

UC Merced

UC Merced Undergraduate Research Journal

Title

A Molecular Characterization of Recombinant HIV-1 Fusin Inhibitors

Permalink

<https://escholarship.org/uc/item/6dd7546p>

Journal

UC Merced Undergraduate Research Journal, 4(2)

Author

Fisher, Christopher

Publication Date

2013

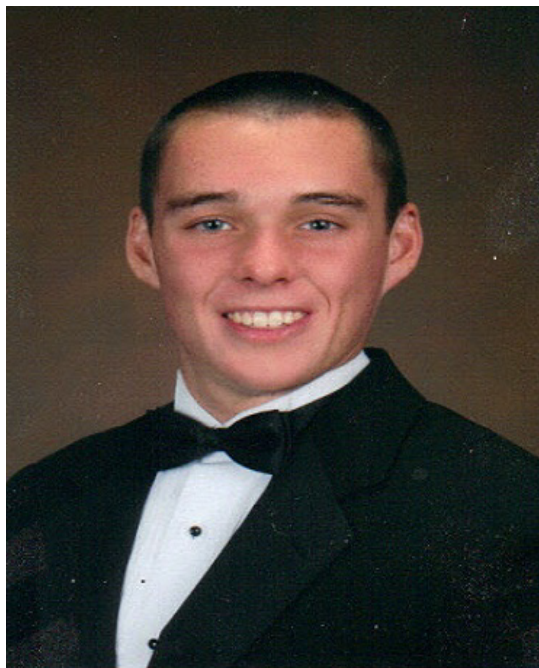
DOI

10.5070/M442018407

Copyright Information

Copyright 2013 by the author(s). All rights reserved unless otherwise indicated. Contact the author(s) for any necessary permissions. Learn more at <https://escholarship.org/terms>

Undergraduate



Christopher Fisher

Currently, I am a senior at UC Merced and will be graduating in May of 2013 with a major in Cellular/Molecular Biology and a minor in Chemical Sciences. I have participated in Physical Organic Chemistry research in the past, but the focus of my current research is the design of more effective HIV cell-entry inhibitors. After graduation, I will pursue my goal of becoming an Orthopedic Surgeon for the US Navy.



A Molecular Characterization of Recombinant HIV-1 Fusin Inhibitors

Chris Fisher

WRI 116

Abstract

Synthetic peptides used as HIV-1 fusion inhibitors are quickly emerging as one of the most promising innovations to combat HIV. Detailed mechanistic insight into the HIV cell-entry process, provided by many research groups, has been pivotal in identifying possible protein-protein interactions that can be targeted by engineered fusion inhibitors. Armed with extensive knowledge of HIV's method of entry, investigators are developing peptides that bind specific sites on viral envelope proteins, thereby interfering with the infection process. The field of synthetic fusion inhibitors has progressed considerably, quickly evolving from designing one-step inhibitors to engineering two-step inhibitors. A persistent problem encountered by researchers is HIV's rapid mutability, allowing the virus to become resistant to the effects of fusion inhibitors. Currently, next-generation fusion peptides are being developed that are less susceptible to induced drug-resistance, thus resulting in greater effectiveness and antiviral potency. The field of fusion inhibitors is transitioning to chimeric molecules that are constructed from multiple peptides that covalently bind their target, in addition to interfering with various other facets of cell entry.

Introduction

There are currently 34 million people worldwide that are infected with HIV, and the most effective treatment approved for clinical use is the highly-active antiretroviral therapy (HAART), which primarily targets the components of HIV that work at the intracellular level using inhibitors such as: nucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, chemokine receptor antagonists, and a very limited selection of entry inhibitors.^{[1][2]} Although the HAART therapy attempts to interfere with HIV infection at multiple stages, the virus quickly develops a resistance toward the cocktail of drugs ^[1], thus requiring a change in the combination of drugs about every three months. Most components of antiviral treatment target the intracellular aspects of HIV infection, but strides have been made in the field of HIV fusion inhibitors, in which synthetic proteins are engineered to effectively interfere with HIV entry. These newly-designed inhibitors have shown to be less susceptible to HIV-resistance, so these drugs remain effective *in vivo* for longer durations. This review discusses the current understanding of fusion inhibition and the progression of R5-tropic HIV-1 fusion inhibitors, including the recent development of chimeric peptides and those inhibitors that utilize covalent binding to inactivate their target.

