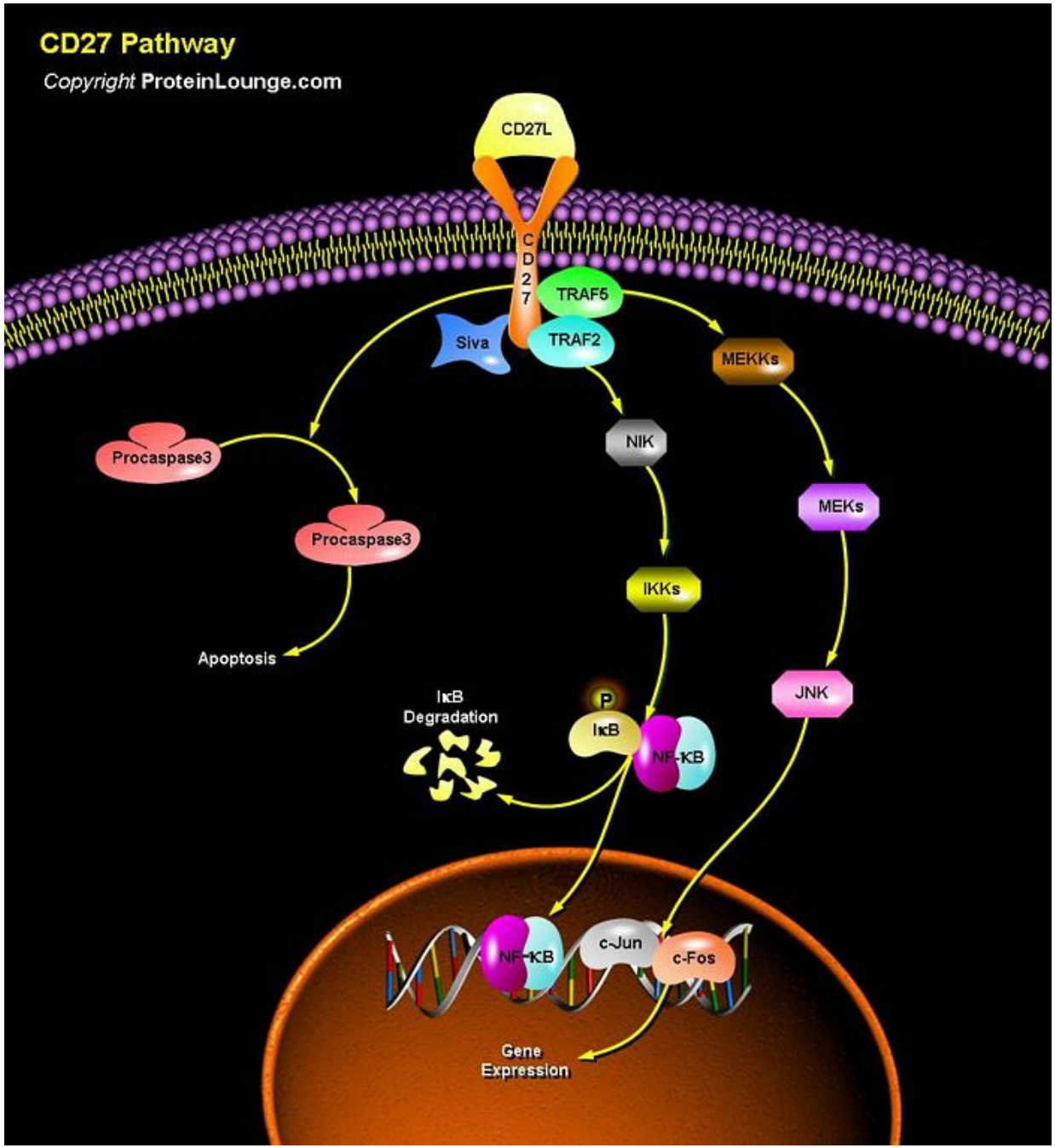
Celldex therapeutics

Antibody Targeting of Human CD27 with Varlilumab (CDX-1127) identifies genes and pathways related to inflammation



