1. Executive summary – Progress Report – 6th-12th month period

Lysosomal storage diseases (LSDs) are genetic disorders causes by mutations in genes encoding specific proteins crucial for lysosomal function. The almost 60 LSDs are individually rare, however, collectively, their incidence is ~1/4,000-7,000, making LSDs a prevalent group of genetic diseases (Meikle *et al.* 1999; Pinto R *et al.* 2004). Patients' symptoms are not manifested unless mutant enzyme functions below approximately 90% of its normal activity. Thus, increasing the residual enzyme activity above the critical threshold (~10%) will prevent lysosomal dysfunction, and thus arrest disease process both at a cellular and clinical level.
Current therapies available for LSDs, including enzyme replacement therapy (ERT), have been able efficacious in the treatment of non-neurological symptoms. Therapies based on small molecules, which could cross the blood-brain barrier, are an attractive approach to complement the ERT by addressing the neurological manifestations of these disorders. Small molecules function enhancing the enzymatic activity by physically binding, and stabilizing misfolded mutant enzymes in the endoplasmic reticulum (ER), thus allowing them to escape the ER associated degradation (ERAD), and reach the lysosome. **The main hypothesis of the project is that small molecules are able to enhance the residual activity of deficient lysosomal enzymes in patients with LSDs by two modes: (i) direct interactions with the mutant enzyme and (ii) indirect effect on different cellular pathways resulting in increased levels of the mutant and partially functional enzyme in the lysosomal compartment.** The latter compounds (ii) are most likely to be identified through cellular assays, which offer a more physiological environment including the full constellation of regulatory networks involved in protein folding, maturation and trafficking. **To test this hypothesis, I plan to identify classes of small molecules able to rescue mutant forms of arylsulfatase A (ASA), the lysosomal enzyme deficient in metachromatic leukodystrophy (MLD) - a neurodegenerative LSD for which specific treatment is not available. To achieve this goal, I aim to: (i) develop a robust and reproducible cell-based high throughput screening (HTS) assay to identify small molecules that function as "enzyme enhancers" for ASA by both mechanisms described above; (ii) to adapt the ASA assay to a quantitative HTS assay in a 1,536-well plate format.**
As demonstrated in last progress note, we are developing a cell-based HTS assay using transformed cultured skin fibroblasts from patients with MLD. The candidate compounds identified in this screening will then be validated using different assays we have also been developing for the characterization of these compounds and how they function as enzyme enhancement agents for the mutant ASA.
I have been miniaturizing the quantitative cell-based assay into 1,536-well plate. The pilot screening using a molecular and structurally diverse 1,286-compound library was performed as in previous report.
This grant funding has been also utilized to develop validation and secondary assays, which are crucial for the characterization of small molecules identified in the primary screening. **As part of this project, during the 6th -12th month period, we have developed an ASA-cell expression system and the immuno-affinity column for purification of wild type and different ASA mutants.** These purified ASA from normal cells and also mutants will be essential to characterize potential ASA interactions of the candidate small molecules identified in the primary screening.