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REVIEW

Treatment of Niemann–Pick Type C Disease by Histone Deacetylase Inhibitors

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Abstract Niemann–Pick type C disease (NPC) is a devastating, recessive, inherited disorder that causes accumulation of cholesterol and other lipids in late endosomes and lysosomes. Mutations in 2 genes, *NPC1* and *NPC2*, are responsible for the disease, which affects about 1 in 120,000 live births. About 95 % of patients have mutations in NPC1, a large polytopic membrane protein that is normally found in late endosomes. More than 200 missense mutations in *NPC1* have been found in NPC patients. The disease is progressive, typically leading to death before the age of 20 years, although some affected individuals live well into adulthood. The disease affects peripheral organs, including the liver, spleen, and lungs, but the most severe symptoms are associated with neurological disease. There are some palliative treatments that slow progression of NPC disease. Recently, it was found that histone deacetylase (HDAC) inhibitors that are effective against HDACs 1, 2, and 3 can reduce the cholesterol accumulation in fibroblasts derived from NPC patients with mutations in *NPC1*. One example is vorinostat. As vorinostat is a Food and Drug Administration–approved drug for treatment of cutaneous T-cell lymphoma, this opens up the possibility that HDAC inhibitors could be repurposed for treatment of this rare disease. The mechanism of action of the HDAC inhibitors requires further study, but these drugs increase the level of the NPC1 protein. This may be due to post-translational stabilization of the NPC1 protein, allowing it to be transported out of the endoplasmic reticulum.

Keywords Histone deacetylase inhibitors · Cholesterol · Lysosomes · Lipids · Proteostasis

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Niemann–Pick Type C Disease

Incidence and Pathology

Niemann–Pick type C disease (NPC) is an autosomal recessive, inherited lysosomal storage disorder that leads to abnormal accumulation of cholesterol and other lipids in late endosomes and lysosomes (LE/Ly) [1]. The lipid accumulation can be seen in tissues throughout the body, but the major disease-associated pathologies are due to progressive neurological deterioration [1]. NPC is a typical example of a large family of lysosomal storage disorders. Other members of this class of diseases include the closely related Niemann–Pick types A and B, as well as Wolman, Gaucher, Fabry, and Tay–Sachs diseases [2]. First described in the early twentieth century [3, 4] and more thoroughly characterized in the 1950s [5], the molecular processes involved were uncovered beginning with the work of Pentchev and coworkers, starting in the 1980s [6–8].

The incidence of NPC disease has been estimated to be approximately 1:120,000 live births [1]. However, patients are frequently misdiagnosed or undiagnosed for several years after the onset of symptoms, and it seems likely that the incidence is actually substantially higher than this [9–11]. The age of onset can be from infancy to adulthood, but the majority of patients develop symptoms in early childhood,

with death frequently occurring before the age of 20 years. The rate of progression of the disease is somewhat variable [9], although some reports indicate that once a threshold of disability is reached there is a fairly uniform rate of progression that is apparently independent of age of onset [12]. A slowly progressing form of the disease has been reported with a very small number of patients as old as 68 years [13].

The health of NPC patients is negatively affected by progressive neurodegeneration and inflammatory events in the brain and visceral organs, with effects on the liver, spleen, and lung being most pronounced. Typical neurological manifestations include vertical supranuclear gaze palsy [14], saccadic eye movement abnormalities, cerebellar ataxia, dystonia, dysmetria, dysphagia, and dysarthria [1]. The central nervous system (CNS) deficits are associated with the presence of meganeurites, ectopic dendrites, and axonal spheroids [15]. There is also extensive neurodegeneration, neuroinflammation, and a patterned loss of cerebellar Purkinje cells [15]. Oropharyngeal dysphagia can be particularly problematic as it can often lead to food or fluid aspiration and subsequent pneumonia.

Molecular and Cellular Characterization of NPC Disease

Two genes (*NPC1* and *NPC2*) have been linked to NPC disease in humans [1]. Approximately 95 % of cases are associated with mutations in *NPC1*. The NPC1 protein is a large membrane protein that is localized in the LE/Ly and is predicted to have 13 transmembrane domains. Most of the *NPC1* mutations are missense point mutations, and more than 200 different *NPC1* mutations have been found in patients [1]. The NPC2 protein is a small globular protein that is targeted to LE/Ly by a mannose-6-phosphate modification that causes it to bind to mannose-6-phosphate receptors, which direct it to late endosomes [16].

