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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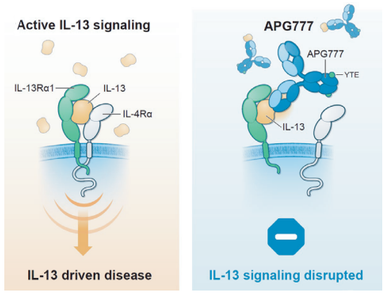
Introduction

- 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-13Rα1/IL-4Rα and interrupts downstream inflammatory 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 ablate 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-13Rα1/IL-4Rα signaling complex and downstream signaling by APG777 was assessed in multiple *in-vitro* assays and compared with dupilumab, lebrikizumab, and tralokinumab.

Figure 1: APG777 is designed to bind IL-13, thereby disrupting Th2 signaling by preventing formation of the IL-13Rα1/IL-4Rα heterodimer



- IL-13 signaling begins with binding of IL-13 to IL-13Rα1.
- This forms an inactive complex that then binds to IL-4Rα to create a complete, active heterodimer.
- Active IL-13Rα1/IL-4Rα heterodimer sets off a signaling cascade that leads to:
  - Skin barrier defects.
  - Immune cell recruitment.
  - Tissue inflammation.
  - Lichenification.
  - Pruritus.

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 and ELISA, respectively.
- Blockade of the signaling complex of IL-13/IL-13Rα1/IL-4Rα and downstream signaling was assessed in cell-line based assays, including:
  - Inhibition of STAT6 phosphorylation in HT-29 cells.
  - Inhibition of TARC release of A549 cells.
  - Inhibition of TF-1 cell-proliferation.
- Potency was also evaluated in lymphocyte-based assays, including:
  - Inhibition of STAT6 phosphorylation.
  - Inhibition of CD23 expression.

Results

- When measured by SPR, APG777 had an affinity of 78 pM compared with 131 pM and 116 pM for lebrikizumab and tralokinumab, respectively (**Figure 2**).
- In binding kinetics studies, APG777 demonstrated an expected YTE-dependent increase in FcRn binding and a LALA-dependent ablation of Fc-dependent binding. (**Table 1**).

