View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by IntechOpen

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

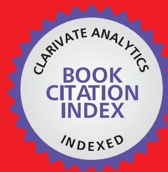
122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

7

Non Viral Gene Transfer Approaches for Lysosomal Storage Disorders

Ursula Matte, Guilherme Baldo and Roberto Giugliani
Hospital de Clinicas de Porto Alegre
Brazil

1. Introduction

The lysosomal storage disorders (LSDs) are a group of almost 50 genetic diseases, characterized by mutations and loss of activity of lysosomal enzymes or, less frequently, non-lysosomal proteins that are involved in protein maturation or lysosomal biogenesis (Meikle et al, 2004). Most LSDs have an autosomal recessive inheritance, with some exceptions as Hunter syndrome (X-linked recessive), Danon disease (X-linked dominant) and Fabry disease (X-linked with a high proportion of heterozygous affected females).

Storage of distinct undegraded or partially degraded material, usually the substrate of the defective enzyme, occurs in the lysosome. The substrate type is used to group the LSDs into general categories (table 1), including mucopolysaccharidoses (characterized by the storage of mucopolysaccharides, also called glycosaminoglycans), lipidoses (storage of lipids), glycogenoses (storage of glycogen) and oligosaccharidoses (storage of small sugar chains). Despite this categorization, many clinical similarities are observed between groups as well as within each group. Generally these diseases are multisystemic, and clinical features of many LSDs include organomegaly, central nervous system dysfunction and coarse hair and faces. Most LSDs are characterized by their progressive course with high morbidity and increased mortality, although there are significant variations between different diseases, and even among patients with the same disease (Walkley 2009). Lysosomal enzymes are ubiquitously distributed, but substrate storage is usually restricted to cells, tissues and organs with higher substrate turnover.

Recently, it has been suggested that the primary gene defect and substrate storage are triggers of a complex cascade of events that lead to many of the disease manifestations (Bellettato & Scarpa, 2010). In this context, secondary substrate storage, perturbations of Calcium homeostasis and lipid trafficking would contribute to disease pathogenesis. Other manifestations, related to the lysosome's role in vescicle trafficking, including antigen presentation, innate immunity, and signal transduction would cause inflammatory and auto-immune disturbances observed in the LSD (Parkinson-Lawrence et al., 2010). In addition, general mechanisms such as unfolded protein response, reticulum stress, oxidative stress and autophagy blockade would also play a role in the pathogenesis (Vitner et al., 2010).

The incidence and prevalence of these diseases varies from different countries and regions. For example, the overall incidence of GM1 Gangliosidosis is considered to be 1:100,000-

1:200,000, however in some countries as Malta (1:3,700) and the South of Brazil (1:13,317) it is considerably higher (Baiotto et al, 2011). Large population studies suggest that the overall incidence of the LSDs vary from 1:5,000- 1:7,700 (Fuller et al, 2006).
The treatment options available for the LSDs were restricted to support measures until a few decades ago. Nowadays, specific treatments are available for a certain number of LSDs, even though some of them are still in the experimental phase or have limited effects. Treatment options listed in table 1 consider those already approved or under clinical trial, including compassionate use. Support measures and palliative care are not considered treatment options in this context.

Disease	OMIM	Gene	Enzyme	Available Treatments
Aspartylglucosaminuria	208400	*AGA*	N-aspartyl-beta-glucosaminidase	HSCT
Canavan disease	271900	*ASPA*	Aspartocylase	None
Cystinosis	219800	*CTNS*	Cystinosin	Cysteamine (drug)
Danon disease	300257	*LAMP2*	Lysosomal-associated membrane protein 2	None
Fabry disease	301500	*GLA*	A-galactosidase A	ERT
Farber disease	228000	*ASAH1*	Ceramidase	HSCT
Fucosidosis	230000	*FUCA1*	α-L-fucosidase	HSCT
Galactosialidosis	256540	*CTSA*	Cathepsin A	None
Gaucher disease	230800	*GBA*	acid β-glucosidase	ERT, GT, HSCT, PCT, SSI
GM1 gangliosidosis	230600	*GLB1*	β-Galactosidase	HSCT
Krabbe disease	245200	*GALC*	galactocerebrosidase	HSCT
Lysosomal Acid Lipase Deficiency	278000	*LIPA*	Lysosomal acid lipase	HSCT
α- mannosidosis	248500	*MAN2B1*	α -D -mannosidase	HSCT
β- mannosidosis	248510	*MANBA*	β -D-mannosidase	None
Metachromatic leucodystrophy	250100	*ARSA*	Arylsulphatase-A	HSCT
Metachromatic leucodystrophy	249900	*ARSA*	Saposin-B	HSCT
Mucolipidosis type I	256550	*NEU1*	Sialidase	None
Mucolipidosis types II/III	252500	*GNPTAB*	N-acetylglucosamine-1-phosphotransferase	None
Mucolipidosis type IIIC	252605	*GNPTG*	N-acetylglucosamine-1-phosphotransferase γ-subunit	None
Mucolipidosis type IV	252650	*MCOLN1*	Mucolipin 1	None
Mucopolysaccharidosis type I	607014	*IDUA*	α-L-iduronidase	ERT, HSCT
Mucopolysaccharidosis type II	309900	*IDS*	Iduronate sulfatase	ERT, GT, HSCT
Mucopolysaccharidosis type IIIA	252900	*SGSH*	Heparan-N-sulfatase	None
Mucopolysaccharidosis type IIIB	252920	*NAGLU*	α- N-acetylglucosaminidase	None
Mucopolysaccharidosis	252930	*HGSNAT*	AcetylCoa-glucosamine-N-	None

type IIIC			acetyltransferase	
Mucopolysaccharidosis type IIID	252940	*GNS*	N-acetylglucosamine-6-sulfatase	None
Mucopolysaccharidosis type IVA	253000	*GALNS*	N-acetylgalactosamine-6-sulphatase	ERT
Mucopolysaccharidosis type IVB	253010	*GLB1*	β-Galactosidase	None
Mucopolysaccharidosis type VI	253200	*ARSB*	N-acetylgalactosamine-4-sulphatase	ERT
Mucopolysaccharidosis type VII	253220	*GUSB*	β-Glucuronidase	None
Mucopolysaccharidosis type IX	601492	*HYAL1*	Hyaluronidase	None
Multiple sulphatase deficiency	272200	*SUMF1*	Formylglicine-generating-enzyme	None
Neuronal ceroid lipofuscinosis 1	256730	*PPT1*	Palmitoyl protein thioesterase-1	HCST
Neuronal ceroid lipofuscinosis 2	204500	*TPP1*	Tripeptydil-peptidase I	GT
Neuronal ceroid lipofuscinosis 3	204200	*CLN3*	CLN3 protein	None
Neuronal ceroid lipofuscinosis 5	256731	*CLN5*	CLN5 protein	None
Neuronal ceroid lipofuscinosis 6	601780	*CLN6*	CLN6 protein	None
Neuronal ceroid lipofuscinosis 8	600143	*CLN8*	CLN8 protein	None
Niemann-Pick disease A/B	257200	*SMPD1*	Acid sphingomyelinidase	None
Niemann-Pick disease C1	257220	*NPC1*	NPC1 protein	SSI
Niemann-Pick disease C2	607625	*NPC2*	NPC2 protein	SSI
Pompe disease	232300	*GAA*	Alpha-glucosidase	ERT, GT
Prosaposin deficiency	176801	*PSAP*	Prosaposin	None
Pycnodysostosis	265800	*CTSK*	Cathepsin K	Hormone therapy
Sandhoff disease	268800	*HEXB*	Hexosaminidase B	PCT
Schindler disease	609241	*NAGA*	Alpha-N-acetylgalactosaminidase	None
Sialic acid storage disease	269920	*SLC17A5*	Sialin	None
Sialuria	269921	*GNE*	UDP-N-acetylglucosamine-2-epimerase	None
Tay-Sachs disease	272800	*HEXA*	Hexosaminidase A	PCT, SSI

Abbreviations: GT – Gene Therapy; HSCT – Hematopoetic Stem Cell Transplantation; PCT – Pharmacological Chaperone Therapy; OMIM – Online Mendelian Inheritance in Man; SSI – Specific Substrate Inhibition; ERT – Enzyme Replacement Therapy

Table 1. List of lysosomal storage diseases with their respective OMIM accession number, gene and enzyme deficiency and treatment options (including experimental ones).

The two most widely used treatment options are Hematopoietic Stem Cell Transplantation (HSCT) and Enzyme Replacement Therapy (ERT). HSCT has proven to be

efficient to some of these diseases, especially if performed early enough to prevent irreversible lesions. Nevertheless, limitations such as the need of an early diagnosis, the difficulties to find a compatible donor in short time, and the high rates of morbidity and mortality associated with the procedure still limit this type of treatment (Malatack et al, 2003). Therefore, despite the many advances in this treatment over the last 30 years (Boelens et al 2010), its use has been deferred in favor of ERT whenever it is available. Enzyme replacement therapy is approved for a growing number of LSD, especially those without CNS involvement. It has proven to reduce some visceral symptoms as hepatosplenomegaly and improve respiratory function (Sifuentes et al, 2007), however difficult-to-reach organs such as the brain and the bones are still a major challenge. Innovative routes of enzyme delivery have been tested to achieve the CNS, such as intrathecal ERT (Munoz-Rojas et al, 2008; Munoz-Rojas et al, 2010).
Other treatment approaches already under clinical use or experimentation are Specific Substrate Inhibition (SSI) and Pharmacological Chaperone Therapy (PCT). SSI aims to decrease the storage by reducing substrate synthesis through an inhibitor. PCT uses small molecules able to stabilize the mutant enzyme and help it escape proteasomal degradation, thus restoring some residual enzyme activity. All such treatments have limitations (table 2), that justify the development of gene therapy approaches for these diseases.

