

New insights into CD8+ T cell function and regulation

Pam Ohashi
Princess Margaret
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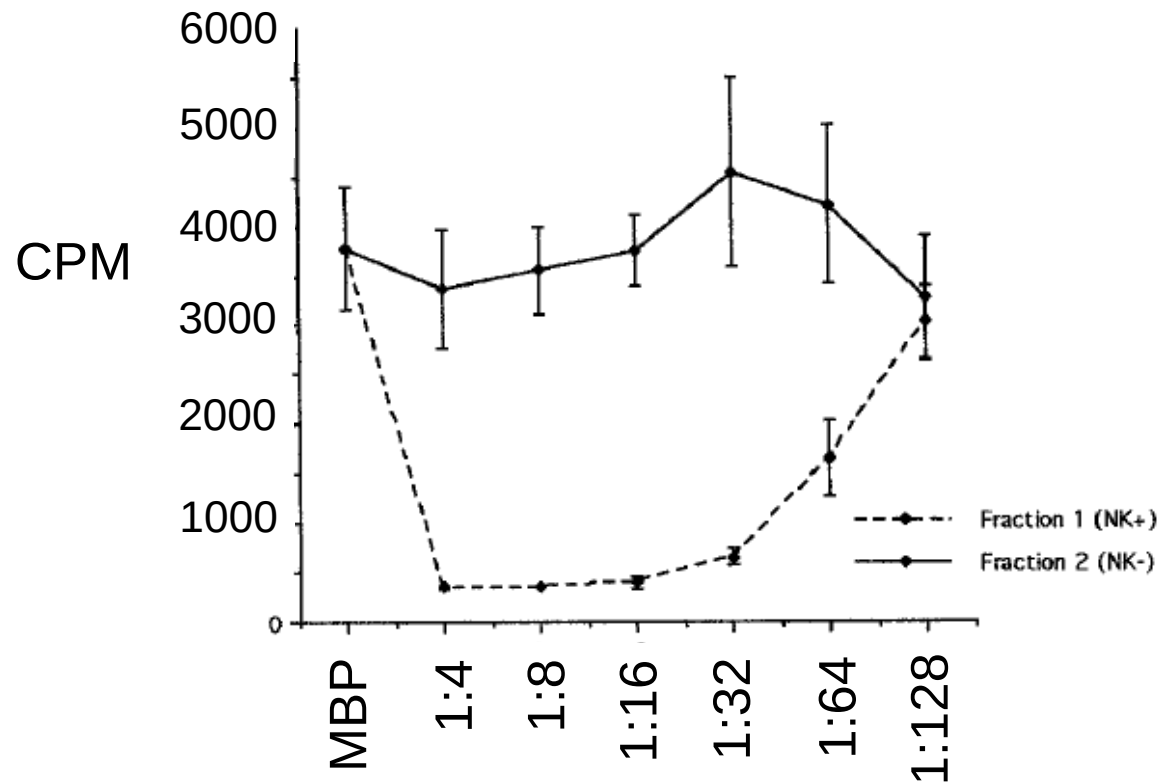
No Disclosures



Role for NK cells in regulating T cell responses

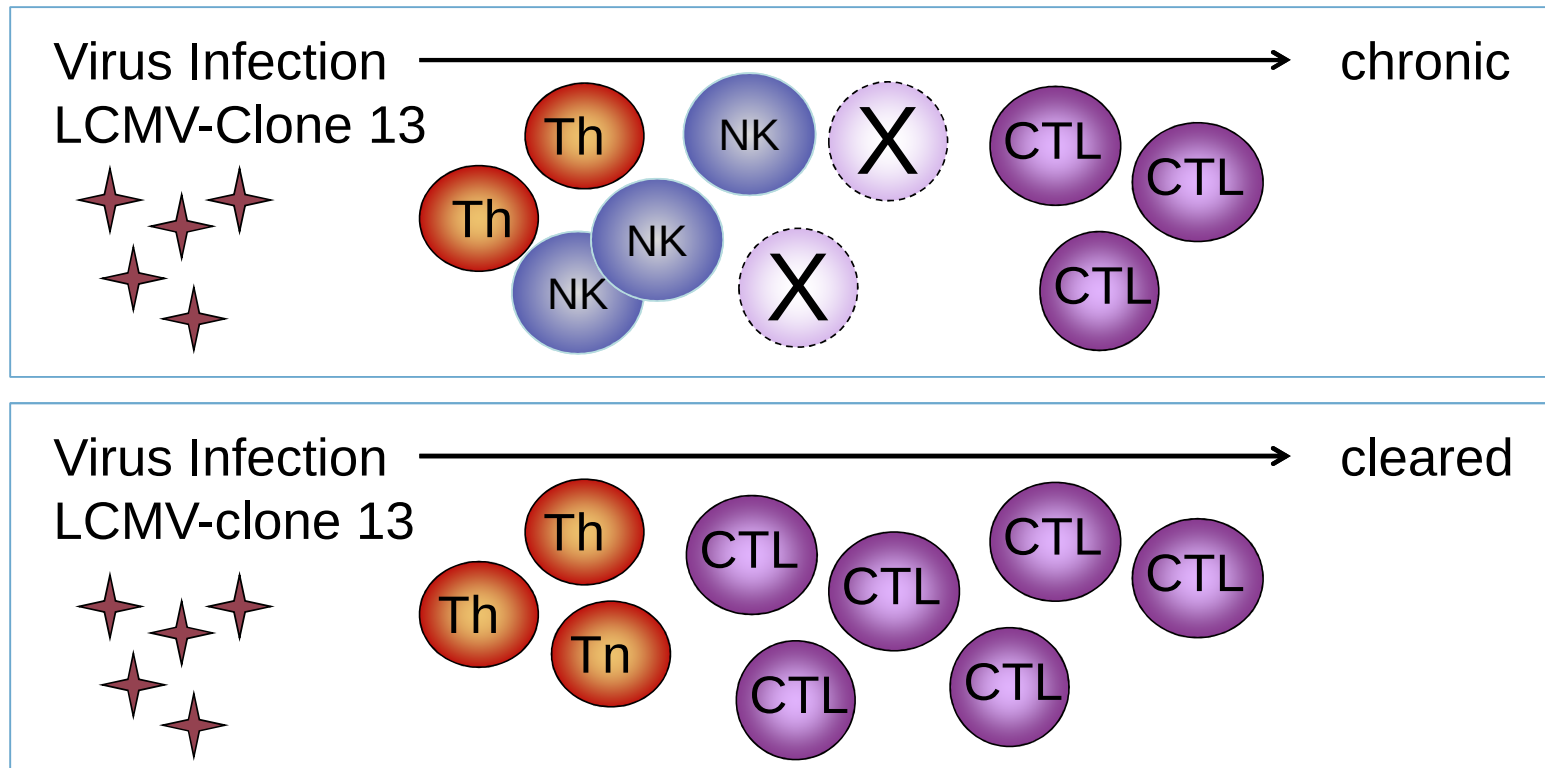
- Biron lab/Bielekova lab – NK cells inhibited T cell survival via contact dependent mechanisms
- In lpr mice - disappearance of NK cells upon the development of autoimmunity (Takeda/Dennert JEM 1993)
- decreased NK cell frequency/function associated with systemic juvenile RA (Villanueva J (2005) Arthritis Res Ther

Innate Immunity: NK regs?



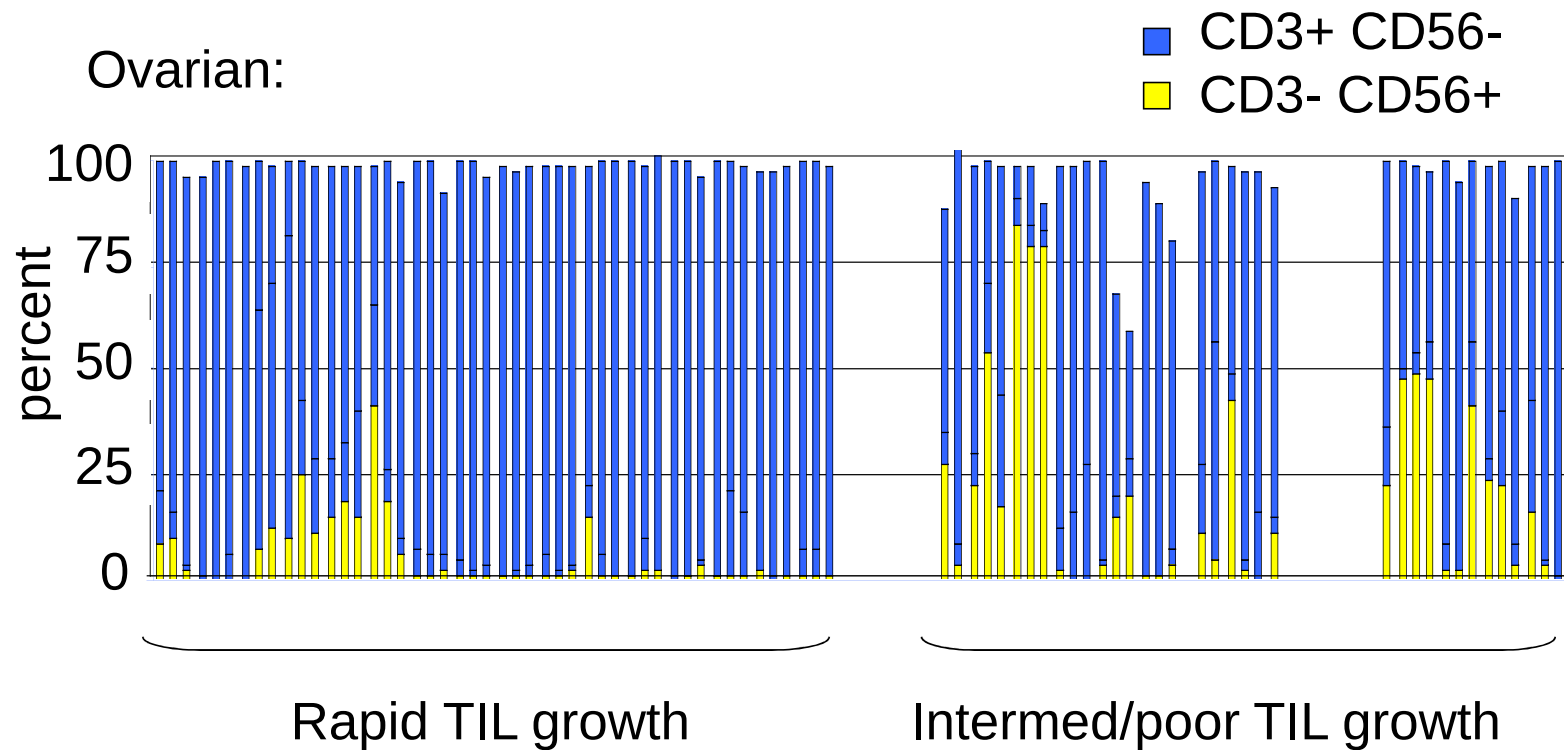
Smeltz RB/ Swanborg R., J Immunol 163:1390 (1999)

NK cells limit CD8 immunity leading to chronic viral infection



Waggoner Nature (2012) 481:394
Lang et al PNAS (2012)109(4):1210

Presence of NK cells are associated with slow growth of tumor infiltrating lymphocytes



Do NK cells inhibit TIL growth ?

