Synergistic Inhibition of R5 HIV-1 by Maraviroc and CCR5 Antibody HGS004 in Primary Cells: Implications for Treatment and Prevention

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Abstract

CCR5 blockers inhibit CCR5-tropic (R5) HIV-1, including strains resistant to other antiretrovirals. We demonstrate that the CCR5 antibody HGS004 and the CCR5 antagonist Maraviroc have potent antiviral synergy against R5 HIV-1, translating into dose reductions of >10-fold for Maraviroc and >150-fold for HGS004. These data, together with the high barrier of resistance to HGS004, suggest that combinations of Maraviroc and HGS004 could provide effective preventive and therapeutic strategies against R5 HIV-1.

CCR5 serves as the main coreceptor for the predominantly transmitted and most persistent HIV-1, the R5 strains. Maraviroc (MVC) is the only licensed CCR5 inhibitor [1, 2]. CCR5 antibodies PRO 140 and HGS004 have significantly reduced HIV-1 RNA in clinical trials of patients infected with R5 HIV-1 only [3-6]. However, HGS004 reduced HIV-1 RNA in only 54% of patients. Retrospectively, it was found that sensitivity of the patients' viruses to HGS004 predicted antiviral responses [6]. We tested the hypothesis that the combination of HGS004 and MVC has antiviral synergy, thereby increasing the potency of both drugs and reducing their effective doses. This hypothesis is based on the observation that HGS004 and MVC recognize separate regions of CCR5, with HGS004 binding to the second extracellular loop [6], and MVC to the transmembrane region [7-9].

We tested the antiviral activity of HGS004 alone and in the presence of varying concentrations of MVC. For comparison, we evaluated HGS004 in combination with the integrase inhibitor Raltegravir (RAL). Both MVC and RAL were used at concentrations spanning their EC_{50}s. We performed these assays using PHA-activated peripheral blood mononuclear cells (PBMCs) infected with the R5 HIV-1 strains BaL or CC1/85. Data for HIV-1 BaL are shown in Fig 1a (upper panels). In the absence of MVC or RAL, HGS004 inhibited HIV-1 BaL with an EC_{50} value of 31.3 μg/ml. However, in the presence of increasing concentrations of MVC, HGS004 EC_{50}s were successively lowered to 4.4, 0.7, 0.1, 0.05 and 0.03 μg/ml. In contrast, RAL had little effect on HGS004 potency, with HGS004 EC_{50}s of 26, 24, 22, 15 and 9.8 μg/ml in the presence of increasing RAL. Neither the drug alone nor the drug combinations treatments were toxic to cells as demonstrated by MTT assays (not shown). Together, the marked leftwards shift of the HGS004 viral

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inhibition curves in the presence of MVC compared to RAL suggested that HGS004 and MVC are synergistic against HIV-1 BaL.

To determine whether inhibition by HGS004 and MVC or RAL might indeed be synergistic, we performed a three-dimensional analysis using the method of Prichard and Shipman [10]. For the HGS004/MVC and HGS004/RAL combinations, the 96% confidence synergy plots, after Bonferroni adjustment, are shown in Fig 1a (lower panels). The combination of HGS004 and MVC had antiviral synergy across the entire concentration grid (synergy volume of 522). In contrast, the combination of HGS004 and RAL was mainly additive (synergy volume of only 19).

Similar results to those with HIV-1 BaL were obtained with HIV-1 CC1/85, a R5 HIV-1 primary patient isolate [11] (Fig 1b, upper panels). HGS004 EC_{50}s were 40 μg/ml in antibody alone treatment, but only 3.75, 0.22, 0.04 and 0.03 μg/ml in combinations containing the indicated MVC concentrations. HGS004 EC_{50}s were 30, 26, 59, 9.2 and 1.5 nM in combinations containing RAL, suggesting some increased antibody potency at high concentrations of RAL (Fig 1b, upper panels). Inhibition of CC1/85 by the HGS004/MVC and HGS004/RAL combinations gave synergy volumes of 502 and 54, respectively (Fig 1b, lower panels).

