Defining cancer cell intrinsic signatures that predict response/resistance to immune checkpoint inhibition using single-cell RNA-sequencing in melanoma

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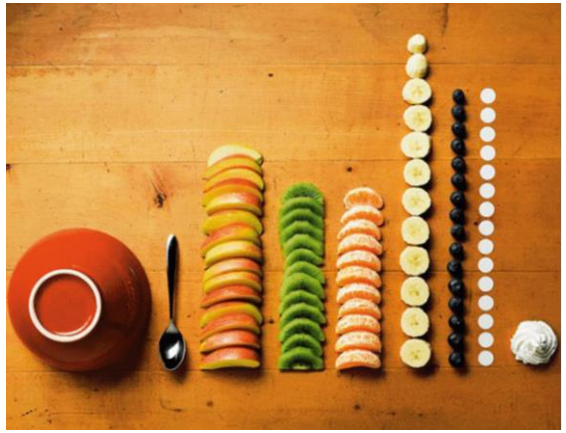
I have no disclosures.

Rationale for single-cell genomics

Example: The Cancer Genome Atlas (TCGA)

Bulk cancer genomics

Large *n, small k (=1)*



Example: Tumor Cell Atlas

Single cell cancer genomics

Small n, large k

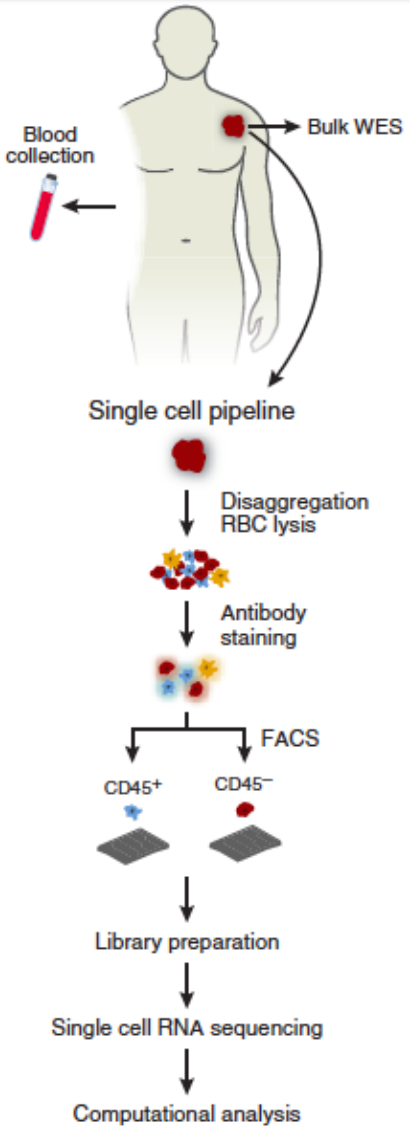
Implementing single-cell RNA-seq as translational tool

Surgeon
Med. Onc.

