CB 2782-PEG: a Complement Factor C3-Inactivating Protease and Potential Long-Acting Treatment for Dry AMD

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Results

- **CB 2782-PEG is a 68 kDa engineered protease to specifically cleave C3 into inactive fragments. It has a ~500 kDa MW on SEC due to the large hydrodynamic radius**
- **The PEGylated enzyme activity was indistinguishable from the unmodified protein**
- **The increased size of CB 2782-PEG resulted in enhanced ocular half-life in rabbits (3.9 vs 1.9 days for the unmodified CB 2782) and in non-human primates (3.7 vs. 1.7 days)**
- **Single intravitreal injection of 125 µg CB 2782-PEG achieved complete, rapid and sustained PD inhibition (>99%) of vitreous C3 for at least 28 days in African green monkeys.**

Conclusions

- **CB 2782-PEG has potential for anti-C3 best-in-class efficacy and convenience in geographic atrophy dry AMD with anticipated intravitreal human dosing three or four times a year**

Introduction

Eleven to 15 million people in the US have age-related macular degeneration (AMD) (NEI estimates, Pennington and DeAngelis 2017). Vision is most affected in late stage wet AMD and geographic atrophy (GA). Wet AMD accounts for approximately 10 – 15% of AMD (AMD alliance) and is well treated by intravitreal VEGF blockade yielding a >$8B global market. GA is nearly as prevalent as wet AMD (amdbook.org), yet has no approved drugs.

Mutations in numerous genes in the complement cascade, in particular those of the alternative pathway, result in increased risk of AMD e.g. Factor H, Factor I, C9, and C3 (Fritsche et al., 2016). Drusen, which is the key marker of early dry AMD, contain alternative pathway and terminal pathway complement components, including products of activation and degradation (Bradley et al. 2010, Hageman et al. 1999 and Johnson et al. 2001).

Despite multiple clinical failures in treating GA by blocking the alternative pathway or the downstream C5 pathways, interest in more completely blocking complement activation in the eye by inhibiting C3, continues. In Phase 2, APL-2, injected once per month intravitreally, statistically significantly inhibited the progression of GA. While this clinical data provides validation for inhibition of C3 to treat GA, improvements in efficacy and reducing the frequency of administration are highly desirable.

We describe a PEGylated long acting engineered protease, CB 2782-PEG, which specifically and catalytically eliminates C3, providing a potential avenue for low frequency intravitreal administration with high efficacy.

Methods

CB 2782 was engineered from a human serine protease (matriptase) to rapidly and specifically degrade human C3 into inactive fragments. A single unpaired cysteine was added to CB 2782 and modified with a 40 kDa linear PEG by maleimide coupling to generate CB 2782-PEG. Both constructs are produced in *E. coli*. Molecular weight and purity were established by SDS-PAGE and size-exclusion chromatography. Primary sequence and single-site PEG modification was verified with mass spectrometry.

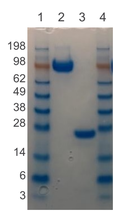
Enzymatic activity and specificity of CB 2782 and CB 2782-PEG were evaluated with peptide hydrolysis assays and with human C3 cleavage. Inhibition of complement activation was evaluated in the standard sheep red blood cell hemolysis assays.

The pharmacokinetics of CB 2782 and CB 2782-PEG were evaluated in a rabbit model in comparison to aflibercept. Both eyes of eight animals were injected with 100 µg of test article. Two animals were sacrificed at each time point. Drug concentrations were determined by enzyme activity and aflibercept by ELISA.

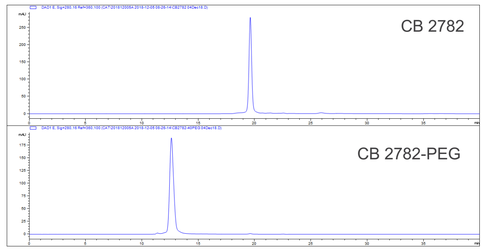
The pharmacokinetics and pharmacodynamics (C3 level) of CB 2782-PEG were evaluated in an African green monkey primate model. The right eye received 125 µg test article, while the left eye received vehicle, N = 12 animals. Vitreous humor was sampled from three animals at multiple time points, and three others were sacrificed at 1, 14, and 28 days. Drug concentrations were determined by enzyme activity, while C3 levels were determined by ELISA.

Results

CB 2782-PEG was produced with good purity by SDS-PAGE and size-exclusion chromatography

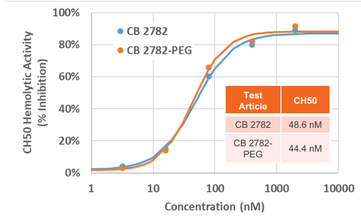


Lane	Sample
1	Ladder
2	CB 2782-PEG
3	CB 2782
4	Ladder



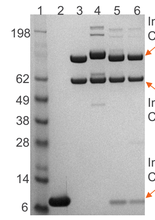
CB 2782-PEG Enzyme Activity is Indistinguishable from the Unmodified Protein

