

# Gaining the hard yard: pre-clinical evaluation of lentiviral-mediated gene therapy for the treatment of $\beta$ -thalassemia

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See related article in EMBO Mol Med (Roselli et al. (2010) EMBO Mol Med 2: 315–328)

Gene therapy is one potential novel therapeutic avenue for the treatment of inherited monogenic disorders. Diseases of the blood are frequent targets for gene therapy because it is relatively easy to harvest haematopoietic stem cells (HSCs) from the bone marrow, genetically modify the cells *ex vivo*, and then re-administer the corrected cells back into the patient via intra-venous injection. In this Closeup, Milsom and Williams discuss the work of Roselli et al, who describe the pre-clinical evaluation of the treatment for  $\beta$ -thalassemia in erythroid cells via the genetic correction of patient HSCs using a lentiviral vector.

Efforts at developing viral vectors and gene transfer methods for molecular therapeutic purposes continue to show steady progress. Challenges to the field continue to stimulate innovation and

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translational studies are key to moving the technology forward. In this issue, Roselli et al describe an extensive pre-clinical assessment of a lentiviral-mediated gene therapy approach for the treatment of  $\beta$ -thalassemia (Roselli et al, 2010). The HIV1-based vector that they describe contains elements of the  $\beta$ -globin promoter and locus control region, which drive the expression of an exogenous  $\beta$ -globin gene. Thus, even though their methodology involves the *ex vivo* transduction of long-lived CD34<sup>+</sup> haematopoietic stem and progenitor cells, the vector payload will only be transcribed in the erythroid progeny of these cells. In order to prepare for an initial clinical trial utilizing this vector, the authors have performed a range of *in vitro* assays to evaluate the efficacy and safety of their approach using primary CD34<sup>+</sup> bone marrow cells collected from a large cohort of  $\beta$ -thalassemia patients and normal donors. The content of their manuscript can be summarized as three key messages. Firstly, they demonstrate that the *ex vivo* manipulations which are

required for the successful transduction of haematopoietic stem cells (HSCs) with a lentiviral vector do not adversely impact the functional capacity of patient CD34<sup>+</sup> cells, nor their global gene expression profile. Secondly, they present data which show that at low copy number, the lentiviral vector they are utilizing is able to correct the deficiency of adult globin expression characteristic of  $\beta$ -thalassemia and restore erythropoiesis in the *in vitro*-differentiated progeny of transduced patient CD34<sup>+</sup> cells. Finally, they analyse the pattern of lentiviral integration sites in transduced patient CD34<sup>+</sup> cells and conclude that the vector they are utilizing has preferences for genomic integration sites similar to those described for other lentiviral vectors, with no bias towards integration in the proximity of proto-oncogenes. Taken together, these data suggest this vector is safe and effective and support further clinical development. The work represents the kind of translational studies that are required to move human investigations forward but are often very difficult to fund and publish.

What many consider the first successful trial involving the use of recombinant retroviral vectors to correct an inherited monogenic disorder in bone marrow-derived HSCs was reported 10 years ago by the group of Alain Fischer in Paris (Cavazzana-Calvo et al, 2000). This

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study, and a second nearly identical study from Adrian Thrasher's group in London (Gaspar et al, 2004), involved the use of a gammaretroviral vector to deliver a complementary DNA (cDNA) encoding the common gamma chain of the interleukin-2 (IL-2) receptor into CD34<sup>+</sup> cells isolated from patients suffering from X-linked severe combined immunodeficiency (SCID-X1). These trials undeniably demonstrated the therapeutic efficacy of this approach. They were however, afterwards noted for the subsequent iatrogenic leukaemias that occurred as a result of retroviral vector-mediated insertional mutagenesis (Hacein-Bey-Abina et al, 2008; Howe et al, 2008). Another set of gene therapy trials using a similar vector backbone in SCID due to adenosine deaminase deficiency has reported no serious adverse events in 20 patients, some now 10 years post-treatment. In neither disease, were serious adverse events predicted during the pre-clinical evaluation of gene transfer. For the past 7 years, the scientists in the gene therapy field have strived to develop new pre-clinical model systems in order to evaluate and then minimize the risk of insertional mutagenesis while maintaining therapeutic efficacy.

