T CELL RECEPTOR SPECIFIC FOR NOVEL TUMOR-RESTRICTED TARGET ROPN1 TO TREAT TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer has high unmet medical need

1 in 7 women develops breast cancer

>200,000 yearly diagnosed* with TNBC

1 in 10 survives metastatic stage

- Young patient population with limited treatment options
- Frequently infiltrated with T cells yet poor responses to immune checkpoints
- Lacks expression of currently exploited targets for TCR-T cells (i.e., NY-ESO1, MAGE-A4)

*US & 5EU, global Data 2022

Target & TCR discovery pipeline

TARGET: >250 intracellular targets
- Healthy tissues (RNAseq, qPCR, IHC)
- Primary TNBC (RNAseq, qPCR, IHC)
- Metastatic TNBC (RNAseq, IHC)
- Pre-treated TNBC (RNAseq)

ROPN1

EPITOPE: >250 theoretical epitopes
- *in silico* predictions
- Immunopeptidomics
- HLA-A2 binding assay
- Immunogenicity

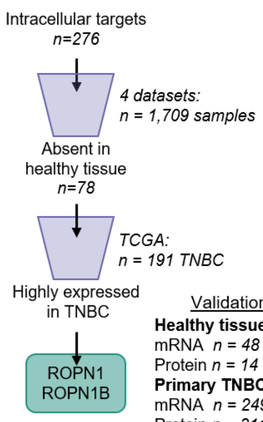
11 epitopes

TCR: >25 identified TCRs
- Expression and function
- Safety (x-scan, peptide library, mispairing)
- Efficacy *in vitro* (2D- & 3D killing)
- Advanced models (pt-derived 3D, *in vivo* NSG)

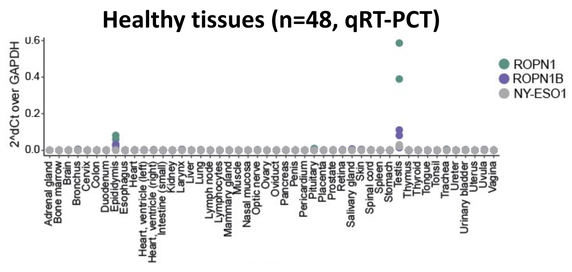
1 Lead TCR (FLY-1A)

ROPN1 is absent in healthy tissues

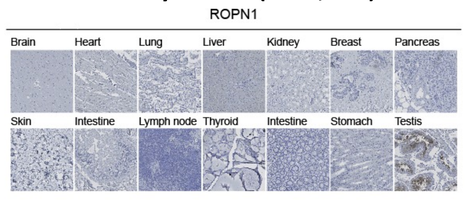
Target discovery workflow



Healthy tissues (n=48, qRT-PCT)



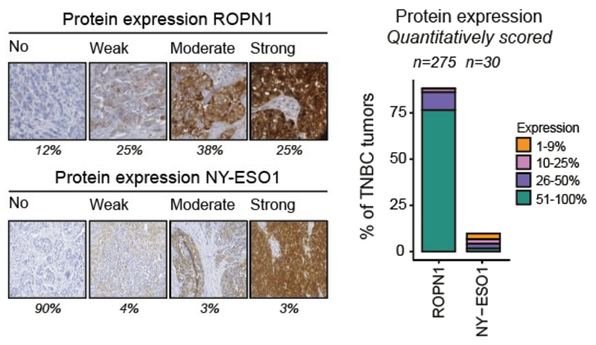
Healthy tissues (n=14, IHC)



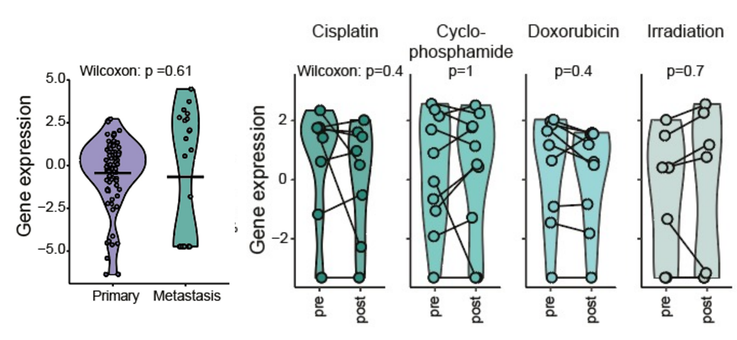
ROPN1 is absent from mature healthy tissues except for immune privileged testis and epididymis which are not present in women

