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Authors

Ferrua, Francesca
Marangoni, Francesco
Aiuti, Alessandro
et al.

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Brief review article – Paradigms and Perspectives

Gene Therapy for Wiskott-Aldrich Syndrome: History, New Vectors, Future Directions

Francesca Ferrua[1]* MD PhD, Francesco Marangoni[2]* PhD, Alessandro Aiuti[1,3#] MD PhD, Maria Grazia Roncarolo[4#] MD

[1] San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) and Pediatric Immunohematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy.
[2] Department of Physiology and Biophysics and Institute for Immunology, University of California, Irvine, CA, United States.
[3] Vita-Salute San Raffaele University, Milan, Italy.
[4] Division of Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics and Institute for Stem Cell Biology and Regenerative Medicine, Stanford School of Medicine, Stanford, CA, United States.

*equal first author contribution, [#] equal last author contribution

Corresponding author's name:

Maria Grazia Roncarolo, MD

Lorry I. Lokey Stem Cell Research Building
265 Campus Drive West, Room G3021A
Stanford, California 94305-5461
T: +1-650-498-0297
E-mail address: mg1@stanford.edu

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Introduction

Wiskott-Aldrich Syndrome (WAS, OMIM 301000) is a severe X-linked disorder characterized by thrombocytopenia, eczema, immunodeficiency, and increased risk of autoimmunity and cancer. Affecting 1-10 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 based on the gravity of thrombocytopenia (score 0.5 to 1), eczema and immunodeficiency (score 2 to 4), and presence of autoimmunity or malignancy (score 5)[1].

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 gold standard treatment for WAS patients is hematopoietic stem/progenitor cell (HSPC) transplantation (HSCT) from an HLA-identical donor[2].

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 to allogeneic HSCT, GT is an autologous procedure that bears negligible risk of rejection or graft-versus-host disease and does not require immunosuppression 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 WAS patients and two independently generated *was*$^{-/-}$ (wko) mouse strains displaying most features of WAS patients, excluding severe thrombocytopenia. Knowledge of WAS pathophysiology (summarized in Figure 1) was crucial in informing several features of clinical gene therapy.

Study of WAS patients 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 utilized γ-retroviral vectors (RV) with strong viral promoters. However, due to the nature of the disease and risk of oncogenicity, most groups moved to lentiviral vectors (LV), likely safer alternatives to RV, as they do not show preference for integration close to transcription start-sites and can incorporate cellular physiological promoter for regulated specific expression of the transgene.

Gene correction using a self-inactivating lentiviral vector (LV) to drive expression of WASp cDNA controlled by a 1.6kb (w1.6W) endogenous WAS promoter restored WASP expression in T, B, and $CD34^+$ cells from patients. It also corrected T-cell dysfunction, DC cytoskeletal abnormalities, and thrombocytopenia in wko mice treated with non-myeloablative irradiation and GT[3]. LV transduced $CD34^+$ cells retained the ability to engraft and differentiate in immunodeficient mice. The w1.6W LV did not cause tumors in GT-treated mice that were followed up on for a year, nor in recipients of secondary transplantation[3], establishing its safety in preclinical models.

Clinical gene therapy

The proof of concept of GTs efficacy in WAS patients was provided by a clinical trial using a γ-RV bearing a strong viral promoter. Long-term engraftment of RV-transduced HSPC led to restoration of WASP expression and improved platelet count and T-cell function, resulting in clinical amelioration of disease phenotype. However, 9/10 patients for which GT was successful developed acute leukemia due to RV integrations close to oncogenes and activation of their expression[4], including LMO2. This further prompted the need for viral vectors with better safety profiles.

Various clinical trials based on LV-engineered autologous HSPC began in 3 centers in Europe (SR-Tiget in Milan, Great Ormond Street Hospital in London, Necker Children's Hospital in Paris) and in 1 center in the US (Boston Children's Hospital) (Table 1). The LV and transduced CD34+ 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 $CD34^+$ cells after conditioning. SR-Tiget adopted a reduced intensity-conditioning regimen (RIC) to minimize toxicity and fully exploit the selective growth advantage of gene corrected cells, while the other centers adopted a more intense regimen (Table 1). 34 WAS patients (Zhu score: 3-5) were treated worldwide, with a median follow up ranging from 3.3 to 7.8 years, depending on the center [5–8] (Table 1). Three out of 34 patients died of morbidities unrelated to the GT product (Table 1). 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 (31/34, 91%) had sustained multi-lineage 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 RIC, sustained and robust *in vivo* BM engraftment of gene corrected progenitors (median 49%, range 22-85%) was achieved[7]. Conditioning is not the only factor influencing engraftment since patients who received a more myeloablative regimen reached a VCN of 0.01 to 0.4 (equivalent to 1% to 40%) in myeloid cells[5]. 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 TPO agonists (Figure 2). This may also be a result of improved platelet function and phenotype after treatment[9]. Autoimmunity improved after GT[5,8],

possibly due to restoration of normal T regulatory cell function and B-cell tolerance. However, in contrast with the results of other centers, two subjects treated in Boston with pre-existing autoimmunity had no resolution after-GT, in association with poor recovery of lymphocytes, including Tregs[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 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 immunological reconstitution provides protection from infections and control of autoimmunity in most patients. On the other hand, 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 HSPC seems to correlate with degree of myeloid cell engraftment and improvement in 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; 3) refinement of the conditioning regimen to increase myeloid gene corrected engraftment while sparing conditioning-related toxicity, such as with 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 $CD34^+$ HSPC (OTL-103) in subjects with WAS (NCT03837483). The use of cryopreserved product aims to increase safety, as 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 WAS patients, but also a possible treatment for patients with milder disease forms.

