isoplexis

Precision Profiling

…predictive single-cell response.

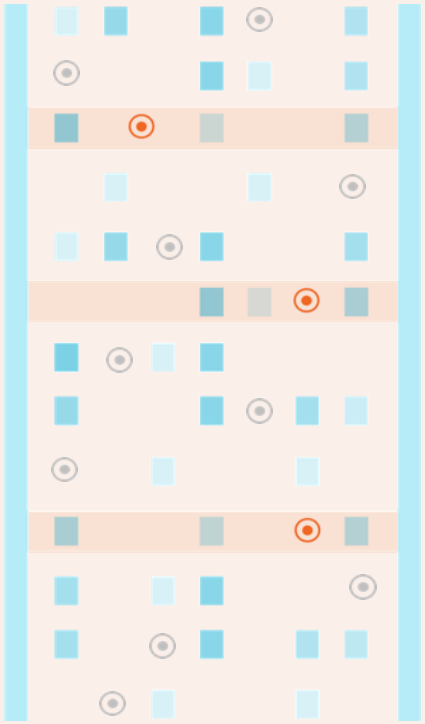
November 9th, 2017

This presentation is not available for CME/CE credit

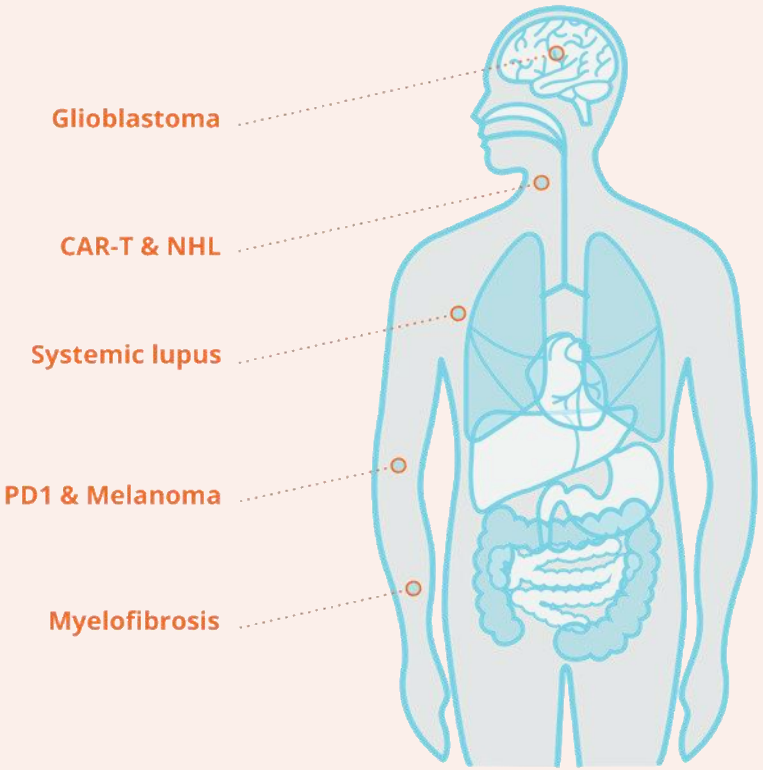
Target the right therapies, as early as possible

to highest urgency cancer patients

Predictive Single Cell Response

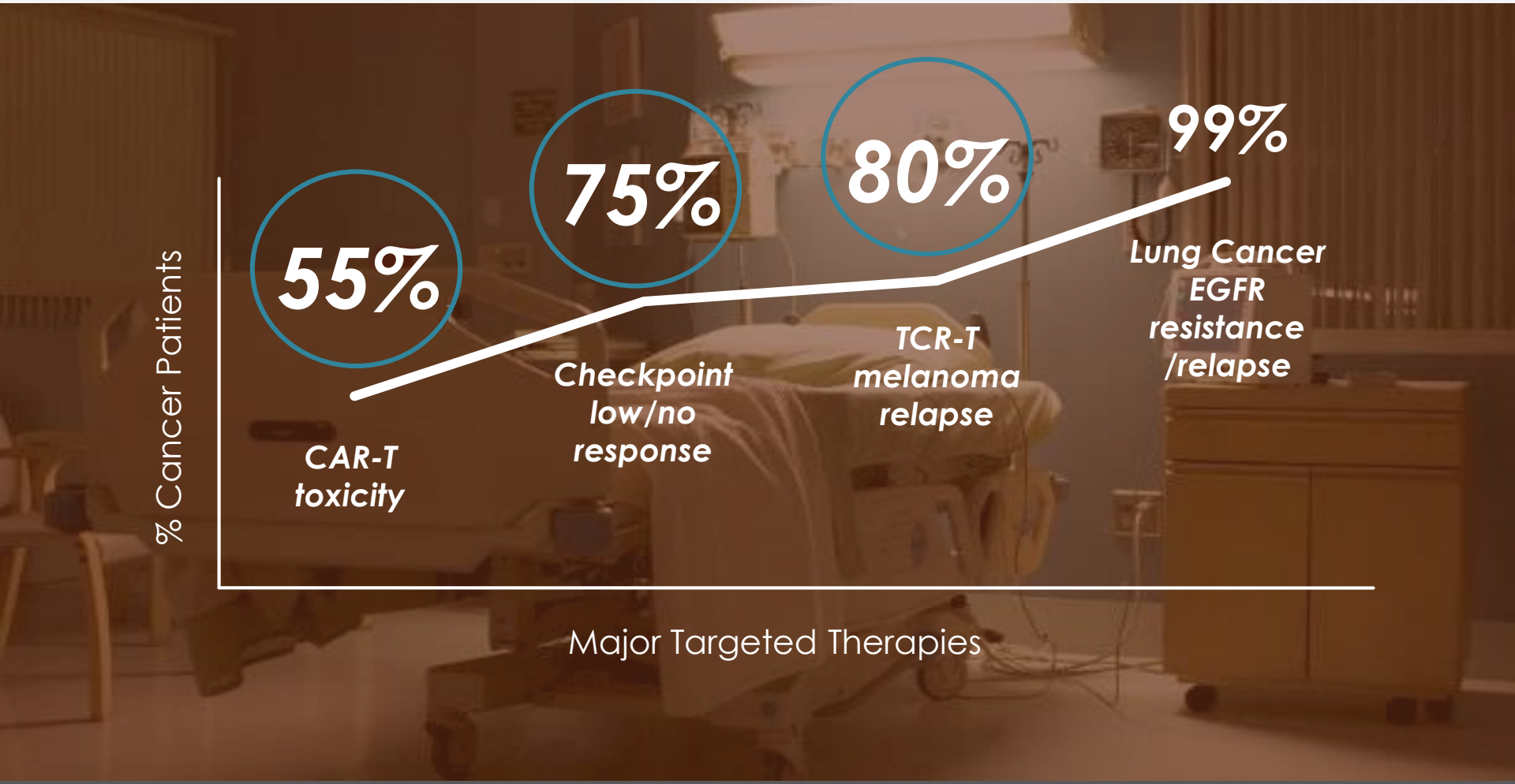


Precision Profiling



Three broader challenges in cancer immunotherapy

IsoPlexis aims to lower three major statistics in immuno-oncology



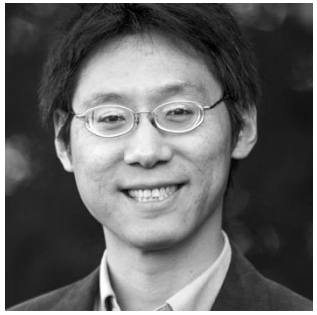
IsoPlexis founding scientists & mission

Lead immuno-oncology clinical and engineering areas, urgent applications of predictive T-cell response

Antoni Ribas, MD, PhD
UCLA

Arnie Levine, PhD
Princeton

Jim Heath, PhD
Caltech



Rong Fan, PhD
Yale

Ross Levine, MD
MSK

David Ho, MD
ADARC

Functional capacity of antigen-specific T cells

Polyfunctionality correlates with quality and durability in patient responses

Nature Reviews Immunology **8**, 247–258 (1 April 2008) | doi:10.1038/nri2274

T-cell quality in memory and protection: implications for vaccine design

Robert A. Seder , Patricia A. Darrah & Mario Roederer

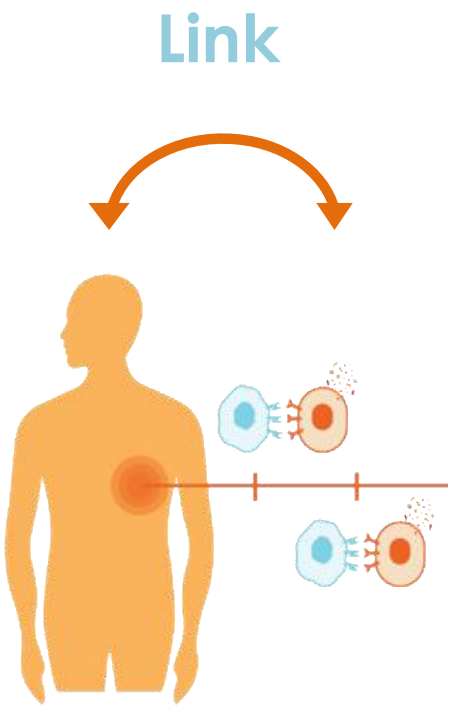
T cells mediate effector functions through a variety of mechanisms. Recently, multiparameter flow cytometry has allowed a simultaneous assessment of the phenotype and multiple effector functions of single T cells; the delineation of T cells into distinct functional populations defines the quality of the response. New evidence suggests that the quality of T-cell responses is crucial for determining the disease outcome to various infections. This Review highlights the importance of using multiparameter flow cytometry to better understand the functional capacity of effector and memory T-cell responses, thereby enabling the development of preventative and therapeutic vaccine strategies for infections.

nature REVIEWS IMMUNOLOGY

Proposes that **polyfunctionality**, the ability for a T cell to co-produce multiple cytokines, is likely a better correlate to the quality of T cells in memory and protection.

