Targeting Chemokine Receptors in HIV: A Status Report

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Abstract

Since the identification of CCR5 and CXCR4 as HIV coreceptors a little over a decade ago, there has been hope that coreceptor inhibitors will be able to make an impact on the HIV epidemic, both as novel therapeutic drugs and as agents used in prevention. Significant progress has been made in the understanding of how coreceptor choice might impact HIV pathology and how coreceptor blockade may affect health. In this review, we focus on some of the key issues that are emerging now that CCR5 has been validated as a promising target for HIV prevention strategies and at a time when a CCR5 inhibitor has been approved in the United States as the first in a new class of anti-HIV therapeutic drugs.

INTRODUCTION

According to the latest estimates (1), 39.5 million people are currently living with HIV/AIDS; this worldwide epidemic continues to grow at a rate of 4.3 million new infections and 2.9 million deaths per year. HIV coreceptor inhibitors are at the forefront of current research directed toward prevention of further spread of the epidemic and control of the disease in infected individuals. They are also of interest because they are the first anti-HIV drugs developed that target a host-encoded structure rather than a virus-encoded structure. In this article, we describe the emergence of the coreceptor inhibitor as a new anti-HIV drug class. We will then review the current status and future perspectives for the development of coreceptor inhibitors, paying attention to both preventative and therapeutic applications.

The Developed World: New Strategies for HIV Therapy

In the developed world, the HIV epidemic has largely been brought under control through the widespread adoption of effective HIV prevention methods, and while the disease remains incurable, infected individuals have access to highly effective drug combinations that provide long-term control of the disease. Nonetheless, current treatment strategies, which can give rise to significant side effects and against which resistant viral strains can emerge (2), are not considered optimal; the pharmaceutical industry is active in the development of new classes of anti-HIV drugs that could either replace or augment existing treatment regimens (3). HIV coreceptor inhibitors are among the new classes of drugs being brought forward; indeed, during the preparation of this review, maraviroc has become the first coreceptor inhibitor to be approved as an anti-HIV therapeutic (4).

The Developing World: HIV Prevention Strategies

Most of the HIV epidemic is taking place in the developing world (1), where young women are now most at risk of infection (5). In these regions, not only has it proved difficult to implement the same prevention approaches that were successful in the developed world, access to anti-HIV medicines remains very limited, and generating and sustaining a sufficient supply of effective medicines to such a large population of infected people represents an enormous economic and logistical challenge (6). As a consequence, the development of new prevention methods is considered a priority. Although an anti-HIV vaccine would be the ideal solution, progress toward the development of an effective strategy has been slow, and alternative prevention methods are being sought in parallel (7). Prominent among the prevention methods currently being evaluated is the prophylactic use of anti-HIV drugs to prevent transmission of HIV, either in preexposure prophylaxis (8, 9) or as part of topical prevention strategies, commonly referred to as microbicides (8, 10), and coreceptor inhibitors are prominent among the drugs currently being evaluated for these purposes.

CORECEPTOR INHIBITORS AND THE HIV ENTRY PROCESS

Coreceptor inhibitors block HIV at the level of entry into target cells, and as such, they make up part of a larger group of drugs known as HIV entry inhibitors (11). The HIV entry process is understood in some detail at the molecular level (**Figure 1**) (12). It is coordinated by the HIV envelope glycoprotein complex (**Figure 1**, inset), a trimer of three gp120 surface glycoproteins, each noncovalently attached to three gp41 transmembrane glycoprotein subunits. HIV must first engage CD4, a host cell surface protein, via the CD4 binding site on gp120 (**Figure 1*a***). Interaction between gp120 and CD4 induces a significant conformational change in the envelope glycoprotein, exposing a previously hidden site (**Figure 1*b***, indicated in yellow)

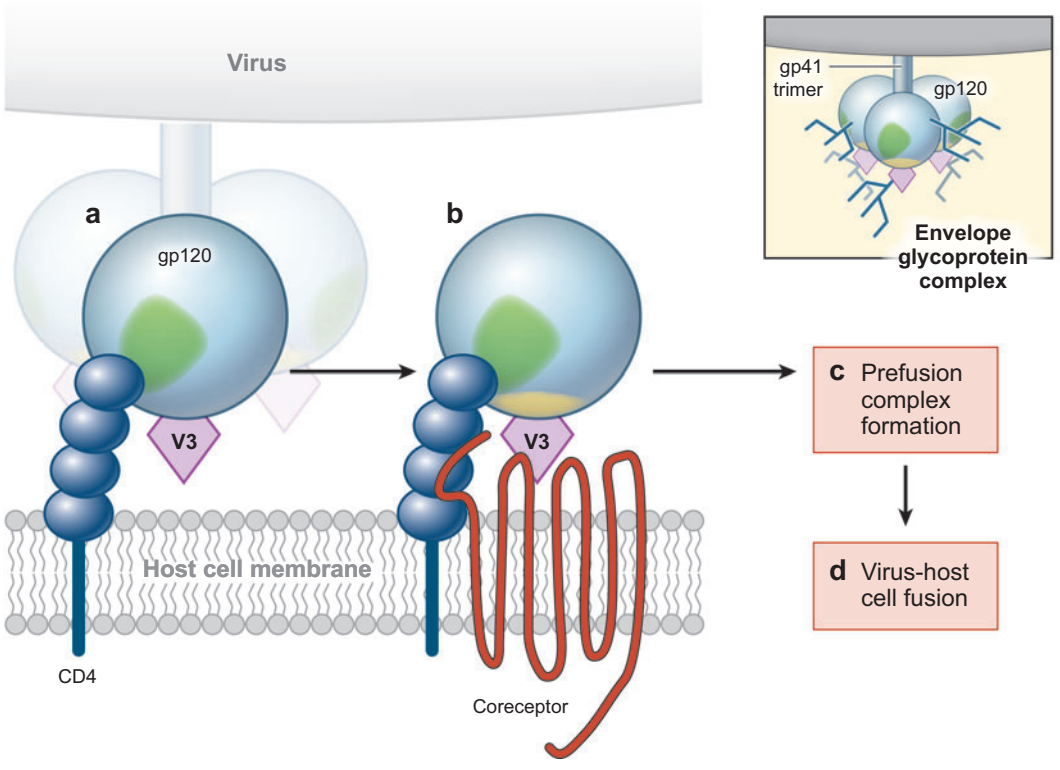

Figure 1

The HIV envelope glycoprotein complex (*inset*) drives viral entry into target cells. (*a*) HIV first engages CD4, a host cell surface protein, via the CD4 binding site on gp120 (*indicated in green*). (*b*) Interaction between gp120 and CD4 induces a conformational change in gp120, revealing the coreceptor binding site (*indicated in yellow*), that, together with the V3 region, is necessary for coreceptor engagement. (*c*, *d*) Events downstream of coreceptor engagement result in the fusion of viral and host cell membranes.

that, together with the V3 region, is necessary for coreceptor engagement (13–16). Binding to the coreceptor induces the insertion of gp41 into the host cell membrane, forming a molecular bridge between the virus and the host cell leading to the formation of a prefusion complex (**Figure 1*c***), which undergoes further conformational rearrangement that is sufficient to drive membrane fusion, allowing the contents of the virion to be ejected into the host cell (12, 17) (**Figure 1*d***).

THE HIV CORECEPTORS CCR5 AND CXCR4

Discovery of the Coreceptors

The mid-1990s saw a series of breakthroughs that led to the identification of the HIV coreceptors as chemokine receptors. In 1995, a group of anti-HIV "suppressive factors" were identified as the chemokine proteins MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 (18). Soon after, a pioneering functional screening study led to the identification of fusin as an HIV coreceptor (19). Fusin turned out to be the chemokine receptor that is now known as CXCR4, with its ligand, SDF-1/CXCL12, endowed with anticoreceptor activity (20, 21). The final key discovery in the series was made when a second chemokine receptor, CCR5, was identified as the HIV coreceptor whose activity is blocked by MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 (22–25). Hence, the HIV coreceptors were identified as chemokine receptors, with the cognate chemokine ligands acting as natural entry inhibitors.

Coreceptor Usage and HIV Tropism

Prior to the identification of the HIV coreceptors, differences in cellular tropism had been noted, and HIV isolates were classified according to whether they could replicate in laboratory T cell lines (T-tropic) or in macrophages (M-tropic). Coreceptor usage provided a molecular explanation for these phenotypes because the laboratory-derived T cell lines used to define the T-tropic phenotype express CXCR4 only, and hence will only support growth of viruses capable of using CXCR4 as a coreceptor. The macrophages used to define the M-tropic phenotype express both coreceptors but the levels of CXCR4 are low relative to CCR5, hence they strongly favor replication of viruses capable of using CCR5 as a coreceptor (26, 27). These observations led to the adoption of a new classification for HIV based on coreceptor usage (28), with R5 used to describe viruses that use CCR5 alone as a coreceptor and X4 to describe viruses that use CXCR4 alone. The term R5X4 was put forward to describe viruses capable of using either CCR5 or CXCR4 to enter target cells, but it is becoming evident that HIV isolates that consist entirely of genuine clonal viruses capable of using both coreceptors are rare, and many isolates that show an R5X4 phenotype are in fact mixtures of R5 plus X4 or, less commonly, either R5 plus dual (R5X4) plus X4 or R5 plus dual (R5X4) clones. Hence, the terminology that has been adopted for dual-tropic isolates is R5+X4 or D/M (dual/mixed) in cases where tropism has not been determined at the clonal level, with the term R5X4 reserved for clones genuinely

capable of using both coreceptors (29, 30). Coreceptor choice is determined exclusively by structures contained on the gp120 subunits of envelope glycoprotein complex, and a number of studies have shown that substitution of the V3 region alone is often sufficient to switch the coreceptor tropism of a viral clone from R5 to X4 and vice versa (reviewed in 31).

Coreceptors Other than CXCR4 and CCR5?

At the time that CCR5 and CXCR4 were identified as coreceptors, a number of articles were published suggesting that other chemokine receptors may function as HIV coreceptors (reviewed in 26, 27). Although these receptors showed HIV coreceptor activity when overexpressed in cell lines, subsequent work has shown that infection of primary cells via receptors other than CCR5 and CXCR4 is rare, and infection of primary cells can normally be fully blocked by either CCR5 or CXCR4 inhibitors (27, 32). Hence, at present, CCR5 and CXCR4 can be considered as being the only HIV coreceptors of physiological significance, and they are the only chemokine receptors against which anti-HIV drug development has been initiated so far.

CORECEPTORS AND HIV-HOST INTERACTIONS

CCR5 Is the Principal Coreceptor for HIV Transmission

The R5 phenotype is almost exclusively present in isolates or clones from primary infection (27, 33), irrespective of the route of infection, e.g., across genital mucosa, from mother to child, or via intravenous drug use (27). Factors limiting the establishment of infection by X4 viruses are not completely understood, although it has been suggested that suppression of X4 transmission may result from an aggregate of the partial inhibition of several different mechanisms at multiple levels, which together provide a highly stringent "gatekeeper" function (27, 34).

The key role of CCR5 as the principal coreceptor for HIV transmission has been underscored by epidemiological studies related to a null allele for CCR5, *CCR5Δ32* (35), which is prevalent in the Caucasian population (36). Several cohort studies (35, 37–41) have demonstrated that delta32 homozygosity provides an almost complete barrier against HIV acquisition. Although studies of the few known cases where *CCR5Δ32* homozygotes have been infected (42–45) indicate that infection by X4 viruses is not impossible, the available data support a model in which CCR5 has a central role in HIV transmission, and suggest that use of coreceptors to achieve CCR5 blockade might provide considerable protection from HIV transmission.

