Modulation of Immune Effector and Regulator Cells by Sunitinib: Potential for Combination Therapy in Renal Cell Carcinoma Patients

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Outline

1) Modulation of Immune Cells by sunitinib in RCC patients

2) Sunitinib combined with vaccine in B16.OVA Tumor Model

3) Suppressive and angiogenic activity of G-MDSC and neutrophils

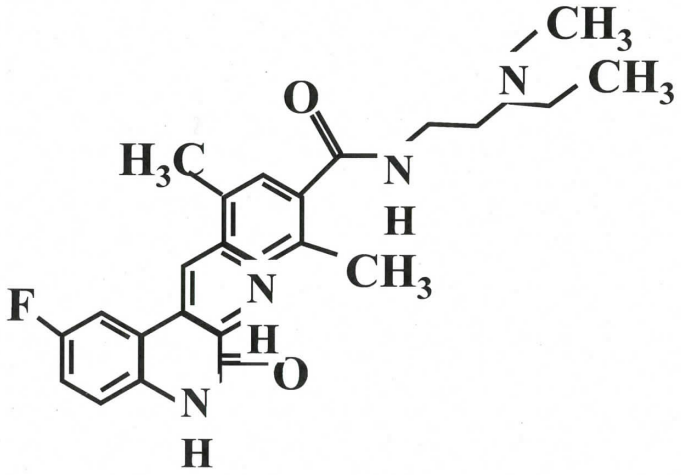
4) MDSC role in sunitinib resistance

Renal Cell Carcinoma (RCC)

- ~50,000 new cases in US/year with ~13,000 deaths
- Incidence rising >2.5%/year
- Renal cell carcinoma arising from the renal cortex is responsible for 80-85% of all kidney cancers.
- It is an aggressive tumor that often has spread beyond the kidney before being diagnosed.
- Most common histological form of RCC is clear cell (>85%)
- Common to have mutation/inactivation of the VHL tumor suppressor gene.
- Unresponsive to modern chemotherapy or radiotherapy
- Anti-angiogenic receptor tyrosine-kinase inhibitors (RTKIs) now first-line therapy
  - **sunitinib (Sutent)**
  - **sorafenib (Nexavar)**

Multitargeted Approaches in mRCC: Sunitinib (Sutent)

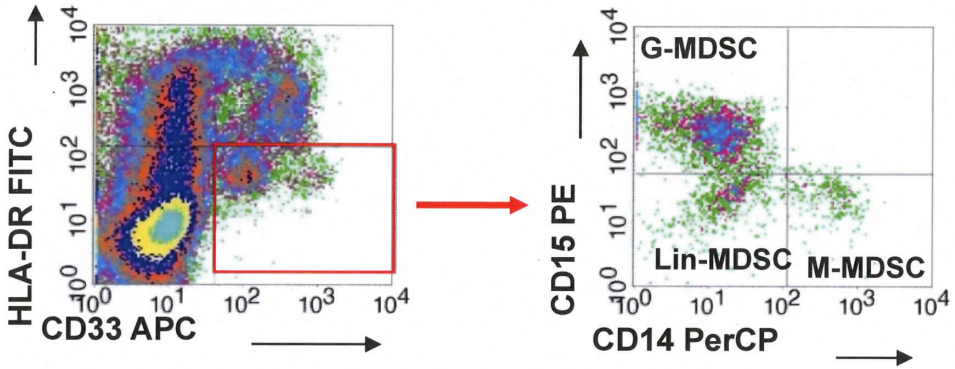
- Small-molecule receptor tyrosine kinase inhibitor[1]
- Inhibits all VEGFRs, PDGFR-A, PDGFR-B, c-KIT, and FLT-3[1]
- Oral administration[1]
- Both antitumor and antiangiogenic activity[1]
- FDA approved January 26, 2006 for treatment of advanced RCC[2]
- 45% response rate in mRCC



- Treatment schedule-50mg daily for 4 weeks + 2 weeks off = one cycle

MDSC Isolated from RCC Patient's Tumor

A.



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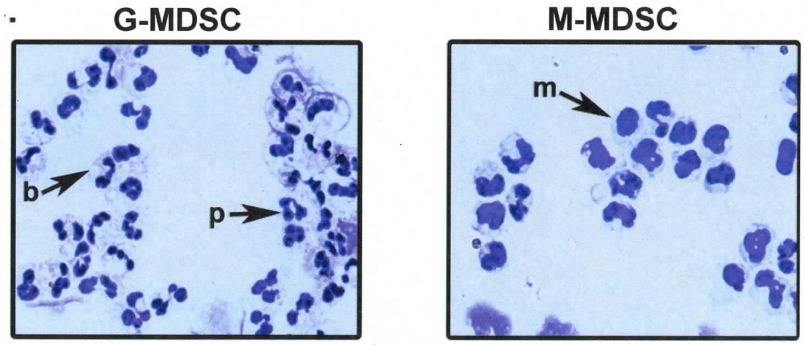
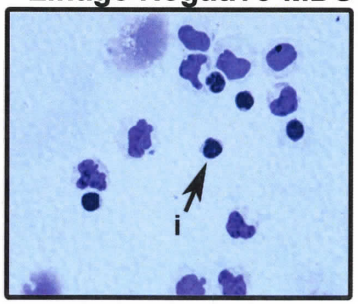
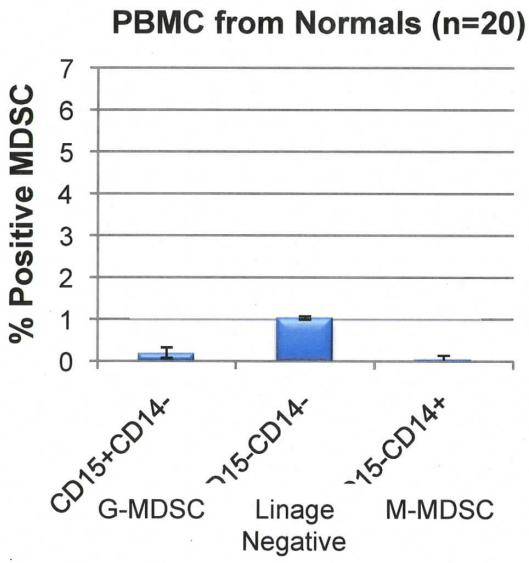


