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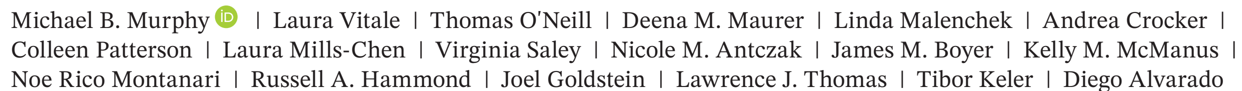
Allergen-Specific Immunotherapy and Biologics

# Dual Inhibition of Mast Cells and Thymic Stromal Lymphopoietin Using a Novel Bispecific Antibody, CDX-622



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## ABSTRACT

**Background:** Mast cells (MCs) respond to an array of allergens that drive allergic and inflammatory diseases. Stem cell factor (SCF), the ligand for the receptor KIT, is required for MC survival and function. Thymic stromal lymphopoietin (TSLP) is an alarmin that promotes Type 2 inflammation in asthma and other inflammatory diseases. We describe CDX-622, a bispecific antibody (bsAb), that targets both SCF and TSLP to neutralize these distinct cytokines.

**Methods:** The bsAb CDX-622 was developed from novel antagonist monoclonal antibodies (mAbs) to SCF (SCF-12) and TSLP (1D10). CDX-622 encodes the full-length 1D10 mAb and the single-chain variable fragment of SCF-12, linked to the C-terminus of the 1D10 heavy chain. CDX-622 was modified to prevent Fcγ receptor interactions and enhance FcRn binding. CDX-622 was tested using in vitro assays of MC and dendritic cell (DC) activation, an ex vivo human skin model, and in vivo studies in non-human primates.

**Results:** Novel SCF and TSLP mAbs with neutralizing activity were generated. The bsAb CDX-622 potently inhibited SCF-driven MC degranulation and TSLP-mediated CCL17 release by DCs. In human skin samples treated with SCF and TSLP, CDX-622 markedly reduced proinflammatory, MC, and DC-related RNA signatures. Additionally, CDX-622 and SCF-12 mAb administered to cynomolgus macaques (*Macaca fascicularis*) had a profound effect on MCs without any observed toxicity.

**Conclusions:** CDX-622 is a potent inhibitor of MCs through the neutralization of SCF and effectively blocks Type 2 inflammatory responses driven by TSLP. Dual inhibition of these cytokines may lead to improved clinical outcomes in certain inflammatory disorders.

## 1 | Introduction

Biologic-based therapies targeting cytokine pathways have resulted in marked clinical benefit in many inflammatory and autoimmune disorders [1]. However, many of these diseases are heterogeneous in nature and are generally driven by a multitude of cell types, pathways, or mechanisms, which inherently limits the effectiveness of most therapies directed at single targets. Using multispecific antibodies to target nonredundant pathways known to play a role in a given disease can be a valuable approach to drive improved disease outcomes [2, 3].

Mast cells (MCs) are tissue-resident innate immune cells located at the interface of the external environment, such as skin, airways, gastrointestinal tract, and vasculature, and are poised to rapidly respond to external or intrinsic stimuli [4–7]. MCs have

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evolved to recognize and respond to invading pathogens, allergens, venoms, and toxins [8–12]. Once activated, MCs rapidly degranulate, releasing a variety of prestored mediators including proteases, histamine, and serotonin, and produce lipid mediators including prostaglandins and leukotrienes, all of which induce vasodilation and itch, and facilitate the influx of other immune cells [4, 13–15]. Furthermore, activated MCs produce a wide array of cytokines and chemokines through de novo synthesis that further promote immune cell infiltration into tissues and their activation [16, 17]. These responses can be sufficient to drive acute events such as anaphylaxis and chronic diseases such as chronic spontaneous urticaria (CSU) [18, 19]. Furthermore, MCs can shape both Type 1 and Type 2 adaptive responses and potentiate many allergic, fibrotic, and inflammatory diseases [20–23].

One approach to inhibiting the contribution of MCs to disease pathogenesis is to antagonize the receptor tyrosine kinase KIT (c-KIT, CD117), which plays an important role in various MC functions and is required for their survival in tissues [24, 25]. This concept is emerging as a promising therapeutic option in CSU and potentially other MC-driven diseases with the recent development of the KIT antagonist mAb, barzolvolimab [26, 27]. KIT activity is controlled by its only ligand stem cell factor (SCF), which is produced either as a predominantly secreted form (sSCF) or as a membrane-associated form (mSCF) depending on the isoform expressed [28]. Both sSCF and mSCF contribute to KIT activation, with variable effects depending on the physiological context. For example, mice engineered to predominantly express mSCF appear to have normal hematopoiesis but a significantly reduced number of MCs, suggesting tissue MCs are more reliant on sSCF, while hematopoietic stem cells rely more heavily on signaling through mSCF [29, 30].

Thymic stromal lymphopoietin (TSLP) is an alarmin that drives potent Type 2 inflammatory responses [31, 32]. TSLP is primarily produced in epithelial cells of the skin, gut, and lung, and its expression is driven by a wide variety of environmental stimuli, including injury, TLR ligands, infection, and cytokines [33–36]. TSLP drives strong Type 2 dendritic cell (DC) polarization [37, 38] and directly activates innate lymphoid cells (ILC2) [39], as well as $CD4^+$ [40, 41] and $CD8^+$ T cells [42, 43], and MCs under inflammatory conditions [34] by engaging a heterodimeric receptor complex comprising TSLP receptor (TSLPR) and the IL-7Rα receptor [44, 45]. TSLP plays a strong pathogenic role in both eosinophilic and noneosinophilic asthma, and its role in other inflammatory diseases is currently being investigated in the clinical setting with neutralizing monoclonal antibodies (mAbs), including tezepelumab [46].

While MC- and TSLP-directed therapies have demonstrated promising or significant clinical efficacy in inflammatory disorders, their combined inhibition may lead to meaningfully improved outcomes in diseases where each pathway contributes uniquely to disease pathogenesis. Here, we describe CDX-622, a novel bispecific antibody (bsAb), that simultaneously depletes MCs through SCF neutralization and potently inhibits Type 2 inflammatory responses via TSLP inhibition.

