A Comprehensive *In Silico* and *In Vitro* Immunogenicity Risk Assessment of Dalcinonacog Alfa Shows No Increased Risk Compared with Wild-Type FIX

Grant E. Blouse, Ph.D., M.Sc.
VP Translational Research
Dalcinonacog alfa

Dalcinonacog alfa, a novel clinical stage SQ FIX product candidate differentiated from IV market leaders:

+ Simpler, less painful, small dose
+ SQ enhances pharmacokinetics
+ Potential to maintain continuous protective levels
+ Disruptive to all current intravenous products
+ Especially well suited for children

Three point mutations in two loops within the FIX protein:

+ Catalytic activity increased
+ Affinity for activated factor VIII increased
+ Resistance to inhibition by antithrombin improved

Best-in-class high-potency recombinant FIX product

+ 22-fold more potent than BeneFIX in man

Orphan Drug Designation in US & EU
Retrospective immunogenicity assessment

A comprehensive assessment of immunogenicity addressed several key hypotheses

**Key Hypotheses Tested**

- The DalcA molecule is inherently immunogenic
- Drug product quality or formulation induce ADAs
- HLA and genotype are risk factors
- ISRs increased the risk of ADAs

“Considering our global and regional *in silico* analysis alongside whole protein and peptide *in vitro* experiments … we find the risk that wildtype FIX and therapeutic candidate DalcA will create or contribute to anti-therapeutic immune response to be minimal.”
Dalcinonacog Phase 1/2 open label design

Subcutaneous treatment of hemophilia B

DalcA IV vs BeneFIX IV

Cohort 1 (n=3)
- BeneFIX IV 70 IU/kg
- DalcA IV 70 IU/kg

Cohort 2 (n=3)
- DalcA IV 70 IU/kg

Cohort 3 (n=3)
- DalcA SQ 70 IU/kg
- DalcA SQ 140 IU/kg

DalcA IV to SQ crossover ascending dose cohorts 2 - 3

DalcA IV multi-dose SQ

Cohort 5 (n=5)
- DalcA SQ 140 IU/kg
  6 daily-doses

DalcA IV + multi-dose SQ

Cohort 6 (n=2)
- DalcA IV 70 IU/kg
- DalcA SQ 140 IU/kg
  9 daily-doses

3-month interval
Phase 1/2: Cohort 5 & 6 FIX activity results

6/7 patients had trough levels >12%, sufficient to protect against spontaneous joint bleeds
Phase 1/2: Cohort 6 FIX nAb development timeline

Time course of neutralizing antibody development after prior exposure in Cohort 5

C5-01-S01

C5-01-S02
The two subjects in cohort 6 that developed the nAbs are cousins and have the same genotype:

- Genotype is an Arg to Gln mutation at amino acid -4 (defective propeptide cleavage site)

Only common HLA type is DPB1 02:01
**In silico immunogenicity assessment shows low risk**

EpiMatrix Protein Scores reflect an excess or shortfall in putative T-cell epitope content relative to random expectation (predicted using the EpiMatrix system).

In vitro DC-T cell assays demonstrated minimal response above unstimulated control background for both sequences, confirming *in silico* prediction of low immunogenicity.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>EpiMatrix Score</th>
<th>Average Response Intensity</th>
<th>Percent of Donors Responding</th>
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*OB: Over Background

*ADA in > 5% exposed patients†ADA in < 5% exposed patients

**EpiMatrix Score**

- Increasing immunogenic potential
- Decreasing immunogenic potential

**Molecules**

- Interferon-Beta
- Erythropoietin
- Thrombopoietin
- Human Growth Hormone
- Thymus-Associated Protein
- Immunogenic Antibodies*
- GMCSF
- Albumin
- Non-Immunogenic Antibodies†
- Beta-2-Microglobulin
- Folitropin-Beta

*ADA in > 5% exposed patients
†ADA in < 5% exposed patients
DalcA shows a similar *in silico* risk as BeneFIX at R318Y

*In Silico* immunogenicity assessment at the R318Y site

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<th>Frame</th>
<th>AA Sequence</th>
<th>Frame Stop</th>
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DalcA shows a similar risk as BeneFIX at R338E

**In Silico immunogenicity assessment at the R338E site**

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DalcA shows a similar risk as BeneFIX at T343R