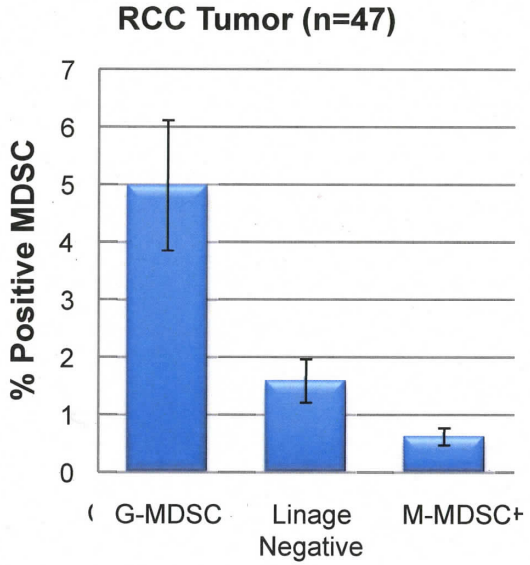
Linage Negative-MDSC

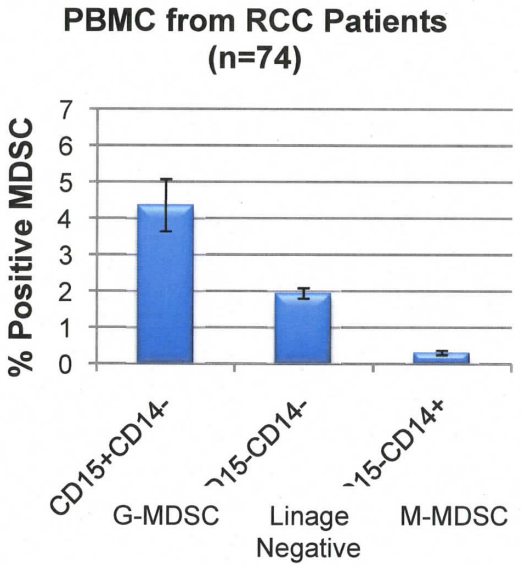


b= band cell
p= polymorphoneuclear
m=monocyte
i=immature

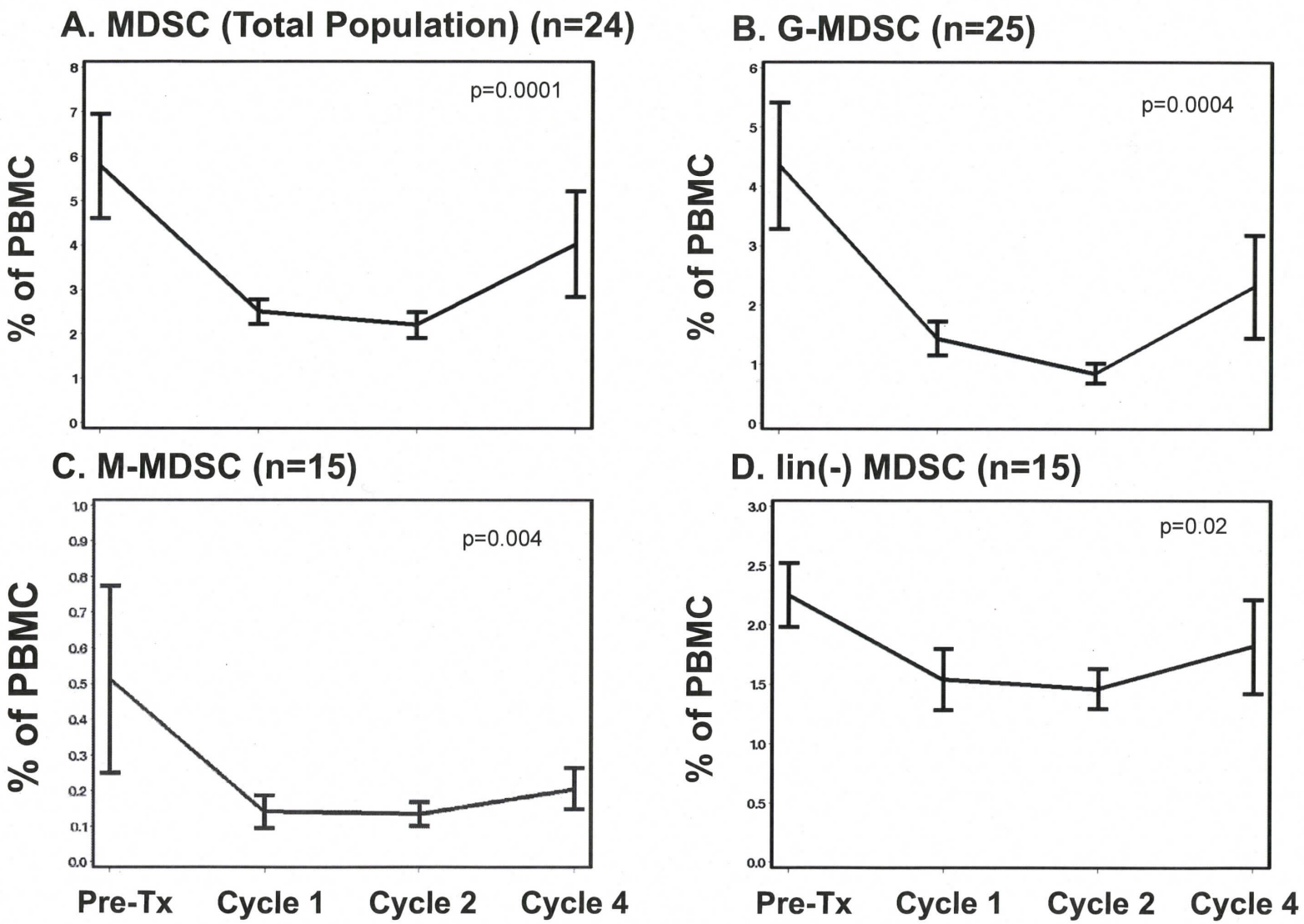
MDSC subsets RCC patients



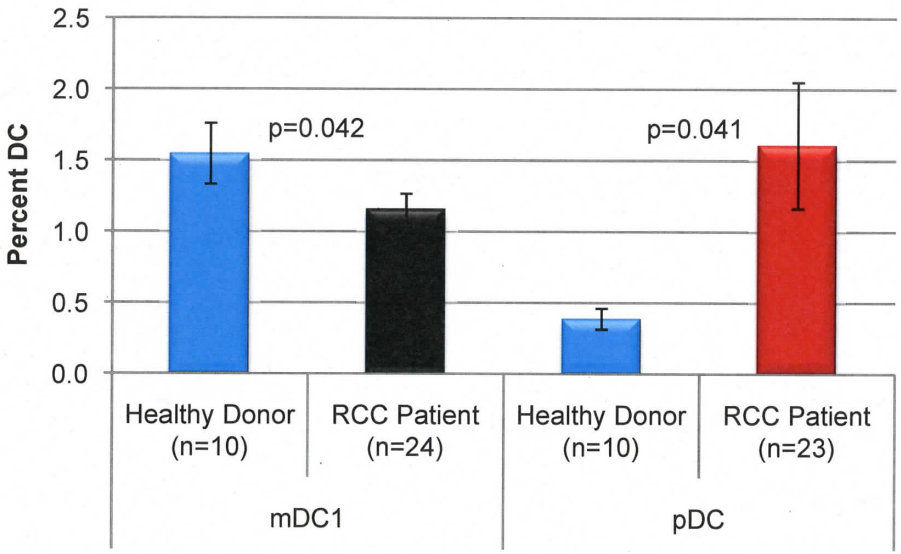




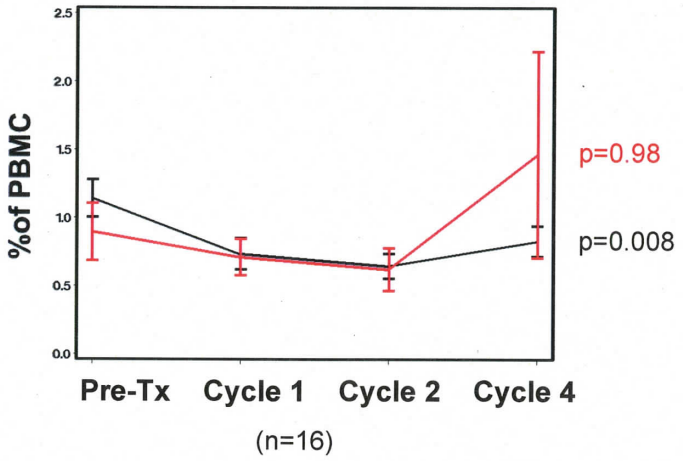
Changes in MDSC and MDSC Subpopulations Following 1, 2, and 4 Cycles of Sunitinib



Modulation of Myeloid DC by Sunitinib

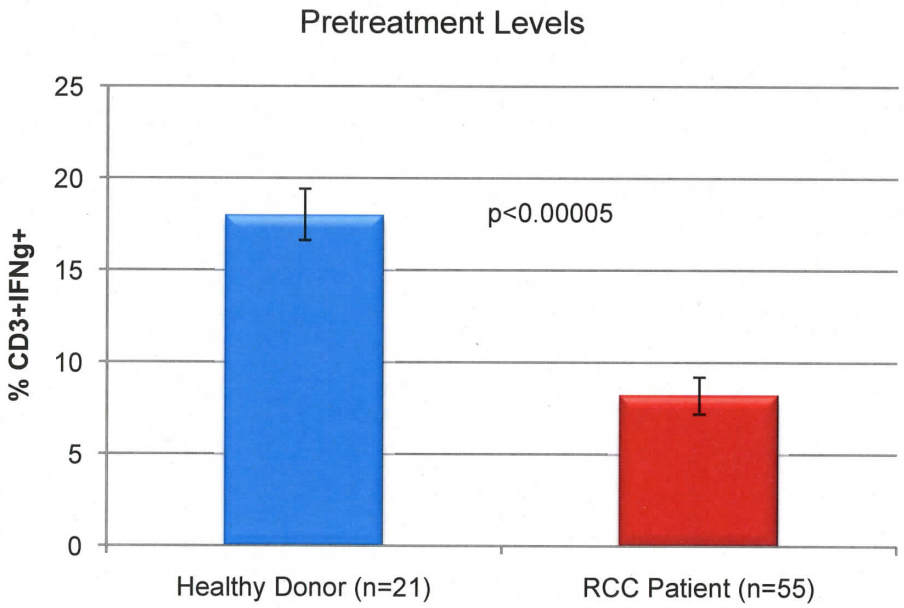


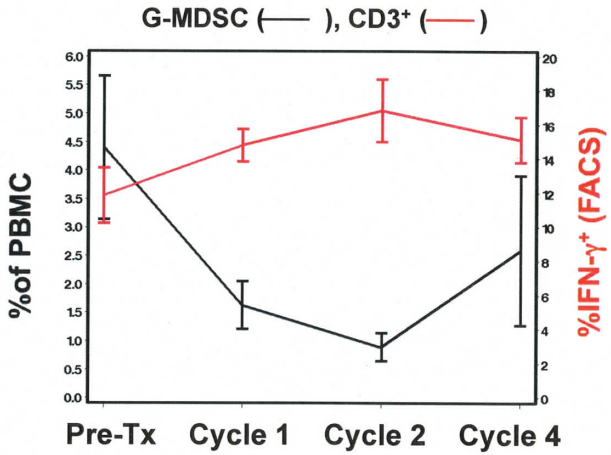
Myeloid DCs (CD1c)(BDCA1+CD19- (——),
Plasmacytoid DCs (pDC)BDCA2+CD14-BDCA1- (——)



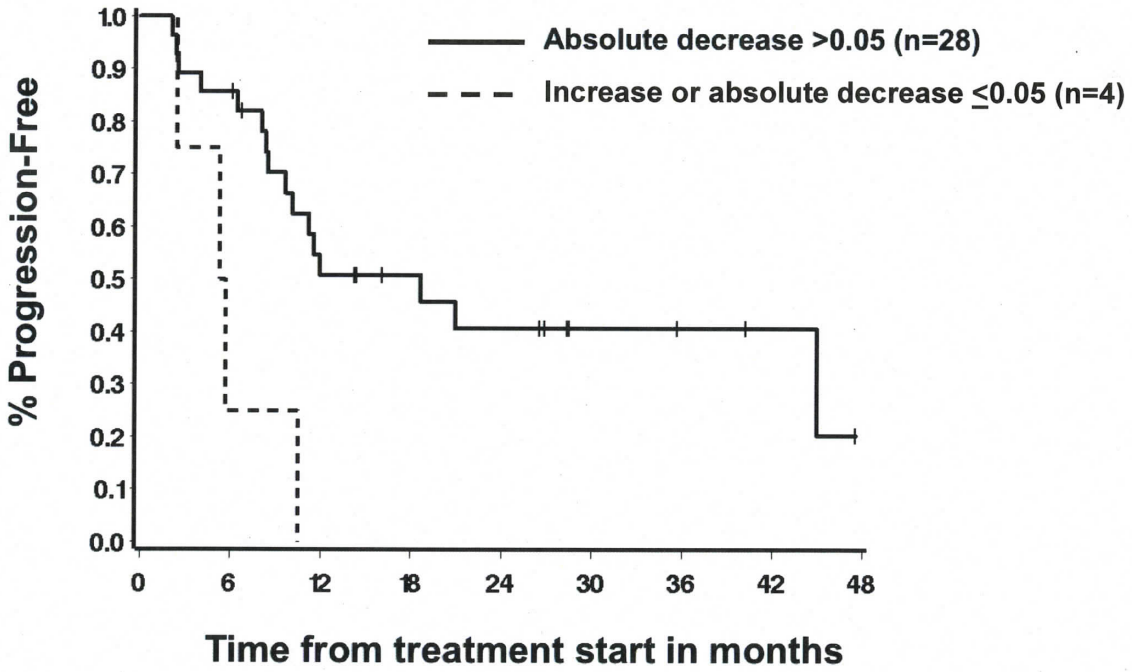
(n=16)

