

Drug Discovery targeting the chemokine system – where are we?

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1. ABSTRACT

The identification of a large cytokine sub-family responsible for the control of the directional migration of leukocytes over 20 years ago brought much excitement to the pharmaceutical industry with the promise of a new family of targets to treat inflammatory diseases. This family of small proteins, subsequently named chemokines, were identified as acting on seven transmembrane spanning (7TM) G protein-coupled receptors – one of the most druggable classes of receptors in the pharmaceutical industry. The interest in chemokines and their receptors as therapeutic targets subsequently evolved beyond inflammation to include cancer and infectious disease such as AIDS, as chemokine biology progressed to demonstrate their role in these processes. The first inhibitors entered the clinic some 7 years ago. However progress and success has not been as rapid as hoped for: both in the identification of candidate molecules as well as their efficacy in the clinic. We will address the chemokine system as drug targets, issues involved in the development of therapeutic candidates and attempt to address the pitfalls and potential routes to success.

2. INTRODUCTION

The chemokine system consists of a large number of ligands and receptors, which have been covered extensively in several excellent reviews (1-3). The feature that separates chemokines from other cytokines is that they act on seven transmembrane spanning (7TM) G protein-coupled receptors. Whilst they interact with their receptors with high affinity, they also interact with low affinity to cell surface glycosaminoglycans (GAGs) to enable their immobilization on the endothelium. There are over 40 ligands identified to date in the human system, and 19 receptors. Both the ligands and receptors have been classified according to the first two cysteines in the canonical four cysteine motif possessed by the majority of chemokines, which creates a fold shared by all chemokines through 2 disulfides, although as with all rules, there are exceptions. The α or CXC class have a single amino acid between the amino terminal cysteines, of which there are 16, whereas 28 have the first two cysteines which are adjacent, giving rise to the β or CC class. A single chemokine, CX3CL1 or fractalkine/neurotactin, has 3 amino acids spacing the amino terminal residues, , whereas

Naïve T cell

CCR7
CXCR4



Eosinophil

CCR1,3,4
CXCR4



Memory T cell

CCR1-10
CXCR3,4,6
CX3CR1



Basophil

CCR2,3,4
CXCR4



NK cell

CCR1,2,5
CXCR3,4
CX3CR1
XCR1



Monocyte

CCR1-5, 8
CXCR4
CX3CR1



Neutrophil

CXCR1,2,4



Dendritic cell

CCR1,5-8
CXCR4



Mast cell

CXCR1,2,3,4



B cell

CCR6,7
CXCR4,5



Figure 1. Chemokine receptor expression on the major leukocyte subsets..

two other chemokines only have a single disulfide. The receptors are also classified according to the ligands that they bind, and promiscuity is rife in the chemokine system, since several ligands bind to more than one receptor, and few receptors bind a single ligand. However chemokine biology has identified another delineating, although again not rigid classification, that of the ligand-receptor pairs involved in basal trafficking and homing, and those which are inducible, and therefore considered as inflammatory. Initially the ligands were named according to their activity initially described, but due to the concomitant identification of ligands by different laboratories resulting in several names for the same ligand, a systemic nomenclature was introduced in 2000 (4). Lastly the systemic nomenclature, based on the chromosomal location was only intended for the human system, but unfortunately it has also been misused for murine chemokines, even by editors of journals who require that the systemic nomenclature is used for the murine ligands!

The initial hope more about 15 years ago was that there would be a single receptor on each leukocyte type, allowing targeting acute inflammatory processes through the receptor on the neutrophil, asthma through the eosinophil receptor, arthritis through the monocyte/macrophage receptor and so on. However as shown in Figure 1, most

leukocytes express multiple receptors. Therefore, as discussed below, target validation is the first task in the drug discovery process.

3. TARGET VALIDATION

In order to identify the relevant target for a drug discovery program, it is essential to delineate which ligands/receptors are important in the disease of interest. This may be achieved by several methods, outlined below, and summarized in Figure 2.

3.1. Expression of protein target in the disease

This may be measured directly at the protein level by immunohistochemistry or ELISA techniques, or indirectly by measuring the mRNA that translates to the protein in question. Immunohistochemistry on lesions in autopsy samples taken from brains of multiple sclerosis (MS) patients has identified the inflammatory chemokines CCL5/RANTES, CCL2/MCP-1, CXCL10/IP-10 and CXCL9/Mig as being upregulated (5,6). This implies that these chemokines and their receptors, CCR1 and CCR5, CCR2 and CXCR3, play a role in either the development or the resolution of the disease process. Elevated levels of chemokines such as CCL5/RANTES and CCL11/eotaxin

