

# An Insight on the Leading HIV Entry Inhibitors

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Received: July 03, 2005; Accepted: August 17, 2005; Revised: September 14, 2005

**Abstract:** The main strategies nowadays to fight AIDS rely on chemical therapy to inhibit the reverse transcriptase or protease of HIV. However, a synthetic 36 amino-acids peptide that blocks the entry of the virus in the target cells (enfuvirtide) has recently reached approval for clinical application. This molecule may probably be just the leader of a new generation of drugs that is about to emerge to interrupt the first step in the HIV life cycle, i.e. preventing the virus from actually entering cells. This paper reviews the enfuvirtide path from clinical trials to the attempts to detail its molecular-level mode of action. It is commonly accepted that this peptide would block the fusion between viral and cell plasma membrane through binding to the N-terminal heptad repeat (NHR) region of the viral protein gp41. However, there has been growing evidence that this model of action may be unrealistic, the action of enfuvirtide being more complex and diverse than initially thought. Membrane-assisted local concentration increase and interference with gp120/co-receptor docking may also contribute for the inhibitory action of the peptide. Selected HIV-entry inhibitors on clinical trials are presented to characterize the future drugs in the market in this class.

**Keywords:** Entry, attachment, fusion, binding, entrance inhibitor, HIV, AIDS, virus, chemokine, peptide, receptor, co-receptor, synergism, clinical trial, enfuvirtide.

## 1. INTRODUCTION

Combination therapy with reverse transcriptase and protease inhibitors is the most common current treatment of HIV-1 infection [1,2]. Despite the success of this therapy, namely reducing morbidity and mortality of HIV-1 infected patients [3-6], it has adverse effects and drug resistant HIV-1 strains emerged [7-11]. A new class of antiviral agents is in development, the entry inhibitors. These molecules target the first step in the HIV-1 replication cycle, the viral entry [12-15]. Unlike reverse transcriptase and protease inhibitors, which target post-entry steps, entry or fusion inhibitors act extracellularly preventing viral entry into target cells. The most advanced and already approved by the Food and Drug Administration fusion inhibitor is enfuvirtide (T20; DP-178) from Trimeris/Roche [16].

### HIV-1 Entry

The HIV-1 envelope glycoproteins complex is expressed on the surface of the viral membrane as an oligomeric protein (trimer) and mediates the viral entry. The complex is composed of two subunits noncovalently associated: gp120, the surface glycoprotein, which interacts with cellular receptors, and gp41, the transmembrane glycoprotein, responsible for fusion of the viral and cellular membranes [e.g. 12,17,18]. HIV-1 entry into target cells is a multi-step process that is initiated by gp120 binding to the CD4 receptor, present in the target cells surface. This contact

induces a conformational change in gp120 enabling it to bind to a cellular co-receptor, usually CCR5 or CXCR4. gp120 binding to CD4 and co-receptor induces conformational changes in gp41 exposing the N-terminal fusion peptide and allowing its insertion into target cell membrane, thereby connecting both membranes. Subsequent changes within the gp41 ectodomain involve the interaction of two heptad repeat sequences (HR1, next the fusion peptide and HR2, preceding the transmembrane domain) and a six-helix bundle structure (also called hairpin structure) is formed. The hairpin formation brings the viral and cell membrane into closed proximity, allowing fusion of the membranes and then entry of the virus [12,13,17-19].

Each of the main steps in the HIV-1 entry process can be a target for entry inhibitors. The ones currently under development fall into three categories: gp120-CD4 binding inhibitors, gp120-co-receptors binding inhibitors, and membrane fusion inhibitors, which interfere with gp41 conformational changes [2,14,16,20]. Fusion inhibitors are the leading compounds, one of them, enfuvirtide, being already approved for clinical applications.

## 2. ENFUVIRTIDE CLINICAL TRIALS

The publication in 1998 of the results of the TRI-001 clinical trial [21] is considered as the proof-of-concept that the entrance of the virus in the cell of the host can be blocked *in vivo* by enfuvirtide. This study evaluated the use of enfuvirtide subcutaneous infusion, as monotherapy, during 14 days. The TRI-001 allowed concluding that the administration, during a reduced period, is safe and induces a significant (1.14 log<sub>10</sub>) viral charge decrease in the group that received a 90 mg deliverable dose of enfuvirtide (100 mg nominal dose) twice daily.