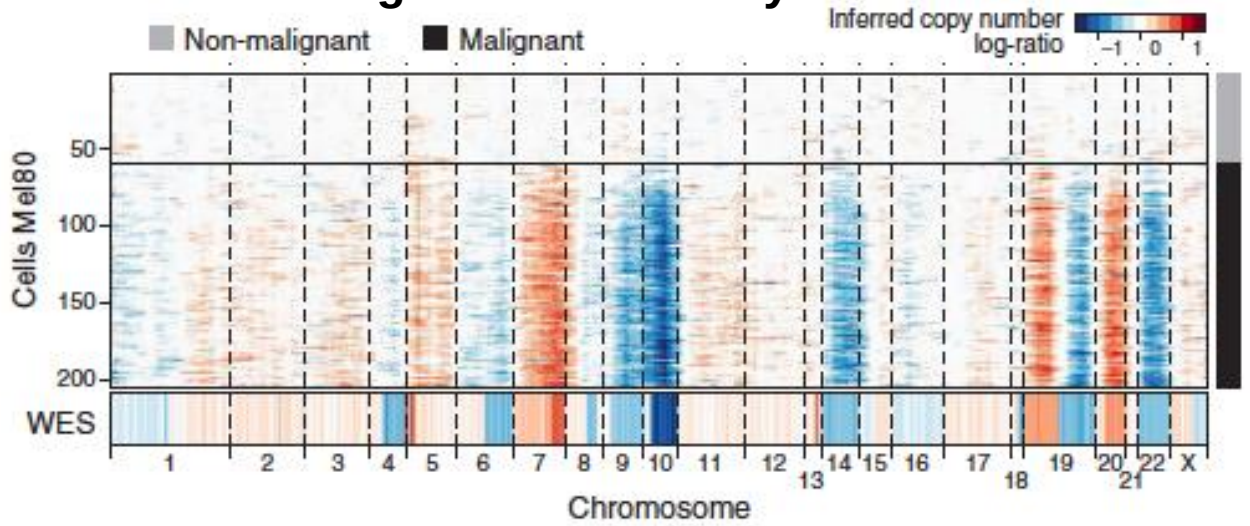
CRC

Lab
Scientist

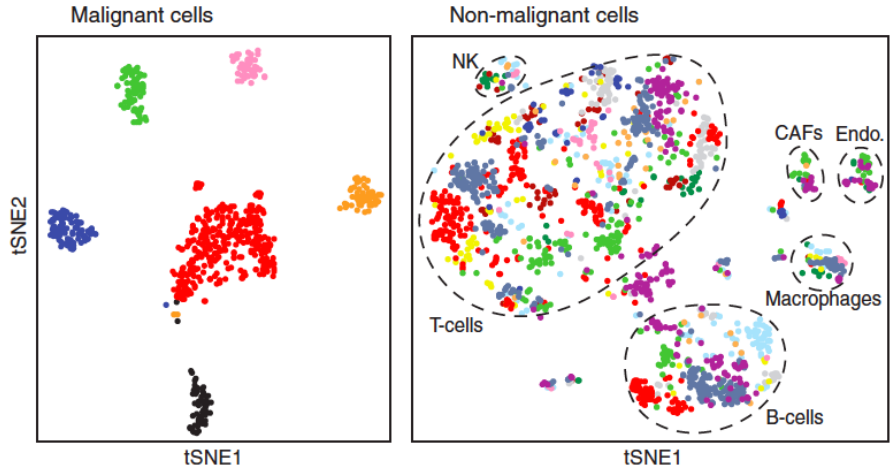
Comp. Bio



Inferring CNVs to identify cancer cells

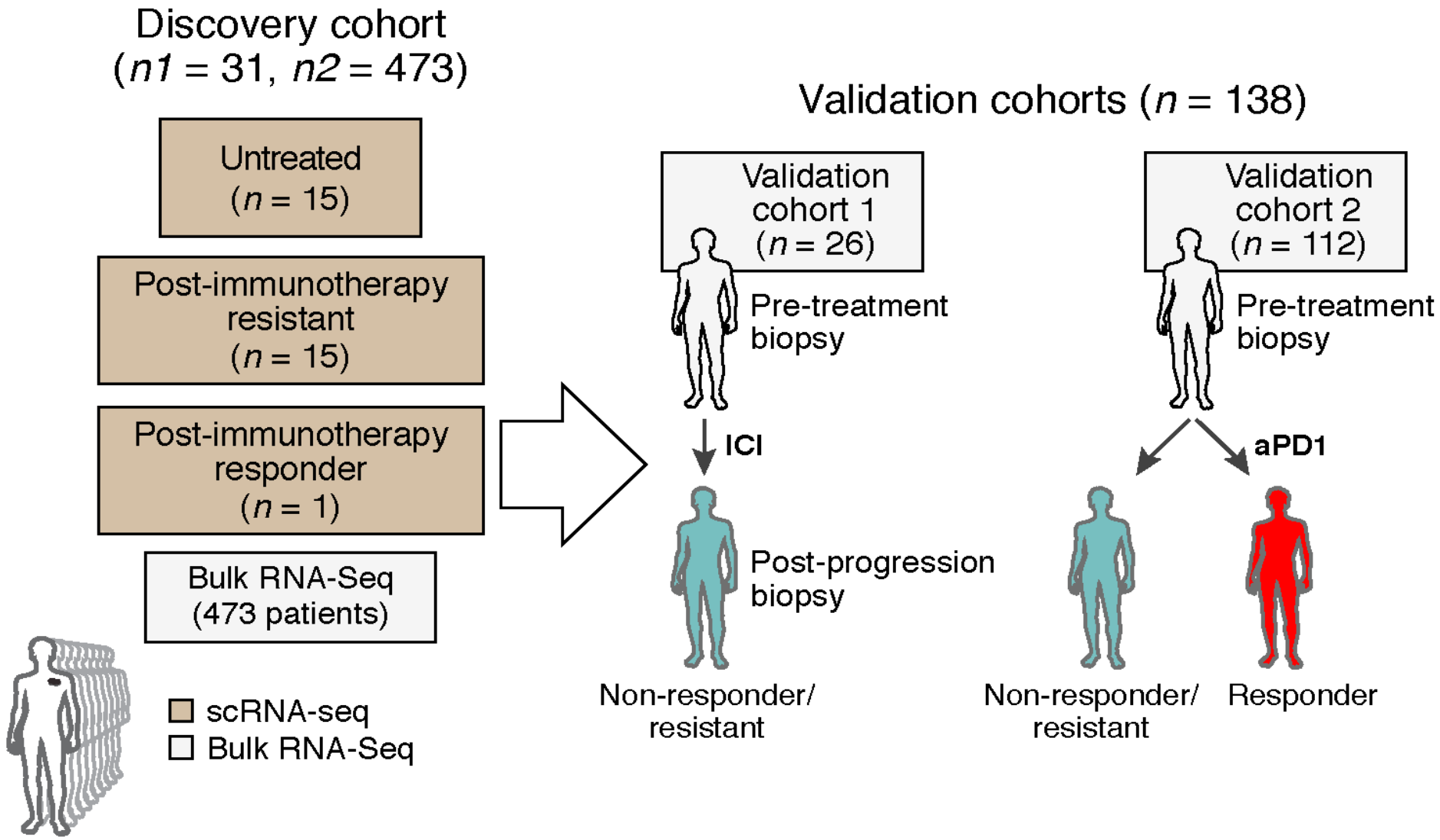


Malignant, non-malignant variability



Tirosh I*, Izar B*#, *Science*, 2016; Oral presentation at ASCO, AACR 2016 by Ben Izar

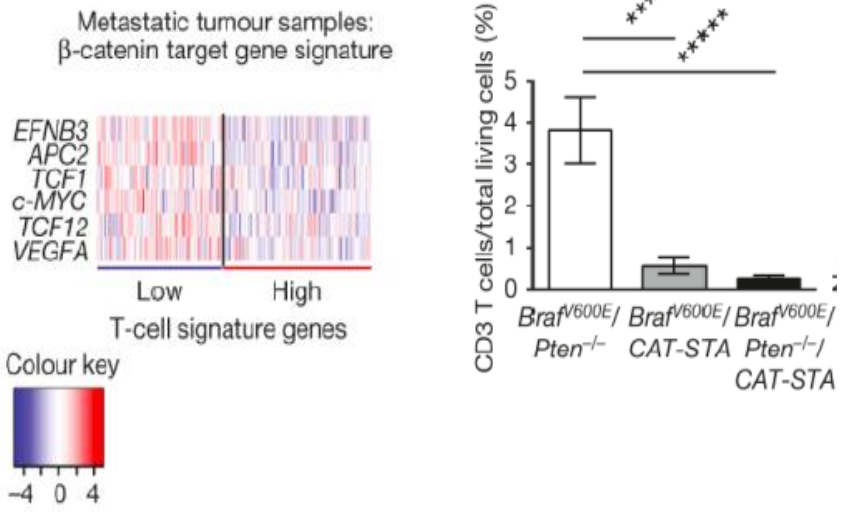
Developing predictive signatures for ICB response and resistance



Jerby,…Izar#, under review; Oral presentation at SMR 2017 and Poster at ASCO 2018, by Ben Izar

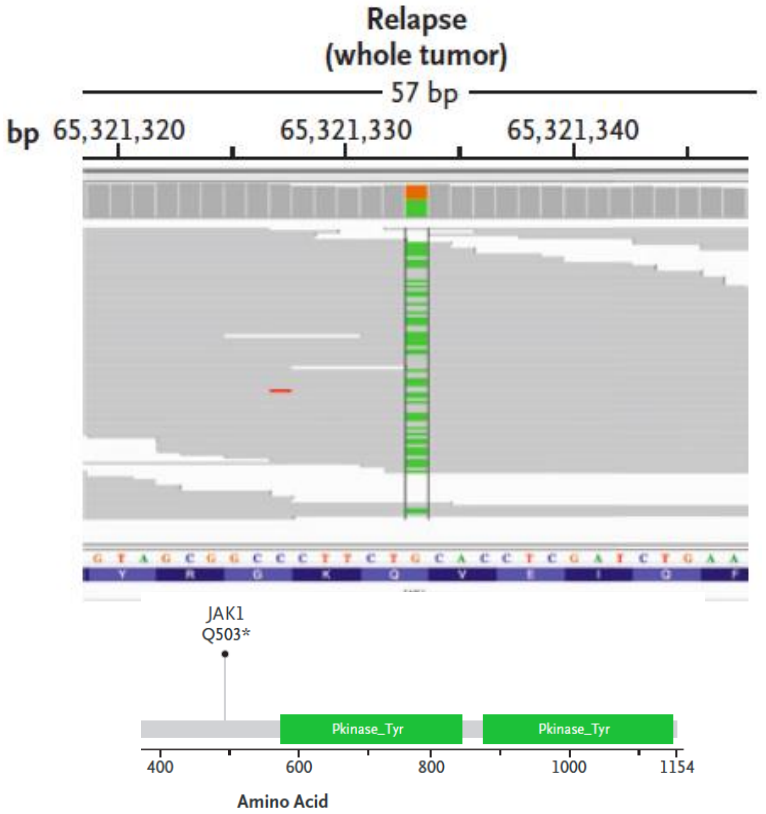
Concepts of ICB resistance (ICR)

