Flow Cytometry Platform for Preclinical Studies and Discovery

WuXi AppTec Research Service Division, Oncology & Immunology Unit

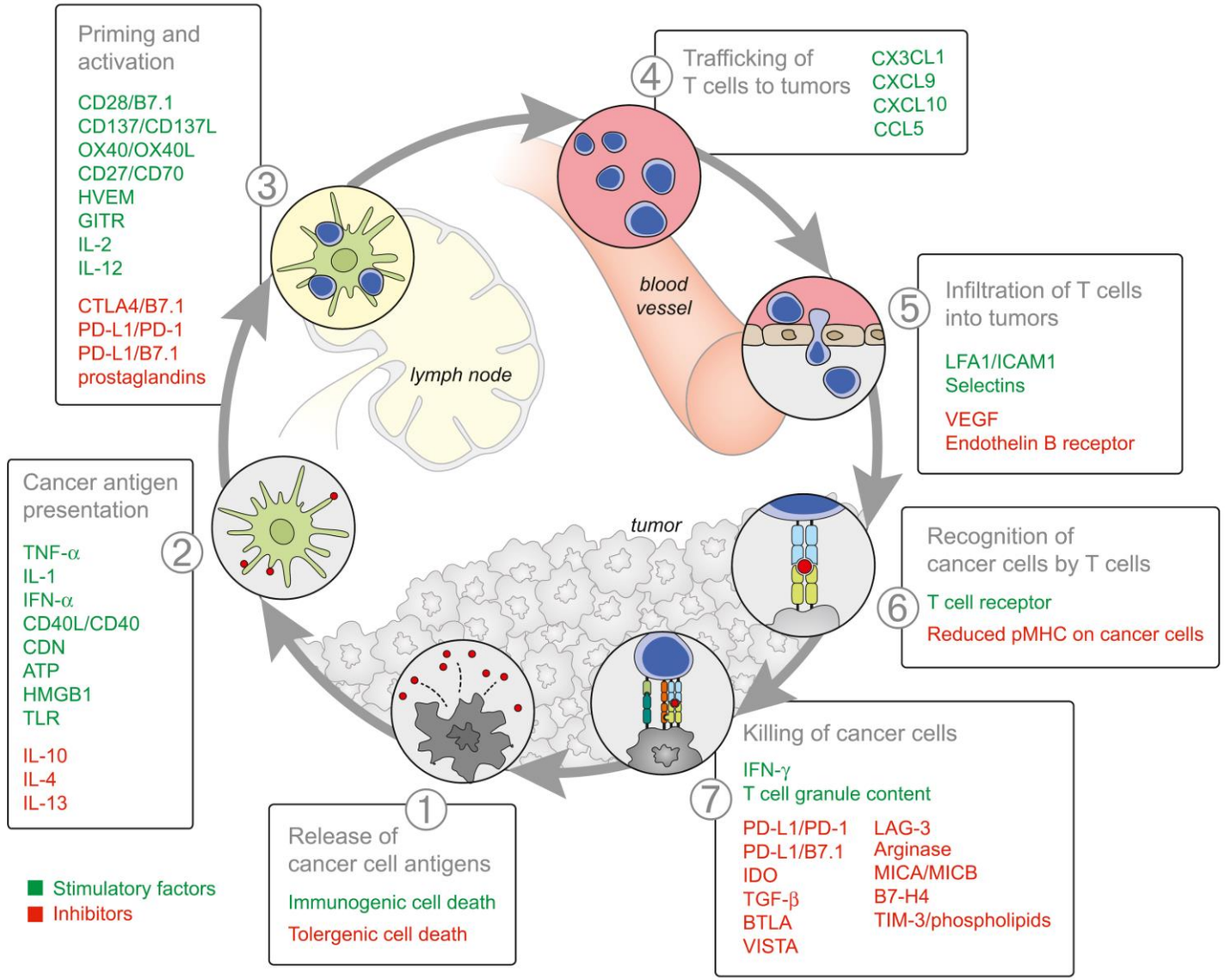
2021.05

OncoWuXi Newsletter

Outline

- **Immuno-oncology background**
- **Flow cytometry lab facility**
- **Flow cytometry lab services**
  - TIL immune profiling, biomarkers and baseline data
  - Functional assay by cell-specific intracellular cytokine analysis
  - Multiplex analysis of secreted cytokine/chemokine by CBA
  - *In vitro* and *in vivo* phosphoflow
  - Multi-dimensional data mining of flow data
- **Case studies**
  - TIL analysis after checkpoint inhibitor blockade treatment
  - Verification of driver genes by flow cytometry
  - Immune-profiling and biomarker detection in tumor tissue across various cancer types

The Cancer-Immunity Cycle



Immunity 39, July 25, 2013

Using flow cytometry to track the change /response in each of the seven steps in cancer-immunity cycle :

- cancer development
- immune response
- drug PD
- status (activation /suppression) of a specific cell type
- biomarker expression

WuXi OIU flow cytometry lab 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)

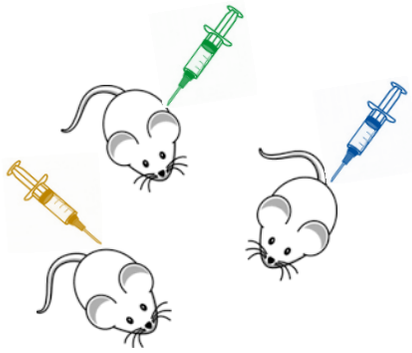
BD FACSCanto plus
(3 Laser, 10 channels)

BD LSRFortessa X20
(5 Laser, 18 channels)

Cytek Aurora
(3 Laser, > 24 colors)

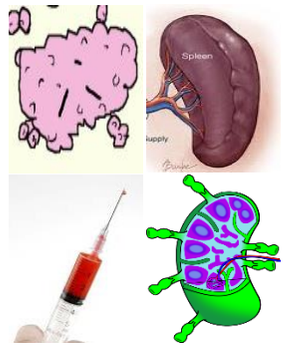
Mouse TIL analysis workflow after *in vivo* drug efficacy study

In vivo efficacy study with syngeneic or humanized models



Collect tissue at different time points

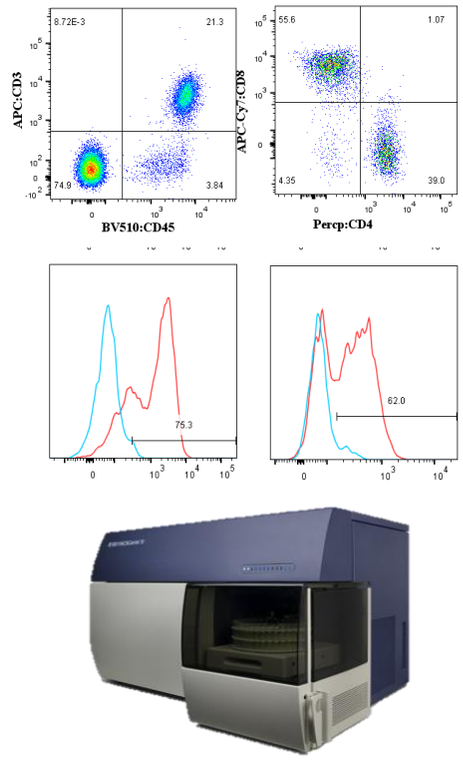
Tumor Spleen



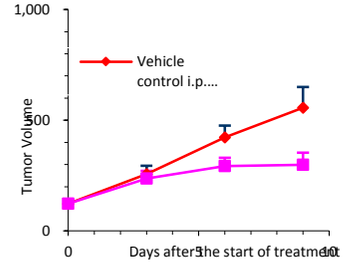
PBMC Lymph node

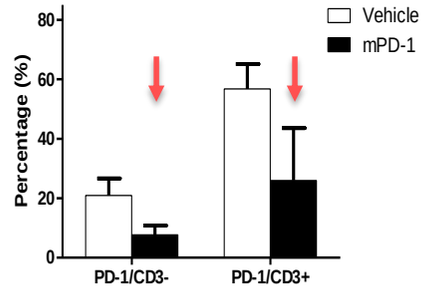
(Optional)
FFPE
TCRseq
GEP

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)

