

UC Irvine

UC Irvine Previously Published Works

Title

Gene therapy for Wiskott-Aldrich syndrome: History, new vectors, future directions

Permalink

<https://escholarship.org/uc/item/4zn8f8bg>

Journal

The Journal of Allergy and Clinical Immunology, 146(2)

ISSN

0091-6749

Authors

Ferrua, Francesca

Marangoni, Francesco

Aiuti, Alessandro

et al.

Publication Date

2020-08-01

DOI

10.1016/j.jaci.2020.06.018

Peer reviewed

1 Brief review article – Paradigms and Perspectives

2

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

4

5 Francesca Ferrua^{1*} MD PhD, Francesco Marangoni^{2*} PhD, Alessandro Aiuti^{1,3#} MD PhD,
6 Maria Grazia Roncarolo^{4#} MD

7

8 ¹ San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) and Pediatric
9 Immunohematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific
10 Institute, Milan, Italy.

11 ² Department of Physiology and Biophysics and Institute for Immunology, University of
12 California, Irvine, CA, United States.

13 ³ Vita-Salute San Raffaele University, Milan, Italy.

14 ⁴ Division of Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics
15 and Institute for Stem Cell Biology and Regenerative Medicine, Stanford School of Medicine,
16 Stanford, CA, United States.

17

18 *equal first author contribution, # equal last author contribution

19

20 **Corresponding author's name:**

21 Maria Grazia Roncarolo, MD

22 Lorry I. Lokey Stem Cell Research Building

23 265 Campus Drive West, Room G3021A

24 Stanford, California 94305-5461

25 T: +1-650-498-0297

26 E-mail address: mg1@stanford.edu

27

28 **Keywords and abbreviations:**

29 Gene therapy (GT), Wiskott-Aldrich Syndrome (WAS), Lentiviral vector (LV), Hematopoietic
30 Stem/Progenitor Cells (HSPC), Reduced-Intensity Conditioning (RIC).

31

32 **Conflicts of interest:** FM is supported by grant NIH NIAMS R21 AR072849. The San Raffaele

33 Telethon Institute for Gene Therapy (SR-Tiget) is a joint venture between the Italian Telethon

34 Foundation and Ospedale San Raffaele (OSR). WAS gene therapy was licensed to

35 GlaxoSmithKline (GSK) in 2014 and then transferred Orchard Therapeutics (OTL) in April

36 2018. AA and FF are investigators of trial #NCT01515462.

37 **Introduction**

38 Wiskott-Aldrich Syndrome (WAS, OMIM 301000) is a severe X-linked disorder
39 characterized by thrombocytopenia, eczema, immunodeficiency, and increased risk of
40 autoimmunity and cancer. Affecting 1-10 males per million, WAS is caused by mutations in
41 the *WASp* gene, which lead to impaired or abolished expression of the WAS protein (WASP),
42 a hematopoietic-specific regulator of actin cytoskeleton remodeling. The severity of WAS is
43 scored based on the gravity of thrombocytopenia (score 0.5 to 1), eczema and
44 immunodeficiency (score 2 to 4), and presence of autoimmunity or malignancy (score 5)¹.

45 Historically, WAS has been treated with splenectomy and immunoglobulin replacement to
46 prevent infections, the former of which may improve platelet counts but further weakens
47 immunity. The gold standard treatment for WAS patients is hematopoietic stem/progenitor cell
48 (HSPC) transplantation (HSCT) from an HLA-identical donor².

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

57

58 **Preclinical data**

59 The pathophysiology of WAS has been studied using cells from WAS patients and two
60 independently generated *was*^{-/-} (*wko*) mouse strains displaying most features of WAS patients,
61 excluding severe thrombocytopenia. Knowledge of WAS pathophysiology (summarized in
62 Figure 1) was crucial in informing several features of clinical gene therapy.

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

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

71 Gene correction using a self-inactivating lentiviral vector (LV) to drive expression of WASp
72 cDNA controlled by a 1.6kb (w1.6W) endogenous WAS promoter restored WASP expression
73 in T, B, and CD34⁺ cells from patients. It also corrected T-cell dysfunction, DC cytoskeletal
74 abnormalities, and thrombocytopenia in wko mice treated with non-myeloablative irradiation
75 and GT³. LV transduced CD34⁺ cells retained the ability to engraft and differentiate in
76 immunodeficient mice. The w1.6W LV did not cause tumors in GT-treated mice that were
77 followed up on for a year, nor in recipients of secondary transplantation³, establishing its safety
78 in preclinical models.

