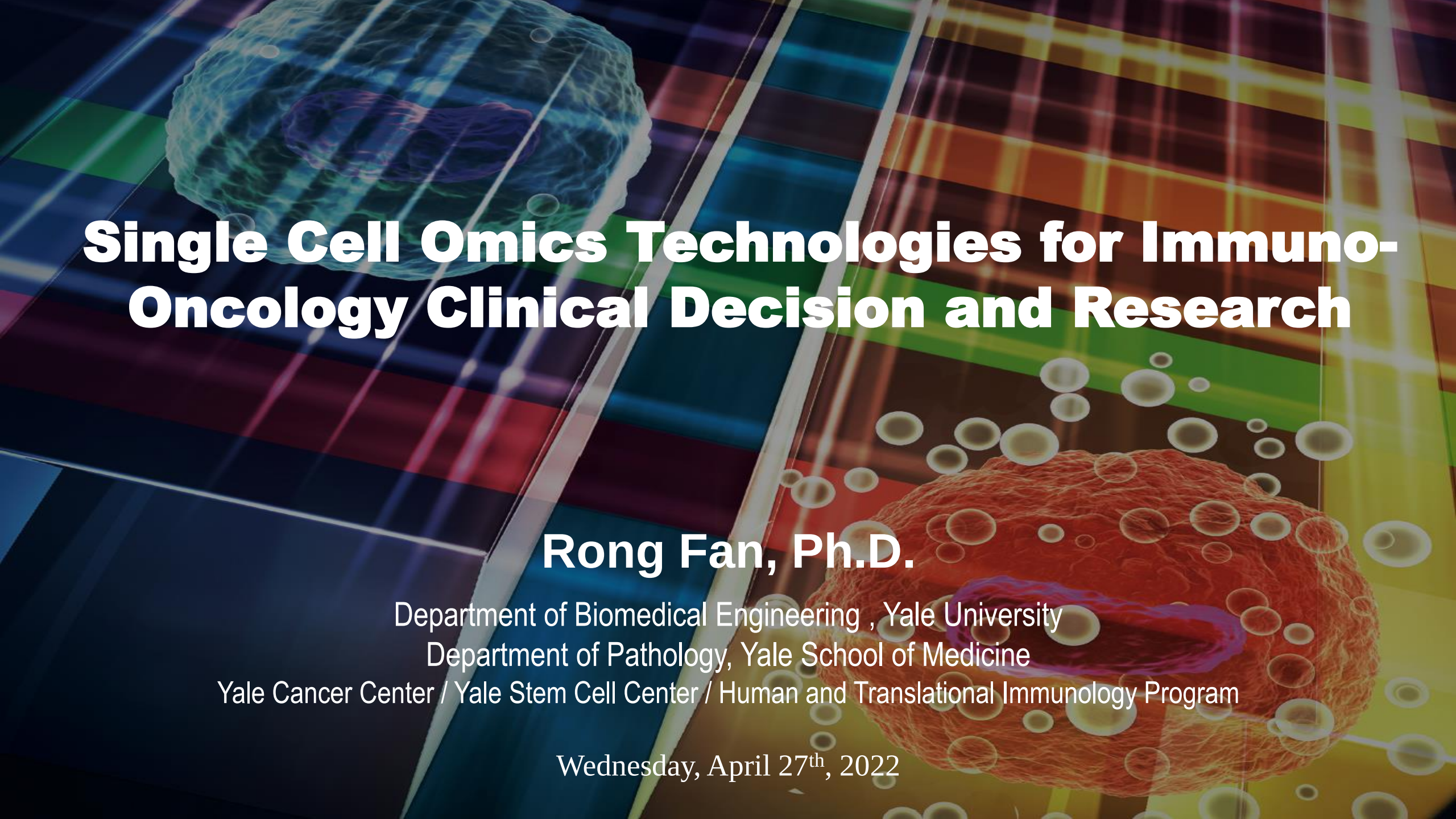


Single Cell Omics Technologies for Immuno-Oncology Clinical Decision and Research

Rong Fan, Ph.D.

Department of Biomedical Engineering , Yale University
Department of Pathology, Yale School of Medicine
Yale Cancer Center / Yale Stem Cell Center / Human and Translational Immunology Program

Wednesday, April 27th, 2022

Conflict of Interest (COI) Disclosure

IsoPlexis
Dr. Fan is Co-founder & SAB member of IsoPlexis Corporation.

Singleron
Dr. Fan is Co-founder & SAB member of Singleron Biotechnologies Co., Ltd

AtlasXomics
Dr. Fan is Co-founder & SAB member of AtlasXomics, INC.

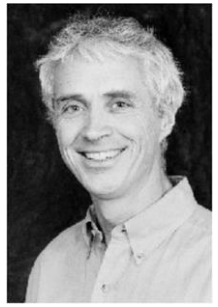
Bio-Techne
Dr. Fan served on the Scientific Advisory Board (SAB) of Bio-Techne. cv

2006 – my cancer biology 101

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Review



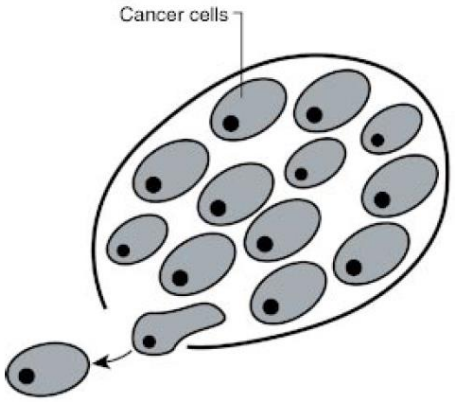
Douglas Hanahan

Douglas Hanahan* and Robert A. Weinberg†
*Department of Biochemistry and Biophysics and Hormone Research Institute
University of California at San Francisco
San Francisco, California 94143
†Whitehead Institute for Biomedical Research and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142

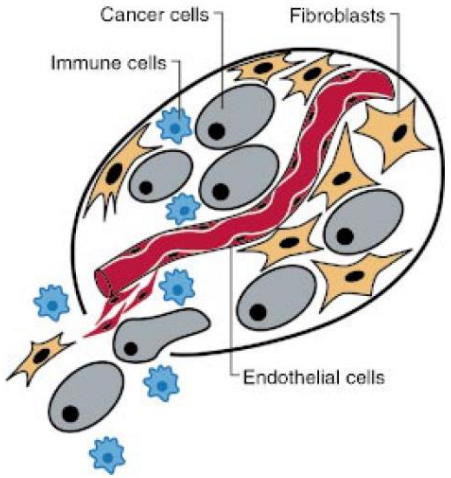
evolve progressively from normalcy via a series of premalignant states into invasive cancers (Foulds, 1954).

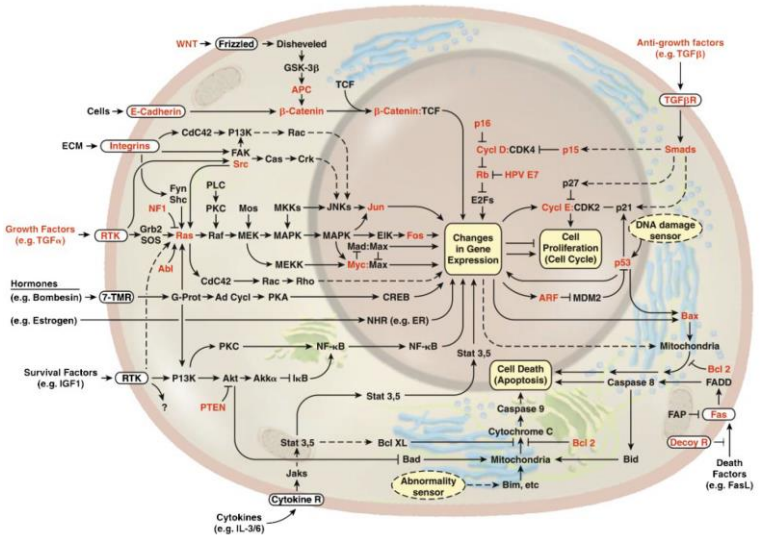
These observations have been rendered more concrete by a large body of work indicating that the genomes of tumor cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement (e.g., Kinzler and Vogelstein, 1996). Transformation of cultured cells is itself a multistep process: rodent cells require at least two introduced genetic changes before they acquire tumorigenic

The Reductionist View



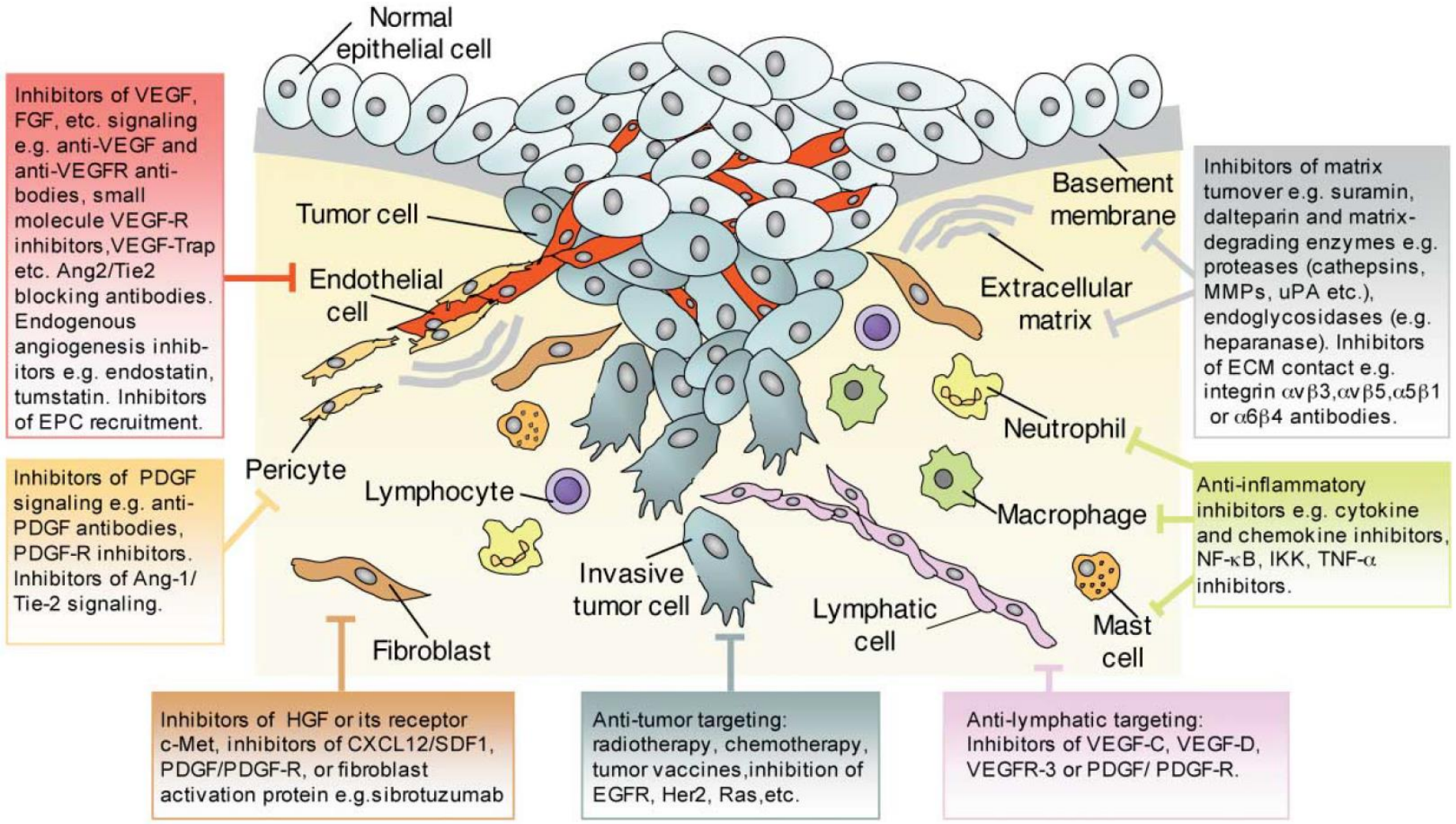
A Heterotypic Cell Biology





Tumor Microenvironment and Paracrine Signaling Between Tumor and Immune Cells

Professor Johanna Joyce (MSKCC/Univ Lausanne)



Cancer Cell(2005)

2006 – first time I learned ELISA

Jim Heath
Caltech/ISB

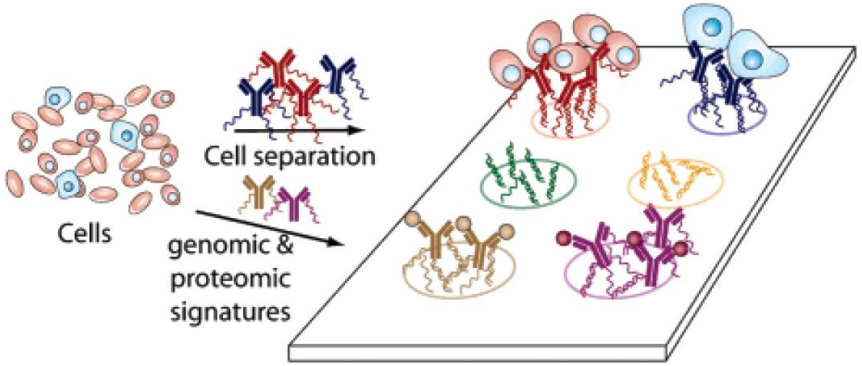
J|A|C|S
ARTICLES
Published on Web 01/30/2007

DNA-Encoded Antibody Libraries: A Unified Platform for Multiplexed Cell Sorting and Detection of Genes and Proteins

Ryan C. Bailey,[†,⊥] Gabriel A. Kwong,[†] Caius G. Radu,[‡] Owen N. Witte,[‡,∥,§] and James R. Heath*[,†]

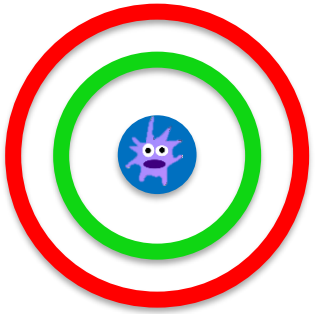
Contribution from the NanoSystems Biology Cancer Center: Division of Chemistry and Chemical Engineering, MC 127-72, California Institute of Technology, Pasadena, California 91125, and Department of Molecular and Medical Pharmacology, Departments of Microbiology, Immunology, and Molecular Genetics, and Howard Hughes Medical Institute, University of California, Los Angeles, California 90095

Received August 15, 2006; E-mail: heath@caltech.edu

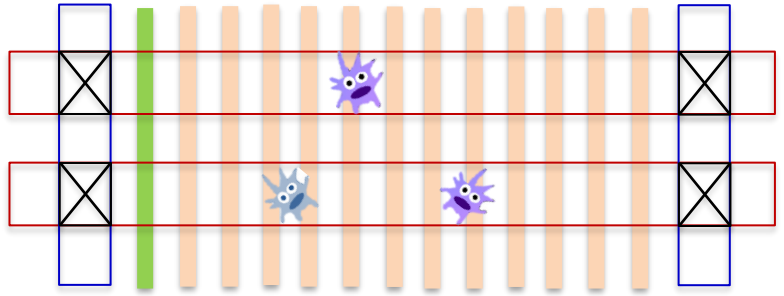


DEAL multiparameter platform for analysis of cells, genes, and proteins

Can we measure all cytokines at single cell level to decipher the secret conversation between tumor and immune cells?



First design: microcontact print or stamping of a Bull's eye pattern for single-cell 3-plex cytokine secretion assay



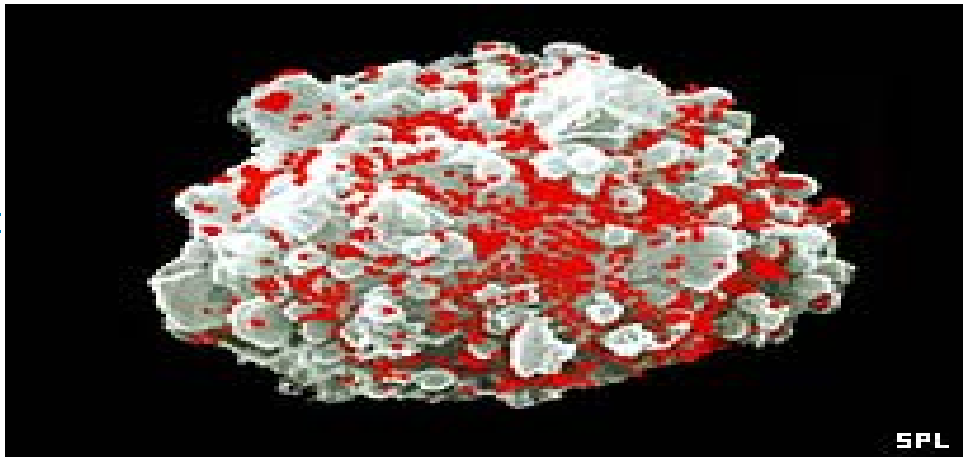
This is the way to go: flow patterned DNA "barcode" linear array for single-cell 12-plex cytokine secretion assay

Lili Yang David Baltimore

Last Updated: Friday, 21 September 2007, 21:52 GMT 22:

Merck abandons HIV vaccine trials

International drug company Merck has halted trials on an HIV vaccine that was regarded as one of the most promising in the fight against Aids.



The vaccine was loaded with copies of three HIV genes

Merck stopped testing the vaccine after it was judged to be ineffective.

In trials, the vaccine failed to prevent HIV infections among volunteers who were at risk of catching the virus, including gay men and sex workers.

Merck had previously expressed high hopes for the drug, which it spent 10 years developing.

'Headed for failure'

Merck's international trial, called Step, began in 2004 and involved 3,000 HIV-negative volunteers from diverse

J Exp Med. 2008 Jan 21;205(1):7-12. doi: 10.1084/jem.20072681. Epub 2008 Jan 14.

The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development?

Sekaly RP.

Université de Montréal, CR-CHUM, Institut National de la Santé et de la Recherche Médicale U743, Montréal, Québec H2X1P1, Canada. rafick-pierre.sekaly@umontreal.ca

Abstract

The world of human immunodeficiency virus (HIV) vaccines has suffered a baffling setback. The first trial of a vaccine designed to elicit strong cellular immunity has shown no protection against infection. More alarmingly, the vaccine appeared to increase the rate of HIV infection in individuals with prior immunity against the adenovirus vector used in the vaccine. A new study in this issue suggests that a different vaccine approach-using a DNA prime/poxvirus boost strategy-induces polyfunctional immune responses to an HIV immunogen. The disappointing results of the recent vaccine trial suggest that a more thorough assessment of vaccine-induced immune responses is urgently needed and that more emphasis should be placed on primate models before efficacy trials are undertaken.

IMMUNE RESPONSES TO VACCINES: QUALITY VERSUS QUANTITY

Back to Top

The Merck candidate vaccine showed good HIV-specific immunogenicity in Phase I and II studies (see http://www.hvtn.org/science/1107.html for the recently released STEP trial results) as measured mostly by a single parameter: the IFN-γ ELISPOT assay. The

POLYFUNCTIONALITY AND ITS SCOPE

Back to Top

Polyfunctional T helper (Th)1 cells that make higher levels of cytokines on an individual cell basis (e.g., as assessed by mean fluorescence intensity for IFN-γ staining) have been associated with protection in vaccine trials against other microbes as well as in HIV-infected elite controllers (31, 32). Both adenovirus-based vaccines and the DNA/NYVAC vaccine trigger T cells that produce at least three different cytokines in response to the HIV immunogen. The DNA/NYVAC vaccine elicited a greater frequency

Review

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.

Mario Roederer (VRC/NIAID/NIH)

TECHNICAL REPORTS

nature medicine

A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells

Chao Ma[1,2,5], Rong Fan[1,2,4,5], Habib Ahmad[1,2], Qihui Shi[1,2], Begonya Comin-Anduix[3], Thinle Chodon[3], Richard C Koya[3], Chao-Chao Liu[2], Gabriel A Kwong[1,2], Caius G Radu[1,3], Antoni Ribas[1,3] & James R Heath[1,2]

Nature Medicine 17, 738–743 (2011)

Professor Antoni Ribas
A true believer of cancer immunotherapy even back to early 2000. I learned a lot from Toni about TCR-T therapy, CTLA-4, and T cell biology

Cellular immunity has an inherent high level of functional heterogeneity. Capturing the full spectrum of these functions requires analysis of large numbers of effector molecules from single cells. We report a microfluidic platform designed for highly multiplexed (more than ten proteins), reliable, sample-efficient (~1 × 10^4 cells) and quantitative measurements of secreted proteins from single cells. We validated the platform by assessment of multiple inflammatory cytokines from lipopolysaccharide (LPS)-stimulated human macrophages and comparison to standard immunotechnologies. We applied the platform toward the *ex vivo* quantification of T cell polyfunctional diversity via the simultaneous measurement of a dozen effector molecules secreted from tumor antigen–specific cytotoxic T lymphocytes (CTLs) that were actively responding to tumor and compared against a cohort of healthy donor controls. We observed profound, yet focused, functional heterogeneity in active tumor antigen–specific CTLs, with the major functional phenotypes quantitatively identified. The platform represents a new and informative tool for immune monitoring and clinical assessment.

