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Immunotherapy persister cells uncovered by dynamic single-cell RNA-sequencing

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NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

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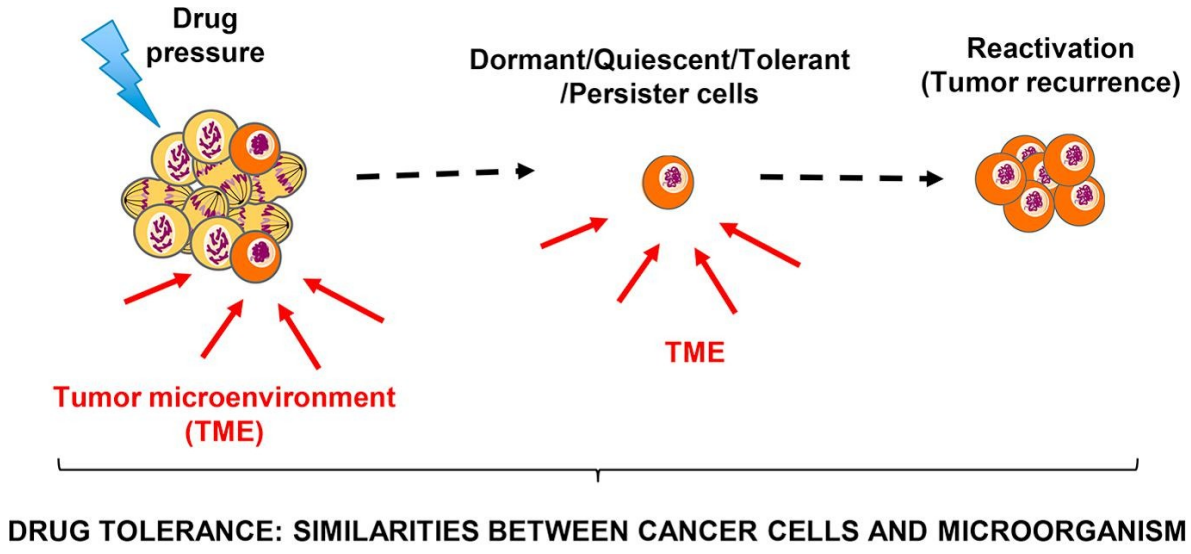
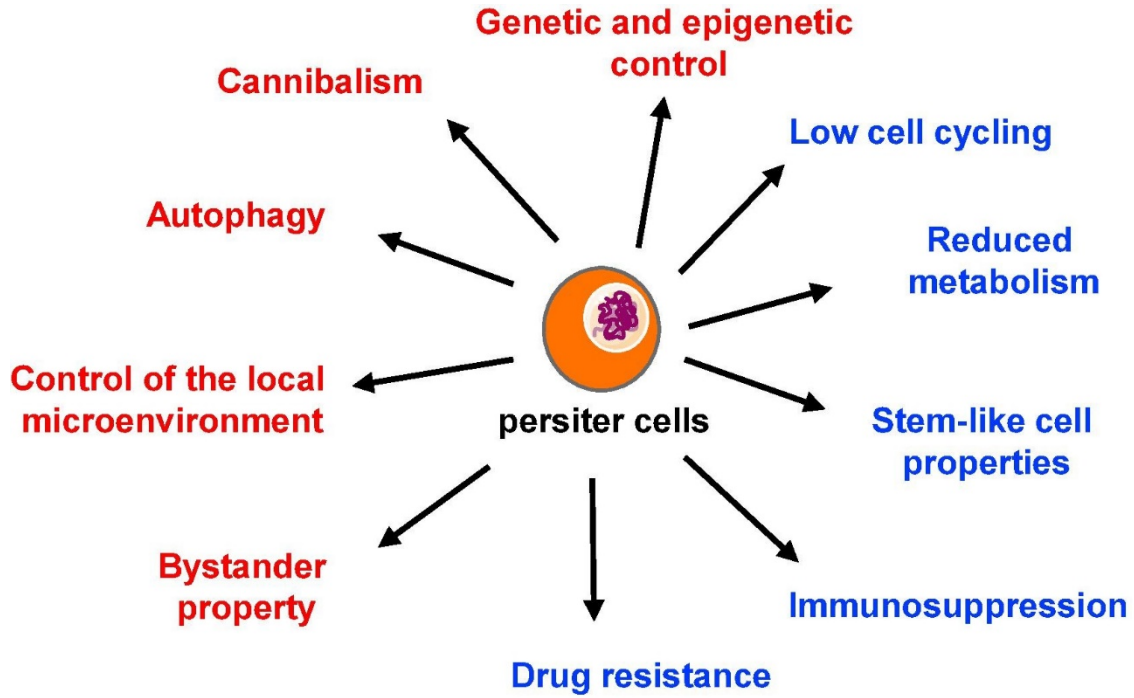
Persister cells: From Tolerance to Resistance

Well described in context of tyrosine kinase inhibitors.
Do similar immunotherapy persister cells (IPCs) exist in context of PD-1 blockade?