CCR5 Plays a Central Role After Infection

R5 viruses also tend to dominate in the early, asymptomatic years of infection (46). Recent studies of large cohorts of treatment-naïve (i.e., yet to receive any antiretroviral

therapy) HIV-infected individuals have shown that the vast majority (between 81% and 88%) have undetectable levels of D/M or X4 viruses (47–49). In contrast, D/M or X4 viruses are detectable in up to 50% of treatment-experienced patients (49–51), but even in these cases, it is uncommon for X4 viruses to dominate the overall viral load (46, 48, 49). The importance of CCR5 as a coreceptor after infection is underlined by epidemiological studies concerning genotypes that lead to lower CCR5 expression levels on target cells (39, 52), including *CCR5Δ32* heterozygosity, which indicate a link between reduction of CCR5 levels and slower disease progression. Hence, blockade of CCR5 is likely to be an effective strategy for HIV therapy.

X4 Viruses: A Clinical Concern

When emergence of detectable levels of X4 viruses does occur in nontreated individuals, it arises within approximately five years of initial infection (46). In other words, X4 viruses are almost completely absent during transmission and early stages of HIV disease and only emerge later in the disease course of a proportion of cases. Despite this apparently marginal role in HIV disease, X4 viruses represent a clinical concern because their emergence correlates with a more rapid progression to AIDS (48). However, it is not yet clear whether the emergence of X4 viruses is a cause or a consequence of this phenomenon. On one hand, X4 viruses are apparently more pathogenic than R5 viruses in vitro (53–55), and experiments in macaques suggest that they may also be more pathogenic in vivo (56, 57). Increased pathogenicity in vivo may relate to the ability of X4 viruses to target CD4 T cell subsets that are different from those available to R5 viruses (57); whereas naïve CD4 cells express high levels of CXCR4, but very little CCR5, CCR5 is upregulated in activated and memory cells (reviewed in 58). On the other hand, there is little doubt that R5 viruses alone are clearly capable of destroying the immune system, as demonstrated by the observation that approximately 50% of individuals progress to AIDS without the emergence of detectable levels of X4 viruses (48). It is therefore conceivable that X4 viruses are simply the less-fit cousins of R5 viruses, which can only emerge opportunistically in cases where destruction of the immune system by R5 viruses is almost complete (27). Work concerning both the causes and the consequences of X4 virus emergence is ongoing, and the subject remains a key issue in the development of coreceptor inhibitors.

PHYSIOLOGICAL ROLES OF CORECEPTORS

CCR5 and CXCR4 are chemokine receptors. The main physiological role of chemokines is to activate and induce the migration of leukocytes. As we know it today, the human chemokine–chemokine receptor system comprises 18 different receptors (59) and 41 different ligands (60). The chemokine ligands are small (8–10 kDa) proteins, and their receptors are seven-transmembrane-spanning G protein–coupled receptors (GPCRs).

Physiological Role of CCR5

The biology of CCR5 and the potential consequences of its inhibition have been covered in detail in a recent review (10). CCR5 is expressed not only on several effector T cell subsets but also on antigen-presenting cells, including macrophages, immature dendritic cells, and Langerhans cells. This expression profile indicates a likely role in both the initiation of adaptive immune responses and the trafficking of effector cells to sites of inflammation and infection.

Studies involving mouse CCR5 knockouts and human *CCR5Δ32* homozygotes have made it possible to draw inferences about the requirement of CCR5 for normal immune function and the possible consequences of its blockade. The earliest reports concerning *CCR5Δ32* homozygotes (37, 38) indicated that congenital absence of CCR5 does not lead to any overt pathology in humans. However, a number of subsequent studies have revealed a link between loss of the receptor and a relatively mild form of immunodeficiency that can nonetheless be exposed under certain circumstances (reviewed in 10, 61).

Although the effects of congenital absence of CCR5, where the immune system has had years to adapt to the loss of the receptor, do not necessarily equate to the results of acute pharmacological blockade, these data suggest that pharmacological blockade of CCR5 is likely to be largely well tolerated. Stringent safety studies will nonetheless be required to carefully monitor effects related to the use of CCR5 inhibitors, with particular attention paid to the consequences of infection by certain pathogens, effects on responses to immunization, and the emergence of opportunistic infections and malignancies (62).

CXCR4 Physiology

CXCR4 is a homeostatic chemokine receptor with a single known ligand, SDF-1/CXCL12. Both the receptor and its ligand appear to play an essential role in embryonic development, and this may explain why no CXCR4 null alleles, analogous to *CCR5Δ32*, have been discovered. In the adult, CXCR4 is expressed on a wide range of cell types and is a key player in several major processes, including hematopoiesis, leukocyte trafficking in the adaptive immune system, and vascularization (reviewed in 63). A number of CXCR4 inhibitors were identified soon after CXCR4 was identified as an HIV coreceptor (64), including a monoclonal antibody directed against CXCR4 (65), and AMD 3100, the first CXCR4 inhibitor to be clinically tested (66). Since these early breakthroughs, relatively little effort has been put into the clinical development of CXCR4 ligands as coreceptor inhibitors, however, and this is likely due to a combination of two factors: the apparently marginal role of X4 viruses in HIV disease and the likelihood that drugs would exhibit significant side effects related to blockade of the physiological function of CXCR4. With regard to the latter point, both of the CXCR4 inhibitors that have been tested in humans, AMD 3100 (66) and AMD 070 (67), caused significant leukocytosis, and it is perhaps significant that the envisaged use for AMD 3100 is now

in hematopoietic stem cell isolation rather than in HIV therapy. For the remainder of this review, we therefore focus on issues relating to the development of CCR5 inhibitors.

DEVELOPING CCR5 INHIBITORS FOR THERAPY AND PREVENTION: KEY CONCERNS

Before discussing the different kinds of CCR5 inhibitor that have been developed in more detail, it is relevant to address the differences in the requirements of drugs according to whether they are destined for use in therapy or prevention. Of key importance is the need to take into account the logistical, financial, and cultural dimensions of the HIV epidemic in the development of effective prevention strategies. These particular issues, which are outlined briefly below, have been described in detail elsewhere (see 8, 68, 69, and references therein).

Efficacy

For therapeutic applications, new drugs need to show demonstrable benefits compared with existing drug regimens. In prevention, although mathematical models suggest that a partially effective microbicide would be sufficient to make an impact on the HIV epidemic (70), higher potency is not only an advantage, but may be essential for demonstrating proof-of-concept in clinical trials (68).

Safety Profile

Because CCR5 inhibitors represent a new drug class that targets a host-encoded target, coreceptor inhibitors need to be carefully scrutinized not only for compound-specific toxicity, but also for class-specific toxicity, i.e., toxicity related to blocking the physiological function of CCR5. For prevention applications, where otherwise healthy people could be using the drug for the whole of their sexually active lives, tolerance thresholds for adverse side effects would be expected to be lower than for drugs used in therapy.

Additional Safety Concerns for Topical Microbicides

The failure of the first large-scale clinical trial of a microbicide candidate (the detergent product nonoxynol-9) led researchers to the stark conclusion that use of agents that can block HIV but which are also capable of damaging the integrity of genital mucosa will lead to enhanced susceptibility to HIV infection (71). It is therefore essential that any new drug under consideration for development as a microbicide show no capacity to elicit structural damage, cytotoxicity, or inflammation on the surfaces to which it will be applied. In addition to not damaging epithelial integrity, it is important that drugs destined for use in vaginal microbicide products do not

interfere with the vaginal environment, which in itself provides a natural defensive barrier (68, 69).

Escape Mutants

As with all anti-HIV drugs used in therapy, mechanisms of viral resistance need to be understood, and effective tests to detect the emergence of escape mutants in treated individuals must be devised. In prevention, the major risk of escape is at the level of the population, namely that a population using a drug for prevention will become exposed to resistant viruses, thereby compromising or even negating its protective effect (69).

Bioavailability

The ability to achieve oral dosing is a major goal in most drug development strategies, including those directed against HIV, and indeed all but one of the currently licensed therapeutic anti-HIV drugs is orally bioavailable. Oral bioavailability is not necessary for topical microbicides, and may even be a disadvantage: The physical properties of a drug that give rise to oral bioavailability, i.e., facility to cross cell membranes, would also be expected to generate a certain level of systemic exposure after topical application. Such exposure is intrinsically undesirable and could also lead to the development and spread of resistant viruses in people who do not know they are infected (69).

Drug-Drug Interactions

Because it is likely that CCR5 antagonists developed for therapy will be used to complement existing therapeutic regimens, the issue of drug-drug interactions, both at the level of efficacy (e.g., synergy, additive effect, antagonism) and metabolism, needs to be considered. Similar issues will apply to CCR5 antagonists implicated in prevention strategies involving cocktails of different inhibitors.

X4 Viruses

In a therapeutic setting, R5 viruses could potentially escape from CCR5 inhibitors by switching coreceptor use to CXCR4. Alternatively, suppression of R5 viruses could lead to breakthrough of X4 viruses. In prevention strategies, the key concern is the potential for transmission, rather than selection or emergence of X4 viruses.

Stability

For drugs used in prevention to make an impact on the HIV epidemic, they will need to be effective after storage in tropical countries where a cold chain cannot be guaranteed. Hence stability, in particular to storage at elevated temperature, is a key concern.

Acceptability

Prevention strategies that show high levels of efficacy will not make an impact on the HIV epidemic unless they are widely acceptable. Coital independence, i.e., having the option of administering the drug at any time, not necessarily immediately prior to sex, is likely to be key to the acceptability of new HIV prevention strategies (8, 68, 69). Coitally independent protection could presumably be achieved by preexposure prophylaxis strategies (8), but strategies based on topical microbicides will need to be carefully devised to ensure that long-term protection can be achieved after a single dose (68, 69).

Affordability

To make an impact on the HIV epidemic, drugs destined for use in prevention strategies will need to be provided continuously over many years to a population of tens of millions of people. Clearly, drugs that are too expensive to produce will not make suitable candidates for development, and production costs of lower than $1 per dose have been put forward as a theoretical limit for feasibility (68).

CHEMOKINE ANALOGS

A First Generation of Coreceptor Inhibitors

The native chemokine ligands of CCR5 function as natural HIV entry inhibitors in vitro (18), and this is likely to be the case in vivo as well, because it has been shown that increased expression of MIP-1α/CCL3 in humans, which occurs as a result of gene duplication, also provides protection from HIV acquisition (72). Could the natural chemokines themselves be used as anti-HIV drugs? Concern has been expressed about adverse effects that could potentially be elicited by an agonist of an inflammatory receptor like CCR5 (73), as well as the limited efficacy of native CCR5 ligands (74).

Chemokines can be engineered to enhance their natural anti-HIV properties (reviewed in 75). The first engineered anti-HIV chemokine to be described was an N-terminally truncated variant of RANTES/CCL5 with modest anti-HIV activity, RANTES (9–68, 76). RANTES (9–68) is a CCR5 antagonist (77), and its identification as an HIV entry inhibitor helped establish the concept that the agonist activity on coreceptors is not a component of the anti-HIV mechanism of native chemokines. Soon after, AOP-RANTES, a RANTES/CCL5 variant carrying a hydrophobic N-terminal extension, was the first coreceptor inhibitor with significantly improved potency over the native chemokines to be described (78). Unlike native RANTES, AOP-RANTES proved to be effective in blocking infection of macrophages as well as T cells.