79

80 **Clinical gene therapy**

81 The proof of concept of GTs efficacy in WAS patients was provided by a clinical trial using a
82 γ -RV bearing a strong viral promoter. Long-term engraftment of RV-transduced HSPC led to
83 restoration of WASP expression and improved platelet count and T-cell function, resulting in
84 clinical amelioration of disease phenotype. However, 9/10 patients for which GT was
85 successful developed acute leukemia due to RV integrations close to oncogenes and
86 activation of their expression⁴, including LMO2. This further prompted the need for viral vectors
87 with better safety profiles.

88 Various clinical trials based on LV-engineered autologous HSPC began in 3 centers in
89 Europe (SR-Tiget in Milan, Great Ormond Street Hospital in London, Necker Children's
90 Hospital in Paris) and in 1 center in the US (Boston Children's Hospital) (Table 1). The LV and
91 transduced CD34+ cells were manufactured at different sites, but vector design was the same.
92 Treatment consisted of a single infusion of LV-transduced autologous bone marrow or
93 mobilized peripheral blood-derived CD34+ cells after conditioning. SR-Tiget adopted a
94 reduced intensity-conditioning regimen (RIC) to minimize toxicity and fully exploit the selective
95 growth advantage of gene corrected cells, while the other centers adopted a more intense
96 regimen (Table 1). 34 WAS patients (Zhu score: 3-5) were treated worldwide, with a median
97 follow up ranging from 3.3 to 7.8 years, depending on the center⁵⁻⁸ (Table 1). Three out of 34
98 patients died of morbidities unrelated to the GT product (Table 1). No severe GT-related
99 adverse events occurred and no treated patients developed clonal selection, insertional
100 mutagenesis, leukemia, or replication-competent LV to date.

101 All surviving patients (31/34, 91%) had sustained multi-lineage engraftment of gene-
102 corrected cells, with higher gene marking and WASP expression in T cells and other lymphoid
103 cells, consistent with their strong selective advantage. Despite the use of a RIC, sustained
104 and robust *in vivo* BM engraftment of gene corrected progenitors (median 49%, range 22-
105 85%) was achieved⁷. Conditioning is not the only factor influencing engraftment since patients
106 who received a more myeloablative regimen reached a VCN of 0.01 to 0.4 (equivalent to 1%
107 to 40%) in myeloid cells⁵. Even in the presence of variable levels of reconstitution, immune
108 function improved enough to provide a clinical benefit with reduced severe infection rate.
109 Humoral immune deficiency ameliorated, allowing for discontinuation of immunoglobulin
110 supplementation in several patients. All subjects showed improvement or resolution of
111 eczema. Platelet count variably improved after GT, but remained below normal range in most
112 patients. Amelioration of thrombocytopenia resulted in protection from severe bleeding, as
113 well as freedom from transfusions and TPO agonists (Figure 2). This may also be a result of
114 improved platelet function and phenotype after treatment⁹. Autoimmunity improved after GT^{5,8},

115 possibly due to restoration of normal T regulatory cell function and B-cell tolerance. However,
116 in contrast with the results of other centers, two subjects treated in Boston with pre-existing
117 autoimmunity had no resolution after-GT, in association with poor recovery of lymphocytes,
118 including Tregs⁶.

119 Although most initially treated patients were children, clinical benefit has now been
120 demonstrated in older subjects (overall age range: 0.8-35.1 years), who are considered at
121 higher risk when treated with allogeneic HSCT^{7,10}.

122

123 **Current challenges and future directions**

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

139 In contrast to the long-lasting experience with HSCT in WAS, there is limited information on
140 the very long-term safety of GT (>10 years). As of today, no patient treated with LV-GT has

141 developed malignancies, the longest follow up being 8.8 years. Despite this timeline being
142 well beyond the reported time of occurrence of leukemia in the RV trial (range: 1.3-5 years),
143 life-long monitoring of all LV-treated patients will be crucial.

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

151

152

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 “x10³ ng x h/mL”.

