Presenter Disclosure Information

<James Finke>

The following relationships exist related to this presentation:

Pfizer- Research Grant

Regulatory Immune Cells

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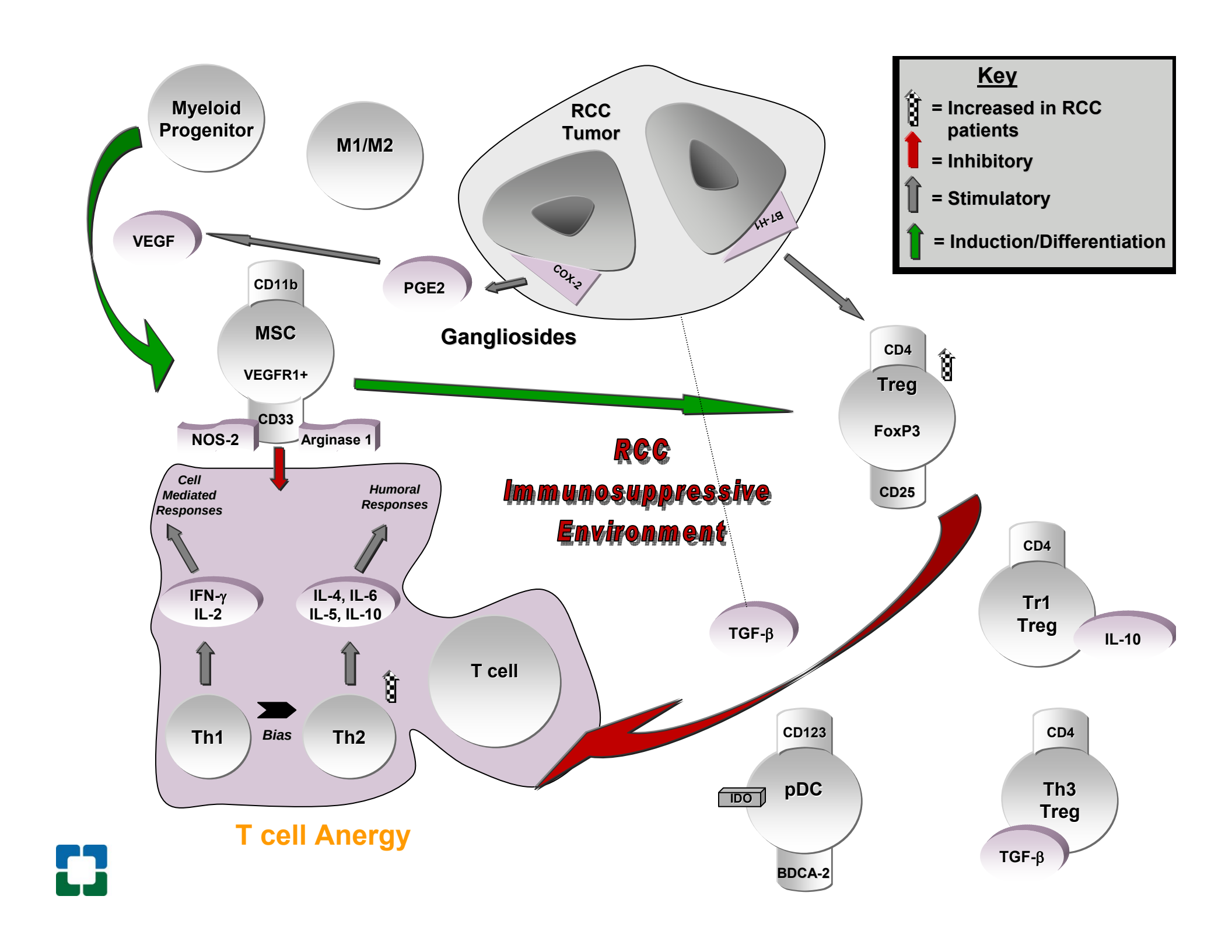
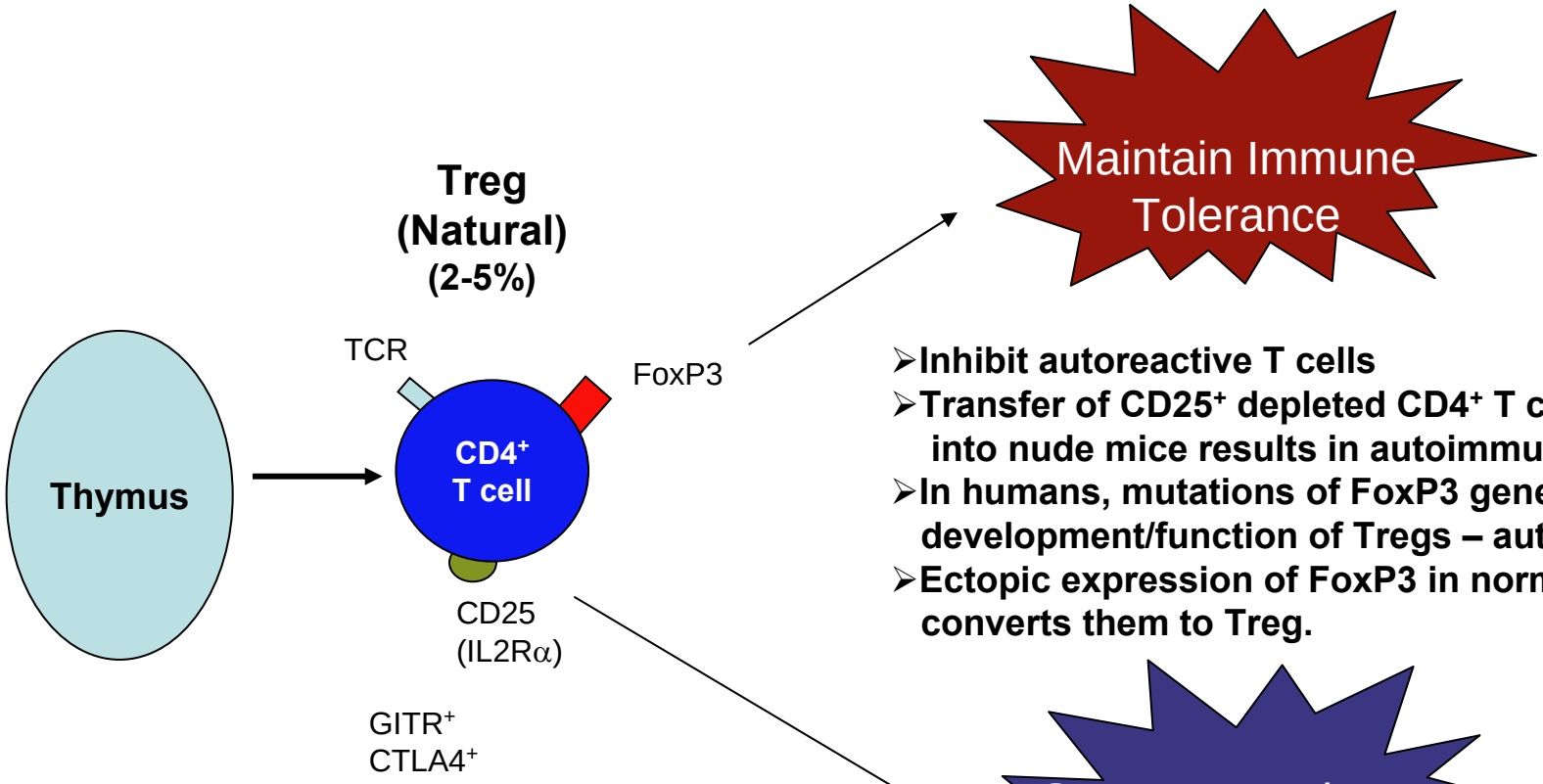

Key
= Increased in RCC patients
= Inhibitory
= Stimulatory
= Induction/Differentiation
Myeloid Progenitor
M1/M2
RCC Tumor
B7-H1
COX-2
VEGF
PGE2
Gangliosides
CD11b
MSC
VEGFR1+
CD33
NOS-2
Arginase 1
CD4
Treg
FoxP3
CD25
RCC Immunosuppressive Environment
Cell Mediated Responses
Humoral Responses
IFN-γ
IL-2
IL-4, IL-6
IL-5, IL-10
T cell
Th1
Bias
Th2
TGF-β
CD4
Tr1 Treg
IL-10
CD123
pDC
IDO
BDCA-2
CD4
Th3 Treg
TGF-β
T cell Anergy

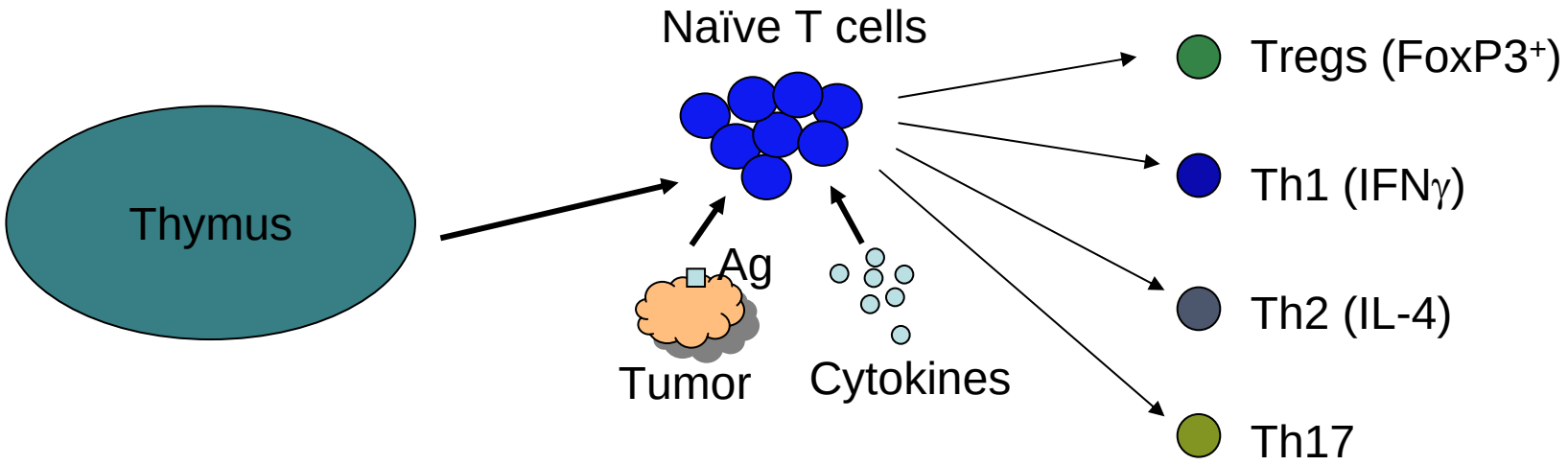
Regulatory T cells in Tumor Immunity



- **Inhibit autoreactive T cells**
- **Transfer of $CD25^+$ depleted $CD4^+$ T cells into nude mice results in autoimmune disease.**
- **In humans, mutations of FoxP3 gene impairs development/function of Tregs – autoimmunity.**
- **Ectopic expression of FoxP3 in normal T cells converts them to Treg.**

