SPARC GIANT Proposal

Project Title - "Tuning Ceramide Routing to Modulate Membrane Stress: A Cross-Disease Study of ASMase in Sickle Cell Disease and Niemann–Pick"

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Proposal Details

"Tuning Ceramide Routing to Modulate Membrane Stress: A Cross-Disease Study of ASMase in Sickle Cell Disease and Niemann–Pick"

Background & Rationale

Sphingolipid metabolism, particularly the acid sphingomyelinase (ASMase)–ceramide axis, is central to membrane homeostasis. In Sickle Cell Disease (SCD), stress and inflammation hyperactivate ASMase, leading to ceramide-driven vesiculation and inflammation. While in Niemann–Pick Disease (NPD), loss of ASMase activity causes sphingomyelin accumulation, trafficking defects, and cell stress. Thus, SCD and NPD represent two ends of the ASMase activity spectrum. By leveraging our models and tools, we will test whether modulating ceramide routing with CERT inhibitors or supplementing with recombinant ASMase can restore balance in both contexts. We will further extend this rationale in *in-vivo* zebrafish models generated via CRISPR, enabling rapid genetic validation

Objectives

Objective 1.

- **a.** Define stress induced ASMase – ceramide dynamics in WT vs SCD erythroid precursors.
- **Approach**: Use Bristol erythroid precursor lines (WT and SCD) exposed to hypoxia/ reoxygenation ± inflammatory cytokines.
- **b.** Define genetically induced ASMase mutation-ceramide dynamics in WT & SCD erythroid precursors.
- **Approach:** Transfect the erythroid precursor cells with plasmid carrying ASMase mutation and generate ASMase-mutant SCD BEL-A clones.
- **Intervention:** CERT inhibitor and ASM inhibitor analogues.
- **Readouts:** Live-cell FRET probes for ASMase activity, ceramide trafficking (BODIPY-ceramide), membrane stress (Annexin V/PI, LDH release, vesiculation).
- **Expected outcome:** SCD cells show exaggerated ASMase activation and vesiculation under stress, which is normalized by CERT inhibition. Further, SCD precursor cells with

ASMase mutation exhibit reduction in ceramide content in plasma membrane, exposure of PS on membrane, and hypoxia-induced sickling.

Objective 2. Establish complementary NPD-like models and test recombinant ASMase rescue.

- **Approach:** Transiently express CRISPR-edited ASMase mutant plasmid in HEK/HeLa cells.
- **Intervention:** Treat with recombinant ASMase.
- **Readouts:** FRET-ASMase activity, ceramide redistribution (BODIPY-ceramide).
- **Expected outcome:** Recombinant ASMase restores enzyme activity and normalizes trafficking defects in mutant-expressing cells.

Objective 3. Demonstrate bidirectional therapeutic modulation of the ASMase–ceramide axis.

- **Approach:** Integrate SCD (gain-of-function) and NPD (loss-of-function) data to test whether CERT inhibition (dampening ceramide stress) and rASMase supplementation (replacing enzyme activity) act as reciprocal corrective levers.
- **Expected outcome:** Proof-of-principle that a single sphingolipid routing node (ASMase–ceramide–CERT) can be targeted in opposite ways to correct distinct disease phenotypes.

Objective 4. Engineer zebrafish lines carrying SCD and NPD mutations to enable precise modeling of disease phenotypes and therapeutic rescue strategies.

- **Approach**: Synthesize sgRNA by in vitro transcription, microinject sgRNA + Cas9 protein (or Cas9 mRNA) ± donor ssODN into **1-cell embryos**, assess mutagenesis/knock-in efficiency in pooled embryos (T7E1/HMA/sequencing), Raise F0 mosaics and outcross/intercross; screen offspring for phenotypes; genotype F1 by Sanger/NGS to confirm precise edits and mosaicism; establish stable homozygous lines through F1 inbreeding or allele crossing.

