66. Multi-specific antibody-serum albumin fusion protein (MIT)

Asset Overview

Product Type	Antibody
Disease Area	Inflammatory Disease
Indication	Rheumatoid Arthritis
Current Stage	Lead Optimization
Target	Human and Murine CXC(ELR+) Chemokines
MoA	Ab binds multiple CXC chemokines to block the CXC inflammatory cascades
Brief Description	• This technology uses an antibody that promiscuously binds multiple CXC chemokines to block the CXC inflammatory cascades associated with RA. A panel of promiscuous antibodies was developed through three sequential rounds of PCR-mutagenesis and directed evolution by selecting for antibodies with both promiscuous binding tendencies (binding to multiple CXCLs), and high binding affinity against multiple CXCLs. • The antibodies were then fused to the serum albumin protein, which stabilizes the antibody in circulation and increases the in vivo half-life. The top three antibody hits from this directed evolution scheme blocked receptor activation in response to 2-5 different CXC ligands in vitro. • The inventors performed proof of concept experiments in an in vivo mouse model of RA that demonstrated a complete resolution of RA disease burden in only 10 days in response to the top candidate antibody.
Intellectual Property	US20210246199A1
Publication	Directed Evolution of Broadly Crossreactive Chemokine-Blocking Antibodies Efficacious in Arthritis. Nature Communications, (2018)
Inventors	Alessandro ANGELINI, Karl Dane Wittrup, Andrew David LUSTER

Highlights

- Promiscuous antibodies allow targeting of multiple CXC ligands with a single antibody formulation
- Highly specific to CXC family ligands
- Effectively reversed disease in a mouse *in vivo* rheumatoid arthritis model
- Potential to translate to other diseases with CXC chemokine dysregulation such as irritable bowel disease and cancer

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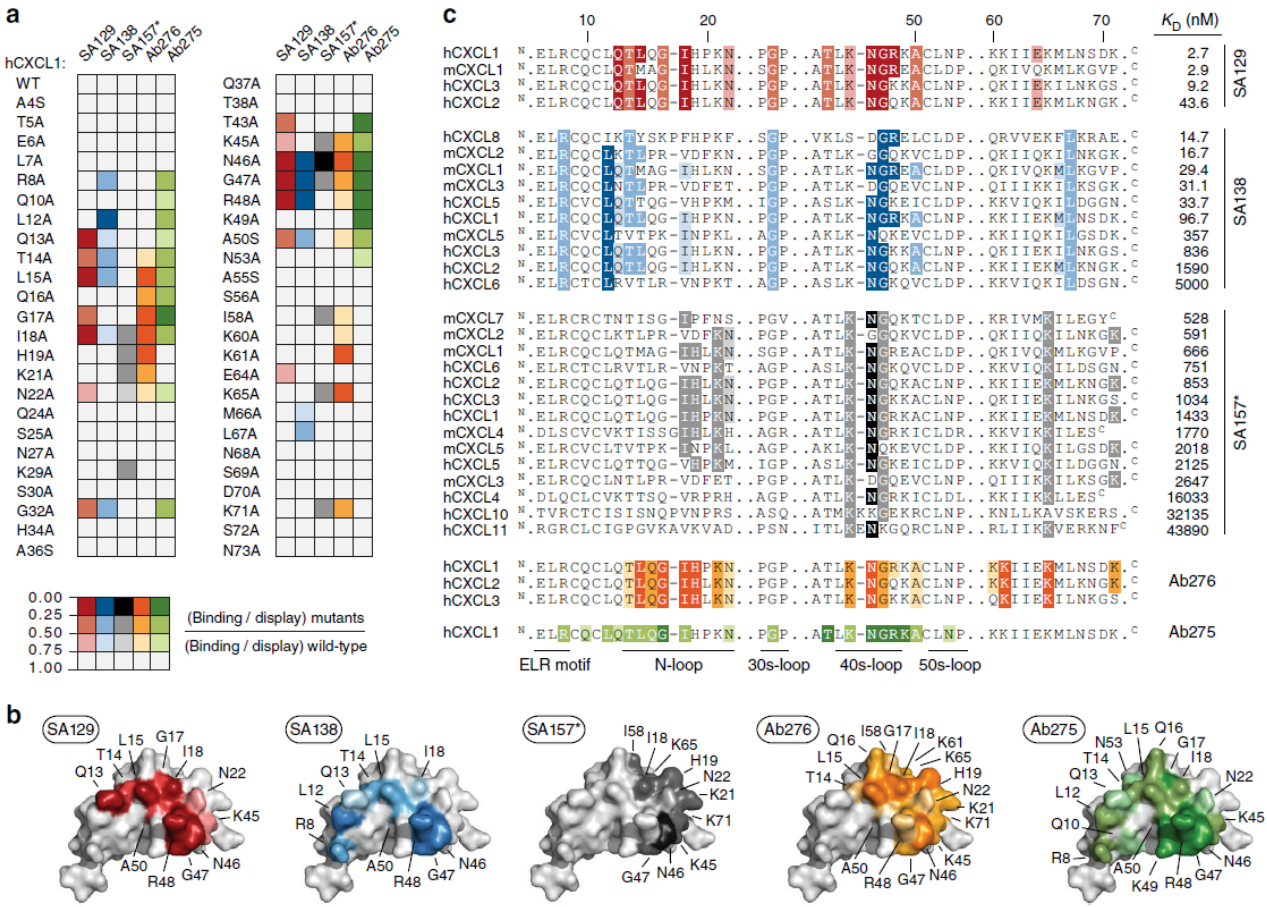
Key Data

