The Immune Escape Game 2012: Targeting Tumor-derived Death Ligands, Exosomes and Treg

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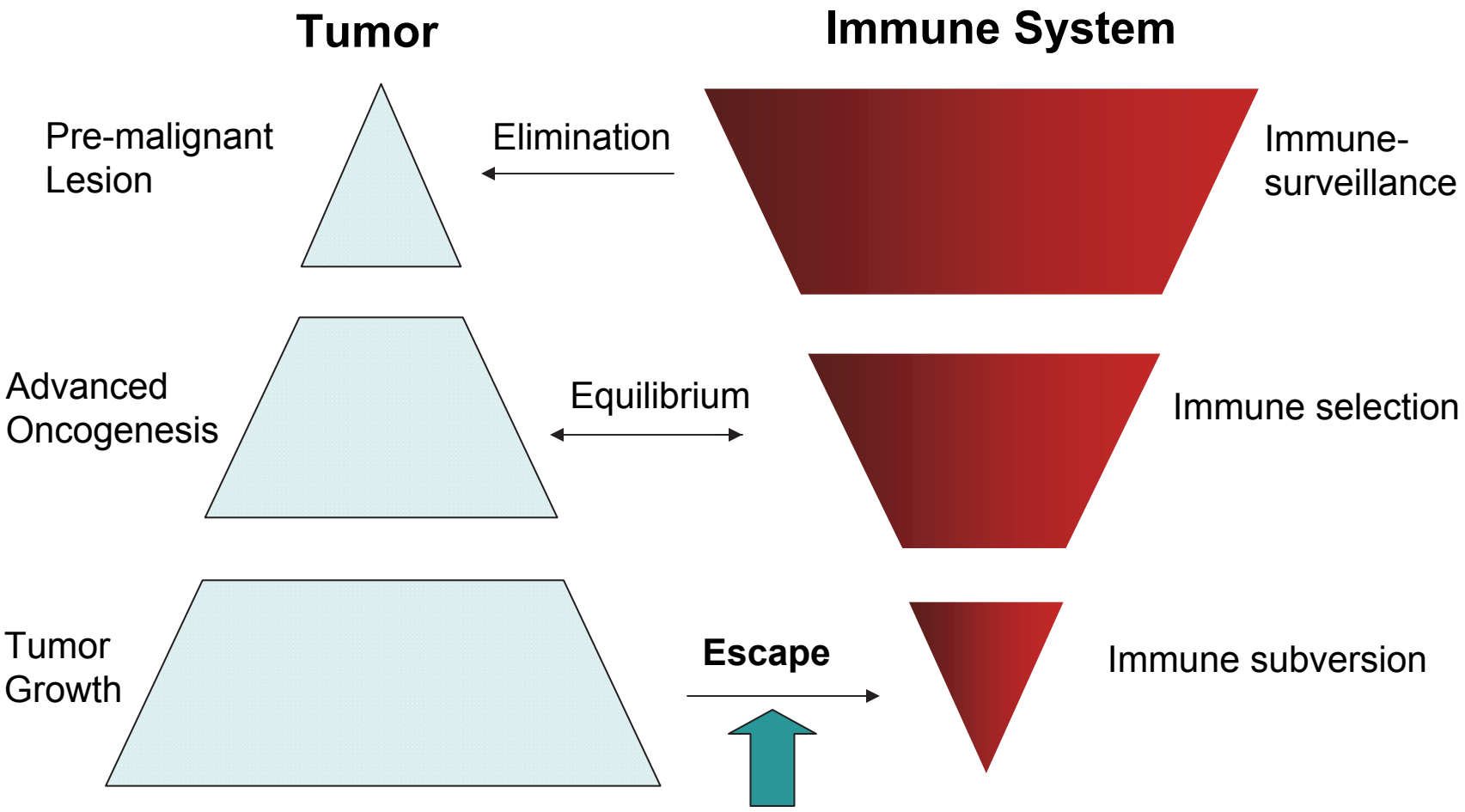
Richard V. Smalley, MD Memorial Award Lecture 2012

Disclosure slide

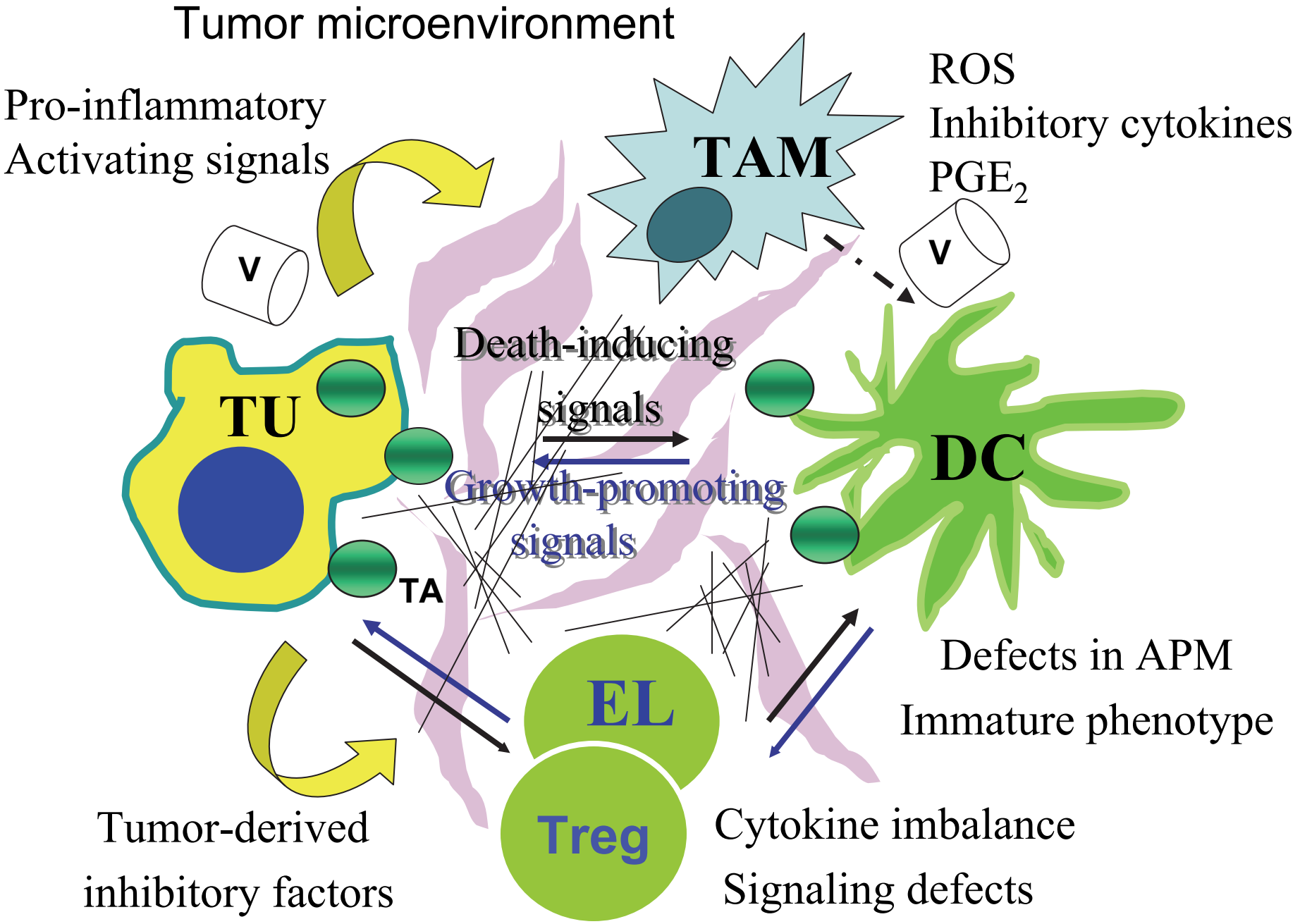
- The presenter of the Richard V. Smalley, MD Memorial Award Lecture 2012 at the Annual SITC Meeting has no disclosures and no conflicts to report



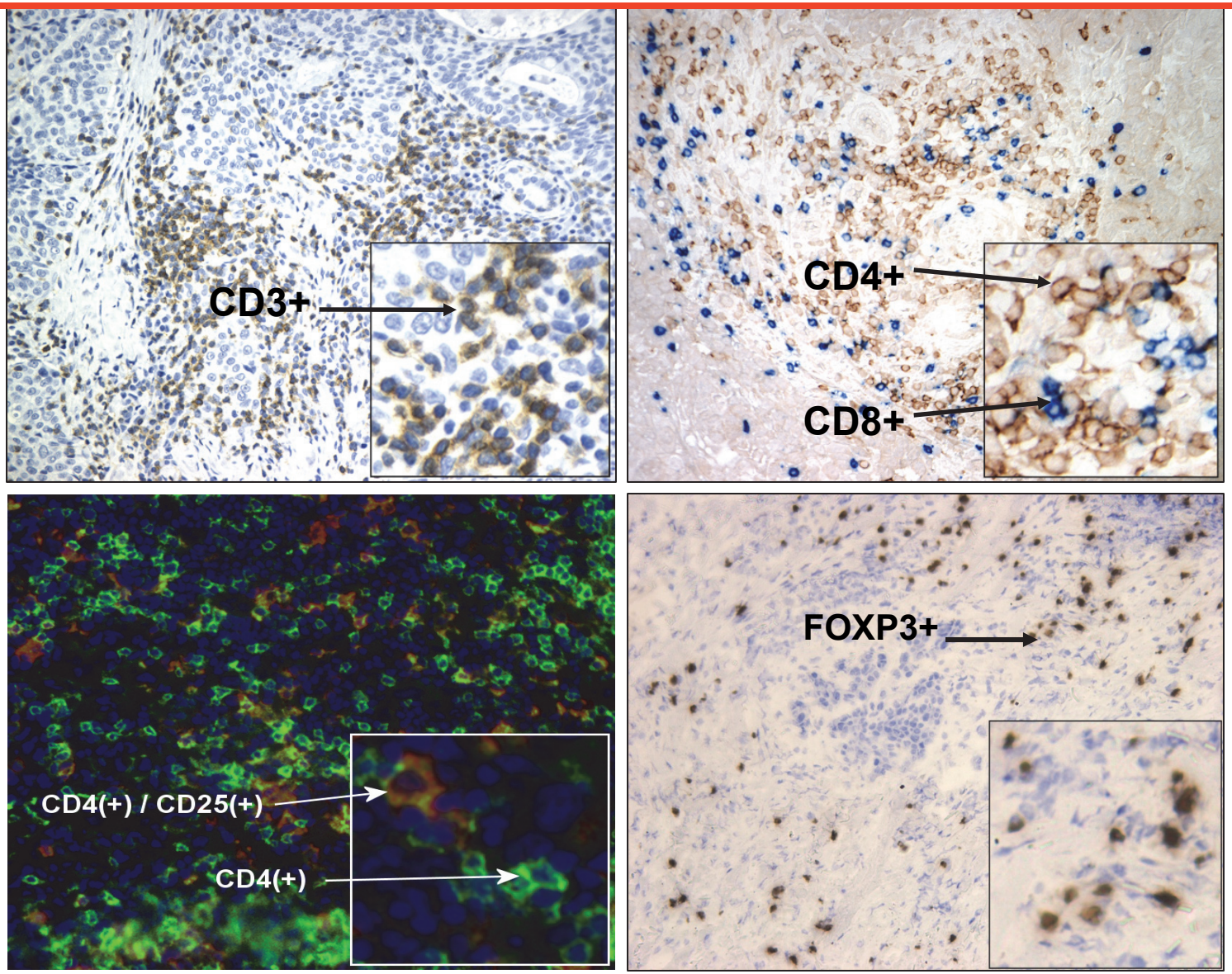
Modified from Zitvogel et al. Nat. Rev. Immunol. 2006 6(10):721-727

Tumors are not passive targets for immune cells: they escape, prosper and fight back

- T-cells, especially TIL, are functionally impaired in tumor-bearing hosts
- Tumor-induced apoptosis of T/NK cells is seen in situ and in the circulation
- Tumors engineer immune escape and hide from the host immune system: appearance of “epitope-loss” variants which are resistant to immune cells
- Tumors create a unique microenvironment which promotes tumor growth and blocks anti-tumor activities of immune cells

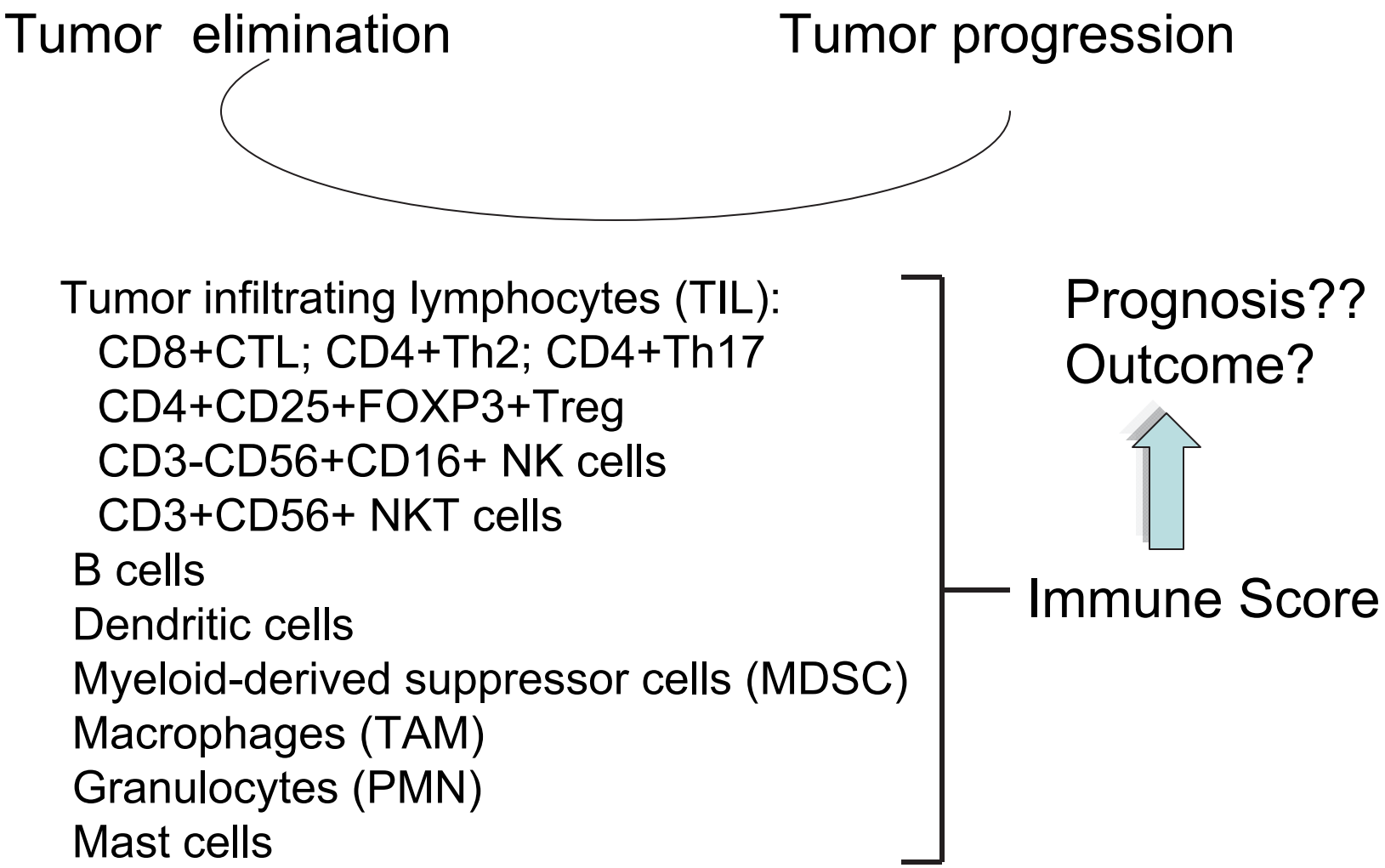

Tumor microenvironment
Pro-inflammatory
Activating signals
TAM
ROS
Inhibitory cytokines
PGE2
V
V
TU
Death-inducing
signals
Growth-promoting
signals
DC
TA
EL
Treg
Defects in APM
Immature phenotype
Tumor-derived
inhibitory factors
Cytokine imbalance
Signaling defects

