Melanoma Challenges and Opportunities and the Need for Rational Combinations of Agents

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Society for Immunotherapy of Cancer (SITC) Annual Meeting 2016

Plenary Session: State-of-the-Art Immunotherapies: Challenges and Opportunities
Friday, November 11, 2016

Disclosures

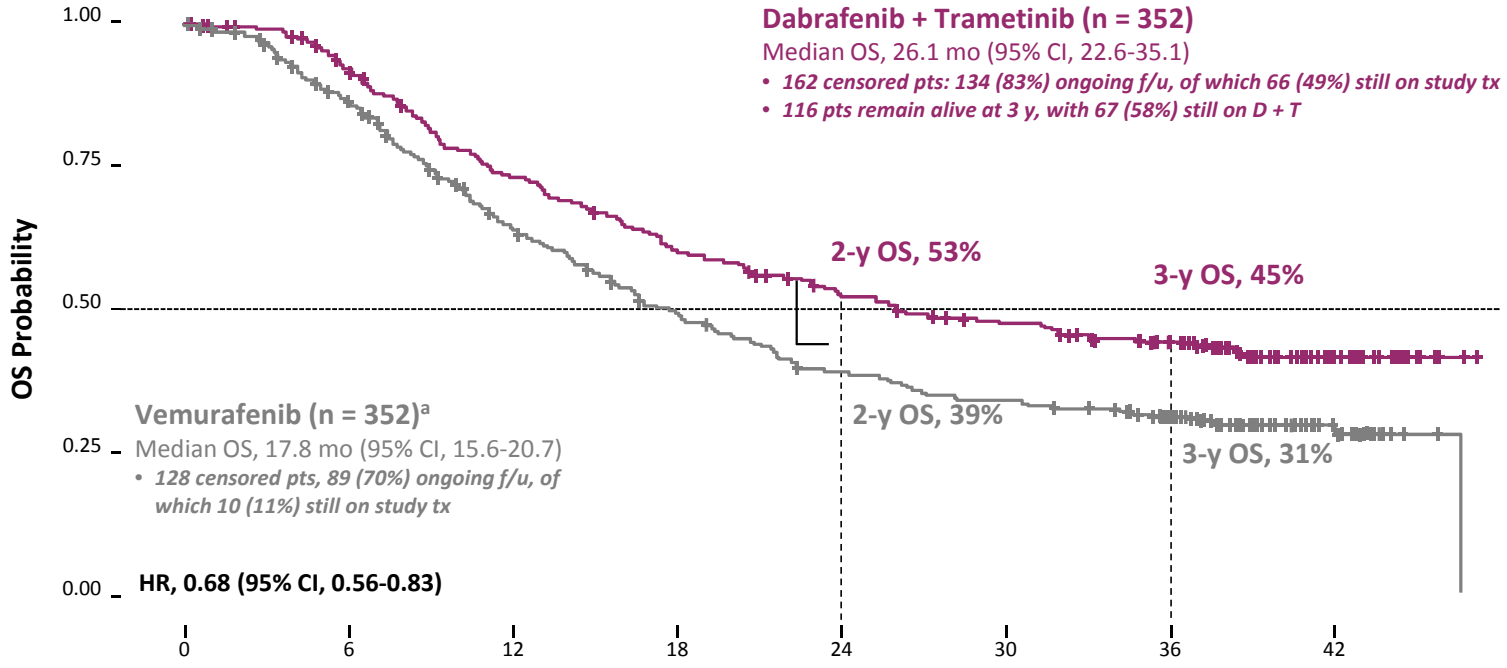
- **SAB for Lion, Immatics, Dragonfly**

Recent Agents That Have Been FDA Approved for Metastatic Melanoma

- **Vemurafenib (Zelboraf) for BRAF mutant late-stage melanoma - Aug 17, 2011.**
- **Ipilimumab (MDX-010/Yervoy) for late-stage melanoma that has spread or cannot be removed by surgery - Mar 2011.**
- **Dabrafenib (Tafinlar) for BRAF mutant metastatic melanoma that cannot be surgically removed – May 2013.**
- **Trametinib (Mekinist) for metastatic melanoma that cannot be surgically removed – May 2013.**
- **Trametinib + Dabrafenib for BRAF mutant metastatic melanoma-2014**
- **Pembrolizumab (Keytruda) for advanced melanoma that no longer responds to other drugs - Sept 2014.**
- **Nivolumab (Opdivo) for advanced melanoma that no longer responds to other drugs – Dec 2014.**
- **Nivolumab (Opdivo) in Combination with Ipilimumab (MDX-010/Yervoy) for the treatment of patients with BRAF V600 wild-type, unresectable or metastatic melanoma – Sept 2015.**
- **Ipilimumab (MDX-010/Yervoy) for adjuvant therapy of melanoma for patients with Stage III disease – Oct 2015**
- **Cobimetinib (MEKi) + Vemurafenib (BRAFi) for BRAF mutant melanoma-Nov 2015**
- **Talimogene Laherparepvec (T –Vec/Imlygic) for patients with advanced melanoma – Oct 2015**
- **Pembrolizumab (Keytruda) as Up-front Therapy for patients with unresectable or metastatic melanoma– Dec 2015**

Targeted Therapy for Melanoma has a Tail on the Survival Curve

COMBI-v: Overall Survival (ITT Population)



Patients at risk, n								
D + T	352	311	245	201	171	150	127	33
Vem	352	289	203	154	119	103	81	22

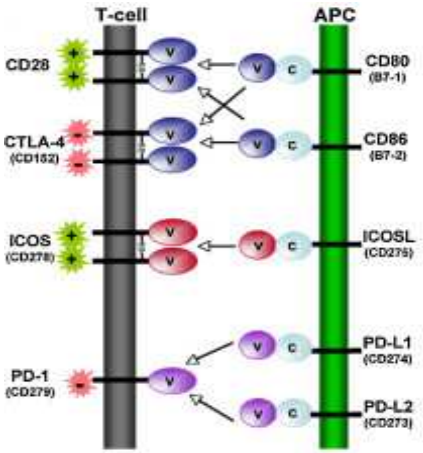
[a] Vemurafenib arm includes 34 patients (10%) who crossed over to the dabrafenib + trametinib arm.

D + T, dabrafenib + trametinib; f/u, follow-up; ITT, intent-to-treat; pts, patients; tx, treatment; Vem, vemurafenib.

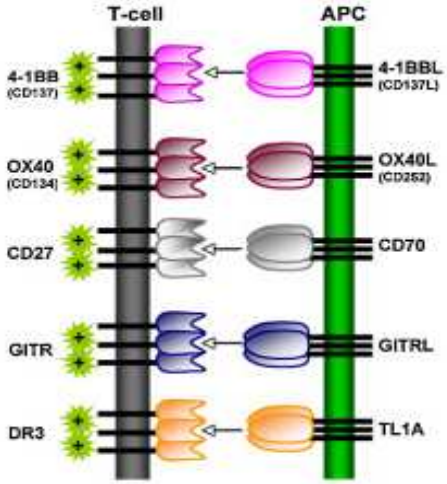
C. Robert

Receptor-ligand Pairs that Play a Role in Regulating T-cell Function

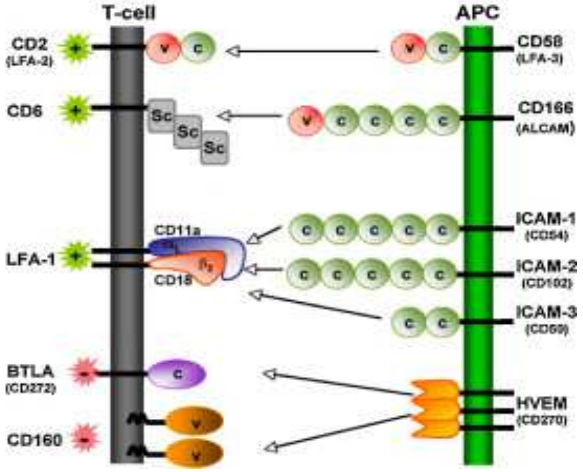
B7-CD28 family



TNF-TNFR family

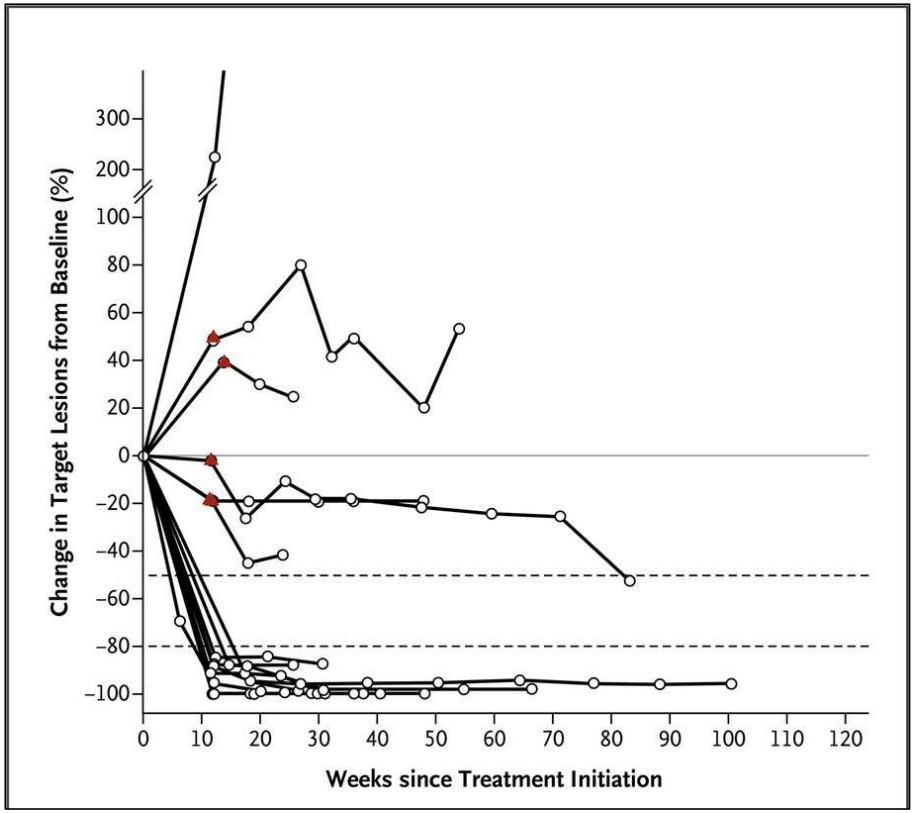


Additional molecules



Adapted from *Immunology Letter* 128:89-97, 2011

Clinical Activity in Patients Who Received the Concurrent Regimen of Nivolumab and Ipilimumab: Immunotherapy Responses Can Be Fast



Wolchok JD…Sznol M.
NEJM 369:122-33, 2013

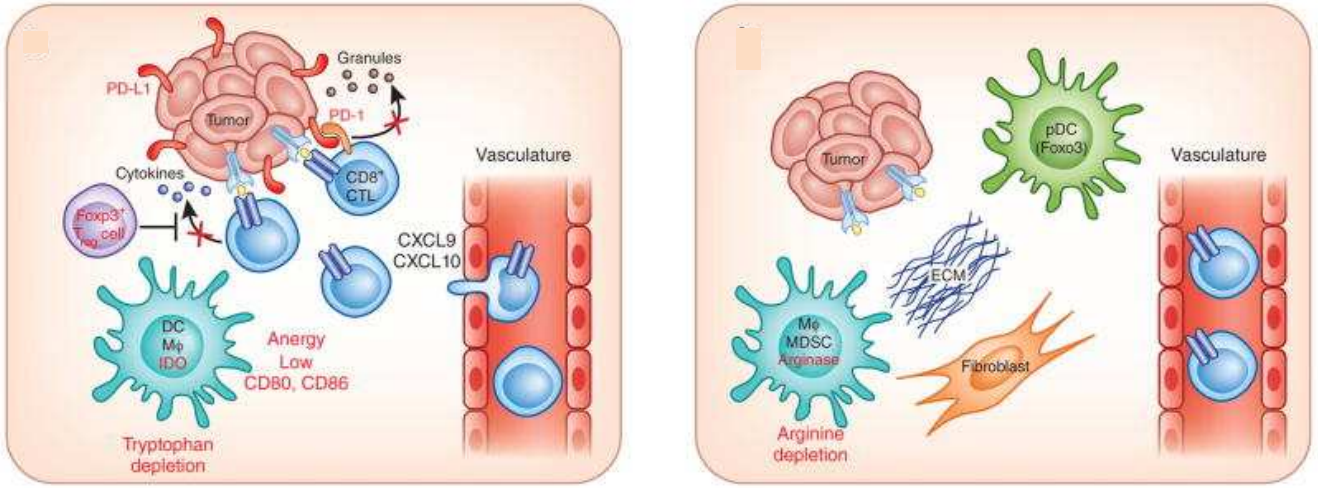
Current Challenges in Melanoma Therapy

- Sequence of targeted vs immunotherapy
- Biomarkers of response to targeted and immunotherapy
- Optimal combinations of agents
- Toxicity vs Benefit of combination therapy
- Mechanisms of resistance to targeted and immunotherapies?
- CNS metastases

Factors that Predict Response to Immune Checkpoint Blockade

- **Number of mutations** (but it's not the whole story)
- **PDL-1 levels**
- **Tumor infiltration by T-cells**
- **Other factors in the tumor micro-environment**
- **Specific signaling pathways in tumor cells**

Working Model for the Segregation of Tumors Based on Immune System Regulatory Pathways in the Tumor Microenvironment

