A Comprehensive *In Silico* And *In Vitro* Immunogenicity Risk Assessment of Dalcinonacog Alfa Shows No Increased Risk Compared With Wild-type FIX

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**Conclusions**

+ *In silico* risk analyses of the DalcA and wild-type FIX sequences showed similar and low predicted immunogenicity
+ T-cell responses to DalcA and BeneFIX⁶ were comparable, showing a low response and frequency of stimulation
+ B-cell epitopes were DalcA specific with no cross-reactivity to wild-type FIX
+ Our analyses suggest that the likelihood of an immune response to DalcA should be similar to wild-type FIX products

**Introduction**

Catalyst Biosciences developed a next-generation coagulation Factor IX, dalcinonacog alfa (DalcA) using rational protein design, (R318Y, T343R and R338E) that provides 22-fold enhanced potency compared with wild-type FIX enabling effective administration by subcutaneous injection for routine prophylaxis. A phase 1/2 study clearly demonstrated the safety and efficacy of DalcA, however two subjects developed neutralizing antibodies (nAbs) specific to DalcA that did not cross-react with wild-type FIX.

**Table 1: HLA and genotype of the subjects who developed nAbs**

<table>
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<tr>
<th>Subject ID</th>
<th>DRB1</th>
<th>DQB1</th>
<th>DPB1</th>
<th>Genotype</th>
<th>Phenotype</th>
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<td>C5-01-S01</td>
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<td>04.01</td>
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</tr>
</tbody>
</table>

**Results**

**Figure 1: DalcA drug product shows low immunogenicity risk**

- Dendritic T-cell responses to DalcA and BeneFIX⁶ were comparable, showing a low response and frequency of stimulation (ProScern⁶ - Prolmmune)
- Two independent analyses were performed using two lots of BeneFIX⁶ and two clinical lots of DalcA (Phase 1/2 and Phase 2b lots)

**Figure 2: 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 in which normal donor cells were exposed to each protein demonstrated minimal response above unstimulated control background for both sequences, confirming *in silico* prediction of low immunogenicity

**Figure 3: MAPPS shows comparability for DalcA and BeneFIX⁶**

- A major histocompatibility complex ("MHC")-associated peptide proteomics ("MAPPS") assay directly identified peptides presented by antigen-presenting cells when loaded with DalcA or BeneFIX⁶ (ProPresent⁶ - Prolmmune)
- Only a single peptide in 1/12 donors was identified for HLA-DQ (173–186 region)

**Figure 4: Correlation of HLA status and and T-cell response**

- Proliferation of HLA-typed donor cells from 50 normal donors exposed to a library of DalcA-derived peptides showed an overall low response to peptide stimulus (ProMap⁶ - Prolmmune)
- 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)
- Patient who developed a high titer nAb has DRB¹*04:01 and DQB¹*03:01 alleles

**Figure 5: B-cell epitope mapping identified the T343R region**

- B-cell epitope mapping using single site variants of DalcA identified the R338E/T343R region to be targeted by neutralizing antibodies in both subjects and confirmed the absence of cross-reactivity to wild-type FIX