T cell exclusion



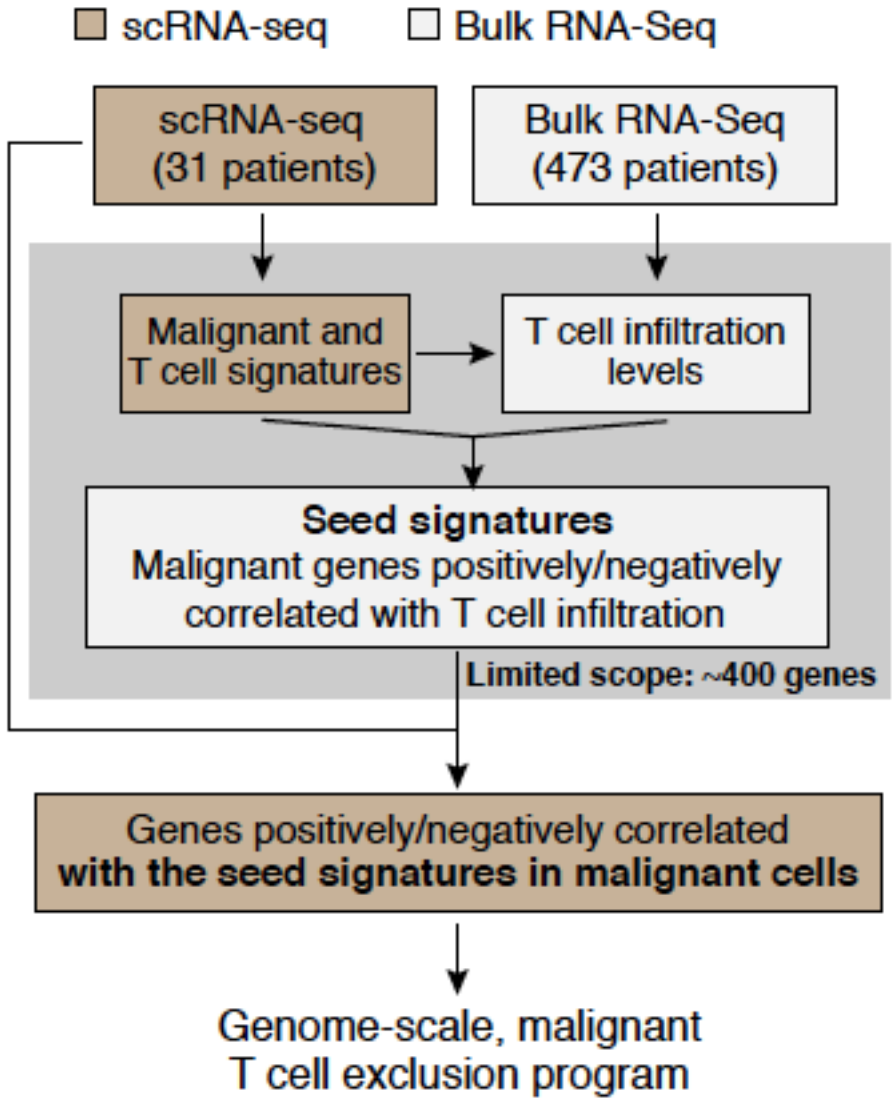
Spranger, Nature, 2015

Cancer cell intrinsic immune evasion



Zaretsky, NEJM, 2016

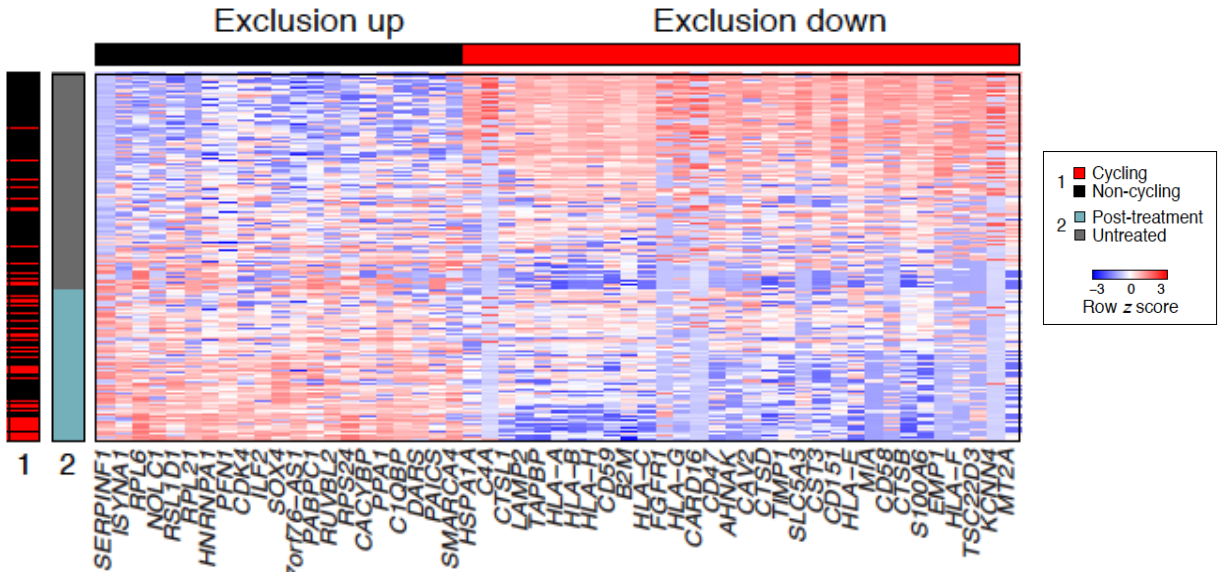
Genome-scale inference of T cell exclusion



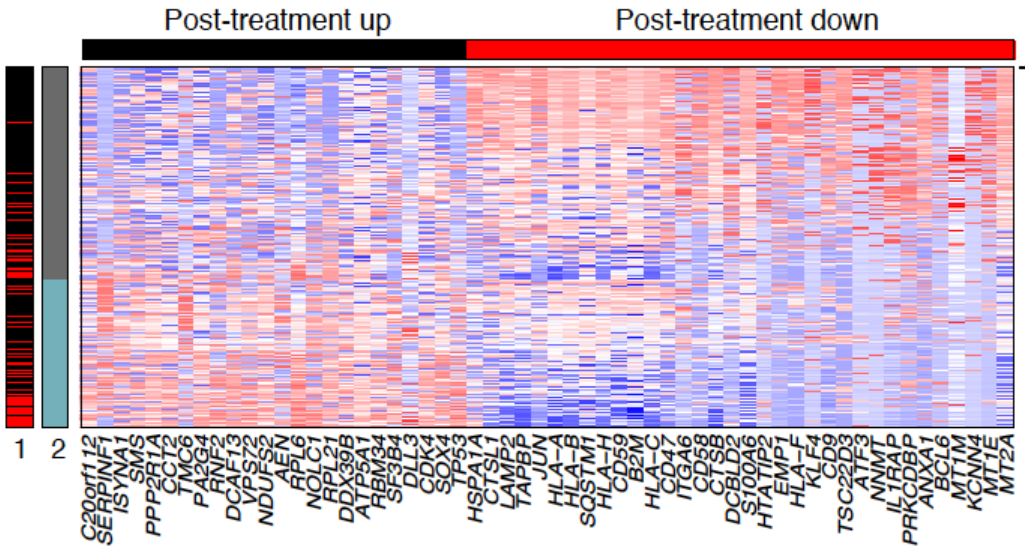
Enables discovery of cancer cell intrinsic expression of genes that promote T cell exclusion.

Genes involved in T cell exclusion and clinical resistance

Cancer cell mediated T cell exclusion



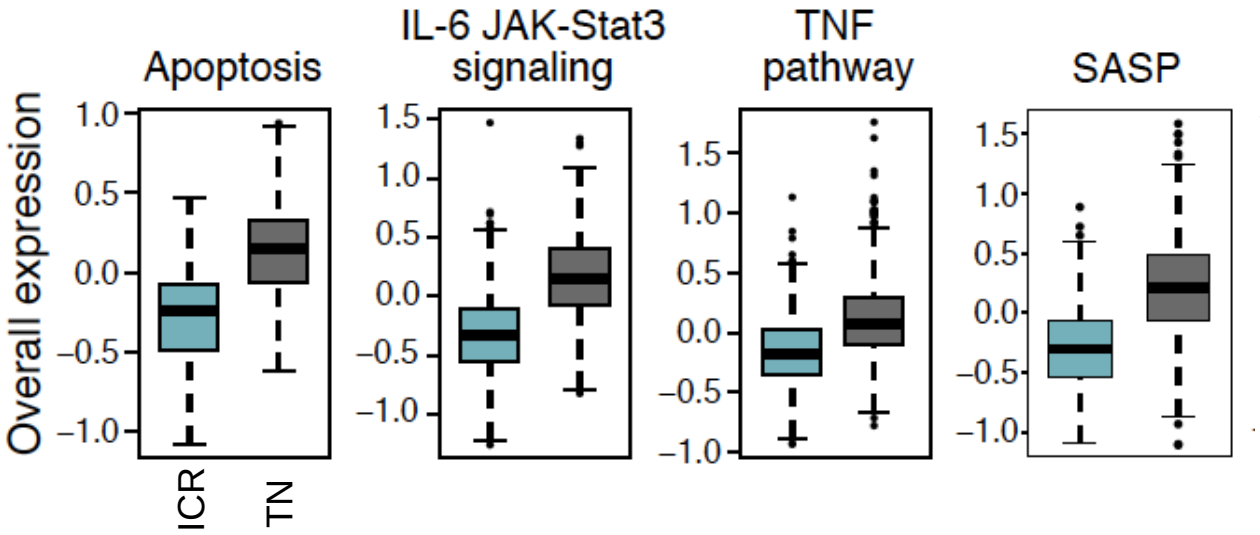
Cancer cells from ICR lesions



Union of T cell exclusion and clinical drug resistance (immune evasion)

= <u>resistance program.</u>

Biology reflected in the resistance program



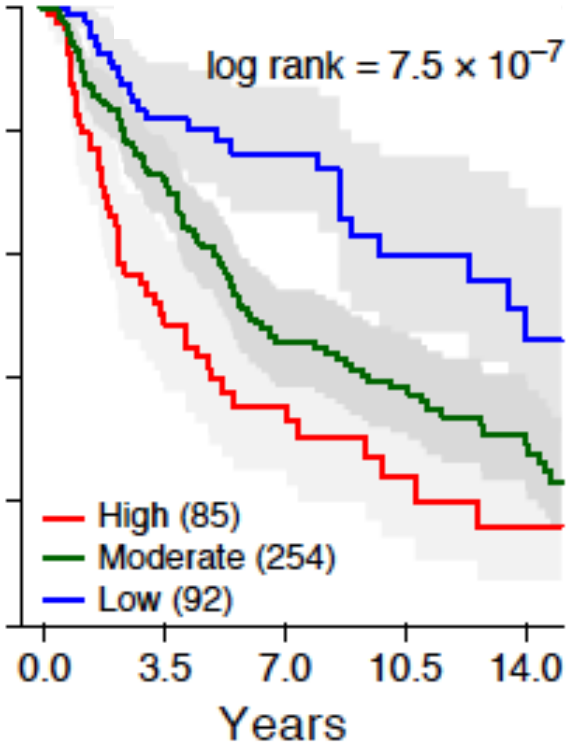
=Senescence-associated secretory phenotype