IsoPlexis uniquely addresses challenges in I-O

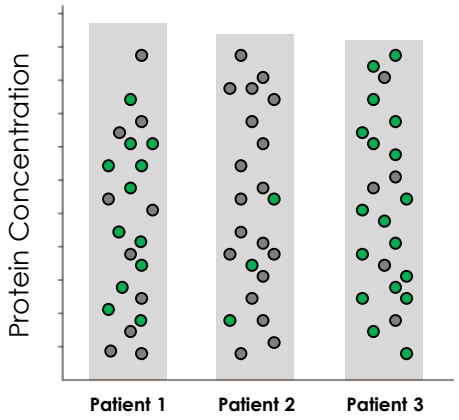
IsoCode assay links T-cell function to patient outcome



IsoCode's more sensitive detection can lead to

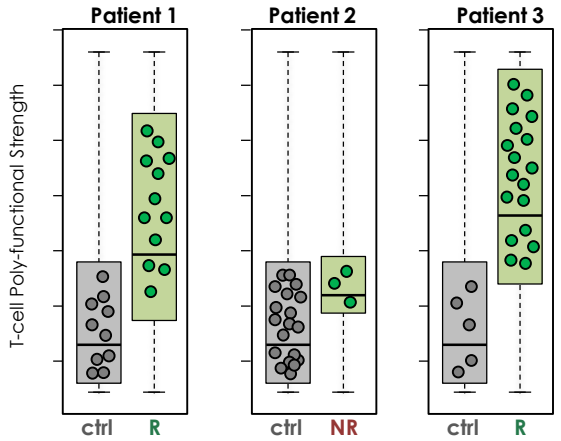
- Development feedback and excellence
- Improvement in patient management: Predictive biomarkers

Bulk blood or T-cell proteins



T-cell function looks similar, despite differences in outcome

IsoPlexis single T-cell function



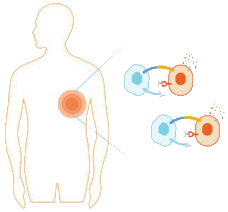
Detect patient T-cell functional differences, correlated to outcome

IsoPlexis recent data: T-cell function links to outcome

Predictive single-cell response applied to I-O

ASCO-SITC Clinical Immuno-Oncology Symposium — Yale — Caltech

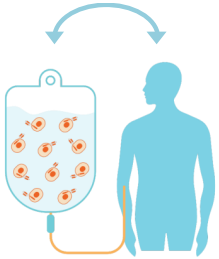
pg. 30



TIL function predicts response and non-response in PD1/CTLA4 treatment

AACR ANNUAL MEETING 2017 WASHINGTON, DC — Kite Pharma — National Cancer Institute

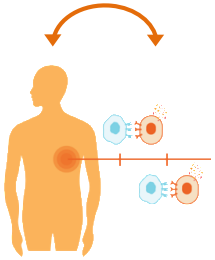
pg. 34



Pre-infusion product profiles predict CAR-T Patient Response

CANCER DISCOVERY — Caltech — UCLA

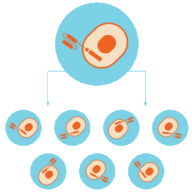
pg. 42



TCR-T product tracks / predicts response and relapse

BioMAN BioMANufacturing Program — NOVARTIS

pg. 45



CAR-T Product Characterization for Manufacturing

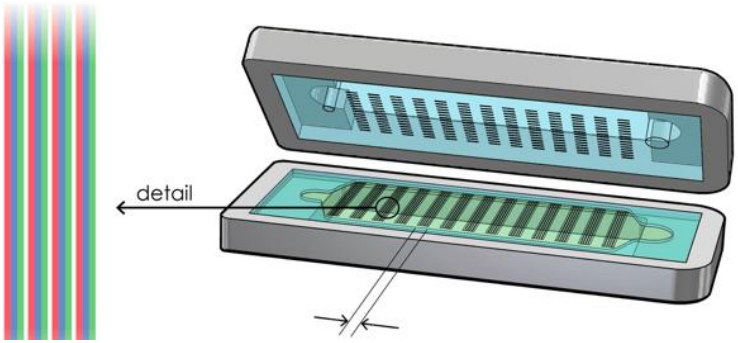
IsoCode Platform Technical Overview & Validation

IsoPlexis: **Iso**lated single-cells, Multi**Plex**ed data

Precision T-cell Profiling enabled by the IsoCode Chip

Sensitivity captured

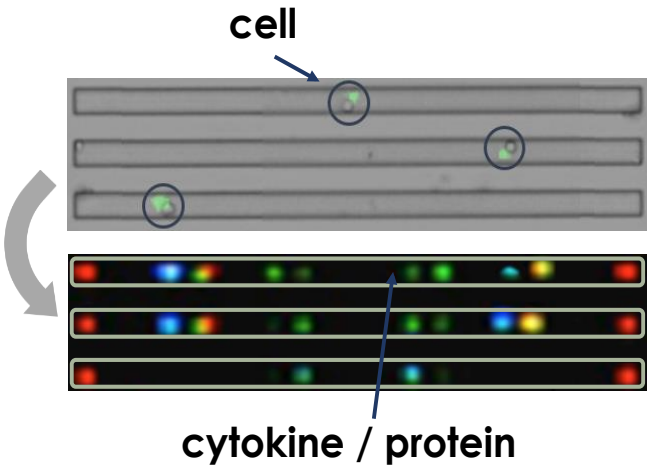
IsoCode Single-cell Chip



Single T-cell & high throughput

Depth of data required

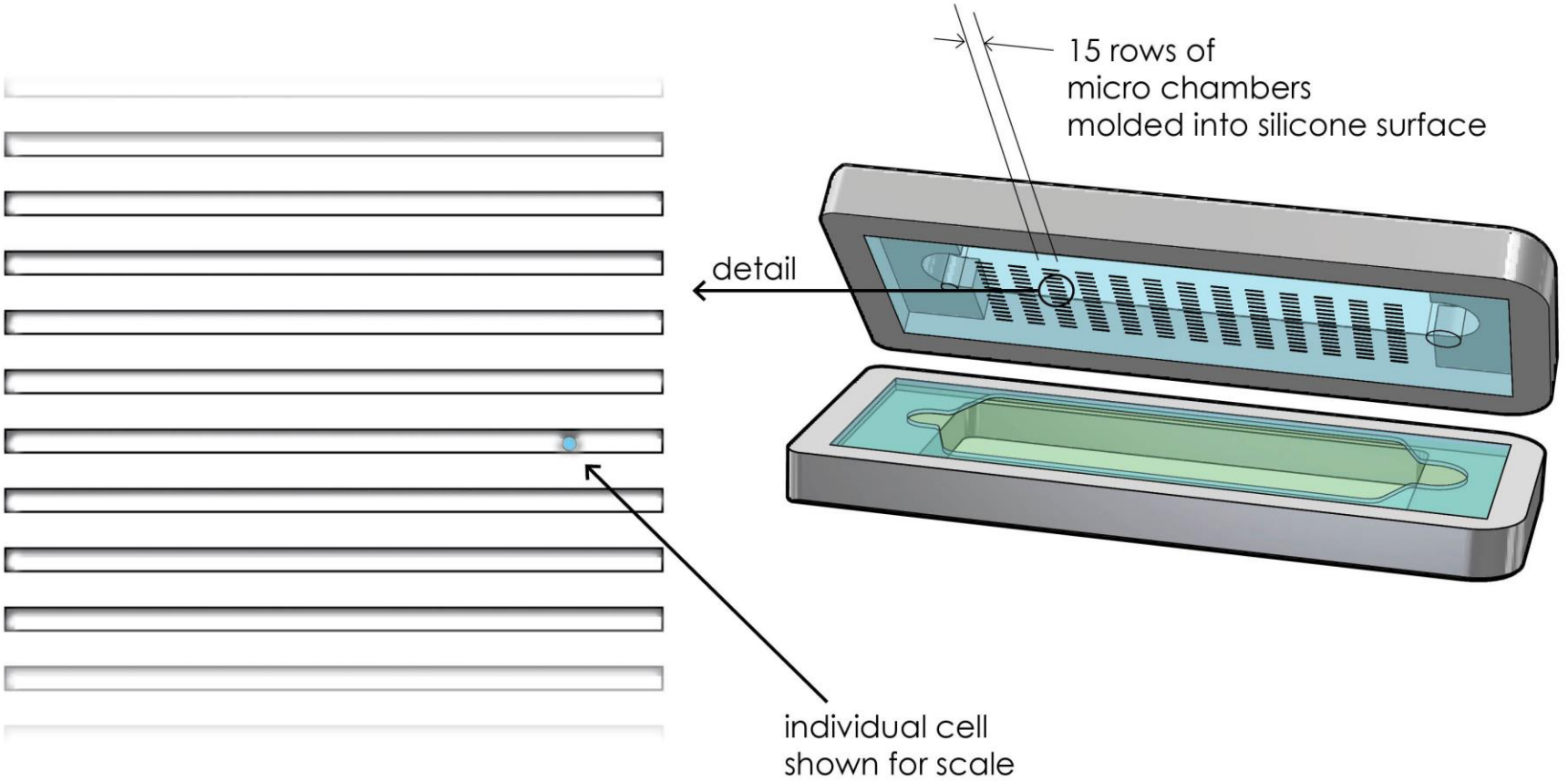
ELISA 40+plex secreted proteins, per cell



