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ABSTRACT

Background

Wiskott Aldrich Syndrome (WAS) is a rare X-linked primary immunodeficiency. This complex disease is characterised by micro-thrombocytopenia, recurrent infections, eczema and is associated with a high incidence of auto-immunity and of lymphoid malignancies. WAS is attracting growing attention not only because it highlights the rich cellular- and systems-biology revolving around cytoskeletal regulation but also because it is candidate for a hematopoietic stem cell gene therapy indication.

Objectives

As several groups are developing this novel approach, the present review will discuss the state-of-the art and challenges in clinical development of gene therapy for WAS, with particular regard to biosafety.

Methods and results

In spite of the current successes of hematopoietic gene therapy for genetic immune deficiencies, there is a need for more efficient transduction protocols and for vectors with a superior safety profile. Preclinical studies have provided reasonable expectations that hematopoietic gene therapy with a self-inactivated HIV-1-derived vector utilising the native gene promoter for expression of the *WAS* transgene will be safe and will lead to the restoration of WASp in the hematopoietic and immune system at levels sufficient to provide an improvement in the condition of WAS patients.

Conclusions

Phase I/II clinical studies will soon be initiated in several European centres to assess the safety and efficacy of this lentiviral vector in WAS patients.

ABBREVIATIONS

WAS: Wiskott Aldrich Syndrome
OMIM: Online Mendelian Inheritance in Man
WASp: Wiskott Aldrich Syndrome Protein
EBV: Epstein-Barr virus
XLT: X-linked thrombocytopenia
XLN: X-linked neutropenia
NK: natural killer cells
Arp2/3 complex: actin-related proteins 2 and 3 complex
BMT: bone marrow transplantation
MLV: Moloney Leukaemia Virus
SCID-X1: X-linked severe combined immunodeficiency
ADA-SCID: adenosine deaminase deficiency
CGD: chronic granulomatous disease
EMEA: European Medical Agency
LTR: long-terminal repeats

INTRODUCTION

In the arena of biological therapies, there is growing interest for so-called "hematopoietic gene therapy". This procedure is used to correct genetic blood or immune diseases by integrating a therapeutic gene into the hematopoietic system through autologous bone marrow transplantation. According to pharmaceutical paradigms, hematopoietic gene therapy has already undergone several cycles of evolution. The first clinical attempts of the early 1990s in primary immune deficiencies were ineffective but they have led to vector improvements and to the use of new clinical protocols. Studies became clinically successful ten years later but also revealed the unexpected complications of insertional mutagenesis [1]. Nowadays, a new generation of safer self-inactivating vectors is entering the clinic with more varied applications. Several studies are ongoing in Europe and United States with self-inactivating HIV-derived lentiviral vectors to treat HIV infection, neurodegenerative disease, or thalassemia. A new candidate disease to be treated by an advanced lentiviral vector is Wiskott Aldrich syndrome (WAS). We have designed a therapeutic lentiviral vector which has obtained an Orphan Drug designation from the European Medical Agency (EMEA) in December 2005. This vector is currently in development for clinical studies that are planned in several European countries. Independently, Germany has approved the first gene therapy study in WAS using a conventional gamma-retroviral vector. Multiple approaches for the same disease are not uncommon in gene therapy given the preliminary stage of the field. However, integrative vectors induce a permanent modification of the genome of somatic cells and their safety profiles must be evaluated carefully, especially when used in children. To provide the reader with a better understanding of issues at stake in the gene therapy for WAS, this review will therefore present the background information in the pathophysiology of the WAS disease and the preclinical data that have led to vector development. State-of the art knowledge in the field of integrative vectors will be discussed and we will argue that self-inactivating lentiviral vectors present several safety advantages for the treatment of WAS.

