



Killing Two Birds with One Drug: A New Application for HIV-1 Cell Entry Inhibitors in the Treatment of Metastatic Cancer

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Abstract

The chemokine receptors *CCR5* and *CXCR4* serve as co-receptors for the human immunodeficiency virus 1 (HIV-1) and thus, are important cellular components during HIV-1 cell entry. In recent years, a new biological role for chemokine receptors has emerged in assisting the spread of primary tumors to distant secondary sites within the human body (metastasis). This review highlights some of the HIV-1 cell entry inhibitors (antagonists), which are currently in development and/or under evaluation in clinical trials, and discusses the therapeutic use of these new antagonists for the treatment of certain forms of metastatic cancer.

deletion mutant in the human *CCR5* gene (*CCR5Δ32*) that renders homozygotes highly resistant to HIV-1 infection⁸.

Chemokines and chemokine receptors

The chemokines belong to a large family of small, chemotactic cytokines, which play an important role in mediating inflammatory responses, leukocyte homing, and cell migration (chemotaxis) in specific types of leukocytes. Chemokines are divided into four subfamilies (CC, CXC, C, and CX3C) characterized by a distinct pattern of conserved cysteine residues^{1,2}. Chemokines mediate their biological effects by binding to chemokine receptors, a family of seven transmembrane (7TM) receptors, which belong to the G protein-coupled receptor (GPCR) superfamily (Figure 1). The chemokine receptor family is divided into two main subclasses, which consists of eleven CC (*CCR1-11*) and six CXC receptors (*CXCR1-6*), and also includes lymphotactin receptor *XCR1* and the fractaline receptor *CX3CR1*^{3,4}. The binding of chemokines (agonists) to the receptor triggers a series of intracellular events mediated by heterotrimeric G proteins, which ultimately leads to actin polymerization and chemotaxis (Figure 1)⁵.

