State of the Art 3: Immunotherapy and Modulators of Apoptosis

James Finke, PhD - Cleveland Clinic, Immunology
Crystal Mackall, MD - NCI, Pediatric Oncology
James Mier, MD - BIDMC, Medical Oncology
Craig Slingluff, MD - UVA, Surgical Oncology

Introduction: define goals
[Immunotherapy and Modulators of Apoptosis]

- Why would it work?
  - Scientific rationale for combinations
- Why it may not work
  - Potential pitfalls and complications
- Data on use of combinations
  - with examples.
- Next steps to advance the described combination therapies

Why would it work?
Scientific rationale for combinations

- Apoptosis resistance is a common cause of failure of immunotherapy
- Selective destruction of tumor before immune activation
- Tumor cell apoptosis may increase antigen presentation
- Cross-reactivity of kinase inhibitors on pathways of immune function
  - Advantages of lymphodepletion
  - Selective destruction of T regs
  - Unexpected immune effects of kinase inhibitors
- Cytokine effects on tumor and on immune cells

Why it may not work.
Potential pitfalls and complications

- Pathways for induction of tumor cell death also mediate immune cell death
  - AKT/NF-KB
  - Negative effects on T cells
- Immune dysfunction after lymphodepletion
- Proteasome inhibitors: cell death, Ag proc
- Autocrine growth factors from tumor mediate escape from cell death
- Dose-related effects are complex to work out
- Complexity of cross-talk, nonspecificity
- Complexity of experimental trial designs

Data on use of combinations, with examples

Apoptosis modulation

- Protease inhibitors
- TRAIL- Caspases
- HDAC inhibitors
- NF-KB
- AKT
- MAPK/BRAF inhibition
- Cox 2 inhibition

Immune therapy

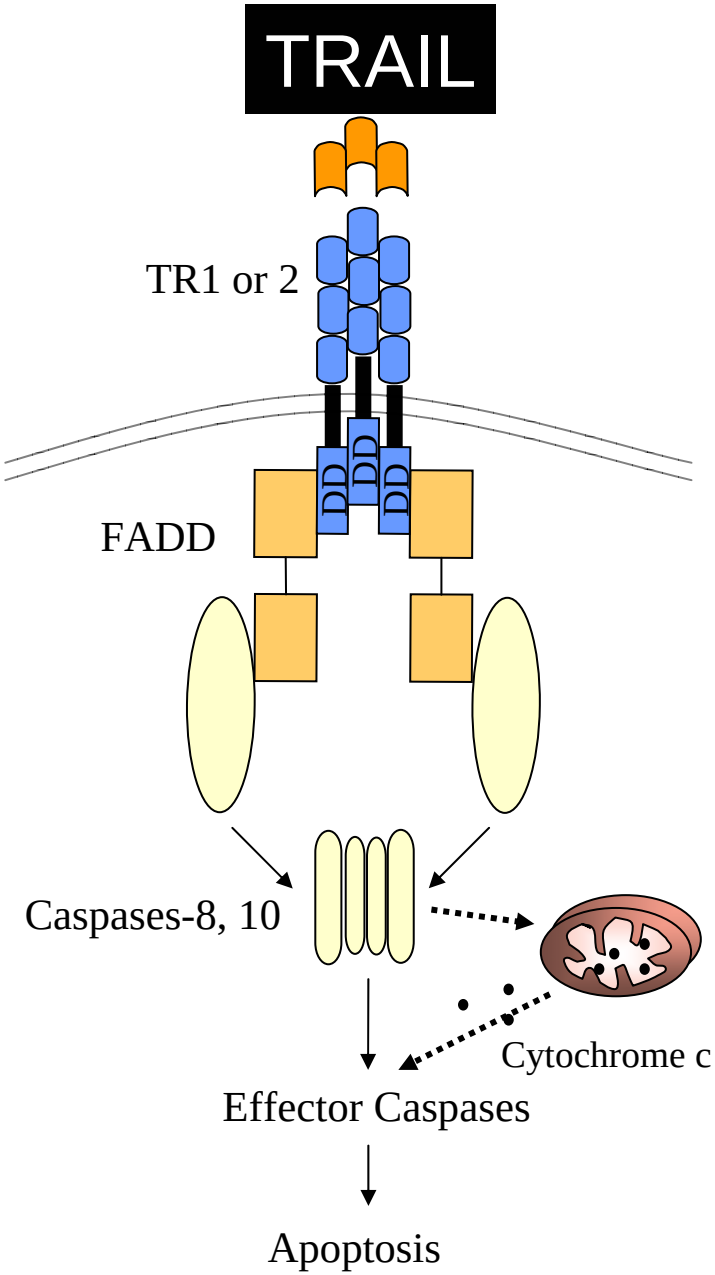
- Vaccines
- Cytokines
- Adoptive therapy
- Immune regulation
- Co-stimulation
- Antibody
- Combination immunotherapies

Data on use of combinations, with examples

- TRAIL, death receptors, and a role for IFN-gamma
  - pediatric sarcomas (Mackall)
- Effects of multikinase inhibitors on Th1/Th2 responses and T-reg cells.
  - renal cell cancer (Finke)
- Sorafenib, survivin and STAT3 – antitumor and immunologic effects
  - melanoma (Mier)
- Selective T reg depletion with low-dose kinase inhibitors
  - melanoma (Slingluff)

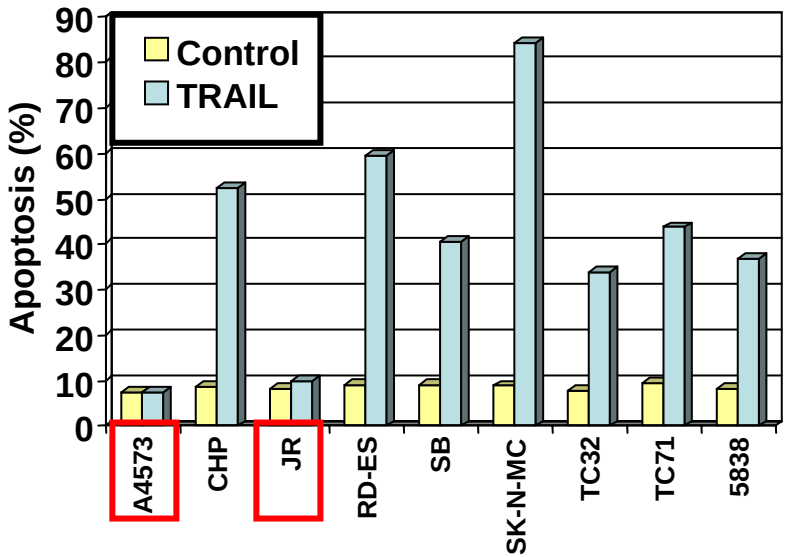
TRAIL, death receptors, and a role for IFN-gamma