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The TRI-003 phase II trial [22], conducted during 28 days with 78 patients, lead to the adoption of subcutaneous injection as the preferential route of administration of enfuvirtide, and evaluated the pharmacokinetics of this and other forms of administration. This study results showed that subcutaneous injection presents significant advantages when compared with subcutaneous infusion, in terms of administration simplicity, tolerability, plasma pharmacokinetics and antiviral response predictability.

The phase II multicenter trial T20-205 [23] tested the administration of 45 mg of enfuvirtide twice daily by subcutaneous injection, in association with conventional optimized therapy, to 71 patients, during 48 weeks, for tests of genotype resistance. 56% of the patients who had kept the treatment presented significant improvements, with a decrease for less than 400 copies of HIV-1 RNA/mL.

The T20-206 assay had the purpose of comparing different enfuvirtide therapeutic doses in addition to conventional antiretroviral therapies [24]. The results of this random study indicated the dose of 90 mg twice daily as the one that, combined with other antiretrovirals, reduces the viral load more efficiently.

The T20-208 phase II trial [25] had the purpose of studying the pharmacokinetics and tolerability of the administration of enfuvirtide in different doses and in formulations of different strengths. This study enabled the optimization of the formulation and dosage to be used in the subsequent phase III trials: twice daily administration of 90 mg enfuvirtide in a single injection of a high-strength formulation in carbonate (instead of the administration of the same 90 mg in two 45 mg injections of a lower-strength formulation, used in most of the previous clinical trials).

A study carried out with 12 HIV-infected patients previously enrolled on the phase II T20-205, T20-206 or T20-208 trials evaluated the pharmacokinetics and relative bioavailability of enfuvirtide following subcutaneous injection at three separate anatomical sites: abdomen, thigh and arm [26]. The relative bioavailabilities of enfuvirtide, taking abdomen as a reference site, were 101% for thigh and 117% for arm. Independently of the slightly higher bioavailability observed for the arm injection, and of some differences on the injection site reactions, the authors considered that the comparability among the three injection sites allows to the infected patients the freedom to choose (and to rotate, if necessary) among these three possible sites of injection.

The publication in 2001 of a study involving the follow-up of 39 patients during 3 years demonstrated that the primary resistance to enfuvirtide treatment is a rare event [27]. However, the authors warned that the emergence of future variants of the virus resistant to this therapy cannot be ruled out. More recently, another study [28] also indicated that primary genotypic enfuvirtide resistance seems to be rare, regardless of subtype or prior antiretroviral therapy. The emergence of enfuvirtide resistant virus in patients receiving enfuvirtide monotherapy was identified by the first time by Wei *et al.* [29], in patients enrolled in the TRI-001 clinical trial [21], receiving an antiretroviral dosage

considerably lower than the later approved for clinical use, during only 14 days.

The enfuvirtide absorption and disposition pharmacokinetics after both intravenous and subcutaneous administration were evaluated in the controlled clinical pharmacokinetic study T20-501 [30]. The same work evaluated the dose proportionality of enfuvirtide pharmacokinetic parameters at higher subcutaneous doses. The 12 patients received randomly four different single doses of enfuvirtide (90 mg intravenous and 45, 90, and 180 mg subcutaneous) separated by a one week washout. Based on the results, a structural pharmacokinetic model was developed, allowing the description of the absorption and disposition kinetics of enfuvirtide plasma concentration after subcutaneous administration. The study also indicated that enfuvirtide is almost completely absorbed from subcutaneous depot, and that the pharmacokinetic parameters were linear up to a dose of 180 mg.

