APL-2 and complement inhibition; a potential treatment of PNH and other complement-mediated diseases

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Boulder Peptide Symposium

September 27, 2016
Apellis Highlights

- **Mission**: to create disease-modifying therapies in chronic inflammatory and orphan disease indications via complement inhibition

- Lead product candidates **APL-1 and APL-2**
  - Potent and selective C3 inhibitors of the *compstatin* family

- **Clinical pipeline** targets autoimmune and inflammatory diseases
  - Initially PNH, AMD and COPD
Lead candidates target C3 central in the complement cascade

Activation Pathways

- Classical pathway: Activated by antibody-antigen complex
- Lectin pathway: Activated by lectin and mannose complex
- Alternative pathway: Spontaneous C3 convertase activation

Broad inhibition of complement cascade

- APL-1
- APL-2

C3

- C3a: Inflammation
- C3b: Cell removal

C5

- C5a: Inflammation
- C5b: Cell destruction

MAC
Evidence of safety of C3 inhibition

- A small population of individuals lack functional levels of C3 and C5*.
  These individuals are susceptible to infection by certain bacterial species

  C5-deficient individuals
  Neisseria meningitidis

  C3-deficient individuals
  Neisseria meningitidis,
  Streptococcus pneumoniae
  Haemophilus influenzae

  INFECTION RISK
  MANAGEABLE WITH
  VACCINATION

- No cases of drug-related infections following experiments involving >300 non-human primates
  - Multiple compounds (APL-1, APL-2 and others)
  - Acute and chronic exposure

- No cases of infections with subcutaneous APL-2 to date – triple vaccination

- No cases of infections with intravitreal APL-2 to date – no vaccination

- Two cases of fever with nebulized APL-1 (resolved with antibiotics) – single vaccination

Overview of complement-related indications

- **Haematological**
  - Paroxysmal nocturnal haemoglobinuria, cold agglutinin disease, and warm antibody autoimmune hemolytic anemia

- **Ophthalmology**
  - Age-related macular degeneration, dry eye, glaucoma, uveitis, and diabetic retinopathy

- **Respiratory**
  - Chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, asthma, bronchiolitis obliterans syndrome and cystic fibrosis

- **Neurological**
  - Myasthenia gravis, neuromyelitis optica, Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, Guillain-Barré syndrome, cerebral lupus, multifocal motor neuropathy, and stroke

- **Renal**
  - Lupus nephritis, membranoproliferative glomerulonephritis, membranous nephritis, immunoglobulin A nephropathy, goodpasture syndrome, post-streptococcal glomerulonephritis, dense deposit disease, C3 glomerulonephritis, and atypical haemolytic uraemic syndrome

- **Rheumatological**
  - Systemic lupus erythematosus, lupus arthritis, rheumatoid arthritis, Sjögren’s syndrome, Behçet’s syndrome, cryopyrin-associated autoinflammatory syndrome and systemic sclerosis

- **Vascular**
  - Myocardial infarction and atherosclerosis

- **Allergic**
  - Anaphylactic shock, allergy and asthma

- **Dermatology**
  - Vasculitis, pemphigus, bullous pemphigoid, phototoxic reactions and psoriasis

- **Other**
  - Inflammatory bowel disease, thyroiditis, Crohn’s disease, cryoglobulinaemia, foetal loss, organ graft rejection, sepsis and trauma (including spinal cord injury)
## Apellis Broad Pipeline

<table>
<thead>
<tr>
<th></th>
<th>2016</th>
<th></th>
<th>2017</th>
<th></th>
<th>2018</th>
<th></th>
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<tr>
<td></td>
<td>1H</td>
<td>2H</td>
<td>1H</td>
<td>2H</td>
<td>1H</td>
<td>2H</td>
</tr>
</tbody>
</table>

### PNH – rMG - NMO (Rare Indications)

- Preclinical Studies
- Ph 1b Soliris (USA; n=8-16)
- Ph 1b Tx NZ; n=6)
- Ph 3 (PNH; TBNaïve (D))
- Ph 3 (rMG; TBD)
- Ph 3 (NMO; TBD)

### GA (Ophthalmology)

- Ph 2 (Aus, USA; n=240)

### COPD (Pulmonary)

- Ph 1 HV (n=18)
Compstatin-based Peptides
Discovery of Compstatin

The sequence of compstatin was identified from a phage library experiment using a 27-mer library.

Summary

- Compstatin was first identified from a phage display experiment by Prof. John Lambris at the University of Pennsylvania.

- A pIII phage 27-mer library of $2 \times 10^8$ recombinants expressing the peptide sequence $SR X_{12} \ (S, P, T, \text{ or } A) \ A (V, A, D, E, \text{ or } G) \ X_{12} \ SR$ was screened against human C3b.