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There is a phase one lentiviral-mediated gene therapy trial for  $\beta$ -thalassaemia directed by Philippe Leboulch currently underway in Paris using a similar vector configuration to the one described by Roselli et al (discussed in Kaiser, 2009). While that work is clearly at a more advanced stage, we believe that there are several compelling reasons for why it is important to publish extensive pre-clinical studies such as Roselli et al. Some of these are outlined below.

Detailed pre-clinical studies act as a template which others may follow to devise a protocol for additional clinical

trials. Developing appropriate protocols and reagents for clinical studies is an extremely costly and time-consuming exercise. Therefore the publication of successful methodologies is of particular interest to those in the field with aspirations to translate their basic studies into the clinic.

For a number of orphan diseases that are, in particular, the target of gene transfer protocols, it is difficult to obtain material from a large cohort of patients with which to perform efficacy and safety studies. It is therefore imperative that the groups who have access to these resources are able to disseminate their findings to others.

The data presented in this manuscript can act as a yardstick against which to gauge the effectiveness of future technical modifications. Since this study reports detailed experimental findings such as the degree of correction per vector copy number; the results of this approach across a range of  $\beta$ -thalassaemia subtypes; and gives an extensive integration site profile, it will be imperative for others to directly compare their findings against those within this publication.

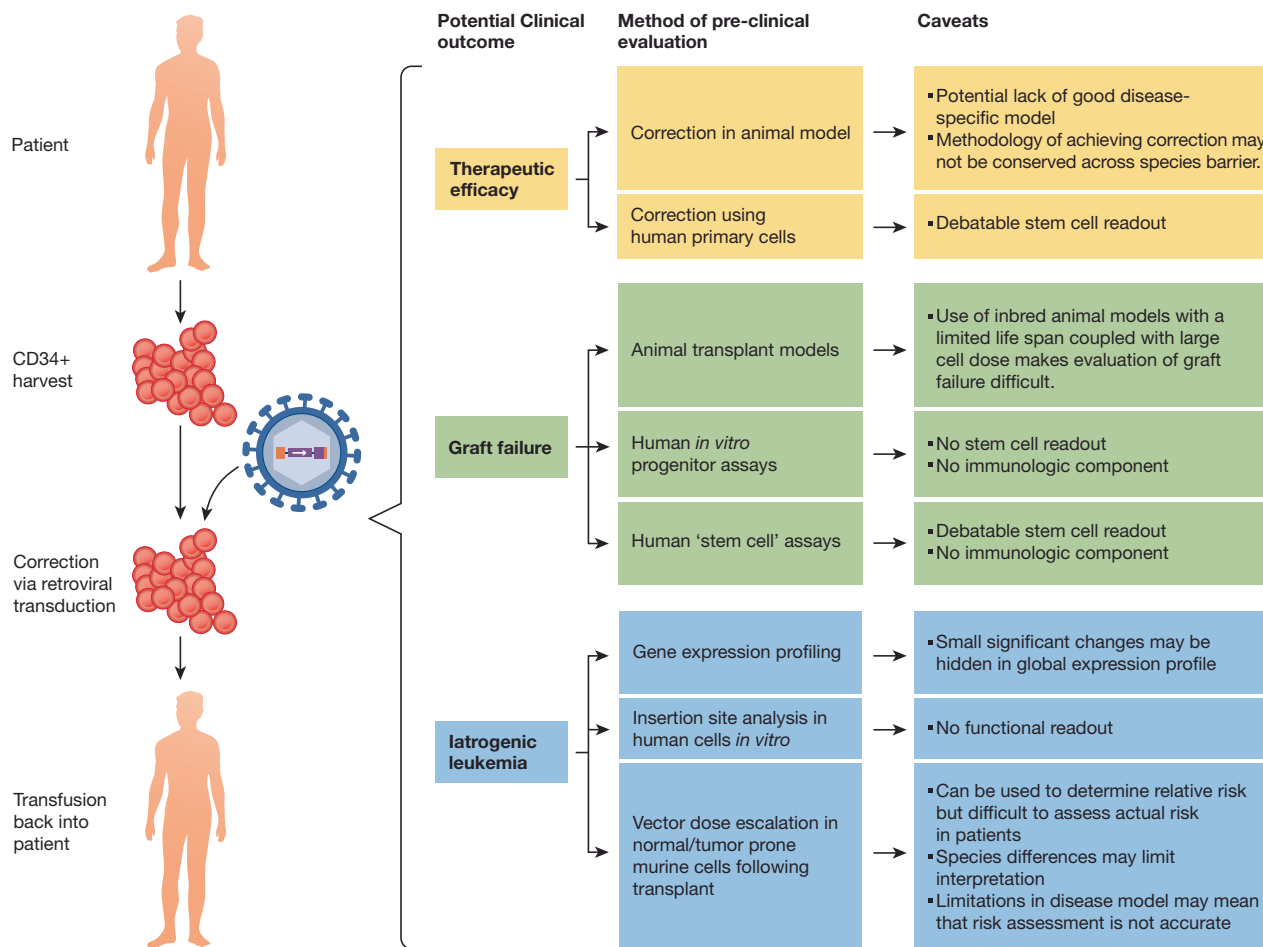
Publications such as this can contribute to more effective and efficient regulation of gene therapy trials. From the point of view of the various regulatory bodies, well-executed pre-clinical studies can act as a guide for recommendations to other investigators developing trials in the same disease. From the point of view of the researcher, the studies can act as a citable example of why their studies have been designed in a specific manner.

