

# 2010 Research Grants

The National MPS Society awarded \$471,000 for research grants in 2010. The funding the Society provides has been and continues to be crucial as we move forward with our mission to find the cures.

We received 45 letters of intent from researchers around the world for the seven grants offered in 2010. After reviewing those letters, our Scientific Advisory Board review committee requested full grant proposals from 14 researchers.

All new grant recipients were awarded \$60,000 for the two year grant, with half of the total provided each year. We received \$60,000 from the Caterina Marcus Foundation, [www.caterinamarcusfoundation.org](http://www.caterinamarcusfoundation.org), to fund a general research grant and \$52,000 from Insieme per Gabriel, an ML family foundation in Graglia, Italy, for an ML Partnership Grant. We are honored that both foundations selected the National MPS Society as partners to fund this very important research.

An additional \$15,000 will support the work of Brains for Brain. The Society will fund \$25,000 to support the Lysosomal Disease Networks NIH grant research goals. The funding is designed for the Neuroimaging Core, which will benefit the four MPS projects. The International MPS Network will announce a grant in September for treatment of CNS in MPS III. The Society has allocated \$5,000 for that Partnership Grant.

## MPS I

**Mark J. Osborn, PhD**

**University of Minnesota, Minneapolis, MN**

### **Gene therapy for the central nervous system pathology of MPS I**

The lysosome acts as the acidic stomach of the cell and contains multiple enzymes responsible for the breakdown of glycosaminoglycans that are normal constituents of the cell that must be turned over and recycled. A loss of a lysosomal enzyme results in accumulation of glycosaminoglycans resulting in swelling of the lysosome and loss/alterd function of the cell resulting in system wide pathology. MPS I (Hurler syndrome) is caused by a mutation to the IDUA gene causing a loss of the IDUA enzyme that acts as a critical enzyme in the breakdown of glycosaminoglycans. One of the most severely affected organs in patients with Hurler syndrome is the brain that shows widespread pathology resulting in severe mental retardation. The available treatment options for Hurler syndrome do not effectively correct the brain pathology therefore we have proposed to test the ability of a novel protein we have developed to reduce the brain pathology of this disease. Our protein is a hybrid comprised of transferrin and IDUA and is able to cross from the blood into the brain and therefore is able to be delivered in a minimally invasive fashion. We will test this protein by delivering a gene encoding it into MPS mice that have been engineered to mimic the human disease. Our pre-clinical testing will provide proof of concept for pursuing similar studies in humans.

## MPS II

**Brett E. Crawford, Ph.D.**

**Zacharon Pharmaceuticals Inc., La Jolla CA,**

### **Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II**

The proposed research project aims to produce a new drug for treating mucopolysaccharidosis (MPS). MPS occurs due to the toxic buildup of cellular carbohydrates (glycosaminoglycans) in cells which lead to serious symptoms ranging from physical deformity, cardiac, joint, and neurological dysfunction. Glycosaminoglycan buildup occurs due to mutations that inactivate enzymes that normally degrade these glycans. Through our previously supported research, we have identified compounds that can alter the synthesis of

glycosaminoglycans so that they can be cleared from patients with MPS. Our most advanced compounds have demonstrated efficacy in MPS models and are able to enter the central nervous system. These compounds represent a critical starting point for the development of a treatment for the neurological aspect of these diseases. Additionally, due to the mechanism of action (by targeting the biosynthesis of glycosaminoglycans), it is possible that this drug will be effective in multiple classes of MPS. The studies we propose here are aimed at identifying the most promising compound for future clinical development.