Required T-cell functions

IsoCode Chip: Cells

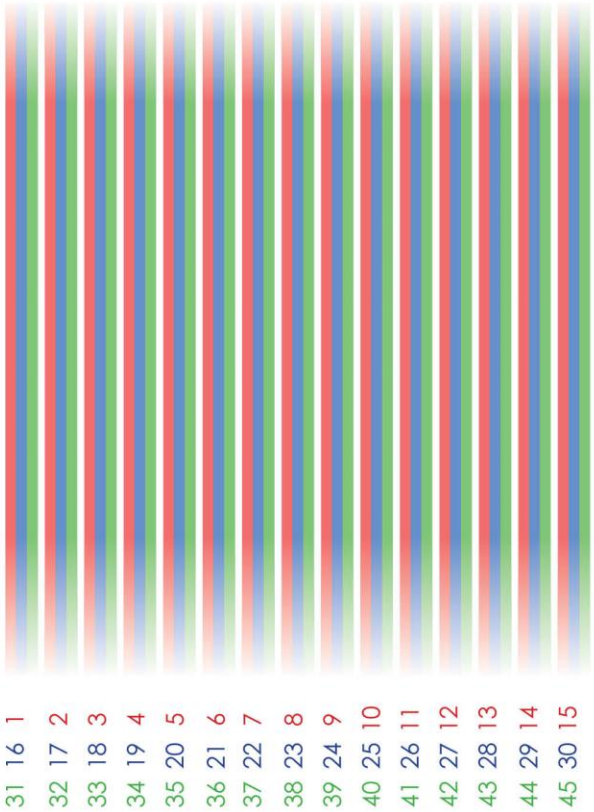
Micro-chamber cell capture

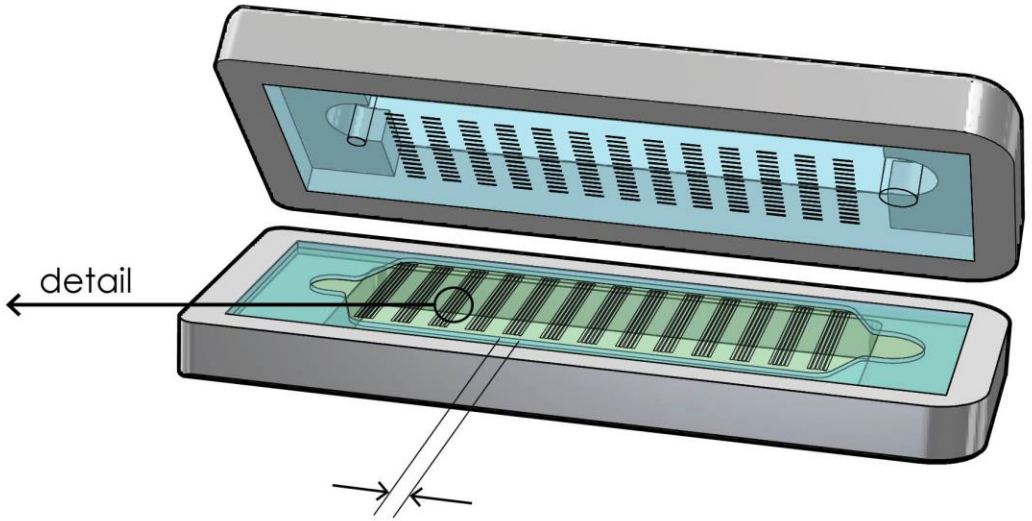


micro-chambers are molded into silicone top surface

IsoCode Chip: Array

High-density antibody barcode array





antibody barcodes
printed onto glass slide surface

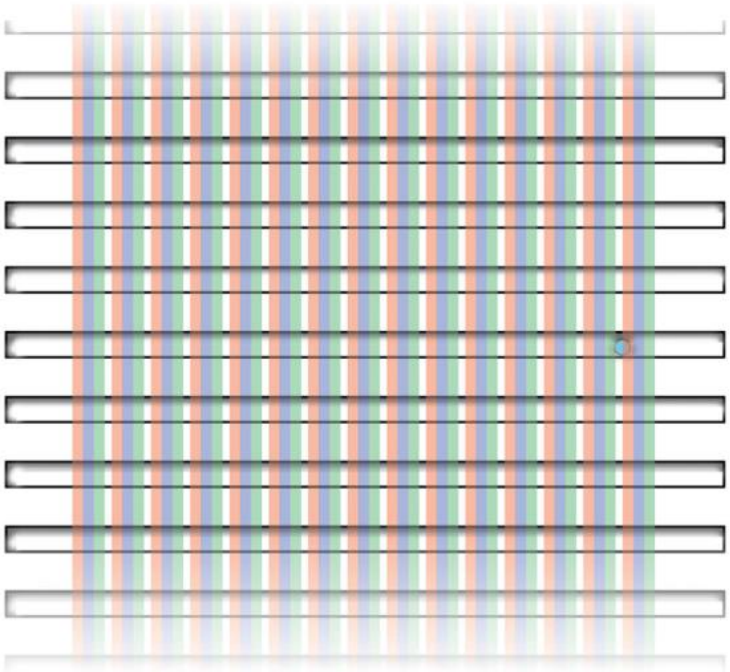
total 45 antibodies printed and able to detect
45 proteins (quantity shown as example)

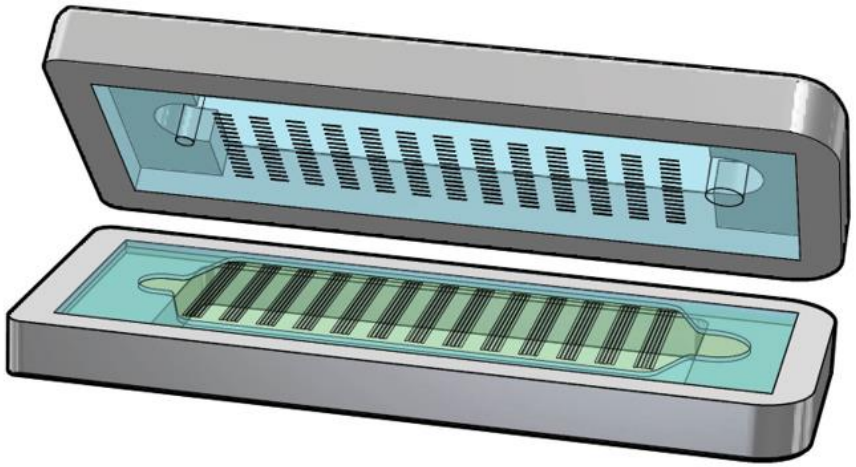
antibody bars printed with **green** fluorescing agent

antibody barcodes are printed onto glass bottom surface

IsoCode Chip: Cell Enclosure

Combining micro-chambers with antibody barcode

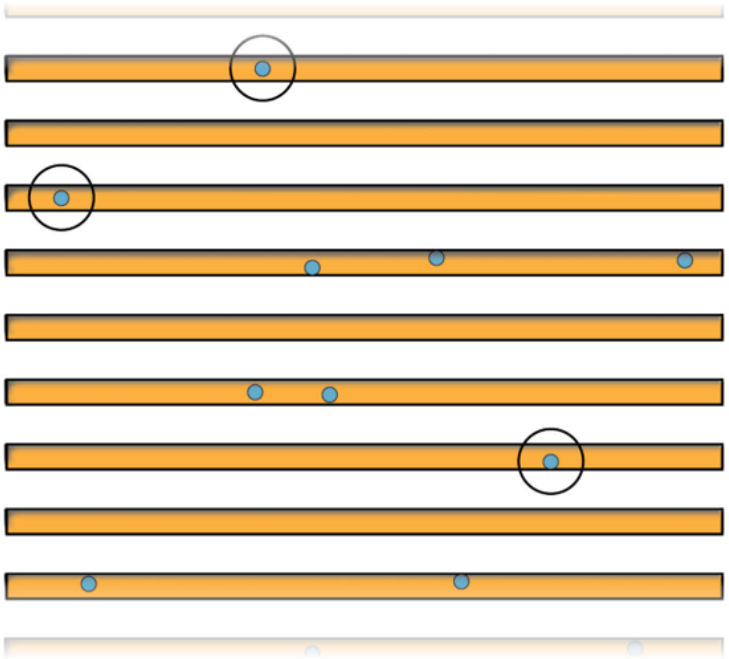


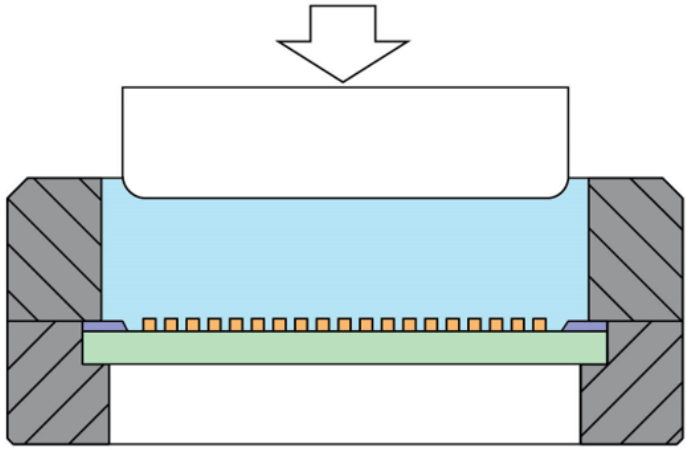


micro-chambers are combined with the antibody barcode for highly multiplexed detection

IsoCode Chip: Cell Identification – Imaging Step 1

Automatic cell detection and counting: 3 surface markers per cell

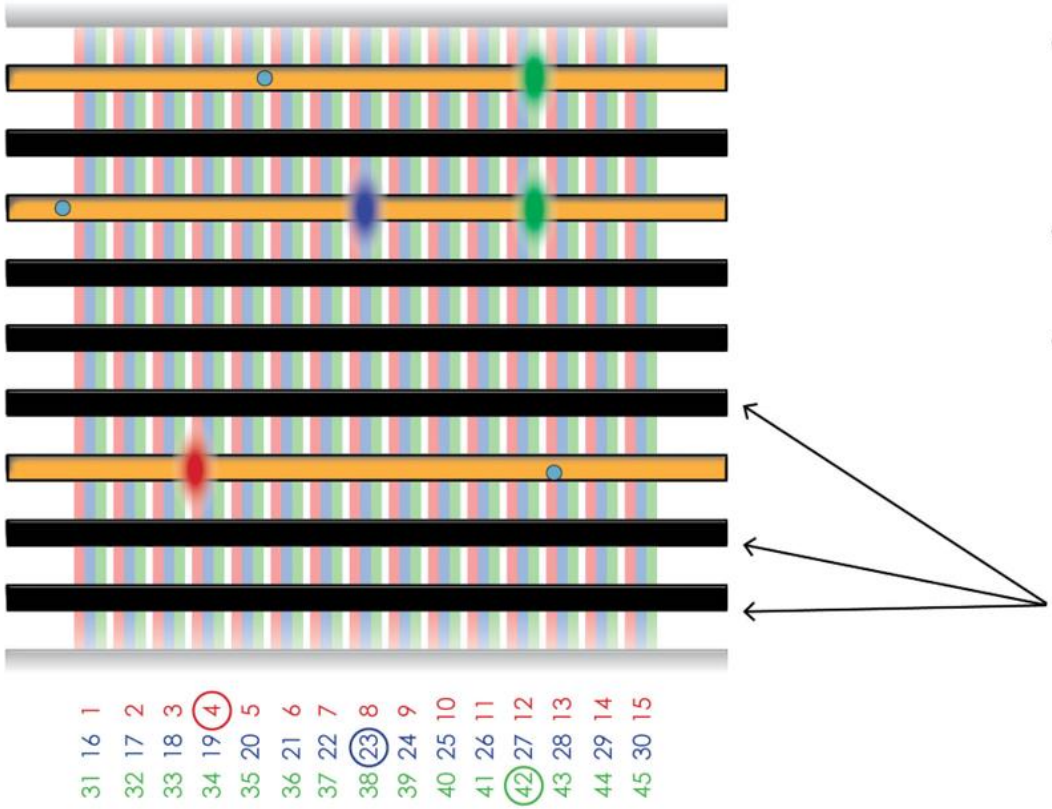




Scan identifies micro-chambers containing single cells

IsoCode Chip: Protein Quantitation – Imaging Step 2

Single cell protein profile: 32+ secreted proteins per cell



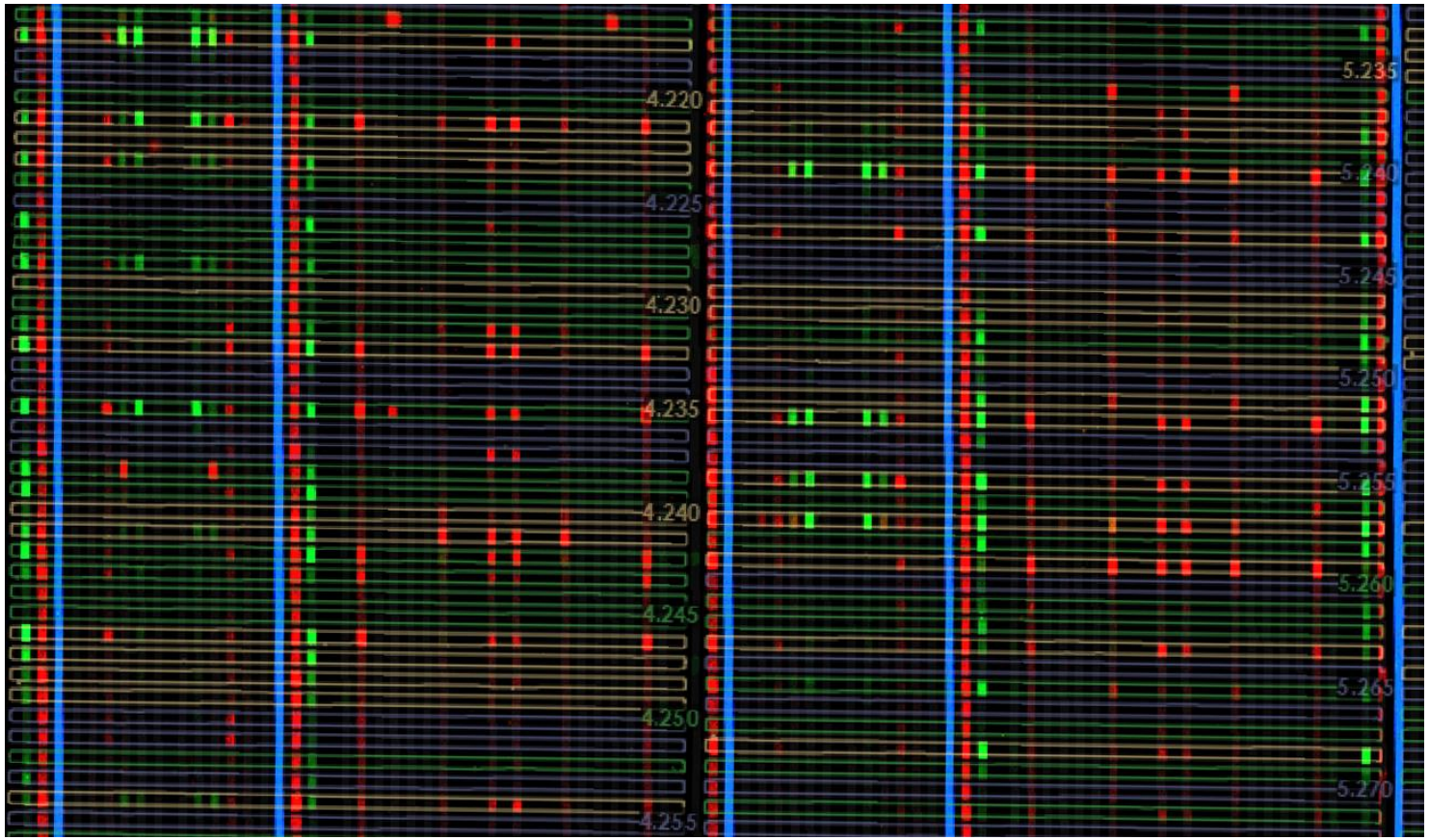
- IsoCode Chips are incubated 12 - 24 hours, causing each captured cell to secrete proteins throughout each individual micro-chamber
- each secreted protein is captured onto a corresponding antibody bar
- reagent is then introduced into the flow path, causing the captured proteins to fluoresce

