

Gene therapy for the Wiskott–Aldrich syndrome

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Background

Wiskott–Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency (PID) characterized by micro-thrombocytopenia, recurrent infections, eczema, which is associated with a high incidence of auto-immunity and lymphoreticular malignancy. One of the first diseases to be successfully treated by allogeneic hematopoietic stem cell transplantation, WAS is currently the subject of several phase I/II gene therapy trials for patients without HLA-compatible donors.

Purpose of review

This article reviews the preclinical and clinical data leading to the development of gene therapy of WAS with lentiviral vectors.

Recent findings

A recent clinical trial using a conventional gammaretroviral vector has demonstrated the proof of principle of gene therapy in WAS, but has also highlighted a common limitation of the technology. Encouraging preclinical efficacy and safety results using refined lentiviral vectors, and the development of robust clinical-grade manufacturing processes have supported the initiation of several phase I/II new studies.

Summary

WAS is amenable to hematopoietic stem cell gene therapy. New trials using lentiviral vectors are expected to improve efficacy and safety profiles. Beyond proof of principle, ongoing international efforts to coordinate trials of gene therapy for the WAS may also provide a model for the expedited development of new treatments for other rare diseases.

Keywords

gene therapy, hematopoietic stem cells, lentiviral vector, rare disease, Wiskott–Aldrich syndrome

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Introduction

Wiskott–Aldrich syndrome (WAS) (OMIM 301000) is a rare X-linked immunodeficiency caused by mutations in a single gene, *WAS* coding for the Wiskott–Aldrich syndrome protein (WASp). At present over 295 unique mutations have been reported in the *WAS* gene (http://rapid.rci.riken.jp/RAPID/mutation?pid_id=AGID_144) resulting in several overlapping but distinct phenotypes [1,2]. The classical WAS is a primary immunodeficiency disease (PID) characterized by micro-thrombocytopenia, recurrent infections, eczema and associated with a high incidence of auto-immunity and of lymphoid malignancies. WAS is found in a wide geographic area and in many diverse ethnic groups. The overall incidence is estimated to be 4.0 per million live male births. Mutations of the *WAS* gene also cause a milder allelic variant that has been described as hereditary X-linked thrombocytopenia (XLT) (OMIM 313900), or occasionally intermittent X-linked thrombocytopenia (IXLT). Whereas mutations causing WAS or XLT result in absent or

severely diminished expression of WASp, extremely rare *WAS* gain-of-function gene mutations are now recognized to cause X-linked neutropenia (XLN) (OMIM 300299), without the platelet defect or any of the other findings characteristic for WAS/XLT. A simple clinical scoring system has been developed to delineate these strikingly different clinical phenotypes, and to evaluate the variable severity in patients with loss of function gene mutations [3].

WAS in its classical form is a life-threatening illness in the absence of definitive therapy [4]. Fatal complications may arise from hemorrhage (particularly when the intrinsic thrombocytopenia is complicated by super-added autoimmunity), infection, and lymphoreticular disease. Clinical manifestations suggestive of WAS or XLT are often present at birth and consist of petechiae, bruising, and bloody diarrhea. Eczema is a common feature of classical WAS during infancy and childhood. Frequent bacterial and viral infections reflect the presence of complex multilineage immunodeficiency [5,6].

Patients with XLT have fewer problems with eczema, infections or auto-immunity [7] and have an overall survival rate which is close to that of the normal male population.

The WAS gene encodes the WASp which is a hematopoietic-specific member of a family of cytoskeletal regulators (reviewed by [8–10]). WASp is found in the cytoplasm of all leukocytes in which it acts essentially as an actin nucleation factor through the Arp2/3 complex, causing dynamic cytoskeletal changes that are essential for multiple cellular functions including adhesion, migration, phagocytosis, immune synapse formation, and receptor-mediated cellular activation processes. These are important for many immune cell types in the context of pathogen recognition and elimination, and also for maintenance of homeostatic mechanisms, including regulatory T-cell function. Therefore, the lack of WASp impairs a wide variety of cellular functions that are involved in both innate and cellular adaptive immunity. The origin of the microthrombocytopenia which is a universal feature of the WAS and XLT phenotypes is not fully understood, although there is likely to be a contribution from defective platelet formation in the bone marrow in addition to excessive destruction of platelets in the spleen.

