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Editorial

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Risks and benefits of gene therapy for immunodeficiency: a reality check

In the first week of October 2002, the French Medicine Agency (AFSSAPS) announced that the clinical trial of gene therapy led by Professors Marina Cavazzana-Calvo and Alain Fischer for patients with X-linked severe combined immunodeficiency (X-SCID) had been voluntarily suspended. This action was taken in response to the news that one of the children treated with retrovirally transduced bone marrow in the trial had developed an unusual form of leukaemia.

Following the blaze of positive publicity that had greeted the original announcement of clinical success in the trial's first cohort just two years ago,[1] this news understandably sent shock waves around the world. Within hours, the USA's Food and Drug Administration (FDA) had suspended similar SCID gene therapy trials at the Children's Hospital in Los Angeles and at the National Institutes of Health. However, the UK's Gene Therapy Advisory Committee took a different view: SCID gene therapy trials can continue to recruit patients, but will be reviewed on a case-by-case basis.

Where does this case leave the field of gene therapy?

The SCID trial at Hôpital Necker-Enfants Malades in Paris, which had treated a total of 11 patients from five countries, was the showpiece success story of the field. All patients participating in the trial, including the boy who has now developed leukaemia, were essentially cured: they showed sustained restoration of normal immunity.[2] For a disease that is otherwise usually fatal in the first year of life, this was a remarkable advance and rightly hailed as the first unequivocal success for gene therapy. The only other treatment option for SCID is bone marrow transplantation. However, for a number of reasons this is not an ideal therapy: a perfectly matched sibling donor is required (available in only 20% of cases), one in four transplantations fail and there can be both short- and long-term complications (including lymphoid malignancy) from this procedure.

The problems first became apparent in the boy in August, 30 months after the gene therapy, when routine tests indicated that his white blood cell count had climbed to abnormally high levels. The initial rise appears to have been associated with a varicella zoster infection. However lymphoproliferation (in γ/δ T cells) continued and it became obvious that it was neoplastic.

Molecular analysis of the leukaemic clone revealed that the therapeutic retrovirus had integrated at 11p13 in the region of LMO2, an oncogene frequently overexpressed in T cell leukaemias. It is still unclear exactly how this retroviral integration led to the development of neoplasia and documenting this process is an important future goal.

The FDA halted a trial led by Dr Donald Kohn (President-elect of the American Society of Gene Therapy) at the University of California Los Angeles involving four children with ADA-SCID, as well as two others due at UCLA and NIH for X-SCID, while the case is investigated. 'The clinical halt, I think, is the only ethical course of action until we have more answers', Kohn said.

An urgent meeting of experts was convened at Bethesda to review the position for these and other US trials involving retroviral vectors. The consensus of the panel was a recommendation to the FDA to reopen the trials of those without a well-matched bone marrow donor. Authorities in Germany had already halted trials there this year after scientists found mice used in experiments had developed leukaemia-like symptoms. However, in Britain, the trials for X-SCID (involving four patients so far) and another immunodeficiency, chronic granulomatous disease will go on. 'It's an ethical dilemma', said Professor Norman Nevin, chair of the UK Gene Therapy Advisory Committee that met to consider the ramifications of the French case. 'The [French] investigation into what happened will take 12 to 18 months. During that time, one could be faced with the situation where you're presented with children with this illness who don't have a bone marrow match, who could die in two to three years. To deny gene therapy to them would be unethical, provided the parents are cognisant of the associated risks.'

The risk of insertional mutagenesis from retroviral integration was well recognised before this unfortunate demonstration of its reality. Indeed, the regulatory bodies recognise that participants in the each of the trials for immunodeficiency were fully briefed about the possibility before giving informed consent to entry. 'GTAC is satisfied that all parents and children treated were informed of this risk and received appropriate counselling prior to treatment', Professor Nevin said.

Defining risk through studying adverse events is recognised as one of the main reasons for doing clinical trials in the first place. Acting director of the FDA's new Office of Cellular, Tissue, and Gene Therapies Philip Noguchi, highlighted the proven benefit of the treatment, as well as the possible risks. 'We now are in a situation of balancing the potential risks versus the potential benefits. This is where gene therapy is right now', he said.

