Defining The Role of TKI in Reducing Immune Suppression While Improving T cell Responsiveness and Efficacy of Immunotherapy For the Treatment of Tumors

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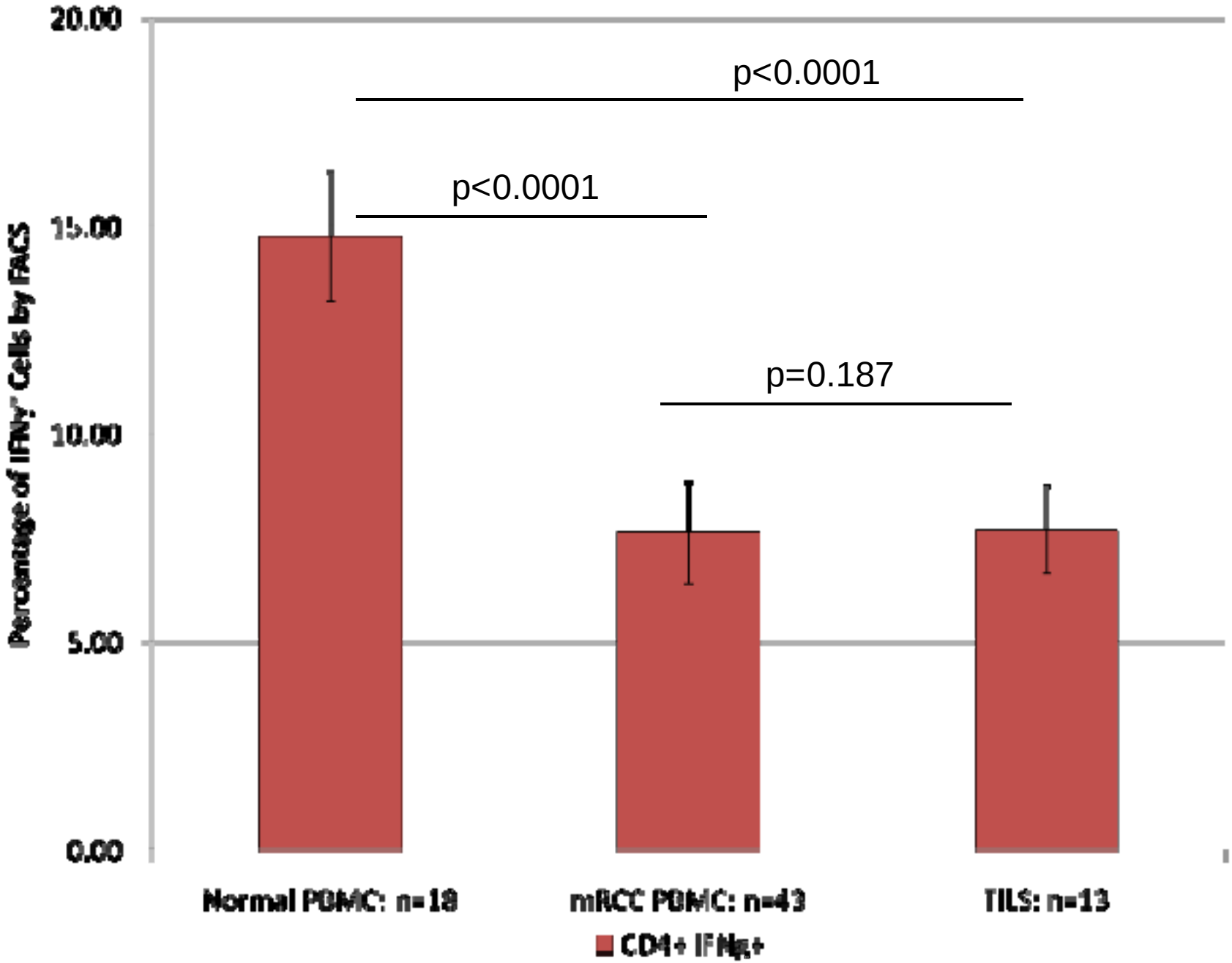
Immune Dysregulation in Cancer

- Increased suppression of a Th1 immune response and increased Th2 cytokine bias
- Increased apoptosis of T lymphocytes
- Impaired DC maturation and function
- Observed in many tumor types including; RCC, Gliomas (GBM), Squameous cell carcinoma of head and neck, Ovarian and Melanoma.

Reduced T cell IFNγ response in RCC patients



Sunitinib-mediated modulation of tumor-induced immune suppression

- **Sunitinib is a small molecule receptor tyrosine kinase inhibitor which was designed to limit angiogenesis.**
- **It promiscuously targets VEGFR, ckit (SCF receptor), flt3, PDGFR, and M-CSFR receptor tyrosine kinases (rTKs).**
- **Sunitinib has major therapeutic impact against renal cell carcinoma and ckit^{pos} GIST tumors.**

Sunitinib Modulation of Immune Cells

- Sunitinib reverses immune suppression and decreases T –regulatory cells in RCC patients. Finke J et al Clinical Cancer Research 2008

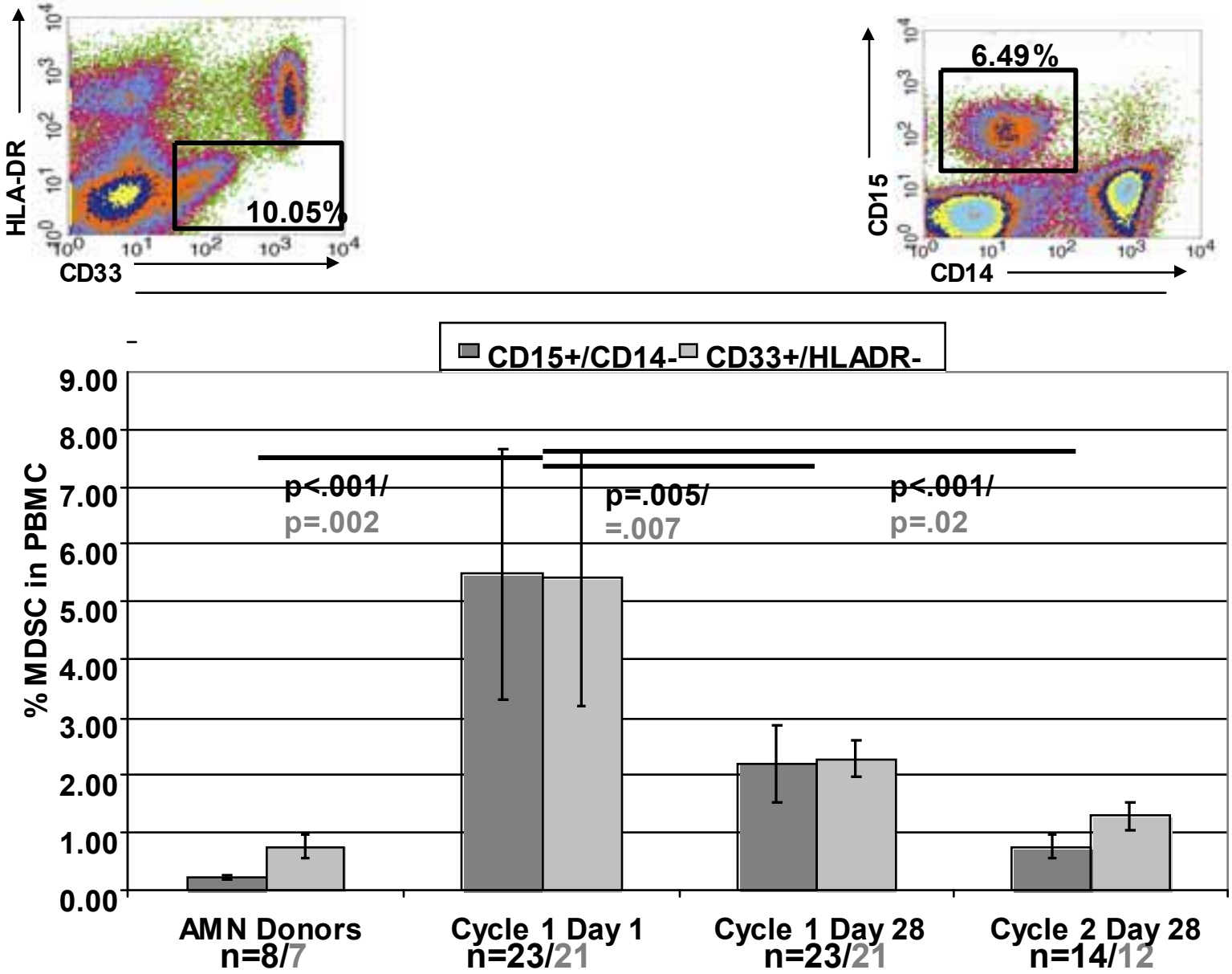
- Sunitinib-induced myeloid linage redistribution in RCC patients: CD1c dendritic cell frequency predicts progression-free survival. Van Cruijsen H. Clinical Cancer Research 2008

- The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and Modulation of tumor microenvironment for immune-based cancer therapies. Ozao-Choy J et al Cancer Research 2009

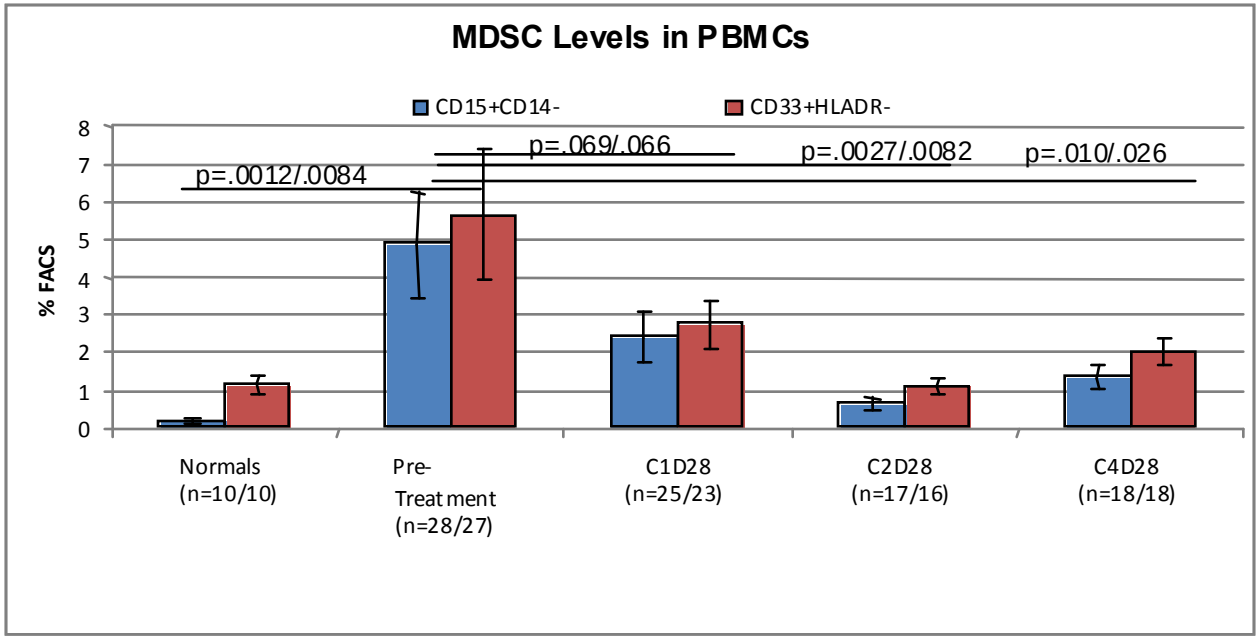
- Sunitinib inhibition of stat2 induces renal cell carcinoma tumor cell apoptosis and reduces Immune suppression. Xin H. et al Cancer Research 2009

