Stem Cell Biology and Regenerative Medicine

Jaap Jan Boelens
Robert Wynn *Editors*

Stem Cell Therapy in Lysosomal Storage Diseases

Humana Press

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Editors

Stem Cell Therapy in Lysosomal Storage Diseases

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During the execution of this book the world has lost a leader in the field of Lysosomal Storage Disorders. Ed Wraith, one of the key contributors to the book - died suddenly in Manchester in April 2013. Ed was a pioneer in every aspect of LSDs including cellular therapy. Indeed Jaap Jan Boelens and Rob Wynn first met each other at a meeting attended and chaired by Ed to facilitate collaboration between European metabolic disease transplant centres. It is a testament to Ed and to his vision for LSD patients and their families that this collaboration has taken root and borne the fruit of hugely improved knowledge about transplant and of course much better patient outcomes. As we look to the future in this book then we remember Ed for all that he taught us and the shining example that he was of the complete physician for his patients. We dedicate this book to his memory.

Rob Wynn, Manchester. May 2013
Jaap Jan Boelens, Utrecht. May 2013

Preface

Inborn errors of metabolism (IEM) are a diverse group of diseases arising from genetic defects in lysosomal enzymes or peroxisomal function. Lysosomal enzymes are hydrolytic and are stored in cellular organelles called lysosomes. Peroxisomes are subcellular organelles involved in lipid metabolism. These diseases are characterized by devastating systemic processes affecting neurologic and cognitive function, growth and development, and cardiopulmonary status. Onset in infancy or early childhood is typically accompanied by rapid deterioration and results in early death in the most severe phenotypes.

Therefore, timely diagnosis and immediate referral to an IEM specialist are essential steps in the management of these disorders. Nowadays various treatment modalities are available for these devastating disorders. Initially only hematopoietic cell transplantation (HCT) was a treatment option in a selected group of disorders. Later (early 2000s) intravenous enzyme replacement therapy became available for some diseases, e.g., MPS-1, 2, 6, Gaucher, Fabry. Furthermore, substrate deprivation therapy is being trialed as well and major progress is made in the development of gene therapy (GT), of which the first trials are currently running. In the future iPS and ES therapies may reach the clinic.

Treatment recommendations are based on the disorder; its phenotype including age at onset, rate of progression, and severity of clinical signs and symptoms; family values and expectations; and the risks and benefits associated with available therapies such as HCT or more recent experimental GT trials. HCT for IEM is performed using donor cells from bone marrow (BM), umbilical cord blood (CB), or growth factor-mobilized peripheral blood (PB). Donor cells are infused into the patient after myelo-suppression and immunosuppression, using chemotherapeutic agents.

To study the effect of the various treatment modalities in these rare diseases, international collaborative efforts are of utmost importance, and they began in the late 1980s and continue till today. Large multi- and single-center reports on the outcomes of HCT have been published on MPS IH (Boelens, Peters, etc.), cerebral X-ALD

(Peters, Beams, Orchard), and GLD (Escolar). This book will focus on stem cell therapies in IEM; an international perspective on progress, limitations, and future directions (e.g., gene therapy, iPS, ES) in the field is provided.

Utrecht, The Netherlands — Jaap Jan Boelens
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Chapter 1
Lysosomal Diseases and Therapeutic Options: An Overview

Eveline J. Langereis and Frits A. Wijburg

1.1 Introduction

Lysosomal storage diseases (LSDs) comprise a group of more than 50 distinct inherited metabolic diseases, each of which is caused by a specific deficient function of a lysosomal enzyme or transporter or by defects in lysosomal biogenesis or vesicular trafficking.

Since the discovery of the lysosome as a cellular organelle in 1955, enormous advances have been made in the understanding of the complex lysosomal biology. This knowledge led to the first pioneering studies on treatment of LSDs, by Hobbs and colleagues using bone-marrow transplantation in a patient with Hurler's disease [1] and by Brady and co-workers on intravenous enzyme supplementation in patients with non-neuronopathic Gaucher disease [2].

In this chapter a concise overview of the history of lysosomal storage diseases, lysosomal biology, lysosomal storage diseases and current and future therapies is presented.

1.2 History

In 1955, the Belgium biochemist Christian de Duve described a new intracellular compartment which he later called the "lysosome" (Greek: *lysis* = to split; *somos* = body) [3, 4]. De Duve was awarded the Nobel Prize in Physiology and Medicine for his work in 1974.

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The first description of a disease that was recognized as an LSD only much later is Gaucher disease. Philippe Gaucher was a French medical student who, in 1882, reported a patient with an enlarged spleen [5]. Microscopic studies revealed abnormal cells which were later called "Gaucher cells". In 1924, a German medical doctor succeeded in isolating a special fatty substance from the spleen of patients with this disorder, which was already named "Gaucher disease". Ten years later additional studies showed that this fatty substance was a glucocerebroside, a component of the plasma membrane of the red and white blood cells.

A significant number of other diseases of which it later became clear that they were all to be classified as LSDs, were already described at the end of the nineteenth and the beginning of the twentieth century. For instance, Fabry disease was reported for the first time independently by two dermatologists, Johannes Fabry from Germany and William Anderson from the UK [6, 7]. The British army doctor C.A. Hunter reported the first cases of what later became known as Hunter disease (mucopolysaccharidosis type II) in 1917 [8] and in 1919 the German paediatrician Gertrud Hurler reported the first cases of patients with the disorder later known as Hurler's disease (mucopolysaccharidosis type I) [9].

The mechanism of lysosomal storage diseases was first discovered in 1963 in Pompe disease. This disease was reported for the first time by the Dutch pathologist J.C. Pompe in 1932 in a 7-month-old infant who had died from cardiac hypertrophy [10]. Dr Pompe discovered that this disease involved accumulation of large quantities of glycogen in almost all studied tissues. He called the disease "cardiomegalia glycogenica diffusa". Gerty Cori, who, together with her husband Carl Ferdinand Cori, had received the Nobel Prize in Physiology or Medicine in 1947 for their work on glycogen degradation, recognized in 1954 that Pompe disease involved abnormal glycogen degradation and classified Pompe disease as "glycogen storage disease type 2" [11]. It was the Belgian biochemist Henri-Géry Hers, a former colleague of de Duve, who finally discovered that the glycogen storage in patients with Pompe disease was caused by a deficiency of the lysosomal enzyme acid-alpha-glucosidase [12]. His discovery of an inherited deficiency of a lysosomal enzyme as the cause of a disease thus marks the development of the concept of LSDs and the start of many new discoveries relating clinical diseases to dysfunction of the lysosomal machinery.

1.3 Lysosomal Biology

The lysosome is a membrane enclosed organelle, containing many unique hydrolytic enzymes, including proteases, nucleases, glycosidases, phosphatases, sulphatases, lipases and phospholipases. In addition, numerous lysosomal transmembrane proteins are recognized and involved in the complex lysosomal biology. Together they transport a large number of biological molecules and hydrolases degrade proteins, nucleic acids, phospholipids and oligosaccharides. These macromolecules can be derived from intracellular material, such as "worn out" organelles or from extracellular material transported to the lysosome after endocytosis or

phagocytosis. The lysosomal enzymes are optimally active in an acidic environment with a pH of about 4.5–5. The low pH optimum of the hydrolases compared to the average cytosolic pH of 7.2 requires a gradient over the unique lysosomal membrane. This gradient is maintained by an ATP-driven H^+-pump, pumping H^+ ions into the lysosome. While this V-type ATPase pump uses the energy derived from converting ATP to ADP, the H^+ gradient thus created also serves as a source of energy for transport of small metabolites across the membrane.

Both the encapsulating membrane and the pH gradient protect the cell from the multiple degradation effects of lysosomal enzymes. Naturally, the digestive enzymes themselves also need to be protected from digestion. For the membrane bound proteins, like transporters, this is realized through an unusual high glycosylation. These sugars form a protective layer keeping the lysosomal proteases from the proteins [13].

The substrates for the lysosomal machinery can reach the lysosome in three ways. Through endocytosis, the cell takes up macromolecules from the extracellular fluid. This way, early endosomes are formed where a selection takes place in the products that should be broken down and those that can be recycled without a full degradation. By sorting the macromolecules and receiving newly formed hydrolases from the Golgi apparatus a late endosome is formed, where hydrolytic digestion begins. Through fusion with existing lysosomes, the pH drops and digestion is facilitated more readily. Once the slowly digestible residues are left over, the organelle most resembles the classic lysosome; compact and dense.

A second pathway for degradation is called autophagy. In this process the cell forms a double membrane around an obsolete intracellular structure or organelle. Once a closed sack is formed, it fuses with a lysosome and digestion starts. In a state of low extracellular nutrient supply such as starvation, autophagy can provide the cell with metabolites needed for survival.

Some specialized cells have the ability to form phagosomes. This way, a cell is able to take up large particles and microorganisms. The processing of the phagosomal contents is similar to that of the autophagosome [14].

The lysosomal hydrolases and membrane proteins are composed and folded in the endoplasmatic reticulum. They are subsequently transported to late endosomes through the Golgi apparatus and especially the *trans*-Golgi-network (TGN). In the TGN the proteins are selected for their specific destination. The lysosomal hydrolases are recognized because they carry specific mannose-6-phosphate (M6P) markers, which are added in the Golgi network to the N-linked oligosaccharides of these enzymes. In the TGN, transmembrane M6P-receptors recognize and bind the flagged enzymes. While binding takes place at a pH of around 6.7, the receptor releases the oligosaccharide at a lower pH of around 6. On average, this is the pH in late endosomes. This leads to a gradual release of the enzymes as the environment gets more acidic in the process of maturation into a lysosome. After this dissociation, the M6P receptors are returned to the TGN, where they enter the cycle once more [15].

Once the lysosome has degraded its contents, transmembrane transporters facilitate relocation of metabolites to the cytosol, and other waste products leave the cell through exocytosis.

Lysosomes and endosomes have several other important functions besides processing cellular waste material. Among these functions are antigen presentation, signal transduction, cell division and neurotransmission. This wide range of tasks might aid our understanding why storage of an indigestible waste product might lead to cell and organ dysfunction in lysosomal storage diseases.

In lysosomal disease it can be either a soluble hydrolytic enzyme that is deficient or any of many regulatory and transport enzymes. The classic hypothesis of cytotoxicity stated that accumulation would eventually overwhelm the cell, impair its normal functions and eventually lead to cell death [16]. Ongoing research in cellular processes learns that the mechanism leading from the relatively simple single enzyme defect with accumulation of material to clinical disease is much more complex. It is now clear that many secondary effects contribute to cell and organ damage. Some of these effects involve ER stress, impaired pH regulation, insufficient M6P recycling, disturbed Ca^{2+} homeostasis, impaired signal transduction and reduced ability of autophagy [17–19].

1.4 Clinical Aspects of Lysosomal Storage Diseases

The lysosomal storage diseases (LSDs) comprise a quantitatively important group within the field of inherited metabolic disorders. The diseases of this heterogeneous group might be classified by the storage products or by the affected cellular mechanism. An extensive overview of lysosomal storage disorders and inherited defects in lysosome-like organelles is presented in Table 1.1.

LSDs are considered rare genetic disorders, but taken together they have an incidence of more than 1 in 8000 births [18]. Incidence of any LSD also highly relies on the ethnicity of the population studied. For example Gaucher disease has an incidence of 1:450 to 1:1,000 is Ashkenazi Jewish families, compared to a general incidence of 1:60,000 [20]. For mucopolysaccharidosis type I (MPS I) the incidence in the Republic of Ireland is around 1:26,000 births. Within this cohort, the incidence among the Irish Traveller Community is even much higher, namely 1:371 [21]. In other countries the incidence of MPS I can be significantly lower, ranging from 1.19:100,000 in the Netherlands [22] to 0.11:100,000 in Taiwan [23].

In general, the characteristic signs and symptoms of any LSD reflect the cell type that is the principal site of substrate deposit. Which cells are affected mainly depends on the availability of substrate for the deficient enzyme. For example, in Pompe disease α-glucosidase is deficient, which is an enzyme involved in glycogen breakdown. Consequently the skeletal muscles, the heart and the liver are the most affected organs in Pompe disease, as these are the most active organs in glycogen metabolism. But as lysosomal proteins are distributed ubiquitously, ultimately any cell type may be involved [24].

Most of the LSDs display a remarkable clinical heterogeneity with a spectrum ranging from severe disease to an attenuated phenotype. In many LSDs, an underlying genetic defect has been identified and although no perfect concordance in the relationship between phenotype and genotype has been found, null-mutations have

Table 1.1 Lysosomal storage disorders and inherited defects in lysosome-like organelles

Disease	Eponyme	OMIM	Clinical phenotype	Locus Gene	Gene product	Storage
Mucopolysaccharidoses (MPS)						
MPS I	Hurler (MPS IH)	607014	*Severe* (MPS IH): organomegaly, heart disease, skeletal abnormalities, mental retardation, death in childhood	4p16 IDUA	α-L-Iduronidase	DS, HS
	Hurler/Scheie (MPS IH/S)	607015	*Intermediate* (MPS IH/S)			
	Scheie (MPS IS)	607016	*Attenuated* (MPS IS): skeletal abnormalities, normal intelligence, normal lifespan			
MPS II	Hunter	309900	*Severe:* organomegaly, mental retardation, death before 15 years *Less severe:* normal intelligence, short stature, survival age to 20–60	Xq28 IDS	Iduronate sulfatase	DS, HS
MPS IIIA	Sanfilippo A	252900	Profound mental deterioration, hyperactivity, relatively mild somatic manifestations	17q25 SGS	Heparan *N*-sulfatase	HS
MPS IIIB	Sanfilippo B	252920	Similar to III A	17q21 NAGLU	*N*-Acetyl glucosaminidase	HS
MPS IIIC	Sanfilippo C	252930	Similar to III A	8p11 TMEM76 HGSNAT	α-Glucosaminide acetyl-CoA transferase	HS
MPS IIID	Sanfilippo D	252940	Similar to III A	12q14 GNS	*N*-acetyl glucosamine 6-sulfatase	HS
MPS IVA	Morquio A	253000	Distinctive skeletal abnormalities, corneal clouding, odontoid hypoplasia, milder forms exist	16q24 GALNS	Galactosamine 6-sulfatase	KS, CS

(continued)

Table 1.1 (continued)

Disease	Eponyme	OMIM	Clinical phenotype	Locus Gene	Gene product	Storage
MPS IVB	Morquio B	253010	Similar to IV A	3p21 GLB1	Acid β-galactosidase	KS
MPS VI	Maroteaux-Lamy	253200	Corneal clouding, normal intelligence, survival to teens in severe form, milder forms exist	5q12 ARSB	Arylsulfatase B	DS
MPS VII	Sly	253220	Wide spectrum of severity, including hydrops fetalis. *Milder*: bone deformities, organomegaly, coreal clouding, mental retardation	7q21 GUSB	Glucuronidase	DS, HS, CS
MPS IX	Hyaluronidase deficiency	601492	Short stature, organomegaly, dysmorphic craniofacial features, skeletal lesions	3p21 HYAL1	Hyaluronidase 1	HA
Sphingolipidoses						
Farber	Lipogranulo-matosis	228000	Subcutaneous nodules, arthritis, hepatomegaly, variable severity	8p22 ASAH	Acid ceramidase	Cer
Fabry	Anderson-Fabry	301500	Acroparasthesias, angiokeratoma, cornea verticillata, cardiac hypertrophy, renal and cerebrovas-cular disease. Atypical variants	Xq22 GLA	α-Galactosidase A	GB3
			Manifestations in heterozygotes			
Gaucher	Glucosyl-ceramidosis	608013	"Collodion": ichthyosis, neonatal death	1q21 GBA	Glucocerebrosidase	GlcCer
		230900	Type II: acute neuronopathic form, death < age of 2			
		231000	Type III: chronic neuronopathic form			
		230800	Type I: child/adult onset, no CNS disease, hepatosplenomegaly, pancytopenia, skeletal disease			
GM1 gangliosidosis		230500	Type I: infantile form. Progressive neurological disease and skeletal dysplasia	3p21 GLB1	β-Galactosidase	GM1
		230600	Type II: late infantile-juvenile form			

Tay-Sachs	GM2-gangliosidosis B	272800	Severest form: neurodegenerative with death by 4 years Less severe: slower onset of symptoms of the nervous system	15q23 HEXA	β-Hexosaminidase α-subunit	GM2
Sandhoff	GM2-gangliosidosis O	268800	Same as Tay-Sachs	5q13 HEXB	β-Hexosaminidase β-subunit	GM2
Tay-Sachs AB variant	GM2 gangliosidosis AB	272750	Same as Tay-Sachs	5q32 GM2A	GM2 activator protein	GM2
Krabbe	Globoid cell leukodystrophy	245200	Early onset with progression to severe mental and motor deterioration	14q31 GALC	β-Galactosyl-ceramidase	GalCer
Metachromatic leukodystro-phy	Arylsulfatase A deficiency	250100	Infantile, juvenile, and adult forms can include mental regression, peripheral neuropathy, seizures, dementia	22q13 ARSA	Arylsulfatase A	Sulfatide
Prosaposin deficiency	Combined saposin deficiency	611721	hyperkinetic behaviour, myoclonus, respiratory insufficiency, and hepatosplenomegaly	10q22 PSAP	Prosaposin	Multiple lipids
Saposin A deficiency	Krabbe variant	611722	Similar to Krabbe disease	10q22	Saposin A	GalCer
Saposin B deficiency	Metachrom-atic leukodystrophy variant	249900	Similar to metachromatic leukodystrophy	10q22 PSAP	Saposin B	Sulfatide
Saposin C deficiency	Gaucher variant	610539	Similar to type III Gaucher, occasionally milder	10q22 PSAP	Saposin C	GlcCer
Niemann-Pick types A and B		257200	Type A: hepatosplenomegaly, lung involvement, mental retardation, death < age of 3 years	11p15 SPMPD1	Acid sphingomyelinase	SM
		607616	Type B: visceral form without neurological manifestations			

(continued)

Table 1.1 (continued)

Disease	Eponyme	OMIM	Clinical phenotype	Locus Gene	Gene product	Storage
Glycoproteinoses						
Aspartyl glucosaminuria		208400	Progressive coarse facies, mental retardation	4q32 AGA	Glycosyl-asparaginase	Aspartyl-glucosamine
Fucosidosis		230000	Spectrum of severities includes psychomotor retardation, coarse facies, growth retardation	1p34 FUCA	α-Fucosidase	Oligosaccharides
α-Mannosidosis		248500	Type I: infantile onset, progressive mental retardation, hepatomegaly, death between 3 and 12 years Type II: juvenile/adult onset, milder, slowly progressive	19q12 MAN2B1	α-Mannosidase	Oligosaccharides
β-Mannosidosis		248510	Severe quadriplegia, death by 15 months in most severe; mild cases have mental retardation, angiokeratoma, facial dysmorphism	4q22 MANBA	β-Mannosidase	Oligosaccharides
Sialidosis	Mucolipidosis type I Sialidase deficiency	256550	Progressive, severe MPS-like features, mental retardation Type I: (cherry red spot-myoclonus syndrome) is the milder form Type II: the more severe form with earlier onset	6p21 NEU1	α-Sialidase Neuraminidase	Oligosaccharides
Schindler	NAGA deficiency	609241	Type I: infantile onset, neuroaxonal dystrophy, severe psychomotor and mental retardation, cortical blindness neurodegeneration	22q13 NAGA	α-*N*-Acetyl-glucosaminidase	Oligosaccharides
	Kanzaki disease	609242	Type II: mild intellectual impairment, angiokeratoma			

Other lipidoses						
Niemann-Pick type C1		257220	Variable phenotype: ataxia, seizures, loss of speech, dystonia, vertical supranuclear gaze palsy, dementia and psychiatric manifestations	18q11 NPC1	NPC1	Cholesterol, GSL
Niemann-Pick type C2		607625	See NPC type I	14q24 NPC2	NPC2	Cholesterol, GSL
Wolman	Cholesteryl ester storage disease	278000	Xanthomatous changes in lungs, liver, spleen, adrenal gland, lymph nodes, bone marrow, small intestine, thymus. Variable severity and age of onset	10q24 LIPA	Acid lipase	Cholesterol-ester
Glycogen storage disease						
Pompe	Glycogen storage disease type II	232300	Infantile, juvenile and adult onset forms. Cardiomyopathy (infantile form), progressive muscle weakness	17q25 GAA	α-Glucosidase	Glycogen
Multiple substrate storage due to multiple enzyme deficiencies						
Multiple sulfatase deficiency	Austin disease	272200	Hypotonia, psychomotor retardation, quadriplegia	3p26 SUMF1	Formyl-glycine generating enzyme	Sulfatide, muco-polysaccharides
Galactosialidosis	Goldberg syndrome	256540	Coarse facies, skeletal dysplasia, early death	20q13 PPCA	Protective protein/ Cathepsin A	GSL, polysaccharides
Mucolipidosis type II	I-cell disease	252500	Hurler-like features but without mucopolysaccharidurie	12q23 GNPTAB	UDP-GlcNac Phospho-transferase alfa/beta unit	Multiple (lipids and oligosaccharides)
Mucolipidosis type IIIA	Classic pseudo-Hurler polydystrophy	252600	Similar but with slower clinical evolution	12q23 GNPTAB	UDP-GlcNac Phospho-transferase alfa/beta unit	See above
Mucolipidosis type III	Pseudo Hurler polydystrophy-	352605	Similar to above	16p GNPTG	UDP-GlcNac Phospho-transferase Gamma-subunit	See above

(continued)

Table 1.1 (continued)

Disease	Eponyme	OMIM	Clinical phenotype	Locus Gene	Gene product	Storage
Mucolipidosis type IV		252650	Psychomotor retardation and ophthalmologic abnormalities	19p13 MCOLN1	Mucolipin-1 cation channel	See above
Neuronal ceroid lipofuscinoses (NCL)						
CLN1	Haltia-Santavuori	256730	Progressive dementia, seizures and visual failure associated with accumulation of autofluorescent storage material	1p32 CLN1	PPT1 palmitoyl protein thioesterase 1	SAPs
CLN2	Janský-Bielschowsky	204500	Late infantile onset neuronal ceroid-lipofuscinosis	11p15 CLN2	TPP1 tripeptidyl peptidase I	SCMAS
CLN3	Spielmeyer-Sjögren Batten	204200	Juvenile onset neuronal ceroid-lipofuscinosis	16p12 CLN3	CLN3	SCMAS
CLN4A	Kufs	204300	Adult onset neuronal ceroid-lipofuscinosis	CLN6	?	SCMAS
CLN4B	Parry disease	162350	Adult onset neuronal ceroid-lipofuscinosis (autosomal dominant)	DNAJC5	?	SAPs
CLN5	vLINCL Finnish	256731	Variant late onset neuronal ceroid-lipofuscinosis	13q22 CLN5	CLN5	SCMAS
CLN6	Lake-Cavanagh	601780	Variant late onset neuronal ceroid-lipofuscinosis	15q21 CLN6	CLN6	SCMAS
CLN7	vLINCL Turkish	610951	Variant late onset neuronal ceroid-lipofuscinosis	CLN7/ MFSD8	?	SCMAS
CLN8	Northern epilepsy	600143	Epilepsy with progressive mental retardation. Variant: late onset neuronal ceroid-lipofuscinosis	8p32 CLN8	CLN8	SCMAS
CLN9	Variant Batten disease	609055	Juvenile onset	CLN1/ CLN3	Regulator of dihydroceramide synthase	SCMAS

CLN10	Congenital NCL	610127	Congenital neuronal ceroid-lipofuscinosis	11p15 CTSD	Cathepsin D	SAPs
Disorders in the extended lysosomal apparatus (melanosomes, lamellar bodies)						
Chediak-Higashi		214500	Partial albinism, susceptibility to neutropenia and lymphoma	1q42 LYST	LYST	Enlarged vacuoles/ melano-somes
MYOV	Griscelli type1	214450	Hypomelanosis with neurological deficits	15q21 MYO5A	Myosin 5A	Melanin granules
RAB27A	Griscelli type2	607624	Hypomelanosis with immune deficit	15q21 RAB27A	RAB27A	Melanin granules
Melanophilin	Griscelli type3	609227	Hypomelanosis with neurological or immune deficits	2q37 MLPH	Melanophilin	Melanin granules
HPS	Hermansky-Pudliak	203300	Type 1: oculocutaneous albinism, bleeding, and lysosomal ceroid storage resulting from defects of melanosomes, platelet-dense granules, and lysosomes	10q23 HPS-1	HPS-1	Multiple vacuoles
			Type 2: see above	5q14 AP3B1	AP3 β-subunit	See above
			Type 3: see above	3q24 HPS3	HPS-3	See above
			Type 4: see above	22q11 HPS-4	HPS-4	See above
			Type 5: see above	11p15 HPS-5	HPS-5	See above
			Type 6: see above	10q24 HPS-6	HPS-6	See above
			Type 7: see above	6p22 DTNB1	Dysbindin	See above

(continued)

Table 1.1 (continued)

Disease	Eponyme	OMIM	Clinical phenotype	Locus Gene	Gene product	Storage
			Type 8: see above	19q13	BLOC1S3	See above
Surfactant metabolism dysfunction-3	SMPD3	610921	Severe neonatal respiratory distress, death in first week	16p13 ABCA3	ABCA3	Alveolar protein-sis
Congenital and lamellar ichthyosis	Harlequin foetus	242500 601277	Usually rapidly fatal ichthyosis	2q34 ABCA12	ABCA12	Abnormal keratin

DS dermatansulfate, *HS* heparansulfate, *KS* keratansulfate, *CS* chrondoitinsulfate, *HA* hyaluronan, *Cer* ceramide, *GlcCer* glucosylceramide, *GB3* globotriaosylceramide, *GM1 and GM2* gangliosides, *GalCer* galactosylceramide, *SM* sphingomyelin, *GSL* glycosphingolipids, *SAPs* sphingolipid activator proteins, *SCMAS* subunit c mitochondrial ATP synthase

been associated with more severe disease and young age of onset. Phenotypes are often biochemically indistinguishable. Prognosis is thus mainly based on clinical presentation.

Different from most inborn errors of metabolism, the LSDs do not present with "intoxication type" symptoms with acute decompensation with altered mental status, but have a gradual onset of symptoms and an invariably progressive course. With the development of disease modifying treatments options, some previously severe and fatal LDSs have now turned into more chronic conditions, with survival well into adulthood, displaying a new phenotype of the disease [25].

As much as two-thirds of the LSDs may present with neurological disease. This can range from central nervous system involvement leading to developmental delay and behavioural problems to peripheral neuropathy [26]. Also organomegaly, corneal disease, skeletal involvement and connective tissue disease are often seen.

1.5 Current Therapeutic Options in Lysosomal Storage Diseases

During the last decades, much progress has been made in the development of therapies for lysosomal storage diseases (LSDs). For some disorders, disease modifying treatment is now available. As none of the disease can yet be completely cured, there is still a large role for supportive care. Main goals in the treatment of LSDs are to ameliorate symptoms and prevent complications.

1.5.1 Supportive Care

Regardless of the type of LSD, as long as there is no curative therapy available, supportive care is and will be the corner stone of treatment. Supportive care should be coordinated by a physician with experience in lysosomal storage disorders. Depending on the expected complications, other specialists such as pulmonologists, cardiologists, otorhinolaryngologists, anaesthesiologists, orthopaedic surgeons, physiotherapists and neurosurgeons should be involved in the management and prevention of symptoms and complications. Besides providing "standard care" every specialist thus involved should be aware of the typical problems that may occur in patients with a lysosomal storage disease. These issues can be life threatening complications, such as cervical instability and cardiac failure, or may primarily affect quality of life, such as immobility, loss of vision and hearing impairment. Arising from a completely unique pathophysiology, these complications require special and multidisciplinary attention [27, 28].

In many LSDs, surgical interventions are frequently needed. Surgical interventions may include carpal tunnel release, ventriculoperitoneal shunting, heart valve replacement or orthopaedic interventions [29]. It is advised that surgical procedures take place in specialized centres. Especially general anaesthesia might be challenging as some of the lysosomal storage diseases such as mucopolysaccharidosis may

present with a distorted anatomy due to clinical features as an enlarged tongue, a short stiff neck and odontoid dysplasia [30, 31].

Aside from somatic care, patients and families with lysosomal storage disorders in general require psychosocial support. The decrease in health related quality of life is not only associated with disease severity, but also with the inability to maintain friends or a hobby [32]. Educational programmes aimed to improve knowledge and promote self support in patients and their care takers may substantially improve quality of life.

1.5.2 Disease Modifying Treatment Options

1.5.2.1 Hematopoietic Stem Cell Transplantation

Disease modifying therapies have been available for lysosomal storage diseases only since the early 1980s, after Hobbs and colleagues performed the first bone marrow transplantation in a patient with Hurler disease. This was successful, with engrafted survival as well as decrease in glycosaminoglycans excretion, reduction in liver size and an arrest in the deterioration of cognitive development [1]. After this encouraging result, bone marrow transplantations have been performed in many other LSDs. It was the discovery of the mechanism of "cross-correction" that led to the hypothesis that this procedure may benefit patients with a lysosomal storage disease.

In 1968 Elisabeth Neufeld and her group reported that the biochemical defects in co-cultured skin fibroblasts of Hunter and Hurler patients were corrected [33]. In addition, they showed that co-culturing with control fibroblasts also resulted in correction of the biochemical defect. Later studies demonstrated that this process of cross-correction was based on the phenomenon that lysosomal enzymes can be excreted and imported by cultured cells. Most of the lysosomal enzymes are targeted for their lysosomal destination by a residue which is recognized by a mannose-6-phosphate receptor (M6P receptor). For enzymes that are not transported to the lysosome, but is instead secreted from the cell, a surface M6P receptor facilitates their re-uptake. These enzymes can subsequently reach the lysosome through the endocytic pathway (see Fig. 1.1).

Cross-correction uses this physiologic process and the essential fact that secreted enzymes are not just re-captured by the excreting cell, but might also be captured by any neighbouring cell with a surface M6P receptor.

As it appears that in most of the LSDs an enzyme activity of only 10–20 % of normal may already substantially improve the clinical outcome, cross-correction is an important mechanism in developing therapeutic strategies [34].

So, through performing bone marrow transplantations, enzyme producing and secreting cells become available in patients with a lysosomal storage disease. Due to its size, secreted enzymes are not able to pass the blood barrier, but surprisingly neurodegeneration is halted in transplanted patients with some types of LSDs. In addition, a decrease in white matter lesions on brain MRI has been reported [35]. This is attributed to the fact that microglia cells in the brain are derived from bone

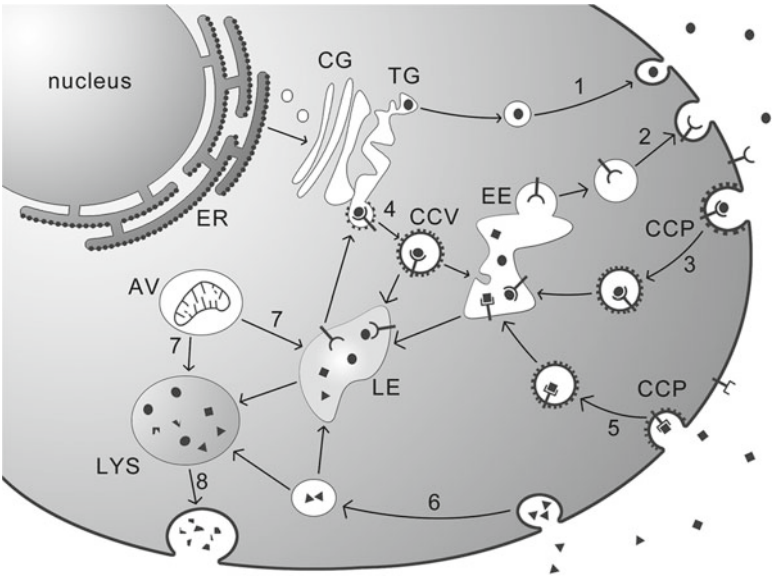

Fig. 1.1 Schematic overview of the lysosome and related intracellular processes. 1: Exocytosis of proteins from the Golgi network. 2: Mannose-6-Phosphate (M6P) receptors return to the cell membrane. 3: M6P receptors facilitate uptake of labelled hydrolytic enzymes. 4: M6P receptors regulate transport of newly formed hydrolytic enzymes from the *trans*-Golgi-network (TGN) to the endosomal/lysosomal system. M6P receptors can be recycled from the LE to the TGN. 5: Receptor mediated endocytosis. 6: Non-receptor mediated endocytosis. 7: Autophagy. 8: Exocytosis. *AV* autophagosome, *CG cis*-Golgi-network, *TG trans*-Golgi-network, *ER* endoplasmatic reticulum, *CCV* clathrin coated vesicle, *EE* early endosome, *LE* late endosome, *LYS* lysosome, *CCP* clathrin coated pit

marrow precursors. After a haematopoietic stem cell transplantation (HSCT), the deficient microglia cells are gradually replaced by enzyme secreting cells, providing the surrounding neuronal cells with the deficient enzyme. Partial or late onset neurological benefits can be due to the slow rate of replacement for microglia cells. Unfortunately, neurological damage already present before HSCT is often irreversible. As a consequence, early—preferably presymptomatic—diagnosis and early initiation of treatment is of utmost importance to improve the outcome of HSCT.

As the brain is populated with microglia cells, other tissue macrophages also infiltrate organs throughout the body. These haematopoietic precursors migrate to the liver to form enzyme producing Kupffer cells and in the lungs they replace deficient alveolar macrophages. Due to their wide tissue distribution they increase the amount of available enzyme [36].

Because of the risks of morbidity and mortality associated with HSCT, the procedure was initially indicated only for patients with the more severe phenotypes of the diseases. Currently most experience is in patients with severe MPS I, also known as Hurler phenotype. In 2005 the European Group for Blood and Marrow Transplant (EBMT) developed transplantation guidelines for these patients.

Based on the success of HSCT, this was subsequently tried in many other LSDs with often disappointing results. However, recent advances in treatment regimen and the donor source may chance these policies in the near future [36].

1.5.2.2 Enzyme Replacement Therapy

The ability of cells to reuptake lysosomal hydrolases is not just the mechanism on which haematopoietic stem cell transplantation relies; it is also the basis of enzyme supplementation or replacement therapy (ERT). With ERT the enzyme is not produced endogenously, but infused at regular intervals as a recombinant enzyme.

As early as 1973, the first attempts to use enzyme replacement therapy (ERT) in LSDs were made by intravenous injection of urine-derived hexosaminidase A in a patient with Sandhoff disease. Biochemically this resulted in a significant reduction of the accumulated globoside in the circulation, but no clinical improvement was noted [37].