Crystal Mackall, NCI



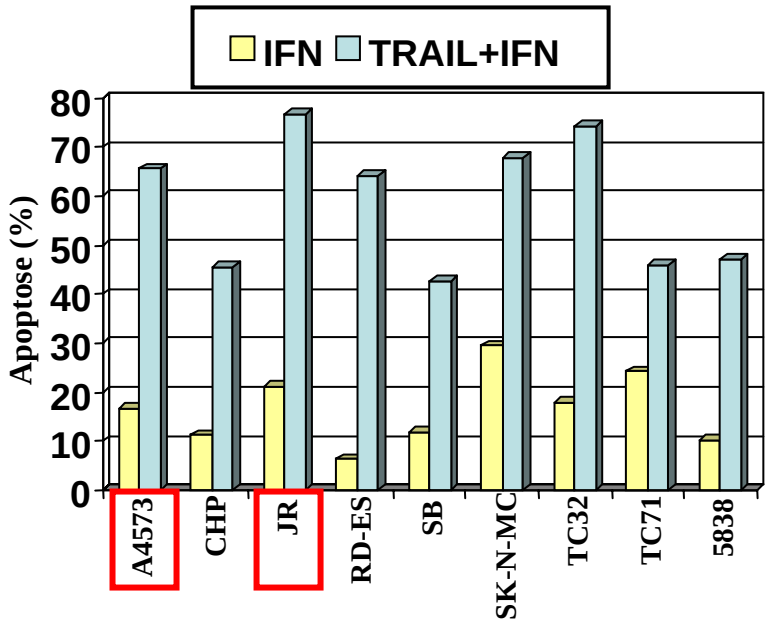
- **Member of the TNF superfamily** (Wiley 1995)
  - Naturally forms homotrimer and binds **TR1, TR2**, TR3, TR4, and OPG
- **Ligation of death domain containing receptors triggers caspase-dependent apoptosis**
- **Critical role for TRAIL in immune surveillance**
  - TRAIL knockout mice susceptible to carcinogen induced sarcomas (Cretney 2002)
  - NK Cells utilize TRAIL for killing in vivo
- **Utilized by Activated Immune Effectors**
  - **B cells are capable of making TRAIL**
    - CpG stimulation (Kemp, 2004)
  - **Monos stimulated by group B strep or IFN** (Halaas, 2004)
  - **Neutrophils in urine of bladder CA patients following BCG** (Ludwig, 2004)
- **TRAIL mediated GVT effect of T cells** (Schmaltz 2003)
- **TRAIL receptor agonists have been developed for clinical application**
  - Agonist mAbs and soluble synthetic TRAIL

TRAIL Kills Most Ewing's Sarcoma Cell Lines *In Vitro*



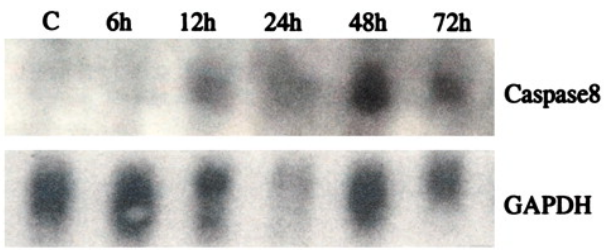
Kontny, Cell Death Diff, 2001

IFNγ Reverses TRAIL Resistance *In Vitro*

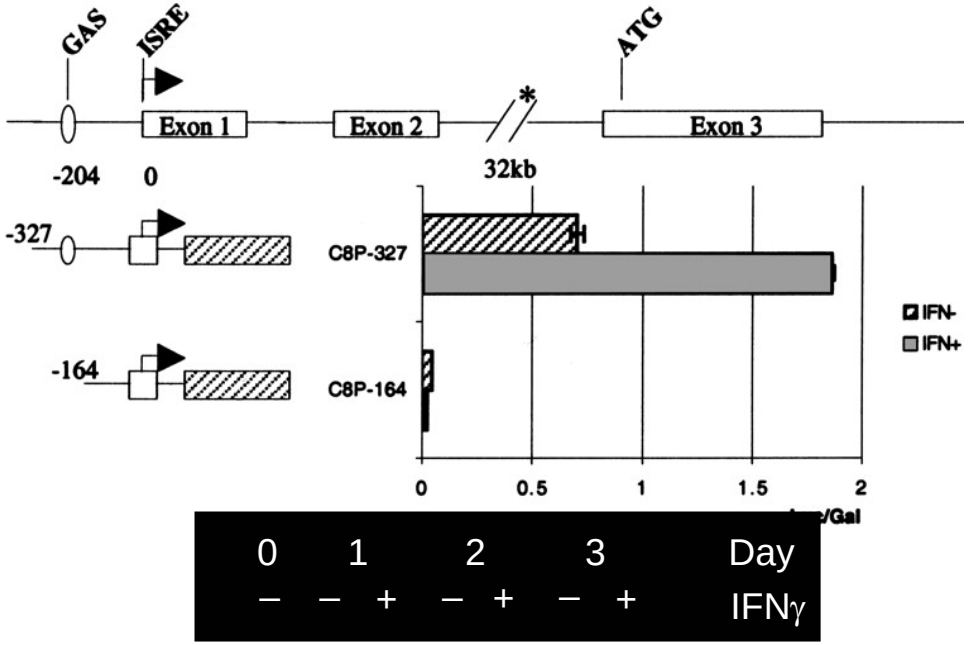


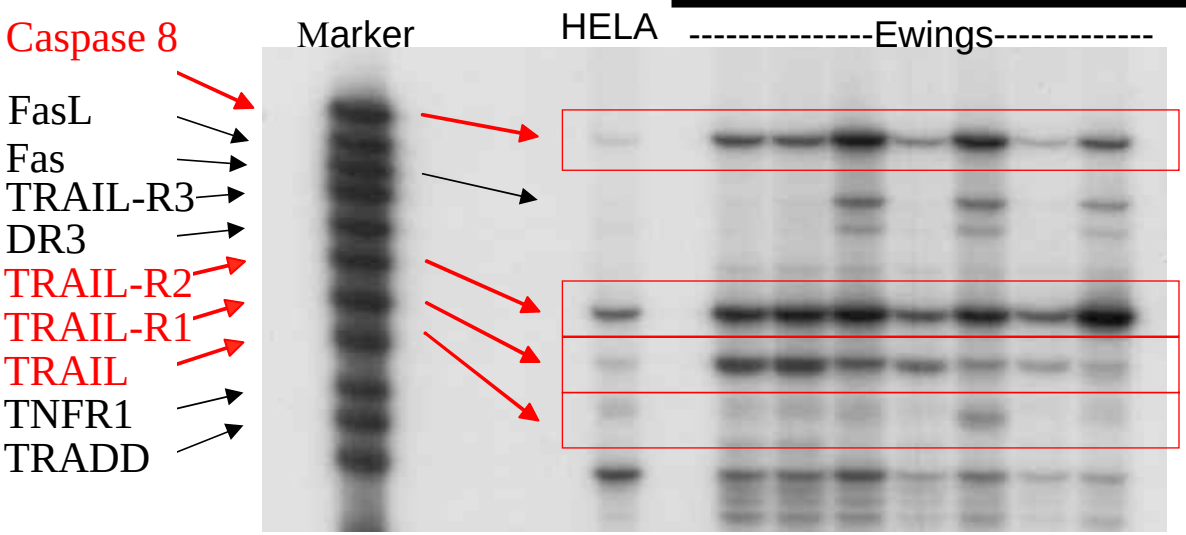
IFNγ Modulates Several Components of the TRAIL Mediated Death Pathway