ROPN1 shows high and homogenous expression in primary and metastatic TNBC

Primary TNBC (n=311, IHC)



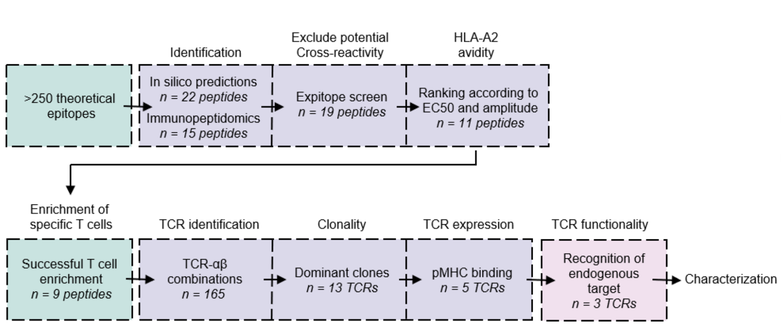
Metastatic TNBC (gene-expression)



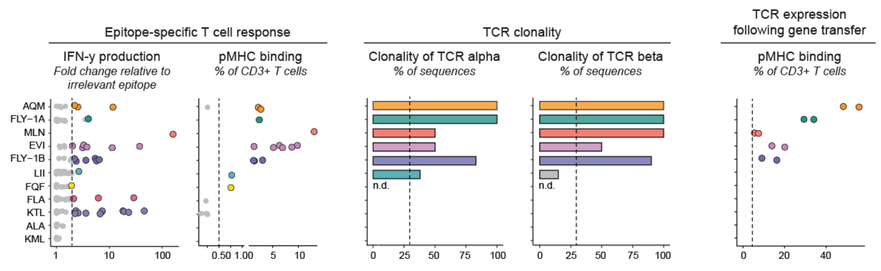
- >90% of TNBC express ROPN1 (mRNA n=440); 90% in melanoma (n=471); 45% in multiple myeloma (n=1437)
- >75% of TNBC show homogenous expression of ROPN1 (protein n=311)
- ROPN1 is maintained in metastatic TNBC lesions (mRNA n=101)
- Metastatic TNBC show similar ROPN1 expression levels compared to primary TNBC (mRNA n=66, protein n=15)
- ROPN1 expression is not affected by chemotherapy nor irradiation (mRNA n=52)

Discovery pipeline yields functional TCRs that recognize endogenously presented ROPN1 epitopes

Epitope & TCR discovery workflow

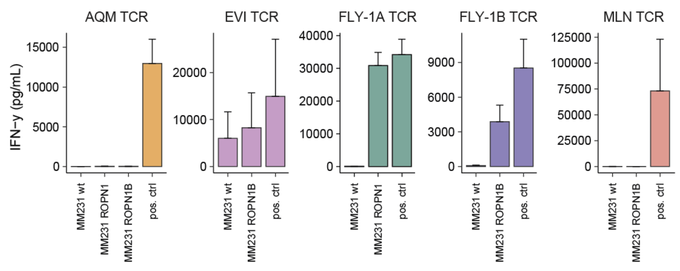


TCR identification



- 9 out of 11 epitopes elicit T cell responses in healthy donors (n=2 donors per epitope)
- TCRs directed against 7 epitopes were HLA-A2 restricted
- TCRs directed against 5 epitopes were functionally expressed upon gene transfer

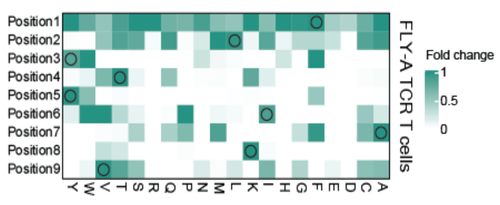
Recognition of endogenously presented epitopes