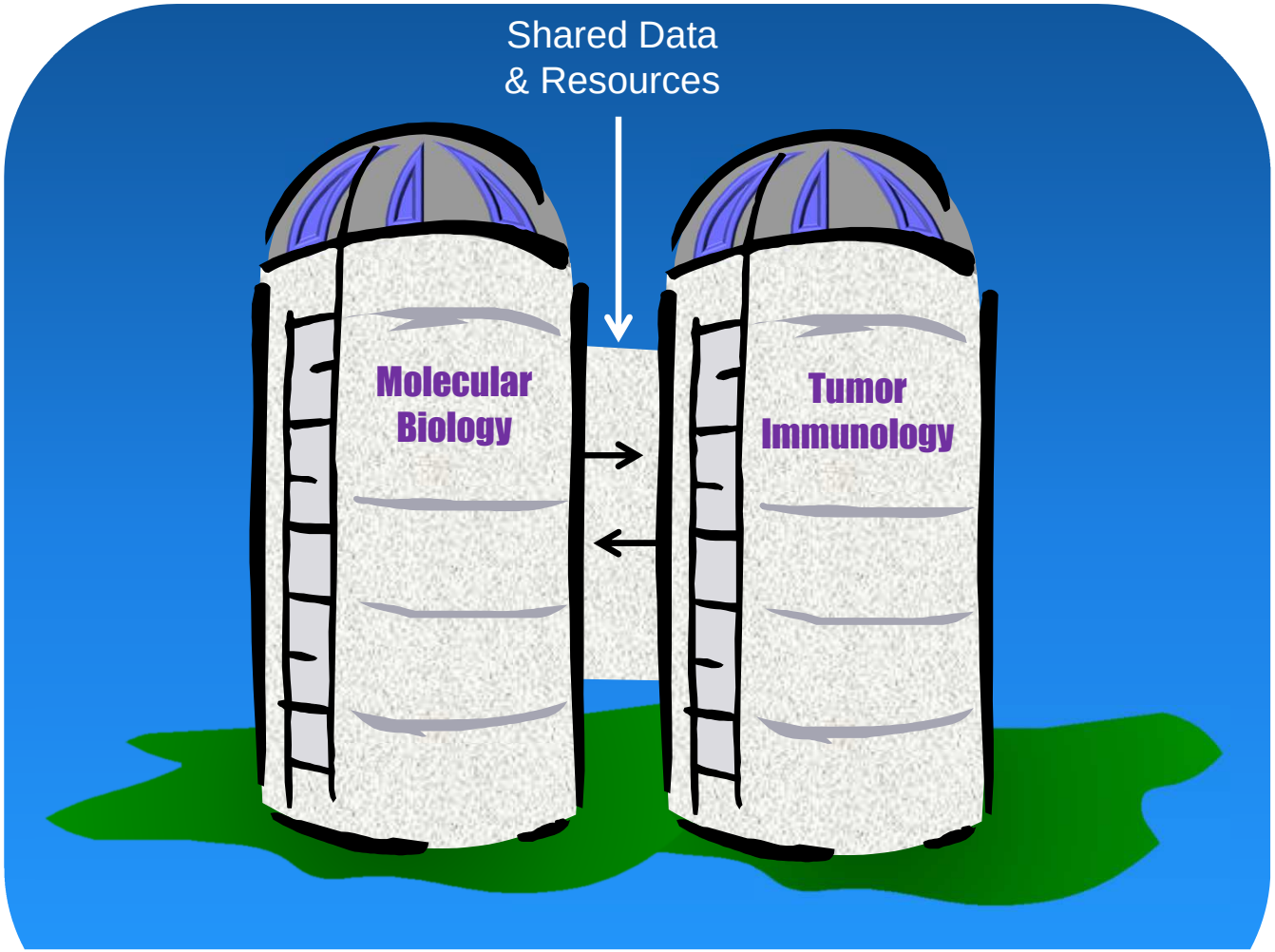


Gajewski TF et al.
Natl Immunol 14(10):1014-22, 2013

What Are the Most Promising New Combinations for Immunotherapy?

- Immuno-immuno combinations
- Targeted Therapy + Immunotherapy
- Standard Chemotherapy + Immunotherapy
- Epigenetic Modulators + Immunotherapy

Bringing Silos Together

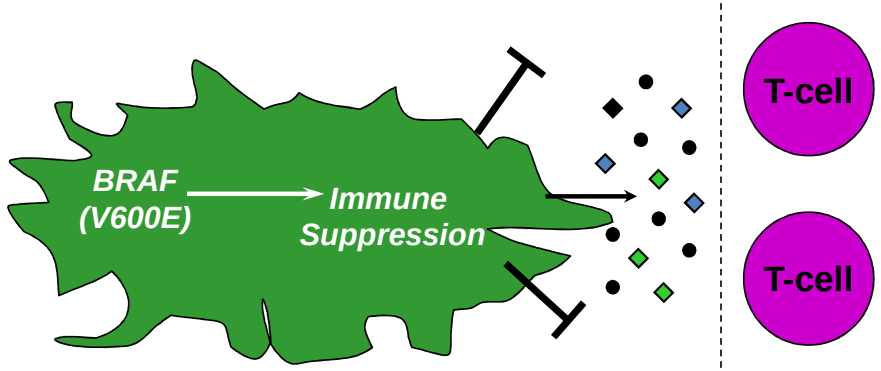


Targeted Therapy + Immunotherapy

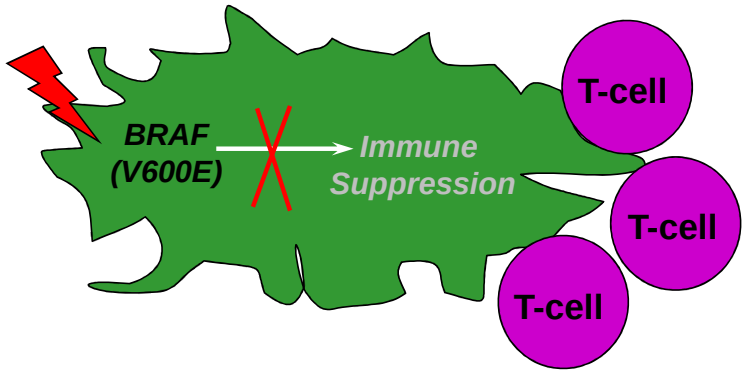
- **What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?**
  - **BRAF/MAPK**
  - **β-catenin**
  - **PI3K**

Combining BRAF(V600E) Inhibition and Immunotherapy

Immunotherapy Alone

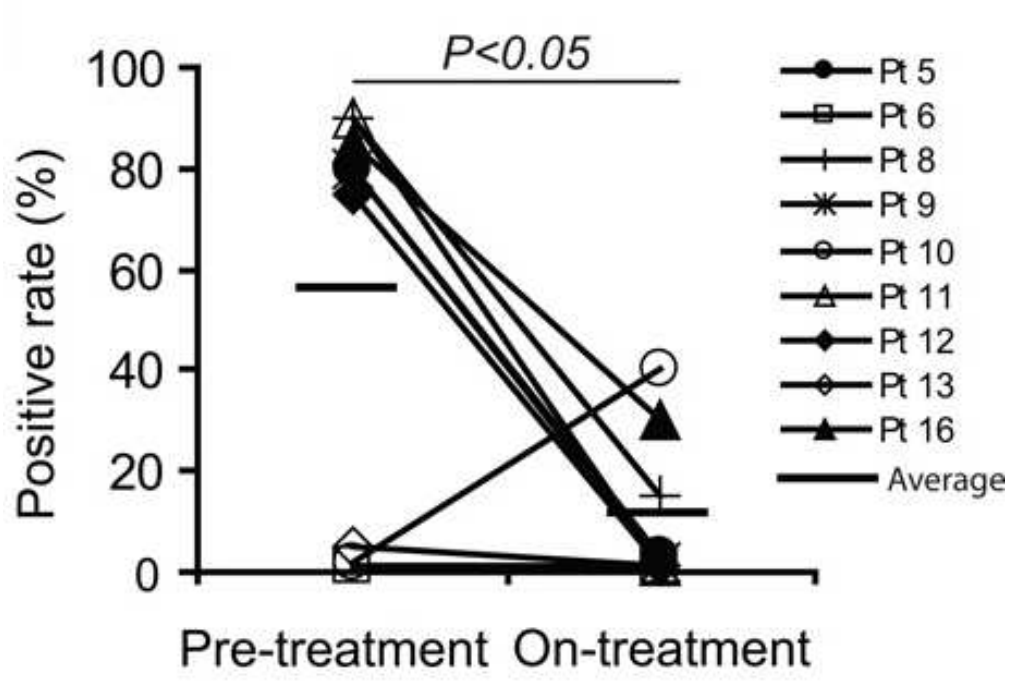


Immunotherapy Plus BRAF(V600E) Inhibition



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

BRAF Inhibition Down-regulates VEGF at the Tumor Site



Liu C…Wargo JA…Flaherty KT… Hwu P. *Clin Cancer Res* 19:393-403, 2013

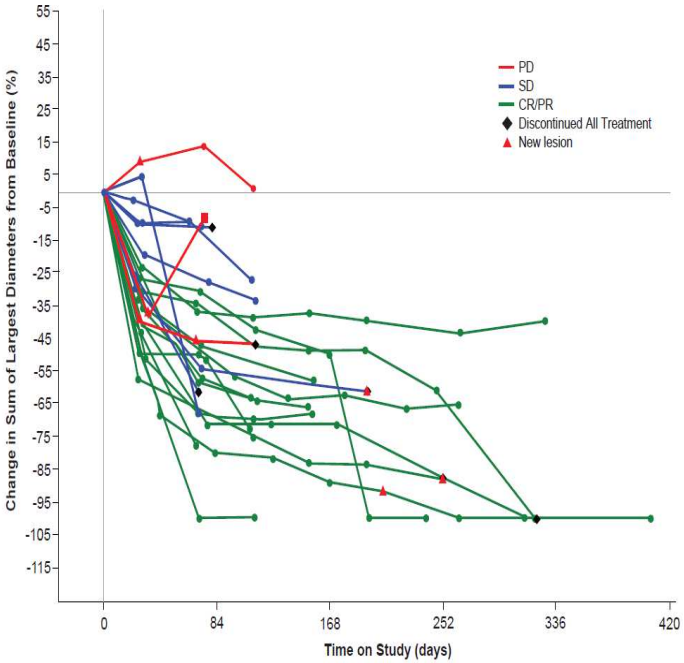
Current and Projected Clinical Trials of BRAF Inhibitors + Immunotherapy

- **Protocol GP28384: A Phase Ib, Open-Label Study of the Safety and Pharmacology of Atezolizumab (PDL3280A), Anti-PD-L1 Antibody) Administered in Combination with Vemurafenib or Vemurafenib Plus Cobimetinib in Patients with Previously Untreated BRAFV600-Mutation Positive Metastatic Melanoma**

- **A Phase II Study of the Anti-PD-1 Antibody Nivolumab in Combination with Dabrafenib and/or Trametinib in Patients with BRAF or NRAS-mutated Metastatic Melanoma**

Clinical Activity of Atezo + Cobi + Vem

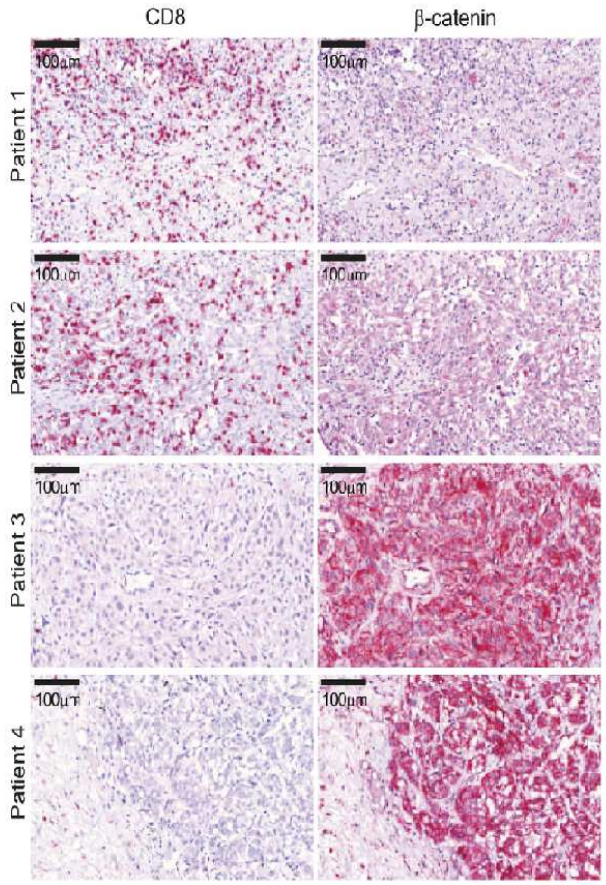
Response (per RECIST v1.1) N = 29	Unconfirmed		
	n	%	(95% CI)
ORR	24	83%	(64.2, 94.2)
CR	3	10%	(2.2, 27.4)
PR	21	72%	(52.8, 87.3)
SD	3	10%	(2.2, 27.4)