Approach	Brief Description	Pros	Limitations
Intravenous enzyme replacement therapy	Intravenous injection of a recombinant version of the missing enzyme	Ameliorates several visceral symptoms	High cost Does not correct difficult-to-reach sites Need of repeated injections
Intrathecal enzyme replacememnt therapy	Intrathecal injection of a recombinant version of the missing enzyme	Reaches the CNS	High Cost Need of repeated injections Efficacy uncertain
Hematopoietic stem cell tranplantation	Non-autologous transplantation of CD 34+ cells	Able to correct visceral and CNS symptoms if performed early	Limited efficacy Relatively high morbidity and mortality rates
Specific substrate inhibition	Use of drugs that can inhibit the synthesis of the undegraded material	Orally administered Reaches the CNS	Limited efficacy High cost
Pharmacological chaperone therapy	Drugs that can stabilize the mutated protein, allowing some enzyme activity	Orally administered Reaches the CNS	Works only in patients with specific mutations
Stop-codon read-through therapy	Use of molecules that can suppress stop-codon mutations	Orally administered Reaches the CNS Low cost	Works only in stop-codon mutations Clinical trials yet to be performed
Gene Therapy	Administration of a normal copy of the mutated gene	Potentially effective with single injection	Safety concerns Efficacy uncertain

Abbreviations: CNS – Central Nervous System

Table 2. Pros and limitations of different therapeutic approaches for lysosomal storage diseases.

2. Rationale for gene therapy in LSDs

The rationale for gene therapy and other enzyme-based approaches for treatment of LSDs was first introduced almost five decades ago by Christian de Duve, and can be summarized in the following sentence from his original work "In our pathogenic speculations and in our therapeutic attempts, it may be well to keep in mind that any substance which is taken up intracellularly in an endocytic process is likely to end up within lysosomes." (de Duve, 1964). His work and other pioneer studies showing cross-correction between fibroblasts from patients with Mucopolysaccharidosis type I and type II (deficient in alpha-L-iduronidase and iduronate-sulphatase, respectively) established that the enzyme produced in one cell could be uptaken by a deficient cell, thus restoring its phenotype (Fratantoni et al, 1968). Later studies identified that this uptake was a saturable, receptor-mediated process, and the main actor of this process was the mannose-6-phosphate (M6P) receptor localized in the plasmatic membrane. The post-translational modification of addition of the M6P to the protein was discovered to be a signal not only to endocytosis but also for targeting nascent hydrolases to lysosomes (Fisher et al, 1980; Sly et al., 1981).

These pivotal discoveries in the field of endocytosis and targeting of lysosomal enzymes provided the basis for treatments like HSCT and ERT. In the same way, LSDs may be considered good targets for gene therapy, despite their multisystemic involvement. The correction of a few cells could lead to the enzyme being secreted into the circulation and uptaken by the deficient cells, resulting in widespread correction of the biochemical defect (figure 1).

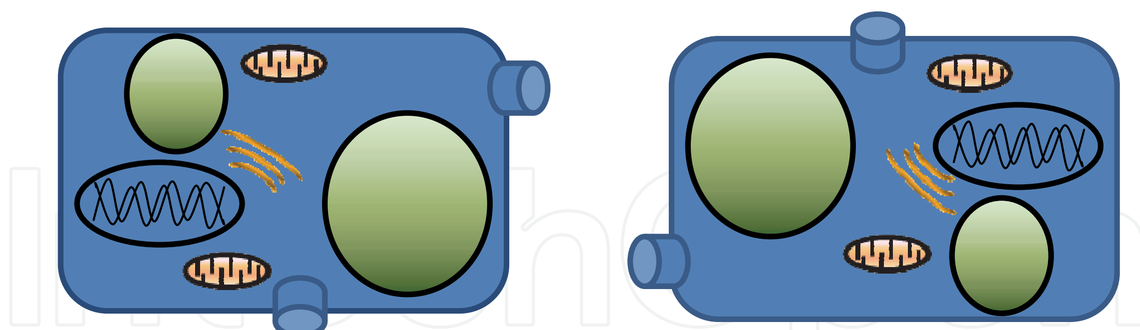
Since long term gene expression is desirable, most clinical and preclinical trials used viral based vectors. Initial studies on fibroblasts showed promising data using retroviruses (Anson et al, 1992). However, when tests in animal models started, it became clear that some organs as the brain would not be easily corrected, as the enzyme could not cross the blood-brain-barrier (Elinwood et al, 2004). This is a major hurdle as most LSD shows some degree of CNS involvement. Nevertheless, CNS targeted approaches could be envisaged to overcome this obstacle. For instance, Worgall et al. (2008) showed a slowing of progression of Neuronal Ceroid Lipofuscinosis 2 in ten children treated with serotype 2 adeno-associated virus expressing *CLN2* cDNA. Another clinical trial, for Pompe disease, also used adeno-associated-based vector, but in this case serotype 1 (NCT00976352-www.clinicaltrials.gov). Other two trials performed in the late 1990s used retroviral vectors for Gaucher (Dunbar et al., 1998) and Mucopolysaccharidosis type II (NCT00004454 - www.clinicaltrials.gov).

Safety issues related to immune response of the adenoviral vectors (Wilson, 2009) and the possibility of insertional mutagenesis of the retroviral vectors (Hacein-Bey-Abina S et al., 2008) led researchers to develop a series of studies in parallel using non-viral approaches to treat lysosomal storage disorders.

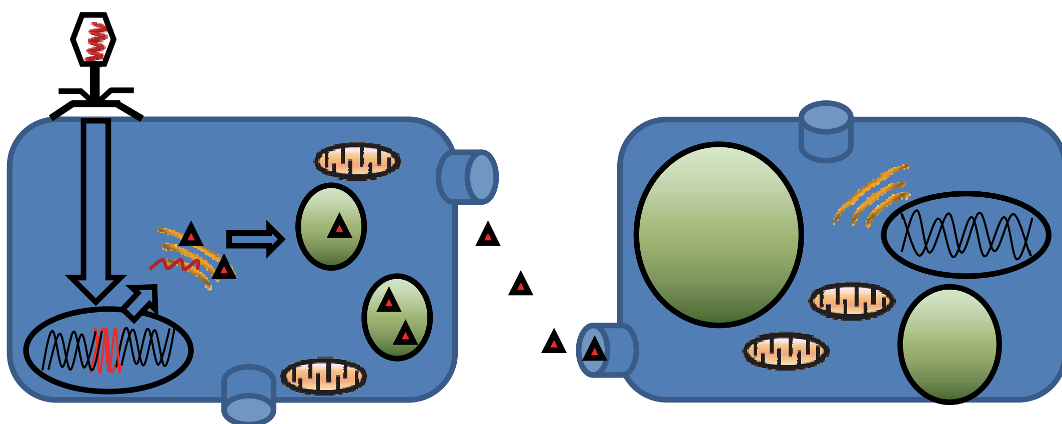
3. Non-viral approaches

Non-viral vectors have important safety advantages over viral approaches, including their reduced pathogenicity and capacity for insertional mutagenesis, as well as their low cost and ease of production (Fraga et al., 2008). The application of non-viral vectors to humans has, however, been held back by the poor efficiency of their delivery to cells and the transient expression of the transgenes. As new strategies are being developed for the

Untreated LSDs cells



Gene Transfer



Cross Correction of neighbor cells

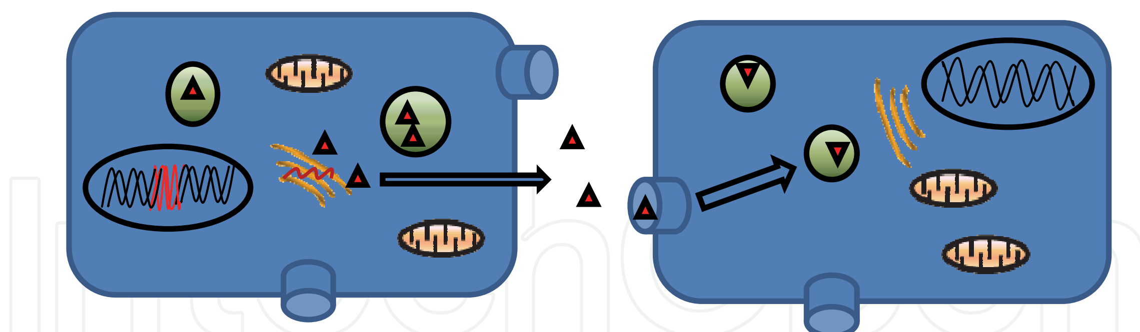


Fig. 1. Rationale for Gene Therapy in Lysosomal Storage Disorders: Cross-correction. The upper part of the figure shows two cells from a patient with LSD, with large lysosomes (green) due to accumulation of undegraded material. The gene transfer of a normal copy of the missing enzyme (red structure in the nucleus), allows the enzyme (red triangles) to be produced, and degrade the material accumulated in the lysosomes. Part of the enzyme is secreted from the recombinant cell and is captured by neighbouring cells via the mannose-6-phosphate receptors (light blue structures located on the cell membrane), reaching the lysosomes and being able to correct their phenotype, in a process called cross-correction. Note that the cell on the right was never transduced, but is able to capture the enzyme from the circulation or from the neighbouring cell.

application of non-viral vectors as nucleic acid delivery systems (Resina et al., 2009; Wasungu and Hoekstra, 2006), progress is being made in the application of this kind of therapy to LSDs.

3.1 Naked plasmid transfection

The direct injection of a plasmid containing the gene of interest and the regulatory mechanisms to ensure its expression is the simplest form of gene therapy. It has some advantages over the virus mediated gene transfer systems. First, DNA preparation is simple and can be performed at relatively low cost. In addition, the safety concerns are much lower and large amounts of DNA can be transferred. However, the major limitation of this method is that it requires a local administration and the level of transgene expression is relatively low and restricted to the injection site (Glover et al., 2005).

Hydrodynamic injection (Liu et al, 1999) is an experimental method capable to achieve efficient gene transfer and high level of transgene expression by systemic administration. In this procedure, a large volume of saline containing plasmid DNA is injected in a short period of time. The large volume and high injection rate forces the DNA solution into the liver, probably due to the permeability of liver fenestrae. A small hepatotoxicity, probably due to the large volume of saline, is observed and resolves within a few days. Even though this procedure is widely used in mice by tail vein injection, its feasibility has been demonstrated in larger animal models using a balloon occlusion catheter-based method to mimic hydrodynamic injection (Brunetti-Pierri et al, 2007; Kamimura et al, 2009).

This approach has been used in a proof-of-concept study to show the importance of coexpression of the formylglycine-generating enzyme for synthesis and secretion of functional Arylsulfatase A in a mouse model of Metachromatic Leukodystrophy (Takakusaki et al., 2005). This enzyme is a posttranslational modifying enzyme essential for activating multiple forms of sulfatases including Arylsulfatase A and therefore limits the amount of functional enzyme that can be secreted from transduced cells.

