Niemann-Pick Type C Disease—
Pathophysiology and Future Perspectives for Treatment

Cristin D Davidson[1] and Steven U Walkley, DVM, PhD[2]

1. Graduate Student; 2. Professor, Department of Neuroscience, Pathology, and Neurology, Albert Einstein College of Medicine

Abstract

Niemann-Pick type C (NPC) disease is a rare and fatal inherited metabolic disorder that results in intracellular accumulation of cholesterol and glycolipids within the endosomal/lysosomal system. Central nervous system (CNS) involvement is especially prominent as evidenced by ataxia and progressive decline of motor skills and intellectual function. Defects in either the NPC1 or NPC2 protein, both of which are thought to be involved in the egress of cholesterol and other lipids out of the endosomal/lysosomal system, leads to NPC disease. The pathogenic cascade is not well understood, and currently there is no corrective therapy for NPC patients. However, some compounds tested in animal models of NPC disease have shown promise in slowing disease onset and progression, and administration of these drugs in combination has provided evidence of synergy and enhanced clinical benefit. This favorable outcome indicates that combinatorial therapies using small molecules likely represent the best therapeutic option available to NPC patients at this time.

Lysosomal diseases are rare metabolic disorders that result from inherited defects in the endosomal/lysosomal system and often lead to severe clinical disease and premature death. The endosomal/lysosomal system is a vital recycling center that plays a critical role in intracellular signaling and homeostatic events, including a primary role in the breakdown and salvage of most cellular components. Endosomal/lysosomal dysfunction leads to a complex disease cascade, with the most recognizable feature being the intracellular accumulation of unmetabolized products. Since the precise role of the lysosomal system is not the same in cells with different metabolic signatures, the overall pathologic features typically vary by both disease and cell type. In approximately two-thirds of lysosomal diseases there is significant central nervous system (CNS) involvement. Niemann-Pick Type C (NPC) disease is one such lysosomal disorder and is characterized by prominent intracellular accumulation of unesterified cholesterol and several glycosphingolipids (GSLs).[1,2] While many cell types are affected in NPC disease, the storage of unmetabolized products is especially severe in cells of the CNS and leads to a variety of pathologic changes and to neuronal dysfunction. Intraneuronal storage is accompanied by many other well-known pathologic changes, including the formation of meganeurites, the growth of ectopic dendrites, and the widespread occurrence of neuroaxonal dystrophy.[3] Patients with NPC disease typically present with vertical supranuclear gaze palsy (VSGP) and learning difficulties, followed by ataxia, seizures, dystonia, respiratory dysfunction, and progressive mental decline.[1,4–6] Most patients succumb to NPC disease in adolescence, although disease onset is variable and adult-onset cases of NPC disease have been reported.[7]

Niemann-Pick Type C Disease—A Brief History

The discovery of Niemann-Pick disease is attributed to Albert Niemann, a German pediatrician, and Ludwig Pick, a German pathologist. In 1914, Niemann provided a report of a child with CNS impairment and enlarged liver and spleen (hepatosplenomegaly).[8] Several years later in the 1920s, Pick analyzed tissues from children with these clinical signs and established this as a new, distinct disease. Niemann-Pick disease was subsequently separated into four different types (A, B, C, and D) by Crocker in the late 1950s based on varying clinical and biochemical data.[9] Subsequently, it was shown that Niemann-Pick types A and B (NPA and NPB) are caused by mutations in the *SMPD1* gene, which codes for acid sphingomyelinase and, when deficient, leads to intracellular accumulation of

sphingomyelin.[10] NPA and NPB are classically defined based on the onset and severity of disease. Type A typically presents in infants and is characterized by severe neurologic involvement, while type B presents later (juvenile) and has little to no neurologic involvement.[10,11] In reality, many patients lie somewhere along a continuous spectrum of the two disease types.[12] While both NPA and NPB are pan-ethnic and have an incidence of approximately 0.5–1:100,000, NPA is known to occur more frequently among individuals of Ashkenazi Jewish decent.[13]

Niemann-Pick types C and D (NPC and NPD) have recently been determined to be genetically equivalent (now collectively known as NPC disease) and distinct from types A and B. NPC disease is also pan-ethnic, with an incidence of approximately 1:150,000, and is the result of mutations in one of two genes, NPC1 or NPC2.[1] Approximately 95% of cases of NPC disease can be attributed to genetic defects in NPC1, while the remaining 5% of patients have defects in NPC2.[1] Although mutations in either of these two genes cause NPC disease, the resulting phenotypes are clinically indistinguishable.[6] NPC1 codes for a 13 transmembrane domain protein localized to late endosomes and lysosomes. While the NPC1 protein has homology to proteins involved in cholesterol homeostasis and is thought to possess a cholesterol-binding site,[14–16] evidence of direct involvement in cholesterol/lipid transport has yet to be shown in vivo. The NPC2 gene codes for a small, soluble, cholesterol-binding protein that is also found in late endosomal/lysosomal compartments.[17,18] The exact functions of NPC1 and NPC2 are not certain, although there are data supporting the concept that these two proteins act co-operatively within late endosomes and lysosomes to traffic cholesterol and other lipids from these organelles to cellular locations such as the endoplasmic reticulum and plasma membrane. Co-operativity was first suggested by the generation of double knock-out mice lacking both proteins. These mice exhibited a clinical phenotype and cellular pathology similar to that of the most severe of the single mutants, NPC1, consistent with both NPC1 and NPC2 functioning in the same metabolic pathway.[19] A second line of evidence for co-ordinated interaction comes from biochemical studies by Infante et al., in which the NPC2 protein was shown to facilitate the transfer of cholesterol between NPC1 and liposomes in vitro.[20]