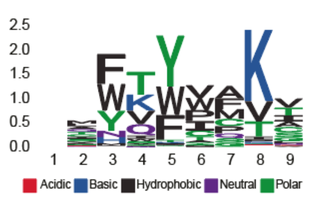
- TCRs directed against 3 epitopes recognized endogenously presented target (overexpression)
- EVI TCR showed off-target reactivity against parental antigen-negative cell line

Lead TCR is ROPN1-specific

Positional amino acid scanning (x-scan)

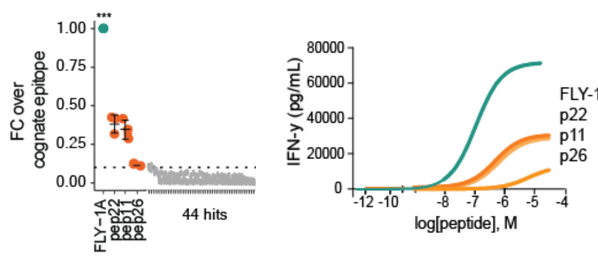


Recognition motif

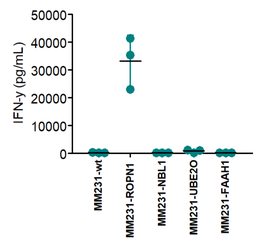


In silico motif screen & HLA-mapping

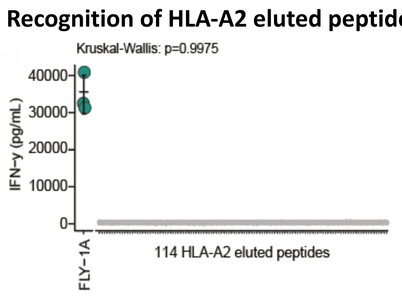
Recognition of screen hits (peptides)



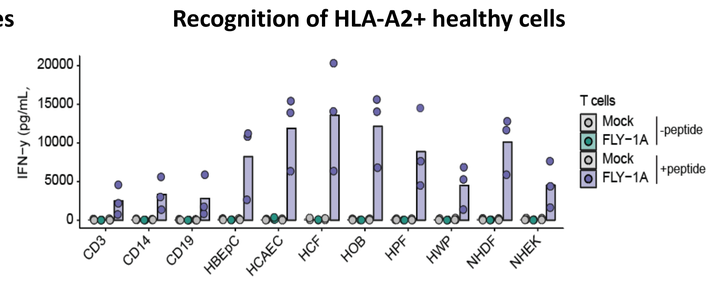
Recognition of screen hits (overexpression of source protein)



Recognition of HLA-A2 eluted peptides

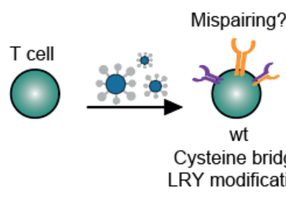


Recognition of HLA-A2+ healthy cells

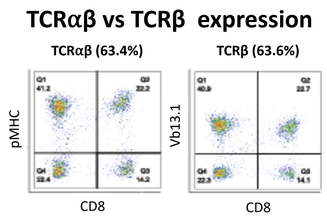


- FLY-1A TCR has stringent recognition motif
- Alternative peptides that map to motif are either not recognized or not endogenously presented
- FLY-1A TCR does not recognize alternative peptides eluted from HLA-A2 nor HLA-A2+ normal cells

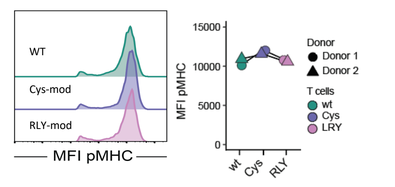
Lead TCR is not prone to mispairing



TCRαβ vs TCRβ expression



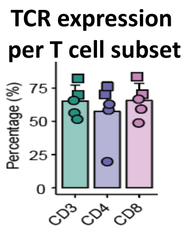
Modifications to enhance expression

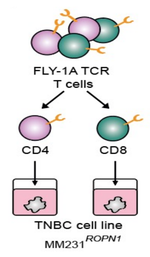


- FLY-1A shows equal expression of TCRαβ (pMHC) and TCRβ (Vβ13.1) indicating lack of mispairing with endogenous TCRα chains
- Known modifications that enhance TCR surface expression do not improve expression of FLY-1A

