WuXi OIU FACS Platform for Drug Discovery

WuXi AppTec Research Service Division, Oncology & Immunology Unit

2020.09

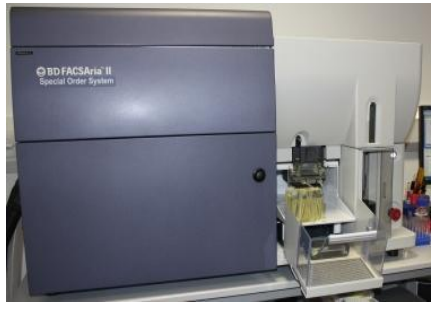
OncoWuXi Newsletter

Contents

WuXi OIU FACS facility

BD FACSCanto II
(3 Laser, 8 channels)

BD LSRFortessa
(5 Laser, 18 channels)



BD FACS Aria II
(4 lasers, 18 channels
2-4 way sorting)

BD FACSCanto plus
(3 Laser, 10 channels)

BD LSRFortessa X20
(5 Laser, 18 channels)

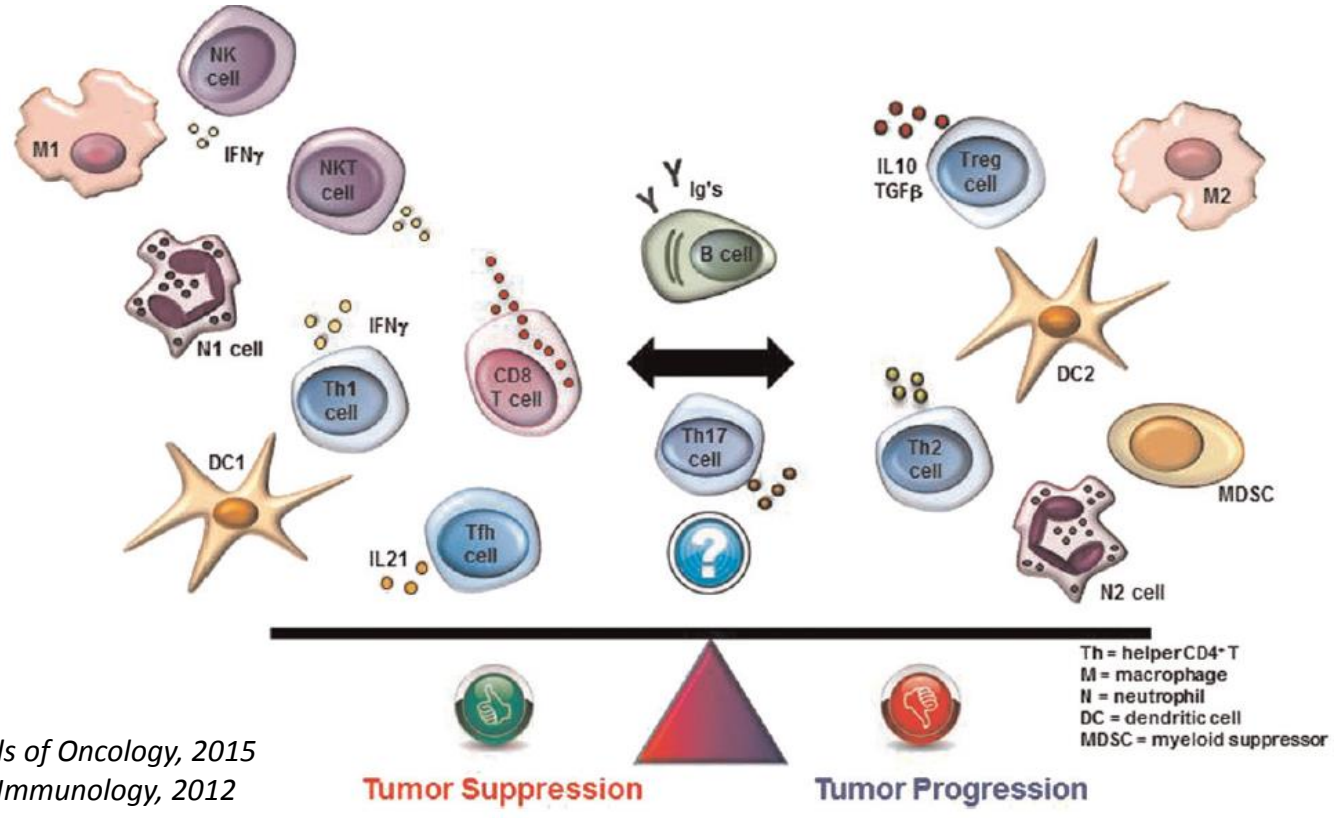
Cytek Aurora
(3 Laser, >24 colors)

WuXi FACS services for preclinical discovery

- **Phenotypic analysis of tumor infiltrating leukocytes (TILs) across different models**
- **Checkpoint/co-stimulatory marker analysis**
- **Function-related intracellular cytokine analysis**
- **Multiplex analysis of secreted cytokine/chemokine by CBA**
- ***In vivo* phosphoflow**
- **Multi-dimensional data mining of flow data**

Tumor infiltrating leukocytes (TILs)

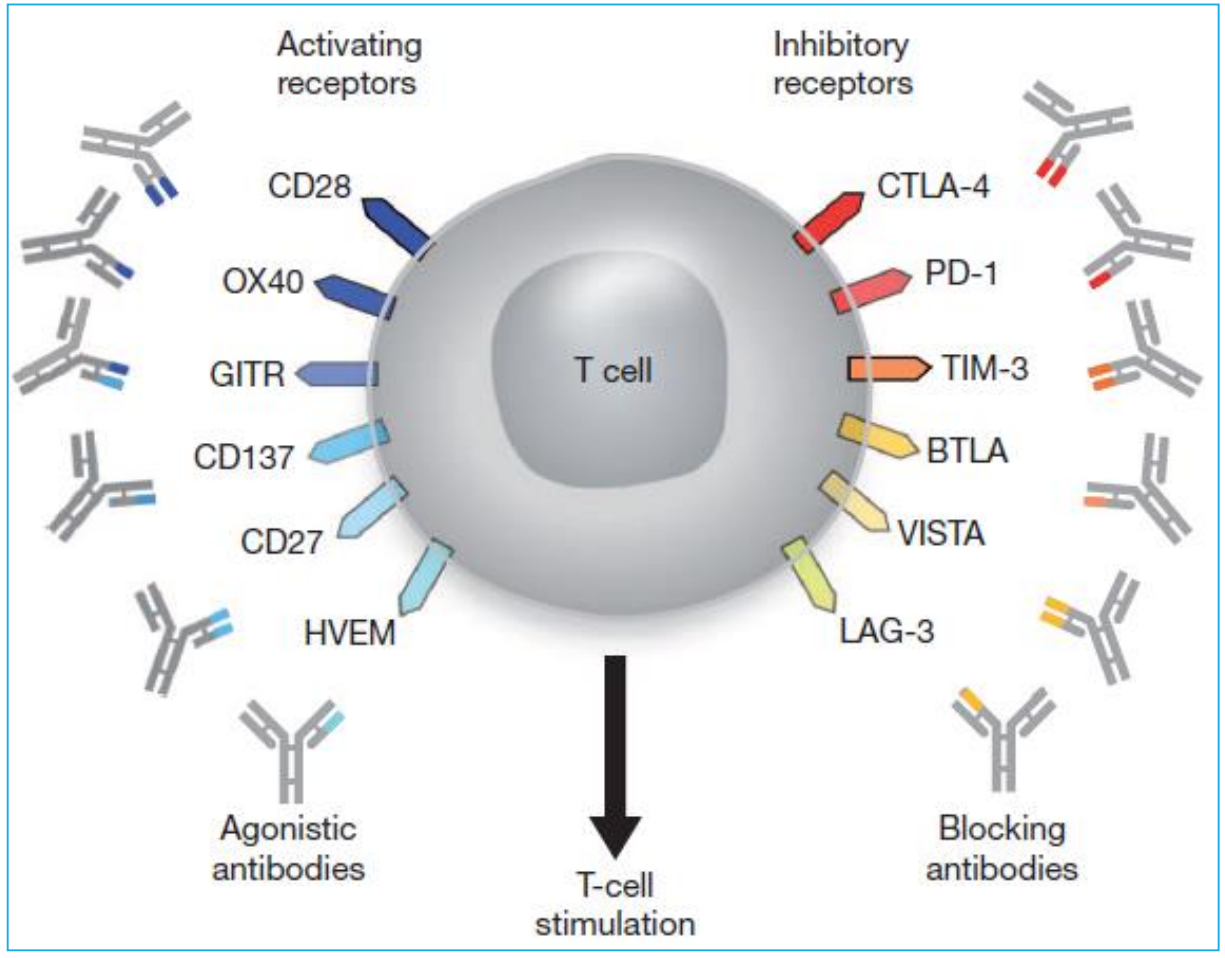
- Balance of pro- and anti- tumor immune cells in the tumor microenvironment (TME) greatly influences tumor growth.
- As a clinical biomarker: Correlation of TIL subpopulations and clinical outcomes (e.g., Tregs in ovarian cancer).



Annals of Oncology, 2015
OncoImmunology, 2012

Regulation of T cell function

T cell exhaustion in tumor microenvironment.



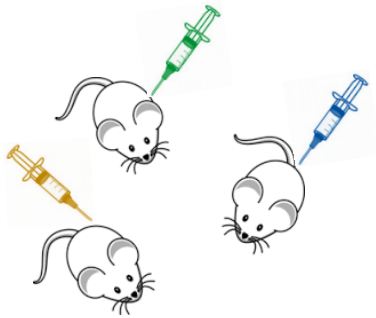
Nature. 2011

Advantages of FACS-based TIL analysis

- **“Global”, thus unbiased**
  - The entire tumor environment can be analyzed
- **Specific and precise**
  - Very specific cell subtypes can be analyzed by employing multi-color FACS analysis

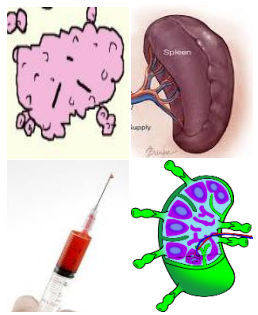
Mouse TILs analysis workflow after *in vivo* drug efficacy study

In vivo efficacy study with syngeneic or humanized models



Collect tissues at different time points

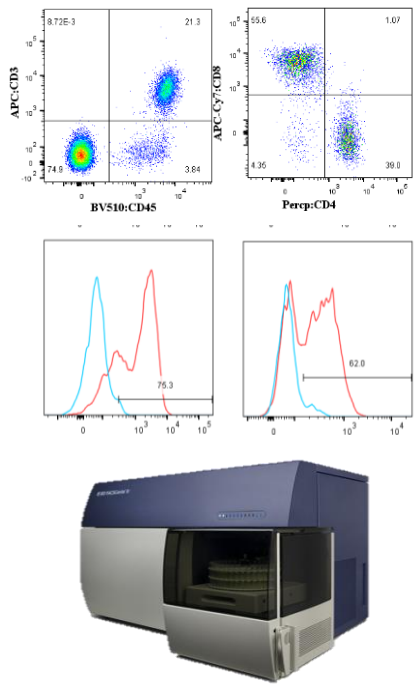
Tumor Spleen



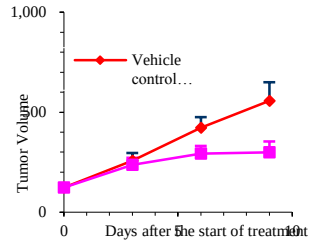
PBMC Lymph node

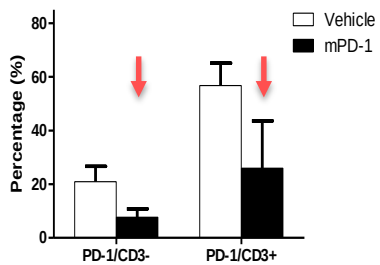
(Optional)
FFPE
TCRseq
GEP

Flow Cytometry Analysis



Information Integration and Summarization





Mouse TIL composition and biomarker analysis

Subtype	Markers
Lymphocytes (T, B, NK)	CD3, CD4, CD8, FoxP3, CD25, B220, CD19, NK1.1/CD49b (DX5)/CD335, CD44, CD62L, CD69
Myeloid (Granulocytes, Mono/Mϕ) and DC	CD11b, Gr1, Ly6G, Ly6C, F4/80, CD206, MHCII, CD11c
Immune Checkpoint-related	PD-1, PD-L1, Tim-3, Lag-3, CTLA-4
Co-stimulator-related	4-1BB (CD137), OX40, CD80/CD86, ICOS, TIGIT, CD226, CD69
Functional	IL-2, GranB, IFNγ, TNFα, Ki-67

Immune cell markers (Mouse)