Expression of Caspase 8 via Direct Effects on the Promoter - Neuroblastoma



Yang, Cancer Res, 2003

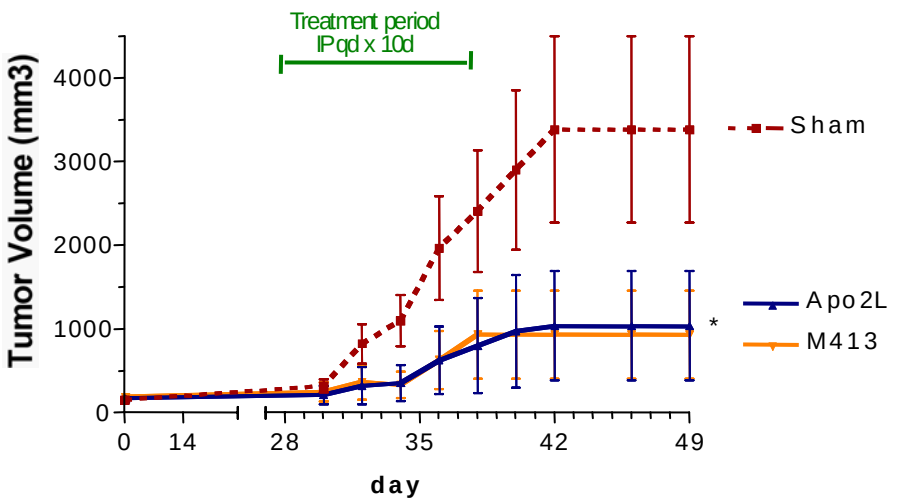




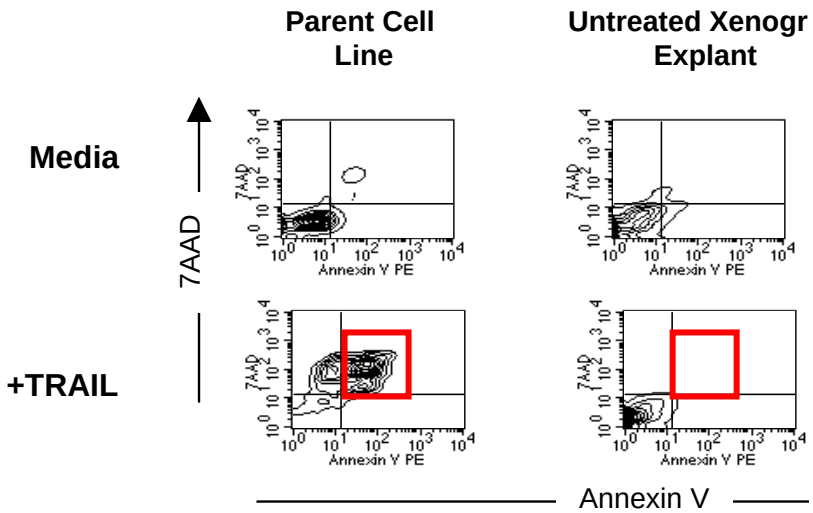
Merchant, Cancer Res, 2004

Ewing's Sarcoma Xenografts Develop TRAIL Resistance *in vivo*

Slowing of growth of some but not all Xenografts

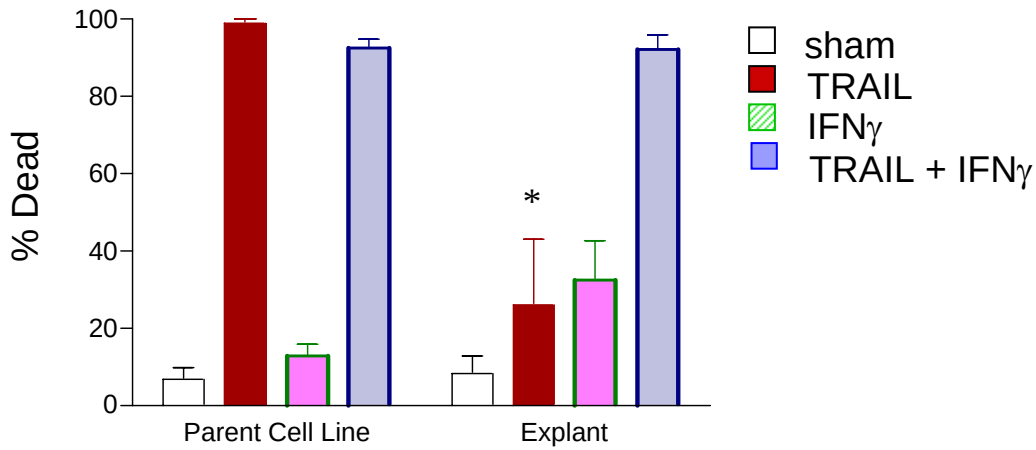


Even cells recovered from untreated mice became resistant



Merchant, Cancer Res, 2004

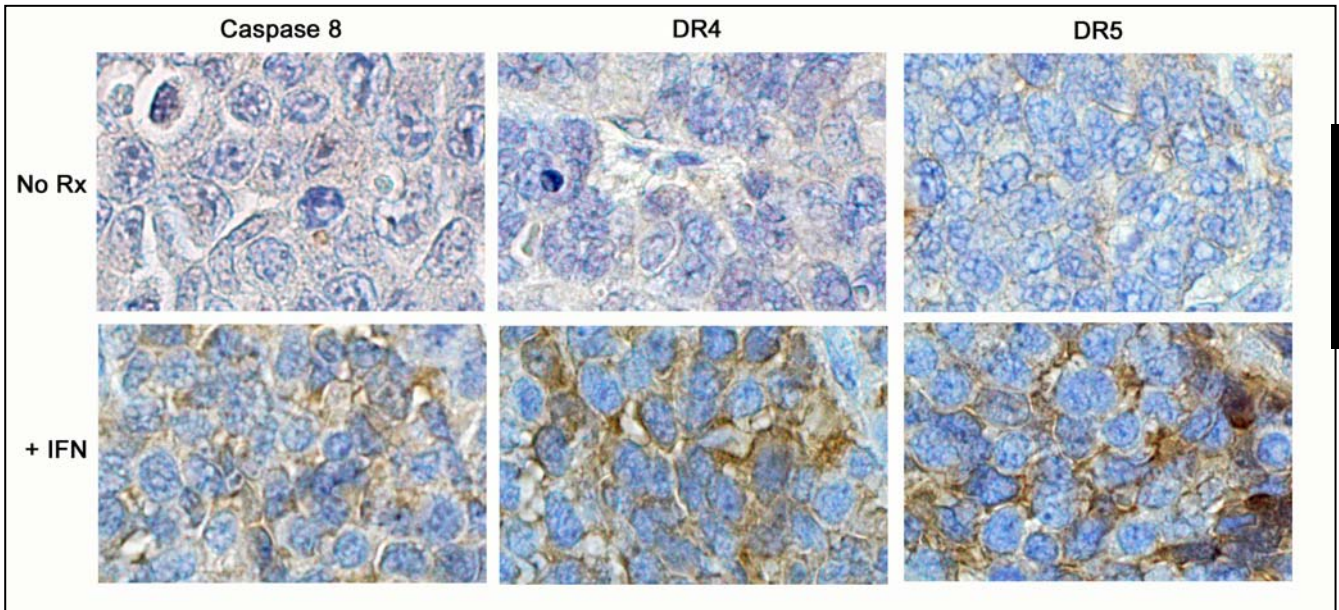
TRAIL Resistance In Explants is Reversed by IFNγ Treatment



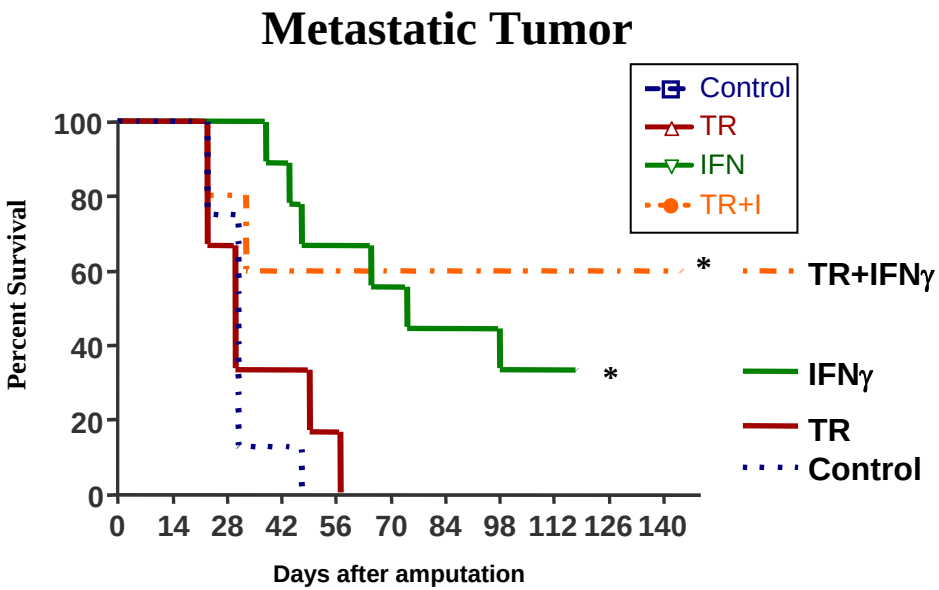
25000 IU IFNγ IP
Daily x5

Resection of tumor
12-16hr after last dose

Tissue fixed, mounted, assayed by IHC



IFNγ Treatment of Mice Induces Caspase 8 And TR Expression



TAKE HOME POINTS:

-Tumor resistance to immune mediated killing remains an issue

-IFNγ modulates several mediators in the caspase dependent cell death pathway

-Effective cellular immunotherapy will deposit IFNγ into the tumor microenvironment

-Immunotherapy would be predicted to enhance the efficacy of TRAIL receptor agonists

Effects of TKIs on Th1/Th2 Response and T-Regulatory cells.