- **Tumor rejection in mouse models correlated inversely with suppressive activity of $CD4^+$ T cells.**
- **Depletion of Treg in vivo ($CD4^+CD25^+Foxp3^+$) reduces tumor growth in mouse models.**

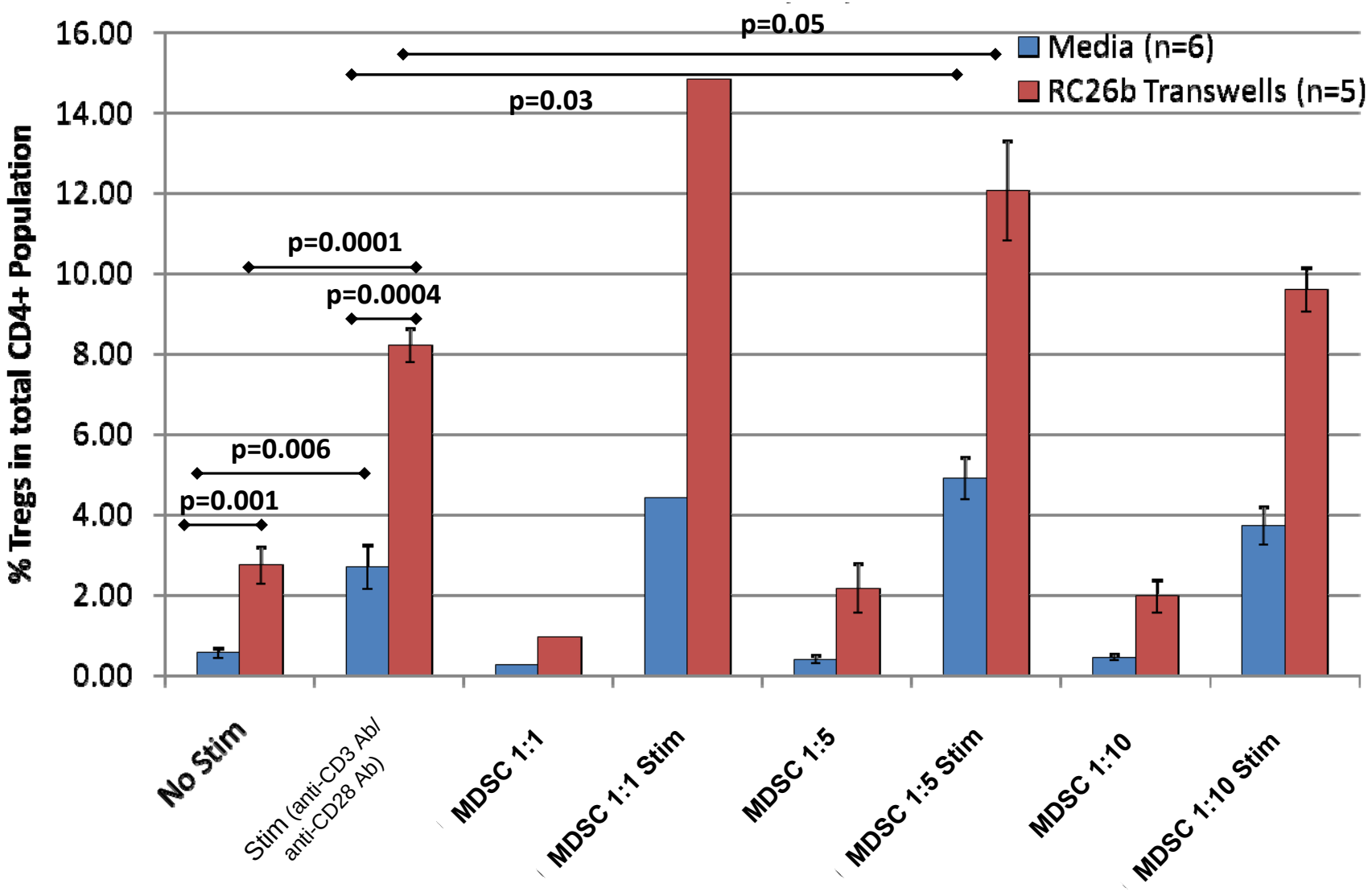
Inducible Tregs (Foxp3^{+})
(Differentiated outside of the Thymus)



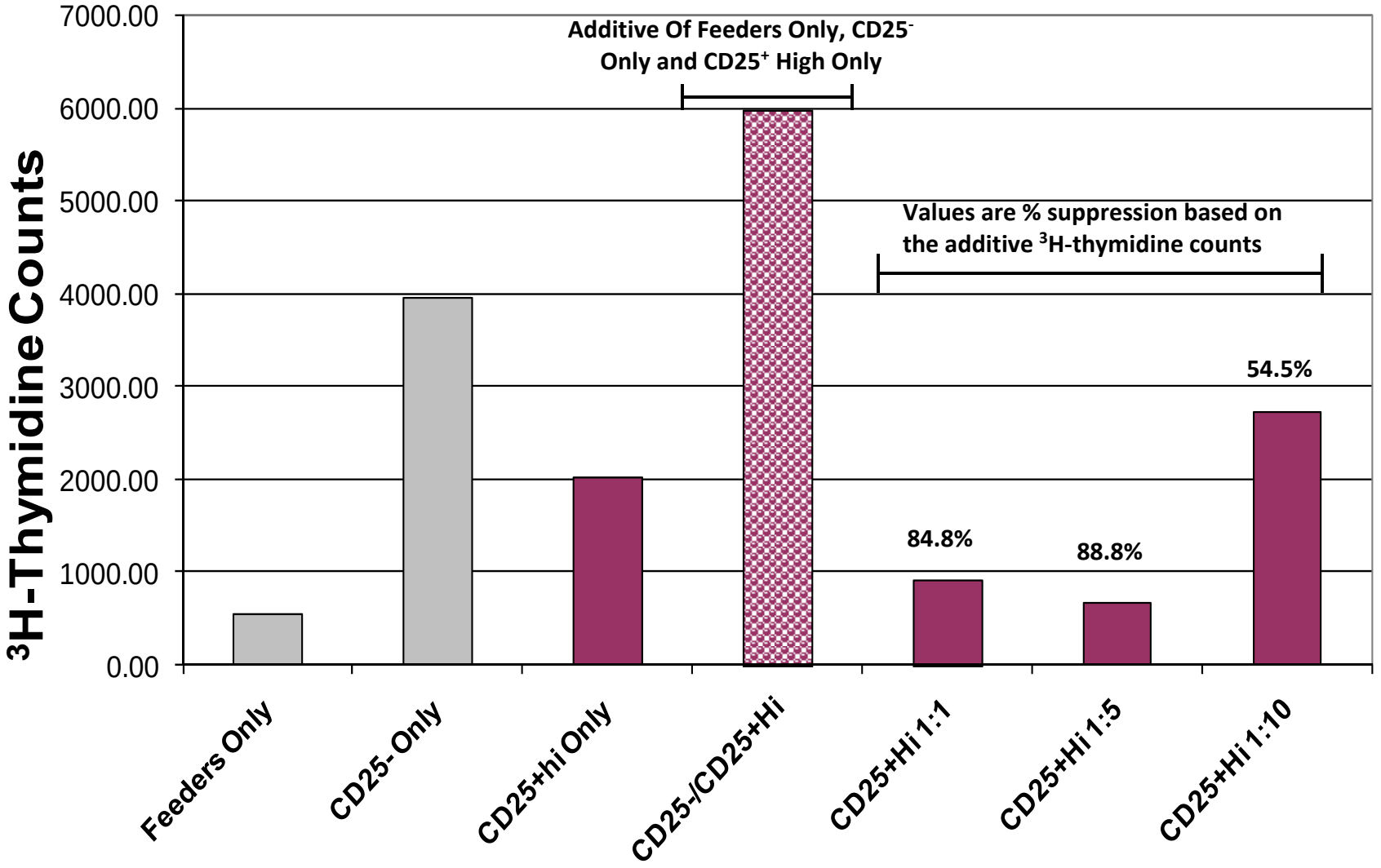
Origin: Naïve T cells CD4+CD25-
Activated effector/memory T cell (CD4^{+}CD25^{-})

Induction signals: TCR stimulation
IL-2, TGFβ
DC/MDSC/Tumor

Treg Induction with RCC cell line (SK-RC-26b), MDSC and anti-CD3/CD28 Stimulation



The CD4+CD25+ High Cells Induced by CoCulture of CD4+CD25- Cells with Tumor Cells are Functionally Suppressive



Treg Numbers in Cancer Patients

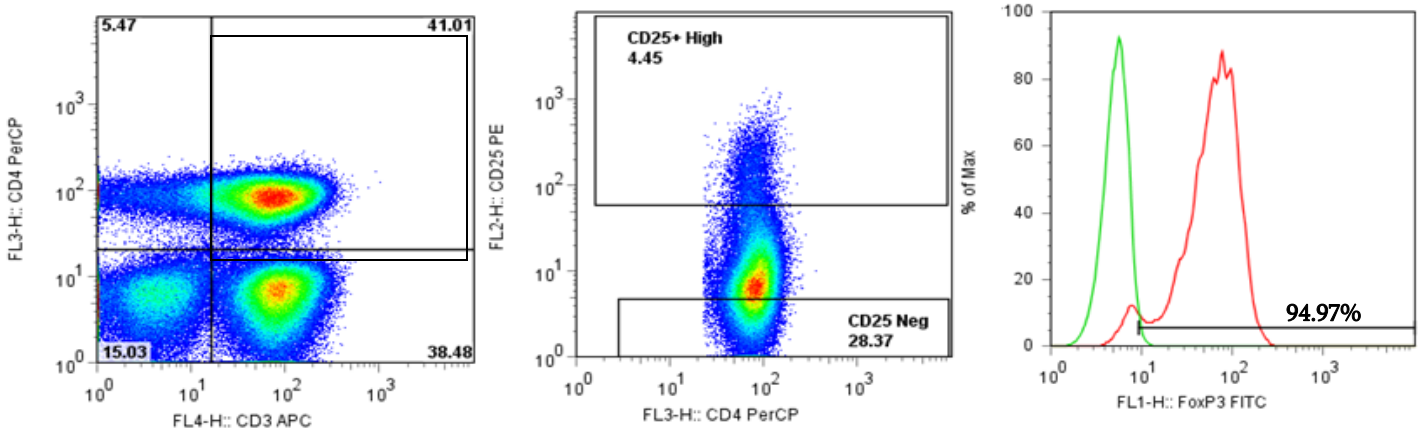
- ✓Increased number of tumor infiltrating FoxP3+ cells (by immunostaining) associated with poor prognosis (Ovarian, Heptocellular, cervical and Head and Neck Squamous Cell Carcinomas)

