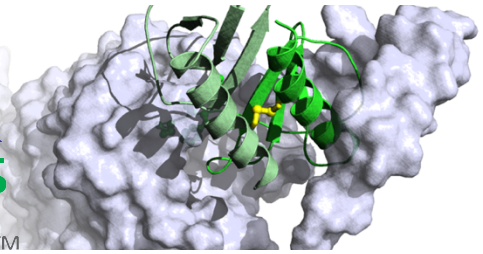
XLock BIOSCIENCES

Novel Biologics for Chemokine Driven Diseases™



XLock Biosciences is a pre-clinical stage biotechnology company overcoming the challenges of chemokine-targeted drug development with protein engineering.

Our current lead molecule, CCL20LD, is an inhibitor of CCL20-driven TH17 diseases. We believe ocular GVHD is the most valuable initial clinical indication for CCL20LD.

We are developing a new CXCL12 molecule for use in treating CXCR4-expressing malignancies.

THE HUMAN CHEMOKINE NETWORK, consisting of 23 GPCRs and 46 chemokines, orchestrates cellular immune function by stimulating leukocyte chemotaxis. Chemokines are small, secreted proteins that oligomerize when enmeshed in the extracellular matrix but only the chemokine monomer induces cell migration when it binds to its cognate receptor. As molecular homing beacons for leukocytes and other motile cells, chemokines can promote inflammatory diseases and malignant metastasis among other functions. Despite their accepted role in several pathologies, drug development against this class of therapeutic targets has been difficult due to the complexity and redundancy of the chemokine network.

Two decades of chemokine structure-function research by XLock founders has shown that obligate dimerization of several chemokines *dramatically* changes the *in vivo* pharmacology. For at least two chemokines, CCL20 and CXCL12, obligate chemokine dimerization results in suppression of ligand-driven cell chemotaxis, ligand-driven chemotaxis otherwise being the hallmark activity of the chemokines. XLock's protein engineering strategy preserves the exquisite specificity of the chemokine-receptor interface, avoids the unwanted poly-pharmacology common to small molecule GPCR antagonists, and exploits the novel power of biased agonism to arrest pathogenic cell populations and treat chemokine-mediated disease.

BIOLOGY OF CHEMOKINE DIMERS – Chemokines typically bind and activate their GPCR targets with low nanomolar potency. Assays that simply measure receptor activation (e.g. second messengers like Ca^{2+}, IP_3 or cAMP) exhibit a sigmoidal dose-response that saturates at chemokine ligand concentrations around 10-100 nM. However, cellular chemotaxis measured in a Boyden chamber or transwell migration apparatus has a biphasic concentration dependence, producing the now classic 'bell-shaped' curve that accompanied the discovery of the first chemokines.

For example, data in the upper panels of **Figure 1** show that an increasing concentration of CXCL12, a pro-metastatic chemokine, leads to an expected sigmoidal response in Ca^{++} flux but leads to a diminishment of chemotaxis at higher concentrations. This observation inspired XLock's scientist founders to probe the effect of

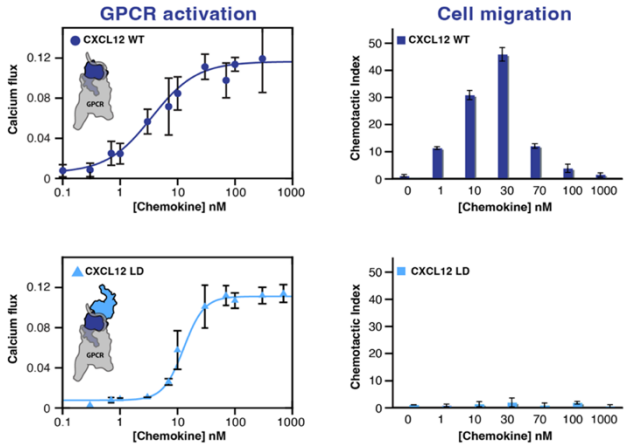


Figure 1. Biological activity of chemokine monomers and dimers. Upper panels: Wild-type CXCL12 shows a sigmoidal dose-response profile for intracellular Ca^{2+}-flux, and a biphasic "bell-shaped" curve for transwell chemotaxis. Lower panels: An engineered covalently linked homodimer of CXCL12 (CXCL12 locked dimer; CXCL12LD) induces no cell migration while leaving the Ca^{2+}-flux response intact.

chemokine dimerization on chemotaxis. They used their knowledge of chemokine structure to design a disulfide-locked CXCL12 homodimer (CXCL12LD), and the lower panels of **Figure 1** show that CXCL12LD binds and activates the receptor but fails to stimulate chemotaxis of THP-1 cells, a human cell line derived from a patient with acute monocytic leukemia.