WISKOTT ALDRICH SYNDROME: CURRENT KNOWLEDGE

The disease Wiskott Aldrich Syndrome was listed as a primary immuno-deficiency about 45 years ago, and is named after two physicians in Germany and United States who had earlier described the family inheritance and clinical features of this condition. About 13 years ago, genetic studies determined that the disease was caused by mutations in the *WAS* gene [2]. The gene was mapped to the X chromosome at Xp11.23-p11.22. Nowadays we know that a variety of mutations in this gene cause not only the

eponymous condition (Online Mendelian Inheritance in Man (OMIM) N° 301000) but also X-linked thrombocytopenia (XLT) which is a milder form of WAS. More recently X-linked neutropenia (XLN) or X-linked neutropenia myelodysplasia (OMIM 300299) was found to be an excessively rare condition due to specific mutations in the WAS gene. Altogether, a wide variety of mutations on the *WAS* gene have been reported in more than 270 unrelated families (more than 160 different mutations found in all exons of the gene). Throughout the world, WAS occurs rarely with an incidence estimated to be around 4 per million live births. Patient mutation databases can be found either in the European Society for Immunodeficiencies web site or at [3]. The most frequently referred-to database which contains more than 400 cases, can be found at [4].

WAS is generally diagnosed early in life. Severely affected patients are multi-symptomatic, suffer from haemorrhages due to a micro-thrombocytopenia and from various immune dysfunctions including eczema, high levels of IgE and recurrent pyogenic viral or fungal infections. In addition, WAS patients frequently develop auto-immunity or lymphoid malignancies especially Epstein-Barr virus (EBV)-induced lympho-proliferative disease [5-8]. Many severe patients therefore do not survive past the age of 10 years without definitive treatment [9]. In contrast patients with XLT or attenuated WAS usually live to adulthood. Patients with *WAS* gene mutations therefore present a broad spectrum of disease severity which can be objectively scored [10]. An important determinant of disease severity is the ability to express, or not, the mutated WAS protein (WASp) in blood leukocytes. Failure to do so correlates with a severe condition, with aggravated immune problems and a significantly worse prognosis than in patients capable of expressing a mutated WASp even at low levels [9, 10]. By correlating the mutation with the levels of expression and the predicted molecular structure of WASp, some studies have revealed a good proteotype-genotype linkage in WAS [11]. The WAS disease is therefore relatively well characterized and objective molecular criteria exist that permit therapeutic development and the evaluation of risks/benefits to the patient.

WASp is the founding member of a family of conserved cytoskeletal regulators called the WASp/Scar (suppressor of G-protein-coupled cyclic-AMP receptor) family of proteins. The various structural domains of WASp have been functionally defined [8, 12, 13]. Unlike other family members, WASp is expressed only in hematopoietic cells and is present in all types of leukocytes [14]. This peculiar pattern of expression is regulated by a proximal promoter [15, 16]. Proximal promoter sequences can be cloned into expression vectors and permit the expression of the downstream cassette at high levels in hematopoietic cells but very poorly in a variety of non-hematopoieitic cell lines and thus strongly suggest that transcriptional regulation is the principal reason for the tissue

specificity of *WAS* expression [17-19]. WASp is located in the intra-cytoplasmic compartment with highest density along the cell membrane. It functions as an effector of signalling pathways, downstream of numerous receptors; including antigen receptors, costimulatory receptors, as well as receptors for integrins and chemokines [13, 20]. A major regulator of the actin cytoskeleton through its C-terminal domain, WASp is also an adaptor molecule via an N-terminal domain region which regulates NFAT-dependent transcription in T cells and natural killer (NK) cells [21, 22]. The best understood function of WASp is in the regulation of actin polymerisation. When activated, WASp adopts an open molecular configuration that permits the binding and the activation of the actin-related proteins 2 and 3 (Arp2/3) complex, leading to the assembly of branched actin structures [23]. Actin remodelling plays an important role in many cellular responses allowing changes in shape or motility, enabling endocytosis, vesicular trafficking and membrane reorganization for efficient cell signalling. In addition, the cellular actin cytoskeleton is also exploited by several microbial species for their motility [24]. As a major regulator of actin polymerisation, WASp is therefore intricately linked with multiple and complex biological processes in leukocytes.

