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Characterization of the Response of Human T cells to an Agonistic Anti-CD27 mAb

Karuna Sundarapandiyan, Biwei Zhao, Laura Vitale, Tom O'Neill, Venky Ramakrishna, Henry Marsh, Tibor Keler

Celldex Therapeutics Inc., Phillipsburg NJ & Needham MA

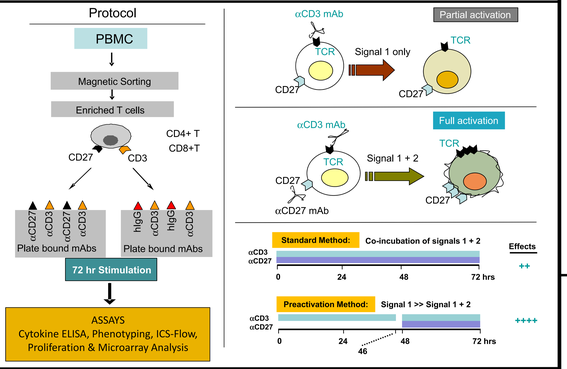
Celldex therapeutics

CD27 Background

Member of tumor necrosis super family of receptors (TNFRSF7). It is constitutively expressed on T cells, B cells and a subset of NK cells. It plays a key role in T cell activation, survival, proliferation and cytotoxicity upon interaction with ligand CD27L (CD70). Monoclonal antibodies to the CD27 molecule have been shown to be useful in effectively modulating immune responses including antitumor immunity in preclinical models (Roberts, DJ et al. 2010 Journal of Immunother.; French RR et al.2007 Blood; Keller AM et al. Immunity 2008)

CDX 1127 (Human anti-CD27 mAb, 1F5)

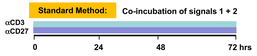
Anti-CD27 mAb 1F5 is a fully human antibody previously described in preclinical models with anti-tumor, proliferation and cytokine inducing agonistic properties (Vitale et al. 2012 Clin. Cancer Res.). In this study we analyze the in vitro activation of human T cells with 1F5 in the context of TCR signaling and present data that support a consistent pattern of immune regulation at the mRNA, protein and cellular level. CDX 1127 is currently in Phase I trials as monotherapy to treat Lymphoma and Solid tumors.



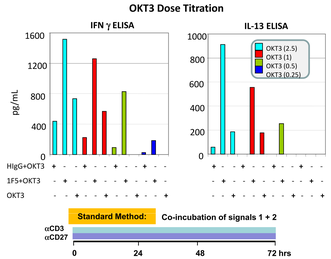
Multi-cytokine analysis of CD27 activated T cells with cytokine multiplex Luminex bead arrays

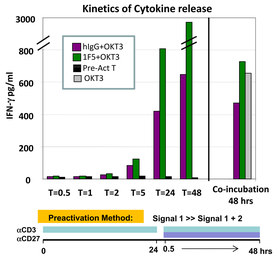
Cytokines	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8
IFN-γ								
IL-2								
IL-4								
IL-5								
IL-6								
IL-10								
IL-12p70								
IL-13								
IL-17A								
TNFα								
IL-9	NA	NA	NA	NA	NA	NA		

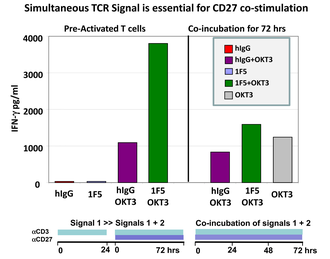
1F5 specific response with controls subtracted

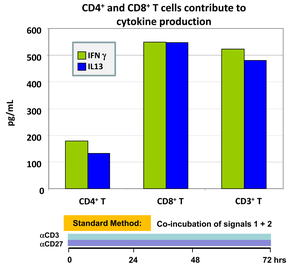


Th1/Th2 cytokine induction: TCR-dependence & kinetics

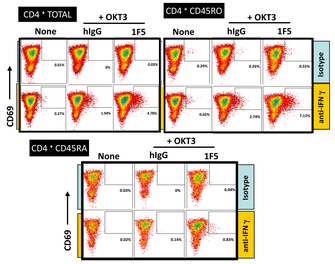


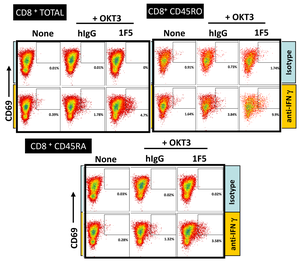




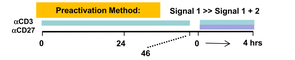


Activated T subset analysis following TCR/CD27 costimulation

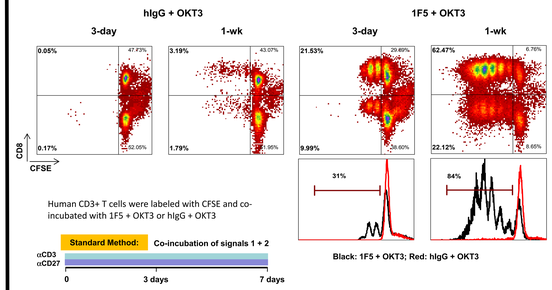




Isolated CD3+ T cells were pre-activated with plate-bound OKT3 for 46 hrs, followed by 4-hr incubation with plate-bound 1F5 + OKT3 or hIgG + OKT3, then stained with ICS for IFN-γ