Venky Ramakrishna PhD
Celldex Therapeutics, Hampton NJ, USA

www.celldextherapeutics.com



Celldex therapeutics

Targeting Cell Surface Molecules for Immune Modulation with mAbs

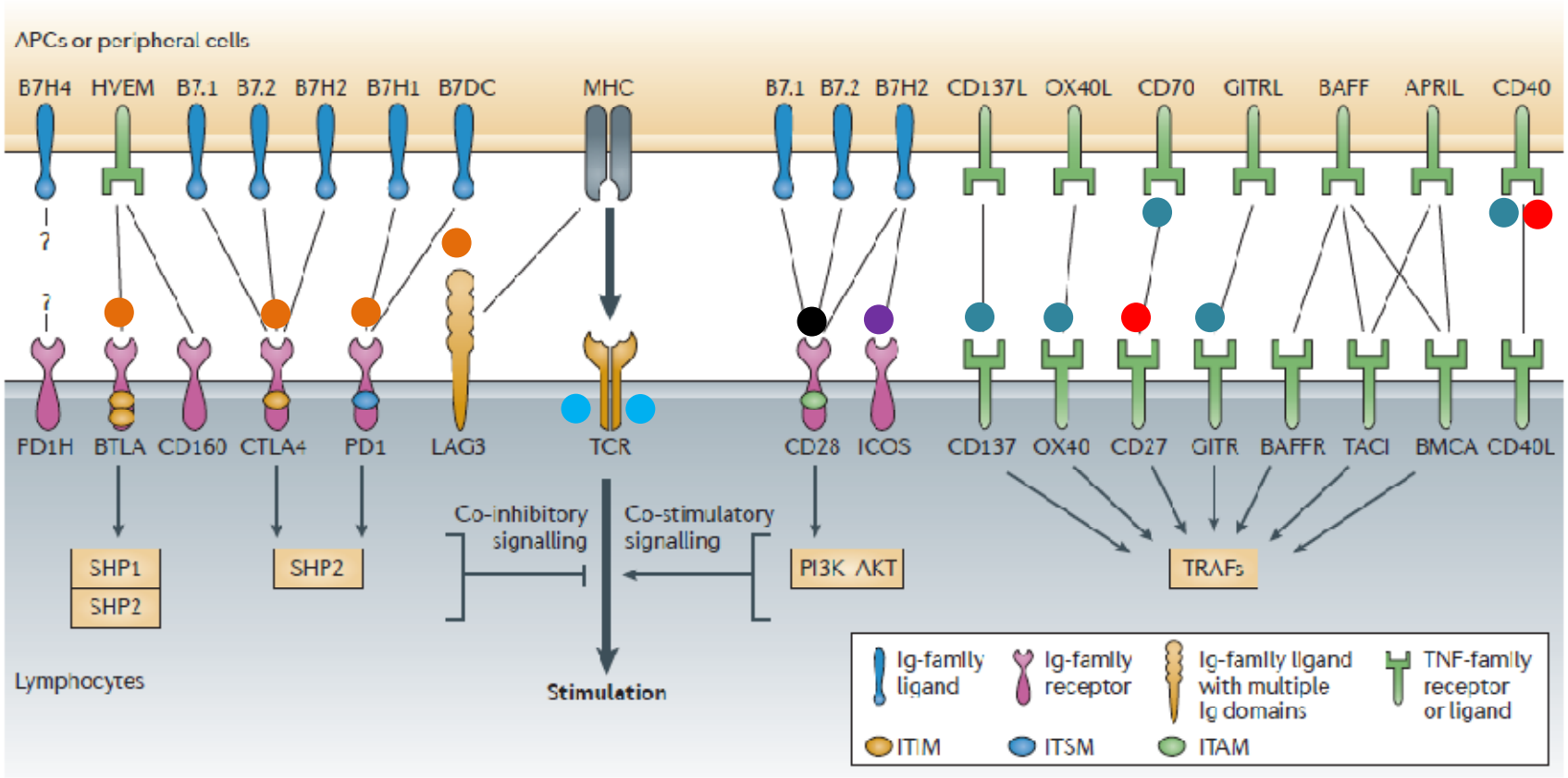


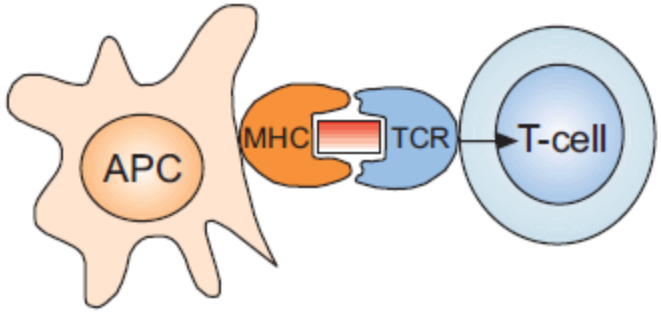
Figure 1 | **Cell surface signalling molecules as important therapeutic targets.** Co-signalling is a complex event that is coordinated through a network of ligand–receptor interactions on the cell surface, with both co-stimulatory and co-inhibitory capacities. The direction and outcome of immune responses are ultimately decided by the interplay of these complicated and often counterbalancing network interactions. Co-signalling pathways are thus important targets for therapeutic intervention. The immunoglobulin (Ig) superfamily and the tumour necrosis factor (TNF)–TNF receptor (TNFR)

Sheng Yao, Yuwen Zhu and Lieping Chen Nat. Rev. Drug Disc. 2013; ***130***

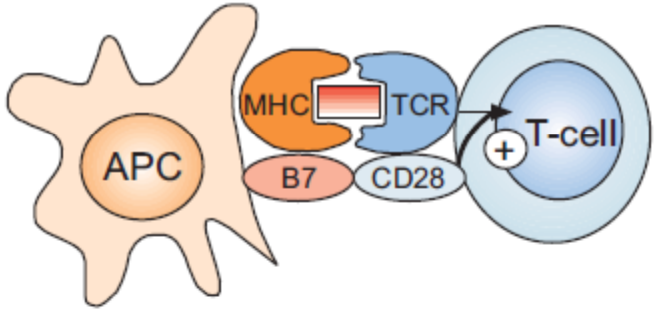
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To Costimulate or Not to Costimulate?

a)

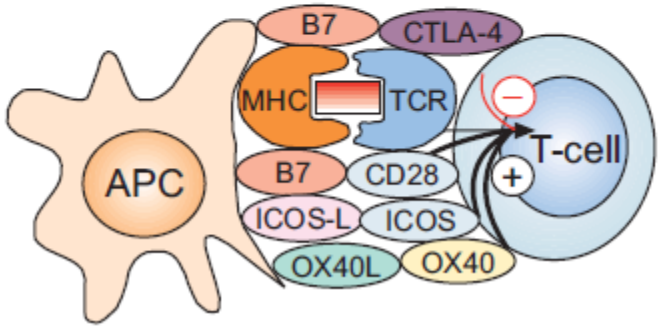


Signal 1 alone:
anergy



Signal 1 + Signal 2:
proliferation
differentiation
cytokine production

b)



Integration of multiple
positive and negative signals

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BJP British Journal of Pharmacology

RESEARCH PAPER

Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells

D Eastwood, L Findlay, S Poole, C Bird, M Wadhwa, M Moore, C Burns, R Thorpe and R Stebbings

Biotherapeutics Group, NIBSC, Potters Bar, Hertfordshire, UK

BJP British Journal of Pharmacology

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DOI:10.1111/j.1476-5381.2010.00922.x
www.bjpharmacol.org

Correspondence
Dr Richard Stebbings, NIBSC, Potters Bar, Hertfordshire EN6 3QG, UK. E-mail: Richard.Stebbings@nibsc.hpa.org.uk

Keywords
TGN1412; cytokine storm; CD28; monoclonal antibody; effector memory T-cells; pre-clinical safety testing; pro-inflammatory cytokines

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J. Immunol. 2007, 179: 3325-31

"Cytokine Storm" in the Phase I Trial of Monoclonal Antibody TGN1412: Better Understanding the Causes to Improve PreClinical Testing of Immunotherapeutics

Richard Stebbings, Lucy Findlay, Cherry Edwards,[1] David Eastwood, Chris Bird, David North, Yogesh Mistry, Paula Dilger, Emily Liefooghe, Isabelle Cludts, Bernard Fox, Gill Tarrant, Jane Robinson, Tony Meager, Carl Dolman, Susan J. Thorpe, Adrian Bristow, Meenu Wadhwa, Robin Thorpe, and Stephen Poole[2]