- ✓No association in Renal Cell Carcinoma

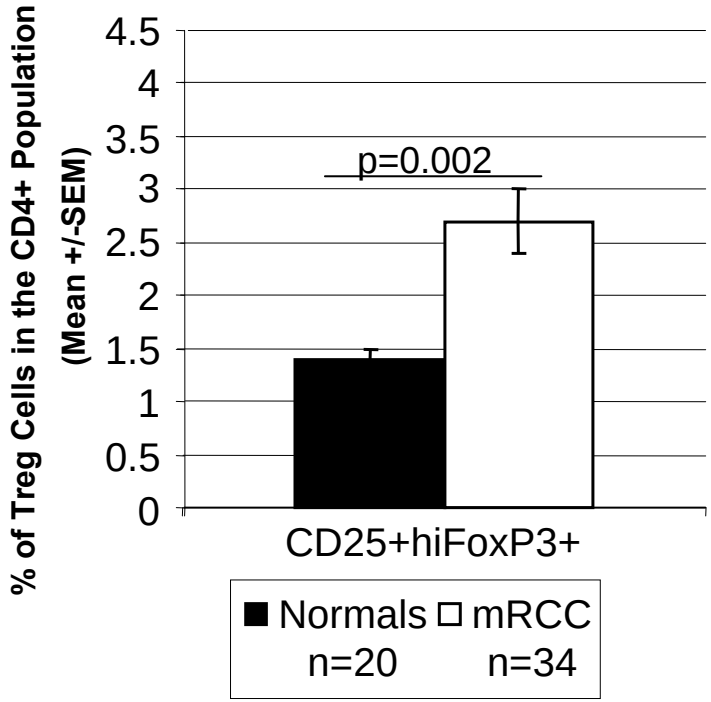
- ✓Frequently increased in the blood of some cancer patients (RCC etc. –no correlation with poor prognosis)

- ✓Suppressive *in vitro*

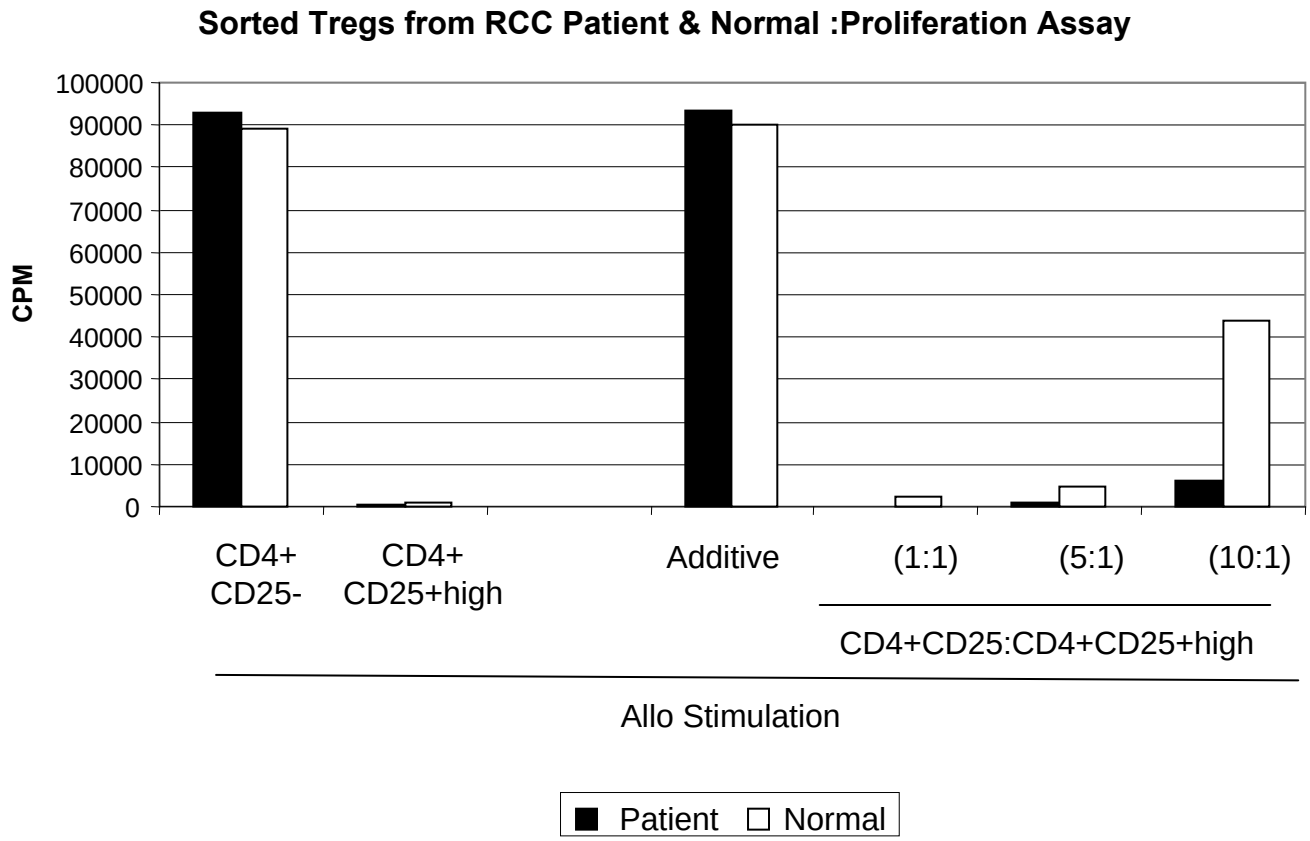
Treg Cell Analysis



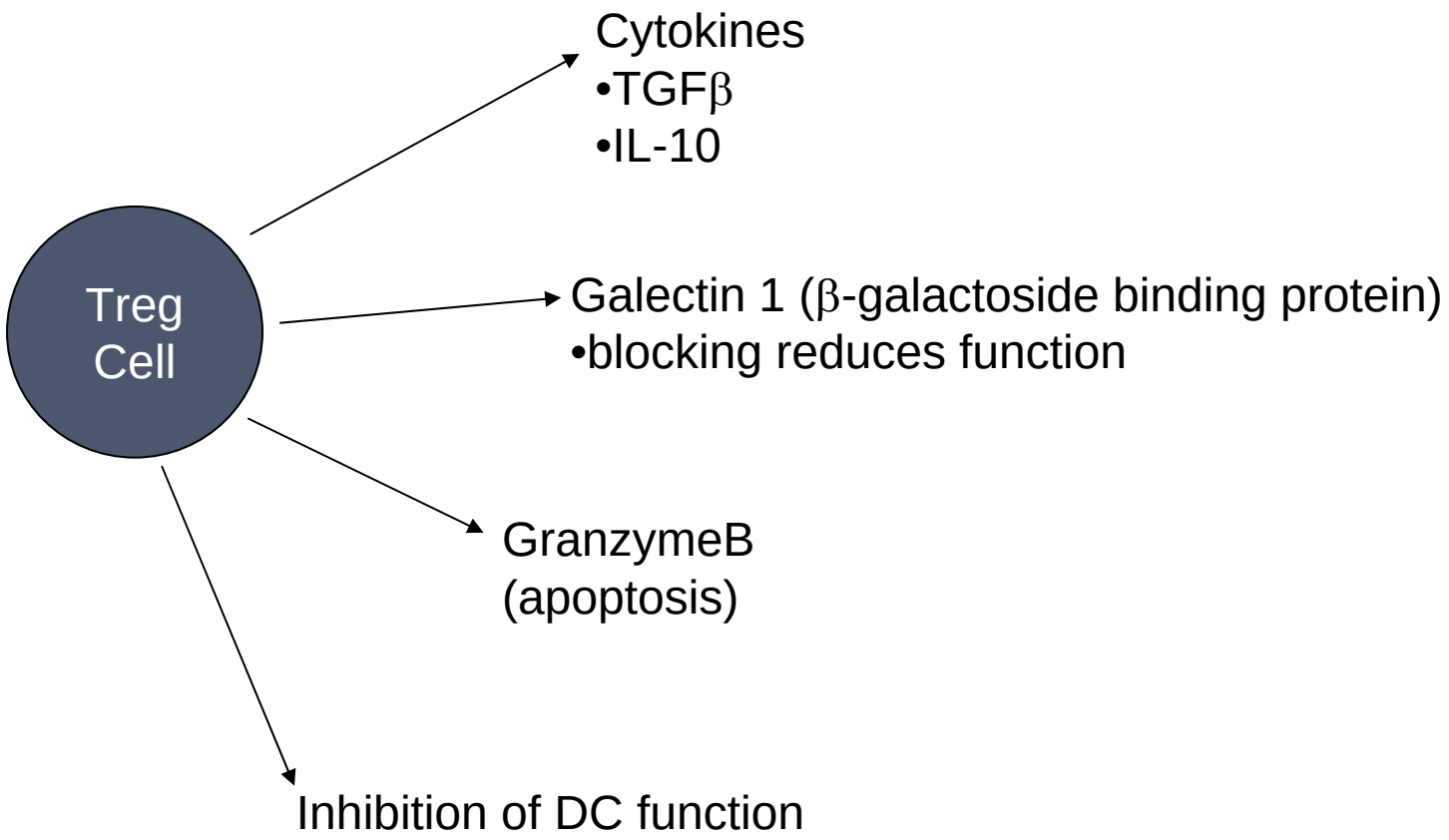
Increased Treg in Peripheral Blood of RCC Patients



Assay for Treg Suppressor Function Using RCC Patient PBMC



Mechanisms of Treg Function



Other Regulatory T cells

Tr1/Tr3

- Antigen Induced
- Tr1 secrete IL-10, Tr3 secrete TGFβ
- No Specific Markers
- FoxP3 not constitutively expressed
- $CD4^+CD25^{int}$ T cells secreting IL-10, not IFN, detected in some human tumors (Gastric Cancer, Renal Cell Carcinoma).