scan disregards micro-chambers containing multiple or no cells

Incubated cells secrete proteins which bond to antibody bars for identification

IsoCode Chip: Actual Protein Readout, Per Chamber

Representative IsoCode Chip data

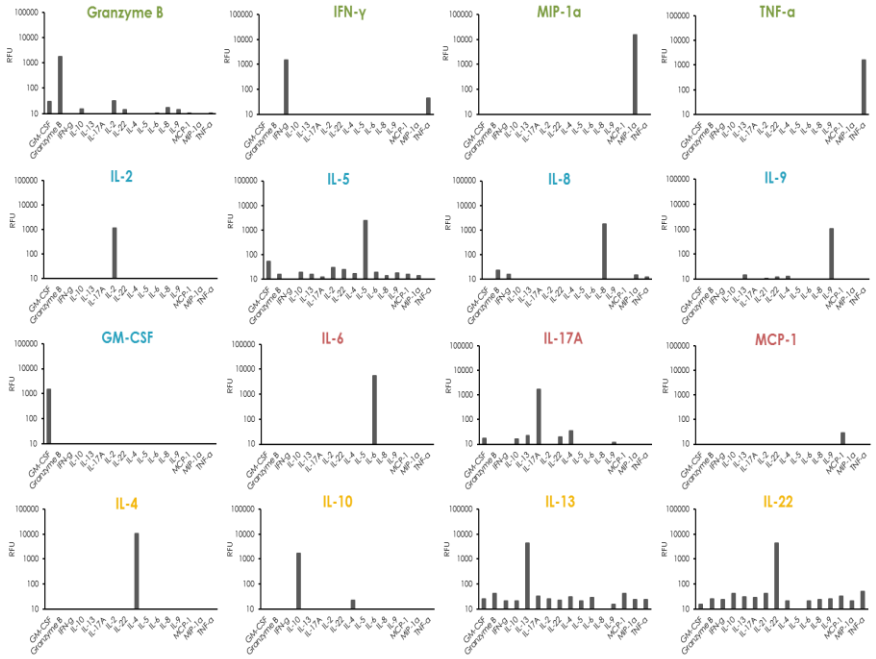


scaled detail of actual IsoCode Chip with fluorescing proteins

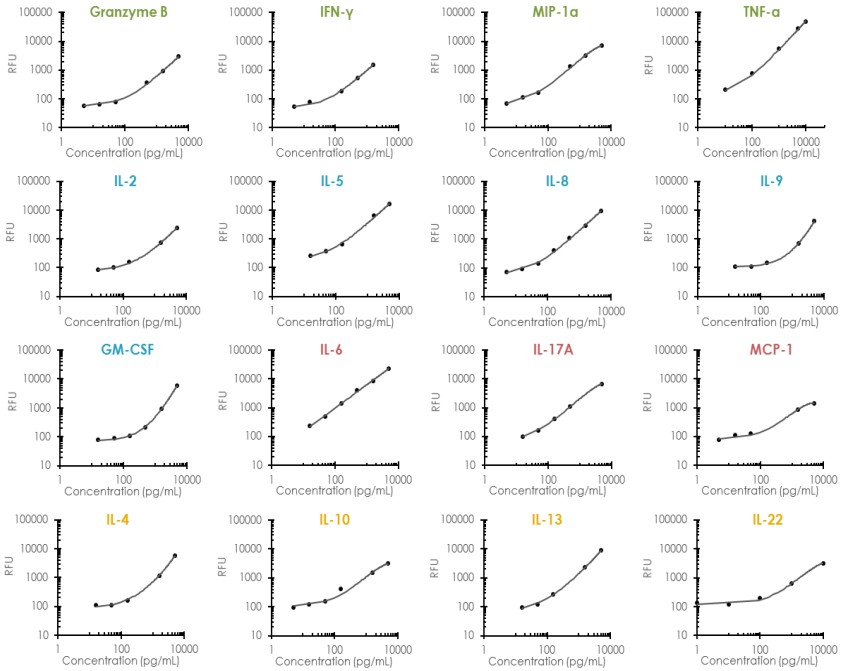
Validation: QC and calibration of antibody panels

Antibody panels are rigorously validated for both sensitivity and specificity

A) rigorous specificity of antibody capture
no cross reactivity of antibodies; SNR of 10



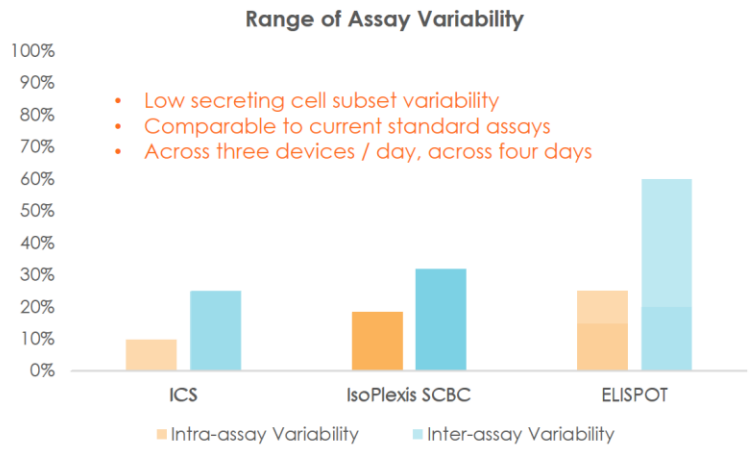
B) sensitivity and linearity of antibody pairs
dynamic range of ~5 to 5000 pg / ml



Validation: Low T-cell subset CV across metrics

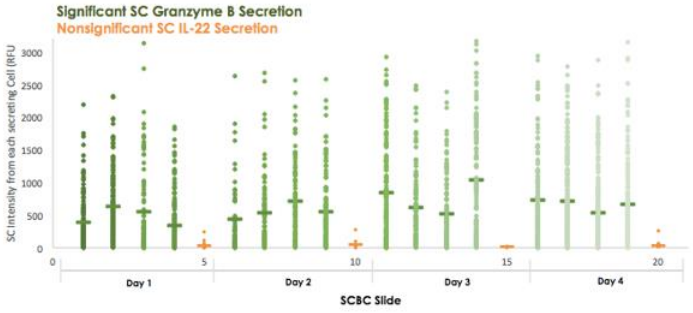
Low variability leads to consistent data

A) low secreting cell subset variability: 18.5% - 32%

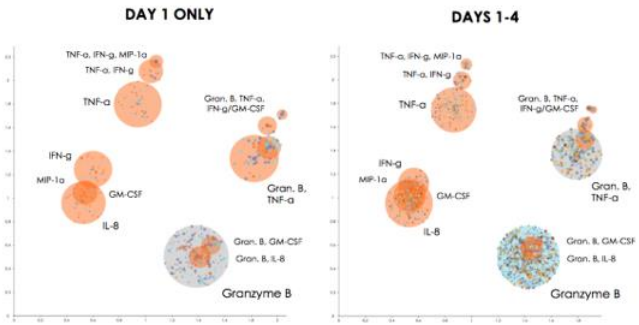


B) poly-functionality cell subset CV of 28.8%
across samples & days

C) cell subset signal CV of 28.5%
across 12 tests of same samples



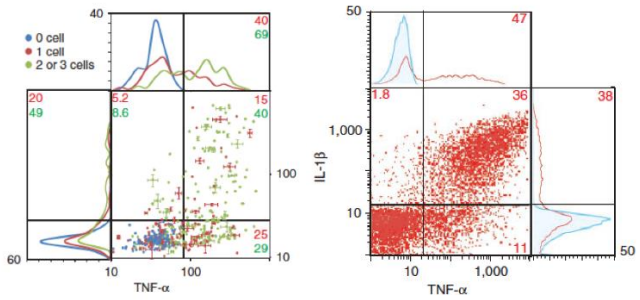
D) PAT PCA subsets conserved as well
across 12 tests of same samples



Validation: IsoCode compared to other immunoassays

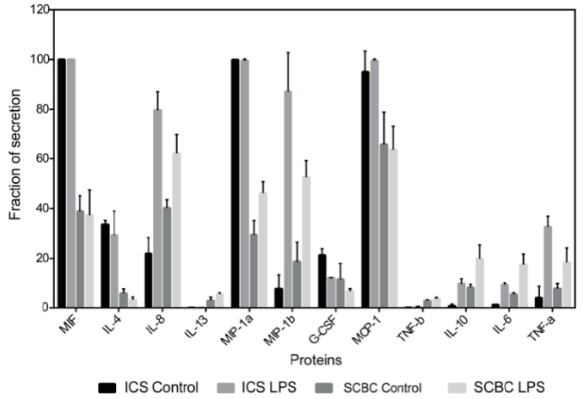
IsoCode has strong correlation with traditional low-plex single cell assays

SCBC vs Flow ICS



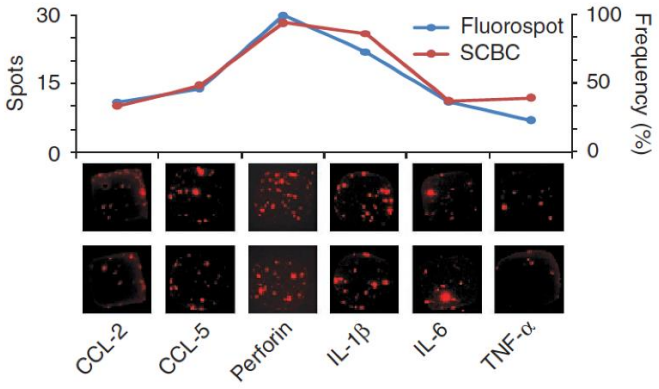
*Ma, Fan, et al., **Nature Medicine**, 17, 738-743 (2011).*

SCBC vs Flow ICS



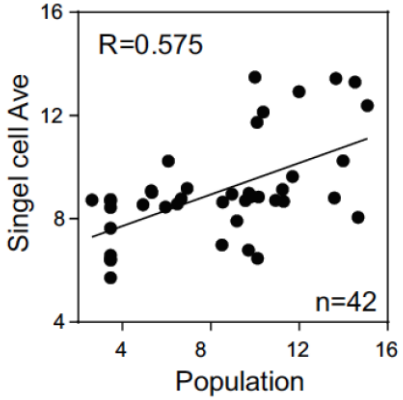
*Lu, Xue, et al., **Proc. Natl. Acad. Sci. U.S.A.**,112(7), 607-615 (2015).*

SCBC vs FLUOROSpot



Ma, Fan, et al., ***Nature Medicine***, 17, 738-743 (2011).

