CAR-T development in subtypes of prostate cancer: how mechanistic biology impacts future therapies

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Human Biology and Clinical Research Divisions
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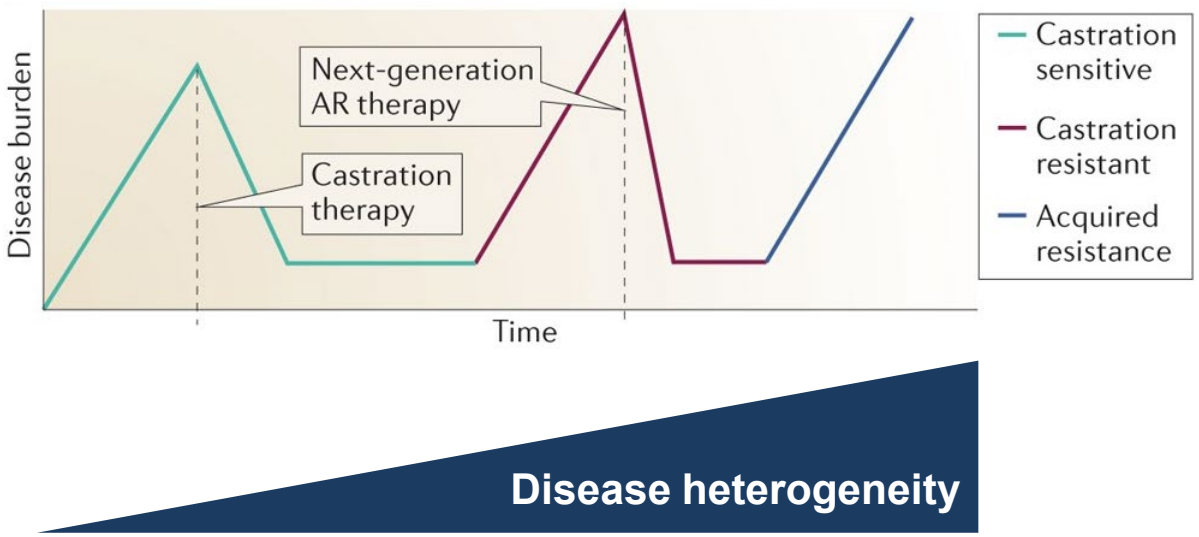
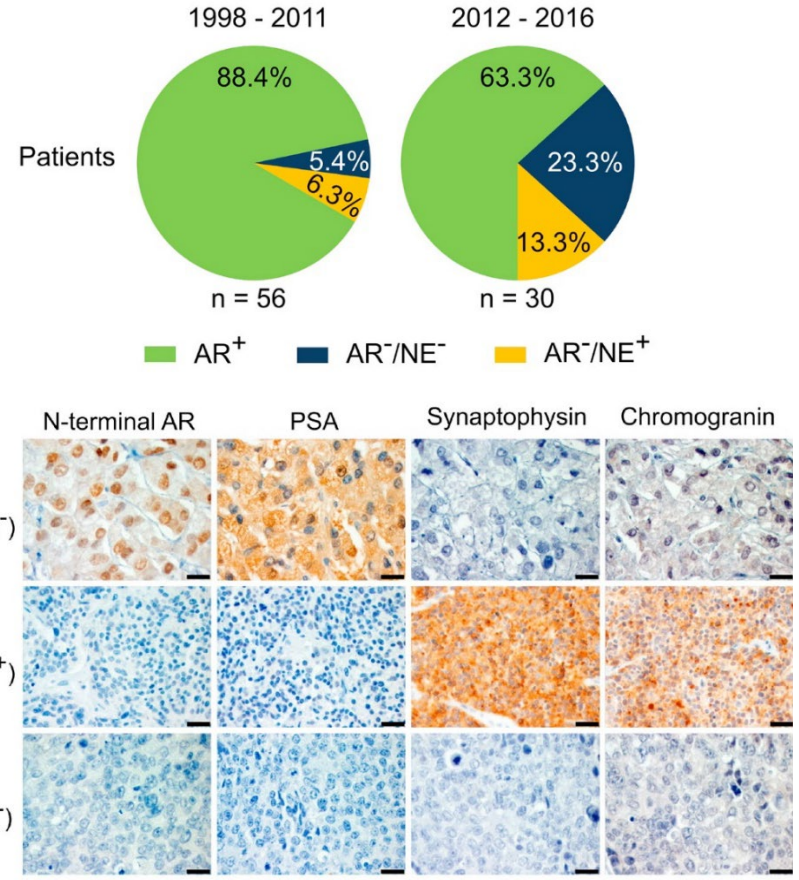
The modern, natural history of prostate cancer – evolving diversity

Increasing frequency of AR$^-$ lethal mCRPC after FDA approval of abiraterone and enzalutamide



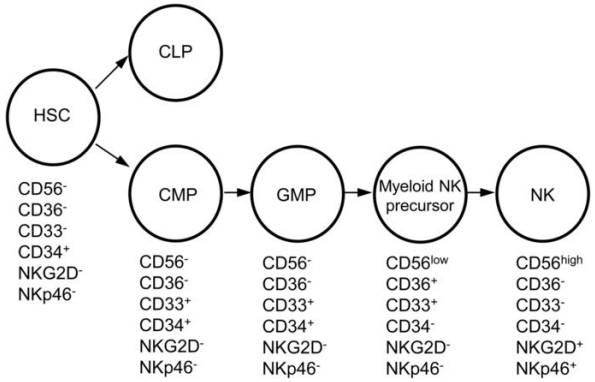
Adapted from Watson PA, Arora VK, Sawyers CL. *Nat Rev Cancer.* 2015.



Bluemn E, et al. *Cancer Cell.* 2017.

Cell surface phenotypes and molecular subtypes of cancer

- Cell surface phenotypes reflect specific differentiation states in normal development



Chen Q, et al. *Sci Rep.* 2015.

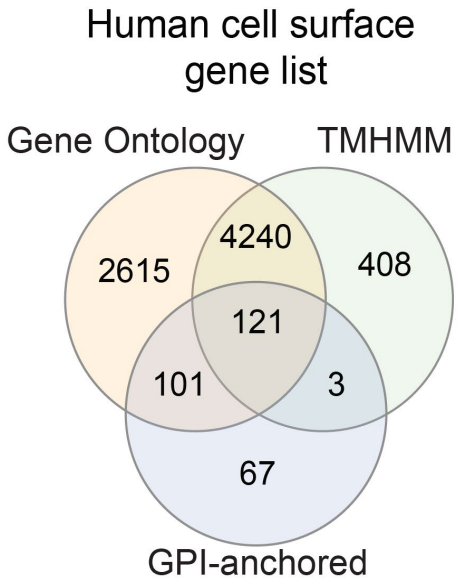
- Molecular subtypes of cancer have been defined by distinct differentiation or activation states
  - Breast cancer (Luminal A, Luminal B, Basal-like, etc.)
  - Diffuse large B-cell lymphoma (GCB, ABC)

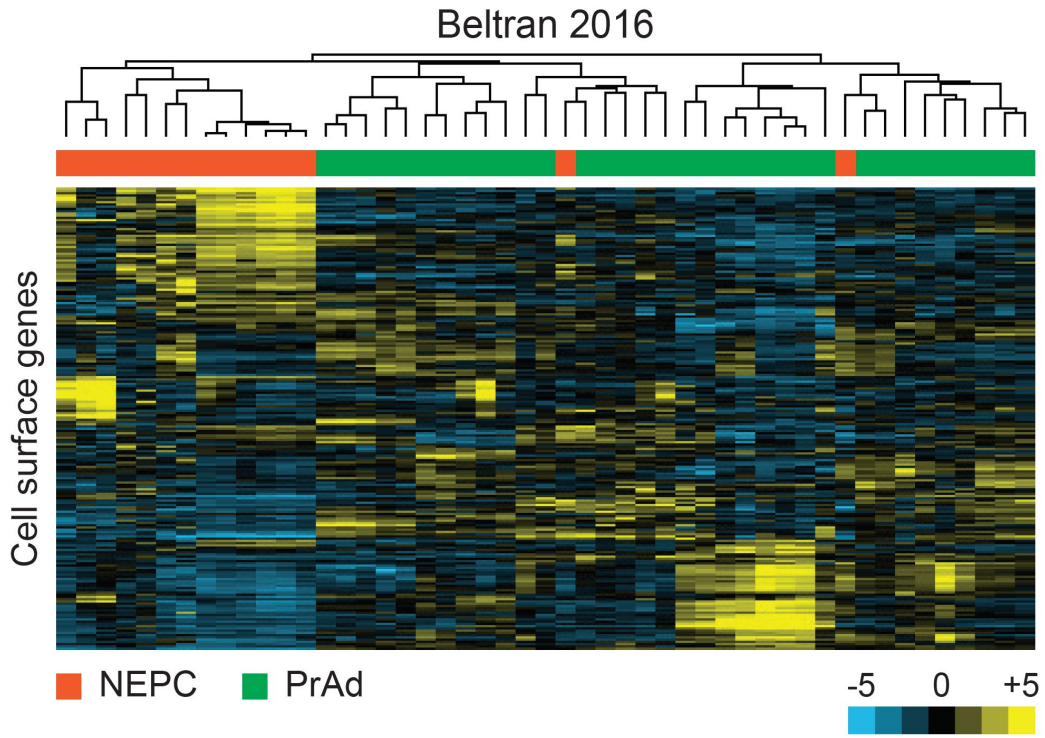
- Cell surface antigens are amenable to Ab-based therapies: ADCs, BiTEs, CAR-Ts

- *Are distinct cell surface markers expressed in subsets of CRPC?*

Cell surface phenotypes and molecular subtypes of CRPC

Unsupervised hierarchical clustering of a CRPC gene expression dataset based on the expression of a bioinformatically derived set of genes encoding cell surface proteins distinguishes subtypes of prostate cancer

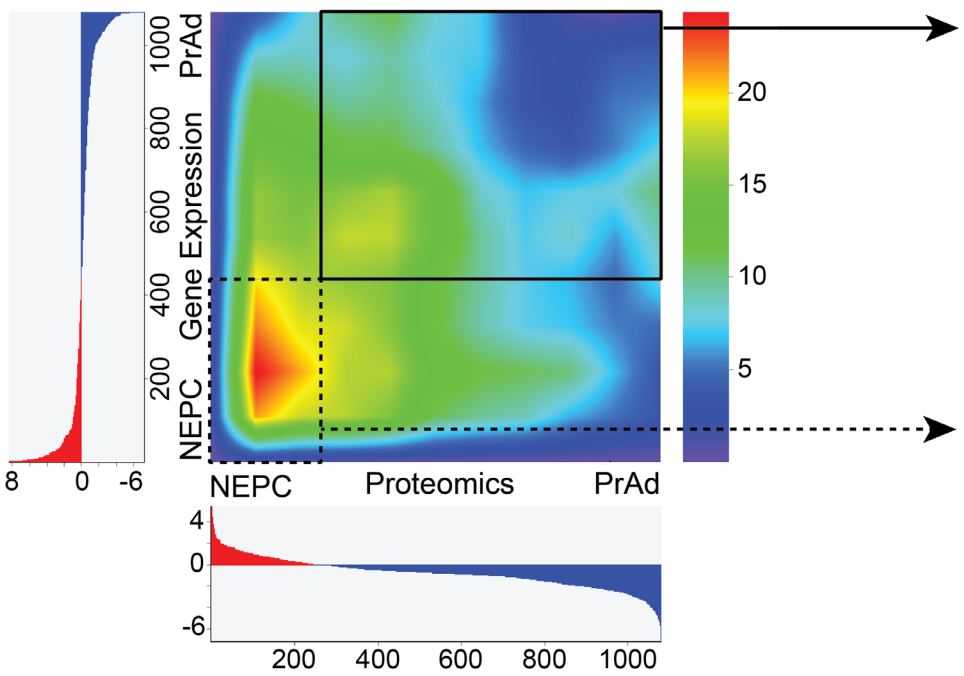




Beltran H, et al. *Nat Med.* 2016.
Lee JK, et al. *Proc Natl Acad Sci USA.* 2018.

