

Single Cell Functional Analysis Provides Insights into Immunotherapy Cancer Patient Responses

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Prof. David Baltimore (Dr. Michael Bethune)

Dr. Steve Rosenberg (NIH)

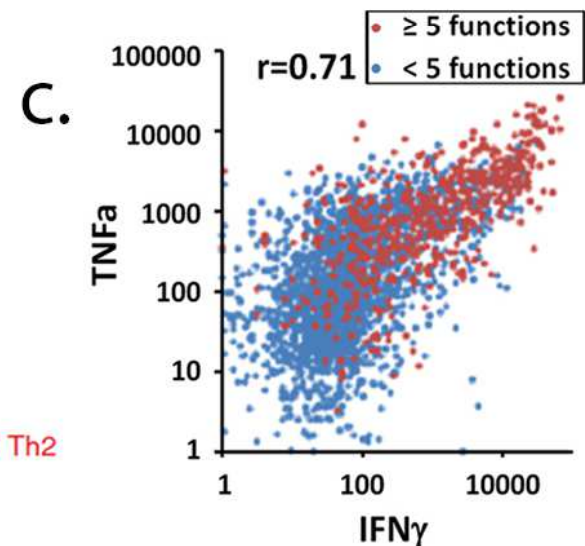
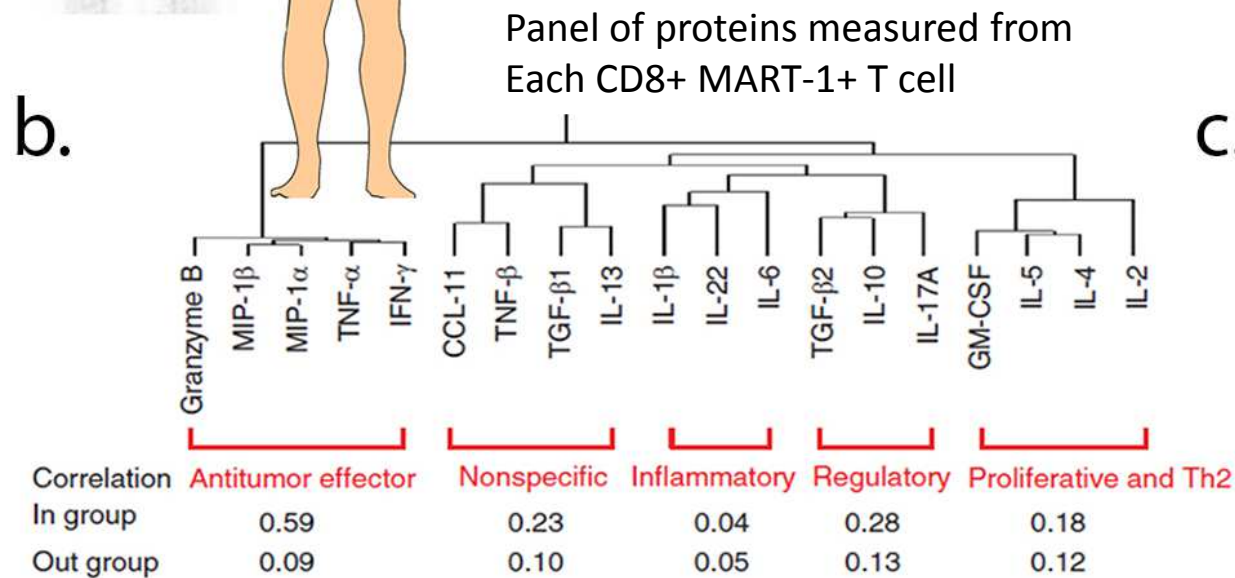
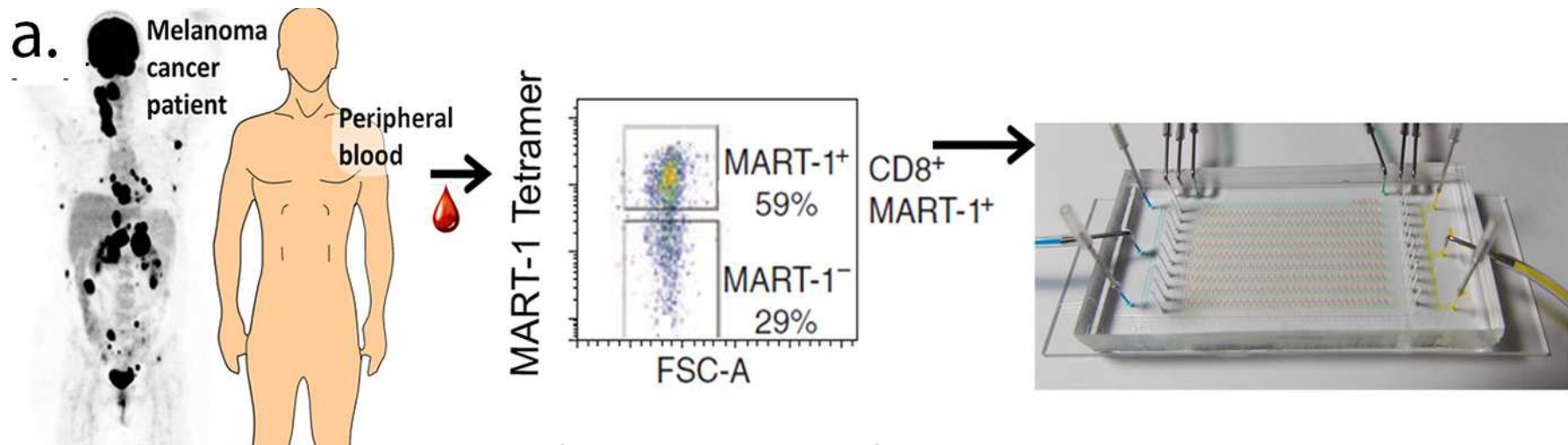
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Melanoma
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Proteomic Analysis of MART-1+ tumor-antigen-specific CD8+ T Cells from a melanoma patient



Ma, et al., Nat. Med. 2012; Ma, et al., *Canc Disc* 2013, Chodon, et al., *Clin. Canc. Res.* 2014

