Analysis of gene expression in adoptively transferred TCR-engineered T cells

SITC Annual Meeting
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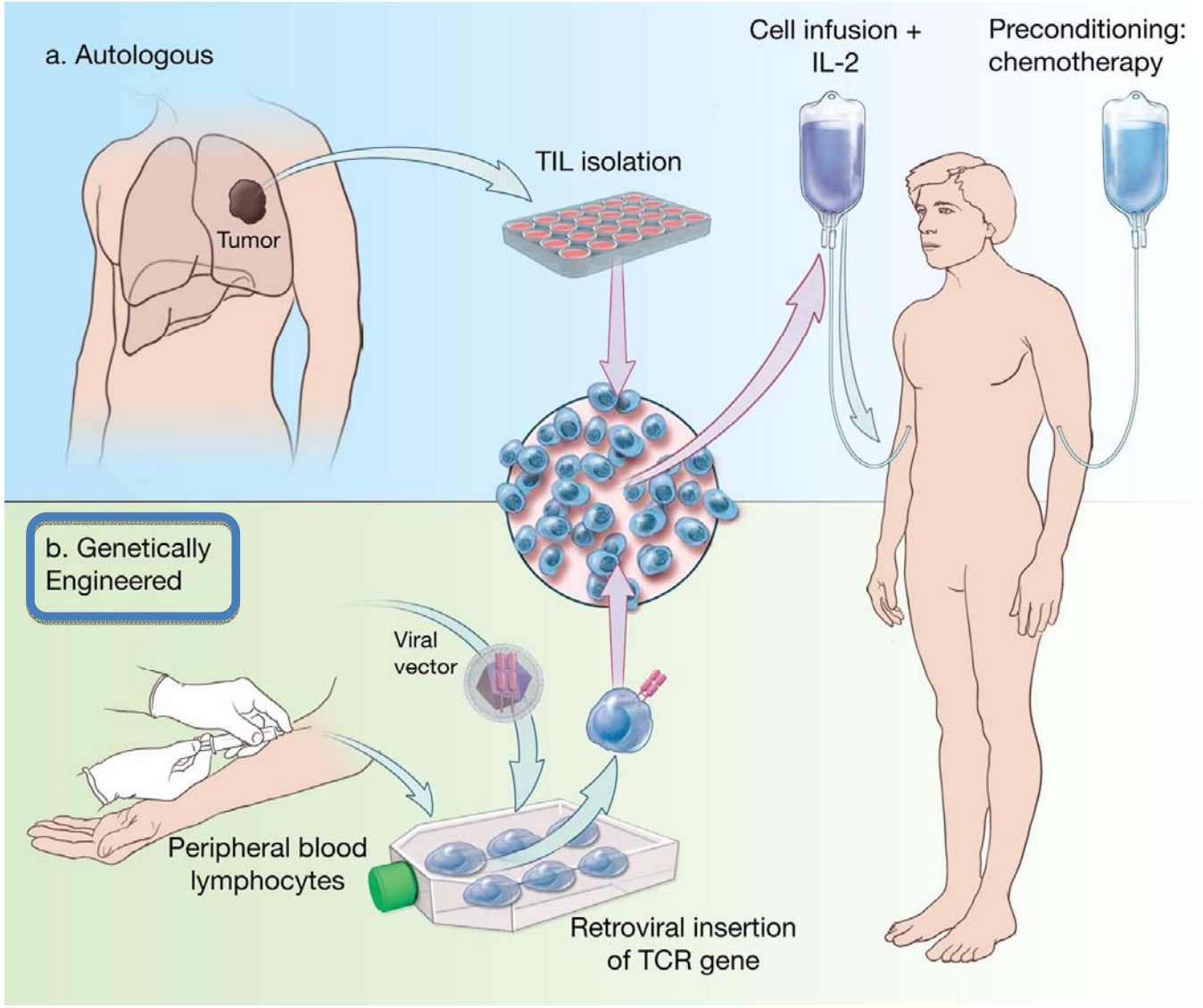
Presenter Disclosure Information

Daniel Abate-Daga, Ph.D.

The following relationships exist related to this presentation:

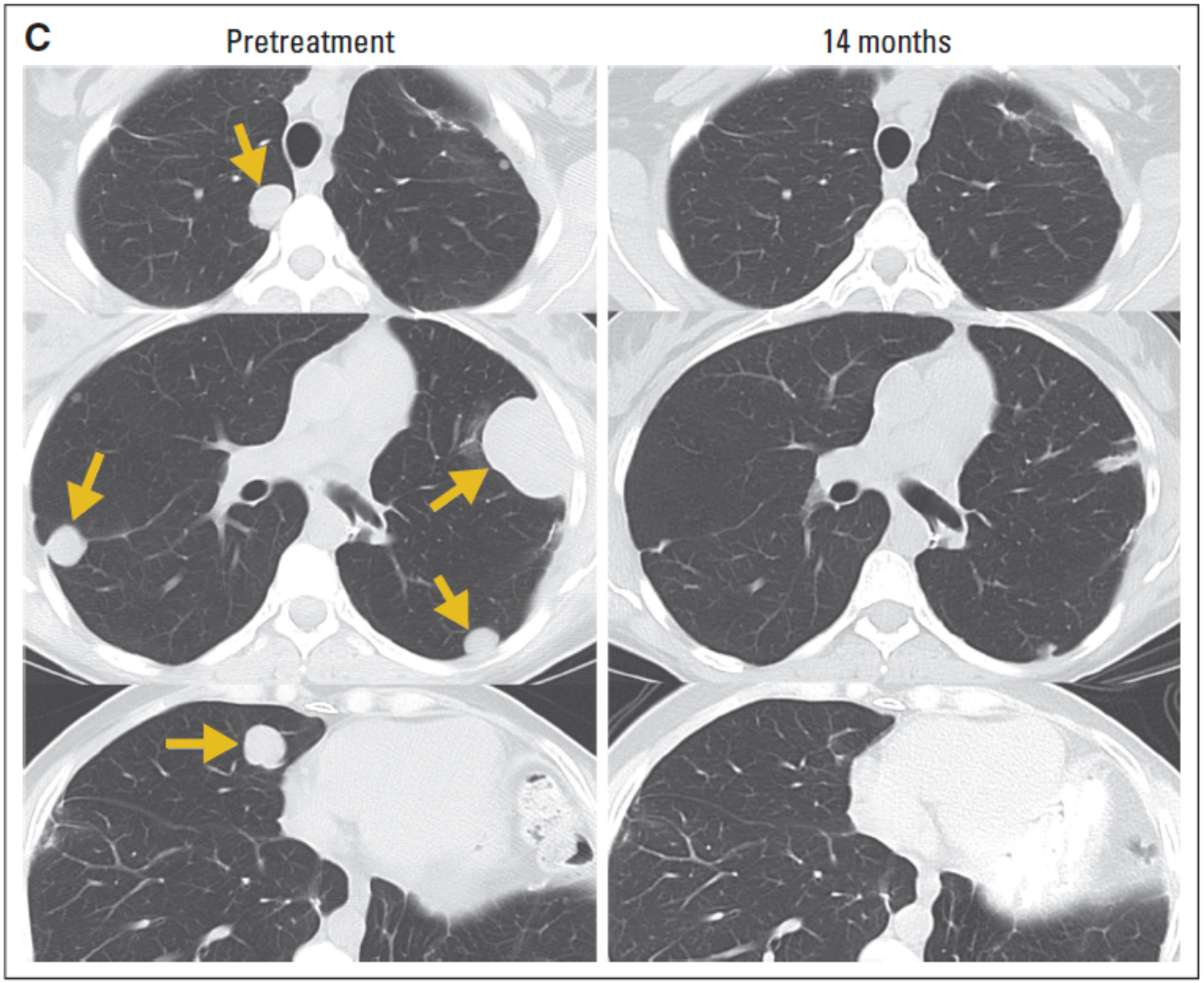
No Relationships to Disclose

Gene-modified PBL are able to mediate objective tumor regression



Park *et al*, Trends in Biotechnology, 2011

Gene-modified PBL are able to mediate objective tumor regression

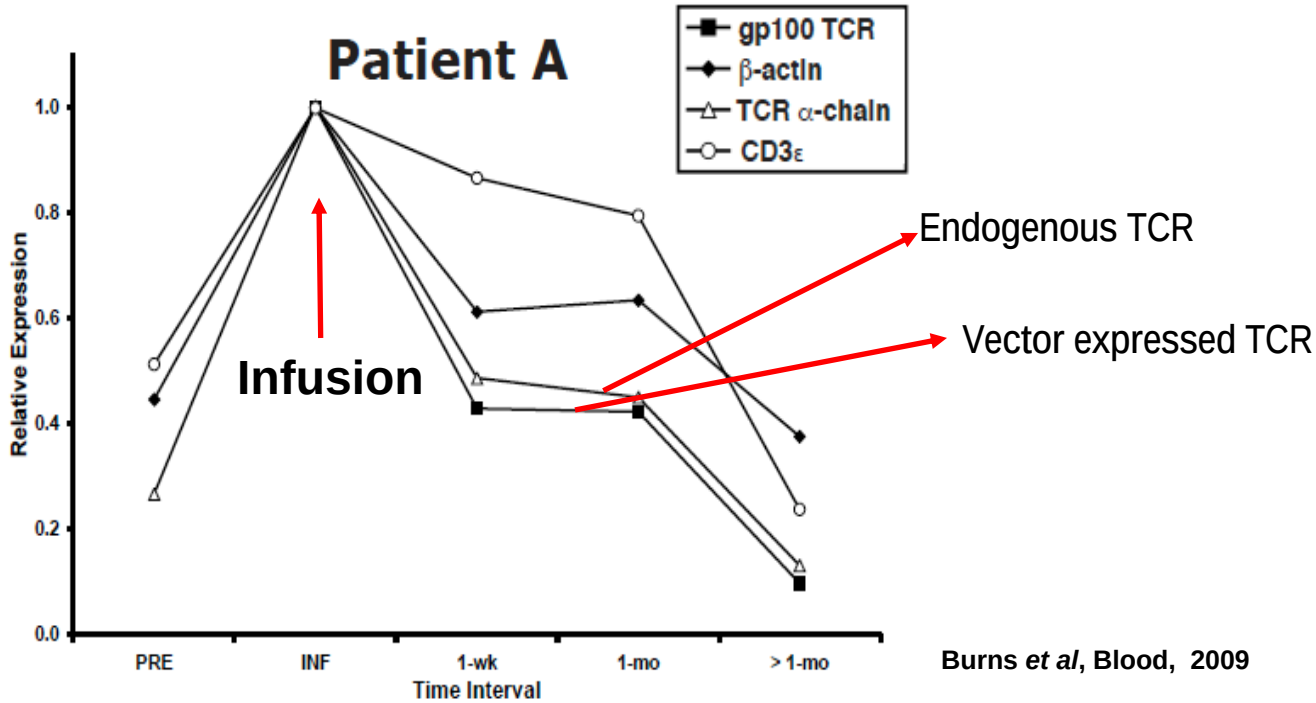


Response rate NY-ESO1 TCR: 45% for melanoma, 67 % for SCS

Robbins *et al*, J. Clin. Oncol., 2011

TCR-transduced T cells persist after transfer but TCR gene expression decreases due to a global down-regulation in gene expression.

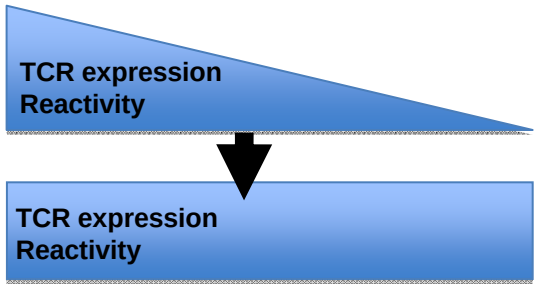
Relative expression in T cells



Burns *et al*, Blood, 2009

Highly activated TCR+ cells

Resting TCR+ cells

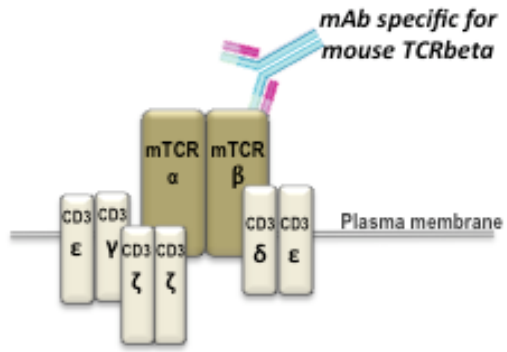


Goal:

Analysis of gene expression: Sample processing

≈-21 — **Transduction and REP** — **0** — **IL-2** — **≈30** — Time (days)

Leukapheresis — **Infusion** — **Leukapheresis**



Pre-infusion

Thawed and magnetically sorted for CD3+

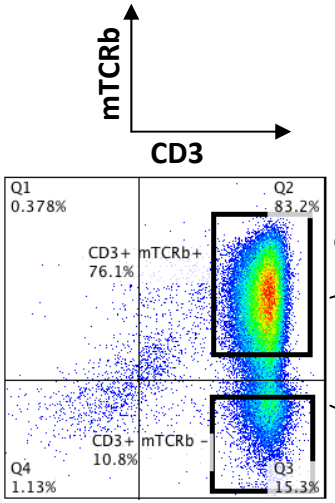
Infusion

Thawed

1 month post-infusion samples

Thawed and FACS-sorted

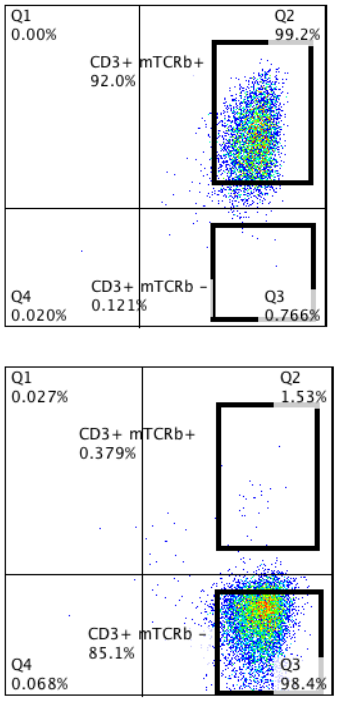
FACS SORT



CD3+ mTCR+

CD3+ mTCR-

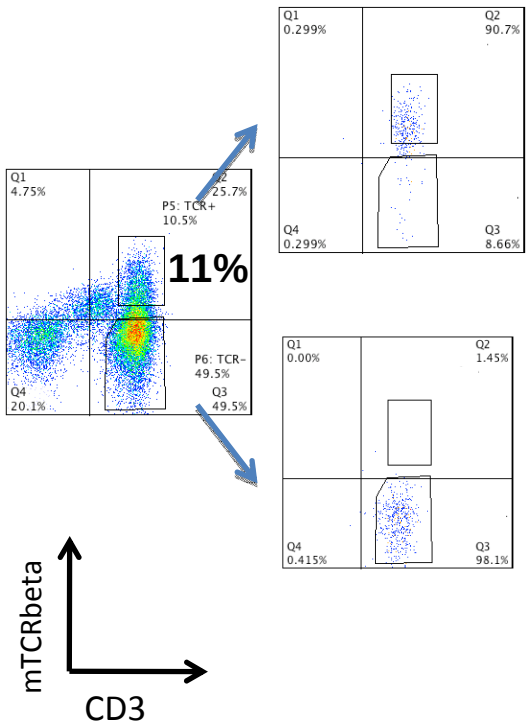
Post-SORT analysis



Donor: patient from gp100 (154) trial

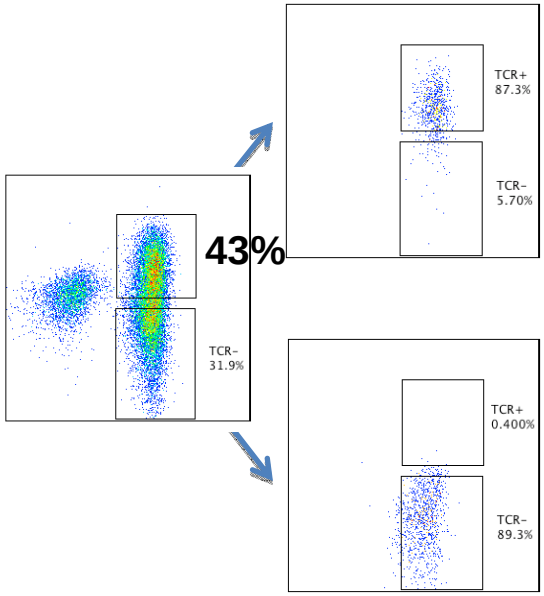
Representative sorting results from patients included in analysis

Patient 1
35 days post-infusion



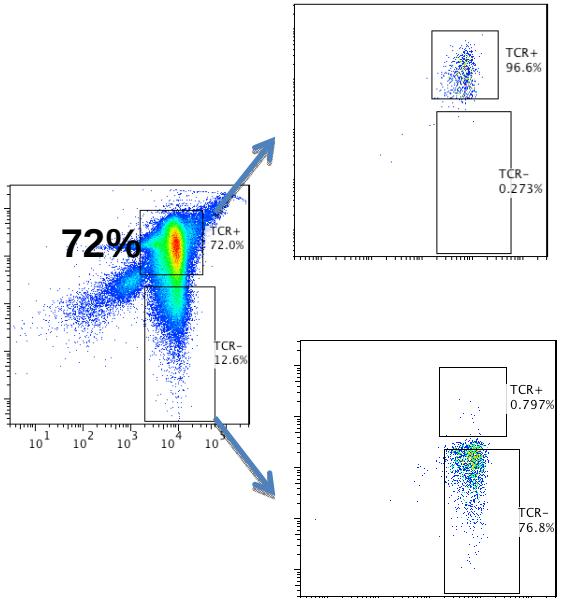
-Received $55.8e^9$ cells.
-No response.

Patient 2
25 days post-infusion



-Received $23.5e^9$ cells.
-Partial response

Patient 3
31 days post-infusion



-Received $68e^9$ cells.
-Complete response.

Screening for genes involved in down regulation of TCR expression by transcriptomic analysis

Pathway-focused PCR array

Pros:

-Accurate quantification

-No technical validation required

Cons:

-Low throughput

Genes analyzed

Lymphocyte Regulatory genes: BTLA, CBLB, CD27, CD28, FAS, FOXP3, IL15, IL2, IL2RA, IL4, LAT, TGFB1, TNFRSF14, TNFSF14, BTLA, CD27, CD40, CD40LG, FOXP1, FOXP3, HDAC9, IL4.