BJP British Journal of Pharmacology

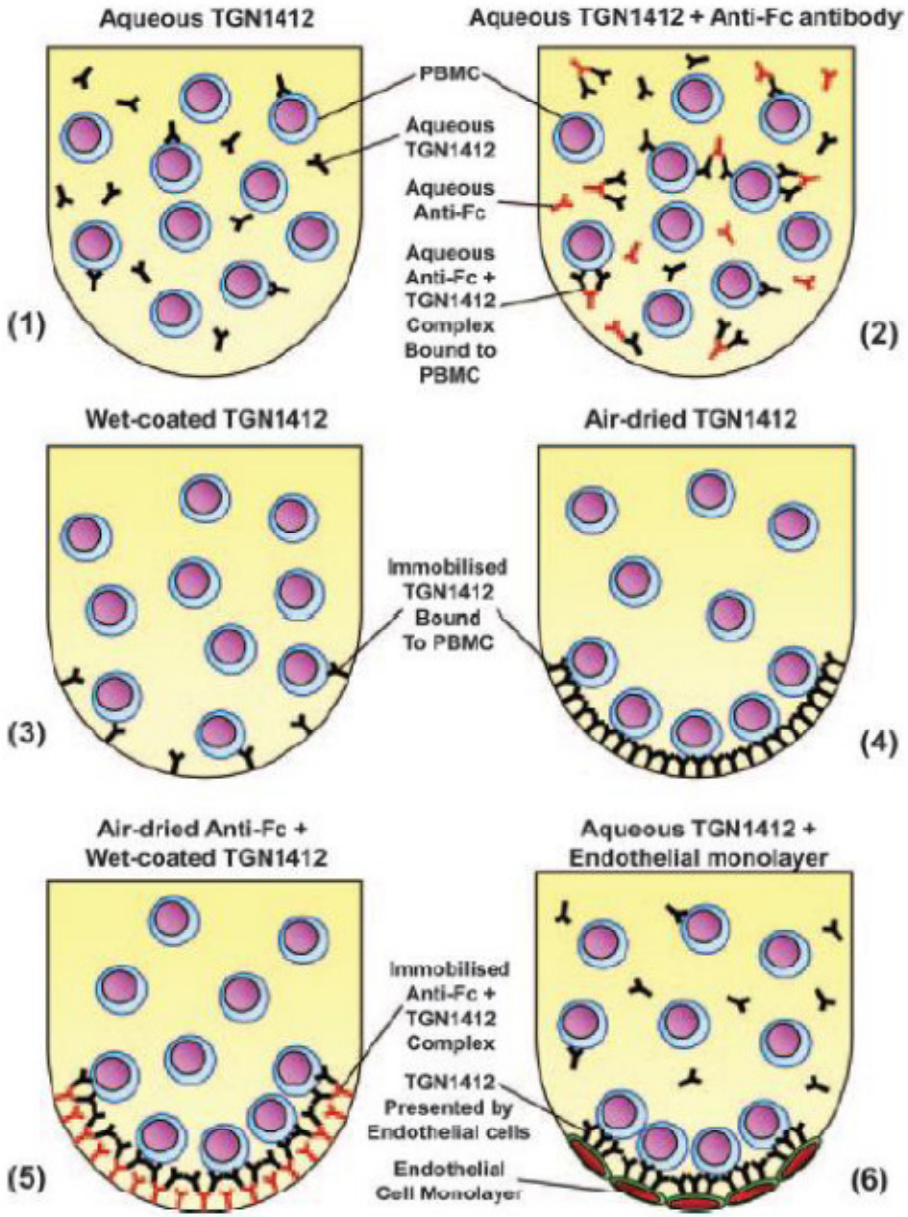
COMMENTARY

Primate testing of TGN1412: right target, wrong cell

M Pallardy[1] and T Hünig[2]

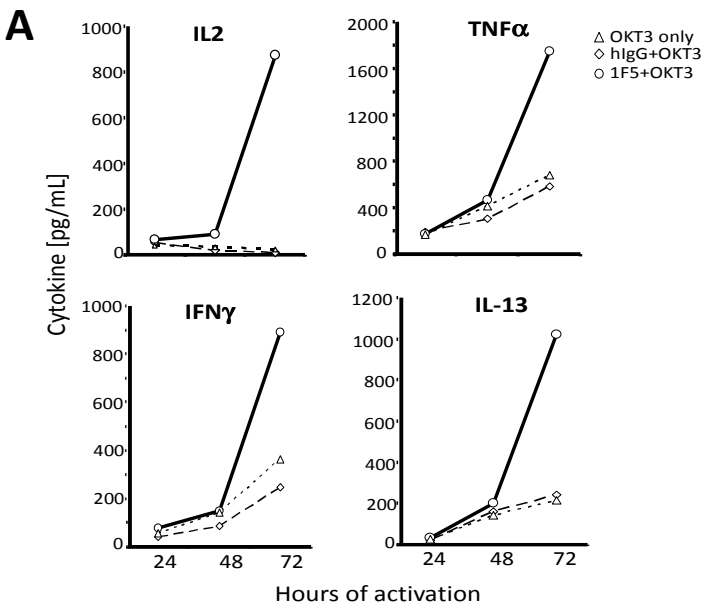
[1]Universud, Faculty of Pharmacy, INSERM UMR 996, Chatenay-Malabry, France, and [2]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany

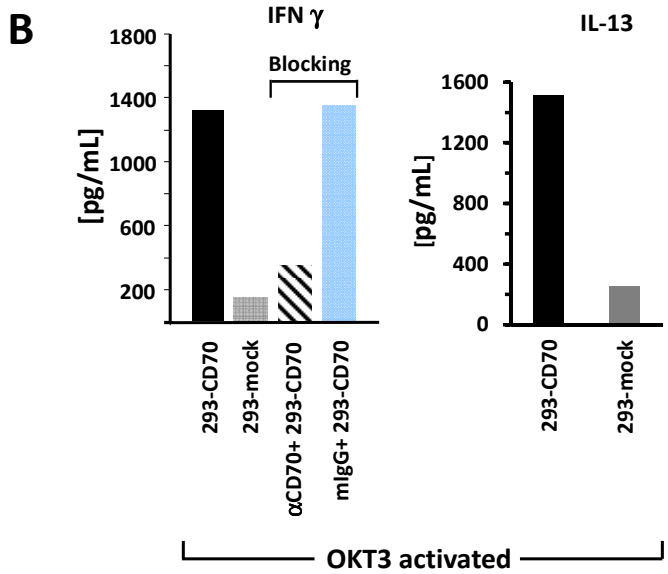
Predicting the Storm

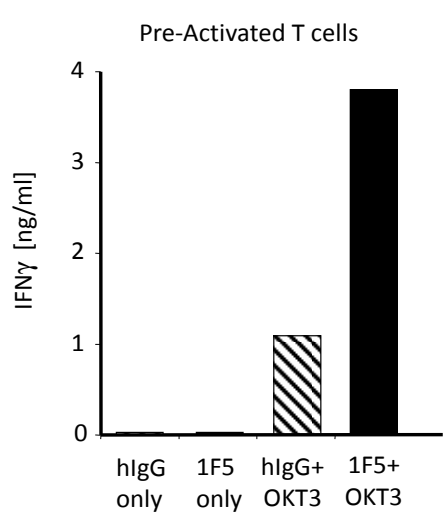


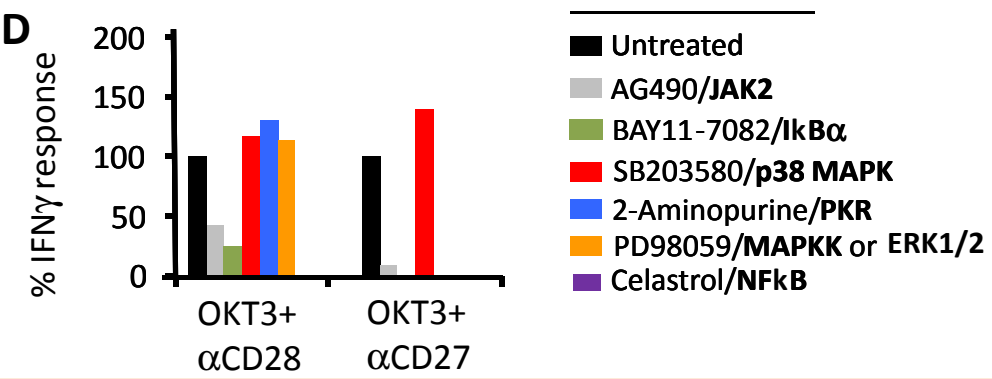
Celldex therapeutics

Costimulation with Varlilumab- Kinetics, TCR-dependence & Signaling Pathways









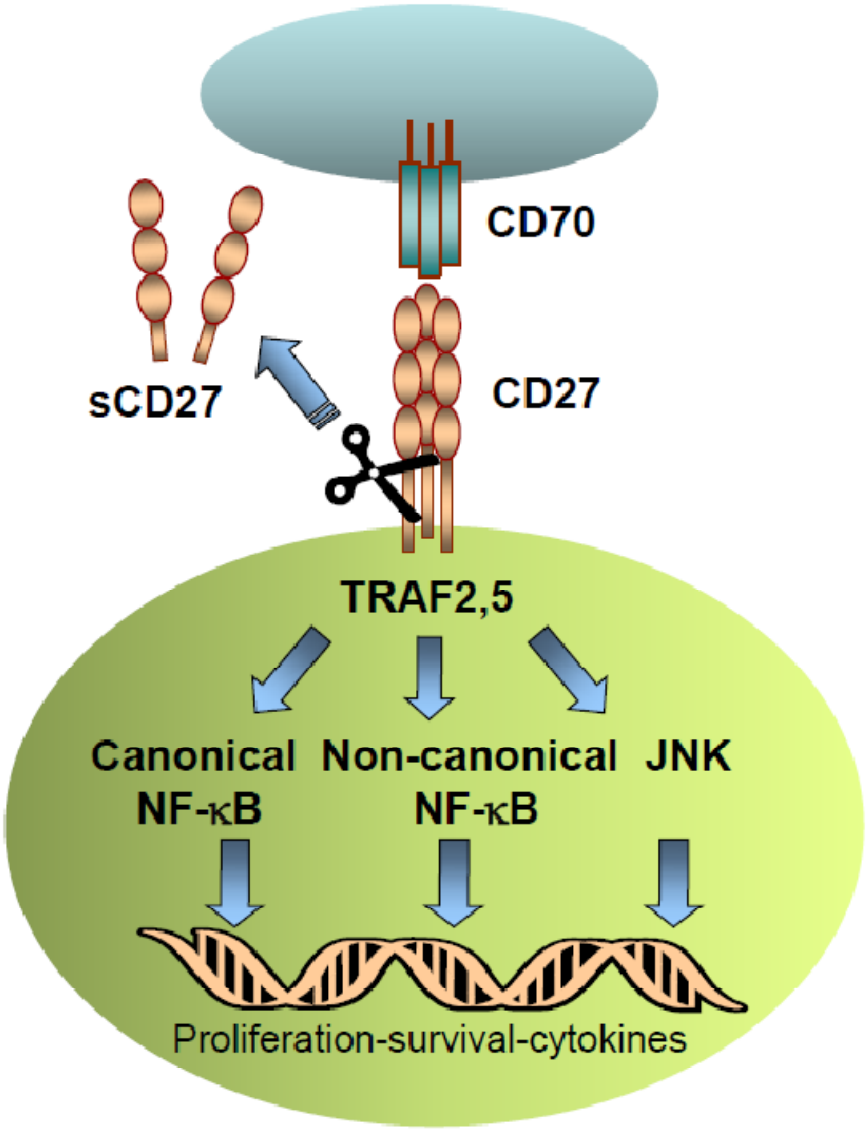
- Costimulation with Varlilumab parallels that with natural ligand- CD70.
- Removal of signal 1 (OKT3) abolishes Varlilumab effect.
- CD28 and CD27 costimulation follow distinct downstream pathways

Celldex therapeutics

sCD27 shedding

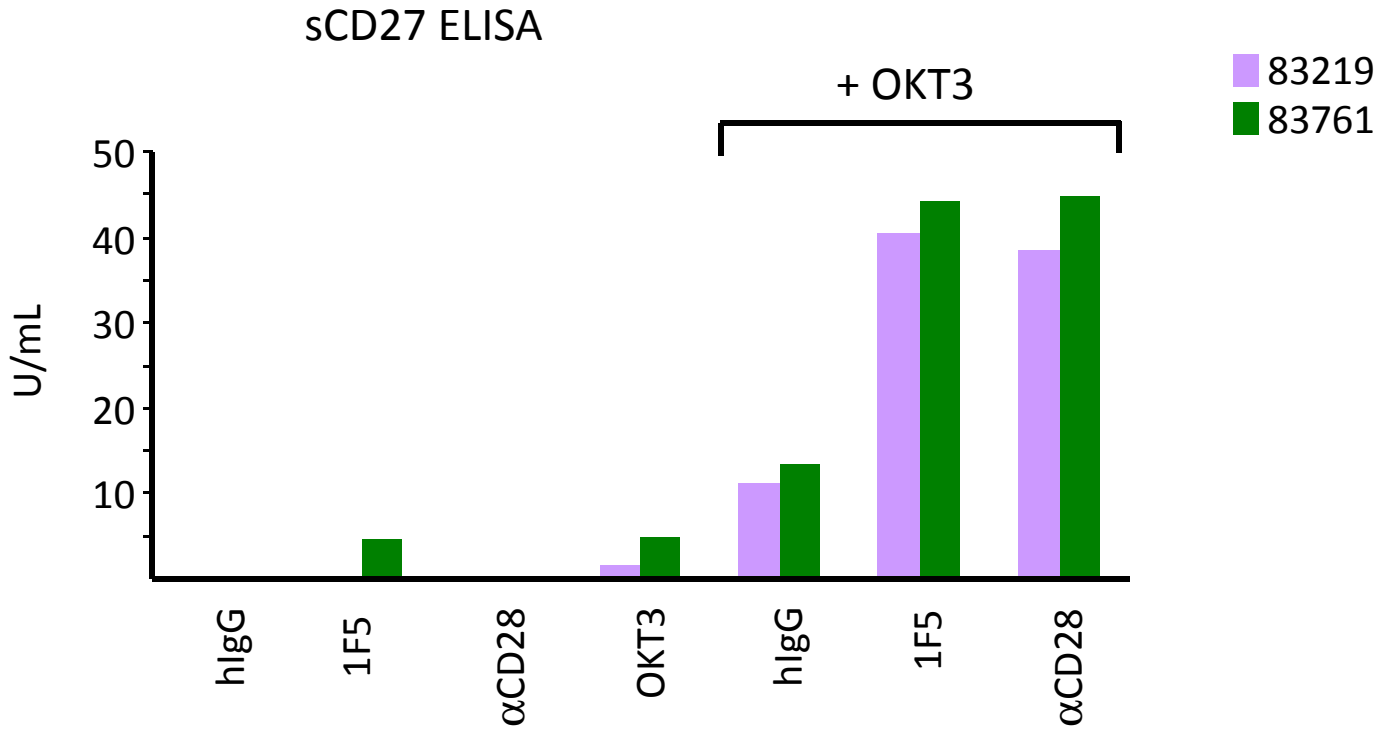
- Circulating biomarker
- Cell culture Supes

- Activation of T cells
- Marker of favorable immune response



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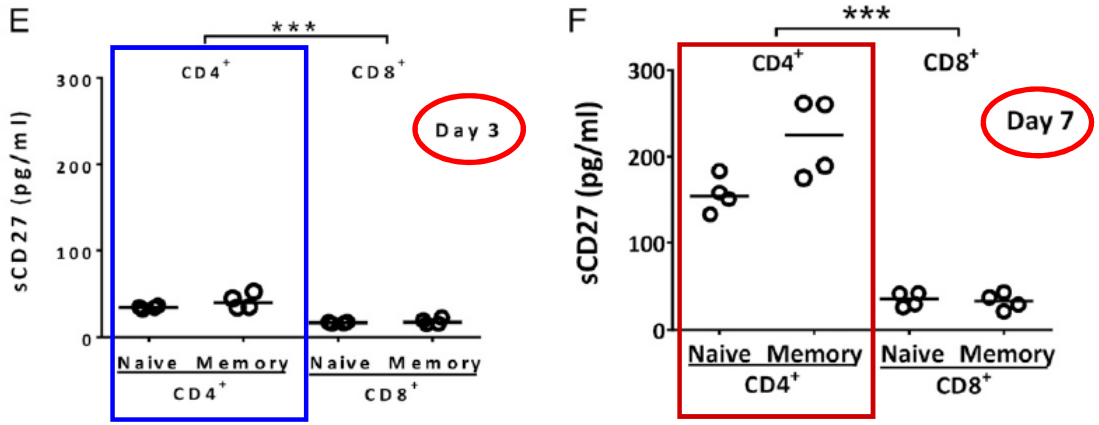
Does 1F5 per se cause sCD27 shedding?

