Improving Neutralization Potency and Breadth by Combining Broadly Reactive HIV-1 Antibodies Targeting Major Neutralization Epitopes

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Introduction

The isolation of broadly neutralizing HIV-1 monoclonal antibodies (mAbs) to distinct epitopes on the viral envelope glycoprotein (Env) provides the potential to use combinations of mAbs for prevention and treatment of HIV-1 infection. Since many of these mAbs have been isolated in the last few years, the potency and breadth of mAb combinations have not been well characterized.

Methods

The neutralizing activities of double, triple and quadruple mAb combinations targeting four distinct epitopes (Fig. 1), including the CD4-binding site, the V1V2-glycan region, the V3-glycan supersite and the gp41 membrane-proximal external region (MPER) were examined in two parallel experiments in TZM-bl neutralization assay, using a panel of 125 Env-pseudotyped viruses. To investigate the potential interactions of mAbs in the combination, experimentally observed IC_{50} titers were compared to the IC_{50} titers predicted based on a simple additive model using the following formulas:

a and b = 1/(1/a + 1/b)

a and b and c = 1/(1/a + 1/b + 1/c)

a and b and c and d = 1/(1/a + 1/b + 1/c +1/d),

where a, b, c, d are the IC_{50}s of the single mAb.

Of note, only viruses with measurable IC_{50}, i.e. $IC_{50} < 25$ µg/ml, to all component mAbs are qualified for this approach.

Number of mAbs		1			2				3			4
Total mAb concentration (µg/ml)		25			50				75			100
Set I	VRC07	+			+	+	+		+	+	+	+
	PG9		+		+			+	+	+		+
	PGT128			+		+		+		+	+	+
	10E8				+		+	+		+	+	+
Set II	3BNC117	+			+	+	+		+	+	+	+
	PG9		+		+			+	+	+		+
	10 -1074			+		+		+		+	+	+
	10E8				+		+	+		+	+	+

Figure 1. Experimental design of mAb combinations. In two parallel experiments (set I and II), mAbs were combined in equal concentrations and tested for HIV-1 neutralization. The starting concentration of single component mAb was 25 µg/ml.

Results

mAb combinations showed improved neutralization breadth

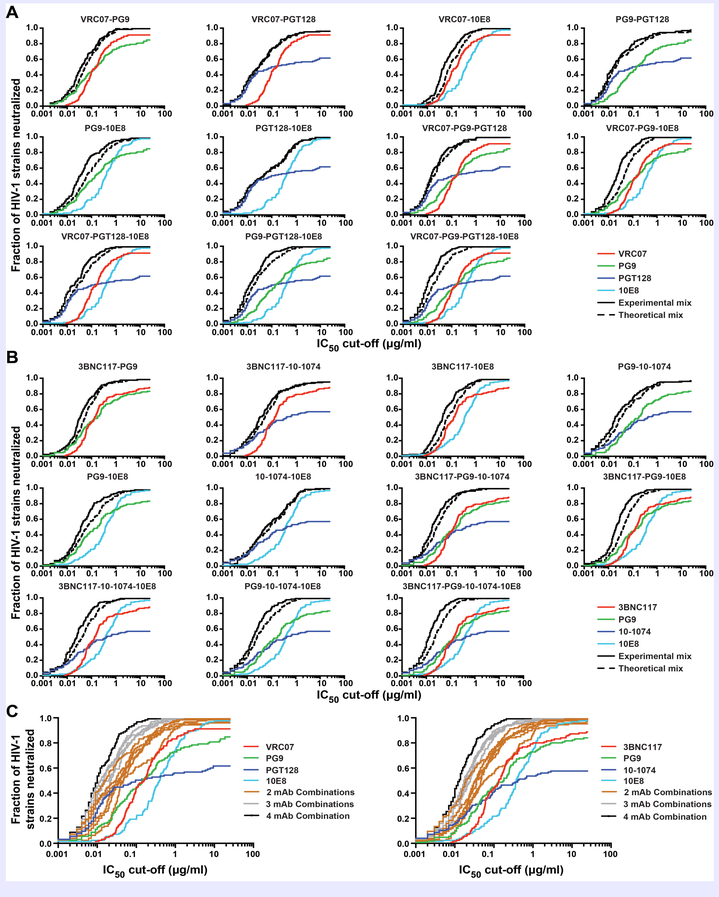


Figure 2. Potency-breadth curves. (A) set I mAb combinations. The theoretical mix curve of the mAb combination (black dotted) is the curve predicted by using the best individual mAb titer as representative of the titer of the mAb combination, while the experimental mix curve is generated based on the titer of physical antibody mix (black solid). (B) set II mAb combinations. (C) All combinations of set I (left) and set II (right) mAbs.