## 2 | Results

### 2.1 | Characterization of a Novel SCF Neutralizing mAb SCF-12

mAbs against SCF were generated by immunizing mice with recombinant human sSCF protein, and candidate mAbs were identified using a single B cell screening approach. The SCF mAb, designated SCF-12, was selected based on its neutralizing potency and its unique properties, and subsequently humanized. In these assays, SCF-12 generally exhibited similar potencies as the anti-KIT mAb barzolvolimab [26]. As shown in Figure 1A, SCF-12 completely blocked the binding of sSCF to the purified human KIT extracellular domain (huKIT-ECD), with low nanomolar potency ($IC_{50} = 2.1 \pm 0.01$ nM). The anti-KIT mAb barzolvolimab, which prevents SCF binding through an allosteric mechanism, is shown as a comparator. The addition of serially diluted SCF-12 and barzolvolimab on CHO-KIT cells stably transfected to express human KIT–inhibited sSCF-dependent KIT phosphorylation ($IC_{50} = 359 \pm 18$ pM and $IC_{50} = 641 \pm 133$ pM, respectively; Figure 1B). Also, SCF-12 and barzolvolimab inhibited sSCF-dependent proliferation of the myeloid leukemia cell line M-07e ($IC_{50} = 1.1 \pm 0.1$ nM and $IC_{50} = 1.2 \pm 0.1$ nM, respectively; Figure 1C). The activity of SCF-12 was evaluated in human peripheral blood mononuclear cell (PBMC)–derived MCs, and consistent with previously published data, SCF addition to human MCs significantly enhanced IgE/FcεR1-dependent MC degranulation as measured by β-hexosaminidase release [47]. In this assay, SCF-12 and barzolvolimab fully inhibited sSCF-enhanced β-hexosaminidase release ($IC_{50} = 5.3 \pm 0.6$ nM and $IC_{50} = 5.4 \pm 0.6$ nM, respectively; Figure 1D).

We discovered that SCF-12 preferentially bound to and inhibited sSCF relative to mSCF. SCF-12 bound to plate-immobilized sSCF with a similar potency as huKIT-ECD ($IC_{50} = 800 \pm 156$ pM and $IC_{50} = 350 \pm 72$ pM, respectively; Figure 1E). However, SCF-12 only bound weakly to Sl/Sl4 cells transfected to express the membrane-associated $SCF^{220}$ variant (Sl/Sl4 $SCF^{220}$) (Figure 1F). Additionally, when KIT-expressing M-07e cells were stimulated with parental Sl/Sl4 cells (expressing no SCF) spiked with recombinant sSCF, SCF-12 potently inhibited sSCF-dependent KIT phosphorylation. By contrast, SCF-12 only partially inhibited KIT phosphorylation induced by Sl/Sl4 $SCF^{220}$ cells (Figure 1G).

SCF-12 mAb cross-reacted with cynomolgus macaque (*Macaca fascicularis*) sSCF (Table S1) and a chimeric IgG1 variant with an unmodified Fc region was tested in a study to investigate its effect on MCs in vivo and to perform a preliminary assessment of toxicity. Barzolvolimab, a fully humanized mAb bearing half-life extending modifications, was used as a comparator [26]. Animals were dosed at 75 mg/kg on Day 1 (D1) and Day 8 (D8) with either SCF-12 ($n = 3$) or barzolvolimab ($n = 2$). In this study, there were no SCF-12 or barzolvolimab-related clinical changes observed, including body weights and body temperature. Clinical chemistry and hematological parameters remained unchanged or were considered nonadverse. Importantly, RNA isolated from skin biopsies taken from the ear prior to treatment (D1), on Day 30 (D30), and Day 57 (D57)



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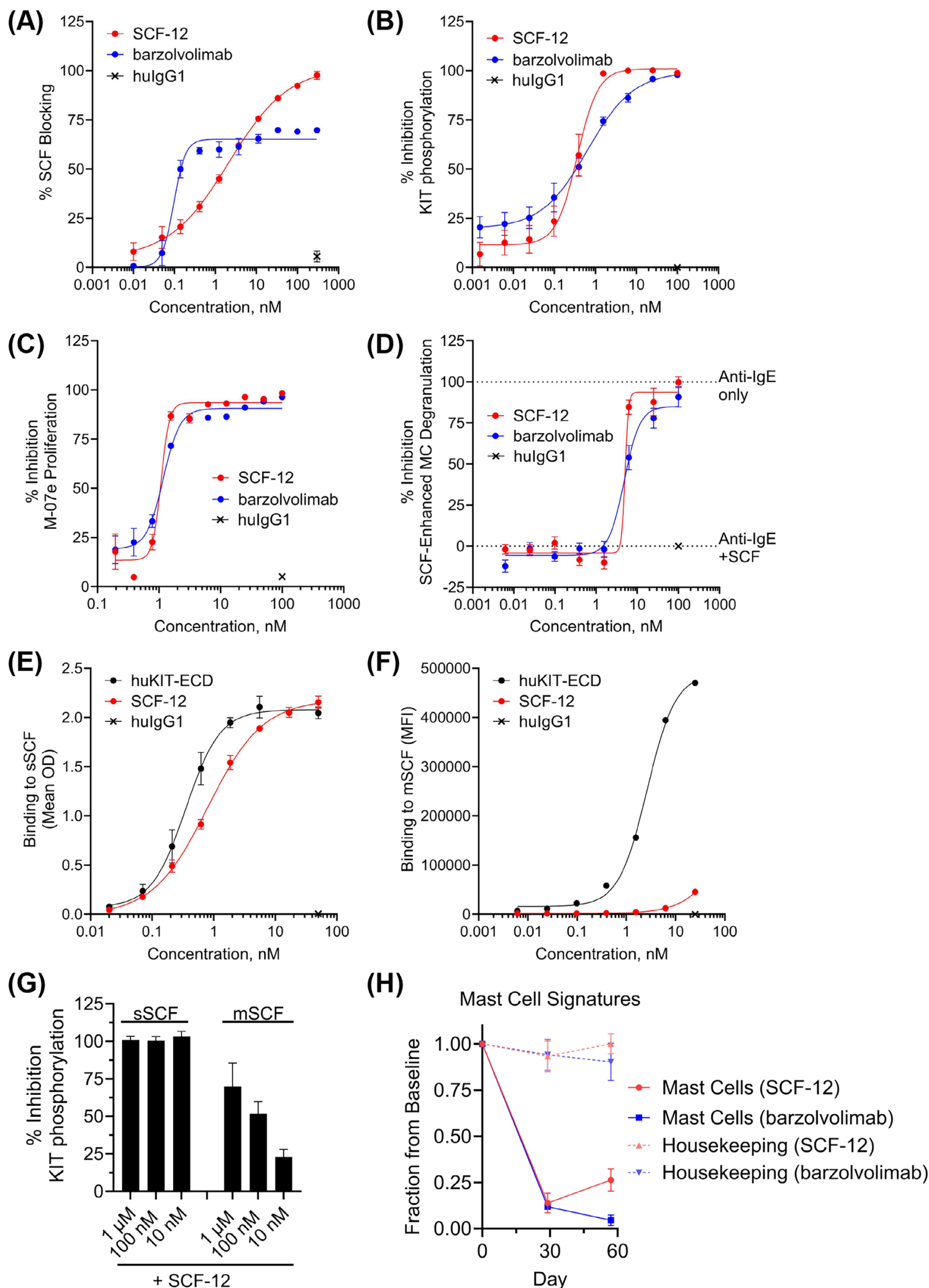