## MPS III

**Elizabeth F. Neufeld, Ph.D.**  
**UCLA, Los Angeles, CA**

### **Making a minigene suitable for gene therapy for MPS IIIB**

MPS IIIB is caused by mutations in the NAGLU gene, causing deficiency of the enzyme alpha-N-acetylglucosaminidase, storage of heparan sulfate and (in brain) of many additional substances. If proved safe, administration of the normal NAGLU gene would be the most effective therapy. Like many other lysosomal storage diseases, MPS IIIB is a candidate for gene therapy, requiring only that the normal gene be introduced into a small number of cells, which would manufacture the enzyme and provide it to neighboring cells, a process known as correction. The gene that is used in gene therapy is not the version found in nature, which is too large to administer to cells or animals. The therapeutic gene is the cDNA version, which is smaller. Ten years ago, we cloned NAGLU cDNA and were disappointed to find that the resulting alpha-N-acetylglucosaminidase was poorly corrective. Nevertheless, this cDNA has been used by four laboratories for gene therapy in MPS IIIB mice. Although all reported therapeutic results, two noted that the results were less than expected. Yet there are plans to use this only partially effective cDNA for clinical trials. Our hypothesis is that segments of DNA taken out of the native NAGLU gene may be important to make an enzyme that will be processed the normal way. This proposal is to make a minigene, which would yield a corrective enzyme and therefore be better suited for gene therapy.

## MPS IV

**Calogera M. Simonaro PhD., Associate Professor**  
**Mount Sinai School of Medicine, New York, New York**

### **A Novel Approach for the Growth & Expansion of Bone Marrow-Derived Mesenchymal Stem Cells in Mucopolysaccharidoses Type IV and Other Mucopolysaccharidoses**

The overall goal of our research is to develop and evaluate new treatment approaches for two important organ systems in the mucopolysaccharidoses (MPS), the bones and joints. The current project is based on recent work showing that an enzyme, recombinant acid ceramidase (rAC), can be used to maintain and expand a unique population of stem cells from the bone marrow. Bone marrow transplantation (BMT) and related gene therapy procedures have been extensively evaluated in MPS patients and/or animal models, with varying degrees of success. While various factors have influenced this outcome, an important limitation is the very low frequency of stem cells within the bone marrow, leading to very low levels of transplanted cells at the disease sites. Direct injection of these cells into these sites helps, however even here the number of surviving cells is very small. Despite these limitations, clinical improvements have been observed, and there is a general agreement in the field that the approach is beneficial, but needs to be enhanced. In this project we will evaluate whether rAC can be used to improve the outcome of BMT, particularly in the bones and joints. Due to the unavailability of a suitable MPS IV animal model that mimics the severe bone and joint disease seen in patients, we will focus our efforts on the MPS VI rat. We will also study the effect of rAC on the growth and transplantation of cells obtained directly from normal and MPS cartilage. If successful, we believe that this approach could greatly improve the outcome of cell-based transplantation procedures in all of the MPS disorders, including MPS IV, and have general applicability to other genetic disorders as well.

## ML

**Dr. Katrin Kollmann, PhD Partnership Grant with Insieme per Gabriele**

**University Medical Center Hamburg-Eppendorf , Hamburg, Germany**

**Skeletal abnormalities in mucopolipidosis II alpha/beta Pathomechanisms and therapeutic strategies**

Skeletal abnormalities are common symptoms in mucopolipidosis II (ML II) and ML III patients leading to a decline in mobility, stiffness and chronic joint pain. In patients bone cells the transport of multiple lysosomal enzymes to lysosomes is altered impairing the function of bone-forming osteoblasts, bone-resorbing osteoclasts and of chondrocytes of the cartilage resulting in osteoporosis. In this study the expression of proteins and genes will be analyzed in cultured bone cells and chondrocytes of a novel ML II mouse model to understand the mechanisms of osteoporosis and to identify novel targets for therapeutical strategies in this disease. Furthermore, ML II knock-in mice will be treated with inhibitors of bone resorption to reduce the osteoporotic phenotype. These experimental approaches might be of relevance especially for ML III and related lysosomal storage diseases with skeletal abnormalities such as MPS VI.

## General

**Dr. Andrea Ballabio Caterina Marcus Foundation Grant**

**TIGEM (Telethon Institute of Genetics & Medicine)**

**Naples, Italy**

**Modulating lysosomal function to treat mucopolysaccharidoses**

We recently discovered that a master gene controls the function and biogenesis of organelles called lysosomes, structures inside cells which breaks down materials into compounds which can be used or discarded by the cell, as needed. This gene, named TFEB, activates lysosomal genes, induces lysosomal biogenesis and increases the ability of cell to degrade complex molecules. In this grant, we plan to build on this discovery and test novel therapies in vivo for the treatment of Mucopolysaccharidosis (MPS). The possibility of achieving global control of lysosomal function, if successful, would represent a paradigm shift in biology and have enormous implications on the therapy of several lysosomal storage disorders, including MPS.

