DNA LAUNCHED BROADLY NEUTRALIZING KILLERS-A DOUBLE-EDGED THERAPEUTIC AGAINST HIV-1

0316

Mansi Purwar[1] , **Sukanya Ghosh**[1], Samson Adeniji[1], Abhijeet Kulkarni[1], Zhe Yuan[1], Pratik Bhojnagarwala[1] Giselle Lopez[1], Luis J. Montaner[1], Mohamed Abdel-Mohsen[1], David Weiner*[1]
[1]Vaccine & Immunotherapy Center, The Wistar Institute, Philadelphia, PA, USA

BACKGROUND

CD8+ T cells play important role in control of HIV-1 viremia. We developed bispecific molecules that are a combination of Broadly Neutralizing Antibody binding regions (bNAbs) and link these to Fab targeting human CD3 and encode these as optimized DNA expression vectors to study their ability to target HIV+ cells as a DNA launched therapeutic intervention for HIV-1

METHODS

We developed a library of broadly neutralizing killers (bNKs) which are single chain molecules, these engage CD3+ T cells from one arm and HIV-1 infected target cells using the other arm. The expression profiles and functionality of these DNA launched bNKs were characterized by binding, neutralization and recruitment of effector cells for killing. Pseudo-neutralization assays were used to test neutralizing potential of these therapeutics against global virus panel. Novel cell based Xcelligence assay was designed to use impedance as readout for killing activity of bNKs in presence of effector T cells and their potencies were defined.

DNA launched broadly neutralizing killers (bNKs) exhibit *potent neutralization* HIV viruses and *killing* of *HIV infected target cells* by *engaging human effector T cells.*

RESULTS

PGDM1400-bNK and 3BNC117-bNK were optimized for expression in vivo. These bound CD3 expressing T cells along while maintaining their capacity to neutralize HIV-1 Tier2/3 viruses with high potency/specificity. These were determined to be potent killers of HIV-1 infected target cells (IC50 @ pg levels). IM DNA delivery resulted in bNK expression in animal serum which exhibited cytolysis and killing of HIV-1 infected cells in novel cell based Xcelligence assay.

CONCLUSIONS

We demonstrate potent killing Our data highlights the design and characterization of novel DNA launched bNKs which are potent double-edged therapeutics capable of broadly neutralizing difficult to neutralize Tier2/3 viruses and engaging effector T cells to their targets and mediate killing of HIV-1 infected cells with high potency and specificity. Further designs are being currently explored to use these molecules as combination therapies to engage various other effector cells of the immune system and target arresting HIV-1 virus escape using our robust HIV therapy.

AUTHOR CONTACT INFORMATION

Luis J. Montaner: montaner@wistar.org
Mohamed Abdel-Mohsen: mmohsen@wistar.org
David B. Weiner* : dweiner@wistar.org (* Corresponding author)

ACKNOWLEDGEMENTS:

The Wistar Institute, Centers for AIDS Research (CFAR) Collaborations, Delaney, WW Smith distinguished professor in cancer research

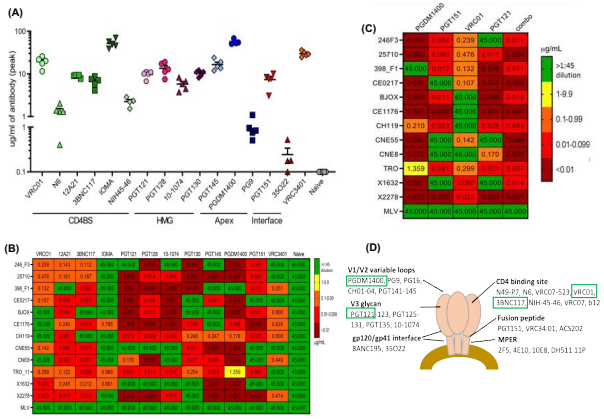
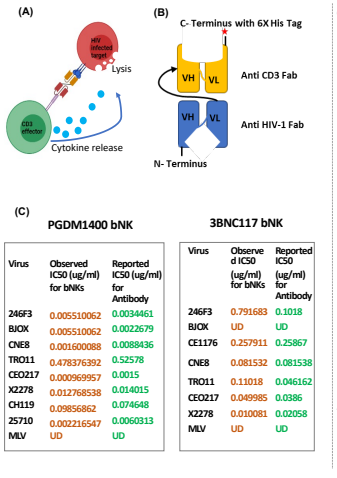


