Enfuvirtide antiretroviral therapy in HIV-1 infection

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Abstract: It has been over 25 years since the first diagnosis of what would be known as AIDS. Although great strides in anti-HIV therapeutics have been made, there is still a great need for antiretrovirals that are effective against drug-resistant HIV. Enfuvirtide (ENF) is the first of a new class of fusion inhibitors to be approved by the US Food and Drug Administration for use in combination with other antiretroviral agents among HIV-1 infected patients with previous treatment experience. The inclusion of enfuvirtide in an optimized antiretroviral background regimen for the treatment of HIV-1 infected (treatment-experienced) patients followed the success of two critical clinical trials (TORO: T20 vs Optimized Regimen Only I and II). Even though injection-site reactions persisted in these trials, improved virological and immunological responses were observed among patients. Challenges associated with ENF treatment include the high cost of the drug, injection-site reactions, determining the optimal time to initiate treatment, and the potential for the selection of drug resistant mutants and viral evolution. ENF is a promising novel treatment for HIV infected individuals whose choices for effective treatment are limited by previous treatment and resistance. Understanding the implications of viral fitness and evolution in the presence of ENF treatment is crucial in determining effective and safe treatment regimens, particularly among treatment-experienced patients.

Keywords: enfuvirtide, HIV, salvage therapy, drug resistance, gp41, evolution

Introduction

It has been over 25 years since what would be known as AIDS was first recognized (Gottlieb et al 1981; Gottlieb 2006). Since then, antiretroviral therapy has greatly increased the lifespan of patients infected with HIV-1. However, drug resistance readily emerges during treatment, and new classes of drugs are needed to combat the virus. Enfuvirtide (also known as Fuzeon® or T-20; Roche) is the first of a novel class of antiretrovirals called fusion inhibitors to receive approval for clinical use. Enfuvirtide (ENF) is a 36 amino-acid synthetic peptide that mimics the amino acids 127–162 present in heptad repeat-2 (HR-2) found in the HIV gp41 envelope glycoprotein subunit (Wild et al 1994; Chen et al 2002). ENF binds to residues in HR-1, blocking a conformational change in gp41 required for fusion of the lipid envelope of HIV with the cytoplasmic membrane of CD4 T cells, thus preventing viral entry (Chen et al 1995; Moore and Doms 2003). ENF was approved in 2003 for use in patients who are treatment-experienced and have advanced HIV-1 infection (Fletcher 2003; Matthews et al 2004) adding a much-needed fourth class of drugs to treat HIV infection.

In the current review we will detail the results from ENF clinical trials. We will then review the clinical management of ENF based regimens including dosing, clinical usage, resistance and cross-resistance and usage pertaining to non-clade B infections. We will then discuss the evolutionary consequences of ENF treatment and we will finish with a summation of these subjects.

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Clinical studies

TORO

ENF was approved in 2003 as a salvage therapy agent following the success of the Phase III TORO (T-20 vs Optimized Regimen Only) clinical trials. TORO 1 (United States, Canada, Mexico, and Brazil) and TORO 2 (Australia and Europe) assessed the safety and efficacy of ENF plus an optimized background (ENF + OB) versus optimized background alone (OB) in approximately 1000 treatment-experienced patients (n = 663 ENF + OB and n = 334 OB) (Lalezari et al 2003; Lazzarin et al 2003). Entry criteria for these trials included previous treatment with at least one nucleoside reverse-transcriptase inhibitor, at least one non-nucleoside reverse-transcriptase inhibitor, and at least one (TORO 2) or two (TORO 1) protease inhibitors, documented resistance to these drugs, or both. After 48 weeks, 30% of the ENF + OB arm had HIV-1 RNA levels <400 copies/mL versus 12% of the OB alone. The ENF + OB arm also had greater decreases in viral load and greater increases in CD4+ T-cell counts than OB alone (Nelson et al 2005). The TORO trials are also important in that they established the importance of using an optimized background as the basis for comparison in clinical trials. Based on this evidence, ENF was granted accelerated approval by the FDA in 2003.

