

Immune Profiling in Drug Discovery



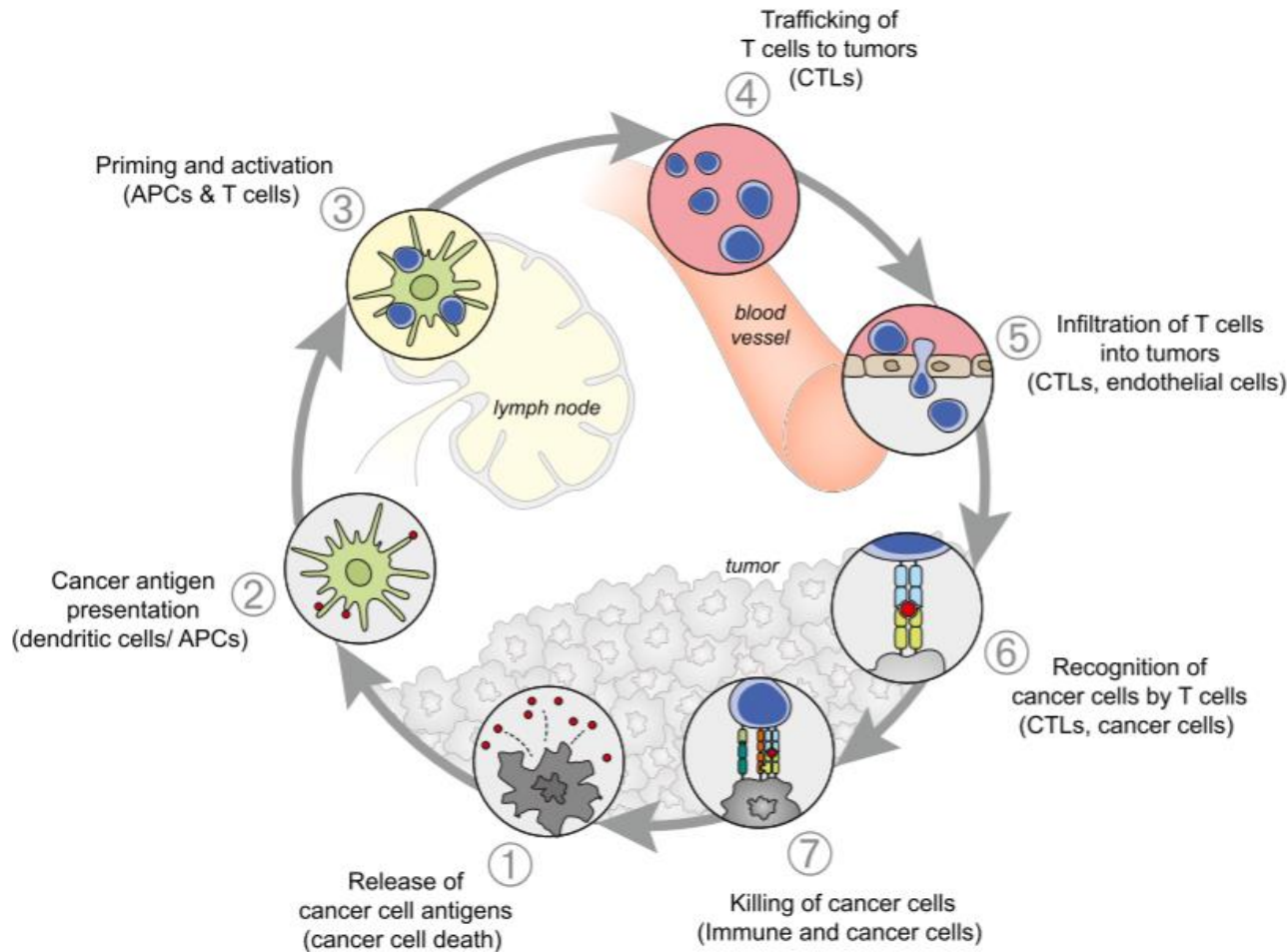
Oncology & Immunology Unit, WuXi AppTec Research Service Division



2021.08

- Immuno-Oncology: a tug-of-war
- The evolution of immune profiling
- OIU immune profiling core facility
- Case studies
 - IHC/Multiplex IHC-based immune profiling
 - FACS-based immune profiling
 - scRNAseq-based immune profiling

The Immuno-Oncology Cycle: A tug-of-war



Immune stimulators (+)

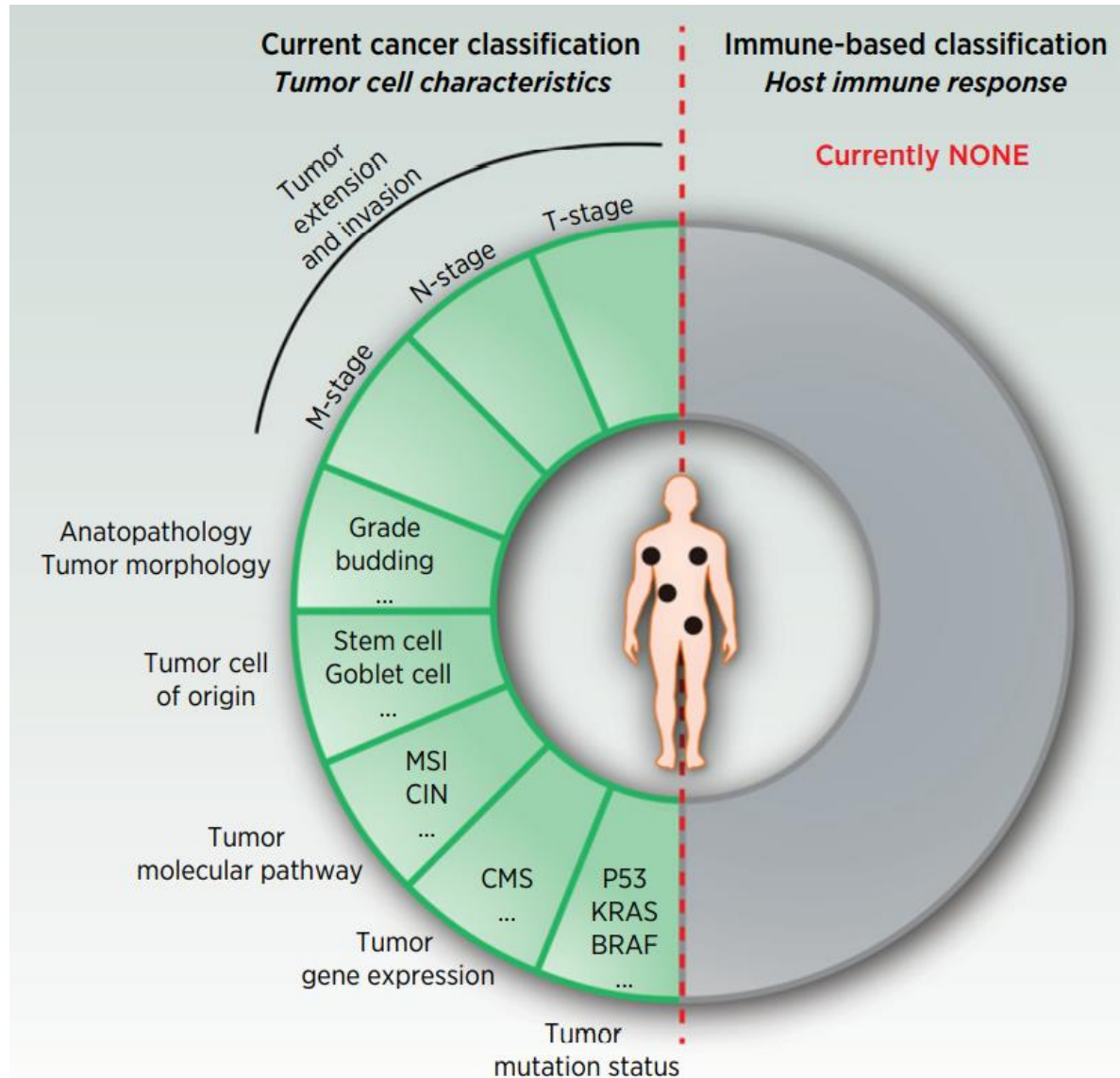
Necrotic cell death
Pro-inflammatory cytokines
Enhanced Ag presentation
Co-stimulatory signal
Immune effector cell

Apoptotic cell death
Anti-inflammatory cytokines
Hindered Ag presentation
Immune checkpoint signal
Immune regulatory cell

Immune Inhibitors (-)

Immunity 39, July 25, 2013

Immune contexture: a novel prognostic approach superior to TNM classification?



Current classification:
Tumor cell characteristics

- AJCC/UICC-TNM system
- Tumor morphology
- Tumor cell of origin
- Deregulated molecular pathways
- Specific tumor signatures
- Mutational status

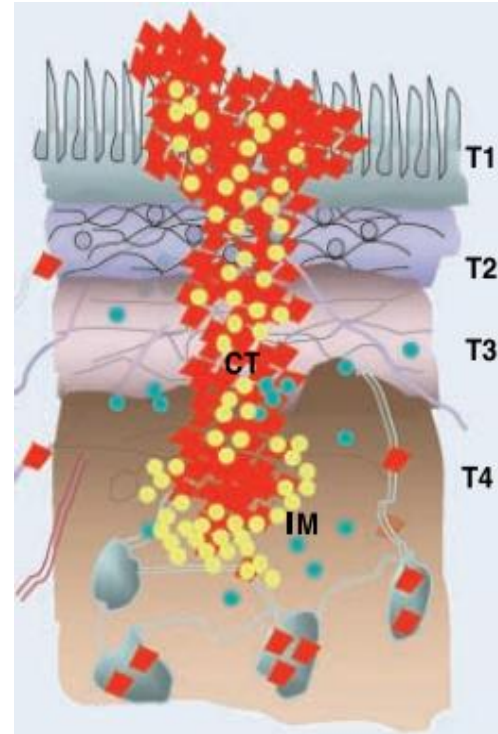
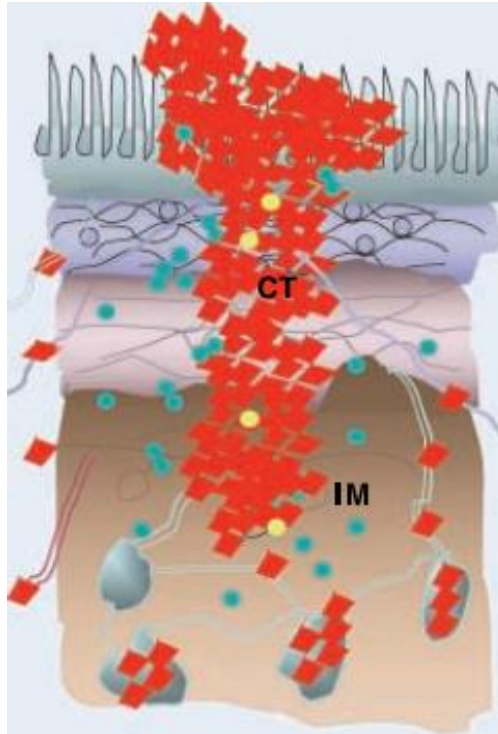
Immune contexture: a better diagnosis approach than TNM classification?