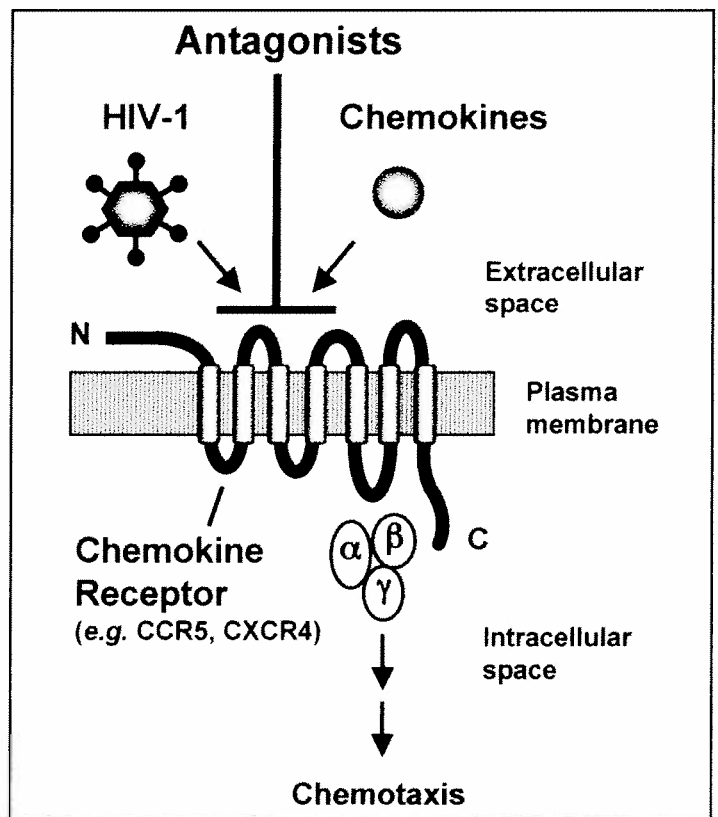
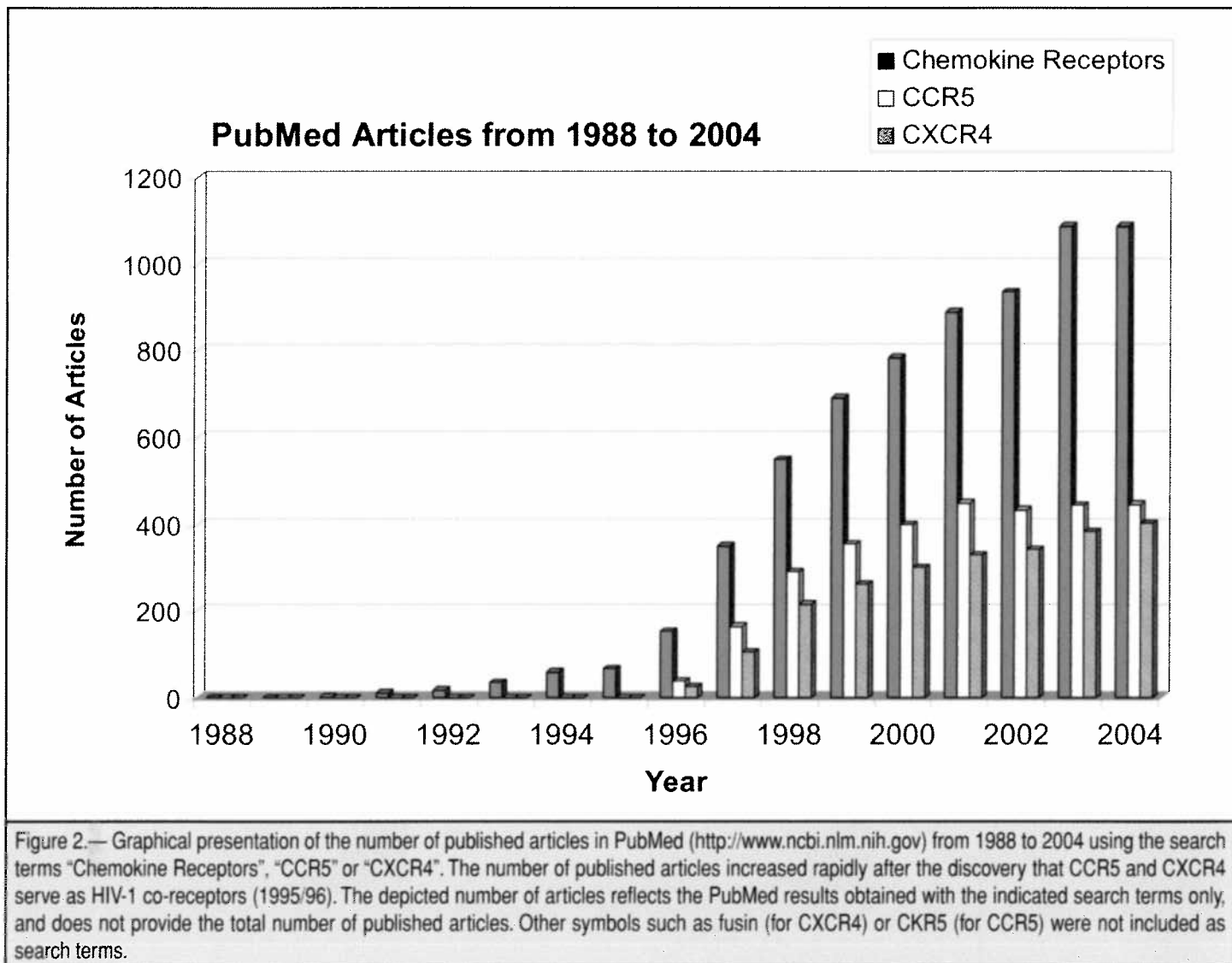


Figure 1.— Simplified diagram showing the proposed seven transmembrane-spanning topography of a chemokine receptor, which belongs to the G protein-coupled receptor (GPCR) superfamily. The chemokines are natural ligands of chemokine receptors and induce intracellular signaling through the heterotrimeric G proteins (α , β , and γ subunits). This ultimately leads to actin polymerization and cell migration (chemotaxis). The human immunodeficiency virus 1 (HIV-1) utilizes chemokine receptors *CCR5* and *CXCR4* as co-receptors, along with the primary receptor *CD4*, to enter the target cells. The recently developed HIV-1 cell entry inhibitors (chemokine receptor antagonists) could also prove useful in preventing chemokine-induced cell migration and immune cell infiltration in certain forms of metastatic tumors. C, Carboxy-terminus; N, Amino-terminus.