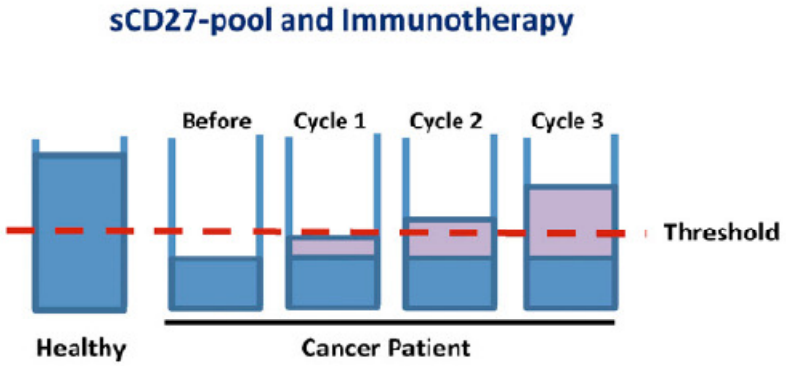


Shedding is a consequence of T cell activation provided by TCR triggering or signal 1 (OKT3) and further augmented in the presence of costimulatory signals via CD27 or CD28 (signal 2). Circulating CD27 as a prognostic serum biomarker in clinically responding patients to immunotherapy has been addressed by Schlom et al. (next slide)

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Patients responding to immunotherapy have elevated sCD27 levels





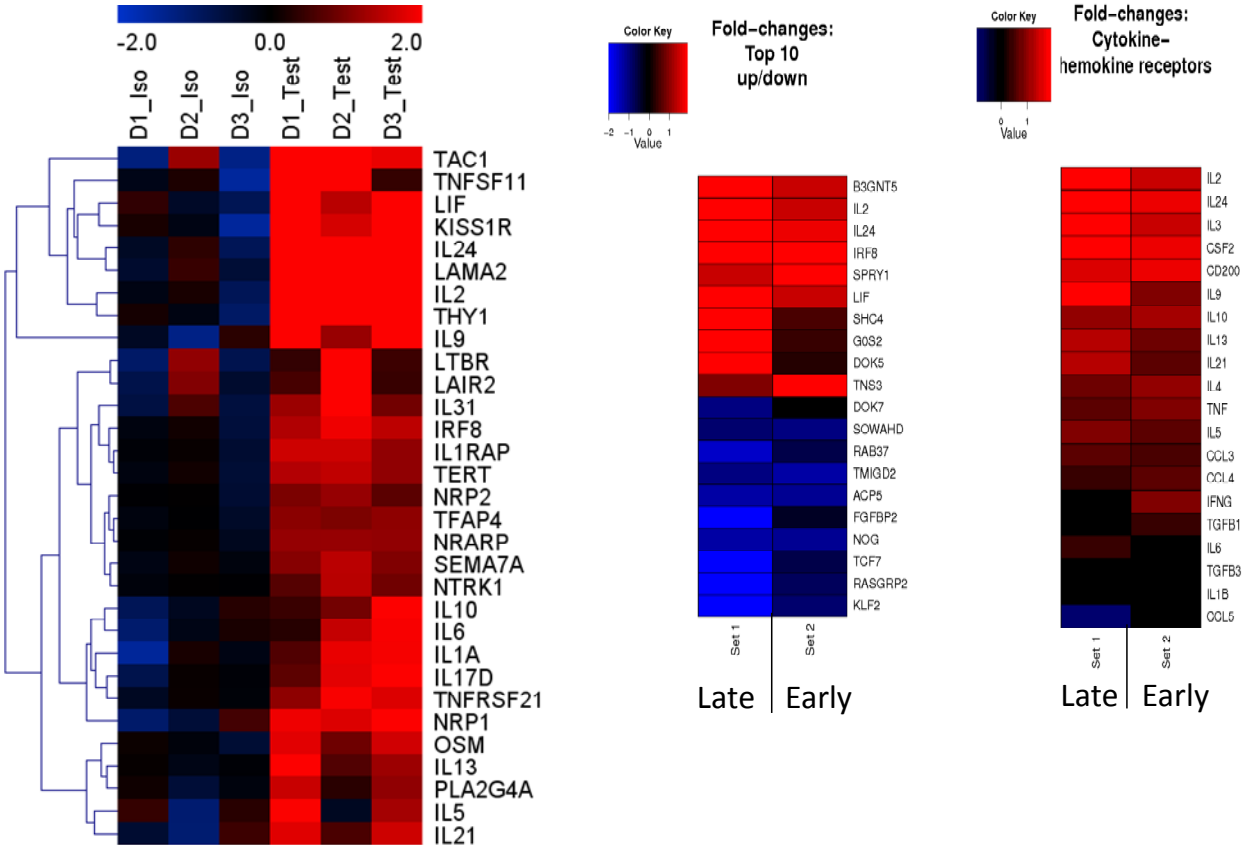
ELISA assays. (**E**) A schematic depiction of the sCD27-pool in healthy donors and cancer patients, and its elevation after immunotherapy. The level of the sCD27-pool is >3-fold higher in healthy individuals than in cancer patients before immunotherapy. After the therapy, the pool was cumulatively refilled as more treatment cycles were given, and some patients reached above a putative threshold (a red dashed line), which is the level required for proper immune function. *$p = 0.022$ (a trend).

Huang J. et al. (2013) sCD27 pool in humans may contribute to T cell activation and tumor immunity. ***Journal of Immunology 190: 6250-6258***

Celldex therapeutics

Molecular footprint of Gene Expression changes in Varlilumab-treated T cells

A **B** **C**



Heatmap showing hierarchical clustering of highly enriched annotated select gene transcripts of cytokines transcription factors and interferon response elements contributing to inflammation - upregulated (red) or downregulated (blue) in Varlilumab-treated and control-treated lymphocytes (**A**); Early and Late changes in genes with Varlilumab treatment- Top 10 genes Up (red) or Down (blue) or changes in cytokine chemokine receptors shown as fold changes over control (**B** and **C**). (*Total probes on chip 29,833 with 320 genes **Up** and 441 **Down**)*