Based on the findings of the previous phases I and II clinical trials, it were the two TORO (T-20 vs. Optimized Regimen Only) phase III clinical trials that set the conditions for the approval of enfuvirtide for clinical use. The TORO 1 trial [31] was conducted with patients from North-America and Brazil, while TORO 2 [32] enrolled patients from Europe and Australia. Both TORO studies compared the efficacy and safety of 24 weeks of combined treatment with enfuvirtide (90 mg twice daily) and an optimized background antiretroviral regimen with the efficacy and safety of the optimized background regimen alone. The patients (491 in TORO 1 and 504 in TORO 2) had at least six months of previous treatment with agents in three classes of antiretroviral drugs, resistance to drugs in these classes, or both, and at least 5000 viral copies/mL. At the end of the 24 weeks, the groups taking enfuvirtide had a least-squares mean decrease from baseline in the viral load of 1.696 and 1.429  $\log_{10}$  copies/mL in TORO 1 and TORO 2, respectively, compared with a decrease of 0.764 and 0.648  $\log_{10}$  copies/mL in the TORO 1 and TORO 2 control groups ( $P < 0.001$  for both pairs). The mean increases in  $CD4^+$  cell counts were 76 and 65.5  $\text{mm}^{-3}$  (TORO 1 and TORO 2 enfuvirtide groups, respectively), compared with 32 and 38.0  $\text{mm}^{-3}$  for their control groups ( $P < 0.001$  for both pairs). These studies demonstrated the significance of the antiretroviral and immunologic benefits of the addition of enfuvirtide to optimized antiretroviral regimens in patients with HIV-1 infections resistant to previously established therapies, and in patients who had previously received multiple antiretroviral drugs.

The most common adverse events associated with enfuvirtide administration are injection-site reactions. The TORO studies reported injection-site reactions in 98% of the patients, including pain, discomfort, erythema, induration, nodules, cysts, pruritus and ecchymosis associated with the injection-site [31,32]. Despite this extremely high incidence, only 4.4% of the patients abandoned the treatment due to injection-site reactions [e.g. 33]. The biopsies carried out in a study of injection-site reactions in 7 patients receiving enfuvirtide revealed inflammatory responses consistent with a localized hypersensitivity reaction, regardless of the type of clinical lesion and even in its absence [34].

The incidences of most of the other adverse events reported in the TORO study for the group of patients treated with enfuvirtide combined with background antiviral therapy were not significantly different from those observed for the control group, treated with the background antiretroviral therapy only [31,32]. However, the incidences of pneumonia (mainly bacterial) and lymphadenopathy were significantly higher in the group using enfuvirtide, as indicated by the combined analysis of the TORO studies after 48 weeks of treatment [e.g. 33]. The statically significant increase on pneumonia incidence in the group of patients taking enfuvirtide combined with optimized background therapy may be related with enfuvirtide administration, or it can also result of an unexplained abnormally low incidence of pneumonia in the control group. Studies carried out with HIV-infected patients on highly active antiretroviral therapy (HAART) indicate pneumonia incidences considerably higher than those reported for the control groups of the TORO studies, and comparable with the observed for the groups taking enfuvirtide combined with optimized background therapy [e.g., 35,36]. The results of the TORO studies, at the 24th week of the treatment, also indicated a higher incidence of some laboratory parameters outside the upper limit of normal in the group taking enfuvirtide; namely, eosinophilia, increased triglycerides, creatine phosphokinase, amylase, lipase, alanine aminotransferase, aspartate aminotransferase and -glutamyl transferase levels, and decreased hemoglobin concentration. However, at the 48th week of treatment the incidences of these laboratory abnormalities were similar in the enfuvirtide and control groups, or even lower in the enfuvirtide group.

