Key words: Gene therapy, Wiskott-Aldrich syndrome, lentiviral condition

Wiskott-Aldrich syndrome (WAS, OMIM 300100) is a severe X-linked disorder characterized by thrombocytopenia, eczema, immunodeficiency, and increased risk of autoimmunity and cancer. Affecting 1 to 10 males per million, WAS is caused by mutations in the WASp gene, which lead to impaired or abolished expression of the WAS protein (WASP), a hematopoietic-specific regulator of actin cytoskeleton remodeling. The severity of WAS is scored on the basis of gravity of thrombocytopenia (score 0.5-1), eczema and immunodeficiency (score 2-4), and presence of autoimmunity or malignancy (score 5).

Historically, WAS has been treated with splenectomy and immunoglobulin replacement to prevent infections, the former of which may improve platelet counts but further weakens immunity. The standard treatment for patients with WAS is hematopoietic stem/progenitor cell (HSPC) transplantation (HSCT) from an HLA-identical donor.

Because related identical donors are rare and a matched-unrelated donor may be untimely, especially within certain ethnicities, *ex vivo* gene therapy (GT) represents a valuable therapeutic alternative. Compared with allogeneic HSCT, GT is an autologous procedure that bears negligible risk of rejection or graft-versus-host disease and does not require immunsuppression or fully myeloablative conditioning, which is associated with increased risk of infection and organ toxicity. On the other hand, GT may present limitations due to gene correction efficiency, levels of WASP expression, and potential occurrence of insertional mutagenesis.

### PRECLINICAL DATA

The pathophysiology of WAS has been studied using cells from patients with WAS and 2 independently generated *was*<sup>−/−</sup> (wko) mouse strains displaying most features of patients with WAS, excluding severe thrombocytopenia. Knowledge of WAS pathophysiology (summarized in Fig 1) was crucial in informing several features of clinical GT.

Study of patients with WAS developing revertant mosaicism, heterozygous wko female mice, gene-corrected patients’ T-cell lines, and wko mice clearly showed a proliferative/survival advantage for WASP-expressing T cells, B cells, and less prominently for platelets.

Initial GT approaches used γ-retroviral vectors (RVs) with strong viral promoters. However, because of the nature of the disease and the risk of oncogenicity, most groups moved to lentiviral vectors (LVs), likely safer alternatives to RVs, because they do not show preferre for integration close to transcription start-sites and can incorporate endogenous cellular promoters for regulated and specific expression of the transgene.

Gene correction using a self-inactivating LV to drive expression of WASp cDNA controlled by a 1.6-kb (w1.6WAS) endogenous WASp promoter restored WASP expression in T, B, and CD34<sup>+</sup> cells from patients. It also corrected T-cell dysfunction, dendritic cell cytoskeletal abnormalities, and thrombocytopenia in wko mice treated with nonmyeloablative irradiation and GT. LV-transduced CD34<sup>+</sup> cells retained the ability to engraft and differentiate in immunodeficient mice. The w1.6WAS LV did not cause tumors in GT-treated mice that were followed up for a year, nor in recipients of secondary transplantation, establishing its safety in preclinical models.

### CLINICAL GT

The proof of concept of efficacy of GT in patients with WAS was provided by a clinical trial using a RV bearing a strong viral promoter. Long-term engraftment of RV-transduced HSPCs led to restoration of WASP expression and improved platelet count and T-cell function, resulting in clinical amelioration of disease phenotype. However, 9 of 10 patients for whom GT was successful developed acute leukemia due to RV integrations close to oncogenes, including LMO2, and activation of their expression. This further prompted the need for viral vectors with better safety profiles.

Various clinical trials based on LV-engineered autologous HSPCs began in 3 centers in Europe (SR-Tiget in Milan, ...
Great Ormond Street Hospital in London, and Necker Children’s Hospital in Paris) and in 1 center in the United States (Boston Children’s Hospital) (Table I).5-8 The LV and transduced CD34<sup>+</sup> cells were manufactured at different sites, but vector design was the same. Treatment consisted of a single infusion of LV-transduced autologous bone marrow or mobilized peripheral blood–derived CD34<sup>+</sup> cells after conditioning. SR-Tiget adopted a reduced-intensity conditioning regimen to minimize toxicity and fully exploit the selective growth advantage of gene-corrected cells, whereas the other centers adopted a more intense regimen (Table I). Thirty-four patients with WAS (Zhu score 3-5) were treated worldwide, with a median follow-up ranging from 3.3 to 7.8 years, depending on the center<sup>5-8</sup> (Table I). Three of 34 patients died of morbidities unrelated to the GT product (Table I). No severe GT-related adverse events occurred and no treated patients developed clonal selection, insertional mutagenesis, leukemia, or replication-competent LV to date.