SOX4 (Pan, Science, 2018)
SMARCA4 (Pan, Science, 2018)
RNF2 (Liu, Nat Immunol, 2018)
PTPTN2 (Manguso, Nature, 2017)
CD58 (Patel, Nature, 2017)
DLL3 (Rudin, Lancet Oncol, 2017)

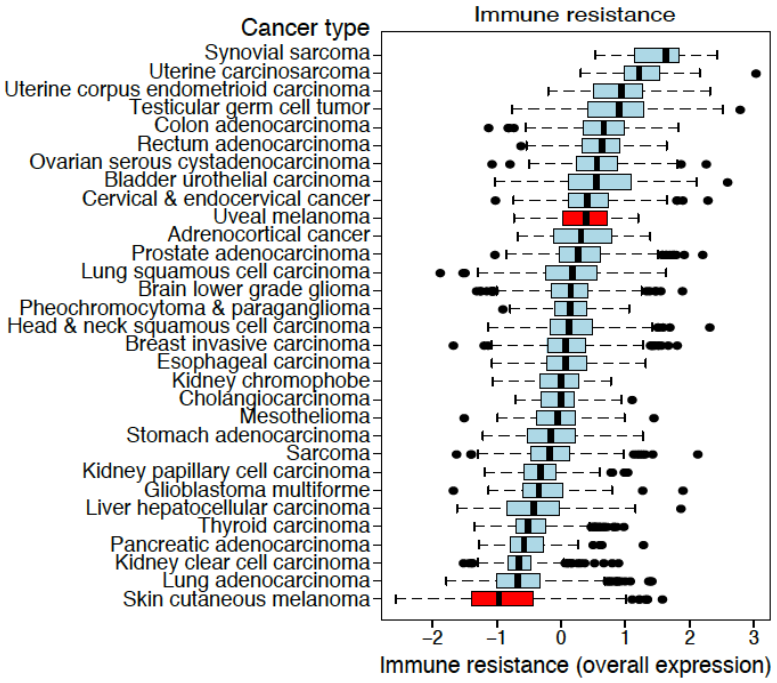
The resistance signature includes known and novel biology, and is coherently regulated.

Expression, prognostic, predictive value in external data

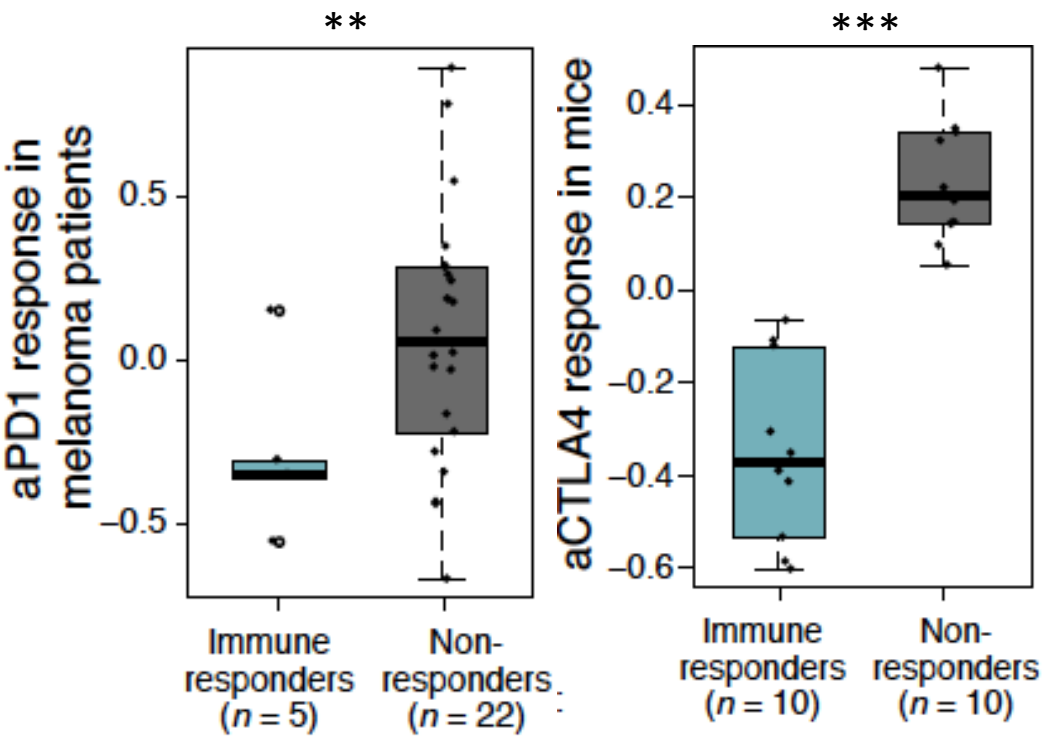
Prognostic value in TCGA



Pan-cancer expression



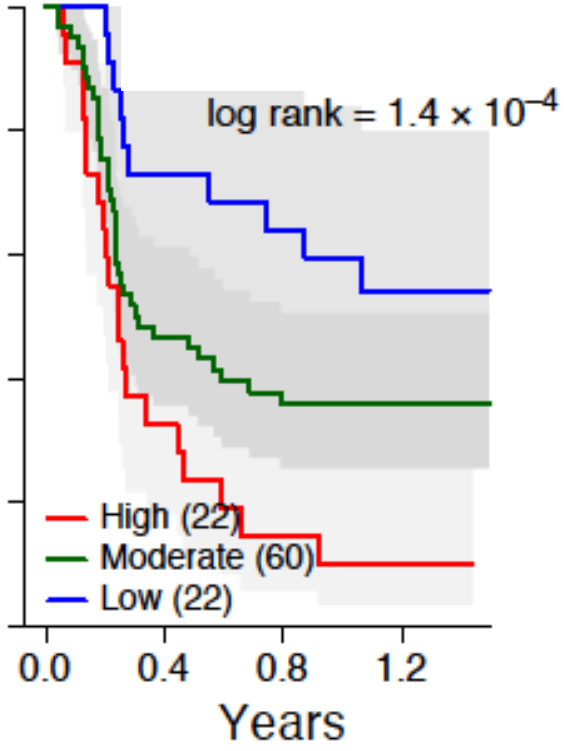
Predictive value in external data



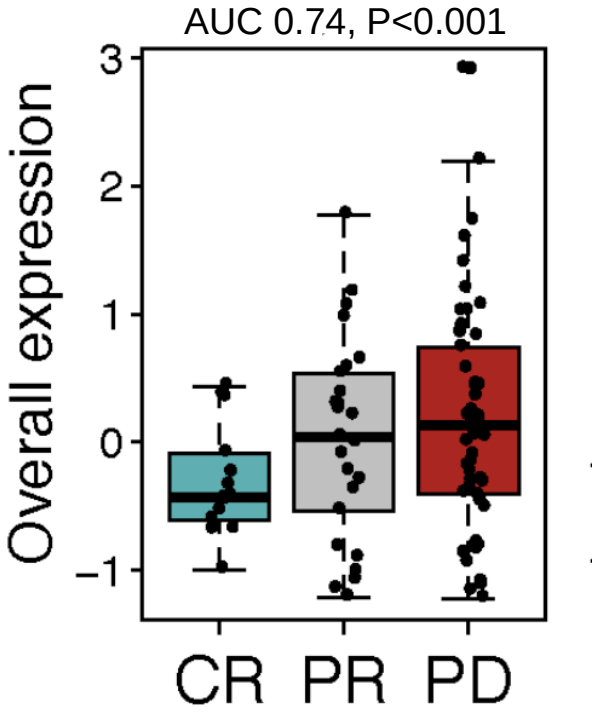
Data sets: Hugo, Cell, 2017; Lesterhuis, Sci Rep, 2015