The immune pathophysiology of the WAS disease has unravelled rapidly in the past few years, because it has illustrated in a relevant manner the importance of the actin cytoskeleton in the activation of hematopoietic and immune cells as reviewed [13]. The lack of WASp perturbs the localization, activation and function of leukocytes creating spatio-temporal defects in adaptive and innate immune responses. Lymphocytes and antigen-presenting cells do not migrate and do not localize appropriately. The cytoskeletal defects are evident by lack of podosomes, which are highly-dynamic actin-rich structures forming at the basal membrane of motile cells such as dendritic cells [25]. The various immune synapses are not assembled correctly, generating non-productive contacts as shown for instance in T cells [26] or between mature dendritic cells lacking WASp and normal natural killer (NK) cells [27]. In T cells, it is well documented that the abnormal synapses perturb the signalling cascade downstream of the T cell receptor [23, 26, 27] and reduces TH1 T helper cell subset development [28]. The regulatory T cell function is clearly impaired as shown by several recent studies, providing an explanation for the auto-immunity prevalent in this condition [29-32]. In addition, in the absence of WASp, macrophages have a reduced ability to phagocytose microbial organisms and apoptotic cells. The migration of neutrophils and the IgE-mediated degranulation of mast cells is also reduced [33, 34].

Overall, the deficiency in WASp creates a complex immune dysfunction which is relatively well explained at the pathophysiological level although some points remain not

understood. The reasons for the progression to high-score disease (complications by auto-immunity and malignancy) in some WASp+ or WASp- patients remain unclear [9, 10]. This may be in part because WASp is not needed for all aspects of immune responses which can therefore evolve in various alternative ways. For example, as WASp regulates the activity of both effector- and regulatory T lymphocytes, one can speculate that resulting immune responses will be complex and variable upon additional factors. The high incidences of lympho-proliferative disease in WAS may have several causes. It may result from a defective tumor immuno-surveillance but it may also involve the cytoskeletal dysregulation in pre-malignant or malignant lymphoid cells. So, although the immune pathophysiology of WAS is complex, there are important molecular and cellular clues that explain how the lack of WASp can cause immunological deficiency and dysregulation.

Much less is understood about the cause of thrombocytopenia in WAS. Low numbers of platelets of small size are found in the circulation while large amounts of platelets are sequestered in the spleen [13]. Unfortunately, the murine WASp-null models are poor phenocopies of the disease having near normal levels and normal aggregation of platelets. In patients, the thrombocytopenia appears to be related to two primary mechanisms: a defect in platelet production and the destruction of platelets in the spleen (which may be aggravated by autoimmunity). The latter mechanism may be circumvented by splenectomy which is capable of augmenting platelet counts in WAS patients [35]. However the thrombocytopenia may recur and the platelet volume is only transiently normalized after splenectomy suggesting that the micro thrombocytopenia cannot solely be explained by destruction. Although an auto-immune mechanism may be partly involved since platelet antibodies have been detected in WAS, it is rarely the case in XLT. Thus, abnormal platelet production is likely the predominant mechanism and there is strong evidence that WASp deficiency results in defects in the formation of proplatelets from megakaryocytes [36]. In addition functionally-defective platelets are produced since platelet aggregation defects are observed in patients and may be due to defective integrin binding of N-terminal mutated WASp [37].

For a rare disease, WAS has been relatively well studied because of the interest in the cell biology of WASp and related proteins. In a little over 10 years following the discovery of the *WAS* gene, significant insights have been gained on the structure function of the protein and improved our understanding of cytoskeletal regulation and intracellular signalling in hematopoietic cells and immune responses. Furthermore, this has facilitated the development of improved diagnostics, and also the identification of factors that may guide prognosis for individual patients. The comprehensive genetic, molecular-based and

clinical knowledge of WAS has naturally prompted efforts to develop gene replacement strategies to treat this disease. Several groups in Europe and United States are currently actively developing retroviral and lentiviral vectors for this approach. It is therefore remarkable that less than 15 years following the identification of the gene responsible for WAS, a molecular gene-based therapy is entering the clinical arena.

RATIONALE FOR GENE THERAPY IN WAS, AND MOVING FORWARD

a) Medical plausibility of hematopoietic gene therapy

The WAS has been one of the first conditions ever to be successfully treated by allogeneic bone marrow transplantation (BMT) nearly 40 years ago. BMT remains the only proven cure for this disease and outcome is reasonably good for those patients with HLA-matched donors. However, a significant number of patients lack this option and mortality or morbidity arising from mismatched transplantation is significant as recently evaluated [38]. For these patients, autologous hematopoeitic gene therapy could represent a novel therapeutic option.

