Hemophilia B Gene Therapy in Mice using a Novel Chimeric AAV Capsid Combined with a Potency Enhanced FIX Variant
Essential Medicines – Superior Outcomes

**Late-Stage Asset**
SQ Marzepacog alfa (activated)
MarzAA (FVIIa)
Phase 3 Ready

**Hemophilia**
SQ MarzAA (FVIIa)
SQ Dalcinonacog alfa – DalcA (FIX)
Factor IX Gene Therapy
Factor Xa

**Complement**
IVT Anti-C3 Dry AMD
CB 2782-PEG
SQ Systemic Complement Inhibitors

Protease Engineering Platform
Presentation outline

New approaches to FIX gene therapy in hemophilia B

+ What patients are looking for in hemophilia treatment
+ The need for improved gene therapy delivery
+ Taking a combined approach to improving FIX gene therapy
+ The origin of the CB 2679d-GT candidate
+ Proof of concept for CB 2679d-GT vs Padua: Collaboration with Vrije University, Brussels
+ A novel chimeric capsid combined with CB 2679d-GT: Collaboration with Stanford University
+ Conclusions
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Goal of gene therapy for hemophilia: Normal bleeding

Patients are seeking sustained clotting factor activity to normalize phenotype.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Bleeds Annually</th>
<th>Protection from Spontaneous Hemarthrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>~30</td>
<td>Protection at &gt;12% Activity</td>
</tr>
<tr>
<td>Moderate</td>
<td>~15-20</td>
<td>Protection at &gt;12% Activity</td>
</tr>
<tr>
<td>Mild</td>
<td>~5</td>
<td>Protection at &gt;12% Activity</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>Protection at &gt;12% Activity</td>
</tr>
</tbody>
</table>
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The next generation of gene therapy in hemophilia B

First generation gene therapies are moving to approval yet there is room for innovation

- Risk of vector dose-limiting toxicity
- Immunogenicity to the vector
- Higher transduction efficiency
- Liver inflammation

Need for more efficient vectors & lower dosing regimen
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How do we achieve a normal bleeding phenotype?

Combining optimized capsid + transgene = improved therapy

**Engineered Capsid**
- High liver tropism
- Transduction efficiency
- Translatable from preclinical to clinic

**Novel Transgene**
- High potency
- Improved efficacy

**Lower AAV Dose**
- Achieve clinically relevant levels
- Reduced viral load

Lower Immunogenicity
Decrease Liver Toxicity
Lower Manufacturing Costs
Lower AAV Dose
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Dalcinonacog alfa: a novel SQ FIX product

Three substitutions within the FIX protein
+ Increased catalytic activity
+ Higher affinity for FVIIIa
+ Resistance to antithrombin inhibition
+ 22-fold increased potency over BeneFIX

Differentiated from marketed IV FIXs
+ Simple, small volume SQ administration
+ Enhanced pharmacokinetics with prolonged half-life
+ Excellent extravascular distribution
+ Potential to maintain continuous protective levels

Orphan Drug Designation in US & EU
Capitalizing on the gene behind dalcinonacog alfa

**Dalcinonacog alfa**
- Recombinant FIX
- Subcutaneous delivery
- Currently in Phase 2b
- Sustained factor levels

**CB 2679d-GT**
- Gene therapy
- AAV delivery
- Preclinical
Improved functionality of CB2679d-GT drives high potency

When combined these properties provide a 22-fold enhanced potency in SQ clinical trials

(**** P<0.0001, *** P<0.001, ** P<0.01 and * P<0.05)
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AAV vector design of CB 2679d-GT in a DJ/8 capsid

- Padua (R338L)
- CB 2679d-GT (R318Y/R338E/T343R)

FIX minigene constructs were packaged into a DJ/8 AAV capsid
Dose dependent and stable FIX levels observed for 20 weeks

(Activity levels determined by OSA with HemosIL and a WHO standard)
CB 2679d-GT reduces total blood loss more than Padua