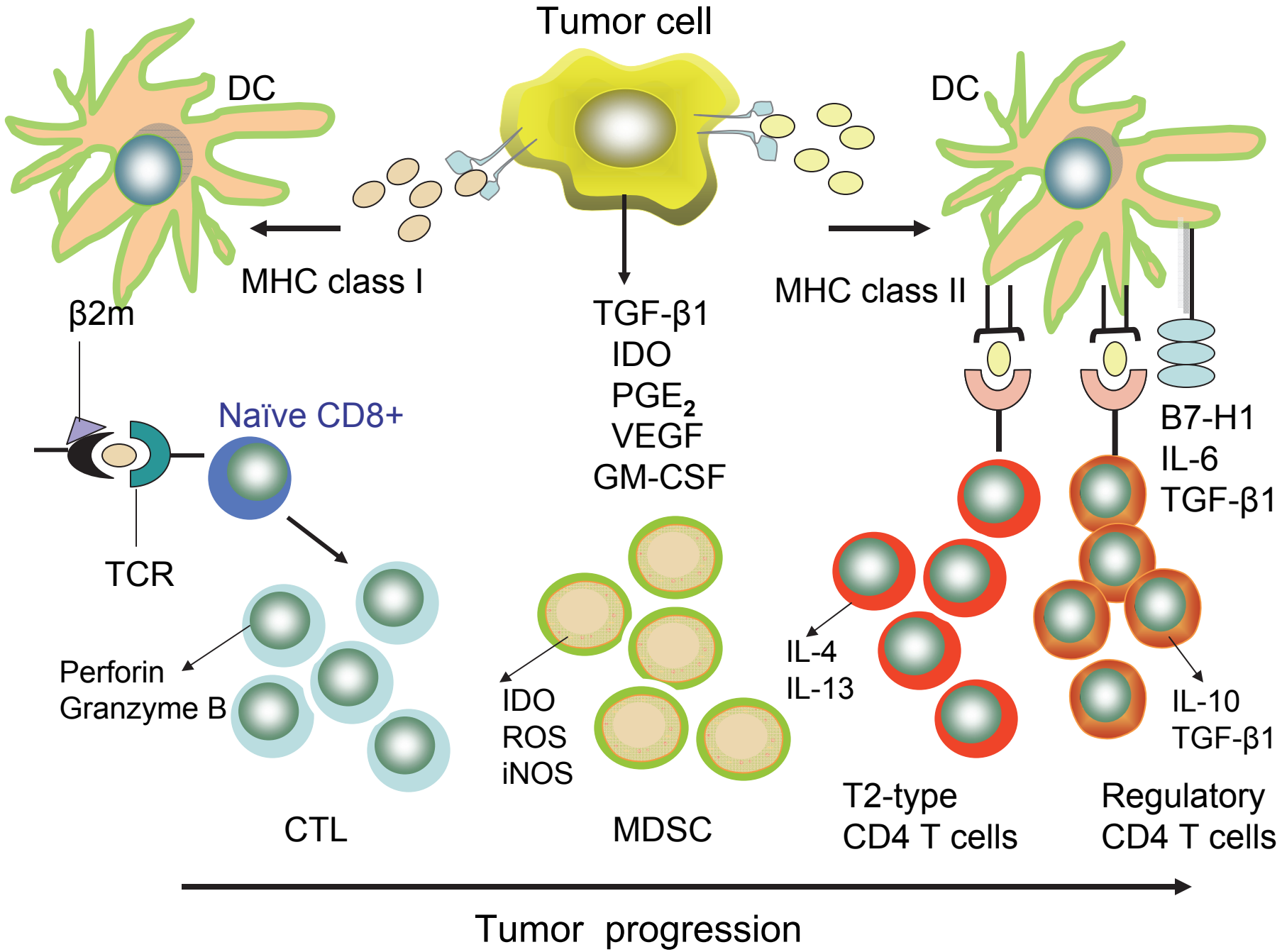
Lymphocytic infiltrates in human tumors



x200 (inset x600)

Immune cells infiltrating a human tumor create its unique "**immune signature**" and mediate pro-tumor or anti-tumor functions depending on locally-generated signals




Tumor cell
DC
DC
MHC class I
MHC class II
β2m
TGF-β1
IDO
PGE2
VEGF
GM-CSF
Naïve CD8+
TCR
B7-H1
IL-6
TGF-β1
Perforin
Granzyme B
IDO
ROS
iNOS
IL-4
IL-13
IL-10
TGF-β1
CTL
MDSC
T2-type
CD4 T cells
Regulatory
CD4 T cells
Tumor progression

Mechanisms used by tumor cells to suppress anti-tumor immunity are many

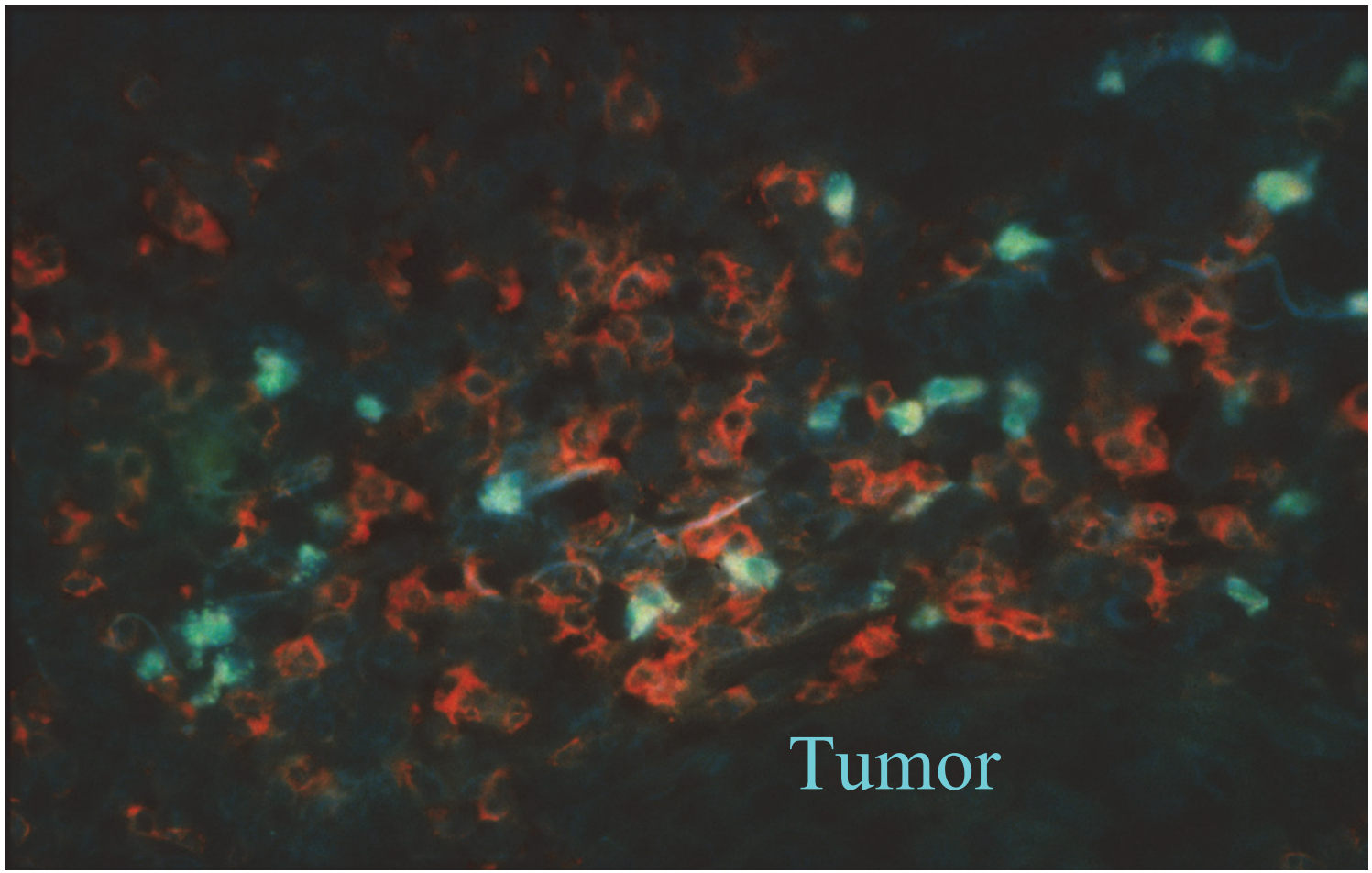
- Elimination of anti-tumor effector Tcells in situ or in the periphery by using the death receptor-ligand signaling pathways
- Tumor-derived exosomes (TEX) as vehicles for the delivery of immunosuppressive signals
- Expansion of Treg in the tumor microenvironment and their impact on anti-tumor responses

What is happening to anti-tumor effector T cells during cancer progression ?

Clues from studies of TIL isolated from human solid tumors:

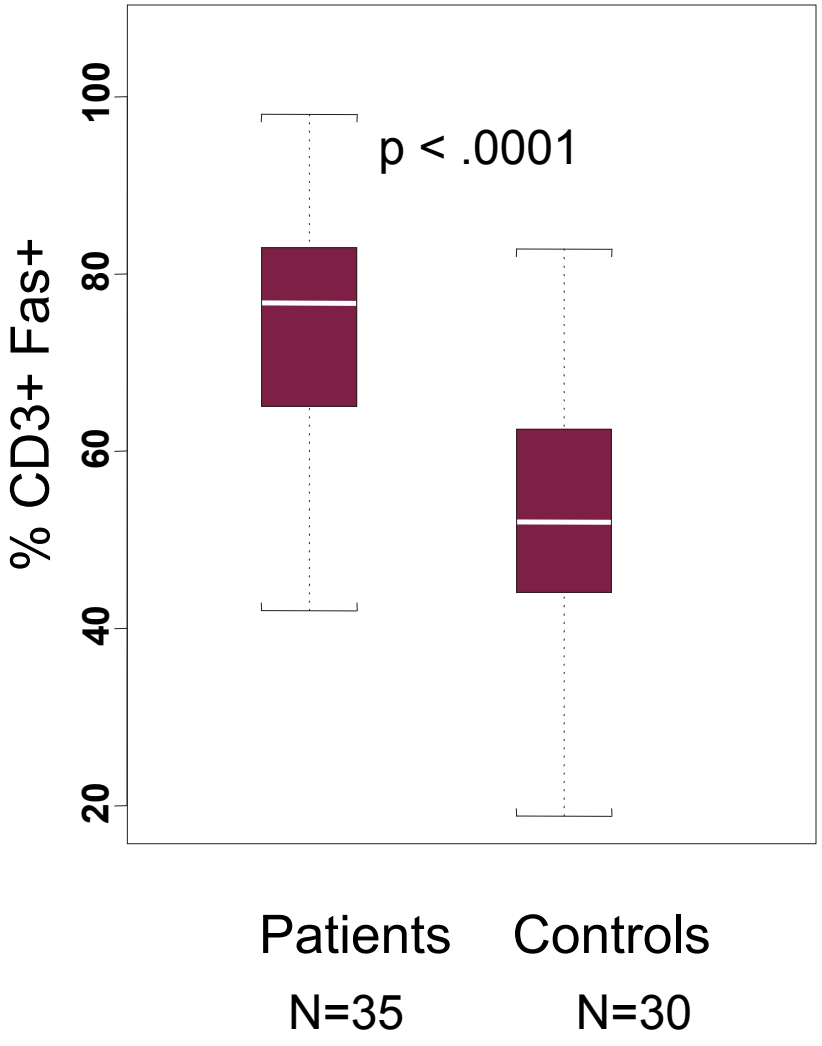
- Low or absent ζ chain expression
- Suppressed NFκB activation
- Depressed locomotion, proliferation, cytotoxicity
- Altered cytokine profile: a lack of IL-2 or IFN-γ
- Increased levels of caspase-3 activity
- Apoptosis of CD8+ T effector cells *in situ*

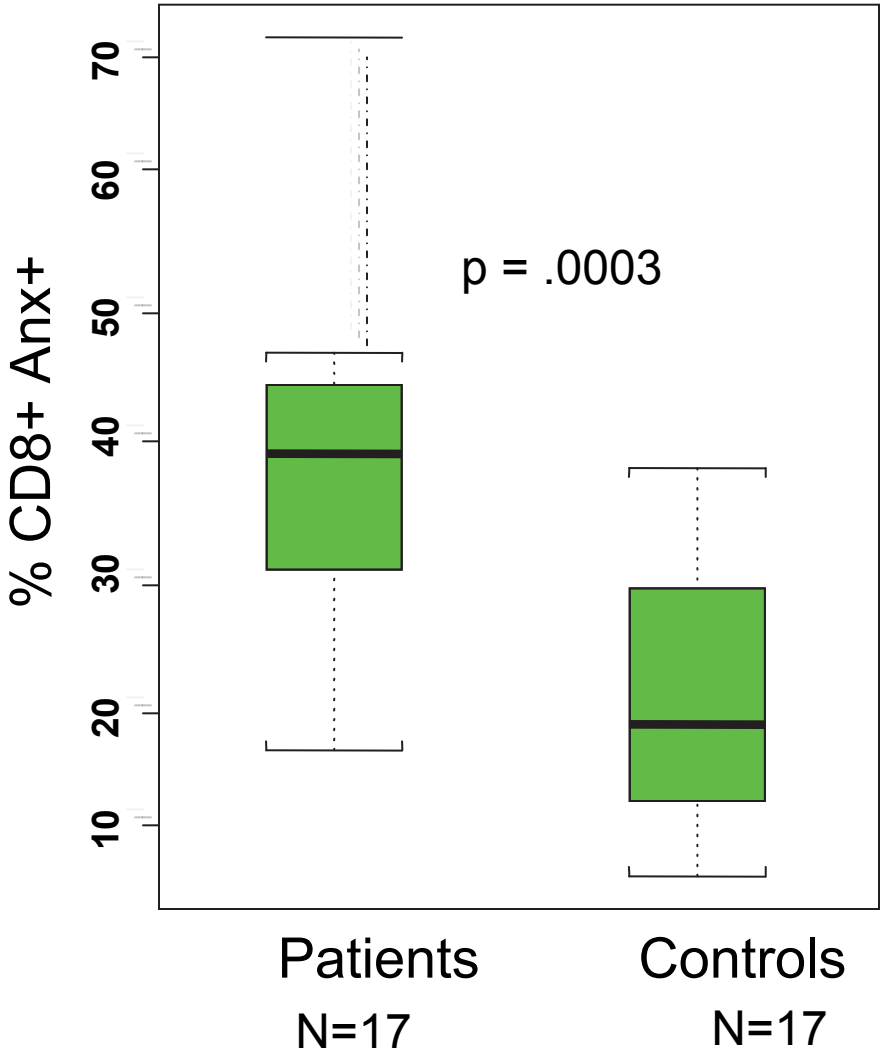
Apoptotic CD8+ T cells in the nest of lymphocytes at the tumor site



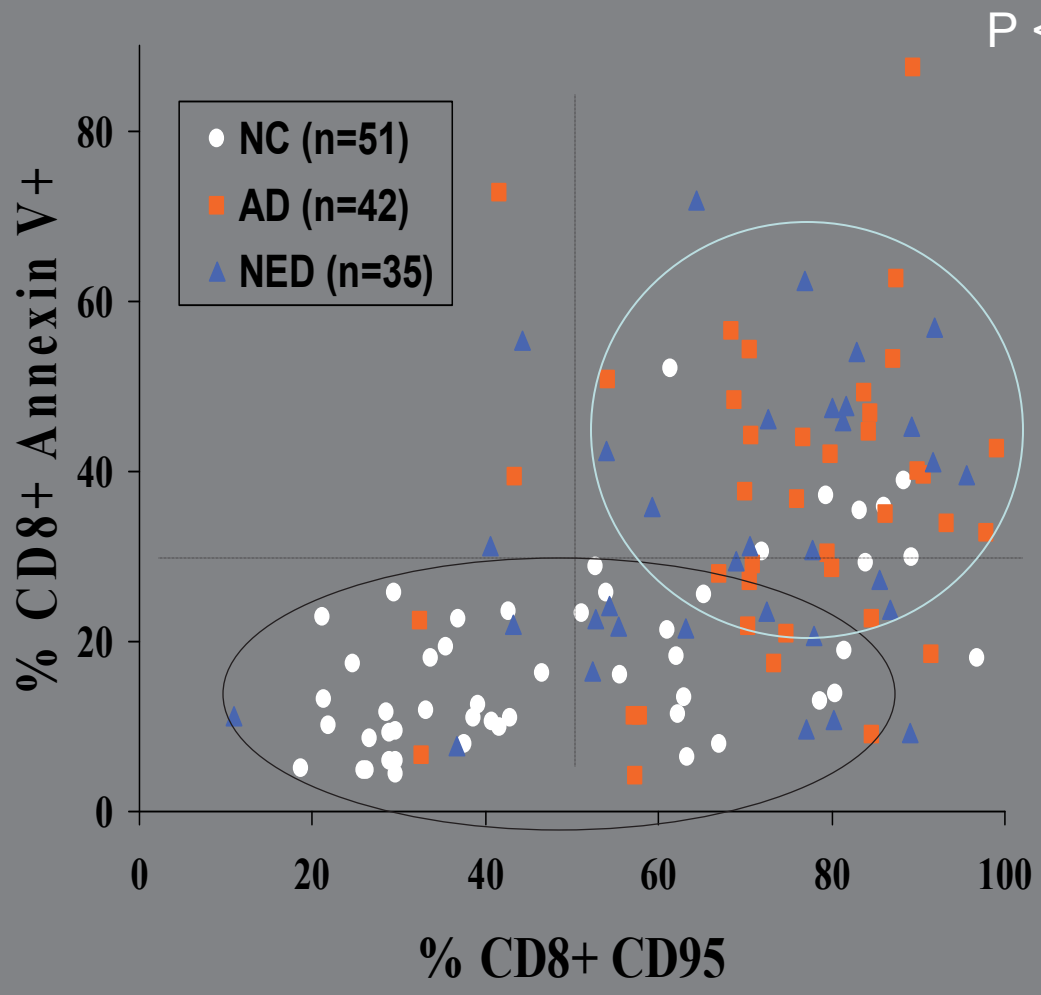
Red = alive CD8+ T cells Blue = dying CD8+ T cells

