Optimal Combinations of bnAbs for Prevention and Treatment of HIV-1 Clade C Infection

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Introduction

The identification of a new generation of potent broadly neutralizing HIV-1 antibodies (bnAbs) has generated substantial interest in their potential use for the prevention and/or treatment of HIV-1 infection (*1*). While combinations of bnAbs targeting distinct epitopes on the viral envelope (Env) will likely be required to overcome the extraordinary diversity of HIV-1, a key outstanding question is which bnAbs, and how many, will be needed to achieve optimal clinical benefit. We assessed the neutralizing activity of 15 bnAbs targeting four distinct epitopes of Env, including the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3-glycan region, and the gp41 membrane proximal external region (MPER), against a panel of 200 acute/early clade C HIV-1 Env pseudoviruses. A mathematical model was developed that predicted neutralization by a subset of experimentally evaluated bnAb combinations with high accuracy. Using this model, we performed a comprehensive and systematic comparison of the predicted neutralizing activity of over 1,600 possible double, triple, and quadruple bnAb combinations. Best-in-class bnAb combinations were identified based not only on breadth and potency of neutralization, but also other relevant measures, such as the extent of complete neutralization (*2*) and instantaneous inhibitory potential (IIP) (*3*).

Methods

A panel of 15 particularly broad and potent human monoclonal antibodies was selected based on prior data from testing against large multiclade panels of HIV-1 pseudoviruses. Neutralizing antibody titers of bnAbs were determined using a luciferase-based assay in TZM.bl cells as previously described (*4*).

The Bliss-Hill model was developed to overcome limitations of the Additive Model for predicting neutralization titers of bnAb combinations (*5*). The Bliss-Hill model combines the single bnAb Hill curves (with arbitrary slopes) within the framework of the Bliss independence model for the binding of multiple species of ligands to a substrate (*6,7*), and incorporates a correction for multiple ligands independently attaching to a substrate. Bliss-Hill model was also used for predicting maximum percent inhibition and IIP for bnAb combinations.

References

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Results

Figure 1

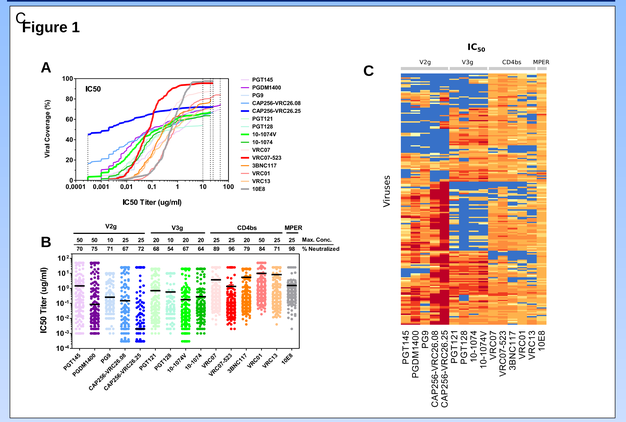


Figure 1:Potency and breadth of single bnAbs against a 200 clade C Env pseudovirus panel. **Fifteen bnAbs were selected that target four distinct epitope regions: the CD4 binding site (CD4bs: 3BNC117, VRC01, VRC07, VRC07-523, VRC13), the V3-glycan supersite (V3g: 10-1074, 10-1074V, PGT121, PGT128), the V1/V2-glycan site (V2g: PG9, PGT145, PGDM1400, CAP256-VRC26.08, CAP256-VRC26.25), and the gp41 MPER epitope (10E8). BnAbs were tested against a panel of 200 early/acute clade C HIV-1 Env pseudoviruses. Neutralizing activities were evaluated using potency-breadth curves (A), scatter plots (B) and heatmaps (C).**

Figure 2

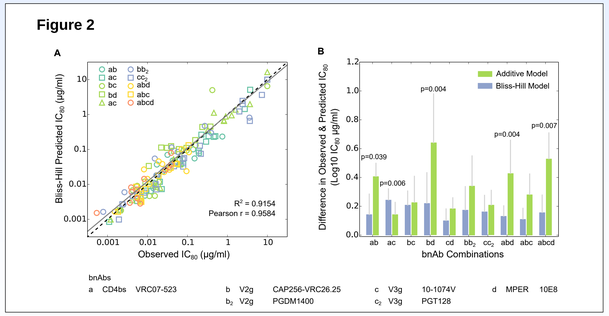


Figure 2: Comparison of Additive and Bliss-Hill Models for predicting neutralization by bnAb combinations. **(A) Bliss-Hill model predictions are plotted against experimental IC_{80} titers for 20 viruses. (B) Comparison of Additive and Bliss-Hill models for predicting bnAb combination IC_{80} titers**

