

Entry and Fusion Inhibitors: an Update

Joseph J. Eron, MD

Associate Professor of Medicine, University of North Carolina at Chapel Hill
Director, Clinical Core, UNC Center for AIDS Research

Christine M. Hogan, MD

Research Associate, The Aaron Diamond AIDS Research Center
Rockefeller University

SUMMARY BY TIM HORN

EDITED BY CALVIN COHEN, MD, AND JOHN MOORE, PhD

Reprinted from *The PRN Notebook*, MARCH 2002.

Dr. James F. Braun, Editor-in-Chief. Tim Horn, Executive Editor.

Published in New York City by the Physicians' Research Network, Inc.®

John Graham Brown, Executive Director. For further information and other articles available online, visit <http://www.PRN.org> All rights reserved. © MARCH 2002.

E RADICATION OF HIV HAS NOT BEEN POSSIBLE with currently available reverse transcriptase inhibitors and protease inhibitors; and multiclass drug-resistant mutants of HIV—along with a multitude of disabling and sometimes life-threatening side effects—are a growing threat. Thus, there is a great need for compounds that target non-protease and reverse transcriptase elements of the HIV lifecycle. Not only might such novel therapies increase the potency of initial HIV treatment, but they may also provide hope for patients who have exhausted current treatment options.

This article, based on PRN lectures delivered by Drs. Joseph Eron and Christine Hogan in January 2002, reviews one of the most promising areas of HIV drug development: inhibitors of HIV fusion and entry. As reviewed by both investigators, two fusion inhibitors targeting HIV's gp41 envelope protein (T-20 and T-1249) continue to advance through clinical trials and have both received fast-track designations by the U.S. Food and Drug Administration. As for other entry inhibitors targeting cellular proteins in the earlier stages of development (e.g., inhibitors of CD4, CCR5, and CXCR4), Drs. Eron and Hogan noted some of the advances and setbacks that have occurred over the past few years.

HIV Fusion and Entry

SINCE 1996, WHEN THE ELUSIVE CD4+ CELL coreceptors CCR5 and CXCR4 were discovered, the process by which HIV gains entry into these cells has been a major focus of research (see "The HIV Envelope Glycoproteins: Their Roles in Virus Entry and as a Vaccine Target," published in the March 1999 issue of *The PRN Notebook*). What we now know about this process is much more

complete, and understanding the details involved has led to the development of inhibitors for each step of HIV entry.

The HIV fusion and entry process begins with interactions between the trimeric envelope complex—a cluster of proteins on HIV's outer coat, sometimes referred to as the gp160 spike—and both CD4 and a chemokine coreceptor (either CCR5 or CXCR4) on the cell surface. This complex is made up of three transmembrane glycoproteins (gp41), which anchor the cluster to the virus, and three extracellular glycoproteins (gp120), which contain the binding domains for both CD4 and the chemokine receptors.

As illustrated in Figure 1, the first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to the N-terminal membrane-distal domain of CD4. Once bound, the envelope complex undergoes a structural change, bringing the chemokine binding domains of gp120 into proximity with the chemokine receptor on the CD4+ cell, allowing for a more stable two-pronged attachment.

With the virus now latched on to both CD4 and the chemokine receptor, additional conformational changes take place in the envelope complex, whereby gp41, originally tucked away within the trimeric complex, is exposed and the N-terminal fusion peptide of gp41 enters the CD4+ cell's membrane. From there, two heptad repeat sequences (HR1 and HR2) of gp41 interact, resulting in collapse of the extracellular portion of gp41 to form a hairpin, which is sometimes referred to as a coiled-coil bundle. The hairpin structure brings the virus and cell membrane close together, allowing fusion of the membranes and subsequent entry of the virus core.

While additional details of this multistep process are still being worked out in the

lab, enough has been learned about the three major steps involved in HIV fusion and entry—the binding of gp120 to CD4, the binding of gp120 to either CCR5 or CXCR4, and the formation of gp41 fusion determinants that allows the virus to mesh with its cellular target—to develop therapeutic strategies targeting each of these steps. Indeed, a number of compounds are in various stages of development. For individuals with HIV resistant to any or all of the current inhibitors of reverse transcriptase and protease, these fusion and entry inhibitors represent truly unique classes of drugs.

Attachment Inhibitors (Agents Targeting CD4)

THE INITIAL INTERACTION OF HIV GP120 WITH CD4 on the cell surface offers the highly conserved CD4 binding site on gp120 as a therapeutic target. Unfortunately, this approach is not without caveats. As explained by Dr. John Moore in the March 1999 *Notebook* article referenced above, the conserved CD4 binding sites of gp120 are protected by glycosylation—a flexible layer of sugars that can occlude the peptide backbone of gp120, alter its antigenicity, and reduce its immunogenicity. This has been one of the main concerns regarding the potential effectiveness of the gp120 vaccine (Vaxgen) for the prevention and possibly immune-based treatment of HIV infection. Similarly, studies employing a soluble form of CD4 (rSCD4) as a therapeutic compound showed promise *in vitro* but had little noticeable activity against primary HIV isolates and was rapidly cleared from the body (Daar, 1990; Schooley, 1990; Schacker, 1995).