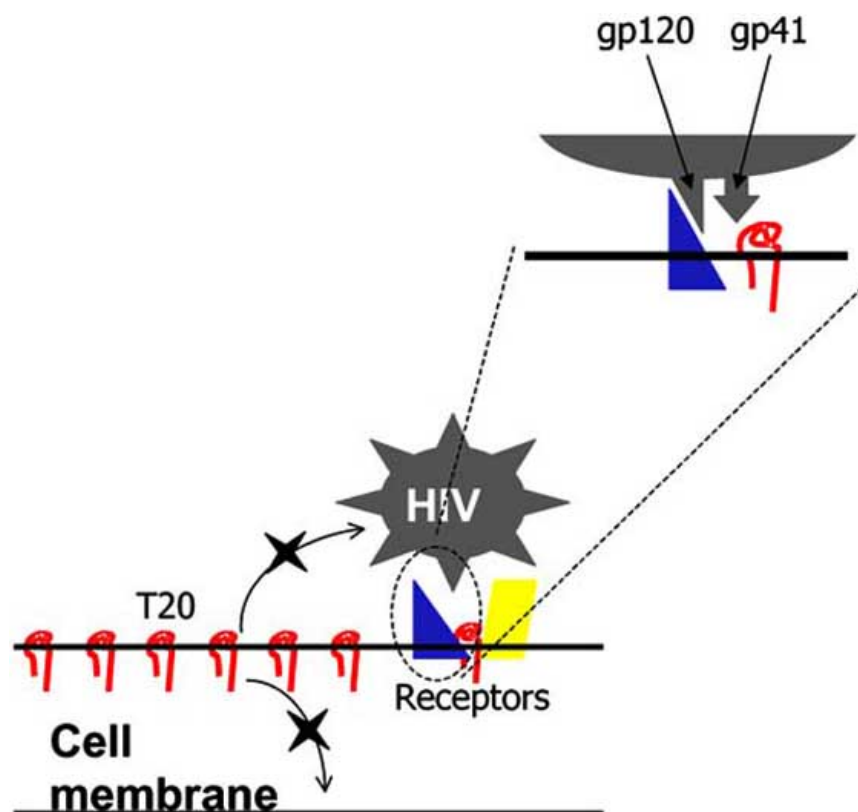
The safety, tolerability, antiretroviral activity, dosage and pharmacokinetics of the pediatric use of enfuvirtide were evaluated in some phase I/II clinical trials [37-40], which will not be described here.

### 3. RECENT HIGHLIGHTS IN THE MOLECULAR-LEVEL ACTION OF THE FUSION INHIBITORS ENFUVIRTIDE AND T-1249: A PLETHORA OF SITES OF ACTION

Enfuvirtide is a synthetic 36 amino-acids peptide derived from a C-terminal sequence of HIV-1 gp41 [41,42]. There are several proposals for the enfuvirtide inhibitory action mechanism involving target sites in gp41 and gp120. In the most accepted mechanism of action model, enfuvirtide binds to the HR1 region of gp41 preventing the formation of the six-helix bundle structure and thereby fusion is blocked [16,41,43-45]. Viral resistance to enfuvirtide is associated with mutations in the GIV sequence of the HR1 region [45]. However, enfuvirtide lacks some amino-terminal residues present in other gp41 HR2 sequence based peptides (C-peptides) thought as essential for the binding to the HR1 region and HIV-1 entry inhibition [41,46]. Also it was suggested that enfuvirtide binding affinity to the HR1 region cannot justify its strong inhibitory activity [47], and most recently Liu *et al.* showed that enfuvirtide cannot interact with HR1 region derived peptides to form a stable six-helix bundle [48]. The existence of a second binding site for enfuvirtide on gp41 was proposed by Muñoz-Barroso *et al.* [49], involving the contact site of gp41 oligomers cluster to form a fusion pore, which is required for the occurrence of