Hematopoietic gene therapy has now been applied in the clinic for over 15 years in attempts to treat primary immunodeficiencies. However, it has only been in the last 10 years that clinical successes have been obtained [39-43]. The current concept is to introduce a therapeutic transgene into the patients' own hematopoietic stem/progenitor cells *ex vivo* using a retroviral vector and to transplant these cells back into the patient. Through the engraftment of stem cells, their controlled differentiation and self-renewal, a constant production of corrected and functional immune effector cells is obtained in theory for the lifetime of the individual. Gammaretroviral vectors derived from Moloney Leukaemia Virus (MLV) were the first effective gene transfer vectors developed to treat patients with X-linked severe combined immunodeficiency (SCID-X1), adenosine deaminase deficiency (ADA-SCID) and chronic granulomatous disease (CGD). Conditioning the patient with a cytoreductive regimen was found to be a critical factor for the successful engraftment of gene-corrected cells, particularly in the case of ADA-SCID and CGD [40]. The transfer of a functional gene in all of these immune deficient patients has resulted in marked improvement of their conditions. In spite of the adverse events that have occurred in some of these trials the large majority of patients have benefited and the approach can be considered highly successful with more than 6 years follow-up in some cases.

b) pre-clinical proofs of principles in WAS hematopoietic gene therapy

The realization of gene therapy for WAS benefits from several positive factors. The *WAS* transgene has proven to be easy to manipulate and to express. The 1.5 kb full-length cDNA (12 exons) can be expressed successfully from various retroviral and lentiviral gene transfer cassettes without the need for complex regulatory elements. The feasibility of the gene replacement concept is supported by initial studies *in vitro* with WAS B cells [44] and by multiple subsequent studies. Several laboratories have independently shown that following gene transfer with various retroviral or lentiviral vectors, the protein WASp can be expressed at physiologically effective levels in cells of WAS or XLT patients including T, B, macrophages, dendritic cells [17, 18, 45-47]. Importantly, gene transfer vectors have effectively transduced the target WAS patients $CD34^+$ cells, expressing the protein in these cells and their daughter cells [19, 47]. Although gene repair using gene targeting technology is an attractive future option [48], at present this approach is limited by efficiency and also by the heterogeneous nature of the genetic mutations in WAS. Therefore, at present, gene replacement appears to be a readily applicable strategy with a good chance of success.

The feasibility of reconstituting a functional immune system *in vivo* with hematopoietic gene therapy has been validated in two murine knock-out models of WAS [49, 50]. Initial proofs of principle demonstrated that the transfer of the *WAS* gene into WASp-null cells could generate functional T lymphocytes *in vivo* following transplantation into RAG-2KO immuno-deficient mice [51]. Subsequently, transplantation experiments into WASp-null recipient mice demonstrated evidence of transgene expression in both the lymphoid and myeloid lineages (lymphocytes, myeloid cells and platelets) and over long periods of time *in vivo* [46, 52, 53]. In murine models, the levels of WASp obtained from the transgene expression can be close to that of control wild-type cells and these levels can be obtained with 1-4 copies of vector integrated per cell [46, 53]. Functional restoration was obtained, notably the correction of T lymphocyte responses, the correction of the cytoskeletal anomalies in dendritic cells and a resolution of the histological signs of colitis which develops in some WASp-null mice. Because patients with *WAS* gene mutations that express even low levels of protein have a better survival and fewer infections compared to protein-null individuals [9], it is reasonable to believe that a therapeutic benefit would be obtained from gene therapy even if normal levels of protein expression are not achieved.

c) Selective advantage

It is generally recognised that a selective advantage for the corrected cells is an important factor that can facilitate the success of gene therapy. Several pieces of evidence suggest that this could apply to WAS. First, in female mice heterozygous for the

WAS gene inactivation, the pattern of X chromosome inactivation becomes less random during the migration of early hematopoietic progenitor cells from fetal liver to bone marrow, suggesting a migratory (or survival) advantage for hematopoietic stem/progenitor cells able to express the un-mutated allele [54]. Second, somatic mosaïcism has often been observed to occur in T lymphocytes (including in regulatory T cells) and more exceptionally in B and NK cells of WAS patients [29, 55-57]. A selective advantage of gene-corrected cells over the null-cells has also been observed in T lymphocytes both *in vitro* and in bone marrow reconstitution experiments in mice [17, 51]. Thus, a proliferative advantage is expected to be conferred by WASp expression in T cells following gene therapy in WAS patients.

