Dual Targeting of Mast Cells and TSLP with a Bispecific Antibody

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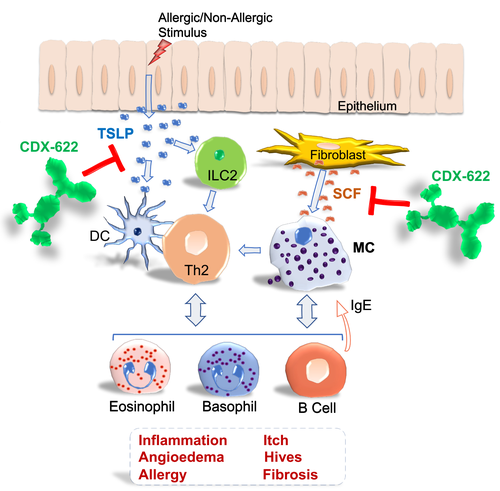
BACKGROUND

- 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 through a KIT-directed inhibitory antibody (barzolvolimab) has show early promising clinical activity in chronic urticarias[1]
  - 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 and has been implicated in fibrosis
  - TSLP neutralization has demonstrated clinical activity in both eosinophilic and non-eosinophilic asthma
- Combined neutralization of SCF and TSLP with a bispecific antibody is expected to simultaneously reduce tissue MCs and inhibit Type 2 inflammatory responses
- CDX-622 is a humanized tetravalent bispecific antibody that simultaneously depletes MCs and neutralizes TSLP, and may offer enhanced therapeutic benefit in inflammatory and fibrotic disorders



RESULTS

Discovery of a Unique SCF Neutralizing Antibody

- To target cytokine-driven pathways with a bispecific antibodies, we undertook discovery of monoclonal antibodies (mAbs) that neutralize SCF
- Neutralizing antibodies that preferentially inhibit soluble SCF over membrane SCF were pursued as they may drive more directed depletion of MCs relative to other KIT-expressing cells[2]
- SCF mAbs were generated through B cell cloning following immunization of mice with purified human SCF

- One mAb, SCF-12, potently inhibited soluble SCF-dependent KIT activation in *in vitro* assays with potency comparable to the clinical anti-KIT mAb barzolvolimab
- SCF-12 was humanized and incorporated into different bispecific constructs co-targeting other cytokines

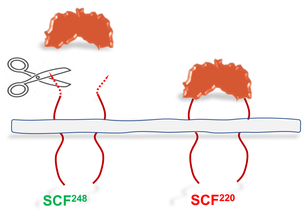
Inhibition of Soluble SCF-Dependent Activities (Mean IC50* nM ± SEM)

mAb	KIT Phosphorylation	MC Degranulation	M-07e Cell Proliferation
SCF-12	1.15 ± 0.20	3.86 ± 1.17	3.96 ± 0.44
Barzolvolimab	0.43 ± 0.11	3.51 ± 0.90	2.86 ± 0.59

*Average of 8-10 independent experiments

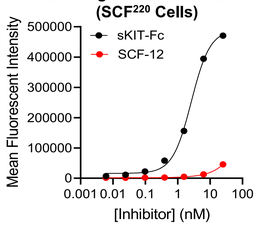
Anti-SCF mAb SCF-12 Preferentially Neutralizes Soluble Over Membrane-Associated SCF

Soluble vs. Membrane-Associated SCF



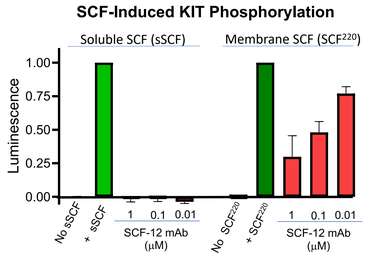
- SCF^{248} isoform is readily cleaved by proteases and secreted
- SCF^{220} isoform is slowly cleaved and largely membrane-bound
- Soluble and membrane SCF reported to play distinct roles in different tissues[2]

Binding to Membrane SCF (SCF^{220} Cells)



- SCF-12 binds very weakly to membrane-associated SCF, relative to sKIT-Fc (SCF trap) or other anti-SCF antibodies (not shown)

SCF-Induced KIT Phosphorylation

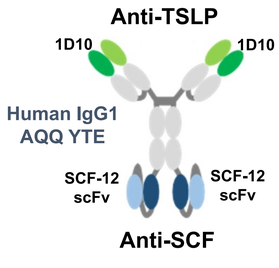


- SCF-12 blocks KIT phosphorylation in M-07e cells that have been stimulated with soluble SCF with greater potency than KIT stimulated with SCF^{220}-expressing cells