Rationale supporting the development of a novel treatment for the Wiskott–Aldrich syndrome disease

A combination of clinical observations, molecular genetics and biochemical tests is used to identify WAS patients with severe disease or with high probability of developing severe disease and who need treatment. A phenotype/genotype correlation exists when patients are divided into WASp-positive (+) patients expressing a mutated protein of normal size in their blood cells, even at low levels and WASp negative patients with absent or truncated protein [4]. With some exceptions, WASp+ patients have the less severe phenotype (predominantly XLT), whereas WASp-negative patients are more likely to have classical disease.

The first successful demonstration that allogeneic hematopoietic stem cell transplantation (HSCT) could be used to cure inherited hematological diseases was obtained almost simultaneously in two patients with WAS and a severe combined immunodeficiency (SCID) more than 40 years ago [11]. Nowadays, HSCT is considered a standard curative procedure for WAS, although success is primarily dependent on the availability of human leukocyte antigen (HLA)-matched donors and presence of a low clinical score at the time of treatment (reviewed by [12]). Long-term outcome of HSCT in WAS has consistently revealed that HLA-mismatched HSCT for

Key points

- Patients with severe Wiskott–Aldrich syndrome (WAS) without HLA-compatible donors are candidates for treatment by gene therapy.
- Defined objective criteria for disease severity and prognosis enable the development of novel treatment options for WAS.
- Conventional gammaretroviral vectors have demonstrated the feasibility of hematopoietic gene therapy in WAS but with technological limitations.
- Lentiviral vectors derived from HIV-1 have a satisfactory efficacy and safety profile in preclinical studies, supporting testing in phase I/II clinical trials.
- International multicenter gene therapy studies in WAS represent a model to accelerate the development of treatments for rare disease.

this condition is associated with substantial morbidity and mortality, and that mixed chimerism may be a predictor of future complications [13•].

Natural correction of WAS mutations through spontaneous ‘somatic reversions’ have been frequently reported in WAS patients. Up to 11% of WAS patients have presented with somatic mosaicism due to reversion to normal of the original mutation or alternatively with second-site compensatory mutations that restore production of a functional WAS gene product. The vast majority of these events occur in T cells [14], although they have also been reported in B cells and natural killer (NK) cells. In general, however, reversions do not provide significant therapeutic benefit and to date no reversions have been detected in myeloid cells suggesting that they are occurring in late progenitors or mature cells [15].

The WAS is therefore a relatively well characterized entity at the clinical and molecular level, also in terms of natural evolution of the disease and outcome of treatment by HSCT or more conservative strategies. Objective criteria exist to provide a reasonable evaluation of disease severity and prognosis which are important elements for therapeutic development and allow reasonable evaluation of risks and benefits to the patient. Patients likely to benefit most from development of gene therapy (at least in initial trials) are those without HLA-matched donors and with mutations predictive of severe disease or with high clinical scores. The fact that XLT patients have minimal immunological disturbance in the presence of low levels of WASp activity, and that WASp expression is associated with a survival advantage particularly for mature lymphocytes suggests that even low level correction may be therapeutically effective. Proof of concept for the feasibility and efficacy of gene transfer has been obtained in various models of the WAS disease and recently in clinical trials.

