

*Highlights from the Society for Medicines Research meeting held March 11, 2004, in Horsham, United Kingdom.*

# Chemokines and Drug Discovery

by *Simon Hodgson, Steven Charlton and Peter Warne*

The one-day Society for Medicines Research meeting held at the Novartis Horsham Research Centre on March 11, 2004, was opened by Prof. John Westwick (Novartis, U.K.), who underscored the importance of chemokine research in the search for new therapies for numerous diseases. Steve Kunkel (University of Michigan Medical School, U.S.A.) set the scene, with an introduction to the field of chemokine biology and the importance of chemokines in many immunological and physiological events. The role of chemokines in recruiting specific leukocyte populations from the vasculature to areas of inflammation has made them an important protein target class for the development of new anti-inflammatory therapeutics. Despite their initial classification as chemotactic proteins, however, a growing body of data supports their role in many other biological processes, including activating cytokine networks, altering the expression of adhesion molecules, increasing cell proliferation, regulating

## Summary

Chemokines and Drug Discovery was a one-day meeting organized by the Society for Medicines Research, held at the Novartis Horsham Research Centre in Horsham, United Kingdom, on March 11, 2004. More than 100 scientists, mostly from industry, attended this meeting. © 2004 Prous Science. All rights reserved.

angiogenesis, promoting viral-target cell interactions, increasing hematopoiesis, stimulating mucus production, increasing the metastatic potential of tumor cells and activating the innate immune system. As such, these proteins and their receptors are likely to play an important role in the progression of chronic immune responses leading to disease.

Gurdip Bhalay (Novartis, U.K.) described a library-based approach to discovering multiple CC-chemokine receptor antagonists. The hypothesis behind the design of this library came

from analysis of the significant patent literature existing for CCR1, CCR2, CCR3 and CCR5 receptor antagonists, where it was found that many, if not all, shared a common pharmacophore. One common feature revealed was a hydrophobic region linked to a basic nitrogen atom, which is separated by a carbon chain from a second hydrophobic region connected via an amide, sulphonamide, urea or an isostere thereof (Fig. 1).

A library based on this pharmacophore was synthesized using fragments described in the patent literature,

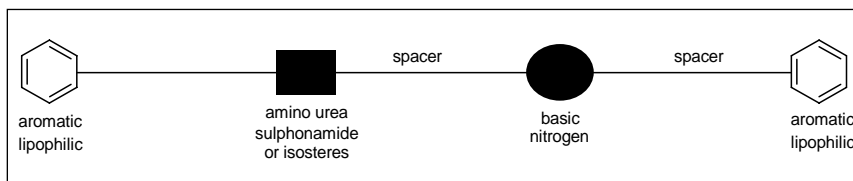
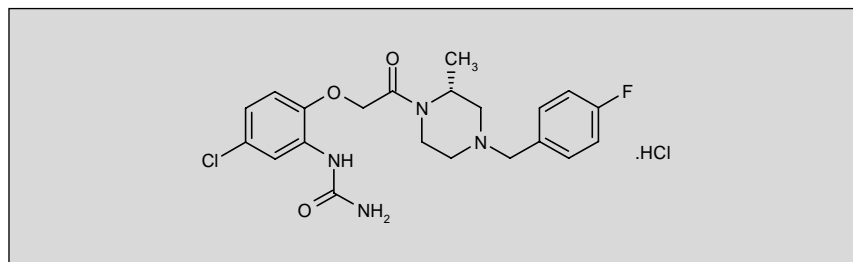


Fig. 1. Putative pharmacophore model for CC-chemokine receptors.

supplemented by commercially available building blocks. Compound activities at CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7 and CCR8 receptors were then tested, and many potent (below 1  $\mu$ M) compounds were discovered. Interestingly, many compounds showed activity at two or more receptors. In most cases, multiple receptors can be activated by a single chemokine, and it is anticipated that these multiple chemokine receptor antagonists may be more effective than compounds acting at a single receptor. In closing, Dr. Bhalay suggested that at present it appears that combinations of CCR2–CCR3, CCR1–CCR3 and CCR3–CCR5 were the most feasible.