the complete fusion process [18,50]. Kliger *et al.* showed that enfuvirtide can bind membranes and oligomerize, but unlike in aqueous solution, it cannot interact with the HR1 region in the membrane environment [51]. Thus, in agreement with these ideas, there are two enfuvirtide binding sites in gp41, contributing to fusion inhibition. By interacting with the HR1 region in aqueous solution enfuvirtide inhibits the formation of the six-helix bundle structure and by interacting with the gp41 C-terminal region in the membrane environment fusion pore formation is prevented [47,49,51]. Other studies suggest that membranes play a role in enfuvirtide action, namely in increasing concentration in its site of action [52,53]. A theoretical analysis of the hydrophobicity and interface affinity of enfuvirtide amino acids sequence clearly suggests that it may interact with biological membranes, which is confirmed experimentally. Enfuvirtide interaction with membranes has peculiar features [53]: i) incorporation into neutral liquid-crystal lipid membranes is extensive ( $K_p = (1.6 \pm 0.1) \times 10^3$ ;  $G = -6.6$  kcal mol<sup>-1</sup>); ii) cholesterol and/or physiological concentrations of negatively charged lipids modulate the incorporation in lipid membranes; iii) a shallow position in the lipid membrane makes it readily available for interaction with gp41; and iv) it has similar secondary structure (random coil) whether in aqueous or lipid environment, therefore no conformational energetic barrier can prevent enfuvirtide from being active both in aqueous solution and lipid membranes. An alternative or additional model for the enfuvirtide action at the molecular level was proposed (Fig. 1). It was also proposed that enfuvirtide may bind to the gp41 fusion peptide in aqueous medium preventing its insertion into the target cell membrane. Thus, concomitant to the HR1 binding, association with the fusion peptide may contribute to block viral entry [54,55]. It appears that sensitivity to enfuvirtide can be also modulated by co-receptor specificity, determined by the V3 loop region of gp120. X4 viruses, that utilize CXCR4 co-receptor for entry, are much more sensitive to enfuvirtide than R5 viruses, which utilize CCR5 [56]. Reeves *et al.* showed that co-receptor binding affinity and co-receptor expression levels are related with enfuvirtide sensitivity [57]. More recently, it was proposed that enfuvirtide can interact with the gp120 co-receptor binding site, blocking gp120 binding to the co-receptor and thus inhibiting viral infection [48,58,59]. This interaction occurs in a CD4-induced manner [58,59] with the gp120 of X4 viruses, which could explain the increased sensitivity of these viruses to enfuvirtide [58]. In this way gp120 can be another target site contributing to enfuvirtide inhibitory activity. However, different studies carried out with isolates from different clinical trials have not identified significant differences in enfuvirtide susceptibility for patients harbouring CCR5, CXCR4 or dual tropic viruses, indicating that viral tropism has no clinically relevant effect on the enfuvirtide therapy efficiency [e.g. 60,61]. New studies are needed to further clarify this matter of debate.

T-1249, a second generation fusion inhibitor that follows enfuvirtide, is a 39 amino-acids peptide composed of sequences derived from HIV-1, HIV-2 and simian immunodeficiency virus (SIV) [62]. More potent than enfuvirtide, this fusion inhibitor also retains activity against most enfuvirtide-resistant HIV-1 strains [2,19,63,64].



**Fig. (1).** A model for the enfuvirtide action at the molecular level. Enfuvirtide (T20) attaches to membranes in an interfacial position, reaching high local concentrations. Translocation is prevented by phospholipid charge effects (among others). When the virus approaches the cell surface, its outer membrane will not compete for enfuvirtide uptake due to its higher cholesterol content. Upon binding, gp41 is exposed near the membrane interface, where enfuvirtide is present in high local concentrations (adapted from [53]).

Although its mechanism of action is not well known, it is believed that T-1249 acts like most C-peptides, interacting with HR1 region preventing hairpin structure formation [16,60]. It was suggested that T-1249 ability to bind both the target cell and virus membranes may play an important role in T-1249 mode of action and provide a peptide reservoir at the action site [65]. At variance with enfuvirtide, T-1249 adsorbs to rafts and gel-like platforms in lipid membranes, this difference being one possible explanation for its improved activity.

In spite of the optimistic results *in vitro*, T-1249 clinical trials were put on hold (Table 1).

#### 4. TOWARDS THE FIRST GENERATION OF A NEW CLASS OF DRUGS

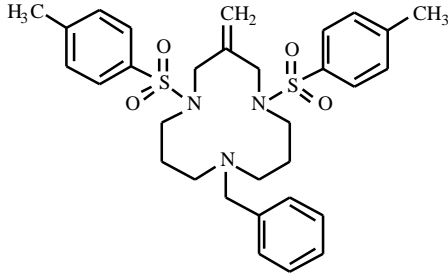
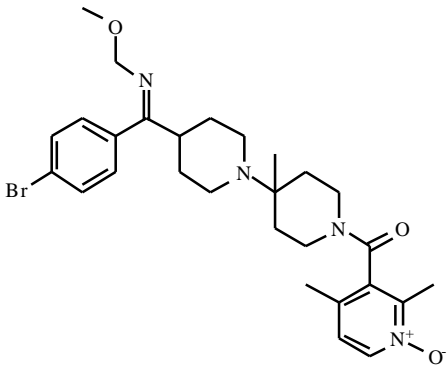
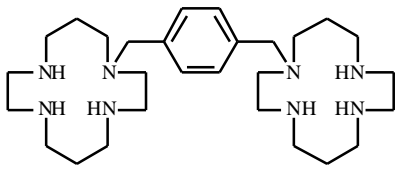
While gp41/gp120-related inhibitors, mainly enfuvirtide, are undoubtedly in the front line of clinical applications of HIV-entry inhibitors, an overview of the recent patents in this field reveals an additional trend for the future. Chemokine receptors are clearly the focus of attention [66-80]. Table 1 lists some of the CCR5 and CXCR4 antagonists currently under clinical trials. Altogether with CD4-targeted strategies and enfuvirtide related peptides, these molecules form a promising pool of candidate drugs to keep the pace in the fight against HIV. A detailed analysis of their action can be found in references 81-83. AMD3100 is a very specific