2.5 x 10^{11} \text{ vg/kg}

5.0 x 10^{11} \text{ vg/kg}

CB 2679d-GT has an ~4-fold reduction in blood loss

(Student’s T-Test: *** P<0.001, ** P<0.01, * P<0.05 and NS – Not Significant)
CB 2679d-GT reduces bleeding time more than Padua

2.5 x 10^{11} \text{ vg/kg}

CB 2679d-GT has an ~5 to 8-fold reduction in bleeding time

(Student's T-Test: *** P<0.001, ** P<0.01, * P<0.05 and NS – Not Significant)

5.0 x 10^{11} \text{ vg/kg}
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DNA shuffling to create a novel AAV vector

DNA shuffling of 8 serotypes

Select for tropism & increased transduction efficiency

Chimeric Capsids

High performing AAV capsid candidates
AAV vector design of CB 2679d-GT in a novel capsid

- Wild-Type FIX
- Padua (R338L)
- CB 2679d-GT (R318Y/R338E/T343R)

FIX minigene constructs were packaged into a novel AAV capsid designed through DNA shuffling of 8 AAV serotypes and showing a high tropism for liver transduction.
FIX antigen levels remained stable

Dose dependent and stable FIX antigen observed for up to 18 weeks

- 8.0 x 10^9 vg/kg
- 8.0 x 10^{10} vg/kg
- 8.0 x 10^{11} vg/kg

(Activity levels determined by OSA with HemosIL and a WHO standard)
FIX activity levels remained stable

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Activity Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0 x 10⁹ vg/kg</td>
<td>8.0 x 10ⁱ⁰ vg/kg</td>
</tr>
</tbody>
</table>

Dose dependent and stable FIX activity levels observed for up to 18 weeks

(Activity levels determined by OSA with HemosIL and a WHO standard)
CB 2679d-GT in combination with a novel chimeric capsid provides a significant improvement in FIX activity levels

Mid-dose data at $8.0 \times 10^{10}$ vg/kg

<table>
<thead>
<tr>
<th>FIX Transgene</th>
<th>AAV Capsid</th>
<th>Study Dose (vg/kg)</th>
<th>FIX Activity (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB 2679d-GT</td>
<td>Novel Chimeric</td>
<td>$8.0 \times 10^{10}$</td>
<td>20</td>
</tr>
<tr>
<td>Padua</td>
<td>TAK-748$^2$</td>
<td>$7.4 \times 10^{11}$</td>
<td>20</td>
</tr>
<tr>
<td>Padua</td>
<td>TAK-748$^2$</td>
<td>$7.4 \times 10^{10}$</td>
<td>1</td>
</tr>
<tr>
<td>CB 2679d-GT</td>
<td>DJ/8$^1$</td>
<td>$2.0 \times 10^{11}$</td>
<td>4</td>
</tr>
<tr>
<td>CB 2679d-GT</td>
<td>DJ/8$^1$</td>
<td>$4.0 \times 10^{10}$</td>
<td>1</td>
</tr>
</tbody>
</table>


What could account for the shortened bleeding time with CB 2679d-GT?

+ Does CB 2679d-GT improve clot structure?
+ Is the clot more stable?
+ Does CB 2679d-GT have a faster time to clot?
+ What functional properties of CB 2679d-GT contribute to the shorter bleeding time?
  - Resistance to antithrombin inhibition?

What is the durability of CB 2679d-GT when delivered in a novel AAV vector?

+ Durability in non-human primates
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AAV CB 2679d-GT is superior to AAV Padua

CB 2679d-GT demonstrates superior preclinical potency vs Padua

- CB 2679d-GT differentiated from FIX Padua by further increased FIX activity
- A novel chimeric capsid demonstrates high FIX levels with lower AAV dose
- FIX antigen/activity levels stable and durable for up to 20 weeks in hemophilia B mice
- CB 2679d-GT achieved a more rapid and robust hemostatic correction than Padua
- Program now progressing to non-human primate studies
THANK YOU