GENE THERAPY FOR ADA-DEFICIENT SCID

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Severe combined immunodeficiencies (SCID) are inherited diseases caused by mutations in crucial genes controlling the development and functions of immune cells. Adenosine deaminase (ADA)-deficiency is a rare autosomal recessive form of SCID caused by an accumulation of adenosine and adenine deoxyribonucleotides (dAXP) in plasma and tissues leading to a complex immune and metabolic phenotype. ADA-SCID patients typically present with lymphopenia, impaired immune cellular and humoral responses, recurrent infections and failure to thrive (1, 2). In addition, the increased levels of purine metabolites cause also multi-system pathologic changes, including skeletal, liver, lung and neurological abnormalities.

Transplant of hematopoietic stem/progenitor cells (HSC) from an HLA-identical sibling donor is a curative treatment, but available for a minority of patients. In the absence of an HLA-identical sibling donor, bone marrow (BM) transplantation from haploidentical or matched unrelated donors is a high risk procedure (3). Enzyme replacement therapy with bovine ADA (PEG-ADA) corrects the metabolic alterations of the disease but the variable degree of immune recovery, the high costs, and the occurrence of neutralizing antibodies or autoimmunity limit its therapeutic impact (1, 3).

Since the first pilot trials of the early 90's, the development of gene therapy approaches for ADA-SCID was supported by a strong rationale (1). ADA gene is a housekeeping gene, expressed in all tissues, which can be inserted into gene transfer vectors under constitutive promoters such as the one present in standard gamma-retroviral vectors. Because as low as 10% of ADA activity can allow normal immune functions in healthy individuals, it was hypothesised that even relatively low amount of correction and/or of engrafted HSC would have resulted in successful therapy. Moreover, wild type or gene corrected cells were shown to carry a strong selective survival advantage over deficient cells in hematopoietic cell transplantation and preclinical gene therapy model. Our Institute was among the pioneers in the development of gene therapy protocols for ADA-SCID using peripheral blood lymphocytes (PBL) or bone marrow HSC. These pilot gene therapy trials showed that retroviral-mediated transfer of the ADA gene was safe and feasible, and resulted into

engraftment of genetically corrected cells (1, 4). The available follow up extends now to over 13 years in the first treated children, with no adverse events or toxicity observed. However, it is only recently that the efficacy of PBL or HSC-based gene therapy has been investigated in the absence of PEG-ADA treatment. We first showed that, following discontinuation of PEG-ADA, engineered PBL persisted long-term after infusion, allowing the correction of the T-cell defect and the generation of antibody responses to neoantigen (5). However, the infusion of transduced PBL alone was not sufficient to provide adequate systemic detoxification, likely because of the limited mass of ADA producing cells. Thus, we designed a new clinical trial based on infusion of autologous transduced $CD34^+$ cells combined with low dose busulfan to "make space" in the BM and to provide an initial developmental advantage to HSC (6). The transduction protocol for $CD34^+$ cells was validated in preclinical models showing efficient gene transfer into multipotent progenitors, including B, NK, and T cell progenitors (7). Furthermore, we chose to avoid the use of PEG-ADA after gene therapy to fully exploit the selective advantage for gene-corrected cells and to evaluate the clinical efficacy of gene therapy alone. Using this improved protocol in two patients, we obtained the first demonstration of clinical efficacy of gene therapy alone for ADA-SCID, with correction of both the immune and metabolic phenotypes.

We have now enrolled in the clinical trial six children affected by early onset ADA-SCID who lacked an HLA-identical sibling donor. Previous therapy included haploidentical BM transplant or PEG-ADA associated with insufficient immune reconstitution or side effects. Autologous BM $CD34^+$ cells were transduced with an MLV retroviral vector encoding ADA and reinfused in the patients following a non-myeloablative conditioning with busulfan (4 mg/Kg total dose), in the absence of PEG-ADA. At present, all patients are alive and well with a follow-up after gene therapy ranging from one year to more than 5 years. The degree of myelosuppression after conditioning ranged from mild (Pt2, Pt4, and Pt6), to short-term neutropenia (Pt1, Pt5), or more prolonged thrombocytopenia and neutropenia (Pt3). Multilineage, stable engraftment of gene corrected HSC was achieved in the BM of all patients, at higher levels in patients 1, 3 and 5 (5-10%). In all patients the vector-ADA+ cells became over time the large majority of T, B and NK lymphocytes. This led to the progressive increase of peripheral blood lymphocyte counts, restoration of polyclonal thymopoiesis, and normalization of proliferative responses to mitogens and antigens. Pt2, who received the lowest dose of transduced HSC, displayed a partial immune reconstitution. Serum Ig levels improved in all patients and production of specific antibodies after IVIG discontinuation and antigen vaccination was observed in three patients. Sustained ADA activity in lymphocytes and RBC resulted in the correction of purine metabolism (dAXP<30 nmoles/ml RBC in 5 patients) and amelioration of systemic toxicity. None of the patients experienced severe infections or adverse

events after gene therapy. All the children are healthy and thriving, in the absence of enzyme replacement therapy. A systematic analysis to identify and map vector insertion sites is ongoing to define the pattern of retroviral integrations, their potential influence on gene expression and the analysis of the clonal composition of transduced HSC and their progeny. This study has revealed a profile of polyclonal integrations in T cells, oligoclonal integrations in myeloid cells, and no evidence of clonal expansion. Importantly, integration analysis in distinct hematopoietic lineages (T cells, B cells, granulocytes, erythroid precursors) has revealed the presence of common integrants detected at different time points, further demonstrating the engraftment of multipotent transduced HSC.

In summary, these results show that gene therapy is a safe and efficacious procedure which allows to correct both the immune and metabolic defect of ADA-deficiency. These results were instrumental for the recent designation by the EMEA of our $CD34^+$ cell based gene therapy as an Orphan Medicinal Product for the treatment of ADA-SCID. Overall, these studies are helping the transition of gene therapy from the experimental phase to an established treatment for ADA-SCID and are providing crucial information for the development of gene therapy approaches for other diseases.

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