Evaluating Broadly Neutralising Antibodies as Prevention for Paediatric HIV-1

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INTRODUCTION

Hypothesis: *Broadly neutralising antibody (bNAb) cocktails, in double or triple combinations synergistically enhance suppression of diverse HIV-1 variants.*

The LIFE study investigated preventive and therapeutic treatment strategies in neonates exposed to and diagnosed with HIV-1. Fifty-five HIV-1 envelope (Env) glycoprotein variants from Mozambique and Tanzania isolated from vertical transmission cases from 2019-2024 were included in this study. Additionally, historic cases from Zambia and Rwanda were also investigated.

PLAIN LANGUAGE SUMMARY

- HIV-1 specific bNAbs have emerged as next generation paediatric interventions with potential to prevent and treat HIV infection by neutralising a wide spectrum of HIV-1 Env glycoprotein variants.
- Neutralising breadth and potency against circulating strains can be enhanced by selecting appropriate bNAb combinations.

CONCLUSIONS

- Selecting suitable bNAb combinations substantially enhanced suppression of diverse HIV-1 variants.
- Triple bNAb combinations were particularly effective, suggesting both the potency and the breadth of multi-bNAb regimens, and supporting their potential as preventative or therapeutic strategies for neonates at risk of vertical HIV-1 transmission.

ACKNOWLEDGEMENTS

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Additional Key Information

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Abbreviations: Pseudovirus particles (PVPs), Non-neutralising PVPs (N-N PVPs), Broadly neutralising antibodies (bNAbs)

VRCO7-523LS (VRCO7), CAP526V2LS (CAP), PGT121.414LS (PGT), 10E8v4-5RLS (10E8v4), 007 Batch 29 (007), 04-A06-WT-B3 (A06)

Abbreviations for bNAbs in Figure 4 heatmap had to be modified due to "R" software default settings: 10E8v4 as TenE8v4, 10-1074 as Ten1074, 007 Batch 29 as Batch29007 and 04-A06-WT-B3 as A06WTB3.

METHODS

- bNAbs targeting specific epitopes on HIV-1 Env glycoprotein were used to neutralise diverse HIV-1 Env variants (Figure 1).
- Pseudo virus particles (PVPs) were generated to represent paediatric vertically transmitted HIV-1 strains (Figure 2).
- Neutralisation assays were performed to characterise single bNAb profiles from a panel of 10 monoclonal antibodies (mAbs) against 33 Env glycoprotein variants (Figure 3, 4 and 5).
- Dual and triple bNAb combinations were tested to assess bNAb breadth and potency (Figure 5 and Figure 6A-D).

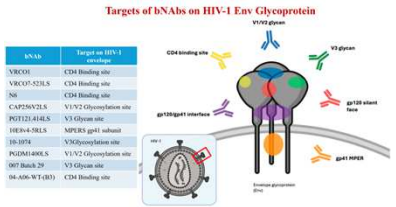


Figure 1: Table showing a list of bNAbs (left), and a cartoon representation of various regions of HIV-1 Env glycoprotein targeted by bNAbs.

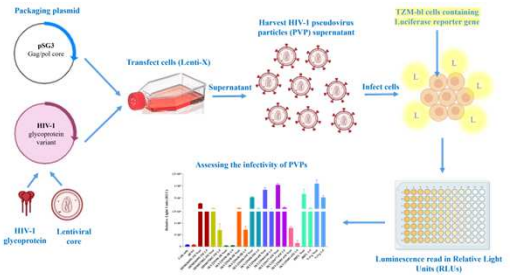


Figure 2: Schematic diagram of pseudovirus particles (PVPs) generation and infection protocol. PVPs were generated by employing a two-plasmid system, 1) A vector plasmid containing the Gag and Pol regions of HIV genome, necessary for virus packaging and 2) A plasmid expressing the HIV-1 Env glycoprotein variant (n=55). The two plasmids were incubated with a producer cell line (Lenti-X cells) in polyethylenimine (PEI) media for efficient transfection. Following 16 hr incubation, PEI media was replaced with regular culture media for further 48 hrs. The supernatants containing the newly formed PVPs were harvested. Infectivity of PVPs was assessed by infecting TZM-bl cells with PVP supernatants for 72 hrs. Luciferase activity indicating infection was measured in relative light units (RLUs). The results were plotted using GraphPad Prism software. RLUs >10^6 demonstrated robust infectivity.

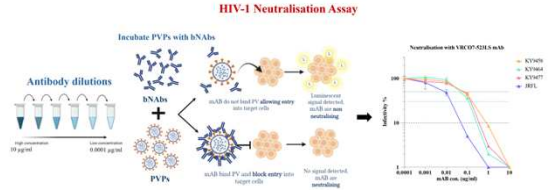


Figure 3: Schematic diagram demonstrating HIV-1 neutralisation assay. Profiles of bNAbs were generated by preparing bNAbs serial dilutions (10 µg/ml – 0.0001 µ/ml). Diluted bNAbs were incubated with PVP supernatant (1:5 diluted in culture media) for 30 min to allow neutralisation. The PVPs+bNAb mixture was incubated with TZM-bl cells to assess dose dependant infectivity of PVPs. Lower RLUs indicated efficient neutralisation whereas higher RLUs indicated incomplete or no neutralisation. Half maximal inhibitory concentrations (IC_{50} values) were determined from neutralisation plots. PVPs exhibiting IC_{50} values >10 µg/ml were considered resistant PVPs.

