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Recent Advances in Gene Therapy for Severe Congenital Immunodeficiency Diseases

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Abstract

Purpose of Review—To discuss new data on safety and efficacy of the ongoing gene therapy trials for primary immune deficiencies, the first reports of new trials and the preclinical developments that are likely to be translated to the clinic in the near future.

Recent findings—Both clinical successes and severe adverse events continue to be reported in trials of gammaretroviral gene therapy for SCID-X1, ADA-SCID and CGD. Insertion site analyses of recently reported trials in all of these diseases have discovered preferential insertion in the 5' ends of genes, including potentially dangerous genes such as proto-oncogenes, and signal transduction and proliferation genes. Preclinical work in rodent and canine models has tested novel vectors, including lentiviruses and foamy viruses.

Summary—Gene therapy for the most common forms of SCID can lead to immune reconstitution in most patients, although a minority of patients has derived minimal clinical benefit and some have suffered severe adverse events, including death. Ongoing pre-clinical work attempts to address the latter shortcomings. In the meantime, in the presence of a careful risk-benefit assessment, gene therapy remains an appropriate subject of clinical investigation.

Keywords

Primary Immunodeficiency; Gene Therapy; Clinical Trials; Insertional Oncogenesis; Insertion Site Analysis

Introduction

This review will cover recent clinical advances in gene therapy for primary immune deficiencies, including the X-linked and adenosine deaminase-deficient forms of severe combined immune deficiency (SCID-X1 and ADA-SCID), chronic granulomatous disease (CGD) and Wiskott-Aldrich Syndrome (WAS). We will briefly review the historical

background to the recently published and currently ongoing trials of gene transfer for these illnesses. We will also cover the current problems facing clinical gene therapy and some of the pre-clinical work that may address these issues.

Recent advances in clinical Gene Therapy

In less than 20 years, the application of gene therapy for primary immune deficiency has gone from minimal success to a potentially completely curative procedure, albeit with substantial risks. Early gene therapy trials conducted in the 1990s for adenosine deaminase (ADA)-deficient severe combined immune deficiency (SCID)[1], chronic granulomatous disease (CGD) [2] and leukocyte adhesion deficiency type I (LAD I) [3] did not achieve long-lasting clinical benefits. However, following on technical advances achieved through extensive pre-clinical studies [4], subsequent trials culminated in the first irrefutably successful results described in the 21st century.

Successful Gene Therapy Trials for SCID-X1

The first successful clinical gene therapy trial was reported by Drs. Fischer and Cavazzana-Calvo in 2000[5]. This initial report described two patients with the X-linked form of SCID (SCID-X1) due to deficiency of the γ c cytokine receptor. No myelosuppressive preparatory regimen was used. Additional data from this trial were reported in 2002 [6], 2003 [7] and 2007 [8•]. Overall, ten patients with classic SCID-X1 were treated, and achieved long-lasting immune reconstitution. Most patients derived clinical benefit. Signs and symptoms of infection resolved. T-cell counts, proliferative responses and diversity normalized. All patients had proviral integration in ~ 100% of their T-cells. Most of the successfully treated patients had normal levels of IgG, IgA and IgM. One of the two patients who did not achieve immune reconstitution was a 10-month-old boy with disseminated bacille Calmette-Guérin (BCG) infection and splenomegaly [6]. This patient ultimately required allogeneic hematopoietic cell transplantation (HCT).

The potentially curative outcome of gene therapy for SCID-X1 was confirmed by Drs. Gaspar and Thrasher in Great Britain. They reported successful reconstitution of immunity in ten patients with classic SCID-X1 [9,10•]. As of the time of the last full report, all patients showed increased lymphocyte counts, most to the normal range. T-cell function and diversity were mostly normalized as well. Six patients no longer need intravenous immunoglobulin (IVIG). Taken together, the French and British reports show, for the first time, clear efficacy of gene therapy for a human genetic disease and also point out some of the limitations of the approach, including the failure of gene therapy in two older patients (age 15–20 years) [11].

Older patients who failed transplant were the targeted population of a recent United States trial evaluating the efficacy of gene therapy for SCID-X1 as rescue therapy after a failed allogeneic HCT. In this report, a team of investigators from the National Institutes of Health, described the treatment of three patients aged 10, 11 and 14 years. After a follow-up of one to two years, only one patient was found to have improvement in T-cell number and proliferation, with increased numbers of CD45RA⁺ T-cells and T-cell receptor excision circles. No patient was able to discontinue IVIG, but all three have reported subjective improvements in well-being, and all have shown increased growth rates [12].

Overall, the current experience indicates that, provided sufficient numbers of transduced hematopoietic stem cells (HSC's) are engrafted, gene therapy is a highly successful therapeutic modality for SCID-X1 infants, while its efficacy decreases in older patients.

