Preclinical Potency, Affinity, and Pharmacokinetics of APG990, a Half-Life Extended Antibody Against OX40L

Grant Wickman,[1] Byong Ha Kang,[2] Jessica Meinke,[1] Steve Raso,[1] Jason Oh,[2] Hussam Shaheen,[2] Lukas Dillinger[1]

[1]Apogee Therapeutics, Inc., Waltham, MA, USA, [2]Paragon Therapeutics, Inc., Waltham, MA, USA

Introduction

- The OX40 ligand (OX40L) and OX40 are immune-signaling molecules that increase inflammatory signaling.[1]
- OX40L is a type II transmembrane protein of the TNF family that is expressed on antigen-presenting cells (APCs). OX40 is a type I cysteine-rich transmembrane protein that belongs to the TNFR superfamily and is expressed on T-cells.[2]
- The OX40/OX40L interaction promotes inflammatory T-cell responses and contributes to exacerbation of symptoms in atopic dermatitis (AD) (**Figure 1**).[1]
- APG990 is a fully human IgG1 monoclonal antibody that was designed to bind to OX40L and disrupt the OX40/OX40L-mediated inflammatory signaling cascade (**Figure 1**).

Figure 1. Role of APG990 in blocking the OX40/OX40L axis

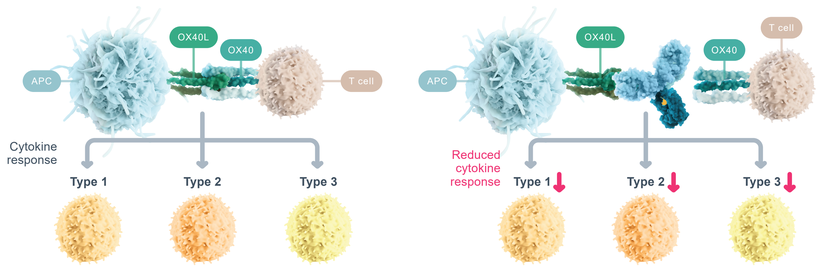
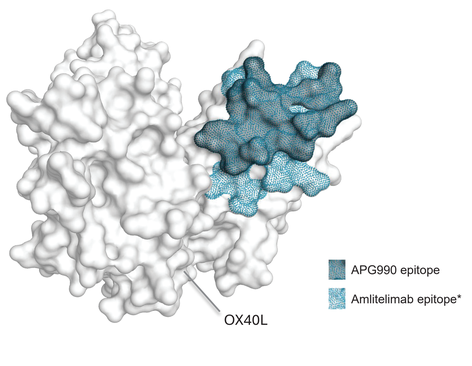


Figure 2. OX40L binding sites (epitopes) for APG990 and amlitelimab



- The OX40L binding epitope for APG990 partially overlaps with that of amlitelimab (**Figure 2**), a monoclonal antibody targeting OX40L[3] that is currently in Phase 3 development for the treatment of atopic dermatitis (AD).
- APG990 was engineered with several modifications to **increase plasma half-life** and **ablate Fc function** of the antibody:
  - APG990 contains a triple amino acid modification M252Y/S254T/T256E (referred to as a 'YTE' modification), in the fragment crystallizable (Fc) region that extends half-life in humans by increasing binding to neonatal Fc receptor (FcRn) under acidic pH conditions.
  - APG990 also contains two additional amino acid modifications L235A/L236A (referred to as a 'LALA' modification) in the Fc region, designed to ablate Fc-mediated functions.

Objective

- The objective of the studies reported here were:
  - To evaluate the binding affinity of APG990 for OX40L and blockade of the OX40/OX40L interaction and downstream inflammatory signaling.
  - To evaluate the pharmacokinetics of APG990 after a single SC or IV dose in non-human primates.