Nominating cell surface antigens in prostate cancer subtypes

Integration of RNA-seq gene expression and cell surface proteomics of a diverse prostate cancer cell line panel by rank-rank hypergeometric overlap



AR^{+}/NE^{-} (PrAd)

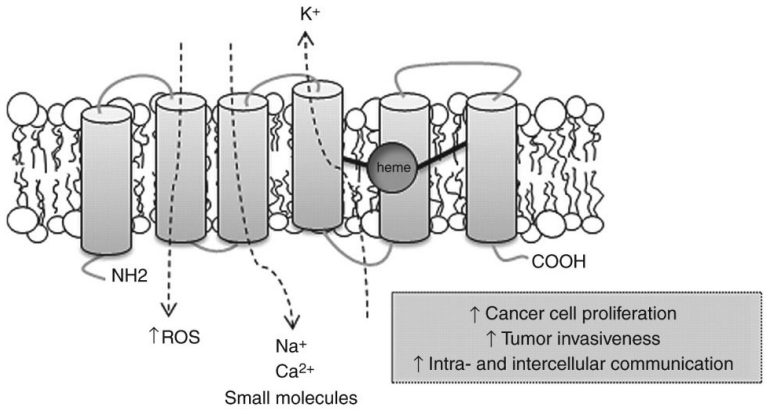
STEAP1
FOLH1 (PSMA)
STEAP2
TACSTD2 (Trop2)

AR^{-}/NE^{+} (NEPC)

CEACAM5
L1CAM
NCAM1
MUC1

Lee JK, et al. *Proc Natl Acad Sci USA.* 2018.

Six transmembrane epithelial antigen of the prostate 1 (STEAP1) in prostate cancer



Gomes IM, Maia CJ, Santos CR. *Mol Canc Res.* 2012.

Cryo-EM structure of STEAP1 as a homotrimer bound to Fab fragments of vandortuzumab

Oosterheert W and Graus P. *J Biol Chem.* 2020.

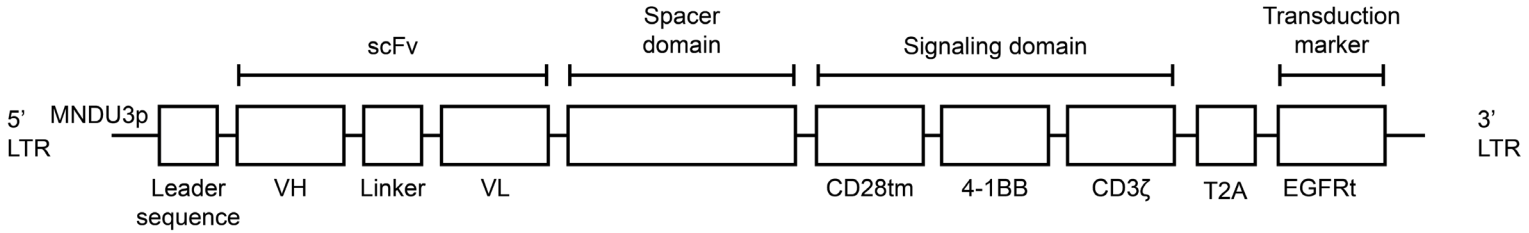
- STEAP proteins are metalloreductases
- STEAP proteins form a homo-/hetero-trimeric structure
- STEAP1 is expressed in >80% of mCRPCs and demonstrates limited expression in normal tissues except the prostate
- STEAP1 has been the target of therapeutic development for prostate cancer for many years:
  1. Vandortuzumab vedotin – ADC
  2. AMG 509 – bispecific T cell engager, under investigation in a phase I clinical trial for mCRPC

Engineering STEAP1 CAR-T cell therapy for prostate cancer

Nik Kamat, MD
Medical Oncology Fellow

Tiffany Pariva
Research Technician
MS1 at Loma Linda

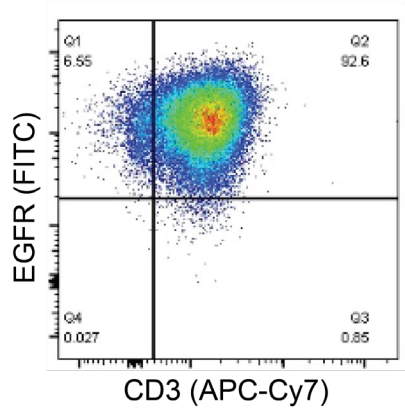
Second-generation CAR construct



Variants based on scFv and spacer length

Clone #	scFv	Hinge/Spacer
1	1	IgG4
2	1	IgG4 CH3
3	1	IgG4 CH2-CH3
4	2	IgG4
5	2	IgG4 CH3
6	2	IgG4 CH2-CH3
7	3	IgG4
8	3	IgG4 CH3
9	3	IgG4 CH2-CH3

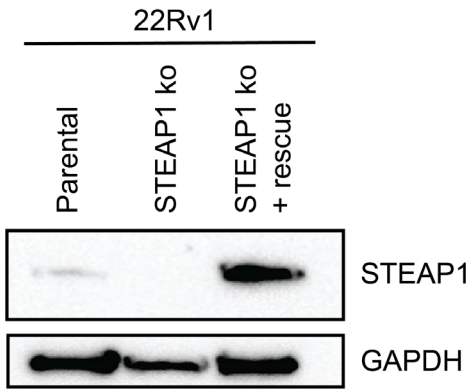
Transduction of human T cells



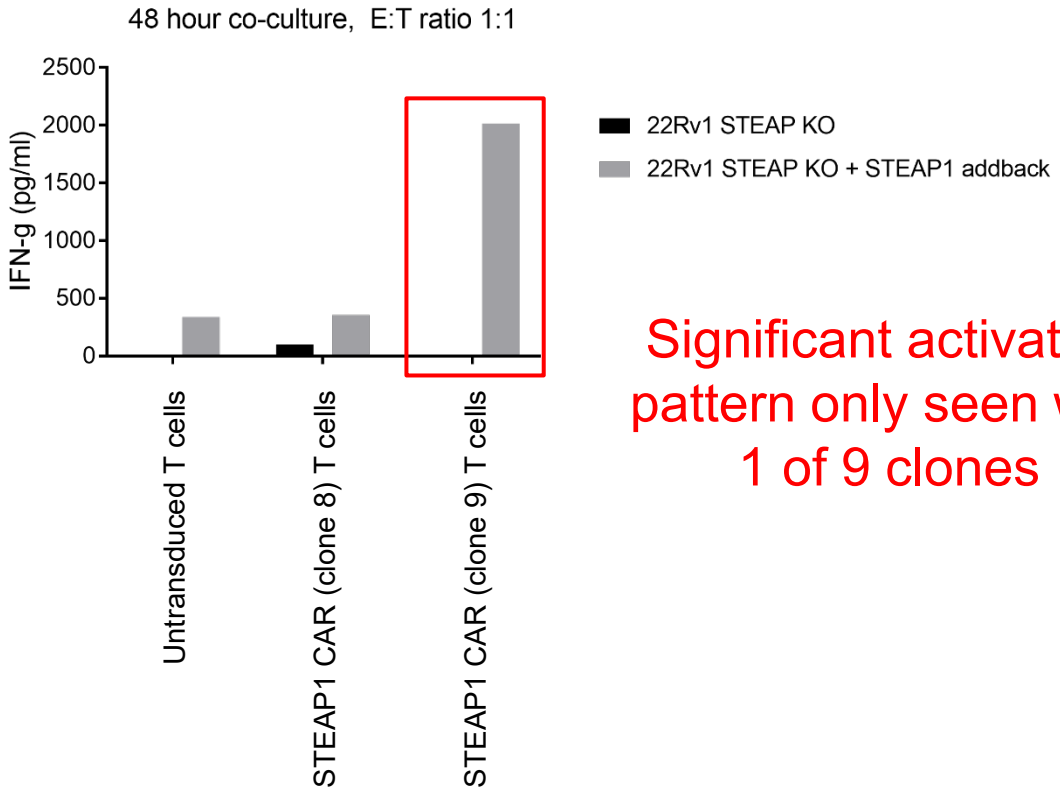
Kamat N, et al. *In preparation.* 2021.

Screening STEAP1 CAR clones for antigen-selective activation of modified T cells

Isogenic 22Rv1 cell lines with defined STEAP1 expression



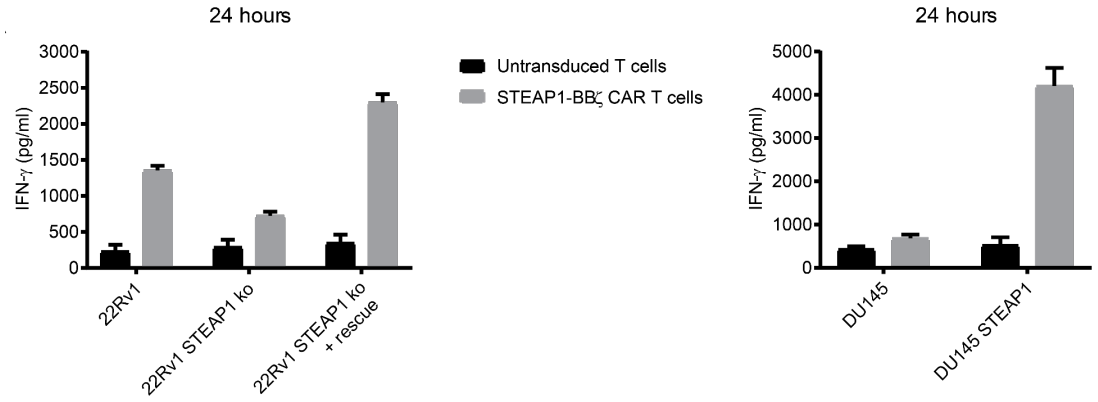
Co-cultures with STEAP1 CAR-T to evaluate for antigen-selective IFNγ release



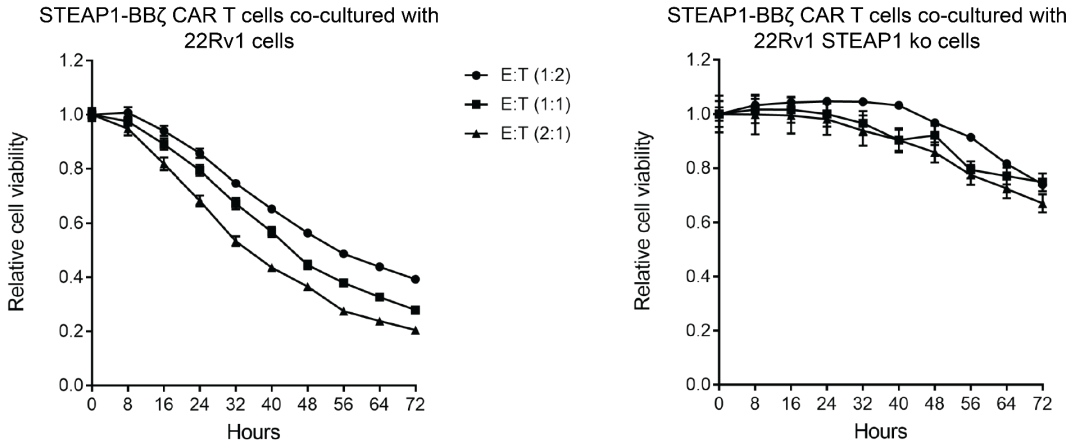
Significant activation pattern only seen with 1 of 9 clones