Both NPC1 and NPC2 have been shown to bind cholesterol [16, 17]. In NPC1 there is a well-characterized cholesterol binding site in the N-terminal domain, which faces the lumen of the LE/Ly. X-ray crystallography shows that cholesterol is bound in a pocket in the NPC1 N-terminal domain with the hydroxyl moiety oriented inward away from the surface of the protein [18, 19]. In contrast, cholesterol binds to NPC2 with the hydroxyl oriented outward toward the surface of the protein [20]. It is relatively difficult to directly load the NPC1 N-terminal domain with cholesterol [17], but the rate of loading is greatly increased when cholesterol is first bound to NPC2 and then transferred to NPC1 [18]. This led to a model in which lipoprotein cholesterol esters are hydrolyzed in LE/Ly, and the cholesterol, which is very insoluble in water, binds to NPC2. The NPC2 would then transfer cholesterol to the NPC1 N-terminal domain in the limiting membrane of the LE/Ly [19]. By a process that is yet to be characterized, cholesterol can then exit the LE/Ly and be delivered to other cell membranes. This model of a cholesterol hand-off is strongly supported by *in vitro* studies showing that NPC2:cholesterol binds to the first lumenal loop of the NPC1 protein, while apo-NPC2 does not bind [21]. Additionally, some NPC1 disease-associated missense mutations found in the first lumenal loop abrogate the binding of NPC2:cholesterol to NPC1 [21], which prevents the efficient transfer of cholesterol from NPC2 to NPC1. These data support a directional transfer of cholesterol from NPC2 to NPC1 in the lumen of the LE/Ly.

The biochemical studies of NPC1 and NPC2 strongly support the hypothesis that defects in cholesterol transport are the underlying mechanism responsible for NPC disease. However, several other lipids accumulate in the lysosomal storage organelles found in NPC mutant cells, including sphingomyelin and bis(monoacylglycero)phosphate, which is also known as lysobisphosphatidic acid [2, 22]. In the CNS the most prominent stored lipids are glycosphingolipids, but cholesterol accumulation is also seen [2].

It has been proposed that the secondary accumulation of sphingolipids may relate to the biophysical properties of cholesterol and sphingolipids as they associate in lipid bilayers. Cholesterol is stabilized in lipid bilayers by the presence of sphingolipids either because of specific interactions or because the structures of the sphingolipids with their large head groups and saturated acyl chains effectively shield the cholesterol from the aqueous phase [23]. As a consequence of these interactions, accumulation of cholesterol may lead to an enrichment of sphingolipids in the internal membranes of lysosomal storage organelles. Similarly, high levels of sphingolipids would be predicted to stabilize the cholesterol in these membranes. Consistent with this model, augmenting the lysosomal hydrolysis of sphingolipids by increasing acid sphingomyelinase levels in NPC mutant fibroblasts leads to a decrease in the cholesterol storage [24]. It is generally unclear if the cellular pathology associated with NPC disease is caused by the accumulation of the cholesterol itself or by the dysregulation of other lipids, including sphingolipids. Miglustat, a drug that inhibits an early step in glycosphingolipid synthesis and is approved for use in NPC patients in several countries, has been found to provide some benefit to NPC patients [25].

Studies on the $NPC1^{I1061T}$ mutant, the most common missense mutation observed in NPC1 patients that represents 15–20 % of all disease alleles, provided insights into the molecular mechanisms of NPC [26]. In NPC patient-derived fibroblasts, the $NPC1^{I1061T}$ protein is synthesized, but is mislocalized and present at reduced levels. In wild-type fibroblasts, NPC1 protein predominantly distributes to the late endosomal compartment, while in human fibroblasts homozygous for the $NPC1^{I1061T}$ the mutant protein fails to localize to the late endosomes. Even though *NPC1* mRNA transcript levels are elevated 1.4–2.4-fold in $NPC1^{I1061T}$ *vs* WT fibroblasts, NPC1 protein levels are reduced by 85 % in the NPC^{I1061T}

