

Fourth case of leukaemia in the first SCID-X1 gene therapy trial, and the diversity of gene therapy.

**Commentary from the Board of the European Society of Gene and Cell Therapy
(ESGCT)**

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As reported by Professor Alain Fischer at 33rd Annual Meeting of the European Group for Blood and Marrow Transplantation (EBMT) in Lyon, France on March 25-28, 2007, a fourth case of a malignant cell expansion has been observed in the clinical phase 1 trial conducted at the Necker Hospital in Paris to treat newborns and young infants suffering from a rare form of X-linked severe combined immunodeficiency disease (SCID-X1). Thus, to date four of the eight patients of this trial who have experienced long-term correction of their disease by the infusion of vector-modified autologous bone marrow cells have developed a malignant complication, which, at present, was lethal in one case. In the first three patients, the vector was inserted next to a known cellular proto-oncogene in the malignant cell clones (insertional mutagenesis), likely representing a driving force of the subsequent uncontrolled expansion [1, 2]. Probably a similar constellation of insertional mutagenesis will be observed in the fourth case. Recently obtained molecular profiles of vector insertion sites in this trial [3], and the long 60 months latency from tumor development in the latest case, compared to about 30 months latency in the three earlier cases, raise concerns for the remaining patients. Alain Fischer's team and collaborators will make detailed data available to the scientific community as soon as analysis is completed.

The retroviral gene transfer vectors used in this clinical trial may express the therapeutic cDNA, IL2RG, at higher levels than the natural gene and certainly in a transcriptionally unregulated mode. Unphysiologic expression of IL2RG has been suspected to contribute to the development of the complications [4, 5]. Recent studies have provided indirect genetic evidence for a specific role of unregulated expression of the IL2RG transgene in inducing tumor formation [6, 7]. Indeed, in all other clinical trials with retroviral vectors conducted since 1990, no leukemic transformation related to the use of retroviral vectors has been observed in hundreds of patients treated in over 15 years [8]. However, studies conducted in transgenic mice and human cells have found no evidence for a transforming potential of over-expressed IL2RG [9-11], and there is no overexpression of IL2RG protein either on resting or activated polyclonal T cells of healthy patients, or on leukemic cells of any of the patients. A puzzling and encouraging finding is that there have been no malignant clonal proliferations in a similar SCID-X1 gene therapy clinical trial conducted in London, although the sequence of the integrated expression vector was almost identical to the one used in Paris.

These observations even open the possibility of other protocol-specific cofactors contributing to the occurrence of transformed cell clones in gene therapy of SCID-X1. Such cofactors could be related to the cytokines and other components of the medium used to cultivate the cells during the exposure to the gene vector, cell dose, and vector copies, as these may influence the survival and proliferation of cells with specific integrations, the kinetics of reconstitution, and levels of transgene expression in transduced cells. Alternatively, differences in the envelope protein of the vector may play a role, by increasing the likelihood to hit a specific subset of cells and/or cells in a different metabolic state. Elucidating the nature and mechanisms of these unanticipated cofactors will be of utmost importance for the future of gene therapy in this disease. In addition, patient-specific issues, such as age and

clinical status, need to be considered. Thus, the combination of transgene, disease, and protocol-specific factors may influence the inherent risk associated with integrating vectors.

As insertional mutagenesis cannot be questioned as a driving force of the severe adverse events in gene therapy of SCID-X1, and in the absence of insight into the nature of the cofactors, a reasonable attempt to prevent the induction of malignant cell proliferation is to use improved vector technology in the context of the cell culture and envelope conditions used in London. Redesigning the expression vector encompassing the therapeutic gene that is to be inserted in the cellular genome may reduce the likelihood to activate randomly hit neighbouring genes. Here, both principal investigators in Paris and London are developing vectors with self-inactivating long terminal repeats (SIN-LTR), starting from new generations of lentiviral or gammaretroviral vectors. Accumulating experimental evidence suggests that carefully redesigned SIN vectors may reduce the risk of insertional mutagenesis. Future technological developments, not yet available for clinical use, are: targeted insertion into safe chromosomal locations or specific correction of the mutated gene. Colleagues in Paris, London and many other places in the world are working hard to implement the clinical use of novel, validated technology with an improved safety profile. If further supported by scientific studies showing an improved risk profile, redesigned integrating vectors may be envisaged as useful tools in clinical trials.

As highlighted by the differences observed between the SCID-X1 clinical trials conducted in Paris and London, there is increasing evidence that multiple disease and protocol-specific factors underlie the induction of a secondary cancer after the use of gene vectors. The elucidation of the co-factors will be critical to improve the long-term prospects of gene therapy using vectors that integrate into the genome of the patient's cells

The diversity of technologies and clinical situations encountered in gene therapy protocols is indeed tremendous, considering only the most obvious variables such as type and numbers of cells treated, disease-specific milieu conditions, and, last but not least, type, dose and content of the gene vectors used. As in other fields of therapy development, this results in the challenge to develop a qualitative and – eventually – quantitative risk profile for each individual protocol, avoiding the temptations of both belittlement and generalization. While we highly encourage the development of preclinical assays addressing individual risk factors, we acknowledge that in most cases the combination and interdependence of multiple factors may play a dominant role, which will ultimately be addressed by appropriate clinical trials.

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