Major Question

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

Correlation Between Active β-catenin and CD8 T-cell Infiltrate in Human Patient

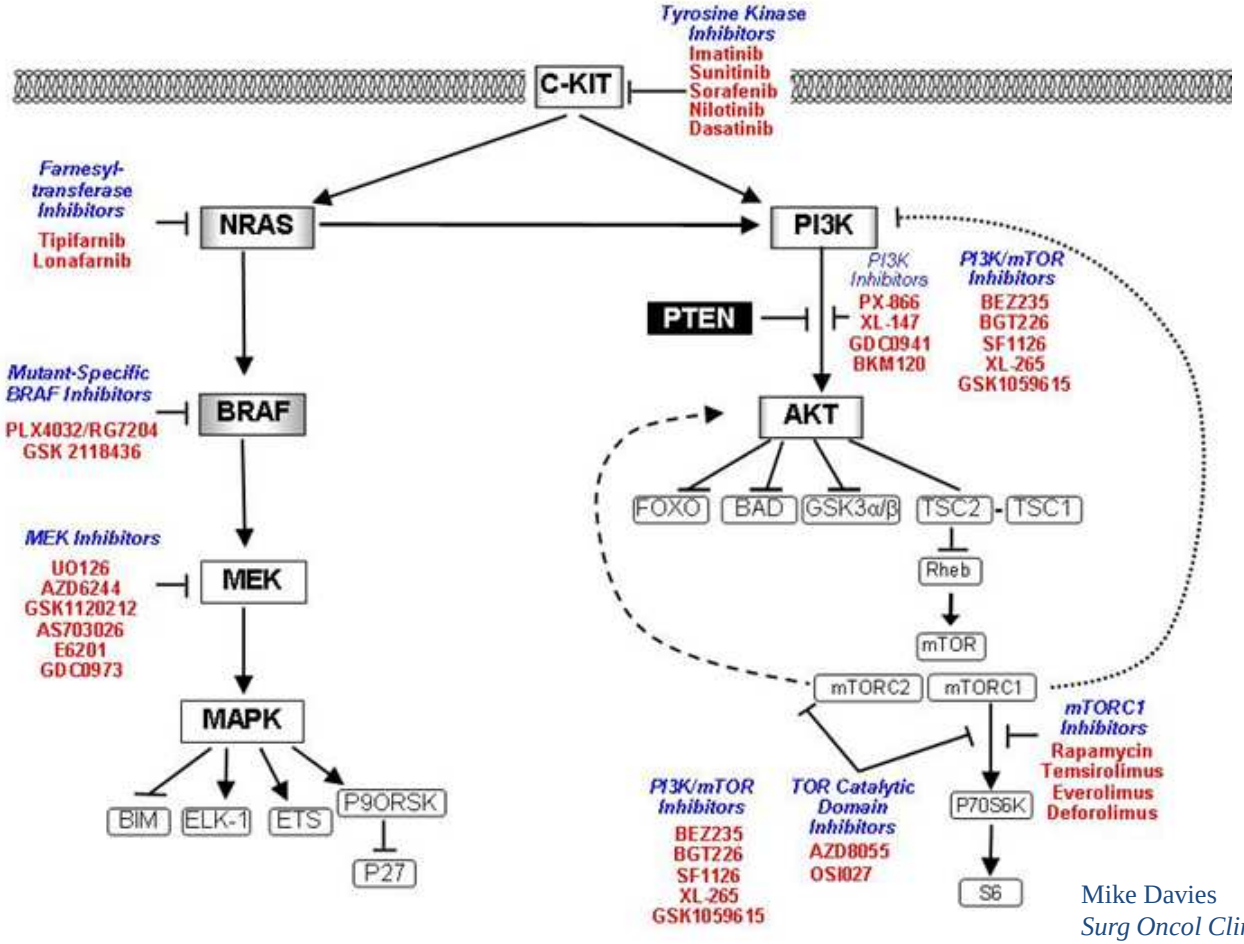


Spranger S…Gajewski TF.
Nature 523(7559):231-5, 2015

Major Question

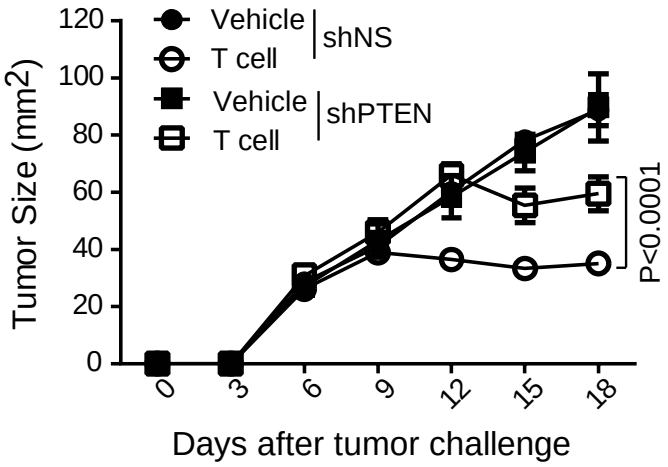
- **What are the signaling pathways in the tumor that modulate the immune microenvironment and sensitivity or resistance to immunotherapy?**
  - **BRAF/MAPK**
  - **β-catenin**
  - **PI3K**

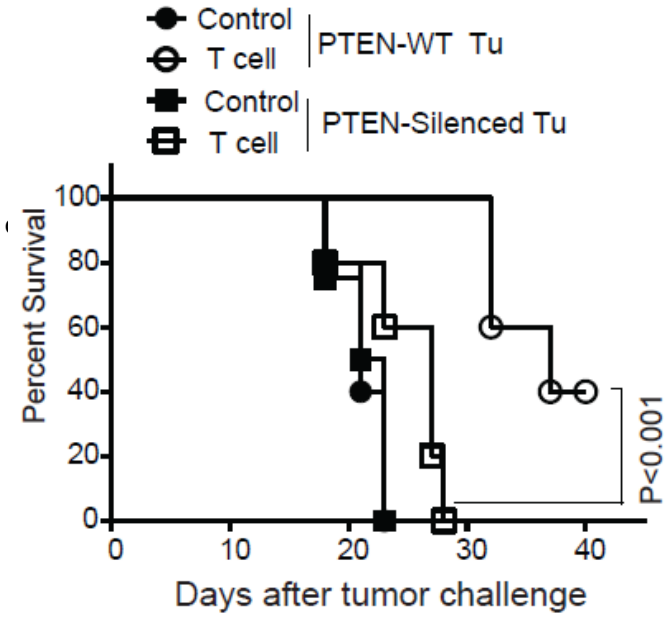
Common Mutations in Cutaneous Melanoma



Mike Davies
Surg Oncol Clin N Am
20(1):165-80, 2011

PTEN-silenced Tumor Poorly Responds to T-cell Therapy





Weiyi Peng MD, PhD
Asst Prof, MD Anderson

Peng W … Hwu P
Cancer Discov
6(2):202-16, 2016

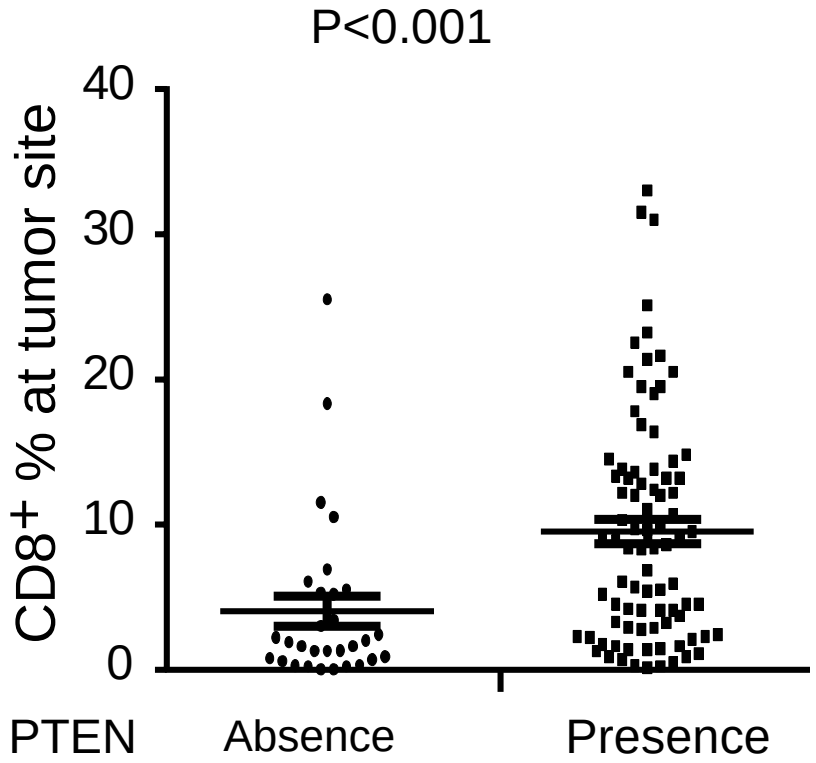
Increased Percentage of PTEN Loss in Tumors from Melanoma Patients with Failed Initial Expansion of TILs

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

P = 0.0405

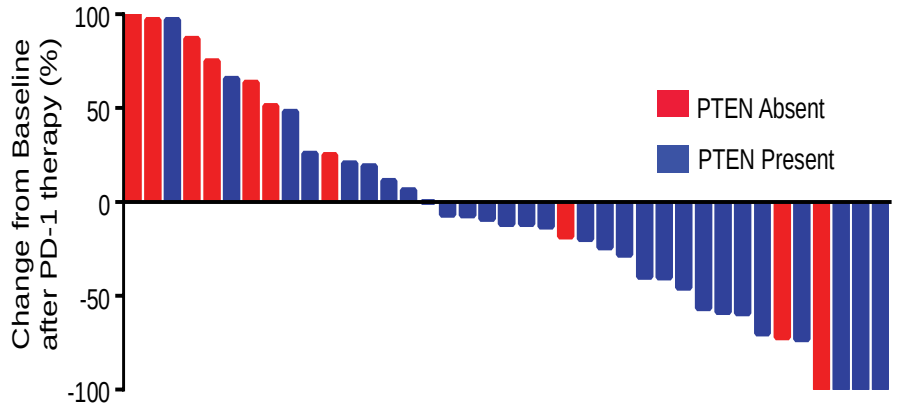
Peng W … Hwu P
Cancer Discov
6(2):202-16, 2016

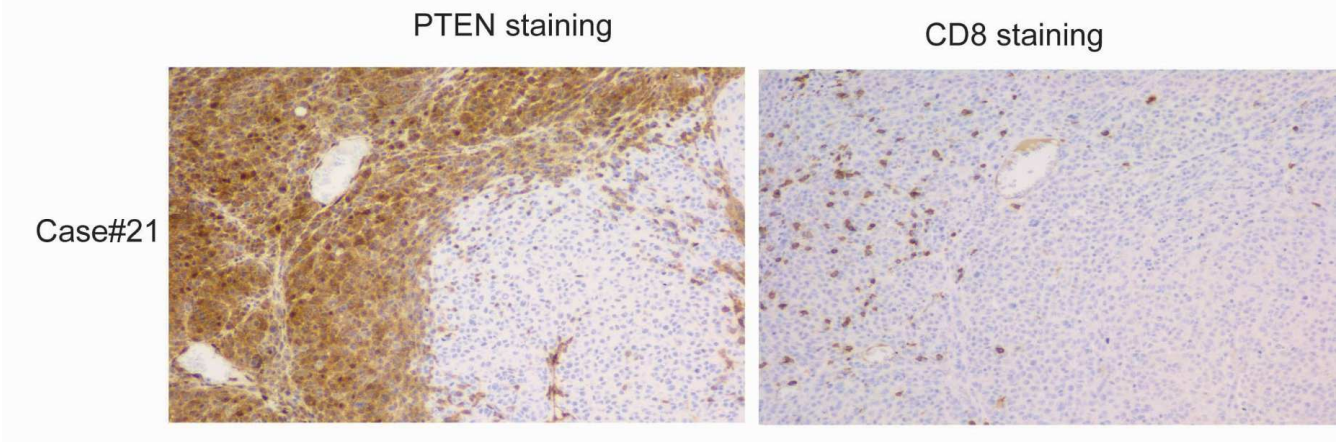
Less T-cell Infiltration in PTEN-loss Tumor in Stage IIIB/C Melanoma Patients



Peng W … Hwu P
Cancer Discov
6(2):202-16, 2016

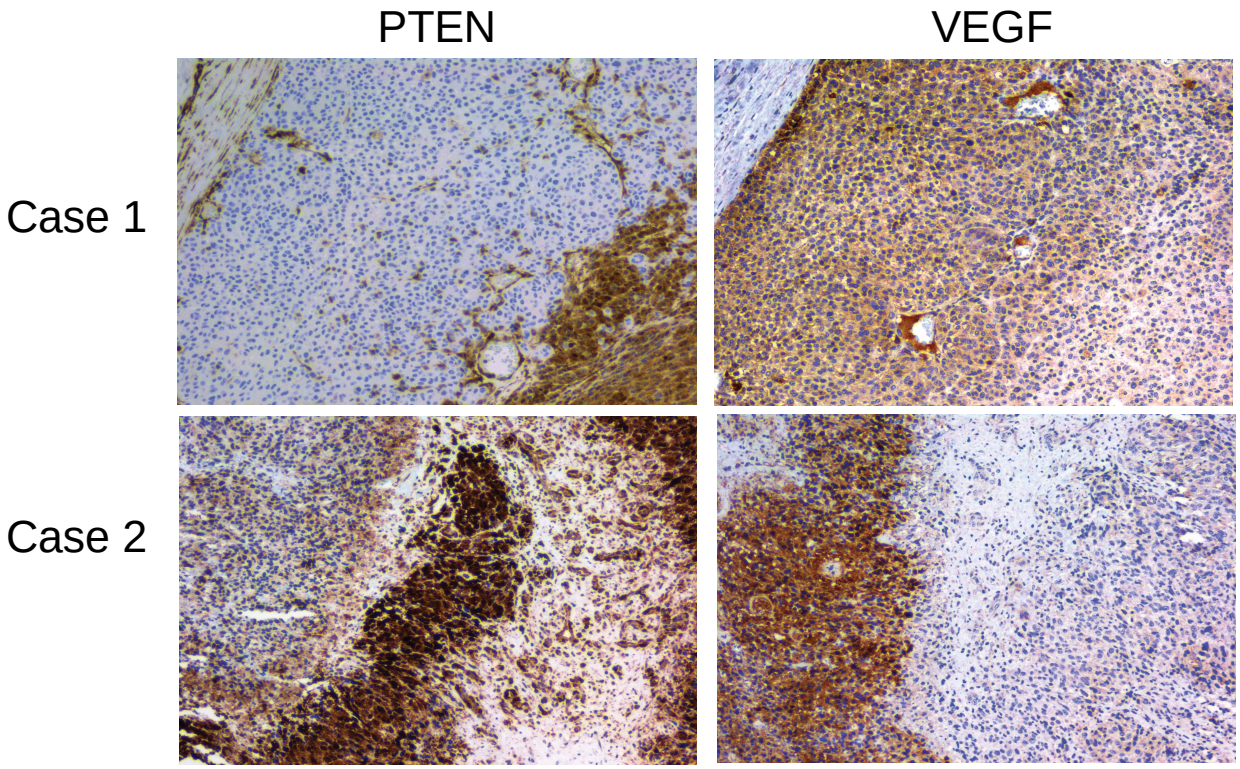
PTEN Loss Inhibits T-cell Infiltration into Tumor