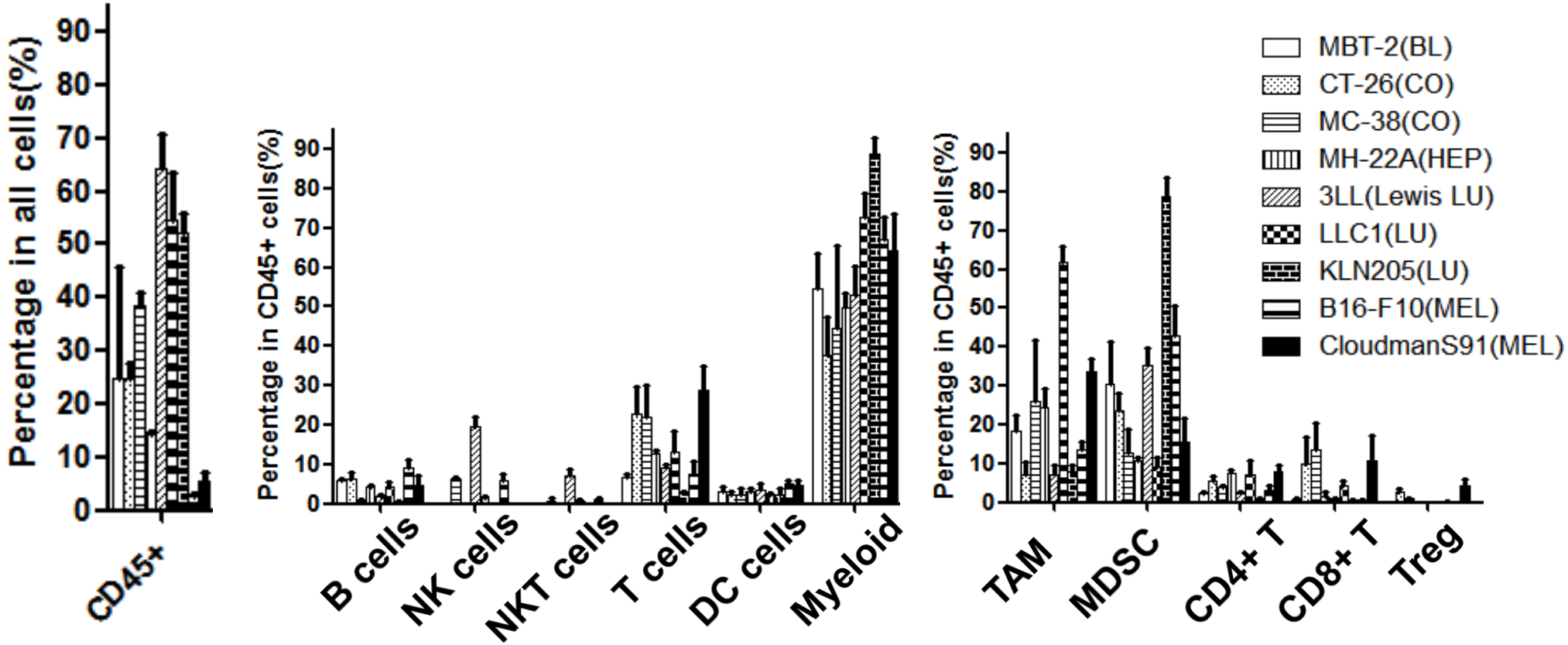
Population	Marker signature	Population	Marker signature
T cell	$CD3^+$	panNK	$CD49b\ (DX5)^+$ or $CD335^+$
CD4 T cell	$CD3^+\ CD4^+\ CD8^-$	panDC	$CD11c^+$
CD8 T cell	$CD3^+\ CD4^-\ CD8^+$	Myeloid	$CD3^-\ CD11b^+$
Treg	$CD3^+\ CD4^+\ (CD25^+)\ Foxp3^+$	MDSC	$CD3^-\ CD11b^+\ Gr1^{+/mid}$
Naïve T cell	$CD3^+\ CD44^{lo}\ CD62L^{hi}$	gMDSC	$CD3^-\ CD11b^+\ Ly6G^+\ Ly6C^{lo}$
Effector T cell	$CD3^+\ CD44^{hi}\ CD62L^{lo}$	mMDSC	$CD3^-\ CD11b^+\ Ly6G^{lo}\ Ly6C^+$
Memory T cell	$CD3^+\ CD44^{hi}\ CD62L^{hi}$	M1	$Gr1^-\ CD11b^+\ F4/80^+\ CD206^{lo}\ MHCII^{hi}$
B cell	$B220^+$ or $CD19^+$	M2	$Gr1^-\ CD11b^+\ F4/80^+\ CD206^+\ MHCII^{lo}$

Basic panel options for flexible design

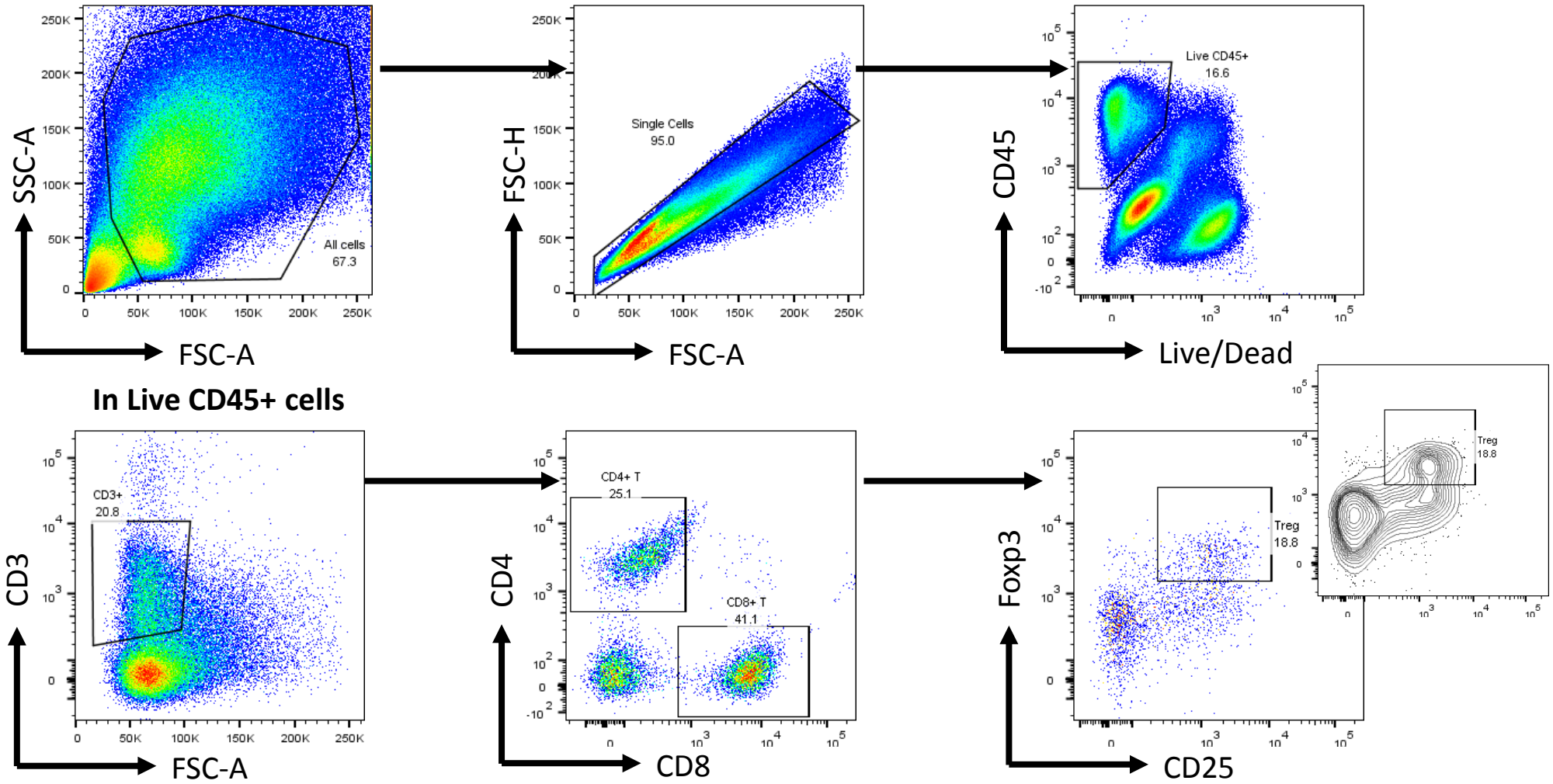
Option1	Option2	Option3	Option4	Option5
L/D	L/D	L/D	L/D	L/D
CD8	CD8	CD8	CD8	CD8
CD44	CD19	MHCII	CD19	CD3
L/D	CD11b	Ly6C	CD335	CD4
CD19	CD335	CD11b	CD11b	CD45
CD62L	PD-L1	F4/80	CD3	FoxP3
CD11b	CD3	CD11c	FoxP3	CD25
CD335	FoxP3	CD3	PD-1	CD335
PD-L1	PD-1	CD206	CD45	CD19
CD25	CD45	Ly6G	CD4	CD44
FoxP3	CD4	CD45		CD62L
PD-1		CD4		CD11b
CD45				Ly6C
CD3				Ly6G
				F4/80
				CD206
				MHCII
				CD11c
				PD-1
				PD-L1
				Ki67

Panels	Detection purpose	Cytometer
Option1	Lymphocyte and biomarkers	Fortessa
Option2	Major cell types and biomarkers	Fortessa
Option3	Myeloid cells	Fortessa
Option4	Major cell types and biomarkers	Canto
Option5	Myeloid cells, lymphocyte and biomarkers	Aurora

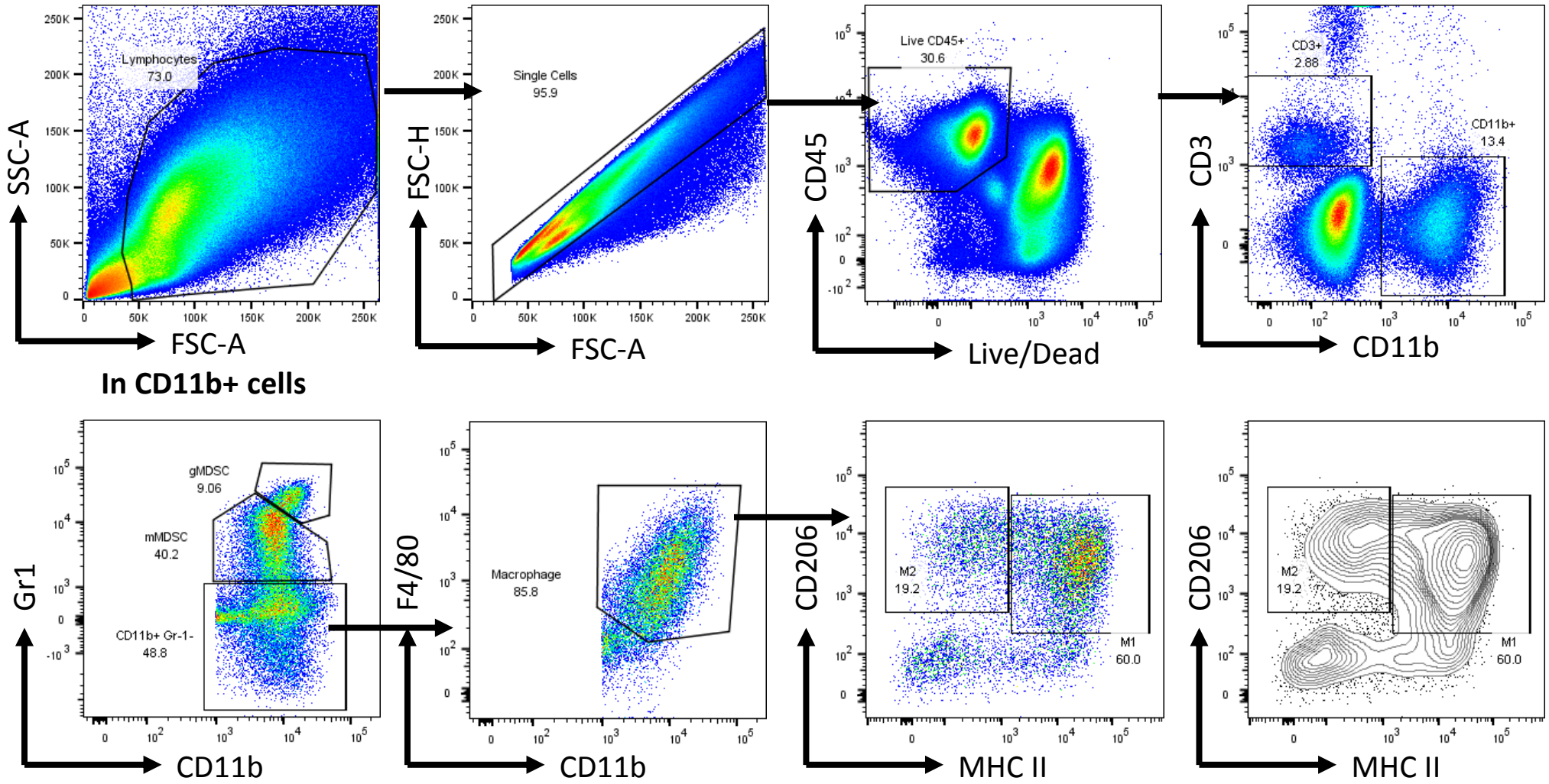
Baseline of TIL subpopulations in different syngeneic models



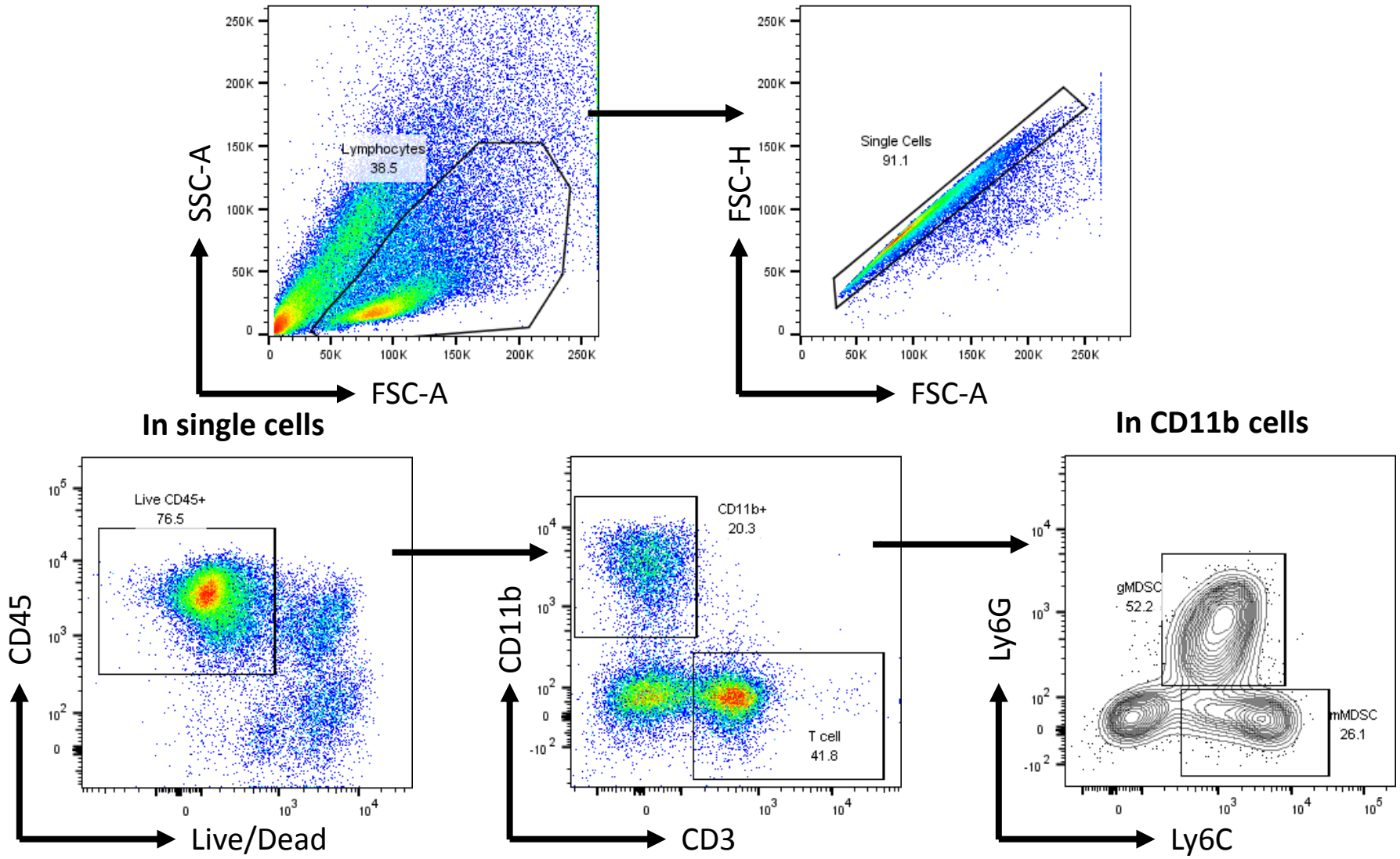
Treg staining (CT-26, TIL)