fibroblasts. Thus, the *NPC1*^{I1061T} substitution affects steady-state levels of endogenously expressed NPC1 protein, possibly by impairing translation of the NPC1 protein or by rendering the protein unstable. Metabolic labeling studies demonstrate that the wild-type protein has 2 distinct rates of degradation [26]. About half of the protein exhibited a $t_{½}$ of 9 h, after which the remaining protein exhibits a glycosylation pattern shift from an endoglycosidase H-sensitive to an endoglycosidase H-resistant species, extending the $t_{½}$ to 42 h. In contrast, NPC1^{I1061T} protein remains almost exclusively endoglycosidase H-sensitive and exhibits a $t_{½}$ of 6.5 h. These data indicate that about half of the wild-type NPC1 and nearly all of the NPC1^{I1061T} protein is degraded in the endoplasmic reticulum (ER). Overexpression of NPC1^{I1061T} or treatment with general protein stabilizers, such as glycerol, led to partial correction of the NPC phenotype [26].

An interesting observation is that siblings with identical mutations in *NPC1* can have very different ages of onset and rates of progression of the disease. This observation suggests that factors outside of the NPC1 protein itself can profoundly affect the disease progression, and therefore changes in other genes or epigenetic changes might alter the susceptibility to mutations in NPC1 or NPC2. It also opens the possibility that drugs might be used to alter the sensitivity to these mutations. As discussed in the next section, histone deacetylase inhibitors (HDACi) appear to be good candidates for therapy of most NPC1 patients. Preliminary findings suggest that this role may be due to an increase in the fraction of mutant NPC1 proteins that leave the ER and are delivered to LE/Ly.

HDACi and Treatment of NPC Disease

Therapies Tested Previously for Treatment of NPC Disease

Over the last few decades, several small molecule agents have been investigated as potential treatments for NPC. These studies have been performed mainly in cell culture and mouse models, and, to a lesser extent, in human patients, as summarized in previous reviews [1, 27]. The choices of many of these agents have been based upon previously known effects of these compounds on sterol absorption, biosynthesis, and metabolism, or, alternatively, upon agents involved in sphingolipid pathways. These studies have largely had limited effect on the NPC disease phenotype. The compounds that have been examined include statin drugs (lovastatin and pravastatin) [28–30], a squalene synthesis inhibitor (CP-340868) [31], cholestyramine [28, 29], nicotinic acid [28], ezetimibe (Zetia) [32], peroxisomal inducers (clofibrate, perfluorooctanoic acid, dehydroepiandrosterone, and diethylhexylphthalate) [33, 34], neurosteroids or their mimics (allopregnanolone, ganaxolone, and T-0901317) [35, 36], oxysterols (25- and 27-hydroxycholesterol, 7-ketocholesterol, and 17b-estradiol) [17, 37–40], synthetic liver X receptor ligands (T-0901317 and bexarotene) [40–43], sphingolipid pathway-targeting agents (miglustat and *n*-butyldeoxygalactonojirimycin) [44–46], calcium regulators (curcumin, thapsigargin, and myriocin; note that curcumin is also an HDACi) [47–49], apoptosis inhibitors (imatinib, minocycline, and B-cell lymphoma 2 protein) [50, 51], a neurodegeneration inhibitor (N^{ω}-nitro-L-arginine methyl ester) [52], α-tocopherol (vitamin E) [53, 54], tamoxifen [53], nitrovin (difurazone) [55], and several members of 3 heterocycle series consisting of highly substituted pyrrolinones, triazines, and thiadiazoles [56–59]. Low cholesterol diets have been found to be ineffective [60].

With respect to the current state of available therapies, miglustat (Zavesca; Fig. 1) was approved in 2009 for treatment of NPC in the European Union and, subsequently, in Canada, Brazil, Turkey, Australia, and Japan [44, 61]. Although it has been reviewed by the Food and Drug Administration (FDA), it has not been approved for NPC in the USA. Miglustat functions as a glucosylceramide synthase inhibitor and therefore affects the glycosphingolipid pathway. It had previously been approved by the FDA for treatment of Gaucher disease, which is another lysosomal storage disorder characterized by accumulation of glucosylceramide [62].The past several years of experience with miglustat indicate that it is able to stabilize NPC patients and is likely to extend their life spans [44, 61].

The most effective treatment for NPC disease in animal models is injection of 2-hydroxypropyl-β-cyclodextrin (HPBCD) (Fig. 1), a cholesterol chelating cyclodextrin [63, 64]. Unfortunately, HPBCD is excreted rapidly and crosses the human blood–brain barrier very poorly; therefore, this treatment requires infusion of high-dose HPBCD for several hours a few times per week and intrathecal injections for delivery into the CNS. A clinical trial for HPBCD therapy is currently underway [65]. Significant side effects that have been observed in cat and mouse models of NPC include hearing impairment and lung complications [66].

