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Gene therapy of primary T cell immunodeficiencies

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ABSTRACT

Gene therapy of severe combined immunodeficiencies has been proven to be effective to provide sustained correction of the T cell immunodeficiencies. This has been achieved for 2 forms of SCID, i.e SCID-X1 (γc deficiency) and adenosine deaminase deficiency. Occurrence of gene toxicity generated by integration of first generation retroviral vectors, as observed in the SCID-X1 trials has led to replace these vectors by self inactivated (SIN) retro(or lenti) viruses that may provide equivalent efficacy with a better safety profile. Results of ongoing clinical studies in SCID as well as in other primary immunodeficiencies, such as the Wiskott Aldrich syndrome, will be thus very informative.

1. Introduction

1.1. The rationale for gene therapy in primary T cell immunodeficiencies

There are many reasons why gene therapy has been developed in the field of primary immunodeficiencies (PIDs) over the last 20 years. Many PIDs are life-threatening conditions — notably severe combined immunodeficiencies (SCIDs) affecting T cell development and function, Wiskott Aldrich syndrome (WAS), hemophagocytic lymphohistiocytosis (HLH), innate immune deficiencies (such as chronic granulomatous disease or Mendelian susceptibility to mycobacterial disease) and inherited autoimmune syndromes. The remarkable progress in treating PIDs has mostly been based on allogeneic hematopoietic stem cell transplantation (HSCT).(Gennery et al., 2010) However, this approach is far from perfect and serious adverse events (SAEs) that can still occur (such as graft-versus-host disease (GVHD)). In particular, GVHD can damage the thymus and compromise the reconstitution of T cell immunity. The limitations of HSCT are necessarily more pronounced in patients who lack HLA-compatible donors. Conversely, the success of HSCT provides a rational basis for the autotransplantation of transduced stem cells — the current approach in gene therapy for PIDs. Most PIDs display Mendelian inheritance, so that introduction of a normal copy of the mutated gene into the patient's cells should (in principle) be effective. The fact that disease-related genes have now been found for most PIDs (Notarangelo et al., 2009) makes gene therapy a feasible approach for many of these conditions.

For some PIDs (e.g. T cell immunodeficiencies), it has become clear that transduced precursor cells can have a selective growth advantage. In several T cell PIDs, the occurrence of somatic mutations positively modifies the mutated genes and leads to the development of functional T cells; the observed attenuation of disease phenotypes strongly supports this concept. This growth advantage is based on (i) the tremendous ability of T cell precursors in the thymus to divide in an interleukin-7-dependent manner and, following expression of the pre-T cell receptor (pre-TCR), (ii) positive selection and (iii) the very long life span of mature T cells. One can thus expect a few transduced T cell precursors to give rise to a full, stable T cell pool in a given individual. Hence, SCID is considered to be an optimal model for assessing the feasibility of gene therapy.

1.2. Gene transfer technology

In the meantime, significant advances in viral vector technology have enabled the transduction of dividing cells and thus replication of the transgene in progeny cells. Replication-defective retroviral vectors have been based on murine oncoretroviruses (the γ retrovirus), simian and human lentiviral viruses, spuma viruses and transposons (Verma and Weitzman, 2005). A key advance was the creation of "self-inactivating" (SIN) viruses in which the absence of enhancer elements in their long terminal repeats (LTRs) makes them less able to transactivate endogenous genes after genome integration (see below) (Yu et al., 1986). In the absence of enhancers, several internal promoters can be used to

Abbreviations: ADA, adenosine deaminase; GVHD, graft versus host disease; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplantation; IPS, induced pluripotent cells; LV, *Lentivirus*; PIDs, primary immunodeficiencies; RV, retrovirus; SAE, serious adverse event; SCID, severe combined immunodeficiency; SIN, self inactivated; WAS, Wiskott Aldrich syndrome.

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drive transgene transcription. Culture conditions for the transduction of hematopoietic progenitor cells have been improved by selecting the best cytokine cocktails and promoting virus/cell interaction by the addition of fibronectin fragments.

1.3. Gene therapy in SCIDs

Following the advent of this vector technology, clinical trials were successfully initiated for SCID-X1 (γc deficiency) in 1999 and then adenosine deaminase (ADA) deficiency. To date, gene therapy results are available for 20 patients with typical X-linked SCIDs, five patients with atypical SCIDs (n = 5) and 38 patients with ADA deficiency (Aiuti et al., 2002, 2009; Candotti et al., 2012; Cavazzana-Calvo et al., 2000; Gaspar et al., 2011a, 2011b; Hacein-Bey-Abina et al., 2010).