Unusual Inhibitory Mechanisms

The inhibitory mechanism of native chemokines was shown to be determined by two factors: occupancy of the receptor, resulting in steric blockade (**Figure 2**) (79),

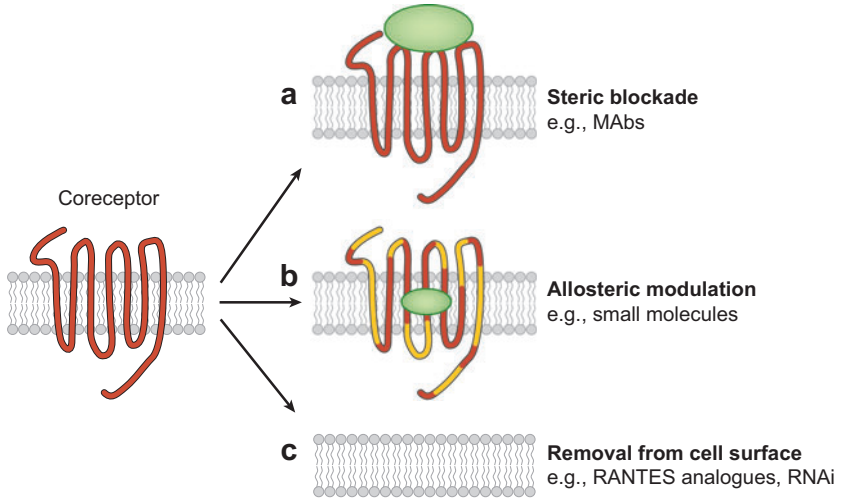

Figure 2

Inhibitory mechanisms of CCR5 inhibitors. Inhibition can either be achieved by (*a*) steric blockade of the coreceptor, i.e., engaging the receptor and thereby blocking viral access; (*b*) allosteric inhibition, i.e., altering the conformation of the receptor (*indicated by yellow/red hatching*) so that it is not usable as a coreceptor; or (*c*) removal of the receptor from the cell surface, either by inducing receptor sequestration or by blocking receptor expression.

and intracellular sequestration, resulting in physical removal of the coreceptor from where it can be accessed by the envelope glycoprotein (**Figure 2**) (80, 81). Receptor sequestration is a consequence of agonist-induced receptor internalization, a component of the desensitization/resensitization process that is broadly conserved across the superfamily of GPCRs (82). N-terminally modified chemokine analogs with potent anti-HIV activity, such as AOP-RANTES (78) and PSC-RANTES (83), have been shown to act via induction of receptor sequestration that is more profound and more prolonged than that seen with the native chemokines (83–85). This inhibitory mechanism may present certain advantages for HIV inhibition. First, it might provide a significant barrier to the generation of escape mutants, and second, it may lead to long-term protection from infection after a single dose. However, all of the potent anti-HIV chemokines that have been described so far also behave as CCR5 agonists (75, 86).

Issues Related to the Clinical Development of Chemokine Analogues as Anti-HIV Drugs

As proteins, chemokines would not be expected to be orally bioavailable, and because they readily bind to and form aggregates on cell surface proteoglycans (87), they are likely to show unsuitable pharmacokinetics after injection. Hence, it is unlikely that chemokine analogues would be suitable for use as anti-HIV therapeutics unless the

capacity to bind to proteoglycans could be engineered out without affecting anti-HIV activity (75). Concerns related to bioavailability would not hinder the development of chemokine analogues as topical microbicides, however, and their unsuitability for development as therapeutics may actually be an advantage with respect to the generation and spread of escape mutants. Another major concern relates to the production costs of proteins, in particular those that cannot be produced directly by biosynthesis (68, 69, 88). Finally, if chemokine analogs with CCR5 agonist activity are to be taken forward into clinical development, they will need to be monitored not only for the potential for adverse effects via inhibition of the physiological role of CCR5 but also for possible inflammatory activity mediated through CCR5 activation.

SMALL-MOLECULE CORECEPTOR INHIBITORS

Small-Molecule CCR5 Inhibitors

The first small-molecule CCR5 antagonist described in the literature was TAK-779 (89). Since then, a number of other pharmaceutical companies have added to a growing range of small-molecule CCR5 inhibitors (**Table 1**), some of which have entered clinical trials, and one, maraviroc from Pfizer, has recently been approved for clinical use.

Inhibitors Occupy a Common Binding Pocket on CCR5

Site-directed mutagenesis studies and molecular modeling of CCR5 have identified a putative binding site that is shared by several small-molecule CCR5 inhibitors in a cavity between the transmembrane helices on the extracellular face of CCR5 (90–95). Competitive binding studies indicate that this site is shared by various small-molecule inhibitors (95, 96). It does not overlap with the binding site for HIV gp120, which is located on the extracellular domains of CCR5 (93).

Table 1 Selected small molecule CCR5 inhibitors that have been evaluated in vitro

Compound	Comments
CMPD-167 (127, 142, 144, 147)	From Merck; used in proof-of-principle studies in macaques; licensed to the International Partnership for Microbicides for development as a topical microbicide.
TAK-779 (89)	From Takeda; first in class; no longer in clinical development
TAK-220 (150)	From Takeda; in clinical development
TAK-625 (151)	From Takeda; in clinical development
AD101 (119)	From Schering-Plough; no longer in clinical development
SCH-C (152)	From Schering-Plough; no longer in clinical development
Vicriviroc (153)	From Schering-Plough; in Phase 2/3 clinical trials
Aplaviroc (99)	From GlaxoSmithKline; Phase 2/3 trials halted due to hepatotoxicity
Maraviroc (132)	From Pfizer; recently approved in the United States for treatment-experienced patients

CCR5 Inhibitors Act Via Allosteric Mechanisms

The GPCR superfamily is now well known for its susceptibility to allosteric modulation (97), and three principle lines of evidence suggest that small-molecule CCR5 inhibitors act against HIV through an allosteric mechanism, i.e., induction or stabilization of a conformation of CCR5 that is not compatible with HIV gp120 binding (**Figure 2**). First, the binding sites on CCR5 for the inhibitors do not overlap with that of the virus; second, detailed biochemical studies have shown that the small molecules block engagement of CCR5 by chemokines via a mechanism that is noncompetitive (96); and third, certain mutations introduced into CCR5 will allow some inhibitors to bind to CCR5 but prevent them from inhibiting coreceptor activity, presumably by preventing conformational changes (98).

Several Different Ways to Achieve Allosteric Inhibition of CCR5

The subtleties of allosteric inhibition of CCR5 have been revealed by analysis of the properties of certain CCR5 mutants. The CCR5 I198M variant prevents SCH-C from inhibiting HIV infection, but does not disrupt its ability to block signaling mediated by RANTES/CCL5, nor affect the anti-HIV activity of another small-molecule inhibitor, AD101 (98). At the same time, another variant, CCR5 F113A, blocks inhibition by AD101 but does not affect the inhibitory activity of SCH-C (98). Different inhibitors can also show selective effects on different CCR5 ligands. Whereas most CCR5 inhibitors are potent antagonists of all of the natural CCR5 ligands, aplaviroc efficiently antagonizes MIP1α/CCL3 binding, but is much less potent against MIP1β/CCL4 or RANTES/CCL5 (96, 99). Hence, whereas all the small-molecule CCR5 inhibitors tested appear to occupy overlapping binding pockets on the receptor and act via a similar mechanism, the conformational details of the inhibitory effect may differ between molecules. These differences are significant because they may affect the extent to which signal transduction through CCR5 is suppressed as well as having an impact on the nature of the escape mutants that are selected in the presence of a given inhibitor.

ANTI-CCR5 ANTIBODIES

Monoclonal antibodies are becoming an increasingly predominant drug class, with applications in a wide range of diseases (100). Although intact antibodies are relatively expensive to produce and normally need to be administered via injection, their beneficial features include typically long circulatory half-lives and a lack of compound-specific toxicity. Many mouse anti-CCR5 monoclonal antibodies (MAbs) with anti-HIV activity have been identified and extensively characterized for their binding sites on CCR5 and their capacity to modulate receptor pharmacology (101–104).

Binding Site and Mechanism of Action

The footprint of an antibody binding site is sufficiently large to ensure overlap with the gp120 binding site on CCR5. It is therefore probable that the inhibitory mechanism

of anti-CCR5 MAbs involves steric blockade of the receptor (**Figure 2**). It should be noted, however, that there is no apparent correlation between the potency with which a number of different anti-CCR5 MAbs inhibit the binding of gp120:sCD4 complexes to CCR5 and the potency with which they inhibit the infection process (101, 102). This may be due to experimental conditions (e.g., the use of monomeric, not virion-associated gp120), but a recent study has indicated that one anti-CCR5 MAb may function at a step that occurs after CCR5 engagement by the virus (105). None of the inhibitory MAbs have proven to be CCR5 agonists or induce internalization of CCR5 (101, 102, 104). The majority have been described as chemokine antagonists (101, 102, 106), although exceptions with relatively weak antagonist properties for some chemokines have been noted (101, 104, 106).

Human and Humanized Antibodies: Genuine Clinical Candidates for Therapy

Although rodent antibodies are useful for studying inhibitory mechanisms, they are not suitable for use in therapy because of the human antimouse antibody response (100). More recently, human [CCR5mAb004 (107)] and humanized [PRO 140 (108)] anti-CCR5 antibodies have been developed. These antibodies, which have been tested clinically, are discussed in more detail below.

Suitability for Use in Prevention

The production costs of intact MAbs, as well as their need for cold chain storage, makes them somewhat unsuitable candidates for development as candidates for use in prevention (68, 69), but the use of plants to produce intact MAbs "plantibodies" (108a), or yeast to produce antibody fragments (109) have been proposed as routes to large-scale, low-cost production.

RNA INTERFERENCE

RNA interference (RNAi) is an evolutionarily conserved posttranscriptional gene-silencing mechanism in which small interfering double-stranded RNA (siRNA) mediates sequence-specific degradation of mRNA (110, 111). Exogenously delivered siRNA is capable of triggering RNAi in mammalian cells, and this phenomenon has been exploited by several groups to generate strategies to block HIV replication (reviewed in 112). One such strategy involves RNAi targeting of CCR5, an approach that has been shown to provide long-duration protection of differentiated primary target cells for HIV via the reduction of cell surface levels of coreceptor (**Figure 2**) (113, 114).

Key Issues: Delivery, Acceptability, and Cost

As is the case for other CCR5 inhibition strategies, RNAi targeting of CCR5 could lead to class-specific toxicity, but an inhibitory mechanism that involves reducing

levels of CCR5 at the cell surface may present a formidable barrier against the development of resistant escape mutants. RNAi strategies could potentially be used for both therapy and prevention, although efficient delivery systems will be key to success, particularly for therapeutic strategies where systemic exposure is required. In this regard, viral vector-mediated delivery of siRNA targeted to CCR5 has been demonstrated in vitro (114), but gene delivery approaches using viral vectors remain largely conceptual and may be many years away from regulatory approval. Furthermore, the acceptability of all approaches using RNAi may be compromised if they are classified, however inappropriately, as gene therapy. Finally, while the potential for achieving long-term protection of target cells after a single dose makes RNAi-based strategies particularly promising for further development as topical microbicides, it will be necessary to demonstrate that an economically feasible cost per dose can be achieved.

GENERATION OF RESISTANCE TO CCR5 INHIBITORS

CCR5 Inhibitors: A New Paradigm for the Development of HIV Resistance

HIV is well known for its ability to rapidly generate resistant escape mutants to antiviral drugs. Escape is generally achieved through the evolution of structural variants of the virus-encoded target that block the effect of the inhibitor while retaining function (115). This escape route will not be open to the virus when it is faced with drugs that are targeted to a host-encoded structure like CCR5, however, so CCR5 inhibitors might be expected to present a more substantial barrier to the development of escape mutants than previous drug classes. Although HIV might benefit from an increased capacity to escape inhibition because it uses its most "plastic" gene product, the envelope glycoprotein complex, to engage coreceptors, the malleability of the HIV envelope has almost certainly evolved owing to selection pressure from the humoral immune system (116), and simultaneous selection pressure driven by neutralizing antibodies may reduce the ability of HIV to introduce or maintain sequence changes required for resistance to coreceptor inhibitors in vivo (117, 118). The situation is further complicated by the fact that HIV could, in principle, respond to CCR5 inhibition by switching coreceptor usage over to CXCR4, a scenario that is of significant concern given the link between the emergence of X4 viruses and rapid disease progression. For these reasons, a great deal of emphasis has been placed on the generation of resistance to CCR5 inhibitors in both in vitro and in vivo settings.