**FIGURE 1** | Legend on next page.

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**FIGURE 1** | Characterization of a novel SCF neutralizing mAb SCF-12. (A) SCF-12 (red) and barzolvolimab (blue) block binding of SCF to plate-coated human KIT extracellular domain (huKIT-ECD). (B) Both antibodies inhibit secreted SCF (sSCF)-dependent KIT phosphorylation in KIT-expressing CHO cells. (C) In a longer term (6 day) assay, SCF-12 and barzolvolimab similarly inhibit sSCF-dependent proliferation of M-07e cells. (D) IgE-dependent MC degranulation as measured by β-hexosaminidase release is enhanced by the addition of sSCF. SCF-12 shows potent inhibition of sSCF-enhanced degranulation of human primary MCs as determined by β-hexosaminidase release. (E) Titrated SCF-12 or huKIT-ECD (black) show similar binding kinetics to plate-coated sSCF as determined by ELISA. (F) SCF-12 binds weakly to membrane-associated SCF (mSCF) expressed on $SCF^{220}$ expressing Sl/Sl4 cells as measured by flow cytometry. (G) SCF-12 fully inhibits KIT phosphorylation induced by Sl/Sl4 parental cells spiked with recombinant sSCF, but only partially inhibits KIT phosphorylation induced by Sl/Sl4 $SCF^{220}$ cells in KIT-expressing M-07e cells. (H) SCF-12 ($n=3$) and barzolvolimab ($n=2$) administration results in marked reductions of mast cell (MC)–related RNA signatures in cynomolgus macaques. Housekeeping transcripts remained largely unchanged throughout dosing with SCF-12. Experiments (A–G) were performed for at least three independent times; mean values and SEMs are shown. HuIgG1, human IgG1 isotype control.

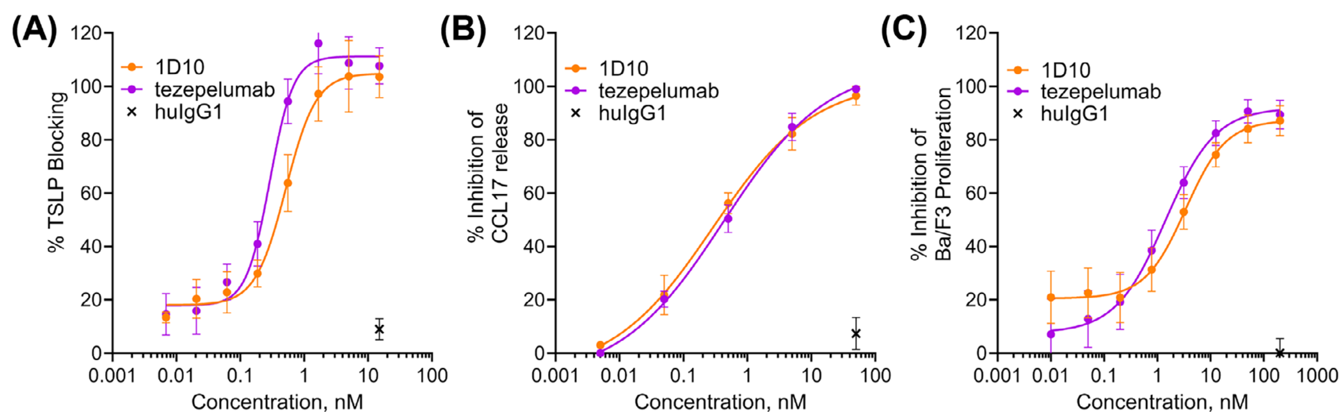


**FIGURE 2** | Characterization of a novel TSLP neutralizing mAb 1D10. (A) 1D10 (orange) and tezepelumab (purple) block binding of TSLP to plate-coated human TSLP receptor (TSLPR) by ELISA. (B) Dendritic cells (DCs) isolated from peripheral blood mononuclear cells were treated overnight with TSLP in the presence of mAbs, and TSLP-mediated CCL17 release by DCs was evaluated by ELISA. 1D10 and tezepelumab similarly inhibit TSLP-dependent CCL17 release from DCs isolated from human PBMCs. (C) 1D10 and tezepelumab inhibit TSLP-dependent proliferation of Ba/F3 cells transfected to express TSLPR/IL-7Rα with similar potency. All experiments were performed for at least three independent times; mean values and SEMs are shown. HuIgG1, human IgG1 isotype control.

and subjected to NanoString analysis showed that SCF-12 treatment resulted in a marked decrease of a composite MC signature composed of transcripts highly enriched in MCs (*KIT*, *TPSD*, *TPSAB*, *CPA3*, and *CMA1*), comparable to barzolvolimab at D30 (Figure 1H). Differences in the durability of MC transcript suppression between SCF-12 and barzolvolimab at D57 are likely attributed to the varying serum exposure levels of the two mAbs. Only barzolvolimab contains the half-life extending YTE modifications, which resulted in greater exposure, as seen in Figure S1A, and therefore more durable MC transcript suppression. A breakdown of individual MC transcripts can be found in Figure S1B,C.

