# Development of a Novel Encapsulated Non-Viral Cell-Based Therapy for MPS VI

**Introduction**

- Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome) is caused by a deficiency of the lysosomal enzyme *arylsulfatase B* (ARSB).
- ARSB deficiency results in incomplete or blocked degradation of glucosaminoglycans (GAGs), which accumulate in the lysosome and disrupt normal cell function.
- Disruption of cell function manifests in symptoms of MPS VI:
  - Short stature, coarse facial features, stiff joints, breathing problems, difficulty walking, hip pain
  - Photo on the right shows rapidly progressing 16yr old male patient

**Hypothesis**

Better outcomes could be achieved with sustained, long-lasting hARSB levels via administration of hARSB-secreting allogeneic human cells shielded within spheres designed to avoid immune rejection and pericapsular fibrotic overgrowth (PFO) in the patient.

**Methods**

1. **Engineer cells to express hARSB**
2. **Evaluate hARSB secreted from cells**
3. **Encapsulate hARSB-secreting cells**
4. **Implant in MPS VI mice**

**Results**

**Establishing In Vitro Assays to Assess hARSB Function**

- **A** Optimizing hARSB activity in culture medium assay buffer matrix
  - 0% medium
  - 12.5% medium
  - 25% medium
  - 50% medium
  - 100% medium
- **B** hARSB Km
  - Vmax
  - Km = 0.38 mM
- **C** Relative dermatan sulfate levels in MPS VI patient fibroblasts
  - Unconditioned Control
  - Conditioned medium containing hARSB

**Panels A&B: Biochemical Analysis of hARSB**

- Optimization of enzymatic assay helped to establish Km for hARSB = 0.38 mM of substrate
- Panel C: hARSB uptake into MPS VI patient fibroblasts
  - Dermatan sulfate (DS) levels are reduced after exposure to culture medium containing hARSB produced by engineered cells

**Encapsulated Cell Line Secretes Active hARSB**

**Results (cont’d)**

<table>
<thead>
<tr>
<th>Strategies to Enhance Secretion of hARSB</th>
<th>Signal Peptide</th>
<th>Point Mutations in M6P Glycosylation Sites</th>
<th>Co-Expression with PTM Enzymes</th>
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<tbody>
<tr>
<td>hARSB production in engineered cell lines</td>
<td>hARSB production in engineered cell lines</td>
<td>hARSB production in cell lines engineered to co-express Post-Translational Modifying Enzyme</td>
<td>hARSB production in engineered cell lines</td>
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</tbody>
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- The secretion was assessed by Western Blotting and found to be consistent with the production results above (data not shown)
- Removing M6P glycosylation sites did not result in improved hARSB production and secretion
- Co-expression of hARSB with enzymes affecting post-translation modifications (PTM, right panel), yielded cell lines with highest production and secretion levels of hARSB

**Substrate Reduction in MPS VI Mouse Tissues**

- Treatment of MPS VI mice with an encapsulated cell line secreting active hARSB results in substrate reduction 7 days after administration