SCBC vs Population



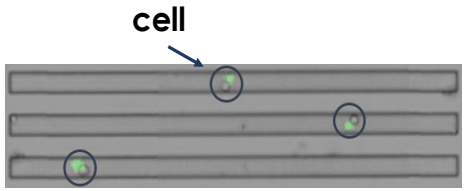
Lu, Xue, et al., ***Proc. Natl. Acad. Sci. U.S.A.***,112(7), 607-615 (2015).

* IsoCode is known as "SCBC", or Single-Cell Barcode Chip, in journal publications

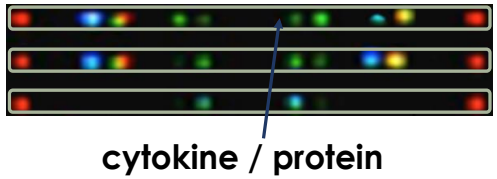
Informatics: Ease of understanding, implementation

The IsoPlexis workflow enables validated analysis in larger trials

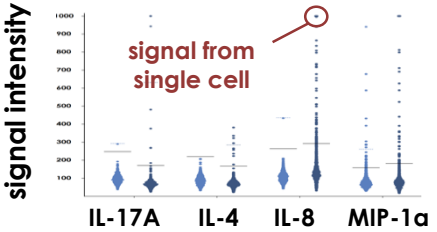
DETECT CELLS



DETECT PROTEINS

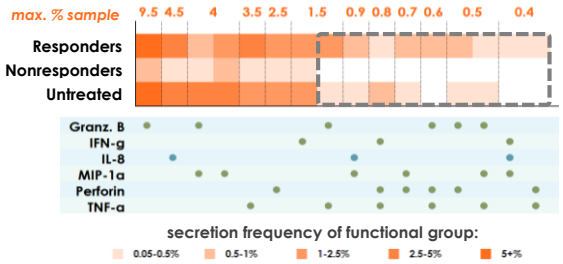


QUANTIFY READOUT



PROFILE DONORS

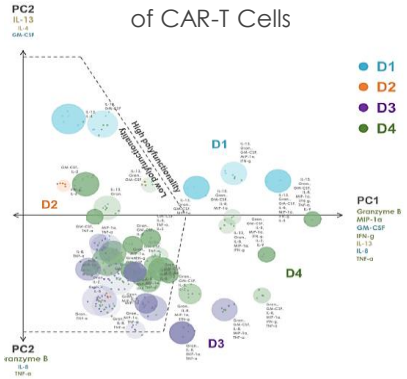
Functional Heat Map of Anti-PD-1 Treated and Untreated Patient Groups



Presented at ASCO 2017

IDENTIFY DIFFERENCES

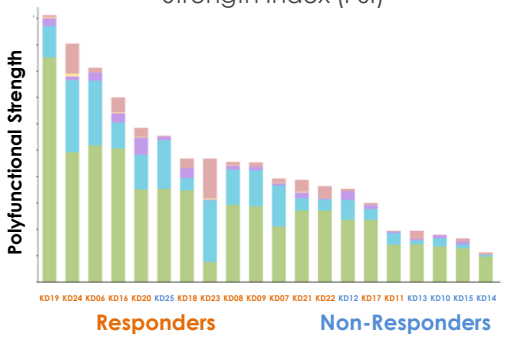
Functional Characterization of CAR-T Cells



Presented at BioMAN Workshop 2016

CORRELATE TO OUTCOME

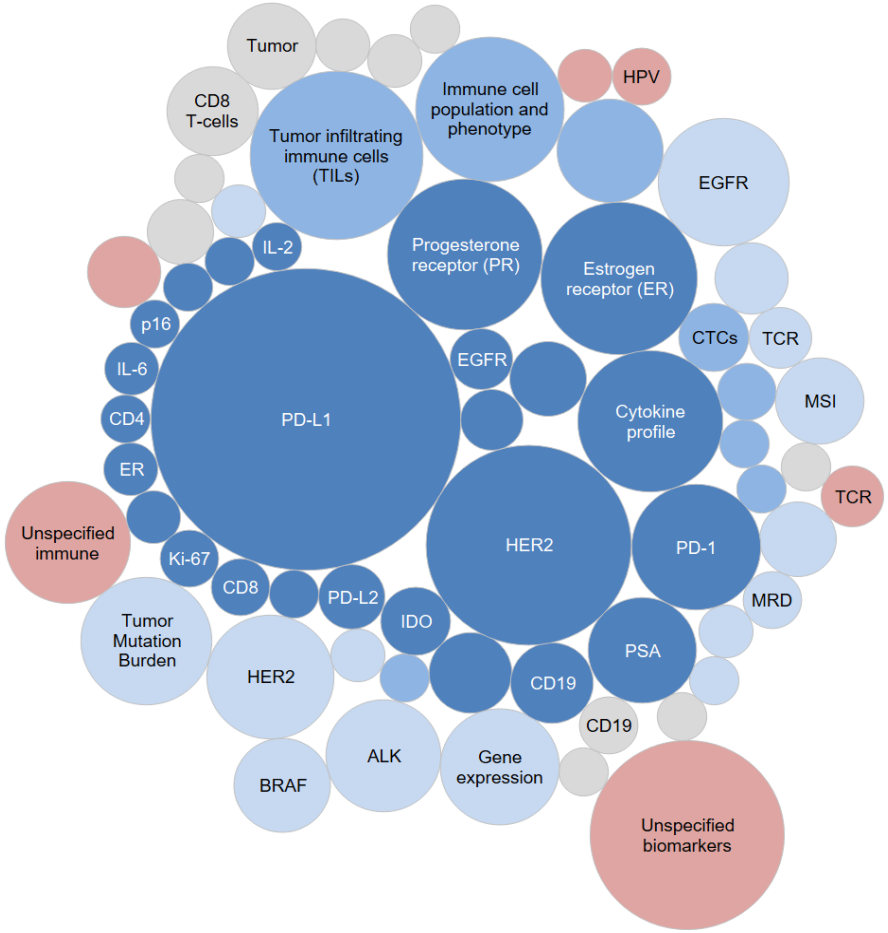
Single-Cell CAR-T Polyfunctional Strength Index (PSI)



Presented at AACR 2017

Immuno-Oncology Biomarkers: Landscape Summary

DeciBio Report



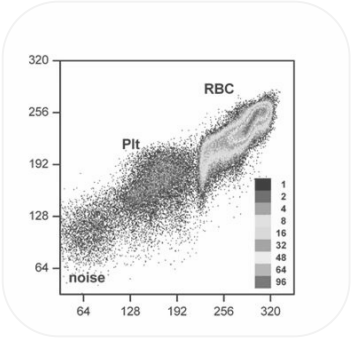
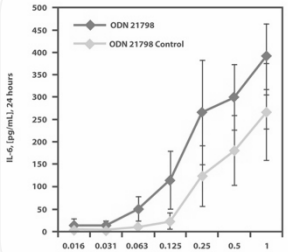
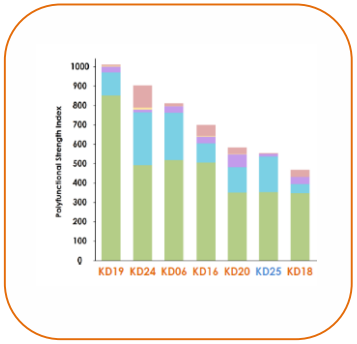
Immuno-Oncology Biomarkers mentioned in at least 5 different clinical trials.

The top "novel" immuno-oncology biomarkers identified in this analysis are:

- Tumor-infiltrating immune cells (mentioned 70 times in 61 different trials)
- Immune cell populations / phenotypes (mentioned 60 times in 45 different trials)
- **Cytokine profiles** (mentioned 52 times in 43 different trials)
- Tumor mutation burden / genomic mutation profiling (mentioned 45 times in 35 different trials)
- Gene expression signatures (mentioned 33 times in 29 different trials)

Technology used for T-cell characterization

IsoPlexis links T-cell functional data to mode of action

	Patient Gene Profile	**Cell Type Frequency**	**Blood Levels**	**T cell Functional Capacity**
				
System	Gene expression	Flow and mass cytometry	ELISA and Luminex	IsoCode
Correlation to MOA in T cells	x	x	x	✓
Single Cell	✓	✓	x	✓
Multiplexed secreted protein data	x	x	✓	✓

I-O Case Studies

Case 1: IsoCode TILs Workflow Overview

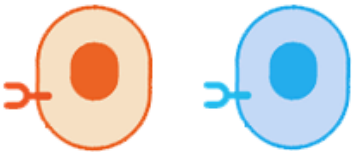
Yale Caltech

Tumor dissociation and stimulation to ensure specific readouts

Sample Enrichment

Sample: Fresh melanoma samples are immediately processed upon arrival to single-cell suspensions.

CD8+ and CD4 TILs are enriched by anti-CD8 and anti-CD4 microbeads respectively.