‘Cold’ tumor

‘Hot’ tumor

N+

N+



Same TNM classification

Different clinical outcome

Relapse
Death

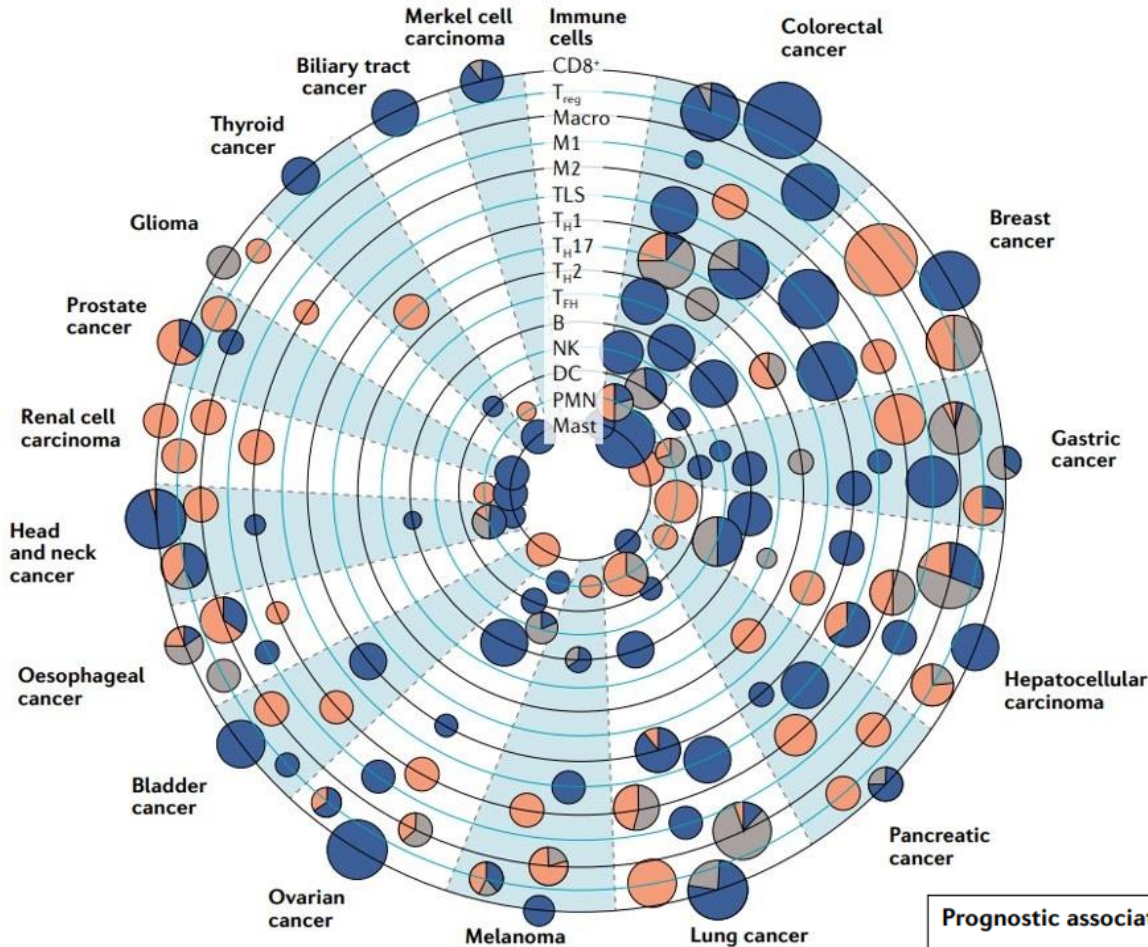
No relapse
Survival



Cancer Res 67, Mar 1, 2007

Immune contexture on the prognosis of patients with cancer

Cancer view



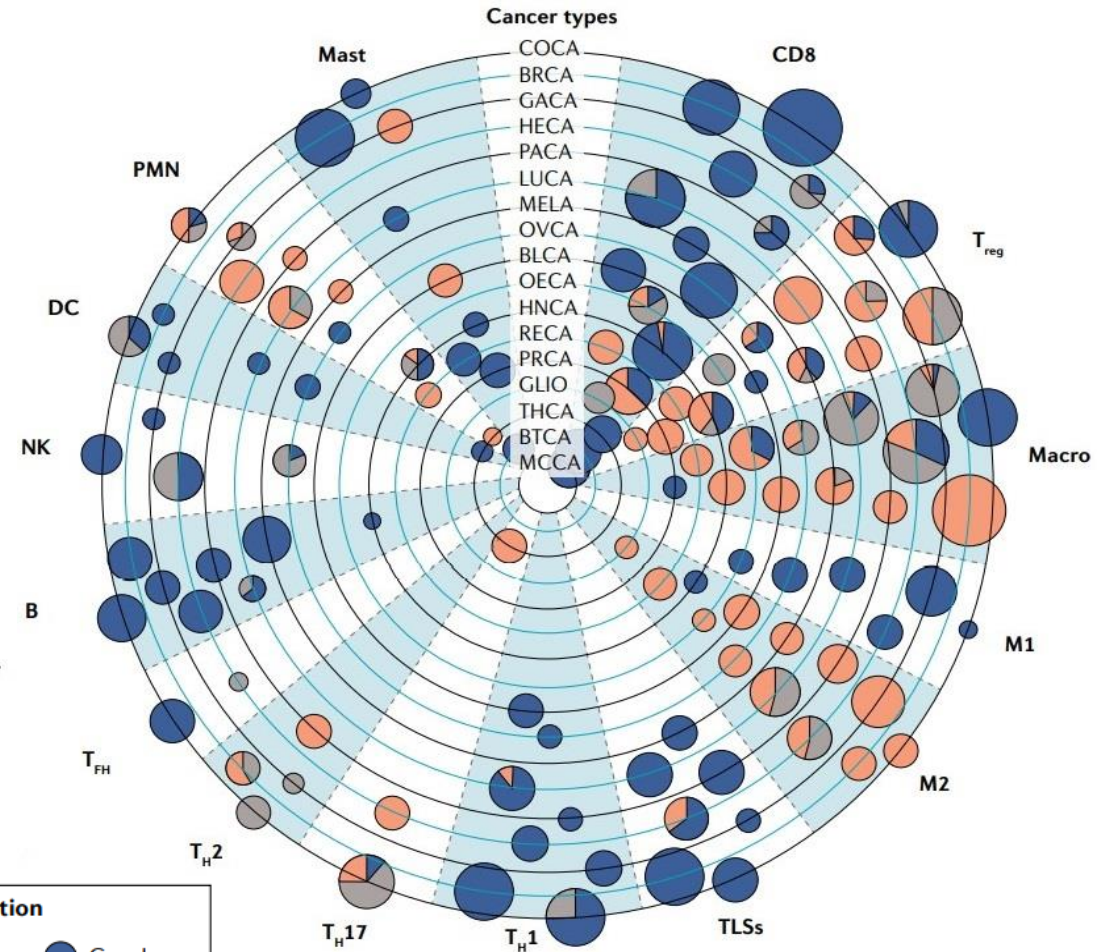
Prognostic association

Bad None Good

Number of patients

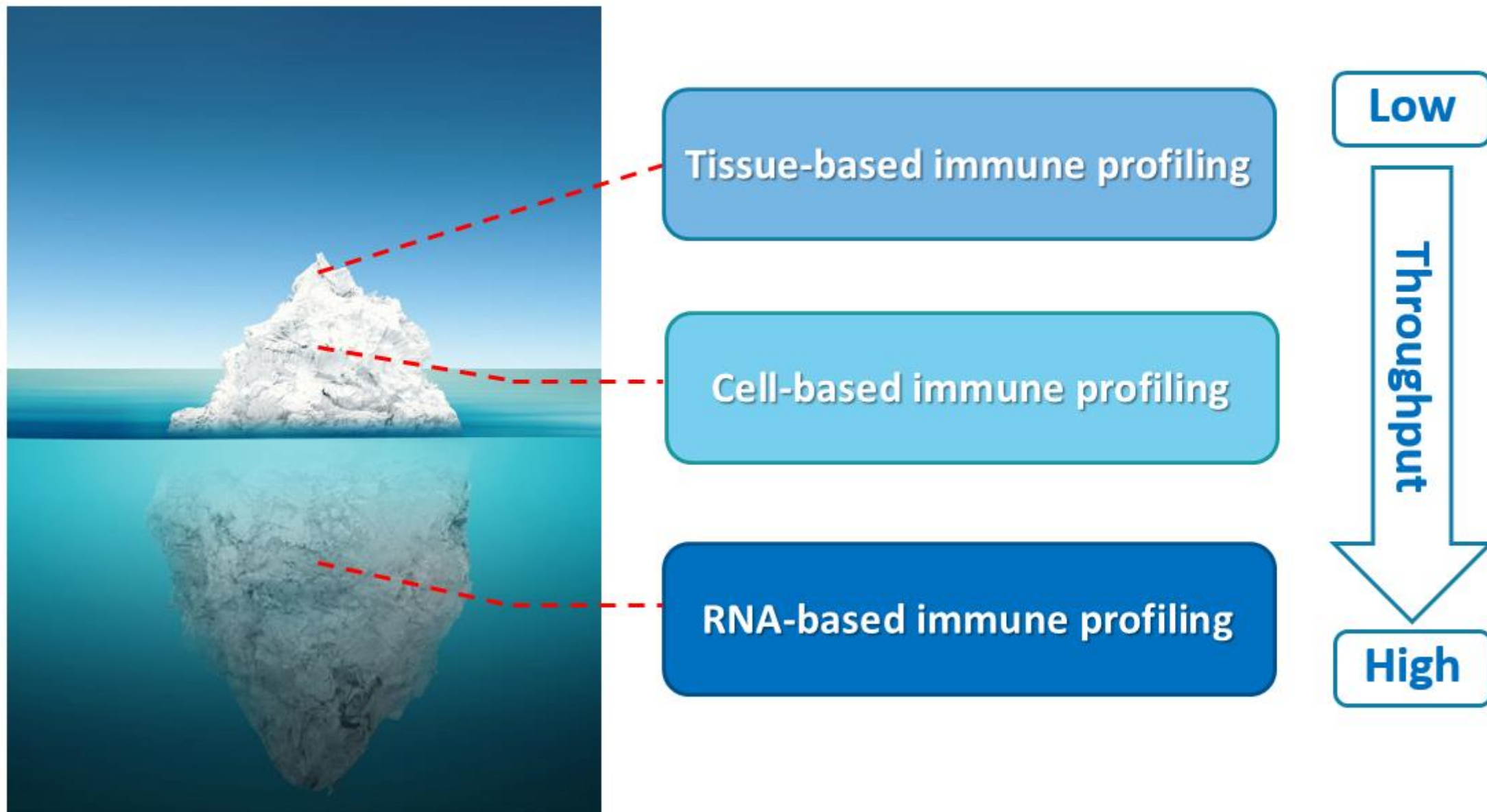
≥5,001 1,001–5,000 501–1,000 101–500 ≤100