Fortunately, newer compounds continue

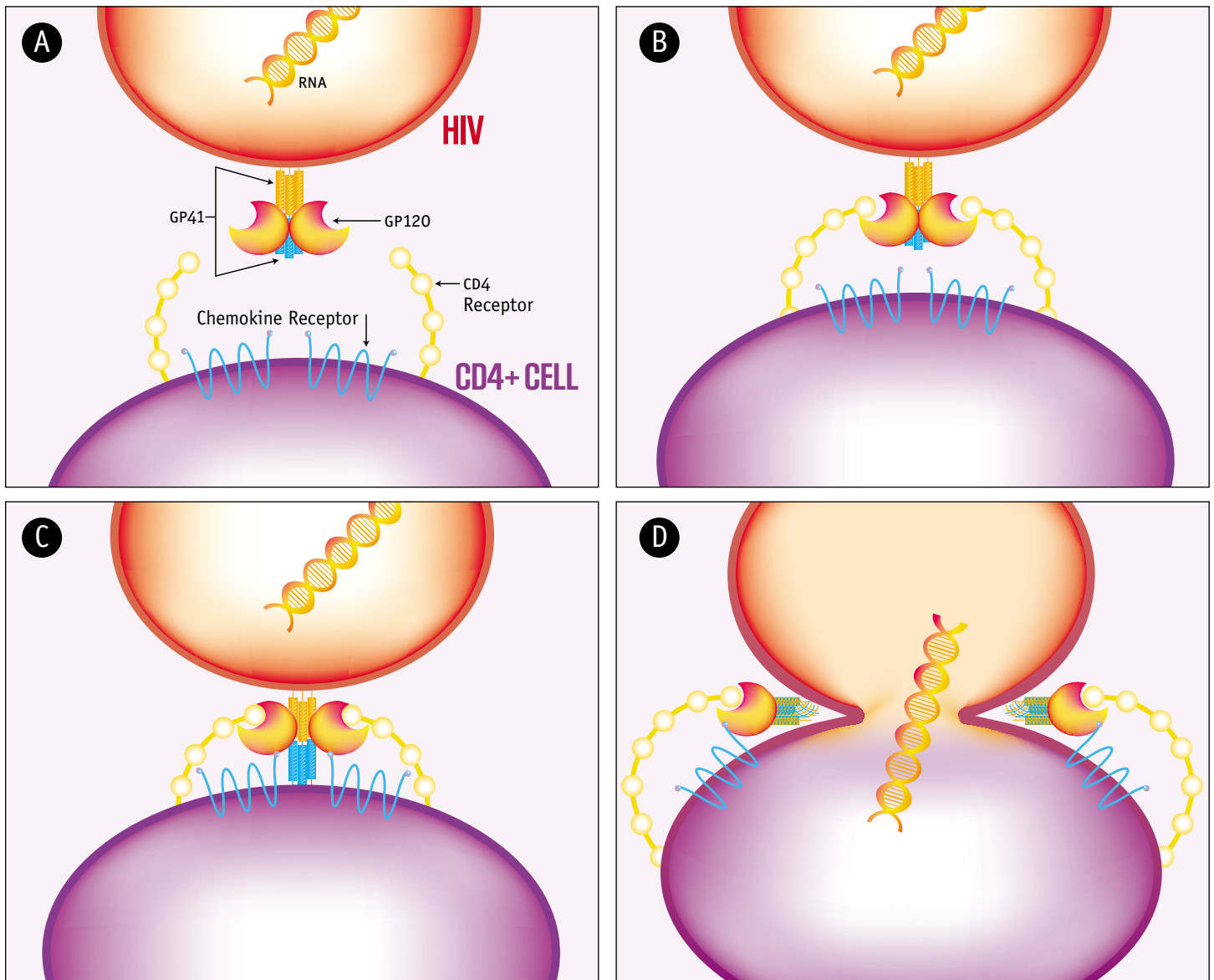


FIGURE 1. HIV Fusion and Entry.

PANEL A: The process begins with interactions between the trimeric envelope complex—a cluster of proteins on HIV’s outer coat, sometimes referred to as the gp160 spike—and both CD4 and a chemokine receptor (either CCR5 or CCR4) on the cell surface. This complex is made up of three transmembrane glycoproteins (gp41), which anchor the cluster to the virus, and three extracellular glycoproteins (gp120), which contain the binding domains for both CD4 and the chemokine receptors.

PANEL B: The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to the N-terminal membrane-distal domains of CD4. CD4 attachment inhibitors (e.g., PRO 542) act here.

PANEL C: Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, bringing the chemokine binding domains of gp120 into proximity with the chemokine receptor, allowing for a more stable two-pronged attachment. Antagonists of CCR5 (e.g., SCH-C) and CXCR4 act here. If the virus latches on to both CD4 and the chemokine receptor, additional conformational changes allow for the N-terminal fusion peptide of gp41 to enter the CD4+ cell membrane.

PANEL D: Two heptad repeat sequences—HR1 (blue) and HR2 (orange)—of gp41 interact, resulting in collapse of the extracellular portion of gp41 to form a hairpin, which is sometimes referred to as a coiled-coil bundle. The fusion inhibitors T-20 and T-1249 act here by mimicking HR2, resulting in a botched formation of the hairpin. In the absence of an inhibitor, the hairpin structure brings the virus and cell membrane close together, allowing fusion of the membranes and subsequent entry of viral RNA.

to hold promise as attachment inhibitors. Of particular interest are two vaginal microbicides: PRO 2000 and Cyanovirin-N. PRO 2000, a naphthalene sulfonate polymer that is active against a wide range of HIV isolates, is currently in phase II clinical trials being conducted around the world. Cyanovirin-N, a mannose binding protein derived from the blue-green algae *Nostoc ellipsosporum*, is active against a diverse range of viruses with mannose-rich glycoproteins, including HIV-1, HIV-2, and human herpes virus-6 (HHV-6). Both compounds are applied topically and are being followed closely by advocates for women's health and HIV prevention.

