Lentiviral Hematopoietic Stem Cell Gene Therapy in Inherited Metabolic Disorders

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Abstract

After more than 20 years of development, lentiviral hematopoietic stem cell gene therapy has entered the stage of initial clinical implementation for immune deficiencies and storage disorders. This brief review summarizes the development and applications, focusing on the lysosomal enzyme deficiencies, especially Pompe disease.

Introduction

RARE DISEASES, OF WHICH 80% are inherited disorders, affect some 6% of the human population, amounting in Europe to around 30 million people. Since many result in chronic disability and cost-intensive care, the impact is disproportional and may well be over 20% of healthcare costs, although the current lack of registration, contrary to cancer, makes exact estimates difficult. Most of the approximately 7500 known inherited diseases lack a curative intervention and are managed by symptomatic treatment and, in case of a family history, by prenatal diagnosis. A small minority is included in newborn screening programs. At the state of the art, gene therapy is within reach for diseases in which (1) the genetic defect is identified, (2) the diagnosis is made sufficiently early for a meaningful therapeutic intervention, (3) a specific animal model is available for efficacy and safety evaluation, (4) strict regulation of transgene product levels is not required, (5) the transgene produces levels sufficient for sustained alleviation of symptoms or cure, and (6) adverse immune responses to the transgene product are not expected, do not interfere with efficacy, or can be successfully counteracted or circumvented. Many of the more than 100 primary immune deficiencies and around 40 lysosomal storage disorders meet those criteria.

Initial Development of Hematopoietic Stem Cell Gene Therapy

Hematopoietic stem cell gene therapy has been developed for more than 20 years. The pioneering trials for X-linked severe combined immune deficiency (SCID) using gammaretroviral gene transfer vectors resulted in successful restoration of T cell immunity (Gaspar *et al.*, 2004; Hacein-Bey-Abina *et al.*, 2010) in 18 patients and in long-term survival for 17 patients out of 20, a survival rate similar to HLA-identical BM transplantation (Rocha *et al.*, 2000).

Unfortunately, in five patients autonomous T cell clones developed into leukemia, among which one patient did not survive. In the context of European collaborative projects, the pathogenesis was rapidly elucidated, resulting in a series of publications on mechanisms involved in gammaretroviral mutagenesis and oncogenesis (Baum *et al.*, 2004; Pike-Overzet *et al.*, 2006, 2007; Deichmann *et al.*, 2007, 2011; Kustikova *et al.*, 2007; Schwarzwaelder *et al.*, 2007). Briefly, gammaretroviral vectors generally integrate near the transcription start sites of expressed genes with a preference for proto-oncogenes, which results in aberrant expression driven by the promoter/enhancer of the therapeutic transgene and may result in a preleukemic state. It is not excluded that the phenotypes of the treated diseases predispose to leukemia development (Shou *et al.*, 2006), given the absence of leukemia in the ADA-SCID trial (Aiuti *et al.*, 2007; Biasco *et al.*, 2011), a 25% incidence in the X-linked SCID trials, and an over 75% incidence in a gammaretroviral Wiskott–Aldrich trial (Braun *et al.*, 2014).

Development of Lentiviral Vector Gene Therapy in Inherited Storage Disorders

The gammaretroviral vectors have been replaced by HIV-1-derived lentiviral vectors (Naldini *et al.*, 1996; Schambach *et al.*, 2006; Zhang *et al.*, 2007), which lack the propensity for integration near proto-oncogenes and have the added advantage of integrating into quiescent cells, such as long-term repopulating stem cells. In addition, third-generation lentiviral vectors made self-inactivating (SIN) by deletion of enhancer regions from the long terminal repeat sequences reduce the risk of influencing nearby genes, resulting in favorable safety profiles (Cesana *et al.*, 2014). Systematic disease-specific efficacy and safety evaluations, including codon optimization and careful promoter selection, have enabled initial clinical trials using these vectors for selected

TABLE 1. HEMATOPOIETIC STEM CELL GENE THERAPY FOR METABOLIC STORAGE DISORDERS

<i>Disorder</i>	<i>Deficient gene/enzyme</i>	<i>References</i>
In clinical trial		
Adrenoleukodystrophy	<i>ABCD1</i>	Cartier <i>et al.</i> , 2009
Metachromatic leukodystrophy	Arylsulfatase A	Biffi <i>et al.</i> , 2013
Preparing for clinical trial		
Hurler syndrome (MPS I)	α -Iduronidase	Visigalli <i>et al.</i> , 2010
Pompe disease (GSD II)	Acid α -glucosidase	van Til <i>et al.</i> , 2010
Fabry disease	Alpha-galactosidase A	Yoshimitsu <i>et al.</i> , 2007
Preclinical efficacy and safety evaluation in progress		
Krabbe disease (globoid cell leukodystrophy)	Galactosylceramidase	Gentner <i>et al.</i> , 2010
Farber disease	Ceramidase	Walia <i>et al.</i> , 2011
Gaucher disease	Glucocerebrosidase	Enquist <i>et al.</i> , 2006

metabolic storage disorders (Table 1), which include adrenoleukodystrophy (ALD), metachromatic leukodystrophy (MLD), and Hurler (MPS I), Pompe (GSD II), and Fabry diseases. We have focused on Pompe disease, the only disorder so far developed for stem cell gene therapy (van Til *et al.*, 2010) in which allogeneic stem cell transplantation has not been applied because of lack of enzyme expression in the hematopoietic system (Hoogerbrugge *et al.*, 1986).