$CD8^+$ Treg Cells

Immunosuppressive populations include:

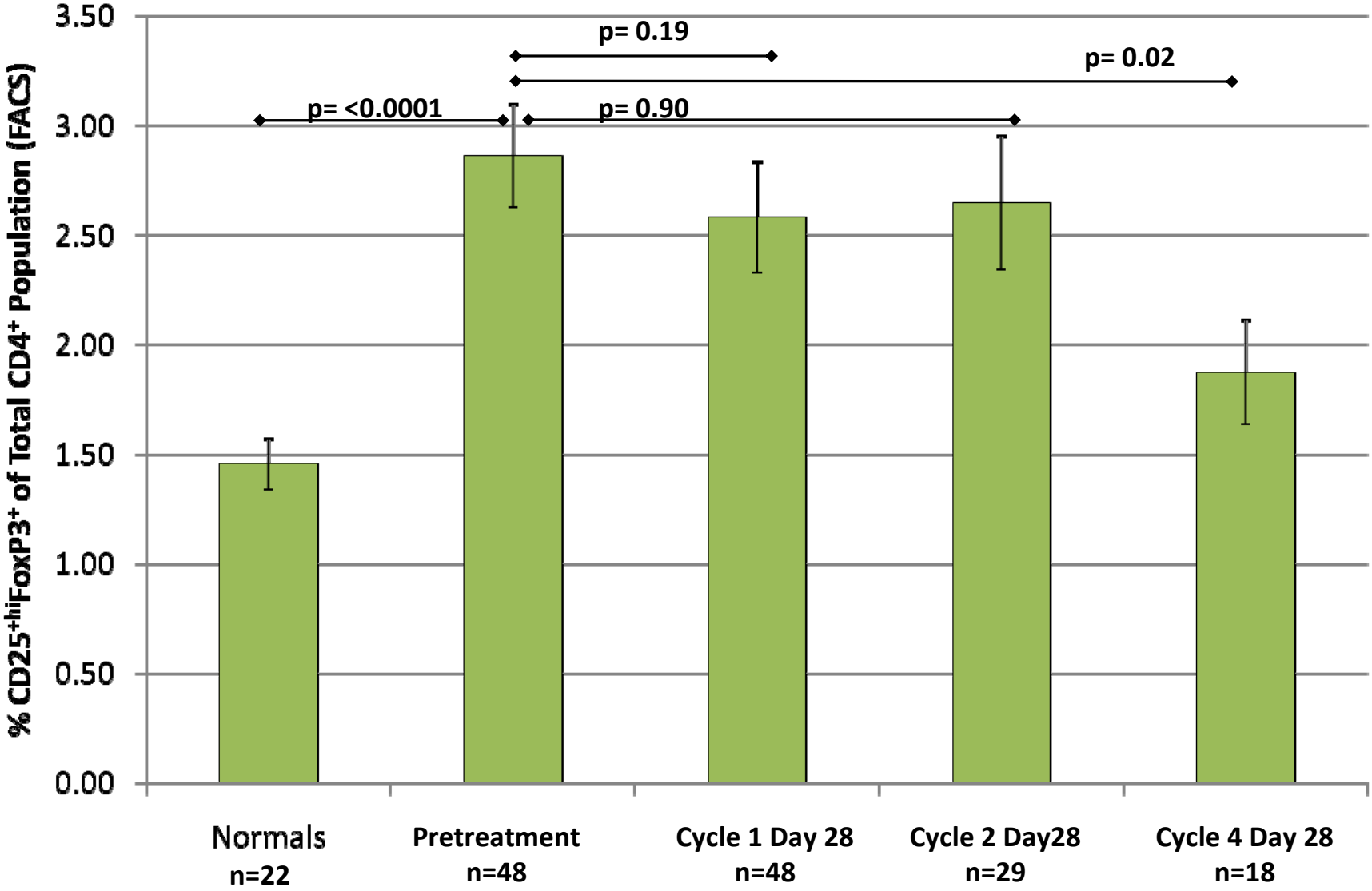
- $CD8^+CD25^+FoxP3^+$
- $CD8^+IL10^+$

NKT regulatory cells

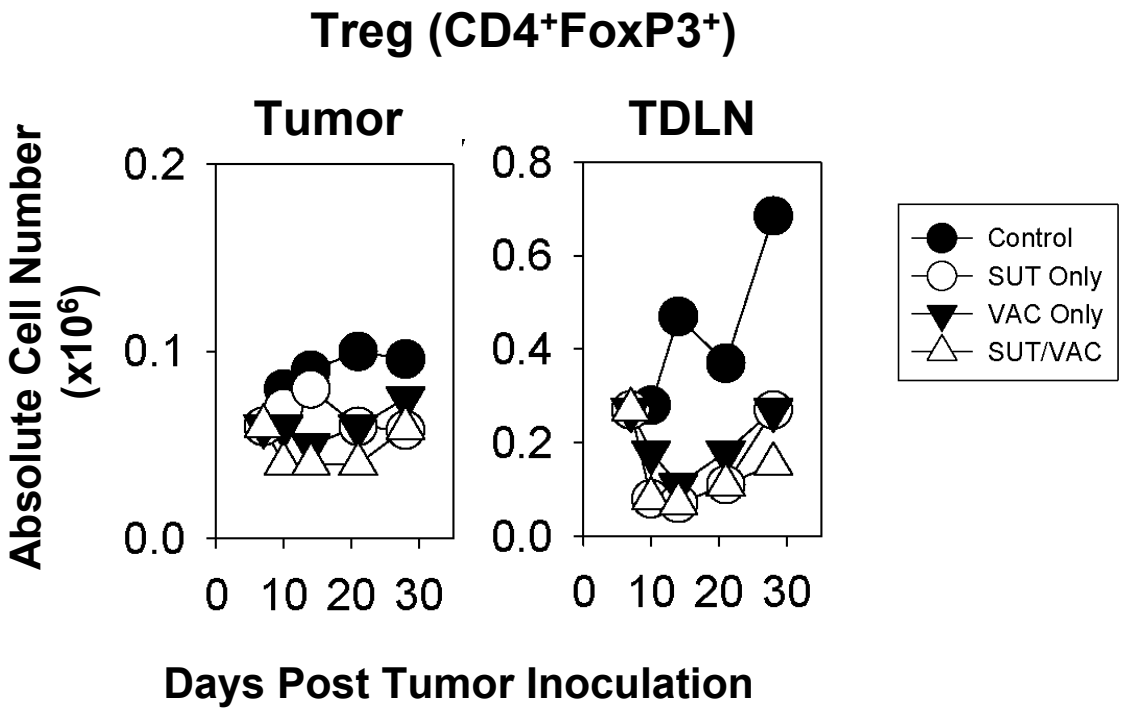
Targeting Tregs

- Targeting CD25 Receptor
  - •Ontak (Denileukin Diftitox, IL-2/Diphtheria toxin fusion protein) +/- vaccine
  - •Recombinant anti-CD25 immunization
  - •Immunized IgG1 monoclonal antibody to CD25 +/- vaccine
    - ▪Varying degrees of decreasing Tregs
    - ▪Increase Th1 response
- Cyclophosphamide
  - •Augments cellular and humoral regions
  - •Deplete Treg and boost efficacy in mouse models
- CpG
  - •Lowers Foxp3+ T cells in lymph nodes of Melanoma patients
- Block Treg Function
  - •Stat 3 decreases function (TKI Sunitinib)
  - •Ox40
- Block Treg Differentiation
- Block Trafficking

Sunitinib Treatment Reduces Treg in RCC Patients Peripheral Blood.



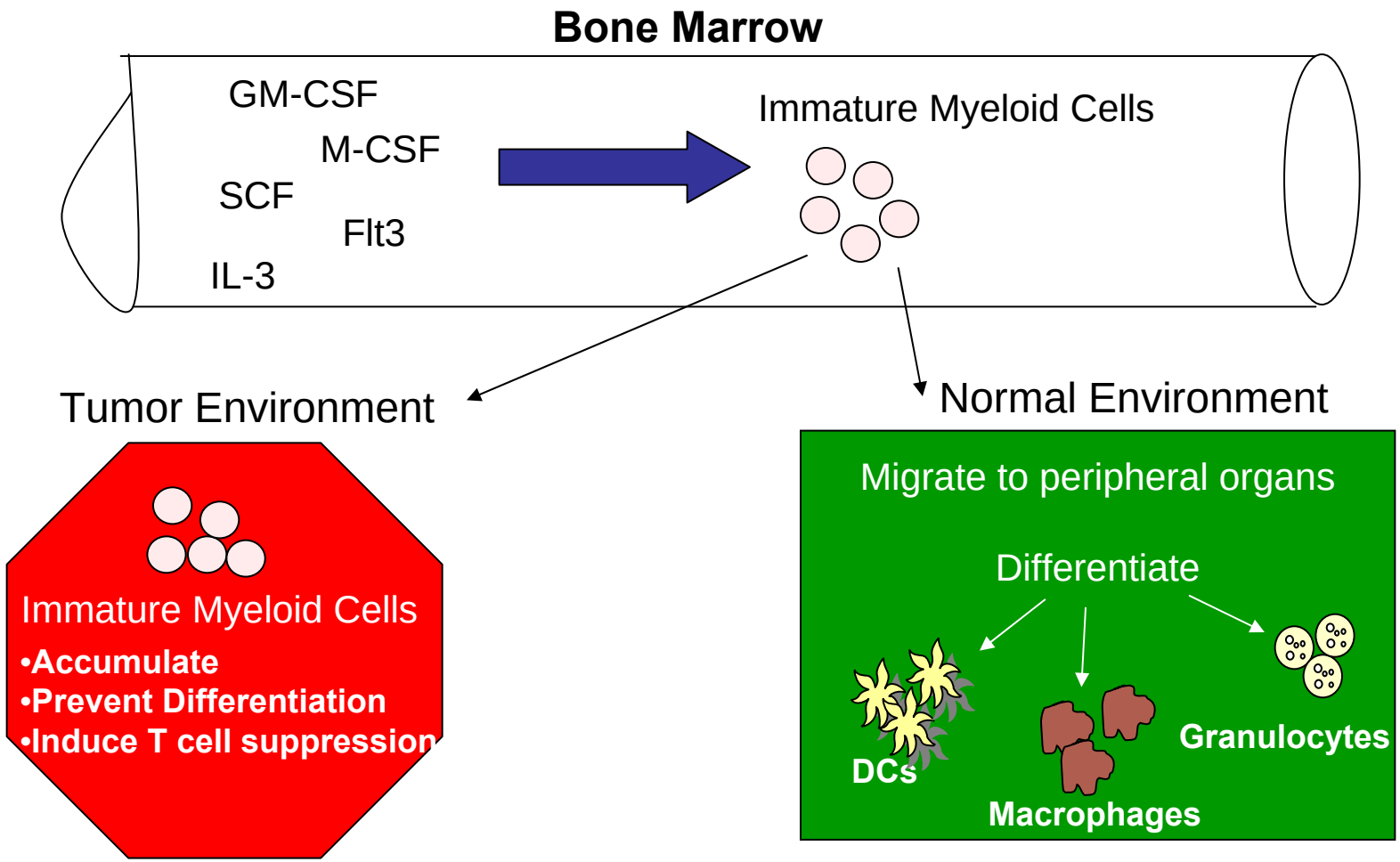
Reduction in CD4^{+}Foxp3^{+} Treg cells in tumor and draining lymph nodes after treatment (B16) with sunitinib, vaccine or both.