## 2.2 | Characterization of a Novel TSLP Neutralizing mAb 1D10

The TSLP mAb designated 1D10 was generated by standard hybridoma technology and selected based on its potent neutralizing properties. 1D10 was subsequently humanized and purified as an IgG1 mAb and shown to have neutralizing activity comparable to tezepelumab. 1D10 and tezepelumab blocked the binding of TSLP to immobilized TSLPR by ELISA ($IC_{50}=513\pm27$ pM, and $IC_{50}=290\pm10$ pM, respectively; Figure 2A). Next, we evaluated the ability of 1D10 to inhibit TSLP-mediated CCL17 release by DCs. Human DCs isolated from PBMCs were treated overnight with TSLP in the presence of mAbs, and CCL17 release was measured by ELISA. The addition of serially diluted 1D10 to human DCs inhibited TSLP-dependent CCL17 release with an $IC_{50}=290\pm95$ pM, comparable to tezepelumab ($IC_{50}=423\pm200$ pM; Figure 2B). Similarly, 1D10 and tezepelumab blocked TSLP-mediated Ba/F3 cellular proliferation ($IC_{50}=3.4\pm0.7$ nM and $IC_{50}=1.4\pm0.3$ nM, respectively; Figure 2C). These data indicate that 1D10 potently neutralizes TSLP-dependent activity in in vitro functional assays.

## 2.3 | Development of the CDX-622 bsAb

We generated a bsAb that incorporates both SCF-12 and 1D10 to neutralize both pathways using a single molecule. We selected a whole IgG1-single-chain variable fragment (scFv) format (Figure 3A) for the CDX-622 bsAb construct, which maintains high-affinity binding to SCF and TSLP and retains mAb-like characteristics [48]. CDX-622 encodes the full-length 1D10 mAb and the scFv of SCF-12 genetically linked in the $V_H$-$V_L$ orientation to the C-terminus of the 1D10 mAb heavy chain. Also, the IgG1 constant region of CDX-622 was modified to introduce AQQ (L234A/L235Q/K322Q) and YTE (M252Y/S254T/T256E) mutations. AQQ mutations were introduced to prevent Fcγ receptor (FcγR) interactions, effector function [26], and C1q receptor binding [49], while YTE mutations increase FcRn binding, leading to improved pharmacokinetics (PK) by reducing the rate of in vivo clearance [50].



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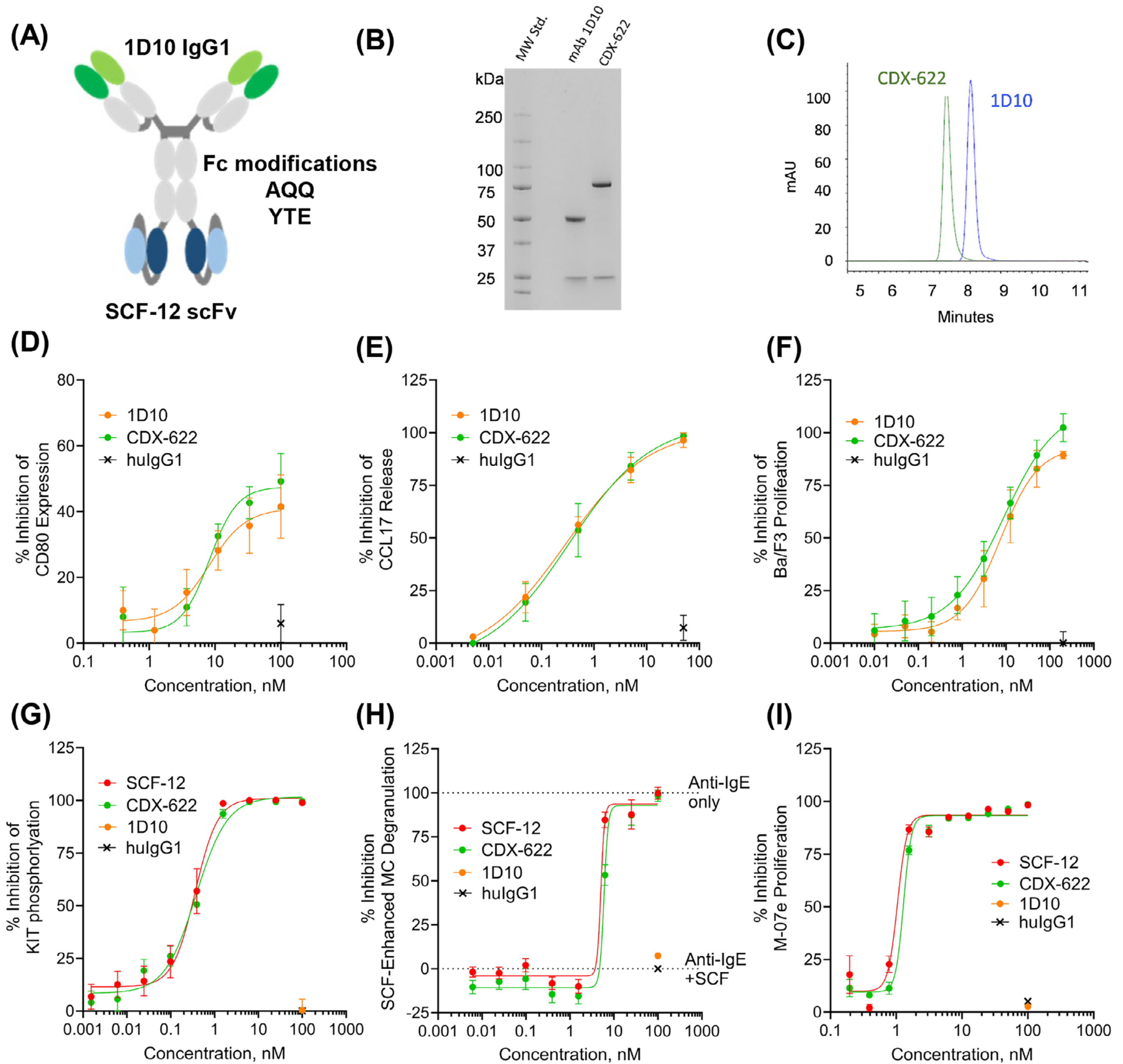


**FIGURE 3** | Characterization of CDX-622. (A) Schematic of CDX-622. Purified CDX-622 is composed of heavy and light chains consistent with their expected size by reducing SDS-PAGE (B) and SEC-HPLC (C). mAb 1D10 is included as a reference. CDX-622 (green) and 1D10 (orange) similarly inhibit TSLP-mediated DC activation as determined by (D) CD80 cell surface expression (FACS) and (E) CCL17 release (ELISA). (F) CDX-622 inhibits TSLP-dependent proliferation of Ba/F3 cells transfected to express TSLPR/IL-7Rα. (G) CDX-622 and SCF-12 (red) similarly inhibit sSCF-dependent KIT phosphorylation in KIT-expressing CHO cells. (H) CDX-622 shows potent inhibition of sSCF-enhanced degranulation of human PBMC-derived MCs as determined by β-hexosaminidase release. (I) In a longer term (6 day) assay, CDX-622 and SCF-12 similarly inhibit sSCF-dependent proliferation of M-07e cells. For CDX-622, all experiments utilized construct 5.7. All experiments were performed for at least three independent times; mean values and SEMs are shown. HuIgG1, human IgG1 isotype control; mAU, milli-Absorbance Units; MW Std., molecular weight standard.

