NOVEL BISPECIFIC ENGAGER REDIRECTS CMV-SPECIFIC CTL TO ELIMINATE HIV-INFECTED CELLS

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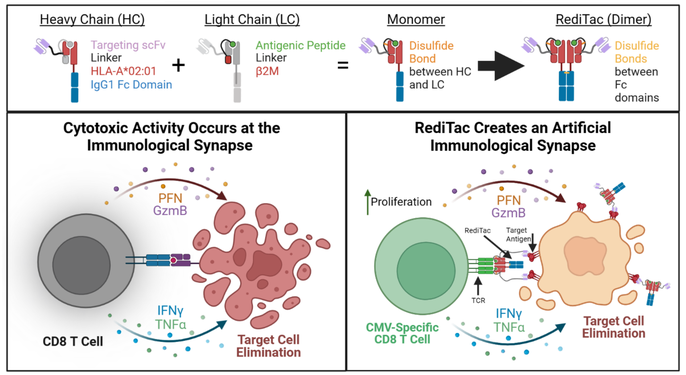
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BACKGROUND

We developed a novel biologic (RediTac) to selectively activate and expand virus-specific memory CD8 T cells (CTLs), redirect them to eliminate HIV-infected cells, and thereby eliminate reactivated latent HIV-infected cells. RediTac employs a bispecific Fc-fusion protein scaffold to dimerize HLA-A2 pMHC linked to HIV bNAb scFvs. The pMHC presents a tethered viral peptide for selective binding and stimulation of cognate TCR on virus-specific CTLs, while the scFv redirects the CTL to eliminate HIV-infected cells. As a proof-of-concept, we constructed RediTac with pMHC presenting a cytomegalovirus (CMV)-derived peptide, NLV, which is associated with a highly functional phenotype. This was linked to an scFv derived from VRC01, an HIV-1 Env-specific bNAb. This NLV-VRC01 RediTac was tested to determine its ability to redirect CMV-specific CTLs to eliminate HIV-1 Env-expressing cells and suppress HIV-1 infection.

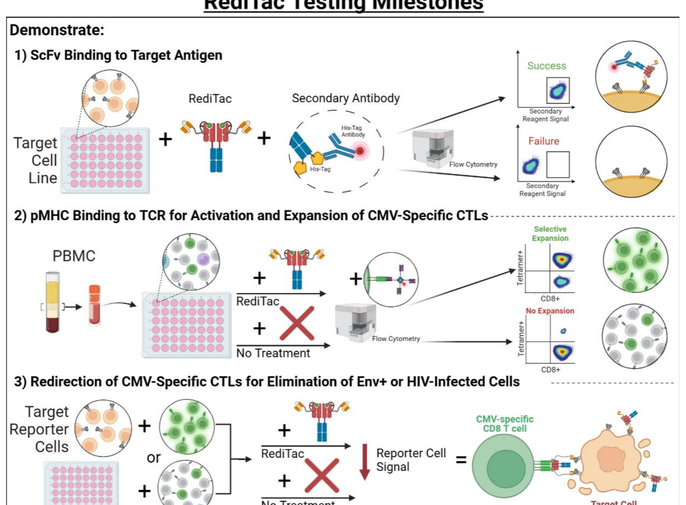
RediTac Architecture and Function



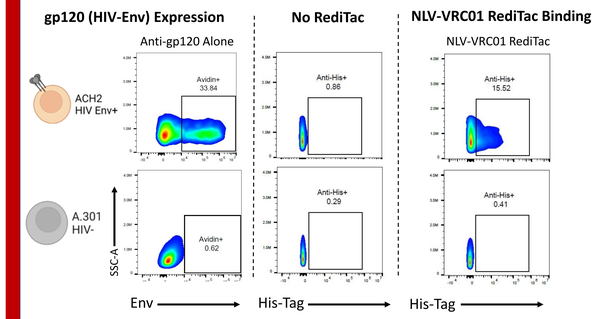
METHODS

Donor PBMC treated with NLV-VRC01 RediTac to expand NLV-specific memory CTLs were cocultured with either an Env-expressing 293T cell line or autologous CD4 T cells infected with an HIV infectious molecular clone expressing a luciferase reporter. NLV-VRC01 RediTac or control treatments were administered and Env-expressing 293T cell killing or luciferase activity reduction was measured as a readout for anti-HIV function.

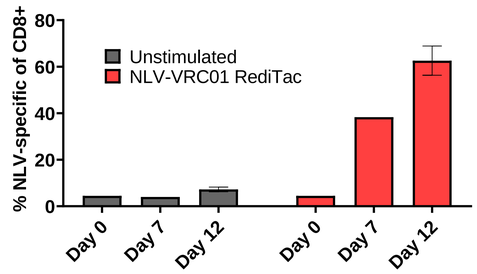
RediTac Testing Milestones



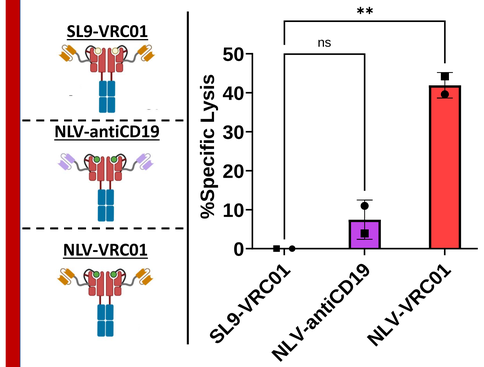
1) NLV-VRC01 Binds to the Surface of HIV-Infected Cells