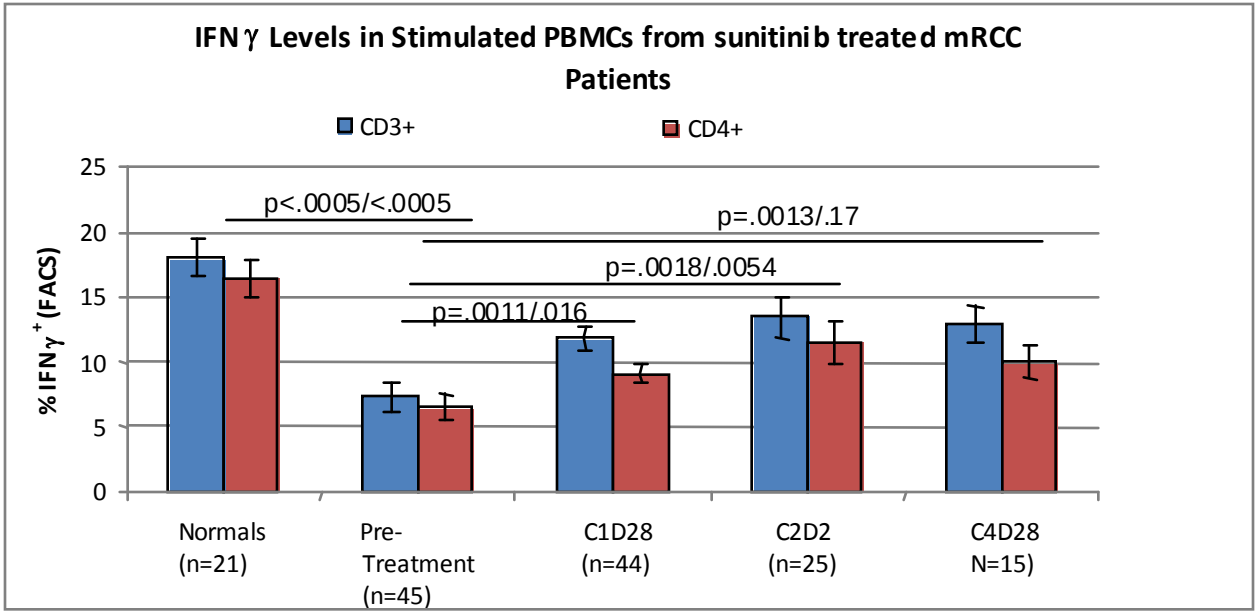
- Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell patients carcinoma Ko J et al Clinical Cancer Research 2009

Sunitinib reverses MDSC accumulation in mRCC patients

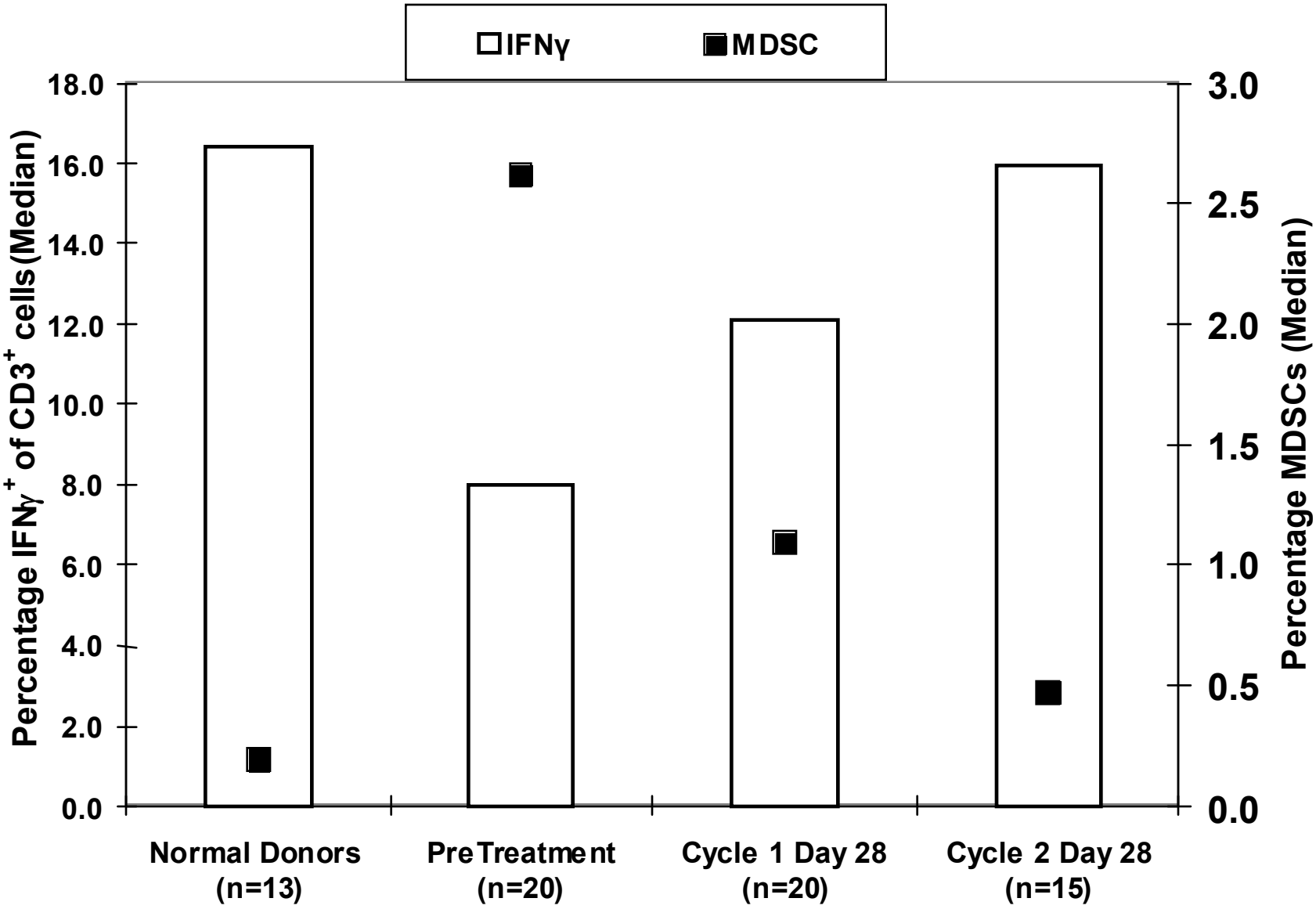


-Ko, JS. et. al. Clin Cancer Res. 2009 Mar 15;15(6):2148-57. Epub 2009 Mar 10.

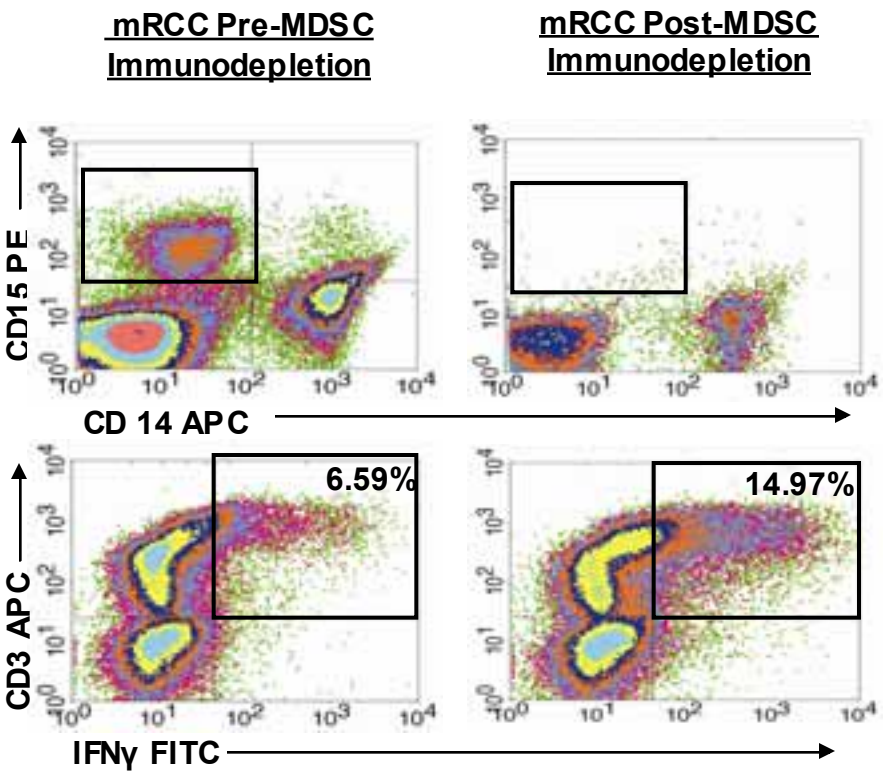

MDSC Levels in PBMCs
CD15+CD14-
CD33+HLADR-
% FACS
8
7
6
5
4
3
2
1
0
p=.0012/.0084
p=.069/.066
p=.0027/.0082
p=.010/.026
Normals (n=10/10)
Pre-Treatment (n=28/27)
C1D28 (n=25/23)
C2D28 (n=17/16)
C4D28 (n=18/18)

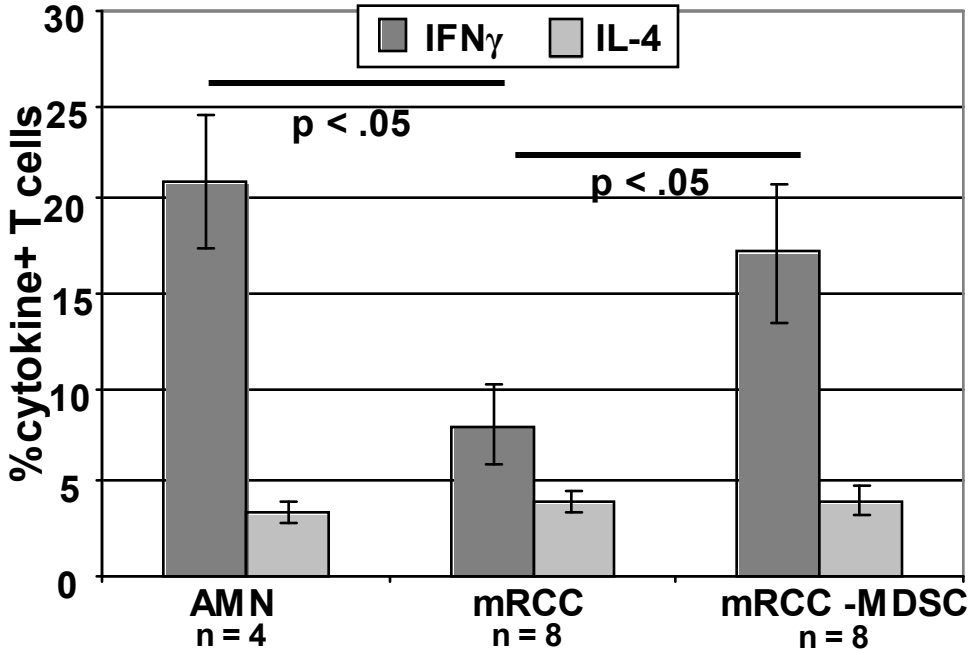

IFNγ Levels in Stimulated PBMCs from sunitinib treated mRCC Patients
CD3+
CD4+
% IFNγ+ (FACS)
25
20
15
10
5
0
p<.0005/<.0005
p=.0013/.17
p=.0018/.0054
p=.0011/.016
Normals (n=21)
Pre-Treatment (n=45)
C1D28 (n=44)
C2D2 (n=25)
C4D28 N=15)

MDSC decline is associated with T effector IFNγ recovery with Sunitinib Treatment

