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New Antiretroviral Agents in the Management of Drug Resistant HIV



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Management of drug resistant HIV

Background

Antiretroviral therapy: There are now six classes of antiretroviral agents available to treat HIV infection – nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitor (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), integrase inhibitors (IIs) and co-receptor antagonists (Table 1). For most patients, potent combinations of three or more of these agents, commonly known as highly active antiretroviral therapy (HAART) reduce HIV replication to levels that make the virus undetectable in the blood. Durable control of viral replication in this way prevents the deterioration of the immune system associated with HIV infection and minimizes the evolution of drug resistant viral strains.



Table 1. Antiretroviral drug names and manufacturers

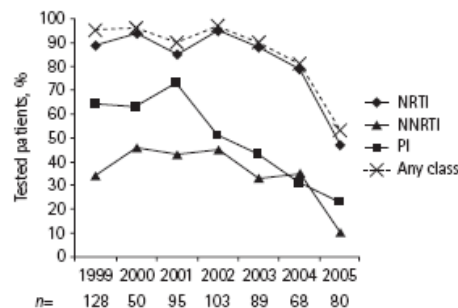
Generic Name	Trade Names	Other Names	Manufacturer
Reverse transcriptase inhibitors (RTIs)			
Abacavir	Ziagen [®]	ABC	GlaxoSmithKline
Abacavir/Lamivudine	Epzicom [™] , Kivexa [®]	Abacavir+3TC, Ziagen+3TC	GlaxoSmithKline
Didanosine	Videx [®]	ddl	Bristol-Myers Squibb
Emtricitabine	Emtriva [®]	FTC	Gilead Sciences
Lamivudine	Epivir [®]	3TC	GlaxoSmithKline
Stavudine	Zerit [®]	d4T	Bristol-Myers Squibb
Tenofovir	Viread [®]	TDF	Gilead Sciences
Tenofovir/emtricitabine	Truvada ^{®™}	TDF+FTC, Viread+Emtriva	Gilead Sciences
Zalcitabine	Hivid [®]	ddC	Discontinued
Zidovudine	Retrovir [®]	AZT, ZDV	GlaxoSmithKline
Zidovudine/lamivudine	Combivir [®]	AZT+3TC	GlaxoSmithKline
Zidovudine/lamivudine/ abacavir	Trizivir [®]	AZT+3TC+Abacavir	GlaxoSmithKline
Non-nucleoside reverse transcriptase inhibitors (NNRTIs, NON-NUKES)			
Delaviridine	Rescriptor [®]	DLV	Pfizer/Agouron
Efavirenz	Sustiva [®] , Stocrin [®]	EFV	Bristol-Myers Squibb/Merck
Etravirine	Intelence [™]	TMC-125	Tibotec/Johnson & Johnson
Nevirapine	Viramune [®]	NVP	Boehringer Ingelheim
Combinations			
Emtricitabine/tenofovir/ efavirenz	Atripla [™]		Gilead Sciences / Bristol Myers Squibb
Protease inhibitors			
Amprenavir	Agenerase [®]	APV	GlaxoSmithKline
Atazanavir	Reyataz [®]	ATV	Bristol-Myers Squibb
Darunavir	Prezista [®]	DRV	Tibotec/Johnson & Johnson
Fosamprenavir	Lexiva [®] , Telzir [®]	FPV	GlaxoSmithKline
Indinavir	Crixivan [®]	IDV	Merck
Lopinavir/ritonavir	Kaletra [®] , Aluvia [®]	LPV/r	Abbott
Nelfinavir	Viracept [®]	NFV	Pfizer/Roche
Ritonavir	Norvir [®]	RTV	Abbott
Saquinavir	Invirase [®]	SQV	Roche
Tipranavir	Aptivus [®]	TPV	Boehringer Ingelheim
Integrase inhibitors			
Raltegravir	Isentress [™]	MK-0518, RAL	Merck
Fusion inhibitors and co-receptor antagonists			
Enfuvirtide	Fuzeon [®]	T-20, ENF	Trimeris/Roche
Maraviroc	Selzentry [™] , Celsentri [®]	UK427857, MVC	Pfizer



HAART, comprising of nucleoside reverse transcriptase inhibitors (NRTIs) combined with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) first became available in 1996. Since then, continuing improvements in antiretroviral drugs and experience with their use for more than a decade has made initial HAART increasingly robust. Nevertheless, a major barrier to successful long-term treatment of HIV infection is the emergence of drug resistant virus, which threatens the durability of HAART regimens and is associated with an increased risk of death [Hogg, 2006, 1574-5].

Prevalence of drug resistance: Drug resistance is most common among patients who were taking antiretroviral therapy prior to the introduction of HAART and those initiating HAART in the early years of the HAART era. Studies of these patients in the US found one in two of those with poorly controlled HIV infection (i.e., virologically failing) harbored virus with resistance to more than one class of drug [Richman, 2004, 1395]. However, HAART has led to a steady decline in the prevalence of resistance to all three drug classes (Figure 1) [Di Giambenedetto, 2007, 836]. A more recent analysis using a large database has confirmed this finding [Sista, EHDRW, 2008]. In particular, starting therapy with a ritonavir-boosted PI (PI/r) has had a major impact on the risk of developing HIV drug resistance, reducing it 5-fold compared to that of patients initiating a non-boosted PI- or NNNRT-based regimen [Gill, 2007, 1].

Figure 1. Evolution of the prevalence of resistance to antiretroviral drugs during the HAART era [Di Giambenedetto, 2007, 836]





Triple-class antiretroviral drug resistance: This refers to HIV with simultaneous resistance to NRTIs, NNRTIs and PIs. Fortunately, triple-class drug failure remains relatively uncommon, with estimates ranging from 6% to 8% among patients taking HAART for 2.5 years to over 9 years [Hogg, 2006, 1574; Napravnik, 2007, 827]. This is consistent with recent estimates of cumulative risk of triple-class virological failure of 3.5% by 5 years and 9% by 10 years post HAART initiation [Phillips, 2007, 1925].

Management of treatment-experienced patients: The goal of treatment for patients virologically failing antiretroviral therapy and with drug resistance is to re-establish maximal viral load suppression (HIV RNA <50 copies/mL) [DHHS Guidelines, 2007, 30]. Current guidelines recommend adding at least two (and preferably three) fully active agents to an optimized background regimen based on treatment history and drug resistance testing [DHHS Guidelines, 2007, 30]. In general, adding a single fully active agent in a new regimen is not recommended because of the risk of development of rapid resistance [DHHS Guidelines, 2007, 32].

Weighted phenotypic sensitivity scores (wPSS) that use a continuous (rather than binary) scale to quantify drug susceptibility of each drug in a given regimen have been found to correlate with virological outcome [Swanstrom, 2004, 890]. Although adding two or three fully active drugs is ideal, in cases where this is not an option, selecting the best combination of partially active agents in combination with a single new active agent can lead to significant viral suppression. For example, 14% of patients with a PSS of 0 receiving enfuvirtide plus an optimized background regimen (OBR) achieved <400 HIV RNA copies/mL compared to those receiving OBR alone [Nelson, 2005, 408].