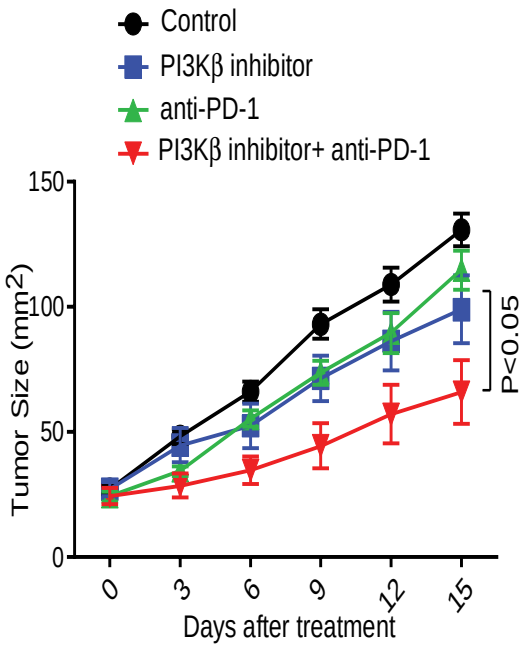
Peng W … Hwu P
Cancer Discov
6(2):202-16, 2016

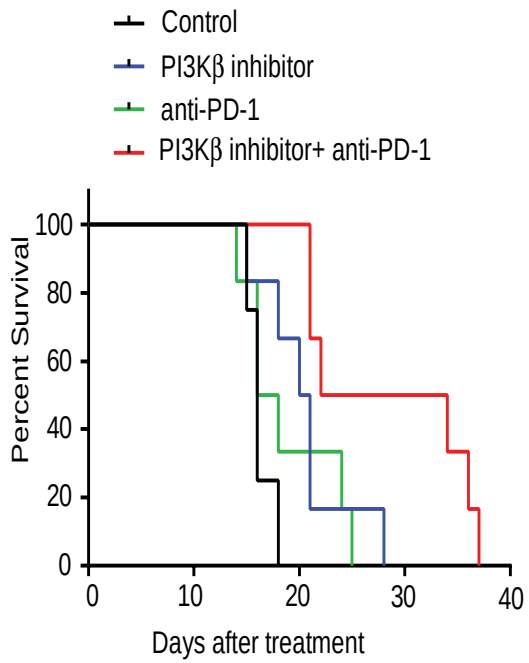
Increased Production of VEGF by Melanoma with PTEN Loss



Peng W … Hwu P
Cancer Discov
6(2):202-16, 2016

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





Peng W…Hwu P.
Cancer Discov
6(2):202-16, 2016

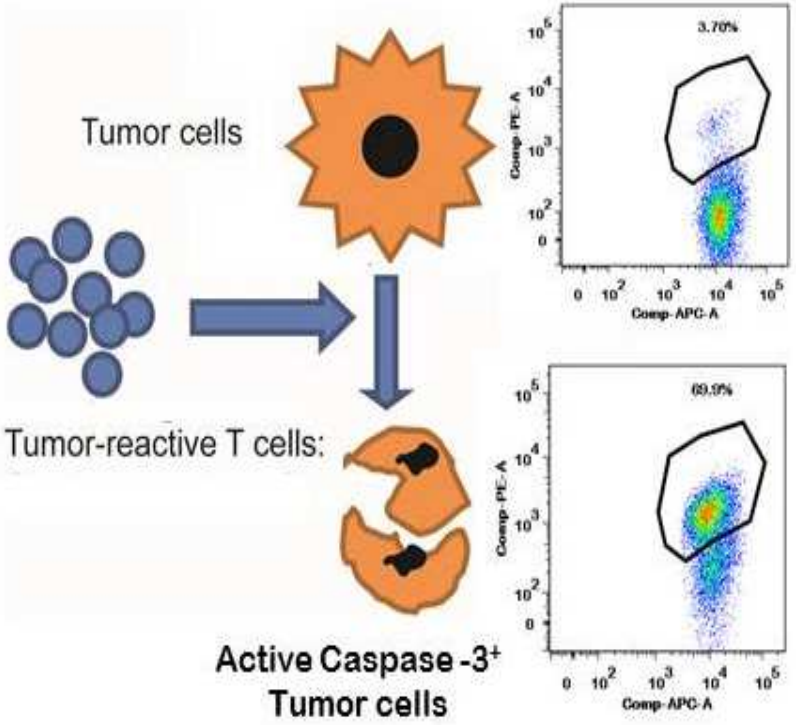
Upcoming Clinical Trial

Anti-PD1 + PI3Kbeta inhibitor in patients with metastatic melanoma (GSK/Merck; Hussein Tawbi/Weiyi Peng**)**

Major Question

Can we use high throughput screening methods to identify molecular markers of resistance to immune therapies?

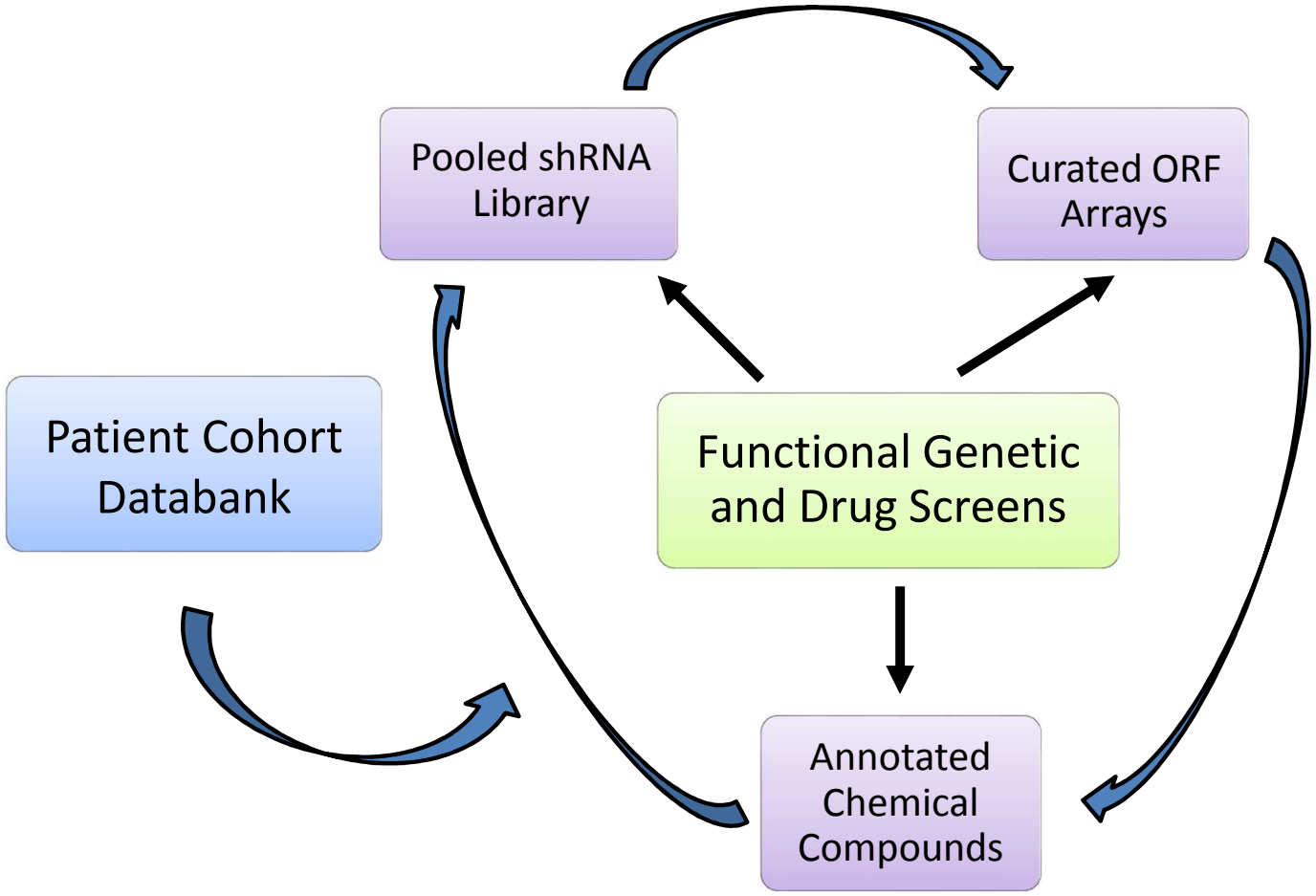
System to Perform Large Scale Screens Using Autologous Tumor / TIL Pairs and T-cell Mediated Cytotoxicity as a Read Out



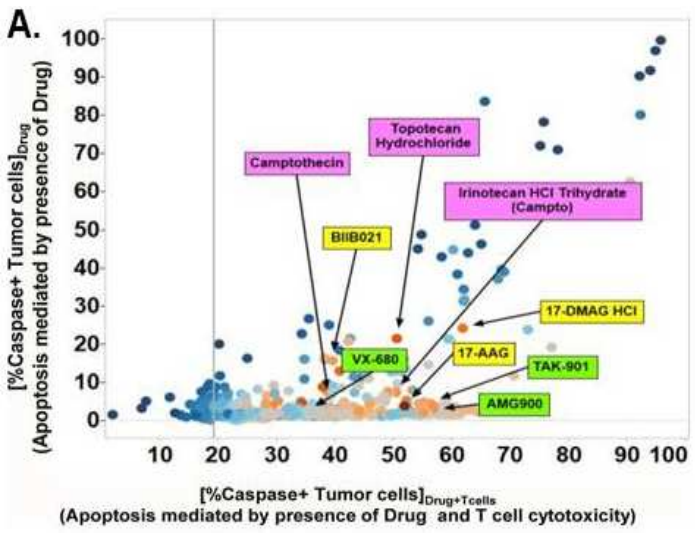
Flow cytometry based T cell cytotoxicity assay for high throughput screen. Depiction of the methodology of T cell cytotoxicity assay. The dot plots for gating and flow cytometric analysis are depicted on the right. Briefly, patient derived melanoma tumor cells are co-cultured with reactive autologous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

Shruti Malu, PhD, Sr. Research Fellow
Dana-Farber Cancer Institute

Principle


Pooled shRNA Library
Curated ORF Arrays
Patient Cohort Databank
Functional Genetic and Drug Screens
Annotated Chemical Compounds

Unbiased Screen #1: Large Scale Drug Screen



B.

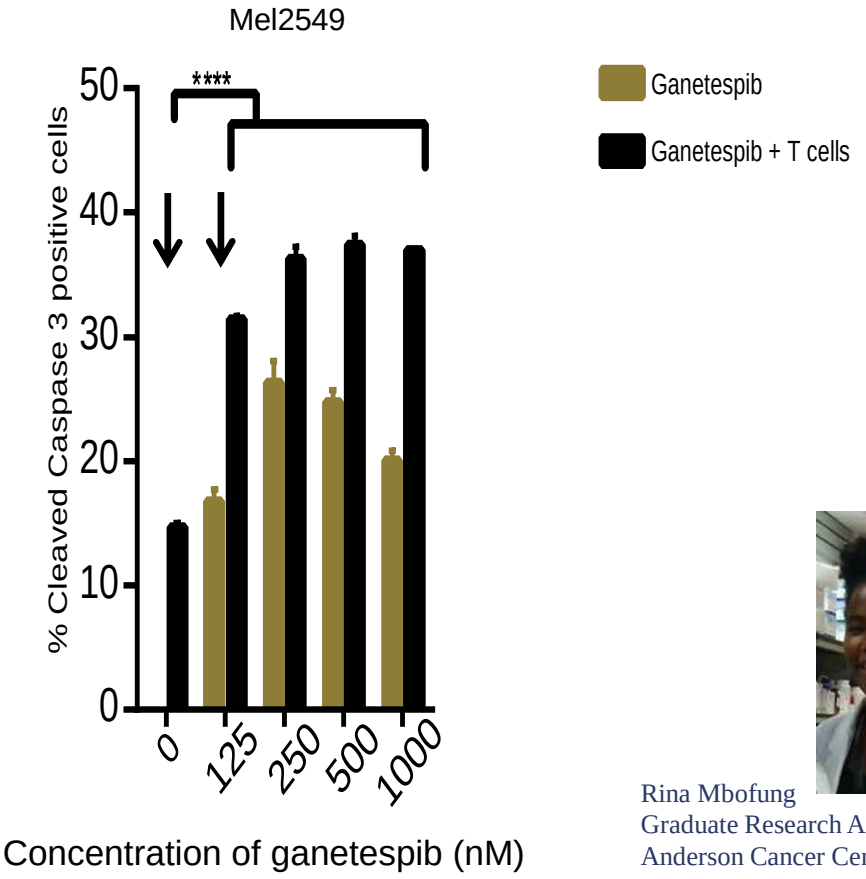
$$\left\{\frac{[\%\text{Caspase}^+\ \text{Tu}]_{\text{Drug+T cell}}-[\%\text{Caspase}^+\ \text{Tu}]_{\text{Drug}}}{[\%\text{Caspase}^+\ \text{Tu}]_{\text{Control+T cell}}-[\%\text{Caspase}^+\ \text{Tu}]_{\text{Control}}}\right\}^2$$

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 ( ).