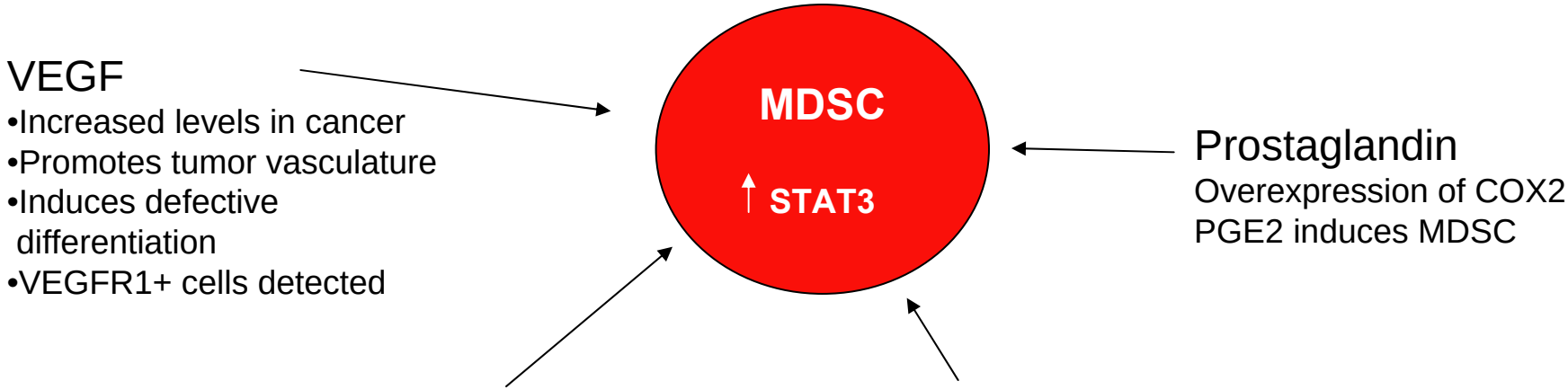
Myeloid-derived Suppressor Cells

- Immunosuppressive myeloid cells
- Normally present in very small amounts but systemically accumulate under pathologic conditions – tumor-bearing
- Accumulation associated with:
  - -VEGF, SCF, GM-CSF, G-CSF, S100A9, and M-CSF

- MDSC depletion in murine tumor models:
  - Inhibits/slows tumor formation
  - Allows for immune-mediated tumor destruction
  - Reduces tumor metastasis
  - Adoptive transfer of MDSC into tumor bearing mice promotes tumor growth and inhibits T cell activation.


Bone Marrow
GM-CSF
M-CSF
SCF
Flt3
IL-3
Immature Myeloid Cells
Tumor Environment
Normal Environment
Immature Myeloid Cells
•Accumulate
•Prevent Differentiation
•Induce T cell suppression
Migrate to peripheral organs
Differentiate
DCs
Macrophages
Granulocytes

MDSC Expansion



VEGF
- Increased levels in cancer
- Promotes tumor vasculature
- Induces defective differentiation
- VEGFR1+ cells detected

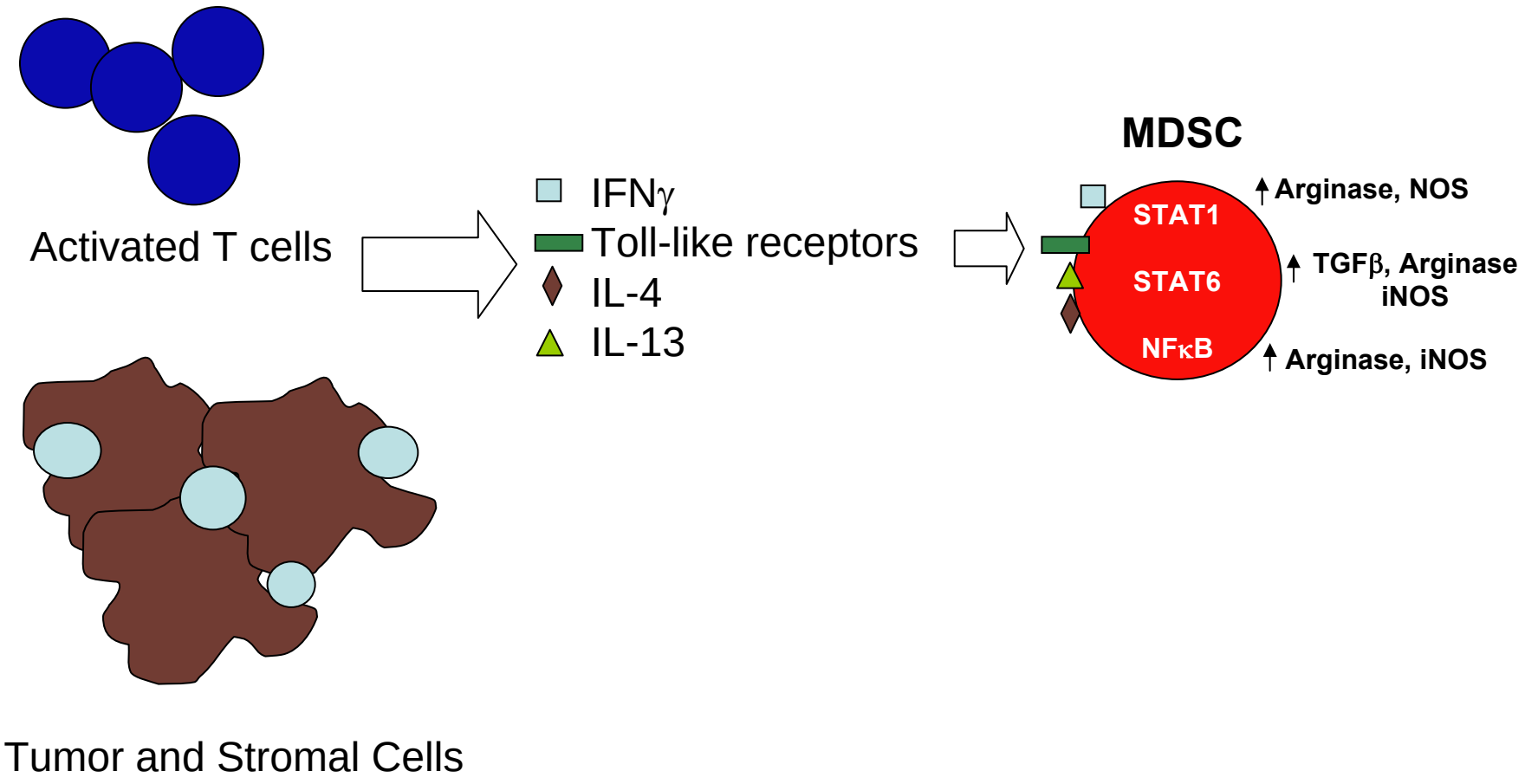
Prostaglandin
Overexpression of COX2
PGE2 induces MDSC

Stem Cell Factor,
IL-6, M-CSF

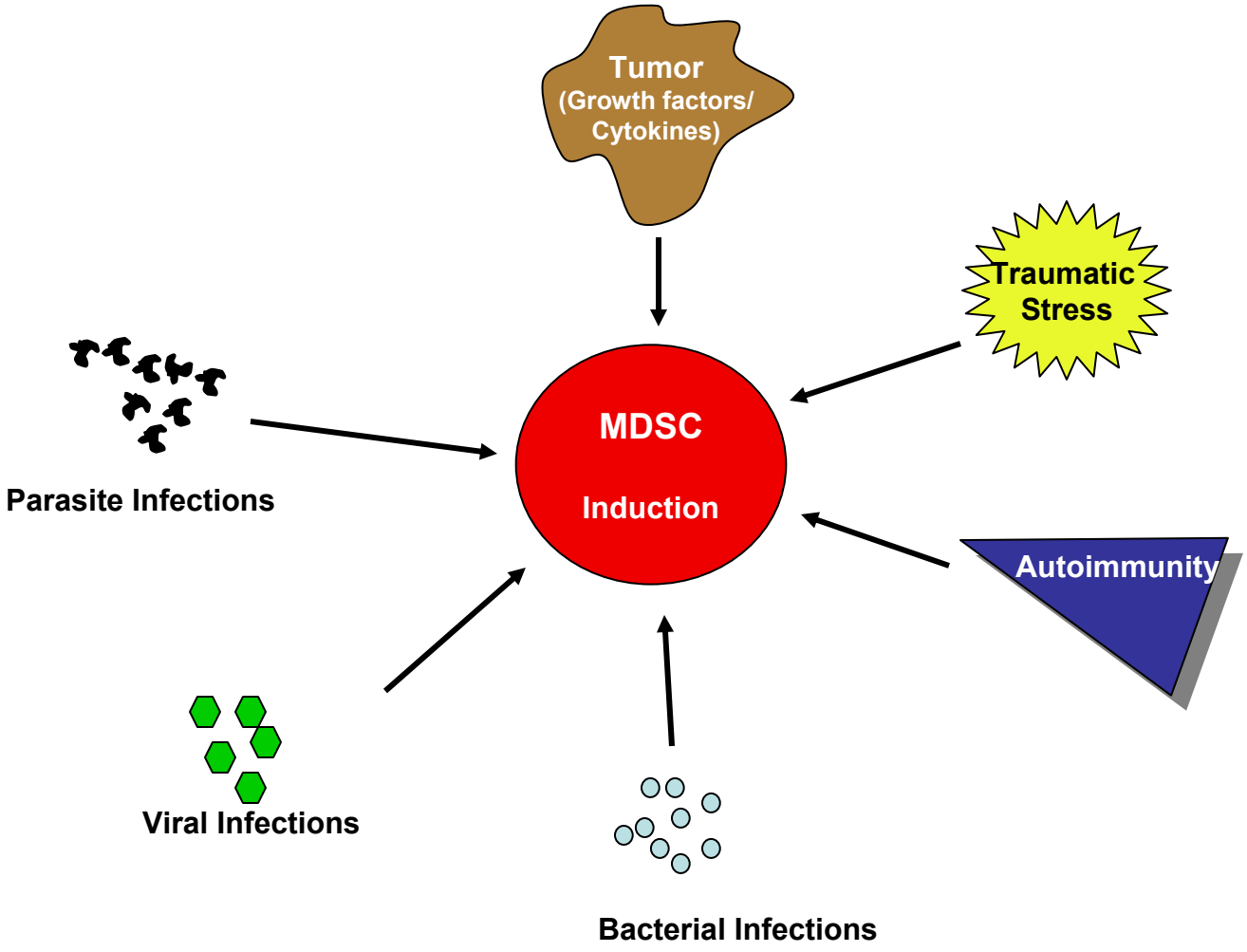
GM-CSF
- Produced by human and mouse tumors
- Promotes differentiation of myeloid precursors
- Bone Marrow cells + GM-CSF become immunosuppressive cells
- Not sufficient alone
- Excessive amounts may be responsible for induction of MDSC, Administration of GM-CSF-based vaccine

Increased MDSC numbers.