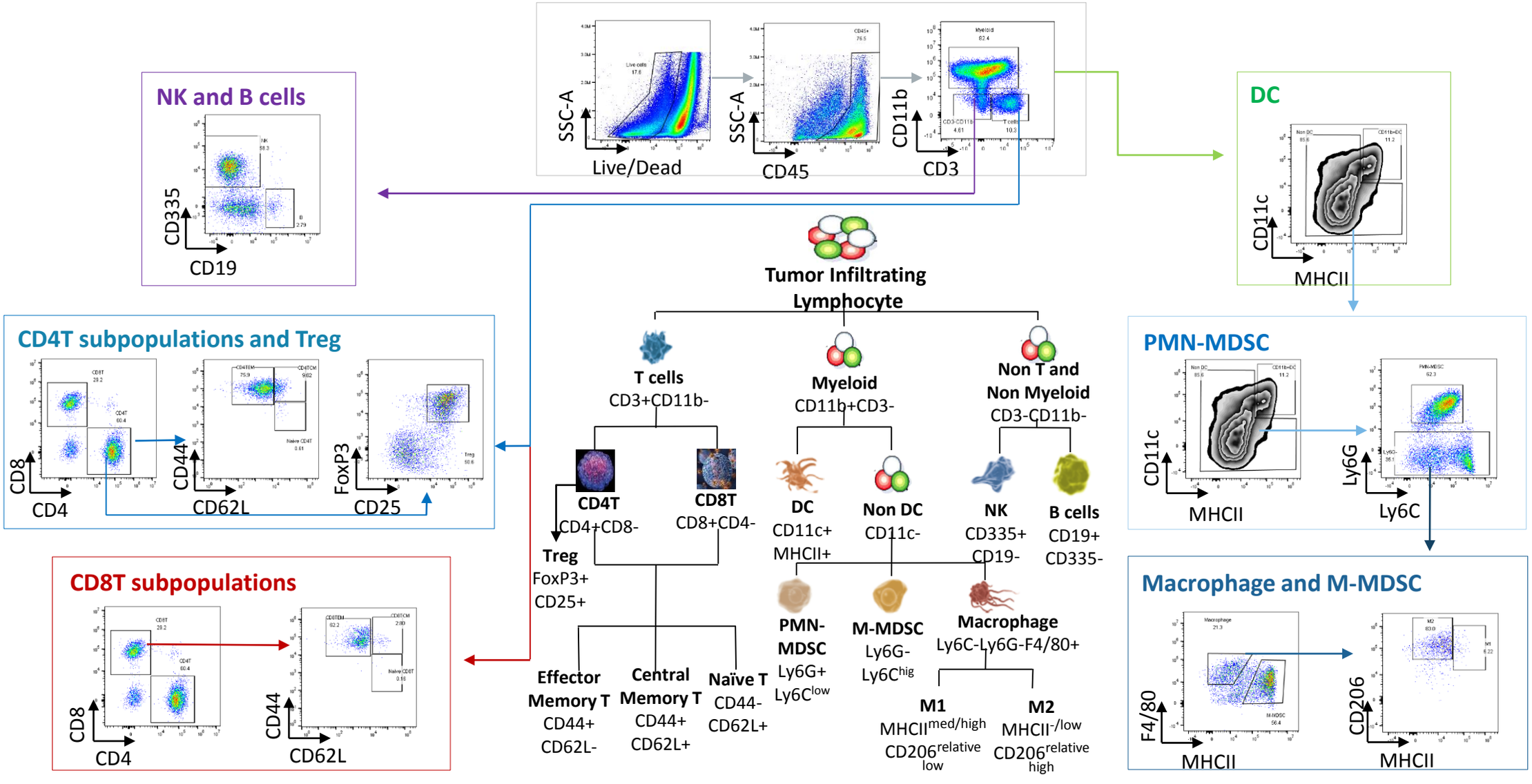
Population	Marker signature	Population	Marker signature
T cell	$CD3^+$	panNK	$CD49b(DX5)^+$ or $CD335^+$
CD4 T cell	$CD3^+$ $CD4^+$ $CD8^-$	panDC	$CD11c^+$
CD8 T cell	$CD3^+$ $CD4^-$ $CD8^+$	Myeloid	$CD3^-$ $CD11b^+$
Treg	$CD3^+$ $CD4^+$ ($CD25^+$) $Foxp3^+$	MDSC	$CD3^-$ $CD11b^+$ $Gr1^{+/mid}$
Naïve 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 to support flexible design

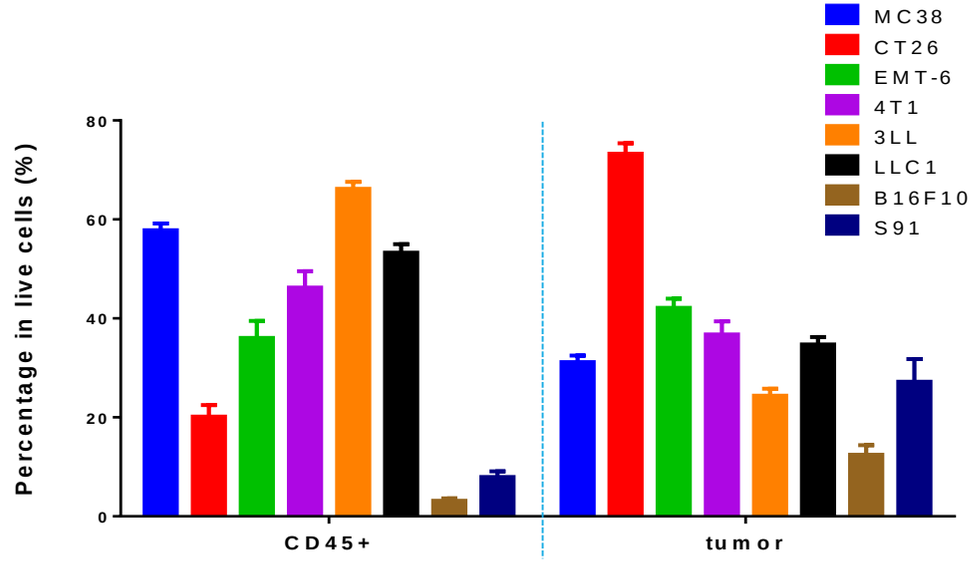
Panels	Purpose	Cytometer
Option1	Panel focus on lymphocyte and biomarkers	Fortessa
Option2	Panel for major cell types and biomarkers	Fortessa
Option3	Panel focus on myeloid cells	Fortessa
Option4	Panel for major cell types and biomarkers	Canto
Option5	Panel for myeloid cells, lymphocyte and biomarkers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

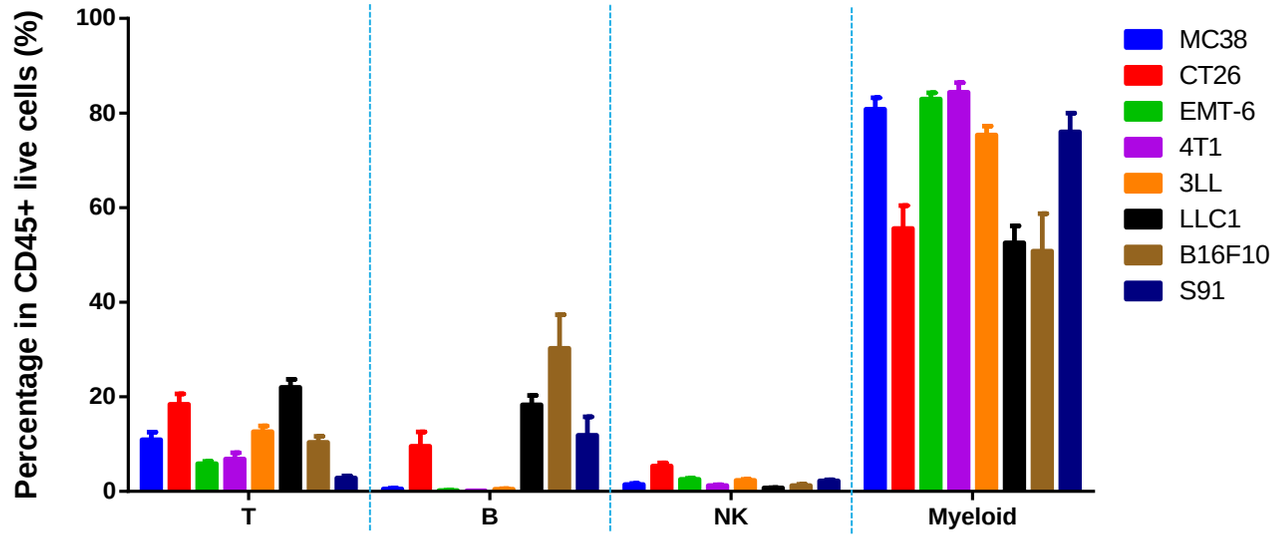
	Option1	Option2	Option3	Option4	Option5	Option6
1	CD8				L/D	L/D
2	CD4				CD45	CD45
3	CD44				CD3	CD3
4		L/D	L/D	L/D	CD4	CD4
5	L/D	CD8	CD8	CD8	CD8	CD8
6	CD19	CD19	MHCII	CD19	FoxP3	FoxP3
7	CD62L		Ly6C		CD25	CD25
8	CD11b	CD11b	CD11b		CD335	CD335
9	CD335	CD335	F4/80	CD335	CD19	CD19
10	PD-L1	PD-L1	CD11c	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

