Gene Therapy's New Era:
A Balance of Unequivocal Benefit and Unequivocal Harm

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During the past several decades, the field of human gene therapy has been buffeted by impressive advances in gene transfer technology, promising preclinical results in model studies, and waves of unsettling reversals—all underscoring the obvious immaturity of the underlying science. Much has been written and discussed about a disappointing series of clinical studies in the mid-1990s and the scientific, policy, and administrative difficulties associated with the death of Jesse Gelsinger in 1999. Those events brought into question some of the procedures and policies involved in the human clinical application of gene-based therapies. It now seems clear that many of the controversies that plagued those early stages of gene therapy research reflected an inability to conduct a convincing risk-benefit calculus. Therapeutic benefits were unconvincing or ambiguous and, with the exception of the death of Jesse Gelsinger, perceived risks were usually intangible.

Ironically, just as the review and regulatory policy of human gene transfer studies were being extensively re-evaluated after the death of Mr. Gelsinger, the first evidence for a truly effective therapeutic outcome in a clinical gene therapy study emerged in studies with children suffering from X-linked severe combined immunodeficiency disease (X-SCID), conducted by Alain Fischer and his colleagues at the Hôpital Necker-Enfants Malades in Paris. Fischer and his group reported in 1998 that retroviral vector-mediated transfer of the cDNA encoding the common γ-chain (γc)—part of a family of cytokine receptors required for lymphocyte development—into bone marrow "stem-cell" preparations from X-SCID patients led to genetic and phenotypic correction of the immunodeficiency disease, and to a virtually total immunological reconstitution of the humoral and cellular immune systems in these very sick patients. Children who were otherwise destined to suffer life-threatening infections and who were not suitable candidates for bone marrow transplantation—the only effective existing therapy—were able for the first time to forego strict isolation and preventive antibiotic treatments and to assume normal childhood lives. They played with friends, went to school, recovered from the usual intercurrent childhood infections, including even chicken pox which would ordinarily have been fatal in such immunocompromised children. Of eleven X-SCID patients treated by the French group, and three patients treated with similar methods by the team of Adrian Thrasher at the Hospital for Sick Children at Great Ormond Street in London, all but two experienced a major clinical improvement. One additional patient, treated in Australia by Ian Alexander with the same vector and protocol employed in the French study, has also shown a partial immune reconstitution.

Sadly, in September 2002, approximately thirty months after treatment, one of the first children treated by the French group began to develop signs of T-cell leukemia. Although there was a family history of cancer—two close relatives had died in childhood of medulloblastoma—it was difficult to avoid the presumption that the genetic manipulation was the immediate trigger for the leukemia. Fischer and his colleagues quickly found that the leukemic cells consisted of a single, clonally expanded population of γδ T cells that contained a single copy of the retroviral provirus integrated in reverse transcriptional orientation in the first intron of the *LMO-2* oncogene. *LMO-2* is known to be associated with some forms of childhood T-cell leukemia. In this case, the integrated provirus had apparently introduced the strong retroviral long terminal repeat enhancer element near enough to the *LMO-2* gene to activate it to a level and time inappropriate for its stage in T-cell development. By mechanisms that are still not entirely understood, the combination of a potential growth-promoting function (γc) with an activated oncogene (*LMO-2*) in one of thousands of transduced cells may have provided that cell with a growth or survival advantage that led it, after almost three years, to outgrow all other T cells in the patient's hematopoietic system and produce a clinically evident leukemia.

To many observers, this terrible outcome seemed to confirm the long-held expectation that randomly integrating vectors could by chance hit upon or activate an oncogene and initiate tumorigenesis. Integration of retroviruses is generally thought to occur by stochastic mechanisms into fairly random or quasi-random sites. If that is so, a very high efficiency of initial transduction of a large enough population of target cells could virtually guarantee an integration event within enhancer-activation proximity to any one of the hundreds of known oncogenes or proto-oncogenes in the human genome. Furthermore, the integration event into a known oncogene in the first patient could have compounded the potential cancer predisposition in the patient's family, thereby representing a "second hit" in a multistep process of leukemogenesis. Because the precise mechanisms of leukemia induction in this child were still not understood, the French investigators and their regulatory agency suspended the study until more information could be gath-