J. Finke PhD, B.I. Rini MD, A. Richmond, R. Suppiah MD, L. Wood RN, P. Elson ScD, P. Shaheen MD, J. Garcia MD, R. Dreicer MD, R.M. Bukowski MD
Divisions of Hematology/Oncology and Immunology,
Cleveland Clinic, Cleveland, OH
Walter Storkus PhD Univ. of Pittsburgh

Th1 and Th2 Responses (n=22)

CD4+ cells (medians)

	Day 1	Day 28	Absolute Change[1]	p-Value[2]
% IFN-γ cells	1.7%	9.4%	5.9	.001
% IL-4 cells	8.6%	4.5%	-1.1	.35
Th2 Bias	4.69	0.83	-4.46	<.001

[1] Day 28 minus Day 1

[2] p-values are from Wilcoxon signed rank test

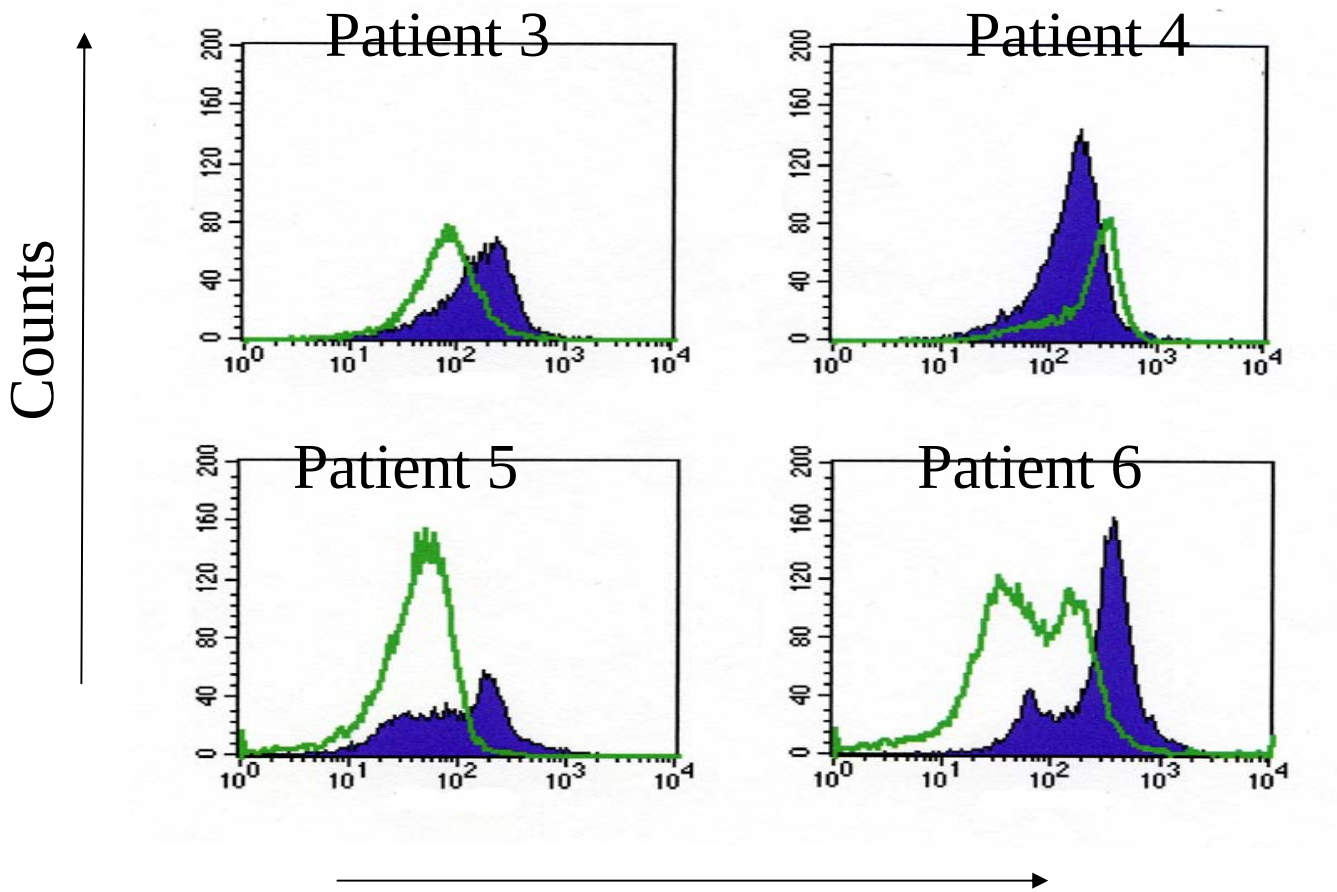
[3] Proportion of cells producing IL-4 divided by the proportion of cells producing IFN-γ; values >0 imply a Th2 (IL-4) bias and values <0 imply a Th1 (IFN-γ) bias.

Treg - Medians (n=23)

	Day 1	Day 28	Absolute Change[1]	p-Values[2]
CD3+/CD4+/CD25hi+ As % of PBMC	3.7%	3.7%	-0.1	0.81
% of CD3+/CD4+/CD25hi+ That are FoxP3+	78.7%	48.5%	-22.4	<.001

1 Day 28 minus Day 1
2 p-values are from Wilcoxon signed rank test

FoxP3 Expression in Tregs after Sunitinib in mRCC Patients



Combination Therapy in Metastatic RCC

Phase I/II Trial

DC/EGF-R peptides plus anti-EGF-R mAb (IMC-255)

Sutent

Trial of Type-1 Polarized DC in Patients with mRCC.

