COMPARABILITY CONSIDERATIONS IN THE DEVELOPMENT OF GENE THERAPY PRODUCTS

CASSS Cell & Gene Therapy Products (CGTP):
Manufacturing, Quality and Regulatory Considerations

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Clinical Results

• Safety
• Reduction in Annualized Bleeding Rate and Use of Recombinant Factors
• Increase in Quality of Life (QOL) Measures
• Sustained Factor VIII Levels 2 Years After a Single Infusion of BMN 270
• Summary and Next Steps

Process Comparability: Factors That Impact Potency

• Capsid Protein Content
• Deamidation of Capsid Proteins
• Presence of Empty Capsids
HEMOPHILIA IS THE OLDEST DESCRIBED GENETIC DISORDER

“. .if the first son of a woman is circumcised and he dies and the second son is circumcised and he dies, you must not circumcise the third son. . additionally, the sons of the woman’s sister should not be circumcised but the sons of her brother can be circumcised.” Talmud (Yebamot 64b)
Therapeutic Value

Evolution of Products

**EVOLUTION OF HEMOPHILIA TREATMENT**

**Plasma-Derived Clotting Factors (1969)**

**Recombinant Clotting Factors**

- FVIII, FIX, FVIIa (1990s)
- EHL clotting factors (2014 - )
- Gene Therapy (2015 – )

**Biosimilars**
- Humanized
- Prolonged Half-Life (FVIII/FIX)

**Improved Safety**
- Eliminated Transmission of Blood Borne Pathogens

**Widespread Viral Contamination**

**Potential for a “One and Done” Treatment**

**Recombinant Era**
FAVORABLE SAFETY PROFILE FOR BMN 270

• Transient transaminitis
  • 73% of subjects; now all resolved
    • 93% Grade 1, 7% Grade 2; Alanine Aminotransferase (ALT) elevation
    • Median onset at 7.6 weeks; duration <25.3 weeks post onset

• All patients are off corticosteroids

• No patients developed inhibitors to FVIII; no subject withdrew

• 2 SAEs, both self-limited
  • Pyrexia, resolved overnight
  • Total knee replacement for pre-existing arthropathy

• Most common other AEs across all dose cohorts:
  • Arthralgia (60%); headache (47%), back pain (40%)
  • Viral upper respiratory tract infection (40%), fatigue, insomnia, pain in extremity (33%)
SUBSTANTIAL REDUCTION IN TREATED ANNUALIZED BLEED RATE (ABR)
STARTING FROM 4 WEEKS POST-INFUSION

6e13 Dose Through 104 Weeks; N=7

97% REDUCTION in MEAN ABR

<table>
<thead>
<tr>
<th>ABR (episodes/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
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<tbody>
<tr>
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<td>median</td>
<td>mean</td>
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<tr>
<td>Pre-infusion</td>
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<td>0</td>
</tr>
<tr>
<td>Post-infusion</td>
<td>16.3</td>
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% Patients Bleed Free

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<tr>
<th></th>
<th>Baseline</th>
<th>Year 1</th>
<th>Year 2</th>
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<tbody>
<tr>
<td>%</td>
<td>15%</td>
<td>71%</td>
<td>86%</td>
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All patients off prophylaxis
100% resolution in target joints

4e13 Dose Through 52 Weeks; N=6

92% REDUCTION in MEAN ABR

<table>
<thead>
<tr>
<th>ABR (episodes/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
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<td>median</td>
<td>mean</td>
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<tr>
<td>Pre-infusion</td>
<td>8</td>
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</tr>
<tr>
<td>Post-infusion</td>
<td>12.2</td>
<td>1</td>
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% Patients Bleed Free

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Year 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>17%</td>
<td>83%</td>
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</table>

All patients off prophylaxis
SUBSTANTIAL REDUCTION IN MEAN ANNUALIZED FVIII USAGE
STARTING FROM 4 WEEKS POST-INFUSION

6e13 vg/kg Dose Through Week 104; N=7

96% REDUCTION in MEAN FVIII USAGE

4e13 vg/kg Dose Through Week 52; N=6

98% REDUCTION in MEAN FVIII USAGE
BMN 270 SUBSTANTIALLY IMPROVED QUALITY OF LIFE

QOL improvement observed in all 6 domains:
- Consequences of Bleeding
- Emotional Impact
- Physical Functioning
- Role Functioning
- Treatment Concern
- Worry

