Moving T-cell Therapy Forward: Understanding Immune Resistance to Optimize Combination Therapy

Patrick Hwu, MD, Professor and Chairman
Melanoma and Sarcoma Medical Oncology
Leader CCSG Immunotherapy Program
Co-Director Center for Cancer Immunology Research
The University of Texas MD Anderson Cancer Center

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
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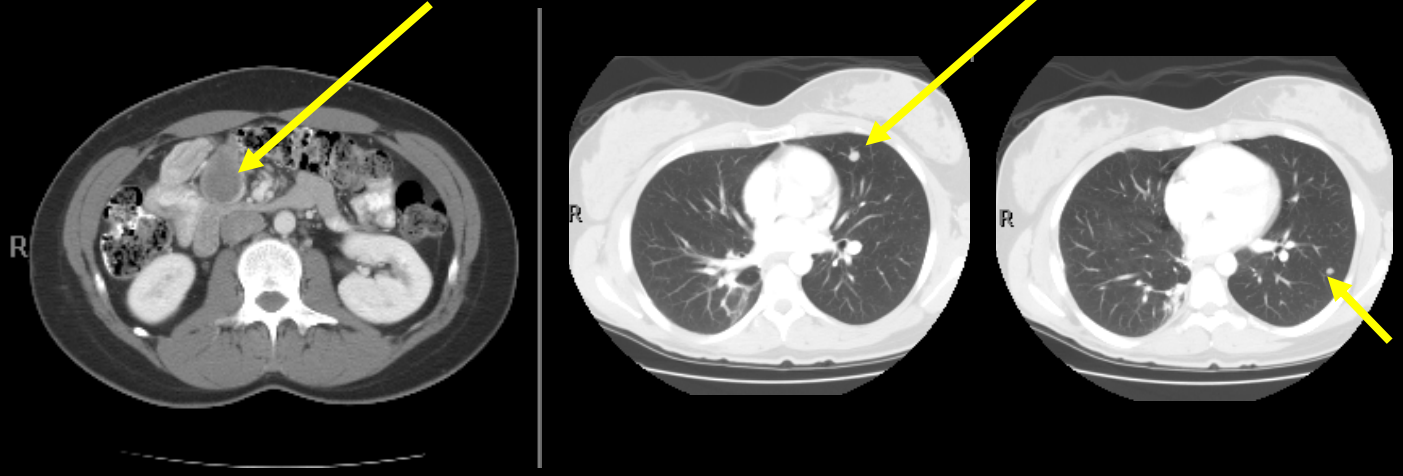
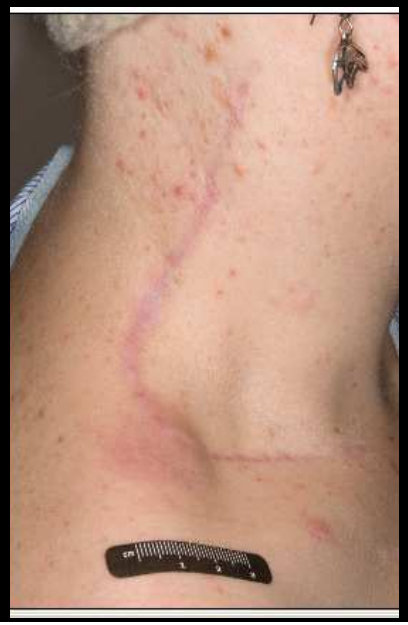
Disclosures

- **Member of Scientific Advisory Board, Lion Biotechnologies**

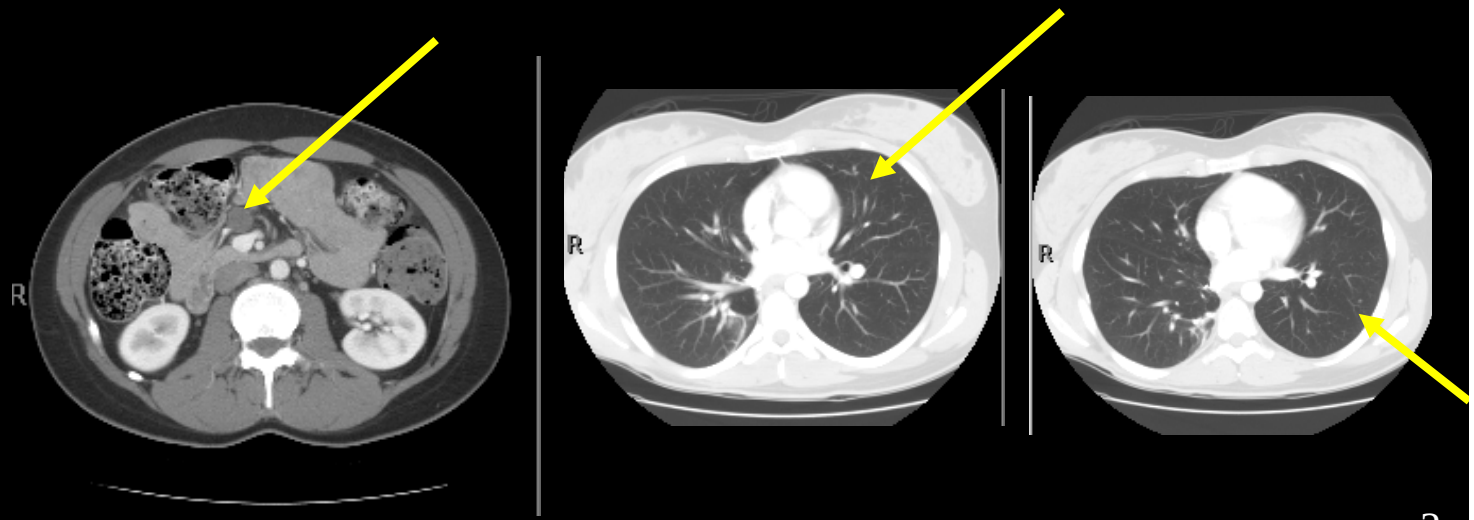
Clinical Response following Lymphodepletion + T-lymphocyte Infusion

Before TIL Infusion





After TIL Infusion



Clinical Response Data from MDACC TIL Clinical Trial

Best overall response:

Number of patients	CR*	PR*	Total
79	4 (5%)	31(39%)	35 (44%)

*Some patients are still undergoing clinical response

Update to data published in
Clin Cancer Res 18: 6758-6770, 2012
Radvanyi … Hwu

Objective Tumor Regression in Patients Receiving Autologous TIL Therapy

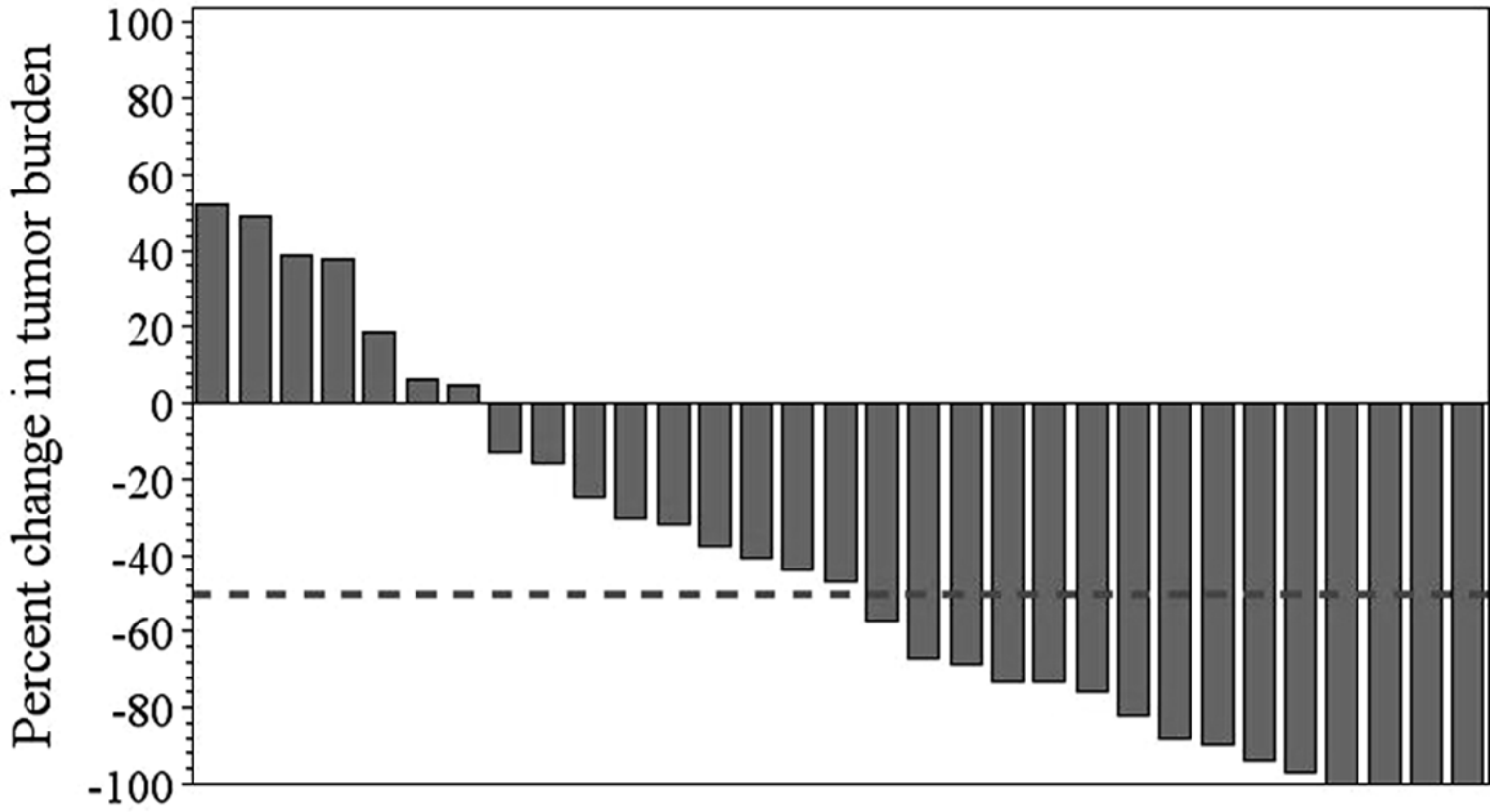


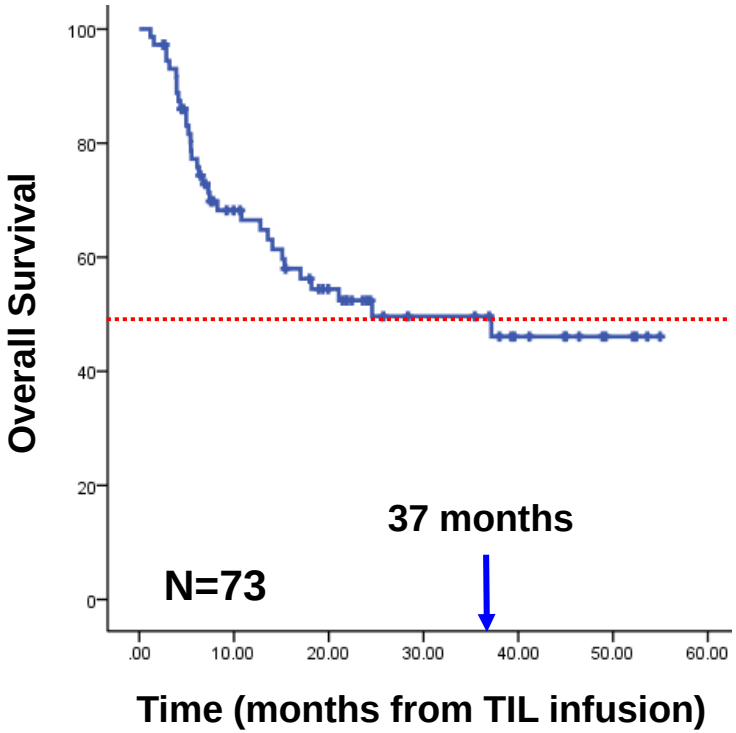
Fig. 1 Waterfall plot of change in tumor burden in treated patients (n=31). Clinical responses were evaluated using irRC from whole body CT scans. The best overall irRC response is shown for all patients. The patients were treated between August 23, 2007 and October 25, 2010.

Radvanyi LG…Hwu P. *Clin Cancer Res* 18(24):6758-70, Dec 2012

Overall Survival After TIL at MD Anderson

Overall survival

(median follow-up 21 months)

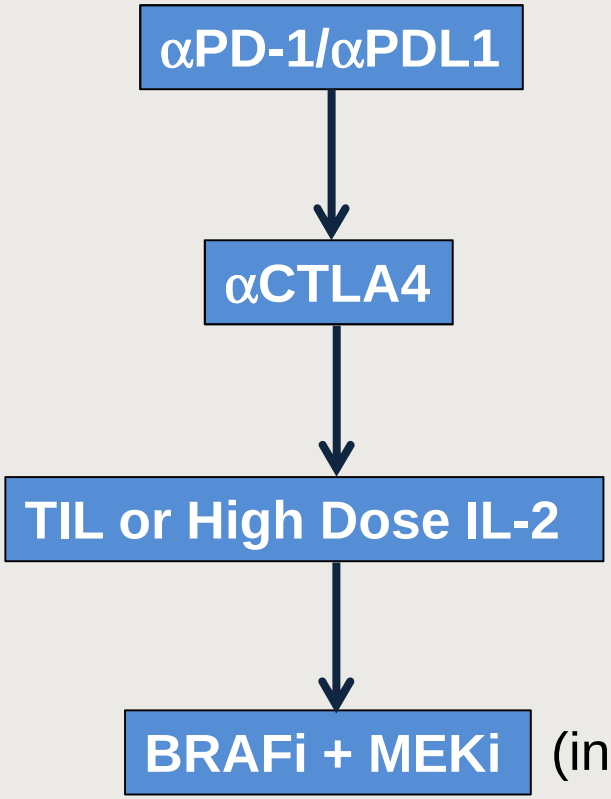


Median OS ~37 months (>3 years)

Major Questions

- Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?
- How can we increase the throughput for this treatment?
- How do we take T-cell therapy to other cancers?
- What distinguishes responders from non-responders?
- What are the best combinations of therapies?

