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Dual Targeting of Mast Cells and TSLP with the Bispecific Antibody CDX-622

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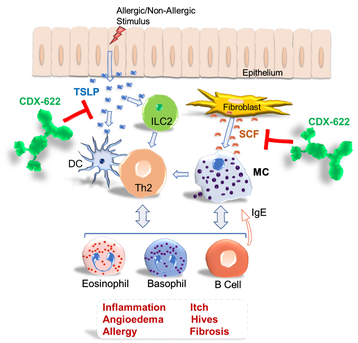
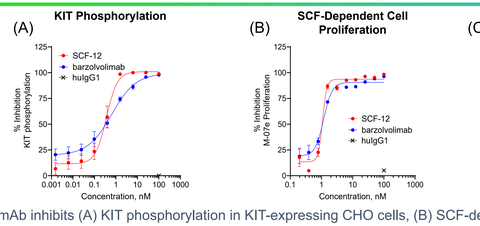
Background

- **Simultaneous neutralization of complementary pathways that drive chronic inflammation may result in improved clinical activity over single target inhibition**
- **Mast cells (MCs) are tissue-resident innate immune cells that drive or contribute to the pathophysiology of allergic, inflammatory, auto-immune, and fibrotic disorders**
  - Activation of the KIT receptor by its sole ligand, stem cell factor (SCF) is required for MC survival and plays a key role in their activation, maturation, and tissue recruitment
  - Reduction of tissue MCs with a KIT-directed inhibitory antibody (barzolvolimab) has show early promising clinical activity in chronic urticarias
  - SCF neutralization is expected to similarly decrease MC numbers
- **The alarmin thymic stromal lymphopoietin (TSLP) drives potent Type 2 inflammation by acting on dendritic cells, T lymphocytes and ILC2 cells**
  - TSLP has been implicated in other the pathogenesis of other disorders, including COPD and fibrosis.
  - TSLP neutralization has demonstrated clinical activity in both eosinophilic and non-eosinophilic asthma
- **Dual neutralization of SCF and TSLP with a bispecific antibody (bsAb) is expected to simultaneously reduce tissue MCs and inhibit Type 2 inflammatory responses**



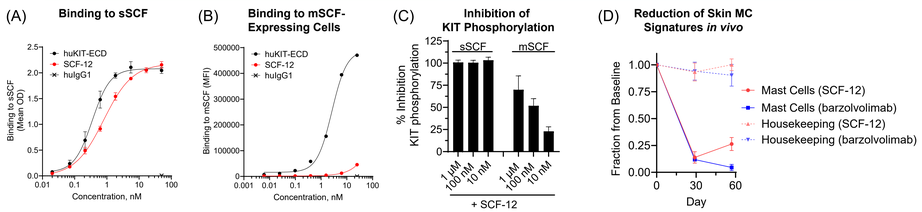
Results

The SCF-neutralizing mAb SCF-12 inhibits SCF/KIT activity *in vitro* with similar potency to barzolvolimab



SCF-12 mAb inhibits (A) KIT phosphorylation in KIT-expressing CHO cells, (B) SCF-dependent M-07e cell proliferation, and (D) SCF-dependent β-hexosaminidase release from IgE-crosslinked human mast cells.

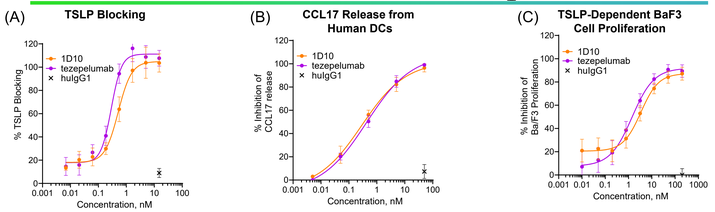
SCF-12 preferentially inhibits soluble over membrane SCF and reduces mast cell signatures *in vivo*



SCF-12 binds potently to (A) soluble SCF (sSCF) and weakly to (B) membrane-associated SCF (mSCF) in SI/SI4-SCF^{220} cells, relative to sKIT-ECD-Fc (SCF trap). (C) KIT-expressing M-07e cells stimulated with either soluble sSCF or SI/SI4 cells expressing mSCF. SCF-12 inhibits sSCF-dependent KIT phosphorylation more potently than KIT phosphorylation elicited by mSCF. (D) Reduction in mast cell RNA signatures in skin biopsies from cynomolgus macaques following administration of two 75 mg/kg of barzolvolimab (blue) or a chimeric SCF-12 variant lacking half-life extending (YTE) mutations (red) at days 1 and 8.

