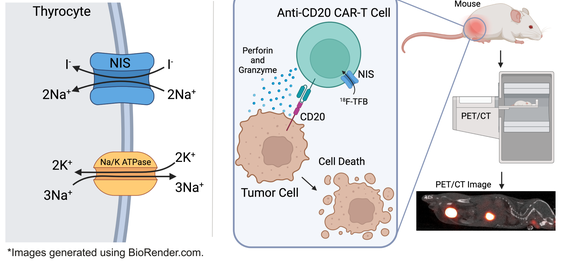
Using the Sodium Iodide Symporter to Visualize CAR-T Cell Trafficking

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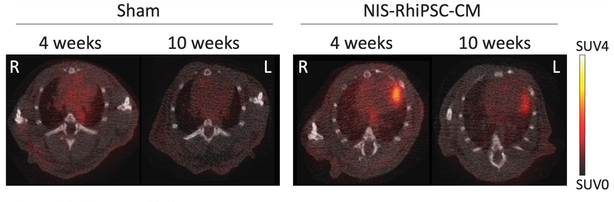
Background

- Chimeric antigen receptor (CAR) T cells are genetically modified T cells that harness the antigen-specificity of antibodies and the cytotoxic potential of T cells.
- Tracking CAR-T cells in patients is currently limited to peripheral blood sampling and does not provide information on CAR-T cell trafficking, expansion, and persistence.
- The role of costimulatory endodomains, with demonstrated profound influence on T cell phenotype, efficacy, and persistence, remains unclear with regards to trafficking.
- The Sodium Iodide Symporter (NIS) is a membrane glycoprotein that actively uptakes iodide and its analogs, and it can be transduced into various cell types in preclinical models allowing sensitive and specific PET/SPECT imaging following administration of a relevant tracer.



*Images generated using BioRender.com.

- Our group previously published work demonstrating the feasibility of using NIS as a sensitive reporter for induced pluripotent stem cell (iPSC) derived cardiomyocytes (CM) in NSG mice.[1]



*Images included courtesy of S. Hong.

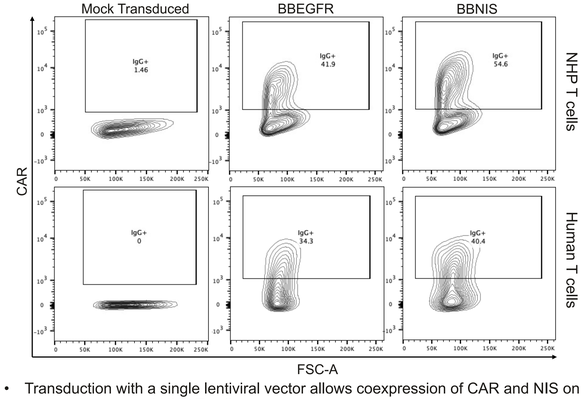
Materials & Methods

CAR Construct Maps

CD20 scFv	IgG4-hinge-CH_2mut-CH3	CD28 TM	4-1BB Costim	CD3ζ	T2A	EGFRt
CD20 scFv	IgG4-hinge-CH_2mut-CH3	CD28 TM	4-1BB Costim	CD3ζ	T2A	rhNIS
CD20 scFv	IgG4-hinge-CH_2mut-CH3	CD28 TM	CD28 Costim	CD3ζ	T2A	rhNIS
CD20 scFv	IgG4-hinge-CH_2mut-CH3	CD28 TM	CD27 Costim	CD3ζ	T2A	rhNIS

- *Lentiviral vector construction*: Golden Gate Assembly was used to generate various CAR constructs from the second generation anti-CD20 CAR previously used in NHPs to study cytokine release and neurotoxicity.[2]
- *T cell transduction*: T cells from healthy human or NHP donors were transduced with lentiviral vectors using spinoculation on days 2 and 3 of culture with target MOI of 2-3. Cells were cultured with 200IU/mL IL-2. Transduction efficiency was determined on day 5-6 by flow cytometry. Cells were sorted for CAR+ IgG+ cells and expanded an additional 2-3 days prior to cryopreservation or experimental use.
- *Iodide uptake experiments*: CAR-transduced cells were cultured in the presence of iodide with or without $KClO_4$. Iodide uptake measured via absorbance or CPM.
- *Mice*: in vivo experiments used NSG mice. Cells were injected subcutaneously at various cell doses. 18F-TFB tracer was administered IV 10-15 minutes following cell injections and mice were imaged with PET/CT 1 hour later. Raji$^{Luc+GFP+}$ tumor bearing mice served as a control.