ered. In the United States, the FDA placed two X-SCID studies and one study on adenosine deaminase deficiency (ADA)-SCID-on "clinical hold". Regulatory agencies in Italy and Japan took similar steps. The German regulatory authority recommended a "clinical hold" on all clinical studies involving live, retrovirus-transduced cells. In contrast, the retrovirus-based X-SCID study in Great Britain was allowed to proceed, because a risk-benefit analysis led to the conclusion that the marked improvement in the quality of life of the treated children outweighed the potential risks. At an FDA Biologic Response Modifiers Advisory Committee (BRMAC) meeting in October 2002, it was concluded that the gene therapy caused the leukemia-like event. Despite placing the studies on "clinical hold," the FDA invited investigators to submit documentation for revised procedures to monitor patients for the emergence of clonally expanding cells and to ensure that the informed-consent procedures included a description of the newly recognized risk of leukemia.

In early December 2002, the US National Institutes of Health Recombinant DNA Advisory Committee (NIH RAC) concluded at its public discussion of the T-cell leukemias in the French study that the "gene transfer was a cause of the leukemia," that "predisposing factors may have contributed to this result" but "that it is too early to know whether this will be a rare event or a common one." According to the evidence available at the time, it seemed that the combination of several individually unlikely events-might not readily recur in other participants of the study. The RAC therefore supported a case-by-case resumption of X-SCID studies "contingent upon appropriate informed consent and monitoring plans." In addition, because of newly emerging evidence for "mild to moderate" clinical improvement in one patient in an Italian study of ADA-SCID, the RAC supported resumption of similar ADA-SCID gene transfer studies, again on the basis of "appropriate informed consent and monitoring plans."

Then came a second case of T-cell leukemia in the French study, reported in mid-December 2002. To be sure, there were important differences in these two cases. In the second case, the leukemic cells comprised several different αβ T-cell clones, rather than a single clonal population of γδ cells. A single integration event seemed to have occurred in a precursor cell before TCR rearrangement, leading to several clonally expanding cell populations with the same integration event. Strikingly, the *LMO-2* gene was again implicated in the presumed mechanisms of leukemogenesis. In the new case, the provirus had integrated near, but not within, the *LMO-2* gene, suggesting decisively that the presumed stochastic mechanisms of leukemia development of the first case were either incorrect or incomplete. The first case suggested a pathogenesis based on improbable and rare events coupled with other improbable and rare events. The second case suggested that although the initial integration events may indeed be stochastic, other nonstochastic mechanisms intrinsic to the gene transfer procedure or the growth or survival functions of the γc-transgene product cooperated to produce leukemia. The interactions, if any, of the activated *LMO-2* gene, the selective advantages provided by *g*c, and the role of other cancer-predisposing genes in the patients are not understood, but it is evident that some or all of these variables interacted to allow the transduced cells to expand as a clonal leukemic cell population. It seems clear now that, under the appropriate selection pressures, even the very rarest of integration events in a large population of efficiently transduced initial target cells may assume unregulated cell growth or favored survival properties.

Why *LMO-2* again and not a different oncogene? It seems likely that integration near any particular site in the genome may not be improbable in a large population of transduced cells. After finding the *LMO2*-associated insertions, Dr. Christof von Kalle, in a presentation to the NIH RAC in December 2002, determined through simple estimation of the target locus size and presumed random integration throughout the genome, that the initial population of transduced bone marrow $CD34^+$ cells in these patients had a considerable probability of containing at least one cell with an integrated provirus within enhancer-activating distance of *LMO-2* or any of the several hundred oncogenes, proto-oncogenes, and tumor suppressor genes in the human genome. Because there is no reproducible evidence for site-specific integration of retroviruses, and because purely coincidental integration into this same region is otherwise unlikely, it seems evident that there must be something particularly advantageous to the growth properties or survival of a T cell containing an activated *LMO-2* gene. Ironically, with this view, the pressure in many gene transfer studies to achieve maximal transduction efficiency may be counterproductive and invite such undesirable cell advantages. Future studies involving integrating viruses may wish to define minimally effective levels of gene transfer efficiency rather than maximal transduction efficiencies, to minimize the possibility of integration into potentially deleterious sites.

