Discovery and Planned Optimization of a Complement Factor C3-Inactivating Alterase, CB-2782

Eric S Furfine[1], Martin Stanton[1], Brajesh Kumar[2], Nassim Usman[2], David Sloane[2] and Mikhail Popkov[2], and Edward Madison[2]

[1]Mosaic Biosciences, Boulder, CO and [2]Catalyst Biosciences, South San Francisico, CA

Introduction

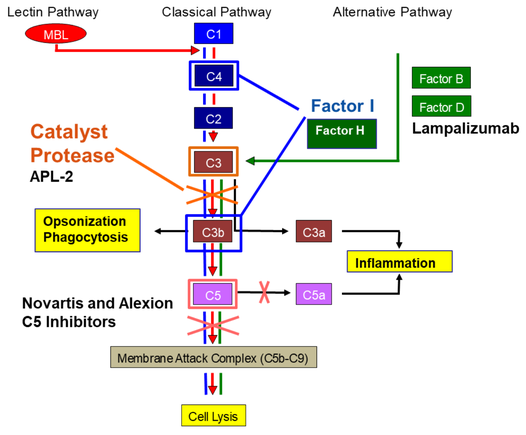
Eleven to 15 million people in the US have AMD (NEI estimates, Pennington and DeAngelis 2017). Vision is most affected in the late stages of the disease, wet age-related macular degeneration (AMD) and geographic atrophy (GA). Wet AMD accounts for approximately 10 – 15% of AMD cases (AMD alliance). Wet AMD is well treated by intravitreal VEGF blockade yielding a >$4B market. Geographic atrophy 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 increase 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 (Figure 1), 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.

Herein we describe an engineered protease, CB2782, which catalytically eliminates C3, providing a potential avenue for low frequency of administration and high efficacy with additional protein engineering.

Figure 1: C3 is at the Center of All Three Complement Pathways



- All three complement pathways funnel through C3
- Genentech (lampalizumab) focused on the alternative pathway (blocking Factor D)
  - Has no effect on classical pathway
  - Only limits cascade "amplification"
- Novartis and Alexion tried to block C5
  - Does not block C3a mediated inflammation or C3b opsonization
- **Apellis (APL-2) and Catalyst target C3 – the center point of the complement cascade**

Figure 2: CB2782 Specifically Cleaves C3, Even When in Vast Catalytic Excess

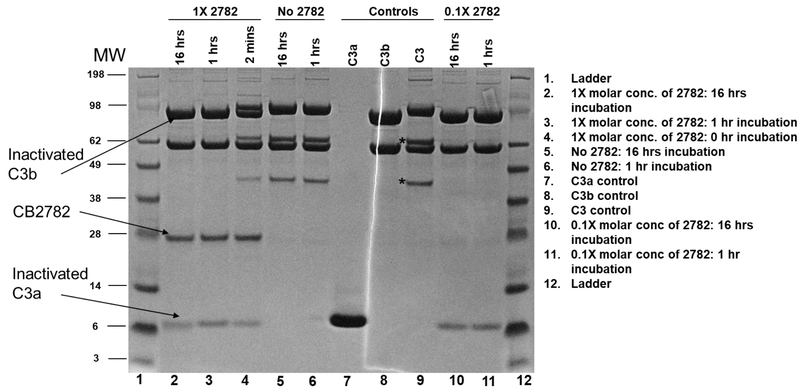


Figure 3: Single 125 µg Dose of CB-2782 has a NHP Half-life of 1.7 Days & Suppresses Vitreal Complement C3 for ≥ 7 Days