TECHNICAL REPORTS

nature medicine

A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells

Chao Ma[1,2,5], Rong Fan[1,2,4,5], Habib Ahmad[1,2], Qihui Shi[1,2], Begonya Comin-Anduix[3], Thinle Chodon[3], Richard C Koya[3], Chao-Chao Liu[2], Gabriel A Kwong[1,2], Caius G Radu[1,3], Antoni Ribas[1,3] & James R Heath[1,2]

Article

pubs.acs.org/ac

High-Throughput Secretomic Analysis of Single Cells to Assess Functional Cellular Heterogeneity

Yao Lu,[†,⊗] Jonathan J. Chen,[†,⊗] Luye Mu,[†,‡] Qiong Xue,[†] Yu Wu,[†] Pei-Hsun Wu,[§] Jie Li,[∥] Alexander O. Vortmeyer,[∥] Kathryn Miller-Jensen,[†] Denis Wirtz,[§] and Rong Fan[*,†,⊥]

2011

2015

CrossMark

PNAS PLUS

PNAS

Highly multiplexed profiling of single-cell effector functions reveals deep functional heterogeneity in response to pathogenic ligands

Yao Lu[a,1], Qiong Xue[a,1], Markus R. Eisele[a,b], Endah S. Sulistijo[a], Kara Brower[c], Lin Han[a], El-ad David Amir[d], Dana Pe'er[d], Kathryn Miller-Jensen[a,e,f,2], and Rong Fan[a,f,g,2]

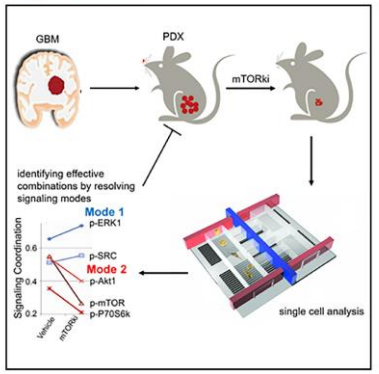
[a]Department of Biomedical Engineering, Yale University, New Haven, CT 06520; [b]Institute for System Dynamics, University of Stuttgart, D-70563 Stuttgart, Germany; [c]IsoPlexis, New Haven, CT 06511; [d]Department of Biological Sciences, Columbia University, New York, NY 10027; [e]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520; [f]Yale Comprehensive Cancer Center, New Haven, CT 06520; and [g]Yale Stem Cell Center, Yale School of Medicine, New Haven, CT 06520

Cancer Cell

Article

Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma

Graphical Abstract



Authors

Wei Wei, Young Shik Shin, Min Xue, ..., Paul S. Mischel, James R. Heath, Beatrice Gini

Correspondence

pmischel@ucsd.edu (P.S.M.), heath@caltech.edu (J.R.H.)

In Brief

Wei et al. utilize single-cell phosphoproteomic analysis of patient-derived glioblastoma models to identify shifts in signaling coordination following short-term treatment with kinase inhibitors, which facilitates the design of combination therapy approaches with reduced resistance and improved efficacy.

Science Signaling

AAAS

CYTOKINE SIGNALING

Analysis of single-cell cytokine secretion reveals a role for paracrine signaling in coordinating macrophage responses to TLR4 stimulation

Qiong Xue,[1*†] Yao Lu,[1*] Markus R. Eisele,[1,2‡] Endah S. Sulistijo,[1] Nafeesa Khan,[1] Rong Fan,[1§] Kathryn Miller-Jensen[1,3§]

Cancer Discovery

JAK-STAT Pathway Activation in Malignant and Nonmalignant Cells Contributes to MPN Pathogenesis and Therapeutic Response

Maria Kleppe[1], Minsuk Kwak[2], Priya Koppikar[1], Markus Riester[3,4], Matthew Keller[1], Lennart Bastian[1], Todd Hricik[1], Neha Bhagwat[1,5], Anna Sophia McKenney[1,5,6], Efthymia Papalexi[1], Omar Abdel-Wahab[1,7], Raajit Rampal[1,7], Sachie Marubayashi[1], Jonathan J. Chen[2], Vincent Romanet[8], Jordan S. Fridman[9], Jacqueline Bromberg[10], Julie Teruya-Feldstein[11], Masato Murakami[8], Thomas Radimerski[8], Franziska Michor[3,4], Rong Fan[2,12], and Ross L. Levine[1,7]

High throughput single-cell cytokine function profiling

Single-Cell 42-Plex Protein Secretion Profiling

PNAS

Highly multiplexed profiling of single-cell effector functions reveals deep functional heterogeneity in response to pathogenic ligands

Yao Lu[a,1], Qiong Xue[a,1], Markus R. Eisele[a,b], Endah S. Sulistijo[a], Kara Brower[c], Lin Han[a], El-ad David Amir[d], Dana Pe'er[d], Kathryn Miller-Jensen[a,e,f,2], and Rong Fan[a,f,g,2]

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Dalian Inst.of Chemical Physics

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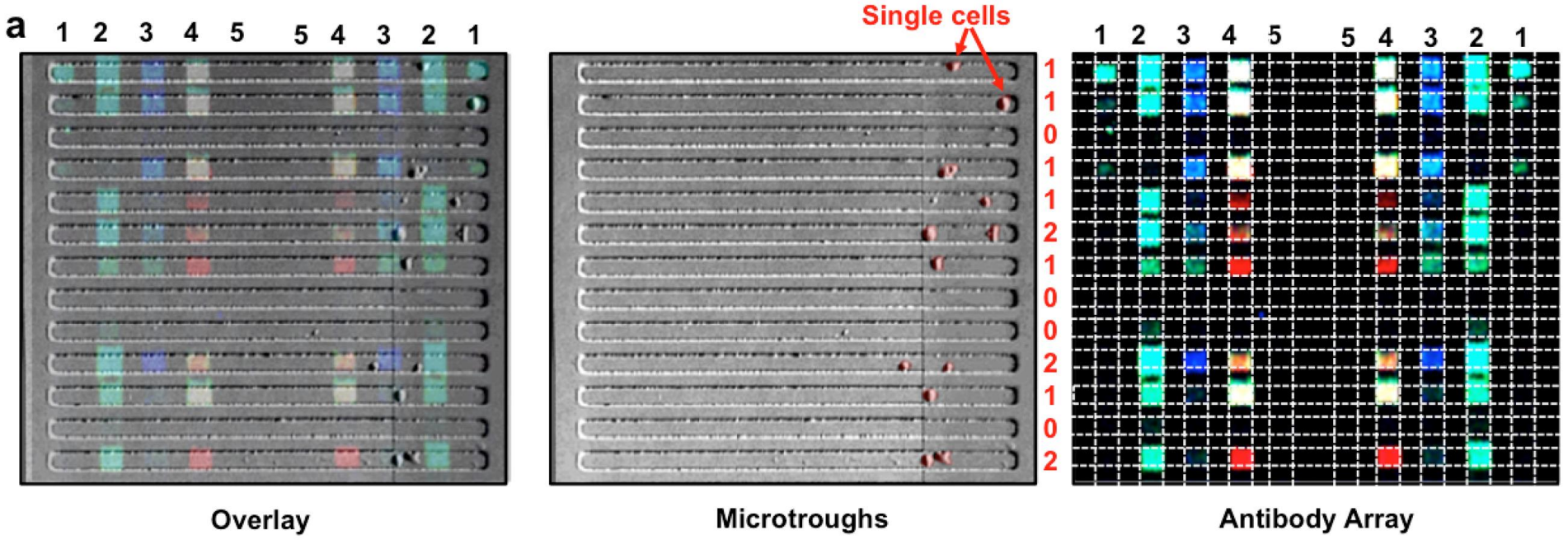
Director of Analytics, Cell Therapies, Takeda

Kathryn Miller-Jensen
Yale BME

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



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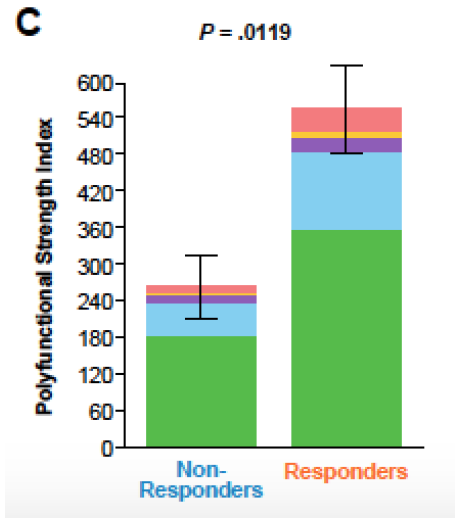
2018 132: 804-814
doi:10.1182/blood-2018-01-828343 originally published online June 12, 2018

Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL

John Rossi, Patrick Paczkowski, Yueh-Wei Shen, Kevin Morse, Brianna Flynn, Alaina Kaiser, Colin Ng, Kyle Gallatin, Tom Cain, Rong Fan, Sean Mackay, James R. Heath, Steven A. Rosenberg, James N. Kochenderfer, Jing Zhou and Adrian Bot

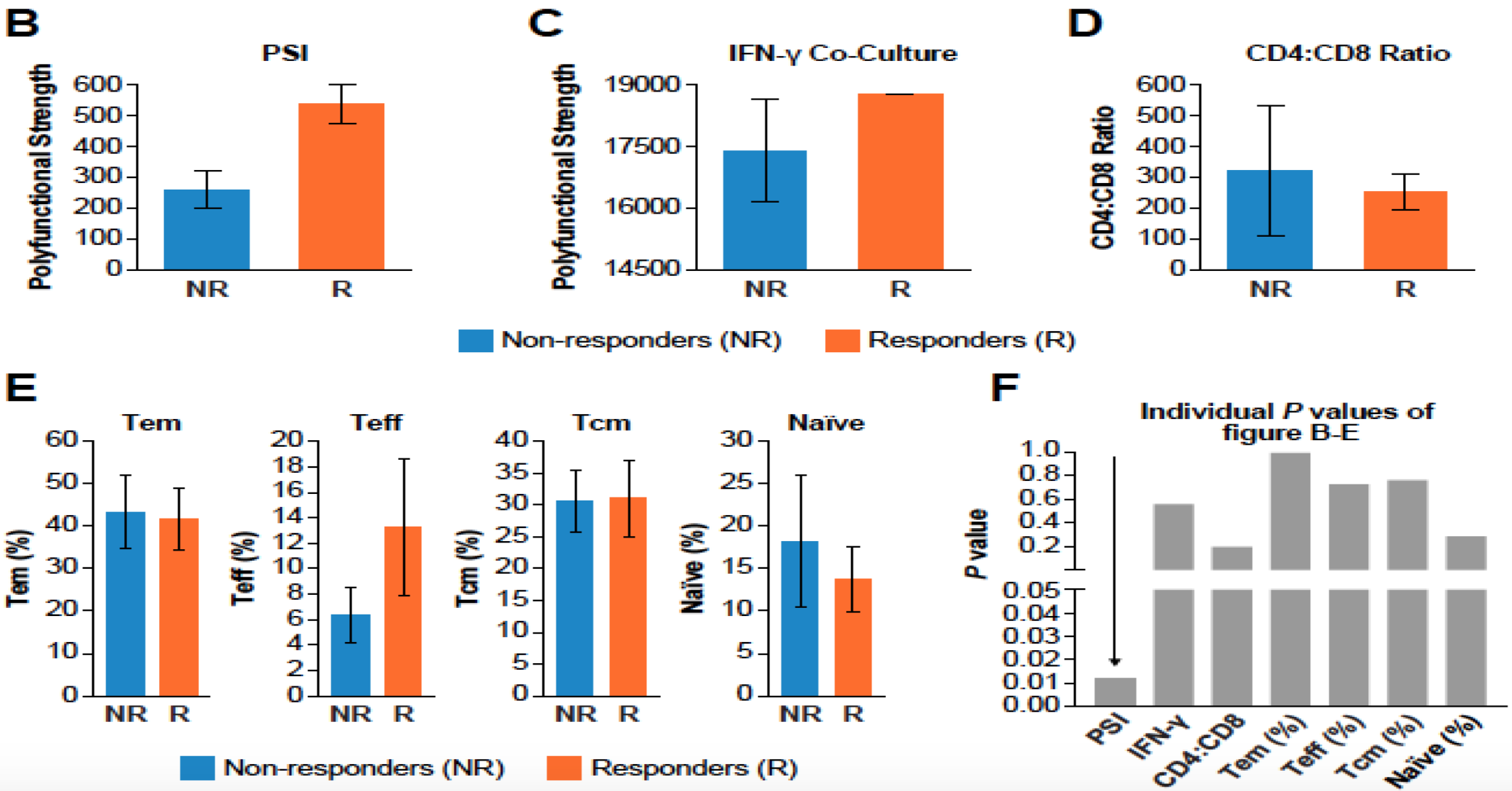
Adrian Bot

John Rossi



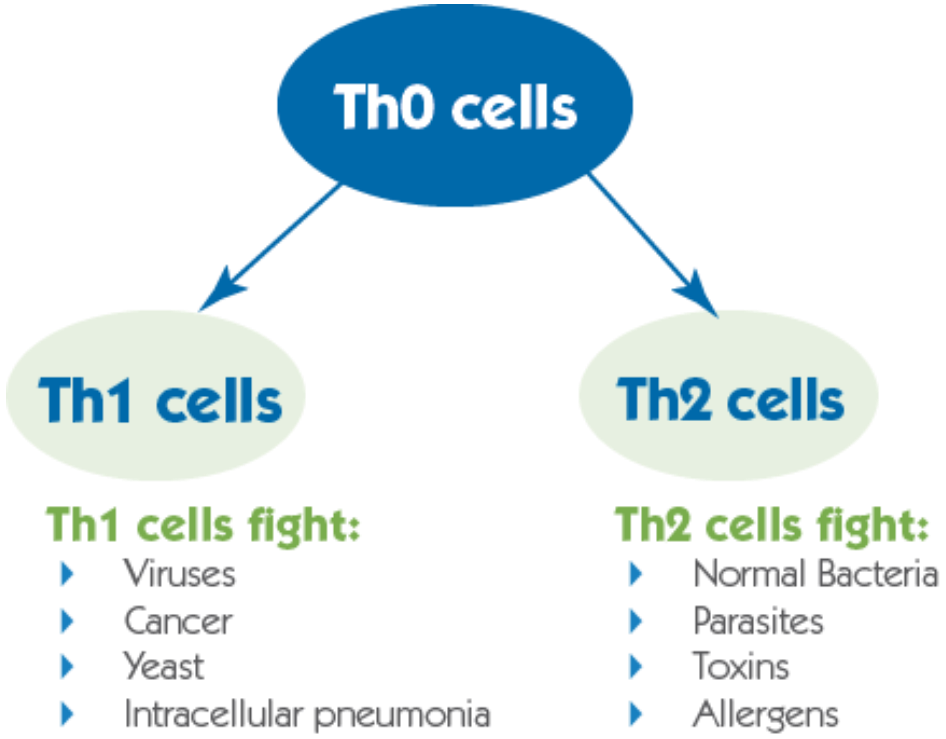
Rossi et al., ***Blood*** (2018)

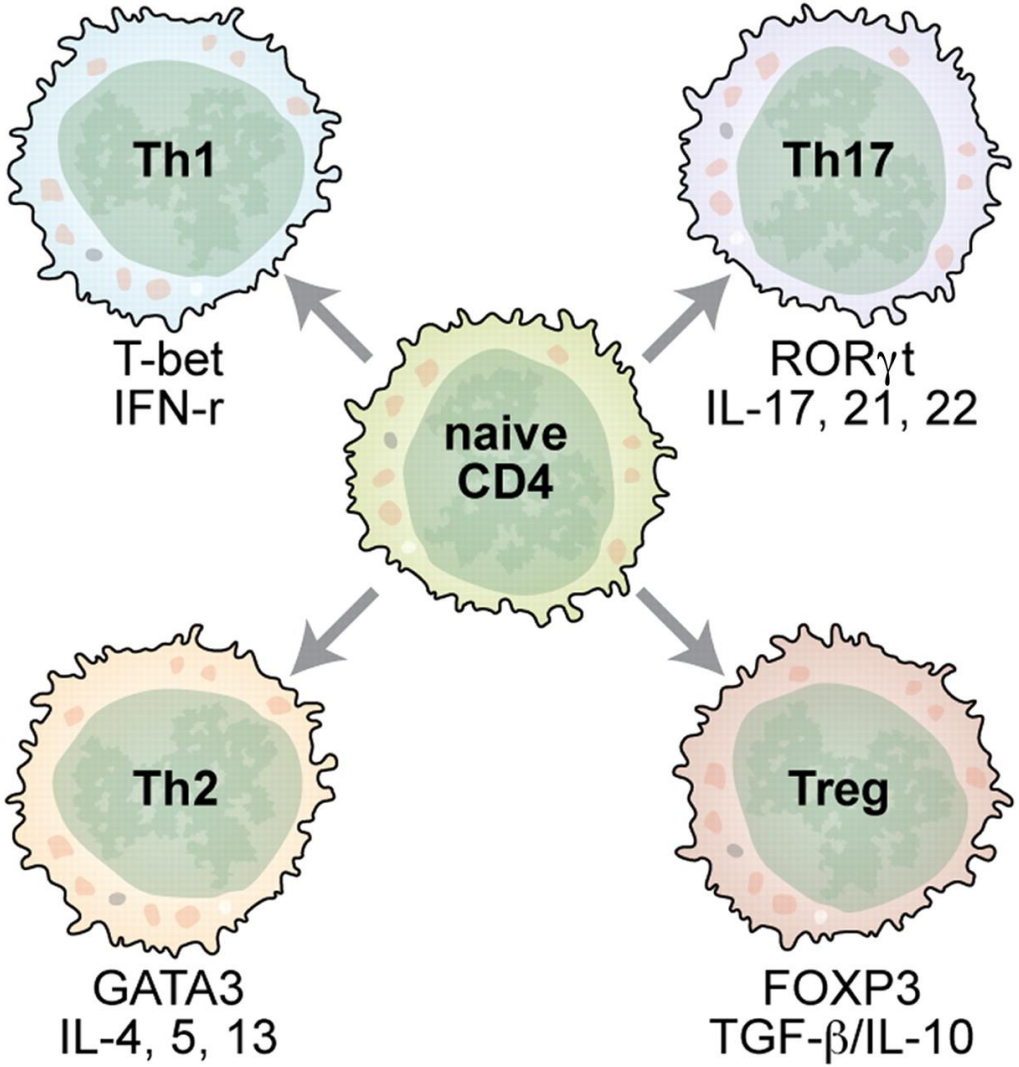
IFNg, phenotype and differentiation status do not correlate with patient responses



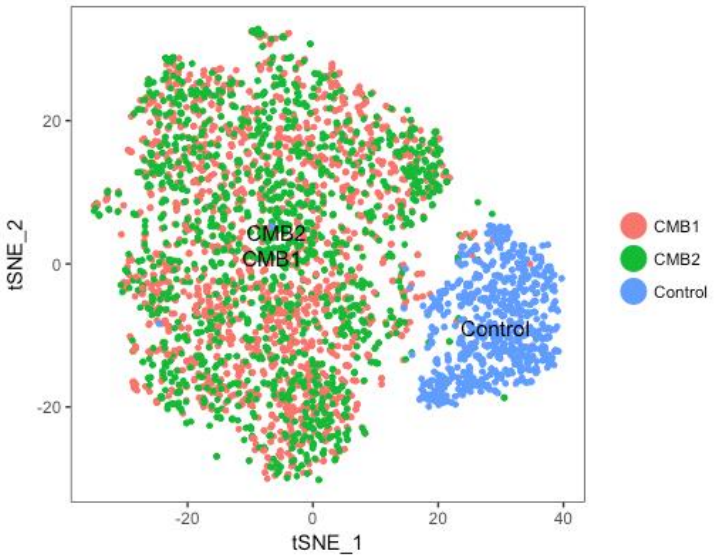
CD4+ T cell polarization and subtypes

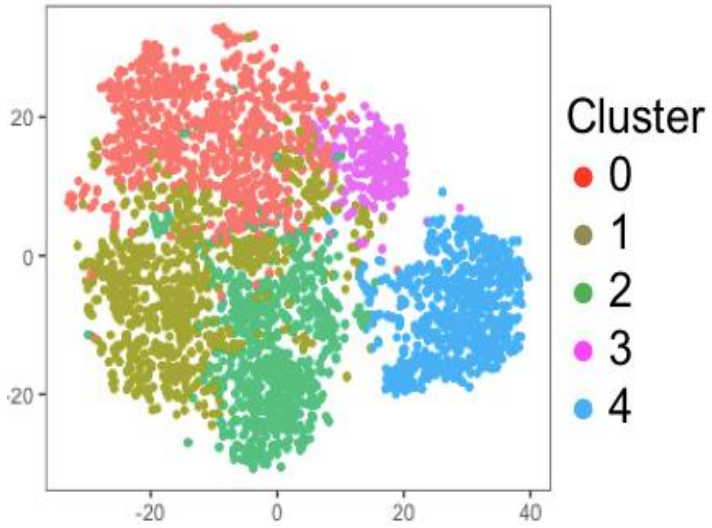
CD4+ T cell polarization





Preliminary Study: single-cell integrative omics analysis of CAR-T cell activation





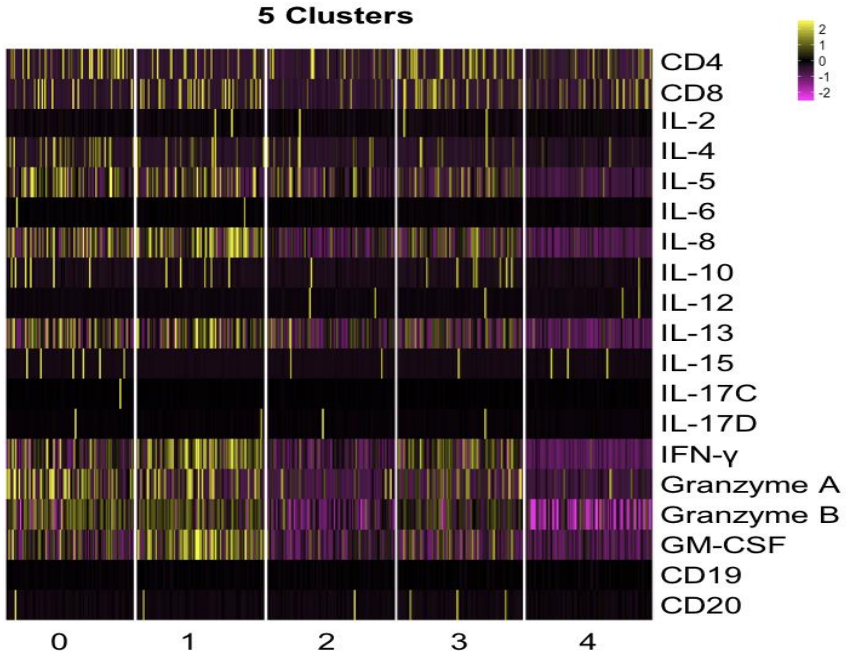
ORIGINAL RESEARCH

Single-cell Analysis of CAR-T Cell Activation Reveals A Mixed T_H1/T_H2 Response Independent of Differentiation