Analytical characterization was performed on CDX-622 purified from stably transfected CHO cells. Reducing SDS-PAGE of CDX-622 showed the expected greater molecular weight of the heavy chain with the genetically fused scFv (approximately 75 kDa) (Figure 3B). Size exclusion chromatography (SEC) by HPLC showed the bsAb product to be > 95% monomeric and an elution profile consistent with an increased mass relative to 1D10 (Figure 3C). CDX-622 retained high-affinity binding to recombinant human and cynomolgus macaque sSCF (Table S1) and TSLP (Table S2), as evaluated by bio-layer interferometry. CDX-622 did not cross-react with any other species of sSCF or TSLP tested (Figure S2). The bsAb showed minimal binding to FcγRs, as expected based on the incorporation of AQQ mutations (Figure S3A). Additionally, CDX-622 showed enhanced affinity to the neonatal FcRn under low pH conditions, which was consistent with the engineered YTE modifications (Figure S3B).



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## 2.4 | CDX-622 Potently Neutralizes the Effects of Human SCF and TSLP In Vitro

To determine whether CDX-622 can effectively block TSLP-dependent activities, we incubated human DCs with recombinant human TSLP in the presence of either CDX-622 or its parental mAb 1D10. CDX-622 potently inhibited TSLP-driven upregulation of DC CD80 cell surface expression ($IC_{50} = 8.2 \pm 1.6$ nM; Figure 3D) and production of CCL17 ($IC_{50} = 341 \pm 90$ pM; Figure 3E). Similarly, CDX-622 prevented TSLP-mediated cell proliferation of Ba/F3 cells transfected to express the TSLPR/IL-7Rα receptor complex ($IC_{50} = 8.8 \pm 1.9$ nM; Figure 3F). These data indicate that CDX-622 can effectively block the functional effects of TSLP binding to its receptor with potencies comparable to the parental mAb 1D10.

CDX-622 also blocked sSCF-mediated activities in vitro with similar potency to SCF-12. Titration of CDX-622 on CHO-KIT cells inhibited sSCF-dependent KIT phosphorylation with an $IC_{50} = 378 \pm 55$ pM (Figure 3G), as well as sSCF-enhanced MC degranulation ($IC_{50} = 6.0 \pm 1.4$ nM; Figure 3H). Additionally, CDX-622 inhibited sSCF-driven M-07e cell proliferation with an $IC_{50} = 1.3 \pm 0.05$ nM (Figure 3I).

These data indicate that CDX-622 can effectively block the functional effects of both TSLP and sSCF in vitro with potencies comparable to the parental mAbs.

## 2.5 | CDX-622 Neutralizes SCF- and TSLP-Dependent Functions

Although TSLP has primarily been implicated in the activation of DCs, T cells, and ILC2s, it can also contribute to MC activation under inflammatory conditions [34, 51]. PBMC-derived MCs primed with an inflammatory cocktail (10 ng/mL of IL-1α, 10 ng/mL of IL-3, and 25 ng/mL of TNF-α) and treated with a combination of sSCF and TSLP resulted in enhanced release of Type 2 cytokines (IL-4, IL-5, IL-13) and monocyte chemoattractant protein-3 (MCP-3) relative to sSCF or TSLP treatment alone. Treatment in the presence of CDX-622 led to complete or near-complete suppression of cytokine release (Figure 4). Additionally, treatment of the mastocytic cell line LAD2 with both sSCF and TSLP led to enhanced release of monocyte chemoattractant protein-1 (MCP-1) compared to individual cytokines alone (Figure S4A). Importantly, the addition of CDX-622 to LAD2 cells in the presence of both cytokines resulted in more potent neutralization of MCP-1 release than treatment with SCF-12 or 1D10 parental mAbs alone, and to a similar extent as the combination of the mAbs (Figure S4B). Together, these data indicate that CDX-622 significantly neutralizes the effects exerted by the addition of sSCF and TSLP to human MCs.

To further investigate the ability of CDX-622 to inhibit TSLP- and SCF-driven inflammatory responses, we utilized an ex vivo human skin model. Live skin biopsies from five healthy donors were treated intradermally with vehicle control (PBS), or a combination of human sSCF and TSLP alone, or the cytokines in the presence of CDX-622 or its parental mAbs (SCF-12 and 1D10). Following injections, skin samples were collected at 6 h (Figure 5) and 24 h (Figure S5) and subjected to RNA sequencing. Administration of SCF and TSLP led to significant upregulation of transcripts previously shown to be regulated by these cytokines [52–67], including markers of myeloid cell activation (*CCL17*, *MRC1*, *CLEC4A*), epithelial barrier function (*CAPN14*, *GJB1*, *AREG*), lymphocyte markers (*CD19*, *GZMB*, *KLRF1*), pruritus (*IL31RA*, *P2RX1*), and inflammatory cytokines (*IL20*) (Figure 5A and Figure S5A). Cytokine co-injection with each parental mAb (SCF-12 or 1D10) revealed that sSCF and TSLP generally induce distinct transcriptional programs, demonstrating a large measure of nonredundancy between these cytokines. Importantly, CDX-622 restored the levels of both sSCF- and TSLP-dependent transcriptional changes to or near baseline levels, consistent with our findings in in vitro assays. Gene set enrichment analysis (GSEA) confirmed the enrichment of numerous pathways including MC activation, acute inflammatory response, and lymphocyte signaling (Figure 5B and Figure S5B). In many cases, the administration of CDX-622 resulted in strong downregulation of many of these inflammatory pathways. Together, these data provide further evidence that CDX-622 can simultaneously neutralize two important and nonoverlapping mechanisms that lead to inflammation.