Patients with Slow to Moderate Growing Melanoma with Good Performance Status



(in BRAF V600 mutants)

Clinical Response to TIL After Immune Checkpoint Blockade

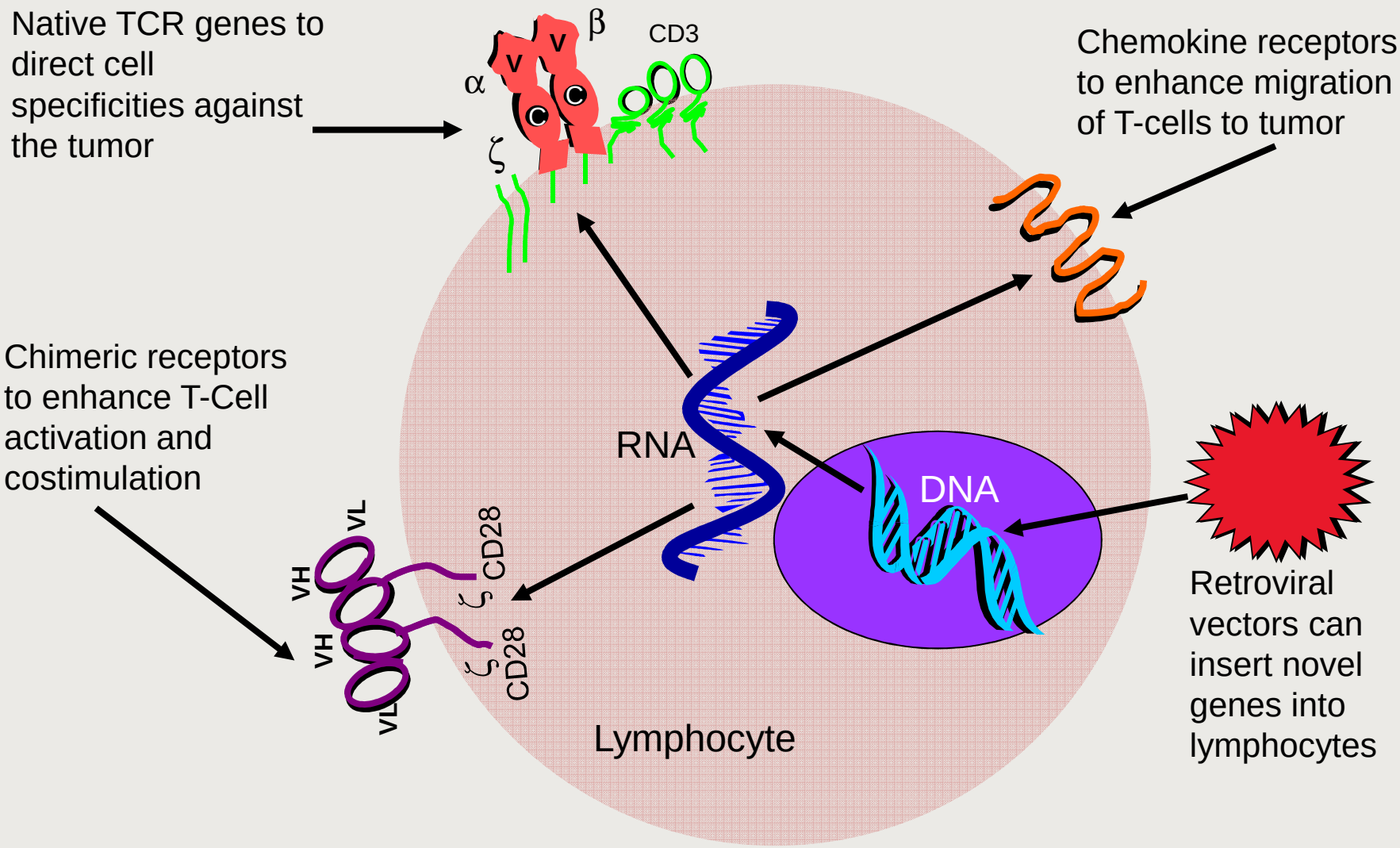
No. Patients	Prior anti-CTLA4	Prior anti-PD1	CR (%)	PR (%)	CR + PR (%)
52	No	No	3	24	27 (52%)
21[1]	Yes	No	1	5	6 (29%)
4[1]	Yes	Yes	0	1	1
2	No	Yes	0	1	1

1 Of the 25 patients treated after anti-CTLA4 therapy, 16 had TIL harvest after anti-CTLA4 (31% response) and 9 had TIL harvest before anti-CTLA4 (22% response)

Major Questions

- **Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?**
- **How can we increase the throughput for this treatment?**
- **How do we take T-cell therapy to other cancers?**
- **What distinguishes responders from non-responders?**
- **What are the best combinations of therapies?**

Insertion of Genes into Lymphocytes to Enhance Antitumor Properties



Major Questions

- **Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?**
- **How can we increase the throughput for this treatment?**
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- **What distinguishes responders from non-responders?**
- **What are the best combinations of therapies?**

Immune Gene Expression Analysis in FFPE Tissues Using NanoString Probe Assay

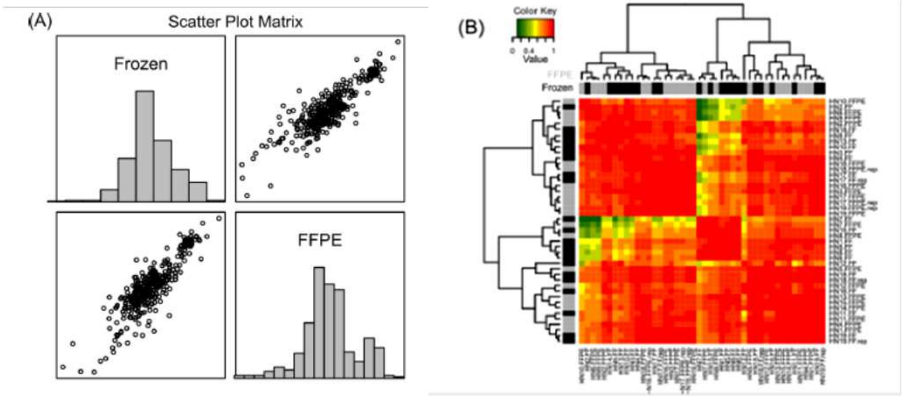
Reis *et al. BMC Biotechnology* 2011, **11**:46
http://www.biomedcentral.com/1472-6750/11/46

BMC Biotechnology

METHODOLOGY ARTICLE **Open Access**

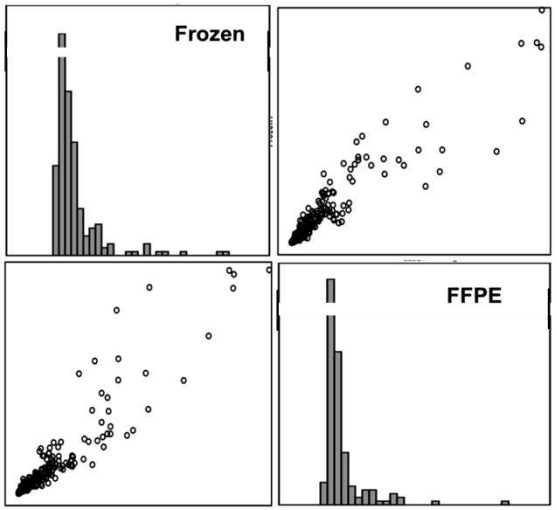
mRNA transcript quantification in archival samples using multiplexed, color-coded probes

Patricia P Reis[1], Levi Waldron[2], Rashmi S Goswami[1,8], Wei Xu[5], Yali Xuan[1], Bayardo Perez-Ordonez[6], Patrick Gullane[7], Jonathan Irish[7], Igor Jurisica[2,3,4] and Suzanne Kamel-Reid[1,5,6,8*]

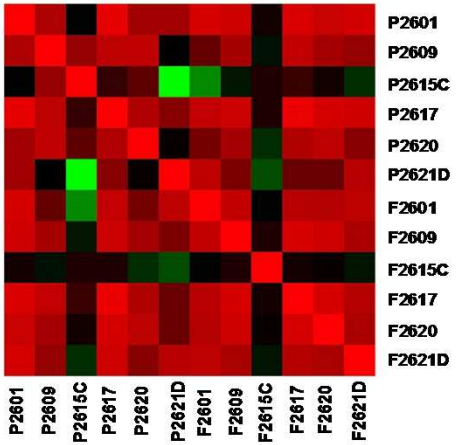


Gene expression in FFPE highly correlated to fresh-frozen tissue

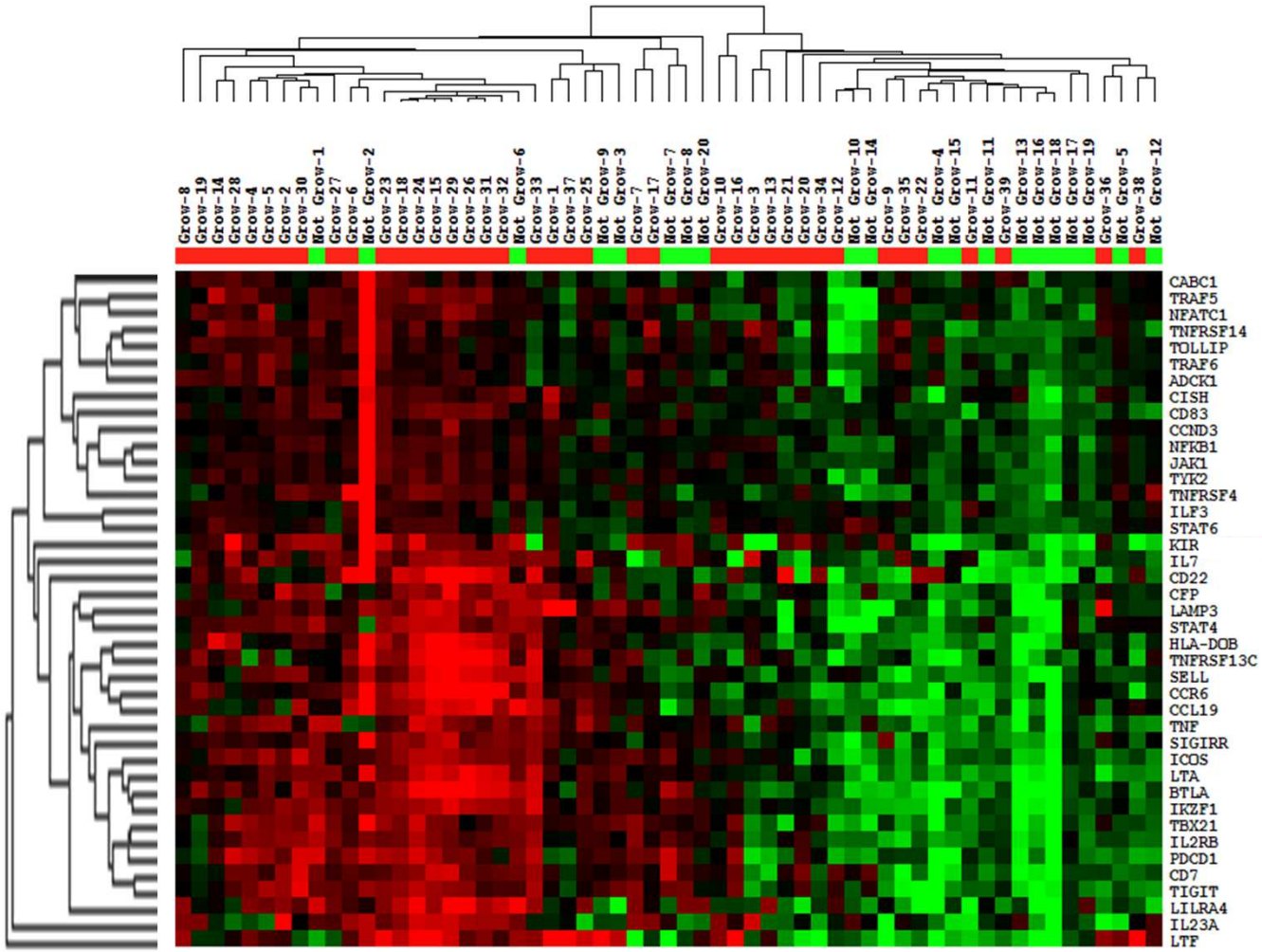
511 Immune gene Scatter plot matrix



$P < 0.0001$; $r^2 = 0.977$



Differentially-expressed Genes in TIL+ vs. TIL- (595 immune gene probe set)



T-cell markers (up):
BTLA
ICOS
PDCD1 (PD-1)
IL-2Rb
TNFRSF14 (LIGHT)
TNFRSF4 (OX40)
CD7

Immune suppression (up):
PDCD1 (PD-1)
BTLA
TIGIT

APC (up):
CD83
HLA-DQB

Innate immunity (down):
TRAF6
TOLLIP
TNF
NFKB1

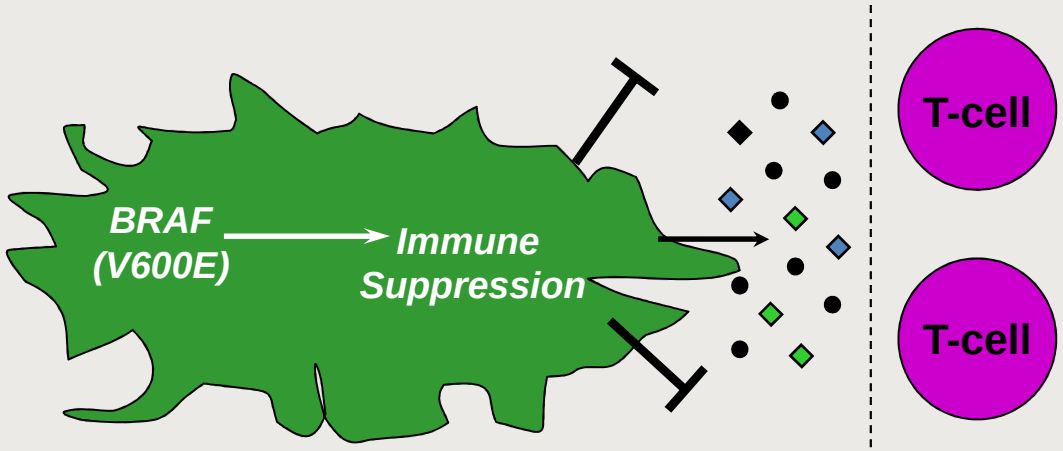
Source: Laszlo Radvanyi/Jie Qing Chen

Major Question

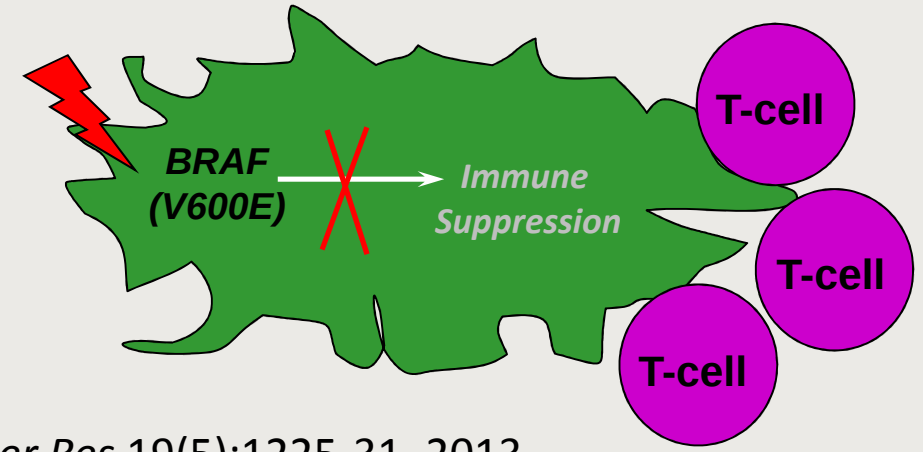
- **What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?**
  - **BRAF/MAPK**
  - **PI3K**
  - **Aurora Kinase**

