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Synergistic Inhibition of HIV-1 Envelope-Mediated Membrane Fusion by Inhibitors Targeting the N and C-Terminal Heptad Repeats of gp41

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The human immunodeficiency virus type-1 (HIV-1) envelope (Env) proteins that mediate membrane fusion represent a major target for the development of new AIDS therapies. Three classes of Env-mediated membrane fusion inhibitors have been described that specifically target the pre-hairpin intermediate conformation of gp41. Class 2 inhibitors bind to the C-terminal heptad repeat (C-HR) of gp41. The single example of a class 3 inhibitor targets the trimeric N-terminal heptad repeat (N-HR) of gp41 and has been postulated to sequestrate the N-HR of the pre-hairpin intermediate through the formation of fusion incompetent heterotrimers. Here, we show that N_{CCG}-gp41, a class 2 inhibitor, and $N36^{Mut(e,g)}$, a class 3 inhibitor, synergistically inhibit Env-mediated membrane fusion for several representative HIV-1 strains (X4 and R5) in both a cell fusion assay (with membrane-bound CD4) and an Env-pseudo-typed virus neutralization assay. The mechanistic, as well as potential therapeutic, implications of these observations for HIV-Env-mediated membrane fusion are discussed.

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Keywords: HIV-1; Env-mediated cell fusion; HIV-1 fusion inhibitors; synergism; gp41

Human immunodeficiency virus (HIV) envelope (Env)-mediated membrane fusion represents the first step of HIV-1 infection. Env comprises two non-covalently interacting proteins, gp120 and gp41, derived by proteolytic cleavage of gp160.[1–3] Fusion is triggered by the binding of gp120 on the virus with first CD4 and, subsequently, a chemokine receptor (CXCR4 or CCR5) on the surface of the target cell. These interactions result in a series of conformational changes that lead to the formation of a pre-hairpin (PHP) intermediate of gp41[4–9] in which the internal trimeric coiled-coil of N-helices (residues 542–591, comprising the N-heptad repeat or N-HR) is exposed and the N-terminal fusion peptide is inserted into the target membrane (Figure 1(a)). gp41 is tethered to the viral membrane *via* a transmembrane domain that lies C-terminal to the C-heptad repeat (C-HR, residues 623–663). Subsequently, apposition of the viral and target cell membrane is driven by the formation of a trimer of hairpins in which the N-HR trimeric coiled-coil is surrounded by helices comprising the C-HR (Figure 1(a), left-most diagram).[2,10] The trimer of hairpins is the structure of the gp41 ectodomain that has been solved by both crystallography[11–13] and NMR.[14]

Three classes of fusion inhibitors directed against the PHP conformation of gp41 have been described (Figure 1(a)). Class I targets the N-HR trimeric coiled-coil. The first examples of class 1 inhibitors comprised peptides derived from the C-HR, such as C34 (residues 628–661) and T20 (residues 638–673, also known as Fuzeon™ and currently used in clinical practice).[15–17] More recently, both polyclonal[18] and monoclonal[19,20] antibodies to the N-HR trimeric coiled-coil have been generated that also inhibit HIV-1 Env-mediated membrane fusion. Class 2 inhibitors target the C-HR. Examples of class 2 are various chimeric proteins in which the complete N-HR trimeric coiled-coil is exposed and stabilized by engineered disulfide bridges,[18,21] such as N_{CCG}-gp41

Abbreviations used: HIV, human immunodeficiency virus; PHP, pre-hairpin; N-HR, N-heptad repeat; C-HR, C-heptad repeat; Env, envelope.

