vehicles for correcting OI [42].

In future, it is likely that, where a clear mutation has been associated with a genetic rheumatic or orthopedic disease, a gene therapy approach will be tried [43].

### Clinical trials

Five clinical trials for the gene therapy of rheumatoid arthritis (RA) have been initiated. Two of these were described at previous meetings of this group. Haim Burstein described the preclinical data leading up to a phase I protocol recently initiated by Targeted Genetics Inc. This protocol uses a serotype 2 AAV carrying what is essentially etanercept cDNA. An equivalent vector has shown efficacy in rat streptococcal cell wall induced arthritis [44], a model of RA, and the clinical vector has proved safe in monkeys. During the phase I study, the vector is injected into individual joints of subjects with RA to establish dosing and safety. At the time of the meeting, the vector had been administered safely to three individuals, but no clinical data were available.

### Conclusion

Rheumatology and orthopedics provide valuable niches for gene therapy. Indeed, these disciplines may find themselves



in the forefront when it comes to clinical applications. Many of these applications are well suited to gene transfer approaches, and the potential patient population is very large. Because most conditions are debilitating rather than lethal, safety is a dominating issue that determines the types of vectors that are acceptable. Impressive data have been generated in various animal models of arthritis, tissue repair, disc degeneration, and OI. It is encouraging that several clinical trials have been initiated for the gene therapy of RA, and thus far these are proving to be safe. However, only one such study has appeared in the literature [45] and we must await further peer-reviewed publication before getting excessively optimistic.

The next meeting in this series will be held in Amsterdam, The Netherlands in 2006. Enquires may be directed to Paul-Peter Tak (P.P.Tak@amc.uva.nl)

### Competing interests

CHE and PDR are members of the Scientific Advisory Board of TissueGene Inc. and Orthogen AG.

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