No Coreceptor Switch to CXCR4 In Vitro

Several published studies have described the in vitro selection of R5 viruses for resistance to the selection pressure of small-molecule CCR5 inhibitors (119–122). In all of these studies, primary R5 isolates were cultured with physiological target cells that are permissive for replication of both R5 and X4 viruses in the presence of increasing concentrations of small-molecule CCR5 inhibitors. Despite the availability

of CXCR4 and the fact that other studies have shown that engineering mutations into a few key positions in the envelope glycoprotein is sufficient to confer X4 phenotype in some R5 viruses (31), all of the resistant viruses retained usage of CCR5 (119–122). The most likely explanation for this phenomenon is that the intermediates involved in the evolution from R5 to X4 are less fit than the starting virus and/or more sensitive to the selecting CCR5 inhibitor (123, 124).

Evidence for Coreceptor Switch and X4 Breakthrough In Vivo

When a human peripheral mononuclear cell–SCID mouse model was used to test the in vivo efficacy of a chemokine analog, NNY-RANTES, coreceptor switch mutants were readily obtained (125). One explanation for this result, which contrasts with results obtained in in vitro tests using small-molecule inhibitors, was the choice of the HIV-1 242 isolate as the R5 challenge virus, which is likely to have increased the probability of coreceptor switch variants because it is known that only a single amino change will generate the 241 sequence, which is an R5X4 virus (126).

In a macaque model of D/M infection, the CCR5 inhibitor CMPD167 was used to treat macaques infected with an R5 SIV and an X4 SHIV. During treatment, all animals showed reductions in R5 viral load, as well as in total viral load. Some animals showed a rise in the X4 component immediately after the initiation of treatment, but X4 levels were seen to return to baseline before the end of the treatment period (127). This suggests that treatment with CCR5 inhibitors may not lead to expansion of X4 viruses in D/M infections.

Coreceptor Switch or X4 Breakthrough: Does Not Appear to Be a Significant Risk

Available preclinical evidence from both in vitro and in vivo models suggests that neither coreceptor switch of R5 viruses to use of X4 nor expansion of X4 viruses present in low levels in mixed isolates is likely to take place during therapy with CCR5 inhibitors (119–122, 127). Although following this parameter very carefully during clinical studies remains a priority (see below), at present it appears that the main viral escape route from small-molecule CCR5 inhibitors is adaptation to enable continued use of CCR5 in the presence of the inhibitor.

Mechanisms of Resistance by Viruses That Retain Usage of CCR5

There are two likely mechanisms of resistance to coreceptor inhibitors, termed competitive resistance and noncompetitive resistance by analogy with competitive and noncompetitive inhibition (128) (**Figure 3**).

Competitive resistance is defined as resistance that results in a shift in the IC_{50} of an inhibitor to a higher concentration, although complete inhibition may still be achieved at sufficient inhibitor concentrations. It would arise from more efficient use of inhibitor-free CCR5 to gain access to target cells, enabling HIV to scavenge low levels of inhibitor-free CCR5. Competitive resistance could arise from the envelope glycoprotein either by developing a higher affinity for CCR5 or from acquiring more

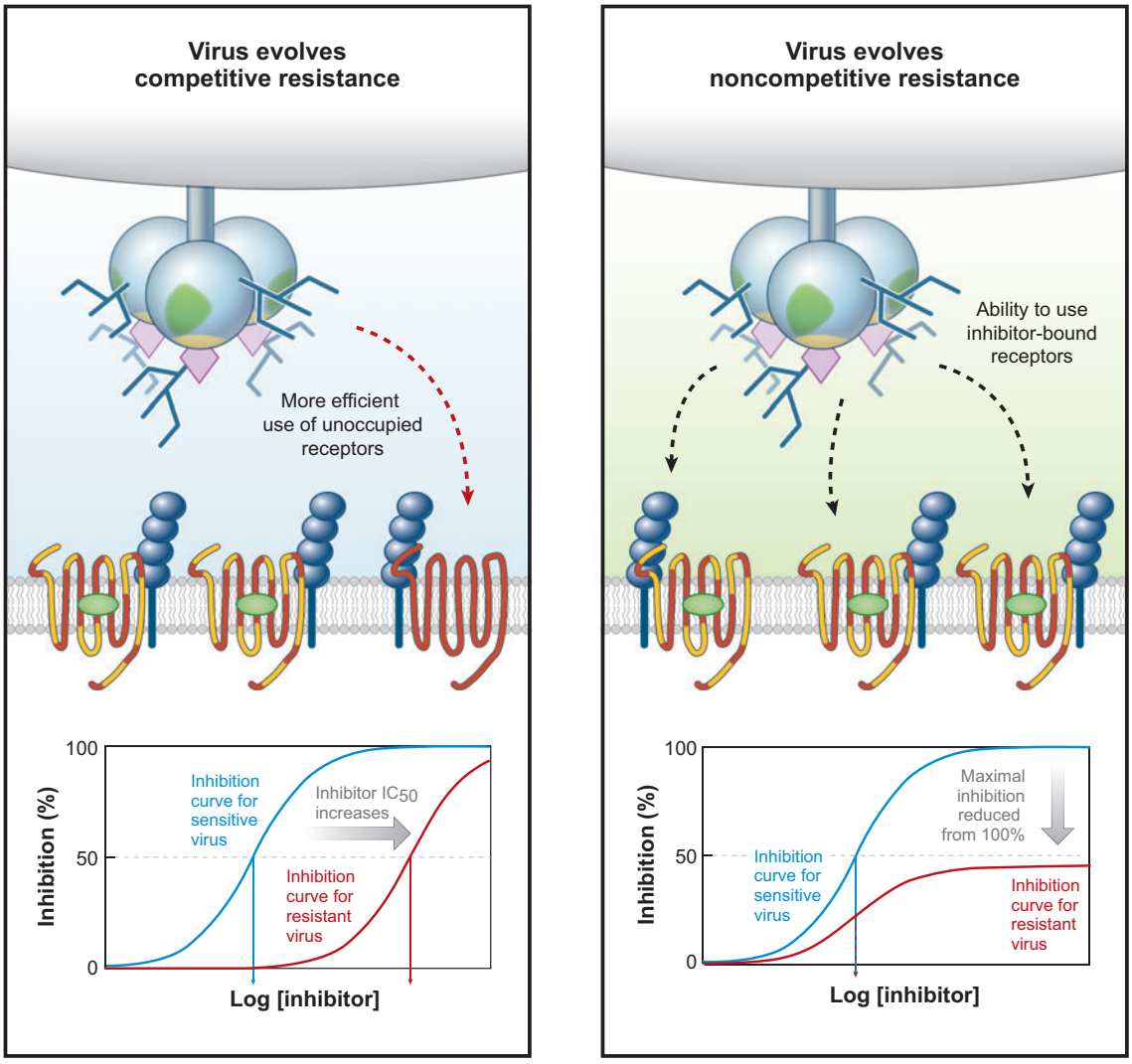

Figure 3

Proposed resistance mechanisms for viral escape from CCR5 inhibitors. Graphs indicate model dose-inhibition curves for sensitive viruses (*blue curves*) or resistant viruses (*red curves*). (*a*) Competitive resistance. Changes in Env structure allow the virus to make more efficient use of unoccupied receptors, hence higher concentrations of inhibitor are required to achieve levels of receptor occupancy necessary for inhibition. This has the effect of increasing the IC_{50} value of the inhibitor (*gray arrow*), but maximal inhibition is still possible (both curves plateau at 100%). (*b*) Noncompetitive resistance. Changes in Env structure enable the virus to use occupied receptors as coreceptors, albeit with lower efficiency than unoccupied receptors. This has the effect of reducing maximal inhibition from 100% (*gray arrow*), but because residual inhibition is related to receptor occupancy, the IC_{50} value is unchanged. Adapted from Reference 128.

rapid fusion kinetics after CCR5 engagement. In theory, the competitive resistance mechanism could apply to either competitive or allosteric inhibitors. However, the extremely slow off-rates of the small molecules targeting CCR5 [$t_{1/2} \approx 50$ to ≥ 150 h (96)] suggest that scavenging free CCR5 in the presence of these inhibitors would be a challenge. Competitive inhibitors, such as MAbs, which are complexed to CCR5 at the cell surface and may transiently dissociate, are more likely to be affected by this mechanism.

In noncompetitive resistance, inhibitor IC_{50} values are equivalent to those for the fully sensitive virus, but at maximum (plateau) levels inhibition remains incomplete. This implies that the resistant envelope glycoprotein complex has adapted to use the inhibitor-bound form of CCR5 as a coreceptor. The level of residual inhibition once the effect has saturated reflects the efficiency with which the virus can use the inhibitor-CCR5 complex relative to inhibitor-free CCR5. It is improbable that this type of resistance would arise from the selection pressure of a competitive inhibitor that acts via steric blockade (i.e., MAbs, see **Figure 2**). Instead, it would seem more probable for inhibitors with allosteric mechanisms that induce CCR5 conformations, which are not usually compatible with coreceptor activity but which nonetheless remain accessible to HIV. Evidence for noncompetitive resistance can be provided in cross-antagonism experiments using pairs of inhibitors that block each other's inhibitory mechanisms, where the virus is sensitive to one inhibitor ("active inhibitor") and resistant to the other ("inactive inhibitor"). Blockade of active inhibitor in the presence of the inactive inhibitor is an indication that the virus is capable of using receptors occupied by the inactive inhibitor as coreceptors (121, 128).

What would happen in approaches that lead to removal of CCR5 from the cell surface? Chemokine analogs such as PSC-RANTES, which act in this way, would seem to pose additional challenges to the development of competitive resistance. Not only do these molecules induce drastic reduction in cell surface levels of CCR5 (83), it is likely that they also remain available to sterically block any receptor molecules that are remaining at the surface or which have been recycled back to the surface after internalization (129). In preliminary in vitro studies using standard approaches it has proved impossible to generate viable escape mutants resistant to PSC-RANTES (130). RNAi approaches targeting CCR5 may also provide a major barrier to the development of escape mutants. Viruses escape from RNAi targeted to viral genes by simply modifying the sequence of the transcript (112), but this escape route would not be open when the target is a host gene such as CCR5. Future work should help establish whether it is possible for HIV to escape from the effects of inhibitors that eliminate CCR5 from the cell surface, and if so, how escape is achieved.

Generation of Resistance to Small-Molecule Inhibitors: Implications for Clinical Use

A number of in vitro studies of the generation of resistance to small-molecule inhibitors have been carried out (**Table 2**). The escape pathways all involved continued use of CCR5 to replicate in lymphocytes in the presence of the inhibitor (119–122). The mechanism of inhibition has been shown to be noncompetitive in all cases where it has been examined (121, 128), either by the demonstration of a plateau

Table 2 Studies of in vitro resistance generated against CCR5 inhibitors

Inhibitor	Starting isolate	Changes to env	Cross-resistance to CCR5 inhibitors		Resistance mechanism (how determined)	References
			Resistant	Sensitive		
AD101	CC1/85	V3 region: H308P, K305R, A316V, G321E	AD101, SCH-C, vicriviroc, maraviroc, aplaviroc	PSC-RANTES, PRO 140	noncompetitive (plateau, antagonism of PSC-RANTES by vicriviroc)	(119, 128, 154); SEK, T.J. Ketas, J.P. Moore, unpublished results
vicriviroc	CC1/85	All outside V3 region	vicriviroc, AD101, SCH-C, maraviroc, aplaviroc	PSC-RANTES, PRO 140	noncompetitive (antagonism of PSC-RANTES by vicriviroc)	(120, 128); SEK, T.J. Ketas, J.P. Moore, unpublished results
	CC1/85 early passage from AD101 selection; H308P present	V3 region: H308P retained; other mutations outside V3 region	vicriviroc, AD101, SCH-C, maraviroc, aplaviroc	PSC-RANTES, PRO 140	ND	
maraviroc	CC1/85	V3 region: A316T, I323V	maraviroc	SCH-C, vicriviroc, aplaviroc	noncompetitive (plateau, antagonism of aplaviroc by maraviroc)	(121, 155)
	RU570	V3 region: deletion at positions 315–317	maraviroc	SCH-C, vicriviroc, aplaviroc	noncompetitive (plateau)	
TAK-652	KK	Multiple changes both within and outside V3 region	TAK-625 TAK-779	TAK-220	ND	(122)

in single-cycle assays in cell lines (see **Figure 3**) or cross-antagonism studies (see above). The results of these studies have important implications for the clinical use of small-molecule inhibitors, including the generation of phenotypic and genotypic assays to predict the emergence of resistant viruses and the likelihood that resistant viruses selected against one small molecule inhibitor will show cross-resistance to other CCR5 inhibitors.