Reduction in G-MDSC Coincides with Increased T cell IFNγ Production

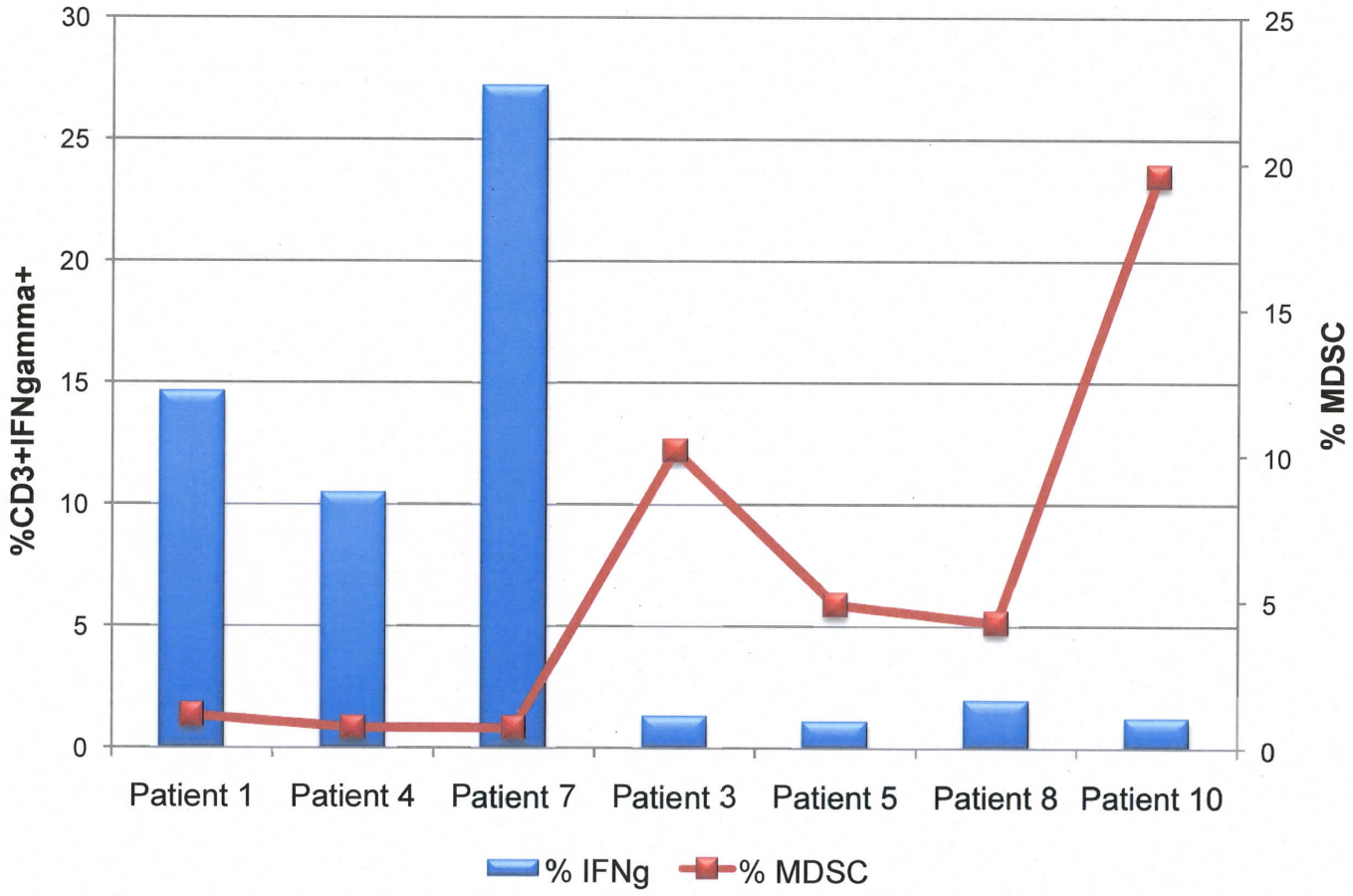




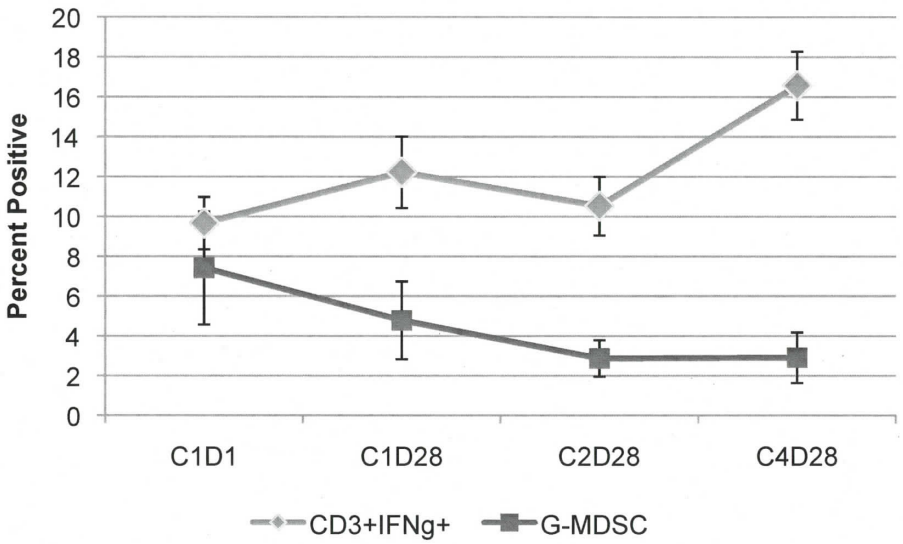
Absolute change in MDSC after 2 cycles of treatment



MDSC and T cells ability to produce IFNγ in RCC Tissue Post Sunitinib Treatment



Pazopanib Treated Patients



p=0.003

p=0.13

n=12 pts

Conclusions:

1. Sunitinib mediates reversal of MDSC accumulation in peripheral blood of RCC patients and enhances T cell IFNγ production.

2. Sunitinib at concentrations achievable in plasma do not inhibit T cell proliferation or production of cytokine/chemokines *in vitro*.

3. Sunitinib decreases the number of myeloid DC in the peripheral blood.

4. Sunitinib-mediated MDSC decline in RCC patients was not correlated with changes in tumor volume. However, preliminary findings suggest that reduced levels of MDSCs after two cycle of therapy was associated with progression-free survival (p=.005).

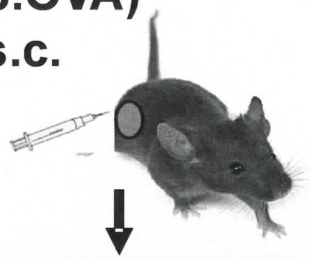
5. Preliminary analysis of tumors from sunitinib treated patients in a neoadjuvant setting show variability in MDSC reduction and T cell function.

6. Pazopanib is also effective at reducing MDSC levels and promoting T cell IFNγ production.

Combining Sunitinib with Immunotherapy

Superior Anti-Tumor Efficacy of Vaccine + TKI Co-Therapy

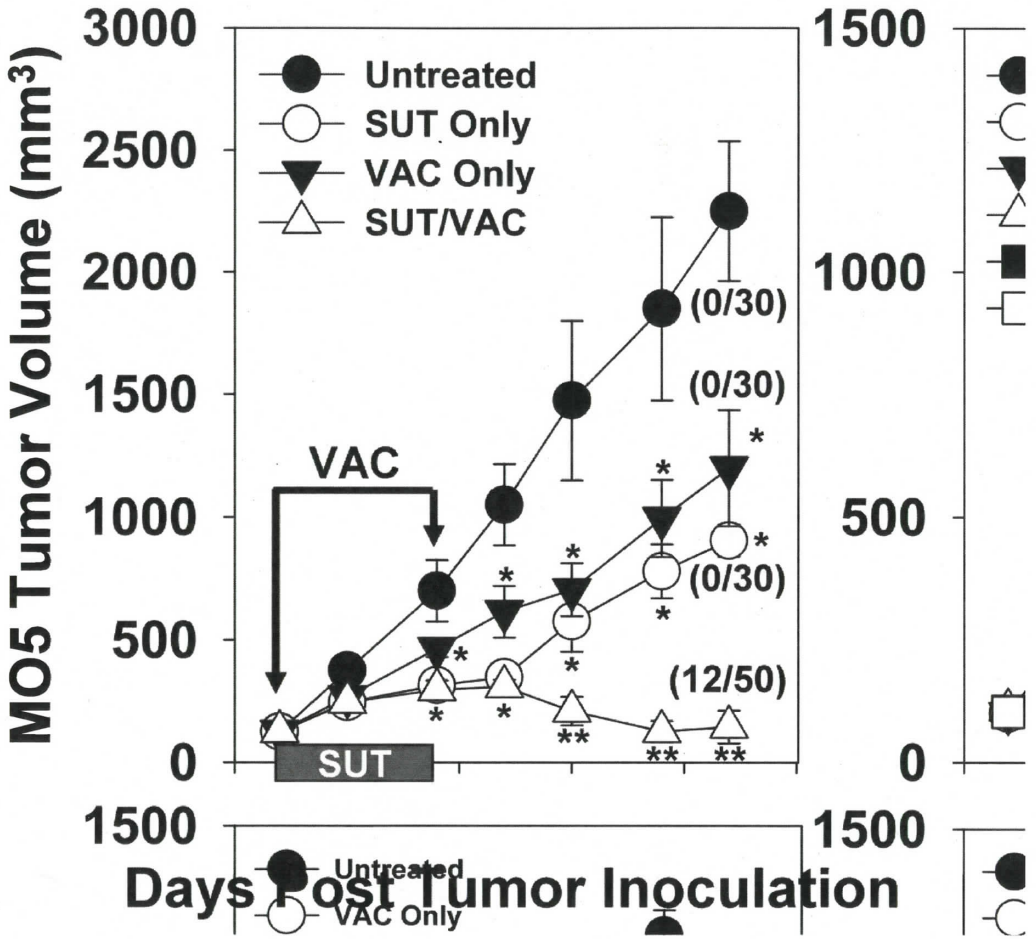
MO5 (B16.OVA) injected s.c. (2×10^5)



+/- oral Sunitinib
(0.1 mg/day, d10-16)