Iva Xhangolli [1,2,a], Burak Dura [1,2,b], GeeHee Lee [1,2,c], Dongjoo Kim [1,2,d], Yang Xiao [1,2,e], Rong Fan [1,2,3,*,f]

[1] *Department of Biomedical Engineering, Yale University, New Haven, CT 06520, USA*
[2] *Yale Cancer Center, Yale School of Medicine, New Haven, CT 06520, USA*
[3] *Yale Stem Cell Center, Yale School of Medicine, New Haven, CT 06520, USA*

Received 10 March 2019; accepted 19 March 2019
Available online 20 June 2019



Iva Xhangolli
Takeda

Single-cell CITE-seq to dissect CAR-T Cell Activation States

Journal for ImmunoTherapy of Cancer

Single-cell multiomics dissection of basal and antigen-specific activation states of CD19-targeted CAR T cells

Zhiliang Bai

Zhiliang Bai,[1,2] Stefan Lundh,[3] Dongjoo Kim,[1] Steven Woodhouse,[4] David M Barrett,[5,6] Regina M Myers,[5] Stephan A Grupp,[5,6] Marcela V Maus ,[7,8] Carl H June,[3,9] Pablo G Camara,[4] J Joseph Melenhorst,[3,9] Rong Fan [1,10]

Carl June

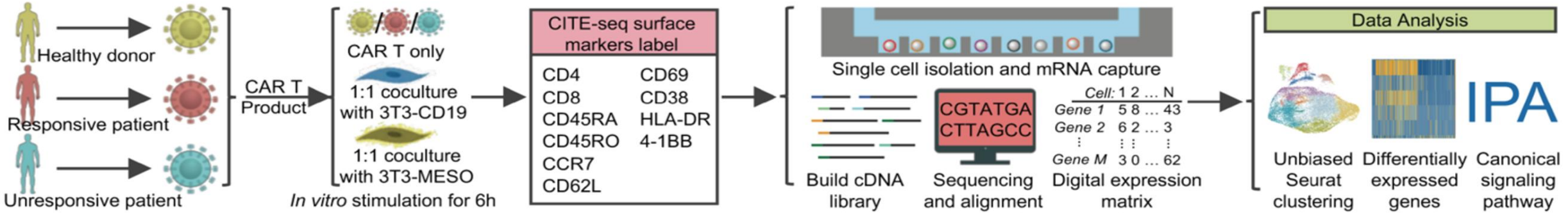
Jos Melenhorst

Marcela Maus (MGH)

Steve Grupp

Pablo G. Camara

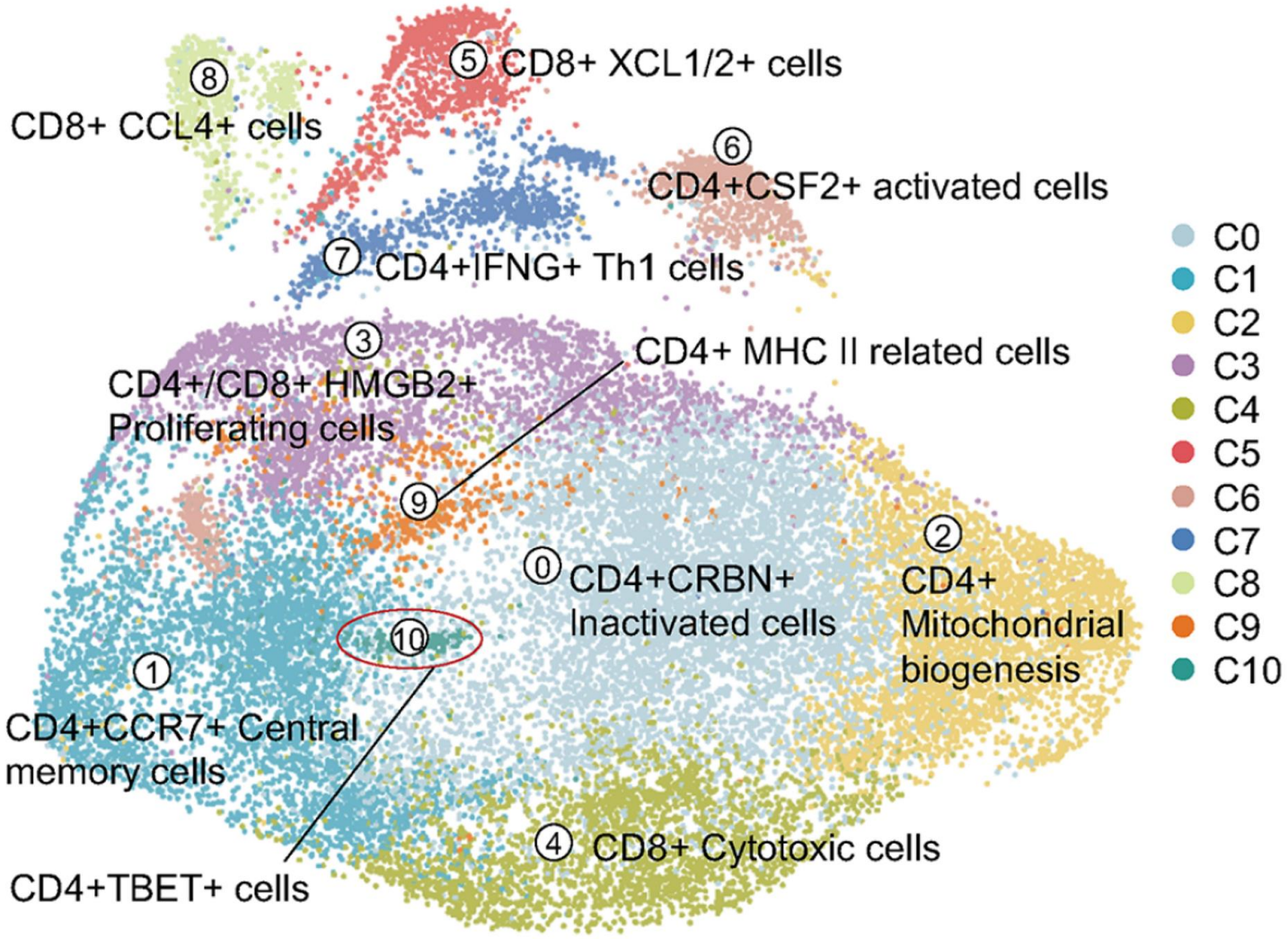
Bai Z, Lundh S, Kim D, *et al*. *Journal for ImmunoTherapy of Cancer (JITC)*, 2021;9:e002328.

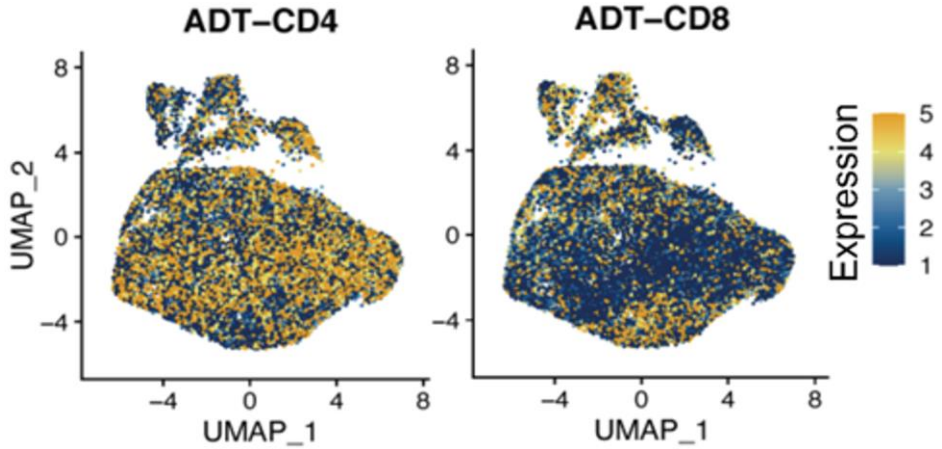


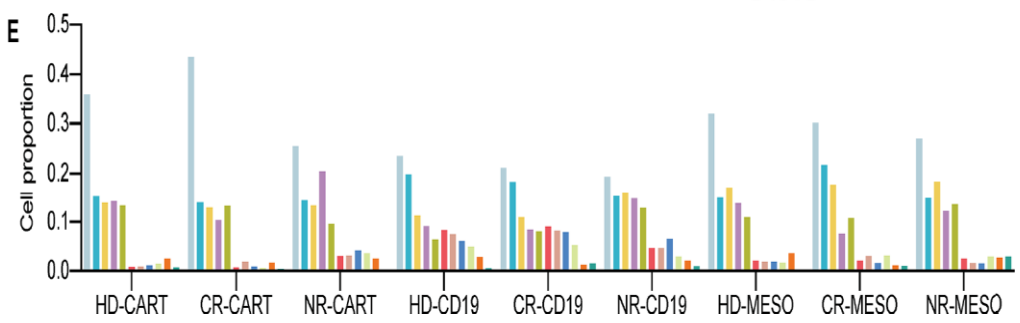
Using single cell mRNA sequencing in conjunction with CITE-seq, we performed multi-omics characterization of CAR T cells generated from healthy donor and acute lymphoblastic leukemia (ALL) patients. CAR T used in this study were manufactured at the University of Pennsylvania through lentiviral transduction with a CD19-4-1BB-CD3ζ construct. Besides unstimulated baseline condition, we engineered NIH-3T3 cells with human CD19 or mesothelin expression to conduct *ex vivo* antigen-specific or non-antigen stimulation of CAR T cells through 6 hours coculture at a 1:1 ratio.

Single Cell Atlas of CAR T Cell Basal and Activation States

Healthy Donor (HD), Complete Response (CR), and Non-Responder (NR)

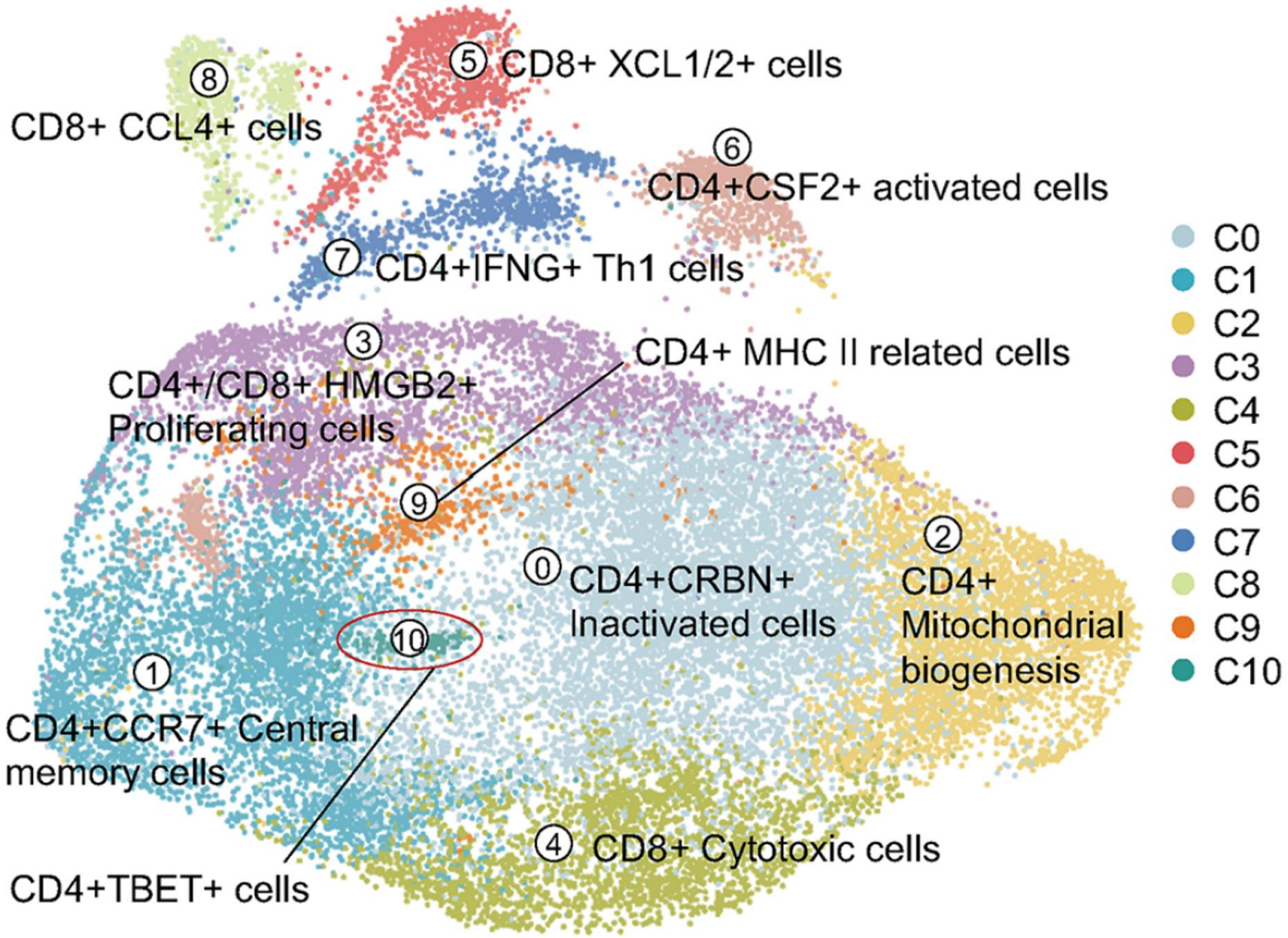






Single Cell Atlas of CAR T Cell Basal and Activation States

Healthy Donor (HD), Complete Response (CR), and Non-Responder (NR)



CRBN (Cereblon), the E3 ubiquitin ligase substrate receptor, is a negative regulator of T cell activation.

HMGB2 correlates with G2/M and S phase proliferating T cells. HMGB2 expression is strongly associated with transition from the quiescent to the proliferative state of NSCs

CSF2, encoding GM-CSF, a cytokine increasingly recognized as an important mediator of T cell function balance., the exact role in different pathologies remains unclear.

XCL1/2, chemokines (lymphotactin), produced mainly by activated CD8 T cells. Its chemotactic activity seems primarily controlling movement of CD4 and CD8 T cells

CCL4, also known as Macrophage inflammatory protein-1β (MIP-1β) is a CC chemokine with specificity for CCR5 receptors. It is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells.

CAR-T Clinical Trials (ALL, CLL, and NHL)

Type	Patients	Trial Phase	CAR Type	CR rate	Midian follow-up	Relapse Rate	Severe CRS rate	Ref
ALL	Ped/Adult	I	4-1BB	79% (22/28)	7 mo	26% (7/27)	27%	[16]
ALL	Adult	I	CD28	67% (32/48)	29 mo	61% (25/41)	26%	[7]
ALL	Pediatric	II	4-1BB	81% (61/75)	13mo	33% (20/61)	47%	[17]
ALL	Pediatric	I	CD28	60% (12/20)	10mo	17% (2/12)	29%	[18]
ALL	Adult	I	4-1BB	93% (27/29)	N/A	33% (9/27)	23%	[19]
ALL	Pediatric	I	4-1BB	93% (40/43)	9.6mo	45% (18/40)	23%	[20]
CLL	Adult	I	4-1BB	17% (4/24)	N/A	N/A	8%	[21]
CLL	Adult	I	4-1BB	29% (4/14)	19 mo	0%	43%	[22]
NHL	Adult	II	4-1BB	40% (37/93)	14 mo	N/A	22%	[25]
NHL	Adult	II	CD28	54% (55/101)	15.4mo	N/A	13%	[26]
NHL	Adult	I	4-1BB	57% (16/28)	28.6mo	0%	18%	[27]
NHL	Adult	I	CD28	55% (12/22)	N/A	8% (1/12)	18%	[28]
NHL	Adult	I	4-1BB	33% (10/30)	N/A	11% (1/9)	13%	[29]

Patient Demographics and Clinical Response

Supplementary Table 1. Patient demographics and response documentation

ID	Infusion date	Age	Sex	Response to CART19	B cell aplasia (BCA)	BCA duration (months)	Relapse	Time to relapse (days)	Relapse type	Last contact days
112	4/16/13	9.6	F	MRD neg CR	Yes	72	No	N/A	N/A	2171
139	6/11/14	15.2	F	MRD neg CR	Yes	4	Yes	1807	CD19 negative	2000
151	9/9/14	15.5	F	MRD neg CR	Yes	61	No	N/A	N/A	1833
158	2/25/15	4.9	F	MRD neg CR	Yes	4	No	N/A	N/A	1696
165	6/23/15	14.5	M	MRD neg CR	Yes	54	No	N/A	N/A	1637
157	2/10/15	12.3	M	MRD neg CR	Yes	6	Yes	632	CD19 positive	1135
161	4/7/15	9.7	F	MRD neg CR	Yes	3	Yes	287	CD19 positive	602
171	9/8/15	9.8	M	MRD neg CR	Yes	3	Yes	595	CD19 positive	944
110	3/19/13	10.0	F	MRD neg CR	Yes	3	Yes	79	CD19 positive	221
111	4/1/13	9.9	F	MRD positive	Yes	1	Yes	44	CD19 positive	69
117	5/9/13	16.2	M	No response	N/A	N/A	N/A	N/A	N/A	79
167	7/9/15	21.5	M	No response	N/A	N/A	N/A	N/A	N/A	31