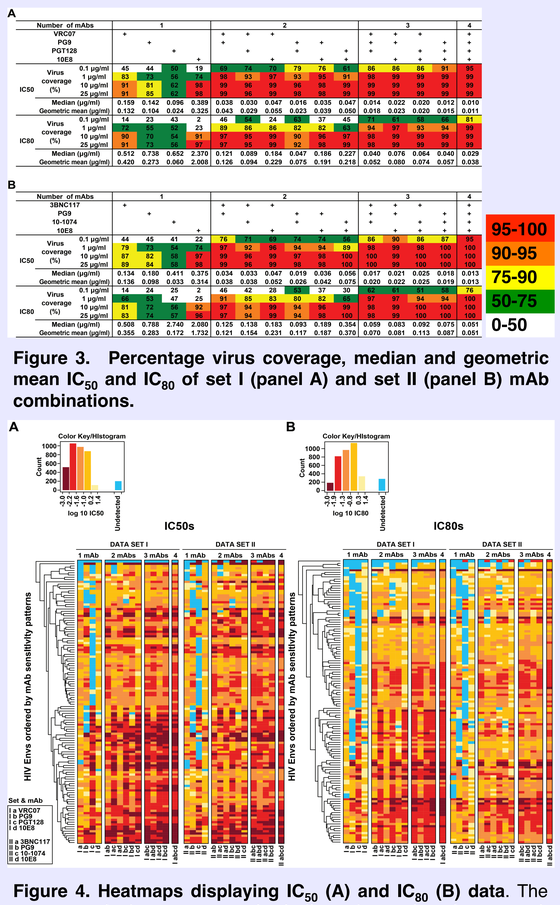


Figure 3. Percentage virus coverage, median and geometric mean IC_{50} and IC_{80} of set I (panel A) and set II (panel B) mAb combinations.

Figure 4. Heatmaps displaying IC_{50} (A) and IC_{80} (B) data. The potency of the response is indicated ranging from weak neutralization (light yellow) to potent (dark red). Blue indicates a titer of >25 µg/ml. The 125 HIV-1 Envs are indicated by rows that are organized according to like-patterns of neutralization sensitivity, using hierarchical clustering of the $\log_{10}(IC_{50})$ or $\log_{10}(IC_{80})$ to aid the eye in resolving patterns.

Subtle but consistent favorable mAb combination effect

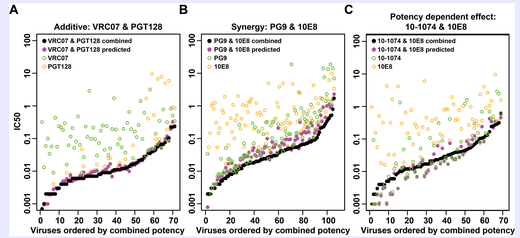


Figure 5. Three patterns of mAb combination effect. (A) Additive effect. (B) Synergy. (C) Potency dependent effect. Only viruses that are sensitive to each single component mAbs are shown.

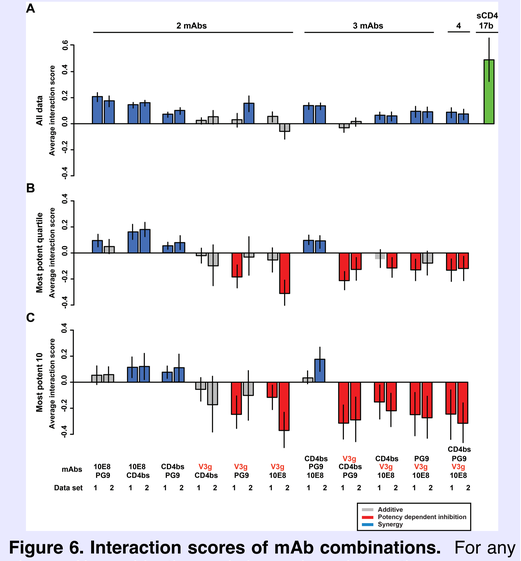


Figure 6. Interaction scores of mAb combinations. For any given mAb combination and virus pair, an interaction score was defined as: $\log_{10}$(predicted IC_{50}/observed IC_{50}). (A) The mean interaction scores of each mAb combination on all qualified viruses. The error reflects the 95% CI. (B) The mean interaction scores on most potent quartile viruses. (C) The mean interaction scores on 10 most potent viruses.

Conclusion

- **All mAb combinations showed substantially improved neutralization breadth compared to the corresponding single mAbs, while the neutralization potency of individual mAbs was maintained**
- **Overall the improvement of neutralization breadth was closely predicted by an additive effect model and explained by complementary neutralization profiles of antibodies recognizing distinct epitopes**
- **Subtle but consistent favorable interactions were observed in some mAb combinations whereas less favorable interactions were observed on a small subset of viruses that are highly sensitive to V3-glycan mAbs**