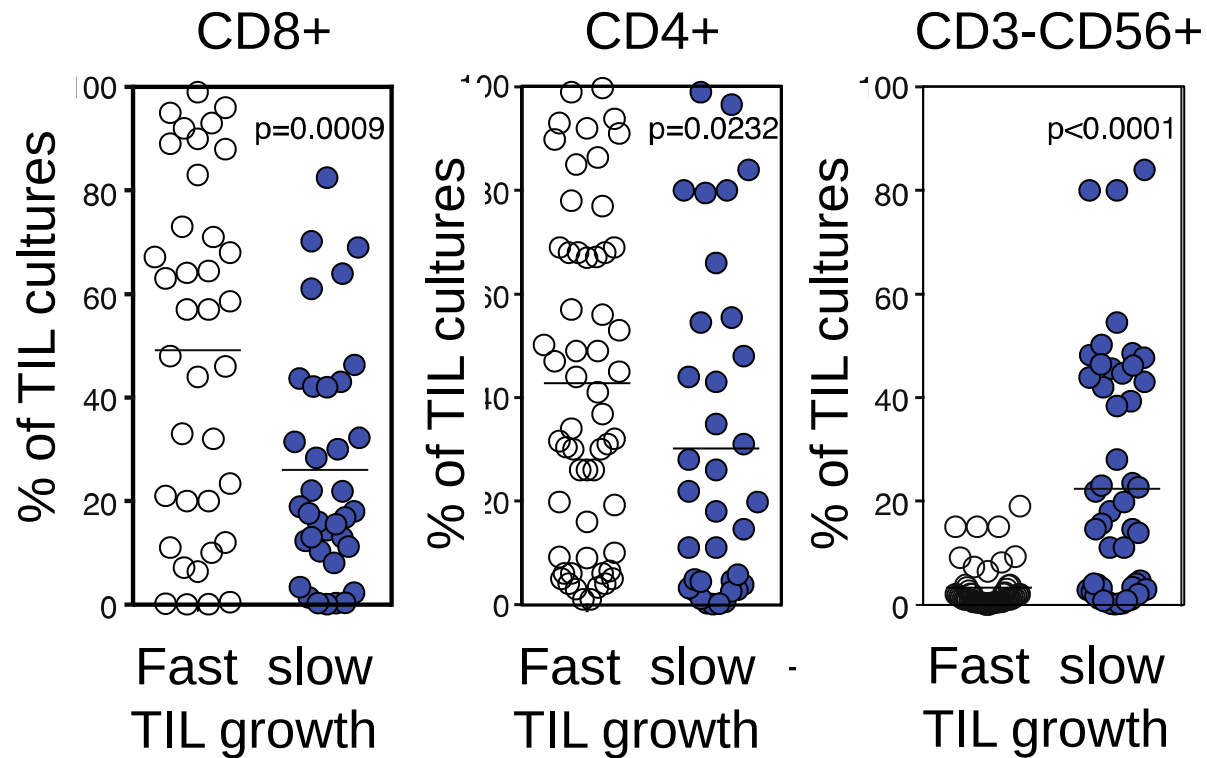


Sarah Crome

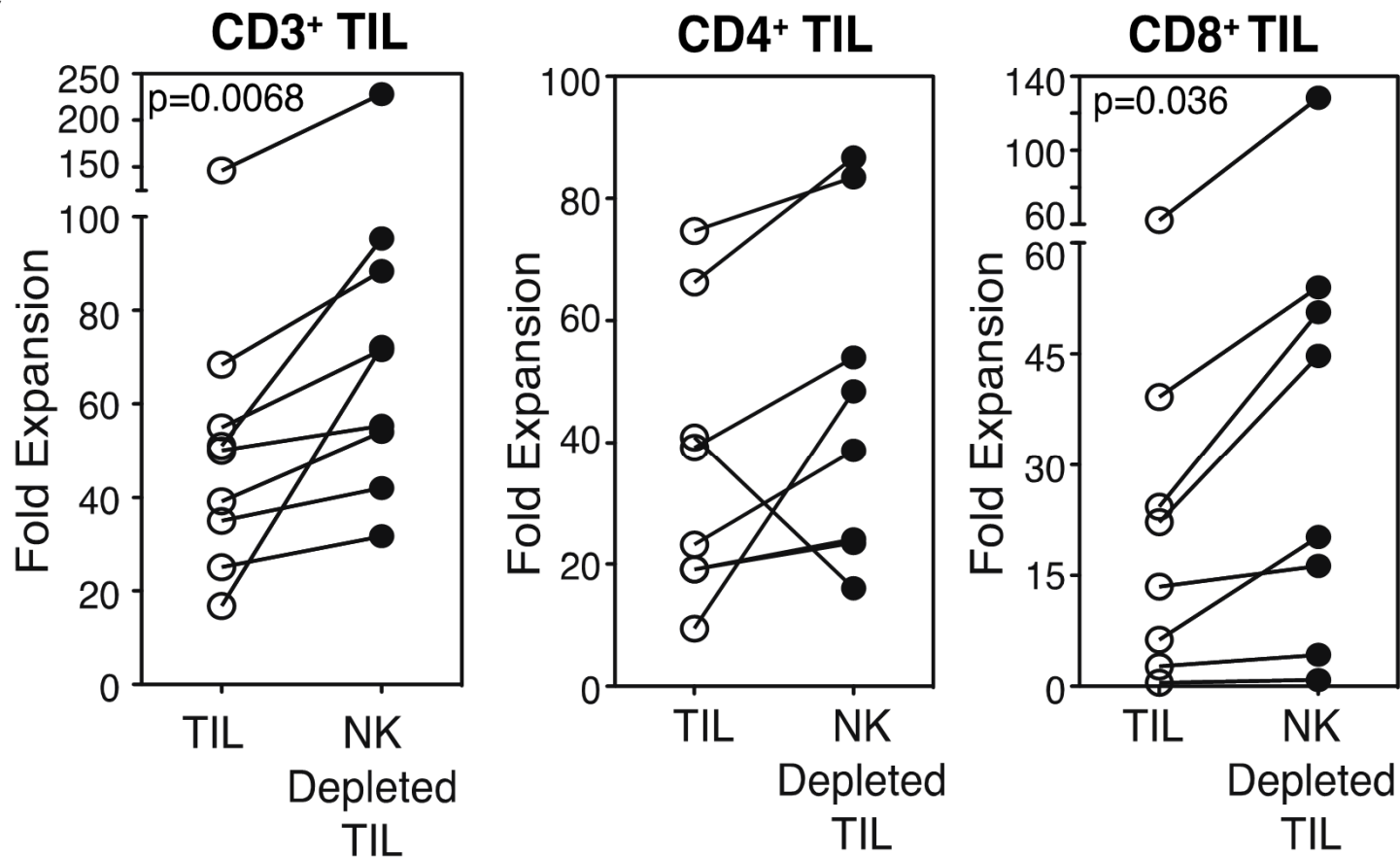


Linh Nguyen

Slow growing cultures have increased % CD56+ cells



NK cells inhibit the expansion of TILs



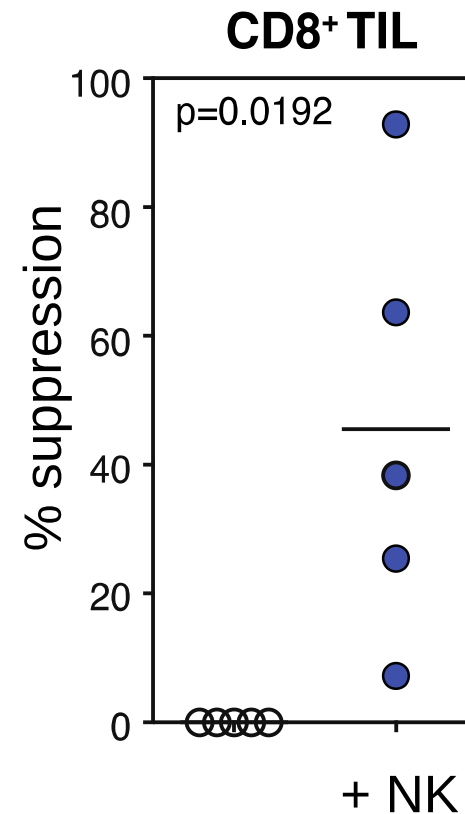
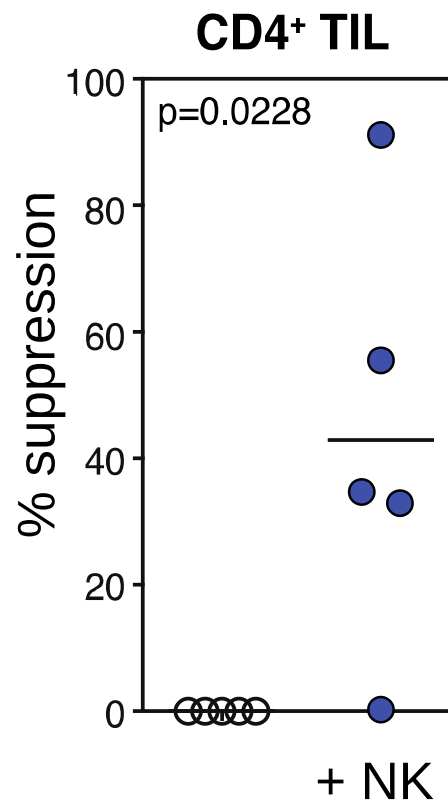
Regulatory NK cells suppress TIL expansion

TIL
(Cell trace)

+ anti-CD3
+ anti-CD28

+/- NK
(Slow
growing)

Proliferation



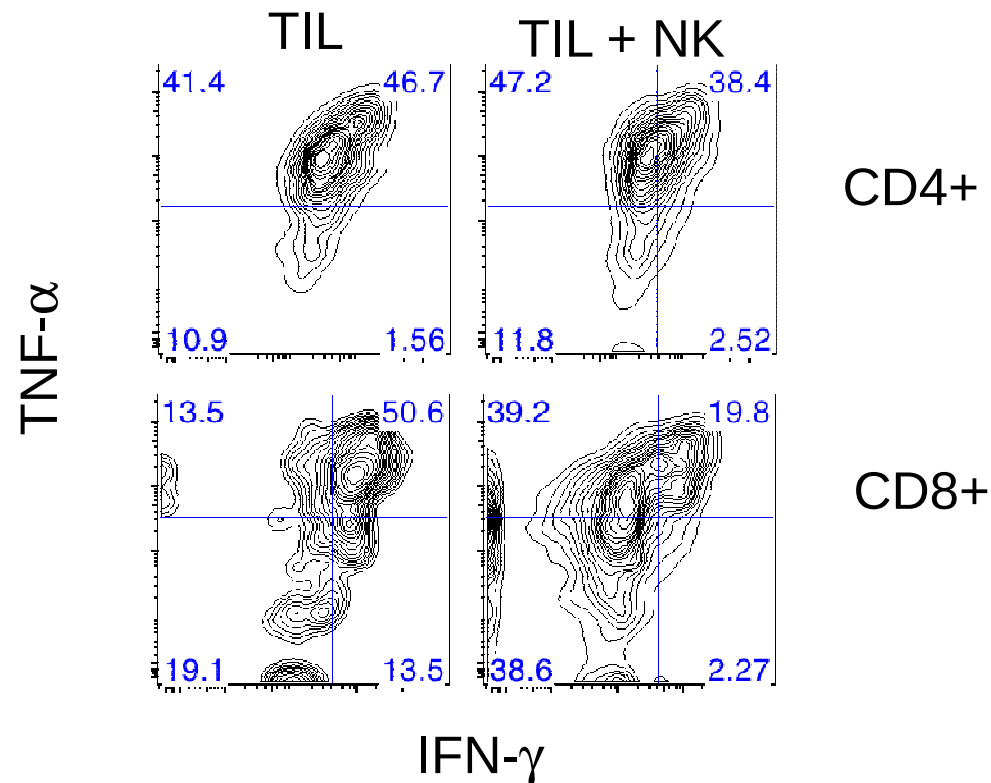
NK cells alter cytokine production by TILs

TIL
(Cell trace)

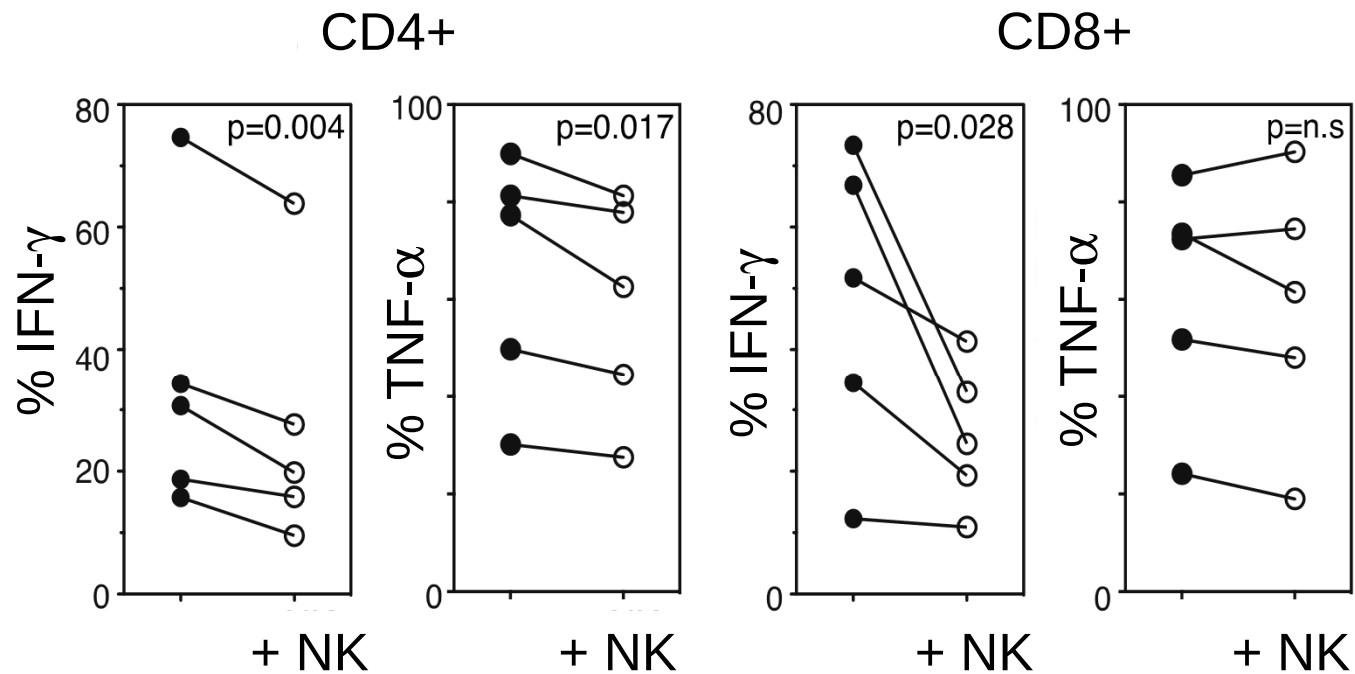
+ anti-CD3
+ anti-CD28

+/- NK
(Slow
growing)
3 days

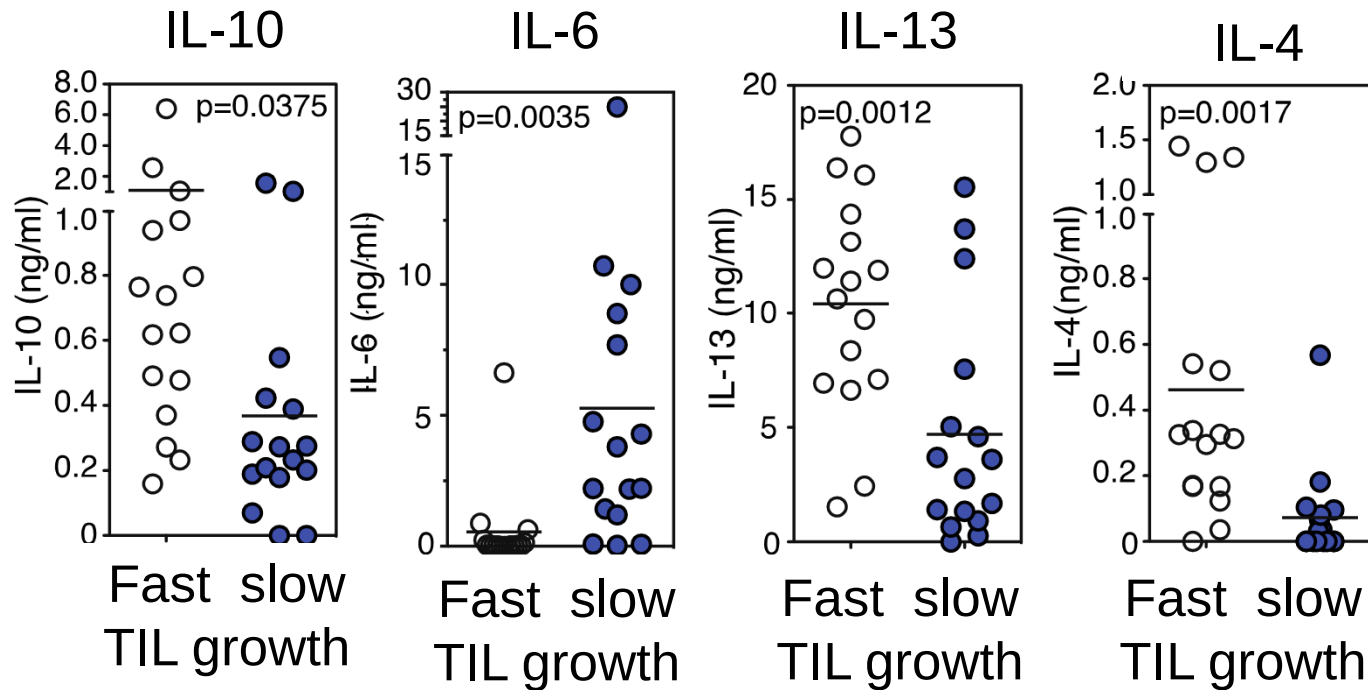
cytokines



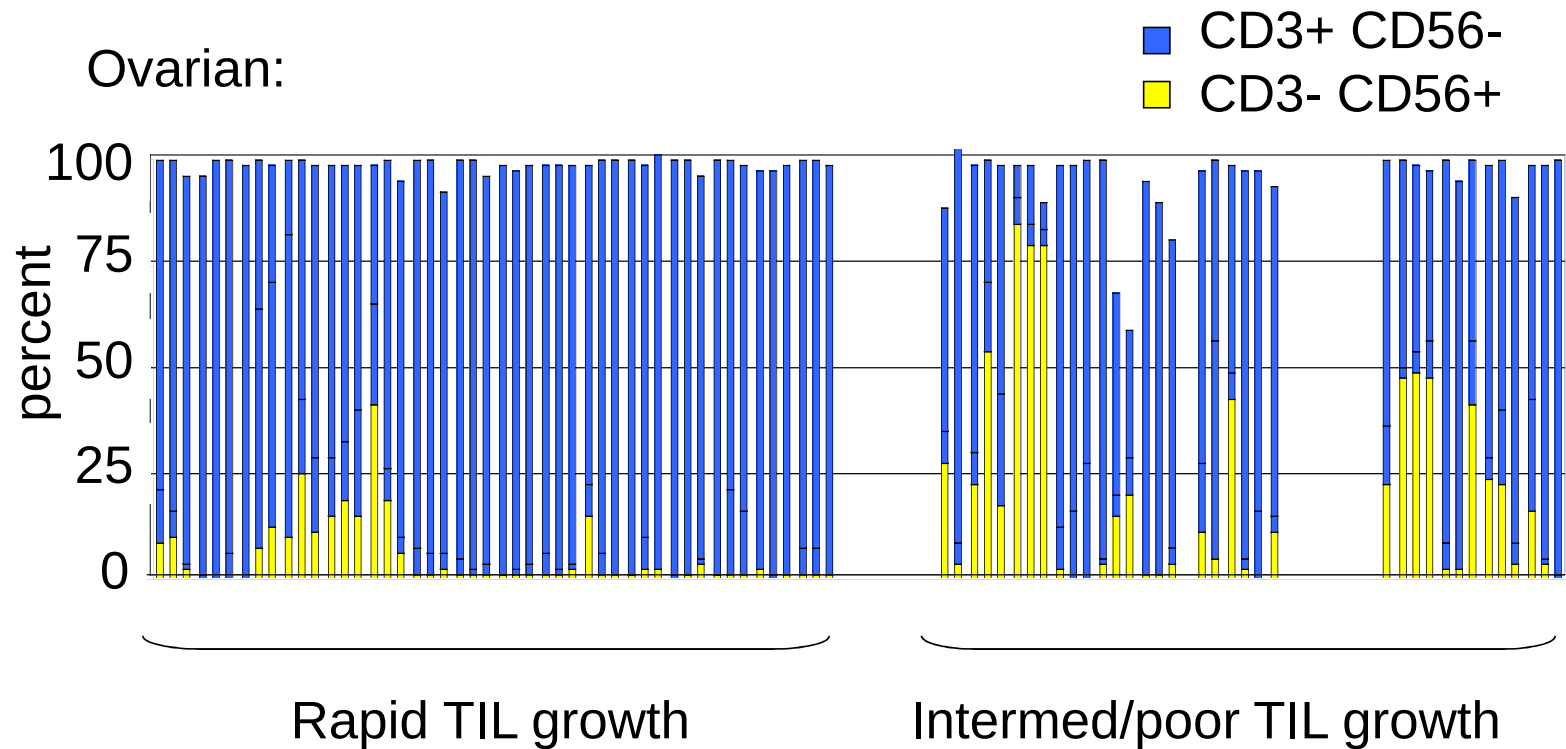
NK cells suppress IFN- γ production by TILs



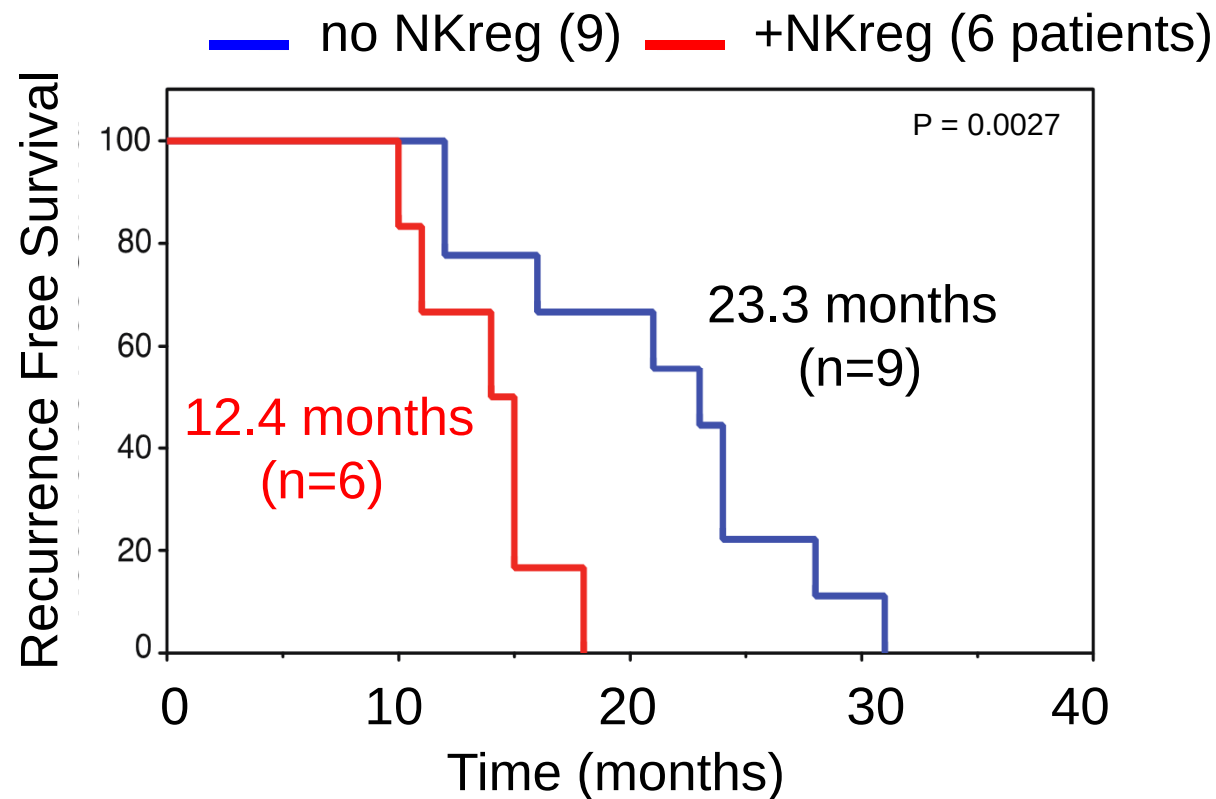
TIL cultures with NKreg cells produce an altered cytokine profile



Does the presence of inhibitory NK cells correlate with disease progression?



