Development of Druggable ON-switch anti-PD-1 CAR T cells for repeated SIV/HIV reservoir depletion

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BACKGROUND

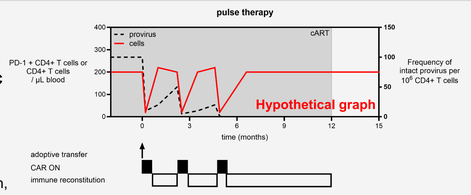
The immune checkpoint marker programmed cell death protein 1 (PD-1) is commonly expressed on memory T cells and enriched in latently HIV-infected CD4+ T cells, including T follicular helper (TFH) cells. Chimeric antigen receptor (CAR) T cells are a cell-based technology that depletes cells expressing a target antigen. To determine whether CAR T cells can deplete reservoir-harboring, PD-1 expressing cells during ART, we previously tested anti–PD-1 CAR T cells in SIV-infected, ART-treated rhesus macaques. These CAR T cells expanded in vivo, depleted TFH cells, and reduced viral replication in germinal centers after interruption of ART. However, long-term CAR T cell persistence led to increased SIV replication in extrafollicular portions of lymph nodes and a loss of immunocompetence potentially due to CD8+ memory T cell depletion. These findings highlight the utility of CAR T cells in studying the SIV/HIV reservoir in this model to inform HIV cure interventions.

METHODS

To enable repeated, temporary depletion of PD-1+ cells, we engineered a novel CAR using a Hepatitis C virus protease NS3-based ON switch (anti-PD-1 CAR NS3), allowing control of cytotoxic activity and cytokine secretion via administration of an NS3 protease inhibitor (grazoprevir; GZV) in vitro. This system enables investigation of “pulse therapy,” a strategy involving repeated reservoir-targeting cycles alternating with CAR T cell inactivity to promote immune reconstitution.

Pulse therapy – repeated reservoir depletion during ART

Background: The immune system can reconstitute after an insult, a characteristic that can be leveraged for pulse therapy.
Homeostatic proliferation can replenish the T cell compartment after lymphodepletion (e.g. Cytoxan, viral infection).



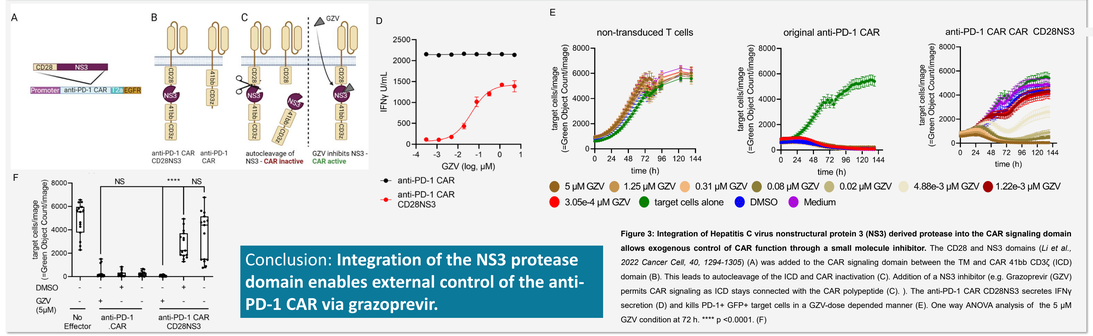
The idea: **Repeated, controlled depletion of CD4+ T-cell subsets using a drug-controlled CAR may reduce the reservoir during ART and allow intermittent phases of immune recovery.**

PLAIN LANGUAGE SUMMARY

We developed a “remote-controlled” living drug made from engineered immune cells to deplete HIV reservoirs during ART. In an animal model, this approach showed signs of working. However, we still need to improve both the drug that provides the remote control and how the engineered cells respond to it for this strategy to work.