The role of chemokine receptors in HIV-1 cell entry

The human immunodeficiency virus 1 (HIV-1) enters its host cell by sequential interaction with receptor *CD4* and one of the chemokine receptors/HIV-1 co-receptors *CCR5* or *CXCR4* (Figure 1)^{6,7}. While R5-tropic HIV-1 strains initiate infection through interaction with *CCR5* and predominate during the period of clinical latency, the more virulent *CXCR4*-interacting X4-tropic HIV-1 strains are found late in infection and after AIDS symptoms appear⁸. However, many primary clinical isolates are dual-tropic and can use either *CCR5* or *CXCR4* to infect the host cells⁹. Although many other chemokine receptor subsets also have HIV-1 co-receptor activity *in vitro*, a function *in vivo* has primarily been demonstrated for *CCR5* and *CXCR4*. Most impressively, a clear role for *CCR5* in HIV-1 pathogenesis has been found through the discovery of a naturally occurring, 32-bp



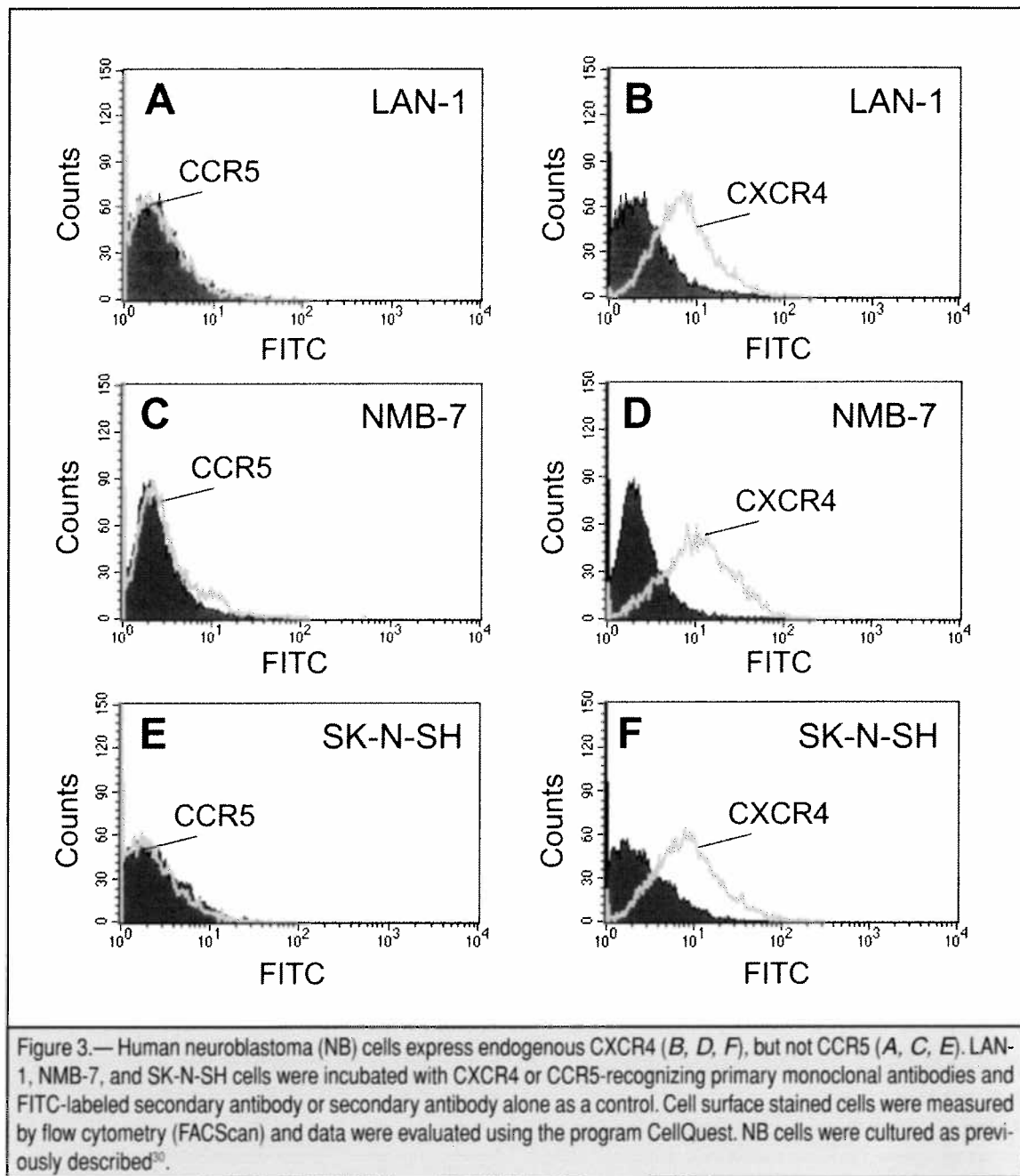
Development of new HIV-1 cell entry inhibitors