Combining BRAF(V600E) Inhibition and Immunotherapy

Immunotherapy Alone

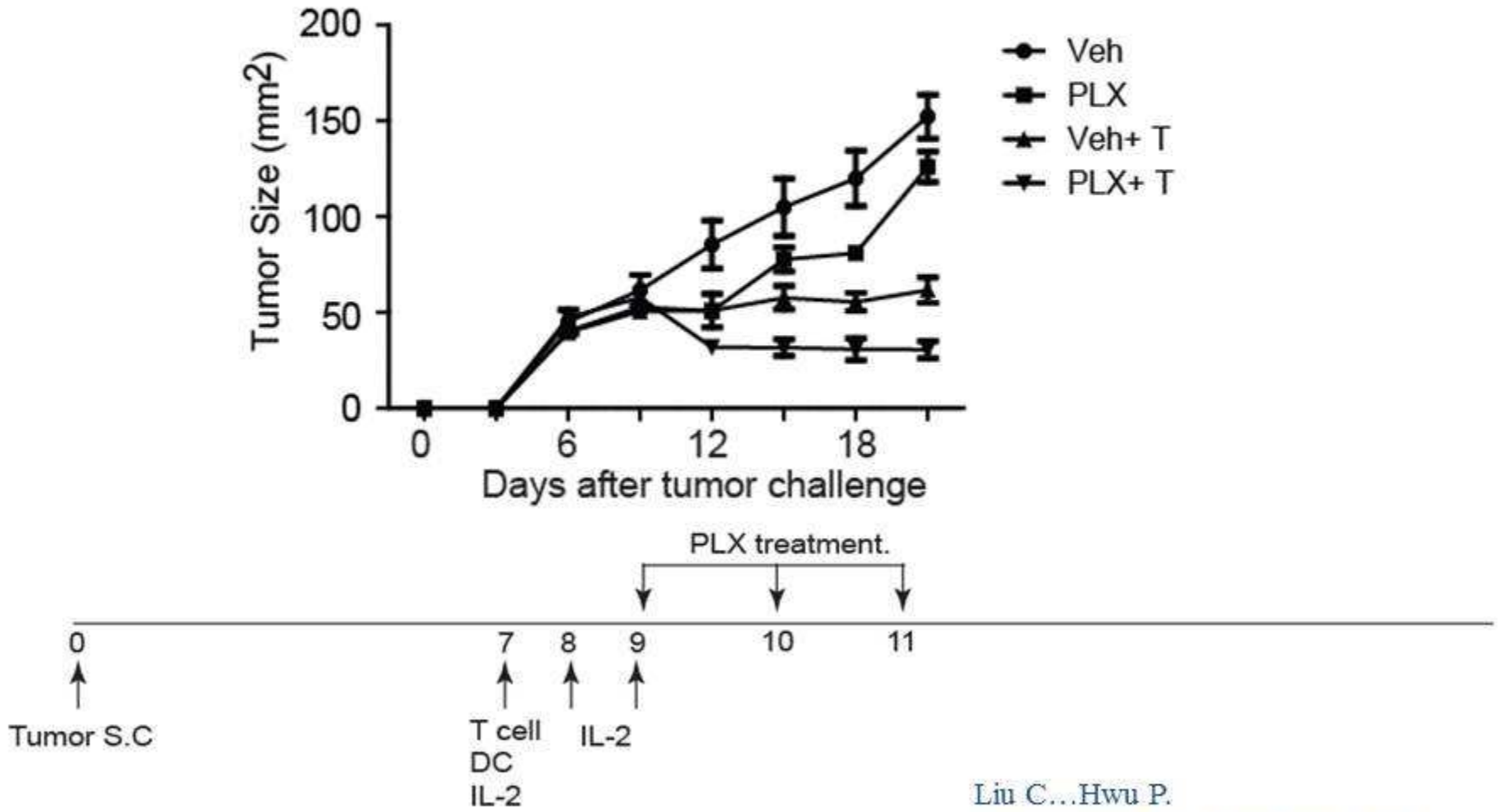


Immunotherapy Plus BRAF(V600E) Inhibition



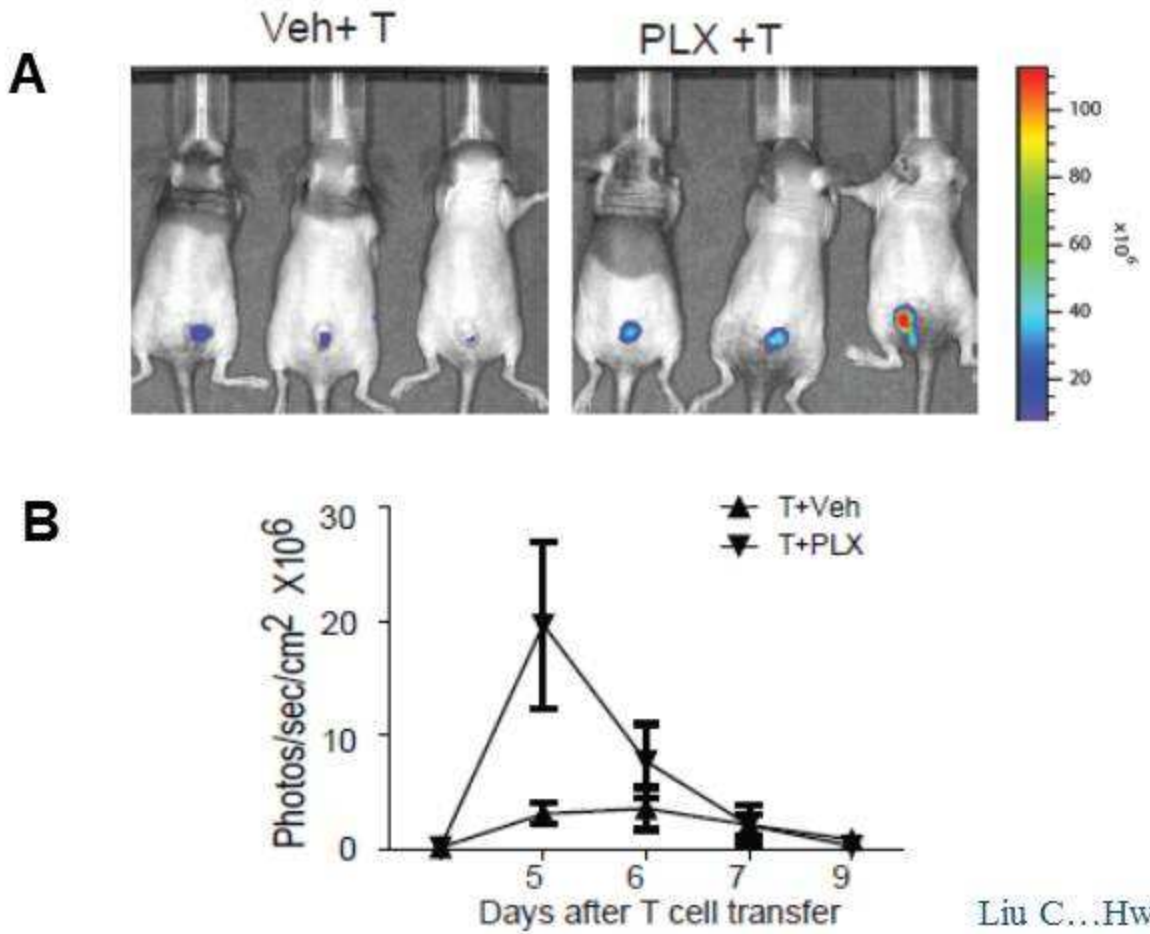
Frederick DT, et al. *Clin Cancer Res* 19(5):1225-31, 2013
Liu C, et al. *Clin Cancer Res* 19:393-403, 2013
Khalili JS, et al. *Clin Cancer Res* 18(19):5329-40, 2012

Combination of PLX4720 with Adoptive T-cell Therapy Leads to Enhanced Anti-tumor Activity (B6 nude mice)



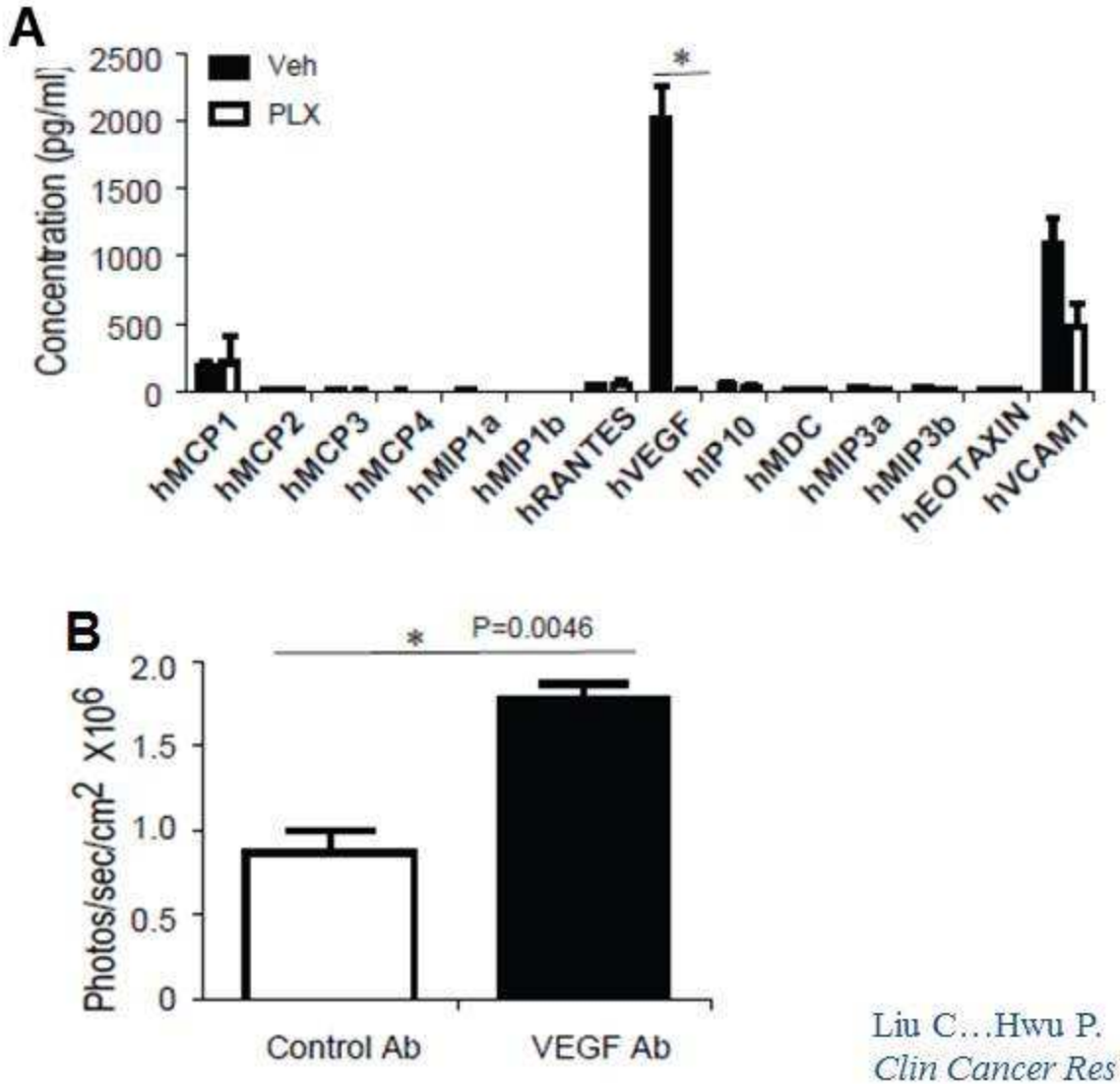
Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

Administration of PLX4720 Increases Tumor Infiltration of Adoptively Transferred pmel-1 T-cells *in vivo*



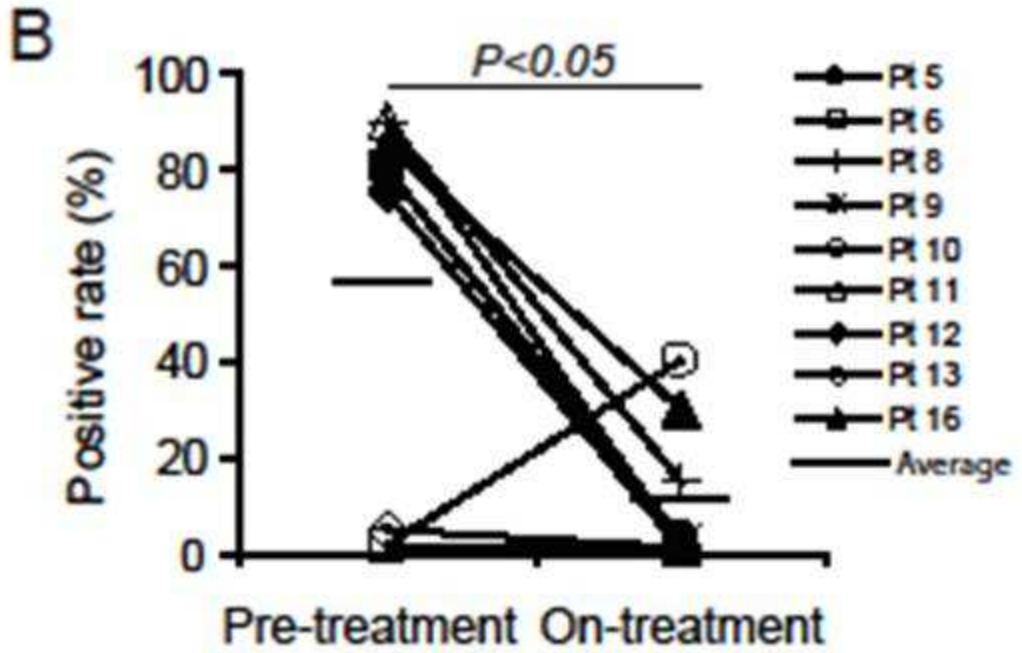
Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

Increased T-cell Infiltration may be Mediated by Inhibition of VEGF Production of Melanoma Cells Treated with PLX4720



Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

BRAF Inhibition Downregulates VEGF at the Tumor Site



Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

Major Question

- **What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?**
  - **BRAF/MAPK**
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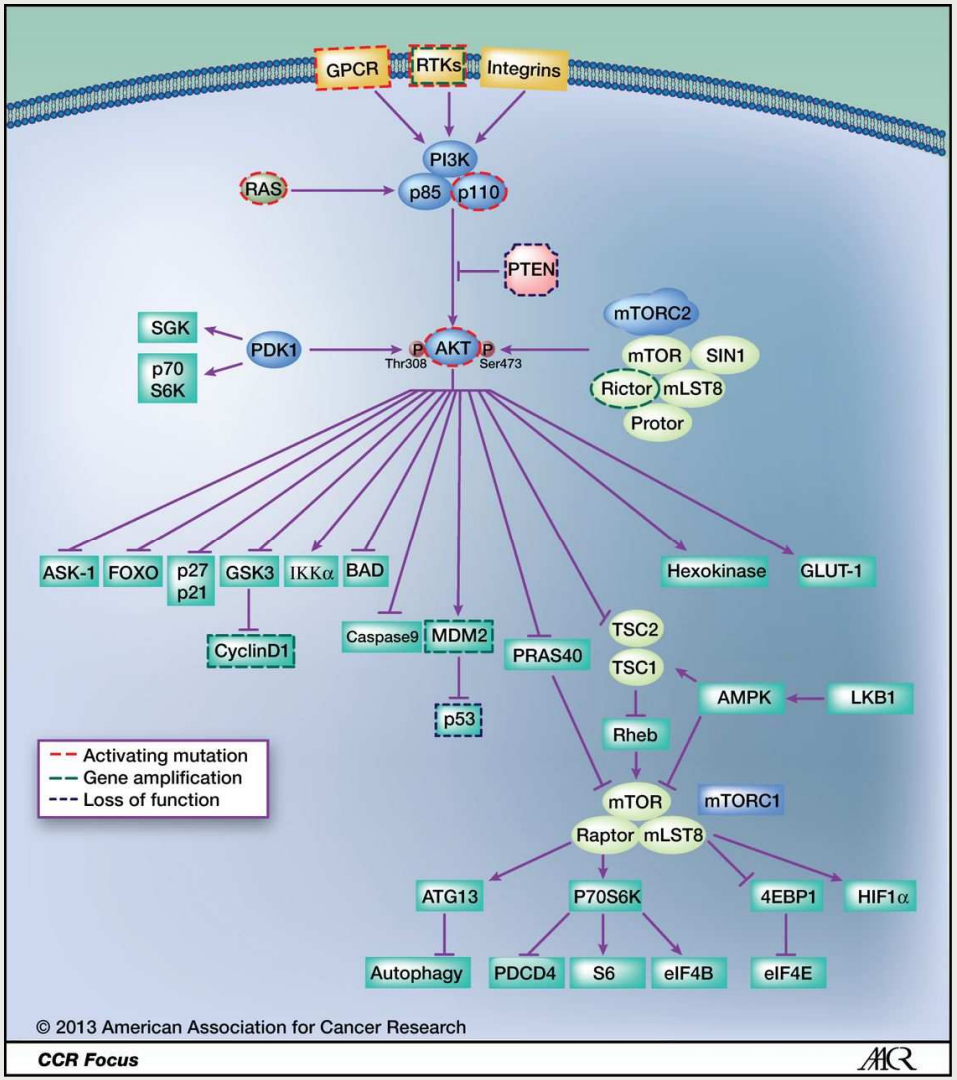
PI3K Pathway Signaling