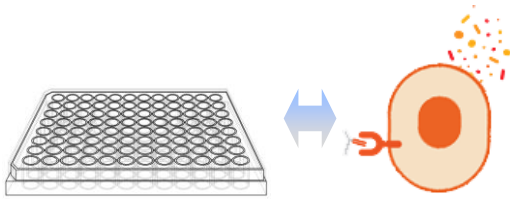
CD8 TIL

CD4 TIL

Number of cells dependent on donor, tumor, and viability

Antigen Stimulation

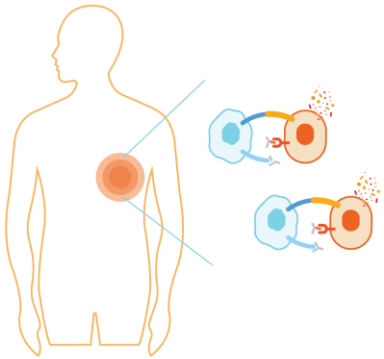
Stimulated: anti-CD3 stimulation at 37°C, 5% CO2 for 20 hours.



anti-CD3 stimulation on plate

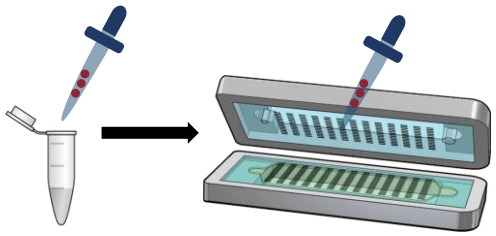
stimulated TIL

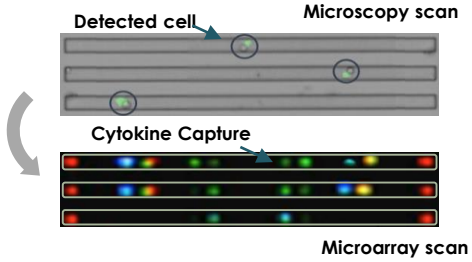
Treated vs. Untreated Patients



TILs Loading & Analysis

Loading: Stimulated TILs are collected, then pipetted from single-cell suspension and loaded onto IsoPlexis' IsoCode system

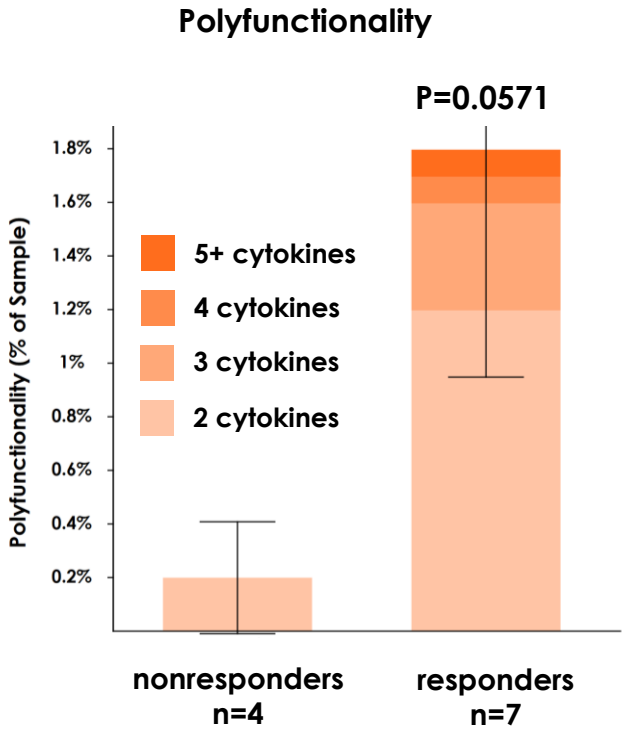


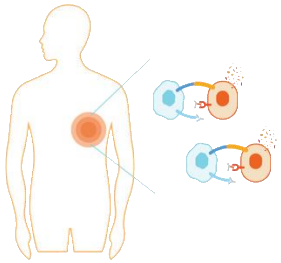


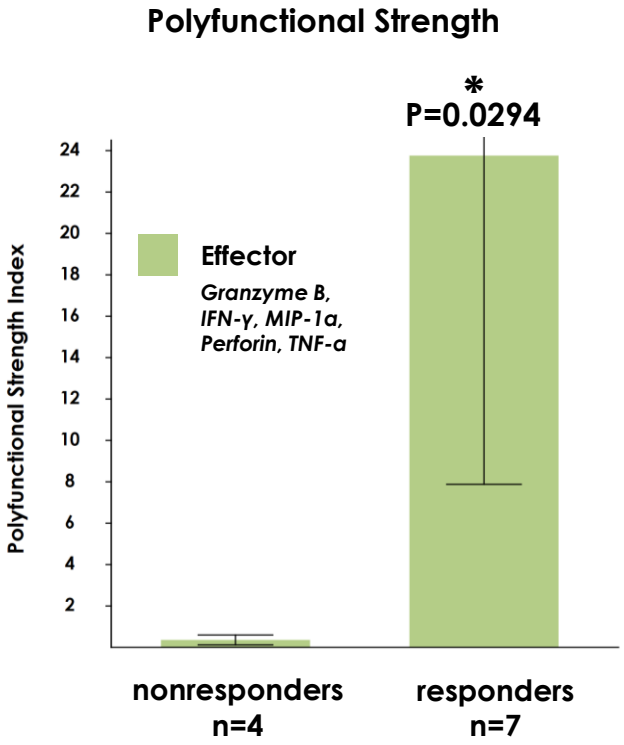
Case 1: checkpoint biomarkers

Yale Caltech

Profiling T Cells for Improved Patient Response Markers to anti PD-1 and/or anti CTLA4 therapy







Objective: Clarity of mechanism & markers of patient selection

* P<0.05, Mann-whitney U test)

- All detail on type of response is available in appendix, and that response includes mixed responders and resistant disease in otherwise responders

Case 1: checkpoint biomarkers

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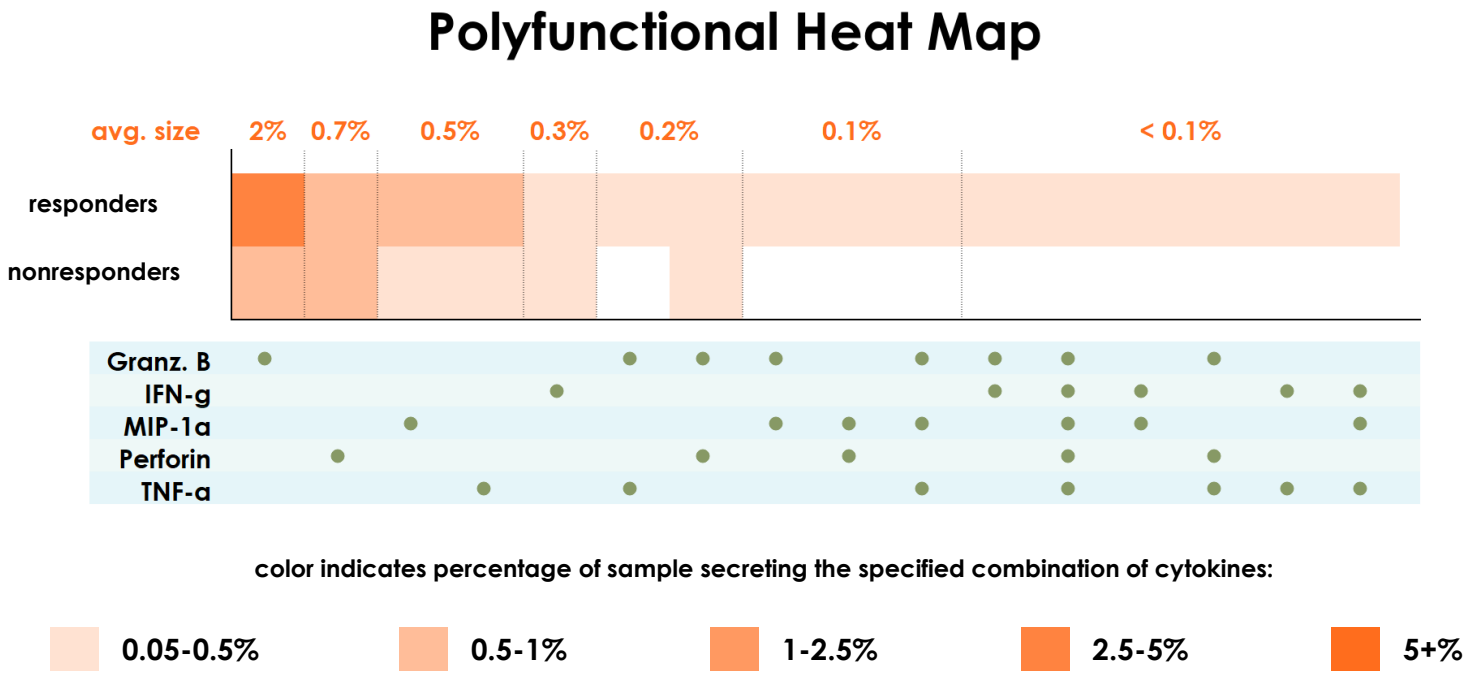
Emergence of unique polyfunctional cell subsets

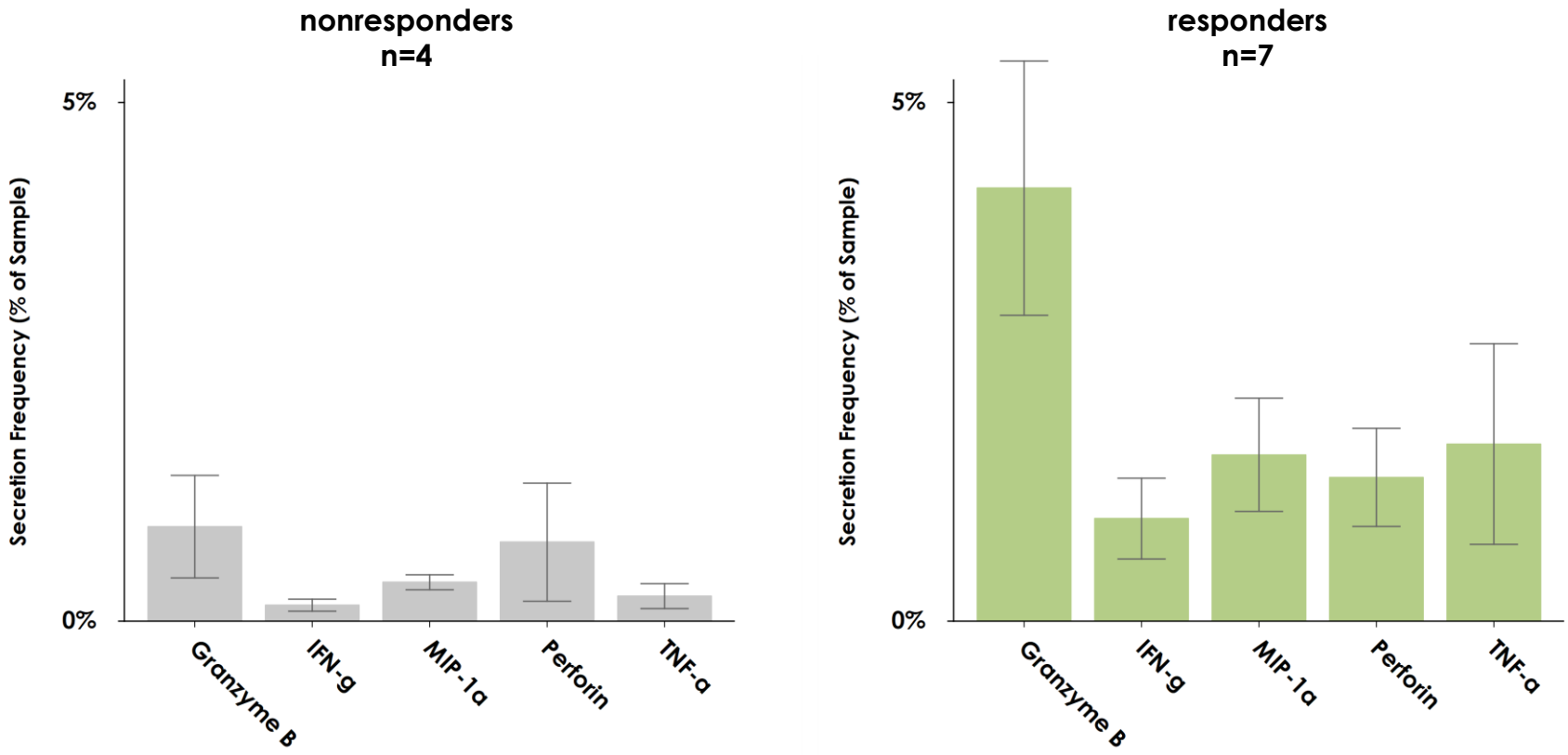


Identify unique polyfunctional cell subsets in patients who responded to anti PD-1 and/or anti CTLA4 therapy

