Efficacy of Functionally Blocking Antibodies Against C-C chemokine receptor 2 (CCR2)

SORRENTO THERAPEUTICS

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Abstract

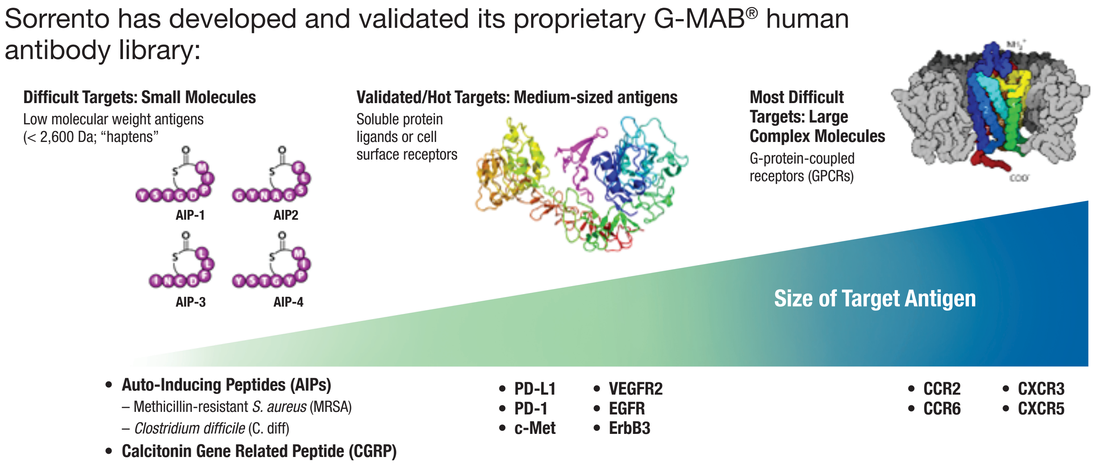
The desire to selectively inhibit a specific G protein-coupled receptor (GPCR) as a therapeutic target has led to greater interest in discovering antibodies directed against GPCRs. One family of these receptors, the chemokine receptors, possesses therapeutic potential for treatment of inflammatory diseases and cancer. One of these GPCRs, namely the C-C chemokine receptor 2 (CCR2), is expressed on the "inflammatory" subset of monocytes, dendritic cells and memory Th1 cells, and has been shown to drive inflammation in a number of animal models. It has also been implicated in bone remodeling and cancer cell metastasis.

We are currently developing antagonistic fully human anti-CCR2 monoclonal antibodies (mAbs) discovered from our proprietary G-MAB® antibody library. Several of these mAbs have demonstrated sub-nanomolar EC_{50} binding values for the human CCR2 receptor expressed on immune cells. The mAbs also exhibit antagonism against five known CCR2 ligands in both calcium flux and chemotaxis assays with IC_{50} values in the sub-nanomolar range. One of Sorrento's antibodies, STI-B0201, is cross-reactive to murine CCR2 and blocks murine CCR2 expressed on cultured WEHI cells. This antibody has been expressed in CHO cells, purified and evaluated in a mouse model of multiple sclerosis. The treated animals showed reduced disease scores compared to control animals during a four week study. To our knowledge, this is the first description of potent anti-CCR2 mAbs obtained from a human antibody library rather than from animal sources.

The human anti-CCR2 mAbs generated by Sorrento have demonstrated outstanding functional characteristics in vitro as well as in vivo and thus, might hold great potential as future anti-inflammatory and/or anti-cancer therapeutics.

Introduction

- Sorrento Therapeutics, Inc. (OTC QB: SRNE) is a development stage biopharmaceutical company engaged in the acquisition, discovery, development and commercialization of proprietary drug candidates for the treatment of cancer, inflammation, metabolic disorders and infectious diseases.
- Sorrento has developed and validated its proprietary G-MAB® human antibody library:



CCR2 Background

CCR2 is a chemokine Type I GPCR that binds to multiple ligands, resulting in receptor dimerization and signaling through multiple pathways. CCR2 is important for the development of peripheral inflammatory diseases, monocyte trafficking to an inflamed CNS and cancer cell survival (for a review, see Ref 1) and thus, is a promising therapeutic target.