As part of the long term follow-up for the TORO trials, at week 48 patients in the OB arm were required to switch to the ENF + OB arm to stay in the study but were allowed to switch prior to week 48 if they experienced virologic failure (Reynes et al 2007). A total of 230 (68.9%) patients from the OB arm switched, of these 222 switched prior to week 48 due to virologic failure. These patients along with the original ENF + OB arm were followed up to 96 weeks where 114 (49.6%) of the switch patients and 362 (55%) of the original ENF + OB remained on ENF + OB at week 96. At 96 weeks, 26.5% of patients on ENF + OB had a viral load less than 400 copies/mL and 17.5% achieved viral suppression below 50 copies/mL. Patients in the original ENF + OB arm had a viral load decrease of 2.1 $\log_{10}$ copies/mL from baseline and an increase of 166 CD4+ T-cells. In the switch arm, patients had a mean decrease of 1.1 $\log_{10}$ copies/mL from baseline and an increase of 116 CD4+ T-cells. The fact that the switch patients had a reduced immunologic and virologic response compared to patients who started with ENF + OB suggests that ENF should be initiated earlier in salvage therapy.

RESIST and POWER

Phase III clinical trial studies RESIST 1 (Randomized Evaluation of Strategic Intervention in multi-drug reSistant patients with Tipranivir-North America and Australia) and RESIST 2 (Europe and Latin America) involved 1509 patients who experienced treatment failure with at least two PI-based regimens (Hicks et al 2006). All patients in the studies were randomized to take ritonavir-boosted tipranivir plus an optimized background (tipranivir/r + OB) or a ritonavir-boosted comparator PI plus OB (cPI). After 48 weeks, patients in the tipranivir/r + OB group had a greater proportion of responders (33.6%) versus cPI (15.3%). The treatment response was greater in both groups if patients had ENF as part of their OB. At 48 weeks, 58.5% of ENF-naïve patients in tipranivir/r + OB who received ENF had a treatment response compared to 21.6% of ENF-naïve patients in cPI who also received ENF. After 96 weeks, 26.4% (197/746) of patients in the tipranivir/r + OB groups had at least a 1 $\log_{10}$ reduction in their plasma viremia from baseline, compared to 10.7% (79/737) of patients in the cPI group (Gazzard et al 2006). Again, this result was more pronounced in patients taking ENF with 45.2% (56/124) in the tipranivir/r + OB group showing a 1 $\log_{10}$ reduction in viral load below their pre-RESIST levels, compared to 16.5% (16/97) in the cPI group.

The phase III clinical trials POWER 1 (Performance of TMC114/r When evaluated in treatment-Experienced patients with PI Resistance) and POWER 2 randomized highly treatment-experienced patients to ritonavir-boosted darunavir plus OB (darunavir/r + OB) or a comparator PI plus OB (cPI) (Clotet et al 2007). After 48 weeks, 61% (67/110) of patients in the darunavir/r + OB had a reduction in plasma viral load of 1 $\log_{10}$copies/mL from baseline versus only 15% (18/120) of patients in the cPI group. This result was also more pronounced in patients who were ENF-naive. In the darunavir/r + OB arm, 81% (29/36) of ENF-naive patients taking ENF had a 1 $\log_{10}$ decrease in viral load from baseline compared to 56% (34/61) of patients on darunavir/r + OB not taking ENF.