Results

Figure 3

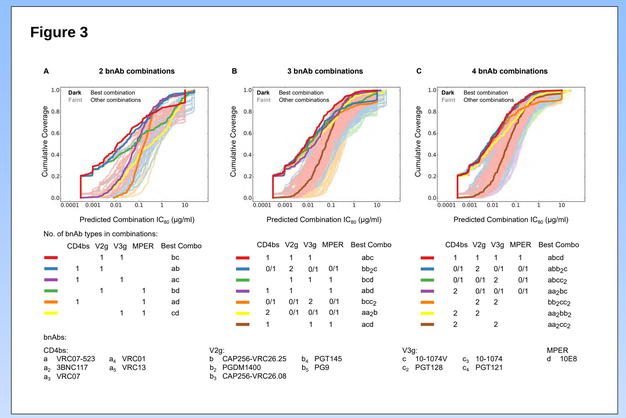


Figure3: Bliss-Hill predicted potency-breadth curves for all candidate 2, 3, and 4 bnAb combinations. **Potency-breadth curves for all candidate 2(A), 3(B), and 4(C) bnAb combinations are shown (1,622 combinations total). Best-in-category bnAb combinations are highlighted in darker colors. Tables indicate best combinations for bnAbs targeting multiple epitopes. In (B) and (C), "0/1" indicates combination categories in which the indicated epitope may or may not have been covered by a representative bnAb.**

# of bnAbs in combination	Best % Coverage (IC_{80})			Best % Coverage (IC_{50})		
	2 or more bnAbs active	3 or more bnAbs active	4 or more bnAbs active	2 or more bnAbs active	3 or more bnAbs active	4 or more bnAbs active
	IC_{80} < 1 μg/ml			IC_{50} < 1μg/ml		
2	44			64.5		
3	67.5	19.5		87	44.5	
4	73.5	26.5	2.5	95	68.5	27.5
	IC_{80} < 5 μg/ml			IC_{50} < 5 μg/ml		
2	57			91.5		
3	80.5	36.5		97	65.5	
4	89.5	62.5	17.5	99.5	87	40
	IC_{80} < 10 μg/ml			IC_{50} < 10 μg/ml		
2	80			92.5		
3	91	49.5		97.5	66.5	
4	95.5	77	26	100	89	40.5
	IC_{80} < 50 μg/ml			IC_{50} < 50 μg/ml		
2	93			93		
3	97.5	68		98	68.5	
4	100	90.5	41.5	100	91	44

Figure4: Viral coverage when requiring multiple bnAbs in combinations to be active. **The percentage of viruses neutralized at various IC_{50} and IC_{80} threshold titers are indicated when requiring at least 2, 3, or 4 bnAbs in best combinations to be simultaneously active.**

Results

Figure 5

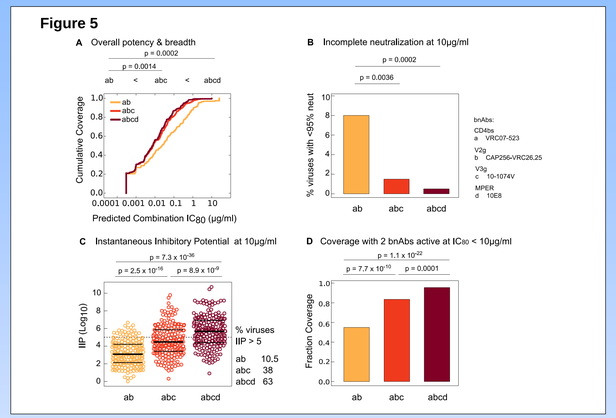


Figure5: Comparison of best 2, 3, and 4 bnAb combinations. **(A) Potency-breadth curves for the best combinations are shown (Wilcoxon rank sum test). (B) Fraction of 200 viruses predicted to have <95% neutralization at 10 μg/ml for the best combinations (Fisher's exact test). (C) Instantaneous Inhibitory Potential calculated at 10 μg/ml for the best combinations against 200 viruses. (Wilcoxon rank sum test). (D) Fraction of viruses neutralized by at least 2 bnAbs in the best combinations at single bnAb IC_{80} thresholds of < 10 μg/ml (Fisher's exact test).**

Conclusion

Best-in-class single bnAbs for potency and breadth were CAP256-VRC26.25 (V2-glycan), 10-1074V (V3-glycan), VRC07-523 (CD4bs) and 10E8 (MPER). Optimal bnAb combinations were identified based on breadth and potency of neutralization, as well as other relevant measures such as the extent of complete neutralization and instantaneous inhibitory potential. By these criteria, VRC07-523 + CAP256-VRC26.25, VRC07-523 + CAP256-VRC26.25 + 10-1074V and VRC07-523 + CAP256-VRC26.25 + 10-1074V + 10E8 were identified as the best 2, 3, and 4 bnAb combinations, respectively. Triple and quadruple combinations of bnAbs were significantly more effective than the best double combinations. Quadruple bnAb combinations were predicted to be similar to triple combinations by some metrics, and significantly better by others. Importantly, increasing the total number of bnAbs in combinations improved the probability of having multiple bnAbs simultaneously active against a given virus, a requirement that may be critical for countering escape *in vivo*. These results provide a rationale for advancing bnAb combinations with the best *in vitro* predictors of success into clinical trials for both the prevention and treatment of HIV-1 infection.