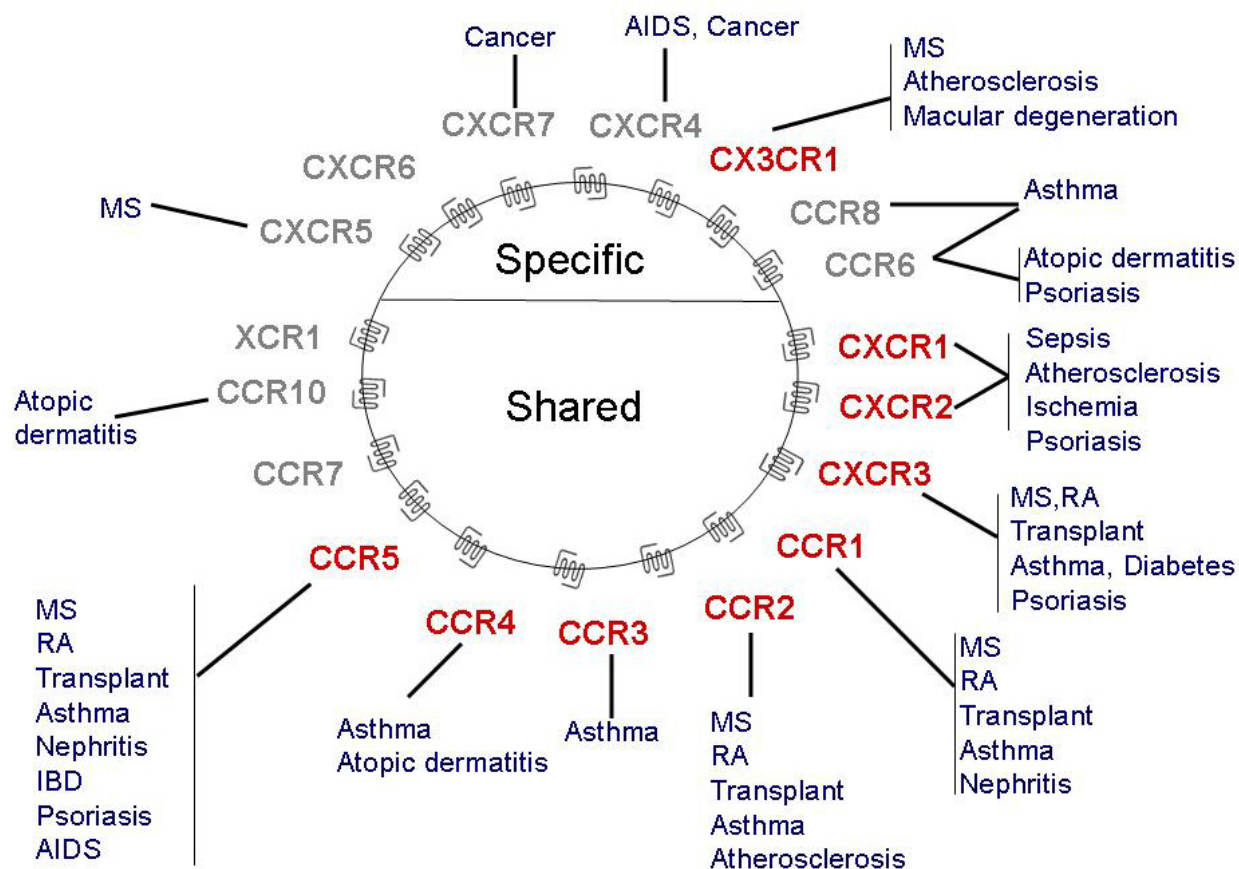


Figure 2. Association of diseases with chemokine receptors. The association of receptors with disease has been largely drawn from investigation in animal models of the diseases, using genetically modified animals, or blockade of either the receptors or the ligands.

have been found in the bronchiolar lavage (BAL) of patients with allergic rhinitis and asthma (7,9). Four unusual CC chemokines in that they have an extra disulfide, CCL6, CCL9, CCL15 and CCL23, and which were described as low affinity CCR1 ligands, are found at elevated levels in synovial fluid of rheumatoid arthritis patients as truncated proteins which are 1000 fold more potent on CCR1 (10).

3.2. Neutralizing antibodies

Neutralization of CCL3/MIP-1 α and CCL2/MCP-1 allowed the delineation of these two chemokines, and by deduction their receptors, in the course of relapsing remitting Experimental Autoimmune Encephalomyelitis (EAE), the rodent model for MS (11). This study demonstrated that MIP-1 α was implicated in the onset of the disease, whilst MCP-1 played a key role in the relapses. The use of antibodies against different chemokines, along with a receptor antagonist, Met-RANTES that neutralizes several receptors, demonstrated a hierarchy of chemokine usage in the ovalbumin-induced lung inflammation model, the murine model of asthma (12). Neutralization of CXCR3 prevented the rejection of cardiac transplants, showing the same phenotype as the CXCR3 $^{-/-}$ mice – an excellent example of two approaches

arriving at the same conclusion (13). However animal models can be misleading as was unfortunately demonstrated in EAE models where neutralizing IFN γ exacerbated disease (14) but in man administration of recombinant IFN γ also worsened the disease (15) and TNF α blockade in EAE reduced clinical score (16) but in humans anti-TNF treatment actually exacerbated disease (17).

3.3. Genetic targeting of chemokine/chemokine receptors

Mice deficient in the ligand or receptor in question have proved to be an excellent approach to study the function of specific chemokine ligand-receptor interactions *in vivo*, particularly in models of inflammatory and infectious disease. Knockout of CCR1, CCR2 and JE (murine MCP-1), support their potential as targets for therapeutic intervention in MS (18-21). The unequivocal role of CCL2/MCP-1 and CCR2 in the pathogenesis of atherosclerosis was demonstrated using knockout mice (22,23). Recently, a chemokine believed to be involved in homeostasis, CXCL13, was demonstrated to be implicated in the development of EAE, contradicting the belief that only pro-inflammatory chemokines were involved in inflammatory diseases (24).

However these approaches are not always successful. Eosinophils are often the predominant inflammatory cell population present in allergic diseases. CCL11/eotaxin was first identified as an eosinophil specific chemoattractant in a guinea pig model of allergic asthma (25) and the unique functional receptor for CCL11/eotaxin, CCR3, is expressed at high density on both eosinophils and basophils. Whilst CCR3 and its ligand eotaxin were found to be highly expressed in the bronchial biopsies and lavages of asthmatics (26), and serum eotaxin levels have been demonstrated to be a marker of disease severity in patients (27) the mice in which these genes were deleted did not express the phenotype that had been predicted, i.e. a resistance to experimental allergic asthma. The true contribution that eosinophils make to disease progression is, however, still constantly debated (28,29) and in this case, the initial optimism of finding ‘the receptor’ to control allergy, which was present in the mid 1990s has now disappeared as a much more complex picture of chemokine involvement in this disease has emerged. However, a CCR3 ligand, eotaxin-3, has recently been shown to be implicated in a specific allergic condition, eosinophilic oesophagitis, suggesting targeting CCR3 for this indication (30). However a flaw in this approach is the difference between the murine and human immune systems. An example is the function of CCR1 between the two systems – in humans CCR1 principally mediates monocyte recruitment whereas in the mouse CCR1 and its ligands recruit neutrophils.

3.4. Use of receptor antagonists in disease models.

The most widely used receptor antagonists have been chemokines with modified amino termini. Chemokines are believed to interact with their receptors in two phases – the first involves interaction with the extracellular loops of the receptor, and the second is believed to be an interaction of the amino terminal region deeper in the receptor, probably in the transmembrane spanning helices which triggers receptor activation and the ensuing signal transduction cascades. Thus chemokines which are modified to retain high affinity binding but which cannot induce signal transduction, will act as antagonists. The majority of such antagonists have been created by N-terminal truncation (31,32) but there are also examples of N-terminal extension (33,34). These variants have been used extensively in animal models of disease demonstrating that blockade of chemokine receptors was an effective anti-inflammatory strategy.

