Design and characterization of ADX-097: A C3d targeted antibody – fH_{1-5} fusion protein for the treatment of complement alternative pathway driven disease

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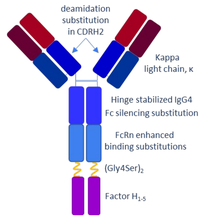
INTRODUCTION

Currently approved anti-complement therapies are directed at systemic blockade of complement. To provide more effective and safer options for patients requiring chronic treatment, we developed a tissue targeting anti-complement therapy to preserve systemic complement activity. A panel of tissue-targeted complement regulators were designed and characterized, providing a range of potency[1] and pharmacokinetic profiles to address multiple complement mediated diseases[2]. One of these, ADX-097, offers a unique targeted therapeutic approach to inhibit alternative pathway (AP) activation in diseased tissue while minimizing systemic complement blockade. ADX-097 is a bifunctional fusion protein containing two moieties of the first five consensus repeats of human factor H (fH_{1-5}) linked to a humanized anti-C3d antibody. ADX-097 is designed to target diseased tissue via binding to C3d, which is deposited at sites of complement activation, and provide localized blockade.

ADX-097
Humanized C3d-mAb fH_{1-5}
Tissue Targeted Inhibitor of the Complement Alternative Pathway



METHODS & RESULTS

Anti-C3d antibody humanization:

1. A human IgG4 isotype was selected for its low Fc gamma receptor III (FcγRIII) and C1q binding potential, minimizing effector function and complement activation.
2. Structure-based CDR grafting into human germline gene acceptor frameworks was used to reduce the potential for immunogenicity.
3. Engineered substitutions enhance its drug-like properties

Biochemical characterization of humanized variants and ADX-097:

1. Binding to FcγRs.
2. pH dependent binding to neonatal Fc Receptors (FcRn)
3. Epitope mapping of mouse C3d mAb and human Fab
4. Binding to C3 fragments
5. Binding to human, cynomolgus monkey and mouse C3d
6. Functional activity in biochemical and complement activation assays

Target Engagement of mouse surrogate ADX-118:

1. Tissue targeting and measurement of complement inhibition in *Cfh-/-* mice

ANTI-C3d HUMANIZATION & ENGINEERING

Humanization – CDR Grafting and Framework Backmutations

Variant (VH, Vκ)	Germline Sequence Identity (GSI)	Framework Backmutations	
VH7*	Highest	Y91F, A93S, R94S	
VH6		R71V, V78A	Variable Heavy Chain
VH5		M48I, V67A, M69L	
VH4		Q1E, T73K, T75S	
VH3		E10V, V20M, R38K	
VH2		A9P, K12V, P41H	
VH1	Lowest	Parental	
Vκ3	Highest	F36L	Variable Light Chain
Vκ2		Q37L, R45K, V104L	
Vκ1	Lowest	Parental	

Heavy and light chain variants selected for humanized mAb scaffold

*VH7 was considered unlikely to binding antigen and was not carried forward for expression

- Variable domains of the 3d8b VH5/Vκ3 antibody have Germline Sequence Identity (GSI) values that are higher than for most FDA-approved humanized antibodies.
- This antibody is therefore likely to have a lower risk of immunogenicity

Engineered Substitutions to Enhance Drug-like Properties

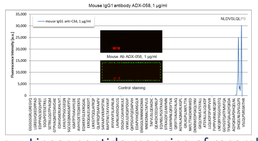
Humanized C3d mAb was engineered to incorporate well-described substitutions that increase stability, PK parameters like half-life and decrease effector function and de-risk residues predicted to result in post-translational modifications.

Substitution	Purpose
VH5/Vk3	Antibody humanization from mouse 3d8b
S228P	Stabilization of the human IgG4 hinge region/abrogation of Fab arm exchange
L235E	Reduction/elimination of FcγR interactions
M428L/N434S	Increase binding to FcRn at pH 6.0
N54S	Removal of asparagine and potential deamidation site

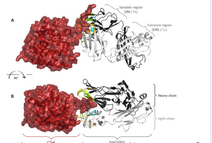
- None of these substitutions affected binding affinity or stability relative to parent C3d humanized mAb (VH5/Vκ3).

Epitope Mapping of mouse C3d mAb and humanized C3d Fab

Linear peptide mapping of mouse mAb to human C3d



Co-crystal structure of humanized Fab in complex with human C3d



- Linear peptide mapping of recombinant hC3d and crystallization of Fab(3d8b):hC3d identify the same amino acid sequence at the C-terminal end of human C3d.
- Humanized and mouse 3d8b bind same C3d region, which is present in C3 cleavage fragments, iC3b, C3dg and C3d and confirmed by SPR (below)
- Crystal structure shows engagement of all six CDRs.

