



Human Hematopoietic Stem Cells and Gene Transfer: Lessons from CGD Human Trials

Feb. 19 , 2009 (Thu.) / 2:00 PM ~ 5:20 PM

1st Floor Conference Room,
Clinical Research Institute,
Seoul National University Hospital,
Korea

Organizer: Korean Society of Gene Therapy,
The Korean College of Pediatric Clinical Immunology,
and The Korean Society of Pediatric Hematology & Oncology

Sponsor: ViroMed Co., Ltd. 

- 02:00 – 02:10 **Opening Address**

Dr. Je-Ho Lee
President of the Korean Society of Gene Therapy and
Professor, Samsung Medical Center
- 02:10 – 02:30 **Overview on Gene Modification of Hematopoietic Stem Cells
Using CGD as Model Case**

Dr. Sunyoung Kim
Professor, Seoul National University and CEO, ViroMed Co., Ltd.

Chairman: Dr. Sunyoung Kim
Professor, Seoul National University and CEO, ViroMed Co., Ltd.
- 02:30 – 03:00 **Gene Therapy for CGD: The Frankfurt Experience and Beyond**

Dr. Manuel Grez
Head, Gene Therapy Unit, Georg-Speyer-Haus, Germany
- 03:00 – 03:30 **Gene Therapy for CGD: Current Studies and Future Approaches**

Dr. Harry L Malech
Head, Genetic Immunotherapy Section, National Institute of Allergy and Infectious Diseases,
National Institutes of Health, USA
- 03:30 – 03:50 **Coffee Break**
- 03:50 – 04:20 **UK Experiences of Gene Therapy for CGD**

Dr. Adrian J Thrasher
Director, Centre for Immunodeficiency, Institute of Child Health, UK
- 04:20 – 04:50 **Gene Therapy for CGD: Korean Experiences**

Dr. Joong Gon Kim
Professor, Seoul National University College of Medicine, Korea
- 04:50 – 05:20 **Open Discussion**

Moderator: Dr. Sunyoung Kim
Professor, Seoul National University and CEO, ViroMed Co., Ltd.

We have previously reported on a gene therapy protocol for the treatment of X-linked Chronic Granulomatous Disease (X-CGD), a primary immunodeficiency characterized by a non-functional NADPH oxidase and defective phagocytic killing. Two young adult X-CGD patients were treated with gene modified cells in combination with non-myeloablative conditioning. Successful engraftment of gene transduced cells resulted in the eradication of pre-existing life-threatening bacterial and fungal infections. However, insertional activation of MDS1/EVI1, PRDM16 and SETBP1 triggered an increase in gene transduced cells in the peripheral blood of both patients.

After the initial benign polyclonal expansion gene marked hematopoiesis in both patients was restricted to a few clones overexpressing MDS1/EVI1 and EVI1 leading to chromosomal instability and ultimately to hematological abnormalities. P1 died 27 months after treatment due to a severe sepsis with multiorgan dysfunction. Although gene marking was still high at this time point, expression of the therapeutic gene, gp91phox, was severely reduced due to CpG methylation at the viral promoter. Similar events were also observed in P2. This patient underwent stem cell transplantation from a matched unrelated donor and is currently recovering from the treatment.

Despite vector inactivation and the severe adverse effects observed in our trial, the initial eradication of preexisting life-threatening infections and other clinical benefits experienced by both patients for more than two years after reinfusion of their autologous gene modified cells, are clear evidence that gene therapy can efficiently provide a long-term cure and encourages the further development of gene therapy for the long-term correction of CGD. Advances in vector design which reduce genotoxicity and enhance efficacy can thus enable the safe and effective application of gene therapy for the correction of immunodeficiencies and other inherited hematological disorders.