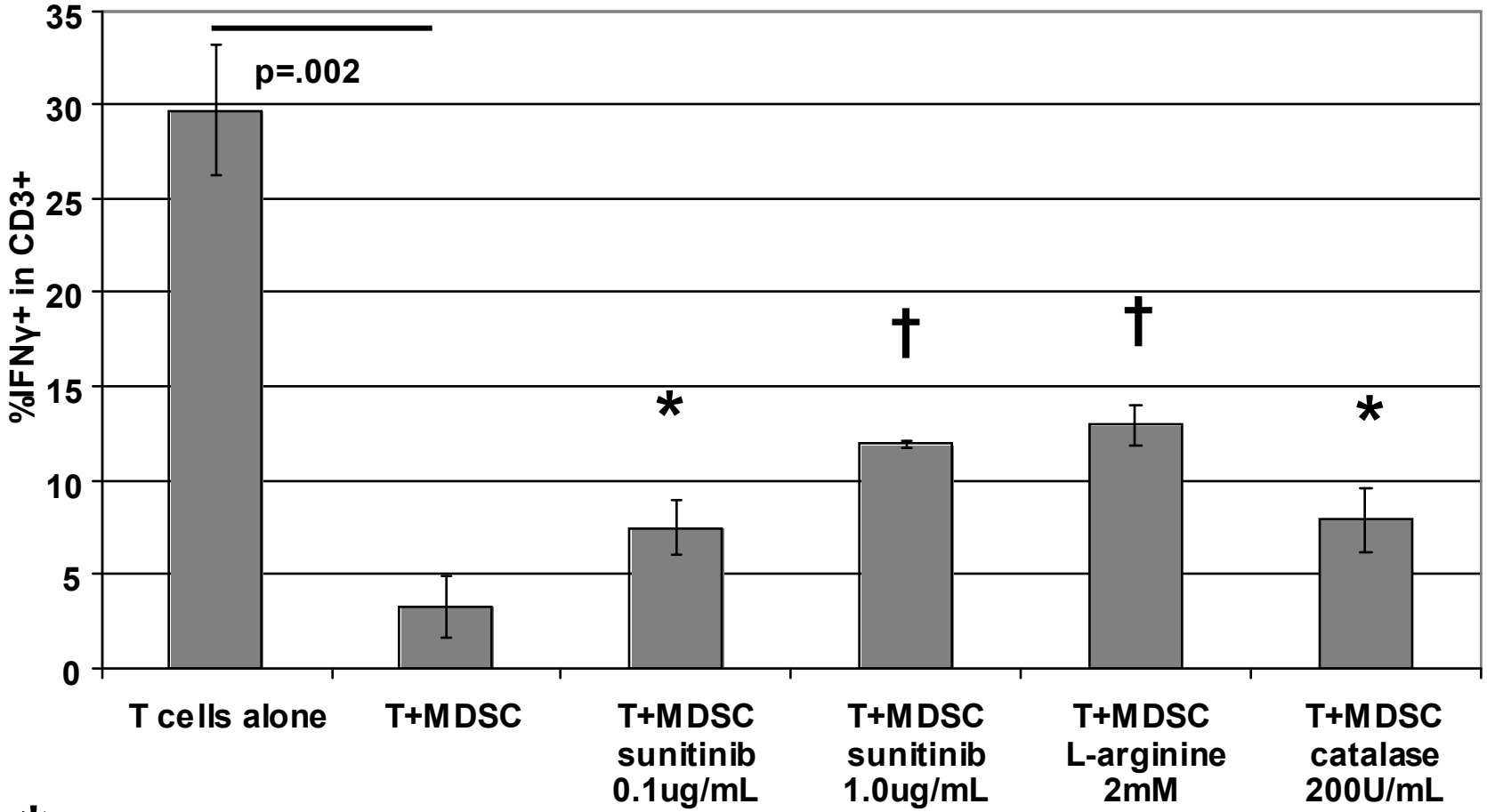


Sunitinib-meditated improvements in T cell function are reproduced by *in vitro* MDSC depletion





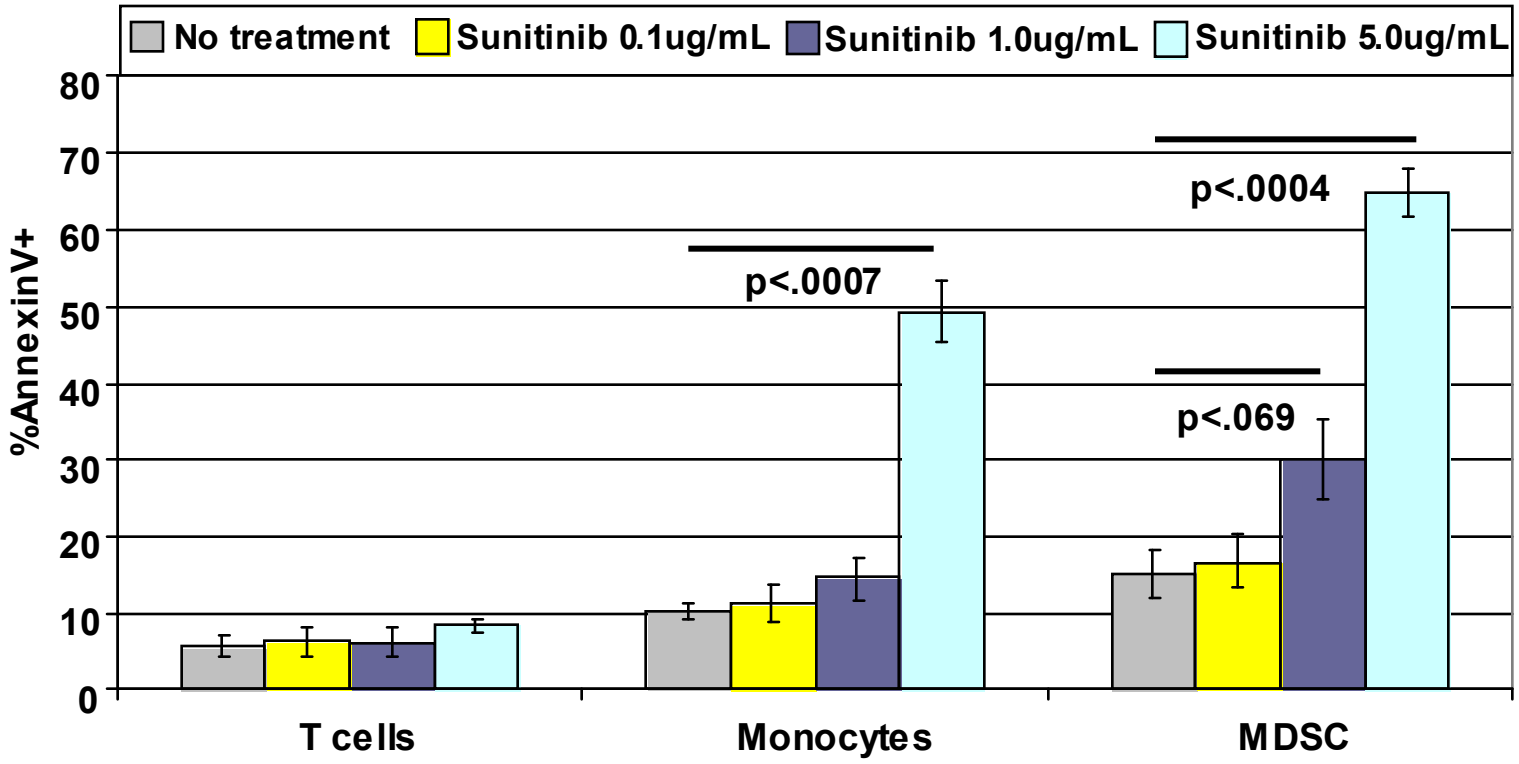
Sunitinib modulates MDSC suppressive effect *in vitro*



* p<0.12 versus T+MDSC

† p<.007 versus T+MDSC

Sunitinib induces myelo-specific apoptosis and patient MDSC-specific apoptosis at 1ug/mL



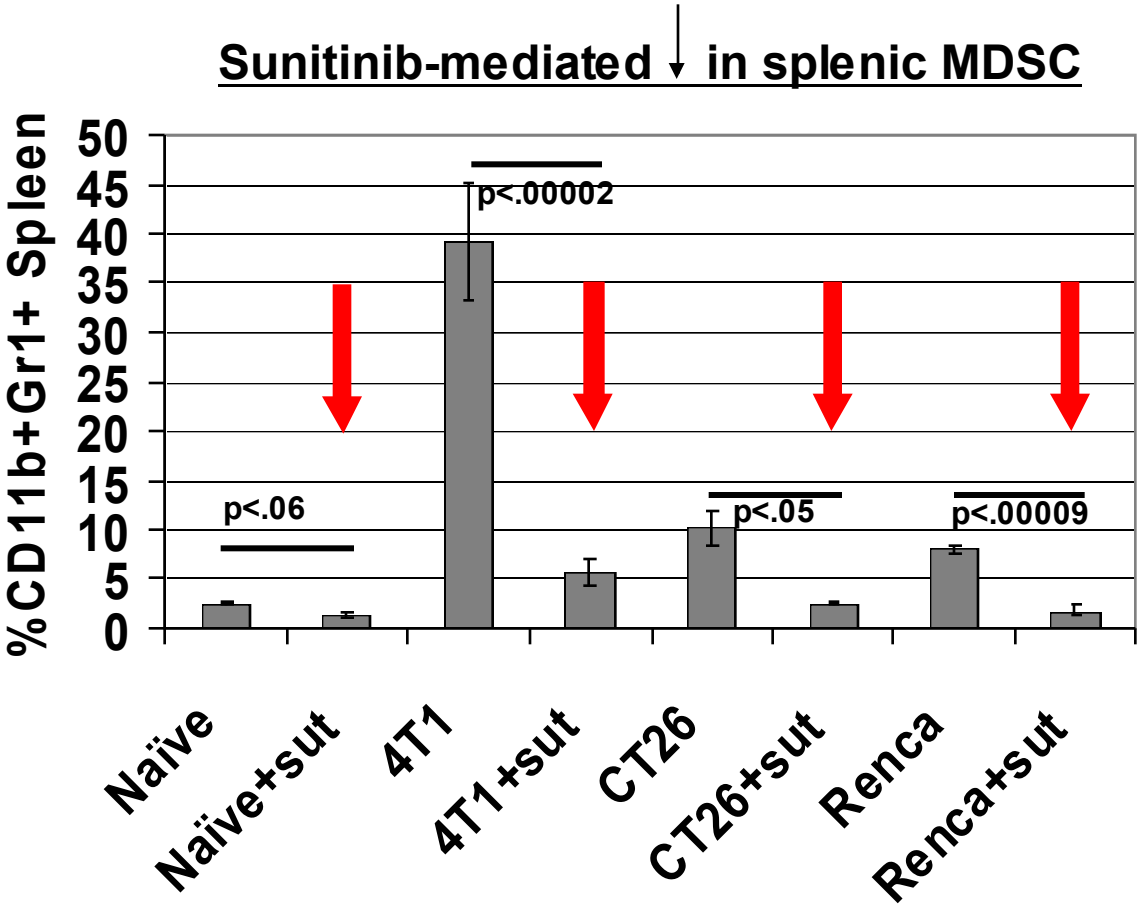
Conclusions:

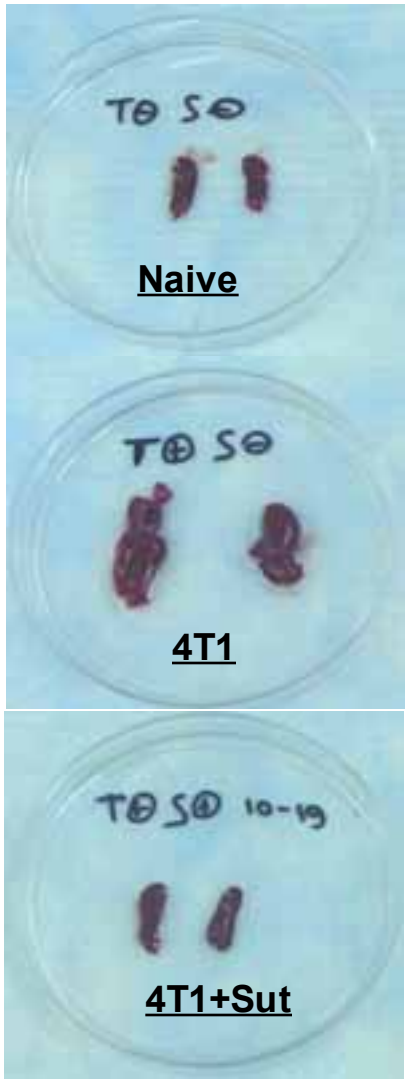
- **Sunitinib mediates reversal of MDSC accumulation in RCC patients and thereby restores patient T cell function.**

- **Sunitinib has a toxic, rather than DC-differentiating effect on RCC patient MDSC in vitro, which may account for its partial inhibition of MDSC suppressive effect in vitro.**