PRO 542

THERE IS ALSO PROGENICS PHARMACEUTICALS' PRO 542, also known as CD4-IgG2. Like rSCD4, PRO 542 is a soluble CD4 receptor that binds to and neutralizes gp120 before binding can occur. The CD4 receptor region is integrated into an immunoglobulin molecule to form a tetrameric protein that can be synthesized using monoclonal antibody technology. This structure has allowed for a prolonged half-life of PRO 542 in blood plasma which, according to the manufacturer, has been associated with better *in vivo* responses than those seen with rSCD4. *In vitro* data also conclude that PRO 542 is active against primary HIV isolates.

Results from phase I/II clinical trials of PRO 542 involving HIV-positive adults and children were published in 2000. In the adult phase I study, volunteers were treated with a single intravenous infusion of PRO 542 at doses of 0.2-10 mg/kg (Jacobson, 2000). PRO 542 was well tolerated, and no dose-limiting toxicities were identified. AUC and peak serum concentrations increased linearly with dose, and a terminal serum half-life of three to four days was observed. No patient developed antibodies to PRO 542. Transient HIV-RNA decreases were reported after single-dose administration.

In the phase I/II study enrolling 18 HIV-positive children, PRO 542 was evaluated by single and multidose intravenous infusions (Shearer, 2000). The drug was well tolerated, and, as with the adults, dose proportionality was observed in terms of AUC and peak serum concentrations. Noticeable decreases of approximately 0.7 log copies/mL in plasma HIV-RNA levels were seen in four of the six children treated with four weekly 10 mg/kg doses. After two weeks of treatment, three children

had sustained reductions in serum HIV-RNA; the other children had rebounded to baseline levels.

A second set of phase II clinical trials, which are also being conducted in adults and children, were kicked off in 2000. These studies will include patients with HIV resistant to current antiretroviral options. The drug is currently being evaluated in an improved formulation for subcutaneous administration.

Chemokine Receptors (CCR5 Antagonists)

ANOTHER SITE IN THE HIV ENTRY PROCESS that has been the focus of therapeutic research is the CD4-dependent interaction between HIV gp120 and the CCR5 chemokine receptor. It has been established that CCR5 is the receptor responsible for the fusion of macrophage-tropic HIV—now usually referred to as an R5 strain—the most common primary strain of the virus transmitted from one person to another. Between 1% and 2% of Caucasians are homozygous for the 32 base-pair deletion in the CCR5 gene, which effectively blocks the expression of CCR5 on the surface of their CD4+ cells and ultimately affords significant protection against HIV infection. Similarly, between 15% and 20% of individuals of Western European descent who are heterozygous for this mutation express reduced levels of CCR5 and, consequently, have exhibited delayed progression to AIDS in most studies.

Anti-HIV protection seems to result from a combination of reduced expression of CCR5 on cells and increased production of the chemokines (RANTES, MIP-1 α and MIP-1 β) that are the natural binding agents (or "ligands") for this receptor. In other words, not only are there fewer CCR5 receptors for HIV to latch on to, there are fewer receptors accessible to HIV that are not already being used by these binding proteins. "Based on these observations," Dr. Hogan explained, "along with those suggesting that the genetic absence of CCR5 is of little consequence to overall health, there has been a significant push in recent years to develop therapeutic strategies that block the binding of HIV to this receptor."

This is not to say that CCR5 antagonists are without their potential caveats. As discussed above, HIV phenotypes responsible for establishing infection in a new host are predominantly CCR5-tropic, whereas the

emergence of CXCR4-tropic variants is widely considered to be associated with accelerated loss of CD4+ cells and progression to AIDS (Koot, 1993). Although the evolution from CCR5- to CXCR4-tropic viruses is slow in HIV-infected individuals, there has been some concern that suppression of HIV infection with a CCR5 antagonist might accelerate the selection for CXCR4- or dual-tropic (CCR5/CXCR4) strains. "It still remains unclear whether or not this phenotypic switch is a consequence or a cause of disease progression," added Dr. Hogan. "Is it that the virus changes tropism and induces disease progression? Or is it that disease progression is already happening, resulting in loss of immune control of CXCR4-tropic virus? We are not really sure yet."

At least one report, published in the *Journal of Virology* in 1999, has documented a switch from R5 to X4-tropic viruses using CCR5 antagonists (Mosier, 1999). In this study, two chemokine analogues—AOP-RANTES and NNY-RANTES—were tested for their ability to prevent HIV-infection and to select for coreceptor switch variants in the hu-PBL SCID mouse model of HIV infection. Mice were infected with an R5-tropic HIV isolate that requires only one or two amino acid substitutions to use CXCR4 as a coreceptor. Even though it achieved lower circulating concentrations than AOP-RANTES, NNY-RANTES was more effective in preventing HIV infection. However, in a subset of treated mice, these levels of NNY-RANTES rapidly selected viruses with mutations in the v3 loop of envelope that altered coreceptor usage. This process led to the emergence of CXCR4-utilizing escape variants within one week of treatment with NNY-RANTES. While *in vitro* replication analysis suggested that the X4 variants were less fit than the parental R5 virus, the variants persisted *in vivo* for three weeks after the chemokine analogue was removed from the system.