Given its ability to induce CXCR4 in a non-chemotactic manner, XLock's founders reasoned that CXCL12LD ought to inhibit CXCL12-stimulated cell migration, as shown for transwell chemotaxis of THP-1 cells in **Figure 2A**. To prove its *in vivo* efficacy as an inhibitor of CXCR4-mediated chemotaxis, they then used CXCL12LD to block the metastatic spread of human colon cancer and melanoma cells in mice (**Figure 2B**).

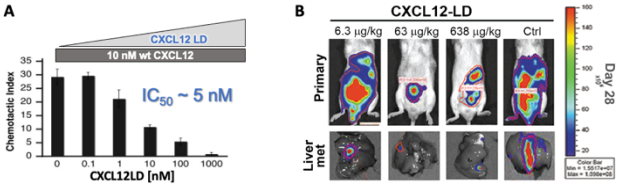


Figure 2. CXCL12 interferes with colonic carcinoma and melanoma tumor metastasis. (A) CXCL12LD blocks native CXCL12-induced transwell chemotaxis of THP-1 cells with low nanomolar potency. **(B)** Systemic administration of CXCL12LD inhibited metastatic migration of orthotopically administered fluorescently labelled HCT-116 hepatocarcinoma cells *in vivo*.

This concept of creating obligate homodimers of chemokines to alter their pharmacology was then applied to the chemokine CCL20. Even while using the CCL20 crystal structure of the dimer as a guide, several early designs yielded unstable, misfolded or non-functional molecules. However, a bioactive CCL20 locked dimer was ultimately created with a single-atom substitution (S64C) (**Figure 3A**) in the native CCL20 molecule. Purity, chemical identity, and structural integrity of the engineered CCL20 locked dimer were characterized exhaustively by several analytical methods. The intermolecular disulfide crosslink that stabilizes CCL20LD was visualized in the high-resolution 3D structure solved by X-ray crystallography (**Figure 3B**), which was superimposable with previously solved crystal structures of native CCL20.

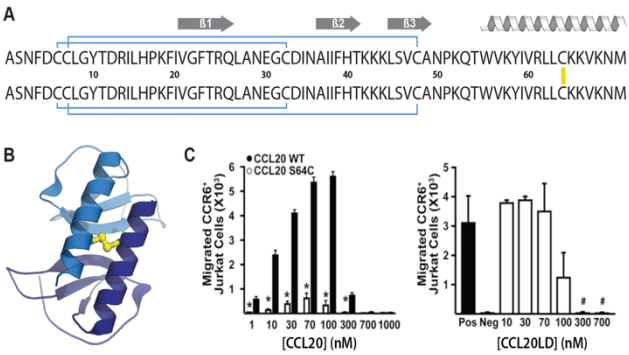


Figure 3. Design and characterization of an engineered CCL20 locked dimer. (A) Amino acid sequence of CCL20 S64C (CCL20LD) with native (intramolecular) and engineered (intermolecular) disulfide bonds indicated with blue and yellow connecting lines, respectively. **(B)** Three-dimensional structure of CCL20LD solved by X-ray crystallography. Intermolecular disulfide shown in yellow. **(C)** Transwell chemotaxis of CCR6-expressing Jurkat T cells shows that CCL20LD induces minimal cell migration compared to native CCL20 and inhibits CCL20-induced chemotaxis.

Recombinant CCL20LD produced in *E. coli* was shown in cell-based assays to retain nanomolar affinity for the CCR6 receptor and activate G protein signaling with no chemotactic function (**Figure 3C**). Furthermore, as previously observed for the CXCL12 locked dimer, CCL20LD inhibited transwell chemotaxis of CCR6+ T cells in response to native CCL20.

Commensurate to our interest in developing novel biologics to treat chemokine-driven diseases, CCL20 and its cognate receptor CCR6 are known to be critical drivers of TH17 processes and overexpression of CCR6 and CCL20 has been shown in several TH17-driven diseases, of which there is a growing list including, psoriasis, psoriatic arthritis, chronic graft versus host disease, Sjögren's syndrome, and many others.