## 2.6 | CDX-622 Shows mAb-Like PK and Inhibits MCs In Vivo

CDX-622 was administered as a single 10 mg/kg slow bolus i.v. injection in four cynomolgus macaques. This study used two nearly identical humanized variants of CDX-622 (referred to as 5.7 and 5.2) varying only by utilizing different humanized variable regions for the SCF-12 scFv. Both bsAbs behaved similarly in all in vitro studies and were used in this pilot study to assess any potential differences in vivo. CDX-622 was well tolerated without any observed adverse effects on clinical or hematological parameters. PK analysis showed prolonged drug exposure in the two animals with construct 5.7 ($t_{1/2}$ approximately 14 days), whereas one of the animals treated with construct 5.2 developed antidrug antibodies that clearly impacted exposure after Day 15 (Figure 6A). Ear punches were taken at Day 1 (baseline), Day 15 (D15), and Day 29 (D29). Similar to our prior observations with SCF-12, CDX-622 led to marked decreases in MC-related RNA signatures as early as D15 and was sustained through D29 (Figure 6B). These data led to the selection of construct 5.7 for further development, demonstrate that CDX-622 has a promising product profile, and suggest it can lead to profound inhibition of MCs consistent with its expected mechanism.

## 3 | Discussion

The use of bsAbs to simultaneously target two complementary immunological pathways has become common in oncology and has recently started to gain traction in treating inflammatory diseases [3]. We describe the development and characterization of CDX-622, a first-in-class bsAb designed to deplete MCs through SCF starvation and inhibit TSLP-mediated Type 2 inflammatory responses.

To build the MC depleting part of our bsAb, we chose to target the ligand SCF rather than KIT, despite the promising clinical data to date with the anti-KIT mAb barzolvolimab [26, 27]. We



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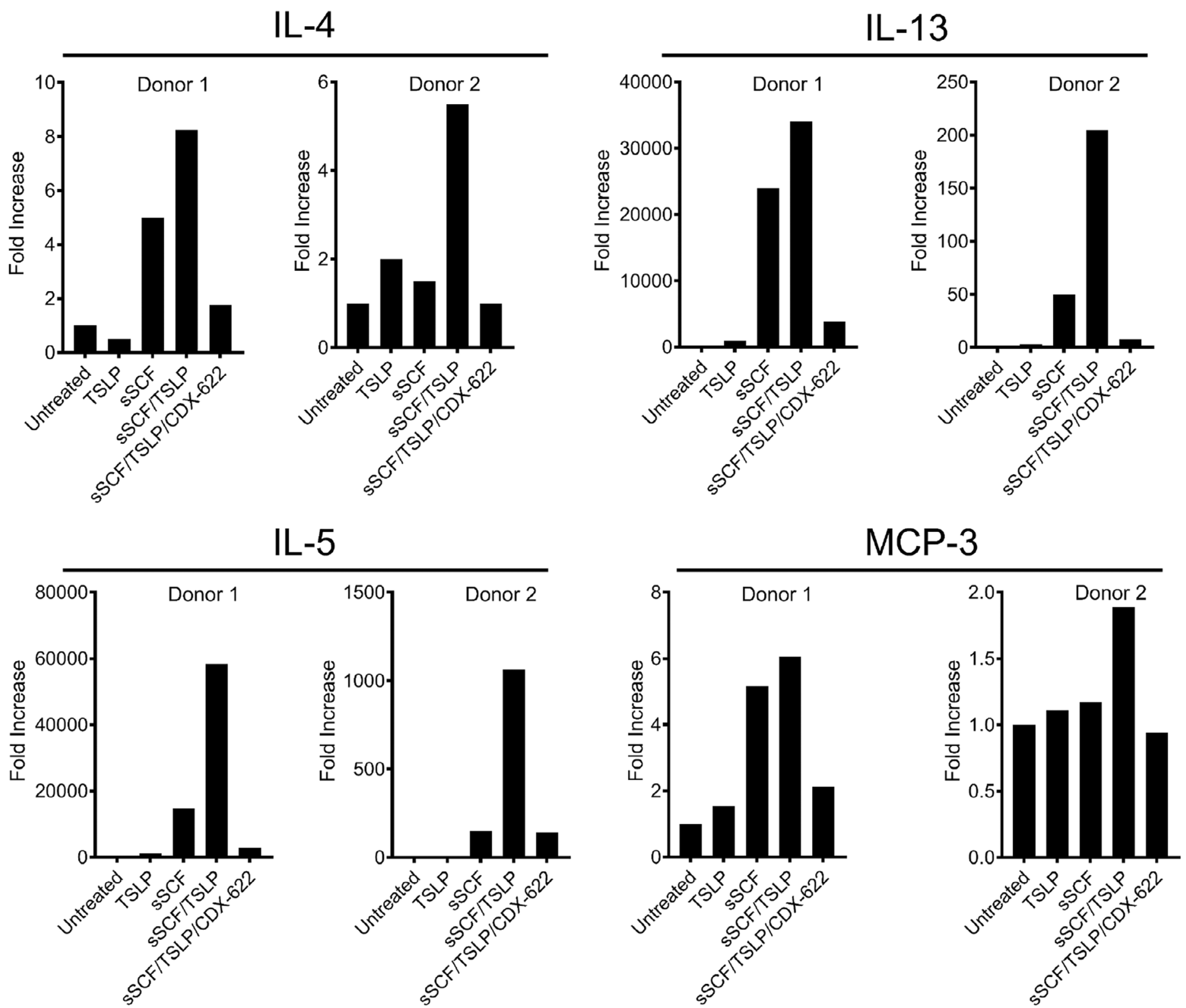


**FIGURE 4** | CDX-622 neutralizes SCF- and TSLP-mediated MC activation. Human PBMC-derived MCs (two independent donors) were primed under inflammatory conditions (IL-1α (10 ng/mL), IL-3 (10 ng/mL), and TNF-α (25 ng/mL)), followed by overnight treatment with sSCF, TSLP, or a combination of both cytokines in the presence of either media or CDX-622 for 24 h. Combination of sSCF with TSLP leads to increased release of IL-4, IL-5, IL-13, and MCP-3 relative to each cytokine alone, while CDX-622 administration inhibits cytokine release to baseline levels. For CDX-622, all experiments utilized construct 5.7.

hypothesized that targeting SCF may circumvent the unlikely possibility of inadvertent MC activation through KIT crosslinking with a bsAb. Targeting MCs by neutralizing SCF is a unique and unprecedented approach. Our antibody discovery efforts yielded SCF-12, a mAb with high-affinity binding to sSCF and potent neutralizing activity of sSCF-mediated MC activation in vitro comparable to barzolvolimab. However, through multiple discovery efforts, SCF-12 was the only mAb found to have highly potent neutralizing activity. Importantly, SCF-12 also demonstrated a dramatic decrease in skin MC-related RNA signatures in cynomolgus macaques, providing strong proof-of-concept evidence of robust neutralization and MC depletion.