Figures and tables

Table 1. GT in WAS: Worldwide experience with w1.6W LV

Center *(courtesy of)*	**Conditioning regimen**	**Clinical trial.gov identifier**	**Patients treated** *(n)*	**Patients alive** *(n)*	**Years of follow up^** *(median and range)*	**References**
London *(A. Thrasher)*	**Bu** 12 mg/kg *(target AUC ~60)* **Flu** 120 mg/m^2	#NCT01347242	7	6[#]	3.5 (1.5 – 8.0)	[5] *and unpublished data*[§]
Paris *(A. Magnani, M. Cavazzana)*	**Bu** 12 mg/kg *(target AUC ~82)*° **Flu** 120 mg/m^2 Anti-CD20 mAb (1 pt*)	#NCT01347346	5	4°°	7.8 (6.0 – 8.8)	[5] *and unpublished data*[§]
Boston *(S-Y. Pai)*	**Bu** 12 mg/kg *(target AUC 70-85)*** **Flu** 120 mg/m^2	#NCT01410825	5	5	5.0 (2.7 - 6.1)	[6] *and unpublished data*[§]
Milan	**Bu** 6.4 – 9.6 mg/kg *(current target AUC 48+/-10%)* **Flu** 60 mg/m^2 **Anti-CD20 mAb**	#NCT01515462 + EAP (HE/CUP)	17	16^^	3.3 (0.1 – 8.8)	[7,8]
Total	-	-	34	31	-	-

Bu, Busulfan; Flu, Fludarabine; AUC, area under the curve; mAb, monoclonal antibody; EAP, early access program; HE, hospital exemption; CUP, compassionate use program.
Busulfan AUC reported in the table is expressed as "x10^3 ng x h/mL".
^In surviving patients. [#]One patient died 3 years after GT due to post-splenectomy sepsis post-influenza (splenectomy performed early in life, many years before GT). °Target Busulfan monitoring was performed in 4 out of 5 treated patients. *With autoimmunity. °°One patient died 7 months after GT due to pre-existing drug-resistant herpes virus infection. **1 pt AUC= 48, not targeted. ^^One patient died 4.5 months after treatment, due to deterioration of an underlying neurodegenerative condition, not related to GT.
[§]Unpublished data have been kindly provided by Adrian Thrasher (UCL, London), Marina Cavazzana and Alessandra Magnani (Hôpital Necker-Enfants malades, Paris) and Sung-Yun Pai (Boston Children's Hospital), who gave their permission to include them in this table and in the present review.

Figure 1: Mechanisms of disease in WAS. (to be replaced with new version by artist)

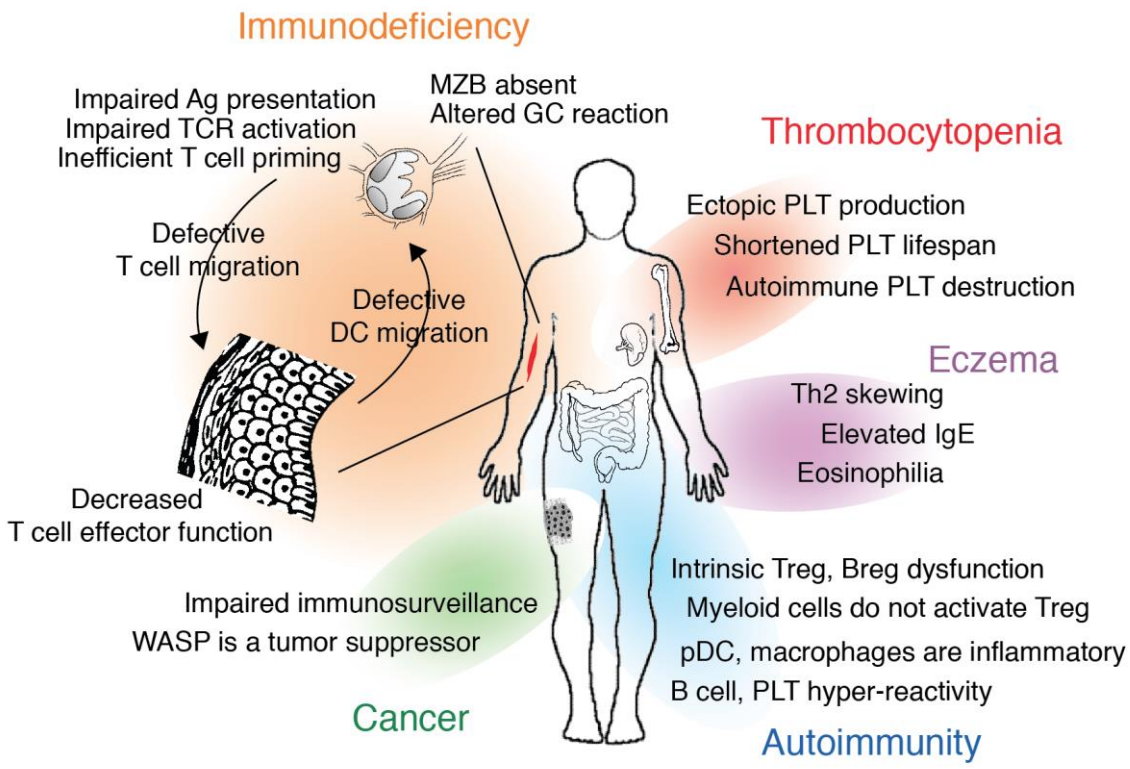
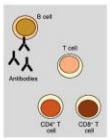
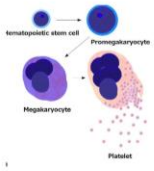


