

Lentiviral Hematopoietic Stem Cell Gene Therapy in Inherited Immune and Lysosomal Enzyme Deficiencies

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Summary

Rare diseases affect millions of people worldwide. Many of those are inherited disorders resulting in chronic disability and requiring cost-intensive care. Hematopoietic stem cell gene therapy has been developed over more than 20 years. At the state of the art, gene therapy is within reach for diseases in which (i) the genetic defect is identified, (ii) the diagnosis is made sufficiently early for a meaningful therapeutic intervention, (iii) a specific animal model is available for efficacy and safety evaluation. Appropriate therapeutic transgenes should also comply with certain biological criteria. Third-generation lentiviral vectors have been made self-inactivating (SIN) by deletion of enhancer regions from the LTR sequences thus reducing the risk of

influencing nearby genes, resulting in favorable safety profiles. At the present time, lentiviral hematopoietic stem cell gene therapy has entered the stage of initial clinical implementation for immune deficiencies and lysosomal storage disorders. We discuss initial clinical trials using these vectors for selected metabolic storage disorders, which include adrenoleukodystrophy, metachromatic leukodystrophy, Hurler (MPS I), Pompe (GSD II), and Fabry diseases. This brief review summarizes the development and current clinical implementation of these approaches.

Keywords

Hematopoietic stem cells, lentivirus vector, gene therapy, inherited immune deficiencies, lysosomal diseases.

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 health care 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, 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 (i) the genetic defect is identified, (ii) the diagnosis is made sufficiently early for a meaningful therapeutic intervention, (iii) a specific animal model is available for efficacy and safety evaluation, (iv) strict regulation of transgene product levels is not required, (v) the transgene produces levels sufficient for sustained alleviation of symptoms or cure, and (vi) 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.

This brief review covers development and prospect of gene therapy for inherited immune deficiencies and lysosomal storage disorders, extending an earlier review [45].

Initial development of hematopoietic stem cell gene therapy

Hematopoietic stem cell gene therapy has been developed over more than 20 years. The pioneering trials for X-linked severe combined immune deficiency (SCID) using gamma-retroviral gene transfer vectors resulted in successful restoration of T cell immunity [17, 22] in 18 patients and in

long-term survival for 17 patients out of 20, a survival rate similar to HLA-identical BM transplantation [35]. Unfortunately, in 5 patients autonomous T cell clones developed into leukemia, among which 1 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 [3,12,13,28,31,32,37]. 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 co-predispose to leukemia development [38], given the absence of leukemia in the ADA-SCID trial [1, 4], a 25% incidence in the X-linked SCID trials and an over 75% incidence in a gammaretroviral Wiskott-Aldrich trial [6].

Development of lentiviral vector gene therapy in inherited storage disorders

The gammaretroviral vectors have been replaced by HIV-1 derived lentiviral vectors [29,36,48], 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 [8]. Systematic disease specific efficacy and safety evaluations, including codon optimization and careful promoter selection have enabled initial clinical trials using these vectors for selected 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 [42] in which allogeneic stem cell transplantation has not been applied due to lack of enzyme expression in the hematopoietic system [25] and in addition studied stem cell gene therapy for Hurler syndrome.

Hurler Syndrome and Pompe disease

Hurler Syndrome (Mucopolysaccharidosis type I, OMIM # 252800) is a lethal autosomal recessive storage disorder caused by a deficiency of the lysosomal enzyme α -L-iduronidase (IDUA; EC 3.2.1.76). The deficiency leads to insufficient degradation of glycoaminoglycans (GAGs) that interferes with normal cellular function and causes a multisystem disorder affecting the CNS, liver, skeleton, lungs and sensory organs (corneal clouding and deafness), average expected life-span approximately 5-10 years. Treatment consists of alloSCT, currently the only therapeutic option to establish long-term survival and protection of the CNS. Enzyme replacement has a systemic effect but does not reach the brain or the skeletal bones. AlloSCT does not improve the skeleton pathology either. Although clinical outcome of SCT has improved by the use of umbilical cord stem cells, progressive bone disease persists, leading to severe handicaps. An international long term clinical follow-up cohort of Hurler patients treated with alloSCT including >80% of the patients successfully transplanted worldwide [2] showed that certain genotypes, age at HSCT, and poor performance at HSCT are predictors for poor skeletal and neurodevelopmental outcome. These findings warrant the development of a single curative gene therapy approach.

Pompe disease (glycogen storage disease type II, acid maltase deficiency, OMIM # 232300) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the gene-encoding acid α -glucosidase (EC 3.2.1.20) [40]. Severe mutations cause complete enzyme deficiency, resulting in the classic infantile form of Pompe disease, which was first

Table 1. Hematopoietic stem cell gene therapy for metabolic storage disorders

Disorder	Deficient gene/enzyme	References
In clinical trial Adrenoleukodystrophy Metachromatic leukodystrophy	<i>ABCD1</i> arylsulfatase A	Cartier <i>et al.</i> , 2009 Biffi <i>et al.</i> , 2013
Preparing for clinical trial Hurler syndrome (MPS I) Pompe disease (GSD II) Fabry disease	α -iduronidase acid α -glucosidase α -galactosidase A	Visigalli <i>et al.</i> , 2010, 2016 van Til, Wagemaker, 2014 Yoshimitsu <i>et al.</i> , 2007
Preclinical efficacy and safety evaluation in progress Krabbe disease (globoid cell leukodystrophy) Farber disease Gaucher disease	galactosylceramidase ceramidase glucocerebrosidase	Gentner <i>et al.</i> , 2010 Walia <i>et al.</i> , 2011 Enquist <i>et al.</i> , 2006

described by the Dutch pathologist J.C.Pompe [33]. 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 one year [39]. Older children and adults may have up to 20-30% residual enzyme activity and show a more slowly progressive phenotype. The 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 [16,40] (Myozyme[®]) is currently the only effective treatment, requiring high dose bi-weekly administration. Although of considerable benefit to many patients, ERT is not curative, requires life-long administration, may result in immune responses to the recombinant enzyme [41] and, partly due to the high doses required for clinical efficacy, the costs are extremely high. Therefore, a corrective intervention with curative intent represents an unmet medical need.