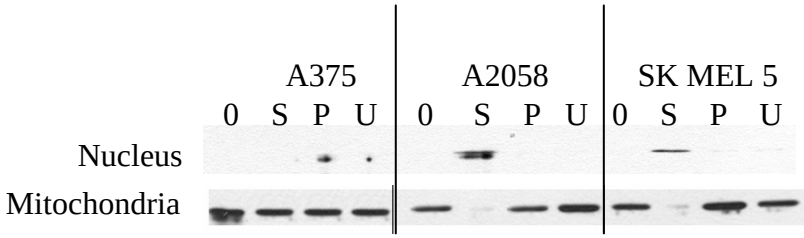
MAGE-6, EphA2 and G250 peptides
Sutent

Sorafenib, survivin and STAT3 – antitumor and immunologic effects

James Mier - BIDMC

Sorafenib induces the nuclear translocation of AIF in A2058 Melanoma Cells

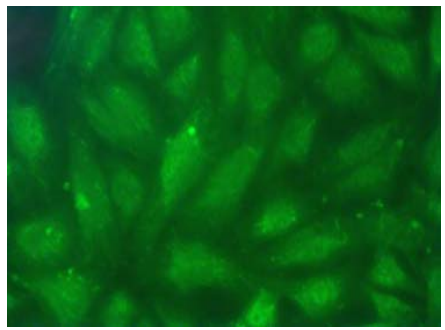
A



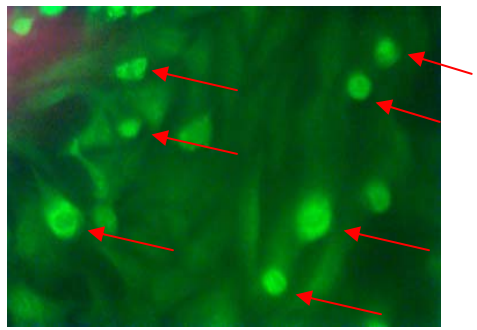
B

AIF/nuclear overlay

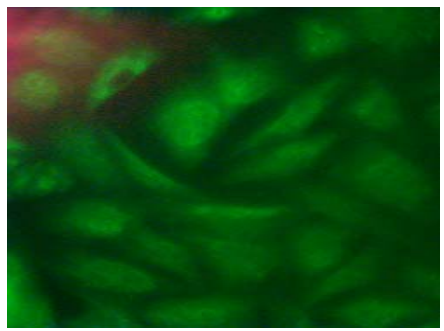
untreated



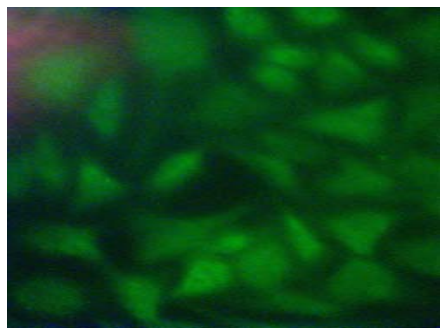
Sorafenib

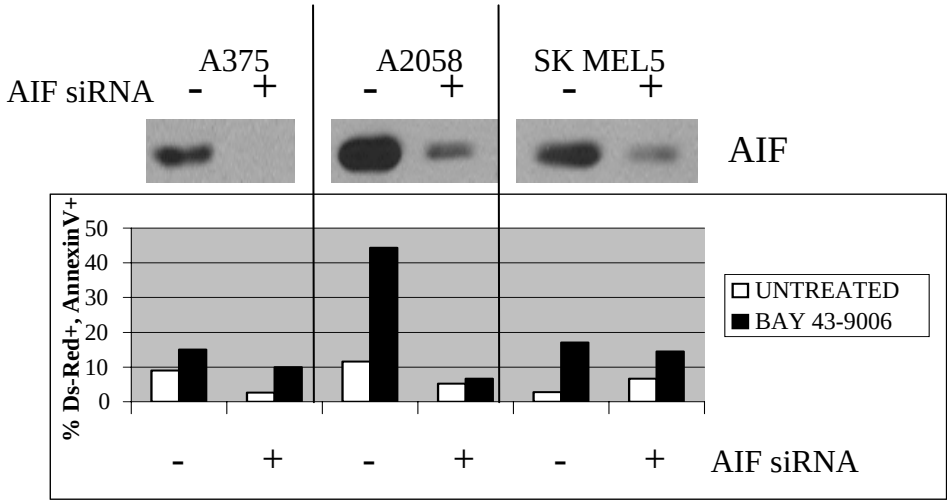


PD 98059



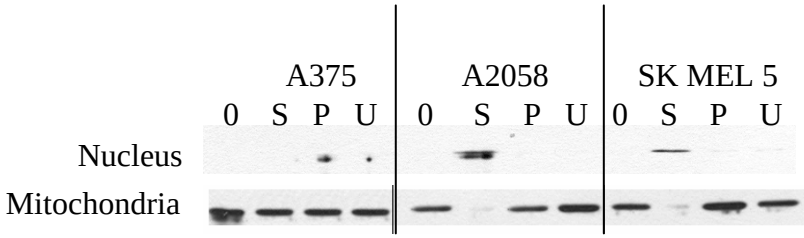
U0126




A375
A2058
SK MEL5
AIF siRNA - + - + - +
AIF
% Ds-Red+, AnnexinV+
50
40
30
20
10
0
UNTREATED
BAY 43-9006
- + - + - +
AIF siRNA