ANIMAL MODELS – One of our founders, Dr. Sam Hwang, was among the very first to delineate the role of CCR6/CCL20 in psoriasis. Thus, we tested CCL20LD in Dr. Hwang's IL23-induced model of psoriasis and found that it prevented TH17-mediated inflammation (**Figure 4**). In this model psoriasis was induced with intradermal injection of recombinant IL23 protein and CCL20LD was started on an alternate day schedule (IP) at the time of psoriasis induction.

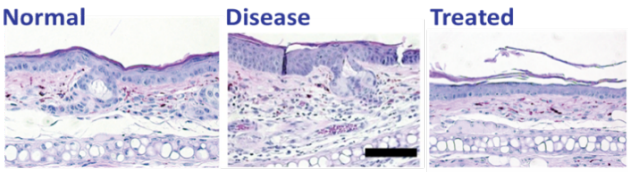


Figure 4. CCL20 locked dimer blocks the recruitment of γδ-low–expressing T cells to the epidermis. Histopathology of the murine ear in uninduced control (left), IL-23 induced disease (center); and IL-23 induced disease treated with CCL20LD (right).

Prevention of disease is interesting, but any clinically relevant molecule should be able to *treat* established disease, as this is what clinicians see. Thus, Dr. Hwang induced psoriatic arthritis, a much more difficult TH17-mediated disease to treat in mice, and *AFTER* the establishment of the disease process began therapeutic treatment with CCL20LD. Results from this study, which were published in a leading rheumatology journal, are shown in **Figure 5**. As noted, these therapeutic studies showed a 50-90% reduction in inflammatory

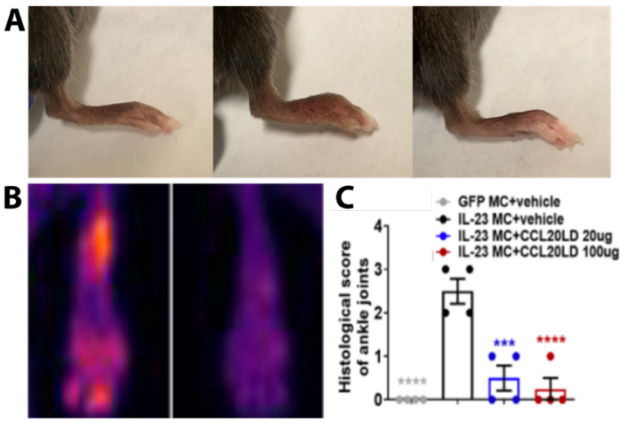


Figure 5. CCL20 locked dimer (CCL20LD) ameliorates IL-23 MC–mediated joint inflammation in a therapeutic manner. (A) Photographs of the hind paw of a representative mouse in each treatment group. B10.RIII mice were treated with phosphate buffered saline (PBS) vehicle or CCL20LD at 100ug for 7 days. **(B)** FDG- PET images of hind paw: right vehicle, left CCL20LD. **(C)** Histological score of ankle joints.

markers, a 70% reduction of disease severity scores and a dramatic reduction in FDG-PET evidence of joint inflammation. These in vivo studies showed the effectiveness of CCL20LD in treating CCl20-driven TH17 diseases. **However, we are not targeting psoriasis or psoriatic arthritis for our initial indications as there is an (over) abundance of therapies for these diseases. Instead, we are targeting complex, and very poorly treated, TH17-driven diseases such as Sjorgrens syndrome and ocular graft versus host disease.**

Thus, we have recently collaborated with investigators at City of Hope to determine the effects of CCL20LD in chronic graft versus host disease, now understood to be significantly TH17 driven. In a murine minor mismatch model, CCL20LD was able to **slightly** prolong survival and to dramatically reduce the number of TH17 cells in target organs such as lung, liver and skin. As shown in Figure 6, the marked reduction of TH17 cells in the treated population is evident in FACS of the liver in a murine model of cGVHD.

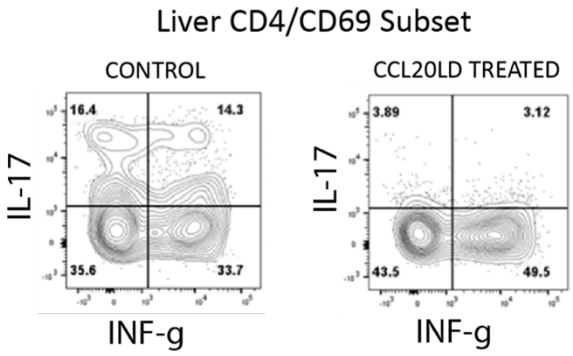


Figure 6. CCL20 locked dimer reduces TH17 recruitment in murine cGVHD. When treated with CCL20LD, analysis of liver cells in a murine minor-mismatch bone marrow transplant setting shows a 78% decrease in IL-17/IFN-g dual positive TH-17 cells.