Epitope mapping of crossreactive antibodies



A Binding of SA129 (red), SA138 (blue), SA157 (gray), Ab275 (green), and Ab276 (orange) to a defined panel of hCXCL1 alanine mutants was assessed by flow cytometry. Obtained median values were normalized to the display median fluorescence intensities of each single yeast surface displayed mutant (binding/display). Normalized values represent the means of at least three independent experiments. Mutations that do not significantly affect binding (0.75–1.0) are shown in white, while mutations that weakly (0.5–0.75), moderately (0.25–0.5), or strongly (0.0–0.25) disrupt binding are shown respectively in light, intermediate, and dark colors.

B The identified contact residues of hCXCL1 (PDB ID: 1MGS) to each antibody as defined by epitope mapping are shown in red (SA129), blue (SA138), gray (SA157*), green (Ab275), and orange (Ab276). The color intensity correlates with the strength of the interaction, with weak and strong interactions shown as light and dark colors, respectively. c Sequence alignment of various CXC chemokine proteins. Positions of conserved solvent-exposed residues that appear to be involved in the interaction with SA129 (red), SA138 (blue), SA157* (gray), Ab275 (green), and Ab276 (orange) based on residues identified using hCXCL1 alanine mutants are shown. Aminoacid sequences have been listed based on binding affinity (KD), with the tightest CXC chemokine protein at the top and the weakest at the bottom. Upper case N and C letters indicate the N- and C-terminus of the amino-acid sequence, respectively. Regions including residues that are not involved in binding are not reported for space reasons. The regions denoting the ELR-motif, N-loop, 30s-loop, 40s-loop, and 50s-loop that are known to be crucial for the
binding of ELR+ CXC chemokines to the cognate CXCR2 receptor are indicated at the bottom. Residues have been highlighted according to the strength of interaction determined using soluble antibodies against hCXCL1 alanine mutants, as shown in panel a