Table 1 Referenced hematopoietic gene therapy studies conducted for primary immune deficiencies in the past 10 years

Disease	Type of vector	Centers	No. of patients	Efficacy	Toxicity	References
ADA-SCID	Gamma retroviral vector LTR-driven transgene expression	Italy, UK, US, Japan	36	Yes for most patients receiving conditioning prior to gene therapy	No	[16,17]
SCID-X1	Gamma retroviral vector LTR-driven transgene expression	France, UK, US	22	Yes (patients <10 year old)	Yes, insertional mutagenesis in 5 patients	[18–21]
CGD	Gamma retroviral vector, LTR-driven transgene expression	Germany, UK, Switzerland, US, South Korea	17	Initial benefit only, 1 patient died of sepsis	Yes, insertional mutagenesis in 3 patients	[22–26]
Wiskott–Aldrich syndrome	Gamma retroviral vector, LTR-driven transgene expression	Germany	10 included, 2 reported	Yes	Yes, insertional mutagenesis in 1 patient	[27**]

LTR, long-terminal repeat.

First trials of gene therapy for Wiskott–Aldrich syndrome

For more than 10 years now, gene-corrected autologous hematopoietic stem cells have been used as an alternative to HLA mis-matched HSCT, to treat certain bone marrow diseases and more recently WAS (Table 1) [16–26,27**]. Gammaretroviral vectors derived from Moloney leukaemia virus (MLV) and expressing a therapeutic transgene from the retroviral 5' long-terminal repeat (LTR) were the first effective gene transfer vectors developed for these clinical applications. Such vectors were successfully used to treat patients with X-linked SCID, adenosine deaminase deficiency (ADA-SCID), chronic granulomatous disease (CGD) and also more recently WAS (see Table 1). Gene therapy has been highly successful for restoration of T-cell homeostasis and T-cell function in SCID patients, follow-up in some patients now being more than 10 years [16–19].

Gene therapy for WAS has been attempted more recently and presents additional challenges, notably the need to correct multiple lineages of cells including platelets. A first phase I/II study [27**] conducted in Hannover included 10 patients treated with a WASp-expressing LTR-driven gammaretroviral vector following myelosuppressive conditioning. The 2.5 years' follow-up results for the two first patients treated with this approach were recently reported, demonstrating stable engraftment of gene-corrected cells in multiple lineages of cells but with a clear advantage for corrected lymphoid cells. Biological parameters were partially restored. Platelet cell numbers increased, leukocyte function was restored as shown by TCR-induced proliferative capacity in T cells, NK cell cytolytic function and dendritic cell cytoskeletal dynamics. In addition, eczema was ameliorated in one patient. Immunoglobulin levels were not fully normalized but both patients were successfully vaccinated against tetanus, diphtheria and hemophilus influenza. This pioneering trial demonstrates the feasibility of

hematopoietic gene transfer in WAS patients, and also confirms that the success of gene therapy in this condition is facilitated by a selective advantage of gene-corrected lymphocytes (as observed in SCID patients previously treated by similar methodologies).

Drawbacks of long-terminal repeat-driven gammaretroviral vectors

In spite of the initial success of the Hannover trial, vector-induced T-cell leukemia has subsequently occurred in one of the cohorts (see press release from Hannover Medical School, http://www.asgct.org/UserFiles/file/Genethrapy_WAS_final_english.pdf). Vector insertion was found near the T-cell oncogene LMO2, which is reminiscent of other cases of vector-induced T-cell leukemias that have been reported in SCID-X1 patients [20,21]. The process of vector-induced insertional mutagenesis is still incompletely understood, but several factors seem to contribute to provide the first hit that ultimately leads to cell transformation [28]. The presence of strong viral enhancers in the vector and the insertional preferences of the vector are thought to contribute by influencing the expression of genes in the host genome. Cellular factors and replicative stress may also be involved [29]. As well as their propensity to dysregulate host gene expression, LTR-driven gammaretroviral vectors are susceptible to epigenetic silencing leading to loss of therapeutic efficacy, as observed in patients with chronic granulomatous disease treated by gene therapy [26]. From the pharmaceutical viewpoint, gammaretroviral vectors are relatively poorly characterized starting materials. Vectors are produced in unpurified cell culture supernatants harvested from packaging cell lines. Multiple rounds of infection of hematopoietic cells in the presence of cell division-inducing cytokines are needed to achieve high levels of gene transfer with MLV vectors, and are associated with a loss of long-term engrafting multipotent HSCs. These issues have prompted several technological advances in the field of

gene therapy. High-performance techniques have been developed to rapidly identify genomic insertion sites of the vector and to detect clonal dominance in patients [30]. Novel preclinical assays have been developed to model the risk of insertional mutagenesis [31]. Finally and most significantly, vector design and production standards have been improved as reviewed recently [32].