HDACi and NPC Disease

HDACi were tested as part of a high-throughput screen for compounds that would reduce cholesterol accumulation in LE/Ly of human fibroblasts derived from patients with mutations in NPC1 or NPC2 [67]. A collection of HDACi was specifically included in the screen for several reasons. Cells with the NPC1^{I1061T} mutation could be partially corrected by protein overexpression or by glycerol, which acts as a nonspecific protein chaperone [26]. A basic hypothesis is that up-regulation of mutant, but functional, NPC1 or NPC2 protein or up-regulation of chaperones to overcome protein misfolding may serve to restore normal cholesterol trafficking. By altering chromatin structure, HDACi change the expression of a large

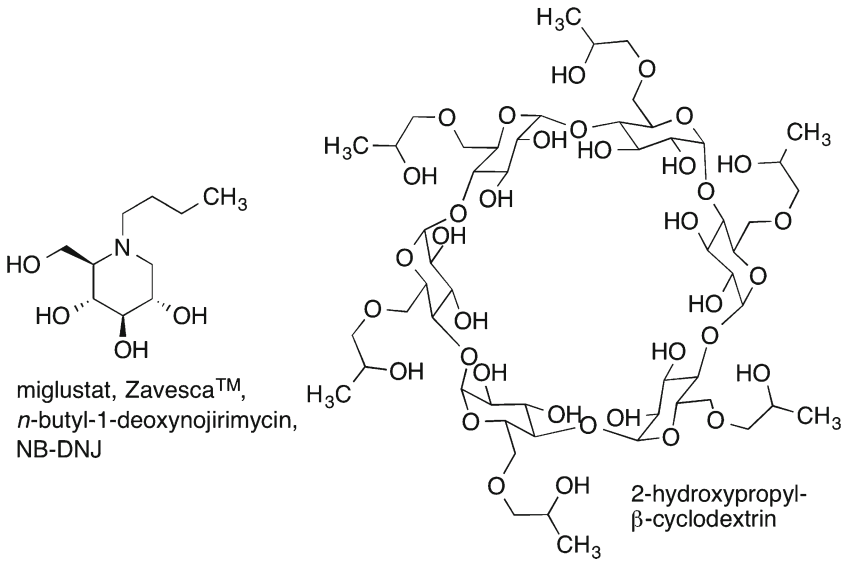

Fig. 1 Niemann–Pick type C disease therapeutics. Miglustat (*left*) inhibits the synthesis of glycosphingolipids, which accumulate along with cholesterol in late endosomes and lysosomes of neurons. 2-Hydroxypropyl-β-cyclodextrin (*right*) binds cholesterol and solubilizes it. This can lead to efflux of stored cholesterol from late endosomes and lysosomes. NB-DNJ = *n*-butyldeoxygalactonojirimycin

number of proteins. HDACi have also been shown to increase production of protein chaperones, which may, in turn, assist transport of the mutant NPC1 protein out of the ER and to avoid protein destruction by ER-associated degradation of misfolded proteins [68]. Preliminary studies of valproic acid supported the hypothesis that HDACi may have a therapeutic effect in NPC disease [69]. Trichostatin A has also been shown to be a repressor of the cholesterol biosynthetic pathway [70], which might provide a secondary benefit. For these reasons, the Maxfield, Wiest, and Helquist laboratories obtained and screened a small set of well-known HDACi representing a range of chemical structural types, potencies, and isoform selectivities. (To avoid duplication, the reader is referred to [71] where the range of HDACi structures, potency, and selectivity are described in greater detail than is the intent here.)

The initial test set consisted of vorinostat (Fig. 2), trichostatin A, panobinostat, PCI-34051, CI-994, and thiophene benzamide (Fig. 3) [67]. The screening was accomplished using filipin staining for fluorescent determination of lysosomal cholesterol in NPC1 mutant GM03123 human fibroblasts carrying heterozygous *P237S* and *I1061T* mutations, and GM18443 carrying the homozygous *I1061T* mutation on both alleles. GM05659 wild-type cells were used as a control. For both of the NPC1 mutants, a dose and time response for lowering of cholesterol levels was observed for 5 of the 6 compounds, with PCI-34051 being the exception.