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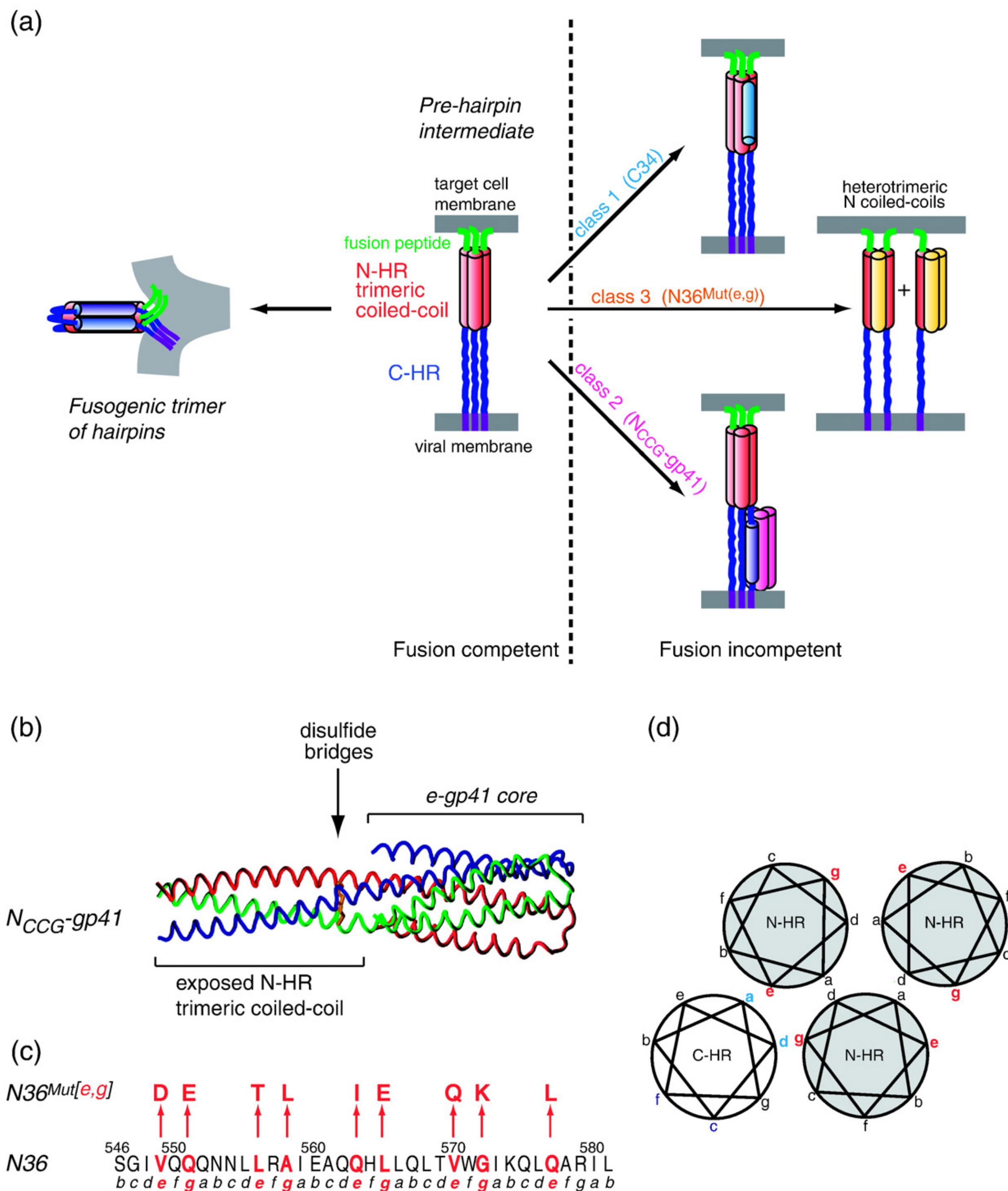
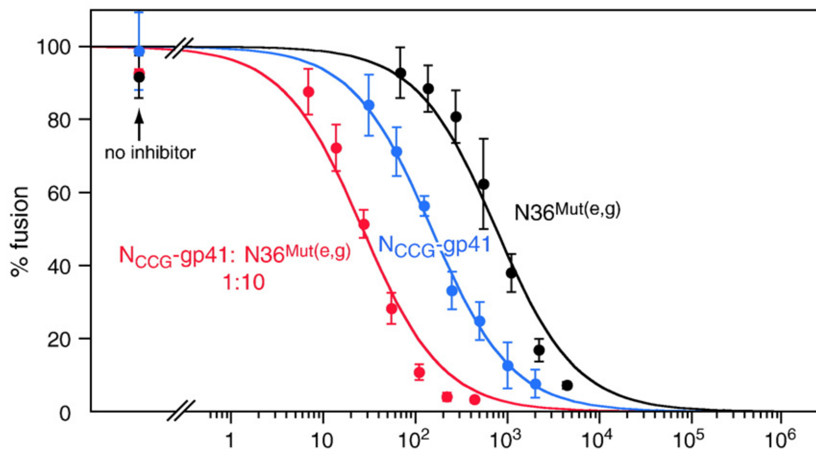