**Dr. Alisdair B. Boraston, PhD**

**University of Victoria, Victoria, BC, Canada**

**Discovery and assessment of inhibitor-based chemical chaperones as potential agents for the treatment of mucopolysaccharidosis IIIB.**

The mucopolysaccharidoses are a group of devastating genetic diseases for which there are currently no cures or even effective treatments. Mucopolysaccharidosis IIIB (MPS IIIB), or Sanfilippo syndrome, is one of these diseases that usually results in death by early adulthood. Our ability to study the cause of MPS IIIB at the atomic level will allow us to develop new medicines to treat MPS IIIB and improve the lives of people suffering from this disease.

## 1st Year Research Reviews - 2010

**MPS II**

**Brett E. Crawford, Ph.D.**

**Zacharon Pharmaceuticals Inc., La Jolla CA,**

**“Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II”**

With the support from the National MPS Society, we have made significant progress toward developing a new therapeutic approach designed to treat both the neurological and non-neurological symptoms of MPS. This approach is based on chemical compounds that modify

glycosaminoglycan synthesis so that certain lysosomal enzymes are not required to degrade them. The following is a brief description of our progress over the last year:

**Improve the Potency of the Lead Compounds.** The first goal of our proposed research is to improve the potency of the inhibitors to a level needed for robust efficacy in experimental models and in patients. This is a very important stage in drug development to ensure that the drug can be administered at effective doses and is sufficiently selective to be safely used in humans. Using the cellular assay of MPS that we developed through our previous MPS Society funding, we have successfully increased the potency of the compound by over 100-fold.

**Identify Safe and Effective Drug Candidates.** With significant progress on the potency, we are now focused on improving other drug like properties required for clinical development. These features include the selectivity, pharmacokinetics, stability, and formulation. Through these studies we aim to identify a potent analog that has all of the characteristics in a drug candidate that is suitable for clinical studies in MPS patients.

Through the next year of support, we are excited to test these optimized compounds in the mouse models of MPS. Due to the broad efficacy of this therapeutic approach we expect to test these compounds in models of MPS I, II, and III.

**Partnership for Further Development.** Based on the progress that we have made through MPS Society grants, NIH grants, and private investors, we are also happy to report that we have entered into a strategic research collaboration with Pfizer to jointly develop these compounds as novel therapeutics for the treatment of MPS.

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## 2nd Year Research Reviews - 2010

### MPS I

Mark J. Osborn, PhD (eight month extension awarded)

University of Minnesota, Minneapolis, MN

Gene therapy for the central nervous system pathology of MPS I

### MPS II

Brett E. Crawford, Ph.D.

Zacharon Pharmaceuticals Inc., La Jolla CA

**Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II**

With the continued support from the National MPS Society, the National Institute of Neurological Disorder and Stroke and Pfizer, we have made significant progress toward developing a new therapeutic approach designed to treat both the neurological and non-neurological symptoms of MPS. This approach is based on compounds that modify glycosaminoglycan synthesis so that certain lysosomal enzymes are not required to degrade them.

The funding from the National MPS Society has provided critical support that has helped us progress this drug development from an idea to an NIH grant supported program and recently to a partnership with Pfizer. The following is a brief description of our progress over the last year:

**Testing the In Vivo Efficacy of Lead Compounds.** Through the first year of support, we identified a series of analogs with improved potency in cellular models of MPS. Our most potent analogs are active in the 100 to 500 nM range, a 100-fold improvement from the original compound. Over the last year, these compounds have been evaluated for their drug-like properties and pharmacological characteristics in a wide range of enzymatic, cellular, and rodent models. Three of these potent compounds with acceptable pharmacological properties were recently tested in

the MPS IIIA mouse model. These in vivo studies demonstrated that all three compounds reduced the lysosomal accumulation of glycosaminoglycans in the brain. Future studies will explore the minimal effective dose and dose required to achieve a phenotypic benefit in the mouse model.