As an alternative source for enzyme, the human placenta was later investigated as option for ERT, first for Fabry disease, later for Gaucher disease [2, 38]. Glucocerebrosidase isolated from human placentae was made available in sufficient quantities and the first clinical trials were initiated. In order to target the exogenous enzyme to macrophages (the most affected cells in Gaucher disease) the glycosylation signal on the enzyme was modified to contain mannose-terminal oligosaccharide side chains. In the early 1990s the first patients with non-neuronopathic Gaucher disease were treated with this modified glucocerebrosidase and an excellent clinical response was observed with a decrease in spleen and liver size and resolution of anaemia and thrombocytopenia and improvement of skeletal disease [39]. In 1991 this first clinically effective ERT was approved by the regulatory authorities in the USA and later in many other countries. Later, Chinese hamster ovary cells were genetically modified to produce glucocerebrosidase which is subsequently processed to express a mannose terminated oligosaccharide residue. This enzyme, imiglucerase, was approved in 1994 for the use in patients with Gaucher disease. At present, over 4,000 patients are treated with this product.

The remarkable success of ERT in Gaucher disease led to increased attention for new therapeutic strategies in lysosomal storage disease in general and ERT in particular. This resulted in the development more recombinant enzymes, as shown in Table 1.2.

It is important to realize that where in Gaucher disease the recombinant enzyme is effectively targeted for only one cell type, this is insufficient in other LSDs as they encompass many more cell types. In addition, it is increasingly recognized that antibody formation may be a great challenge in some of the enzyme replacement therapies, as this might lessen the available enzyme and its effects. Despite these challenges, ERT has proven to significantly reduce some of the somatic signs and symptoms in LSDs and improve the quality of life of the patients.

Table 1.2 Approved enzyme replacement therapies in lysosomal storage diseases

Disease	Deficient enzyme	Year of introduction	Recombinant enzyme
Gaucher disease	Glucocerebrosidase	1994	Imiglucerase (Cerezyme®)
		2010	Velaglucerase α (VPRIV®)
Fabry disease	α-Galactosidase A	2001	Agalsidase α (Replagal®) and
		2003	Agalsidase β (Fabrazyme®)
Pompe disease	Acid α-glucosidase	2006	Alglucosidase α (Myozyme®)
MPS I (Hurler, Hurler/Scheie, Scheie)	α-L-iduronidase	2003	Laronidase (Aldurazyme®)
MPS II (Hunter)	Iduronate sulfatase	2006	Idursulfase (Elaprase®)
MPS VI (Maroteaux-Lamy)	Arylsulfatase B	2005	Galsulfase (Naglazyme®)

1.5.2.3 Substrate Reduction Therapy

A different approach to the treatment of LSDs is substrate reduction therapy. By partially inhibiting the synthesis of the substrate for the deficient enzyme, the influx into the lysosome and subsequent accumulation may be reduced. Any residual activity of the deficient enzyme might be enough for the lysosome to cope with the amount of macromolecules still synthesized. At present, *N*-butyldeoxynojirimycin (miglustat) is the only registered substrate reduction therapy in LSDs. This iminosugar inhibits the synthesis of glucosphingolipids and is registered for Gaucher disease and Niemann Pick type C. Clinical trials are being performed to confirm its beneficial effects and safety in other LSDs [40, 41].

1.6 Future Therapies

As more knowledge on the pathophysiology processes involved in lysosomal storage diseases (LSDs) is gained, new treatment options are considered and studied. Although a number of successful therapies have been introduced during the last decades, many LSDs remain without a proper disease modifying option. Also, there are limitations to the existing therapies. Often, the central nervous system involvement is not or only partially treated, resulting in ongoing brain disease.

New therapeutic options are therefore still very much needed. Several promising possibilities will be discussed in some detail.

1.6.1 Gene Therapy

Lysosomal storage diseases appear to be ideal candidates for gene therapy. Firstly, most LSDs are caused by a single genetic defect. Secondly, the involved genes are not subject to a complex regulatory system. Various experiments with both microencapsulation and viral vector delivery systems have been performed. Although the in vitro and non-human in vivo results are often promising, clinical experience is still limited.

In gene therapy, the aim is to restore enzyme availability in one of two ways. The first technique allows genes to be transfected into the subject's cells by viral vectors such as adeno-associated or lentiviruses. Once the gene is expressed, these cells will produce the specific enzyme which will not only provide for their own lysosomal degradation of the storage product, but through cross-correction will also treat cells in their vicinity.

Another form of gene therapy consists of the so called microencapsulation technique. In this situation, genetically modified cell are encapsulated in a semipermeable membrane and then brought into the host. The membrane allows for exchange of molecules such as nutrients and metabolites between the cell and its environment, while preventing the access of the immune system. In microencapsulation cross-correction is again the mechanism with which the surrounding cells receive their deficient enzyme [42, 43].

1.6.2 Small Molecules Therapy

Enzyme deficiency is the result of a misbalance between synthesis, degradation and functionality of the enzyme. Misfolding, for instance, leads to premature degradation of enzymes. Small molecule therapies aim to restore the balance.

In chaperone therapy, enzyme stabilization is facilitated by administering a low dose of hydrolase inhibitory molecules. The otherwise degraded enzyme might in this way bypass the cell's quality control mechanism and still perform its function in the lysosome. No human in vivo trials have been published to date [43, 44].

Substrate reduction therapy (SRT) is a form of small molecule therapy. The clinical success of miglustat use in Gaucher and Niemann Pick type C disease has prompted more research. Both miglustat's efficacy in other LSDs and alternative substrate reducing agents are currently under investigation. At present, phase 2 clinical trials with eliglustat are performed, which might prove a more potent form of SRT in Gaucher disease.

1.6.3 Alternative Uses of Known Therapies

In addition to the search for new therapies, new utilities for known therapies are studied. For example, combining therapies in order to target different aspects of disease at the same time might improve clinical outcome in LSDs. Both additional and synergistic effects have been noted in murine models [45]. No clinical data is available at this point. The combined therapies might be LSD-specific such as ERT and SRT for Fabry disease, but also commonly used drugs as non-steroidal anti-inflammatory drugs [43].

As well as combining two or more therapies, an alternative route of administration might also improve efficacy of known drugs. For instance, direct intra-thecal

injection allows for bypassing the blood–brain barrier. Intrathecal enzyme injection has been tried in a patient with MPS I and MPS VI, with some clinical improvement [46] and is currently studied in MPS II and MPS III patients. Another way of combining two therapeutic strategies may consist of genetic modification of autologous bone marrow. This method was used by Aubourg and colleagues [47]. They performed an autologous HSCT in two patients with the peroxisomal inborn error X-linked adrenoleukodystrophy. Before re-infusion the patients CD34+ cells were genetically corrected ex vivo to produce the deficient enzyme. Thus genetic modification and transplantation techniques may decrease the risks and side effects of these techniques, and improve efficacy.

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Chapter 2
Alternative Treatment Options: Enzyme Replacement and Small Molecule Therapies

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2.1 Enzyme Replacement Therapy

Initial attempts at enzyme replacement therapy (ERT) for lysosomal storage disease (LSD) began in the 1970s using enzyme extracted from placenta or urine. Although some proof of concept was obtained, it quickly became apparent that there were a number of problems that needed solution before this approach could be developed further into a useful therapy for affected patients [11].

These initial problems included an inability to produce pharmacological quantities of enzyme necessary to treat all patients, an inability to target the infused enzyme to all tissues and a lack of animal models on which to evaluate this new approach to therapy. Consequently interest in this approach to treatment declined and other forms of therapy were pursued culminating in the introduction of bone marrow transplantation in the early 1980s. The identification of the mannose-6-phosphate receptor in the late 1970s was a pivotal moment for subsequent development of ERT but one that could not be exploited at that time [25].

A breakthrough of importance in ERT was made in the early 1990s when it was demonstrated that 2–3 mg/kg of placental derived, mannose-terminated, β-Glucosidase markedly improved haematological indices and reduced hepatosplenomegaly in patients with type I Gaucher disease [3, 4]. The demonstration that fortnightly infused enzyme was well tolerated, apparently free from side-effects and efficacious in reversing many years of substrate accumulation, confirmed that this clinical approach to treatment for non-neurological LSDs was worthy of pursuit.

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Genzyme, a (then) small biotechnology company founded in June 1981, purified and modified β-glucosidase from human placenta collected on an industrial scale. The resulting enzyme, marketed as Ceredase, revolutionized the treatment and outcome for many sufferers of this progressive disorder. Advances in gene identification and cloning led to the subsequent production of and demonstration of equal efficacy of recombinant human enzyme (produced in Chinese hamster ovary (CHO) cells. The introduction of Cerezyme® [16] led to the withdrawal of the placental product. As a parallel development, the identification and availability of animal models (especially domestic species, e.g. MPS I dog and MPS VI cat) allowed for the first time new therapies to be studied carefully in a preclinical setting.

Currently available therapies include three ERTs for Gaucher disease (Imiglucerase, Genzyme; Velglucerase alfa, Shire Human Genetic Therapies and Taliglucerase alfa, Protalix) two products for Fabry disease (Agalsidase alfa, Shire Human Genetic Therapies and Agalsidase beta, Genzyme) as well as products for MPS I (Laronidase, BioMarin), MPS II (Elaprase, Shire Human Genetic Therapies), MPS VI (Galsulfase, Biomarin), Pompe disease (Aglucosidase alfa, Genzyme). A treatment for MPS IV A is in clinical trial and recombinant enzymes for deficiencies of sphingomyelinase, arylsulfatase A and alpha-mannosidase are in the pipeline.

2.1.1 Goals of Treatment

Before considering individual disorders it is important to have a clear idea of what one hopes to achieve from therapy. In LSDs these aims can be summarized as follows. The treatment should be safe and the level of storage within the cells or organs of the individual should be reduced and as a consequence the natural history of the disease should be altered favourably. Effective treatment should leave a minimal residual disease burden and the burden of treatment should be less than the burden of the illness. Finally, the treatment should be affordable [61].

2.1.2 The Expected Response to Treatment

It would be hoped that if ERT or any other therapy were to be instituted before symptoms the patient would remain asymptomatic. For treatment commencing after the patient has begun to show clinical signs it would again be hoped that the patient could be returned to an asymptomatic state. In reality for most LSDs there is a residual disease burden as with time most disorders are associated with irreversible damage. The size of the residual disease burden is critical in assessing the success or failure of an individual therapy. To give an example, some patients with infantile Pompe disease will continue to exhibit marked skeletal muscle dysfunction despite dramatic improvement in cardiomyopathy following ERT. If the skeletal muscle disease is severe enough to require full-time ventilation because of respiratory

muscle weakness, the residual disease burden is enormous and many would doubt the efficacy of the underlying therapy, especially as it has to be maintained regularly at great expense.

The end result of a variable residual disease burden in a group of heterogeneous disorders is the production of multiple, different, new clinical phenotypes whose natural history is unknown.

2.1.3 Individual Disorders Treated by ERT

2.1.3.1 Gaucher Disease

Gaucher disease (GD) is an autosomal recessive disorder resulting from a deficiency of β-Glucocerebrosidase (β-Glucosidase, GBA, EC 3.2.1.45). The enzyme deficiency results in an accumulation of glucosylceramide (GlcCer, glucosylcerebroside) primarily within cells of the macrophage–monocyte system leading to the characteristic "Gaucher cell". The disorder is particularly common in individuals of Ashkenazi Jewish origin [62] and has an estimated worldwide prevalence of around 1:75,000 births [17].

The disorder produces a clinical spectrum of disease that has for convenience been traditionally described as three types depending on the presence or absence of neurological involvement:

- Type I non-neuronopathic
- Type II acute neuronopathic
- Type III chronic neuronopathic

There is no evidence that ERT can influence favourably the neurological components of GD II or III. It can however improve the visceral disease seen in GD III and ERT should be prescribed for these patients. Some groups also favour the use of ERT in GD II as part of a package of palliative care aimed at improving symptoms but this approach has not been met with uniform approval. The majority of patients on treatment will however have type I disease and it is in this group that the major benefits of therapy are seen.

Early treatment with Cerzyme® (Imiglucerase, Genzyme) in GD I results in an improvement in anaemia, thrombocytopenia, organomegaly, bone pain and bone crises. Treatment regimens have varied but most are now based on the recommended licensed dosage of 60 Units/kg/2 weeks. Many patients show significant improvements on a lower dose and a compelling case can be made for a more individualized approach to dosage regimens [20]. Therapeutic goals have been established against which therapy can be monitored [37] and long term safety of ERT has been assessed from patient registry data [50].

The success of Cerezyme® therapy in the treatment of GD I has encouraged the development of two further enzyme products. The first product Velglucerase alfa (Shire Human Genetic Therapies) is made by gene activation in a human cell and as

a consequence is believed to be more favourably glycosylated resulting in increased cellular uptake when compared to Cerezyme® [7]. The third product, Taliglucerase alfa (Uplyso®, Protalix) is produced in a plant based expression system. Velaglucerase (Vpriv) has now been licensed for use [63]. The worldwide shortage of imiglucerase (Cerezyme®) beginning in late 2009 highlighted the need for alternative products to be available even for very rare disorders [22].

2.1.3.2 Fabry Disease

Fabry disease is an X-linked disorder caused by deficiency of the lysosomal enzyme α-Galactosidase A (GLA, EC3.2.1.22). The resulting accumulation of globotriaosylceramide leads to a wide spectrum of clinical signs and symptoms that affect many organs, including the brain, heart and kidney [8]. Unlike most other X-linked disorders carrier females often exhibit signs and symptoms of organ damage similar to affected males [56]. The incidence of Fabry disease has been estimated to be 1 in 40,000 to 1 in 117,000 worldwide although data from newborn screening programmes suggests that late onset variants may be much more common than this [49].

HSCT has never been suggested as a potential therapy for Fabry disease. Although untreated patients die much younger than average the mainstays of treatment until the introduction of ERT were palliative therapies including medications to control pain, renal replacement therapy and cardiac medications [31].

Outside of the USA both agalsidase alfa (Replagal®, Shire Human Genetic Therapies) 0.2 mg/kg every other week and algasidase beta (Fabrazyme®, Genzyme) 1 mg/kg every other week are available as ERT to treat Fabry disease, whereas in the USA only agalsidase beta has been approved so far.

Clinical trials have suggested efficacy of both products and the evidence supporting this has recently been reviewed [46]. In brief, the timely introduction of ERT can prevent disease progression in many patients with Fabry disease, but in some patients with advanced disease ERT may slow down but not prevent end organ failure [2].

2.1.3.3 Pompe Disease

Glycogen storage disease type II (GSD II, Pompe disease) is an autosomal recessive disorder caused by a deficiency of acid alpha-Glucosidase (GAA, EC 3.2.1.20). The classic infantile form of the disease (Pompe disease) is associated with muscular hypotonia and a rapidly progressive hypertrophic cardiomyopathy that leads to death in the first year of life in the majority of affected patients [27]. Late onset forms (juvenile or adult onset GSD II, also known as acid maltase deficiency) are primarily a disorder of skeletal muscle (often mistaken for limb girdle muscular dystrophy) leading to progressive problems with mobility and respiratory function. Cardiomyopathy is not a feature of late onset variants [59]. The history of ERT for Pompe disease, culminating in the development of Myozyme® (Alglucosidase alfa, Genzyme), has been reviewed recently [53].

Unlike other LSDs the dosage of enzyme required to effect an improvement in clinical condition is much higher in Pompe disease (20 mg/kg/dose compared with 0.2–1.0 mg/kg/dose). In addition, ERT is much more effective in clearing glycogen from cardiac as opposed to skeletal muscle. In the latter type II fibres are particularly difficult to treat [44]. Despite these limitations ERT has demonstrated long term efficacy in infantile Pompe disease [28] as well as leading to improvements in late onset variants monitored over a 12 month period [51].

2.1.3.4 Mucopolysaccharidoses

Successful clinical trials have led to the approval of ERT for MPS I, II and VI [18, 35, 60]. Modest improvements in respiratory function, growth and endurance are common after the institution of therapy but ERT cannot influence the development of CNS disease as the enzymes are unable to cross the blood–brain barrier. Attempts have been made to establish criteria or guidelines for therapy but these still generally remain nationally based and differ from country to country. A major challenge is deciding on the role of ERT in patients with significant cognitive impairment. The use of ERT in severely affected patients as part of a general package of palliative care is favoured by some groups whilst others refuse or are not allowed to prescribe for patients with significant learning disability. Whatever the degree of cognitive impairment is present it is important to have clearly defined treatment goals and be prepared to discontinue therapy in patients who appear to be gaining no benefit from treatment.

In patients with spinal cord compression due to dural hyperplasia intra-thecal ERT (as well as intravenous) may be an appropriate approach to therapy and prevent the need for invasive surgery [36]. The fact that enzyme can be delivered safely by this route also allows the consideration of using this method of treatment to try and obtain brain penetration in those disorders associated with cognitive impairment e.g. Mucopolysaccharidosis type III (San Filippo disease). This approach has already been tried in MPS III dogs and a clinical trial in human patients with MPS III is underway [19]. A clinical trial of intra-thecal idursulfase (Shire HGT) for cognitive impairment in MPS II is also underway.

The currently approved preparations with their recommended dosages are Aldurazyme® (Laronidase, Biomarin) 100 units (0.58 mg)/kg/week for MPS I, Elaprase® (Idursulfase, Shire Human Genetic Therapies) 0.5 mg/kg/week for MPS II and Naglazyme® (Galsulfase, Biomarin) 1 mg/kg/week. A Phase III clinical trial of recombinant galactosamine 6-sulfatase (GALNS, BMN 110, Biomarin) for MPS IVA (Morquio disease) has been completed and met the primary endurance related end point.

2.1.3.5 Other Disorders

ERT is in development for a number of other disorders including sphingomyelinase deficiency (Niemann–Pick disease type B, NPB, Genzyme), arylsulfatase A

deficiency (metachromatic leucodystrophy, MLD, Shire Human Genetic Therapies), alpha mannosidase deficiency (alpha mannosidosis, Zymenex), lysosomal acid lipase deficiency (Wolman disease, Synageva corp) and there are likely to be others.

2.1.4 Limitations of Enzyme Replacement Therapy

The clearly documented safety and efficacy of ERT in GD has led Cerezyme® to be regarded as the "gold standard" against which all other products are compared even though the disorders being treated are diverse. Although there is no doubting the efficacy of Cerezyme® in GD, there are clearly some limitations [12]. First, the treatment is intravenous, although it can be delivered safely in the patient's home. Second, the therapy is ineffective in neuronopathic disease even if instilled directly into the cerebrospinal fluid and has relatively poor efficacy against pre-existing bone and lung disease. Finally like all recombinant protein products, the treatment is expensive and therefore unless funded by charitable means, is unavailable to patients in countries that have more pressing health care concerns. Despite Cerezyme's clear shortcomings none of the subsequently developed ERTs approach its efficacy due to the nature of these other disorders and due to limitations of the enzyme products themselves.

2.1.4.1 Limitations of Therapy Due to the Nature of the Disease

For most of the disorders under treatment we lack a comprehensive knowledge of the natural history of the disease. This is not surprising as most clinicians, even in very big clinics, will not see the full range of presentations of these individually rare disorders. Furthermore there is increasing evidence that some of the clinical symptoms associated with LSDs may be initiated by processes secondary to the storage of substrate. It is by no means certain that ERT can "switch-off" these secondary effects, and the resulting pathogenic cascades could go on to produce further damage to tissues and organs despite timely introduction of ERT. This whole area of lysosome biology and LSD pathogenesis has been reviewed recently [54].

With the exception of Gaucher disease, where Cerezyme® has been available for a number of years, our knowledge about the efficacy of the products is based on clinical trials performed on patients with relatively advanced disease. The reasons for this are understandable—the disorders are rare, there is a need to demonstrate a clinical effect quickly and the patients that are able to cooperate with sophisticated testing are therefore older and inevitably have more advanced disease than those younger patients who would perhaps be too immature to cooperate fully with testing. Until we are able to judge the ability of the various enzymes to *prevent* disease, by being used in a prophylactic manner following early diagnosis of the LSD, we will remain unaware of the full potential of this therapy.

Finally, for most of the disorders, we lack biomarkers and severity scores to allow us to judge the stage of the disease in the individual and also the efficacy of the therapy in reducing the disease burden. The role of biomarkers in LSDs has recently been reviewed [10] and a number of severity scores have also been produced recently to try and address these deficiencies and to aid with assessing efficacy [15, 58].

2.1.4.2 Limitations of the Treatment Itself

Unfortunately, none of the currently prescribed ERTs is able to treat all aspects of the disorders equally. Animal studies have revealed organ-specific variations in response to ERT. Among other studies showing similar results, ERT in the dog model of MPS I, for example, produced no significant changes histologically in cartilage and heart valve despite high-dose therapy for a prolonged period [24]. Experience has shown that bone, cartilage, heart valves and brain remain especially resistant to correction by intravenous ERT, and alternative methods of delivery or targeting will be needed if these problems are to be overcome. There is some evidence that very early (presymptomatic) treatment by ERT may lead to a much better outcome [13]. However, until ERT can be linked to a newborn screening programme for LSDs, this question will remain unanswered as the majority of patients present with a significant disease burden.

Of increasing concern is the development of specific antibodies against the infused proteins. Whilst severe infusion associated reactions are fortunately rare and usually manageable [26, 34] antibodies that inhibit enzyme activity or block uptake of enzyme into the cell are of more concern. The clearest clinical example of this is seen in infantile Pompe disease where patients with mutations that lead to the production of no natural protein (CRIM negative, Cross Reactive Immunologic Material negative) have a much worse outcome than patients that are CRIM positive and this is directly related to the early onset and very high antibody titres seen in the CRIM negative patient [29]. Successful attempts at eliminating antibodies to Myozyme® in Pompe disease has been reported [32, 33] but in most patients with very high titres and deteriorating clinical disease this has proved impossible and most patients will succumb to their disease. What is becoming clear is that for a small number of patients with all the disorders under treatment with enzyme replacement therapy significant immunological reactions will limit benefit and indeed may put the patients at risk [45]. Immune tolerance regimens and better understanding of the role played by these antibodies need to be developed quickly if these patients are to benefit from this major advance in therapy [55].

The final limitation to treatment is the financial burden, which makes therapy difficult to obtain for those in countries that have more pressing health care needs. As an example the approximate cost *per year* for a 70 kg adult patient with Gaucher, Fabry and Pompe disease on licensed dosages of Cerezyme®, Fabrazyme®, Replagal® and Myozyme® is £200,000, £118,000, £130,000 and £269,000 (taken from prices in British National Formulary [bnf.org], March 2009 and using dosages

of 60 mg/kg, 1 mg/kg/, 0.2 mg/kg and 20 mg/kg respectively). For patients with a mucopolysaccharide disorder, who are generally smaller, the approximate costs for a 40 kg patient with MPS I (Aldurazyme®), II (Elaprase®) and VI (Naglazyme®) are £192,000, £310,000 and £255,000 (taken from prices in British National Formulary [bnf.org], March 2009 and using dosages of 100 units/kg/week, 0.5 mg/kg/week [rounded down to take into account vial size] and 1 mg/kg/week) respectively.

2.2 Conclusions

ERT has been a major advance in the treatment of LSDs. Many disorders now have therapy and our efforts should be aimed at improving targeting to brain and bone, where considerable limitations still remain. The full potential of ERT has not yet been realized because the majority of patients treated either in clinical trials or on commissioned therapy have had advanced disease and we will only understand the full impact of this therapy when it is used on a cohort of patients identified soon after birth.

For the majority of patients the treatment will be safe but there is increasing concern about immune reactions leading to treatment failures in a significant minority of patients with most diseases with the possible exception of Gaucher disease.

Cost remains an issue for many countries. The treatment is expensive and, as more complicated and less prevalent disorders are targeted for therapy, costs have risen, so that ERT remains impossible to obtain for many affected patients.

2.3 Substrate Reduction Therapy

Substrate reduction therapy (SRT) offers a different approach to the therapy of LSDs by reducing the rate of synthesis of macromolecules (for example glycosphingolipids, GSL) to a level where any residual enzyme activity in the cell is sufficient to prevent substrate accumulation. It should be possible over time to reverse storage and storage related pathologies. This approach has been exploited with the development of Zavesca® (Miglustat, Actelion) an *N*-alkylated imino sugar and a synthetic analogue of D-glucose. Zavesca® is an inhibitor of the enzyme glucosylceramide synthase, which is a glucosyl transferase enzyme responsible for the first step in the synthesis of most GSLs. An advantage of Zavesca® is that it is of low molecular weight as well as being active orally. It is known to cross the blood–brain barrier and therefore offers a potential therapy for LSDs with a CNS component due to an accumulation of GSLs.

The most common adverse effects of Zavesca® seen in clinical trials and confirmed in post marketing surveillance are diarrhoea (mild to moderate) and weight loss (6–7 % of body weight) occurring in 80 % and 65 % of treated patients respectively. More serious complications such as peripheral neuropathy have been reported

and Zavesca® is also contraindicated in pregnancy, but on the whole the product has been well tolerated. More details about safety and tolerability can be found on the Zavesca® website (www.zavesca.com).

2.3.1 Gaucher Disease

In Gaucher disease the primary disease pathology is associated with the storage of specific GSLs and therefore would seem the ideal disorder to respond to SRT with Zavesca®. Subsequent clinical trials indeed led to the approval of Zavesca® by both the EMEA and the FDA for use in Gaucher disease type I patients felt to be unsuitable for ERT. This would include patients with very difficult venous access, needle phobia or a history of allergic reactions to Cerezyme®. Detailed guidance on the use of Zavesca® in type I Gaucher disease has been published [57] and long term post authorisation follow up studies have confirmed a favourable safety profile [21].

Most of the therapeutic goals in Gaucher disease (including improvements in bone disease) that have been established for ERT can also be met by Zavesca® but at a slower rate. This is to be expected given the differing modes of action of Cerezyme® and Zavesca® [38].

The attractiveness of an oral therapy for Gaucher disease has led to the development of other possible substrate inhibitors. Genz-112638 is a novel glucosylceramide analogue and when given orally partially inhibits glucosylceramide synthase, resulting in reduced production of glucosylceramide. The drug is claimed to have high potency and favourable results were obtained in a Phase II study [40]. A definitive Phase III study started enrolling patients in late 2009.

2.3.2 Niemann–Pick Disease Type C

Niemann–Pick Disease type C (NPC) is an inherited neurodegenerative disorder characterized by an intracellular lipid-trafficking defect with secondary accumulation of glycosphingolipids. A clinical trial of Zavesca® in NPC showed improvement or stabilization of a number of clinically relevant markers of disease activity over a 12 month period [39]. A number of subsequent observational studies have confirmed disease stabilization (especially in patients with more slowly progressive disease) but longer studies are needed to confirm whether or not the stabilization represents a temporary or permanent state [14, 41].

A major difficulty is deciding when to start Zavesca® in NPC patients. About a third of affected patients present with liver disease in the newborn period and of these the majority go on to make a full recovery only to re-present later with neurological signs and symptoms. The pre-symptomatic period may be many years, even decades in this group of patients [23]. It would be tempting to start Zavesca® as soon as the liver disease resolves but that would mean potentially many years on therapy

in the absence of symptoms with the additional uncertainty of not knowing whether the absence of symptoms was due to the drug or the natural history of the disease in the particular individual. In addition the long term effects of inhibiting GSL production in the presence of an immature CNS are unknown.

2.3.3 Other Disorders

Unfortunately Zavesca® has not shown the same degree of efficacy in other neurodegenerative LSDs. Clinical trials in Gaucher disease type III [47], Juvenile Sandhoff disease [30], late onset Tay-Sach's disease [48] have all failed to demonstrate a disease stabilizing effect from Zavesca®.

2.3.4 Other Substrate Reduction Therapies

Genistein (4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is the most abundant isoflavone in soy and one of its many actions is to inhibit the synthesis of glycosaminoglycans (GAGs) demonstrated in skin fibroblasts from patients with mucopolysaccharidoses [42]. Genistein has also been shown to cross the blood–brain barrier achieving levels of approximately 10 % of those attained in blood [52]. This combination of features make genistein an attractive candidate for treating mucopolysaccharidosis type III (San Filippo disease) and double blind, placebo controlled, clinical trials are anticipated in the near future.

Substrate optimization therapy (SOT) is a newly suggested approach based on making substrates more amenable to degradation by modifying their chemical structure without reducing the overall amount produced. It is claimed that this can be achieved in MPS disease by small molecules modifying the glycan sulfation pattern of the GAG biosynthetic enzymes [6].

2.3.5 Conclusions

Oral substrate reduction therapy currently has a limited role to play in the management of LSDs. There are clear indications for use of Zavesca® in type I Gaucher disease in patients unable to tolerate ERT. Other indications are less certain with the exception of Niemann–Pick disease type C where a disease stabilizing effect has been demonstrated. In animal models SRT has shown a synergistic effect with bone marrow transplantation and anti-inflammatory treatment and therefore in the future role of SRT may be as an adjunct to other modalities of treatment rather than being a primary therapy in isolation [43].

2.4 Chaperone Mediated Enzyme Enhancement

Certain DNA missense mutations lead to the production of proteins that are misfolded but which often retain active functional domains. The misfolded proteins produced in this way are usually blocked within the endoplasmic reticulum where they are degraded by the proteosomal system. Some low molecular weight ligands that are usually competitive inhibitors of these proteins can bind to the functional domains and in sub-inhibitory concentrations act as chaperones, rescuing the protein and allowing its transfer to the lysosome where the active functional domains can initiate some hydrolytic activity. The end result is a small increase in residual enzyme activity which in the individual patient may be enough to convert a severe disorder into a more attenuated form. The advantages of this approach are similar to those of SRT, namely that these chaperones are active orally and as they are of low molecular weight they can generally cross the blood–brain barrier. A major disadvantage is that this form of therapy is very mutation dependent and will not be suitable for the many patients that have disease caused by large DNA deletions or nonsense mutations associated for instance with no protein or the production of very truncated proteins.

As yet, no products of this type have fulfilled the regulatory requirements for licensing.

Amicus Therapeutics Inc. (Cranbury, NJ, USA) have led the way with this form of therapy and have a number of clinical trials in progress aimed at developing chaperone therapy for Fabry, Gaucher and Pompe disease. Although the respective products are at different stages of development the Pompe programme (AT 2220) ran into difficulties when two of the patients on the Phase II study deteriorated significantly on therapy. The FDA subsequently terminated this trial and Amicus plan to initiate a further phase I study of AT2220 specifically to gather more pharmacokinetic data and dependent on the results of this study further trials in patients may recommence (http://ir.amicustherapeutics.com/releasedetail.cfm?ReleaseID=412487).

Late onset Hexosaminidase A (Hex A) deficiency is another disorder often associated with misfolded and prematurely degraded protein. Cell culture studies subsequently revealed evidence of enzyme enhancement after exposure of the cells to the antimalarial pyrimethamine a competitive inhibitor of Hex A. An open-label Phase I/II study has recently reported encouraging preliminary results [9].

Once again combination therapies utilizing chaperones as adjuncts to more definitive therapies are likely to be the main role for this form of treatment.

2.4.1 Conclusions

Like SRT, chaperone therapy is likely to play a small role in the treatment of LSDs. The need for compliant mutations limits its use in many disorders and combinations of ERT, SRT, chaperones and cell based therapies perhaps offer the best chance of successful outcomes in disorders associated with CNS disease.

2.5 Stop Codon Read Through

In contrast to both SRT and chaperone therapy the efficacy of stop codon read through therapy depends on the presence of nonsense mutations within the gene leading to premature stop codons. A number of LSDs, especially the more severe variants, are caused in this way. PTC124® (Ataluren, PTC Therapeutics) is the first investigational product to be developed aimed at providing a mechanism for the ribosome to ignore the premature stop codon and allow some full length protein to be produced. This effect has previously been noted with aminoglycosides as well as some other compounds but the doses required in cell culture to gain an appreciable effect on residual enzyme activity are too high to be used in humans without the risk of severe side effects [5]. As yet there are no clinical trials of PTC124® in human LSDs although studies in cystic fibrosis, haemophilia and Duchenne muscular dystrophy have been initiated (http://clinicaltrials.gov/ct2/results?term=PTC+124).

2.6 Final Conclusions

ERT has made a contribution to improving the quality of life for many patients with LSDs. The positive clinical benefits are seen most keenly in Gaucher disease and whilst the other ERTs have not reached the same degree of efficacy, clinical improvements have been seen in many patients with Fabry, Pompe and mucopolysaccharide disease. Clinical efficacy depends on the stage of the disease when treatment commences (some disease elements are irreversible once established) and whether or not the patient generates neutralizing or blocking antibodies to the infused protein. Oral, small molecule therapies, have made less of an impact on patient outcome so far. There is a small, but clearly defined, place for the use of Zavesca® in Gaucher disease and Niemann–Pick disease type C, but other indications are so far lacking. Other substrate inhibitors are at a much earlier stage of development as are chaperone and other small molecule therapies. It may be that these treatments will never be stand-alone but will be destined to play a part in multi-modality therapy in combination with more definitive treatments such as transplantation or gene transfer.

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Chapter 3
Hematopoietic Cell Transplantation in Inborn Errors of Metabolism

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3.1 Introduction and Historical Perspective

Timely diagnosis and immediate referral to an IEM specialist are essential steps in management of these devastating disorders. Treatment recommendations are based on: the disorder; its phenotype including age at onset, rate of progression, severity of clinical signs and symptoms; family values and expectations; and the risks and benefits associated with available therapies such as hematopoietic stem transplantation (HCT). In this chapter we focus on HCT in IEM. HCT for an IEM is performed using donor cells from bone marrow (BM), umbilical cord blood (CB) and in a rare case with a growth factor mobilized peripheral blood (PB). Donor cells are infused into the patient after myelosuppression and immunosuppression using a chemotherapy containing regimen.

The rational for HCT in IEM is based on the cross-correction. The concept of cross-correction of metabolic defects with transferable lysosomal enzymes was described in 1968, when fibroblasts of patients with Hurler (MPS IH) and Hunter (MPS II) syndromes were co-cultured in the laboratory [1]. Metabolic correction of lysosomal storage diseases occurs by mannose-6-phosphate receptor-mediated endocytosis of secreted enzyme and by direct transfer of enzyme from adjacent cells [2].

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Both mechanisms are likely after HCT. The mechanism by which HCT halts cerebral demyelination of the peroxisomal disorder cerebral X-linked adrenoleukodystrophy (X-ALD) is threefold: immunosuppression, replacement with metabolically competent cell populations leading to decreased perivascular inflammation and metabolic correction. Migration, distribution, and growth of donor-derived metabolically competent cells into host tissues including the central nervous system (CNS) are critical to the success of transplant. Microglia, the hematopoietically derived mononuclear phagocytes of the CNS [3, 4], account for 5–10 % of non-neuronal cells in brain and, when activated, participate in antigen presentation, inflammation, and response to CNS injury [5]. The kinetics of microglial replacement after HCT is slower than that of other tissue macrophages (e.g., alveolar macrophages, Kupffer cells) [6], partially explaining the limited ability of HCT to stabilize the CNS and neurologic function with rapidly progressing cerebral disorders.

HCT in IEM started in the early 1980s when a 9-month-old boy with MPS IH received a related bone marrow being the first allogeneic HCT recipient for an IEM. The HCT led to normal development and intelligence, despite his having mutations (homozygous W402X) that were predictive of a severe phenotype [7]. After this first HCT, for various other IEM indications HCT were performed; Maroteaux–Lamy, Metachromatic Leukodystrophy, X-Linked Adenoleukodystrophy, Krabbe Disease [8–11]. The success in these diseases highly depended on disease, disease status, donor availability, etc. Currently, HCT from an HLA-matched, enzymatically normal related donor and unrelated donor cord blood transplant (CBT) are the most common modalities of HCT for IEM.