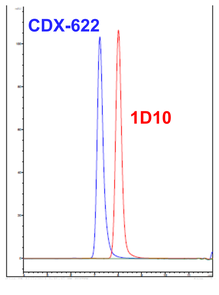
Discovery and Characterization of CDX-622, a TSLP x SCF-Neutralizing bsAb

- TSLP neutralizing mAbs were generated through hybridoma technology, following immunization of mice with purified human TSLP
- Resulting antibodies were screened for TSLP binding and potent neutralization in *in vitro* functional assays
- The lead candidate mAb for TSLP (1D10) was humanized
- Bispecific (bsAb) tetravalent antibody variants with an IgG1 backbone were constructed from the lead TSLP and SCF humanized mAbs and screened for expression in CHO cells, biophysical properties, and neutralization activity against each target
- CDX-622 emerged as the best bsAb variant and was selected for further development
- The Fc domain of CDX-622 was engineered to eliminate effector function (AQQ mutations) and extend serum half-life (YTE mutations)

CDX-622



SEC-HPLC



Affinity for Human and Cynomolgus TSLP and SCF (Bio-layer Interferometry)

TSLP Affinity		
CDX-622	Human TSLP	Cyno TSLP
K_D (nM)	0.540	33.7
K_{on} (1/Ms)	1.87E+06	6.38E+05
K_{dis} (1/s)	1.01E-03	2.15E-02
R^2	0.9603	0.9948

SCF Affinity		
CDX-622	Human SCF	Cyno SCF
K_D (nM)	5.21	1.16
K_{on} (1/Ms)	1.32E+04	4.38E+04
K_{dis} (1/s)	6.87E-05	5.08E-05
R^2	0.9989	0.9992

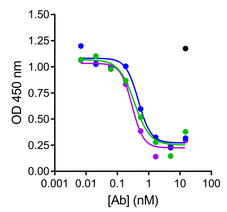
- CDX-622 exhibits good biophysical properties
- High affinity for human TSLP and SCF, and significant cross-reactivity to cynomolgus targets

References
1. Terhorst-Molawi, et al. Allergy. 2022.
2. Tajima et al. PNAS. 1998.

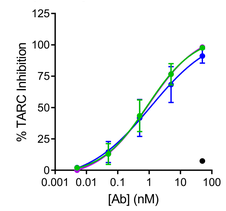
RESULTS

CDX-622 Inhibits TSLP-Dependent Activity *In Vitro*

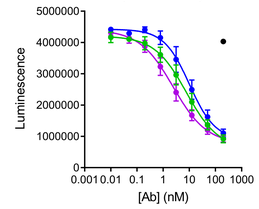
Inhibition of TSLP Binding to TSLP-R (ELISA)



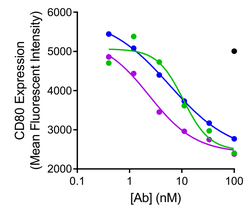
Inhibition of TSLP-Mediated TARC Release (Primary Human Dendritic Cells)



Inhibition of TSLP-Mediated Cell Proliferation (BaF3 Cells)



Inhibition of CD80 Expression (Primary Human Dendritic Cells)

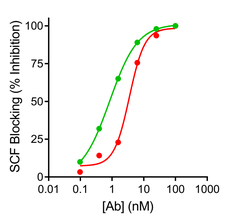


- Anti-TSLP mAb 1D10 neutralizes TSLP-dependent activity in *in vitro* functional assays with a potency comparable to tezepelumab
- CDX-622 retains comparable activity to its parental mAb 1D10

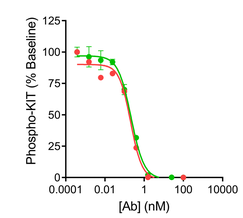
1D10 (Anti-TSLP mAb)
CDX-622
Tezepelumab
Control Human IgG1

CDX-622 Neutralizes Soluble SCF Activity *In Vitro*

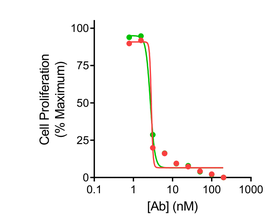
Inhibition of SCF Binding to KIT (ELISA)



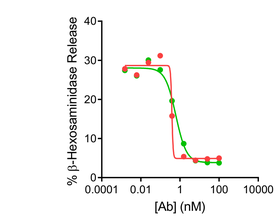
Inhibition of SCF-Dependent KIT Phosphorylation (CHO-KIT Cells)



Inhibition of SCF-Dependent Cell Proliferation (M-07e Cells)



Inhibition of SCF-Dependent Degranulation (Primary Human MCs)