It has also been used in Mucopolysaccharidosis (MPS) type I (Camassola et al, 2005) and type VII (Richard et al, 2009). In the MPS I animals, storage content was reduced and enzyme activity was elevated in the liver and spleen. For the MPS VII model, a beneficial effect on the pathology was also observed, as liver-directed gene transfer led to the correction of secondary enzymatic elevations and to the reduction of GAGs storage in peripheral tissues and brain, as well as to histological correction in many tissues.

3.2 Liposomes and nanotechnology

Liposomes are lipid particles that resemble the cell membrane. Liposome-based gene delivery was first reported by Felgner in 1987, and is still one of the major techniques for non viral gene delivery into cells (Niidome & Huang, 2002). Lipoplexes are formed by the interaction of anionic nucleic acids binding to the surface of cationic lipids, forming multilamellar lipid–nucleic acid complexes where the negatively charged nucleic acid remains trapped inside the lipid bilayer (Dass, 2004). Since its discovery, different lipid formulations have been tested and modified. For example, stealth liposomes sterically stabilized with methoxypoly(ethylene glycol)distearoylphosphatidylethanolamine conjugates (PEG-DSPE) have long circulation half-lives following intravenous injection (Moreira et al., 2001). In addition, linear polycations such as linear, branched and dendritic vectors based on poly(ethylenimine) (PEI), poly(L-lysine) (PLL) and a range of

poly(ethylene glycol) (PEG) were also developed (Hunter, 2006). Although linear PEI shows greater *in vivo* efficiency because of a dynamic structure change of the complex under high salt concentrations as found in blood (Niidome & Huang, 2002), it also possesses greater toxicity (Morille et al., 2008).

Cationic nanoemulsions have been more recently considered as potential systems for nucleic acid delivery. The interest in these systems is justified by the fact that they are biocompatible and able to form complexes with DNA protecting it from enzymatic degradation (Nam et al., 2009). Other practical advantages include ease of production and the potential for repeated administration (Al-Dorsari and Gao, 2009). We have investigated the influence of phospholipids on the properties of cationic nanoemulsions/pDNA complexes. Complexes containing the phospholipids DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and DSPE (1,2- distearoyl-sn-glycero-3- phosphoethanolamine) were less toxic in comparison with the formulations obtained with lecithin, DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine). In addition, higher amounts of reporter DNA were detected for the formulation obtained with the DSPC phospholipid (Fraga et al., in press).

These cationic macromolecules can readily condense DNA or RNA into stable nanostructures for use in gene delivery (Hunter, 2006) but also for other nanotechnology-based approaches useful for the treatment of LSDs (Muro, 2010). Nanomaterials, as a result of their small size and their large surface area offer great promise for neuro-therapeutics (Ragnail et al., 2011) and thus may be a valid option for a large number of LSDs that affect the CNS.

Liposome-mediated gene transfer for LSD has been performed *in vitro* using patient's fibroblasts as target cells. Estruch et al. (2001) delivered therapeutic genes by integrin-mediated uptake into fibroblasts from patients with Fucosidosis and Fabry disease. The vectors consisted of a complex of lipofectin and a peptide containing an integrin-targeting domain and a poly-lysine domain to which plasmid DNA was bound. Transfected cells produced the corresponding enzyme at levels which were 10-40% of the total activity in cultures of normal fibroblasts. Although 95-98% of this activity was secreted, it did not appear to affect the viability of the cells. Our group used Lipofectamine to transduce fibroblasts from GM1 Gangliosidosis patients with the beta-galactosidase gene. Treated cells showed 33 to 100- fold increases in enzyme activity compared to untreated fibroblasts. However, after seven days enzyme activity was back to uncorrected values (Balestrin et al., 2008). When Geneticin was added to the medium (figure 2), stable expression at therapeutic levels was observed (mean 300 nmoles/h/mg prot) for 30 days, although at values lower than the normal range (mean 1,300 nmoles/h/mg prot).

In vivo, PEG-coated liposomes have been modified with monoclonal antibodies in order to reach the CNS. A liposome is coated with peptidomimetic monoclonal antibodies that undergo receptor-mediated transcytosis across the blood-brain barrier on the endogenous peptide receptor transporters (Pardridge, 2007). These Trojan horses (figure 3) may use the insulin or transferrin receptor, and since the MAb binding site is different from the binding site of the endogenous ligand, there is no interference of endogenous ligand transport (Skarlatos et al, 1995).

This approach has been used to deliver a non-viral plasmid DNA to the brain across the blood-brain-barrier after intravenous administration of liposomes coated with monoclonal antibody to the mouse transferrin receptor in a mouse model of Mucopolysaccharidosis type VII (Zhang et al, 2008). The enzyme activity was increased greater than ten-fold in brain, liver, spleen, lung, and kidney, but not in heart. A similar strategy has been used by Osborn

et al (2008) for Mucopolysaccharidosis type I, although they used a plasmid bearing a fusion gene consisting of Transferrin (Tf) and α-L-iduronidase. The fusion product consisted of an enzymatically active protein that was transported into the CNS by TfR-mediated endocytosis. Short-term treatment resulted in a decrease in GAGs in the cerebellum of Mucopolysaccharidosis type I mice.

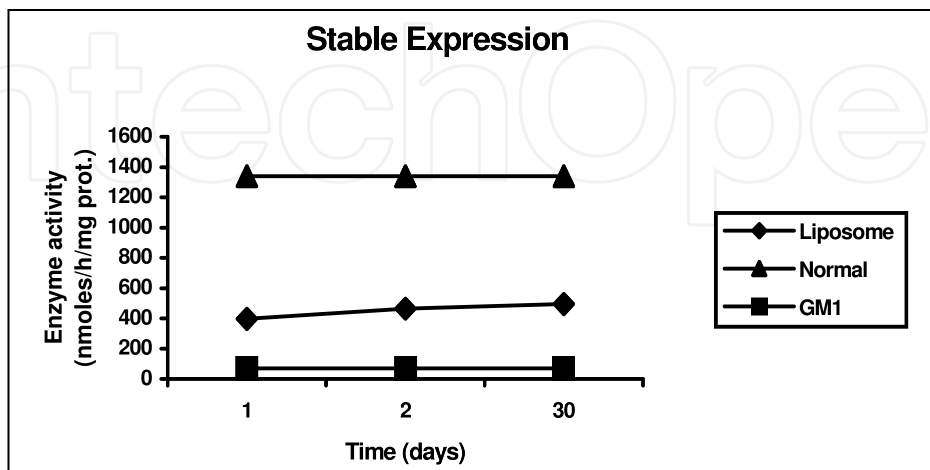


Fig. 2. Stable expression of β-Galactosidase in fibroblasts from G1 Gangliosidosis patients after *in vitro* liposome-based gene transfer. Mean values of liposome-treated cells: 300 nmoles/h/mg prot.; mean values of normal fibroblasts: 1,300 nmoles/h/mg prot.; mean values of affected fibroblasts: 68 nmoles/h/mg prot. (Balestrin et al., 2008).

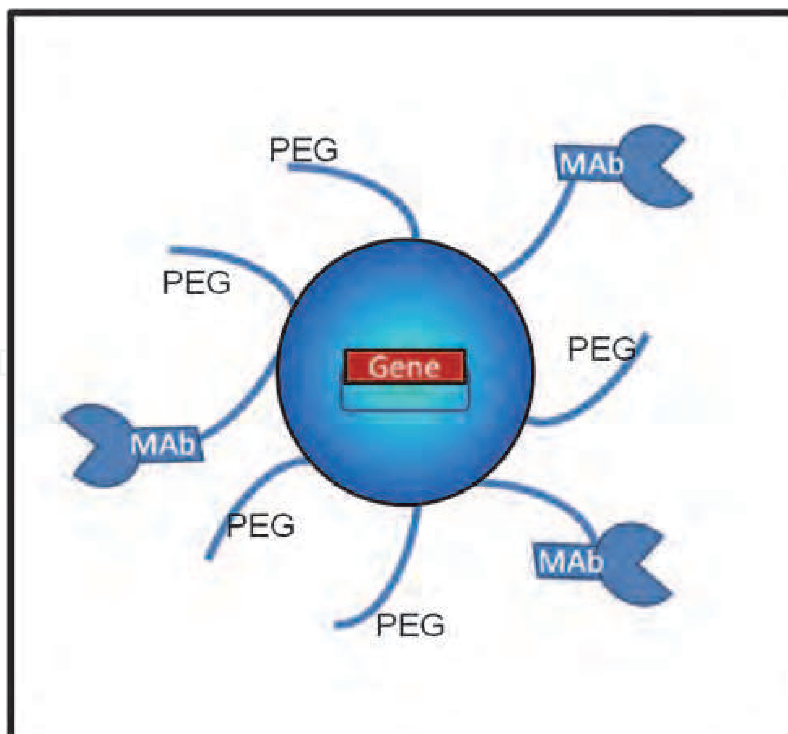


Fig. 3. Schematic view of a Trojan horse liposome. A stealth PEGylated liposome is complexed with monoclonal antibodies (MAb) that undergo receptor-mediated transcytosis across the blood-brain barrier.

3.3 Cell microencapsulation

Cell microencapsulation is an approach in which cells are trapped in a semipermeable membrane, allowing the exchange of metabolites and nutrients between them and the external environment. The membrane prevents the access of the immune system to the cells, without the need for continued immunosuppression of the host (Uludag et al., 2000). Furthermore, this technique allows the localized and controlled release, and long term duration of therapeutic products derived from the microencapsulated cells (Orive et al., 2002). Microencapsulation has become an important system for cellular preservation (Mayer et al., 2010) and a potential strategy for the controlled delivery of therapeutic products (Orive et al., 2003). Alginate has been the most important encapsulation polymer due to its abundance, easy gelling properties and biocompatibility. Agarose, chitosan, and hyaluronic acid are other polymers used for microencapsulation (Orive et al., 2003).

Cell microencapsulation presents the potential to deliver the therapeutic product of interest directly to the Central Nervous System (CNS). This has been achieved by different groups for brain tumors (Kuijlen et al 2007) and neurodegenerative diseases (Spuch et al 2010). A phase I clinical trial was conducted in Huntington's patients without signs of toxicity (Bloch et al 2004). This approach delivers the gene of interest in the spinal fluid, similar to the intrathecal enzyme replacement therapy. Thus, cell encapsulation can be suitable for the treatment of LSD, once the deficient enzyme could be released for long term directly in the CNS (Matte et al., 2011).