Safer and more efficient vector technology for Wiskott–Aldrich syndrome gene therapy

Taking advantage of newer technology in gene transfer vectors, we and others have developed a recombinant HIV-1-derived (rHIV) lentiviral vector for the gene therapy for WAS [33]. Vectors derived from HIV-1 present several advantages in terms of efficiency and lack of dependence on cell division for transduction. As a consequence, cell culture periods can be minimized, and HSC activity can be more easily preserved during the transduction process. Potential interference between the integrated vector and neighboring gene expression can be substantially reduced by the use of a self-inactivated (SIN) design in which viral enhancers and promoters in the retroviral LTR are deleted [34]. In SIN vectors, the gene of interest is expressed from an internal promoter which can be chosen to be a physiological promoter allowing endogenous gene regulation and minimizing the risk of insertional mutagenesis [35]. The genomic integration profile of HIV-derived vectors is also possibly more favorable than that of MLV in terms of likelihood of activating proto-oncogenes [36]. For WAS, we employed a 1.6 kb native *WAS* gene proximal promoter sequence to express the *WAS* full cDNA in hematopoietic-restricted manner (as is naturally the case for the *WAS* gene). A modified woodchuck post-transcriptional regulatory element (WPRE) sequence was incorporated in the vector to enhance expression of the *WAS* transgene [37]. The vector was pseudotyped with vesicular stomatitis virus G glycoprotein (VSVg) which has several advantages, in particular a broad tropism and adequate stability from a manufacturing stand-point. Since high concentrations of rHIV-1 vector particles are usually required for efficient gene transfer in primary cells the stability of VSVg-pseudotyped rHIV vectors enables their purification through removal of about 3 log₁₀ values of protein and DNA contaminants

from the culture medium harvested from producer cells [38[•]]. The purification of HIV-1-derived VSVg-pseudotyped *WAS* vectors was successfully implemented for manufacture according to good manufacturing process (GMP) standards.

Preclinical data supporting the use of lentiviral vectors for treatment of Wiskott–Aldrich syndrome

The *WAS* rHIV vector described above was found to be efficacious and safe in preclinical studies. Human CD34+ cells were transduced efficiently without toxicity and using clinically applicable protocols. In mice, ex-vivo HSC gene therapy resulted in significant levels of engraftment of *WAS*Sp-expressing T cells, B cells, platelets, and myeloid cells [39,40[•]]. T cells harboring one or two vector copies displayed partial to full correction of T-cell receptor-driven IL-2 production and proliferation. Polymerization of F-actin and localization of *WAS*Sp at the site of the immunological synapse was restored. Defects of dendritic cell architecture were corrected and numbers of splenic B cells were partially restored [37]. Interestingly, a large proportion of *WAS*Sp-deficient mice develop diarrhea as a result of an inflammatory colitis. In animals successfully engrafted with gene corrected cells, the incidence and severity of colitis were markedly reduced or eliminated [37]. The treatment was well tolerated in mice, and engraftment of transduced long-term repopulating HSC was documented with no adverse pathology detected by systematic blood analysis and autopsy after long observation periods [40[•]]. The vector genomic insertion profile conformed to that expected from rHIV-derived vectors [41], and there was no evidence for toxicity arising from insertional mutagenesis or unexpected effects of transgene expression *in vivo* [40[•]] or *in vitro* [42[•]].