Predictive value for PFS and OR in 112 melanoma patients treated with anti-PD-1

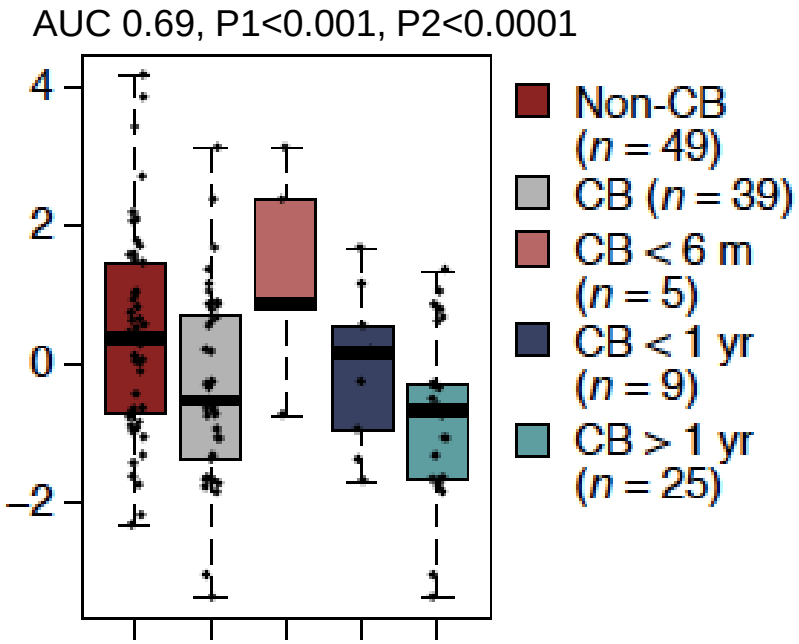
Progression-free survival



RECIST responses



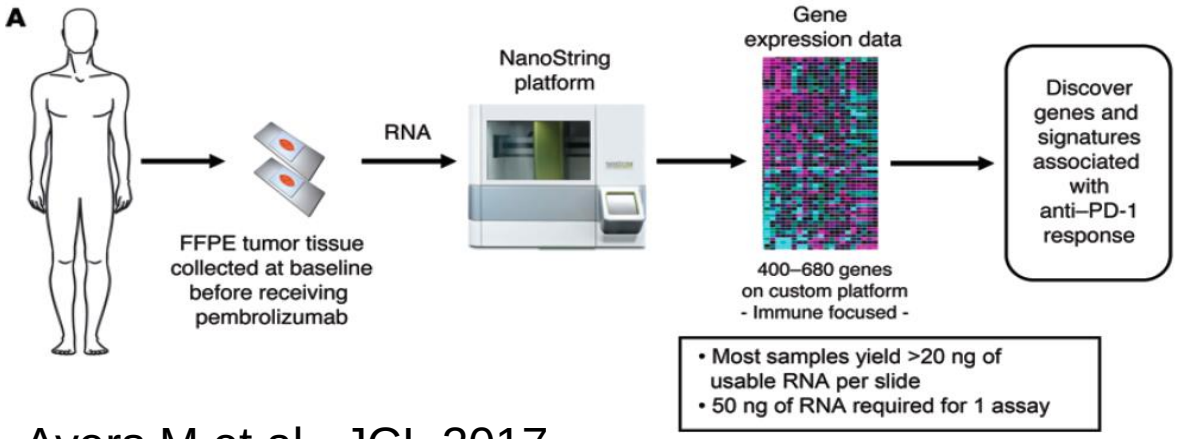
Clinical Benefit (CR/PR)



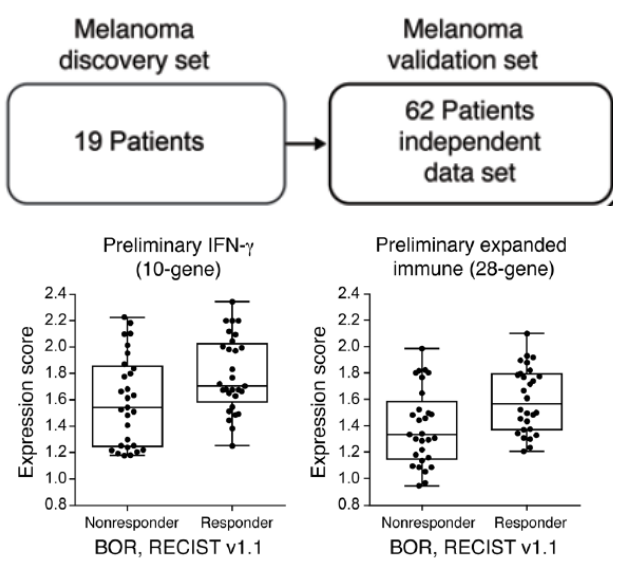
The resistance signature has predictive value in a large validation cohort.

1. **Identifies "rapid progressors"**
2. **Complete responders**

Other RNA-based predictors for response to ICB

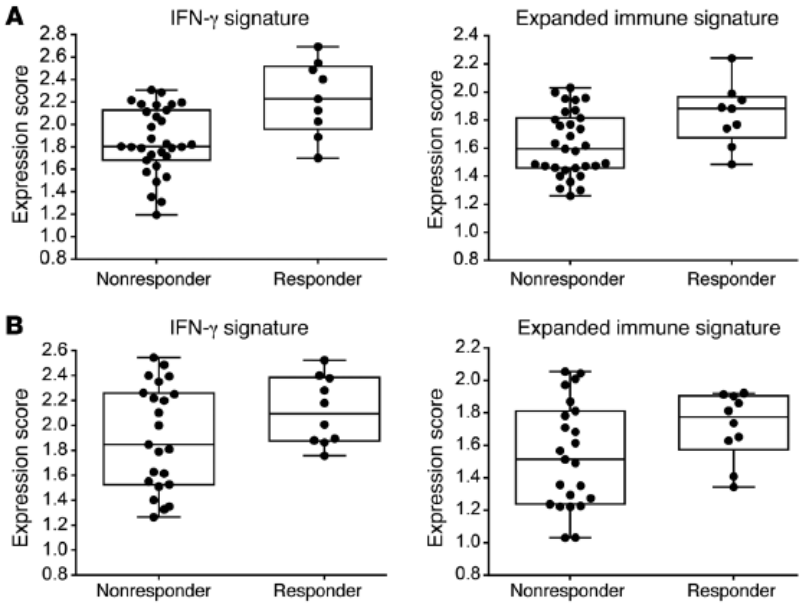


Ayers M et al., JCI, 2017



Final signatures

IFN-γ	Expanded immune gene signature	
IDO1	*CD3D*	*IL2RG*
CXCL10	*IDO1*	*NKG7*
CXCL9	*CIITA*	*HLA-E*
HLA-DRA	*CD3E*	*CXCR6*
STAT1	*CCL5*	*LAG3*
IFNG	*GZMK*	*TAGAP*
	CD2	*CXCL10*
	HLA-DRA	*STAT1*
	CXCL13	*GZMB*



Signature	Nominal 1-sided *P* value[A] BOR[B]	PFS
Head and neck cohort	*n* = 40	*n* = 43
IFN-γ signature (6 genes)	0.005[C]	<0.001[C]
Expanded immune signature (18 genes)	0.015	<0.001
Gastric cohort	*n* = 33	*n* = 33
IFN-γ signature (6-gene)	0.077	0.032
Expanded immune signature (18-gene)	0.062	0.049

Biologically plausible RNA-based signatures are useful predictors for ICB.