Adverse events in the SCID-X1 trials

Unfortunately, despite successful results, the gene therapy trials for SCID-X1 highlighted the substantial risks of the procedure. About two and a half years after treatment, the fourth patient treated on the French trial developed a monoclonal lymphoproliferation caused by the insertion of the therapeutic vector near *LMO2*, a known T-cell oncogene. Dysregulation of *LMO2* induced by the murine leukemia virus (MLV) enhancer was concluded to be the basis of the leukemic event in this patient and in a second case that occurred shortly thereafter [7,13]. Over the last five years, additional reports have described other patients with T-cell lymphoproliferations [7,13–16] (and www.asgt.org, posted 12-18-07, accessed 1-29-08). Two of the patients were very young (1 and 3 months of age at the time of gene therapy), and received cell doses in excess of 10 million cells/kg. Very young age, however, was not common to all of the cases of leukemia [10,14] and (www.asgt.org, posted 12-18-07, accessed 1-29-08)}. Overall, as of the last reports, twenty classic SCID-X1 patients have been treated in the French and British versions of this trial. Three of these patients had poor immune reconstitution and one of these died after allogeneic hematopoietic cell transplantation. Five patients developed leukemia and one of these patients died as well [17]. Thus, the overall mortality was 10% and the overall morbidity was 30%.

The question of why five out of 20 patients treated on the French and British SCID-X1 trials developed leukemia is one of the most pressing current problems in clinical gene therapy. Some controversy exists over the relative roles of *IL2RG*, *LMO2* and/or their combination in leukemogenicity [15,18•,19•,20,21••]. A recent report noted that, in addition to frequently integrating near the 5' ends of genes, the vector used in the French trial preferentially integrated into common fragile sites [22••]. Because *LMO2* itself is a fragile site, this finding may help to explain why targeting of this particular oncogene was seen so frequently. In addition to these considerations, it is generally accepted that the preference of retroviral vectors for integration in gene-rich regions [23,24], and particularly near the transcriptional start sites of genes has likely contributed to severe adverse events. Recent surveys of clinical samples from the British and French SCID-X1 trials [8••,10••] have confirmed this tendency, a finding that was described earlier *in vitro* and in experimental animal systems [23–26].

Success of gene therapy for ADA-SCID

Uniquely among the primary immunodeficiencies, ADA-SCID can be treated with enzyme replacement therapy (ERT) using pegylated bovine ADA (PEG-ADA) [27]. Early trials of gene therapy for ADA-SCID [1,28,29] treated patients who, for ethical reasons, were kept on PEG-ADA. The initial attempts at targeting HSC's resulted in low transduction efficiency [28,30,31] and low engraftment of gene-corrected cells [29].

In a landmark study published in 2002, Aiuti and colleagues treated ADA-SCID patients who did not have access to PEG-ADA with low dose busulfan prior to infusion of autologous bone marrow CD34+ cells that had been genetically corrected using a retroviral vector, with the goal of facilitating the engraftment of corrected cells [32]. The initial report showed convincingly that immunity could be restored with this approach. To date, data on twelve patients have been reported in various forms [32,33••,34•] and indicated long-term multilineage engraftment of gene-corrected cells, associated with improvement in T-cell counts and proliferation to exogenous stimuli, leading to clear clinical benefits. No serious adverse events have been observed in the trial. An extensive analysis of the pattern of retroviral integration in five patients has recently been published and showed that integrations have occurred within and/or near the *LMO2*, *BLM*, *CCND2* and *BCL2* genes, among others, although these events did not lead to clonal selection [33••].

The efficacy of these trials surpassed those of a concurrent collaborative trial conducted by or own group and that of Dr. Kohn at Childrens Hospital Los Angeles (CHLA). The emphasis in this trial was on vector design. Patients were kept on PEG-ADA and myelosuppressive chemotherapy was not used [35].

A Japanese trial recently reported in abstract form [36•] also did not use marrow conditioning, but involved discontinuation of PEG-ADA before reinfusion of gene-corrected cells. Patients on this trial showed slow immunological improvement compared to the Milan study.

The initial results of a British study of gene therapy for ADA-SCID that also included withdrawal of PEG-ADA as well as myelosuppressive chemotherapy were also published in 2006 and showed immune recovery manifested by increasing T-cell numbers and T-cell proliferative responses in the first treated patient [37••].

More recently, our own group has used a similar protocol with PEG-ADA withdrawal and low dose myelosuppressive chemotherapy. Four patients with ADA-SCID have been treated with this approach to date. The first treated patient was discovered after gene therapy to have had bone marrow trisomy 8 that pre-dated chemotherapy. The patient suffered from persistent myelosuppression post-chemotherapy and ultimately required hematopoietic stem cell transplantation[38]. Two other patients have been followed for at least six months and remain off PEG-ADA treatment without opportunistic infection, and one of these is also off IVIG. The patients have shown general clinical improvement as well as improvement in T-cell counts and in T-cell proliferative responses. Finally, a fourth child recently treated remains off PEG-ADA, but otherwise has not had enough time since treatment to evaluate the outcome[39].