	C3 and C3 Cleavage Products				
Antibody	C3	C3b	iC3b	C3d	C4d
3d8b (mouse)	–	–	+	+	–
ADX-093 (humanized)	–	–	+	+	–

SPR binding of mouse and humanized C3d mAb to Complement fragments shows specificity remains intact and correlates with identified epitopes

Fc Receptor and C1q Binding of ADX-097

ADX-097 was evaluated for FcRn, FcγR and C1q binding compared to IgG1 (positive) IgG4 (negative) controls:

- SPR: pH-dependent binding to FcRn at pH 6 and pH 7.4
- SPR: FcγRI, FcγRIIA (167R), FcγRIIA (167H), FcγRIIB, FcγRIIIA (176F), FcγRIIIA (176V), FcγRIIIB
- ELISA: C1q binding

Fc Receptor Binding (K_D, µM)

	FcRn pH 6	FcRn pH 7.4	FcγRI	FcγRIIA (Arg167)	FcγRIIA (His167)	FcγRIIB*	FcγRIIIA (Phe176)*	FcγRIIIA (Val176)*	FcγRIIIB
Screening Conc (µM)	0.016-2	0.5-2	0.0004 – 0.033	0.296 - 24	0.296 - 24	0.296 - 24	0.099 - 8	0.099 - 8	0.099 - 8
Control IgG1	0.56	NB	0.0027	2.12	1.4	18.8	4.1	1.6	6.24
Control IgG4	0.88	NB	0.0096	NB	13.8	14.4	NB	NB	NB
ADX-097	0.48	NB	NB	NB	NB	20.6	NB	NB	NB

*Reported values are from assays performed in 300mM NaCl to reduce non-specific binding, Control antibodies are unchanged at increase salt concentrations.
NB = No binding

C1q Binding (ELISA)

Test Article	EC_{50}, µg/mL
Rituximab (IgG1), positive control	1.4
ADX-097 (IgG4)	No EC_{50} determined
ADX-152* (IgG1), positive control	3.1
IgG4 – S228P/L235E	No EC_{50} determined
IgG4 – Wild-type	No EC_{50} determined

*ADX-152 was expressed as an Fc competent IgG1 C3d mAb-fH_{1-5} to provide the most equivalent control for ADX-097

- ADX-097 binds with nM affinity to FcRn at pH 6 and not at pH 7.4 suggesting that it should be recycled in the endosome.
- ADX-097 shows no binding to C1q or activating FcγRs and binding to the immune cell inhibitory receptor, FcγRIIB, indicating low risk of effector function in humans.

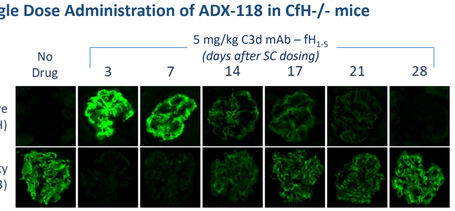
Comparison of Binding Affinity and Functional Activity for Humanized and Mouse C3d-mAbs and Humanized and Mouse Surrogate C3d mAb – fH_{1-5} Fusion Proteins

	C3d binding affinity (species cross-reactivity)					C3d binding affinity and complement inhibitory activity			
ID	ADX-058[a]	ADX-093[b]	ADX-118	ADX-097	**ID**	ADX-048 3d8b-fH_{1-5}	ADX-097 3d8b-fH_{1-5}	ADX-118 3d8b-fH_{1-5}
Species/ Isotype Fusion	Mouse IgG1	Human IgG4	Mouse IgG1 mouse fH_{1-5}	Human IgG4 human fH_{1-5}	**Species/ Isotype Fusion**	Mouse IgG1 human fH_{1-5}	Human IgG4 human fH_{1-5}	Mouse IgG1 mouse fH_{1-5}
Human C3d binding K_D, M	7.10E-09	1.00E-08	9.40E-09	1.20E-08	**C3d binding K_D, nM**	-	**11.9**	**9.4**
Mouse C3d binding K_D, M	6.9E-09	NT	**7.16E-09**	3.15E-09	**Wieslab (C5b-9) IC_{50}, nM**	73 ± 8.3	82 ± 4.2	NT
Cyno C3d binding K_D, M	NT	NT	1.33E-09	6.32E-09	**Hemolysis, AH_{50}, nM**	260 ± 34	300 ± 5.7	NT
					Zymosan IC50, nM	-	**25 ± 4.2**	**4.4 ± 0.4**

[a]ADX-058 is the mouse parent 3d8b that binds C3d with similar affinities as ADX-118
[b]ADX-093 is the humanized parent 3d8b antibody that binds to C3d with similar affinities as ADX-097.

- Assay not performed, orthogonal assay used
NT= Not Tested due to incompatibility of assay

Immunostaining with Anti-fH and Anti-C3 Fragment Antibodies After Single Dose Administration of ADX-118 in CfH-/- mice



- CfH-/- mice used as an in vivo model system to assess in vivo effects of ADX-097 and its mouse surrogate ADX-118.
- No anti-fH detected prior to dosing and active C3 fragments were present.
- At early timepoints (Days 3 and 7), substantial anti-fH immunostaining was evident and little or no active C3 fragment was observed, indicating homing of ADX-118 to tissue leading to complement inhibition.
- At later time points (Days 14, 17, and 21), anti-fH immunostaining decreased while anti-C3 fragment immunostaining gradually returned, indicating gradual clearance of ADX-118 from tissue and return of complement activity.

CONCLUSIONS

ADX-097 was designed and engineered to optimize binding affinity, functional activity and drug developability properties. It's biochemical and functional profile demonstrate excellent tissue localization, potent inhibition of the alternative complement pathway and attractive drug-like properties. ADX-097 has therapeutic potential in multiple indications with unmet need. Further validation of tissue-targeting and demonstration of complement inhibition *in vivo* can be found at Poster B24: C3d-Targeted fH Achieves Potent Tissue-Directed Complement Inhibition and Disease – Modifying Efficacy Without Affecting Systemic Complement

REFERENCES

[1]Fahnoe et al., Design and characterization of C3d targeted fusion proteins for tissue localized inhibition of complement activation. **Poster A85**

[2]Liu et al., C3d-Targeted fH Achieves Potent Tissue-Directed Complement Inhibition and Disease – Modifying Efficacy Without Affecting Systemic Complement. **Poster B24**

CONTACT

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