The recognition that chemokine receptors function as co-receptors and assist the cell entry of HIV-1 fueled the interest in this family of receptor proteins, which resulted in a significant increase in published literature after 1995 (Figure 2). The exploration of chemokine receptors as potential therapeutic targets followed soon after and led to the development of a number of HIV-1 cell entry inhibitors (*e.g.* TAK-779 and AMD3100) and derivatives (*e.g.* TAK-220 and AMD070), which are currently in development or entering clinical phase trials (Table 1)¹⁰⁻¹³. These antagonists specifically block the binding of the HIV-1 envelope protein (Env) to either CCR5 or CXCR4 and thus form a new generation of anti-HIV drugs, which may be used in combination with existing protease and reverse-transcriptase (RT) inhibitors to treat HIV-1 infection⁷. A number of pharmaceutical companies are leading in the discovery of cell entry inhibitors, including Schering Plough, Glaxo Smith-Kline, Pfizer, Millennium, Merck, Takeda, Novartis, and Astra Zeneca.

Receptor	Antagonist	Clinical Trial ^b	Comments
CCR5	TAK-779	Phase I	First non-peptidic inhibitor, discontinued
	TAK-220	Phase I	Derivative of TAK-779, orally bioavailable
	SCH-C	Phase I	Also called SCH 351125, orally bioavailable, discontinued
	SCH-D	Phase II	More potent in vitro than SCH-C, orally bioavailable
	AD101	NA	Also called SCH 350581, overlapping bindings sites with SCH-C
	E913	NA	Inhibits multidrug-resistant HIV-1, orally bioavailable
	CMPD 167	NA	Also called MRK-1, effective in SIV-infected rhesus macaques
	GW873140	Phase II	Orally bioavailable
	UK-427857	Phase II/III	Derivative of UK-107543, orally bioavailable
CXCR4	AMD3100	Phase II/III	Prototype, bicyclam
	AMD070	Phase I/II	Derivative of AMD3100, higher stability, orally bioavailable
	AMD3465	NA	Derivative of AMD3100
	KRH-1636	NA	Anti-HIV profile similar to AMD3100

^aReviewed by De Clercq (2005)^{10,11}, Horuk (2003)¹², and Ribeiro & Horuk (2005)¹³.

^bCurrent development stage according to published literature and/or disclosed information on the internet; NA, information on current development stage not available or status unclear.



A role for chemokine receptors in cancer metastasis

In many cancers, metastasis is the leading cause of mortality. Despite extensive research, the precise mechanisms by which cancer cells are disseminated to sites distant from the primary tumor are not fully understood. While the chemokine-mediated cell migration of leukocytes has long been known, it has only recently been found that tumor cells may utilize similar mechanisms during cancer metastasis¹⁴. It has been proposed that subsets of chemokine receptors are expressed by certain tumor cells and that specific chemokines are highly expressed at sites of cancer metastasis, thus suggesting that specific combinations of chemokines and chemokine receptors determine

the final destination of metastatic tumor cells¹⁵⁻¹⁷.