Fig 1. We developed a library of DMAbs for broadly neutralizing HIV antibodies. These were expressed in vivo up to 75ug/ml for some antibodies **(A)**. The DMabs neutralize similarly to parental antibodies **(B)**. Combinations delivered in vivo neutralize 100% of isolates in this tier 2 global panel **(C)**. Targeting multiple epitopic regions of HIV-1 env for combination therapy **(D)**.



PGDM1400 bNK

Virus	Observed IC50 (ug/ml) for bNKs	Reported IC50 (ug/ml) for Antibody
246F3	0.005510062	0.0034461
BJOX	0.005510062	0.0022679
CNE8	0.001600088	0.0088436
TRO11	0.478376392	0.52578
CEO217	0.000969957	0.0015
X2278	0.012768538	0.014015
CH119	0.09856862	0.074648
25710	0.002216547	0.0060313
MLV	UD	UD

3BNC117 bNK

Virus	Observed IC50 (ug/ml) for bNKs	Reported IC50 (ug/ml) for Antibody
246F3	0.791683	0.1018
BJOX	UD	UD
CE1176	0.257911	0.25867
CNE8	0.081532	0.081538
TRO11	0.11018	0.046162
CEO217	0.049985	0.0386
X2278	0.010081	0.02058
MLV	UD	UD

Fig 2. We developed PGDM1400 and 3BNC117 as broadly neutralizing killers. Human CD3 Fab engaging CD8+ cells **(A)**. These are designed as single chain DNA launched molecules **(B).** Neutralization potency was maintained as that of the parent Abs **(C)**.

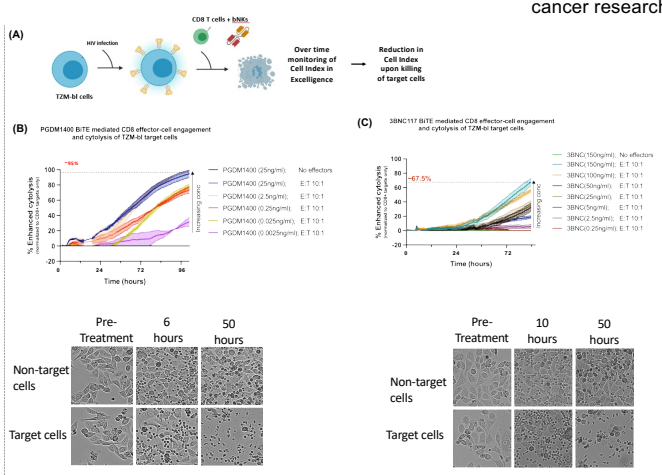


Fig 3. Novel cell impedance base killing assay **(A)** demonstrates concentration dependent killing of HIV infected TZM-bl cells by engaging human effector CD8+ T cells by PGDM1400 bNKs **(B)** and 3BNC117 bNKs **(C)**.

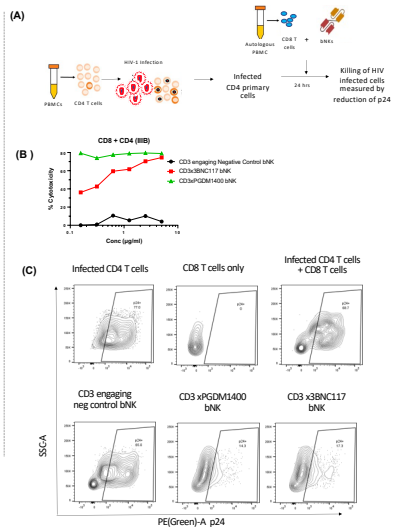


Fig 4. DNA launched bNKs significantly reduces HIV+ primary CD4 T cells