Agents that target drug resistant HIV

Substantial numbers of HIV infected individuals harbor drug resistant HIV. Drugs that target resistant virus, and/or drugs from new classes may be of significant benefit to these patients. A variety of new agents that fulfill these criteria have been identified and developed for treatment of HIV infection. These include novel PIs and NNRTIs that exhibit a high genetic barrier to reduced susceptibility of HIV (i.e., the accumulation of multiple resistance mutations is required to confer high level resistance) as well as agents from new drug classes:

Novel PIs

Tipranavir (TPV, Aptivus, Boehringer Ingelheim, FDA approved June, 2005, EMEA approved July, 2005)
Darunavir (DRV, Prezista, Tibotec/Johnson & Johnson, FDA approved June 2006, EMEA approved February, 2007)

Novel NNRTIs

Etravirine (TMC 125, Intelence, Tibotec, FDA approved January, 2008. Applications for approval of Intelence have also been submitted to the EMEA and with regulatory authorities in Canada, Switzerland, Russia and Australia.)
Rilpivirine (TMC 278, Tibotec) Phase IIb ongoing

Co-receptor antagonists

Maraviroc (Celsentri, Pfizer, FDA and EMEA approved July, 2007)
Vicriviroc (Schering Plough) Phase III underway

HIV integrase inhibitors

Raltegravir (Isentress, Merck, FDA approved October, 2007, EMEA approved November, 2007)
Elvitegravir (Gilead Sciences) Phase II ongoing

Other antiretroviral drug candidates that are currently in early development:

- *PIs*: MK-8122 (PPL-100 Merck)
- *NNRTIs*: BILR 355 BS (Boehringer Ingelheim), RDEA806 (Ardea Biosciences) and calanolide A (Sarawak Medichem Pharmaceuticals).
- *Fusion inhibitors and co-receptor antagonists*: PRO 140 (fusion inhibitor, Progenics) and INCB9471 (CCR5 inhibitor, Incyte Corporation).
- *Maturation inhibitors* (drugs that specifically block a late step in HIV processing): Bevirimat (PA-457, Panacos) and Vivecon (MPC-9055, Myriad Pharmaceuticals).



Agents with new mechanisms of action

1. Novel PIs: tipranavir and darunavir

Mechanism of action

PIs inhibit HIV protease an enzyme that cleaves polyproteins into its component proteins preventing the formation of mature virions (Figure 2). The HIV protease inhibitors fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir are small peptidomimetic agents that mimic HIV polyproteins. These agents are reversible competitive inhibitors that displace HIV polyproteins, thereby preventing cleavage.

The novel PIs, tipranavir and darunavir, are non-peptidic inhibitors. Although tipranavir has a similar mechanism of action to the earlier PIs, it is active against PI-resistant HIV strains [Larder, 2000, 1944]. Increased flexibility of tipranavir binding to HIV protease, compared to that of the peptidomimetic inhibitors, is thought to contribute to its capacity to maintain activity against broadly PI cross-resistant HIV variants [Larder, 2000, 1947]. Darunavir, on-the-other hand, binds extensively to the protease backbone within the active site, interactions that are maintained with mutant proteases [Ghosh, 2008, 81-2]. Darunavir also effectively suppresses viral replication of PI-resistant and broadly PI cross-resistant HIV variants [Ghosh, 2008, 81].

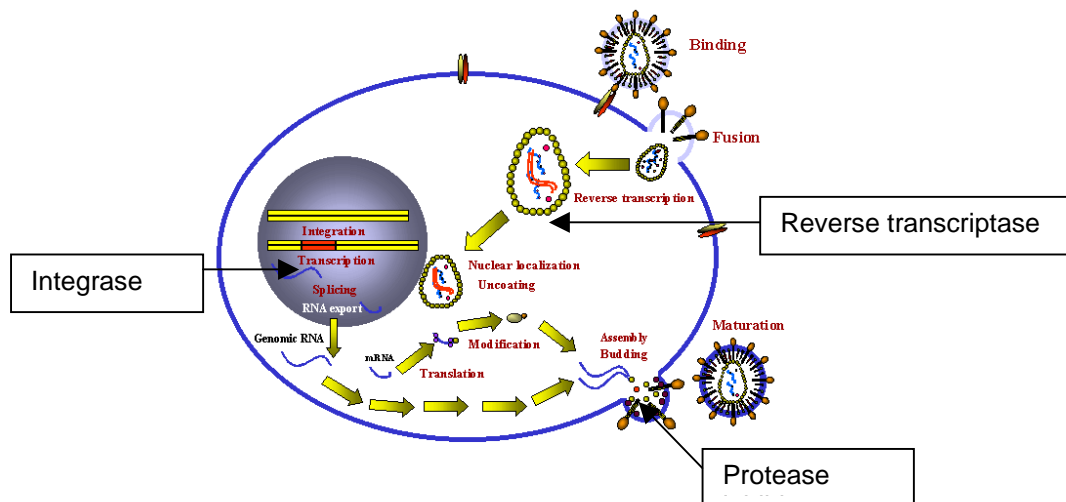


Figure 2. The HIV replication cycle. Upon entering a new host, HIV binds to target cell receptors, which initiates processes that allow the viral envelope to fuse with the cell membrane. HIV RNA is released into the cell, where HIV reverse transcriptase (RT) uses the viral RNA as a template to produce viral DNA. HIV DNA is transported to the host cell nucleus where it is integrated into the cell's genome by HIV integrase. When activated, the cell transcribes and translates HIV DNA as it does human genes, producing new copies of HIV RNA and proteins. The viral components assemble into viral particles and are released from the cell into the body fluids and blood stream. [Adapted from USC School of Medicine]

PI-based regimens

Currently preferred PI-based regimens use combinations of two NRTIs and a ritonavir-boosted PI (PI/r) [DHHS Guidelines, 2008, 17]. PI-based regimens have different side effect profiles than NNRTI-based regimens, but generally cause more gastrointestinal symptoms and lipid abnormalities [DHHS Guidelines, 2008, 17]. However, PIs have a high genetic barrier to resistance, with multiple mutations required before significant loss of susceptibility occurs, and resistance to protease therapy seldom develops following early virological failure [DHSS Guidelines, 2008, 17].

Efficacy

Tipranavir: Phase III trials (RESIST-1 and -2) in triple-class treatment-experienced patients and PI resistance have demonstrated superior efficacy of ritonavir-boosted tipranavir (TPV/r) plus an optimized background regimen compared to a comparator PI/r after 48 weeks, as well as showing the benefit of adding a second active agent (Table 2) [Hicks, 2006, 471].