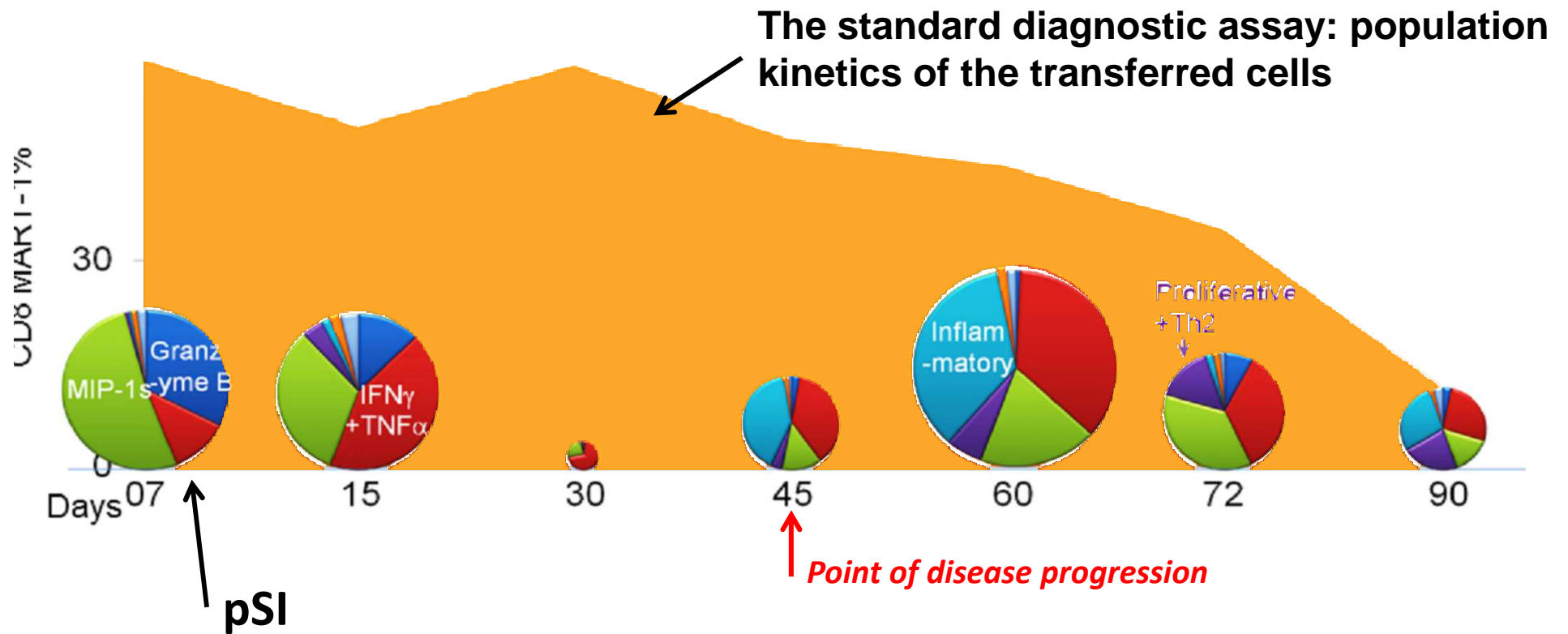


Immune cell functionality is a better measure of the quality of an immune response than is immune cell abundance

The polyfunctional strength index (pSI)

= (# of functional proteins secreted per cell x copy #'s of those proteins)

Provides a quality metric

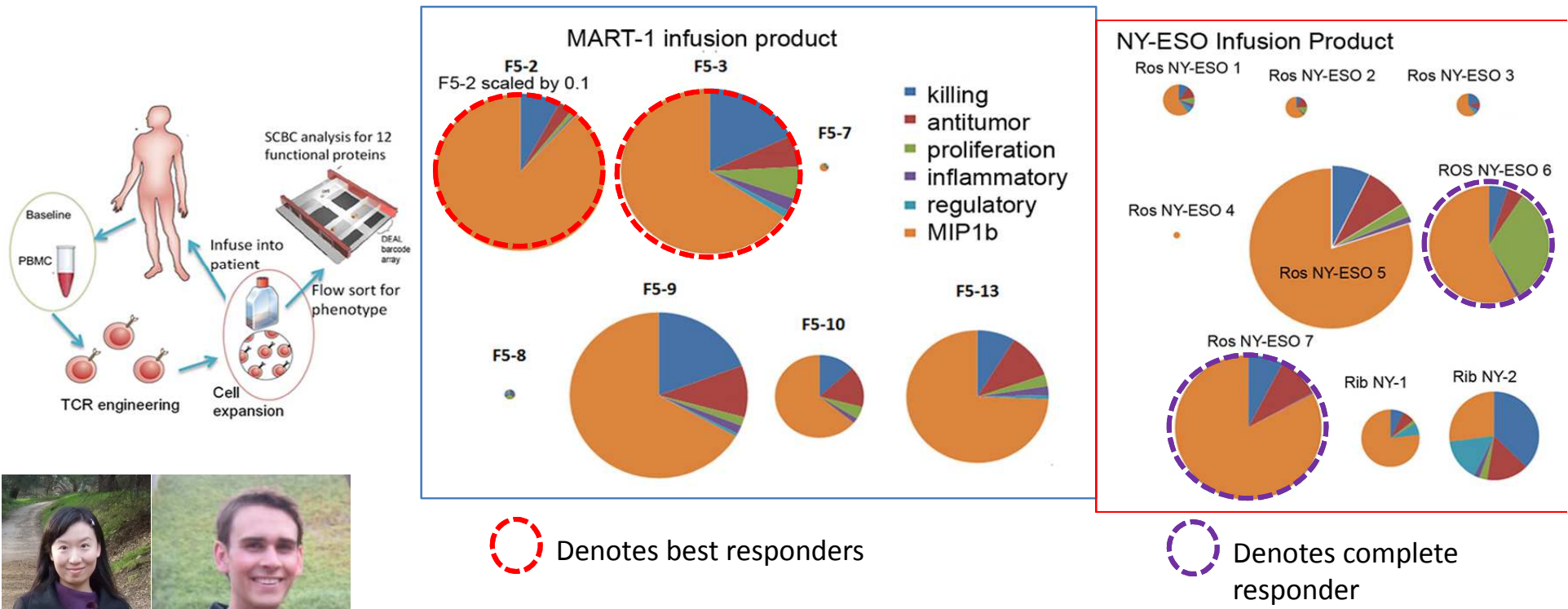


Ma, et al., *Cancer Discovery* 2013, Chodon, et al., *Clin. Canc. Res.* 2014
Also, see Seder R, Darrah P, Roederer M: *Nat Rev Immun* 2008, 8:247.

Characterization of Infusion Product T cells for TCR-engineered ACT

T cell functionality is a better indicator of the quality of an immune response than is the abundance of those cells. (Seder R, Darrah P, Roederer M: *Nat Rev Immun* 2008, 8:247. Ma, et al., *Canc. Disc.* 2013)

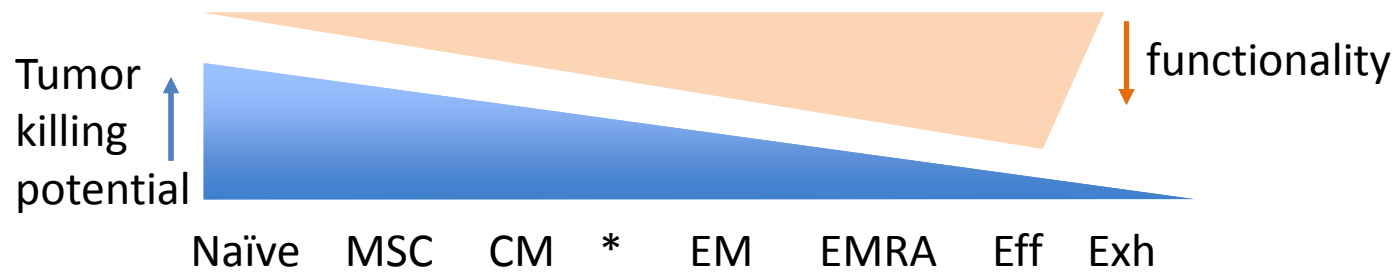
The polyfunctionality index of pre-infusion products for e-ACT therapy is a highly uncontrolled parameter (data from 3 trials).



The polyfunctionality index may be prognostic for best responders



T cell functionality, phenotype, and tumor killing capacity are interrelated



*= a characterized phenotype that doesn't fall neatly into any the listed categories

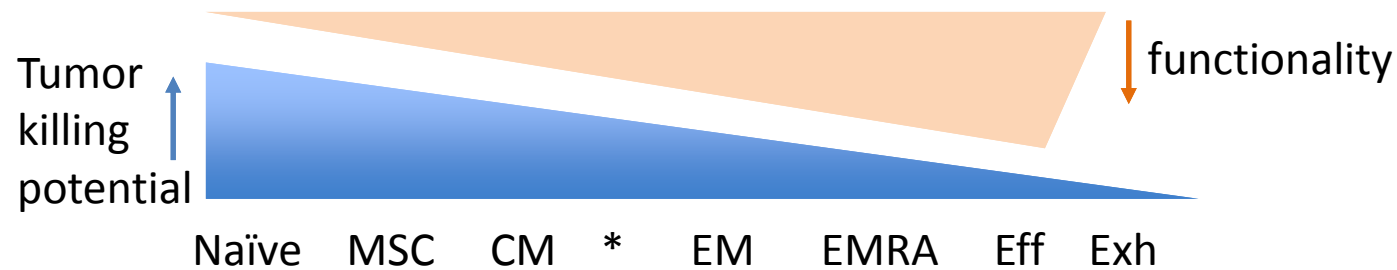
T cell – T cell interactions (of stimulated T cells) push cells too far to the right on this plot

Compton, Sukumar, Restifo, *Immunological Rev.*, 2014

Wolde ye bothe eate your cake, and haue your cake?
(John Heywood, 1546)