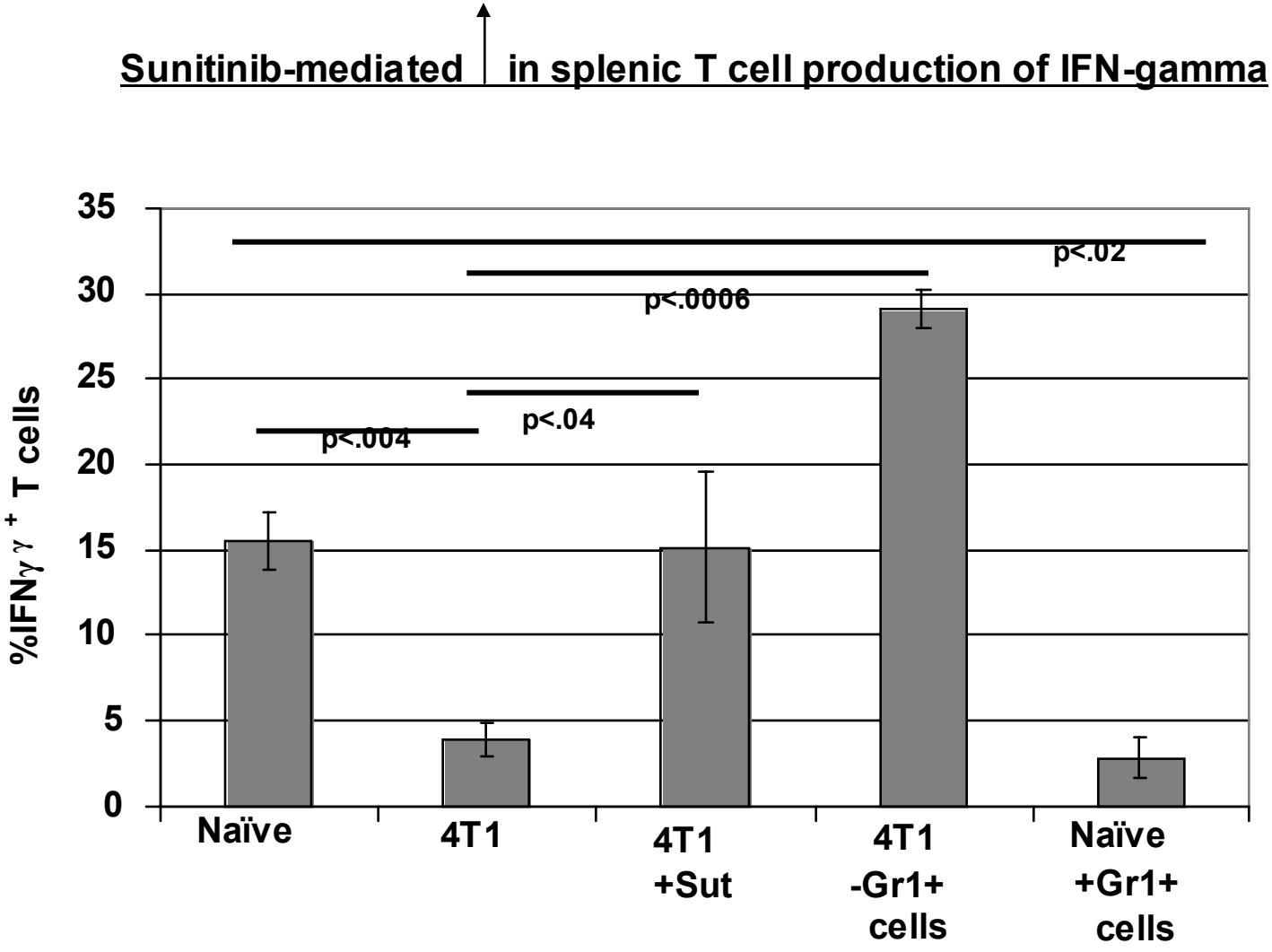
- **Sunitinib-mediated MDSC declines in RCC patients were not correlated with changes in tumor volume.**

Sunitinib significantly reverses MDSC-mediated immune suppression in mice bearing RCC and non-RCC tumors

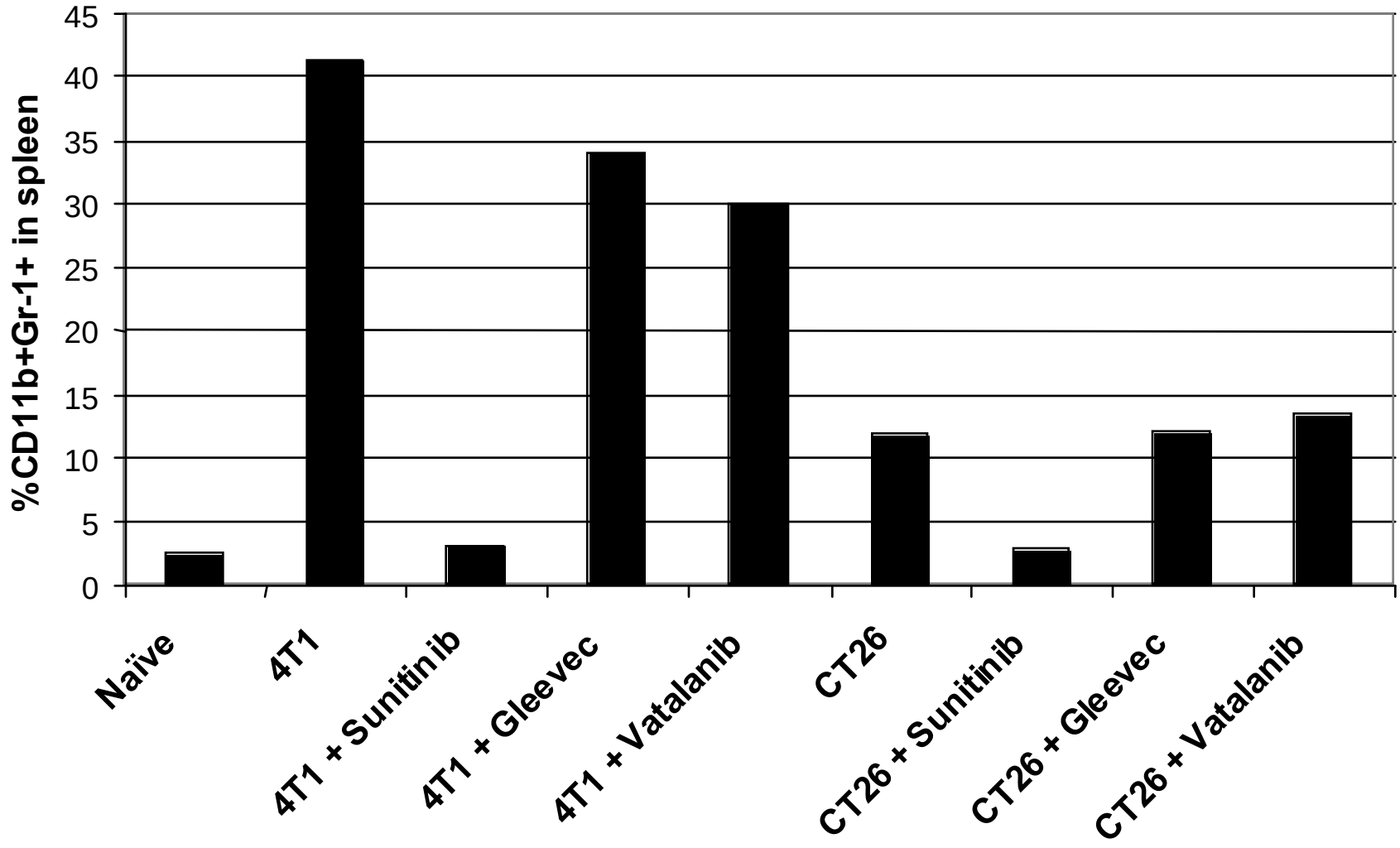




Sunitinib significantly reverses MDSC-mediated immune suppression in mice bearing RCC

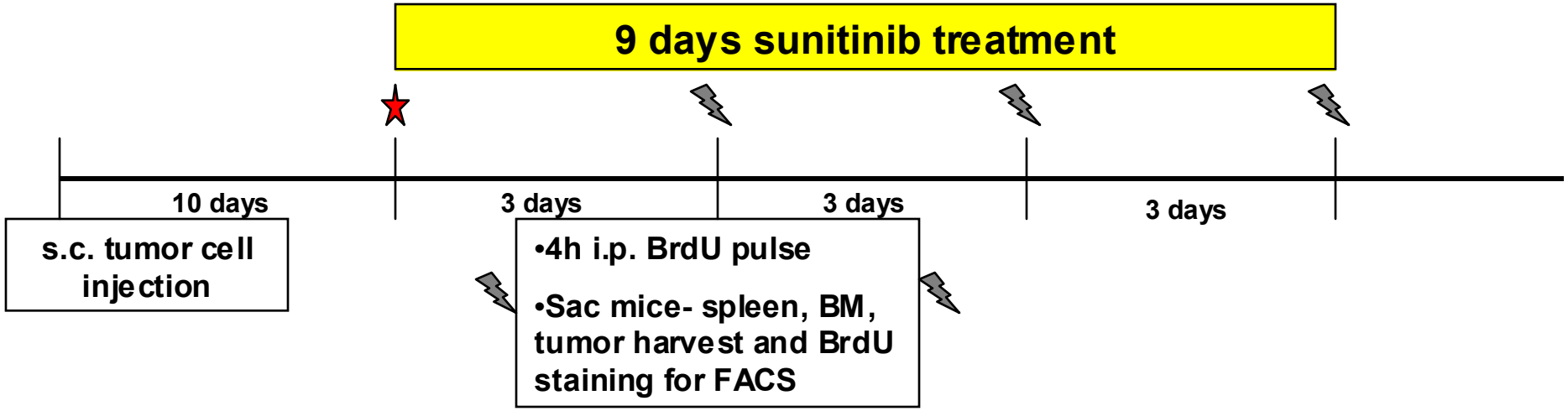


Sunitinib-mediated MDSC decline may not be attributed to single target



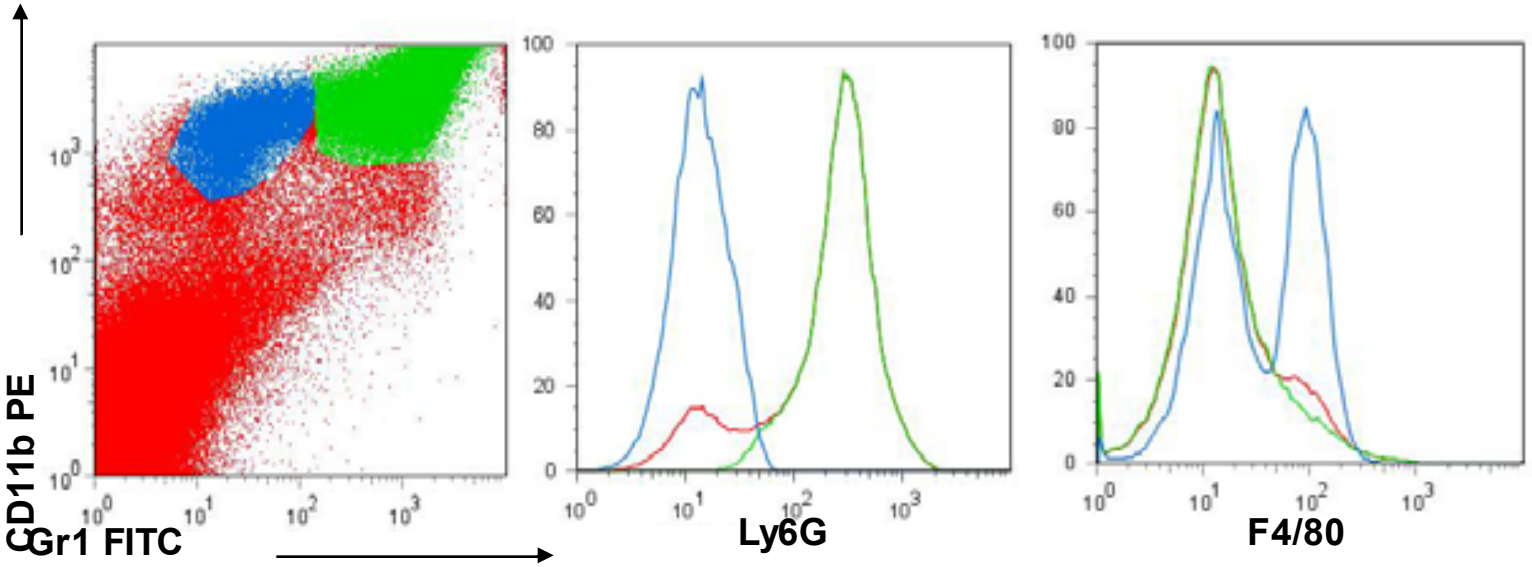
Method to evaluate sunitinib's impact on MDSC expansion in vivo