Immune view



Nat Rev Cancer 20, Nov, 2020

The evolution of immune profiling



Tissue-based immune profiling at WuXi OIU

Pathology platform



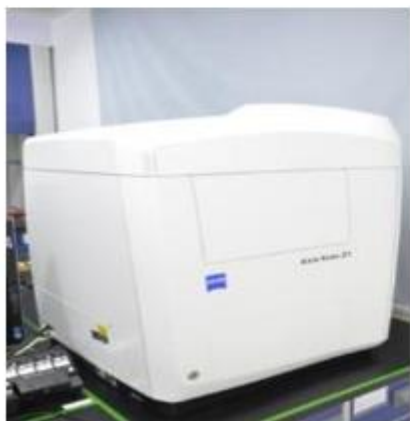
Ventana BenchMark ULTRA



Dako Link48



Leica BOND RX



ZEISS-Axio Scanner Z1-100



PE Vectra



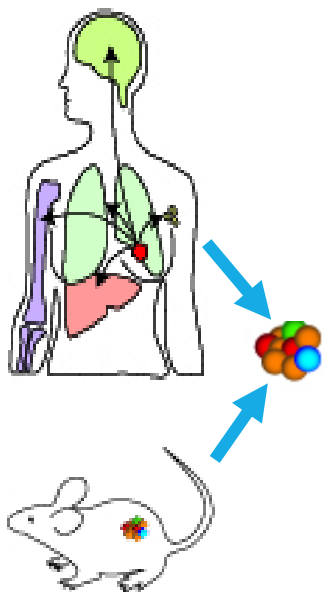
Aperio VERSA 8



HALO Digital Analysis

Schematic workflow of IHC/IF staining

Surgical resection of tumors

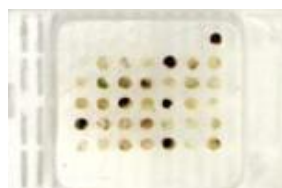


In vivo efficacy study

Tissue processing and section

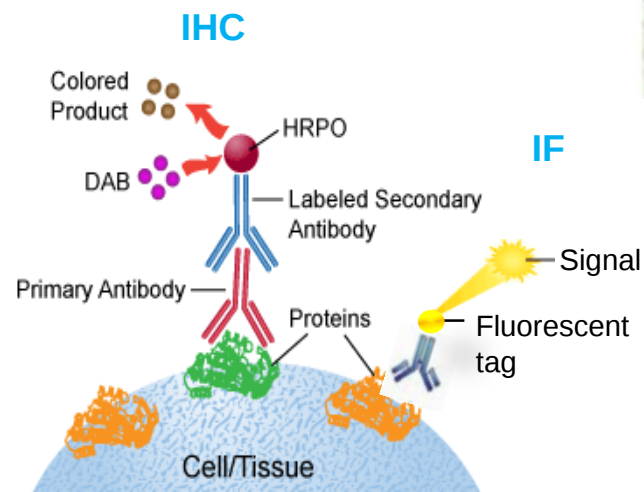


FFPE blocks

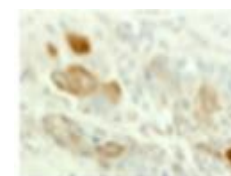


TMA block

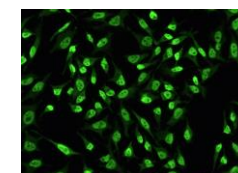
Staining with antibodies



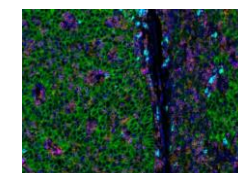
Capture/scan images and analysis



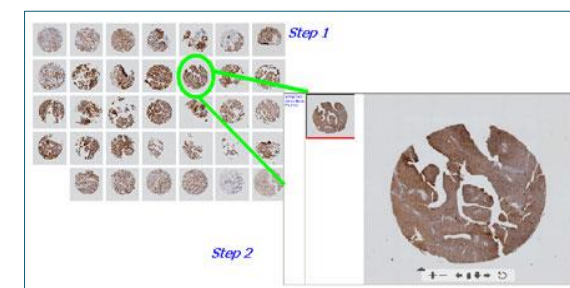
IHC



IF



Multiplex IF



TMA

Validated antibodies for regular IHC staining

	No.	Antibody ID	No.	Antibody ID		No.	Antibody ID
Tumor-associated biomarkers	1	Anti-ALK antibody	19	Anti-FIX IX antibody	Immuno-Oncology markers	1	Anti-CD11B antibody
	2	Anti-AR antibody	20	Anti-GFP antibody		2	Anti-CD11C antibody
	3	Anti-ATM antibody	21	Anti-GLUT antibody		3	Anti-CD19 antibody
	4	Anti-AXL antibody	22	Anti-Glypican 3 antibody		4	Anti-CD20 antibody
	5	Anti-Beta Amyloid antibody	23	Anti-Her2 antibody		5	Anti-CD25 antibody
	6	Anti-CA9 antibody	24	Anti-Iba1 antibody		6	Anti-CD3 antibody
	7	Anti-Cas 9 antibody	25	Anti-IRAK4 antibody		7	Anti-CD30 antibody
	8	Anti-Caspase-3 antibody	26	Anti-KI67 antibody		8	Anti-CD33 antibody
	9	Anti-Cleaved caspase-3 antibody	27	Anti-p-EGFR antibody		9	Anti-CD4 antibody
	10	Anti-c-Met antibody	28	Anti-p-Erk antibody		10	Anti-CD8 antibody
	11	Anti-CXCL-12 antibody	29	Anti-p-Histone antibody		11	Anti-F4/80 antibody
	12	Anti-CYLD antibody	30	Anti-p-MET antibody		12	Anti-FOXP3 antibody
	13	Anti-cytokeratin 7 antibody	31	Anti-PR antibody		13	Anti-Grazyme B antibody
	14	Anti-E-cadherin antibody	32	Anti-p-STAT-3 antibody		14	Anti-PD-1 antibody
	15	Anti-EGFR antibody	33	Anti-pan-Trk antibody		15	Anti-PD-L1 antibody
	16	Anti-ER antibody	34	Anti-PAR antibody		16	Anti-TDO2 antibody
	17	Anti-Erk antibody	35	Anti-PARP antibody		17	Anti-Tim3 antibody
	18	Anti-FAP antibody	36	Anti-PCNA antibody			

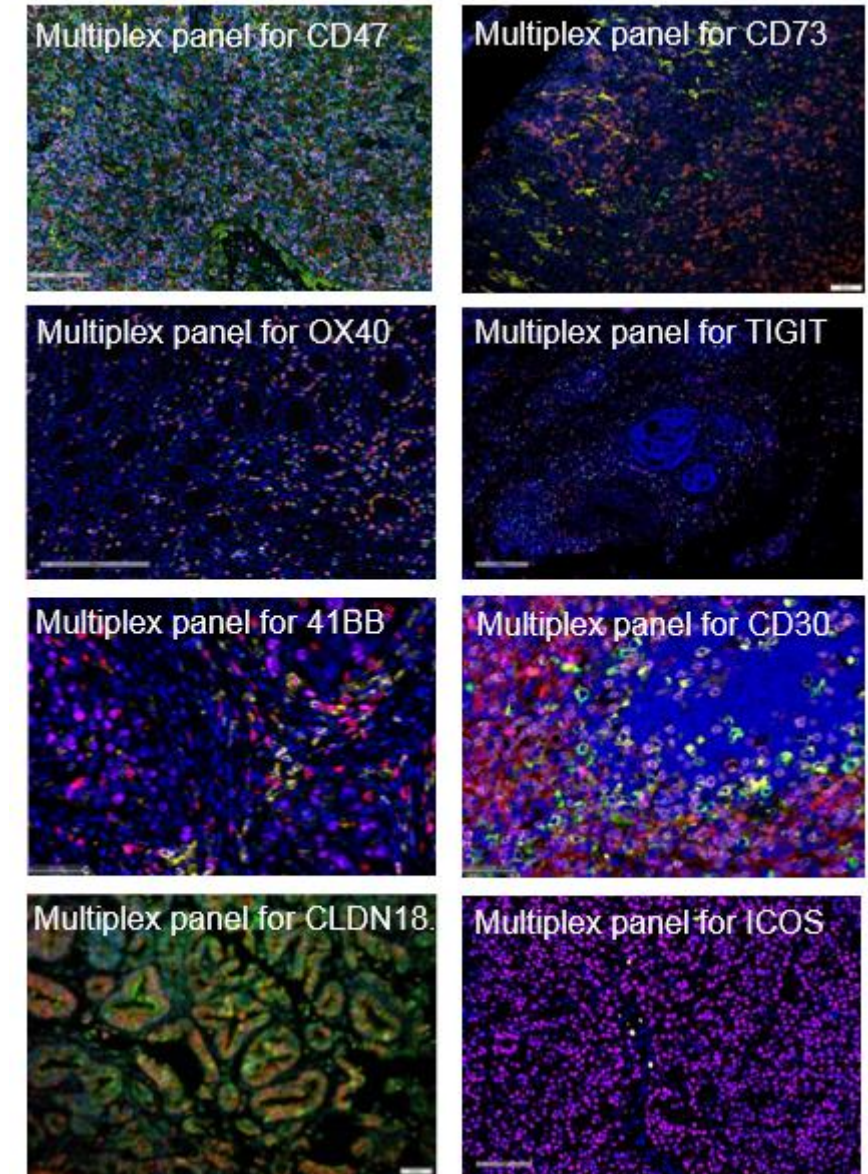
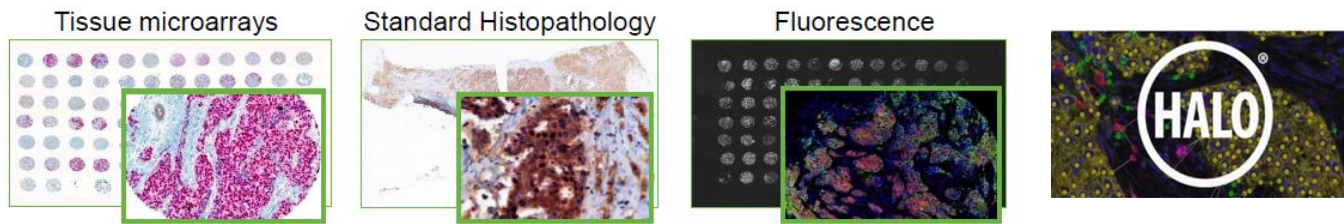
Validated antibodies for multiplex IF staining

■ High-plex Panel Content

- Over 35 validated panels, over 120 markers for selection
- Up to 12-Plex analysis on one slide

■ Establishing various tissue staining and whole slide image (WSI) analysis

- HE/IHC/multiplex IF/RNAscope/FISH/ISH+IHC
- Quantitative pathology to interrogate protein/RNA expression in cancer
- With Direct labeling and TSA multiplex IF staining technology
- HALO vs inForm digital pathological analysis
- Invested World-class scanners— multispectral imaging, single Cell Resolution



FACS platform



BD FACSCanto II
(3 Laser, 8 channels)



BD LSRFortessa
(5 Laser, 18 channels)



BD FACS Aria II
(4 lasers, 18 channels)



BD FACSCanto plus
(3 Laser, 10 channels)



BD LSRFortessa X20
(5 Laser, 18 channels)

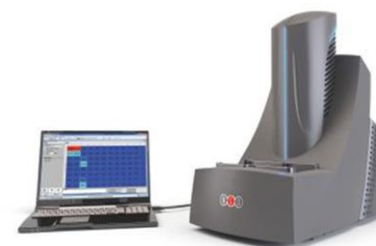


Cytek Aurora
(3 Laser, > 24 colors)

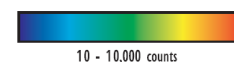
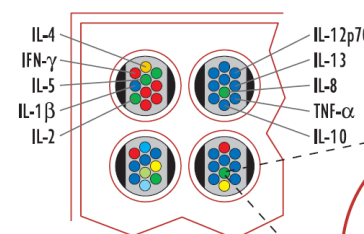
Cytokine Analysis



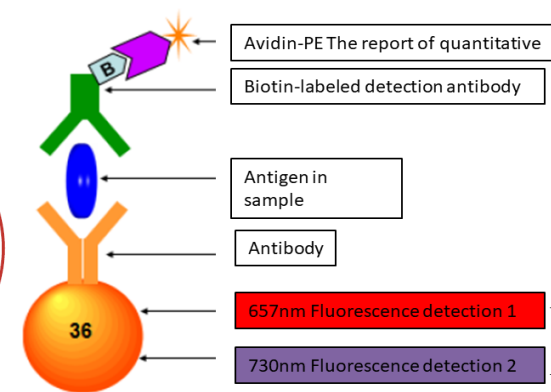
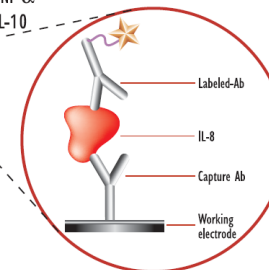
Meso Scale Discovery (MSD)



Luminex Multiplex System



Colormap display demonstrates different patterns of cytokine responses in different wells of a 96-well 10-spot assay plate.



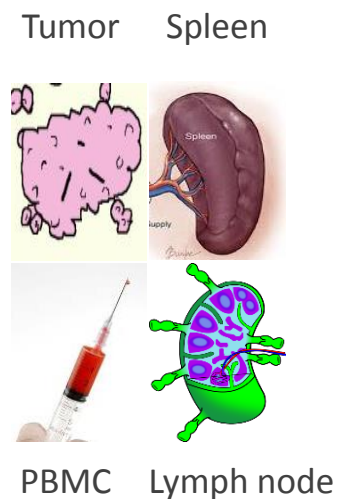
Schematic workflow of TIL analysis

From *in vivo* efficacy study to leukocytes profiling

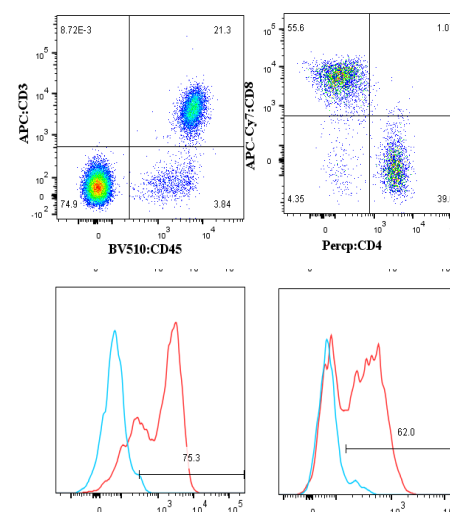
In vivo efficacy study with tumor-bearing mice



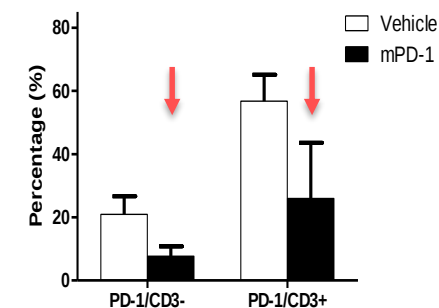
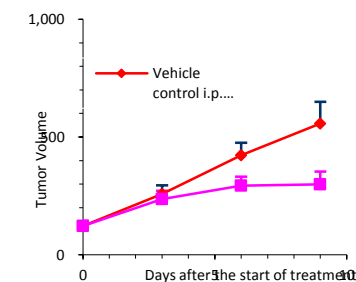
Specimen collection and processing