This reduction of TH17 cells in a target organ of GVHD, the liver, raises our confidence that CCL20LD will be effective in ocular GVHD given either topically in the eye, systemically or, quite possibly, both systemically and topically. We plan to begin studies in a murine model of ocular GVHD in October of 2023.

Taken together, XLock's published and proprietary results demonstrate efficacy of the CCL20LD molecule in several TH17 pre-clinical models including psoriasis, psoriatic arthritis, and a murine model of cGVHD. Our findings suggest that CCL20LD is an extremely promising new therapy for a range of TH17-induced diseases.

DEVELOPMENT STATUS – XLock has demonstrated the ability to produce CCL20LD at scale and with purity and activity in E. coli. We understand that many companies now prefer to produce biologics in a eukaryotic system. Thus, working with a CRO we have demonstrated that CCL20LDcan be produced in CHO cells. We have also demonstrated that an Fc-linked version of CCL20LD can be produced in CHO cells.

We have done a 2-week safety / toxicology study in mice at Charles River Lab. This study was done to log order higher doses than the therapeutic studies shown above. No adverse histopathology of chemical signatures was found even at these significant overages.

INTELLECTUAL PROPERTY – CCL20LD is patented and granted composition of matter IP in the US (2020), Australia (2022), Canada (2023), Europe (nationalized in 5 countries) (2022), and Japan (2022). Additional method of use patents for treatment of specific clinical indications are being reviewed by the USPTO.

DEVELOPING A NEW PLATFORM TECHNOLOGY – The founding scientists of XLock continue their basic research to understand the structural mechanisms of chemokine receptor-ligand interactions. Using Alpha Fold and other advanced methods for protein modeling and design, XLock scientists can create novel GPCR ligands with any chosen activity profile. **Figure 7** shows an example of an entirely new recombinant protein technology that has been successfully engineered to elicit the full spectrum of pharmacologic responses from a chemokine receptor.

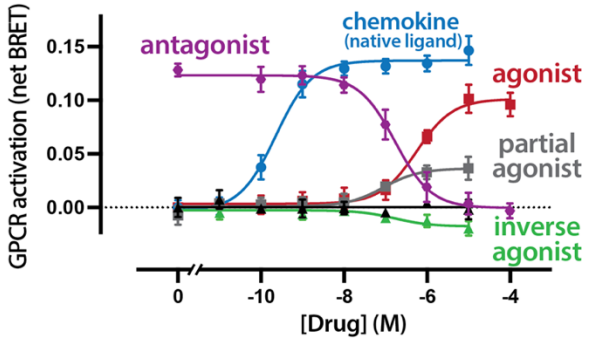


Figure 7. Protein design of GPCR ligands with customizable pharmacology. The parent molecule, a soluble recombinant protein, functions as a neutral antagonist (violet) for a member of the chemokine receptor family. Amino acid substitutions at one or a few sequence positions convert it to a full agonist (red), partial agonist (gray) or inverse agonist (green).

With these results as a starting point, XLock is developing a proprietary platform technology designed to enlarge the range of druggable targets in the chemokine network. This entails both the 'locked dimer' approach and an entirely novel approach which may well enable us to completely tailor the response of chemokine receptors.

PEOPLE – The company consists of physicians and scientists from Medical College of Wisconsin and UC Davis who together have studied chemokine biophysical chemistry and immunology for over twenty years, invented CXCL12LD and CCL20LD, delineated their molecular mechanisms of action, performed initial pre-clinical models, and lead active NIH-funded basic and translational research programs to develop other chemokine-focused treatments for human disease.

CEO Bill Clarke is a physician-scientist (molecular pharmacology and anesthesiology) with 20 years of industry experience including: Director of Biological Sciences at GlaxoWelcome (target ID and validation); Global Head of R&D at Amersham; CMO of GE Healthcare (briefly, after the GEHealthcare acquisition of Amersham); and CEO of a successful early phase biotech, Cellectar, in WI. Most recently Dr. Clarke was the Director of the Drug, Device and Diagnostic Accelerator at Boston Children's. Dr. Clarke now devotes himself entirely to leading XLock Biosciences through the IND process for its most promising lead molecule.

Contact Dr. Clarke (bill.clarke@xlockbio.com or +1 262-501-4800) for additional information.

Visit our website at xlockbio.com

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