**Expanded Drug Discover Effort.** With the additional support of Pfizer over the last year, we have also expanded our efforts to identify additional therapeutic approaches to MPS. We have used our cellular model of MPS and the Sensi-Pro® assay to screen hundreds of compounds from Pfizer's collections that are potent inhibitors of known drug targets. Our goal is to identify new drug targets that could accelerate the clinical testing of active agents in MPS patients. These studies have revealed a number of potentially active compounds that we are currently characterizing further.

In the next year we will continue to optimize the drug-like properties of the most potent inhibitors, explore novel approaches to MPS, and test the improved compounds in the MPS mouse models. We are optimistic that a novel therapy will emerge from these studies and move closer to clinical testing in the near future. We sincerely appreciate the dire need for effective therapies for these devastating diseases and are committed to bring a new therapy to the clinic as soon as possible.

### **MPS III**

**Elizabeth F. Neufeld, Ph.D.**

**David Geffen School of Medicine at UCLA, Los Angeles, CA**

**Making a minigene suitable for gene therapy for MPS IIIB**

### **MPS IV**

**Calogera M. Simonaro, PhD**

**Mount Sinai School of Medicine, New York, NY**

**A Novel Approach for the Growth & Expansion of Bone Marrow-Derived Mesenchymal Stem Cells in Mucopolysaccharidosis Type IV and Other Mucopolysaccharidoses**

The overall goal of this research project was to evaluate the use of a recombinant enzyme (acid ceramidase, rAC) produced and studied in our laboratory to improve the outcome of cell-based therapies for the MPS diseases. An important and debilitating feature of MPS is progressive cartilage destruction leading to the development of severe arthritic joint disease. At the present time there are no suitable methods to prevent these abnormalities in MPS, and current enzyme replacement (ERT) and bone marrow transplantation (BMT) therapies have limited effects. We have previously found that glycosaminoglycan (GAG) storage in MPS activates numerous inflammatory and other signaling pathways, leading to cartilage cell (i.e., chondrocyte) death and cartilage destruction. Among the many changes in MPS, there is an elevation of the pro-death fat or lipid called ceramide.

Based on this we proposed that rAC could be used to improve the survival and integrity of MPS chondrocytes, both in the laboratory (cell culture) and in animal models of MPS. rAC is the enzyme that degrades the pro-death lipid ceramide, providing a basis for this hypothesis. During the course of this project we determined that the addition of rAC to normal animal (human, rat, horse etc) chondrocytes maintained in the laboratory improved their growth and quality, as determined by several established methods to assess cartilage integrity (e.g., expression of cartilage-specific collagen etc). Moreover, we found that these effects were even more pronounced using chondrocytes obtained from several animal models of MPS. Due to the GAG accumulation in MPS cells and subsequent downstream changes, these cells grow very poorly and lose their cartilage-like properties, even more than normal cells. In the presence of rAC, these features were significantly improved.

We also tested the effects of rAC on the growth and properties of stem cells obtained from the bone marrow of MPS animals (i.e., bone marrow mesenchymal stem cells, MSC). We found that

addition of rAC to normal and MPS bone marrow cells increased the production of MSC about 2-fold, and also significantly improved their ability to become chondrocytes. We tested this using bone marrow from several MPS animal models, and found similar results.

We also obtained bone marrow from mice expressing a protein called green fluorescence protein (GFP), and also found similar results. Based on these observations we have begun to evaluate whether cells (MSC or chondrocytes exposed to rAC) grow better after they are transplanted into MPS animals, and whether they have an enhanced effect on repairing or preserving the cartilage disease. These studies have been initiated and are ongoing.

Thus, our ongoing goal continues to be to develop improved methods to repair the defective cartilage in MPS patients. Funds from this grant have provided essential information that has moved us closer to this goal.