The RESIST and POWER studies were similar in terms of average age, gender, race, pre-study viral loads and CD4+ T-cell counts, prior treatment experience, and evidence of drug resistance, however, there are several significant differences (Hill and Moyle 2006). First, the POWER studies involved only 255 individuals while the RESIST study included 1,509 patients. Second, more people, 23%, in POWER used "double-boosted" comparator PIs (two PIs combined with ritonavir) compared to 0% in the RESIST studies. Third, ENF was more likely to be used in POWER than in RESIST trials (47% vs 25%, respectively). After 48 weeks in both studies, approximately 46% in the darunavir/r + OB POWER groups had viral loads below 50 copies/mL,

compared to 10% in the cPI POWER groups. Conversely, in the RESIST studies, 23% of patients in the tipranivir/r + OB groups had viral loads below 50 copies/mL after 48 weeks, compared to 10% of patients in the cPI groups. In the darunavir/r + OB group 81% (29/36) of the ENF-naïve patients taking ENF had a viral load reduction of at least 1 log copies/mL at week 48 versus 58.5% (72/123) of the tipranavir/r + OB group. However, it must be noted that in the POWER studies, ENF use was stratified, not randomized, and patients receiving ENF were more likely to have higher baseline CD4+ T-cell counts, lower baseline viral loads and shorter duration of infections. ENF was not randomized or stratified in the RESIST studies and thus interpretations must be made with caution when comparing these studies.

Pediatrics

The safety and efficacy of ENF has been examined in two small open-label studies involving treatment experienced children and adolescents (Church et al 2004; Wiznia et al 2007). As in the TORO studies involving adults, ENF was combined with OB therapy consisting of inhibitors of the HIV-1 reverse transcriptase and protease enzymes. These pediatric patients were followed for periods of up to 96 weeks, with frequent evaluation of T cell counts and viral load measurements. Suppression of plasma HIV RNA to undetectable levels (<50 copies/mL) was uncommon, occurring in about 10% of participants. However, in the larger study, viral load was reduced by a median of 1.17 log10 copies/mL, and the median increase in T cell number was more than 100 cells/mm^3 and the CD4 T cell percentage rose by 4.7% (Wiznia et al 2007). The treatment responses were greater in children than in adolescents, perhaps reflecting problems with adherence among the older participants. Although injection site reactions were very common in pediatric patients of all ages, no patients were forced to discontinue ENF therapy because of these or other adverse events.

Clinical management

Dosing

ENF must be administered by subcutaneous injection. ENF is in powder form and must be reconstituted with sterile water. Standard dosing for adults and adolescents greater than 16 years of age or older is 90 mg twice daily. Among children, the pharmacokinetics of ENF do not appear to be affected by age and children aged 6–16 receive two daily doses of 2 mg/kg, not to exceed 90 mg per day (Zhang et al 2007). ENF is not approved for children less than 6 years of age. There are no food restrictions and no known clinically significant interactions between ENF and other medications (Boyd et al 2003).

Side effects

ENF is generally well tolerated in both pediatric and adult patients (Lalezari et al 2003; Lazzarin et al 2003; Nelson et al 2005; Reynes et al 2007). Injection site reactions are the most commonly reported side-effect and occur in almost all patients. These are typically mild to moderate in severity and are rarely treatment-limiting. Other side effects include nausea, diarrhea, and fatigue. Eosinophilia has also been reported. At 96 weeks in the TORO trials, there were no new reported ENF safety issues in adults or children (Reynes et al 2007).

Adherence

As noted before, ENF is associated with injection site reactions (ISR) in nearly all patients, including redness, itching, pain, and development of nodules at the injection site. These ISR represent the most common adverse events associated with the agent that when combined with the inconvenient route of administration, represent potential barriers to adherence. The inferior treatment responses seen in adolescents compared to younger children in one recent trial (Wiznia et al 2007) may reflect problems with adherence (more reports of missed doses) seen in the former. Massaging injection sites, the use of analgesics, and other simple measures may limit ISR and improve adherence (Hu et al 2000; Clotet et al 2004).

Clinical usage

A consensus of recommendations concerning the use of ENF includes key points regarding resistance testing, initiation and discontinuation of therapy, safety concerns, and patient education and support is reviewed by Clotet et al (2004). The implementation of these recommendations into the day-to-day clinical practice of providers involved in the care of HIV can be critical in improving patient care and compliance with ENF therapy.