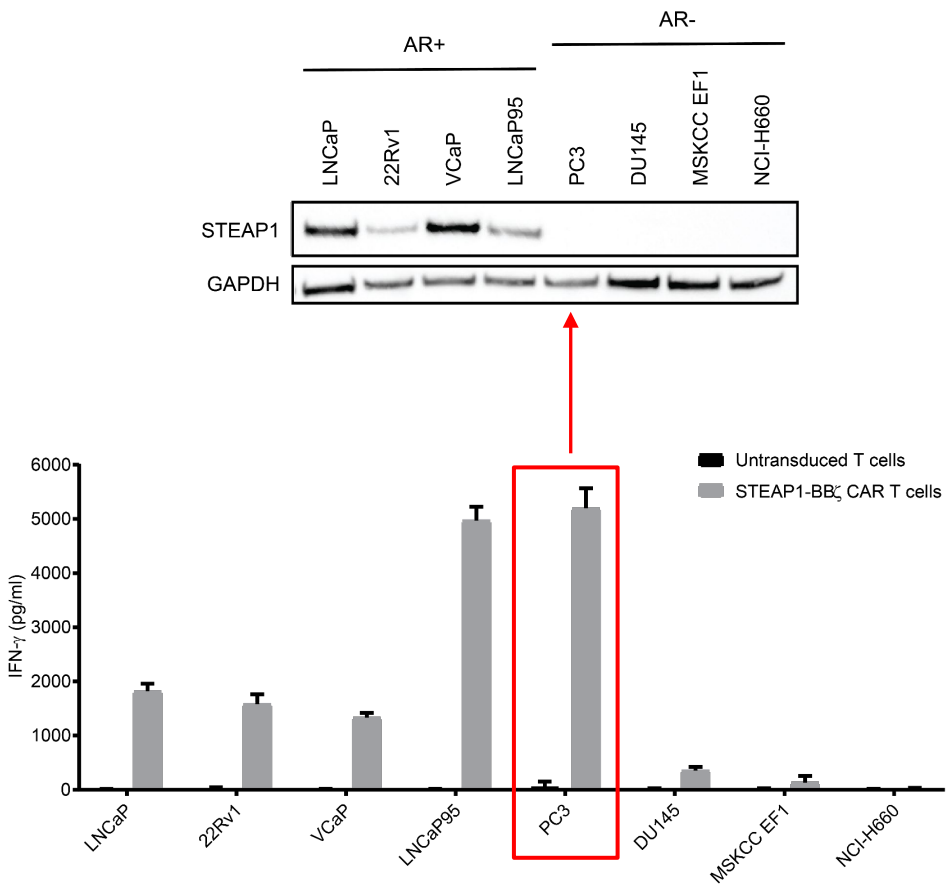
Kamat N, et al. *In preparation.* 2021.

Further validation of the antigen-selective activation and cytotoxicity of STEAP1 CAR-T cells *in vitro*



Enumeration of target cell killing by live cell imaging

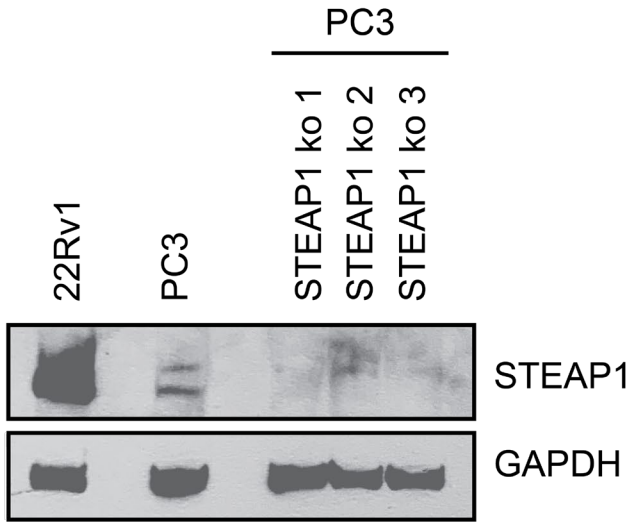


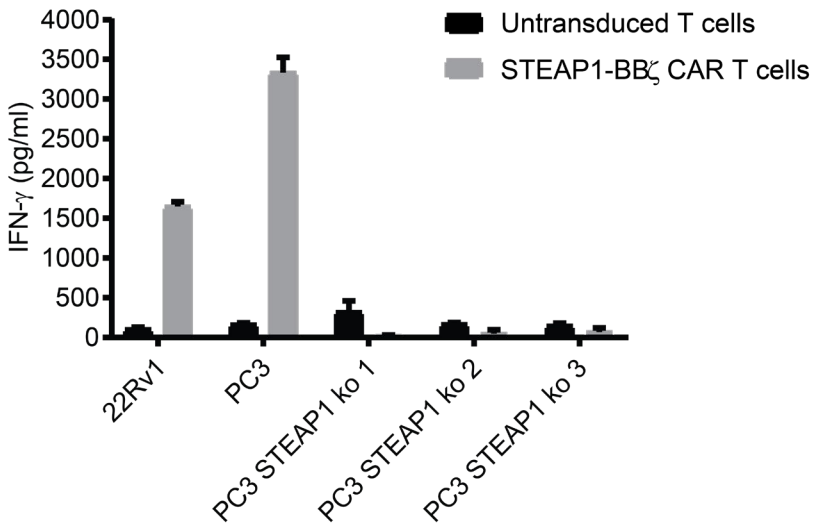


Discrepant co-culture result relative to STEAP1 expression in PC3?

Kamat N, et al. *In preparation.* 2021.

STEAP1 CAR-T cells are responsive to very low STEAP1 expression in PC3

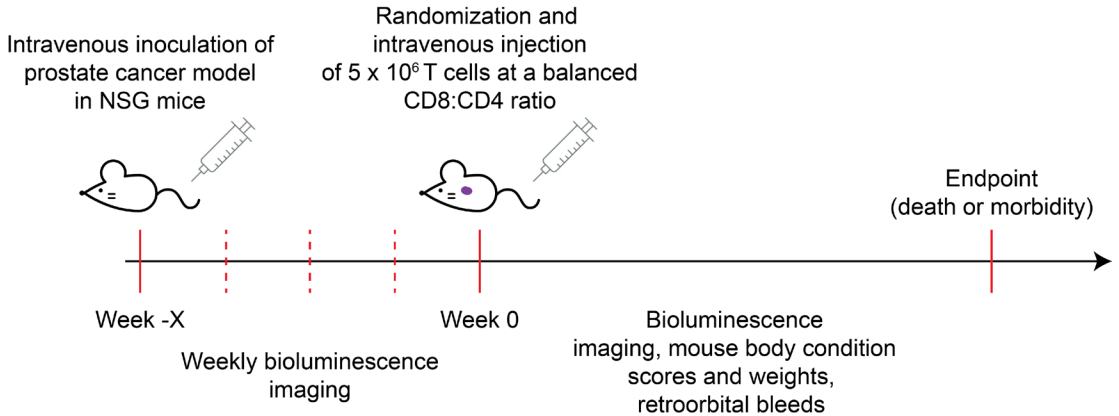


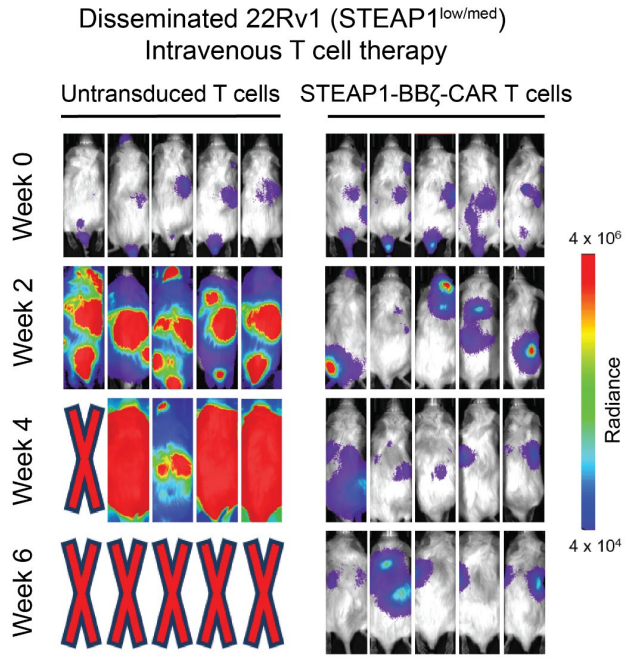


Potential “double-edged sword” of potency vs. off-tumor, on-target toxicity

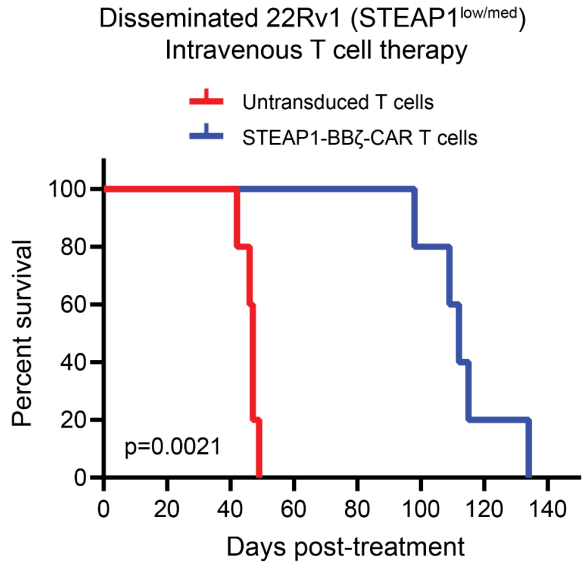
Kamat N, et al. *In preparation.* 2021.

STEAP1 CAR-T cells significantly inhibit disease progression in a mouse model of disseminated 22Rv1 with native STEAP1 expression



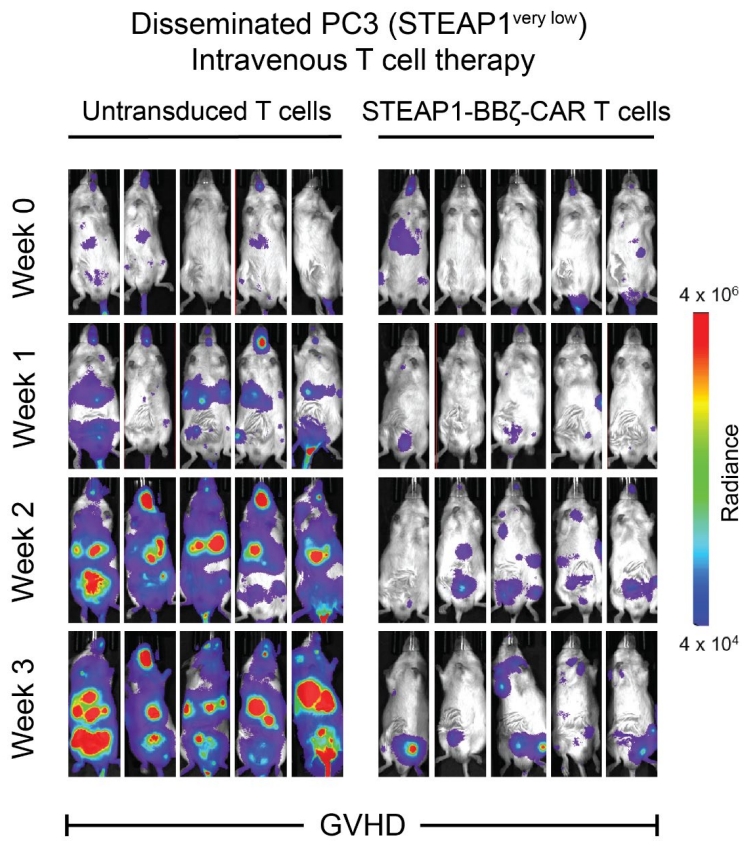


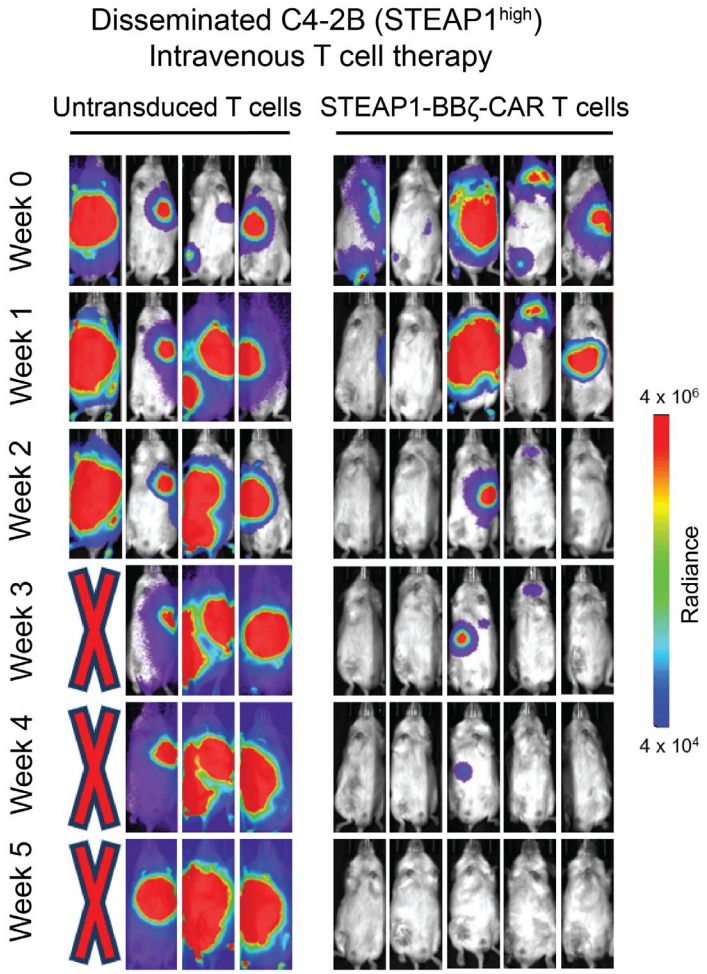
Weekly and additional imaging timepoints after week 6 are not shown for brevity



Kamat N, et al. *In preparation.* 2021.