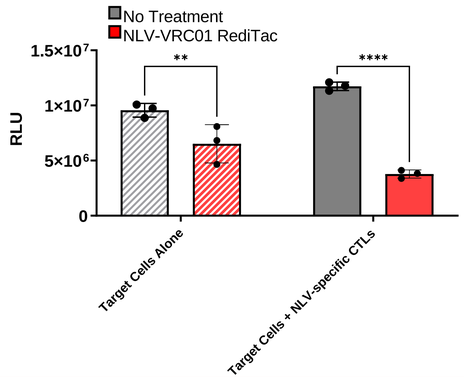
2) NLV-VRC01 Activates and Expands NLV-specific CTLs



3) NLV-VRC01 Redirects NLV-specific CTLs to Eliminate HIV Env+ Cell Line



4) NLV-VRC01 Redirects NLV-specific CTLs to Suppress HIV Infection in Donor T Cells



RESULTS

1. **NLV-VRC01 Binds to the Surface of HIV-Infected Cells**. Secondary staining found that NLV-VRC01 RediTac was detectable on the surface of ACH2 cells that contain an integrated HIV provirus, but not HIV-negative parental A.301 cells, indicating selective binding to HIV Env (**Figure 1**).
2. **NLV-VRC01 Activates and Expands NLV-specific CTLs**. Two weeks after NLV-VRC01 RediTac treatment, NLV-specific CTLs expanded by >50-fold compared to untreated controls (**Figure 2**). N=1 representative donor with technical duplicates.
3. **NLV-VRC01 Redirects NLV-specific CTLs to Eliminate HIV Env+ Cell Line**. Following a two-day coculture at a 10:1 effector to target cell ratio, expanded CTLs eliminated ~40% of Env-expressing 293T cells when treated with fresh NLV-VRC01 RediTac. No elimination was observed when control non-cognate SL9-VRC01 RediTac or irrelevant CD19-targeting NLV-antiCD19 scFv RediTac was added instead (**Figure 3**). N=2 donors with mean displayed as independent shapes.
4. **NLV-VRC01 Redirects NLV-specific CTLs to Suppress HIV Infection in Donor T Cells**. The addition of NLV-VRC01 RediTac without NLV-specific CTLs suppressed an ongoing HIV infection of donor CD4 T cells by ~35%, indicating neutralizing activity of the VRC01 scFv domain. When NLV-VRC01 RediTac was administered to HIV-infected donor CD4 T cells with 1:1 autologous NLV-specific CTLs, the infection was suppressed by ~70% as compared to untreated cultures (**Figure 4**). Infection was measured by relative luciferase units (RLU) to quantify the number of cells infected by a luciferase reporter infectious clone. N=1 donor with technical triplicates.

Statistical Testing: 1way (Fig. 3) 2way (Fig. 4) ANOVA with multiple comparisons. p<.01 = **, p<0.0001 = ****

CONCLUSIONS

CMV-specific CTLs can display a well-described phenotype, termed memory inflation, in which intermittent antigen exposure during CMV infection accumulates a large population of highly functional effector memory cells. However, during chronic HIV-1 infection, prolonged antigen exposure can lead to T cell exhaustion in HIV-specific CTLs that impedes their functional activity. This phenomenon partially explains why HIV-specific CTL responses fail to control untreated HIV-1 infection in nearly all individuals. Without a robust CTL response, cure strategies that implement immune-mediated control or elimination of HIV may not be feasible. As such, there are considerable efforts to investigate immunotherapies that harness functional CD8 T cell activity for an HIV cure. One class of immunotherapy that shows promise is bispecific T cell redirectors. These protein technologies use anti-CD3 TCR engagers paired with targeting scfvs to override the endogenous antigen-specificities of T cells, thus enabling global redirection of T cell cytotoxic activity against cell populations that are defined by the targeting specificity of the scFv. However, this global response can cause inflammatory toxicities and may not harness the most efficacious sub-populations of effector CTLs. In this poster, we described the proof-of-concept development of NLV-VRC01 RediTac, a novel T cell redirector that can focally activate and redirect potent CMV-specific memory CTLs for the elimination of HIV-infected cells.

- We demonstrated that NLV-VRC01 RediTac selectively expands CMV-specific memory CTLs to generate a large population of effector CTLs (Figure 2).
- Additionally, NLV-VRC01 could redirect the cytotoxic activity of these CTLs to eliminate HIV-infected cells while retaining the ability of a bNAb to inhibit HIV infection via neutralization in the absence of effector cells (Figure 4).
- These results support antigen-specific CTL redirection as a novel immunotherapy strategy that can be combined with latency reversing agents to target and deplete the HIV viral reservoir, allowing a path towards a functional cure.

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