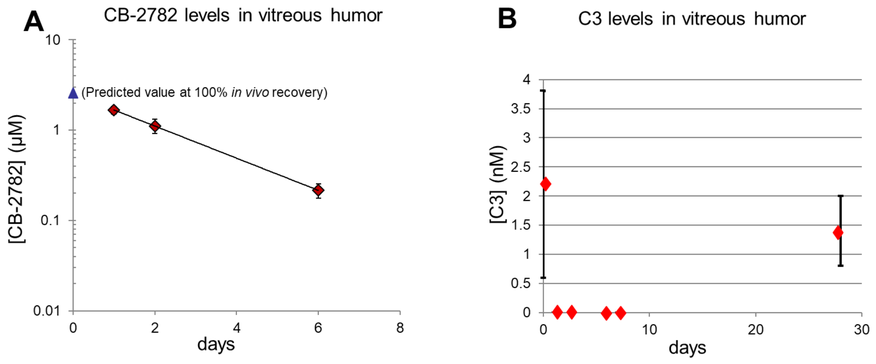


Figure 5: Modeled NHP C3 Levels in Fit to Observed PD Data

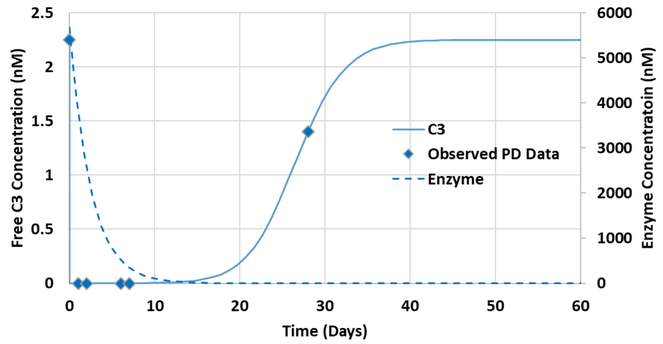
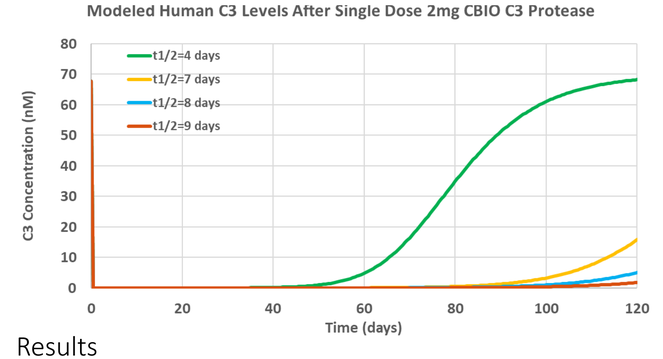


Figure 6: 7-Day Half-life and 2 mg Dose Suggest > 3-Month Frequency of Administration in Humans



Methods

C3 inactivation by CB2782 *in vitro*: 5 µg of C3 (CompTech) cleaved by varying concentrations of 2782 at 37 ºC. SDS PAGE under reducing conditions

IVT CB2782 PK/PD Study in cynomolgus monkeys: PK: 7 days, PD: 28 days, One dose: 125 µg of test article in right eye, vehicle injection in left eye. 12 total animals injected, four animals sacrificed at each time point. Vitreous humor collected and analyzed for variant concentration, activity, and [C3]. Vitreal C3 detection in primate PK/PD utilized an ELISA that detects C3 and C3a (time points for experiment 2 were 0, 1, 7, and 28 days). Vitreal CB2782 concentrations in primate PK/PD study determined by ELISA and enzyme activity (time points for experiment 1 were 0, 1, 2, and 6 days).

IVT toxicology and tolerability study in cynomolgus monkeys: Three intravitreal doses (up to 1000 µg/eye) with three cynomolgus monkeys per dose (no re-dosing). Right eye received test article; left eye injected with vehicle control. "Clinical observations", food consumption, etc. Ophthalmic examinations: slit-lamp biomicroscopy and indirect ophthalmoscope observations, followed by color fundus photography or optical coherence tomography (OCT) prior to dosing and on days 2, 8, and 15 post-dosing. Any observations followed for up to 4 weeks until resolution.

Enzyme Model: Fit to Observed Primate PD Data

Steady state concentration of C3 in vitreous (nM)
[C3] = 2.25 nM

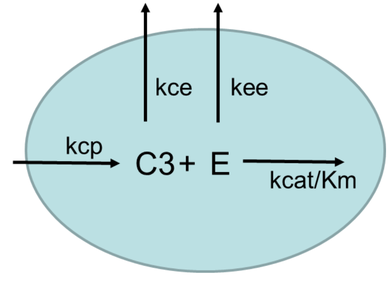
Starting drug concentration (nM)
Dose = 0.125mg, 2.2ml volume, MW=25000
Dose = 2272 nM

kce : Rate of C3 elimination from eye. (day^{-1})
Assume $t_{1/2}$ = 3 day

kcp : Rate of C3 production in eye.

kee : Rate of drug elimination from eye.
$t_{1/2}$ = 1.7 days from PK measurements

kcat/Km : Fit to observed PD data = 1.8 / nM /day
or 2.1×10^4 /M /sec



Results

- CB2782 completely cleaves C3 at one site despite vast catalytic excess, suggesting a selectivity for the C3 cleavage site of > 2400-fold over any other site in C3.
- Intact C3 was undetectable (<10 pM) in CB2782-treated eyes through 7 days
- There were variable levels of C3 (0.4 – 50 nM) in vehicle-injected eyes (2 outliers - probable blood contamination of vitreous)
- Half-life of CB2782 was 1.7 days by both ELISA and enzyme activity
- CB2782 was well-tolerated at single doses up to 1 mg/eye. Severity of observed ocular inflammation was similar or less than that observed with a single intravitreal injection of other intravitreal biologics in monkeys; Lucentis, Eyelea
- PK/PD Modeling indicates increasing the NHP half life of CB2782 would correlate with a doubling in humans and predict suppression of C3 for 3 months or more.

Conclusions

- NHP repeat dose selection up to 1000 µg/eye is justified based on single dose study results
- PK PD Modeling indicates an 2-fold enhancement of vitreal half life in monkeys would result in a product with at least quarterly frequency of administration
- Straightforward Pegylation or fusion (e.g. Fc fusion) protein engineering in progress to sufficiently enhance vitreal PK of CB2782.
- Given target validation of C3 blockade in GA, modest PK enhancement of CB2782 has potential for best in class efficacy and convenience