HIV Mechanism of Entry

The innovation of HIV fusion inhibitors has been facilitated by the in-depth elucidation of the HIV cell-entry mechanism. The key steps in this process are a series of crucial protein-protein interactions that take place between surface proteins on the virus and CD4⁺ Helper T-cells. Evidence heavily supports the hypothesis that the relevant proteins involved in the cell entry process are gp120 and gp41, on the surface of HIV-1, and CD4 and CCR5 on the surface of the T-cell.^[3] As HIV contacts its target, the gp120 homotrimer binds to CD4, causing a conformational change in the structure of gp120 exposing the CCR5 binding domain on an adjacent subunit of gp120.^[3] Upon binding to CCR5, gp120 undergoes further conformational change to expose the gp41 glycoprotein, which extends outward toward the T-cell membrane.^[3] The N-terminal heptad repeat region of gp41 inserts into the T-cell membrane, triggering the N-terminal and C-terminal regions of gp41 to fold on top of one another forming what is known as a six-helix bundle.^{[3][4]} Formation of the gp41 six-helix bundle pulls the viral membrane and T-cell membrane together, allowing the fusion of the two membranes and entry of HIV-1.^[3] Although many studies have been devoted to revealing the mechanism of HIV membrane fusion, spectroscopic analyses and protein-binding assays continue to refine the details of the viral cell-entry.

The mechanism of HIV-1 cell entry is considered to be a generally well-understood process that has been studied and reviewed by many researchers. The goal, now, is to identify specific aspects of this mechanism that can be targeted by various types of fusion inhibitors. Research suspects that inhibiting one step of HIV cell entry should be sufficient to prevent HIV-1 from infecting the T-cell.

Inhibition of One Step of HIV Cell Entry

There are several interactions during the HIV-1 cell-entry mechanism that are susceptible to attack. These potential points of inhibition include: gp120, gp41, CCR5, and CD4. Inhibition of the CCR5 coreceptor has been easily accessed due to the presence of endogenous chemokines that already bind this coreceptor. Experimental evidence has confirmed that the interaction of gp120 with CCR5 can be prevented by the CCR5-antagonist, RANTES.^[5] Using multiple optimization assays, the Hartley group agreed that the RANTES derivative, 5p12-RANTES, demonstrated the most promising results: strong binding, but neither induction of G-protein signal transduction, nor CCR5 sequestration by the T-cell.^[5] This compound was shown to prevent

HIV-1 infection by interfering with a key protein-protein interaction in the cell-entry mechanism.^[5] The utility of 5p12-RANTES is obvious in that its interaction with the CCR5 coreceptor is benign, but its presence prevents a protein-protein interaction that is crucial for HIV cell entry.

Although RANTES derivatives were demonstrated to decrease HIV-1 cell entry, it has its flaws. Kuritzkes et al. (2012) experimentally determined that, in some patients, the two domains of the CCR5 coreceptor, the N-terminal domain and the extracellular loop, show variability in their molecular composition.^[6] The effect of these deviations from the predicted homogeneity of CCR5 receptors presents a problem for CCR5 antagonists, but the preliminary nature of this study requires that more data be gathered using spectroscopic analyses and binding-affinity assays to provide greater insight into the specific deviations within the CCR5 coreceptor.