Potent activity of STEAP1 CAR-T cells also observed in mouse models of disseminated PC3 and C4-2B with native STEAP1 expression



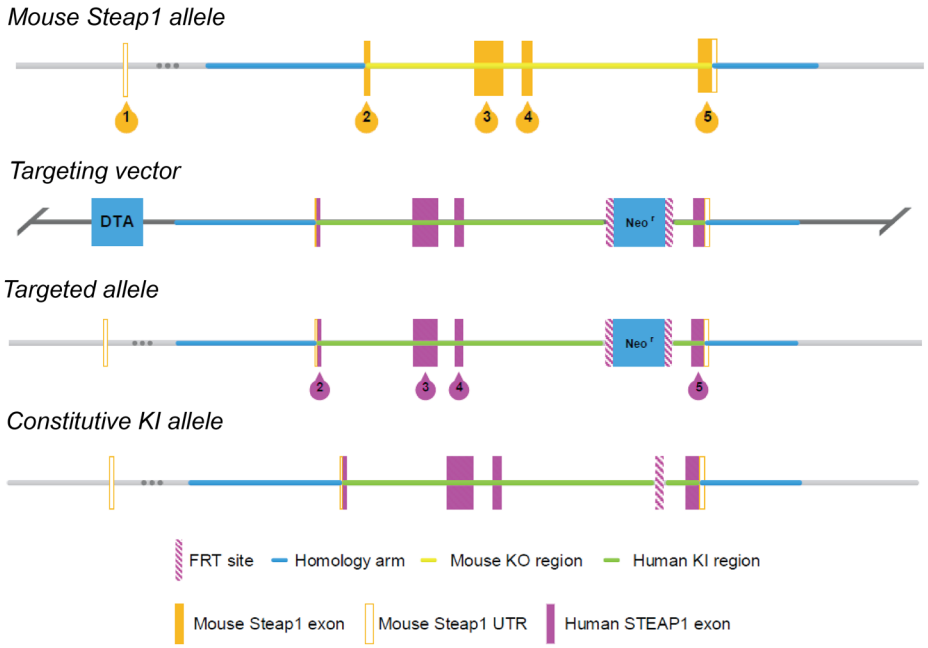


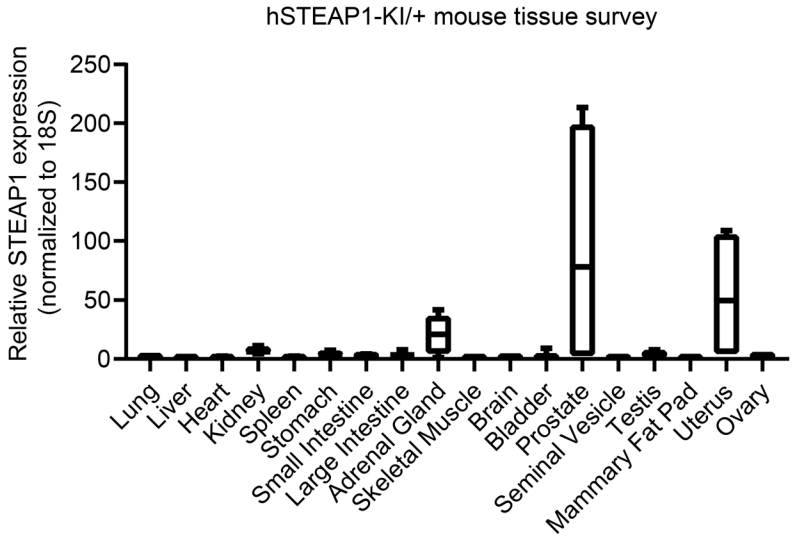
Disease also not detectable by *ex vivo* bioluminescence imaging of harvested organs/tissues

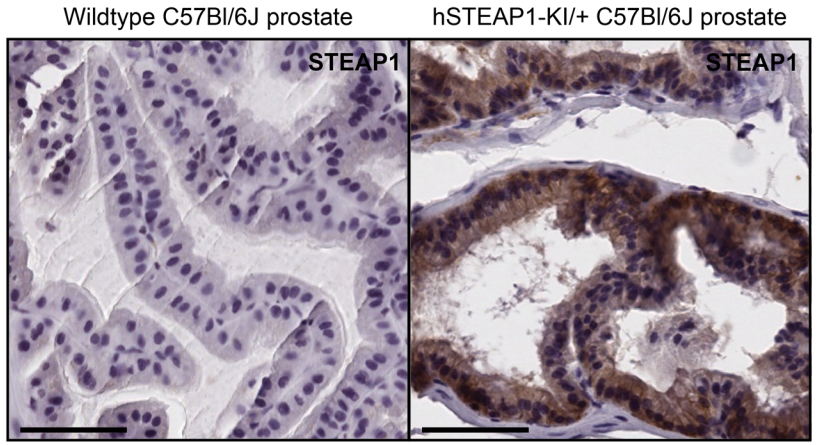
Kamat N, et al. *In preparation.* 2021.

Generation of a human STEAP1 knock-in (hSTEAP1-KI) mouse on the C56Bl/6J background to study safety and efficacy in the immune-competent setting

Targeted knock-in strategy



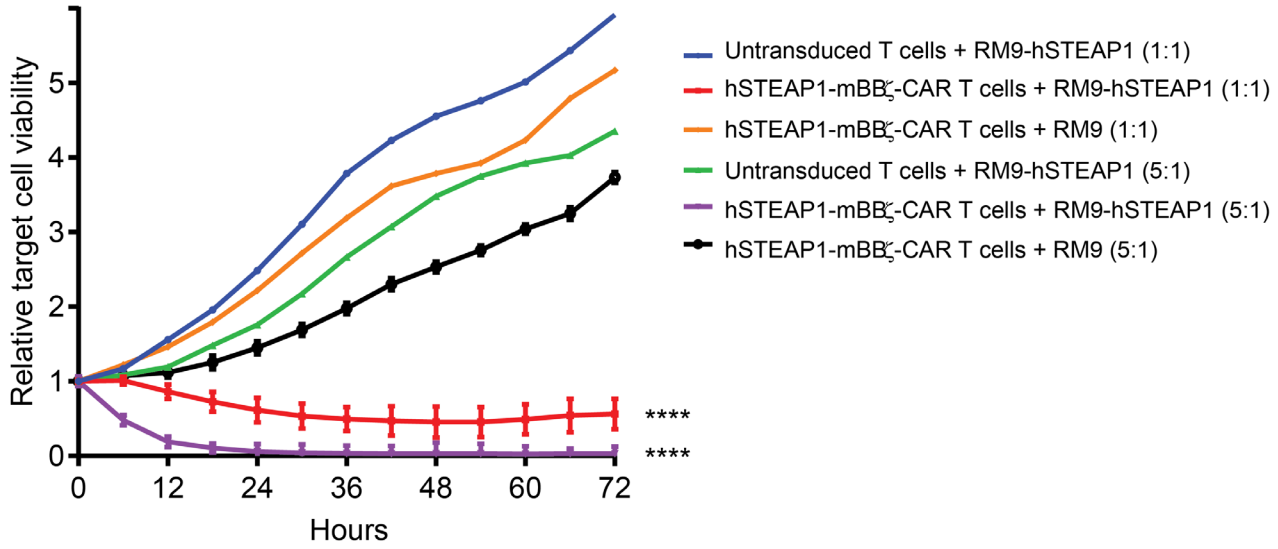




Unpublished data.

Generation and validation of a retroviral murinized STEAP1 CAR construct

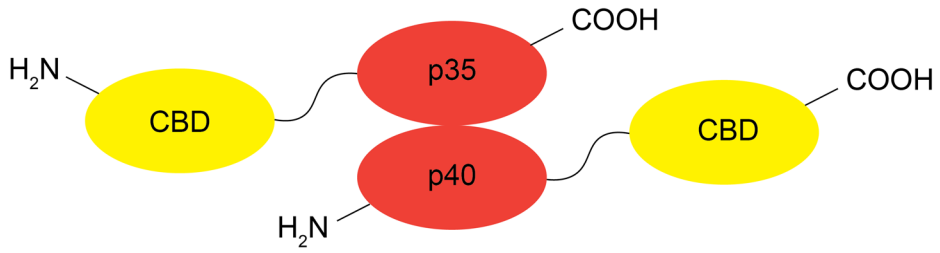
Co-cultures of murine splenocytes with RM9 cells



In vivo studies in hSTEAP1-KI/+ mice with disseminated RM9-hSTEAP1 treated with mouse STEAP1 CAR-T have just commenced

Unpublished data.

An example of ongoing efforts to convert the “cold” tumor microenvironment of prostate cancer to a “hot” state as an adjunct to CAR-T cell therapy