Changes to Env: No Clear Genotypic Signature that Accompanies Development of Resistance

The studies carried out so far have not revealed a common resistance pathway against small-molecule CCR5 inhibitors, as have been seen previously for drug classes targeting virus-encoded structures (131). The observation that the same primary virus isolate, CC1/85, can generate resistance to three different small-molecule inhibitors in three different ways (**Table 2**) may be a reflection of subtle differences in the inhibitory mechanisms of the different molecules and an indication that HIV can use its highly flexible envelope glycoprotein to find a number of different solutions to the same problem. Significantly, if a complex network of escape pathways from individual CCR5 inhibitors is available to HIV, this will make monitoring of the generation of resistant mutants by genotyping impossible, and reliable phenotypic assays using viruses pseudotyped with envelope glycoproteins will be required instead. In this regard, recent evidence suggests that the apparent levels of sensitivity to CCR5 inhibitors exhibited by escape viruses that exhibit noncompetitive resistance can vary to a significant extent according to the target cell (primary cells versus cell line) and assay type (replication versus single cycle) used (121, 128). These differences need to be taken into account in the design and interpretation of phenotypic assays to monitor the development of resistance to CCR5 inhibitors.

Generation of Cross-Resistance to Other Small-Molecule CCR5 Inhibitors

Given the apparent similarities between small-molecule CCR5 antagonists, both in terms of CCR5 binding sites and inhibitory mechanisms, it is perhaps not surprising that acquisition by viruses of resistance to one molecule can lead to broad cross-resistance to the other molecules in the class. Two of the four in vitro studies did not lead to the generation of broadly cross-resistant viruses, however, indicating that while cross-resistant escape is certainly a possibility, it is not inevitable. Selective resistance may be a reflection of the subtleties of the allosteric inhibitory mechanisms—while having broadly overlapping binding sites on CCR5, different molecules may be capable of inducing different inhibitory conformations of CCR5.

The potential for viruses to develop, under certain circumstances, broad cross-resistance to small-molecule CCR5 inhibitors may become a clinically relevant parameter. First, will maraviroc, as in vitro studies have suggested (**Table 2**), prove to be less prone to the development of cross-resistant escape mutants than the other small-molecule inhibitors? Second, would the development of viruses resistant to a given CCR5 inhibitor in a treated individual mean other CCR5 inhibitors will be ruled out as options for therapy? Third, in light of the fact that that CMPD 167 is a small-molecule CCR5 inhibitor currently being developed for use as a topical microbicide, would the development of viruses resistant to a CCR5 inhibitor used for therapy ultimately undermine the performance of another small-molecule drug that has been developed for use in HIV prevention?

Table 3 CCR5 inhibitors that have been clinically tested as HIV therapeutics

Molecule	Class	Status	Comments	Reference
Aplaviroc	Small molecule	Clinical development terminated in 2005	Safety issues: several cases of severe hepatotoxicity	(135)
Maraviroc	Small molecule	Phase 2b/3 treatment naïve: ongoing	One case of severe hepatotoxicity not considered drug related.	(137)
		Phase 2b/3 treatment experienced: completed	Well tolerated, potent antiretroviral effect through 24 weeks. Approved for use in treatment-experienced individuals.	(4, 138)
Vicriviroc	Small molecule	Phase 2b/3 treatment naïve: Terminated in 2006	Lack of sufficient efficacy	(136)
		Phase 2b/3 treatment experienced: ongoing	Phase 2b trial unblinded due to malignancies. Well tolerated, potent antiretroviral effect through 24 weeks. Open label follow-up continues.	(49a)
PRO 140	MAb	Phase 1b	Tolerated, prolonged reduction of viral load after a single dose	(156)
CCR5mAb004		Phase 1b	Tolerated, prolonged reduction of viral load after a single dose	(157)

EVALUATION OF CCR5 INHIBITORS AS THERAPEUTICS IN RECENT CLINICAL TRIALS

Results from large-scale efficacy studies of three small-molecule CCR5 inhibitors, as well as from early clinical studies of two anti-CCR5 MAbs have recently been reported (**Table 3**).

Phase 2b/3 Trials of Small-Molecule Inhibitors: Some Success, Some Attrition

Although successful Phase 2a trials were reported for three small-molecule CCR5 inhibitors (132–134), some obstacles were subsequently encountered during the Phase 2b/3 trials that followed. Clinical development of aplaviroc was terminated in late 2005 owing to safety fears after a number of severe cases of hepatotoxicity were reported (135), and a few months later, the large-scale trial of vicriviroc in treatment-naïve patients was halted owing to insufficient efficacy (136). There have also been some very encouraging results from the Phase 2b trials, however. Vicriviroc is still under evaluation for use in treatment-experienced individuals, and maraviroc, which has recently been approved for use in this setting (4), may yet be approved for use in treatment-naïve patients.

No Clear Signs of Class-Specific Toxicity

The toxicity issues that have emerged so far in trials involving small-molecule CCR5 inhibitors appear to have been either compound-specific, in the case of aplaviroc

(severe hepatotoxicity), or have not been linked to the use of the drug, in the case of vicriviroc [malignancies, (49a) and maraviroc [severe hepatotoxicity, (137)]. Long-term monitoring for class-specific toxicity is nonetheless imperative, and five-year follow-up studies, including monitoring for class-specific toxicity, have been recommended for all CCR5 inhibitors approved for use in therapy (62).

No Escape by Coreceptor Switching to CXCR4; No Breakthrough of X4 Viruses

As predicted by the preclinical data obtained in vitro and in vivo, the clinical trials carried out with the small-molecule CCR5 inhibitors have not suggested that their use leads to harmful virological effects related to the selection for or emergence of X4 viruses. In trials involving treatment-experienced patients using an optimized background regimen, patients who failed treatment with maraviroc were found to be more likely to have detectable X4 viruses than those taking placebo. The maraviroc-treated patients were much less likely to fail treatment overall, however. Genotypic analysis has shown that the emergence of X4 viruses during treatment was due to expansion of a preexisting, but undetectable, population of X4 viruses. In a trial in patients with D/M virus, mean viral load in the maraviroc group did not differ from the placebo group, and other virologic parameters were only marginally improved. On the other hand, clinical parameters such as CD4 and CD8 counts were significantly improved in the maraviroc-treated group (138). Thus, it appears that although treatment of the X4 component of D/M infections with a small-molecule CCR5 inhibitor is unlikely to have significant virologic benefit, it is unlikely to be harmful.

In Vivo Resistance to Small-Molecule CCR5 Inhibitors Involving Continued Use of CCR5

For approximately 45% of treatment-experienced patients experiencing treatment failure on maraviroc, only R5 virus was detectable. Of a subset of these viral isolates that were analyzed, approximately half showed resistance in a phenotypic assay using envelope glycoprotein pseudotyped viruses. These in vivo–selected, R5-tropic drug-resistant viruses all had properties consistent with noncompetitive resistance (lower plateaus, unchanged IC_{50} values; see **Figure 3**). Clonal analysis revealed mutations in V3 that were shown to be involved in resistance in all of the cases studied, although other uncharacterized mutations outside of V3 were also implicated in some cases. As was the case for the in vitro escape mutants, no pattern to the V3 mutations involved was apparent (138).

Anti-CCR5 MAbs Performing Well in Early Clinical Trials

Results from Phase 1b studies of two monoclonal anti-CCR5 antibodies, PRO 140 and CCR5mAb004, have been recently reported. The antibodies were well tolerated at the highest doses tested, and because the safety studies were performed in patients with R5 HIV, it was possible to obtain preliminary data related to their efficacy.

Both of the antibodies showed promising efficacy, producing viral load reduction that was sustained for several weeks following a single dose. Hence, the clinical evaluation of strategies using anti-CCR5 MAbs for HIV therapy is encouraging at this stage, and there is additional preclinical evidence to suggest that not only could anti-CCR5 MAbs provide synergy if used clinically together with small-molecule CCR5 inhibitors (105, 139, 140) but they could also be deployed in cases where individuals had developed R5 viruses resistant to small molecule CCR5 inhibitors (**Table 2**). Although the MAbs are injectables rather than orally available, the data available suggest that dosing as infrequent as twice monthly may provide a satisfactory outcome, and this compares well with the twice-daily injection regime that is necessary for Fuzeon (141), the only injectable anti-HIV drug that is currently registered.

PRECLINICAL EVALUATION OF CCR5 INHIBITORS DEVELOPED FOR USE IN PREVENTION

Microbicides: To Target CCR5 Alone or in Combination with Other Targets?

Two CCR5 inhibitors, CMPD-167 and PSC-RANTES, have shown efficacy in a macaque model of vaginal transmission. In the first reported test, CMPD-167 provided only moderate levels of protection from challenge with an R5-tropic SHIV (two out of eleven animals protected) at the highest dose tested [1 mM (142)]. The results from this challenge experiment supported the argument that to successfully prevent transmission using topical strategies, it will be necessary to simultaneously block a number of different infection routes for HIV (143). The inability of CMPD-167 alone to block transmission in this study may have been due to insufficient efficacy at the dose used, however, because in subsequent experiments using the same model it was shown that use of CMPD-167 at a higher concentration (5 mM) can provide substantial protection [eight of ten animals protected (144)]. More compelling evidence was provided in a third study, where PSC-RANTES provided full protection (five out of five animals) at a 1 mM concentration (145). While these latter results suggest that topical targeting of CCR5 alone may be a valid HIV prevention strategy, it is noteworthy that high levels of protection are achievable when combinations of drugs with different inhibitory targets are used together (144), indicating that it would be prudent to explore microbicide cocktails comprising combinations of different inhibitors, including drugs that block CCR5.

Large Quantities of Drug are Likely to be Required for Effective Doses

A key concept that has emerged from a range of different studies in the macaque model is that to demonstrate efficacy in this model entry inhibitors are required at concentrations hugely in excess of their in vitro IC_{50} values (68, 69). While there are a number of potential explanations for this phenomenon, the likelihood is that it does not simply relate to species differences between humans and macaques, and

that similarly high doses of entry inhibitors will be required for effective protection in humans. Crucially, this implies that drugs that are expensive to produce are unlikely to be feasible for development as topical microbicides. Commentators have pointed out (68, 69, 88) that it will be a challenge to find low-cost production methods for intact MAbs, which are normally produced in relatively expensive expression systems, as well as molecules like PSC-RANTES, whose production requires chemical synthesis steps (83). With respect to the latter point, a recently described group of fully recombinant RANTES analogs with in vitro potency comparable with that of PSC-RANTES may provide an efficacious and cost-effective alternative to the chemically modified molecule (146).

In-Depth Safety Studies Will Be Required

Studies of CCR5 inhibitors for prevention are currently at the preclinical stage, and little data relating to their safety is available. The observations from the clinical trials of small-molecule CCR5 inhibitors provide some reassurance that targeting CCR5 systemically will not lead to toxicity, but additional studies focused on the impact of topical inhibition of CCR5 at genital mucosa will be required to assess the safety of new microbicide candidates. Local immunosuppression is a potential class-specific effect, as is the potential to provoke local inflammation in the case of chemokine agonists such as PSC-RANTES (10). It should be noted that because chemokine receptor genes are not highly conserved between species, animal models for safety may fail to pick up effects directly related to receptor activity. It may therefore be prudent to place more emphasis on carefully planned Phase 1 safety studies in populations not at risk from HIV acquisition.

Getting Coitally Independent Protection

Coital independence is likely to be a key parameter for a successful prevention approach. This could, in principle, be addressed by preexposure prophylaxis, i.e., maintenance of protective systemic levels of a drug through oral dosing; indeed, CMPD-167 has shown some efficacy in a macaque model of PrEP (147). The drug doses required for an effect were significantly higher than those necessary for detectable efficacy when topical administration was used, however, so it would seem that any advantages with respect to coital independence might be offset by the disadvantage of the necessity for increased doses.