More recent data, published earlier this year in the *Proceedings of the National Academy of Sciences*, casts some doubt on the likelihood that CXCR4-tropic HIV variants will emerge with the use of clinically relevant CCR5 inhibitors (Trkola, 2002). While this may be interpreted as good news, these data show that, even *in vitro*, HIV was able to escape inhibition by a CCR5 antagonist and still use the CCR5 receptor—an observation that is on a par with drug resistance seen with current antiretroviral agents. As a result, the use

of these chemokine-receptor inhibitors as monotherapy is not likely to be successful.

In this study, the team passaged the virus several times in PBMCs with increasing concentrations of the CCR5-specific small molecule inhibitor AD101. By 19 passages, an escape mutant emerged with greater than 20,000-fold resistance to AD101. However, the escape mutant was unable to use CXCR4 or any other tested coreceptor (e.g., CCR2 or CCR3) to enter cells. Instead, HIV apparently acquires the ability to use CCR5 despite the inhibitor, first by requiring lower levels of CCR5 for entry and then probably by using the drug-bound form of the receptor.

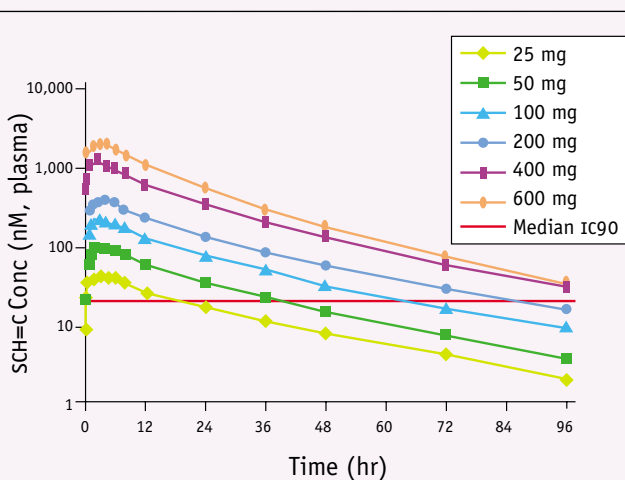
Several inhibitors of CCR5-mediated HIV entry have been shown to prevent R5 virus infection *in vitro*. These include chemokines themselves, modified chemokines (e.g., AOP-RANTES, NNY-RANTES, 9-68 RANTES, 3-68 RANTES, Met RANTES), monoclonal antibodies to CCR5 (e.g., PRO 140), and small-molecule antagonists.

SCH-C

OF PARTICULAR INTEREST TO BOTH DRs. ERON and Hogan was the orally bioavailable CCR5 antagonist Schering C (SCH-C), which is being developed by Schering-Plough Research Institute. SCH-C is one of several small-molecule agents that have been studied as potential antagonists of CCR5. Others include TAK-779, which will no longer be developed because of bioavailability and formulation concerns, and SCH-D, a second CCR5 antagonist from Schering-Plough currently in the preliminary stages of laboratory testing.

A detailed overview of preclinical data surrounding SCH-C was provided by Dr. Hogan and is summarized in a research paper appearing in the October 21, 2001, issue of the *Proceedings of the National Academy of Sciences* (Strizki, 2001). In short, SCH-C is an oxime-piperidine compound that, according to several *in vitro* evaluations, is a bona fide antagonist of CCR5 receptor binding and signal transduction. *In vitro* observations also suggest that SCH-C is CCR5-exclusive—it has no effect on infection of CXCR4-expressing cells—and has broad and potent antiviral activity against primary CCR5-tropic HIV isolates.

FIGURE 2. SCH-C Plasma Profiles Following Single Oral Dose in Humans



Source: Schering-Plough Research Institute

Antiviral and pharmacokinetic properties of SCH-C have been evaluated in laboratory animals. In SCID-hu mice, the compound strongly inhibited the replication of a CCR5-tropic isolated. In rats and primates, SCH-C had a favorable pharmacokinetic profile, with an oral bioavailability of 50% to 60% and a serum half-life of five to six hours.

Dr. Hogan also reviewed some unpublished pharmacokinetics and safety data from single-dose and multiple-dose studies conducted in HIV-negative volunteers. The single-dose study enrolled 54 individuals who were allotted to receive one of six doses: 25mg, 50mg, 100mg, 200mg, 400mg, and 600mg. Six volunteers in each group received the compound; three received placebo. While the pharmacokinetics of SCH-C varied, depending on the dose administered, plasma levels were above the IC_{90} for most of the doses employed. Twenty-four hours after SCH-C was administered, the drug's C_{min} was still above the IC_{90} , suggesting that once-daily dosing is possible.

Of particular concern in this single-dose study was QT prolongation observed in the individuals receiving the highest dose of SCH-C. According to data released by Schering-Plough, the mean maximal increase in the QT interval, among individuals who received the 600 mg dose, was 60 msec. There were no symptomatic events (e.g., arrhythmias), although more extensive follow-up testing (e.g., Holter monitoring) was generally not conducted. These observations led the U.S. Food and Drug Administration to put the SCH-C development pro-

gram on hold. However, the hold on development has since been lifted.