Figure 1. gp41 targeted inhibitors of HIV-1 Env-mediated membrane fusion. (a) Diagrammatic representation of the sites of action of the three classes of inhibitors that target the pre-hairpin intermediate state of gp41. Class 1 binds to the N-trimeric coiled-coil formed by the N-heptad repeat (N-HR); class 2 binds to the C-heptad repeat (C-HR); class 3 forms heterotrimeric coiled-coils with the N-HR of gp41. (b) Backbone tube representation of the chimeric construct N_{CCG}-gp41 in which the trimeric coiled-coil of N-HR helices is fused in helical phase onto the minimal ectodomain core of gp41 and held together by engineered disulfide bridges. (c) Sequence of the N36 peptide comprising residues 546–581 of HIV-1 Env that constitute the N-HR, and the mutations introduced to generate the class 3 inhibitor $N36^{Mut(e,g)}$. (d) Helical wheel representation of the interaction between N-HR trimeric coiled-coil and a C-HR helix in the fusogenic trimer of hairpins; the main site of interaction occurs between positions *e* and *g* (red) of two N-HR helices and *a* and *d* (blue), respectively, of one C-HR helix. Mutation of the residues at positions *e* and *g* of the N-HR in $N36^{Mut(e,g)}$ preclude any interaction with the C-HR. N_{CCG}-gp41 was expressed and purified as described.[21] $N36^{Mut(e,g)}$, purchased from Biopeptide Co, Inc. (San Diego, CA), was synthesized on a solid phase support, purified by reverse phase high performance liquid chromatography, and verified for purity by mass spectrometry. $N36^{Mut(e,g)}$ bears an acetyl group at the N terminus and an amide group at the C terminus. N_{CCG}-gp41 is an obligate trimer, since the chains are held together by engineered intermolecular disulfide bridges.[21] $N36^{Mut(e,g)}$ forms a stable, monodisperse trimer in solution.[23]

(Figure 1(b)) and $N35_{CCG}$-N13, as well as a construct known as 5-helix in which only one of the N-helices in the trimeric N-HR coiled-coil is exposed.[22] Finally, one example of a class 3 inhibitor has been described which also targets the N-HR trimeric coiled-coil but has been postulated to form fusion incompetent, inactive heterotrimers with the N-HR (Figure 1(a)).[23] This particular inhibitor, known as $N36^{Mut(e,g)}$, was derived from the N-HR sequence comprising residues 546–581 of HIV-1 Env where all residues located at positions *e* and *g* of the helical wheel of the N-HR trimeric coiled-coil were mutated (Figure 1(c)). Because these two positions are principal sites of interaction with the C-HR in the fusogenic trimer of haipins conformation of gp41 (Figure 1(d)), $N36^{Mut(e,g)}$ can no longer interact with the C-HR yet forms monodisperse trimers in solution.

Since the N-HR and C-HR represent non-overlapping sites in the PHP conformation of gp41, one might speculate that inhibitors that target these two regions might inhibit HIV-1 Env-mediated membrane fusion synergistically. However, class 1 and 2 inhibitors are mutually antagonistic, since they strongly interact with one another to form a six-helix bundle analogous to the trimer of hairpins structure of the fusogenic form of gp41.[22] Class 2 and 3 inhibitors, however, do not interact with one another, and if the postulated mechanism of the class 3 inhibitor $N36^{Mut(e,g)}$ is correct, synergistic inhibition by combination of class 2 and 3 inhibitors should occur. Here, we show, using two different assays with Env from several representative HIV-1 strains that the class 2 inhibitor NCCG-gp41 and the class 3 inhibitor $N36^{Mut(e,g)}$ do indeed