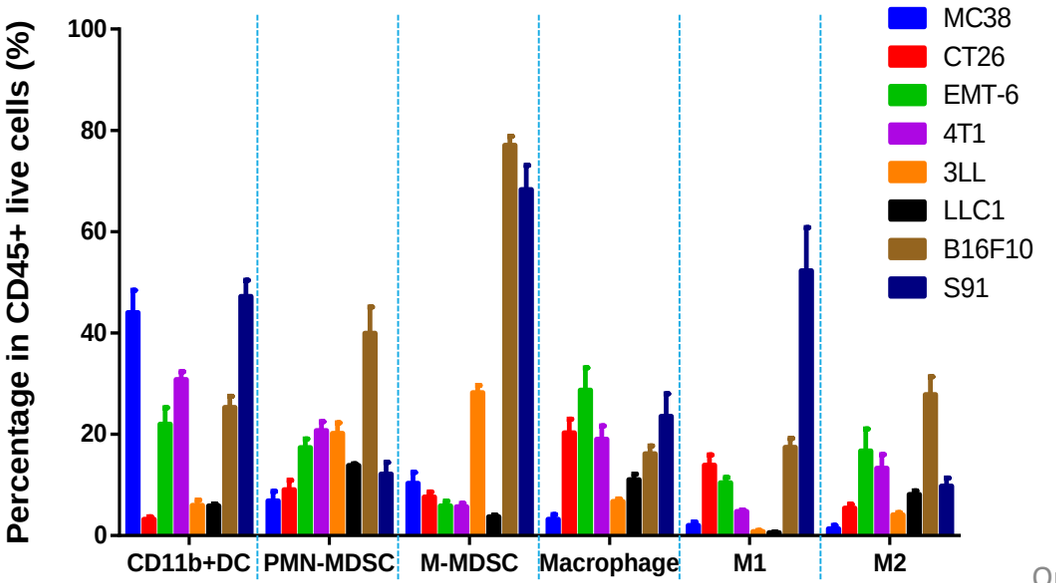
Immune-profiling by flow cytometry in a single panel

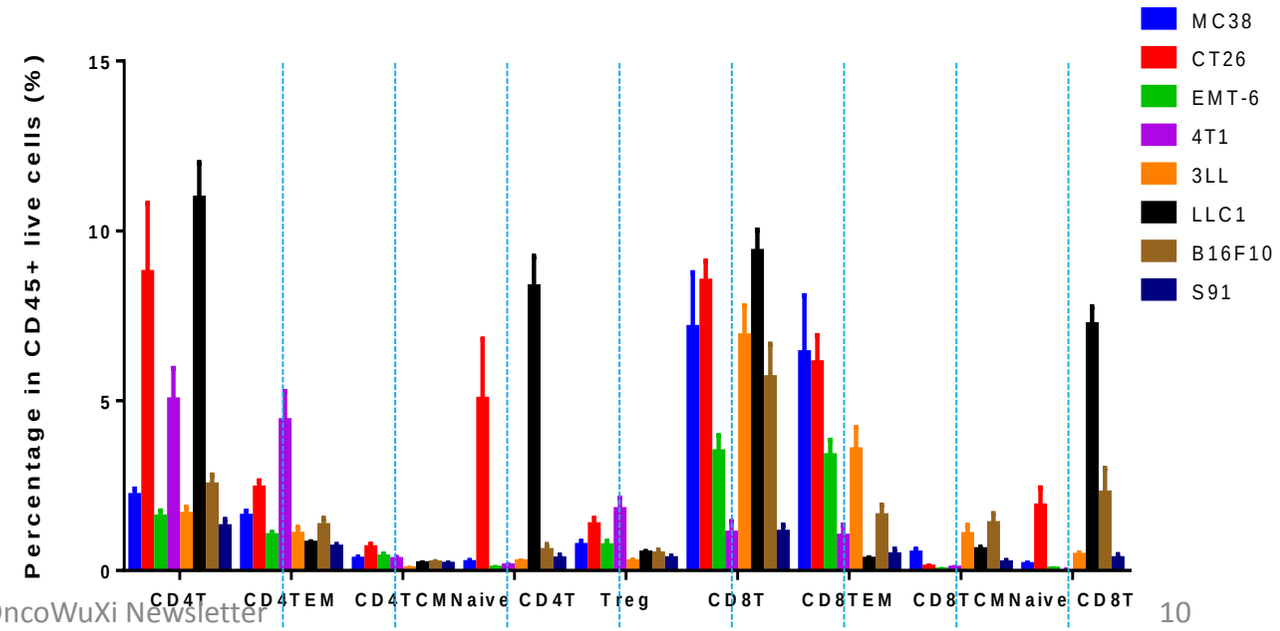


TIL baseline of syngeneic models



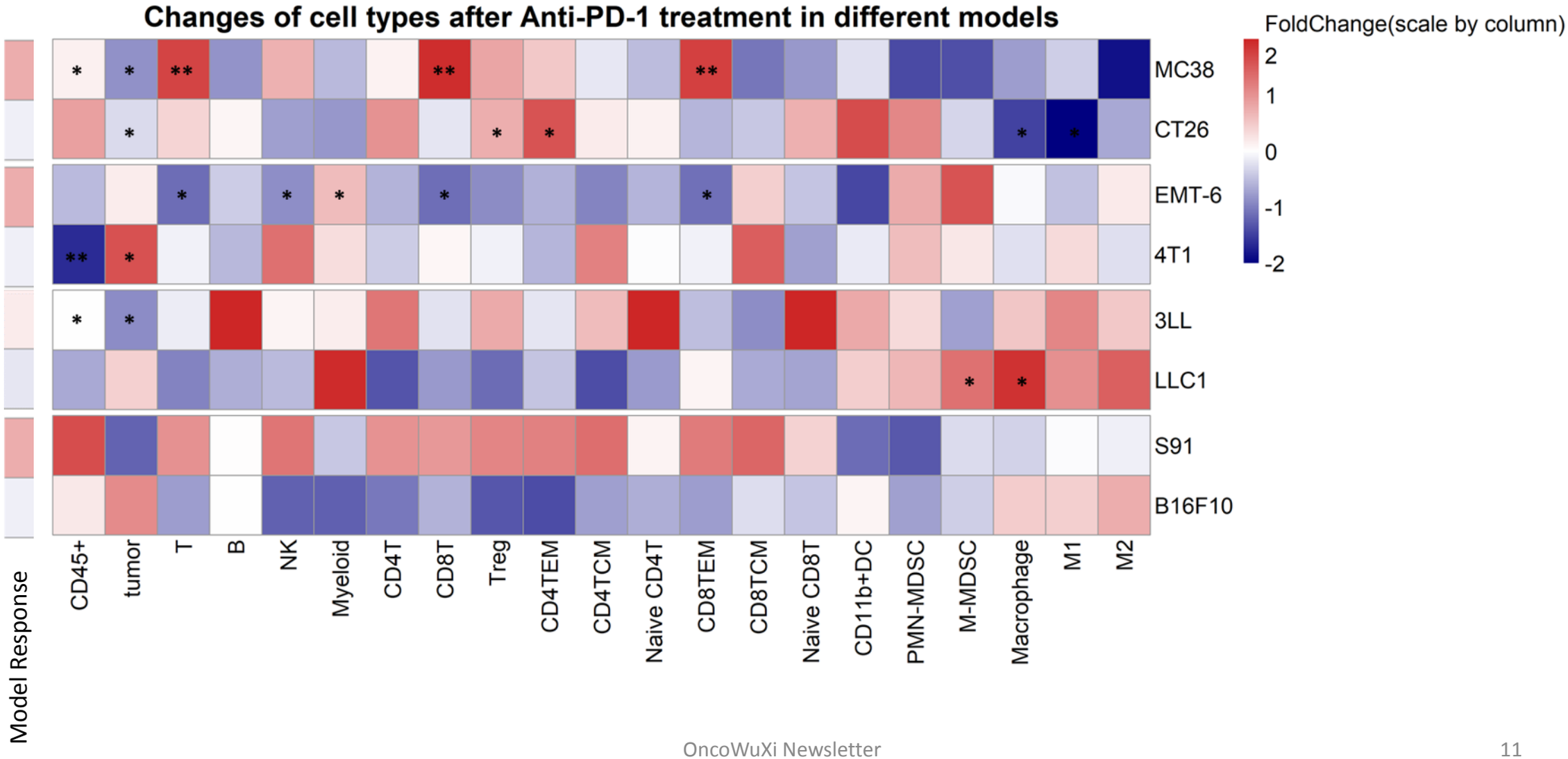






TIL response to anti-PD-1 treatment

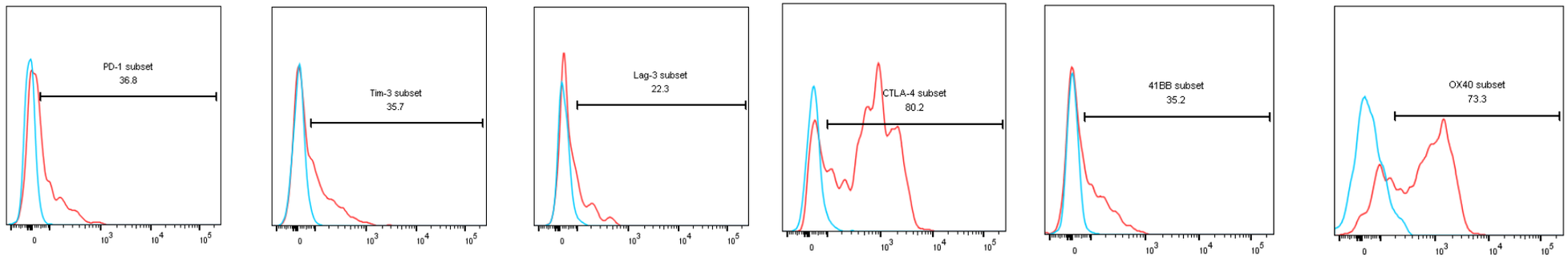
- TIL analysis shows great correlation with drug efficacy across various model