Materials and methods

- Monoclonal antibodies were produced by transient expression as research-grade material.
- Comparator antibodies were generated based on the published sequence for amlitelimab.
- The affinity of APG990 for human OX40L was measured by surface plasmon resonance (SPR).
- Blockade of the OX40/OX40L interaction was evaluated with an ELISA that examined competitive binding of APG990 and OX40 to human OX40L.
- The ability of APG990 to inhibit human OX40L-induced signaling was assessed by examining the effects of increasing concentrations of APG990 on a human OX40 reporter cell line.
- Activated human primary CD4+ T cells were used to assess the APG990 concentration-dependent blocking of OX40L-induced IL-2 release.
- The pharmacokinetics of APG990 were evaluated following a single bolus (SC or IV, ~50 mg/kg) of APG990 in cynomolgus monkeys.

Results

Preclinical characterization of APG990

- APG990 had a binding affinity of 106.4 pM to human OX40L compared with 70.9 pM for amlitelimab.
- In binding kinetics studies, APG990 demonstrated an expected YTE-dependent increase in FcRn binding and a LALA-dependent ablation of Fc-dependent binding (**Table 1**).
- APG990 inhibited human OX40L binding to OX40 in a concentration-dependent manner, with an IC_{90} of 6.5 nM vs 4.7 nM for amlitelimab (**Figure 3**). The IC_{50} was 1.8 nM for APG990 and 1.3 nM for amlitelimab.
- APG990 blocked signaling mediated by the OX40/OX40L axis, as measured by quantification of OX40L-induced IL-2 release. The IC_{90} was 2.9 nM for APG990 and 2.3 nM for amlitelimab (**Figure 4**). The IC_{50} values were 1.6 nM for APG990 and 0.9 nM for amlitelimab.
- APG990 blocked human OX40L-induced activation of OX40 reporter cells with an IC_{90} of 4.5 nM vs 7.8 nM for amlitelimab (**Figure 5**). The IC_{50} was 1.3 nM for APG990 and 1.7 nM for amlitelimab.
- In NHPs, APG990 exhibited a mean half-life of 25.5 (SC) and 26.9 (IV) days, versus 22.2 (SC) and 19.8 (IV) for amlitelimab (**Figure 6**; SC data shown).
  - APG990 had a clearance rate of 2.1 (IV) and 2.4 (SC) mL/day/kg, and was well-absorbed, with an average bioavailability of 96%.
  - Amlitelimab had a clearance rate of 2.8 (IV) and 3.5 (SC) mL/kg, with a bioavailability of 92%.

Figure 3. APG990 inhibits binding between OX40L and OX40

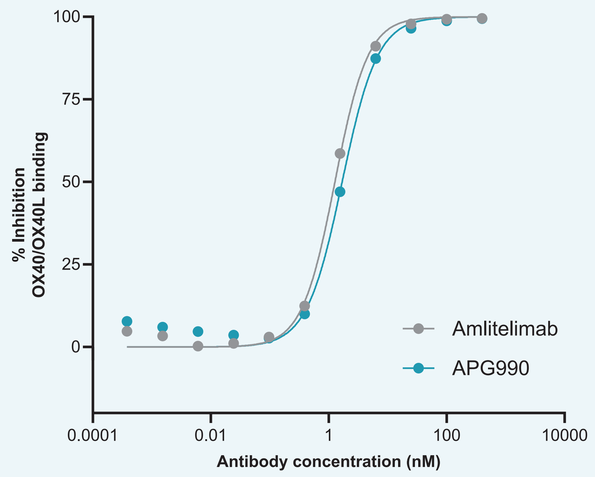
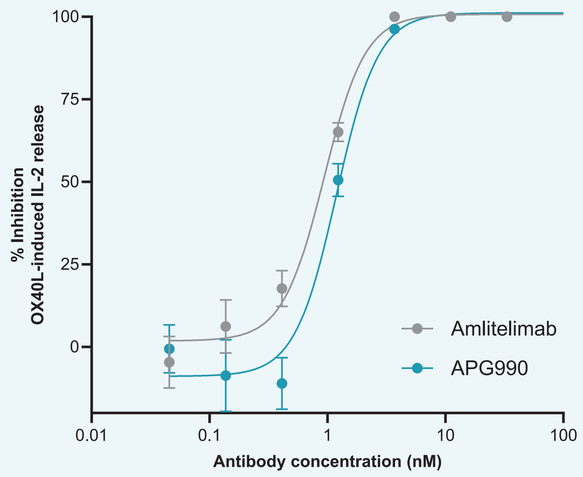


Figure 4. APG990 blocks OX40L-induced IL-2 release*



*Representative data from primary human T-cells isolated from four donors.