ENF should be administered in conjunction with an optimized background HIV regimen. Resistance testing is recommended in patients receiving enfuvirtide in order to select an appropriate therapeutic background regimen. To this end, genotyping is currently favored as the preferred method of resistance testing for patients with chronic HIV infection (Clotet et al 2004). Susceptibility testing for ENF before initiation is not currently recommended as viruses with wild-type HR-1 can vary as much as 500-fold in susceptibility

and these differences were not associated with differences in clinical response (Clotet et al 2004, 2007).

ENF is only FDA approved for patients who have failed treatment with other agents, and thus is not recommended for initial therapy. The best possible initiation time for ENF treatment is when the patient can be predicted to have a strong, sustained virologic response Treatment with ENF is optimized when there are at least two other active agents besides ENF in the OB regimen, CD4 T-cell counts are greater than 100 and plasma HIV-1 RNA burden is less than 100,000 copies/mL (Clotet et al 2004). In general, patients who have higher CD4 counts, lower viral loads and greater number of active agents are more likely to have durable favorable virologic and immunologic response to ENF + OB (Lalezari et al 2003; Lazzarin et al 2003; Nelson et al 2005; Hicks et al 2006; Clotet et al 2007; Reynes et al 2007). The fact that the TORO switch patients had a reduced immunologic and virologic response compared to patients who started with ENF+OB reinforces this observation and suggests that ENF should be initiated earlier in salvage therapy. Several substudies in addition to the RESIST and POWER studies suggest that not all OB regimens are equal when used with ENF. Negredo et al found that patients who received both tenofovir and didanosine in their OB had little increase in CD4 counts at 8 weeks compared to patients with neither or one of these antiretrovirals in their OB regimen (Negredo et al 2005). If a boosted PI such as lopinavir/ritonavir (Nelson et al 2005), tipranivir/ritonavir (Hicks et al 2006) or duranavir/ritonavir (Clotet et al 2007) is the sensitive agent, then a boosted PI should be considered in the OB (Youle et al 2006). Patients may also initiate ENF in situations where resistance to all available treatment options is present, although this is clearly a less than optimal approach. Fortunately, additional antiretroviral agents, including integrase inhibitors, are nearing approval for use that may be combined to create successful salvage regimens for patients with advanced disease.

Further concerns with ENF treatment involve safety issues of injection site reactions. Good injection technique, rotation of injection sites, and massage of the site post-injection may all aid in reducing the occurrence of injection site reactions.

Further recommendations for the management of ENF-treated patients should include a multidisciplinary approach to patient education, training, and support. ENF has high viral specificity and low toxicity and has been shown to achieve a sustained virologic and immunologic response in three-class experienced patients. The main drawbacks of ENF are the high cost of the drug, the need for parenteral administration, and the potential for resistance and viral evolution.