In vivo BrdU assay

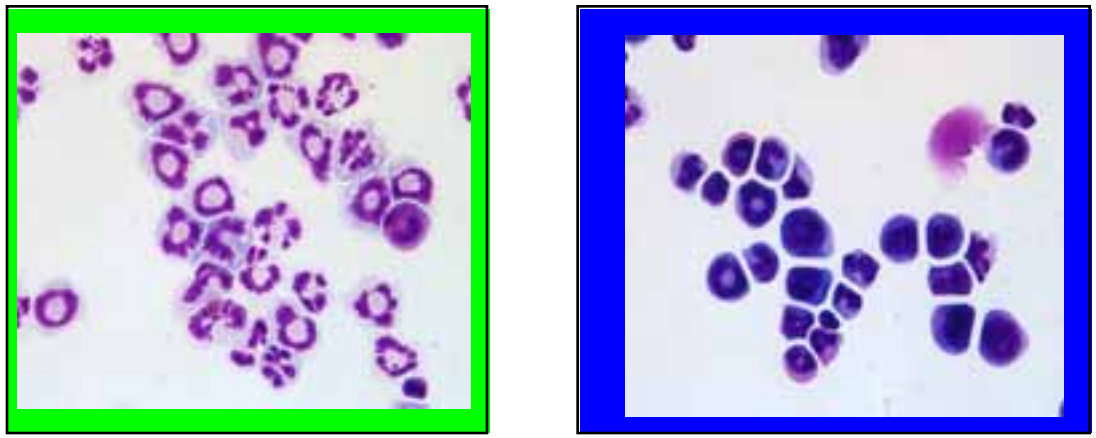


Ly6G^{hi} Neutrophilic MDSC are Gr1^{hi} and Ly6G^{lo} Monocytic MDSC are Gr1^{lo}

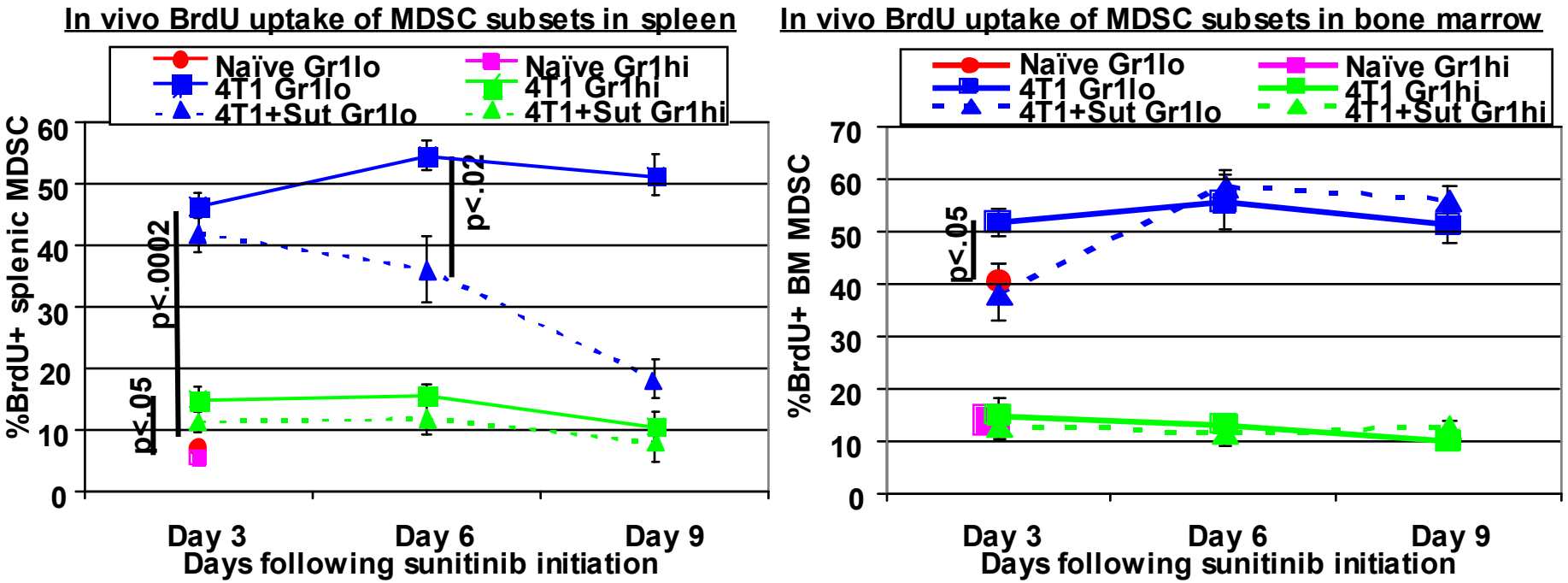
Gr1^{hi}N-MDSC= green, Gr1^{lo}M-MDSC= blue

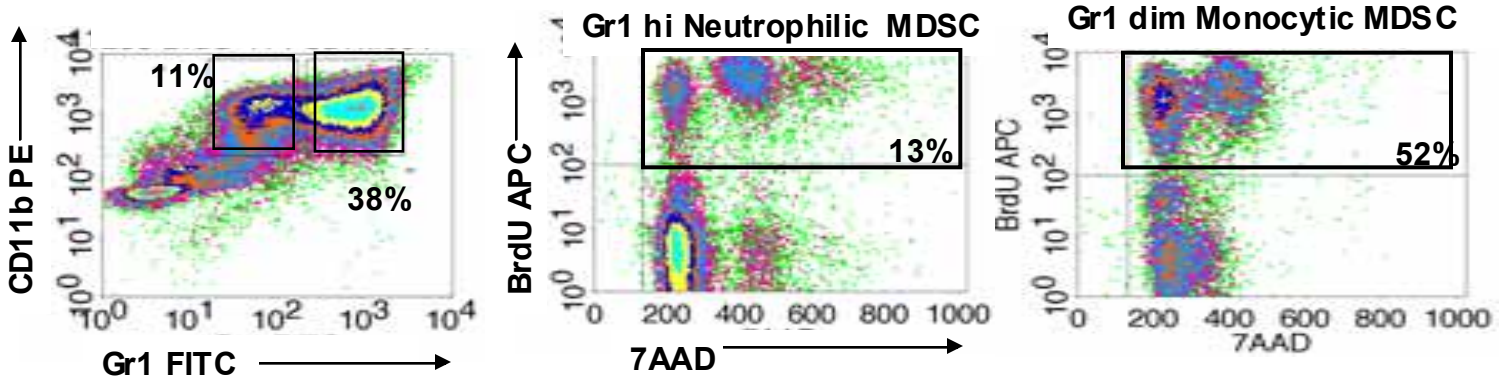


Gr1^{hi} N-MDSC=green Gr1^{lo} M-MDSC=blue

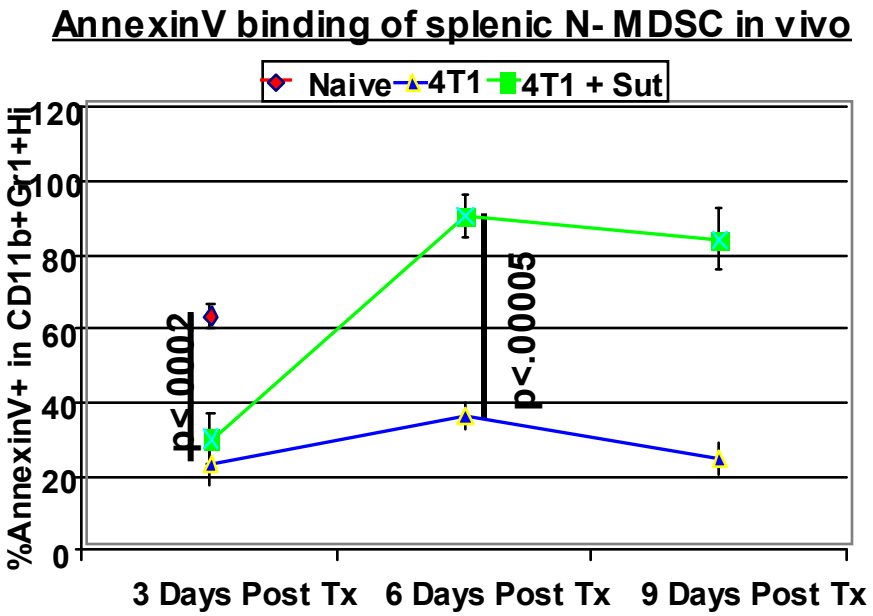


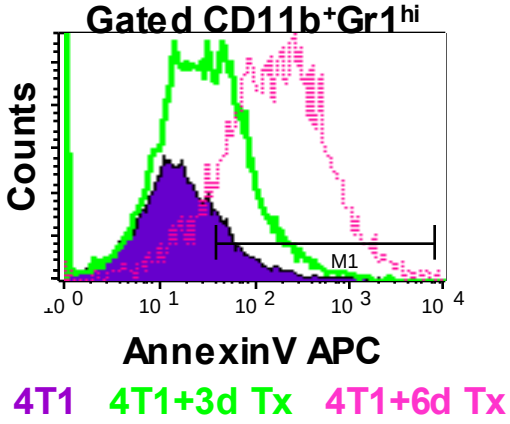
Sunitinib inhibits pathological proliferation of M-MDSC in the spleen but not in the bone marrow

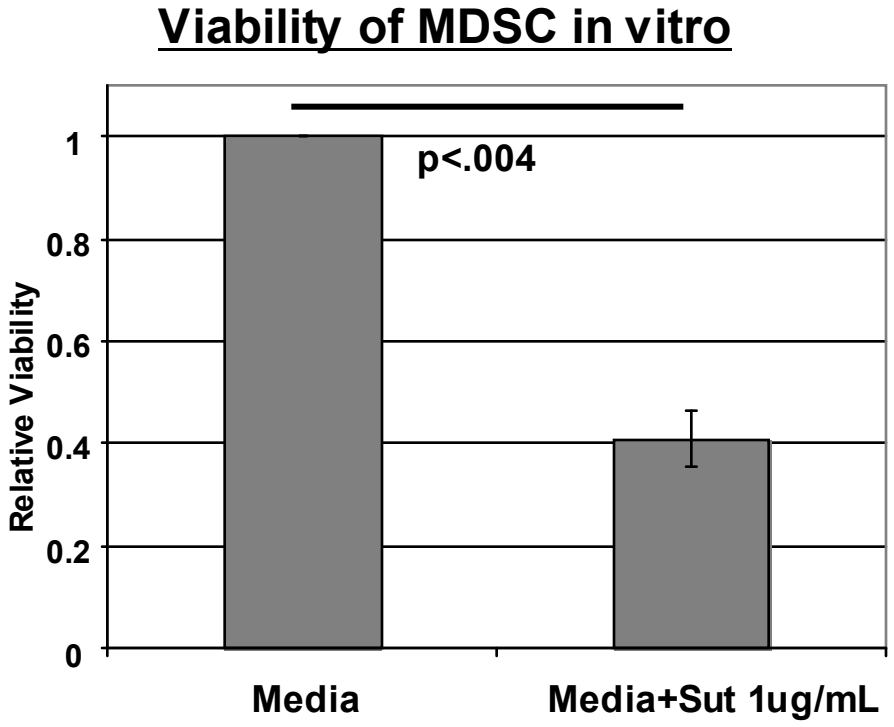




Sunitinib impairs N-MDSC viability in vivo and in vitro







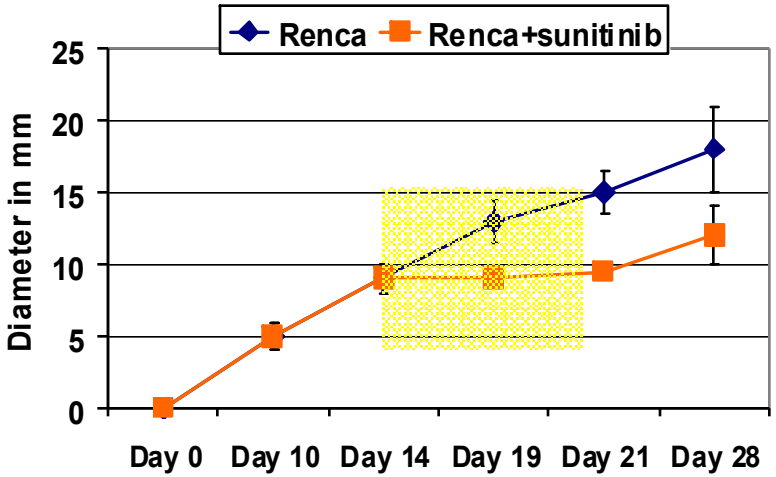
Conclusions:

- **Similar to the human studies, sunitinib treatment reduces MDSC levels and restored T cell response in several mouse tumor models.**

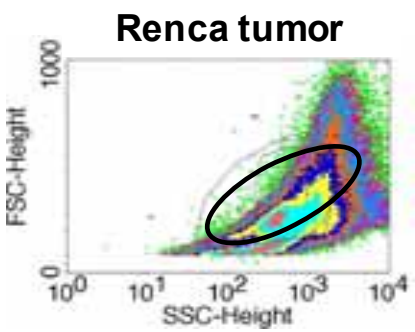
- **Sunitinib inhibits the pathological expansion in the spleen of proliferative Gr1lo M-MDSC.**

