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Oral session

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Effective limb transduction with phenotypic correction after injection of rAAV8-U7snRNA in GRMD dogs

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In the Duchenne Muscular Dystrophy (DMD) the selective removal by exon skipping of exons flanking an out-of frame mutation in the dystrophin messenger can result in in-frame mRNA transcripts that are translated into shorter but functionally active dystrophin. The goal of our project is to determine in GRMD, the effective dose of our therapeutic product defined as a recombinant Adeno-Associated Virus serotype 8 (rAAV8) expressing a modified U7 snRNA specific for the exon skipping of the dystrophin transcript. The mode of delivery is the locoregional high-pressure intravenous (IV) injection in a forelimb. Three groups of GRMD dogs were exposed to 3 different rAAV8-U7snRNA doses. Each dog was followed&3 months after injection. The primary outcomes are the restoration of dystrophin expression and the improvement of the tissue pathology in the injected limb compared to the controlateral. The secondary outcomes are the muscle strength correction,the biodistribution and shedding patterns as well as the immune response against rAAV8 capsid and dystrophin. We built a unique network of laboratories with complementary skills to deliver a GLP-compliant set of preclinical data to further define the regulatory toxicology studies. The organization of our network and the results obtained in our GRMD dogs study will be presented. This project is supported by AFM (Association Franc¸aise contre les Myopathies) and by ADNA (Advanced Diagnostics for New Therapeutic Approaches), a program dedicated to personalized medicine, coordinated by Institut Me´rieux and supported by research and innovation aid from the French public agency, OSEO.

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Poster sessions

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Development of a reliable blood test for the detection of rAAV-mediated gene doping

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The goal of our project is to develop a test for the detection of the presence of a vector delivered transgene of interest encoding a protein capable of enhancing athletic performance. Several in vivo studies have shown that vectors derived from Adeno-Associated Virus (AAV) are able to provide long-term expression of a transgene after a single administration. It has been demonstrated especially after one intramuscular injection of a recombinant AAV (rAAV) vector that vector genomes are detectable in blood cells several months or years after injection. These results suggested that a sensitive-PCR technique can be used to detect rAAV-mediated gene doping from a simple blood sample. Our project consisted of a single intramuscular injection of macaques with two different rAAV serotypes known for their efficiency to transduce skeletal muscle cells: the rAAV1 and rAAV8. Various clinically relevant doses were tested. Each vector carried the expression cassette CMV-cmEpo-SV40polyA that,codes the cynomologous Erythropoietin gene. Our initial goal was to establish the minimal dose of vector that can cause an increase of&15% of the hematocrit compared to the normal level. Once the minimum dose was determined, an optimized PCR method was used to highlight the presence of the transgene in blood. We validated Taqman real-time PCR sensitive conditions that allow us to detect at least 5 copies of the rAAV Epo transgene in the background of endogenous genomic DNA.
Data generated in our nonhuman primates will be the basis for developing a legally defensible commercial real-time PCR assay.

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Histidine-rich amphipathic peptides promote efficient delivery of nucleic acids into mammalian cells

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Besides being a useful tool in research, gene transfer has a high potential as treatment for a variety of genetic and acquired diseases. However, in order to enable a gene to become a pharmaceutical, efficient and safe methods of delivery have to be developed. We found that cationic amphipathic histidine-rich peptide antibiotics can efficiently deliver DNA into mammalian cells. Our lead compound, LAH4 (KKAL LALALHHLAHLALHLALALKKA), demonstrated in vitro transfection efficiencies comparable to those of commercially available reagents. Synthesis and evaluation of LAH mutants provided evidence that the transfection efficiency depends on the number and positioning of histidine residues in the peptide as well as on the pH at which the in-plane to transmembrane transition takes place. Our results also suggest a mechanism of selective destabilization by LAH4 of anionic lipids in the membranes of cells during transfection. Further results indicate that acidification of the endosome results in high local concentrations of free peptide in this organelle.
These peptides become then available to interact with the endosomal membranes and thereby are responsible for the delivery of the plasmid DNA complex to the cytoplasm. When these data are taken together, they indicate a

dual role of the peptide during the transfection process, namely DNA complexation and membrane permeabilization. Finally, we will report that peptides of the LAH family are efficient siRNA delivery vehicles.