Current gene therapy trials for Wiskott–Aldrich syndrome

Encouraging results in preclinical studies, together with the development of processes to manufacture lentiviral vectors under defined GMP conditions, have supported the initiation of several phase I/II gene therapy trials with the *WAS* rHIV vector (Table 2). Currently, three trials are open in London, Paris and Boston to treat *WAS* patients with a similar protocol involving the same source of vector. A fourth similar study is ongoing in Milan to

Table 2 Current ongoing phase I/II gene therapy studies in Wiskott–Aldrich syndrome

Centers	No. of patients to be included	Vector	Vector manufacture	Sponsor of study	Date of start trial
UK (London)	5	Lentiviral vector 1.6 kb <i>WAS</i> promoter VSVg pseudotype	Genethon	Genethon	2009
France (Paris)	5		Genethon	Genethon	2010
US (Boston)	5		Genethon	DA Williams (supported NHLBI)	2011
Italy (Milan)	6		MolMed	Fondazione Telethon	2010

VSVg, virus G glycoprotein; *WAS*, Wiskott–Aldrich syndrome.

test the same vector configuration but from a different manufacturing source. This multicenter approach is designed to facilitate the accrual of data with the same product in a rare disease indication such as WAS. These trials include patients with severe WAS and lacking alternative treatment options. Autologous CD34+ hematopoietic cells are collected from bone marrow or peripheral blood following mobilization. During ex-vivo transduction of these cells, patients undergo a cytoreductive and immunosuppressive conditioning based on fludarabine and busulfan administered over 3 days. The transduced cells are immediately reinfused and patients are monitored at regular intervals for at least 2 years. These phase I/II studies are designed to demonstrate safety, restoration of multilineage immunological function, and correction of microthrombocytopenia. A fully successful treatment of WAS by gene therapy is also expected to improve the overall health of the patient in terms of augmented immunity, reduction in frequency of infections, resolution of autoimmunity, improvement in eczema, and reduction in bruising and bleeding episodes. The level of conditioning required for effective engraftment of transduced HSC, and the level of chimerism (corrected versus non-corrected cells) resulting in clinical recovery will require long-term follow-up but at least for lymphocytes will benefit from selective advantage imparted to mature cells in particular.

Conclusion

The WAS is a well characterized PID which is amenable to cure by allogeneic HSCT if suitable HLA-compatible donors are available. However, outcome for patients without good donors remains suboptimal, and has driven the development of alternative therapies based on gene transfer. The first clinical trial in WAS using a gammaretroviral vector has shown that in spite of the severity and complexity of this disease, restoration of cellular function and immunological mechanisms is feasible. However, this and similar studies in other hematological disorders have highlighted limitations in technology and the need to develop enhanced methods. Multicenter international gene therapy trials for WAS are ongoing using a physiologically regulated lentiviral vector following extensive preclinical development. The aims of these studies are to implement high standards in vector safety and product quality ultimately leading to registration of a novel treatment for classical WAS patients. If safety is verified, a similar strategy may be equally applicable to milder forms of the disease. Results obtained in WAS are therefore expected to move forward the field of gene therapy beyond proof-of-concept studies and towards the realization of gene therapy as a recognized treatment for a broad spectrum of genetic diseases.

