

# The Twelfth Annual Meeting of the European Society of Gene Therapy

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The 2004 annual meeting of the European Society of Gene Therapy (ESGT) was held in Tampere, Finland from the 4<sup>th</sup> to the 7<sup>th</sup> of November. With more than 500 attendees, 66 oral presentations, over 200 posters, and a full day of educational symposia, the topics covered ran the gamut, including cancer, stem cells, cardiovascular disease, infectious disease, skeleto-muscular systems, promoter targeting and genome editing, siRNA and zinc finger proteins, and included recent advances in viral and nonviral vector development and regulatory issues.

RNAi continues to play a major role in the discovery of gene function and application and was featured prominently at the meeting. Reuven Agami (National Cancer Institute, Amsterdam) reported on the use of RNAi library screening to identify tumor suppressor genes. Using this screen, Dr. Agami identified the pituitary homeobox 1 (*PITX1*) gene, which was subsequently shown to function upstream of *RAS* in the control of cell proliferation in the later stages of tumorigenesis. Mark Kay (Stanford University) reported on the use of RNAi to knock down gene expression as a tool for gene therapy. Dr. Kay previously published a report showing that hydrodynamic tail vein injection of shRNA could suppress transient hepatitis B virus (HBV) replication in mouse liver. At this meeting, Kay described the effect of shRNA in HBV transgenic mice. These mice simulate human HBV infection more effectively by generating viral particles that are secreted into the serum. The liver was targeted with an AAV2/8 chimeric virus carrying an shRNA expression cassette. Although mice that received the shRNA demonstrated significantly reduced viral titers, they eventually died. However, decreasing the size of the expressed siRNA from 21-23 nt to 19 nt maintained the antiviral effect while preventing death of the mice. The authors concluded that it was possible to overcome HBV replication in transgenic mice with RNAi. They further speculated that the death of mice expressing the longer RNAi might be attributed to off-target effects of the siRNA, although no direct evidence was provided in favor of this hypothesis.

Kazunari Taira (Tokyo University) reported on the effects and use of different types of ribozymes for gene therapy. In addition, Taira showed evidence suggesting that it was possible to express relatively long (~ 50 nt) shRNAs without stimulating the innate immune system. These expressed shRNAs harbored mutations in the sense strand, generating bulges in the shRNA. These bulges lead to structures that

resemble microRNAs. Obviously, this observation could open new therapeutic avenues.

Zeger Debyser (Rega Institute for Medical Research, Leuven) presented new insight into the process of genomic integration by lentiviral vectors. HIV integrase (IN) has previously been shown to interact with human lens epithelium-derived growth factor (LEDGF/p75), a DNA-binding protein involved in the cellular stress response. LEDGF/p75 is essential for nuclear accumulation and chromosomal association of HIV-1 IN and therefore has been postulated to tether the HIV-1 IN to DNA. Debyser presented data based on fluorescence correlation spectroscopy measurements suggesting that LEDGF/p75 promoted binding of HIV-1 IN to DNA. Furthermore he showed that knockdown of LEDGF/p75 expression by siRNA blocked nuclear localization and chromosomal association of HIV-1 IN and decreased viral replication by two- to threefold. The interacting domain in HIV-1 IN has been mapped to the C-terminus of the protein. Debyser showed that mutations within the IN-binding domain (Q168L) abolished binding of IN to DNA and inhibited HIV replication due to an integration defect. These observations could give rise to new therapeutic strategies to treat HIV-1 infection and could also facilitate the development of strategies to target HIV-1 integration to selected chromosomal sites. This may be relevant in the near future, since major advances were presented using lentiviral vectors for the correction of genetic diseases like Wiskott-Aldrich Syndrome (F. Marangoni *et al.*, Milan), human factor IX deficiency (B.W. Bigger *et al.*, London), ADA-SCID (A.R. Mortellaro *et al.*, Milan) and childhood cerebral adrenoleukodystrophy (N. Cartier *et al.*, Paris).