FAS expression on and ANXV binding to CD8+ T cells in patients with HNC and NC





Apoptosis of circulating CD8+ T cells discriminates patients with HNC from normal controls



However, this study also shows that Annexin binding does not discriminate between HNC patients with AD vs. NED

Which subsets of circulating CD3+ T cells undergo apoptosis? (i.e, are ANXV+)

CD8+ T cells preferentially over CD4+ (> 35% vs. 5%)

CD8+CD95+ (>50% vs. <20%)

CD8+CD45RO-CD27- (>12% vs. <2%)

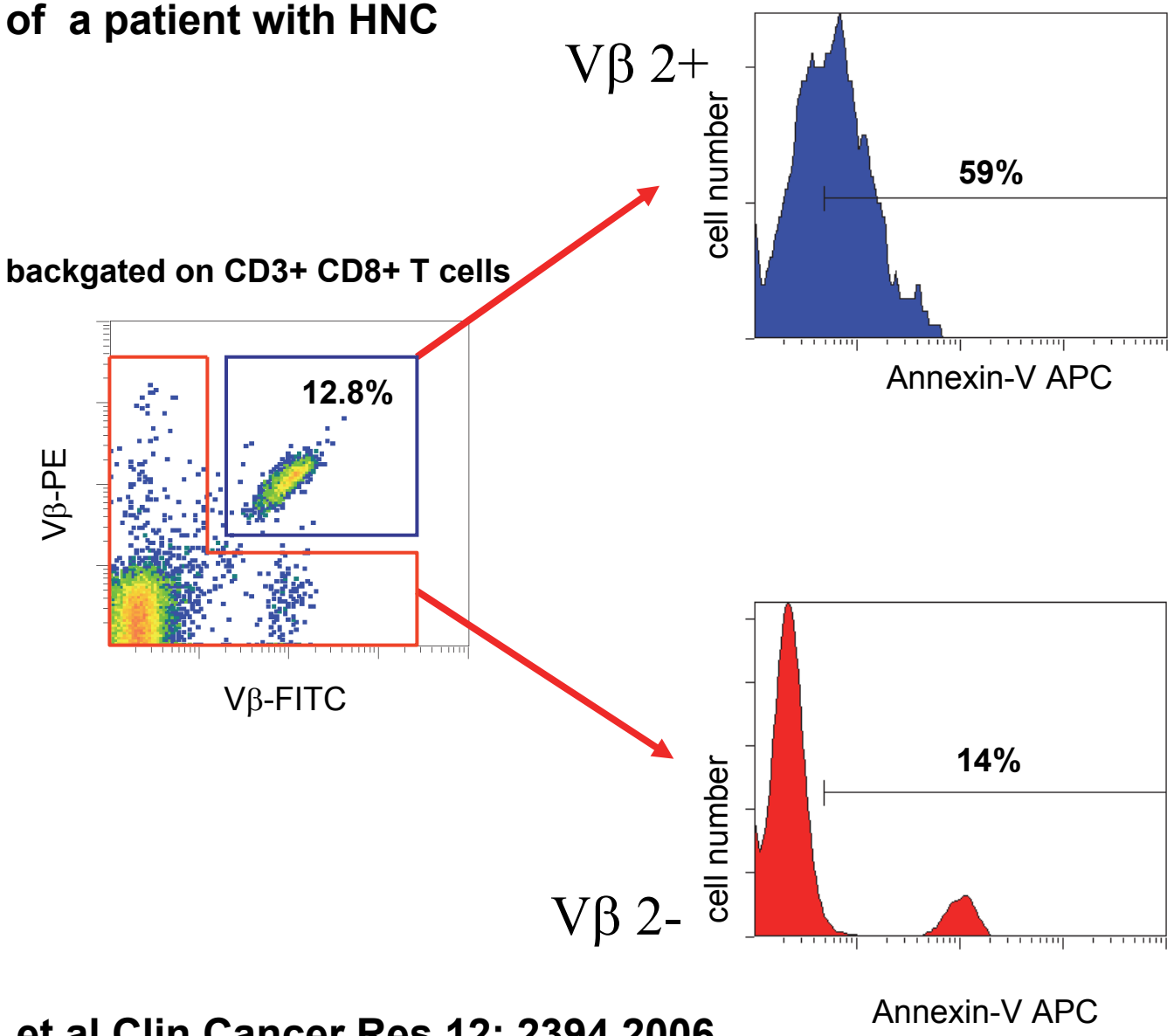
CD8+CD28- (>15% vs. <5%)

CD8+CCR7+ (>35% vs. <5%)

The data are mean values from various cohorts of HNC patients vs. NC
All differences were statistically significant

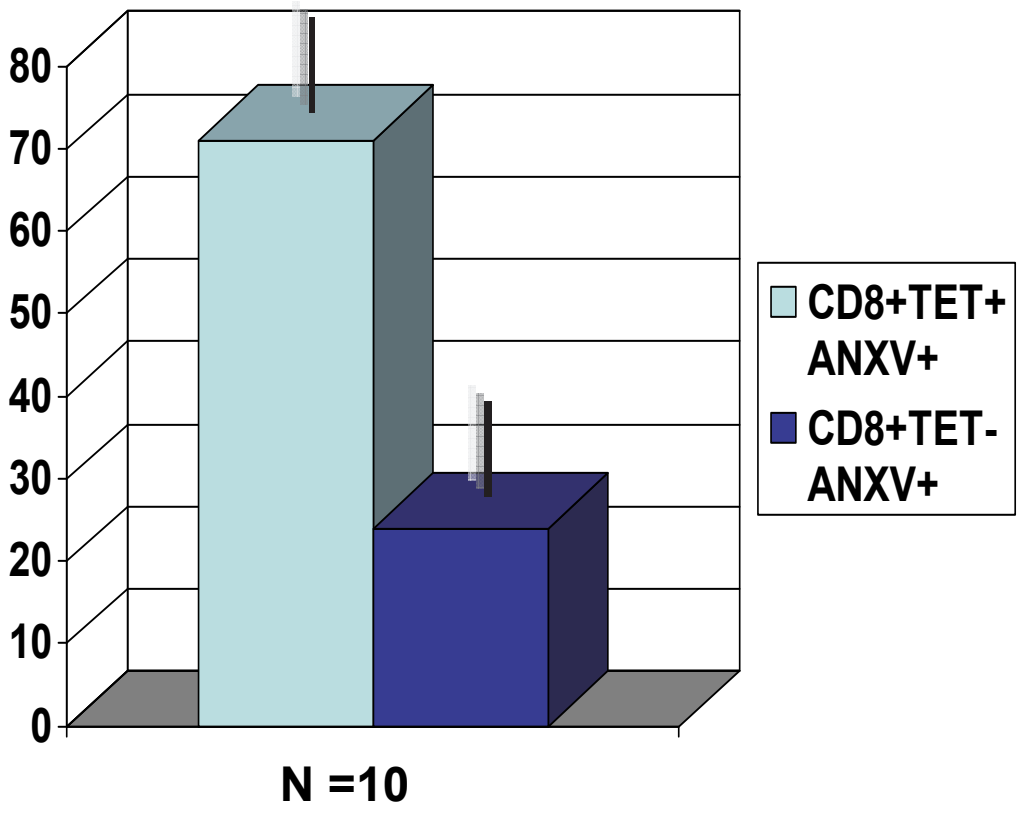
Is apoptosis CD8+ T cells directed at clonally-restricted T cells mediating anti-tumor responses?

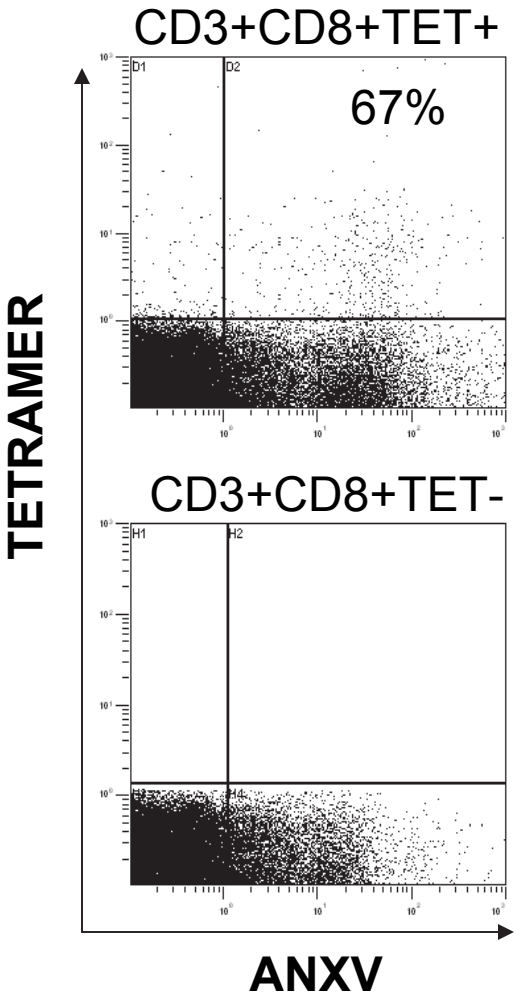
Restricted Vβ2 subset and ANX V binding to CD8+T cells in the circulation of a patient with HNC



Albers A, et al Clin Cancer Res 12: 2394,2006

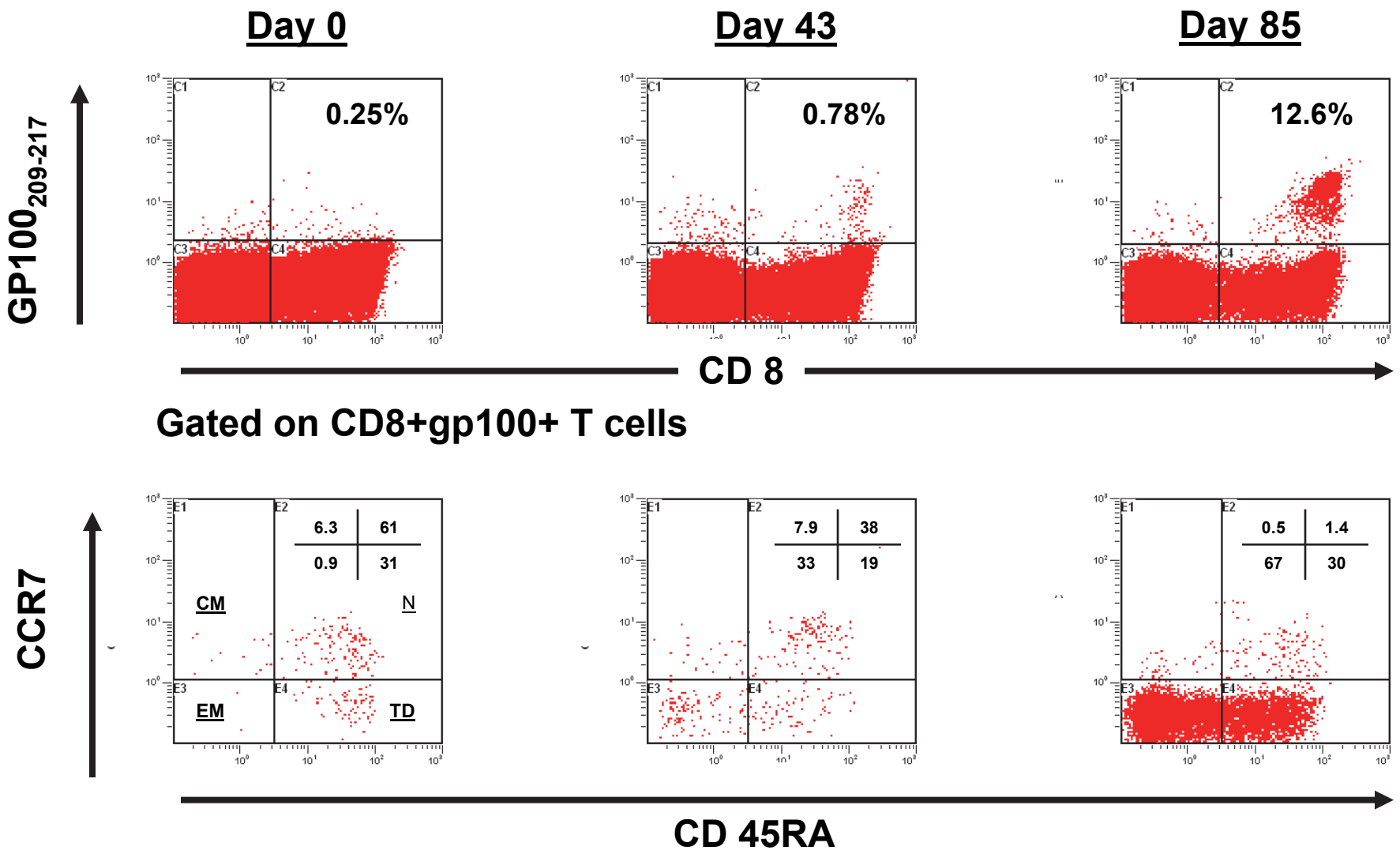
ANXV binding to circulating CD8+ $p53_{264}$TET+ or CD8+TET- cells in patients with HNC





Albers A et al. Head & Neck , 2008

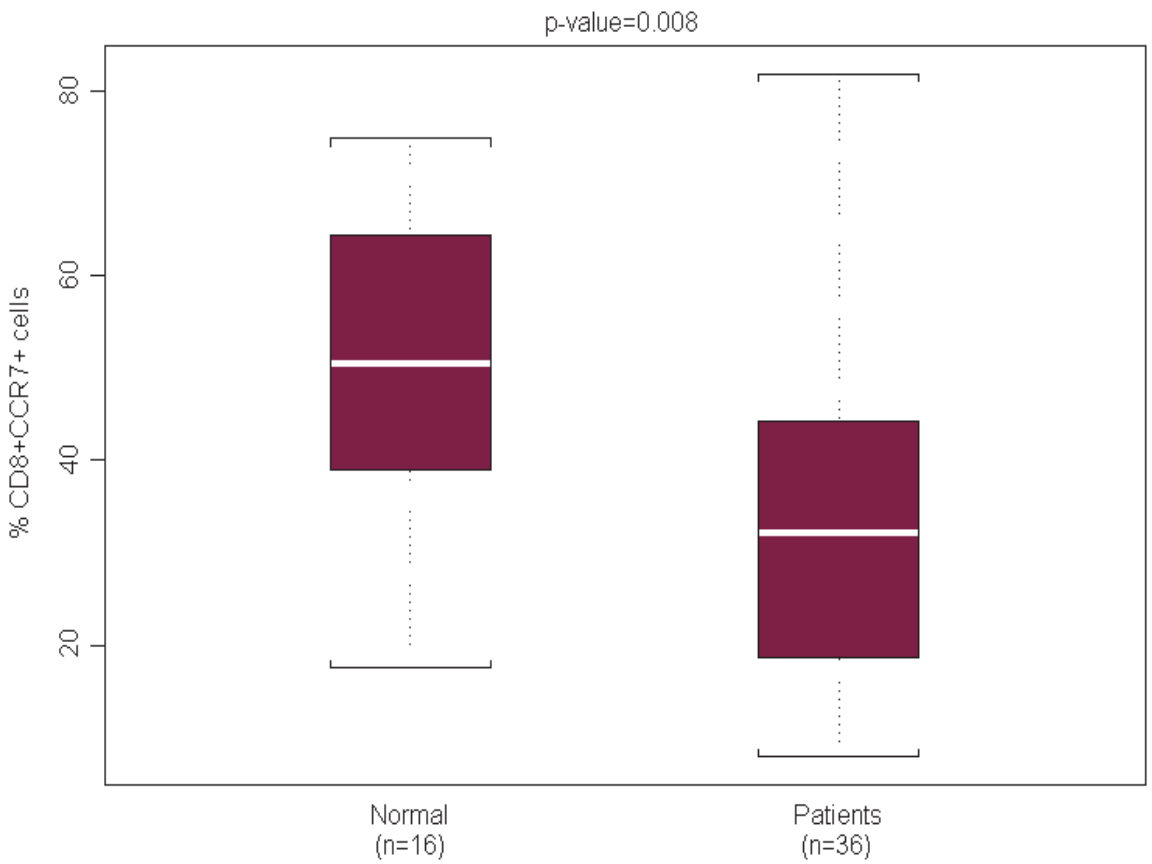
Expansion of $CD8^{+}GP100_{209-217}^{+}$- T cells and change of differentiation status in this subset seen in one melanoma patient treated with a multi-epitope vaccine



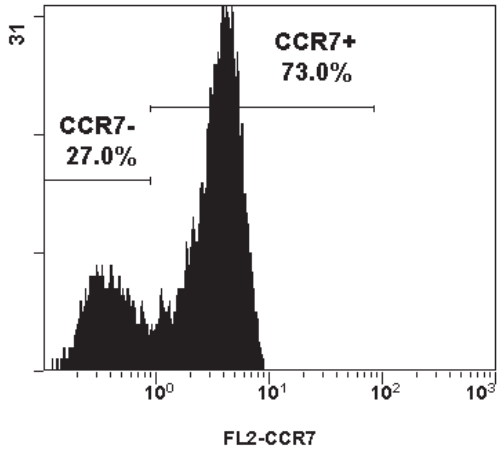
Schaefer C et al, Int. J. Cancer 2012

In HNC patients, the frequency of circulating CCR7+ CD8+ T cells is decreased, while the frequency of CCR7-CD8+ cells is increased