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
IASLC 18TH WORLD CONFERENCE ON LUNG CANCER
October 15–18, 2017 | Yokohama, Japan
WWW.IASLC.ORG

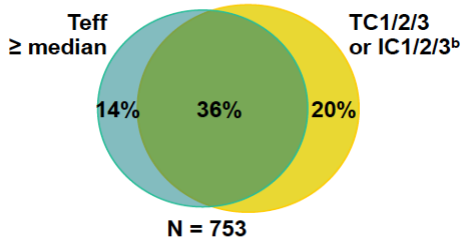
Pre-Existing Immunity Measured by Teff Gene Expression in Tumor Tissue Is Associated With Atezolizumab Efficacy in NSCLC

Marcin Kowanetz,[1] Wei Zou,[1] Mark McCleland,[1] David R. Gandara,[2] Shirish Gadgeel,[3] Achim Rittmeyer,[4] Fabrice Barlesi,[5] Keunchil Park,[6] David Shames,[1] Hartmut Koeppen,[1] Marcus Ballinger,[1] Alan Sandler,[1] Priti Hegde[1]

Teff Gene Signature	
PDL1	PD-L1 expression on TC and IC
IFNG	Pre-existing immunity
CXCL9	Pre-existing immunity

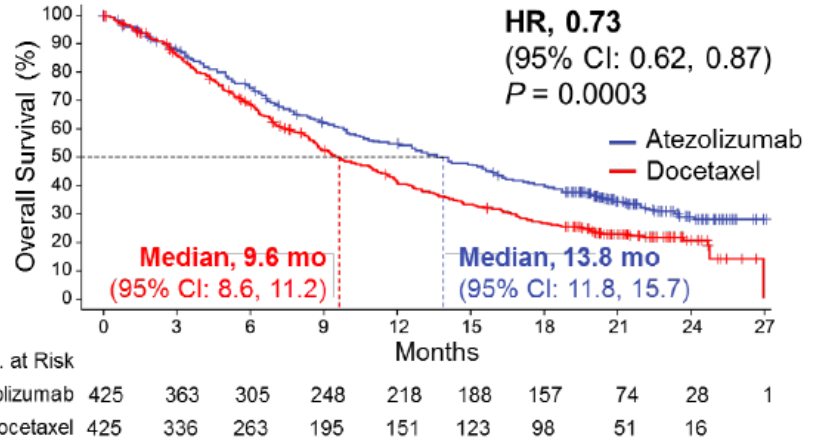
Derived from a 9-gene-signature from Fehrenbacher L, et al. Lancet. 2016

Teff Gene Signature vs PD-L1 IHC (SP142)[a]



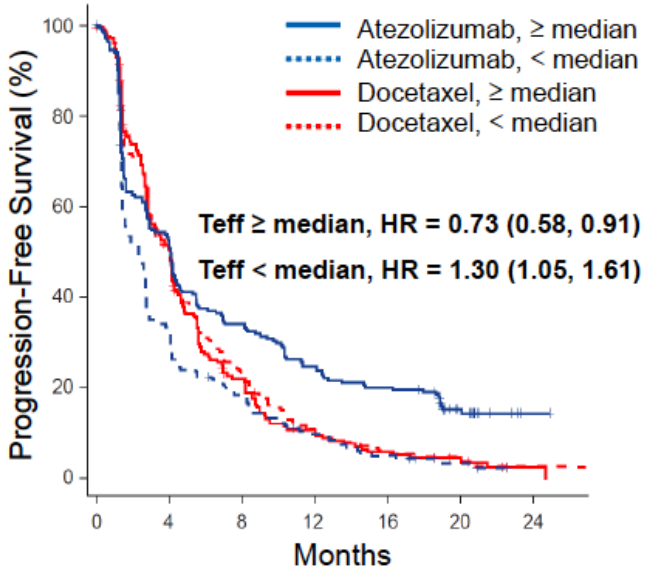
Surrogate for PD-L1 IHC and "pre-existing" immunity.

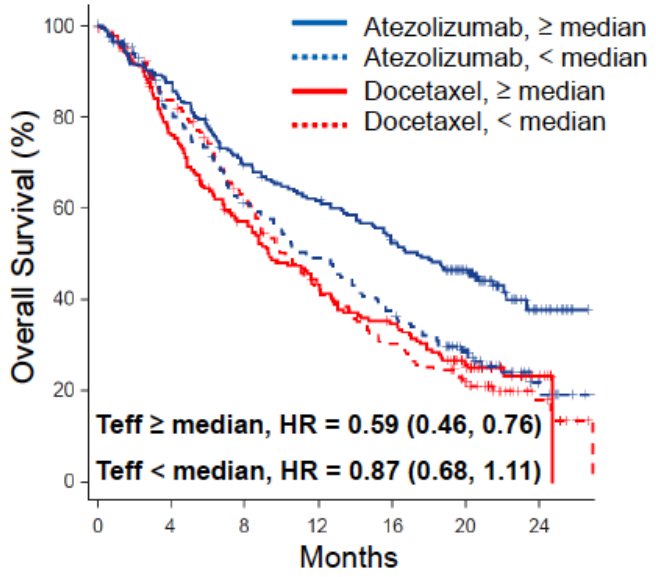
OAK Primary Analysis



Improved OS irrespective of PD-L1 IHC (>1% on TC *or* IC)

Rittmeyer A et al., Lancet, 2017





RNA-based signatures enrich for patients more likely to respond to ICB.

Summary

- Identification and validation of a prognostic and predictive signature by integration of single-cell RNA-seq and bulk-RNA sequencing

- mRNA-based signatures may be applicable across lineages.

- mRNA based predictive signatures are entering clinical trials.

Challenges and opportunities

- Comparison of data generated on different platforms
- Correcting for increasingly recognized confounders (i.e. TIL infiltration)
- **Integration with imaging platforms and use of spatial transcriptomics**
- **Pharmacologically targeting the transcriptome**

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- Chris Rodman
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- **Kai Wucherpfennig**
- Shruti Malu (Eli Lilly)
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- Meri Rogava*
- **Andrew Aguirre**
- Marios Giannakis
- Eli van Allen
- Adam Cartwright
- Charles Thomas

