Pioneering a New Class of Genomic Medicine: Design and Characterization of a Programmable Epigenomic CXCL1-8 mRNA Therapeutic

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BACKGROUND

Therapeutic modulation of the epigenome presents significant opportunities to leverage natural mechanisms to control and resolve gene dysregulation pre-transcriptionally. Omega Therapeutics is designing programmable epigenomic mRNA therapeutics through an innovative platform capable of specifically, controllably and durably modifying epigenetic chromatin state to correct aberrant gene expression and treat disease.

Leveraging 3D chromatin architecture, we identify Insulated Genomic Domains (IGDs), target sequences driving epigenetic gene control within these IGDs, and rationally design Epigenomic Controllers (ECs), mRNA-encoded proteins that precisely target the epigenome for tunable and durable effect that specifically modulate local epigenetic state (Fig 1). Using endogenous modifications, ECs induce changes processed by cellular machinery to tune expression levels of one or more genes.

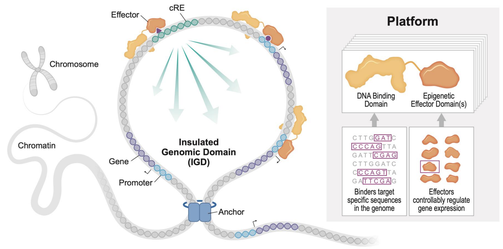

Figure 1. Composition and Mechanism of Action of Epigenomic Controllers

We have designed a single epigenomic controller (EC) inhibiting the multigenic CXCL1-8 locus. CXCL1-8 are functionally similar chemo-attractants that regulate neutrophil migration by binding to CXCR1 and CXCR2 and their dysregulation is implicated in a wide array of diseases including inflammatory disorders and cancers. A library of ECs were designed and tested with the goal to downregulate the entire cytokine locus response to stimulation, including a reduction of CXCL8 (IL-8) of greater than 90%. By targeting the entire locus along with its response to NF-kB signaling, we avoid compensatory upregulation of CXCL genes that can occur when targeting a single gene alone.

In vitro models identified two ECs, CXCL-EC$_1$ which inhibits CXCL8 95% and CXCL-EC$_2$ which inhibits all of the CXCL1-8 genes 30-60%. Molecular mechanism of action studies (ChIP) validated on-target controller/chromatin association and establishment of an inhibitory epigenetic signature, loss of activating epigenetic signature, and loss of NF-kB-related transcription factor binding for both CXCL-EC$_1$ and CXCL-EC$_2$.

A single EC, CXCL-EC, designed to target multiple locations, demonstrated similar activity in vitro in multiple lung cell lines. In an assay using human lung fibroblasts, a significant reduction in neutrophil migration was observed. Mouse surrogate ECs were efficacious in vivo in a mouse LPS challenge model, demonstrating significant reduction in neutrophil migration to the inflamed lung.

The CXCL1-8 Insulated Genomic Domain (IGD)

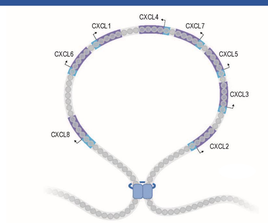

Figure 2: The CXCL1-8 IGD is a jointly regulated locus implicated in a wide array of diseases through its role in neutrophil migration. CXCL1-8 are colocalized and coordinately regulated within a single IGD. Omega's approach enables multigenic modulation of CXCL1-8 utilizing a single therapeutic.

CXCL-EC$_1$ and CXCL-EC$_2$ were designed to inhibit CXCR1/2 related cytokine responses

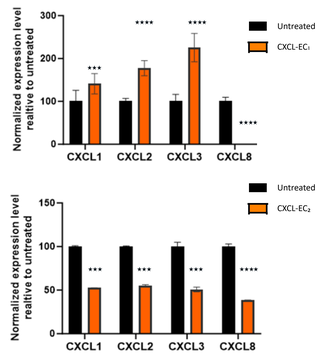

Figure 3: Stimulated IMR90 cells treated with CXCL-EC$_1$ and CXCL-EC$_2$ demonstrate different gene expression profiles.

Top panel: CXCL-EC$_1$ demonstrates a 95% reduction in CXCL8. As anticipated, compensatory increases in CXCL1, CXCL2 and CXCL3 expression observed when only targeting a single gene.

Bottom panel: CXCL-EC$_2$ demonstrates a 45-60% reduction in CXCL1, CXCL2, CXCL3 and CXCL8 expression.