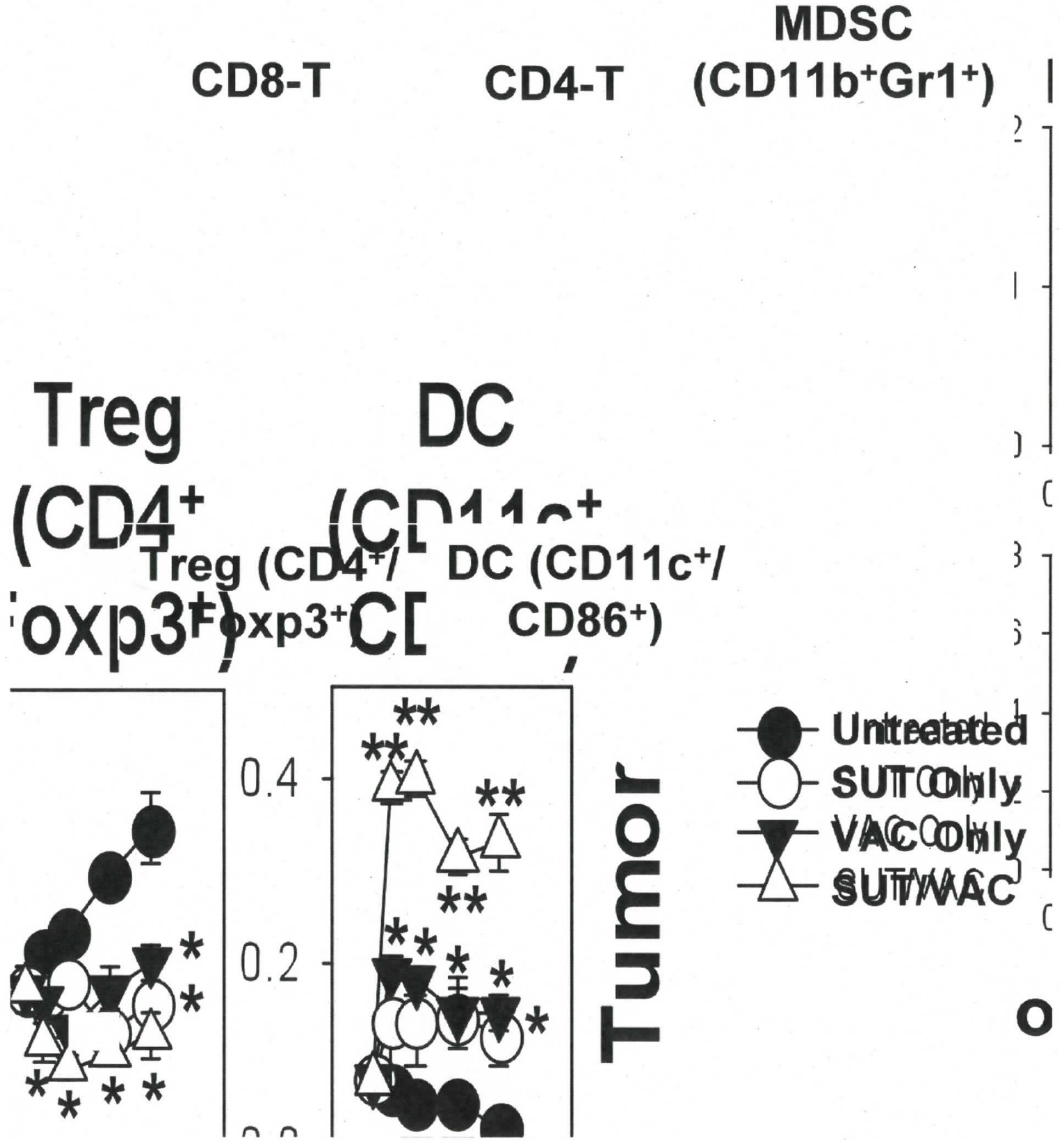
+/- s.c. DC1/$OVA_{257-262}$ vaccines d10, d17

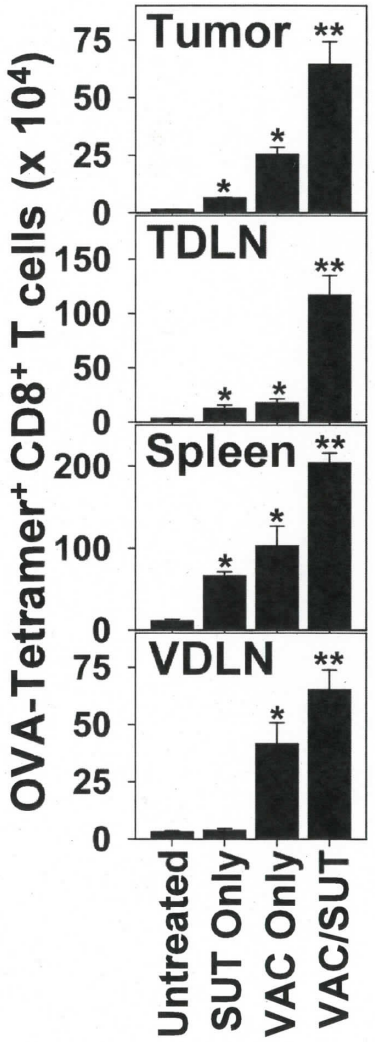
Monitor tumor size
TME analysis (d34)
Immune monitoring (d34)



Bose et al. *Int. J. Cancer* 2011; 129: 2158 - 2170.

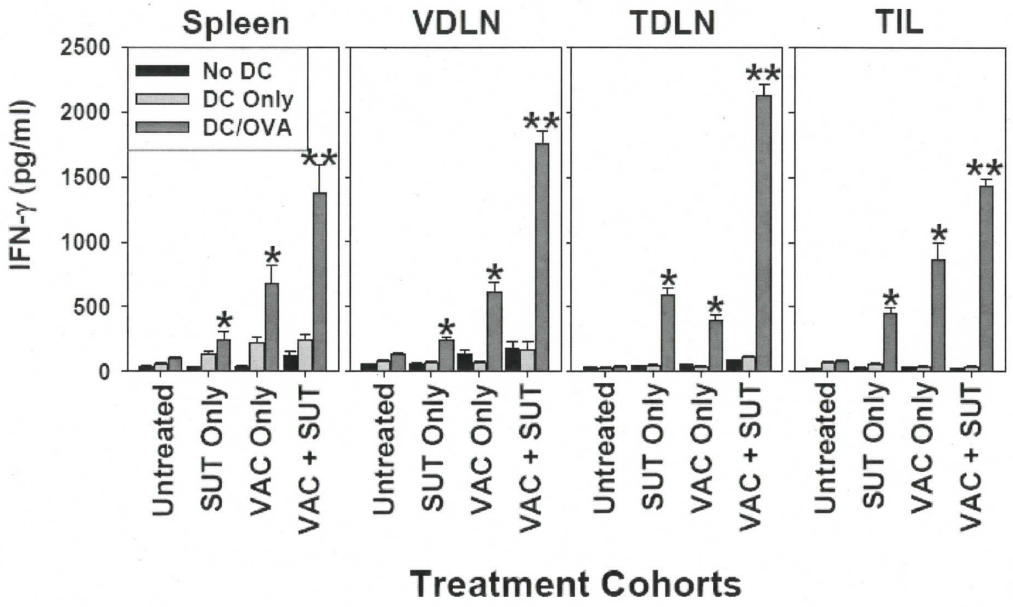
Vaccine/TKI Co-Therapy Promotes the Inhibition of Suppressor Cells and the Activation/Recruitment of Protective CD8⁺ T cells





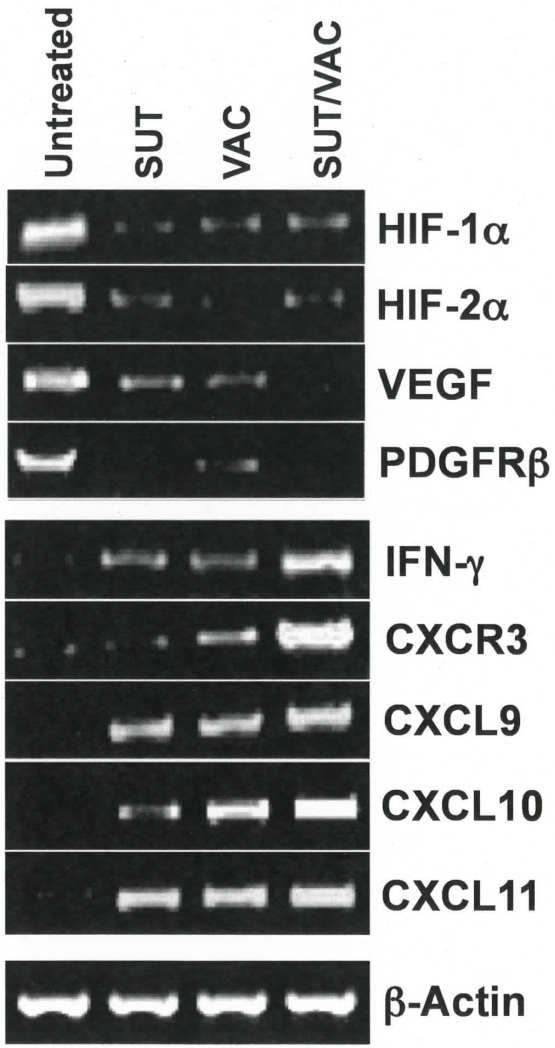
Bose et al. *Int. J. Cancer* 2011; 129: *2158* - 2170.

Combination Treatment with Vaccine and Sunitinib Improved T cell Response



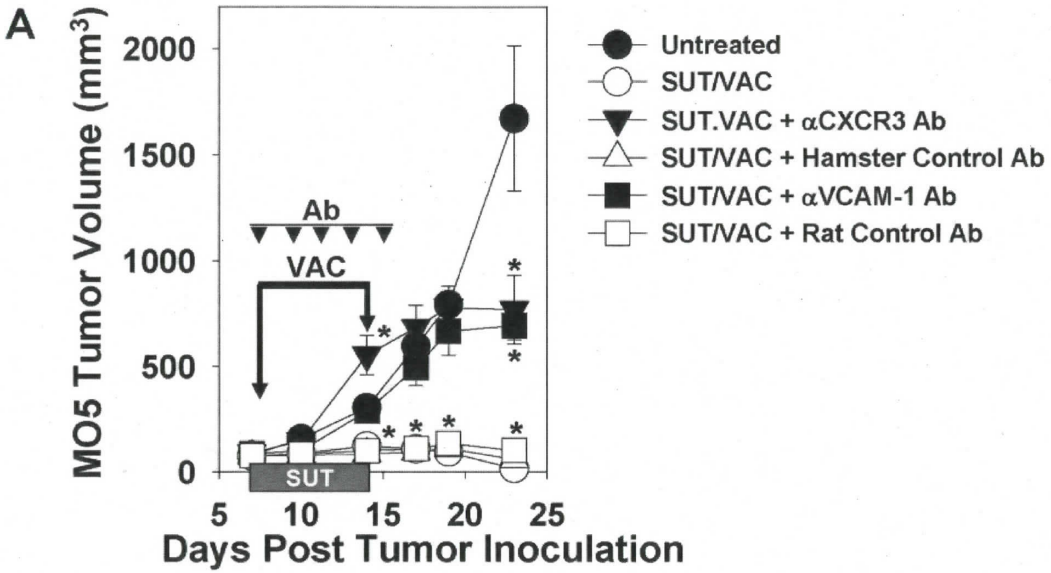
Bose et al. *Int. J. Cancer* 2011; 129: *2158* - 2170

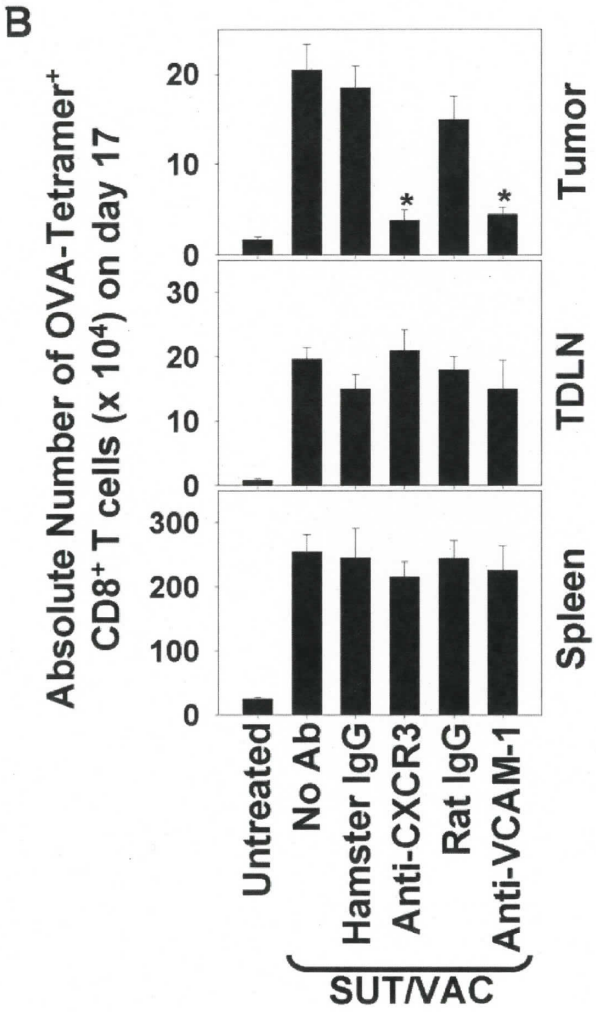
Combination Therapy Results in a Type-1 Biased Immune Profile in the Tumor



Bose et al. *Int. J. Cancer* 2011; 129: 2158 - 2170.