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Conflicts of interest

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There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Jin Y, Mazza C, Christie JR, *et al.* Mutations of the Wiskott–Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood* 2004; 104:4010–4019.
- 2 Ochs HD. Mutations of the Wiskott–Aldrich syndrome protein affect protein expression and dictate the clinical phenotypes. *Immunol Res* 2009; 44:84–88.
- 3 Zhu Q, Zhang M, Blaese RM, *et al.* The Wiskott–Aldrich syndrome and X-linked congenital thrombocytopenia are caused by mutations of the same gene. *Blood* 1995; 86:3797–3804.
- 4 Imai K, Morio T, Zhu Y, *et al.* Clinical course of patients with WASP gene mutations. *Blood* 2004; 103:456–464.
- 5 Ochs HD, Filipovich AH, Veys P, *et al.* Wiskott–Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. *Biol Blood Marrow Transplant* 2009; 15 (1 Suppl):84–90.
- 6 Thrasher AJ. New insights into the biology of Wiskott–Aldrich syndrome (WAS). *Hematol Am Soc Hematol Educ Program* 2009; 132–138.
- 7 Albert MH, Notarangelo LD, Ochs HD. Clinical spectrum, pathophysiology and treatment of the Wiskott–Aldrich syndrome. *Curr Opin Hematol* 2010. Epublication
- 8 Blundell MP, Worth A, Bouma G, Thrasher AJ. The Wiskott–Aldrich syndrome: the actin cytoskeleton and immune cell function. *Dis Markers* 2010; 29:157–175.
- 9 Thrasher AJ, Burns SO. WASP: a key immunological multitasker. *Nat Rev Immunol* 2010; 10:182–192.
- 10 Bouma G, Burns SO, Thrasher AJ. Wiskott–Aldrich syndrome: immunodeficiency resulting from defective cell migration and impaired immunostimulatory activation. *Immunobiology* 2009; 214:778–790.
- 11 Good RA. Bone marrow transplantation for immunodeficiency diseases. *Am J Med Sci* 1987; 294:68–74.
- 12 Pai SY, Notarangelo LD. Hematopoietic cell transplantation for Wiskott–Aldrich syndrome: advances in biology and future directions for treatment. *Immunol Allergy Clin North Am* 2010; 30:179–194.
- 13 Moratto D, Giliiani S, Bonfim C, *et al.* Long-term outcome and lineage-specific chimerism in 194 Wiskott–Aldrich syndrome patients treated by hematopoietic cell transplantation between 1980–2009: an international collaborative study. *Blood* 2011. [Epub ahead of print]
- Important study showing long-term outcome in a large cohort of patients. Although outcome has improved progressively, mixed chimerism was shown to associate with persistence of thrombocytopenia, and also with post-transplantation autoimmunity.
- 14 Davis BR, Candotti F. Revertant somatic mosaicism in the Wiskott–Aldrich syndrome. *Immunol Res* 2009; 44:127–131.
- 15 Stewart DM, Candotti F, Nelson DL. The phenomenon of spontaneous genetic reversions in the Wiskott–Aldrich syndrome: a report of the workshop of the ESID Genetics Working Party at the XIth Meeting of the European Society for Immunodeficiencies (ESID). Budapest, Hungary October 4–7, 2009. *J Clin Immunol* 2007; 27:634–639.
- 16 Aiuti A, Cattaneo F, Galimberti S, *et al.* Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009; 360:447–458.
- 17 Gaspar HB, Cooray S, Gilmour KC, *et al.* Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction. *Sci Transl Med* 2011; 3:97ra79.

