Tri-Specific Killer Engagers in macaques potently induce NK cell responses in blood and tissues

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BACKGROUND

Strategies to enhance immune responses towards HIV-infected cells are needed for eliminating HIV reservoirs. Natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC) plays a key role in HIV protection. In cancer, Tri-specific Killer Engagers (TriKEs), consisting of a CD16-binding domain, an antigen-targeting domain, and an IL-15 moiety, enhance NK cell-mediated ADCC by forming immune synapses through CD16 and antigen engagement. Here, we developed a gp120-targeting TriKE (anti-CD16/IL15/CD4ECD1) by utilizing the extracellular domain 1 of the CD4 molecule, which binds to gp120 but has no interaction with MHC class II, to activate NK cells for targeting HIV-infected cells (Figure 1). A version specific for rhesus macaques (RMs) has been generated to conduct an *in vivo* dose-response study in uninfected RMs to evaluate TriKE safety and its impact on NK cell responses.

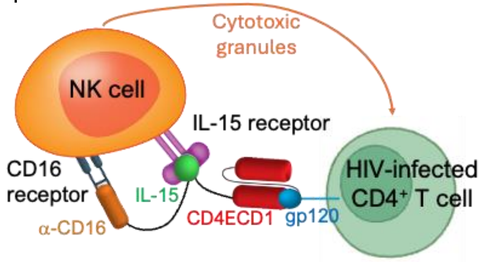


Figure 1. Schematic of anti-HIV Tri-specific killer engagers. The TriKE comprises an anti-CD16 (clone 3G8) linked to the extracellular domain-1 of CD4 (CD4ECD1) by two short peptide linkers and an IL-15 molecul

METHODS

TriKE was administered subcutaneously 5 times per week for 3 weeks in 3 uninfected RMs, with doses escalating from 100 to 300 µg/kg (Figure 2). Complete blood counts and blood chemistry tests were performed; whole blood and lymph nodes (LNs) were collected longitudinally for immunophenotyping.

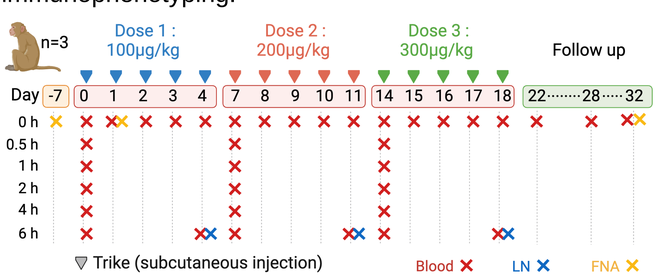
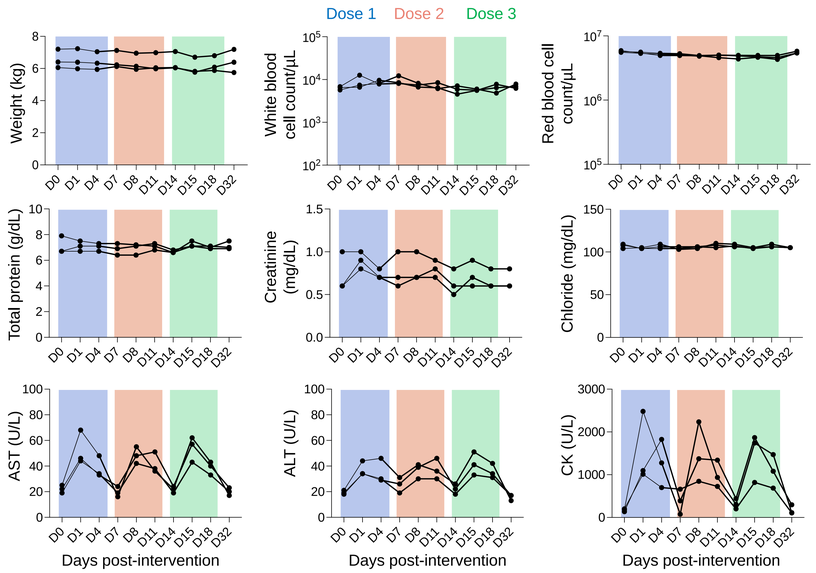


Figure 2. Study design of TriKE intervention in uninfected rhesus macaques. LN: lymph node; FNA: fine needle aspirate used to collect lymph node cells.

The gp120-targeting TriKE effectively and safely engaged NK cells in RMs, leading to their robust activation, proliferation, and expansion in blood and lymph nodes.