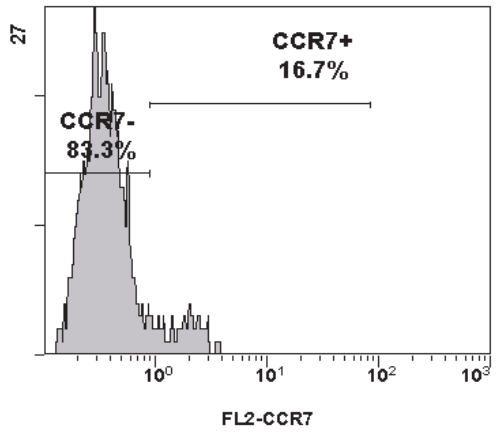
Gate set on CD3+ CD8+ T cells

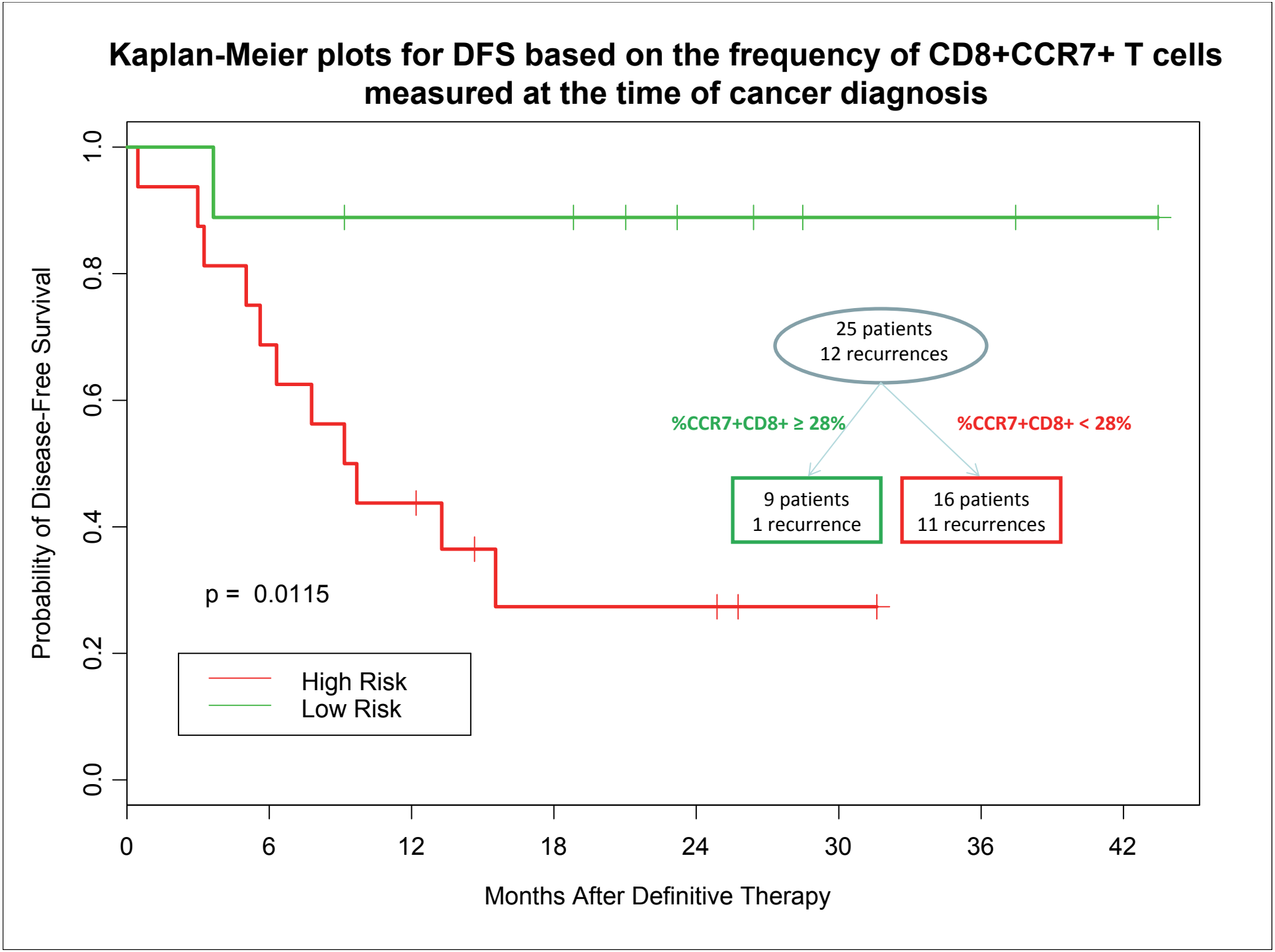


Normal control



Patient

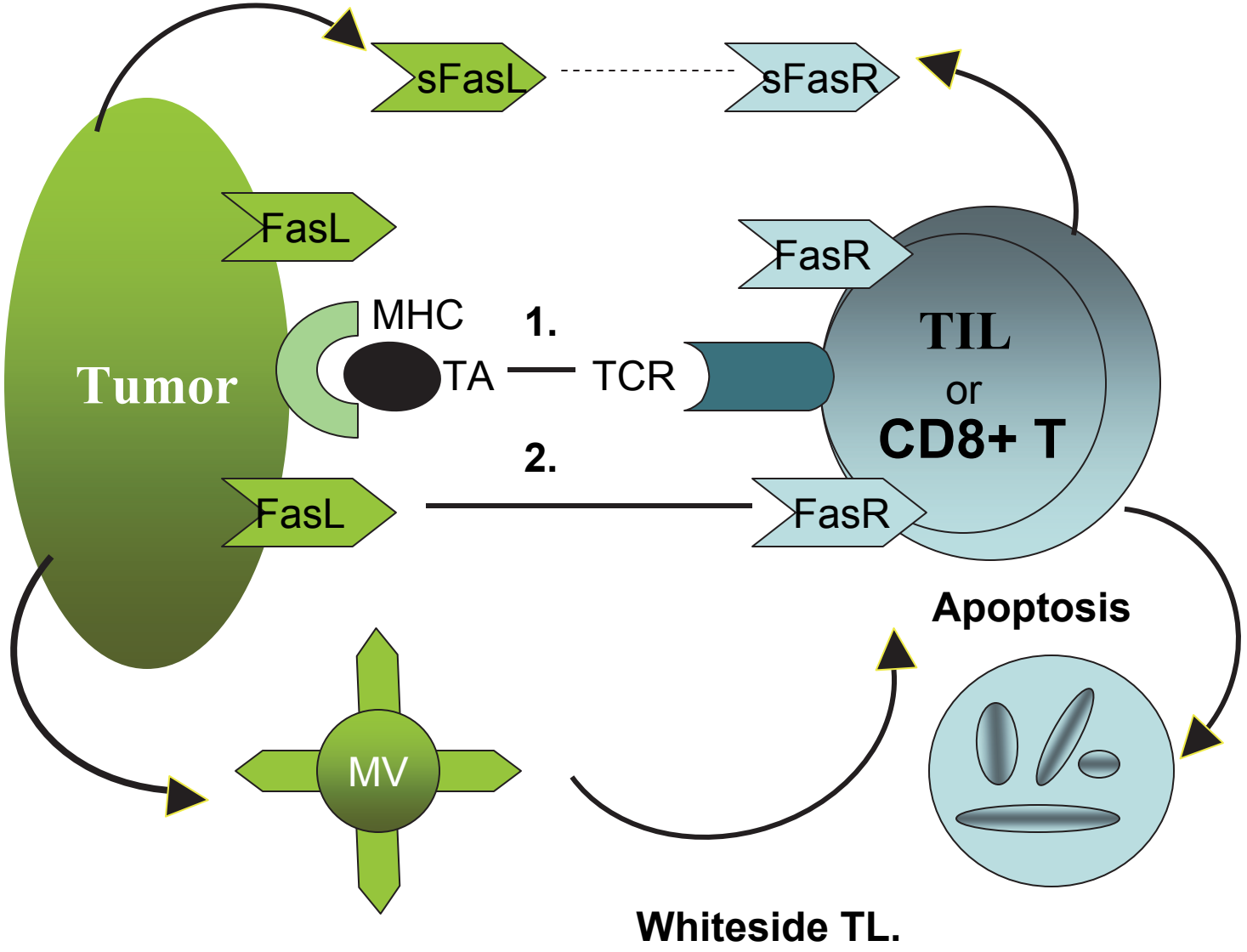


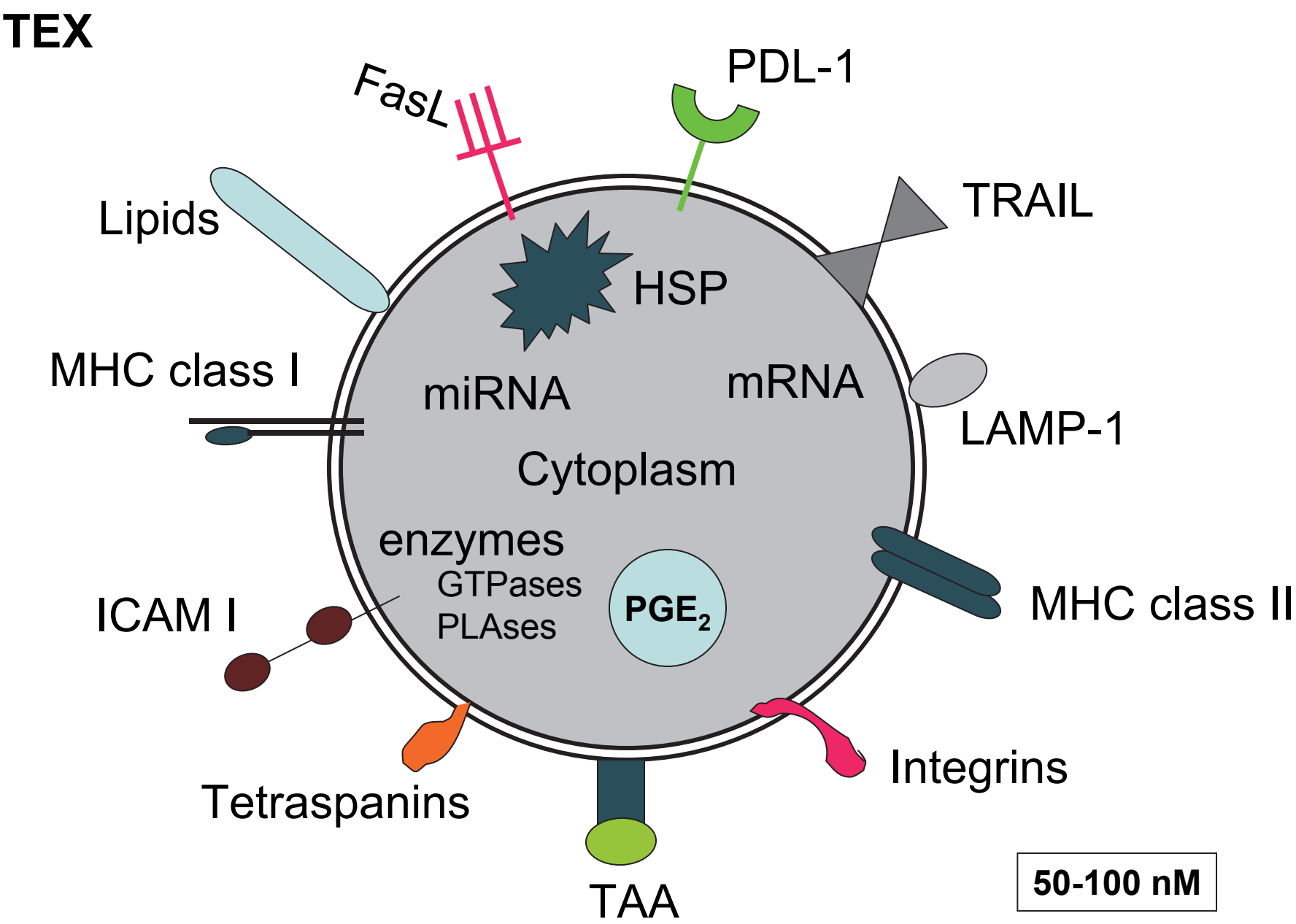

Kaplan-Meier plots for DFS based on the frequency of CD8+CCR7+ T cells measured at the time of cancer diagnosis
Probability of Disease-Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30
36
42
Months After Definitive Therapy
25 patients
12 recurrences
%CCR7+CD8+ ≥ 28%
%CCR7+CD8+ < 28%
9 patients
1 recurrence
16 patients
11 recurrences
p = 0.0115
High Risk
Low Risk

Fate of immune cells in patients with cancer

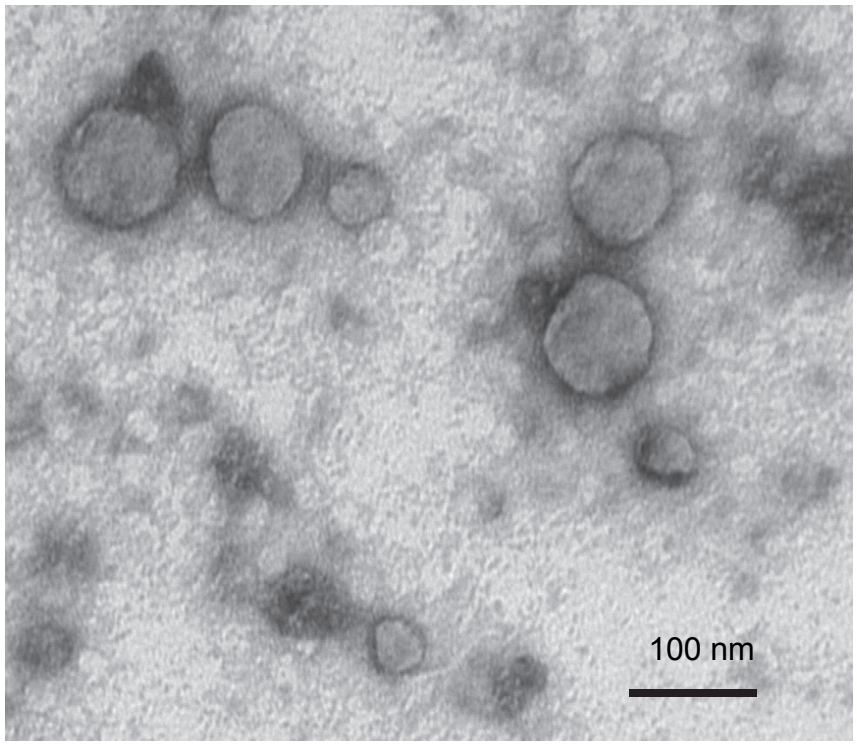
- Imbalance of lymphocyte subsets
- Selective demise of CD8+ effector T cells
- Accelerated apoptosis in tumor antigen-specific (tetramer+) T cells
- Persistent alterations in lymphocyte homeostasis: a rapid turnover
- Presence in serum of microvesicles “armed” with biologically-active FasL

The Fas/FasL pathway and T-cell apoptosis in situ or in the periphery





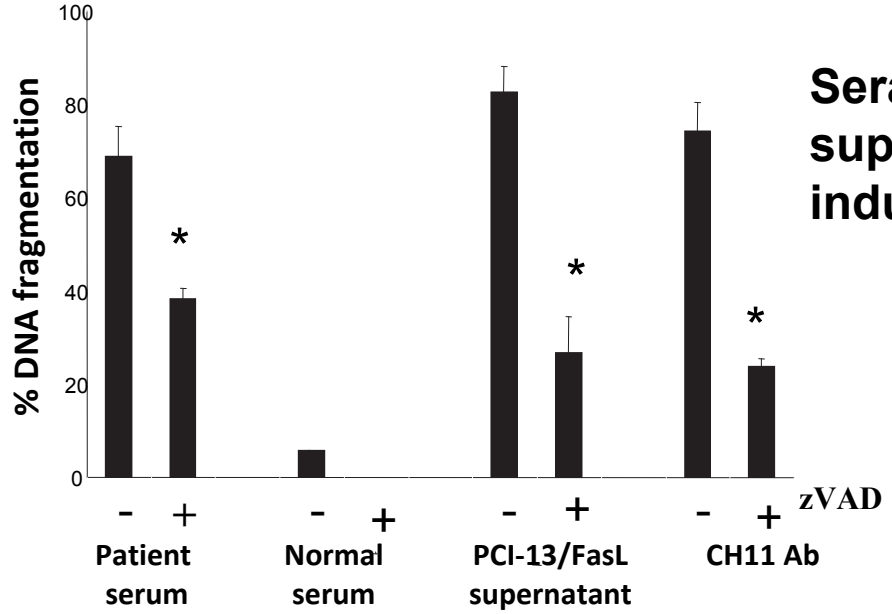
Whiteside TL. Bochem Soc. Transactions (London), 2012, In Press



Exosomes isolated from the serum of a patient with cancer, fixed with gluteraldehyde embedded in Epon and sectioned

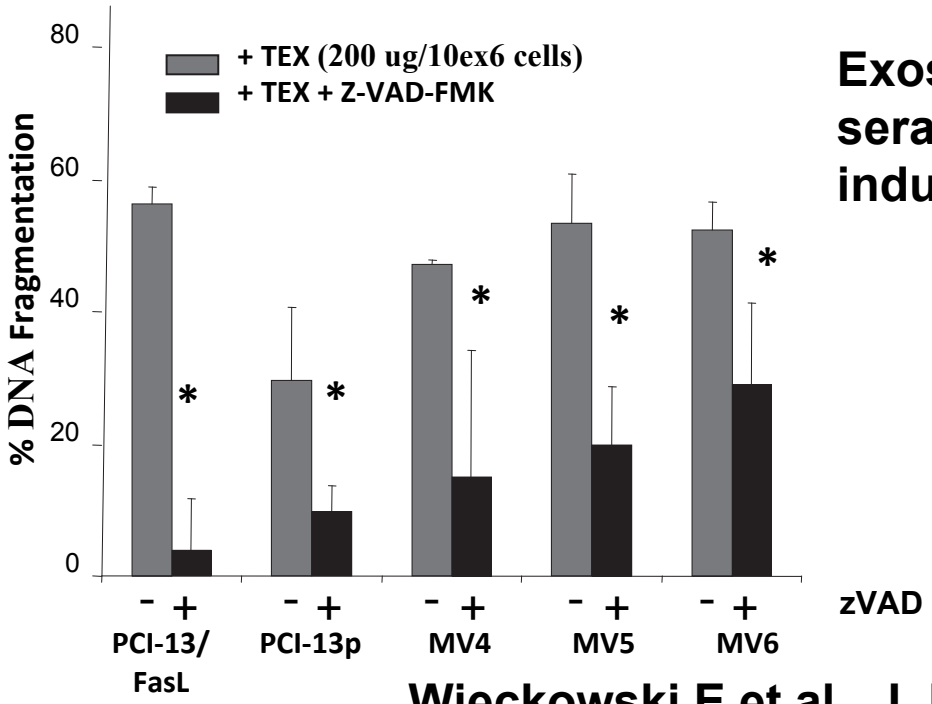
Transmission electron microscopy

A.