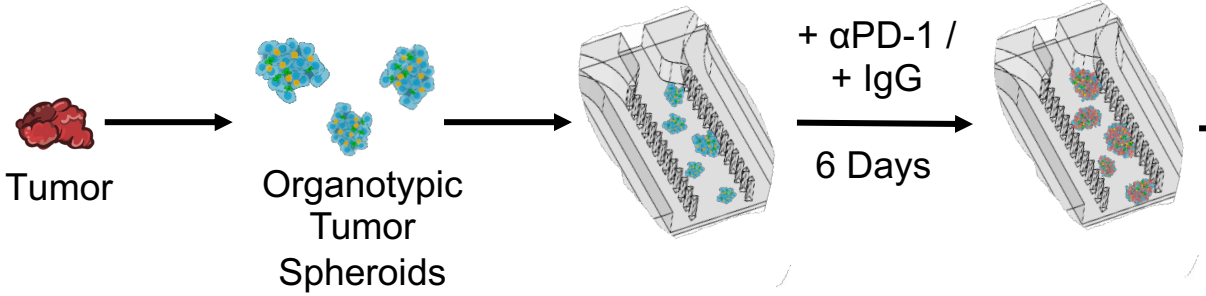


Ref: Vallette et al. Biochem Pharmacol. 2019

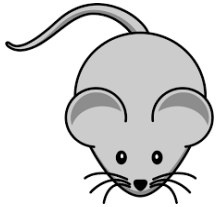
Profiling transcriptional signature of anti-PD-1 resistant cells

Ex vivo platform which
- recapitulates tumor along with its microenvironment
- recapitulates *in vivo* results

MDOTS = Murine-derived Organotypic Tumor Spheroids



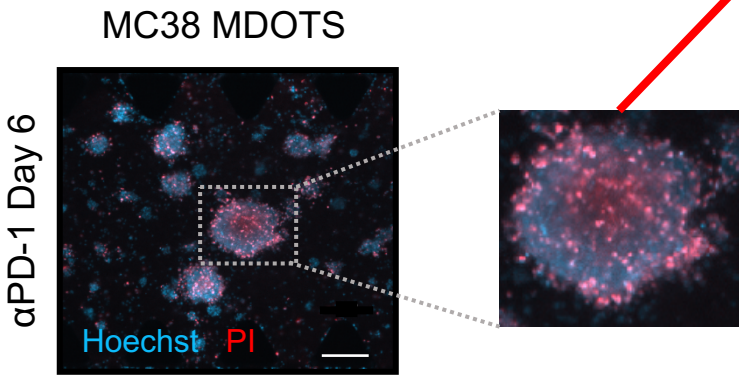
Isolate and Profile Cellular Trancriptomes
- Bulk RNA-seq
- Single-cell RNA-seq



MC38 Colorectal Cancer	CT26 Colorectal Cancer
Microsatellite-instability HIGH	Microsatellite STABLE
αPD-1 therapy: SENSITIVE	**αPD-1 therapy: INTERMEDIATE SENSITIVITY**

αPD-1 = Anti-PD-1 therapy
IgG = Isotype control

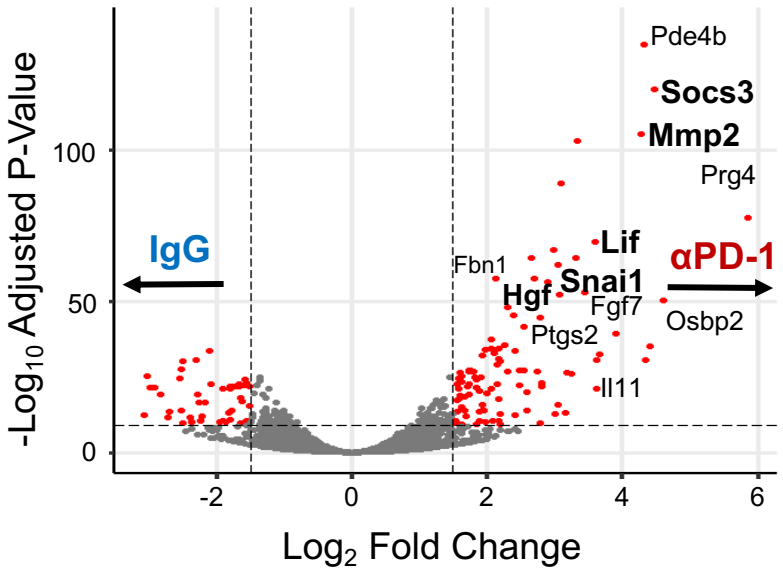
MC38 MDOTS



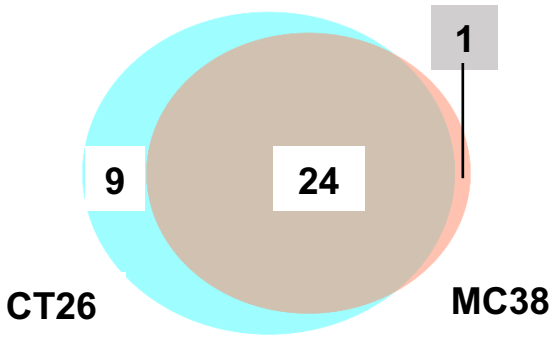
Ref: Jenkins et al (Barbie DA), Cancer Discovery, 2018

Bulk RNA-sequencing of anti-PD-1 resistant cells reveals a distinct transcriptional signature

MC38 MDOTS Bulk RNA-seq



Overlap of Hallmark Pathways (αPD-1 vs. IgG Bulk RNA-seq)