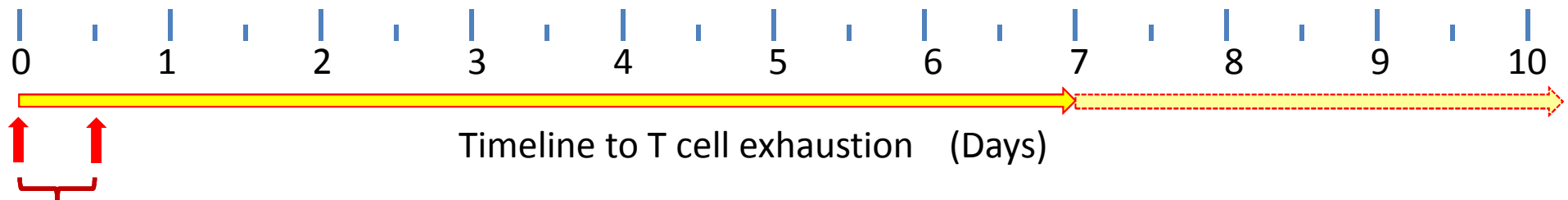


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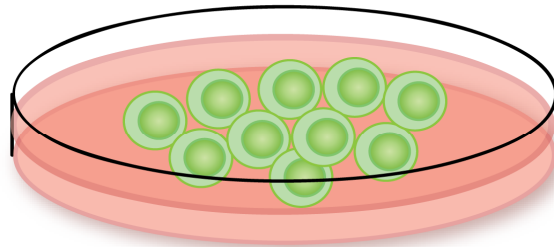


Our hypothesis: short time (0.5 day) interactions of tumor antigen stimulated T cells strongly promote tumor-killing functions, while not leading to terminal differentiation of the T cells.

T cell-T cell interactions following antigen stimulation yield strong activation

Step 1

Stimulate in **population**
Stimulation (Training) Time: T_1

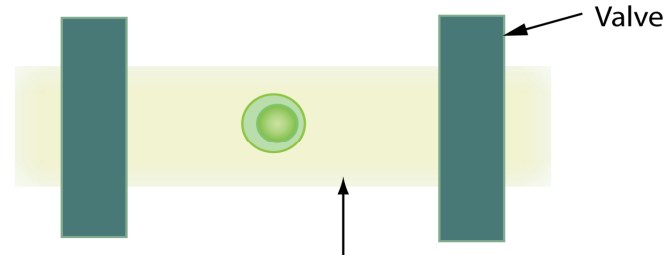


Traditional ensemble assay

Wash out supernatants
Resuspend

Step 2

Stimulate in **isolation**
Stimulation Time: T_2

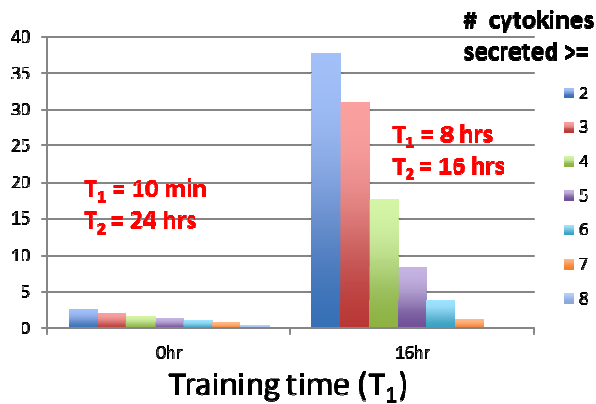


One isolated micro-chamber from SCBC

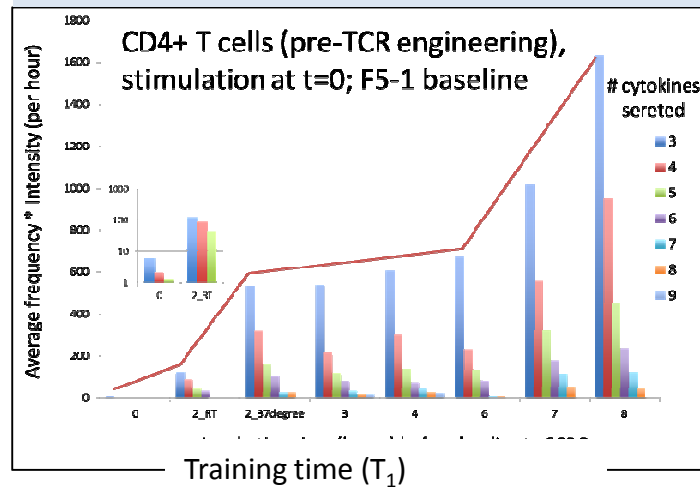
Total stimulation time $T = T_1 + T_2$ is constant

T (human patients) = 13 hours; T (OT-1 mouse) = 24 hours

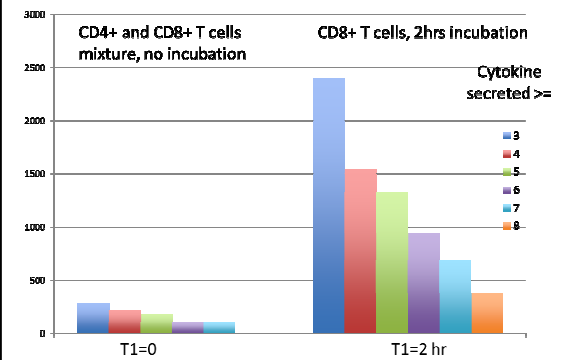
OT-1 antigen specific T cells
from OT-1 mouse models
(tetramer stimulation)

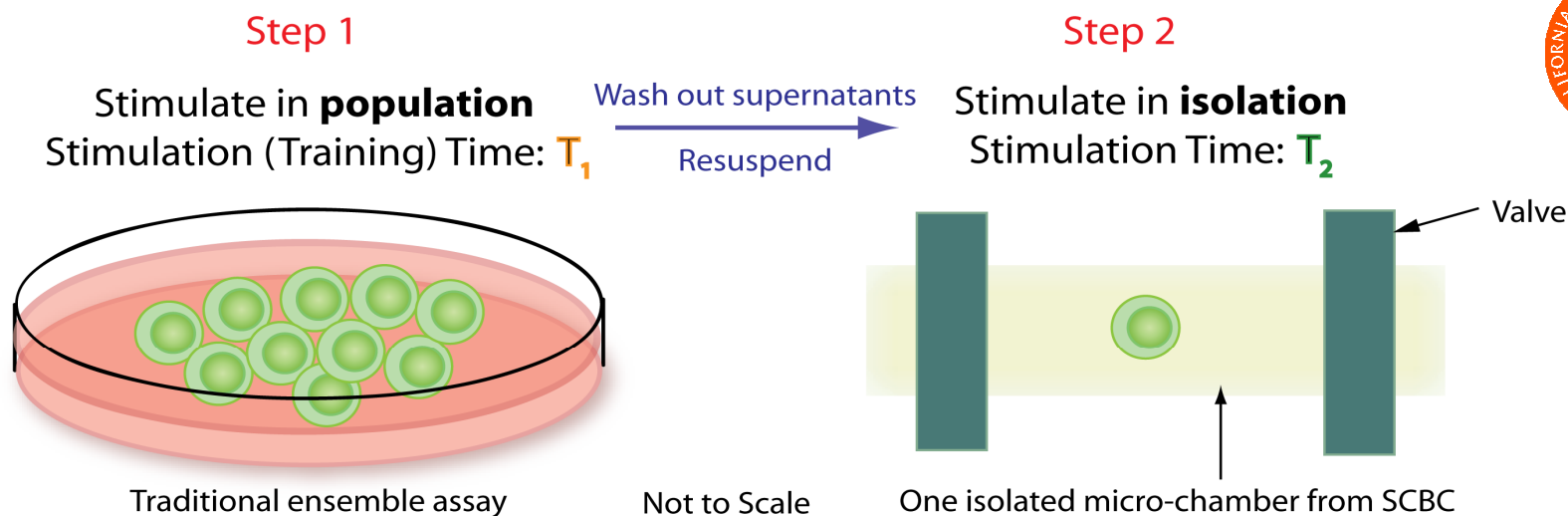


Patient Baseline T cells
(PMA + ionomycin stimulation)