In a multiple-dose study involving HIV-negative volunteers, the doses selected were 50mg, 100mg, 200mg, and 400mg—all to be administered twice daily. In compliance with requests from the FDA, a 600mg dose was abandoned and all volunteers were to be hospitalized for close monitoring for the 14 days of on-treatment evaluation. The four groups were evaluated sequentially, as opposed to collectively, to gather preliminary safety information before studying a higher dose in a new group of individuals.

While there were no QT interval problems reported in the individuals who received either the 50mg or 100mg BID doses of SCH-C, there were signs of QT prolongation at the 200mg dose, thus prompting the investigators to drop the 400mg group. The QT prolongation observed in the 200mg group was rapidly reversed upon stopping therapy.

Dr. Hogan pointed out that a clinical trial evaluating SCH-C monotherapy in HIV-infected individuals is currently open to enrollment. One site is open in Montpellier, France, under the direction of Professor Jacques Reynes, and another site is open at the Aaron Diamond AIDS Research Center in New York. Other expected research sites in this, and perhaps other, SCH-C studies include the Institute of Human Virology in Baltimore and the University of Pennsylvania in Philadelphia.

Three doses have been selected for evaluation—25 mg, 50 mg, and 100 mg, all administered twice daily—and a safety review will be conducted for each dose before moving on to the next higher dose. Data involving the first 12 patients to receive SCH-C 25 mg BID were reported recently in Seattle, at the 9th Conference on Retroviruses and Opportunistic Infections (Reynes, 2002). After ten days, the average reduction in HIV-RNA was approximately 0.7 log. The most common adverse events were mild headaches and altered taste; no toxicity-related discontinuations were reported. Some QT prolongation was observed—the mean increase was 11.5 msec after ten days of treatment. Antiviral activity and safety data involving the 50 mg dose will likely be presented this Spring.

Chemokine Receptors (CXCR4 Antagonists)

APPROXIMATELY 40% OF HIV-POSITIVE people develop phenotypic strains that can use CXCR4 in addition to, or sometimes instead of, CCR5 as a coreceptor (Simmons, 1996; Berger, 1997). As stated above, CXCR4 is associated with HIV disease progression, a finding that raises the possibility of using inhibitors of this chemokine to halt the pathogenic activity of CXCR4-tropic virus.

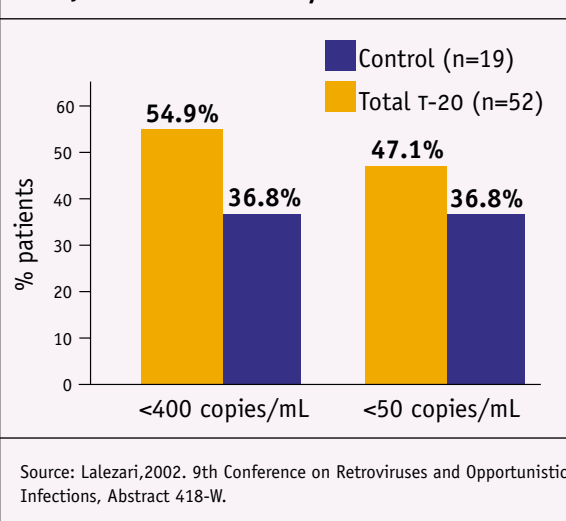
There has been some concern that, because CXCR4 is expressed on a much wider range of cell types than CCR5, the potential for adverse effects resulting from CXCR4 inhibition may be greater. In fact, CXCR4 knockout mice have been shown to have multiple birth defects, including: abnormal B-cell lymphopoiesis and bone-marrow myelopoiesis, abnormal heart development, poor CNS development, decreased vascularization of the gastrointestinal tract, and prenatal or neonatal death.

Even without these very serious concerns, initiatives to develop an effective CXCR4 antagonist have been plagued by formulation shortcomings. ALX40-4C, a 9 D-amino acid peptide developed by Allelix Pharmaceuticals, was pulled by the manufacturer in 1997 because of limited efficacy and formulation problems. There was also AMD3100, a small molecule in the bicyclam family that demonstrated potent antiviral activity *in vitro*. *In vivo* experiments in Thy/Liv SCID-hu mice showed that AMD3100 prevented the replication of CXCR4-tropic virus, but not CCR5-tropic HIV. Interestingly, AMD3100 partially suppressed dual-tropic CCR5/CXCR4 isolates in the mice; the recovered virus was unable to use CXCR4 and had lost its syncytia-inducing phenotype.

Development of AMD3100 was halted in May 2001 because of a possible link between cardiac toxicity and high doses of the drug (lower, potentially safer doses of AMD3100 had unimpressive antiviral activity in preliminary studies involving HIV-infected patients). Moreover, AMD3100 treatment resulted in a threefold increase in white blood cells in some clinical trial participants, probably because of WBC mobilization from tissues into the blood (Hendrix, 2000). Anormed, however, is still moving forward with an orally bioavailable analogue of AMD3100, dubbed AMD8664.

Other CXCR4 antagonists in the preclin-

FIGURE 3. T20-206: 48-Week HIV-RNA Responses to HAART +/- T-20



ical stages of development include: variants of SDF-1, a natural ligand of CXCR4; T-22, an injectable 18 amino acid peptide; and T-134 and T-140, two 9 D-amino acid peptides.

