Preclinical Experience with rhASM for Niemann-Pick Disease

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Shani  Niemann-Pick disease Type B  UK
Acid Sphingomyelinase Deficiency (ASMD)

- Includes Niemann-Pick disease types A and B
- Autosomal recessive inheritance
- Birth prevalence ~1/250,000
- Panethnic
- Accumulation of sphingomyelin in monocyte/macrophage cell lineage of reticuloendothelial system (foam cells)
- Premature death from cirrhosis, respiratory compromise, bleeding, heart disease
- Serious and life-threatening with unmet medical need
ASMD Clinical Spectrum

Type A
- <1% residual ASM activity
- acute neurodegeneration
- severe somatic disease
- Ashkenazi Jewish predilection
- death by age 3

Type B
- ~5-10% residual ASM activity
- no neurological disease
- variable onset and features
- hepatosplenomegaly
- thrombocytopenia
- interstitial lung disease
- liver fibrosis
- pro-atherogenic lipid profile
- fatigue, pain, dyspnea
- death in teens/adulthood

Type A/B - chronic neurological disease
Recombinant Human Acid Sphingomyelinase (rhASM) Enzyme Replacement Therapy

Sphingomyelin

\[
\text{CH}_2\text{OPO}_3\text{CH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3
\]

Ceramide

\[
\text{CH}_2\text{O}
\]

Acid Sphingomyelinase

\[
\text{PO}_3\text{CH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3
\]

Phosphorylcholine
Acid Sphingomyelinase Knock-out Mouse ASMKO – Preclinical Efficacy

- No residual ASM activity leads to phenotype between NPD A and NPD B
- Ataxia and tremor at 4 mo, death by 6-8 mo
- Accumulates sphingomyelin in relevant organs, e.g. liver, spleen, and lungs, but less than in patients - no hepatosplenomegaly or hematological abnormalities
- rhASM reduced sphingomyelin to a lesser extent in lung than spleen and liver

Liver | Lung | Spleen
--- | --- | ---
A | B | C
D | E | F
G | H | I

5 mg/kg rhASM QOD x 4 doses

Miranda, FASEB J 2000;14:1988-95
Proof of Concept Confirmed in ASMKO Mouse

Dose-responsive reductions in liver sphingomyelin following biweekly dosing of rhASM in AMSKO mice for 13 weeks.
Preclinical IND Safety Package in 2003

• Conducted 2 single IV dose toxicity studies
  - Sprague-Dawley rat – NOAEL > 30 mg/kg
  - Beagle dog – NOAEL > 30 mg/kg

• Conducted 26 week IV repeat dose toxicity study
  - Sprague-Dawley rat – NOAEL > 30 mg/kg

• Conducted a safety pharmacology study
  - Beagle dog – NOAEL > 30 mg/kg

➢ No significant findings in any “normal” animals
Can Lung Efficacy be Improved with Higher Doses of rhASM?

• Prior to filing the IND in 2003, an effort was made to increase the efficacy in the lung of ASMKO mice

• Research studies conducted in ASMKO mice with high doses of rhASM resulted in dose-responsive lethargy, unwillingness to move, coldness to touch, and death

• Dose-responsive deaths in ASMKO mice
  - 30 mg/kg – all died at 4-6 hours post-dose
  - 20 mg/kg – all died at 12-24 hours
  - 10 mg/kg - 50% died at 48 -72 hours
Is Toxicity Due to rhASM or Breakdown Products?

- Hypothesized that rapid degradation of substrate and production of downstream mediators are responsible for the toxicity

- Ceramide and sphingosine
  - Inflammation
  - Pro-apoptotic
  - Direct cardiovascular effects

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Sphingomyelin

enzyme
acid sphingomyelinase

Ceramide

enzyme
ceramidase

Phosphorylcholine

caspases
apoptosis

cytokines

Sphingosine

Cardiotoxic effects
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Single-dose Toxicity Studies Conducted in ASMKO and C57BL/6 (Wild-type) Mice

• Single IV doses – 0, 0.1, 0.3, 1, 3, 10 mg/kg

• C57BL/6 (Wild-type)
  – No clinical observations of toxicity
  – No adverse effects noted during histopathological examination

• ASMKO
  – Death and shock-like observations at highest dose (10 mg/kg)
  – Dose-responsive histopathology in liver and adrenal glands at doses of ≥0.3 mg/kg
    – Findings reversible at doses up to 10 mg/kg
  – Single dose NOAEL = 0.3 mg/kg supported a starting dose of 0.03 mg/kg in patients with 10-fold safety margin
Dose-Responsive Histopathological Changes in Liver of ASMKO Mice After 10 mg/kg rhASM

Ballooning degeneration

Inflammatory foci
Dose-responsive Histopathological Changes in Adrenals of ASMKO Mice after 10 mg/kg rhASM

Adrenal hemorrhage (B) and renal tubular necrosis lesions (not shown) may have resulted from reduced blood flow to these organs, indicating that a hypotensive event or shock occurred.
Can Debulking with Low Doses of rhASM Prevent Toxicity of a High Dose in ASMKO Mice?