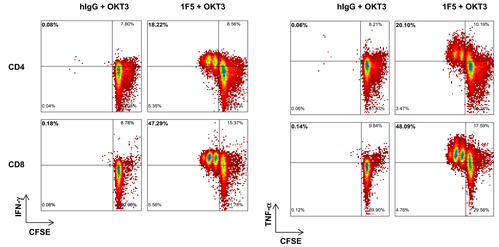
CD27 mAb 1F5 triggers proliferation of CD4 & CD8 T cells

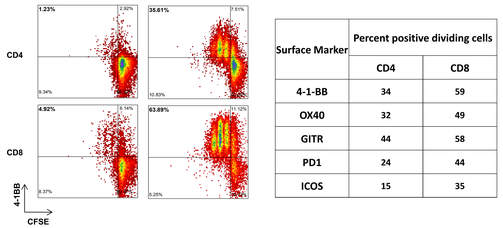


Human CD3+ T cells were labeled with CFSE and co-incubated with 1F5 + OKT3 or hIgG + OKT3

Black: 1F5 + OKT3; Red: hIgG + OKT3

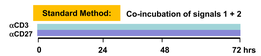
Characterization of TCR & CD27 activated T cells



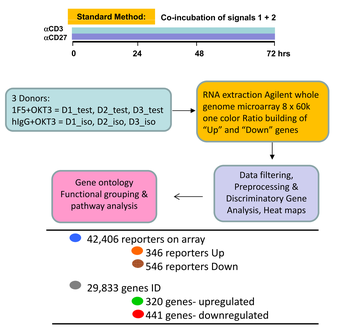


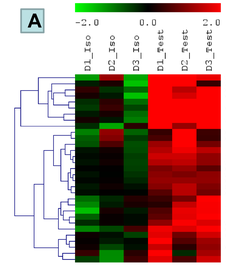
Surface Marker	Percent positive dividing cells	
	CD4	CD8
4-1-BB	34	59
OX40	32	49
GITR	44	58
PD1	24	44
ICOS	15	35

Human CD3+ T cells were labeled with CFSE and co-incubated with 1F5 + OKT3 or hIgG + OKT3 for 72 hrs and then stained for surface expression or intracellular cytokine staining for TNF-α and IFN-γ

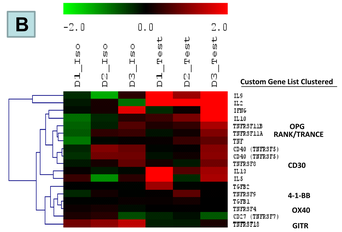


Expression Profiling with Whole Genome Microarrays

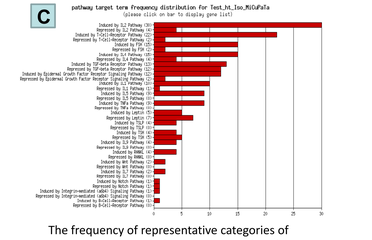




Highly enriched gene lists differentially expressed "test" and "iso"



Modulation of related TNFRs and cytokine genes in "test" and "iso"



The frequency of representative categories of proposed transcriptional targets of signaling pathways potentially induced or repressed by CD27 activation in "test" relative to "iso".

Summary & Conclusions

- Anti-CD27 mAb, 1F5 can provide co-stimulatory signals to human T cells in a TCR-dependent manner. Concomitant TCR signaling is required for 1F5 induced effects. Removal of TCR signaling abolishes any subsequent signaling by 1F5.
- The T cell cytokine response to OKT3 and 1F5 has a dominant Th1-like pro-inflammatory signature (IFNγ, IL-2, TNFα) accompanied by a delayed Th2 cytokine production, IL-13, suggesting a regulatory role like the related TNFR, 4-1-BB (CD137) to limit overt Th1 activity.
- T cells proliferating in response to stimulation with OKT3 and 1F5 are IFNγ and TNFα producing CD4+ and CD8+ T cells. Proliferating T cells also express markers consistent with activated phenotype.
- Subset analysis of OKT3 and 1F5 responding cells further point to effector memory IFNγ+ CD4 and CD8 T cells.
- Gene expression microarray analysis reveal modulation of signaling, protein kinases, growth and cytokine-chemokine pathways.
- These in vitro characterizations will be used to guide the biomarker analysis of CDX 1127 Phase I trial subjects to demonstrate in vivo T cell activation.