Pathogenic Cascades

The pathophysiology of NPC disease is complex and the disease cascade not fully understood. On a cellular level, NPC disease results in improper trafficking and accumulation of unesterified cholesterol along with gangliosides and other glycolipids.[1] While the significance of this storage (for NPC and other lysosomal diseases) was originally thought to be its massive overabundance and thus burden on the cell, more recent studies suggest that cells may actually be deficient in specific metabolites normally derived from these storage compounds. This potential deficit may in turn lead to an increase in de novo synthesis in the affected metabolic pathways, thus ultimately exacerbating storage. For example, cholesterol synthesis is increased in the liver in NPC disease, apparently in response to lack of cholesterol egress from the late endosomal/lysosomal system.[21] For brain, studies have shown that the overall cholesterol content in the brain remains unchanged in NPC disease compared with normal brain,[22] although the cellular localization

of cholesterol is different in diseased versus normal cells.[23,24] Filipin labeling, a histochemical method commonly employed to visualize unesterified cholesterol, reveals conspicuous intracellular accumulation principally within the endosomal/lysosomal system of NPC neurons.[23] Additionally, immunohistochemical (IHC) labeling of GM2 and GM3 gangliosides yields a neuronal staining pattern similar to that seen with unesterified cholesterol. Cellular accumulation of these unmetabolized products is manifested as polymembranous cytoplasmic bodies, a common hallmark of NPC disease.[3]

Several other morphologic changes are present in many neurons of NPC patients, although the pathogenic cascades leading to these alterations are unclear. For example, the formation of meganeurites, or swellings at the axon hillock, is commonly seen in NPC disease as well as other lysosomal diseases.[3] Meganeurites often exhibit the growth of abnormal dendritic spines and neurites, a phenomenon known as ectopic dendritogenesis that is unique to lysosomal disease.[3] Axonal spheroids, or focal swellings along axons, are another abnormality frequently observed in NPC disease.[3] The impact of axonal spheroids on the normal function of neurons is not well understood, but it is likely that these focal swellings interfere with both transport of materials between the cell body and axon terminals and with propagation of action potentials.[25] Neurofibrillary tangles (NFTs), which consist of paired helical filaments and are a hallmark of Alzheimer's disease (AD) and other tauopathies, are also routinely observed in NPC disease.[3] In a few patients, even the presence of Lewy bodies has been documented.[3] Lewy bodies are protein aggregates of α-synuclein and are a pathologic hallmark of Parkinson's disease (PD) and Lewy body dementias.[26] Since there is evidence indicating that disruption of the ubiquitin-proteasome system (UPS) may be involved in altered protein degradation leading to NFTs and Lewy body formation,[27–29] the presence of these two pathologies suggests that compromise of UPS function, in addition to the well-known endosomal/lysosomal abnormalities, may be occurring in NPC disease.

Besides the above abnormalities in cell morphology, there are many other cellular consequences of the NPC defect, some of which are well known and others that are just being discovered. For instance, NPC disease is known to have a prominent neuro-inflammatory component, similar to many other neurodegenerative diseases.[30] NPC1 mice have also been shown to be deficient in the production of neurosteroids, specifically allopregnanolone, presumably as a result of altered cholesterol egress from lysosomes.[31] Recently, work on NPC disease and alpha-tocopherol (vitamin E) has revealed an accumulation of vitamin E in cultured hepatocytes and fibroblasts with a loss of NPC1 or 2 function, as well as altered levels in the liver and some CNS regions of NPC mice (Manor D, Ulatowski L, personal communication). These observations suggest the NPC proteins play a role in the transport of vitamin E that may be similar to that of cholesterol. Another recent study has reported intracellular accumulation of copper present in hepatoma cells with an induced NPC phenotype, suggesting a link between copper metabolism and the NPC1 defect.[32] Additionally, copper is known to play an important role in brain function, principally as a cofactor of metabolic enzymes, and copper-transporting ATPases are present in Purkinje cells (PCs)

and Bergmann glia of the cerebellum.[33] Alterations in copper metabolism may disrupt cellular homeostasis, potentially by leading to mitochondrial dysfunction, impaired neurotransmitter biosynthesis, and other detrimental changes[34] within PCs and other neurons found to exhibit copper accumulation.