Fusion Inhibitors (gp41 Binders)

FURTHEST ALONG IN DEVELOPMENT ARE THE fusion inhibitors—compounds that block infection by preventing HIV from fusing with and inserting its genetic machinery into host cells. “We’ve had our eyes on these compounds for quite awhile,” commented Dr. Eron. “Now that T-20 has been studied in a few phase II studies and is currently in phase III trials, we’re getting a better sense of what we can expect from this drug.”

T-20

T-20, A PEPTIDE FUSION INHIBITOR, IS BEING developed by Trimeris, Inc., in collaboration with Hoffmann-LaRoche. It is a conserved 36 amino acid synthetic C-peptide that corresponds to a region of the C-helix structure (HR-2) of gp41. The C-peptide goes to work after gp120 has successfully docked with the CD4 receptor. T-20 mimics the activity of HR2 and binds with HR1. This, in turn, effectively blocks the conformational changes that are required to secure viral/cellular membrane fusion.

Because T-20 is a peptide, it must be infused intravenously (IV) or injected subcutaneously (SC). Initial proof-of-principle (TRI-001) and dose-escalation (TRI-003) studies found that twice-daily SC injections of T-20 (100 mg BID) were superior to continu-

ous infusion. “Using an insulin pump to deliver T-20 was not successful,” commented Dr. Eron. “The infusion lines kept clogging up, setting off the pump alarms.” Pharmacokinetic analyses showed a half-life of 2.3 to 2.4 hours and bioavailability levels between 60% to 70% following SC administration. Viral load decreases of up to 1.5 log were reported in both studies. “We really have beautiful pharmacokinetics with the SC injections,” he added. “Nice and steady flat PK curve from dose to dose.”

The original formulation of T-20 required two 50 mg injections twice daily. The manufacturers have improved the formulation such that a single injection can deliver 90 mg of T-20. Twice-daily administration will still be required (Lalezari, 2001).

Volunteers who participated in either TRI-001 or TRI-003 were permitted to enroll in a larger and longer safety and efficacy trial (T20-205) (Lalezari, 2000). Prior to entering the study, the 71 patients who enrolled had failed, on average, 10 antiretrovirals—97% had failed at least one protease inhibitor and 79% had failed at least one drug in all three currently available classes. The median baseline viral load was 4.8 log, and the median baseline CD4+ count was 135 cells/mm³.

Patients received twice-daily 50 mg injections of T-20 in combination with other antiretroviral drugs that were chosen on the basis of treatment history and genotype. After 48 weeks of follow-up, only 41/71 (58%) remained on therapy. Of those individuals who completed the trial (on-treatment analysis), 56% had viral loads either below 400 copies/mL or tenfold below baseline, and 22% had HIV-RNA levels below 50 copies/mL. Evaluating the results using an intent-to-treat analysis, 33% of the study population had HIV-RNA levels below 400 copies/mL or tenfold below baseline, and 13% had viral load levels below 50 copies/mL after 48 weeks.

Another component of the T20-205 study was a post-trial quality-of-life questionnaire, discussed last year by Dr. Calvin Cohen at the First International AIDS Society Conference on HIV Pathogenesis and Treatment in Buenos Aires (Cohen, 2001). Roughly half (51%) of the patients rated the ease of injection as “very easy/easy;” 36% rated the twice-daily injections as “not bad,” and 13% rated them “some-

what difficult.” The majority of participants felt that sc injections did not interfere with their normal daily life, and 94% said that they would continue to use the injections.

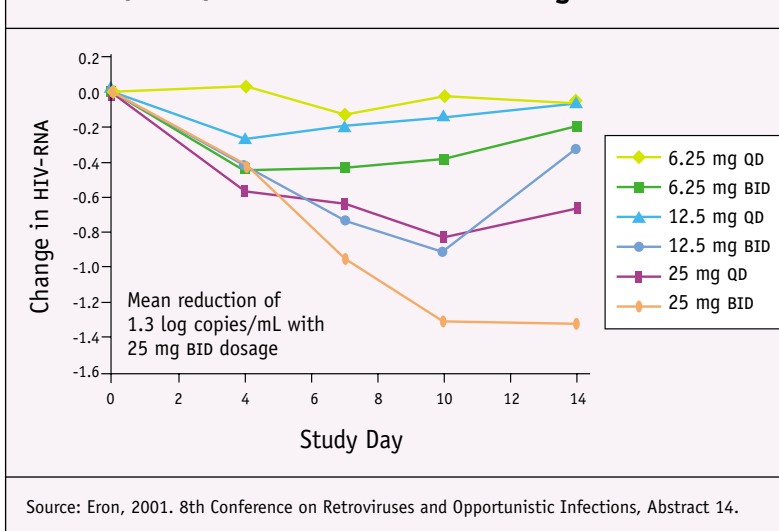
Forty-eight-week data from the first randomized and controlled clinical trial of T-20 were reported recently at the 9th Conference on Retroviruses and Opportunistic Infections in Seattle (Lalezari, 2002). The study compared three doses of T-20 in combination with a regimen of approved anti-retrovirals—abacavir (Zi-

agen), amprenavir (Agenerase), low-dose zidovudine (Retrovir), and efavirenz (Sustiva)—in 71 patients who were naive to non-nucleoside reverse transcriptase inhibitors but experienced with protease inhibitors and nucleoside reverse transcriptase inhibitors. The subjects were randomized in an open-label fashion to one of four treatment groups: control regimen without T-20 (arm A) or control regimen with either 50 (arm B), 75 (arm C), or 100 mg (arm D) administered twice daily via sc injection.