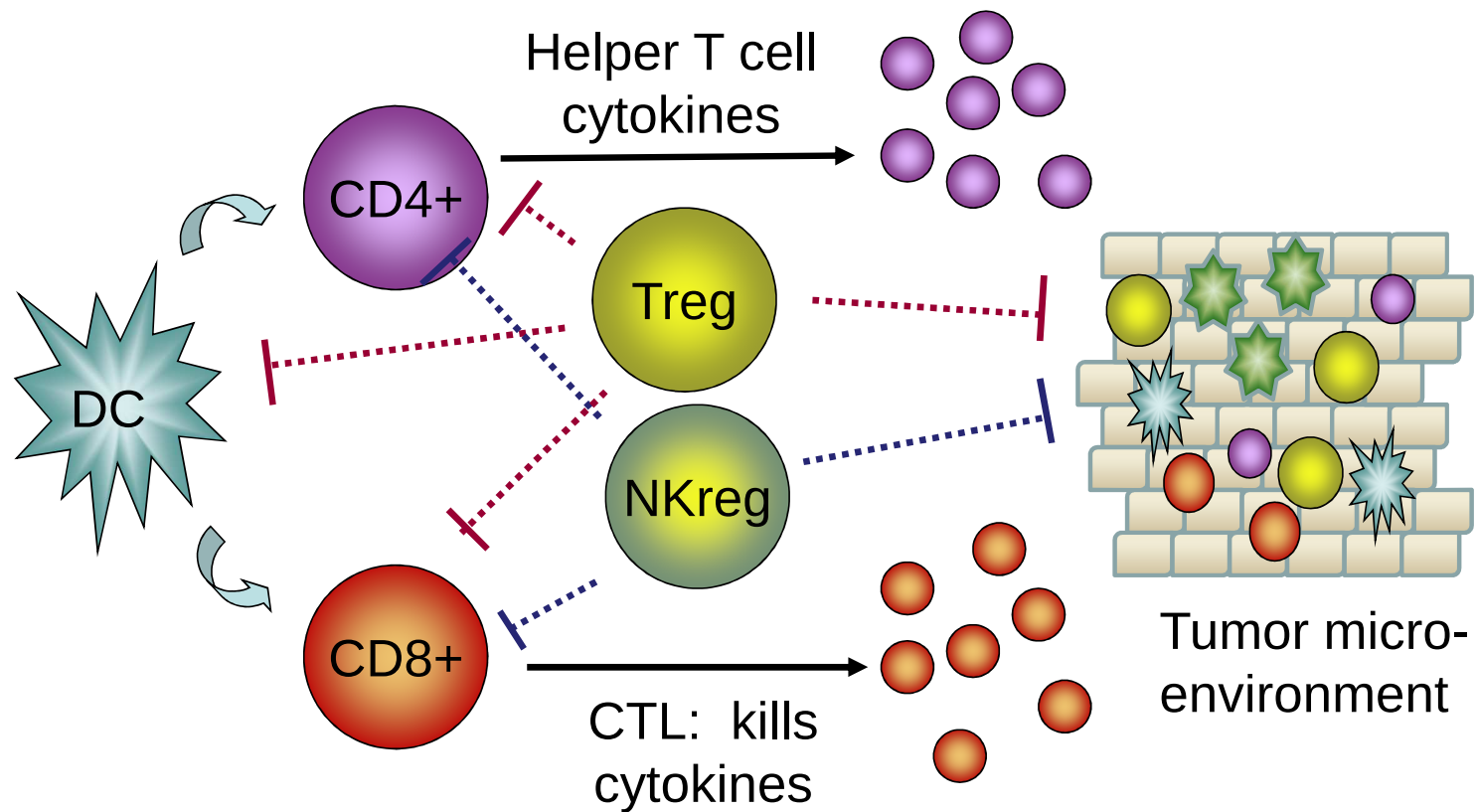
Recurrence free survival correlates with presence of NKregs



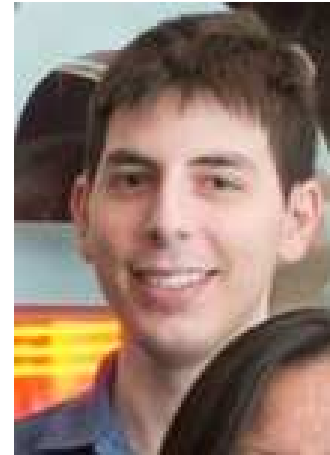
Summary: Subset of NK /ILCs inhibit T cell immunity

- Increased proportion of NK cells in slow growing TIL cultures
- NK cells limit TIL expansion
- NK cells alter T cell cytokine production
- Presence of NK regs in TIL cultures correlate with faster time to recurrence in ovarian cancer

Importance of NK cells in regulating Immunity

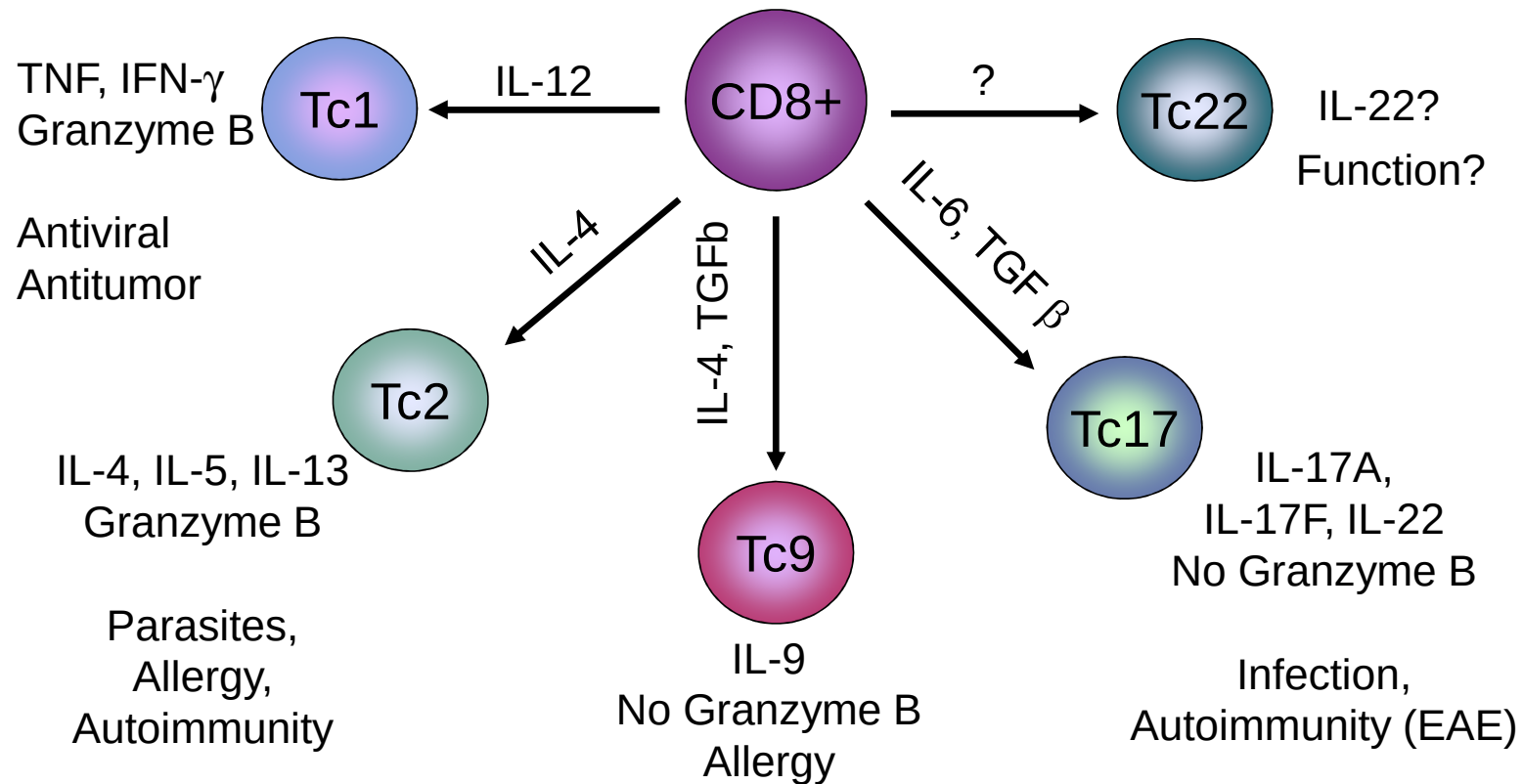


Role of novel T cell subsets in anti-tumor immunity

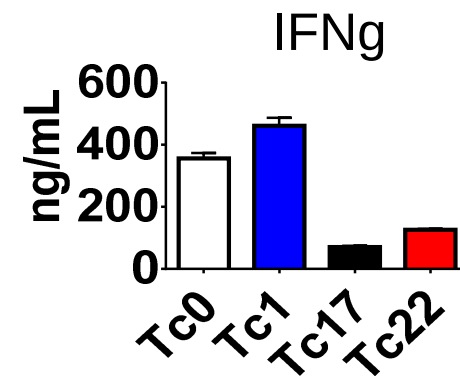
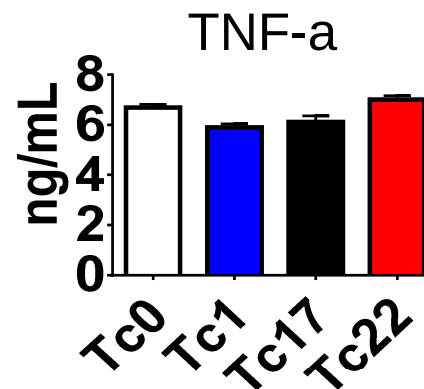
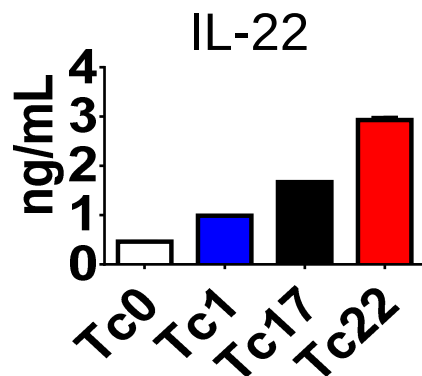
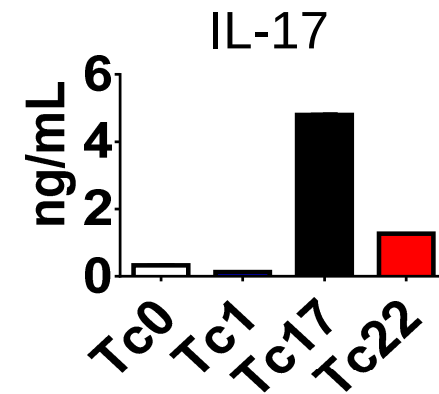
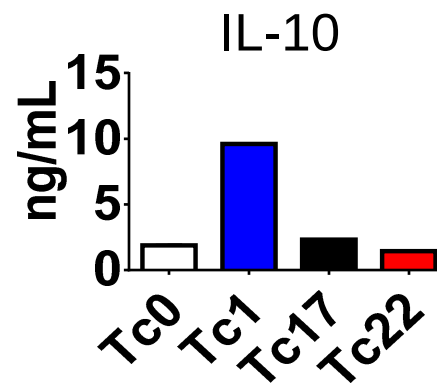
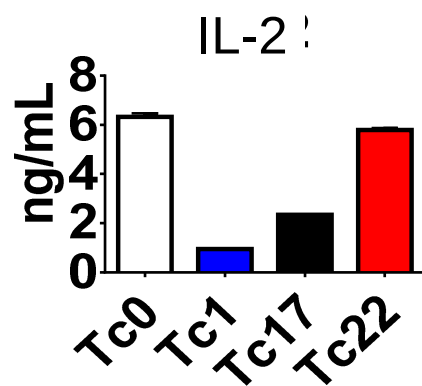


Michael Saint Paul

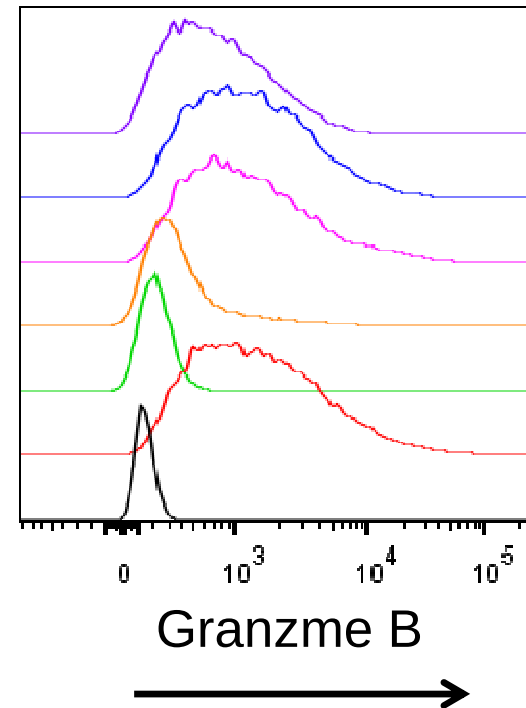
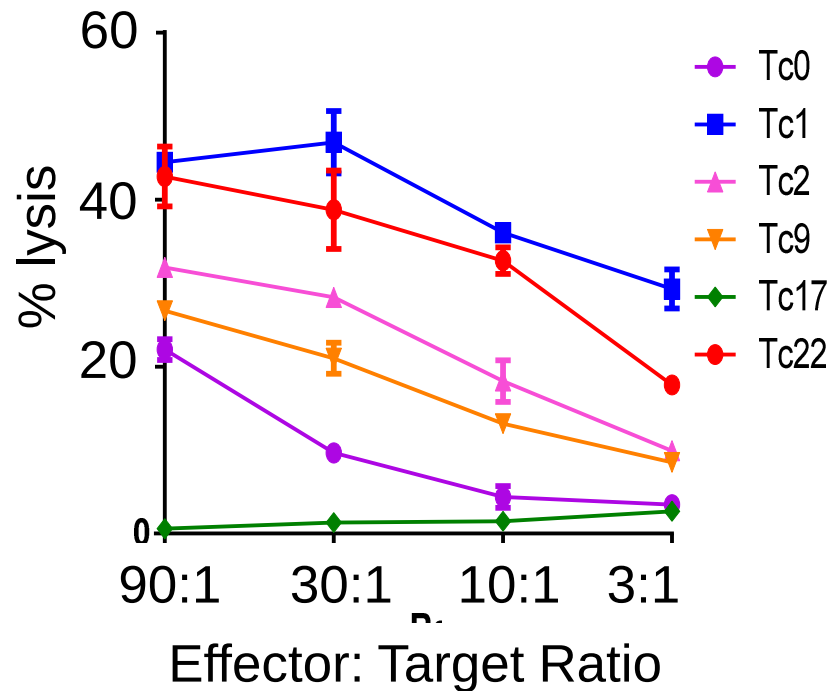
Multiple different CD8+ T cell lineages