Immune profiling



Data Integration and Summarization

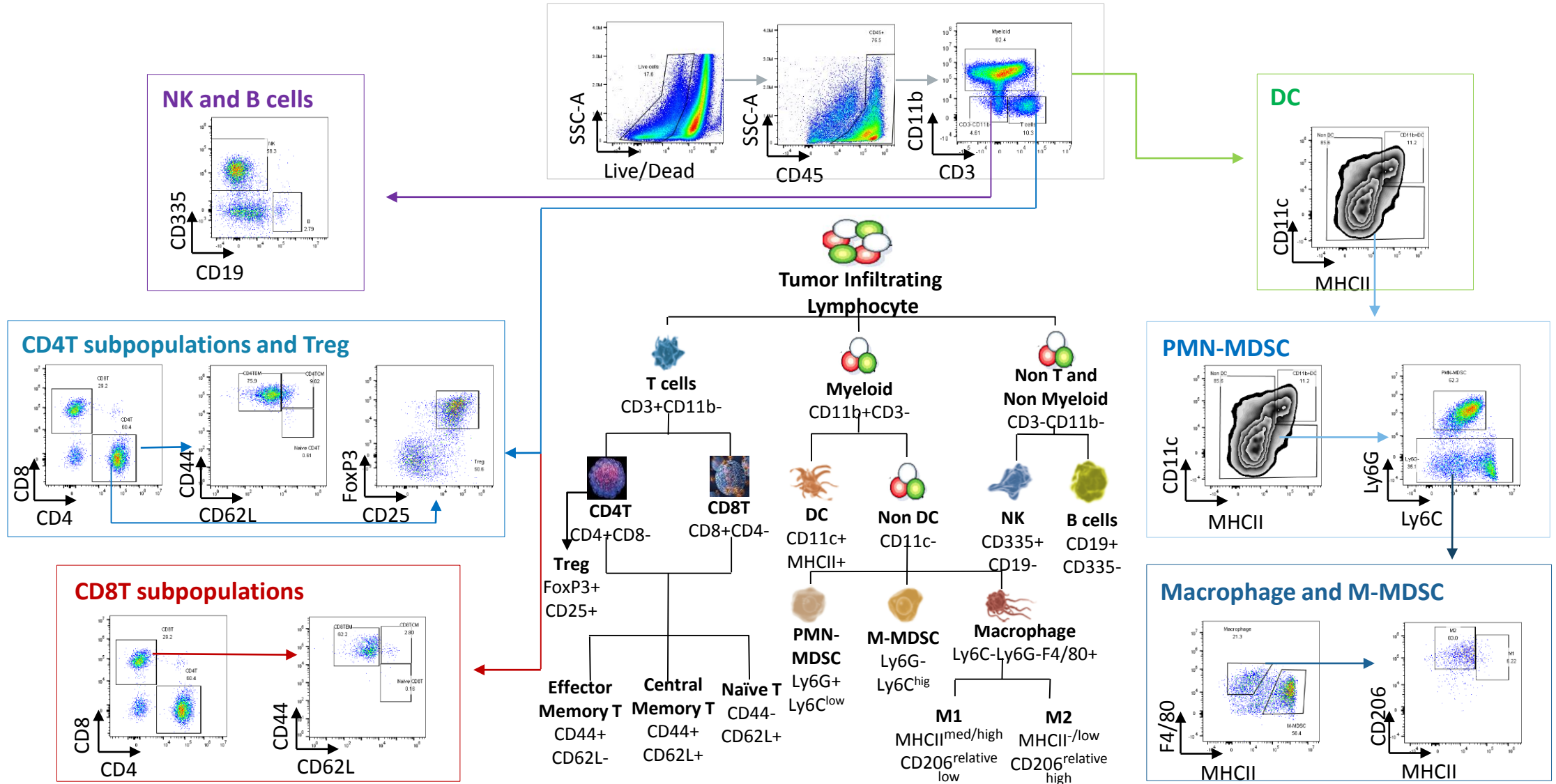


Validated panel to support flexible design

Panels	Purpose	Cytometer
Option1	Panel focus on lymphocyte and biomarkers	Fortessa
Option2	Panel for major cell types and biomarkers	Fortessa
Option3	Panel focus on myeloid cells	Fortessa
Option4	Panel for major cell types and biomarkers	Canto
Option5	Panel for myeloid cells, lymphocyte and biomarkers	Aurora
Option6	Panel for myeloid cells, lymphocyte and more biomarkers	Aurora

	Option1	Option2	Option3	Option4	Option5	Option6
1	CD8				L/D	L/D
2	CD4				CD45	CD45
3	CD44				CD3	CD3
4		L/D	L/D	L/D	CD4	CD4
5	L/D	CD8	CD8	CD8	CD8	CD8
6	CD19	CD19	MHCII	CD19	FoxP3	FoxP3
7	CD62L		Ly6C		CD25	CD25
8	CD11b	CD11b	CD11b		CD335	CD335
9	CD335	CD335	F4/80	CD335	CD19	CD19
10	PD-L1	PD-L1	CD11c	CD11b	CD44	CD44
11	CD25	CD3	CD3	CD3	CD62L	CD62L
12	FoxP3	FoxP3	CD206	FoxP3	CD11b	CD11b
13	PD-1	PD-1	Ly6G	PD-1	Ly6C	Ly6C
14	CD45	CD45	CD45	CD45	Ly6G	Ly6G
15	CD3	CD4	CD4	CD4	F4/80	F4/80
16					CD206	CD206
17					MHCII	MHCII
18					CD11c	CD11c
19					PD-1	PD-1
20					PD-L1	PD-L1
21					Ki67	Ki67
22						LaG3
23						OX40

TIL immune-profiling in a single panel



RNA-based immune profiling at WuXi OIU

scRNAseq



Chromium Single
Cell Controller
(10x Genomics)

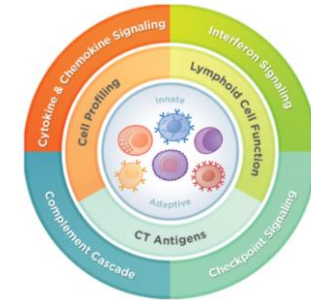


NovaSeq 6000
Illumina platform

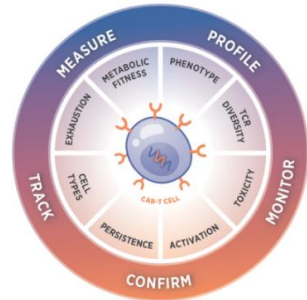
NanoString



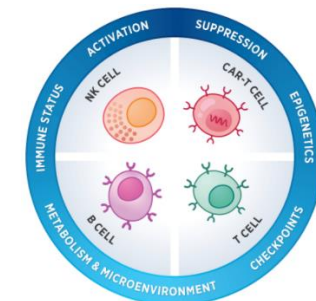
nanoString
TECHNOLOGIES



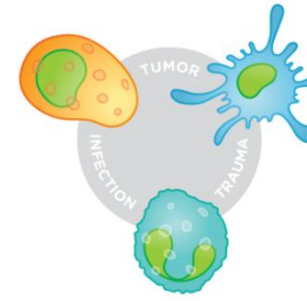
PanCancer Immune
Profiling Panel



CAR-T
Characterization
Panel



Immune Exhaustion
Panel



Myeloid Innate
Immunity Panel

Schematic workflow of scRNA sequencing

- How to decide the dosage for a scRNA-seq study?
- How to select the time points?

Pilot Study

In Vivo Treatment

- Fit-for-purpose design and processing optimization

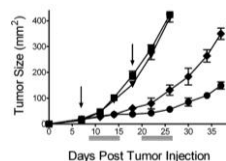
Sample Collection & Single Cell Prep

Library Prep & Sequencing

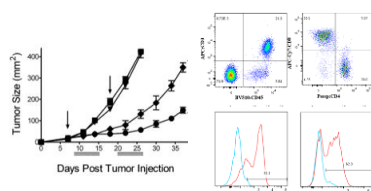
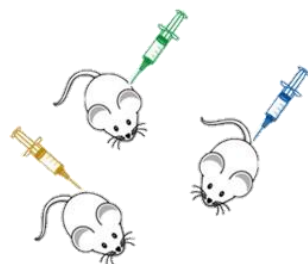
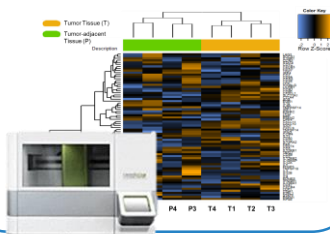
- What cell types are affected by the treatment?
- What's the MOA?

Bioinformatics Analysis

Dose Selection by Efficacy Study



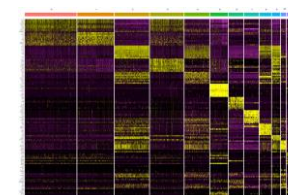
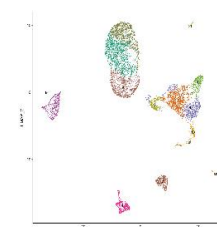
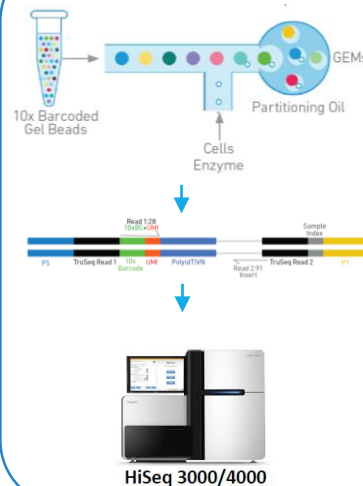
Time Points Selection



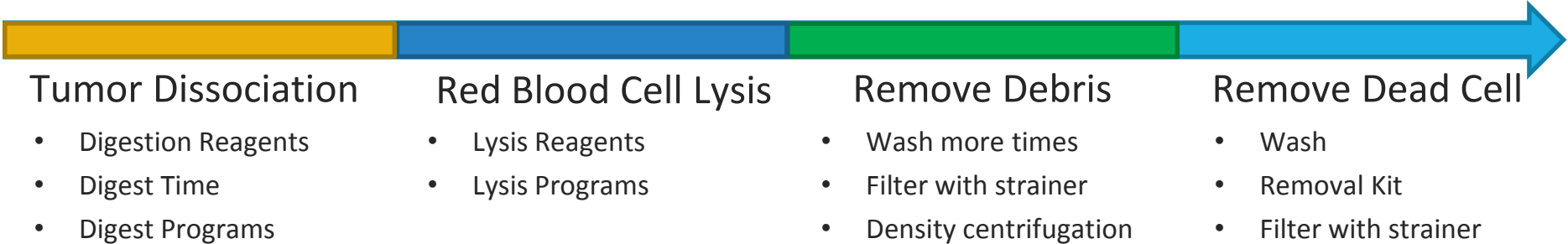
Complex tissue



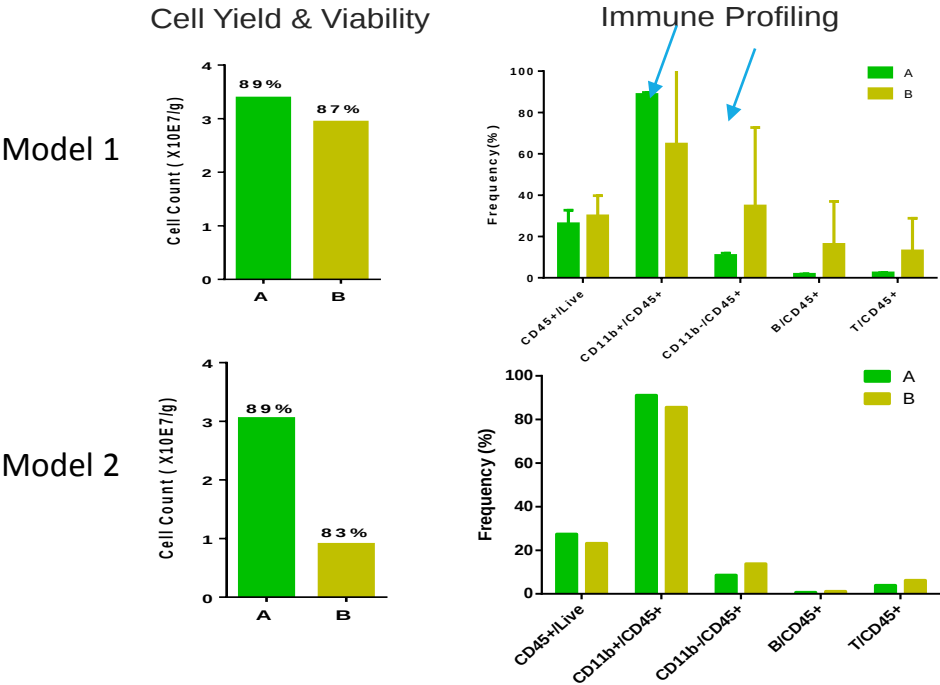
Cell isolation



Fit-for-purpose sample processing optimization



Tumor Dissociation Optimization



RBC Lysis Optimization

Model 1

Method	A	B	C	D	E	F
Cell Yield	Green	Green	Green	Green	Green	Green
RBC Residue	Green	Yellow	Green	Green	Green	Green
Cell Viability	Green	Green	Yellow	Green	Green	Yellow
Cell Type Bias	Green	Green	Green	Green	Green	Green
Time Cost	Yellow	Green	Green	Green	Green	Green

Model 2

Method	A	B	C	D	E	F
Cell Yield	Yellow	Yellow	Green	Yellow	Green	Green
RBC Residue	Green	Green	Green	Green	Yellow	Yellow
Cell Viability	Green	Yellow	Green	Green	Green	Green
Cell Type Bias	Green	Green	Green	Green	Green	Green
Time Cost	Yellow	Green	Green	Green	Green	Green

Example of quality control

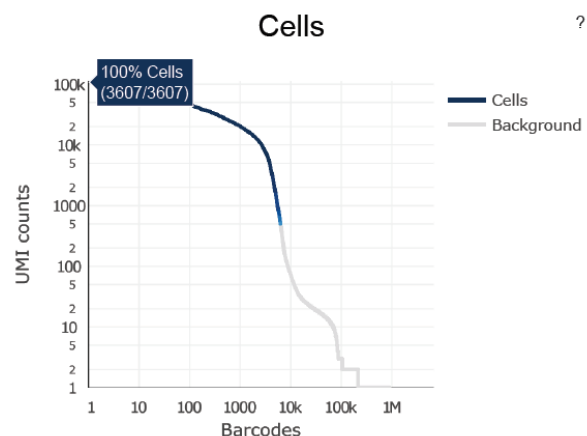
Sample Preparation

Model ID	Live Cells (Counts) /g	Viability (Mean ± SD)
Model 1	(6.15 ± 4.24)E+07	87.96 ± 1.92%
Model 2	(2.8 ± 0.54)E+07	86.40 ± 4.13%