The strongest responses were seen for the most potent HDACi used in this survey, namely panobinostat (LBH-589) and trichostatin A, with EC_{50} values of <5 nM and ~60 nM, respectively. The dose–response curve for vorinostat is shown in Fig. 4. Essentially, complete correction of the NPC phenotype was seen with 10 μM vorinostat (Fig. 5). Based upon the known isoform selectivities of the HDACi in this survey (see Figs 2 and 3), the relevant target for correction of the NPC1 phenotype is likely to be HDAC1, 2, or 3, whereas HDAC 8 is ruled out by lack of response to PCI-343051. These HDACi were also screened in the NPC2 mutant GM18445 human fibroblast cell line having the homozygous *V39M* mutation, but there was no significant response. The HDACi examined here did not show cytotoxicity, but they did show cytostatic activity in the NPC1 mutant GM03123 cells.

With respect to mechanism of action, treatment of the NPC1 mutant cells with the HDACi led to increased production of NPC1 protein in parallel with correction of the NPC phenotype. Also observed was decreased uptake of low-density lipoprotein, reduced proteolytic processing of sterol-responsive element binding protein-2 transcription factor, and increased cholesterol esterification by acyl Co-A:cholesterol acyl transferase—all consistent with restoration of normal cellular cholesterol homeostasis. Studies are in progress to determine if the effect of HDACi treatment is mainly due to

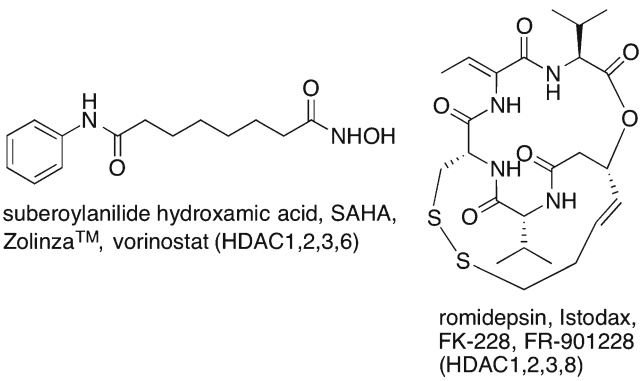

Fig. 2 Histone deacetylase (HDAC) inhibitors previously approved by the Food and Drug Administration. The various names used for the compounds are provided. Entries in *parentheses* indicate the HDAC for which the strongest inhibitory effects are seen.

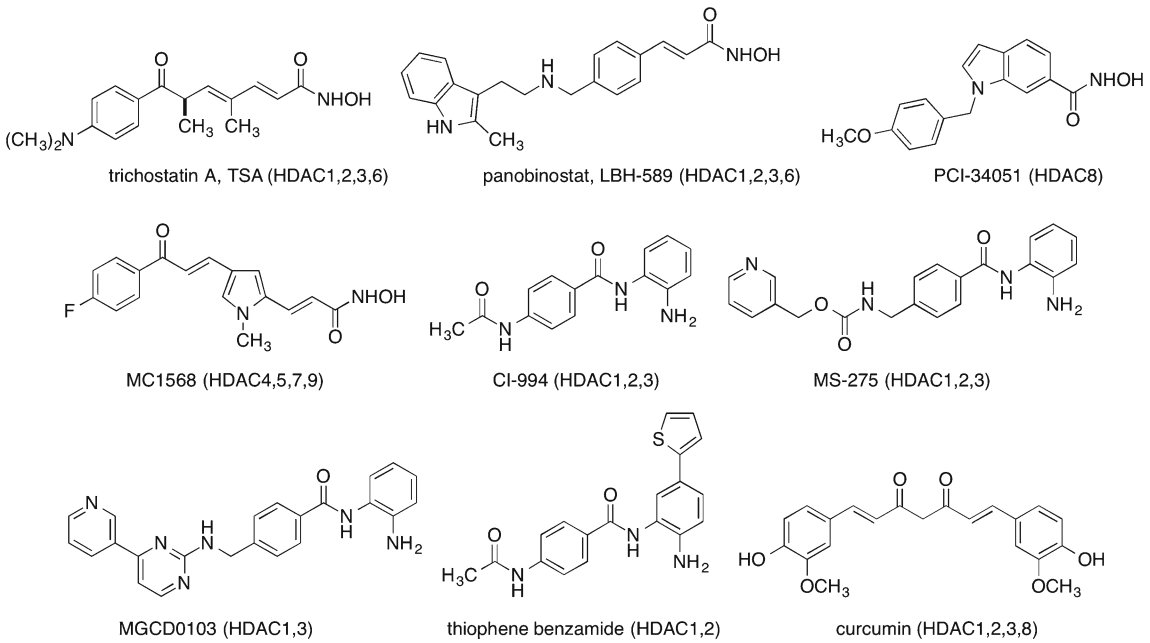

Fig. 3 Histone deacetylase (HDAC) inhibitors. Entries in *parentheses* indicate the HDAC for which the strongest inhibitory effects are seen

