Engineered TCR-redirected Clearance of Gag-positive Reservoir Cells from ART-treated Subjects

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Abstract

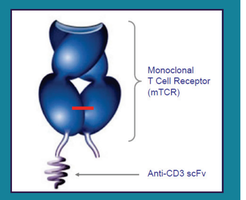
BACKGROUND: Combination ART is unable to eradicate infection due to rapid establishment of a reservoir of long-lived latently infected CD4+ T cells. Immunotherapeutic strategies that target viral reservoirs are needed to achieve a functional cure. Immune-mobilising monoclonal T cell receptors have been used successfully to kill cancer cells without the need for ex vivo cellular manipulation. We report on the antiviral efficacy of TCRs that re-direct CD8+ T cells to kill HIV-infected CD4+ T cells (termed ImmTACs).

METHODS: TCRs specific for the immunodominant HIV gag epitope, SLYNTVATL (SL9), and its common escape variants were engineered to achieve picomolar affinity and fused to a humanised CD3-specific single chain antibody fragment (scFv). The potency of these ImmTACs against CD4+ T cells from 10 HLA-A*0201+ patients on ART for >1 year was assessed using CD8+ T cell viral inhibition and killing assays.

RESULTS: Replication of endogenous HIV in CD4+ T cells was significantly reduced by ImmTAC-mediated re-direction of ex vivo autologous CD8+ T cells at CD8+/CD4+ cell ratios of 1:1 and 1:10 (mean, SD: 72%, 14%, p = 0.008; 55%, 15%, p = 0.004 respectively) and at nanomolar ImmTAC concentrations (10^{-8} – 10^{-11} M). More profound inhibition (mean, SD: 85%, 8%, p < 0.0001) was achieved using CD8+ T cells from healthy donors, implying a persistent global functional defect in autologous CD8+ T cells despite long-term ART. No significant effect on HIV replication was observed with CD8+ T cells alone or with an irrelevant TCR. ImmTAC-mediated killing was indicated by specific upregulation of caspase-3 in infected CD4+ T cells and by a lack of viral recrudescence after removal of the ImmTAC from cell cultures. Of note, ImmTACs enabled rapid and efficient killing of resting as well as activated CD4+ T cells and their potency was highly correlated with the level of intracellular HIV gag expression (r^2 = 0.48, p <0.0001). These data demonstrate that resting CD4+ T cells from patients were susceptible to immune killing. ImmTAC potency was dependent on CD8+ T cell phenotype, with effector memory (CCR7-/CD45RA- and CCR7-/CD45RA+) subsets showing the greatest antiviral efficacy.

CONCLUSIONS: ImmTACs can re-direct CD8+ T cells to kill primary CD4+ T cells carrying diverse HIV isolates, even when in the resting state. Their potency at low effector/target cell ratios and low epitope densities, coupled with the capacity to harness a large number of effector cells simultaneously, addresses major limitations in adaptive immunity to HIV. ImmTACs have the potential to accelerate clearance of HIV reservoirs.

ImmTAC Technology



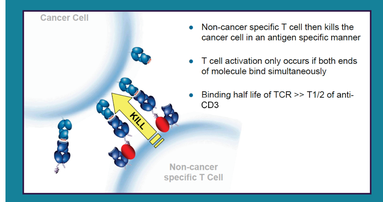
Key features of ImmTAC technology (immune-mobilising monoclonal TCR against cancer)

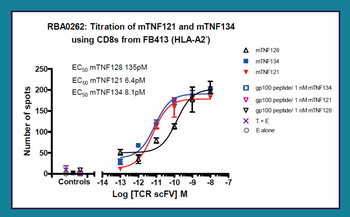
• Engineered soluble high affinity monoclonal T cell receptors against cancer and viral antigens – in vivo administration of reagents does not require cellular manipulation ex vivo

• Bi-specific reagents: TCR provides antigen specificity; scFv specific for CD3 provides effector function

ImmTAC Production:

1. Create stable soluble TCRs
2. Affinity enhancements: modify CDRs by phage display
3. Effector capability: attach CD3-specific scFv to β chain – enables recruitment of entire effector repertoire





Biophysical properties of HIV ImmTACs:

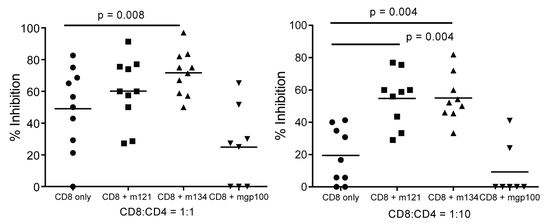
• ImmTACs with affinity for HLA-A*02-restricted HIV-1 gag p17 epitope