Recent data on gene therapy for CGD and WAS

A recent trial of gene therapy for CGD was conducted by Drs. Grez and Seger in Germany and Switzerland. The retroviral vector used in this study carries gp91^{phox} under the control of the spleen focus forming virus long terminal repeat (LTR), a promoter that is highly active in myeloid cells. High levels of functionally corrected phagocytes were observed, resulting in reduction of infections in three treated patients. Oligoclonal expansion of gene-corrected myeloid clones was also observed in the first two treated patients. This likely was due to insertional activation of the cancer-associated genes MDS1-EVI1, PRDM16 and SETBP1 [40••,41]. Recent findings from this trial indicate that both of these patients developed monosomy 7, presumed to be related to these insertional events [42]. Clearly, these results raise serious concerns regarding the vector LTR used in this trial.

More recently, Malech and colleagues have reported on the use of genetically modified cells to treat infections in CGD, rather than attempting immune reconstitution per se [43,44]. Conditioning consisted of busulfan 10 mg/kg given over two days. One of two treated patients had resolution of multiple staphylococcal liver abscesses and retained 1% correction of myeloid cells for more than one year. The second patient was felt to have an immune-mediated rejection of the transgene product that is currently being characterized.

Finally, at the 2007 meeting of the American Society of Hematology a preliminary report was given on a trial of retroviral gene therapy for WAS [45]. Based on prior optimization *in vitro* [46], Dr. Klein and colleagues treated two patients, both of whom have derived clinical benefit from the procedure. Both patients' genetic marking is increasing in T and NK cells. No evidence of adverse events or clonal dominance have been noted on this trial to this time.

Implications of Recent Clinical Results for Pre-Clinical Development of Gene Therapy

The occurrence of severe adverse events in SCID-X1 gene therapy patients [47] has stimulated extensive preclinical research efforts aimed at reducing the risk of insertional oncogenesis in future applications of gene therapy to this and other primary immune deficiencies. The tendency of gammaretrovirus vectors to integrate near transcription start sites of transcriptionally active genes [8•,10•,23,24,26,33•] and the intrinsic promoter enhancing activity of retroviral LTR's are seen as critical risk factors. To overcome the latter, Thornhill, Thrasher, et al have developed self inactivating (SIN) gammaretroviral vectors for gene therapy of SCID-X1 [48]. SIN vectors lack the enhancer sequences of retroviral LTR's and are therefore expected to be less prone to activating promoters near the integration sites [49,50]. However, these vectors necessarily require the use of an internal promoter, and this element must also need to be devoid of significant enhancer properties if ectopic expression of genes neighboring the integration site is to be avoided.

Vectors based on lentiviruses are an appealing alternative to gammaretrovirus vectors because their LTRs have no meaningful activity in the absence of the TAT transcriptional regulator, and their pattern of integration has been shown to be less biased towards transcription start sites [23]. At least one paper [51•] suggests that lentiviral vectors are less likely than are MLV-derived vectors to integrate into sites related to cancer, cell growth or oncogenesis. For these reasons, lentiviral vectors are being developed and tested for a variety of primary immunodeficiencies, including the X-linked [52•], ADA-deficient [53•,54•] and Artemis-deficient [55•] forms of SCID, as well as CGD [56] and WAS [57•,58•].

Vectors based on foamy virus [59] offer another alternative that is being evaluated for gene therapy of primary immunodeficiencies. Foamy vectors offer a number of advantages, including the observation that foamy virus is not known to be a human pathogen [59] and that the integration pattern of foamy viruses has been described as having a less strong preference for integrating near genes [25,60•]. Foamy vectors have been developed for gene therapy of LAD I [25], a disease that has also been the object of efforts to increase the efficiency of retrovirus vectors in the canine model of the human condition [61•,62].

Despite advances made in the above-mentioned viral vectors, they all suffer from the drawback that their use implies gene addition strategies that are less desirable than genetic repair approaches. Of the various approaches to genetic repair being studied [63–66], the zinc finger nuclease approach has been applied for SCID-X1 [67], as an example of a disease where the strong selective advantage of gene-corrected cells could compensate for the low efficiency that at present characterizes all gene repair methods.

Conclusions

After extensive theoretical and preclinical work, human gene therapy for SCID was translated to the clinic in 1990. Within twelve years, this form of treatment was shown to be efficacious. This rapid and successful progress can be attributed to advances in vectorology, cell culture conditions and the use of preparative myelosuppressive therapy. While still incompletely characterized, the risks of gene therapy for the primary immunodeficiencies remain high, and warrant a careful analysis of the risk-benefit ratio. Pre-clinical and clinical advances continue to be made in the safety and efficacy of gene transfer vectors and gene correction approaches. While it is likely that future gene therapy for primary immunodeficiencies will evolve beyond the use of integrating vectors for gene addition approaches. However, integrating vectors currently remain the state of the clinical science. Carefully monitored clinical trials of gene therapy are appropriate for patients with these severe illnesses who lack of a matched related

donor. In some cases, gene therapy for immune deficiency has brought clinical benefit, and can be considered to have been life-saving despite the serious complications that have been reported in some patients. Based on the documented clinical successes, further progress in the development of gene therapy for primary immune deficiency can be expected.

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