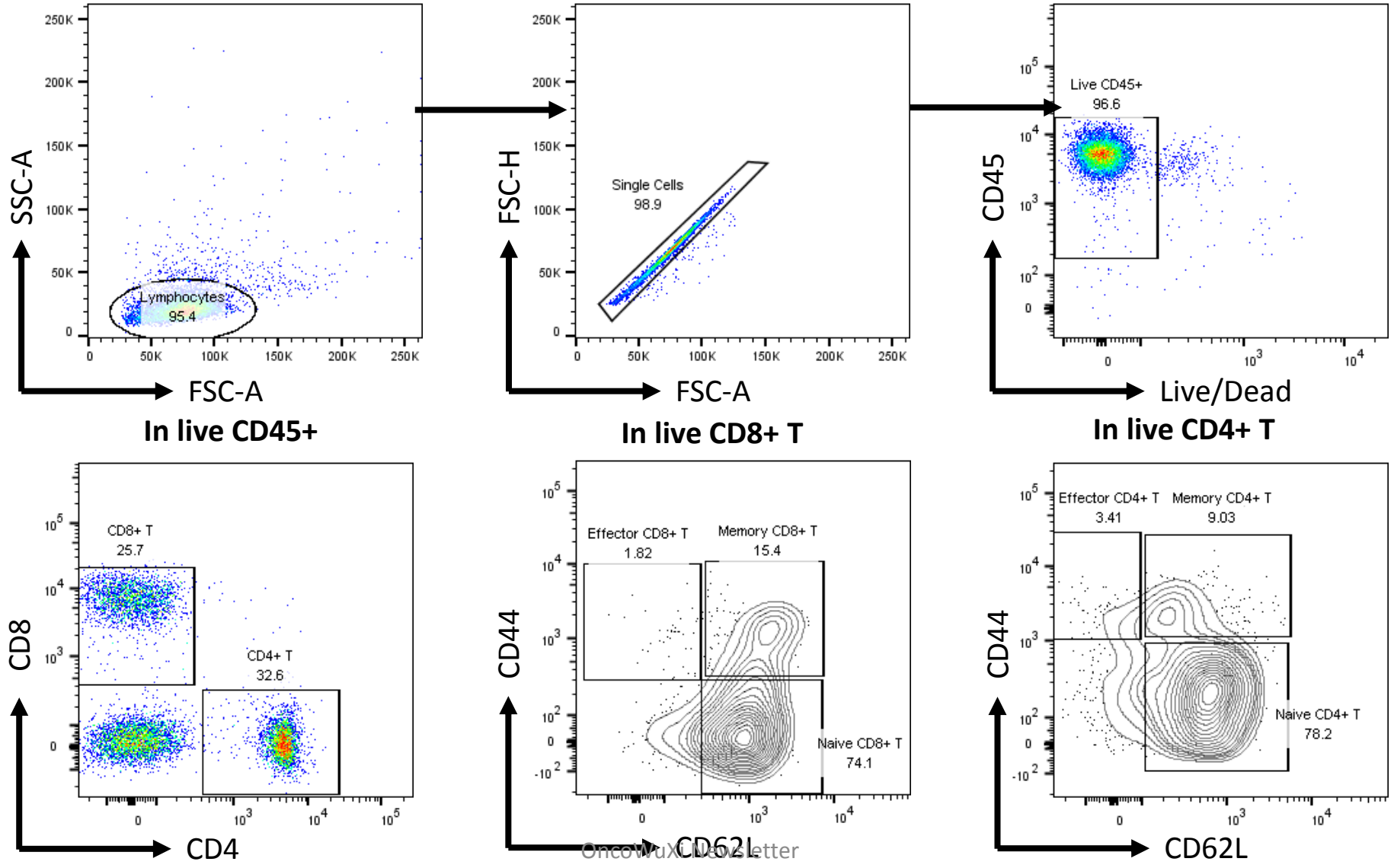
TAM and MDSC staining (P815, TIL)



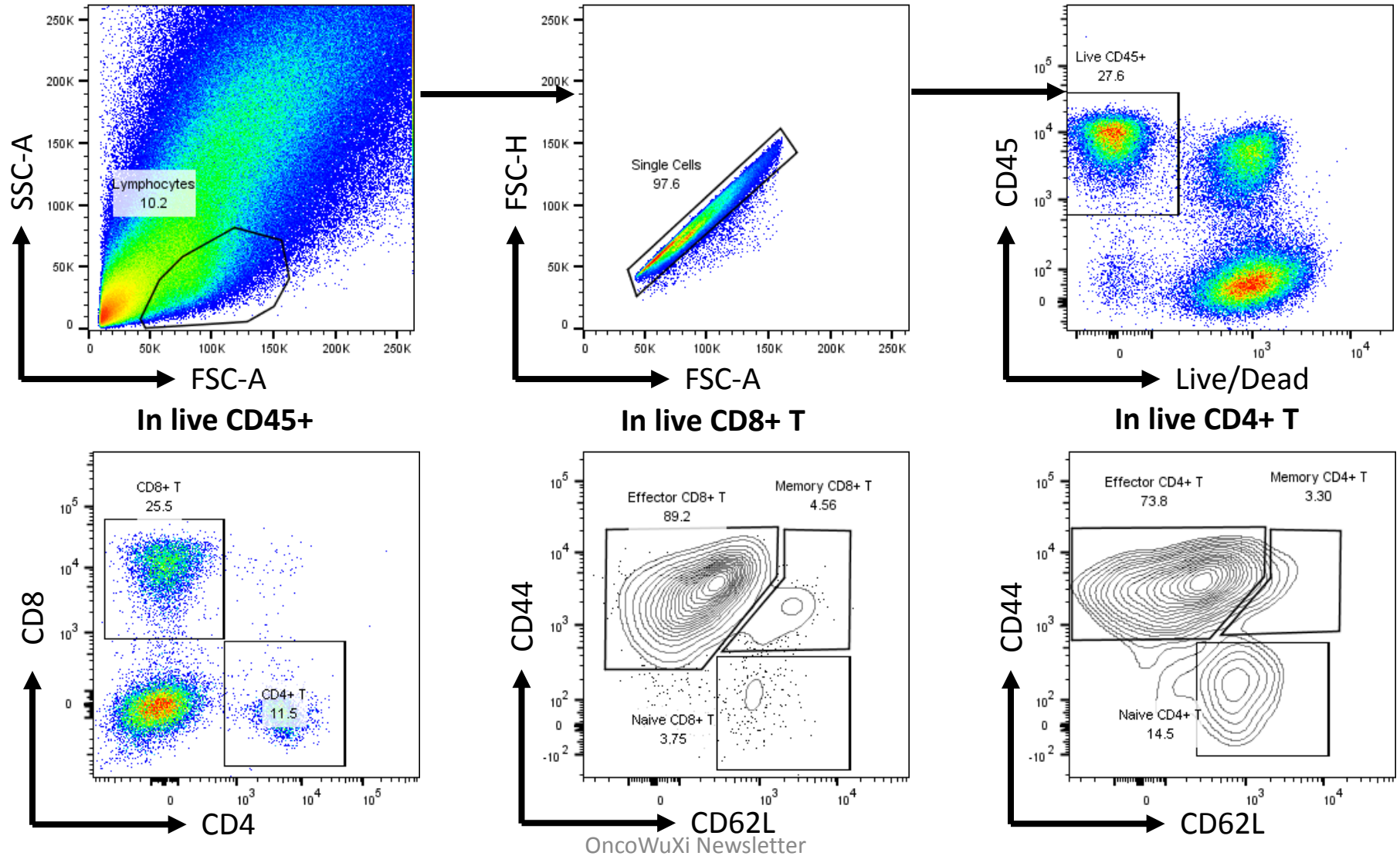
MDSC staining (MC38, TIL)



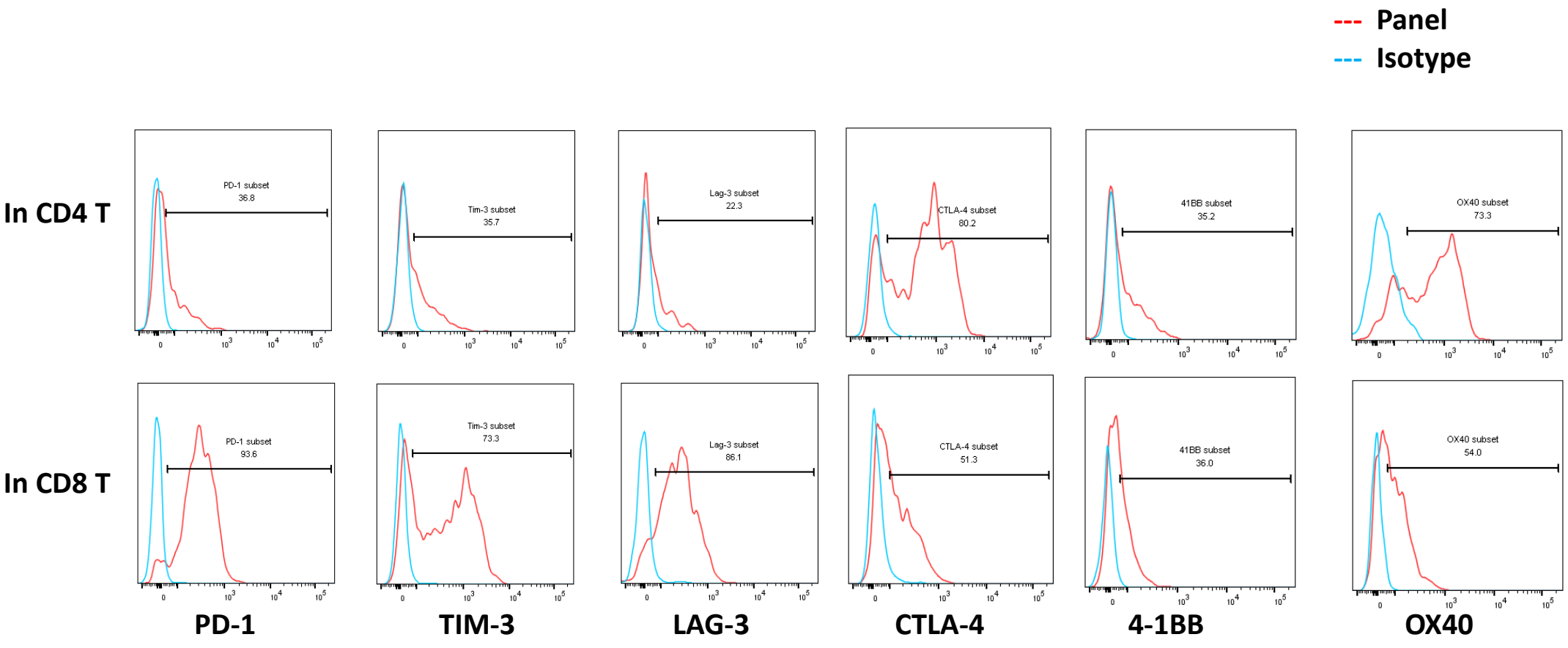
Naïve/Memory/Effector T staining (MC38, Lymph node)



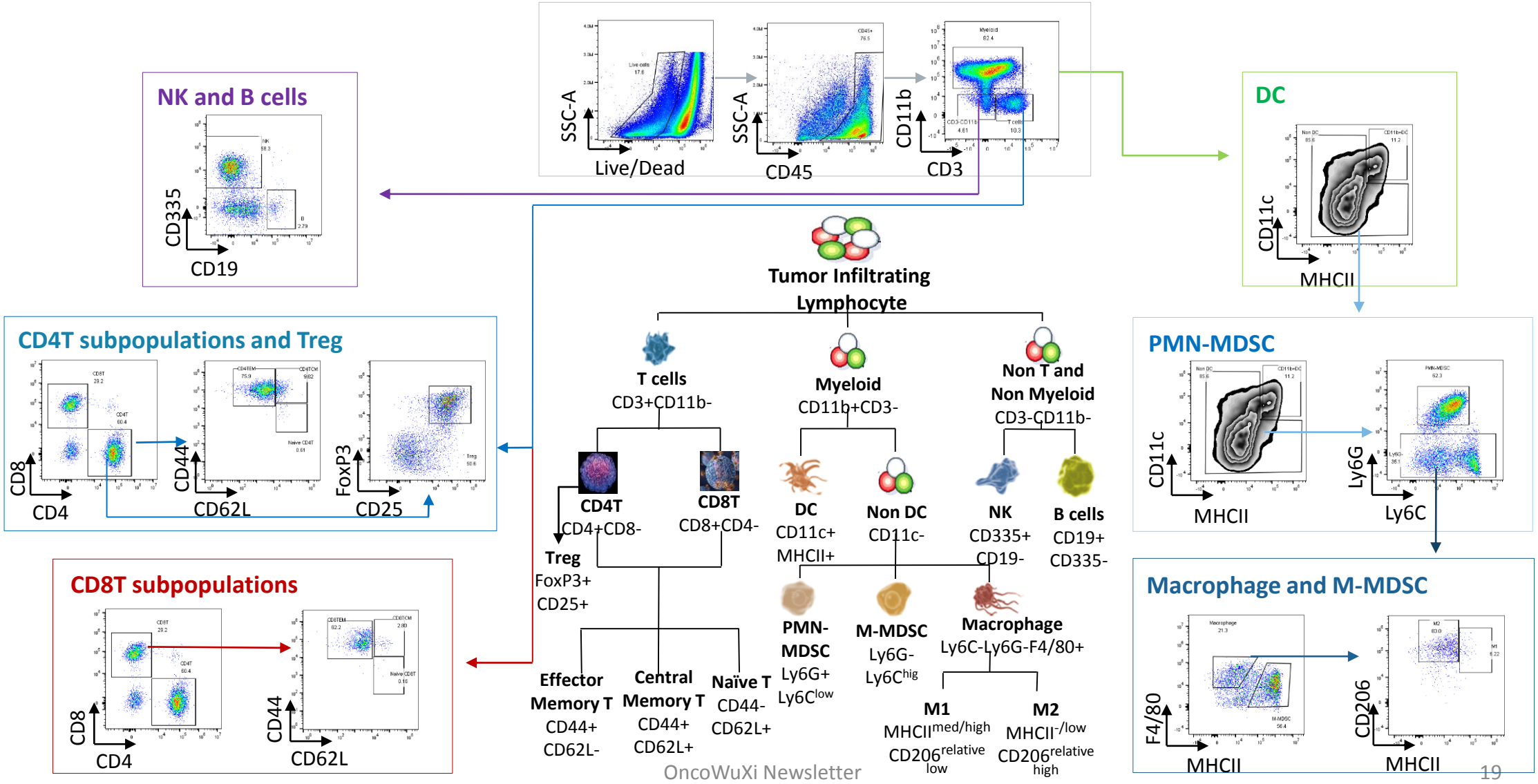
Naïve/Memory/Effector T staining (MC38, TIL)



