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# Gene Therapy for Primary Immunodeficiencies

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## 1. Introduction

Primary immunodeficiencies (PID) are caused by mutations in genes involved in the normal development or activity of the immune system [1, 2]. PIDs include B- and T-cell defects, phagocytic disorders, and complement deficiencies with the common feature of frequent life-threatening infections. The phenotypes vary from asymptomatic (IgA deficiency) to severe PIDs (such as Severe combined immunodeficiencies). Treatment of patients with severe PIDs relies in intravenous injection of immunoglobulins, bone marrow transplantation (BMT) and antibiotics. Identical and haploidentical BMT are the only curative treatment, however, the lack of a HLA-matched donor in over 70% of the patients make necessary the development of new therapeutic strategies [3, 4]. Gene therapy (GT) could be the best alternative for the treatment of patients with severe PID that lack a HLA-matched donor [5]. The aim of GT strategies is the stable correction of the mutated gene on the patient's own haematopoietic stem cells (HSCs).

The first successful gene therapy clinical trial used gamma-retroviral derived vectors expressing common cytokine-receptor gamma chain ( $\gamma_c$ ) cDNA in HSCs from X-linked severe combined immunodeficiency (SCID-X1) patients [6]. So far, using a very similar vector platform, over 50 PID patients treated with GT can be considered "cured" from SCID-X1, adenosine deaminase deficiency (ADA) and Wiskott-Aldrich syndrome (WAS) PID [7-13]. However, in six children, GT treatment resulted in clonal T-cell proliferation (leukaemia-like disease) [9].

The results obtained in the SCID-X1, ADA and WAS clinical trials clearly showed the importance to improve vector's safety and efficiency [8,14, 15]. Lentiviral-based vectors have been the vector of choice to enhance efficiency and, at the same time, reduce the side effects of gammaretroviral vectors (see below). Several GT clinical trials for SCID-X1, chronic granu-

lomatous disease (CGD) and WAS PID using lentiviral vectors (LVs) have started in the last few years.

This chapter intend to illustrate the past, present and near future of GT for the treatment of severe PIDs

## 2. Gamma-retroviral vector based gene therapy clinical trials for primary immunodeficiencies

### 2.1. Gammaretrovirus-based vectors

Gammaretrovirus, also named oncoretrovirus, are efficient, integrative, easy to manipulate and poorly immunogenic. Vector derived for these retroviruses are often named “retroviral vectors” and “oncoretroviral vectors”. All the clinical data that will be presented in this section was obtained using a similar gammaretroviral backbone: **LTR---ψ-----transgene-----LTR**. As consequence the therapeutic gene is expressed through the promoter and enhancer sequences present at the viral LTR. Another common aspect of all the GT strategies presented in this section is the modification of the patient’s hematopoietic stem cells (HSCs). However HSCs are quiescent or very slowly dividing cells and gammaretroviral-based vectors require active cell division for transduction [16]. Therefore HSCs transduction protocols require cytokine “pre-stimulation” to induce cell proliferation [17], a process that can modify the characteristics of the haematopoietic precursors [18]. However, since LTR-driven gammaretroviral vectors were the only integrative vectors available at the time, several clinical trials started on SCID-X1, ADA CGD and WAS. An overall conclusion of these clinical trials was that GT is as efficient and safe as haploidentical BMT. However it was also evident the necessity of improving the vector system before GT of PID could be of general use in clinic.

### 2.2. X-linked Severe Combined Immunodeficiency (SCID-X1)

SCID-X1 is a monogenic disease caused by mutations in the interleukin-2 receptor gamma chain gene ( $\gamma c$ ). Patients with SCID-X1 deficiency do not have T nor NK cells, consequently B-lymphocyte function is also intrinsically compromised [19]. SCID-X1 has been an attractive GT target because patient’s cells expressing the transgene have a growth advantage over non-expressing cells [20, 21]. Therefore, GT could, in theory, achieved complete immune reconstitution with a relatively low number of gene-corrected cells. The Fischer group at the “Unité d’Immunologie et d’Hématologie Pédiatriques, Hôpital Necker” in France achieved the first unequivocal success of gene therapy in the two patients treated [6]. The authors transduced patients HSCs (CD34<sup>+</sup>) with a Murine Leukaemia Virus (MLV) based vector expressing the  $\gamma c$  cDNA following pre-activation with stem cell factor (SCF), polyethylene glycol-megakaryocyte differentiation factor (PG-MDF), IL-3 and Flt3-L. The continuation of this work and other clinical trials in other countries enrolled a total of 20 SCID-X1 patients [7, 8, 22, 23]. Between 5 and 12 years after GT, 17 of the 20 treated patients are alive and display full or nearly full correction of the T cell deficiency [24, 25]. The GT treatment led to clear benefits since patients

recover from ongoing infections with poor prognosis (disseminated infections) and live in a normal environment without evidence of increased susceptibility to infection.