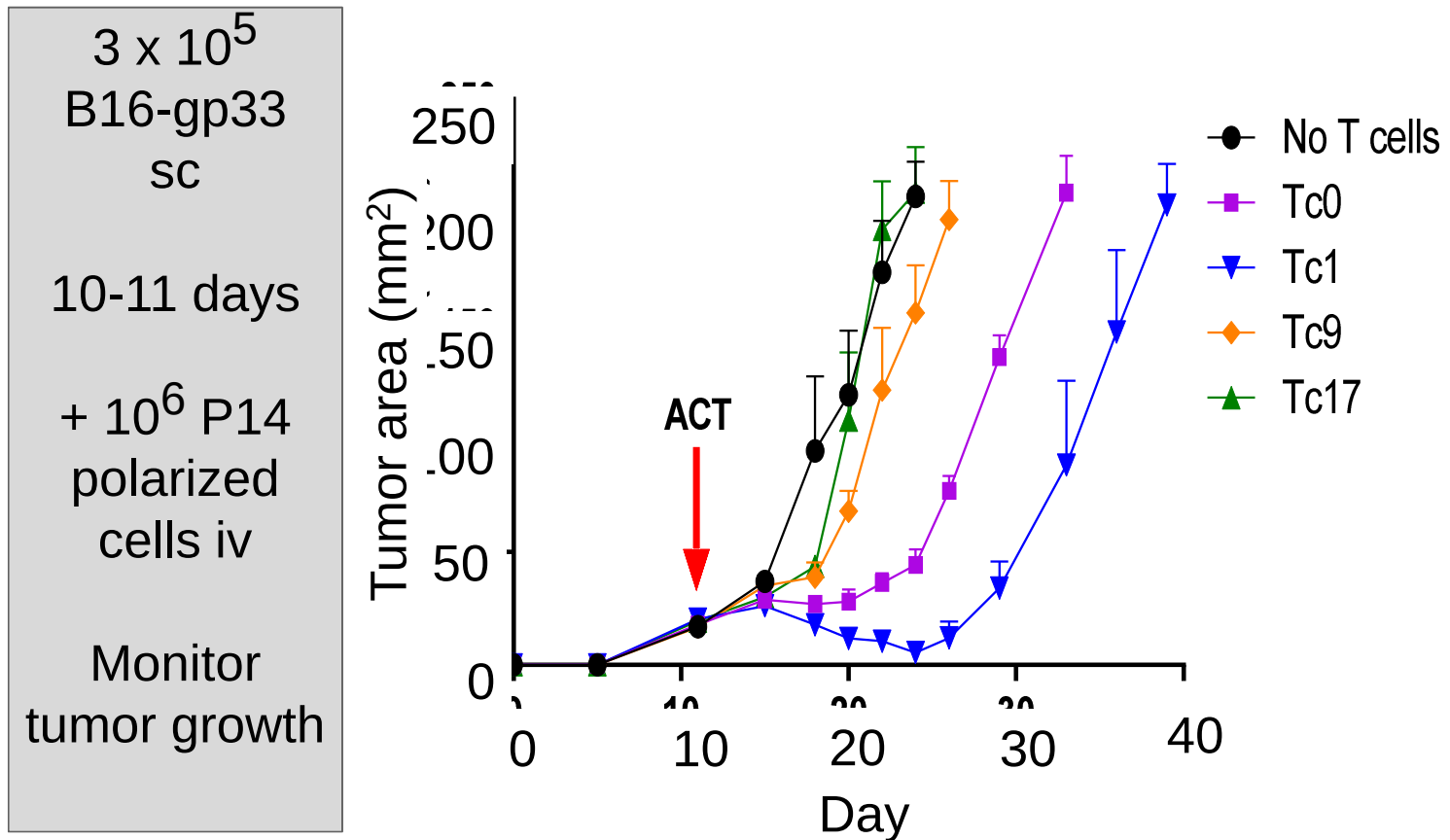
Cytokine Production by different CD8+ T cell lineages



Differential ability for Tc subsets to lyse target cells



The Tc1 lineage can limit the growth of B16 melanoma cells



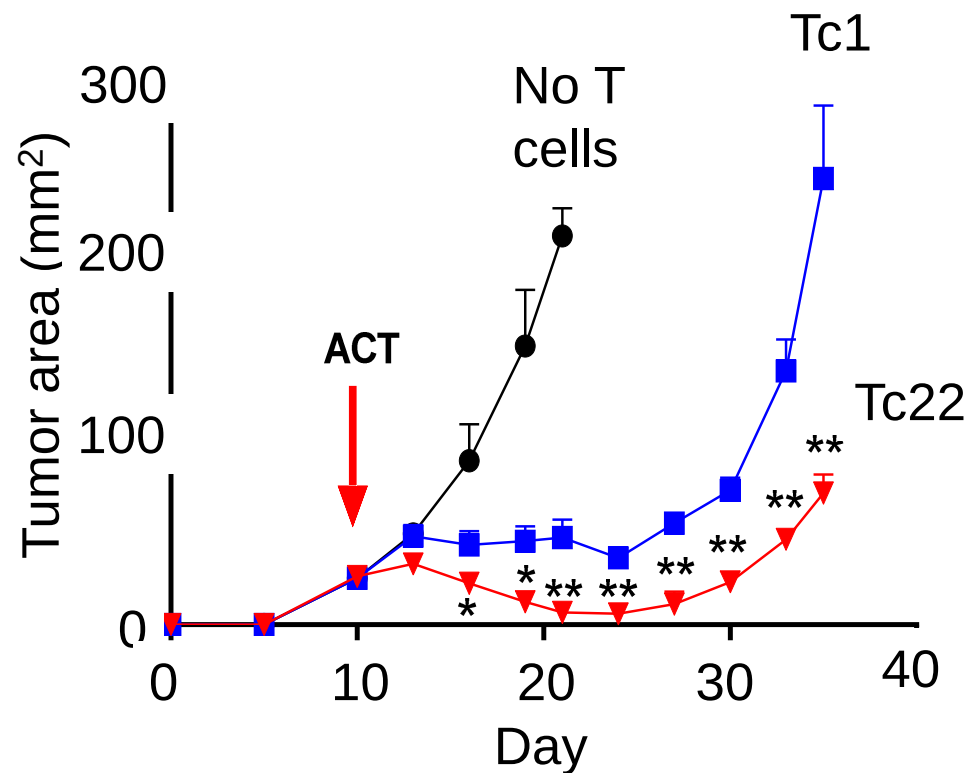
The Tc22 subset can also limit tumor growth

3×10^5
B16-gp33
sc

10-11 days

+ 10^6 P14
polarized
cells iv

Monitor
tumor growth



No T cells
Tc1
Tc22

Summary: Tc lineages

- can skew Tc 1, 2, 9,17, 22 in vitro
- Tc22 make IL-2, TNF α , IL-22 but IFN- γ lo
- Tc 1 and Tc22 show high cytotoxic activity in vitro
- Tc1 and Tc22 can limit tumor growth



Sarah Crome
 Philipp Lang
 Marcus Bernardini
 Golnessa Mojtahedi
 Pat Shaw
 Anca Milea

Michael St Paul



Lewis Lanier
 Sandra Lopez-Verges



Linh Nguyen
 Michael Pniak
 Jessica Nie
 Patty Yen

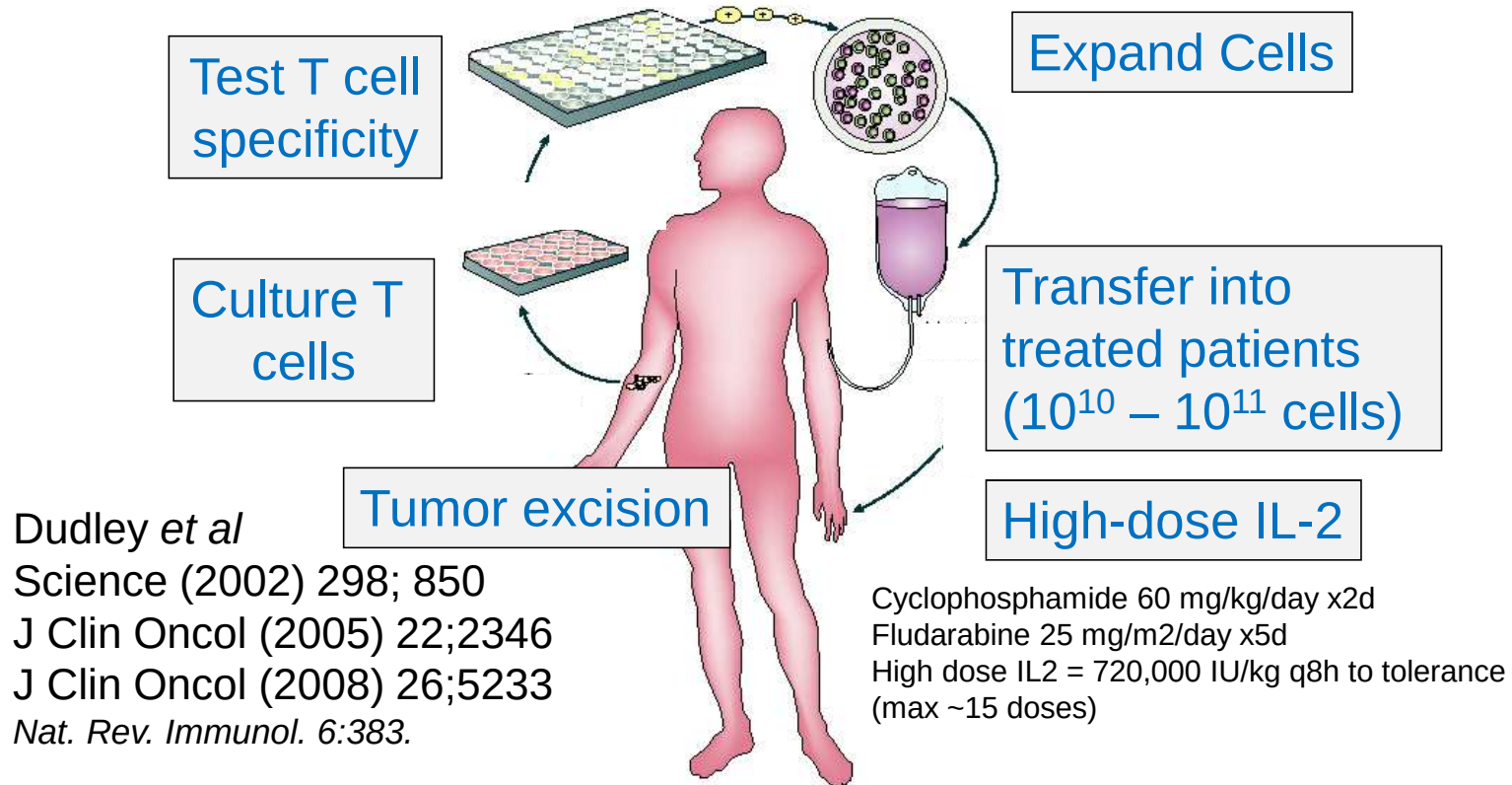


Lab Focus: Basic mechanisms of immune regulation – relevance/impact on immunotherapy

Tumor infiltrating lymphocytes (TILs):

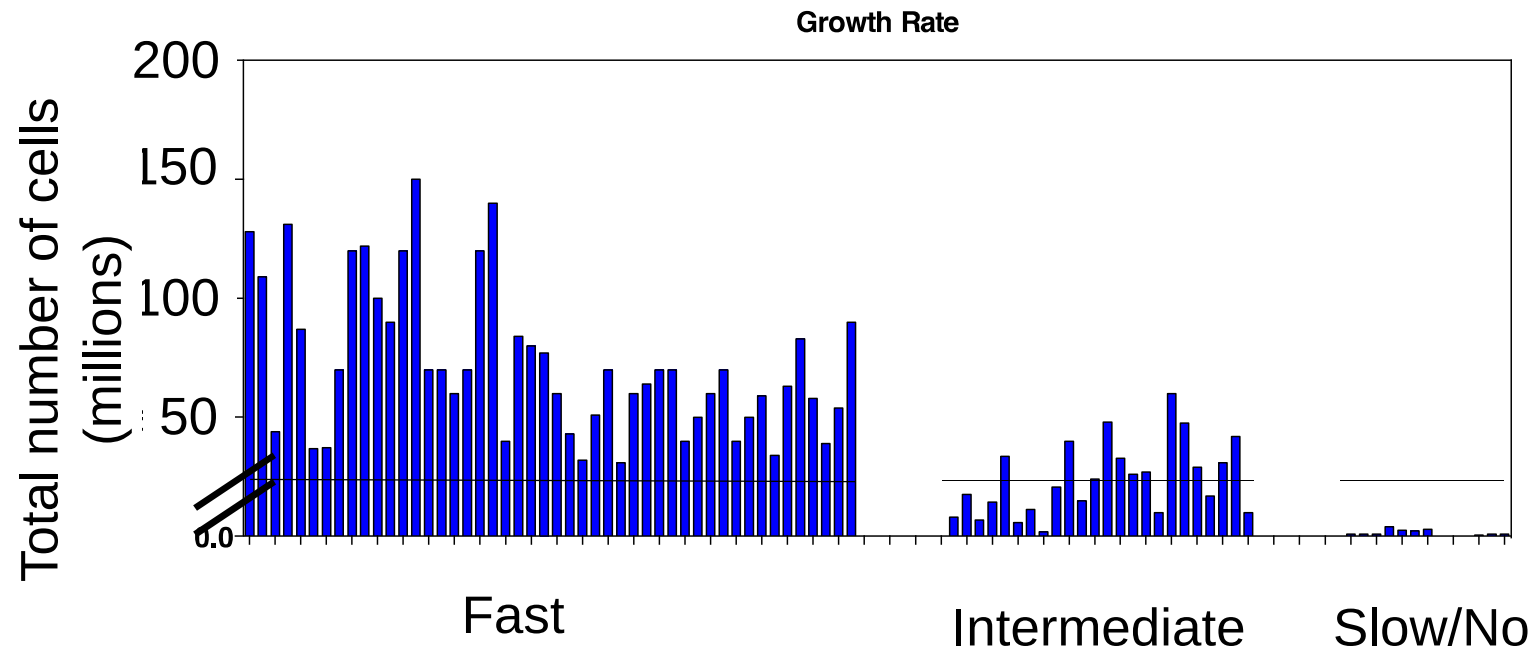
- 1) TIL therapy, melanoma, mesothelioma ovarian cancer
- 2) Biology of the Tumor Microenvironment

Adoptive T cell Therapy

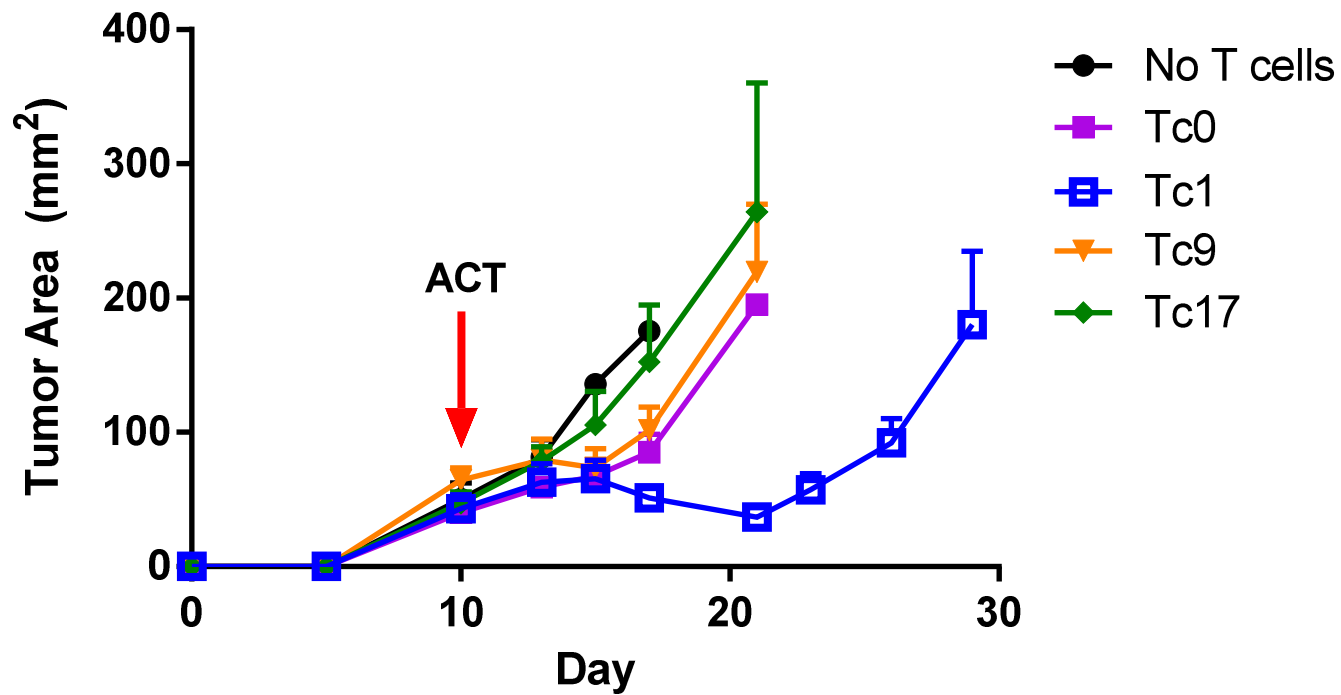


22% of patients durable CR of 93 patients
49% 59% 72% response rate

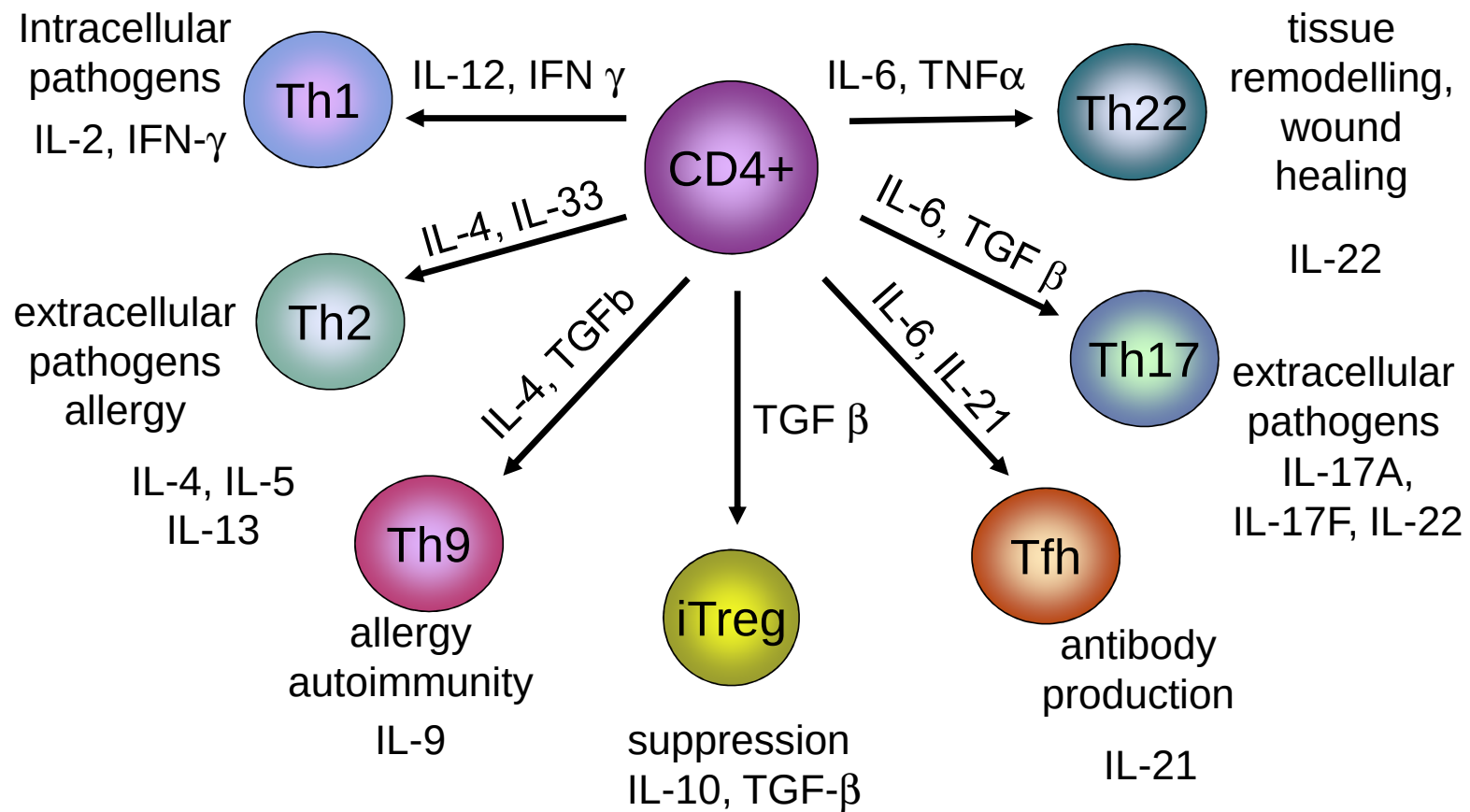
TIL from ovarian cancer specimens show variable rates of expansion