In addition to CCR5, glycoproteins gp120 and gp41 on the surface of HIV-1 are obvious sites for potential inhibition. The LiWang and Klasse groups have completed extensive research testing the effects of a recombinant lectin, Griffithsin, on binding gp120. Results of site-directed mutagenesis experiments and spectroscopic analyses indicate that upon binding to the CD4 binding-site on gp120, Griffithsin (GRFT) prevents the conformational change that allows progression through the cell entry mechanism and subsequent infection of the CD4⁺ T-cell.^{[7][8]} Valuable structural insight was provided for GRFT, leading researchers to identify key residues that could possibly play key roles in gp120-binding.^[8] LiWang showed that the mutation of any of multiple carbohydrate-binding sites on gp120 appeared to significantly reduced GRFT's binding affinity to gp120, indicating that this lectin is easily nullified by HIV-1 mutation.^[8] The ease with which drug-resistance is induced implies that Griffithsin may not be as promising a fusion inhibitor candidate as once thought, but this peptide has shown to have other features that contribute to its significance as an antiviral peptide. Investigation by Klasse et al. (2012) suggests that GRFT has a high affinity for binding to gp120. Upon binding, the complex of GRFT-gp120 elicited a more robust immune response than just gp120 alone.^[7] Provoking an immune response could increase the effectiveness of GRFT and similar fusion inhibitors, but this hypothesis

must be supported by more data from assays testing the effect of increased antibody concentration on HIV-1 cell entry.

Multiple studies have also shown that gp41 is an excellent site to target with engineered inhibitors. The N-terminal heptad repeat region of this glycoprotein is the most commonly targeted site due to its crucial activity.^[1] T20, also known as enfuvirtide, was the earliest of the engineered C-peptides, and its mechanism of action is to bind gp41, thereby preventing the formation of the 6-helix bundle that is necessary for membrane fusion.^{[9][10]} Next generation derivatives of T20 have been designed, with T1249 and T2635 being most recently developed and tested.^{[9][11]} The Egnick group provided convincing data suggesting that both T1249 and T2635 show greater resistance to amino acid substitutions than T20^{[9][11]}, thus indicating that HIV-1 cannot as easily mutate around these next-generation C-peptides. Using multiple virological and biophysical analyses, specific amino acid substitutions were identified that were necessary for gp41 to escape inhibition by T1249 and T2635 ^{[9][11]}, and the electrostatic interactions between these C-peptides and gp41 were rigorously characterized. ^{[9][11]} Furthermore, support has been provided suggesting that T2635 is even more resistant to gp41 mutation than T1249^[11], making this C-peptide the best candidate for future studies.

Other research has developed another fusion inhibitor, CP32M, that binds gp41 via a series of salt bridges and hydrogen bonds.^[12] The potency of this peptide was determined to be greater than that of T20, and through cell assays using pseudovirus entities, it was shown to be comparable to T2635 in its susceptibility to gp41 amino acid substitutions.^[12] The formation of salt bridges and weak intermolecular forces, rather than covalent bonds, used by the aforementioned C-peptides, may lead to questions about their validity – especially because the incidence of induced drug resistance involving these inhibitors is so high. Future research will work to develop C-peptides that demonstrate a higher affinity for gp41, and these studies will undoubtedly focus on identifying amino acids that are necessary for gp41 functioning.

The field of fusion inhibitors is rapidly progressing by engineering peptides that are mosaics of the drugs that have been mentioned thus far. It has been hypothesized that the cumulative effect of combining

multiple inhibitors will significantly increase inhibition of HIV-1 cell entry and lead to decreased rates of induced drug resistance.

Inhibition of Two Steps of HIV Cell Entry

While inhibiting only one surface protein on HIV-1 proved to be effective at interfering with membrane fusion, investigators generally agree that these peptides quickly induce drug resistance. Researchers have suggested multiple methods of engineering recombinant inhibitors to be more resistant to HIV-1 mutation, and it was hypothesized that HIV-1 would be less able to evade a peptide impeding two steps of membrane fusion.