So what studies need to be done to help define the risks?

One direction is to examine the density and location of integration events in the target cell population. Until recently, retroviral insertion had been thought to be essentially random, affecting any region of the genome.

Methods employed to study retrovirus integration sites *in vivo* include restriction enzyme digestions and blotting, fluorescence *in situ* hybridisation, PCR-based assays, as well as cloning and sequencing the virus/host integration junctions. Most studies have analysed only a small number of integration sites, or focused on selected genomic regions. However, the availability of the human genome sequence[3] means the pattern of integration can be studied on a global genome scale. It appears that HIV-1 preferably integrates into the human genome at the location of active genes, genome regions with increased gene density, cytogenetic light bands, and GC-rich regions.[4] The data point to unexpectedly strong biases in integration site selection, with regional hotspots including a 2.4 kb region containing 1% of sites.[5]

Among other retrovirus species, Moloney murine leukaemia virus has been reported to integrate into transcriptionally active regions of the genome in five of nine cases analysed.[6] In contrast, avian sarcoma leukosis virus RAV-1, transcriptional activity of one locus was found to be associated with a decrease in integration frequency.[7]

What is needed now is a comprehensive analysis of the integration characteristics of the retroviral vectors, and tracking of insertion sites in target cells. It may not be possible to extrapolate from observations on the parental (wild-type) viruses, particularly with the advent of composite vectors with elements of several different origins. The possibility that such vectors may acquire genetic material from their packaging systems also needs to be carefully investigated in clinical applications.

More work is needed on alternative approaches to achieving stable insertion of genes into stem cell (or long-term repopulating) cells. Plasmid-based approaches generally have poor efficiency of stable gene transfer. A recent report proposed the use of the phiC31 bacteriophage integrase, which stably integrates large DNA sequences containing a specific 285-base-pair attB sequence into genomic 'pseudo-attP sites'.[8] The investigators used phiC31 integrase-based gene transfer to integrate the COL7A1 cDNA stably into genomes of primary epidermal progenitor cells and skin regenerated using these cells displayed stable correction of disease features. This approach may be further enhanced by directed evolution of integrases, such as C31, toward even more selective targeting of site-specific genomic integration.[9]

Where does this leave the field of gene therapy?

Director of the Pittsburgh Human Gene Therapy Center and President of the American Society of Gene Therapy Joe Glorioso said that gene therapy for immunodeficiency through manipulation of bone marrow still looks 'exceedingly promising'. 'The field of gene therapy remains vigorous and robust', says Professor Glorioso. All involved with the journal *Gene Therapy* support this view, and overall we should remain optimistic. As Dr Bobby Gaspar of Great Ormond Street Hospital says, 'Five years ago there were no successful gene therapies, now we have cures. This is the first step to gene therapy for a wide range of diseases.'

NR Lemoine
Faculty of Medicine, Imperial College London, UK

References

1 Cavazzano-Calvo M *et al*. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000; **288**: 669–672.
2 Hacein-Bey-Abina S *et al*. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med* 2002; **346**: 1185–1193.
3 Lander ES *et al*. Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860–921.
4 Elleder D, Pavlicek A, Paces J, Hejnar J. Preferential integration of human immunodeficiency virus type 1 into genes, cytogenetic R bands and GC-rich DNA regions: insight from the human genome sequence. *FEBS Lett* 2002; **517**: 285–286.
5 Schroder AR *et al*. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 2002; **110**: 521–529.
6 Scherdin U, Rhodes K, Breindl M. Transcriptionally active genome regions are preferred targets for retrovirus integration. *J Virol* 1990; **64**: 907–912.
7 Weidhaas JB, Angelichio EL, Fenner S, Coffin JM. Relationship between retroviral DNA integration and gene expression. *J Virol* 2000; **74**: 8382–8389.
8 Ortiz-Urda S *et al*. Stable nonviral genetic correction of inherited human skin disease. *Nat Med* 2002; **8**: 1166–1170.
9 Sclimenti CR, Thyagarajan B, Calos MP. Directed evolution of a recombinase for improved genomic integration at a native human sequence. *Nucleic Acids Res* 2001; **29**: 5044–5051.