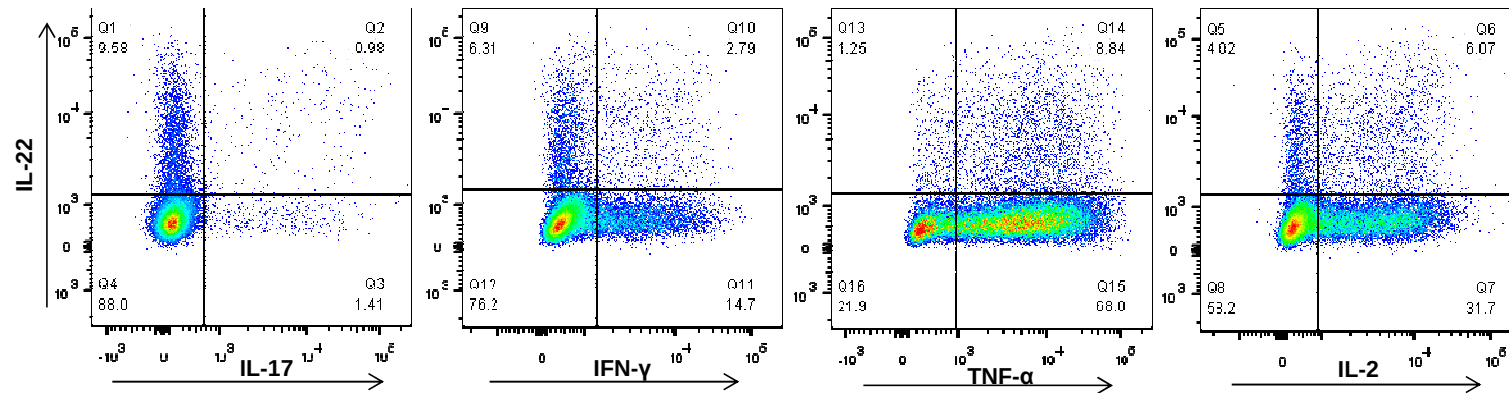
Tc1 cells are superior for limiting tumor growth



Multiple different CD4+ T cell lineages



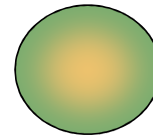
Cytokine profile of Tc22s



Cells of the Adaptive Immune System

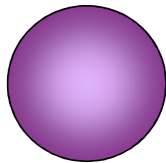
FEATURE:
antigen specific receptors

B Cells



Antibody
production

T cells CD4+



cytokines
regulatory



IL2



IL4



IL9



IL17



22



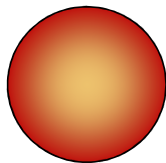
TFH



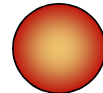
Treg

Treg

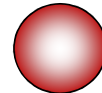
T cells CD8+



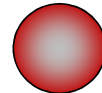
cytotoxic
cytokines



IL2



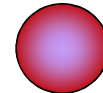
IL4



IL9

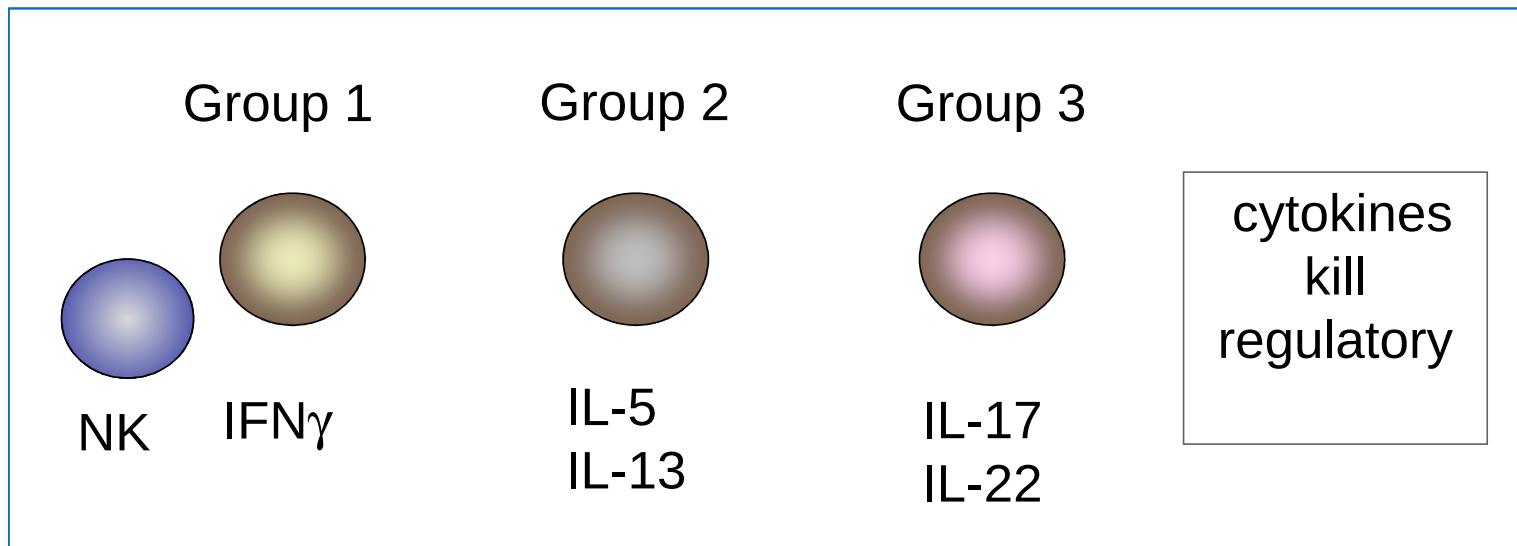


IL17

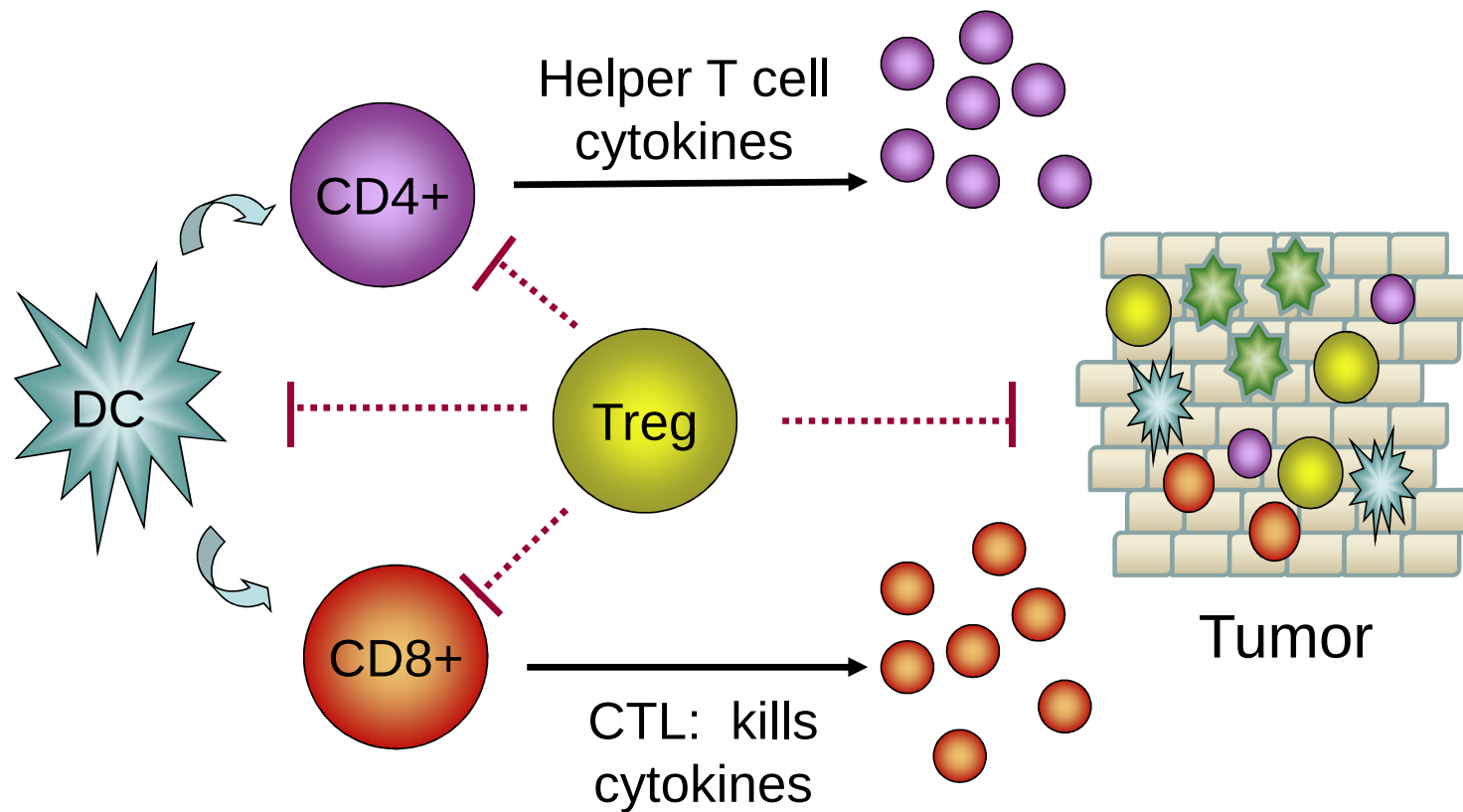


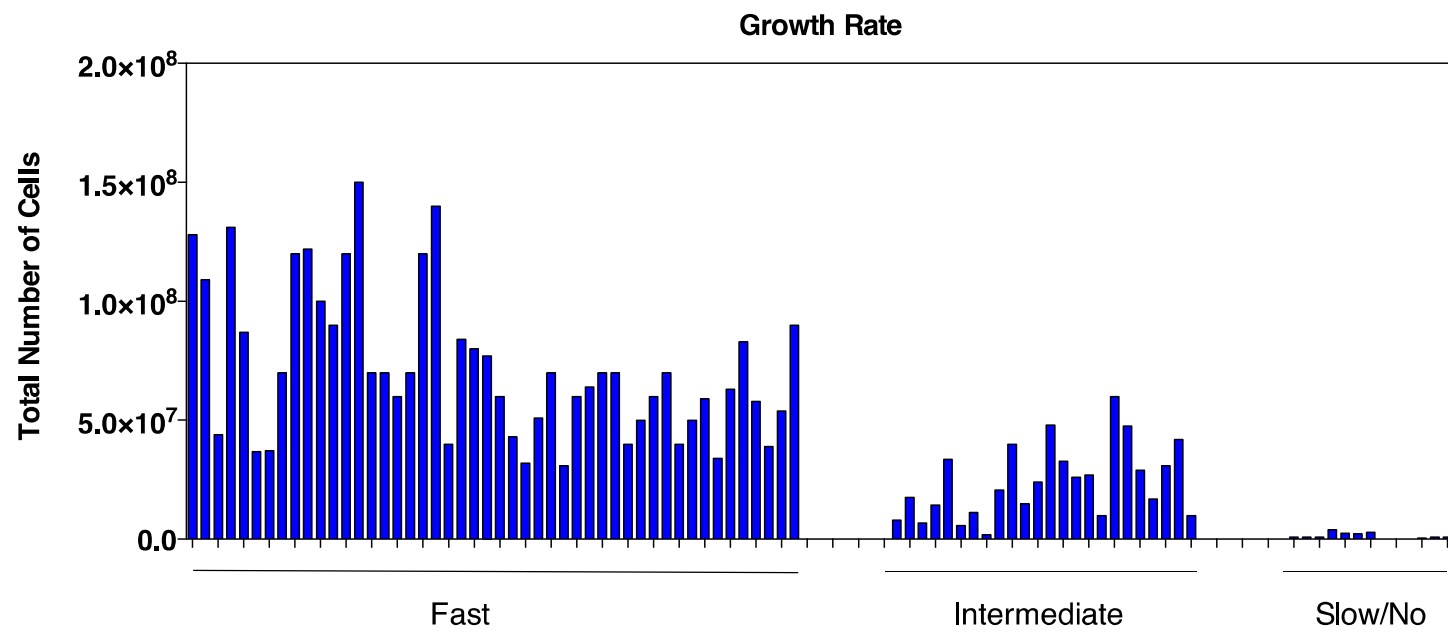
IL22

Innate Lymphoid Cells (ILCs)