Library Preparation

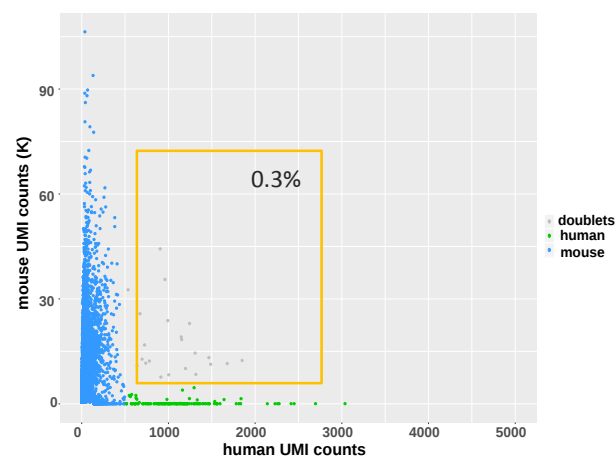
Step	RNA Conc ng/ul	Fragmentation (nt)
cDNA	15 ± 5 ng/ul	-
Library	25 ± 5 ng/ul	450 ± 50 bp

Cell Count QC- Cell Ranger



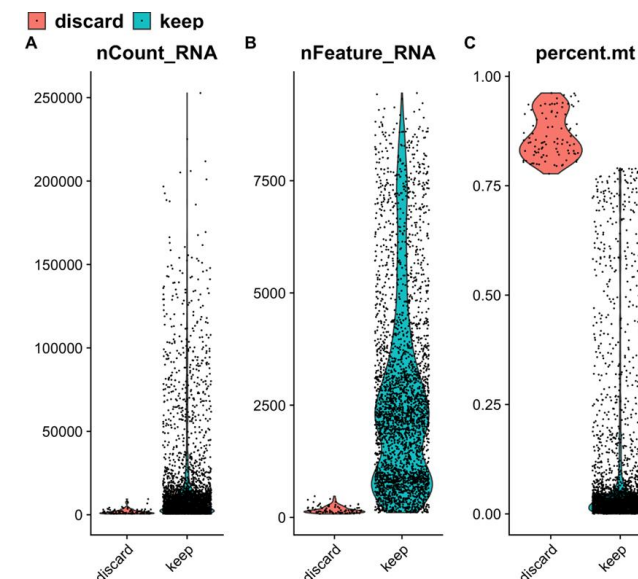
Estimated Number of Cells	5,969
Fraction Reads in Cells	95.6%
Mean Reads per Cell	27,451
Median Genes per Cell	2,632
Total Genes Detected	19,346
Median UMI Counts per Cell	8,349

Doublers Rates Analysis



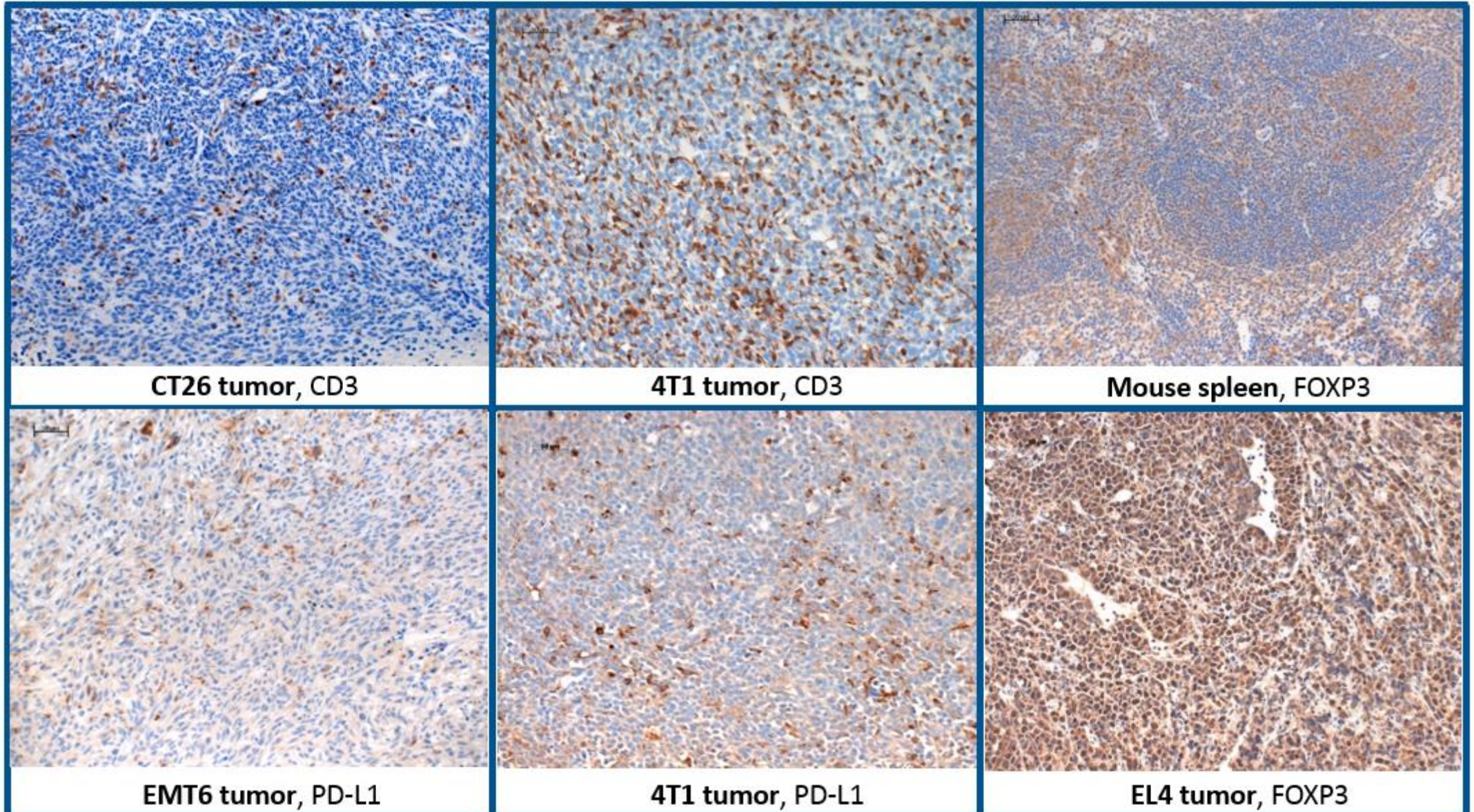
Data Processing

QC metrics



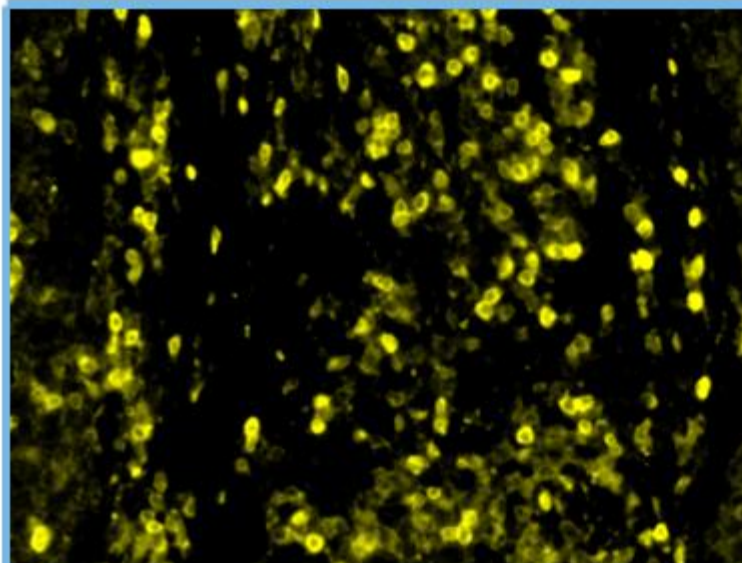
Case study of tissue-based immune profiling

Regular IHC staining with anti-mouse antibodies in syngeneic models

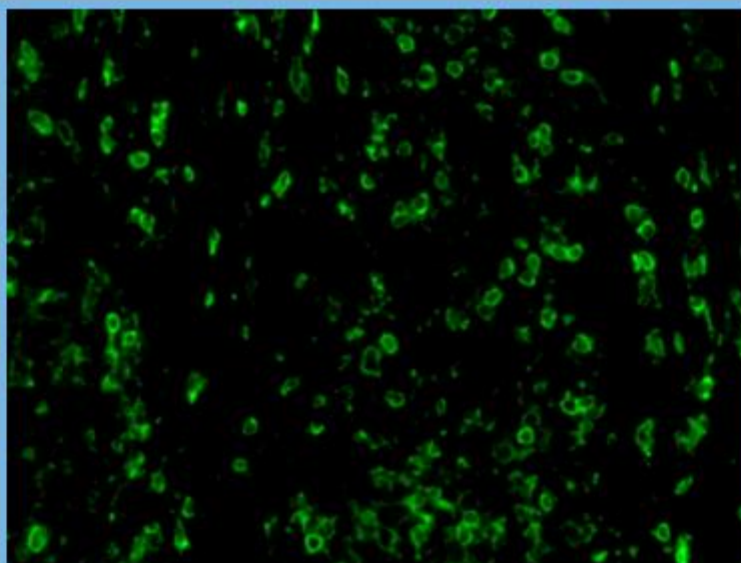


Case study of tissue-based immune profiling

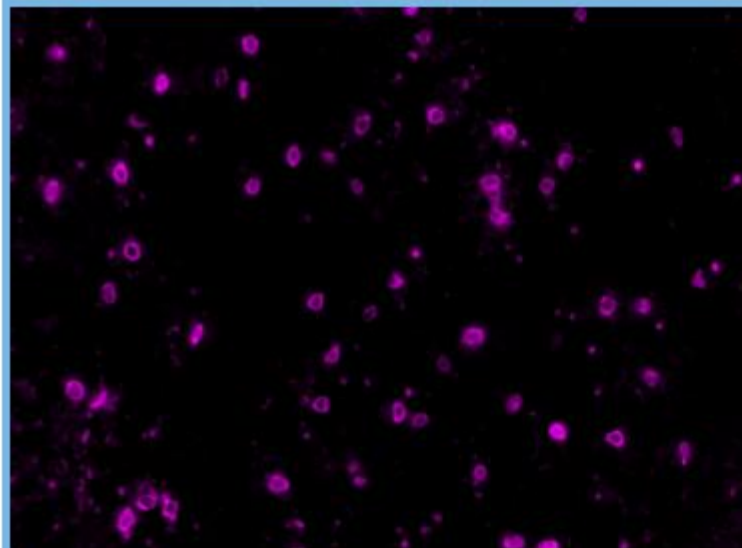
Multiplex IF in patient esophageal tumor sample