[^]1n 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)

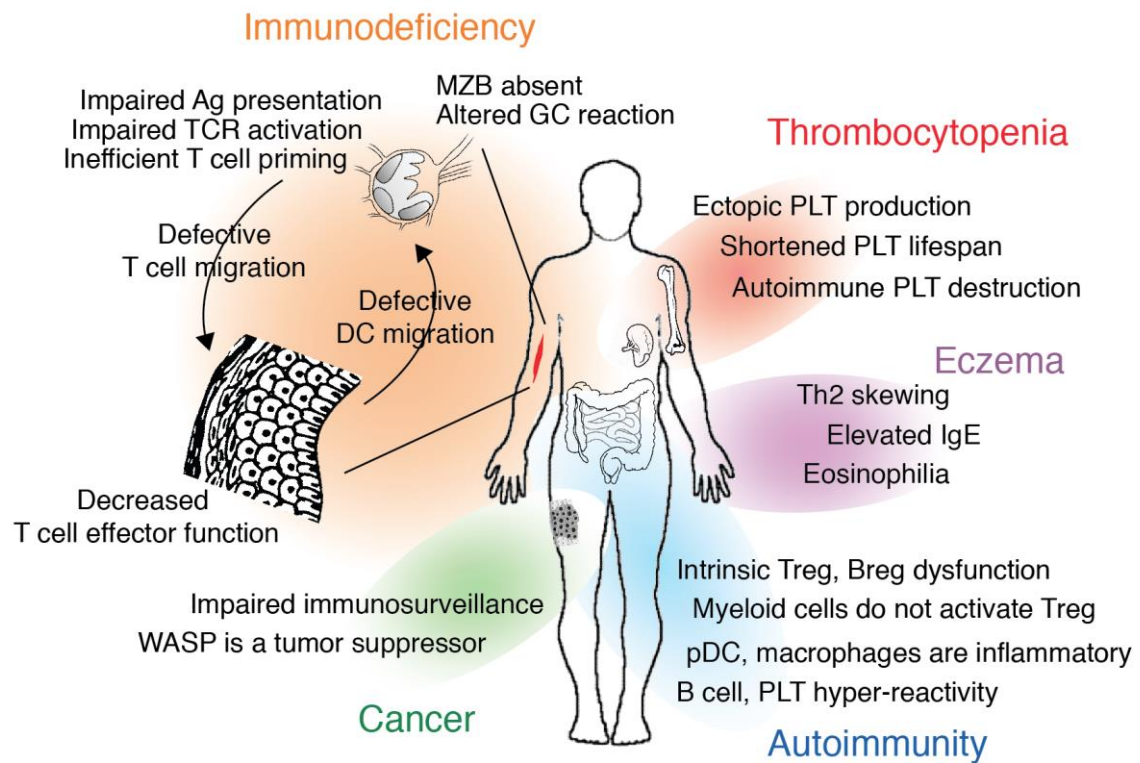
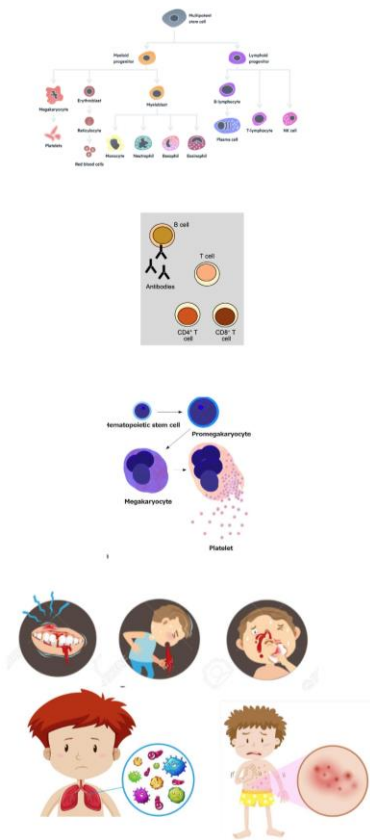


Figure 1: Mechanisms of disease in WAS. WAS-associated immunodeficiency is characterized by defective T cell priming and effector functions due to deficits in antigen presentation by APC, T cell activation through the TCR, and migration of T cells and APC. B cell responses are also defective because 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 (adhesion 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.