Two well-known peptides, GRFT and C37, have been extensively studied in their effectiveness as fusion inhibitors, and the potential for a substantial increase in potency led to the suggestion of combining the two peptides.^[13] Experiments by Dr. LiWang's lab led to the development of GRFT-linker-C37, GRFT physically linked to C37, forming what she theorized would be a more effective inhibitor due to simultaneous binding of gp120 and gp41.^[13] Evidence from viral assays indicated that the combination of these two peptides led to a nine-fold decrease in HIV-1 activity compared to unlinked GRFT and C37, signifying greater inhibition.^[13] The elevated antiviral activity can be attributed to the proximity and localization of the two inhibitors near the surface of viral membrane, in addition to the fact that there are two fusion inhibitors present.

The success of GRFT-linker-C37 led to the construction of another chimeric peptide that consisted of 5p12-RANTES and C37. Through a series of biochemical assays testing for HIV-1 infection and receptor binding, studies have shown that 5p12-RANTES-linker-C37 demonstrated a nearly 100-fold reduction of HIV-1 activity compared to the individual peptides.^[14] The 5p12-RANTES portion of this inhibitor is believed to localize this chimeric peptide to the surface of the T-cell membrane by binding to CCR5, ensuring that C37 will be very near its target, gp41. The significant decrease in HIV-1 activity was attributed to the close proximity of C37 to the site of protein-protein interactions.^[14] Experiments involving both GRFT-linker-C37 and 5p12-RANTES-linker-C37 have provided strong support for the notion that targeting two steps of membrane fusion with one molecule is more effective than using multiple peptides by themselves. Due to interfering

with two steps of HIV-1 cell entry, the virus must mutate at least twice as much in order to avoid these proteins, which suggests that chimeric inhibitors are much more efficient in terms of avoiding drug resistance. Furthermore, overwhelming evidence supporting the increased antiviral potency of linked fusion inhibitors has led to the general consensus that chimeric inhibitors are more effective than their individual constituents. Nonetheless, more research involving mutagenesis of specific amino acids on gp120 and gp41 is required to further elucidate the molecular interactions that occur and to identify more powerful combinations of synthetic peptides.

The research being performed by the LiWang group is at the forefront of designing more powerful HIV-1 fusion inhibitors. In future experiments, Dr. LiWang is interested in exchanging C37 with T2635 in both chimeric inhibitors, and as described, T2635 has been shown to be more difficult for HIV-1 to evade by mutating. [13][14]

What Is In Store For Fusion Inhibitors?

Synthetic peptides that are engineered to sequester specific sites on HIV surface proteins have been shown to be effective in preventing HIV cell entry. Recent publications suggest alternative designs of fusion inhibitors which incorporate naturally-occurring elements in the human body such as antibodies. Antibodies are known to have high affinity for the epitopes that they recognize, and this tropism is being exploited to deliver anti-HIV drugs to precise locations. Chang et al. (2012) demonstrated, by cell-cell fusion assays, that antibodies linked to multiple copies of T20 potentially decreased HIV-1 activity and prevented cell entry.[10] Results of biochemical assays from other studies appeared to show decreased HIV-1 activity when exposed to monoclonal antibodies that were specific for domains on gp120, suggesting that using antibodies acting as fusion inhibitors could be feasible.[15] In stark contrast, the results of other research has indicated that anti-gp120 and anti-gp41 monoclonal antibodies exhibit such modest antiviral activity that their use as fusion inhibitors is unreasonable.[16] There seems to be disagreement in this area of fusion inhibitors, but by far the most widely accepted consensus is that antibodies are not feasible as HIV fusion inhibitors. The results of recent studies

indicate two completely contrasting results, which emphasizes the need for more research that will provide more in-depth insight into the neutralizing ability of antibodies.

Researchers continue to confront the question, “How can we design a fusion inhibitor that HIV cannot avoid by mutation of its surface proteins?” In addition to chimeric fusion inhibitors^{[13][14]}, this question has begun to be answered by the initial development of fusion inhibitors that covalently, rather than electrostatically, bind to viral surface proteins. The Liu and Li groups have provided preliminary evidence suggesting the significant increase in binding affinity and antiviral activity of fusion inhibitors that use covalent interactions to bind gp41.^{[4][17]} By attaching reactive chemical groups to a synthetic inhibitor, these peptides have shown to irreversibly bind specific amino acid residues of gp41^{[4][17]}, which should lead to significant reductions in instances of induced drug resistance. Once HIV interacts with these inhibitors, the residues are irreversibly bound, thereby not giving HIV the opportunity to mutate to avoid inhibition. The use of covalent binding has provided promising, albeit early, results, but more data from cell-cell fusion and pseudoviral assays are necessary to add credibility to these preliminary results.