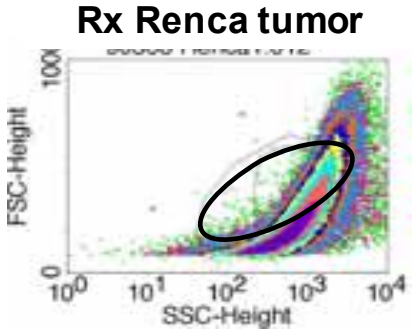
- **Sunitinib has an apoptotic, rather than DC-differentiating effect on N-MDSC.**

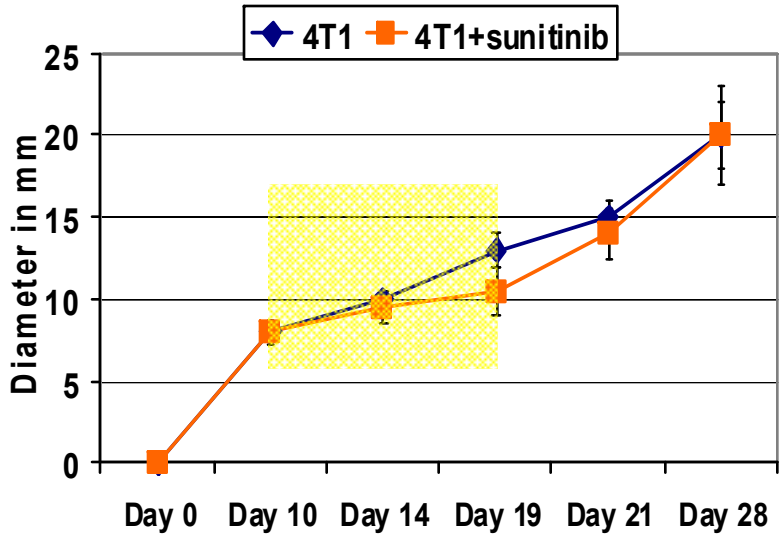
Sunitinib's impact on splenic MDSC is independent of sunitinib's demonstrable anti-tumor effect



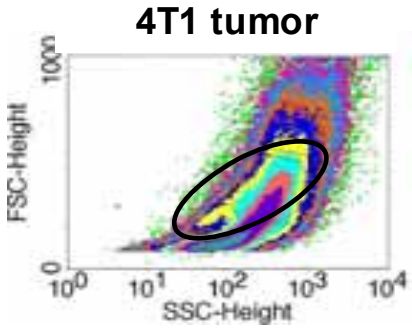
Renca tumors are extremely sensitive in vivo

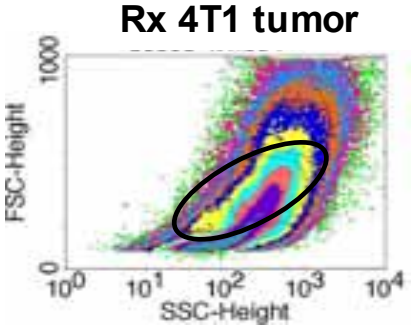






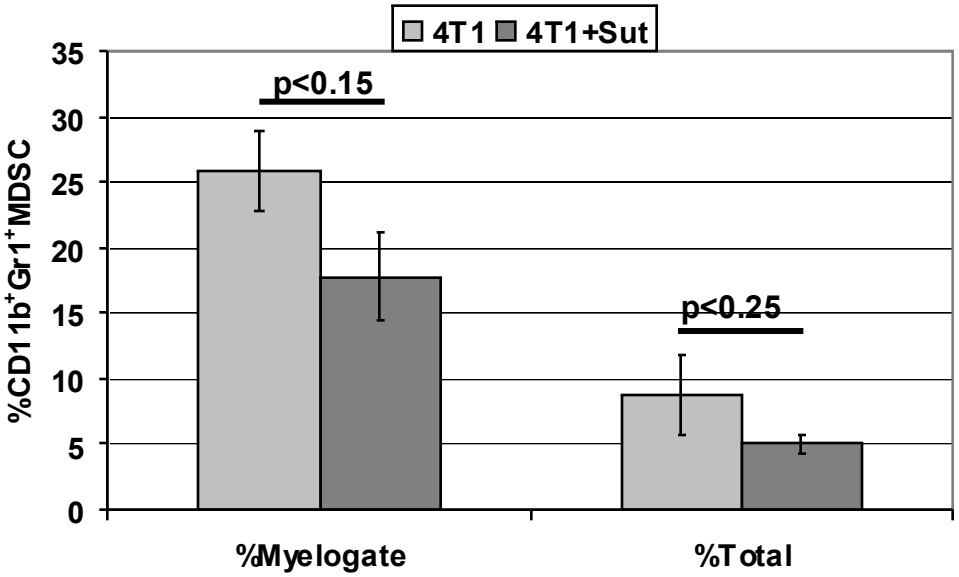
4T1 tumors are relatively insensitive in vivo

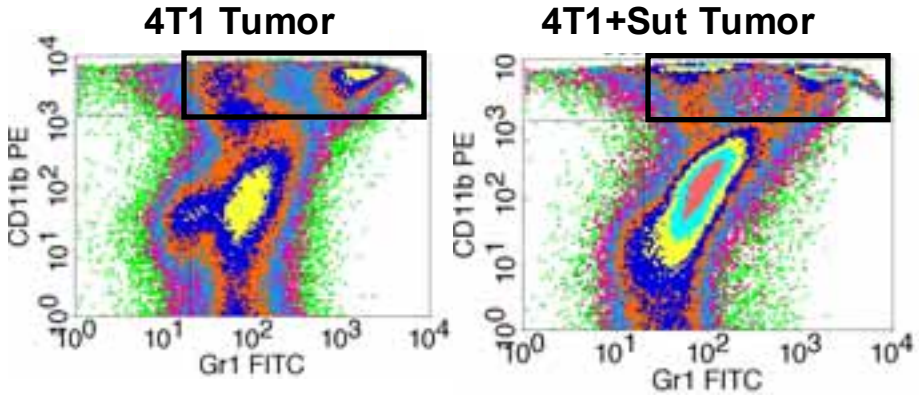




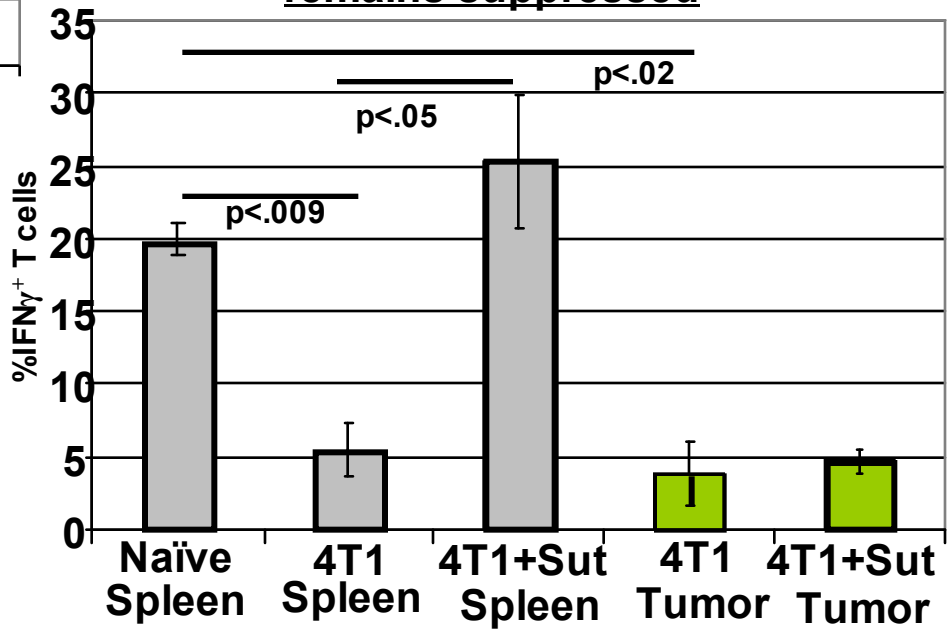
Tumor-associated MDSC in resistant 4T1 tumor model are relatively resistant to sunitinib

