APG777, a humanized IgG1 mAb, binds to IL-13 with high affinity and potently blocks IL-13 signaling in multiple in vitro assays

Eric Zhu1, Hussam Shaheen1, Carl Dambkowski2, and Jason Oh1

1Paragon Therapeutics, Inc. Waltham, MA, USA; 2Apogee Therapeutics, Inc. Waltham, MA, USA

Introduction

IL-13 is a T helper type 2 (Th2) cytokine that plays a key role in the pathogenesis of atopic dermatitis, asthma, and other allergic and immunologic conditions.1-3 APG777 is a humanized IgG1 monoclonal antibody (mAb) engineered to have high affinity for IL-13. It works by blocking heterodimerization of the signaling complex of IL-13/IL-13Rα/IL-4Rα and interrupts downstream inflammatory signaling (Figure 1): APG777 contains a triple amino acid modification, typically MG2S/V524/S7256 (referred to as a YTE modification), in the fragment crystallizable (Fc) region designed to extend its half-life in humans by increasing binding to neonatal Fc receptor (FcRn) and a LALA-dependent ablation of Fc-dependent binding. (Figure 1) In binding kinetics studies, APG777 demonstrated an expected YTE-dependent increase in binding affinity. Figure 2

Materials and methods

Monoclonal antibodies were produced by transient expression as research grade material. The affinity of APG777 for human IL-13 was measured by surface plasmon resonance (SPR) and compared with monoclonal antibodies published with established sequences of lebrikizumab and tralokinumab. The binding kinetics of APG777 to human Fc-receptors and C1q were determined by SPR and ELISA, respectively. Blockade of the signaling complex of IL-13/IL-13Rα/IL-4Rα and downstream signaling was assessed in cell-based assays, including:

- Inhibition of IL-13 binding to IL-13Rα/IL-4Rα
- Inhibition of pSTAT6 induction
- Inhibition of TARC secretion
- Inhibition of IL-13 binding to IL-13Rα/IL-4Rα

Table 1: Binding kinetics of APG777 to human Fc-receptors and C1q

<table>
<thead>
<tr>
<th>Ligand</th>
<th>APG777 EC50 (nM)</th>
<th>IgG1 pos. control EC50 (nM)</th>
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<tbody>
<tr>
<td>FcRβ</td>
<td>9.44 x 10^-6</td>
<td>1.28 x 10^-6</td>
</tr>
<tr>
<td>FcRβ (H135)</td>
<td>Not determinable</td>
<td>2.9 x 10^-5</td>
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<tr>
<td>FcRβ (R135)</td>
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<td>5.9 x 10^-6</td>
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<td>FcRβ (L235A/L236A)</td>
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<td>FcRβ (L108)</td>
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<td>9.57 x 10^-4</td>
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<td>FcRβ (V138)</td>
<td>1.21 x 10^-4</td>
<td>1.93 x 10^-4</td>
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<th>IgG1 pos. control EC50 (nM)</th>
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<tbody>
<tr>
<td>C1q</td>
<td>Not determinable</td>
<td>16</td>
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In cell-based assays (Figure 3), APG777 exhibited an IC50 of:
- 0.89 nM inhibiting IL-13 binding to an IL-13Rα/IL-4Rα overexpressing cell line compared with 1.11 nM for lebrikizumab.
- 0.28 nM inhibiting phosphorylation of STAT6 in H29 cells compared with 0.56 nM for dupilumab, 0.23 nM for lebrikizumab, and 0.41 nM for tralokinumab.
- 0.86 nM inhibiting release of TARC in A549 cells compared with 1.11 nM for dupilumab, 0.74 nM for lebrikizumab, and 4.14 nM for tralokinumab.
- 0.16 nM inhibiting proliferation of TF-1 cells compared with 0.19 nM for dupilumab, 0.20 nM for lebrikizumab, and 0.59 nM for tralokinumab.

In primary human lymphocytes (Figure 4), APG777 blocked IL-13 activity as exhibited by an IC50 of:
- 0.46 nM inhibiting phosphorylation of STAT6 compared with 0.38 nM for lebrikizumab.
- 0.86 nM in inhibiting CD23 expression compared with 0.81 nM for lebrikizumab.

Conclusions

- APG777 demonstrated similar affinity for IL-13 compared with monoclonic antibodies based on published sequences of lebrikizumab and tralokinumab.
- The enhanced binding to human FcRn and ablated binding to Fc-receptors and C1q confirmed the function of the YTE and LALA amino acid modifications, respectively. These findings support an expected half-life extension and Fc-silencing, and therefore increased safety in vivo.
- These data provide preclinical evidence of APG777’s clinical potential for the treatment of a variety of diseases where IL-13 signaling is the main driver of the inflammatory response, including atopic dermatitis.
- These data support the initiation of a Phase 1 study of APG777 in healthy volunteers, which has been initiated in Australia.

References

5. Zhu E, et al. EVRO 2023 (poster PR155)

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For further information please contact Carl Dambkowski (carl.dambkowski@apogeetherapeutics.com)