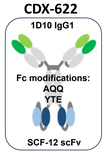
Results

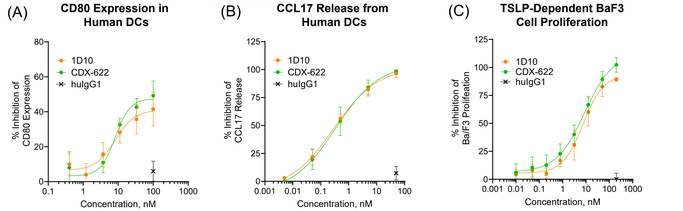
Characterization of the TSLP-neutralizing mAb 1D10



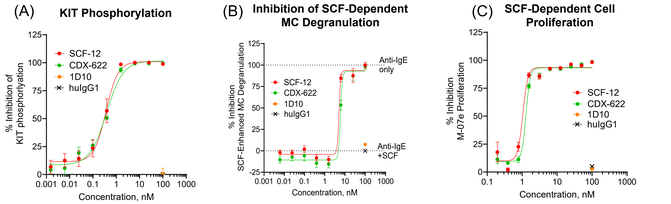
(A) 1D10 blocks binding to plate-coated TSLP receptor (TSLPR). 1D10 inhibits TSLP-dependent release of CCL17 from primary human dendritic cells (DCs) (B) and proliferation of BaF3 cells expressing TSLPR and IL17Rα (C) with similar potency as the approved anti-TSLP mAb tezepelumab

The bsAb CDX-622 inhibits TSLP and SCF with similar potency as its parental mAbs



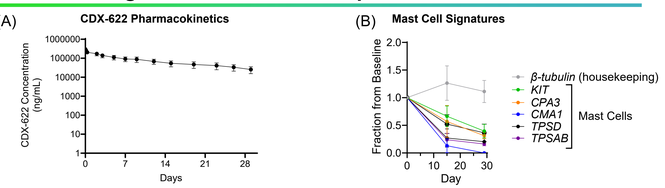


1D10 and CDX-622 similarly inhibit TSLP-mediated induction of (A) CD80 expression and (B) CCL17 secretion from human DCs, and (C) TSLP-dependent cell proliferation



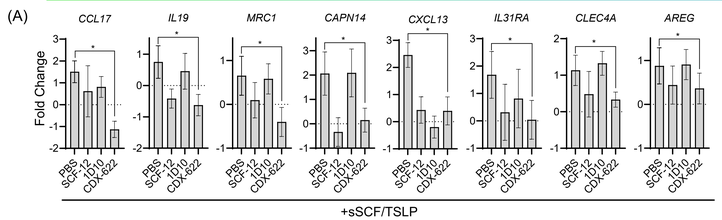
SCF-12 and CDX-622 similarly inhibit SCF-dependent (A) KIT phosphorylation, (B) human MC degranulation and (C) M-07e cell proliferation

CDX-622 exhibits mAb-like pharmacokinetics and reduces MC signatures in non-human primate skin



(A) CDX-622 exhibits mAb-like pharmacokinetics after a single 10 mg/kg intravenous infusion in cynomolgus macaques (n=3; mean ± stdev is shown). (B) CDX-622 reduces mast cell RNA signatures from ear punch biopsies from the same study.

CDX-622 inhibits SCF and TSLP-dependent inflammatory signatures in human skin



(A) Administration of SCF+TSLP in live human skin samples leads to upregulation of transcripts associated with myeloid cell activation (CCL17/TARC, MRC1, CLEC4A), epithelial barrier function (CAPN14, AREG), pruritus (IL31RA,) and inflammatory cytokines (IL19). Addition of CDX-622 leads to suppression of these signatures to a similar or greater extent than its parental mAbs.

(B) Gene Set Enrichment Analysis (GSEA) confirmed the enrichment of numerous pathways upon SCF and TSLP administration, including MC activation, acute inflammatory responses, and lymphocyte signaling. CDX-622 broadly inhibits pathways induced by SCF or TSLP.

Methods:
Live skin samples from 5 donors were treated with a combination of SCF+TSLP alone or in the presence of CDX-622 or its parental mAbs for 6 hours. Samples were subjected to RNA sequencing. (A) Selected transcripts implicated in distinct SCF and TSLP-related biological functions are shown. In all cases, CDX-622 treatment induces statistically significant reductions (p-value < 0.05)
(B) Gene set enrichment analysis (GSEA) was performed on the normalized data for each comparison (antibody treatment versus PBS-treated control) to produce a normalized enrichment score (NES). NES values > 0 and < 1 or > 1 are represented by small and large dots, respectively. Significant p-values (< 0.05) are shown in red, while unsignificant p-values (> 0.05) are represented by gray dots

Discussion

- **We report the discovery and characterization of CDX-622, a novel bsAb that neutralizes TSLP and leads to mast cell suppression by inhibiting SCF. CDX-622 exhibits the following properties:**
  - inhibits TSLP and SCF-dependent activities *in vitro* with similar potency as its parental mAbs as well as tezepelumab or barzolvolimab
  - preferentially inhibits the soluble over the membrane form of SCF, which may lead to differential impact on KIT-dependent processes
  - inhibits SCF and TSLP-dependent inflammatory signatures in a human skin explant model
  - exhibits mAb-like PK properties and leads to significant reduction in skin mast cell signatures in non-human primates
- **In a GLP toxicology study, CDX-622 was well tolerated at all dose levels, with a NOAEL established at the high dose level of 75 mg/kg/dose and led to a profound MC depletion in several tissues.**
- **CDX-622 may lead to improved outcomes in disorders where TSLP and SCF play complementary roles.**
- **A two-part single and multiple ascending dose study in healthy participants is ongoing (NCT06650761)**

This work was funded by Celldex Therapeutics