Bispecific Antibodies Promote NK Cell-mediated Elimination of the HIV Reservoir

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Background

- The persistence of long-lived HIV-infected cells comprising the latent reservoir is the main barrier to a cure. “Shock and kill,” a strategy for clearing the latent reservoir, involves selective reactivation of HIV gene expression through treatment with a latency reversing agent (LRA) followed by immune-mediated elimination of HIV-infected cells.
- To date, LRA-based clinical interventions in PLWH have failed to achieve a significant reduction in the latent reservoir despite observed transient increases in plasma and cell-associated viral RNA[1-3]. A potential explanation for this discrepancy may be that the cytolytic immune effector cells of PLWH exhibit compromised responses and are unable to efficiently recognize and eliminate cells expressing viral gene products[4-6]. To ensure effective elimination of HIV-infected cells in the context of “shock and kill,” novel immunotherapies must be developed to enhance HIV-specific cell-mediated cytolytic activity.
- Here, we generated novel bispecific antibodies and evaluated their ability to promote NK cell-mediated elimination of the infected cells comprising the HIV reservoir.

Generation of NK cell-engaging HIV-1-specific scDbs

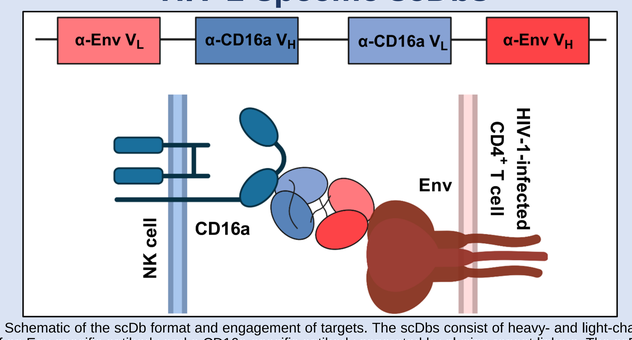


Figure 1: Schematic of the scDb format and engagement of targets. The scDbs consist of heavy- and light-chain variable regions of an Env-specific antibody and a CD16a-specific antibody separated by glycine repeat linkers. The scDbs bind to CD16a expressed on the surface of cytolytic NK cells and to Env expressed on the surface of HIV-1-infected CD4$^+$ T cells. This interaction promotes NK cell activation, release of cytotoxic factors, and lysis of the infected cell.

scDbs promote NK cell-mediated elimination of HIV-infected cells

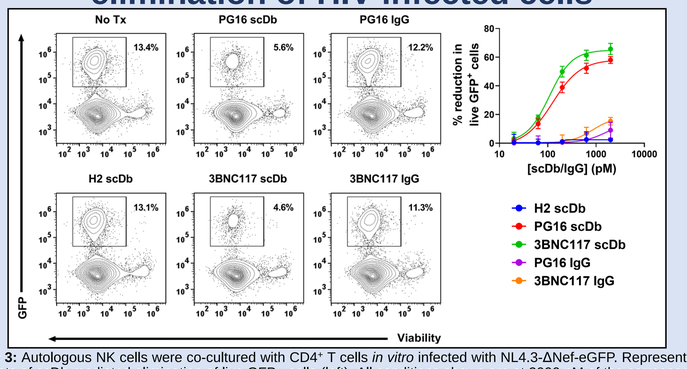


Figure 3: Autologous NK cells were co-cultured with CD4$^+$ T cells *in vitro* infected with NL4.3-ΔNef-eGFP. Representative flow plots of scDb-mediated elimination of live GFP+ cells (left). All conditions shown are at 2000 pM of the corresponding scDb or IgG. H2 scDb represents an isotype control scDb that targets an irrelevant epitope.

Participant characteristics

Subject ID	Age	Sex	Race	Time since diagnosis (months)	ART regimen	Time on ART (months)	Time on suppressive ART (months)
SCOPE 1713	37	M	White	130	FTC/TDF/BIC	130	124
SCOPE 2256	66	M	White	437	FTC/TDF/RPV/TCV	347	248
SCOPE 2274	60	M	White	235	FTC/TDF/NVP	222	220
SCOPE 2286	56	M	White	233	FTC/TDF/RPV	233	229
SCOPE 2761	49	M	Hispanic	289	FTC/TDF/BIC	289	275
SCOPE 2947	56	M	Hispanic	368	3TC/ABC/TDF/NVP	344	79
SCOPE 3147	65	M	Hispanic	342	FTC/TDF/BIC	320	172
SCOPE 3171	69	M	Mixed	329	FTC/TDF/RGV/DRV/RTV	326	193
DEL-SPC-040	34	M	Mixed	153	3TC/ABC/TCV	146	135
DEL-SPC-044	55	M	AA	238	FTC/TDF/BIC	216	176
DEL-SPC-045	68	M	White	384	FTC/TDF/ATV/RTV	360	86

Figure 4: Leukapheresis samples from PLWH on suppressive ART were obtained from UPenn (BEAT-HIV Delaney, DEL-SPC) and from UCSF (SCOPE). Selection criteria included viral suppression for >72 months and undetectable plasma HIV-1 RNA levels (<50 copies/mL). Abacavir, ABC; African American, AA; antiretroviral therapy, ART; atazanavir, ATV; bictegravir, BIC; darunavir, DRV; dolutegravir, TCV; emtricitabine, FTC; lamivudine, 3TC; nevirapine, NVP; raltegravir, RGV; rilpivirine, RPV; ritonavir, RTV; tenofovir, TDF.