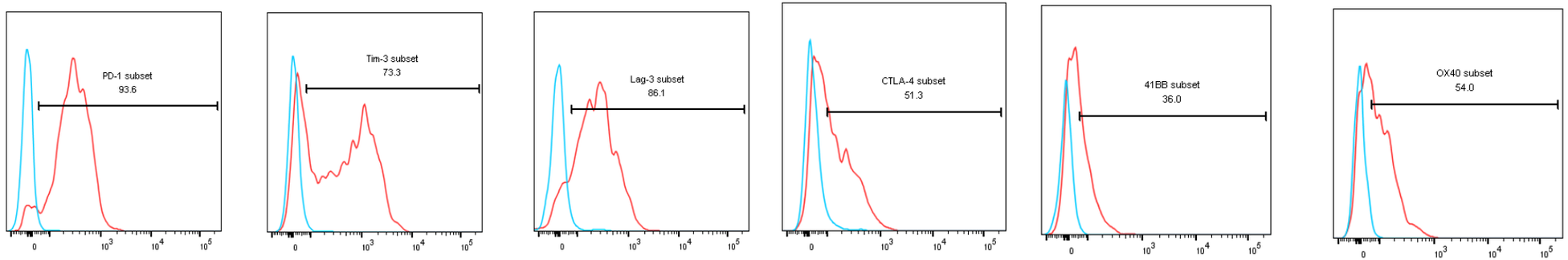
Checkpoint/co-stimulatory markers (CT-26)

--- Panel
--- Isotype

In CD4 T



In CD8 T

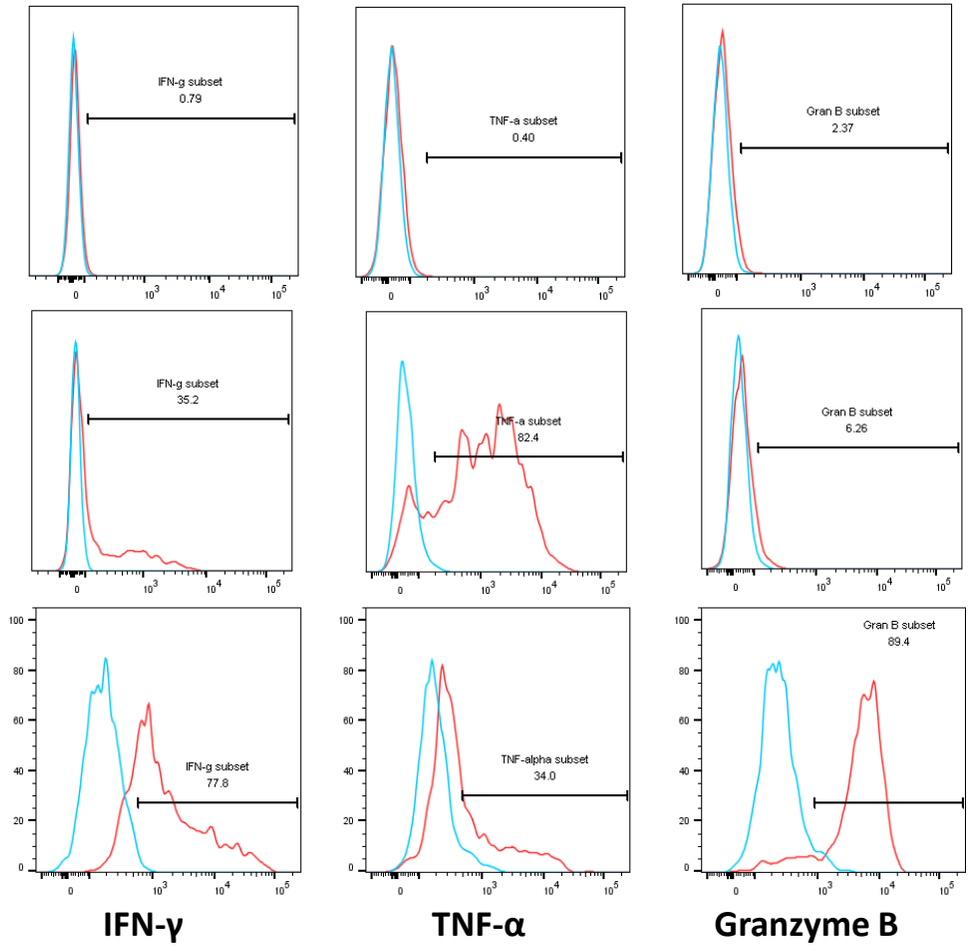


PD-1 **TIM-3** **LAG-3** **CTLA-4** **4-1BB** **OX40**

Intracellular cytokine staining

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

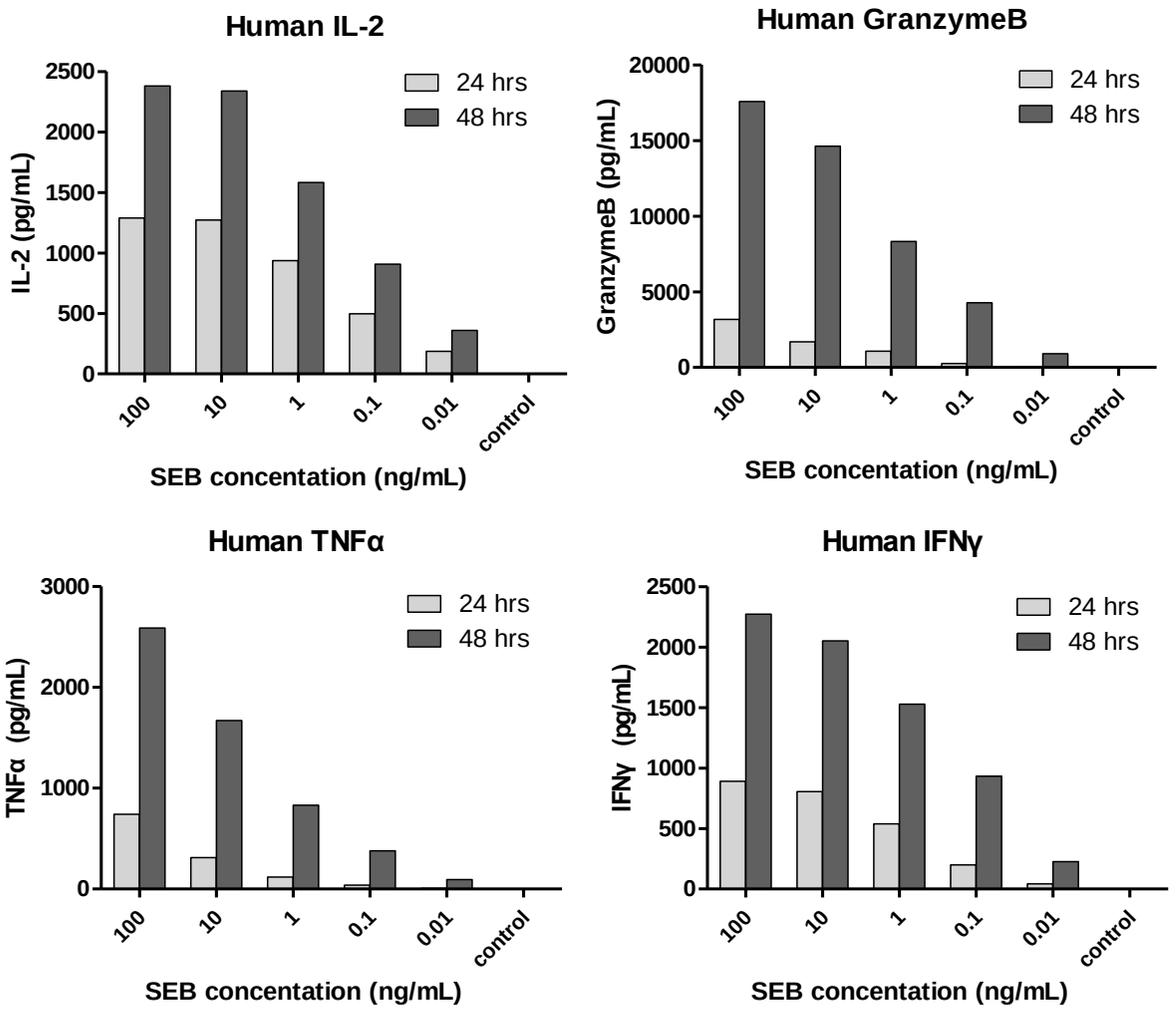
--- Panel
--- Isotype



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 hrs. 4-6 hrs before collection for FCM analysis, Protein Transport inhibitors were added, and intracellular cytokines in CD8+ T were analyzed by BD FACSCanto.