The SCID-X1 trials were associated with clinical events caused by vector genotoxicity and shall be discussed first.

1.4. Genotoxicity in SCID-X1 trials

Five of the 20 patients (four in the Paris trial and one in the London trial) developed T cell leukemia 2 to 5.5 years after gene therapy (Hacein-Bey-Abina et al., 2008; Howe et al., 2008). Interestingly enough no events occurred subsequently, suggesting reduced risk overtime. Following chemotherapy, four patients survived and showed sustained remission and T cell immunity (Gaspar et al., 2011a; Hacein-Bey-Abina et al., 2010) (see below). One patient died from refractory leukemia (Hacein-Bey-Abina et al., 2010). In all cases, it was found that the abnormal clone had one or two provirus integrations within a proto-oncogene locus. Many other genomic abnormalities were found (Hacein-Bey-Abina et al., 2008; Howe et al., 2008). Accordingly, the clinical trials were discontinued. Considerable effort was then devoted to investigating the mechanism underlying these SAEs. It was clearly shown that retroviruses do preferentially integrate within genes (especially actively transcribed ones). Epigenetic signatures which favor retroviral integration have been recently identified (Dave et al., 2009; Santoni et al., 2010). It turned out that the LMO2 locus in hematopoietic progenitors contains several of the features that favor frequent local integration. In parallel, it became clear that the viral LTRs' enhancer activity could permanently turn on transcription of the target gene and thus trigger the leukemic process (Cattoglio et al., 2010; Kustikova et al., 2010; Santoni et al., 2010). It is noteworthy that despite the use of a similar gene transfer technology in the ADA trials, none of the successfully treated patients (n = 28) developed leukemia — a result that significantly differs from that of the SCID-X1 trials (Aiuti et al., 2009; Candotti et al., 2012; Ferrua et al., 2010; Gaspar et al., 2011a). These findings strongly suggest that one or more disease-associated factors interfere with retroviral integration, e.g. the nature of progenitor cells in the bone marrow above the differentiation block, the possibly convergent effects of transgene and oncogene expression and an inadequate in vivo milieu for cell growth why gene therapy of ADA deficiency has not led to leukemia could be related to the toxic effects of (deoxy) adenosine metabolites, that accumulate because of the ADA deficiency. These effects may partially affect the epithelial component of the thymus. A putative diminution in cell division rate could reduce the risk of secondary genomic alterations that are required to induce leukemia. The fact that a similar, LMO2-associated leukemic event was also observed in 4/9 WAS patient efficiently treated with ex vivo retrovirally mediated gene transfer into CD34 cells also indicates that the ADA deficiency setting should be regarded as unfavorable for the occurrence of leukemia (Boztug et al., 2010).

Researchers have made huge efforts to construct safer vectors, with the development of enhancer-deleted LTR-SIN vectors containing an internal promoter. This type of vector has been shown to be less genotoxic in in vitro assays of the clonogenicity of myeloid precursors (Cattoglio et al., 2010; Kustikova et al., 2010; Yu et al., 1986). Despite efforts to set up predictive in vivo assays in murine models, an absolute demonstration of safety can only be provided by the ongoing, recently initiated clinical trials. Furthermore, use of insulators (for functional isolation of the integrated provirus from the genomic environment) and addition of a suicide gene might be useful. Nevertheless, these measures will probably be only partially effective and have their own pitfalls. The use of HIV-derived lentiviral vectors might constitute an additional safeguard, since this type of vector only integrates into genes (and not upstream of the transcription start site). This advantage might, however, be counterbalanced by greater transduction efficacy and thus more frequent vector integration into the patient's cells. Other potential improvements for the future include gene targeting to neutral ("safe harbor") genome regions and gene repair by target-specific nucleases (Lombardo et al., 2007; Papapetrou et al., 2011).

1.5. Efficacy in the SCID-X1 trial

At present, 18 of 20 SCID-X1 patients treated in the Paris/London trials are alive 5.7 to 13.5 years after treatment (median: 10.3 years). Seventeen patients show the sustained presence of transduced lymphocytes (Cavazzana-Calvo et al., 2000; Gaspar et al., 2011a; Hacein-Bey-Abina et al., 2010). Blood T cell counts are in the normal or close-to-normal range, while phenotype and functional characteristics are also satisfactory. This includes detection of distinct T cell subsets including innate like T cells (γδ T cells, NK T cells) and FoxP3 (+) CD4 (+) regulatory T cells. Antigen-specific T cell activation can be evidenced in vitro following in vivo immunization. Immunoscope analysis detects a fully diversified TCRVB repertoire including in patients who had leukemia and received chemotherapy. Remarkably, most patients (including the 4 who received chemotherapy) have some naïve T cells characterized by the detection of T cell receptor excision circles (TRECs) — indicating the presence of ongoing, long-term thymopoiesis from transduced progenitor cells.