At baseline, the median viral load was 4.27 log and the CD4+ count was 232 cells/mm³. After 48 weeks of follow-up, HIV-RNA levels were -2.39 log below baseline in patients receiving 100 mg T-20 in conjunction with standard antiretroviral therapy. In the control group, viral load was -1.87 log below baseline, compared to -2.10 log and -2.62 log in the 50 mg and 75 mg T-20 groups, respectively. The percentages of patients with HIV-RNA levels below 400 copies/mL and 50 copies/mL are reported in Figure 3.

As for immunologic responses at 48 weeks, there was a total CD4+ count gain of 90 cells/mm³ in the control group, compared to a gain of 124 CD4+ cells/mm³ in the 100 mg T-20 group. In terms of side effects, mild to moderate local injection site reactions have been the most frequently reported T-20-related adverse events, occurring in approximately two-thirds of patients who have received the treatment in clinical trials. “The most common adverse effect of T-20 has been nodules that form at the injections site in some patients,” Dr. Eron said. “Despite these nodules, some of our patients have been receiving injec-

FIGURE 4. T1249-101: Mean HIV-RNA Change from Baseline



tions, twice daily, for more than two years. The appearance of these nodules can be frustrating for some of these individuals, when the nodules occur with every injection. A few patients have opted to discontinue therapy in part because of this adverse effect.”

The incidence of other clinical adverse events and laboratory abnormalities was similar between the T-20 arms and the control arm in T20-206 and did not appear to be dose-dependent in any of the preliminary study results reported thus far.

Both *in vitro* and *in vivo* data indicate that HIV resistance to T-20 can and does occur. HIV variants with decreased sensitivity to T-20 contain mutations in the HR1 region of the gp41 complex. The most significant mutations are those found in the GIVQQ sequence, located near the N-terminus of the HR1 region. “The fact is, we do have resistance to T-20,” explained Dr. Eron. “We’re still going to need new agents, including new fusion inhibitors like T-1249.” Fortunately, *in vitro* data suggest that T-20 does not induce cross-resistance to T-1249, which binds to a partially overlapping but distinct region of HR1. Dr. Eron also mentioned that several companies with drug-resistance assays are fine-tuning their tests to allow for the genotypic and phenotypic assessment of plasma samples, once T-20 is approved for general use.

There has been some consternation regarding the possibility that, because T-20 (and its potential successor T-1249) is a peptide, it will elicit a humoral response *in vivo*. T-20 is immunogenic in animals and humans, although no data reported thus far indicates that antibody responses to either

drug—even those present in high levels—have any effect on the molecules’ pharmacokinetics or effectiveness.

T-20 has been evaluated in HIV-positive children. Twelve treatment-experienced or treatment-naive children ages three to 12 were evaluated in a Phase I/II study being conducted in collaboration with the Pediatric AIDS Clinical Trials Group (PACTG 1005) (Church, 2001). The preliminary findings suggest that short-term (up to 12 weeks) subcutaneous dosing with T-20 is well tolerated by children,

and that in the highest dose group (60 mg/m²) the drug resulted in rapid suppression of HIV-RNA of approximately tenfold average reduction from baseline levels in seven days.

When standard antiretroviral drugs were added to T-20, this tenfold reduction was maintained in the majority of children (three of four subjects in the 30 mg/m² group and six of seven subjects in the 60 mg/m² group) who had crossed the eight-week time point. In the study, T-20 was well tolerated. One child discontinued treatment due to aversion to injections but no child discontinued due to adverse events. Mild to moderate injection site reactions (erythema, induration, pruritis, discomfort) occurred in eight of 12 children but were usually mild.

With respect to the phase III development program initiated by Trimeris/Roche, two studies are currently under way: T20-301 and T20-302. The T20-301 trial is evaluating T-20 as a component of salvage therapy in the United States, Canada and Latin America. HIV-positive individuals with greater than six months of experience with all three of the currently approved antiretroviral classes are eligible. The T20-302 study is similar to the T20-301 study, but will be conducted in Europe and Australia. Both studies are now fully enrolled—approximately 525 individuals are participating in each trial—and will gather at least 48 weeks of on-treatment follow-up data.

“If I were to guess, I expect that T-20 will go before the Food and Drug Administration this fall,” Dr. Eron speculated. “If the stars and planets line up just right, this drug might be approved by late 2002. I’d be shocked if the phase III studies did not

mirror the encouraging results we saw in earlier studies, so I really do think we have this drug to look forward to.”

T-1249

T-1249, ANOTHER COMPOUND BEING DEVELOPED by Trimeris, Inc., is the second HR-2 peptide analogue fusion inhibitor to enter clinical trials. This molecule, a 39 amino acid peptide, is active against HIV strains resistant to both standard antiretrovirals and variants resistant to T-20.


Animal studies have determined that the bioavailability of T-1249 averages 90% for the subcutaneous formulation. Doses ranging from 0.8 to 1.6 mg/kg yield plasma concentration in excess of 6 mg/ml, which is higher than the target concentration needed to maintain antiviral activity.