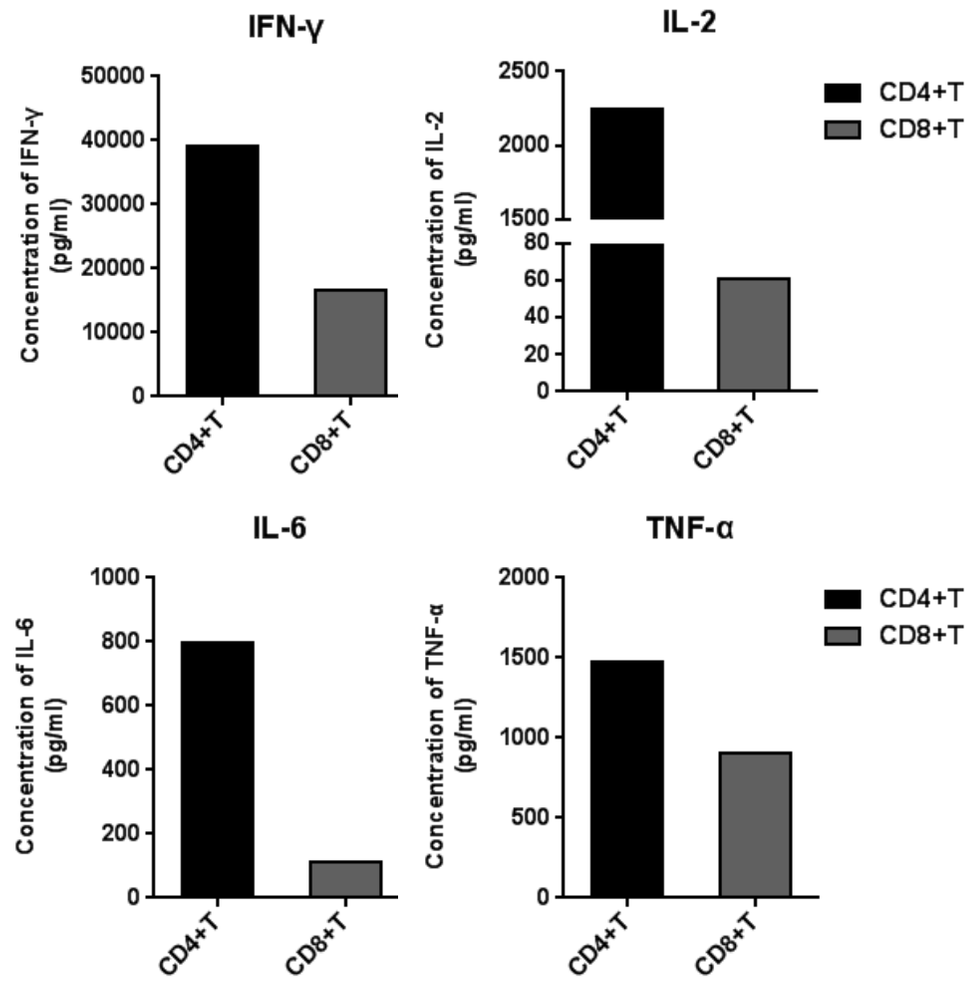
Secreted Cytokine Analysis by CBA and ELISA

Secreted Cytokine Analysis by CBA on SEB-stimulated PBMC



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

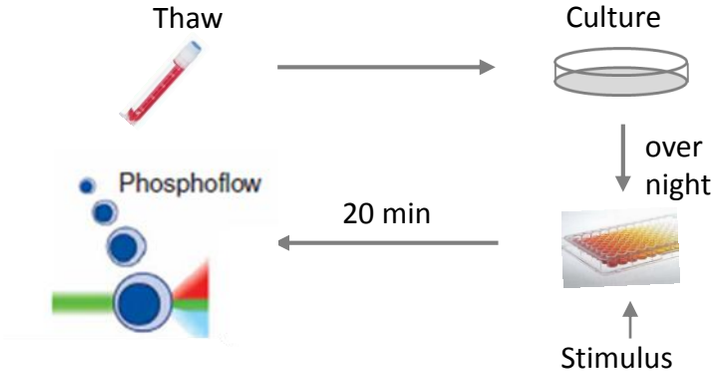
Secreted Cytokine Analysis by ELISA on sorted CD4 T and CD8 T from PBMC

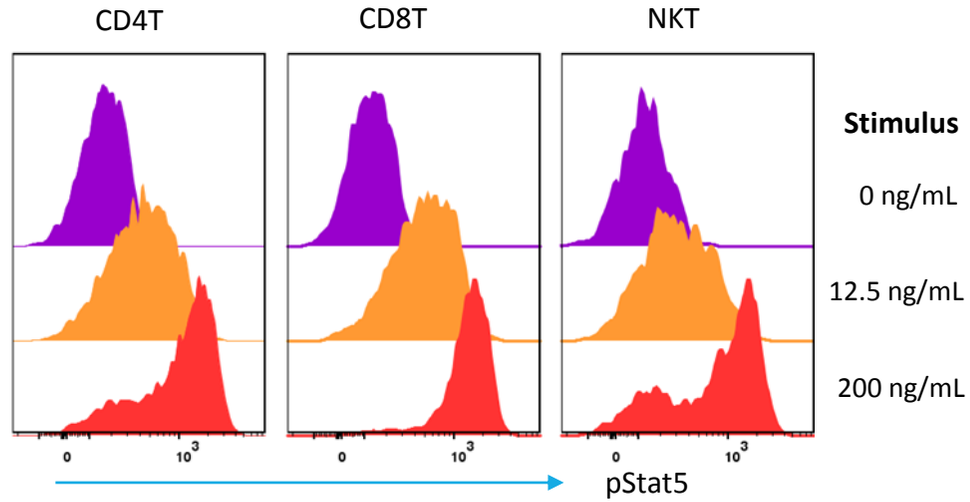


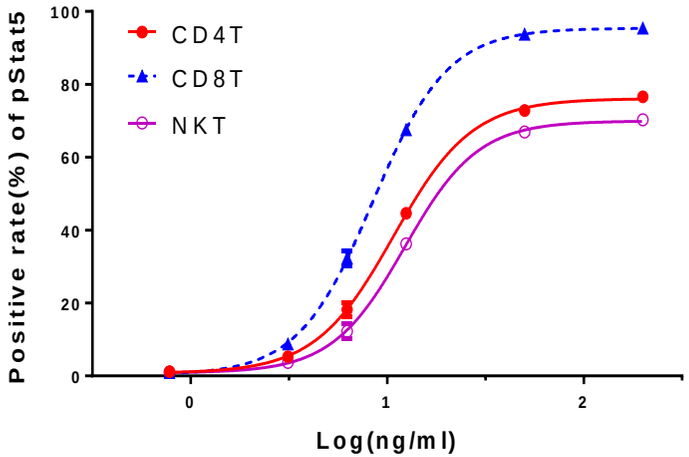
PBMCs were sorted for CD4 T and CD8 T, stimulated with anti-CD3 and anti-CD28 antibody. The secreted cytokines in the cell culture medium were quantified by ELISA.

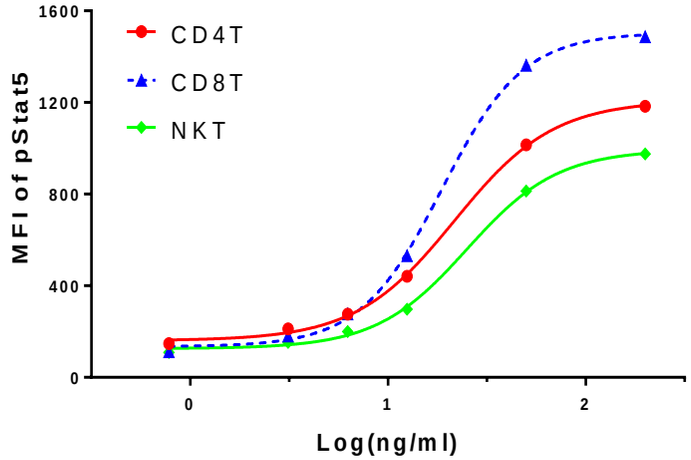
In Vitro Phosphoflow

Screen drugs based on phosphorylated protein change in target cell types in PBMC







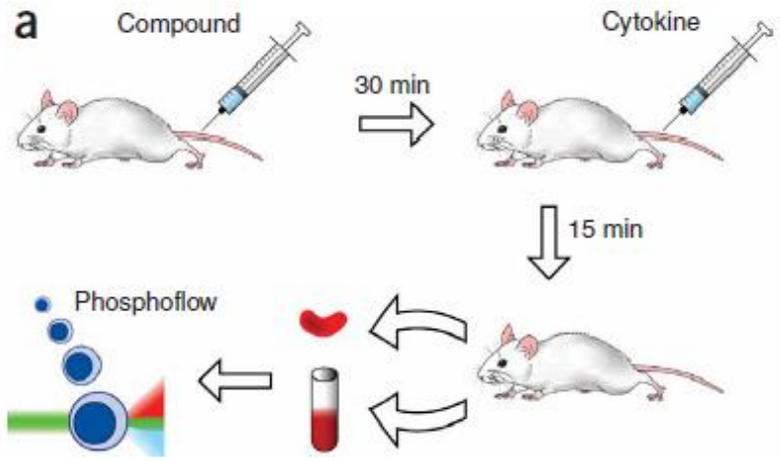


Cell Type	Drug Concentration(ng/mL)	
	EC50	**EC90**
CD4T	10.79	29.63
CD8T	8.46	22.18
NKT	12.31	31.45

pStat5 was detected in CD4T, CD8T and NKT after drug stimulation, and stimulation curve was drawn against drug concentration. EC50/EC90 of each drug was calculated accordingly.