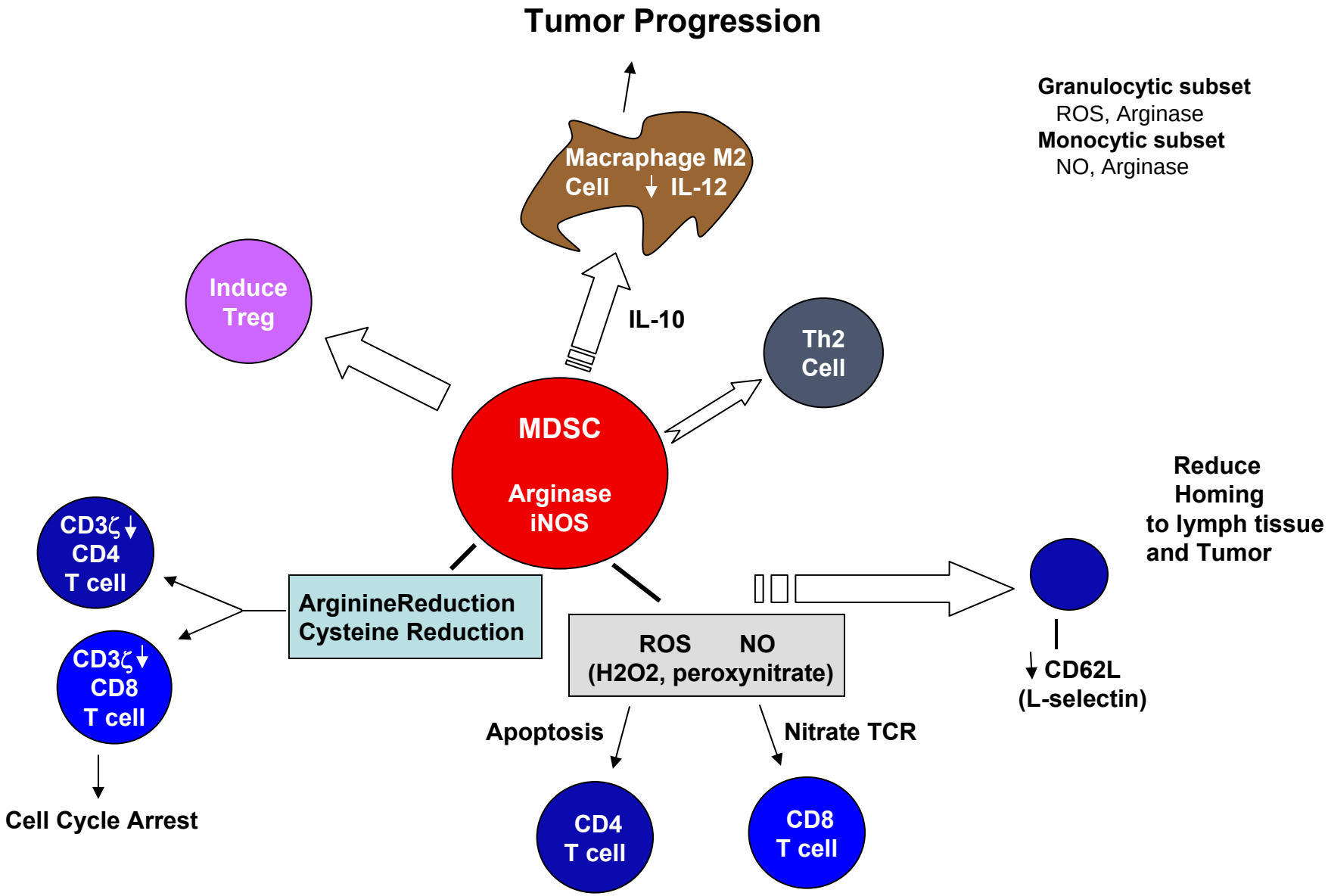
Mechanisms of MDSC Activation



MDSC Induction



MDSC –Mechanisms of Suppression



Markers Expressed by Murine and Human MDSC

Mice

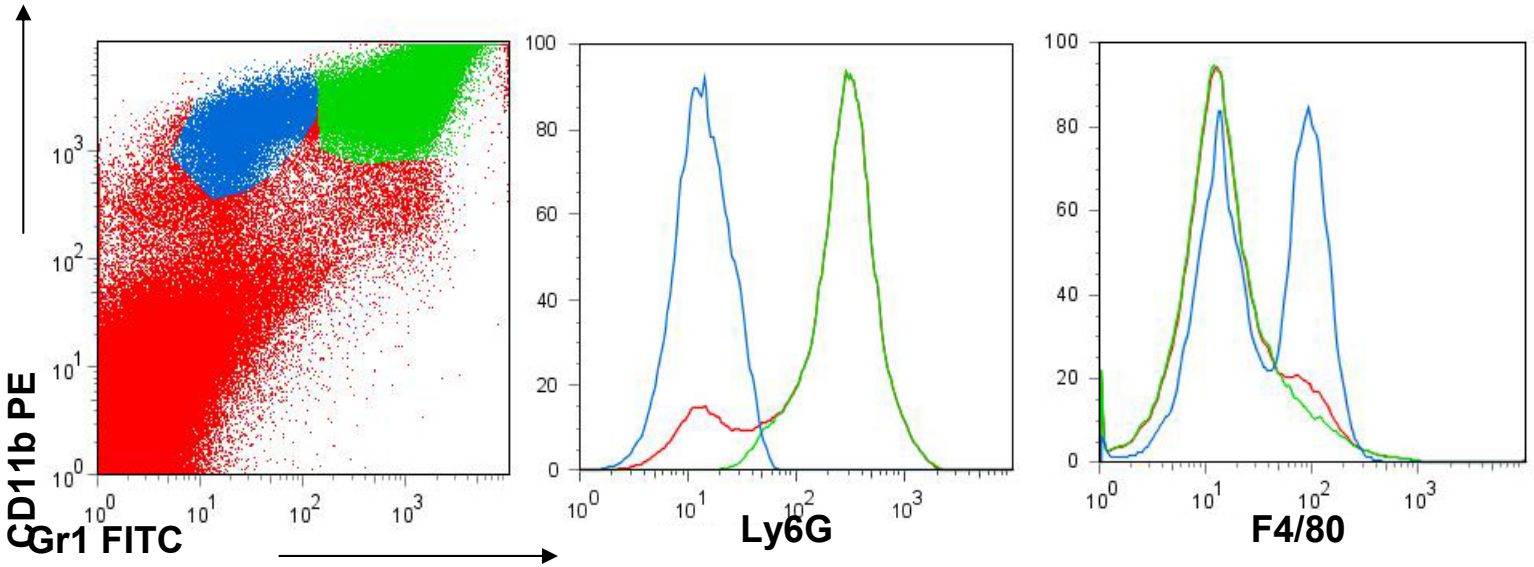
Monocytic-MDSC		**Granulocytic-MDSC**	
Gr1	(+)	Gr1	(+)
CD11b	(+)	CD11b	(+)
F4/80	(+)	F4/80	(-)
Ly6C	(+hi)	Ly6C	(+low)
Ly6G	(-)	Ly6G	(+)

Human

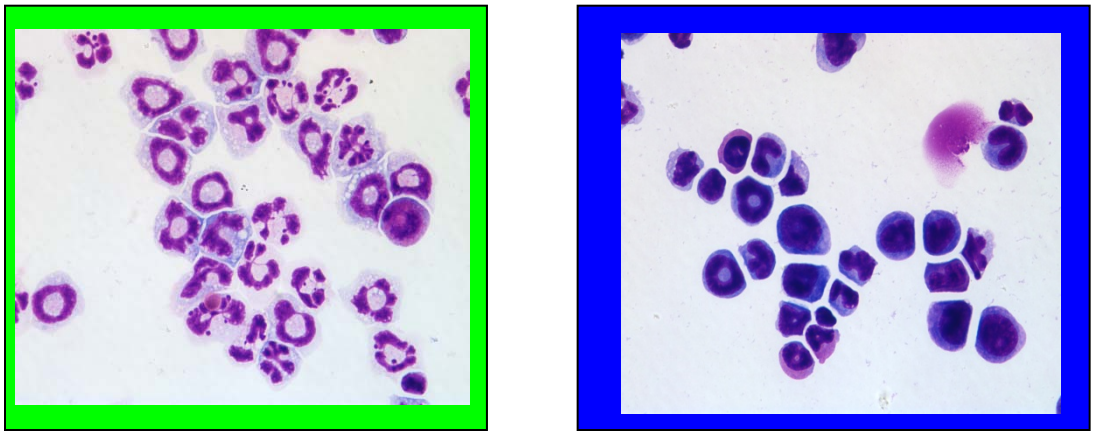
Monocytic-MDSC		**Granulocytic-MDSC**	
CD33	(+)	CD33	(+)
CD11b	(+)	CD11b	(+)
CD66b	(+/-)	CD66b	(+)
CD14	(+)	CD14	(-)
CD15	(+/-)	CD15	(+)
CD124	(+)	CD124	(+)

Ly6G^{hi} Neutrophilic MDSC are Gr1^{hi} and Ly6G^{lo} Monocytic MDSC are Gr1^{lo}

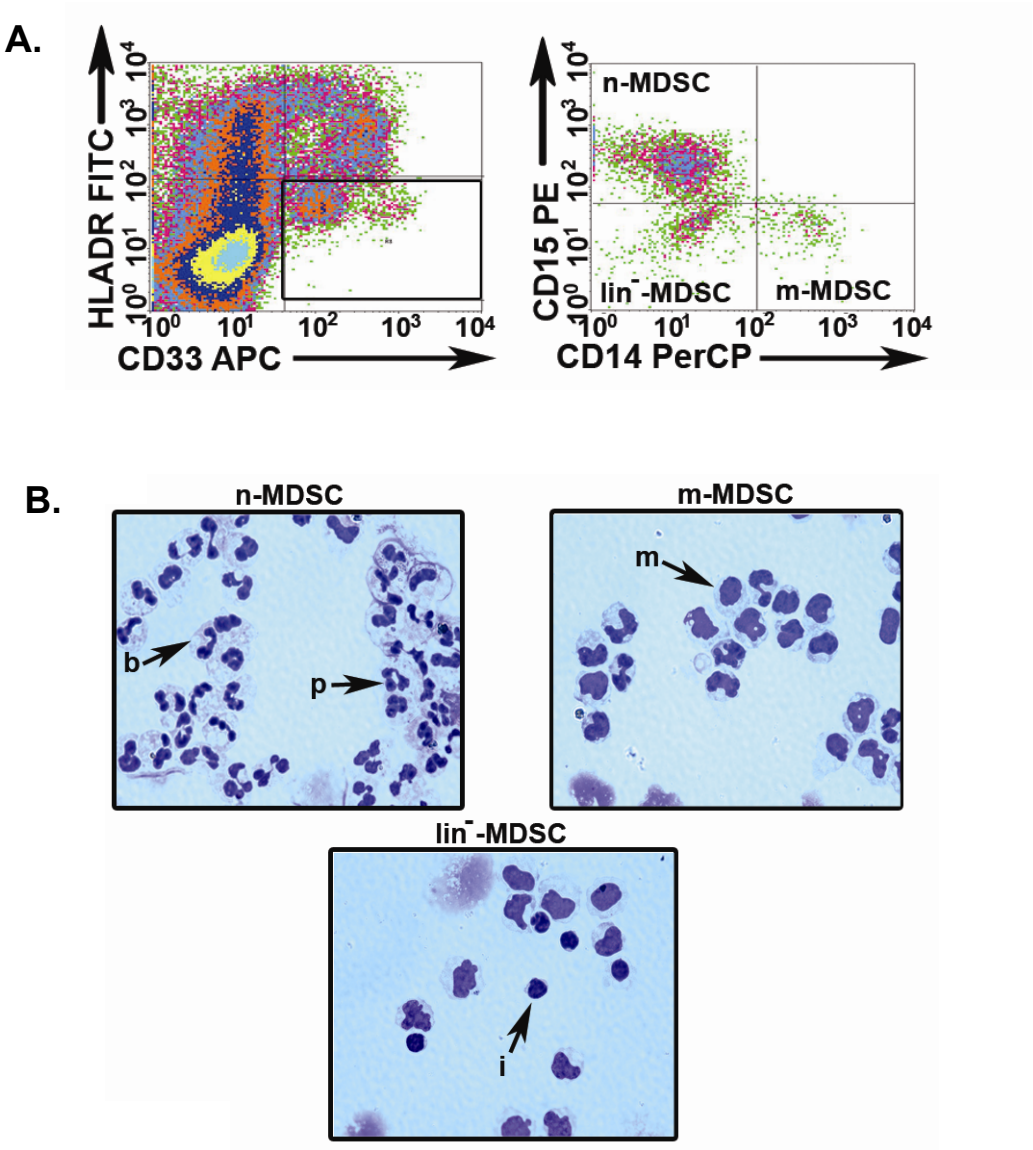
Gr1^{hi}N-MDSC= green, Gr1^{lo}M-MDSC= blue