Although many neurons are able to survive the above myriad morphological changes and cellular insults for years, neurodegeneration does occur in NPC disease, the most notable example being the patterned cell death of PCs in the cerebellum.[35] As PCs represent the sole output of the cerebellar cortex, PC dysfunction and death likely plays a key role in ataxia and other motor impairments present in NPC patients. Why PCs exhibit this selective vulnerability to neurodegeneration is unknown, but it has recently been hypothesized that the early and prominent development of neuroaxonal dystrophy affecting these neurons may be responsible by depriving them of essential growth factors for survival.[25]

Clinical Manifestations

Clinical signs and progression of NPC disease vary from patient to patient. It has generally been considered that disease progression in NPC and other lysosomal diseases correlates to age of onset, with earlier clinical onset normally leading to a more rapid progression and earlier death.[1] However, one group of researchers recently designed a clinical severity scale to analyze disease progression in NPC patients and reported a linear clinical progression regardless of age at onset.[36]

The hallmark clinical signs of NPC disease are considered to be VSGP and ataxia, although not all patients present with these two abnormalities. In fact, patient phenotypes can be loosely categorized based on age at disease onset.[1] Peri-natal-onset patients usually present with fetal ascites, neonatal jaundice, and hepatosplenomegaly. Early infantile patients may also exhibit hepatosplenomegaly in addition to hypotonia and a general delay in development of normal motor function. Late-infantile-onset generally presents with clumsiness and ataxia and occasionally organomegaly and VSGP. Juvenile patients most commonly experience behavioral problems, learning difficulties, and ataxia, as well as VSGP, dysarthria, dystonia, seizures, and cataplexy. Finally, adolescent- and adult-onset disease is characterized by ataxia, dementia, psychosis, and a general progressive neurologic decline. VSGP may or may not be present in older patients. Sleeping problems and/or narcolepsy as well as respiratory distress are other issues commonly encountered in NPC patients and pulmonary complications are commonly the cause of death.

Animal Models

Animal models have been and continue to be an invaluable resource in the quest to understand the NPC defect and resulting pathogenic cascades, as well as to test the efficacy and safety of possible treatments. There is an established feline colony of NPC1 disease, as well as three murine (BALB/cNctr-Npc1[m1N]/J, C57BLKS/J-Npc1[spm]/J, and Npc2^{-/-}) models of NPC disease. With the exception of the NPC2 murine model, which was generated by targeted disruption of NPC2 and resulted in a severe hypomorph,[19] all models arose from spontaneous mutations in the *Npc1* gene.[37–39] These animal models replicate many of the human pathologies, including the presence of polymembranous cytoplasmic bodies and prominent intraneuronal storage, axonal spheroids, meganeurites, ectopic dendritogenesis (less severe in the murine models), and PC degeneration. They also exhibit some of the classic clinical features seen in NPC patients, such as ataxia and loss of motor co-ordination. The *NPC* genes have also been identified in non-mammalian model systems, such as *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cervisiae*, indicating evolutionary conservation of the NPC proteins and providing simple systems for analysis of NPC protein function.

Therapeutic Options

A treatment for NPC disease remains elusive and the search for successful therapies has been difficult for several reasons. In most cases, NPC patients appear to be healthy at birth and undergo a seemingly normal early childhood development. For many patients, it is only years after birth that the first clinical signs are observed and diagnosis is made. This delay in clinical onset provides a window of opportunity during which therapeutic intervention prior to disease manifestation is likely to be most successful in preventing and/or delaying clinical disease. The fact that diagnosis is most often made after the onset of clinical signs means that a successful therapy needs to not only prevent further pathological changes but, ideally, reverse those changes already present. A second obstacle lies in the fact that, while cells of the CNS are most severely affected, they are also the most difficult to reach due to the protective blood–brain barrier (BBB). Therapeutic agents must bypass or cross the BBB while exerting minimal toxic effects on the patient. Finally, the exact functions of the NPC1 and NPC2 proteins remain uncertain, making it difficult to rectify a problem that is not completely understood.

Over the course of the last two decades, much time and effort was invested in the development of therapies that reach beyond simple supportive care to better the lives of NPC patients. Of these treatment strategies, some have proved ineffective or have not yet been fully developed, while others have been shown to beneficially impact NPC disease progression (see *Table 1*). One of the first therapies tested in NPC patients was the administration of cholesterol-lowering drugs and implementation of reduced cholesterol diets. This approach was expected to be beneficial as NPC disease was thought of as a storage disorder with an overabundance of cholesterol, suggesting that reduction of cholesterol intake or inhibition of cholesterol synthesis via administration of statins would provide some relief. While clinical studies did show that cholesterol-lowering agents reduced hepatic and plasma cholesterol levels, they did not appear to affect storage in the CNS or disease progression in either NPC patients or the NPC1 mouse model.[40,41] This earlier notion that NPC disease leads to cellular levels of cholesterol in excess of normal levels is contrary to more recent views in which intracellular storage is suggested to deprive cells of lysosomally-derived metabolites, such as cholesterol and simple GSLs. Based on this latter theory, statins and cholesterol-lowering diets would not be expected to benefit NPC patients.