Pompe Disease

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM No. 232300) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the gene-encoding acid α -glucosidase (EC 3.2.1.20) (van der Ploeg and Reuser, 2008). Severe mutations cause complete enzyme deficiency, resulting in the classic infantile form of Pompe disease, which was first described by the Dutch pathologist J.C. Pompe (Pompe, 1932). Symptoms are caused by glycogen accumulation, mainly in skeletal, cardiac, and smooth muscle, but also in other tissues, including the central and peripheral nervous system. In the first months of life, patients present with progressive muscle weakness, hypertrophic cardiomyopathy, respiratory problems, and feeding difficulties. If untreated, this leads to death before the age of 1 year (van den Hout *et al.*, 2003). Older children and adults may have up to 20–30% residual enzyme activity and show a more slowly progressive phenotype. Symptoms generally result from weakness of the (proximal) skeletal muscles. These patients eventually become wheelchair bound and ventilator dependent in late childhood or adulthood.

Enzyme replacement therapy (ERT) by administration of recombinant acid α -glucosidase (Fuller *et al.*, 1995; van der Ploeg and Reuser, 2008) (Myozyme) is currently the only effective treatment, requiring high-dose biweekly administration. Although of considerable benefit to many patients, ERT is not curative, requires life-long administration, and may result in immune responses to the recombinant enzyme (van Gelder *et al.*, 2014), and partly due to the high doses required for clinical efficacy, the costs are extremely high. Therefore, a corrective treatment with curative intent represents an unmet medical need.

Efficacy and Safety Evaluation of Lentiviral Vector Gene Therapy in the Pompe Mouse Model

In the initial evaluation of lentiviral stem cell gene therapy for Pompe disease (van Til *et al.*, 2010), using an efficient

overnight transduction protocol (van Til and Wagemaker, 2014), we demonstrated that approximately 30% successfully transduced cells present in the bone marrow after sublethal total body irradiation as conditioning for transplantation resulted in high levels of α -glucosidase. Restoration of α -glucosidase activity in target tissues by uptake through the mannose-6-phosphate receptor reduced glycogen storage proportional to the enzyme levels achieved, with full correction of glycogen storage in liver and spleen, correction of the life-threatening cardiomyopathy, significantly improved respiration, and improved, but not fully normalized, skeletal muscle function. Of particular interest was the demonstration of robust immune tolerance to the recombinant transgene product.

In the follow-up study (manuscript in preparation), codon optimization of the therapeutic transgene resulted in full correction of the phenotype, including skeletal muscles. Pompe disease does not result in mental retardation or other neuronal problems. Remarkably, brain glycogen levels normalized entirely, with all astrocytes, which play a key role in glycogen storage and glycogenolysis in the brain, showing active acid α -glucosidase activity. Apparently, the microglia descendants of hematopoietic stem cells, which are capable of passing the blood–brain barrier as we originally demonstrated in Krabbe disease (Hoogerbrugge *et al.*, 1988), provided sufficient acid α -glucosidase to normalize glycogen levels also in neuronal tissue. Up till now, hematopoietic stem cell gene therapy is the only approach to achieve both robust immune tolerance to the transgene product and efficacy in bypassing the blood–brain barrier, as has also been observed by others (Visigalli *et al.*, 2010).

Further Development

The future development of stem cell gene therapy efficacy and safety obviously would benefit considerably from noncytoreductive preparation of the patients to enable engraftment of the gene-corrected cells, *ex vivo* stem cell expansion both to promote engraftment of transduced cells and to enable selection of stem cells for transplantation, lineage-specific expression of the therapeutic transgene, targeted gene delivery, and eventually gene editing of the mutated deficient genes. Promoting engraftment by temporary mobilization of endogenous stem cells to open the stem cell niches in the bone marrow has been proposed (Chen *et al.*, 2006) and applied successfully to the X-SCID mouse model

(Huston *et al.*, in review). An initial success has recently been reported in gene editing (Genovese *et al.*, 2014). If the current clinical trials using lentiviral stem cell gene transfer prove efficacious and safe, its rapid clinical implementation in a variety of eligible inherited disorders becomes within reach in the interest of the patients involved and thereby of healthcare and its costs.

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