protein stabilization in the ER [72]. Ongoing studies in the Maxfield laboratory have shown that many different NPC1 mutations found in several patient-derived fibroblast cell lines respond to HDACi with a dose-dependent reduction in stored cholesterol [72]. Testing is also underway using an engineered cell line expressing many different *NPC1* mutations, and preliminary results are encouraging in that a high fraction of *NPC1* mutations are responsive (F. Maxfield, unpublished data).

Subsequent studies by Munkacsi et al. [73] confirmed that vorinostat and trichostatin A reduce lysosomal cholesterol levels in human NPC fibroblasts along with lowering sphingolipid accumulation and increasing esterification of free cholesterol, which is deficient in untreated NPC cells. The cholesterol lowering effect was seen to be less pronounced with the more isoform-selective HDACi MC1568 (HDACs 4, 5, 7, 9) and MGCD0103 (HDACs 1, 2, 3, 11) compared with the less selective vorinostat and trichostatin A. Wehrmann et al. [74] have also confirmed the cholesterol lowering effect of vorinostat and panobinostat in human NPC fibroblasts. This effect was enhanced in the presence of HPBCD.

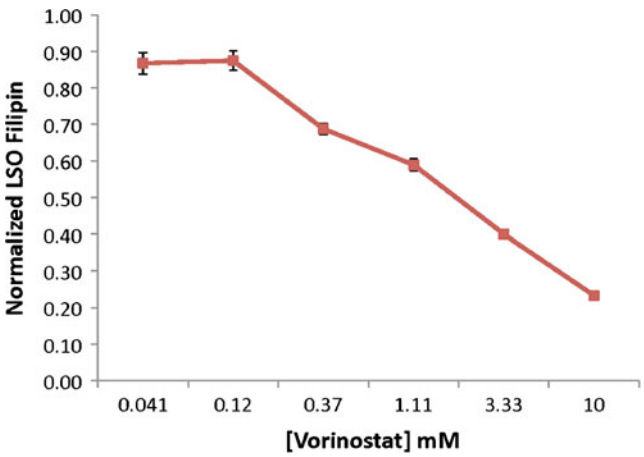

Fig. 4 Dose–time response for treatment of GM03123 Niemann–Pick type C disease 1 mutant cells with vorinostat. The vertical axis measures the fluorescence response of filipin staining of cholesterol in lysosomal storage organelles (LSO). Cells were treated for 48 h with varying concentrations of vorinostat. The cells were then fixed and stained with filipin

Considerations for Use of HDACi as Therapies for NPC1 Disease

In recent years, a very large number of compounds have been designed and developed specifically as HDACi. The first 2 to receive FDA approval for clinical use were suberoylanilide hydroxamic acid (vorinostat, Zolinza) in 2006 and romidepsin (Istodax, FK-228, FR-901228) in 2009, both for treatment of cutaneous T-cell lymphoma (Fig. 2) [75, 76].

Although HDACi have been developed most prominently for treatment of cancers, their effectiveness in many other diseases has also been investigated [71, 77]. 4-Phenylbutyric acid and valproic acid are among the simplest and also the weakest HDACi (Fig. 6), although their function as HDACi was not recognized until long after early studies of their therapeutic properties. Regardless of its simplicity, the sodium salt of the former was approved by the FDA in 1996 and commercialized as Buphenyl (Ucyclyd Pharma) for the treatment of urea cycle disorders—another group of rare diseases [78, 79]. It was subsequently studied in thalassemia [80], sickle cell anemia [81], and cystic fibrosis [82]. The derivative, glycerol phenylbutyrate (Ravicti), has also been