d) Clinical application

Altogether, multiple points can be put forward to argue that gene therapy for WAS should be feasible and efficacious. Ethical considerations indicate that the risk benefit ratio for this new procedure is acceptable in patients with severe disease and no bone marrow donor. Given the widespread expression and role of WASp within the hematopoietic tissue, gene therapy should attempt to correct extensive numbers and types of cells both in the lymphoid and myeloid lineages. While effective correction in T and NK lymphocytes is expected on the basis of the proliferative advantage discussed above, the degree of transgene-positive cells in the myeloid lineages may depend on multiple factors including conditioning regimen. Therefore, it will remain a challenge to design optimal patient conditioning regimens for maximal myeloid engraftment, removal of potentially auto-reactive pre-existing lymphocytes, while at the same time minimizing toxicity to the patient. The first gene therapy study in WAS has been initiated last year in Germany with the use of an MLV-derived retroviral vector encoding the full WAS cDNA and pseudotyped with the Gibbon Ape Leukemia Virus envelope ([58], Trial ID: DE-053) [59]. Preliminary results from this trial were recently reported by C. Klein at the 15th annual meeting of the European Society for Gene and Cell Therapy in Rotterdam (Oct 27-30, 2007). Data from 2 patients are encouraging in terms of the feasibility of gene therapy in WAS. In addition, there is clear evidence of transduced T and NK lymphoid cells and platelet counts have risen following this treatment, which hopefully will benefit these children.

SAFETY ISSUES IN HEMATOPOIETIC GENE THERAPY: PROBLEMS ENCOUNTERED WITH GAMMA-RETROVIRAL VECTORS

Gammaretroviral vectors have provided the first demonstration of clinical efficacy of hematopoietic gene therapy, although some of these first studies encountered

unexpected serious side-effects that revealed the potential genotoxicity of retroviral integration in hematopoietic cells [60]. In the past 4.5 years, vector insertion has been incriminated in the occurrence of T cell leukemias in X-SCID patients and in the appearance of dominant myeloid clones in patients treated for CGD [42]. While the oncogenic potential of the parental onco-retroviruses was known, the genotoxic effects of the MLV vectors were not obvious in the initial pre-clinical studies. Nowadays, several new experimental models have been developed to evaluate the mutagenic potential of non-replicative retroviral insertion in hematopoietic cells. For instance, cells in which the vector insertion provokes a conversion into growth-factor independence can be selected in culture through intense pressure for growth [61]. In animal models, the genotoxicity of vector insertion can be revealed when in combination with other oncogenic factors for instance in tumor-prone murine models [62]. These new experimental models are sensitive and are able to show that differences in vector design can have an important impact on genotoxicity [63]. Databases including an ever-growing number of vector insertion sites are also providing excellent insight into the molecular events and pathway interactions following retroviral insertion [64]. Studies in humans, monkeys and mice show that retroviruses preferentially insert themselves into coding regions but MLV prefer to integrate close to the start of transcriptional units [65-67]. In this position, there is increased likelihood for interactions between retroviral regulatory sequences and promoters, as shown in trapping studies [67]. The powerful duplicated enhancer sequences present in the native long-terminal repeats (LTRs) may be particularly potent for dysregulation of neighbouring genes over relatively large distances. To date, nearly all reported cases of mutagenic events in animal models or in clinical trials have involved conventional gamma-retroviral vectors using intact LTRs. However, it must be recognized that vectors with attenuated LTRs have been used less frequently in preclinical models and have yet to enter the clinic. It is also important to note that not all patients treated with full LTR gammaretroviral vectors have developed complications. Insertional mutagenesis has occurred in two trials (X-SCID and CGD) but not in other trials. Thus, other factors related to the treatment protocols, to the transgene and to the type of disease being treated may also therefore be influential.

Finally, a significant problem encountered with full LTR MLV retroviral vectors is their loss of transcriptional activity or silencing *in vivo* as demonstrated in various experimental systems [68] [62]. This has also recently been observed in a clinical trial for patients with CGD, where the loss of transgene expression in the patient's leukocytes correlates with the gradual methylation of the proviral LTR (M. Grez, personal communication May 5, 07).