Selected Shared Hallmark pathways (αPD-1 vs. IgG Bulk RNA-seq)

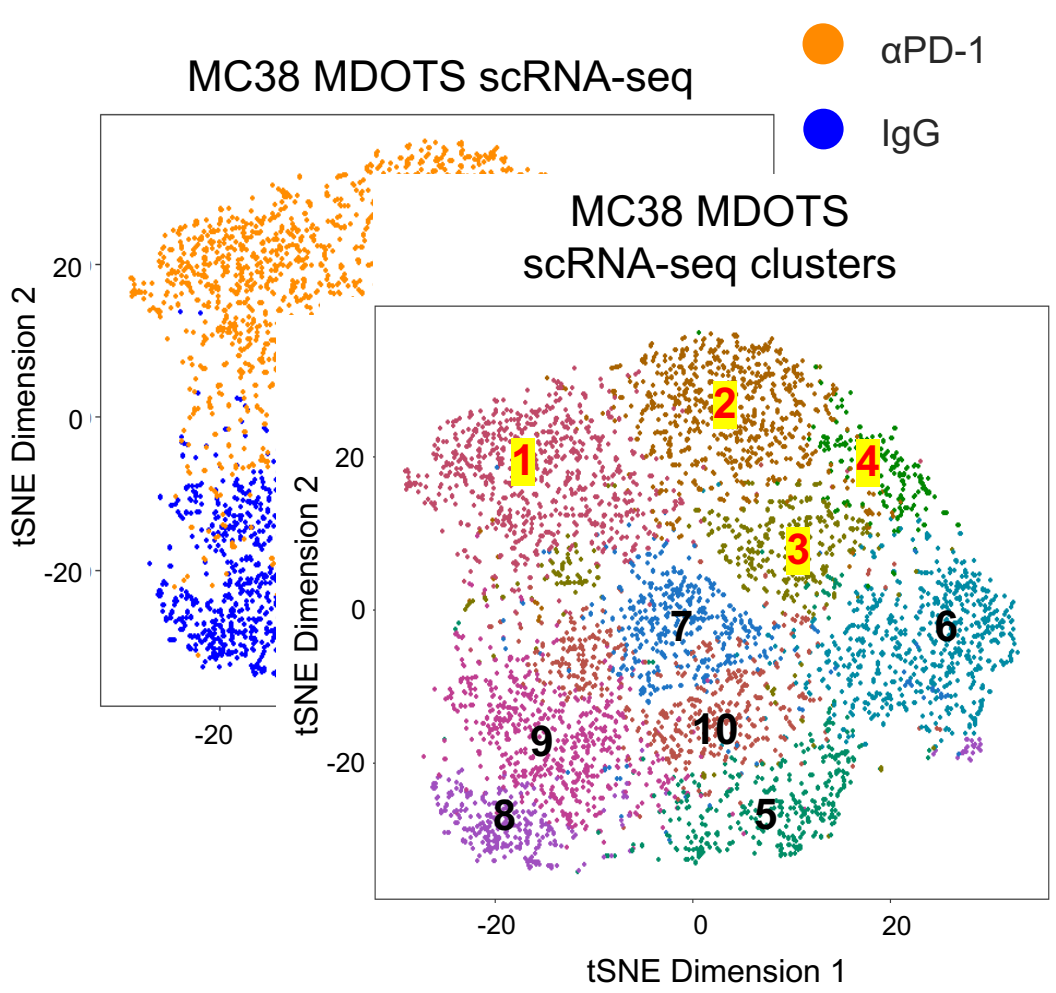
UP:

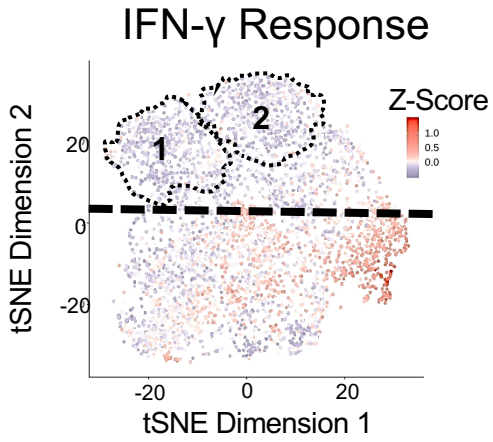
- **TNF-α signaling via NFκβ**
- **Epithelial-Mesenchymal Transition (EMT)**
- Hypoxia
- IL6 JAK STAT3 signaling
- Angiogenesis
- TGFβ Signaling

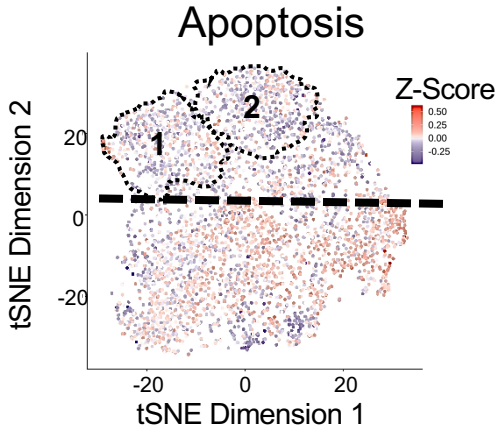
Down:

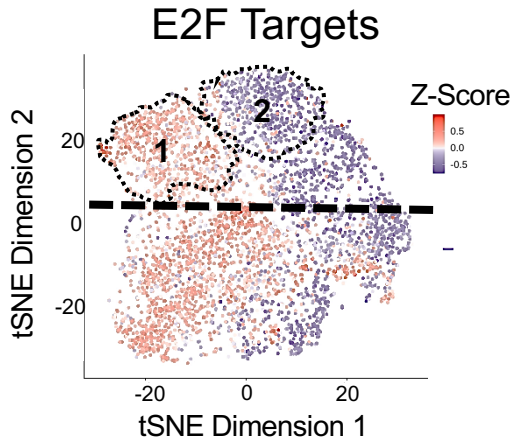
- **E2F targets**
- **Myc targets V1**
- Myc targets V2
- G2M checkpoint
- Interferon-alpha response
- Interferon-gamma response

Single RNA-sequencing of anti-PD-1 resistant cells reveals four distinct clusters

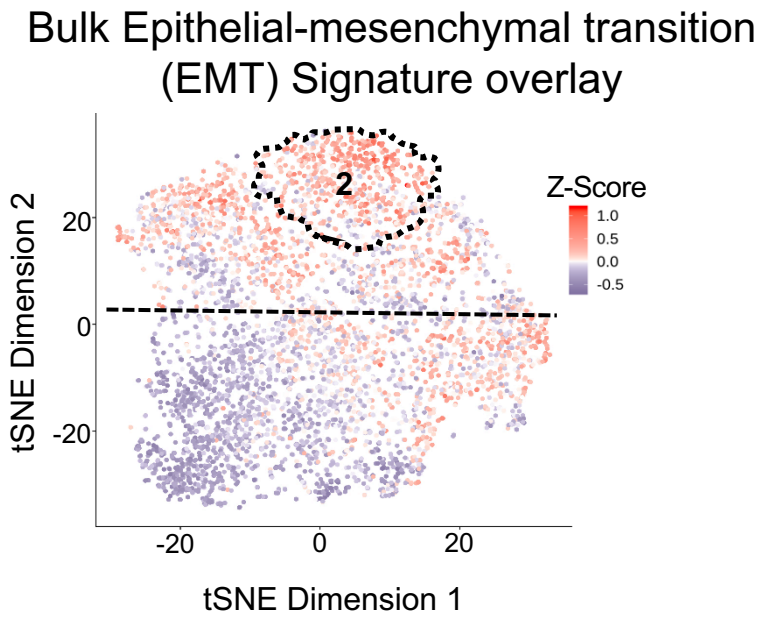


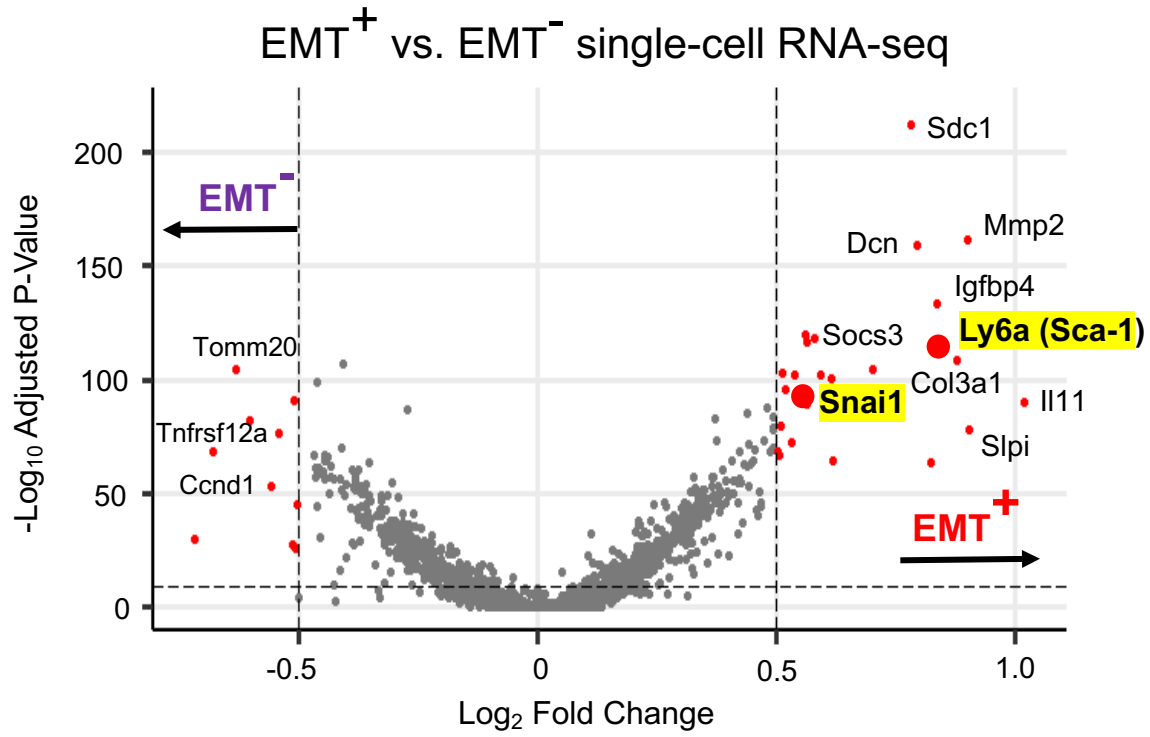






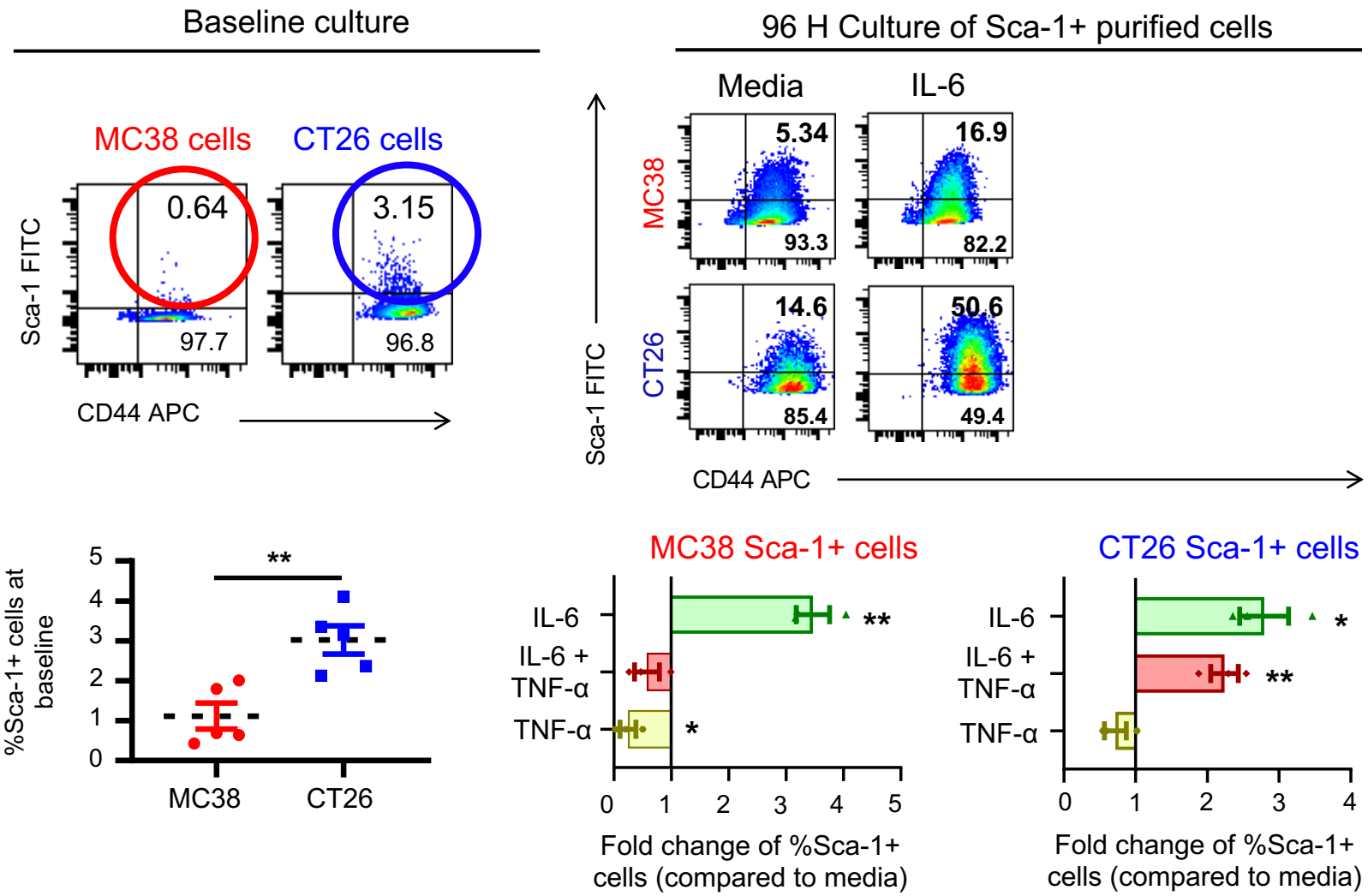
Single-cell RNA-sequencing of anti-PD-1 resistant cells uncovers a ‘stem cell-like persister’ phenotype and markers of IPCs

