

An Antibody-Recruiting Small Molecule That Targets HIV gp120

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In recent years, antibody-based therapeutics have become important instruments in treating human disease.¹ These approaches suffer from certain limitations, including severe side effects, lack of oral bioavailability, and high cost.² Thus, alternative small-molecule-based methods that exploit the powerful cytolytic potential of antibodies already present in the human bloodstream could address many of these disadvantages.

Here we report that a rationally designed bifunctional small molecule,³ called “antibody-recruiting molecule targeting HIV” (ARM-H), is capable of redirecting anti-dinitrophenyl (anti-DNP) antibodies, a population of antibodies present in high concentrations in the human bloodstream,⁴ to the HIV gp120 *env* gene product (Figure 1). The Env glycoprotein, a complex between gp120 and membrane-bound gp41, is expressed on both the surface of the HIV virus and on virus-infected cells.⁵ The gp120 component of Env mediates the first step in viral entry into human cells by binding the protein CD4. We demonstrate here that a ternary complex formed between anti-DNP antibodies, ARM-H, and Env-expressing cells can mediate the complement-dependent destruction of these cells. Further, since ARM-H binds gp120 competitively with CD4, it also inhibits the entry of live HIV virus into human T-cells. Thus, ARM-H has the potential to interfere with the survival of HIV through multiple complementary mechanisms.

Our design of ARM-H began with the small molecule BMS-378806 (**1**, Figure 1b), a known inhibitor of the CD4–gp120 interaction.⁶ On the basis of previously reported structure–activity relationships in which the carbon atom of the C4 methoxy group could be replaced with various bulky substituents,⁷ we hypothesized that it might be possible to derivatize **1** at this site with a linker attached to DNP without sacrificing the compound’s ability to inhibit viral entry. This hypothesis was supported by an analysis of a published computational docking model suggesting that the C4 methoxy group in **1** points toward the solvent in the complex.⁸ Thus, we reasoned that BMS-378806 could be re-engineered to recruit anti-DNP antibodies to gp120-expressing particles (infected cells or viruses), increasing their “visibility” to the human immune system. On the basis of this strategy, we prepared ARM-H (**4**) in high yield (38% overall) via azide–alkyne cycloaddition^{9,10} of **2** and **3**, which were derived in turn from known intermediates (Schemes S2 and S3 in the Supporting Information).⁶

The ability of ARM-H to inhibit CD4 binding to HIV-1 gp120 was assessed first in an enzyme-linked immunosorbent assay (ELISA) (Figure 2a).¹¹ Here ARM-H was able to out-compete the CD4–gp120 interaction with a mean inhibitory concentration (IC₅₀) of ~8.7 μ M, and it was only slightly less potent than the parent

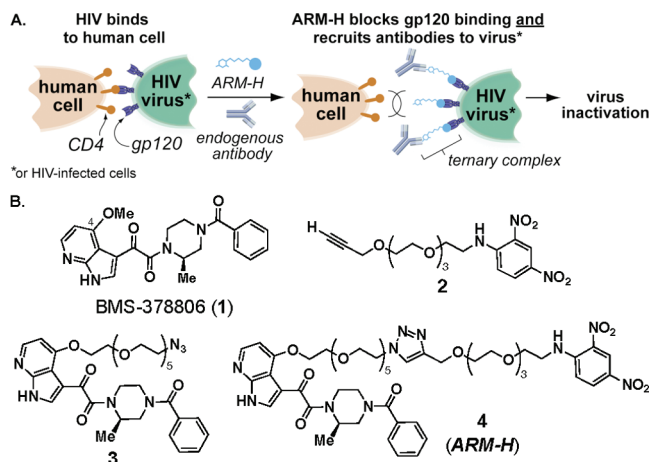


Figure 1. (A) Schematic depiction of the reported approach to HIV targeting. The synthetic bifunctional small molecule ARM-H is designed to function through two autonomous mechanisms: (1) by binding the viral glycoprotein gp120, inhibiting its interaction with CD4, and (2) by recruiting antibodies to the HIV virus, and to HIV-infected cells, for destruction by the human immune system. (B) Chemical structures of small molecules employed in these studies.

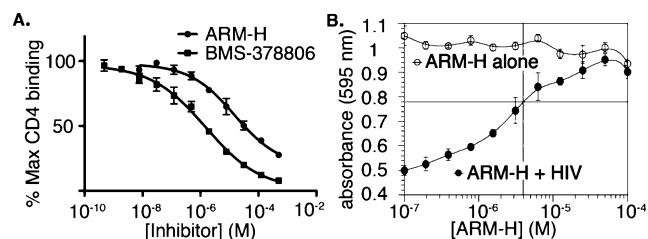


Figure 2. ARM-H outcompetes the gp120–CD4 interaction in vitro and with a live HIV-1 virus assay. (A) Competition ELISA monitoring the binding of sCD4 to immobilized gp120. (B) HIV-1 viral replication assay. UV absorption at 595 nm, increased by the metabolic action of live MT-2 cells on an assay reagent,¹³ was monitored as a surrogate for cell viability in the presence of increasing concentrations of ARM-H alone (white circles) or ARM-H plus live HIV-1 virus (black circles).