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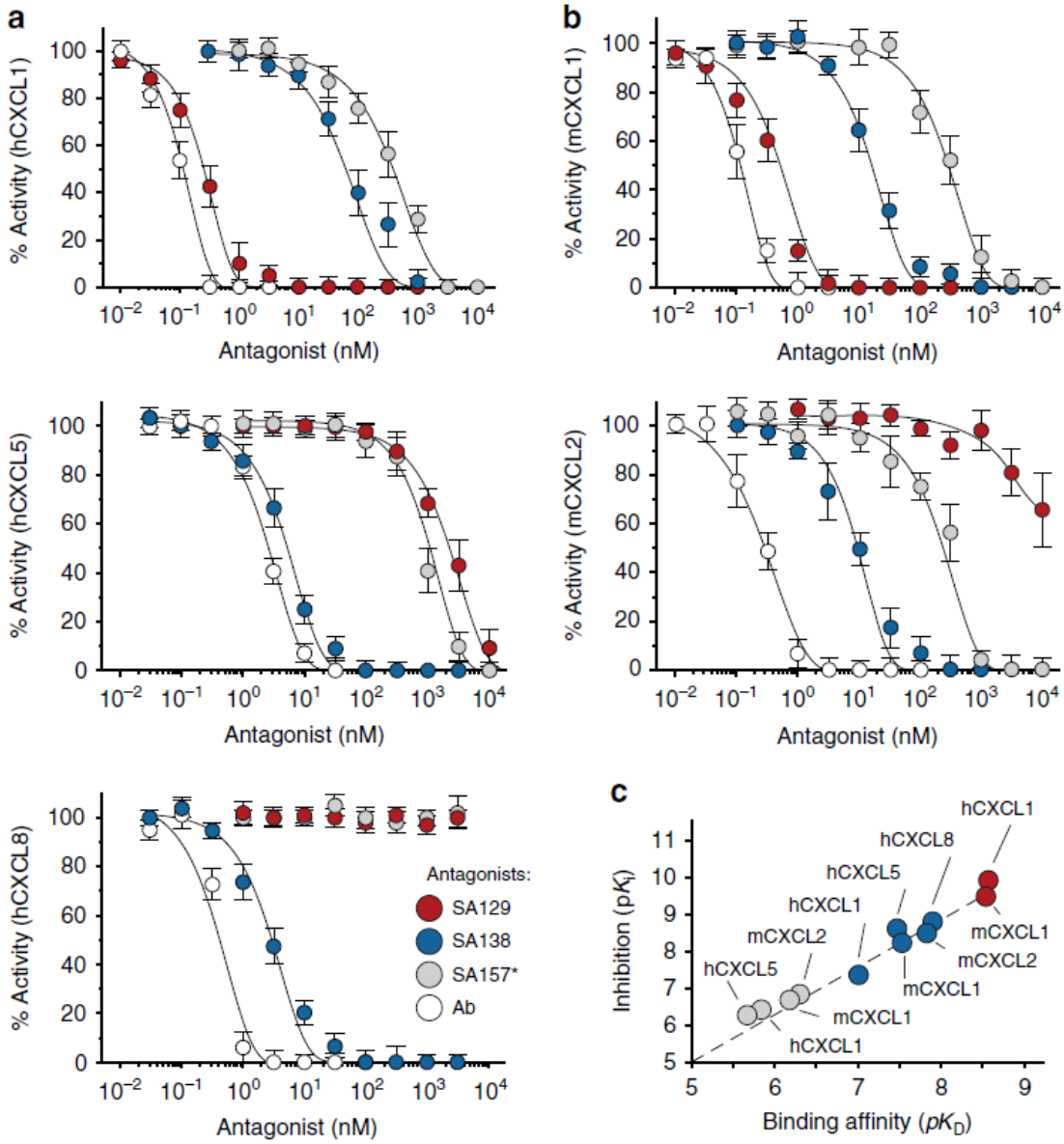
Crossreactive antibodies inhibit ELR+ CXC chemokine signaling in vitro



Residual activity of a human hCXCL1, hCXCL5, and hCXCL8 and b mouse mCXCL1 and mCXCL2 chemokines incubated with varying concentrations of SA129 (red), SA138 (blue), and SA157* (gray) fusions, and commercial neutralizing antibodies (Ab, white). The indicated values are the means of three independent experiments. c Plot displaying pKi versus the calculated pKD of SA129 (red), SA138 (blue), and SA157* (gray) fusions. Data are presented as mean (dots) ± s.e.m. (bars)