Pre-Infusion Product
(Patient F5-13); tetramer
stimulation



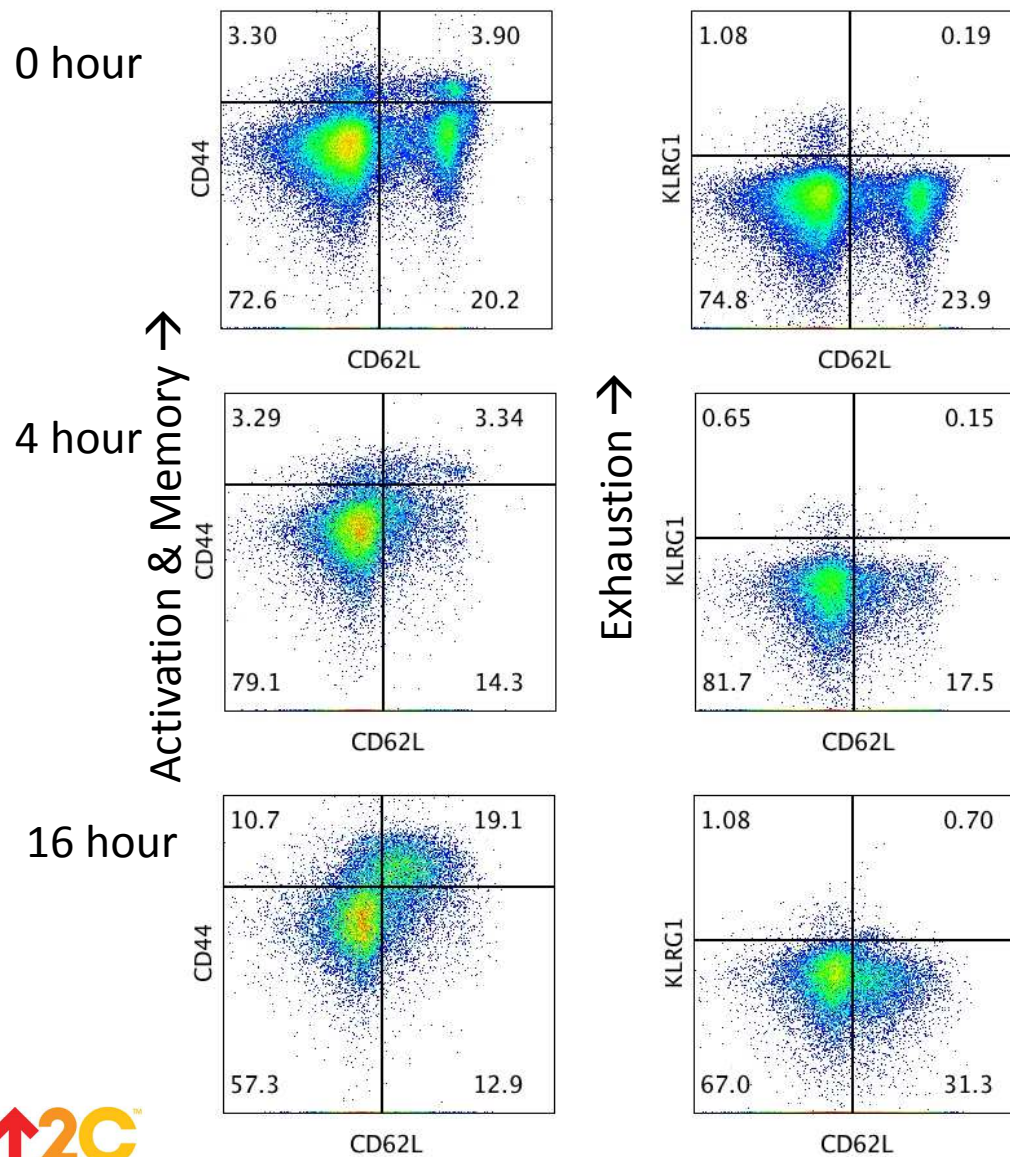


Total stimulation time $T = T_1 + T_2$ is constant

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T Cell Source	Functional kinetics $T_1=0-18$ hrs post stimulation	Phenotype kinetics	Transcriptome kinetics	Influence of T_1 on In vivo tumor killing
OT-1 mice (analogous to Restifo)	Measured	Measured	Measured	Measured
Patient baseline T cells	Measured	Measured	Measured	N.A.
Patient pre-infusion TCR-engineering T cells (n=20; 3 trials)	Measured	Can't Measure on current patient samples; new experiment planned	Measured	Functionality correlated w/ patient responses

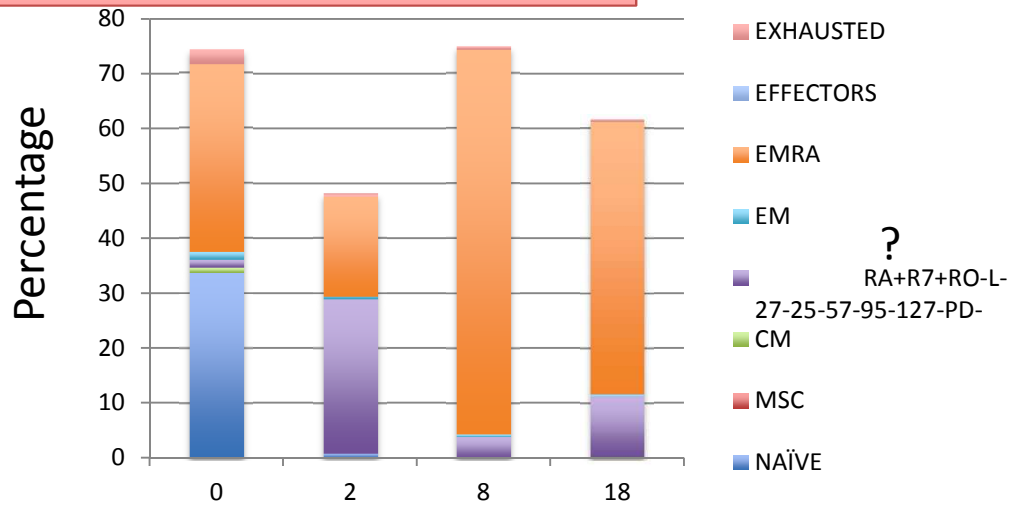
OT-1 mouse T cell phenotype dynamics reveal only minor changes in phenotype over time-course of T cell conditioning regimen



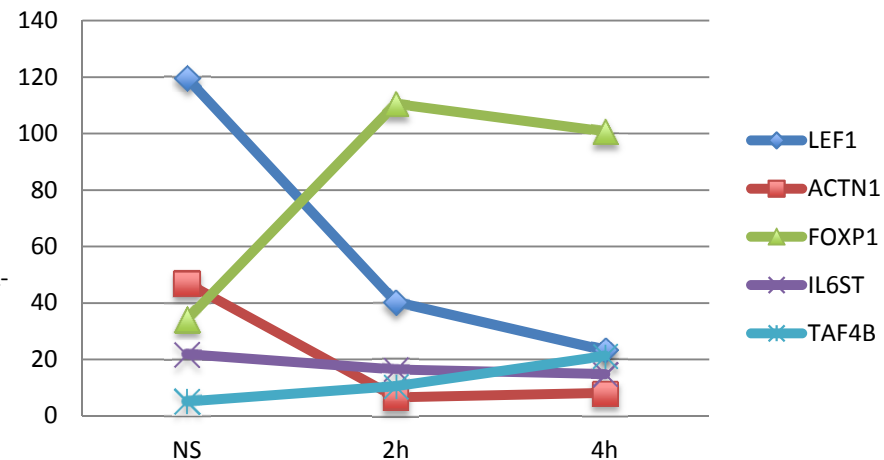
KLRG1: exhaustion marker
 CD44: Activation and memory marker
 CD62L: +++: naïve T cells
 ++/+ : central memory
 - : effector
 memory/effector/exhausted

Patient baseline CD8+ T cells: loss of naïve phenotype, no evidence of Effector or Exhausted phenotype