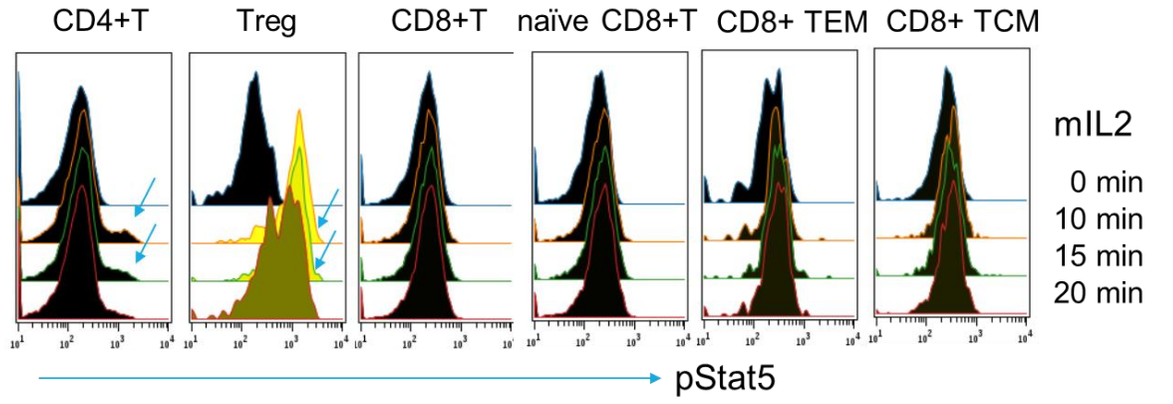
In Vivo Phosphoflow

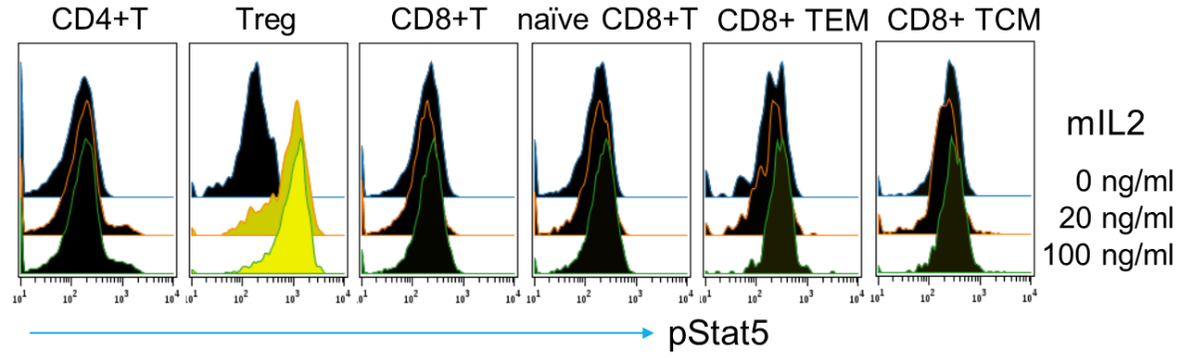
Accurate and timely detection of changes in phosphorylation levels *in vivo*



Method Optimization

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



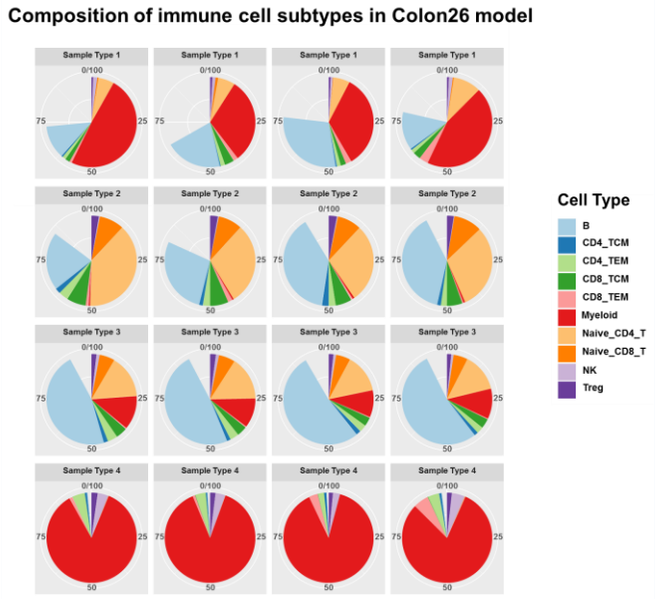


Due to the fast turnover of some phosphorylated protein, sample should be fixed in less than 5 minutes after Stimuli on in vivo model. Methods were optimized for each phosphoflow. Optimization includes selection of markers, antibodies, reagents of fix and perm, etc.

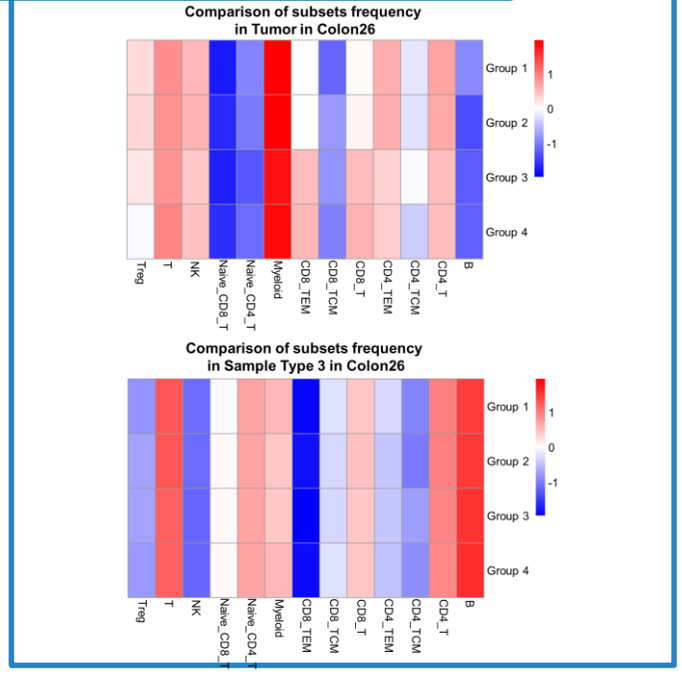
Flow Data Analysis

Covering from traditional gating to unsupervised learning

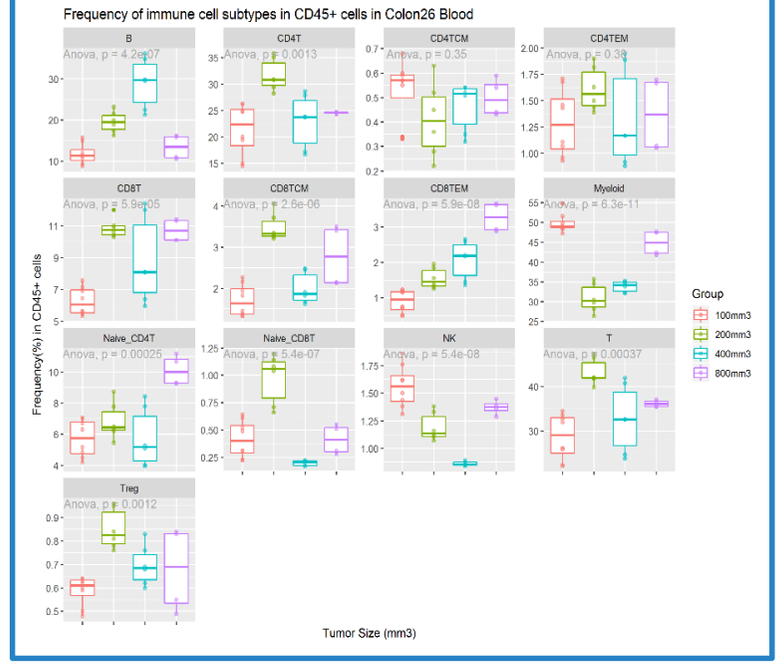
Composition of immune cell subtypes in Model 3 (by piechart)



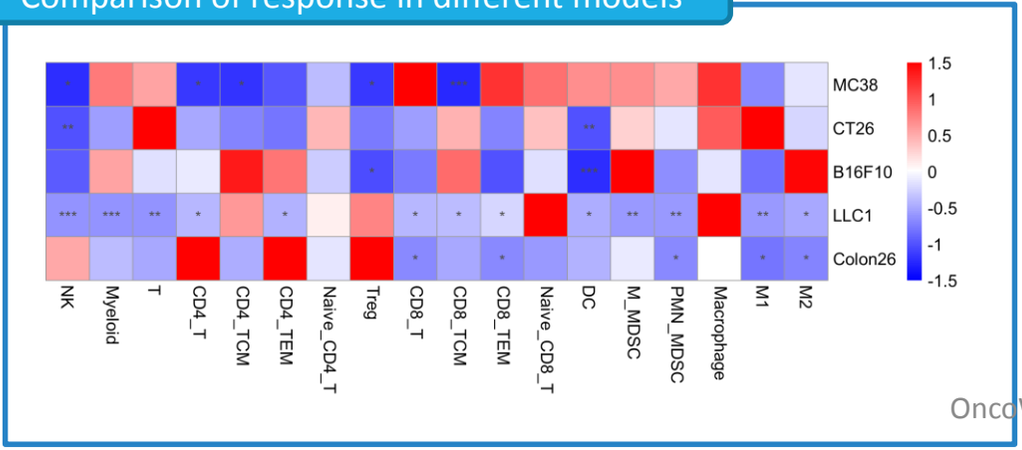
Comparison of immune cells in different groups (by heatmap)



