heme biosynthetic pathway. The severity of the disease, the lack of specific treatment except for allogenic bone marrow transplantation (BMT) and the knowledge of the molecular lesions are strong arguments towards gene therapy. We have recently developed a knock-in mouse model of CEP (Ged *et al.*, Genomics, 2006) in order to evaluate the feasibility of gene therapy in haematopoietic stem cells (HSCs). This novel mouse model closely mimics the CEP disease in humans (porphyrins accumulation in urine, blood and spleen, erythrodontia, moderate photosensitivity, hepatosplenomegaly, and haemolytic anaemia). Here we develop a self-inactivating lentiviral vector containing human UROS cDNA driven by the human ankyrin-1/b-globin HS-40 chimeric erythroid promoter/enhancer. Murine HSCs from CEP donor mice were transduced by the erythroid-specific lentiviral vector and injected them into CEP recipient mice conditioned with high doses of busulfan. We observed a high transduction efficiency of HSCs (88 ± 14% $PCR^+$ CFC, 20 weeks after BMT) resulting in an effective gene therapy of primary recipient CEP mice without any selectable system. A full restoration of enzymatic activity in BM, spleen and peripheral blood cells was obtained (2 fold increased UROS activity versus normal mice) resulting in skin photosensitivity correction and disappearance of splenomegaly. Furthermore, we observed a dramatic decrease of porphyrin accumulation in red blood cells (RBCs) and urines as well as a full correction of haemolytic anemia. In addition, a complete disappearance of fluorescent RBCs (fluorocytes) was observed by FACS. We are currently investigated the existence of a natural selective advantage of corrected cells in CEP mice. We demonstrate for the first time the high efficiency of HSCs gene therapy using a SIN erythroid-specific lentiviral vector in a murine model of CEP. This study forms the basis of gene therapy clinical trial for this severe congenital erythropoietic porphyria disease.

## 62. Proteasome Activity Restricts Lentiviral Gene Transfer into Hematopoietic Stem Cells and Is Down-Regulated by Cytokines That Enhance Transduction

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The therapeutic potential of hematopoietic stem cell (HSC) gene therapy can be fully exploited only by reaching efficient gene transfer into HSCs without compromising their biological properties. HIV-derived lentiviral vectors (LVs) are good candidates for this purpose. Although HSCs can be transduced by LVs in short *ex vivo* culture, they display low permissivity to the vector, requiring cytokine stimulation to reach high-frequency transduction.

Using stringent assays of competitive xenograft repopulation, we show that early-acting cytokines (SCF, IL6, TPO, Flt3L) synergistically enhanced human HSC gene transfer by LV without impairing engraftment and repopulation capacity. Using S-phase suicide assays, we show that transduction enhancement by cytokines was not dependent on cell cycle progression and demonstrate that LVs can transduce quiescent HSCs. Cytokines enhancement of LV gene transfer was not dependent on the envelope pseudotype, indicating that cytokine stimulation did not affect a specific entry pathway.

We then evaluated factors influencing the post-entry steps of viral infection. Pharmacological inhibition of the proteasome during transduction dramatically enhanced HSC gene transfer. HSCs transduced in the presence of proteasome inhibitors showed a dramatic increase in the levels of vector integration, without apparent adverse effects on engraftment capacity in NOD/SCID mice. This indicates that: i) a large excess of vector particles is uptaken by HSCs but does not become integrated; ii) LVs are effectively restricted at a post-entry step by proteasome activity; iii) proteasome inhibition may represent a new strategy to overcome the low HSC permissiveness to LV gene transfer.

By measuring the proteasome activities in cytokine-stimulated and unstimulated hematopoietic progenitors, unexpectedly we found that they were significantly down-regulated by exposure to the cytokines. Importantly, this response was rapid, with a maximal effect at early time points corresponding to the period in which the first steps of LV transduction take place, and synergistic for SCF, TPO and Flt3L. These findings highlight one of the mechanisms by which cytokines may enhance permissiveness to LV gene transfer. Interestingly, we did not observe a similar extent of proteasome-mediated LV restriction in other primary cells, suggesting that it represents a specific feature of HSCs, both human and murine. Effective restriction correlated with high proteasome activity.

We are currently investigating the molecular mechanism of restriction, whether the proteasome targets uncoating vector cores, directly or through the action of host restrictive factors, or rather acts by targeting factors required for the early steps of transduction.

Our findings demonstrate that antiviral responses ultimately mediated by the proteasome strongly limit the efficiency of HSC transduction by LVs, and help to establish improved conditions for HSC-based gene therapy.

## 63. Long-Term Constitutive Expression of the Therapeutic Gene, IL2RG, Causes T-Cell Lymphomas in a Gene Therapy Model for XSCID

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The successful gene therapy clinical trials, where the immune function of patients with X-linked severe combined immune deficiency (XSCID) was restored, demonstrated the utility for vector mediated gene transduction of hematopoietic stem cells in the treatment of a life threatening disease. However, the occurrence of T-cell leukemias in three patients, with the suspected oncogenic event resulting from upregulation of expression of the known oncogene, LMO2, by insertional mutagenesis, has led to the re-evaluation of the safety of this procedure. To model the gene therapy adverse events and to better understand the mechanism of generation of leukemia in the patients, we expressed the therapeutic gene, IL2RG, used to treat XSCID patients, in combination with and without LMO2 in a murine model of XSCID, and followed the fates of transplanted mice up to 1.8 years post transplantation. In this study, we unexpectedly found that the therapeutic gene IL2RG alone is a major contributor to the genesis of T-cell lymphomas in mice, with a prevalence of 33%, n=15. We found that IL2RG-/- mice transplanted with IL2RG vector transduced IL2RG-/- cells or similarly transduced wild type cells developed the T-cell lymphomas. As expected, T-cell lymphomas were also detected in the positive control LMO2 transduced mice with a prevalence of 50%, n=12, with cell phenotype similar to those previously published. In contrast, none of the mock transduced mice (n=16) nor LV-GFP vector transduced mice (n=17) developed the T-cell lymphoma in this study. These findings were generated from 7 independent experiments totaling 99 mice ranging from 71 to 91 weeks posttransplant. Interestingly, the average time of onset of the T-cell lymphomas in IL2RG transduced mice was faster than the LMO2 transduced mice, 10.1 months versus 12.2 months post transplant,