Carl June

Jos Melenhorst

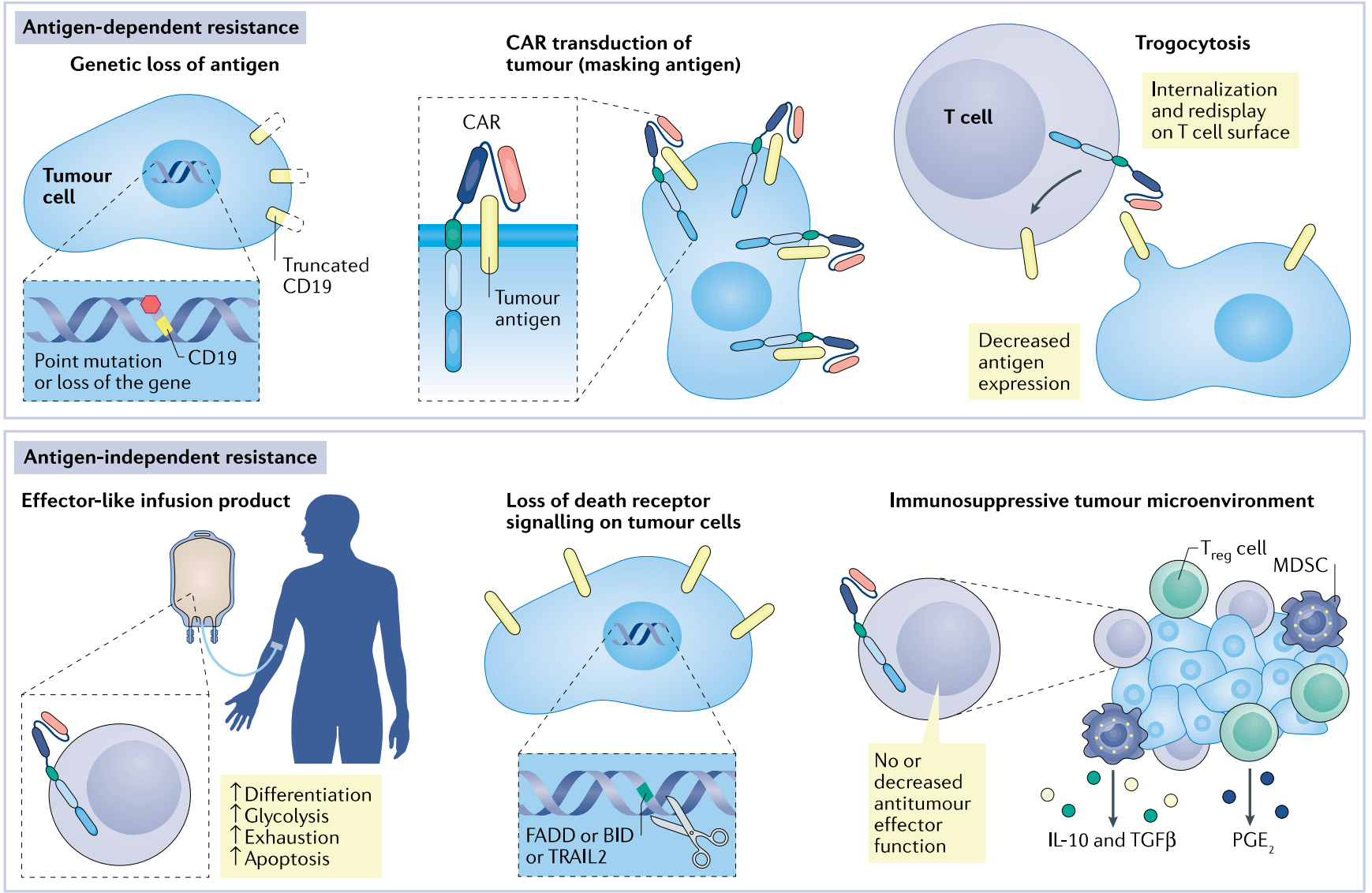
Steve Grupp

Pablo G. Camara

Penn Medicine

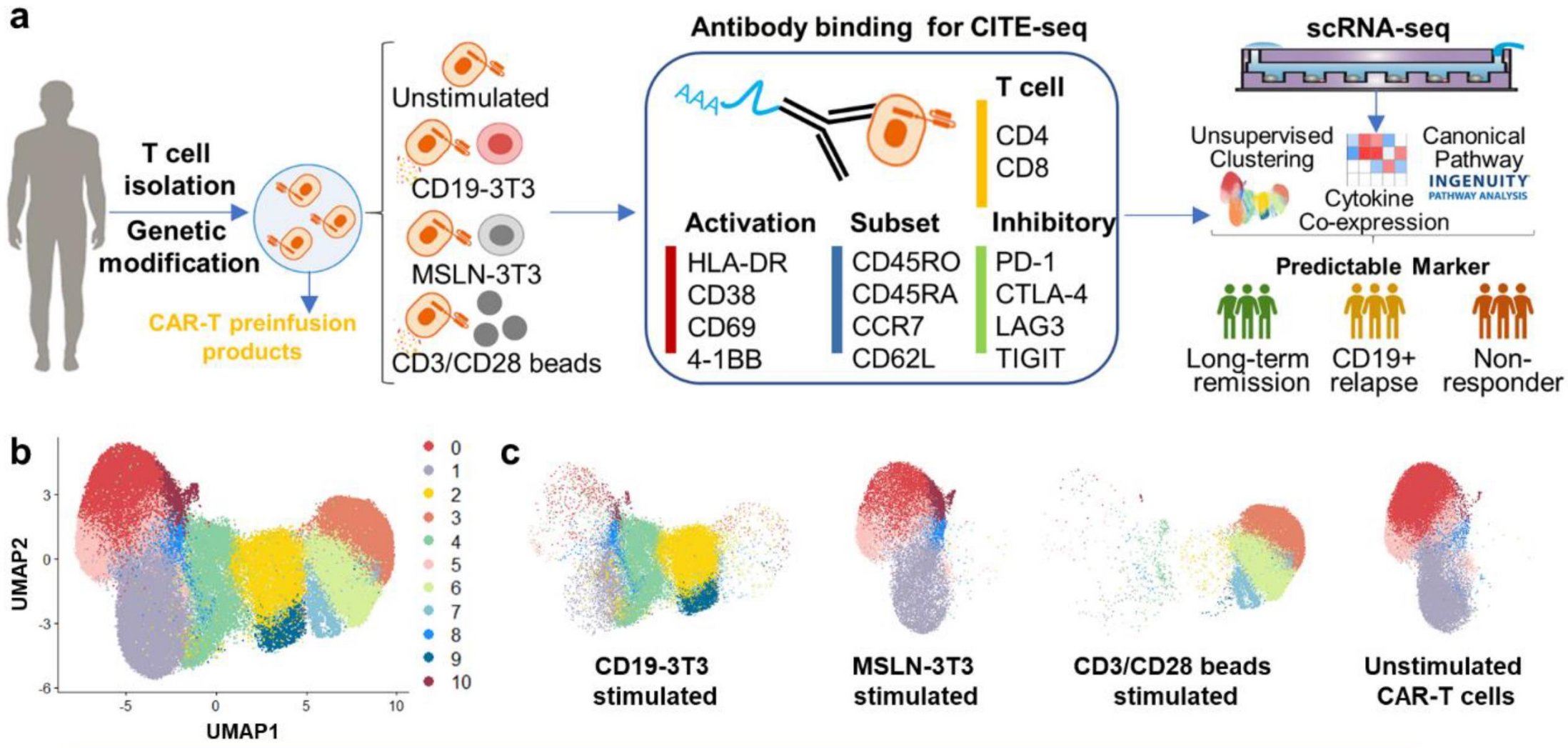
The mechanisms of CD19-positive relapse remain elusive

- Chimeric antigen receptor (CAR) T-cell therapy has been a breakthrough in cancer therapy, yet failure to achieve long-term CAR T-cell persistence remains a major barrier to sustained remission in many patients.
- The remissions in a significant fraction of subjects are short-lived and 30-60% of treated patients relapse within one year.
- However, the molecular mechanisms that underlie the acquired resistance to the CAR T treatment are still unclear, especially for CD19-positive relapse.
- We hypothesize that the functional capacity of CAR T cells in the infusion product could be an essential factor determining long-term therapeutic response.



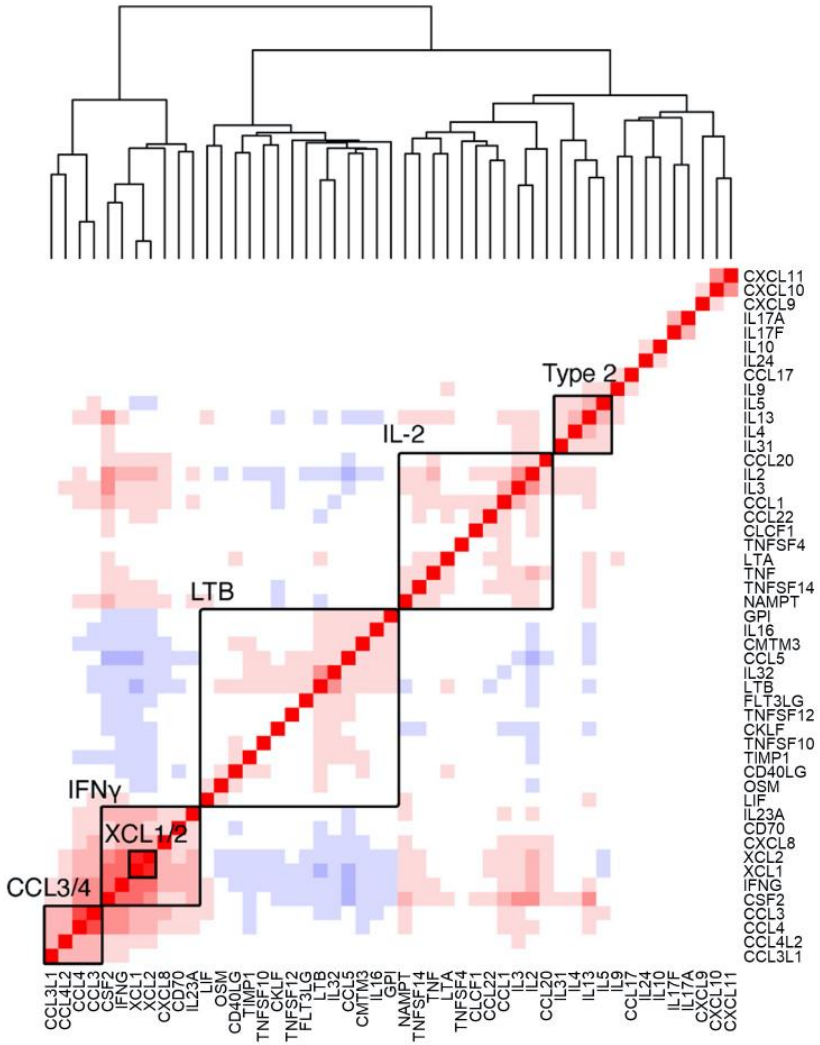
Rebecca C. Larson and Marcela V. Maus. Nat Rev Cancer, 2021 Mar.

Single-cell multi-omics profiling of CAR-T cell activation states and therapeutic responses in ALL Patients

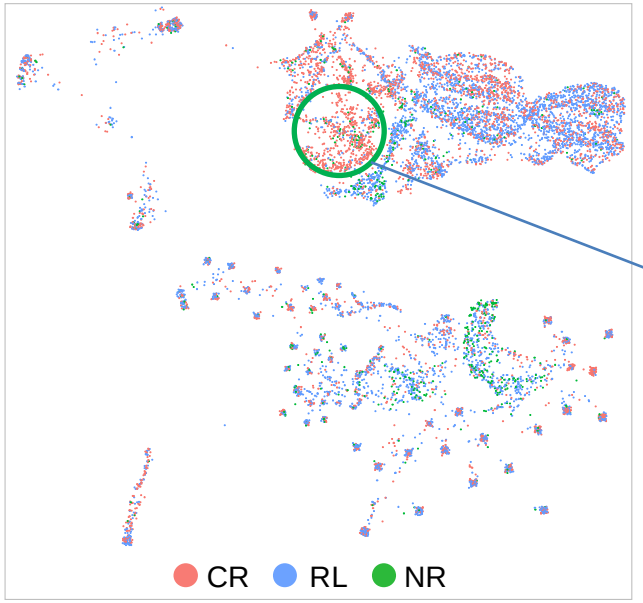


Bai ZL, et al., *Science Advances,* in press (2022)

Failure to sustain a long-term response to the therapy is associated with a deficit of Th2 function in antigen-specific CAR T activation

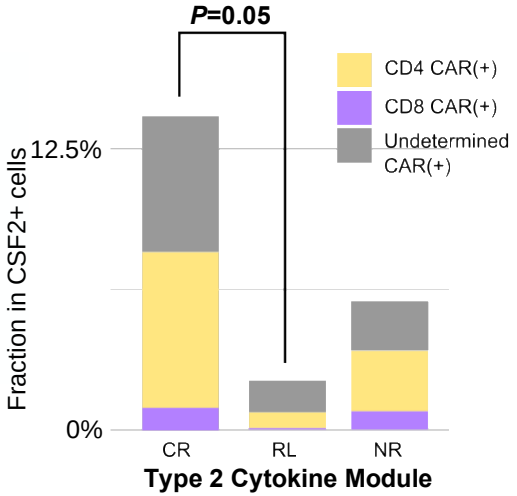


Unsupervised analysis identified co-expressed cytokine modules



The distribution of Th2 module expression

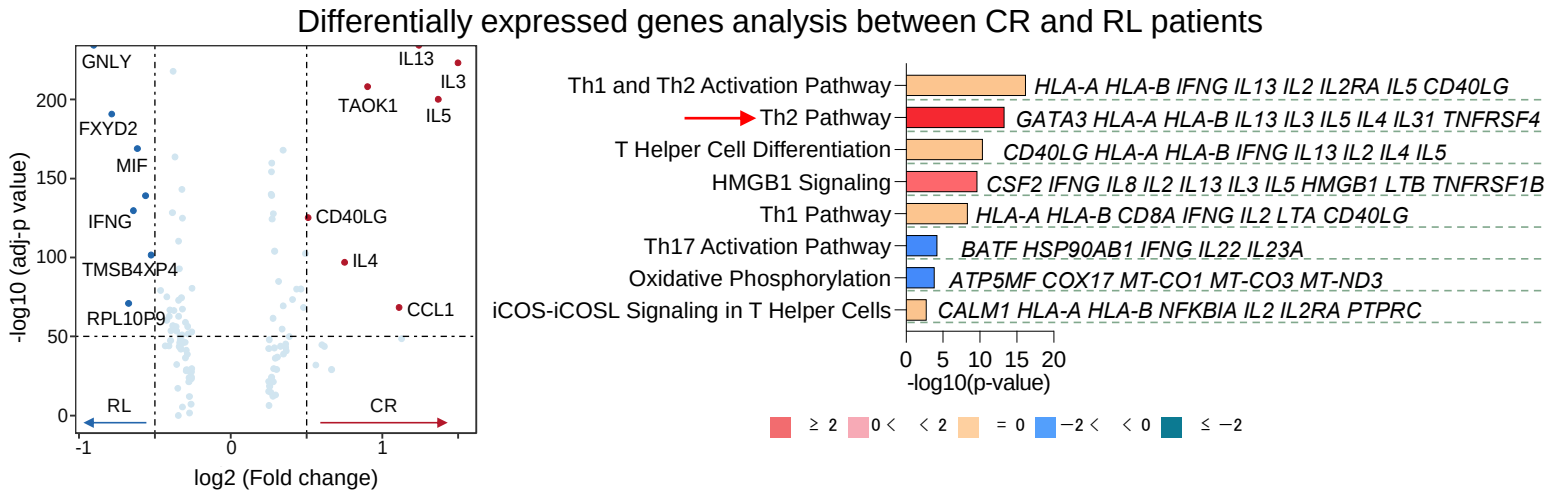
- The type-2 (Th2) module was found to be enriched in a region containing mostly cells from CR patients, implying that Th2 function might be indispensable for maintaining a long-term remission in CAR T therapy.

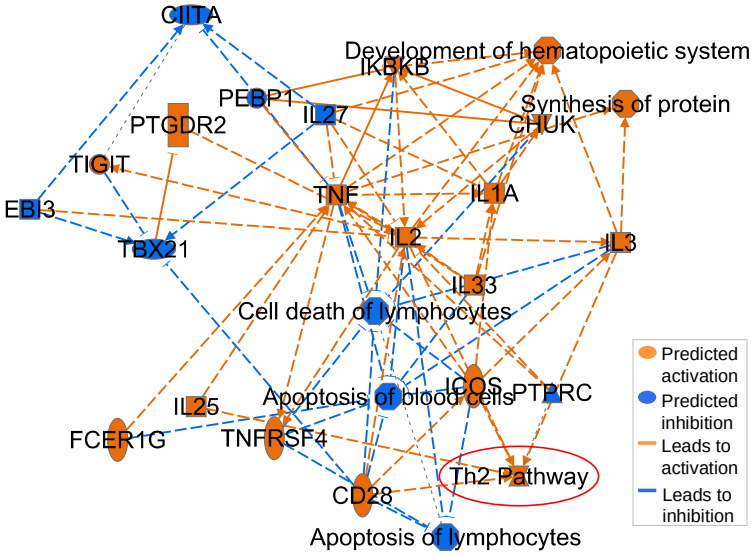


- A quantitative comparison between the landscape of CD19-3T3 stimulated CAR T cells identified a significant depletion of CAR+ cells expressing the type-2 cytokine module in RL patients compared to CR patients (MFC=4.14, Mann-Whitney test p-value=0.05)

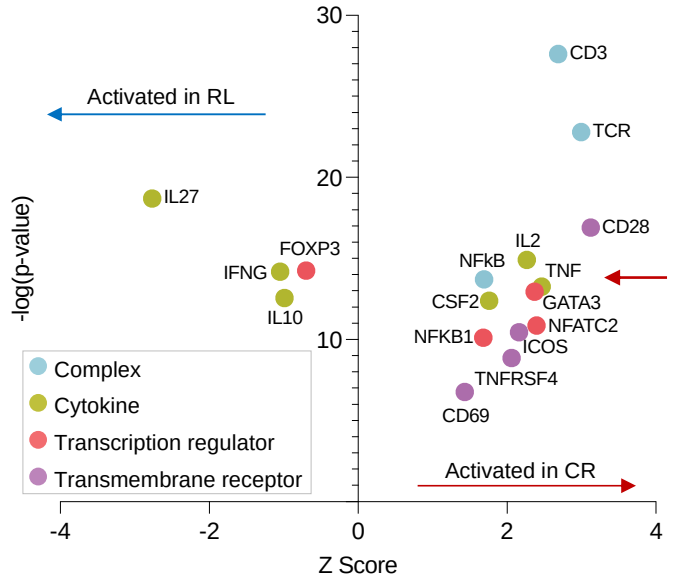
Bai ZL, et al., *Science Advances,* in press (2022)

Functional immune programs in activated CAR+ cells do not distinguish CD19-positive relapse from CR subjects, except for Th2 related pathways, genes and upstream regulators

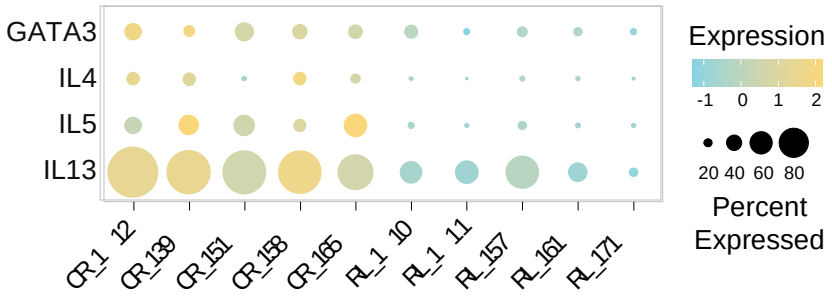


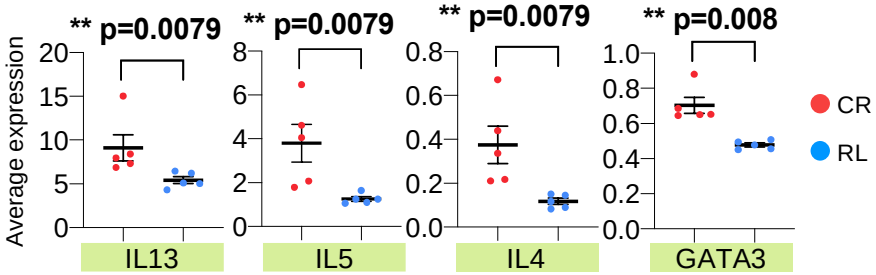


Network analysis of canonical pathways, upstream regulators, and biological functions



IPA upstream analysis



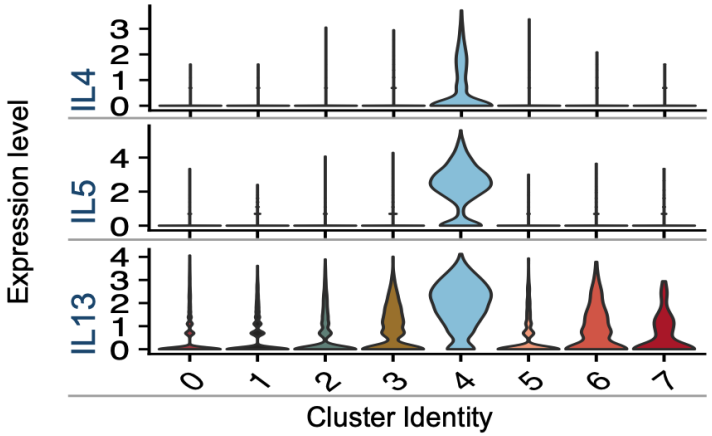


- To ensure that the above observations were not biased by any single subject, we assessed the expression of IL13, IL5, IL4 and GATA3 in all the CR and RL patients. The results of this analysis showed ubiquity of expression enrichment of the four genes in CR patients compared with relapsed subjects.

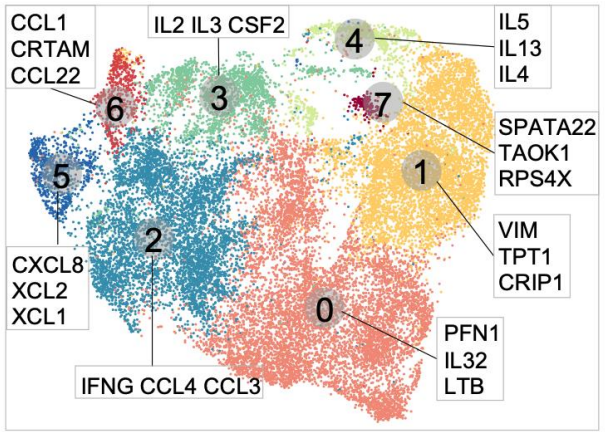
Bai ZL, et al., *Science Advances,* in press (2022)

Whether the proportion of cells with specific functional signatures affects the sustainability of CAR T cells?

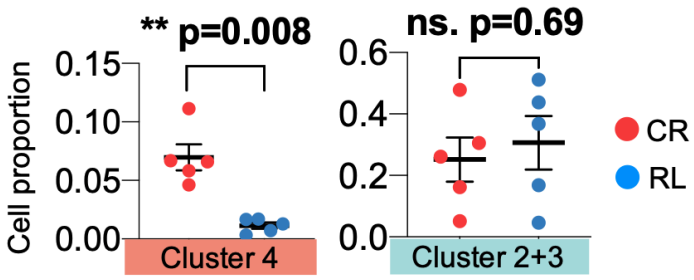
Single cell transcriptomic clustering of CAR T cells identifies comparable functional immune profiles between CR and RL patients, except for Th2 function.