Ian Anderson (Cambridge Antibody Technology) offered a different approach to modulating chemokine function, namely the use of human monoclonal antibodies directed toward either the chemokines themselves or their cognate receptors. The important recent success of *Xolair*<sup>®</sup> (omalizumab; anti-IgE antibody) in the treatment of asthma exemplifies the importance and value of the use of biological agents as drugs. Dr. Anderson talked in detail about **CAT-213**, a high-affinity ( $K_D$  8.8 pM) anti-eotaxin1 IgG<sub>4</sub> generated using phage display techniques. Eotaxin1 (also known as CCL11) is an important regulator of eosinophil and mast cell function, and is thought to play a role in the pathogenesis of asthma. CAT-213 is able to inhibit the biological function of eotaxin1 both *in vitro* and *in vivo*. It has a good safety profile and an elimination half-life of 8.5 days in humans. Importantly, a randomized, double-blind clinical study showed that CAT-213 was able to inhibit eosinophilia and mast cell numbers following intranasal allergen challenge in hay fever sufferers. These early successes suggest that CAT-213 may provide control of airway eosinophilia and therefore reduce exacerbation rates in patients with severe asthma.

The focus of the meeting then switched. Several speakers described results of work on small-molecule chemokine antagonists that has pro-



**Fig. 2.** Chemical structure of BX-471, a 2 nM ( $K_i$ ) inhibitor of the CCR1 receptor.

gressed beyond the lead identification stage. Richard Horuk (Berlex Biosciences, U.S.A.) described the potential importance of chemokines implicated in a variety of diseases, most notably autoimmune disease. Chemokines are commercially attractive in that they are G-protein-coupled receptors, a group of receptors that are the targets for more than 45% of all medicines. Since the first cloning of the CCR1 receptor and the subsequent maturation of cytokine biology, several CCR antagonists have been described in the patent literature. Pfizer is developing a phase II candidate for the treatment of rheumatoid arthritis (**CP-481715**) and a CCR5 inhibitor for AIDS (see below), and there are CCR3 modulators targeted toward asthma.

**BX-471** (also known as **ZK-811752**; Fig. 2) is a 2 nM ( $K_i$ ) inhibitor of the CCR1 receptor, which is active in the EAE model of multiple sclerosis in the rat and shows promise in models of transplantation rejection. The product passed through phase I trials during 2001 and 2002 and is currently in phase II trials in multiple sclerosis patients, which are scheduled to complete in July 2004.

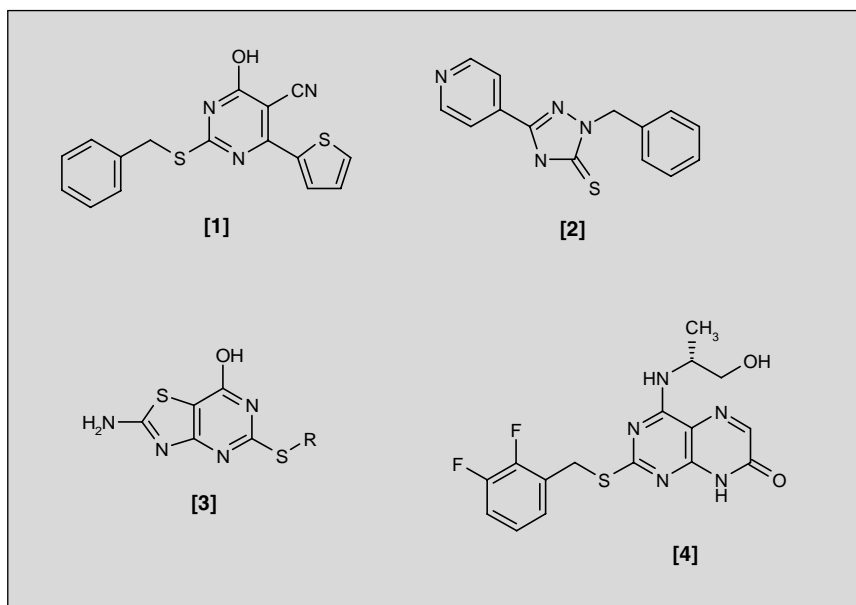
Owing to a two-order difference in species specificity, **BX-471** is considerably more active against the human CCR1 receptor than those of either the rat or the guinea pig. *In vitro*, low nM activity against the human receptor converts to  $K_i$  values in the region of 120 to more than 200 nM when tested against the corresponding rodent proteins. And high doses (5–50 mg/kg) have been necessary to show activity in the EAE model.