Table 2. Efficacy of TPV/r at 48 weeks [Hicks, 2006, 471]

Clinical Trial	HIV RNA <50 copies/mL		P
	TPV/r N	Comparator PI/r N	
RESIST-1 and -2	170 (22.8%)	75 (10.2%)	<0.0001
Combined analysis			
No enfuvirtide	122 (21.1%)	56 (9.3%)	<0.0001
Enfuvirtide	48 (28.4%)	19 (14.1%)	<0.0001

P value for use of enfuvirtide = 0.0093

Darunavir. Phase IIb clinical trials (POWER 1 and 2) of ritonavir-boosted darunavir (DRV/r) in combination with an optimized background regimen in treatment-experienced patients demonstrated superior viral suppression compared to comparator PI/r after 48 weeks (Table 3) [Clotet, 2007, 1174]. As for TPV/r, addition of a second active agent in the background regimen, was beneficial [Clotet, 2007, 1175].

Table 3. Efficacy of ritonavir-boosted darunavir at 48 weeks [Clotet, 2007, 1174-5]

Clinical Trial	HIV RNA <50 copies/mL		P
	DRV/r N	Comparator PI/r N	
POWER-1 and -2	50 (45%)	12 (10%)	<0.0001
Combined analysis			
No enfuvirtide	27 (44%)	7 (10%)	<0.0001
Enfuvirtide	21 (58%)	4 (11%)	<0.0001



Resistance

Tipranavir: In many cases, HIV isolated from highly PI-experienced patients remains susceptible to tipranavir suggesting little cross-resistance with other PIs [Larder, 2000, 1944]. Multiple mutations are required to confer high level tipranavir resistance in vitro [Doyon, 2005, 30] and variants isolated from patients with reduced susceptibility to tipranavir have been utilized to generate tipranavir resistance scores that may help predict reduced response to tipranavir [Baxter, 2006, 10798]. The most common mutations developing in patients failing TPV/r are shown in Table 4.

Table 4. HIV protease mutations developing on TPV/r treatment [Naeger, 2007, 183]

Frequency	Mutations
>20%	L33V/I/F, V82T, I184V
10–20%	L10V/I/S, I13V, E35D/G/N, I47V, K55R, V82L and L89V/M/W

The number of baseline PI mutations (any change at protease positions 30,32, 36, 46, 47, 48, 50, 53, 54, 82, 84, 88 and/or 90) is correlated with virological response to TPV/r. For example, a median change in HIV RNA from baseline of 1.5 log₁₀ by weeks 2-4 has been observed regardless of the number of baseline PI mutations [Naeger, 2007, 182]. However, in the absence of enfuvirtide, viral rebound occurred in patients with five or more protease resistance mutations after 4–8 weeks [Naeger, 2007, 182]. Reduced virological responses to TPV/r were also observed when viral isolates from treatment-experienced patients had a baseline substitution at positions I13, V32, M36, I47, Q58, D60, or I84 and, depending on the substitution, V82 [Naeger, 2007, 182]. Recently, a tipranavir weighted mutation score has been developed consequently improving the prediction of virologic response to this drug. The PI resistance associated mutations with the highest weight determining TPV resistance were 74P and 47V, followed by 58E, 82L/T, 83D, 54A/M/V, 36I, 43T, 84V, 10V and 46L [Scherer, EHDRW, 2008].

TPV/r responses have also been assessed by baseline phenotype. The proportion of responders (confirmed >1 log₁₀ decrease in HIV RNA at week 24) was 45% among those with a ≤3-fold reduction in TPV susceptibility (EC₅₀), dropping to 21% among those with a >3- to 10-fold reduction in susceptibility [Naeger, 2007, 182-3]. Thus, baseline phenotype appears to be a predictor of treatment response.

Darunavir: This compound was designed to bind tightly to the protease backbone of drug resistant HIV variants [Ghosh, 2007, 81-2]. It has an excellent antiviral profile and is active against a broad range of PI-resistant isolates [De Meyer, 2005, 2316]. Eleven protease mutations that when present at baseline and in a background of at least 10 IAS-USA defined PI mutations were associated with a diminished response to darunavir (Table 5). A clear association between diminished virological response and the accumulation of three or more of these mutations has been observed [De Meyer, 2008, 383].

Table 5. Darunavir resistance-associated mutations [De Meyer, 2008, 383]

V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, L89V
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Mutations developing during virological failure of DRV/r include I15V, V32I, L33F, M46I, I47V, I54L and L89V, with V32I and I54L occurring in >20% of cases. However, susceptibility to darunavir is only reduced when these mutations occur in association with a high number of IAS-USA protease resistance mutations [De Meyer, 2008, 384, 386]. Darunavir has demonstrated a high genetic barrier to resistance outside of clinical trials, exhibiting antiviral activity against HIV variants with up to 16 protease resistance mutations [Poveda, 2007, 1411]. Furthermore, the prevalence of baseline darunavir resistance mutations among



patients failing other PI-based regimens is low (<10% of clinical isolates from patients failing PIs had three or more darunavir-associated mutations) [Poveda, 2007,886]. Baseline phenotype is a strong predictor of DRV/r responses with virological response maintained up to a 10-fold decrease in susceptibility; above this, virological response gradually declined [De Meyer, 2008, 381].

Clinical considerations

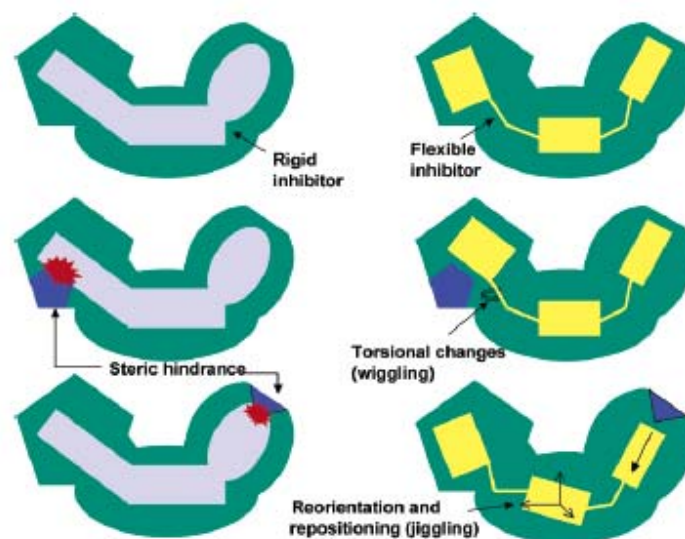
TPV/r and DRV/r are approved for patients who are highly treatment-experienced or have multiple PI-resistant HIV. These agents are most effective when used in combination with other active agents such as enfuvirtide, a CCR5 inhibitor, or an integrase inhibitor [DHHS Guidelines, 2008, 35] and offer important new options for second- and third-line regimens. Baseline resistance profiles, safety profiles and drug-drug interaction profiles should be considered in order to select the most suitable PI on an individual basis.

2. Novel NNRTIs: etravirine and rilpivirine

Mechanism of action

NNRTIs inhibit HIV reverse transcriptase (RT) (Figure 2). These agents bind within a hydrophobic pocket of HIV RT near the active site of the enzyme, which induces a conformational change that inhibits reverse transcription of HIV RNA [Deeks, 2001, S25]. Three NNRTIs (efavirenz, nevirapine and delavirdine) are currently available. These compounds are rigid molecules that fit in the hydrophobic pocket like a key in a lock, blocking RT activity. New novel NNRTIs etravirine and rilpivirine, on the other hand, have torsional flexibility that allows them to “wobble” and “jiggle” into numerous positions and orientations within the binding pocket (Figure 3), which may account for their activity against NNRTI resistant virus [Das, 2004, 2557].

