Long Half-Life Broadly Neutralizing Killer Bispecifics Against HIV-1: Harnessing the Immune System

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BACKGROUND

HIV-1 remains significant global health challenge, requiring continuous efforts to develop innovative therapeutic approaches. Clinical use of mAb needs multiple infusions and for BiTEs is limited by short half-life in blood, demanding continuous infusion resulting in high costs and inconvenience. We describe the development of DNA-launched, single-chain bispecific molecules that redirect T cells towards killing target HIV infected cells. These are highly specific Broadly neutralizing Killers (BnKs) that demonstrate long half-lives (LHL) in-vivo.

METHODS

We designed DNA-launched LHL- BnKs with bispecific scFvs and a Fc fragment having Fc effector-null mutations to abrogate Fcγ receptor binding. We evaluated in-vitro and in-vivo expression of DNA launched LHL-BnKs and characterized functionality. ELISA was performed for binding and pseudovirus-neutralization assays to test neutralizing potential against a global virus panel. A novel cell based Xcelligence assay was used for killing activity. In-vivo pharmacokinetics were evaluated by inoculating therapeutic molecule encoding optimized plasmid DNA.

DNA launched single-chain broadly neutralizing killers (bNKs) have high and long-term expression with *potent neutralization of* HIV-1 viruses and *killing* of *HIV-1 infected target cells* by *engaging human effector T cells.*

RESULTS

DNA launched PGDM1400, 3BNC117 and PGT121 LHL-BnKs respectively were designed and optimized for expression both in-vitro and in-vivo. Binding studies demonstrate that these molecules bind to CD3 in a concentration dependent manner. They also demonstrated binding to CD3+T cells and neutralized a global panel of HIV-1 Tier 2/3 viruses with high potency and specificity. The LHL-BnKs displayed potent killing of HIV-1 infected target cells with nanogram/ml IC50 for killing. Combination therapy of 10-1074 and 3BNC117 did significantly reduce viral rebound in clinical trials where the groups received up to 8 infusions of 3BNC117 during the 24-week period. Our data suggests that after single inoculation of 50ug DNA/site (total 200ug), plasma concentration of 10-38 ug/ml was achieved in vivo with peak serum expression around day 21 and expressed for extended duration.

CONCLUSIONS

Our data highlights the design and characterization of novel DNA launched immunotherapeutic against HIV-1, which possess significant characteristics of a promising immunotherapy having broad neutralization capacities of a Tier 2/3 global virus panel, specific engagement of effector cells and killing of the infected target cells and longer serum half-life in comparison to conventional bispecific T cell engaging molecules. Delivery of LHL-BnKs as combination therapy.

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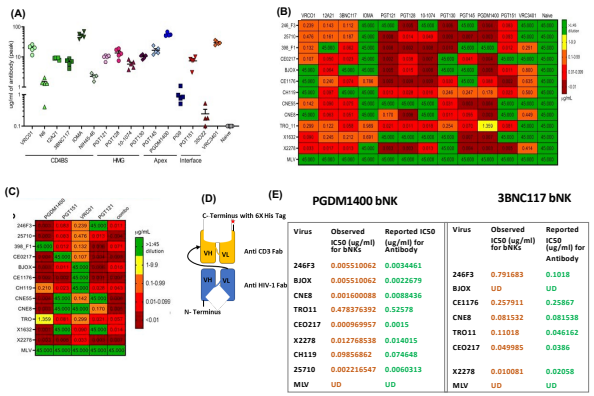


Fig 1. We developed a library of DMAbs for broadly neutralizing HIV antibodies. These were expressed in vivo up to 75ug/ml for some antibodies **(A)**. The DMabs neutralize similarly to parental antibodies **(B)**. Combinations delivered in vivo neutralize 100% of isolates in this tier 2 global panel **(C)**. Schematics of DNA launched HIV-1 targeting bNKs **(D)**. bNKs neutralize similarly to parental antibodies **(E)**.

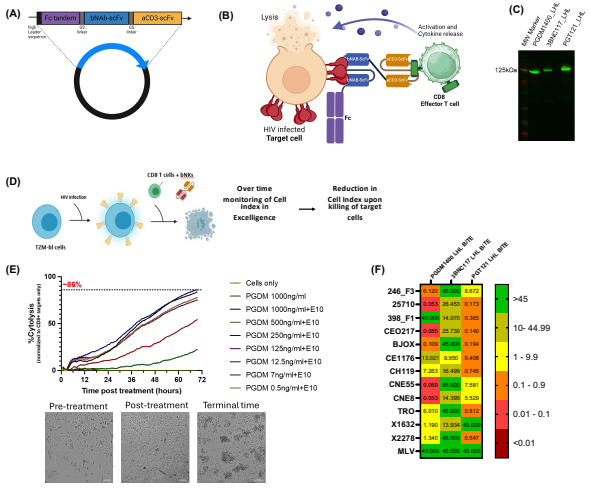


Fig 2. We developed PGDM1400, 3BNC117 and PGT121 as DNA launched single-chain broadly neutralizing killers **(A).** Schematics of effector and HIV-1 target cell engagement and killing **(B)**. Confirmation of LHL-bNKs by western blot **(C)**. Novel cell impedance base killing assay **(D)** demonstrates concentration dependent killing of HIV infected TZM-bl cells by engaging human effector CD8+ T cells by PGDM1400 bNKs **(E).** Neutralization potency was maintained as that of the parent Abs **(F)**.

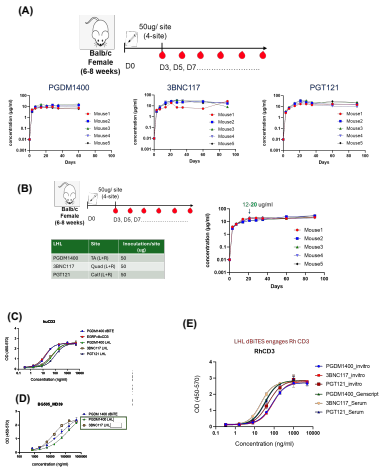


Fig 3. We expressed DNA launched LHL- bNKs in-vivo. LHL-bNKs expressed for 90 days post inoculation **(A).** High expression of LHL-bNKs were observed for inoculation of bNKs targeting 3 vulnerable sites in single animal **(B).** Binding of LHL bNKs to hu CD3 **(C)**, HIV-1 trimer **(D)** and cross species–rhesus CD3 binding **(E).**

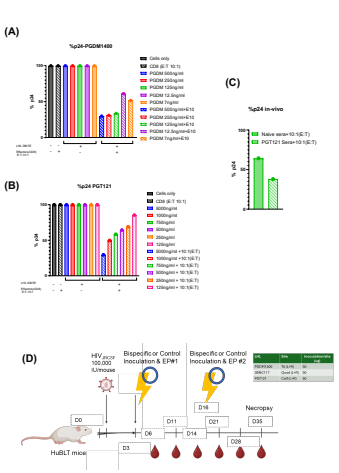


Fig 4. p24 estimation of HIV-1 infected TZM-bl cells post CD8+ T cells and PGDM1400 **(A)**, PGT121 **(B)** and PGT121 serum **(C)** treatment. Schematics of HIV-1 JRCSF infected hu-BLT mice that were inoculated with combination therapy for further estimation of reduction in viral load **(D).**