**In Silico** immunogenicity assessment at the T343R site

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<td>-0.03</td>
<td>0.95</td>
<td>0.13</td>
<td>0.55</td>
<td>0</td>
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<tr>
<td>342</td>
<td>FTVYNMFC</td>
<td>350</td>
<td>0.61</td>
<td>1.21</td>
<td>0.24</td>
<td><strong>1.37</strong></td>
<td>1.14</td>
<td>2.25</td>
<td>1.44</td>
<td>0.62</td>
<td>2.53</td>
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<tr>
<td>343</td>
<td>TIYNNMFC</td>
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<td>0.5</td>
<td>-0.19</td>
<td>-0.66</td>
<td>-0.87</td>
<td>-0.72</td>
<td>-0.82</td>
<td>0.20</td>
<td>-0.69</td>
<td>-0.40</td>
<td>0</td>
</tr>
</tbody>
</table>

**Frame Start** indicates the start of the frame, **AA Sequence** shows the amino acid sequence, **Frame Stop** indicates the end of the frame, and **Hydrophobicity** is the hydrophobicity score. The **Z-Score** values indicate the immunogenicity assessment at the T343R site.
Preclinical toolkit for evaluation of immunogenicity

Effector T-Cell

DC T-Cell Assay

T-Cell Proliferation

Antigen Presenting Cell

Processing

In Silico Analysis

Uptake

Display

MAPPS Peptide Presentation

EpiVax
DalcA drug product shows low immunogenicity risk

Clinical therapeutics with low risk have Response Index values (RI) between 0.1 and 0.4

- Dendritic cell T-cell responses to DalcA and BeneFIX were comparable, showing a low response and frequency of stimulation (ProScern - ProImmune)
- Overall immunogenicity risk profile risk is low and on par with BeneFIX
A major histocompatibility complex ("MHC")-associated peptide proteomics ("MAPPS") assay directly identified peptides presented by antigen-presenting cells when loaded with DalcA or BeneFIX (ProPresent - ProImmune).

+ Only a single peptide in 1/12 donors was identified for HLA-DQ (173–186 region)
### T-cell epitope clusters identified by *in silico* screening were presented in MAPPS assays

<table>
<thead>
<tr>
<th>Input Sequence</th>
<th>Cluster Address</th>
<th>Cluster Sequence</th>
<th>Hydrophobicity</th>
<th>EpiMatrix Hits</th>
<th>EpiMatrix Cluster Score</th>
<th>JanusMatrix Human Homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIX (WT)</td>
<td>112 - 126</td>
<td>TEGYRLAENQKSCEP</td>
<td>-1.51</td>
<td>7</td>
<td>17.74</td>
<td>4.43</td>
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<tr>
<td>FIX (WT)</td>
<td>191 - 207</td>
<td>QFPWQVVLNGKVDAFCG</td>
<td>0.3</td>
<td>7</td>
<td>12.47</td>
<td>1.00</td>
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<td>FIX (WT)</td>
<td>256 - 277</td>
<td>HHHYNAAINKYNHDIALLEDE</td>
<td>-0.83</td>
<td>9</td>
<td>12.64</td>
<td>1.22</td>
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<tr>
<td>FIX (WT)</td>
<td>296 - 311</td>
<td>TNIFLKFGSYVSGWG</td>
<td>0.29</td>
<td>6</td>
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<td>1.29</td>
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<tr>
<td>FIX (WT)</td>
<td>311 - 334</td>
<td>GRVFHKGRSALVQLYLRVPLVDRA</td>
<td>0.08</td>
<td>19</td>
<td>33.49</td>
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</tr>
<tr>
<td>FIX (WT)</td>
<td>310 - 326</td>
<td>WGRVFHKGRSALVQLY</td>
<td>0.06</td>
<td>11</td>
<td>16.82</td>
<td>1.73</td>
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<tr>
<td>DalcA</td>
<td>310 - 326</td>
<td>WGRVFHKGYSALVQLY</td>
<td>0.25</td>
<td>9</td>
<td><strong>12.03</strong></td>
<td>0.67</td>
</tr>
<tr>
<td>FIX (WT)</td>
<td>330 - 351</td>
<td>LVDRATCLRSTKFTIYNNMFCA</td>
<td>0.22</td>
<td>4</td>
<td>-2.77</td>
<td>1.5</td>
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<tr>
<td>DalcA</td>
<td>330 - 351</td>
<td>LVDRATCLESTKFTIYNNMFCA</td>
<td>0.09</td>
<td>5</td>
<td><strong>-1.12</strong></td>
<td>0.20</td>
</tr>
</tbody>
</table>

**JanusMatrix Human Homology >3:** Elevated degree of cross-conservation with epitopes derived from human proteome.

+ 4/5 T-cell epitope clusters identified by *in silico* screening in wild-type FIX were presented by DalcA and BeneFIX in the MAPPS assays (top panel) with some overlap to peptides containing substituted residues (lower panel)
Preclinical toolkit for evaluation of immunogenicity