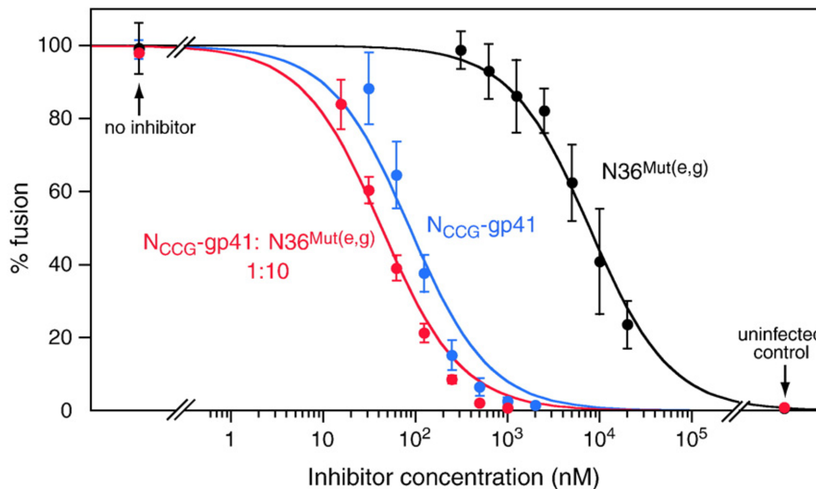


Figure 2. Synergistic inhibition of HIV-1 Env-mediated membrane fusion by combination of N_{CCG}-gp41 and $N36^{Mut(e,g)}$. (a) Dose-response curves from a quantitative vaccinia virus-based reporter gene assay with Env from HIV-1 LAV expressed on the surface of the effector cells and membrane bound CD4 and CXCR4 co-receptor expressed on the surface of the TMZ-b1 target cells. (b) Dose-response curves from an HXB2 Env-pseudo-typed virus neutralization assay. The data for N_{CCG}-gp41 and $N36^{Mut(e,g)}$ alone are shown in blue and back, respectively; the data for N_{CCG}-gp41 and $N36^{Mut(e,g)}$ in a 1:10 combination are shown in red. The experimental data (shown as filled circles) represent the averages of four to eight independent experiments and the standard deviations in the measurements are shown as vertical bars. The continuous curves represent best-fits to the experimental data using the simple activity relationship %fusion = 100/(1+[inhibitor]/IC_{50}). The concentration of $N36^{Mut(e,g)}$ is expressed as monomer, and the concentrations for the combination curves refer to the concentration of N_{CCG}-gp41. The IC_{50} values for N_{CCG}-gp41 and $N36^{Mut(e,g)}$ alone are summarized in Table 1, and dose reduction and combination indices for both inhibitors acting in combination are listed in Table 2. Experimental details for both assays are given in the footnotes to Table 1. Although some curves may appear to exhibit a steeper concentration dependence than that predicted from the simple activity relationship, fitting the data to a more complex relationship is not warranted given the absence of a detailed mechanism, as well as uncertainties in the data. More importantly, the simple activity relationship fit provides an excellent estimate of the IC_{50}. For example, the red curve in (a) obtained with a combination of N_{CCG}-gp41 and $N36^{Mut(e,g)}$ in a ratio of 1:10 can be fit perfectly to the expression % fusion = $100/(1+[\text{inhibitor}]/K)^n$ with a value of K = 71(±8) nM (in N_{CCG}gp41), and the Hill coefficient n set equal to 2. The relationship between K and IC_{50} is given by[34] $IC_{50} = (\sqrt[n]{2}-1)K$ yielding a calculated value of 29(±3) nM for the IC_{50}; this compares to the near identical value of 27(±6) nM for the IC_{50} obtained by fitting the same data to the simple activity relationship (i.e. $n=1$).

inhibit Env-mediated membrane fusion in a synergistic manner.