Figure 3. Schematic representation depicting how a flexible inhibitor is more effective than a rigid inhibitor in overcoming the effects of a resistance mutation [Das, 2004, 2557]



NNRTI-based regimens

NNRTI-based regimens containing either efavirenz or nevirapine are commonly prescribed as initial therapy and have the advantage of lower pill burden compared with most PI-based regimens [DHHS Guidelines, 2007, 17]. In addition, starting with NNRTIs may also reduce exposure to adverse events more commonly associated with PIs [DHHS Guidelines, 2007, 17].

The major disadvantage of currently available NNRTIs is the low genetic barrier for the development of resistance – a single mutation can confer high level NNRTI resistance [DHSS Guidelines, 2007, 17]. The prevalence of NNRTI resistant HIV among treatment naïve patients, estimated at around 3%, is also a concern [Novak, 2005, 471].



Efficacy

Efavirenz and nevirapine: These drugs have been shown to provide similar levels of virological suppression in treatment naïve patients (HIV RNA <50 copies/mL 70% and 65.4%, respectively) [van Leth, 2004, 1258]. Efavirenz is currently recommended as the preferred NNRTI, as nevirapine has been associated with increased risk of hepatic toxicity, particularly in patients with higher CD4 cell counts (women >250 cells/mm³ men >400 cells/mm³) [DHHS Guidelines, 2007, 18]. Delaviridine appears to have less potent antiretroviral activity and is not recommended for initial therapy [DHHS Guidelines, 2007, 18].

Etravirine: Ongoing phase III trials (DUET I and DUET II) in treatment-experienced patients with NNRTI and PI resistance have demonstrated etravirine provides superior viral suppression (HIV RNA <50 copies/mL) compared to placebo at 24 weeks (Table 6), despite NNRTI resistance [Madruga, 2007, 32-3; Lazzarin, 2007, 42-3].

Table 6. Efficacy of etravirine at 24 weeks [Madruga, 2007, 32-3; Lazzarin, 2007, 42-3]

Clinical trial	HIV RNA <50 copies/mL		
	Etravirine N (%)	Placebo N (%)	Difference % (95% CI)
Duet I	170 (56)	119 (39)	17 (9–25)*
Duet II	183 (62)	129 (44)	18 (11–26)**

CI: confidence interval. *p=0.005. **p=0.0003. Etravirine 200mg twice daily.

Rilpivirine: A phase IIb dose-finding study of this agent in combination with Combivir or Truvada in treatment-naïve HIV patients showed that at 48 weeks, 77% to 81% of patients receiving 25mg, 75mg or 150mg of rilpivirine achieved viral suppression (HIV RNA <50 copies/mL) [Pozniak, 2007, 1]. This was not significantly different from treatment with efavirenz in similar regimens (81%) [Pozniak, 2007, p1].

Resistance

Nevirapine, efavirenz and delaviridine: These compounds primarily select resistance mutations in two regions between codons 98 and 108 and codons 179 and 190 (Table 7) [Deeks, 2001, S27]. However, selection of a single mutation can interfere with their binding in the hydrophobic pocket, conferring resistance to the drug and often cross-resistance to the other NNRTIs [Bacheler, 2001, 5002]. As a result, failure with any one of these drugs may cause loss of utility of the others and may lead to transmission of NNRTI resistant HIV strains [DHHS Guidelines, 2007, 17].

Table 7. Common mutations associated with reduced susceptibility to first generation NNRTIs [Deeks, 2001, S27].

Compound	Mutations
Nevirapine	K103N, V106A, V108I, Y181C/I, Y188C, G190A
Efavirenz	L100I, K103N, V108I, Y188L, G190E/S, P225H
Delaviridine	K103N, Y181C/I, P236L

Etravirine and rilpivirine: The flexibility of these compounds allows them to adopt multiple conformations that can overcome the effects of drug resistance mutations within the NNRTI binding pocket [Das, 2004, 2557].

Etravirine appears to have a higher genetic barrier to the development of resistance than the first generation NNRTIs, requiring multiple mutations to confer high losses in susceptibility. It can suppress the selection of resistant virus from NNRTI-resistant virus population in vitro and is active in HIV infected patients with documented NNRTI resistance [Vingerhoets, 2005, 12780; Vingerhoets, CROI, 2006]. Virologic response to etravirine is a function of the number



of baseline NNRTI mutations. Patients with ≤ 2 NNRTI mutations achieved a viral load reduction of ≥ 1 log, while patients, with ≥ 3 mutations achieved a mean 0.66 log reduction (at 24 weeks) [Vingerhoets, CROI, 2006]. Clinical cutoffs of 1.6 fold-change (20% loss of etravirine response) and 27.6 fold-change (80% loss of etravirine response) have been established [Winters, CROI, 2008].

Fourteen mutations at 8 codons in the RT are associated with reduced in vivo response to etravirine (Table 8) [Vingerhoets, 2007, S34]. It is worth noting that K103N, which confers high level resistance to all three first generation NNRTIs is not one of them [Bacheler, 2001, 5002]. Among patients harboring virus with NNRTI resistance mutations, the presence of Y181V, G190S and V179F/T has been associated with the poorest virological response to etravirine; however, this combination of mutations is rare, occurring in $< 5\%$ of patients [Vingerhoets, 2007, S34].

Table 8. Etravirine-resistance associated mutations [Vingerhoets, 2007, S34]

V90I, A98G, L100I, K101E/P, V106I, V179D/F/T, Y181C/I/V and G190A/S

Rilpivirine is a potent inhibitor of wild-type HIV-1 that retains activity against over 85% of clinical isolates harboring NNRTI-resistance mutations [de Bethune, CROI, 2005]. Among engineered strains with the most prevalent NNRTI mutations, the greatest loss of response to rilpivirine was observed with the double mutant L100I+K103N [de Bethune, CROI, 2005]. Like etravirine, rilpivirine demonstrated a high genetic barrier to the development of resistance in vitro, with viral breakthrough only occurring after the accumulation of as many as 8 mutations, including L100L/I, V106V/I, Y181Y/C and M230M/I [de Bethune, CROI, 2005].

Clinical considerations

The FDA has recently approved etravirine (January, 2008). Prior to this, it was available through an expanded access program in the US, as well as a number of European countries, Australia and Canada, for patients with limited treatment options and with resistance to the currently approved NNRTIs.