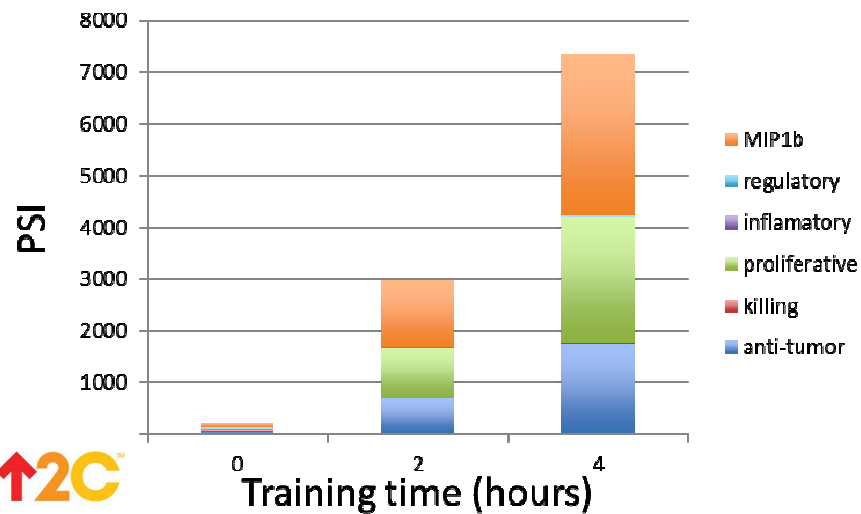
Phenotype dynamics



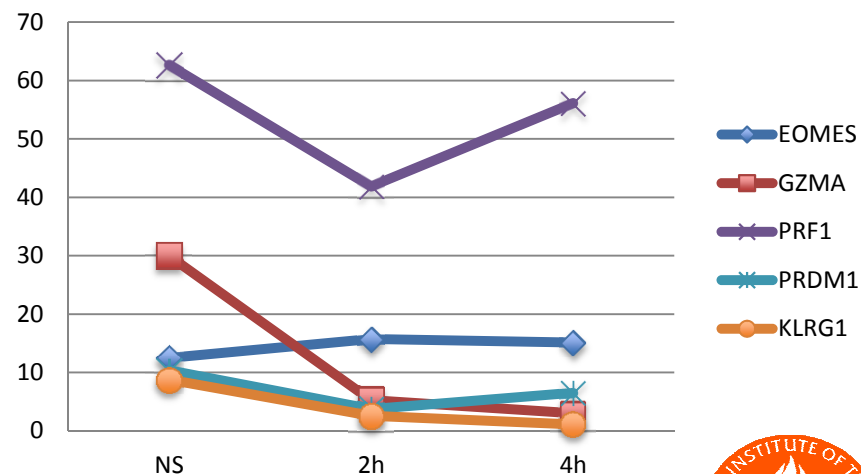
Transcripts, naïve associated



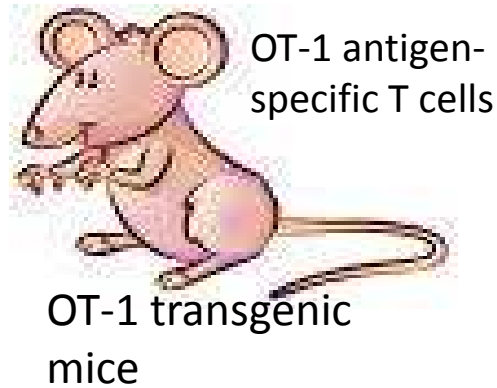
Functional dynamics



Effector associated

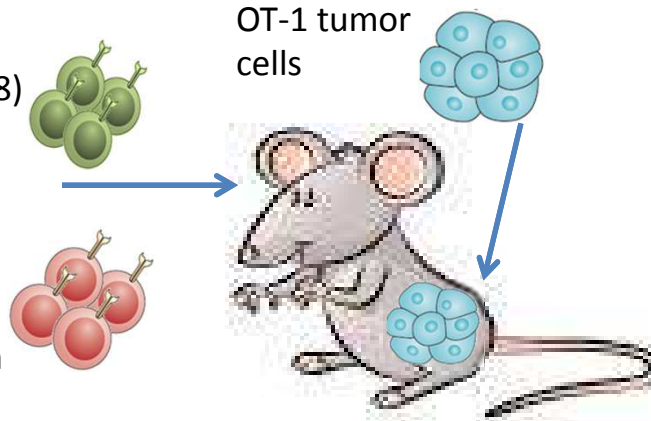


In vivo tumor killing: the role of stimulation strength and T_1 (model analogous to Restifo, et al.)

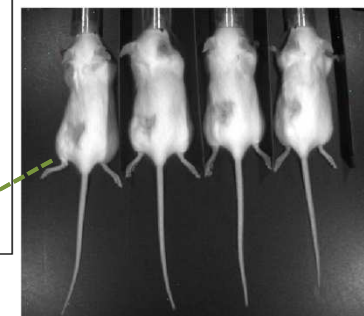
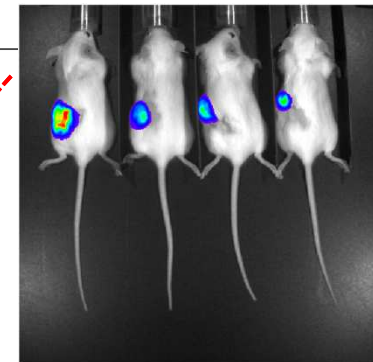
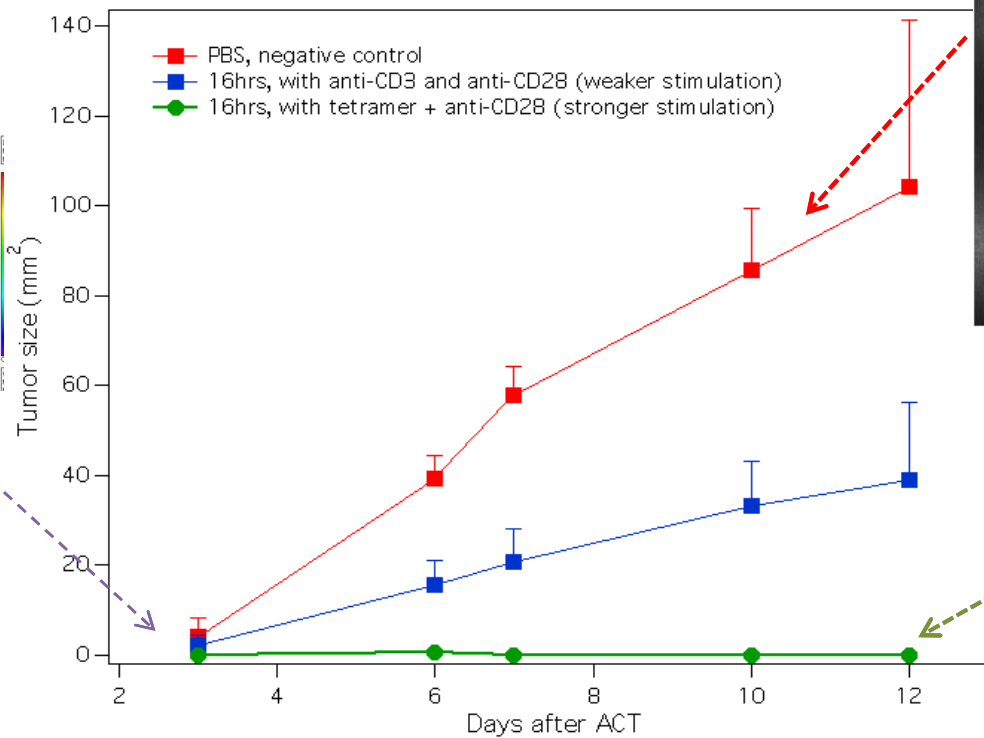
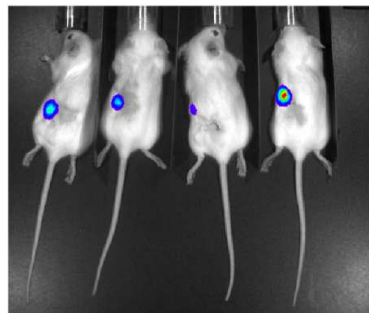