RESULTS

- Ten bNAbs were profiled against 33 PVPs representing Mother-to-Child (MTCT) vertically transmitted HIV-1 strains from varying subtypes (Figure 4).
- All bNAbs demonstrated inhibitory activity across a broad spectrum of HIV-1 Env variants, with potency increasing in a dose-dependent manner (Figure 5).
- Triple antibody combinations targeting CD4 binding site (bs), V3 glycans and MPERS gp41 subunit of the HIV-1 Env glycoprotein efficiently neutralised mixture of multiple PVPs demonstrating significantly reduced IC_{50} values ($p<0.05$) (Figure 5 and 6 A).
- Non-neutralising resistant strains targeted with dual and triple bNAbs demonstrated IC_{50} values significantly below the cut-off value of 1 µg/ml (Figure 6 B, C and D).

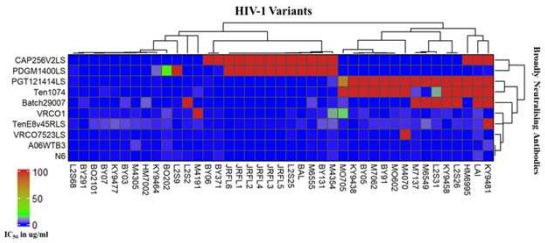


Figure 4: Overview of bNAb profile against 33 HIV-1 Env glycoprotein variant PVPs. Most PVPs exhibited IC_{50} values below 1 µg/ml, a widely accepted high potency concentration indicating efficient neutralisation (blue squares) . PVPs exhibiting higher IC_{50} values of ≥10 µg/ml were considered resistant PVPs (red squares).

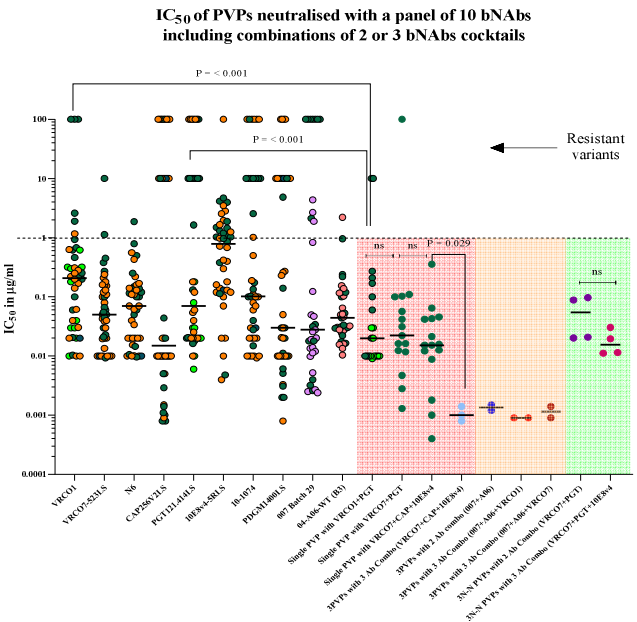


Figure 5: Dot plot exhibiting profiles of 10 bNAbs against 33 HIV-1 Env glycoprotein variant PVPs. Orange dots in first 8 columns (left-right) indicate 33 PVPs neutralised with 8 bNAbs. Purple and pink dots exhibit neutralisation of the same PVPs with newly acquired bNAbs (007 and A06). Pink shaded area shows neutralisations of single PVP (green dots) with a combination of 2 or 3 bNAb. Light blue dots (pink shaded area) represent 3 experiments performed on different occasions demonstrating neutralisation of 3 PVPs with 3 bNAb combo. Light orange shaded area shows neutralisation of 3 PVPs with a combo of 2 new bNAbs and a combo of 3 bNAbs. Each dot in the green shaded area represents neutralisation a of 4 different sets of 3 N-N PVPs with 2 (dark purple dots) and 3 (dark pink dots) bNAb combos.

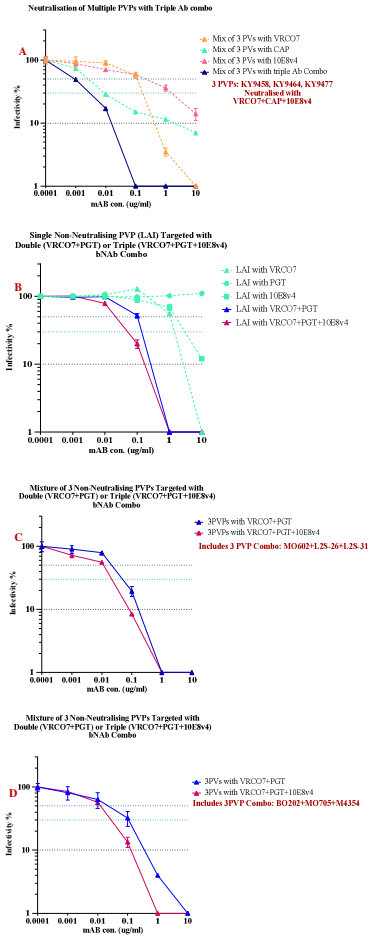


Figure 6: Representative neutralisation plots. **(A)** Neutralisation of 3 PVPs with single bNAb VRCO7 (orange dotted line), CAP (green dotted line), 10E8v4 (pink dotted line) and combo of 3 bNAbs (blue solid line). **(B)** Neutralisation of single N-N PVP (LAI) with single bNAb VRCO7 (green triangle dotted line), PGT (green circle dotted line), 10E8v4 (green square dotted line) and combo of 2 and 3 bNAbs (blue and red solid lines respectively). **(C)** and **(D)** Representative neutralisation plots for 2 different sets of 3 N-N PVPs targeted with combos of 2 (blue lines) and 3 (red lines) bNAbs.