CBP-201, a novel and differentiated IL-4Rα targeting antibody being evaluated in Th2 inflammatory diseases

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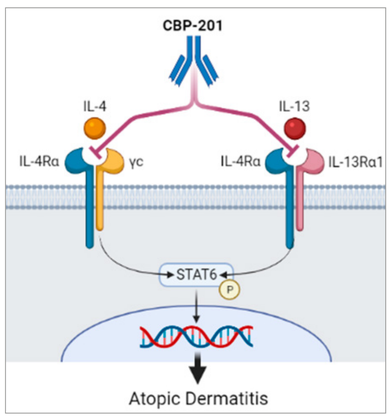


Fig 1: IL-4 and IL-13 cytokines contribute to atopic dermatitis pathogenesis; CBP-201 inhibits the signaling pathway

Introduction

Atopic dermatitis (AD) lesional skin is characterized by overexpression of inflammatory Th2 cytokines, including IL-4 and IL-13.[1]

Pharmacological evidence clearly demonstrates that binding of IL-4 and IL-13 to their cognate receptors (Fig. 1) significantly contributes to AD pathogenesis.[2,3]

Patients with AD also have elevated IgE levels, owing to IL-4 production by Th2 T cells in peripheral blood and skin.[4]

CBP-201 is a novel IgG4 targeting IL-4Rα (Fig. 1).

In a Phase 2b trial (WW001, NCT04444752), all dose regimens of CBP-201 met the primary endpoint in the treatment of moderate-to-severe AD - statistically significant reductions in Eczema Area and Severity Index (EASI) scores at Week 16.[3]

Here, we report the first description of the immunological profile of CBP-201 from our in-house preclinical experiments, including all comparisons to dupilumab.

Methods

Binding affinity to IL-4Rα

One of two surface plasmon resonance protocols was used. *Protocol A*: Anti-histidine antibody was immobilized onto a sensor chip, facilitating histidine tagged soluble (s)IL-4Rα to capture CBP-201 or dupilumab at different concentrations. *Protocol B*: Anti-Human IgG(Fc) was immobilized onto a sensor chip, then CBP-201 or dupilumab was captured. sIL4Rα was analyzed at different concentrations. The data were acquired with the Biacore 8K (GE).

IL-4Rα epitope mapping

Site-specific mutations and alanine scanning mutations were incorporated into sIL-4Rα to identify epitopes that bind to CBP-201 or dupilumab antibodies. ELISA was used to evaluate the binding activity of CBP-201 or dupilumab to the sIL-4Rα mutants using a Flex Station 3 (Molecular Devices) and half maximal effective concentration (EC_{50}) calculated (GraphPad Prism).

Binding to human IL-4Rα vs monkey and mouse IL-4Rα

The binding of CBP-201 to human, monkey, and mouse IL-4Rα was evaluated by ELISA. Soluble IL-4Rα proteins were coated onto the plates and a CBP-201 concentration range evaluated. Data were acquired with the Flex Station 3 (Molecular Devices) and EC_{50} calculated (GraphPad Prism).

Cytokine-induced intracellular signaling

Human embryonic kidney (HEK) Blue™ IL-4/IL-13 cells containing a STAT6 reporter gene (Invivogen) were stimulated with 0.5 ng/mL IL-4 or 2.5 ng/mL IL-13, either alone or in the presence of CBP-201 or dupilumab. Per the manufacturer's instructions, STAT6 activation was quantified via secreted alkaline phosphatase hydrolysis of QUANTI-Blue™ substrate colorimetric change.

Cytokine-induced cell proliferation

TF-1 human erythroid leukemia cells (ATCC) proliferate following IL-4 (0.5 ng/mL) or IL-13 (5 ng/mL) stimulation. Proliferation was quantified after 72 hours using a cell counting kit-8 (CCK-8, Beyotime) as per the manufacturer's instructions.

Cytokine-induced TARC release

Primary human peripheral blood mononuclear cells (PBMCs) were stimulated with IL-4 (2 ng/mL, Sino Biological) or IL-13 (1 ng/mL, Sino Biological). CBP-201 or dupilumab were added to the culture for 72 hours and supernatants collected. Human thymus and activation regulated chemokine (TARC) concentrations were quantified by ELISA (Abcam) as per the manufacturer's instructions. Data were acquired with the Flex Station 3 (Molecular Devices) and half maximal inhibitory concentration (IC_{50}) calculated (GraphPad Prism).