## ML

**Katrin Kollmann, PhD (Partnership grant with Insieme per Gabriel)**

**University Medical Center Hamburg-Eppendorf, Hamburg, Germany**

**Skeletal abnormalities in mucopolidosis II alpha/beta - Pathomechanisms and therapeutic strategies**

The lysosomal storage disease mucopolidosis type II (MLII) is caused by defects in the GlcNAc-1-phosphotransferase. The phosphotransferase is an enzyme complex composed of six subunits ( $\alpha_2\beta_2\gamma_2$ ) that catalyzes the first step in the formation of the mannose 6-phosphate (M6P) recognition markers on lysosomal hydrolases. The M6P recognition marker is important for the efficient transport of newly synthesized lysosomal proteins/hydrolases to lysosomes. In MLII with mutations in the gene encoding the alpha/beta subunits of the complex (MLII alpha/beta), lysosomal hydrolases are not modified with M6P residues, and therefore many lysosomal hydrolases are missorted and do not reach lysosomes. The deficiency of hydrolases in the lysosomes leads to lysosomal dysfunction and the accumulation of undegraded material in different cell types of the body. Severe skeletal abnormalities accompanied by a decline in mobility and chronic joint pain are features of MLII alpha/beta.

We generated a mouse model for MLII alpha/beta by the insertion of a mutation into the murine *Gnptab* gene (c.3082insC) that is homologous to the mutation *GNPTAB* c.3145insC detected in MLII patients. The MLII mice show all characteristic biochemical alterations and clinical features found in the human MLII disease and allow the analysis of underlying pathogenetic cellular mechanisms. MLII mice show an increased lethality, reduced mean body weight and body length, and display skeletal alterations like abnormal spine curvature and osteoporosis<sup>1</sup>. We investigated the bone pathology in detail by biochemical, histomorphometric, histochemical and immunological methods to characterize alterations and identify pathomechanisms affecting the bone metabolism.

Electron microscopic analyses demonstrated the formation of storage lysosomes in osteocytes and osteoblasts but not in osteoclasts. To analyze the targeting defect in the bone we cultured primary cells like osteoblasts and osteoclasts of wildtype and the MLII mice and determined the steady state expression level, sorting, proteolytic processing and the half-life of several enzymes such as tartrate resistant acid phosphatase (TRAP), cathepsin D, Z and K. Pulse-chase and real-time PCR experiments on cultured fibroblasts, osteoblasts and osteoclasts indicated that the rate of synthesis is similar in MLII cells compared to wildtype cells whereas the sorting efficiency to lysosomes was affected resulting in their hypersecretion into the medium. The extent of missorting, however, depends on the lysosomal enzyme examined. Thus,  $\beta$ -hexosaminidase and TRAP, were found to be highly reduced in MLII fibroblasts, osteoclasts and osteoblasts whereas the steady state expression level of proteins like cathepsin D were unchanged.

Our data indicate that subpopulations of lysosomal hydrolases appear to be more affected by the loss of M6P residues than others transported to lysosomes via M6P-independent pathways. Whereas in osteoclasts the missorting of lysosomal proteins lead to an increased bone resorption capacity *in vitro* the consequences of their mistargeting in osteoblasts are unclear. Microarray analyses carried out in cultured osteoblasts and osteoclasts from wildtype and MLII mice revealed changes in the expression of several genes which have to be confirmed by independent methods. Current studies are focussed on i) the isolation and identification of osteoblast-specific M6P-containing proteins that are directly involved in the regulation of bone remodelling, and ii) the pharmacological intervention of altered bone metabolism in MLII.

1 Kollmann K, Damme M, ... (2012) Lysosomal Dysfunction Cause Neurodegeneration in Mucopolipidosis II “Knock-in” Mice. *Brain*, in press

This work was presented on the ESGLD (European Study Group on Lysosomal Diseases) workshop in Helsinki 2011, where it was selected for oral presentation. At the annual conference of the APS 2012 (Working group for paediatric metabolic disorders in the german society for children medicine) it was awarded the poster prize.