Efficacy and safety evaluation of lentiviral vector gene therapy in the Hurler and Pompe mouse models

The natural course of Hurler's disease is invalidating and lethal, and the drawbacks of the current therapeutic modalities justify a gene therapy approach. An IDUA knock-out mouse model has been developed suitable to study gene therapeutic approaches and made available to our research. A gene therapy study with HSC in the IDUA KO mice showed improvement of disease pathology, including the brain, but bone remodeling was not reported [14]. Using a codon optimized IDUA gene driven by a cellular promoter in a lentiviral vector to transduce hematopoietic stem cells, we demonstrated long-term expression in the hematopoietic system resulting in full correction of the Hurler phenotype (unpublished) without any severe adverse effects. Similar re-

sults and a further safety analysis were obtained in a parallel study [43,44], completing the preclinical analysis enabling an initial clinical trial in selected patients.

In the initial evaluation of lentiviral stem cell gene therapy for Pompe disease using an efficient overnight transduction protocol [42], 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. Remarkably, although Pompe disease does not result in mental retardation or other neuronal problems, also 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 the mouse model of Krabbe disease [24], provide 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 [43].

Clinical implementation of stem cell gene therapy in primary immune deficiencies

A summary of the current developments is provided in Table 2. Briefly, successful clinical trials are ongoing for X-linked SCID, ADA-SCID and Wiskott-Aldrich syndrome, while

Table 2. Hematopoietic stem cell gene therapy for inherited immune deficiencies

Disorder	Deficient gene/protein	References
In clinical trial SCID-X1 ADA-SCID Wiskott-Aldrich Syndrome	IL2-RG Adenosine deaminase WASP	Gaspar <i>et al.</i> , 2011a; Hacein-Bey-Abina <i>et al.</i> , 2014 Gaspar <i>et al.</i> , 2011b; Cicalese <i>et al.</i> , 2016 Pala <i>et al.</i> , 2015
Preparing for clinical trial Chronic Granulomatous Disease RAG2 deficiency Artemis	gp91phox RAG-2 Artemis	Kaufmann <i>et al.</i> , 2014; De Ravin <i>et al.</i> , 2016 van Til, Wagemaker, 2014 Rivera-Munoz <i>et al.</i> , 2016

clinical trials are being prepared for RAG2 deficiency, chronic granulomatous disease and Artemis. Gene therapy for ADA-SCID, which is difficult to treat with allogeneic stem cell transplantation, has recently been registered as an advanced therapy medicinal product and will soon be considered as the standard treatment for this disease.

Further developments

The future development of stem cell gene therapy efficacy and safety would obviously benefit considerably from non-cytoreductive 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 deficient mutant genes. Promoting engraftment by temporary mobilization of endogenous stem cells to open the stem cell niches in the bone marrow has been proposed [9] and applied successfully in the X-SCID mouse model [26]. An initial success has recently been reported in gene editing [20]. 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 will become within reach in the interest of the patients involved and thereby of health care and its costs.

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Генная терапия лентивирусными векторами в гемопоэтических стволовых клетках при врожденных дефицитах иммунитета и лизосомных энзимов

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Резюме

Редкие болезни поражают, в целом, миллионы людей во всем мире. Многие из этих являются наследственными заболеваниями, ведущими к инвалидности и требующими дорогостоящего ухода. Генная терапия гемопоэтическими стволовыми клетками (ГТГСК) разработана за последние 20 лет. На современном уровне генная терапия выполнима при заболеваниях, для которых (1) есть идентифицированный генетический дефект, (2) диагноз ставится достаточно рано для действенного терапевтического вмешательства, (3) имеется специфическая экспериментальная модель для оценки эффективности и безопасности лечения. Соответствующие терапевтические трансгены должны также отвечать определенным биологическим критериям. Лентивирусные векторы третьего поколения выполнены самоинактивирующимися (SIN), путем делеции энхансерных участков из LTR-последовательностей, тем самым снижая риск воздействия на соседние гены, что

приводит к достаточным уровням безопасности. В настоящее время лентивирусная ГТГСК вступила в фазу начального клинического внедрения для лечения иммунодефицитов и лизосомных болезней накопления. Мы обсуждаем начальные клинические испытания с применением этих векторов для некоторых метаболических болезней накопления, которые включают адренолейкодистрофию, метахроматическую лейкодистрофию, синдром Гурлер (MPS I), Помпе (GSD II), и болезнь Фабри. Данный краткий обзор обобщает развитие и современное клиническое внедрение этих подходов.

Ключевые слова

Гемопоэтические стволовые клетки, лентивирусный вектор, генная терапия, врожденные иммунодефициты, лизосомные болезни.