Readout in vivo

- Hematologic indices, RBC morphology/sickling under hypoxia/reoxygenation.
- **Live FRET sensors**: FRET-ASMase activity, ceramide redistribution (BODIPY-ceramide).

Interventions

- **CERT inhibitors** to down-route ceramide transfer; **rASMase** to modulate enzyme node; hypoxia paradigms.

Expected outcomes

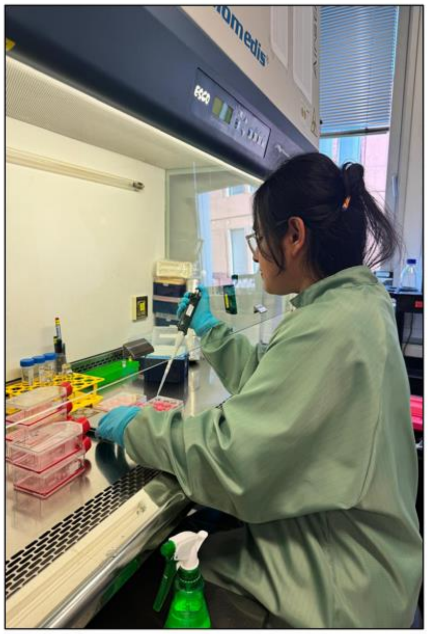
- Precise SCD knock-in recapitulates stress-provoked sickling and membrane stress; **CERT inhibition** reduces vesiculation/sickling in vivo.
- Smpd1 mutants display low ASMase activity, sphingomyelin accumulation, and trafficking stress; **rASMase** normalizes enzyme activity and rescues membrane phenotypes.

Innovation

- First direct comparison of SCD and NPD as mirror disorders of sphingolipid flux.
- Uses live-cell FRET ASMase probes, CERT inhibitors, and recombinant ASMase in a cohesive experimental system.
- Provides a rapid, mechanistic platform to evaluate therapeutic interventions targeting sphingolipid pathways.

Impact

This project will reveal how tuning ceramide routing either up or down can normalize erythroid stress phenotypes. The findings will provide a unified mechanistic framework for two seemingly distinct disorders and highlight novel intervention strategies.

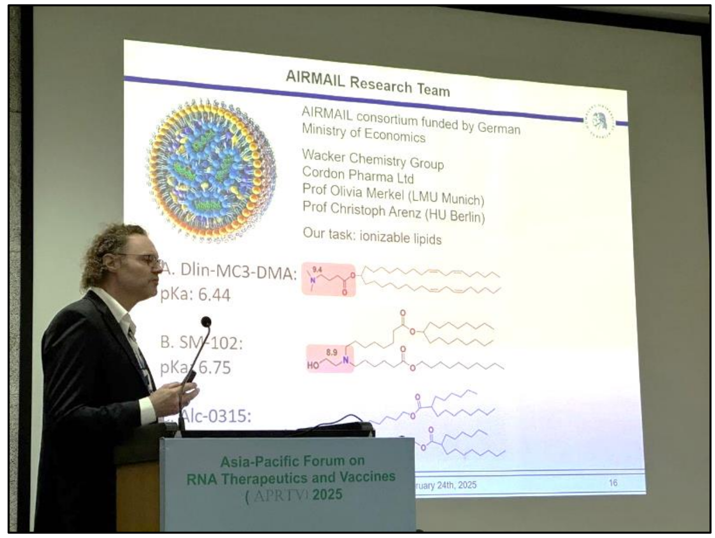


Humboldt University of Berlin, Germany

Special Center for Molecular Medicine JNU, India

Prof. Shailja Singh with her students at International Conference at Convention Centre, Jawaharlal Nehru University, New Delhi

Lab meeting of students at Special Centre for Molecular Medicine, JNU with PI Prof. Shailja Singh



Prof. Christoph Arenz (International PI) delivering a lecture at International Conference at Convention Centre, Jawaharlal Nehru University, New Delhi