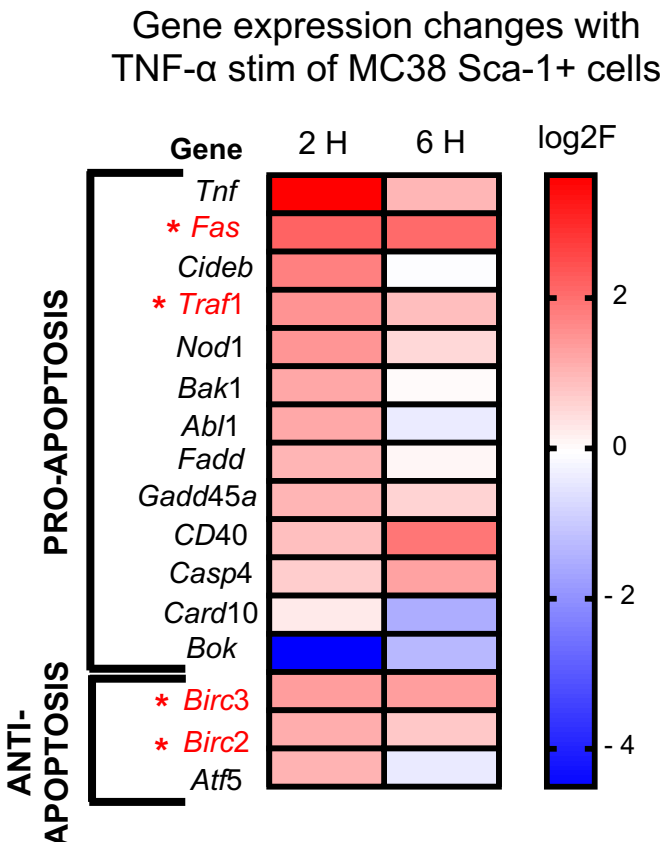




Ly6a/Sca-1 (Stem cell antigen-1)	Marker of Hematopoietic stem cells
Snai1	Master EMT transcription factor

Sca-1+ cell sub-population pre-exists in syngeneic cancer models, with differential effects of IL-6 versus TNF-α stimulation

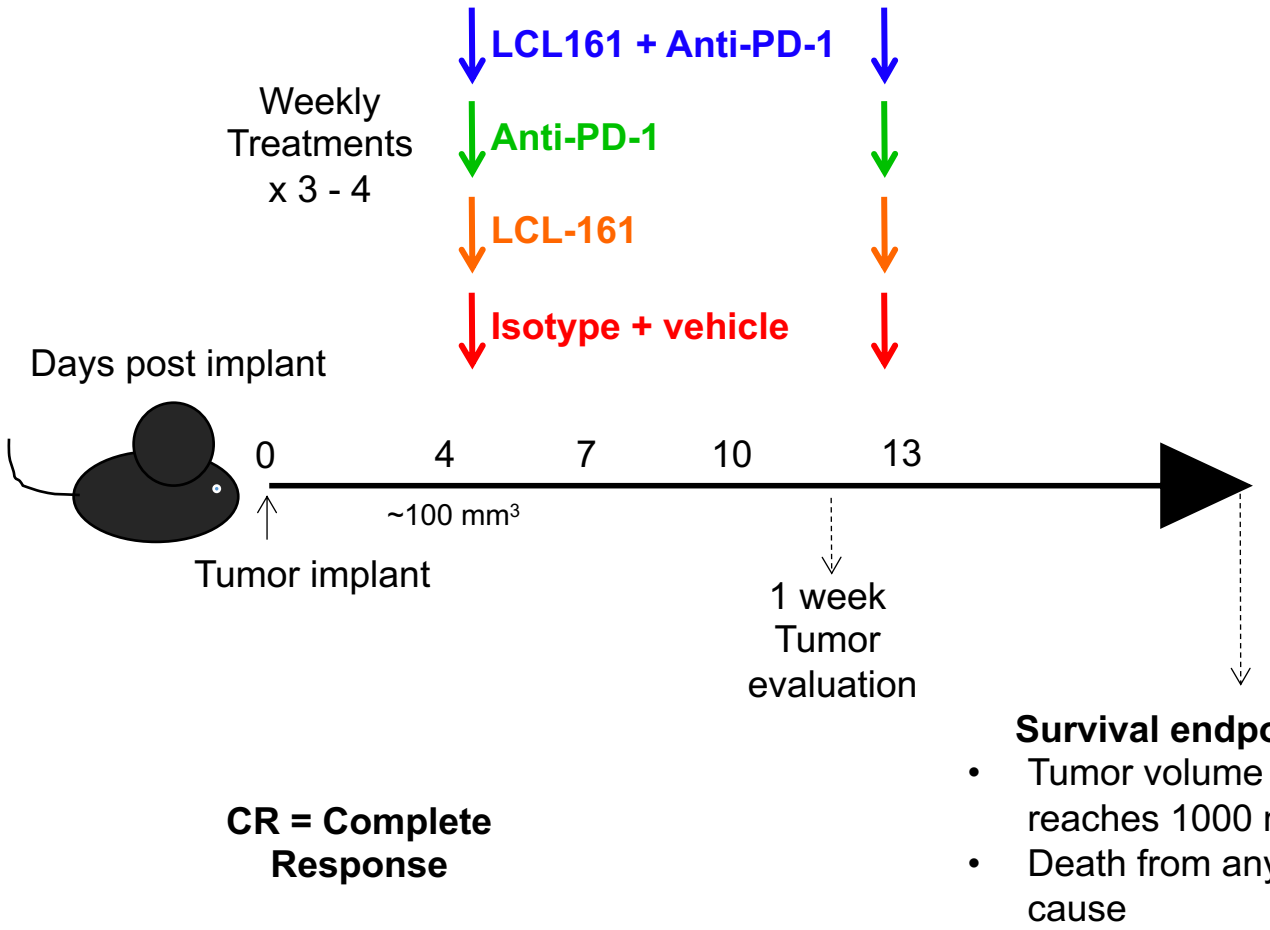




Birc2 identified as a target in loss-of-function CRISPR/Cas9 screen in cancer cells treated with anti-PD-1 therapy

Ref:
Manguso et al, Nature 2017;
Vredevoogd et al, Cell 2019.