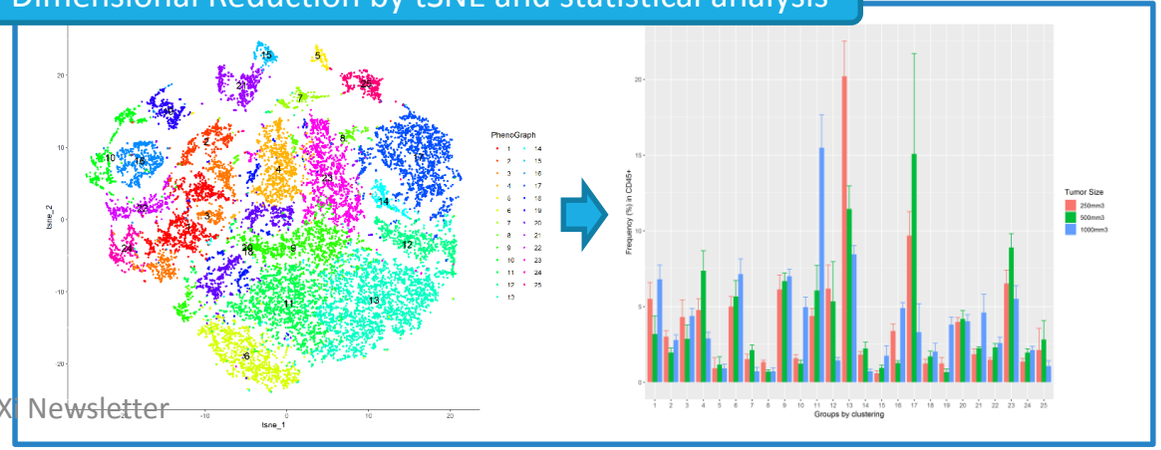
Comparison of immune cell subtypes in different groups (by boxplot)



Comparison of response in different models



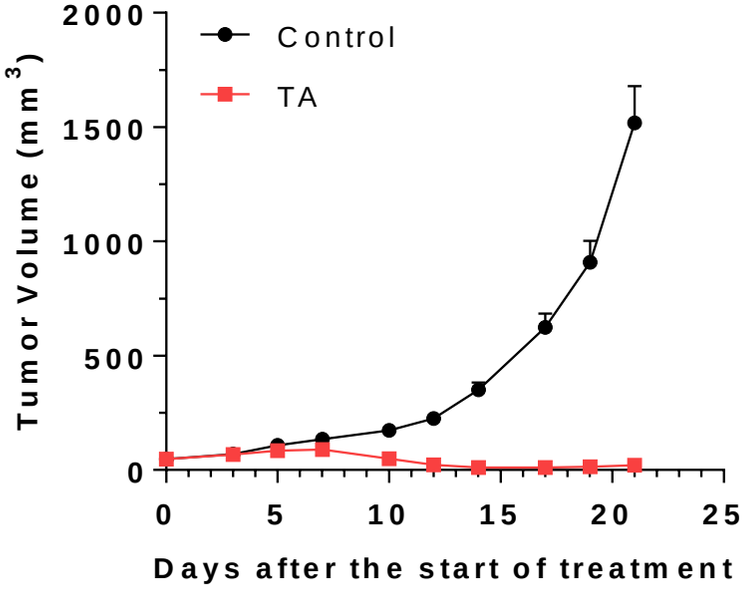
Dimensional Reduction by tSNE and statistical analysis



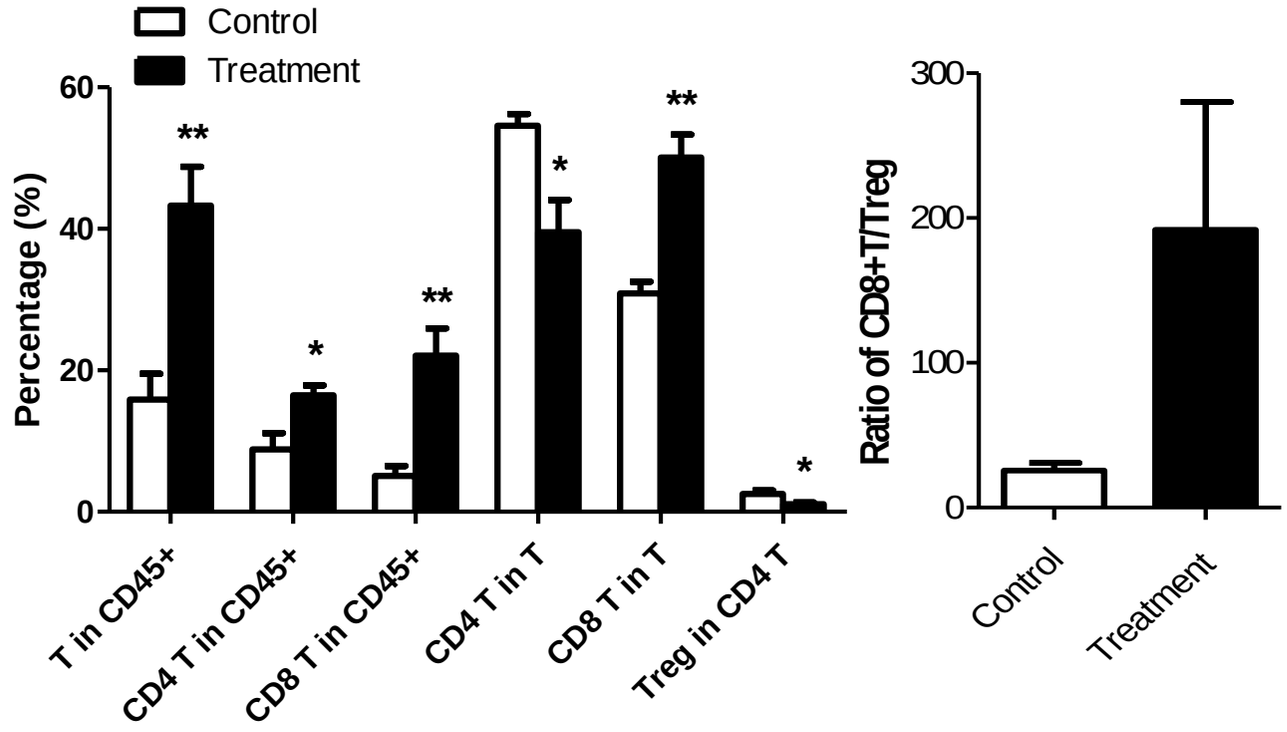
Case study: TIL analysis after checkpoint inhibitor blockade treatment

(MC38 in humanized mouse model)

In vivo efficacy



TIL analysis



The MC38 model was established in humanized immune checkpoint mice. Mice were treated with a single dose of control antibody or a checkpoint inhibitor antibody and TILs were analyzed by FACS 5 days later. Student's t-test were used for statistical analysis.
N=4. *, p <0.05; **, p <0.01.

Case study: verification of driver genes by flow cytometry

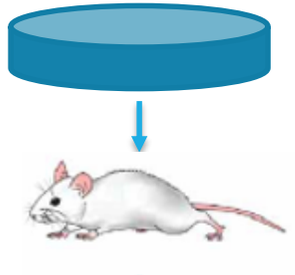
Target gene that involved in immunotherapy was found by CRISPR