Sorafenib induces the nuclear translocation of AIF in A2058 Melanoma Cells

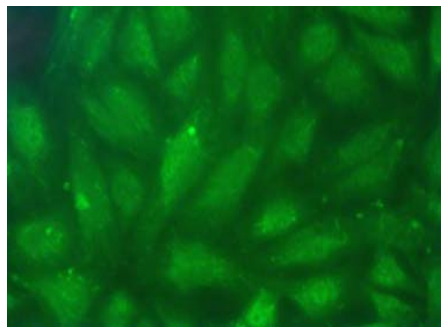
A



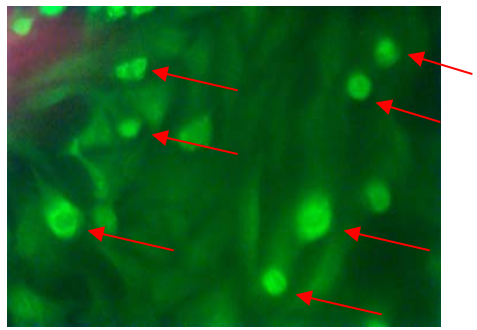
B

AIF/nuclear overlay

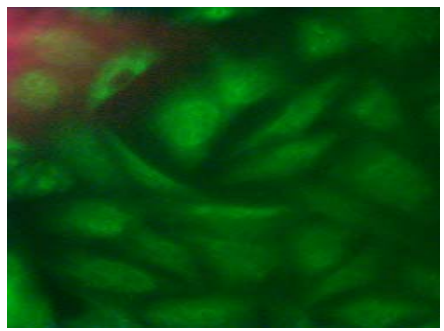
untreated



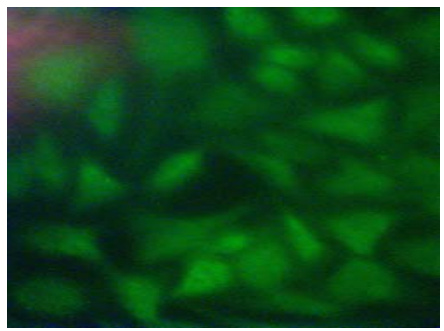
Sorafenib



PD 98059

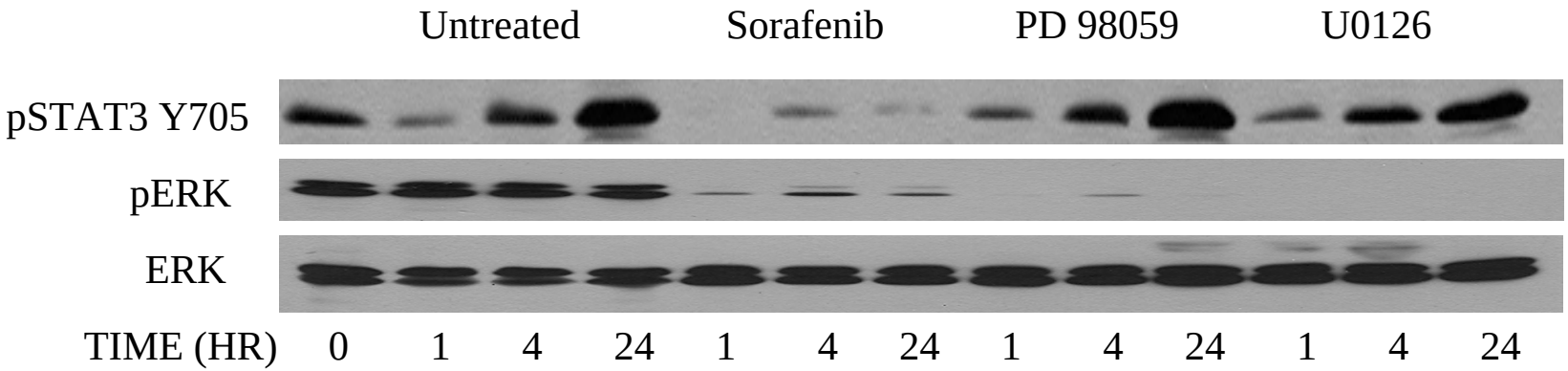


U0126

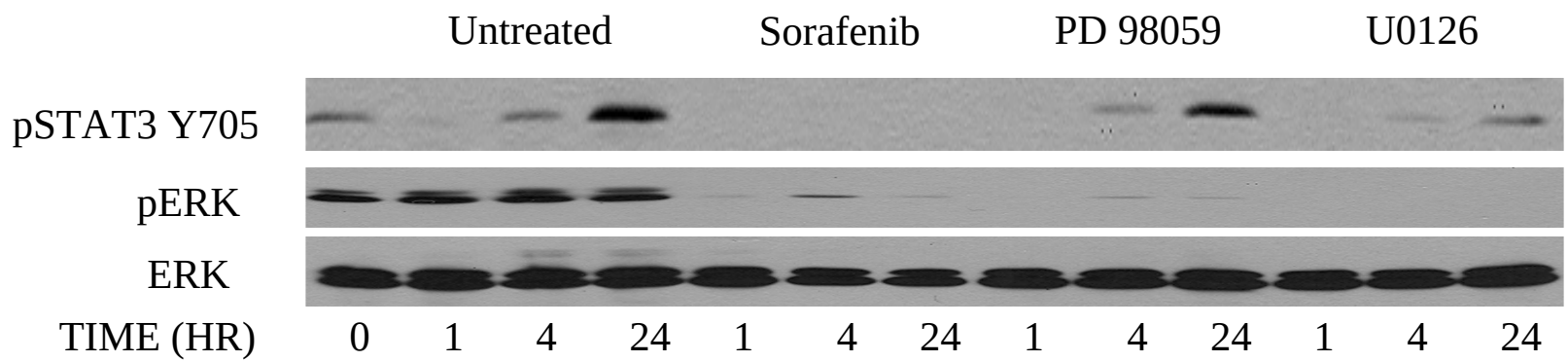


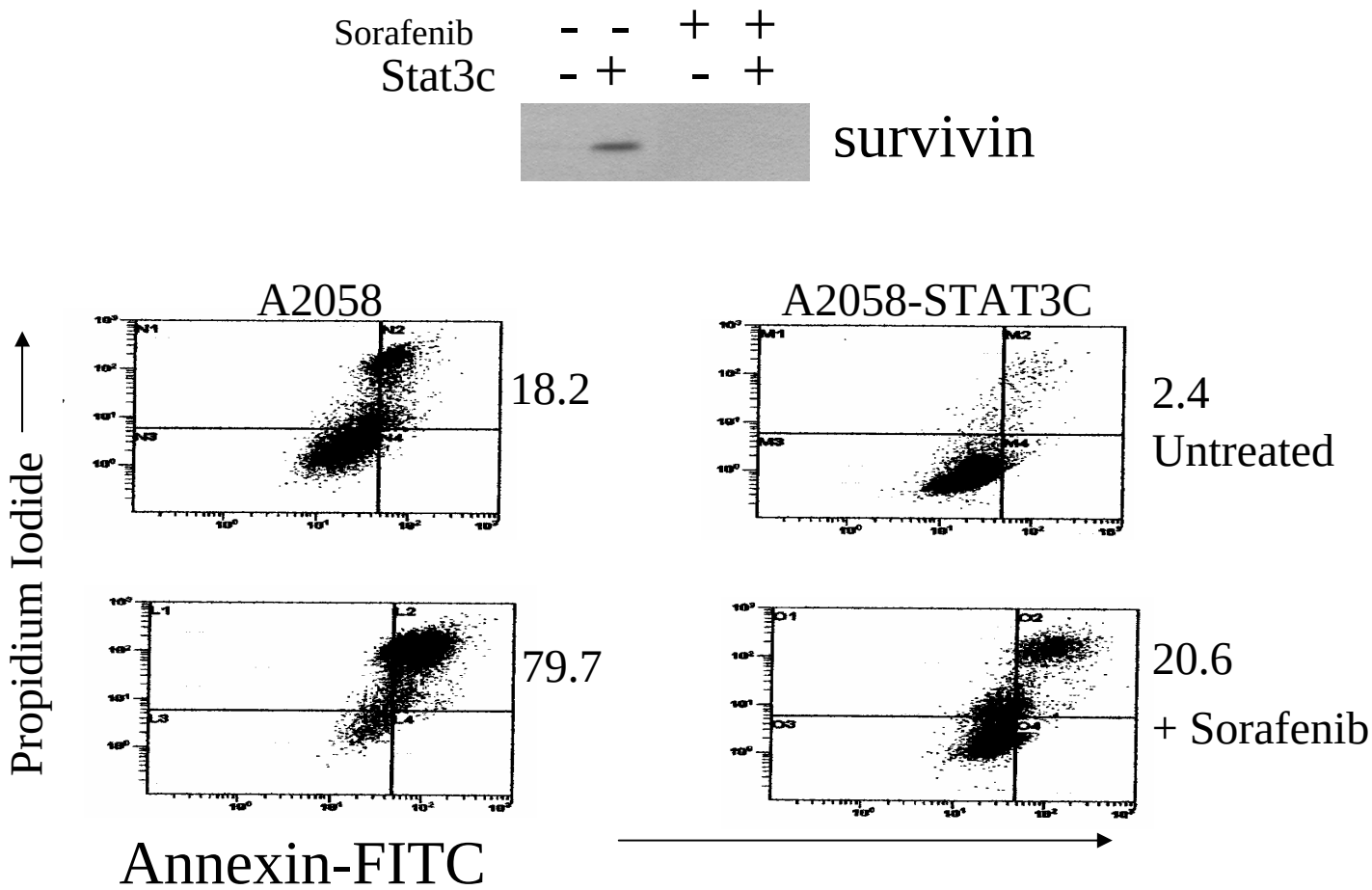
Sorafenib inhibits the activation of STAT 3

A375



A2058




Sorafenib - - + +
Stat3c - + - +
survivin
A2058
A2058-STAT3C
18.2
2.4
Untreated
79.7
20.6
+ Sorafenib
Propidium Iodide
Annexin-FITC

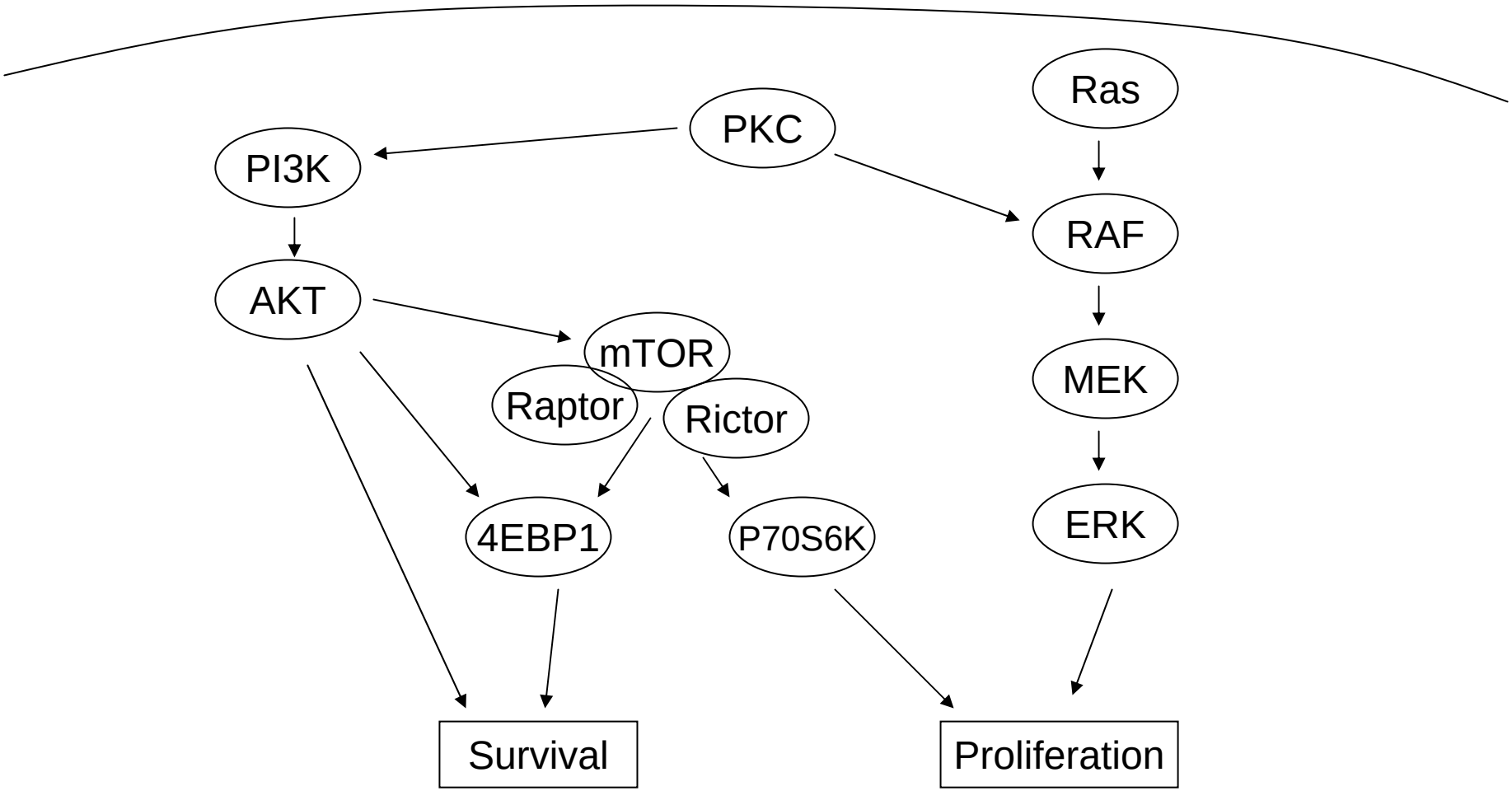
Selective T reg depletion with low-dose kinase inhibitors melanoma