To confirm the synergy data, we analyzed the drug combinations by the Median Effect Principle [12], which evaluates drug combinations at fixed ratios and allows calculation of Combination Indices (CI). CI=1 indicates additivity, CI>1 indicates antagonism, and CI<1 indicates synergy. At 50% viral inhibition, the CI values for the HGS004/MVC combinations ranged 0.070-0.154 for HIV-1 BaL, and 0.060-0.117 for HIV-1 CC1/85. In contrast, CI values for the HGS004/RAL combination ranged 0.898-1.230. As CI values are proportional to the amount of synergy, the obtained CIs for the HGS004/MVC combinations demonstrate a quite potent synergistic interaction. These CI values translated into 10-fold lower doses of MVC and 230-fold lower doses of HGS004 against BaL, and 14-fold lower doses of MVC and 155-fold lower doses of HGS004 against CC1/85. Overall, these data are consistent with those obtained by Three-dimensional modeling analysis, demonstrating that HGS004 has antiviral synergy with MVC, but mostly additivity with RAL. The low level synergy between HGS004 and RAL identified by Three-dimensional analysis was not identified by the Median Effect Principle analysis, likely reflecting that synergy occurs only at a restricted concentration range (see synergy plots in Fig 1).

We also evaluated the HGS004/MVC combination against R5 primary isolates 92BR020 (Subtype B, from Brazil) and 92UG031 (Subtype A, from Uganda). Antiviral synergy was higher than for BaL and CC1/85, with synergy volumes of 811 and 1100 for 92BR020 and 92UG031, respectively. We could not calculate CI values because drugs were not used at fixed ratios. We also evaluated HGS004 and MVC against R5X4 dual tropic HIV-1 89.6. Neither HGS004 nor MVC, alone or in combination, inhibited 89.6, in agreement with previous data suggesting that this virus primarily uses CXCR4 in PBMCs [7].

In summary, we demonstrate that MVC and HGS004 have potent synergy against R5 HIV-1 in PBMCs, significantly reducing HGS004 and MVC effective doses. Our results are consistent with previously described synergies between MVC and CCR5 antibodies PRO 140 [13] or RoAb13 [14]. These data, together with HGS004 long-acting activity, high barrier to drug resistance, and favorable antiviral interactions with reverse transcriptase, protease and integrase inhibitors ([6, 15]; this report), suggest that combinations of MVC and HGS004 could provide effective and durable treatment options for patients infected with R5 HIV-1. In addition, since viral transmission is mainly due to R5 HIV-1 [16], HGS004 could improve the potency of MVC in HIV-1 prevention strategies [17].

In flow cytometry studies, binding of HGS004 to CCR5 was higher in the presence than in the absence of MVC, suggesting that MVC induces conformational changes increasing exposure of the HGS004 epitope [18]. As such, HGS004 and MVC may act synergistically by reducing the number of unoccupied CCR5 molecules available to the virus [19, 20]. Consistent with this, HGS004 restored MVC sensitivity of MVC-resistant R5 HIV-1 [18], whose reduced affinity for MVC-bound CCR5 presumably requires the engagement of more coreceptor molecules for formation of a fusion pore [18, 21-23]. Thus, the combination of HGS004 and MVC may also reduce the risk of developing drug resistance.