International collaborative efforts to examine HCT outcomes began in the late 1980s and continue today. Large multicenter and single-center reports on the outcomes of HCT have been published on MPS IH [12–14], cerebral X-ALD [57], MLD [15], and GLD [16]. These collaborations have resulted in international guidelines and significantly better outcomes. This chapter will discuss indications (Table 3.1) for HCT and outcomes of HCT for selected IEM. An international perspective on progress, limitations, and future directions in the field is provided.

Table 3.1 Inborn errors of Metabolism for which hematopoietic cell transplantation may be indicated

Disorder	Enzyme/Protein	Indication	Comments
Mucopolysaccharidoses			
Hurler (MPS IH)	Alpha-L-iduronidase	Standard	
Hurler/Scheie (MPS IH/S)	Alpha-L-iduronidase	Option	ERT first-line therapy
Scheie (MPS IS)	Alpha-L-iduronidase	Option	ERT first-line therapy
Hunter: Severe (MPS IIA)	Iduronate-2-sulfatase	Investigational	Only early or asymptomatic
Hunter: Attenuated (MPS IIB)	Iduronate-2-sulfatase	Option	ERT first-line therapy
Sanfilippo (MPS IIIA)	Heparan-*N*-sulfatase	Investigational	Only early or asymptomatic
Sanfilippo (MPS IIIB)	*N*-acetylglucosaminidase	Investigational	Only early or asymptomatic
Sanfilippo (MPS IIIC)	AcetylCoA:*N*-acetyltransferase	Investigational	Only early or asymptomatic

(continued)

Table 3.1 (continued)

Sanfilippo (MPS IIID)	*N*-acetylglucosamine 6-sulfatase	Investigational	Only early or asymptomatic
Maroteaux–Lamy (MPS VI)	Arylsulfatase B	Option	ERT first-line therapy
Sly (MPS VII)	Beta-glucuronidase	Option	
Leukodystrophies			
X-ALD, cerebral	ALD protein	Standard	Not for advanced disease
MLD: Early infantile	Arylsulfatase A	No	
MLD: late-infantile/Juvenile	Arylsulfatase A	Option/Standard	Asymptomatic
MLD: Adult onset	Arylsulfatase A	Standard	Only early or asymptomatic
GLD: Early onset	Galactocerebrosidase	Standard	Neonate, screening diagnosis, or second case in known family; not for advanced disease
GLD: Late onset	Galactocerebrosidase	Option	
Glycoprotein metabolic and miscellaneous disorders			
Fucosidosis	Fucosidase	Option	
Alpha-mannosidosis	Alpha-mannosidase	Option	
Aspartylglucosaminuria	Aspartylglucosaminidase	Option	
Farber	Ceraminidase	Option	
Tay-Sachs: Early onset	Hexosaminidase A	Unknown	Neonate, screening diagnosis, or second case in known family
Tay-Sachs: Juvenile	Hexosaminidase A	Unknown	
Sandhoff: Early onset	Hexosaminidase A and B	Unknown	Neonate, screening diagnosis, or second case in known family
Sandhoff: Juvenile	Hexosaminidase A and B	Unknown	
Gaucher I (non-neuronopathic)	Glucocerebrosidase	Option	ERT first-line therapy
Gaucher II (acute neuronopathic)	Glucocerebrosidase	Unknown	
Gaucher III (subacute neuronopathic)	Glucocerebrosidase	Unknown	Limited benefit of ERT
Pompe	Glucosidase	Investigational	ERT available
Niemann Pick: Type A	Acid sphingomyelinase	Unknown	
Niemann Pick: Type B	Acid sphingomyelinase	Option	ERT first-line therapy
Niemann Pick: Type C	Cholesterol trafficking	Option for C-2	
Mucolipidosis: Type II (I-cell)	*N*-acetylglucosamine-1-phosphotransferase	Investigational	Only early or asymptomatic
Wolman Syndrome	Acid lipase	Option	May be viewed as Standard
Multiple sulfatase deficiency	Sulfatases	Investigational	
MNGIE (mitochondrial neurogastrointestinal enchephalomyopathy)	Thymidine phosphorylase	Option	Not in advanced disease

Inborn errors of metabolism for which hematopoietic cell transplantation (HCT) may be indicated
Table does not include diseases where HCT is not indicated
Standard: HCT applied routinely. Considerable published research evidence from registries and institutions demonstrates efficacy. Delayed diagnosis and/or advanced disease may preclude transplant for individual patients. Option: HCT is effective but other therapy is increasingly considered first choice. Or, insufficient published evidence for HCT to be considered Standard. Investigational: Possible a priori reason for HCT. Further published evidence needed to support the use of HCT in clinical practice. Unknown: No published evidence that HCT is beneficial

3.2 Hurler Syndrome, MPS IH

Hurler syndrome (MPS IH), the most severe phenotype of alpha-L-iduronidase deficiency, is an autosomal recessive disorder characterized by progressive accumulation of storage material termed glycosaminoglycans (GAGs). Hurler and other phenotypes of MPS I-Scheie (MPS IS, attenuated) and Hurler–Scheie (MPS IH/S, intermediate) are a broad, continuous clinical spectrum. Accumulation of GAGs results in progressive, multisystem dysfunction including psychomotor retardation, severe skeletal malformations, life threatening cardiopulmonary complications, and premature death [2]. According to registries such as the Center for International Blood and Marrow Transplant Research (CIBMTR) and the European Group for Blood and Marrow Transplant (EBMT), over 600 HSCTs have been performed worldwide for children with MPS IH since 1980, making it the most commonly transplanted IEM.

HCT for children with MPS IH is effective, resulting in increased life expectancy and improvement of clinical parameters [17]. HCT must be performed early in the disease course before the onset of irreversible damage to derive maximum long-term benefit in children with MPS IH. Donor cell engraftment after HCT has resulted in rapid reduction of obstructive airway symptoms and hepatosplenomegaly. Hearing, vision, and linear growth improve in many cases. Hydrocephalus is either prevented or stabilized and cardiovascular pathology after HCT is altered beneficially. Although cerebral damage already present before HCT appears to be irreversible, successful HCT is able to prevent progressive psychomotor deterioration and improve cognitive function [14, 17].

Recent HCT experience for MPS IH demonstrates significantly improved graft outcomes when compared to earlier findings [Peters, Boelens]. Poorer historical results such as engrafted survival rates of 25–70 % [14, 18, 19] have been attributed to a clinical learning curve, restricted donor availability, graft failure, mixed chimerism and transplant-related morbidity and mortality. Predictors for graft-failure were identified as; T-cell depleted grafts and reduced intensity conditioning, while busulfan with therapeutic drug monitoring appeared to be predictor for higher "event free survival" [12, 13]. These data led to the development of an EBMT transplant protocol (EBMT/EHA Handbook 2008 and 2012). These guidelines included a standardized busulfan (BU)/cyclophosphamide (CY) conditioning regimen and the use of CB as a preferred graft source, second only to enzymatically normal matched sibling BM. This transplant protocol resulted in a significant improved survival rate to over 90 % compared to 53 % of the historical cohort (1994–2004) (REFs: north am. Clin and BMT 2007). In the most updated version of the handbook the conditioning was modified to BU (+ therapeutic drug monitoring) + Fludarabine (instead of cyclophosphamide) (EBMT/EHA Handbook 2012).

Over the past decade, unrelated CB has been used with increasing frequency as a graft source for HCT in children with an IEM. CB offers several advantages over BM or PB including better availability, greater tolerance for HLA mismatch, lower incidence and severity of graft versus host disease (GVHD), and reduced likelihood

of transmitting viral infections [16, 20]. Furthermore, laboratory studies suggest that CB stem cells may be capable of trans-differentiation into osteoblasts, chondroblasts, and neurons [21, 22]. A recent EUROCORD-Duke University MPS IH collaborative study showed that predictors for higher EFS were, (1) faster transplant (i.e., <4.6 months from diagnosis to transplant) with CB and BU/CY conditioning [12]. More recently the outcomes of an international cell source comparison study have been published [23] and extended and confirmed earlier reports. Early referral for HCT, with the best available HLA-matched donor offers the best EFS. The highest EFS rates were found in patients who received an identical matched sibling donor or an identical (6/6) unrelated cord blood, followed by 5/6 matched CB or 10/10 matched unrelated donor. Interestingly, all CB recipients had normal enzymes compared to mixed chimerism, associated with lower enzyme levels, in matched sibling and MUD donors. UCB units are particularly attractive as the unit is readily available and it is important to recognize most MSDs are carriers and influence post-transplant enzyme levels. Enzyme levels after HCT appears to be important for long-term outcomes, including neurocognitive outcomes [14, 17] and are currently analyzed in an international long-term outcome study, including most American and European MPS-1 patients. Completion of the ongoing study, an international collaborative effort to correlate enzyme levels with functional outcome may further refine donor selection for clinical practice.

A suggested alternative treatment is intravenous “enzyme replacement therapy” (ERT), which became available for patients with MPS I in 2003. While ERT is not the primary treatment for MPS IH, it is hypothesized that ERT prior to HSCT can improve the medical condition of the child and decrease the frequency of HSCT- and MPS IH-related complications [24]. Intravenously administered enzyme does not cross the blood–brain barrier (BBB), so ERT is not able to prevent CNS deterioration [25, 26]. The ERT trials in patients with attenuated forms of MPS I (Scheie, Hurler–Scheie) have demonstrated reduced organomegaly, decreased sleep apnea/hypopnea, improved pulmonary function, and increased physical ability [26, 27]. More recent more data is becoming available that neutralizing antibodies are a serious concern influencing the clinical effect.

The combination of ERT and HCT for MPS IH children has been evaluated in single and multicenter studies. These studies found that although ERT was well tolerated, the combination of ERT and HCT did not significantly affect rates of survival, engrafted survival, or HCT-associated morbidity [28, 29]. However, in the EUROCORD-Duke University study, as well as in the recent cell source comparison study ERT was not found to be a predictor influencing either EFS or transplant-related toxicities [12, 23]. But important to mention is that some patients, in a very poor clinical condition improved significantly on ERT, making them eligible for HCT. Currently, many transplant centers are administering ERT to MPS IH patients prior to HCT and continuing it until either start of the conditioning or achievement of donor-derived engraftment.

Despite these areas of success, some disease manifestations persist and even progress after HCT. Of note, musculoskeletal features often require orthopedic surgical interventions [17, 27]. Additionally, many conditions present at diagnosis or at

HCT may be irreversible, including neurocognitive dysfunction and corneal clouding [17]. Use of improved and reduced toxicity HCT techniques at an earlier age and the achievement of full donor chimerism with normalization of enzyme activity may enhance outcomes.

Overall, the long-term clinical outcome for MPS-1H children receiving HCT appears to be promising, yet variable from child to child. This variability is presumably due to: genotype, age and clinical status at HSCT, donor enzyme activity level, donor chimerism (mixed or full), stem cell source (CB, BM or PB), and the enzyme level in the recipient [17, 30]. As mentioned above, an international long-term follow-up study involving European and North American centers to evaluate the natural course and the influence of various patient, donor and transplant characteristics is underway.

Important progress has been made in the field in recent years, in part, due to international collaborations. HCT for children with MPS IH has become a safer procedure with recent survival rates exceeding 90 %. Greater attention is being paid to timely diagnosis, prompt HCT, and use of better procedures. CBT is now routinely used to perform early HCT and to foster higher rates of full-donor chimerism and normal enzyme levels. Although HCT is the procedure of choice for MPS IH, ERT is increasingly used as an adjuvant treatment prior to HCT, although the scientific basis for this practice lacks, except for patients in a poor clinical condition.

3.3 Other Mucopolysaccharidosis Syndromes

3.3.1 Hunter's Syndrome

Mucopolysaccharidosis II (MPS II), also known as Hunter's Syndrome is an X-linked, lysosomal storage disease caused by the deficiency of iduronate-2-sulfatase (I2S) that results in the accumulation of the glycoaminoglycans (GAG), dermatan and heparan sulfate, reviewed [31]. Glycoaminoglycans accumulate in various tissues and organs including the brain. Hunter syndrome occurs mostly in males, with a reported incidence of about 1 in 170,000 male births. Hunter's patients typically have normal appearance at birth, with the initial signs and symptoms emerging between 2 and 4 years of age but the onset can be heterogeneous and in a subgroup of patients symptoms may appear up to 2 years later [31–35]. The majority of patients exhibit the severe form of MPS II and these children display profound mental retardation, communicating hydrocephalus, hearing impairment, coarse facial features, hepatomegaly, obstructive airway disease, cardiac dysfunction, joint stiffness, and skeletal involvement. They typically die in their mid teens secondary to severe neurological involvement and/or airway obstruction complicated by cardiac disease [35].

A phase I/II clinical trial with recombinant human I2S (Elaprase®, idursulfase, Shire Human Genetic Therapies, Inc., Cambridge, MA, USA) tested the clinical efficacy and safety of enzyme replacement therapy (ERT) [36]. Recombinant enzyme was shown to ameliorate somatic symptoms and resulted in improved exercise tolerance. Over 80 % of patients had normal liver volume. While only 40 %

normalized urinary GAGS, the majority of treated patients were approaching the upper end of normal range. Although the treatment with weekly infusions has proven safe, a significant portion of patients develop antibodies. Importantly, idursulfase is not able to cross the BBB and therefore children with neurological impairment continue to deteriorate [31]. Hematopoietc cell transplantation (HCT) has been attempted since the early 1990s and it resulted in decreased urinary glycosaminoglycan excretion and increased iduronate-2-sulfatase activity in serum and leukocytes [37]. Nevertheless, despite measurable improvements in all tested somatic organ function, these early reports reported no obvious neurocognitive benefits in children over age 2 years after HCT [38, 39]. A significant limiting factor in outcome analysis was the prohibitive treatment related mortality (TRM) that is well illustrated in a series of ten boys reported in 1999 of whom only three survived long-term after bone marrow transplantation. Nevertheless, one of the three, a 10-month-old boy maintained normal intellectual development 7 years after matched sib HCT suggesting the possible neurocognitive benefits of HCT supported by other case reports [19, 40]. Favorable outcome after HCT was also reported on patients inflicted with the neurocognitively attenuated form of the disease [40, 41]. These early reports, in concert with the disappointing HCT outcome report by Guffon for neurocognitive outcome in the severe form of the disease [42], contributed to the prevailing judgement by many in the genetic and metabolic disease community to declare transplantation an inferior option to enzyme replacement therapy based on the latter's superior safety profile [43]. However, these position statements reflected on transplantation experience performed predominantly at age 2.5 years and above when children afflicted with the cognitively severe form have demonstrated progressive cognitive decline. A landmark survey from Japan is the first to bring forth the comparative long term-neurocognitive benefits of transplantation if the procedure was performed relatively early, before brain atrophy became obvious on MRI and before developmental delays became evident [44]. The medical records of the transplanted cohort ($n=21$ from 8 centers) was compared to records on 66 patients from the Japanese MPS Family Society. The cognitively severe subtypes of MPS II were categorized into groups C and D based on whether speech deterioration started after age 2 years or before. In HCT untreated category C patients, 12 out of 19 children showed deterioration of speech while this was found in only one of seven of transplanted kids. Brain MRI findings also improved after transplantation leading to an overall conclusion by a broad consensus that HCT is a worthwhile intervention if performed before brain atrophy appears on MRI and before cardiac valvular regurgitations develop [45].

Supporting data for HCT efficacy positively impacting neurocognitive outcome is emerging from elsewhere too. Between December 2002 and December 2008, nine children at a median age of 2.0 (range 0.1–4.0) were treated with unrelated donor umbilical cord blood transplantation at Duke University Medical Center (DUMC). The first eight received myeloablative therapy while the last boy received reduced intensity conditioning. Two patients were diagnosed prenatally and they were transplanted at 0.1 and 0.3 years of age respectively after Busulfan/Cyclophophomide/ATG conditioning. Altogether, there were ten transplants performed in nine children.

The youngest one was re-transplanted for mixed chimerism and poor graft function associated with immune cytopenias and chronic GVHD. He is one of the three children who died. One boy died 2 years after CBT secondary to presumed bacterial sepsis associated with chronic GVHD, while another one 11 months post CBT due to EBV PTLD (Post Transplantation Lymphoprolifirative disease) and fungal infection associated with chronic GVHD. The infant transplanted at age 0.3 years has the longest follow up, exceeding 9 years with continued gain in neurocognitive function (Paul Szabolcs personal communication). The index case in his family was his older brother who died at an early age with seizures and other complications of Hunters. Seven of the nine boys received 5/6 HLA-matched grafts and two received 4/6 HLA-matched UCBT. The quality of life and neurocognitive outcome of the six surviving children is encouraging compared to their untransplanted siblings and natural history cohorts (Maria Escolar personal communication), most notably when the intervention was performed early, within the first year of life.

3.3.2 Sanfilippo Syndrome

3.3.2.1 Sanfilippo A Syndrome

All four subtypes of MPSIII or Sanfilippo syndrome (A, B, C, D) are autosomal recessive lysosomal storage disorders (LSD) characterized by impaired degradation of heparan sulfate. This mucopolysaccharidosis syndrome was named after Sylvester Sanfilippo who first reported on the disorder in 1963. There have been no clinical trials yet with ERT or gene therapy even though in a mouse model of MPS IIIB there was reduced pathology in the brain following transplantation with gene transduced transplanted bone marrow [45].

MPSIII Type A is considered [46] to be the most severe of the four MPS III gene defects, with earlier onset and rapid progression of symptoms leading to shorter survival. MPS IIIA is caused by deficiency in heparan *N*-sulfatase, also called *N*-sulfoglucosamine sulfohydrolase (SGSH; MIM 605270), or briefly as "sulfamidase." The SGSH gene is localized on chr17q25.3. Severe central nervous system degeneration is associated with relatively mild somatic disease rendering it hard to diagnose before age 18–24 months when speech delay and recurrent middle ear infections typically prompt a more extensive workup.

The first report on transplant outcome came from investigators at the University of Minnesota analyzing patients transplanted at four US centers and in Manchester, England, all having received bone marrow as graft source (Klein et al. BMT 1995, I5;S 176). Follow-up was reported on five children were with MPS IIIA aged between 2.8 and 5.7 years and despite successful engraftment all continued to progress with neurocognitive decline.

There have been 16 children with MPS IIIA transplanted at DUMC between 2001 and 2009 at a median age of 2.7 years (range 1.2–5.0), most reported previously [20]. All but one were Caucasian and all received single unit unrelated cord

blood grafts following a myeloablative regimen (MAC) with a Busulfan/Cyclophophamide/ATG regimen. Three of the 16 received 6/6 HLA-matched grafts 9 received 5/6 and 4 received 4/6 HLA-matched UCBT with a median cryopreserved cell dose of 8.9×10^7 TNC/kg. The thawed and infused CB units delivered a median CD34+ cell dose of 2.6×10^5/kg. Twelve of the 16 patients are alive, ranging 9 months to 9 years. Two children failed to engraft and both eventually died. One received three separate cytoreductive regimens and successful engraftment was achieved after his third attempt. He died due to adenovirus and multiorgan failure. Notably, the two children who died post-UCB engraftment, both died at or beyond 2 years after transplant with extensive chronic GVHD and severe neurocognitive decline. Other than the last three children transplanted who received Cyclosporine A + Mycophenolate for graft versus host disease prophylaxis the other 13 children received Cyclosporine A + methylprednisolone as described in the COBLT trial [47]. Interestingly, even though majority of the children received 5–6/6 HLA-matched grafts acute GVHD was common and more prevalent than described amongst all children transplanted at the same institution for other lysosomal storage disorders, excluding non-MPS disorders [20]. Amongst the 14 children eligible for aGVHD analysis 10 patients experienced grade II–IV aGVHD, however, in severe manifestation only 1 child had grade III GVHD. The median onset of aGVHD was 13 days, mean 26 days ± 13 days. The relatively early onset is associated with a relatively early engraftment. Median neutrophil engraftment occurred after 21 days, mean 22 ± 7 days. Platelet transfusion independence was achieved at a median 54 days mean 64 ± 28 days. The relatively frequent occurrence of grade II acute GVHD led to chronic GVHD in 10 of 14 children evaluable. Eight of ten involved skin, 6/10 had gut involvement and overall 7/10 was deemed "extensive" and 6/10 scored as severe according to the recently implemented NIH "global scoring scale" [48].

Sanfilippo B syndrome is characterized by deficiency of α-*N*-acetylglucosaminidase (NAGLU). Neurocognitive decline is reported to start later and at a possibly slower pace when compared to MPSIIIA. The gene encoding NAGLU is located on chr17q21. The aforementioned study by Klein et al. reported on three patients with MPS IIIB who received bone marrow grafts and Vellodi from London, UK reported on two twins both reported to be less handicapped as their untreated brothers 9 years after BMT. At DUMC there have been seven children transplanted at a median age of 3.2 years of age (range 1.4–4.5 years) all have received unrelated CB units. While five of seven received the traditional Busulfan/Cyclophosphamide/ATG regimen, two patients were enrolled on an in-house "Reduced Intensity Conditioning (RIC)" trials based on a modified Fludarabine, Melphalan, Campath-1H (FMC) backbone. Hydroxyurea and Thiotepa are in addition to FMC. Notably, 2/5 children after MAC regimen failed to engraft, both died subsequent to a second UCBT attempt from viral infectious complications, while both children after the RIC engrafted although one with mixed chimerism detecting ~50 % donor cells prior to her unexpected death due to transfusion reaction. Overall survival is surprisingly poor in this small cohort. Only 2/5 MAC and one of two RIC recipients are surviving rendering this cohort too small for further analysis. Notably, three of the four children had adenovirus infections preceding death and in two it was the primary cause.

3.4 Adrenoleukodystrophy

X-ALD is a metabolic disorder characterized by the impaired peroxisomal beta-oxidation of very long chain fatty acids (VLCFA) and there is accumulation of these VLCFA in all tissues [49]. This is an X-linked disease and mutation is in the ABCD1 gene and there is absence or dysfunction of ALDP, a peroxisomal membrane transporter protein. More than 500 mutations in the gene are described (http://www.x-ald.nl).

X-ALD may be diagnosed in an asymptomatic subject or an affected individual by the finding of elevated levels of VLCFA in plasma followed by molecular analysis of the ABCD1 gene. There are other causes of elevated VLCFA and such levels are not by themselves therefore diagnostic of X-ALD [50, 51]. For the detection of carrier status in females the preferred method is mutation analysis since the VLCFA may be within the normal range in known female carriers.

3.4.1 Patterns of Clinical Presentation in X-ALD

X-ALD is an interesting metabolic disease and several patterns of clinical illness are recognized:

1. Asymptomatic individual: Diagnosis is made either by mutation analysis within a known family kindred or by the finding of elevated VLCFA in an asymptomatic individual.
2. Adrenal insufficiency—this presentation affects 70 % of genetically affected males. X-ALD is a frequent cause of Addison's disease in boys and adult males and must be therefore investigated as a cause of such cases. Recognition in a boy that adrenal failure is caused by X-ALD has important implications both for genetic counseling and extended family studies and for that patient management as other presentations of X-ALDS should be specifically looked for in that boy [52]. Usually glucocorticoid function is affected before mineralocorticoid function. Replacement therapy under the direction of an endocrinologist is appropriate management.
3. Adrenomyeloneuropathy (AMN)—Virtually all male patients with X-ALD develop AMN, usually in their 20s or 30s. Patients develop progressive spastic paraparesis, sensory ataxia, sphincter disturbance, and impotence. Pathologically there is a non-inflammatory distal axonopathy. Such an illness can affect carrier females although their presentation is usually at a more advanced age. Cerebral involvement (see below) may accompany AMN in affected males but rarely females [53, 54].
4. Cerebral X-ALD—this is the most devastating presentation of this disorder and is most commonly seen in affected boys during the childhood years but can occur in adolescence and adult life also. This is a rapidly progressive, intensively inflammatory myelinopathy which most commonly begins in the corpus callosum and typically progresses to the parieto-occipital region. Clinical presentation may be initially difficult to pick up with insidious, progressive defects in visuospatial and visuomotor functions or in attention and reasoning. The affected boy, especially an index case, may have a decline in school performance and be misdiagnosed—often as attention deficit hyperactivity disorder. With time then

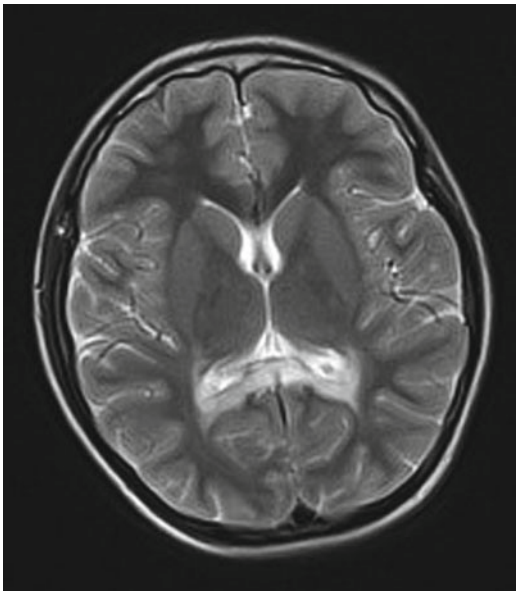

Fig. 3.1 Extensive changes in a clinically affected boy

more clinically obvious neurological signs will become apparent but further progression to devastating disability can be rapid.

The clinical manifestations of X-ALD in an affected individual are not stable. Asymptomatic boys can develop adrenal failure or cerebral X-ALD. Boys with stable and controlled adrenal failure can develop X-ALD. MR imaging can be used to detect cerebral disease before clinical neurology and this observation is at the heart of current management of boys with the genetic markers of X-ALD but without clinical cerebral X-ALD.

Brian MRI shows abnormal signal intensities (increased signal on T2 and FLAIR sequences, decreased on T1 sequence) in the affected areas. Figures 3.1 and 3.2 are MRI images from two genetically affected boys within the same family. Figure 3.1 shows extensive changes in a clinically affected boy. The scan in Fig. 3.2 shows the same changes of cerebral X-ALD in his brother who was clinically unaffected. This boy has since undergone Hematopoietic Stem Cell Transplantation as discussed below. A 34-point MRI scoring system specific for X-ALD that was designed by Loes and colleagues is used for diagnosis and progression [55, 56].

3.4.2 Clinical Management of X-ALD

The following are important summary statements about the principles of management of X-ALD within a multidisciplinary team:

1. Genetic counseling must be given to the parents of affected boys and to adult males and women with X-ALD and their family to detect carriers that they may

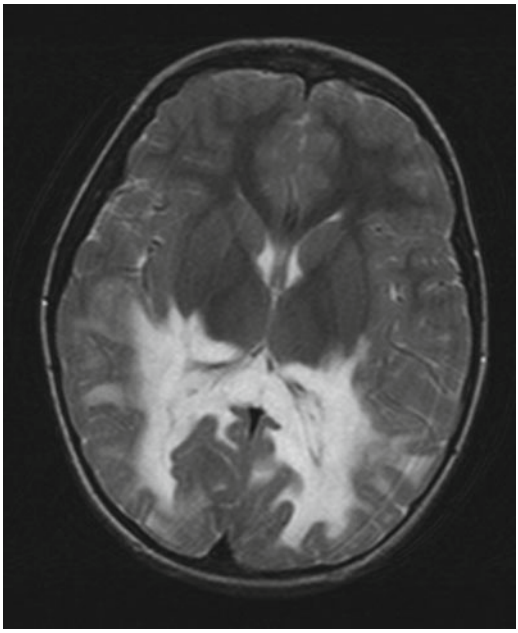

Fig. 3.2 The same changes of cerebral X-ALD in his brother who was clinically unaffected

be offered prenatal diagnosis (using a fresh chorionic villus biopsy taken at 11–13 weeks of gestation) and to detect presymptomatic or asymptomatic males who might benefit from therapeutic intervention.
2. Genetically affected males should be seen by an endocrinology to investigate and manage adrenal dysfunction.
3. Genetically affected boys who have no neurological illness should have an MRI every 6 months during childhood and yearly thereafter to detect presymptomatic cerebral X-ALD. These boys should be offered Hematopoietic Cell Transplantation.
4. For boys with established neurological disease of cerebral X-ALD or AMN then management should be with a clinical neurologist.
5. Lorenzo's oil (LO) is a 4:1 mixture of glycerol trioleate and glyceryl trierucate that normalizes the elevated level of VLCFA and has been advocated to delay the onset of AMN in genetically affected boys. There is evidence that AMN occurs even where the VLCFA are suppressed into the normal range by LO although there are also small retrospective studies suggesting delay in neurological symptom onset in boys taking LO. There is no placebo randomized trial in this field. LO is difficult to take.

3.4.3 Stem Cell Transplantation in X-ALD

Hematopoietic Cell Transplantation (HCT) has an important role to play in the management of boys who are genetically affected with X-ALD [8, 57, 58]. It is not clear

how HCT is efficacious in arresting the development of cerebral disease in X-ALD. It is not cross correction of a deficient enzyme as in the lysosomal storage disorders where transplant is effective. However it is not simply an effect of intensive immune suppression. The current role of HCT is to prevent progression of cerebral X-ALD that is present on scan but before it is clinically advanced disease. Genetically affected boys should have a suitable donor identified so that the interval between first abnormal MRI and actual transplant is not delayed by a donor search that could already have been undertaken.

Review of the literature on HCT and X-ALD supports the following statements:

1. Most boys have been transplanted from the best available donor using full intensity, chemotherapy only preparative regimens [57].
2. Most unrelated donors have been adult BM donors, but some CB donors have been employed [59].
3. Peters et al. described donor-derived engraftment rates that were higher than MPS IH registry data—86 % of 93 evaluable patients at a median follow-up of 11 months (93 % of related donor transplants, and 80 % of unrelated donor transplants [14, 57]).
4. Outcome is affected by disease status, donor source and HLA matching. The commonest causes of death were progressive cerebral X-ALD disease and GVHD. Transplant related mortality in the Peters series was 10 % in related donors and 18 % in unrelated donors. Five year survival rates for related donors and unrelated donors in this series were 64 % and 53 %, respectively [57].
5. Survival is clearly affected by disease status at time of transplant as assessed by the number of neurologic deficits and the MRI severity score. In those with 0 or 1 neurologic deficit and MRI score of less than 9, the 5 year survival was 92 %; in other patients it was 45 % [57].

The ability of HCT to alter the natural history of cerebral X-ALD was seminally described in 12 boys who were at least 10 years post BMT by Shapiro et al. [60]. MRI abnormalities reversed completely in 2 boys, improved in 1 and were unchanged in 1. In the remaining 8 boys there was early progression and subsequent stabilization of MRI changes. Motor function remained normal or improved in 10, verbal intelligence remained within the normal range in 11 patients, and performance (non verbal) abilities were improved or stable in 7 patients, and declined and then stabilized in 5. In boys with clinical illness and advanced MRI changes, the disease-specific outcome has been very poor with many patients dying of progressive ALD [55]. For survivors, there are permanent, severe neurologic and neuropsychological sequelae and life quality is compromised.

It is unclear whether successful transplant for cerebral X-ALD in early life will alter the incidence of later AMN as insufficient time has elapsed to allow the survivors of HCT to be fully assessed for AMN, the onset of which may be beyond the third decade of life. HCT is not currently offered to asymptomatic boys as prophylaxis against future disease progression. In view of the natural history of the disease such a practice would mean that some boys would undergo HCT—with its short-term mortality and long-term morbidity risks—who might otherwise have been

healthy. There is no evidence yet that HCT will prevent the other neurological manifestations of the disease. Boys who definitely need HCT (as identified by MRI lesions) will have a good outcome from HCT and this observation underpins current HCT practice. Recently, peri-transplant antioxidative therapy with *N*-acetyl-L-cysteine has been shown to be protective against fulminant demyelination in advanced cerebral X-ALD. Its role is under continued investigation [61].

HSCT has no effect on adrenal dysfunction. VLCFA levels decline but remain elevated above the normal range. Adrenal dysfunction may influence cerebral dysfunction and must be monitored closely and managed promptly. Adrenal crisis can precipitate a profound decline in CNS function in these boys, as can significant acute GVHD. Lorenzo's Oil has no impact on the natural history of boys with MRI imaging changes or clinical disease and it is therefore of limited interest or relevance to the HCT team. It may cause thrombocytopenia. In some institutions, Lorenzo's oil is given in the weeks prior to HCT in order to normalize VLCFA, but this is not universal practice and there is no evidence that it is beneficial to HCT outcomes [62].

3.4.4 Changing Cellular Therapy of ALD

There are, of course, limitations to allogeneic HCT. A donor is required. Where a mismatched donor is used, the toxicity of the procedure—infections, Graft Versus Host disease—increases. There has been success with gene therapy approaches in this disease using a lentiviral gene delivery system to autologous hematopoietic stem cells [63, 64]. There are preclinical data and early clinical data to suggest that this approach may be successful. Clearly more experience and longer follow-up of transplanted patients is required but this is a likely safer approach to stem cell transplantation than current allogeneic stem cell transplantation techniques.

Increasingly in many diseases—metabolic, immunodeficiency—HCT is offered to children at early stage of their disease in order to prevent future likely complications. These future likely complications of the disorder are known from natural history studies and include cerebral disease and AMN in X-ALD. In order for HCT to be offered in this fashion in X-ALD in order to prevent AMN etc. then there needs to be clearer evidence that transplant prevents AMN. As AMN is a delayed complication of the disease this question is only now becoming amenable to study as the boys transplanted in the past reach an age at which AMN is part of the natural history of the disorder. Only now therefore can we ask whether HCT is able to alter this aspect the natural history of the disorder. If it is able to then transplant might be offered more widely to X-ALD individuals and not just to prevent early cerebral X-ALD as it currently is. Whether sibling, carrier donors offer the same protection as wild type donors is also unclear. The sibling donor might be the safer transplant but we know from the Hurler experience that the disease outcome might be better where a wild type donor is used, although the transplant is more toxic and has higher risk. This question is also more amenable to retrospective study as there are more individuals transplanted for X-ALD and the time since their transplant is now considerable in many cases.

3.5 Globoid Cell Leukodystrophy

Globoid cell leukodystrophy (GLD) is often referred to Krabbe Disease, named after it's discoverer who identified this syndrome in young infants as a familial form of brain sclerosis [65]. Rapid progression to death associated with spasticity and hyperreflexia was noted. The genetic cause of GLD was identified in 1971 and is one of several monogenic lysosomal hydrolase enzyme defects that may lead to neurodegeneration as a result of reduced or altered myelination reflecting progressive apoptosis of oligodendrocytes, the major myelin producing cells in the brain. The affected gene, galactocerebroside β-galactosidase (GALC) is mapped to chromosome 14q31. GLD is inherited in an autosomal recessive fashion with an incidence approximately 1:100,000, however, there may be variations based on geographical and ethnic determinants. The gene is approximately 56 kb long and consists of 17 exons. The number of mutations associated with clinical manifestations is rapidly approaching 100, notably these are widely dispersed throughout the gene. Many affected children represent the cumulative effect of being compound heterozygotes with dramatically reduced GALC enzyme activity. In fact, the recently implemented newborn screening in New York State and others subsequently has already identified several new mutations and polymorphisms leading to decreased enzyme level [65]. The recently identified crystal structure of GALC in complex with its substrate identifies three domains including a novel lectin domain that renders GALC unique [66]. Mutations described so far in affected children may not only impact substrate binding but disease causing missense mutations can impact the folding and stability of the enzyme. The encoded enzyme exerts its function during normal lysosomal degradation of galactoshingolipids, including the principal substrate, galactosylceramide to cleave it into galactose and ceramide. However, there are other substrates of GALC and it is widely accepted that toxic accumulation of another metabolite, galactosylsphingosine, also called psychosine is critical in the pathogenesis of the clinical disease with toxic effects on oligodendrocytes. Interestingly, the total brain content of galactosylceramide is not increased, nevertheless the widespread globoid cell reaction reflects on the aberrant turnover of galactolipids. Globoid cells reflect on the toxic morphologic alterations seen by pathologists.