Shruti Malu, PhD, Sr. Research Fellow
Dana-Farber Cancer Institute

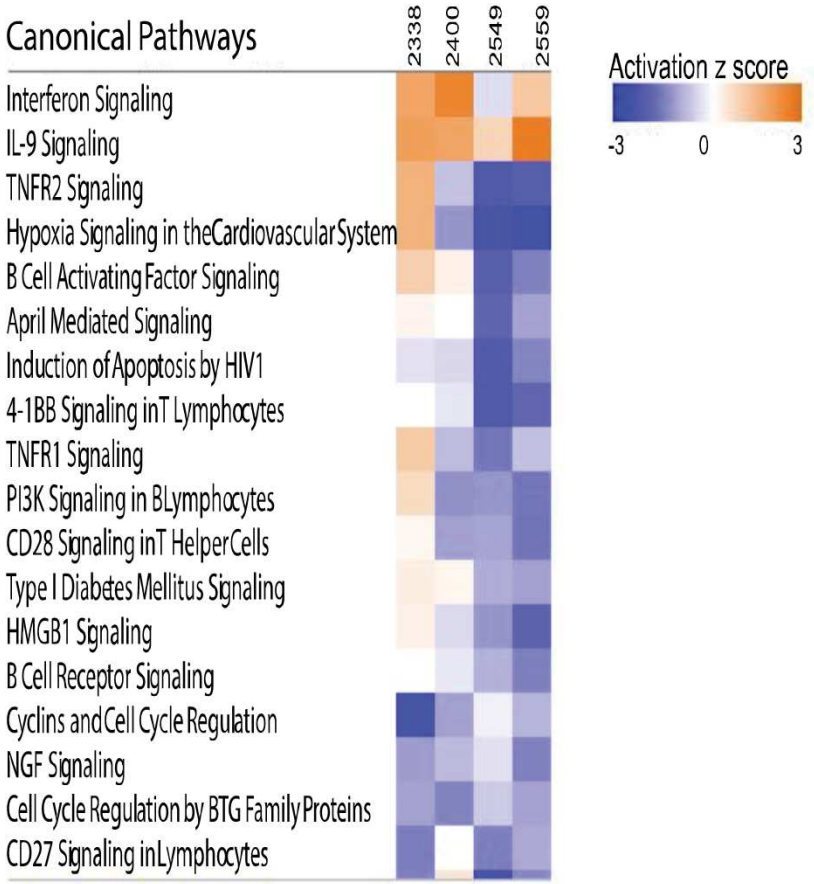
Leila Williams, Sr. Research Assistant
MD Anderson Cancer Center

HSP90 inhibitor, Ganetespib, Enhances T-cell Mediated Killing of Melanoma

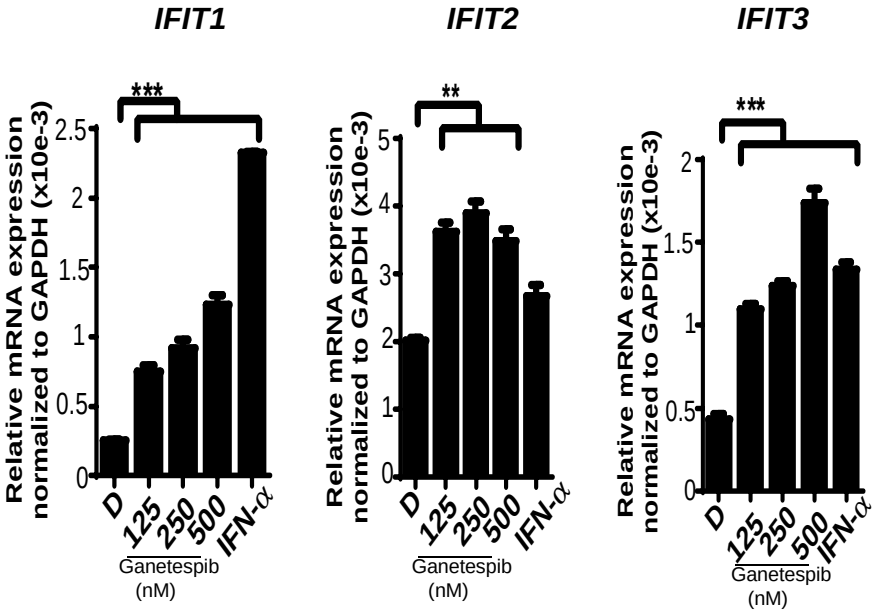


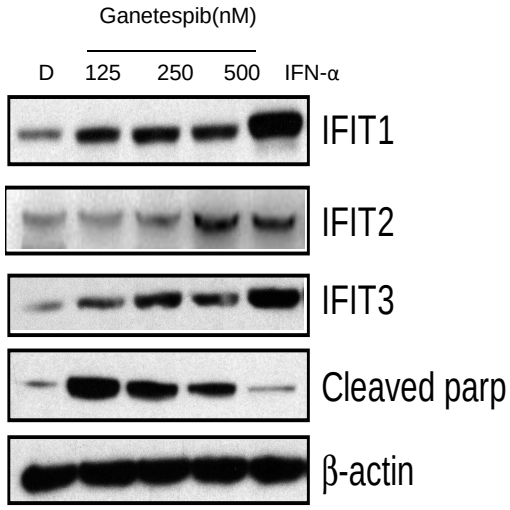
Rina Mbofung
Graduate Research Asst GSBS, MD Anderson Cancer Center

Interferon Signaling is the Top Pathway Upregulated following Treatment with Ganetespib

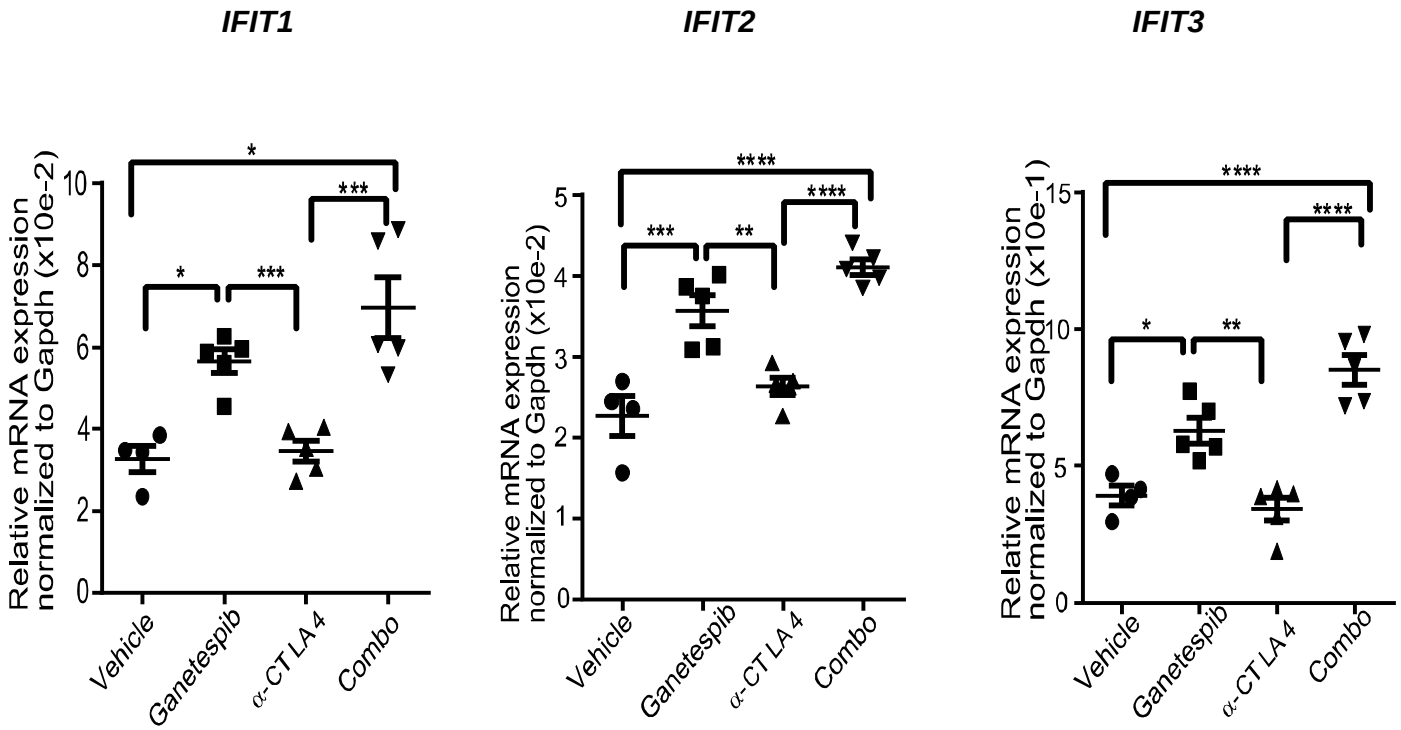


IFIT1, IFIT2 and *IFIT3* Genes are Consistently Upregulated in Melanoma Cell Lines following HSP90 Inhibition

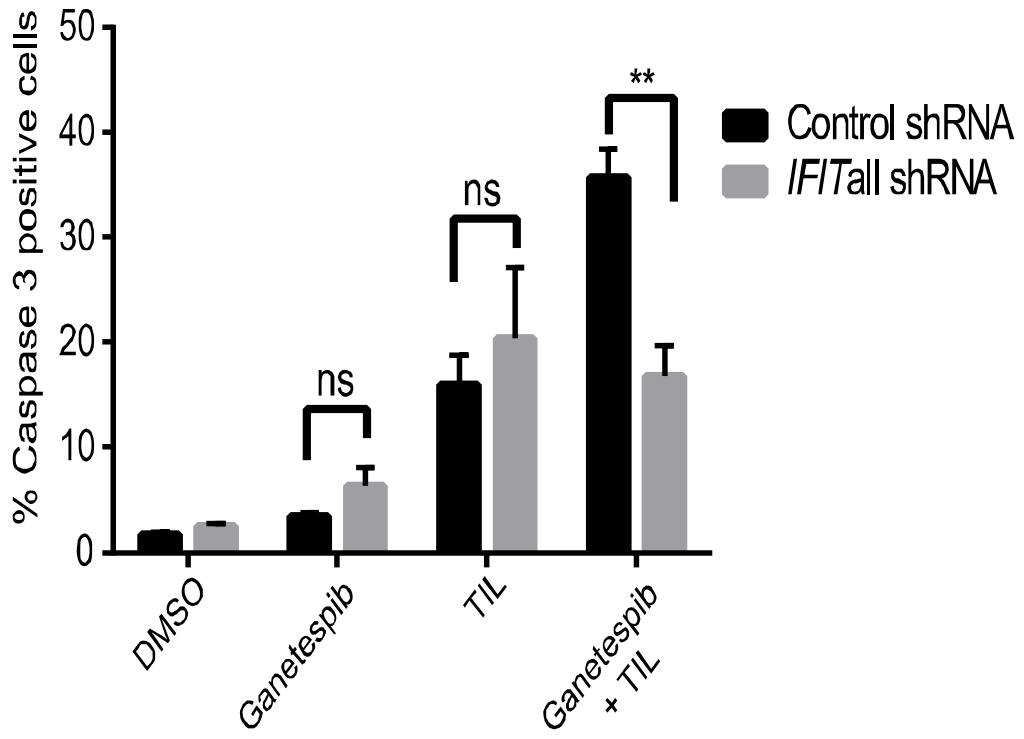




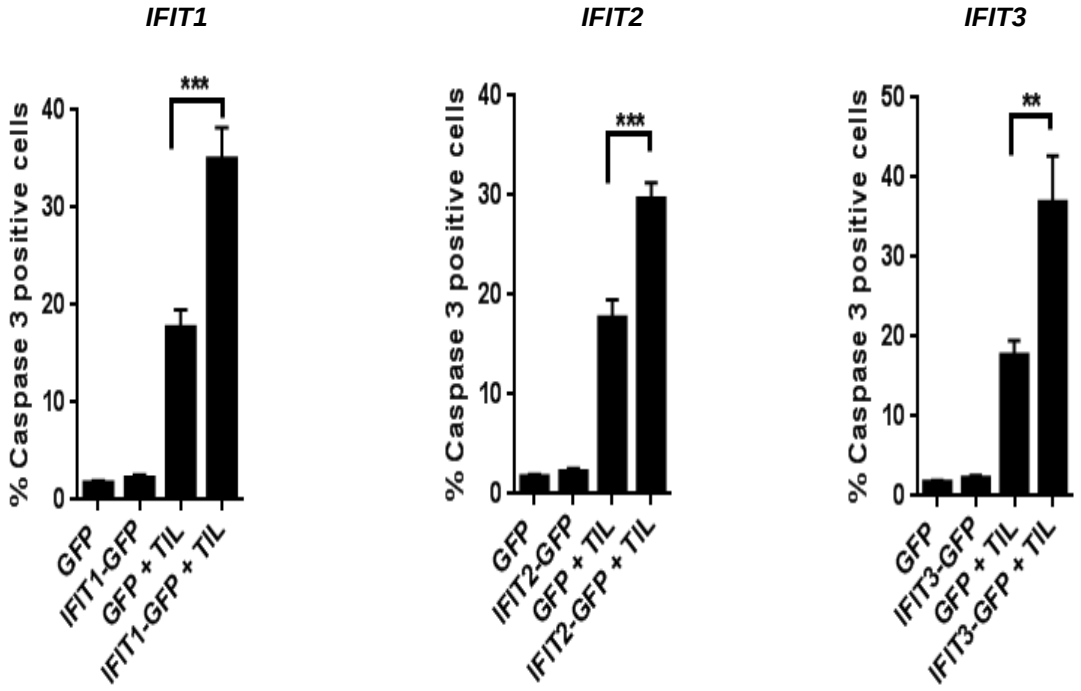
HSP90 Inhibition Upregulates *IFIT1, IFIT2* and *IFIT3* Genes *in vivo*