Conclusion

HIV affects millions of people worldwide, and efforts to subdue the morbidity of this virus have led to significant research geared toward the development of more effective fusion inhibitors. Progress in the development of more effective HIV-1 cell-entry inhibitors will undoubtedly focus on identifying conserved regions of gp120 and gp41 that are necessary for function; such domains cannot accommodate mutations without experiencing a loss of function, and any such elimination of activity would result in a non-functional viral particle. Maiden results provide very convincing evidence supporting the efficacy of covalent fusion inhibition^{[4][17]}, and future experiments will certainly focus on the design of covalent inhibitors that bind to indispensable residues in order to achieve the highly sought after “escape-proof antiviral.”

Fusion inhibitors have rapidly advanced from interfering with one step of membrane fusion to now, interfering with two steps; innovations such as the implementation of covalent binding and the improvement of

C-peptides, have also resulted in the development of synthetic peptides that display increased antiviral activity. Presently, recombinant, chimeric peptides that target multiple steps of HIV cell-entry have demonstrated the greatest antiviral potency, and researchers have tended to agree that inhibition of multiple steps of viral cell-entry with a single drug will be the future of fusion inhibitor design.

References:

1. Hartman TL & Buckheit RW Jr. The continuing evolution of HIV-1 therapy: Identification and development of novel antiretroviral agents targeting viral and cellular targets. - *Mol Biol Int.*2012;2012:401965.Epub 2012 Jul 10.
2. Rathburn, Chris R. "Antiretroviral Therapy for HIV Infection." *Antiretroviral Therapy for HIV Infection*. Medscape Reference, 4 Sept. 2012. Web. 20 Oct. 2012. <<http://emedicine.medscape.com/article/1533218-overview>>.
3. Klasse PJ. The molecular basis of HIV entry. - *Cell Microbiol.*2012 Aug;14(8):1183-92.Doi: 10.1111/j.1462-5822.2012.01812.x.Epub 2012 Jun 5.
4. Bai Y, Xue H, Wang K, Liu K. Covalent fusion inhibitors targeting HIV-1 gp41 deep pocket. - *Amino Acids.*2012 Sep 9.
5. Gaertner H, Cerini F, Escola JM, Hartley O. Highly potent, fully recombinant anti-HIV chemokines: Reengineering a low-cost microbicide. - *Proc Natl Acad Sci U S A.*2008 Nov 18;105(46):17706-11.Epub 2008 Nov 12.
6. Henrich TJ, Lewine NR, Lee SH, Kuritzkes DR. Differential use of CCR5 by HIV-1 clinical isolates resistant to small-molecule CCR5 antagonists. - *Antimicrob Agents Chemother.*2012 Apr;56(4):1931-5.Epub 2012 Jan 17.
7. Banerjee K, Michael E, Eggink D, Klasse PJ. Occluding the mannose moieties on human immunodeficiency virus type 1 gp120 with griffithsin improves the antibody responses to both proteins in mice. - *AIDS Res Hum Retroviruses.*2012 Feb;28(2):206-14.Epub 2011 Jul 27.
8. Xue J, Gao Y, Hoorelbeke B, Liwang PJ. The role of individual carbohydrate-binding sites in the function of the potent anti-HIV lectin griffithsin. - *Mol Pharm.*2012 Sep 4;9(9):2613-25.Doi: 10.1021/mp300194b.Epub 2012 Aug 21.
9. Eggink D, Langedijk JP, Bonvin AM, Sanders RW. Detailed mechanistic insights into HIV-1 sensitivity to three generations of fusion inhibitors. - *J Biol Chem.*2009 Sep 25;284(39):26941-50.Epub 2009 Jul 17.
10. Chang CH, Hinkula J, Loo M, Wahren B. A novel class of anti-HIV agents with multiple copies of enfuvirtide enhances inhibition of viral replication and cellular transmission in vitro. - *PLoS One.*2012;7(7):E41235.Epub 2012 Jul 23.
11. Eggink D, Bontjer I, Langedijk JP, Sanders RW. Resistance of human immunodeficiency virus type 1 to a third-generation fusion inhibitor requires multiple mutations in gp41 and is accompanied by a dramatic loss of gp41 function. - *J Virol.*2011 Oct;85(20):10785-97.Epub 2011 Aug
12. Yao X, Chong H, Zhang C, Cui S. Structural basis of potent and broad HIV-1 fusion inhibitor CP32M. *J Biol Chem.*2012 Aug 3;287(32):26618-29.Epub 2012 Jun 7