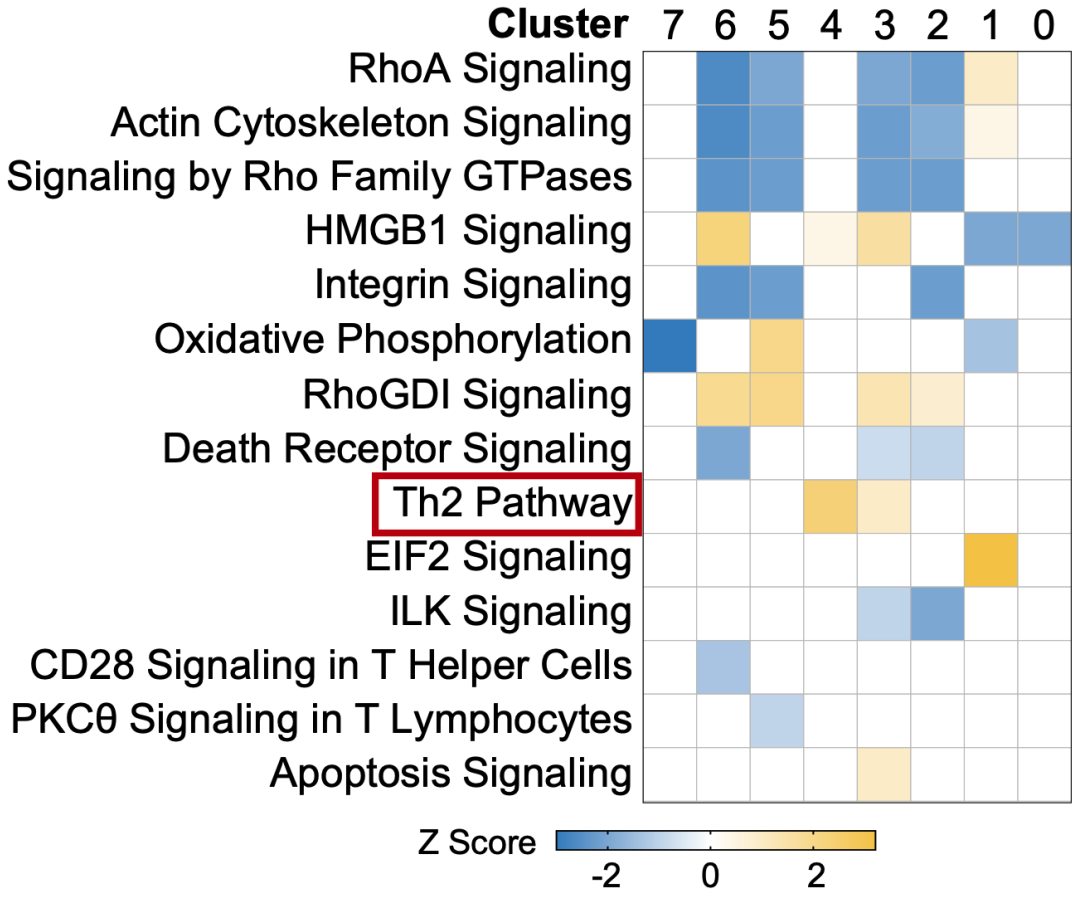
The expression of *IL4*, *IL5*, *IL13* in each identified clusters.



UMAP clustering of CAR+ cells from CR and RL patients and the top 3 dominant genes defining each cluster.



Comparison of cell proportion in particular cluster between CR and RL patients. The P values were calculated with two-tailed Mann-Whitney test. Scatter plots show mean±s.e.m.



Canonical signaling pathway comparison between all the identified clusters. Differentially expressed genes of each cluster are used to identify the biological pathways. A statistical quantity, called z score, is computed and used to characterize the activation level. z score reflects the predicted activation level ($z < 0$, inhibited; $z > 0$, activated; $z \geq 2$ or $z \leq -2$ can be considered significant).

Bai ZL, et al., *Science Advances,* in press (2022)

Validation in a cohort of 49 patients using high-plex cytokine profiling and flow assay confirmed the role of Th2 function in long-term response

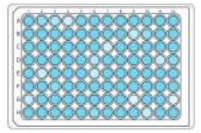
Cohort 1
CHP959
(n=22)

7 CR and 15 RL

Cohort 2
16CT022
(n=27)

19 CR and 8 RL

Stimulation of CAR T infusion products

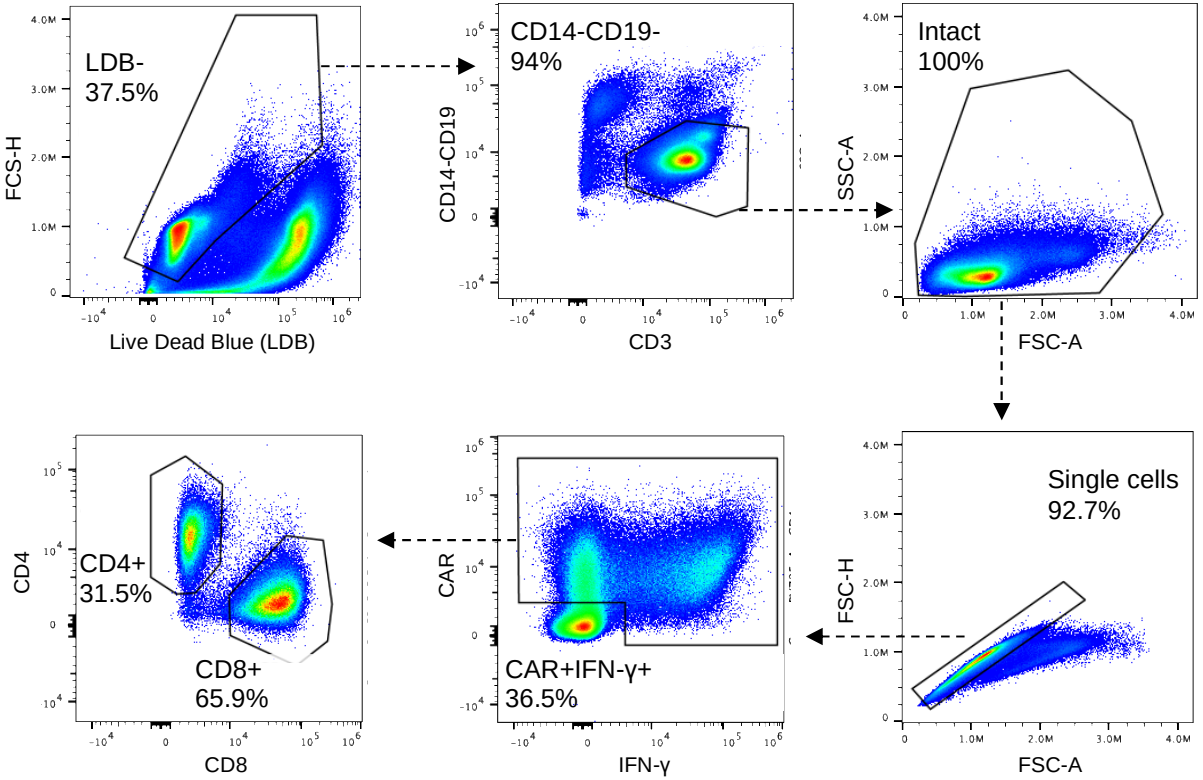


CD19-3T3
MSLN-3T3
CD3/CD28 beads

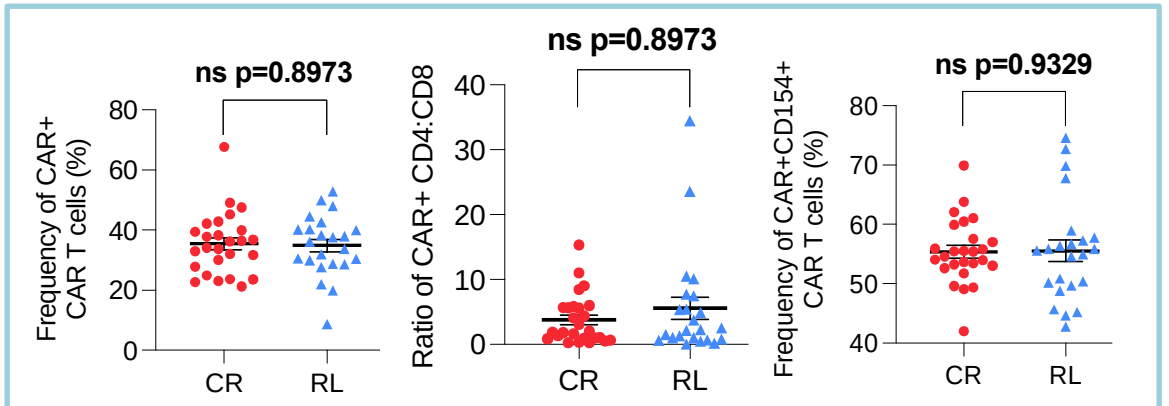
Intracellular flow cytometry (at UPenn)

Multiplexed secretomic assay (at Yale)

To further validate and refine our findings, we expanded our study to 49 B-ALL patients enrolled in two anti-CD19 CAR T-cell therapy trials. 26 patients had robust CR response with median relapse-free remission duration of 33.7 months, and the other 23 patients developed CD19-positive relapse at a median timepoint of 9.3 months

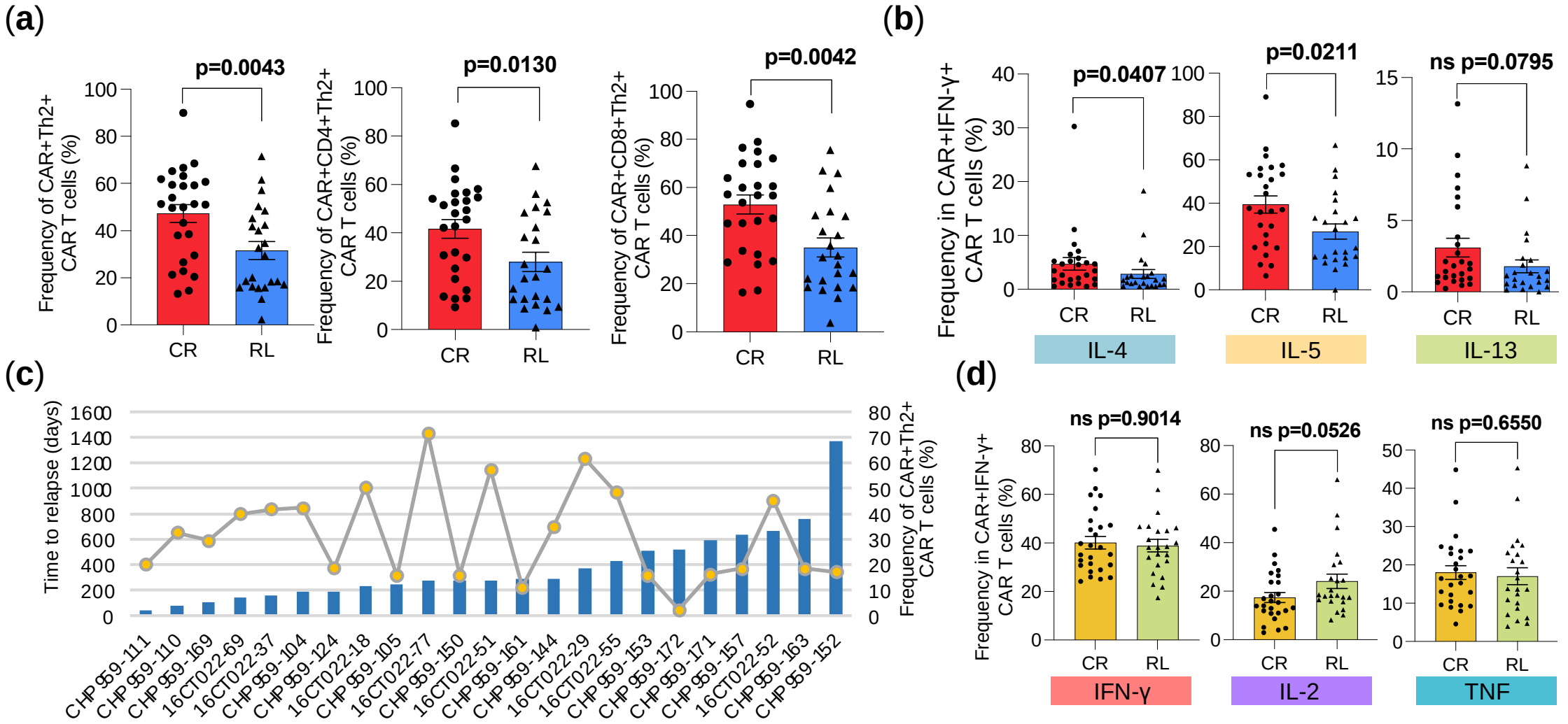


Gating strategy of flow cytometry data analysis. The Live Dead Blue (LDB) was used to select live cells, followed by CD14-CD19−/CD3+ subset, intact and single cell selection. CAR+ IFNG+ were applied to select fully activated CAR+ CAR T cells, and CD4+ or CD8+ subpopulation was analyzed separately.



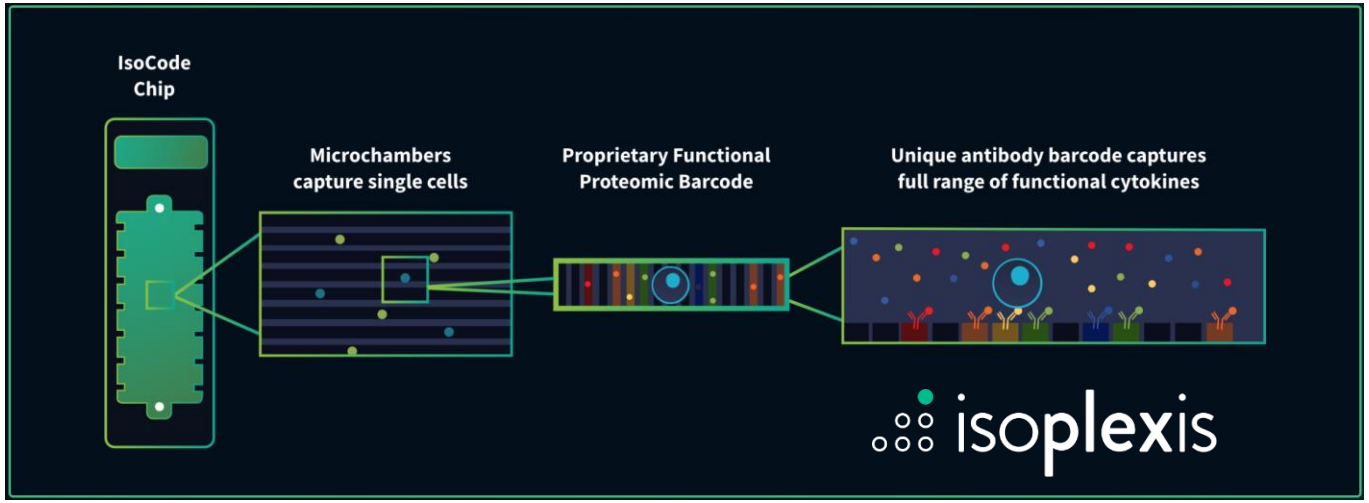
Quantification of the CAR transduction efficiency, CD4+/CD8+ ratio, and activation level in the CAR T cells did not reveal any significant differences between CR and RL patients

Validation in a cohort of 49 patients using high-plex cytokine profiling and flow assay confirmed the role of Th2 function in long-term response



Bai ZL, et al., *Science Advances,* in press (2022)

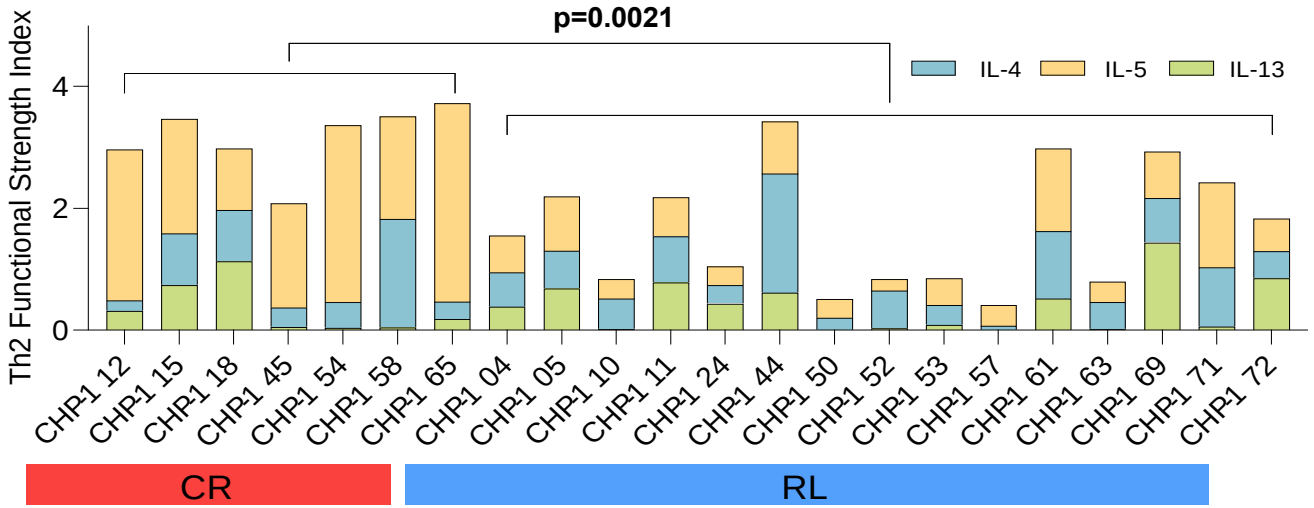
Validation in a cohort of 49 patients using high-plex cytokine profiling and flow assay confirmed the role of Th2 function in long-term response

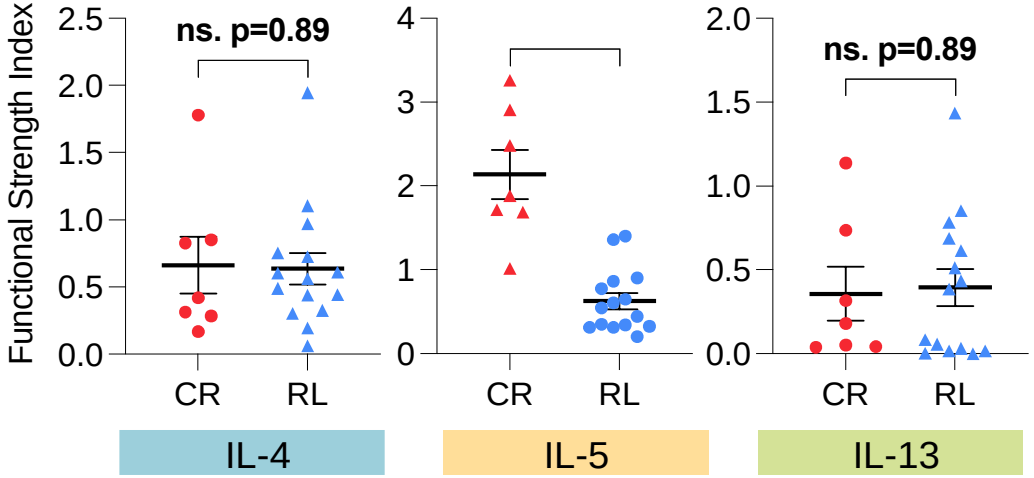


Multiplexed single-cell cytokine secretomic assay

Total chambers: 12,000
Total cells loaded: 30,000 cells

The **functional strength index (FSI)** was used to describe the specific functionality profile of CAR T cells, which was defined as the frequency of cells secreting a cytokine multiplied by the average signal intensity of the cytokine.

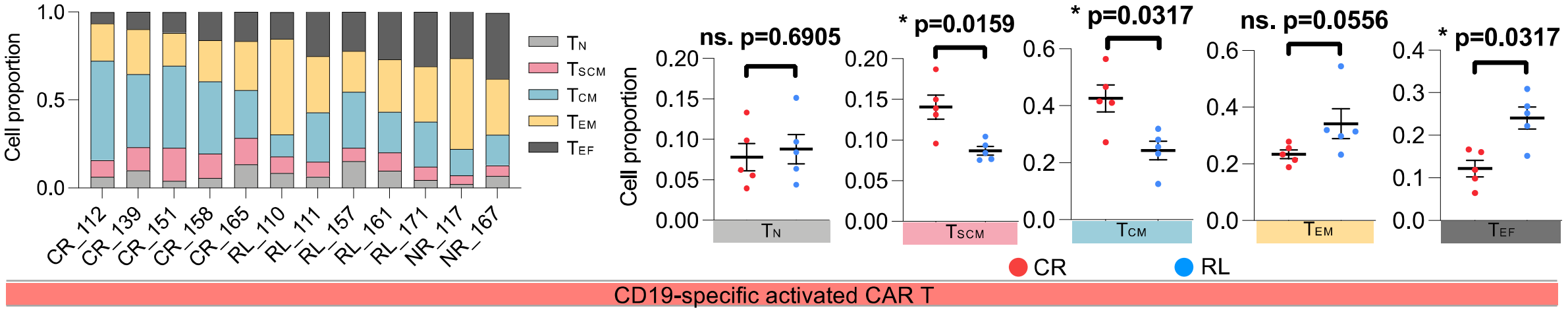




We found a significantly higher Th2 FSI in CR patients as compared with RL patients.
In in particular, IL-5 FSI has the highest predictive power.

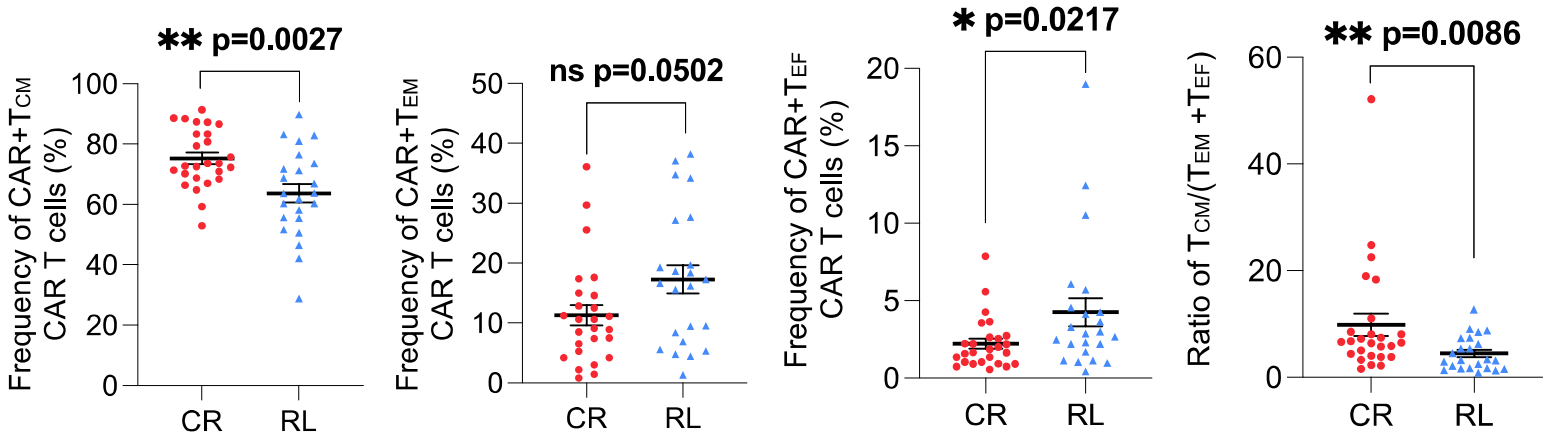
Bai ZL, et al., *Science Advances,* in press (2022)

Single-cell CITE-seq based phenotypic proteomic profiling revealed that early memory state is negatively associated CD19-positive relapse



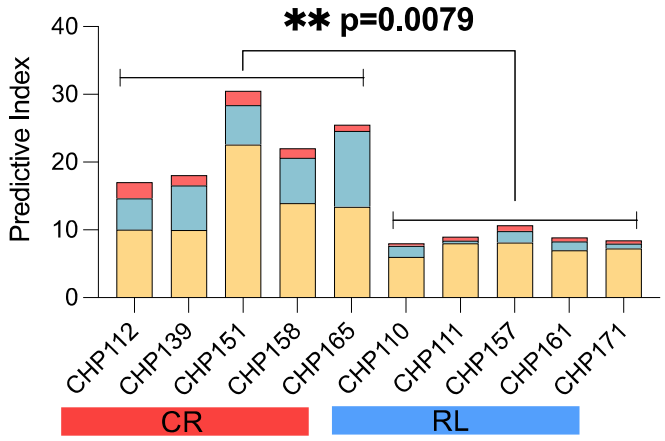
Cellular stemness and memory formation of CAR T cells in mediating the efficacy and persistence of the CAR T therapy.