Sera of cancer patients and supernatants of tumor cells induce apoptosis of T cells

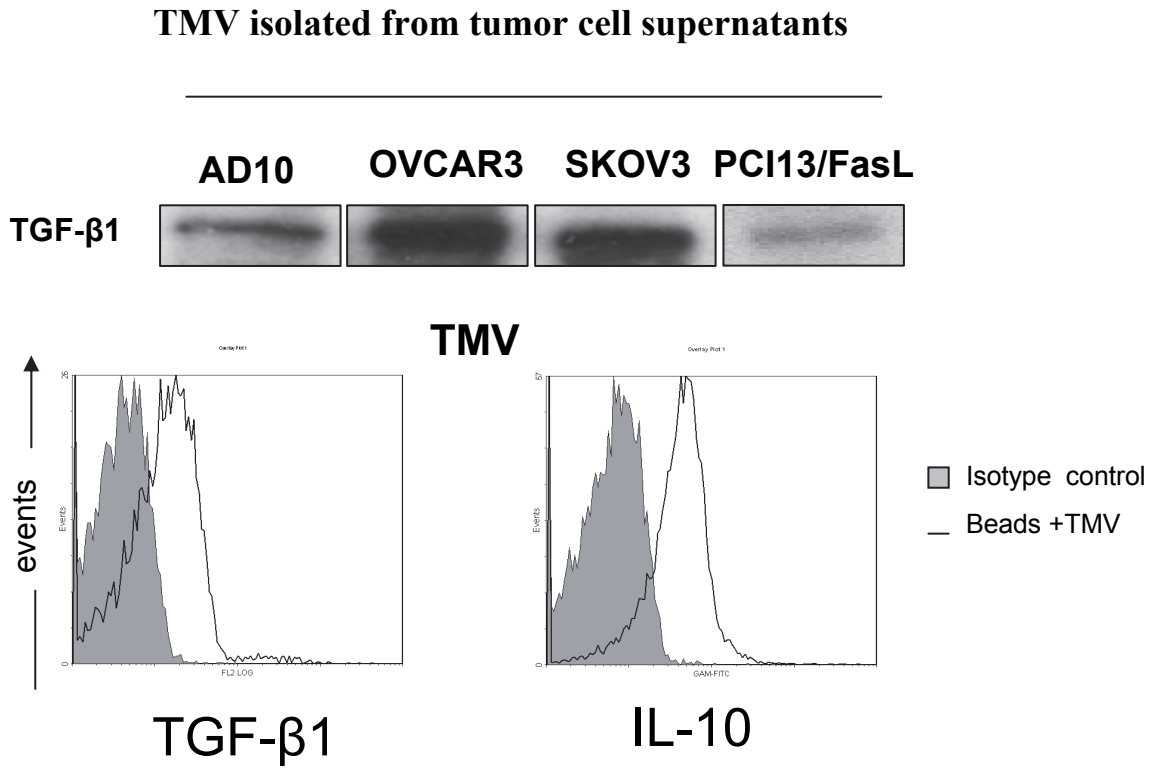
B.



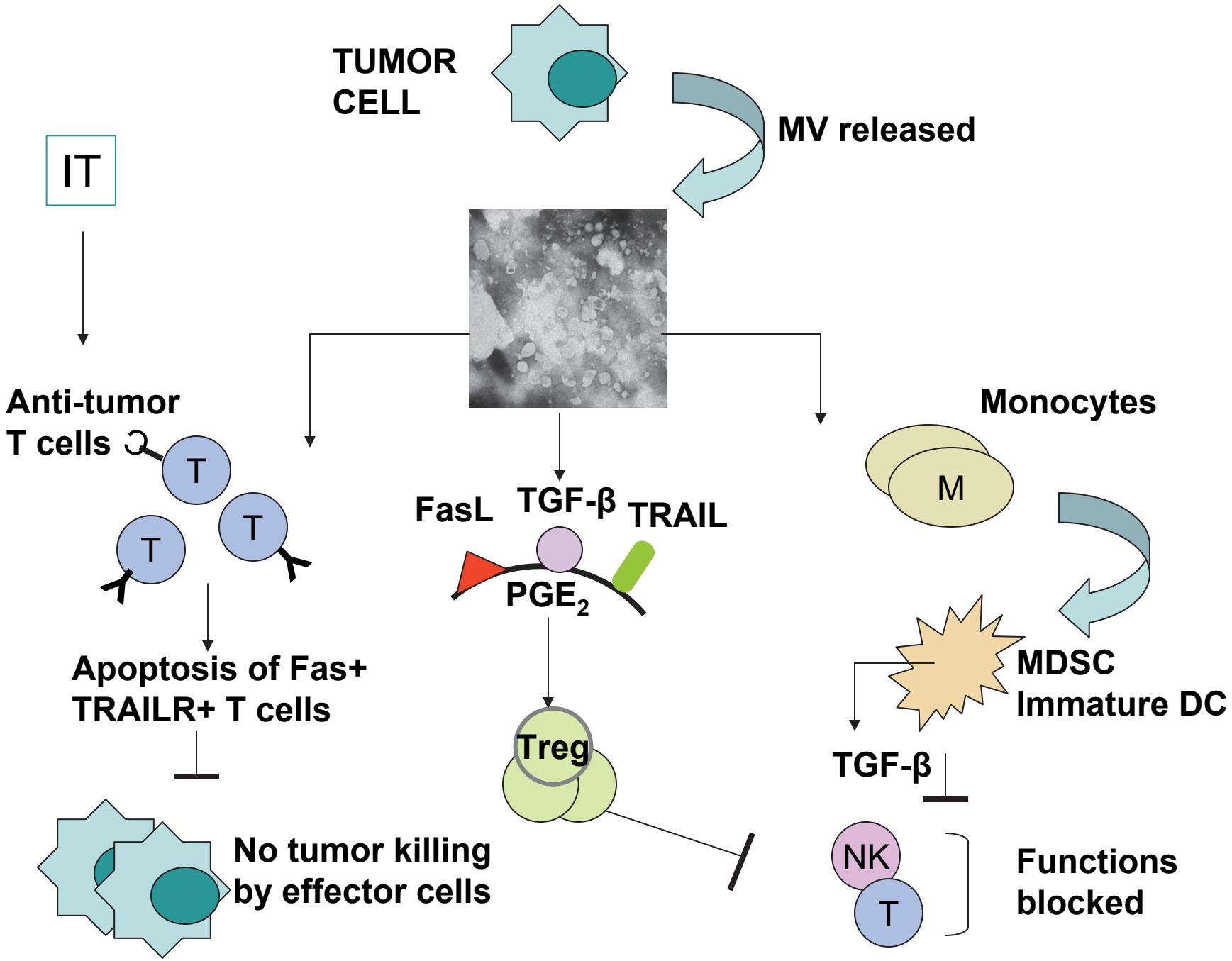
Exosomes isolated from sera of cancer patients induce apoptosis of T cells

Wieckowski E et al., J. Immunol 183:3720,2009

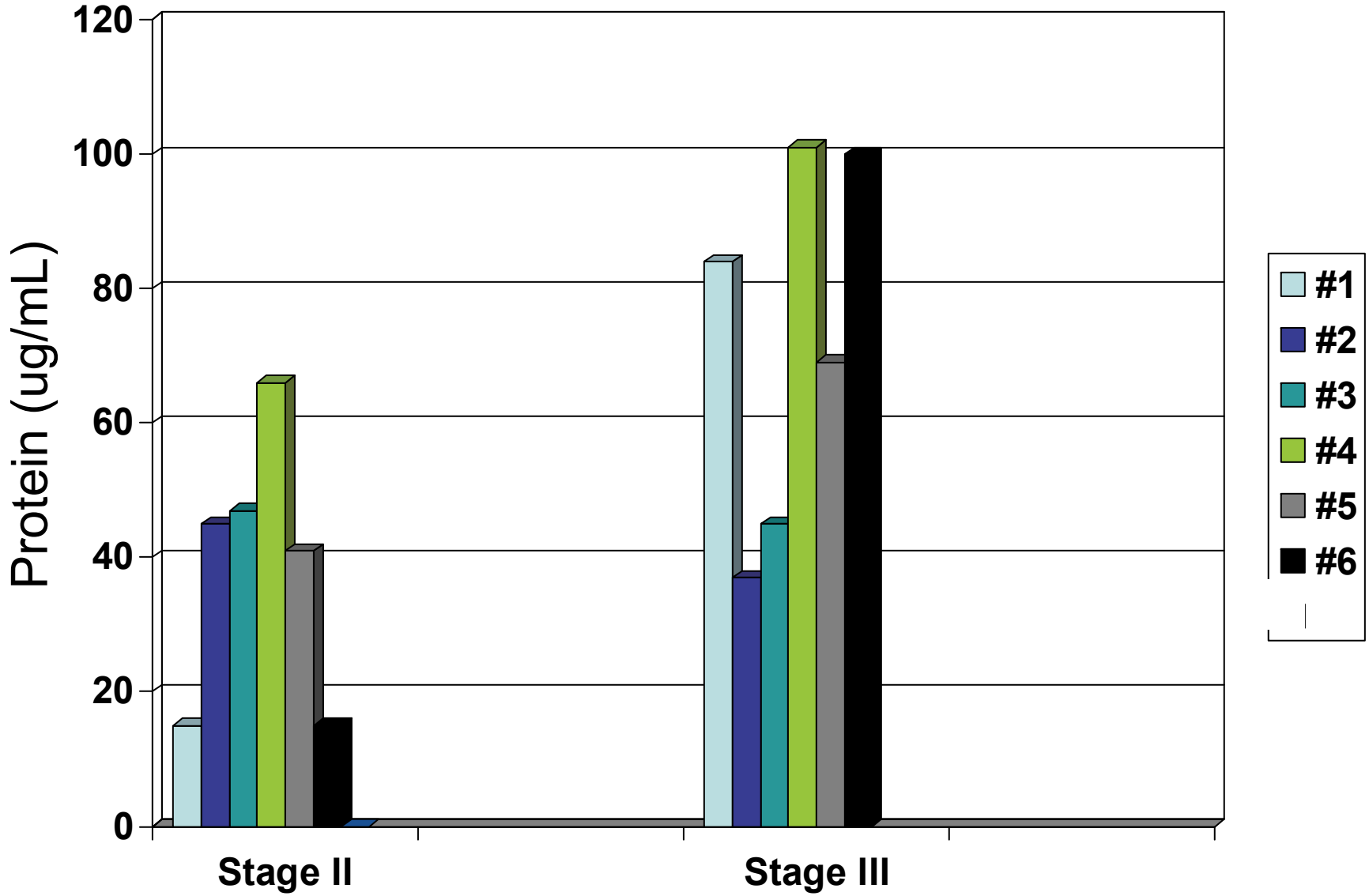
TMV carry immunosuppressive cytokines TGF-β1 and IL-10



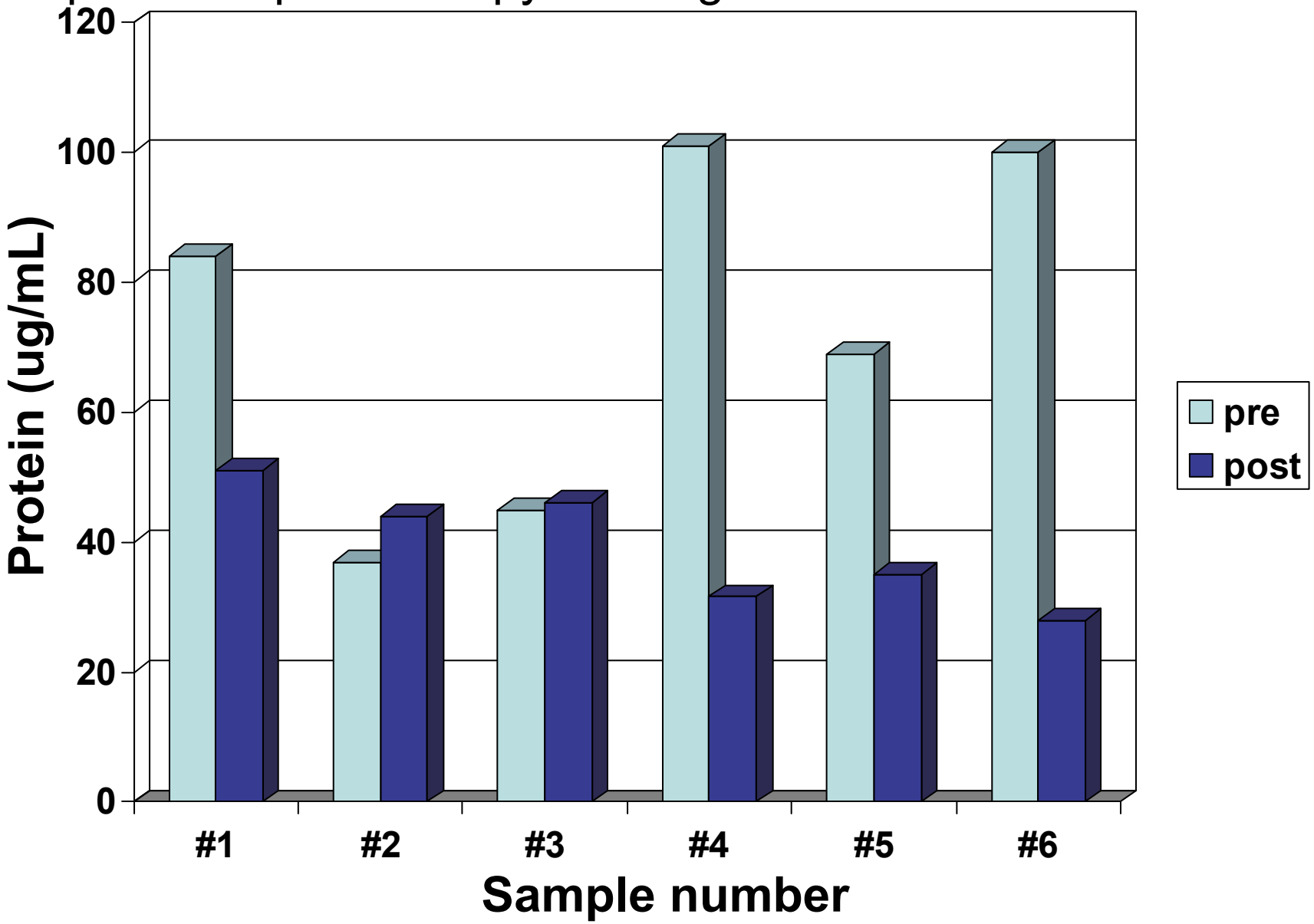
Szajnik M, et al, PLoS ONE 5, 2010:


TUMOR CELL
MV released
IT
Anti-tumor T cells
T
T
T
Apoptosis of Fas+ TRAILR+ T cells
No tumor killing by effector cells
FasL
TGF-β
TRAIL
PGE2
Treg
Monocytes
M
MDSC
Immature DC
TGF-β
NK
T
Functions blocked