- One clone (Clone 9) was isolated that was shown to bind selectively to C3, C3b, and C3c.

Identification of Compstatin

A 13-mer complement inhibiting domain of Clone 9 was identified and named Compstatin.

<table>
<thead>
<tr>
<th>Peptide/Clone</th>
<th>Amino Acid Sequence</th>
<th>Classical Pathway $^{a}$</th>
<th>Alternative Pathway $^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC$_{50}$ (µM)</td>
<td>IC$_{50}$ (µM)</td>
</tr>
<tr>
<td>Peptide IV</td>
<td>I-C-V-V-Q-D-W-G-H-H-R-C-T</td>
<td>63</td>
<td>12</td>
</tr>
<tr>
<td>Peptide V$^{c}$</td>
<td>I-C-V-V-Q-D-W-G-H-H-R-C-T</td>
<td>&gt;600</td>
<td>ND</td>
</tr>
<tr>
<td>Peptide VI</td>
<td>C-V-V-Q-D-W-G-H-H-A-C</td>
<td></td>
<td>70</td>
</tr>
</tbody>
</table>

$^{a}$ Classical pathway activity was determined using EA lysis assay.

$^{b}$ Alternative pathway activity was measured using Er lysis assay.

$^{c}$ In peptide II and V, cysteines were reduced and alkylated (see Materials and Methods). Bold-face residues were fixed in all library clones.


The complement inhibitory domain was identified as a 13-mer cyclic subsequence (peptide IV) using standard hemolysis-based classical and alternative complement inhibition assay (with an IC$_{50}$ of 63 µM and 12 µM respectively) and was named Compstatin.
Cyclization and acetylation were shown to slow the degradation and enhance the activity of Compstatin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Inhibition of complement</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Baboon</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Marmoset</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Squirrel monkey</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Aotus</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>&gt;600$^b$</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>&gt;600$^c$</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>&gt;600</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>&gt;600</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>&gt;600</td>
<td></td>
</tr>
</tbody>
</table>


- Compstatin was shown to be active only against primate C3 (above).
- Non acetylated and linear compstatin degrades very quickly by cleavage at the N- terminus and has reduced activity (right)
Mechanism of Action of Compstatin

Compstatin binds to a pocket of C3 and sterically hinders its binding.

Summary

- Crystal structure of compstatin and C3c was elucidated in 2007.
- Compstatin was shown not to induce significant structural changes to C3 upon binding.
- Compstatin is believed to sterically interfere with C3 binding to C3-convertase complexes of the complement cascade.

APL-1 is an improved compstatin derivative.

Overview

- APL-1
  - 3rd generation compstatin derivative optimized using rational design and peptide library screening.
  - At least 250x more potent than the original compstatin

<table>
<thead>
<tr>
<th>Kd (using SPR)</th>
<th>C3</th>
<th>C3(H2O)</th>
<th>C3b</th>
<th>iC3b</th>
<th>C3c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compstatin</td>
<td>0.13 µM</td>
<td>0.06 µM</td>
<td>2.6 µM</td>
<td>ND</td>
<td>9.6 µM</td>
</tr>
<tr>
<td>APL-1</td>
<td>260 pM</td>
<td>ND</td>
<td>174 pM</td>
<td>435 pM</td>
<td>ND</td>
</tr>
</tbody>
</table>

APL-1 Structure
APL-2 Program
APL-2 Program

This program aims to extend the circulating half-life of APL-1 and improve its solubility in serum in order to address chronic, systemic complement-mediated indications.

Design of New APL-1 Conjugates with Extended Serum Half-Life

- Several life extensions technologies were tested, among them were PEGylation, albumin conjugation and some proprietary technologies.
Extended Half-Life Candidates Pharmacokinetics

Extended half-life candidates have a terminal half-life 10x higher and a significantly higher Cmax than APL-1 in Cynomolgus monkeys at comparable dose.
PK vs Conjugate Size in Cyno

Larger conjugates have longer half-life as expected. Balance between size and half-life is important.
Coupling Chemistry Stability

Simple stability experiment outlines very significant differences between coupling chemistry.
Different coupling chemistries are available during conjugation. Some of these chemistries are more stable in vivo than others.