In order to obtain larger amounts of secreted enzyme, the encapsulated cells should be genetically modified to over-express the enzyme of interest. This enzyme would then be released to the extracapsular space (Bressel et al 2008) and uptaken by adjacent deficient cells (figure 4). This strategy has been used experimentally for different LSD, especially the Mucopolysaccharidosis (MPS).

Three *in vitro* studies were performed in LSDs other than the MPS, one in Fabry disease and the other two in Metachromatic Leukodystrophy (MLD). Naganawa et al (2002) co-cultured fibroblasts from patients with Fabry disease with microencapsulated recombinant Chinese Hamster Ovary cells (CHO) over-expressing alpha-galactosidase. The deficient cells were able to uptake the enzyme decreasing their levels of globotriaosylceramide storage. A similar approach was used by our group to test the ability of Baby Hamster Kidney (BHK) cells over-expressing ARSA to correct the deficiency of this enzyme in human skin fibroblasts from MLD patients. Fibroblasts co-cultured with the encapsulated cells for four weeks showed levels of enzyme activity higher than normal. Transmission electron microscopy showed evidence of normalization of the lysosomal ultra structure, suggesting that the secreted enzyme was able to degrade the substrate (Lagranha et al., 2008). Consiglio et al (2007) collected the conditioned media of C2C12 cells over-expressing ARSA encapsulated in polyether-sulfone polymer and used it to treat oligodendrocytes from MLD mice. The deficient cells internalized the enzyme and it was normally sorted to the lysosomal compartment, reaching 80% of physiological levels and restoring sulfatide metabolism.

Both *in vitro* and *in vivo* studies have been performed in the MPS types I, II and VII. For MPS II two studies were performed. The first was a proof of principle in which Hunter primary fibroblasts were co-cultured with alginate microcapsules containing C2C12 cell clones over-expressing IDS. After 5 days of co-culture this strategy was able to increase IDS activity inside the deficient fibroblasts to levels similar to normal (Tomanin et al 2002). The second study was a pre-clinical experiment in which APA (alginate-poli-L-lysine-alginate) microcapsules containing 1.5 x 10^6 allogeneic C2C12 myoblasts over-expressing IDS were

implanted in the peritoneum of the MPS II mouse model. An increase in IDS activity in plasma was observed, along with a reduction on urinary GAG between the fourth and the sixth week of treatment. After 8 weeks, a reduction of 30% in the amount of GAG accumulated in the liver and 38% in the kidney were shown (Friso et al 2005).

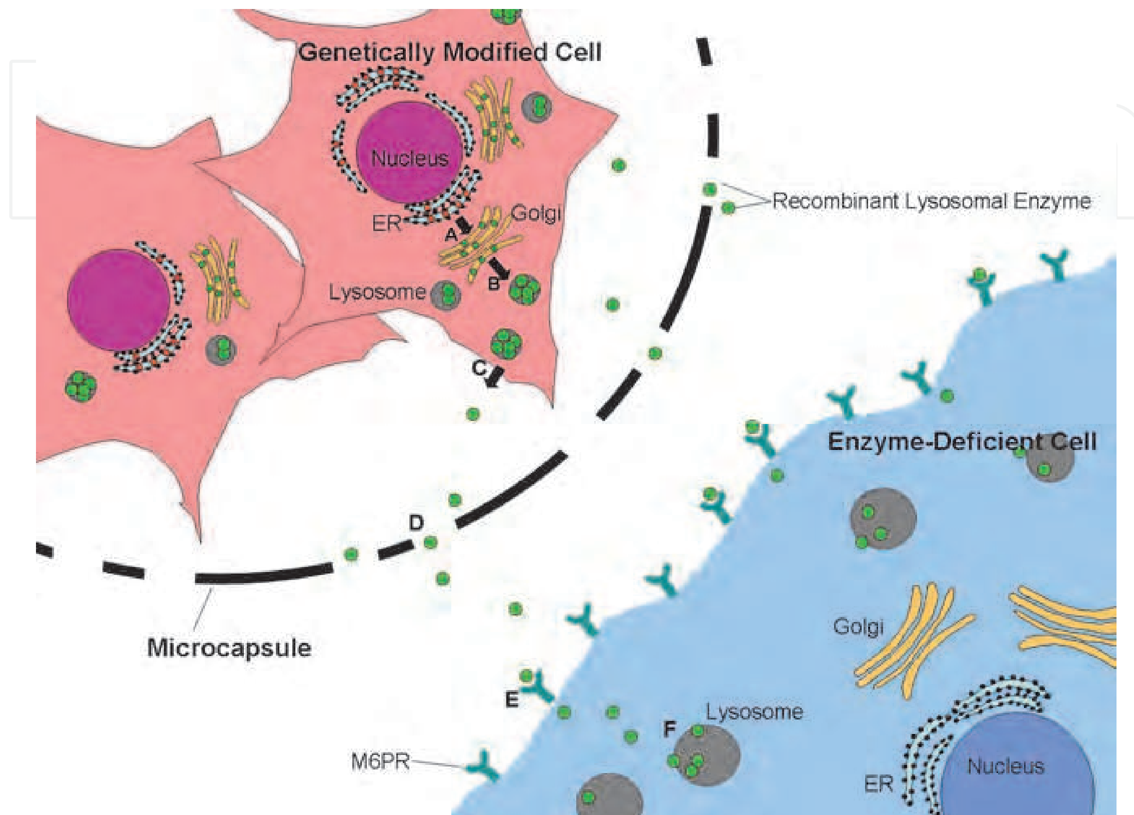


Fig. 4. Traffic of lysosomal enzymes throughout the encapsulated cells. The nascent lysosomal enzymes are glycosylated in the endoplasmic reticulum (ER) of the genetically modified cells. (A) The enzymes are phosphorylated at the residue of mannose-6 in the Golgi apparatus. (B) Most enzymes are transported to mature lysosomes. (C) Some, however, are secreted to the extracellular environment and (D) to outside of the microcapsules. (E) Phosphorylated enzymes bind to mannose-6-phosphate receptors (M6PR) of the enzyme-deficient cells (F) where they are endocyted and subsequently targeted to the lysosomes (Matte et al., 2011).

To evaluate the usefulness of this technique to treat MPS VII, Ross et al (2000a) injected APA encapsulated non-autologous cells overexpressing Gusb in the peritoneum of MPS VII mice. The results showed the presence of Gusb in the plasma 24 hours after implantation, reaching 66% of physiological levels by 2 weeks post implantation. Activity of Gusb was also detected in liver and spleen for the duration of the 8-week experiment. Accumulation of GAG was significantly reduced in liver and spleen sections and urinary GAG content reached normal levels. In another study, enzyme released by APA encapsulated 2A50 fibroblasts implanted directly into the lateral ventricles of the brain of MPS VII mice was delivered throughout most of the CNS, reversing the histological pathology (Ross et al., 2000b). *In vitro* studies were performed by Nakama et al (2006) who encapsulated immortalized recombinant human amniotic epithelial cells with MPS VII human and mouse fibroblasts and high GUSB

activity was detected in the medium. Addition of mannose-6-phosphate led to decreased enzyme activity, suggesting that enzyme uptake was mediated by mannose-6-phosphate receptor.

Our group has shown that the correction of MPS I fibroblasts by recombinant encapsulated BHK cells is also mediated by mannose-6-phosphate receptor. The effect of the ratio fibroblasts:encapsulated cells was also analysed (figure 5). The amount of enzyme uptaken by the fibroblasts is essentially the same under the different ratios (5:1; 1:1; 1:5) although the enzyme activity in the medium increases, as more enzyme is released.

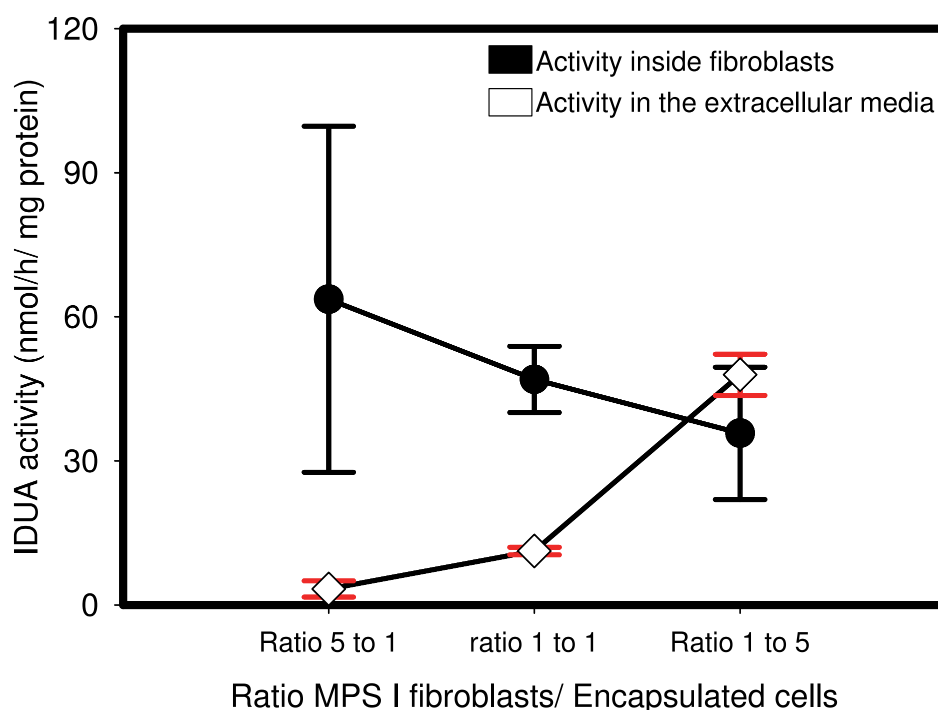


Fig. 5. IDUA enzyme activity in MPS I fibroblasts co-cultured with recombinant BHK cells overexpressing IDUA at different ratios.