13. Kagiampakis I, Gharibi A, Mankowski MK, LiWang PJ. Potent strategy to inhibit HIV-1 by binding both gp120 and gp41. - *Antimicrob Agents Chemother.*2011 Jan;55(1):264-75.Epub 2010 Oct 18.
14. Zhao B, Mankowski MK, Snyder BA, Liwang PJ. Highly potent chimeric inhibitors targeting two steps of HIV cell entry. - *J Biol Chem.*2011 Aug 12;286(32):28370-81.Epub 2011 Jun 9.
15. Chen W, Y., Feng Y, Wang Y, Dimitrov DS. Fusion proteins of HIV-1 envelope glycoprotein gp120 with CD4-induced antibodies showed enhanced binding to CD4 and CD4 binding site antibodies. - *Biochem Biophys Res Commun.*2012 Sep 7;425(4):931-7.Epub 2012 Aug 11
16. Yee M, Konopka K, Balzarini J, & Duzgunes N. Inhibition of HIV-1 env-mediated cell-cell fusion by lectins, peptide T-20, and neutralizing antibodies. - *Open Virol J.*2011;5:44-51.Epub 2011 may 12.
17. Zhao L, Tong P, Chen YX, Li YM. A multi-functional peptide as an HIV-1 entry inhibitor based on self-concentration, recognition, and covalent attachment. - *Org Biomol Chem.*2012 Aug 28;10(32):6512-20.Epub 2012 Jul 4.

Annotated Bibliography:**1. Bai Y, Xue H, Wang K, Liu K.**

The purpose of this study was to test the effectiveness of adding a reactive chemical group to the C-peptide fusion inhibitor C34. Using the standard cell-cell fusion assays and pseudoviral assays, this experiment demonstrated an increase in antiviral potency presumably due to the addition of the thioester group to C34. This research is significant because it suggests an alternative method for designing fusion inhibitors. Most, if not all, C-peptide inhibitors that bind gp41 are very susceptible to drug resistance, but by covalently binding gp41, the proposed fusion inhibitor appears to have successfully addressed the problem of HIV mutability with regards to developing drug resistance.

2. Banerjee K, Michael E, Eggink D, Klasse PJ.

The purpose of this study was to investigate the Griffithsin's (GRFT) effect on the humoral response to gp120 on HIV-1. Using DC-SIGN binding assays and multiple variations of ELISA, the researches gathered data that suggested that the GRFT-gp120 complex caused a greater and more effective humoral response to gp120 than GRFT or gp120 alone. This study is significant because it identifies an immunological response that could further enhance GRFT's antiviral activity. This research is significant to this review because it suggests that antibodies, in conjunction with GRFT, could play a profound role in preventing HIV cell-entry; this implication has identified the possibility of using antibodies as neutralizing agents to increase the effectiveness of synthetic fusion inhibitors.