3.5. Target validation in clinical trials.

The best validation will obviously come from testing in humans. So far the results are disappointing, as will be addressed in the remainder of this review, although conclusions from the first trials targeting such a complex system should be drawn with care, as multiple factors could be involved. However the patient selection is critical in this analysis, very well illustrated by the fact that HER-2 is only upregulated in 25% of women with breast cancer, so had Herceptin been tested in patients who were not over expressers we would have a failed clinical trial for Herregulin, which is clearly an effective drug in treating this disease.

3.6. Lessons from evolution.

Pathogens such as viruses or parasites that require evasion of the immune system have evolved many mechanisms to avoid the host's immune response – including that of interference with the chemokine system. This certainly enforces that the chemokine system is a well validated target! For the readers interested in the viral mechanism of chemokine antagonism, comprehensive reviews are recommended (35,36). Eukaryotic chemokine binding proteins have only recently been described, although evidence for their existence has been reported a few years ago (37,38). The first to be molecularly identified was from the parasite *S. mansoni* (39), but a new family has been recently identified from thick salivary glands (40)

4. STRATEGIES

A major difference between the chemokine family and other cytokines is that the ligand-receptor interaction for cytokines has proven to be almost impossible to block with small molecule inhibitors, whilst this is not the case for chemokines. The reason for this is simple – chemokines are the only members of the cytokine family that interact with 7TM receptors. This class of receptors is one of the cornerstones of the pharmaceutical industry as more than half of marketed drugs target these receptors which provide small binding sites amenable to blockade with small molecules. Cytokines on the other hand have large surface areas involved in their ligand-receptor interaction, which is well exemplified by IL-1 where 7 amino acids equally contribute to the interaction with the receptor, covering a large surface area, rendering the blockade almost impossible by a small molecule with a mass ≤ 600 Da, a requirement for orally available medicines.

As mentioned above, chemokines are thought to interact with their receptors in two steps. Although the first interaction, that of the body of the chemokine with the extracellular loops may pose a similar problem as that of cytokines, the second interaction can be considered as amenable to blockade with small molecules. In this respect chemokines differ from other 7TM ligands, which are often small molecules such as serotonin and histamine which can be screened against for competitive inhibitors of the ligand binding site. Chemokines are in fact rather large 7TM ligands, despite the fact that they are small proteins, generally around 8 kDa.

Thus the chemokine system lends itself to both the pharmaceutical and biotechnology sectors where in the former the receptors provide targets for high throughput screening campaigns and in the latter, to approaches such as neutralizing antibodies against either the receptor or the ligand. The former approach has been that which has been most widely adopted in the search for drug candidates. The rationale for this approach is certainly sound, but was perhaps colored by the belief that the development of neutralizing monoclonal antibodies, by no means a trivial task, may simply have served as proof of concept in the clinic for the orally available small molecules that would shortly follow. In hindsight this strategy may not have been the wisest of choices in view of the difficulty of

identifying small molecules, as discussed below, and several monoclonal antibodies against both receptors and ligands, are in development, and have entered clinical trials.

However these two approaches are certainly not exhaustive. Modified chemokines as pure receptor antagonists have been considered, but to our knowledge, are not currently in development. A combination of the relevance of the two essential interactions of chemokines required for their activity *in vivo*, the interaction with the receptor, as well as the low affinity interaction with GAGs is being developed as a therapeutic approach (41). Selected chemokines are being screened first for mutations which abolish receptor activation and then for mutations which will increase their avidity for GAGs, whereby they will prevent the active chemokine from forming the chemotactic gradient on the endothelial surface.

Another approach is to identify natural binding proteins that negate the ligand. There are several examples of such proteins for the cytokine family in humans where the body appears to upregulate these proteins in response to the over-production of the inflammatory molecules, presumably in a disease situation. These include the cytokine binding proteins TNBP, the soluble TNF receptor, IFNAR and IL-18BP – the first example is in fact a highly successful bio-therapeutic on the market today – ENBREL, a soluble TNF receptor fusion protein. To date no such binding proteins for chemokines have been identified in humans. However parasites, in particular viruses, appear to have understood the importance of the chemokine system in regulating the immune response, since several chemokine binding proteins have been characterized. Two of these are currently in pre-clinical development, T1 and T7, isolated from the myxoma pox virus.

All members of the cytokine family require signal transduction pathways, which provide points of potential intervention for pharmaceutical therapeutics. To date the most sought after target in the chemokine field is PI3K γ , which was originally shown to be required for cellular recruitment in gene deleted mice (42). Several companies are actively pursuing this target as demonstrated by the number of published patents. The concept of inhibiting this enzyme has been demonstrated in two disease models, collagen induced arthritis (43) and systemic lupus (44).

5. MAJOR HURDLES

One of the major hurdles in drug development is identifying a potent drug that targets the human protein of interest but that does not recognize the equivalent protein in rodents or worse still in any other species. This problem has in particular bedevilled the field of GPCR therapeutics and the landscape is littered with examples of these development problems. Drug substances that are limited in specificity to human target proteins can be problematic during drug development since they are difficult to test in surrogate animal efficacy models and without such data it can become very difficult to justify further development of the drug, given the considerable risks and costs involved. Even more problematic is that no measure of target induced

safety problems can be measured. Many a project has been stalled or delayed because of these types of problems.

A prime example of such a problem was highlighted in the development of the CCR5 antagonists. With the discovery of CCR5 as an essential co-receptor for HIV infectivity over 10 years ago, many pharmaceutical companies engaged in such programs but not one company has ever identified an antagonist that could block rodent CCR5 receptors, and even worse, many of these antagonists did not cross-react with CCR5 receptors from non-human primates, despite their extremely high similarity with human CCR5. In the case of the first CCR1 antagonist BX471, the situation was less severe because BX 471, a potent inhibitor of human CCR1 (1 nM) was able to block rodent CCR1, albeit with weaker affinity, with K_i values for inhibition of CCL3 binding to rat and mouse CCR1 of 121 nM and 215 nM respectively (45,46). This poor affinity of potent small molecule inhibitors of human chemokine receptors for non-human chemokine receptors has represented one of the more challenging problems in drug development and numerous examples abound in the literature. For example, the quinoxaline CCR1 antagonist CP-481,715 from Pfizer that has good activity for the human receptor (IC_{50} for displacement of ^{125}I -CCL3 binding = 74 nM and K_D for displacement of 3H- CP-481,715 binding = 9.2 nM) does not inhibit the binding of CCR1 ligands to mouse, rat, guinea pig, dog, rabbit or monkey CCR1 receptors (47). However, a solution to circumvent the species cross-reactivity problem is the creation of mice expressing the human receptor, which was the approach adopted by Pfizer.