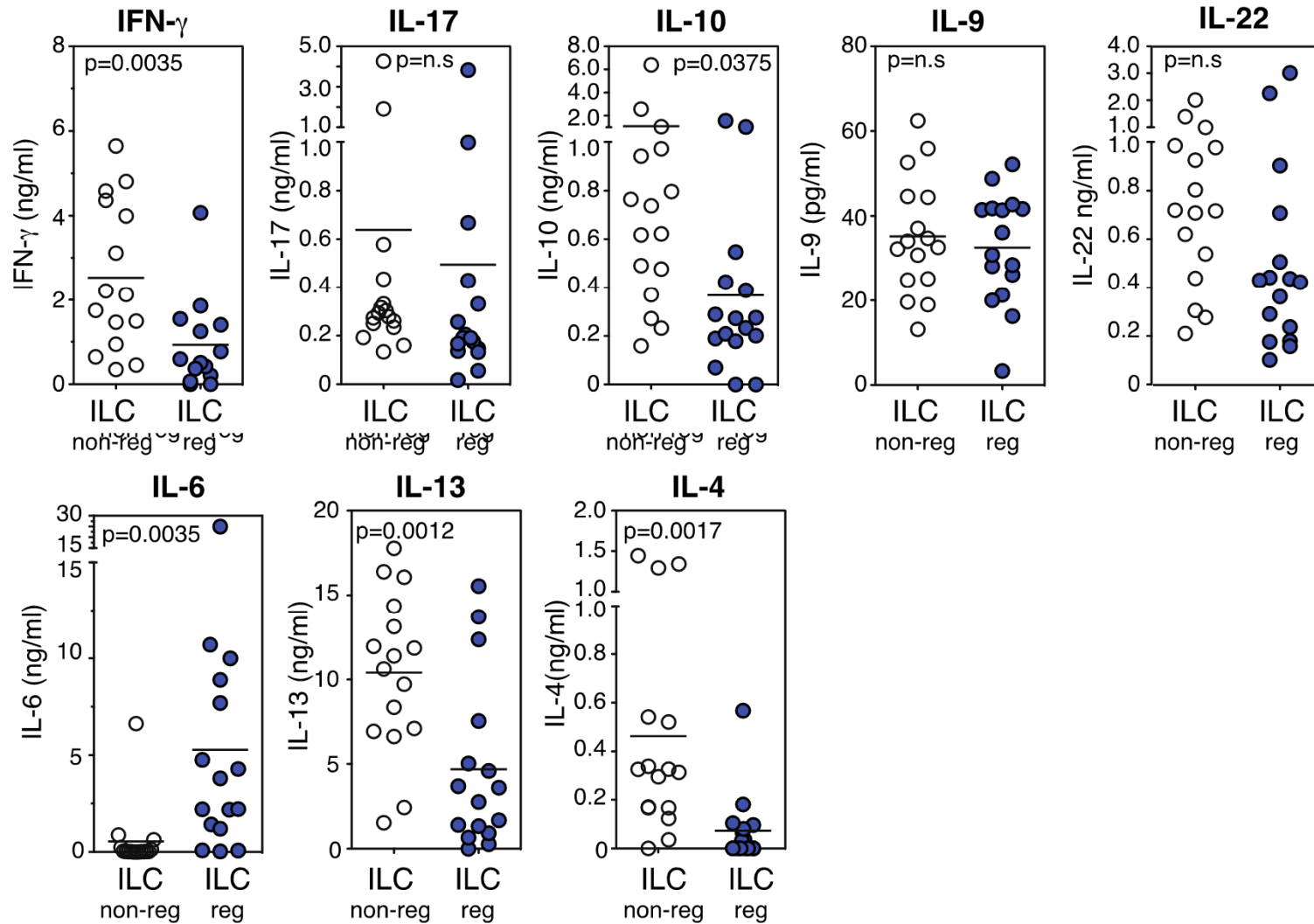


Players in the Immune Response





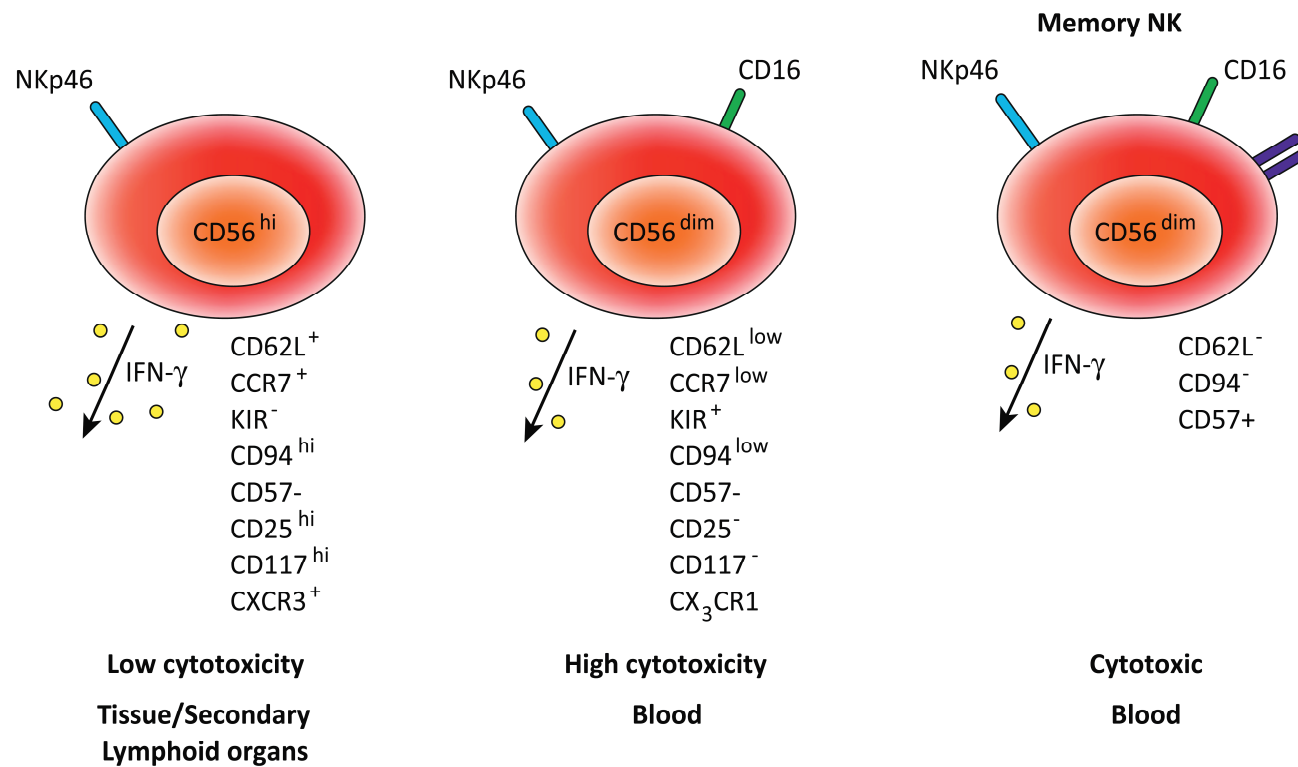
Altered Cytokine-Profile of TIL With IL-Cregs



Innate Immunity: NK cells

- regulated via a series of activating / inhibitory receptors
- cytolytic function – virus infected targets, tumor cells
- important role for controlling virus infections and tumor growth
- NK cell memory
- have inhibitory function – regulatory subpopulation
 - produce IL-10, limit CD8 responses
(SH Lee JEM 206:2235 (2009))
 - 2B4 regulates NK cell function: absence of 2B4, NK cells killed activated CD8 + T cells, perforin dependent
(Waggoner SN JCI 120;1925 (2010))

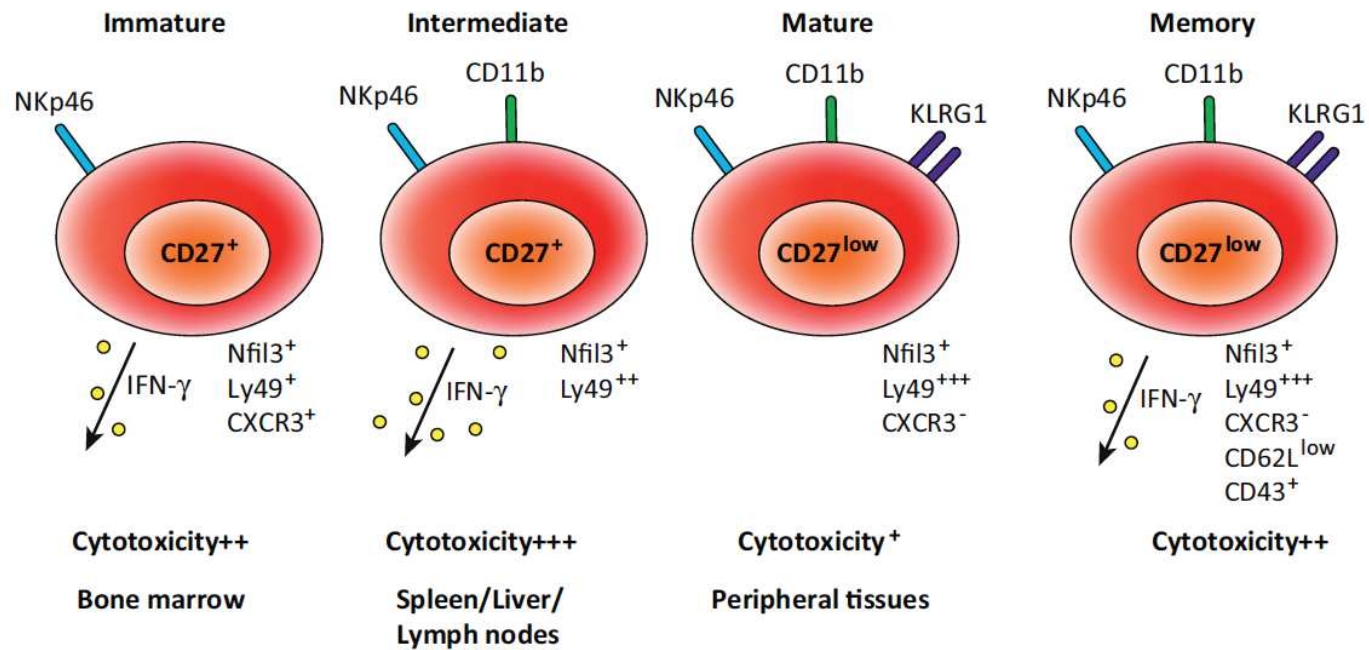
Human NK Cells



TRENDS in Immunology

Crome et al, 2013

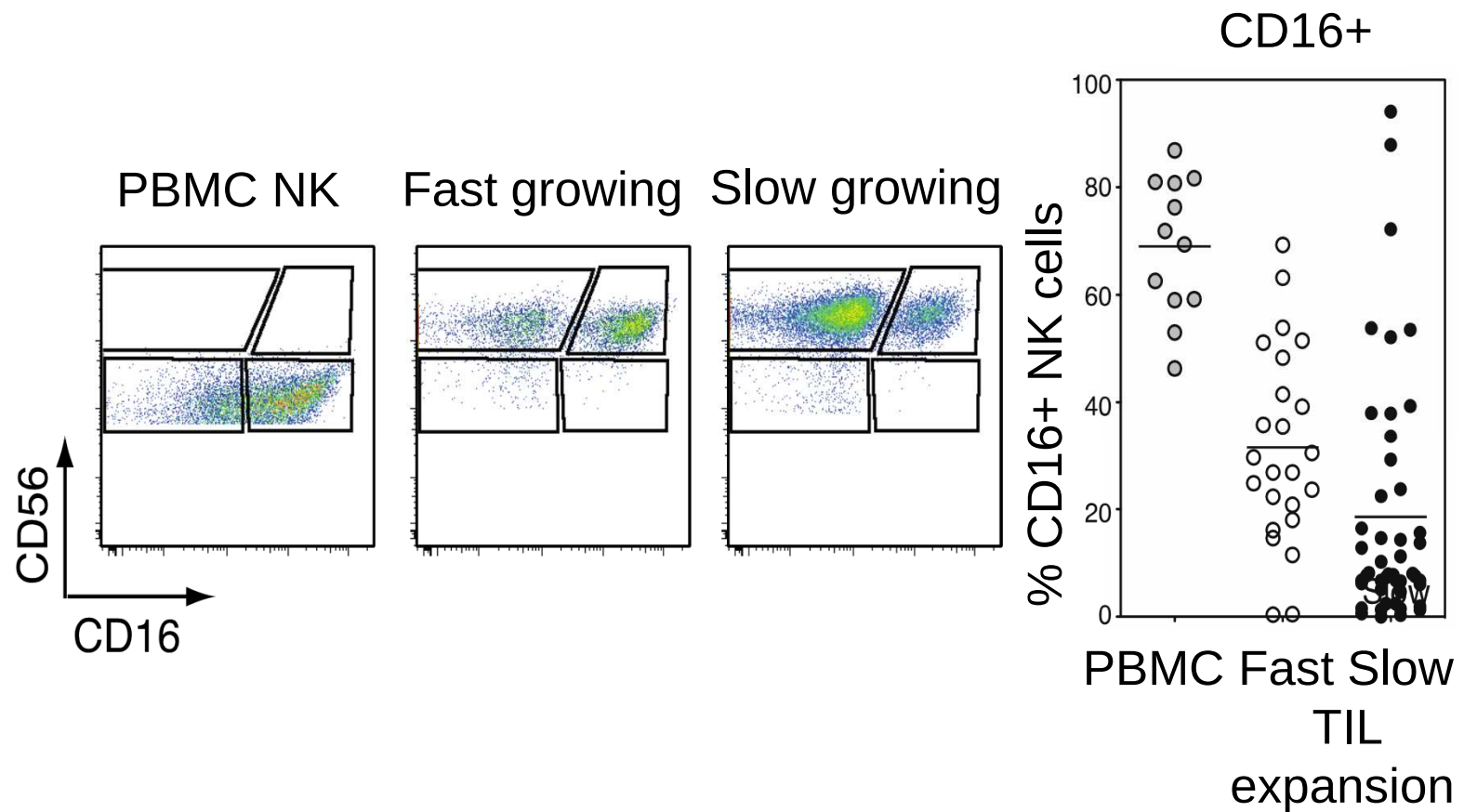
Mouse NK Cells



TRENDS in Immunology

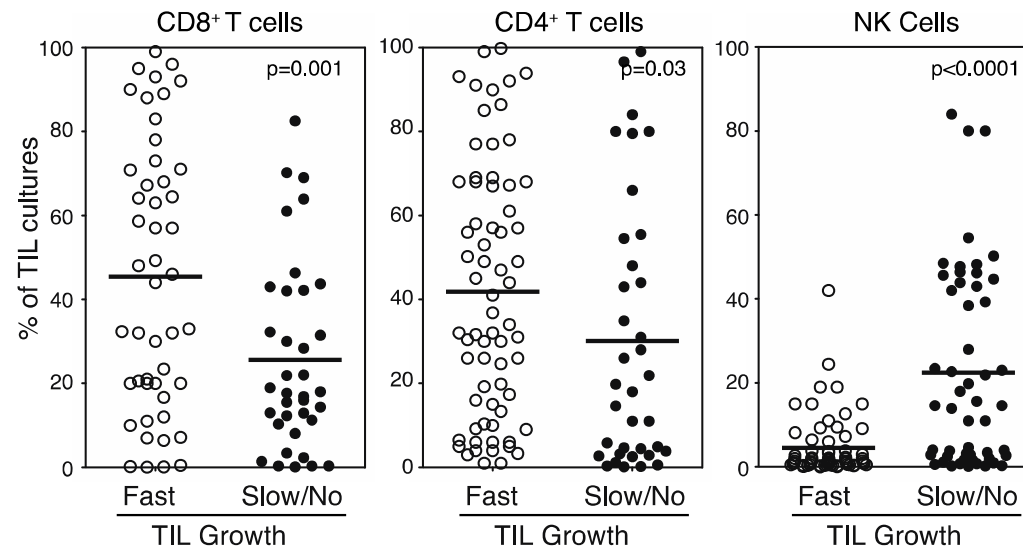
Crome et al, 2013

CD16 expression distinguishes NK cells from fast versus slow growing TIL cultures