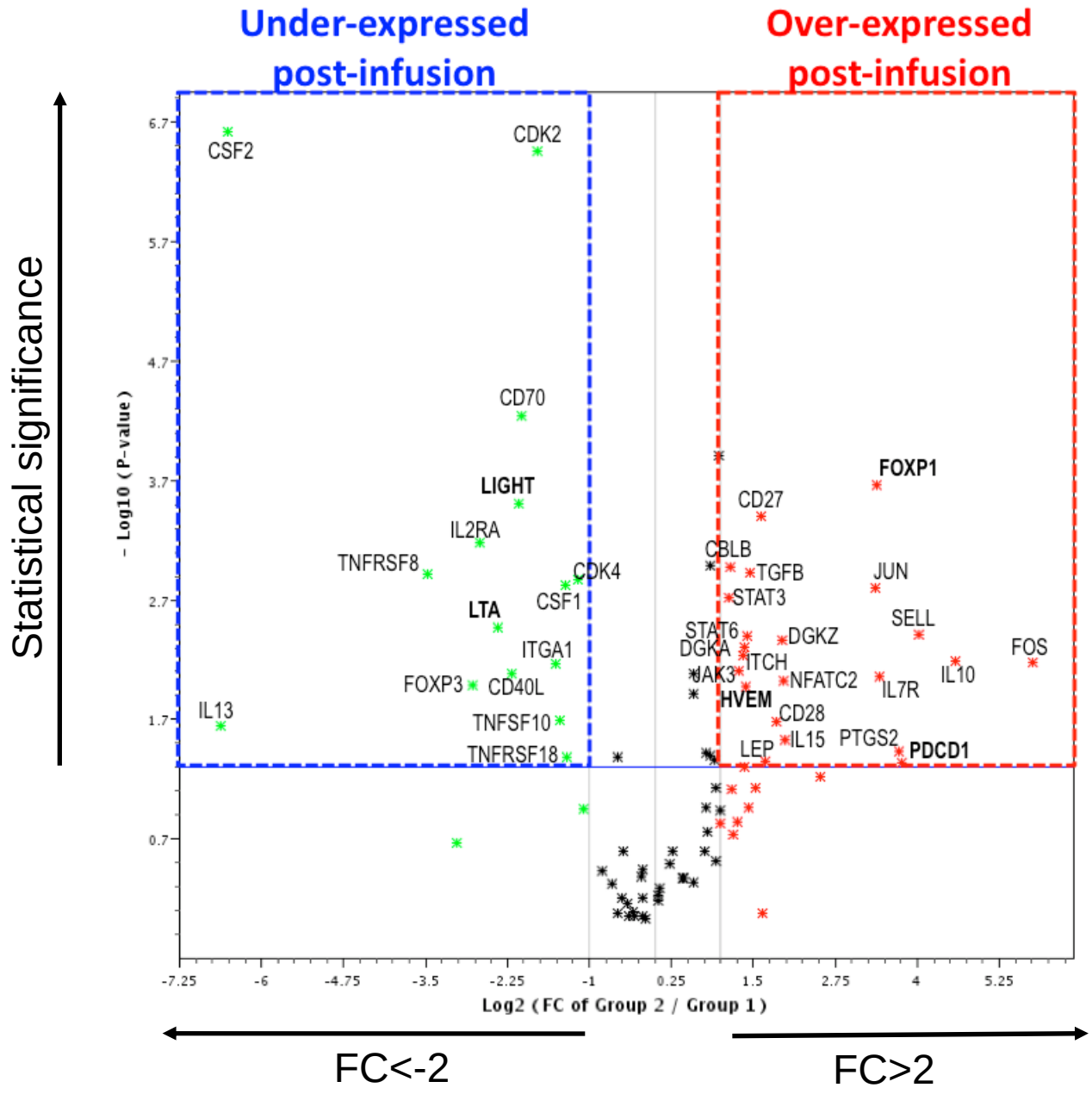
Cytokines, Receptors and their related Proteins: CCL3L1, CCR4, CD40LG, CD70, CSF1, CSF2, FASLG, IFNG, IL10, IL10RA, IL13, IL15, IL17A, IL1A, IL2, IL2RA, IL2RB, IL31, IL4, IL5, IL6, IL7R, LEP, LTA, PRF1, PTGER2, PTGS2, TGFB1, TNFSF10, TNFSF14, TNFSF8.

TNF Superfamily Members and their Receptors: CD40LG, CD70, FAS, FASLG, LTA, TNFRSF10A, TNFRSF4, TNFRSF18, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF10, TNFSF14, TNFSF8.

Transcriptional Regulators: CDK2, CDK4, EGR2, EGR3, EOMES, FOS, FOXP1, FOXP2, FOXP3, GATA3, HDAC9, IFNG, ING4, IRF4, JAK3, JUN, MEF2A, NFATC1, NFATC2, NFATC3, NFKB1, NHLH2, NOTCH1, STAT3, STAT6, TBX21, TGFB1.

Other Genes involved in T-cell Anergy: CMA1, CTLA4, DGKA, DGKZ, GZMB, ICAM1, ICOS, ITCH, ITGA1, JAK1, LGALS3, PDCD1, PRKCG, RNF128, SELL.