A striking observation at the meeting was the number of successful studies in mice that are currently being translated into preclinical evaluation in large animal models. Aurélie Goyenvallé and colleagues from Genethon (Evry, France) achieved persistent exon skipping that removed the mutated exon in the dystrophin mRNA of the *mdx* mouse, by a single administration of an AAV1 vector expressing antisense sequences linked to a modified U7 short nuclear RNA (snRNA). They showed that the components of the associated glycoprotein complex, including  $\alpha$  and  $\beta$  sarcoglycans and  $\beta$  dystroglycan were expressed along with the rescued dystrophin at the periphery of fibers in the treated mice. Importantly, the treated animals exhibited normal contractile and mechanical properties, measured by resistance to

tetanic contractions accompanied by forced lengthening. Obviously, translating this remarkable finding to the *GRMD* dog model will be an exciting challenge in the coming year.

Karl Tryggvason (Karolinska Institute) presented an overview of gene therapy for Alport syndrome (AS). AS arises from mutations in the gene encoding the type IV collagen  $\alpha 5$  chain, resulting in a defective glomerular basement membrane. Clinically, AS-affected patients present a progressive kidney disease with hematuria, proteinuria and renal failure, often accompanied by hearing loss and ocular defects. Tryggvason and colleagues initiated gene transfer studies in Navasota dogs with AS by perfusing recombinant adenovirus into the renal artery. A surgical protocol was previously validated in a swine model that resulted in a high rate of selective transduction of glomeruli. Preliminary data presented at the meeting suggested that the type IV collagen  $\alpha 5$  chain could be expressed in the AS-affected dog, leading to the expression of other associated chains ( $\alpha 3$  and  $\alpha 4$ ). Hopefully, next year's meeting will confirm this stimulating finding with functional recovery and alternative delivery vectors.

Fatima Bosch (Universitat Autònoma, Barcelona) demonstrated correction of glycemia in a double diabetic KO mouse after electrotransfer and AAV1-mediated transfer of both the insulin and glucokinase cDNAs in skeletal muscle. Bosch's group also achieved adenovirus-mediated gene transfer to the pancreas in healthy and diabetic dogs after *in situ* delivery. Clearly, treatment of large animal models of type 1 diabetes by gene transfer is moving forward.

Robin Ali (Moorsfield Hospital, London) has previously investigated AAV-mediated reporter gene transfer to the retina of wild-type dogs. He is now developing therapeutic approaches to treat two dog models of inherited retinal degeneration. The first is a form of autosomal recessive *retinitis pigmentosa* due to a defect in a photoreceptor-specific gene encoding  $\beta$  phosphodiesterase (*PDE*). The second is a form of Leber's congenital amaurosis due to a defect in *RPE65*, a retinal pigment epithelium-specific gene. He and his colleagues are planning a clinical trial for *RPE65*-deficient patients in the near future.

Alain Fischer (Necker Hospital, Paris) presented an update

on the Phase I SCID-X1 clinical trial. With a follow up of more than five years for the first treated patients, this study has clearly shown the potential of gene therapy for the treatment of immunodeficiencies. Fisher presented evidence supporting gene transfer into pluripotent hematopoietic stem cells. This evidence was based on the persistent detection of naïve T cells, low but detectable levels of gene marking in granulocytes and monocytes, and common proviral integration sites in T and B lymphocytes, granulocytes and monocytes. Fischer also discussed the two cases of lymphoproliferative disease that occurred in two of the treated patients. Both patients were allotransplanted after chemotherapy and while one of them is well and alive (P5), the other died a few weeks prior to the meeting. Despite this unfortunate outcome, the French scientists have decided to continue with the treatment of patients with certain modifications to their protocol. These include the treatment of only those patients older than six months of age and limitation of the administered cell dose to ten million CD34<sup>+</sup> cells per kg.

A consortium of scientists from Frankfurt, Zurich and London reported the treatment of chronic granulomatous disease (CGD) by gene transfer. They presented data on two patients in which gene modified cells were clearly detectable in the peripheral blood four to five months after transplantation. The difference between this study and previously conducted studies for CGD was the use of a myelosuppressive conditioning regime (busulfan) to provide space in the bone marrow for the incoming gene-modified cells. Although the follow-up of these patients is yet too short to assess long-term persistence of gene-modified cells, this protocol may pave the way for the treatment of diseases for which gene-modified cells will not have a selective advantage over nontransduced cells.

A remarkable presence as always, Inder Verma was the keynote speaker at the opening ceremony. Bernd Gänsbacher (Germany) stepped down from his four-year term as President of the ESGT Board after an outstanding job. David Klatzmann (France) was named as his successor. The 2005 meeting of the society will be held in Prague.