CB 2782 and CB 2782-PEG inhibit complement-mediated hemolysis *in vitro*



Test Article	CH50
CB 2782	48.6 nM
CB 2782-PEG	44.4 nM

Sub-stoichiometric CB 2782 and CB 2782-PEG specifically cleave C3 into inactive species

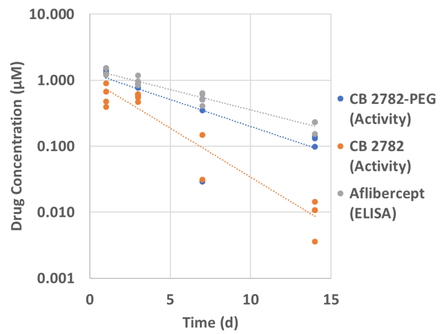


Reduced SDS-PAGE	
Lane	Sample
1	Ladder
2	C3a
3	C3b
4	C3
5	2 µM C3 treated with 0.2 µM CB 2782-PEG
6	2 µM C3 treated with 0.2 µM CB 2782

CB 2782-PEG and CB 2782 have similar activity on a human C3 peptide substrate

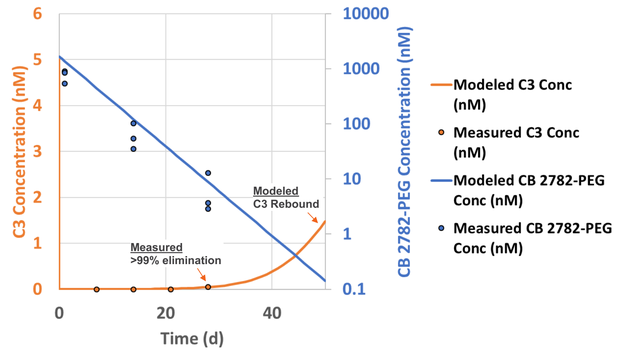
Test Article	Specific Activity (nmol/min/mg)	Relative Activity to CB 2782
CB 2782	5565	100%
CB 2782-PEG	5699	102%

PEGylation of CB 2782 extends the ocular half-life in rabbits and has similar pharmacokinetics to aflibercept



Parameter	CB 2782-PEG	CB 2782	Aflibercept
t-half-terminal (d)	3.85	1.90	4.82
Mean residence time (d)	5.63	3.21	7.24
Cmax (µM)	1.52	0.90	1.52
Tmax (d)	1	1	1
AUC 0-inf (µM-d)	7.79	3.14	9.66
AUC 0-t (µM-d)	7.07	3.12	8.06

Intravitreal CB 2782-PEG eliminates at least 99% of vitreous humor complement C3 for at least 28 days in African green monkeys



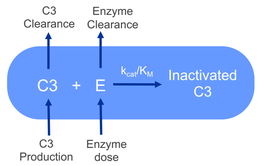
Parameter	CB 2782-PEG
t-half-terminal (d)	3.7
Mean residence time (d)	3.37
Cmax (µM)	0.90
Tmax (d)	1
AUC 0-inf (µM-d)	6.94
AUC 0-t (µM-d)	6.92

Modeling of CB 2782-PEG NHP Results Enables Prediction of Human PK/PD

Molecule	Size	Vitreal Clearance Half-life (d)		
		NHP	Human	Human/NHP Ratio
Lucentis[1,3,5,6]	48 kD	2-4	5-9	~2.3x
Avastin[3,5,7]	149 kD	3	7-10	~2.8x
Eylea[2,3,4]	115 kD	2-3	7	~2.8x

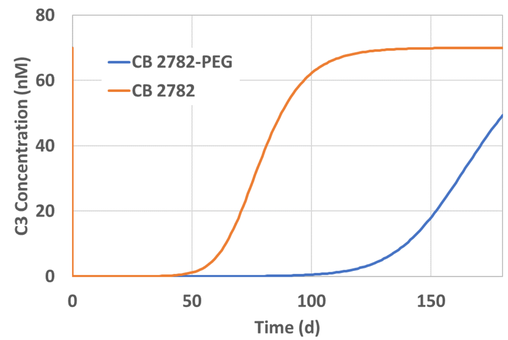
[1] Gaudreault 2005
[2] Eylea EMA guidance
[3] Steward 2011
[4] Struble 2011
[5] Krohne 2012
[6] Xu 2013
[7] Miyake 2010

- NHP CB 2782-PEG half-life measured at 3.7 d scales to 8.5 days in human
- NHP CB 2782 half-life measured at 1.7 d scales to 3.9 days in human



Model Parameter	African Green Monkey		Human	
	Value	Source	Value	Source
Vitreous Volume (mL)	3.0	Measured	4.4	Literature
C3 Steady State Conc (nM)	5.0	Measured	70	Literature
C3 Vitreous Half-Life (d)	4.4	Literature	8.2	Literature
Enzyme Dose (mg)	0.125	Known	2	Known
Enzyme Half-Life (d)	3.7	Measured	8.5	2.3X scaling from AGM to human
Enzyme k_{cat}/K_M ($nM^{-1}d^{-1}$)	1.88	Fit	1.88	AGM Model

A CB 2782-PEG 8.5 day half-life and 2 mg dose suggests intravitreal administration three or four times a year in humans



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