Enzyme replacement therapy (ERT), or intravenous administration of functional enzyme to patients, is currently used to treat

Table 1: Therapeutic Options for Niemann-Pick Type C Disease

Therapy	Pathological Target	Major Benefits	Models Tested	References
HPBCD (SRT)	Cholesterol and ganglioside storage	• ~3-week delay in onset of clinical signs • ~125% (~14.5-week) increase in lifespan • Reduced cholesterol and GSL storage • Increased PC survival	NPC1 and NPC2 murine models	66
		• ~37% increase in lifespan* • Improved liver function • Increased PC survival	NPC1 murine model	67
Miglustat (SRT)	Ganglioside storage	• Delay in onset of clinical signs • ~25% (~3.1-week) increase in lifespan (murine model) • Qualitative reduction of GSL storage • Increased PC survival	NPC1 murine model, NPC1 feline model	60
		• Stabilization or improvement of clinically relevant markers of disease	NPC patients	63, 64
Curcumin	Altered calcium homeostasis	• Reduced tremors, increased activity • ~35% (~3.5-week) increase in lifespan • Reduced GSL storage	NPC1 murine model	58
Imatinib	Neurodegeneration	• ~1-week delay in onset of clinical signs • ~12% increase in lifespan • Increased PC survival	NPC1 murine model	57
Ibuprofen (NSAIDs)	Neuroinflammation	• Delayed onset of clinical signs • ~1.7-week increase in lifespan • Reduced neuroinflammation in the cerebellum	NPC1 murine model	52
Tamoxifen (antioxidant properties) vitamin E (antioxidant)	Oxidative damage	• Modest improvement on rota-rod • ~1-week increase in lifespan (sex- and treatment-dependent)	NPC1 murine model	53
Vitamin C (antioxidant)	Oxidative damage	• No benefit seen	NPC1 murine model	52
Overexpression of Rab9	Defective vesicle trafficking	• Reduced GSL storage • ~22% increase in lifespan	NPC1 murine model	51
LXR agonist T0901317	Defective sterol metabolism	• Modest improvement on rota-rod • ~11% (~1.4-week) increase in lifespan • Increased PC survival • Reduced neuroinflammation in the cerebellum	NPC1 murine model	49
BMT	Defective NPC1 protein	• Improved liver, spleen, bone marrow, and lung function • No benefit seen in CNS	NPC1 patient	44
Combination of lovastatin, cholestyramine, and nicotinic acid (statins)	Cholesterol storage	• Reduced plasma and hepatic levels of cholesterol • No benefit seen in CNS	NPC patients	40
Probucol (lowers cholesterol in bloodstream by increasing rate of LDL catabolism; may inhibit cholesterol synthesis) Nifedipine (calcium-channel blocker proposed to have a downstream effect of increasing excretion of cholesterol from cells)	Cholesterol storage	• Reduced plasma and hepatic levels of cholesterol • Very slight delay in onset of clinical signs • No benefit seen in CNS or on longevity	NPC1 murine model	41

*Estimate of percent increase calculated from graphical data provided in article. BMT= bone marrow transplantation; CNS = central nervous system; GSL = glycosphingolipid; HPBCD = 2-hydroxypropyl-beta-cylcodextrin; LDL = low-density lipoprotein; LXR = liver X receptor; NPC1 = Niemann-Pick type C1 protein; NPC2 = Niemann-Pick type C2 protein; NSAIDs = non-steroidal anti-inflammatory drugs; PC = Purkinje cell; SRT = substrate-reduction therapy.

several lysosomal diseases with enzymatic deficiencies such as mucopolysaccharidosis I and II and Pompe, Gaucher, and Fabry diseases.[42] This therapy makes use of purified or recombinantly produced functional enzymes to replace those deficient in cells and allow the catabolism of stored metabolites to take place. As lysosomal hydrolases are subject to degradation and turnover themselves, patients on ERT require repeated administration of the enzyme over their lifetime. Successful ERT requires that the enzyme is targeted to specific organs and cells and then endocytosed and trafficked to the late endosomes/lysosomes, meanwhile eliciting no significant immune response. Since approximately 95% of NPC cases are due to defects in the non-soluble, transmembrane protein NPC1, patients are unlikely to benefit from a protein replacement therapy similar to ERT. While the NPC2 protein is secreted, it has no enzyme activity; therefore, ascertaining the functionality of a recombinantly produced NPC2 protein is more difficult than a simple enzyme assay. For these reasons, an ERT-like therapy involving protein replacement is not likely to represent a successful treatment for NPC disease.

Besides ERT, bone marrow transplantation (BMT) or stem cell therapy also offers potential cell-mediated correction of some lysosomal diseases.[43] The therapeutic efficacy of BMT relies heavily on cross-correction of diseased cells by donor cells. Again, it is unlikely that NPC1 disease would be corrected since this therapy requires protein secretion and uptake. BMT was performed in a NPC1 patient who was 2.5 years of age, resulting in full engraftment that led to reduced pathology in the liver, spleen, and lung[44] (likely a reflection of a normalization of macrophages). However, there were no improvements in neurologic function, consistent with the view that BMT is unlikely to correct the NPC1 defect.[44] While BMT studies in the mouse model of NPC2 disease are ongoing (Dobrenis K, personal communication), this approach must confront the difficulties of achieving efficient cross-correction and directly affect the CNS in order to be successful.