A drug-inducible ON-switch CAR T cell designed for repeated HIV reservoir depletion expanded temporarily in a pilot adoptive-transfer study. Additional PK studies and CAR construct optimization are warranted.

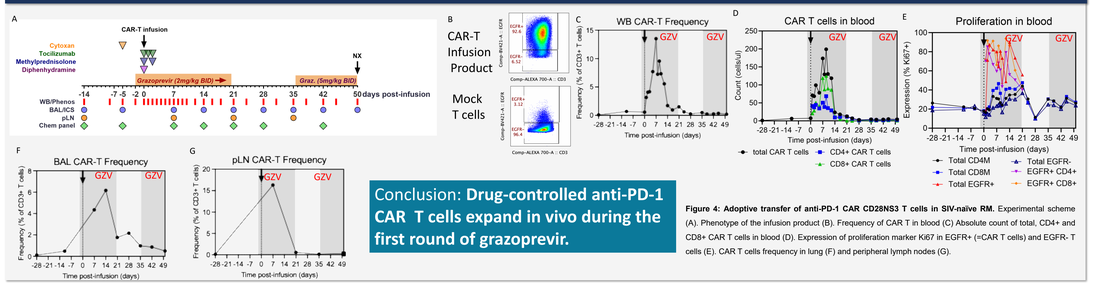
A remote-controlled CAR: Integration of a drug-controlled NS3 protease ON switch into the anti-PD-1 CAR signaling domain



Conclusion: **Integration of the NS3 protease domain enables external control of the anti-PD-1 CAR via grazoprevir.**

Figure 3: Integration of Hepatitis C virus nonstructural protein 3 (NS3) derived protease into the CAR signaling domain allows exogenous control of CAR function through a small molecule inhibitor. The CD28 and NS3 domains (*Li et al., 2022 Cancer Cell, 40, 1294-1305*) (A) was added to the CAR signaling domain between the TM and CAR 41bb CD3ζ (ICD) domain (B). This leads to autocleavage of the ICD and CAR inactivation (C). Addition of a NS3 inhibitor (e.g. Grazoprevir (GZV) permits CAR signaling as ICD stays connected with the CAR polypeptide (C).). The anti-PD-1 CAR CD28NS3 secretes IFNγ secretion (D) and kills PD-1+ GFP+ target cells in a GZV-dose depended manner (E). One way ANOVA analysis of the 5 µM GZV condition at 72 h. **** p <0.0001. (F)

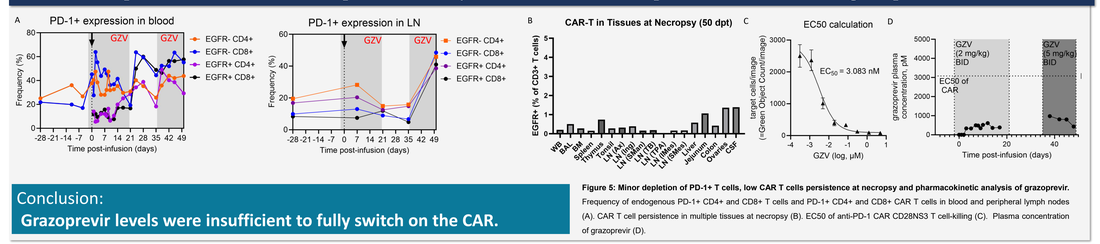
Drug-controlled anti-PD-1 CAR CD28NS3 T cells expand in presence of grazoprevir in blood and tissue in vivo in a rhesus macaque



Conclusion: **Drug-controlled anti-PD-1 CAR T cells expand in vivo during the first round of grazoprevir.**

Figure 4: Adoptive transfer of anti-PD-1 CAR CD28NS3 T cells in SIV-naïve RM. Experimental scheme (A). Phenotype of the infusion product (B). Frequency of CAR T in blood (C) Absolute count of total, CD4+ and CD8+ CAR T cells in blood (D). Expression of proliferation marker Ki67 in EGFR+ (=CAR T cells) and EGFR- T cells (E). CAR T cells frequency in lung (F) and peripheral lymph nodes (G).

