HIGH AFfINITY ANTIBODIES TARGETING THE CHEMOKINE CXCL12

- In vivo efficacy demonstrates therapeutic potential
- Excellent in vitro inhibition of CXCL12-induced cell migration
- Fully human IgG2 therapeutic antibody
- Potential for inclusion into combination strategies directed at tumour angiogenesis and immune control

COMMERCIAL OPPORTUNITY

The anti-CXCL12 antibodies come with a package of data demonstrating potent inhibitory activity both in vitro and in vivo. The CXCL12 signalling axis is well recognised as a target for therapeutic intervention in the treatment of cancer, with CXCL12 and its receptors (CXCR4 and CXCR7) over-expressed in numerous cancers (such as breast, pancreatic, glioblastoma and ovarian) and in many cases correlates with metastasis and patient prognosis [1]. CRT seeks a commercial partner for the further development of these therapeutic anti-CXCL12 antibodies.

THE TECHNOLOGY

Two lead anti-CXCL12 antibodies (114_3H1 and 113_1H12) are the result of a collaborative research programme between Prof Frances Balkwill, Prof Gerard Graham and Dr John McCafferty; experts in the fields of chemokine biology and antibody discovery. Developed through multiple rounds of phage-display library selection followed by affinity maturation, the two lead antibodies possess low nanomolar $K_D$ binding to CXCL12 and are highly effective at inhibiting CXCL12-induced migration (Figure 1). The lead antibodies have been converted, and tested, in several different antibody formats (scFv-Fc fusion, human IgG2 and murine IgG2a) and bind to novel epitopes within CXCL12, including areas involved in receptor binding. The antibodies are able to inhibit both human and murine CXCL12, crucial for further pre-clinical development ahead of transition into the clinic.

A preliminary in vivo experiment in a CXCR4-dependent experimental metastasis model has shown that the lead anti-CXCL12 antibodies are effective at reducing pulmonary metastasis following tail vein injection of melanoma cells (Figure 2). These data demonstrate that the lead antibodies can reach their target and effectively inhibit signalling induced by CXCL12 in vivo. The next key development stage is to investigate the antibodies in a primary tumour model and we are currently exploring options to progress this work.

![Figure 1](https://example.com/fig1.png)

**Figure 1:** The lead anti-CXCL12 antibodies reduce the migration of CXCR4-positive TOV21G ovarian cancer cells towards human CXCL12 in a concentration dependent manner. Fluorescent TOV21G cells were incubated in the upper portion of a Boyden chamber in the presence or absence of the anti-CXCL12 antibodies 114_3H1 and 113_1H12 (human IgG2 format). CXCL12 was added to the lower chamber and the level of migration towards the chemokine was quantified. The IC50 of 114_3H1 is 5nM and of 113_1H12 is 9nM.

The lead anti-CXCL12 antibodies have the potential for development into either single agent therapeutics or as part of a combination treatment approach including, for example, through incorporation of the antibodies into a bispecific product.
BACKGROUND AND THERAPEUTIC RATIONALE

Figure 2: The lead anti-CXCL12 antibody 113_1H12 (Murine IgG2a antibody format) reduces pulmonary metastasis in an experimental metastasis model. B16F10 melanoma cells were introduced through tail vein injection on day 0 and treatment commenced on day 1. Treatment regimes were either 5 mg/kg of the clinical CXCR4 inhibitor AMD3100 (Plerixafor) twice daily or twice a week with either 10, 15 or 20 mg/kg of the anti-CXCL12 antibody. Mice in the control arm were treated twice a week with 20 mg/kg of a control antibody. All mice were culled on day 14 and the number of metastatic colonies in the lungs quantified. A level of inhibition equivalent to that of AMD3100 was achieved with the 20 mg/kg dose of 113_1H12.

Upon activation by CXCL12, CXCR4 and CXCR7 stimulate multiple signal transduction pathways, such as those involving ERK1/2, JNK, JAK/STAT and PI3K, to modulate cell proliferation, cell survival, migration and angiogenesis [1]. All of these biological processes play important roles in the development and progression of cancer and hence an antibody that inhibits CXCL12 has the potential to be an effective anti-cancer treatment.

CXCL12-CXCR4 signalling pathway has been reported as the main regulator of the biological features of cancer stem cells in glioblastoma and CXCR4 is expressed on cancer stem cells in multiple cancers [2]. CXCL12 has also been shown to promote angiogenesis in cancer through several means including up-regulation and increased secretion of VEGF, up-regulation of angiogenic genes and the recruitment of endothelial progenitor cells [1].

CXCL12 induced migration affects tumour development and progression through recruitment of CXCR4-positive cancer and stromal cells as well as regulating immune cell infiltration. CXCL12 may aid the formation of pre-metastatic niches through the recruitment of regulatory T cells, to produce an immunosuppressive environment [3]. In prostate cancer, cancer associated fibroblasts (CAFs) engage monocyte recruitment and M2 polarization through CXCL12. The interplay between M2 macrophages and CAFs ultimately increases the malignancy of prostate cancer [4]. High levels of CXCL12 are associated with low numbers of CD3+ T cells in a pancreatic cancer model and it was possible to increase T cell infiltration through combined treatment with PD-L1 and CXCR4 inhibitors. This increase in T cell infiltration was accompanied by a significant reduction in tumour volume, highlighting the role of the CXCL12/CXCR4 axis in immune control of cancer [5].

Certain chemotherapeutics, anti-angiogenic agents and irradiation have been shown to cause additional upregulation of CXCL12/CXCR4, which aids tumour recurrence post-treatment. An increased level of CXCL12 triggers mobilisation of endothelial progenitors [6] and the recruitment of monocytes to the tumour [7], which stimulate tumour invasion, neoangiogenesis and metastasis as well as suppress anti-tumour immune responses. Combination treatment including CXCL12/CXCR4 inhibitors has been shown to enhance the anti-tumour activity of the original treatment and reduce post-treatment recurrence in both lung cancer and glioblastoma [8, 9].

These studies highlight a potential path to clinic for our anti-CXCL12 antibodies would be in combination strategies with new and existing therapies.

There are several agents targeting the CXCL12-CXCR4/CXCR7 pathway in development, the majority of which are inhibitors of CXCR4. CXCR4 inhibitors have shown efficacy in both pre-clinical and clinical testing, however the discovery that CXCR7 is also a receptor for CXCL12 brings into question the rationale behind selective CXCR4 receptor inhibition. Indeed blockade of CXCR4 only partially inhibited migration towards CXCL12 gradients in several animal models and CXCL12-induced cell proliferation has been shown to be mediated by either CXCR7 or CXCR4, indicating functional redundancy between the receptors. Furthermore, CXCR7 and CXCR4 can form heterodimers, which exhibit distinct functional properties [10]. Therefore, targeting CXCL12 rather than either CXCR4 or CXCR7 should result in a more complete shutdown of the signalling pathway and hence provide a competitive advantage over receptor selective inhibitors.

INTELLECTUAL PROPERTY

A patent application was filed in December 2014 to protect the lead anti-CXCL12 antibodies along with claims to targeting the novel epitopes to which they bind.

REFERENCES

1. Guo et al, 2015, Oncogene, doi: 10.1038/onc.2015.139
2. Wurth et al, 2014, Frontiers in Cellular Neuroscience, 8:144

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