Case 1: checkpoint biomarkers

Yale Caltech

Functional differences in responding patients

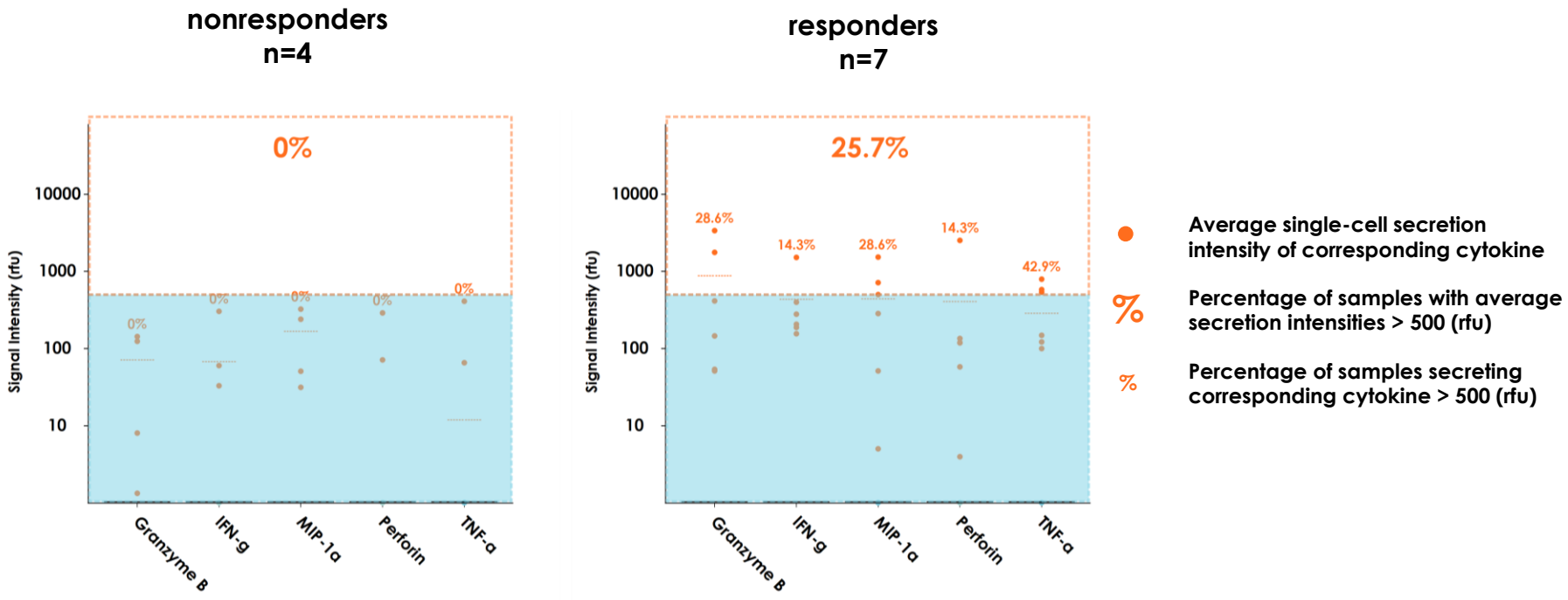


Increased antitumor protein secretions in patient responding to anti PD-1 and/or anti CTLA4 therapy

Case 1: checkpoint biomarkers

Yale Caltech

Enhanced secretion intensity in responding patients

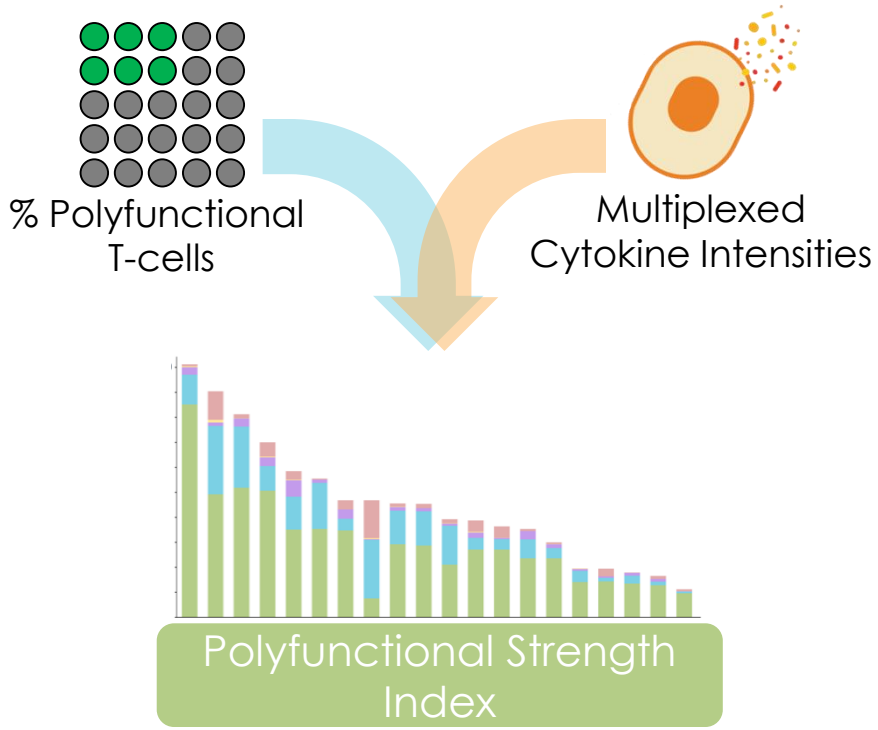


Enhanced average secretion intensities of proteins associated with antitumor immunity in patients who responded to anti PD-1 and/or anti CTLA4 therapy

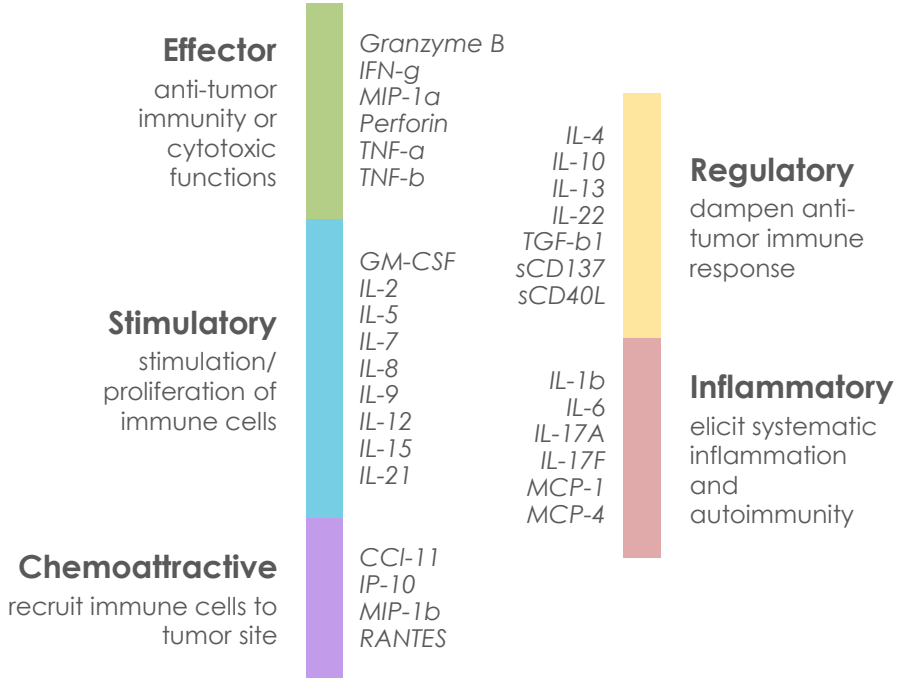
Case 2: CD19 CAR-T Polyfunctionality for Kite Product

Polyfunctional Strength Index (PSI)[1] metric links to patient outcomes

Product Metric: Requires IsoCode



IsoCode Single-Cell Panel



1. IsoPlexis IsoCode Technology and Ma et al 2013

CCL, chemokine ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL., interleukin; IP, interferon-gamma-inducible protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PSI, polyfunctional strength index; RANTES, regulated on activation, normal T cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor.

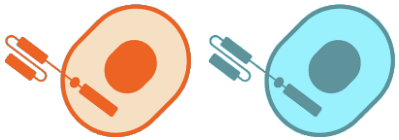
Case 2: IsoCode CAR-T Workflow Overview

Stimulation & loading to ensure antigen specific readouts

Sample Enrichment

Sample: Cryopreserved samples are thawed upon arrival to RT.

CD8+ and CD4 CAR-T cells are enriched by anti-CD8 or anti-CD4 microbeads respectively.



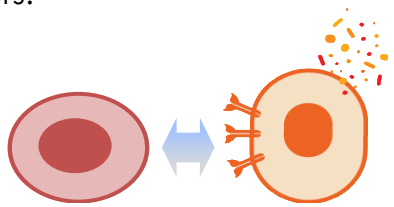
CD8 CAR-T cell

CD4 CAR-T cell

2 million cells requested per donor, viability

Antigen Stimulation

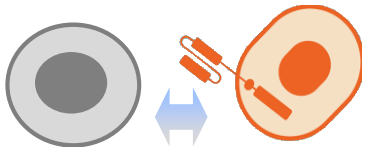
Stimulated: CD19-specific response at 37°C, 5% CO2 for 20 hours.



CD19-K562

stimulated CAR-T cell

Control: Non CAR specific (allogeneic) response at 37°C, 5% CO2 for 20 hours.

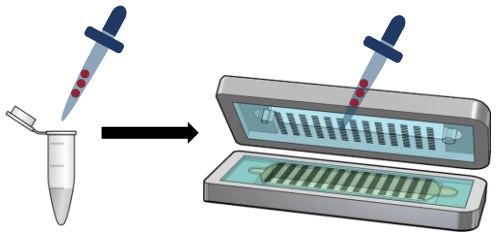


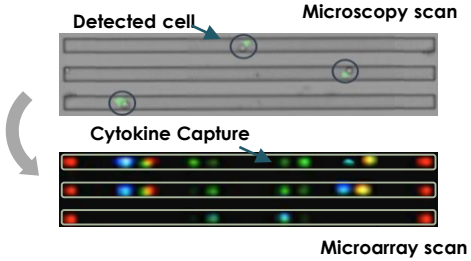
NGFR-K562

non-stimulated CAR-T cell

Depletion & CAR-T Loading

Depletion: Cancer target cells are depleted, then stimulated CAR-T cells are pipetted from single-cell suspension is loaded onto IsoPlexis' IsoCode system