BIOSAFETY FEATURES OF AN ADVANCED-GENERATION LENTIVIRAL VECTOR ENCODING WAS

a) Reduced impact of LV transduction on hematopoiesis

We have developed a lentiviral vector for the gene therapy of WAS (WAS-LV) both for efficacy and safety reasons. In general, lentiviral vectors derived from HIV (HIV-LV) provide stable long-term gene expression *in vitro* and *in vivo*. They present a number of advantages over gammaretroviral vectors (RV) for the transduction of hematopoietic stem cells (HSC) [69]. Unlike retroviruses, lentiviruses rely on active transport of the pre-integration complex through the nucleopore by the nuclear import machinery of the target cell. This phenomenon is independent of the cell cycle status, therefore, HIV-LV can integrate in the nucleus of non-dividing cells (i.e. lymphocytes, neurons, glial cells) and more effectively transduce quiescent HSC than RV. Practically, the transduction of HSC with HIV-LV can be achieved with no or very rapid cytokine pre-stimulation which is an advantage compared to retroviral transduction procedures [70]. Minimizing the *ex vivo* manipulation of HSC has the merit of preserving their self-renewing properties, essential for sustained multi-lineage hematopoietic reconstitution.

b) Safety design of the vector

The WAS-LV possesses important general safety features. As an advanced-generation vector, the gene transfer cassette is deleted of all HIV accessory proteins (vpu, vpr, nef, vif) and of Tat [71]. Packaging is therefore conditional on the Rev and gag-pol genes which are expressed in trans on separate plasmids. An envelope pseudotype is also encoded separately. The glycoprotein of the vesicular stomatitis virus (VSV-g) is a useful LV pseudotype that has a broad tropism, in particular the ability to target hematopoietic stem cells. The minimal homology between the various cassettes for packaging and gene transfer minimize the likelihood of homologous recombination events and the generation of replication-competent HIV particles (RCL). The presence of RCL can be assessed with several types of assays, notably by measuring p24 production in target cells [72].

The WAS-LV is a self-inactivating (SIN) construct. A 400 bp deletion was introduced in the 3'LTR to remove major transcription factor binding sites [73]. Reverse transcription which generates both U3 regions from the 3' of the viral genome transfers the deletion to the 5' LTR of the proviral DNA. The viral particle conceived with a SIN configuration enables the production of infective particles only in the first run of infection, minimizing the risk of producing RCL. Consequently, the expression of the transgene must be controlled from an internal promoter.

Cellular promoters can be used as internal promoters and may have less long range enhancer activity than viral LTRs. For the WAS-LV vector, we found that a variety of cellular promoters could be used to express the transgene [19, 46]. We used the native *WAS* gene proximal promoter which provides a hematopoietic-restricted pattern of expression of the transgene as is naturally the case for the *WAS* gene. The *WAS* gene promoter limits the possibility of transgene toxicity or genotoxicity as it is not as transcriptionally potent as a typical gammaretroviral LTR [19]. It is nevertheless functional in lymphoid and myeloid cells. Indeed, WASp-null mice can be safely treated by *ex vivo* gene therapy with this vector and reconstitute a hematopoietic system that expresses the transgene for long periods of time without signs of toxicity (up to 16 months) [53]. The presence of WASp can be documented in all lineages of cells including platelets and the proliferation of T cells can be corrected following gene transfer with this vector with an average of about 1 copy per cell. Our more recent observations also indicate a functional restoration in myeloid cells with the demonstration of restored podosome formation in dendritic cells, as well as the resolution of colitis in some animals (A. Galy, unpublished observations). The WAS-LV using a *WAS* promoter is also active in human patient cells, inducing near normal levels of WASp in $CD34^+$ cells and B cells. The cytoskeletal function of WAS-DC was augmented by this vector to the same extent as other LV encoding WASp [19]. Thus, without compromising the efficacy of the vector we were able to design a potentially less toxic and more physiological construct.

In SIN vectors, the reduced number of enhancer sequences in the LTR that are capable of long distance interactions, and the low transcriptional strength of the internal promoter are two major reasons concurring to reduce the genotoxicity. Like other retroviruses, the pattern of integration of LV into the host genome is not random but it is distinctly different from that of RV. Unlike MLV-derived RV, HIV-1-derived vectors integrate anywhere in transcriptional units not particularly near transcriptional start [67]. Integration site analyses and experimental testing of insertional transformation of target cells, suggest that SIN HIV-derived LV have a more favourable safety profile compared to full LTR MLV-derived gammaretroviral vectors [62, 74, 75].