Upon CAR-specific stimulation, the lower abundance of T_{SCM} and T_{CM} cells was significantly associated with the relapsed subjects. There was no difference in the frequency of T_{EM} CAR T cells, yet a higher proportion of cells in the RL group differentiated into a T_{EF} state compared with the CR group.



The flow cytometry analysis in the expanded cohort confirmed a significant increase of the T_{CM} and a decrease of the T_{EF} cell proportion in CR patients as compared with RL patients, thereby providing further validation of our findings.

An integrative model establishes the predictive power of the combined Th2 functionality strength and early memory potential



Th2 Expression

Average single cell expression level of IL4+IL5+IL13

Th2 Frequency

Single cell frequency in Th2-gene dominated functional cluster

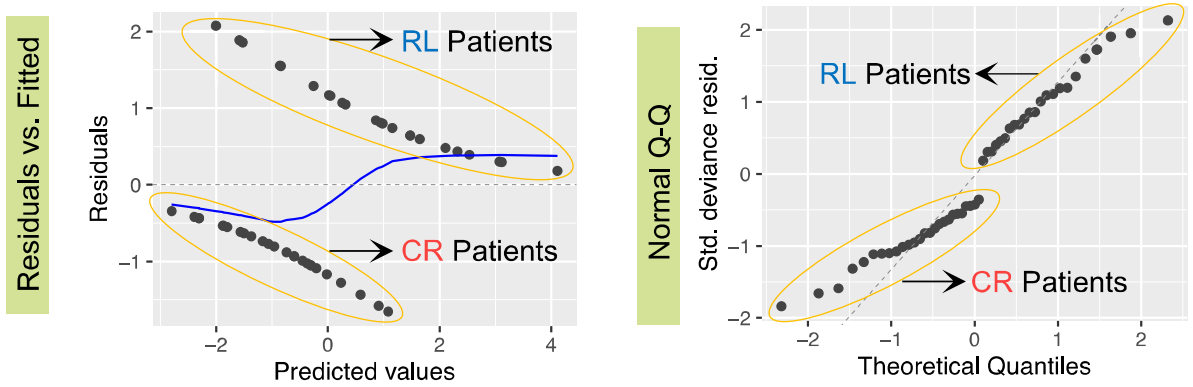
Memory-to-effector Ratio

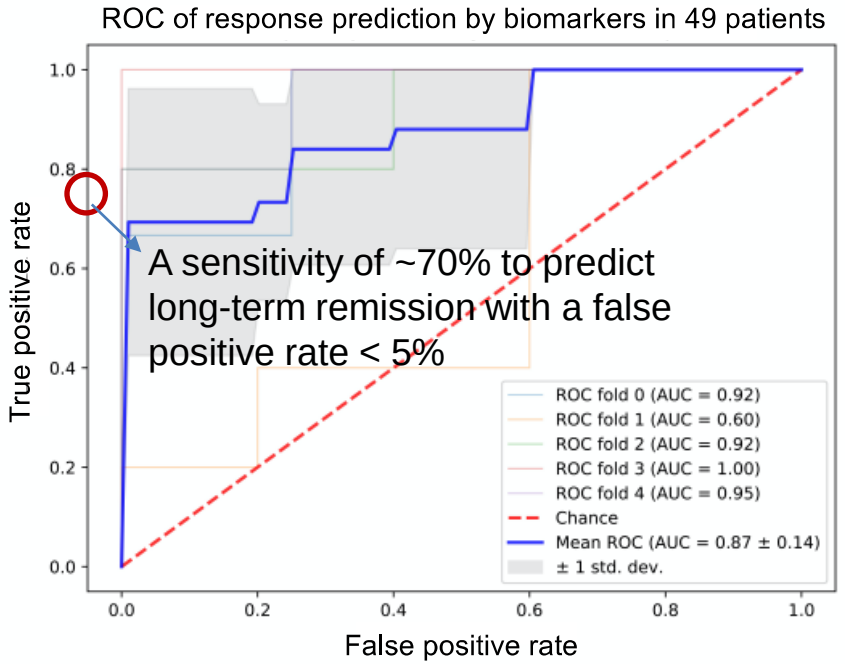
The ratio of (T_{SCM}+ T_{CM}) / (T_{EM}+ T_{EF})

To evaluate if the combination of some of these biomarkers into a single predictive index could be a viable strategy, we aggregated three of the major biomarkers identified in the discovery cohort into a single index. The values of the index were strongly associated with the clinical response in the initial cohort, suggesting that these markers could indeed be efficiently combined to predict durable responses.

We devised a similar index that was exclusively based on flow cytometry inputs so that we could train and test it in the larger validation cohort of patients.

$$\text{Response}\sim -0.0558\times Th2 - 0.0643\times T_{CM} + 0.0649\times(T_{EM}+T_{EF}) + 5.564$$

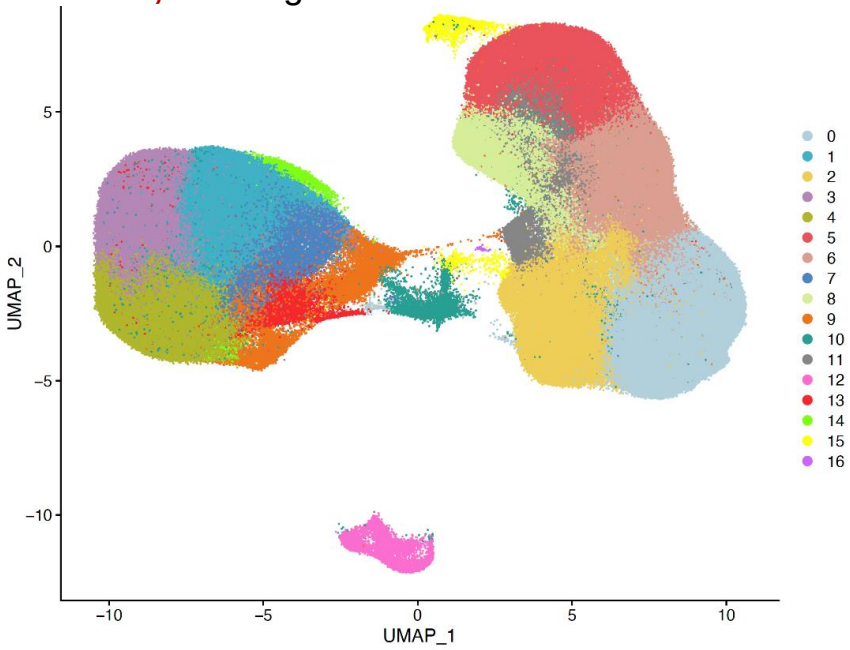




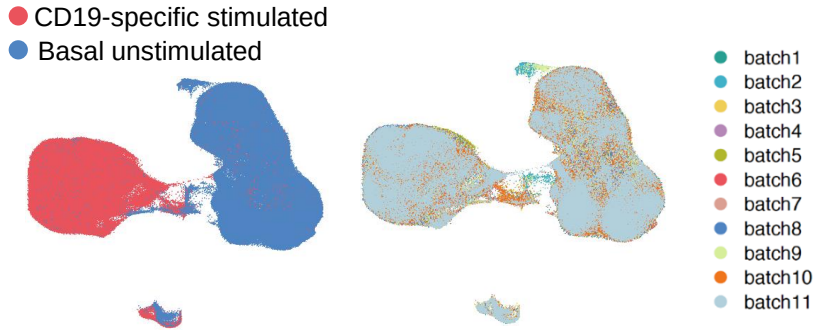
A binomial logistic regression was used to fit the model with CR or RL as the response variable, and a stratified 5-fold cross-validation was implemented to compute the ROC and AUC.

Ongoing: Single-Cell CITE-Seq of ~1M CAR T cells from 87 patients

695,820 single cells



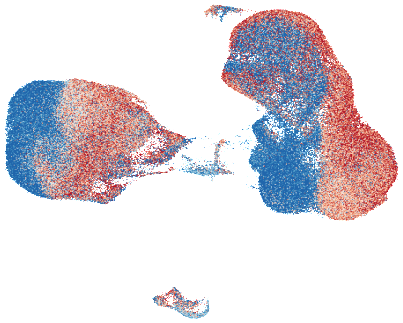
Response	Number
Complete remission	26
CD19 pos relapse	23
CD19 neg relapse	26
Non-responder	6
Healthy donor	6
Total	**87**



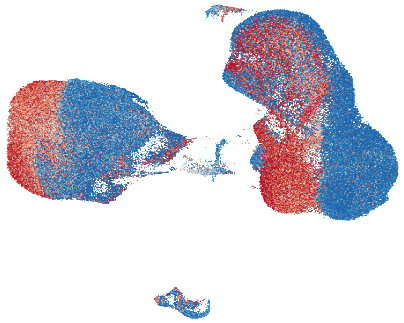
CAR T cells from two experimental conditions can be geographically separated

Integrated dataset suggests minimum batch effect

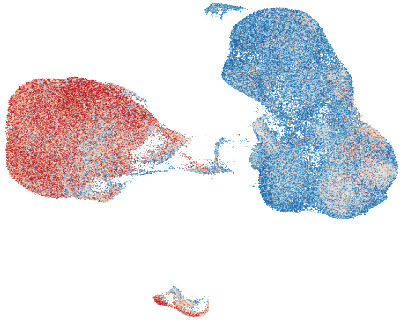
Protein-CD4



Protein-CD8



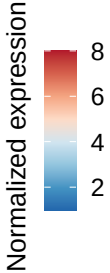
Protein-CD69

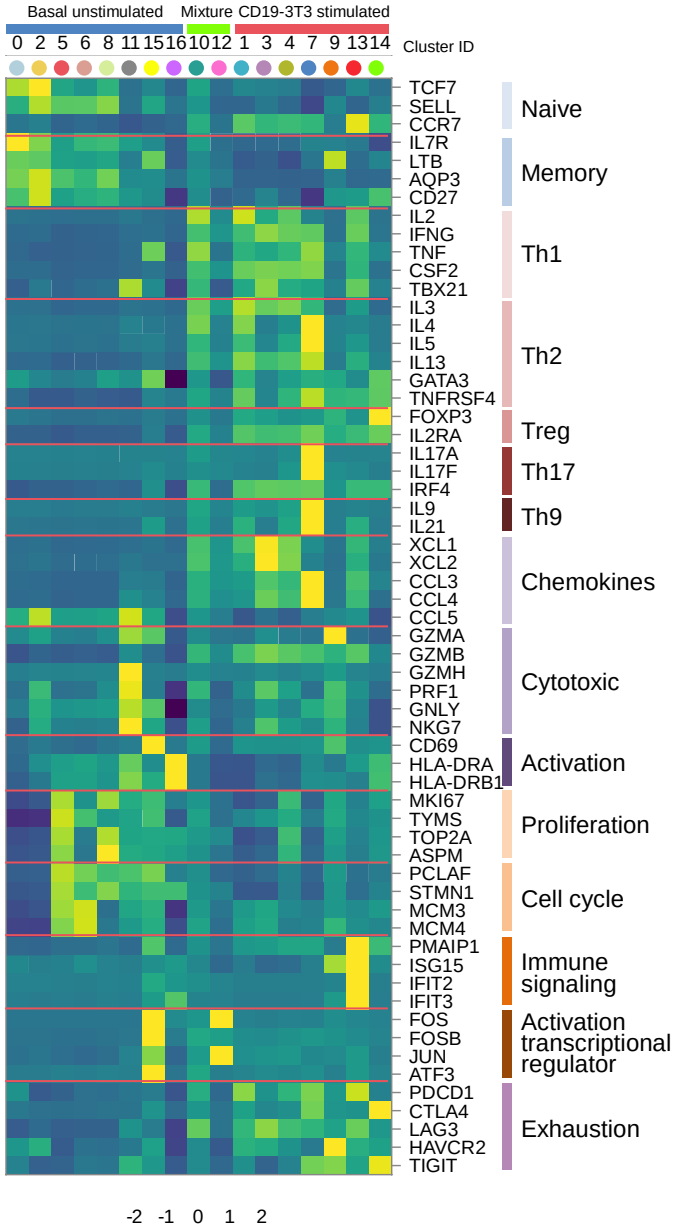


Activated T cell marker

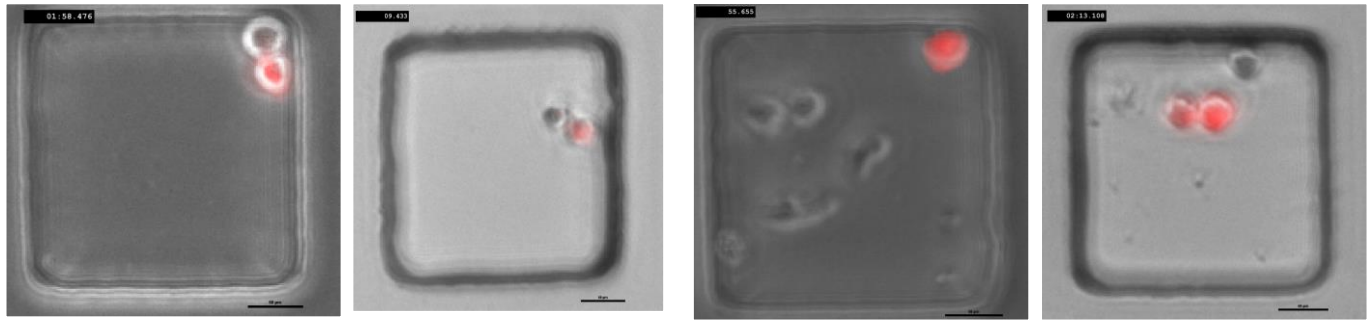
Protein-CD62L

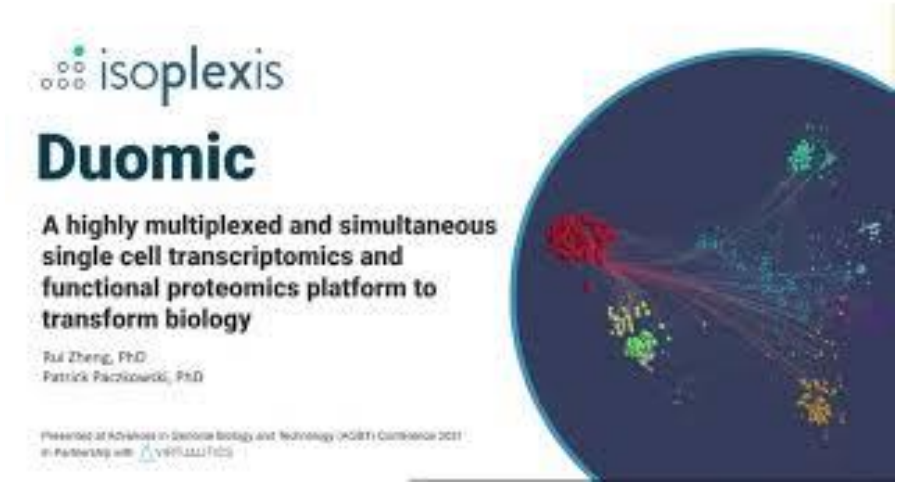
Naive T cell marker



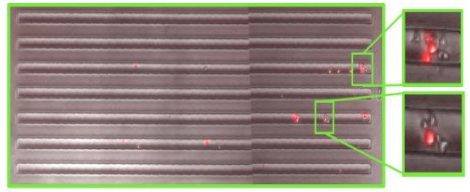


Ongoing: linking single-cell tumor-killing activity to cytokine functions as well as transcriptomics upon antigen-specific activation





Target (tumor) : Effector (CAR-T) Co-incubation



Fluorescent signal represents cytokines secreted

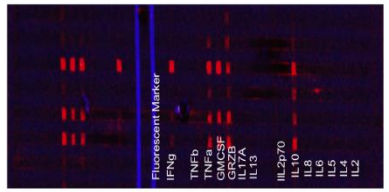
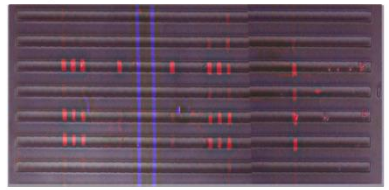
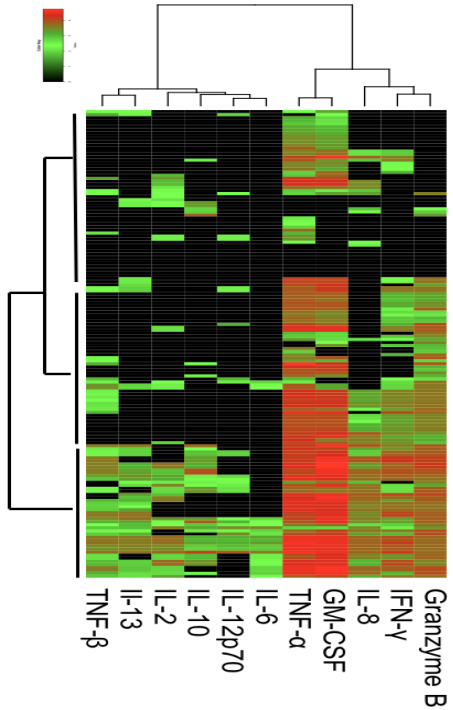
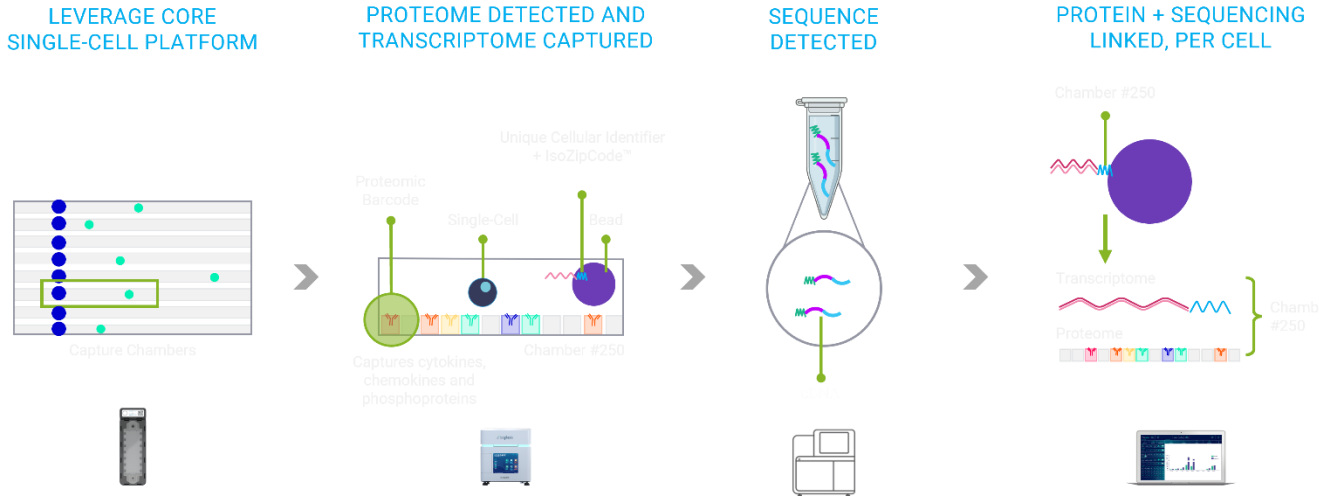