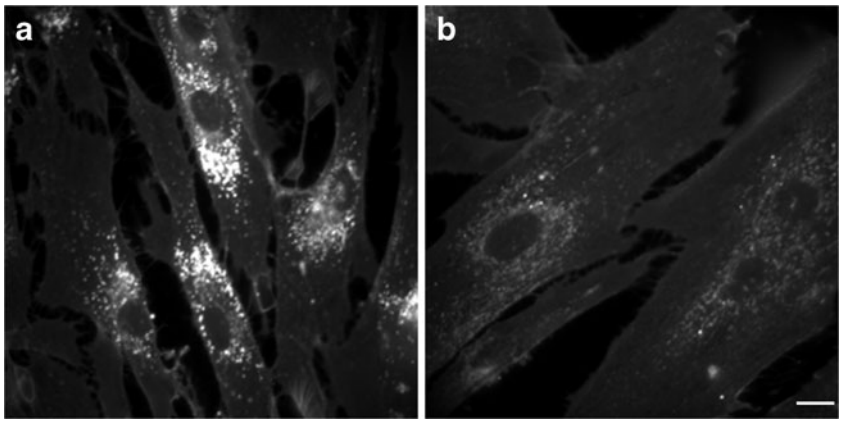

Fig. 5 Effect of vorinostat on cholesterol storage. Fluorescence microscopy images of filipin-stained GM03123 Niemann–Pick type C disease 1 mutant cells treated with dimethyl sulfoxide vehicle (**a**) or 10 μM vorinostat (**b**) for 2 days. Scale bar=15 μm

approved for the treatment of urea cycle disorders [83]. The likewise simple valproic acid is used to treat epilepsy, other seizures, and bipolar disorder [84]. Also of relevance in a neurodegenerative disease such as NPC is that HDACi have been studied for treatment of cognitive disorders such as Alzheimer's, Huntington's, and Parkinson's diseases [85]. HDACi are also reported to function as cognitive enhancers [86]. Whereas increased histone acetylation in the brain is associated with improved memory, histone deacetylation correlates with poorer memory [87, 88].

For an ultra-rare disease such as NPC, the most reasonable strategy may be to identify a treatment from among previously approved drugs or, at the least, other previously known compounds that have already been through extensive studies, including clinical trials. Developing a new drug *de novo* would likely encounter enormous costs that could not be recouped from the very small NPC patient population.

Prospective

Although the study of HDACi for treatment of NPC is at a relatively early stage, this therapeutic strategy shows significant promise. In order to accelerate such a treatment into the clinic, the selection of a previously approved drug is a reasonable choice. Of the HDACi screened to date for NPC, vorinostat meets this criterion, whereas romidepsin, another approved HDACi has apparently not been screened yet for this disease. This latter compound is also more chemically complex than vorinostat and may be less accessible.

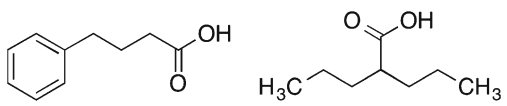

Fig. 6 Simple, weak histone deacetylase inhibitors with therapeutic applications

The advantages of seeking clinical trials of vorinostat are numerous. There has been considerable prior human experience with its use, albeit as a cancer treatment. A search of the MedLine, Web of Science, and Biosis Previews databases shows 81 journal citations beginning with Phase I studies in cancer patients monitoring the pharmacology and pharmacokinetics following intravenous and oral drug administration. The level of acetylated histones in human peripheral blood mononuclear cells (PBMCs) was established as a reliable biomarker of drug action [89, 90]. The acetylation of PBMC histones by vorinostat paralleled its pharmacodynamic effects in tissue. Rapid induction of histone acetylation in PBMCs occurs after intravenous and oral dosing in a time- and concentration-dependent manner [89, 90]. Elevation of acetylated histones was consistently observed 2 h after a single oral dose of vorinostat ranging from 200 to 600 mg. With an increasing vorinostat dose, the duration of the elevated levels of acetylated histones in PBMCs was maintained up to 8 h. In patients who were under study for 6 months or longer, vorinostat was found to continue to stimulate an increase in PBMC acetylated histones, indicative of a sustained pharmacological effect over time [89–91]. The major pathways of vorinostat metabolism involve glucuronidation and hydrolysis followed by α-oxidation [92]. The major metabolites, O-glucuronide and 4-anilino-4-oxobutanoic acids, are both pharmacologically inactive. The clinical pharmacology profile of vorinostat is favorable, exhibiting dose-proportional pharmacokinetics and modest food effect. There appear to be no major differences in the pharmacokinetics of vorinostat in special populations, including varying demographics and hepatic dysfunction. Combination therapy pharmacokinetic data indicate that vorinostat has a low propensity for drug interactions [93].