Checkpoint/co-stimulatory markers (CT-26)

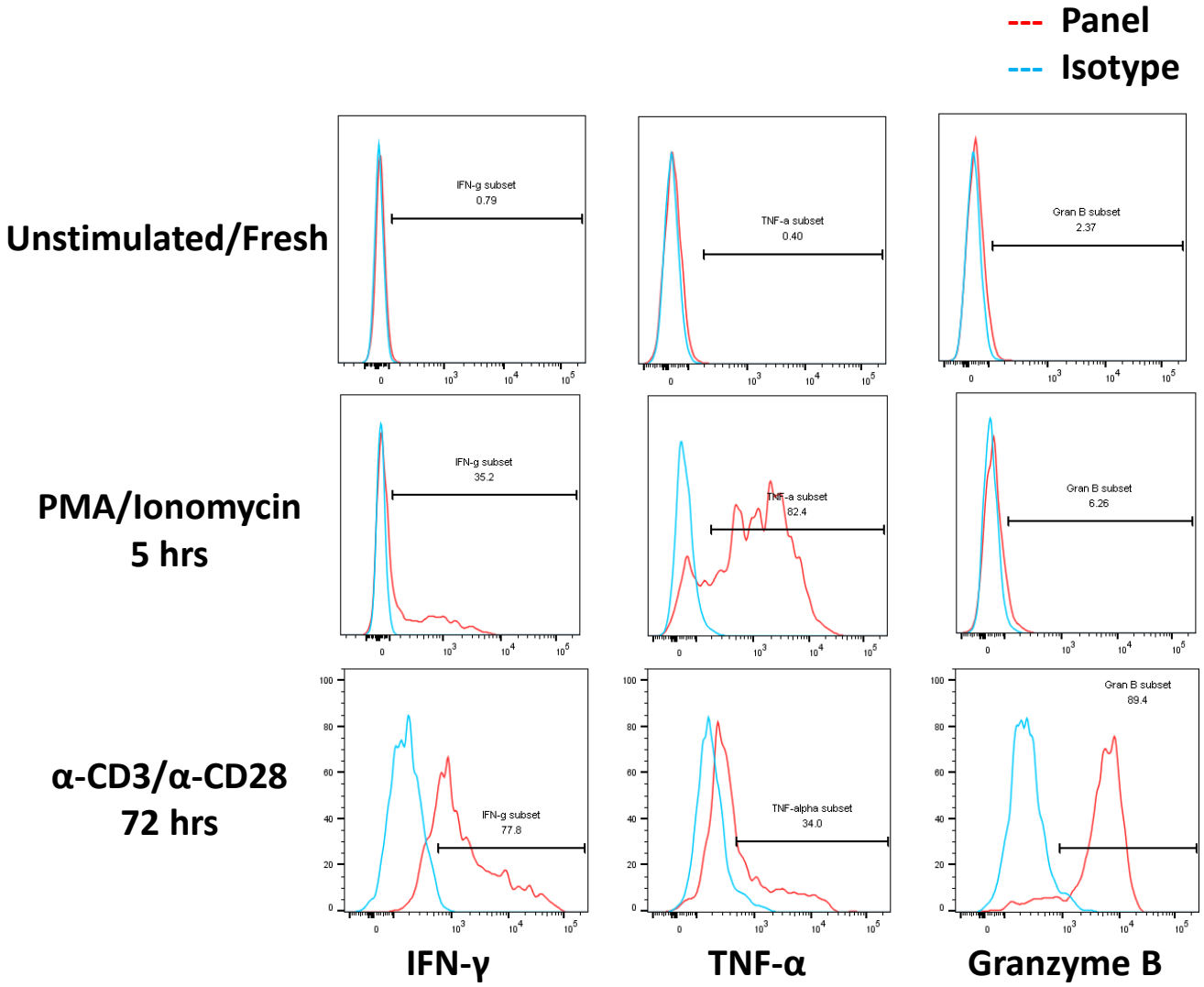


Immune-profiling by flow cytometry in a single panel



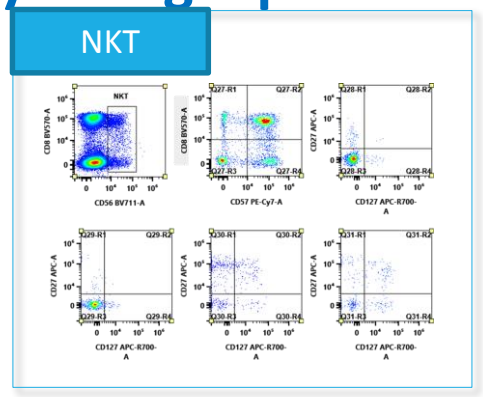
Intracellular cytokine staining

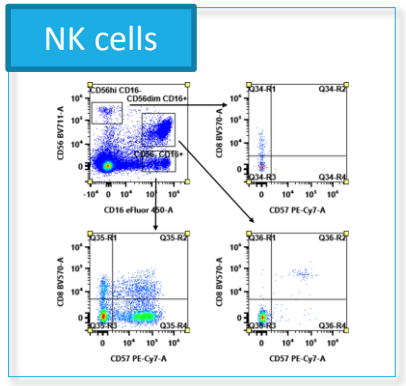
IFN-γ, TNF-α and GranB in spleen CD8+ T cells

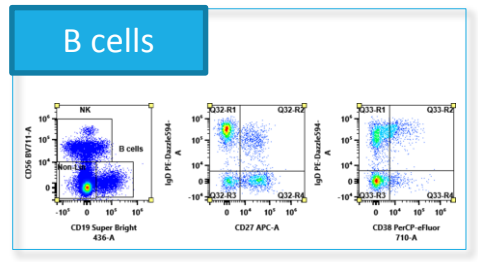


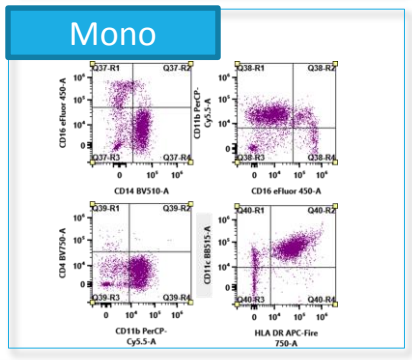
- Spleen cells from Naive C57 mice were stimulated with PMA and Ionomycin for 5 hrs or with coated-α-CD3 and α-CD28 for 72 hours. 4-6 hours before collection for FCM analysis, protein transport inhibitors were added, and intracellular cytokines in CD8+ T were analyzed by BD FACSCanto.

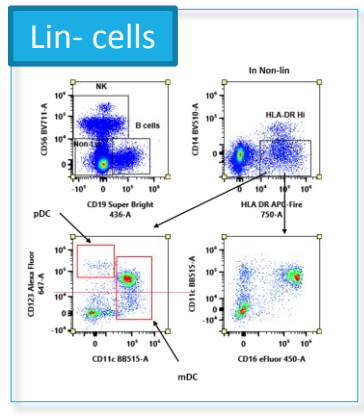
Analysis on major cell populations (24 markers) in peripheral blood by a single panel