A phase I/II clinical trial of T-1249 was conducted in 72 HIV-positive, treatment-experienced adults who received no other HIV therapy over the 14-day treatment period (Eron, 2001). In this study (T-1249 101) patients received T-1249 as monotherapy for 14 days at doses ranging from 6.25 mg/day to 50 mg/day on a once or twice daily regimen. The results indicated that T-1249 was well tolerated over 14 days and conferred dose-related suppression of HIV (see Figure 4). On day 14, the median decrease in HIV-RNA levels from baseline ranged from 0.10 logs for the 6.25 mg/day dose to 1.4 log₁₀ reduction in viral copies/mL for the 50 mg/day dose. These encouraging data provide the basis to continue the clinical development of T-1249.

Conclusion

AS ILLUSTRATED BY DRs. ERON AND HOGAN, the development of fusion and entry inhibitors has come a long way in recent years. There are, in essence, three distinct classes of fusion and entry inhibitors in development: CD4-binding inhibitors, coreceptor-binding antagonists, and fusion inhibitors. Continued development and—thinking optimistically—eventual approval of these compounds will increase the antiretroviral arsenal to six available classes, a much needed improvement for patients at the end of their therapeutic rope.

“We still have some challenges to deal with,” Dr. Eron offered in his concluding remarks. “The parenteral administration of T-20 and T-1249 can be frustrating and there may be problems with the CCR5 inhibitors,

including the potential for cardiac toxicity with SCH-C, as well as theoretical concerns surrounding the CXCR4 inhibitors. But there’s a lot to be encouraged about. It would be great to have these new classes available now. But we’re just not there yet.” 

References

Berger EA. HIV entry and tropism. *AIDS* 11(Supplement A):S3-S16, 1997.

Cohen C, Dusek A, Green J, et al. Patient satisfaction and activities of daily living (ADL) in HIV infected adults using T-20 given by subcutaneous injection (SC) over 48 weeks [Abstract 708]. First IAS Conference on HIV Pathogenesis and Treatment, Buenos Aires, 2001.

Church J, Cunningham C, Palumbo P, et al. Safety and antiviral activity of chronic subcutaneous administration of T-20 in HIV-1 infected children [Abstract 681]. 8th Conference on Retroviruses and Opportunistic Infections, Chicago, 2001.

Daar ES, Li XL, Moudgil T, et al. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. *Proc Natl Acad Sci USA* 87:6574-8, 1990.

Eron J, Merigan T, Kilby M, et al. A 14-Day Assessment of the Safety, Pharmacokinetics, and Antiviral Activity of T-1249, a Peptide Inhibitor of Membrane Fusion [Abstract 14]. 8th Conference on Retroviruses and Opportunistic Infections, Chicago, 2001.

Hendrix CW, Flexner C, MacFarland RT, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother* 44(6):1667-73, 2000.

Jacobson JM, Lowy I, Fletcher CV, et al. Single-dose safety, pharmacology, and antiviral activity of the human immunodeficiency virus (HIV) type 1 entry inhibitor PRO 542 in HIV-infected adults. *J Infect Dis* 182(1):326-9, 2000.

Koot M, Keet I, Vos A, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. *Ann Intern Med* 118:681-8, 1993.

Lalezari J, DeJesus E, Northfelt D, et al. A week 48 assessment of a randomized, controlled, open-label phase II trial (T20-206) evaluating three doses of T-20 in PI-experienced, NNRTI-naive patients infected with HIV-1 [Abstract 418-W]. 9th Conference on Retroviruses and Opportunistic Infections, Seattle, 2002.

Lalezari J, Wheeler D, Kilby M, et al. Improved carbonate (CO₂) formulation of T-20 reduces number of injections required to administer 100 mg BID dose [Abstract LB-P20]. First IAS Conference on HIV Pathogenesis and Treatment, Buenos Aires, 2001.

Lalezari J, Cohen C, Eron J, et al. Forty-eight week analysis of patients receiving T-20 as a component of multi-drug salvage therapy [Abstract LBP116]. 13th International Conference on AIDS, Durban, 2000.

Mosier DE, Picchio GR, Gulizia RJ, et al. 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(5):3544-50, 1999.

Reynes J, Rouzier R, Kanouni T, et al. SCH-C: Safety and antiviral effects of a CCR5 receptor antagonist in HIV-1 infected subject [Abstract 1]. 9th Conference on Retroviruses and Opportunistic Infections, Seattle, 2002.

Schacker T, Collier AC, Coombs R, et al. Phase I study of high-dose, intravenous rSCD4 in subjects with advanced HIV-1 infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 9(2):145-52, 1995.

Schooley RT, Merigan TC, Gaut P, et al. Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. A phase I-II escalating dosage trial. *Ann Intern Med* 112:247-53, 1990.

Shearer WT, Israel RJ, Starr S, et al. Recombinant CD4-IgG2 in human immunodeficiency virus type 1-infected children: phase 1/2 study. The Pediatric AIDS Clinical Trials Group Protocol 351 Study Team. *J Infect Dis* 182(6):1774-9, 2000.

Simmons G, Wilkinson D, Reeves JD, et al. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J Virol* 70:8355-60, 1996.

Strizki JM, Xu S, Wagner NE, et al. SCH-C (SCH351125), 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(22):12718-23, 2001.

Trkola A, Kuhmann SE, Strizki JM, et al. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. *Proc Natl Acad Sci USA* 99(1):395-400, 2002.