At present, the basis of antagonist selectivity is not well understood at the molecular level. However, we should remember that the difference between high affinity (1nM) and low affinity (1 μ M) is only a few kilocalories in free energy terms - a little less than the energy gained from breaking a hydrogen bond. Thus, it is obvious that even very small changes in the receptor binding pocket for an antagonist could impart sufficient differences to totally prevent it from binding. Recent efforts at molecular modeling coupled with receptor mutagenesis may shed some light upon this and facilitate the rational design of antagonists (48,49).

6. CLINICAL SUCCESSES – AND FAILURES

Recently the CCR5 antagonist maraviroc, marketed by Pfizer for the treatment of AIDS, was granted approval by the FDA. This represents the first example of a chemokine receptor antagonist successfully realizing its potential as a therapeutic. In addition the CXCR4 antagonist Plerixaflor (MozobilTM, AMD3100) was recently granted FDA approval for mobilizing haematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma. The drug is currently used in combination with granulocyte-colony stimulating factor (G-CSF). Today there are a large number of molecules in various stage of development ranging from pre-clinical to Phase I, II and III,

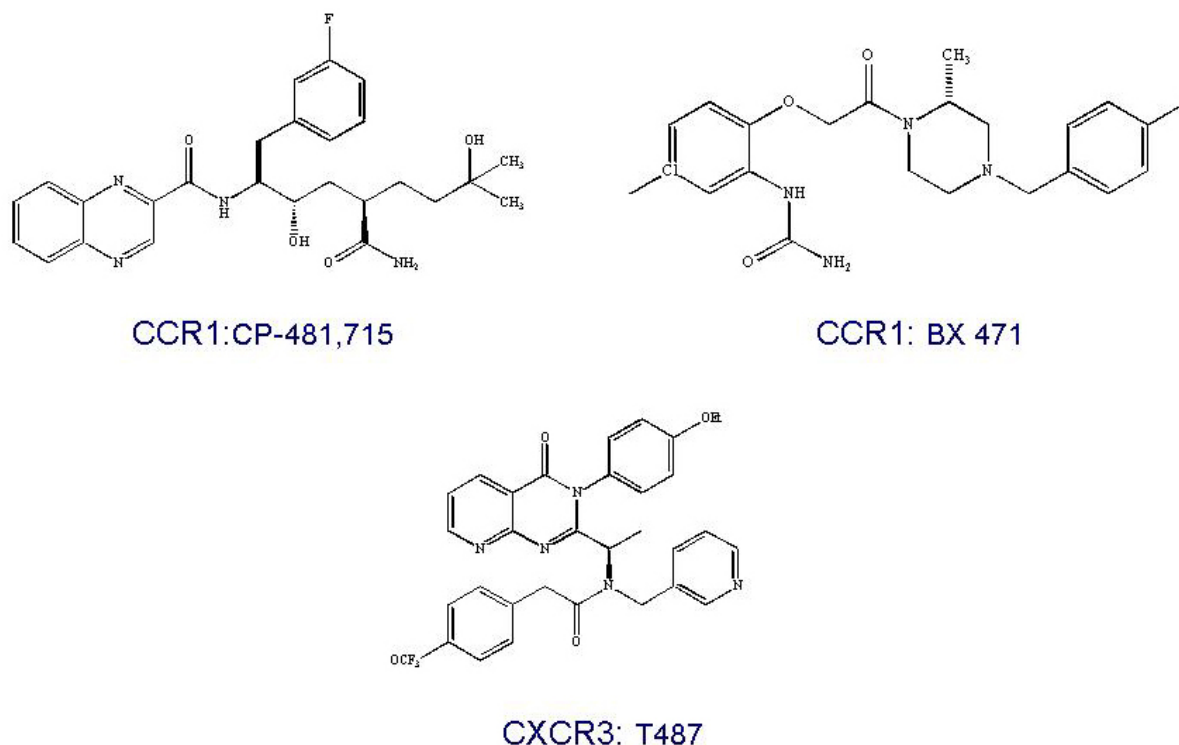


Figure 3. Structures of some chemokine receptor antagonists that have failed in clinical trials. CP-481,715 and BX 471 are specific CCR1 antagonists from Pfizer and Schering AG, and both failed to show efficacy for rheumatoid arthritis and multiple sclerosis, respectively in Phase II. T487 is a specific CXCR3 antagonist from Amgen/Turalik which failed in Phase IIa psoriasis clinical trials due to lack of efficacy.

but it is beyond the scope of this review to discuss them in detail, and we will discuss a representative selection.

In contrast to the positive experience of maraviroc, clinical trials of chemokine receptor antagonists for autoimmune diseases have so far been disappointingly unsuccessful. Several trials with small molecule antagonists have been reported (50) as well as trials with neutralizing antibodies. The Pfizer CCR1 antagonist CP-481,715 entered Phase II trials in February 2004. Data suggested that it did not exhibit efficacy in patients with rheumatoid arthritis after 6 weeks of treatment based on any sign of efficacy and the clinical trial was therefore terminated. The Berlex/Schering AG oral CCR1 antagonist BX 471 entered phase II clinical trials for multiple sclerosis in early 2004. The antagonist was administered orally to 105 patients with relapsing/remitting MS in a 16-week, randomized, double-blind, placebo-controlled trial. The primary endpoint was the cumulative number of newly active lesions on serial Magnetic Resonance Imaging (MRI) scans. Although the drug was well tolerated and showed no safety concerns its development was stopped after the clinical Phase II study failed to show a reduction in the number of new inflammatory CNS lesions detected by MRI (51). A CXCR3 antagonist from Amgen recently entered a Phase II trial for psoriasis but again the inhibitor unfortunately failed to demonstrate any signs of efficacy and the trial was terminated. Figure 3 shows the structures of these development candidates.