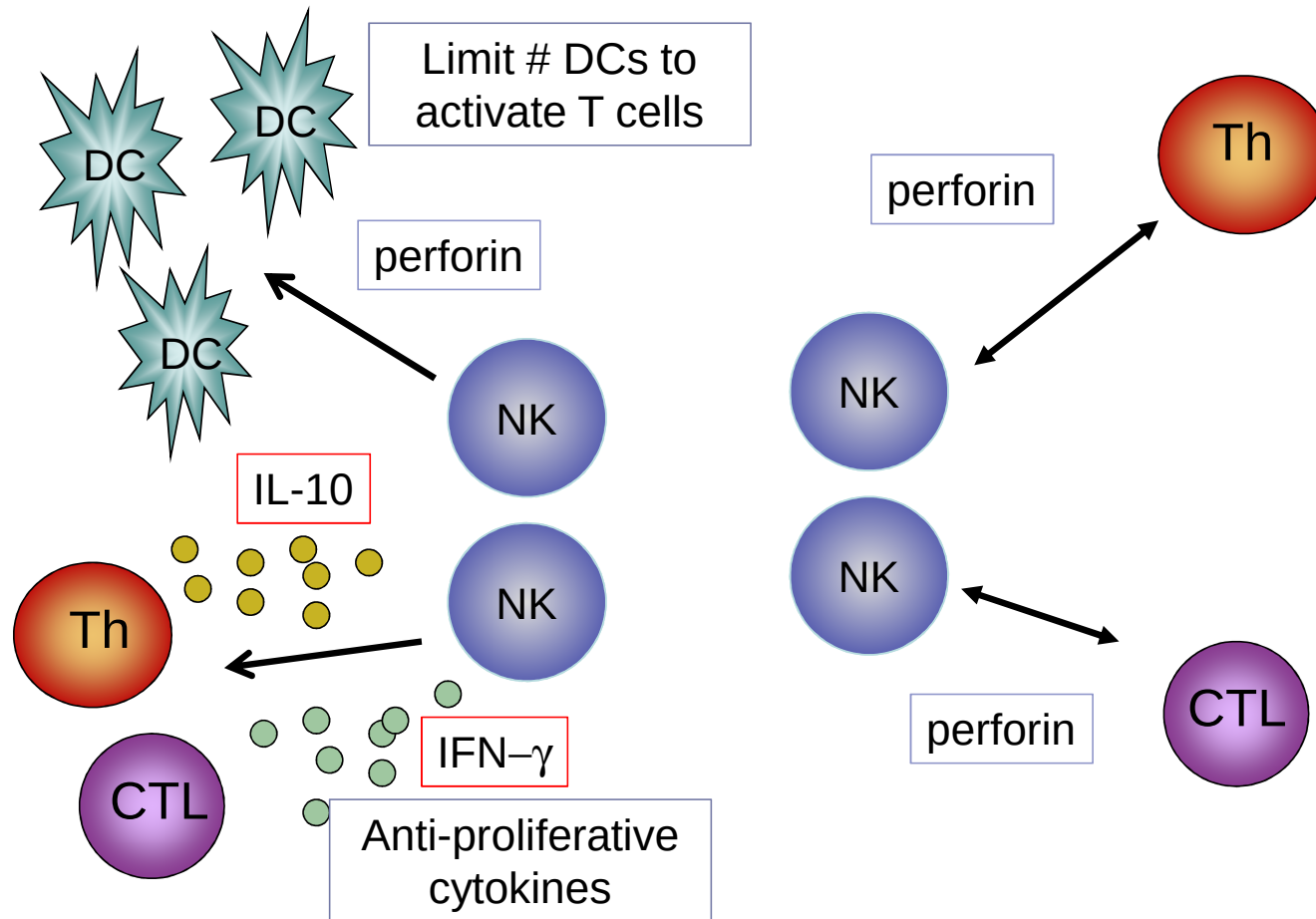


Ovarian TIL Expansions Correlate with NK Ratio

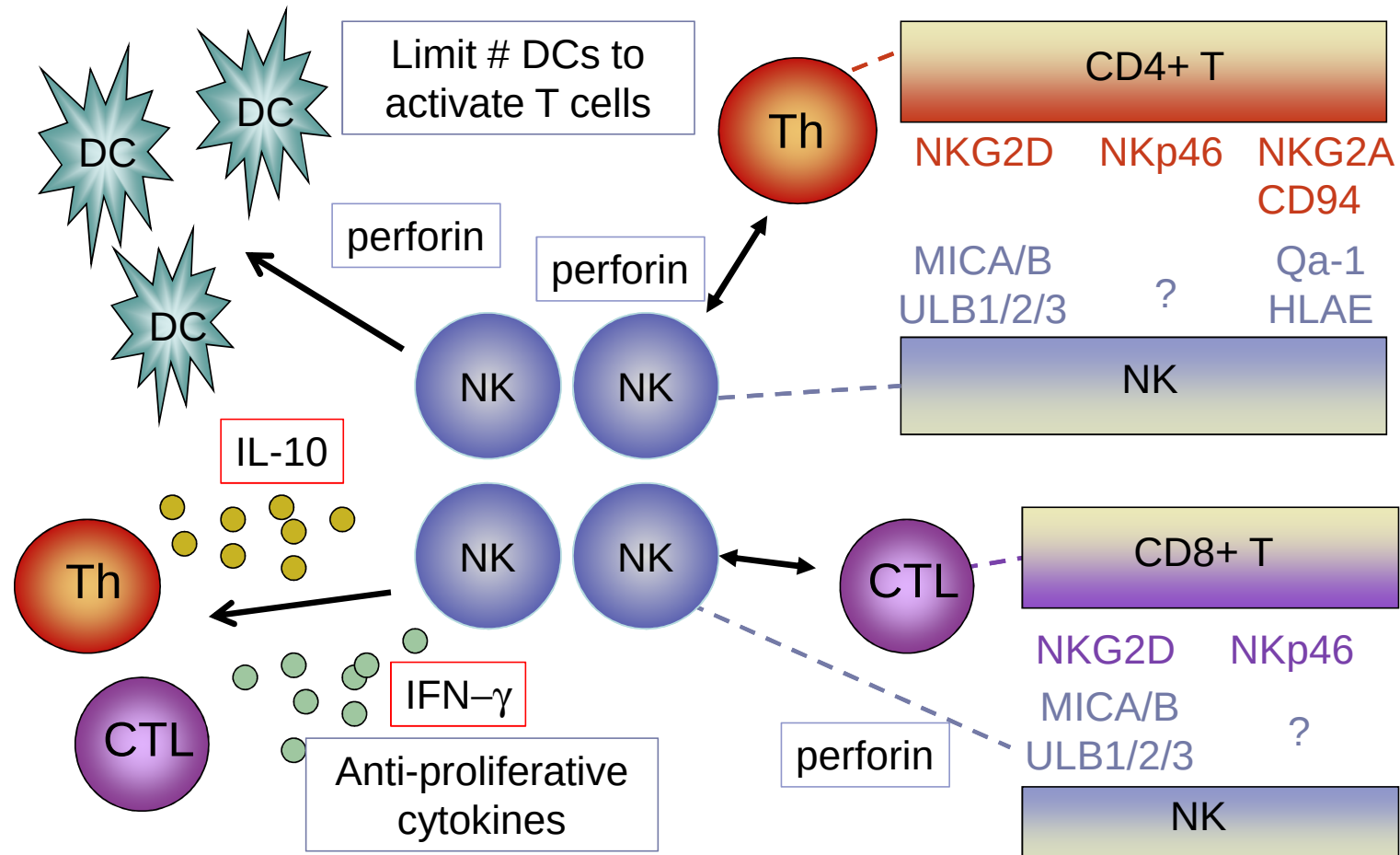
c)



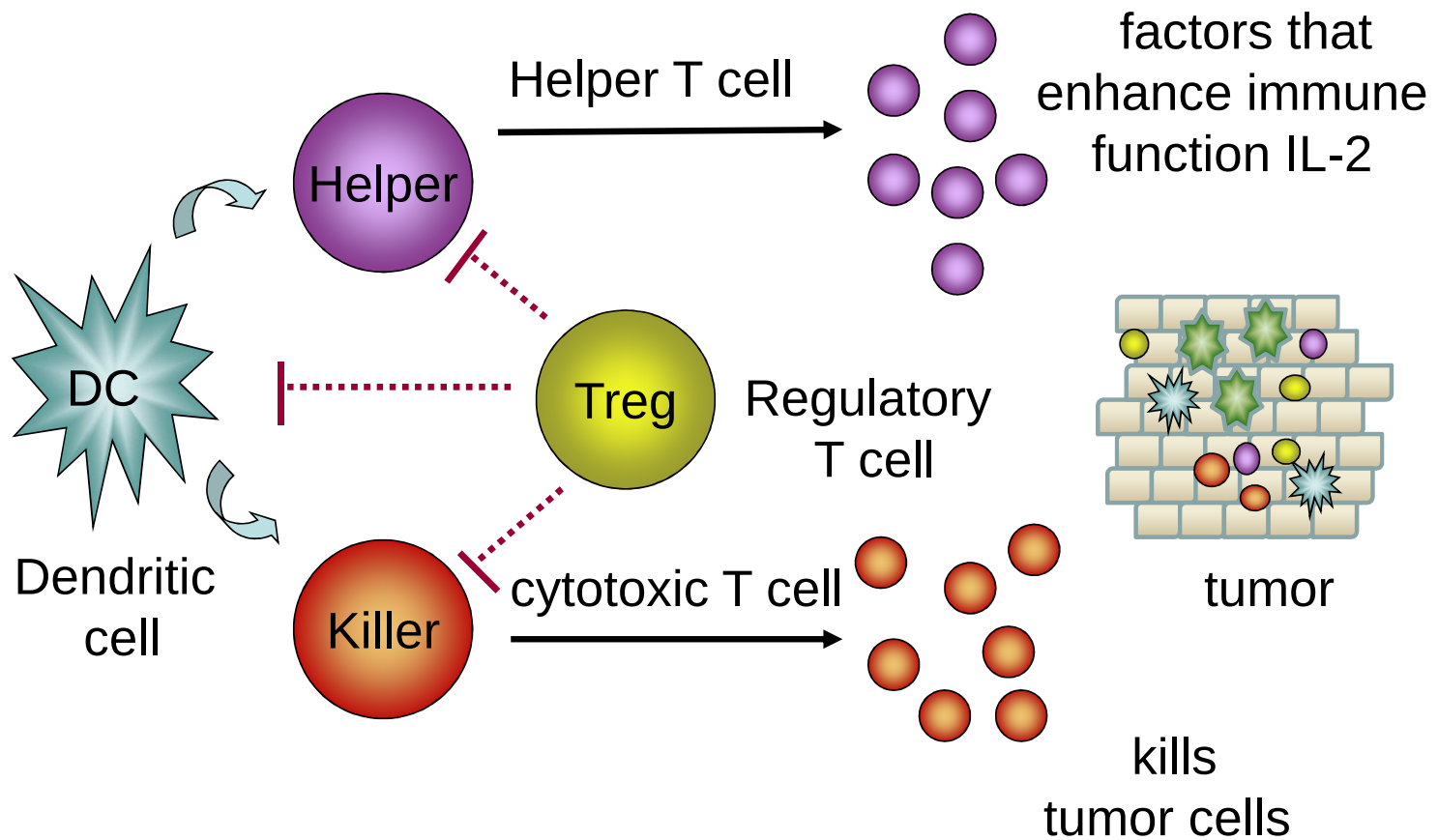
NK cells inhibit adaptive immunity through a variety of mechanisms



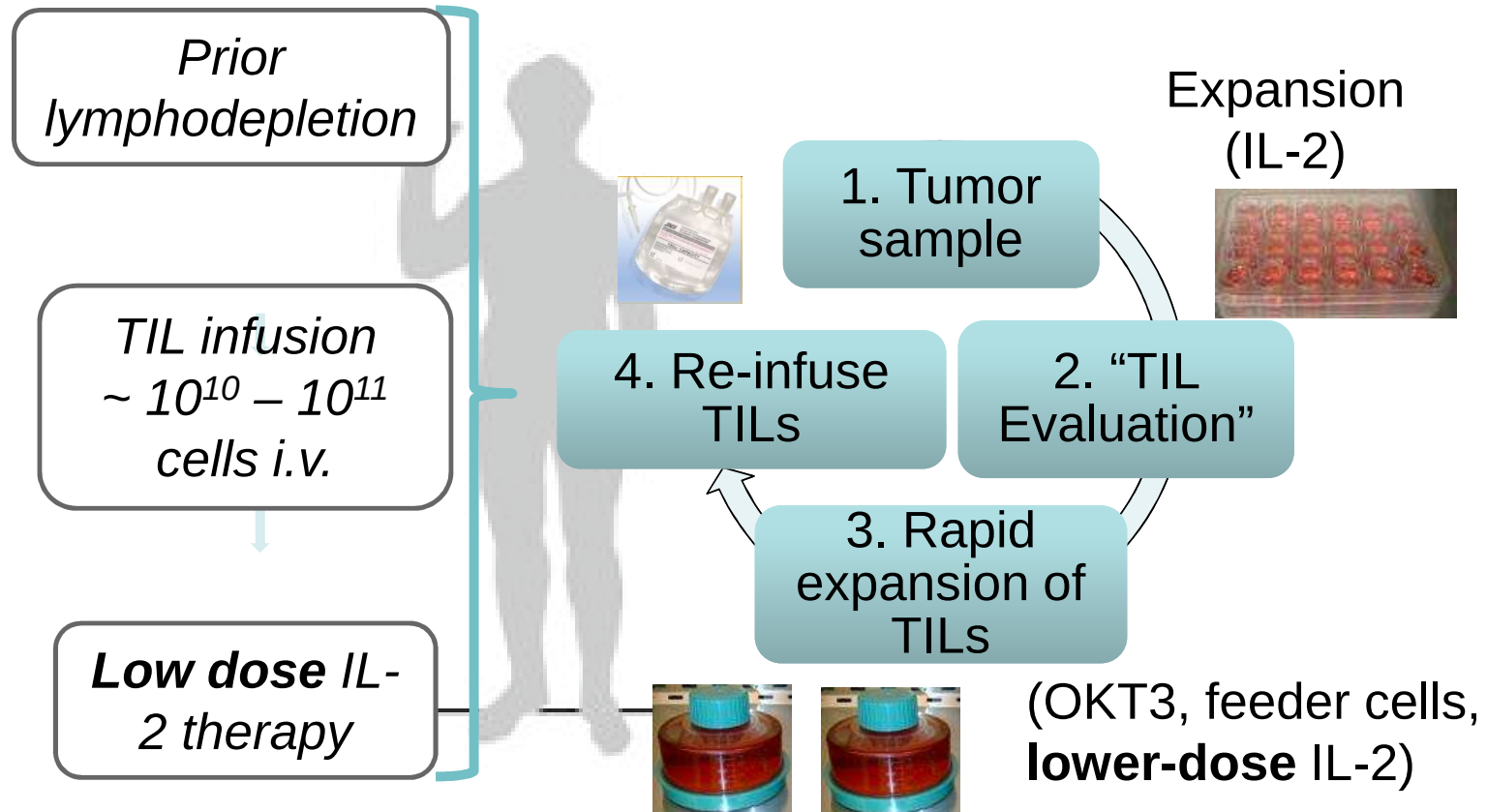
NK cells inhibit adaptive immunity through a variety of mechanisms



Players in the Immune Response

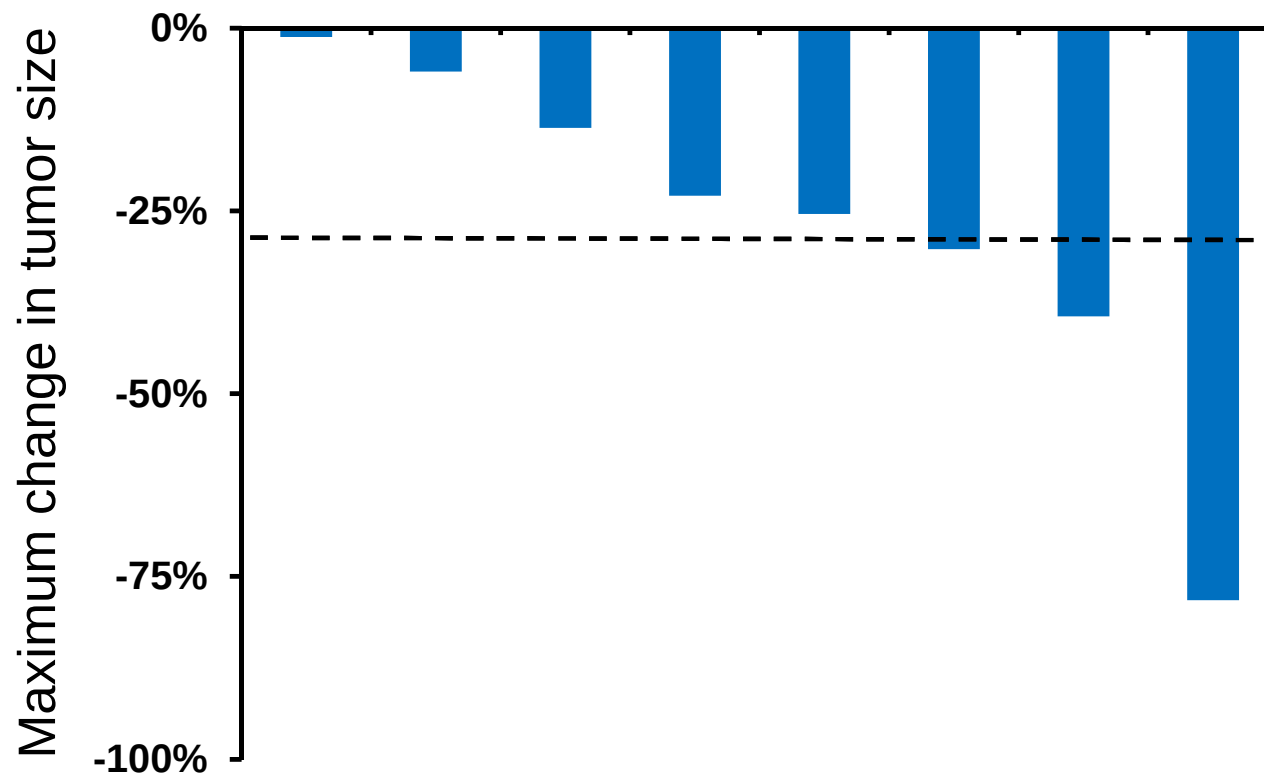


TIL based adoptive T cell therapy



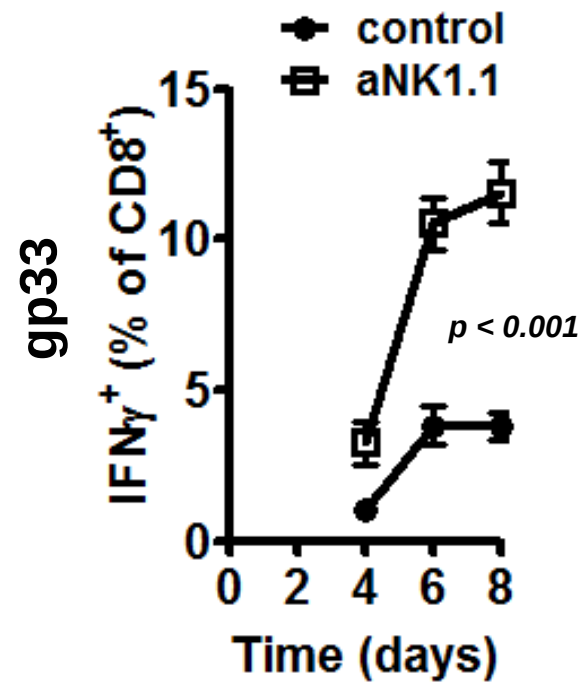
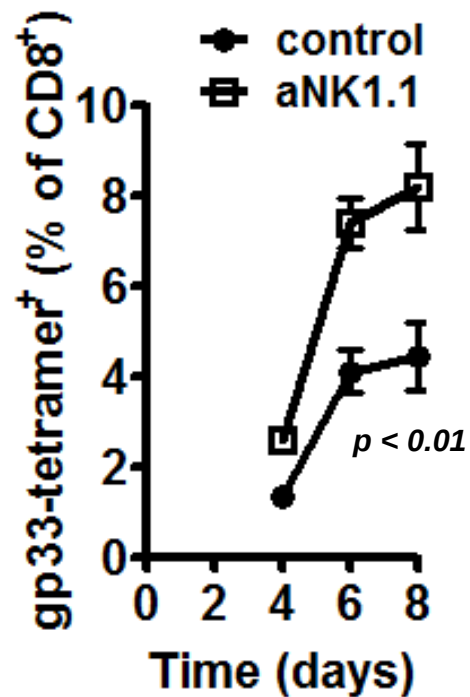
Nguyen et al PLoS ONE 2010