- All mice appeared normal for duration of study and survived to designated time points with no significant histopathological findings.
- Toxicity related to the amount of sphingomyelin present and its rate of degradation.

Day 1: 3 mg/kg QOD
Day 8: 20 mg/kg
Day 11: SAC 4 days post-dose n=4
Day 14: SAC 7 days post-dose n=2
What is Causing rhASM Toxicity?

• Explored mechanism of toxicity including the role of ceramide

• Identified biomarkers to monitor toxicity in the clinic
  - Cytokine studies
  - Telemetry
Does rhASM Increase Plasma Ceramide Levels in ASMKO Mice?

Biphasic rise in ASMKO mice, but not C57 mice

First rise was similar between ASMKO and C57 mice, while the second rise in ASMKO mice was sustained through 540 minutes.
Does rhASM Stimulate Pro-inflammatory Cytokines in ASMKO Mice?

- ASMKO Mice (Female), 8-10 weeks of age
  - Single doses of rhASM - 0.3, 3, 10, and 20 mg/kg
  - Cytokines evaluated 2, 3, 4, 6, and 9 hours post-dose
- Key cytokines were up-regulated in the ASMKO mouse following rhASM administration (≥ 3 mg/kg rhASM)
  - IL-6, IL-1
    - Induce acute phase inflammation responses
    - Increase cellular response to other cytokines
    - Pro-inflammatory
    - Known cardiac effects
  - G-CSF
    - Stimulates neutrophil proliferation
  - KC (human IL-8 homologue)
    - Proinflammatory
  - MIP-1α
    - Chemoattractant, neutrophils
rhASM Causes Dose-responsive Increases in Cytokine Levels

Rises from baseline in IL-6 (1000x), G-CSF (500x), and KC (100x)
Does Debulking with Low Doses of rhASM Prevent Cytokine Response with High Dose in ASMKO Mice?

Ceramide levels also became attenuated after first dose.
Does rhASM Alter Cardiovascular Hemodynamics in ASMKO Mice?

Telemetered ASMKO mice demonstrated dose-responsive reductions in heart rate and blood pressure.

- Reduction in heart rate after 45 min coincided with lethargy and reduced activity.
- Prolonged reduction in blood pressure coincided with histopathological changes in adrenals and kidneys.

Murray, 2014, Mol Genet Metab, epub
Phase 1 Single-Dose Study Demonstrated Dose-Dependent Rises in Ceramide in NPD B Patients

Ceramide, mg/mL

Timepoint

Pre-Inf 15m EOL 15m 30m 45m 60m 2h 3h 4h 6h 8h 12h 18h 24h 48h 72h 14 d

012112-1.0
012010-0.6
012313-0.6
010807-0.3
011509-0.3
010202-0.03
010503-0.03
010401-0.03
010304-0.1
010605-0.1
010906-0.1
Phase 1 Single-Dose Study Demonstrated Dose-Dependent Rises in CRP in NPD B Patients

Acute phase response observed in patients receiving ≥ 0.3 mg/kg rhASM
Can Debulking with Low Doses of rhASM Prevent Toxicity after Multiple High Doses?

- In ASMKO mice, repeat-dose NOAEL = 3 mg/kg, which supported a maximum dose of only 0.3 mg/kg QOW x 13 weeks with 10-fold safety margin.
- Study demonstrated that repeat doses of rhASM at 3, 10, or 30 mg/kg could be safely administered to ASMKO mice following a rhASM debulking regimen.
- After debulking, NOAEL ≥30 mg/kg in male and female ASMKO mice.
- Supports chronic dosing in patients up to 3 mg/kg with 10-fold safety margin.
Conclusions

• While normal animals are pharmacologically relevant, the ASMKO mouse proved to be the most sensitive and most relevant animal because of substrate-based toxicity.

• Toxicity in ASMKO mice could be prevented by gradual debulking, leading to within-patient dose escalation strategy.

• Several safety biomarkers were identified from ASMKO mouse studies and incorporated into patient studies:
  – Ceramide, inflammatory mediators (CRP, cytokines)
  – 72-hour inpatient telemetry

• Patient studies have shown some acute phase reactions:
  – Phase 1a: single dose MTD = 0.6 mg/kg
  – Phase 1b: all 5 patients successfully escalated to 3 mg/kg
Limitations of ASMKO Animal Model

- Disease not exactly recapitulated
- Not possible to complete 26-week chronic tox study in ASMKO mice because of fragility and limited lifespan
- Cross-species differences in lipid metabolism
- Administration differs - bolus injections of rhASM in mice compared to infusions in dogs, monkeys, and patients
- Hypersensitivity reactions, formation of antibodies to human protein, after 2nd dose