3. Chang CH, Hinkula J, Loo M, Wahren B.

The purpose of this research was to study the antiviral activity of antibody-T20 inhibitor complexes constructed using Dock-and-Lock technology. Data from cell-cell fusion and viral activity assays suggested that the antibody-T20 complexes were delivered to the surface of HIV, and that the T20 C-peptide displayed significant antiviral activity in its characteristic manner. This study is significant because it provides data that appears to support the notion that antibodies increase the potency of fusion inhibitors. This publication is significant to this review because it proposes a future direction for the development of novel fusion inhibitors through the combination inhibitory peptides and antibodies.

4. Chen W, Y., Feng Y, Wang Y, Dimitrov DS.

The purpose of this study was to investigate the utility of monoclonal antibodies in the neutralization of HIV. Data from pseudoviral assays and ELISA suggest that binding of monoclonal antibodies to the CD4 binding sites on gp120 cause a conformational change in gp120 resulting in the exposure of the CCR5 coreceptor-binding site. Once this coreceptor-binding site is exposed, it is susceptible to inhibition by other synthetic peptides. This study is significant because it suggests and appears to support the use of antibodies as agents that contribute to inhibition of viral cell-entry. This research is significant to this review because it suggests possible avenues for future development of next-generation fusion inhibitors using the specificity of monoclonal antibodies.

5. Eggink D, Langedijk JP, Bonvin AM, Sanders RW.

The purpose of this research was to determine the effectiveness of next-generation T20 derivatives and to characterize their interaction with gp41. Through cell-cell fusion assays and infectivity assays the

antiviral potency and relative antiviral activity of T1249 and T2635 was determined as compared to T20. This research is significant because it characterized the interaction of T1249 and T2635 with gp41, as well as provided data that indicates that both of these C-peptides demonstrated increased anti-HIV activity. This study pertains to this review because it describes the search to identify conserved residues on gp41 that are immutable, and this investigation also demonstrates the progression of fusion inhibitor design toward peptides that HIV cannot mutate to avoid.

6. Eggink D, Bontjer I, Langedijk JP, Sanders RW.

The purpose of this study was to identify the specific amino acid mutations in gp41 that are required for HIV-1 to become resistant to the effects of T2635. Results from infectivity assays identified the residues that must be mutated in order for T2635 to not bind to gp41, and the data also strongly indicates that this third-generation C-peptide requires greater gp41 mutation than the previous C-peptides. This study is significant because it characterizes the interaction between gp41 and T2635, and the specific mutations that allow gp41 to become resistant to T2635 inhibition. This research is significant to this review because it identifies that there is a gap in knowledge about which amino acids in gp41 are conserved and cannot be mutated.

7. Gaertner H, Cerini F, Escola JM, Hartley O.

The purpose of this study was to develop an antiretroviral drug that interferes with HIV-1 cell entry by binding to the CCR5 coreceptor on CD4+ Helper T-cells. Using multiple rounds of optimization assays, cell-cell fusion assays, and viral activity assays this research eventually converged on a synthetic peptide that showed significant antiviral activity by interfering with membrane fusion. This research is significant because it identifies another method of preventing viral cell-entry, has been used by subsequent investigators in attempts to construct effective fusion inhibitors. This study is significant to this review because it describes the detailed activity of the engineered fusion inhibitor and its relevance toward the progression of HIV-1 inhibition.

8. Henrich TJ, Lewine NR, Lee SH, Kuritzkes DR.

The goal of this investigation was to characterize the nature of the molecular heterogeneity of the CCR5 coreceptor and describe how this variability affects CCR5-antagonist binding. This research presents an observation that is overlooked by the many studies in the field of fusion inhibitors. Mutagenesis techniques and ELISA assays have indicated that, indeed, there is considerable variation in multiple domains of the CCR5 coreceptor, and this dilemma suggests that the general consensus in this field should be re-evaluated to better understand the CCR5 coreceptor. This study describes one of the few gaps in understanding in this field. It was thought that the CCR5 coreceptor had been well-characterized, but the data from this report suggests otherwise.

