Molecular Evolution and Design of Pegylated CB 2782 as a Complement Factor C3-Inactivating Protease for Dry AMD

ASBMB Symposium on Serine Proteases and Extracellular Proteolysis

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VP Translational Research
Age-Related Macular Degeneration (AMD)

- Wet and dry AMD are distinct diseases of which both lead to vision loss and blindness
- Geographic atrophy (GA) results in progressive loss of photoreceptors and irreversible central vision loss
- Unlike wet AMD, no marketed treatment is available for dry AMD

C3 is the only validated target for GA in dry AMD

Advanced dAMD, or geographic atrophy (GA), has a devastating impact on vision and leads to blindness
+ No currently approved therapies

C3 is the only clinically validated target in GA
+ Apellis APL-2 (anti-C3 PEGylated cyclic peptide) completed P2
+ 15 mg intravitreal injection in randomized P2 (n=246)
  • Qmo - 29% inhibition of GA (p=0.008)
  • Q2mo - 20% inhibition of GA (p=0.067)

Proteases provide superiority to peptides or antibodies
+ Sub-stoichiometric dosing and a catalytic mechanism

Catalyst’s long acting anti-C3 protease is best-in-class
+ Provide superior efficacy and better convenience
+ Q3mo or Q4mo dosing
Selection of a specific “inactivating” cleavage site

Schematic of C3 structure and the C3 convertase cleavage site

- CB 2782 was engineered to specifically cleave a single site in C3
  - Divergent from that which is cleaved by the C3 convertases
- Cleavage of C3 results in an inactive C3a and C3b-related species
  - Cannot be further activated by the C3 convertases
Using SERPINs as a “kinetic” trap to select for catalysis

Phage library presented to a plate coated with biotinylated serpin-AT “Bait”

Catalytic activity of MTSP-1 variant drives cleavage of the unique site in the bait

Serpin-mediated trapping of phage variant that cleaves the unique site

MTSP-1 Displaying Phage

Unique Site “Bait Loop”

AT Serpin Scaffold

Plate surface coated with streptavidin

Trapped Selective MTSP-1
Catalyst Biosciences: Alterase™ Protease Platform

Proprietary Technology for Protease Discovery

- Based on cleavage activity and not on binding by using a serpin-mediated trapping approach
- Allows rapid discovery of new proteases with tailored catalytic activity
- Allows rapid lead optimization by screening for enhanced activity & specificity simultaneously

protein production
DNA mutagenesis
Creation of new MTSP-1 variant libraries
DNA extraction and sequence
Positive hits for scale up
Cloning in expression plasmids
Pick colonies and grow large quantity of phages
Infect bacteria and grow colonies on plates
Grow MTSP-1 variant phage library
Purify MTSP-1 phage library
Add MTSP-1 phage library to immobilized serpin bait
Re-selection and counterselection for phage enrichment
Remove unbound phages by washing plate
Elute phages “trapped” by Serpin
Biotinylated Serpin bait
Streptavidin coated plate
catalystbiosciences.com
Molecular evolution of CB 2782 for C3-specific cleavage

MTSP-1 → Select Coordination → Catalysis → Human Plasma (counter selection)

CB 2013

CB 2426

CB 2470

CB 2782 (lead candidate)

MTSP-1 mature

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Directed Engineering → α2-Macroglobulin (counterselection)

Evolved Candidate → CB 2782
CB 2782 shows significant improvement in cleavage of C3

<table>
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<th></th>
<th>MTSP-1 (nM)</th>
<th>CB 2782 (nM)</th>
<th>Ratio</th>
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<tr>
<td>Buffer</td>
<td>13.9</td>
<td>6.9</td>
<td>2</td>
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<tr>
<td>Human Plasma</td>
<td>2800</td>
<td>92</td>
<td>30</td>
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<tr>
<td>Cyno Plasma</td>
<td>3500</td>
<td>25</td>
<td>140</td>
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4.2 nM
CB 2782 shows high specificity

Cleavage of PentaXv2 Library

- Essential no detectable cleavage of the PentaXv2 library by CB 2782
- Near complete cleavage by MTSP-1
- Complete cleavage by trypsin
- Very little cleavage by uPA
Moving from CB 2782 to CB 2782-PEG

+ Despite attractive safety profile the PK parameters did not support a clinical candidate
+ Cysteine specific 40 kDa PEG (maleimide) conjugated
  • Site specific labeling on the free Cys122
CB 2782-PEG has indistinguishable activity vs CB 2782

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

Sub-stoichiometric CB 2782 and CB 2782-PEG specifically cleave C3 at a single site into inactive fragments
CB 2782-PEG eliminates vitreous C3 for at least 28 Days

Intravitreal CB 2782-PEG has a half-life of 3.7 days and eliminates at least 99% of C3 in vitreous humor of African green monkeys for at least 28 days

<table>
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<th>Parameter</th>
<th>CB 2782-PEG</th>
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<tr>
<td>t-half-terminal (d)</td>
<td>3.7</td>
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<tr>
<td>Mean residence time (d)</td>
<td>3.37</td>
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<tr>
<td>Cmax (µM)</td>
<td>0.90</td>
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<td>Tmax (d)</td>
<td>1</td>
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<td>AUC 0-inf (µM-d)</td>
<td>6.94</td>
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<tr>
<td>AUC 0-t (µM-d)</td>
<td>6.92</td>
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Predicted 2.0 mg human dose three to four times a year

Enzyme Model: Fit to observed primate PK/PD data and scaled to the human condition

**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 | 2.0 | 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 |

**Legend:**
- CB 2782-PEG
- CB 2782

**Graph:**
- C3 Concentration (nM) vs. Time (days)
- Predicted >90% elimination of C3 at 4 months

**Equation:**
- $C3 + E \xrightarrow{k_{cat}/K_M} \text{Inactivated C3}$
- C3 Production
- Enzyme dose
- C3 Clearance
- Enzyme Clearance

**Sources:**
- Nasdaq: CBIO
- Mosaic Biosciences
- catalystbiosciences.com

**Additional Information:**
- 13
Engineered novel specificity through molecular evolution of MTSP-1

Significantly improved catalysis and stability in a biological milieu

Intravitreal injection resulted in at least 99% elimination of C3 for at least 28 days

CB 2782-PEG has potential for best-in-class efficacy and convenience in dry AMD
Acknowledgements

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