Due to the overlapping resistance profiles of etravirine with the first generation NNRTIs, a large proportion (70%) of HIV strains from clinical samples with mutations associated with resistance to nevirapine or efavirenz (K103N, Y181C, G190A, or V108I) also have one or more mutations associated with etravirine resistance [Llibre, 2007, S74]. However, the high genetic barrier of etravirine suggests it will retain considerable activity in most patients failing NNRTIs and while low-to-intermediate resistance is likely to be common, complete resistance will be rare [Llibre, 2007 S74]. This is consistent with an observational cohort study of HIV infected patients with prior NNRTI experience in which almost two-thirds of those with NNRTI resistance-associated mutations had etravirine-associated resistance mutations but, of the latter, $< 5\%$ of patients had ≥ 3 etravirine mutations [Povedas, 2007, 1410]. This study also found the mean number of etravirine-associated resistance mutations was significantly higher in patients with prior exposure to nevirapine than to efavirenz, with Y181C, K101E and G190A more prevalent in nevirapine- than efavirenz-treated patients [Povedas, 2007, 1410].

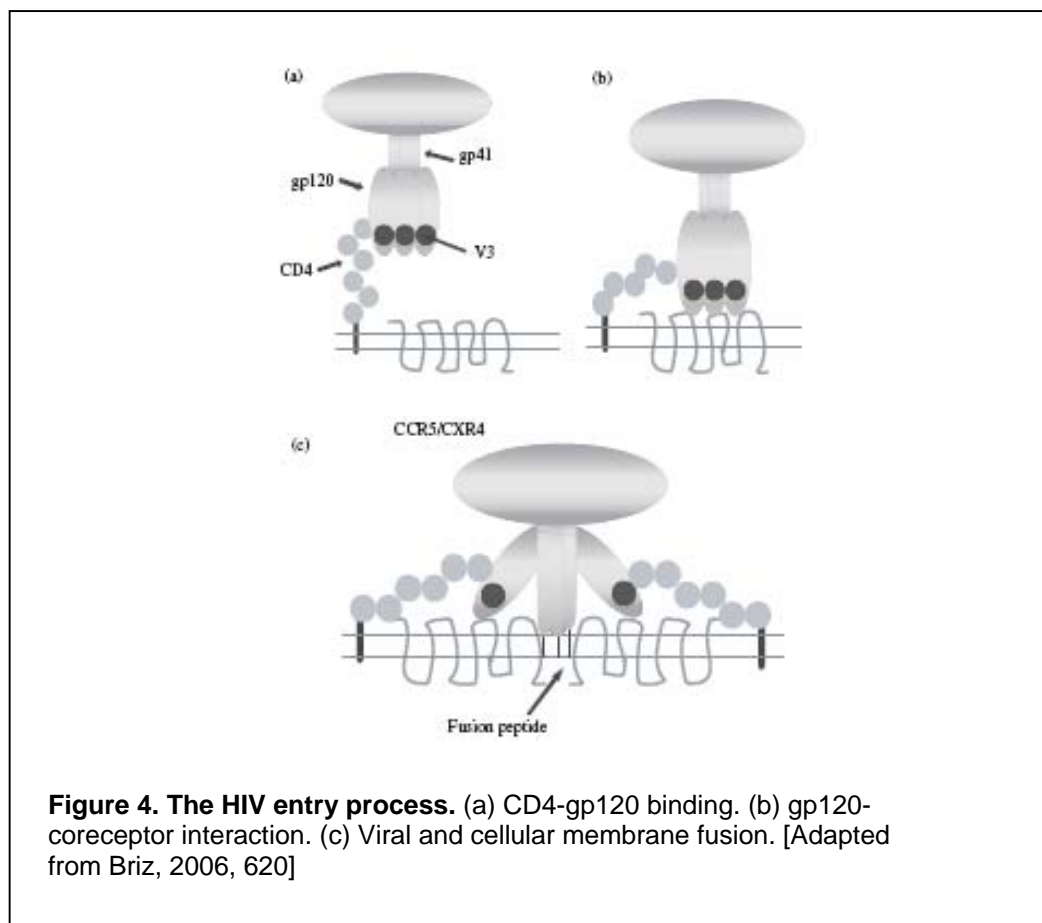
These data support the strategy of withdrawing first generation NNRTIs from failing regimens as soon as possible in order to prevent the accumulation of NNRTI-associated resistance mutations and preserve the activity of etravirine and other new agents for use in future regimens [Llibre, 2007, S74].

3. Co-receptor antagonists: maraviroc and vicriviroc

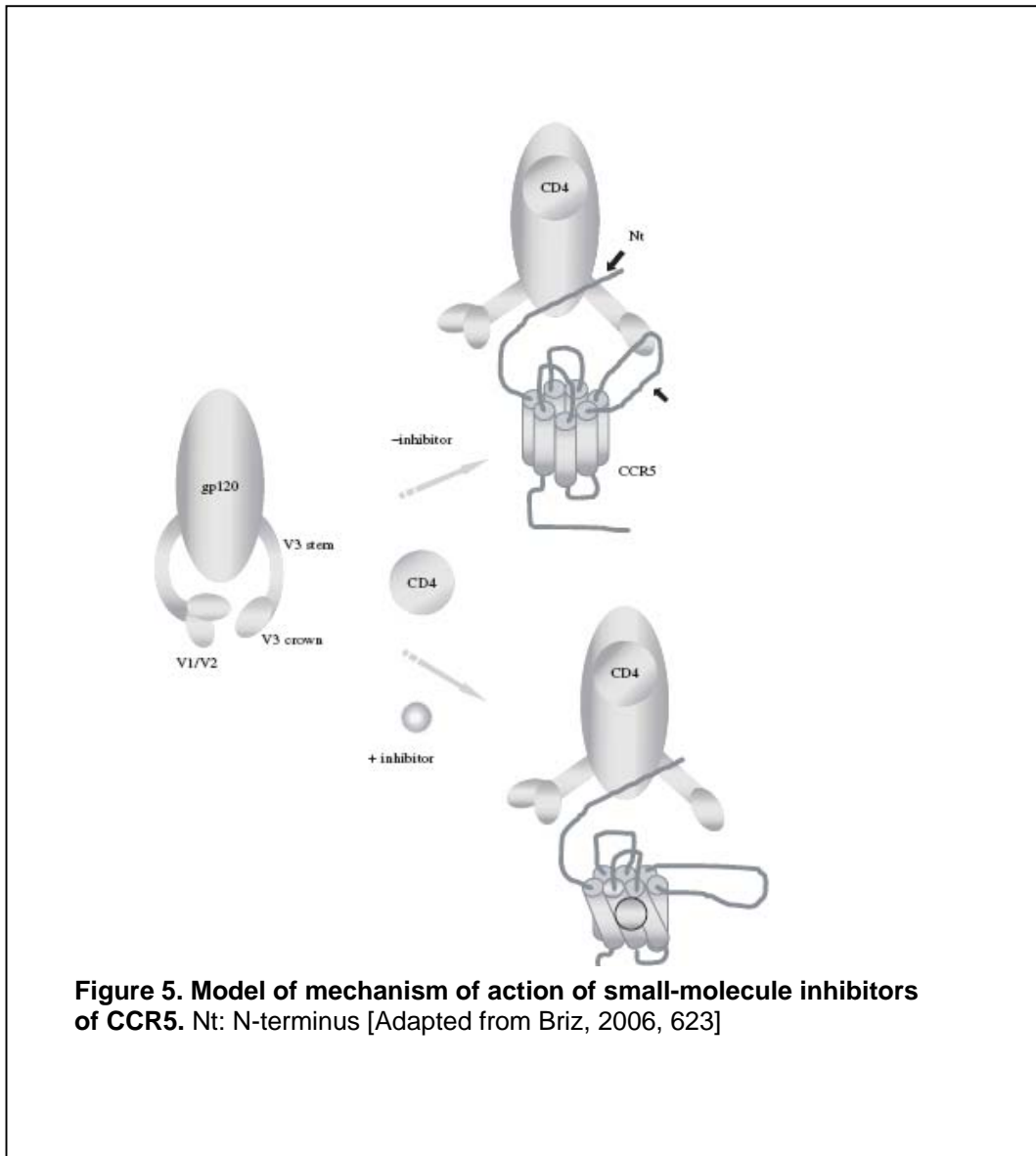
HIV uses receptors on the cell surface to attach to and gain entry into a host cell. CD4 is a glycoprotein that is expressed on T-cells, macrophages and other cells of the immune system that is involved in activation of an immune response and that is also the primary receptor for HIV entry. However, CD4 alone is not sufficient as HIV also requires one of several secondary co-receptors (chemokine receptors). The two major co-receptors enabling HIV entry are CCR5 and CXCR4 [Deng, 1996, 662; Feng, 1996, 875]. Specific HIV variants may use either CCR5 or CXCR4 or, more rarely, both of these co-receptors [Zhang, 1996, 768].

