APG777, a high-affinity humanized IgG1 monoclonal antibody targeting IL-13, demonstrates prolonged half-life and pSTAT6 inhibition in non-human primates

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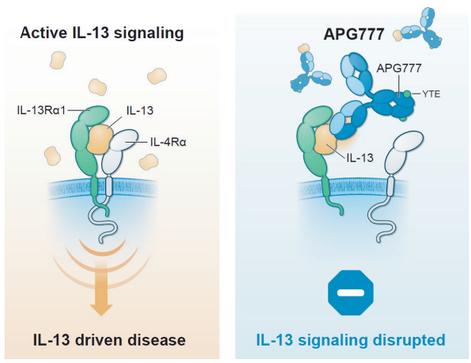
Introduction

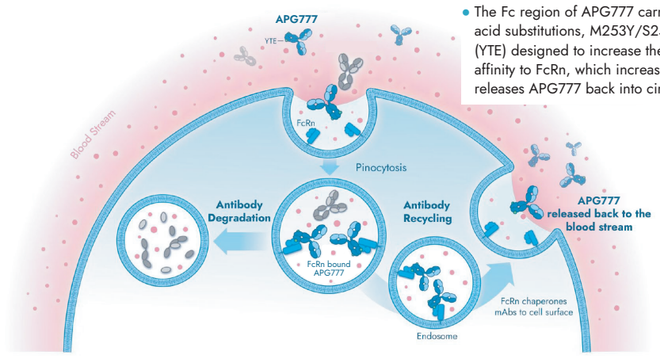
- APG777 is a high-affinity humanized anti-IL-13 IgG1 monoclonal antibody (mAb) that blocks formation of the IL-13/IL-13Rα1/IL-4Rα signalling complex, preventing receptor heterodimerization and downstream signalling (**Figure 1**).[1]
- APG777 contains a triple amino acid substitution, M253Y/S255T/T257E (referred to as a 'YTE' substitution), in the fragment crystallizable (Fc) region designed to extend its half-life in non-human primates (NHPs) and humans by increasing binding to the neonatal Fc receptor (FcRn) under acidic pH conditions (**Figure 2A**).
- In these experiments, the pharmacokinetics of APG777 and inhibition of downstream IL-13-mediated signaling (**Figure 2B**) was evaluated in NHPs.

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



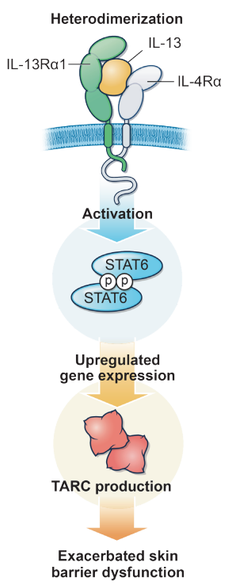
- IL-13 signaling begins with binding of IL-13 to IL-13Rα1.
- This forms an inactive complex that then binds to IL-4Rα to create a complete, active heterodimer.
- Active IL-13Rα1/IL-4Rα heterodimer sets off a signaling cascade that leads to:
  - Skin barrier defects.
  - Immune cell recruitment.
  -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

Figure 2A: Mechanism of APG777 half-life extension



- The Fc region of APG777 carries triple amino acid substitutions, M253Y/S255T/T257E (YTE) designed to increase the binding affinity to FcRn, which increases recycling and releases APG777 back into circulation.[2]

Figure 2B: pSTAT6 is a marker of IL-13 receptor activation



IL-13 signals through the IL-13Rα1 and IL-4Rα heterodimer. The bound receptor/ligand complex leads to phosphorylation (p) of Signal Transducer and Activator of Transcription 6 (STAT6), which can then enter the nucleus to drive transcription of inflammatory mediators, including Thymus and Activation Regulated Chemokine (TARC, also known as CCL17).[3]

Methods

Pharmacokinetics:

