

# Is it going to be SIN?

## A European Society of Gene Therapy commentary. Phasing-out the clinical use of non self-inactivating murine leukemia virus vectors: initiative on hold

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### Abstract

Insertional mutagenesis resulting in a leukaemia-like lymphoproliferative disease, as observed in the X-SCID (severe combined immunodeficiency) clinical trial using a  $\gamma$ -retroviral vector that transferred a functional copy of the defective gene into hematopoietic precursor cells of affected children, sparked a debate about a ban on conventional  $\gamma$ -retroviral vectors. This commentary summarizes the relevant data on this topic and concludes that there is no preclinical or clinical evidence as yet that SIN vectors, which self-inactivate the retroviral long terminal repeats (LTRs), will indeed show an improved safety profile. Conventional murine leukaemia virus (MLV) vectors can thus be used further in clinical gene therapy trials but require a thorough case-by-case risk-benefit analysis. Copyright © 2006 John Wiley & Sons, Ltd.

**Keywords** gamma-retroviral vectors; murine leukaemia virus; self inactivating; SIN; insertional mutagenesis; gene therapy clinical trials

The Gene Therapy Advisory Committee (GTAC), which is the 'United Kingdom National Research Ethics Committee for Gene Therapy Clinical Research', initiated a discussion in 2003, that extended into 2005, about the phasing out of non-SIN (self-inactivating long terminal repeat (LTR)) retroviral vectors in clinical trials. In a circular letter, which was published at the time of the 12th Annual Conference of the European Society of Gene Therapy (ESGT) in Prague in November 2005, and directly communicated to the attendants, interested parties were invited to contribute to this discussion. SIN vectors distinguish themselves from conventional  $\gamma$ -retroviral vectors by deletions in the 3' LTR covering the LTR promoter and enhancer. Upon reverse transcription and genomic integration, the  $\gamma$ -retroviral vector will carry the SIN LTR at both vector flanks, i.e. at the 3' and at the 5' end. The transcription of the therapeutic gene encompassed by SIN vectors is driven by an internal promoter, which typically contains a weaker and/or cell-type-specific enhancer. Based on our current scientific understanding of the molecular mechanisms described, it has been proposed that the elimination of the LTR enhancer sequences would minimize the risk of activating the promoter of a cellular gene located adjacent to the vector integration site. Consequently, this should reduce the risk of insertional mutagenesis, i.e. activation of cellular genes (including proto-oncogenes) adjacent to the vector integration site.

The GTAC initiative was preceded by reports about a leukaemia-like lymphoproliferative disease in two children late in 2002 as well as



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in a third child in January 2005, all having received gene therapy for X-linked SCID (severe combined immunodeficiency) in Paris [1]. The leukaemia-like complication correlated in all three patients with the occurrence of retroviral vector insertions near the *lmo2* gene, which is known to affect cellular lymphoproliferation. In addition, a recent publication by Woods *et al.* [2] suggests a contribution of the therapeutic  $\gamma$ -c chain gene to the leukemias. While one of the three patients, having received chemotherapy, died from a relapse of this complication in October 2004, the two others were in complete remission after chemotherapy, as reported in several meeting presentations from the Fischer group.