Another potential approach to treating NPC disease is gene therapy. Like ERT and BMT, successful gene therapy requires that the gene reach targeted areas in addition to producing a sufficient quantity of gene product to impact the disease, often via cross-correction, while minimizing immune response to vector and protein product. In theory, gene therapy has the potential to correct many different lysosomal diseases, namely those deficient in soluble proteins and lysosomal enzymes.[45] Designing and implementing successful gene therapy for NPC1 is difficult as the gene must be incorporated into every cell for efficient correction of the defective membrane-bound protein to take place. On the other hand, NPC2 may be more amenable to gene therapy because as a soluble protein it may benefit from cross-correction. Studies are currently under way investigating gene therapy in NPC1 disease, with emphasis being placed on successful penetration of the BBB as well as targeting of neuronal cell populations (Dobrenis K and Lippell R, personal communication).

A different strategy, chaperone-mediated therapy, relies on the assistance of small molecules or upregulation of endogenous chaperones and related molecules to properly fold, stabilize, and/or traffic partially functioning or misfolded proteins.[46,47] Although there are no harmacologic chaperones currently used for the treatment of

NPC disease, there is evidence that some mutated NPC proteins are targeted for endoplasmic reticulum-associated degradation (ERAD) due to protein misfolding.[48] This suggests that potent small-molecule chaperones may be of benefit to patients harboring specific mutations in which the NPC protein is misfolded. Alternately, small-molecule therapy designed to correct defective metabolic pathways may also be a viable therapeutic option for NPC disease. For example, administration of the synthetic ligand T0901317, which was shown to increase liver X receptor-β (LXR-β) activity in the brain of NPC1 mice, was reported to provide modest slowing of neurodegeneration and to prolong life.[49] This small molecule was suggested to enhance net sterol excretion from the brain, as LXR-β is a ligand-activated transcription factor involved in regulation of sterol metabolism.[49] In other studies, upregulation of Rab9, a small GTPase protein involved in vesicle trafficking, demonstrated a reduction in intracellular cholesterol storage in NPC2 patient fibroblasts as well as NPC1 murine neurons.[50] *In vivo* overexpression of Rab9 in a transgenic murine model of NPC1 affected disease progression by modestly reducing ganglioside accumulation and increasing lifespan by up to 22%.[51]

While the potential therapies outlined above either require further scientific and/or technological advances or, in one case has proven ineffective, several newer drugs targeted at specific pathologic consequences have shown promise in animal models by delaying the progression of NPC disease. For instance, neuroinflammation is known to occur in NPC disease and treatment with ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), has prolonged lifespan and slowed onset of clinical signs in a mouse model of NPC1.[52] Additionally, the treatment of NPC1 mice with compounds possessing antioxidant properties (tamoxifen or vitamin E) showed limited benefit in one study, suggesting that oxidative damage may play a role in NPC pathology.[53] Although a later study in NPC1 mice concluded no benefit from the administration of vitamin C,[52] the potential role of oxidative stress has been further supported by additional recent findings. In two studies, both comparing NPC patient fibroblasts with normal fibroblasts, researchers showed differences in gene-expression profiles indicative of oxidative stress[54] as well as increases in reactive oxygen species and lipid peroxidation.[55] Furthermore, administration of another antioxidant, N-acetyl cysteine (NAC), to NPC1 mice has generated data indicating a delay in the onset of clinical disease and a modest increase in longevity (Ory D, personal communication). Encouraging findings with the use of antioxidants have led to a clinical trial designed to test the efficacy and safety of NAC in NPC patients.[56]

Neurodegeneration is another potential target for treatment of NPC disease. In an effort to prevent neuronal cell death in NPC1 mice, researchers administered imatinib, a c-Abl-specific inhibitor that reduces apoptosis. Treated NPC1 mice were reported to show improved neurological symptoms, moderately increased lifespan, and rescue of PCs in the cerebellum.[57] In other work, sphingosine, one of several lipids believed to accumulate in NPC disease, has been suggested to cause abnormally low calcium levels in the acidic compartments of NPC1 cells. Treatment of NPC1 mice with curcumin, a drug thought to be capable of elevating cytosolic calcium levels and purportedly overcoming late endosomal/lysosomal transport defects,

showed benefits in terms of reduced tremors and increased activity, as well as a modest increase in lifespan.[58]

As NPC disease is characterized by the storage of unmetabolized cellular components, reducing or preventing the accumulation of these offending metabolites offers a means by which cellular metabolic burden can be decreased. This method of treatment is known as substrate reduction therapy (SRT).[59] In theory, this goal can be achieved by partially inhibiting the biosynthesis of selected cellular compounds so that the rate of synthesis is more closely matched to the rate of degradation.

The most notable example of SRT to date is N-butyldeoxynojirimycin, or miglustat, a small amino sugar that partially inhibits the synthesis of GSLs, a known storage product in NPC and other lysosomal disorders such as Gaucher disease.[60,61] In both murine and feline studies, administration of miglustat has led to a delay in the onset of clinical signs and to an increase in lifespan.[60] Qualitative studies examining GM2 and GM3 gangliosides also suggested a reduction in storage of these GSLs in brain with treatment of miglustat. Likewise, human clinical trials with miglustat have shown stabilization or improvement of several clinical markers of NPC disease in both pediatric[62,63] and juvenile/adult[64] patients. The European Medicines Agency (EMEA) recently approved miglustat for the treatment of NPC disease and it is currently prescribed off-label in the US and is under consideration for approval by the US Food and Drug Administration (FDA).