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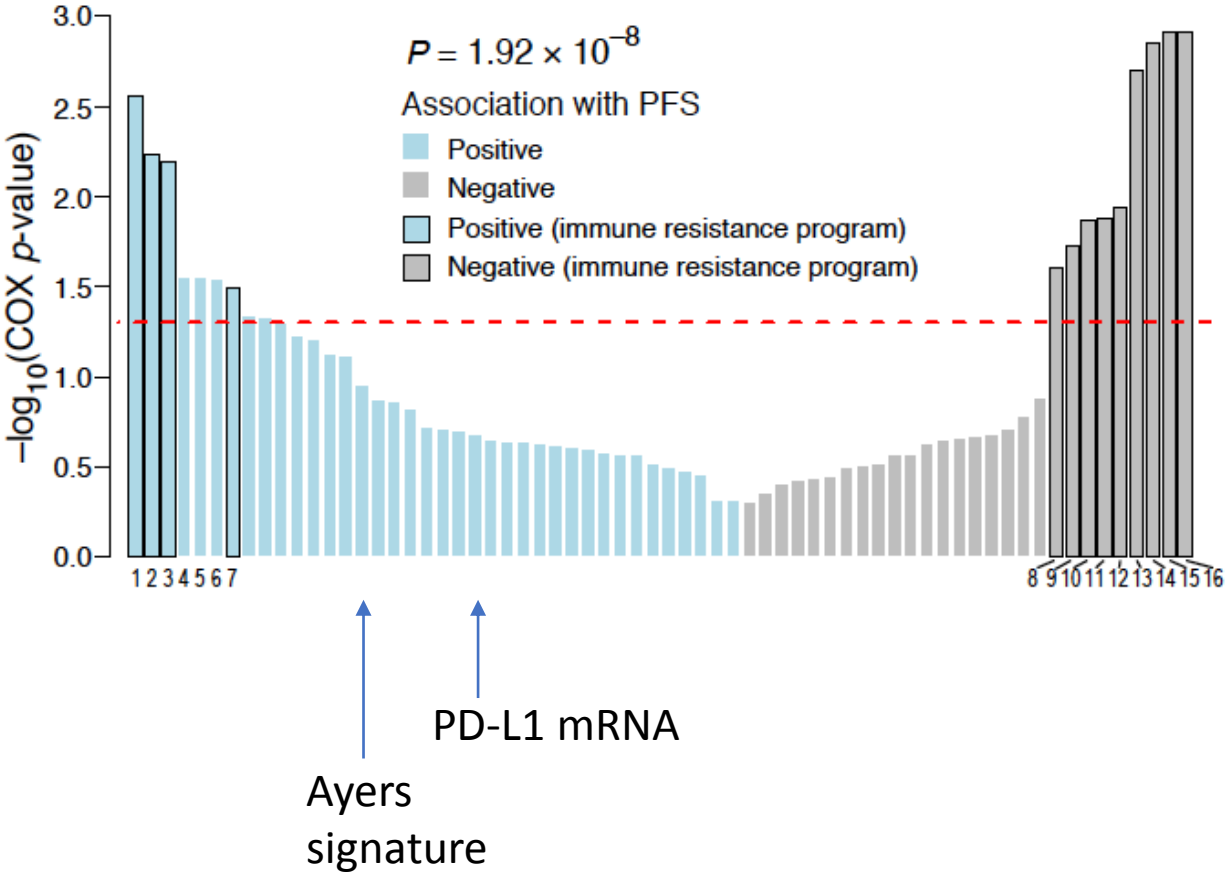
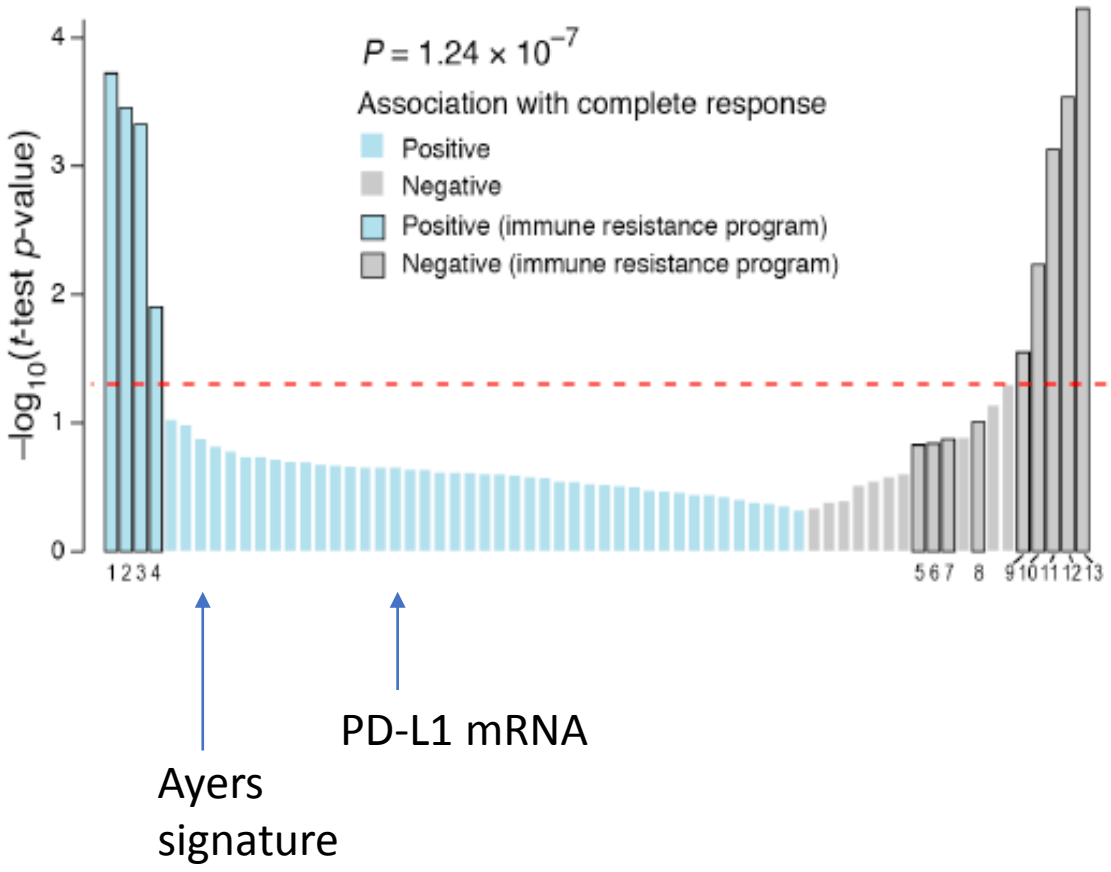
BMS-SITC Translational Immunotherapy Fellowship
Ludwig Center for Cancer Research at Harvard
Burroughs Wellcome Fund Career Award for Medical Scientists
Broad*Next*10 Program
Klarman Cell Observatory

Comparison of other signatures in our validation cohort

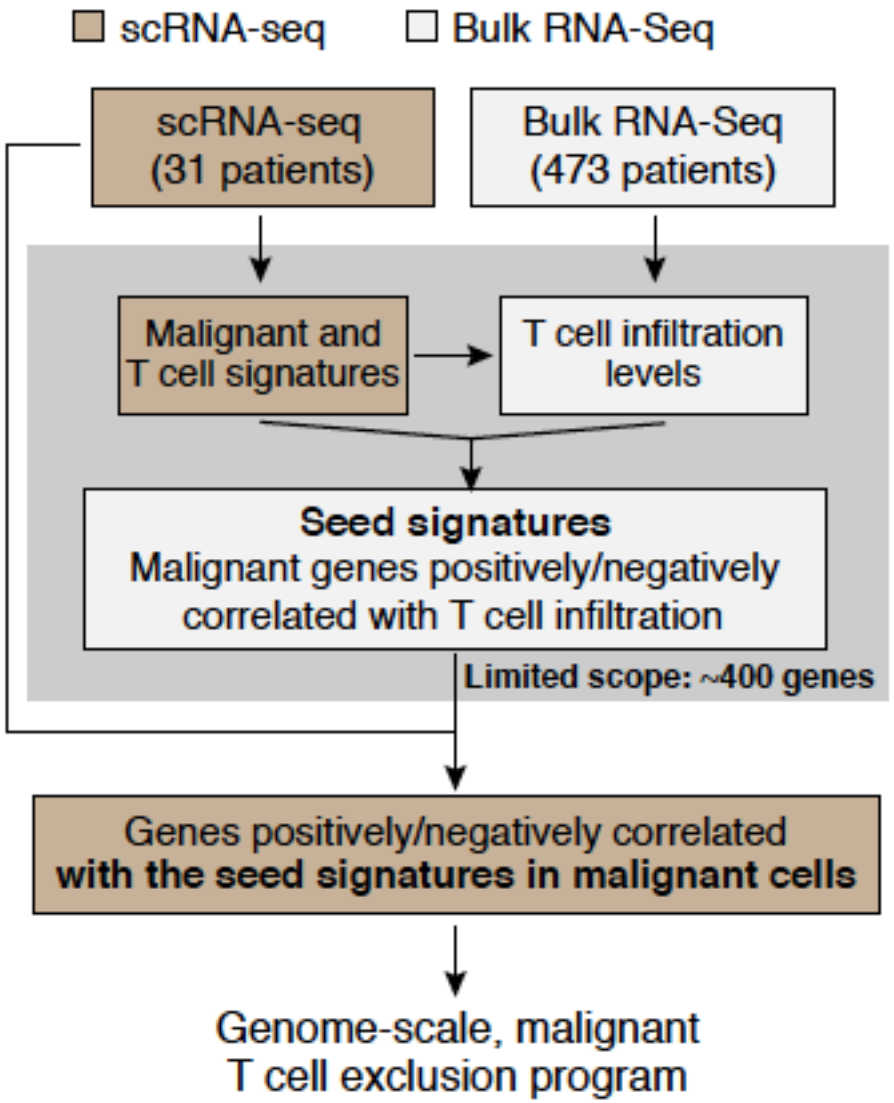
Association with PFS compared to other RNA signatures

