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# POSTER ABSTRACTS

## 651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

### Cael-101 Enhances the Clearance of Light Chain Fibrils and Intermediate Aggregates By Phagocytosis

*Matt Costello, MS*[1], *Nina Park, BS*[1], *Alyssa Neill, MS*[1], *Edmond Wong, PhD*[2], *Karin Müller, PhD*[3], *Andrew Proffitt, MS*[1], *Richie Huynh, BS*[1], *Vishwa Rao, PhD*[1], *Fang Sun, MD*[1], *Aaron Bozzi, PhD*[1], *Meera Ramanujam, PhD*[1], *Sahar Usmani-Brown, PhD*[1]

[1] Alexion AstraZeneca Rare Disease, New Haven, CT
[2] AstraZeneca R&D Biologics Engineering, Biomedical Campus, Cambridge, United Kingdom
[3] Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom

**Background**

Light chain amyloidosis (AL) is a rare, systemic disease characterized by amyloid fibril deposition in various organs due to overproduction of light chains from plasma cell dyscrasia (PCD). Current treatment strategies target PCD rather than clearing amyloid deposits directly from organs. CAEL-101 is a chimeric monoclonal IgG1 antibody that targets these amyloid fibrils. It binds to a cryptic epitope at the N-terminus of both kappa ($\kappa$) and lambda ($\lambda$) light chain misfolded proteins. In this study we tested the role of CAEL-101 in mediating the phagocytosis of synthetic fibrils and their intermediates by macrophages and neutrophils, as a way of elucidating its mechanism of action.

**Methods**

Two human VL proteins, named LEN and AL09, were recombinantly expressed in mammalian cell systems. Synthetic LEN and AL09 fibrils were generated using purified recombinant VL protein. Fibril formation was assessed by Thioflavin T (ThT) fluorescence emission fold change over time, change in radius of protein sample via Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). The binding of CAEL-101 to fibrils was determined using an immunoassay performed on an MSD® platform and SPR (Biacore™ T200).

To generate fibril intermediates (light chain aggregates), recombinant AL09 VL protein was processed per a fibril formation protocol. Samples were taken at 48- and 72-hour timepoints wherein the ThT signal had not yet achieved saturation. DLS readings showed these samples to be smaller in size than samples acquired with fully formed synthetic fibrils. TEM revealed samples to be aggregated and lacking a beta-pleated fibrillar structure. Phagocytosis of synthetic fibrils and intermediates were demonstrated using PMA differentiated THP1 macrophages and neutrophils isolated from healthy donors. Synthetic fibrils and intermediates were labeled with pHrodo™ iFL Red STP Ester dye and incubated with various dilutions of CAEL-101, IgGk1 isotype control, and 10% normal human serum (NHS). Fucoidan, a macrophage scavenger receptor A inhibitor, was included in assays using THP-1 cells. An Fc blocking reagent was included to evaluate whether the observed phagocytosis is Fc$\gamma$R dependent. Phagocytosis assays were also done with complement depleted human serum to evaluate the impact of complement activation on CAEL-101 mediated amyloid clearance.

Phagocytosis at 3hr timepoint was assessed using an Incucyte® S3 system, as indicated by presence of pHrodo signal emitted from cells.

**Results**

CAEL-101 bound to synthetic fibrils dehydrated onto an MSD plate with an $EC_{50}$ of 6.346E-010 M for LEN fibrils and 9.21E-07 M for AL09 fibrils.

CAEL-101 bound to LEN fibrils via SPR with an affinity of 2.66E-11 KD (M) and to AL09 fibrils with 3.62E-07 KD (M) at pH 7.4.

Phagocytosis assays demonstrated that incubation of synthetic fibrils with CAEL-101 led to statistically significant enhancement of phagocytosis in both the differentiated THP-1 cells and neutrophils, as compared to phagocytosis observed with IgGk1 isotype control antibody or fibril alone. Phagocytosis was enhanced in the presence of 10% normal human serum compared to complement depleted serum. At 3hr timepoint, CAEL-101 significantly enhanced LEN fibril phagocytosis ($p<0.05$, two-way ANOVA) at antibody concentrations of 0.41 nM and above, with no phagocytosis observed above background in the presence of Fc block.

CAEL-101 significantly enhanced the phagocytosis of intermediate AL09 light chain aggregates (48- and 72- hour timepoint collections), as compared with IgGk1 isotype control antibody or fibril alone.

## Conclusions

The current *in-vitro* study demonstrated that CAEL-101 binds to light chain amyloid fibrils and upon binding causes the phagocytosis of fibrils via macrophages and neutrophils in a FcγR dependent manner. The phagocytotic activity is further enhanced in the presence of complement. In addition, for the first time we demonstrated that CAEL-101 was able to phagocytose intermediate soluble light chain aggregates, known precursors of amyloid fibril formation.

**Disclosures Costello:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Park:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Neill:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Wong:** *AstraZeneca:* Current Employment, Current equity holder in publicly-traded company. **Müller:** *University of Cambridge:* Other: Imaging services . **Proffitt:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Huynh:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Rao:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Sun:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Bozzi:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Ramanujam:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company. **Usmani-Brown:** *Alexion AstraZeneca Rare Disease:* Current Employment, Current equity holder in publicly-traded company.

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