To test the hypothesis that class 2 and 3 inhibitors may act synergistically to inhibit viral fusion we tested the respective, representative inhibitors, N_{CCG}-gp41 and N36$^{Mut(e,g)}$, individually and in constant ratio combinations against several representative HIV-1 strains (two R4 strains, LAV and HXB2, and one R5 strain, SF162). In addition, to address potential differences that may arise between different fusion assays, the same inhibitors or combinations thereof were tested in two different fusion assays. The first assay was a quantitative vaccinia virus-based reporter gene assay[21,24–26] for cell-cell fusion that employs robust cell surface expression of HIV-1 Env on effector cells and constitutive cell surface expression of membrane-bound CD4 and chemokine co-receptors on target TZM-b1 cells. The total duration of the fusion assay is ~2.5 h. The second assay, which is ~48 h in duration, was a neutralization assay employing Env-pseudo-typed virus and TZM-b1 target cells.[27,28] Representative data for each of these assays is shown in Figure 2 and the complete data are summarized in Tables 1 and 2. Figure 2(a) displays the results of testing N_{CCG}-gp41 and N36$^{Mut(e,g)}$ individually and in combination (ratio of 1:10) in the cell fusion assay with Env from HIV-1 LAV, while Figure 2(b) illustrates the corresponding data obtained using the HXB2 Env-pseudo-typed virus in a neutralization assay. The IC_{50} values for N_{CCG}-gp41 and N36$^{Mut(e,g)}$ are 150(±16) nM and 753(±152) nM, respectively, in the fusion assay, and 85(±18) nM and 7399(±713) nM, respectively, in the HXB2 Env-pseudo-typed virus neutralization assay. When N_{CCG}-gp41 and N36$^{Mut(e,g)}$ are used in combination in a ratio of 1:10, the IC_{50} (expressed in concentration of N_{CCG}-gp41) value is reduced to 27 (±6) nM and 43(±7) nM in the cell fusion and Env-pseudo-typed virus neutralization assays, respectively (Figure 2).

The formalism used to ascertain the effects of inhibitors in combination has been described in detail by Chou & Talalay.[29] In the context of the present assays, the dose reduction index (DRI_x) of a fusion inhibitor x in the presence of a fusion inhibitor y is given by $DRI_x = IC_{50,x}/IC_{50,x}(y)$, where $IC_{50,x}$ and $IC_{50,x}(y)$ are the IC_{50} values of inhibitor x alone and in the presence of inhibitor y, respectively. Assuming simple ligand binding, the

Table 1. IC_{50} values for inhibition of HIV-1 Env-mediated membrane fusion for N_{CCG}-gp41 and N36$^{Mut(e.g)}$ alone obtained for several HIV-1 strains using cell fusion and Env-pseudo-typed virus neutralization assays

	IC_{50} (nM)	
	N_{CCG}-gp41	N36$^{Mut(e,g)a}$
A. *Cell-cell fusion assay with membrane-bound CD4*[b]		
LAV HIV-1 Env	150±16[c]	753±152
SF162 HIV-1 Env	472±113	3606±578
B. *Env-pseudotyped virus neutralization assay*[d]		
HXB2 HIV-1 Env	85±18	7399±713
SF162 HIV-1 Env	183±24	ND[e]

Notes to Table 1:
The following were obtained from the National Institutes of Health AIDS Research and Reference Research program: recombinant vaccinia viruses vCB41 (encoding HIV-1 LAV Env protein; catalog no. 3375) and vCB21R-LacZ (encoding β-galactosidase; catalog no. 3365); the HIV-1 expression plasmid SG3Δenv (catalog no. 11051); the HIV-1 Env molecular clone pCAGGS SF162 gp160 (catalog no. 10463); and the TZM-b1 (JC53BL-13) indicator cells, a HeLa-derived cell line genetically modified to constitutively express CD4, CCR5 and CXCR4 (catalog no. 8129). 293T cells were obtained from the American Type Culture Collection. B-SC-1 cells and recombinant vaccinia viruses vP11T7gene1 (encoding phage T7 polymerase) and vCB32 (encoding HIV-1 SF162 Env protein) were gifts from P. Kennedy, E.A. Berger and C. Broder. The HXB2 gp160 expression plasmid pSVIII HBXc2 was kindly provided by J.Sodroski.[33]

[a] IC_{50} values for N36$^{Mut(e,g)}$ are reported as monomer concentration.