Kerrington Molhoek

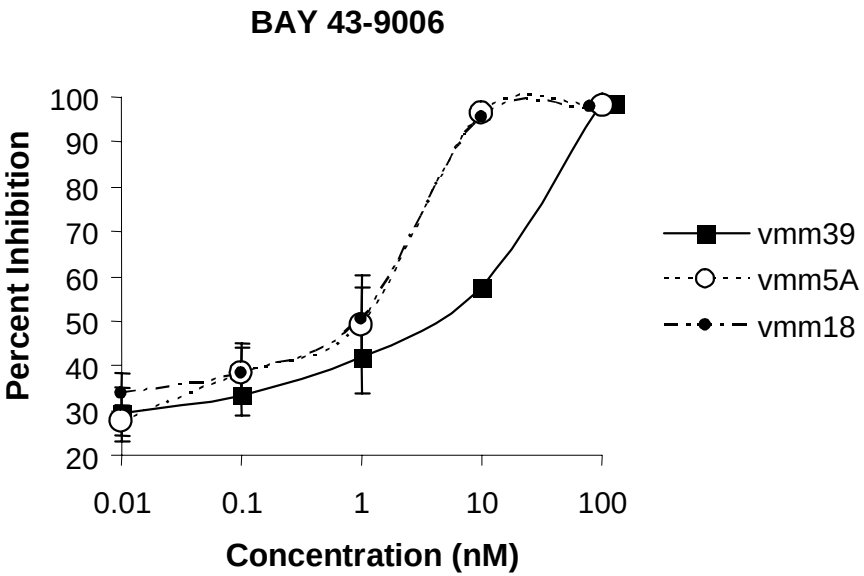
David Brautigan

Craig Slingluff

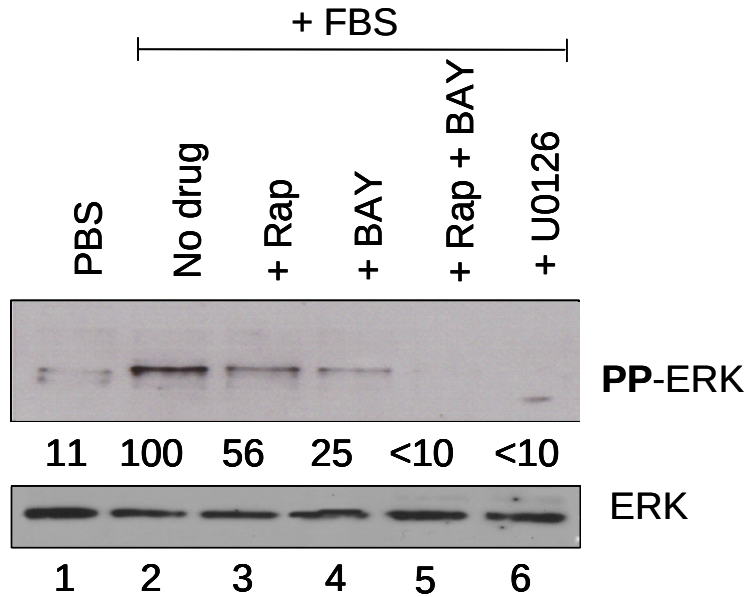
Survival and Proliferation signaling pathways in cancer and in lymphocytes



Inhibition of serum-stimulated proliferation of human melanoma cells



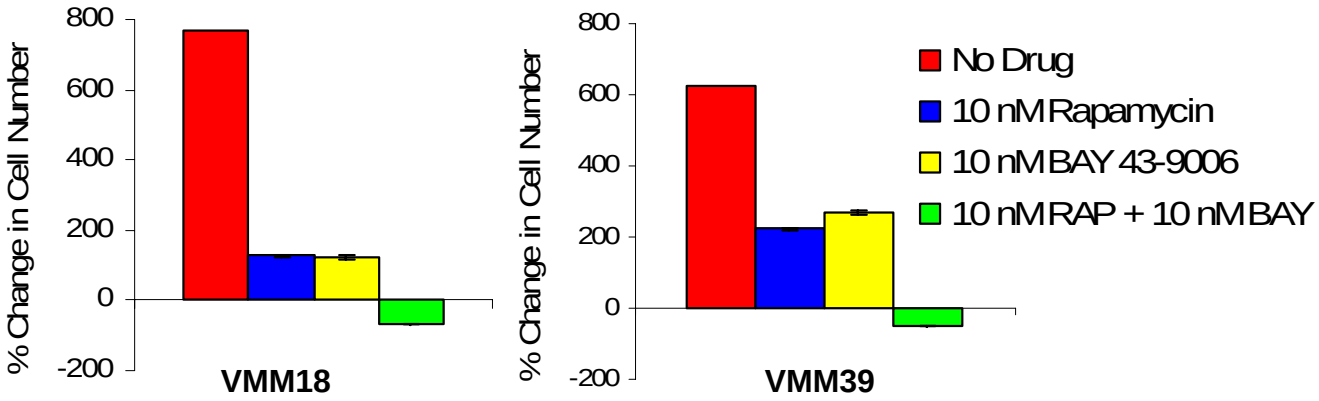
Synergistic inhibition of ERK phosphorylation in human melanoma cells



10nM
24h

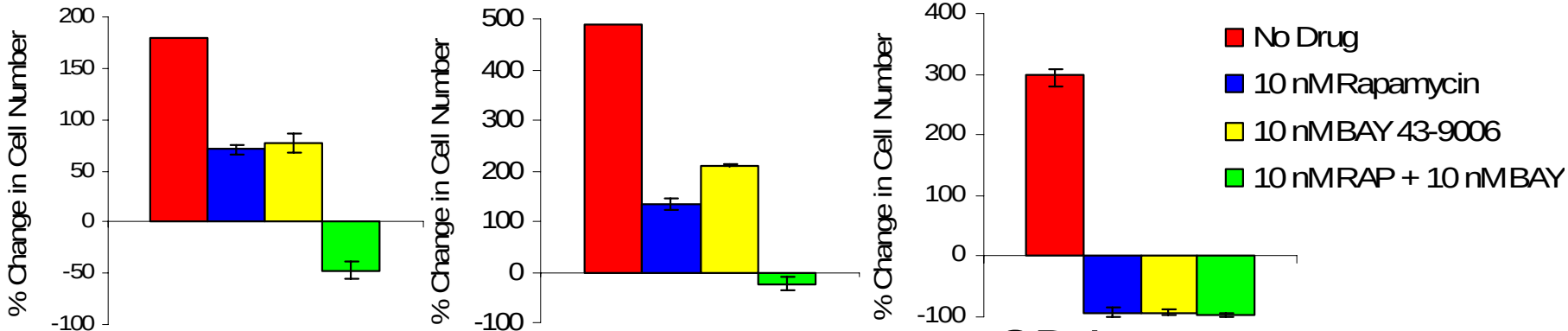
Molhoek KR, Brautigan DL. Slingluff CL.
J. Trans. Med. 2005, 3:39.