Kwong LN and Davies MA. *Clin Cancer Res* 19:5310-19, 2013

Generation of PTEN-deficient BRAF Mutated Human Tumor Cell Line

A375

hgp100/H2-Db

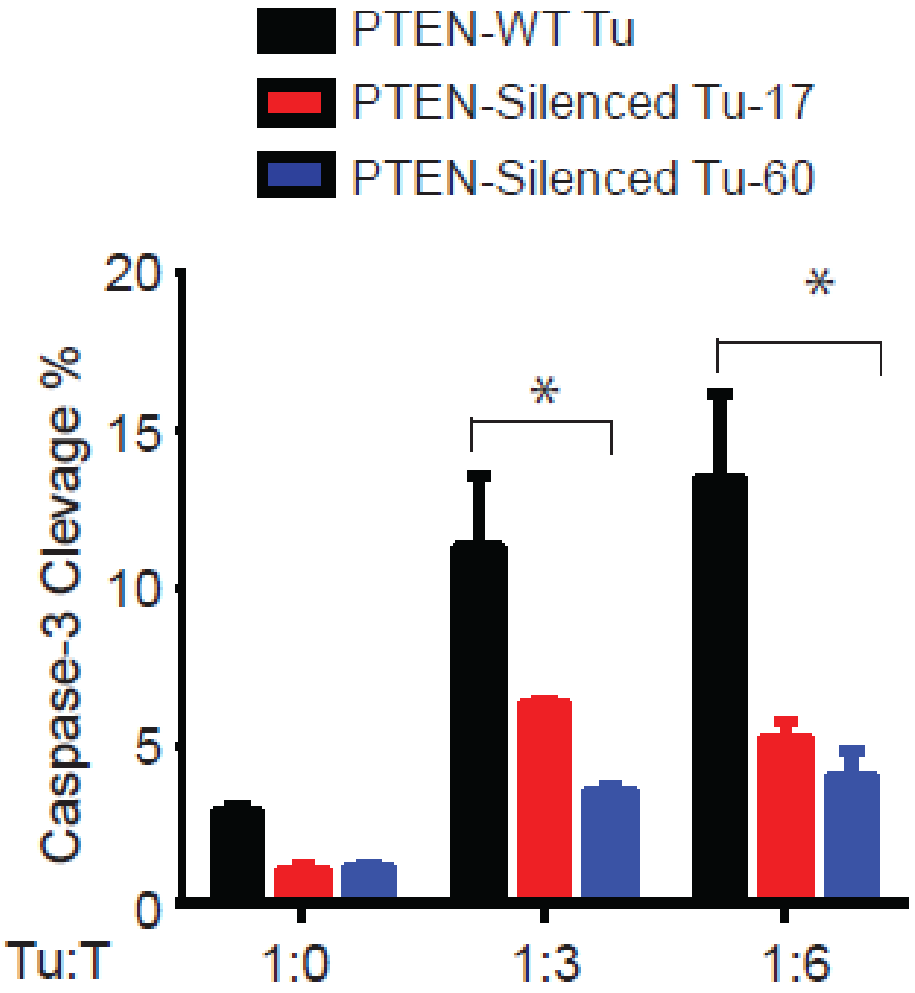
A375/gp

Pten specific shRNA

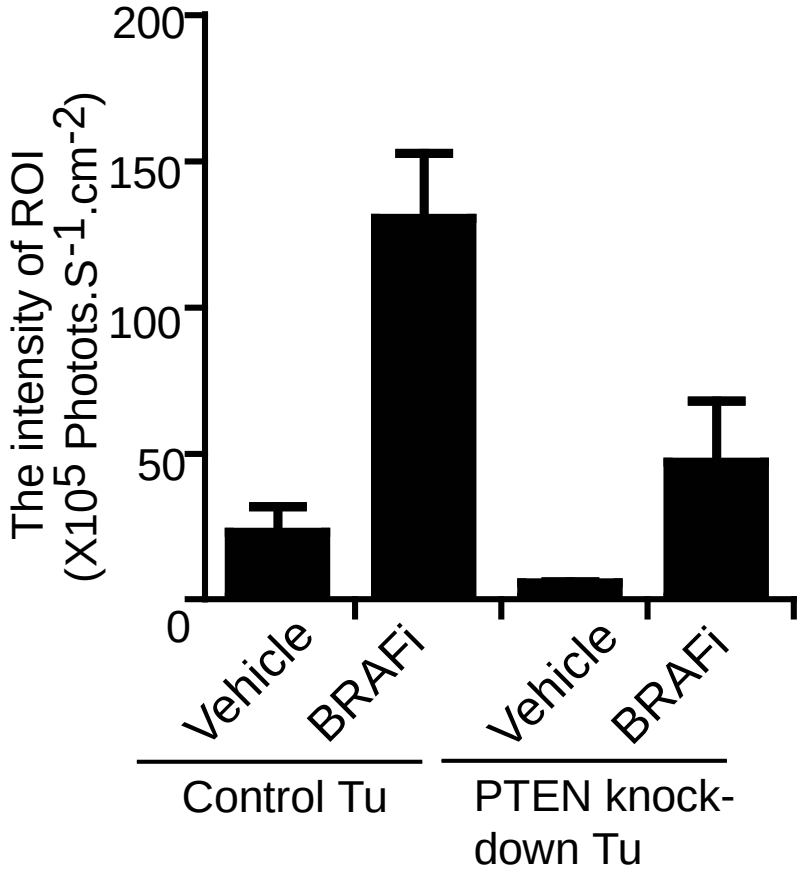
A375/shPten-17 or A375/shPten-60

Weiyi Peng MD, PhD
Instructor, MD Anderson

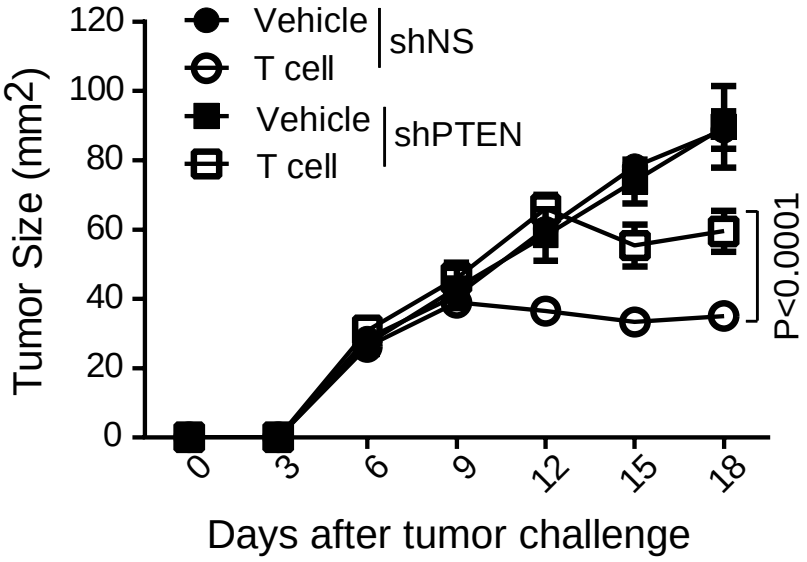
PTEN-specific shRNA Knock Down Induces Resistance of Human Mlanoma Cells to T-cell Killing

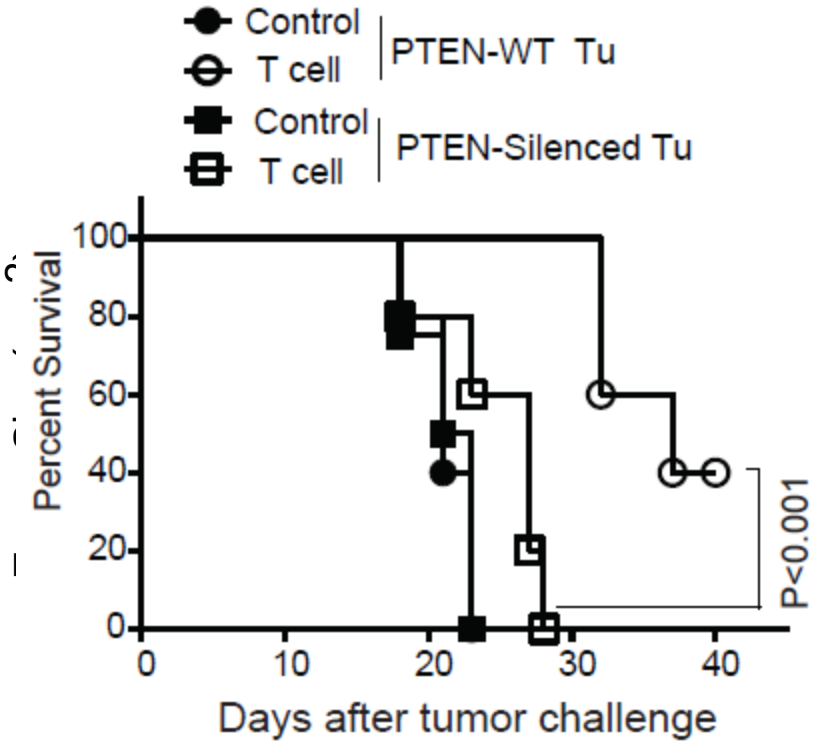


Decreased Infiltration of Transferred T-cells into PTEN-null Tumor



PTEN-silenced Tumor Poorly Responds to T-cell Therapy



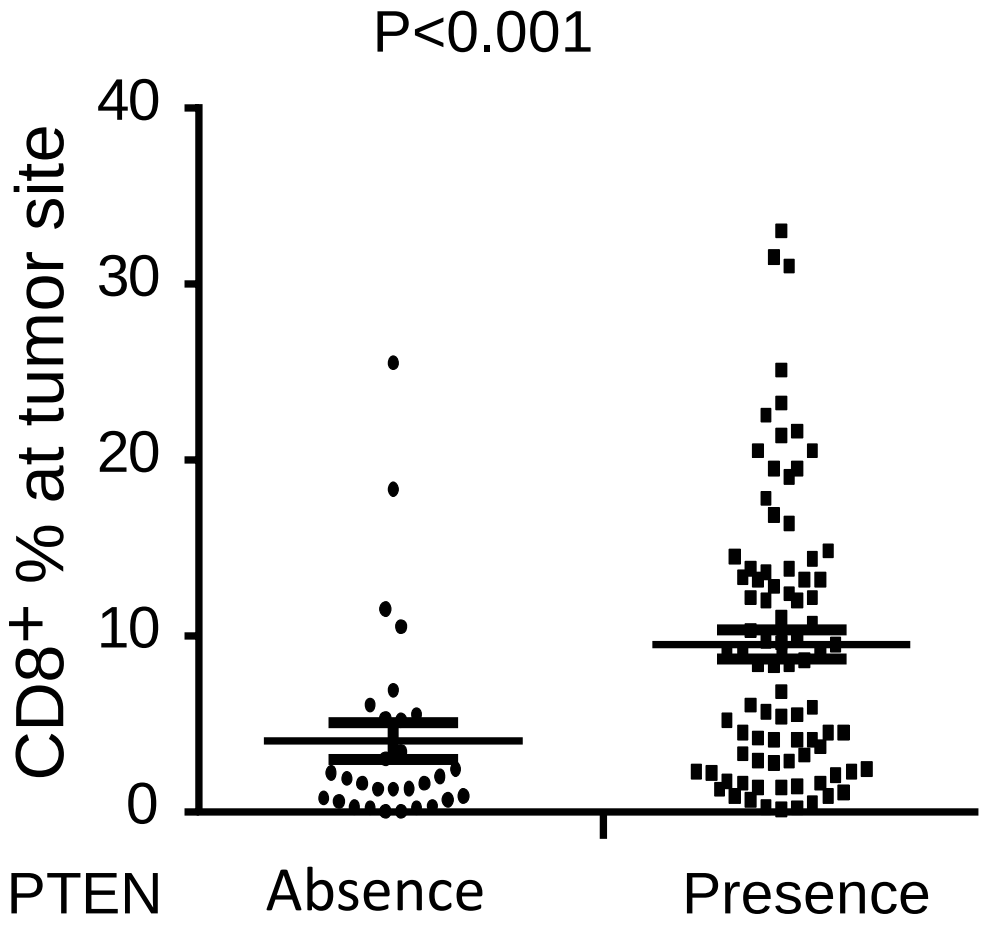


Increase Percentage of PTEN Loss in Tumors from Melanoma Patients with Failed Initial Expansion of TILs

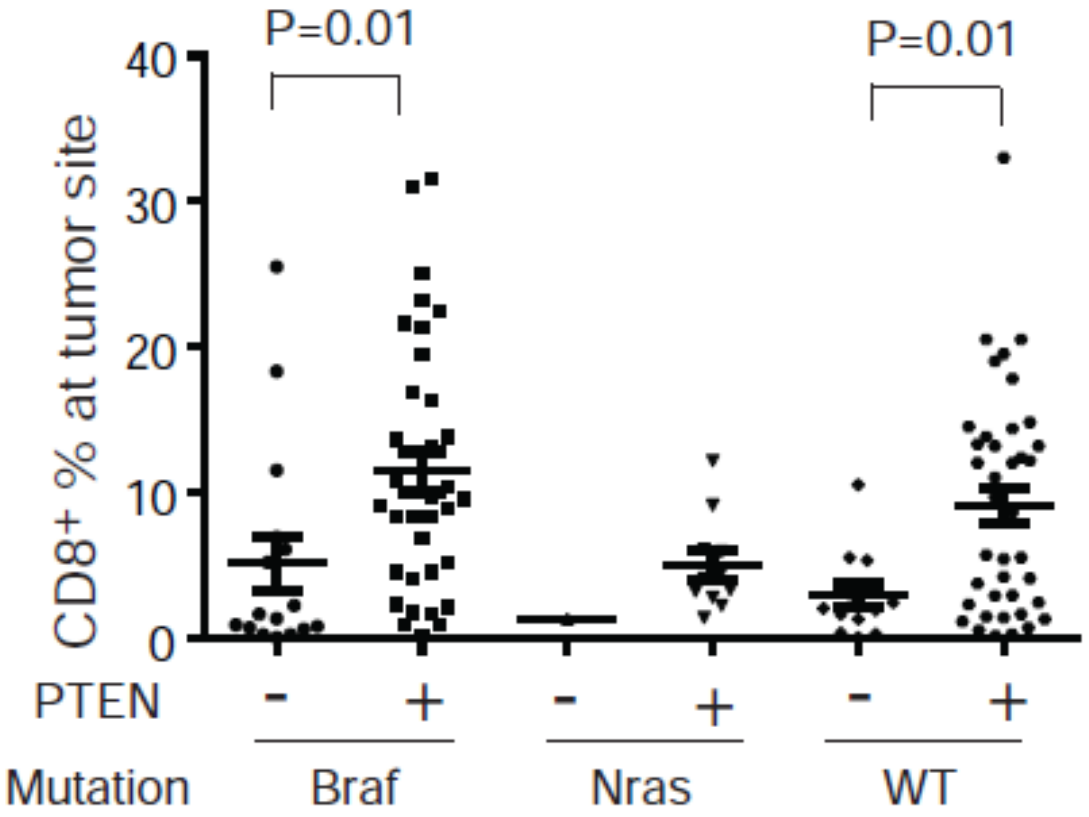
	TIL Growth	No TIL Growth
PTEN Absent	9	11
PTEN Present	72	31
Percentage without PTEN	11%	26%