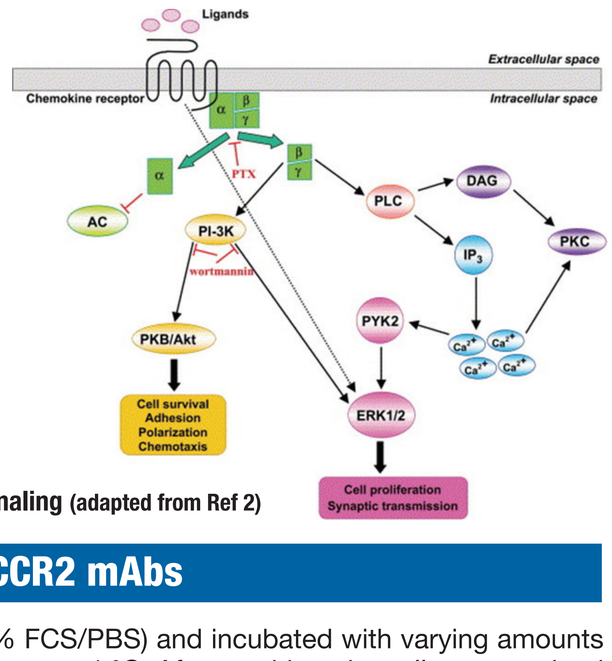


Figure 1. GPCR signaling (adapted from Ref 2)

In vitro Cell Binding by Anti-CCR2 mAbs

Cells were resuspended in FACS buffer (2% FCS/PBS) and incubated with varying amounts of anti-CCR2 mAb (IgG1) in triplicate for 1 hour at 4 °C. After washing, the cells were stained for 25 min with an anti-IgG antibody labeled with PE. The cells were washed and analyzed by FACS. The EC_{50} values were calculated from the generated curve. NR = no reaction, n.d.= not determined. The competitor antibody was produced in-house as IgG1.

Table 1. EC_{50} values for anti-CCR2 antibodies

mAb	CHO-CCR2 (human) EC_{50} (nM)	THP-1 (human) EC_{50} (nM)	WEHI (mouse) EC_{50} (nM)	RBC (Cynomolgus) EC_{50} (nM)
STI-B0201	0.17	0.8	2	5
STI-B0211	0.13	n.d.	5	n.d.
STI-B0221	0.15	n.d.	2	30-60
STI-B0232	0.34	n.d.	NR	n.d.
STI-B0234	0.36	n.d.	NR	n.d.
Competitor mAb	21	n.d.	NR	n.d.

In vitro Functional Antagonism by Anti-CCR2 mAbs

Chemotaxis: Human THP-1 cells or murine WEHI cells were added to the top compartment of a 96-well chemotaxis chamber with a 5 micron polycarbonate barrier. A range of ligand concentrations was added to the lower chamber and the apparatus incubated at 37 °C and 5% CO_2. The number of cells migrating to the lower chamber in each well were counted and plotted to obtain the IC_{50} for each ligand.

Table 2. IC_{50} values for chemotaxis studies for anti-CCR2 antibodies using human monocytes or murine WEHI cells

mAb	MCP-1 EC_{50} (pM)	MCP-2 EC_{50} (pM)	MCP-3 EC_{50} (pM)	MCP-4 EC_{50} (pM)	MCP-5 EC_{50} (pM)	MCP-1 IC_{50} (nM) (WEHI)
STI-B0201	170	170	110	140	190	66
STI-B0211	130	30	100	n.d.	110	35
STI-B0221	280	n.d.	470	80	840	13
STI-B0232	80	160	70	60	260	n.d.
STI-B0234	90	60	40	50	200	n.d.
Competitor mAb	310	460	1830	370	370	—

Calcium flux: THP-1 cells were incubated with varying amounts of anti-CCR2 antibody at room temperature for 20 min before added to a 384-well plate and incubated with FLIPR calcium 4 reagent (Molecular Devices) and probenecid (3 mM) for 1 h (37 °C and 5% CO_2) and then room temperature for 10 min. A FlexStation® 3 was used to measure the calcium response upon addition of ligand to the cells and the IC_{50} value was calculated for each ligand.