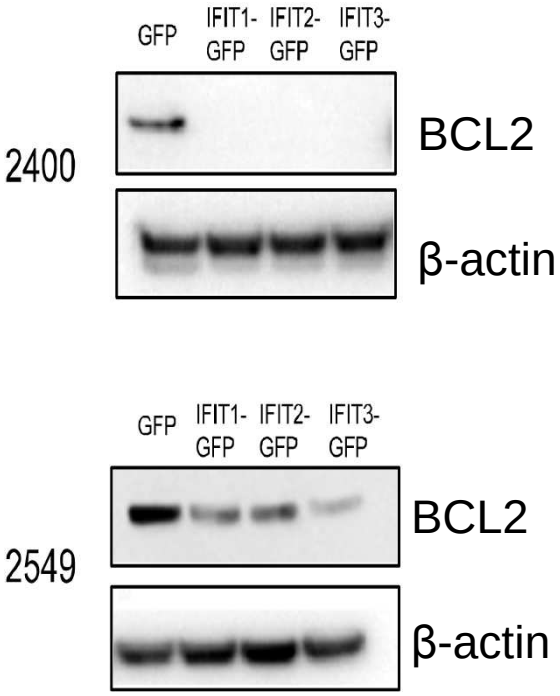
Silencing IFIT Genes Diminishes the Synergy of HSP90 Inhibition and T-cell Killing *in vitro*



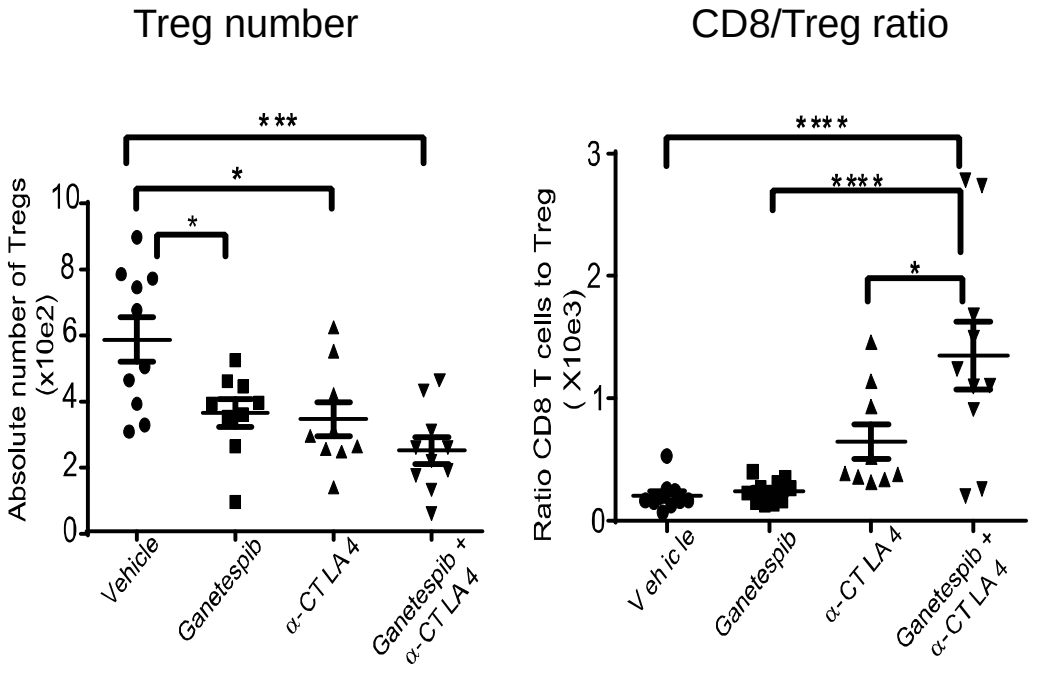
Overexpression of IFIT Genes Enhances T-cell Killing of Melanoma Cells *in vitro*



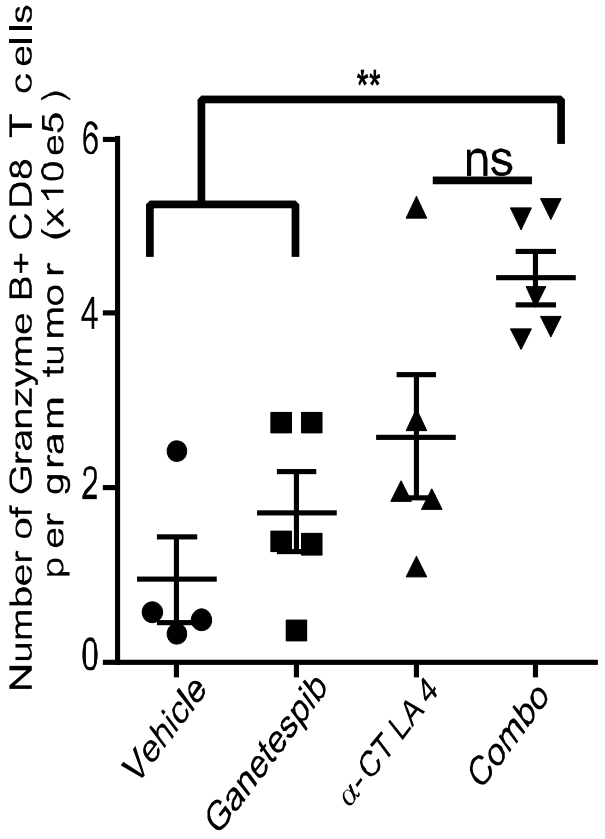
Overexpression of *IFIT* Genes Decreases BCL2 Expression



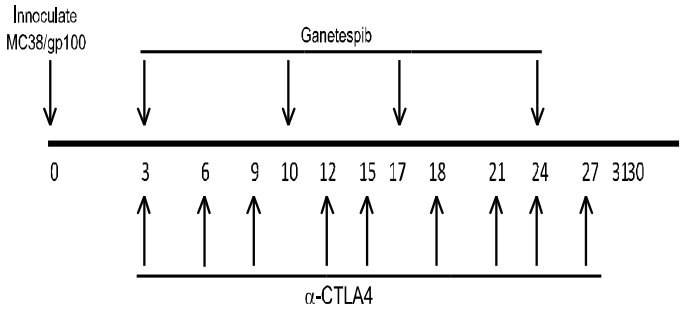
Combination of Ganetespib and α-CTLA4 Increases the CD8 T-cell/Treg Ratio

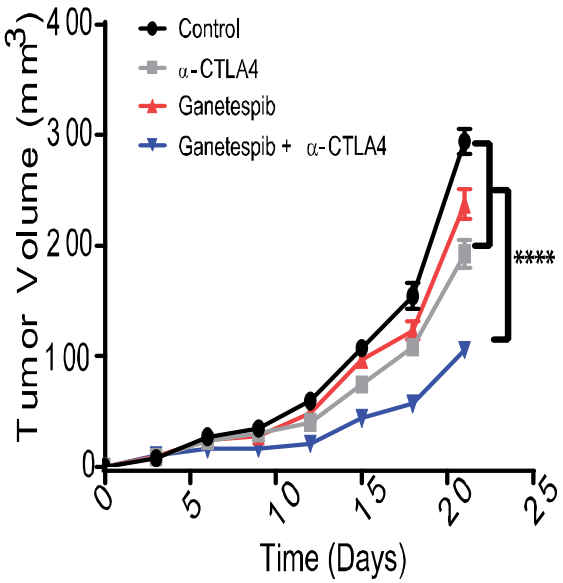


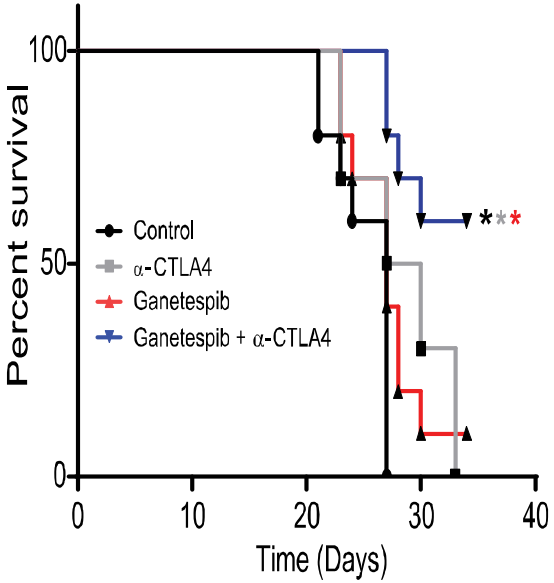
Combination of Ganetespib and α-CTLA4 has the Potential to Increase the Cytolytic Ability of CD8 T-cells



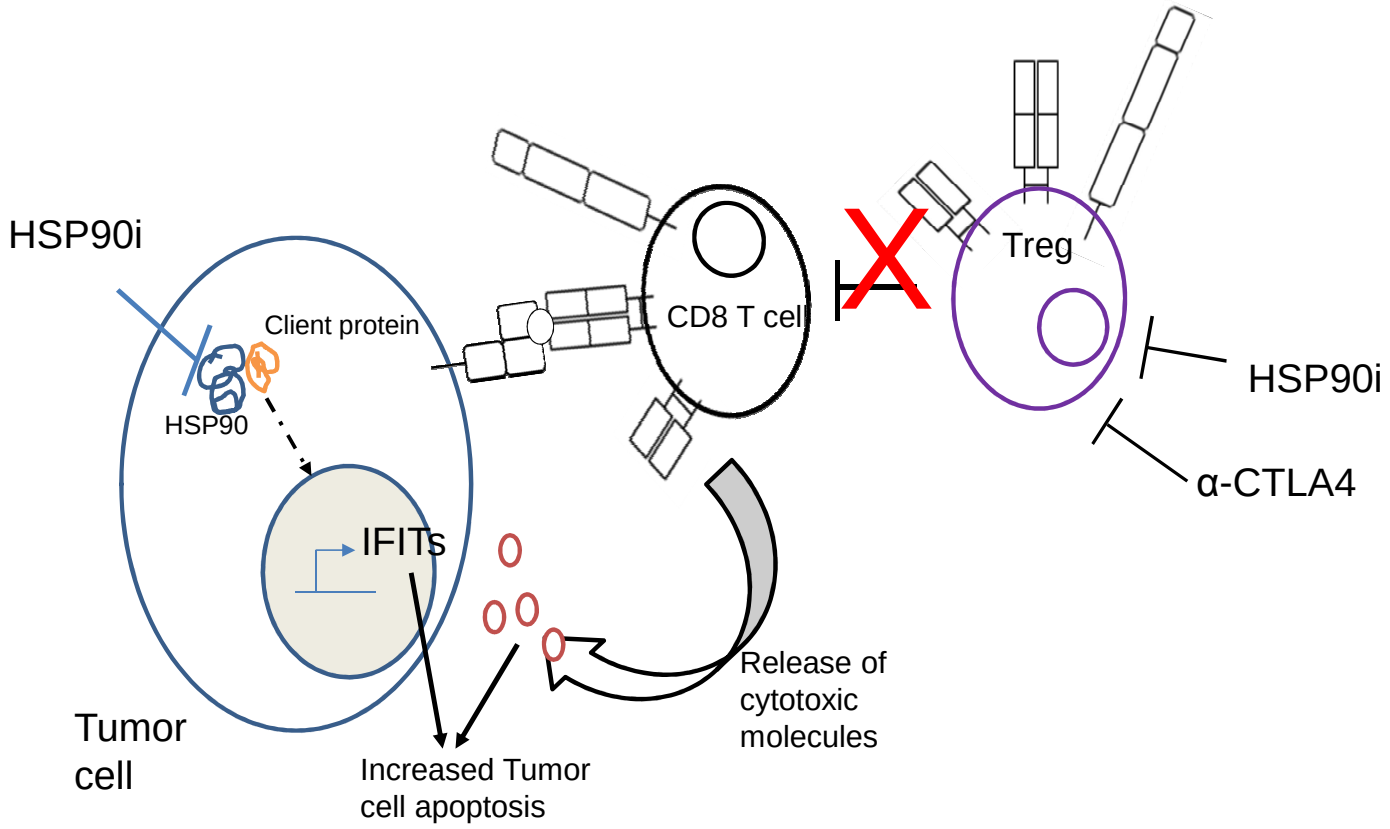
HSP90 Inhibition Enhances Response to α-CTLA 4 *in vivo*