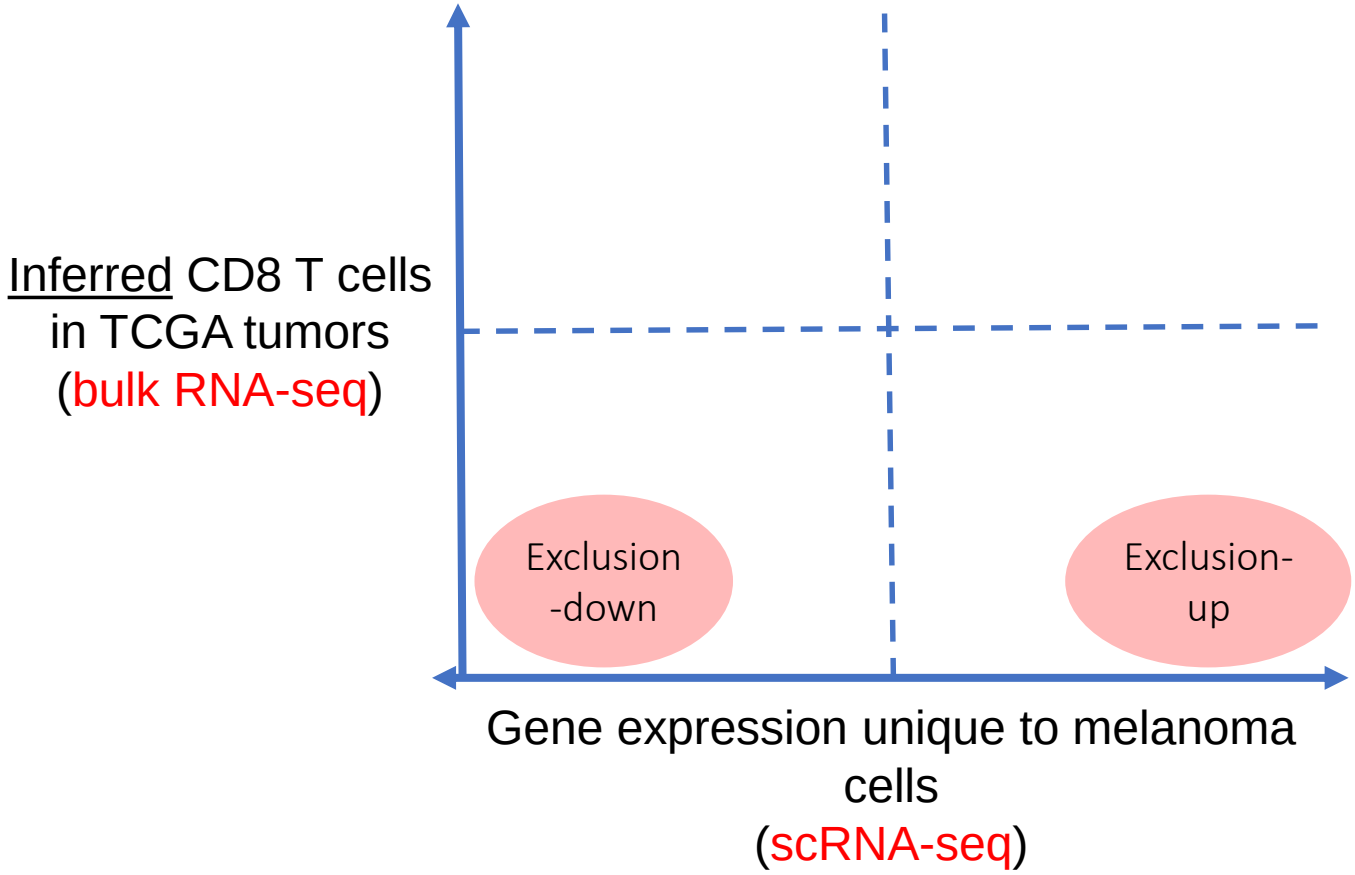


Association with CR compared to other signatures



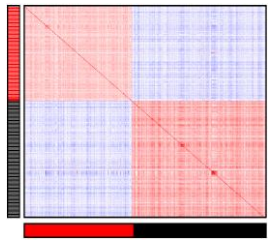
Genome-scale inference of T cell exclusion





Target the resistance transcriptome

Published large-scale screen with matched GE



X

= reduction in cell viability

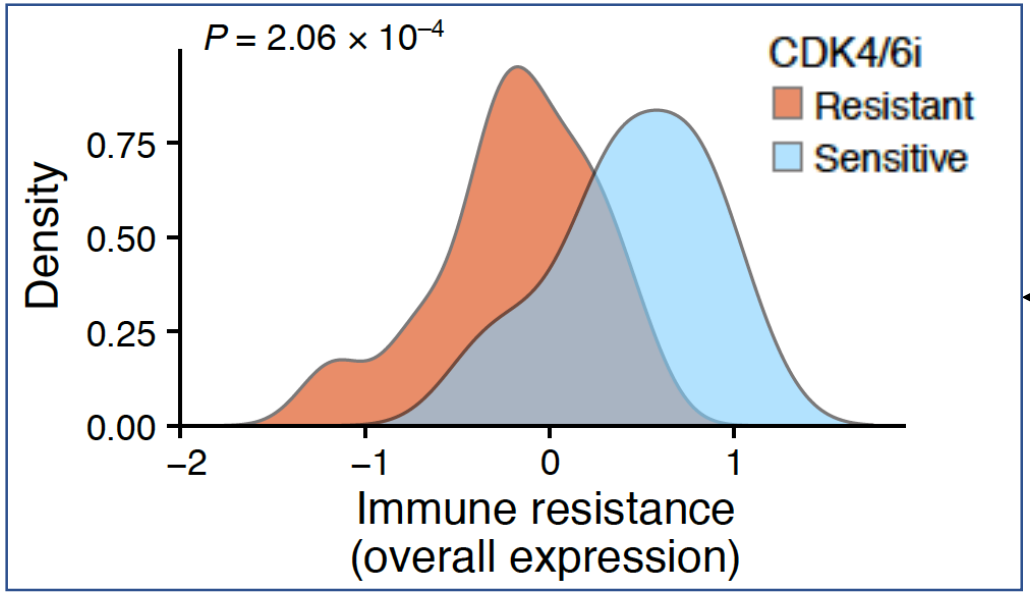
Gene expression
> 600 cell lines

n=131 compounds

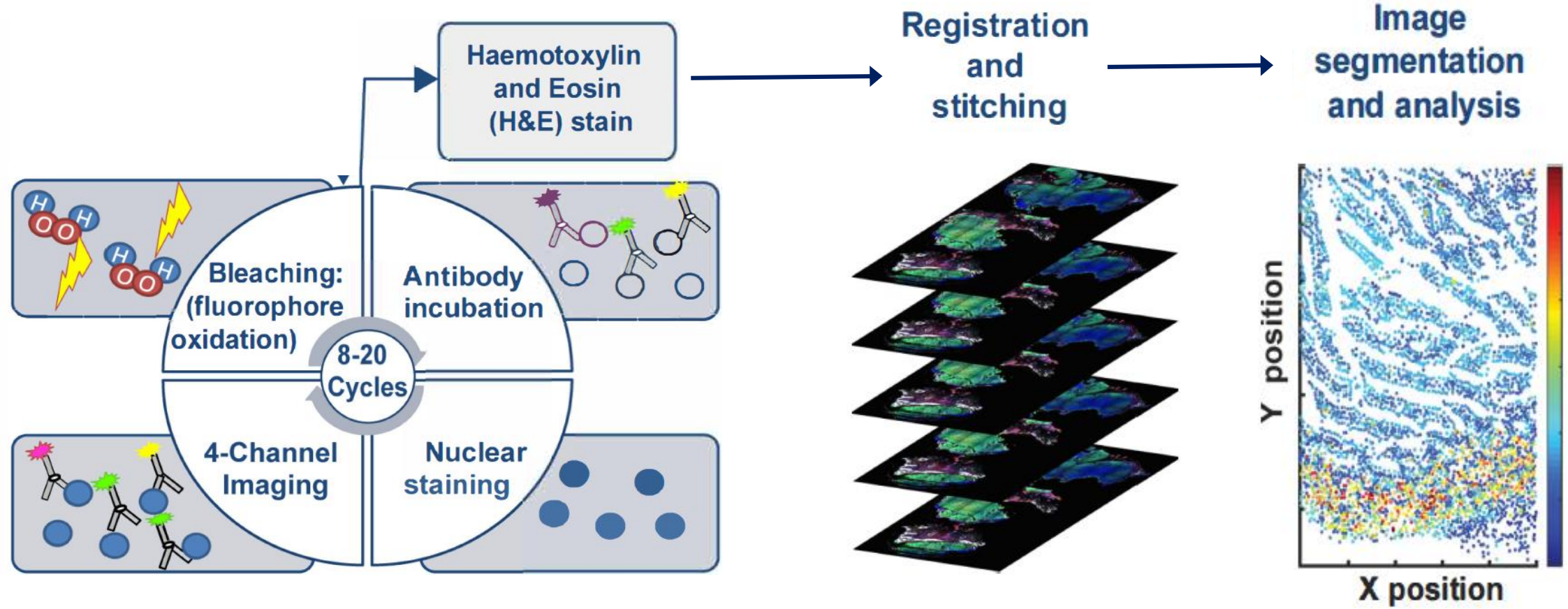
Garnett, Nature, 2012

1. Measured expression of resistance program
2. Identified compounds that selectively reduce viability in high-expressing cell lines

CDK4/6 inhibition is predicted to reduce viability in cell lines with high expression of the resistance program.



Highly-multiplexed imaging for single-cell analyses of FFPE specimens



With Jerry Lin and Peter Sorger, Laboratory for Systems Pharmacology, Harvard Medical School

Lin*, Izar*, eLife, 2018 (accepted)
Presented at SMR 2017 by Ben Izar

Tissue cyclic immunofluorescence (t-CyCIF)