6 Primary immune deficiency disease

- 18 Hacein-Bey-Abina S, Hauer J, Lim A, *et al.* Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010; 363:355–364.
- 19 Gaspar HB, Cooray S, Gilmour KC, *et al.* Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. *Sci Transl Med* 2011; 3:97ra80.
- 20 Hacein-Bey-Abina S, Garrigue A, Wang GP, *et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008; 118:3132–3142.
- 21 Howe SJ, Mansour MR, Schwarzwaelder K, *et al.* Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008; 118:3143–3150.
- 22 Malech H, Maples PB, Whiting-Theobald N, *et al.* Prolonged production of nadph oxidase-corrected granulocytes after gene therapy of chronic granulomatous disease. *Proc Natl Acad Sci U S A* 1997; 94:12133–12138.
- 23 Ott MG, Schmidt M, Schwarzwaelder K, *et al.* Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat Med* 2006; 12:401–409.
- 24 Kang HJ, Bartholomae CC, Paruzynski A, *et al.* Retroviral gene therapy for x-linked chronic granulomatous disease: results from phase I/II trial. *Mol Ther*. 2011. [Epub ahead of print]
- 25 Kang EM, Choi U, Theobald N, *et al.* Retrovirus gene therapy for x-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. *Blood* 2010; 115:783–791.
- 26 Stein S, Ott MG, Schultze-Strasser S, *et al.* Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nat Med* 2010; 16:198–204.
- 27 Boztug K, Schmidt M, Schwarzer A, *et al.* Stem-cell gene therapy for the • Wiskott–Aldrich syndrome. *N Engl J Med* 2010; 363:1918–1927.
Proof-of-principle demonstration that hematopoietic gene therapy is biologically and clinically effective in patients. This reports outcome on the first two treated, although subsequently development of T-ALL has been reported in one of the cohort of 10 treated in this study.
- 28 Baum C. Parachuting in the epigenome: the biology of gene vector insertion profiles in the context of clinical trials. *EMBO Mol Med* 2011; 3:75–77.
- 29 Ginn SL, Liao SH, Dane AP, *et al.* Lymphomagenesis in SCID-X1 mice following lentivirus-mediated phenotype correction independent of insertional mutagenesis and gammac overexpression. *Mol Ther* 2010; 18:965–976.
- 30 Paruzynski A, Arens A, Gabriel R, *et al.* Genome-wide high-throughput integrome analyses by nrLAM-PCR and next-generation sequencing. *Nat Protoc* 2010; 5:1379–1395.
- 31 Modlich U, Bohne J, Schmidt M, *et al.* Cell-culture assays reveal the importance of retroviral vector design for insertional genotoxicity. *Blood* 2006; 108:2545–2553.
- 32 Dropulic B. Lentiviral vectors: their molecular design, safety, and use in laboratory and preclinical research. *Hum Gene Ther* 2011; 22:649–657.
- 33 Galy A, Roncarolo MG, Thrasher AJ. Development of lentiviral gene therapy for Wiskott–Aldrich syndrome. *Expert Opin Biol Ther* 2008; 8:181–190.
- 34 Zufferey R, Dull T, Mandel RJ, *et al.* Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol* 1998; 72:9873–9880.
- 35 Zychlinski D, Schambach A, Modlich U, *et al.* Physiological promoters reduce the genotoxic risk of integrating gene vectors. *Mol Ther* 2008; 16:718–725.
- 36 Cattoglio C, Pellin D, Rizzi E, *et al.* High-definition mapping of retroviral integration sites identifies active regulatory elements in human multipotent hematopoietic progenitors. *Blood* 2010; 116:5507–5517.
- 37 Zanta-Boussif MA, Charrier S, Brice-Ouzet A, *et al.* Validation of a mutated PRE sequence allowing high and sustained transgene expression while abrogating WHV-X protein synthesis: application to the gene therapy of WAS. *Gene Ther* 2009; 16:605–619.
- 38 Merten OW, Charrier S, Laroudie N, *et al.* Large scale manufacture • and characterisation of a lentiviral vector produced for clinical ex vivo gene therapy application. *Hum Gene Ther* 2011; 22:343–356.
Development of robust clinical grade manufacturing techniques for a lentiviral vector used in current WAS gene therapy trials.
- 39 Dupre L, Marangoni F, Scaramuzza S, *et al.* Efficacy of gene therapy for Wiskott–Aldrich syndrome using a WAS promoter/cDNA-containing lentiviral vector and nonlethal irradiation. *Hum Gene Ther* 2006; 17:303–313.
- 40 Marangoni F, Bosticardo M, Charrier S, *et al.* Evidence for long-term efficacy • and safety of gene therapy for Wiskott–Aldrich syndrome in preclinical models. *Mol Ther* 2009; 17:1073–1082.
Demonstration of effective lentiviral-mediated gene therapy for WAS in an animal model.
- 41 Mantovani J, Charrier S, Eckenberg R, *et al.* Diverse genomic integration of a lentiviral vector developed for the treatment of Wiskott–Aldrich syndrome. *J Gene Med* 2009; 11:645–654.
- 42 Modlich U, Navarro S, Zychlinski D, *et al.* Insertional transformation of • hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. *Mol Ther* 2009.
Demonstration that SIN vector configurations are associated with reduced mutagenesis *in vitro*.