P = 0.0405

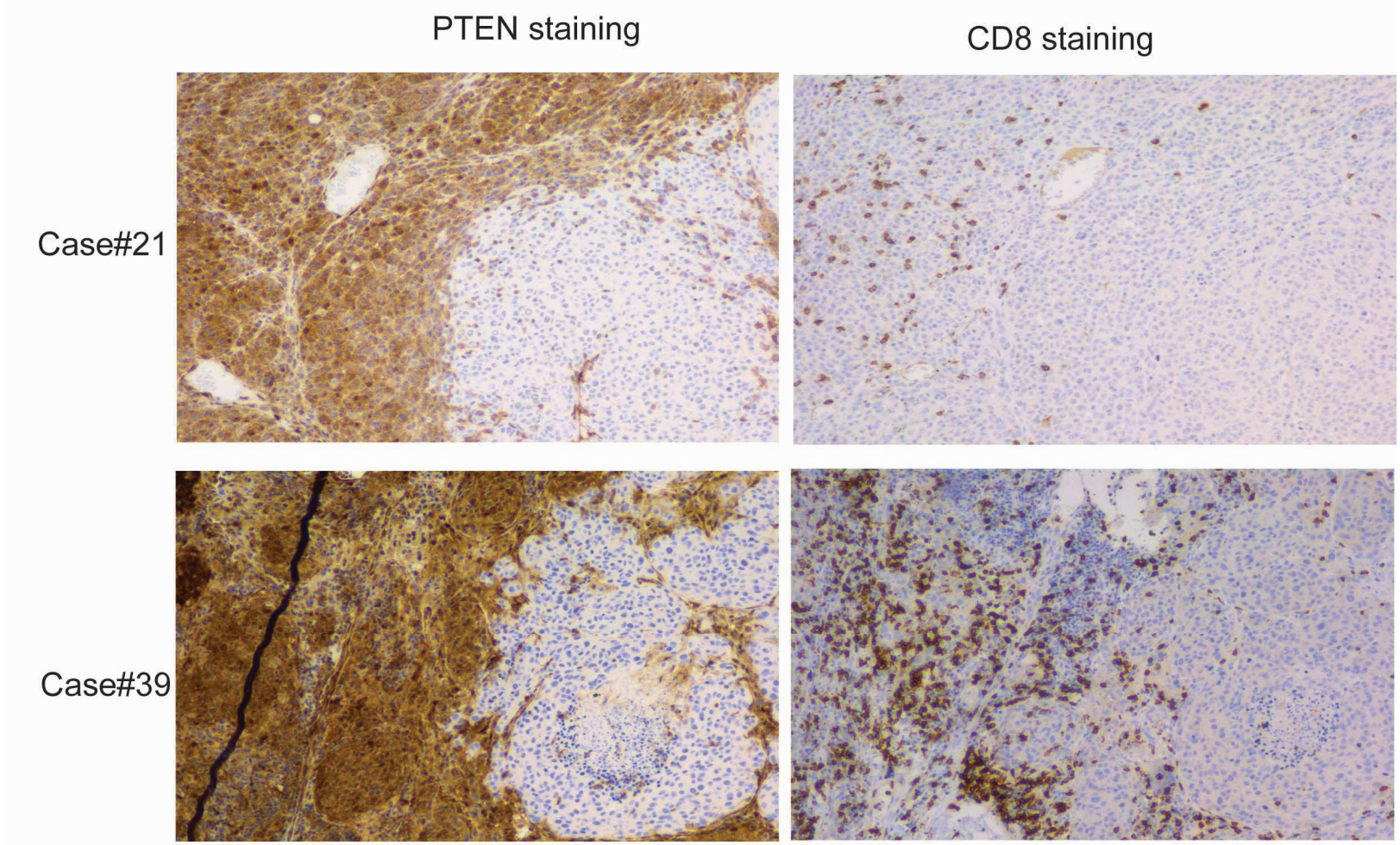
Less T-cell Infiltration in PTEN-loss Tumor in Stage IIIB/C Melanoma Patients



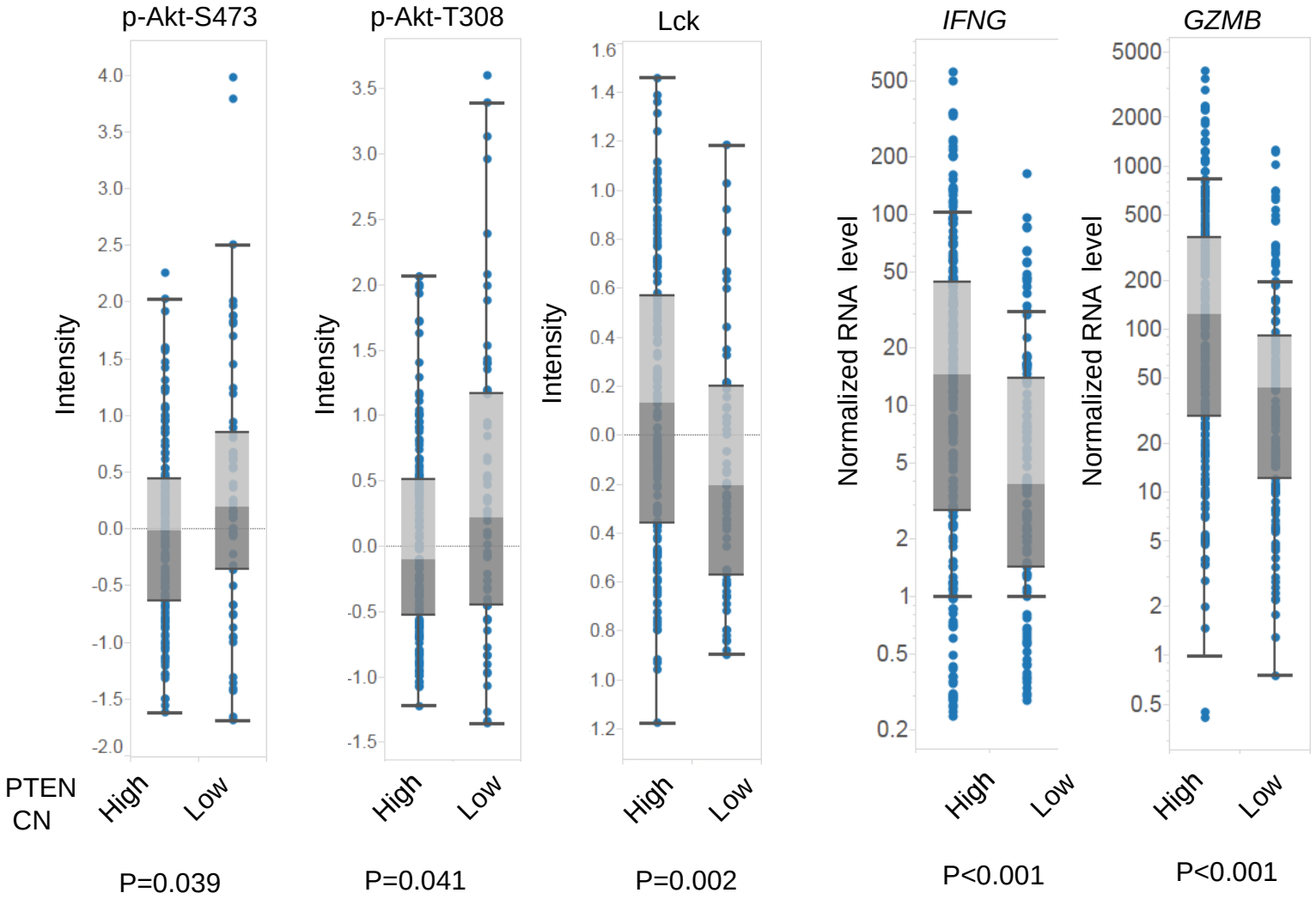
T-cell Infiltration to Tumor is Decreased in Melanomas Lacking PTEN