Until then, gene therapy using retroviral vectors had been considered safe. Hundreds of patients had been treated with retroviral vectors in more than 200 clinical trials by 2002 (Journal of Gene Medicine Gene Therapy Clinical Trials Worldwide Database: <http://www.wiley.co.uk/genmed/clinical/>). Although a substantial number of these trials were based on the genetic modification of cells from the haematopoietic system and were thus related to the treatment scheme of the SCID-X1 trial, no serious adverse events had been reported [3]. However, in these 'early trials' the proportion and number of engrafting vector-positive haematopoietic cells were low and insufficient for full clinical benefit. Successful X-SCID gene therapy has been reported by the French team of Alain Fischer and Marina Cavazzano-Calvo as well as by the British team of Adrian Thrasher, to full clinical correction with  $\gamma$ -retroviral gene transfer using a conventional non-SIN MLV (murine leukaemia virus)-based vector [4,5]. In this type of disease, high-level transduction followed by expression of the functional gene product resulted in a selective advantage allowing the gene-corrected cells to overgrow the gene-deficient cells in the patient. This point seems to be especially crucial, as it turned out to be a key issue in two additional successful gene therapy trials. Adenosine deaminase deficient (ADA-SCID) and chronic granulomatosis (CGD) patients also suffer from inherited immunodeficiencies. Here, however, transfer of the functional gene is believed not to result in a selective growth advantage of the gene corrected CD34-positive cells after transplantation. Only when gene therapy was combined with a non-myeloablative pre-conditioning did full correction of both the immune and metabolic defects of ADA-deficiency become possible [6]. The Swiss-German gene therapy clinical trial on CGD has led to the correction of up to 60% of circulating phagocytes in two adults out of three treated patients. Here again, non-SIN MLV vector-modified CD34-positive blood cells were infused after non-myeloablative pre-conditioning [7]. However, a longer observation period will be required to show whether this benefit will be maintained without severe adverse events related to the vector.

Combining all these clinical data, the gene therapy experience in SCID treatment comprises successful

treatment in 23 patients, with all of this being based on the use of non-SIN MLV vectors. The available data show that genetic correction of lymphocytes and blood stem cells using non-SIN MLV vectors, even in conjunction with non-myeloablative conditioning regimens, resulted in very little or no short-term toxicity while providing sustained clinical benefit. Given the severe morbidity and high mortality of patients suffering from immunodeficiencies in the absence of a perfectly HLA-matched haematopoietic stem cell donor, a positive risk-benefit can currently be concluded in favor of gene therapy using non-SIN MLV vectors.

Despite the plausible, but yet unproven, hypothesis that retroviral SIN vectors will be safer, we have to acknowledge that SIN vectors require additional internal enhancers and promoters to drive transgene expression. Although it seems reasonable to assume that 'weaker' or 'cell-specific' enhancers exert less influence than LTRs on neighboring genes, there is no preclinical or clinical evidence yet that this will be the case. Platform studies *in vitro* and in suitable animal models will show if retroviral SIN vectors will decrease insertional gene activation and, concomitantly, the risk of insertional oncogenesis. Once this has been demonstrated and subsequent clinical studies will have provided evidence for a reduction of the oncogenic risk in conjunction with retained clinical efficacy, phasing out of conventional retroviral vectors can be justified. For the moment, however, phasing out non-SIN vectors would be a step taken too early.

This was also the conclusion of the GTAC survey published in December 2005, which basically stated that there was currently insufficient evidence for a consensus on phasing out non-SIN vectors. Conventional MLV vectors can thus be used further in clinical gene therapy trials, depending on a case-by-case risk-benefit analysis. SIN vectors, based on MLV or lentiviruses, will soon be used in the clinic. It will then become clear if their expected enhanced safety profile can be confirmed by clinical data and whether the SIN configuration influences the therapeutic outcome.

## Acknowledgements

This commentary was read and approved by the board of the European Society of Gene Therapy (ESGT) and represents their views on recent progress in this area of gene therapy research. The primary objectives of the ESGT are to promote basic and clinical research in gene therapy, to promote education and the exchange of information and technology related to gene transfer and therapy, and to serve as a professional adviser to the community and to regulatory bodies in Europe.

## References

1. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**: 415–419.

2. Woods NB, Bottero V, Schmidt M, von Kalle C, Verma IM. Gene therapy: therapeutic gene causing lymphoma. *Nature* 2006; **440**: 1123.
3. Bonini C, Grez M, Traversari C, Ciceri F, *et al.* Safety of retroviral gene marking with a truncated NGF receptor. *Nat Med* 2003; **9**: 367–369.
4. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, *et al.* Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000; **288**: 669–672.
5. Gaspar HB, Parsley KL, Howe S, *et al.* Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet* 2004; **364**: 2181–2187.
6. Aiuti A, Slavin S, Aker M, Ficara F, *et al.* Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 2002; **296**: 2410–2413.
7. Ott MG, Schmidt M, Schwarzwaelder K, *et al.* Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EV11, PRDM16 or SETBP1. *Nat Med* 2006; **12**: 401–409.