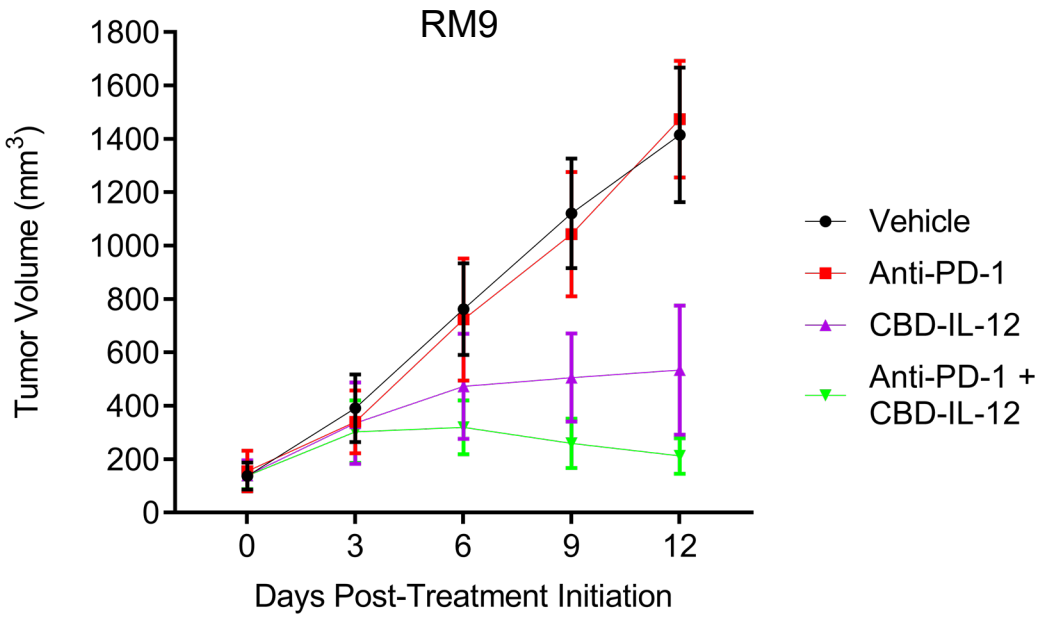


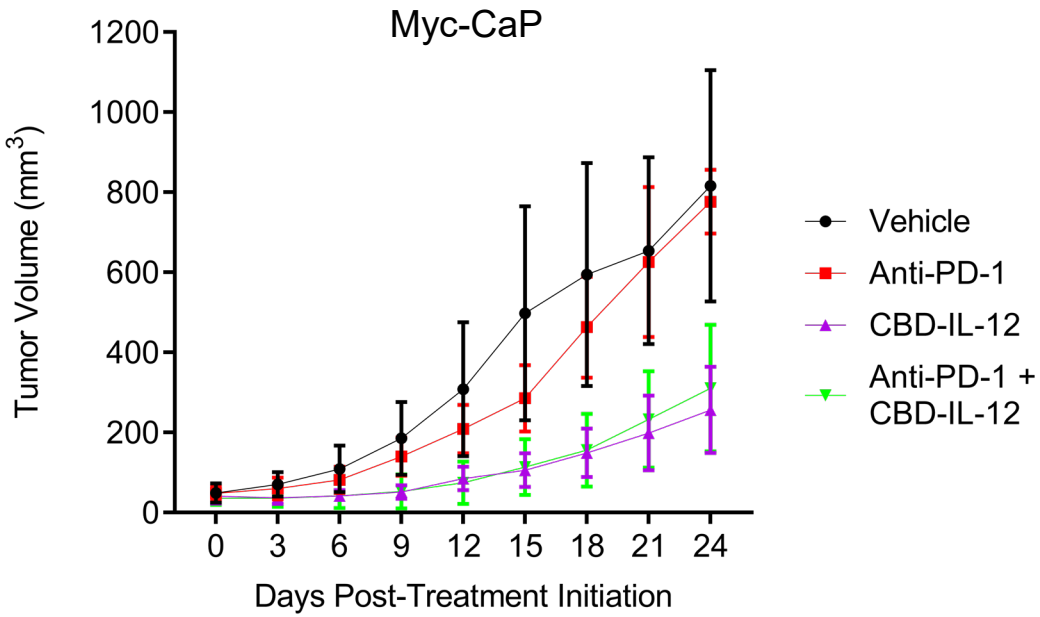
Collagen-binding domain (VWF A3)-IL-12 fusion cytokine

Mansurov A, Ishihara J, et al. *Nat Biomed Eng.* 2020.

Treatment of syngeneic subcutaneous tumors in C57BL/6J mice

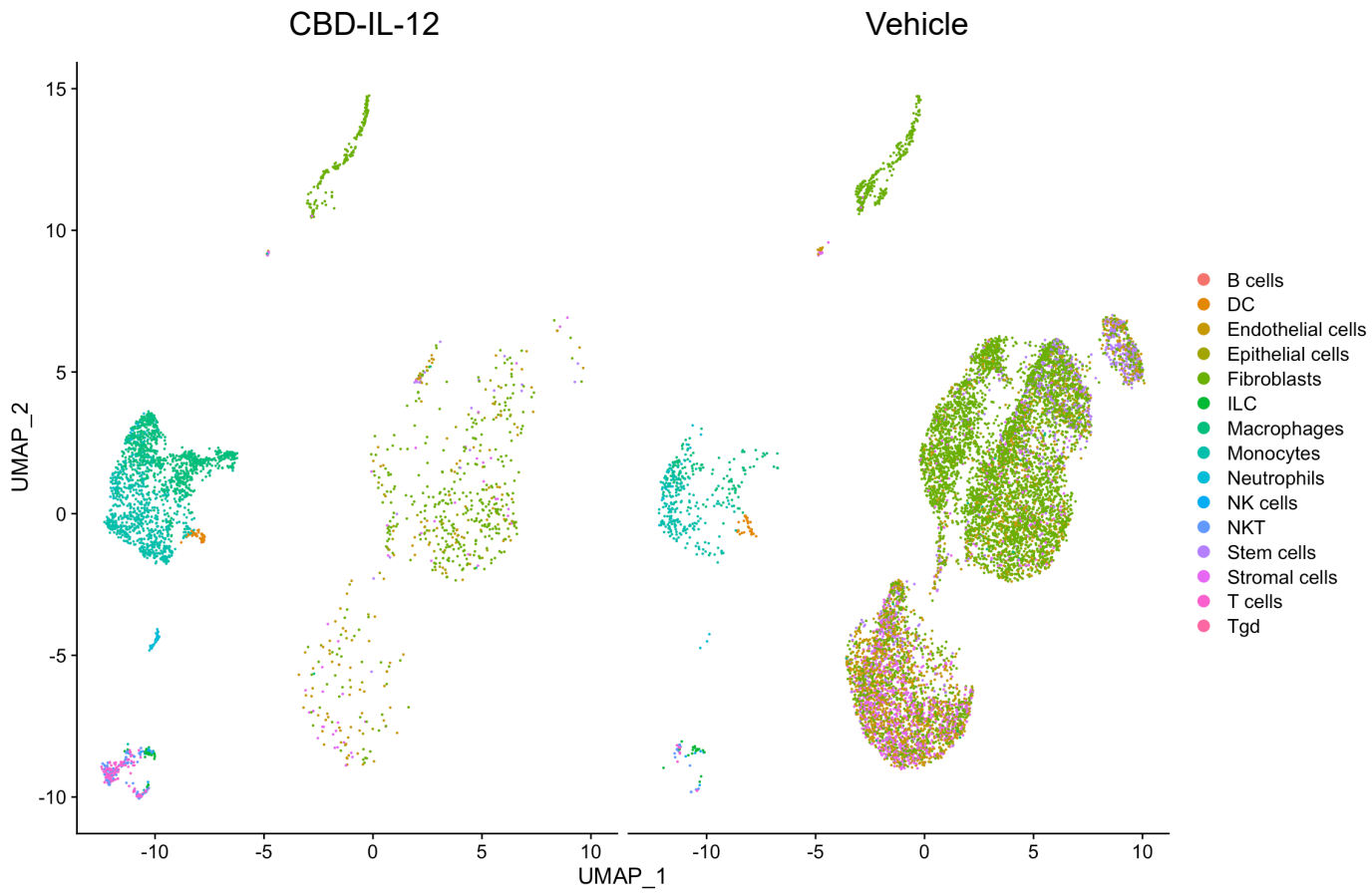
n=7-8 mice/group
CBD-IL-12 25 ug iv every 5 days
Anti-PD-1 (29F.1A12) ip every 5 days



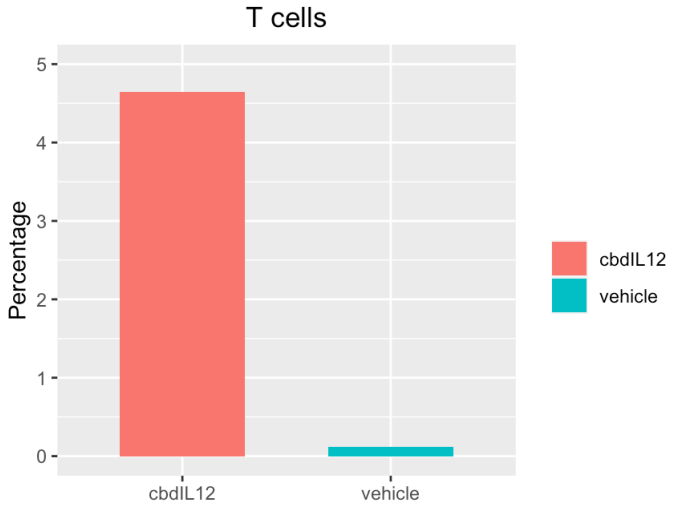


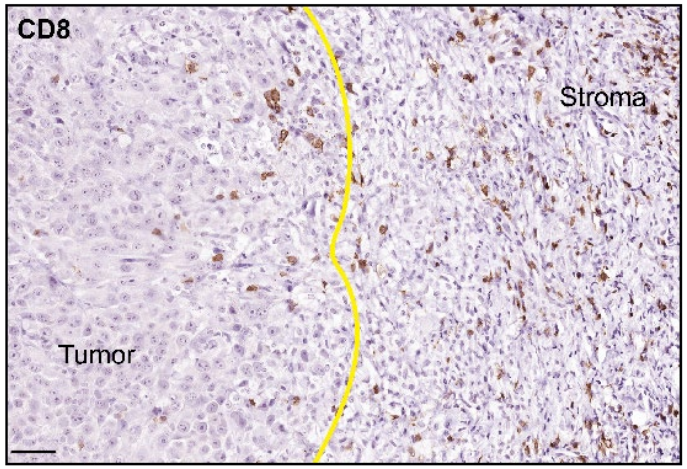
Unpublished data.
Collaboration with Jun Ishihara, Imperial College London.

Increased intratumoral T cell ($CD8^+ > CD4^+$) and monocyte and macrophage frequencies with CBD-IL-12 treatment of RM9-bearing mice



Aggregate data from 5 tumors each, >30,000 cells per condition



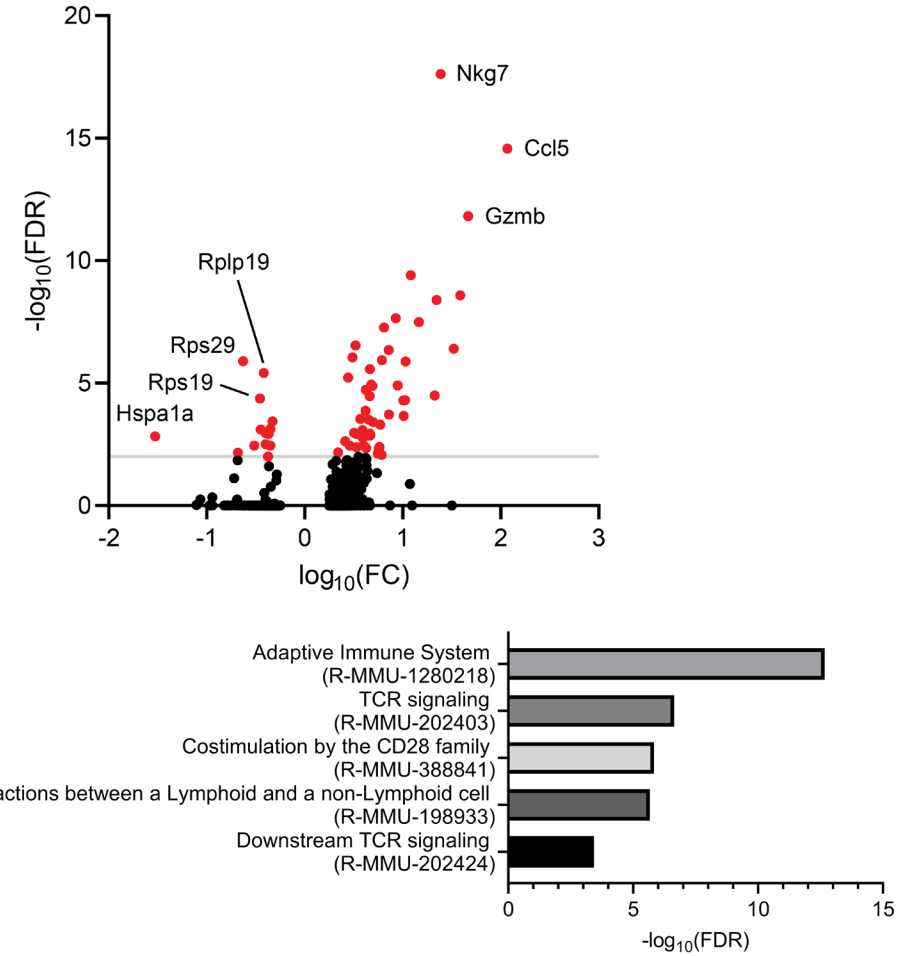


RM9 tumor treated with CBD-IL-12

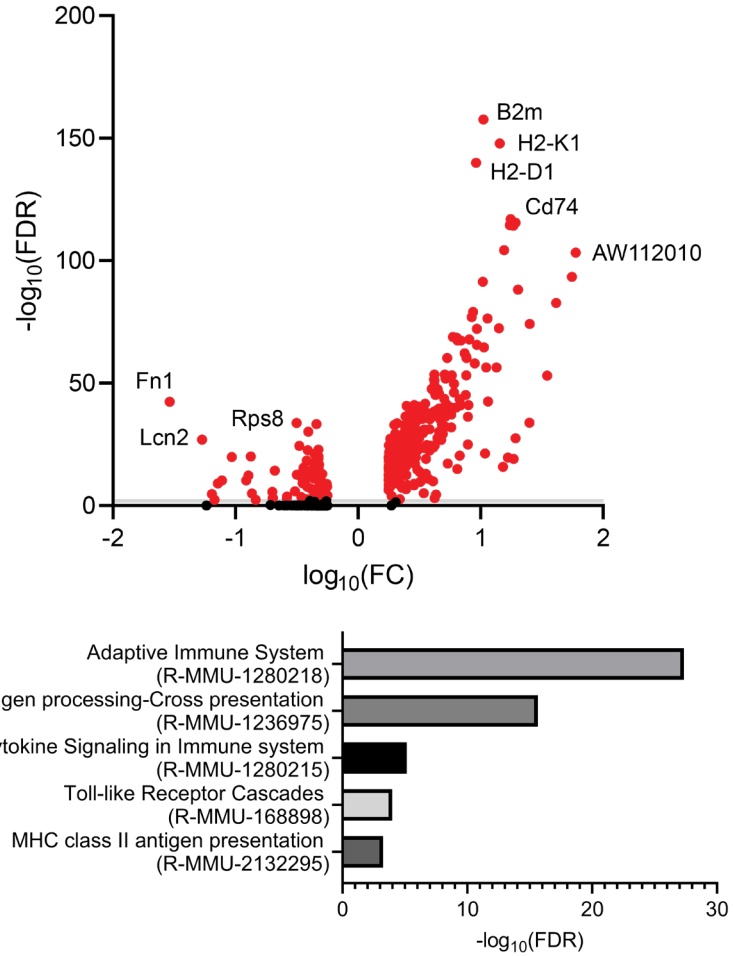
Unpublished data.
Collaboration with Jun Ishihara, Imperial College London.