An anti-IL-8 antibody, ABX-IL8, has been tested in three indications, psoriasis, chronic obstructive pulmonary disease (COPD) and metastatic melanoma, but development was discontinued due to lack of efficacy. Although differences in breathing as assessed by the transition dyspnea index in the antibody treated group were greater than the placebo group in the COPD trial, there were no significant differences observed for lung function (52). It could be hypothesized that the reason for lack of efficacy of this antibody was that it does not recognize bound IL-8 (53) since immobilized chemokine on the endothelium is the active form *in vivo*. Development of another monoclonal, ABN912, targeting a ligand, MCP-1, has also been discontinued following insufficient clinical benefit in a trial on rheumatoid arthritis patients – this antibody resulted in an unexpected dose-related increase up to 2,000 fold in levels of free and bound MCP-1 (54). However, an anti-receptor antibody, MLN1012, is currently in Phase II trials for multiple sclerosis.

The reason for the clinical failures of the small molecule trials is not immediately obvious since only in the case of the Schering CCR1 inhibitor have any details of the clinical trials been reported. What is apparent, however, is that the discovery process for these three development candidates was similar. For example, all were low nM antagonists discovered through high throughput competition binding assays and the antagonists were able to inhibit receptor function monitored either by GTPγS,

calcium flux assays and chemotaxis (45,47). The complexity of autoimmune diseases, however, cannot be simulated with any known *in vitro* assays with successful outcomes. Chemotaxis although an important component of these diseases and an indicator of the ability of these compounds to inhibit migration of PBMCs (from patients or healthy individuals) towards a specific chemokine, certainly cannot predict the activity of these compounds *in vivo*. In contrast the successful chemokine antagonist maraviroc had the advantage that it blocked viral entry and this certainly is directly applicable to the clinical disease AIDS.

The reason for the failure of the Schering CCR1 clinical trial is not obvious. It was determined that the drug was present in sufficient quantity to fully inhibit the clinical biomarker CD11b ruling out the reason for failure of this trial due to the bioavailability of the molecule itself. Could failure therefore be related to the role of the receptor CCR1 in the pathogenesis of the disease targeted? The heterogeneity of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis coupled with the promiscuity of chemokines and their receptors, could therefore play some role in the failure of these therapeutic approaches. From animal models as well as human disease expression data, it has been shown that CCR1, CCR2, CCR5 and CXCR3 are all implicated in the pathophysiology of multiple sclerosis. Thus depending on the expression and activation of these receptors in the disease, inhibition of a single receptor may not be sufficient to show efficacy.

As we have discussed above the regulation and expression of these receptors can be influenced by a variety of factors and since specific biomarkers to predict the stage of the disease and receptor expression during disease progression are not available it might be more effective to target multiple chemokine receptors to obtain efficacy. The question can be addressed from an evolutionary point of view – as discussed above viruses have developed chemokine binding proteins with broad specificity in that they inhibit a wide range of chemokines. A recent report demonstrates the effectiveness of targeting multiple chemokines/ receptors (55). In this study, a model of intimal hyperplasia in response to femoral arterial injury was examined in transgenic mice that were induced to express M3, a herpesvirus protein that binds and inhibits multiple chemokines of all classes. Induction of M3 expression resulted in a 67% reduction in intimal area and a 68% reduction in intimal/medial ratio after femoral artery injury. In contrast, to these data, targeted deletion of CCL2 alone resulted in only a 29% reduction in intimal hyperplasia (56) suggesting that multiple chemokines and receptors are involved in the pathophysiology. Consistent with these results, in a mouse dextran sodium sulfate (DSS) model of colitis TAK-779, a dual antagonist of CC chemokine receptors CCR2 and CCR5, which also inhibits CXCR3, inhibited the recruitment of monocytes/macrophages into the colonic mucosa and delayed the onset disease (57).

It follows from the discussion above that the design of promiscuous chemokine receptor antagonists that

target several receptors could be quite advantageous in a number of situations, for example, joint CXCR4 and CCR5 inhibitors would be advantageous over a single receptor inhibitor, by circumventing viral escape mutants (58), and therefore more useful therapeutically as fusion inhibitors to treat AIDS patients. Whether such an approach is feasible obviously depends on the similarity of the binding pockets of the receptors of interest. A positive example of this type of approach is provided by dual antagonists of the angiotensin II receptor AT1 and the endothelin receptor ETA, which should be of greater benefit in the treatment of pulmonary hypertension, congestive heart failure and arteriosclerosis. Although the overall sequence identity of AT1 and ETA is low, only 19%, it has proven to be possible to design antagonists that bind both receptors with high affinity in the low nM range (59).

In line with this idea, targeting multiple chemokine receptors with one drug is certainly feasible. We already know that small molecule drugs can target CCR1 and CCR3 and several classes of drugs can target both CCR2 and CCR5. From this simple analysis it really is only a simple step away from designing small molecules that can target more than one chemokine receptor either by standard high throughput screening or even through drug design approaches (60). It is standard practice in the pharmaceutical industry that during the screening of a biological targets we often find drugs that hit more than one target, the tendency in the past has been for the medicinal chemist to design away from this to identify a compound that only hits one specific target. Perhaps it is now time to reconsider this approach in favor of new paradigms. as Brian Roth put it: “go for a magic shotgun approach rather than a magic bullet” (61). In closing, we should consider a successful example of this approach, the drug Olanzapine which targets 14 different GPCRs and was one of the most successful blockbuster drugs in its class (62).

7. PROSPECTS FOR THE FUTURE

The heterogeneity of autoimmune diseases could be one of the root causes for the failure of many of the current therapeutic approaches that are aimed at treating them. The success of any drug as a potential therapeutic will thus in the end depend on being able to develop clinical markers that more accurately delineate these different subtypes of disease. Failure to do so will lead not only to poorly designed clinical trials in which potentially useful therapeutic approaches fail, but also to a continuing major unmet medical need. In addition, to this the pharmaceutical industry as a whole will also need to invest heavily in studies aimed at obtaining a more complete understanding of how the chosen drug target is involved in mediating disease in the animal models that are used to gauge the efficacy of the drug and how this correlates to the human disease that the drug is supposed to treat. Careful attention to these points are not only important if chemokine receptor antagonists are ever to reach their potential as marketed drugs but also for the future of drug discovery as a whole. To this end, different approaches in this area of research including promiscuous antagonists that target more than one receptor may be needed if we are to

see chemokine receptor antagonists become the valuable new therapeutics that they were initially promised to be. However, we should not exclude that for reasons that are not yet apparent, chemical entities that block a single receptor may be efficacious, the evidence of which will become apparent as more clinical trials are conducted. Finally, chemokine receptors may have their place in diseases in which a single receptor plays a predominant role, such as CCR9 which targets T cells to the intestine – the ongoing trials conducted by ChemoCentryx /GSK will answer this question.