## General Grants

**Dr. Andrea Ballabio (Partnership grant with Caterina Marcus Foundation)**

**TIGEM, Naples, Italy**

**Modulating lysosomal function to treat MPS**

**A. SPECIFIC AIMS**

*Aim 1: Development of tools for in vivo TFEB activation*

*Aim 2: Evaluation of the therapeutic effects of in vivo TFEB overexpression in MPSIIIA mice.*

To study the effects of TFEB overexpression *in vivo* in both wild-type mice and in the mouse models of MPSIIIA, we generated a conditional gain-of-function (TFEB-COND-GAIN) mouse line. Time-and/or tissue-specific expression of the transgene can be obtained by crossing the transgenic mouse line with a strain carrying the CRE recombinase. As a first test of the system we generated two founder lines specifically overexpressing Tcfef in the liver using the Albumin-CRE strain that expresses CRE in the hepatocytes. These lines show different levels of TFEB overexpression. One overexpresses TFEB approximately 3-fold normal levels, while the other approximately 20-fold. We observed that TFEB overexpression in liver resulted in the activation of TFEB target genes. To generate brain specific expression of TFEB, the TFEB-COND-GAIN mice were crossed with a NESTIN-CRE strain, that expresses CRE in the brain and central nervous system. Unfortunately, we observed that the first line tested showed embryonic lethality. We believe this could be due a wider expression pattern of NESTIN-CRE in the developing embryo, and by the high levels of TFEB overexpression. We are repeating the experiments with the founder line that shows lower expression levels of TFEB and by using a different brain specific CRE line, GFAP-CRE that has a more restricted expression pattern.

In the meantime, we had obtained very encouraging results on the function of TFEB overexpression in cellular models of lysosomal storage disorders (LSDs), both MPSIIIA and multiple sulfatase deficiency (MSD). We had evidence that TFEB overexpression increased lysosomal exocytosis in cultured HeLa cells, we tested whether we observed the same effect in mouse embryonic fibroblasts derived from the murine models of MSD and MPSIIIA. TFEB overexpression in these cells types resulted in a significant increase of LAMP1 on the plasma membrane and of lysosomal enzymes into the culture medium, hallmarks of lysosomal exocytosis. This indicates that LSD cells efficiently respond to TFEB-mediated induction of lysosomal exocytosis. Therefore, we evaluated the effect of TFEB overexpression on the clearance of GAGs in glia differentiated neuronal stem cells (NSCs) isolated from mouse models of MSD and MPSIIIA. TFEB overexpression resulted in a striking reduction of alcian blue-stained GAGs in both MSD and MPSIIIA NSC-derived glial cells (Figure 1A). The latter result was further confirmed by pulse-and-chase experiments using H3 glucosamine to label GAGs, showing a significant reduction of the levels of labeled GAGs

after 48 hr of chase in both MSD and MPSIIIA NSC-derived glial cells overexpressing TFEB (Figure 1B). Finally, EM analysis revealed that TFEB-mediated clearance of GAGs in TFEB-overexpressing MSD and MPS-III A cells was associated with both significant reduction of cellular vacuolization and recovery of normal cellular morphology (Figure 1C). These results indicate that TFEB overexpression results in an increased lysosomal exocytosis that leads to increased cellular clearance.

We have completed the generation of an adeno-associated type 2/9 virus (AAV2/9) that carries TFEB-3xflag under the control of a strong TBG promoter. As a first pilot study we injected this vector systemically into adult multiple sulfatase deficiency (MSD) mice. This mouse model allowed us to treat the mice with a systemic injection, that is technically less challenging than direct intra-cerebral injections. To this end, we injected systemically AAV2/9-TFEB-3xflag into adult MSD mice. One month after injection, several tissues were collected to monitor transduction efficiency and GAG storage. AAV-mediated TFEB delivery resulted in efficient TFEB transduction and significant reduction of GAG staining in liver and skeletal muscles, as detected by alcian blue staining and GAG quantification (Figure 2A,B). Subsequently, we investigated whether TFEB-mediated clearance of GAGs resulted in the reduction of the pathologic hallmarks of MSD, such as macrophage infiltration and apoptosis. We found a striking reduction of CD68-positive cells in AAV-TFEB injected MSD mice compared with untreated mice (Figure 2C). Most importantly, we also observed a significant reduction of TUNEL-positive cells (Figure 2D) (Medina et al. *Development Cell* [Volume 21, Issue 3](#), 421-430). These results indicate that TFEB activation of lysosomal exocytosis reduced both primary accumulation of GAGs and secondary pathological processes associated with LSDs such as inflammation and cell death. Our next challenge is to treat MPSIIIA mice with this vector in the brain directly.