All surviving patients (31 of 34 [91%]) had sustained multilineage engraftment of gene-corrected cells, with higher gene marking and WASP expression in T cells and other lymphoid cells, consistent with their strong selective advantage. Despite the use of a reduced-intensity conditioning, sustained and robust in vivo bone marrow engraftment of gene-corrected progenitors (median, 49%; range, 22%-85%) was achieved.<sup>7</sup> Conditioning is not the only factor influencing engraftment because patients who received a more myeloablative regimen reached a vector copy number of 0.01 to 0.4 (equivalent to 1%-40% WASP expression) in myeloid cells.<sup>5</sup> Even in the presence of variable levels of reconstitution, immune function improved enough to provide a clinical benefit with reduced severe infection rate. Humoral immune deficiency ameliorated, allowing for discontinuation of immunoglobulin supplementation in several patients. All subjects showed improvement or resolution of eczema. Platelet count variably improved after GT, but remained below normal range in most patients. Amelioration of thrombocytopenia resulted in protection from severe bleeding, as well as freedom from transfusions and thrombopoietin agonists (Fig 2). This may also be a result of improved platelet function and phenotype after treatment.<sup>9</sup> Autoimmunity improved after GT,<sup>5,8</sup> possibly due to restoration of normal regulatory T-cell function and B-cell
tolerance. However, in contrast to the results of other centers, 2 subjects treated in Boston with preexisting autoimmunity had no resolution after GT, in association with poor recovery of lymphocytes, including regulatory T cells.6

Although most initially treated patients were children, clinical benefit has now been demonstrated in older subjects (overall age range, 0.8–35.1 years), who are considered at a higher risk when treated with allogeneic HSCT.7,10

### CURRENT CHALLENGES AND FUTURE DIRECTIONS

GT has proven to be an effective treatment for WAS. Available data from recent GT clinical trials using LV demonstrate the safety and efficacy of this therapeutic approach in the short- and medium-term. The experience from this cohort of patients indicates that an adequate immunologic reconstitution provides protection from infections and control of autoimmunity in most patients. However, thrombocytopenia persists in several patients after GT, although in a milder and mostly asymptomatic form. This also occurs, albeit less frequently, after allogeneic HSCT and is usually associated with low myeloid chimerism. In line with this, the dose of gene-corrected drug product and in vivo correction of HSPCs seems to correlate with the degree of myeloid cell engraftment and improvement of thrombocytopenia. Strategies to achieve full correction of thrombocytopenia could be based on (1) improvement of vector construct to increase transgene expression; (2) optimization of gene transfer efficiency and LV copy number by transduction enhancers; and (3) refinement of the conditioning regimen to increase the engraftment of gene-corrected myeloid cells while sparing conditioning-related toxicity, for instance using stem cell–depleting antibody-drug conjugates. These changes over the current protocols will however mandate a careful reassessment of risks.

In contrast to the long-lasting experience with HSCT in WAS, there is limited information on the very long-term safety of GT (>10 years). As of today, no patient treated with LV-GT has developed malignancies, the longest follow-up being 8.8 years. Despite this timeline being well beyond the reported time of occurrence of leukemia in the RV trial (range, 1.3–5 years), life-long monitoring of all LV-treated patients will be crucial.

In 2019, a new clinical study started at SR-Tiget to evaluate the use of a cryopreserved formulation of w1.6W-transduced autologous CD34+ HSPCs (OTL-103) in subjects with WAS (NCT03837483). The use of cryopreserved product aims to increase safety, because it allows for quality testing of the medicinal product before infusion. If comparable to its fresh counterpart, the cryoformulation may increase the availability of GT worldwide, making it not only a standard option in the clinical management of patients with WAS but also a possible treatment for patients with milder disease forms.
REFERENCES