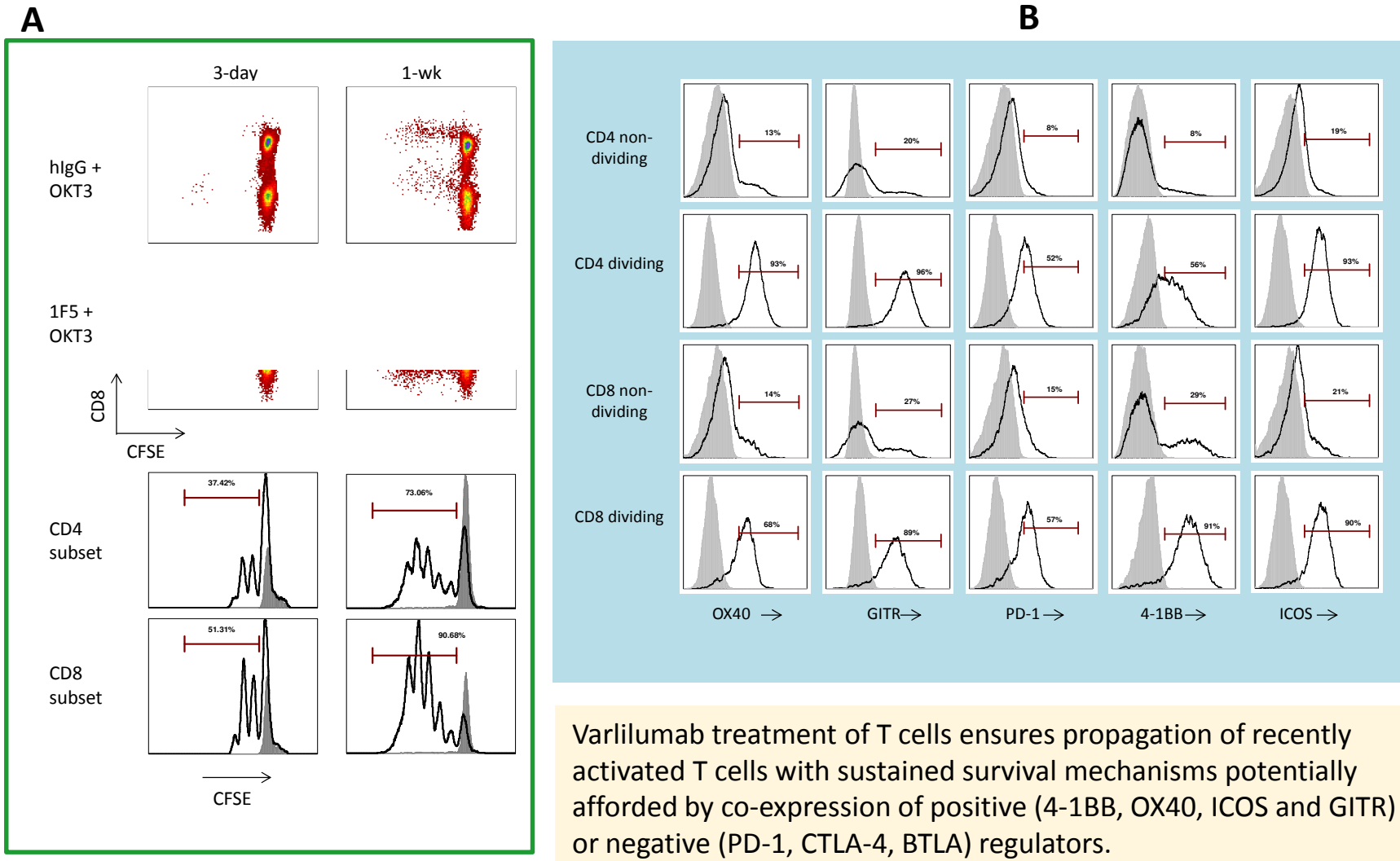
Celldex therapeutics

Biomarkers associated with CD27 costimulation of TCR-triggered T cells – microarray analysis

- **Costimulatory**
  - **4-1BB↑**
  - **GITR↓**
  - **OX40↓**
- **Cell signaling**
  - **Sprouty-1**
    - **MAPKK/ ERK1/2**
    - **JAK2**
    - **FGF-2**
  - **TNFRSF21 [DR6]**
    - **IL-13 production**
    - **B cell differentiation**
- **Coinhibitory**
  - **CTLA-4↓**
  - **Tim-3↑**
  - **LAG-3↓**
  - **BTLA↑**
  - **ICOS↓**
  - **PD-1↑**

Celldex therapeutics

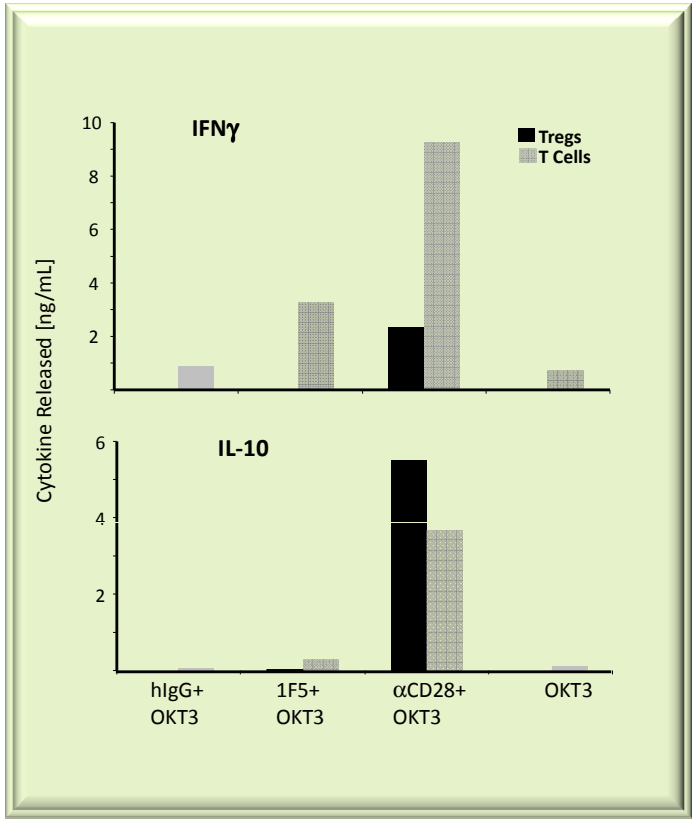
Varlilumab-induced expression of co-signaling molecules in proliferating CD8+ and CD4+ lymphocytes



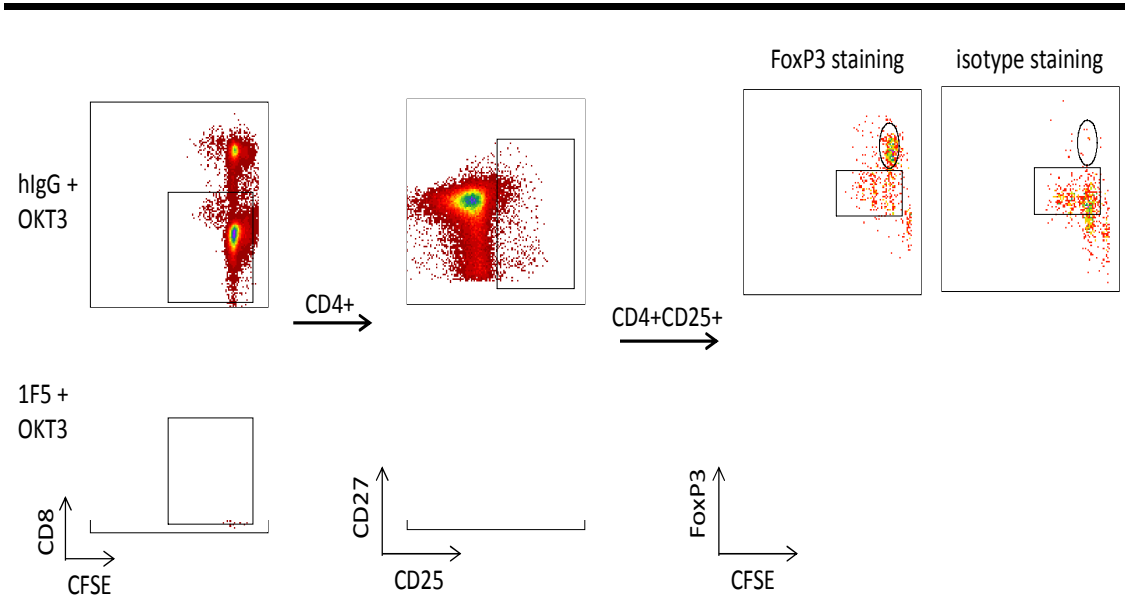
Varlilumab treatment of T cells ensures propagation of recently activated T cells with sustained survival mechanisms potentially afforded by co-expression of positive (4-1BB, OX40, ICOS and GITR) or negative (PD-1, CTLA-4, BTLA) regulators.

Celldex therapeutics

Costimulatory requirements of Tregs may substantially differ from those of non-Tregs (i.e. effector T cells)



Purified Tregs (MACS kit)- CD4/CD25hi/FoxP3 (nTregs)



Detection of Tregs in a Pan T cell prep; CFSE stained T cells in a 3 day Varlilumab culture

Crosslinking CD27 with Varlilumab on Tregs is akin to crosslinking GITR with GITR-Abs. Understanding how closely receptors interplay requires specific knowledge of receptor availability, Ab affinity, cells and tissues that express them and status of inhibitory circuits (CTLA-4, PD-1, BTLA, Tim-3, KLRG1, LAG-3 etc.).