8. REFERENCES

- Rollins, B.J. Chemokines. *Blood* 90:909-928 (1997)
- Luster, A.D. Chemokines--chemotactic cytokines that mediate inflammation. *New England Journal of Medicine* 338:436-445. (1998)
- Moser, B., M. Wolf, A. Walz, and P. Loetscher. Chemokines: multiple levels of leukocyte migration control. *Trends Immunol.* 25:75-84. (2004)
- Zlotnik, A. and O. Yoshie. Chemokines: a new classification system and their role in immunity. *Immunity* 12:121-127. (2000)
- Sorensen, T.L., M. Tani, J. Jensen, V. Pierce, C. Lucchinetti, V.A.Q. Folcik, J. Rottman, F. Sellebjerg, R.M. Strieter, and J.L.R. Frederiksen. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest.* 103:807-815. (1999)
- Balashov, K.E., J.B. Rottman, H.L. Weiner, and W.W. Hancock. CCR5 (+) and CXCR3 (+) T cells are increased in multiple sclerosis and their ligands MIP-1 alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. U.S.A.* 96:6873-6878. (1999)
- Lilly, C.M., H. Nakamura, H. Kesselman, C. Nagler-Anderson, K. Asano, E.A. Garcia-Zepeda, M.E. Rothenberg, J.M. Drazen, and A.D. Luster. Expression of eotaxin by human lung epithelial cells - induction by cytokines and inhibition by glucocorticoids. *J. Clin. Invest.* 99:1767-1773. (1997)
- Holgate, S.T., K.S. Bodey, A. Janezic, A.J. Frew, A.P. Kaplan, and L.M. Teran. Release of RANTES, MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. *Am J Respir Crit Care Med* 156:1377-1383. (1997)
- Brown, J.R., J. Kleimberg, M. Marini, G. Sun, A. Bellini, and S. Mattoli. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin. Exp. Immunol.* 114:137-146. (1998)
- Berahovich, R.D., Z. Miao, Y. Wang, B. Premack, M.C. Howard, and T.J. Schall. Proteolytic activation of alternative CCR1 ligands in inflammation. *J. Immunol.* 174:7341-7351. (2005)
- Kennedy, K.J., R.M. Strieter, S.L. Kunkel, N.W. Lukacs, and W.J. Karpus. Acute and relapsing experimental autoimmune encephalomyelitis are regulated by differential expression of the CC chemokines macrophage inflammatory protein-1alpha and monocyte chemoattractant protein-1. *J. Neuroimmunol.* 92:98-108. (1998)
- Gonzalo, J.A., C.M. Lloyd, J.P. Albar, D. Wen, T.N.C. Wells, A.E.I. Proudfoot, C. Martinez-A, T. Bjerke, A.J. Coyle, and J.C. Gutierrez-Ramos. The Coordinated Action of CC Chemokines in the Lung Orchestrates Allergic Inflammation and Airway Hyperresponsiveness. *J. Exp. Med.* 188:157-167. (1998)
- Hancock, W.W., B. Lu, W. Gao, V. Csizmadia, K. Faia, J.A. King, S.T. Smiley, M. Ling, N.P. Gerard, and C. Gerard. Requirement of the chemokine receptor CXCR3 for acute allograft rejection. *J. Exp. Med.* 192:1515-1520. (2000)
- Duong, T.T., J. St Louis, J.J. Gilbert, F.D. Finkelman, and G.H. Strejan. Effect of anti-interferon-gamma and anti-interleukin-2 monoclonal antibody treatment on the development of actively and passively induced experimental allergic encephalomyelitis in the SJL/J mouse. *J. Neuroimmunol.* 36:105-115. (1992)
- Panitch, H.S., R.L. Hirsch, A.S. Haley, and K.P. Johnson. Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet* 1:893-895. (1987)
- Glabinski, A.R., B. Bielecki, J.A. Kawczak, V.K. Tuohy, K. Selmaj, and R.M. Ransohoff. Treatment with soluble tumor necrosis factor receptor (sTNFR):Fc/p80 fusion protein ameliorates relapsing-remitting experimental autoimmune encephalomyelitis and decreases chemokine expression. *Autoimmunity* 37:465-471. (2004)
- Wiendl, H. and R. Hohlfeld. Therapeutic approaches in multiple sclerosis: lessons from failed and interrupted treatment trials. *BioDrugs.* 16:183-200. (2002)
- Rottman, J.B., A.J. Slavin, R. Silva, H.L. Weiner, C.G. Gerard, and W.W. Hancock. Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent. *Eur. J. Immunol.* 30:2372-2377. (2000)
- Hromas, R., P.W. Gray, D. Chantry, R. Godiska, M. Krathwohl, K. Fife, G.I. Bell, J. Takeda, S. Aronica, M. Gordon, S. Cooper, H.E. Broxmeyer, and M.J. Klemsz. Cloning and characterization of exodus, a novel beta-chemokine. *Blood* 89:3315-3322. (1987)
- Izikson, L., R.S. Klein, I.F. Charo, H.L. Weiner, and A.D. Luster. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J. Exp. Med.* 192:1075-1080. (2000)
- Huang, D.R., J. Wang, P. Kivisakk, B.J. Rollins, and R.M. Ransohoff. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1

