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Molecular Therapy

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Lentiviral Vector Gene Therapy: Effective and Safe?

Over the past decade, more than 30 patients with different immunodeficiency disorders have been treated successfully using murine leukemia virus (MLV)-based γ-retroviral vectors to transfer therapeutic genes to autologous hematopoietic cells.[1–3] However, this approach has been complicated by adverse events caused by γ-retroviral vector integration into the target cell genome. Five cases of lymphoid leukemia have been reported from two different X-linked severe combined immunodeficiency (SCID-X1) trials,[4,5] while a recent report now documents two cases of myelodysplasia occurring in a clinical trial for X-linked chronic granulomatous disease.[6] A common finding in these patients was transcriptional activation of nearby proto-oncogenes by the powerful enhancer elements contained within the γ-retroviral long terminal repeats (LTRs) of the vector—a feature that has been difficult, in the case of SCID-X1, to recapitulate in animal models (see the Commentary by Sorrentino in this issue[7]).

These events fostered the development of several new *in vitro* and *in vivo* models to study vector genotoxicity. One study reported the apparent enhanced safety of lentiviral vectors based on HIV.[8] Two major factors seem to be important for the initial observation of decreased genotoxicity of lentiviral vectors, relative to a standard γ-retroviral vector. The first is that lentiviral vectors were effectively developed early on without viral LTR enhancer elements and that these so-called self-inactivating (SIN) vectors could be made without loss of titer. In contrast, early versions of the comparable SIN γ-retroviral vectors exhibited low titers and were thus not extensively pursued for clinical application. Thus, γ-retroviral vectors with intact LTRs were utilized in the early trials. Second, the overall insertion site selection of lentiviral vectors is different from that of γ-retroviral vectors, probably due in part to differences between the integrases of the two type of viruses and the presence of the viral LTRs in the γ-retroviral vectors.[9,10] Although lentiviral vectors target active transcription units, they integrate somewhat randomly throughout the entire gene. In contrast, the integrase and LTRs of γ-retroviral vectors seem to promote preferential interaction with active host cell promoters and enhancers—regions that are enriched for transcription factor binding sites (TFBS).[10] Because many cancer-related genes are often highly expressed in primitive hematopoietic cells and thus are rich in such regions, this might at least partly explain differences in the safety profiles of the two vector types.

Therefore, the first report of a clinical trial utilizing an HIV-based, lentiviral vector has been eagerly awaited from the standpoints of both efficacy and safety. A few months ago, Cartier *et al.* reported the successful treatment of two children with X-linked adrenoleukodystrophy (X-ALD) using a lentiviral vector encoding the deficient peroxisomal adenosine triphosphate–binding cassette transporter protein ABCD1 (ref. 11). Nearly 2 years after transplantation with transduced autologous $CD34^+$ cells, 9–14% of peripheral blood cells of the patients express the ABCD1 protein and neurological function has stabilized. An extensive analysis of vector insertion sites thus far shows a highly polyclonal hematopoiesis by the transduced cells. Importantly, despite the use of a lentiviral vector that contained an internal viral LTR to drive transgene expression, no clustering of vector insertions in oncogenes or growth-related genes was observed. This contrasts with the γ-retroviral clustering in such sites that was noted even in SCID-X1 patients who did not develop malignancy or clonal dominance.[12,13]

The clinical efficacy together with the pattern of vector insertions reported in this trial thus seemed quite encouraging for the use of lentiviral vectors. Unfortunately, a report of clonal dominance, despite clinical improvement, in a different clinical trial using a β-globin lentiviral vector has injected a note of caution into this early positive assessment. Although this study has not yet been formally published in a peer-reviewed format, we learned last summer—from a memo issued by the National Institutes of Health's Office of Biotechnology Activities, via public presentations by

the investigators, and through a news article in *Science*—that a relative clonal dominance occurred in a patient treated with gene therapy for a form of β-thalassemia.[14] The patient was treated in June 2007 with autologous CD34+ cells transduced with a lentiviral vector encoding a variant β-globin gene transcribed from a promoter under the control of a strong erythroid enhancer. The dominant clone that emerged approximately a year after transplant contained an activating insertion within the HMGA2 proto-oncogene. Aberrant expression of HMGA2 was likely caused by the erythroid enhancer carried by the vector. Although this clone comprises approximately one half of the progeny of the transduced cells, its abundance has been stable for many months since being discovered, implying that the clone is benign. Importantly, the patient is transfusion independent and exhibits no evidence of hematologic abnormality. At this point, cause, effect, and clinical significance remain unclear and the field awaits more details in a peer-reviewed format about this important trial and its implications for lentiviral vector safety.

It seems reasonable to conclude for now that we need results from additional patients treated with lentiviral vector gene transfer before we can draw solid conclusions regarding the relative safety of this vector system. In this regard, two new upcoming trials for SCID-X1 that have recently undergone review by the Recombinant DNA Advisory Committee should prove highly informative (http://oba.od.nih.gov/oba/RAC/meetings/dec2008/RAC_Minutes_12-08.pdf; http://oba.od.nih.gov/oba/RAC/meetings/mar2009/March%202009%20minutes.pdf). Both trials propose the use of vectors devoid of viral LTRs or strong enhancers, which clearly elevate genotoxic risk. Building on recent improvements in the design of SIN γ-retroviral vectors that can be produced with effective titers,[15] one trial proposes the use of a SIN MLV-based γ-retroviral vector that utilizes the elongation factor (EF)-1α cellular promoter to direct transgene expression. Recent preclinical studies have shown that such a design results in a vector that compares favorably to a lentiviral vector in genotoxicity studies and gives rise to significantly reduced clustering of integration sites at TFBS-rich regions in the genome.[10,16] The second trial proposes to use a SIN lentiviral vector that incorporates an insulator element and also exploits the EF-1α promoter to drive gene expression. Comparisons of clinical outcomes and insertion-site profiles will be interesting and most assuredly instructive.

Currently, the strategy of these trials to eliminate enhancer sequences from the vector would seem to be the best approach to minimize genotoxicity of vector integration. However, this is dependent upon the ability to achieve therapeutic transgene expression in the target cells using a promoter of relatively weak to moderate strength. For disorders in which high-level transgene expression is required for a therapeutic effect (e.g., β-thalassemia), inclusion of some type of enhancer element in the vector will be unavoidable. One potential solution to minimize the risk of such vectors is to target their integration to "safe" regions within the genomes. However, realization of this goal is years away. For now, the most practical solution is to include an effective insulator element (enhancer blocker) in the vector, which can prevent the interaction of the enhancer with nearby genes. Although some elements have been identified in various assays that reduce the risk of viral enhancer–mediated transcriptional activation of cellular genes, thus far none has shown the ability to do so absolutely. It is likely that an optimal insulator will need to be identified on a case-by-case basis for each specific enhancer-containing vector, taking into account the cellular context. This should be possible as methods advance to identify novel enhancer-blocking elements[17] for testing in gene transfer models. Exciting days are ahead for hematopoietic cell–directed gene therapy.

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