Responses with TIL based adoptive T cell therapy – Melanoma



NK cells limit CD8 immunity

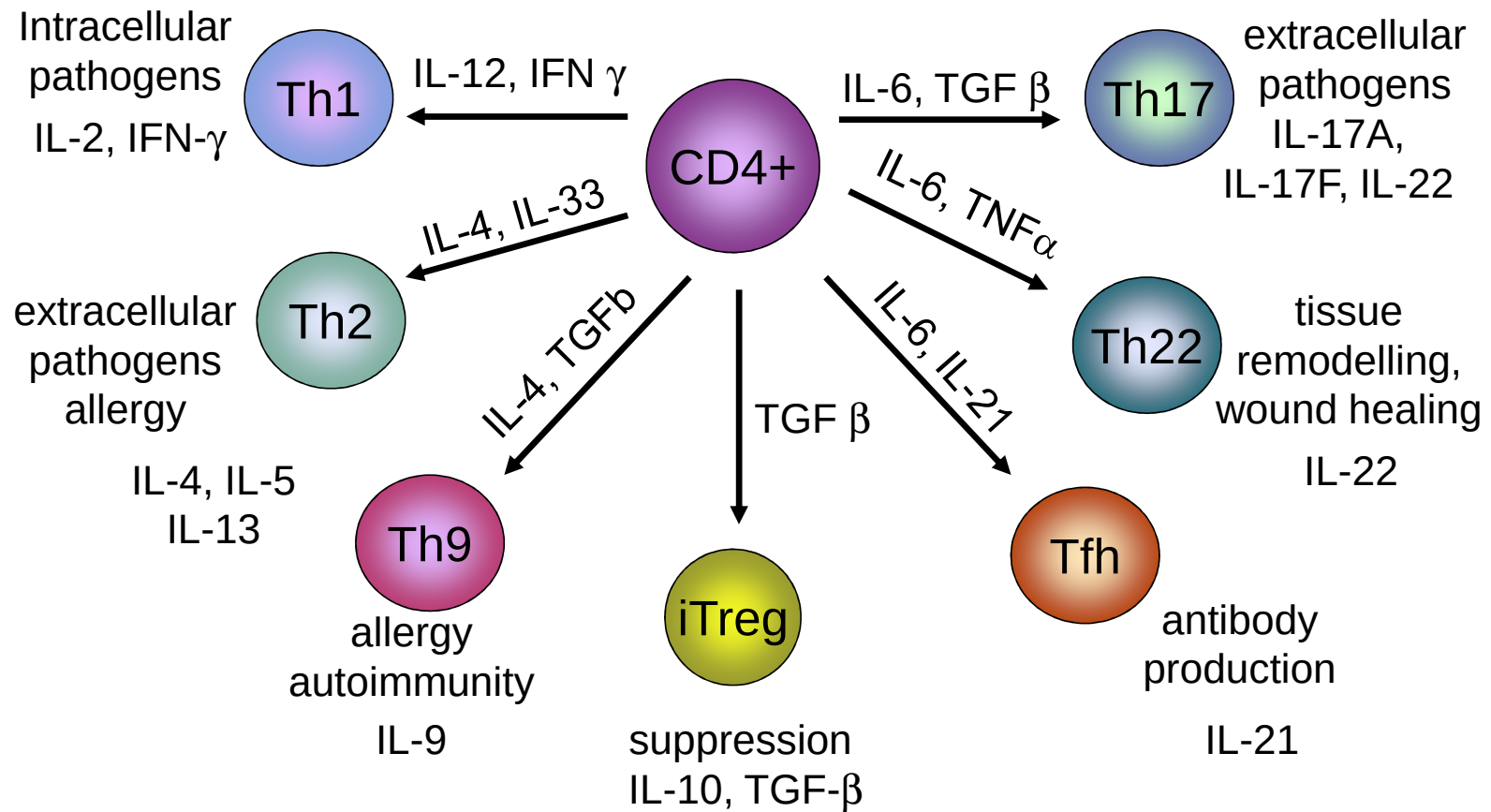
WT mice → LCMV → tetramer
NK depleted mice → cytokine



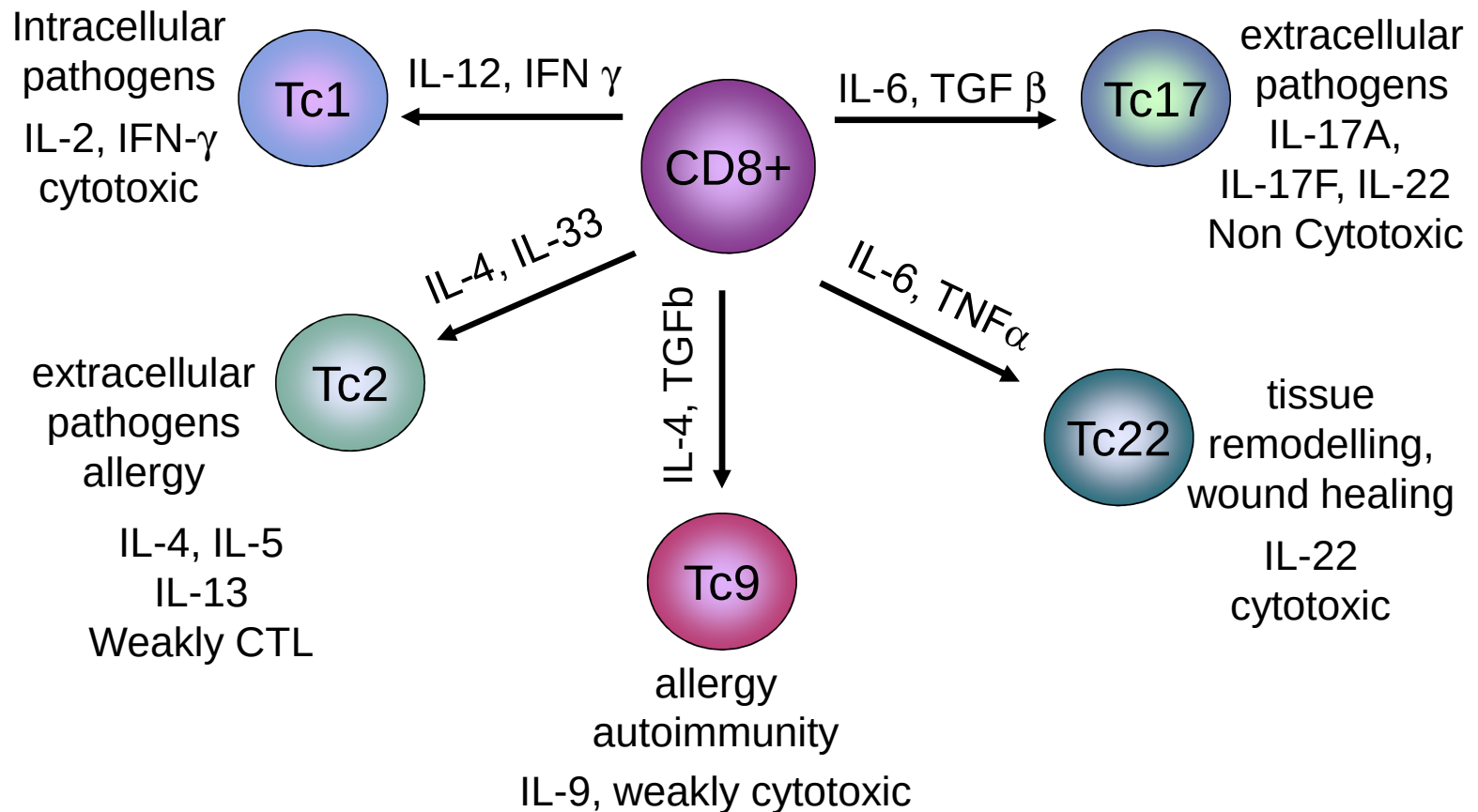
Big questions:

- 1) How is the TME set up? Mutations, inherent properties of immune response in patients
- 2) Dissect cell types, function, markers
- 3) Predictive value: treatments, responses?
- 4) Need to include live cell analysis

Multiple different CD4+ T cell lineages



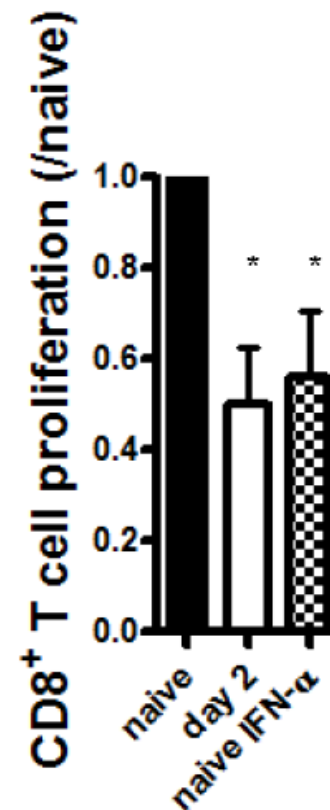
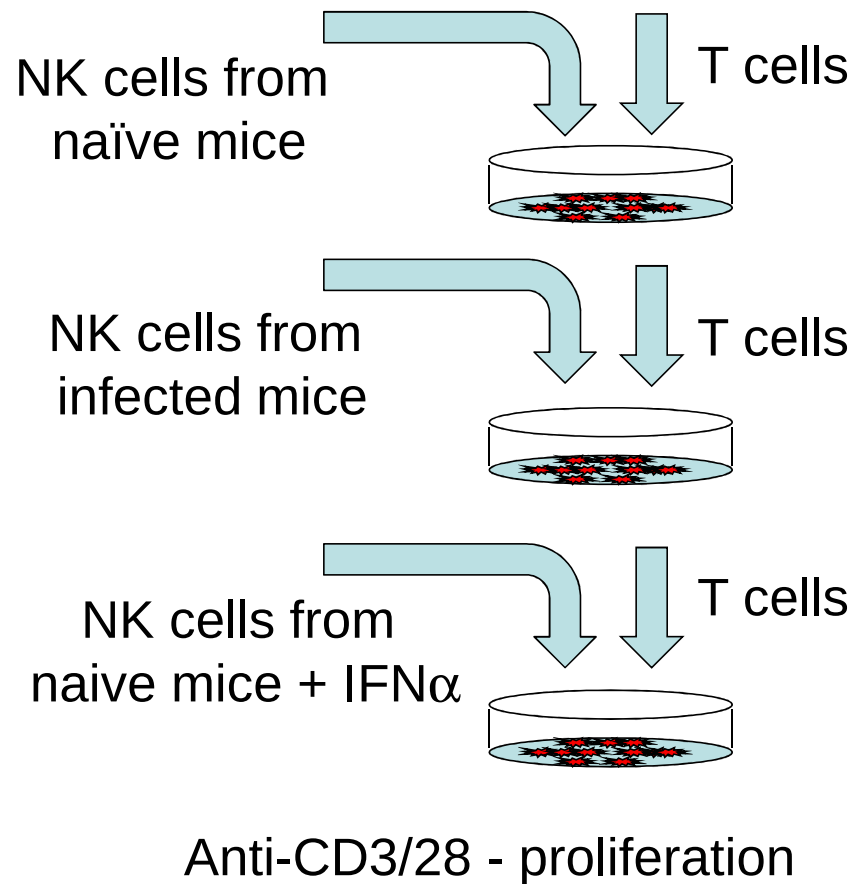
Multiple different CD8+ T cell lineages



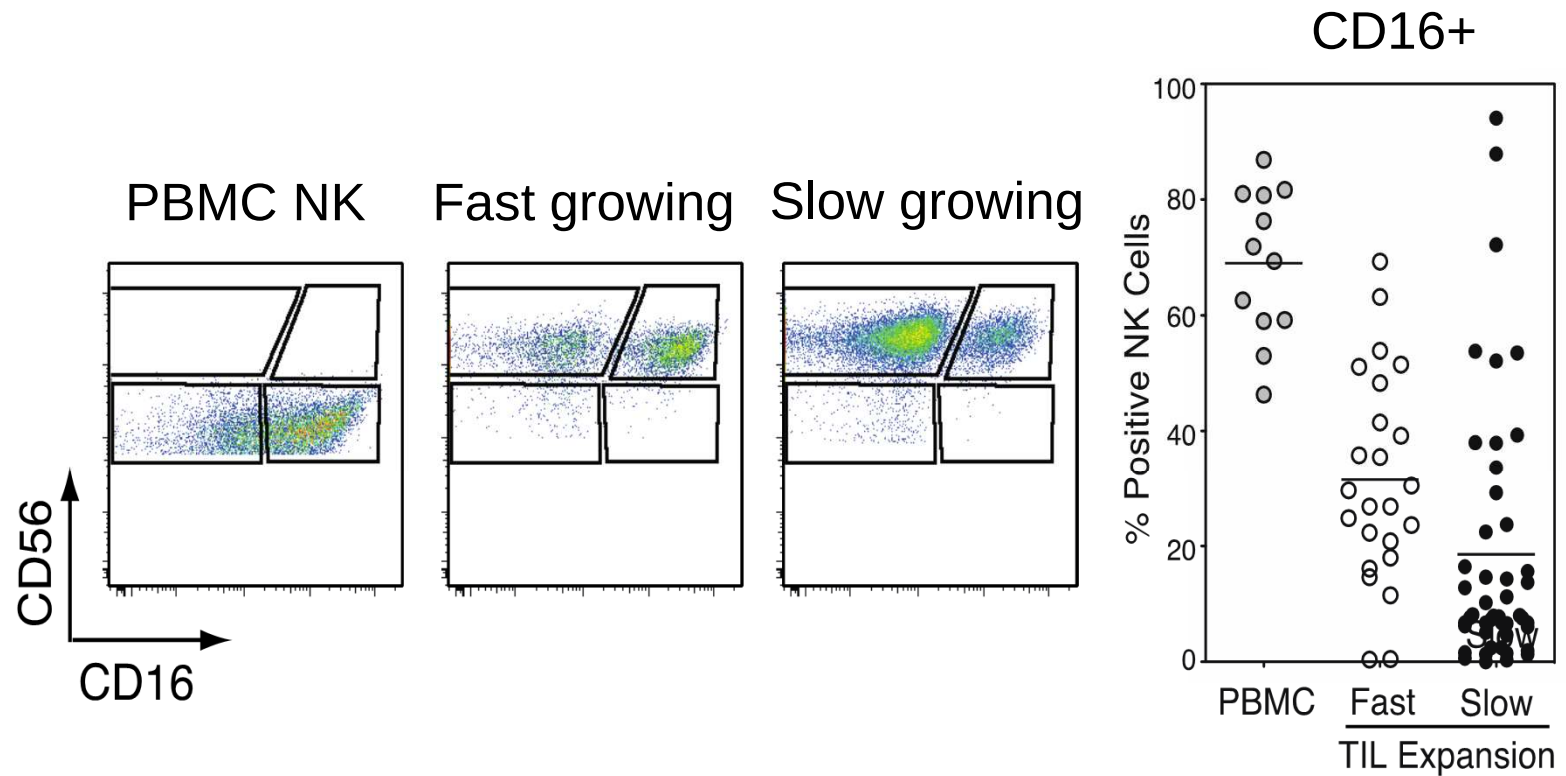
Team TILs



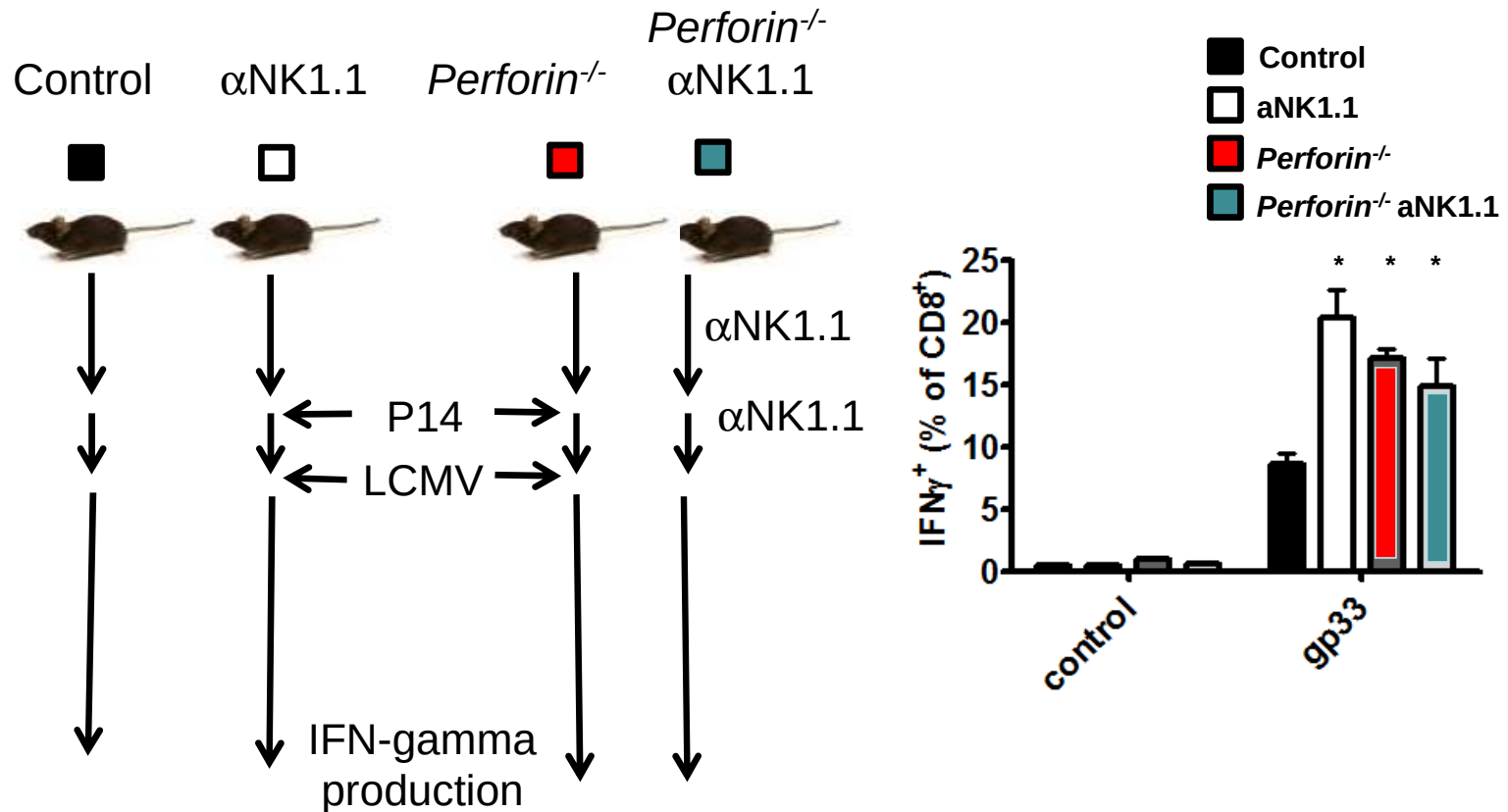
NK cells limit T cell proliferation in vitro



CD16 expression distinguishes NK cells from fast versus slow growing TIL cultures



NK cells limit T cell function via perforin



Points for discussion:

- 1) Define markers/signature for this NKreg/ILC population.
- 2) What triggers the development of NKregs in vivo, in the tumor setting?
- 3) Do they have prognostic value?
- 4) Do they play a role in other cancers?