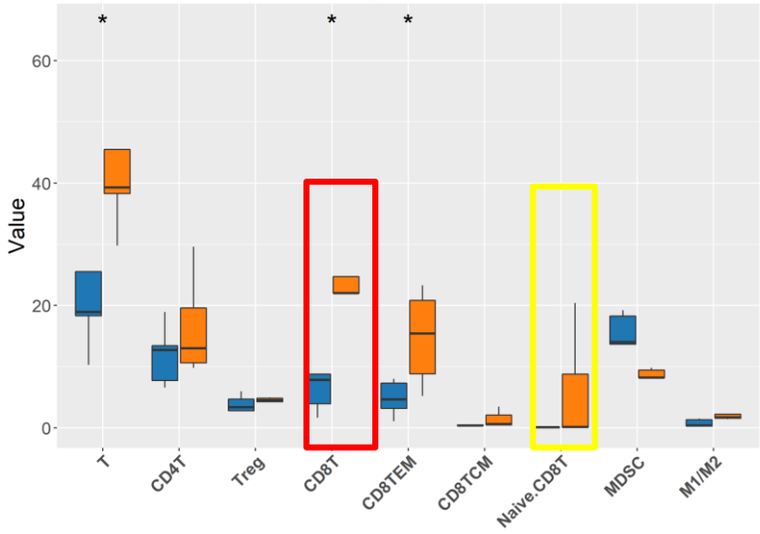
Cell line engineering

Mouse model establishment and efficacy study

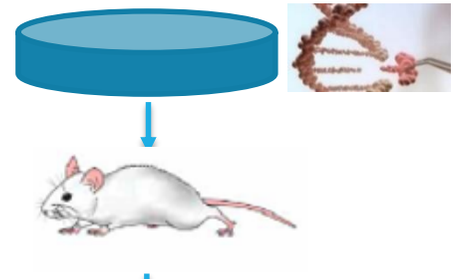
Analysis by flow cytometry

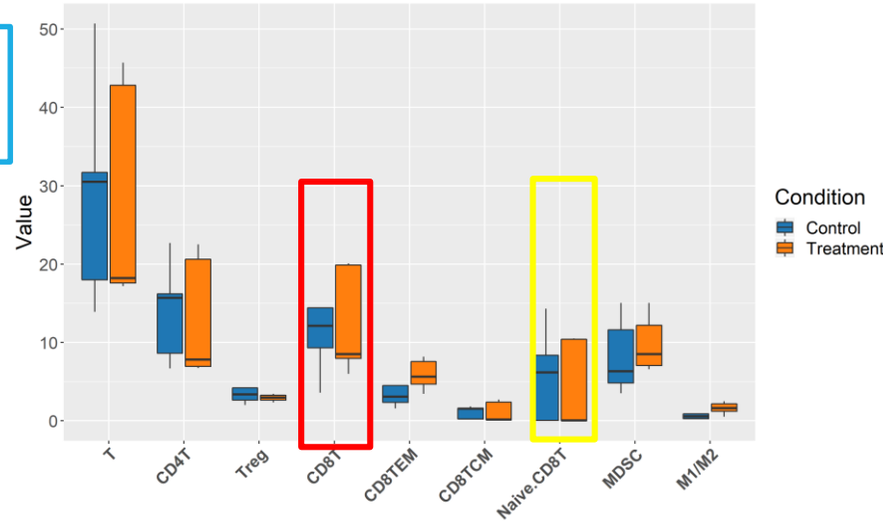
Cell line with wild type target gene



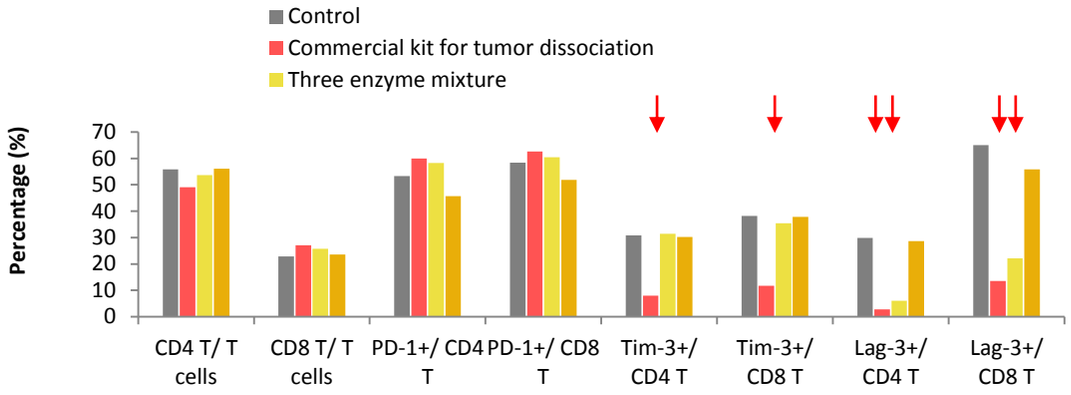


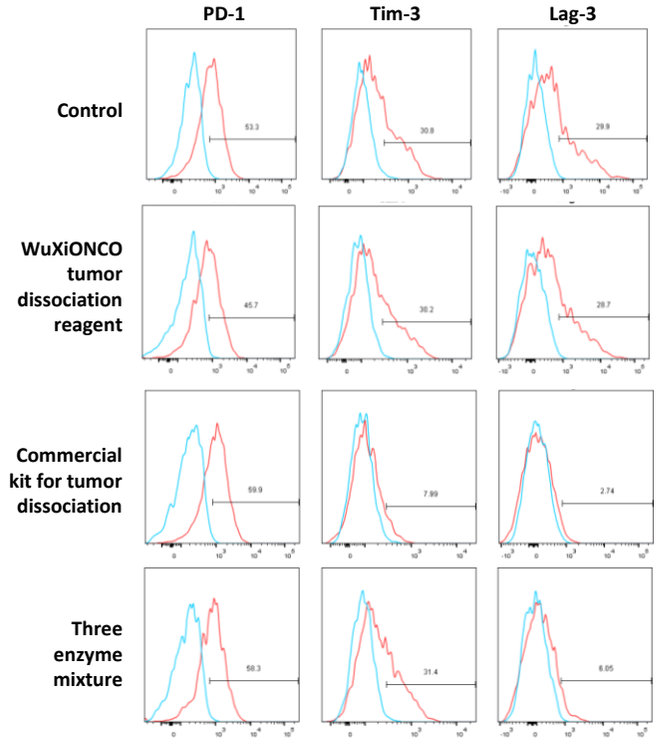
Cell line with mutated/ dysfunctional target gene

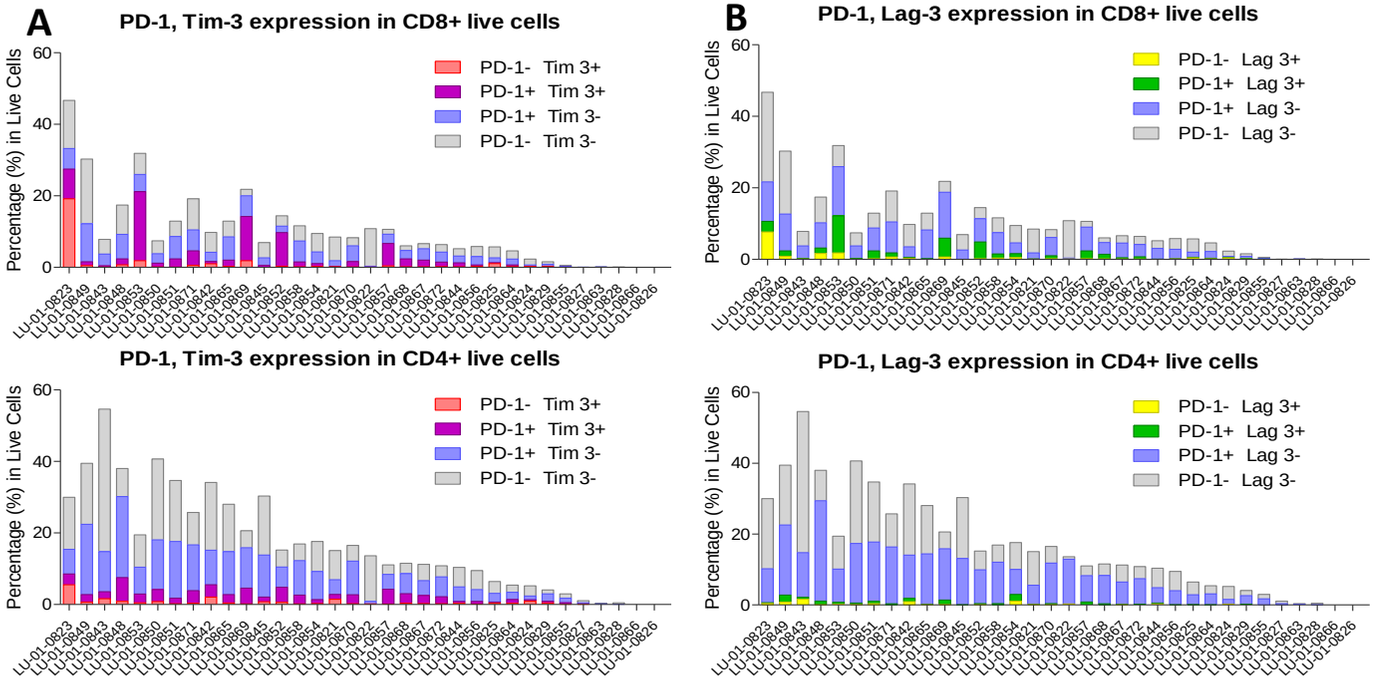


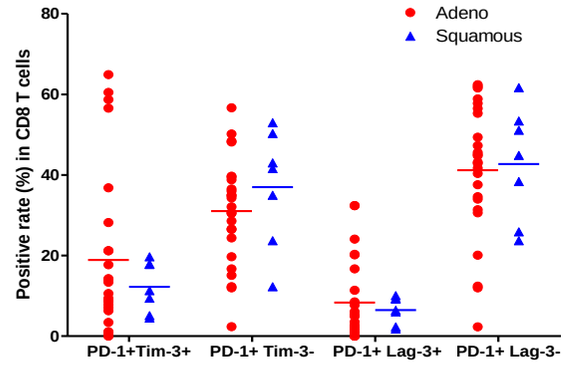


Case study: Immune-profiling and biomarker detection in tumor tissue









OUR COMMITMENT

Improving Health. Making a Difference.

For questions and requests, please email to info_onco@wuxiapptec.com

https://onco.wuxiapptec.com

Mobile App