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An "I" (Immunogenicity Risk Assessment) for an Eye (Ophthalmology Biologics)

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PURPOSE

As new products are brought forward to the clinic for treatment of eye diseases, immunogenicity needs to be carefully considered, despite the reputation of the eye as an immune-privileged site. An Immunogenicity Risk Assessment is now considered to be an integral part of IND (Investigational new drug) applications to the FDA.

Various strategies are employed to assess immunogenicity risk. In this context, three prominent approaches stand out:

1) in silico prediction models

2) in vitro T cell assays

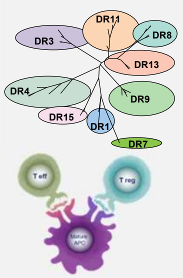
3) MAPPs assays

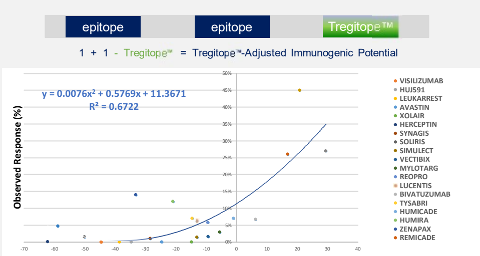
We have developed and applied an in silico risk assessment platform called ISPRI, that can rapidly assess the immunogenic potential of a biologic, identifying key 'clusters' of immunogenic or tolerogenic potential that may be relevant to immune responses and for immunogenic clusters, that may also be modified to reduce immunogenicity. Here we apply ISPRI analysis to an ophthalmologic biologic that has been associated with intra-ocular inflammation (IOI) and anti-drug antibody responses (ADA). Brolucizumab is a humanized monoclonal single chain variable fragment (scFv) for the treatment of neovascular age-related macular degeneration.

METHODS

We retrieved the amino acid sequence of brolucizumab, an scFv consisting of a variable heavy chain domain and a variable light chain domain. We then used ISPRI to assess its potential for immunogenicity. For each input sequence and domain of the scFv, ISPRI provides Tregitope-adjusted Immunogenicity Scores.

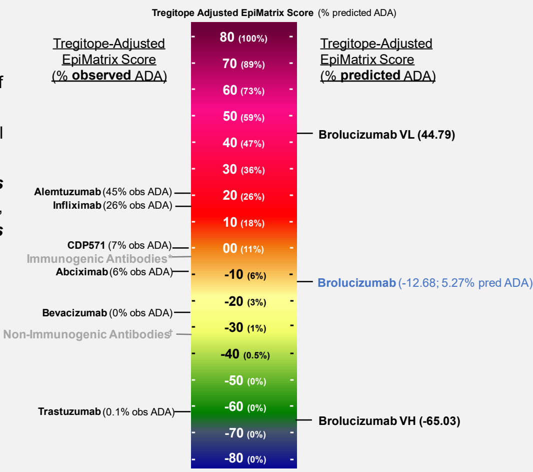
The Tregitope-adjusted Immunogenicity Score can be used to predict the ADA response rate for novel antibody products using a regression model based on a set of clinical benchmark monoclonal antibodies whose observed ADA response rates are known.





RESULTS

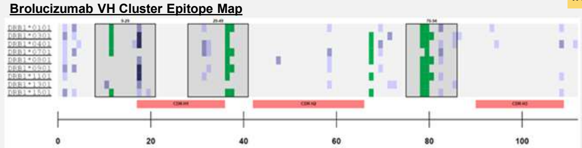
- The variable light chain domain has a very high score as compared to many non-immunogenic antibodies.
- As a result, the ISPRI-generated combined score of the variable heavy and variable light chain domains of brolucizumab is in the upper range of the antibody immunogenicity scale.
- This score indicated that the biologic would likely be associated with ADA, as was observed in several clinical trials.
- According to the FDA label for this product, "***After initiation of dosing, anti-brolucizumab antibodies were detected in at least one serum sample in 53% to 76% of patients treated with …***" the biologic, and "***Intraocular inflammation was observed in 6% of patients with anti-brolucizumab antibodies detected during … clinical trials***."