Resistance

As with all inhibitors of HIV, variants resistant to ENF have been reported. Resistance to ENF usually occurs due to mutations in the N-terminal heptad repeat region of gp41 (HR-1) although mutations in HR-2 have also been reported (Rimsky 1998; Roman et al 2003; Cabrera et al 2006; Lu 2006; Melby et al 2006; Johnson et al 2007; Peuchant et al 2007). ENF resistance is uncommon in patients who are naïve to ENF (Roman et al 2003; Melby et al 2006) where the most common polymorphism observed was N42S (16.2% of patients). Transmission of ENF resistance has been reported in treatment naïve patients (Peuchant et al 2007). In vitro studies have shown resistance to ENF conferred by mutations in gp41 HR-1 amino acids 36–38 (Rimsky et al 1998). In vivo resistance associated mutations were seen in gp41 amino acids 36–45 within HR-1. In the TORO trials the most common mutations (greater than 10% of the viruses) were G36D, V38A, V38M, Q40H, and N43D. Lu et al in a clonal analysis of serial samples of patients with ENF failure found rapid emergence of ENF resistance (Lu et al 2006). At week 2 they found mutations at codons 36 (G36E/D/S) and 38 (V38A/G/M), while at week 4 mutations and codon 40 (Q40H) and 43 (N43D) were found. In a separate study of 13 heavily pre-treated patients receiving ENF, mutations in HR-1 positions 36, 38, and 43 were found as well as mutations further upstream from HR-1 and in HR-2 (Cabrera et al 2006). They observed that mutations at V38E + N42S resulted in a 513 fold change in ENF susceptibility while mutations at V38A + N42D/T had approximately a 140 fold change in ENF susceptibility and N42T + N43K/S resulted in a 32–61 fold change in susceptibility. Single mutations V38A, G36D, G36S, and N43D conferred a 16-, 8-, 7-, and 18-fold change in ENF susceptibility respectively. According to the International AIDS Society 2007, mutations associated with resistance to ENF include: G36D/S, I37V, V38A/M/E, Q39R, Q40H, N42T, N43D (Johnson et al 2007). Furthermore these guidelines caution that mutations in HR-2, as well as coreceptor usage and density could affect ENF susceptibility.

Cross-resistance

Reeves et al introduced ENF resistance mutations in HR-1 into both a CCR5 and a CXCR4 utilizing strain to test for viral sensitivity to other classes of entry inhibitors (Reeves et al 2005). They found that ENF-resistance did not confer cross-resistance to other inhibitors including fusion inhibitors,

chemokine receptor blocking agents, and agents that target CD4 binding. Other studies found that clinical resistance to ENF did not affect susceptibility to other classes of inhibitors (Fikkert et al 1003; Rimsky et al 1998; Greenberg and Cammack 2004; Ray et al 2007).

Coreceptor usage

HIV fusion is a multi-step process that involves conformational changes in both gp41 and gp120. Several in vitro studies have suggested that ENF susceptibility may be linked to coreceptor usage (Derdeyn et al 2000; Reeves et al 2002). However, data from a large number of clinical isolates from the TORO trials showed that virologic response to ENF was independent of baseline coreceptor usage (CCR5, CXCR4, and dual tropic-viruses) (Melby et al 2006). This study also showed that changes in viral load were similar across baseline coreceptor usage.

Non-clade B infection

Although ENF was originally developed based on the gp41 sequence of clade B, antiviral activity has been shown against non-B viral subtypes (Villahermosa et al 2003; Greenberg and Cammack 2004; D'Arrigo et al 2007; Holguin et al 2007). Although there was substantial variability in gp41 within and between subtypes, primary resistance to enfuvirtide was rare (Villahermosa et al 2003; D'Arrigo et al 2007; Holguin et al 2007) in untreated non-B individuals. Baseline IC_{50} values for ENF-naïve individuals were similar to other reports for ENF (Holguin et al 2007).

Although ENF has shown susceptibility to non-B strains, it is not part of the current WHO approach to treatment guidelines for resource-limited settings (Gilks et al 2006). Current limitations for ENF in resource-limited settings include cost, availability of sterile syringes and water, and availability of medical staff to instruct proper injection technique and to monitor and treat injection site reactions. However, Brazil, the first developing country to implement policies that provide universal access to free antiretrovirals, has included enfuvirtide in its treatment guidelines (Nunnet al 2007). Although the cost of this drug has been implicated as a major component of the doubling Brazil's total drug expenditures, it should provide more treatment options for salvage therapy for patients who have limited treatment options (Nunn et al 2007). It remains unclear if other developing countries will follow the Brazil model and adopt enfuvirtide in their treatment guidelines.