Figure 5. APG990 blocks human OX40L-induced activation of OX40 reporter cells

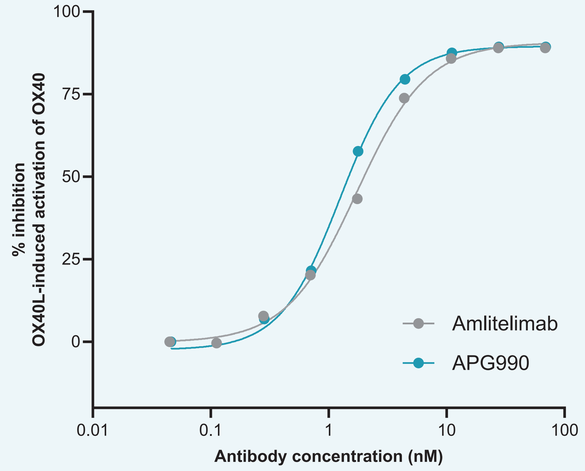
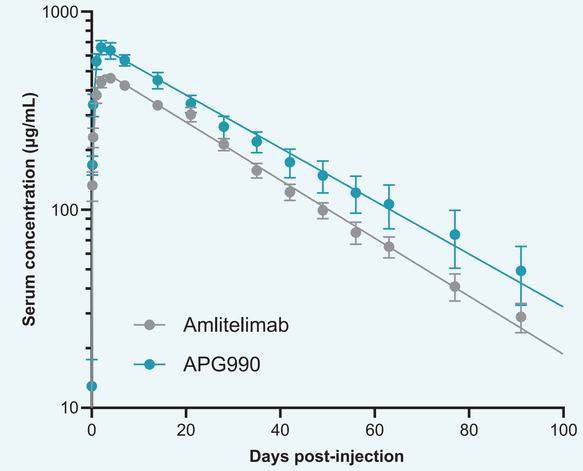


Figure 6. Pharmacokinetics of APG990 in NHP following a single SC bolus



Results

Table 1. Binding affinity of APG990 to human Fc-receptors and C1q

Ligand	APG990 KD (M)	IgG1 Positive Control KD (M)
FcRn	1.79×10^{-7}	1.49×10^{-6}
FcγRI	1.08×10^{-5}	2.11×10^{-9}
FcγRIIb	No or weak binding	1.22×10^{-5}
FcγRIIIb	No or weak binding	7.55×10^{-6}
FcγRIIa (H131)	No or weak binding	1.37×10^{-6}
FcγRIIa (R131)	No or weak binding	3.15×10^{-6}
FcγRIIIa (F158)	No or weak binding	1.33×10^{-6}
FcγRIIIa (V158)	1.15×10^{-5}	3.12×10^{-7}
C1q	No or weak binding	4.66×10^{-8}

FcRn binding was conducted at pH 6.0. FcRn/C1q binding was assessed using surface plasmon resonance (SPR).

Conclusions

- In preclinical assays, APG990 demonstrated strong binding affinity for OX40L and effectively blocked inflammatory signaling mediated by the OX40/OX40L complex.
- In NHPs, APG990 had a longer half life than amlitelimab.
- These data support continued development of APG990, which is currently being investigated in an ongoing Phase 1 trial.

References

1. Guttman-Yassky E, et al. *Br J Dermatol* 2024;191:488-96.
2. Croft M, et al. Regulation of T Cell Immunity by OX40 and OX40L. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013.
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- **GW**, **JM, LD** and **SR** are employees of Apogee Therapeutics, Inc. and may hold company stock/stock options. **BHK**, **JO**, and **HS** are current or former employees of Paragon Therapeutics, Inc. and may hold company stock/stock options.
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