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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Grant E. Blouse[1], Howard Levy[1], Andrew Hetherington[1], Anju Chatterji[1], Chur Woo You[2], Frances Terry[3]
[1]Catalyst Biosciences, South San Francisco, CA, USA, [2]Eulji University Hospital Seo-gu Daejeon, Republic of (South) Korea, [3]EpiVax, Providence, RI, USA

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 BeneFIX® 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

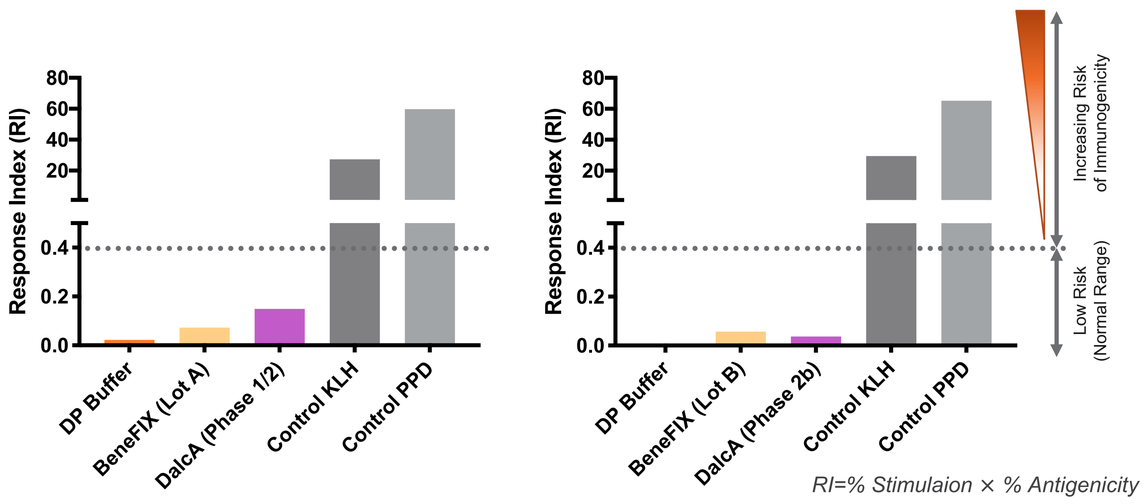
Catalyst Biosciences developed a next-generation coagulation Factor IX, dalcinonacog alfa (DalcA) using rational protein design, (R318Y, T343R and R338E) that provides 22-fold enhanced potency compared with wild-type FIX enabling effective administration by subcutaneous injection for routine prophylaxis. 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

Subject ID	DRB1		DQB1		DPB1		Genotype	Phenotype
C5-01-S01	03:01	04:01	02:01	03:01	02:01	02:01	128G>A	Arg43Gln: propeptide
C5-01-S02	01:01	13:01	05:01	06:01	02:01	04:01	128G>A	Arg43Gln: propeptide