Viral fitness and evolution

Understanding the mechanisms by which resistance mutations affect viral fitness and evolution, particularly among patients with significant antiretroviral experience is critical in determining effective therapy regimens (Deeks et al 2005). Viral fitness or replication capacity measures the association between mutations that confer resistance and changes in the ability of the virus to infect cells and reproduce. Fitness levels for a given mutation may vary depending on the presence of other resistance-associated mutations or compensatory mutations as well as other viral characteristics such as coreceptor usage and experimental conditions. Viruses that harbor mutations at positions 36–45 were found to be less virulent than wild-type in the absence of ENF, however in the presence of ENF these viruses displayed greater replicative fitness than wild-type (Lu et al 2004). Although ENF confers selective pressure against gp41, the *env* region must retain its resistance against neutralizing antibodies or it will be selected against in vivo (Moore and Doms 2003). Reeves et al introduced HR-1 mutations that have been shown to confer ENF resistance into both CCR5-tropic and CXCR4-tropic strains (Reeves et al 2005). They found that these mutations reduced infection and fusion efficacy, and delayed fusion kinetics indicating reduction in viral fitness. However, they also found that some of these mutations conferred enhanced sensitivity to neutralizing monoclonal antibodies, suggesting that these mutations may make the virus more susceptible to humoral immune responses. Labrosse et al found that in 5/6 patients who failed ENF therapy, the emergence of resistance mutations did not reduce *env* replicative capacity (Labrosse et al 2006). Further, they found that during ENF therapy the dominant *env* quasi-species differed from the dominant quasi-species circulating before ENF was initiated and persisted even after ENF interruption. Deeks et al examined the level of continued antiviral activity of ENF in the presence of incomplete viral suppression and ENF resistance mutations (Deeks et al 2007). Interruption of ENF only while remaining on a stable optimized background regimen resulted in a small but significant increase in plasma viremia and a small but significant decrease in CD4+ T cell counts suggesting a modest residual antiviral activity by ENF. ENF resistance mutations decreased rapidly during the interruption and in most patients were undetectable by week 16 in a clonal analysis. Kitchen et al examined the viral evolution of these clones from 9 of these patients taken at three time points: before ENF, during ENF failure and after interruption of ENF therapy (Kitchen et al 2006). They found strong evidence supporting ongoing viral evolution during ENF therapy and interruption, suggesting that the loss of ENF resistance mutations was due to back-mutation and not recall of presumed archive ENF-susceptible strains. This result is

consistent with the findings in Labrosse et al (2006). These observations that the dominant quasispecies arising during ENF therapy persists even after interruption suggests that this virus is relatively more fit that the presumed archived ENF-susceptible virus. This may reflect continued evolution in *env* to the host immune system.

Conclusions

ENF is a novel therapeutic agent, and represents the first member of the fusion inhibitor class of antiretroviral agents. It represents a valuable choice for treatment-experienced patients who otherwise have few options for effective treatment. ENF-resistance has not been shown to confer cross-resistance to other inhibitors including fusion inhibitors, coreceptor inhibitors, and agents that target CD4 binding. ENF has been successfully used to produce durable reductions in viremia even in patients with multi-drug resistance. The likelihood of a sustainable virologic and immunologic responses is maximized when the patient has at least 2 other active agents besides ENF in the OB, CD4 counts are greater than 100 cells/mm^3, and plasma viral burden is less than 100,000 copies/ml. Based on the results from several clinical trials, this likelihood may be even greater when used with an active boosted PI regimen. The differing results from in vivo and in vitro tests of viral fitness of ENF mutations along with the finding of continued evolution of *env* during ENF treatment and interruption suggests that there exists a stronger selective pressure, perhaps the immune system, that is driving the evolution of the virus forward. This, coupled with the high cost and inconvenience of parental administration of ENF, and the limited residual antiviral activity may limit the long-term use of the drug in patients who have ENF-resistance. Fortunately, with the introduction of new novel antiretroviral agents, including integrase inhibitors, that are nearing approval for use, options for the treatment of patients with multi-class resistant HIV-1 are improving. Overall, ENF treatment has been proven effective among treatment-experienced individuals infected with HIV-1.

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