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LV mediated Gene therapy for Wiskott-Aldrich Syndrome: *in vitro* and *in vivo* preclinical studies

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Wiskott-Aldrich Syndrome (WAS) is an X-linked immunodeficiency characterized by thrombocytopenia, infections, autoimmunity and lymphomas. We previously demonstrated that a lentiviral vector (LVV) encoding for humanWAS under the control of endogenous 1.6 kb promoter efficiently corrected ESGCT 2010 POSTER PRESENTATIONS 1437 human and mouse cells. We then set up a clinically applicable and efficient transduction protocol based on 60 hours of culture and 2 hits of gene transfer (MOI 100) on CD34þ cells from normal donors. The selected protocol was applied to WAS patients bone marrow CD34þ cells for the validation of the GMP grade LVV. Transduced cells showed a vector copy number per cell of 1.4±0.3 and about 80% of transduced colonies.WAS cells proliferated less than normal donors (with or without LVV exposure) but there was no toxicity of LVV on clonogenic progenitors. Following gene transfer, WASp expression was restored in patients' differentiated cells, including megakaryocytes.Analyses of vector integrations on in vitro transduced CD34þ cells showed polyclonal integrations with the expected bias of LVV for transcriptional units. Pharmacokinetic of transduced CD34þ cells was studied by injection in sub-lethally irradiated neonate $Rag2^{-/-}$ $\gamma c^{-/-}$ mice. Transduced cells showed a normal biodistribution to hematopoietic organs and differentiation capacity

in the absence of vector shedding and germline transmission.In conclusion, we demonstrate that our GMP grade LVV allows robust and reproducible transduction of CD34þ cells leading to restoration of WASp expression with no toxicity. A phase I=II gene therapy protocol based on infusion of transduced CD34þ cells combined with a reduced intensity conditioning has recently started.

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Gene therapy trial with lentiviral vector transduced CD34þ cells for the treatment of Wiskott-Aldrich Syndrome

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Wiskott-Aldrich Syndrome (WAS) is an X-linked immunodeficiency characterized by thrombocytopenia, infections, autoimmunity and lymphomas. Gene therapy with autologous hematopoietic stem cells (HSC) could represent a valid alternative to allogeneic transplant for patients lacking an HLA-identical donor or who are at high risk of complications. We have developed a novel approach based on a self-inactivating lentiviral vector (LVV) encoding for human WAS under the control of the endogenous 1.6 kb promoter.We previously showed that this vector is safe and efficiently corrects the WAS defect in the murine model of the disease and in human cells. A highly purified, GMP grade, LVV transduced at high efficiency human CD34þ cells from healthy donors and patients, allowing restoration of WASp expression in multiple lineages without toxicity. A phase I=II protocol aimed at studying safety, biological activity, and efficacy of gene therapy in 6 WAS patients was opened in April 2010. Patients will receive preconditioning with anti-CD20 monoclonal antibody and reduced intensity busulfan and fludarabin; ATG will be included in case of autoimmune manifestations. The first patient was recently enrolled and treated with autologous CD34þ cells obtained from bone marrow (BM) and mobilized peripheral blood (MPB), achieving an optimal target HSC dose. Transduced cells met all quality specifications and showed a vector copy number per cell of 1.4 in the MPB and 1.9 in the BM, respectively, with high gene transfer efficiency in clonogenic progenitors (88-92%). The patient did not experience toxicity or adverse events, recovered well from transient neutropenia and is currently independent from platelet transfusions, 3 months after gene therapy. Initial engraftment analyses is showing the presence of vector transduced cells in multiple lineages of peripheral blood and BM, with evidence of WASp expression. Long-term assessment study will provide key information on the safety and efficacy of gene therapy for WAS patients using lentiviral-vector transduced HSC in combination with reduced intensity conditioning.

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