CBD-IL-12 treatment is associated with enhanced TCR signaling and antigen presentation in the RM9 tumors

T cells, CBD-IL-12 vs. Vehicle



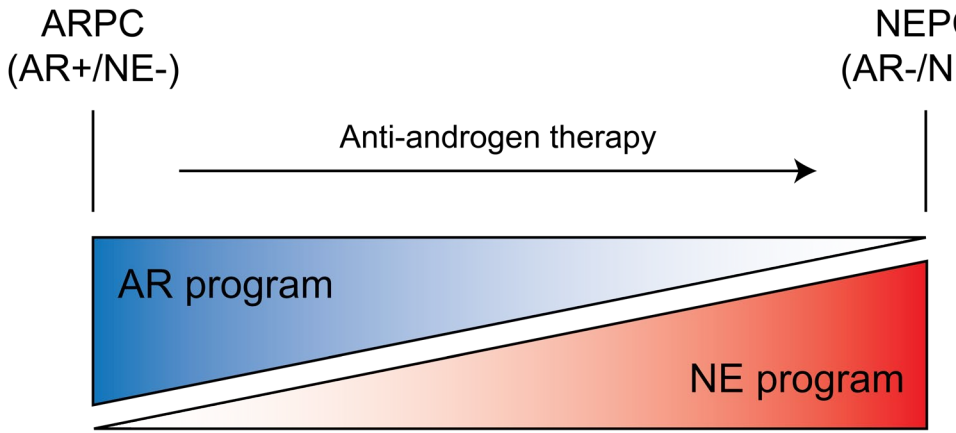
Monocytes and macrophages, CBD-IL-12 vs. Vehicle



Unpublished data.

Collaboration with Jun Ishihara, Imperial College London.

Understanding NE transdifferentiation as a resistance mechanism in mCRPC



Loss of TP53 and RB1
MYCN amplification
Loss of REST
Neuronal transcription factor expression
Others

Association - - - - - - - - - - - - ➤ Function ?

Beltran H, et al. *Cancer Discov.* 2011.
Lee JK, et al. *Cancer Cell.* 2016.
Dardenne E, et al. *Cancer Cell*. 2016.
Bishop JL, et al. *Cancer Discov.* 2017.
Mu P, et al. *Science*. 2017.
Ku S, et al. *Science.* 2017.
Park JW, et al. *Science*. 2018.
And many others!

NEPC is made up of transcriptionally distinct subtypes

Labrecque M, et al. *JCI.* 2019.

NEPC is not an obligate clinical outcome of the loss of TP53 and RB1 in prostate cancer

Loss of TP53 and RB1 in human prostate cancer cell lines does not induce fulminant NE differentiation

Nyquist MD, et al. *Cell Rep.* 2020.

Loss of AR expression/signaling, even in the context of PTEN, TP53, and RB1 loss, does not enforce a NEPC phenotype but rather a DNPC (AR^-/NE^-) phenotype

Brennen WN, et al. *JCI Insight.* 2021.

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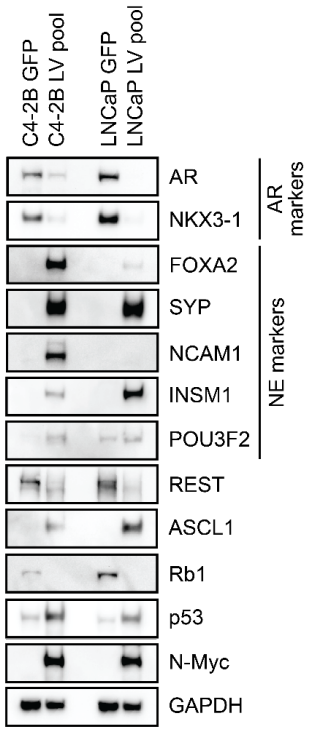
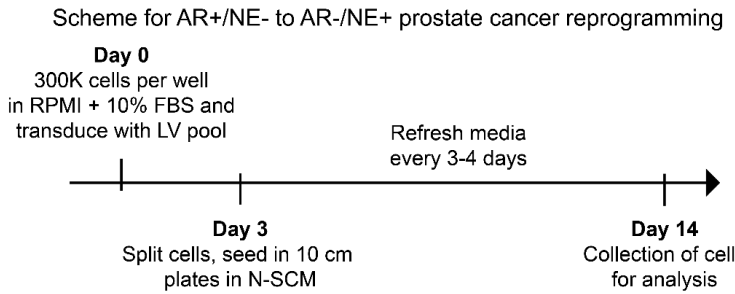
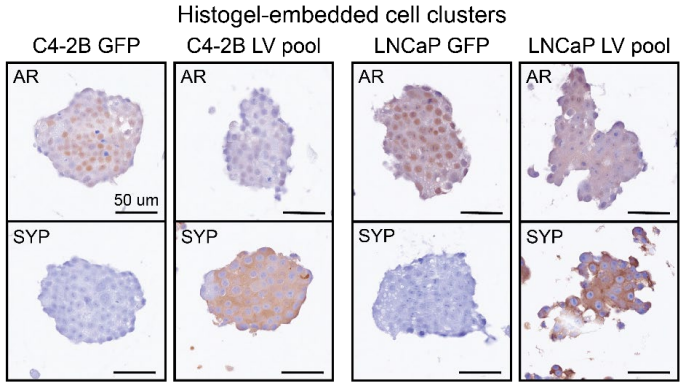
Defining the contribution of defined factors to *in vitro* reprogramming of AR^{+}/NE^{-} to AR^{-}/NE^{+} prostate cancer

Shan Li, PhD
Post-doctoral Fellow
DoD PCRP EIRA

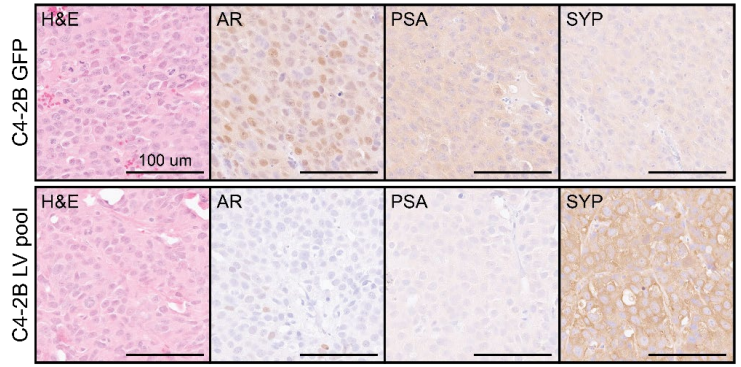
Candidate Factor LV Pool
shRB1
TP53 R175H
MYCN
ASCL1
SRRM4
NR0B2
BCL2
KRAS G12V





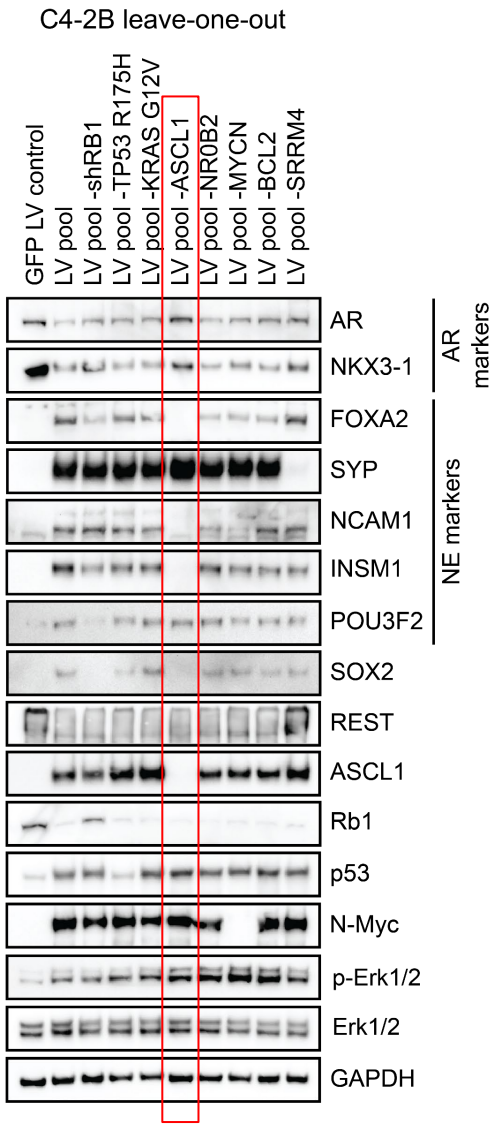


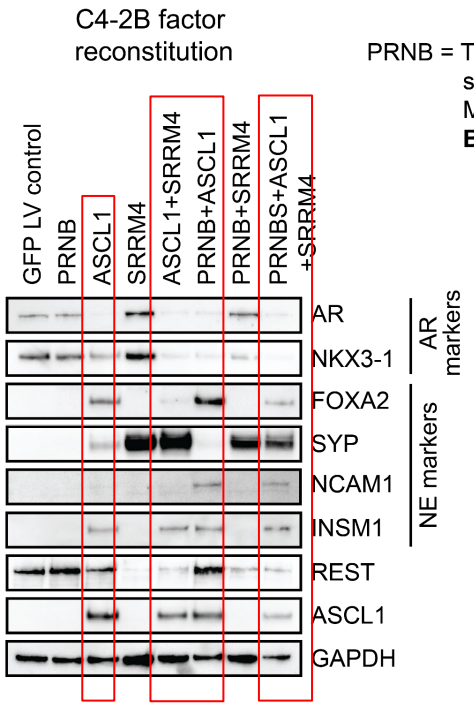
Tumor xenografts
in NSG mice

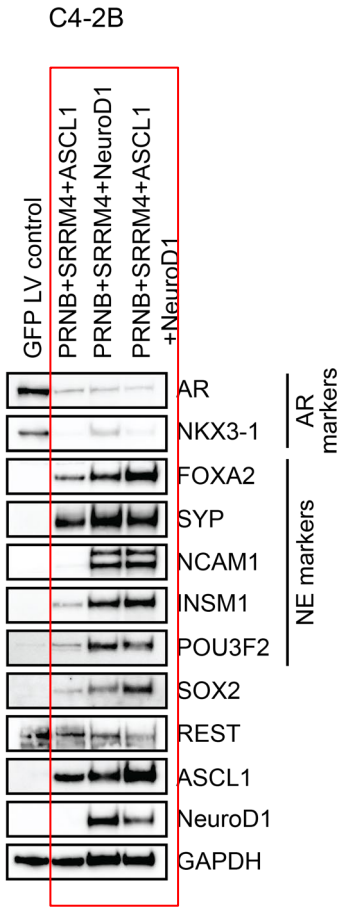


Unpublished data.