Weak stimulation
(anti-CD3 + anti-CD28)
No incubation

or
Strong stimulation
(tetramer, anti-CD28)
+ 16 hours incubation

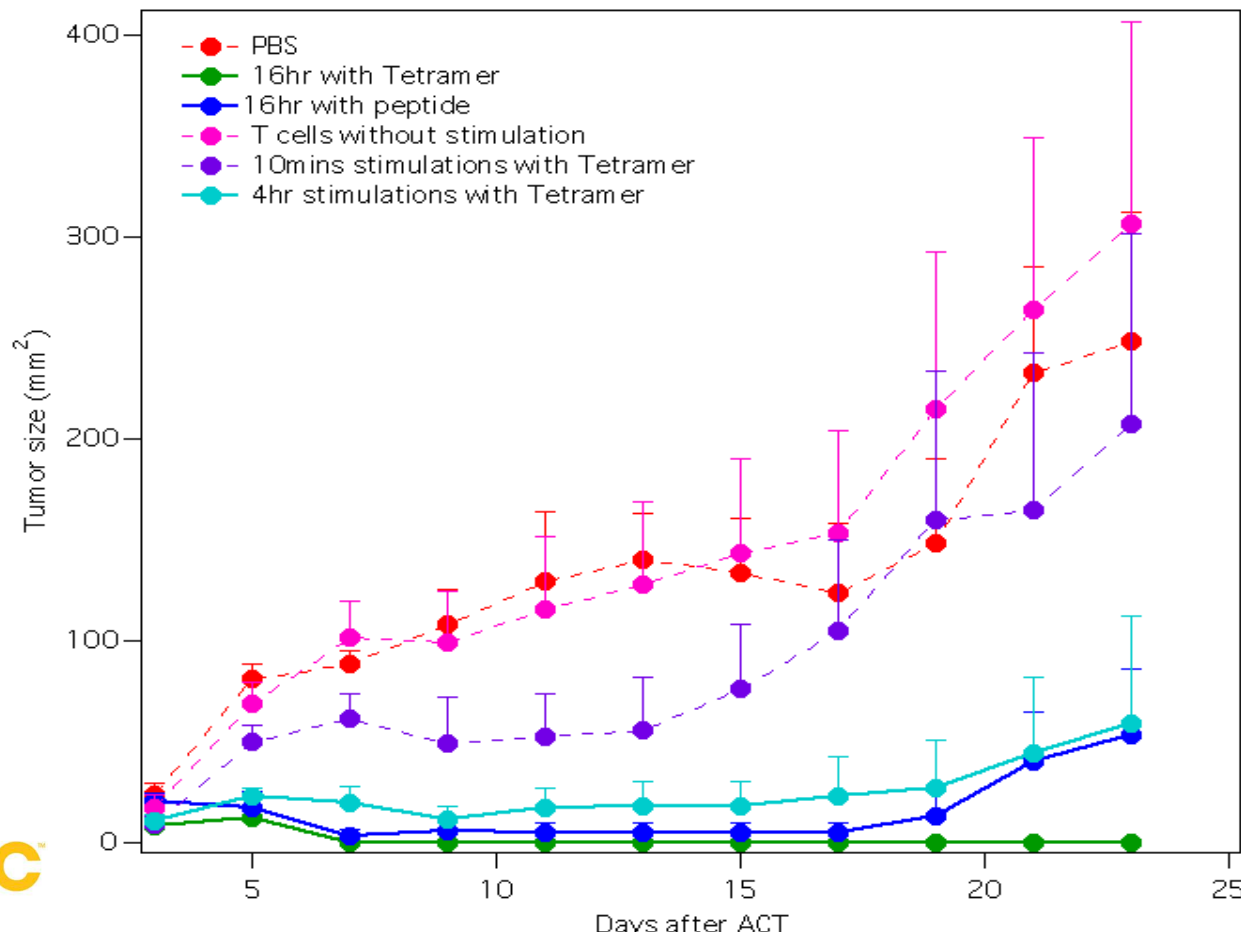
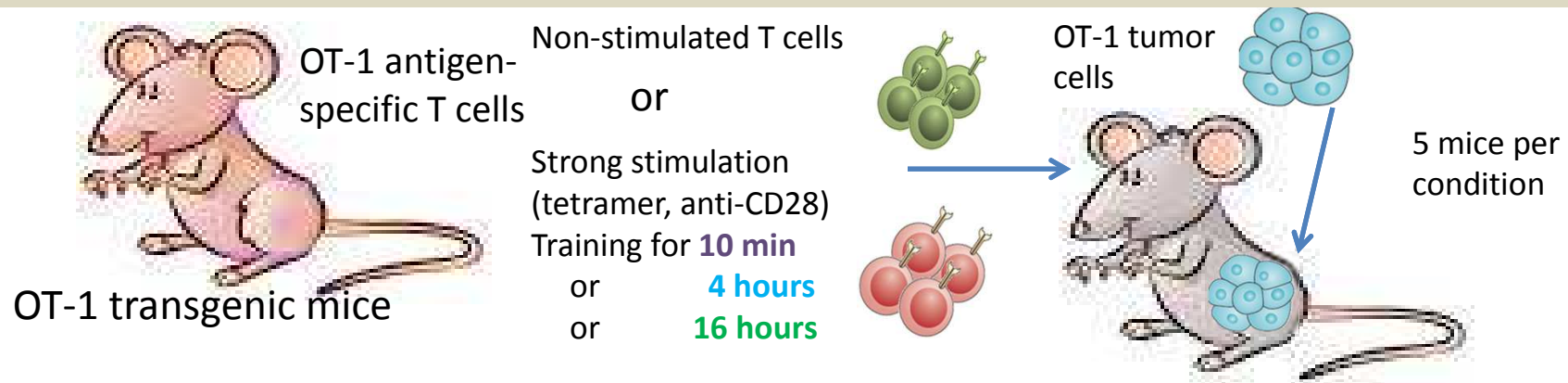


Jing Zhou



Testing the kinetics of T_1 (training time)

(model is analogous to that of Restifo, et al)

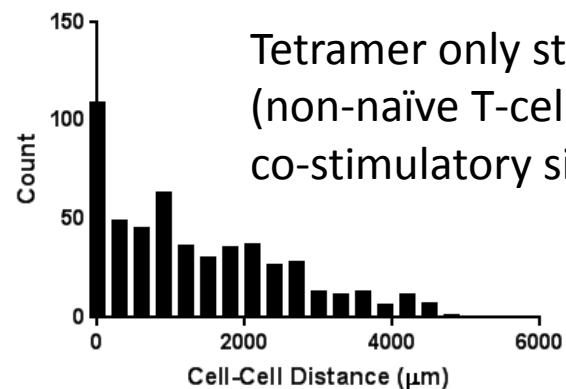


**Tetramer stimulation +
16 hrs training time is
best protocol
(repeated 3 separate
times)**

T Cell Training occurs via Pairwise Contact Interactions Between stimulated T Cells



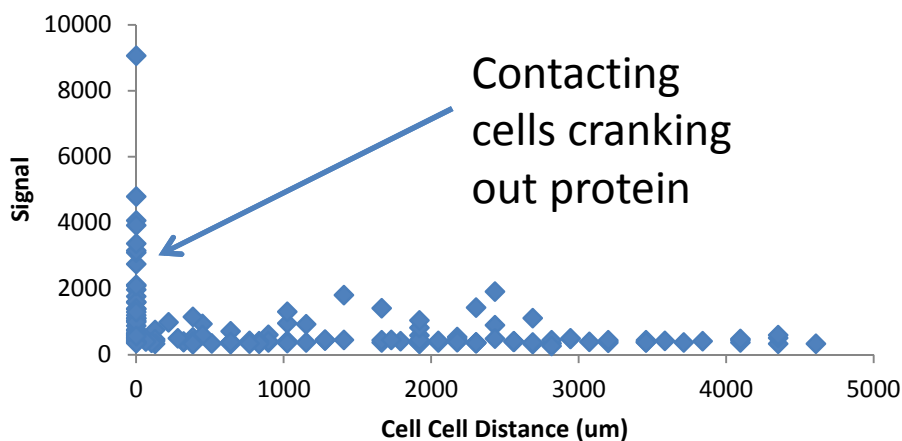
Distance Distribution in Two Cell Chambers