For topical microbicides, it is hoped that optimized formulation strategies will generate products that provide coitally independent protection (68), but the drug component could also make a positive impact on this parameter if its pharmacological characteristics led to long-duration inhibition. The pharmacological properties of certain CCR5 inhibitors, e.g., very slow receptor off-rates exhibited by the small-molecule inhibitors and sustained intracellular sequestration of the receptor induced by RANTES analogs, may prove to be significant in this regard.

The Threat of Resistant Mutants

HIV can undoubtedly generate escape mutants during exposure to small-molecule CCR5 inhibitors, and in some cases this leads to the generation of cross-resistance to other drugs in the same class. Could the initiation of therapeutic strategies based on targeting CCR5 lead to the dissemination of broadly cross-resistant viruses that would render prevention strategies based on targeting CCR5 ineffective? The pertinence of this question is underlined by the fact that maraviroc, a small-molecule CCR5 inhibitor, is likely to enter use as a therapeutic in the near future, while the most advanced CCR5 inhibitor in development for prevention applications is another small-molecule CCR5 inhibitor, CMPD-167. Although the probability of generating broadly resistant viruses in an individual is quite high, the probability of these viruses spreading into an uninfected population will be reduced by a number of factors. First, the size of the population having access to the CCR5 inhibitor for therapy is likely to be quite small, at least for the first years of its use. Second, the location of the population having access to the CCR5 inhibitor for therapy is likely to be geographically distinct from the populations most in need of prevention strategies. This is principally because the immediate requirement of people in populations worst affected by the HIV epidemic is not a new drug class, but access to standard first-line antiretroviral therapy. Furthermore, it may be inadvisable to use CCR5 inhibitors for therapy in tropical regions where pathogens against which CCR5 may be an essential component of the host defense mechanism (e.g., West Nile Virus) are prevalent. Third, although there is no clear information with respect to clinical CCR5 resistance at present, viruses resistant to other drug classes are typically relatively unfit, and this may reduce their potential to spread into uninfected populations (148).

The Threat of Attrition During Clinical Development

The mixed fortunes of the small-molecule CCR5 inhibitors in Phase 2b/3 trials provide yet another example of a well-established concept: Very promising drug candidates can drop out of the pipeline relatively late in the clinical development process. The pharmaceutical industry has the resources to partially cover this risk by generating and maintaining a series of candidate drugs at earlier stages in development. This is not the case for the development of drugs for HIV prevention, however. While the pharmaceutical industry does participate by occasionally out-licensing molecules that are no longer in development, e.g., Merck's provision of CMPD-167 to the International Partnership for Microbicides (149), it cannot be expected to donate and maintain entire research and development programs to insure against the risk of attrition. There is an unquestionable need for new and effective HIV prevention strategies, and a clear scientific rationale for achieving effective HIV prevention via CCR5 blockade. But the current pipeline of CCR5 inhibitors for development as microbicides, which is limited to CMPD-167 and a small group of RANTES analogs, is certainly not free from the threat of attrition during the clinical development process.

CONCLUSIONS

With CCR5 inhibitors now available as a new class of therapeutic anti-HIV drugs, as well as showing promise in preclinical models for HIV prevention, this is an exciting period in the history of HIV coreceptor inhibitors. New challenges, related to managing the risks of toxicity and novel viral escape mechanisms, need to be met to ensure that maximum benefits are reaped from the therapeutic use of CCR5 inhibitors. The development of CCR5 inhibitors for HIV prevention presents an even bigger challenge, all the more so because it relates to issues that are not generally concerns in drug development. HIV coreceptors were first identified slightly more than ten years ago; the next ten years will reveal whether coreceptor inhibitors can fulfill their promise as effective tools to combat the HIV epidemic through both therapy and prevention.

SUMMARY POINTS LIST

1. To combat the HIV epidemic, new drugs for both prevention and therapy are required. CCR5 is a promising anti-HIV target for both therapy and prevention.
2. In addition to maraviroc, other orally available small-molecule CCR5 inhibitors are likely to enter clinical use as therapeutics in the near future, and monoclonal antibodies directed against CCR5 are showing promise in early clinical trials.
3. New therapeutic drugs targeting CCR5 will have to be monitored carefully for the development of escape mutants, the promotion of viruses using CXCR4 as a coreceptor, and any effects on health related to suppression of the physiological function of CCR5.
4. The key concerns for the development of HIV prevention strategies based on CCR5 inhibition are different from those related to the development of therapeutics and must be properly addressed to make an impact on the HIV epidemic.
5. CCR5 inhibitors have shown promise in preclinical studies related to HIV prevention.

DISCLOSURE STATEMENT

Oliver Hartley is the inventor of several patents related to anti-HIV chemokines; is the cofounder and vice-president of the Mintaka Foundation for Medical Research, a non-profit organization whose aims include developing anti-HIV chemokines for use in microbicides; and is the holder of several academic grants related to the evaluation of anti-HIV chemokines as candidates for development in topical microbicide strategies.

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LITERATURE CITED

1. UNAIDS/WHO. 2006. *AIDS Epidemic Update*. Geneva, Switz.: UNAIDS/WHO
2. Hammer SM, Saag MS, Schechter M, Montaner JS, Schooley RT, et al. 2006. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *JAMA* 296:827–43
3. Stephenson J. 2007. Researchers buoyed by novel HIV drugs: will expand drug arsenal against resistant virus. *JAMA* 297:1535–36
4. Food Drug Adm. (FDA), Center Drug Eval. Res. 2007. *Summary Minutes of the Antiviral Drugs Advisory Comm. Meet.*
5. Quinn TC, Overbaugh J. 2005. HIV/AIDS in women: an expanding epidemic. *Science* 308:1582–83
6. Ruxin J, Paluzzi JE, Wilson PA, Tozan Y, Kruk M, Teklehaimanot A. 2005. Emerging consensus in HIV/AIDS, malaria, tuberculosis, and access to essential medicines. *Lancet* 365:618–21
7. 2007. Newer approaches to HIV prevention. *Lancet* 369:615
8. Shattock RJ, Moore JP. 2003. Inhibiting sexual transmission of HIV-1 infection. *Nat. Rev. Microbiol.* 1:25–34
9. Grant RM, Buchbinder S, Cates W Jr, Clarke E, Coates T, et al. 2005. AIDS. Promote HIV chemoprophylaxis research, don't prevent it. *Science* 309:2170–71
10. Lederman MM, Penn-Nicholson A, Cho M, Mosier D. 2006. Biology of CCR5 and its role in HIV infection and treatment. *JAMA* 296:815–26
11. O'Hara BM, Olson WC. 2002. HIV entry inhibitors in clinical development. *Curr. Opin. Pharmacol.* 2:523–28
12. Gallo SA, Finnegan CM, Viard M, Raviv Y, Dimitrov A, et al. 2003. The HIV env-mediated fusion reaction. *Biochim. Biophys. Acta* 1614:36–50
13. Huang CC, Tang M, Zhang MY, Majeed S, Montabana E, et al. 2005. Structure of a V3-containing HIV-1 gp120 core. *Science* 310:1025–28
14. Chen B, Vogan EM, Gong H, Skehel JJ, Wiley DC, Harrison SC. 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. *Nature* 433:834–41
15. Cocchi F, DeVico AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P. 1996. The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. *Nat. Med.* 2:1244–47
16. Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, et al. 1998. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* 280:1949–53

17. Weiss CD. 2003. HIV-1 gp41: mediator of fusion and target for inhibition. *AIDS Rev.* 5:214–21
18. Cocchi F, DeVico AL, Garzino DA, Arya SK, Gallo RC, Lusso P. 1995. Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by $CD8^+$ T cells [see comments]. *Science* 270:1811–15
19. Feng Y, Broder CC, Kennedy PE, Berger EA. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872–77
20. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, et al. 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 382:833–35
21. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, et al. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 382:829–33
22. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, et al. 1996. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272:1955–58
23. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, et al. 1996. The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85:1135–48
24. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, et al. 1996. Identification of a major coreceptor for primary isolates of HIV-1. *Nature* 381:661–66
25. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, et al. 1996. HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381:667–73
26. Berger EA, Murphy PM, Farber JM. 1999. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.* 17:657–700
27. Moore JP, Kitchen SG, Pugach P, Zack JA. 2004. The CCR5 and CXCR4 coreceptors–central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retrovir.* 20:111–26
28. Berger EA, Doms RW, Fenyo EM, Korber BT, Littman DR, et al. 1998. A new classification for HIV-1. *Nature* 391:240
29. Whitcomb JM, Huang W, Fransen S, Limoli K, Toma J, et al. 2007. Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. *Antimicrob. Agents Chemother.* 51:566–75
30. Spudich SS, Huang W, Nilsson AC, Petropoulos CJ, Liegler TJ, et al. 2005. HIV-1 chemokine coreceptor utilization in paired cerebrospinal fluid and plasma samples: a survey of subjects with viremia. *J. Infect. Dis.* 191:890–98
31. Hartley O, Klasse PJ, Sattentau QJ, Moore JP. 2005. V3: HIV's Switch-Hitter. *AIDS Res. Hum. Retrovir.* 21:171–89
32. Zhang Y, Moore JP. 1999. Will multiple coreceptors need to be targeted by inhibitors of human immunodeficiency virus type 1 entry? *J. Virol.* 73:3443–48

33. Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. 1997. Change in coreceptor use correlates with disease progression in HIV-1–infected individuals. *J. Exp. Med.* 185:621–28
34. Margolis L, Shattock R. 2006. Selective transmission of CCR5-utilizing HIV-1: the 'gatekeeper' problem resolved? *Nat. Rev. Microbiol.* 4:312–17
35. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, et al. 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86:367–77
36. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. 1997. Global distribution of the CCR5 gene 32-basepair deletion. *Nat. Genet.* 16:100–3
37. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, et al. 1996. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat. Med.* 2:1240–43
38. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, et al. 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemoph. Growth Dev. Study, Multicent. AIDS Cohort Study, Multicent. Hemoph. Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* 273:1856–62. Erratum. 1996. *Science* 274(5290):1069
39. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik J, et al. 1997. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. *Mol. Med.* 3:23–36
40. Salkowitz JR, Purvis SF, Meyerson H, Zimmerman P, O'Brien TR, et al. 2001. Characterization of high-risk HIV-1 seronegative hemophiliacs. *Clin. Immunol.* 98:200–11
41. Michael NL, Chang G, Louie LG, Mascola JR, Dondero D, et al. 1997. The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. *Nat. Med.* 3:338–40
42. Gorry PR, Zhang C, Wu S, Kunstman K, Trachtenberg E, et al. 2002. Persistence of dual-tropic HIV-1 in an individual homozygous for the CCR5Δ32 allele. *Lancet* 359:1832–34
43. Michael NL, Nelson JA, KewalRamani VN, Chang G, O'Brien SJ, et al. 1998. Exclusive and persistent use of the entry coreceptor CXCR4 by human immunodeficiency virus type 1 from a subject homozygous for CCR5δ32. *J. Virol.* 72:6040–47
44. Naif HM, Cunningham AL, Alali M, Li S, Nasr N, et al. 2002. A human immunodeficiency virus type 1 isolate from an infected person homozygous for CCR5Δ32 exhibits dual tropism by infecting macrophages and MT2 cells via CXCR4. *J. Virol.* 76:3114–24
45. Sheppard HW, Celum C, Michael NL, O'Brien S, Dean M, et al. 2002. HIV-1 infection in individuals with the CCR5-Δ32/Δ32 genotype: acquisition of syncytium-inducing virus at seroconversion. *J. Acquired Immune Defic. Syndr.* 29:307–13