c) Absence of hematopoietic toxicity

The expression of WASp in cells is normally tightly controlled, presumably to avoid detrimental effects of inappropriate actin polymerisation. One key factor is the binding of a chaperone protein WASp-interacting protein (WIP), which is essential for WASp stability. Attempts to significantly over-express WASp in haematopoietic cells have generally failed, presumably because the levels of WIP are limiting. Similarly, on direct testing in various *in vitro* or *in vivo* settings, we have found no evidence of toxicity of the

WAS-LV in human or murine hematopoietic cells. The transduction of normal or WAS $CD34^+$ cells with this vector does not affect their clonogenic activity, growth potential or viability [19]. Using quantitative real-time PCR on individual colonies we could demonstrate the presence of integrated vector in several types of colonies i.e. CFU-GM, BFU-E and CFU-mix, thus confirming that normal hematopoietic differentiation can take place following transduction with the WAS-LV (A. Galy et al., unpublished observations). So far, in our laboratories, more than 100 WAS knock-out mice have been treated with the WAS-LV using either lethal or sub-lethal conditioning and durable engraftment has been observed in almost all cases. Secondary bone marrow transplants have also been performed and suggest that long-term HSC are transduced with the WAS-LV (A. Galy et al. unpublished observations).

d) Purified product

A detailed description of the bioprocess of production of the WAS-LV is beyond the scope of this review. However it is important to note that the VSV-g pseudotype confers a relative resistance to the particle, thus permitting its extensive purification from the components of the production medium through several chromatographic steps adapted to good manufacturing practices (GMP). The resulting product is enriched in infectious particles while depleted by several logs in protein and DNA from the cell culture medium of the vector packaging cells. In pharmaceutical and regulatory terms, a purified and characterized vector presents obvious safety advantages compared to the use of raw cell culture supernatants which have been employed in prior clinical transduction protocols with gammaretroviral vectors.

CONCLUSION

In spite of the current successes of hematopoietic gene therapy for genetic immune deficiencies, the need for more efficient transduction protocols and mounting concerns about the safety of LTR-driven vector systems are prompting an interest in the development of alternative vectors for gene transfer. Our pre-clinical data demonstrate that a SIN HIV-derived advanced generation WAS vector is both efficacious and safe. A purified WAS-LV vector will therefore be tested in the next years for WAS indications in several European centers. Clinical protocols utilising this WAS-LV will vary the modalities of patient conditioning as this remains an important parameter in our ability to safely treat this complex disease with gene therapy. It will be several years before we can analyze the outcome of any clinical study of gene therapy in WAS given the low prevalence of the disease and the length of time imposed for the evaluation of adverse events with integrative vectors. Due to its design, the WAS lentiviral vector may have

significant advantages for clinical application in humans in terms of natural gene regulation, and reduction in the potential for adverse mutagenic events.

EXPERT OPINION

The lack of satisfactory therapeutic options for many patients, together with experimental proofs of concept and a relatively good understanding of the molecular basis of disease, provide a strong rationale for designing a gene therapy approach for WAS. Currently, hematopoietic gene therapy has proven successful in other diseases, but the occurrence of unexpected adverse events due to insertional mutagenesis demands a careful approach to maximise safety. With that in mind, we believe that a SIN lentiviral vector utilising the native gene promoter for expression of the *WAS* transgene will have a superior safety profile. Preclinical studies have also provided reasonable expectations that hematopoietic gene therapy with such type of vector will lead to the restoration of WASp in the hematopoietic and immune system at levels sufficient to provide an improvement in the condition of patients. Phase I/II clinical studies are therefore now warranted to address the safety and efficacy of this treatment.

The gene therapy of WAS represents a new challenge compared to other immune deficiencies. The degree of conditioning of the patient will test our ability to extensively gene-modify engrafting hematopoietic stem cells. The study will also contribute important data on the use of lentiviral gene transfer vectors in human subjects and will facilitate the development of similar strategies for other haematopoietic and immunological disorders. In the next 5 to 10 years, we foresee that the initial gene therapy studies in WAS will lead to widespread approval of a novel gene-based drug for the treatment of this orphan condition. Successful gene therapy procedures will increase the availability of a cure for the patients and may reduce the potential for side effects associated with mismatched allogeneic transplantation. As a one-time procedure, gene therapy could also prove to be economically-advantageous.

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