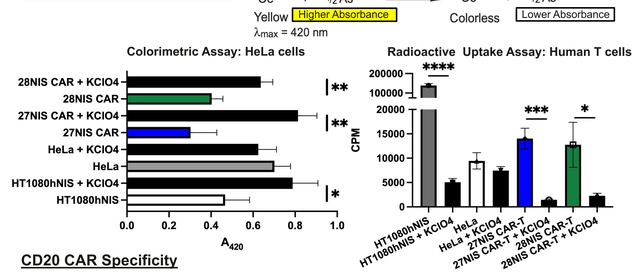
In Vitro Results



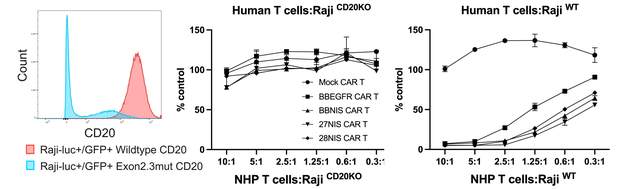
- Transduction with a single lentiviral vector allows coexpression of CAR and NIS on primary human and NHP T cells.

In Vitro Results

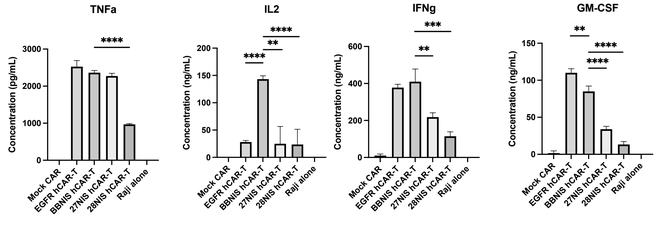
NIS-dependent Iodide Uptake



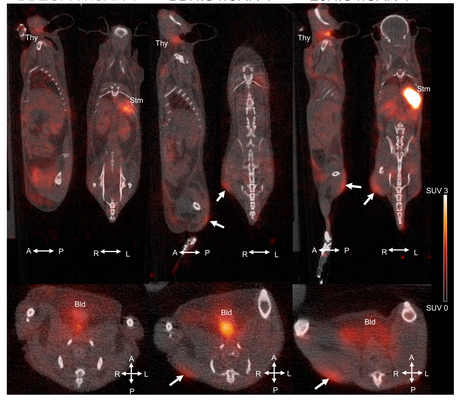
CD20 CAR Specificity



Cytokine Release



In Vivo Results

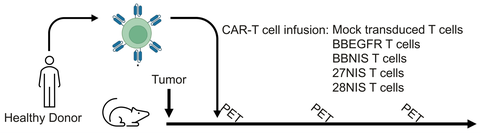


Thy = Thyroid, Stm = Stomach, Bld = Bladder; A = Anterior, P = Posterior.

- Subcutaneous CAR-T cells without NIS are not visualized by PET/CT after IV injection of ^{18}F-TFB.
- Background signal is detected in salivary glands, thyroid, stomach, and bladder, as expected.
- As few as 1x10^6 CAR-T cells expressing NIS can be visualized in mice following subcutaneous injection using PET/CT imaging.

Summary & Future Directions

- NIS is a nonimmunogenic reporter molecule that can be incorporated into various CAR-containing expression cassettes and transduced into human and non-human primate T cells.
- NIS-containing CAR-T cells demonstrate antigen-specific cytotoxicity, cytokine release, and iodide analog uptake *in vitro* and can be localized *in vivo* using PET/CT imaging.



References

1. Ostrominski, JW, et al. (2020). Stem Cells Transl Med **9**: 1203-1217.
2. Taraseviciute, A., et al. (2018). Cancer Discov **8**(6): 750-763.
3. Background images generated using BioRender.com.

Original anti-CD20 CAR lentiviral vector was generously provided by Dr. Leslie Kean (Dana Farber). Raji$^{Luc+GFP+}$ cells were generously provided by Dr. Kazusa Ishii (NCI/NIH).