Mechanism of action

The first step of HIV entry into a host cell is the binding of the HIV envelope protein gp120 to a CD4 receptor on the target cell surface (see Figure 2). This induces a conformational change that allows gp120 to interact with either CCR5 or CXCR4 (Figure 4), and facilitates fusion of the virus with the host cell membrane [Reviewed in Briz, 2006].



Maraviroc and vicriviroc are CCR5 antagonists that block the interaction of gp120 with the CCR5 co-receptor (Figure 5), preventing HIV infection of the target cell [Dorr, 2005, 4724-5; Strizki, 2005, 4913-5].





HIV tropism

HIV tropism refers to HIV phenotype based on co-receptor use. HIV strains that use the CCR5 co-receptor are called “CCR5 tropic” or R5 viruses, while those using CXCR4 co-receptors are “CXCR4 tropic” or X4 viruses. Variants able to use both co-receptors are “dual tropic” or R5X4 viruses [Berger, 1998, 240]. R5 viruses are generally transmitted and, in the absence of treatment, predominate during the first years of infection after which X4 viruses begin to appear and typically exists as a minority species [Poveda, 2007, 1042]. The prevalence of X4 HIV reportedly ranges from 18%–25% in antiretroviral-naïve patients compared to 40%–50% in antiretroviral-experienced patients (i.e., those with detectable viral load who are failing therapy) [Poveda, 2007, 1042; Hunt, 2007, 927; Wilkin, 2007, 592]. The increased prevalence in patients exposed to antiretroviral drugs has been associated with lower pretreatment nadir CD4 cell counts, higher viral load, and low CD4 cell count [Hunt, 2007, 928; Poveda, 2007, 1042; Wilkin, 2007, 592]. The presence of X4 variants has been associated with faster disease progression and the onset of AIDS [Connor, 1997, 623].

Tropism Tests

Current guidelines recommend that co-receptor antagonists should not be used unless co-receptor tropism has been determined [DHHS Guidelines, 2007, 9]. Three approaches have been taken towards developing sensitive and specific tropism tests (Table 9).

Table 9. HIV tropism tests for the detection of X4 variants

Assay system	Methodology
Phenotypic assays	These assays are cell-culture based assays that utilize recombinant virus containing the envelope region from the test isolate. Recombinant virus is then used to infect indicator cells expressing either R5 or X4 co-receptors.
Genotypic assays	Genotypic predictors have been designed utilizing algorithms that incorporate sequence data, and in some cases other clinical data
Other:	
Heteroduplex tracking	This methodology detects X4 tropic HIV by forming heteroduplexes with R5 V3 probes that can be identified by electrophoretic separation
Flow cytometry	This technique utilizes fluorescent-tagged HIV-1 mRNA to detect whether or not test HIV is replicating in X4 or R5 cells.

Phenotype assays: Trofile from Monogram Biosciences is currently the only commercially available co-receptor tropic phenotype assay. The Trofile assay takes about two-to-three weeks to get a result and requires a plasma viral load of at least 1,000 copies/mL [DHHS Guidelines, 2007, 9]. It is worth noting that the sensitivity of the Trofile test assays may not be sufficient to detect low levels of X4 virus (<10%), which could be clinically relevant [Whitcombe, 2007, 571]; a newer version of this assay with enhanced sensitivity may be capable of detecting lower level viral subpopulations [Hunt, CROI, 2008]. Another phenotype assay, PHENOSCRIPT ENV (BioAlliance/Eurofins) that is capable of detecting X4 at levels of 5% to 10% is in development [Roulet, CROI, 2008].

Genotype assays: Various bioinformatic approaches for predicting tropism from V3 sequence data are under investigation. Predictor algorithms include SVM, C4.5, PART, Charge Rule, geno2pheno co-receptor, PSSMX4R5 and PSSMsinsi. However, upon testing with HIV



variants of known tropism according to the Trofile assay, it appears the sensitivity of these methods is inadequate for predicting HIV X4 co-receptor usage in clinical samples at this time [Low, 2007, F18-19].

Other approaches: Two other assays are commercially available, SensiTrop HIV Co-Receptor Tropism Assay from Pathway Diagnostics and Virotest Tropism Assay for HIV-1 from Invirion Diagnostics (a flow-cytometry based assay, for which no published data is currently available). SensiTrop utilizes heteroduplex tracking and reportedly detects X4 when present at only 1% of R5 HIV levels [Li, CROI, 2008]. However, when tested head-to-head against the Trofile assay using samples from the maraviroc expanded access program, it was found to be lacking in sensitivity to detect X4 (<50%) in these clinical samples [Tressler, CROI, 2008].

Co-receptor inhibitors as a component of HAART

Maraviroc is the first co-receptor antagonist approved. It is indicated, in combination with other antiretroviral agents, for treatment-experienced adult patients who have evidence of viral replication, HIV-1 strains resistant to multiple antiretroviral agents and have only R5 virus [Maraviroc, 2007, 2]. It is important to note that maraviroc is not active in HIV infected patients with dual/mixed (R5/X4) tropic infection or against X4 virus [Mayer, IAC, 2006].

Efficacy

Maraviroc: This drug is being tested in combination with an optimized background regimen in two phase III trials MOTIVATE 1 and MOTIVATE 2, in antiretroviral treatment-experienced patients infected with R5 HIV-1 [Lalezari, CROI, 2007; Nelson, CROI, 2007]. A pooled analysis of interim data at 24 weeks shows that compared to placebo, maraviroc provides superior virological control (HIV RNA <50 copies/mL, 61% versus 28%, respectively) and superior increases in mean CD4 cell counts (106 cells/mm³ versus 57 cells/mm³) (Table 10) [Maraviroc, 2007, 16].