T-cell Infiltration in Tumor from Patients with PTEN Clonal Expression

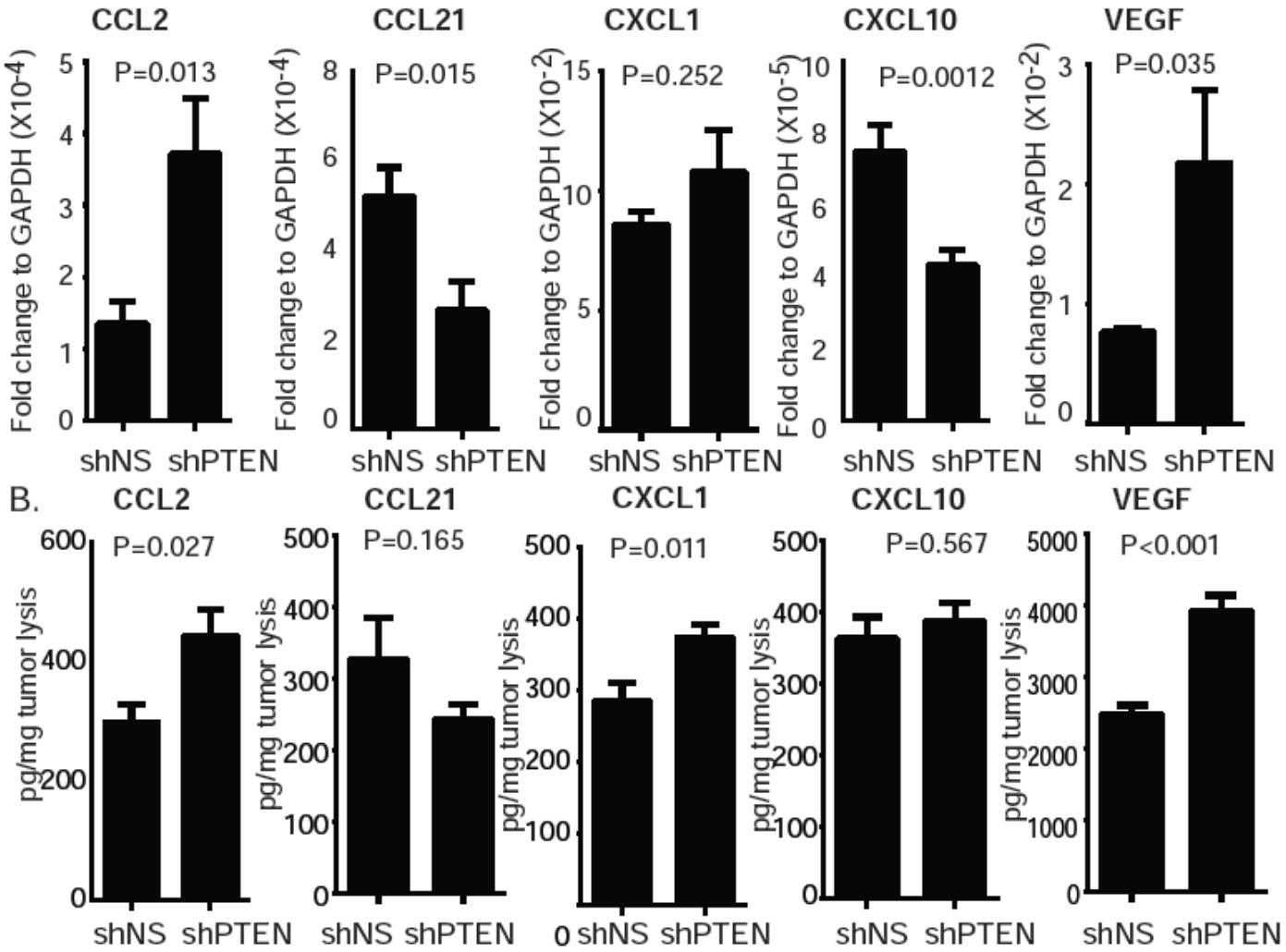


Decreased Number of Infiltrating T-cells in Patients with Low PTEN Copy Number

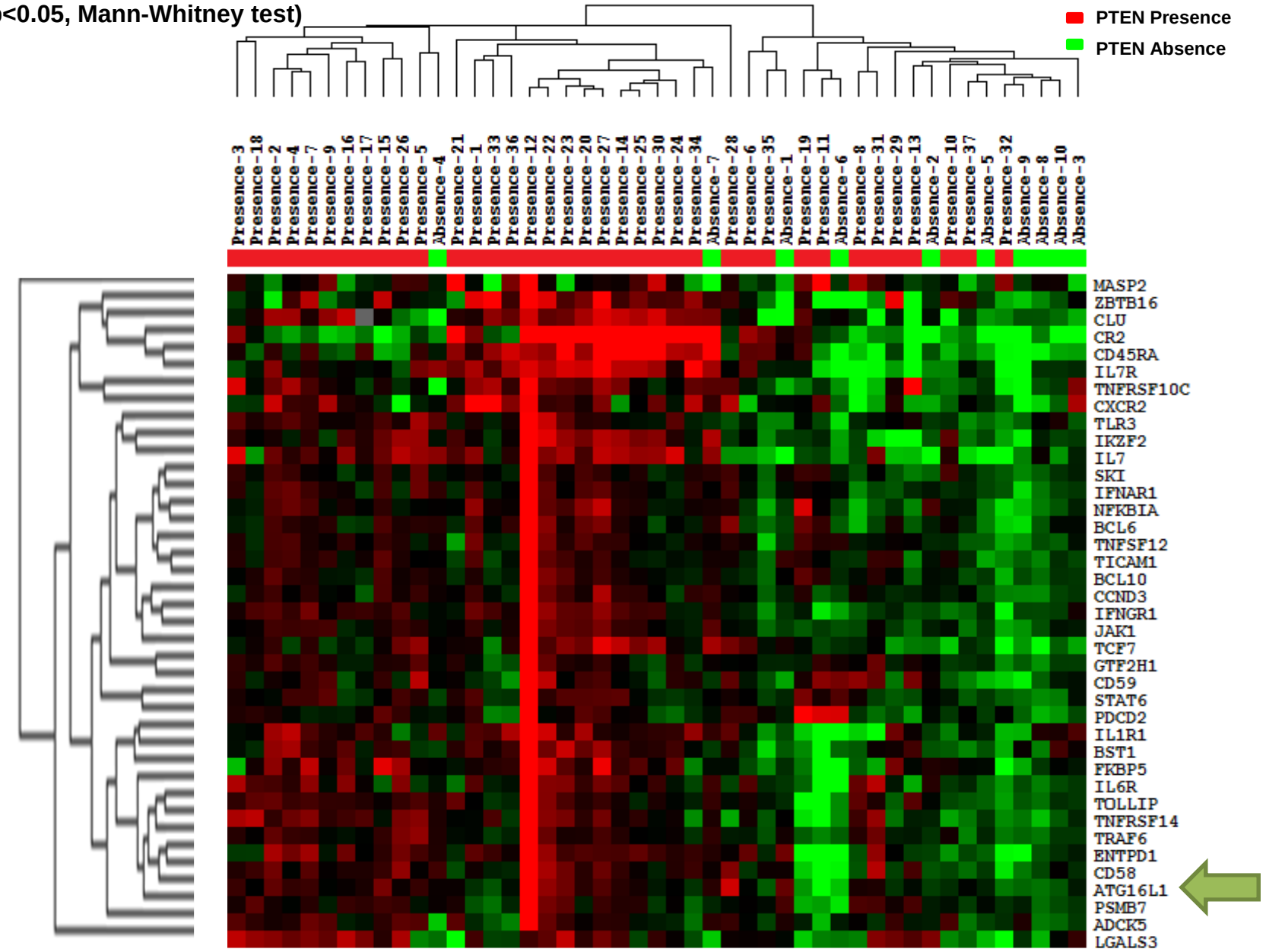


PTEN copy number(CN) low <=0.4
PTEN CN high >0.4

In Vivo Changes in Chemokine Expression following PTEN Knockdown

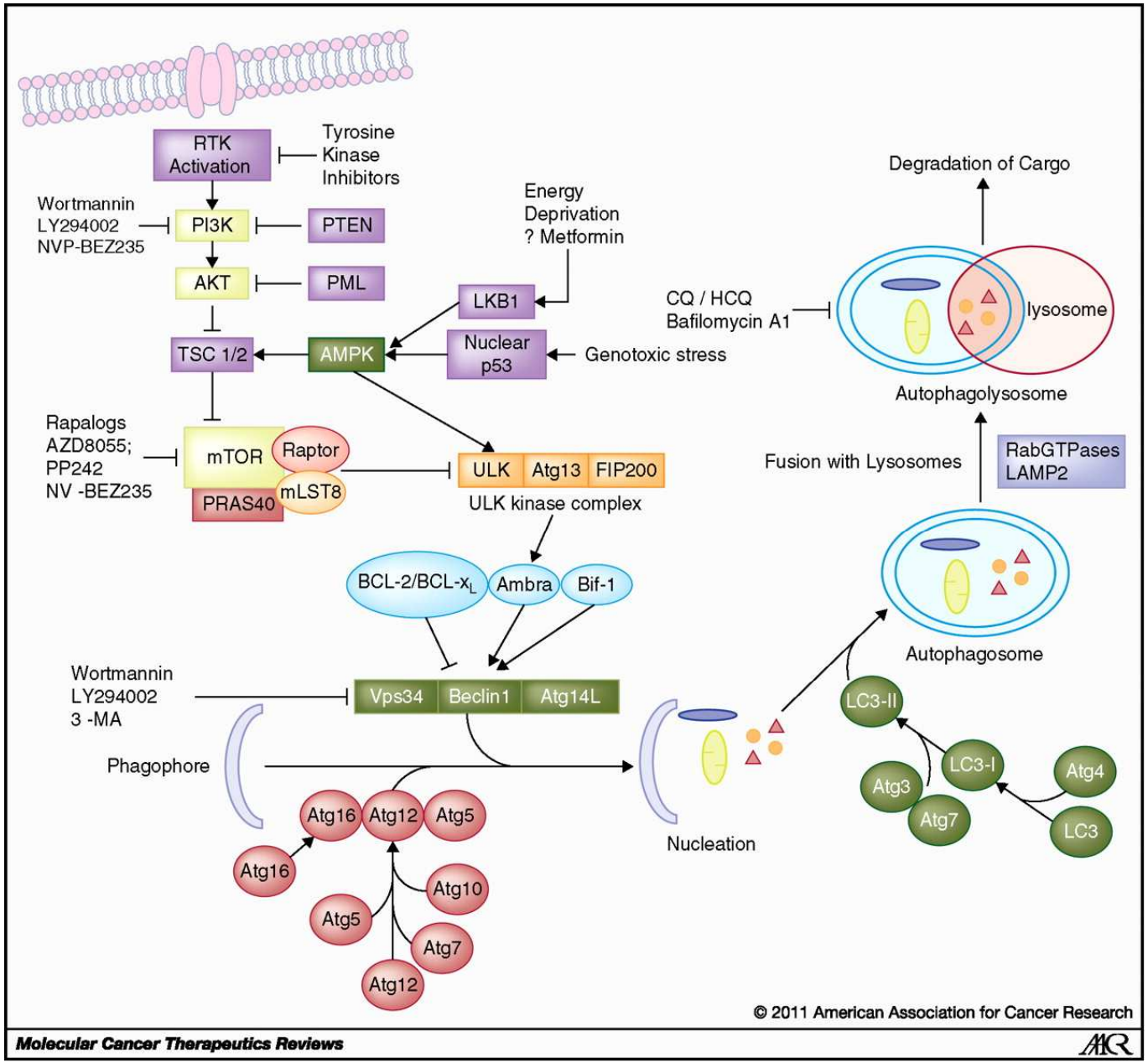


Hierarchical clustering of gene expression using Nanostring data comparing melanomas from 37 PTEN positive and 10 PTEN negative tumors in patients without systemic treatment for the past 2 months (p<0.05, Mann-Whitney test)

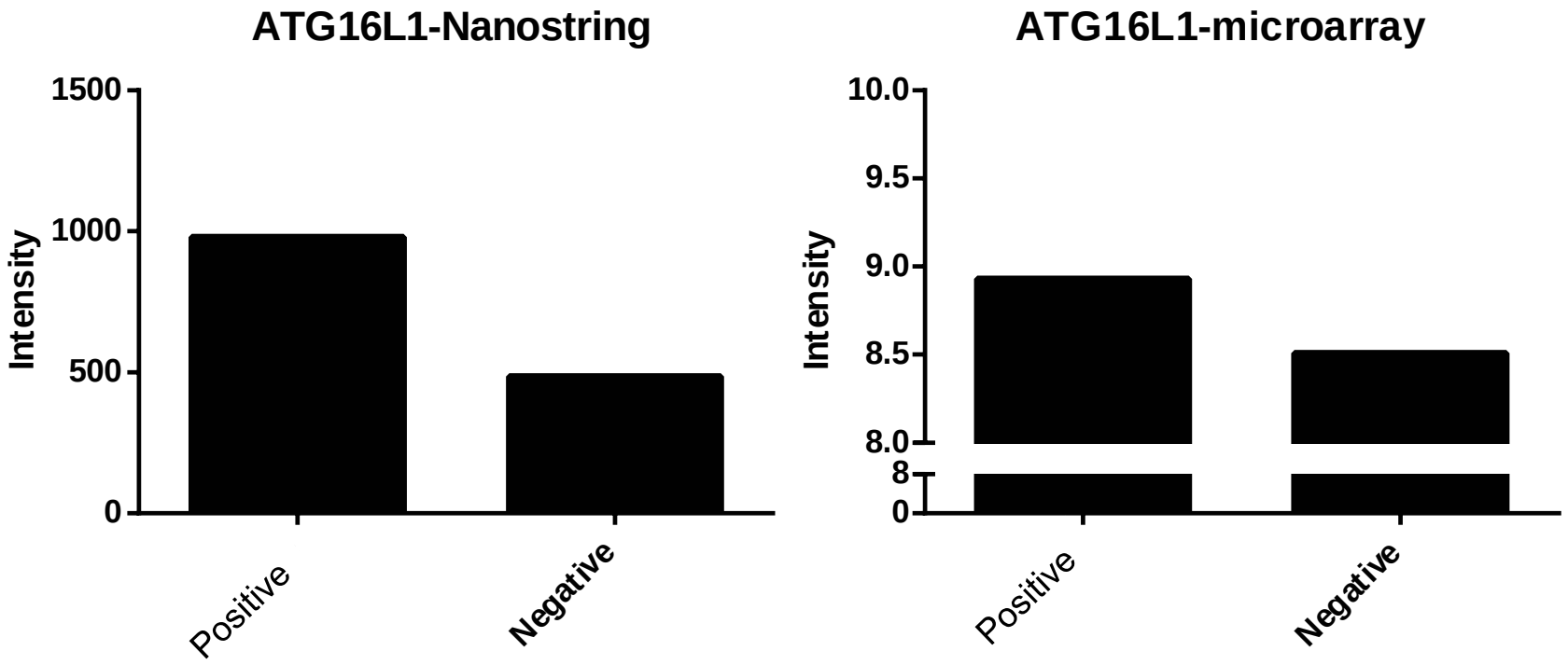


Source: Laszlo Radvanyi/Jie Qing Chen

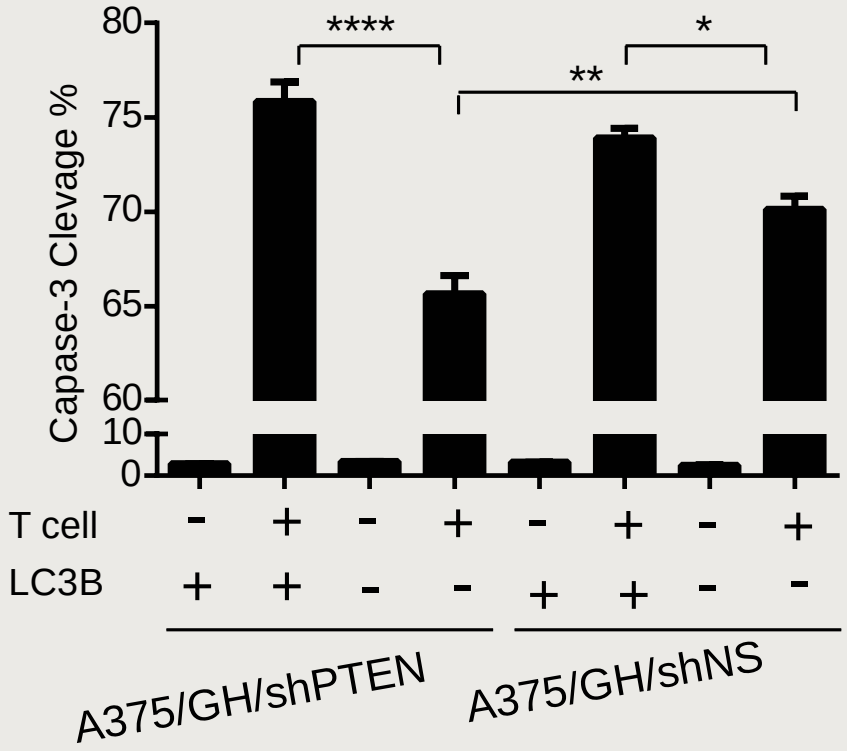
The Autophagy Pathway



Decreased ATG16L Expression in PTEN-loss Tumor



Increased T-cell Induced Tumor Apoptosis by Overexpressing Autophagy Related Genes



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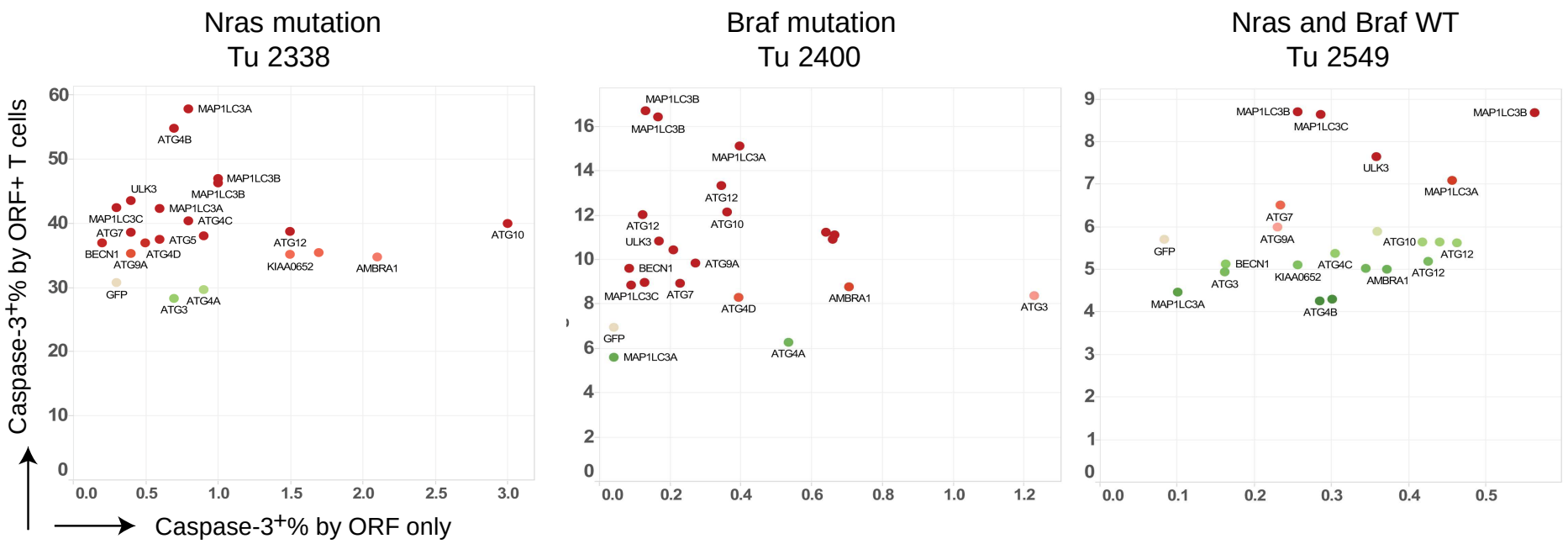
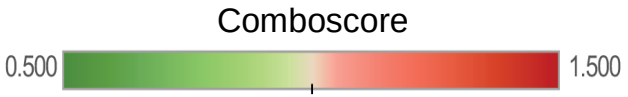
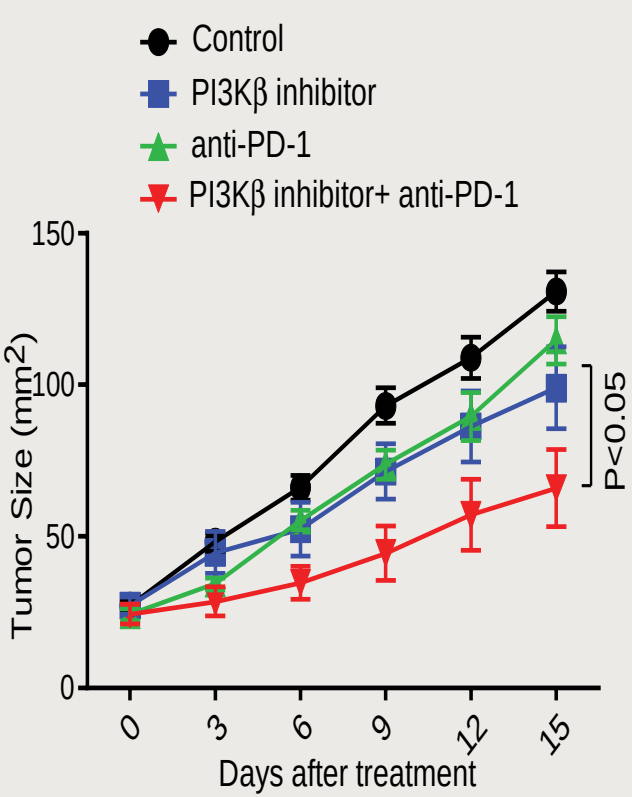
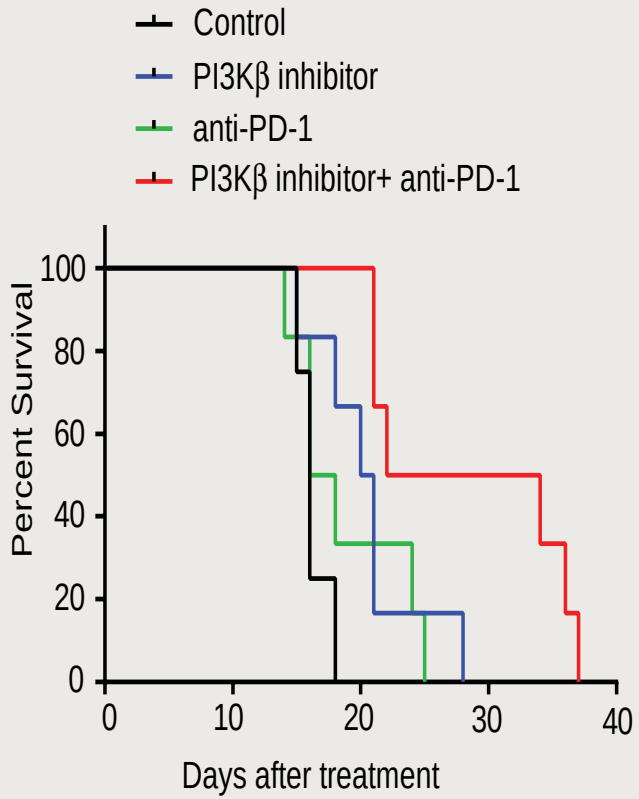


