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Anti-HIV CAR+ lymphocytes protected from HIV-infection by CCR5 disruption as a strategy to cure HIV

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Background: A cure for HIV remains an important treatment goal. A previous Phase II randomized clinical trial of anti-HIV chimeric antigen receptor (CAR)-expressing T-cells was partially effective. We hypothesize that a limitation of that strategy was that the anti-HIV CAR+ lymphocytes were susceptible to HIV infection. We sought to produce and test anti-HIV CAR expressing lymphocytes that are protected from HIV infection.

Methods: We designed novel anti-HIV CARs based on the scFV of a series of broadly neutralizing HIV antibodies. A CCR5 megaTAL nuclease (an engineered homing endonuclease and TALEN chimera) was used to disrupt CCR5 as a means of protecting lymphocytes from HIV infection. CAR+ lymphocytes were mixed with HIV-infected and uninfected cell lines (ACH-2 and A3.01) in the presence of ART (AZT/3TC/NVP) or added to active HIV viral culture (JR-CSF). The reduction in the number of HIV infected cells was assessed by flow cytometry and PCR. The reduction in replicating HIV was quantified by HIV capsid protein ELISA. Results were compared to the effect of non-specific (anti-CD19) CAR+ lymphocytes, and to the effect of anti-HIV CAR+ lymphocytes on HIV-uninfected cells.

Results: Depending on the production methods, we achieved 40-60% disruption of the CCR5 gene in CAR+ lymphocytes. Anti-HIV CAR+ lymphocytes appropriately upregulated cell surface CD137 and secreted IFN-γ when mixed with HIV-infected target cells, killed a median of 75% (range 38-94%) of HIV-infected cells over 2-3 days, and reduced HIV DNA by approximately one log over 5 days. Beyond 72 hours of culture the anti-HIV CAR+ lymphocytes with CCR5 disruption resulted in greater reduction in HIV than CAR+ lymphocytes without CCR5 disruption.

Conclusions: This is one of the few therapeutic strategies shown to kill HIV-infected cells in the absence of viral replication. It demonstrates that it is feasible to construct anti-HIV CAR+ T-cells that are protected from HIV infection. This strategy warrants further study using in vivo models of HIV latency.