Image Overlay

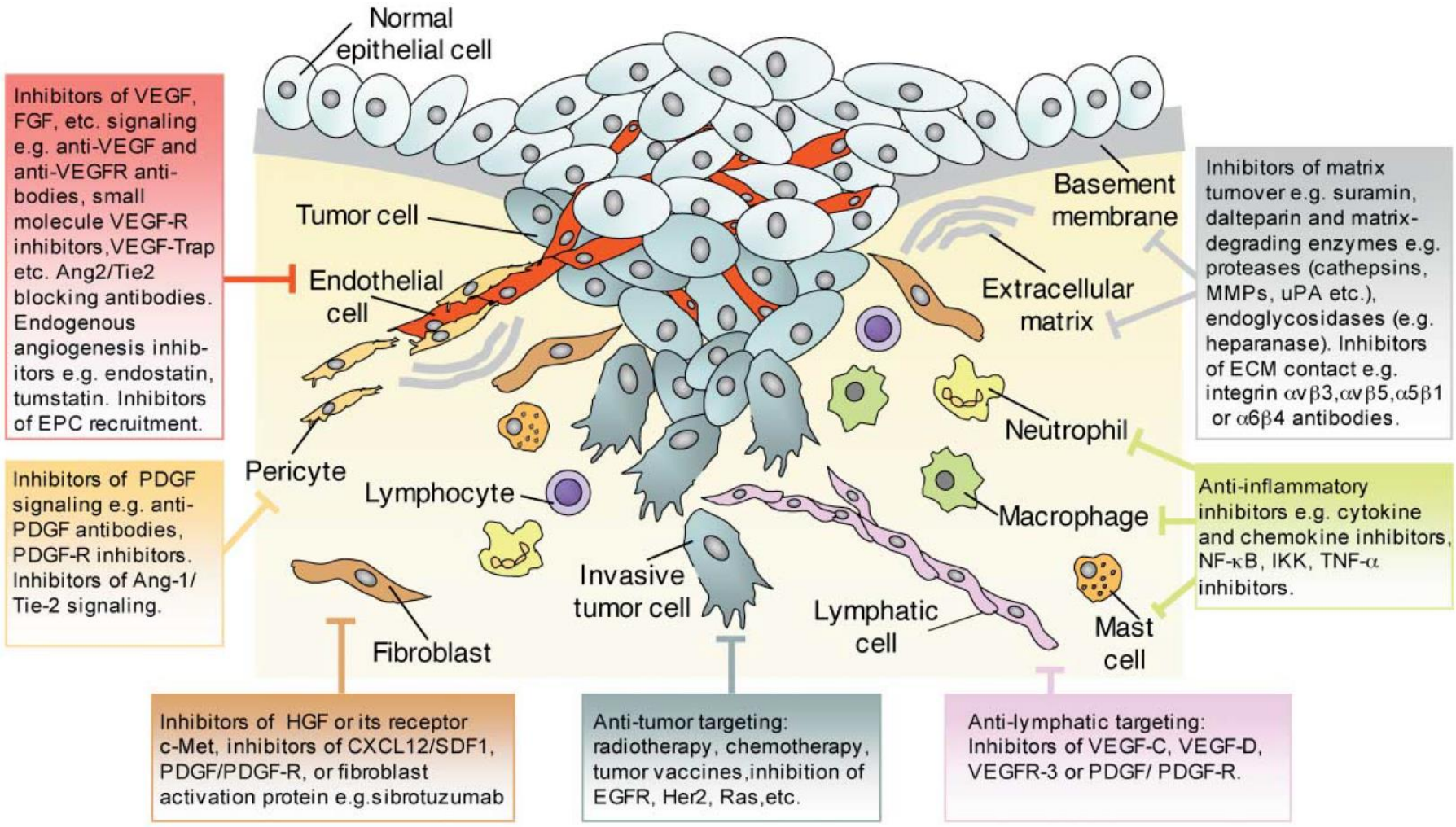






2006 –what I learned about the tumor microenvironment

Professor Johanna Joyce



Cancer Cell (2005)

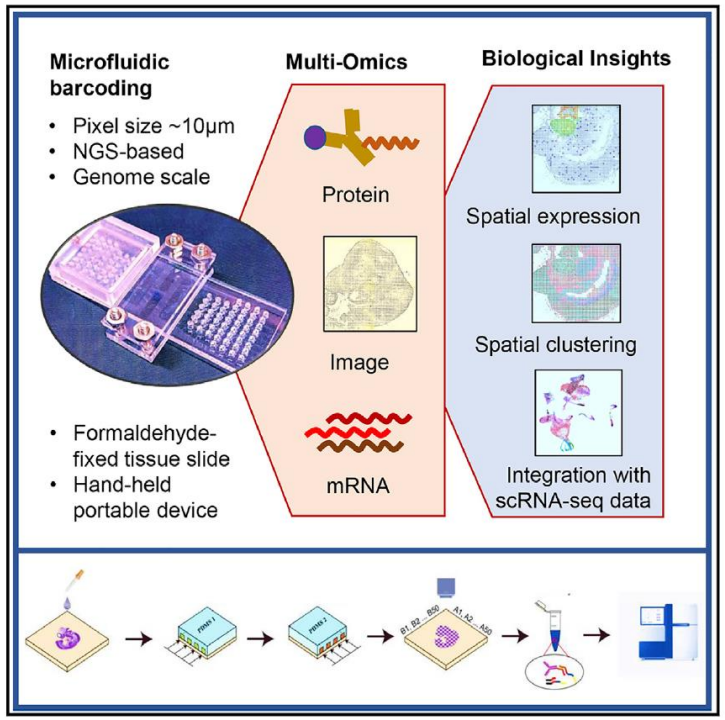
Novel Approach for Spatial Omics Sequencing via Deterministic Barcoding in Tissue (DBiT)

Resource

Cell

High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue

Graphical Abstract



Authors

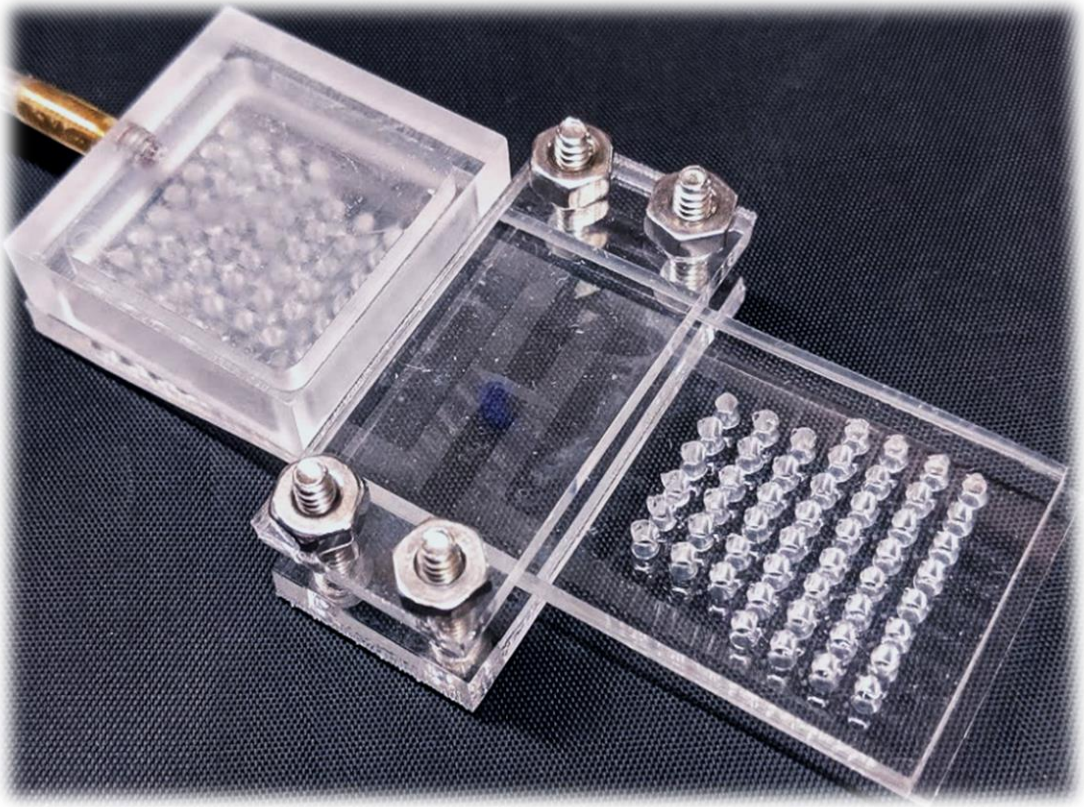
Yang Liu, Mingyu Yang, Yanxiang Deng, ..., Yang Xiao, Stephanie Halene, Rong Fan

Correspondence

rong.fan@yale.edu

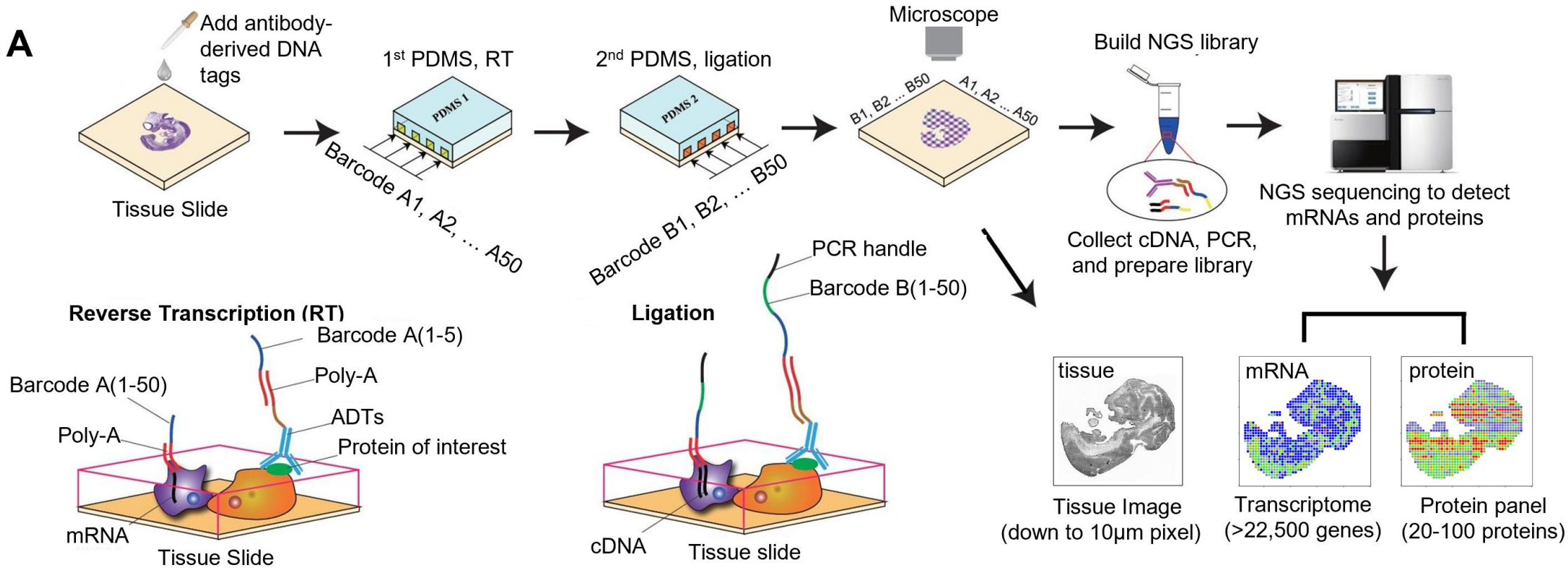
In Brief

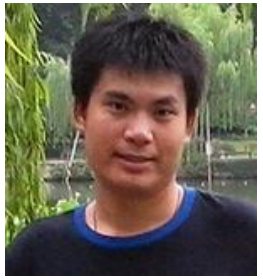
DBiT-seq is a microfluidic-based method to deliver barcodes to the surface of a tissue slide to allow for spatial omics sequencing with 10-μm pixel size.



Liu, Yang, Deng, et al., ***bioRxiv*** 788992 (2019).
Liu, Yang, Deng, et al., ***Cell***, 10;183(6):1665-1681 (2020).

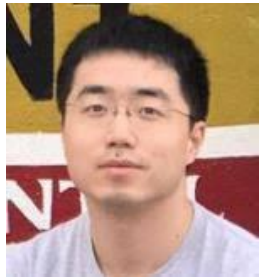
High-Spatial-Resolution Multi-Omics Atlas Sequencing via Deterministic Barcoding in Tissue (DBiT-seq)





Dr. Yang Liu

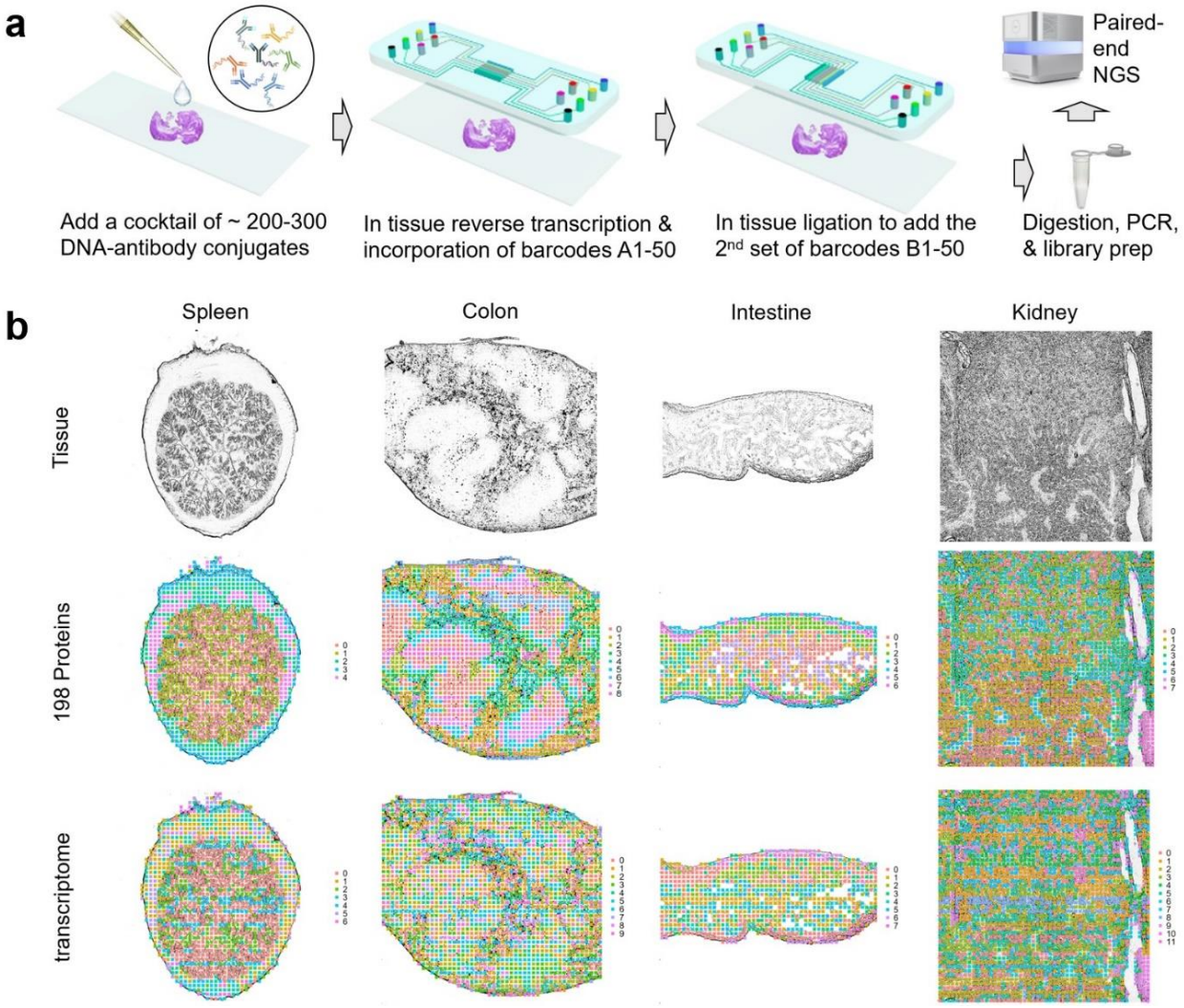
Dr. Mingyu Yang



Dr. Yanxiang Deng

Liu, Yang, Deng, et al., ***bioRxiv*** 788992 (2019).
Liu, Yang, Deng, et al., ***Cell***, 10;183(6):1665-1681 (2020).

Spatial-CITE-seq: spatial co-indexing of transcriptomes and epitopes for multi-omics mapping by NGS



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Spatial-CITE-seq: spatially resolved high-plex protein and whole transcriptome co-mapping

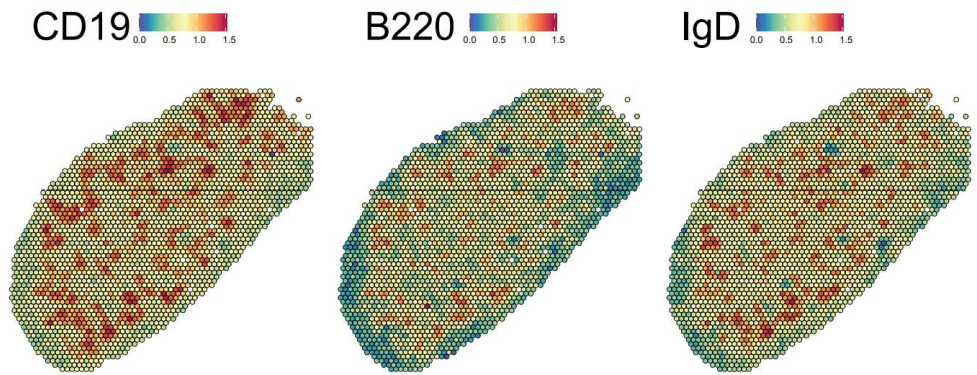
Yang Liu, Marcello DiStasio, Graham Su, Hiromitsu Asashima, Archibald Enninful, Xiaoyu Qin, Yanxiang Deng, Pino Bordignon, Marco Cassano, Mary Tomayko, Mina Xu, Stephanie Halene, Joseph E. Craft, David Hafler, Rong Fan

doi: https://doi.org/10.1101/2022.04.01.486788

Integrated protein and transcriptome high-throughput spatial profiling

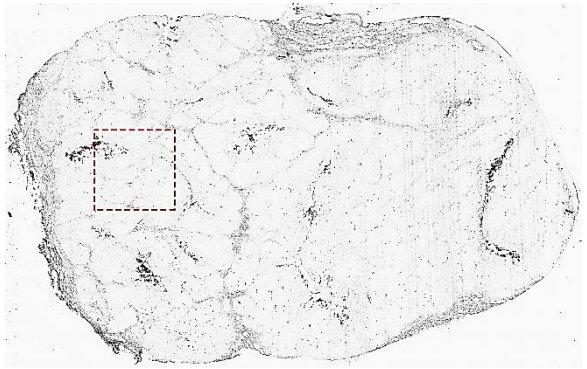
Nir Ben-Chetrit, Xiang Niu, Ariel D. Swett, Jesus Sotelo, Maria S. Jiao, Patrick Roelli, Marlon Stoeckius, Dan A. Landau

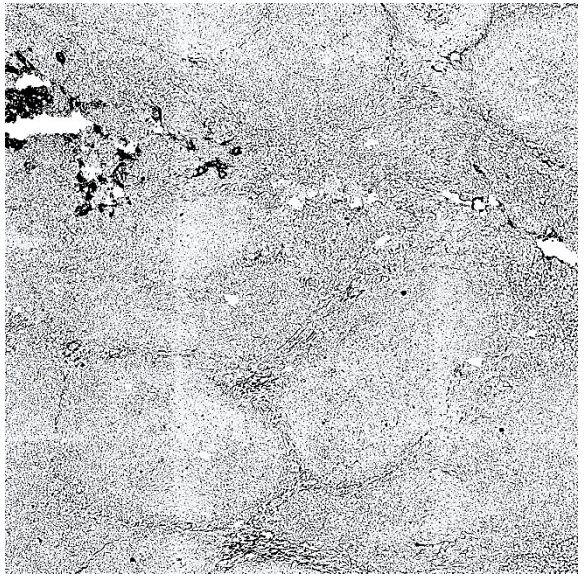
doi: https://doi.org/10.1101/2022.03.15.484516



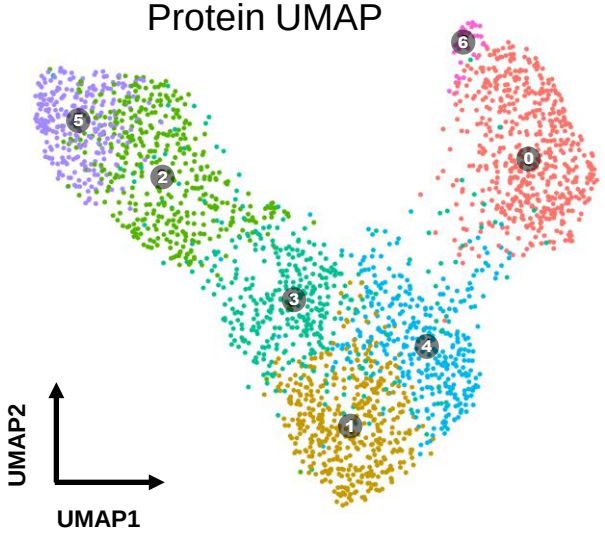
Spatial Co-mapping of whole transcriptome and a panel of ~300 proteins in human tonsil