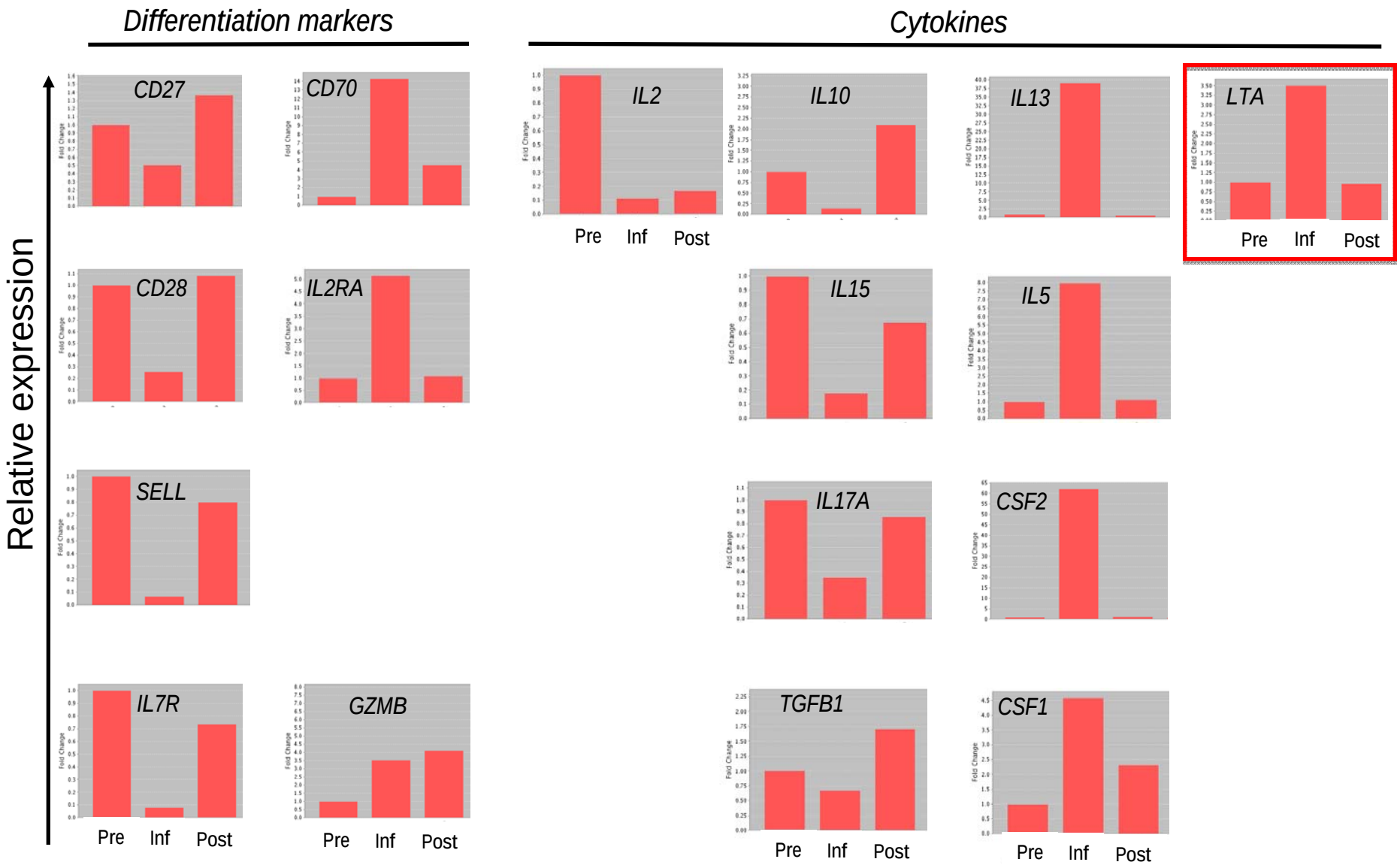
Post-infusion mTCRb+ vs Infusion



(FC>2, <-2; p<0.05)