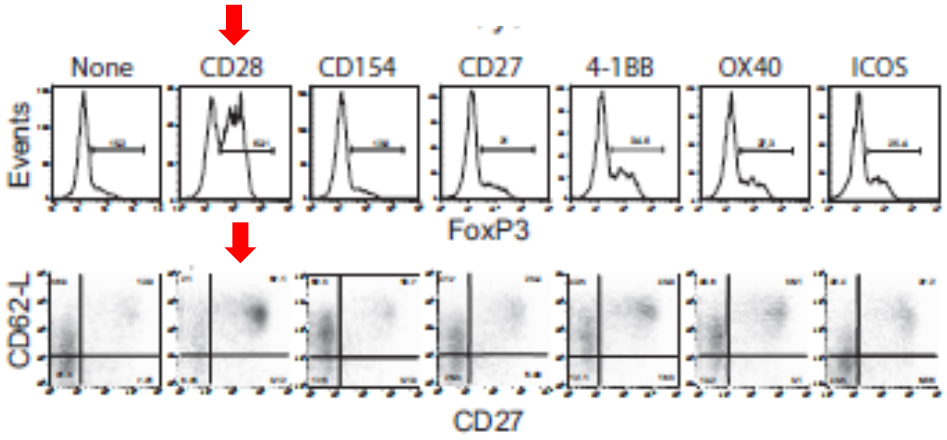
Celldex therapeutics

Costimulation via CD28 is the only way to expand Tregs

CD28 Costimulation Is Essential for Human T Regulatory Expansion and Function[1]

Tatiana N. Golovina,* Tatiana Mikheeva,* Megan M. Suhoski,* Nicole A. Aqui,*
Victoria C. Tai,* Xiaochuan Shan,* Ronghua Liu,* R. Robert Balcarcel,† Nancy Fisher,‡
Bruce L. Levine,* Richard G. Carroll,* Noel Warner,† Bruce R. Blazar,§ Carl H. June,2*
and James L. Riley2,3*

The costimulatory requirements required for peripheral blood T regulatory cells (Tregs) are unclear. Using cell-based artificial APCs we found that CD28 but not ICOS, OX40, 4-1BB, CD27, or CD40 ligand costimulation maintained high levels of Foxp3 expression and in vitro suppressive function. Only CD28 costimulation in the presence of rapamycin consistently generated Tregs that consistently suppressed xenogeneic graft-vs-host disease in immunodeficient mice. Restimulation of Tregs after 8–12 days of culture with CD28 costimulation in the presence of rapamycin resulted in >1000-fold expansion of Tregs in <3 wk. Next, we determined whether other costimulatory pathways could augment the expansive potential of CD28-costimulated Tregs. We observed that while OX40 costimulation augmented the proliferative capacity of CD28-costimulated Tregs, Foxp3 expression and suppressive function were diminished. These studies indicate that the costimulatory requirements for expanding Tregs differ from those for T effector cells and, furthermore, they extend findings from mouse Tregs to demonstrate that human postthymic Tregs require CD28 costimulation to expand and maintain potent suppressive function in vivo. *The Journal of Immunology*, 2008, 181: 2855–2868.

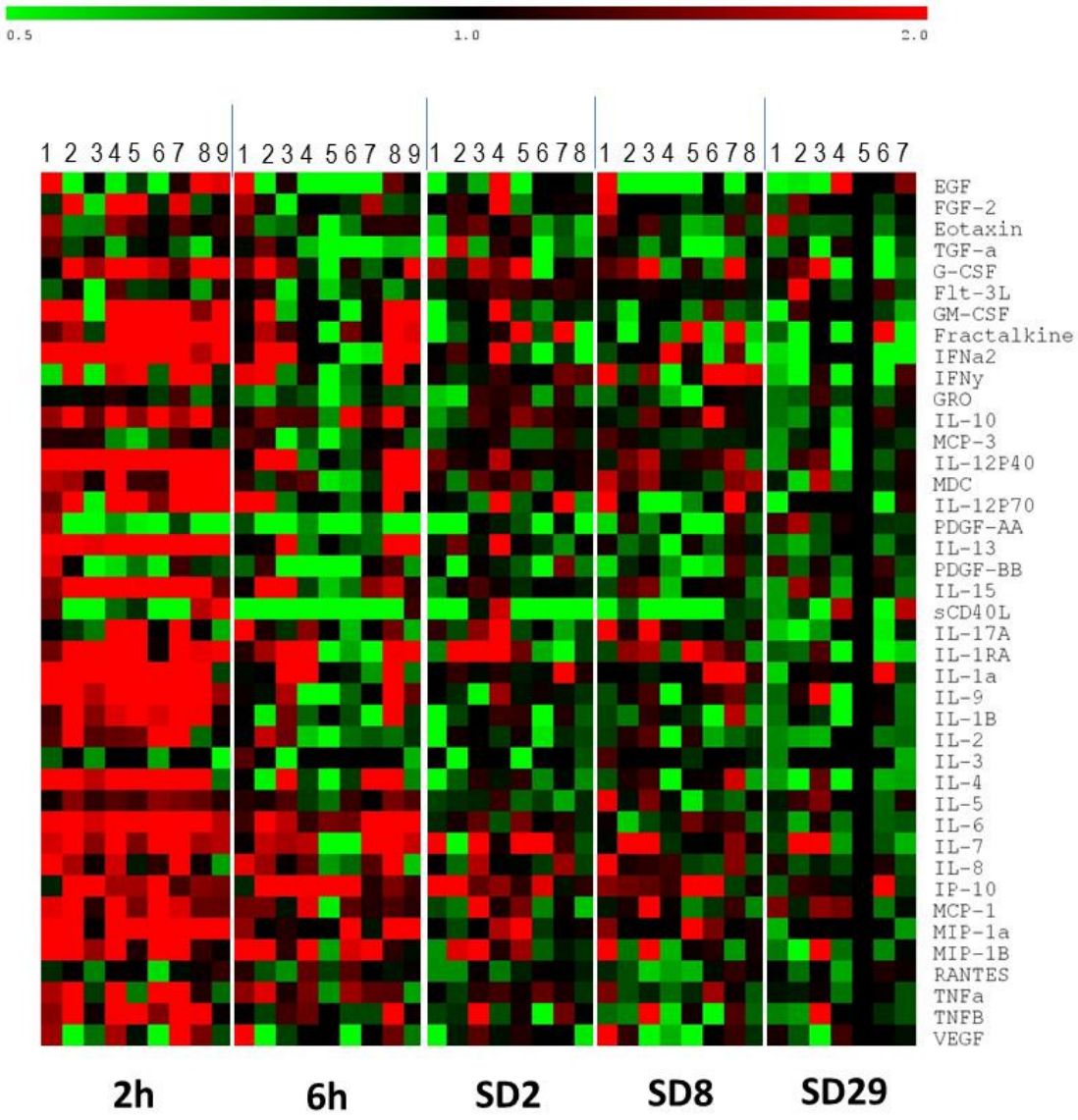


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Clinical Immune Response Modifiers in Patients receiving Varlilumab