Gr1^{hi} N-MDSC=green Gr1^{lo} M-MDSC=blue



MDSC Isolated from RCC Patient's Tumor



Targeting MDSC To Improve Immunotherapy

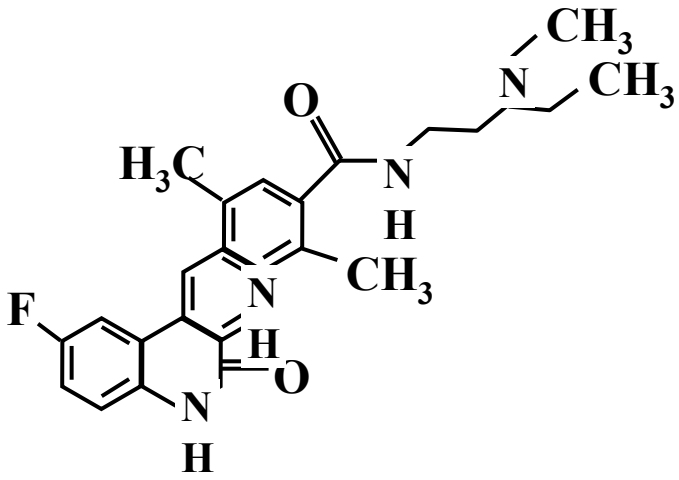
Agents	Mode of Action	Tumor Type	MDSC Reduction (numbers/ function)	T cell Response Improved
VEGF Trap (Fusion Protein)	Binds VEGF	Multiple Types	No	No
Anti-VEGF (bevacizumab)	Binds VEGF	RCC	Mixed (1 yes, 1 NO)	Not Clear
TKI (AZD2171)	Blocks VEGFR Signaling	Multiple Types	Slight Reduction in Numbers	Not Tested
Triterpenoids (CDD0-Me)	Antioxidant Reduced ROS	RCC	No reduction # Reduced Function	Yes
P**hosphodiesterase-5** (Sildanefil)	Reduces Arginase 1 & NOS-2 expression	Head/Neck Myeloma	Reduced Function	Yes

Targeting MDSC To Improve Immunotherapy

Agents	Mode of Action	Tumor Type	MDSC Reduction (numbers/ function)	T cell Response Improved
All-Trans retinoic Acid	MDSC Differentiation (Increased glutathione syn and reduced ROS)	mRCC	Reduced Numbers	Yes
Vitamin D3 Bioactive Metabolite	Promote Differentiation	Head & Neck	Reduced numbers	Yes
Gencitabine + Cyclophosphomide	Chemotherapeutic Drug	Breast Cancer	Reduced numbers	?
Sunitinib	TKI (blocks Proliferation of mMDSC and causes Apoptosis of nMDSC)	mRCC	Reduced Numbers	Yes

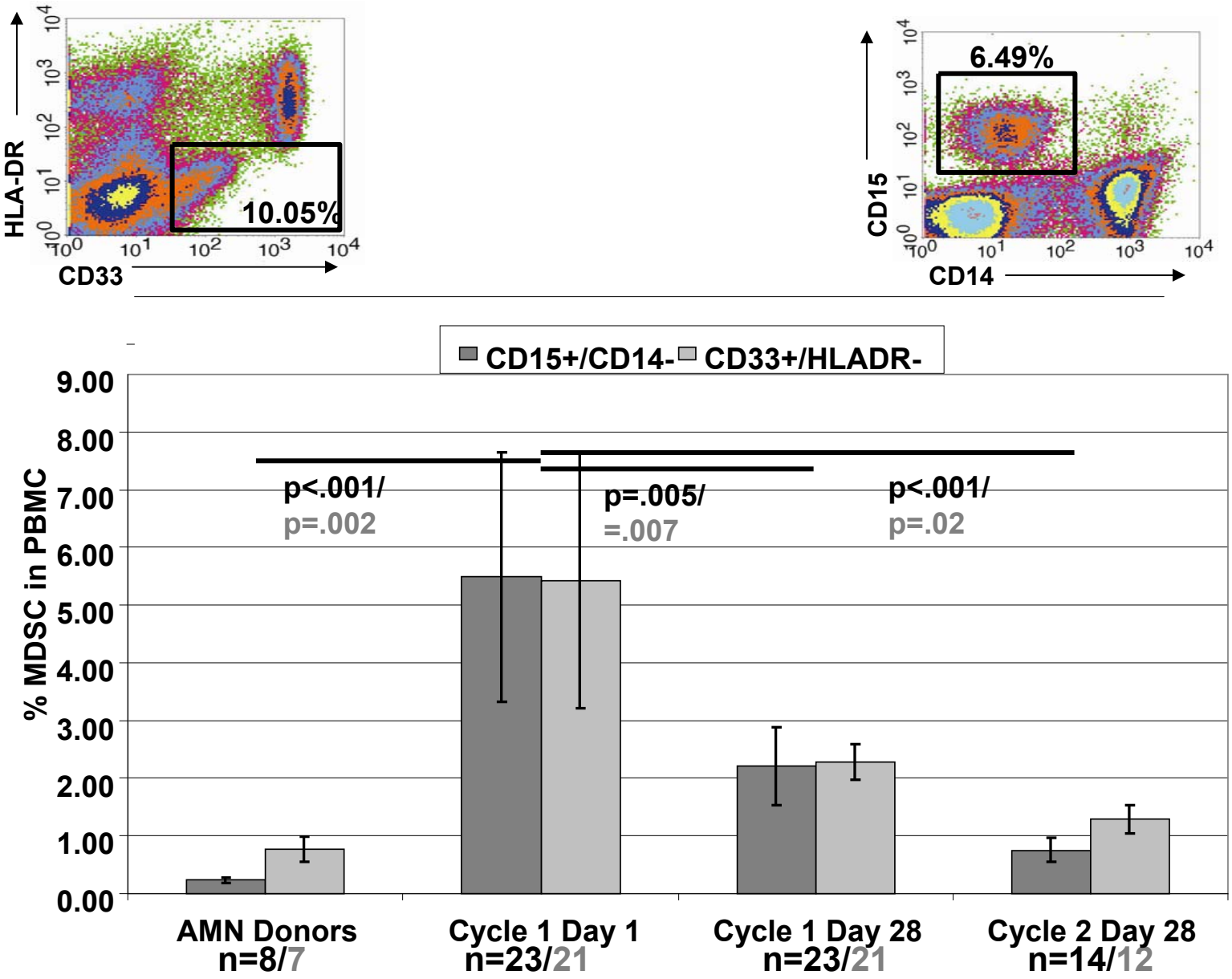
Multitargeted Approaches in mRCC: Sunitinib (SU11248)

- Small-molecule receptor tyrosine kinase inhibitor[1]
- Inhibits all VEGFRs, PDGFR-A, PDGFR-B, c-KIT, and FLT-3[1]
- Oral administration[1]
- Both antitumor and antiangiogenic activity[1]
- FDA approved January 26, 2006 for treatment of advanced RCC[2]
- 50% response rate in mRCC



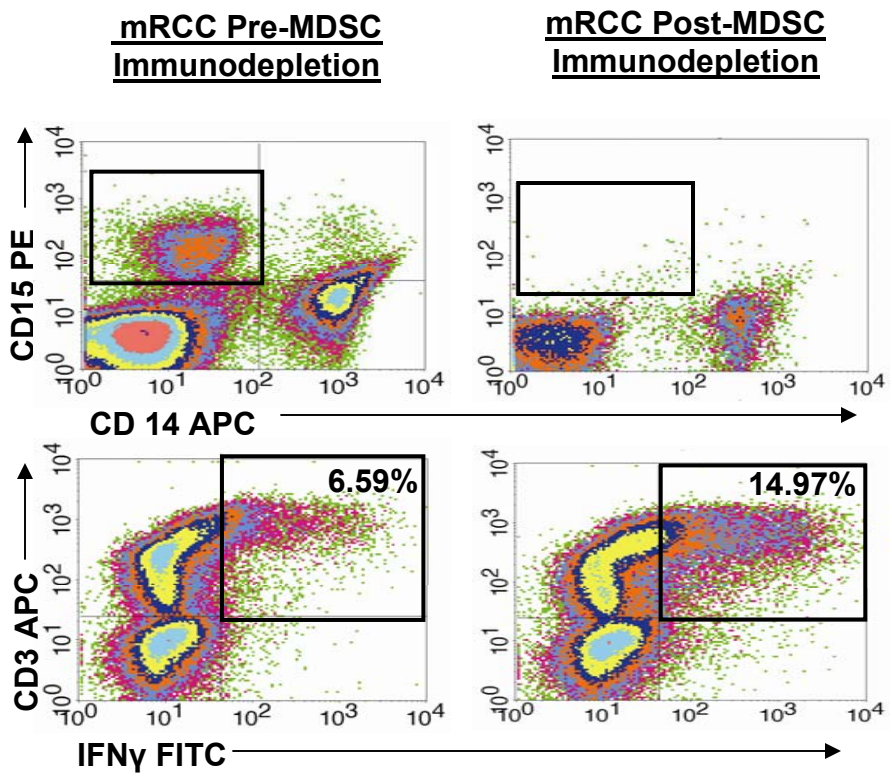
1. Pietras K, Hanahan D. *J Clin Oncol.* 2005;23:939-952.
2. Food and Drug Administration. FDA approves new treatment for gastrointestinal and advanced kidney cancer. Available at: http://www.fda.gov/bbs/topics/news/2006/NEW01302.html. Accessed January 26, 2006.