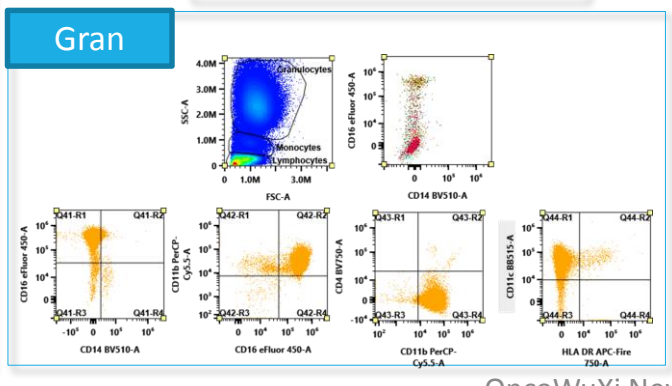


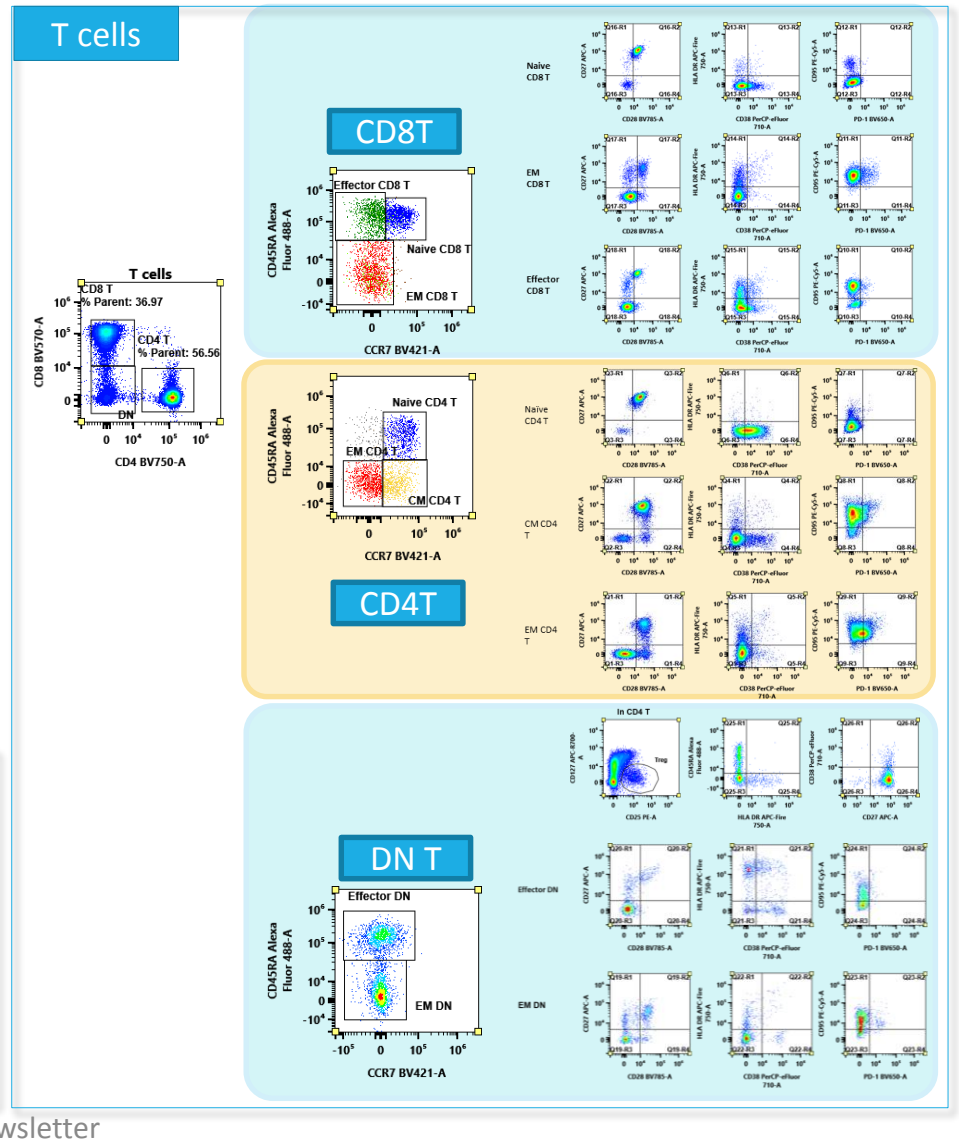






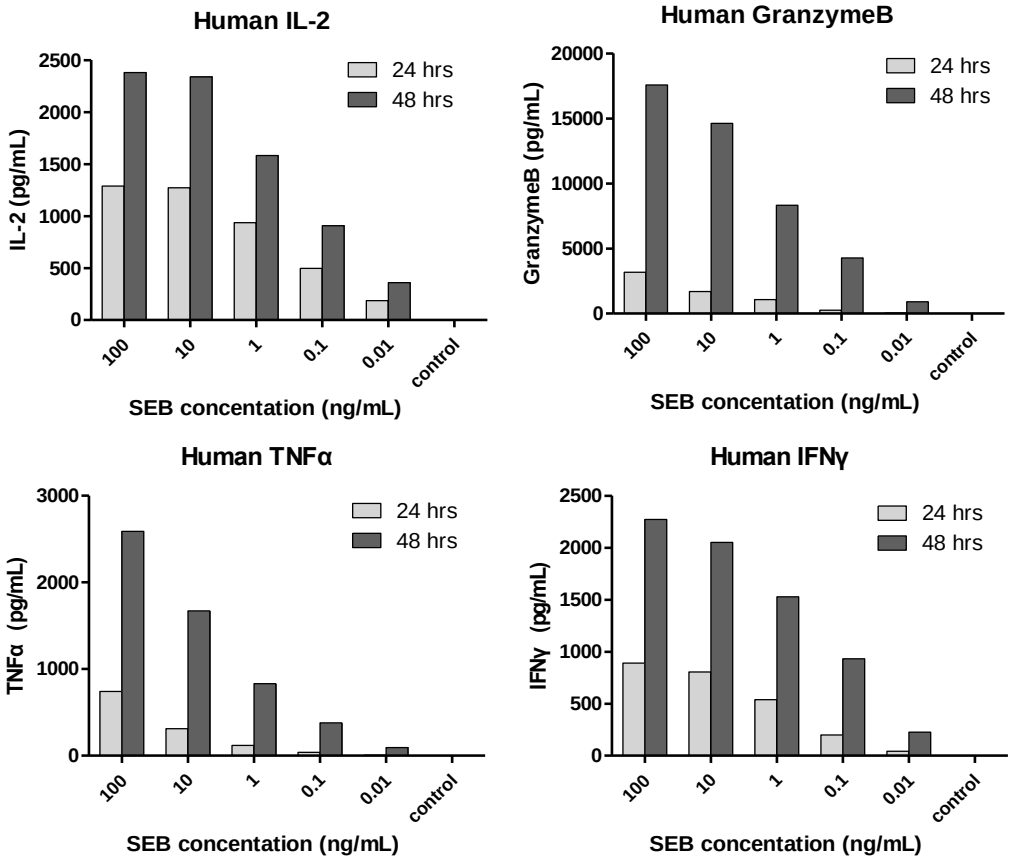






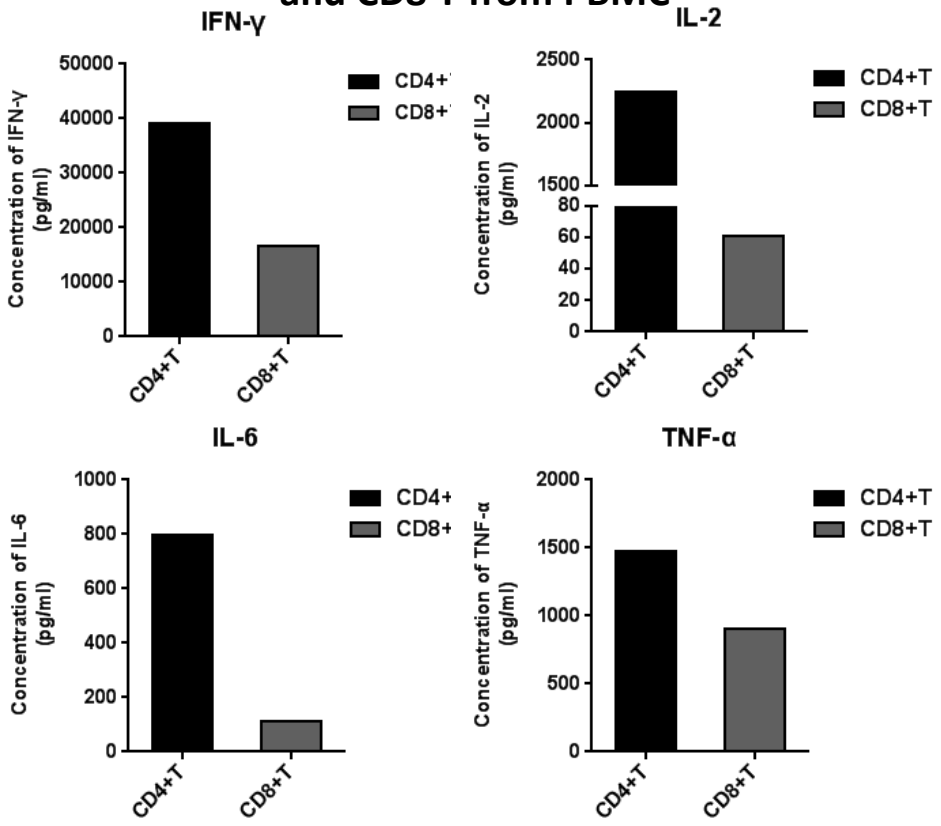
Secreted cytokine analysis by cytometric bead assay (CBA) and ELISA

Secreted Cytokine Analysis by CBA on SEB-stimulated PBMC



Human PBMCs were stimulated with 0 ~ 100ng/ml SEB for 24 hrs and 48 hrs, respectively. The secreted cytokines in the cell culture medium were quantified by CBA.

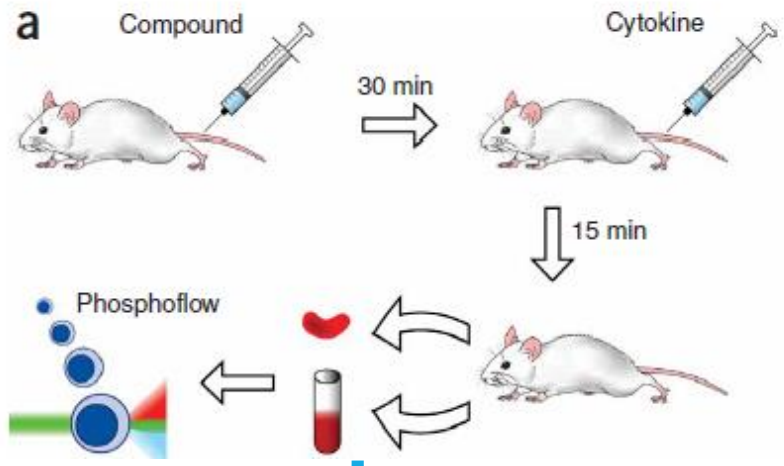
Secreted Cytokine Analysis by ELISA on sorted CD4 T and CD8 T from PBMC



PBMCs were sorted for CD4 T and CD8 T, stimulated with anti CD3 and anti CD28 antibodies. The secreted cytokines in the cell culture medium were quantified by ELISA.

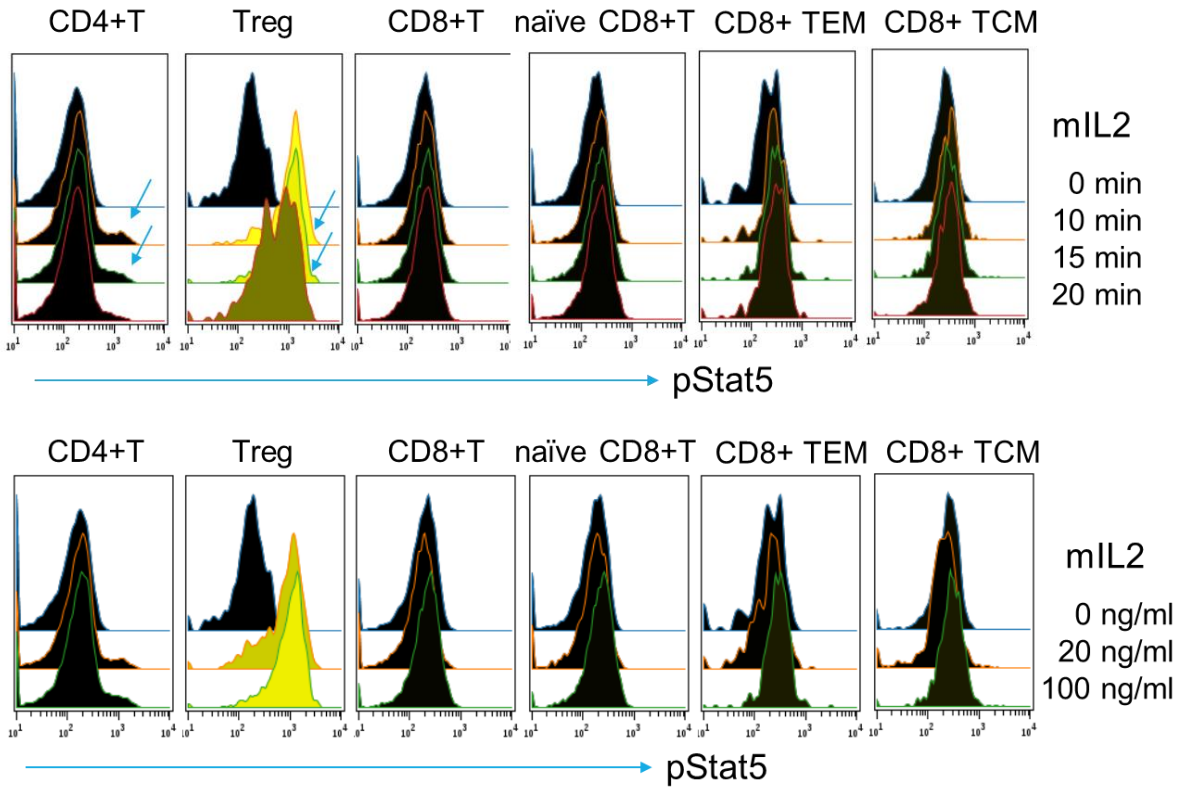
In Vivo Phosphoflow

Accurate and timely detection of changes in phosphorylation levels *in vivo*



Method Optimization

	Fixation	Ab Staining	Perm	Ab Staining
CD3				
CD4				
CD8				
…				
…				
…				
…				
…				
…				
…				

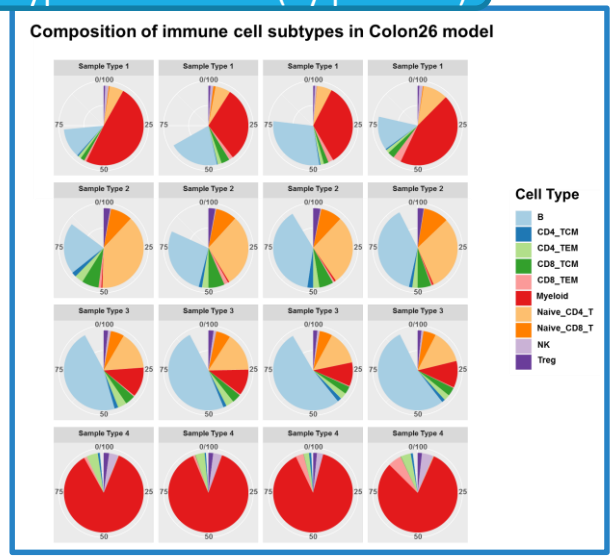


Due to the fast turnover of some phosphorylated protein, sample should be fixed within 5 minutes after stimuli *in vivo*. Methods were optimized for each phosphoflow. Optimization includes selection of markers, antibodies, fixative and permeabilization reagents, etc.

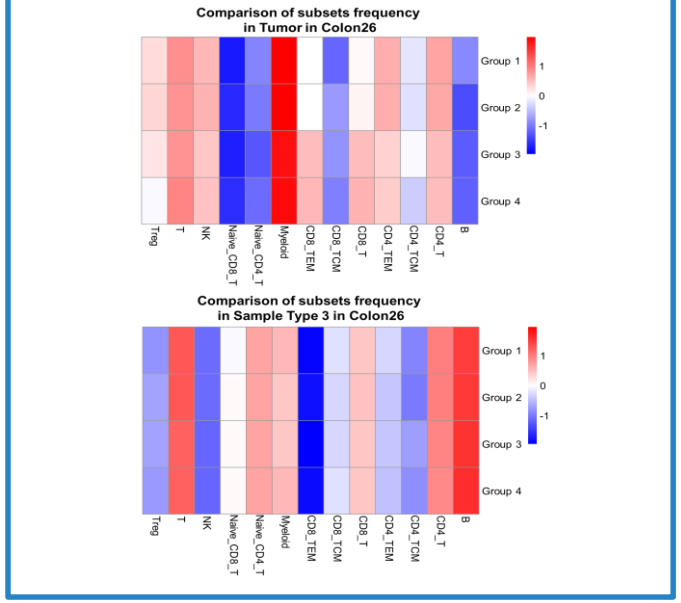
Flow Data Analysis

Covering from traditional gating to unsupervised learning

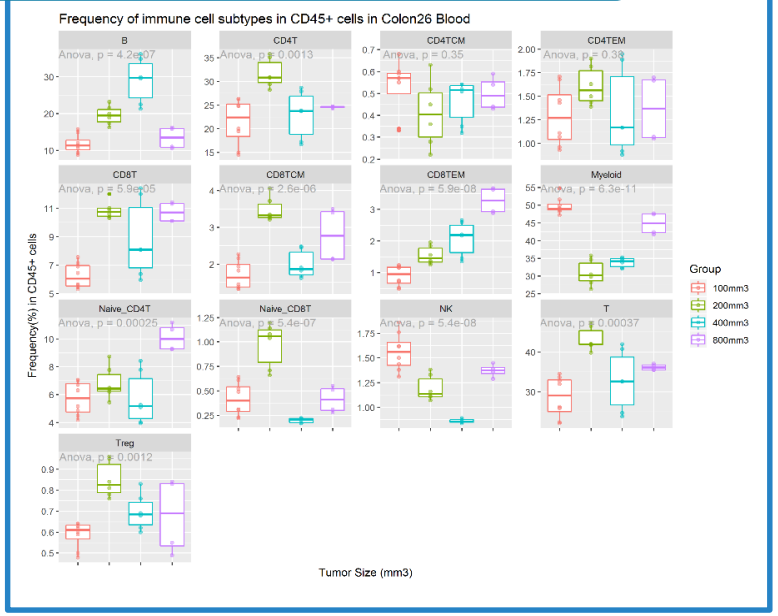
Composition of immune cell subtypes in Model 3 (by piechart)



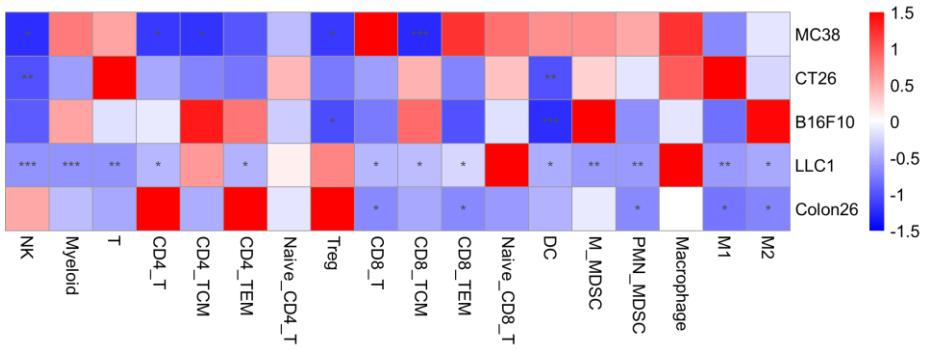
Comparison of immune cells in different groups (by heatmap)



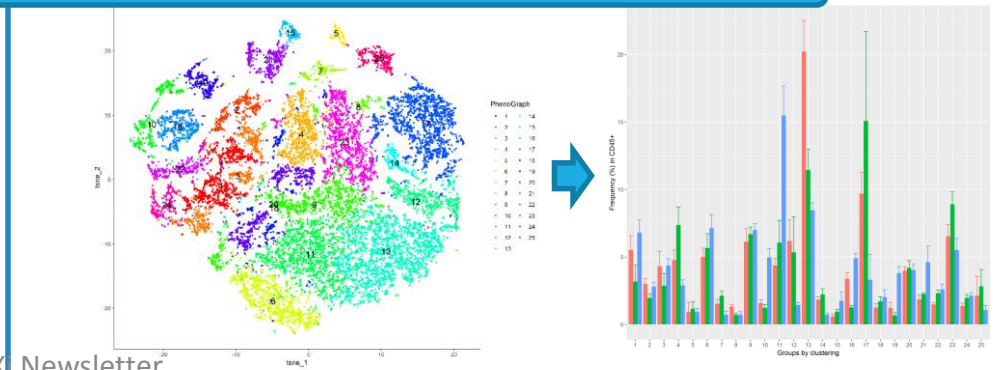
Comparison of immune cell subtypes in different groups (by boxplot)