Leave-one-out and factor reconstitution analyses identify ASCL1 and NeuroD1 as critical factors involved in the modulation of AR and NE programs







Neural transcription factors may coordinate AR downregulation and induction of a neuroendocrine differentiation program

Unpublished data.

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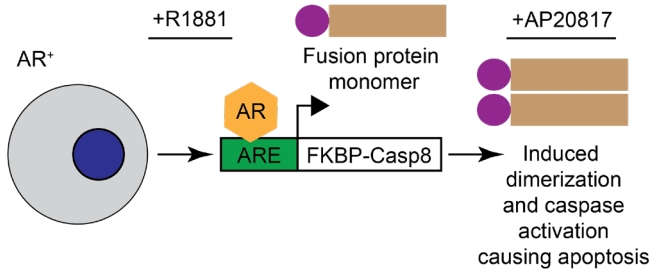
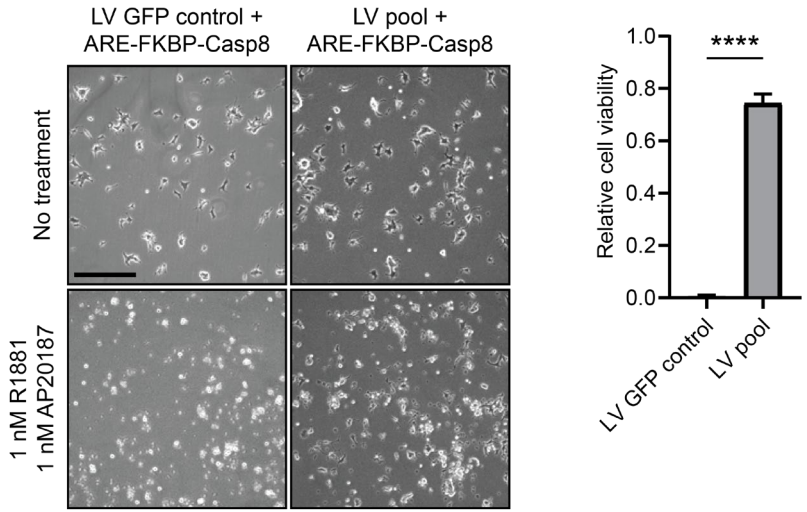
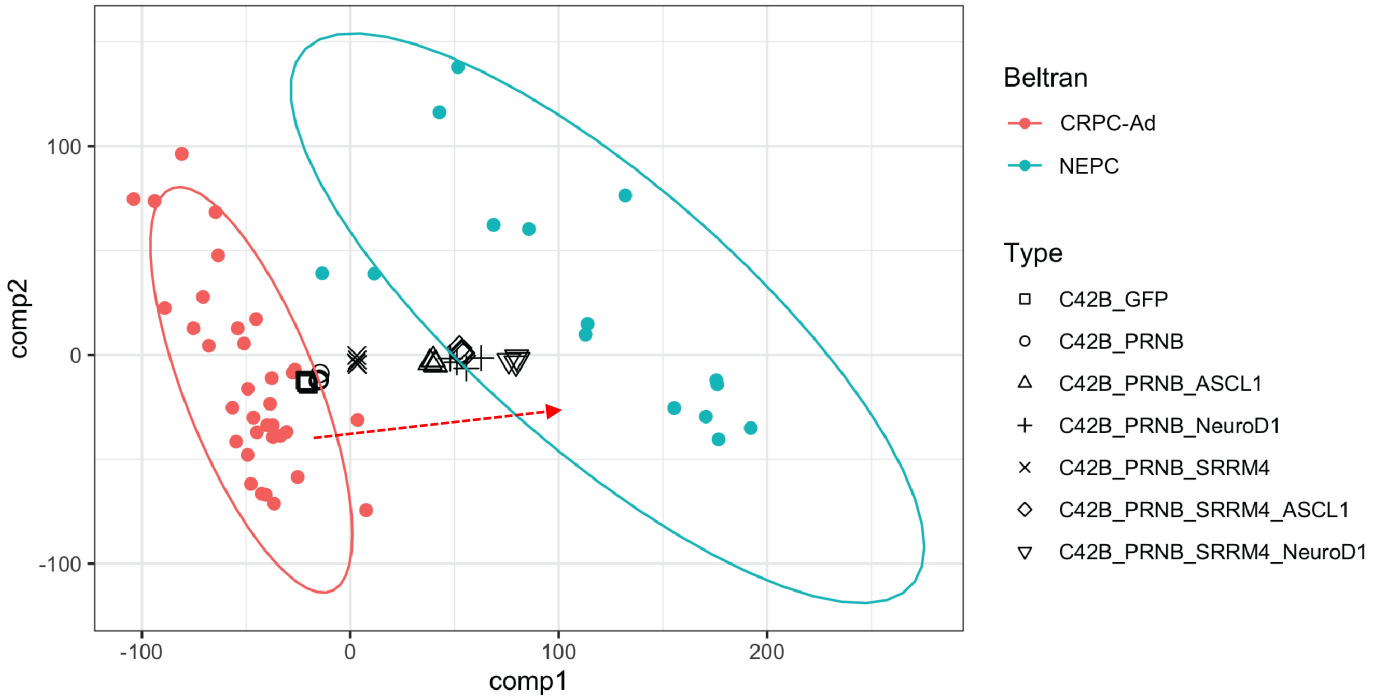
NE reprogrammed C4-2B cells are functionally AR independent and are transcriptionally similar to NEPC





Collaboration with Yong Tao, Nelson Lab at Fred Hutch.

Partial least squares regression analysis projecting NE reprogrammed samples onto CRPC samples



Beltran H, et al. *Nat Med.* 2016.
Collaboration with Graeber Lab at UCLA

Unpublished data.

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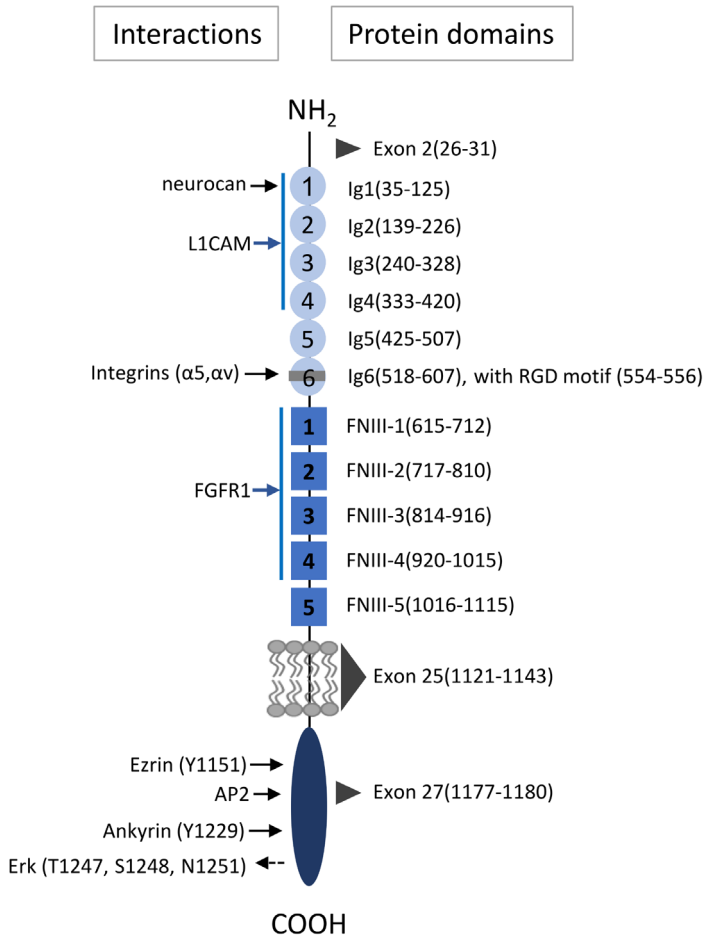
L1 cell adhesion molecule (L1CAM) in cancer

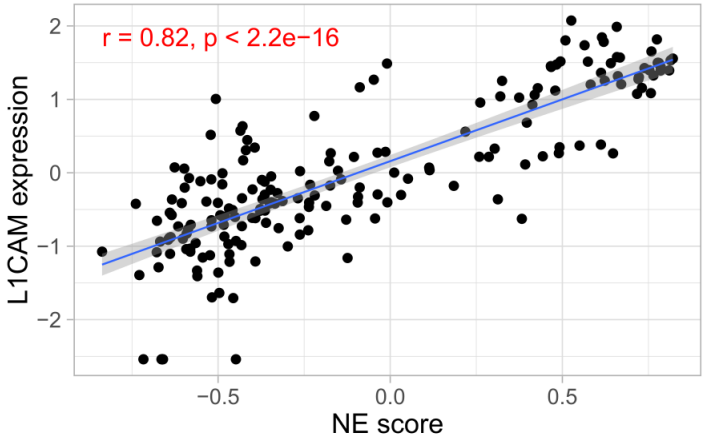


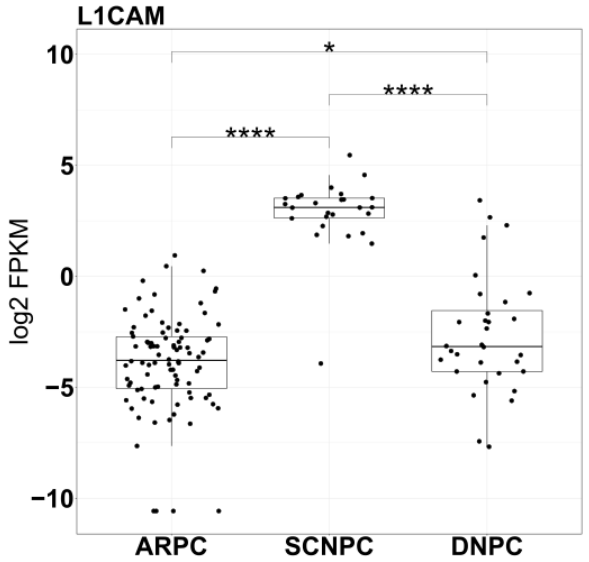
van der Maten M, et al. *Int J Mol Sci.* 2019.

- Increases cancer cell motility and invasion
- Augments cancer resistance to chemotherapy
- Promotes epithelial-to-mesenchymal transition (EMT)
- Expression in a number of cancer has been associated with progression and poor prognoses
- L1CAM is negatively regulated by AR and REST → explains expression in NEPC?