Byproduct replacement therapy is another potential treatment for lysosomal diseases, the objective being to replace cellular products found to be deficient in particular diseases.[65] This concept was based on the finding that allopregnanolone was deficient in NPC1 mice and administration of this neurosteroid in early development was thought to delay onset of clinical symptoms and significantly increase lifespan.[31] Subsequent studies showed that the agent responsible for the beneficial effect seen in NPC1 mice was actually the vehicle 2-hydroxypropyl-beta-cyclodextrin (HPBCD).[66,67] While byproduct replacement therapy has yet to be validated as a treatment for NPC disease, it did lead to the discovery of HPBCD as a therapeutic agent.

Cyclodextrins are cyclic sugar molecules commonly utilized as delivery agents for poorly soluble drugs.[68] The inner hydrophobic pocket can form a guest:host complex with drugs or other compounds of interest that are then easily solubilized due to the hydrophilic outer ring of the cyclodextrin molecule. HPBCD is also known to bind cholesterol and is frequently used to add or extract cholesterol in cultured cells.[68,69] When administered to either NPC1 or NPC2 mice at early ages, significant benefits are seen in terms of the delayed onset of clinical signs, reduced storage of both cholesterol and GSLs, and increased lifespan.[66] Additionally, single injections of HPBCD at post-natal day seven or 49 have been shown to significantly improve liver function and increase lifespan.[67] The mechanism of action of HPBCD is unknown and studies have yet to confirm whether it actually crosses the BBB to exert its effect. However, it is well established that intraneuronal accumulation of both cholesterol and gangliosides is diminished with HPBCD treatment and that lifespan is increased beyond that seen with any other current monotherapy.[66,67]

Figure 1: Pathologic Targets for Treatment of Niemann-Pick Type C Disease
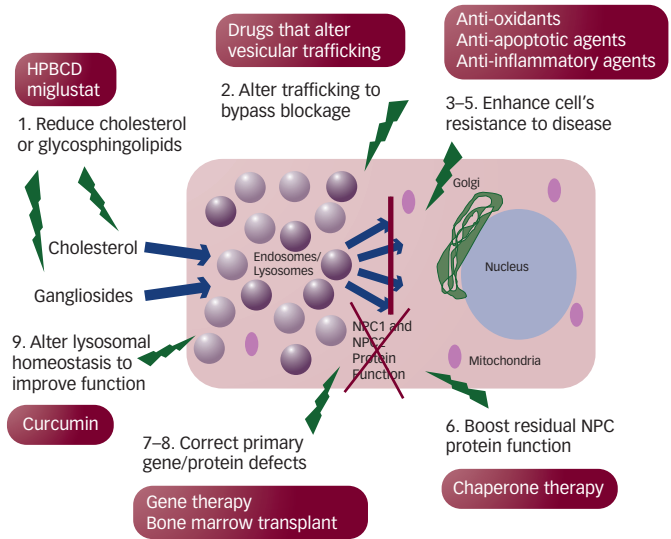
Lack of Niemann-Pick type C1 protein (NPC1) or NPC2 protein is thought to lead to a block in the egress of cholesterol and glycosphingolipids from the endosomal/lysosomal system, as well as many other downstream effects altering normal cellular homeostasis. Currently, therapies are targeted at treating one or more pathologic features of NPC disease, and combinations of these small-molecule drugs have shown enhanced clinical benefit. Individual treatments aim to: (1) reduce the accumulation of offending metabolites, (2) bypass defective metabolic pathways, (3–5) enhance a cell's resistance to disease, (6) increase/rescue function of existing NPC proteins, (7–8) correct the primary metabolic defects, or (9) improve lysosomal function. HPBCD = 2-hydroxypropyl-beta-cylcodextrin. See text for details

Several of the therapies discussed above provide limited benefit to NPC patients or animal models of NPC disease when administered alone. However, they are by no means a cure and each therapy tends to address only one or a few elements in the pathogenic cascade (see *Figure 1*). As such, a logical approach to treatment of NPC disease, as well as many other diseases, is that of combination therapy. By providing two or more therapies to target different pathogenic features, greater benefit can be obtained. This is precisely the case with combinatorial therapies tested in the NPC mouse model. NPC1 mice administered miglustat in conjunction with either HPBCD or NSAIDs resulted in even greater delays in clinical onset, reduction of stored metabolites, and increases in lifespan beyond that of any monotherapy alone.[52,66]

Conclusion

NPC is a devastating disease with a complex and poorly understood pathogenic cascade. The biochemical changes caused by defective NPC1 or NPC2 proteins lead to massive intracellular accumulation of cholesterol and GSLs, adversely affecting many cells of the body. The CNS is especially vulnerable to pathologic changes, resulting in severe phenotypic and behavioral changes, such as ataxia, motor inco-ordination, and progressive mental decline. Although great advances have been made in determining the genetic defects responsible for NPC disease, the putative functions of the mutated proteins and possible approaches for slowing disease progression, still more progress is required to significantly improve both the quality of life and the lifespan of NPC patients. While the greatest amelioration of NPC disease would be expected to come only from successful correction of

the primary gene or protein defects, neither of these therapies is currently available for this disorder.