Combination Therapy for Melanoma – Immunologic Impact
Rapamycin (mTOR inhibition) & Sorafenib (B-Raf inhibition)



Melanoma Cells

Molhoek KR, Brautigan DL. Slingluff CL.
J. Trans. Med. 2005, 3:39.



CD4+ CD25-

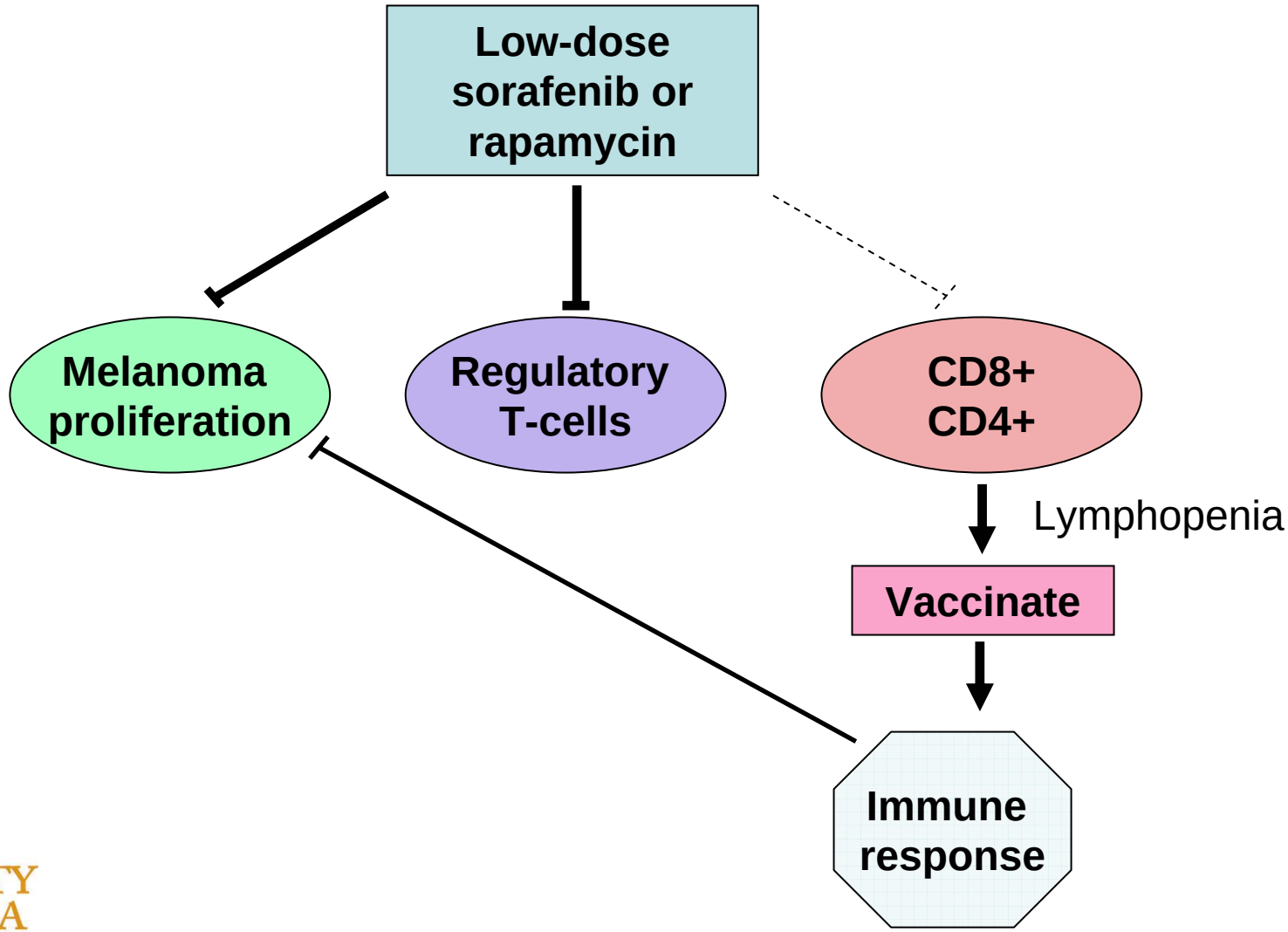
CD8+

CD4+ CD25+

T-Cells

Selective Inhibition of Regulatory T cells

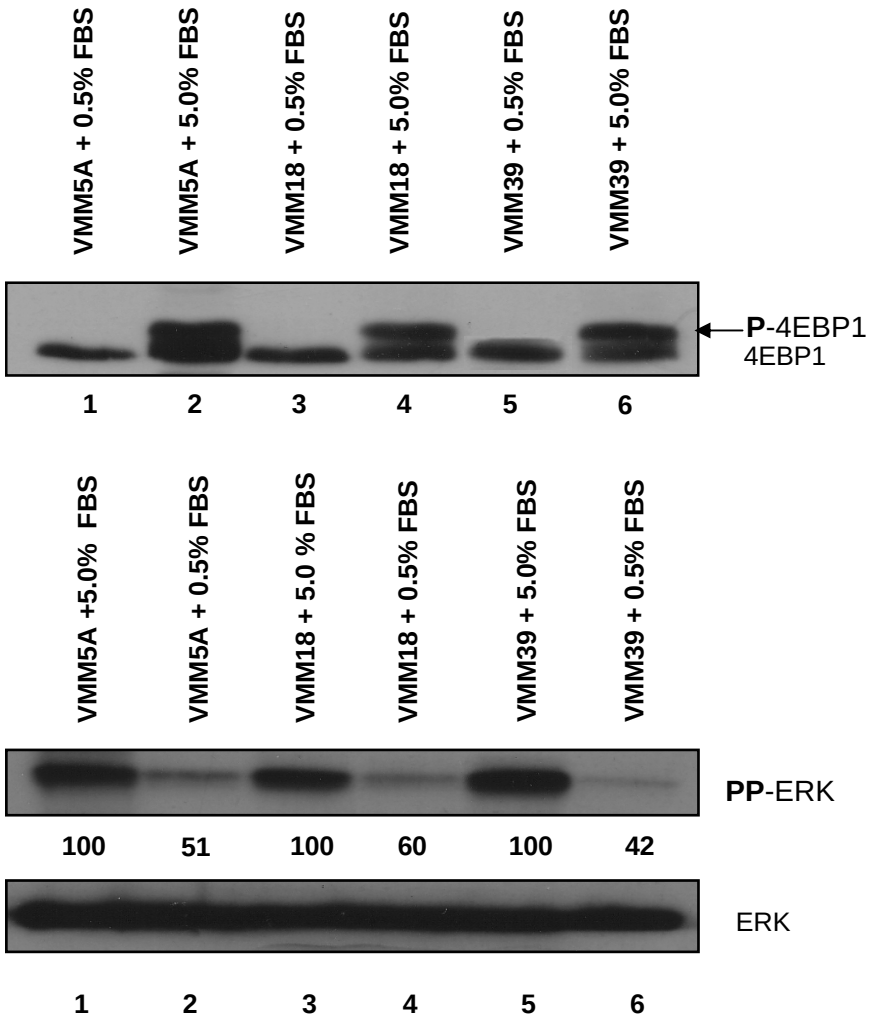
Future Directions: Combination Therapy – low-dose sorafenib or rapamycin prior to vaccine



Discussion

- Next steps to advance the described combination therapies
- Flexible trial designs re: timing and doses
- Rapid translation to clinical trials
- Proof of principle with small trials of specific combinations
- Monitoring biologic effect: Need for clinical trials with tumor collection

Serum-stimulated upregulation of mTOR and MAPK in melanoma cells: Phosphorylation of 4EBP1 and ERK



Molhoek KR, Brautigan DL. Slingluff CL.
J. Trans. Med. 2005, 3:39.

Inhibition of serum-stimulated proliferation of human melanoma cells