- CDX-622 maintains similar neutralizing potency to its parental mAb, SCF-12

SCF-12 (Anti-SCF mAb)
CDX-622

Pharmacokinetics, Tolerability, and MC Impact in a Pilot Non-Human Primate Study

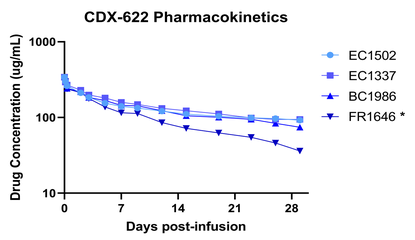
Study Design

- Four cynomolgus macaques were administered 10 mg/kg of CDX-622 on Day 1 by a slow intravenous injection
- Blood samples were taken as indicated for determination of circulating test article levels, ADAs and clinical pathology
- Punch biopsies from each ear pinna, along with a buccal swab, were collected for histology and RNA (Nanostring) analysis

Test Article	Animal ID	Sex	Dose Level	Dose Schedule
CDX-622	EC1502	F	10 mg/kg	Day 1
	EC1337	M		
	BC1986	F		
	FR1646	M		

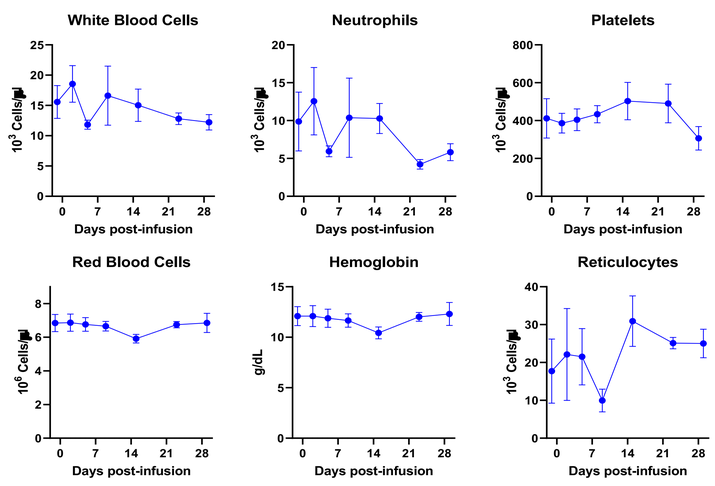
Pharmacokinetics and Immunogenicity

CDX-622 Pharmacokinetics



- Favorable PK profile and high exposure
- T1/2 and clearance consistent with that of IgG1 antibodies
- * Animal FR1646 developed anti-drug antibodies, resulting in decreased antibody exposure

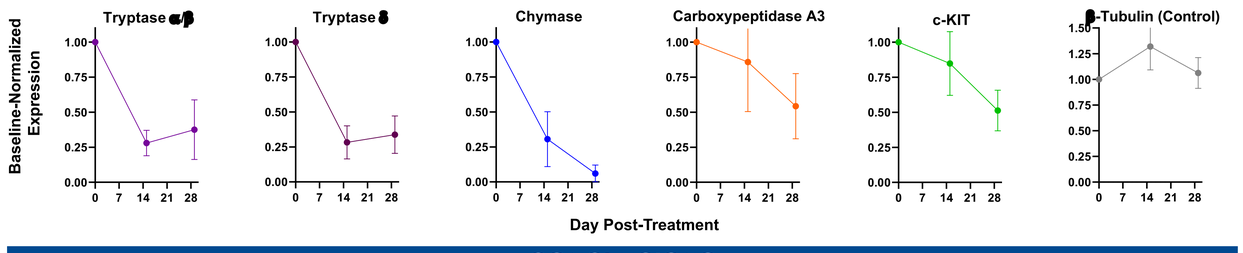
Hematology



- CDX-622 administration did not result in significant decreases in hematological parameters

A Single Dose of CDX-622 Reduces Skin MCs

Baseline-normalized RNA Analysis (Nanostring)



CONCLUSIONS

- CDX-622 is a bsAb that potently neutralizes the alarmin TSLP, and depletes MCs via SCF starvation
  - CDX-622 inhibits TSLP and SCF with similar potency to both its respective parental mAbs and comparator mAbs *in vitro*
  - Preferential inhibition of soluble over membrane-associated SCF may result in differential effects in KIT-expressing cells
- A single dose IV administration of CDX-622 to cynomolgus macaques was well tolerated
  - Favorable pharmacokinetics with a clearance and half-life consistent with IgG1 antibodies
  - No significant clinical pathology findings
    - Hematology parameters remain in the normal range
  - Strong evidence of skin MC depletion, consistent with expected role of SCF/KIT in MC survival
- Simultaneous inhibition of TSLP and MCs with CDX-622 may result in improved efficacy over approaches targeting single pathways in inflammatory, fibrotic, and allergic disorders
- Manufacturing and IND-enabling activities are ongoing

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