DC T-Cell Assay → T-Cell Proliferation
Effector T-Cell

Antigen Presenting Cell

Designed Peptides
Uptake
Peptides from DalcA show low immunogenicity risk

% Responding Donors

DC-T cell stimulation: Peptides

+ Overall immunogenicity risk profile for the individual peptides is low and on par with BeneFIX

+ Peptides covered all three amino acid substitutions and selected from *in silico* data

+ Peptides identified in MAPPS experiment have partial or full overlap with tested peptides

BeneFIX derived peptide

DalC derived peptide

Control peptide or protein

R318Y

- GRVFHKGRSALVLOQY
- GRVFHKGRSALVLOQY
- HKGRSALVLOQYLRV
- HKGRSALVLOQYLRV
- WGRVFHKGRSALVLOQ
- WGRVFHKGRSALVLOQ
- VDRATCLESKTFTIY
- VDRATCLESKTFTIY
- VPLVDRATCLESKT
- VPLVDRATCLESKT
- CLRSTKFITYNNMFC
- CLRSTKFITYNNMFC
- RSTKFITYNNMFCA
- RSTKFITYNNMFCA
- STKFITYNNMFCA
- STKFITYNNMFCA
- ATCLSTKFITYNNM
- ATCLSTKFITYNNM
- YLRVPLDRCRL
- YLRVPLDRCRL
- HA
- PPD
- KLH

R338E

R338E/T343R

T343R

% Responders

0 10 20 30 40 50 60 70 80 90 100

KLH

PPD

HA

17
Peptides from DalcA show low immunogenicity risk

Response Index

DC-T cell stimulation: Peptides

+ Overall immunogenicity risk profile for the individual peptides is low and on par with BeneFIX
+ Peptides covered all three amino acid substitutions and selected from \textit{in silico} data
+ Peptides identified in MAPPS experiment have partial or full overlap with tested peptides

- BeneFIX derived peptide
- DalcA derived peptide
- Control peptide or protein
Positive responses were defined as proliferation (> 1 S.D.) exceeding control.

Only HLA alleles DRB1*04:07, DRB1*04:08 and DQB1*03:01 were significantly associated with an increased odds of positive response (Odds Ratio >1).
B-cell epitope mapping identified the T343R region

B-cell epitope mapping using single site variants of DalcA identified the binding region

- B-cell epitope mapping identified the R338E/T343R region to be targeted by neutralizing antibodies in both subjects
- Confirmed the absence of cross-reactivity to WT FIX
DalcA is comparable to BeneFIX & RIXUBIS

Multiple industry standard characterizations performed

- Potency
- Biological Activity
- Product Purity
- Biophysical and Structural Properties
- Chemical Modifications
- Post Translational Modifications
- Host Cell Impurities
- Product and Process Related Impurities
- Thermal Stability upon Reconstitution

Product quality & stability attributes are comparable to marketed rFIX products
What may have led to the development of nAbs?

The DalcA molecule is not inherently immunogenic – What now to consider in the clinic

- The nAbs were a rare event observed early in the trial within a restricted population
- The nAbs were associated with the rare genotype and/or certain HLA types
- The nAbs did not cross-react with BeneFIX or RIXUBIS so do not present a safety risk

Conclusion – Evaluate further safety & efficacy in a Phase 2b trial

+ Broaden the subject population to have a diverse ethnic and genotypic background
+ Exclude the rare genotype of the two subjects who developed nAbs in the P1/2 trial
+ Consider HLA profile and exclude those with HLA types that may be deemed at risk
+ Execute the P2b trial (28 days of dosing) with careful monitoring for development of nAbs
Ongoing and currently enrolling the phase 2b study: DLZ-201

- Enrollment: 6 patients
- Single IV dose followed by 28 day SQ dosing

+ Primary endpoint: Steady state FIX activity level above 12% with daily dosing
+ Secondary endpoints: no inhibitor formation, pharmacokinetics, pharmacodynamics
Conclusions on the dalcinonacog alfa program

Clinical development after an extensive immunogenicity risk assessment

Preclinical immunogenicity assessment showed that dalcinonacog alfa is equivalent to that of competitors such as BeneFIX

A comprehensive evaluation of the drug product showed comparable quality to marketed rFIX products

KoLs and subject experts agree with the immunogenicity risk assessment and proceeding with the P2b to evaluate the safety and efficacy of dalcinonacog alfa