Gene therapy based on the development of T cell immunity provided clear-cut clinical benefits to these patients, since they can now deal normally with infections and are doing well in the absence of any therapy (apart from immunoglobulin (Ig) substitution in 8 cases, see below). Long-term natural killer (NK) cell reconstitution is not as impressive, with only a few such cells in their blood (as is also observed after allogeneic HSCT in the absence of myeloablative conditioning). These results suggest that NK cell dynamics (precursor expansion and/or progeny life span) differ significantly from T cell dynamics. The patients' B cell functions have been partially restored, despite very low (and decreasing) transduced B lymphocyte counts. Accordingly, 10 out of 18 of the patients do not require Ig substitution. This observation may be due to (i) to competition with normal B cell development in the absence of γc expansion/function and (ii) B cell dynamics. It may well be of value to establish whether plasma cells in the bone marrow express γc or not. Thanks to the development of novel methods and technologies (e.g. ligation-mediated PCR with multiple restriction enzymes and deep sequencing), a wealth of information has been provided by the in-depth analysis of retroviral integration sites in the patients' cell populations. For example, it has been shown that the patients' T cells originate from as few as 300 to 4000 transduced progenitor cells. Given that the T cells display significant diversity in a TCR repertoire analysis, one can deduce that these few cells have divided extensively (thanks to γc receptor expression) prior to TCR rearrangements in the thymus. This finding validates the selective advantage concept on which gene therapy for SCID was launched (Cavazzana-Calvo et al., 2000; Gaspar et al., 2011a; Hacein-Bey-Abina et al., 2010). It has also been noted that there is considerable variation over time in the abundance of clones, with no evidence for long-term selection. Furthermore, there are significant changes over time in the clonal composition of peripheral T cells; this could (as least in part) be explained by non-exhaustive detection of the more rarely represented clones at a certain time point and/or by unexpected variations in the immune system's

"use" of T cell progenitors. Lastly, although detection of the same integration sites in T cells and myeloid cells soon after treatment demonstrated that a least some multipotent hematopoietic progenitors had been transduced, only transduced T cells (including naïve T cells) are found 8–13 years post-gene therapy. The latter result suggests that T cell precursors with self renewal capacity persist in the thymus as recently described in murine models (Martins et al., 2012; Peaudecerf et al., 2012).

In addition to these 20 patients treated soon after diagnosis of a typical SCID-X1 early in life, five other patients were treated later in life (at between 10 and 20 years of age) because of either an atypical SCID-X1 caused by hypomorphic mutation or poorly reconstituted T cell immunity years after HSCT. Despite technically efficient gene transfer, the results have been disappointing — with little or no improvement in T cell immunity (Thrasher et al., 2005). Defective residual thymic function at a later age in SCID patients very probably accounts for these failures and raises the question of how long the thymus remains potentially functional in a patient lacking effective thymopoiesis.

Based on the efficacy of these trials, a new clinical trial has been reinitiated for which a SIN retroviral vector with a satisfactory in vitro safety data (Modlich et al., 2009) containing the γc gene has been designed. This international trial has been initiated two years ago and should provide within the next couple of years the expected informations on its combined safety/efficacy profile (Cavazzana-Calvo et al., 2012).

1.6. Gene therapy in ADA deficiency

Adenosine deaminase deficiency has now been treated with modern gene therapy techniques, following the inclusion of 38 patients in three trials (performed in Italy, the UK and the USA) (Aiuti et al., 2002, 2009; Cavazzana-Calvo et al., 2012; Ferrua et al., 2010). The technology is essentially similar to that used to treat SCID-X1. An important difference was related to the use of a mild conditioning regimen (4 mg/kg busulfan for most patients), in order to improve transduced stem cell engraftment. This choice was motivated by the fact that ADA deficiency is a metabolic disease in which increasing the number of transduced cells within the different cell lineages could be advantageous. This chemotherapy has been well tolerated and (as mentioned above) none of the patients have developed treatment-related genotoxic complications.