compound BMS-378806 (IC₅₀ = 1.3 μ M). On the basis of this observation, we then investigated ARM-H’s ability to inhibit the entry of live HIV-1 virus into the human MT-2 T-cell line (Figure 2b, IC₅₀ = 6.4 μ M). Although in this assay ARM-H once again proved to be less potent than BMS-378806 (IC₅₀ = 0.32 μ M; Figure S1),¹² it demonstrated potency equivalent to that of d4T, which is currently a mainstay in HIV pharmacotherapy (IC₅₀ = 4.2 μ M; Figure S1). Notably, ARM-H demonstrated no observable cytotoxicity in control MT-2 cultures lacking HIV virus (Figure 2b, white circles).

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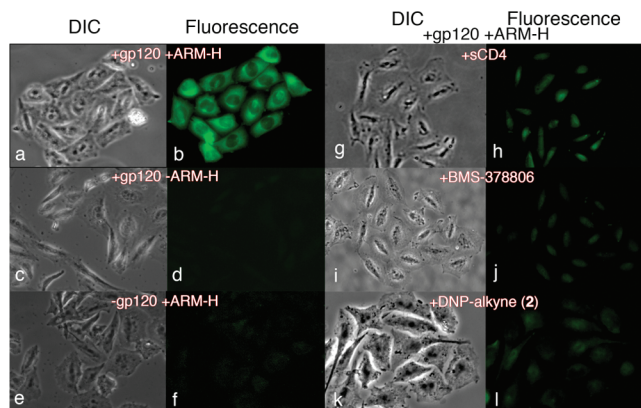


Figure 3. Immunofluorescence microscopy. Chinese hamster ovary cells expressing HIV-1 Envelope (Env) glycoprotein (+gp120) and control cells lacking Env (−gp120) were stained with Alexa Fluor 488-conjugated anti-DNP antibodies (15 $\mu\text{g}/\text{mL}$). Microscopy was then performed after treatment with the following reagents, as indicated: ARM-H (10 μM), sCD4 (4 $\mu\text{g}/\text{mL}$), BMS-378806 (BMS, 500 μM), or DNP-containing alkyne **2** (50 μM). Fluorescence signals indicate ARM-H-mediated recruitment of antibodies to gp120-expressing cells.

We next investigated the ability of ARM-H to recruit antibodies to gp120 both in vitro and in tissue culture. To this end, initial ELISA experiments (Figure S2) demonstrated a concentration-dependent increase in anti-DNP antibody binding to the ARM-H–gp120 complex but not to gp120 alone. Thus, ARM-H is capable of templating a ternary complex that also includes gp120 and anti-DNP antibody.

To demonstrate that this ternary association could form in a complex cellular milieu, we evaluated the ability of ARM-H to recruit Alexa Fluor 488-labeled anti-DNP antibodies to HIV-Env-expressing Chinese hamster ovary cells (CHO-gp120 cells)¹⁴ by immunofluorescence microscopy. As depicted in Figure 3, a strong fluorescence signal was observed when CHO-gp120 cells were incubated with ARM-H and labeled anti-DNP antibodies (Figure 3a,b).¹⁵ This fluorescence was absent from both CHO-gp120 cells not treated with ARM-H (Figure 3c,d) and CHO cells not coding for HIV-*env* gene expression (CHO-WT cells, Figure 3e,f). Furthermore, the intense fluorescence observed in Figure 3b was considerably diminished in the presence of the competing ligands soluble CD4 (sCD4, Figure 3g,h), BMS-378806 (Figure 3i,j), and a DNP-containing alkyne that lacks the gp120-binding motif (**2**, Figure 3k,l). Taken together, these results provide strong evidence that ARM-H is capable of recruiting anti-DNP antibodies to cells expressing the Env glycoprotein in a fashion that depends upon its simultaneous binding to both gp120 and anti-DNP antibodies.

Finally, we explored whether the ternary complex formed from anti-DNP antibody, ARM-H, and a live Env-expressing cell could activate complement proteins and mediate cellular death. Complement proteins are known to lyse cells by forming pores in lipid membranes and have been shown to play a critical role in inactivating HIV in humans.^{16,17} Thus, rabbit complement proteins were added to CHO-gp120 cells in the presence of ARM-H and a fixed concentration anti-DNP antibodies (Figure 4). Substantial cell killing that exhibited a significant dependence on the ARM-H concentration (data in red) was observed.^{18,19} Notably, in the absence of anti-DNP antibody and complement-preserved serum (data in green), in cells lacking the Env glycoprotein (CHO-WT, data in black), or in the presence of **3**, which lacks the DNP group (data in blue), no cell death was