The Anti-tumor Efficacy of Combined Sunitinib/Vaccine Therapy is CXCR3 and VCAM-1 Dependent





Bose et al. *Int. J. Cancer* 2011; 129: 2158 - 2170.

Conclusions

- 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)

Sunitinib Combined with Immunotherapy in Murine Tumor Models

- Ozao-Choy et. al. J Cancer Res 69:2514, 2009
  4-1BBLigand/IL12(adenoviral vector)/sunitinib (MCA26)

- Bose A et. al. Int J Cancer 129: *2158*, 2010
  OVA-DC vaccine /sunitinib (B16-OVA)

- Farsaci et. al. Int J Cancer 130:1948, 2012
  Poxvirus-based vaccine (CEA) /sunitinib

- Kujawski et. al. Cancer Res 70:9599, 2010
  $CD8^+$T cells/sunitinib(Renca)

Therapeutic Tumor Blood Vessel Antigen (TBVA) Targets

	Cells Expressing	*Aliases*	*Comments*
DLK-1	Pericytes "Stemmy" cells	Delta-like 1 homologue Preadipocyte factor 1 Fetal Antigen 1	NOTCH ligand (antagonist) EGF Family Member Shed by ADAM17/TACE Inhibits adipogenesis.
EphA2	VEC	ECK	RTK; binds ephrin A1, A5 Involved in angiogenesis, migration; poor prognosis
HBB	Pericytes	Hemoglobin-β (CD113t-C)	Expressed by pericyte progenitors (from hemangioblasts)
NRP1	Pericytes >> VEC	Neuropilin-1 (CD304)	Co-receptor for VEGF/ semaphorin 3A; Involved in angiogenesis, axon guidance, and cell survival & migration
RGS5	Pericytes	Reg. G-protein signaling 5	Regulates heterotrimeric G proteins as a GTPase activator; Hypoxia-responsive.
TEM1	Pericytes, VEC	Endosialin (CD248)	Binds fibronectin/collagen, Role in cell migration

Zhao X et al J Immunol 188:1782, 2012
Zhao X et al Molecular Therapy 19:805,2011

Overexpression of TBVA Transcripts in Tumor Versus Normal Adjacent Kidney (NAK): Real-Time RT-PCR (Mean +/- SD)

Ratio of RCC/NAK mRNA expression (N = 6)

Antigen	Pericyte	VEC
CD31	-	0.8 ± 0.5
DLK1	52.4 ± 3.1	5.4 ± 1.6
EphA2	-	6.5 ± 2.6
HBB	37.5 ± 4.3	7.6 ± 3.2
NG2	1.3 ± 0.8	-
NRP1	8.8 ± 3.4	0.6 ± 0.5
PDGFRβ	13.7 ± 4.2	-
RGS5	9.5 ± 3.2	1.0 ± 0.6
TEM1	14.6 ± 6.1	0.4 ± 0.6
VEGFR1	3.5 ± 1.1	2.1 ± 0.6
VEGFR2	2.7 ± 0.7	1.7 ± 1.2
αSMA	2.2 ± 1.0	0.2 ± 0.4

Sunitinib-Induced Alterations in Type-1 T Cell Response to Vascular Antigens in HLA-A2$^+$ RCC Patients (d35 versus d0; IFN-γ real-time)

Thaw PBMC
Place in AIM-V
Recovery o.n. 37°C

Add 1 μM Peptide

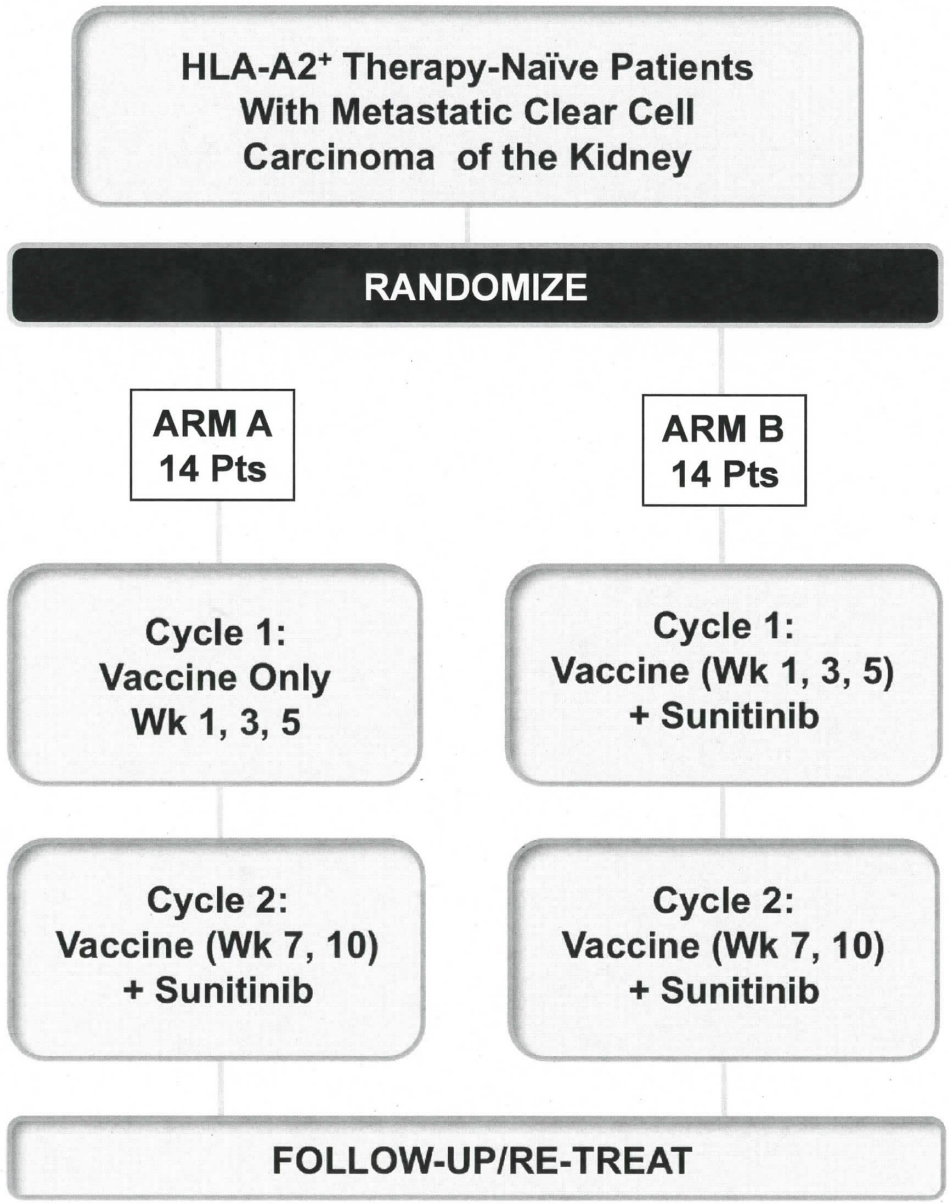
Culture 2h, 37°C

Extract mRNA
Run real-time PCR

T cell Response Against Antigen	Patient 1	Patient 2
HIV-nef [-]	-	-
FluM1 [+]	0.95	1.03
DLK1	4.86	0.97
EphA2	6.49	1.16
HBB	1.87	0.93
NG2	0.96	1.02
NRP1	1.10	0.91
RGS5	1.57	1.13
TEM1	2.12	3.74
VEGFR1	0.91	1.07
VEGFR2	1.16	0.96
G250	2.94	4.18

W Strokus et al

A Randomized Phase II Pilot Trial of Type-1-Polarized Autologous Dendritic Cell Vaccines Incorporating TBVA Peptides In Combination With SUNITINIB (SUTENT®) In Patients with Metastatic Clear Cell Carcinoma of the Kidney



VAC = αDC1 (10^7) + TBVA Peptide Pool (DLK1, EphA2, HBB, NRP1, RGS5, TEM1), s.c.

SUT = 50 mg/day

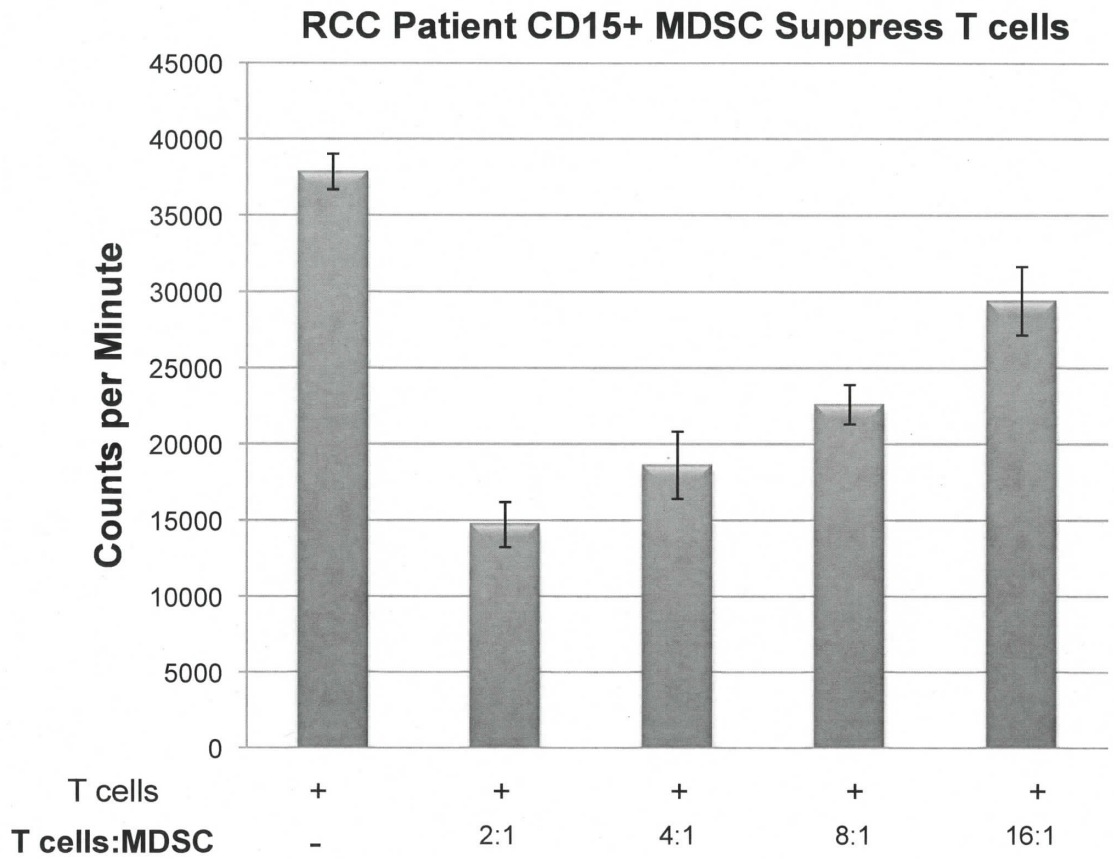
Primary Endpoint

- Specific Immune response rate in PBMC (MHC/peptide dextramers)