The use of available pharmacological therapies such as HPBCD, believed to act on cholesterol,[70] or miglustat for GSL reduction,[60] currently holds the greatest promise as treatment for NPC disease. However, much more needs to be done. It is likely simplistic to believe that modulating the accumulation of these two storage compounds alone will lead to complete normalization of a disease cascade caused by lack of the NPC1 protein. An important caveat is that while many of the pathologic features of NPC disease have been characterized and provide known targets for therapeutic intervention, there are likely other abnormalities that have yet to be discovered or are just now coming to light. For instance, the putative accumulation of copper in cells with an induced NPC1 phenotype[32] may prove to be another pathologic feature present in NPC patients and animal models, representing yet another necessary target for drug therapy. The better our understanding of disease pathogenesis, the more likely it is that an effective combination therapy will be developed, ultimately leading to greater clinical benefit for NPC patients. ■

Cristin D Davidson is a graduate student in the Department of Neuroscience at the Albert Einstein College of Medicine in New York, where she is working on therapies for lysosomal diseases, specifically Niemann-Pick type C (NPC) disease. Her work focuses on understanding the mechanism of action of cyclodextrin, a drug that she and others discovered as having an ameliorating effect on NPC disease. She is also interested in combination therapies in NPC murine models to achieve greater benefit, with the hope that these therapies can benefit children with this disorder.

Steven U Walkley, DVM, PhD, is a Professor in the Departments of Neuroscience, Pathology, and Neurology at the Albert Einstein College of Medicine. He has focused his career on the study of lysosomal diseases affecting the brain, an interest that began with his early training in veterinary and comparative medicine. He trained principally as a neuroscientist and his key research focus is on understanding pathogenesis of brain dysfunction in lysosomal disorders, and on developing therapies for these conditions. Dr Walkley serves on the scientific advisory boards of several lysosomal disease organizations, including the National Mucopolysaccharidosis (MPS) Society and the National Niemann-Pick Disease Foundation (NNPDF).