Most patients with Krabbe disease display the early or late infantile onset characterized by rapidly progressing leuko-encephalopathy with initially dominant upper motor neuron disease and long-tract signs. The distinction between these two variants is somewhat arbitrary; nevertheless the early infantile and most aggressive form presents by age 6 months with irritability, swallowing difficulty, deafness, and seizures, leading to vegetative state and death typically by 2 years unless extensive supportive measures are implemented. Small animal models have been available for some time, in fact in a landmark papers Yeager et al. [67, 68] demonstrated prolonged survival and re-myelination after bone marrow transplantation in the twitcher mouse, that is characterized by absent GALC activity. By the early 1990s a few transplant centers embarked on transplanting the first patients that lead to the seminal report by Krivit et al. in 1998 [10] demonstrating in five children the beneficial effects of donor leukocyte derived GALC activity. In four out of five children symptoms

stabilized and even improved, notably all were minimally affected juvenile onset with one presymptomatic 2 month old infant. Over the ensuing years, benefiting from the rapid availability of cord blood grafts and unrelated cord blood transplantation (UCBT) rapidly emerged as the most frequently chosen methodology. Escolar et al. characterized extensively the outcome of 25 transplanted babies reflecting the largest single center experience, highlighting both the benefits if UCBT is performed early, most receiving transplant within the first month of life ($n=11$) and also the limitations of this procedure when performed in symptomatic babies ($n=14$) all beyond 4 months of age typically between 6 and 8 month of age [69]. Survival was excellent in presymptomatic babies (all 11 survived with >3 year follow-up), while only 6 of 14 symptomatic babies lived beyond 3 years. Not only was survival significantly better in the presymptomatic infants but these babies demonstrated on follow-up evaluation continued ventral myelination and overall gains in most developmental skill with variable delays in expressive language and gross motor function reflecting on less favorable functional impact with cell based replenishment of GALC on the peripheral nervous system. Similarly, nerve conduction studies showed no improvement in the symptomatic babies and worsening was recorded even amongst some of the presymptomatic babies more and more apparent with the progress of time. Four of the eight deaths were attributable to progressive Krabbe disease, while GVHD and infections contributed to the death of four infants [69].

3.6 Metachromatic Leukodystrophy

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal disorder arising from deficiency of arylsulfatase A (ARSA) enzyme activity and characterized by increased urinary sulfatides. Documentation of increased sulfatides is of critical importance to confirm the diagnosis since the ARSA pseudodeficiency allele is common in the general population. Deficiency in ARSA results in defective desulfation of sulfated glycolipids present in myelin sheaths of the central nervous system (CNS) and peripheral nervous system (PNS), and to a lesser extent in visceral organs (kidney, gallbladder, liver). Lysosomal accumulation occurs which manifests itself as meta-chromatic staining. Deficiency in saposin B can cause MLD as well. Furthermore, multiple sulfatase deficiency (MSD) has an overlapping clinical picture with signs of MLD, signs of mucopolysaccharidosis, and ichthyosis. Saposin B deficiency and MSD share with MLD: increased CSF protein, slowed nerve conduction velocity (NCV), and increased urinary sulfatides.

The clinical phenotype is a broad continuous spectrum ranging from early-infantile MLD to adult onset forms. The late infantile (onset 0.5–4 years) presents generally in the first or second year of life due to loss of motor milestones including gait disturbance, abnormal speech, loss of neurocognitive skills, optic atrophy, progressive spastic quadriparesis, increased CSF protein and slowed nerve conduction velocities, culminating in neurodegeneration and death within several years. The

early juvenile form (onset 4–6 years) shows gait and postural abnormalities, emotional and behavioral disturbances, optic atrophy, progressive spastic quadriparesis, increased CSF protein, and slowed NCV. The late juvenile form (onset 6–16 years) exhibits behavioral abnormalities, poor school performance, language regression, gait disturbance, slowly progressive tetraparesis, increased CSF protein, and slowed NCV. Finally, the adult form (late onset, >16 years) is characterized by mental regression, psychiatric symptoms, incontinence, slowly progressive spastic tetraparesis, normal or increased CSF protein, and normal or slowed NCV.

The first HCT for MLD was done more than 20 years ago. According to the EBMT and CIBMTR registries, more than 100 transplants have been performed. Unfortunately, despite this number, the lack of graft-outcome and long-term follow-up studies makes it difficult to draw firm conclusions regarding the efficacy of HCT. It appears from this literature, mostly small series, that the outcomes are less promising than those for MPS IH. Although in presymptomatic juvenile MLD forms and perhaps more clearly in adult-onset MLD it appears that the CNS disease has stabilized, while the effect on the PNS has been uncertain [70–75]. The MRI changes may even become less after successful transplantation [76]. These results derive from the pioneering years of HCT in IEM when a significant proportion of children were transplanted at an older age with advanced, irreversible disease. Additionally, outdated transplant techniques and the use of carrier donors and mixed-chimerism leading to lower enzyme levels may have affected outcomes.

More recent the single center experience of Duke University ($n=27$) with unrelated cord blood (UCB) transplantation was described. They concluded that overall, children with juvenile onset had better outcomes than those with late-infantile onset. As in other leukodystrophies, early intervention correlated with optimal outcomes; UCB transplantation benefits children with presymptomatic late-infantile MLD or minimally symptomatic juvenile MLD [15].

Today, the use of CB grafts (associated with full-donor chimerism and normal enzyme levels) and the development of standardized protocols (e.g., HCT for MPS IH) may result in improved outcomes for MLD [15, 20, 23]. Current preferred practice for HCT in MLD, is to perform transplants only on presymptomatic (or minimally affected older: late-juvenile or adult onset) individuals. Long-term follow-up and multicenter collaborative studies on the efficacy of HCT for MLD since 2000 are highly needed to define better international transplant protocols.

Regarding alternative treatments, an ERT trial for MLD was initiated to examine its impact on the PNS. ERT is not expected to ameliorate CNS disease due to its inability to cross the BBB. The San Raffaele Institute in Milan has an MLD gene-therapy trial open for patients with late-infantile or early-juvenile forms of MLD [63]. The first results, including longer-term follow-up data, are expected soon. Use of mesenchymal stromal stem cells (MSC) to correct residual deficits such as PNS abnormalities was investigated by Koç et al., but no clinical improvement was seen likely due to the inability to obtain sustained engraftment of donor MSCs in the PNS [77].

In summary, the outcomes of HCT for MLD are highly variable and unclear since there have been no large multicenter series performed in the last decade. Even

after more than 20 years of HCT for MLD, it is not exactly clear if MLD patients or which phenotype might benefit from HCT. For presymptomatic juvenile and adult-onset patients there is positive evidence. Improved transplantation techniques and the prompt availability of CB grafts may positively influence long-term outcomes. An international registry would facilitate comparative evaluation of therapeutic options, leading to improved guidelines.

3.7 Miscellaneous Disorders

In contrast to above described IEM; other mucopolysaccharidoses, leukodystrophies and related enzyme deficiencies have a much less uniform treatment history [78]. For example, Wolman disease, caused by deficiency of acid lipase, does respond to early HSCT, and up to 11 years experience with favorable outcome has been described [79]. This study has shown that lasting correction of hepatic function, normal adrenal function and normal neurologic outcome are possible after HCT. Similarly, in alpha-mannosidosis, stabilization of neurocognitive variables and possibly musculoskeletal features has been observed in a recent international series, describing the outcome of [79, 80].

The natural history of I-cell disease (mucolipidosis II, deficiency in GlcNAc-I phosphotransferase) can be altered by HCT, yet significant improvement after HCT has been rare [81]. Equally disappointing are responses to cellular therapy in GM-gangliosidoses (Tay-Sachs disease, Sandhoff disease, as well as GM1 gangliosidosis), acid sphingomyelinase deficient Niemann–Pick disease and in the neuronal ceroid lipofuscinoses [82–84]. There may be some beneficial effect of HCT when it is performed in a timely and appropriate manner for individuals with the juvenile forms of GM-gangliosidoses and possibly Niemann Pick disease (types B and C2). Despite enormous biochemical variation, the common feature of these neuronopathic metabolic disorders is their progressive nature. The ideal time for therapeutic intervention is early in life before damage from substrate accumulation becomes severe and irreversible.

3.8 Conditioning and Preferred Cell Source in IEM

In Table 3.2 the current European transplant guidelines are shown. Mainly data studying predictors for graft-failure performed in MPS-1H patients has let to current guidelines for HSCT in MPS IH. Initially these guidelines include a standardized busulfan (Bu)/cyclophosphamide (Cy)-conditioning regimen and the use of CB as a preferred graft source, second only to enzymatically normal matched sibling-BM. Recent evaluation of this guideline showed significantly higher engrafted survival rates 90 % ($n=43$) [11] compared to the historical EBMT-cohort 53 %

Table 3.2 Donor hierarchy and conditioning

a.	Stem Cell Source Hierarchy in lysosomal storage diseases and peroxisomal disorders:	
	Identical-SIB (not carriers)	
	UD (10/10)* = Unrelated Cord Blood (UCB: 6/6)	
	UCB (5/6)	
	UCB (4/6) = mismatched-UD (non-T-depleted)	
	UCB (3/6) = HAPLO (not recommended)	
	UD (10/10) may be bypassed depending on institutional preference or because of time.	
	For UD: BM preferred cell source	
	Cell dose for UCB: 5–6/6 match: $>3.0\times10^7$ NC/Kg and/or 2×10^5 CD34+/kg, 4/6-match $>5\times10^7$ NC/Kg and/or $>3\times10^5$ CD34+/kg. Matching according to intermediate resolution criteria (low resolution on A and B, high resolution on DR)	
	Unrelated donors are regarded as noncarriers of the mutation	
b.	Serotherapy:	
	id-SIB	No
	UCB	ATG-genzyme 4 × 2.5 mg/kg (day −8 to −5)
	UD	Either Campath-1H 3 × 0.3 mg (day −9 to −7) or ATG-genzyme 4 × 2.5 mg/kg (day −4 to −1)
c.	Conditioning:	
	SIB/UCB/UD	Busulfan weight-based dosing (IV: day −5 to −2) with therapeutic drug monitoring AUC 90 mg h/L (range 85–95) cumulative over 4 days (AUC in µM min = 22, range 20.5–23.5)
		Fludarabine 160 mg/m^2 (day −5 to −2)
d.	GvHD-prophylaxis:	
	SIB	CsA** (+ MTX: 10 mg Msq; day +3, +6 and +11)
	UD (BM):	
	With Campath-1H	CsA
	With ATG	CsA + MTX (10 mg Msq; day +3, +6 and +11)
	UD (PBSC) UD/ Mismatched-UD (BM)	CsA + MMF (30 mg/kg: stop day +28 in case no GvHD)
	UCB donors	CsA + either prednison 1mg/kg/day (till day +28, taper in 2 weeks) or MMF 45mg/kg (stop day +28)

* may be bypassed according to institutional guidlines or preferences
** through levels 200–250 ug/L

(1995–2004) [13]. More recently a reduced toxicity conditioning regimen has been introduced: cyclophosphamide was replaced by fludarabine since in a recent study busulfan with therapeutic drug monitoring combined with fludarabine showed lower toxicity and showed to be least as effective as conventional BuCy. For the upcoming years there will remain room for further reducing toxicity, but studied in well designed and preferably multicenter studies.

Currently the preferred cell dose is unrelated cord blood after a noncarrier matched sibling donor. In a recent cell source comparison study in MPS-1H patients similar survival rates in MSD and 6/6 uCB recipients were noted [23]. While a 10/10 MUD donor showed similar survival as the 5/6 matched uCB. Furthermore uCB is readily available and will result in full donor chimerism in almost all patients. Mixed-chimerism was seen in 30–50 % of the patients receiving either a matched

sibling donor or volunteer unrelated donor. As mixed-chimerism is associated with lower enzyme levels and as lower enzymes are associated with worse long-term outcomes, achieving full donor chimerism is important [17].

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Chapter 4
Leukodystrophies and Lysosomal Storage Disorders

Maria L. Escolar and Mieke Aldenhoven

The leukodystrophies are a group of inherited metabolic disorders in which there is abnormal development or progressive degeneration of the myelin sheath, the fatty covering that acts as an insulator around nerve fibers. Each of the leukodystrophies is the result of a defect in the gene that controls the production or metabolism of one of the many component molecules of myelin. The word leukodystrophy comes from the Greek roots *leuko*, white, *dys*, lack of, and *troph*, growth. Specific leukodystrophies include metachromatic leukodystrophy, Krabbé disease, adrenoleukodystrophy, Pelizaeus–Merzbacher disease, Canavan disease, Alexander disease, Refsum disease, cerebrotendinous xanthomatosis, and childhood ataxia with central nervous system hypomyelination (also known as vanishing white matter disease).

Lysosomal storage disorders (LSDs) comprise a group of heterogeneous disorders caused by the deficiency of a lysosomal enzyme or a disturbance of other lysosomal protein functions. The disrupted lysosomal function results in incomplete breakdown and subsequent widespread and progressive accumulation of undegraded macromolecules (e.g., proteins, polysaccharides, lipids) in the lysosomes of various tissues. It is thought that as these nondegraded toxic substrates accumulate, the lysosomes enlarge, and secondary processes are triggered, leading to cell, tissue, and organ dysfunction, and ultimately resulting in progressive, multisystemic disease.

The natural history of the LSDs is characterized by progressive multiple organ involvement, with a varying range of affected organ systems and a variable degree

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of severity among the disorders, reflecting the sites of storage and the nature of the stored compound. Organ systems that are often affected include the central nervous system (CNS), abdominal viscera (liver and spleen), and skeletal, cardiovascular, respiratory, and sensory (vision and hearing) systems. In 1969, Fratantoni et al. were the first to demonstrate cross-correction of the defective metabolism in cultured fibroblasts derived from patients with Hurler (mucopolysaccharidosis type I or MPS I) and Hunter (MPS type II) [113]. A similar correction was subsequently shown in vivo by Di Ferrante et al., demonstrating induced degradation of glycosaminoglycans (GAGs) in patients with Hurler and Hunter syndrome after administration of normal human plasma [78]. These observations resulted in the first trial of allogeneic hematopoietic stem cell transplantation (HSCT) for Hurler syndrome, reported in 1981 by Hobbs et al. [155]. Biochemical improvement and a dramatic reversal of the clinical manifestations in a 1-year-old boy with Hurler syndrome were demonstrated, making this case a landmark proof-of-principle experiment.

The donor-derived stem cells engrafted after allogeneic HSCT provide a continuous endogenous source of the missing enzyme throughout the body—in the CNS as well as peripheral tissues. Since intravenously administered enzymes are incapable of crossing the blood–brain barrier, enzyme replacement therapy (ERT) is not able to prevent CNS deterioration [173, 414]. Allogeneic HSCT is therefore considered the treatment of choice in several LSDs that are characterized by CNS involvement, whereas ERT is indicated only for the milder phenotypes (i.e., without CNS involvement).

At present, more than 1,000 HSCTs have been performed in leukodystrophy and LSD patients worldwide. However, since HSCT for these diseases is performed only in a limited number of patients, evaluation of the clinical benefits of HSCT has been based mainly on retrospective case series or cohort studies. Hurler syndrome accounts for the large majority of the HSCTs performed in MPS patients, and this syndrome is therefore often used as a prototype. This chapter will focus on clinical outcomes, neurodevelopmental as well as somatic, of the various disorders after HSCT.

Neurodevelopmental delays and deterioration occur in untreated patients with severe leukodystrophies and LSDs, typically after a period of normal development. In some of these diseases, HSCT has been shown to improve or stabilize neurodevelopmental outcomes, including cognitive abilities, adaptive behavior, expressive and receptive language, and fine and gross motor function. Although appropriate tests of neurodevelopment can assess individual domains of function over time to evaluate the effect of treatment, these tools were not developed to test children with neurodegenerative disease. Therefore, results must be interpreted in light of the various complications of the disease, such as behavior problems, sleep disorders, cardiac and respiratory difficulties, speech problems due to enlarged tongue or difficulty swallowing, and motor, visual, audiologic impairment [227, 326].

Although the dominant clinical features of several leukodystrophies and LSDs for which HSCT is a treatment option are neurological dysfunction and neurocognitive decline, the somatic organs are also severely affected in several diseases.

Classic examples showing significant involvement of the somatic organs are the MPS disorders, for which many patients have undergone transplantation. Most of the available data on somatic outcome after HSCT therefore involves MPS disorders.

4.1 Metachromatic Leukodystrophy

Metachromatic leukodystrophy (MLD) is an inborn error of metabolism caused by a functional deficiency of the lysosomal enzyme arylsulfatase A (ARSA). ARSA catalyzes the hydrolysis of sulfated glycosphingolipids, chiefly 3-*O*-sulfogalactosylceramide (sulfatide), which is then targeted to the lysosomal storage compartment for degradation [231]. Sulfatide is the major acidic sphingolipid of myelin, comprising ~5 % of myelin lipids [247]. Functions of sulfatide include ion balance, signal transduction, cell–cell recognition, maintaining the insulating ability of the myelin sheath, preserving paranodal junctions, and regulating enzyme activity and oligodendrocyte differentiation [297, 393, 429]. ARSA-deficient cells are incapable of hydrolyzing sulfatide, which accumulates in intralysosomal storage granules, which appear as distinctive metachromatic inclusions upon histological examination [90, 393]. Because of the abundance of sulfatide in myelin, its storage has a devastating effect on oligodendrocytes and Schwann cells, causing progressive demyelination of the peripheral nervous system (PNS) and central nervous system (CNS) [28, 321]. Sulfatide buildup may occur in other tissues, especially those with excretory functions such as the liver, kidney, gallbladder, and bile ducts, but buildup in these locations does not contribute to the disease's lethality [90, 124, 393].

4.1.1 Pathophysiology

The mechanism by which sulfatide storage leads to demyelination is not well understood. One theory states that the inability to hydrolyze sulfatide in the innermost layer of myelin impedes axonal cross-sectional growth, as experiments have shown that ARSA activity is required both during growth and after the active growth stage is complete [90, 393]. Another theory states that the abnormal lipid composition of myelin in MLD leads to instability and degradation. The rise in sulfatides accompanied by the inability to convert its byproducts to cerebrosides alters the cerebroside:sulfatide ratio, which may be less than 1 in MLD (normal ~3) [374, 393]. This change increases the extracellular negative charge and interferes with formation and maintenance of a stable lipid bilayer [90, 374]. A final explanation for myelin destruction is the accumulation of lysosulfatide, a cytotoxic metabolite of sulfatide that inhibits protein kinase C and cytochrome oxidase C [393]. Other pathophysiological findings include secondary storage of minor

monosialogangliosides (GM_2, GD_3, GM_3, and GD_2), abnormal cell signaling and trafficking, abnormal synaptogenesis, decreased brain cholesterol, increased intracytoplasmic calcium, accumulation of α-synucleins, and inflammation, all of which may contribute to neuronal and glial cell apoptosis [321, 351].

4.1.2 Clinical Disease and Diagnosis

There are four clinical forms of MLD classified by age of disease onset: late infantile (onset between 6 months and 4 years), early juvenile (4–6 years), juvenile (6–16 years), and adult. The late-infantile form is the most common and most severe. Early signs of late-infantile MLD include regression of motor skills, hypotonia, decreased deep tendon reflexes, and ataxia. Later symptoms include decreased or absent speech, optic atrophy and blindness, hearing loss, spastic tetraplegia, pathological reflexes such as a positive Babinski sign, absence of all voluntary function, and death in a decerebrated state within 5–6 years. Early-juvenile MLD initially presents with motor problems and gait abnormalities, similar to the late-infantile form. In late-juvenile MLD, behavioral and attention problems in school precede motor problems. Unlike late-infantile MLD, both juvenile forms are characterized by brisk deep tendon reflexes. Otherwise, symptoms are similar, but patients show a slower progression. Adult MLD presents with psychological disturbances and mental regression, and patients may be misdiagnosed with schizophrenia or psychotic depression. Adult MLD patients may show an extended course of many years or even decades, leading to progressive spastic tetraplegia and death [17, 53, 90, 346, 393, 428].

Following onset of symptoms, diagnosis of MLD is confirmed by assay of ARSA activity in leukocytes or cultured skin fibroblasts. Values of ARSA activity in MLD patients range from undetectable to less than 10 % of normal values (<10 nmol/h/mg) [428]. Nerve conduction studies and neuroimaging are additional diagnostic measures of MLD. Peripheral neuropathy is present in all forms of MLD (except some cases of adult MLD) and often occurs before CNS involvement in the late-infantile and early-juvenile forms [53]. Uniform slowing of nerve conduction velocity correlates with demyelination [186]. Values may range from two standard deviations below normal to completely absent in later stages of the disease [53]. Typically, sensory nerves are affected before motor nerves. Magnetic resonance imaging (MRI) studies of patients with MLD show white matter changes in the periventricular and subcortical areas, centrum semiovale, genu and splenium of the corpus callosum, posterior limb of the internal capsule, descending pyramidal tracts, and claustrum [90]. The affected white matter is reduced, sometimes to the point of cavitation, with characteristic sparing of the U-fibers (arcuate fibers) [428]. In later stages there is cortical atrophy, destruction of the molecular and granular layers of the cerebellum, occasional involvement of the arcuate fibers, and enlargement of the ventricles, corresponding to clinical deterioration [428].

4.1.3 Genetics

Mutations in the *ARSA* gene are by far the most common cause of MLD, which is inherited in an autosomal recessive fashion with a frequency of 1 in 40,000. However, frequency may be much higher in certain ethnic groups such as Habbanite Jews living in Israel, Arabs living in Israel, Eskimos, and Navajo Indians [24, 124, 393]. The *ARSA* gene maps to chromosome 22q, and at least 115 disease-causing mutations have been identified [321, 393]. The high number of identified mutations may contribute to the wide variability in clinical onset and severity of disease. Most mutations are found only in a few or a single family, and only three mutations have been identified in a high number of patients [124]. Mutations precluding any synthesis of functional ARSA are designated null alleles or I alleles. Patients homozygous for null alleles always present with late-infantile MLD [124, 393]. The most common mutation associated with the late-infantile subtype disrupts the exon 2 splice donor site [224]. Conversely, alleles associated with some residual enzyme activity are designated R alleles. Homozygosity for R alleles most often produces adult MLD. Compound heterozygosity (null/R) correlates with juvenile MLD [24, 393]. The two most common mutations associated with later-onset MLD are the Pro426Leu amino acid substitution and Lle179Ser amino acid substitution [224]. Genotype–phenotype relationships verify that the level of residual ARSA activity is a strong predictor of clinical presentation, but other factors must be considered as well, including general genetic features or susceptibilities, environmental factors, other mutations in *ARSA*, mutations occurring on the background of the pseudodeficiency allele, and polymorphisms in ARSA activators [24, 30]. Striking intrafamilial variance in clinical disease has been identified; for example, in a case of dizygotic twins, one patient had late-infantile MLD, and the other displayed the juvenile form [24]. Generally, however, disease progression among affected siblings seems to follow a similar course, unlike many other leukodystrophies.

The genetics of MLD are complicated by a number of factors. A variant form of MLD is caused by a deficiency of sphingolipid activator protein saposin B, which stimulates ARSA activity. Although much less common, MLD caused by saposin B deficiency is clinically identical to MLD caused by ARSA deficiency [393]. Additionally, a pseudodeficiency phenotype that results in a nonpathogenic reduction in ARSA activity (approximately 8 % of normal) has been identified. The pseudodeficiency allele has a polyadenylation defect and a point mutation. The point mutation causes an amino acid substitution that results in a smaller than normal protein and loss of an N-glycosylation site [147]. The disturbance in the polyadenylation signal results in decreased synthesis of ARSA; that is, the pseudodeficiency phenotype is characterized by a decreased protein level, but not defective activity, of ARSA. The ARSA that is present in pseudodeficient individuals is sufficient for sulfatide hydrolysis, and such individuals show no sulfatide storage or urinary excretion. The pseudodeficiency allele is relatively common, with a frequency of 1 in 7 in Europe [393]. Because of these rare cases of MLD caused by saposin B deficiency and the presence of the pseudodeficiency allele, low ARSA activity does not

unequivocally confirm a diagnosis of MLD, nor does normal ARSA activity preclude the diagnosis. As such, extra care must be taken in prenatal testing and screening of asymptomatic relatives.

4.1.4 HSCT

Delivery of treatment to the CNS is a barrier in effectively treating MLD. However, hematopoietic cells traverse the blood–brain barrier and thus may be effective conduits for drug or enzyme delivery. Donor-derived monocytes are capable of differentiating into perivascular and parenchymal microglia; these microglial cells can then cross-correct the deficient glial cells by secreting functional ARSA [321, 393]. As such, HSCT has been the mainstay of treatment for certain LSDs and has been shown to positively influence disease progression in Krabbé disease and more recently in MLD [98, 226, 341].

Two factors must be considered when considering bone marrow transplantation (BMT) as a treatment option for MLD. The first factor is the form of MLD with which the patient is affected; BMT has been shown to stabilize cerebral demyelination in patients with asymptomatic late-infantile, symptomatic juvenile, and adult forms [17, 186, 226, 321]. A single case report by Görg et al. [130] describes stabilization in a patient with the juvenile form for 13 years. Such observations may be due to the fact that the disease-causing mutations in the later-onset forms of MLD result in low levels of residual ARSA expression, making the patient more amenable to treatment [231]. In contrast, symptomatic patients with late-infantile MLD showed delayed but continued disease progression despite BMT [222] or initial deterioration followed by stabilization [77, 192]. The second prognostic factor in evaluating the efficacy of HSCT is the severity of symptoms present at the time of transplant [303]. BMT has been shown to halt disease progression 1 year after transplantation in asymptomatic patients who achieve engraftment prior to the age at which symptoms occurred in an affected, symptomatic sibling [192]. However, patients with mild to moderate disease progression at the time of transplantation continue to deteriorate, leading to severe impairment [222]. Furthermore, HSCT does not prevent degeneration of peripheral nerves; therefore, patients show progressive neurophysiological abnormalities despite achieving 100 % donor hematopoietic chimerism [186, 222, 321].

Until recently it was thought that the late-infantile form of MLD progressed too rapidly to achieve any benefit from HSCT. Studies have found that even when the procedure is performed as early as 12–24 months of age, HSCT is ineffective in arresting demyelination and CNS involvement [267, 321], likely because of the slow turnover of brain microglia after transplantation [67]. During the lag time, patients deteriorate to the extent that they derive little or no benefit from treatment. The use of unrelated umbilical cord blood as a source of donor stem cells poses a major advantage in that cord blood is readily available, eliminating the need to perform a national search for a matched bone marrow donor [98]. Umbilical cord blood

transplantation (UCBT) has proven effective in treating infantile Krabbé disease, X-linked adrenoleukodystrophy, and Hurler syndrome [20, 98, 341]. Longitudinal studies evaluating UCBT for the treatment of MLD indicate benefits for both patients with juvenile onset and those with late-infantile onset if performed very early in the course of the disease [50, 226], suggesting that implementing a newborn screening program would be advantageous. In addition, the simultaneous or subsequent transplantation of allogeneic mesenchymal stem cells, which are able to differentiate into various cell types including neurons and astrocytes, appears to improve outcomes after HSCT [186, 243].

4.1.4.1 HSCT Neurodevelopmental Outcomes

Case studies have reported variable neurodevelopmental outcomes for patients with juvenile MLD, with stabilized cognitive function observed in several patients who were treated in the early stages of the disease [50, 139, 181, 326]. Several children with late-infantile disease appear to have obtained some benefit from BMT, including a baby who showed slower disease progression after treatment [292] and a girl who gained skills but experienced severe motor disability 5 years after transplantation [195]. However, it is thought that in the rapidly progressive late-infantile form of the disease, replacement of macrophages/microglia in the CNS does not occur rapidly enough to prevent neurologic deterioration [30, 43], and BMT may even accelerate disease progression [189]. A recent study evaluating long-term outcomes after UCBT reported that asymptomatic patients with late-infantile or juvenile onset continued to gain cognitive, language, and adaptive behavior skills after treatment, and some of the juvenile-onset patients with minor cognitive delay stabilized after treatment [226]. Mild-to-severe motor disability was observed in all treated patients in this study, and neurodevelopmental outcomes correlated with pre-transplant MRI findings [226].

4.1.4.2 HSCT Sensory Outcomes

In MLD, HSCT has been shown to stabilize or even slightly improve visual and auditory function in some patients, with better outcomes for those who were asymptomatic at the time of transplantation [139, 226, 326].

4.1.5 Other Treatments

Because of the wide variation in clinical outcomes and the toxicity of the transplant regimen, researchers are also investigating the possibility of ERT, which has been successful in treating other lysosomal storage diseases. Lysosomal enzymes such as ARSA are secreted and delivered to cells via the mannose-6-phosphate (M6P)

pathway. Enzymes with an M6P signal that are administered intravenously do not tend to enter the cerebrospinal fluid or the brain to any significant extent [267]. Thus, as is the case for transplantation-based approaches, internalization and correct lysosomal targeting of the therapeutic enzyme to defective neuronal and glial cells is the major obstacle to effective therapy [230, 321]. Nevertheless, studies in animal models other lysosomal storage diseases such as aspartylglucosaminuria, α-mannosidosis, MPS VII, and Krabbé disease demonstrate that intravenous injection of the therapeutic enzyme can reduce cerebral storage, although the mechanisms of cellular uptake are unclear [321].

Studies have shown that injecting ARSA knockout mice with recombinant human ARSA reduces sulfatide storage in the PNS and CNS, ameliorating motor and coordination difficulties [230, 235]. However, antibodies against the recombinant enzyme can reduce cellular uptake [232]. To bypass the blood–brain barrier, continuous intracerebroventricular enzyme infusion was recently tested in the mouse model. This approach reduced sulfatide accumulation in the brain and improved motor function at a much lower dose than that required for intravenous administration and did not stimulate antibody production [347]. Intravenous ERT may still have clinical importance in treating PNS symptoms, which are present in all forms of MLD and often remain even after HSCT. However, the mild phenotype of the ARSA knockout mouse complicates the translation of these results to the much more severe human disease.

ERT may also have clinical applications when administered in the period before HSCT, but caution must be exercised to ensure that formation of noninhibitory antibodies does not impede posttransplant engraftment [232]. To further investigate the promise of ERT for MLD, phase II clinical trials have recently been completed or are currently underway in Europe.

Several cell-based therapies have been tested in a mouse model with the goal of restoring functional oligodendrocytes. Oligodendrocyte progenitors injected into the brain successfully differentiated into myelinating oligodendrocytes and secreted enzyme for uptake by deficient cells [128]. In addition, intracranially injected neural stem cells were able to correct enzyme deficiency and improve sulfatide metabolism but failed to differentiate into oligodendrocytes [127, 174]. In a recent study in which bone marrow cells were transduced with homeobox B4 (HoxB4) to accelerate recovery, a small percentage of the cells (1.3 %) were found to differentiate into oligodendrocytes [246].

Gene therapy is another strategy that clears sulfatide storage and prevents motor disability in the mouse model. After ex vivo transduction, genetically modified HSCs can provide widespread enzyme overexpression with a lower risk of graft-versus-host disease and other side effects associated with allogeneic HSCT [28, 29, 233, 234]. Alternatively, intracranial injections of an adeno-associated viral (AAV) vector provide rapid enzyme delivery to the CNS only, targeting primarily neurons in mice [68, 284, 321, 322]. In addition, a recent study reported that the AAVrh.10 vector is able to transduce oligodendrocytes [284]. Intracranial delivery, which may be more effective at halting rapid progression in late-infantile MLD, also significantly increased enzyme activity (12–38 %) in nonhuman primates, suggesting a therapeutic benefit for patients [65]. Both gene therapy approaches are currently being tested in

clinical trials. Another strategy to increase the efficacy of gene therapy involves modifying the conditions under which the recombinant gene is expressed. For example, co-delivery of the gene encoding sulfatase-modifying factor 1 (*SUMF1*) significantly increases ARSA activity in mice, and maintaining supranormal levels of ARSA does not effect cell function, proliferation, or differentiation [54, 112].

As drug trials for peripheral ERT progress and other treatments such as UCBT, gene therapy (using adenoviral, lentiviral, and retroviral vectors), and mesenchymal stem cell infusion are being investigated, a better understanding of the natural course of MLD is necessary. Presently, the wide variability in clinical disease makes it difficult to differentiate between the disease process and effects of treatment. Furthermore, the paucity of established genotype–phenotype correlations makes it difficult to predict age of onset or expected clinical course. To that end, a standardized scoring system has been suggested to assess gross motor decline late-infantile and juvenile disease [176]. Demyelination load (demyelinated white matter) may be useful for evaluating disease progression and response to treatment in late-infantile MLD [62, 136].

4.2 Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is an X-linked disorder caused by mutations in the *ABCD1* gene and characterized by the accumulation of very long-chain fatty acids (VLCFAs, especially C24:0 and C26:0) in plasma and tissues throughout the body, including the brain and adrenal cortex. It is the most common leukodystrophy, with an estimated incidence of 1 in 17,000 births [27]. Adrenoleukodystrophy has widely variable phenotypes, the most common of which are childhood cerebral ALD (31–35 % of male patients), which is the most severe form, and adrenomyeloneuropathy (AMN; 40–46 % of male patients) [249].

The *ABCD1* gene encodes adrenoleukodystrophy protein (ALDP), a peroxisomal membrane protein belonging to the ATP-binding cassette transporter superfamily. Loss of ALDP function results in impaired transport of VLCFAs into the peroxisome for β-oxidation, resulting in their accumulation in plasma, adrenal glands, white matter of the brain, and other tissues [177]. These VLCFAs are incorporated into cell membranes, altering their structure and function (e.g., reducing the ability of adrenocortical cells to respond to adrenocorticotropic hormone) [404].

The diet is a source of VLCFAs, but most are obtained through elongation of shorter fatty acids by the elongases ELOVL1–7 [264].