Our results also showed an increase in IDUA activity in MPS I fibroblasts after 15, 30 and 45 days of co-culture with the capsules. Cytological analysis showed a marked reduction in GAG storage within MPS I cells (Baldo et al., 2011). Ongoing experiments are under way in the MPS I mouse model. The capsules were implanted in the peritoneum (figure 6) and animals were sacrificed at 4 months later. Histological analysis showed a reduction on GAG storage although plasma and tissue enzyme activity levels were not increased.

These results are quite different from those of Barsoum et al (2003) who implanted genetically modified Madin-Darby canine kidney cells (MDCK) over-expressing canine Idua in the brain parenchyma of one MPS I dog. Enzyme in plasma and cerebrospinal fluid was low but detectable for 21 days. Immunohistochemistry with anti-IDUA antibody showed the presence of the enzyme in various brain regions, however an extensive inflammatory reaction was noted, both at the sites of implantation and in the immediate vicinity. This may be the reason why histological correction of lysosomal inclusions has not been observed.

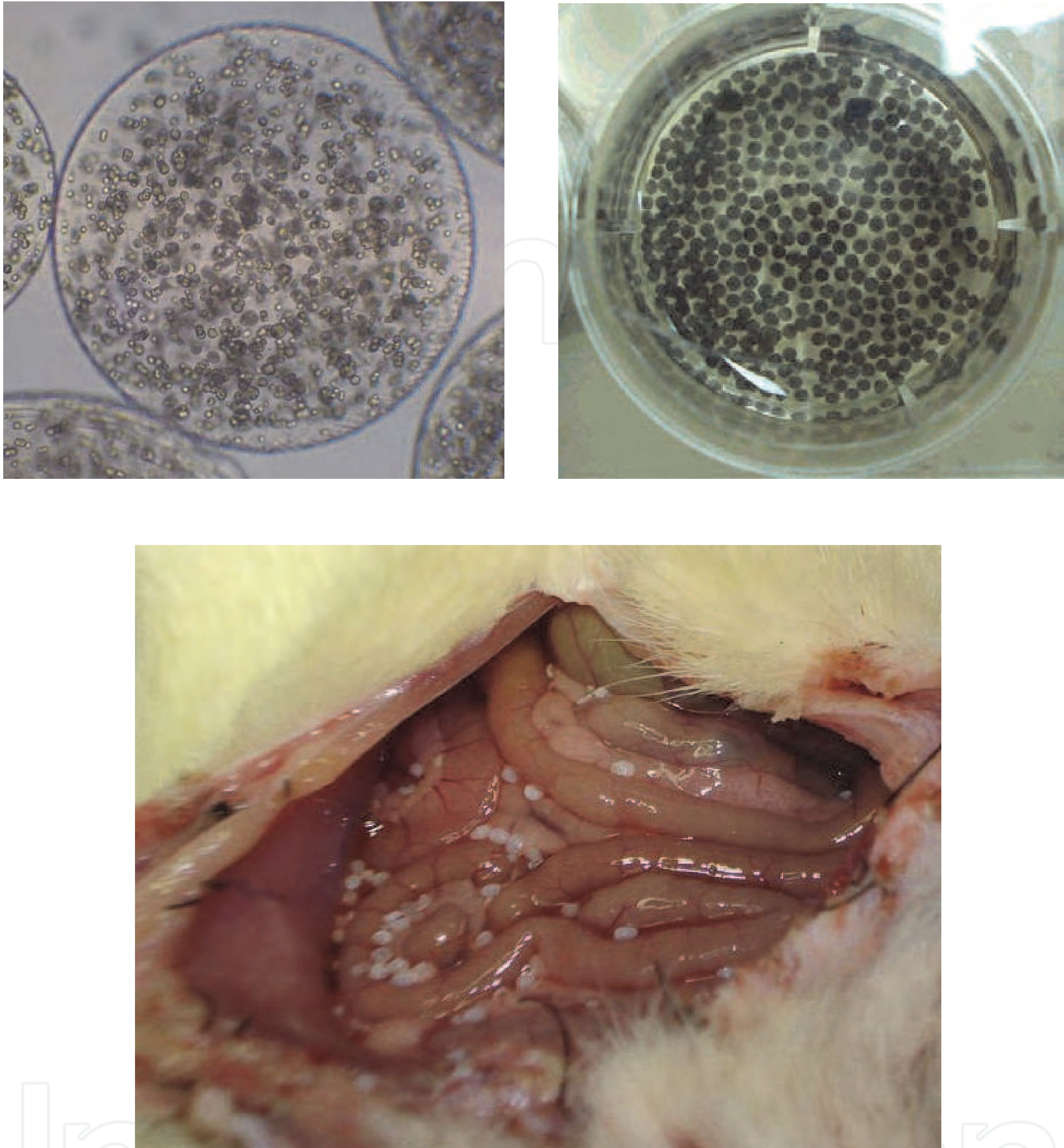


Fig. 6. Microcapsules used for the treatment of MPS I mice. Left upper panel: photomicrography of alginate beads containing recombinant BHK that overexpress IDUA (small round dots). Right upper panel: Macroscopic aspect of the microcapsules (black dots) in a 96-well plate. Capsules were ink stained to help visualization. Lower panel: Aspect of the capsules in the peritoneum ten days after implantation. Note that some capsules are attached to the intestine.

3.4 Transposon-based systems

For human gene therapy we can enumerate some important aspects of transposon systems (special emphasis in this chapter will be given to the *sleeping beauty* transposon) that make them appealing as a vector: (1) the integrated gene has stable expression, providing long-lasting expression of a therapeutic gene, which, as already mentioned, is essential for

lysosomal storage diseases; (2) the transposase directs the integration of single copies of a DNA sequence into chromatin and (3) the system is binary (the transposon is not autonomous or able to transpose on its own) (Hacket et al, 2005).
The most studied transposon system, which has been used in pre-clinical studies for treatment of lysosomal disorders, is the *Sleeping beauty (SB)* system. Sleeping beauty transposon is a type of mobile element that belongs to the *Tc1/Mariner* class and that is able to transpose via movement of a DNA element in a simple cut-and-paste manner. For that, a precise piece of DNA is excised from one DNA molecule and moved to another site in the same or in a different DNA molecule (Plasterk, 1993). This reaction is catalyzed by the protein transposase, which can be supplied *in trans* by another plasmid for gene therapy purposes.
The SB transposon system consists of two components: (i) a transposon, made up of the gene of interest flanked by inverted repeats (IRs), and (ii) a source of transposase (figure 7). For Sleeping Beauty-mediated transposition, the transposase can recognize the ends of the IRs, excises the gene of interest from the delivered plasmid DNA, and then inserts it into another DNA site. Based on studies in about 2,000 integration events in either mouse or human genomes, transposons seem to integrate into random sites, including exons, introns and intergenic sequences (Carlson et al, 2003; Horie et al, 2003; Hacket et al, 2005). This is a potential problem, since it may lead to an event of insertional mutagenesis. A complete list of insertion positions of the SB tranposon can be found at the Mouse Transposon Insertion database at http://mouse.ccgb.umn.edu/transposon/ (Roberg-Perez et al, 2003).
The use of SB transposons as gene therapy approach in LSD is still recent. The first published work was conducted by Aronovich et al (2007) who studied the effects of intravenous hydrodynamic injection of the SB transposon into mice with Mucopolysaccharidosis types I or VII. Without immunomodulation, initial enzyme activities in plasma reached levels higher than 100-fold that of wild-type (WT). However, both GUSB (MPS VII) and IDUA (MPS I) levels fell to background within 4 weeks post-injection.
A second group of animals was performed with immunomodulation only in MPS I mice. Plasma IDUA persisted for over 3 months at up to 100-fold WT activity in one-third of the mice, which was sufficient to reverse lysosomal pathology in the liver and, partially, in distant organs. Histological and immunohistochemical examination of liver sections in IDUA transposon-treated WT mice revealed inflammation 10 days post-injection consisting predominantly of mononuclear cells, which can be seen as a potential side-effect.
A posterior study by the same group (Aronovich et al, 2009) was performed in another MPS I strain, which is immunodeficient (NOD/SCID mice). Using the same approach from the previous experiment, they were able to show a persistent elevation (100-fold normal) in the plasma IDUA levels for 18 weeks. Also, IDUA activity was present in all organs analyzed, including the brain. The SB transposon system proved efficacious in correcting several clinical manifestations of MPS I mice, including bone abnormalities, hepatomegaly, and accumulation of foamy macrophages in bone marrow and synovium. In 2008 the first human clinical trial using the SB transposon was approved in the USA for treatment of cancer (Williams, 2008), however although promising no clinical trials have been conducted in LSDs so far.