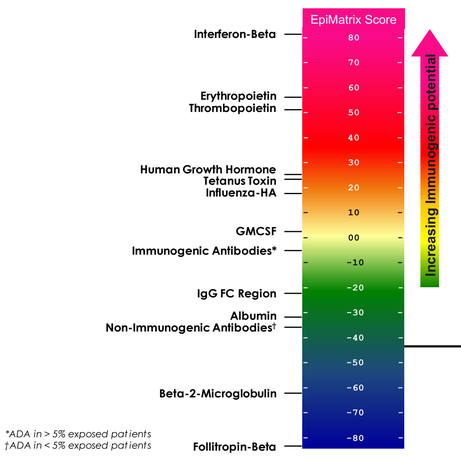
Results

Figure 1: DalcA drug product shows low immunogenicity risk



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

Figure 2: *In silico* immunogenicity assessment shows low risk

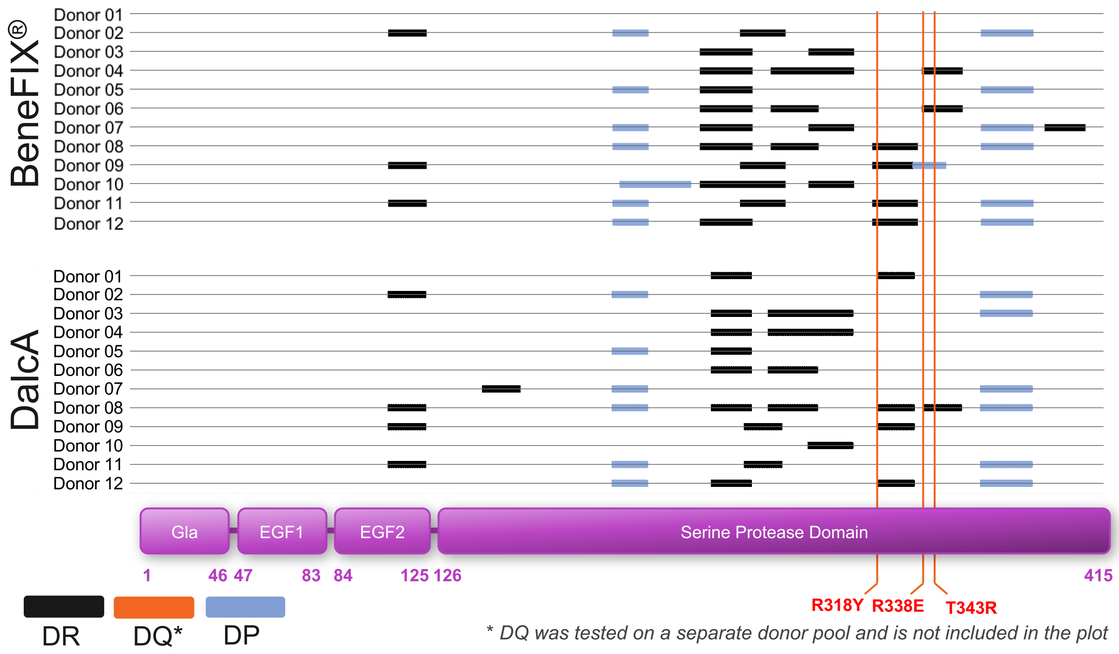


Molecule	EpiMatrix Score	Average Response Intensity	Percent of Donors Responding
BeneFIX Lot A	-41.65	0.76% OB	10%
DalcA Phase 1/2	-42.54	0.97% OB	15%
BeneFIX Lot A	-41.65	2.84% OB	2%
DalcA Phase 2b	-42.54	0.92% OB	4%