Cytokine-induced B cell activation

Splenocytes, isolated from B-hIL4/hIL4RA mice, were incubated with different concentrations of CBP-201, dupilumab, or human IgG4-kappa isotype control (hIgG4) in the presence of 50 pM human IL-4 or 50 nM human IL-13. After 72 hours, B cell activation was analyzed by flow cytometry using CD23 or MHCII as activation markers. Data were acquired with NxT software (Invitrogen) and mean fluorescence intensity (MFI) analyzed (GraphPad Prism).

Ovalbumin-induced Th2 allergy mouse model

Double humanized IL-4/IL-4RA (hIL-4/hIL-4RA) mice (Biocytogen) express human IL-4 and IL-4RA, replacing their murine counterparts. The mice were sensitized with repeated intraperitoneal injections of ovalbumin (OVA). CBP-201 or dupilumab was administered on days 20 and 23. On days 21-25, mice were stimulated by inhaling atomized OVA, triggering a Th2-driven recall response. At termination, alveolar lavage fluid was collected for flow cytometry and serum analyzed for OVA-specific IgE.

Results

CBP-201 binds with high affinity to IL-4Rα via a distinct epitope

CBP-201 demonstrated higher affinity for human IL-4Rα (20.7 pM) than dupilumab (45.8 pM; Table 1A).

In human IL-4Rα, mutation of amino acid residues that are crucial for IL-4 binding[5] had little effect on the affinity for dupilumab; however, the same mutations (A, C, D, E, L, M, O) completely abolished binding of CBP-201 (Table 1B).

Table 1: CBP-201 and dupilumab (A) binding affinity for IL-4Rα and (B) binding activity to IL-4Rα

A.

Ligand	Analyte	1:1 binding		
		K_a (1/Ms)	K_d (1/s)	K_D (M)
CBP-201	sIL-4Rα	6.51E+05	2.92E-04	4.48E-10
Dupilumab	sIL-4Rα	6.41E+05	3.31E-04	5.16E-10
sIL-4Rα	CBP-201	1.54E+07	3.19E-04	2.07E-11
sIL-4Rα	Dupilumab	7.71E+06	3.53E-04	4.58E-11

K_a association rate constant; K_d dissociation rate constant; K_D equilibrium dissociation constant

B.

IL-4Rα Mutation Code	Binding Activity	
	CBP-201	Dupilumab
No mutation		
A		
B		
C		
D		
E		
F		
G		
H		
I		
J		
K		
L		
M		
N		
O		
P		

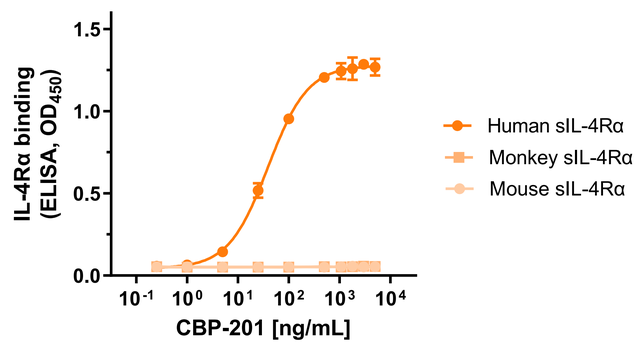
Activity was measured by binding affinity to IL-4Rα mutants or wildtype IL-4Rα

Binding Activity: Full, Moderate, Weak, None

CBP-201 is specific for human IL-4Rα

Cross-species ELISA revealed that CBP-201 bound to human sIL-4Rα with a K_D of 106 pM, but did not cross react with monkey or mouse IL-4Rα (Fig. 2).