Acknowledgments

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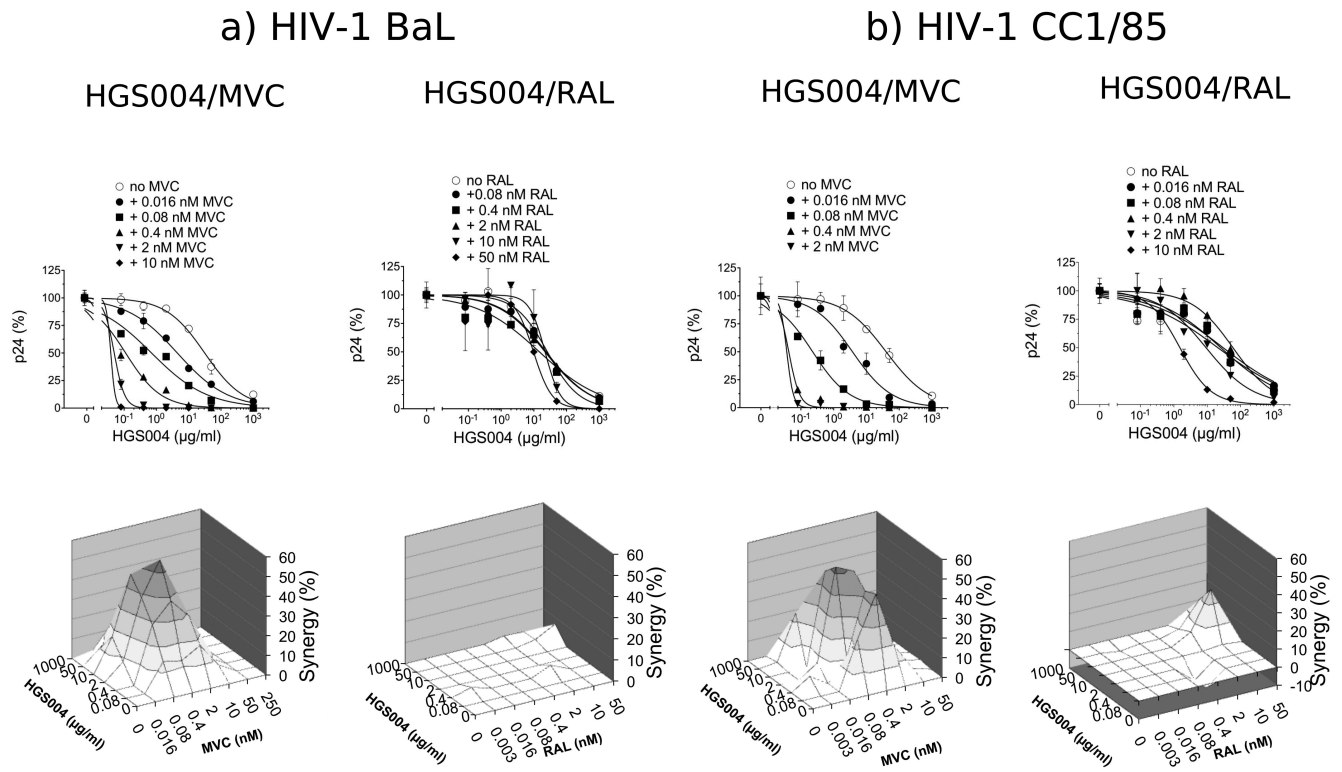## a) HIV-1 BaL

HGS004/MVC   HGS004/RAL

b) HIV-1 CC1/85

HGS004/MVC   HGS004/RAL

Figure 1. Antiviral interactions between HGS004 and MVC versus HGS004 and RAL against R5 HIV-1 in primary cells

PHA-activated PBMCs infected with HIV-1 BaL (*a*) or HIV-1 CC1/85 (*b*) using an MOI of 0.001 were cultured in the presence of the indicated drug concentrations. Virus production was measured on day 7 by p24 ELISA. Inhibition data from replicates were plotted using GraphPad Prism software and EC_{50} values determined using variable slope non-linear regression analysis. MVC EC_{50}s for BaL and CC1/85 were 0.74 and 0.34 nM, respectively; whereas RAL EC_{50}s for BaL and CC1/85 were 4.3 and 1.1 nM, respectively (not shown). *Upper panels*: Dose response curves for HGS004, alone and in combination with MVC or RAL. At each MVC or RAL concentration, p24 inhibition by HGS004 was normalized to inhibition by MVC or RAL alone. Data points are means ± S.D. of duplicates. *Lower panels*: Three-dimensional analysis of viral inhibition by drug combinations. Percentage synergy obtained by each combination was plotted against HGS004 and MVC or RAL concentrations according to Prichard's synergy model. The 96% confidence interval synergy plots are shown. Percentage synergies at each 10% increment are shown in different color intensities. Note that the synergy plots included more drug concentrations than those shown in dose response curves. Data are representative of three independent experiments for BaL and two independent experiments for CC1/85, with PBMCs from different donors in each experiment.