46. Shankarappa R, Margolick JB, Gange SJ, Rodrigo AG, Upchurch D, et al. 1999. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. *J. Virol.* 73:10489–502
47. Brumme ZL, Brumme CJ, Henrick BM, et al. 2005. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naïve individuals. *J. Infect. Dis.* 192:466–74
48. Moyle GJ, Wildfire A, Mandalia S, Mayer H, Goodrich J, et al. 2005. Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection. *J. Infect. Dis.* 191:866–72
49. Coakley E, Benhamida J, Chappey C, Whitcomb J, Goodrich J, et al. 2006. An evaluation of tropism profiles and other characteristics among 3988 individuals screened for the A4001026, A4001027 (motivate 1) and A4001028 (motivate 2) studies for maraviroc. Presented at Int.Worksh. Target. HIV Entry, 2nd, Boston, MA
49a. Gulick RM, Su Z, Flexner C, Hughes MD, Skolnik PR, et al. 2007. Phase 2 study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-infected, treatment-experienced patients: AIDS clinical trial group 5211. *J. Infect. Dis.* 196:304–12
50. Melby T, Despirito M, Demasi R, Greenberg M, Heilek-Snyder G, Graham N. 2006. *HIV-1 coreceptor tropism in triple-class-experienced patients: baseline correlates and relationship to enfuvirtide response*. Presented at 13th Conf. Retrovir. Oppor. Infect., Denver, CO
51. Wilkin T, Su Z, Kuritzkes D, Hughes M, Flexner C, et al. 2006. *Co-receptor tropism in patients screening for ACTG 5211, a phase 2 study of vicriviroc, a CCR5 inhibitor*. Presented at 13th Conf. Retrovir. Oppor. Infect., Denver, CO
52. Ioannidis JP, Rosenberg PS, Goedert JJ, Ashton LJ, Benfield TL, et al. 2001. Effects of CCR5-Δ32, CCR2–64I, and SDF-1 3′A alleles on HIV-1 disease progression: An international meta-analysis of individual-patient data. *Ann. Intern. Med.* 135:782–95
53. Penn ML, Grivel JC, Schramm B, Goldsmith MA, Margolis L. 1999. CXCR4 utilization is sufficient to trigger $CD4^+$ T cell depletion in HIV-1-infected human lymphoid tissue. *Proc. Natl. Acad. Sci. USA* 96:663–68
54. Arthos J, Cicala C, Selig SM, White AA, Ravindranath HM, et al. 2002. The role of the CD4 receptor vs HIV coreceptors in envelope-mediated apoptosis in peripheral blood mononuclear cells. *Virology* 292:98–106
55. Kwa D, Vingerhoed J, Boeser-Nunnink B, Broersen S, Schuitemaker H. 2001. Cytopathic effects of nonsyncytium-inducing and syncytium-inducing human immunodeficiency virus type 1 variants on different CD4(+)-T-cell subsets are determined only by coreceptor expression. *J. Virol.* 75:10455–59
56. Nishimura Y, Brown CR, Mattapallil JJ, Igarashi T, Buckler-White A, et al. 2005. Resting naive $CD4^+$ T cells are massively infected and eliminated by X4-tropic simian-human immunodeficiency viruses in macaques. *Proc. Natl. Acad. Sci. USA* 102:8000–5
57. Nishimura Y, Igarashi T, Donau OK, Buckler-White A, Buckler C, et al. 2004. Highly pathogenic SHIVs and SIVs target different $CD4^+$ T cell subsets in

rhesus monkeys, explaining their divergent clinical courses. *Proc. Natl. Acad. Sci. USA* 101:12324–29
58. Douek DC. 2003. Disrupting T-cell homeostasis: how HIV-1 infection causes disease. *AIDS Rev.* 5:172–77
59. Murphy PM. 2002. International Union of Pharmacology. XXX. Update on chemokine receptor nomenclature. *Pharmacol. Rev.* 54:227–29
60. 2003. Chemokine/chemokine receptor nomenclature. *Cytokine* 21:48–49
61. de Silva E, Stumpf MP. 2004. HIV and the CCR5-Δ32 resistance allele. *FEMS Microbiol. Lett.* 241:1–12
62. Fry J, Bogulavsky Grossman J. 2007. *Collaborative Approaches to HIV Drug Development: Planning for Long-Term Monitoring of Safety in CCR5 Antagonist Development*, ed. V Miller. *Rep. FDA/FCHR Jt. Public Meet.*Washington, DC: Forum Collab. HIV Res.
63. Murdoch C. 2000. CXCR4: chemokine receptor extraordinaire. *Immunol. Rev.* 177:175–84
64. Baggiolini M, Moser B. 1997. Blocking chemokine receptors. *J. Exp. Med.* 186:1189–91
65. Endres MJ, Clapham PR, Marsh M, Ahuja M, Turner JD, et al. 1996. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell* 87:745–56
66. Hendrix CW, Collier AC, Lederman MM, Schols D, Pollard RB, et al. 2004. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J. Acquired Immune Defic. Syndr.* 37:1253–62
67. Stone ND, Dunaway SB, Flexner CW, Tierney C, Calandra GB, et al. 2007. Multiple dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selective CXCR4 receptor inhibitor, in human subjects (ACTG A5191). *Antimicrob. Agents Chemother.* 51:2351–58
68. Klasse PJ, Shattock RJ, Moore JP. 2006. Which topical microbicides for blocking HIV-1 transmission will work in the real world? *PLoS Med.* 3:e351
69. Lederman MM, Offord RE, Hartley O. 2006. Microbicides and other topical strategies to prevent vaginal transmission of HIV. *Nat. Rev. Immunol.* 6:371–82
70. Weber J, Desai K, Darbyshire J. 2005. The development of vaginal microbicides for the prevention of HIV transmission. *PLoS Med.* 2:e142
71. Hillier SL, Moench T, Shattock R, Black R, Reichelderfer P, Veronese F. 2005. In vitro and in vivo: the story of nonoxynol 9. *J. Acquired Immune Defic. Syndr.* 39:1–8
72. Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, et al. 2005. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 307:1434–40
73. Nardese V, Longhi R, Polo S, Sironi F, Arcelloni C, et al. 2001. Structural determinants of CCR5 recognition and HIV-1 blockade in RANTES. *Nat. Struct. Biol.* 8:611–15
74. Schmidtmayerova H, Sherry B, Bukrinsky M. 1996. Chemokines and HIV replication. *Nature* 382:767
75. Hartley O, Offord RE. 2005. Engineering chemokines to develop optimized HIV inhibitors. *Curr. Protein Pept. Sci.* 6:207–19

76. Arenzana-Seisdedos F, Virelizier JL, Rousset D, Clark-Lewis I, Loetscher P, et al. 1996. HIV blocked by chemokine antagonist. *Nature* 383:400
77. Gong JH, Uguccioni M, Dewald B, Baggiolini M, Clark-Lewis I. 1996. RANTES and MCP-3 antagonists bind multiple chemokine receptors. *J. Biol. Chem.* 271:10521–27
78. Simmons G, Clapham PR, Picard L, Offord RE, Rosenkilde MM, et al. 1997. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* 276:276–79
79. Trkola A, Paxton WA, Monard SP, Hoxie JA, Siani MA, et al. 1998. Genetic subtype-independent inhibition of human immunodeficiency virus type 1 replication by CC and CXC chemokines. *J. Virol.* 72:396–404
80. Amara A, Gall SL, Schwartz O, Salamero J, Montes M, et al. 1997. HIV coreceptor downregulation as antiviral principle: SDF-1alpha-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J. Exp. Med.* 186:139–46
81. Alkhatib G, Locati M, Kennedy PE, Murphy PM, Berger EA. 1997. HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines: independence from G protein signaling and importance of coreceptor downmodulation. *Virology* 234:340–48
82. Ferguson SS. 2001. Evolving concepts in G protein-coupled receptor endocytosis: The role in receptor desensitization and signaling. *Pharmacol. Rev.* 53:1–24
83. Hartley O, Gaertner H, Wilken J, Thompson D, Fish R, et al. 2004. Medicinal chemistry applied to a synthetic protein: development of highly potent HIV entry inhibitors. *Proc. Natl. Acad. Sci. USA* 101:16460–65
84. Sabbe R, Picchio GR, Pastore C, Chaloin O, Hartley O, et al. 2001. Donor- and ligand-dependent differences in C-C chemokine receptor 5 reexpression. *J. Virol.* 75:661–71
85. Mack M, Luckow B, Nelson PJ, Cihak J, Simmons G, et al. 1998. Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J. Exp. Med.* 187:1215–24
86. Oppermann M, Mack M, Proudfoot AE, Olbrich H. 1999. Differential effects of CC chemokines on CC chemokine receptor 5 (CCR5) phosphorylation and identification of phosphorylation sites on the CCR5 carboxyl terminus. *J. Biol. Chem.* 274:8875–85
87. Proudfoot AE, Handel TM, Johnson Z, Lau EK, LiWang P, et al. 2003. Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. *Proc. Natl. Acad. Sci. USA* 100:1885–90
88. Moore JP. 2005. Topical microbicides become topical. *N. Engl. J. Med.* 352:298–300
89. Baba M, Nishimura O, Kanzaki N, Okamoto M, Sawada H, et al. 1999. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. USA* 96:5698–703

90. Castonguay LA, Weng Y, Adolfsen W, Di Salvo J, Kilburn R, et al. 2003. Binding of 2-aryl-4-(piperidin-1-yl)butanamines and 1,3,4-trisubstituted pyrrolidines to human CCR5: a molecular modeling-guided mutagenesis study of the binding pocket. *Biochemistry* 42:1544–50
91. Dragic T, Trkola A, Thompson DA, Cormier EG, Kajumo FA, et al. 2000. A binding pocket for a small molecule inhibitor of HIV-1 entry within the transmembrane helices of CCR5. *Proc. Natl. Acad. Sci. USA* 97:5639–44
92. Nishikawa M, Takashima K, Nishi T, Furuta RA, Kanzaki N, et al. 2005. Analysis of binding sites for the new small-molecule CCR5 antagonist TAK-220 on human CCR5. *Antimicrob. Agents Chemother.* 49:4708–15
93. Tsamis F, Gavrilov S, Kajumo F, Seibert C, Kuhmann S, et al. 2003. Analysis of the mechanism by which the small-molecule CCR5 antagonists SCH-3,51,125 and SCH-3,50,581 inhibit human immunodeficiency virus type 1 entry. *J. Virol.* 77:5201–8
94. Seibert C, Ying W, Gavrilov S, Tsamis F, Kuhmann SE, et al. 2006. Interaction of small molecule inhibitors of HIV-1 entry with CCR5. *Virology* 349:41–54
95. Maeda K, Das D, Ogata-Aoki H, Nakata H, Miyakawa T, et al. 2006. Structural and molecular interactions of CCR5 inhibitors with CCR5. *J. Biol. Chem.* 281:12688–98
96. Watson C, Jenkinson S, Kazmierski W, Kenakin T. 2005. The CCR5 receptor-based mechanism of action of 8,73,140, a potent allosteric noncompetitive HIV entry inhibitor. *Mol. Pharmacol.* 67:1268–82
97. May LT, Leach K, Sexton PM, Christopoulos A. 2007. Allosteric modulation of G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 47:1–51
98. Billick E, Seibert C, Pugach P, Ketas T, Trkola A, et al. 2004. The differential sensitivity of human and rhesus macaque CCR5 to small-molecule inhibitors of human immunodeficiency virus type 1 entry is explained by a single amino acid difference and suggests a mechanism of action for these inhibitors. *J. Virol.* 78:4134–44
99. Maeda K, Nakata H, Koh Y, Miyakawa T, Ogata H, et al. 2004. Spirodiketopiperazine-based CCR5 inhibitor which preserves CC-chemokine/CCR5 interactions and exerts potent activity against R5 human immunodeficiency virus type 1 in vitro. *J. Virol.* 78:8654–62
100. Mascelli MA, Zhou H, Sweet R, Getsy J, Davis HM, et al. 2007. Molecular, biologic, and pharmacokinetic properties of monoclonal antibodies: impact of these parameters on early clinical development. *J. Clin. Pharmacol.* 47:553–65
101. Olson WC, Rabut GE, Nagashima KA, Tran DN, Anselma DJ, et al. 1999. Differential inhibition of human immunodeficiency virus type 1 fusion, gp120 binding, and CC-chemokine activity by monoclonal antibodies to CCR5. *J. Virol.* 73:4145–55
102. Lee B, Sharron M, Blanpain C, Doranz BJ, Vakili J, et al. 1999. Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. *J. Biol. Chem.* 274:9617–26