9. Kagiampakis I, Gharibi A, Mankowski MK, LiWang PJ.

The purpose of this research was to investigate the antiviral activity of a novel fusion inhibitor that consisted of the combination of Griffithsin (GRFT) and C37. Results from a series of cell-cell fusion and pseudoviral assays provided convincing evidence that this chimeric peptide had substantially increased antiviral potency compared to its constituent components. This study is significant because it suggests inhibiting two-steps of viral cell-entry and demonstrates that the combination of inhibitory peptides re-

sults in profoundly decreased viral activity. This research is significant to this review because it provides evidence that support the chimeric cell-entry inhibitors as the future direction of fusion inhibition.

10. Xue J, Gao Y, Hoorelbeke B, Liwang PJ.

The purpose of this study was to evaluate the interactions between Griffithsin (GRFT) and gp120 that account for its antiviral potency and to provide conformational insight into the structure of GRFT. Data collected from spectroscopic analysis and the mutation of specific carbohydrate-binding sites identified those active sites that are critical for GRFT's antiviral potency; the disruption of these sites resulted in substantial loss of gp120 binding. This study is significant because it provides evidence of GRFT's antiviral activity, as well as suggests possible mechanisms of gp120 binding. This investigation is significant to this review because it suggests that how GRFT works is not entirely known and that GRFT is easily nullified by HIV mutation; this article also presents data that supports GRFT's potency as a fusion inhibitor.

11. Yao X, Chong H, Zhang C, Cui S.

The purpose of this study was to design a novel HIV fusion inhibitor that binds gp41 and to identify the specific interactions that CP32M makes with residues on gp41. Variations of viral assays and specific mutagenesis techniques elucidated the structural and mechanistic detail of the fusion inhibitor, CP32M. This study is significant because it presents the development of a novel C-peptide that is completely different from the most common C-peptide, T20. Results that strongly indicate gp41 and decreased viral activity further strengthen the argument that gp41 is ideal site to target to prevent HIV infection. This research is significant to this review because it conveys the consensus that gp41 is a prime target for engineered peptide inhibitors.

12. Yee M, Konopka K, Balzarini J, & Duzgunes N.

The purpose of this study was to investigate the effectiveness of monoclonal antibodies in preventing HIV cell entry and infection. Cell-cell fusion assays and fluorescence labeling were used to determine that antibodies result in little, if any, decrease in HIV activity. Antibodies have known to be ineffective against HIV infection, but the significance of this study was that it clearly described the specificity of antibody domains, and it was implied that this tropism could be exploited to deliver fusion inhibitors to the surface of HIV. This research illustrates how the field of fusion inhibitors is exploring the use multiple biological molecules in the design of future cell-entry inhibitors.

13. Zhao B, Mankowski MK, Snyder BA, Liwang PJ.

The purpose of this study was to design and determine the effectiveness of a recombinant peptide that inhibits two steps of HIV cell-entry. Using cell-cell fusion assays and viral fusion assays, results indicated that this synthetic peptide, 5p12-RANTES-linker-C37, displayed up to 100-fold increases in antiviral activity by preventing HIV membrane fusion. This study is significant because it provides further evidence supporting the hypothesis that inhibiting two steps of viral cell-entry will result in exponential decreases in viral activity and infectivity. This study is significant to this review because it demonstrates that inhibiting two steps of membrane fusion is much more effective than targeting only one step, thus helping to solidify the general understanding within the field.

14. Zhao L, Tong P, Chen YX, Li YM.

The purpose of this research was to design a novel fusion inhibitor that had a cholesterol moiety and would covalently and irreversibly bind gp41. Data from HPLC analyses and pseudoviral assays indicated that the isothiocyanate group exhibited a high affinity for gp41 and that the cholesterol group significantly increased antiviral potency by concentrating the inhibitor at the site of membrane fusion. This study is significant because it shows that antiviral effectiveness can be increased by localizing the fusion inhibitor at the T-cell membrane and by covalently binding gp41 as opposed to electrostatically binding gp41. This research is significant to this review because it demonstrates the use of covalent, irreversible binding to inhibit gp41, which seems to be the future direction of inhibitory C-peptides.