[b] Inhibition of HIV envelope-mediated cell fusion was carried out using minor modifications of the vaccinia virus-based reporter gene assay, as described.[21,24–26] B-SC-1 cells, co-infected with recombinant vaccinia viruses vP11T7 gene1 (encoding phage T7 polymerase) and vCB41 (encoding HIV-1 LAV Env protein) or vCB32 (encoding HIV-1 SF162 Env protein) were used as effector cells. TZM-bl indicator cells, expressing both membrane bound CD4 and chemokine co-receptors on their surface, infected with vCB21R-LacZ recombinant vaccinia virus (encoding β-galactosidase under the control of T7 promoter) were used as target cells. A multiplicity of infection of ~1 was used for all vaccinia viruses. Following infection, cells were incubated for 18 h at 32 °C to allow for vaccinia virus-mediated expression of recombinant proteins. For inhibition studies, N_{CCG}-gp41 and N36$^{Mut(e,g)}$, either alone or in combination, were added to an appropriate volume of Dulbecco's Modified Eagle's Medium (DMEM) containing 2.5% (v/v) fetal calf serum (FCS) and phosphate-buffered saline (PBS) to yield identical buffer compositions. 4×10^4 B-SC-1 effector cells (in 20 μl of medium) per well were added to 160 μl of inhibitor solution, followed by 4×10^4 TZM-b1 target cells (in 20 μl medium) per well. Following 2.5 h incubation at 37 °C, assay plates were frozen. Cells were subsequently lysed and β-galactosidase activity of the cell lysates was measured by the absorbance at 570 nm (Molecular Devices; 96 well spectrophotometer) upon addition of chorophenol-red-β-D-galactopyranoside (Roche, Nutley, NJ).

[c] It is worth noting that the IC_{50} for inhibition of LAV Env-mediated cell fusion by N_{CCG}-gp41 in the fusion assay employed here that makes use of CD4 expressed on the surface of the target cells is about five- to seven fold higher than that in the equivalent fusion assay using soluble CD4 and CD4-negative target cells.[21] This is not unexpected, since the fusion activity in the soluble CD4 activated system is lower than that in the membrane-bound activated system.[25]

[d] Env-pseudo-typed virus stocks were prepared as described.[27,28] Exponentially dividing 293T cells were transfected using the FUGENE6 transfection kit (Roche, Nutley, NJ) with the Env-deficient HIV-1 expression plasmid SG3Δenv and the appropriate Env-expressing plasmid in the ratios corresponding approximately to the ratio of the vector sizes (16 μg total DNA per T-150 culture flask). Culture supernatants were collected two days post-transfection, filtered through 0.45 μm filter and stored at −86 °C. Env-pseudo-typed virus neutralization assays were performed essentially as described.[27,28] Serial dilutions of inhibitor solution were added to Env-pseudo-typed virus in DMEM 10% FCS (50 μl), followed by trypsinized TZM-bl indicator cells (10,000 cells in 20 μl of the same medium). Plates were incubated at 37 °C overnight, after which a further 150 μl of growth medium was added. Approximately 48 h post-infection cells were lysed and luciferase activity was measured using the BrightGlo luciferase assay kit (Promega, Madison, WI) with a Wallac 1450 MicroBeta TriLux liquid scintillation and luminescence counter (Perkin-Elmer Life Sciences, Downers Grove, IL). Env-pseudo-typed virus stocks were diluted to yield ~100-fold increase of luminescence over the uninfected cells background. The effect of virus input was examined in preliminary experiments, and IC_{50} values were found to remain stable using virus inputs producing between 20 and 200-fold increases of luminescence over the background (between 2×10^3 and 2×10^4 LCPS units, respectively).

[e] ND, no inhibition of fusion detected.