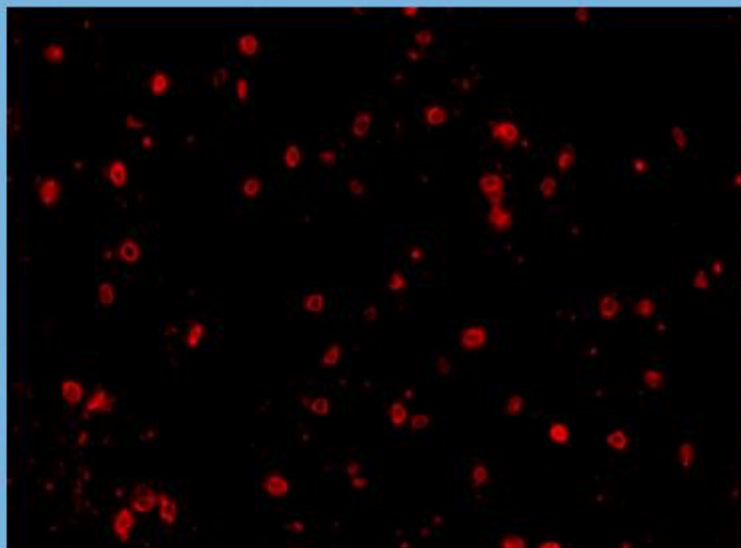
Patient esophageal cancer, CD3



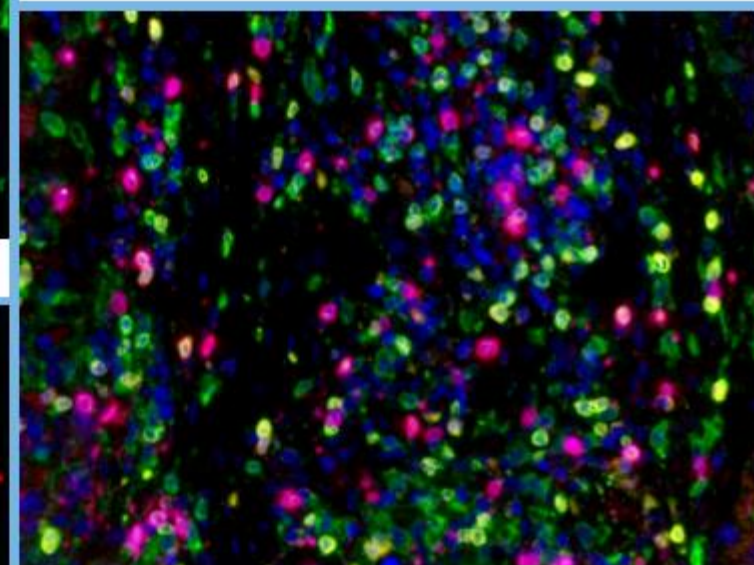
Patient esophageal cancer, CD4



Patient esophageal cancer, CD8



Patient esophageal cancer, PD-1

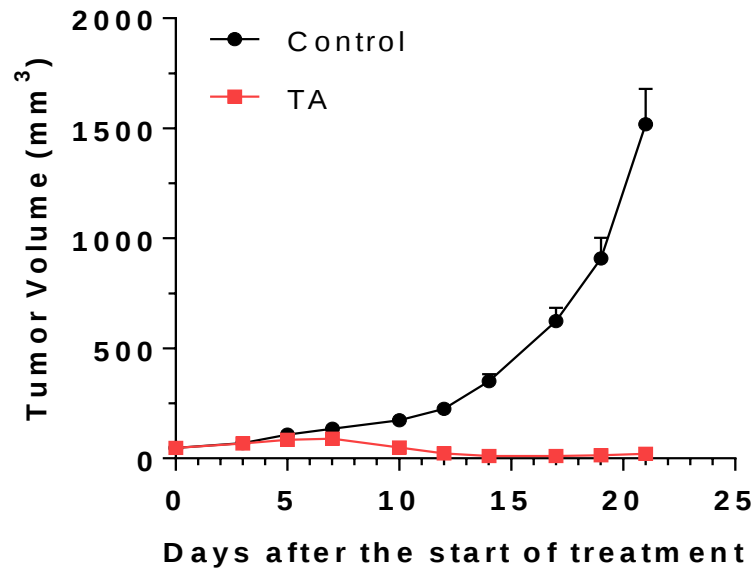


Overlaid, PD-1

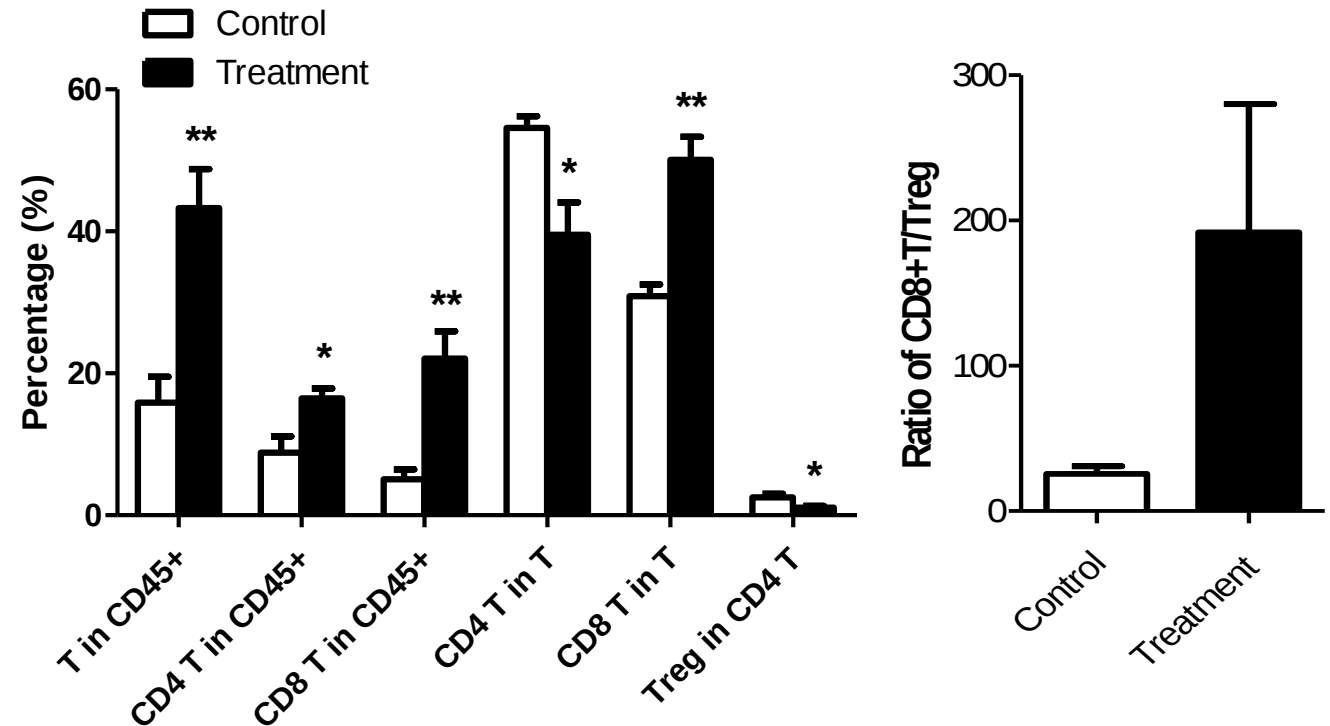
Case study of FACS-based immune profiling

TIL analysis post immune checkpoint inhibitor therapy (MC38 in humanized mouse model)

In vivo efficacy



TIL analysis

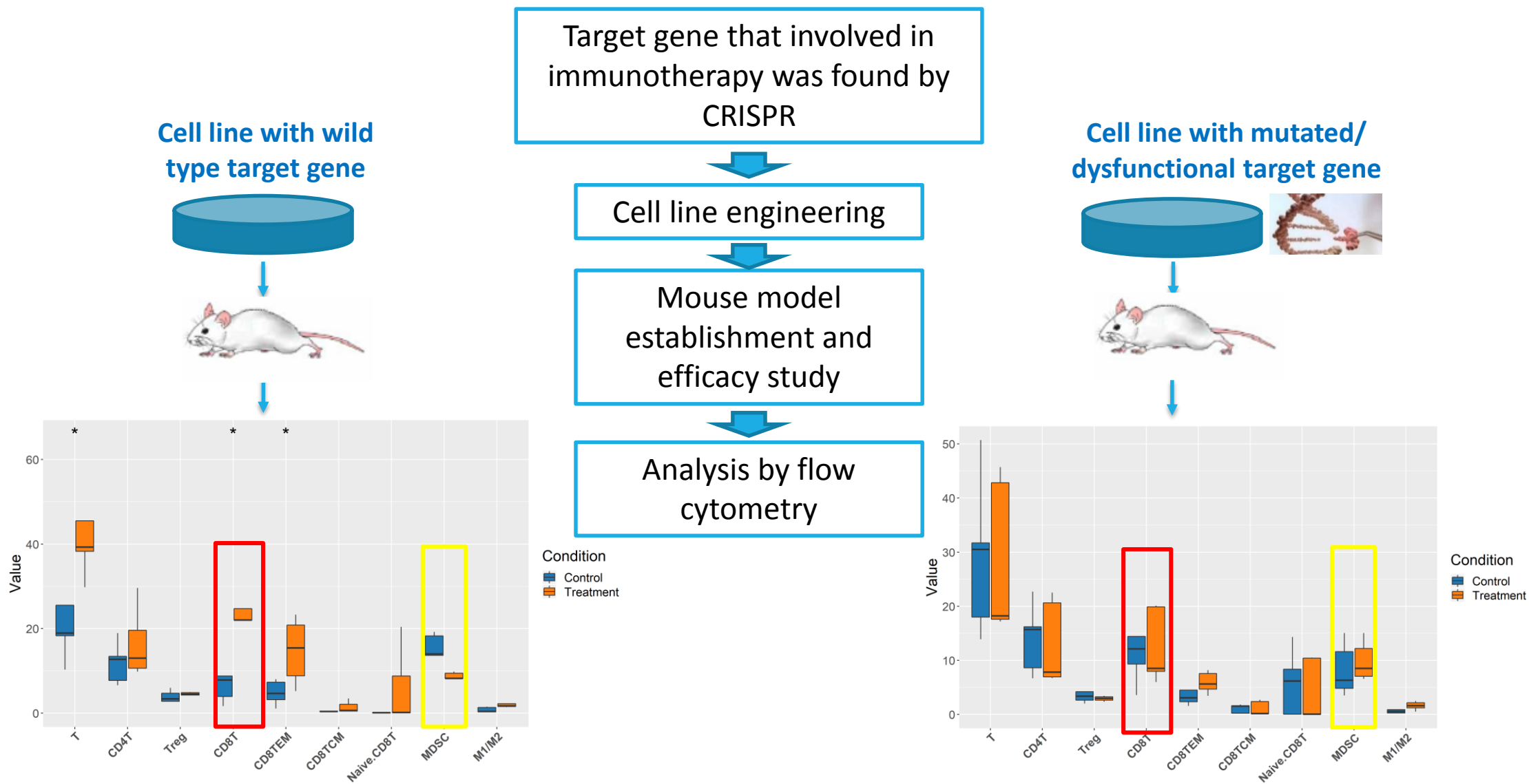


The MC38 model was established in humanized immune-deficient mice. Mice were treated with either a single dose of isotype control or an immune checkpoint-blocking antibody and further TILs were analyzed by FCM. Student's t-test were used for statistical analysis.

N=4. *, $p < 0.05$; **, $p < 0.01$.

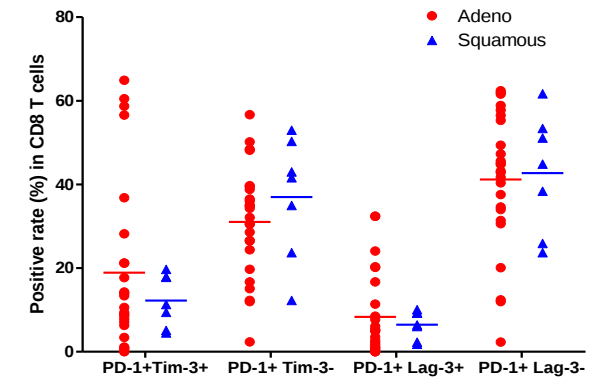
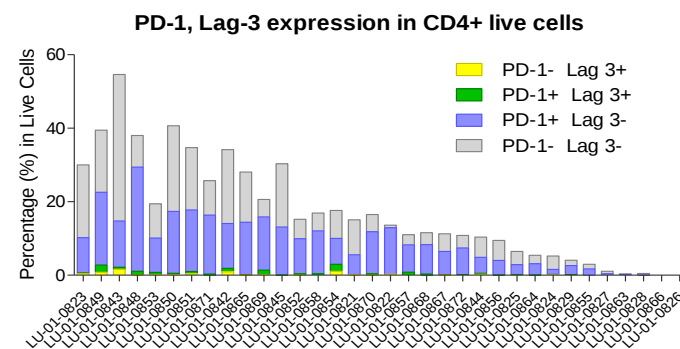
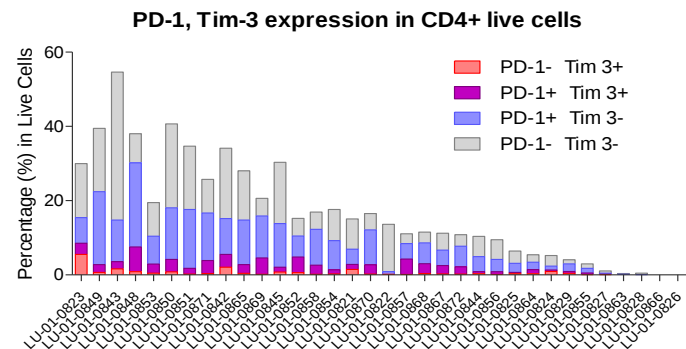
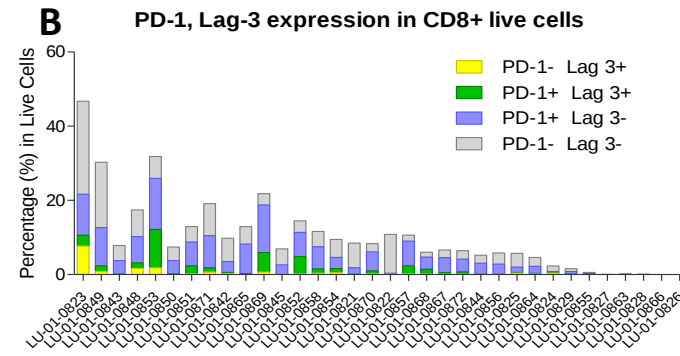
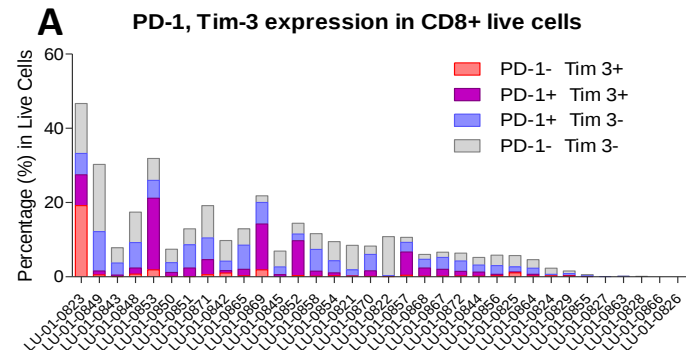
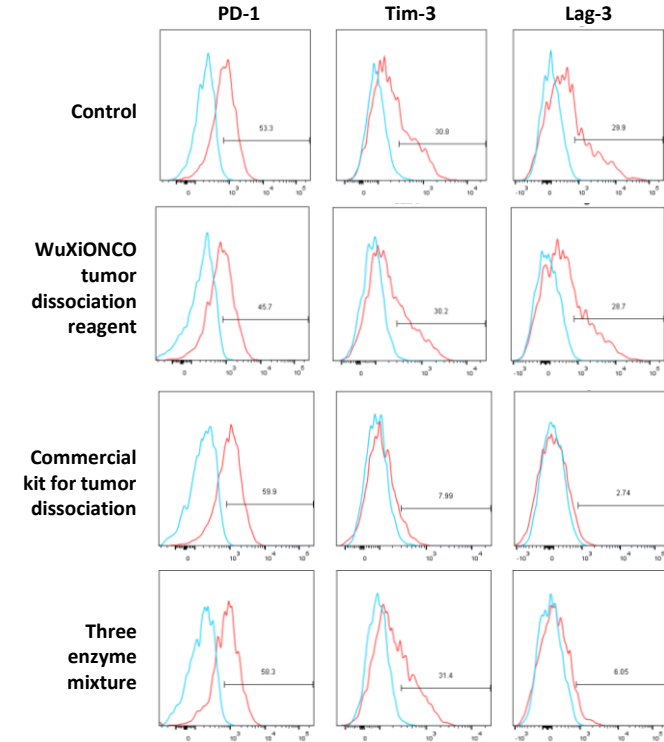
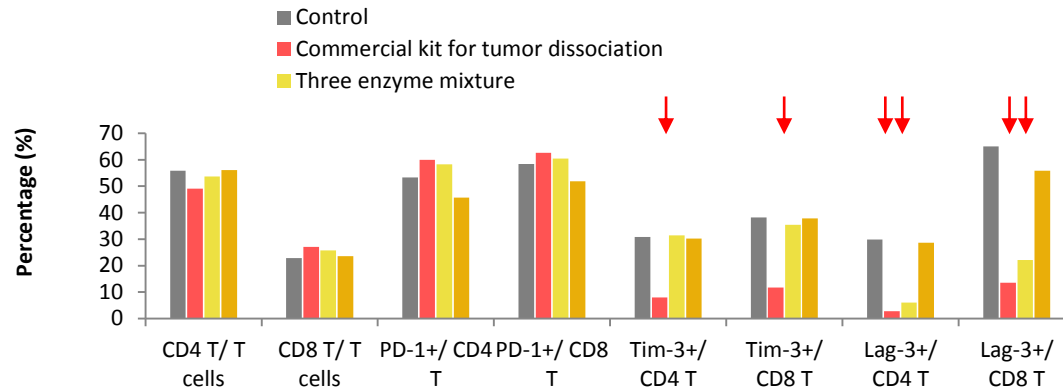
Case study of FACS-based immune profiling