4.2.1 Pathophysiology

Oxidative stress in the spinal cord, brain, and adrenal cortex, primarily from lipid peroxidation, appears to be an important factor early in the course of the disease [109, 289]. Oxidative damage to enzymes involved in glycolysis and the

tricarboxylic acid cycle may underlie the axonopathy seen in all forms of ALD [111]. Higher VLCFA levels in white matter of the brain (but not plasma or fibroblasts) correlate with more severe phenotypes [8], in which VLCFA-induced mitochondrial depolarization and increased intracellular calcium levels lead to cell death in oligodendrocytes, astrocytes, and neurons [148]. The specific events that lead to inflammation and demyelination in cerebral ALD are unclear but may involve genetic polymorphisms in fatty acid elongases, secondary peroxisomal dysfunction, or decreased levels of plasmalogens, which could result in increased oxidation of cholesterol in cell membranes [179].

In cerebral ALD, demyelination typically starts in the splenium of the corpus callosum and extends into the parieto-occipital white matter, or starts in the genu of the corpus callosum and extends to the frontal lobes. As the disease progresses, gadolinium-enhanced MRI reveals inflammation and blood–brain barrier disruption [94], which may be mediated by matric metalloproteinases [360]. A scoring system (Loes score) was developed to quantify the severity of white matter changes observed on MRI [208] and is used to make treatment decisions and evaluate response to treatment.

4.2.2 Clinical Disease and Diagnosis

Childhood cerebral ALD is characterized by progressive, inflammatory brain demyelination; neurologic, cognitive, and behavioral deficits typically appear by 4–8 years of age. Adolescent cerebral ALD is less common (4–7 % of ALD cases), and disease progression is typically slower than in childhood cerebral disease [249]. Symptom onset in AMN typically occurs around 30 years of age and is characterized by noninflammatory axonopathy and slowly progressive demyelination affecting the spinal cord and peripheral nerves. Approximately 30–40 % of men with AMN eventually develop inflammatory cerebral demyelination [249].

Patients with the Addison-only phenotype show no signs of neurologic involvement; however, most of these patients eventually develop AMN. The olivopontocerebellar form of ALD, which affects both adolescent and adult males, comprises only 1–2 % of ALD cases. Although some patients with the diagnosis of ALD show no symptoms at all, asymptomatic male patients older than 40 years old are rare. Approximately 50 % of female heterozygotes develop mild-to-severe myelopathy similar to AMN, typically after the age of 30, but they seldom exhibit cerebral involvement or adrenal insufficiency [249].

Adrenocortical insufficiency is often the presenting symptom of male patients; thus, a diagnosis of ALD should be considered for boys or men with Addison's disease. Early symptoms of cerebral involvement in boys and adolescents include hyperactivity and learning difficulties, which is frequently misdiagnosed as attention deficit hyperactivity disorder. In later stages of the disease, symptoms include auditory and vision impairment, apraxia, gait and motor difficulties, and seizures, eventually leading to a vegetative state and early death (typically within 4 years of

symptom onset). Cerebral involvement in adults may be misdiagnosed as a psychotic disorder such as schizophrenia. In some patients, demyelination arrests spontaneously but can recur, even after a long period of stability [94]. Symptoms of AMN include spastic paraparesis, sensory ataxia, leg pain, sphincter dysfunction, and impotence, reflecting spinal cord involvement; MRI of the spinal cord reveals nonspecific atrophy without demyelination, whereas brain MRI is typically normal or shows only subtle abnormalities [94].

Diagnosis of ALD is based on measurement of plasma VLCFA levels (C26:0 concentration, ratio of C24:0 to C22:0, and ratio of C26:0 to C22:0) [373]. However, hemolytic samples or a ketogenic diet can produce false-positive results. In addition, approximately 20 % of female heterozygotes may have normal VLCFA levels [179], and consumption of oils rich in erucic acid can produce false-negative results [94], indicating the need for mutational analysis for individuals at risk for ALD.

4.2.3 Genetics

More than 1,400 *ABCD1* gene mutations have been identified, and 46 % of those are nonrecurrent mutations (http://www.x-ald.nl/). Most are missense mutations (62 %), frame-shift mutations (22 %), or nonsense mutations (10 %). A recent study reported a de novo mutation rate of 4.1 % for index cases and evidence of gonadal and gonosomal mosaicism (<1 % of patients) [399]. Lack of genotype–phenotype correlation and the wide range of phenotypes, even within the same family, suggest the involvement of other factors contributing to the phenotypic expression of ALD. No modifier genes have been yet identified, but head trauma appears to be an environmental factor that can trigger rapid demyelination in patients with AMN [299].

4.2.4 HSCT

Since the first successful transplantation was described in 1990 [11], several large analyses have confirmed the benefits of HSCT for patients with early-stage disease [216, 244, 282]. Treatment outcomes are dependent on pre-transplantation Loes score and clinical neurologic status, as HSCT is unable to reverse or halt neurologic deterioration. Reduced-intensity conditioning has been used successfully to decrease transplant-related morbidity and mortality [262, 266, 302]; however, long-term follow-up is needed.

Because ALDP is a peroxisomal membrane protein, HSCT cannot provide cross-correction of the missing protein in ALD. The mechanism may involve replacing a portion of the perivascular microglia, and the continued demyelination that occurs for 12–18 months after transplantation may be due to the slow replacement of dysfunctional microglia [56]. Cerebral ALD is a rapidly progressing disease, leaving only a narrow window of opportunity in which transplantation will be effective.

To increase early detection of ALD, a newborn screening method was developed to detect elevated VLCFA levels in dried blood spots [161], and New York State passed legislation in March 2013 to add ALD to its newborn screening program. However, biomarkers are needed to identify which patients are likely to develop cerebral disease. In addition, alternative treatments are urgently needed for patients in later stages of the disease or for whom an HLA-matched donor or cord blood cannot be found.

4.2.4.1 HSCT Neurodevelopmental Outcomes

Early reports of HSCT described stabilized or improved memory, concentration, and language abilities in boys with cerebral ALD [11, 326]. This finding has been confirmed in long-term follow-up; however, most patients have some neurocognitive impairment after transplantation. Neurodevelopmental outcome is dependent on disease progression (e.g., Loes score, nonverbal IQ) and the extent and location of cerebral demyelination at the time of transplantation [20, 244, 282, 328]. In particular, parietal occipital demyelination is associated with poor outcomes [282]. A recent study evaluating adaptive functioning in cerebral ALD after HSCT reported that the one of the four surviving patients was employed full-time, and the other three attended school; all were ambulatory and functioning independently 5–13 years after transplantation [120].

4.2.4.2 HSCT Sensory Outcomes

Posttransplant visual and auditory function is significantly impaired in many patients with cerebral ALD [56, 251, 354], although HSCT has been reported to stabilize or improve function in some patients [20]. Demyelination involving the parietal-occipital lobes, performance IQ < 76, and pretransplant MRI severity score >11 are predictive of vision loss after transplantation [123].

4.2.5 Other Treatments

Alternative approaches for the treatment of ALD include drugs targeting brain inflammation, oxidative damage, VLCFA biosynthesis, and induction of *ABCD2* (a close homolog of *ABCD1*). In addition, most male patients have adrenal insufficiency requiring adrenal hormone replacement therapy, which can be life saving, and many patients require other symptomatic treatments (e.g., for incontinence, pain, spasticity) [94]. Combining a low-fat diet with oral intake of Lorenzo's oil (4:1 mixture of glyceryl trioleate and glyceryl trierucate) has been shown to normalize plasma VLCFAs, likely through competitive inhibition of enzymes involved

in VLCFA formation [380]. This treatment may reduce the risk of cerebral disease in presymptomatic individuals [250] but does not provide clinical benefits for patients with neurologic deficits [380]. Although lovastatin was reported to lower VLCFA levels, prompting its use in patients, findings from a recent randomized, double-blind, placebo-controlled crossover trial indicate that the small decrease in plasma VLCFA levels in patients taking lovastatin is due to a decrease in LDL cholesterol; VLCFA levels in peripheral blood lymphocytes and erythrocytes were unchanged [95].

Induction of *ABCD2* expression with 4-phenylbuyrate showed promise in animal studies, but this approach was not successful in a clinical trial, perhaps due to the short half-life of this compound or onset of tachyphylaxis [178]. Bezafibrate, a pan-peroxisome proliferator-activated receptor activator, lowers VLCFA levels in vitro through direct inhibition of ELOVL1, the enzyme responsible for the biosynthesis of C26:0 [96]. However, bezafibrate did not lower VLCFA levels in plasma or lymphocytes in a recent pilot study in ten men with AMN at the doses used [97].

Attempts to reduce inflammation with immunomodulators and immunosuppressive drugs (e.g., intravenous immunoglobulin, interferon β, cyclophosphamide) have not been successful [25]. However, the use of antioxidants has shown promise. For example, *N*-acetyl-L-cysteine administered intravenously before and after HSCT may stabilize disease progression [363]. For that reason, all patients who undergo transplantation at the University of Minnesota now receive *N-acetyl-l*-cysteine [244]. A more recent study showed that valproic acid, which exerts antioxidant effects and induces *ABCD2*, decreases levels of monounsaturated VLCFA (26:1) and ameliorates oxidative damage in cultured fibroblasts of ALD patients [110]. Treating ALD patients with valproic acid for 6 months reversed oxidative damage in peripheral blood mononuclear cells [110], and 6-month treatment with valproate improved cognitive and behavioral impairment in a 24-year-old man with cerebral ALD [310]. In addition, the combination of NAC, α-lipoic acid, and α-tocopherol reverses oxidative, axonal degeneration, and locomotor deficits in a mouse model of ALD [210] and is currently being tested in patients with AMN.

To overcome some of the problems associated with traditional HSCT (e.g., graft rejection, graft-versus host disease), a lentiviral-based gene therapy approach has been developed involving the collection, genetic correction, and reinfusion of the patient's CD34+ cells. In the first trial, two patients successfully underwent HSC gene therapy after full myeloablative conditioning [58]. Results of follow-up revealed stable ALDP expression in peripheral blood cells, polyclonal hematopoietic reconstitution, reduced plasma VLCFA levels (by 38 %), and arrest of CNS demyelination within 16 months [58]. Besides an initial decline in cognitive function before the arrest of demyelination, the first patient is considered relatively normal with some decline in verbal IQ and spasticity in one arm. The other patient experienced severe loss of visual acuity and cognitive decline due to visual impairment, but school performance is normal. During the 3-year follow-up, no changes in demyelination were observed [57].

4.3 Krabbé Disease

Krabbé disease, also known as globoid cell leukodystrophy, is caused by the deficiency of galactocerebrosidase (GALC), a lysosomal enzyme that degrades galactolipids for the normal turnover of myelin [403]. Rarely, a deficiency of saposin A protein, an activator of GALC, is the defect causing Krabbé disease [340]. The disease is characterized by the infiltration of multinucleated globoid cells, progressive demyelination of the CNS and PNS, and axonal degeneration in the PNS [402].

Disease onset can have an infantile, juvenile, or adult presentation. Infantile disease is the most common form, with an incidence of 1 in 70,000–100,000 [403], and can be divided into early-infantile (birth to 6 months) and late-infantile (6 months to 3 years). Neurologic deterioration in the infantile form is rapidly progressive, ultimately leading to loss of cerebral brain function and early death. Clinical presentation in the later-onset forms of the disease varies widely, and the disease is less relentlessly progressive, with some patients exhibiting no cognitive impairment [403].

4.3.1 Pathophysiology

Undegraded galactosylceramide stimulates the massive infiltration of blood-derived macrophages, which phagocytose the free galactosylceramide, forming globoid cells [352]. Accumulation of psychosine (a toxic galactolipid) results in the loss of myelin-forming cells, progressive demyelination, astrocytic gliosis, and axonal degeneration [402]. Mechanisms underlying the effects of psychosine appear to involve peroxisomal dysfunction [144] and/or regulation of phospholipase A2 [126], apoptotic pathways [145, 335], AMP-activated protein kinase [125], protein kinase C [406], or lipid raft-mediated endocytosis [405]. Neuropathologic changes in Krabbé disease include degeneration of the optic radiation, frontoparietal white matter, and corticospinal tract [402]. Peripheral neuropathy can often be detected by nerve conduction studies, even before clinical symptoms appear [331].

4.3.2 Clinical Disease and Diagnosis

Babies with infantile Krabbé disease generally develop normally during the first few months of life. Initial signs and symptoms include feeding difficulties, excessive crying and irritability, and loss of developmental milestones, followed by stiffness, seizures, apnea, and loss of hearing and vision. Most children with early-infantile disease die within 2 years unless they receive aggressive supportive

care [85]. Signs and symptoms are similar in late-infantile disease, but these children survive longer, with about half living more than 10 years after symptom onset. The most common initial sign of juvenile Krabbé disease is change in gait, followed by loss of milestones and vision loss (in younger children), stiffness, poor feeding, and seizures. Patients with adolescent/adult onset also tend to present with gait changes or leg weakness/stiffness; disease progression is much slower in these cases and sometimes stabilizes [86].

Diagnosis is established by very low GALC levels in peripheral blood leukocytes (0–5 % of the normal mean, 4.2 nmol/h/mg protein with a range of 1–10 nmol/h/mg protein) in peripheral blood leukocytes and identification of mutations in the *GALC* gene located at 14q24.3-34.1 [402]. In 2006, New York State began screening newborns for Krabbé disease with other states soon to follow [88]. Because neither enzyme activity or mutational analysis can predict phenotype in most cases, infants identified through screening are referred for standardized neurologic examinations and neurodiagnostic tests in an attempt to determine which infants have early-infantile disease and thus require urgent treatment [87]. Diffusion tensor imaging with fiber tracking has been used to identify changes in the corticospinal tracts before symptoms appear [100].

4.3.3 Genetics

Krabbé disease is inherited in an autosomal, recessive manner, and more than 75 disease-causing mutations have been identified. The most common mutation is a 30-kb deletion; all patients homozygous for this mutation have infantile Krabbé disease [401]. Mutations affecting protein folding and processing, secretion and reuptake, and localization to the lysosome have been characterized [202]. Sequencing of the *GALC* gene may occasionally identify the 30-kb deletion or a late-onset mutation (809G > A), otherwise no genotype–phenotype relationship has been identified. Genetic background appears to influence the overall phenotypic expression of the disease [402]; therefore, phenotype predictions cannot be made a priori based on the nature or location of a missense mutation. In addition, common polymorphisms can influence the level of circulating enzyme; therefore, enzyme levels do not always correlate with disease severity [401].

4.3.4 HSCT

The only disease-modifying treatment for Krabbé disease is HSCT, which was originally performed using bone marrow [194] but is now more commonly performed using umbilical cord blood. In asymptomatic neonates UCBT improves neurologic outcome; however, symptomatic patients remain devastated after

transplantation [99]. A staging system based on clinical signs and symptoms (feeding problems, abnormal reflexes, seizures) was developed to identify infants likely to benefit from UCBT [99]. Almost all newborns that undergo transplantation successfully maintain normal cognitive function; however, motor outcomes range from mild to severe disability. In addition, many require therapy, augmentative communication devices, and gastrostomy tubes for supplemental feeding after transplantation [98].

4.3.4.1 HSCT Neurodevelopmental Outcomes

In the late-infantile and juvenile forms of Krabbé disease, HSCT has been shown to halt CNS deterioration, even after signs/symptoms become apparent. After engraftment, some patients show normal cognitive ability, improved motor development, and independence in performing activities of daily living [192, 194, 326]. In addition, HSCT improves neurodevelopmental outcomes of patients with early-infantile disease, but because this form is so rapidly progressive, transplantation must be performed in the neonatal period before symptoms of CNS deterioration appear [189, 192]. Asymptomatic newborns that undergo successful HSCT often show normal cognitive function, receptive language, and adaptive behavior after treatment [98]. However, in all forms of the disease, difficulties in motor function and expressive language are common, possibly due to irreversible damage to motor tracts that occurs before treatment [98].

4.3.4.2 HSCT Somatic Outcomes

HSCT is able to preserve normal vision and hearing in presymptomatic newborns with early-infantile Krabbé disease [98], and stabilize or improve vision and hearing in patients with later-onset disease [193, 194, 326].

4.3.5 Other Treatments

Although UCBT prolongs life and provides significant neurologic benefits, it is not a cure for Krabbé disease. To target multiple aspects of this complex disease, alternative treatments are being tested, alone or in combination, in the mouse model of Krabbé disease. For example, CNS-directed ERT [294] and gene therapy [117, 296, 300] have been combined with BMT to provide synergistic effects. Other researchers are taking advantage of the anti-inflammatory and immunosuppressive properties of mesenchymal stem cells [304] or neural stem cells [259] for use in transplantation.

4.4 Mucopolysaccharidoses

The mucopolysaccharidoses are a group of disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down glycosaminoglycans (GAGs), unbranched polysaccharides that are extremely diverse in terms of chain length, sulfation pattern, and other modifications. These complex molecules are involved in inflammatory processes [359], cell signaling [182], recycling of proteins and organelles [320], and intracellular trafficking [185]. In addition, GAGs help build bone, cartilage, tendons, corneas, skin, and connective tissue. Individuals with a mucopolysaccharidosis (MPS) disorder either do not produce enough of 1 of the 11 enzymes required to break down GAG chains or they produce enzymes that do not work properly. Thus GAGs collect in the cells, blood, and connective tissues over time. The result is permanent, progressive cellular damage that affects appearance, physical abilities, organ and system functioning, and in most cases, mental development.

4.4.1 Clinical Disease and Diagnosis

Seven distinct clinical types and numerous subtypes of the mucopolysaccharidoses have been identified. Although MPS disorders differ clinically, most patients generally experience a period of normal development followed by a decline in physical and/or mental function. Mucopolysaccharidoses I, II, and III have been studied to a greater extent than MPS IV, VI, VII, where there is significant bone involvement. For the purpose of this chapter we will focus primarily on MPS I, II, and III.

4.4.1.1 Mucopolysaccharidosis I (Hurler Syndrome)

MPS type I is an autosomal, recessive disorder caused by a deficiency of α-l-iduronidase, a lysosomal enzyme that degrades heparan sulfate and dermatan sulfate [260]. In Hurler syndrome (the most severe form of MPS I), GAG accumulation results in progressive CNS deterioration, skeletal abnormalities, cardiac disease, hepatosplenomegaly, corneal clouding, deafness, and death in childhood [10, 89, 260, 332, 400, 415].

Diagnosis is based on enzyme activity, and mutational analysis can help predict phenotype. The gene encoding α-L-iduronidase (*IDUA*) is located on chromosome 4p16.3, spans 19 kb, and contains 14 exons [315]. More than 100 disease-causing mutations in the *IDUA* gene have been reported in the Human Gene Mutation Database (http://www.hgmd.org). Most mutations are private, and most genotypes are unique combinations of mutations. However, the W402X, Q70X, P533R, and G51D mutations are common in specific populations [22]. Hurler syndrome is

caused by two severe mutations that result in negligible or no enzyme activity (nonsense, frame shift, and splice-site mutations), whereas milder MPS I phenotypes are associated with at least one allele that allows the production of some functional enzyme. However, environmental factors and polymorphisms in *IDUA* and other genes in the degradation pathway limit the ability to predict phenotype from genotype [22].

The accumulation of undegraded heparan sulfate and dermatan sulfate appears to trigger oxidative stress [301] and lysosomal membrane permeabilization [278], and impair the function of other lysosomal enzymes, leading to secondary storage products such as gangliosides [236]. Ganglioside accumulation is thought to contribute to the CNS manifestations in Hurler syndrome by altering axon and dendrite morphology [396]. Products of incomplete heparan sulfate degradation also stimulate toll-like receptor 4, which may be responsible for microglial activation in the CNS [168, 265], and interfere with fibroblast growth factor 2 and bone morphogenetic protein 4 signaling, which may contribute to neurodegeneration and skeletal abnormalities [15].

HSCT

Used widely since 1980, allogeneic BMT can halt disease progression and prolong life, particularly if the procedure is performed early in life [407]. However, many children lack an appropriately matched donor. Unrelated UCBT has been shown to be effective in improving neurological function (Staba 2004). Analysis of a large cohort of patients with Hurler syndrome confirmed the importance of early transplantation and supports the use of UCB, which is readily available and can prevent delays in treatment [34].

Other Treatments

Another treatment option for Hurler syndrome is ERT, but the efficacy of intravenous ERT is limited by the inability of the enzyme to cross the blood–brain barrier. However, combining HSCT with ERT, which is provided before and after transplantation, appears to improve transplant outcomes [91], possibly by reducing the GAG accumulation that occurs before engraftment. Delivering recombinant enzyme directly to the CNS by intrathecal injection was shown to normalize GAG levels in the brain in large animal models [80, 172, 392]. In addition, intrathecal ERT has shown promising results in a patient with Hurler syndrome [257] and is currently being tested in clinical trials. Possible limitations of this approach include its invasive nature, the need for multiple injections, and difficulty in achieving adequate distribution of the enzyme in the brain.

Alternative therapies under investigation include the use of oral aminoglycosides [175, 398], which can cross the blood–brain barrier and may be able to restore enzyme expression in patients with premature stop codons. In addition,

results of preclinical trials have demonstrated the effectiveness of gene therapy using retroviral vectors [242], AAV vectors [92], HSCs transduced with lentiviral vectors (ex vivo gene therapy) [391], and nonviral methods such as a nonintegrating minicircle DNA vector [269].

4.4.1.2 Mucopolysaccharidosis II (Hunter Syndrome)

Hunter syndrome is an X-linked metabolic storage disorder that occurs predominantly in males, with an estimated prevalence of approximately 1 in 100,000 male live births [13, 211, 240, 288, 312, 425]. Although females with Hunter syndrome have been identified, most affected girls are heterozygotes in whom the normal allele is not expressed due to additional genetic events such as skewed X-inactivation [33, 330, 408, 410]. In Hunter syndrome, deleterious mutations in the iduronate-2-sulfatase (*IDS*) gene located at Xq28, result in an insufficiency of the iduronate-2-sulfatase (I2S) enzyme required to initiate degradation of dermatan and heparan sulfates via removal of sulfate [260]. The loss of I2S activity results in excess GAG accumulation within lysosomes of various tissues and organs, including the CNS, with progressive cellular vacuolization and consequent cell death. One outcome of this gradual cellular destruction is the urinary excretion of I2S at high levels and the myriad clinical manifestations of Hunter syndrome [426]. The precise mechanisms by which I2S deficiency leads to the presenting features of the disease are not well understood. However, the pathophysiologic mechanisms are believed to be multifaceted and likely involve one or more of the following: (1) direct storage of GAGs in the lysosome, (2) inhibition of other lysosomal enzymes by GAG fragments, (3) interference of lysosomal and endosomal trafficking of molecules, (4) inflammatory response to the GAG accumulation, (5) interference with the function of cell surface receptors and other proteoglycans, and (6) alterations in the extracellular matrix by GAG accumulation [60].

Two clinical forms of Hunter syndrome are recognized: mild (attenuated, nonneuronopathic) and severe (neuronopathic). The central distinguishing factor between the two forms is the presence, or absence, of progressive intellectual deterioration [205, 424]. Patients with neurologic involvement typically display wide-ranging abnormalities beginning around 3–4 years of age and die during their second decade from obstructive airway disease and cardiac failure. Conversely, patients without signs of cognitive impairment can remain mentally functional into early adulthood but still exhibit cardiorespiratory and skeletal disease [240, 260]. Although Hunter syndrome is often characterized by multiple skeletal deformities (known collectively as dysostosis multiplex), extensive organ and soft tissue involvement, and neuropsychological impairments, the attenuated form is generally characterized by a broad spectrum of joint stiffness and varying degrees of somatic changes [260].

More than 300 different genotypic variations have been documented in *IDS* gene, located at Xq28, patients with Hunter syndrome [225]. Studies have estimated disease-causing mutations as 55–57 % missense, 21 % nonsense, 14–20 % small

deletions (<20 bp), and 4–10 % major structural alterations such as large deletions (>20 bp) and rearrangements [49, 107, 225]. Additionally, it has been reported that in 13 % of Hunter syndrome patients, a homologous chromosomal recombination has occurred that involves *IDS* and the *IDS* pseudogene (*IDS2*) located within 80 bp on the telomeric side of *IDS* [35]. Most of these mutations are private, making evaluation of genotype–phenotype association difficult. However, the severe phenotype often appears to be associated with complete absence of functional enzyme due to total or partial gene deletion or the *IDS*/*IDS2* rearrangement [116, 131]. Enzyme abnormalities resulting from single amino acid mutations are associated with a broad spectrum of phenotypes running the gamut from severe to attenuated clinical presentation [131, 204, 371]. Additionally, atypical presentation of clinical symptoms in association with the severe phenotype occurs in patients with deletions spanning beyond the *IDS* locus [362]. Results of routine diagnostic assays measuring either the amount or activity of I2S do not correlate with phenotypic severity in Hunter syndrome patients [272].

The natural history of Hunter syndrome was recently described [157] and indicates that the neuronopathic form of the disease affects children by 7.3 months on average, leading to severe brain involvement by the age of 5 years. The nonneuronopathic form does not affect cognition but can cause hearing loss. Early markers of disease progression were described to help identify those children at risk for developing brain disease [156].

HSCT

HSCT has been attempted in order to correct the neurological involvement in Hunter syndrome. Results of early studies showed decreased GAG levels and improvement in somatic features after transplantation, but neurologic deterioration continued in patients with the severe form of the disease [138, 383]. A more recent study of 21 patients in Japan suggested some neurologic improvements after HSCT if performed in the early stages of the disease (e.g., ability to perform activities of daily living were maintained, improvements in brain abnormalities on MRI) [357]. The effects of unrelated UCBT are presently under study.

Other Treatments

The following methods have been proposed to replace the I2S enzyme: human amnion membrane implantation [254], fibroblast transplantation [74], white blood cell infusion [184], serum or plasma infusion [421], gene therapy [55, 345, 364], intraperitoneal implantation of myoblasts overexpressing I2S [115], intravenous ERT [253, 255], and intrathecal ERT [81, 336]. Although many of the physical symptoms can be reduced or eradicated via intravenous ERT [256], neurologic deterioration is irreversible, and early diagnosis and treatment may be necessary to ameliorate or prevent CNS damage.

4.4.1.3 Mucopolysaccharidosis III (Sanfilippo Syndrome)

Sanfilippo syndrome is an autosomal, recessive disease caused by the accumulation of heparan sulfate. The disease can be divided into four subtypes based on which enzyme involved in the stepwise degradation of heparan sulfate is deficient: heparan *N*-sulfatase (MPS IIA), *N*-acetyl α-glucosaminidase (MPS IIIB), acetyl-CoA:α-glucosaminide *N*-acetyltransferase (MPS IIIC), or *N*-acetylglucosamine 6-sulfatase (MPS IIID) [379]. All subtypes are characterized by progressive neurologic decline after a period of apparently normal development. Developmental delay and behavioral problems (hyperactivity, aggression) typically appear between 2 and 6 years of age, followed by sleep disturbances, recurrent infections, diarrhea, hearing impairment, epilepsy, developmental regression, and early death. Other than facial dysmorphology, which may be mild, there is typically little or no somatic involvement [379]. Cognitive function varies widely, with patients reaching the maximal developmental age of 3–4 years for more severe phenotypes and up to 10 years without regression in attenuated phenotypes [377]. Sanfilippo syndrome is the most common MPS disorder, with an estimated prevalence of 0.28–4.1 per 100,000 births [379].

More than 75 mutations have been reported for Sanfilippo type A, which appears to be the severe form; most are missense mutations. Five mutations account for 81 % of all mutations (p.R245H, p.S298P, p.Q380R, p.S66W, and c.1080delC), and genotype has some ability to predict phenotype [378]. Of the more than 100 mutations that have been reported for type B, most are missense and most are private mutations. A recent Dutch study showed that most patients with this subtype have attenuated disease; however, this finding may be due to the high degree of awareness of Sanfilippo disease in the Netherlands [376]. More than 50 mutations have reported for type C. Analysis of the 21 missense mutations, which typically cause the severe phenotype, shows that 17 cause protein misfolding, suggesting the potential therapeutic value of pharmacological chaperones [378]. In contrast, only 22 mutations have been reported for type D (3 missense, 4 nonsense, 5 splice site, 5 frame shift, 4 large deletions, and 1 small deletion). Only 31 patients with this rare form of Sanfilippo syndrome have been described in the literature, and these cases have showed variable rates of progression and motor function [375].

HSCT

HSCT has generally proved disappointing in treating Sanfilippo syndrome [291, 379].

Other Treatments

Intravenous ERT reduces levels of heparan sulfate-derived disaccharides and secondary storage products (ganglioside and unesterified cholesterol) in dogs [70], and

intrathecal ERT is currently being studied in clinical trials. To target the CNS, preclinical studies of gene therapy have focused on intracerebral injection [92], intrathecal injection [92], or ex vivo transduction of HSCs [149, 199]. In a mouse model of Sanfilippo type B, combining intracranial injection of an AAV vector with intravenous infusion of lentiviral vector improved motor function and hearing, as well as biochemical and histologic aspects of the disease [149]. A clinical trial investigating the use of intracerebral gene therapy for Sanfilippo type A is currently underway.

Although substrate reduction with genistein has been promising in animal models, doses of 5–10 mg/kg/day in patients with Sanfilippo syndrome have produced mixed results [73, 76, 286]. However, larger doses (10–15 mg/kg/day) appear to improve clinical outcomes (behavior, sleep, developmental regression) in patients with Sanfilippo type A or B [219].

4.4.1.4 Mucopolysaccharidosis IV, VI, and VII

Morquio syndrome (MPS IV) is characterized by severe skeletal dysplasia, hearing impairment, and cardiovascular and respiratory manifestations, but cognitive function is typically normal. In type A Morquio A syndrome, which is caused by galactosamine-6-sulfatase (GALNS) deficiency, the digestive system and vision are also affected. More than 180 disease-causing mutations have been identified in the *GALNS* gene, most of which cause protein misfolding [305] and are associated with the severe phenotype [150]. Type B Morquio syndrome is caused by a deficiency in β-D-galactosidase, which can also cause GM_1 gangliosidosis. Currently, only symptomatic treatment is available for patients with Morquio syndrome. However, ERT was shown to reduce GAG accumulation in a mouse model of Morquio A [366], and intravenous ERT for this subtype is currently being tested in clinical trials.

Clinical symptoms are similar in Maroteaux–Lamy syndrome (MPS VI), which is caused by arylsulfatase B deficiency. Most of the more than 130 mutations in the *ARSB* gene are private mutations, making it difficult to determine genotype–phenotype correlations [372]. Little data is available regarding long-term clinical outcomes after HSCT. Intravenous infusion of recombinant human arylsulfatase B (galsulfase), which has been available since 2005, reduces cardiac abnormalities and improves endurance, pulmonary function, and growth [41, 75, 146].

Mucopolysaccharidosis VII (Sly syndrome) is caused by β-glucuronidase deficiency, which results in bone dysplasia, short stature, mental retardation, and sometimes hydrops fetalis. At least 49 disease-causing mutations gave been identified, and genotype–phenotype correlations have been reported [365]. Several ERT approaches have shown promise in animal models, including infusion of recombinant enzyme modified to prevent clearance by mannose 6-phosphate and mannose receptors [309]. Clinical trials to test intravenous ERT are currently being planned.

4.4.1.5 HSCT Neurodevelopmental Outcomes in MPS Disorders

In untreated patients with Hurler syndrome (MPS I), neurocognitive decline begins within the first few years of life and progresses rapidly. Treatment with HSCT has been successful in some patients with this disease. Poor neurodevelopmental outcome after treatment is associated with lower mental developmental score (cognitive, adaptive, emotional, and social functioning) and age (>2 years) at the time of transplantation, low enzyme activity after engraftment, and acute graft-versus-host disease grade II–IV [281, 283, 326, 386, 407]. Despite initially falling behind, most children transplanted early in the course of the disease stabilize or improve in cognitive function; however, memory and concentration problems are common, and treated children often require special education and speech therapy [135, 220, 339, 341]. Results of an exploratory study suggest that the decreased attention span observed in patients after HSCT may be due to the treatment itself [327]. Chemotherapy-based conditioning regimens appear to affect white matter structure and function in transplanted children, similar to the changes seen in children who undergo chemotherapy for cancer [6]. A recent study reported that combining with HSCT with ERT improves neurocognitive development, particularly in the visual reception domain [91].

In patients with Hunter syndrome (MPS II), neurodevelopmental outcomes have generally been poor after HSCT, particularly in patients with the severe form of the disease who already show cognitive decline at the time of treatment [69, 138, 239, 279, 326, 383]. However, a recent study reported that when performed early in the course of the disease, HSCT can stabilize or improve expressive language, motor function, ability to carry out activities of daily living, and intelligence quotient, in some patients with severe Hunter syndrome, as well as those with attenuated disease [357]. In Sanfilippo syndrome (MPS III), HSCT has been unable to prevent significant developmental delay, behavioral disturbance (extreme aggression and hyperactivity), and eventual dementia, even when performed in the early stages of the disease before neurologic symptoms have appeared [141, 158, 280, 326, 333, 387].

4.4.1.6 HSCT Somatic Outcomes for MPS Disorders

One of the characteristic signs of the MPS diseases, which can lead to diagnosis, is the so-called coarse facial features, which are thought to be caused by GAG accumulation in the soft tissues of the orofacial region and dysostosis of the underlying facial bone. Characteristic facial features include macrocephaly with frontal bossing; short neck; midfacial dysplasia with a depressed nasal bridge and hypertelorism; enlarged tongue; thickening of the alae nasi, lips, and ear lobules; and coarsening of scalp hair and eyebrows. Although often subtle in the early phase, these dysmorphisms progress over time [64]. Few studies have described the outcome of facial characteristics after HSCT. Normalization of facial abnormalities, described as being “less coarse” has been reported in patients with MPS I, II, and VI

after HSCT [138, 152, 220, 239, 339, 386]. In addition, papular skin lesions that are characteristic of Hunter syndrome resolve after treatment [163].

Nearly all untreated patients with MPS disorders appear to have hepatosplenomegaly with preserved hepatic function [260]. After successful engraftment, the metabolism and clearance of GAGs is dramatically improved in the highly perfused visceral organs, resulting in a major reduction of the hepatosplenomegaly within the first months after HSCT [2, 4, 138, 162, 163, 190].

The accumulation of GAGs in the cartilage and subsequent failure of ossification and abnormal bone modeling result in a characteristic pattern of skeletal manifestations called dysostosis multiplex in all types of MPS, except for MPS type IX. These skeletal manifestations include thoracolumbar kyphoscoliosis, odontoid dysplasia, acetabular dysplasia, and genu valgum. In addition, GAG deposition in the joint capsules, tendons, and ligaments causes severe joint stiffness and contractures, carpal tunnel syndrome, and associated trigger fingers. Involvement of the growth plate is thought to be responsible for the significant short stature observed in untreated MPS patients. This growth failure is characterized by a disproportionally short trunk. Because of the highly progressive nature of these skeletal manifestations in most MPS (sub)types, both the severe complications that evolve (e.g., cord compression and joint luxation) and the severely reduced joint mobility will eventually lead to severe disability and discomfort in untreated patients [3].