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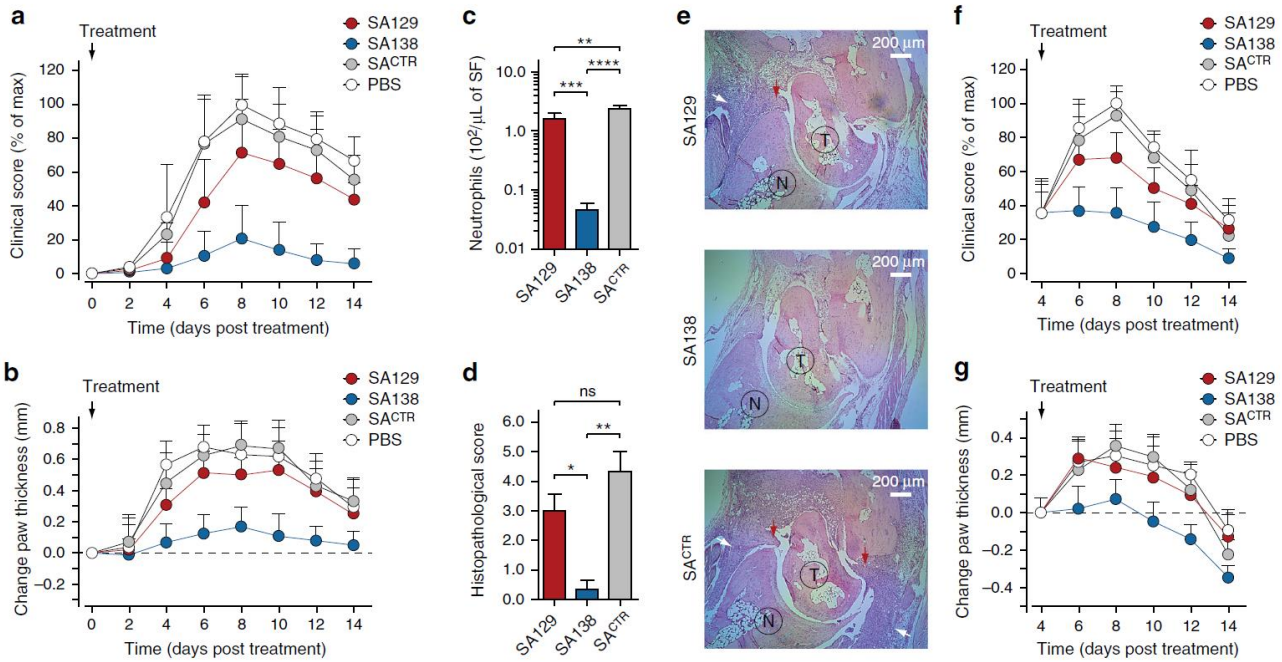
Key Data

Crossreactive serum albumin–antibody fusion reverses inflammation in vivo



A Clinical score (% of max) and b change in ankle thickness (mm) of mice treated with serum albumin–antibody fusion proteins on day 0 (preventative regimen). Arthritogenic serum was injected into C57BL/6J on days 0 and 2. Mice were also treated daily with SA129, SA138, and SACTR fusions (1 mg per mouse in PBS i.p.) beginning on day 0. Paw thickness of ten mice per group (n= 10), pooled from two independent experiments, were measured every 2 days for a total of 14 days. Arrows indicate first day of treatment. Data are presented as mean (dots) ± s.e.m. (bars). c Columns graph reporting the number of infiltrating synovial fluid neutrophils (Ly6G+ cells) from the ankles of serum-transferred arthritic mice measured at day 8 by flow cytometry (n = 3 per condition). Statistical comparisons were made between each group using one-way analysis of variance (ANOVA), followed by Tukey's test to calculate P-values: *P < 0.05, **P < 0.01, ***P < 0.001; ****P < 0.0001. ns: nonsignificant. d Columns graph reporting the histopathological scoring and e representative H&E staining of ankle tissue sections of mice treated with SA129 (top), SA138 (middle), and control SACTR (bottom) on day 8. Scale bar represents 200 µm. White arrows indicate joint-infiltrating inflammatory cells, and red arrows indicate pannus formation. T taulus, N navicular. f Clinical score (% of max) and g change in ankle thickness (mm) of K/BxN serum-induced arthritic mice treated beginning on day 4 with serum albumin–antibody fusion proteins (therapeutic regimen). Arthritogenic serum was injected into C57BL/6J on days 0 and 2, and mice were then treated daily i.p. with SA129, SA138, and SACTR fusions (1 mg per mouse in PBS i.p.) beginning on day 4 after inflammation had developed. Paw thickness of ten mice per group (n = 10), pooled from two independent experiments, was measured every 2 days for a total of 14 days. Arrows indicate the day treatment began. Data are presented as mean (dots) ± s.e.m. (bars)