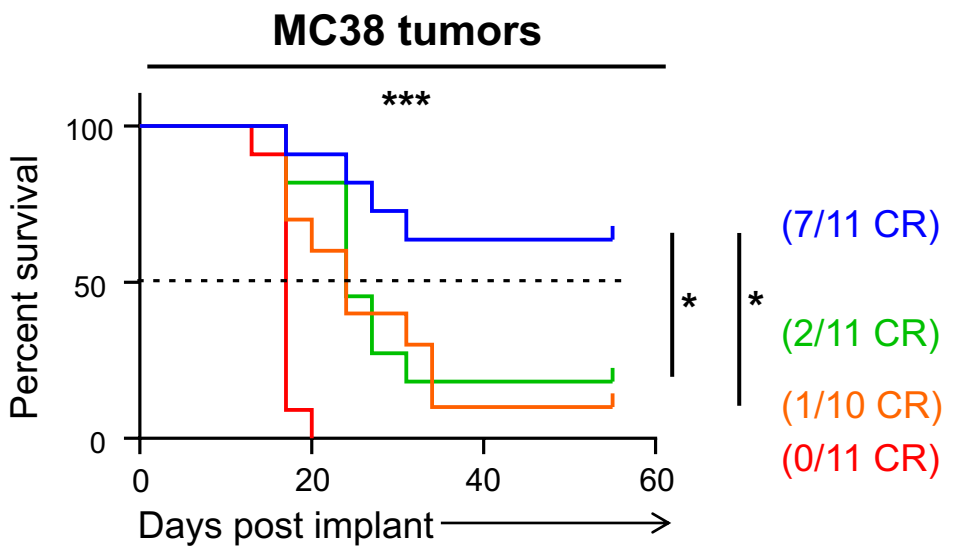
In vivo evaluation of combination of PD-1 blockade with *Birc2/3* antagonist LCL161

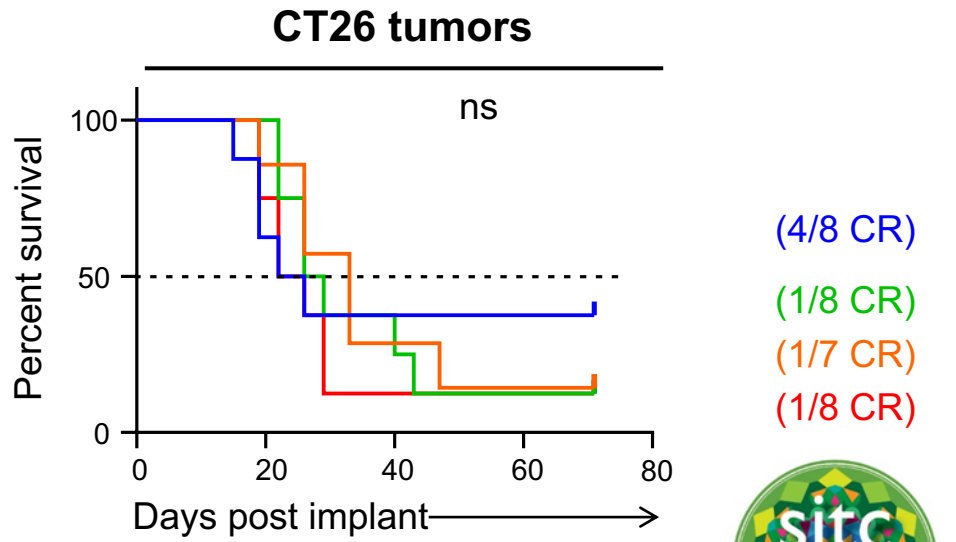


CR = Complete Response

Survival endpoint

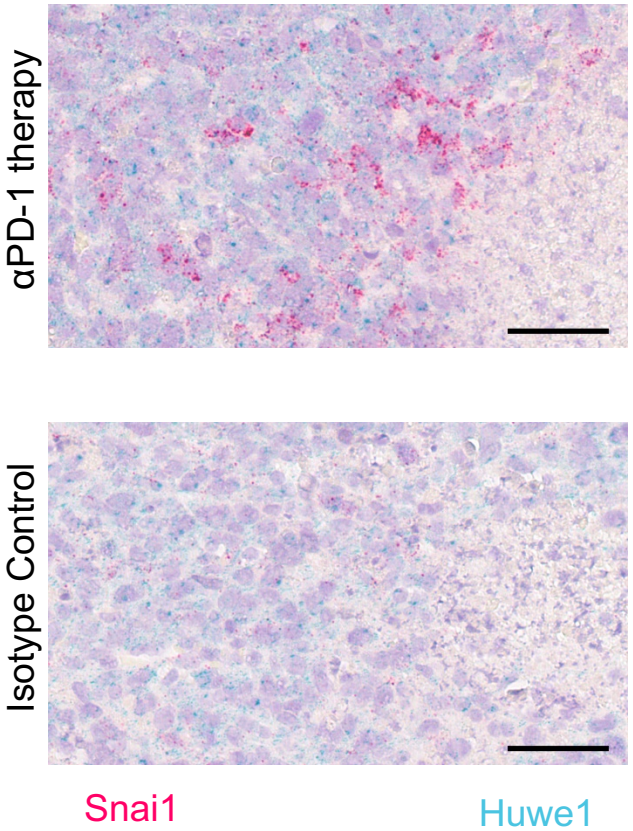
- Tumor volume reaches 1000 mm^3
- Death from any cause