1. Patterson MC, Vanier MT, Suzuki K, et al., *The Metabolic & Molecular Bases of Inherited Disease*, *Eighth edition*, New York: McGraw-Hill, 2001;3611–33.
2. Pentchev PG, Comly ME, Kruth HS, et al., *Proc Natl Acad Sci U S A*, 1985;82:8247–51.
3. Walkley SU, Suzuki K, *Biochim Biophys Acta*, 2004;1685:48–62.
4. Imrie J, Dasgupta S, Besley GT, et al., *J Inherit Metab Dis*, 2007;30:51–9.
5. Garver WS, Francis GA, Jelinek D, et al., *Am J Med Genet A*, 2007;143A:1204–11.
6. Vanier MT, Millat G, *Clin Genet*, 2003;64:269–81.
7. Sevin M, Lesca G, Baumann N, et al., *Brain*, 2007;130:120–33.
8. Niemann A, *Jahrb Kinderheilkd*, 1914;79:1.
9. Crocker AC, *J Neurochem*, 1961;7:69–80.
10. Schuchman EHaD RJ, *The Metabolic & Molecular Bases of Inherited Disease*, New York: McGraw-Hill, 2001;3589–3610.
11. Rodriguez-Lafrasse C, Vanier MT, *Neurochem Res*, 1999;24:199–205.
12. Schuchman EH, *J Inherit Metab Dis*, 2007;30:654–63.
13. Schuchman EH, *Int J Clin Pharmacol Ther*, 2009;47 (Suppl. 1):S48–57.
14. Carstea ED, Morris JA, Coleman KG, et al., *Science*, 1997;277:228–31.
15. Ohgami N, Ko DC, Thomas M, et al., *Proc Natl Acad Sci U S A*, 2004;101:12473–8.
16. Infante RE, Abi-Mosleh L, Radhakrishnan A, et al., *J Biol Chem*, 2008;283:1052–63.
17. Ko DC, Binkley J, Sidow A, *Proc Natl Acad Sci U S A*, 2003;100:2518–25.
18. Naureckiene S, Sleat DE, Lackland H, et al., *Science*, 2000;290:2298–2301.
19. Sleat DE, Wiseman JA, El-Banna M, et al., *Proc Natl Acad Sci U S A*, 2004;101:5886–91.
20. Infante RE, Wang ML, Radhakrishnan A, et al., *Proc Natl Acad Sci U S A*, 2008;105:15287–92.
21. Garver WS, Jelinek D, Oyarzo JN, et al., *J Cell Biochem*, 2007;101:498–516.
22. Vanier MT, *Neurochem Res*, 1999;24:481–9.
23. Walkley SU, Vanier MT, *Biochim Biophys Acta*, 2009;1793:726–36.
24. Karten B, Vance DE, Campenot RB, Vance JE, *J Neurochem*, 2002;83:1154–63.
25. Walkley SU, Sikora J, Micsenyi M, et al., *Biochem Soc Trans*, 2010; in press.
26. Jellinger KA, *J Neurol*, 2009;256(Suppl. 3):270–79.
27. Oddo S, *J Cell Mol Med*, 2008;12:363–73.
28. Leroy E, Boyer R, Auburger G, et al., *Nature*, 1998;395:451–2.
29. McNaught KS, Belizaire R, Isacson O, et al., *Exp Neurol*, 2003;179:38–46.
30. Baudry M, Yao Y, Simmons D, et al., *Exp Neurol*, 2003;184:887–903.
31. Griffin LD, Gong W, Verot L, Mellon SH, *Nat Med*, 2004;10:704–11.
32. Yanagimoto C, Harada M, Kumemura H, et al., *Exp Cell Res*, 2009;315:119–26.
33. Barnes N, Tsivkovskii R, Tsivkovskaia N, Lutsenko S, *J Biol Chem*, 2005;280:9640–45.
34. Frieden E, *Clin Physiol Biochem*, 1986;4:11–19.
35. Sarna JR, Larouche M, Marzban H, et al., *J Comp Neurol*, 2003;456:279–91.
36. Yanjanin NM, Velez JI, Gropman A, et al., *Am J Med Genet B Neuropsychiatr Genet*, 2010;153B:132–40.
37. Lowenthal AC, Cummings JF, Wenger DA, et al., *Acta Neuropathol*, 1990;81:189–97.
38. Miyawaki S, Yoshida H, Mitsuoka S, et al., *J Hered*, 1986;77:379–84.
39. Morris MD, Bhuvaneswaran C, Shio H, Fowler S, *Am J Pathol*, 1982;108:140–49.
40. Patterson MC, Di Bisceglie AM, Higgins JJ, et al., *Neurology*, 1993;43:61–4.
41. Erickson RP, Garver WS, Camargo F, et al., *J Inherit Metab Dis*, 2000;23:54–62.
42. Rohrbach M, Clarke JT, *Drugs*, 2007;67:2697–2716.
43. Bae JS, Han HS, Youn DH, et al., *Stem Cells*, 2007;25:1307–16.
44. Hsu YS, Hwu WL, Huang SF, et al., *Bone Marrow Transplant*, 1999;24:103–7.
45. Haskins M, *ILAR J*, 2009;50:112–21.
46. Okumiya T, Kroos MA, Vliet LV, et al., *Mol Genet Metab*, 2007;90:49–57.
47. Pastores GM, Sathe S, *Drugs R D*, 2006;7:339–48.
48. Gelsthorpe ME, Baumann N, Millard E, et al., *J Biol Chem*, 2008;283:8229–36.
49. Repa JJ, Li H, Frank-Cannon TC, et al., *J Neurosci*, 2007;27:14470–80.
50. Narita K, Choudhury A, Dobrenis K, et al., *FASEB J*, 2005;19:1558–60.
51. Kaptzan T, West SA, Holicky EL, et al., *Am J Pathol*, 2009;174:14–20.
52. Smith D, Wallom KL, Williams IM, et al., *Neurobiol Dis*, 2009;36:242–51.
53. Bascunan-Castillo EC, Erickson RP, Howison CM, et al., *J Appl Genet*, 2004;45:461–7.
54. Reddy JV, Ganley IG, Pfeffer SR, *PLoS One*, 2006;1:e19.
55. Zampieri S, Mellon SH, Butters TD, et al., *J Cell Mol Med*, 2009;13:3786–96.
56. Biomarker Validation for Niemann-Pick Disease, Available at: http://clinicaltrials.gov/ct2/show/study/NCT00975689
57. Alvarez AR, Klein A, Castro J, et al., *FASEB J*, 2008;22:3617–27.
58. Lloyd-Evans E, Morgan AJ, He X, et al., *Nat Med*, 2008;14:1247–55.
59. Platt FM, Jeyakumar M, *Acta Paediatr*, 2008;97:88–93.
60. Zervas M, Somers KL, Thrall MA, Walkley SU, *Curr Biol*, 2001;11:1283–7.
61. Schiffmann R, Fitzgibbon EJ, Harris C, et al., *Ann Neurol*, 2008;64:514–22.
62. Patterson MC, Vecchio D, Jacklin E, et al., *J Child Neurol*, 2010;25:300–305.
63. Patterson MC, Vecchio D, Prady H, et al., *Lancet Neurol*, 2007;6:765–72.
64. Wraith JE, Vecchio D, Jacklin E, et al., *Mol Genet Metab*, 2010;99:351–7.
65. Walkley SU, *Acta Paediatr*, 2007;96:26–32.
66. Davidson CD, Ali NF, Micsenyi MC, et al., *PLoS One*, 2009;4:e6951.
67. Liu B, Turley SD, Burns DK, et al., *Proc Natl Acad Sci U S A*, 2009;106:2377–82.
68. Irie T, Uekama K, *J Pharm Sci*, 1997;86:147–62.
69. Zidovetzki R, Levitan I, *Biochim Biophys Acta*, 2007;1768:1311–24.
70. Rosenbaum AI, Zhang G, Warren JD, *Proc Natl Acad Sci U S A*, 2010;107:5477–82.