scDbs eliminate cells harboring intact and inducible proviruses *ex vivo*

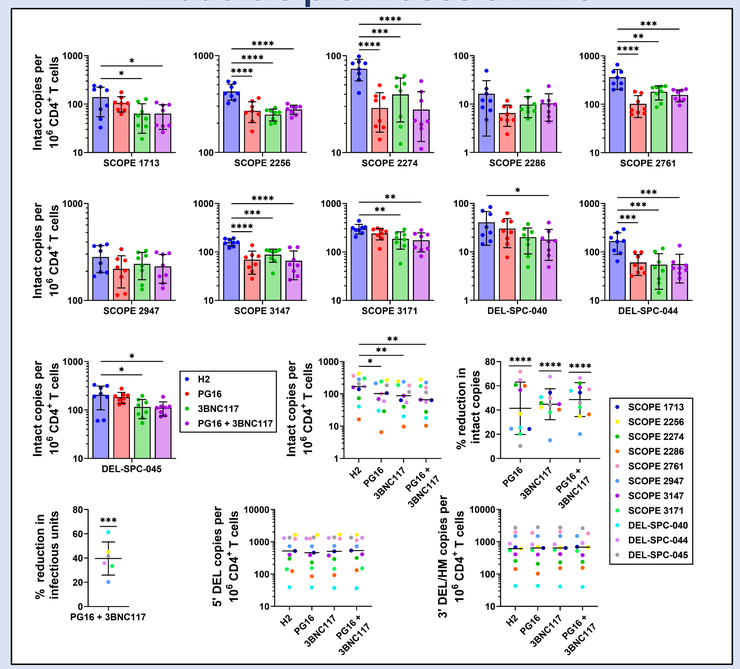


Figure 6: CD4$^+$ T cells from 11 PLWH were treated with PMA/I to reverse HIV latency and co-cultured with autologous NK cells. Live CD4$^+$ T cells were re-isolated from the co-cultures and analyzed by the intact proviral DNA assay[7] or plated at limiting dilution for quantitative viral outgrowth assays[8] (bottom left), one-way ANOVA with Dunnett’s multiple comparisons test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

scDbs induce polyfunctional HIV-specific NK cell responses

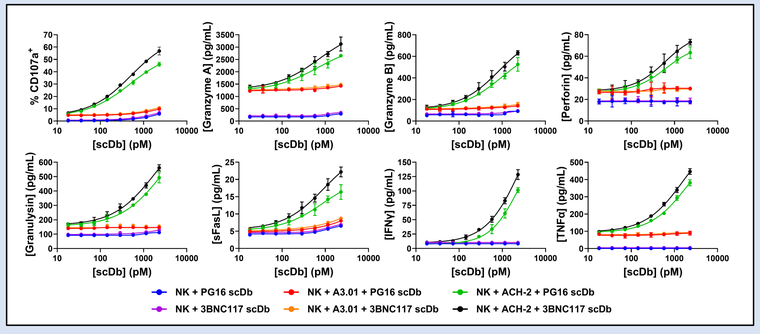


Figure 2: CD107a surface expression measured on CD3$^-$ CD56$^+$ NK cells either alone or in co-culture with uninfected A3.01 cells or HIV-1-infected ACH-2 cells in the presence of the scDbs (top left). Expression of effector molecules (cytolytic granule proteins, a TNF family death ligand, and inflammatory cytokines) released into co-culture supernatant upon NK cell activation.

scDb-induced elimination of HIV-infected cells *ex vivo* depends upon efficient latency reversal

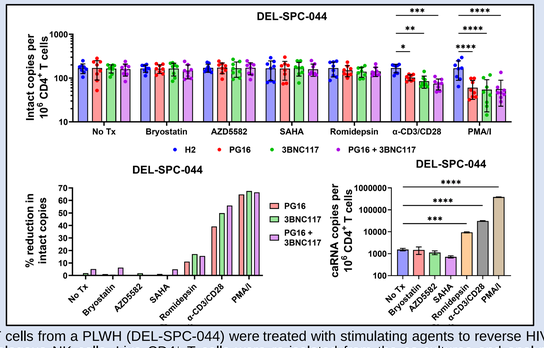


Figure 5: CD4$^+$ T cells from a PLWH (DEL-SPC-044) were treated with stimulating agents to reverse HIV latency and co-cultured with autologous NK cells. Live CD4$^+$ T cells were re-isolated from the co-cultures and analyzed by the intact proviral DNA assay[7] (top), two-way ANOVA with Dunnett’s multiple comparisons test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Paired cell-associated HIV RNA measurements of treated CD4+ T cells prior to co-culture (bottom right), one-way ANOVA followed by Dunnett’s test for multiple comparisons, ***P<0.001, ****P<0.0001.

Conclusions

- Env-directed bispecific antibodies promote potent activation of NK cells and elimination of HIV-infected cells.
- Bispecific antibodies reduce the frequency of CD4$^+$ T cells harboring intact, replication-competent proviruses *ex vivo*.
- Bispecific antibody-induced *ex vivo* elimination of intact provirus harboring cells is highly dependent on efficient latency reversal and single LRA treatments fail to induce sufficient latency reversal for targeting.
- Bispecific antibodies do not eliminate CD4$^+$ T cells harboring defective proviruses with large deletions or extensive hypermutation, likely as a result of impaired Env expression.
- In combination with effective LRAs, the HIV-1-specific scDbs described merit further preclinical evaluation as potential therapeutics for clearance of HIV-1 reservoir cells.

References & Acknowledgements

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