Secondary Endpoints

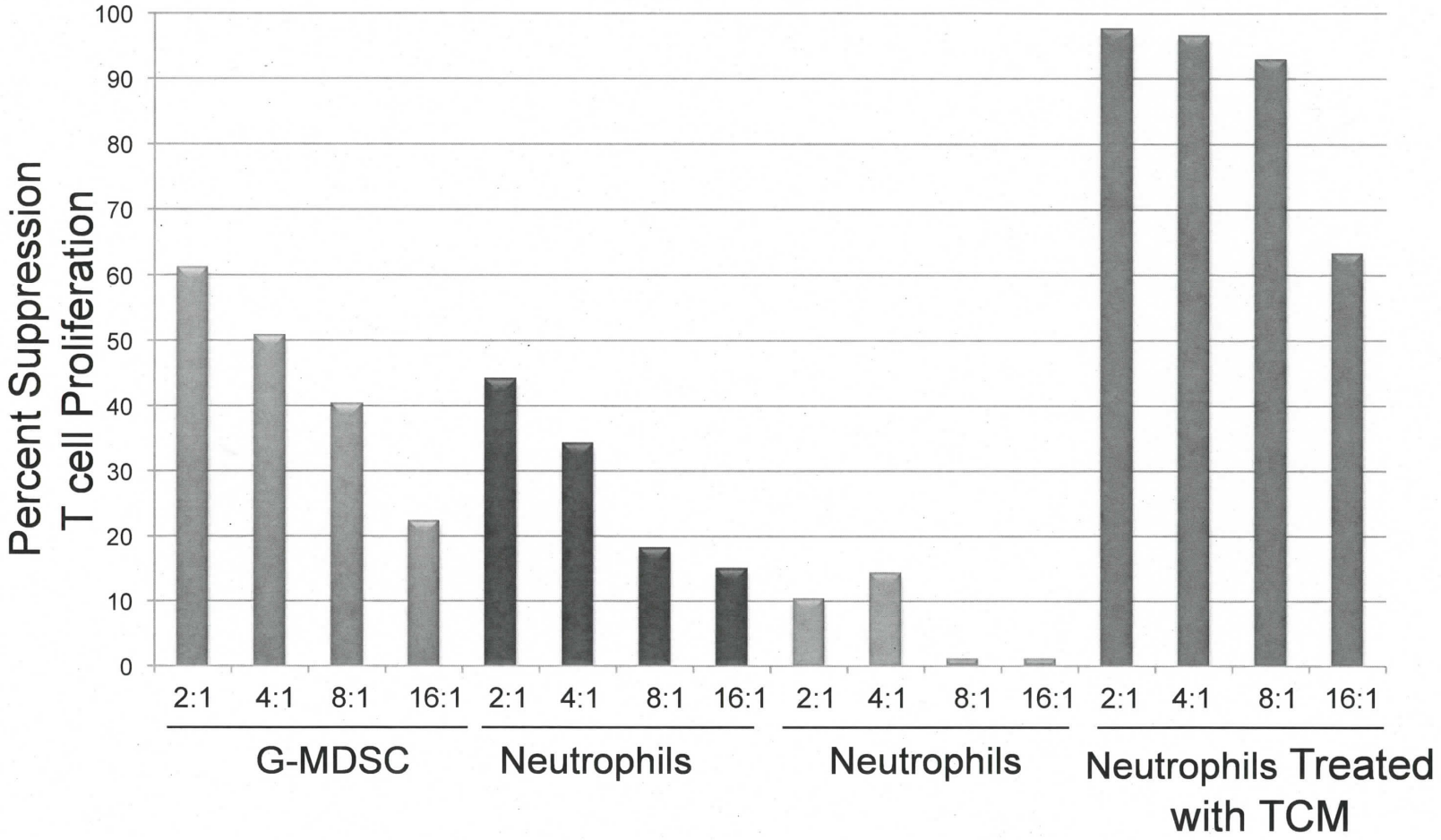
- Clinical response rate
- $CD8^+$ TIL in tumor biopsy pre/post
- Suppressor cell reduction tumor/blood
- Reduction in tumor blood vessel density
- Increased CXCR3 ligand chemokine levels in serum

Suppressive and Angiogenic Activity of G-MDSC in RCC Patients



(Representative of 8 Exp)

Suppression of T cells by G-MDSC & Neutrophils from RCC Patient & Normal



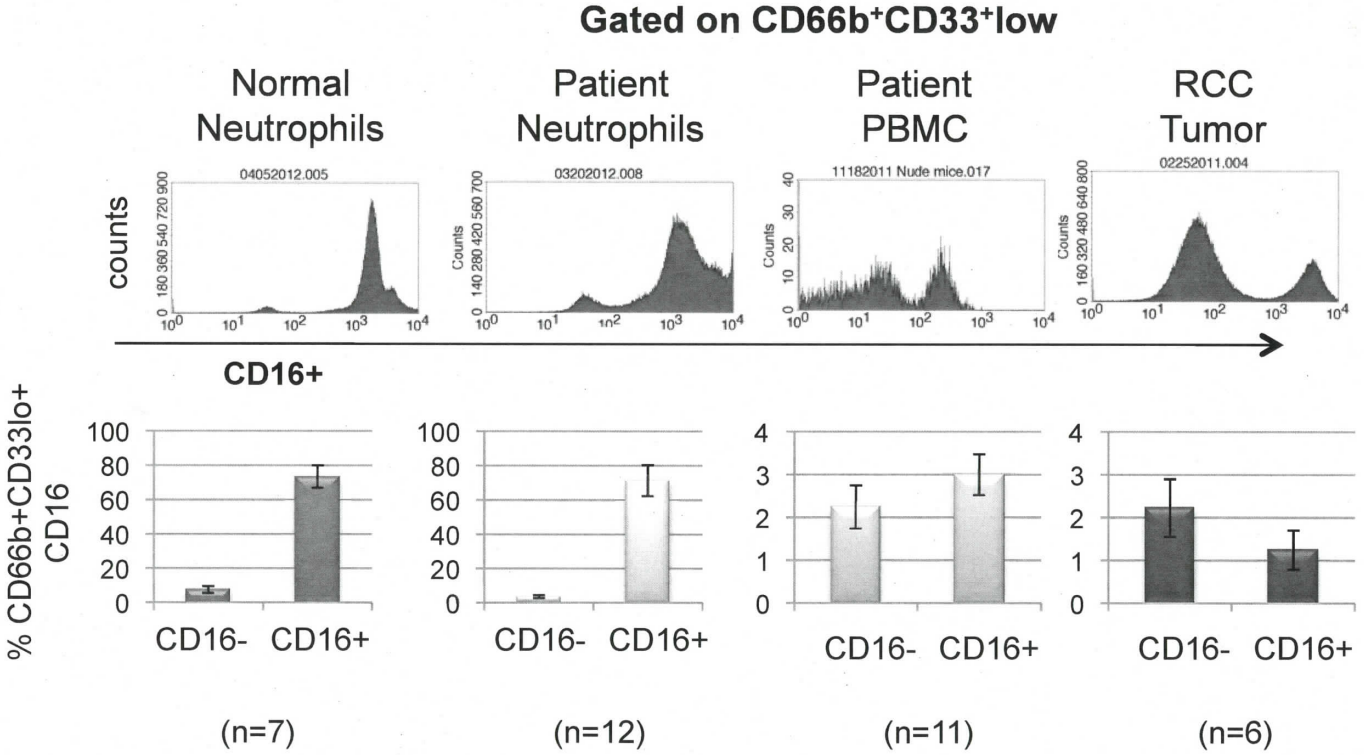
RCC Patient

(Representative of 8 Exp)