Table 10. MOTIVATE 1 and 2: Pooled efficacy analysis at 24 weeks [Maraviroc, 2007, 16]

Outcome	Maraviroc twice daily n=426	Placebo n=209	Mean difference
Mean change in viral load from baseline, log ₁₀ copies/mL	-1.96	-0.99	0.97
<50 HIV RNA copies/mL, n (%)	193 (45.3%)	48 (23.0%)	33.0%

Maraviroc is most effective when combined with at least one other potent fully active antiretroviral agent in the regimen (with no resistance-associated mutations present). This has been demonstrated with maraviroc in combination with first-time use of enfuvirtide (53% of patients with HIV RNA <50 copies/mL at 24 weeks) or Kaletra (70% patients with HIV RNA <50 copies/mL at 24 weeks) [van der Ryst, IAS, 2007].

Maraviroc versus efavirenz: The efficacy of maraviroc has also been compared with that of efavirenz, both in combination with AZT/3TC, in antiretroviral naïve patients for 48 weeks (Table 11) [Saag, IAS, 2007]. The CD4 cell count increase was greater in response to maraviroc than to efavirenz [Saag, IAS, 2007]. However, the proportion of patients achieving HIV RNA <50 copies/mL was significantly higher in response to efavirenz (69.3% versus 65.3%) and the equivalence of the efficacy of these treatments was not proven [Saag, IAS, 2007].



Table 11. Maraviroc versus efavirenz: efficacy at 48 weeks [Saag, IAS, 2007]

Outcome	Maraviroc twice daily + AZT/3TC (n=360)	Efavirenz + AZT/3TC (n=361)
HIV RNA <50 copies/mL, %	65.3	69.3
Mean change from baseline in CD4 cell counts, cells/mm ³	170	143

Vicriviroc: This drug demonstrated potent antiviral activity in a Phase II trial (ACTG 5211), in HIV infected antiretroviral-experienced patients, virological failure and R5 virus (Table 12) [Gulick, 2007, 308]. Furthermore, HIV remained undetectable in 14 (70%) individuals at 48 weeks [Gulick, IAS, 2007].

Table 12. Virologic and immunologic responses to vicriviroc at 24 weeks [Gulick, 2007, 308]

Outcome	Placebo (n=28)	Treatment group		
		5mg (n=30)	10mg (n=30)	15mg (n=30)
Mean change in viral load from baseline, log ₁₀ copies/mL	-0.29	-1.51	-1.86	-1.68
HIV RNA <50 copies/mL, n (%)	2 (7)	6 (26)	12 (40)	8 (27)
Mean change from baseline in CD4 cell counts, cells/mm ³	-9	+84	+142	+142

Higher doses of vicriviroc have subsequently been shown to be well tolerated (VICTOR-E1) [Slim, IAS, 2007], and participants are currently being recruited into two Phase III trials VICTOR-E3 and VICTOR-E4. These trials will test the safety and efficacy of vicriviroc 30 mg administered once daily in treatment-experienced HIV patients with R5 virus. Like maraviroc, there is no evidence that vicriviroc is active against dual/mixed tropic HIV or X4 virus.

Resistance

The genotype of the V3 loop of HIV gp120 (crown and stem; see Figure 5) largely determines whether a variant is R5 or X4 (i.e, non-syncytia [NSI] or syncytia forming [SI]) [Fouchier, 1992, 3183-4]. Specific amino acids, particularly positively charged ones at positions 306 and 320 of the V3 loop have been associated with an X4 phenotype [Fouchier, 1995, 907].

Maraviroc: Mutations associated with resistance to maraviroc that have been identified in vitro include A316T, A319A/S and I323V located in the V3 loop, as well as a deletion in this region equivalent to positions 315 to 317 [Westby, 2007, 2363, 2369]. Mutations outside the V3 loop region were also observed in resistant variants; however, none of the changes were associated with a tropism switch [Westby, 2007, 2363]. V3 loop mutations have also been observed in HIV strains from patients failing treatment with maraviroc, with amino acid changes differing between patients [Mori, 2007, S12].



Vicriviroc: Multiple changes in the V3 loop sequence have been identified in HIV infected patients failing treatment with vicriviroc [Tsibris, 2007, S15]. Resistance to vicriviroc due to the emergence of X4 virus has also been observed [Tsibris, 2007, S15].

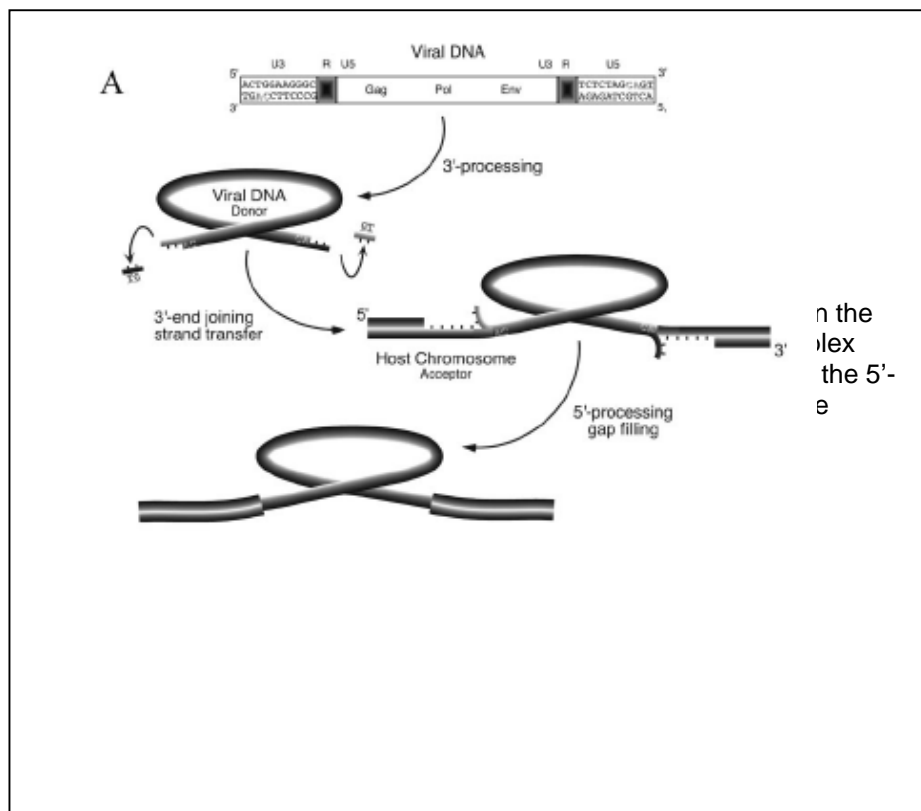
Clinical considerations

There is concern that failure of a regimen containing an R5 inhibitor may drive the selection of more pathogenic X4 variants. In vitro evidence to date suggests that tropism switches of the R5 to the X4 phenotype do not readily occur [Westby, 2007, 2360-1]. Rather, the emergence of X4 virus in HIV infected patients failing maraviroc has been attributed to the presence of pre-existing X4 minority variants that were not detected at screening [Westby, 2006, 4912-5]. Presently maraviroc is approved only for HIV infected patients with drug resistance.