This concept is supported by several reports, which describe the expression of a distinct non-random pattern of subsets of functionally active chemokine receptors, such as CXCR3 and CXCR4 in human melanoma cells^{18,19}, CXCR4 in metastatic breast cancer cells²⁰, and CCR7 and CCR10 in skin metastases²⁰. Other evidence for the role of chemokine receptors in cancer metastasis include the findings that CCR5 is expressed on stromal cells and thereby promotes pulmonary metastasis²¹ and that the upregulation of CXCR4 is essential for HER2-mediated breast tumor metastasis²². Moreover, the relevance of chemokine receptors *in vivo* was demonstrated by using CXCR4-specific antibodies, which significantly reduced the

formation of lymph node and lung metastases in immunodeficient mice²⁰ and by studying CCR5 knockout mice (CCR5^{-/-}), which developed fewer metastases²¹.

Expression of chemokine receptor CXCR4 in human neuroblastoma cells

The expression of chemokine receptors was initially thought to be restricted to leukocytes. However, there is now clear evidence that they are also expressed in neurons²³ as well as neuroblastoma (NB) tumors^{24,25}. NB is a pediatric cancer, in which malignant cells form in the nerve tissue of the adrenal gland, neck, chest, or spinal cord. Metastatic dissemination of NB occurs by hemogenous and lymphatic pathways to lymph nodes, bone, and bone marrow. Recent studies reported the presence of endogenous CXCR4 in NB cells^{24,25}, and our own data with three human NB cell lines confirmed their observations (Figure 3). In contrast, we did not detect any CCR5 protein in these cells, which is in accordance with previously reported findings using the NB cell line SH-SY5Y, a subclone of SK-N-SH²⁶. A biological role for CXCR4 in the development of bone marrow metastases was proposed, thus further underlining the importance of CXCR4^{24,25}.

Potential application of HIV-1 cell entry inhibitors as anti-metastatic drugs

The recognition that CCR5 and CXCR4 play a crucial role in certain forms of cancer metastasis clearly suggests that HIV-1 cell entry inhibitors, which are generally safe and well tolerated in HIV-1 patients²⁷, should be exploited in clinical cancer trials. These anti-metastatic drugs may act in several ways: by preventing the metastatic spread of primary tumor cells, by inhibiting macrophage infiltrates, and by induction of chemokine receptor-mediated tumor cell arrest and programmed cell death (apoptosis)^{15,16}. To gain more insights into the role of CXCR4 my laboratory is interested in exploring the effects of HIV-1 cell entry inhibitors on the migration, differentiation, and apoptosis of human NB cells with the ultimate goal to develop novel therapeutics for high-risk NB patients.

Conclusions

It is intriguing to speculate that HIV-1 cell entry inhibitors could prove to be useful therapeutics for the prevention and treatment of metastasis of CCR5- or CXCR4-associated tumors. Furthermore, the development of antagonists that specifically block the function of other chemokine receptors will further contribute to formulating selective anti-metastatic therapies, which are likely to reduce unwanted side effects in patients. The screening of a larger number of cancer patients will be necessary to better define the expression pattern of chemokine receptors in metastatic tumors. Such information, combined with an effort to determine a cancer patient's individual chemokine receptor profile prior to treatment, will allow a more personalized patient care with improved therapeutic outcome. Finally, chemokines are also associated with a number of autoinflammatory diseases including multiple sclerosis, rheumatoid arthritis, diabetes, and endometriosis, further suggesting a wide range of therapeutic applications for chemokine receptor antagonists^{13,28,29}.

For more information about the Cancer Research Center of Hawaii, please visit our website at www.crch.org.

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Surfer's Medical Association: Conference in Biarritz, France

The first European meeting of the SMA will be held from September 30 to October 9, 2005. The conference would be of interest to physicians and allied health-care professionals who treat surfers as patients and/or who are surfers themselves.

The conference program will include presentations by all attendees to update the status of the health-care/surfing interface plus networking with international colleagues. Additional details are available on the SMA website (www.damoon.net) in the events section.

The conference registration fee of \$2,200 USD includes one oceanfront room (based on double occupancy) for ten nights, three meals per day, and ground transport each day to selected surf spots.

To enroll, make check payable to SMA:BIARRITZ and mail to 1330 Ala Moana Blvd., #2101, Honolulu, HI 96814. Questions may be directed to the conference chairperson, Dr. Bob Speers at speers@lava.net.