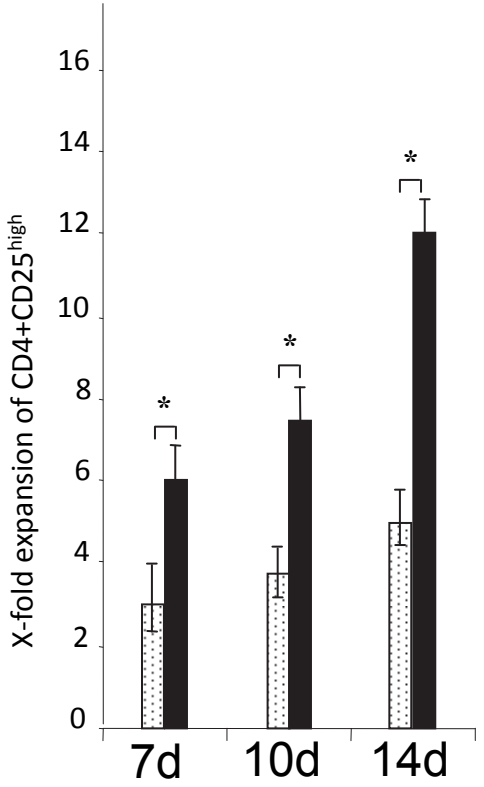
Exosome fractions obtained from sera of patients with Stage II or Stage III melanoma at the time of diagnosis and prior to therapy



Exosome fractions obtained from sera of patients with melanoma prior and post therapy with high-dose interferon-α



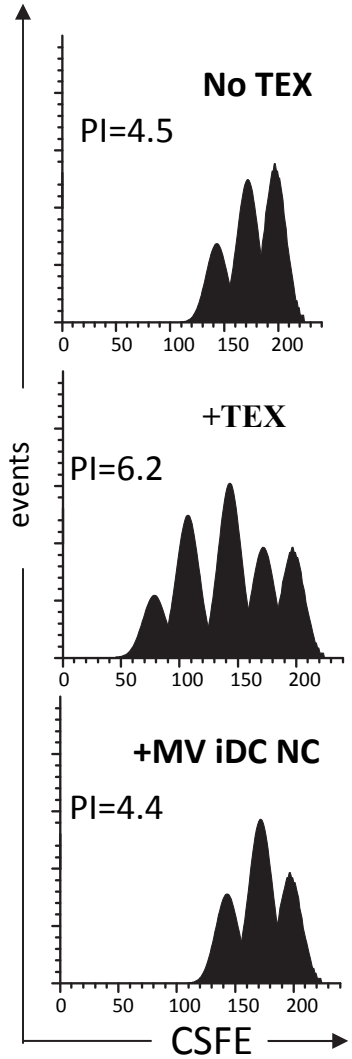
Fresh, sorted CD4+CD25^{high}



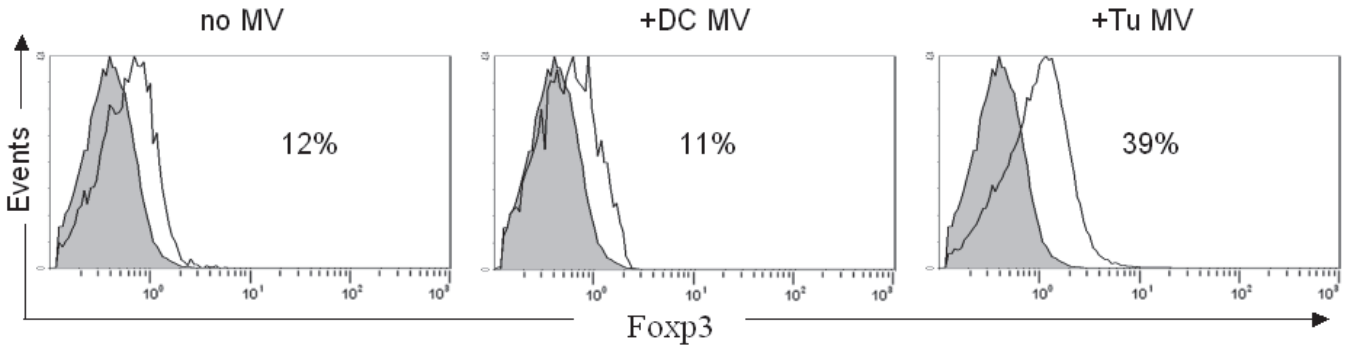
Tumor-derived exosomes (TEX, TuMV) promote proliferation of human Treg

Szajnik M, et al. PLoS ONE 5, 2010

CSFE assay



Gated on CD3+CD4+CD25+



Treg in humans

- Phenotype: **CD4+CD25^{hi}FOXP3+**
  CD4+CD39+CD25+FOXP3+

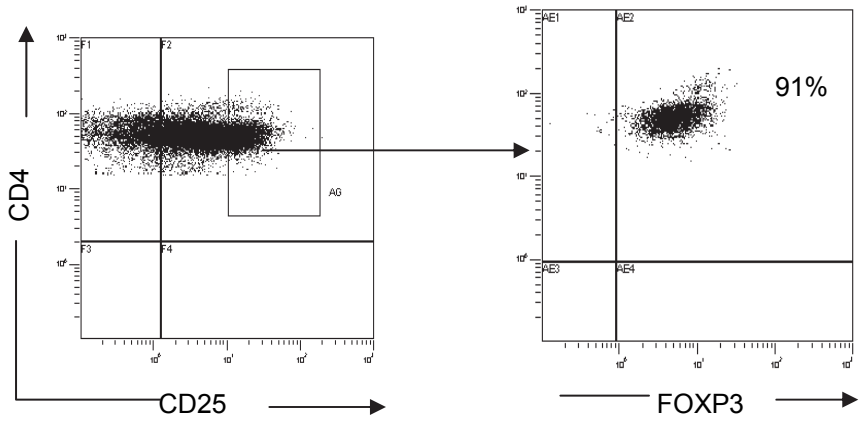
- Functional definition
- Different subtypes: nTreg
  inducible (i)Treg (Tr1)

- Treg accumulate in tumors, and their frequency is increased in the blood of cancer patients

Treg can be isolated from tissues and blood using surface markers such as CD25 or CD39

Gating strategy

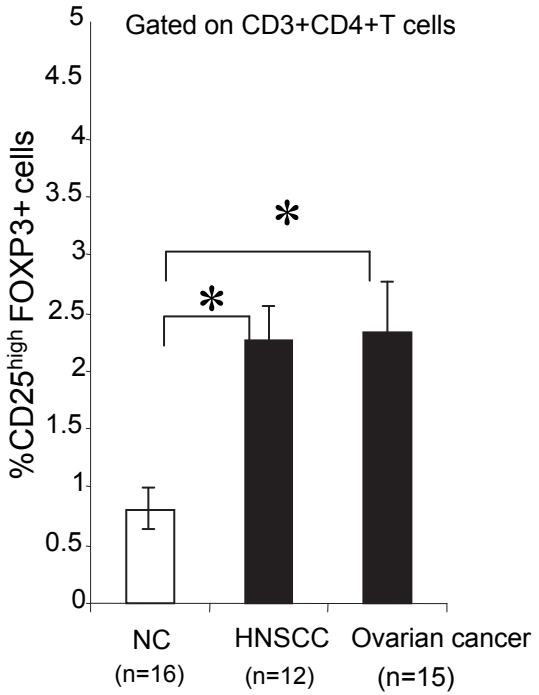
Gate set on CD25^{high}



>90% of CD4+CD25^{high} are FOXP3+

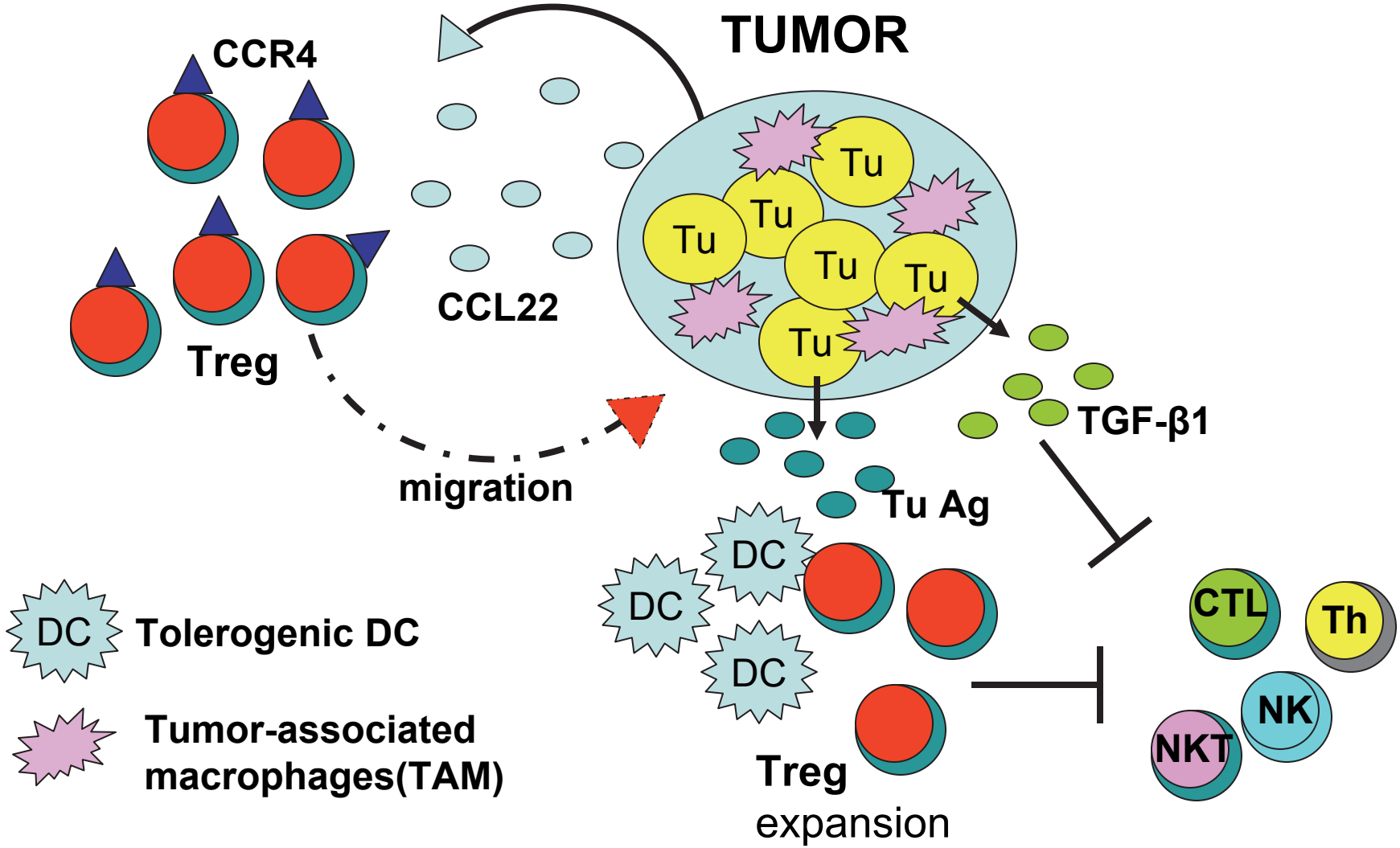
(FOXP3 is an intracellular transcription factor)

% Treg in CD4^{+} PBMC

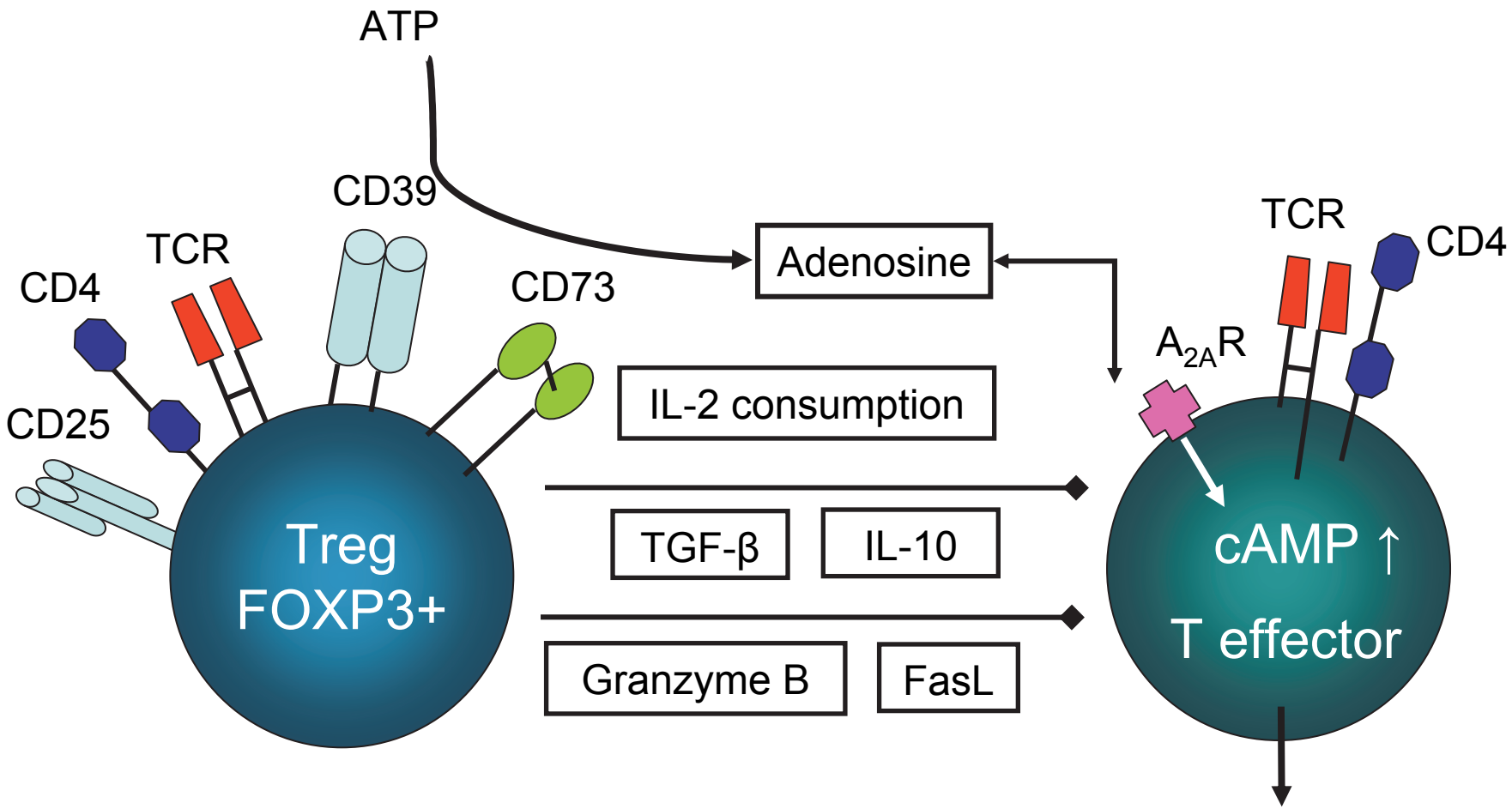


- In OvCa, increased Treg frequency and function are associated with poor prognosis (e.g., Curiel et al, Nature Med:10, 2004 and many others)
- In colon Ca, increased Treg frequency is associated with better prognosis and improved overall survival (Salama et al, J. Clin Oncol. 27, 2009)

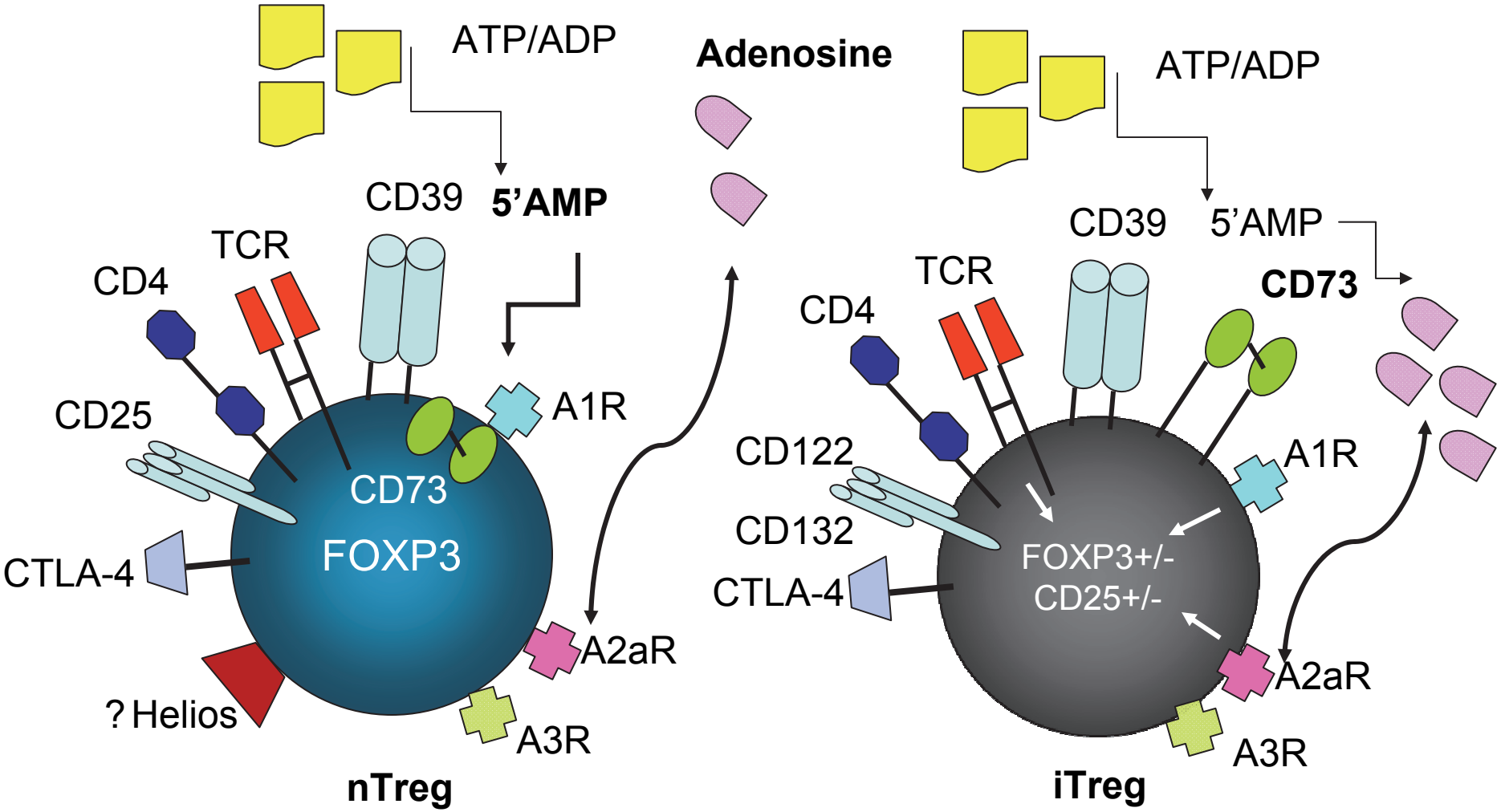
Treg are recruited to the tumor site, they expand and suppress anti-tumor immune responses favoring tumor escape