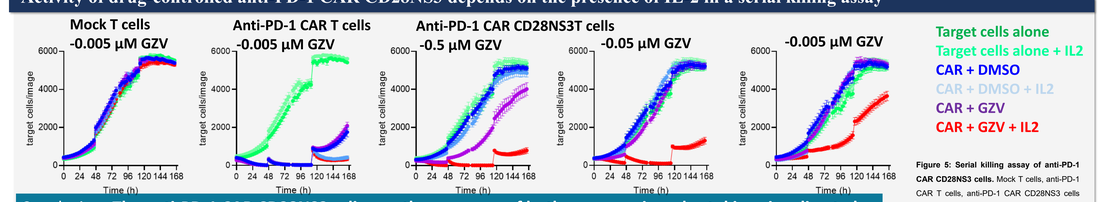
Low PD-1 depletion and low CAR T cell persistence may be associated with low plasma concentration of grazoprevir



Conclusion:
Grazoprevir levels were insufficient to fully switch on the CAR.

Figure 5: Minor depletion of PD-1+ T cells, low CAR T cells persistence at necropsy and pharmacokinetic analysis of grazoprevir. Frequency of endogenous PD-1+ CD4+ and CD8+ T cells and PD-1+ CD4+ and CD8+ CAR T cells in blood and peripheral lymph nodes (A). CAR T cell persistence in multiple tissues at necropsy (B). EC50 of anti-PD-1 CAR CD28NS3 T cell-killing (C). Plasma concentration of grazoprevir (D).

Activity of drug-controlled anti-PD-1 CAR CD28NS3 depends on the presence of IL-2 in a serial killing assay



Conclusion: **The anti-PD-1 CAR CD28NS3 relies on the presence of both grazoprevir and cytokine signaling to be fully functional – redesigning the intracellular domain is ongoing.**

Figure 5: Serial killing assay of anti-PD-1 CAR CD28NS3 cells. Mock T cells, anti-PD-1 CAR T cells, anti-PD-1 CAR CD28NS3 cells were cultured in presence of absence of IL-2 and various concentrations of grazoprevir (0.005 – 0.5 µM).

RESULTS

We tested pulse therapy in an adoptive transfer pilot study using a SIV-naïve rhesus macaque that received anti-PD-1 CAR NS3 T cells and two GZV courses, separated by a 2-week washout phase. During the first GZV course, anti-PD-1 CAR NS3 T cells expanded in vivo, without a noticeable decrease in PD-1+ cell frequency in peripheral blood. CAR T cells proliferated vigorously (up to 80% Ki67+ by day 14) and trafficked to lymphoid tissues and lungs. However, during the second GZV course, CAR T cells did not re-expand and remained at low frequency. Pharmacokinetic analyses of GZV and further studies on CAR function indicated that the GZV drug levels were insufficient to achieve complete CAR T cells functionality and that anti-PD-1 CAR NS3 T cells are more reliant on exogenous interleukin-2 than the original CAR.

CONCLUSIONS

- A drug controlled anti-PD-1 CAR T cell can be controlled by NS3 inhibitor grazoprevir in vitro
- In an adoptive transfer pilot experiment testing the concept of pulse therapy, drug controlled anti-PD-1 CAR T cells expanded only during the first bout of grazoprevir; with no apparent depletion of PD-1+ T cells.
- Drug levels were to low in vivo and we discovered that this CAR is also highly reliant on the presence of IL-2.
- PK studies to improve grazoprevir levels and reengineering of the drug-controlled CAR are underway

These data suggest that pharmacological control of anti-PD-1 CAR NS3 T cells is achievable; however, further refinement is required to optimize pulse therapy for effectively studying SIV/HIV reservoir dynamics during ART.

ADDITIONAL KEY INFORMATION

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