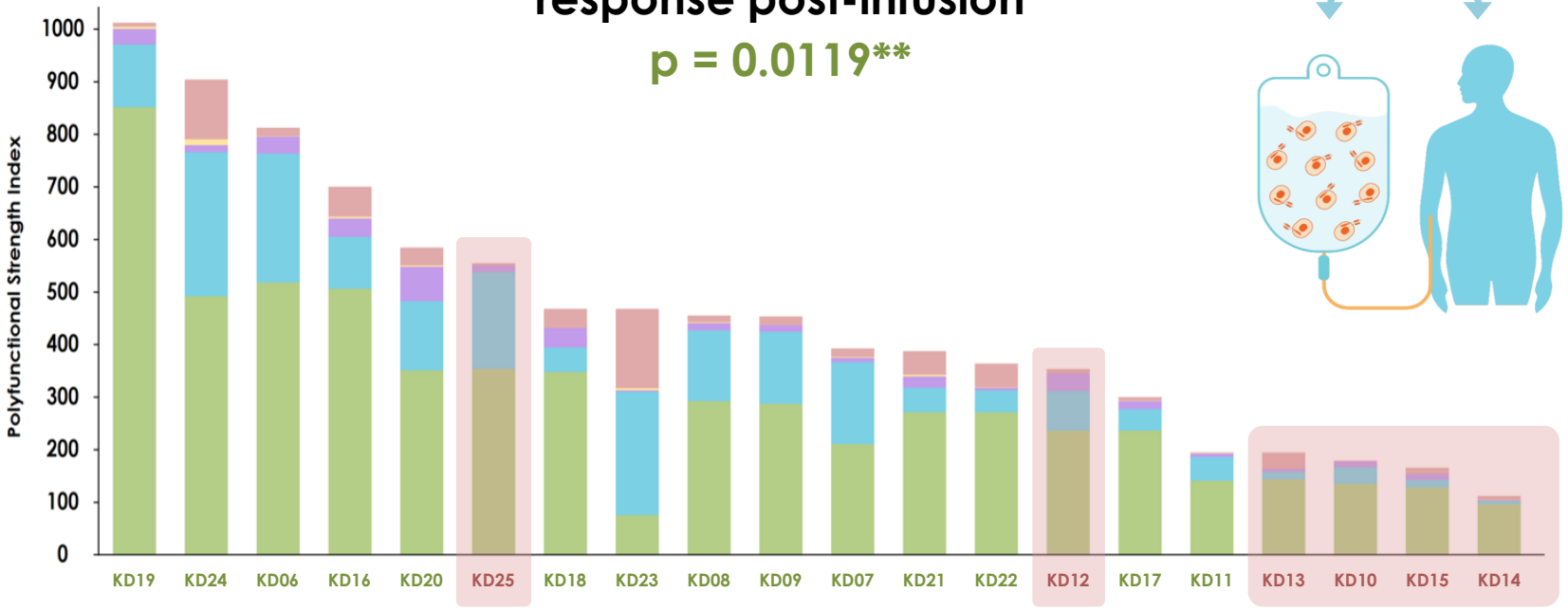


Case 2: ranking CAR-T donors by PSI

Patient pre-infusion product profiles enable early intervention

CAR PSI* pre-infusion predicts response post-infusion

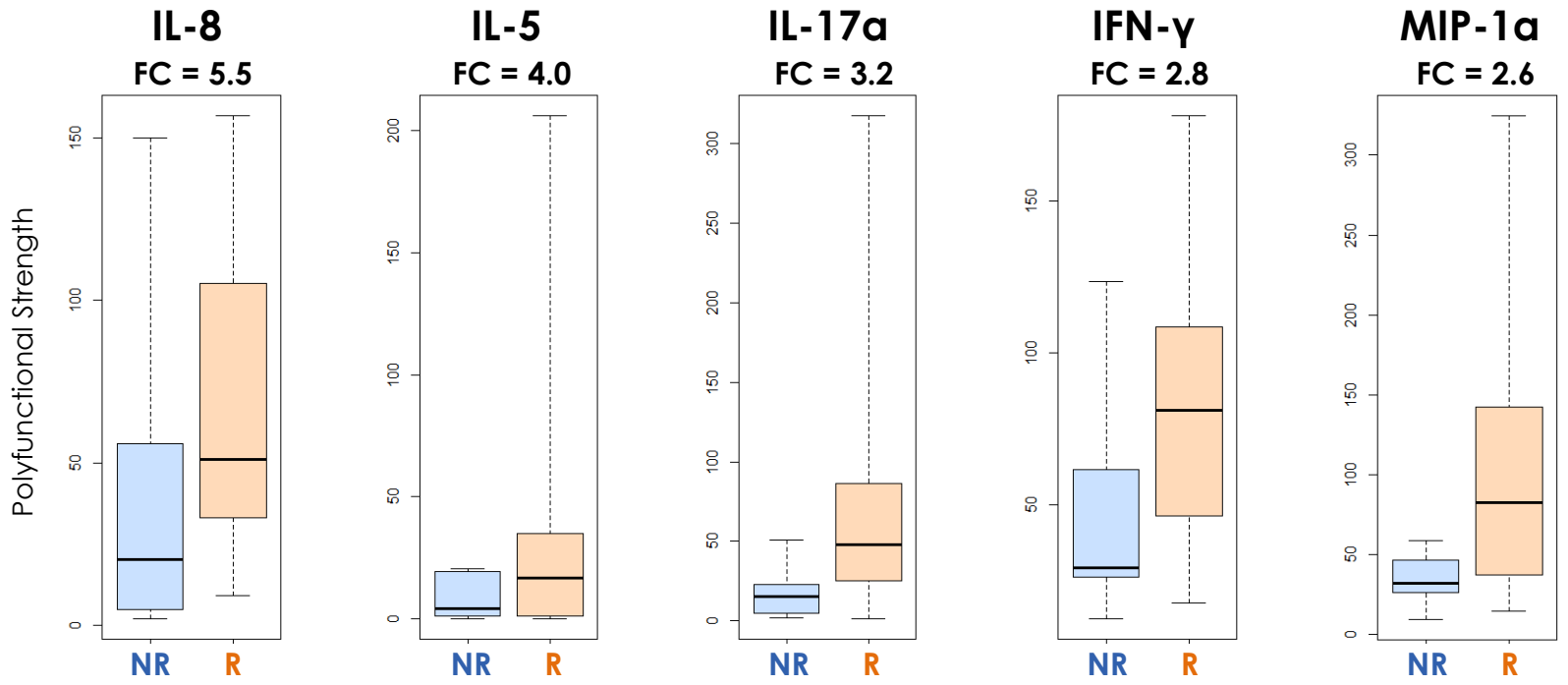
p = 0.0119**



Rossi J, Paczkowski P, Shen Y, Morse K, Flynn B, Kaiser A, Ng C, Gallatin K, Cain T, Fan R, Mackay S, Heath JR, Rosenberg SA, Kochenderfer JN, Zhou J, and Bot A. Polyfunctional Anti-CD19 CAR T Cells Determined by Single-Cell Multiplex Proteomics Associated with Clinical Activity in Patients with Advanced Non-Hodgkin's Lymphoma. Presented at AACR 2017, Session MS.CL10.01 - Clinical Biomarkers.

Case 2: drivers of CAR-T product potency

Further, from the readouts, non-redundant cytokines contributed to these PSI differences (CD4 ex.)



additionally, pre-infusion product is an independent variable vs. CAR-T expansion

Deeper biomarkers for response / non-response

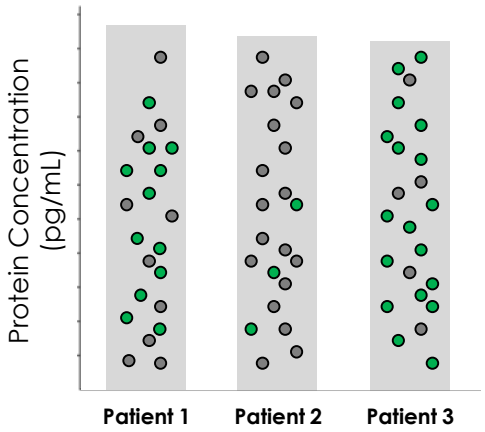
Applying improved sensitivity to patient stratification

Isoplexis technology provides

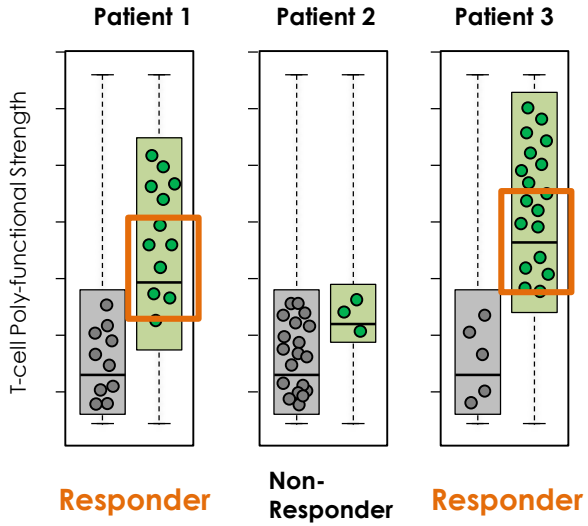
data driven by & related to patient outcome

Current Methodologies

(flow cytometry / bulk assay)



Single T-cell Patient Response



- T-cell biomarkers are masked in current analysis and methodologies.
- Functional differences in cellular sub-populations are unable to be differentiated.

- Improve / optimize development and administration to achieve T-cell poly-functional potency metrics, linked to outcome
- Improve multi-dimensional assessment of patient response to enable stratification

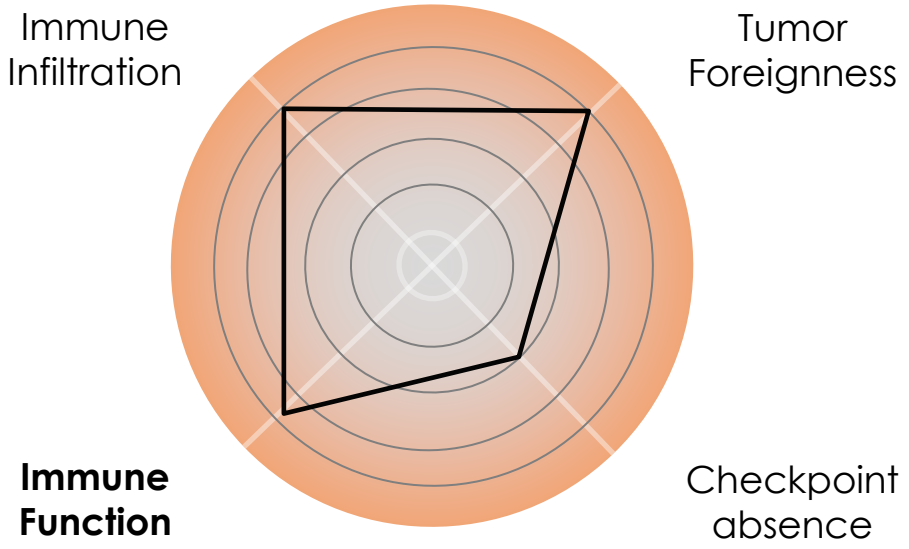
non-functional T-cell **poly-functional T-cell** *(2+ cytokines per cell)*

Patient biomarker methods & differentiation

Predictive solutions add value to characterizing T-cell function

Immunotherapy Assessment
Genetic profiling
Flow and Mass Cytometry
Histology
Serum profiling
IsoCode T cell Functionality

Multiple T-Cell & Tumor Readouts for assessment necessary: ImmunoGram[1]



IsoCode offers critical T cell function signature linked to outcome to complement tumor signature solutions

1. Christian U. Blank, John B. Haanen, Antoni Ribas and Ton N. Schumacher (May 5, 2016). *Science* **352** (6286), 658-660.

Thank You & Appendix

35 NE INDUSTRIAL RD,
BRANFORD, CT 06405, USA
email: info@isoplexis.com