Hurler Syndrome (MPS I)

The benefits regarding the outcome of the skeletal manifestations in Hurler syndrome patients after HSCT are less apparent than outcomes of other organ systems. Although upper extremity joint mobility and odontoid dysplasia improve in patients after successful HSCT, successful engraftment cannot prevent progression of most of the skeletal manifestations [106, 137, 154, 339, 343, 358, 386, 400]. Linear growth is maintained for several years after successful HSCT, but long-term follow-up results show a gradual fall in growth [137, 287, 339, 386]. Later age at HSCT and exposure to total body irradiation have been associated with short stature [287]. Preliminary results indicate that growth hormone treatment post-HSCT increases growth velocity in a subgroup of patients [287]. Furthermore, genu valgum, acetabular dysplasia, thoracolumbar kyphosis, and poor mobility of the lower limbs often progress in successfully transplanted Hurler syndrome patients, although the rate of progression varies. In addition, carpal tunnel syndrome and associated trigger fingers still develop in some patients after successful HSCT [106, 137, 339, 358, 386]. As a consequence, orthopedic and neurosurgical interventions are still necessary in many transplanted Hurler patients. Skeletal deterioration in MPS, despite successful engraftment of hematopoietic stem cells, is presumably caused by the relative avascularity of the skeletal tissues, precluding enzyme penetration into these tissues [106].

Because life expectancy is significantly increased after HSCT, progressing skeletal manifestations are now encountered in long-term survivors. To maintain function and improve quality of life in transplanted MPS patients, an appropriate

approach to the potential skeletal complications is of utmost importance. The benefits and doubts concerning the various surgical interventions therefore need to be further established during longer follow-up.

4.4.1.7 Other MPS Types

Although improved joint mobility and at least stable skeletal disease has been reported in several transplanted patients with Hunter syndrome (MPS I), Maroteaux–Lamy syndrome (MPS VI), and Sly syndrome (MPS VII), the skeletal outcome after HSCT appears to be highly variable, with marked progression in some patients [26, 69, 138, 152, 153, 158, 162, 190, 191, 239, 383, 419]. However, severe complications such as cord compression or atlantoaxial instability have not been reported after HSCT, except for one MPS II patient (mild cord compression at the craniocervical junction) with severely reduced enzyme levels due to mixed donor chimerism of only 8 % and use of a carrier donor [383]. Without HSCT these severe complications occur in a substantial percentage of patients with MPS I, II, IV, VI, and VII [171].

Data on skeletal outcome after HSCT in MPS III are scarce because only a few case reports exist, and the disease is dominated by neuropsychological manifestations, with skeletal manifestations being only of limited interest [63, 192, 333, 387]. A study by Vellodi et al. showed periods of normal growth interspersed with periods of decreased growth in transplanted twin sisters [387].

Cardiovascular manifestations that occur in the various MPS disorders include progressive cardiac valve abnormalities, increasing stenosis of the coronary arteries, increasing stenosis of the coronary arteries, thoracoabdominal aorta and systemic vessels, and primary myocardial involvement. These manifestations may evolve into dilated cardiomyopathy with congestive heart failure, contributing to early death, mainly in the more severe phenotypes [2, 71, 105, 260, 411].

HSCT in patients with MPS I, II, VI, and VII disorders preserves myocardial function and results in regression of hypertrophy, which will remain in long-term survivors [2, 40, 121, 137, 138, 152, 162, 190, 383, 390, 407, 419]. Postmortem examination of a 17-year-old Hurler syndrome patient 14 years after HSCT showed only minimally affected coronary arteries [39], in sharp contrast to the progressive coronary artery disease observed in untreated MPS patients [38, 47]. These improvements have made death resulting from coronary occlusion or severe congestive heart failure exceptionally uncommon in MPS patients after successful HSCT [40]. Yet, mitral and aortic valve deformities persist and may even progress immediately after transplantation, but stabilize with longer follow-up [40, 105, 138, 152, 162, 342, 383, 419]. Although valvular insufficiency evolves in some cases, surgical intervention of the valves is only rarely required, and discontinuation of cardiac medication is sometimes possible [2, 105].

A combination of respiratory manifestations is usually present in MPS patients, including reduced lung capacity and upper respiratory obstruction [203, 318, 324]. These manifestations contribute to the severe obstructive sleep apnea syndrome commonly reported in untreated patients [203, 324]. In addition, recurrent upper and lower respiratory tract infections often occur [324]. Eventually, pulmonary

hypertension and cor pulmonale may develop, contributing to cardiopulmonary failure and early death in untreated MPS patients [318]. Within the first months after HSCT, dramatic relief of obstructive airway symptoms, including persistent rhinorrhea, is seen in patients with MPS type I, II, VI, and VII [4, 26, 108, 137, 138, 152, 158, 339, 386, 407, 419]. Occurrence of obstructive sleep apnea is markedly decreased after HSCT, and pulmonary insufficiency is no longer observed, except for those cases caused by transplantation-related pulmonary complications [23, 152, 223, 339, 407, 419].

The predominant ocular manifestations observed in MPS are progressive corneal clouding and retinal degeneration causing progressively decreasing visual acuity in untreated patients. Optic atrophy, secondary to hydrocephalus, has been documented in untreated patients with the severe phenotype and leads to severe visual impairment [9, 10, 66, 140, 332, 348, 349]. After successful HSCT, the reported outcome of corneal clouding varies among MPS patients, probably due in part to difficulties in the objective grading of corneal opacities. Although clinical improvement of corneal clouding is seen in most MPS patients, a few patients show progression. Most transplanted patients have mild residual corneal clouding [101, 140, 152, 350], but corneal transplantation is only rarely required after HSCT [140, 339, 386]. Additionally, optic nerve edema resolves in most MPS patients [140, 350]. Despite stabilization or improvement in retinal function soon after HSCT [350], progressive retinal degeneration does occur in some transplanted MPS patients and may cause decreased visual acuity in long-term survivors [140, 370]. Importantly, optic nerve edema resolves along with the increased intracranial pressure after successful HSCT, and atrophy is not longer observed [140]. However, graft-versus-host disease and the conditioning regimens and immunosuppressive agents used in HSCT can cause new ocular complications in children with MPS and other metabolic storage disorders. These complications include cataracts, keratoconjunctivitis sicca, ocular hypertension, and visual field deficits [102, 103, 140, 367]

In addition to visual impairment, severe and progressive hearing loss is a universal problem in MPS patients. Hearing loss is usually caused by a combination of conductive and neurosensory problems [260, 332]. After successful HSCT, hearing is gradually normalized or improved or stabilized in most MPS patients [137, 138, 152, 159, 189, 239, 339, 386]. In addition to the rapidly improving conductive component, a late improvement of the neurosensory component has also been observed [137]. Only a small number of successfully engrafted patients require hearing aids [137, 138, 159, 385]. However, repeated grommet insertions due to chronic recurrent middle-ear infections are some times seen after HSCT [42, 152, 383, 386].

4.5 Mucolipidoses

Mucolipidosis (ML) disorders are a group of autosomal, recessive disorders characterized by the lysosomal storage of lipids and water-soluble substances. They are classified according to enzyme/protein deficiency, and symptoms are similar to

those seen in MPS disorders. ML I (sialidosis) is caused by mutations in the *NEU1* gene, which encodes α-neuraminidase (sialidase), a lysosomal enzyme involved in the catabolism of sialylated glycoconjugates and regulation of cell signaling [293]. Although age at onset and clinical severity vary among affected individuals, symptoms generally include progressive visual impairment, myoclonus syndrome, macular, cherry-red spots, seizures, facial coarsening, dysostosis multiplex, mental retardation, and hepatosplenomegaly [293, 344].

ML II (I-cell disease) and ML III (pseudo Hurler polydystrophy) are caused by mutations in the genes encoding different subunits of the enzyme *N*-acetylglucosamine-1-phosphotransferase, a membrane-bound enzyme that modifies lysosomal proteins with the trafficking marker mannose 6-phosphate [82]. Although ML III is less severe, both diseases are characterized by clinical symptoms similar to those seen in Hurler syndrome (MPS I) including psychomotor retardation, short stature, joint stiffness, contractures, hip dysplasia, organomegaly, corneal clouding, facial coarsening, and cardiac disease [3, 165].

ML IV is caused by mutations in *MCOLN1*, which encodes mucolipin 1, a lysosomal ion channel that appears to interact with lysosome-associated protein transmembrane proteins thought to be involved in the regulation of lysosomal function [388]. This disorder, which occurs more frequently in Ashkenazi Jews than in the general population, is characterized by ophthalmological abnormalities (corneal clouding, strabismus, retinal dystrophy, optic atrophy) and severe mental and psychomotor retardation [395]. The initial symptoms typically occur in infancy, followed by a long period of stability in which developmental age is maintained at about 12–15 months; however, milder forms of the disease have been reported [395].

4.5.1 HSCT Neurodevelopmental Outcomes

Several patients with ML I or II have undergone HSCT [132, 180, 290, 313, 420], and the reported results suggest modest effects. A 19-month-old girl with ML II who underwent transplantation initially gained expressive language and fine motor and adaptive skills; however, she showed mild to moderate cognitive impairment 5.5 years after treatment [132]. A patient with severe ML I who underwent HSCT at 9 months of age showed moderate to severe psychomotor retardation 11 years later (developmental quotient = 51) [313].

4.5.2 HSCT Somatic Outcomes

In patients with ML, hepatosplenomegaly is considerably reduced within the first months after successful HSCT [132, 162, 197]. Two ML II patients showed stabilization or worsening of skeletal manifestations after HSCT, even though both

patients achieved only heterogeneous enzyme levels [132, 197]. In addition, HSCT can relieve obstructive airway symptoms and prevent progression of corneal clouding and cardiac dysfunction in ML II [132, 162, 197]. In one patient with ML II, hearing loss was stabilized after transplantation but grommet insertion was needed due to chronic recurrent middle-ear infections [132].

4.5.3 Other Treatments

Results of in vitro studies indicate the potential for pharmacologic chaperones and proteasomal inhibitors in the treatment of ML I [263], and substrate reduction therapy with genistein in the treatment of ML II [271].

4.6 Alpha-Mannosidosis

Alpha-mannosidosis is caused by a deficiency of lysosomal α-mannosidase activity, leading to the accumulation of undegraded oligosaccharides in the CNS, bone marrow, and other tissues. The disease is characterized by progressive neurodevelopmental arrest deterioration, immune system defects, skeletal abnormalities, and hearing impairment [221]. The estimated birth prevalence has been reported as approximately 1 in 1,000,000 [285].

Although this disease shows considerable variability in clinical presentation, most patients exhibit skeletal abnormalities early in the course of the disease; these include kyphoscoliosis, pectus carinatum, genu valgum, joint contractures, and hip dysplasia [21]. Other common symptoms include sensorineural hearing loss and facial coarsening, followed by minor ophthalmologic symptoms (slight corneal opacity or cataract and amblyopia), recurrent infections, ataxia, mental retardation, and psychiatric symptoms (e.g., anxiety, depression, psychosis) [21, 221]. The severe form of α-mannosidosis (type I) is characterized by skeletal abnormalities at birth and early death, typically caused by CNS involvement and severe infections [21, 221]. Symptom onset occurs later in attenuated disease, and these patients typically survive into adulthood [21, 221]. Diagnosis is made by measuring activity of acid α-mannosidase in nucleated cells and can be confirmed by genetic analysis [221].

Alpha-mannosidosis is an autosomal, recessive disease caused by mutations in the *MAN2B1* (*LAMAN*) gene. Most disease-causing mutations that have been identified to date are missense, and impaired transport to the lysosome is the most common defect [143, 196]. No genotype–phenotype correlation has been identified [221].

4.6.1 HSCT Outcomes

Although the best outcomes have been seen in presymptomatic patients and those with relatively mild symptoms at the time of transplantation, HSCT has also been shown to resolve organomegaly, improve skeletal abnormalities, and stabilize or improve neurocognitive function in symptomatic patients [1, 46, 133, 258, 397, 422].

4.6.2 Other Treatments

Preclinical studies of ERT have demonstrated wide distribution of the enzyme and the apparent ability to cross the blood–brain barrier in mice [31, 307]. Preliminary results of a phase 2 clinical trial indicate that ERT may be able to improve both motor and cognitive function in patients with α-mannosidosis [37]. In addition, the calcium channel blockers diltiazem and verapamil have been shown to facilitate the proper folding and trafficking of the mutant enzyme, likely by upregulating the expression of cellular chaperone proteins [252].

4.7 GM_1 Gangliosidosis

GM_1 gangliosidosis is an autosomal, recessive disease caused by mutations in the *GLB1* gene that result in β-galactosidase deficiency and the massive storage of GM_1 ganglioside and related glycoconjugates in various tissues, including the CNS [52]. GM_1 gangliosidosis is characterized by progressive neurologic decline and has an estimated incidence of 1 in 100,000–200,0000 live births [48]. Suggested pathophysiologic mechanisms underlying this disease include the unfolded protein response, neuronal apoptosis, oligodendrocyte loss, inflammation, and mitochondrial dysfunction [48]. Insufficient β-galactosidase activity can also cause MPS IVB (Morquio B disease), but its major storage product is keratan sulfate, and Morquio B disease does not appear to affect the CNS [52].

Symptom onset occurs before 6 months of age for the infantile form of the GM_1 gangliosidosis (type I), between 7 months and 3 years for the late-infantile/juvenile form (type II), and between 3 and 30 years for late-onset disease (type III) [48]. As with other LSDs, age at onset is associated with disease severity. Initial symptoms in type I disease include psychomotor regression and hypotonia, followed by hepatosplenomegaly, skeletal abnormalities, coarsening of facial features, macular cherry-red spot, and early death (by 2 years of age). Patients with type II disease show milder facial and skeletal abnormalities and slower neurologic decline. In type III disease, typical symptoms include cerebellar dysfunction, dystonia, and mild vertebral deformities [52]

4.7.1 HSCT Outcomes

HSCT does not appear to be able to prevent disease progression, even when performed in a developmentally normal patient [329].

4.7.2 Other Treatments

Several small molecule drugs have been investigated for GM_1 gangliosidosis. For example, nonsteroidal anti-inflammatory drugs (indomethacin, aspirin, ibuprofen) and antioxidants (L-ascorbic acid, α-tocopherol acetate) are able to slow disease progression in mice, suggesting their potential use as adjunctive therapy [166]. *N*-butyldeoxynojirimycin (miglustat) inhibits glucosylceramide synthase, the enzyme that catalyzes the first committed step in glycosphingolipids synthesis, and reduces GM_1 ganglioside biosynthesis in a mouse model of juvenile disease [93]. However, miglustat appears to have only limited clinical benefit in patients with GM_1 gangliosidosis, improving gross motor skills and expressive language but not cognitive function in two patients with juvenile disease [361], and effective only during the first 4 weeks of treatment in an 18-month old symptomatic patient with infantile disease [119]. The pharmacological chaperone drug *N*-octyl-4-epi-b-valienamine shows promise, restoring enzyme activity in somatic cells of patients and halting neurologic progression in mice [353].

Gene therapy for GM_1 gangliosidosis is still in the preclinical phase of research. An ex vivo approach using transduced bone marrow cells was shown to reduce inflammation and halt neurodegeneration in mice [311]. Intracranial delivery of a vector encoding β-galactosidase restored enzyme activity, decreased lysosomal storage, and prolonged the lifespan of mice [14, 45].

4.8 GM_2 Gangliosidosis

The GM_2 gangliosidoses are a group of autosomal, recessive neurodegenerative disorders (Tay–Sachs disease, Sandhoff disease, AB variant) caused by insufficient β-hexosaminidase activity, resulting in the accumulation of GM_2 ganglioside. Beta-hexosaminidase is a dimeric enzyme with three isoenzymes, each of which is a different combination of the two subunits (α and β). Tay–Sachs disease is caused by mutations in the α subunit encoded by the *HEXA* gene and has an estimated incidence of 1 in 222,000 live births in the general population and 1 in 3,900 live births among Ashkenazi Jews [104, 217, 240]. Although more than 100 *HEXA* mutations have been characterized, three mutations account for 93 % of identified carriers in the Ashkenazi Jewish population [170]. Sandhoff disease is caused by mutations in the β subunit encoded by the *HEXB* gene and has estimated incidence 1 in 422,000

live births [217, 240]. The extremely rare AB variant is caused by mutations in *GMA2*, which encodes the GM_2 ganglioside activator protein required for β-hexosaminidase activity [217]. Although these diseases result from different mutations, the clinical course is similar, with age at onset, specific symptoms, and severity dependent on residual enzyme activity [218].

Accumulation of GM_2 ganglioside along with other membrane proteins and lipids occurs in various tissues, especially neurons, which form meganeurites and axonal spheroids. Neuronal death occurs due to altered calcium homeostasis and an inflammatory process involving activated macrophages/microglia [188, 384]. Common initial symptoms for infantile GM_2 gangliosidosis, the most common form, include developmental arrest, exaggerated startle response, hypotonia, vision problems, loss of motor skills, and seizures. Death typically occurs before the age of 5 years [32]. In juvenile GM_2 gangliosidosis, common initial symptoms are gait disturbances, incoordination, speech problems, and developmental delay, followed by muscle wasting and proximal weakness, incontinence, and frequently cognitive impairment; neuropathy or psychiatric symptoms occur more frequently in Sandhoff disease [114, 214]. The disease course is similar in the adult form of the disease, but intelligence is generally only minimally affected [214].

4.8.1 HSCT Outcomes

HSCT does not appear to increase survival in patients with GM_2 gangliosidosis, and no patients have gained developmental milestones after transplantation, including those who underwent treatment before 6 months of age [32, 164].

4.8.2 Other Treatments

Several small molecule drugs have shown promise in the treatment of some forms of GM_1 gangliosidosis. Miglustat appears to slow disease progression in juvenile-onset Sandhoff disease [213, 355, 413] but showed no measureable benefits in a randomized, control trial testing its ability to ameliorate progressive muscle wasting in late-onset Tay–Sachs disease [325]. Pyrimethamine acts as a pharmacological chaperone, partially restoring enzyme activity in cell lines with mutations that affect protein stability [215] and is being tested in patients with late-onset GM_1 gangliosidosis [61, 270]. In addition, nonsteroidal anti-inflammatory drugs, alone or in combination with substrate reduction therapy, slowed disease progression and extended lifespan in a mouse model of Sandhoff disease [166].

In mouse models of Tay–Sachs and Sandhoff disease, gene therapy delivered by intracranial injection has been shown to restore enzyme activity, decrease GM_2 ganglioside storage in the CNS [228, 229], reduce inflammation, and increase lifespan [51, 368]. Clinical trials are currently underway to test gene therapy in patients with Tay–Sachs disease.

4.9 Niemann–Pick Disease

Niemann–Pick disease is a group of autosomal, recessive disorders classified according to biochemical defect: (1) deficiency of acid sphingomyelinase, the enzyme required for the hydrolysis of sphingomyelin (types A and B), or (2) defects in intracellular lipid trafficking (type C) [187]. Niemann–Pick types A and B are caused by mutations in the *SMPD1* gene [169], resulting in lysosphingomyelin accumulation in tissues throughout the body in both types [308]. Lysosphingomyelin also accumulates in the brain in the more severe type A disease, which occurs more frequently among Ashkenazi Jews; three mutations (R496L, L302P, fsP330) account for about 92 % of the mutant alleles in this population [187]. Niemann–Pick type C, the most common type, is caused by mutations in *NPC1* or *NPC2*, which encode proteins involved in the lysosomal–endosomal trafficking of cholesterol and other molecules [334]. Disease-causing mutations in either gene result in the same clinical phenotype.

The pathophysiologic mechanisms underlying acid sphingomyelinase-deficient Niemann–Pick disease (types A and B) appear to involve membrane alterations caused by sphingolipid accumulation, leading secondary biochemical and cellular abnormalities [200]. Residual acid sphingomyelinase activity is lower in the more severe neuronopathic phenotype (type A), which is characterized by brain abnormalities including the secondary accumulation of GM_2 and GM_3 gangliosides, demyelination, and alterations in calcium homeostasis, signal transduction, neuronal polarization, and presynaptic plasticity [200].

Patients with type A disease typically present with hepatosplenomegaly in first few months of life. Other signs and symptoms include feeding difficulties, failure to thrive, irritability, macular cherry-red spot, respiratory infections, and sleep disturbance [237]. Neurodevelopment plateaus in the first year and then rapidly declines, with death typically occurring by 3 years [237]. Type B disease has a more variable clinical course with little or no neurologic involvement. Childhood onset is more common than adult onset, with hepatosplenomegaly, bleeding, shortness of breath, pulmonary infections, and joint/limb pain as typical initial signs and symptoms [238]. Most patients show evidence of interstitial lung disease and are at high risk for coronary artery disease [238].

In Niemann–Pick type C unesterified cholesterol, sphingolipids, and other lipids accumulate in the liver and spleen, but GM_2 and GM_3 gangliosides and sphingosine are the primary storage material in the brain [382]. Neuropathologic features of type C disease include neuronal meganeurites, extensive ectopic dendrite growth, neurofibrillary tangles, neuroaxonal dystrophy, and neuronal death [382].

The clinical course of Niemann–Pick type C is heterogeneous in terms of symptoms, age at onset, and disease progression. Most patients experience visceral involvement (e.g., hepatosplenomegaly, lung infiltration with foam cells), which precedes the progressive and severe neurodegeneration [382]. Neurologic symptoms include ataxia, dysarthria, dysphagia, vertical supranuclear gaze palsy, seizures, cataplexy, psychiatric disturbances, and cognitive impairment [382].

Early onset of neurologic symptoms correlates with shorter lifespan [382]. Diagnosis is based primarily on the results of biochemical and genetic tests [276].

4.9.1 HSCT Outcomes

HSCT has not been successful in the treatment of Niemann–Pick type A disease [18, 19, 248]. The first patient with type B disease to undergo HSCT did not achieve normal enzyme levels or obtain clinical benefits, and subsequently experienced neurologic decline [385, 389]. More recent case studies reported that HSCT reversed or improved thrombocytopenia, severe pulmonary disease, hepatomegaly symptoms in two patients with type B disease, but one patient had a mild gross motor delay [314], and the other experienced a decline in memory and visual motor development [323]. In type C disease, HSCT was reported to improve pulmonary disease and hepatosplenomegaly in two patients; one patient made some developmental progress after treatment [36, 44], but the other experienced severe neurologic deterioration [160].

4.9.2 Other Treatments

The neuroprotective effects of other types of stem and progenitor cells have also been investigated. For example, intracerebral transplantation of mesenchymal stem cells reverses lysosomal storage in the brain in a mouse model of type A disease [167] and decreases astroglial activation and loss of cerebellar Purkinje neurons in a mouse model of type C disease [12, 201, 319]. Substrate reduction therapy with miglustat improves or stabilizes symptoms and slows disease progression in children with Niemann–Pick type C [59, 151, 277, 416] and appears to prevent neurologic manifestations if initiated before symptoms appear [79]. Miglustat has been approved for the treatment of type C disease in the European Union and other countries [276].

In mouse model of type A disease, gene therapy reduced lysosomal storage and improved pathology in the visceral organs when delivered by intravenous injection [16], and improved pathology in the brain as well when delivered by intracranial injection [83, 275]. However, the best outcomes were obtained with a combination of brain and systemic injections, which preserved long-term motor and cognitive function and prolonged survival in mice [273].

In a mouse model of type A disease, recombinant acid sphingomyelinase reduced sphingomyelin accumulation in visceral organs when delivered by intravenous injection [245] and also reduced sphingomyelin accumulation in the CNS and improved motor function when delivered by intracerebroventricular infusion [84]. In a mouse model of type C disease, intravenous delivery of NPC2 protein reduced cholesterol storage and pathology in visceral organs but had no effect on CNS manifestations

[261]. In addition, 2-hydroxypropyl-β-cyclodextrin was shown to replace the function of NPC1 and NPC2 in a mouse model of type C disease [381], decreasing accumulation of cholesterol and sphingolipid in the brain and visceral organs, slowing disease progression, and prolonging lifespan [72, 206, 207, 298]. Both ERT and 2-hydroxypropyl-β-cyclodextrin are currently being tested in clinical trials.

4.10 Neuronal Ceroid Lipofuscinosis

Neuronal ceroid lipofuscinosis (NCL) is a family of at least 14 disorders classified according to age at onset, clinical features, and storage material. Collectively known as Batten disease, NCL disorders have an estimated incidence of 1 in 14,000–67,000 live births [142]. All childhood-onset forms are autosomal, recessive disorders, but the less common adult forms can also show autosomal, dominant inheritance [118]. More than 360 disease-causing mutations have been identified in 13 different ceroid lipofuscinosis neuronal (*CLN*) genes, some of which are known to encode soluble lysosomal enzymes or putative transmembrane proteins [142].

Patients with all forms of NCL show progressive neuronal loss and the accumulation of autofluorescent material containing subunit c of mitochondrial ATP synthase and/or sphingolipid activator proteins [129, 142]. Neuronal loss occurs primarily in the cerebral and cerebellar cortices, resulting in brain atrophy, with microcephaly seen in some forms of the disease [7]. Retinal degeneration, microglial activation, and astrocytosis are other common features of NCL [129]. Childhood-onset forms of the disease are characterized by progressive vision loss, cognitive and motor dysfunction, and epileptic seizures, and early death, whereas adult-onset disease is characterized by dementia [142]. Diagnosis is based on enzyme assay, genetic analysis, and clinical features of the disease. In some cases, examination of the ultrastructural features of the storage material by electron microscopy is needed to confirm the diagnosis [409].

4.10.1 HSCT Outcomes

HSCT has been evaluated in both symptomatic and asymptomatic patients with various forms of NCL (infantile, late-infantile, juvenile) but does not appear to improve clinical outcomes [198, 209, 427].

4.10.2 Other Treatments

Gene therapy and ERT show promise in infantile and late-infantile forms of NCL. Intracranial delivery of *CLN2*, which encodes tripeptidyl peptidase I (TPP1), has been successful in animal models [274, 337, 338] and is currently being tested in

clinical trials [412]. In mice deficient in palmitoyl protein thioesterase-1 (PPT1), intracranial delivery of the recombinant enzyme provides some clinical benefits [134], but combining ERT with HSCT produced synergistic effects, increasing lifespan and improving motor function [212]. Intrathecal delivery of TTP1 protein reduces storage material, increases lifespan, and ameliorates neuropathology in animal models of late-infantile disease [394, 418], and there is evidence that the recombinant enzyme enters the brain when delivered it is intravenously [241].

Neural stem cell therapy provides neuroprotective effects in a mouse model of infantile NCL, integrating into the brain, slowing neuron loss, and improving motor function [356]. A phase I clinical trial has demonstrated the feasibility of this approach in children with infantile or late-infantile NCL [317]. Other therapies in clinical trials include the immunosuppressive drug mycophenolate, which decreases neuroinflammation and improves motor function in a mouse model of juvenile NCL [316], and the PPT1 mimetic cysteamine, which decreases lysosomal storage in infantile NCL [430]. Although cysteamine alone does not appear to provide clinical benefits for patients with NCL [122], it has shown promise when combined with gene therapy in mice [306] and is currently being tested in combination with *N*-acetylcysteine in patients with infantile NCL.

4.11 Discussion

The introduction of HSCT for LSDs in 1981 by Hobbs et al. has significantly improved the prognosis of several of these disorders [155]. Improved cardiopulmonary function, reversal of organomegaly, hearing and vision improvement, and preservation of neurocognitive development after HSCT have allowed many of the transplanted patients to survive into adulthood. Yet, residual disease, presumably due to existing irreversible damage at time of HSCT, is still being reported after transplantation. Interestingly, the natural course after successful HSCT has been shown to be highly variable among organ systems, patients, and disorders. Several factors may account for this high variability.

4.11.1 Differences Among Organ Systems

Variable treatment outcomes among organ systems within transplanted patients have been largely ascribed to differences in tissue vascularization. The cardiac muscle, CNS, and abdominal organs (e.g., liver and spleen) have an extensive blood supply, but other tissues such as cartilage, heart valves, and retina are largely avascular or isolated because of a physiological barrier, limiting diffusion of the donor enzyme. Nevertheless, many authors and clinicians have suggested that disease progression in transplanted patients is less severe than that seen in untreated patients. Since HSCT increases life expectancy, some disease manifestations become apparent only in long-term survivors.

4.11.2 Differences Among Disorders and Patients

The impressive results observed in Hurler syndrome (MPS I) led researchers to believe that most LSDs could be alleviated by HSCT. However, the long-term clinical outcome of HSCT in several of LSDs is not as impressive as observed in patients with Hurler syndrome. Benefit from HSCT appears to be limited to select subsets of LSD patients. The main reason for the failure of HSCT in certain LSDs seems to be slow replacement of patient tissue macrophages and microglia populations by the donor hematopoietic cell progeny relative to the rapid progression of the primary disease [30]. Furthermore, there is a high variability among transplanted patients, as well as disorders, suggesting multifarious factors, although few studies have evaluated the influence of the individual factors on outcome. Genotype, age at HSCT, clinical condition at the time of HSCT, and enzyme level achieved (dependent on level of donor engraftment and donor enzyme level) have all been suggested as important factors in the outcome of LSD patients after HSCT [2].

It is important to note that a considerable percentage of the LSD patients that have been reported underwent transplantation at a relatively high age, already significantly affected by the disease. In addition, transplantation techniques such as total body irradiation have been used in the past, and a large percentage of the patients achieved only mixed chimerism or were transplanted using a carrier donor, both of which are associated with lower enzyme levels. The efficacy of HSCT for the various LSD disorders can therefore be accurately determined by evaluating outcomes of transplanted patients who undergo HSCT early in the disease course (before irreversible damage occurs) and achieve optimal enzyme levels.

To achieve optimal long-term clinical outcomes in LSD patients who undergo HSCT, certain recommendations can be given.

- Because early treatment appears to result in superior outcomes, transplantation should be performed as early as possible in the course of the disease, before irreversible tissue damage has occurred.
- It is not clear whether enzyme levels are important for clinical outcome. However, higher levels may be more desirable. Some transplantation centers now prefer an unrelated donor rather than a related heterozygous donor in order to achieve high enzyme levels post-HSCT. The use of unrelated cord blood is associated with a high percentage of full donor chimerism and normal enzyme levels in LSD patients. Furthermore, cord blood is readily available, reducing the time between diagnosis and treatment. Therefore, for those with younger age and rapid progressive disease at transplantation cord blood may be the preferred stem cell source.
- To evaluate clinical outcomes of LSD patients after HSCT and the influence of various patient, donor, and transplantation characteristics on outcomes, multicenter studies or registries are needed. International collaborations are of utmost importance to further optimize transplantation techniques and study new developments. Natural history studies and treatment of minimally symptomatic babies are needed to evaluate more precisely the effects of transplantation.

4.11.3 HSCT Versus ERT

Important historical limitations of HSCT in LSDs were the high mortality and morbidity rates and the nonavailability of suitable HLA-matched donors. Therefore, in the past, clinicians advised against HSCT or postponed HSCT until very late in the course of the disease, making a favorable outcome less likely. However, recent advances in transplantation have expanded stem cell sources and significantly reduced the risks associated with the procedure, achieving survival rates >90 % in recent years.

It is therefore important to reevaluate the risks and benefits of HSCT in comparison with more conservative but noncurative options like ERT in the treatment of patients with LSDs. At present, HSCT is the only treatment that can provide a permanent endogenous source of enzyme for patients with certain LSDs. Furthermore, this treatment is considerably less expensive than ERT, and HSCT is more effective in metabolic correction than ERT at the currently used dosages [417, 423]. Another potential limitation of ERT is the induction of an immune response to the therapeutic enzyme. These antibodies can neutralize the effect of ERT by reducing the efficiency of enzyme uptake and its redirection to other target tissues [5, 80, 173, 183, 295].

If new improvements in HSCT techniques and reduced intensity conditioning protocols further decrease transplantation-related morbidity and mortality rates in LSD patients, HSCT may then be recommended for milder LSD phenotypes, such as MPS I Hurler–Scheie syndrome. However, continuing research on outcomes of ERT and HSCT is needed before a definite conclusion can be drawn. In the meantime, clinicians will need to balance the possible benefits of HSCT against the potential risks in LSD patients.

4.12 Conclusion

Despite variability among organ systems, patients, and diseases, HSCT is clearly effective in improving the prognosis of several LSDs and some leukodystrophies, as evidenced by increased life expectancy and improvement of important clinical outcome parameters. The variability in the natural course of clinical manifestations in LSD patients after HSCT may be due to differences in genotype, age and symptoms at the time of HSCT. Some clinical manifestations appear to be irreversible or continue to progress despite HSCT, resulting in residual disease burden in long-term survivors. Furthermore, since life expectancy is increased after HSCT, several disease manifestations have become apparent in long-term survivors. To improve the prognosis of LSD patients after HSCT, transplantation must be performed as early as possible in the course of the disease, before irreversible tissue damage has occurred. International collaborations are of real importance to evaluate clinical outcomes, optimize transplantation protocols, and study new developments in the field.

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Chapter 5
Hematopoietic Stem Cell Gene Therapy for Lysosomal Storage Disorders: Expected Benefits and Limitations

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5.1 Gene Therapy Approaches in LSDs

Gene therapy is the insertion of genes into an individual's cell and biological tissues to treat diseases, such as genetic disorders, where deleterious mutant alleles are replaced with functional ones. Lysosomal Storage Disorders (LSDs) are excellent candidates for gene therapy. Firstly, they represent generally well-characterized, single gene disorders, and secondly, a partial reconstitution of enzyme activity is usually sufficient for clinical efficacy [1]. Gene therapy approaches for the treatment of LSDs consists of:

1. Direct vector administration into peripheral tissues, such as the liver or skeletal muscles that thus become a source of the functional enzyme to be released into the bloodstream;
2. Direct administration of the vector into the central nervous system (CNS), potentially allowing for metabolic correction of specific cell types representing major disease targets in LSDs with neurological involvement, such as oligodendrocytes and neurons;
3. Replacement of specific cell subsets in the affected tissues by genetically corrected stem cells or progenitors, such as ex vivo transduced autologous hematopoietic stem cells (HSCs) or neural stem cells. Several examples of application of these strategies to LSD animal models exist and, in rare cases, to LSD patients.

Different vector platforms have been employed in the context of these different approaches. However, given the chronic nature and wide trophism of LSDs, vectors capable of high infectivity and long-term persistence in vivo, i.e., integration into the cellular genome, have been generally preferred.

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In particular, the state of the development of HSC-based gene therapy approaches for LSDs based on gamma-retroviral and lentiviral vectors and efforts towards their clinical application are here discussed.

5.2 Hematopoietic Stem Cell Gene Therapy in LSDs

Based on the positive clinical experience with hematopoietic stem cell transplantation (HCT) from healthy donors in some LSDs (see Chap. 2), HSC-based gene therapy has been considered as an attractive alternative for the treatment of these disorders. This is of particular relevance since the amount of enzyme that transplantation from a matched donor can provide to the organism is low, especially since donors are often heterozygous siblings. Indeed, a relationship between circulating enzyme levels after transplant and the urinary substrate has been recently shown in Mucopolysaccharidosis Type I (MPS I) [2]: the low enzyme levels achieved with heterozygote donor transplant lead to less adequate reduction in GAG levels. The impact of HCT on CNS and skeletal disease is suboptimal in many LSDs and disabilities persist in the transplanted patients and this is at least in part likely due to insufficient metabolic correction at disease sites. The relation between metabolic correction and clinical outcome is difficult to study as these disorders are rare and there are many different factors that might influence the clinical outcome to a particular applied therapy.