However, 5 of the 20 patients with SCID-X1 on GT trials developed leukaemia 3-6 years after treatment. Four patients were successfully treated with chemotherapy and they are alive and doing well. However the other patient died from chemotherapy-refractory leukemia [26]. This leukaemia-like disease was a result of vector-mediated up-regulation of host cellular oncogenes (i.e. LMO2) [8, 27]. Several studies have demonstrated that MLV-derived vectors integration favour transcriptionally active genes near transcription start sites (TSSs) [28-30]. Leukemogenesis could also be the result of insertional mutagenesis (activation of the LMO2 oncogene) combined with the acquisition of genetic abnormalities unrelated to vector insertion, such as the increase activity of NOTCH1 or the deletion of CDKN2A gene [8].

However, in spite of the secondary effects observed, the results obtained with GT using first generation MLV-based vectors are comparable to those obtained with HLA-identical HSC transplant (HSCT). It is expected that next generation vectors will certainly improve these results as it will discussed later.

### 2.3. Adenosine Deaminase (ADA) Severe Combined Immunodeficiency (ADA-SCID)

ADA-deficiency has been also considered an important target for GT. The *ADA* gene codify for an enzyme that is expressed in all tissues and catalyses the deamination of 2'-deoxyadenosine and adenosine to 2'-deoxyinosine and inosine. Its absence or malfunction cause the accumulation of purine metabolites that are toxic to the cells. Although the *ADA* gene is expressed in all tissues, the accumulation of purine metabolites in the immune cells is the main problem. As consequence, ADA patients suffer from lymphopenia, reduced (or absent) cellular and humoral immunity, failure to thrive and recurrent infections. Additionally, the accumulation of purine metabolites in other tissues also produces skeletal, hepatic, renal, lung, and neurologic abnormalities [31, 32]. Like for SCID-X1, bone marrow transplantation (BMT) is the best therapeutic alternative. However, contrary to SCID-X1, there are other treatment options that allow ADA patients to have near-normal lives: Enzyme replacement therapy (ERT) with polyethylene-glycol-conjugated bovine ADA (PEG-ADA). However, although ERT treatment is well tolerated and can partially restore immune function, its effect decline over time and, in addition, lifelong treatment is very expensive[33].

ADA deficiency has been successfully treated by GT using a similar approach to that for SCID-X1, but requiring mild bone-marrow chemoablation [34]. The authors showed immunological and metabolic reconstitution after transplantation of gene-modified CD34<sup>+</sup> using ADA-expressing-MLV based vectors. The selective growth advantage of ADA-expressing lymphocytes played an important role in the success of this trial. Similar findings have been reported by Gaspar *et al.* [23] and again by Aiuti *et al*[10]. In total, over 40 patients with ADA have been treated in Italy, UK and USA. At present all patients are alive and 29 of them do not require ERT [9, 10, 23, 25, 34-36].

It is important to remark that no leukaemia-like disease have been observed in the ADA-SCID GT trial. The author propose that the differences between SCID-X1 and ADA might be related

with SCID-X1 genetic background or the role of the therapeutic transgene (*ADA* is a house-keeping enzyme whereas  $\gamma c$  is a potential oncogene growth factor receptor). However, in the last clinical trial some non-life threatening adverse effects have been reported such as neutropenia (2 patients), treatment-related infections (2 patients), Epstein-Barr virus reactivations (1 patient) and autoimmune hepatitis (1 patient).