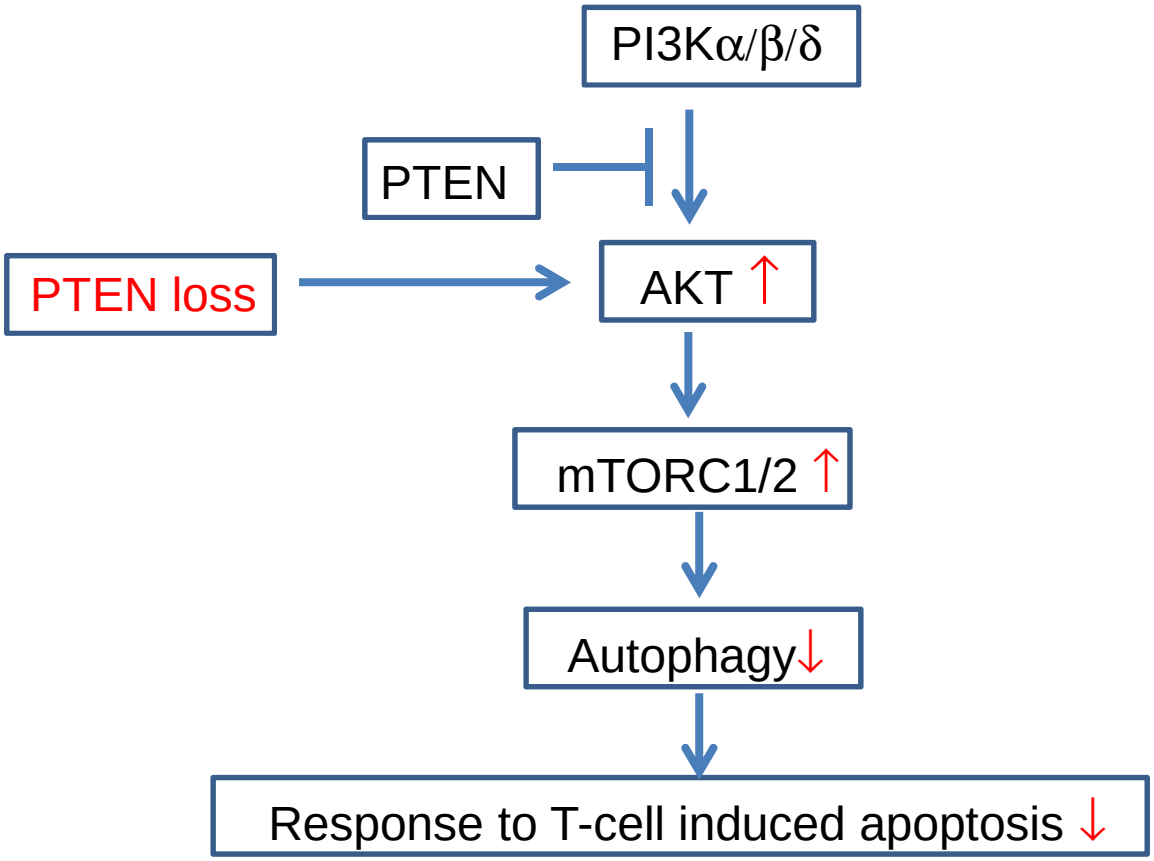


PI3Kβ Inhibitor Improves the Anti-tumor Activity of anti-PD-1 in a Genetically Engineered PTEN Loss Tumor Model





Summary


PI3Kα/β/δ
PTEN
PTEN loss
AKT ↑
mTORC1/2 ↑
Autophagy↓
Response to T-cell induced apoptosis ↓

Major Question

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  - **BRAF/MAPK**
  - **PI3K**
  - **Aurora Kinase**

System to Perform Large Scale Screens Using Autologous Tumor/TIL Pairs and T-cell Mediated Cytotoxicity as a Read Out

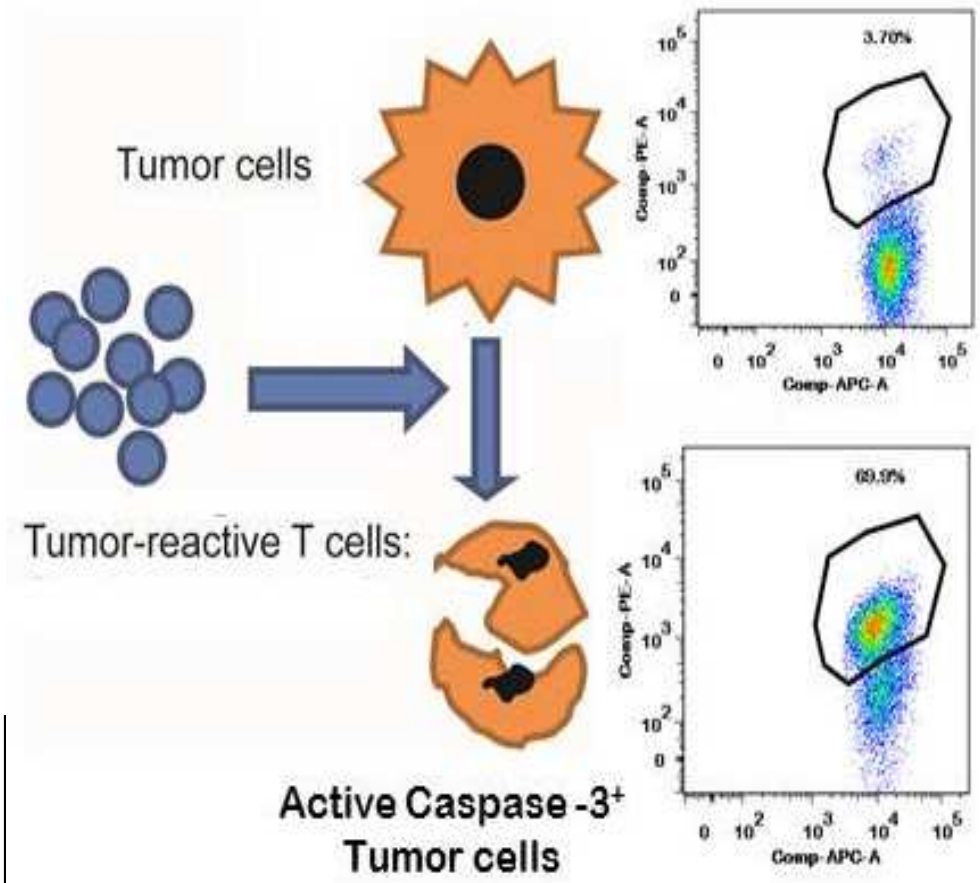


Figure 1: Flow cytometry based T cell cytotoxicity assay for high throughput screen. Depiction of the methodology of T cell cytotoxicity assay. The dot plots for gating and flow cytometric analysis are depicted on the right. Briefly, patient derived melanoma tumor cells are co-cultured with reactive autologous T cells, followed by intracellular staining for active Caspase-3. The % cytotoxicity is measured by % active caspase-3 positive tumor cells.

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research

Unbiased Screen #1: Large Scale Drug Screen

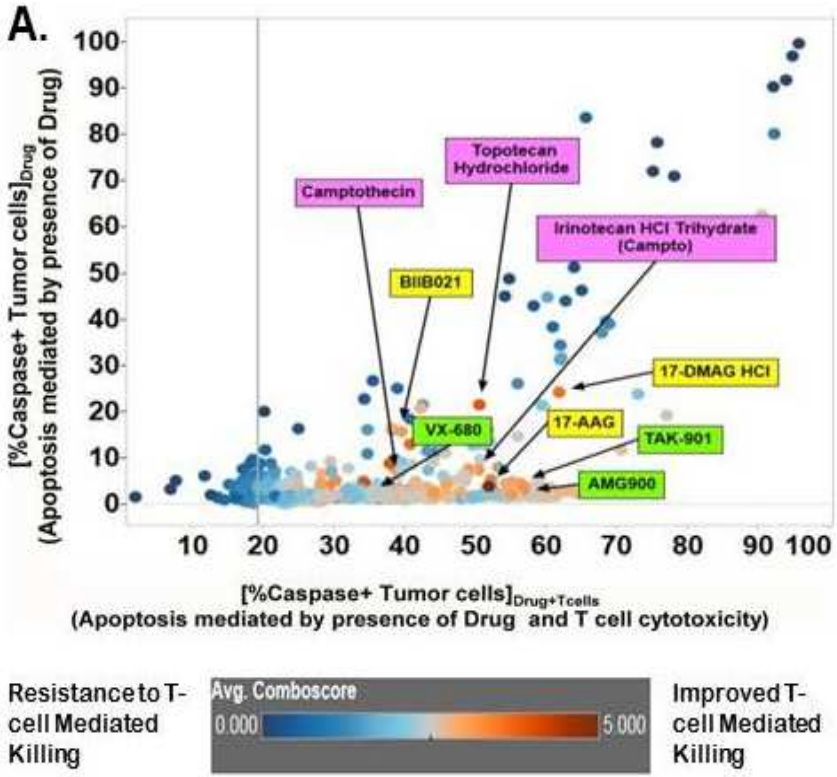


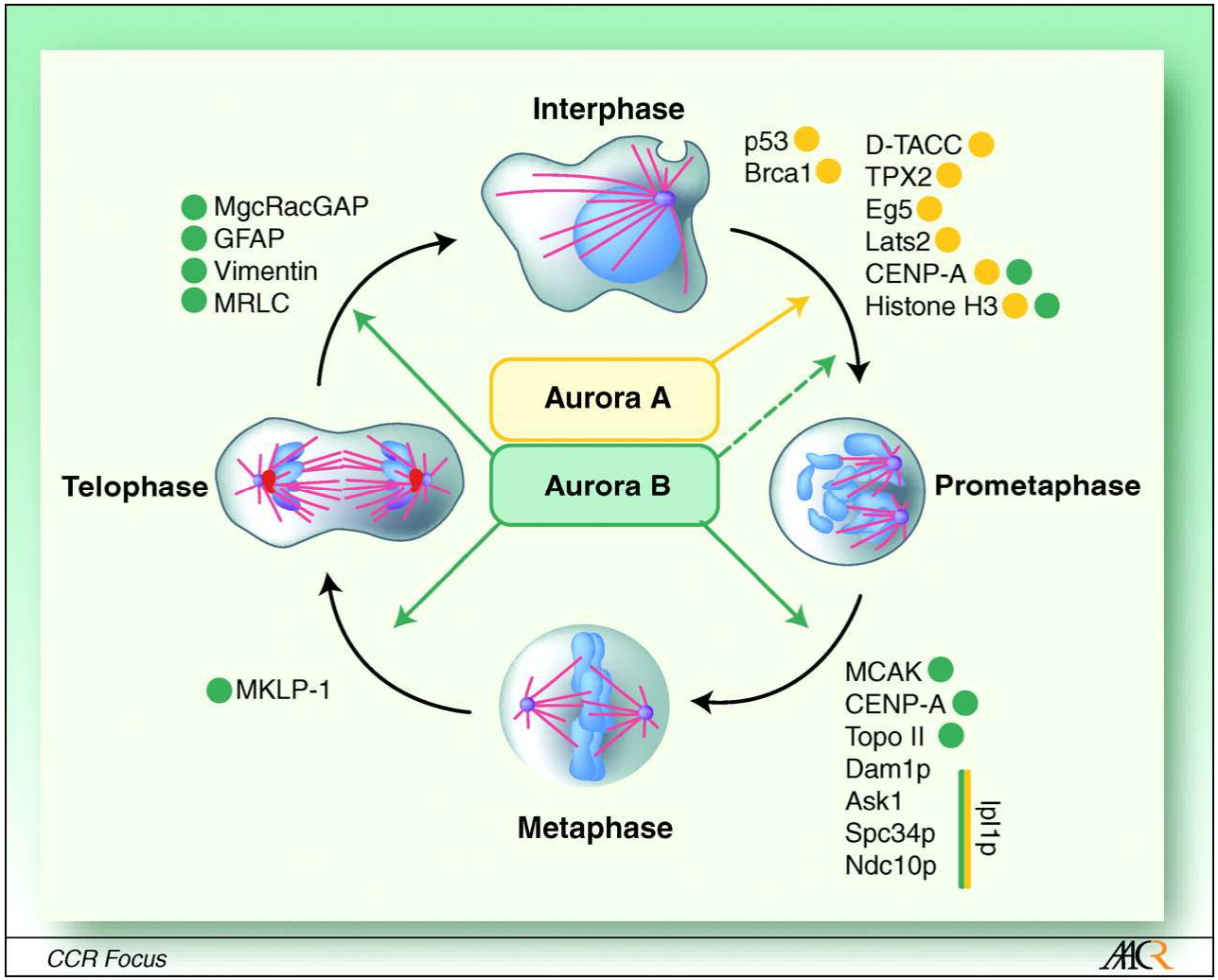
Figure 2: Aurora Kinase inhibitors were identified in an unbiased screen to display synergistic effects with T cell mediated anti tumor cytotoxicity. (A). The comboscores of different bioactive compounds in a representative drug screen using a patient-derived melanoma cell lines. The color bar below is the key for comboscores. **(B)**. Definition of comboscore. The drugs with the highest comboscores i.e. highest synergy potential are indicated by arrows and include Aurora Kinase inhibitors in green ( ).

B.

$$\left\{ \frac{[\%\text{Caspase}^+\ \text{Tu}]_{\text{Drug+T cell}} - [\%\text{Caspase}^+\ \text{Tu}]_{\text{Drug}}}{[\%\text{Caspase}^+\ \text{Tu}]_{\text{Control+T cell}} - [\%\text{Caspase}^+\ \text{Tu}]_{\text{Control}}} \right\}^2$$

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research

Cell Cycle Execution Points and Targets of Aurora A and B Kinases



Gautschi O et al. Clin Cancer Res 2008;14:1639-1648

©2008 by American Association for Cancer Research

Synergistic Response of Melanoma Cells Lines to Aurora Kinase Inhibitors

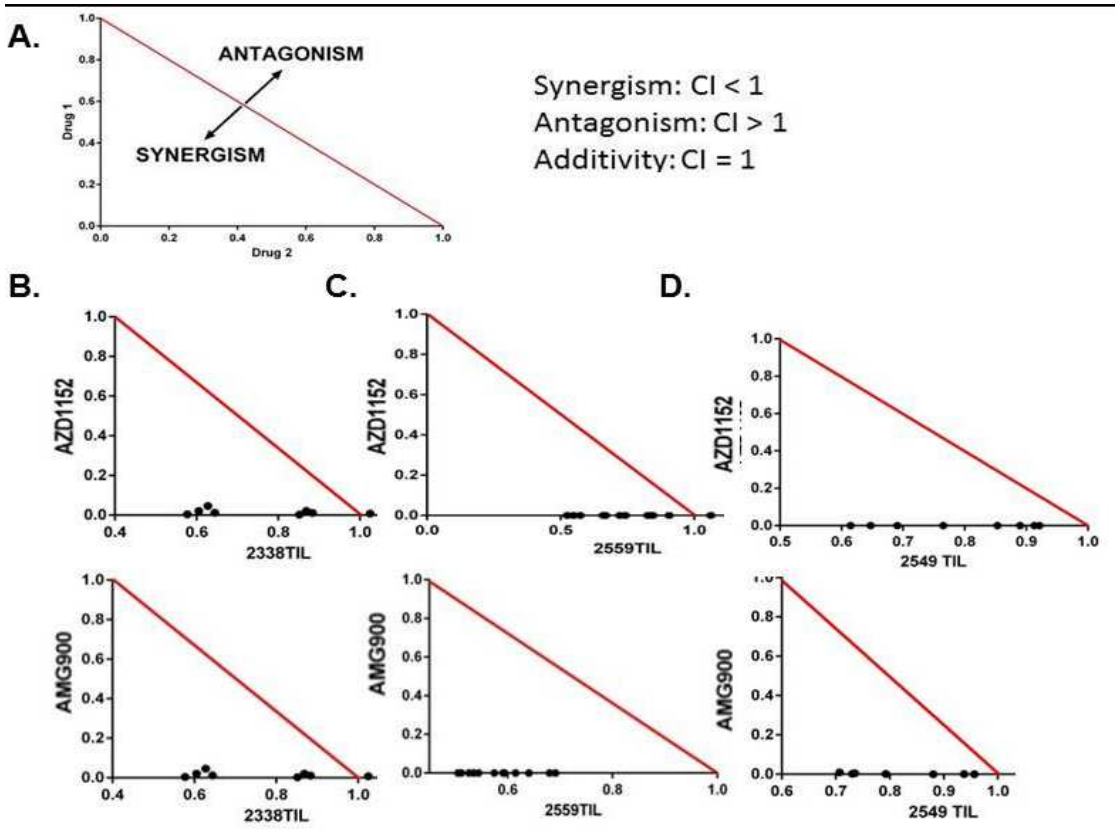
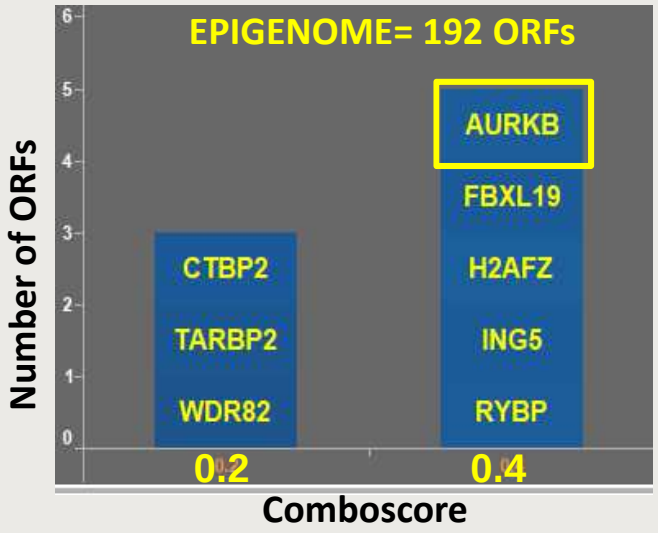


Figure 3: Synergistic response of melanoma cell lines to Aurora Kinase inhibitors with T cell mediated cytotoxicity as determined using Calcusyn™. (A) The curve is depicting combination index for two drugs and areas of synergy and antagonism are shown. **(B).** Synergy of T cell cytotoxicity with Aurora kinase inhibitor AMG900 and Aurora Kinase B specific inhibitor AZD1152 in melanoma line 2338; (**C).** in cell line 2400 and,**(D)** in cell line 2549.