MEAN FVIII ACTIVITY LEVELS SETTLING IN NORMAL RANGE (6e13 VG/KG)

No FVIII Activity Above Upper Limit of Normal at 2 Years

The upper and lower box bounds represent 25th and 75th percentiles. The whisker lines represent the minimum and maximum values.
MEAN FVIII ACTIVITY LEVELS AT HIGH END OF MILD RANGE (4e13 VG/KG)

No FVIII Activity Above Normal at 1 Year

The upper and lower box bounds represent 25th and 75th percentiles. The whisker lines represent the minimum and maximum values.
SUMMARY OF CLINICAL RESULTS

- BMN 270 was Well-Tolerated with Favorable Safety Profile; Currently:
  - No subjects in either cohort have FVIII activity levels above the upper limit of normal
  - ALT levels within normal limits in all subjects
  - All subjects remain off corticosteroids
  - No inhibitors to FVIII
  - Only 2 serious adverse events as previously reported

- Annualize Bleeding Rate: Profound Reduction with Both Cohorts
  - 6e13 vg/kg sustained 2 years, 4e13 vg/kg sustained 1 year and counting

- FVIII Usage: Profound Reduction with Both 6e13 and 4e13 vg/kg Cohorts

- Quality of Life Improvement in Six Domains Reflects:
  - Cessation of Bleeding, Freedom From Worry, Independence From Treatment and Improved Functioning

- FVIII Activity Levels
  - 6e13 vg/kg cohort, settling within normal range
  - 4e13 vg/kg cohort, at upper range of mild hemophilia

- Patterns of FVIII Activity Levels Consistent with Other Clinical and Pre-Clinical Reports

- Gene Therapy Has the Potential to Transform the Standard of Care in Hemophilia A
NEXT STEPS

• New goal is to prove superiority of BMN 270 to prophylactic therapy

• GENEr8-1 (6e13 vg/kg) sample size now powered for superiority
  – 90% power to demonstrate reduction in bleeding events from ABR of 3.5 to 1.0
  – N = 130 (90 additional patients)
  – Expect to complete enrollment in Q1 2019

• GENEr8-2 (4e13 vg/kg) study design unchanged
  – N = 40
  – FVIII primary endpoint
  – Targeted to finish enrollment 1-2 quarters after GENEr8-1

• Comprehensive program underway
  – AAV5+ study initiated
  – Global seroprevalance study
  – Ongoing follow-up of 201 patients
  – Initiating use of full commercial scale material from BioMarin manufacturing facility
Process Comparability: Factors That Can Impact Potency

• Capsid Protein Content

• Deamidation of Capsid Proteins

• Presence of Empty Capsids
Potency Assay Overview

• Method Details
  – Cell-based assay confirms mechanism of action and enables monitoring of consistency and comparability between processes
  – Relies on vg titer to determine assay loading
  – Readout is a Potency Ratio (test sample read-out normalized to reference standard read-out).
  – Potency is assessed via measurement of FVIII activity and protein levels (separate potency ratios for both)

What is Biologically Required to Demonstrate Potency?

1. Effectively enter the cell
   a) Available Receptors
   b) Functional Capsid
2. Traffic DNA to the Nucleus
3. Forms complete Double Stranded DNA
4. FVIII Efficiently Expressed and Secreted (ELISA)
5. FVIII Activity (Chromogenic Assay)
   a) Requires effective expression, correct folding and posttranslational modifications
**Structural Assembly of AAV5 Capsid**

**Functional Role of Different Viral Proteins (VP) Constituents:**

- **Phospholipase A$_2$ (PLA$_2$):** Membrane Disruption of Endosome (VP1)
- **Basic Region (BR):** Putative Nuclear Localization Signals (VP1, VP2)
- **Receptor Binding Domain:** Capsid Interaction with Cell Surface Receptors (VP1, VP2 & VP3)
- **Capsid Assembly:** Icosahedral Structure (VP1, VP2 & VP3)

Mutagenesis of Adeno-Associated Virus Type 2 Capsid Protein VP1 Uncovers New Roles for Basic Amino Acids in Trafficking and Cell-Specific Transduction
Johnson et al. (2010) J.Virol. 84: 8888-8902

Impact of VP1-Specific Protein Sequence Motifs on Adeno-Associated Virus Type 2 Intracellular Trafficking and Nuclear Entry
Viral capsid proteins are detected using UV wavelength 214 and identified using mass spec analysis.
Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking
DEAMIDATION ANALYSIS BY LC/MS

