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

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Introduction

Interleukin-13 (IL-13) is a T helper type 2 (Th2) cytokine that plays a key role in the pathogenesis of atopic dermatitis, asthma, and other inflammatory 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 immunomodulatory signaling (Figure 1):

- APG777 contains a triple amino acid modification, typically M252Y/S254T/T256E (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) under acidic pH conditions.4,5

- APG777 also contains two additional amino acid modifications L235A/L236A (referred to as 'LALA' modification) in the Fc region, designed to stabilize Fc and complement effector functions.

- In this analysis, the affinity of APG777 for IL-13 was compared with lebrikizumab and tralokinumab.

- In addition, blockade of the IL-13/IL-13Rα/IL-4Rα signaling complex and downstream signaling by APG777 was assessed in multiple in vitro assays and compared with dupilumab, lebrikizumab, and tralokinumab.

In primary human lymphocytes (Figure 4), APG777 blocked IL-13 activity as exhibited by an IC50 of:

- 0.10 nM inhibiting proliferation of TF-1 cells compared with 0.19 nM for dupilumab,
- 0.89 nM inhibiting IL-13 binding on an IL-13Rα overexpressing cell line compared with 0.18 nM for dupilumab, 0.23 nM for lebrikizumab, and 0.41 nM for tralokinumab.
- 0.86 nM inhibiting release of TARC in As49 cells compared with 1.11 nM for lebrikizumab and 2.04 nM for dupilumab.
- 0.16 nM inhibiting phosphorylation of STAT6 in TF-1 cells compared with 0.39 nM for dupilumab, 0.20 nM for lebrikizumab, and 0.59 nM for tralokinumab.

In human primary 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 inhibiting CD23 expression compared with 0.81 nM for lebrikizumab.

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 using published sequences of lebrikizumab and tralokinumab.

The binding kinetics of APG777 to human Fc-receptors and C1q were determined by SPR (Figure 2).

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

<table>
<thead>
<tr>
<th>Ligand</th>
<th>IC50 (nM)</th>
<th>IC50 pos. control KD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcRα</td>
<td>9.44 x 10^-6</td>
<td>1.28 x 10^-6</td>
</tr>
<tr>
<td>FcRβ</td>
<td>7.88 x 10^-6</td>
<td>7.55 x 10^-6</td>
</tr>
<tr>
<td>FcRγRIIα (H131)</td>
<td>Not determinable</td>
<td>5.95 x 10^-6</td>
</tr>
<tr>
<td>FcRγRIIβ</td>
<td>Not determinable</td>
<td>1.53 x 10^-6</td>
</tr>
<tr>
<td>FcRγRII (I158)</td>
<td>5.62 x 10^-6</td>
<td>9.57 x 10^-6</td>
</tr>
<tr>
<td>FcRγRII (V158)</td>
<td>1.21 x 10^-5</td>
<td>1.93 x 10^-5</td>
</tr>
</tbody>
</table>

Conclusions

- APG777 demonstrated similar affinity for IL-13 compared with monoclonal antibodies based on published sequences of lebrikizumab and tralokinumab and similar potency in multiple functional assays compared with both antibodies based on published sequences of dupilumab and lebrikizumab.
- The enhanced binding to human FcRn and allotyping 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

4. APG777 is a key role in the pathogenesis of type 2 inflammatory disorders and is expressed in brush skin of patients with atop dermatitis.
5. The IL-13/IL-13Rα heterodimer binds to IL-4Rα, forming an inactive complex.
6. The active heterodimer IL-13/IL-13Rα/IL-4Rα triggers downstream type 2 inflammatory pathways.

Acknowledgements

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