Comparison of response in different models



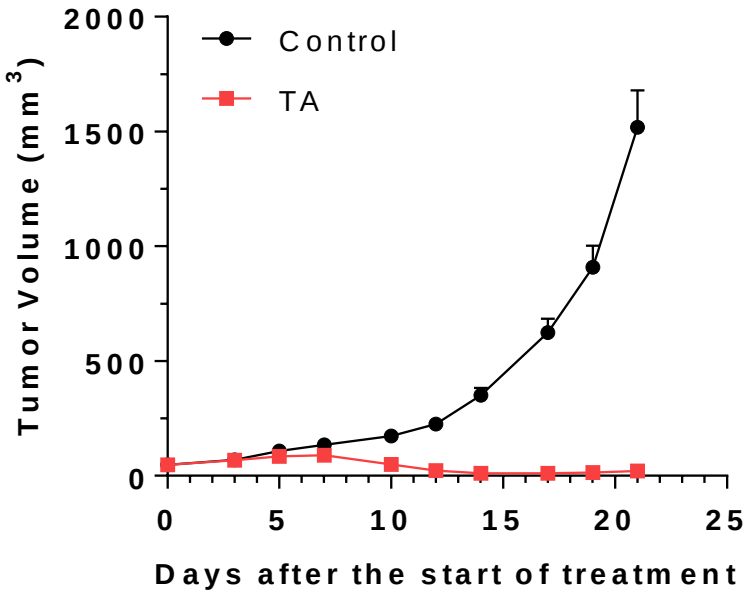
Dimensional reduction by tSNE and statistical analysis



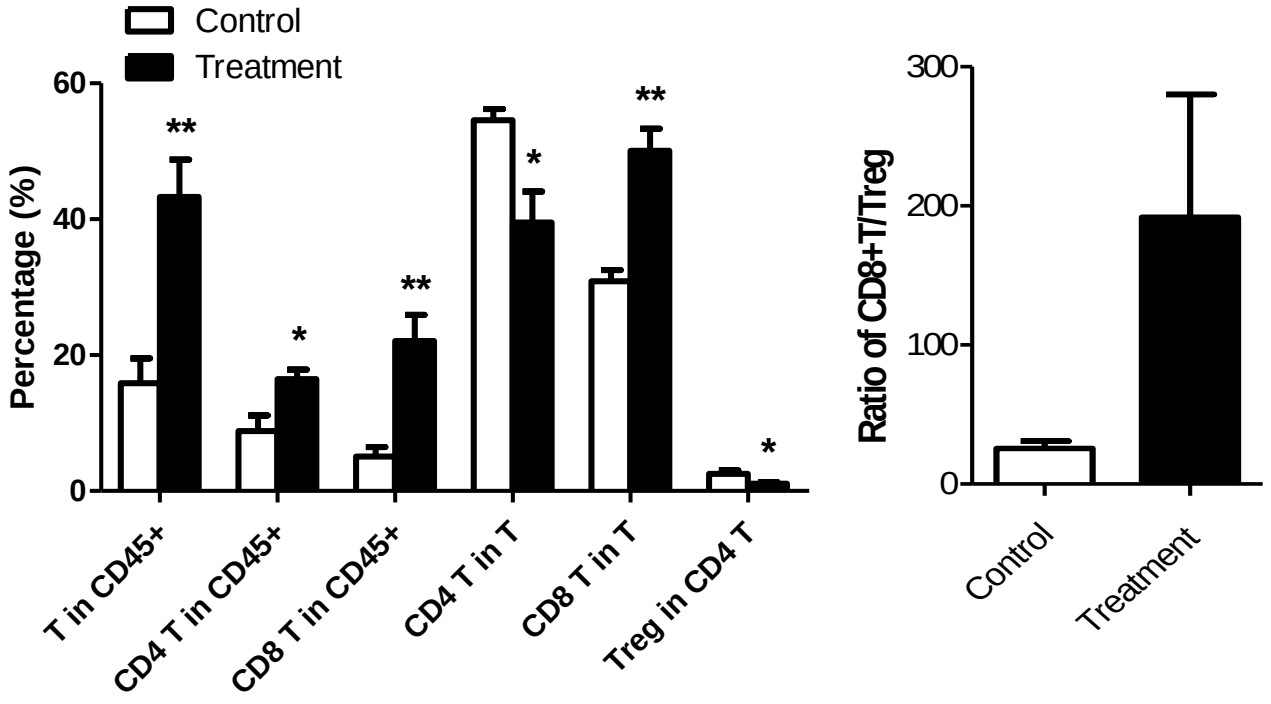
TIL analysis after checkpoint inhibitor blockade treatment

(MC38 in humanized mouse model)

***In vivo* efficacy**



TIL FACS analysis



MC38 model was established in human gene KI mice. Mice were treated with a single dose of control antibody or a checkpoint inhibitor and TILs were analyzed by FACS 5 days later.

Student's t-test was used for statistical analysis.

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

Case study: verification of driver genes by flow cytometry

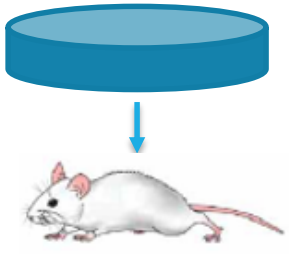
Target gene that involved in immunotherapy was found by CRISPR

Cell line engineering

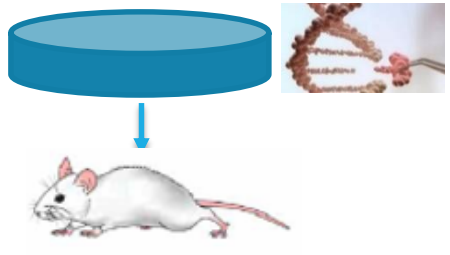
Mouse model establishment and efficacy study

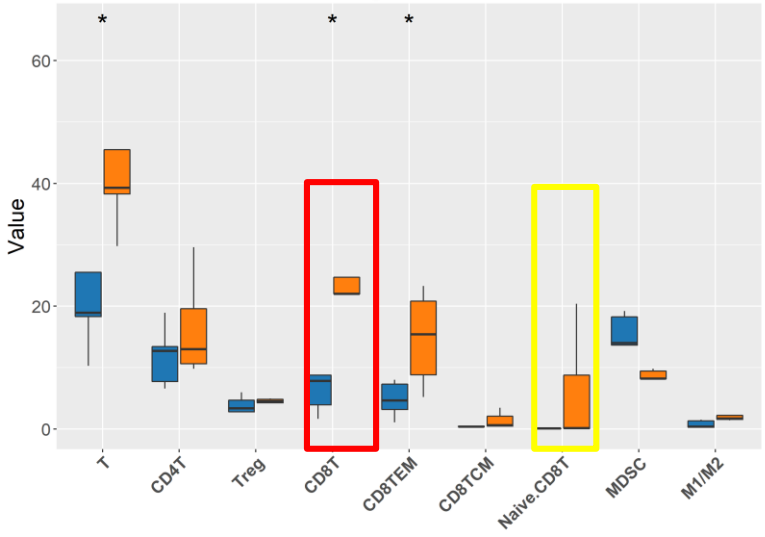
Analysis by flow cytometry

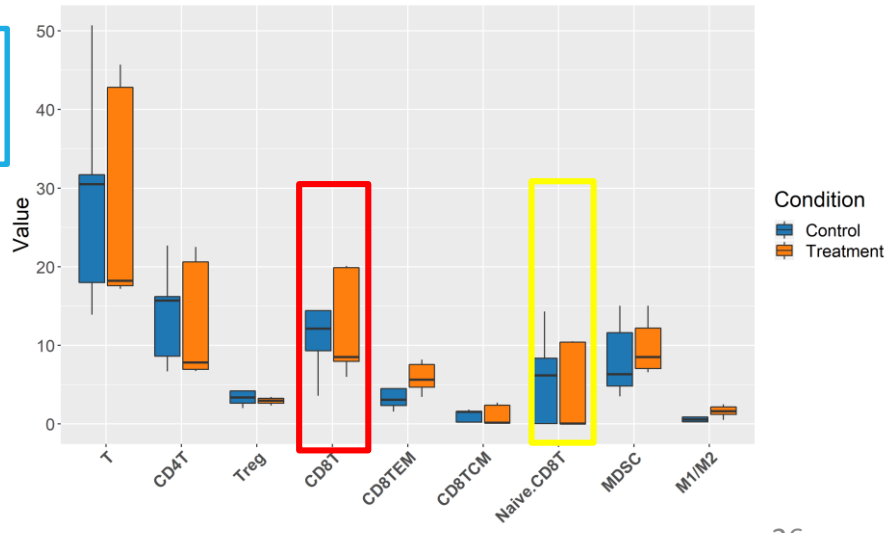
Cell line with wild type target gene



Cell line with mutated/ dysfunctional target gene







Clinically relevant flow cytometry capabilities

- **TBNK (T, B, NK cells) cell numeration in human peripheral blood (CD3, CD4, CD8, CD16/CD56, CD19, CD45; 6-color analysis), FDA-approved test**
- **PD/PK assay for a CAR-T/TCR-T clinical study, LDT**
- **Receptor occupancy assay, LDT**
- **Biomarker detection (surface, intracellular), LDT**
- **Phospho-biomarker detection, LDT**
- **Immune-phenotyping in peripheral blood (T subset, myeloid subset, biomarkers), LDT**
- **Tumor microenvironment immuno-phenotyping, Research**

LDT: lab developed test

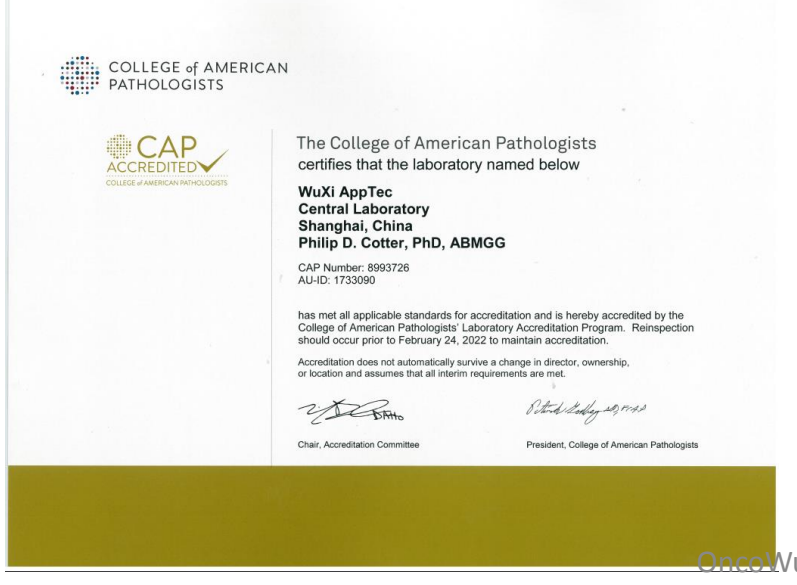
CAP-certified lab introduction

OIU - Oncology and Immunology Unit in WuXi AppTec

Fully compliant with CAP and GCP regulations on PHI (patient health information), clinical testing and sample management, data traceability and accuracy requirements. Passed the 2020 CAP re-inspection with zero defect.

CAP

The College of American Pathologists (CAP), the leading organization of board-certified pathologists, serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide.


COLLEGE of AMERICAN PATHOLOGISTS

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

The College of American Pathologists
certifies that the laboratory named below

WuXi AppTec
Central Laboratory
Shanghai, China
Philip D. Cotter, PhD, ABMGG

CAP Number: 8993726
AU-ID: 1733090

has met all applicable standards for accreditation and is hereby accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to February 24, 2022 to maintain accreditation.

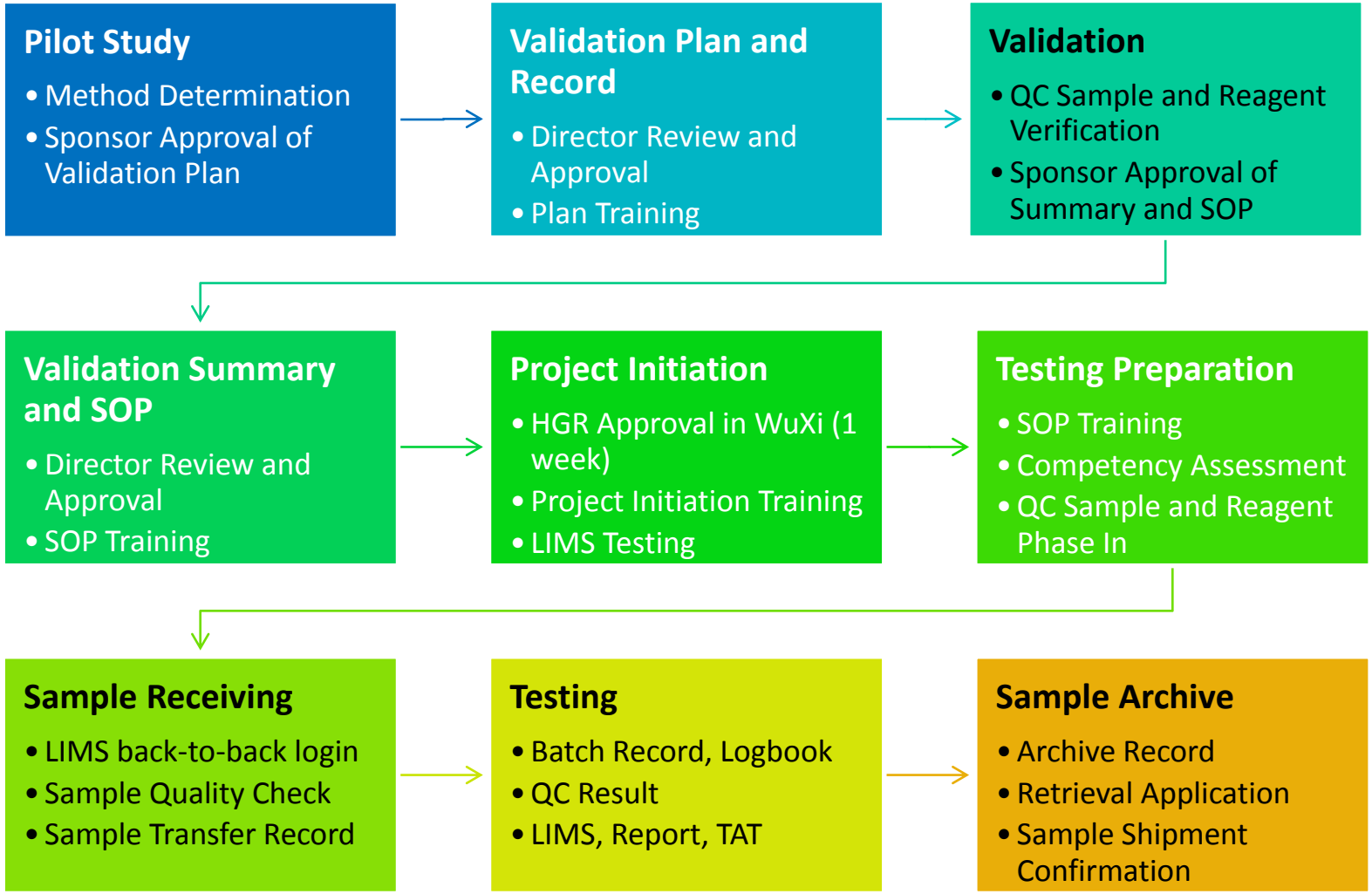
Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Chair, Accreditation Committee