Efficacy (judged in terms of T cell development and the absence of clinical indications for supplementing patients with pegylated ADA) has been seen in 28 of the 30 patients, whereas the 10 others are alive and on enzyme replacement therapy. The median follow-up is 3.5 years (range: 1 to 11.5 years). The quality of T cell reconstitution has not been as good as in SCID-X1 (Aiuti et al., 2009) — probably because of the unfavorable setting of ADA deficiency in non-hematopoietic tissues such as thymic epithelial cells. Nevertheless, T cell reconstitution has been good enough to enable the patients to thrive. The provirus integration profile and characteristics are strikingly similar to those seen in the SCID-X1 trial. Furthermore, significant transduced B, NK lymphocyte and myeloid cell counts have been detected as a consequence of the mild myeloablation and the transduced stem cells' good engraftment. These results are very encouraging and suggest that gene therapy is a coherent therapeutic option for patients with ADA deficiency.

1.7. Gene therapy in Wiskott–Aldrich syndrome

Wiskott–Aldrich syndrome is a life-threatening immunodeficiency. Since lymphocyte development is not perturbed, transduced cells are not expected to have the full selective advantage observed in SCIDs. Nevertheless, this might still be partially the case, given the WAS protein's functional involvement in migration of CD34 cells to the bone marrow. Myeloablation may favor the engraftment of transduced cells.

Lentiviral-mediated transfer of the WASp gene has now been recently initiated, in order to achieve optimal transduction of stem cells. This was observed in the adrenoleukodystrophy (ALD) trial (Cartier et al., 2009), in which up to 10% of all hematopoietic lineages were found to be stably transduced 3 years after therapy. In the meantime, a retrovirus-based trial for WAS was set up. It is also based on ex vivo gene transfer into CD34+ cells following myeloablation. The recently published preliminary results suggest that many aspects of the disease (e.g. T + B cell immunodeficiencies and thrombocytopenia) had been corrected in 9 patients (Boztug et al., 2010). Although longer follow-up is obviously needed, these results are sufficiently encouraging to justify the development of clinical trials with safer vectors (i.e SIN-LV) given the occurrence of leukemia in 4 into treated patients in this trial. SIN-LV vectors with a WASp promoter are being used in present clinical trials (Merten et al., 2011).

1.8. Extending our present experience

The results achieved to date have provided proof of concept for gene therapy of SCIDs and WAS. It will be critical to see whether the SIN vectors are indeed as safe as expected in the ongoing SCID-X1 and WAS trials. Even from a cautious standpoint, extension of gene therapy to other SCID diseases is logical. Encouraging preclinical results have been reported for Artemis and Rag-2 deficiencies and, to a lesser extent, Rag-1 deficiency (Benjelloun et al., 2008; Lagresle-Peyrou et al., 2006; Mostoslavsky et al., 2006). Further development in the treatment of other primary T cell immunodeficiencies (such as HLH, immunodysregulation, polyendocrinopathy, enteropathy and X-linked syndrome) is underway. Two strategies can be considered in such cases: the transduction of hematopoietic stem cells (HSCs) or that of diseased, mature T cells (Fischer et al., 2010).

The fact that the sustained detection of transduced blood cells has been observed in the treatment of one disease (ALD) in which expression of the therapeutic transgene does not provide a competitive advantage suggests that a similar gene therapy strategy can be applied to PIDs characterized by the similar absence of growth/survival activity for the defective protein. This might open up the way to the safe, efficient treatment of PIDs of the myeloid lineages, in which consistent transduction of HSCs will be needed to ensure the daily renewal of neutrophils. Chronic granulomatous diseases and leukocyte adhesion deficiency are obvious target diseases (Grez et al., 2011; Hunter et al., 2011).

Advances in gene therapy for PIDs will undoubtedly stem from technological progress, such as the above-mentioned safe harbor and gene repair strategies. Furthermore, ex vivo stem cell expansion (Boitano et al., 2010) could increase the number of treated cells and hence boost efficacy and (if clone selection can be performed) safety. Lastly, the production of HSCs from other cell sources (as recently achieved with human fibroblasts(Szabo et al., 2010)) opens up further development pathways, together with potential gene mutation correction by genetic engineering based on TALE nucleases (Hockemeyer et al., 2011; Zhang et al., 2011). Reprogramming of cells as achieved by the generation of induced pluripotent stem cells (iPS) provides a tool to engineer repair of correction of a given inherited disorder prior to induce cell differentiation to hematopoietic cells. Potential safety issues that might emerge from incomplete reprogramming as well as robustness of induced hematopoietic cells are the challenges to tackle next.

Conflict of interest

The authors declare that they have no conflict of interest.

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