An interesting effect has been observed in a model of heart transplant rejection. Neither **BX-471** nor **ciclosporin** alone is particularly active in this test (10 mg/kg CysA required for activity), but together BX-471 augments the effects of both low-dose (2.5 mg/kg) and high-dose (10 mg/kg) ciclosporin upon cumulative survival. Mechanistic studies suggest that BX-471 inhibits monocyte adhesion and subsequent infiltration of the heart from the systemic circulation. Similarly, in a model of kidney fibrosis in the rat (ligated ureter), treatment with BX-471 has been shown to reduce CD45 expression and fibroblast influx.

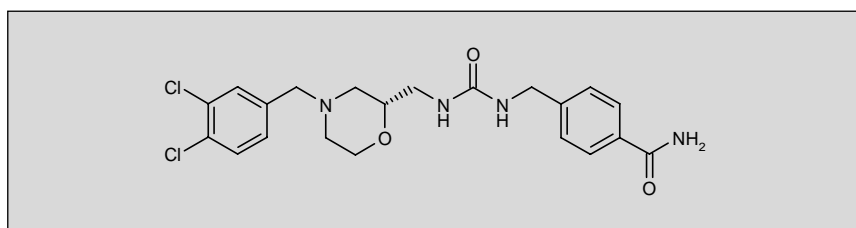
Roger Bonnert (AstraZeneca, U.K.) described his team's work on CXCR2 antagonists with interest in a range of immune and inflammatory diseases. In particular, the work focused on taking the output from high-throughput screening and conducting hits to lead work and lead optimization on three templates, pyrimidines (**[1]**), triazoles (**[2]**) and pteridines (**[3]**; Fig. 3).

The target was  $PIC_{50} = 8$ ,  $pA_2$  in whole blood of 6 and other features of developability (selectivity, P450, solubility, etc.). A 20 x 80 matrix array of the pteridine template gave an example (**[4]**; Fig. 3) with high potency,  $pIC_{50} = 9$  and 40% oral bioavailability in the rat.

Simon Hodgson (GlaxoSmithKline, U.K.) described his team's work on CCR3 antagonists for asthma and allergy. On the basis of pharmacophore generation and array work, an aminomethylmorpholine series was identified. Lead optimization gave a series of acetamides, an example of which had



**Fig. 3.** CXCR2 antagonists being developed by AstraZeneca for the treatment of immune and inflammatory diseases.

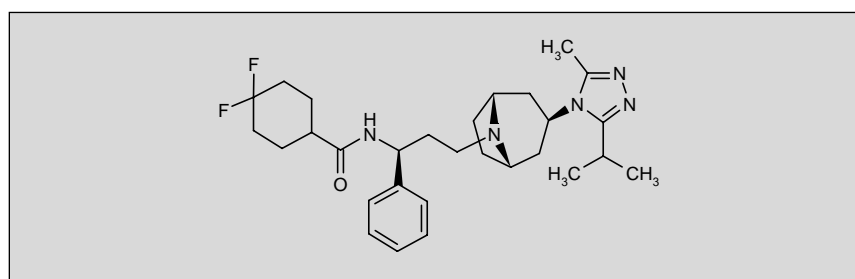


**Fig. 4.** Chemical structure of GW-766994, a CCR3 antagonist being developed by GlaxoSmithKline for the treatment of asthma and allergy.

good PK properties in the rat, guinea pig and dog. However, some P450 inhibition and extensive metabolites were seen. A series on ureas was optimized, resulting in **GW-766994** (Fig. 4). The profile of this compound was described as having good selectivity, good PK properties in the rat and dog and no significant P450 inhibition, and the compound had now entered clinical development. GW-766994 was the first CCR3 antagonist to be described in clinical development. The Glaxo-SmithKline series showed an interesting set of antiinflammatory effects on eosinophils in the rat and guinea pig, and on clinical outcomes and mast cell degranulation in a mouse model of allergic conjunctivitis.

David Price (Pfizer Global Research, U.K.) completed the meeting

with a discussion of the possible application of CCR5 antagonists to address AIDS. In spite of significant progress made in the treatment of AIDS in recent decades, there remains a need for improved therapy. Resistance to existing therapy is greater than 10%, and there are toxicity and tolerance



**Fig. 5.** Chemical structure of UK-427857, an antagonist of CCR5 with antiviral activity being developed by Pfizer.

limitations, so that more than 50% of all patients are forced to change their treatment.