Solid tumors: Melanoma, Ovarian, Colorectal, Renal, NSCLC, Prostate, Pancreatic

Circulating biomarkers in serum



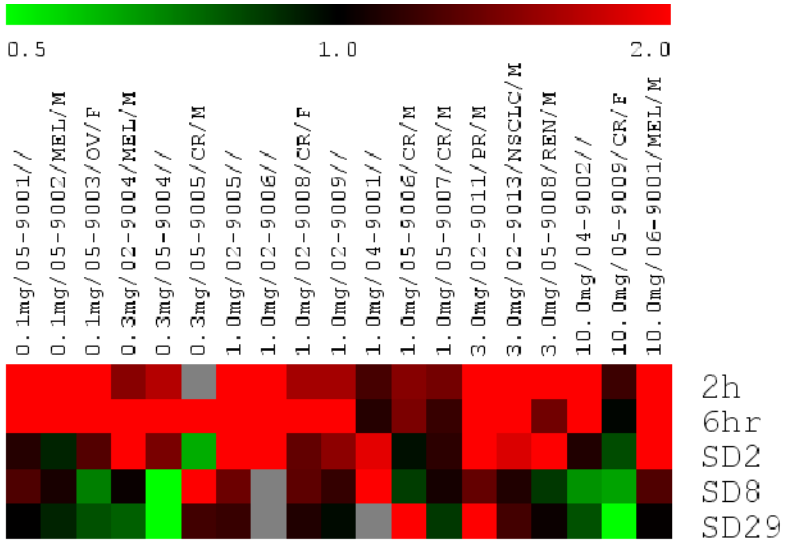
1127 @
1.0 mg/kg

Celldex therapeutics

Screening for serum biomarkers for prognostic/ diagnostic purposes

IP-10 [CXCL10] signature

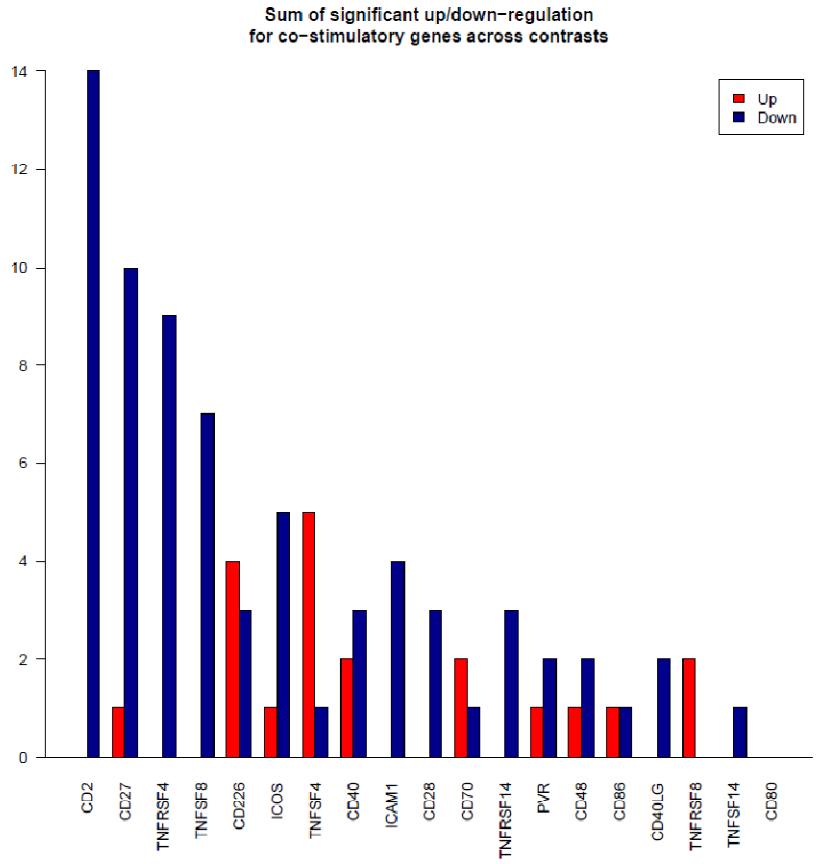
Heatmap of serum IP-10 protein; fold-change over PreVaccine sample

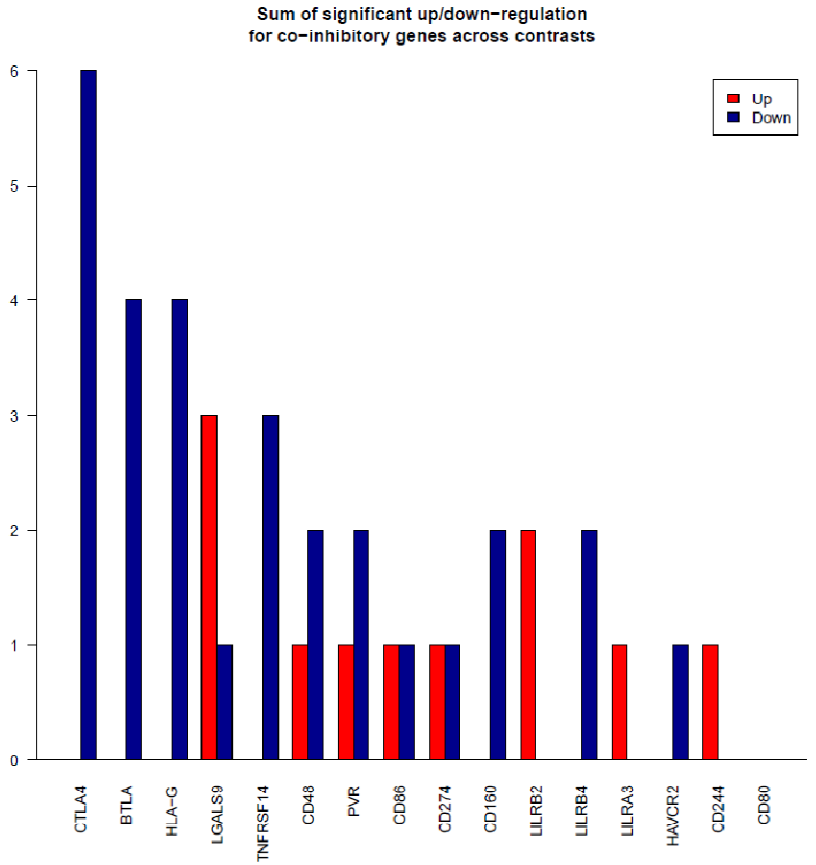


There may be other signatures worth pursuing but this one looks consistent and promising. Currently we're building the infrastructure to have data mining and bioinformatics capability in house.

Celldex therapeutics

CDX-1127 Phase I PAXgene RNAseq Data: Source FiosGenomics, UK





In vitro studies show some degree of overlap with patient data with regard to PD-1/L1, CTLA-4, LIGHT, Spry-1, FGF-2, CD40LG etc.

Celldex therapeutics

Congruence between In vitro and Human Phase I

BRMs	T cells In Vitro*	Patient Serum*
CD27 shedding	+	??
sCD40L	+	+
IL-2	+	+
IL-12p40/p70	--	+
IL-15	--	+
IL-9	--	+
IL-17A	--	+
IL-1b, IL-6	--	+
IFNs	+	+
TNF	+	+
IL-13	+	+
IP-10	+	+
MIP-1	+	+
MCP-1	+	+

*** 72h post Varlilumab**

*** 2h post Varlilumab @ 1.0mg/kg dose**

Celldex therapeutics

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Dr. Henry Marsh VP R&D
Dr. Biwei Zhao
Karuna Sundarapandiyan

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Dr. Michael DeSalvo, Phalanx Biotech, CA
Dr. Max Bylesjo, FiosGenomics, UK
Dr. Jutta Kollett, Miltenyi-Biotec, Germany

Celldex therapeutics