Table 2. Dose reduction and combination indices for inhibition of HIV-1 Env-mediated membrane fusion by N_{CCG}-gp41 and $N36^{Mut(e,g)}$ in combination, using several virus strains and three different assays

Fusion assay	Combination ratio N_{CCG}-gp41: $N36^{Mut(e,g)}$	Dose reduction index[a] N_{CCG}-gp41	Dose reduction index[a] $N36^{Mut(e,g)}$	Combination index[b]
A. *Cell-cell fusion assay with membrane bound CD4*				
LAV HIV-1	1:5	4.5±1.1	4.5±1.3	0.5±0.2
Env	1:10	5.6±1.3	2.8±0.8	0.6±0.2
	1:15	7.0±1.5	2.3±0.7	0.6±0.2
SF162	1:5	4.8±1.4	7.3±1.7	0.4±0.2
HIV-1	1:10	5.9±1.8	4.5±1.1	0.4±0.2
	1:15	7.7±2.2	3.9±0.9	0.4±0.2
B. *Env-pseudo-typed virus neutralization assay*				
HXB2	1:10	2.0±0.5	17.1±3.1	0.6±0.2
HIV-1 Env	1:15	2.3±0.7	13.4±2.8	0.5±0.2
	1:20	2.4±0.7	10.5±2.5	0.6±0.2
	1:30	2.7±0.8	7.8±1.9	0.6±0.2
SF162	1:10	1.7±0.5	ND[c]	–
HIV-1 Env	1:15	1.7±0.5	ND[c]	–

[a] The dose reduction index for N_{CCG}-gp41 is calculated as the ratio of the IC_{50} for the $N36^{Mut(e,g)}+N_{CCG}$gp41 mixture, expressed in terms of the concentration of N_{CCG}-gp41, to the IC_{50} for N_{CCG}-gp41 alone.The dose reduction index for $N36^{Mut(e,g)}$ is calculated as the ratio of the IC_{50} for the $N36^{Mut(e,g)}+N_{CCG}$gp41 mixture, expressed in terms of the concentration of $N36^{Mut(e,g)}$ (in monomer units), to the IC_{50} for $N36^{Mut(e,g)}$ alone.

[b] The combination index is given by equation (1) in the text.

[c] ND, not determined. Since no detectable inhibition of fusion could be obtained with $N36^{Mut(e,g)}$ alone in the virus neutralization assay employing SF162 HIV-1 Env, the dose reduction index for $N36^{Mut(e,g)}$ as well as the overall combination index cannot be determined. Nevertheless, the presence of synergy between N_{CCG}-gp41 and $N36^{Mut(e,g)}$ is evident, since the IC_{50} for N_{CCG}-gp41 is significantly reduced in the presence of $N36^{Mut(e,g)}$.

effect of two inhibitors in combination is quantified by the combination index (*CI*), given by:

$$CI = DRI_x^{-1} + DRI_y^{-1} + (DRI_x\, DRI_y)^{-1} \quad (1)$$

(Note that the last term, which makes only a small contribution to the CI, accounts for the state in which both inhibitors are bound). A value of $CI=1$ is indicative of a purely additive effect of the two inhibitors; that is the inhibition curve of the two inhibitors in combination is simply given by the summation of the two inhibitor curves alone, taking into account the different concentrations of the two inhibitors employed. A value of $CI>1$ indicates that the two inhibitors are mutually antagonistic; that is the effect of two inhibitors in combination is smaller than expected for an additive effect. A value of $CI<1$ indicates that the two inhibitors act synergistically; that is the inhibitory effect is greater than one would predict for a purely additive effect. Typically, a value of *CI* of 0.3–0.7 is considered to represent significant synergism, 0.70–0.85 moderate synergism, and 0.85–0.90 slight synergism.[30] For optimal observation of synergy, the ratio of inhibitor concentrations should be approximately comparable to the ratio of the IC_{50} values of the inhibitors alone.