Importantly, we discovered that SCF-12 preferentially binds and inhibits sSCF over mSCF in vitro. Prior work in mice suggests that both variants may be differentially required in distinct tissues or cell types. For instance, $SCF^{248}$, which is rapidly cleaved by proteases leading to the secretion of sSCF, may play a more prominent role in MC function, while the predominantly mSCF ($SCF^{220}$) may be necessary to support KIT-dependent erythropoiesis [30, 68]. Furthermore, $SCF^{248}$ appears to be differentially upregulated during inflammatory conditions and selectively upregulated in fibroblasts from patients with idiopathic pulmonary fibrosis [69]. Thus, biased inhibition of soluble over membrane SCF with SCF-12 may lead to a more selective impact on MC activity over other KIT-dependent functions; however, more rigorous and comprehensive studies will be necessary to fully understand the effect of SCF-12 or SCF-12 containing bsAbs on KIT activity in various tissues.

To complement SCF-12 for our bsAb, we discovered mAb 1D10, which potently neutralized TSLP activity and DC activation similar to the commercially approved anti-TSLP mAb tezepelumab. CDX-622 was developed as a tetravalent IgG1–scFv construct in



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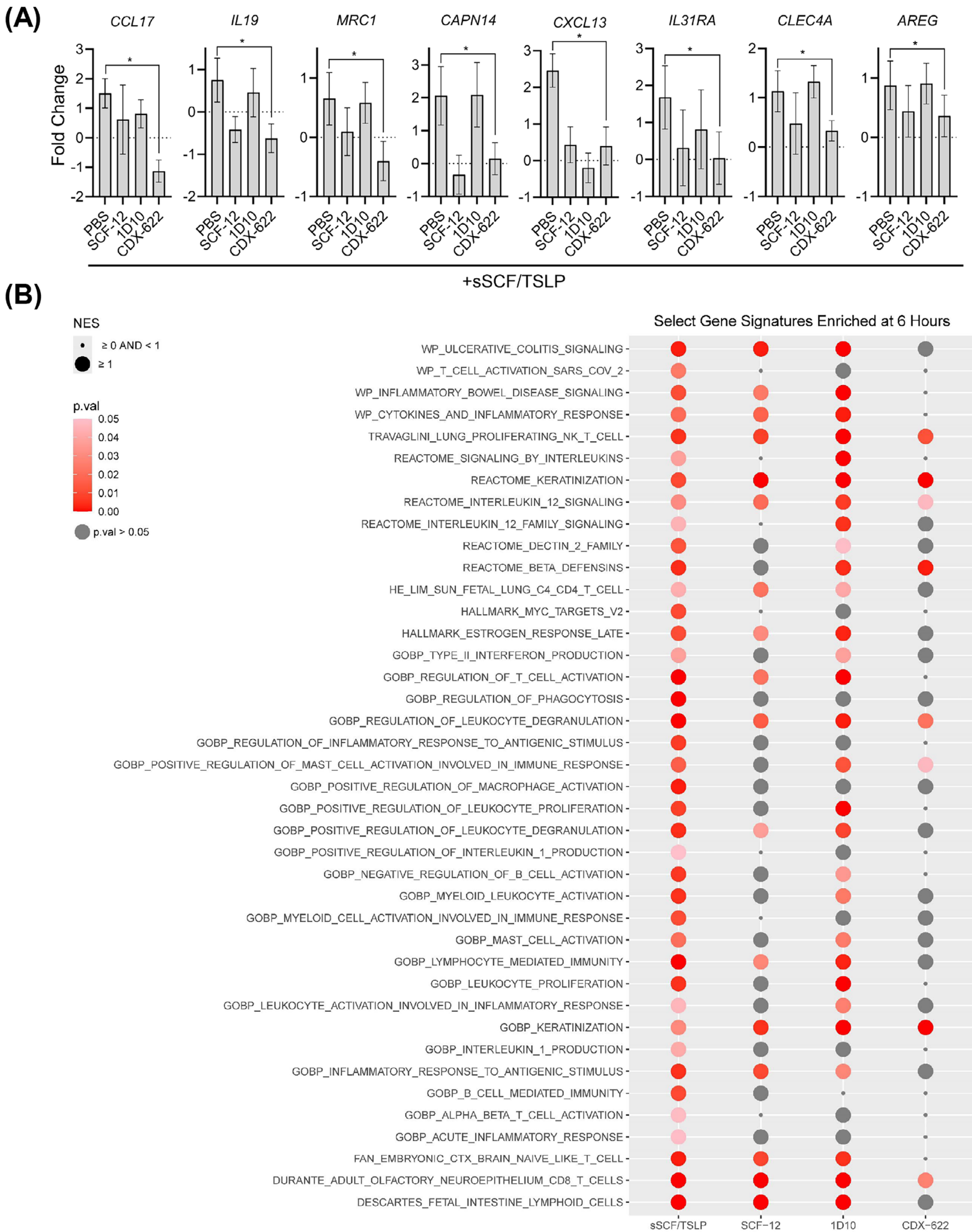


**FIGURE 5** | Legend on next page.

which the IgG is 1D10 and the scFv is SCF-12. CDX-622 exhibited good biophysical properties and preserved comparable in vitro cytokine binding and blocking potency to its parental mAbs. The Fc region of CDX-622 was engineered to eliminate effector function by significantly reducing binding to FcγRs, as the molecule does not benefit from effector function and these interactions could



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**FIGURE 5** | CDX-622 inhibits SCF- and TSLP-induced inflammatory RNA signatures in an ex vivo skin model. Live skin biopsies from five human healthy donors were treated with vehicle control, or a cocktail of human sSCF and TSLP alone, or in the presence of CDX-622 or its parental mAbs (SCF-12 and 1D10). Samples were collected at 6h after injection and subjected to RNA sequencing. (A) Data were analyzed by calculating the fold change of each treatment relative to a PBS-treated control for each donor, using $\log_2$ normalized RNA-Seq data. Selected transcripts implicated in distinct biological functions are shown. In all cases, CDX-622 treatment induces statistically significant reductions ($p$-value $< 0.05$) relative to sSCF + TSLP treatment alone as determined by a paired $t$-test, *$p < 0.05$. (B) Gene set enrichment analysis (GSEA) was performed on the normalized data for each comparison (antibody treatment vs. 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. For CDX-622, experiments utilized construct 5.7.