5.2.1 Therapeutic Efficacy and Dose-Effect Relationship

The level of donor cell engraftment and the enzyme expression by the progeny of those transplanted HSCs will vary between transplants and between different indicated diseases. Furthermore the enzyme delivery sufficient and/or required to achieve therapeutic efficacy in HCT and/or HSC gene therapy could vary between those different diseases. In those LSDs characterized by CNS involvement or bone pathology, the presence of physical barriers affecting enzyme delivery (such as the Blood–Brain Barrier [BBB]), the low expression of the Mannose-6-Phosphate Receptor (M6P-R) on target cells [3] and the poor vascularization of affected tissues such as bone make the case for high donor cell engraftment and high enzymatic expression from the transplanted cells in order to achieve maximal therapeutic potency from such cellular therapies. A prototypical example is that of Metachromatic Leukodystrophy (MLD), an LSD due to the inherited deficiency in Arylsulfatase A (ARSA) activity with a severe and almost exclusive involvement of the CNS and peripheral nervous system (PNS). Indeed, unexpectedly high levels of ARSA expression into HSCs and their progeny are necessary for achieving correction of the metabolic defect in the CNS, as shown in the murine disease model [4], accounting for the poor reported outcome of HCT from healthy donors in the affected patients [5, 6]. Matzner and colleagues showed that gene therapy based on bone

marrow cells expressing the human ARSA cDNA from a gamma-retroviral vector (γRV) resulted in a substantial reduction of lipid storage in visceral organs, but only in partial correction of the lipid metabolism in the brain [7, 8], associated to a modest amelioration of the neuropathology and of the performance of treated animals at behavioral tests. Interestingly, when advanced generation lentiviral vectors (LV) were employed for transduction of HSC, upon efficient transduction and integration of multiple copies of the vector into the HSC genome, a supra-physiological expression of ARSA was achieved in their differentiated progeny. By transplanting these LV-transduced HSCs, efficacious enzyme activity reconstitution was obtained in the nervous system of MLD mice, allowing for prevention of CNS and PNS disease manifestations and correction of the neurologic phenotype upon presymptomatic and symptomatic treatment, respectively [9, 10]. Remarkably, this approach when compared directly in the same model with wild type conventional HSC transplant had a remarkably greater therapeutic impact [9], thus suggesting that the degree of efficacy of gene therapy in this setting is dependent on the levels of enzyme activity in HSC and their progeny. Results from an on going Phase I/II clinical trial of LV-transduced autologous HSC transplantation in early onset MLD patients support this concept (*Biffi et al.*, Science 2013).

The importance of sustained enzyme delivery to the affected tissues was suggested also in mice affected by the LSD galactosialidosis [11]. Knock-out mice that received HCT from transgenic mice over-expressing the functional enzyme showed a better phenotype than mice treated with HCT from wild type donors. A relationship between enzyme level and degree of correction was also observed in preclinical studies on Mucopolysaccharidosis type IIIB (MPS IIIB, also known as Sanfilippo syndrome), caused by the deficiency of *N*-acetyl-α-D-glucosaminidase (NAGLU), a lysosomal enzyme involved in the degradation of heparan sulfate. MPS IIIB mice were transplanted with HSCs transduced with a γRV encoding for the human NAGLU. Only when very high levels of NAGLU were achieved in the blood were the neurologic symptoms ameliorated: treated mice showed an increased number of normal-appearing neurons in the cortex, while microglia with engorged lysosomes had almost completely disappeared [12].

Similarly, when MPS1 mice were transplanted with either mock-transduced HSC from wild type donors or HSC from affected siblings transduced with LV encoding for the functional α-iduronidase (IDUA) enzyme leading to IDUA supranormal expression, reconstitution of IDUA activity in the brain was achieved only in the gene therapy treated mice, in which full correction of behavioral deficits and skeletal abnormalities was also observed. On the contrary, wild type HSC transplantation was unable to deliver detectable amounts of the functional lysosomal enzyme to the nervous system and failed to prevent disease related neurological impairments and skeletal defects (Visigalli, Delai et al, Blood 2010).

The same indication of dose dependence of the benefit on the nervous system was also recently obtained in the animal model of Globoid Cell Leukodystrophy (GLD), on which a sustained survival advantage with amelioration of CNS and PNS disease positively correlated with enzyme delivery to the affected tissues by LV-transduced, galactocerebrosidase (GALC) over-expressing HSC-progeny cells (Gentner, Visigalli et al., Sci Transl Med 2010).

5.2.2 Cross correction

HSC gene therapy as well as HCT could be employed to treat LSDs since they lead to the progressive reconstitution of recipient's macrophage and microglia populations by functional cells, progeny of the genetically corrected/donor-derived and transplanted HSC respectively. These cells of the monocytic lineage act as mini-pumps synthesizing and secreting a portion of their lysosomal enzymes, which can be taken up by neighboring cells [13, 14]. The advantage of gene therapy with respect to allogeneic HCT is the possibility to express at supra-physiological level the functional enzyme in the genetically modified cells, thus possibly enhancing this cross correction of neighboring cells. The occurrence of cross correction of resident non-hematopoietic cells following genetically modified HSC transplantation was indeed proven in relevant tissues, such as the CNS [10].

As mentioned above, the role of donor derived or gene-corrected macrophages and microglia consists not only in enzyme delivery, but also in the clearance of the stored material and debris throughout the affected tissues. This suggests that HSC gene therapy could be efficacious even for those diseases where the missing protein is not a secreted enzyme. The results obtained by HCT and HSC gene therapy in Adrenoleukodystrophy (ALD), an inherited leukodystrophy similar to MLD, but caused by a defect in a non-secreted peroxisomal transporter protein, reinforce this hypothesis. Indeed, HCT is successfully applied in patients showing early neurologic symptoms and affected by the childhood cerebral form of ALD [15]. On the basis of these evidences, a gene therapy trial based on autologous HSC and LV was conducted children affected by ALD. Four patients have been treated thus far, with very encouraging results. Upon significant engraftment of the autologous transduced cells, the patients showed an arrest of their disease progression [16]. The fact that the neurologic benefits of HSC gene therapy, in the presence of a transduced cell chimerism below 15 % in peripheral blood and bone marrow were comparable to those seen with allogeneic HCT upon establishment of full donor chimeras allows speculating of a in vivo selective advantage of the genetically corrected cells in the affected brain, which, if proven, could further strengthen the rationale for HSC gene therapy in ALD and in general in LSDs.

5.2.3 Microglia Reconstitution and Timing for Efficacy

Importantly for those LSDs characterized by a severe involvement of the nervous system, upon HCT, the HSC progeny might contribute significantly to the turnover of CNS microglia [9, 17], which plays a major role in the pathogenesis of nervous tissue damage [18, 19]. Microglia represents the first line of immune defense in the CNS by exerting phagocytosis, antigen presentation and secretion of inflammatory cytokines [20–22]. In the developing CNS, microglia originates from myeloid progenitors deriving from extramedullary sources of hematopoiesis, such as the yolk sac [23] and from the bone marrow before birth. Controversy exists on the

maintenance and renewal of microglia in the adult CNS. It is not clear whether in physiological conditions microglia renew via in situ proliferation from resident progenitors or whether its pool is replenished from circulating progenitors derived from the bone marrow [24]. It is unclear how relevant preclinical studies of microglial reconstitution after experimental HCT are to such reconstitution after clinical HCT or how much they are affected by specific experimental procedures such as the modality of HSC harvest and the conditioning regimen applied prior to the transplantation. Two recent studies highlighted the role of total body irradiation in promoting microglia reconstitution [25, 26]. Interestingly, CNS irradiation induces the local synthesis of cytokines and chemokines, including CCL2, crucial for the recruitment of bone marrow-derived microglia. When myeloablation was achieved by administering chemotherapeutic agents such as the replacement of host microglia with cells of donor origin might be instead enhanced by an ablative effect of bona fide CNS-resident microglia progenitors exerted by the drug (Capotondo et al., PNAS 2012) [25–28]. Recent studies demonstrated that the reconstitution of brain microglia upon HCT might also be influenced by the characteristics of the transplanted cells. In particular, the mechanical flushing of femurs and tibiae used in preclinical studies to collect HSC, and possibly also the mechanical harvesting of bone marrow from iliac crests in humans, could damage the bone marrow niche, thus allowing mobilization of microglia progenitors that could then enter the circulation and possibly cross the BBB in the transplant recipient. In physiological conditions or upon cytokine stimulated mobilization of stem cells, these cells should not be able to leave the bone marrow [25, 26]. These issues might be extremely relevant in the definition of a clinical protocol for HCT in LSDs. In particular, the use of a suitable conditioning regimen should be carefully evaluated considering both the neurologic disease of the patient and the need for a rapid microglia replacement. Finally, the replacement of brain microglia by donor-derived macrophages appears to be favored by neuroinflammation or neurodegeneration [9, 29–32]. Microglia recruitment seems to be enhanced in models characterized by a massive influx of blood circulating leukocytes or by an altered BBB, and in those ones in which a diffuse or a focal neurodegeneration was observed. These finding were confirmed also by studies on the murine model of Globoid Cell Leukodystrophy (GLD), the twitcher mouse, and in Sandhoff mice [33, 34].

Very little evidence is available, but it is likely that microglia replacement following HSC transplantation might take many months or even years to accomplish in humans [35]. Therefore HSC gene therapy as HCT may not be beneficial for rapidly progressing LSDs or for symptomatic patients [36]. However, given this long time likely required for microglia reconstitution to be accomplished, HSC gene therapy might have the advantage, with respect to allogeneic HCT, of enzyme overexpression by those few cells that actually reach the CNS. For this reason, HSC gene therapy might need proportionately lesser microglial reconstitution and a shorter time to be effective compared with conventional HCT and the same transplant conditions. Moreover, in some diseases, gene corrected microglia might have a selective advantage in the CNS, as suggested by the encouraging results obtained in the ALD clinical trial [16].

5.2.4 Safety of HSC Gene Therapy

As discussed, the potential advantage of gene-modified autologous HCT in LSDs compared with conventional HCT requires that a high level of transgene expression by HSCs and their committed progeny. High transgene expression relies on strong promoters and on multiple vector integrations into HSCs, a condition that might increase the risk of integration-dependent adverse events.

Integration of a transgene into the cell chromatin may ensure stable expression of the gene product in the target cell and its progeny. For this reason, integrating vectors, such as retrovirus-based vectors, have been the preferred choice for gene delivery into HSCs. On the other hand, vector integration may significantly affect the expression of host cell genes consequently altering cell growth control and eventually triggering cell transformation.

Retrovirus-based vectors were considered relatively safe, since integration was thought to be randomly distributed in the genome and the chance therefore of accidentally disrupting or activating a gene was considered remote. This assumption was reconsidered when a lymphoproliferative disorder was reported in one patient treated for X-linked Severe Combined ImmunoDeficiency (X-SCID) with γRV-transduced HSC [37]. Mapping of retrovirus-based vector integrations in the predominant T-cell clone revealed a single proviral insertion within the LMO-2 locus, associated with up-regulation of transcript and protein levels. A similar complication was reported in three more patients enrolled in the same clinical study [38] and in patients recruited to an independent X-SCID trial [39] and an X-linked chronic granulomatous disease trial [40]. Recent reports have also challenged the notion that retroviral integration occurs randomly in the target cell chromatin and have indicated specific biases for integration into transcriptionally active genes, for both Moloney retrovirus (MoRV) and HIV [41–43]. These recent findings have prompted the scientific community to study and compare integration site selection among different integrating vectors in greater depth, in order to both evaluate the risk–benefit ratio in gene therapy applications and to develop safer vectors. The use of a tumor-prone mouse model has demonstrated a dose-dependent acceleration of leukemia/lymphoma onset triggered by MoRV upon HSC gene transfer and tranplantation. By contrast, tumorigenesis was unaffected by self-inactivating (SIN) LV [44]. The different integration site selection patterns of the two vectors and the lack of transcriptionally active LTRs in the LV design were recently both shown to contribute to the low genotoxicity of LV observed in this model [45]. Recent studies confirmed the different integration pattern of LV, which unlike MoRV, rarely integrate in proximity to expressed genes [41]. Therefore, these studies and the promising safety follow up of LV gene therapy treated patients (Cartier and Biffi) suggest LV to be a safer vector as compared to MoRV for HSC gene transfer, although it is only from ongoing and future clinical trials that a more precise assessment of the clinical risk associated with the use of LV for gene therapy can be made.

5.2.5 HSC Gene Therapy with Respect to Other Gene Transfer Approaches

Other gene therapy strategies currently under preclinical or clinical investigation for LSDs treatment are systemic gene therapy and direct CNS gene delivery.

In vivo gene therapy in LSDs is designed to establish a sustained source of therapeutic enzyme within the body for metabolic correction via the bloodstream. Intravenous delivery of viral vectors may represent an effective strategy to target gene transfer to tissues, which are readily accessible from the bloodstream and may thus become efficient sources of systemic enzyme distribution, i.e., the liver. The efficacy of this strategy has been evaluated in several LSD animal models, such as MPS I and VII [46, 47]. Despite encouraging preclinical data, this approach is still affected by some relevant limitations:

1. The occurrence of immune responses directed towards the vector or the novel therapeutic protein can cause clearance of the transduced cells and/or loss of enzyme activity [48–52];
2. The poor therapeutic potential of this strategy in LSDs with CNS involvement, since the secreted enzyme is unable or poorly capable to cross the BBB, thus limiting the benefit to peripheral organs, without correcting nervous system manifestations;
3. The concerns of toxicity of parenteral vector administration, also related to the possible risk of inadvertent gene transfer to the gonads and germ-line transmission of the vector.

The development of an immune responses may be reduced by the use of liver-specific promoters capable of restricting transgene expression to parenchymal cells of the liver, thereby reducing the expression within antigen-presenting cells [53]. Moreover, the residual off-target expression of the transgene has been recently prevented by using microRNA regulation, which also allow establishing tolerance to the foreign protein [54]. Several strategies have been investigated in order to overcome the issue of BBB crossing. Good results have been obtained by targeting specific receptors on the BBB: Spencer et al. created a LV coding for a fusion protein between the lysosomal enzyme glucocerebrosidase and the low-density lipoprotein receptor-binding domain of the apolipoprotein B [55]. Other groups exploited the physiological increased permeability of the BBB in the perinatal period. Young et al. have observed an increased number of enzyme-positive cells in CNS upon systemic LV delivery in newborns *twitcher* mice. In this study the permeability of BBB was further increased by the systemic administration of VEGF. The same strategy was coupled also with HCT, in order to improve the microglia reconstitution [56]. These novel approaches are currently being tested in vivo, for therapeutic efficacy in LSD models.

Delivery systems have been developed for direct in vivo gene transfer into the CNS. Efficient transduction of the neurons of adult rodent brains was observed with all generations of LV [57–60] and effective gene transfer was also observed in the

neurons of nonhuman primates [61]. Similarly, efficient targeting of the CNS was observed upon transduction with recombinant AAV [62–64]. Preclinical studies have been performed on animal models for several LSDs, including MLD, MPS I, and late infantile neuronal ceroid lipofuscinosis (LINCL) [65–69]. Overall, these studies demonstrated a reduction of storage material, but significant recovery did not occur when the treatment was applied after the onset of symptoms. Clinical protocols for CNS-directed gene therapy with AAV have been approved for LINCL and for Canavan disease [70, 71]. Unfortunately, some adverse events have been observed: in a LINCL trial, one patient died after developing epileptic seizures, while another one showed a humoral response against the transgene [72]. In the Canavan disease clinical trial, three out of 10 patients developed a low level of neutralizing antibodies. Besides these adverse events, direct CNS gene delivery did not provide overt clinical benefit, likely because of the modest distribution of the vector and/or enzyme throughout the brain [70].

As compared to these strategies, HSC gene therapy provides the opportunity for a widespread distribution of the therapeutic enzyme in the CNS, being of particular relevance for diseases characterized by a diffuse CNS involvement, as well as to peripheral organs. Indeed, the progeny of the genetically corrected HSC could virtually target all the tissues infiltrated by hematopoietic cells. Moreover, HSC gene therapy permits induction of tolerance to the therapeutic protein. Further, some LSDs such as GLD and others, are characterized by neuroinflammation, activation of microglia, astrocytosis and pro-inflammatory cytokines secretion by microglia and astrocytes [73–75]. Differently from systemic and CNS-directed gene therapy approaches, in addition to the favorable metabolic effect exerted by the genetically corrected cells, HSC gene therapy generates a new set of enzymatically competent and not activated microglia, which might be beneficial in down-regulating the inflammation in the CNS [34]. For these reasons, both LSDs with hematopoietic and visceral manifestation and those with also neurologic symptoms might benefit from HSC gene therapy.

5.2.6 Issues for Clinical Translation

When approaching a LSD with gene therapy, the choice of a suitable approach (direct CNS gene delivery, systemic gene delivery, or cell gene therapy) should be taken according to the characteristic of the disease and the tissues involved. Moreover, the cellular (which is the cell population primarily affected by the enzymatic deficit) and the sub-cellular localization of the therapeutic enzyme (secreted enzyme or membrane protein) is also important. Another issue relates to the choice of the best vector and the best transduction protocol for gene transfer. Of course, the suitable vector should be selected according to the target cells or tissue, the existence of a selective advantage of gene corrected cells and the level of gene expression required for efficacy. Once stringent demonstration of the efficacy and safety of the vector and gene transfer protocols of choice are obtained in the most appropriate preclinical models,

then actual clinical translation will require a number of critical issues to be carefully addressed. There must be development of clinical-grade large scale vector manufacture with appropriate quality assays, mobilization of the considerable financial resources required to support such an endeavor and the consensus and/or approval of the scientific and biomedical communities, national and international regulatory bodies and patients associations must be secured. Moreover, a comprehensive knowledge of the natural history of disease manifestations and their evolution will be crucial to allow proper assessment of the risk–benefit ratio and selection of the best candidate patients for testing these new gene therapy strategies.

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Chapter 6
Alternative Future Therapies for Lysosomal Storage Diseases: Embryonic Stem Cell- and Induced Pluripotent Stem Cell Therapy

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6.1 Summary

There are 40 known genetically inherited lysosomal storage diseases, most of which are caused by a mutation(s) in a single gene or enzyme [1]. This category of diseases may provide a good platform for the field of regenerative medicine to apply new therapeutical methods: stem cell therapy and induced pluripotent stem cell therapy. Stem cells are defined by two properties, the ability to self-renew indefinitely and the ability to differentiate into specialized cells. Embryonic stem cells (ESCs) are pluripotent; they have the capacity to generate all cell types of the body. With the more recent advances in this field, somatic cells can now be reprogrammed to induced pluripotent stem cells (iPSCs) which are functionally equivalent to ESCs. These cells hold much promise for future therapeutic applications. In this chapter, we provide an overview of (iPSC) technology and discuss ESC therapy and iPSC therapy in the context of their potential application in lysosomal storage diseases.

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6.2 Introduction

The promise of patient-specific therapy (where immune rejection is not an issue) may be further advanced by the application of induced pluripotent stem cells (iPSCs). iPSCs are somatic cells that have been reprogrammed into pluripotent stem cells, thereby recapturing the ability to differentiate into all types of cells in the body. These cells are functionally equivalent to embryonic stem cells (ESCs), which can also self-renew and differentiate into all cell types.

It has been known since the 1960s that adult (somatic) cells are genetically equivalent to early embryonic cells and that differences in gene expression are due to reversible epigenetic changes that occur during development [2, 3]. Through the more recent technology of somatic cell nuclear transfer (SCNT), the differentiated state a mature cell can be reversed into one characteristic of an undifferentiated embryonic cell that can then be implanted into a foster parent to develop. This was first proven in mammals when Dolly, the sheep, was cloned by Wilmut et al. in 1996 [4]. Reproductive cloning, however, is inefficient and abnormalities are found at all stages of development [5–9].

Study of these developmental defects determined malfunction at the epigenetic level, which manifests itself in altered gene expression. Persistence of donor-cell-specific gene expression indicates a retention of an "epigenetic memory" of the donor nucleus [8, 10].

Faithful reprogramming of the somatic genome and complete elimination of this epigenetic memory of the donor nucleus seem to require passage through the germ line [11]. For the last two decades, creation of pluripotent cell lines through SCNT has been considered a potential method for generating patient-specific cell lines. This procedure, also called therapeutic cloning [12], is plausible in mice [2, 13]; however, its proof-of-principle in humans is yet to be realized.

An alternative to SCNT is the recently developed induced iPSC technology by Takahashi and Yamanaka et al. [14]. iPSCs are somatic cells that have been reprogrammed without SCNT technology, therefore, without the need for an oocyte. By introducing key transcription factors essential for pluripotency, a somatic cell can be obtained from a patient (e.g., via skin biopsy) and transformed back into an ESC-like state and then differentiated into the desired specialized cell.

This new technique looks promising, especially for lysosomal storage disorders (LSDs). LSDs are genetically inherited and currently, there is no cure. Since the 40 known LSDs are caused by mutation(s) in a single gene, iPSC methods have the potential to be efficient and effective.

6.3 Embryonic Stem Cells

6.3.1 Derivation and Characteristics

Embryonic stem cells are pluripotent stem cells derived from the inner cell mass (ICM) of a preimplantation blastocyst. These cells are capable of self-renewing

indefinitely in culture and can generate all three primary germ layers: endoderm, mesoderm, or ectoderm [15].

In 1981, Evans and Kaufman isolated the first mouse embryonic stem cell (mESC) [15] and a decade later, the first human embryonic stem cell lines were derived by Thomson et al. [16]. Although both mESCs and hESCs are derived from the ICM they require different growth factors to maintain their pluripotency. mESCs are dependent on leukemia inhibitory factor (LIF) which maintains the pluripotent state by activating the signal transducer and activator of transcription 3 (STAT3) signalling pathway [17, 18] and bone morphogenetic protein (BMP) signalling [19]. In hESCs, however, LIF does not maintain pluripotency, and BMP-4 induces differentiation indicating involvement of alternative signalling pathways. Moreover, hESCs require the presence of basic fibroblast growth factor (bFGF), and laminin and fibronectin have positive effects on hESC maintenance [20], while they all promote differentiation in mESCs. Although there are more differences between mESC and hESC, they share the expression of the core pluripotency transcription factors: OCT4, SOX2, and NANOG [21–23].

The pluripotency of ESCs is assessed in vitro by their potential to differentiate into all three germ layers through embryoid body (EB) formation. EBs are formed by suspension culture of ESCs which induces differentiation and mimics early embryonic development in an disorganized manner [24]. Pluripotency is also assayed in vivo by teratoma formation in immune compromised mice; this is the most stringent method for measuring pluripotency in hESCs [16], since it is unethical to test pluripotency for human cells via chimeras. In mice, however, the production of chimeras after injection of cells into the blastocyst stage embryo is standard as is the ability of introduced cells into a 4*n* blastocyst to generate a complete organism (tetraploid complementation) [25].

6.4 Therapeutic Potential of ESCs

6.4.1 Cell-Therapy

Although far from realizing cell-based therapies with ESCs, there have been developments in several areas of research that demonstrate their potential. For example, in a model of blindness caused by photoreceptor degeneration, retinal progenitors derived from hESCs migrate into mouse retinas following interocular injection, engraft into the appropriate regions and express markers for rod and cone photoreceptor cells. After transplantation of these cells into the subretinal space of adult Crx(−/−) mice (a model of Leber's Congenital Amaurosis, which results in severe loss of vision or blindness), the hESC-derived retinal cells differentiate into functional photoreceptors and restore light responses to treated animals [26]. These results demonstrate that hESCs can, in principle, be used for photoreceptor replacement therapies.

Another example that highlights the potential clinical utility of hESCs is liver disease. It is possible to differentiate hESCs into hepatocytes and isolate only the cells that express secreted functional human liver-specific genes similar to those of primary human hepatocytes. These enriched cells can be used in drug discovery research and potentially developed as a therapeutic material [27].

6.4.2 Clinical Trials with hESC-Derived Cells

ESC-based therapies are in the first phases of clinical development. The first ever hESC clinical trial was approved in January 2009 by the US Food and Drug Administration (FDA) to Geron Corporation for GRNOPC1 (Geron oligodendrocyte progenitor cells) in the context of acute spinal cord injury. GRNOPC1 contains oligodendrocyte progenitor cells that have been derived from hESCs. In animal models of acute spinal cord injury, Geron has demonstrated properties of remyelination and stimulation of nerve growth 7 days post injury [28] with evidence of long-term engraftment at the lesion and myelinated rat axons 9 months after treatment. The Phase I multicenter clinical trial, which will focus on safety and tolerability of GRNOPC1, involves qualified patients with grade A spinal cord injuries; this category is the most severe with complete loss of locomotor and sensory activity. GRNOPC1 is injected into the spine 7–14 days post injury and measured outcomes include safety and efficacy with sensory and lower extremity motor scores.

This trial, however, has recently been put on hold due to additional preclinical expansion studies in animals by Geron, which revealed a larger than expected occurrence of small cysts. As a result, Geron, further optimized GNROPC1 and developed new candidate markers and assays, and the ban was lifted in July 2010. However, in 2012 the trial was aborted.

A second company, Advanced Cell Technology (ACT) obtained in January 2010 approval to initiate a Phase I/II multicenter study using hESCs derived retinal pigment epithelium (RPE) cells to treat patients with Stargardt's Macular Dystrophy (SMD). SMD is a photoreceptor degenerative disease causing untreatable blindness. ACT's treatment for eye disease uses stem cells to re-create RPE cells, a type of cell in the retina that supports the photoreceptors needed for vision, which are often the first cells to die off in SMD which in turn leads to loss of vision. ACT performed long-term testing to show that differentiated retinal pigment cells can improve the visual performance in a rat model of macular degeneration without adverse effects (e.g., teratomas) in hundreds of treated animals [29].

6.4.3 Gene Correction

Therapeutic application of homologous recombination in ESCs has been proven in a mouse model for human sickle cell anemia, where mESCs were genetically corrected, engrafted and also corrected the associated pathology of sickle cell anemia [30]. In principle, homologous recombination could also be used to repair genetic defects in hESCs; however, there are only few examples and the efficiency is in general extremely low [30]. The HPRT1 locus [31], the POU5F1 locus [32], the Olig2 gene [33], the MIXL locus [34], and the AFM gene [35]. Clonal selection of hESCS after homologous recombination is hampered by their limitation to grow from single cells which is required for selection of rare targeted clones, whereas mESCs can be clonally selected since they can grow from single cells. A transitory

state of hESCs to mESC-like, would overcome this limitation and allow more efficient gene targeting strategies in hESCs [36]. Continuous development of genome editing tools like Zn-fingers and tale like-effector nucleases (TALENs) contribute to more efficient homologous recombination in human ESC and iPSC.

6.4.4 Drawbacks of ESC-Therapy

Transplantation of ESC-derived cells offers the potential to repair damaged organs and restore function. However, several limitations severely hamper the efficacy of this type of treatment: mislocalization or improper integration; transplanted cells may have retained their pluripotent characteristics and immune rejection of transplanted cells and tissues. Mislocalization or improper integration of ESC-derived cells may lead to undesired effects such as malfunctioning of the tissue in which they integrated. Cells that retain their pluripotent characteristics may lead to unwanted expansion of the cells and teratoma formation. Although hESCs express low levels of MHC class I molecules, differentiated hESCs upregulate MHC class I molecules sufficiently to trigger immune rejection [37] and there are there are too many MHC combinations for increased banking of hESC lines to be sufficient [38].

6.5 Therapeutic Cloning Through SCNT

The interest and demand for patient-specific therapy, along with ethical issues associated with deriving hESCs, has created a need for alternatives to transplantation of ESC-derived cells. Genetic correction via somatic cell nuclear transfer (SCNT) was described in the late 1990s [12] and its success demonstrated in a proof-of-principle publication in 2002 [13]. SCNT, however, requires the use of an unfertilized oocyte, thereby generating great concern regarding ethical oversight of this technology.

In the process of SCNT, also referred to as therapeutic cloning, the nucleus of an unfertilized oocyte is extracted and replaced with the nucleus of a donor (patient's) somatic cell, which is reprogrammed by the oocyte. The cell is then induced to develop in vitro to the blastocyst stage, the ICM can then be isolated to generate a new pluripotent ESC line, or in the case of Dolly, the sheep, implanted into a foster mother and allowed to develop into an organism (Fig. 6.1).

6.5.1 Therapy

In 2002, Rideout et al. published a proof-of-principle experiment using SCNT to correct a genetic mutation, which results in severe combined immune deficiency in Rag2(−/−) mice [13]. Immune-deficient Rag2(−/−) mice were used as nuclear

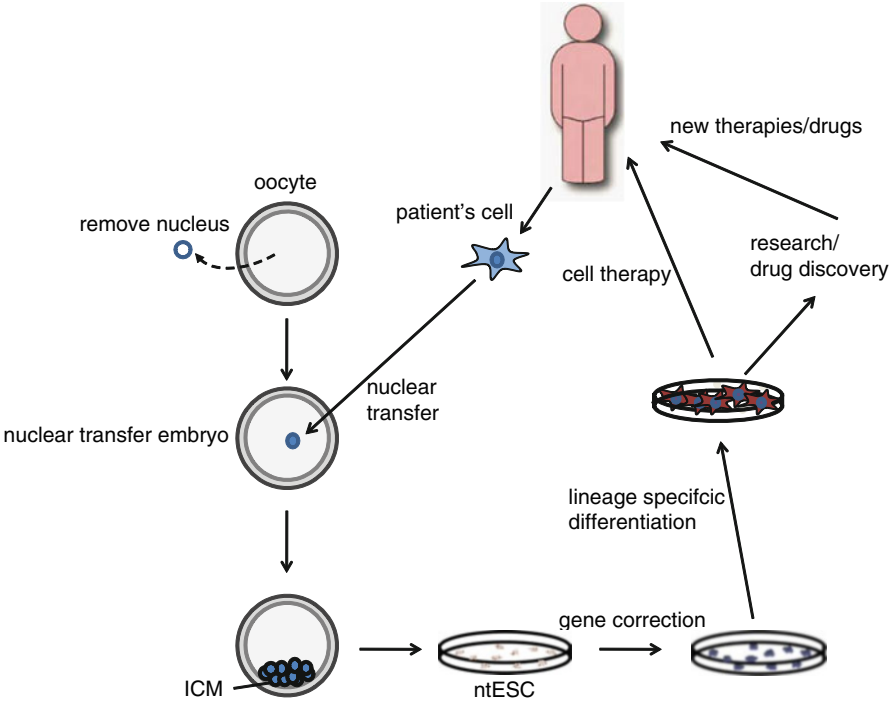

Fig. 6.1 Scheme for hypothetical therapeutic cloning in humans, gene correction, and cell therapy. The nucleus from a patient's cell is isolated and transferred to an enucleated oocyte and cultured to the blastocyst stage. The cells from the ICM are isolated and ESC (ntESC) are derived that can be genetically corrected by homologous recombination. The ntESC can be differentiated to the desired lineage to be used for research and drug screens and transplantation into the patient

donors for transfer into enucleated oocytes, and the resulting blastocysts were cultured to isolate an isogenic ESC line. One of the mutated alleles in the Rag2(−/−) ESCs was repaired by homologous recombination and mutant mice were treated with the repaired ESCs using two strategies. Immune-competent mice were generated from the corrected ESCs by tetraploid embryo complementation and were used as bone marrow donors for transplantation. The second strategy involved derivation of hematopoietic precursors by in vitro differentiation from the repaired ESCs and engraftment into mutant mice. Mature myeloid and lymphoid cells as well as immunoglobulins became detectable 3–4 weeks post transplantation, indicating the repair of the genetic disorder by a combination of therapeutic cloning with gene therapy. This clearly demonstrated the potential of therapeutic cloning and cell therapy in repairing genetic defects.

Cell transplantation with ESC progeny requires immunological compatibility with host tissue. Therapeutic cloning is a strategy to overcome this limitation by generating nuclear transfer (nt) ESCs that are genetically matched to an individual. Highlighting another example, derivation of 187 ntESC lines from 24 mice with Parkinson's

Disease, dopaminergic differentiation, and transplantation into individually matched host mice showed therapeutic efficacy and lack of immunological response, indicating the feasibility of treating individual mice via therapeutic cloning [39].

6.5.2 Limitations for Human Application

Nearly 15 years after the birth of Dolly the sheep [4] and nearly 10 years after the generation of SCNT embryonic stem cells in mice [40, 41], the derivation of (patient-specific) SCNT hESC lines remains elusive. Initial attempts to produce nuclear-transfer human blastocysts from somatic donor cells have been unsuccessful. The primary drawback in SCNT-hESC technology is the need for human eggs, which are difficult to obtain and raise ethical concerns. However, mouse zygotes temporarily arrested in mitosis can, like oocytes, support somatic cell reprogramming and produce ESCs and cloned animals. Thus early human zygotes could perhaps be used as a supplemental source to oocytes in the derivation of patient-specific hESC [42]; however, this still implies destruction of the embryo.

Alternative techniques to create pluripotent stem cells were needed and the development of induced pluripotent stem cell (iPSC) technology in 2006 by Yamanaka and coworkers [14] was a major breakthrough for this area of biomedical research. This work was awarded with a Nobel Prize in 2012.

6.6 iPSC Reprogramming

6.6.1 Derivation and Characteristics

iPSCs are pluripotent stem cells that are created by reprogramming of somatic mouse or human cells by introduction of a key set of pluripotency-related transcription factors. Initially, a selection of 24 ESC genes, believed to be important for pluripotency was introduced in fibroblasts by retroviral transduction and scored for the ability to induce an ESC-like phenotype. Further narrowing down the candidate genes, a selection of just four genes (Oct4, Sox2, c-Myc, and Klf4) seemed to be sufficient to reprogram the fibroblast into ESC-like cells [14]. These iPSC are similar to ESC in pluripotency, morphology, proliferation, teratoma formation, gene expression, and DNA methylation patterns, and improving iPSC selection criteria also resulted in chimera formation of the iPSC, the most stringent criterion for pluripotency [43, 44]. The methods to generate iPSCs are under constant development; however, there is a consensus in the reprogramming factors. Most commonly used are a set of the transcription factors Oct4, Sox2, c-Myc, and KLF4 (the “Yamanaka factors”) or the combination of Oct4, Sox2, Nanog, and Lin-28.

In November 2007 two independent labs, Yamanaka and Thomson, took the iPSCs a step further for application in medicine and created human iPSCs [45, 46]. Yamanaka and colleagues created human iPSC with identical factors used with the murine lines using retroviruses and Thomson and colleagues used Oct4, Sox2, Nanog, and LIN28 using a lentiviral system. The iPSCs created show the same morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and expression of hTERT (human telomerase reverse transcriptase) as hESCs [45, 46]. These human iPSCs could form teratomas when implanted into immunocompromised mice, passing the most stringent assessment of pluripotency possible for human ESCs and iPSCs. Although hESCs differ from mESCs in several aspects, human fibroblasts could thus be reprogrammed with the same set of four factors as mouse fibroblasts.