President, College of American Pathologists

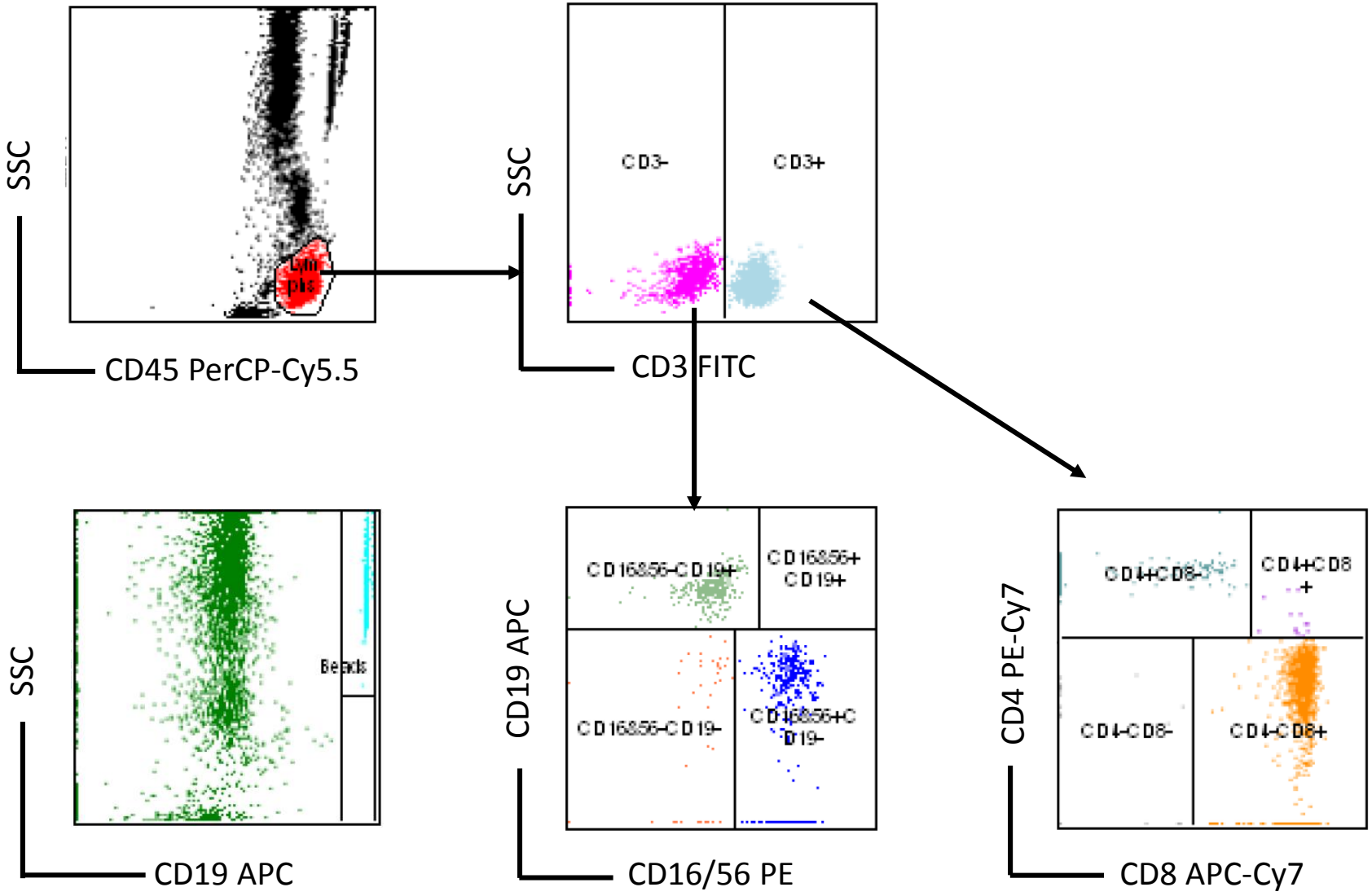
- Regular Proficiency Test from CAP
- Annual Inspection and Audit
- Quarterly Lab Inspection
- Monthly Lab Activity Review
- Monthly Documentation Review
- Equipment Quality Management

Clinical project workflow

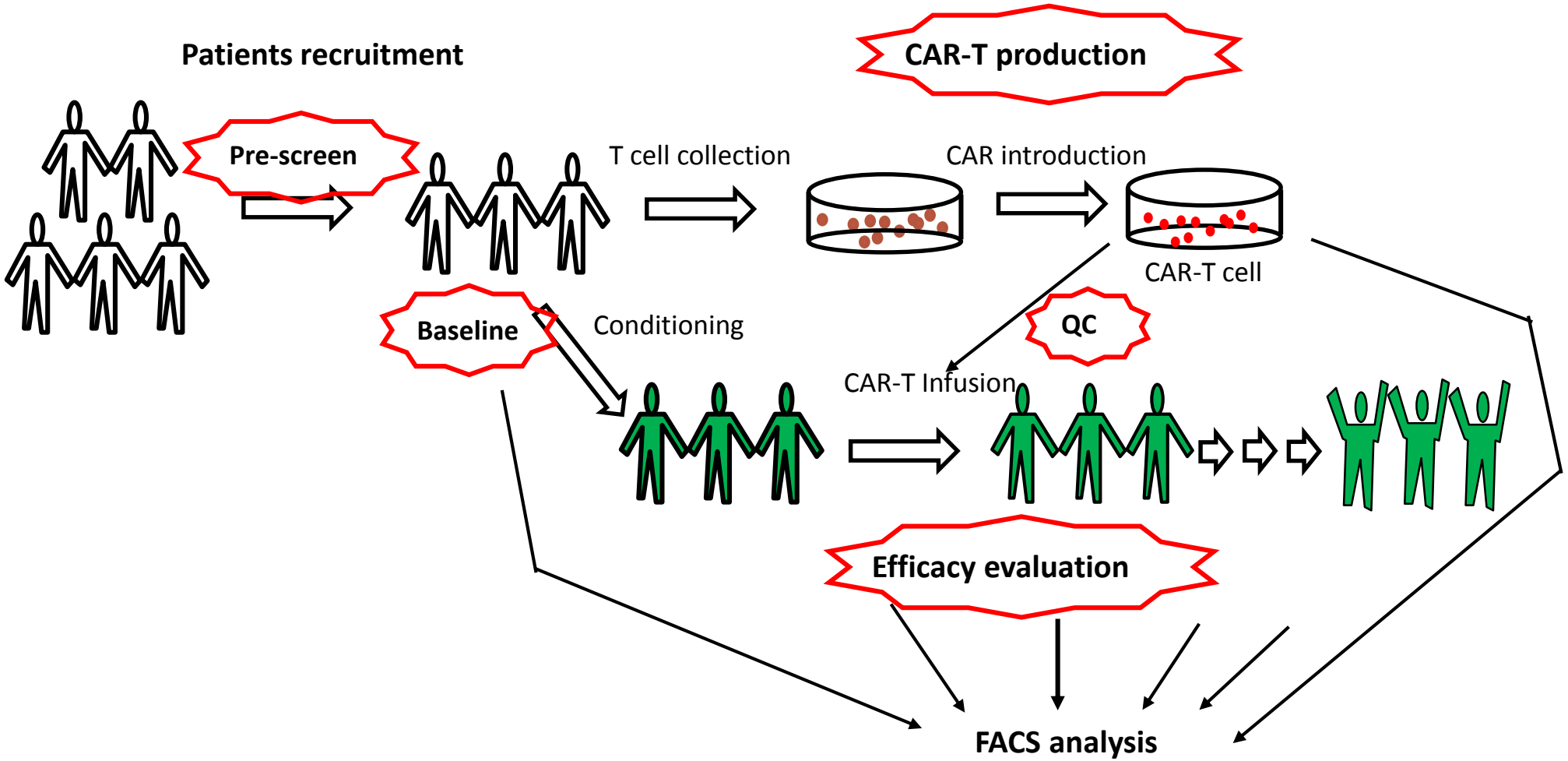


TBNK (T, B, NK cells) cell numeration in human peripheral blood

Case study: 6-Color human PBMC TBNK cell numeration assay

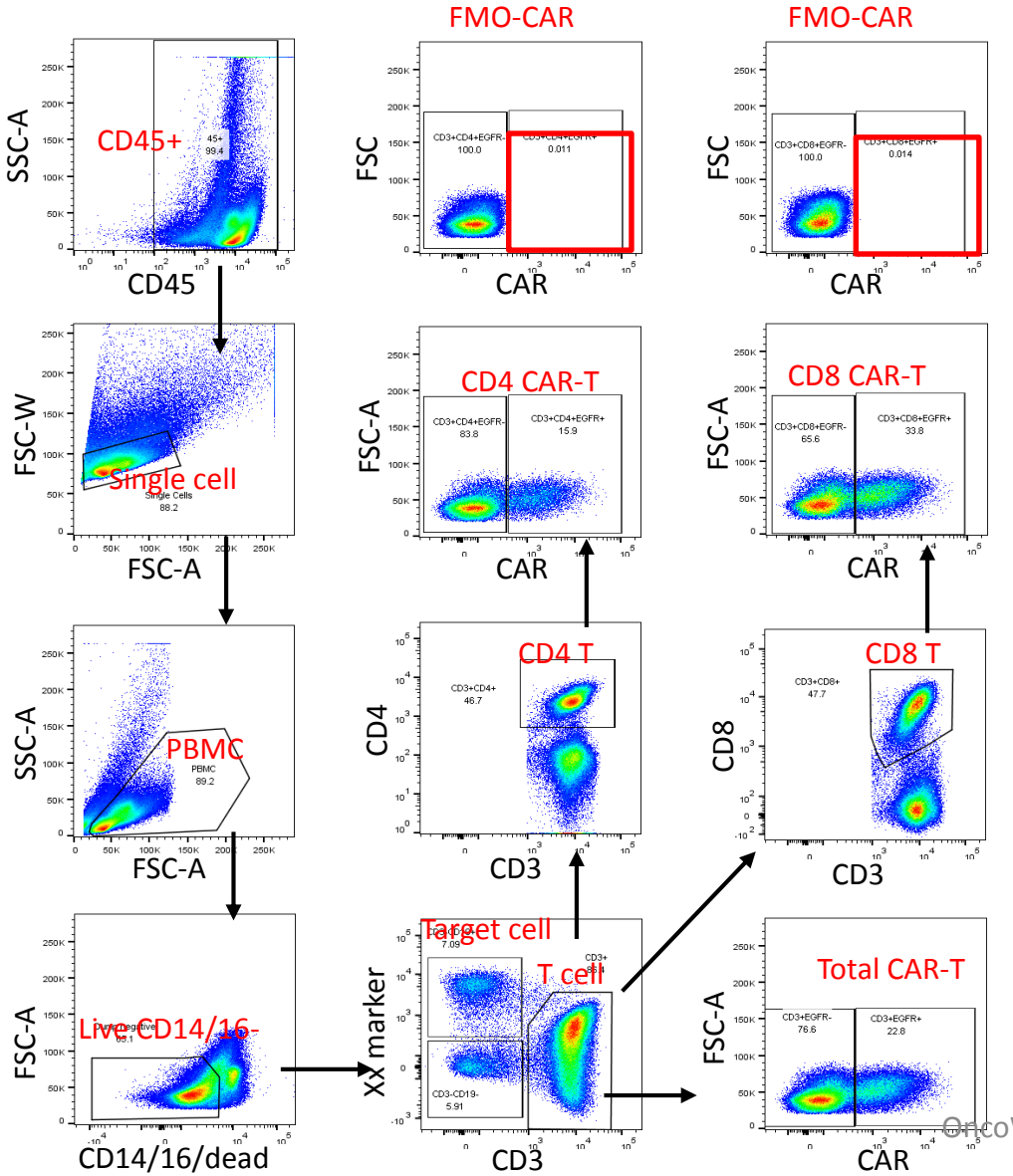


FACS analysis to support CAR-T clinical trials

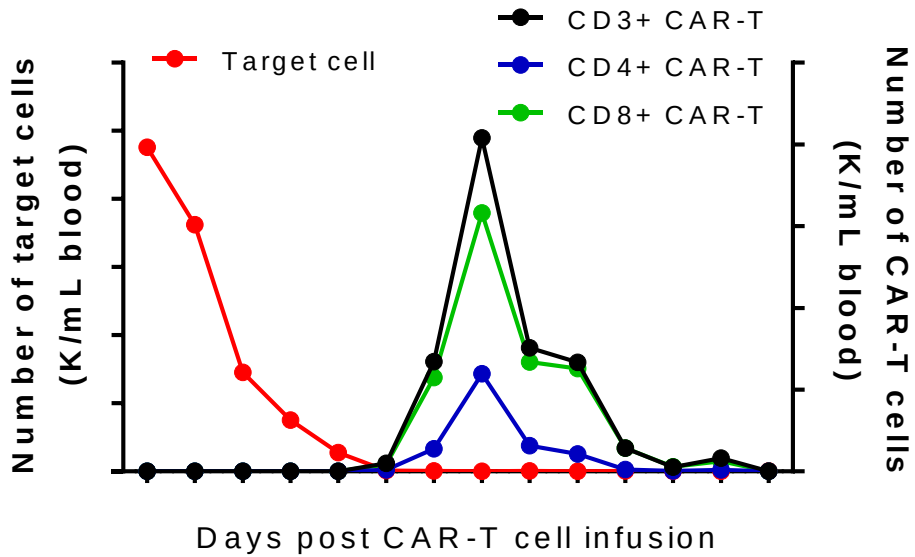


FACS analysis to support CAR-T clinical trials

Case study: PD/PK assay for a CAR-T clinical study (7-color analysis)