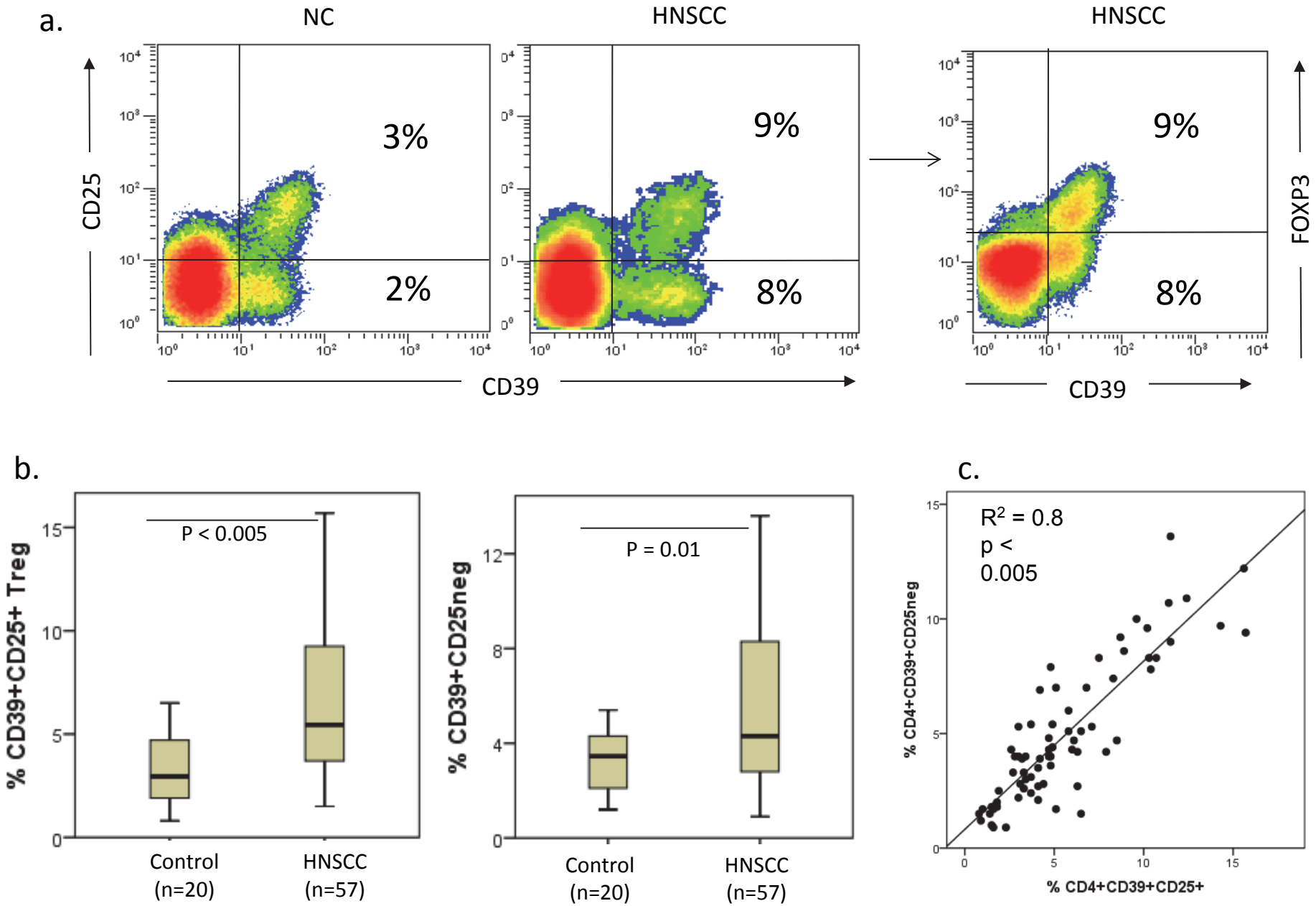


Mechanisms of suppression mediated by human regulatory T cells

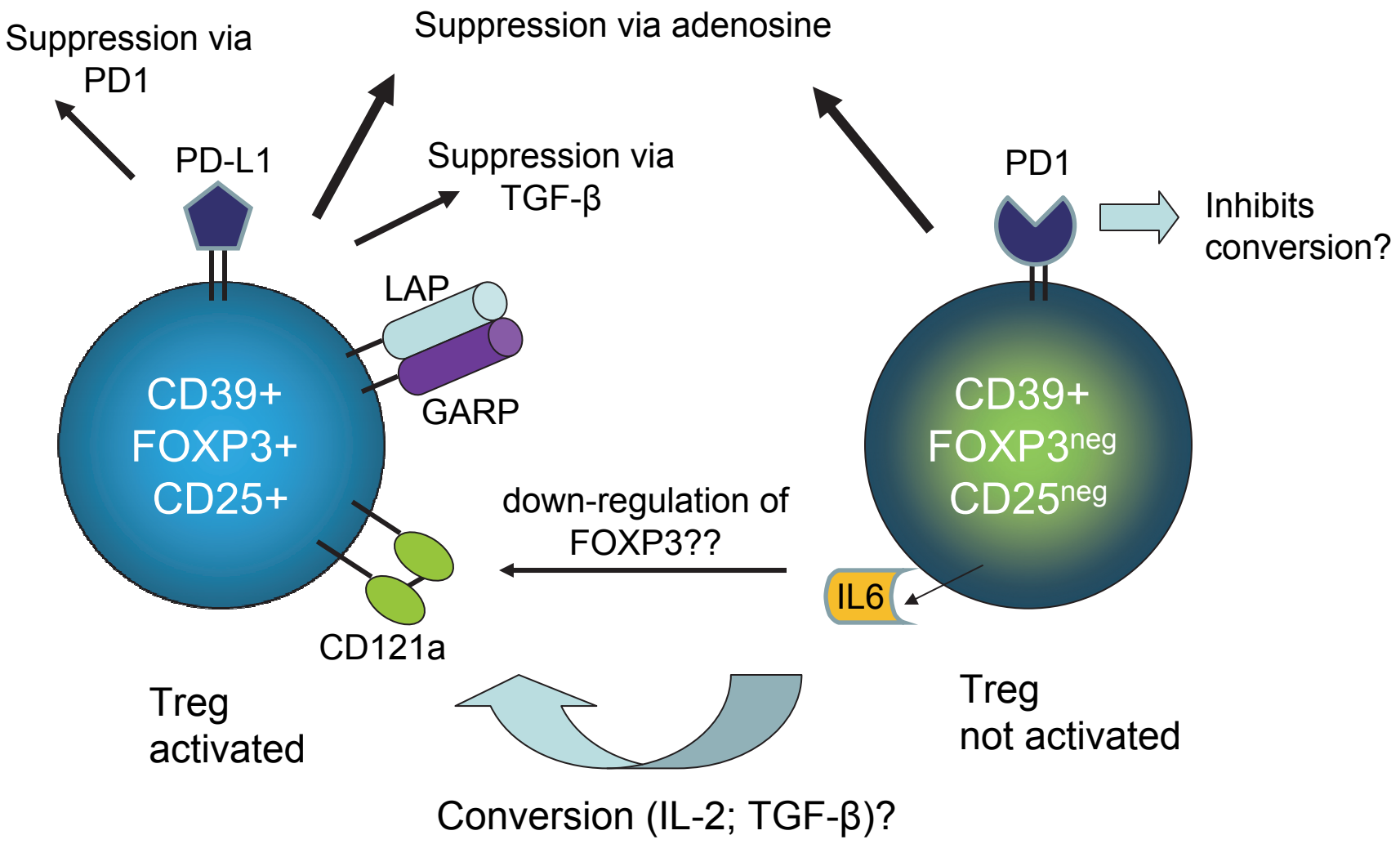


Do all Treg use these mechanisms or are there functionally-specific Treg subsets??


ATP/ADP
Adenosine
ATP/ADP
CD39
5'AMP
TCR
CD4
CD25
A1R
CD73
FOXP3
CTLA-4
A2aR
? Helios
A3R
nTreg
CD39
5'AMP
CD73
TCR
CD4
CD122
CD132
A1R
FOXP3+/-
CD25+/-
CTLA-4
A2aR
A3R
iTreg


a.
NC
HNSCC
HNSCC
CD25
FOXP3
CD39
CD39
3%
2%
9%
8%
9%
8%
b.
% CD39+CD25+ Treg
P < 0.005
Control (n=20)
HNSCC (n=57)
% CD39+CD25neg
P = 0.01
Control (n=20)
HNSCC (n=57)
c.
R2 = 0.8
p < 0.005
% CD4+CD39+CD25neg
% CD4+CD39+CD25+

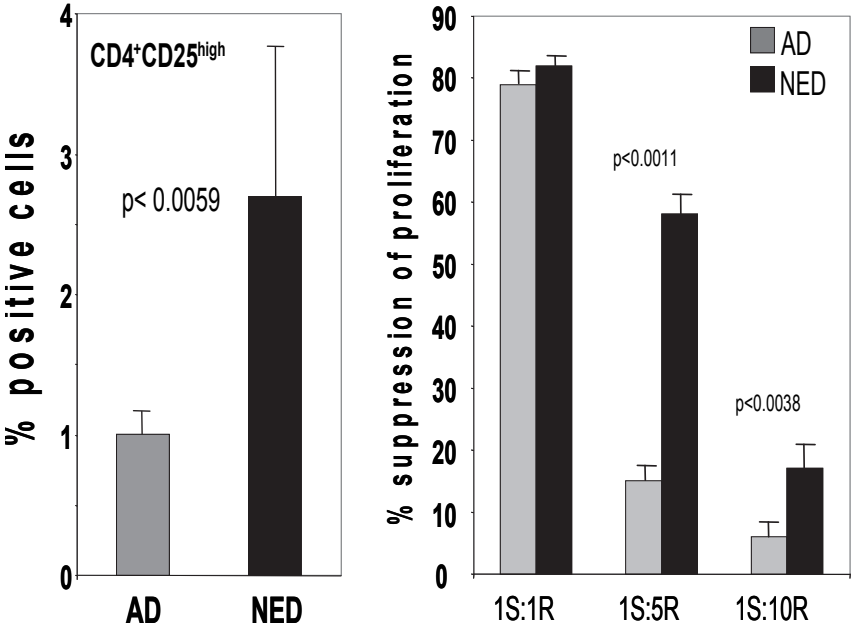
CD4+CD39+ subsets in human peripheral blood

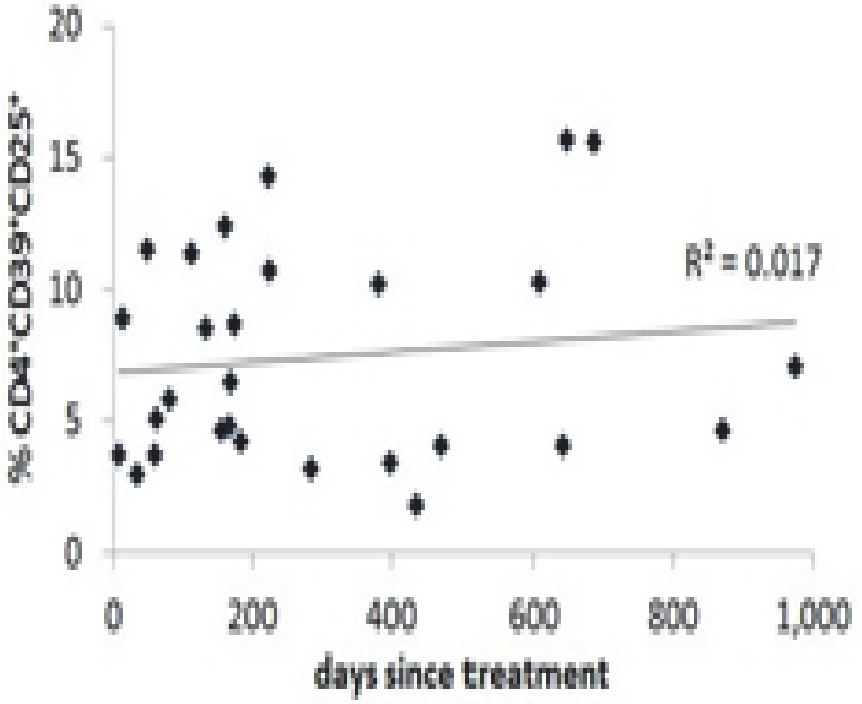


Treg in cancer clinical trials

- Treg (CD4+ CD25+FOXP3+) frequency is often serially monitored in immunotherapy clinical trials
- Attenuation of Treg could serve as measure of favorable response to therapy
- Unexpectedly, Treg were found to be significantly increased in the frequency and activity after various immunotherapies (data from many clinical trials and institutions)

Persistent elevation of Treg in the peripheral circulation of patients with HNC who are NED following chemotherapy or chemo-radiotherapy

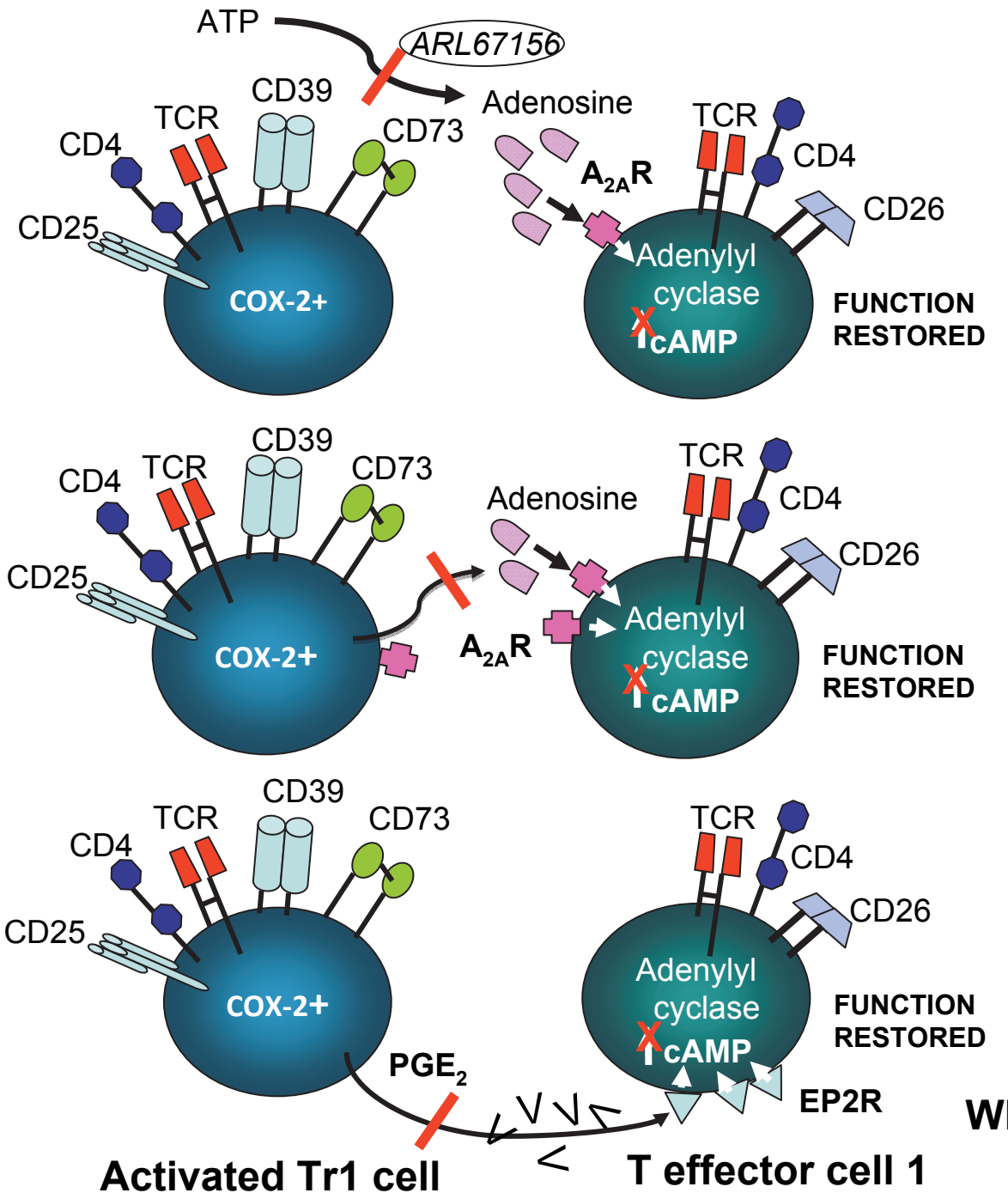




AD =15
NED=25

Circumventing Treg activity in cancer??