- immune response in experimental autoimmune encephalomyelitis. *J.Exp.Med.* 193:713-726. (2001)
22. Peters,W. and I.F.Charo. Involvement of chemokine receptor 2 and its ligand, monocyte chemoattractant protein-1, in the development of atherosclerosis: lessons from knockout mice. *Curr.Opin.Lipidol.* 12:175-180. (2001)
23. Gu,L., Y.Okada, S.K.Clinton, C.Gerard, G.K.Sukhova, P.Libby, and B.J.Rollins. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol.Cell* 2:275-281. (1998)
24. Bagaeva,L.V., P.Rao, J.M.Powers, and B.M.Segal. CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. *J.Immunol.* 176:7676-7685. (2006)
25. Jose,P.J., D.A.Griffiths-Johnson, P.D.Collins, D.T.Walsh, R.Moqbel, N.F.Totty, O.Truong, J.J.Hsuan, and T.J.Williams. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J.Exp.Med.* 179:881-887. (1994)
26. Ying,S., D.S.Robinson, Q.Meng, J.Rottman, R.Kennedy, D.J.Ringler, C.R.Mackay, B.L.Daugherty, M.S.Springer, S.R.Durham, T.J.Williams, and A.B.Kay. Enhanced expression of eotaxin and ccr3 mRNA and protein in atopic asthma - association with airway hyperresponsiveness and predominant colocalization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur.J.Immunol.* 27:3507-3516. (1997)
27. Nakamura,H., S.T.Weiss, E.Israel, A.D.Luster, J.M.Drazen, and C.M.Lilly. Eotaxin and impaired lung function in asthma. *Am J Respir Crit Care Med* 160:1952-1956. (1999)
28. Humbles,A.A., C.M.Lloyd, S.J.McMillan, D.S.Friend, G.Xanthou, E.E.McKenna, S.Ghiran, N.P.Gerard, C.Yu, S.H.Orkin, and C.Gerard. A critical role for eosinophils in allergic airways remodeling. *Science* 305:1776-1779. (2004)
29. Lee,J.J., D.Dimina, M.P.Macias, S.I.Ochur, M.P.McGarry, K.R.O'Neill, C.Protheroe, R.Pero, T.Nguyen, S.A.Cormier, E.Lenkiewicz, D.Colbert, L.Rinaldi, S.J.Ackerman, C.G.Irvin, and N.A.Lee. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 305:1773-1776. (2004)
30. Blanchard,C., N.Wang, K.F.Stringer, A.Mishra, P.C.Fulkerson, J.P.Abonia, S.C.Jameson, C.Kirby, M.R.Konikoff, M.H.Collins, M.B.Cohen, R.Akers, S.P.Hogan, A.H.Assaad, P.E.Putnam, B.J.Aronow, and M.E.Rothenberg. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J.Clin.Invest* 116:536-547. (2006)
31. Zhang,Y. and B.J.Rollins. A dominant negative inhibitor indicates that monocyte chemoattractant protein 1 functions as a dimer. *Mol.Cell.Biol.* 15:4851-4855. (1995)
32. Gong,J.H., M.Ugucioni, B.Dewald, M.Baggiolini, and I.Clark-Lewis. RANTES and MCP-3 antagonists bind multiple chemokine receptors. *J.Biol.Chem.* 271:10521-10527. (1996)
33. Proudfoot,A.E.I., C.A.Power, A.J.Hoogewerf, M.O.Montjovent, F.Borlat, R.E.Offord, and T.N.C.Wells. Extension of recombinant human RANTES by the retention of the initiating methionine produces a potent antagonist. *J.Biol.Chem.* 271:2599-2603. (1996)
34. Simmons,G., P.R.Clapham, L.Picard, R.E.Offord, Rosenkilde, MM, T.W.Schwartz, R.Buser, T.N.C.Wells, and A.E.I.Proudfoot. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* 276:276-279. (1997)
35. Lucas,A. and G.McFadden. Secreted immunomodulatory viral proteins as novel biotherapeutics. *J.Immunol.* 173:4765-4774. (2004)
36. Alcami,A. New insights into the subversion of the chemokine system by poxviruses. *Eur.J.Immunol.* 37:880-883. (2007)
37. Hajnicka,V., P.Kocakova, M.Slavikova, M.Slovak, J.Gasperik, N.Fuchsberger, and P.A.Nuttall. Anti-interleukin-8 activity of tick salivary gland extracts. *Parasite Immunol.* 23:483-489. (2001)
38. Hajnicka,V., I.Vancova, P.Kocakova, M.Slovak, J.Gasperik, M.Slavikova, R.S.Hails, M.Labuda, and P.A.Nuttall. Manipulation of host cytokine network by ticks: a potential gateway for pathogen transmission. *Parasitology* 130:333-342. (2005)
39. Smith,P., R.E.Fallon, N.E.Mangan, C.M.Walsh, M.Saraiva, J.R.Sayers, A.N.McKenzie, A.Alcami, and P.G.Fallon. Schistosoma mansoni secretes a chemokine binding protein with antiinflammatory activity. *J.Exp.Med.* 202:1319-1325. (2005)
40. Frauenschuh,A., C.A.Power, M.Deruaz, B.R.Ferreira, J.S.Silva, M.M.Teixeira, J.M.Dias, T.Martin, T.N.Wells, and A.E.Proudfoot. Molecular cloning and characterization of a highly selective chemokine-binding protein from the tick Rhipicephalus sanguineus. *J.Biol.Chem.* 282:27250-27258. (2007)
41. Potzinger,H., E.Geretti, B.Brandner, V.Wabitsch, A.M.Piccinini, A.Rek, and A.J.Kungl. Developing chemokine mutants with improved proteoglycan affinity and knocked-out GPCR activity as anti-inflammatory recombinant drugs. *Biochem.Soc.Trans.* 34:435-437. (2006)
42. Hirsch,E., V.L.Katanaev, C.Garlanda, O.Azzolino, L.Pirola, L.Silengo, S.Sozzani, A.Mantovani, F.Altruda, and M.P.Wymann. Central role for G protein-coupled

phosphoinositide 3-kinase gamma in inflammation. *Science* 287:1049-1053. (2000)

43. Camps,M., T.Ruckle, H.Ji, V.Ardissone, F.Rintelen, J.Shaw, C.Ferrandi, C.Chabert, C.Gillieron, B.Francon, T.Martin, D.Gretener, D.Perrin, D.Leroy, P.A.Vitte, E.Hirsch, M.P.Wymann, R.Cirillo, M.K.Schwarz, and C.Rommel. Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat.Med.* 11:936-943. (2005)

44. Barber,D.F., A.Bartolome, C.Hernandez, J.M.Flores, C.Redondo, C.Fernandez-Arias, M.Camps, T.Ruckle, M.K.Schwarz, S.Rodriguez, A.Martinez, D.Balomenos, C.Rommel, and A.C.Carrera. PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat.Med.* 11:933-935. (2005)