Figure 1: Mechanisms of disease in WAS. WAS-associated immunodeficiency is characterized by defective T cell priming and effector functions due to deficits in antigen presentation by APC, T cell activation through the TCR, and migration of T cells and APC. B cell responses are also defective because the GC reaction is impaired and MZB cells are absent. Thrombocytopenia results from accelerated platelet destruction as WAS platelets have intrinsically shortened lifespan, as well as from autoimmune attack. Newly generated platelets may also be trapped in the bone marrow space. Both impaired immunosurveillance and a cell-intrinsic role of WASP as a tumor suppressor are at the basis of the development of cancer, especially B cell lymphomas. Th2 skewing, elevated IgE titers and eosinophilia might explain the high incidence of eczema in WAS patients. Autoimmunity results from the cooperation of several defects including dysfunction in regulatory cells, excessive production of inflammatory cytokines by myeloid cells, and intrinsic hyper-reactivity of B cells and platelets.

Figure 2: Summary of WAS LV-GT outcome (to be replaced with new version by artist)





- Multilineage engraftment of gene corrected stem cells
- Restored Wasp expression
- Selective advantage for WASP+ cells

- Improved immune functions
  - T-cell proliferation
  - Treg function
  - Podosome formation
  - B-cell tolerance and development
  - Ig discontinuation and vaccinations

- Increased platelet counts
- Improved platelet structure and function (adehesion and aggregation)

- Reduced rate of severe infections
- Protection from severe bleeding
- Improved/resolved eczema
- Resolution/absence of autoimmunity in most patients
- No malignancy

References

1. Candotti F. Clinical Manifestations and Pathophysiological Mechanisms of the Wiskott-Aldrich Syndrome. J Clin Immunol. 2018;38:13–27.

2. Burroughs L, Petrovic A, Brazauskas R, Liu X, Griffith LM, Ochs HD, et al. Excellent Outcomes Following Hematopoietic Cell Transplantation for Wiskott-Aldrich Syndrome: A PIDTC Report. Blood. 2020;

3. Marangoni F, Bosticardo M, Charrier S, Draghici E, Locci M, Scaramuzza S, et al. Evidence for Long-term Efficacy and Safety of Gene Therapy for Wiskott–Aldrich Syndrome in Preclinical Models. Mol Ther. 2009;17:1073–82.

4. Cavazzana M, Bushman FD, Miccio A, André-Schmutz I, Six E. Gene therapy targeting haematopoietic stem cells for inherited diseases: progress and challenges. Nat Rev Drug Discov. 2019;18:447–62.

5. Hacein-Bey Abina S, Gaspar H, Blondeau J, Caccavelli L, Charrier S, Buckland K, et al. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA. 2015;313:1550–63.

6. Labrosse R, Chu J, Armant M, van der Spek J, Miggelbrink A, Fong J, et al. Outcome of Hematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich Syndrome. Blood. 2019;134:4629.

7. Ferrua F, Cicalese MP, Galimberti S, Giannelli S, Dionisio F, Barzaghi F, et al. Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS): Up to 8 Years of Follow up in 17 Subjects Treated Since 2010. Blood. 2019;134:3346.

8. Ferrua F, Cicalese MP, Galimberti S, Giannelli S, Dionisio F, Barzaghi F, et al. Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical

study. Lancet Haematol. 2019;6:e239–53.

9. Sereni L, Castiello MC, Di Silvestre D, Della Valle P, Brombin C, Ferrua F, et al. Lentiviral gene therapy corrects platelet phenotype and function in patients with Wiskott-Aldrich syndrome. J Allergy Clin Immunol. 2019;144:825–38.

10. Morris E, Fox T, Chakraverty R, Tendeiro R, Snell K, Rivat C, et al. Gene therapy for Wiskott-Aldrich syndrome in a severely affected adult. Blood. 2017;130:1327–35.