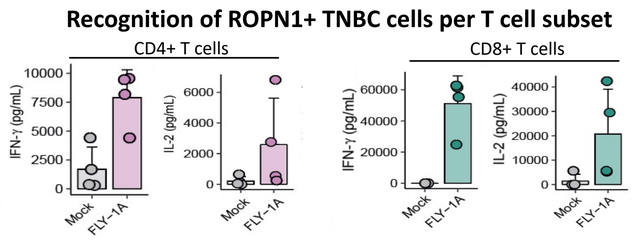
Lead TCR is functionally expressed in CD4+ T cells

TCR expression per T cell subset



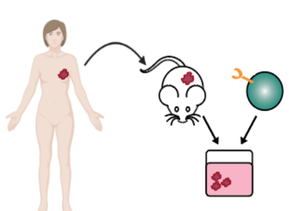


Recognition of ROPN1+ TNBC cells per T cell subset

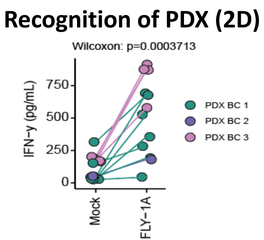


- CD4 T cells show similar expression of FLY-1A when compared to CD8 T cells
- CD4+FLY-1A+ cells produce cytokines when stimulated with ROPN1+ HLA-A2+ TNBC cells

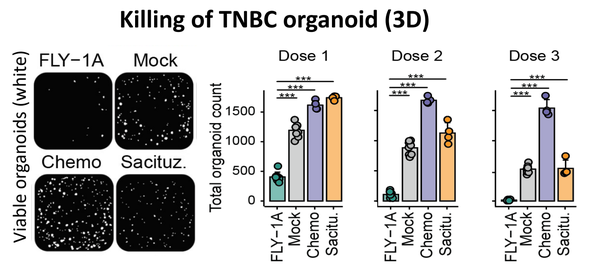
Lead TCR recognizes patient-derived TNBC



Recognition of PDX (2D)

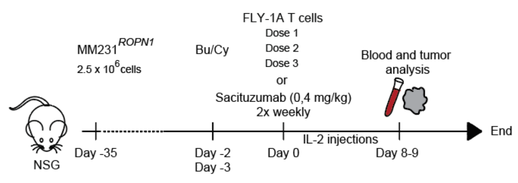


Killing of TNBC organoid (3D)



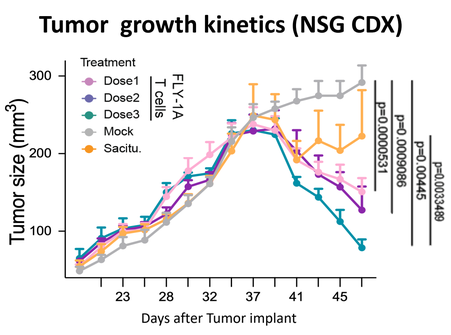
- FLY-1A TCR-T specifically recognize patient-derived TNBC ex vivo
- FLY-1A TCR-T effectively kill patient-derived TNBC organoids in 3D and outperform standard of care treatments within 48h

Lead TCR mediates dose-dependent regression of large tumors and outperforms Sacituzumab-govitecam in vivo

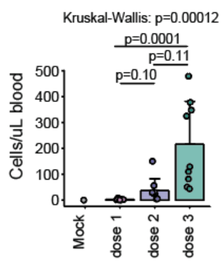


- Single administration of FLY-1A TCR-T cells outperforms bi-weekly administration of Sacituzumab-govitecam
- FLY-1A+ TCR-T cells are found in blood and tumor in a dose-dependent manner

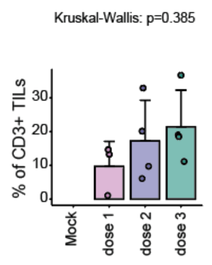
Tumor growth kinetics (NSG CDX)



T cells in blood



T cells in tumor



Summary & Conclusion

- ROPN1 is a promising new target in TNBC
- Lead TCR (FLY-1A) has excellent safety profile and bears low risk for on- and off-target toxicity
- Lead TCR (FLY-1A) is highly effective and outperforms standard of care treatments in advanced preclinical models
- Lead TCR (FLY-1A) has been selected for clinical development

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