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**Dr. Alisdair B. Boraston**

**Department of Biochemistry and Microbiology, University of Victoria, Victoria, Canada**  
**Discovery and assessment of inhibitor-based chemical chaperones as potential agents for the treatment of mucopolysaccharidosis IIIB.**

**HYPOTHESIS** - Mutant forms of human NAGLU, which cause the MPS IIIB phenotype, are destabilized by the mutations, but not rendered non-functional, and can be chaperoned to the lysosome by specific inhibitors of  $\alpha$ -N-acetylglucosaminidase (NAGLU) inhibitors (chemical chaperones) to result in elevated levels of lysosomal NAGLU activity.

**GENERAL APPROACH** - 1) Generation of potent and selective inhibitors of NAGLU. We are combining synthetic chemistry and X-ray crystallographic analysis of a model protein in complex with synthesized inhibitors to generate compounds that are selective for NAGLU. 2) Candidate inhibitors are being assessed in a Chinese Hamster Ovary (CHO) cell model of MPSIIIB. The readout in this model assay is increased NAGLU concentration and activity in lysosomes upon treatment with our compounds.

**BACKGROUND RESULTS** - Prior to receiving funding from NMPSS we established using biochemical, structural, and cellular assays that inhibitory compounds based on piperidine and indolizine scaffolds could be made and would function as reasonably effective and selective chemical chaperones in our cellular assay, providing the basis for this work. Our endeavours were to focus on expanding the number of compounds that display the required properties for chaperones namely affinity, solubility, bioavailability and selectivity as well as expanding the model system to include other *naglu* mutations.

**PROGRESS** - Towards investigating inhibitor scaffolds using synthetic methodologies that potentially could result in compounds that are potent inhibitors of NAGLU, we have prepared a large number of compounds and are at different stages of their evaluation as inhibitors of NAGLU. Efforts to date have centered on the use of scaffolds demonstrated to be important in inhibiting glycosidases in general. Due to the synthetic difficulties that have resulted in some of the scaffolds syntheses, during the study we decided to first focus on hydroximolactone and piperidine scaffolds (see above figure) as these could be prepared in a more robust fashion.

We have completed the synthesis of a library of compounds for these scaffolds and are at different stages of evaluating them as inhibitors of NAGLU. Initial results have shown whilst they are modestly potent against NAGLU, these studies and the evaluation of roughly 20 crystal structures of bacterial NAGLU in complex with these inhibitors reveal them to lack selectivity for the enzyme over a functionally related human enzyme. This information, although disappointing, will guide us in the future preparation of selective compounds for NAGLU.

Despite the compounds above not being selective for NAGLU, we have been assessing the chemical chaperone potential of them in a CHO cell model of MPSIIIB that incorporates a single known mutation of *naglu*. We have assayed the most potent compounds found to date as chemical chaperones in the model against the six mutants that we had prior to receiving funding from NMPSS. Nine new mutants have been prepared in this study, bringing the total to fifteen mutants in our library. Again it was disappointing to find that of the compounds that have been assayed, even though they were not toxic at high concentrations, they were not able to increase the levels of mutant NAGLU activity above control levels with any of our mutants. The nine new mutants were also not active against our initial piperidine and indolizine compounds that we had prior to receiving funding from NMPSS. We are now assessing the observed weaker binding compounds of NAGLU, as well we will assess the compounds that are yet to be evaluated as inhibitors of NAGLU, as chemical chaperones in future work. These results have also guided us in the future development of compounds that display the required properties for chaperones namely affinity, solubility and bioavailability.