RESULTS

Figure 3. Weight, hematological and serum chemistry parameters

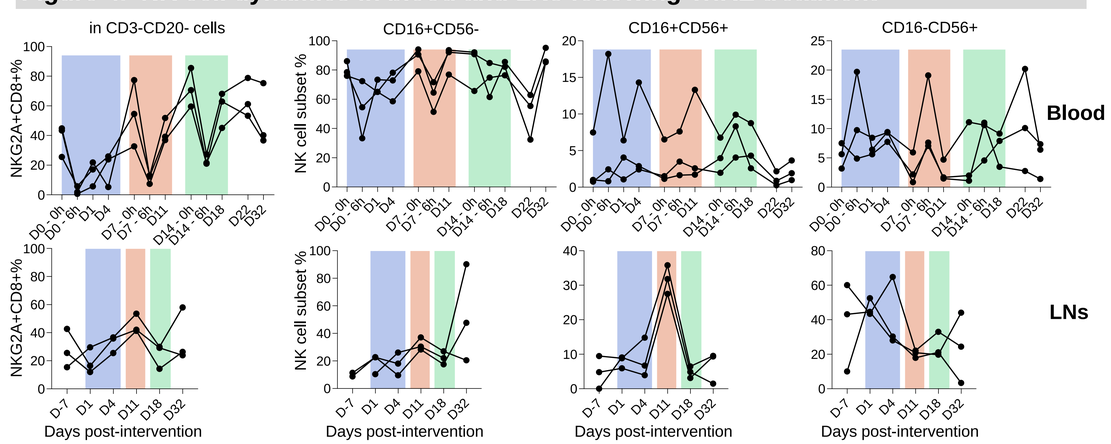


TriKE treatment was well-tolerated.

There was no significant change in weight, white blood cell, red blood cell count.

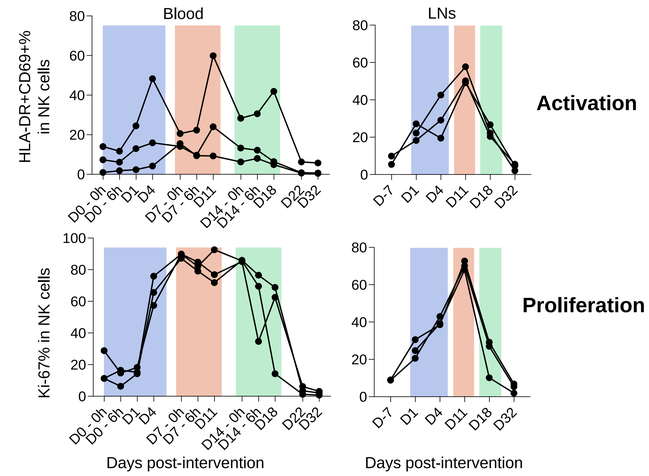
Blood biochemistry parameters remained normal throughout the study, with transient increases in transaminases (AST/ALT) and creatine kinase (CK) levels following the first administration of each cycle.

Figure 4. NK cell dynamics in blood and LNs following TriKE treatment



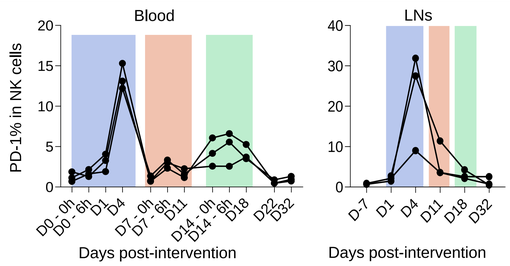
TriKE treatment increases NK cell (NKG2A+CD8+) frequencies both in blood and LNs. CD16+CD56- and CD16+CD56+ NK cell subsets peaked in LNs on day 11, coinciding with the final administration in week 2. During the third week's dosing, the frequency of NK cells decreased in LNs (Day 18).

Figure 5. NK cell activation and proliferation



NK cell expansion was accompanied by increased NK cell activation (HLA-DR+CD69+) and proliferation (Ki-67+). Following the third week's dosing (300 µg/kg, day 14), NK cell activation and proliferation levels decreased.

Figure 6. PD-1 expression in NK cells



Interestingly, PD-1 expression significantly increased on day 4 (end of the first week's dosing) in both blood and LNs, normalizing by day 11. This may reflect an immunological response to prevent excessive NK cell activation.

CONCLUSION

The gp120-targeting TriKE safely and potently induces NK cell responses in RMs, with their substantial activation, proliferation, and expansion in blood and lymphoid compartments. Blocking PD-1 upregulation could synergize with TriKE treatment to enhance TriKE-mediated NK cell function. These findings provide strong rationale to consider TriKE-based therapies as a strategy for targeting HIV/SIV reservoirs, including in lymphoid tissues.

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