6.6.2 Risks and Solutions

The use of iPSC in human cell therapy is limited by the initial transduction protocols, the reprogramming factors and the iPSC themselves. The use of retroviruses or lentiviruses for delivery the reprogramming factors includes the risk of insertional mutagenesis, resulting in tumor formation. The use of the oncogene c-Myc in combination with viral delivery, potentially results in cellular transformation and tumor development [43, 44]. Twenty percent of the iPSC chimeras derived offspring developed tumors over time and showed retroviral expression of c-myc [43, 44]. This indicated that reactivation of c-myc expression was the cause of tumor formation and needs to be circumvented by alternative iPSC strategies. Solutions to these problems include alternative delivery methods or reprogramming techniques, like adenoviral mediated delivery [47], the use of removable reprogramming factors [48] or non-integrating vectors [49, 50] or direct protein [51, 52] or RNA transfection [53], (partial) chemically induced reprogramming [54–56], and exclusion of c-myc as reprogramming factor [57].

6.6.3 Sources for iPSC Reprogramming

The ideal cell type for reprogramming will depend on the application, which will be ranging from fundamental research and drug screens to cell-therapy. Reprogramming was first performed in fibroblasts and was focused on the developing reprogramming methods. However, other cell types, like blood, can also be used and might be preferred since they will be a preferable source of cells to obtain from specific patients [58].

The choice of fibroblasts as the starting cell population for iPSC reprogramming has several advantages. First, adult fibroblasts have been previously shown to be suited for reprogramming by nuclear transfer in mouse [2] and cell fusion in both

mouse and human [59, 60]. Second, the derivation of fibroblasts is simple and the culture conditions are compatible with ESC culture conditions. Furthermore, they are used as feeder layers for ESCs, and disease-specific human fibroblasts are available through cell repositories such as Coriell.

After the success of fibroblast reprogramming, multiple mouse cell types have successfully been reprogrammed into iPSCs. These include stomach cells [61], liver cells [61], pancreatic β cells [62], lymphocytes [63], and neural progenitor cells [64, 65]. Furthermore, human keratinocytes [66] and human peripheral blood cells [58] have also been reprogrammed.

To determine the optimal cell type for a reprogramming approach one has to take into account several factors. These include age and source of the cells, the ease of derivation of the cell type and the reprogramming efficiency of this cell type. For basic research efforts fibroblasts will likely remain the choice of cell type, but for therapeutic applications donor cells would preferably be more easily attainable and less likely to contain genetic aberrations that might occur due to age and UV-induced mutations. One good candidate would be hematopoietic progenitor cells, mobilized from the bone marrow [58].

In humans, iPSCs are commonly generated from dermal fibroblasts; however, the requirement for skin biopsies and the need to expand fibroblast cells for several passages in vitro make it a cumbersome source for generating patient-specific stem cells. Human keratinocytes isolated from plucked hair can be reprogrammed to iPSCs [66]. However, it remains unclear whether hair cells will be a faithful source for reprogramming because the growth and quality of the hair follicles are dependent on the age, genotype, and the medical conditions of the human donors [67, 68]. Human cord blood cells, directly isolated from the umbilical cord after birth, are a source of fetal cells that have had minimal exposure to DNA-damaging influences that occur during natural ageing. CD133+ cells from the cord blood have been reprogrammed using only Oct4 and Sox2 [69] which is a great advantage over using the four factor reprogramming that include c-Myc. This source however is limited to the patients who had their cord blood banked at birth. iPSCs were successfully generated from cord blood that was cryopreserved for 5 years [69] which is of importance for older patients. It remains to be determined if prolonged cryopreservation of cord blood influences the reprogramming efficiency. However, this will most likely not be a problem for correct cryopreserved cord blood since for allogeneic bone marrow transplantations cord blood of up to 25 years old is successfully used and probably even older cord blood (40 years old) will be used in the future.

6.6.4 Cell Source and Memory of iPSC

iPSC, fulfilling al pluripotency requirements, exhibit epigenetic memory [70, 71]. iPSC obtained from fibroblasts (f-iPSC) and blood (bl-iPSCs), ESC derived from nuclear transfer (ntESC) and normal blastocyst derived ESC (ESC) have all been differentiated into blood and bone. Interestingly, the bl-iPSCs generated more

hematopoietic colonies than the f-iPSCs. Differentiation into osteoblasts, a mesenchymal lineage that can be obtained from fibroblasts, showed a better osteogenic differentiation of the f-iPSCs as compared to the bl-iPSCs. In contrast to the iPSCs, the ntESCs differentiated comparable to the ESCs into both lineages. The hematopoietic loci (important for hematopoietic development) were hypermethylated in the f-iPSCs, silencing important hematopoietic genes. In contrast to the f-iPSCs, these loci are not hypermethylated in the bl-iPSC. Similarly, a large number of fibroblast-specific genes are hypermethylated in bl-iPSCs. This specific methylation pattern suggest that iPSCs harbor epigenetic marks similar to the cell type they were derived from and which are detrimental to generation of cell lineages distinct from the donor cell. The methylation pattern of an iPSCs cell can be reset to a different lineage by an extra round of reprogramming. The iPSCs is differentiated to a lineage that is distinct from the original cell type. Thereafter, the differentiated cell is reprogrammed and now carries the epigenetic memory of its recent origin and not that of the original cell type. Interfering with epigenetic gene regulation (histone acetylation, DNA methyation) by trichostatin-A (inhibitor of histone deacetylation) and 5-azacytidine, enhanced the differentiation of the iPSCs (brain derived) into the hematopoietic lineage. The iPSCs created after reprogramming of somatic cells apparently do not reach a fully naïve ground state of pluripotency and calls for improvement of the reprogramming methods [70]. However, through continuous culture of the iPSCs, the transcriptional, epigenetic and functional differences between iPSCs from different origin appear to be abrogated. Most likely, this also applies to human derived iPSCs. Therefore, for therapeutic purposes the choice of donor cell in relation to the desired cell type should be taken into consideration. When a donor cell of the desired type is not available or not the obvious choice, the epigenetic memory of the iPSCs could be modified using drugs like trichostatin-A and 5-azacytidine which possibly allow more efficient differentiation of the iPSCs to a lineage different from the donor cell.

6.6.5 Therapeutic Potential of iPSC

The therapeutic potential of iPSCs was demonstrated in a proof of principle experiment [72]. It was shown in a humanized sickle cell anemia mouse model that mice can be rescued after transplantation with hematopoietic progenitors obtained in vitro from autologous iPSCs. Autologous skin cells were used to derive iPSCs in which the human sickle hemoglobin allele was corrected by gene-specific targeting. Subsequently, the corrected iPSCs were differentiated in vitro to hematopoietic progenitors that were transplanted into the bone marrow of the diseased mouse and could rescue the disease phenotype. This murine experiment suggests that, in principle, human iPSCs could also be used for regenerative and therapeutic applications. Other diseases, such as neural degeneration diseases could also possibly be treated using iPSC technology. iPSCs have been efficiently differentiated into neural precursor cells, giving rise to neuronal and glial cell types in culture [73].

The iPSCs derived neural precursors were separated using fluorescence-activated cell sorting from the pluripotent iPSCs to minimize the risk of teratoma formation after transplantation. Upon transplantation into the fetal mouse brain, the cells migrate into several brain regions and differentiate into glia and neurons, including glutamatergic, GABAergic, and catecholaminergic subtypes. The grafted neurons had mature neuronal activity and were functionally integrated in the host brain showing their therapeutic potential. Furthermore, iPSCs were induced to differentiate into dopamine neurons of midbrain character and were able to improve behavior in a rat model of Parkinson's disease upon transplantation into the adult brain showing the therapeutic potential of iPSC derived neural precursors.

6.6.6 Patient Specific iPSC

The generation of iPSC from an individual patient would enable large-scale production of the cell types affected by that patient's disease. These patient-specific iPSCs could be used for disease modeling, drug discovery and cell therapy (Fig. 6.2). iPSCs offer the potential to generate patient-specific cells that would be recognized as "self" by the immune system thus circumventing the issue of graft-rejection. Therefore, auto-transplantation of relevant cell types derived in vitro from autologous iPSC can take place after correction of genetic defects.

Patient-specific fibroblasts were successfully reprogrammed for the first time in 2008 [74]. Fibroblasts from an 82-year-old woman diagnosed with a familial form of amyotrophic lateral sclerosis (ALS) were reprogrammed to iPSCs. These iPSCs were differentiated into patient-specific motor neurons and glia, the cell types implicated in ALS pathology, creating a model for studying the sporadic form of this disease. This model will provide insight into the intrinsic survival properties, the interactions with other cell types, and the susceptibility to the environmental conditions of motor neurons; all of these features are considered to play an important role in ALS pathogenesis. However, no comparison was made yet to the differentiation capacity of healthy control iPSCs, showing the diseased phenotype of the ALS-iPSCs in vitro.

iPSCs created from spinal muscular atrophy (SMA) patients [74, 75] which progressively lose their motor neurons resulting in muscle weakness, paralysis, and often death, have been differentiated into motor neurons. However, after prolonged time the SMA-iPSC culture showed less and smaller motor neurons compared to the control culture, suggesting a defect in motor neuron generation and/or increase in motor neuron degeneration. This shows that human iPSCs can be used to model the pathology in a genetic inherited disease [75].

Somatic cells from Fanconi anemia patients were reprogrammed to pluripotency to generate patient-specific iPSC. These iPSCs were corrected using lentiviral vectors expressing FANCA or FANCD2 and could give rise to hematopoietic progenitors of the myeloid and erythroid lineages that are phenotypically normal, that is, disease-free [76]. These data offer proof-of-concept that iPSC technology can be used for the generation of disease-corrected, patient-specific cells with potential

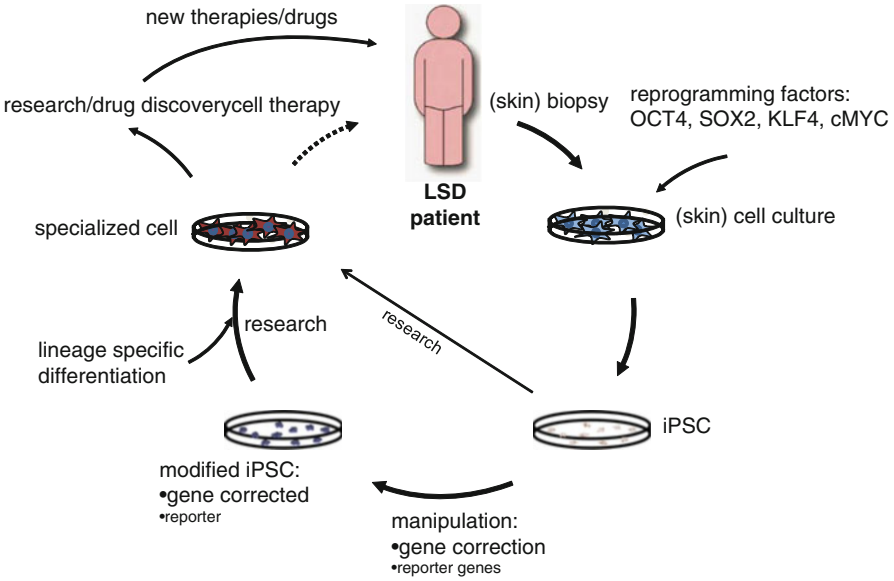

Fig. 6.2 Patient-specific iPSC generation. Differentiated cells are isolated from the patient, cultured and reprogrammed with OCT4, SOX2, KLF4, and c-MYC to iPSCs. These iPSCs can be used for research or manipulated through homologous recombination to correct the genetic defect or genetically manipulated for research applications. These corrected iPSCs can be differentiated to the lineage(s) of choice and used for cell transplantation into the patient (this however still requires optimization of the iPSC protocols). These iPSCs can also be used for research and drug screens that will benefit the patient

value for cell therapy applications. However, here the disease was corrected using lentiviral overexpression, which increases the possibility of insertional mutagenesis and tumor formation.

These patient-specific stem cells create the opportunity to study disease modeling, drug development, and eventually autologous cell replacement therapies (Fig. 6.2).

6.6.7 Cell Therapy and Safety

However, all of these patient-specific iPSC are generated using the three or four factor (Oct4, Sox2, Klf4 and/or c-Myc) reprogramming using retroviruses or lentiviruses making it almost impossible to use these iPSCs for cell replacement therapy [74, 75]. The generation of safe iPSCs, using integration-free reprogramming techniques such as protein-reprogramming is essential for clinical applications. However, since iPSCs are pluripotent and the transplanted cells are differentiated from the iPSCs, it will be essential to purify the differentiated cells to 100 % purity before transplantation to prevent teratoma formation as side-effect due to the remaining pluripotent cells. Recent findings show that cells can also be

reprogrammed from one somatic cell type to the other somatic cell type, the induced somatic cells, without an intermediate pluripotent state [77–79].

Other safety concerns include that most culture conditions include nonhuman animal products, raising the concern of transmission of pathogens. However, total animal component free culture conditions are becoming available for iPSC culture.

6.7 Application of ESC and iPSC for Treatment of Lysosomal Storage Diseases

The recent development of the iPSC technology now potentially allows to study and design alternative therapies for other diseases. Lysosomal storage diseases (LSDs) are a group of approximately 40 rare inherited metabolic disorders that result from defects in lysosomal function and are life-threatening. As a group, the LSD's occur in approximately 1 in 5,000 live births and represent a prevalent group of genetic disorders in humans. Most LSDs are caused by deficiency of a single enzyme, leading to an accumulation of its substrate in all cells (except erythrocytes) and eventually cellular dysfunction. There are no cures for lysosomal storage diseases. Enzyme replacement therapies (ERT) is used but with limited success [80, 81]. The ERT in clinical trials of several LSDs has shown that this procedure could be effective against LSDs that do not have a neuropathic component since the administered enzyme cannot cross the blood–brain barrier. Alternatively, the use of umbilical cord blood transplantations in better defined international guidelines/protocols has resulted in significant better survival rates (to >95 %) [82]. Gene therapy to overexpress the missing enzyme via hematopoietic progenitors or other tissue-specific progenitors that could be transplanted into patients may offer cures in the future and is currently been trialed in MLD (see Chap. 5).

6.7.1 LSD-iPSCs

Since most LSD's are caused by mutation(s) in a single gene, the creation of patient-specific and gene corrected iPSC would open possibilities for gene and cell therapy. In principle, all LSD's would be good candidates for such an approach since all organs are affected and iPSC could be differentiated to multiple desired lineages as source for cell therapy thereby replenishing the organs with enzyme. Especially the central nervous system pathologies would benefit from autologous cells expressing the corrected enzyme.

Recently, LSD-iPSCs from mouse models of Fabry disease, globoid cell leukodystrophy (GLD), and mucopolysaccharidoses type VII (MPSVII) have been generated and showed defects in disease-specific enzyme activities and increased levels of the corresponding substrates [83]. Fabry-iPSCs and GLD-iPSCs could efficiently be differentiated into disease-relevant cell types, such as cardiomyocytes and neural

stem cells indicating their usefulness for therapeutic studies. MPSVII-iPSCs were impaired in the ability to differentiate into embryoid bodies (EB), which could be improved by partial correction of the enzyme levels. Moreover, there is partial lethality of MPSVII mice in utero and in combination with the impaired differentiation capacity into EBs, this might suggest a possible abnormality of embryonic development in patients [83].

Human LSD (Gaucher disease type III) -iPSCs were generated from donor male Gaucher disease type III fibroblasts [74] and Hurler syndrome-iPSCs from patient keratinocytes and mesenchymal stromal cells [84]. The Hurler-iPSCs show elevated GAG content, a hallmark of the defect in Hurler disease, and can be differentiated into hematopoietic cells which are of special interest for autologous hematopoietic stem cell transplantation. The Hurler-iPSCs were corrected using lentiviral expression of IDUA, the enzyme mutated in Hurler and corrected Hurler-iPSCs showed equal hematopoietic colony formation capacity as non-corrected Hurler-iPSCs and wt-iPSCs. However, one of the lentiviral mediated gene corrected iPSC lines showed interference with the function of tumor suppressor genes, oncogenes, or cell cycle and proliferation genes [84], underscoring the potential danger of lentiviral mediated gene correction. Nevertheless, Hurler-iPSCs offer of a new model to study pathogenic cascades underlying the defects in this complex disease.

6.7.2 LSD-Cell Therapy

Moreover, as in general for iPSC, LSD-iPSC would offer new possibilities for cell therapy. Since ERT and bone marrow transplantation are not effective in LSDs that affect the central nervous system, alternative treatments are needed. Analogous to treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin [72] it would be possible to correct patient-specific LSD-iPSCs using homologous recombination to replace the mutated gene with the correct gene and thereby genetically correcting the LSD-iPSCs (Fig. 6.2). The possibility to perform homologous recombination in human iPSCs has recently been shown by Geijsen and colleagues [36]. Alternative methods to repair the lack of sufficient levels of gene product were shown in chronic granulomatous disease (CGD) iPSC. Using Zn-finger mediated recombination, a correct copy of the defected gene was integrated into the AAVS1 locus to produce the correct protein which restored functionality [85]. However, this method does not replace the defected gene and is not under control of the endogenous transcriptional program. Zinc-fingers, like tale like effector nucleases (TALENs) can also be used for genome editing in ESC and iPSC and replace the affected gene with a correct copy [86]. These corrected iPSCs could then be differentiated into neural stem cells to be transplanted into the brain. Brain transplantation of genetically engineered human neural stem cells (NSCs) into a mucopolysaccharidosis type VII mouse showed that brain lesions could be corrected, which makes this approach potentially applicable to treatment of LSD patients suffering from neurological disorders [87]. Moreover, the corrected iPSCs could also

be differentiated into hematopoietic progenitors and mesenchymal stem cells [84, 88] for repopulation of the bone marrow and also other desired lineages (Fig. 6.2). Although the cell therapy as just suggested for treatment of LSD involves many hurdles that need to be taken, it might one day be feasible in the not distant future. Alternatively, engineered polymer embedded cell implants that allow passing of the enzyme but not of cells could [89, 90] could be developed. The optimal cell-type that will be in the implant needs further investigation and will probably be an optimal combination of enzyme production and viability and will probably depend on the site of implantation. Ideally such an implant will be vascularized and supported by nutrients from the host and deliver the produced enzyme to the host. An advantage of such Implants in view of safety is the possibility to surgically remove it and replace it with a new implant.

6.7.3 Drug Development to Treat LSD

Since LSD patients are characterized for their specific mutations, the generation of hESCs carrying these specific mutations would be feasible through homologous recombination. Next to the generation of human LSD-specific iPSC, LSD-hESCs would be greatly beneficial for the understanding of mechanisms that explain disease progression. Since LSD involves the lack or malfunctioning of a specific enzyme, iPSCs could be used in large scale compound screens to identify drugs that will compensate for the (partial) loss of enzyme activity. Such drugs could be applied alone or in combination with cell therapy.

6.8 Concluding Remarks

The ability to generate LSD-patient-specific iPSCs has opened new possibilities to study and possibly find treatments for the patients. Specific mutations could be corrected, disease pathogenesis could be studied and large drug screens could be performed. Once, safe iPSCs can be generated, these cells could be used for cell therapy and restore function in the whole patient including the brain area. The use of LSD-iPSCs in compound screens will hopefully identify new drugs that can be used alone or perhaps in combination with cell therapy.

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Chapter 7
Concluding Comments and Future Directions

Robert Wynn and Jaap Jan Boelens

7.1 Introduction

The chapters of this book describe enormous, almost unbelievable, progress in the last few years in the field of the cellular therapy of lysosomal storage disorders (LSDs). We have learnt much about the diseases themselves whilst we have made parallel great progress in the clinical management of affected individuals. In these concluding notes we, the editors, wish to both summarise the key advances that have been made in this field of cellular therapy of LSD and speculate upon the future of the field in the coming years and decades. Furthermore we wish to indicate how all us in scientific and clinical fields—editors, contributing authors and readers of this book—must act together in order to effect this future.

Allogeneic stem cell therapy of LSDs has been known for 30 years to have some effect on the natural history of certain LSDs. During this time we have learnt that some diseases are more responsive to such transplant therapy than other diseases. Thus it is often stated that children with Hurler Syndrome (MPS IH) are more sensitive to transplant than boys with Hunter Syndrome (MPS II). Within a responding disease such as Hurler we have also learnt over this same time period that some organs are better corrected towards normal than some other organs. Thus in Hurler the somatic manifestations—hepato-splenomegaly, obstructive airways, and

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cardiomyopathy—are better corrected than the orthopedic aspects of the illness, and the neurological aspects sit somewhere in between.

So pronounced are these differences between diseases that only some diseases are even referred for consideration of allogeneic stem cell transplantation. In the UK for example only MPSIH and mannosidosis and in the Netherlands also pre-symptomatic later onset MLD patients, are considered routine indications for allogeneic transplant and transplant in other LSD is considered at best developmental and at worst experimental. So varied are outcomes following transplant, including the variable outcomes between different organs within a single transplanted patient, that we might consider transplanted LSD patients to have a new clinical entity, a unique collection of clinical symptoms and problems. Transplanted Hurler children thus have a different clinical phenotype than children with more attenuated forms of MPSI such as Hurler–Scheie or Scheie.

These are rare disorders and the first thing that we have learnt over the years is that in order to make progress in understanding both the diseases and these rather unique effects of our therapies on these diseases is that children should be looked after in centres with expertise and experience. The second thing is that these centres should collaborate and pool their clinical experiences and together study the large numbers of patients that they collectively look after so that they can learn from that collective experience about how they may better look after future patients.

This book has described the increasing number of therapies that are becoming available in the LSD field. There is allogeneic stem cell transplant. There is pharmacological enzyme replacement therapy (ERT). ERT can be delivered directly into the tissue, e.g. intrathecally for CNS disease, or more usually via an intravenous infusion. There are different formulations, different doses and different schedules of ERT administration. There are small molecule inhibitors and there is gene-therapy in its various forms. This plethora of treatment options only makes it the more important that proper investigation of therapies takes place between large collaborating centres so that we can all learn which treatments are best for which patients so that they can be then given to the patients that we look after.

7.2 Factors Affecting Outcomes Following Treatments for LSD

We have sought to explain the differences in outcomes of different diseases and different organs to our applied therapies. We summarise six factors that influence outcome in an individual patient with a particular LSD in response to a particular therapy.

Two of these we cannot influence—the disease itself and the genotype of that disease.

Two of these factors can be influenced within our current clinics—the complications of an applied treatment and the other therapies that are applied in addition to that treatment within an experienced multidisciplinary clinic.

The remaining two—the age at diagnosis and the effectiveness of our therapies at reversing substrate accumulation—might be possible for us to influence in the future years and it is our contention that the major progress in the treatment of LSD in the coming years will come from systemically addressing these two factors. Substrate accumulation is the initiating pathophysiological event, however incomplete an explanation such an accumulation might be for all disease manifestations and however ineffective reversing such accumulation is in correcting established disease in LDS.

1. The Disease

   Some diseases are more responsive than others to cellular therapy. There are several reasons for this and not all of this difference is understood. Some of the difference might reflect the age at diagnosis. Thus some of the variability in response between MPSI Hurler and MPSII Hunter might reflect the age at diagnosis. Cellular therapy is better applied to prevent progression of disease rather than reverse established disease manifestations. More obvious somatic and bony manifestations therefore in Hurler might allow earlier diagnosis so that cellular therapy is applied earlier and more effective in altering the neurological outcome of the disease when compared to this same manifestation in Hunter.

   Diseases with predominantly bony manifestations of the disease—MPSIV, Morquio for example—will be intrinsically resistant to the effect of enzyme delivered by cellular or pharmacological means.

   In some disease perhaps the enzyme is not secreted by engrafted donor leukocytes so that cellular enzyme delivery is necessarily less efficient than where it is more readily excreted by these cells. Enzyme delivery might be better from "gene-modified autologous cells" as described by Biffi since the promoter included in the gene transfection will facilitate enzyme production by cells of the haemopoietic system. Perhaps the target cells are poor in receptors for the enzyme and so cannot take up sufficient quantities of the delivered enzyme for adequate cross correction.

   Enzyme delivery and substrate reduction are not the whole story in the LSDs: Although enzyme deficiency and substrate accumulation are the initiating events in the LSDs the pathophysiology of the disease is mediated by diverse mechanisms in different diseases including inflammatory events as has been discussed. Thus enzyme delivery and reversal of substrate accumulation might not be sufficient to reverse the course of a particular disease. Better understanding of the events that occur downstream from substrate accumulation and which mediate the pathological events within a disease need to better elucidated in order to optimise therapies of these disorders. These downstream mechanisms of disease are likely different in different LSDs and will influence the response of disease to a particular therapy.

2. Genotype

   Patients with a genotype that predicts for a severe phenotype will have a functionally poorer outcome than those with a genotype that allows production of some enzyme. Transplantation and other therapies including ERT will never compensate for such genotypic differences. It is self-evident that any therapy will have a better outcome in a patient with a milder disease.

3. Complications of the Applied Therapy

   A patient with a complicated therapy will have an inferior outcome to a patient where the therapy is applied without complication. In this respect the cellular therapy of LSD is no different to any other medical discipline. Children who have bad "graft-versus-host disease" after a transplant and prolonged hospitalisation will have an inferior outcome to those who have an uncomplicated transplant. Patients that have antibodies directed at pharmacological ERT will have an inferior outcome to that therapy compared to those that have no such antibody response.

4. Other Applied Therapies

   Before cellular therapy and ERT of LSD there was only symptom-directed care. In the era of these more specific therapies then we should not forget these other interventions in order that we optimise patient outcome. The multidisciplinary management of transplanted patients is best delivered in a centre where many such patients are looked after as the problems these patients face are rather specific and different from patients that are not transplanted (for example those with attenuated phenotype disease).

   Those therapies that optimise patient outcomes and which form part of such multi-disciplinary follow up include orthopedic surgery (to back, hips and knees), physiotherapy, speech and language therapy, and otorhinolaryngology and endocrinology medical specialists. Patient support and advocacy organisations have much to offer patients and their families in these clinics so that the patient experience is seen as uncommon but not unique. There will be shared experiences that will help families to manage.

5. Age at Diagnosis

   There is abundant evidence that when therapy is applied early then the outcome is better. This includes both pharmacological ERT and cellular therapies of LSD. It provides a compelling argument for screening of newborns for LSDs so that therapy can be applied as early as practically possible.

   In ERT there is evidence of improved outcome in the Hurler dog model when ERT is started at birth compared to when it is started at a later time point. There is anecdotal clinical evidence from sibling pairs with attenuated phenotypes of MPSI that the same is true in the clinical setting. The proband is diagnosed with symptomatic disease and ERT is less effective than when the diagnosis is made by directed genetic or enzymatic screening of a subsequently born, affected sibling.

   In cellular therapy there is similar evidence from the clinic that earlier diagnosis affects outcome. In children with MPSIH then it has long been recognised

that the efficacy of the therapy in reversing neurologic progression of the disease is influenced by the age at transplant. It has been suggested that transplant not be offered to subjects over the age of 24 months at presentation. Whilst such an absolute cut off might be disputable it is not difficult to observe in clinics the association of age at transplant and outcome. One of the benefits of cord blood transplant in this condition is that it shortens the interval between diagnosis and transplant compared to adult unrelated donor transplant. This is because the degree of matching is reduced and the cord donation is already frozen and ready to use.

In globoid cell leukodystrophy (Krabbe disease) then a clear relationship has been observed between outcomes of children without transplant, of children with cord transplant at first sign of disease and of children transplanted immediately at diagnosis following newborn genetic screening. The outcome of children with the last intervention have best outcome and the children transplanted at first symptoms—still during infancy—fare significantly better than the untransplanted subjects. Infantile Krabbe is a rapidly progressive disease and for therapy to have any effect it much be promptly applied. At the very least it serves as a "proof of principle" of the efficacy of newborn screening and prompt therapy in improving outcomes in an LSD following transplant therapy.

6. Efficacy of Therapy in Reversing Substrate Accumulation

Different therapies have a different ability to correct the disorder metabolically, if not clinically. There are different measures of substrate accumulation. In MPSIH one might measure total urine glycosaminoglycan (GAGs). Every treatment of the condition must be shown to reduce urine GAGs at the very least and this forms the basis of a screening test for diagnosis. The specific accumulation of dermatan-sulphate (DS) in MPSIH in the GAG might be demonstrated by the rather more specific ratio of accumulated GAG (DS) to non-accumulated GAG, chondroitin sulphate (CS).

We have previously demonstrated that the DS/CS ratio in urine following therapy is better reduced following wild type donor transplant than following heterozygous carrier donor transplant and both have better correction than following pharmacological ERT. The improved outcome of wild type compared to carrier transplant is a description of "gene dose" effect. This has been developed by Biffi in her autologous gene-modified haematopoietic stem cell transplant of Metachromatic Leukodystrophy (MLD). She demonstrates and describes improved outcomes in the autologous gene-modified setting compared to wild type transplant as the former delivers more enzyme still than the wild type transplant does to residual enzyme deficient tissues. This might be because more gene copies per cell are introduced or because the promoter employed favours continued and increased enzyme production and secretion from the mature engrafted progeny (including engrafted tissue macrophages—as microglia in the brain) of the transduced haematopoietic stem cell.

Within clinical transplantation we have demonstrated that there is a relationship between enzyme level after transplant and various clinical measures of

residual disease. Thus those with higher enzyme levels appear to have a reduced need for orthopedic intervention or at least that the time to such surgery is delayed. Cord blood will deliver reliably high enzyme levels after transplant as it is necessarily wild type and there are high levels of donor cells engraftment compared to bone marrow as a cell source. Some units now favour use of an unrelated wild type donor over a carrier family donor, so accepted has the observation become between delivered enzyme dose and clinical as well as metabolic outcome.

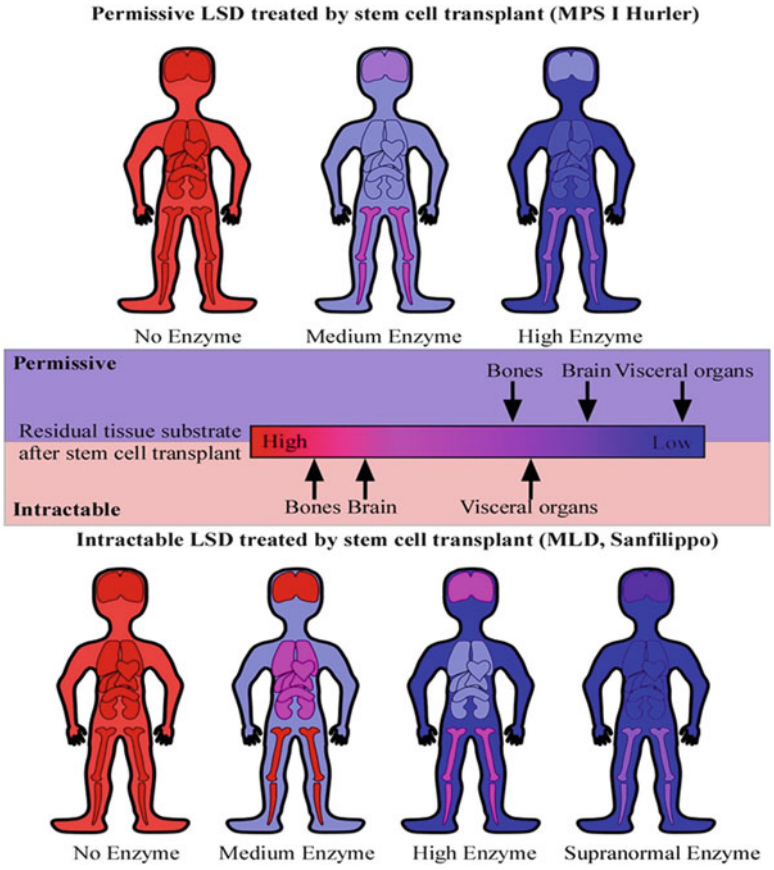
In pharmacological ERT the delivered enzyme to the tissue will be reduced where there is production of an attenuating allo-antibody. We have described the production of such an antibody in children with MPSIH who are receiving ERT prior to allogeneic transplant. Most of these children develop antibodies and with the appearance of antibody then biomarkers of disease activity increase. In ERT—just as in cellular therapies—if the efficacy of treatment is attenuated then the metabolic correction delivered is also attenuated. There is a clinical correlate too of such antibodies and we know in ERT that sustained antibody production is associated with a loss or a reduction in clinical benefit.

7.3 A Working Model of Therapy in LSD

We have developed the following model for therapy in LSD. In the upper caption there is a "transplant sensitive" disorder. Some tissues require more enzyme to be delivered in order that they be corrected—so bone requires more than brain which in turn requires more than the somatic tissues. This model will then explain why all therapies—ERT, carrier donor HSCT and unrelated donor HSCT will correct somatic manifestations of disease but outcomes of other aspects of the disorder are better corrected by those therapies that deliver more enzyme.

In the lower panel we have a "transplant refractory" disease model, such as infantile MLD or MPSIII. In such a disorder unrelated donor transplant is less effective in the clinic than it is in MPSIH. Thus wild type transplant does not correct the neurologic disorder in mice or men. However Biffi's mice and now her patients show that the condition remains correctable but with more enzyme again. It is tempting to hope that the bone disease of MPSIH or indeed of MPSIV might also be better correctable with more enzyme than might be deliverable either with ERT or with conventional wild type donor transplant.

The other factors that we have discussed will still influence outcomes but cannot be so readily illustrated in this cartoon. Of course the age at which therapy can be delivered and those other therapies that need to be given alongside delivered enzyme remain greatly important. Patients with attenuated phenotype will still do better than those with a null mutation. Patients with a complicated treatment course will still have an inferior outcome to these that have an uncomplicated treatment course.



7.4 Conclusion

We complete this chapter and the book with our vision as physicians and scientists in this field for the way forward. We leave the reader with six statements about cellular therapies of LSD for their consideration as this field moves forward and patients look forward to expanding therapeutic options:

- We firstly state again that collaboration between centres that look after large numbers of patients is a necessary prerequisite for continued progress in this area of medicine.
- There must be better understanding of the relationship between enzyme accumulation and disease. Only with such an understanding of the basic science of the disease will it become more possible to reverse established disease in the many patients that we currently care for.

- The future therapies will include multiple modality therapies especially in refractory disease patients and towards treatment of refractory organs in responding patients. We already use ERT in many centres to improve somatic performance prior to SCT but perhaps post-transplant ERT might improve bony outcome or the addition of substrate inhibitors might aid outcomes.
- Diagnosis must be made earlier and there must be development of newborn screening strategies. This is particularly true in those LSDs where there is already available an effective therapy and a clear relationship between outcomes and age at initiation of therapy. Of course the screening strategy must be robust and not generate needless anxieties through the generation of results of uncertain significance.
- The protocols for the "gene-therapy" that are in early clinical trial and have been demonstrated in animal models to be both safe and of increased efficacy must be rigorously tested in the wider clinical setting.
- There must be better biomarkers of LSD disease activity as clinical outcomes are often difficult to measure, heterogeneous and delayed, sometimes over decades. Those biomarkers that we currently have are inadequately related to disease outcomes and disease pathophysiology.

We commend this field to aspiring scientists and clinicians as one of the great potential rewards to both the clinician and scientist and to the patients that they look after.

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