Fig 2: CBP-201 binding to human IL-4Rα, compared with monkey and mouse IL-4Rα



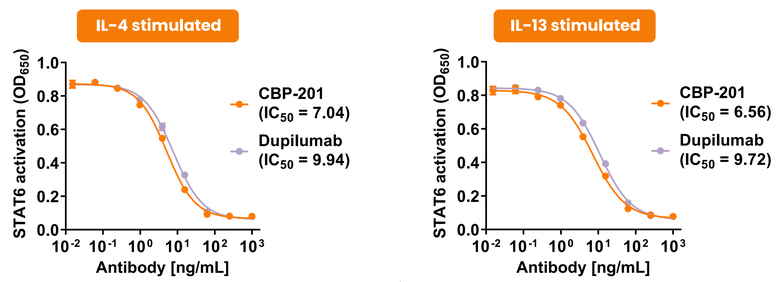
Data presented are mean ± standard error.

CBP-201 inhibits cytokine-induced intracellular signaling

IL-4 and IL-13, when engaged with their cognate receptors, trigger intracellular activation of the Janis kinase (JAK)-signal transducer and activator of transcription 6 (STAT6) pathway[6] (Fig. 1).

In the HEK Blue reporter cell assay, CBP-201 demonstrated concentration-dependent inhibition of STAT6 activation, with numerically more potent IC_{50} when compared head-to-head with dupilumab (Fig. 3).

Fig 3: Inhibition of cytokine-induced intracellular STAT6 signaling by CBP-201 vs dupilumab



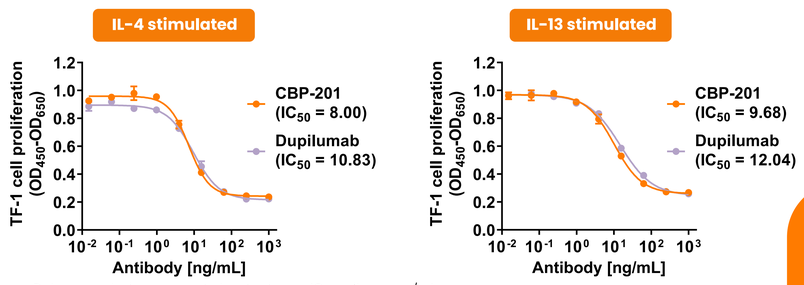
Data presented are mean ± standard error. IC_{50} units are ng/mL.

CBP-201 inhibits cytokine-induced cell proliferation

IL-4 and IL-13 are growth factors for the TF-1 cell line.[7]

Incubation with CBP-201 inhibited cytokine-induced TF-1 cell proliferation in a concentration-dependent manner, with numerically more potent IC_{50} when compared head-to-head with dupilumab (Fig. 4), consistent with findings from the STAT6 activation assay (Fig. 3).

Fig 4: Inhibition of cytokine-induced TF-1 cell proliferation by CBP-201 vs dupilumab



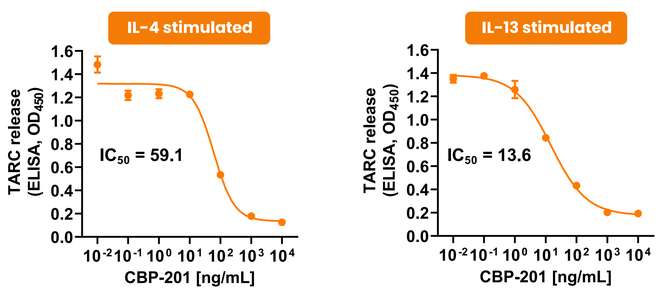
Data presented are mean ± standard error. IC_{50} units are ng/mL.

CBP-201 inhibits cytokine-induced TARC release

TARC, also known as CCL17, recruits Th2 cells to inflammatory sites and is a clinically validated serum biomarker of AD clinical activity.[6]

Healthy donor PBMCs, when activated *in vitro* with either IL-4 or IL-13, release TARC into the culture supernatant. Incubation with CBP-201 resulted in concentration-dependent inhibition of TARC release (Fig. 5).

Fig 5: Inhibition of cytokine-induced TARC release from primary human PBMCs by CBP-201



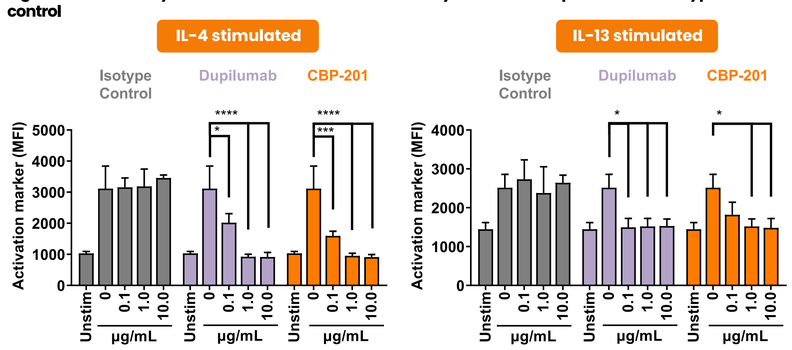
Data presented are mean ± standard error. IC_{50} units are ng/mL.