#### 2.4. X-linked Chronic Granulomatous Disease (X-CGD)

Chronic granulomatous disease (CGD) is a rare PID characterized by severe, life threatening bacterial and fungal infections. Patients with CGD have also defective degradation of inflammatory mediators leading to granuloma formation. All of these defects are caused by mutations in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits in phagocytic cells [37]. *gp91<sup>phox</sup>* mutations occur in up to 70% of the CGD cases and represent the X-linked form of this disorder (X-CGD). Neutrophils, monocytes, macrophages, and eosinophils from CGD patients cannot generate superoxide and other reactive oxygen intermediates to destroy invading bacteria and fungi.

Contrary to SCID-X1 and *ADA*, CGD is a difficult target for GT, since the expression of the correct form of the gene does not provide selective advantage to hematopoietic progenitors. In addition, myeloid cells have a short life span and therefore a large amount of HSC must be corrected to achieve clinical benefits. Myeloablative conditioning is therefore required to increase the amount of gene-modified cells that engraft into the patients. Several GT clinical trials for CGD have been conducted since 1997. Initial studies using retroviral vector to express *p47-phox* into CD34+ cells, resulted in low and short-term engraftment of CGD-corrected cells [38]. More recent GT clinical trials on X-CGD conducted in Frankfurt, Zurich, London, USA and Seoul resulted in higher correction and clinical benefit in several patients. Dr Grez's group showed the most dramatic effects in two children (5 and 8 years old) showing recovery from severe pulmonary and spinal aspergillosis. GT treatment also achieved recovery from paraparesis of both legs in one of the children [39]. However, the efficacy was only partial due to a progressive loss of gene-corrected cells over time [39-41]. The loss of transgene expression was, at least in part, due to inactivation of the vector promoter. However, there are other hypothesis that point to the potential toxicity of ectopic expression of *gp91* gene on HSCs as a potential cause of the loss of gene-corrected cells [42]. In addition, three patients developed a myelodysplastic syndrome (MDS) due to transactivation of the MDS/EVI oncogene by the retroviral enhancer [40]. The MDS was fatal for two of the patients while the third was treated with HSCTs. These results revealed the importance of developing new, safer and more efficient vectors for GT in CGD.

#### 2.5. Wiskott-Aldrich Syndrome (WAS)

Wiskott-Aldrich syndrome (WAS) is a X-linked PID caused by mutation in the *WAS* gene coding for the Wiskott-Aldrich syndrome protein (WASP), a hematopoietic-specific member of regulators of the actin cytoskeleton [43, 44]. The most severe form of WAS (where the mutation cause total absence of protein or function) is characterized by recurrent infections,



microtrombocytopenia, eczema and higher susceptibility to autoimmune diseases and lymphoid malignancies [45].

As for other PID, HLA-identical sibling HSC donor transplantation is considered the treatment of choice (over 80% survival rate). Allogeneic HSCTs is offering nowadays good outcomes due to improvements in HLA-typing and new alternative donor sources and myeloablative conditioning regimens [46]. However, patients lacking a HLA-matched donor still require alternative therapeutic approaches. In this direction GT could be an alternative in the near future for these patients. In fact WAS is an attractive target for GT since expression of WASP confer selective growth advantage [47-52].

Dr Klein group (Hannover Medical School, Hannover, Germany) performed the first clinical trial for WAS GT [53]. 10 patients were enrolled in this trial and they received autologous CD34<sup>+</sup> cells transduced with LTR-driven gammaretroviral vectors expressing WASP. All patients received reduced intensity conditioning with Busulfan. Most of the patients treated gain WASP expression in multiple lineages. Platelet counts increased and clinical condition improved with resolution of eczema and bleeding disorder [54, 55]. However, as occurred in the SCID-X1 clinical trials, four out of 10 of the treated patients developed leukaemia [55, 56]. The presence of the strong LTR enhancer and the patient's predisposition to develop lymphomas could favour the high frequency of leukaemia in this trial.