Dr. Manuel Grez
Head, Gene Therapy Unit, Georg-Speyer-Haus, Germany

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Senior Scientist, Heinrich-Pette Institute für Experimentelle Virologie und Immunologie an der Universität Hamburg (1985-1990)
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Post-doc, Max-Planck-Institute for Molecular Genetics, Berlin, Germany (1978-1980)

X-linked Chronic Granulomatous Disease (X-CGD) is an immune deficiency resulting from mutations in the gp91phox subunit of superoxide-generating phagocyte NADPH oxidase, leading to recurrent infections. Allogeneic bone marrow transplant can cure CGD and improvements in transplant outcomes have resulted in an emerging consensus that X-CGD patients with oxidase function negative phenotype and a fully HLA-matched sibling donor should be considered for transplant. However, most patients lack an HLA-matched sibling. Furthermore, recent significant advances in infection prophylaxis and therapy of CGD have decreased morbidity and mortality for many patients, complicating the decision of which patients should be considered for transplant. Gene therapy may become an alternate management option for some patients.

We conducted clinical trials of *ex vivo* gene therapy for CGD in 1995-2000 using the amphotropic MoMuLV-derived gamma retrovirus vector, MFGS, without chemotherapy conditioning. While fully normal levels of oxidase production were observed on a per gene corrected neutrophil basis, the number of circulating oxidase-normal neutrophils was consistently <0.1%, even at earliest observable times (14 to 28 days), and detection of any gene corrected neutrophils at much lower levels persisted for only two or three months after each treatment. Recently, Ott et al from the Grez laboratory using busulfan 8mg/kg marrow conditioning and an SF71 gamma retrovirus gp91phox vector reported achieving gene marking of >20% at earliest observable times after treatment in two patients. Furthermore, over the next few months they observed a progressive increase in marking of the myeloid lineage to 40-60% in both patients likely due to oligoclonal outgrowth of cells with vector insertions in EVI1/MSD, SetBP1 or PRDM16. These investigators reported oxidase activity of the gene corrected neutrophils on a per corrected cell basis at 15% to 30% of that in normal neutrophils. Clinical benefit was reported for these two patients in the form of resolution of infections that were present at the time of the gene transfer therapy.

In late 2006 we opened a new gene transfer treatment clinical trial using the same amphotropic MFGS-gp91phox murine gamma retrovirus vector we had used in our previous studies, except that our new trial used a conditioning regimen with busulfan 10 mg/kg at the time of gene therapy. We chose this dosing of busulfan based partly upon the experience of Ott et al, but also upon our own recent experience of the safety and efficacy of this dose of busulfan used by us for marrow conditioning to achieve successful full engraftment of allogeneic HLA-matched sibling hematopoietic stem cell transplantation for X-CGD at the NIH. Since our studies in non-human primates with this dose of busulfan conditioning for gene transfer suggested that we likely could achieve clinical benefit, but not a cure of CGD with current vectors and methods, patients eligible for our study had to have life-threatening infection demonstrated to be unresponsive to conventional therapy. The gene transfer treatment in this setting was considered salvage therapy for patients with an infection deemed incurable by conventional management. In all cases state of the art high dose combination antibiotic therapy directed against the etiologic agent was continued in these patients.

Our adult patient #1 in our new trial had very large liver abscesses involving >60% of his liver mass. In November 2006 he received approximately 30 million autologous gene marked CD34 hematopoietic progenitor cells. Transduction efficiency following 4 daily transductions was 73%. In the first few weeks after gene therapy 24% of circulating neutrophils and monocytes in patient #1 were fully oxidase-normal. This level of marking of the myeloid lineage decreased by half about every 1.5 months until gene marking stabilized at about 1% of neutrophils at 9 months after treatment. By this time his liver abscess had fully resolved. At one year post therapy, a bone marrow aspirate demonstrated that 1% of bone marrow CD34 positive cells were gene marked, and could give rise to oxidase positive myeloid colonies and neutrophils when cultured. Now more than two years after treatment, this patient is without active infection; has a normal hematology complete blood count profile; regularly attends work and graduate studies; and 1% of his circulating neutrophils remain oxidase-normal.