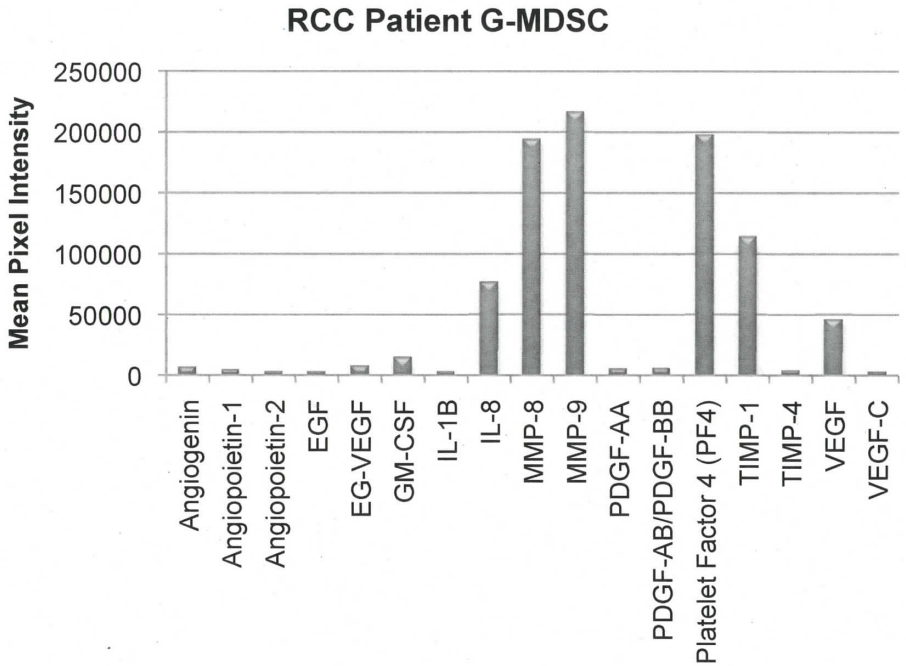
Normal

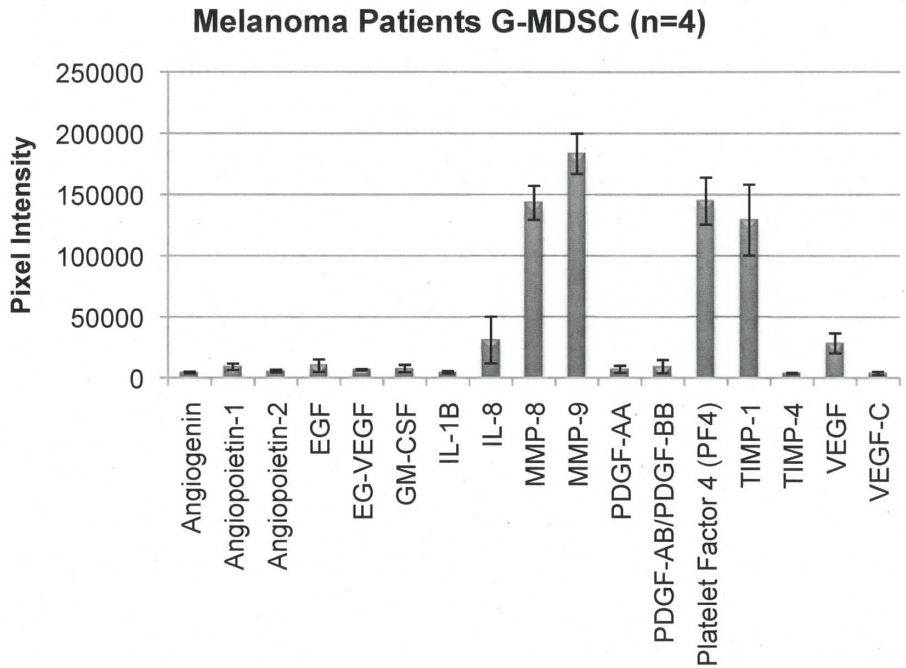
(Representative of 5 Exp)

G-MDSC From RCC Patients Relative to Neutrophils Have a Significant Population of CD16^{+}low population.



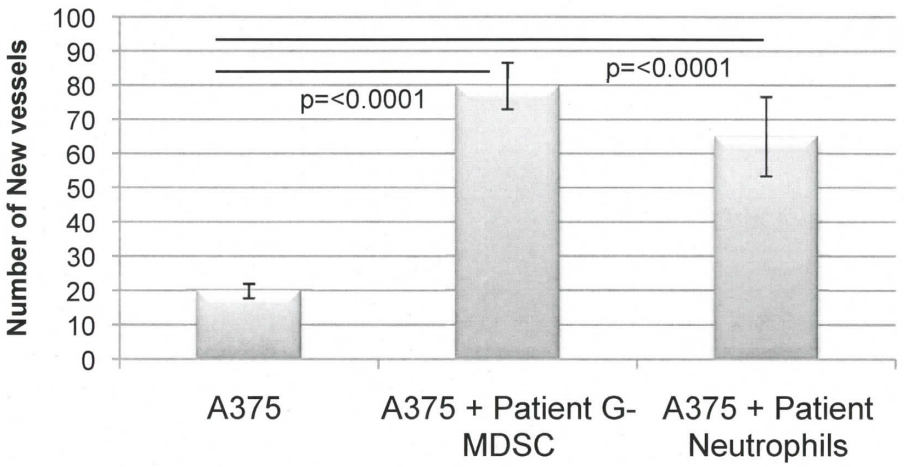
Angiogenic Proteome Profile Array



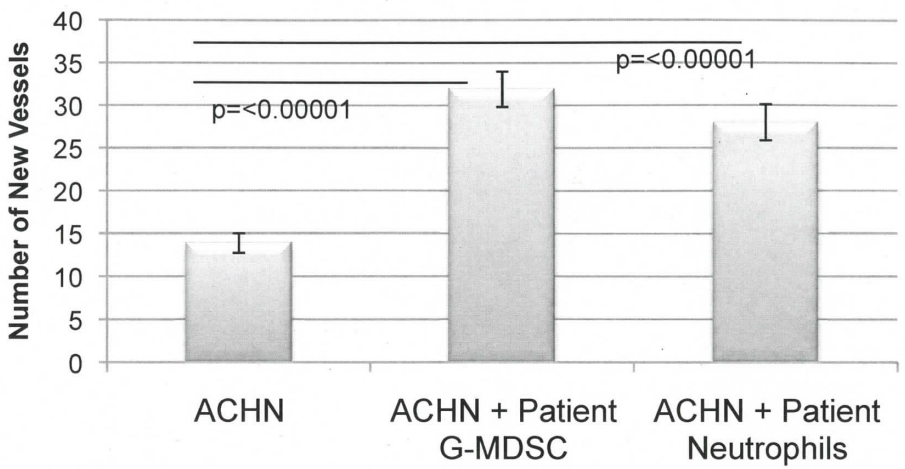


G-MDSC and Patient Neutrophils are Proangiogenic in vivo- Xenograft Nude Mouse Model

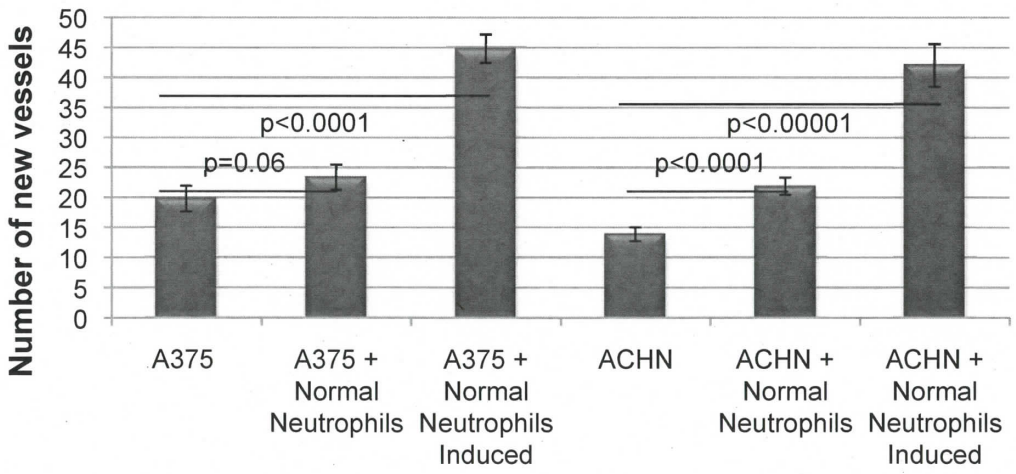
Melanoma Patient



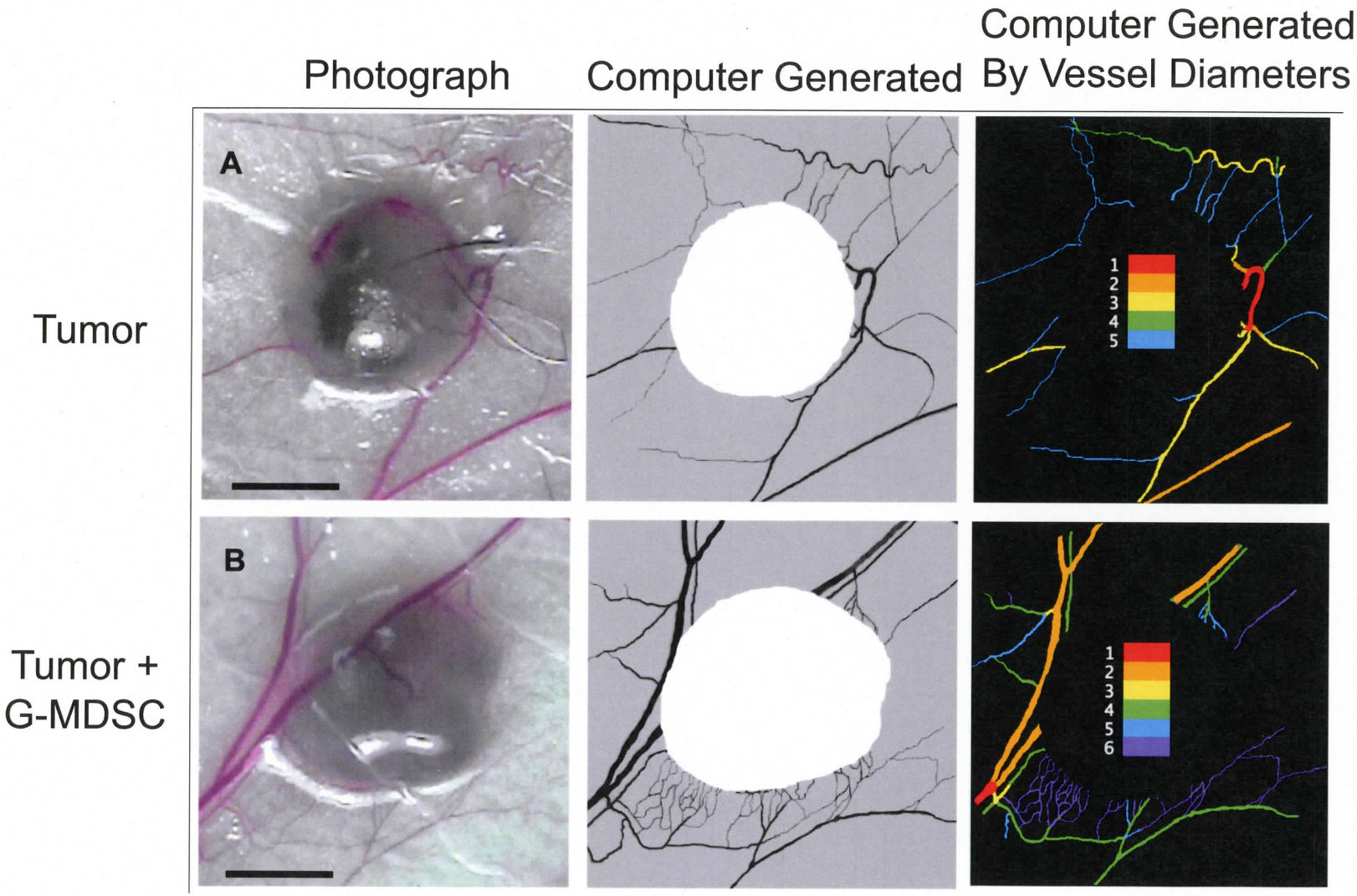
RCC Patient



Induced Neutrophils are Proangiogenic in vivo

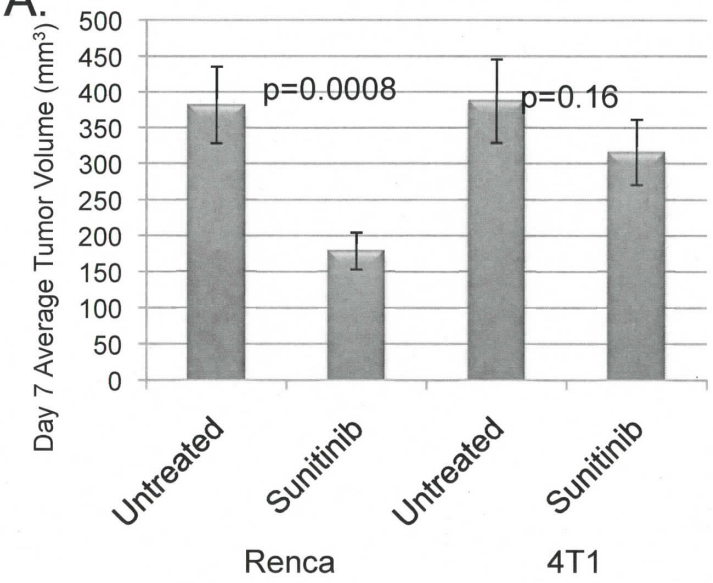


Nude Mouse Xenograft Model of Angiogenesis



MDSC in 4T1 Tumor Bed Are Relative Resistant to Sunitinib Compared to MDSC in Renca