CBP-201 inhibits cytokine-induced B cell activation

Total serum IgE are elevated in patients with AD. B cells play an important role in allergic diseases, owing to secretion of IgE. Moreover, IL-13 promotes IgE synthesis in IL-4Rα-expressing B cells.[8]

Incubation with CBP-201 resulted in concentration-dependent inhibition of IL-4-induced upregulation of CD23 and IL-13-mediated MHCII expression in splenocytes isolated from B-hIL4/hIL4RA mice (Fig. 6). The magnitude of CBP-201 response was similar to dupilumab-treated splenocytes.

Fig 6: Inhibition of cytokine-induced B cell activation by CBP-201 vs dupilumab and isotype control



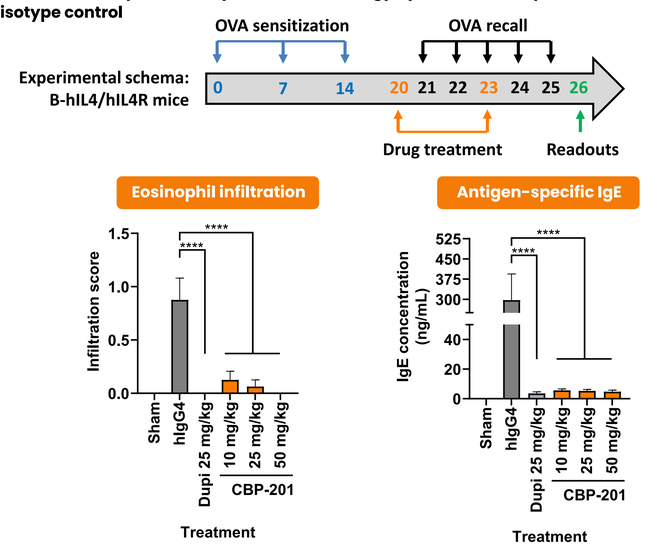
Data presented are mean + standard deviation. The figures show inhibition of IL-4-induced upregulation of CD23 and inhibition of IL-13-mediated MHCII expression. ANOVA: *p<0.05, ***p<0.0001.

CBP-201 inhibits experimentally induced Th2 allergy in a mouse model

Repeated OVA stimulation and recall challenge of double humanized (hIL-4/hIL-4RA) mice results in a model characterized by inflammatory cell infiltration, IgE release, and Th2 cytokine secretion.[9]

Subcutaneous administration of CBP-201 during the OVA recall phase dose-dependently inhibited immune cell tissue infiltration and significantly reduced the generation of circulating antigen-specific IgE (Fig. 7).

Fig 7: Inhibition of experimentally induced Th2 allergy by CBP-201 vs dupilumab and isotype control



Data presented are mean + standard error. ANOVA: ***p<0.0001.

Discussion

- This is the first description of the immunological profile of CBP-201, a novel IgG4, which demonstrates improved target engagement properties and greater inhibition of Th2-driven inflammation compared with dupilumab.
- CBP-201 engages with distinct epitopes and binds with higher affinity to the IL-4Rα target than dupilumab.
- CBP-201 inhibits IL-4/IL-13-dependent activation of the JAK-STAT pathway and cell proliferation in a concentration-dependent manner.
- IL-4/IL-13-mediated release of TARC, an inflammatory Th2 chemokine, is downregulated in the presence of CBP-201.
- CBP-201 effectively inhibits IL-4/IL-13-induced immune cell activation, tissue inflammation and IgE production.
- In a Phase 2b trial (WW001, NCT04444752), all dose regimens of CBP-201 met the primary endpoint in the treatment of moderate-to-severe AD - statistically significant reductions in EASI scores at Week 16.[3]
- CBP-201 is also under evaluation in persistent asthma (NCT04773678).

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