OB: Over Background

+ 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 BeneFIX®



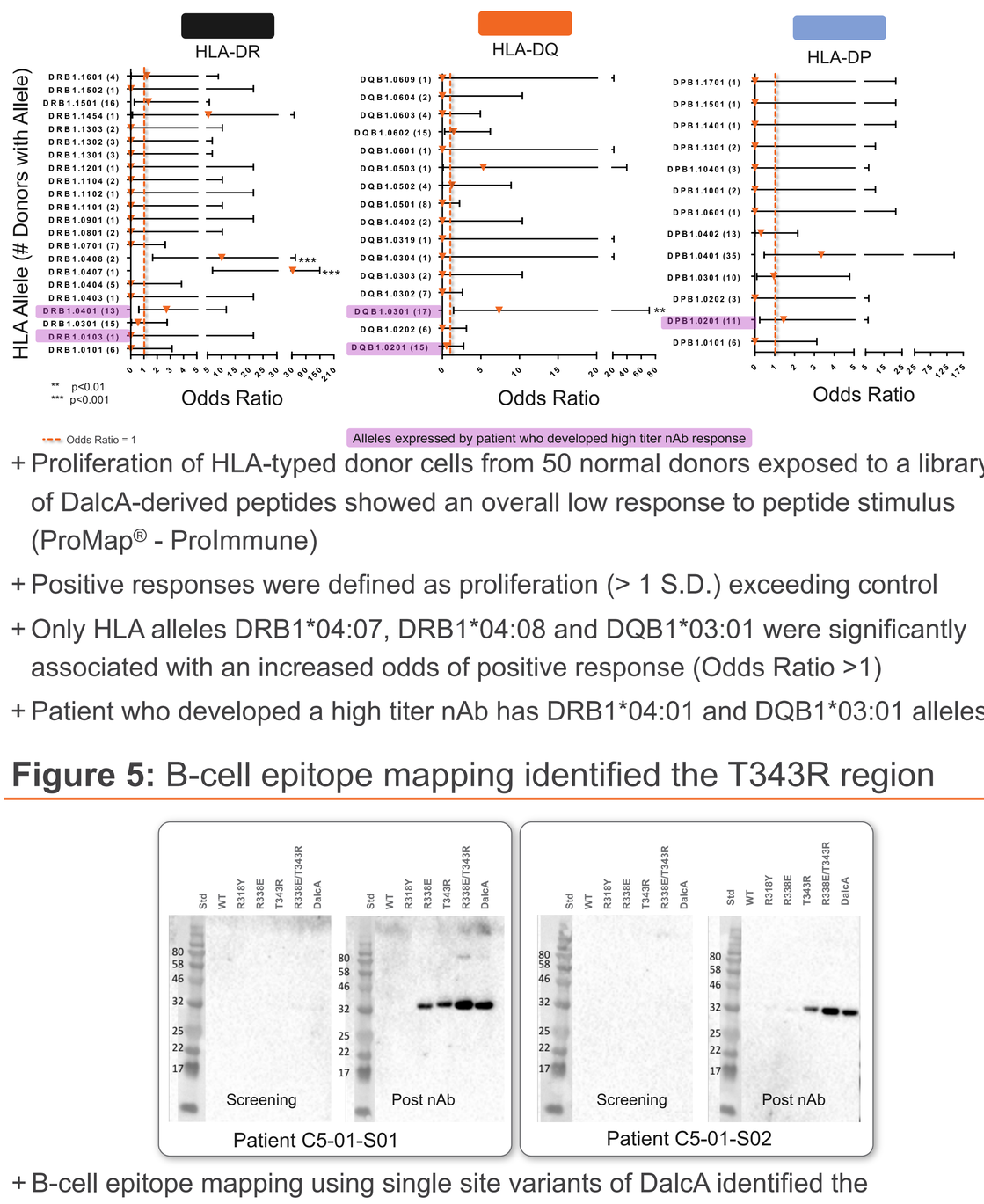
** DQ was tested on a separate donor pool and is not included in the plot*

+ 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)

Input Sequence	Cluster Address	Cluster Sequence	Hydro-phobicity	EpiMatrix Hits	EpiMatrix Cluster Score	JanusMatrix Human Homology	# Donors Presenting WT FIX	# Donors Presenting DalcA
FIX (WT)	112 - 126	TEGYRLAENQKSCEP	-1.51	7	17.74	4.43	3	4
FIX (WT)	191 - 207	QFPWQVVLNGKVDAFCG	0.3	7	12.47	1.00	0	0
FIX (WT)	256 - 277	HHNYNAAINKYNHDIALLELDE	-0.83	9	12.64	1.22	4	2
FIX (WT)	296 - 311	TNIFLKFGSGYVSGWG	0.29	6	11.04	1.29	4	3
FIX (WT)	311 - 334	GRVFHKGRSALVLQYLRVPLVDRA	0.08	19	33.49	2.09	4	4
JanusMatrix Human Homology >3: Elevated degree of cross-conservation with epitopes derived from human proteome.								
FIX (WT)	310 - 326	WGRVFHKGRSALVLQYL	0.06	11	16.82	1.73	4	4
DalcA	310 - 326	WGRVFHKGYSALVLQYL	0.25	9	12.03	0.67		
FIX (WT)	330 - 351	LVDRATCLRSTKFTIYNNMFCA	0.22	4	-2.77	1.5	2	1
DalcA	330 - 351	LVDRATCLESTKFRIYNNMFCA	0.09	5	-1.12	0.20		

+ 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)

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 (ProMap® - ProImmune)
+ 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