Capsid particles → Denaturation → Viral protein mixture → Digestion → Peptides

Analysis → Mass Spectrometry → LC separation

Unmodified → Deamidated
Thermal degradation results in deamidation of the capsid virus proteins

In the RP-HPLC, deamidation results in the decrease of VP1 and VP3 peak areas and an increase of VP2 apparent peak area. It has been determined by various MS methods that deamidated VP3 species (shown here as VP3') co-eludes with VP2.
CORRELATION BETWEEN SITE SPECIFIC DEAMIDATION AND IN VITRO POTENCY

VP1 N-Terminal Site

Surface Site A

Surface Site B

Surface Site C

R² = 0.9742

R² = 0.9663

R² = 0.9908

R² = 0.9466
Transition Temperature Inflections Detected By Fluorescence Emission Increases Are Indicative of Capsid Instability
CAPSID STABILITY ANALYSIS AND CORRELATION WITH DEAMIDATION AND POTENCY

Initial Fluorescence and Transition Temperature Change Over Time Consistent With Capsid Instability

Initial Fluorescence

Transition Temperature

$R^2 = 0.9883$

$R^2 = 0.9457$
Initial Fluorescence Change is Correlated with % Deamidation Increase and Potency Decrease of Capsid Vectors
CAPSID STABILITY ANALYSIS AND CORRELATION WITH DEAMIDATION AND POTENCY

Transition Temperature Change is Correlated with % Deamidation Increase and Potency Decrease of Capsid Vectors

\[ R^2 = 0.9616 \]

\[ R^2 = 0.9390 \]
BIOMARIN PROCESS IS ABLE TO REMOVE EMPTY CAPSIDS TO UNDETECTABLE LEVELS

Electron Microscopy of Full Capsids

Electron Microscopy of Empty Capsids

BioMarin Phase 3/Commercial Process

Removal of Empty Capsids from Type 1 Adeno-Associated Virus Vector Stocks by Anion-Exchange Chromatography Potentiates Transgene Expression, Masashi et al., Molecular Therapy, Volume 13, Issue 4, April 2006, Pages 823-828
Empty capsids mixed with full capsids to generate material with increasing [CP:VG].

Cells were transduced with the same concentration of VG/cell regardless of CP:VG ratio.

Empty capsids result in reduced potency even at a constant viral genome.

Log transformed potency values exhibit a negative association with log transformed [CP:VG].

CP:VG ratios between 1 and 21 shown in graph above.

$R^2 = 0.9927$
SUMMARY OF COMPARABILITY APPROACH

• Biomimetic Cell Based Assays Most Appropriate for Measurement of Potency in Such a Complex Product
  • Cell Based Assays Monitors For:
    • Binding to the Cellular Receptor
    • Internalization of the Vector into the Cell
    • Release and Translocation of the Transgene to the Nuclease (Nucleolus)
    • Expression of Functional Factor VIII as a Clinically Meaningful Readout

• Capsid Protein Content and Potency Assays Used to Monitor Comparability During
  • First In-Human Study
  • Process Robustness Initiatives
  • Facility & Scale Modifications

• Deamidation of Capsid Proteins
  • Strong Negative Correlation Between Deamidation and Potency

• Empty Capsid Content
  • Strong Negative Correlation Between Empty Capsid Content and Potency
  • Empty Capsid Results in Reduced Potency Even at a Constant Viral Genome Level
ELEMENTS AND BENEFITS OF A COMPREHENSIVE COMPARABILITY STRATEGY HAVE INCLUDED

PROCESS DEVELOPMENT
- We Have Developed a Robust Vector Manufacturing Process Consistent With ICH Guidance Facilitating World Wide Registration

ANALYTICAL CHARACTERIZATION
- We Have Developed a Battery of Biochemical, Cell Based and Animal Based Methods Consistent With ICH Guidance
- These Methods Provide Comprehensive Characterization of Purity, Potency, Consistency and Comparability

SCALE
- Fermentation, Purification & Filling Operations Performed at Commercial Scale Demonstrating Comparability

FACILITY
- Constructed, Commissioned and GMP Operational to Support Intended Commercial Production
- Recognized as ISPE 2018 Facility of the Year – Project Execution Category
- Material Generated Currently Supporting Clinical Development and Capable of Supporting Projected Commercial Demand
- Capable of Supporting at Least 3,000 Patients Per Year at the Highest Clinical Dose Tested to Date
THANK YOU