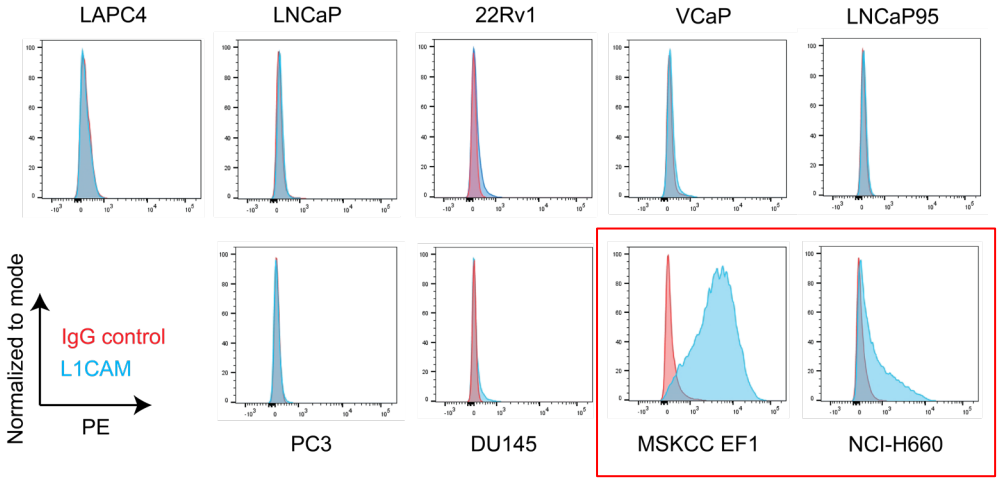
L1CAM expression associates with NEPC

UW Tissue Acquisition Necropsy data

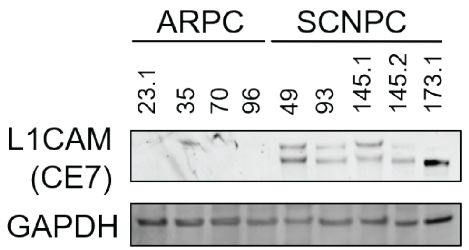


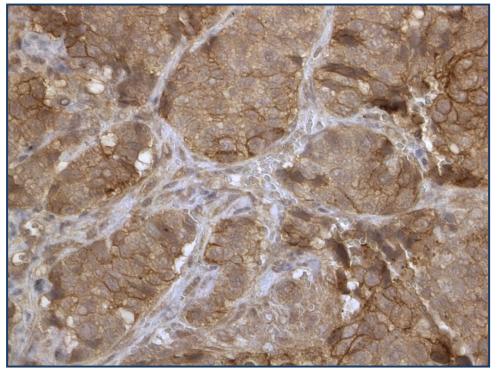


Prostate cancer cell line models



Patient-derived xenograft models



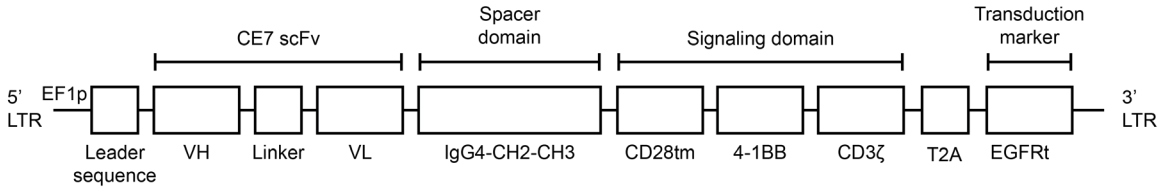


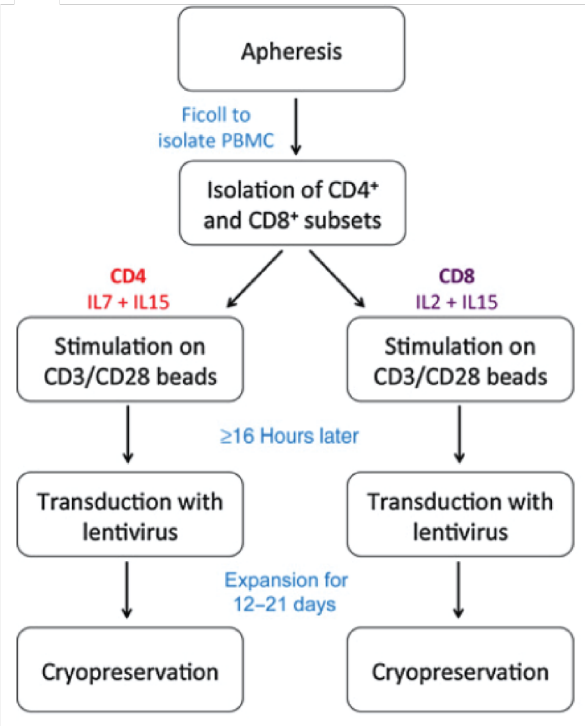
LuCaP 173.1 – L1CAM IHC

Unpublished data.
Collaboration with Nelson Lab, Fred Hutch.

Repurposing L1CAM CE7 CAR T cell therapy for NEPC

CE7 recognizes a glycosylation-dependent, tumor-specific epitope of L1CAM

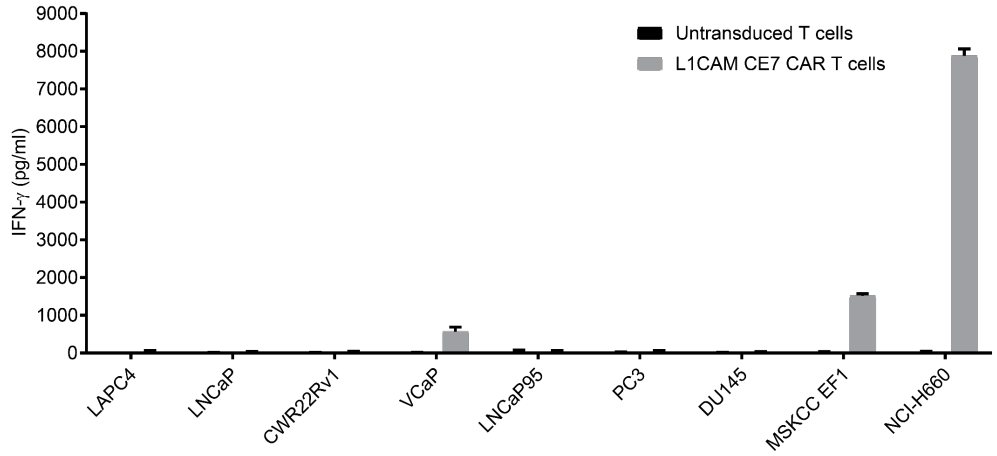


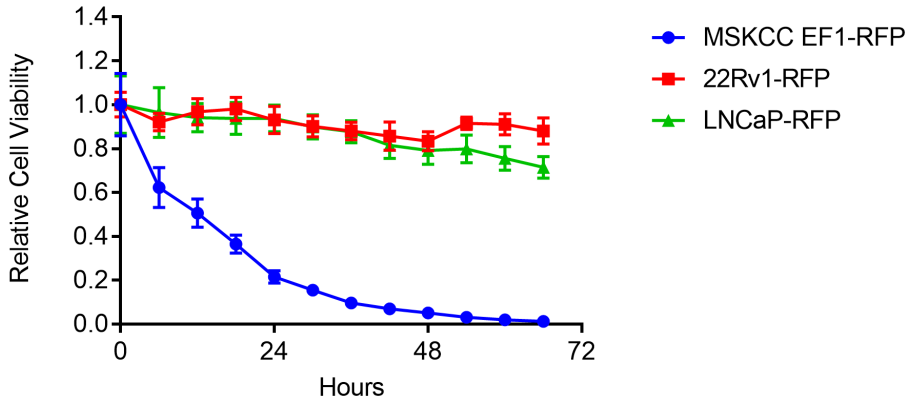


Kunkele A, et al. *Clin Cancer Res.* 2019.

Currently under evaluation in the ENCIT-01 phase I trial for childhood neuroblastoma at Seattle Children's Hospital (PI: Navin Pinto, MD)

Co-cultures with L1CAM CE7 CAR-T suggest selective T cell activation/killing of NEPC



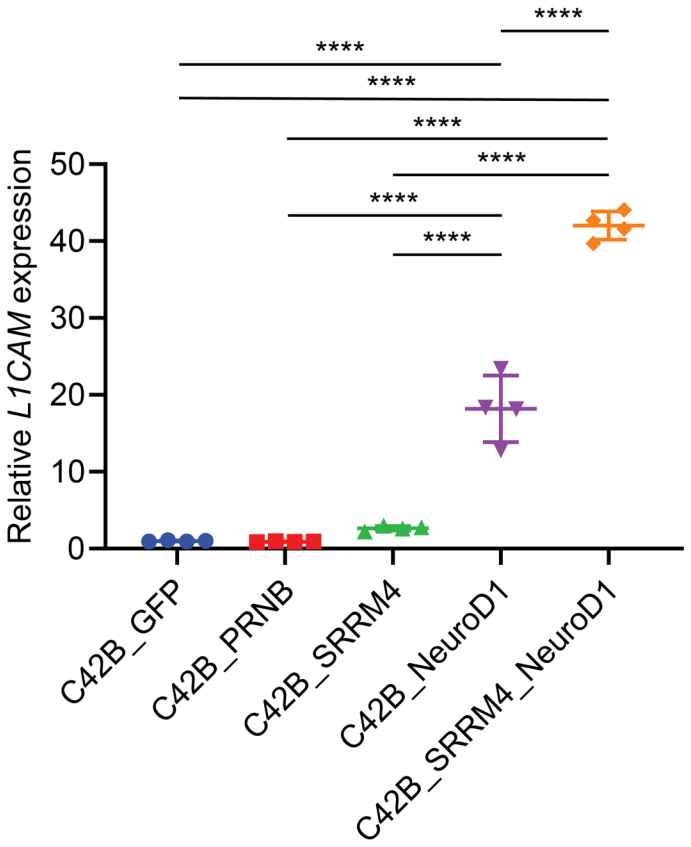


Phase I trial for NEPC currently being planned at SCCA/Fred Hutch (PI: Michael Schweizer, MD)

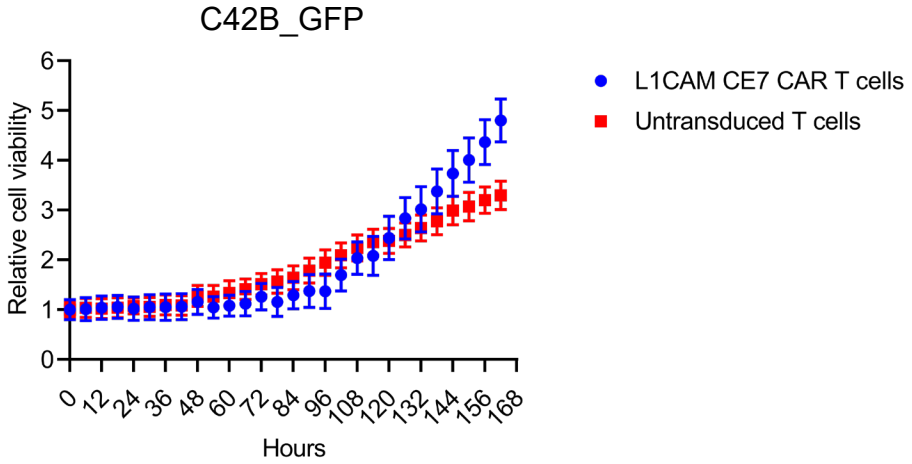
Unpublished data.

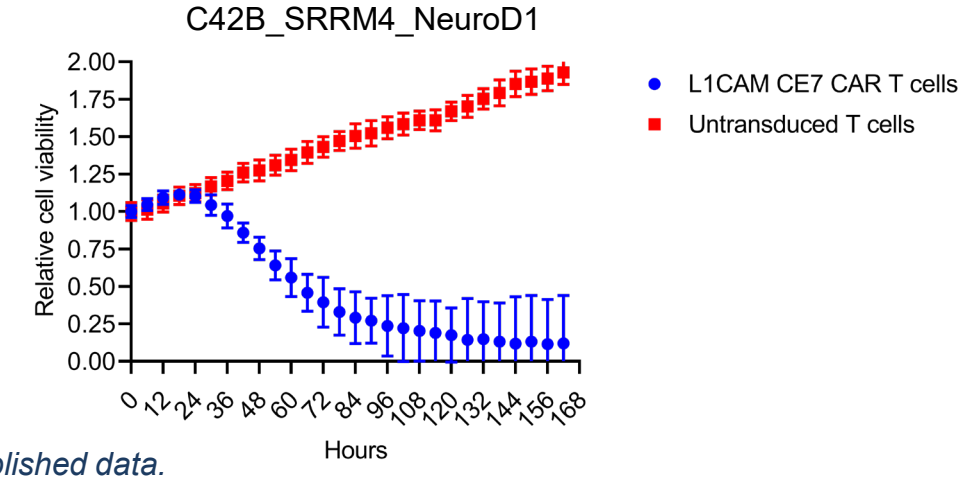
Collaboration with Nelson Lab, Fred Hutch; Navin Pinto, Julie Parks, Michael Jensen, Seattle Children's.

NeuroD1 and SRRM4 drive L1CAM expression during NE reprogramming and susceptibility to CAR-T cell therapy



Enumeration of target cell killing by live cell imaging





Unpublished data.

Conclusions and questions

1. CAR-T development for subtypes of prostate cancer
2. Understanding determinants of transitions in mCRPC disease state may help frame effective CAR-T therapies
3. How do we target prostate cancer heterogeneity (ie. subtypes and subtypes of subtypes) arising with disease progression with CAR-T? Combinatorial immunotherapies?

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