- The pharmacokinetics of APG777 and a monoclonal antibody based on the published sequence of lebrikizumab were studied in female cynomolgus monkeys.
- Cynomolgus monkeys (n=3 per dose group) were given a single bolus dose of 3 mg/kg of APG777 or lebrikizumab, given either intravenously (IV) or subcutaneously (SC).
- Blood samples were collected serially starting with a sample pre-dose and subsequently at 0.167, 1, 4, 8, 24, 48, 96, 168, 336, 504, 674, 840,1334,1680, and 2160 hours post-dose.
- Pharmacokinetic parameters included:
  - Maximum observed serum concentration (C_{max}).
  - Time to maximum observed serum concentration (T_{max}).
  - Area under the serum concentration versus time curve from time 0 extrapolated to infinity (AUC_{0-inf}).
  - Clearance (Cl).
  - Volume of distribution at steady-state (V_{ss}).
  - Half-life ($t_{1/2}$).
  - Absolute subcutaneous bioavailability (F).

Inhibition of pSTAT6:

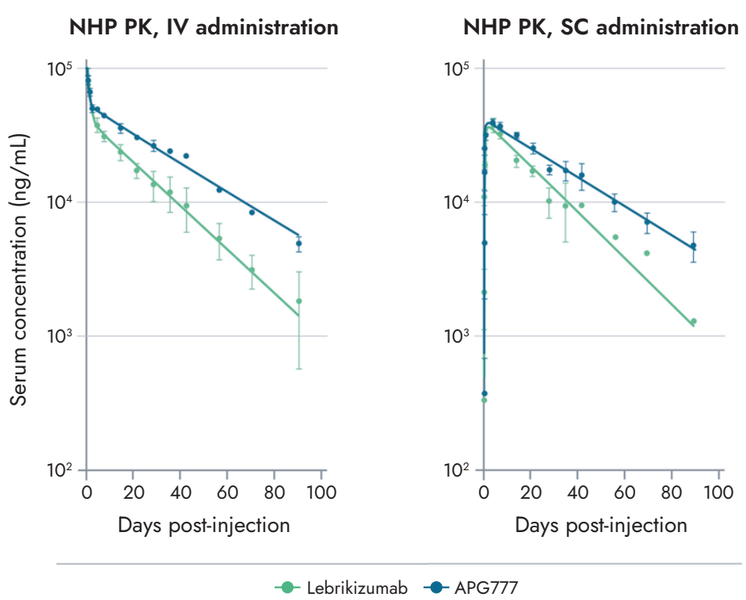
- Inhibition of pSTAT6 was assessed ex vivo in whole blood isolated from cynomolgus monkeys dosed with a tool compound of APG777 (n=4), APG777-LS, or a monoclonal antibody expressed based on the published sequence of lebrikizumab (n=4):
  - APG777-LS contains a half-life extending LS amino acid modification.
  - Cynomolgus monkeys were given a single bolus dose of 1 mg/kg SC.
- Whole blood samples were collected serially starting with pre-dose on Day -2 and subsequently at 0.167, 1, 4, 8, 24 hours and 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 days post-dose. At each time point collection, the freshly obtained blood samples were stimulated with recombinant human IL-13 for 15 minutes at 37C, then fixed and stained for intracellular phosphoSTAT6 and analyzed by flow cytometry gating on lymphocytes and myeloid cells.
- Animals that did not demonstrate a reduction in mAb exposure due to anti-drug antibodies were included in the primary aggregate analysis: 2/4 in the APG777-LS cohort and 3/4 in the lebrikizumab cohort.

Results

Pharmacokinetics:

- APG777 exhibited a $t_{1/2}$ of 28.2 days and Cl rate of 1.43 (mL/day/kg) when injected IV in NHPs (**Table 1, Figure 3**).
- Lebrikizumab exhibited a $t_{1/2}$ of 18.1 days and Cl rate of 2.93 (mL/day/kg) when injected IV in NHPs (**Table 1, Figure 3**).
- Both APG777 and lebrikizumab were well-absorbed, with subcutaneous bioavailability (F) determined to be 81.22% and 75.70% respectively (values for IV and SC administration are presented in **Table 1**).