Two incontrovertible facts emerged from this second case of leukemia and from the unfolding picture of this approach to X-SCID treatment. The first is that the studies of retrovirus-based gene transfer into patients with X-SCID have demonstrated unequivocal and prolonged clinical benefit to patients with lethal disease. There is no doubt that patients have received demonstrable benefit from the gene transfer; not only have they received "gene transfer, they have also received "treatment." It might therefore finally be justified to say that gene therapy has succeeded and that at least in the case of these X-SCID studies, one might legitimately use the phrase "gene therapy" instead of "gene transfer." That this clinical benefit has come with the heavy price of very serious and life-threatening complications induced directly by the therapeutic manipulation, is simultaneously an unassailable fact. In other words, for the first time a study in the field of gene therapy can be evaluated on the basis of indisputable clinical benefits and equally indisputable, treatment-induced harm. Such risk-benefit calculus in previous

gene therapy studies has been hindered by a dependence on a comparison of the two unknown quantities: uncertain benefits and ill-defined or merely theoretical risks. The X-SCID experience has clearly provided an unprecedented opportunity in the field of gene therapy to evaluate a study at this new level of maturity, which characterizes other areas of clinical research.

Regulatory and/or review bodies in the United States such as the FDA and the NIH RAC, and similar bodies in other countries, have already begun to incorporate this new appreciation of technical complexity into revised regulatory and review processes to increase the effectiveness and safety of clinical gene transfer studies. After a 28 February 2003 BRMAC meeting, the FDA responded by placing "clinical holds" on twenty-seven retrovirus-based hematopoietic stem-cell protocols, regardless of the target diseases, and again offered a case-by-case evaluation of the studies on the basis of acceptable revisions of monitoring and informed-consent issues. Similarly, the NIH formally adopted a set of recommendations to investigators and to local institutional biosafety committees on criteria that it recommends should be satisfied before investigators be permitted by their local institutional committees to proceed with some kinds of retrovirus-based gene transfer studies. At its public meeting on 10 February 2003, the NIH RAC concluded:

1. The majority of children in this X-linked SCID gene transfer study have had major clinical improvement to date.

2. Of the nine children in this experimental study who had successful engraftment of their γc-transduced cells, two developed leukemia approximately 3 years after treatment and have required chemotherapy; the overall frequency of this adverse event in this trial cannot be determined at this time.

3. The gene transfer was a cause of both leukemias.

4. The occurrence of leukemia in this protocol is not a random event and constitutes a serious inherent risk in this study.

5. Some subjects in gene transfer studies for non X-linked SCID experienced mild to moderate clinical improvement.

These findings led the NIH to adopt the following recommendations, which will be reviewed and potentially revised as new data become available.

1. Pending further data or extenuating circumstances reviewed on a case-by-case basis, retroviral gene transfer studies for X-linked SCID should be limited to patients who have failed identical or haploidentical stem-cell transplantation or for whom no suitable stem cell donor can be identified.

2. There are not sufficient data or reports of adverse events directly attributable to the use of retroviral vectors at this

time to warrant cessation of other retroviral human gene transfer studies, including studies for non-X-linked SCID. Such studies may be justified contingent upon appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

The conclusion from this experience with X-SCID is that gene therapy research is in an increasingly complex phase of development that requires rigorous standards of clinical research and simultaneously offers exciting potential for true clinical application. It would be a mistake to ignore either the difficulty of the scientific challenge or the urgency of the therapeutic endpoint of much of this work. The daunting challenge is to satisfy the need to translate new basic knowledge as quickly as possible into therapy in ways that are scientifically justifiable, ethically supportable, and that protect and enhance the lives of treated research subjects.

Review and regulatory agencies in this country and abroad, as well as professional societies, investigators, institutional review boards, and biosafety committees, are now addressing these new opportunities and challenges. As part of that effort, review bodies such as the RAC and FDA should continually update their criteria for basic and preclinical studies required during protocol evaluation, work to develop increasingly effective interactions with investigators, and catalyze more effective interactions among the relevant regulatory and review bodies in the United States and abroad. They should ensure that informed-consent procedures be reviewed and amended as necessary to ensure that information available to patients and research subjects is thorough and updated and that methods be developed to ensure effective monitoring of all research participants. New areas of research should be identified to fill technical lacunae that impede the design of optimally effective and safe clinical studies. Finally, effective methods of data sharing and communication between investigators in all countries should be developed to forestall administrative difficulties in implementing gene therapy studies in instances where markedly different oversight structures may exist. With these and additional initiatives, review bodies such as the RAC and FDA will work to ensure that the field of human gene therapy continues to mature effectively and safely through a rigorous collaboration of scientific investigation, clinical application, and constructive oversight mechanisms. Their work has already helped to bring gene therapy to this new level of early clinical reality. These refinements will help to bring more widespread success to many other urgent clinical needs.