The involvement of CCR5 in the transmission of AIDS is derived from a small population of patients in whom there is a 32-base pair deletion in their CCR5 expression. These patients are immune to HIV, even though they continue with high-risk sexual behavior. Similarly, heterozygotes display a delayed progression of the disease compared with homozygous wild types. These findings have now been reconciled by the demonstration that CCR5 activation is essential for the interaction of the lymphocyte CD4 receptor with the gp120 viral protein.

The Pfizer scientists had a clear vision for their product: a potent and durable antagonist of CCR5 suitable for once-daily dosing and devoid of P450 inhibition (an issue pertinent to the use of several of the current products). Starting from a known compound in the company collection (**UK-107543**) and analogues, an early breakthrough in the demonstration of antiviral activity was complemented by modification to remove P450 2D6 inhibitory activity. **UK-382055**, however, inhibited the hERG channel and required structural modification to increase its cardiac safety profile. This was achieved in the shape of **UK-395859**, but although initially exciting, this product suffered from poor cell permeability. Other modifications increased cell permeability but introduced metabolic instability, requiring a change of chemical scaffold before **UK-427857** (Fig. 5) could emerge.

**UK-427857** has an IC<sub>90</sub> antiviral activity of 2.1 nM, and while the product is still poorly orally bioavailable, this potency coupled with a sound safety profile is regarded as acceptable for project progression at a dose of 100 mg bid. Key to the product's efficacy are its slow offset binding kinetics, which contribute to potency and reduce the impact of apparently adverse pharma-

cokinetics. The product is currently undergoing phase II trials.

---

*Simon Hodgson, Steven Charlton and Peter Warne are members of the SMR Committee. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four*

*times a year. These one-day conferences are of a multidisciplinary nature, therapeutically focused and normally staged in or around London. Details about forthcoming meetings can be obtained from: SMR Secretariat, Triangle House, Broomhill Road, London SW18 4HX, U.K. Tel: +44 20 8875-2431; Fax: +44 20 8875-2424; E-mail: secretariat@socmr.org; URL: <http://www.socmr.org>.*

#### **IND CLEARANCE FOR SQUALAMINE IN NEOVASCULAR DISEASES OF THE EYE**

Genaera Corporation announced April 22, 2004, that the U.S. FDA has cleared Genaera's Investigational New Drug (IND) application for its systemically administered antiangiogenic drug **squalamine**. The IND covers treatment of neovascular diseases of the eye, including wet age-related macular degeneration (AMD). Genaera plans to begin phase II trials in AMD this quarter, which will run concurrently with the start of phase III trials in AMD, beginning in early 2005. Three phase II trials are planned. A multicenter, randomized, double-blind, controlled, exploratory trial will evaluate the safety and efficacy of squalamine in 100 patients with AMD over a 2-year period. It will study two dose levels of squalamine (20 or 40 mg) given once weekly for 4 weeks, fol-

lowed by maintenance doses once every 4 weeks until week 48. At the end of 48 weeks of therapy, each patient will be followed for 12 months.

A second exploratory phase II trial will evaluate the effects of two different doses of squalamine in combination with an initial *Visudyne*<sup>®</sup> treatment in 30 patients with AMD. The potential benefits of squalamine pretreatment on the actions of *Visudyne* will be studied, as will the added potential benefit of dosing squalamine after *Visudyne* to inhibit the potentially detrimental effects of the vascular endothelial growth factor (VEGF) burst that commonly occurs after *Visudyne* treatment. The multicenter, randomized, controlled, blinded study includes monthly squalamine maintenance therapy, and 12 months subsequent follow-up for each patient.

In addition, a phase II pharmacokinetic and safety trial is planned in

18 patients with AMD at three different doses of squalamine over 4 months. In the open-label, parallel group study, squalamine will be administered intravenously at three doses, once weekly for 4 weeks. Working within activated endothelial cells, squalamine inhibits growth factor signaling including VEGF, integrin expression, and reverses cytoskeletal formation, thereby resulting in endothelial cell inactivation and apoptosis. Systemically administered squalamine inhibits abnormal angiogenesis in rodent models of retinopathy of prematurity, and the development of choroidal neovascular membranes in rat models of AMD. Preclinical studies show that systemic squalamine administration is effective in reaching abnormal ocular blood vessels in primates, and leads to partial regression and inhibition of new abnormal vessels in the eye.