APG808, a fully human monoclonal IgG1 antibody, binds to IL-4Rα with high affinity and blocks IL-13 and IL-4 mediated signaling in multiple in vitro assays

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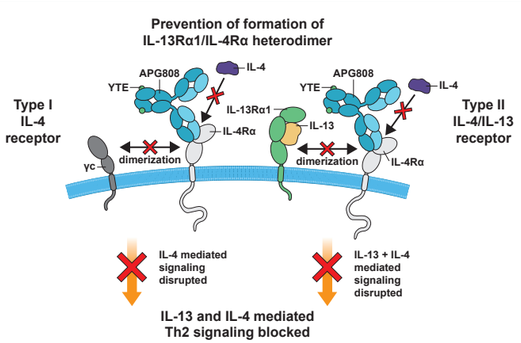
Introduction

- Interleukin-4 receptor-alpha chain (IL-4Rα) is a transmembrane protein that mediates the signaling of interleukin-4 (IL-4) and interleukin-13 (IL-13), which are key cytokines promoting type 2 inflammation.[1]
- Dysregulation of type 2 inflammation plays a central role in several human diseases, such as some forms of chronic obstructive pulmonary disease, asthma, and atopic dermatitis.[2,3]
- APG808 is an optimized, high-affinity, fully human IgG1 monoclonal antibody (mAb) that binds IL-4Rα and prevents formation of the IL-13Rα1/IL-4Rα active heterodimer and disrupts subsequent IL-13 and IL-4 mediated signaling (**Figure 1**).
- The Fc region of APG808 includes amino acid modifications M253Y/S255T/T257E (YTE), designed to extend antibody half-life by increasing binding to the neonatal Fc receptor.[4,5]
- APG808 also contains two additional amino-acid modifications L234A/L235A (LALA), designed to ablate Fc and complement effector functions.
- In these analyses, binding affinity of APG808 to human IL-4Rα was determined and multiple in vitro assays were used to evaluate the inhibition of IL-4Rα signaling by APG808.

Figure 1: APG808 is designed to disrupt IL-13 and IL-4 mediated Th2 signaling by preventing formation of the IL-13Rα1/IL-4Rα heterodimer



Materials and methods

- Binding affinity of APG808 to human IL-4Rα was determined by Kinetic Exclusion Assay (KinExA).
- In vitro assays were used to compare APG808 to a positive control monoclonal antibody based on the published sequence of dupilumab (PAL001-0001-4; herein referred to as dupilumab).
- Multiple cell-line based assays were used to assess APG808 blockade of IL-4 or IL-13 mediated signaling:
  - Inhibition of IL-4 and IL-13 binding to cells co-expressing IL-13Rα1 and IL-4Rα by flow cytometry.
  - Phosphorylation of STAT6 in HT-29 cells by flow cytometry.
  - Release of TARC in A549 cells by ELISA.
  - Proliferation of TF-1 cells.

Results

- Binding affinity (K_D) of APG808 to human IL-4Rα was 421 fM (0.4 pM), as determined by KinExA.
- APG808 and dupilumab potently inhibited formation of the full signaling complex of IL-13/IL-13Rα1/IL-4Rα and downstream signaling in multiple cell-line based assays (**Table 1; Figures 2–5**).

Table 1: Inhibition of full signaling complex of IL-13/IL-13Rα1/IL-4Rα and downstream signaling in multiple cell-line based assays

	IL-4 stimulation IC_{50} (nM) (95% CI)		IL-13 stimulation IC_{50} (nM) (95% CI)	
	APG808	Dupilumab	APG808	Dupilumab
Inhibition of binding to HEK293 cells overexpressing human IL-13Rα1 and IL-4Rα	0.27 (0.26–0.29)	0.25 (0.24–0.26)	0.23 (0.22–0.24)	0.27 (0.26–0.29)
Phosphorylation of STAT6 in HT-29 cells	0.25 (0.22–0.28)	0.30 (0.21–0.41)	1.05 (0.83–1.33)	1.08 (0.90–1.29)
Release of TARC in A549 cells	0.26 (0.23–0.29)	0.38 (0.34–0.43)	0.53 (0.48–0.58)	0.61 (0.57–0.66)
Proliferation of TF-1 cells	0.052 (0.046–0.058)	0.059 (0.052–0.057)	0.16 (0.15–0.18)	0.16 (0.15–0.18)