### 3. Lentiviral-vector based gene therapy clinical trials for primary immunodeficiencies

As soon as the first cases of leukaemia appeared in the SCID-X1 GT trial, it was clear that LTR-driven gammaretroviral vectors were not the vector of choice to go further into clinic. Improvements in the gammaretroviral vectors and the design of new integrative vectors became the main goal in the GT field. Several groups have dedicated considerable effort to understand the mechanism of leukomogenesis upon gammaretroviral transduction. The LMO2 oncogene was found in 4/5 cases in the SCID-X1 trial and it is now clear that retrovirus-mediated gene transfer can deregulate proto-oncogene expression through the LTR enhancer activity. With this in mind, Dr. Naldini's group have developed self-inactivated (LTR mutated) lentiviral vectors (based in HIV-1) which have one of the best efficiency/safety ratio [57-59]. LVs, contrary to gammaretroviral vectors are able to achieve efficient transduction of HSCs with minimal activation [60]. They are also safer than gammaretroviral vectors due to their less genotoxic integration site [61-63]. Several clinical trials for PID have started using HIV-1-based vectors and some promising results have already been shown on international meetings. In most cases, the general structure of the vectors is as follow: **LTRAU3-- ψ ----human promoter ----- transgene----- LTRAU3**

There are at the moment two GT clinical trials on going for SCID-X1 using lentiviral vectors (<http://www.wiley.com/legacy/wileychi/genmed/clinical/>). One is designed for newly diagnose children (St Jude Children's Research Hospital) and other is a Phase I/II non-randomized clinical trial designed to treat 13 patients with SCID-X1 who are between 2 and 30 years of age

and who have clinically significant impairment of immunity. Both cases are based on mice experiments showing a better profile of lentiviral vectors both in term of reconstitution and safety [64].

Dr Gaspar and Dr Kohn have launched two other clinical trials using lentiviral vectors to treat ADA patients in UK and USA respectively. Both groups use EF1 promoter driven lentiviral vectors produced at the same site (Indiana University Vector Production Facility) through a Transatlantic Gene Therapy Consortium. The primary objective of the trial is to examine the safety of the protocol in 10 patients transplanted with LV gene-modified CD34<sup>+</sup> cells. The protocol will involve non-myeloablative conditioning with busulfan and withholding of PEG-ADA ERT. As secondary objectives the trial will aim for the expression of ADA in peripheral blood leucocytes and immune reconstitution.

CGD is probably the PID where the necessity to improve vector efficiency and safety has been more obvious. The absence of the selective advantage of the gene-modified cells and the short life span of myeloid cells reduce the clinical benefits of gammaretroviral vectors but kept all the secondary effects. In addition, the potential toxicity of ectopic expression of gp91<sup>phox</sup> on HSCs required the use of physiologically regulated vectors [65] expressing the transgene specifically in granulocytes. Very encouraging results have been obtained in animal models using transcriptionally regulated LV [66, 67]. The first clinical trial for CGD using LV started on November 2011 directed by Adrian Thrasher at Great Ormond Street Hospital for Children (UK). The primary outcome measures will be overall survival but the trial will also study reduction in frequency of infections and long-term immune reconstitution (<http://clinicaltrials.gov/ct2/show/NCT01381003>).

As SCID-X1 and CGD, GT for WAS has also good reasons to change the therapeutic vectors (see above). There are four clinical trials on going for WAS using LV (FR-0047, UK-0168 and US-1052: journal of gene medicine GT clinical trials data base; NCT01515462: Clinicaltrial.gov). All trials will use a similar construct which drive the expression of the WASP cDNA through its own promoter. The WASp-promoter-driven LVs are haematopoietic-specific [47, 49, 68], physiological [49, 69] and avoid deleterious effects of over-expression in non-target cells[70]. Preliminary data presented at the 20th European Society of Gene and Cell Therapy by the Italian and French groups showed impressive results both, in terms of immune reconstitution and safety profile. It is important to note that integration site analysis in these patients did not show any preference for the proto-oncogenes LMO2 or EVI1. In addition they didn't observe, at the time of analysis, any evidence of clonal dominance (usually indicative of proto-oncogenes activation).

## 4. Future directions

Based on the data shown, it does appear that new generation LVs driving the expression of the transgene through physiological promoters could be a big step toward GT clinical translation. Exciting results are expected on the clinical trials undergoing at the moment. Still, LV integrates randomly at active sites in the cell genome and can therefore alter its normal

expression pattern. New, undesired side effects could appear in the future. New vectors must still consider improving two safety aspects: 1- genotoxicity (genomic alteration due to vector integrations) and 2- ectopic/unregulated expression of the transgene. Strategies to minimize or eliminate genotoxicity problems can be grouped in those based in improving retroviral vectors and those based in the development of non-viral technologies such as gene editing (revised in [14, 65]).

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