One-way ANOVA: *** p <0.001; **** p <0.0001

CXCL-EC$_1$ and CXCL-EC$_2$ engagement induces inhibitory signatures which lead to depletion of NF-kB-related binding

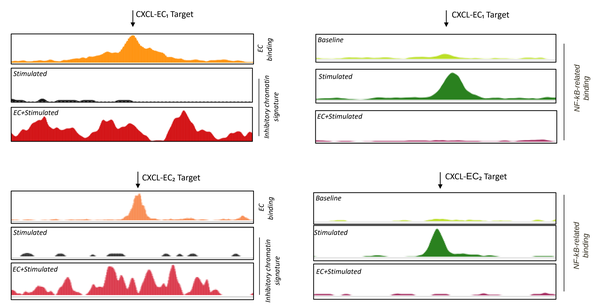

Figure 4: Left panel: Observed on-target controller/chromatin association using HA ChIP-seq for CXCL-EC$_1$ (top orange plot) and CXCL-EC$_2$ (bottom orange plot) and an establishment of the inhibitory signature marks using ChIP-seq (red plot) Right panel: Demonstration of depletion of NF-kB-related binding following treatment with CXCL-EC$_1$ and CXCL-EC$_2$ (purple plots)

CXCL-EC, designed to express both CXCL-EC$_1$ and CXCL-EC$_2$, generates the intended gene expression profile in lung fibroblasts

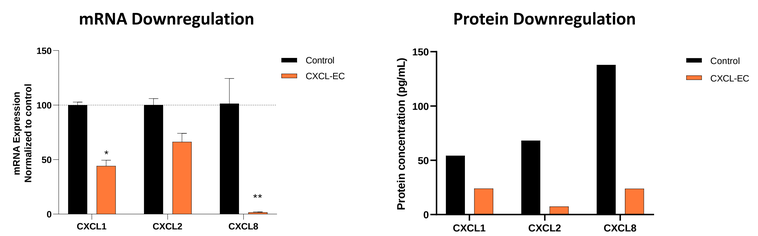

Figure 5: Treatment with CXCL-EC, a bicistronic controller, encoding the epigenomic controllers expressed in CXCL-EC$_1$ and CXCL-EC$_2$, demonstrates a 30-90% reduction in mRNA expression and protein production of CXCL1, CXCL2 and CXCL8. One-way ANOVA: * p <0.01; ** p <0.001

Supernatants from CXCL-EC-treated human lung fibroblasts demonstrate a significant decrease in neutrophil migration in vitro

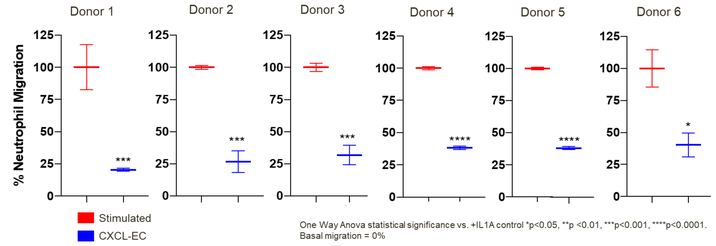

Figure 6: Supernatants from stimulated IMR90 cells treated with CXCL-EC demonstrate significant decreases in neutrophil migration in six individual donors compared to untreated IMR90 cell supernatant.

A mouse surrogate EC, analogous to CXCL-EC, significantly inhibits immune cell recruitment to the lung in an in vivo mouse model

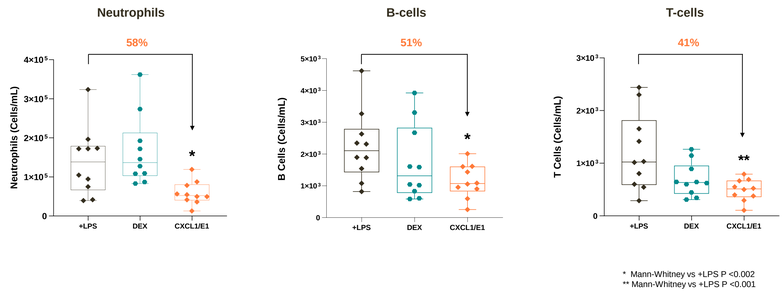

Figure 7: A single prophylactic administration of the MM-CXCL-EC$_1$/MM-CXCL-EC$_2$ co-formulation 8 hours prior to LPS challenge significantly inhibited neutrophil, B-cell and T-cell recruitment into bronchoalveolar lavage fluid 24h post-LPS challenge.

Summary

The CXCL1-8 IGD expresses eight genes which coordinately bind to CXCR1 and CXCR2 in multiple cell types to regulate immune status. Preclinical and clinical evidence have demonstrated that CXCL8 expression is important in most cell lineages with the other genes playing a supporting role in specific cell types.

Our platform enables precise control of multiple genes at once with a single bicistronic epigenomic controller. Rather than targeting CXCL8 alone, which we demonstrate leads to compensatory up-regulation of other CXCL genes within the IGD, our bicistronic epigenomic controller specifically targets the regulatory activity of the entire CXCL1-8 gene locus in a context-dependent manner. By specifically targeting, and epigenetically modifying, regulatory regions controlling CXCL1-8 response to NF-kB signaling, our EC inhibits the complete cytokine response, without compensatory regulatory feedback.

This offers a key advantage to previous approaches targeting individual cytokines. In vitro data validates multigenic control, its mechanism, and a decrease in neutrophil migration, with a murine surrogate demonstrating inhibition of immune cell recruitment in vivo.