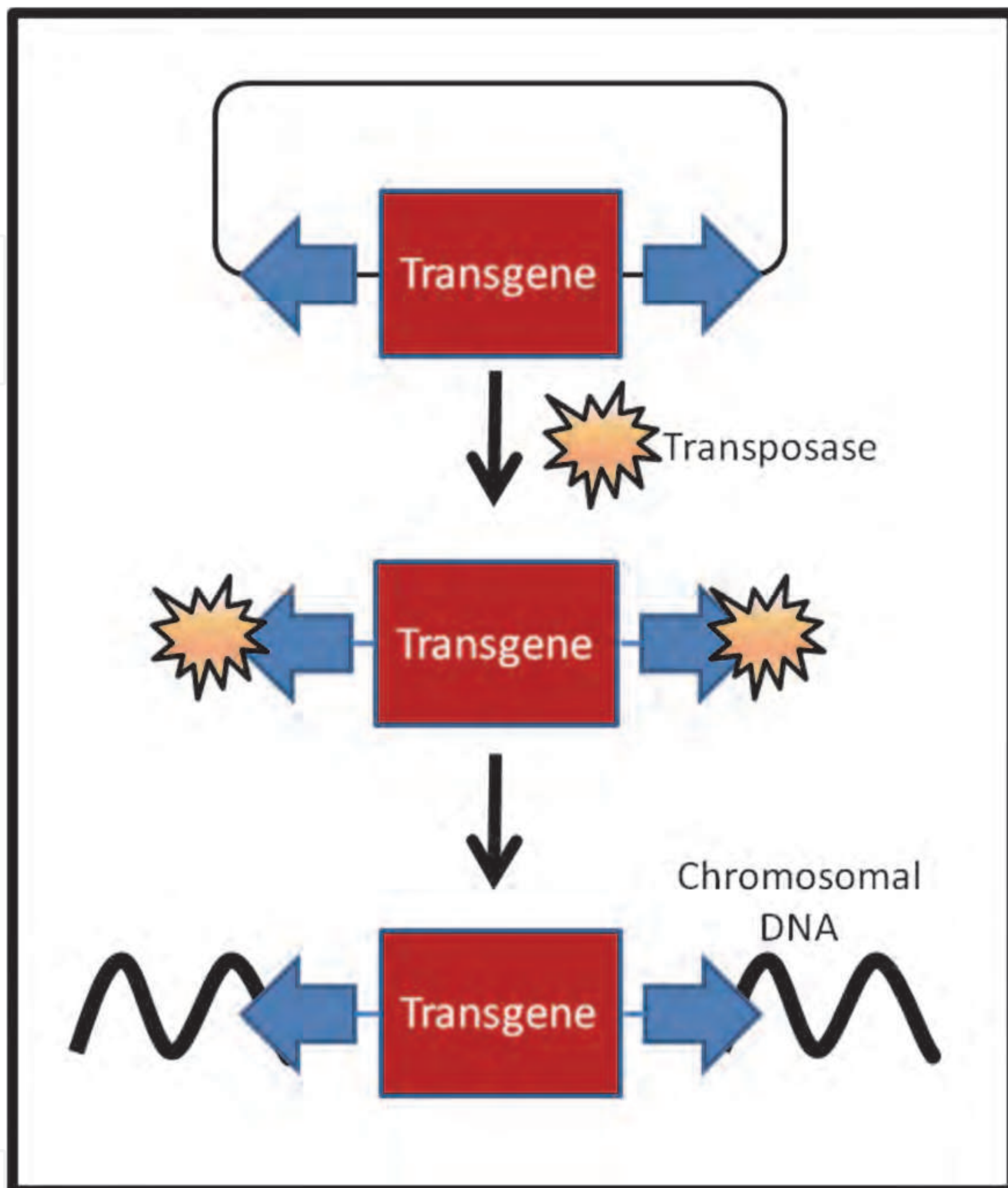


Fig. 7. SB transposon system. This simplified version of the SB transposon system shows the cut-and-paste system used by the SB transposase to insert the DNA into the host genome. The gene of interest is flanked by two inverted repeats regions (IRs, arrows) which will be recognized by the transposase (usually given in *trans* in a second plasmid) and allow the transgene to be inserted into the host genome. This way only the transgene will have prolonged expression, as transposase gene expression is transient. The SB transposon is a non-viral method of gene delivery that allows integration of the transgene in the host cell.

3.5 Minicircle gene therapy

Sustained *in vivo* transgene expression from plasmids can be difficult to achieve due to gene silencing. The mechanism by which this process occurs was postulated to be due to the deposition of repressive heterochromatin on the noncoding bacterial backbone sequences required for plasmid bacterial preparation and propagation (Chen et al, 2008; Riu et al, 2005).

Based on those findings, a new technology has emerged, known as the minicircle (MC) gene therapy. This system uses a phiC31 integrase recombination event to remove the bacterial backbone elements of the plasmid resulting in a DNA circle (the MC), encoding the mammalian expression cassette of choice and a small attR footprint (Chen et al, 2003). This has proven to be resistant to gene silencing *in vivo*, is maintained as an extrachromosomal episome, and therefore represents an interesting platform for gene replacement strategies for lysosomal storage disorders (figure 8).
This technology was recently used for treatment of MPS I mice in a proof-of-concept study (Osborn et al, 2011). In this study, the researchers performed a hydrodynamic injection of a minicircle plasmid containing the IDUA gene combined with immunomodulation, achieving stable expression of the transgene, increased IDUA tissue levels and reduction in GAG storage. As a recent technology, this is the only study performed on LSDs so far, but results are encouraging and should be tested on other diseases soon.

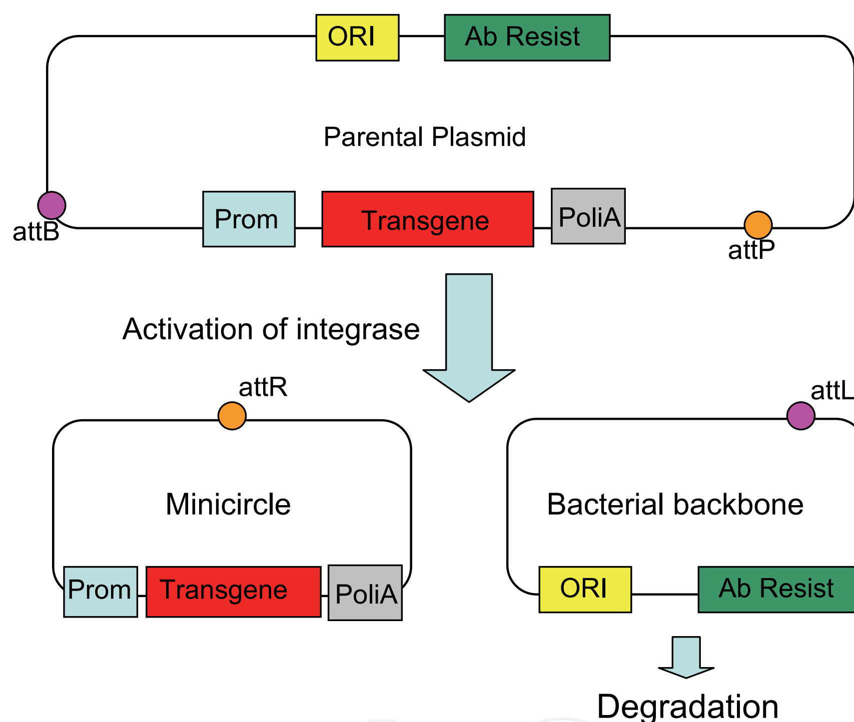


Fig. 8. Production of a minicircle plasmid. This simplified version of the process shows the parental plasmid containing both the gene of interest and the bacterial components, including origin of replication (ORI), genes that confer resistance to antibiotic (Ab resist) and sites that allow attachment of the integrase (att B and P). After activation of the integrase, a cis-recombination event occurs, separating the gene of interest and its regulatory elements from the bacterial backbone, which then is degraded.

4. Conclusions

Still nowadays most lysosomal storage disorders do not have an effective treatment. Moreover, treatments currently available are not able to correct all the manifestations of these multisystemic diseases. Despite the small number of protocols (if compared to other areas, like oncology), gene therapy approaches have shown their potential to be helpful in many of these diseases. Not only proof of concept experiments have been performed, but clinically relevant results were obtained in some cases.

The limitations of non-viral gene transfer, i.e., transient expression of the transgene and low transfection efficiency, are being slowly overcome in the last decade using improved vector design and techniques, such as nanotechnology, transposons, and minicircle approaches (to name a few) as demonstrated throughout this chapter. Novel mechanisms to help the DNA to escape endosomal degradation and pass through the nuclear envelope are also under development but were not in the scope of this chapter. Nevertheless, these improvements help non-viral gene therapy to move towards clinical trials in LSDs, which are expected to happen in the years to come. Non-viral vectors are safer than viral particles, which make them an appealing alternative for treatment of lysosomal storage disorders and even other monogenic diseases. Yet, there is a long way to clinical application but the road is paved and the scientific community advances steadily.

5. Acknowledgements

The authors are thankful to Conselho Nacional de Desenvolvimento Cientifico- CNPq and Fundacao de Amparo a Pesquisa do Rio Grande do Sul- FAPERGS.
We also would like to thank our colleagues Fabiana Quoos Mayer, Carlos Oscar Kieling, Valeska Lizzi Lagranha and Raquel Balestrin for contributing with data to this paper.

6. References

Al-Dosari, M.S., Gao X. (2009). Nonviral gene delivery: principle, limitations, and recent progress. *AAPS J*, Vol 11, No 4, pp 671-681.

Anson, D.S., Bielicki, J., Hopwood, J.J. (1992). Correction of mucopolysaccharidosis type I fibroblasts by retroviral-mediated transfer of the human alpha-L-iduronidase gene. *Hum Gene Ther*, Vol 3, No 4, pp 371-379.

Aronovich, E.L., Bell, J.B., Belur, L.R., Gunther, R., Koniar, B., Erickson, D.C., Schachern, P.A., Matise, I., McIvor, R.S., Whitley, C.B., Hackett, P.B. (2007) Prolonged expression of a lysosomal enzyme in mouse liver after Sleeping Beauty transposon-mediated gene delivery: implications for non-viral gene therapy of mucopolysaccharidoses. *J Gene Med*, Vol 9, No 5, pp 403-415.

Aronovich EL, Bell JB, Khan SA, Belur LR, Gunther R, Koniar B, Schachern PA, Parker JB, Carlson CS, Whitley CB, McIvor RS, Gupta P, Hackett PB. (2009) Systemic correction of storage disease in MPS I NOD/SCID mice using the sleeping beauty transposon system. *Mol Ther.*Vol 17, No 7, pp 1136-1144.

Baiotto, C., Sperb, F., Matte ,U., Silva, C.D., Sano, R., Coelho, J.C., Giugliani, R. (2011) Population analysis of the GLB1 gene in south Brazil. *Gen Mol Biol*, Vol 34, No 1, pp 45-48.

Baldo, G., Quoos Mayer, F., Burin, M., Carrillo-Farga, J., Matte, U., Giugliani, R. (2011) Recombinant Encapsulated Cells Overexpressing Alpha-L-Iduronidase Correct Enzyme Deficiency in Human Mucopolysaccharidosis Type I Cells. *Cells Tissues Organs*, epub.

Balestrin, R.C., Baldo, G., Vieira, M.B., Sano, R., Coelho, J.C., Giugliani, R., Matte, U. (2008) Transient high-level expression of beta-galactosidase after transfection of fibroblasts from GM1 gangliosidosis patients with plasmid DNA. *Braz J Med Biol Res,* Vol 41, No 4, PP 283-288.

Barsoum, S.C., Milgram, W., Mackay, W., Coblentz, C., Delaney, K.H., Kwiecien, J.M., Kruth, S.A., Chang P.L. (2003) Delivery of recombinant gene product to canine brain with the use of microencapsulation. *J Lab Clin Med,* Vol 142, No 6, pp 399-413.