- **APL-1** (IV 200 mg/kg): 
  - \( T_{1/2} \approx 6-8 \text{ hours} \)

- **Chemistry A** (IV 50 mg/kg): 
  - \( T_{1/2} \approx 4-5 \text{ days} \)

- **Chemistry B** (IV 32 mg/kg): 
  - \( T_{1/2} \approx 7-8 \text{ days} \)
Careful Design (Geometry, Linker, Chemistry) Preserved Conjugate Activity

Alternative Complement Inhibition Assay

Complement Activity (Absorbance Units) vs. Concentration (μM)

- APL-1
- APL-2
APL-2: Preclinical Data
APL-2 Preclinical Summary

- APL-2 SC preclinical work conducted in cynomolgus monkeys, New Zealand white rabbits, or CD rats

- Safety Pharmacology (monkeys)
  - hERG assay: no significant drug-related hERG current amplitude change over the concentrations used (1 to 300 µM)
  - Cardiopulmonary telemetry: no drug-related observations (highest dose tested: 140 mg/kg)

- APL-2 non-genotoxic (AMES, In vitro TK6 micronucleus, In Vivo mouse micronucleus)

- Reproductive SC studies (rats and rabbits)
  - Pilot prenatal studies conducted (1, 3.5, 7, 28 mg/kg/d)
  - No drug-related findings at any dose in either species

- Chronic IVT repeated-dose studies (monkeys only)
  - Monkeys: 9-month study with 3-month peel-off
  - IVT→3.1, 12.4, 24.8 mg/eye (every 4 weeks)
  - No significant drug-related observation at any doses tested (NOEL ≥24.8 mg/eye)
APL-2 Preclinical Summary (cont.)

- 28-day SC/IV repeated-dose studies (monkeys and rabbits)
  - Doses tested: SC→0.25, 1, 3, 7, 28, 140 mg/kg/d; IV→2 x 42 mg/kg
  - SC: Macrophage vacuolation at ≥3 mg/kg/d (NOEL ~3 mg/kg/d)
  - SC: Minimal kidney tubule degeneration at ≥28 mg/kg/d (NOAEL between 7 and 28 mg/kg/d)
  - IV: No drug-related observation at 2 x 42 mg/kg (doses on Day 1 and Day 15)
  - PEG and APL-2 mildly antigenic in rabbits and not antigenic in monkeys

- Chronic SC repeated-dose studies (monkeys and rabbits)
  - Monkeys: 9-month study with 3-month peel-off
    - After 3 months of treatment the same effects than the 28-day dosing study were observed.
  - Rabbits: 6-month study with no peel-off
    - Doses tested: SC→1, 7, 28 mg/kg/d
    - No drug-related findings after 6 months of treatment.
APL-2 PK: Subcutaneous Injections

APL-2 demonstrates well-behaved pharmacokinetic behavior in monkeys. T½ of approximately 7.5 days.
APL-2 PK: Intravitreal Injections

APL-2 T½ in monkeys was 3.2 days in vitreous and 10.4 days in serum.

APL-2 PK after intravitreal injections in Cynos (every 4 wks)
APL-2 (subcutaneous):
Paroxysmal Nocturnal Hemoglobinuria
Unmet need in paroxysmal nocturnal hemoglobinuria

DISEASE
- ~4,700 patients in the US
- Severe anemia, thrombotic risk, impaired bone marrow functions
- ~35% 5-year mortality if left untreated (main cause: thrombosis)

STANDARD OF CARE
- Soliris® only approved therapy
  - Controls intravascular hemolysis
  - ~$583,000 / year / adult patient

UNMET NEED
- 35-40% of patients on Soliris continued to be transfusion-dependent for 30 months following the beginning of treatment*
  - Soliris® does not prevent C3b-mediated extravascular hemolysis

(Dr. Hillmen is an advisor to Apellis)
Intravascular vs extravascular hemolysis

Inhibition of C5 prevents C5a and MAC but not C3a-mediated inflammation and C3b deposition
Broader inhibition of complement at C3 may overcome limitations of C5 inhibition

**Potential Benefits APL-2**
- Prevention of blood clot formation
- Reduced anemia and transfusion dependency
- Ease of use (self-administered once daily)
- Disease modifying potential

**Activation Pathways**
- **Classical pathway** Activated by antibody-antigen complex
- **Lectin pathway** Activated by lectin and mannose complex
- **Alternative pathway** Spontaneous C3 convertase activation

**Inflammation**
- C3b on RBC
- C5b MAC
- C3a
- C5a

**CELL REMOVAL**
- Intravascular
- Extravascular

**CELL DESTRUCTION**
- Intravascular
In PNH blood APL-2 prevents C3 deposition and should prevent extravascular hemolysis*

*Abstract at ASH Conference 2013 by Dr. Peter Hillmen, Leeds Teaching Hospitals, NHS Trust, United Kingdom (Dr. Hillmen is an advisor to Apellis)
## Phase 1 Studies

Design: randomized, double-blind, placebo-controlled, single and multiple ascending dose studies to assess the safety, tolerability, PK and PD of subcutaneous APL-2 in healthy adult subjects who have received the triple vaccination.