It is important to note that oxidase function on a per corrected neutrophil basis remains equal to that seen in neutrophils from healthy volunteer as measured in the dihydrorhodamine (DHR) flow cytometry assay. There is ongoing assessment of the profile of vector insertion sites in neutrophils from this patient. Marking remains polyclonal, with no evidence for emergence of dominant clone(s) or preference for insertion near any particular gene or group of genes. However, given that publications from several laboratories have highlighted vector insertions within the EVI1/MDS complex, it could be of significance that one of more than sixty distinct vector insertion sites identified from peripheral blood of this patient was an insert near the EVI1/MDS complex. No vector insertions were detected near SetBP1, PRDM16 or LMO2.

We have treated two additional X-CGD patients, but with new lots of vector that have lower titer than the remaining amounts of a very high titer lot of vector used to treat Patient #1. Patient #2, with lung/chest wall/vertebral body *Paeclomyces* fungus infection, was conditioned with busulfan 10mg/kg and then given autologous CD34 progenitor cells with *ex vivo* transduction efficiency of 42%. Patient #2 had early *in vivo* marking of 5% of circulating neutrophils, where on a per corrected cell basis the neutrophils were oxidase-normal in the DHR assay. However, in this patient there was a precipitous loss of all detectable gene marking at the third week following gene therapy as measured by DHR or real time PCR. We suspect immune clearance and not silencing, since marking became PCR undetectable. However, we have not been able to confirm a cellular or humoral immune response to gp91phox. For subsequent patients we proposed adding rapamycin to the regimen for the first month following gene therapy to suppress immune rejection and encourage development of tolerance to the therapeutic neoantigen. Because gene marking did not persist long enough in this patient to provide significant clinical benefit, the patient was subsequently treated with a prolonged course of granulocyte transfusions to supplement his ongoing antibiotic therapy, but we had to cease these transfusions after a month because of the development of a demonstrated strong anti-HLA response. Though the patient fully recovered normal hematopoiesis cell counts following the gene therapy, and clearly demonstrated clinical benefit during the period of administration of allogeneic granulocyte transfusions, this patient expired more than 5 months after the gene therapy from progression of his primary *Paeclomyces* infection of lung/chest wall/vertebrae.

Patient #3 with long standing progressive *Aspergillus nidulans* infection of lung/chest wall/vertebrae was conditioned with busulfan 10mg/kg and then given autologous CD34 progenitor cells with *ex vivo* transduction efficiency of 24%. Daily oral rapamycin was also started one day before gene therapy and continued for one month following gene therapy aiming for a blood level of 15 ng/ml. At two weeks following gene therapy at a time when the absolute neutrophil count was about 500 cells/ul, 4.2% of circulating neutrophils were oxidase-normal by DHR assay. This marking decreased to 1.1% by 4 weeks at a time when the absolute neutrophil count was above 2000 cells/ul. At the most recent assay at almost 2 months post gene therapy, marking has decreased to about 0.1% of neutrophils detected as oxidase normal by DHR assay. There has been some improvement noted in the extent of lung involvement with this patient's *Aspergillus nidulans* infection, though it is not possible at this time to know if this resulted from the gene therapy treatment or will continue to improve.

Though we are encouraged by the unequivocal clinically beneficial outcome in patient #1 and possible early clinical benefit to patient #3, it is clear to us that additional improvements are needed to achieve higher permanent long term production of oxidase-normal neutrophils while at the same time incorporating additional safety features. In collaboration with the John Gray, Brian Sorrentino, Derek Persons and others at St. Jude Children's Research Hospital in Memphis, TN, we have jointly developed a next generation vector that we hope to bring to the clinic in the form of a self-inactivating insulated lentivirus vector with EF1alpha internal promoter driving production from a codon optimized gp91phox ORF. Preliminary laboratory studies of this next generation lentivector suggest that it can be produced from a permanent cell producer line at high titer, and can induce sufficient levels of production of gp91phox in patient X-CGD CD34 cells differentiating to neutrophils in culture or in a NOD/SCID mouse xenograft model to achieve normal levels of oxidase activity on a per corrected neutrophil basis as measured in the DHR assay.