Table 3. IC_{50} values for calcium flux studies for anti-CCR2 antibodies

mAb	MCP-1-induced calcium flux (IC_{50}; pM)	MCP-3-induced calcium flux (IC_{50}; pM)	MCP-5-induced calcium flux (IC_{50}; pM)
STI-B0201	180	190	180
STI-B0211	340	220	130
STI-B0221	370	190	270
STI-B0232	750	250	560
STI-B0234	920	290	890
Competitor mAb	40	110	180

In vivo Efficacy by Anti-CCR2 mAb

The in vivo efficacy of anti-CCR2 mAb STI-B0201, which is cross-reactive to murine CCR2, was determined in a mouse model for inflammation. The experimental autoimmune encephalomyelitis (EAE) animal model was induced in C57BL/6 mice (10 per group) with MOG (myelin oligodendrocyte glycoprotein) followed by pertussis toxin. Antibody treatment was started on Day 13 and dosed 3x/week (5 mg/kg). An unrelated STI human mAb was utilized as a negative control IgG1 antibody.

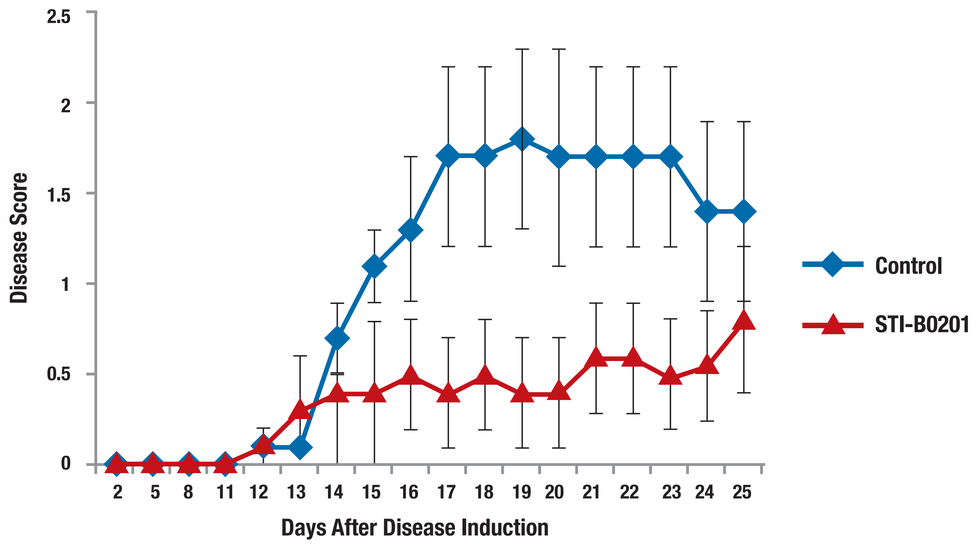


Figure 2. Anti-CCR2 mAb STI-B0201 shows efficacy in a murine EAE in vivo model.

Conclusions

Human anti-CCR2 antibodies generated by Sorrento Therapeutics have demonstrated outstanding characteristics as shown by their high affinity binding to CCR2 on cells, impressive inhibition of functional characteristics using multiple in vitro assays and five CCR2 ligands, and efficacy in a mouse inflammation model. These data indicate that Sorrento Therapeutics' antibodies may possess potential as future anti-inflammatory therapeutics and warrant testing for other indications.

References

1) Yamasaki, R. et al; *Clinical and Experimental Neuroimmunology* 2012, 3:16-29

2) Cartier, L. et al; *Brain Research Reviews* 2005, 48(1): 16-42