45. Liang,M., M.Rosser, H.P.Ng, K.May, J.G.Bauman, I.Islam, A.Ghannam, P.J.Kretschmer, H.Pu, L.Dunning, R.M.Snider, M.M.Morrissey, J.Hesselgesser, H.D.Perez, and R.Horuk. Species selectivity of a small molecule antagonist for the CCR1 chemokine receptor. *Eur.J.Pharmacol.* 389:41-49. (2000)

46. Anders,H.J., V.Vielhauer, M.Frink, Y.Linde, C.D.Cohen, S.M.Blattner, M.Kretzler, F.Strutz, M.Mack, H.J.Grone, J.Onuffer, R.Horuk, Nelson, PJ, and D.Schlondorff. A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation. *J. Clin. Invest.* 109:251-259. (2002)

47. Gladue,R.P., L.A.Tylaska, W.H.Brisette, P.D.Lira, J.C.Kath, C.S.Poss, M.F.Brown, T.J.Paradis, M.J.Conklyn, K.T.Ogborne, M.A.McGlynn, B.M.Lillie, A.P.DiRico, E.N.Mairs, E.B.McElroy, W.H.Martin, I.A.Stock, R.M.Shepard, H.J.Showell, and K.Neote. CP-481,715, a potent and selective CCR1 antagonist with potential therapeutic implications for inflammatory diseases. *J.Biol.Chem.* 278:40473-40480. (2003)

48. Onuffer,J., M.A.McCarrick, L.Dunning, M.Liang, M.Rosser, G.P.Wei, Ng .H, and R.Horuk. Structure function differences in nonpeptide CCR1 antagonists for human and mouse CCR1. *J.Immunol.* 170:1910-1916. (2003)

49. de Mendonca,F.L., P.C.da Fonseca, R.M.Phillips, J.W.Saldanha, T.J.Williams, and J.E.Pease. Site-directed mutagenesis of CC chemokine receptor 1 reveals the mechanism of action of UCB 35625, a small molecule chemokine receptor antagonist. *J.Biol.Chem.* 280:4808-4816. (2005)

50. Pease,J.E. and R.Horuk. CCR1 antagonists in clinical development. *Expert.Opin.Investig.Drugs* 14:785-796. (2005)

51. Zipp,F., H.P.Hartung, J.Hillert, S.Schimrigk, C.Trebst, M.Stangel, C.Infante-Duarte, P.Jakobs, C.Wolf, R.Sandbrink, C.Pohl, and M.Filippi. Blockade of chemokine signaling in patients with multiple sclerosis. *Neurology* 67:1880-1883. (2006)

52. Mahler,D.A., S.Huang, M.Tabrizi, and G.M.Bell. Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. *Chest* 126:926-934. (2004)

53. Yang,X.D., J.R.Corvalan, P.Wang, C.M.Roy, and C.G.Davis. Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states. *J.Leukoc.Biol.* 66:401-410. (1999)

54. Haringman,J.J., D.M.Gerlag, T.J.Smeets, D.Baeten, B.F.van den, B.Bresnihan, F.C.Breedveld, H.J.Dinant, F.Legay, H.Gram, P.Loetscher, R.Schmouder, T.Woodworth, and P.P.Tak. A randomized controlled trial with an anti-CCL2 (anti-monocyte chemotactic protein 1) monoclonal antibody in patients with rheumatoid arthritis. *Arthritis Rheum.* 54:2387-2392. (2006)

55. Pyo,R., K.K.Jensen, M.T.Wiekowski, D.Manfra, A.Alcami, M.B.Taubman, and S.A.Lira. Inhibition of intimal hyperplasia in transgenic mice conditionally expressing the chemokine-binding protein M3. *Am.J.Pathol.* 164:2289-2297. (2004)

56. Kim,W.J., I.Chreshnev, M.Gazdoiu, J.T.Fallon, B.J.Rollins, and M.B.Taubman. MCP-1 deficiency is associated with reduced intimal hyperplasia after arterial injury. *Biochem.Biophys.Res.Comm.* 310:936-942. (2003)

57. Tokuyama,H., S.Ueha, M.Kurachi, K.Matsushima, F.Moriyasu, R.S.Blumberg, and K.Kakimi. The simultaneous blockade of chemokine receptors CCR2, CCR5 and CXCR3 by a non-peptide chemokine receptor antagonist protects mice from dextran sodium sulfate-mediated colitis. *Int.Immunol.* 17:1023-1034. (2005)

58. Mosier,D.E., G.R.Picchio, R.J.Gulizia, R.Sabbe, P.Poignard, L.Picard, R.E.Offord, D.A.Thompson, and J.Wilken. Highly potent RANTES analogues either prevent CCR5-using human immunodeficiency virus type 1 infection *in vivo* or rapidly select for CXCR4-using variants. *J.Virol.* 73:3544-3550. (1999)

59. Murugesan,N., Z.Gu, L.Fadnis, J.E.Tellew, R.A.Baska, Y.Yang, S.M.Beyer, H.Monshizadegan, K.E.Dickinson, M.T.Valentine, W.G.Humphreys, S.J.Lan, W.R.Ewing, K.E.Carlson, M.C.Kowala, R.Zahler, and J.E.Macor. Dual angiotensin II and endothelin A receptor antagonists: synthesis of 2'-substituted N-3-isoxazolyl biphenylsulfonamides with improved potency and pharmacokinetics. *J.Med.Chem.* 48:171-179. (2005)

60. Vaidehi,N., S.Schlyer, R.J.Trabanino, W.B.Floriano, R.Abrol, S.Sharma, M.Kochanny, S.Koovakat, L.Dunning, M.Liang, J.M.Fox, F.L.de Mendonca, J.E.Pease, W.A.Goddard, III, and R.Horuk. 2006. Predictions of CCR1 chemokine receptor structure and BX 471 antagonist binding followed by experimental validation. *J.Biol.Chem.* 281:27613-27620. (2006)

61. Roth,B.L., D.J.Sheffler, and W.K.Kroeze. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat.Rev.Drug Discov.* 3:353-359. (2004)

62. Schlyer,S. and R.Horuk. I want a new drug: G-protein-coupled receptors in drug development. *Drug Discovery Today* 11:481-493. (2006)

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