antagonist for CXCR4 and is a potent inhibitor of X4 HIV-1 replication. AMD070 is an orally bioavailable derivative of AMD3100 as potent as its parent molecule. SCH-C can also be administered orally and preliminary clinical data proved its ability to significantly reduce R5 HIV-1 viral load. Several related molecules allowed equal or better affinity for the CCR5 receptor.

An obvious approach to HIV entry inhibition would be to prevent gp120 to dock CD4 receptors. Soluble CD4 for competitive inhibition of attachment would be the simplest strategy. However, soluble CD4 performs poorly in AIDS patients. CD4-immunoglobulins fusion proteins have better pharmacokinetics, affinity for gp120 and minimal immunogenic effects. PRO542 is CD4-IgG2, a tetrameric CD4-based fusion protein comprising human IgG2 in which the HIV-binding region of CD4 has replaced the Fv portions of both heavy and light chains. In phase I clinical trials, PRO542 showed antiviral activity as well as a favourable safety and pharmacological profile. Enfuvirtide acts synergistically with PRO542 [84].

A more indirect approach is to down-modulate the CD4 receptor. CADA (Table 1) inhibits HIV infection by the specific down-modulation of CD4, probably by means of down-regulation of CD4 expression at the (post)translational level [81]. CADA also exhibits a synergistic action with enfuvirtide, as well as AMD3100.

**Table 1. Short Survey of Some Molecules for their Action Preventing the Entry of HIV into Target Cells. Most Information Regarding Clinical Trials Status can be Found at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) and <http://hiv.net> (Accessed June 2005)**

Name	Molecule	Site of action	Company	Clinical trials
T20 = Enfuvirtide	36 amino-acids peptide [53]	gp41, gp120	Roche / Trimeris	As Fuzeon on the market
T-1249	39 amino-acids peptide [65]	gp41 (only?)	Roche / Trimeris	Stopped; Clinical development on hold <sup>1</sup>
CADA		CD4 (down-modulator)	-----	-----
PRO542	CD4-IgG <sub>2</sub>	CD4/gp120 binding	Progenics	Phase II
SCH-C		CCR5 antagonist	Schering-Plough	Stopped <sup>2</sup>
SCH-D	SCH-C derivative	CCR5 antagonist	Schering-Plough	Phase II <sup>3</sup>
UK427,857	-----	CCR5 antagonist	Pfizer	Phase II <sup>4</sup>
AMD3100		CXCR4 antagonist	AnorMED	Stopped <sup>5</sup>
AMD070 = AMD 11070	AMD3100 derivative	CXCR4 antagonist	AnorMED	Phase I/II

1- <http://trimeris.com/pipeline/t-1249.html> (accessed June 2005): «In January 2004, the clinical development of T-1249 was put on hold due to challenges in achieving the desired technical profile of the current formulation. The compound's safety, efficacy and tolerability were no way related to the decision».

2- Heart rhythm disturbance was reported.

3- No serious side effects yet known.

4- No side effects occurred during Phase I.

5- Cardiotoxic.

## 5. CURRENT & FUTURE DEVELOPMENTS

The synergism among efficient HIV-entry inhibitors is probably the main reason to be optimistic in having powerful

tools to interrupt the HIV life cycle in the first step. Interruption of the entry step in the HIV life cycle may be of comparable importance in the future to reverse transcriptase or protease inhibition. This new front against HIV will

further help controlling AIDS for the sake of the comfort and longevity of the patients.

## ACKNOWLEDGMENTS

ASV thanks Fundação para a Ciência e A Tecnologia (Portugal) for grant SFRH/BD/14336/2003. Mr. Hansjörg Möst is acknowledged for his collaboration.

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