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Gene Therapy Trials: lessons and remaining questions

Basic research tries to decrease the risks of translational medicine

M Cavazzana-Calvo

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Although gene transfer by retroviral vectors has shown its potential for the correction of three different inherited disorders of the haematopoietic system,[1–4] hopes of rapidly available therapeutic solutions have been tempered by the observation of leukaemogenic side effects[5,6] in a small proportion of patients. Frank JT Staal's review in *Leukemia* (entitled '*Sola dosis facit venenum*. Leukemia in gene therapy trials: a question of vectors, inserts and dosage?') nicely summarized the state of our knowledge in the field of γ-retroviral gene transfer and described the research progress achieved over the last 5 years.

The occurrence of the first case of leukaemia in the SCID-X1 (Severe Combined Immunodeficiency-XI) clinical trial[7] prompted the spontaneous constitution of an intensively collaborative international research network, which has since provided many insights into the biological mechanism underlying the observed severe side effects. Herein, Frank JT Staal presents the main data generated by a network comprising virologists, molecular biologists, haematologists and cellular biologists and within which the interplay between fundamental science and medicine has rapidly borne fruit. So, what did we learn and where do we go from here?

Retroviral vectors are very attractive tools for gene therapy of genetic diseases because they enable efficient, stable transgene insertion with a controlled copy number. However, growing evidence (from both animal and clinical studies) indicates that γ-retroviral vector insertions have a significant influence on the nearby growth-regulation genes (that is, insertional mutagenesis) and the *in vivo* behaviour of the affected clones; this may result in leukaemia or clonal dominance.[8] Molecular analysis of retroviral vector-mediated malignant transformation has shown that activation of growth-promoting genes is the prime mechanism behind insertional mutagenesis. Consequently, a huge effort has been taken to enhance the sensitivity of (i) mouse models used for detecting leukaemogenic events;[8–10] and (ii) the *in vitro* vector screening assays used for obtaining quantitative data on vector design-related insertional mutagenesis.[11]

Thanks to the knowledge generated by these studies, self-inactivating vectors lacking the relevant sequences in the U3 region of their long terminal repeats have been shown to be significantly less mutagenic *in vitro* and *in vivo*—supporting the concept whereby the type of vector backbone and the nature of its *cis*-regulatory sequences are crucial variables in the therapeutic index of integrating vectors. A very interesting study by Cornils,[12] recently described the influence of the vector backbone on the characteristics of long-term repopulating stem cells; the work showed unambiguously that the insertion pattern of promoter-deprived-γ-retroviral self-inactivating vectors differs significantly from that of γ-retroviral long terminal repeat vectors and does not induce clonal dominance. However, this neutral vector backbone has not yet been allowed to express the therapeutic transgene at cellular levels capable of correcting the pathological phenotype.

Despite these many important advances, the occurrence of leukaemic events also appears to depend on other less well-characterized factors. For example, the cytokine cocktail and the length of the activation phase may influence the virus integration pattern, given that viral integration is correlated with the expression level of genes in the target CD34+ human stem/progenitor cells.

Other additional factors that may influence the fate of the transduced cells are the target cell population and the nature of the underlying disease. Indeed, the difference between the SCID-X1 and adenosine deaminase forms of severe combined immunodeficiency may account for the observed disparity in the occurrence of severe side effects. This may be linked to the transgene, but a number of studies have confirmed that the 'ectopic' expression of IL2Rγ is not sufficient enough to trigger leukaemia.[13] The second important difference between these two diseases relates to their respective physiopathologies; their completely different bone marrow morphologies give rise to an accumulation of 'blocked' precursors in SCID-X1 patients, and a hypoplastic bone marrow (owing to the toxic death of haematopoietic precursors) in patients suffering from the adenosine deaminase deficiency. Although Shou *et al.* have suggested that 'blocked' precursors could accumulate additional somatic mutations (making them more 'tumour-prone'), there is currently no evidence in favour of this hypothesis.

Another hypothesis is that after retroviral integration, 'affected' precursors can actively transcribe 'dangerous' genes at much higher frequencies than those observed when the steady-state bone marrow is transduced *ex vivo*.

Unfortunately, the contribution of the 'affected' human bone marrow to the occurrence of side effects will be difficult to test—even if (as suggested by Frank JT Staal) careful analysis of human lymphoid and myeloid development in a NOD (non-obese diabetic)-SCID model could reveal preleukaemic conditions.

Despite these unanswered questions, the field of gene therapy continues to move forward cautiously, and the results of the ongoing clinical trials are awaited.[14] Similarly, research into genome engineering with endonucleases (designed to recognize and cleave the DNA sequence of specific genes[15] at the mutation site) is generating much excitement.

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M Cavazzana-Calvo is at the Department of Biotherapy, Clinical Investigation Centre for Biotherapies, Hôpital Necker, APHP, Inserm U-768, Université Paris Descartes, Paris, France.
E-mail: m.cavazzana@nck.aphp.fr

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