Background We constructed RediTac, novel biologics designed to selectively activate and expand virus-specific CD8 T cells (CTL) and redirect them to eliminate cancer cells (figure 1A). RediTac utilize an Fc-fusion protein scaffold to dimerize an HLA-A2 pMHC linked to cancer-targeting scFvs (figure 1B). The pMHC presents a tethered virus peptide to target its cognate TCR and selectively stimulate virus-specific CTL. The scFv redirects virus-specific CTLs to eliminate cancer cells. As a proof-of-concept, we developed RediTac with a pMHC presenting a CMV-derived peptide, NLV, capable of expanding CMV-specific CTLs with potent cytotoxic activity. This pMHC was linked to a CD19-specific scFv capable of targeting malignant B cells.

Methods We first confirmed the functionality of the NLV-pMHC domain and CD19-specific scFv domain by demonstrating NLV-CD19 RediTac-mediated expansion of NLV-specific CTLs and selective binding to a CD19 on cells, respectively. The NLV-CD19 RediTac was then evaluated for its capacity to expand and redirect CMV-specific CTLs to eliminate NALM6, a CD19-expressing leukemia cell-line, in vitro and in vivo. To do this, donor PBMC with expanded NLV-specific CTLs were cocultured with NALM6 cells or coinjected with NALM6 cells into spleens of NSG mice. NLV-CD19 RediTac or control treatments were administered and NALM6 cell elimination was measured as a readout for functional activity.

Results NLV-CD19 was detected binding to the surface of CD19+ NALM6 cells (figure 2A,B). Twelve days after NLV-CD19 RediTac treatment, NLV-specific CTLs expanded by >40-fold as compared to untreated controls (figure 3A). After expansion, treatment with fresh NLV-CD19 RediTac induced IFN\(\gamma\) secretion by these expanded CTLs (figure 3B). Expanded CTLs eliminated ~60% of NALM6 cells when treated with NLV-CD19 RediTac during a two-day coculture, but no significant NALM6 cell elimination was observed when treated with control RediTac, either without a targeting scFv or with a pMHC linked to an irrelevant peptide (figure 4A). Five days after expanded CTLs and NALM6 cells were coinjected into NSG mice and NLV-CD19 was administered intravenously, the NALM6 population in the spleens was reduced by >99% when compared to a vehicle control treatment or a no-effector-cell control (figure 4B).

Conclusions NLV-CD19 RediTac expands CMV-specific CTLs and redirects their cytotoxic activity to eliminate NALM6 leukemia cells. We can use the RediTac modular design to expand and redirect other virus-specific T cells and target other cancers with scFvs. These results support RediTac-mediated virus-specific CTL expansion and redirection as a novel immunotherapy strategy to eliminate cancer cells and an effective platform for future treatments.

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REFERENCE

Ethics Approval All human PBMC samples and animal work was done with ethics approval by the Albert Einstein College of Medicine. All participants gave informed consent. This study was approved by the Albert Einstein College of Medicine institution’s Ethics Board; approval number 2017-8116. Animal use was approved by the AECOM Institute of Animal Studies animal use protocol number 00001105.

Abstract 1170 Figure 1 RediTac is designed to redirect antigen-specific CTL activity against labeled cancer cells. (A) Graphic depicting the designed function of RediTac. RediTac binds cancer-associated antigen via an antibody-derived single chain variable fragment (scFv), which coats the cancer cell in bivalent peptide-major histocompatibility complex (pMHC) modules. These modules trigger antigen-specific T cell receptor (TCR) activation and facilitate redirection of cytotoxic activity against the cancer cell and proliferation of the effector cell. (B) Components of the complete RediTac dimer.
Abstract 1170 Figure 2 NLV-CD19 RediTac can bind CD19 on the surface of NALM6 leukemia cells. (A) Methodology for the detection of scFv-mediated RediTac binding to the surface of antigen-expressing cells (B) CD19+ NALM6 cells or CD19- Jurkat cells were incubated with 80nM of either NLV-CD19 RediTac or NLV-gp120 RediTac with an scFv targeting an irrelevant antigen. Following an incubation with a fluorochrome-conjugated anti-His-Tag secondary antibody, RediTac binding was detected using flow cytometry. Representative dot plots depict one of four replicates from two independent experiments using two different batches of NLV-CD19 RediTac.

Abstract 1170 Figure 3 NLV-CD19 RediTac expands CMV-specific CTLs and activates secretion of IFNy. (A) Donor-derived PBMC with memory for CMV were treated with 1nM NLV-CD19 RediTac or left untreated. Cells were collected and stained before treatment (Day 0) and on days 7 or 12 following treatment. Selective expansion was determined as an increase in the percentage of live CD8+ T cells that were NLV-tetramer+. Data represents mean of N=4 different donor samples depicted as unique shapes. Two-way ANOVA and Sidak’s multiple comparison test was performed. ** = p>0.01. (B) On day 14 following NLV-CD19 RediTac treatment, PBMC with expanded NLV-specific CTLs were treated with 5nM fresh NLV-CD19 RediTac or left untreated in the presence or absence of CD19+ NALM6 cells. IFNy ELISpot was performed two days following secondary treatment of PBMC. Data represents mean and SD of n=3 technical replicates using PBMC from a single donor. One-way ANOVA with Tukey’s multiple comparisons test performed. *** = p> 0.001, **** = p> 0.0001.

Abstract 1170 Figure 4 NLV-CD19 RediTac redirects expanded NLV-specific CTLs to eliminate NALM6 leukemia cells. (A) PBMC with NLV-specific CTLs that were expanded for seven days were cocultured with PKH26 membrane dyed NALM6 cells at a 1:1 Effector:Target (E:T) ratio and were treated with 1 nM fresh NLV-CD19 RediTac, 1nM SL9-CD19 RediTac with an irrelevant pMHC, 1nM RediTac without a targeting scFV (NLV-no scFv RediTac), or nothing. Flow cytometry was performed after two days to detect live PKH26+ NALM6 cell count per well. Data depicts mean of n=3 technical replicates using PBMC from a single donor. One-way ANOVA with Dunnett’s multiple comparisons test performed. ** = p> 0.01. (B) PBMC with NLV-specific CTLs that were expanded for twelve days were coinjected at a 1:1 E:T ratio with CD24+ CD19+ NALM6 cells into the spleen of immunocompromised NSG mice. Some mice received no effector PBMC (0:1 E:T). Mice received a 1 mg/kg retro-orbital injection of NLV-CD19 RediTac or a PBS vehicle control. Spleens were harvested and processed and splenocytes were stained for human CD3, CD24, and CD19 to detect NALM6 cells by flow cytometry. Data is a representative dot plot for n=2 mice gated on CD3- cells, with the mean count of NALM6 cells depicted.

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