Bellettato, C.M., Scarpa, M. (2010) Pathophysiology of neuropathic lysosomal storage disorders. *J Inherit Metab Dis,* Vol 33, No4, PP 347-362.

Bloch, J., Bachoud-Lévi, A.C., Déglon, N., Lefaucheur, J.P., Winkel, L., Palfi, S., Nguyen, J.P., Bourdet, C., Gaura, V., Remy, P., Brugières, P., Boisse, M.F., Baudic, S., Cesaro, P., Hantraye, P., Aebischer, P., Peschanski, M. (2004). Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. *Hum Gene Ther*, Vol 15, No 10, pp 968-975.

Bressel, T., Paz, A.H., Baldo, G., Lima, E.O.C., Matte, U., Saraiva-Pereira, M. (2008) An effective device for generating alginate microcapsules. *Genet. Mol. Biol*, Vol 31, No 1, pp 136-140.

Brunetti-Pierri, N., Stapleton, G.E., Palmer, D.J., Zuo, Y., Mane, V.P., Finegold, M.J., Beaudet, A.L., Leland, M.M., Mullins, C.E., Ng, P. (2007) Pseudo-hydrodynamic delivery of helper-dependent adenoviral vectors into non-human primates for liver-directed gene therapy. *Mol Ther*, Vol 15, No4, pp 732- 740.

Camassola, M., Braga, L.M., Delgado-Cañedo, A., Dalberto, T.P., Matte, U., Burin, M., Giugliani, R., Nardi, N.B. (2005) Nonviral in vivo gene transfer in the mucopolysaccharidosis I murine model. *J Inherit Metab Dis*, Vol 28, No 6, pp 1035-1043.

Carlson, C.M., Dupuy, A.J., Fritz, S., Roberg-Perez, K.J., Fletcher, C.F., Largaespada, D.A. (2003) Transposon mutagenesis of the mouse germline. *Genetics* Vol 165, No 1 pp 243–256.

Chen, Z.Y., He, C.Y., Ehrhardt, A. and Kay, M.A. (2003) Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. *Mol Ther,* Vol 8, No 3, pp 495–500.

Chen, Z.Y., Riu, E., He, C.Y., Xu, H. and Kay, M.A. (2008) Silencing of episomal transgene expression in liver by plasmid bacterial backbone DNA is independent of CpG methylation. *Mol Ther,* Vol 16, No 3, pp 548–556.

Consiglio, A., Martino, S., Dolcetta, D. (2007) Metabolic correction in oligodendrocytes derived from metachromatic leukodystrophy mouse model by using encapsulated recombinant myoblasts. *J Neurol Sci*, Vol 255, No 1-2, pp 7-16.

Dass, C.R.(2004) Lipoplex-mediated delivery of nucleic acids: factors affecting in vivo transfection. *J Mol Med*, Vol 82, No 9, pp 579–591.

de Duve, C. (1964) From cytases to lysosomes. *Fed Proc*, Vol 23, pp 1045–1049.

Dunbar, C.E., Kohn, D.B., Schiffmann, R., Barton, N.W., Nolta, J.A., Esplin, J.A., Pensiero, M., Long, Z., Lockey, C., Emmons, R.V., Csik, S., Leitman, S., Krebs, C.B., Carter, C., Brady, R.O., Karlsson, S. (1998) Retroviral transfer of the glucocerebrosidase gene into CD34+ cells from patients with Gaucher disease: in vivo detection of transduced cells without myeloablation. *Hum Gene Ther*, Vol 9, No 17, pp 2629-2640.

Ellinwood, N.M., Vite, C.H., Haskins, M.E. (2004) Gene therapy for lysosomal storage diseases: the lessons and promise of animal models. *J Gene Med*. Vol 6, No 5, pp 481-506.

Estruch, E.J., Hart, S.L., Kinnon, C., Winchester, B.G. (2001) Non-viral, integrin-mediated gene transfer into fibroblasts from patients with lysosomal storage diseases. *J Gene Med*, Vol 3, No 5, pp 488-497.

Fischer, H.D., Gonzalez-Noriega, A., Sly, W.S., Morre, D.J. (1980) Phosphomannosyl-enzyme receptors in rat liver. Subcellular distribution and role in intracellular transport of lysosomal enzymes. J Biol Chem. Vol 255, No 20, pp 9608 – 15.

Fraga, M., Laux, M., Zandoná, B., Santos, G.R., Giuberti, C.S., Oliveira, M.C., Matte, U., Teixeira, H.F. (2008) Optimization of stearylamine-based nanoemulsions obtained by spontaneous emulsification process as nucleic acids delivery systems. *J Drug Del Sci Tech* Vol 18, No 6, pp 398-403.

Fraga, M., Bruxel, F., Lagranha, V., Teixeira, H.F., Matte, U. Influence of phospholipid composition on DNA/cationic emulsions complexes: Physicochemical properties, cytotoxicity and transfection on Hep G2 cells. *International Journal of Nanomedicine*, *in press*.

Fratantoni, J.C., Hall, C.W., Neufeld, E.F. (1968) Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science*, Vol 162, No 853, pp 570–572.

Friso, A., Tomanin, R., Alba, S. (2005) Reduction of GAG storage in MPS II mouse model following implantation of encapsulated recombinant myoblasts. *J Gene Med*, Vol 7, No 11, pp 1482-1491.

Fuller, M., Meikle, P.J., Hopwood, J.J. (2006) Epidemiology of lysosomal storage diseases: an overview. In *Fabry Disease: Perspectives from 5 Years of FOS*. Mehta A, Beck M, Sunder-Plassmann G, editors. Oxford: Oxford PharmaGenesis.

Glover D, Lipps H, Jans D (2005) Towards safe, non-viral therapeutic gene expression in humans. *Nature Reviews Genetics,* Vol 6, No 4, 299-310.

Hacein-Bey-Abina, S., Garrigue, A., Wang, G.P., Soulier, J., Lim, A., Morillon, E., Clappier, E., Caccavelli, L., Delabesse, E., Beldjord, K., Asnafi, V., MacIntyre, E., Dal Cortivo, L., Radford, I., Brousse, N., Sigaux, F., Moshous, D., Hauer, J., Borkhardt, A., Belohradsky, B.H., Wintergerst, U., Velez, M.C., Leiva, L., Sorensen, R., Wulffraat, N., Blanche, S., Bushman, F.D., Fischer, A., Cavazzana-Calvo, M. (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest*, Vol 118, No 9, pp 3132-3142.

Hackett, P.B., Ekker, S.C., Largaespada, D.A., McIvor, R.S. (2005) Sleeping beauty transposon-mediated gene therapy for prolonged expression. *Adv Genet*, Vol 54, pp 189-232.

Horie, K., Yusa, K., Yae, K., Odajima, J., Fischer, S. E., Keng, V. W., Hayakawa, T., Mizuno, S., Kondoh, G., Ijiri, T., Matsuda, Y., Plasterk, R. H., and Takeda, J. (2003) Characterization of Sleeping Beauty transposition and its application to genetic screening in mice. *Mol. Cell. Biol*, Vol 23, No 24, pp 9189–9207.

Hunter, C. (2006) Molecular hurdles in polyfectin design and mechanistic background to polycation induced cytotoxicity. *Advanced Drug Delivery Reviews,* Vol 58, pp 1523–1531.

Kamimura, K., Zhang, G., Liu, D. (2009) Image-guided, intravascular hydrodynamic gene delivery to skeletal muscle in pigs. *Mol Ther,* Vol 18, No 1, pp 93-100.

Kuijlen, J.M., de Haan, B.J., Helfrich, W., de Boer, J.F., Samplonius, D., Mooij, J.J., de Vos, P. (2006) The efficacy of alginate encapsulated CHO-K1 single chain-TRAIL producer cells in the treatment of brain tumors. *J Neurooncol* Vol 78, No 1, pp 31-39.

Lagranha, V.L., Baldo, G., de Carvalho, T.G. Burin M, Saraiva-Pereira, M.L., Matte, U., Giugliani, R. (2008) In vitro correction of ARSA deficiency in human skin fibroblasts from metachromatic leukodystrophy patients after treatment with microencapsulated recombinant cells. *Metab Brain Dis* Vol 23, No 4, pp 469-84.

Liu, F., Song, Y., Liu, D. (1999) Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Ther*, Vol 6, No 7, pp 1258-1266.

Malatack, J.J., Consolini, D.M., Bayever, E. (2003) The status of hematopoietic stem cell transplantation in lysosomal storage disease. *Pediatr Neurol*, Vol 29, No 5, pp 391-403.

Matte U, de Carvalho T, Mayer F, Lagranha V, Giugliani R. (2011) Cell Microencapsulation: a Potential Tool for the Treatment of Neuronopathic Lysosomal Storage Diseases. *Journal of Inherited Metabolic Diseases,* epub.

Mayer, F.Q., Baldo, G., de Carvalho, T.G., Lagranha, V.L., Giugliani, R., Matte, U. (2010) Effects of cryopreservation and hypothermic storage on cell viability and enzyme activity in

recombinant encapsulated cells overexpressing alpha-L-iduronidase. *Artif Organs*, Vol 34, No 5, pp 434-439.

Meikle, P.J., Fietz, M.J., Hopwood, J.J. (2004) Diagnosis of lysosomal storage disorders: current techniques and future directions. *Expert Rev Mol Diagn.* Vol 4, No 5, pp 677–691.

Moreira, J., Gaspar, R., Allen T. (2001) Targeting Stealth liposomes in a murine model of human small cell lung cancer. *Biochimica et Biophysica Acta,* Vol 1515, pp 167-176.

Morille, M., Passirani, C., Vonarbourg, A., Clavreul, A., Benoit, J.P. (2008) Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. *Biomaterials,* Vol, 29, No 24-25, pp 3477-3496.

Muñoz-Rojas, M.V., Horovitz, D.D., Jardim, L.B., Raymundo, M., Llerena, J.C. Jr, de Magalhães, T.S., Vieira, T.A., Costa, R., Kakkis, E., Giugliani, R. (2010) Intrathecal administration of recombinant human N-acetylgalactosamine 4-sulfatase to a MPS VI patient with pachymeningitis cervicalis. *Mol Genet Metab,* Vol 99, No 4, pp 346-350.