Dr. Harry L Malech

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Chronic Granulomatous Disease (CGD) is caused by mutations in genes encoding components of the phagocyte NADPH-oxidase complex, which is responsible for mediating efficient killing and digestion of many bacteria and fungi. In many ways this has become a model disorder for testing HSC gene therapy strategies, as there is no growth or survival advantage for corrected cells. Enzyme activity is predominantly important in relatively short-lived terminally differentiated effector cells such as neutrophils and macrophages, meaning that long term efficacy is entirely dependent on efficient stable transduction of HSCs.

Important information can be obtained from the study of variant patients, who retain partial NADPH-oxidase activity, and carriers of the X-linked form of the disease. From these it can be predicted that over 10% correction in terms of cell numbers will be therapeutically effective, but that levels of correction per cell (in other words the levels of enzyme activity) probably needs to be more than 30%. Therefore the challenges are to achieve sufficient engraftment of transduced HSC, and efficient gene expression in terminally-differentiated cells.

The natural patterns of gp91phox expression, which is deficient in the X-linked form of the disease, reveal high levels in late myeloid development at the time of secondary granule formation. Vectors that mimic this physiological gene expression programme would be ideal, but so far have been difficult to design. Several clinical studies have been performed using standard gammaretroviral vectors, but in the absence of bone marrow pre-conditioning, only transient low level correction has been achieved. More recently, studies have been initiated using low-intensity conditioning (busulfan or melphalan) to create space for incoming transduced HSC. These have provided good evidence for substantial correction associated with genuine therapeutic effect (clearance of infections), although issues remain relating to the reliability of engraftment, mutagenesis, and silencing of LTR-based vectors. At the same time, optimal conditioning regimens to ensure effective engraftment need to be rationalised. Novel vector designs to restrict gene expression to late myeloid progenitors, to ensure reliability, and to minimise mutagenesis will be discussed.

Dr. Adrian J Thrasher

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X-linked Chronic Granulomatous Disease (X-CGD) is an inherited immunodeficiency disease caused by a defect in the gp91phox gene encoding one of the subunits of the NADPH oxidase complex. NADPH oxidase plays an important role in eradicating the pathogen engulfed by the phagocytes.

CGD patients suffer from recurring life-threatening infections by bacteria or fungi, and die before the age of 30 in most cases. In an effort to treat this life-threatening disease, we initiated a Phase I/II gene therapy trial in 2007. Two X-CGD patients were enrolled in the trial. The retroviral vector used for gene delivery was the MLV-based MT vector containing gp91 phox cDNA (Yu et al., Gene Ther 2000; 7: 797, Hong et al., J Gene Med 2004; 6: 724).

Viral vectors have been produced from PG13 packaging cells in compliance with GMP. The clinical protocol was approved by the Korean FDA. G-CSF mobilized peripheral blood CD34+ cells were obtained from patients, and transduced in retronectin-coated gas-permeable bags containing SCGM media supplemented with SCF, FLT3L, TPO, and IL-3. The transduction efficiency was 10.5% for patient #1 and 28.5% for patient #2 when assessed by gp91 FACS analysis. Before receiving transduced cells, patients were treated with a conditioning regimen consisting of busulfan (3.2 mg/kg/day for 2 days) and fludarabine (40 mg/m2/day for 3 days). No adverse effects were observed from the use of busulfan and fludarabine. The percentage of superoxide-producing cells, as determined by DHR assay, was 6.4% and 14.5% at day 17, and decreased to less than 0.1% and 0.4% after 1 year. Thus far, abnormal cell expansion has not been observed.

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