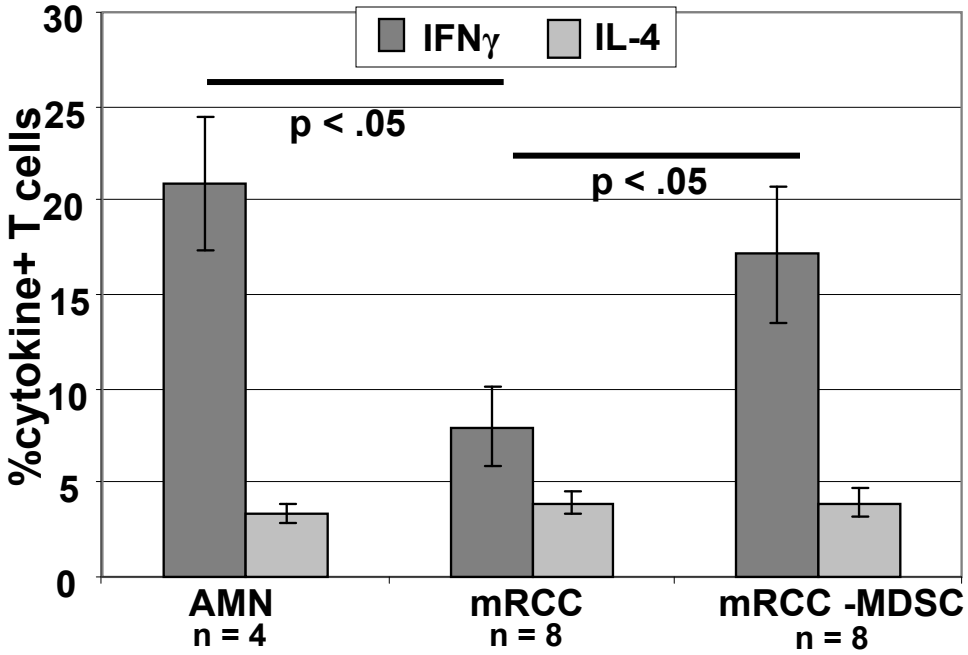
Sunitinib reverses MDSC accumulation in mRCC patients



-Ko, JS. et. al. Clin Cancer Res. 2009 Mar 15;15(6):2148-57. Epub 2009 Mar 10.

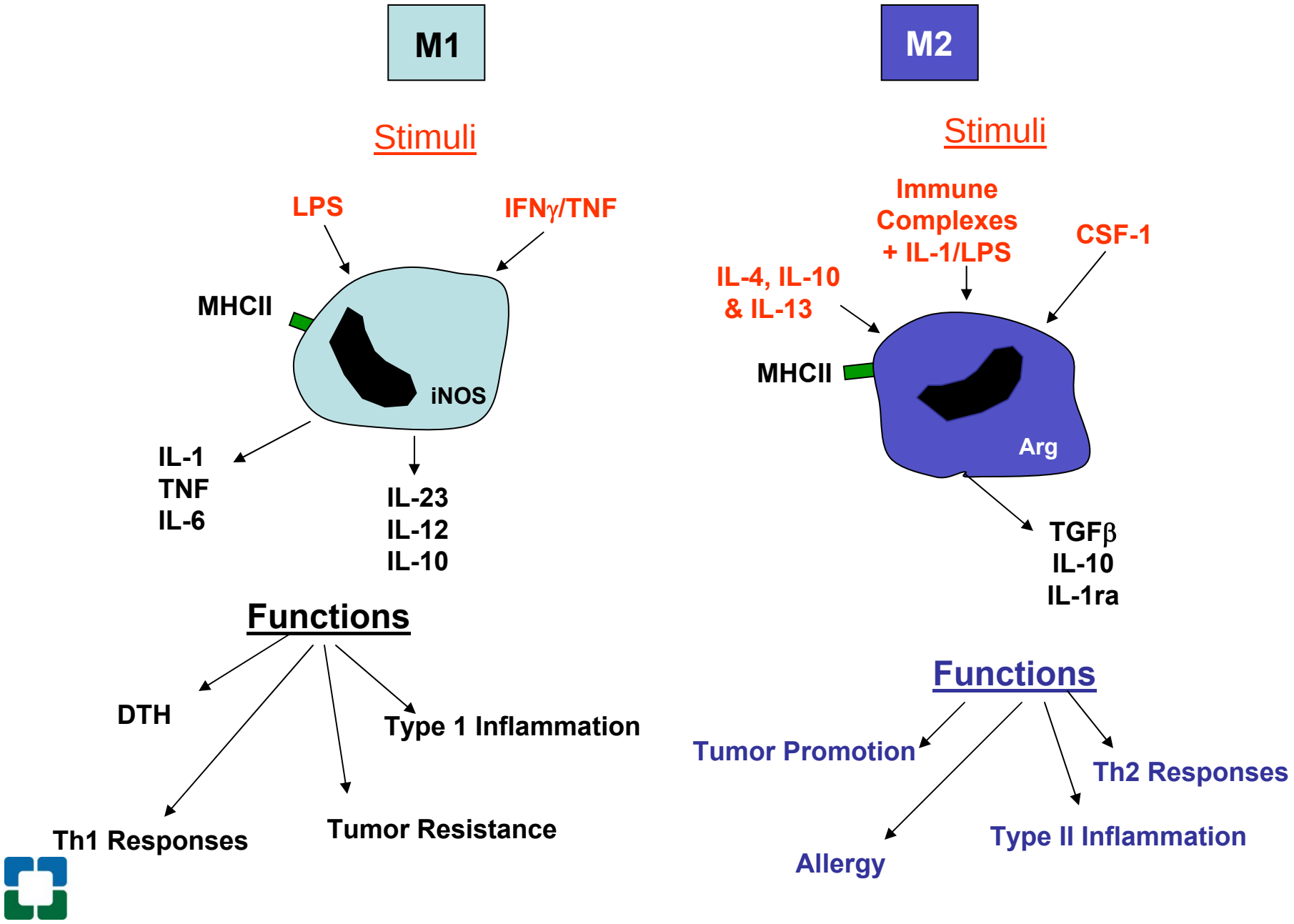
Sunitinib-meditated improvements in T cell function are reproduced by *in vitro* MDSC depletion



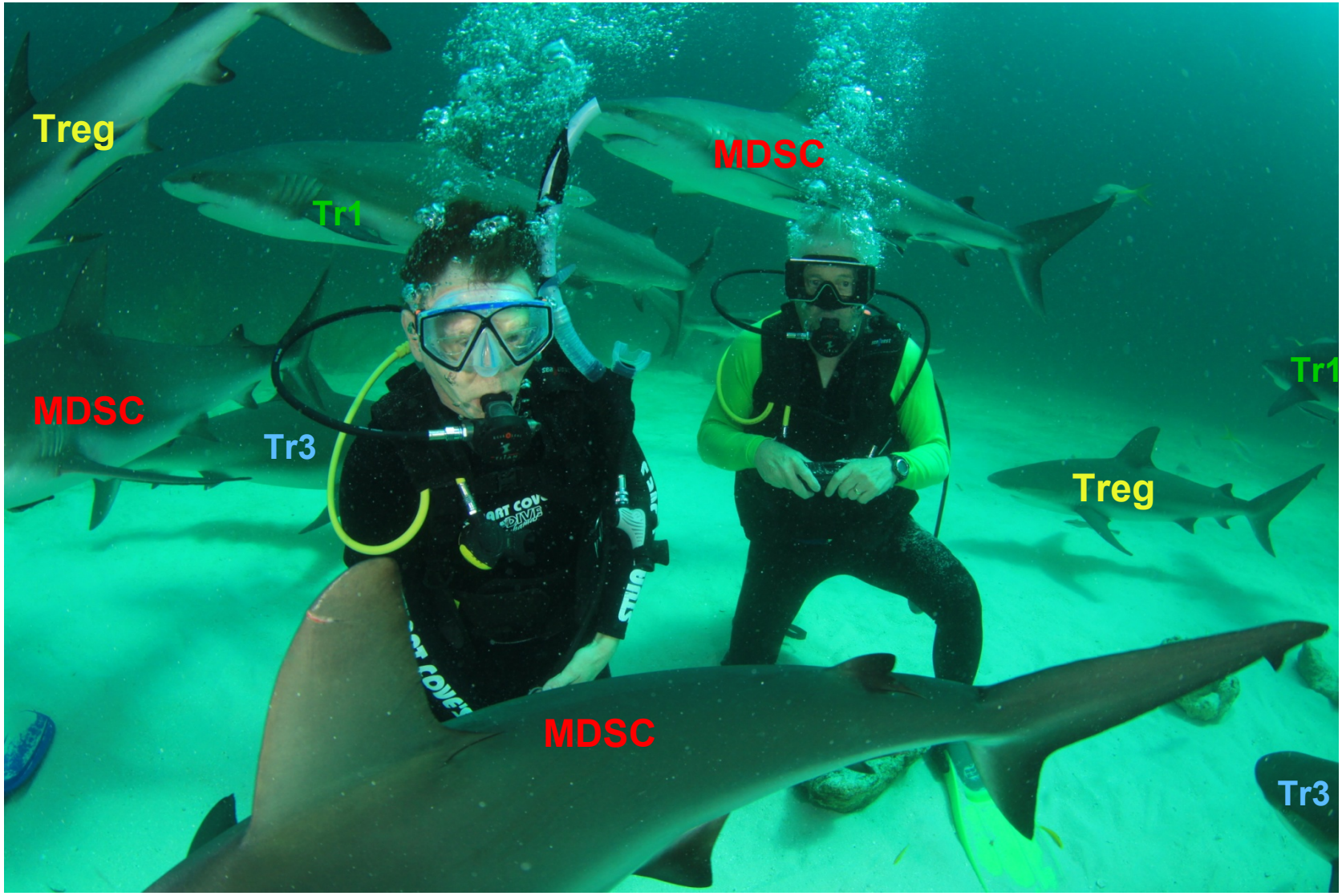


-*Ko. JS. et. al. Clin Cancer Res. 2009 Mar 15:15(6):2148-57*

Tumor Associated Macrophases



Obstacle To Overcome To Promote Immunotherapy in Cancer



Future Directions

• Identify new targets for reducing Treg numbers and/or their suppressive function.

• Better understand the role of other immune suppressive T cell populations (Tr1/ Tr3,CD8) in tumor-induced immune suppression. Identify targets for blocking/deleting them.

• Identify which of the various strategies shown to reduce MDSC in the peripheral blood of patients are also effective within the tumor microenvironment and define which ones promote strong anti-tumor immunity.

• In clinical studies test whether effective blocking of Tregs and MDSC will provide greater efficacy for different forms of immunotherapy (vaccines and adoptive therapy).