Scalable CD64-Engineered Expanded NK Cells Sustain HIV-Specific bnAb Retention and HIV-Directed ADCC.

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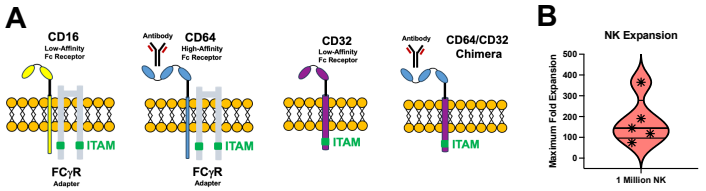
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ABSTRACT

HIV-1 Infected Autologous CD4+ primary T cells remain largely resistant to NK mediated cytotoxicity unless antibodies specific for viral envelope are added to trigger Antibody-dependent cellular cytotoxicity (ADCC). We have previously shown that ADCC activity by NK cells can be augmented by equipping them with the ADAM-17 resistant high-affinity Fc receptor, CD64 (Tomescu, et al. 2025. *J Immunol.* 214(2): 253-264). However, poor persistence of primary NK cells and limited proliferation have constrained scalability of CD64 genetically modified NK cells as a viable immunotherapy approach. Whether HIV-BNAb directed ADCC through CD64 could be improved via inclusion of alternative signaling domains (such as the direct signaling domain of CD32) also remained undetermined. Here, we have developed an *ex vivo* approach to gene-modify NK cells after feeder cell expansion with the full-length CD64 gene sequence or Fc receptor chimeric molecules possessing the extracellular portion of CD64 fused with the intra-cellular signaling domain of CD32 (**Figure 1A**).

FIGURE 1



MATERIALS AND METHODS

Primary human NK cells from peripheral blood were expanded to scale (>100-fold per round of incubation with irradiated K562-mbIL-21 feeder cells, **Figure 1B**) and engineered to express CD64 constructs by mRNA electroporation. Expanded NK cells electroporated with no mRNA, CD64 wild-type mRNA, or CD64/CD32 chimeric mRNA, pre-loaded with 50 μg/mL of the HIV-specific bnAb 3BNC117 for 15 minutes and then washed twice to remove unbound antibody. For antibody retention experiments (**Figure 2**), electroporated and bnAb pre-loaded expanded NK cells were stained daily with antibodies to CD56, CD3, CD16, CD64 and a secondary antibody to detect surface bound human IgG by Flow Cytometry. HIV-specific BNAb Capture is shown on X-axis compared to CD64 expression on Y-axis. For ADCC functional experiments, electroporated and bnAb pre-loaded expanded NK cells were incubated with autologous uninfected or HIV-1 infected CD4+ primary T cells a 1:2 effector to target cell ratio in a standard 3-hour degranulation assay with a PE-conjugated anti-CD107a mAb. CD107a degranulation (x-axis) was measured on live Aqua viability dye negative/CD56+/CD3- gated NK cells. Gates were set based upon NK cells incubated with no target cells (first panels).

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FIGURE 2

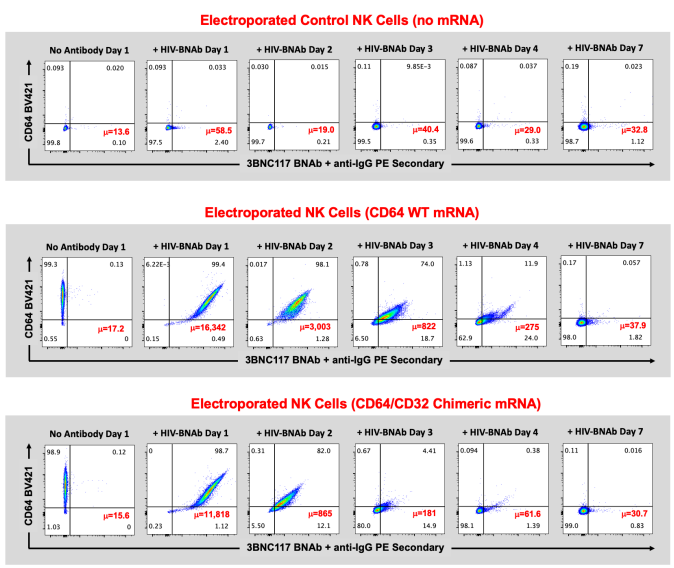


Figure 2. Flow Cytometric Analysis of HIV-specific BNAb Capture and Retention by CD64 Modified NK cells.

RESULTS

- Primary NK cells can be successfully expanded several hundred-fold *ex vivo* with a single exposure to irradiated feeder cells **(Figure 1B)**.
- CD64 engineered NK cells (wild type or CD64/CD32 chimera) bound bnAbs at markedly higher levels than control NK cells expressing only CD16 (>10,000-increase in geometric mean fluorescence) and retained surface-bound antibody for several days following electroporation **(Figure 2).**
- Wild Type CD64 mRNA electroporated and HIV-specific bnAb pre-loaded NK cells showed significantly greater ADCC degranulation against HIV-infected, but not uninfected, autologous CD4+ primary T cells compared to control NK cells or CD64/CD32 chimeric NK cells **(Figure 3).**
- Fusion of the high-affinity antibody extra-cellular domain of CD64 with the transmembrane and intra-cellular portion of CD32 direct signaling domain **(Figure 1A)** is feasible, but results in reduced kinetics of CD64 expression over time **(Figure 2)** as well as diminished ADCC functional activity.

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FIGURE 3

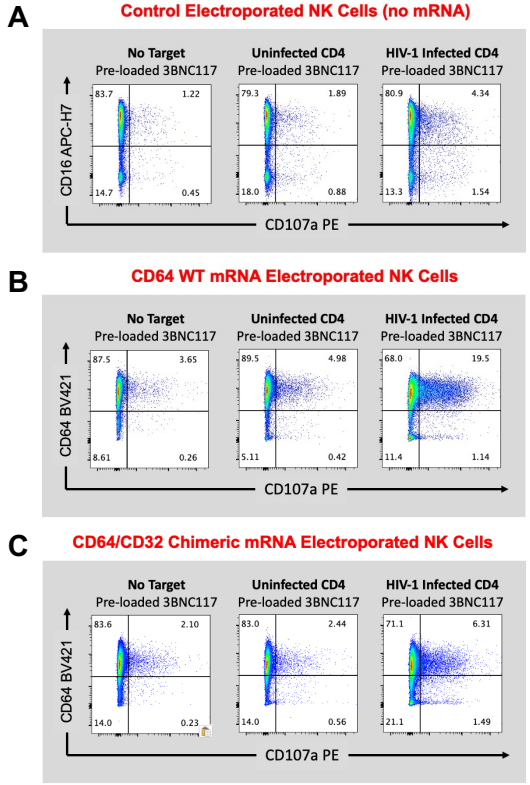


Figure 3. ADCC Functional Analysis of CD64 Electroporated NK Cells Against HIV-1 Infected Autologous CD4 Primary T Cells.

CONCLUSIONS

We demonstrate scalable manufacture level expansion and genetic modification of full-length Fc receptor engineered human NK cells that support advancing CD64 "antibody-armored" autologous NK immunotherapy strategies toward a HIV cure. NK cells expressing wild type CD64 Fc receptors display prolonged CD64 expression, enhanced HIV-specific bnAb retention, and superior HIV-specific ADCC activity when compared to control NK cells or chimeric CD64/CD32 Fc receptor expressing NK cells.