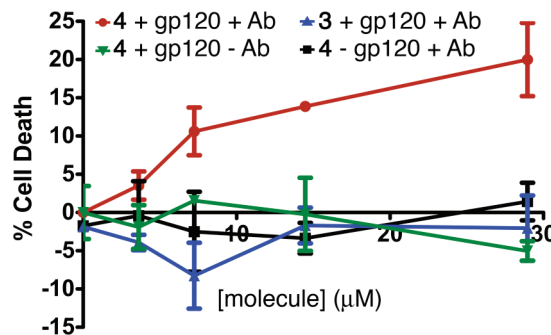


Figure 4. ARM-H-mediated killing of live HIV-Env-expressing CHO cells. Env-expressing CHO cells (+gp120) or control cells lacking Env (−gp120) were treated with ARM-H (**4**) or control compound (**3**) at the indicated concentrations in the presence (+Ab) or absence (−Ab) of 10% complement-preserved rabbit serum and rat anti-DNP IgG (50 $\mu\text{g}/\text{mL}$). Values on the vertical axis correspond to % cell death (vs background). Each data point depicted represents the mean $\pm$ standard deviation of triplicate data from a representative experiment. The depicted trends were reproduced on at least three separate occasions.²⁰

observed, suggesting that ternary complex formation is necessary for complement-dependent cytotoxicity (CDC) and that ARM-H itself is not toxic to cells. Positive control experiments in which cells were directly labeled with 2,4-dinitrobenzenesulfonic acid (Figure S4) were found to yield levels of CDC comparable to those observed for ARM-H, providing a benchmark for the assay results depicted in Figure 4. Thus, ARM-H is capable of recruiting a functional complement-dependent cytotoxic response against Env-expressing cells.

Thus, we have shown that the bifunctional small molecule ARM-H can both recruit anti-DNP antibodies to gp120-expressing cells and inhibit the gp120–CD4 interaction. Data supporting these conclusions include the following: (1) ARM-H binds to gp120 competitively with CD4 and decreases viral infectivity in an MT-2 cell assay. (2) The small molecule can guide the formation of a ternary complex that includes anti-DNP antibodies and Env-expressing cells. (3) Antibodies present in this ternary complex can promote the complement-mediated killing of Env-expressing cells. Critically, no nonspecific cytotoxicity was observed in either MT-2 or CHO cell lines in response to ARM-H. Also, ARM-H-mediated inhibition of HIV entry and CDC activity were both operative at concentrations ranging from 6 to 30 μM , confirming that ARM-H could function simultaneously through dual mechanisms under therapeutic conditions.

While a few methods for recruiting naturally occurring antibodies to cancer cells,^{21–27} bacteria,^{28–31} and viruses,^{32–34} have appeared in the literature, this area remains underexplored. In the HIV realm, all such approaches have relied upon protein- or peptide-based antibody targeting constructs. For example, Shokat and Schultz³² first demonstrated that anti-DNP antibodies could be redirected to immobilized protein targets (gp120 and streptavidin) as a therapeutic strategy toward HIV. More recent work in this vein has employed peptide- α -Gal conjugates to target human anti-Gal antibodies to HIV-infected cells.^{33,34} These peptide conjugates were shown to be effective in killing Env-expressing cells but were also found to exhibit some nonspecific cytotoxicity.²⁸ ARM-H is unique in that it represents a small-molecule-based anti-HIV strategy and targets the virus lifecycle through mutually reinforcing molecular mechanisms: it both prevents virus entry and targets Env-expressing cells for immune recognition and clearance. In general, small molecules have certain advantages over proteins from a therapeutic standpoint

because of their low propensity for immunogenicity, high metabolic stability, ready large-scale production, and relatively low cost. Small-molecule antibody-recruiting therapeutics such as ARM-H would have additional benefits over available treatment approaches to HIV. For example, directing HIV-infected cells and virus particles to Fc γ receptors on antigen-presenting cells could enhance the presentation of viral antigens on MHC proteins and contribute to long-lasting anti-HIV immunity.^{26,35} Furthermore, because anti-DNP antibodies are already present in the human bloodstream, no pre-vaccination would be necessary for ARM-H activity. Also, the binding of bifunctional small-molecule targeting agents to antibodies should prolong their plasma half-life, thus increasing their effectiveness.²⁷ Elucidation of the molecular details governing the interactions among ARM-H, gp120, and anti-DNP antibodies will assist in optimization efforts as well as in the evaluation of this strategy in more complex biological models for HIV infection. These and other investigations are currently ongoing in our laboratories.

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Supporting Information Available: Detailed experimental procedures, compound characterization, and complete author lists for refs 6 and 11. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (19) Characteristic autoinhibition of ternary complex formation at high levels of bifunctional molecule, arising from excess free bifunctional material that drives the equilibria toward formation of binary complexes, was not reliably observed in these assays (see the Supporting Information for more details). For more information on autoinhibitory behavior in ternary complexes, see: Mack, E. T.; Perez-Castillejos, R.; Suo, Z.; Whitesides, G. M. *Anal. Chem.* **2008**, *80*, 5550–5555, and references contained therein.
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