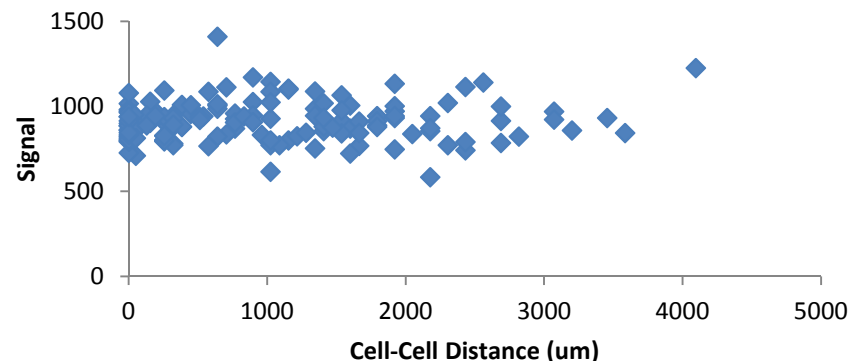
Alex Sutherland

2-cell proteomic data, measured as a function of intercellular separation distance

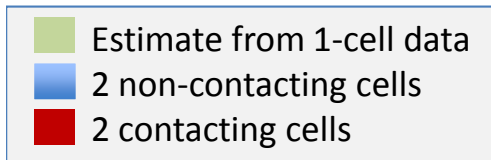
MIP1b secretion (NY-ESO TCR e-ACT patient infusion product)



IL-10 secretion: (NY-ESO TCR e-ACT patient infusion product)

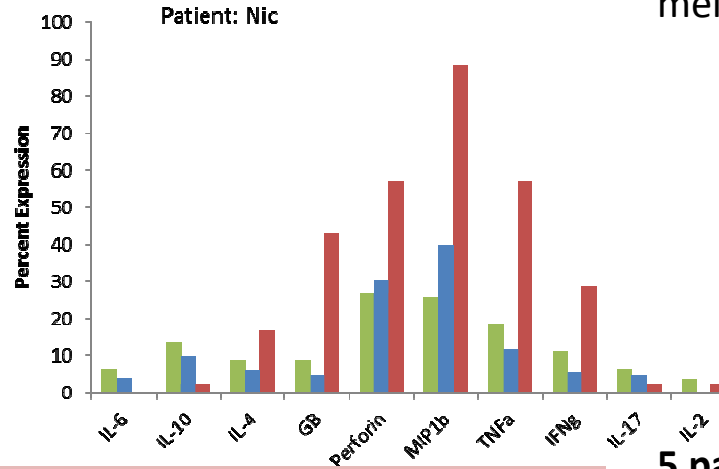
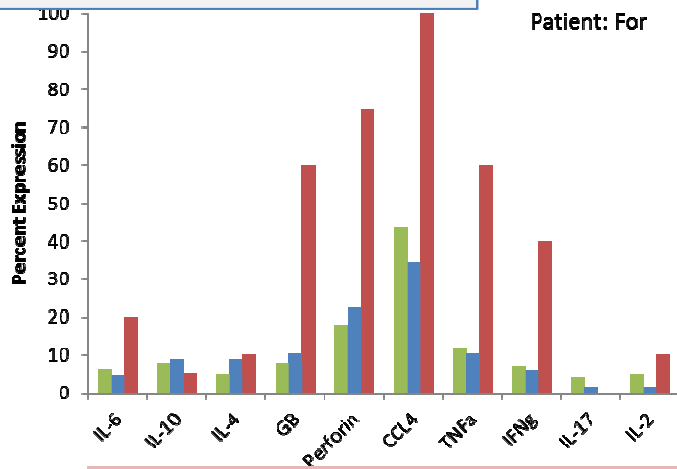


All infusion products are improved when T cells contact after stimulation



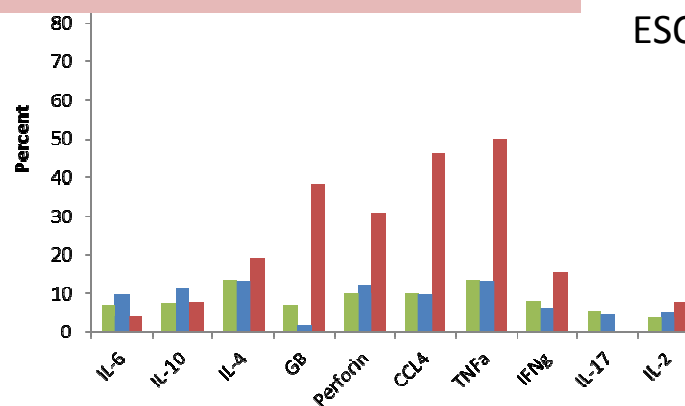
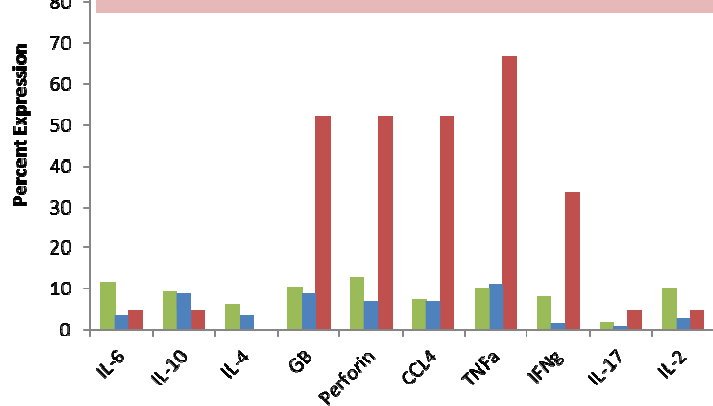
Tetramer only stimulation + incubation of pre-infusion product

Nearly identical data seen for **4 patients** from Steve Rosenberg's NY-ESO e-ACT melanoma trial



All patient samples analyzed exhibit similar behavior
Cell-cell contact induced proteins are almost all anti-tumor

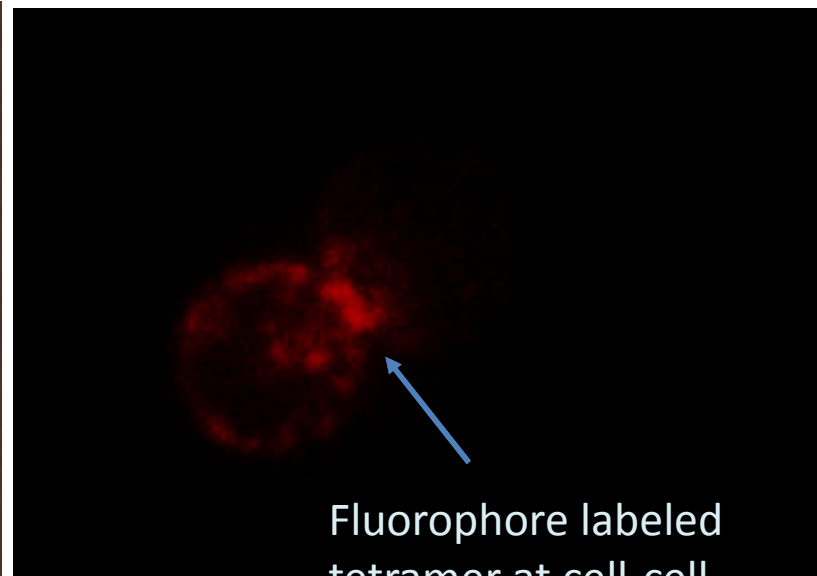
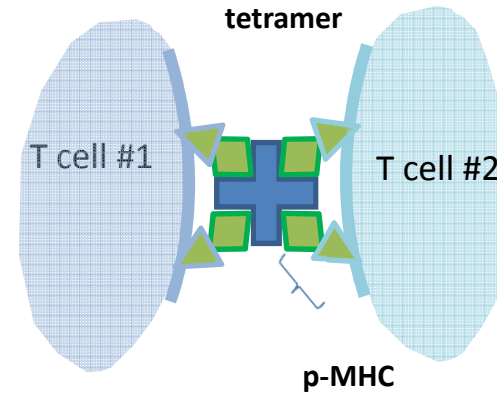
5 patients from Toni Ribas' MART-1 e-ACT trial
And 2 patients from Ribas' NY-ESO trial