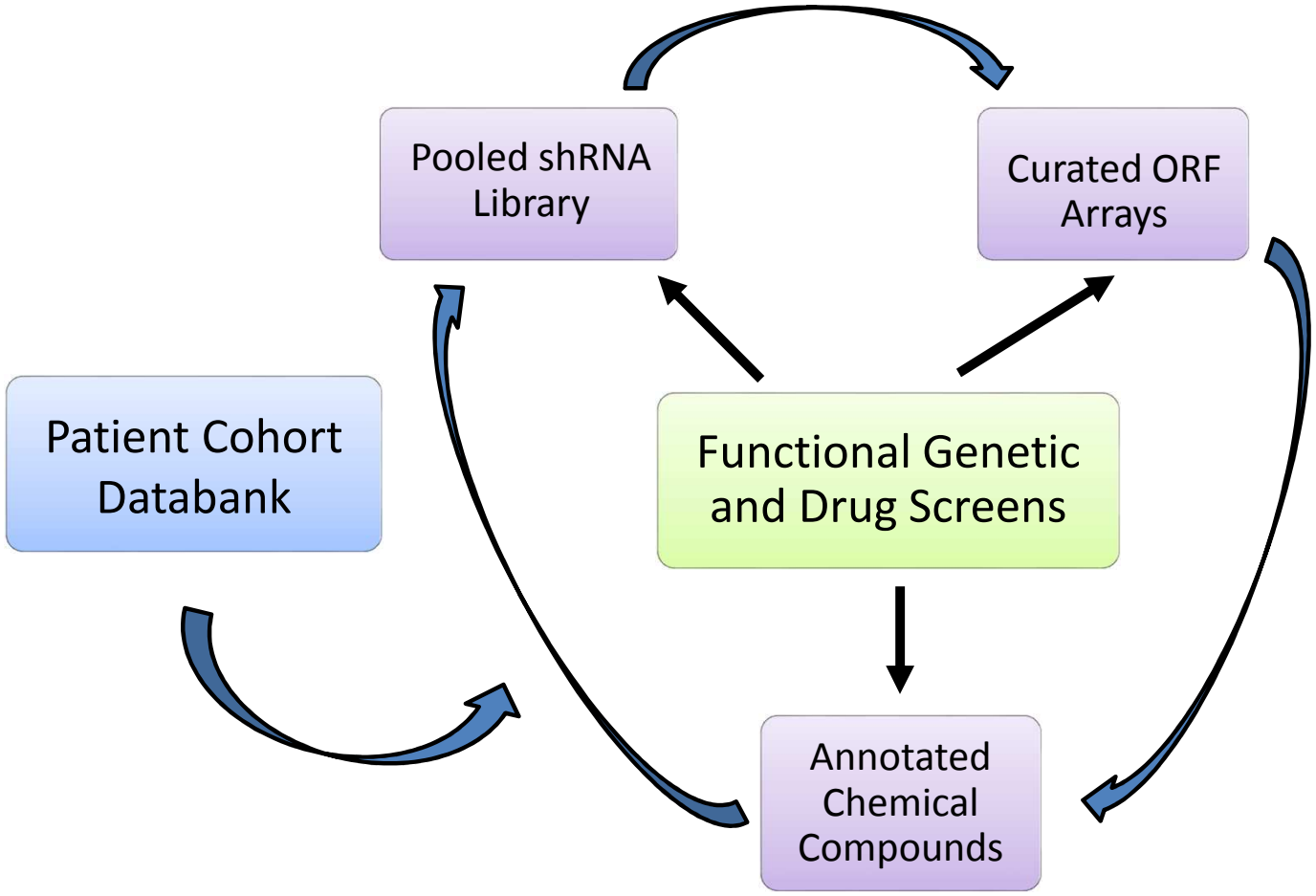




Increase in T-cell Mediated Antitumor Response by HSP90 Inhibition



Principle

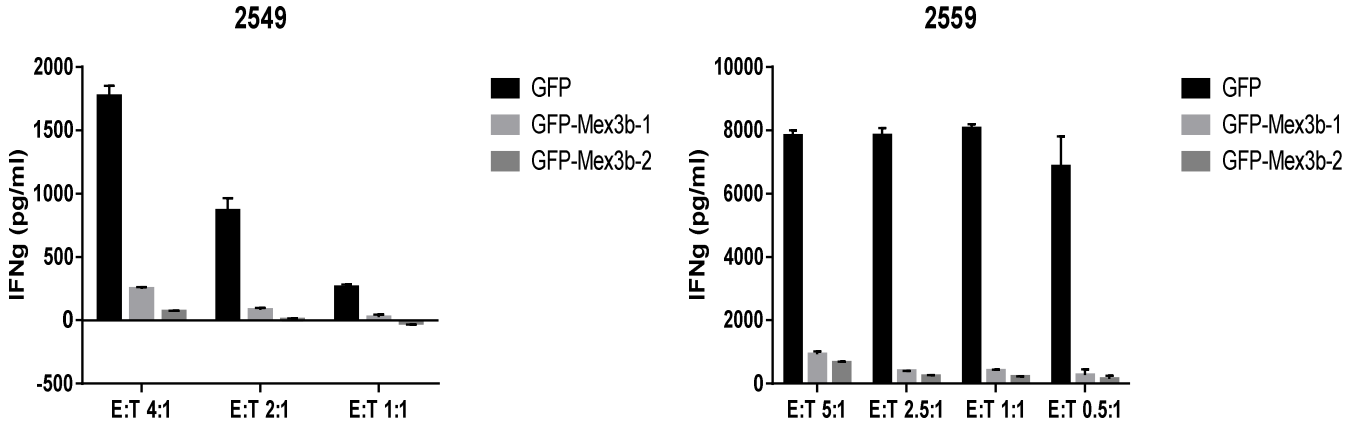


P. Hwu

Overexpression of Mex3b in melanoma cells decreases IFNγ release by autologous TILs

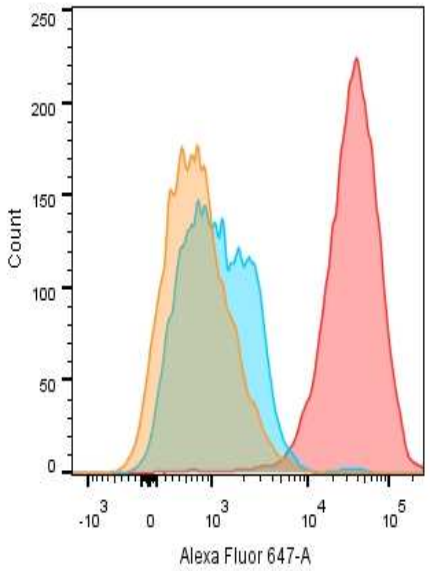
Hypothesis:
Mex3b overexpression in tumor cells inhibits their recognition by autologous TILs, thus inhibiting IFNγ production in autologous TILs

Experimental design:
Melanoma cells overexpressing GFP-Mex3b or GFP were incubated with autologous TILs for 24 hrs, followed by measurement of IFNγ levels in supernatants by ELISA



Lu Huang

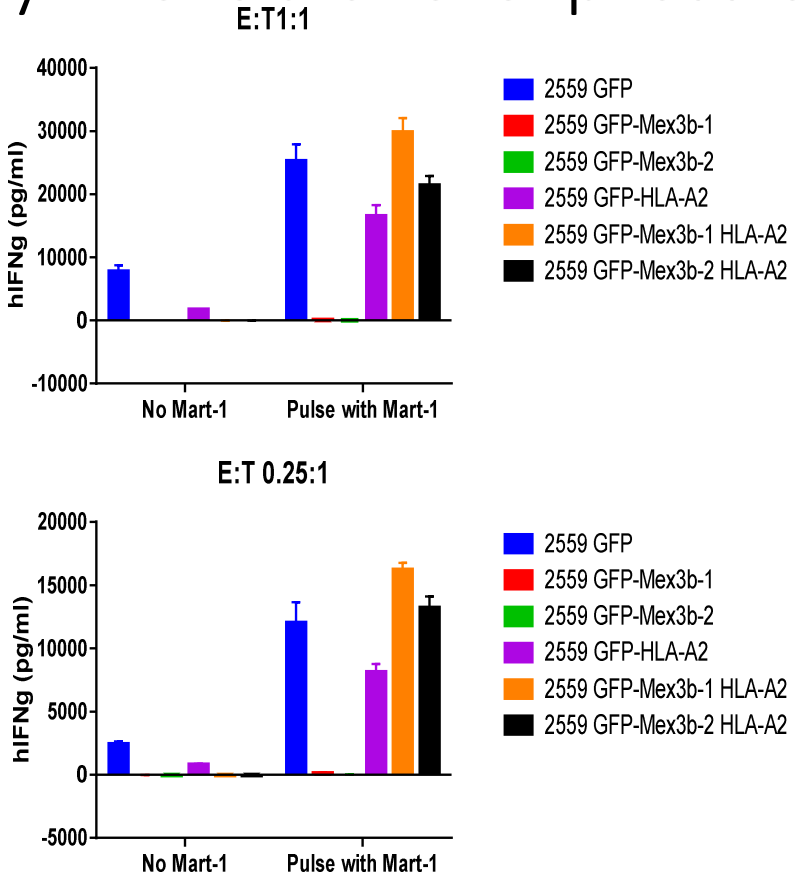
Mex3b inhibits surface HLA-A2 levels in 2559



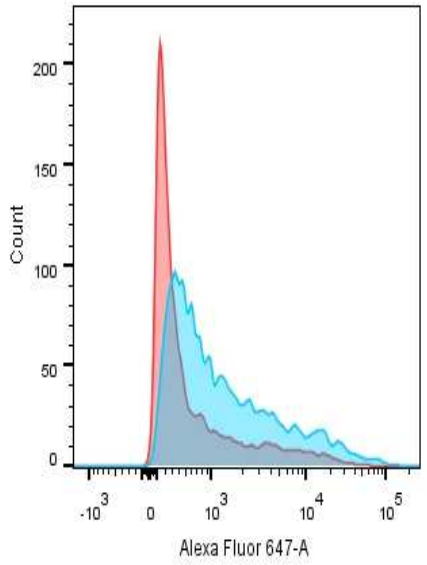
Red: 2559 GFP
Blue: 2559 GFP-Mex3b-1
Orange: 2559 GFP-Mex3b-2

GFP positive cells: **Mean** : *Alexa Fluor 647-A*: 43935
GFP positive cells: **Mean** : *Alexa Fluor 647-A*: 1913
GFP positive cells: **Mean** : *Alexa Fluor 647-A*: 963

Overexpression of HLA-A2 in 2559 melanoma cells rescues the phenotype induced by Mex3b overexpression



Mex3b decreases HLA-A31 levels in 2549

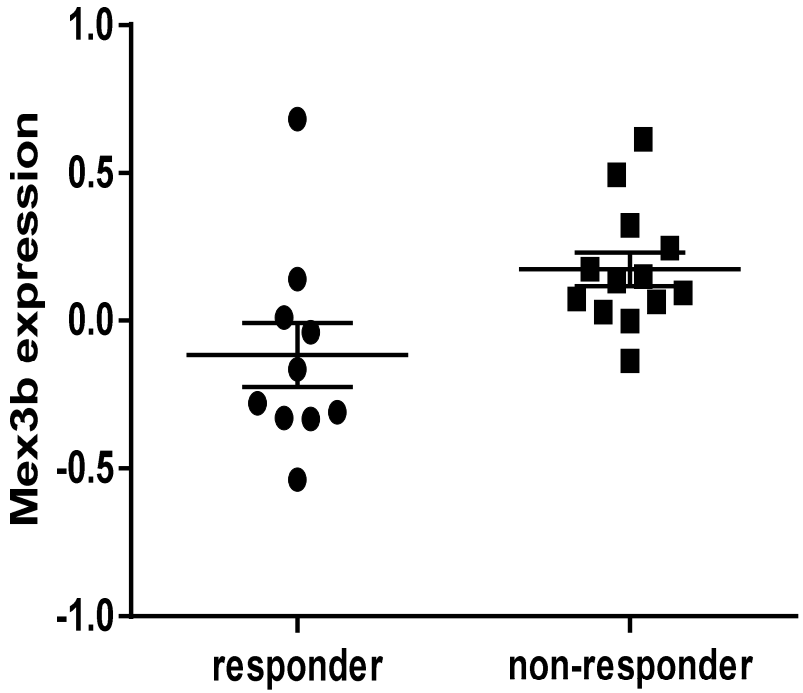


Blue: 2549 GFP
Red: 2549 GFP-Mex3b

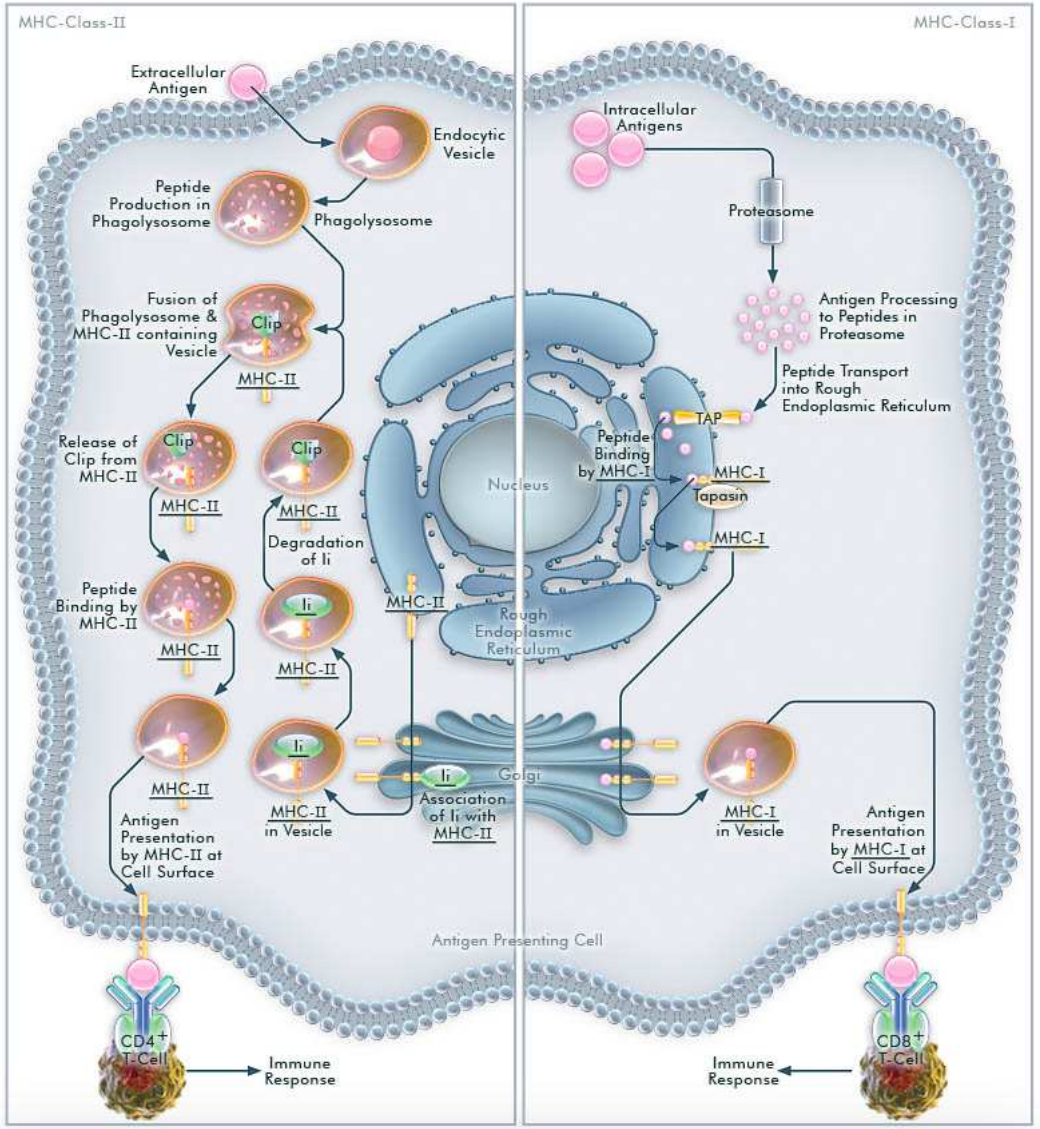
GFP positive cells: **Mean** : *Alexa Fluor 647-A*: 4681
GFP positive cells: **Mean** : *Alexa Fluor 647-A*: 1958