- Elimination of Treg prior to immunotherapy
- Inhibition of Treg function
- Treg depletion:
  - Daclizumab (anti-CD25)
  - Ontak (denileukin diftitox)
  - Anti-CD25/toxins
  - TKIs (Sunitinib)
  - Chemotherapy drugs (?)



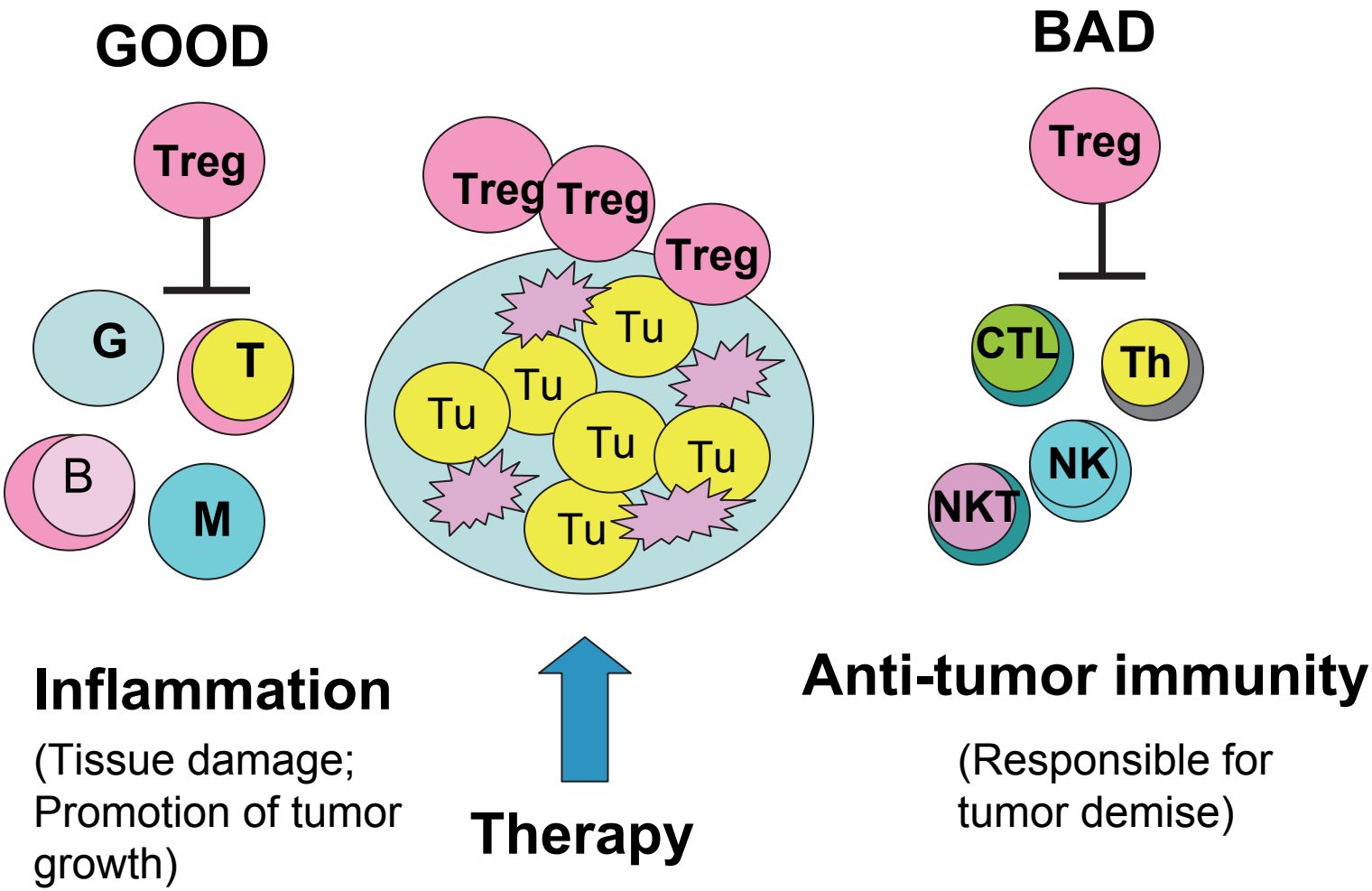
Disarming iTreg by blocking adenosine production

Disarming iTreg by blocking adenosine binding to $A_{2A}R$

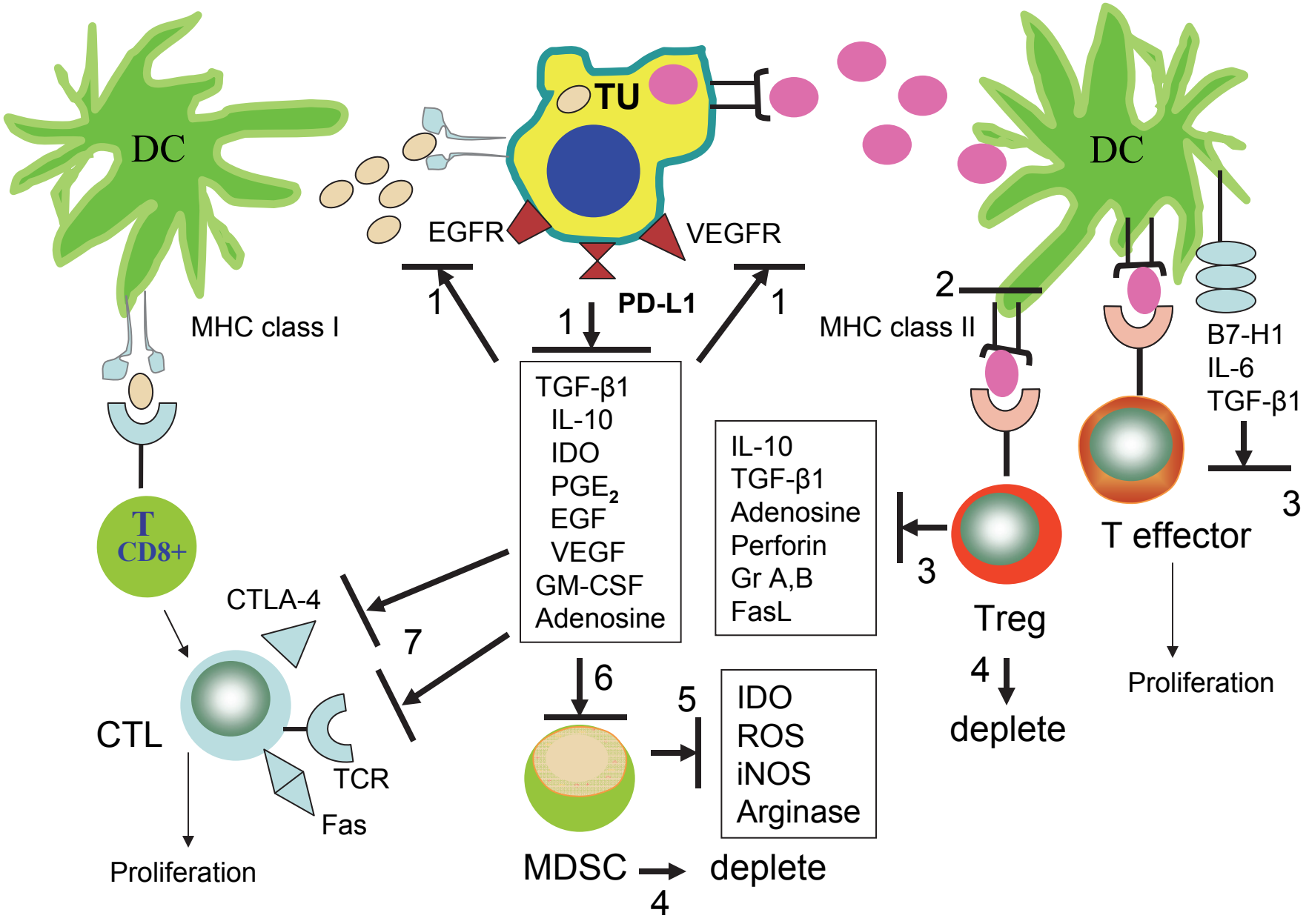
Disarming iTreg by blocking PGE_2 production

Whiteside TL. CII 61: 283, 2012

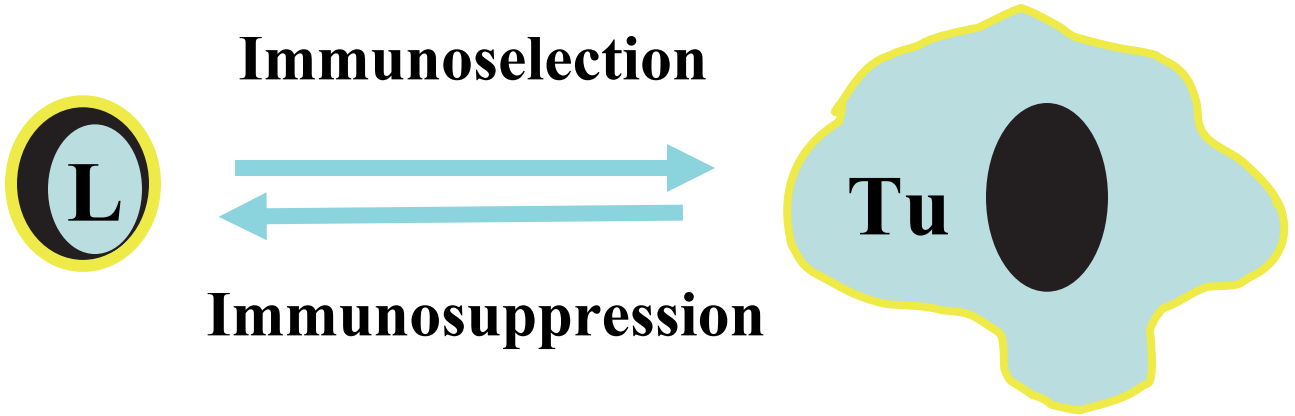
Are Treg good or bad in cancer ?



Strategies for inhibiting the inhibitors



Improving therapeutic effects of immune therapies



- Delivery of immune therapies to patients with less compromised immune responses
- Understanding of molecular pathways responsible for tumor escape and of tumor immune signature
- Improved knowledge of mechanisms engaged by immuno-therapeutic agents
- Rationally selected combinatorial strategies used in the setting of minimal residual disease

And so…the 2012 immune escape games are in full gear, with many promising strategies available for use and in use in the clinic……

"*Why, is it asked, does the supply of new miracle drugs lag so far behind, while the biology continues to move from strength to strength……? There is still the conspicuous asymmetry between molecular biology and, say, the therapy of lung cancer.*"

-Lewis Thomas,
The Lives of a Cell, 1978

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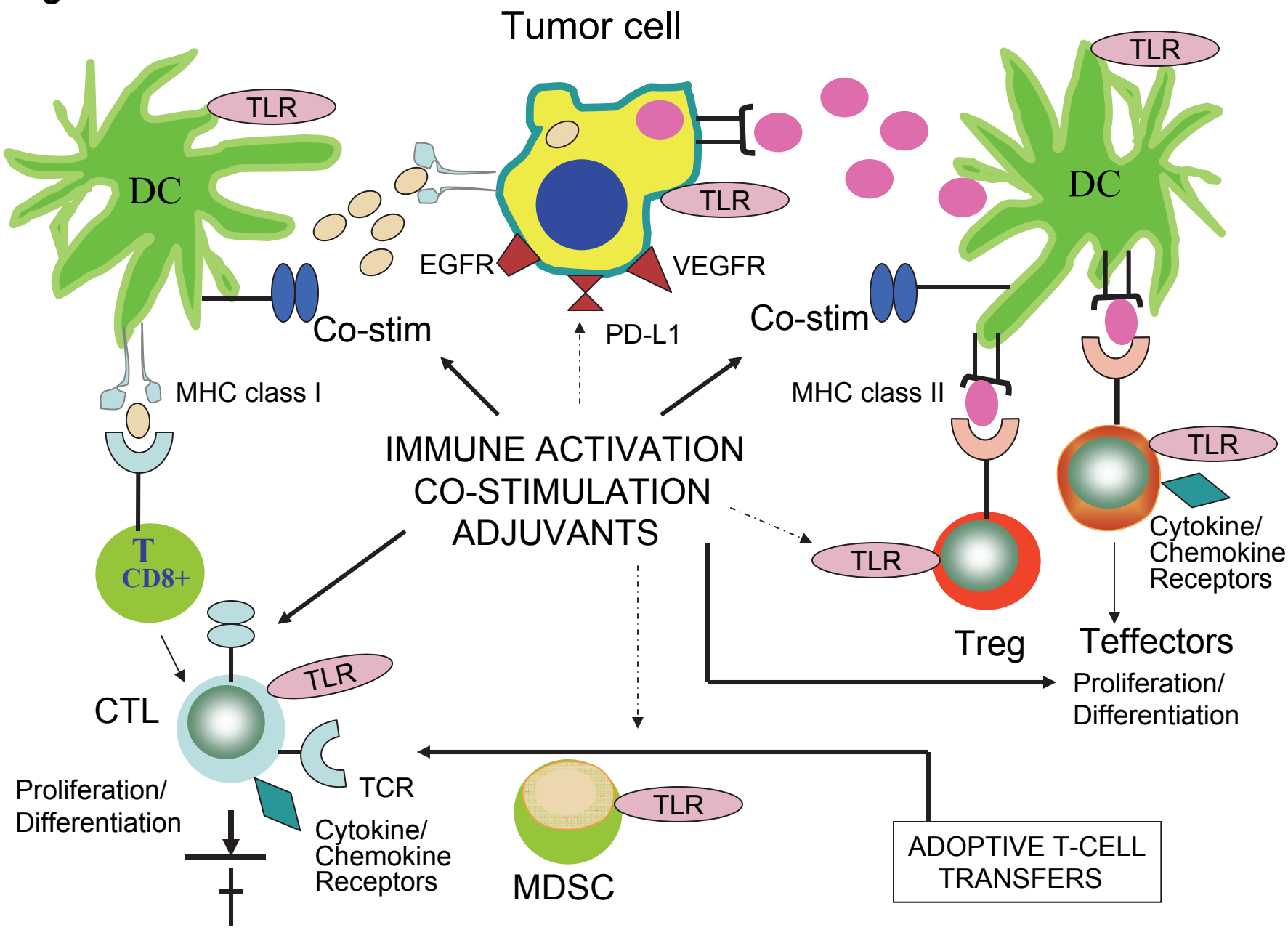
- Colleagues: Butterfield LH; Ferris RL; Johnson JT; Kirkwood JM; Boyiadzis M; DeLeo AB; Ferrone S; Hong C-S; Storkus JW; Lotze M; Jackson EK.
- Dr. Ronald B. Herberman
- NCI: PO-1CA 109688

Figure 1B.


Tumor cell
TLR
DC
TLR
EGFR
VEGFR
TLR
DC
Co-stim
PD-L1
Co-stim
MHC class I
MHC class II
IMMUNE ACTIVATION
CO-STIMULATION
ADJUVANTS
T
CD8+
TLR
TLR
Cytokine/
Chemokine
Receptors
Treg
Teffectors
Proliferation/
Differentiation
CTL
TLR
Proliferation/
Differentiation
TCR
Cytokine/
Chemokine
Receptors
TLR
MDSC
ADOPTIVE T-CELL
TRANSFERS

Immune escape in early 1980s

- T cells obtained from human tumors (TIL) failed to clone vs T cells from the blood or from tissues of normal donors and were ineffective in eliminating tumor targets (e.g., Miescher S., et al, J. Immunol. 138:4004,1987)
- TIL proliferation as well as anti-tumor activity were inhibited by tumor-derived factors whose nature was an enigma at the time
- Concurrently, Dr. Rosenberg at NCI was successfully expanding TIL for IT of patients with melanoma
- Studies in mouse models of tumor growth (~1990s) confirmed the concept of tumor-mediated suppression under the name of “immunoediting” (Dunn et al, Nat Immunol. 3: 991,2002)