Tetramer stimulation + incubation can 'glue' cells together, but it isn't required for this effect

Tetramer stimulated cells appear in non-statistical numbers as cell pairs;

Tetramer stimulated OT-1 cells exhibit superior tumor killing following T1 = few hours, even relative to peptide stimulated OT-1 cells



Fluorophore labeled tetramer at cell-cell adhesion point

Mechanism & Kinetics of "Training"



*Negulescu et al
Immunity, 1996*

Motility is enhanced
upon stimulation

Outcome: Enlargement of
superman T cells

Gerard et al, Nat. Immun. 2013

LFA-1 and ICAM-1 interaction
facilitate contacting, promoting
proliferation and IFN γ secretion

Stimulation

T= 0 hr

Cytokine secretion
& motility
enhancement

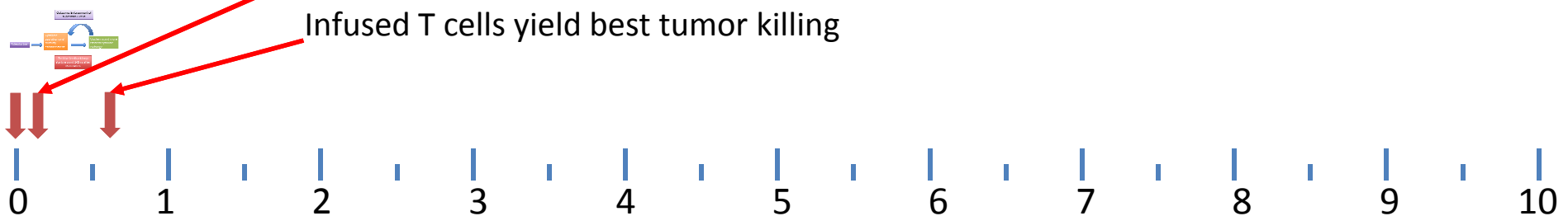
Clustering and more
efficient cytokine
exchange

Positive feedback loop starts
~1-2hrs after stimulation

*Sabatos et al,
Immunity, 2008*

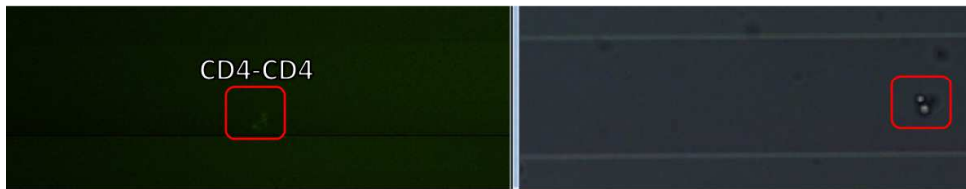
Synapse-mediated cytokine
delivery accelerates responses in
activating T cells

Infused T cells yield best tumor killing



Timeline to T cell exhaustion (Restifo's protocols) (Days)

Phenotype Dependence of Proteins Secreted

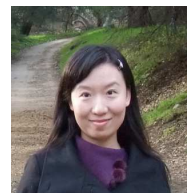
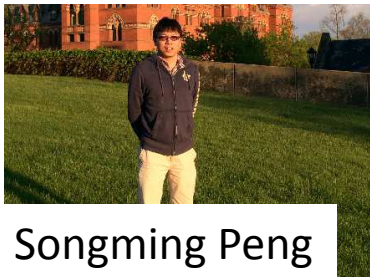
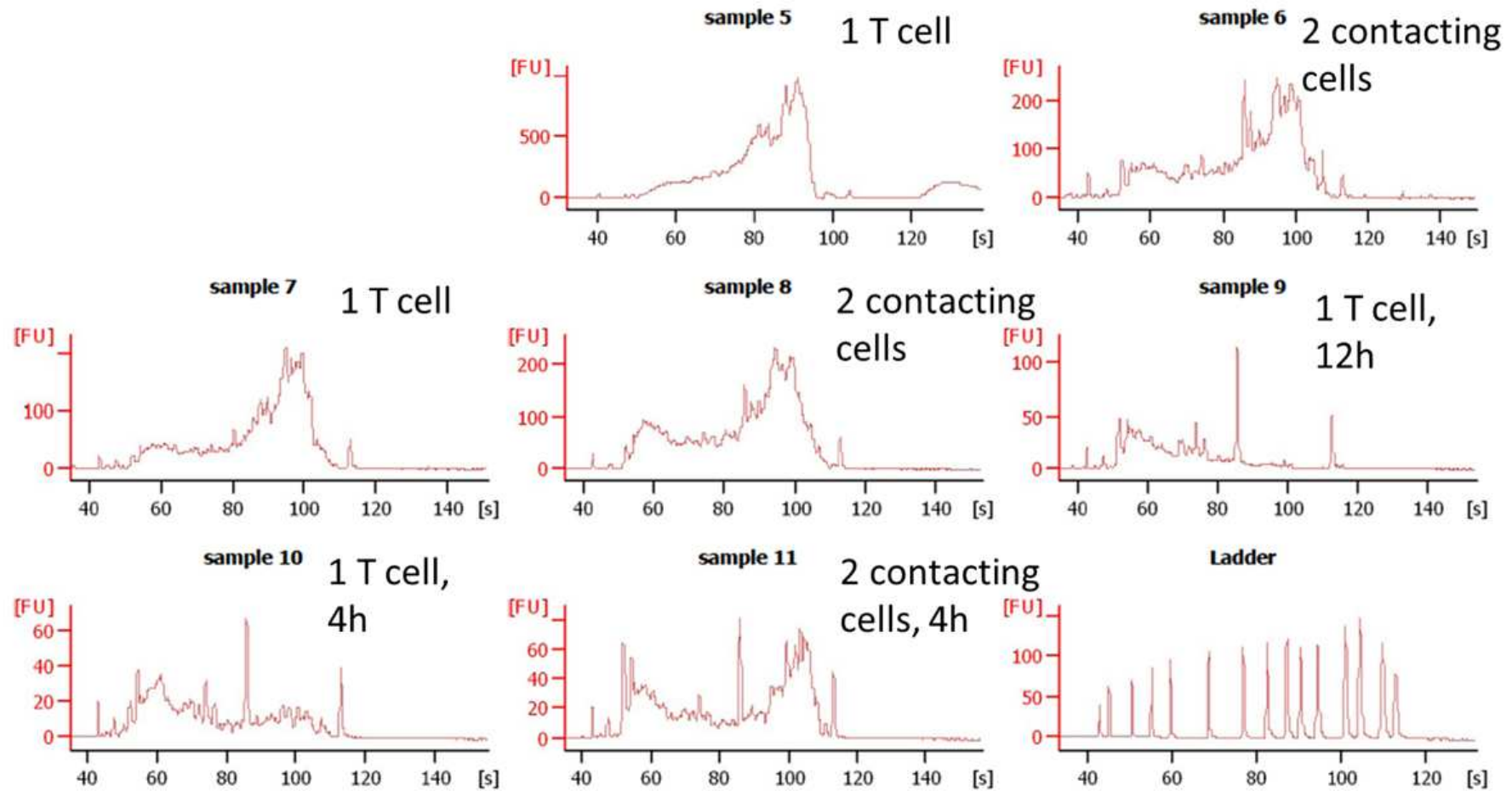


Proteins most commonly upregulated

	CD4	CD8
CD4	IFNg	IFNg, CCL4
CD8		GB, CCL4

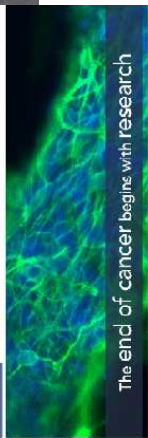


Following Functional Proteomic Analysis of Single T cells and T cell pairs by whole transcriptome analysis of those same cells





UCLA Jonsson Comprehensive Cancer Center



S↑**2C**



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