Verification of driver gene



Case study of FACS-based immune profiling

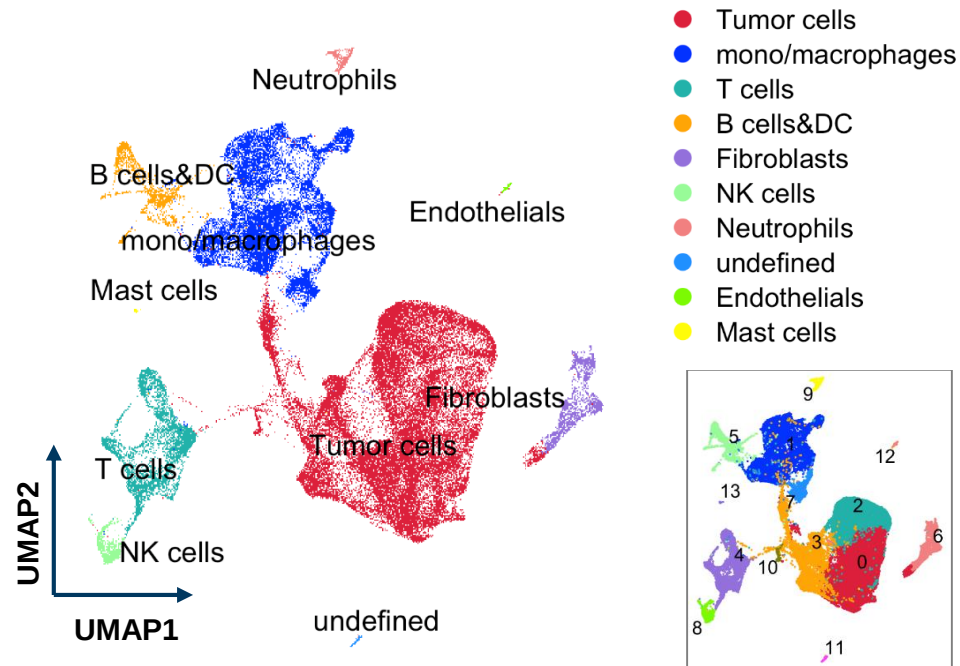
TIL immune profiling and biomarker detection across cancer types



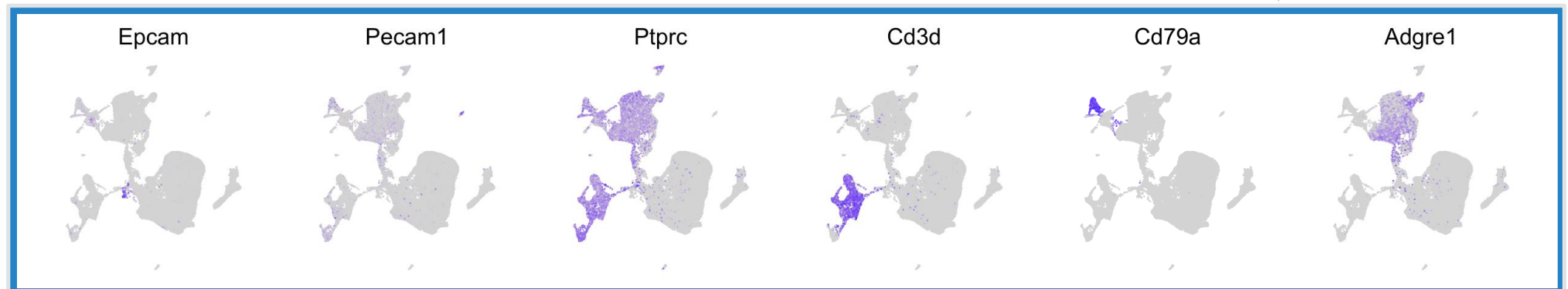
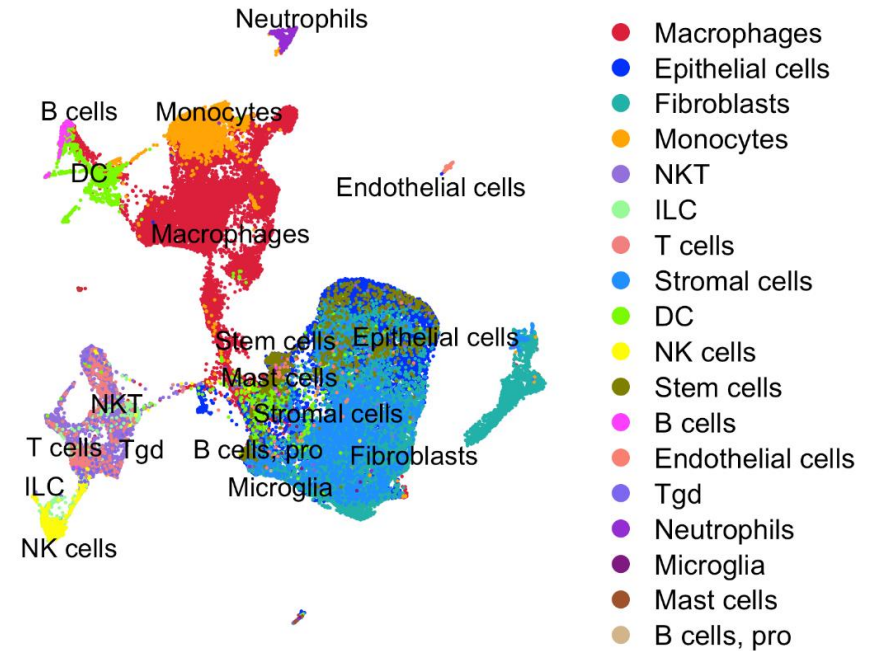
Case study of RNA-based immune profiling

Visualization of cell types and markers of interest on 2D plots

Manually by a multi-step approaches



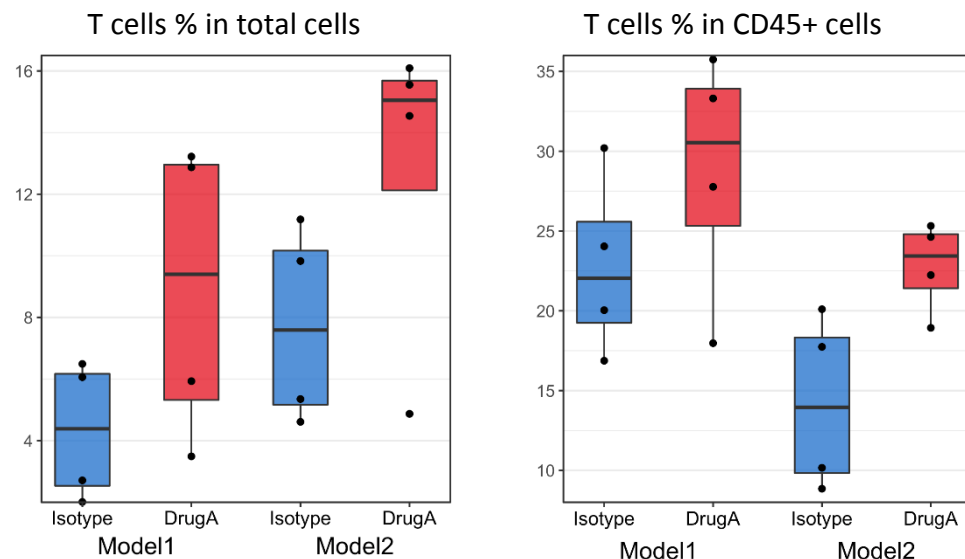
Automatically based on database



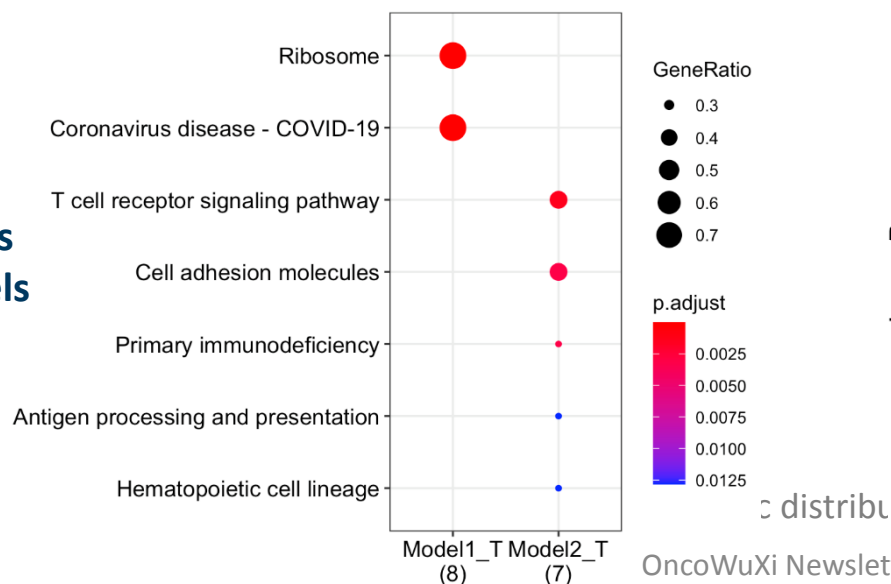
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Treatment-induced immunology changes