4. Integrase inhibitors: raltegravir, elvitegravir

Mechanism of action

HIV integrase catalyses the insertion of viral DNA into the host cell genome (see Figure 2). Integration of viral DNA occurs in three steps 3'-processing, strand transfer and gap filling (Figure 6) [Marchand, 2002, 12596]. Raltegravir and elvitegravir are strand transfer inhibitors with potent in vitro activity against a broad panel of HIV variants including those with resistance to PIs, NRTIs and NNRTIs [Miller, IAC, 2006; Shimura, 2007, 766-7].



Integrase inhibitors as a component of HAART

Raltegravir is the first integrase inhibitor to be approved. It is currently indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-experienced adult patients who have evidence of viral replication and HIV-1 strains that are resistant to multiple antiretroviral agents [Raltegravir, 2007, 2].



Efficacy

Raltegravir. This drug is being tested in combination with an optimized background regimen in two Phase III trials BENCHMRK 1 and BENCHMRK 2, in antiretroviral treatment-experienced adult patients with documented resistance to at least one drug in each of three drug classes (NRTIs, NNRTIs and PIs) [Cooper, CROI, 2007; Steigbigel, CROI, 2007]. A pooled analysis of interim data at 24 weeks showed that compared to placebo, raltegravir provided superior virological control (Table 13), as well as superior increases in mean CD4 cell counts (89 cells/mm³ versus 35 cells/mm³) [Raltegravir, 2007, 12].

Table 13. BENCHMRK 1 and 2: Pooled efficacy data analysis at 24 weeks [Raltegravir, 2007, 12]

Outcome	Raltegravir 400mg twice daily + OBR (n=286)	Placebo + OBR (n=150)
HIV RNA <50 copies/mL, n (%)	179 (62.6)	50 (33.3)
HIV RNA <400 copies /mL, n(%)	216 (75.5)	59 (39.3)

OBR: optimized background regimen

Raltegravir versus efavirenz. The safety and efficacy of raltegravir has also been compared with that of efavirenz, both in combination with tenofovir/3TC, in treatment naïve patients for 48 weeks (Table 14) [Markowitz, 2007, 130]. Raltegravir provided similar antiviral activity to that of efavirenz at 48 weeks, but with maximal viral suppression (<50 copies/mL) achieved more rapidly [Markowitz, 2007, 128-9]. Mean CD4 cell count increases were comparable for both treatments [Markowitz, 2007, 128].

Table 14. Raltegravir versus efavirenz: efficacy at 48 weeks [Markowitz, 2007, 130]

Outcome	Raltegravir (400mg) twice daily + tenofovir/3TC (n=41)	Efavirenz + AZT/3TC (n=38)
HIV RNA <50 copies/mL, n (%) [95% CI]	36 (88 [74, 96])	33 (87 [72, 96])
Mean change from baseline in CD4 cell counts, cells/mm ³ (95% CI)	144 (109, 179)	170 (125, 215)



Elvitegravir: This drug (125mg dose), in combination with an optimized background regimen, demonstrated increased antiviral activity after 24 weeks compared to boosted comparator PIs (cPI), in an ongoing Phase II dose-finding trial, in treatment-experienced patients with HIV infection (Table 15) [Zolopa, CROI, 2007]. Phase III trials of elvitegravir are currently being considered.

Table 15. Virologic and immunologic responses to elvitegravir at 24 weeks [Zolopa, CROI, 2007]

Outcome	Treatment group		
	CPI/r (n=63)	50mg (n=71)	125mg (n=73)
Mean change in viral load from baseline, log ₁₀ copies/mL	-1.2	-1.4	-1.7
Mean difference versus CPI/r (95% CI)		-0.2 (-0.6, 0.2)	-0.4 (-0.8, -0.05)
p-value versus CPI/r		0.27	0.02

CPI/r: ritonavir boosted comparator PI

Resistance

Multiple mutations associated with resistance to raltegravir and elvitegravir are selected for in HIV infected patients failing integrase inhibitor therapy (Table 16) [Stanford, 2007, online].

Table 16. Integrase inhibitor mutations [Stanford 2007, online]

Compound	Mutations
Raltegravir	L74M, E92Q, E138A/K, G140A/S, Q148HRK, N155H, E157Q, G163R
Elvitegravir	H51Y, T66I, E92Q, E138K, G140S, S147G, Q148H/R/K, S153Y, N155H, E157Q, R263K

HIV reverse transcriptase and integrase enzymes interact physically and biochemically during HIV replication [Tasara, 2000, 39-41, Interestingly, it appears that specific integrase mutations may co-evolve with reverse transcriptase mutations, although it is unclear at this point how these affect integrase or RT activity in vivo [Ceccherini-Silberstein, 2007, S6].

Raltegravir: Resistance mutations located near the catalytic site of the integrase enzyme include E92Q, G140S+Q148H and N155H [Malet, 2007, S9]. These changes decrease susceptibility to raltegravir by 2- to 50-fold [Stanford 2007, online], but also compromise enzyme function that results in defects in strand transfer and, in some cases, in 3' processing [Malet, 2007, S9]. Raltegravir resistance appears to arise via two distinct pathways defined by mutations at either N155H or Q148H/R/K, which reduce susceptibility by approximately 10- and 25-fold, respectively [Hazuda, 2007, S8]. The emergence of additional mutations (N155 plus L74M, E92Q, G163R, or Q148H/R/K plus E138K, G140S/A) moderates the replication defects and results in high level raltegravir resistance [Hazuda, 2007, S8]. HIV-1 variants with N155 or Q148 exhibit cross-resistance to other integrase inhibitors [Hazuda, 2007, S8].

Elvitegravir: Mutations associated with resistance to elvitegravir that emerge in vivo include T66I/A/K, E92Q, E138K, S147G Q148H/R/K and N155H [McColl, 2007, S11]. These changes decrease susceptibility to elvitegravir by 1- to 120-fold [Stanford 2007, online]. Several of these (E92Q, E138K, Q148H/R/K and N155H) are also selected in HIV infected patients failing raltegravir therapy (see above). As could be expected, viral strains from patients failing



elvitegravir are cross-resistant to raltegravir (mean elvitegravir fold-change of >150-fold [range 1.02–301], mean raltegravir fold-change of >28-fold [range 0.78–>256]) [McColl, 2007, S11].

Clinical considerations

Treatment guidelines currently indicate that raltegravir, due to its unique mechanism of action, potent antiretroviral activity and good safety profile, represents a fully active agent for antiretroviral-experienced patients who are naïve to integrase inhibitors [DHHS, 2007, 33]. As such, it is likely that this agent will play a major role in the management of drug resistant HIV. Furthermore, the 48-week data indicating similar efficacy to efavirenz-based regimens in treatment-naïve HIV infected patients paves the way for raltegravir to become an alternative component for initial HAART; although, the resistance profiles indicate that as with other antiretroviral drug classes the utility of integrase inhibitors will be limited by cross-resistance between agents.



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