Shruti Malu, Postdoctoral Fellow
Melanoma Medical Oncology - Research

Unbiased Screen #2: ORF Screen



Candidate ORFs that induce resistance to T cell mediated killing : Low Comboscore

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Unbiased Screen #3: shRNA Screen

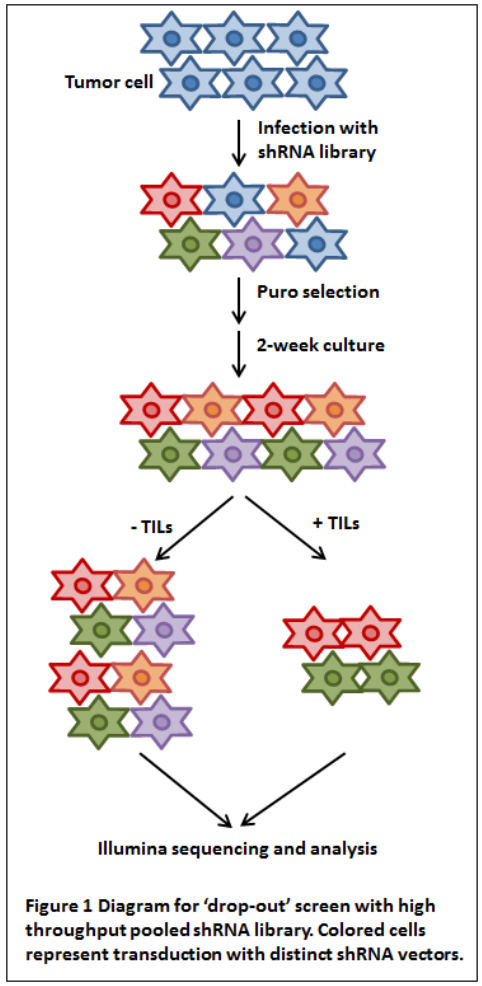

Figure 1 Diagram for 'drop-out' screen with high throughput pooled shRNA library. Colored cells represent transduction with distinct shRNA vectors.

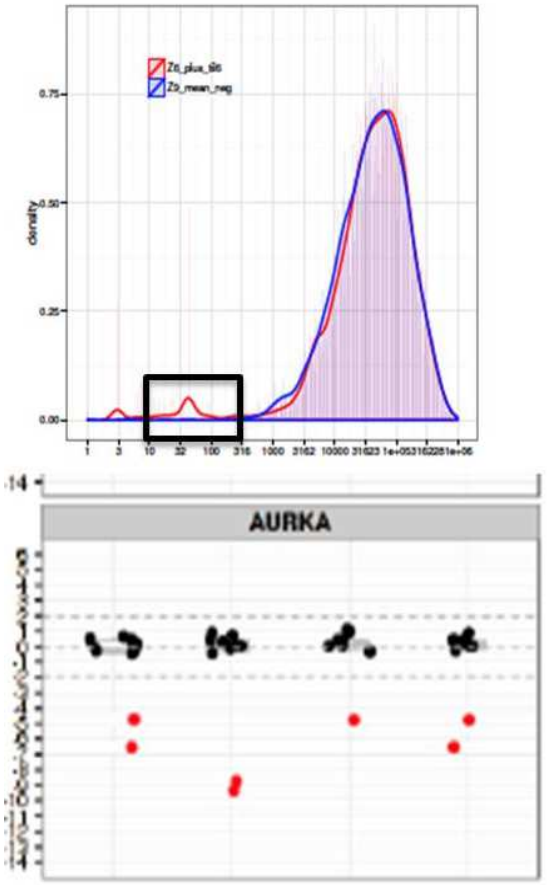

Figure 4: In an unbiased pooled shRNA screen, treatment with shRNA to AURKA results in increased sensitivity to T cell mediated cytotoxicity. In an unbiased pooled shRNA screen, the shRNAs that were deleted on treatment with TILs are depicted in the black box. shRNAs to AURKA were among these depleted from the pooled shRNA expressing cells on treatment with TILs indicating that AURKA is a resistance marker for T cell mediated killing (the individual dots is a single shRNA).

Shruti Malu, Postdoctoral Fellow
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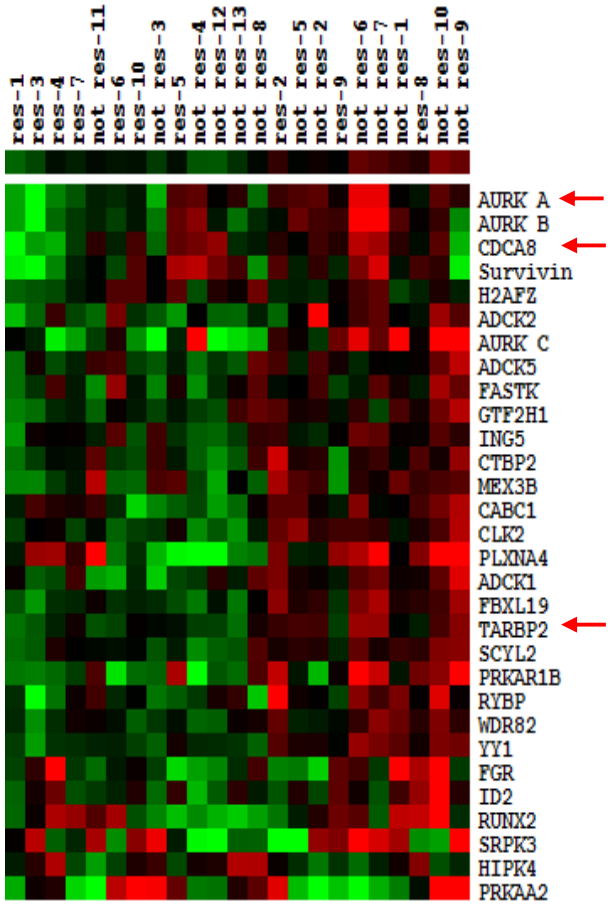
Nanostring™ Analysis of Gene Expression in Tumors from Patients on TIL Therapy



10 Responder vs 13 Non-Responder to TIL therapy

AURKA, CDCA8, TARBP2 have p<0.05
AURKB has p<0.08

Figure 6: Aurora Kinase and CDCA8 have significantly higher expression in tumors of patients non-responding to Adoptive T cell therapy. Hierarchical clustering of expression of 30 genes by Nanostring™ analysis on RNA of tumor samples from patients treated by TIL therapy. **Expression of** Aurora Kinase A (AURK A) and CDCA8 expression was significantly different (* denotes p<0.05) between patients that are responders to TIL therapy (res) and non responders to TIL therapy (non-res) i.e. higher in non responders. * denotes p<0.08

Combination of Aurora Kinase B Inhibitor with Immunotherapy (anti CTLA4) is Highly Efficacious in MC38/gp100 Tumor Model

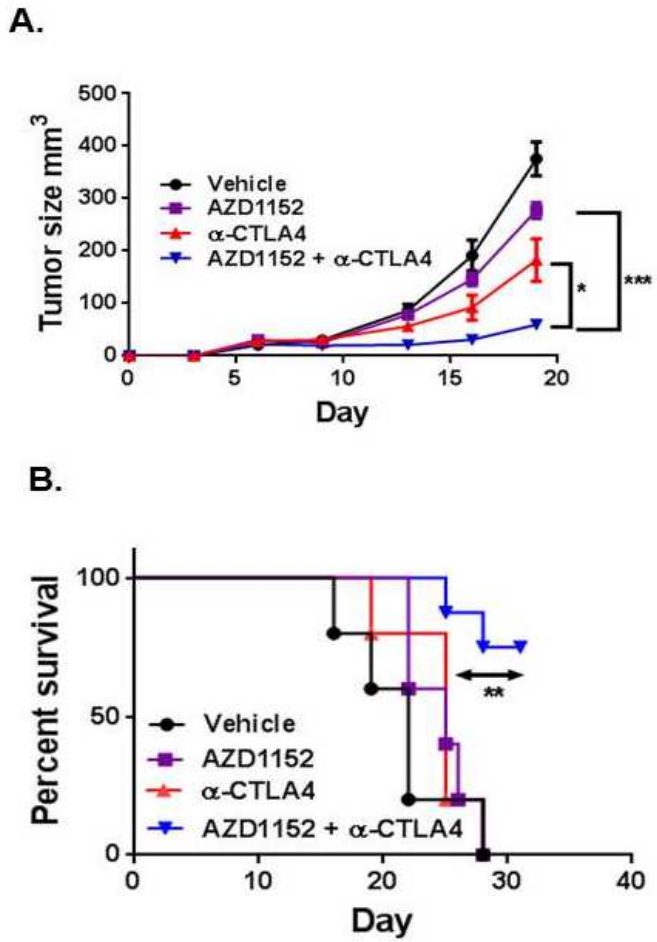


Figure 5: Combination of Aurora Kinase B inhibitor with immunotherapy is highly efficacious in MC38/gp100 tumor model. (A) Mice were inoculated with MC38/gp100 tumor on day (0). On Day 3-6, mice were treated with Aurora Kinase B inhibitor AZD1152 (25mg/kg) and 100μg of anti-CTLA4 antibody on Day 3, 6, 9 and 15. The tumor shrinkage using combination therapy was beyond the response seen for mice treated with either treatments alone, indicating synergy of this combination. *** indicates $p<0.005$ and * indicates $p<0.05$. **(B)** Mouse survival is significantly improved with the combination of AZD1152 and α-CTLA4. ** p-value is < .01.

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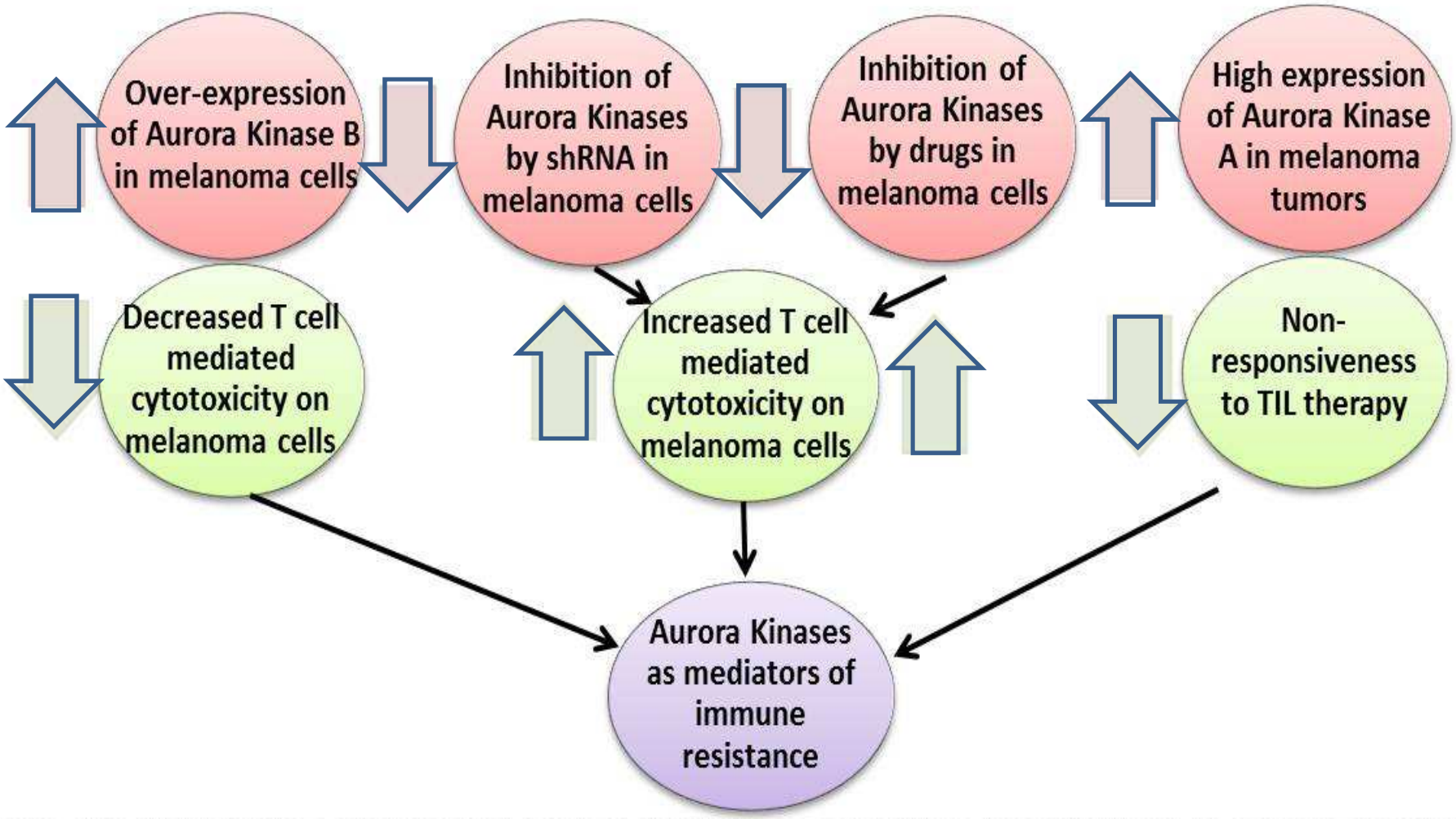
Significance of Studying Aurora Kinases as Mediators of Resistance to Cancer Immune Therapy
A Four-Screen Hit



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Major Question

- **What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?**
  - **BRAF/MAPK**
  - **PI3K**
  - **Aurora Kinase**

Major Questions

- Does TIL therapy for melanoma work in patients who have failed immune checkpoint blockade?
- How can we increase the throughput for this treatment?
- How do we take T-cell therapy to other cancers?
- What distinguishes responders from non-responders?
- What are the best combinations of therapies?

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IND Office
Linda Duggan