Mild ↓ in tumor bed MDSC in treated 4T1 mice

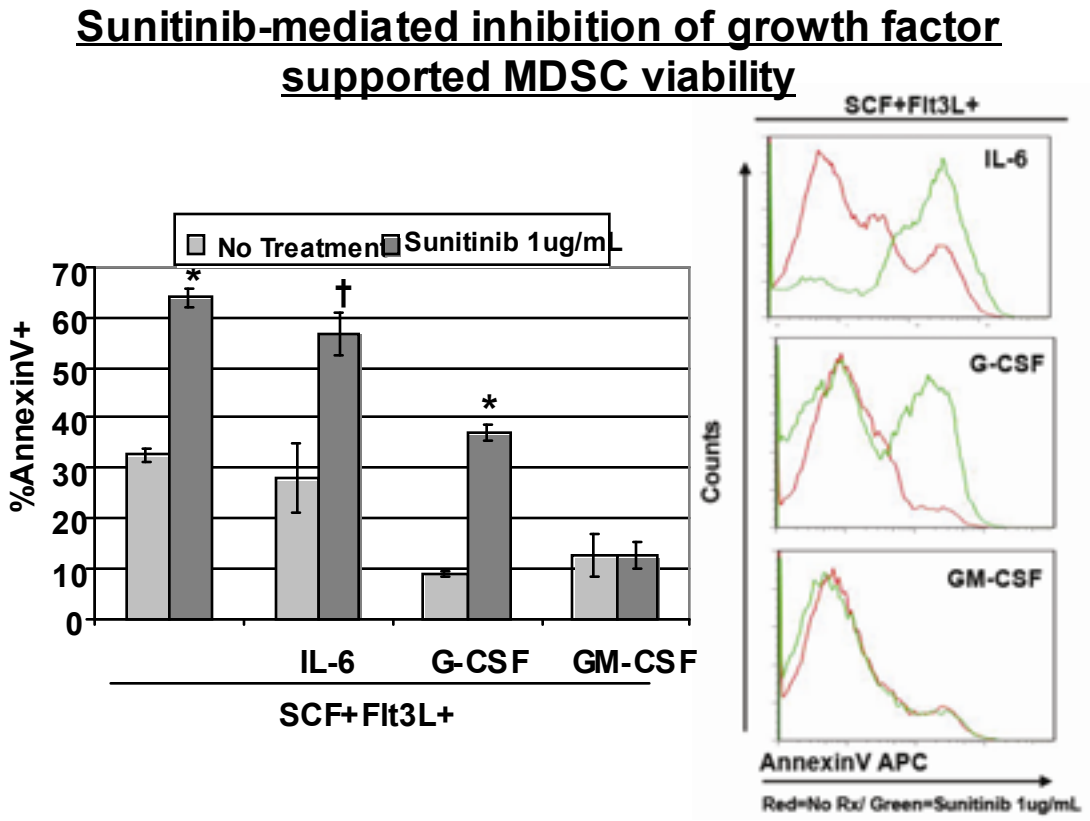




Type 1 function of Tumor Infiltrating T cells remains suppressed

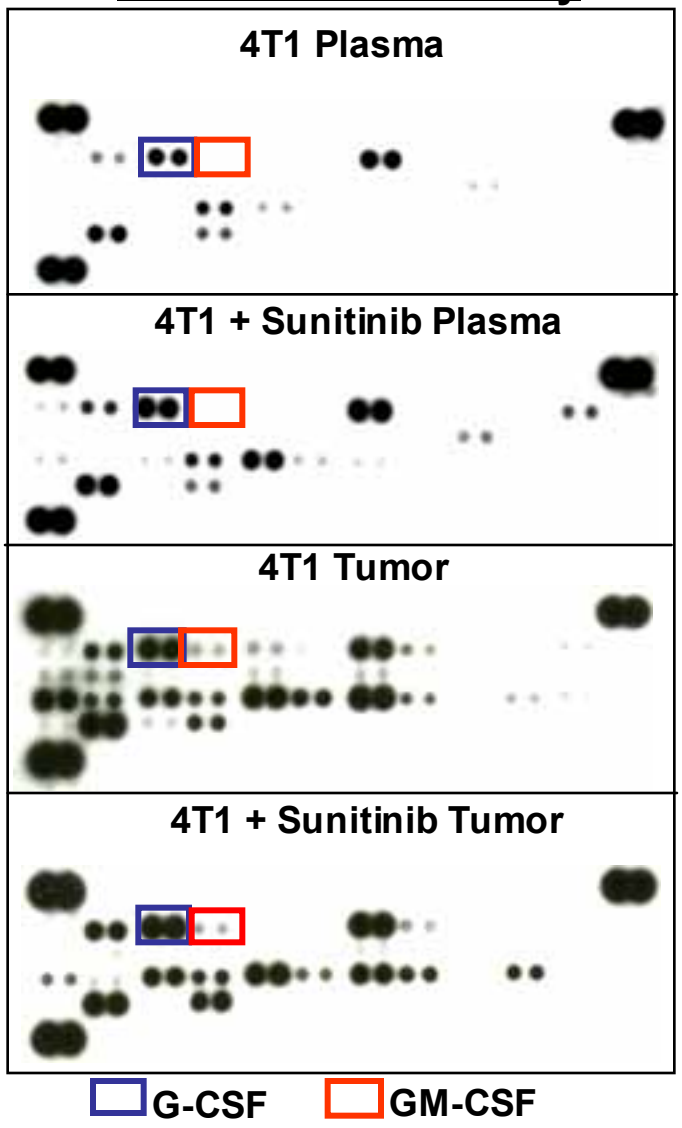


GM-CSF uniquely protects MDSC in the presence of sunitinib

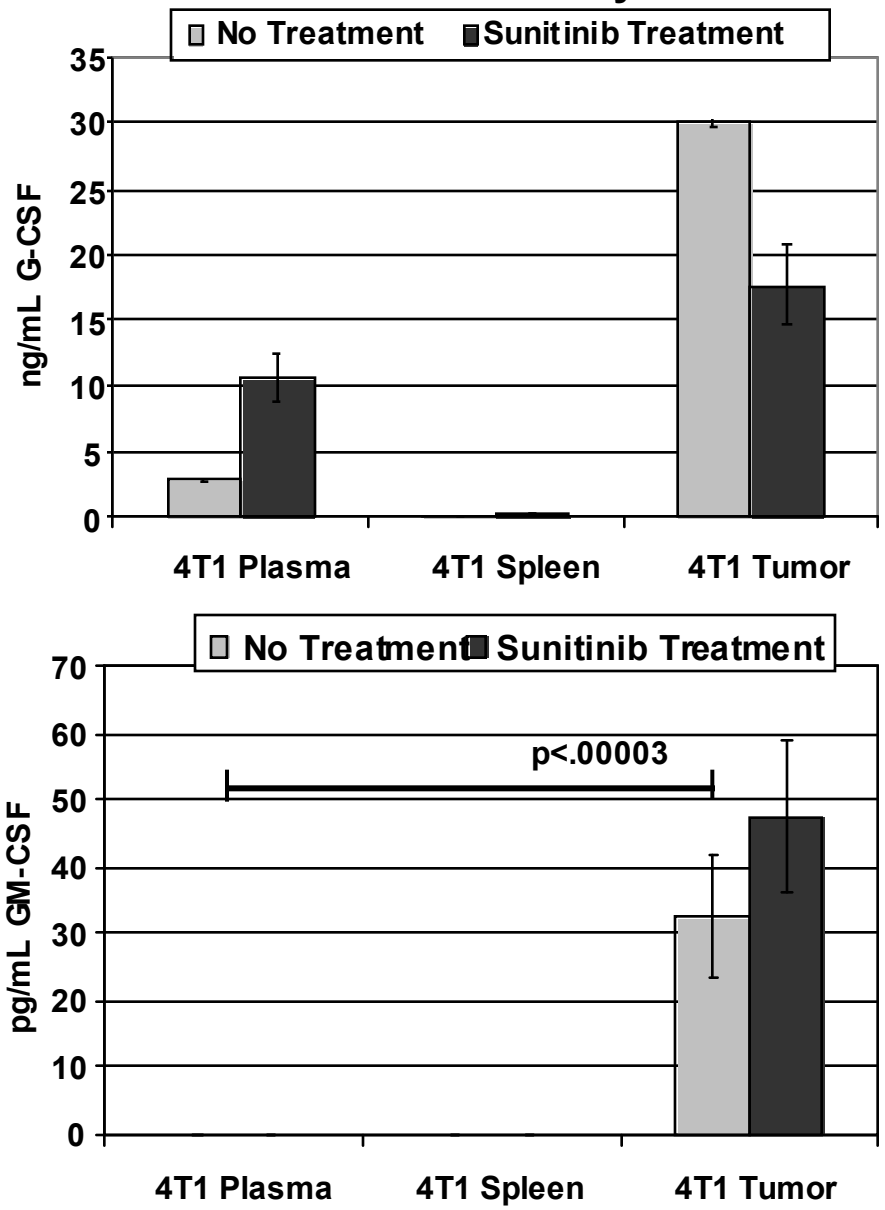


GM-CSF is selectively expressed in tumor microenvironment *in vivo*

Proteome Profile Array



Luminex Array



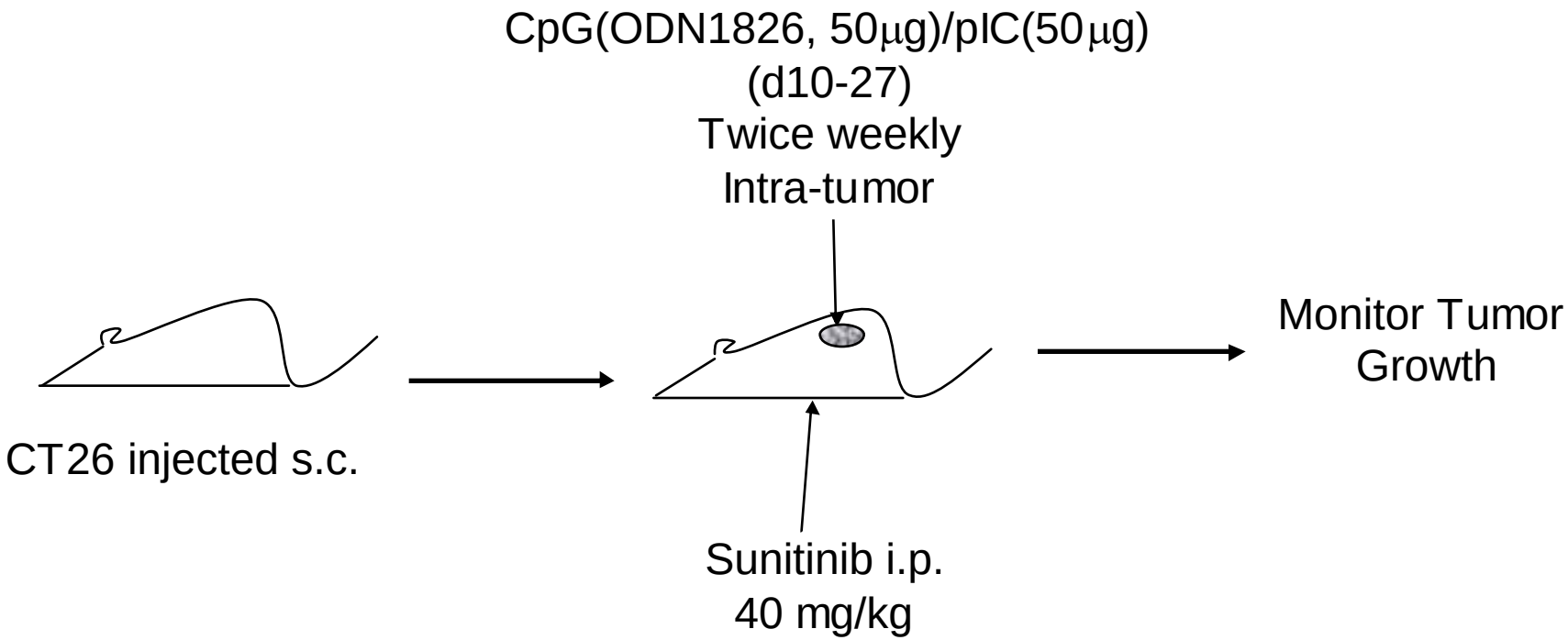
Conclusions:

- **Sunitinib-mediated MDSC declines in RCC patients and TB-mice are direct and independent of anti-tumor effects or consequent changes in cytokines.**

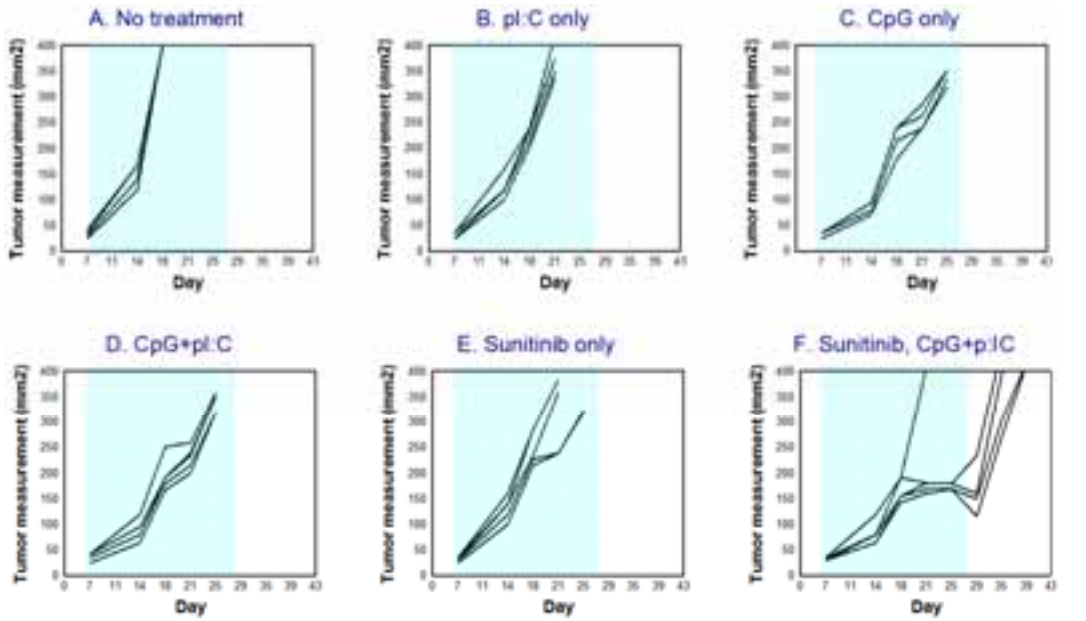
- **GM-CSF may mediate intratumoral resistance to sunitinib in RCC patients and TB-mice.**

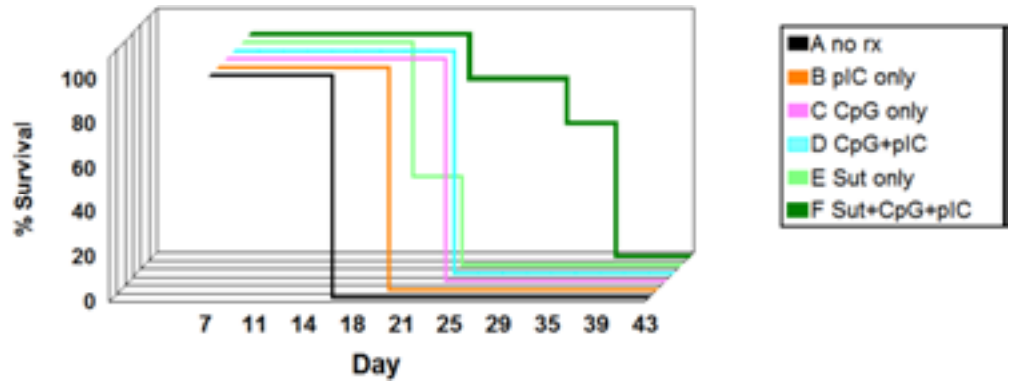
Combining Sunitinib with Immunotherapy

Peter Cohen MD, Mayo Clinic Arizona


CpG(ODN1826, 50μg)/pIC(50μg)
(d10-27)
Twice weekly
Intra-tumor
Monitor Tumor
Growth
CT26 injected s.c.
Sunitinib i.p.
40 mg/kg

Significantly Improved Survival is Associated With Combined Sunitinib and Immunotherapy (CpG and pIC)