Gene expression overview

Pre → Inf → Post

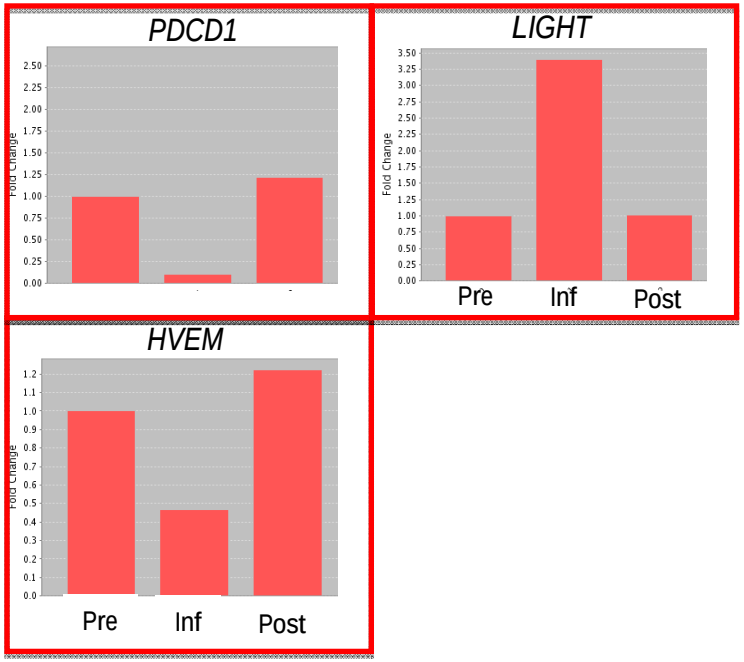


Gene expression overview *(cont.)*

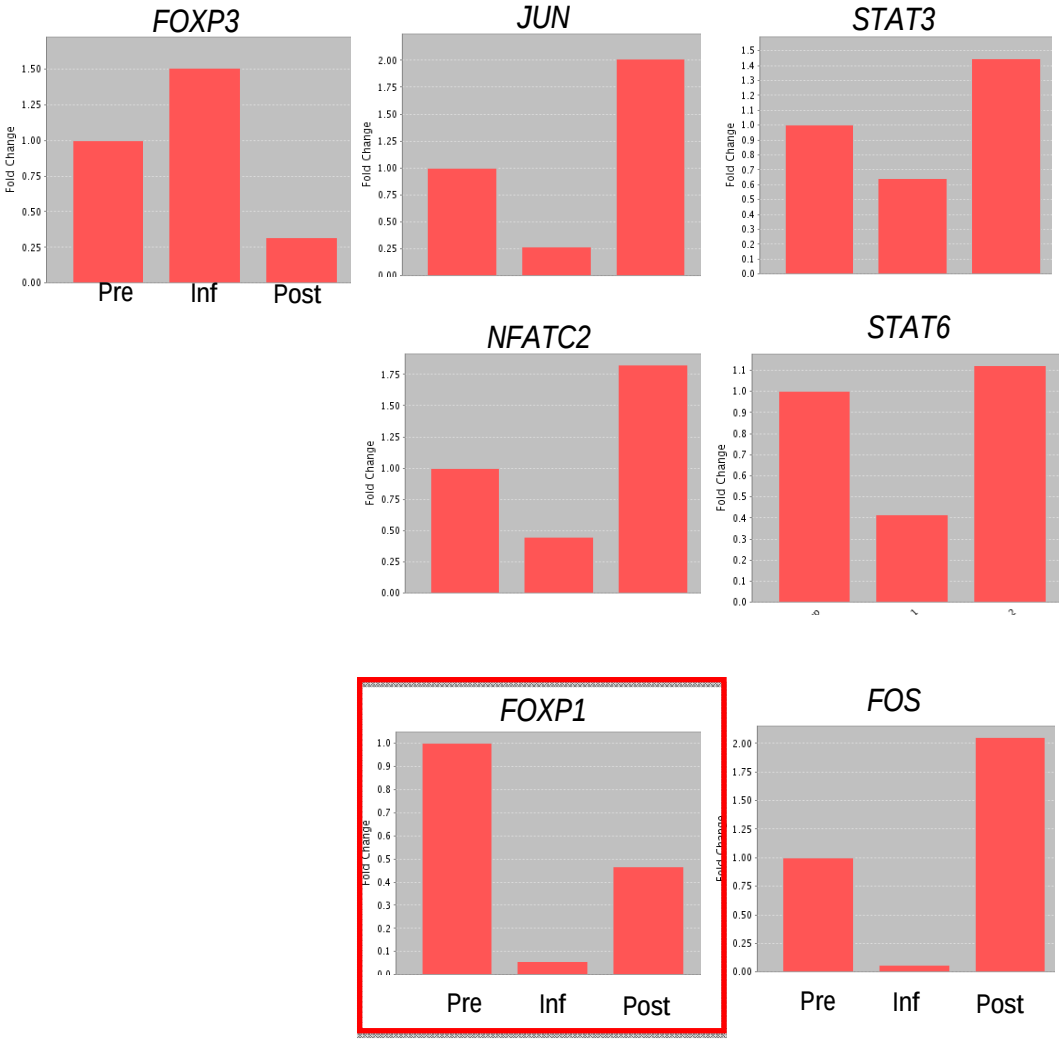
Pre → Inf → Post

Relative expression

Co-stimulatory/co-inhibitory receptors



Transcription factors



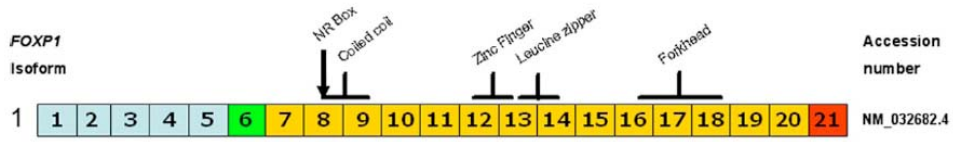
Examples of regulated genes

1- FOXP1

2- PDCD1

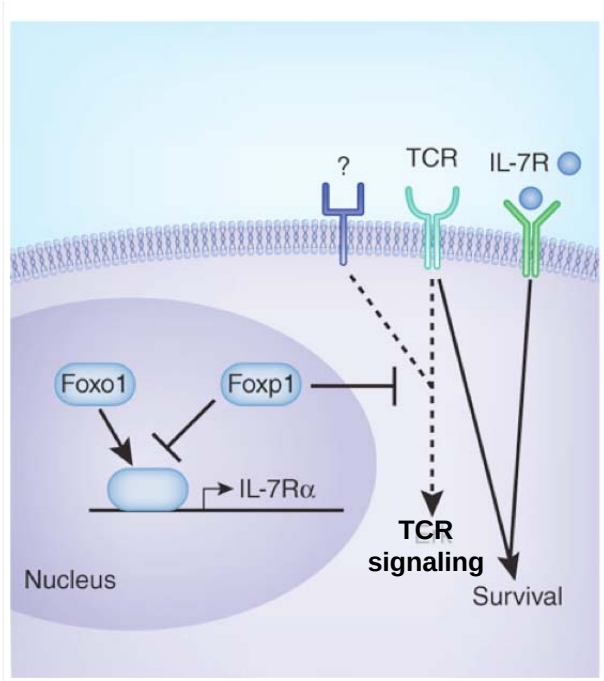
3- HVEM / LTA

1) Forkhead box protein 1 (FOXP1)