Where similar clinical trials have been performed in parallel by different groups with slightly differing methodologies, it is important to have ready access to a wealth of pre-clinical data in any attempt to explain differing outcomes. For instance, in the first successful clinical gene therapy trial of SCID-X1, 4 out of 10 patients treated on the protocol in Paris went on to develop T cell leukaemia (Hacein-Bey-Abina et al, 2008). At the same time, in a similar parallel trial run in London (Gaspar et al, 2004), there had been no reported incidences of adverse events in the 10 patients treated there, although one patient in the London trial

subsequently developed iatrogenic T cell acute lymphoblastic leukaemia (Howe et al, 2008). Thus during the initial time period after the report of leukaemia in the trial in Paris there appeared to be a difference in the clinical outcomes between the two trials. A great deal of effort was focused in an attempt to investigate whether minor differences in methodology such as different vector envelope pseudotypes and culture conditions could explain the lack of leukaemia in the London trial. Since such variables may only have been directly compared in pre-clinical studies, it is possible that the retrospective analysis of published pre-clinical data could be informative about differential outcomes by eliminating or highlighting specific lines of investigation for follow-up. In relation to this, and of direct relevance to the current study, a non-malignant clonal imbalance associated with a vector-mediated insertional event has been described in the ongoing pilot trial for lentiviral-mediated gene therapy of  $\beta$ -thalassaemia (as discussed in Kaiser, 2009). The extensive insertion site analysis performed in the current study may contribute towards the follow up analysis and subsequent interpretation of this event.

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Last and perhaps most importantly, retrospective analysis of pre-clinical data and analysis of how well these data predicted clinical trial outcome, will facilitate the future refinement of such studies. The gene therapy field is particularly lacking in validated assays that one can employ to anticipate whether a specific methodology will be effective and/or safe (Figure 1). For example, a number of surrogate assays have been developed to evaluate the risk of insertional mutagenesis in human HSCs following transplant. Although these



**Figure 1.** Current approaches for pre-clinical modeling of gene therapy strategies using established technologies.

assays may facilitate a comparative analysis of the probability of inducing insertional mutagenesis *in vivo* in mice, it is extremely difficult to extrapolate what these assays mean in terms of actual risk of malignant transformation in patients. We have recently undertaken an extensive pre-clinical assessment of vector toxicity in haematopoietic stem and progenitor cells using a murine transplant model and comparative insertion site identification after *in vivo* engraftment of cells, in preparation for a second generation clinical trial for the gene therapy of SCID-X1 (<http://clinicaltrials.gov/ct2/show/NCT01129544>). Given that the vector configuration which promoted leukaemia in the London and Paris clinical trials would not be predicted to elicit malignant transformation

in the assay (and indeed did not lead to leukaemia in this new set of experiments), we are unsure of how informative the data from this experiment will be. Such experiments are costly, have taken more than one year to complete, and yet there is a real possibility that we have gained no further insight into potential human toxicity in the current trial. With this in mind, we suggest that it is of the utmost importance for the field to develop and validate robust assays in human cells for the pre-clinical assessment of human gene therapy protocols.

Considering the inherent difficulties accompanying human research (low relative number of samples, high biological variability, difficulty in performing timely follow-up experiments to address

new findings and small numbers of repeats), studies like those reported in this issue of *EMBO Molecular Medicine* by Roselli et al are extremely important for moving the field forward.

The authors declare that they have no conflict of interest.

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