Snai1 expression is a human marker for IPCs

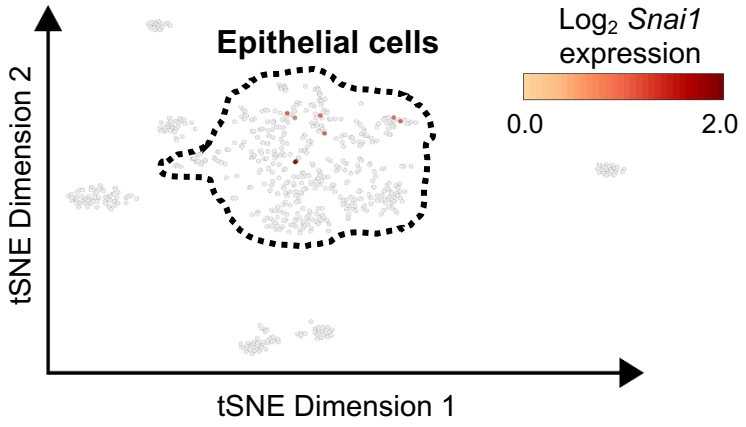
MC38 tumors *in vivo*
(1-week post one treatment)



mRNA ISH = mRNA in-situ hybridization

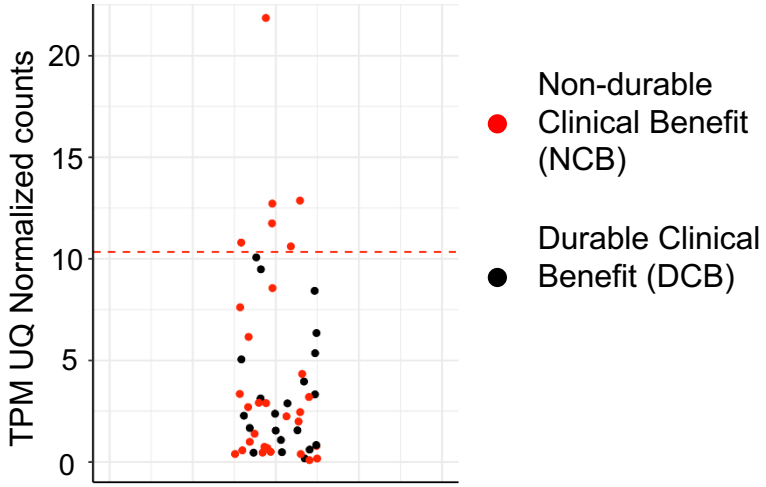
Colorectal cancer patient
Pre-treatment MSI-High tumor
(Resistant to anti-PD-1 therapy)

Single-cell RNA-seq



Riaz cohort (N = 51)
Pre-nivolumab melanoma tumor

***Snai1* expression**



	High *Snai1* expression (>90%ile)	Low *Snai1* expression (<90%ile)
DCB	0	21
NCB	6	24

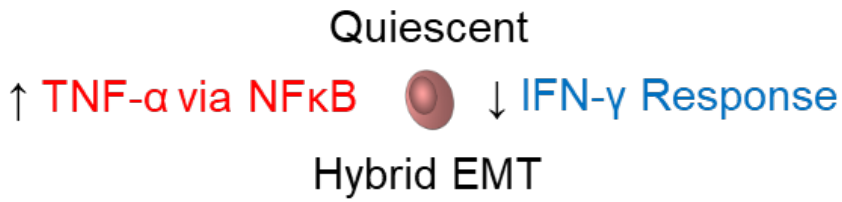
p=0.036

Ref:
Riaz et al, Cell 2017;
Gurjao et al, Cancer Immunol Res 2019.

Conclusions

Immunotherapy Persister cells, marked by Snai1 and Sca-1, represent a stem-like cancer cell subpopulation with therapeutic vulnerabilities to augment PD-1 blockade

- Functional single-cell RNA sequencing of *ex vivo* anti-PD-1 blockade uncovers immunotherapy persister cells (IPCs).
- Stem cell antigen-1 (Sca-1) and Snai1 identify IPCs which exhibit a 'stem-like phenotype'.



- Balance between IL-6 and TNF-α influences expansion of IPCs.
- *Birc*2/3 degradation markedly reduces IPCs and improves durable anti-PD-1 responses *in vivo*.
- *Snai*1 is a marker of immunotherapy persister cells that merits further evaluation as a biomarker.

Full Article available now at: https://www.jci.org/articles/view/135038

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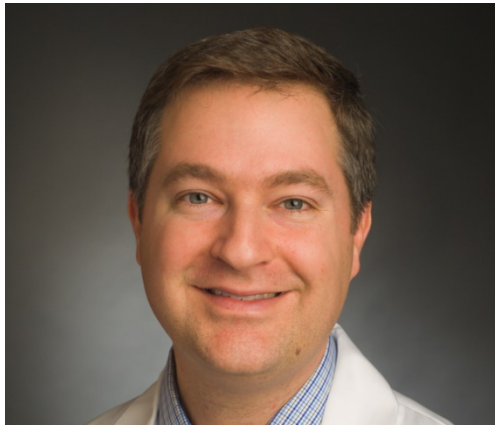

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