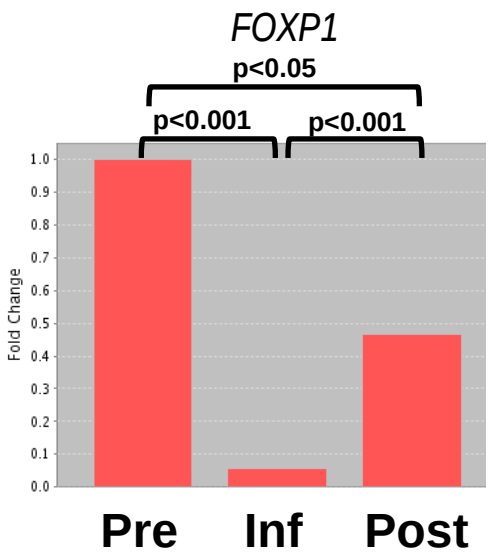


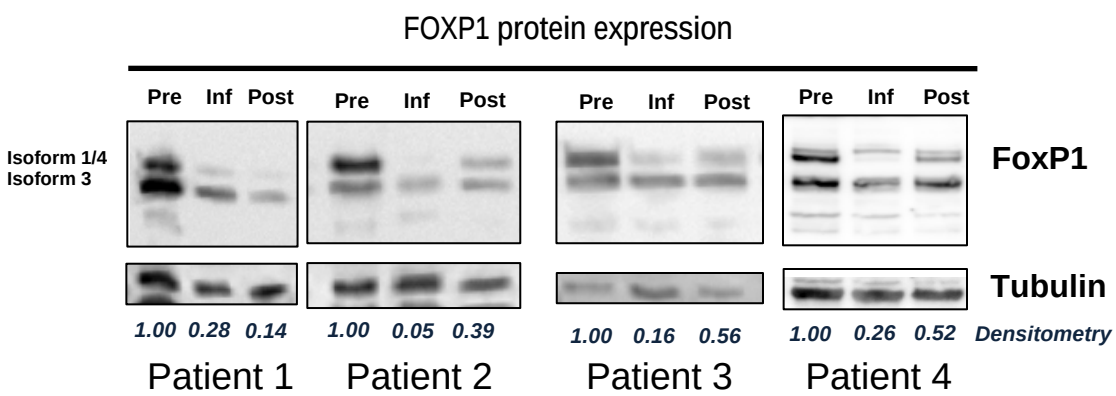
Adapted from Brown *et al*, Blood, 2009

9 FOXP1 isoforms described



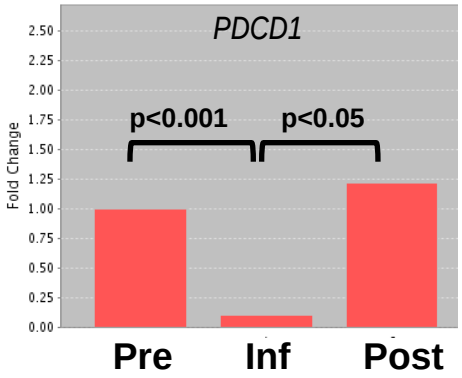
Skon and Jameson, Nat Immunology 2011



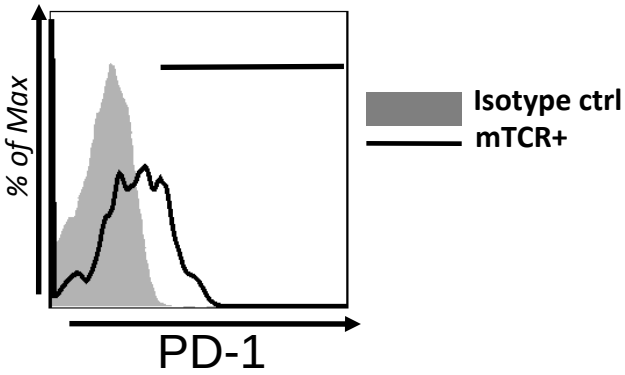


2) PDCD1 (PD-1, CD279)

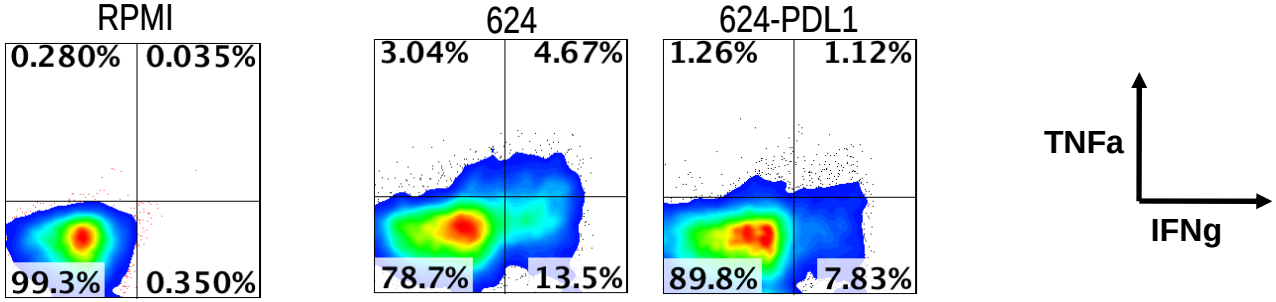
Relative change in gene expression:



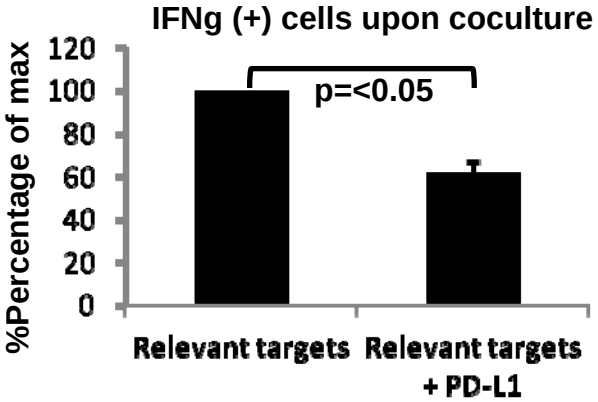
PD-1 expression in post-Inf cells



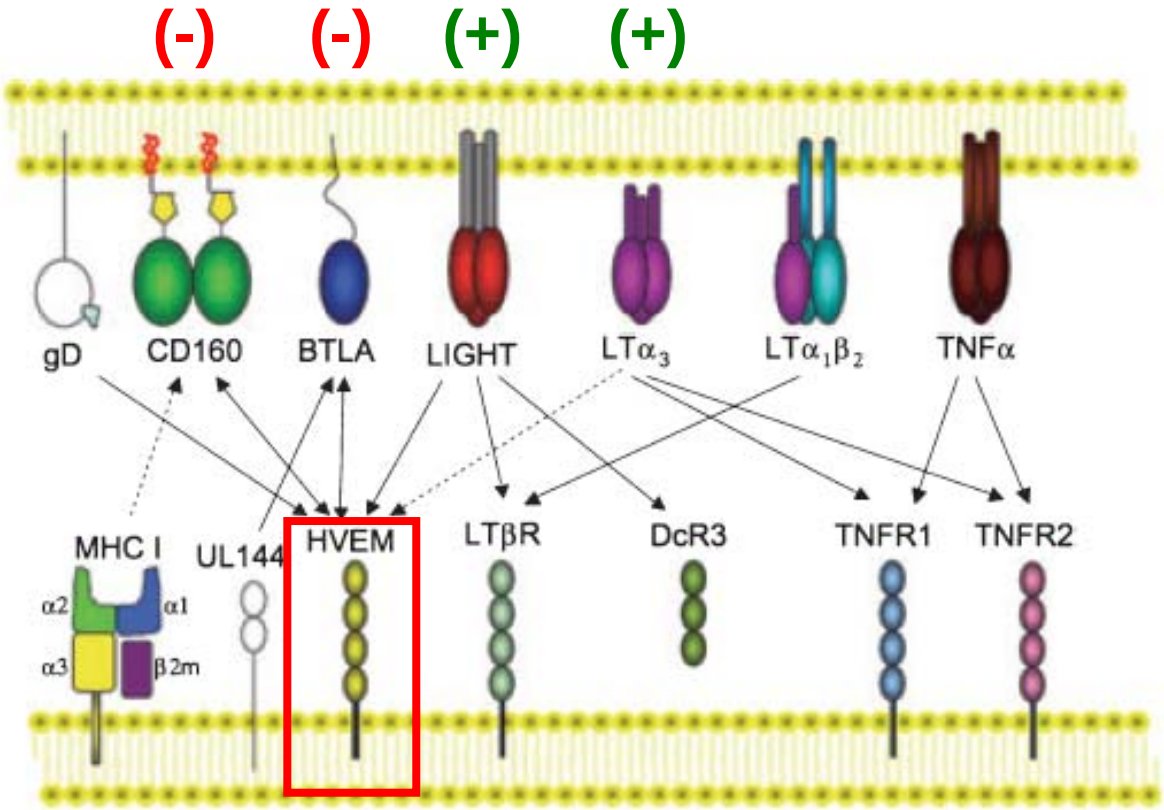
Functional effect:



1 month post-Inf cells are less reactive against PD-L1 targets:



3) HVEM and binding partners

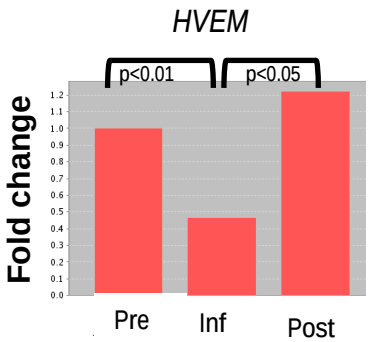


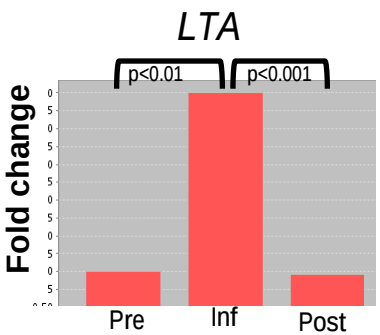
T cell

Adapted from Cai, G. and Freeman, G.J., Immunological Review, 2009

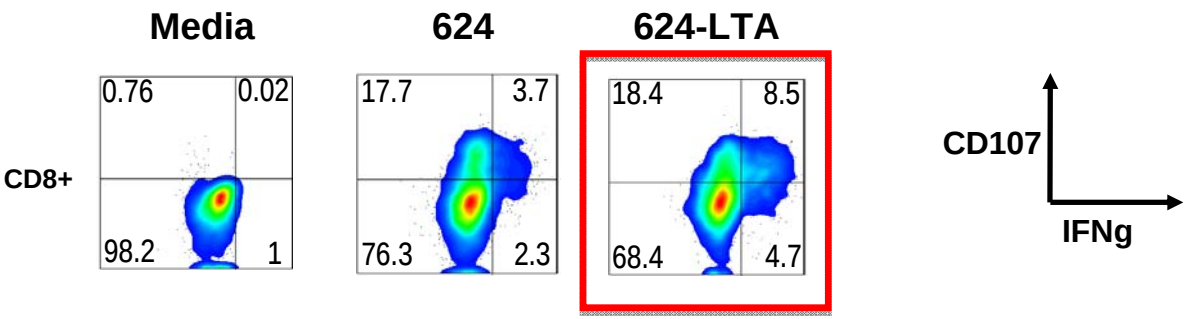
HVEM as a receptor in T cells

Relative change in gene expression:

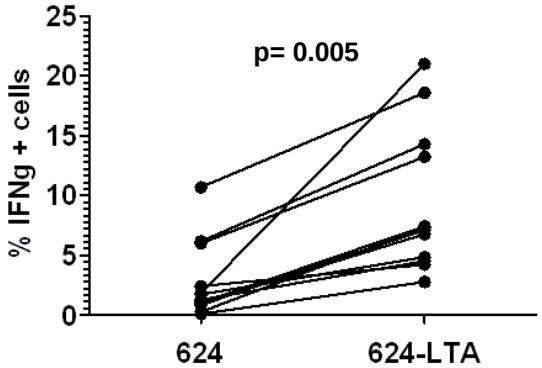




Functional effect:



LTA increases reactivity of infusion TCR cells:



Summary

-Of 84 genes analyzed, 38 were found to be differentially expressed in one month post-infusion engineered T cells as compared to matched infusion samples.

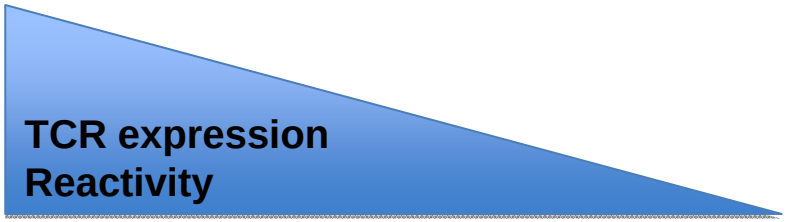
Examples:

Transcription factor FOXP1 was dramatically under-expressed in infusion samples compared to pre-infusion T cells and presented intermediate levels in post-infusion T cells.

-PDCD1 was overexpressed in post-infusion T cells, compared to infusion samples. Surface expression was confirmed by flow cytometry and sensitivity to PD-L1 was observed in coculture experiments.

-HVEM and the genes encoding for its binding partners, LTA and LIGHT, were found to be differentially expressed between post-infusion and infusion T cells. Overexpression of LTA resulted in increased production of IFN gamma by infusion T cells in coculture experiments.

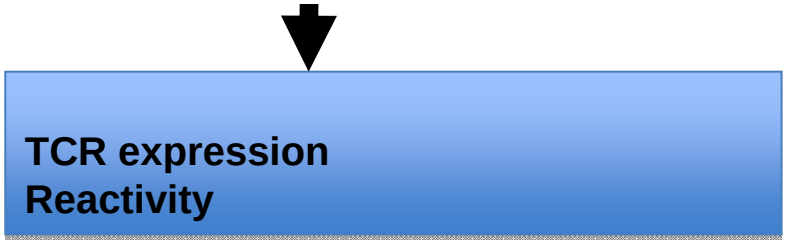
Potential genes for intervention



-Knock-down of FOXP1

-Knock-down of PD-1

-Overexpression of LTA



Acknowledgements

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