A.



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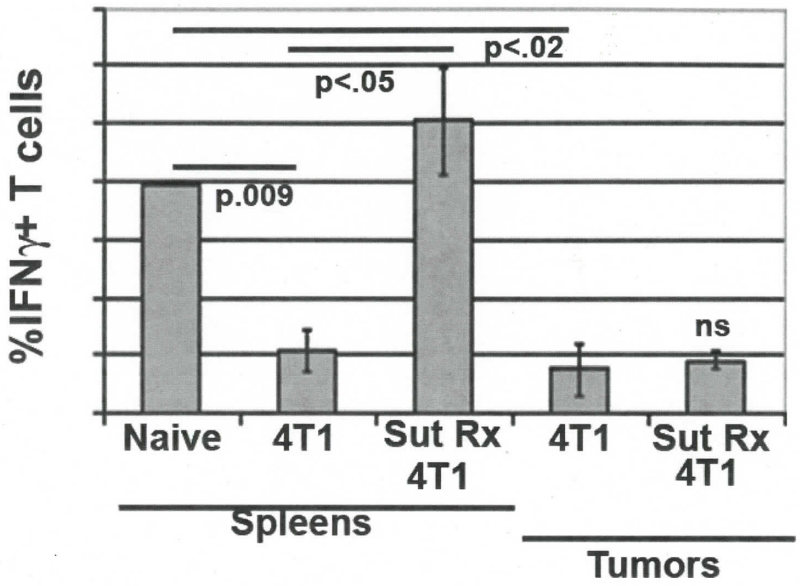

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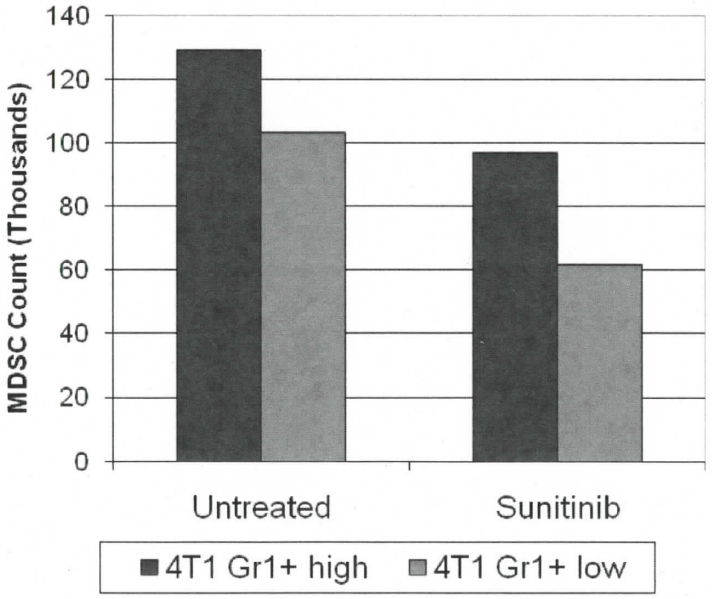
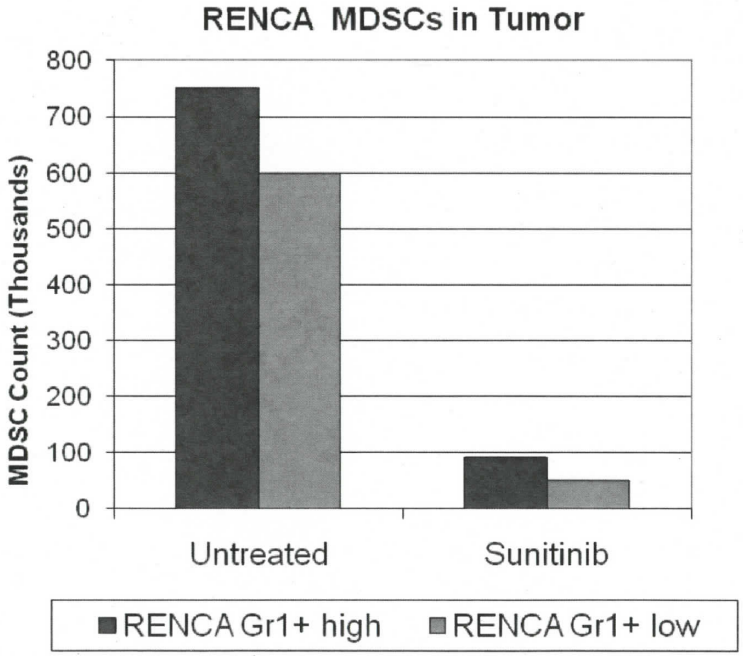
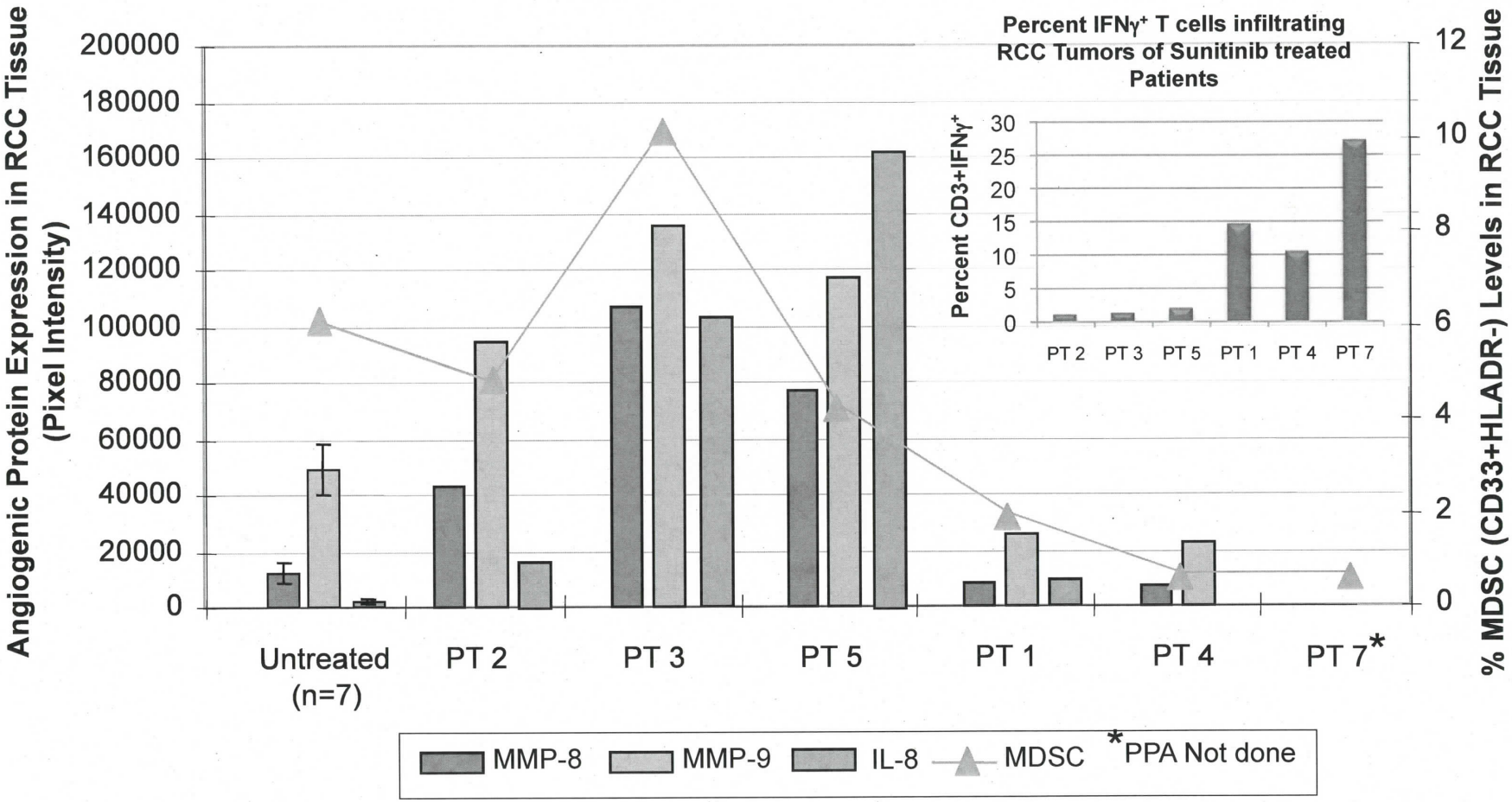




MDSC Persistence in RCC Tissue Post Sunitinib Treatment (Neoadjuvant)



Conclusions:

- G-MDSC and neutrophils isolated from are RCC patients are immunosuppressive.
- Tumor conditioned medium from RCC cell lines can activate neutrophils from healthy donors to acquire suppressive activity.
- This activation causes degranulation of neutrophils with release of T cell suppressive arginase and angiogenic MMP9.
- Granulocytic MDSC and activated neutrophils may promote increase in tumor vasculature.
- Persistence of MDSC after sunitinib treatment in the tumor may promote resistant in some patients and mouse tumor models.
- Tumor derived products including GM-CSF may protect MDSC from sunitinib mediated apoptosis.

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Grant Support - NIH, Pfizer Corporation, and GlaxoSmithKline