103. Wu L, LaRosa G, Kassam N, Gordon CJ, Heath H, et al. 1997. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. *J. Exp. Med.* 186:1373–81
104. Ji C, Brandt M, Dioszegi M, Jekle A, Schwoerer S, et al. 2007. Novel CCR5 monoclonal antibodies with potent and broad-spectrum anti-HIV activities. *Antiviral Res.* 74:125–37
105. Safarian D, Carnec X, Tsamis F, Kajumo F, Dragic T. 2006. An anti-CCR5 monoclonal antibody and small molecule CCR5 antagonists synergize by inhibiting different stages of human immunodeficiency virus type 1 entry. *Virology* 352:477–84
106. Zhang J, Rao E, Dioszegi M, Kondru R, DeRosier A, et al. 2007. The second extracellular loop of CCR5 contains the dominant epitopes for highly potent antihuman immunodeficiency virus monoclonal antibodies. *Antimicrob. Agents Chemother.* 51:1386–97
107. Roschke V, Clark S, Branco L, Kanakaraj P, Kaufman T, et al. 2004. *Characterization of a panel of novel human monoclonal antibodies that specifically antagonize CCR5 and block HIV entry*. Presented at 44th Annu. Intersci. Conf. Antimicrob. Agents Chemother., Washington, DC
108. Olson WC, Tsurushita N, Hinton PR, Xiong J, Ketas TJ, et al. 2002. *Potent, broad-spectrum inhibition of HIV-1 entry with the humanized monoclonal antibody PRO 140*. Presented at XIV Int. AIDS Conf., Barcelona, Spain
108a. Stoger E, Sack M, Nicholson L, Fischer R, Christou P. 2005 Recent progress in plantibody technology. *Curr Pharm Des* 11:2439–57
109. van de Laar T, Visser C, Holster M, Lopez CG, Kreuning D, et al. 2007. Increased heterologous protein production by Saccharomyces cerevisiae growing on ethanol as sole carbon source. *Biotechnol. Bioeng.* 96:483–94
110. Hannon GJ. 2002. RNA interference. *Nature* 418:244–51
111. Dykxhoorn DM, Lieberman J. 2005. The silent revolution: RNA interference as basic biology, research tool, and therapeutic. *Annu. Rev. Med.* 56:401–23
112. Kitabwalla M, Ruprecht RM. 2002. RNA interference–a new weapon against HIV and beyond. *N. Engl. J. Med.* 347:1364–67
113. Song E, Lee SK, Dykxhoorn DM, Novina C, Zhang D, et al. 2003. Sustained small interfering RNA-mediated human immunodeficiency virus type 1 inhibition in primary macrophages. *J. Virol.* 77:7174–81
114. Qin XF, An DS, Chen IS, Baltimore D. 2003. Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5. *Proc. Natl. Acad. Sci. USA* 100:183–88
115. Richman DD. 2006. Antiviral drug resistance. *Antiviral Res.* 71:117–21
116. Pantophlet R, Burton DR. 2006. GP120: target for neutralizing HIV-1 antibodies. *Annu. Rev. Immunol.* 24:739–69
117. Frost SD, Wrin T, Smith DM, Kosakovsky Pond SL, et al. 2005. Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. *Proc. Natl. Acad. Sci. USA* 102:18514–19
118. Wei X, Decker JM, Wang S, Hui H, Kappes JC, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature* 422:307–12

119. Trkola A, Kuhmann SE, Strizki JM, Maxwell E, Ketas T, et al. 2002. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. *Proc. Natl. Acad. Sci. USA* 99:395–400
120. Marozsan AJ, Kuhmann SE, Morgan T, Herrera C, Rivera-Troche E, et al. 2005. Generation and properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH-4,17,690 (SCH-D). *Virology* 338:182–99
121. Westby M, Smith-Burchnell C, Mori J, Lewis M, Mosley M, et al. 2007. Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *J. Virol.* 81:2359–71
122. Baba M, Miyake H, Wang X, Okamoto M, Takashima K. 2007. Isolation and characterization of human immunodeficiency virus type 1 resistant to the small-molecule CCR5 antagonist TAK-652. *Antimicrob. Agents Chemother.* 51:707–15
123. Pastore C, Nedellec R, Ramos A, Hartley O, Miamidian JL, et al. 2007. Conserved changes in envelope function during human immunodeficiency virus type 1 coreceptor switching. *J. Virol.* 81:8165–79
124. Pastore C, Ramos A, Mosier DE. 2004. Intrinsic obstacles to human immunodeficiency virus type 1 coreceptor switching. *J. Virol.* 78:7565–74
125. Mosier DE, Picchio GR, Gulizia RJ, Sabbe R, Poignard P, et al. 1999. 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.* 73:3544–50
126. Speck RF, Wehrly K, Platt EJ, Atchison RE, Charo IF, et al. 1997. Selective employment of chemokine receptors as human immunodeficiency virus type 1 coreceptors determined by individual amino acids within the envelope V3 loop. *J. Virol.* 71:7136–39
127. Wolinsky SM, Veazey RS, Kunstman KJ, Klasse PJ, Dufour J, et al. 2004. Effect of a CCR5 inhibitor on viral loads in macaques dual-infected with R5 and X4 primate immunodeficiency viruses. *Virology* 328:19–29
128. Pugach P, Marozsan AJ, Ketas TJ, Landes EL, Moore JP, Kuhmann SE. 2007. HIV-1 clones resistant to a small molecule CCR5 inhibitor use the inhibitor-bound form of CCR5 for entry. *Virology* 361:212–28
129. Pastore C, Picchio GR, Galimi F, Fish R, Hartley O, et al. 2003. Two mechanisms for human immunodeficiency virus type 1 inhibition by N-terminal modifications of RANTES. *Antimicrob. Agents Chemother.* 47:509–17
130. Ramjee G, Shattock R, Delany S, McGowan I, Morar N, Gottemoeller M. 2006. Microbicides 2006 conference. *AIDS Res. Ther.* 3:25
131. Johnson VA, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, et al. 2005. Update of the drug resistance mutations in HIV-1: 2005. *Top. HIV Med.* 13:51–57
132. Fatkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, et al. 2005. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat. Med.* 11:1170–72

133. Lalezari J, Thompson M, Kumar P, Piliero P, Davey R, et al. 2005. Antiviral activity and safety of 8,73,140, a novel CCR5 antagonist, during short-term monotherapy in HIV-infected adults. *AIDS* 19:1443–48
134. Schuermann D, Pechardscheck C, Rouzier R, Nougarede R, Faetkenheuer G, et al. 2005. *SCH 4,17,690: antiviral activity of a potent new CCR5 receptor antagonist*. Presented at 3rd Int. AIDS Soc. Conf. HIV Pathog. Treatm., Rio de Janeiro, Brazil
135. Crabb C. 2006. GlaxoSmithKline ends aplaviroc trials. *AIDS* 20:641
136. Greaves W, Landovitz R, Fatkenheuer G, Hoffmann C, Antunes F, et al. 2006. *Late virologic breakthrough in treatment-naïve patients on a regimen of Combivir + vicriviroc*. Presented at 13th Conf. Retrovir. Oppor. Infect., Denver, CO
137. Mayer H. 2005. *A case of severe hepatotoxicity*. Presented at 1st Int. Workshop Target. HIV Entry, Bethesda, MD
138. Pfizer Inc. 2007. Maraviroc tablets NDA 22–128. Antiviral Drugs Advisory Committee (AVDAC) Brief. Doc., April 24
139. Murga JD, Franti M, Pevear DC, Maddon PJ, Olson WC. 2006. Potent antiviral synergy between monoclonal antibody and small-molecule CCR5 inhibitors of human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 50:3289–96
140. Ji C, Zhang J, Dioszegi M, Chiu S, Rao E, et al. 2007. CCR5 small molecule antagonists and monoclonal antibodies exert potent synergistic antiviral effects by co-binding to the receptor. *Mol. Pharmacol.* 72:18–28
141. Roche. 2007. Fuzeon prescribing information. **http://www.rocheusa.com/products/fuzeon**
142. Veazey RS, Klasse PJ, Ketas TJ, Reeves JD, Piatak M Jr, et al. 2003. Use of a small molecule CCR5 inhibitor in macaques to treat simian immunodeficiency virus infection or prevent simian-human immunodeficiency virus infection. *J. Exp. Med.* 198:1551–62
143. Davis CW, Doms RW. 2004. HIV Transmission: Closing all the doors. *J. Exp. Med.* 199:1037–40
144. Veazey RS, Klasse PJ, Schader SM, Hu Q, Ketas TJ, et al. 2005. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* 438:99–102
145. Lederman MM, Veazey RS, Offord R, Mosier DE, Dufour J, et al. 2004. Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* 306:485–87
146. 2006. Int. Meet. Inst. Hum. Virol., Baltimore, Md., 17–21 Nov. *Retrovirology* 3(Suppl. 1):S1–109, P1–79 (Abstr.)
147. Veazey RS, Springer MS, Marx PA, Dufour J, Klasse PJ, Moore JP. 2005. Protection of macaques from vaginal SHIV challenge by an orally delivered CCR5 inhibitor. *Nat. Med.* 11:1293–94
148. Turner D, Wainberg MA. 2006. HIV transmission and primary drug resistance. *AIDS Rev.* 8:17–23
149. 2005. Merck and Bristol-Myers Squibb License New AIDS Drugs to IPM for Development as Microbicides to Protect Women from HIV.

http://www.ipm-microbicides.org/news_room/english/press_releases/2005/2005_1031_bms.htm

150. Takashima K, Miyake H, Kanzaki N, Tagawa Y, Wang X, et al. 2005. Highly potent inhibition of human immunodeficiency virus type 1 replication by TAK-220, an orally bioavailable small-molecule CCR5 antagonist. *Antimicrob. Agents Chemother.* 49:3474–82
151. Baba M, Takashima K, Miyake H, Kanzaki N, Teshima K, et al. 2005. TAK-652 inhibits CCR5-mediated human immunodeficiency virus type 1 infection in vitro and has favorable pharmacokinetics in humans. *Antimicrob. Agents Chemother.* 49:4584–91
152. Strizki JM, Xu S, Wagner NE, Wojcik L, Liu J, et al. 2001. SCH-C (SCH 3,51,125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 98:12718–23
153. Strizki JM, Tremblay C, Xu S, Wojcik L, Wagner N, et al. 2005. Discovery and characterization of vicriviroc (SCH 4,17,690), a CCR5 antagonist with potent activity against human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 49:4911–19
154. Kuhmann SE, Pugach P, Kunstman KJ, Taylor J, Stanfield RL, et al. 2004. Genetic and phenotypic analyses of human immunodeficiency virus type 1 escape from a small-molecule CCR5 inhibitor. *J. Virol.* 78:2790–807
155. Westby M, Mori J, Smith-Burchnell C, Lewis M, Mosley M, et al. 2005. *Maraviroc (MVC, UK-4,27,857)-resistant HIV-1 variants, selected by serial passage, are sensitive to CCR5 antagonists (GW873140, Schering-C, Schering-D) and T-20 (enfuvirtide)*. Presented at XIV Int. Drug Resist. Workshop, Québec City, Can.
156. Progenics. 2007. Progenics announces positive results in clinical trial of novel HIV therapy. Press release, May 1
157. Lalezari J, Lederman M, Yadavalli G, Para M, DeJesus E, et al. 2006. *A phase 1, dose-escalation, placebo controlled study of a fully human monoclonal antibody (CCR5mAb004) against CCR5 in patients with CCR5 tropic HIV-1 infection*. Presented at 46th Int. Conf. Antimicrob. Agents Chemother., San Francisco, CA

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Errata

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