Figure 3: Serum concentration-time curves for APG777 and lebrikizumab



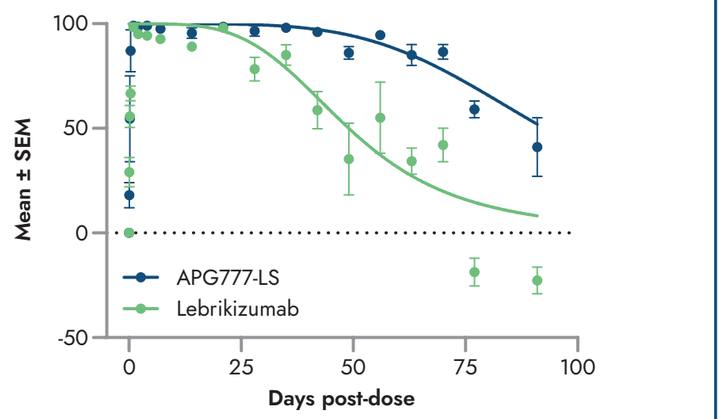
Values represent mean ± SEM serum concentration vs. time
IV, intravenous; NHP, non-human primate; PK, pharmacokinetic; SC, subcutaneous

Table 1: PK parameters of APG777 and lebrikizumab following a single bolus IV or SC dose in cynomolgus monkeys

Mean (SE)	APG777		Lebrikizumab*	
	IV	SC	IV	SC
T_{max} (days)	0	3.33 (0.67)	0	2.33 (0.89)
C_{max} (ng/mL)	1.03×10^5 (4.50×10^3)	4.13×10^4 (1.65×10^3)	9.68×10^4 (4.65×10^3)	4.25×10^4 (1.16×10^3)
AUC_{0-inf} (ng h/mL)	5.05×10^7 (1.99×10^6)	4.10×10^7 (5.39×10^6)	2.66×10^7 (4.76×10^6)	2.01×10^7 (4.18×10^6)
Cl (mL/day/kg)†	1.43 (0.05)	1.48 (0.20)	2.93 (0.61)	2.93 (0.53)
V_{ss} (mL/kg)†	54.06 (1.18)	57.24 (1.92)	59.26 (5.79)	44.95 (4.01)
F (%)‡	N/A	81.22 (13.70)	N/A	75.70 (27.40)
$t_{1/2}$ (days)	28.2 (1.16)	27.0 (2.45)	18.1 (3.87)	13.5 (2.66)

†Both Cl and V_{ss} of subcutaneous administration were dose normalized using the subcutaneous bioavailability (F) indicated in the table;
‡F (%) was calculated by dividing the mean dose-normalized AUC_{0-inf} following subcutaneous administration by the mean dose-normalized AUC_{0-inf} following IV administration.
AUC_{0-inf}, area under the serum concentration versus time curve from time 0 extrapolated to infinity; Cl, clearance; C_{max}, maximum observed serum concentration; F, bioavailability; IV, intravenous; PK, pharmacokinetic; SC, subcutaneous; SE, standard error; T_{max}, time to maximum observed serum concentration; V_{ss}, volume of distribution at steady-state.

Figure 4: Inhibition of pSTAT6



Inhibition of pSTAT6:

- Serum concentration >5 µg/mL of APG777-LS or lebrikizumab maintained complete inhibition of pSTAT6 in NHP whole blood (**Figure 4**).
- pSTAT6 inhibition was sustained for a longer duration with APG777-LS (IC_{90} = 56.8 days post-dose, IC_{50} = 92.6 days) compared to lebrikizumab (IC_{90} = 27.9 days, IC_{50} = 48.9 days) (**Figure 4**).

Conclusions

- APG777 demonstrated increased $t_{1/2}$ and exposure and reduced clearance relative to lebrikizumab in NHPs.
- The sustained inhibition of pSTAT6 supports the downstream anti-inflammatory properties of APG777.
- Together these studies provide preclinical support for an ongoing phase 1 study in healthy volunteers (Poster #LB945) and phase 2 study in moderate-to-severe atopic dermatitis.
- A Phase 2 study of APG777 in adults with moderate-to-severe atopic dermatitis is currently underway where every 3- to 6-month maintenance dosing will be evaluated.

References

1. Zhu E, et al. EADV 2023 (poster #P0437).
2. Dall'Acqua, et al. J Biol Chem 2006;281:23514–24.
3. Catherine & Roufosse. Semin Immunopathol 2021;43:439–58.

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