<table>
<thead>
<tr>
<th>Trial</th>
<th>N</th>
<th>Doses &amp; dosing period</th>
<th>Endpoints</th>
<th>Preliminary Results</th>
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</thead>
<tbody>
<tr>
<td><strong>Phase 1 Healthy SAD</strong></td>
<td>31</td>
<td>24 active 7 placebo</td>
<td>• Safety/tolerability</td>
<td>No SAEs. Well tolerated C max 3 days/ half life 10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• PK/PD</td>
<td>Reduced hemolytic activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 45-1440 mg/day</td>
<td>• Hemolytic activity</td>
<td></td>
</tr>
<tr>
<td><strong>Phase 1 Healthy MAD</strong></td>
<td>20</td>
<td>16 active 4 placebo</td>
<td>• Safety/tolerability</td>
<td>No SAEs. Well tolerated PK profile supports daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• PK/PD</td>
<td>subcutaneous injection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 30 – 270 mg/day</td>
<td>• Hemolytic activity</td>
<td>Reduced hemolytic activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 28 days</td>
<td></td>
<td></td>
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</table>
Phase 1 healthy SAD trial PK data

APL-2 demonstrates well-behaved pharmacokinetic behavior in human subjects. $T_{\text{max}}$ of approximately 4-5 days and long T½ of approximately 9 to 10 days.
Phase 1 healthy MAD trial PK data (28-day)

Cohort 1 – 30 mg/day
Cohort 2 – 90 mg/day
Cohort 3 – 180 mg/day
Cohort 4 – 270 mg/day
Normal hemolytic activity in placebo subject

Hemolysis of Red Blood Cells by the Alternative Pathway
(1:4 Plasma Dilution)

Treatment Period

Effective complement inhibition by Soliris (≤20% hemolytic activity)**,***

*** Hemolytic activity assays used may vary
APL-2 reduces hemolytic activity - 180 mg

** Hemolysis of Red Blood Cells by the Alternative Pathway **

- **Healthy Subject (placebo)**
- **Healthy Subjects (APL-2 treated)**

**Effective complement inhibition by Soliris (≤20% hemolytic activity)**


*** Hemolytic activity assays used may vary
APL-2 reduces hemolytic activity - 270 mg

Rabbit Red Blood Cell Alternative Pathway Hemolysis

**Healthy Subject (placebo)**

**Healthy Subjects (APL-2 treated)**

Effective complement inhibition by Soliris (≤20% hemolytic activity)**,***

*** Hemolytic activity assays used may vary
Phase 1b studies in PNH patients

To assess the safety, preliminary efficacy and pharmacokinetics of subcutaneously administered APL-2

The Paddock study
- Subjects with Paroxysmal Nocturnal Hemoglobinuria (PNH) that have not been treated with Eculizumab in the past
- Total of 6 subjects in 2 Cohorts
- Two doses: 180 and 270 mg/d of APL-2 for 28 days

The Pharoah study
- Subjects with PNH currently receiving Eculizumab
- Total of 8 subjects in 4 Cohorts
- Doses ranging for 30mg/d to 270 mg/d of APL-2 for 28 days

Both Studies
- **Primary Endpoints**: number and severity of TEAEs and pharmacokinetics parameters of APL-2 following administration of multiple SC doses
- **Secondary Endpoints**: Lactate dehydrogenase (LD), Hemoglobin, Haptoglobin, PNH clones
Conclusion

APL-1 Conjugate with Extended Half-Life was Successfully Developed, Optimized, and Is Now Being Tested in Multiple Clinical Trials

APL-2 Development

- Numerous half-life extension technologies were tested.
- The most promising technology was chosen for optimization.

APL-2 Optimization

- The optimal size was determined as the best compromise between serum half-life and molecular weight.
- The most stable coupling chemistry was used.
- The resulting optimized molecule has a half-life that is about 30x the half-life of APL-1 with an activity that is at least 50% higher on a molar basis.

APL-2 Preclinical Testing

- Up to date APL-2 has proven to be safe when administered chronically by SC and IVT administration.

APL-2 Clinical Testing

- APL-2 was tested in healthy volunteers to confirm subcutaneous PK parameters.
- It was confirmed that APL-2 could reduce the hemolytic activity via the Alternative Pathway at doses of 180 and 270 mg/day.
- Testing in PNH patients is ongoing using subcutaneous injections.
- Testing in AMD patients is ongoing using intravitreal injections.
Thank you