Until recently, the mouse models for NPC1 deficiency have been *NPC1*$^{-/-}$, so they would not be good models for testing a drug that worked by increased expression of a mutant, but somewhat functional, NPC1 protein. Recently, a mouse model with a point mutation in NPC1 (D1005G) has been described [94], and mouse models with other mutations are being

developed. These mouse models will provide the capability of testing HDACi therapy in mouse models. In addition, there is a line of cats with a naturally-occurring missense mutation in the feline NPC1 protein [95], and these provide an opportunity for testing in a large mammal. Such animal testing may not be required for regulatory approval of tests of vorinostat in adults, but it would be useful for testing dosing regimens and for testing efficacy in juvenile animals.

In order for vorinostat to be clinically effective for the treatment of NPC, sufficient levels must be achieved in the brain to increase the brain level of acetylated histones leading to pharmacologically correct cholesterol processing and trafficking. The passage of vorinostat across the blood–brain barrier has been observed in laboratory animals using several techniques. An increase of acetylated histones levels in the brain following vorinostat treatment is observed in cancer [96, 97] and noncancer animal models [98, 99]. Following systemic administration, uptake of [^{14}C]vorinostat was significant into normal rodent brain reaching a brain/blood concentration ratio at 30 min that exceeded the brain residual blood volume by 5- to 7-fold [97]. An increase in the level of acetylated histones was measured in biopsies of brain tumor in patients with neuroblastoma treated with vorinostat [100]. Vorinostat is reported to slow neurodegenerative processes in laboratory cellular and animal disease models, indicative of its pharmacological activity in whole animal brain tissue [101].

Inflammation in the brain and visceral organs is also a major pathogenic process in NPC patients that requires therapeutic intervention. The anti-inflammatory activity of vorinostat that has been observed in human cancer patients, and its immunosuppressive properties in multiple laboratory models, may have potential benefits in treated NPC patients. Histone acetylation status has been found to regulate inflammatory gene expression in laboratory models of chronic inflammatory lung diseases and other autoimmune diseases [102].

Since vorinostat was developed as a cancer treatment, most of the safety testing in humans has been in adults, although there have also been trials in pediatric patients. The pediatric studies have, likewise, been for treatment of cancers, including CNS or non-CNS solid tumors, lymphomas, and leukemias [103–106]. Most of the published clinical studies with vorinostat include the tabulation of clinical and laboratory adverse events for patients with recurrent or relapsed carcinoma. For FDA approval of vorinostat to treat cutaneous T-cell lymphoma, the safety and adverse event data came from 73 patients [107]. In a later review of pooled clinical data from 498 patients with solid and hematological malignancies, it was shown that vorinostat was well tolerated as monotherapy or combination therapy [108]. The most commonly reported drug-related adverse events associated with monotherapy were fatigue (61.9 %), nausea (55.7 %), diarrhea (49.3 %), anorexia (48.1 %), and vomiting (32.8 %), and Grade 3/4 drug-related adverse events included fatigue (12.0 %), thrombocytopenia (10.6 %), and dehydration (7.3 %). The clinical use of vorinostat to treat NPC patients will most likely use a cyclic treatment schedule of 3–5 days with intervals of no treatment.

On the whole, the preceding observations are supportive of vorinostat as an epigenetic treatment for NPC, but there are some factors that suggest room for improvement in longer-term drug development efforts. Vorinostat is not nearly as potent as many other HDACi. Its lack of HDAC isoform selectivity likely leads to changes in expression of a large number of genes that have no relevance to NPC. Although there is evidence of some penetration of the blood–brain barrier by vorinostat, other HDACi have measurably superior penetration [109–112].

Much remains to be learned about the function of HDACi in NPC. Which of the several HDAC isoforms is (are) the relevant target for this disease? What is the mechanism by which HDACi correct the NPC phenotype—is it due to up-regulation of NPC protein biosynthesis, a protein chaperone mechanism, or yet additional cellular pathways? Addressing these questions will continue in parallel with medicinal chemists needing to pursue development of a pipeline of other compounds as improved treatments for this disease. Ultimately, the knowledge gained for treatment of NPC will likely translate to other related diseases.

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Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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