Figure 2: Affinity for human IL-13 as measured by SPR

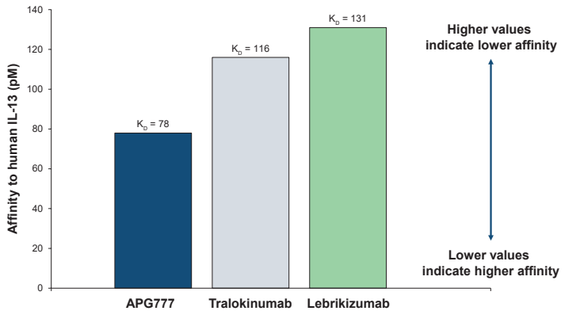


Table 1: Binding kinetics of APG777 to human Fc-receptors and C1q

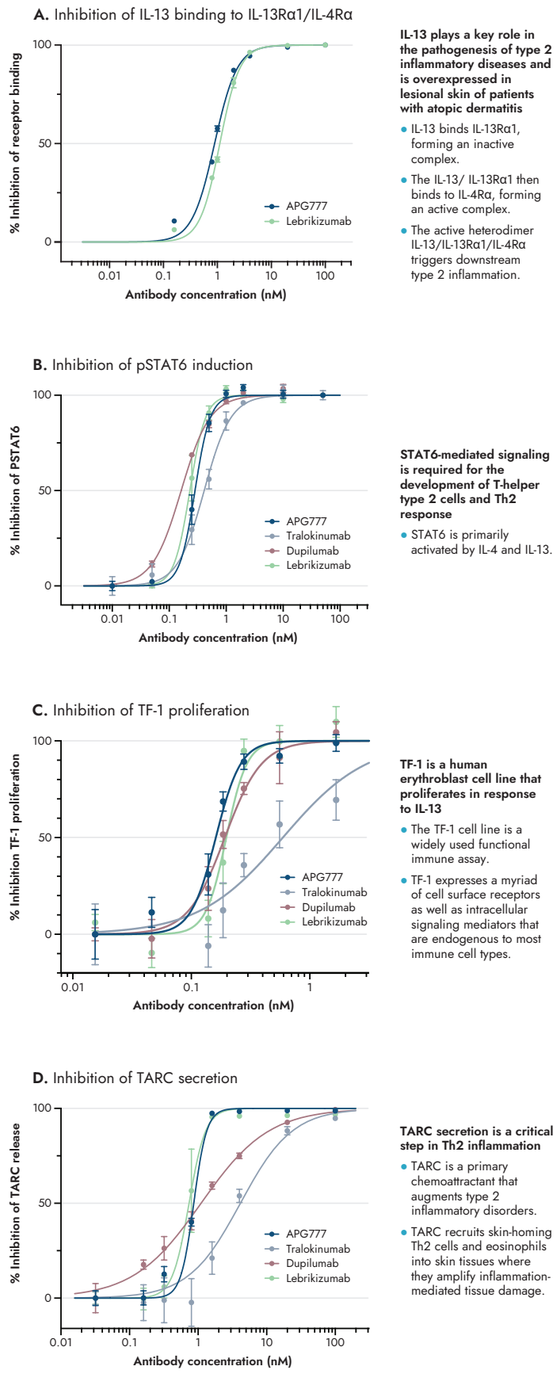
Ligand	APG777 KD (M)	IgG1 pos. control KD (M)
FcRn	9.44 x 10^{-8}	1.28 x 10^{-6}

Ligand	APG777 KD (M)	IgG1 pos. control KD (M)
FcγRI	7.88 x 10^{-6}	7.55 x 10^{-10}
FcγRIIa (H131)	Not determinable	2.93 x 10^{-6}
FcγRIIa (R131)	Not determinable	5.95 x 10^{-6}
FcγRIIb	Not determinable	1.53 x 10^{-5}
FcγRIIIa (F158)	5.62 x 10^{-5}	9.57 x 10^{-7}
FcγRIIIa (V158)	1.21 x 10^{-5}	1.93 x 10^{-7}

Ligand	APG777 EC_{50} (nM)	IgG1 pos. control EC_{50} (nM)
C1q	Not determinable	16

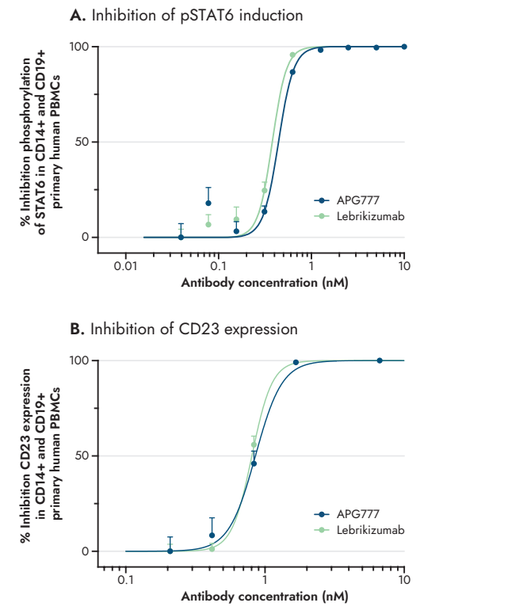
- In cell-line-based assays (**Figure 3**), APG777 exhibited an IC_{50} of:
  - 0.89 nM inhibiting IL-13 binding on an IL-13Rα1/IL-4Rα overexpressing cell line compared with 1.11 nM for lebrikizumab.
  - 0.28 nM inhibiting phosphorylation of STAT6 in HT-29 cells compared with 0.16 nM for dupilumab, 0.23 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 IC_{50} of:
  - 0.44 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.

Figure 3: Cell-line functional assays



Values represent mean ± SEM with non-linear regression fit

Figure 4: IL-13 blockade in primary human lymphocytes



Values represent mean ± SEM with non-linear regression fit

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 monoclonal antibodies based on published sequences of dupilumab and lebrikizumab.
- 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

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Acknowledgements

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