• ImmTAC m121 and m134 showed potent activity against peptide-pulsed targets

EC_{50} (m121) = 6.4 pM

EC_{50} (m134) = 8.1 pM

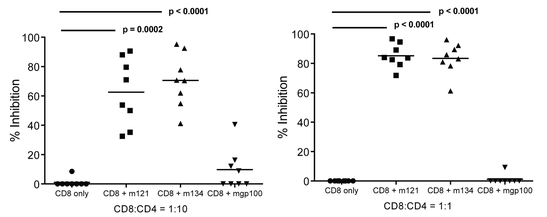
RESULTS 1: ImmTACs enhance control of autologous HIV replication by CD8+ T cells from chronically infected ART-treated subjects



10 HLA-A*0201+ chronically infected patients; 8 on ART > 1 year - VL < 50 copies, CD4+ > 350 cells/µl.

Purified CD4+ T cells activated by PHA then cultured with autologous CD8+ T cells +/- ImmTACs (HIV or irrelevant TCR mgp100) for 7 days. Viral inhibition analysed by intra-cellular p24 staining.

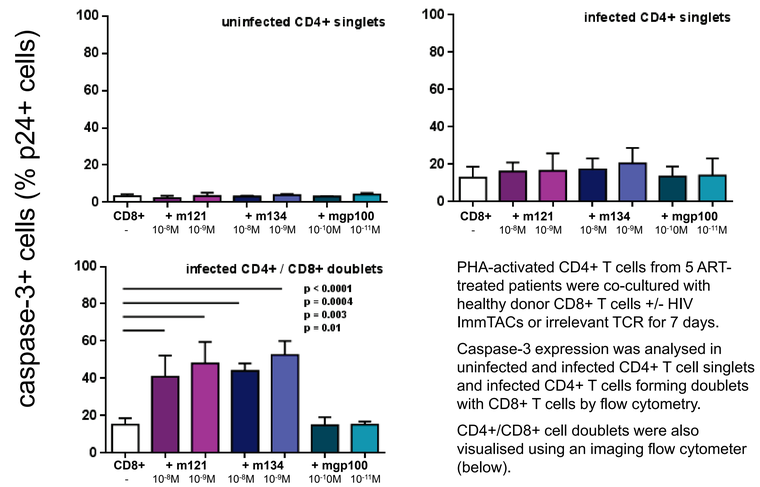
RESULTS 2: ImmTAC-redirected CD8+ T cells from healthy donors potently suppress autologous HIV replication in CD4+ T cells from HIV-positive subjects



CD4+ T cells from 8 HLA-A*0201+ ART-treated patients activated with PHA, then co-cultured with CD8+ T cells from a healthy donor +/- HIV ImmTACs or irrelevant TCR for 7 days.

Viral inhibition analysed by intracellular p24 staining.

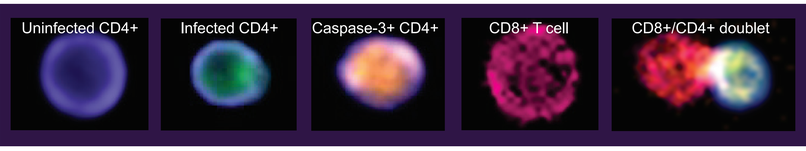
RESULTS 3: Antiviral efficacy of ImmTAC-redirected CD8+ T cells is dependent on rapid killing of infected cells

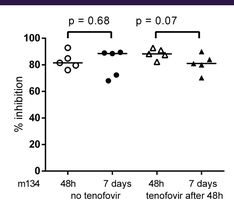


PHA-activated CD4+ T cells from 5 ART-treated patients were co-cultured with healthy donor CD8+ T cells +/- HIV ImmTACs or irrelevant TCR for 7 days.

Caspase-3 expression was analysed in uninfected and infected CD4+ T cell singlets and infected CD4+ T cells forming doublets with CD8+ T cells by flow cytometry.

CD4+/CD8+ cell doublets were also visualised using an imaging flow cytometer (below).

