**Introduction**

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by overexpression of inflammatory Th2 cytokines, including IL-4 and IL-13.

Pharmacologically, evidence clearly demonstrates that binding of IL-4 and IL-13 to their cognate receptors contribute to AD pathogenesis. Patients with AD also have elevated IgE levels, owing to IL-4 production by TH2 T cells in peripheral blood and skin. Dupilumab is a novel IgG4 antibody targeting IL-4Rα (Fig. 1). In a Phase 2b trial (WW001, NCT04444475), 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.

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 to IL-4Rα*

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

*IL-4Rα epitope mapping*

Site-specific mutations and amino acid substitutions were incorporated into sIL-4Ra 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 receptor. The binding affinities were analyzed using a Flex Station 3 (Molecular Devices) and half maximal effective concentration (IC50) calculated (GraphPad Prism).

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

The binding of CBP-201 to human, mouse, and rIL-4Rα was evaluated by ELISA. Soluble IL-4Rα protein was coated onto plates and a CBP-201 binding competition range was determined. The data were acquired with the Flex Station 3 (Molecular Devices) and EC50 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 CBP-201 or dupilumab antibodies. ELISA was used to evaluate the binding activity of CBP-201 or dupilumab to the sIL-4Rα receptor.

*Cytokine-induced cell proliferation*

Primary human peripheral blood mononuclear cells (PBMCs) were stimulated with IL-4 (2 ng/mL, Sino Biological) or IL-13 (5 ng/mL). The magnitude of CBP-201 response was similar to dupilumab-treated splenocytes. The data were acquired with the Flex Station 3 (Molecular Devices) and IC50 calculated (GraphPad Prism).

*Cytokine-induced TARC release*

Primary human peripheral blood mononuclear cells (PBMCs) were stimulated with IL-4 or IL-13 (1 ng/mL, Sino Biological). CBP-201 or dupilumab were added to the culture for 72 hours and supernatants collected. Human TARC and activation regulated chemokine (TARC) concentrations were quantified by ELISA (Abcam) as per the manufacturer's instructions. The data were acquired with the Flex Station 3 (Molecular Devices) and half maximal inhibitory concentration (IC50) calculated (GraphPad Prism).

*Cytokine-induced B cell activation*

Splenocytes, isolated from hIL4/hIL4Rα mice, were incubated with different concentrations of CBP-201, dupilumab, or human IgG4-kappa isotype control (IgG4) in the presence of 50 ng/mL human IL-4 or 50 ng/mL human IL-13. After 72 hours, B cell activation was analyzed by flow cytometry using CD20 or CD23 markers as activation markers. The data were acquired with BD Cytomics FlowLab software (BD) and mean fluorescent intensity (MFI) analyzed (GraphPad Prism).

*Ovalbumin-induced Th2 allergy mouse model*

Double humanized (h4-13A, hIL4-4A) mice (Bicoyogenics) express human IL-4 and IL-13, replacing their murine counterparts. The mice were sensitized with repeated intraperitoneal injections of ovalbumin (OVA). CBP-201 or dupilumab was administered on days 25 and 28. On days 25–28, mice were challenged with intratracheal 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 nM) than dupilumab (48.6 nM). In human IL-4Rα, mutation of amino acid residues that are crucial for IL-4 binding had little effect on the affinity for dupilumab; however, the same mutations (A, G, L, E, M) completely abolished binding of CBP-201 (Table 1).

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

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Affinity (nM)</th>
<th>IC50 (nM)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4Rα</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.58×10^-10</td>
<td>2.31×10</td>
<td>4.48×10</td>
</tr>
<tr>
<td>B</td>
<td>2.31×10</td>
<td>5.58×10^-10</td>
<td></td>
</tr>
</tbody>
</table>

**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, NCT04444475), all dose regimens of CBP-201 met their primary endpoint in the treatment of moderate-to-severe AD—statistically significant reductions in EASI scores at Week 16.

CBP-201 is also under evaluation in persistent asthma (NCT04773678). The magnitude of CBP-201 response was similar to dupilumab-treated splenocytes. The data were acquired with the Flex Station 3 (Molecular Devices) and IC50 calculated (GraphPad Prism).

**Funding:** Connect Biopharm LLC. Additional assistance was provided by Fortis Pharma Consulting.

**References:**


**Poster ID L8945**