The data in Table 2 summarize the *DRI* and *CI* values obtained for a series of constant ratio combinations of N_{CCG}-gp41 and $N36^{Mut(e,g)}$ ranging from 1:5 to 1:30. For N_{CCG}-gp41 alone, the IC_{50} values were lower in the Env-pseudo-typed neutralization assay than in the cell fusion assay for both X4 and R5 strains; for $N36^{Mut(e,g)}$ alone the opposite trend was observed (Table 1). Despite the fact that the IC_{50} values for N_{CCG}-gp41 and $N36^{Mut(e,g)}$ alone display a range of values depending on both the strain of HIV-1 Env employed and the nature of the assay, in every case, clear cut synergistic inhibition of Env-mediated fusion by these two inhibitors in combination is apparent with *CI* values ranging from 0.4 to 0.6 (Table 2). The mean value of the *CI* obtained from all the assays is 0.52 with a standard error of 0.03. In one case, namely inhibition of SF162 Env-mediated membrane fusion observed in the Env-pseudo-typed virus neutralization assay, a *CI* value could not be determined, since no inhibition of fusion could be detected for $N36^{Mut(e,g)}$ alone (Table 1). Nevertheless, even in this instance clear evidence for synergism was obtained, since the combination of N_{CCG}-gp41 and $N36^{Mut(e,g)}$ resulted in a significant reduction in the IC_{50} of N_{CCG}-gp41 ($DRI_{NCCG\text{-}gp41}\sim 1.7$; Table 2).

Control experiments (data not shown) were also carried out using combinations of N_{CCG}-gp41 and cyanovirin-N, a fusion inhibitor that targets high-mannose oligosaccharides on the surface of gp120.[31] In this instance, the effect of these two inhibitors, at a ratio of cyanovirin to N_{CCG}-gp41 of 1:10 was purely additive in the cell fusion assay with a *CI* value of 0.97±0.07. This result is expected, since the two inhibitors act on discrete, unrelated targets of HIV-1 Env that are relevant at different steps during the fusion process.

The synergistic inhibition of HIV-1 Env-mediated membrane fusion by N_{CCG}-gp41 and $N36^{Mut(e,g)}$ has mechanistic, as well as obvious potential practical applications. In the context of inhibition of fusion by class 2 inhibitors, such as N_{CCG}-gp41,

the binding of the inhibitor to the C-HR of the PHP conformation of gp41 must compete with the binding of the C-HR of gp41 to the N-HR of the same molecule that results in the formation of the fusogenic trimer of hairpins configuration. In the intact system the formation of the latter can be considered irreversible, since it is accompanied by concomitant apposition of the viral and cell membranes and subsequent fusion. By forming heterotrimers between $N36^{Mut(e,g)}$ and the N-HR of the PHP of gp41 it is logical that (a) the overall apparent strength of the interaction between the N-HR and C-HR of gp41 will be significantly diminished, since the population of native state in which one C-HR interacts with two adjacent N-HRs in the trimer of hairpins will be reduced as a result of heterotrimer formation (cf Figure 1(a)), and (b) the probability of forming a trimer of hairpins will also be significantly reduced owing to the presence of heterotrimers, which are in relatively slow equilibrium with the homotrimeric coiled-coil of N-HR helices (cf. Figure 1(a)). Concomitantly, the sequestration of the N-HR of the PHP of gp41 into fusion incompetent heterotrimers by $N36^{Mut(e,g)}$ will enhance the probability of complex formation between N_{CCG}-gp41 and the C-HR of the PHP of gp41. Dimitrov *et al.*[32] recently showed that treatment of Env-expressing cell with $N36^{Mut(e,g)}$ reduced the binding of the monoclonal antibody NC-1 which recognizes the N-HR trimer of gp41 and enhanced binding to the class 2 fusion inhibitor 5-helix. Both observations are consistent with the mechanism of $N36^{Mut(e,g)}$ forming stable heterotrimers with wild-type N-HR of Env.[23] Thus, the results presented here further substantiate the mechanism of action of class 3 inhibitors postulated by Bewley *et al.*[23] From a purely practical perspective, the data presented here suggest that combination therapy with class 2 and 3 inhibitors may represent a useful avenue for future development and implementation of novel anti-fusion HIV therapies.

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