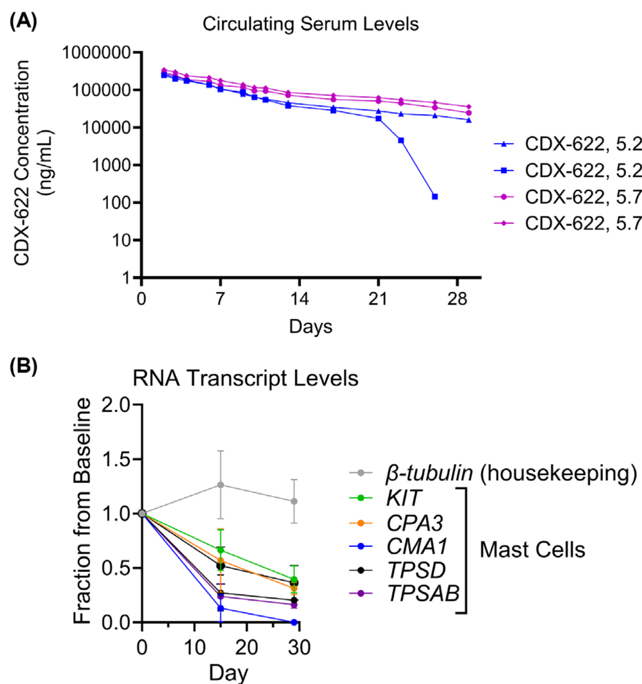


**FIGURE 6** | Pharmacokinetics of CDX-622 in cynomolgus macaques. Four cynomolgus monkeys were administered a 10mg/kg slow bolus intravenous injection of either the 5.2 or 5.7 construct of CDX-622, which utilize different humanized variable regions for SCF-12. (A) Serum concentrations of CDX-622 were measured using a Meso Scale Discovery (MSD)-based assay. (B) CDX-622 administration results in marked reductions of MC-related RNA signatures in cynomolgus monkeys. Housekeeping transcript, β-tubulin, remained largely unchanged throughout dosing with CDX-622. One animal in the 5.2 group with greatly reduced exposure due to the development of antidrug antibodies was removed from analysis. *CMA1*, chymase 1; *CPA3*, carboxypeptidase A3; *TPSAB*, tryptase alpha/beta; *TPSD*, tryptase delta.

potentially have a negative impact on safety and PK. In addition, YTE substitutions that enhance the affinity for FcRn are predicted to reduce antibody clearance and increase drug exposure. This may potentially enable less frequent dosing in patients, as demonstrated with other antibodies [50]. In a pilot study in NHPs, a single dose of CDX-622 at 10mg/kg showed antibody-like PK. No significant safety findings or meaningful changes in hematological parameters were reported. Importantly, a single dose of CDX-622 significantly reduced MC-related RNA signatures as early as 2weeks, indicative of strong SCF neutralization. It is tempting to speculate that sSCF may play a predominant role in cutaneous MC function in NHPs, although binding and inhibition of mSCF cannot be ruled out at these high doses.

As expected, CDX-622 led to greater in vitro inhibition of combined sSCF- and TSLP-dependent activities than its parental mAbs alone, underscoring its potential use in disorders where MCs and TSLP play a pathogenic role, including CSU and asthma [70, 71]. We observed that co-addition of sSCF and TSLP to inflammatory primed human PBMC–derived MCs and immortalized LAD2 MCs led to additive increases in the secretion of IL-4, IL-5, IL-13, and MCP-3, or MCP-1, respectively. While the addition of anti-SCF or anti-TSLP mAbs inhibited cytokine release elicited by their respective targets, CDX-622 reduced their secretion to a greater extent. These data suggest that engagement of sSCF and TSLP with CDX-622 might improve the clinical efficacy of mAb monotherapies in MC- and TSLP-driven diseases.

Historically, research in inflammatory skin diseases has relied heavily on animal models; however, the advent of human skin explant models has become an effective way to directly assess the effects of therapeutics and their targets on live human tissues. Despite our ex vivo skin model being inherently limited by high donor-to-donor variability and the need to inject supraphysiological concentrations of ligands to achieve sufficient target activation, we found that an extensive array of RNA signatures was upregulated in response to sSCF and TSLP intradermal injections. Among these included CCL17, a chemokine upregulated in response to TSLP and known to recruit Th2 cells [72]. Additionally, transcripts implicated in various inflammatory diseases were upregulated. For example, increased expression of CAPN14, which is upregulated by IL-13, has been shown to directly influence epithelial barrier function [73], while IL-31RA upregulation has been associated with airway hyperresponsiveness in asthma patients and is an important driver of chronic itch [74, 75]. GSEA pathway analysis also uncovered a number of pathways that were upregulated, consistent with MC activation, inflammatory responses, T/B cell activation, and transcripts associated with immune cellular migration. Importantly, many of these transcripts and pathways were significantly downregulated upon treatment with CDX-622. Overall, these data suggest that CDX-622 broadly inhibits transcriptional programs induced by sSCF or TSLP and that these pathways regulate generally nonoverlapping immune functions that drive inflammatory disease.

The use of multitargeted bsAbs represents an emerging therapeutic modality to treat immunoinflammatory and autoimmune disorders. MC activation and TSLP released from barrier cells are important contributors to the pathogenesis of multiple allergic and autoimmune diseases, and their combined inhibition may lead to improved disease outcomes. Our data show that



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CDX-622 is a novel bsAb that can inhibit and deplete MCs directly through SCF starvation, while simultaneously inhibiting TSLP-mediated signaling, in turn, silencing two complementary drivers of inflammation. Collectively, these studies support advancing CDX-622 to clinical trials.

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### Author Contributions

M.B.M., L.V., L.J.T., T.K., and D.A. designed experiments. M.B.M., L.V., T.O., D.M.M., L.M., L.M.-C., A.C., C.P., V.S., N.M.A., J.M.B., K.M.M., N.R.M., R.A.H., J.G., L.J.T., T.K., and D.A. performed experiments and analyzed the data. M.B.M., J.G., T.K., and D.A. drafted and revised the manuscript.

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### Conflicts of Interest

M.B.M., L.V., T.O., D.M.M., L.M., A.C., C.P., L.M.-C., V.S., N.M.A., J.M.B., K.M.M., N.R.M., R.A.H., J.G., L.J.T., T.K., and D.A. are full-time employees of Celldex Therapeutics.

### Data Availability Statement

The authors have nothing to report.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.



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