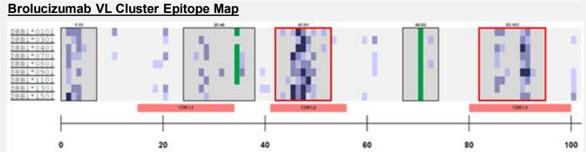
Protein Sequence	Length	EpiMatrix Hits	EpiMatrix Score	tReg Adjusted EpiMatrix Score	Paired tReg Adjusted EpiMatrix Score	Predicted T-Dependent Ab Response[1]
BROLUCIZUMAB_VH	120	56	9.21	-65.03	-12.68	5.27%
BROLUCIZUMAB_VL	110	84	77.1	44.79		

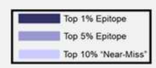
Within the VL domain, the **ISPRI algorithm identified several hot spots, two of which scored very high on the scale**. Deimmunization of these identified hot spots could have resulted in a less immunogenic therapeutic.

Brolucizumab VH Cluster Epitope Map



Brolucizumab VL Cluster Epitope Map





- The Clustimer algorithm identified several "hot spots" especially in the VL domain.
- Two of those "hot spots" contain several promiscuous non-human epitopes.

EpiMatrix Cluster Detail Report

Sequence: BROLUCIZUMAB_VL Cluster: 43

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-score	DRB1*0301 Z-score	DRB1*0401 Z-score	DRB1*0701 Z-score	DRB1*0801 Z-score	DRB1*0901 Z-score	DRB1*1101 Z-score	DRB1*1301 Z-score	DRB1*1501 Z-score	Hits	AbDB[1] Frequency (%)	Germline[2] Count
43	APKLLIYLA	51	1.41	-0.57	-0.75	0.03	-0.07	-0.35	-0.39	0.24	-0.65	-0.20	0	0.06	0
44	PKLLIYLAS	52	1.12	1.47	0.15	1.00	0.59	0.54	0.85	1.16	0.52	1.29	0	0.19	0
45	KLLIYLAST	53	1.22	0.52	-0.37	0.55	0.14	0.24	1.13	1.41	0.20	0.87	0	0.06	0
46	LLIYLASTL	54	2.08	1.58	1.25	1.15	2.32	1.72	2.29	1.02	2.44	2.46	5	0	0
47	LIYLASTLA	55	1.86	2.41	1.58	2.33	1.87	1.63	2.05	1.46	1.78	2.12	6	0	0
48	IYLASTLAS	56	1.34	2.04	2.33	2.99	1.58	1.91	2.41	2.14	2.42	2.37	8	0	0
49	YLASTLASG	57	0.8	1.23	1.85	1.38	0.39	2.03	2.31	2.39	1.70	1.20	5	0	0
50	LASTLASGV	58	1.41	1.50	0.60	1.04	2.14	0.55	1.86	0.26	1.30	1.51	2	0	0
51	ASTLASGVP	59	0.81	-0.41	-0.88	-0.34	-0.05	-0.48	0.21	-0.34	-1.72	-0.22	0	0.06	0
52	STLASGVPS	60	0.52	1.37	1.05	1.34	0.29	0.52	1.17	0.02	1.01	1.09	0	0.06	0
53	TLASGVPSR	61	0.11	0.26	0.81	0.33	-0.09	0.44	0.22	0.42	0.57	-0.55	0	0.06	0
Summarized Results				**DRB1*0101**	**DRB1*0301**	**DRB1*0401**	**DRB1*0701**	**DRB1*0801**	**DRB1*0901**	**DRB1*1101**	**DRB1*1301**	**DRB1*1501**	**Total**		
Maximum Single Z-score				2.41	2.33	2.99	2.32	2.03	2.41	2.39	2.44	2.46	--		
Sum of Significant Z-scores				4.45	4.18	5.32	6.33	5.66	10.92	4.53	8.34	6.95	56.68		
Count of Significant Z-scores				2	2	2	3	3	5	2	4	3	26		
Total Assessments Performed: 99				**Hydrophobicity: 0.68**			**EpiMatrix Score: 46.49**			**EpiMatrix Score (w/o flanks): 52.05**					

CONCLUSIONS

In this study, brolucizumab was evaluated and was shown to have an overall immunogenicity risk assessment performed by ISPRI that was consistent with clinical observations. This analysis of brolucizumab shows that it is necessary to not only consider the immunogenic potential of novel constructs as a whole, but also of individual components that might each have distinct T cell epitope characteristics. The use of in silico tools such as the ISPRI platform proves to be an essential step in the development of biological therapies as evidenced by this case study.

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