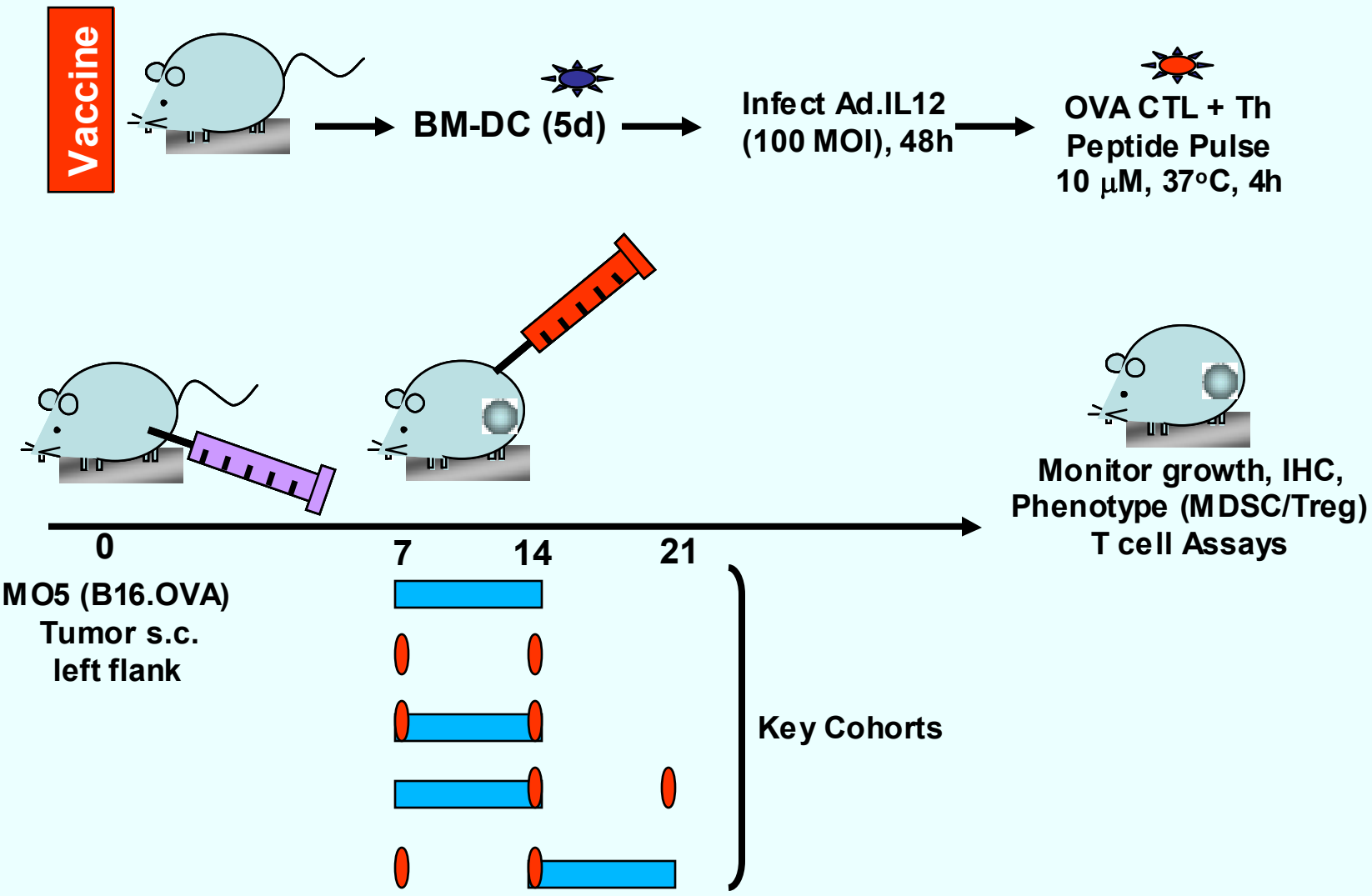
Peter Cohen MD, Mayo Clinic Arizona

Collaborators at University of Pittsburgh Medical Center

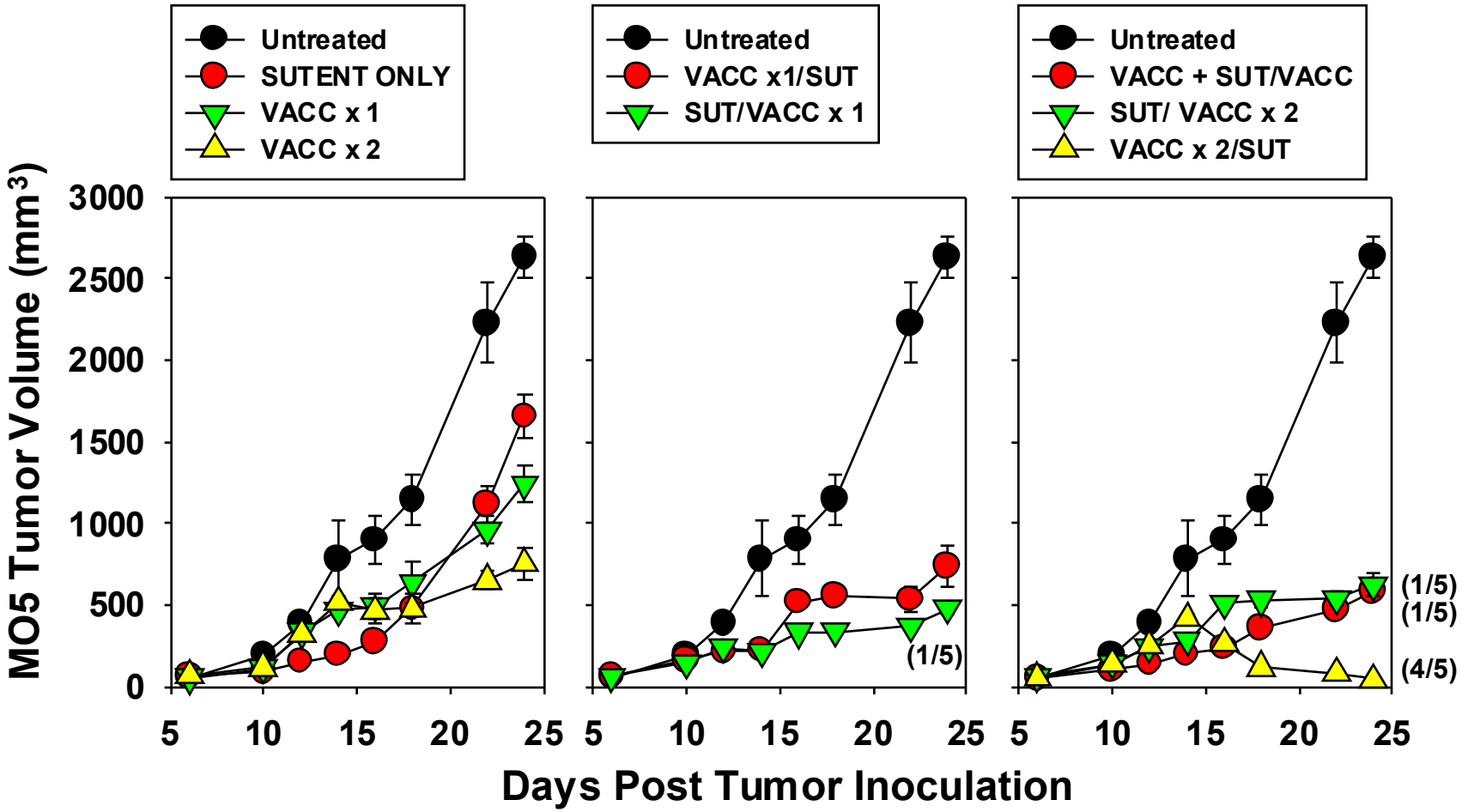
Anamika Bose, Ph.D.

Walt Storkus, Ph.D.

Combination Therapy Design

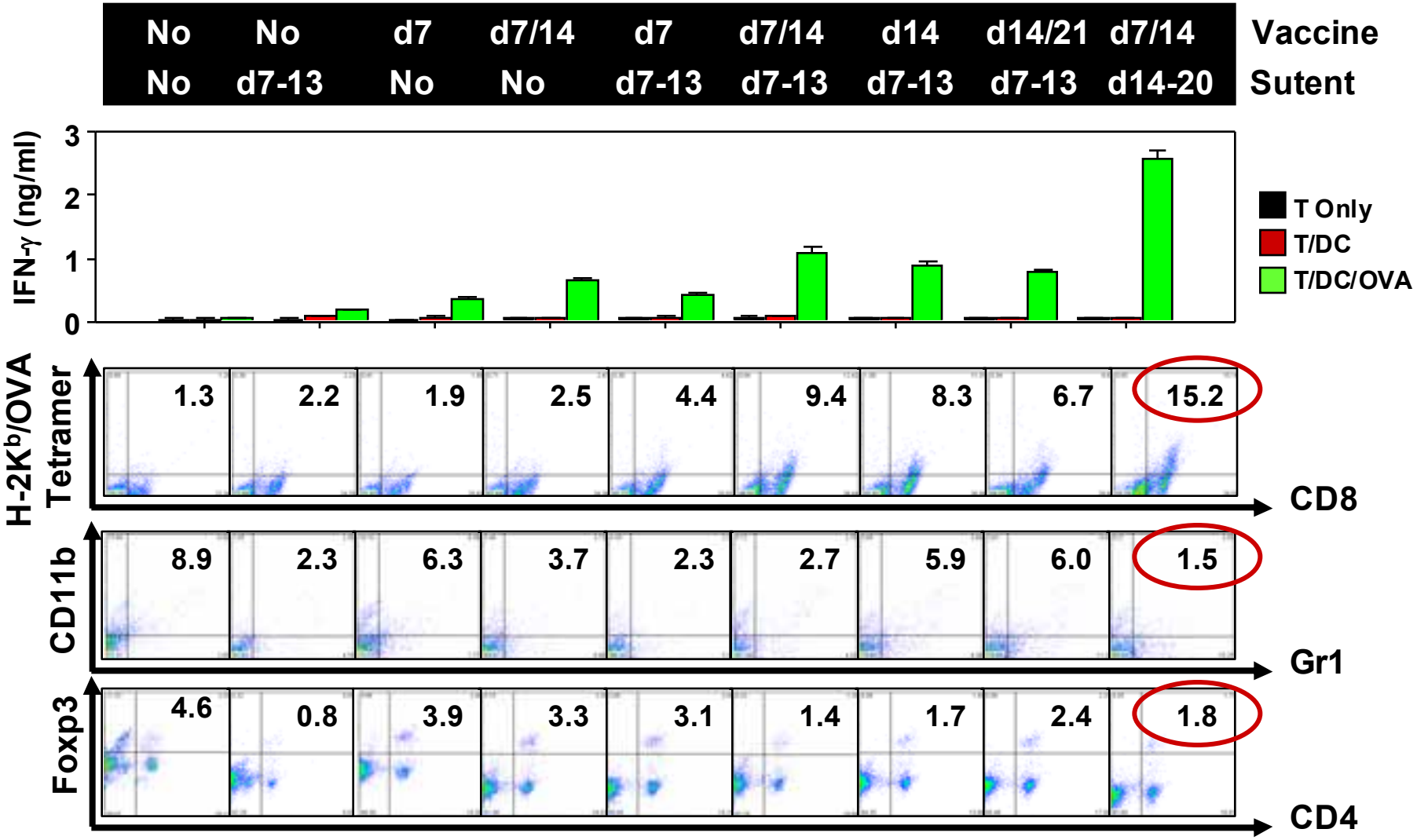


Combination vs. Single Modality Therapy of Day 10 Established M05 (B16.OVA) Melanomas with Sutent and Specific Vaccination



(number tumor-free mice day 24)

SUTENT +/- Vaccine Immunomonitoring: TIL (d24)



Conclusions

- **SUTENT/sunitinib improves anti-tumor efficacy when combined with specific immunization as a combinational therapy.**

- **Combinational therapy associated with reductions in MDSC and Treg frequencies in the TME**

- **Therapeutic benefits correlated with vaccine-induced CD8+ TIL frequencies (tetramer)**

Combinational Therapy

- Sutent, 50mg/daily

- Vaccinate with Type-1-polarized dendritic cell s(aDC-1) loaded with a mixture of 3 RCC-associated T-helper peptide epitopes in HLA-DR4+ patients with mRCC.

  $EphA2_{53\text{-}75}$, $G250_{249\text{-}268}$, $MAGE\text{-}6_{140\text{-}160}$

- RCC expression in situ: EphA2 (97%), G25 (85%+) and MAGE-3/6 (>80%)

- Evaluate changes in the magnitude and function polarization of RCC antigen-specific CD4+ (and CD8+) T cells in the peripheral blood.

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Lab Members

Dr. Jennifer Ko (MD/PhD), Joanna Ireland, Patricia Rayman, Dr. Kausik Biswas (PhD), Soumika Biswas, Leticia Varella (MD), Cynthia Hilston, Yuntao Li.

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-Dr. Bukowski MD

-Dr. Charles Tannenbaum PhD