Munoz-Rojas, M.V., Vieira, T., Costa, R., Fagondes, S., John, A., Jardim, L.B., Vedolin, L.M., Raymundo, M., Dickson, P.I., Kakkis, E., Giugliani, R. (2008) Intrathecal enzyme replacement therapy in a patient with mucopolysaccharidosis type I and symptomatic spinal cord compression. *Am J Med Genet A*, Vol 146A, Vol 19, pp 2538-2544.

Muro, S. (2010) New biotechnological and nanomedicine strategies for treatment of lysosomal storage disorders. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, Vol 2, No 2, pp 189-204.

Naganawa, Y., Ohsugi, K., Kase, R., Date, I., Sakuraba, H., Sakuragawa, N. (2002) In vitro study of encapsulation therapy for Fabry disease using genetically engineered CHO cell line. *Cell Transplant*, Vol 11, No 4, pp 325-329.

Nakama, H, Ohsugi, K, Otsuki, T., Date, I., Kosuga, M., Okuyama, T., Sakuragawa, N. (2006) Encapsulation cell therapy for mucopolysaccharidosis type VII using genetically engineered immortalized human amniotic epithelial cells. *Tohoku J Exp Med*, Vol 209, No 1, pp 23-32.

Nam, H.Y., Park, J.H., Kim, K., Kwon, I.C., Jeong, S.Y. (2009) Lipid-based emulsion system as non-viral gene carriers. *Arch Pharm Res* Vol 32, No 5, pp 639-646.

Niidome, T. and Huang, L. (2002) Gene Therapy Progress and Prospects: Nonviral vectors. *Gene Therapy* Vol 9, No 24, pp 1647–1652.

Orive, G., Ponce, S., Hernandez, R.M., Gascon, A.R., Igartua, M., Pedraz, J.L. (2002) Biocompatibility of microcapsules for cell imobilization alaborated with different type of alginates. *Biomaterials* Vol 23, No 18, pp 3825-3831.

Orive, G., Gascon, R.A., Hernandez, R.M., Igartua, M., Pedraz, J.L. (2003) Cell microencapsulation technology for biomedical purposes: novel insights and challenges. *Trends in Pharmacological Sciences*, Vol 24, No 5, pp 207-210.

Osborn, M.J., McElmurry, R.T., Peacock, B., Tolar, J., Blazar, B.R. (2008) Targeting of the CNS in MPS-IH using a nonviral transferrin-alpha-L-iduronidase fusion gene product. *Mol Ther*, Vol 16, No 8, pp 1459-1466.

Osborn, M.J., McElmurry, R.T., Lees, C.J., Defeo, A.P., Chen, Z.Y., Kay, M.A., Naldini, L., Freeman, G., Tolar, J., Blazar, B.R. (2011) Minicircle DNA-based Gene Therapy Coupled With Immune Modulation Permits Long-term Expression of α-L-Iduronidase in Mice With Mucopolysaccharidosis Type I. *Mol Ther*, 2011 Vol 19, No 3, pp 450-460.

Pardridge, W.M. (2007) Blood-brain barrier delivery of protein and non-viral gene therapeutics with molecular Trojan horses. *J Control Release*, Vol 122, No 3, pp 345-348.

Parkinson-Lawrence, E.J., Shandala, T., Prodoehl, M., Plew, R., Borlace, G.N., Brooks, D.A. (2010) Lysosomal storage disease: revealing lysosomal function and physiology. *Physiology (Bethesda),* Vol 25, No 2, pp 102-115.

Plasterk, R.H. (1993) Molecular mechanisms of transposition and its control. *Cell*, Vol 74, No 5, pp 781–786.

Ragnaill, M.N., Brown, M., Ye, D., Bramini, M., Callanan, S., Lynch, I., Dawson, K.A. (2011) Internal benchmarking of a human blood-brain barrier cell model for screening of nanoparticle uptake and transcytosis. *Eur J Pharm Biopharm*. Vol 77, No 3, pp 360-367.

Resina, S., Prevot, P., Thierry, A.R. (2009) Physico-chemical characteristics of lipoplexes influence cell uptake mechanisms and transfection efficacy. *PLoS One* Vol 4, No 6, e6058.

Richard, M., Arfi, A., Seguin, J., Gandolphe, C., Scherman, D. (2009) Widespread biochemical correction of murine mucopolysaccharidosis type VII pathology by liver hydrodynamic plasmid delivery. *Gene Ther*, Vol 16, No 6, pp 746-756.

Riu, E., Grimm, D., Huang, Z. and Kay, M.A. (2005). Increased maintenance and persistence of transgenes by excision of expression cassettes from plasmid sequences in vivo. *Hum Gene Ther* Vol 16, No 5, pp 558–570.

Roberg-Perez, K., Carlson, C.M., Largaespada, D.A. (2003) MTID: a database of Sleeping Beauty transposon insertions in mice. *Nucleic Acids Res*, Vol 31, No 1, pp 78-81.

Ross, C.J., Bastedo, L., Maier, S.A., Sands, M.S., Chang, P.L. (2000a) Treatment of a lysosomal storage disease, mucopolysaccharidosis VII, with microencapsulated recombinant cells. *Hum Gene Ther,* Vol 11, No 15, pp 2117-2127.

Ross, C.J., Ralph, M., Chang, P.L. (2000b) Somatic gene therapy for a neurodegenerative disease using microencapsulated recombinant cells. *Exp Neurol*, Vol 166, No 2, pp 276-286.

Sifuentes, M., Doroshow, R., Hoft, R., Mason, G., Walot, I., Diament, M., Okazaki, S., Huff, K., Cox, G.F., Swiedler, S.J., Kakkis, E.D. (2007) A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. *Mol Genet Metab*, Vol 90, No 2, pp 171-180.

Skarlatos, S., Yoshikawa, T., Pardridge, W.M. (1995) Transport of [125I]transferrin through the rat blood-brain barrier. *Brain Res*, Vol 683, No 2, pp 164-171.

Sly, W.S., Fischer, H.D., Gonzalez-Noriega, A., Grubb, J.H., Natowicz, M. (1981) Role of the 6-phosphomannosyl-enzyme receptor in intracellular transport and adsorptive pinocytosis of lysosomal enzymes. *Methods Cell Biol,* Vol 23, pp 191-214.

Spuch. C., Antequera, D., Portero, A., Orive, G., Hernández, R.M., Molina, J.A., Bermejo-Pareja, F., Pedraz, J.L., Carro, E. (2010) The effect of encapsulated VEGF-secreting cells on brain amyloid load and behavioral impairment in a mouse model of Alzheimer's disease. *Biomaterials*, Vol 31, No21, pp 5608-5618.

Takakusaki, Y., Hisayasu, S., Hirai, Y., Shimada, T. (2005) Coexpression of formylglycine-generating enzyme is essential for synthesis and secretion of functional arylsulfatase A in a mouse model of metachromatic leukodystrophy. *Hum Gene Ther*, Vol 16, No 8, pp 929-936.

Tomanin, R., Friso, A., Alba, S., Piller Puicher, E., Mennuni, C., La Monica, N., Hortelano, G., Zacchello, F., Scarpa M. (2002) Non-viral transfer approaches for the gene therapy of mucopolysaccharidosis type II (Hunter syndrome). *Acta Paediatr Suppl*, Vol 91, No 439, pp 100-104.

Uludag, H., Vos, P., Tresco, P. (2000) Technology of mammalian cell encapsulation. *Adv Drug Delivery Reviews*, Vol 42, No 1-2, pp 29-64.

Vitner, E.B., Platt, F.M., Futerman, A.H. (2010) Common and uncommon pathogenic cascades in lysosomal storage diseases. *J Biol Chem*, Vol 285, No 27, pp 20423-20427.

Walkley, S.U. (2009) Pathogenic cascades in lysosomal disease-Why so complex? *J Inherit Metab Dis*, Vol 32, No 2, pp 181-189.

Wasungu, L., Hoekstra, D. (2006) Cationic lipids, lipoplexes and intracellular delivery of genes. *Journal of Controlled Release*, Vol. 116, No 2, pp 255-264.

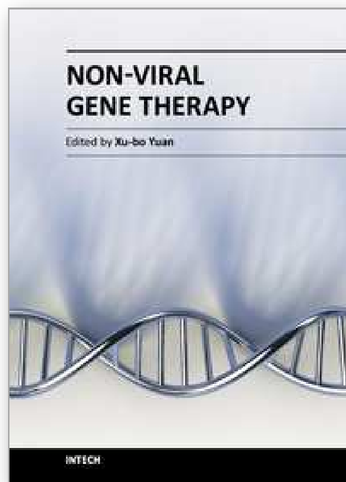
Williams, D.A. (2008). Sleeping beauty vector system moves toward human trials in the United States. *Mol Ther*, Vol 16, No 9, pp 1515-1516.

Wilson JM. (2009) Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. *Mol Genet Metab,*Vol 96, No 4, pp 151-157.

Worgall, S., Sondhi, D., Hackett, N.R., Kosofsky, B., Kekatpure, M.V., Neyzi, N., Dyke, J.P., Ballon, D., Heier, L., Greenwald, B.M., Christos, P., Mazumdar, M., Souweidane, M.M., Kaplitt, M.G., Crystal, R.G. (2008) Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther*, Vol 19, No 5, pp 463-474.

Zhang, Y., Wang, Y., Boado, R.J., Pardridge, W.M. (2007) Lysosomal enzyme replacement of the brain with intravenous non-viral gene transfer. *Pharm Res,* Vol 25, No 2, pp 400-406.

IntechOpen



Non-Viral Gene Therapy
Edited by Prof. Xubo Yuan

ISBN 978-953-307-538-9
Hard cover, 696 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

This book focuses on recent advancement of gene delivery systems research. With the multidisciplinary contribution in gene delivery, the book covers several aspects in the gene therapy development: various gene delivery systems, methods to enhance delivery, materials with modification and multifunction for the tumor or tissue targeting. This book will help molecular biologists gain a basic knowledge of gene delivery vehicles, while drug delivery scientist will better understand DNA, molecular biology, and DNA manipulation.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ursula Matte, Guilherme Baldo and Roberto Giugliani (2011). Non Viral Gene Transfer Approaches for Lysosomal Storage Disorders, Non-Viral Gene Therapy, Prof. Xubo Yuan (Ed.), ISBN: 978-953-307-538-9, InTech, Available from: http://www.intechopen.com/books/non-viral-gene-therapy/non-viral-gene-transfer-approaches-for-lysosomal-storage-disorders

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen