

33. Non-Random Genomic Distribution of Retrovirus Vector Integration in Successful SCID-X1 Gene Therapy

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Retrovirus gene transfer into hematopoietic stem cells is an effective treatment of X-linked severe combined immunodeficiency (SCID-X1). Nevertheless, the recent occurrence of TALL like disease in three SCID-X1 patients led to intensive studies on vector biosafety with focus on insertional mutagenesis. Large-scale mapping of retrovirus integration sites in human cell lines revealed preferential vector integration into genomic coding regions, in particular near transcription start sites. We hypothesized that in clinical applications, the distribution of insertion sites may be influenced by additional factors, including the gene expression profile of the target cell population and the selection of hematopoietic cells capable of engrafting and proliferating *in vivo*. In this trial, six patients have been treated successfully since 2001 using a GALV-pseudotyped MLV-based vector carrying the therapeutic common gamma chain gene for transduction of autologous CD34+ cells. The patients did not receive any conditioning therapy before transplantation. Using a high-volume integration site sequencing approach, we have characterized more than 400 unique integration sites derived from the patients. So far, no vector integration in the LMO2 oncogene could be detected in this trial, and the patients do not reveal any other evidence for malignancy or clonal deformation of their stem cell compartment. We could show that integration of the mammalian gammaretroviral vector in this gene therapy trial is not random. Integration of the vector happens generally within or close to gene coding regions, preferential surrounding the transcription start site. Moreover, we found common integration sites (CIS) in RefSeq gene regions. The targeted RefSeq genes were classified according to the Gene Ontology database. Our data strengthen the hypothesis that curative gene therapeutic treatment requires a sustained polyclonal contribution of *ex vivo* manipulated stem and progenitor cells. The distribution of insertions into the vicinity genes and common integration sites indicates that the observed distribution may also have been influenced by the biological effects these insertions may have caused in their target cells.

MS and CvK hold US-patent on LAM-PCR.

34. Transposon-Based Gene Therapy of Hemophilia A Targeting Endothelial Cells in Neonates

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Hemophilia A is an X-linked bleeding disorder caused by the absence of functional coagulation factor VIII (FVIII). Gene therapy is a promising strategy for the treatment of FVIII deficiency, as only small amounts of protein are needed to phenotypically correct the disorder. Obstacles to non-viral gene therapy of hemophilia A include the lack of prolonged expression and immune responses to the FVIII transgene in the form of neutralizing inhibitory antibodies. In an effort to overcome these obstacles we have utilized the *Sleeping*

Beauty (SB) transposon system to facilitate gene insertion and promote long-term expression. To reduce the likelihood of generating inhibitory antibodies, gene therapy was conducted in neonatal mice, which are more likely to be tolerant of foreign protein expression. While FVIII is thought to be primarily synthesized in the liver, endothelial cells may also be a source for FVIII production. Here we targeted transgene expression to endothelial cells within the lung by a combination of gene delivery approach and a transposon that harbors an endothelial cell specific promoter (Liu et al., (2004) Mol. Ther. 10(1):97-105). Two groups of FVIII deficient mice (n=7) were treated with a transposon plasmid expressing FVIII (pMSZ-FVIII) and an expression plasmid encoding either a defective SB transposase (mutant), or a hyperactive transposase (HSB#17) (2:1 molar ratio). A total of 5 µg of plasmid DNA was complexed with polyethyleneimine (PEI) and injected via the superficial temporal vein in one-day-old mice (total volume 30 µl). Chromogenic FVIII activity assays (Coatest) revealed ~80% of normal activity at day +3 after injection. Immunohistochemical studies at day +3 showed FVIII immunoreactivity in blood vessels within the lung and scattered throughout the liver. FVIII activity assays and Bethesda titers were monitored over the ensuing 6 months. The activity of FVIII in mice that received the hyperactive SB transposase reached a plateau of approximately 10% by 8 weeks and remained stable thereafter. The FVIII activity in mice receiving the mutant transposase fell to around 2-4%. Bethesda titers for both groups ranged from 0.2 to 0.8 BU, with the higher titers within the hyperactive transposase group. Immunohistochemistry at 24 weeks showed the presence of FVIII protein within endothelial cells of the lung, while there was no detectable immunoreactive protein within the liver. Phenotypic correction of the bleeding disorder was confirmed by tail bleeding times, which were significantly reduced in the gene therapy treated animals. These results suggest that endothelial cell targeting of transposon-based gene therapy can result in long-term correction of the bleeding disorder in hemophilia A.

35. Generalized Detoxification Associated with Engraftment of Gene-Corrected Repopulating Cells Achieved in ADA-SCID Patients by Stem Cell Gene Therapy without Myeloablative Pre-Conditioning

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Severe combined immunodeficiency due to genetic defects of *adenosine deaminase* (ADA-SCID) has been a target disease of retroviral-mediated gene therapy. Recently, great success was reported in one of such clinical trials conducted in Italy aiming for the correction of patients' hematopoietic stem cells (HSC). In contrast, the US trial which is similar to, but differs significantly from that of Italy has not lead to visible clinical improvement. Considering the differences in protocol between two, absence of concomitant enzyme replacement therapy (PEG-ADA) and/or mild pre-conditioning seem to be critical for the efficacy of stem cell gene therapy (SCGT) for ADA-SCID. However, since immune reconstitution has been achievable by SCGT for untreated XSCID patients, it is still unknown whether pre-conditioning is absolutely necessary for ADA-SCID.