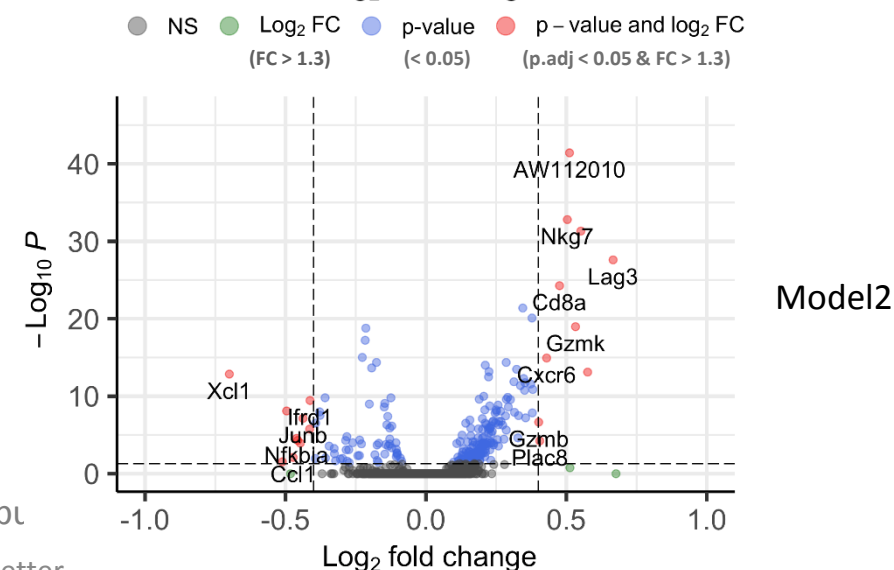
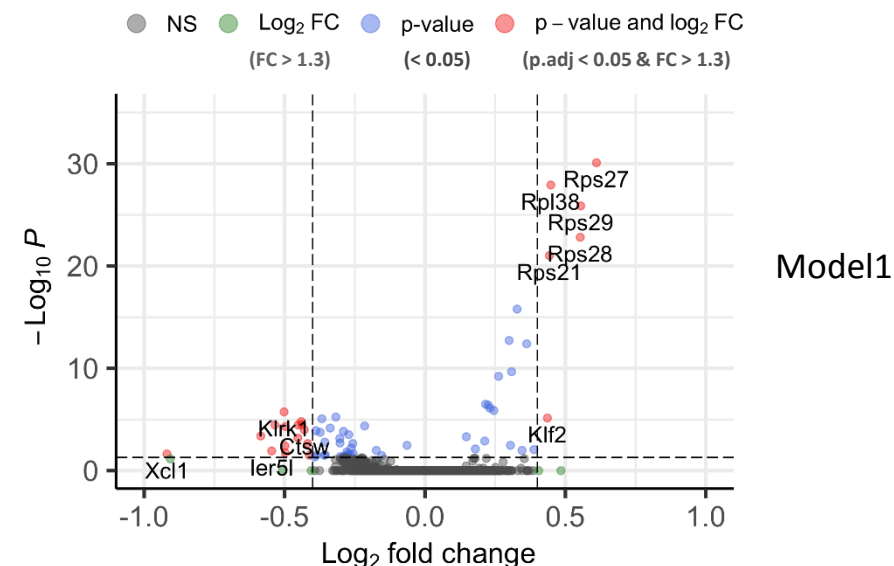
T cell frequency changes



Pathway changes in different models



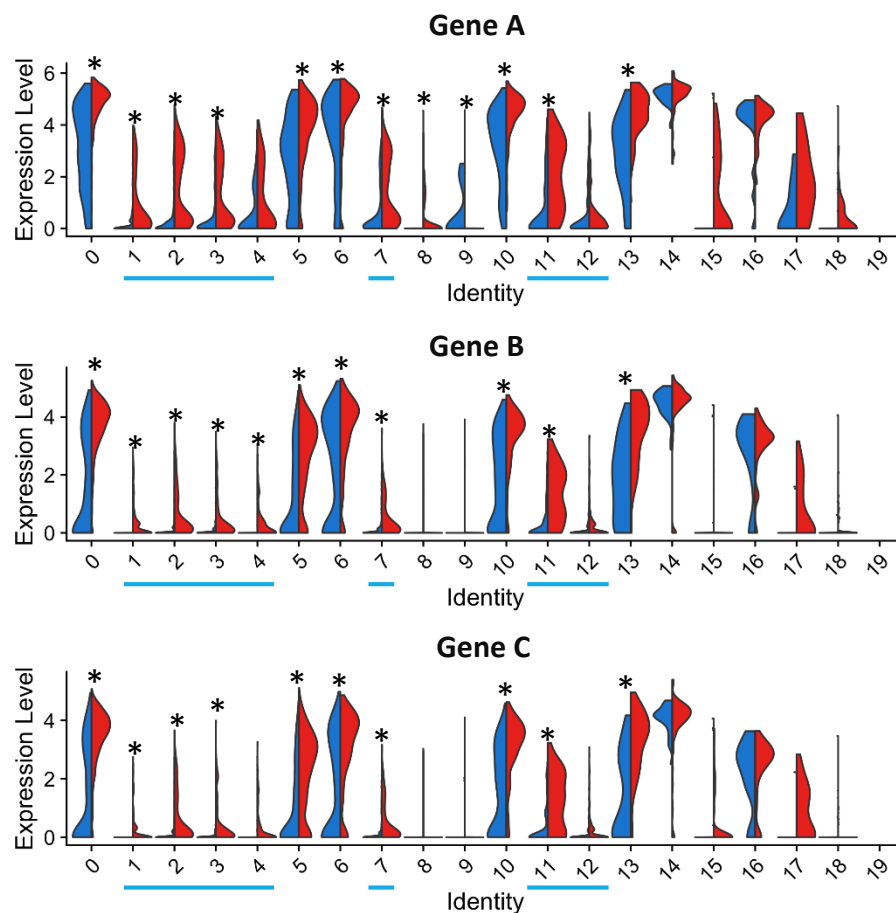
Differential gene expression across models



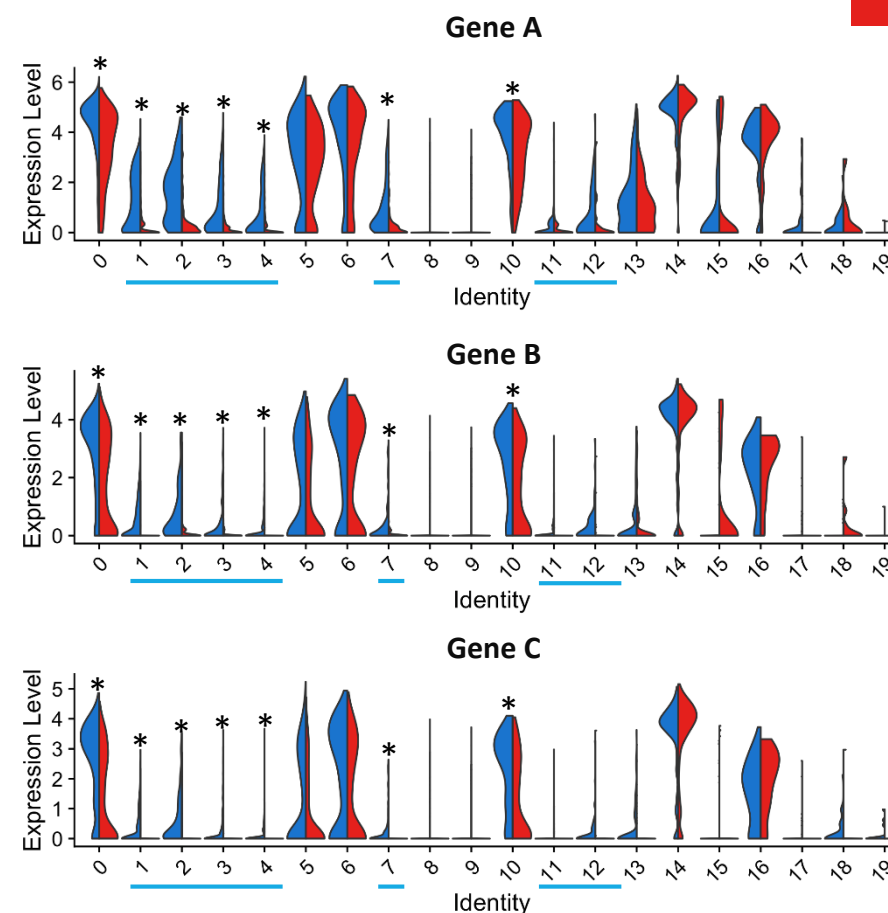
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Treatment-induced gene expression alternation in tumor cells

Model1 (responsive)



Model2 (non-responsive)



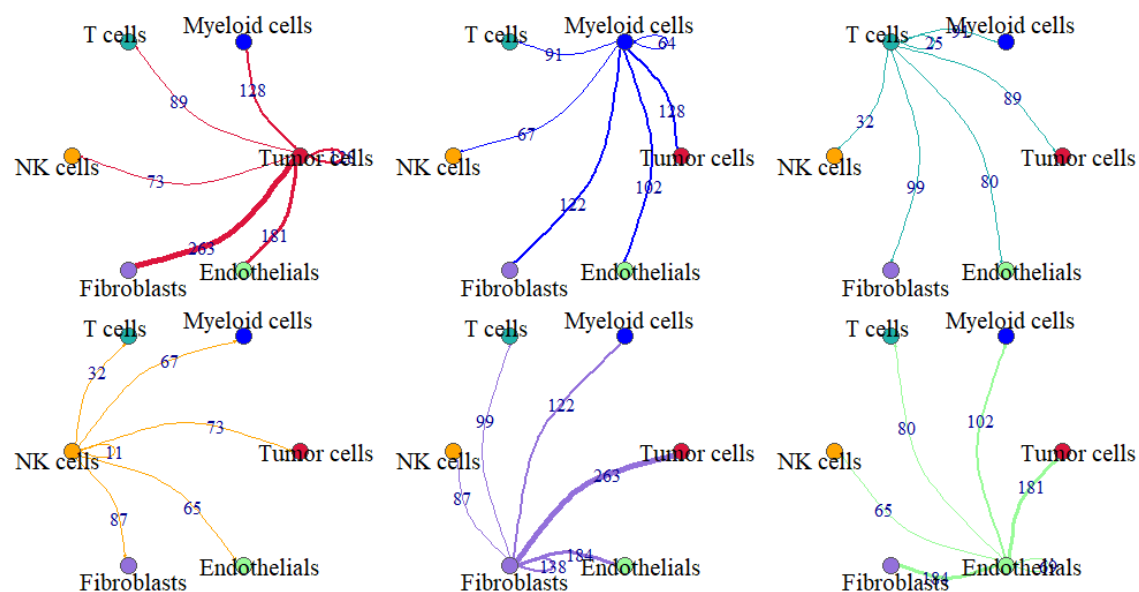
Treatment

isotype control
drug A

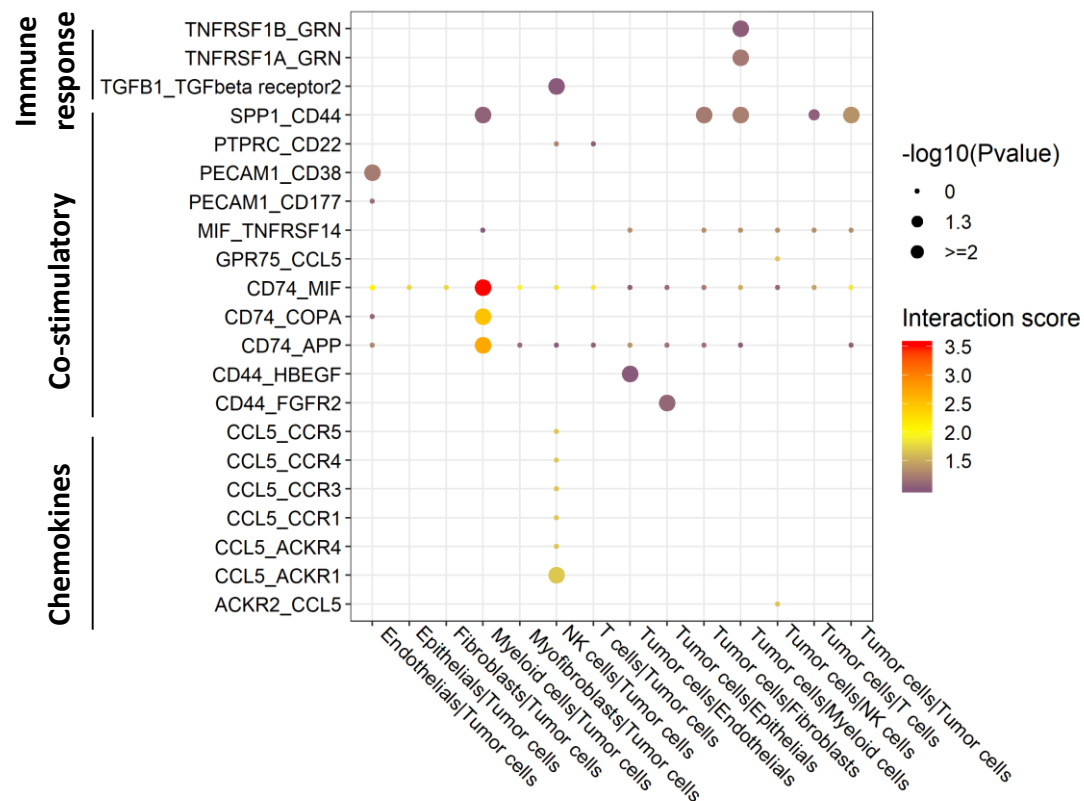
Case study of RNA-based immune profiling

The potential key ligand-receptor pairs functioning in TME

Cell-cell communication analysis based on ligand-receptor pairs database



Overview of selected ligand-receptor interactions of tumor cells





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