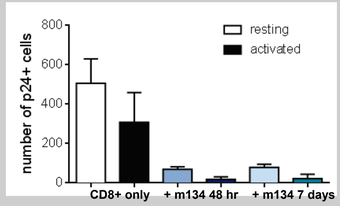


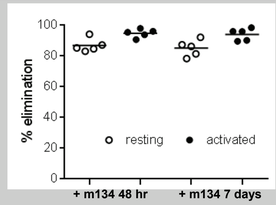


We confirmed that ImmTACs were able to mediate killing of infected CD4+ T cells by washing them out of the co-culture after 48 hours. There was no increase in viral replication after removal of ImmTACs.

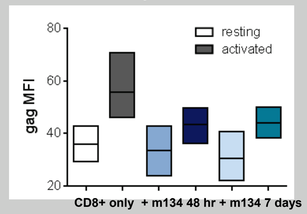
PHA-activated CD4+ T cells from 5 ART-treated patients were co-cultured with healthy donor CD8+ T cells and ImmTAC (m134) for 48 hours or 7 days. Infected cells were quantified by intracellular staining after 7 days' culture.

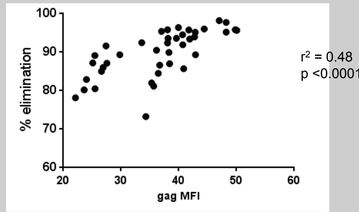
RESULTS 4: ImmTACs mediate highly efficient killing of resting gag-positive CD4+ T cells despite lower epitope expression than activated cells





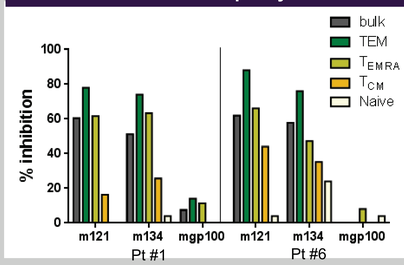
Same results when tenofovir added at 48hr





PHA-activated CD4+ T cells from 5 ART-treated patients co-cultured with healthy donor CD8+ T cells plus ImmTACs for 48 hours or 7 days. After 7 days' culture, residual infected resting (CD25-/CD69-/ HLA-DR-) and activated (CD25+/CD69+/HLA-DR+) CD4+ T cells were quantified. Bottom panels indicate that susceptibility to ImmTAC killing was dependent on the level of gag expression, irrespective of the activation state of the cell.

RESULTS 5: CD8+ T cell effector memory subsets show the most potent antiviral capacity when redirected by HIV ImmTACs



PHA-activated CD4+ T cells from 2 patients cultured with sorted healthy donor CD8+ T cells plus HIV ImmTACs or irrelevant TCR (mgp100):

• CCR7-/CD45RA-

• CCR7-/CD45RA+

• CCR7+/CD45RA-

• CCR7+/CD45RA+

• CD8+/CD4+ cell ratio = 1:2

Methods

HIV infection: Exogenous HIV infection was achieved by stimulating CD8-depleted PBMC (CD4+ T cells) with PHA (5 µg/ml) in medium ('R10') for three days. The cells were then washed and infected with HIV IIIB ($1x10^{-2}$) by spinoculation for two hours. Endogenous HIV infection in PBMC from HIV+ subjects was achieved by reactivation of CD4+ T cells with PHA (5 µg/ml) in R10 medium for three days.

Viral inhibition assay: HIV-infected CD4+ T cells ($5x10^4$ cells/well) were cultured in R10 with IL-2 (20 IU/ml) either alone or with CD8+ T cells with or without ImmTACs. In all experiments an irrelevant TCR, mgp100, was used as a negative control. After 7 days the cells were stained for intracellular p24 Ag to determine the frequency of infected cells. Viral inhibition was calculated using the formula:

[(fraction of p24+ cells in CD4+ T cells cultured with CD8+ T cells alone) – (fraction of p24+ in CD4+ T cells cultured with CD8+ T cells plus ImmTAC)]/[fraction of p24+ cells in CD4+ T cells cultured with CD8+ T cells alone] x 100

Conclusions

• Engineered bi-specific immune-mobilising TCRs targeting a single HIV gag epitope can redirect ex vivo CD8+ T cells from chronically infected ART-treated subjects to kill autologous infected CD4+ T cells.

• Healthy donor CD8+ T cells are more effective than autologous CD8+ T cells, suggesting that global defects in CD8+ T cell function persist despite ART.

• HIV ImmTACs eliminated majority of activated and resting CD4+ T cells and were potent at low CD8+/CD4+ cell ratios and low epitope density.

In vitro efficacy at 10^{-9} M concentration - which would be suitable for in vivo use

ImmTACs can overcome major limitations faced by adaptive immune response: antigenic variability, low epitope expression, time