Figure 2: APG808 blocks IL-4 and IL-13 binding to their receptors

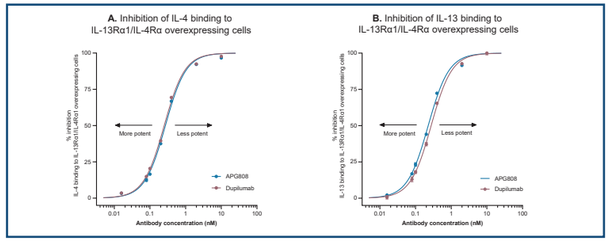
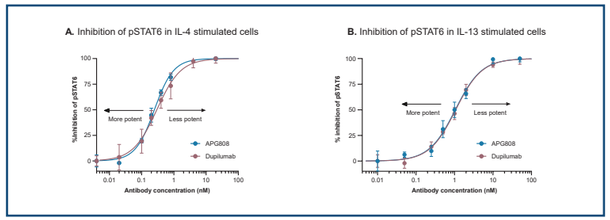
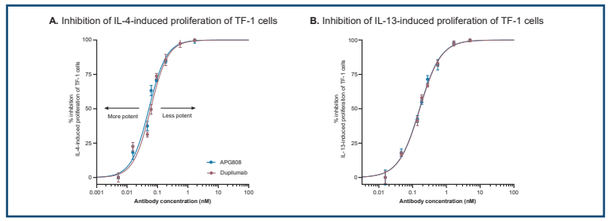


Figure 3: APG808 blocks STAT6 phosphorylation stimulated by IL-4 and IL-13



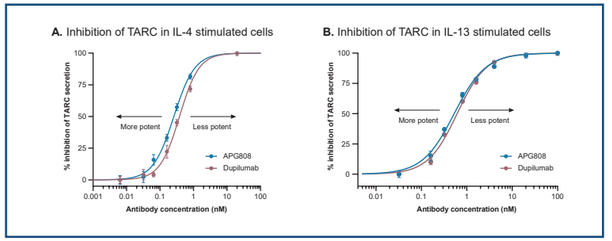
- STAT6-mediated signaling is required for the development of Th2 response.
- STAT6 is primarily activated by IL-4 and IL-13.

Figure 4: APG808 inhibits proliferation of TF-1 cells stimulated by IL-4 and IL-13



- TF-1 cells proliferate in response to IL-4 or IL-13.
- The TF-1 cell line is widely used in functional immune assays.
- TF-1 expresses a myriad of cell surface receptors as well as intracellular signaling mediators that are endogenous to most immune cell types.

Figure 5: APG808 inhibits TARC secretion stimulated by IL-4 and IL-13



- TARC secretion is a critical step in Th2 inflammation.
- TARC (also known as CCL17) is a primary chemoattractant that augments type 2 inflammatory disorders.
- TARC recruits skin-homing Th2 cells and eosinophils via chemokine receptors, CCR4 and CCR8, into skin tissues where they amplify inflammation-mediated tissue damage.

Conclusions

- APG808 demonstrated high affinity for human IL-4Rα and similar potency in multiple functional assays compared with a monoclonal antibody based on the published sequence of dupilumab.
- These data provide preclinical evidence of APG808's therapeutic potential in a variety of diseases, in which IL-4 and IL-13 induced signaling is the main driver of type 2 inflammation.
- The enhanced binding to human FcRn due to the YTE modification offers the potential for reduced dosing frequency with APG808 compared with currently available therapies.
- These data support the ongoing Phase 1 study of APG808 in healthy volunteers, which is currently enrolling in Australia.

References

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