Human Tonsil

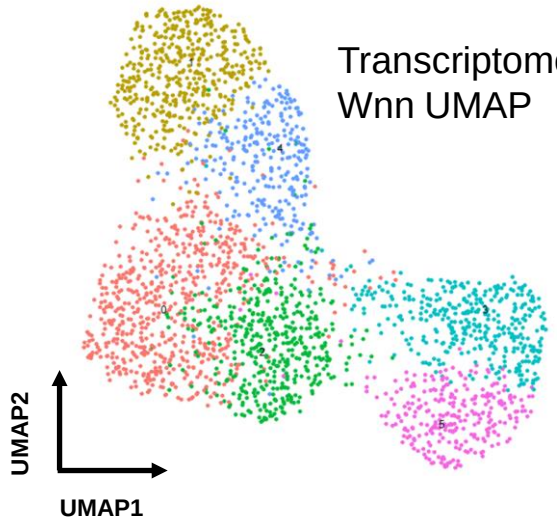


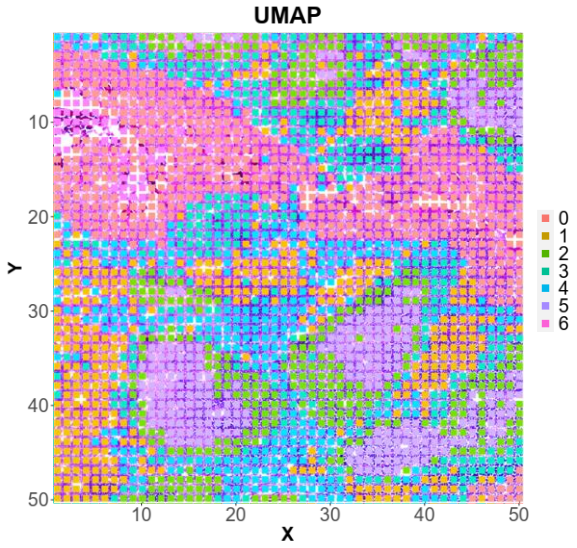


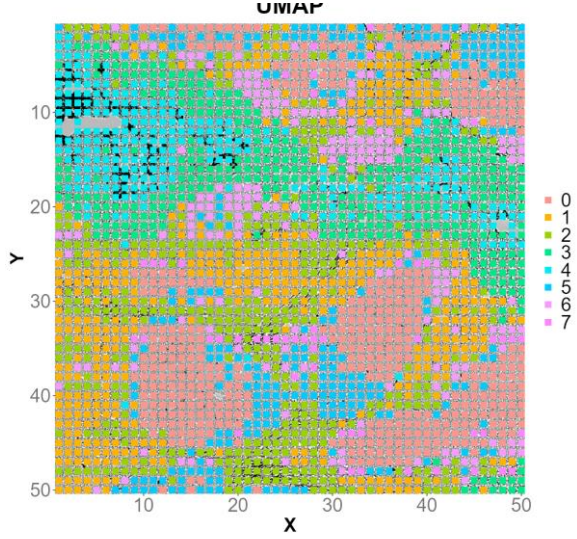
Protein UMAP

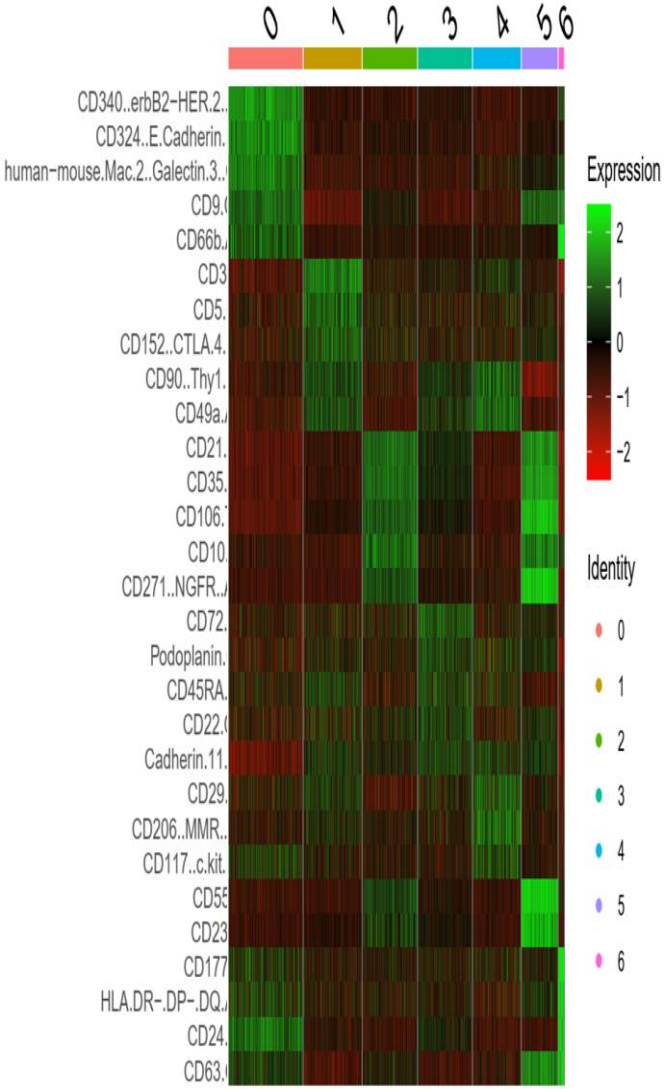


Transcriptome
Wnn UMAP

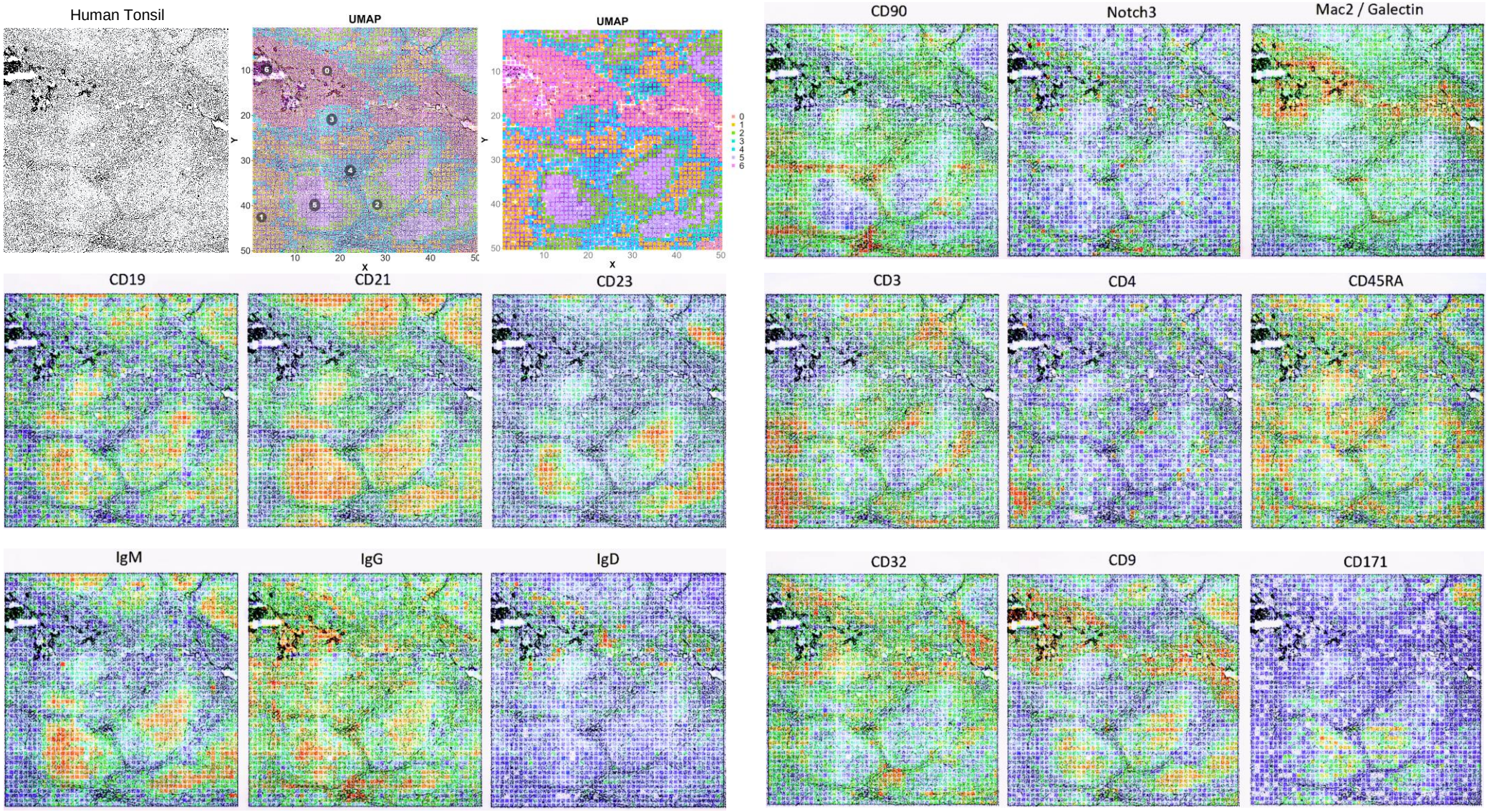




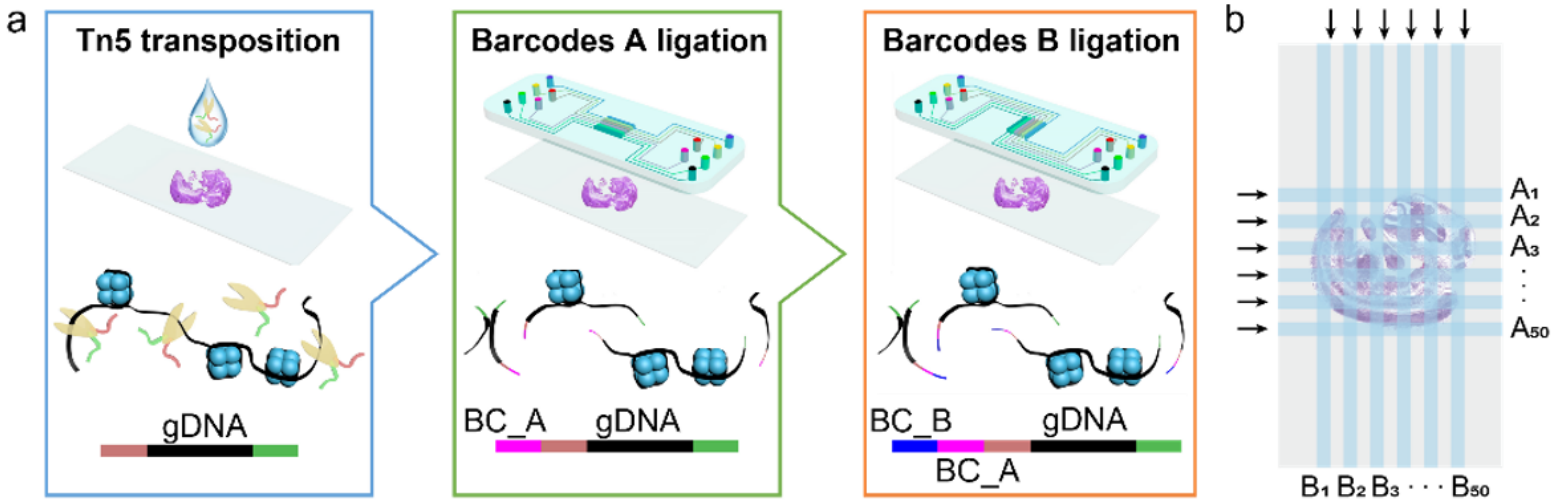


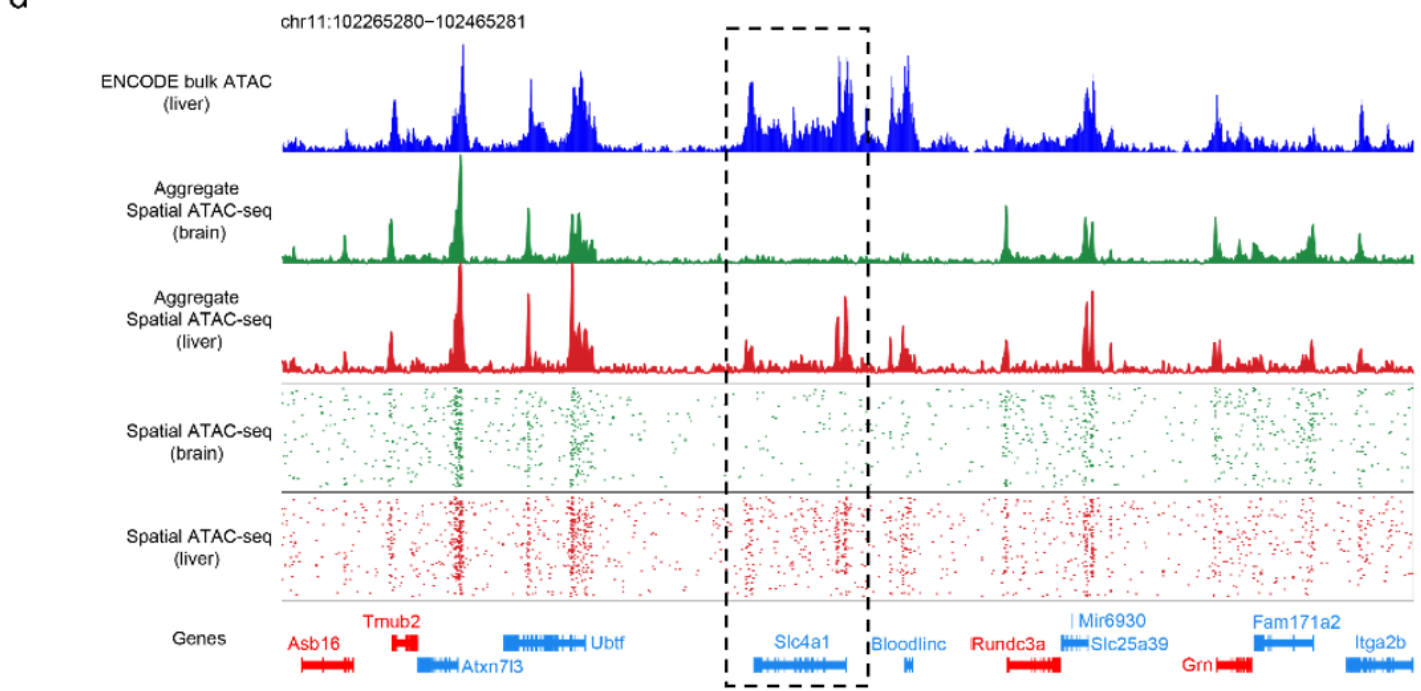


High-Plex Protein Mapping of Germinal Center (GC) Reaction and T-B Interaction



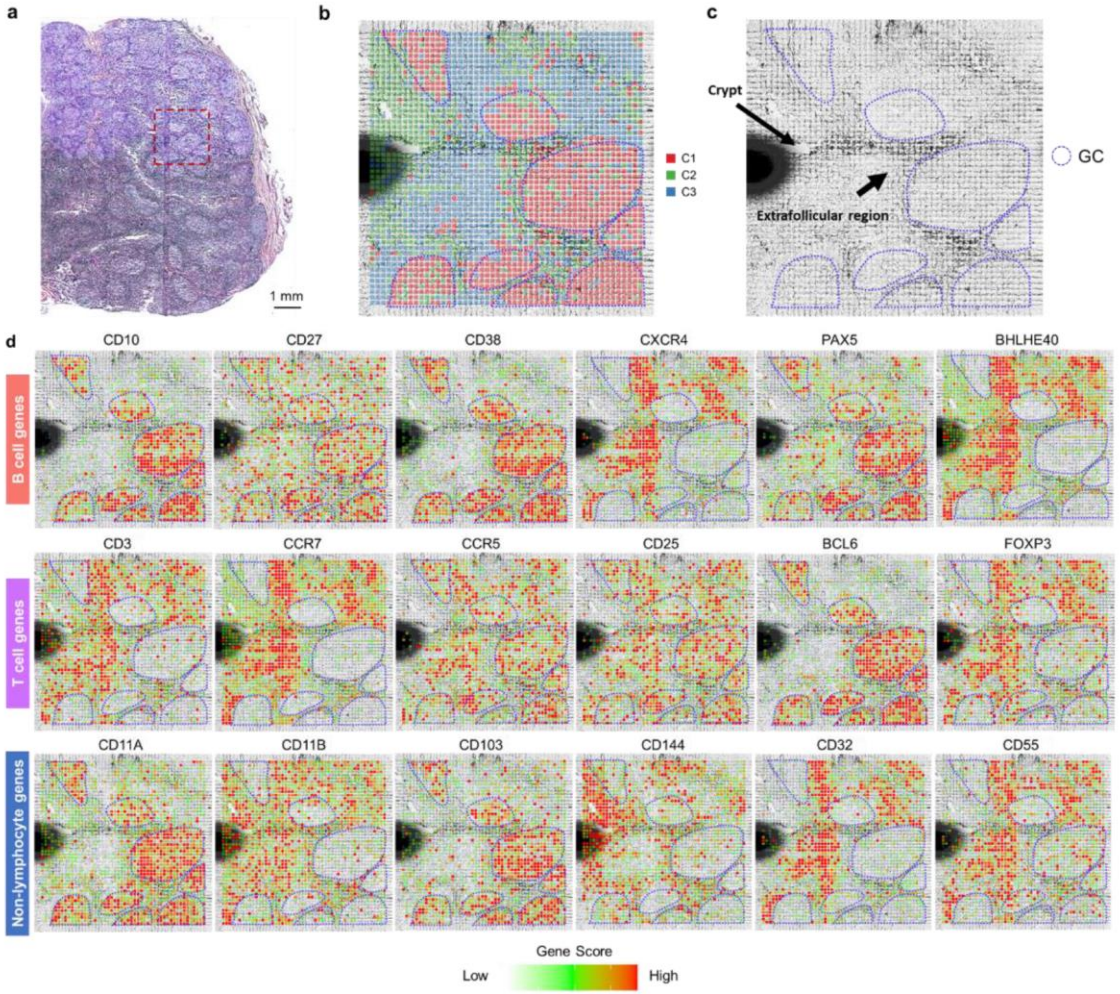
Spatial Epigenomics (spatial-ATAC-seq)

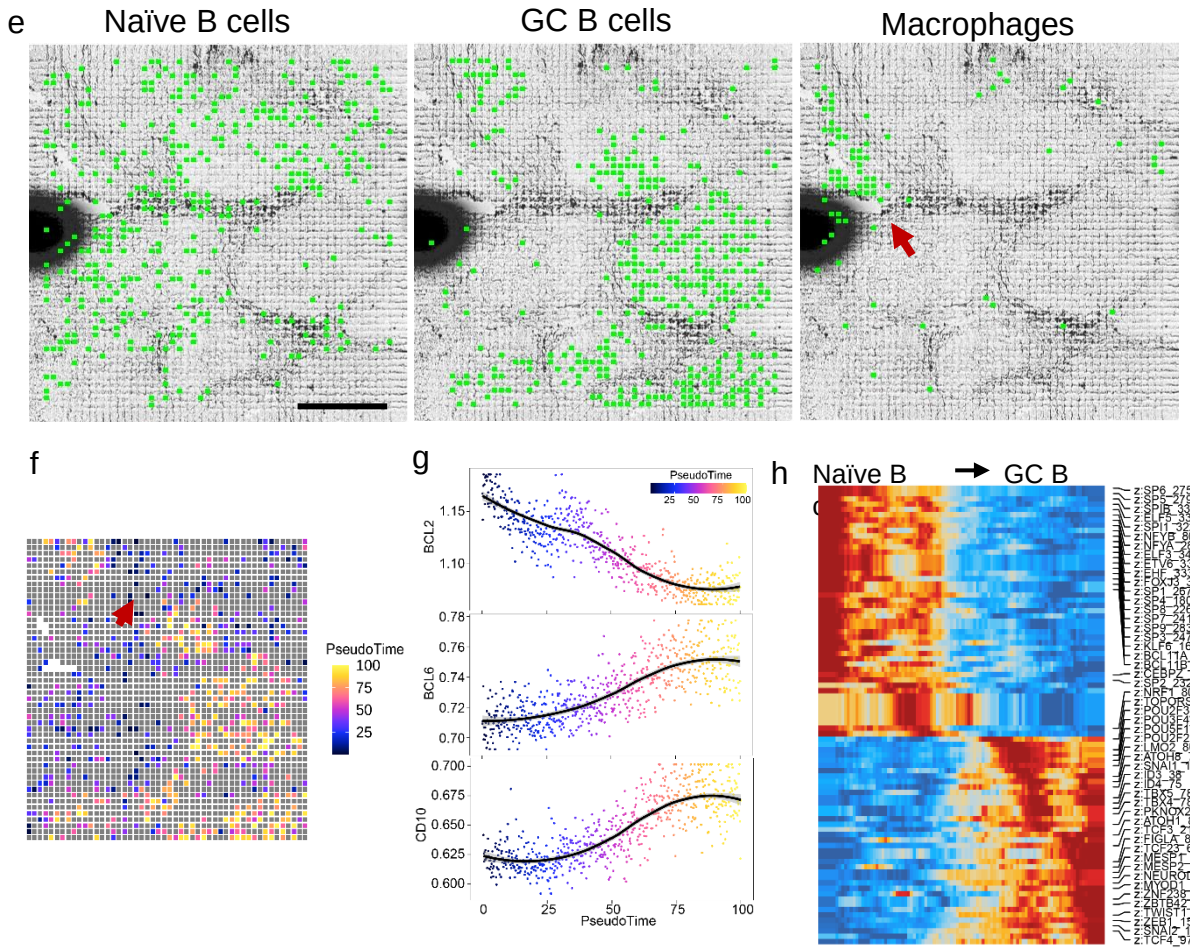




Deng et al, revision submitted (2021)

Spatial Mapping of Open Chromatin States in Germinal Center Reaction and T-B Cell Interaction

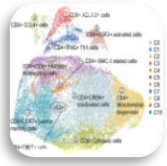




Deng et al, revision submitted (2021)

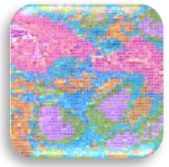
Summary

Single-cell high-plex cytokine profiling for relatively unbiased, comprehensive, and full-spectrum dissection of T cell effector functions.



Single-cell CITE-seq integrated with single-cell cytokine profiling allowed for examining the mechanism of CAR T cell activation.

Th2 functional module is associated with long-term remission in ALL patients treated with CD19-targeted CAR T cell therapy.



Spatial multi-omics technologies emerge to revolutionize the study of immune cell function in the tissue microenvironment for future immunotherapy development.

Single-Cell and Spatial Omics Techniques Unlock New Opportunities in Immuno-Oncology Research, Therapeutics Discovery, and Clinical Decision Making

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Thank you!