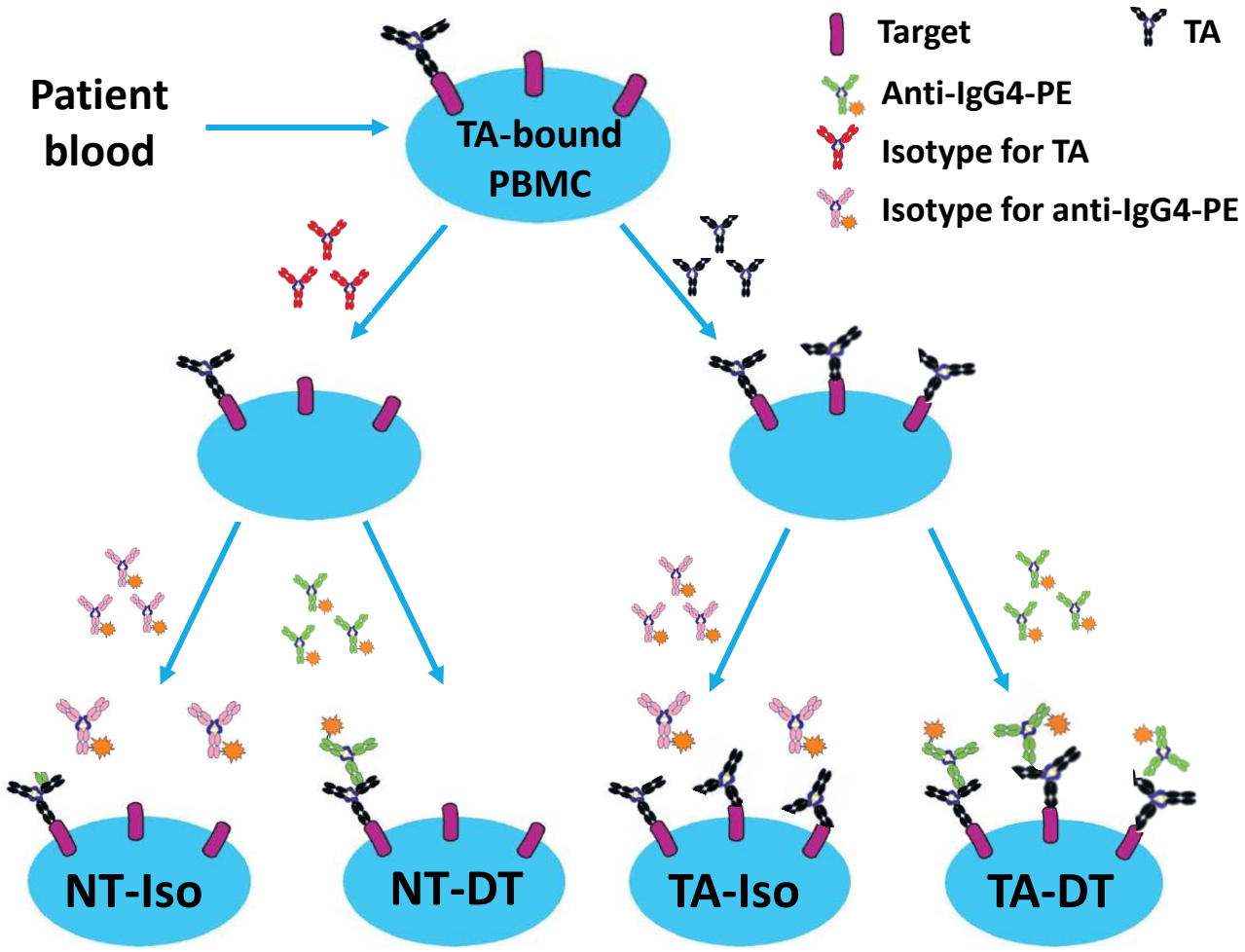


Compiled analysis for 1 patient



Receptor occupancy assay

Procedure

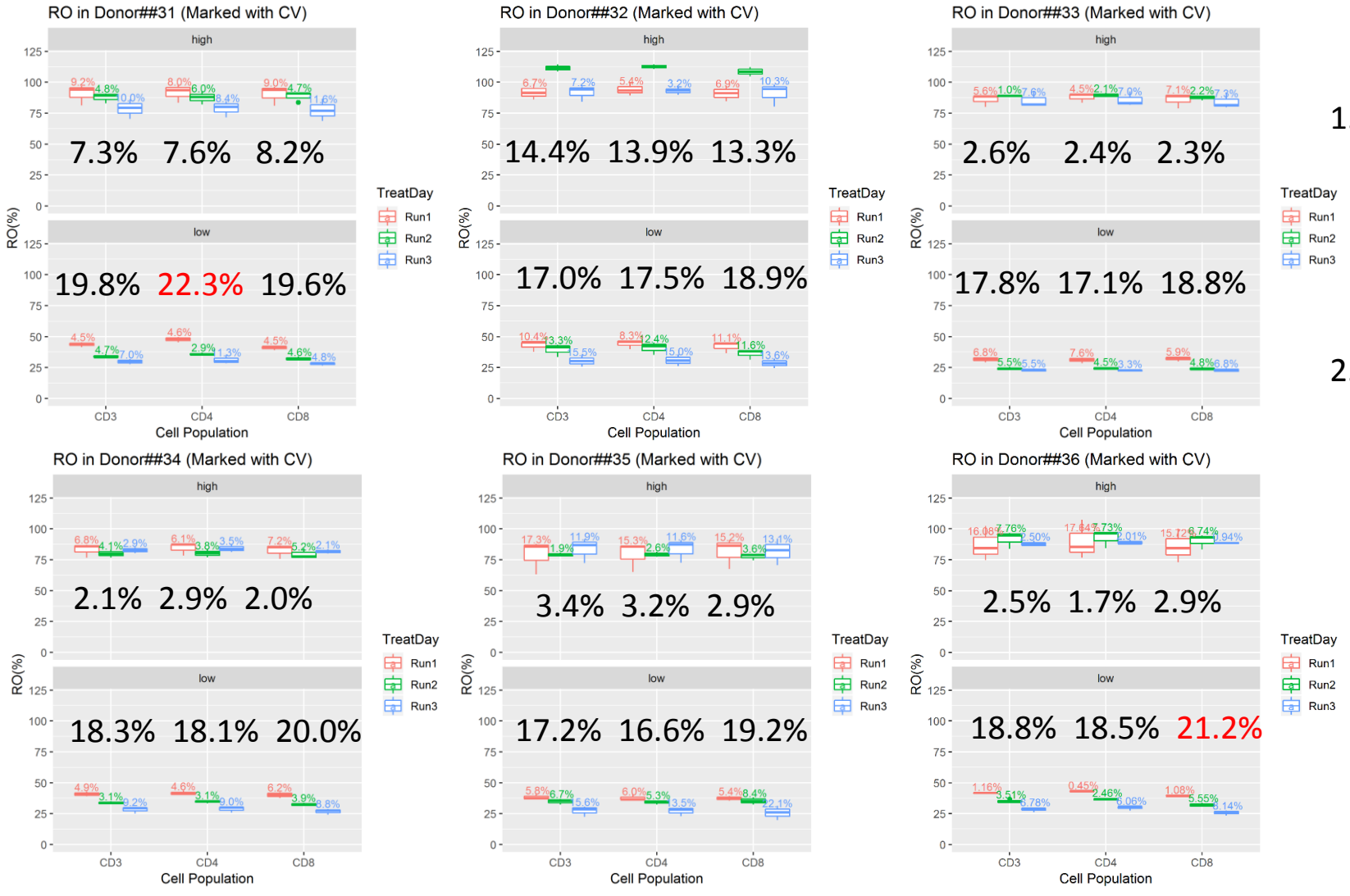


1. PBMCs are purified from whole blood collected from patient.
2. PBMCs are incubated with isotype antibody for TA or with saturation concentration of TA.
3. PBMCs are then detected with detection antibody or isotype detection antibody.

$$RO = \frac{NT_{(DT)} - NT_{(Iso)}}{TA_{(DT)} - TA_{(Iso)}}$$

Receptor occupancy assay

Validation results _ Precision

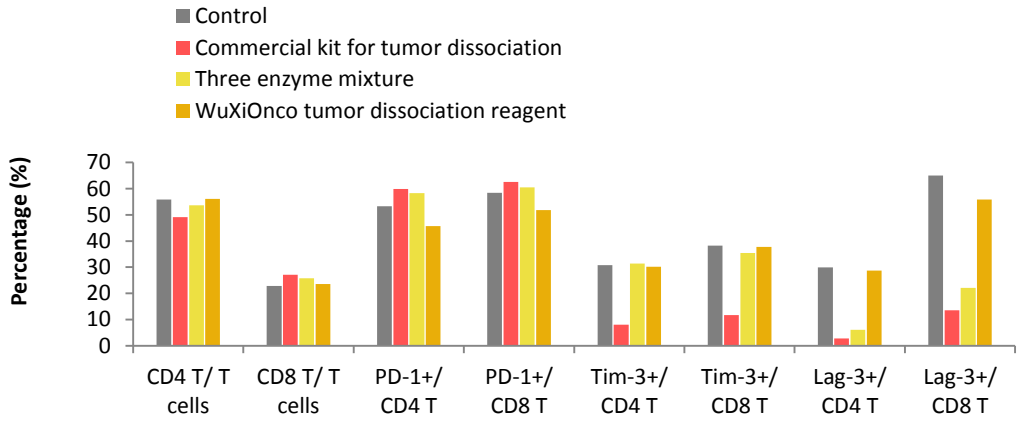


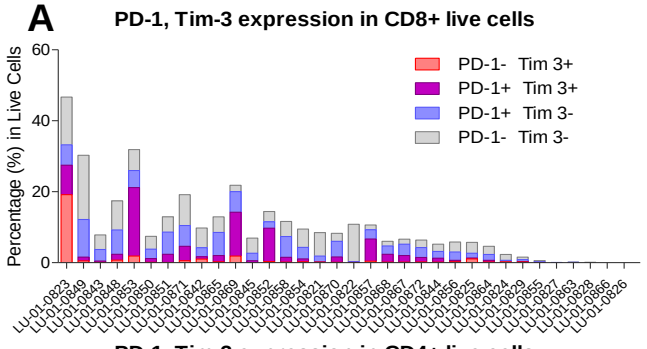
1. Triplicate tubes were prepared for each treatment. The same experiment was performed 3 times (Run1, Run2 and Run3) and inter-run CVs and inter-run CVs were calculated.
2. 6 PBMCs were used for this experiment

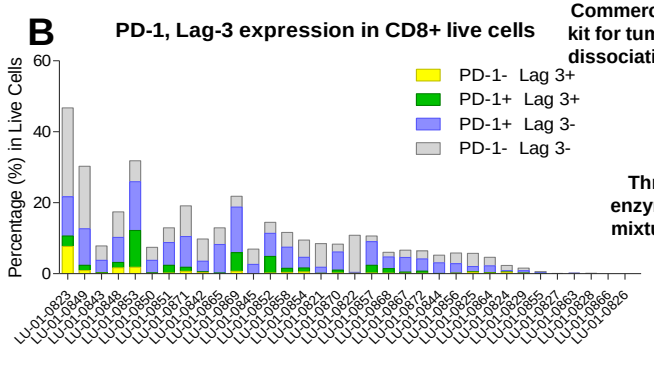
The inter-run CVs for only 2 out of 108 data points are >20%.

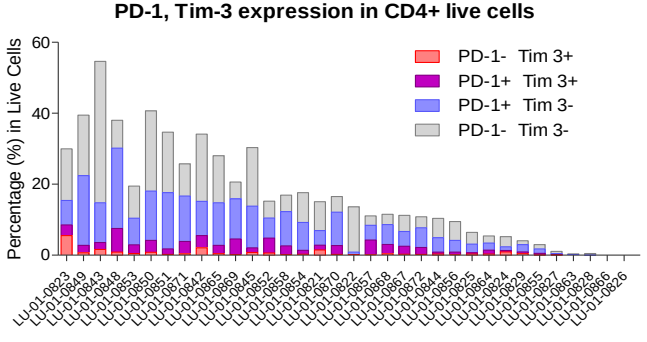
Inter-run CVs are labeled with bigger front size letters;
Intra-run CVs are labeled with smaller front size and colored letters.

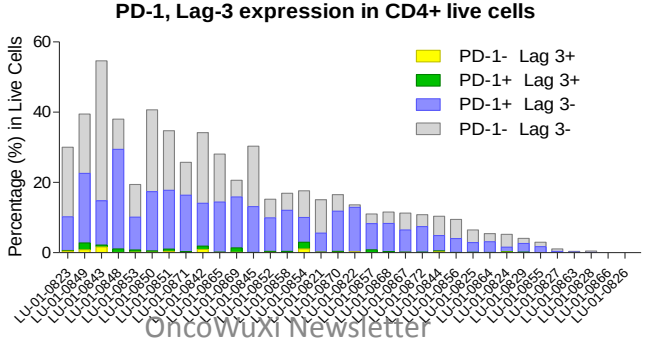
Immune-profiling and biomarker detection in lung cancer PDX model tumor tissue

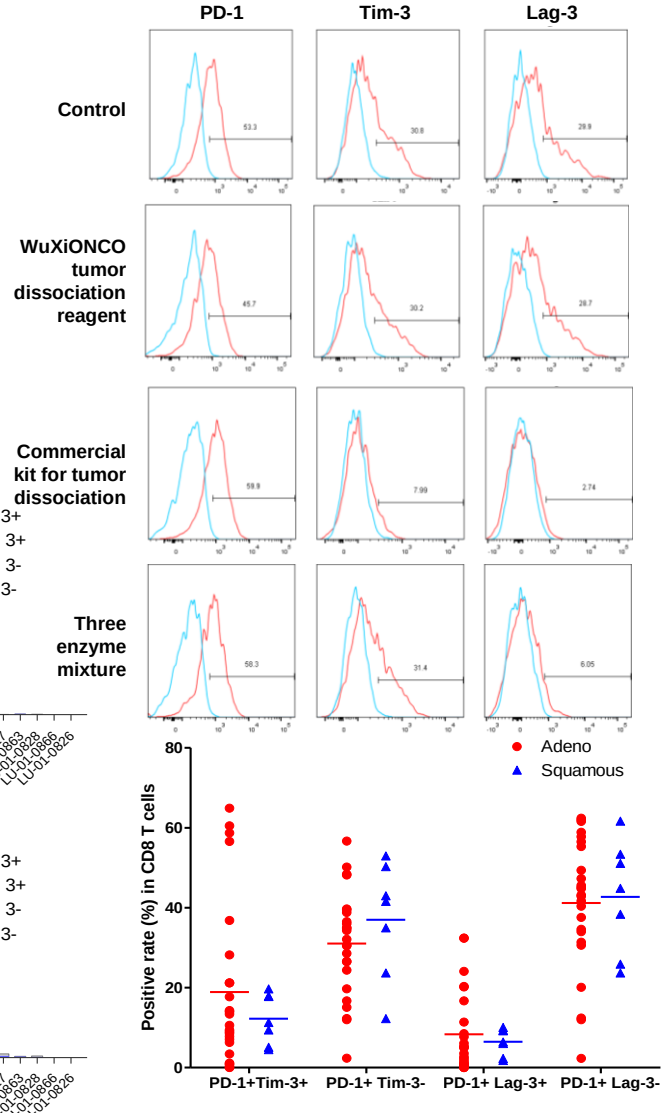












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