Mex3b and patient response to anti-PD1

Gene
ALDH1L2
MFAP2
CDH1
OLIG1
TRAF3IP2
TDRD10
CILP2
MEX3B
SLC45A1
RASL11B



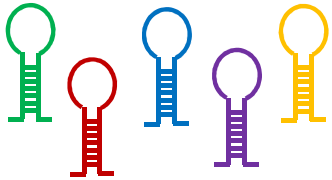
Hugo et al., *Cell* 2016



Antigen Processing and Presentation by MHCs

shRNA Screen

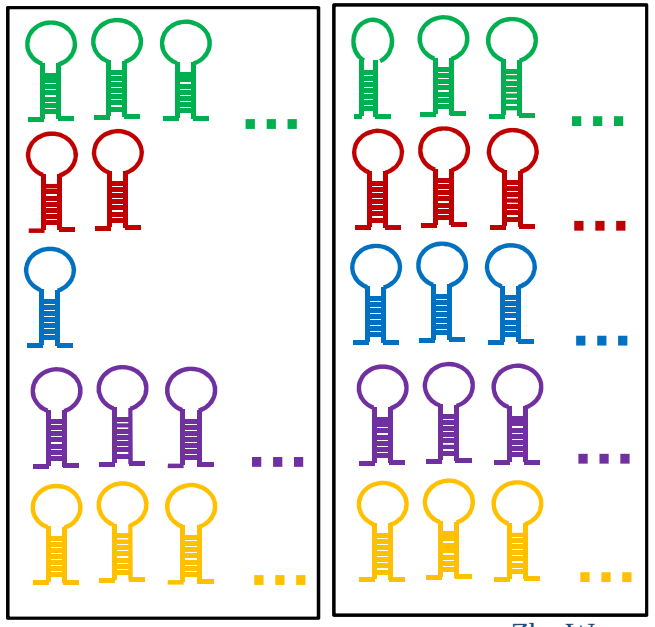
Pooled Human Lentiviral
shRNA Library
(~ 10 shRNA hairpins per gene)



Tumor
Cell
Infection

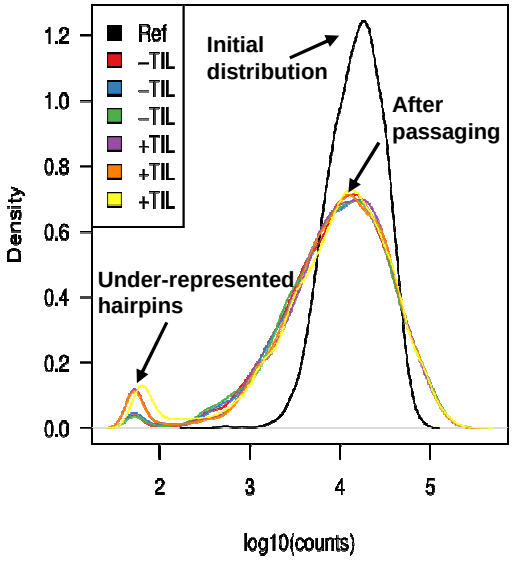
NGS

+ TIL - TIL

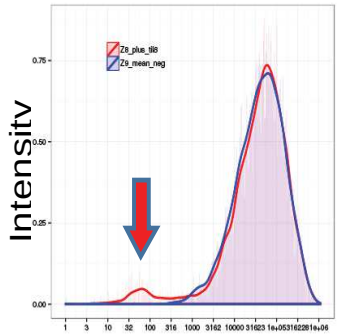


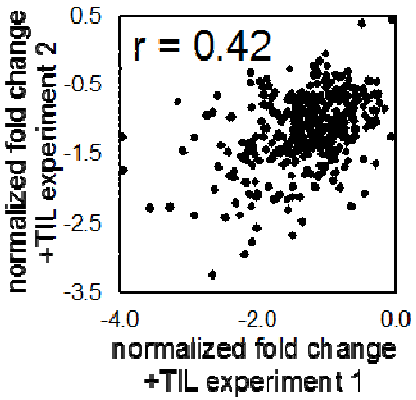
Statistical
analysis

Distribution of hairpins



Several Glycolysis-related Genes Were Identified as Candidate Genes Promoting Immune Resistance





Glycolysis Pathway

fructose-1,6P
ALDOA
ALDOC
glyceraldehyde-3P
TKTL2
2-phosphoglycerate
erthrose-4P
phosphoenolpyruvate
PKM
pyruvate
LDHA
PDHA1
ACAT1
lactate
acetyl-CoA
acetoacetyl-CoA

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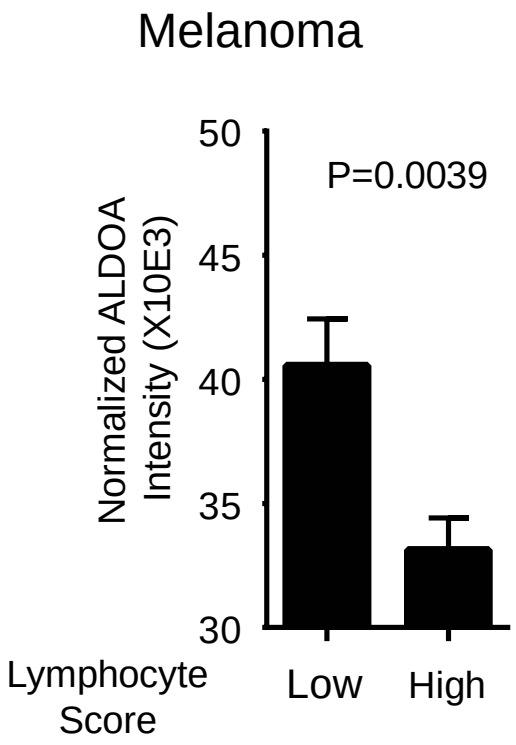
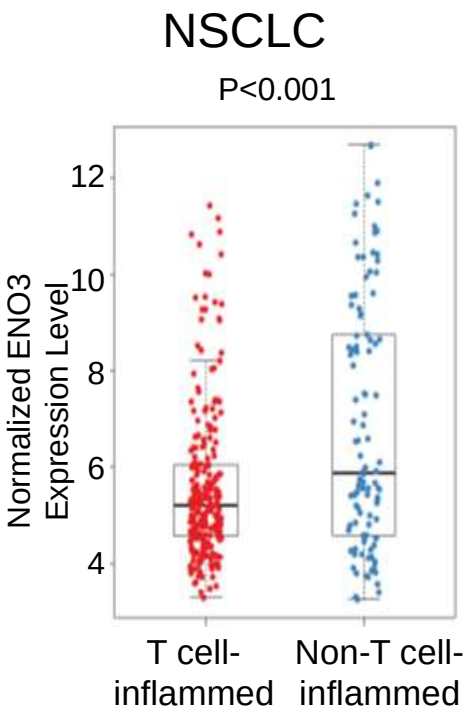
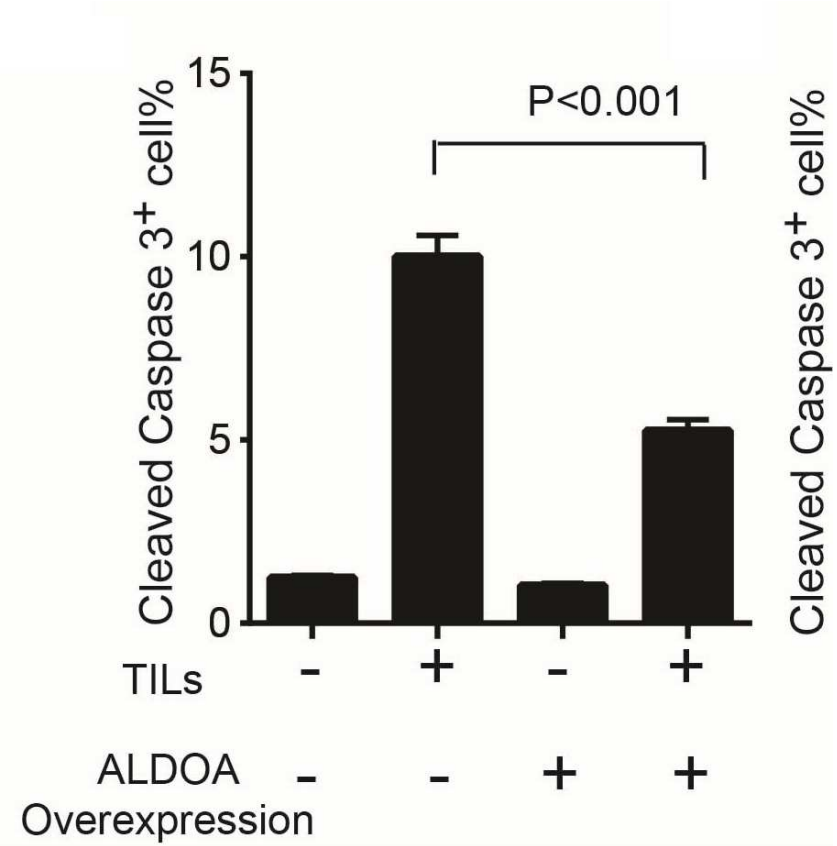
Increased Expression Levels of Glycolysis-related Genes in Melanoma Tumors and NSCLCs with Poor T-cell Infiltration





Overexpressing Glycolysis-related Genes in Melanoma Cells Reduces the Susceptibility of Tumors to T-cell Mediated Killing



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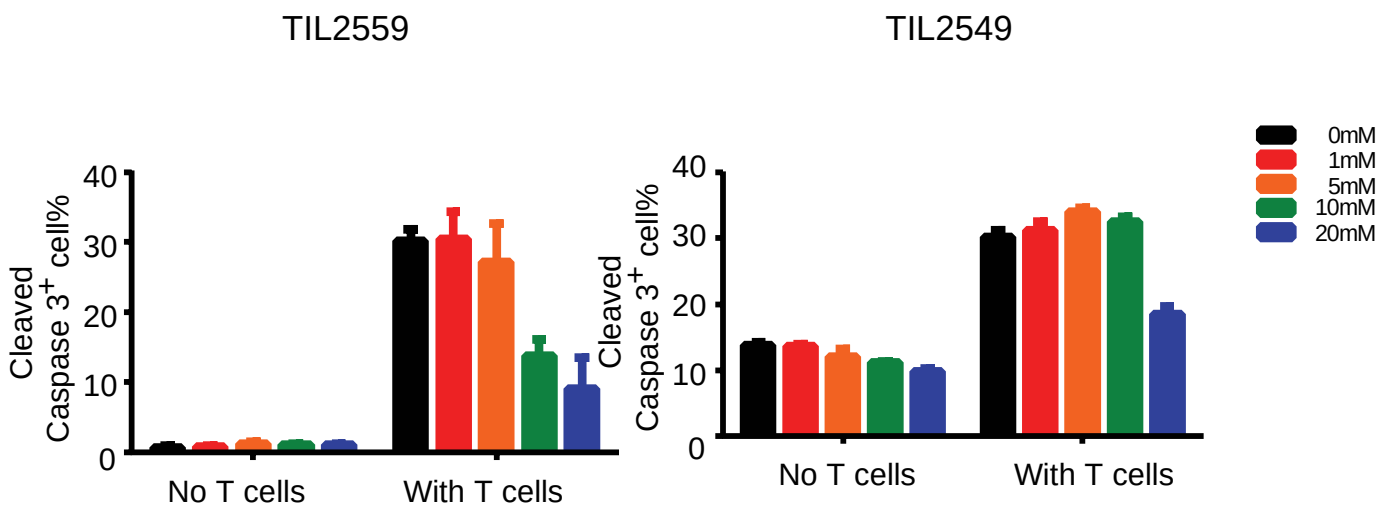
Lactic Acid, the End Product of Glycolysis, Impairs Effector Function of Tumor-infiltrating T-cells (TILs)



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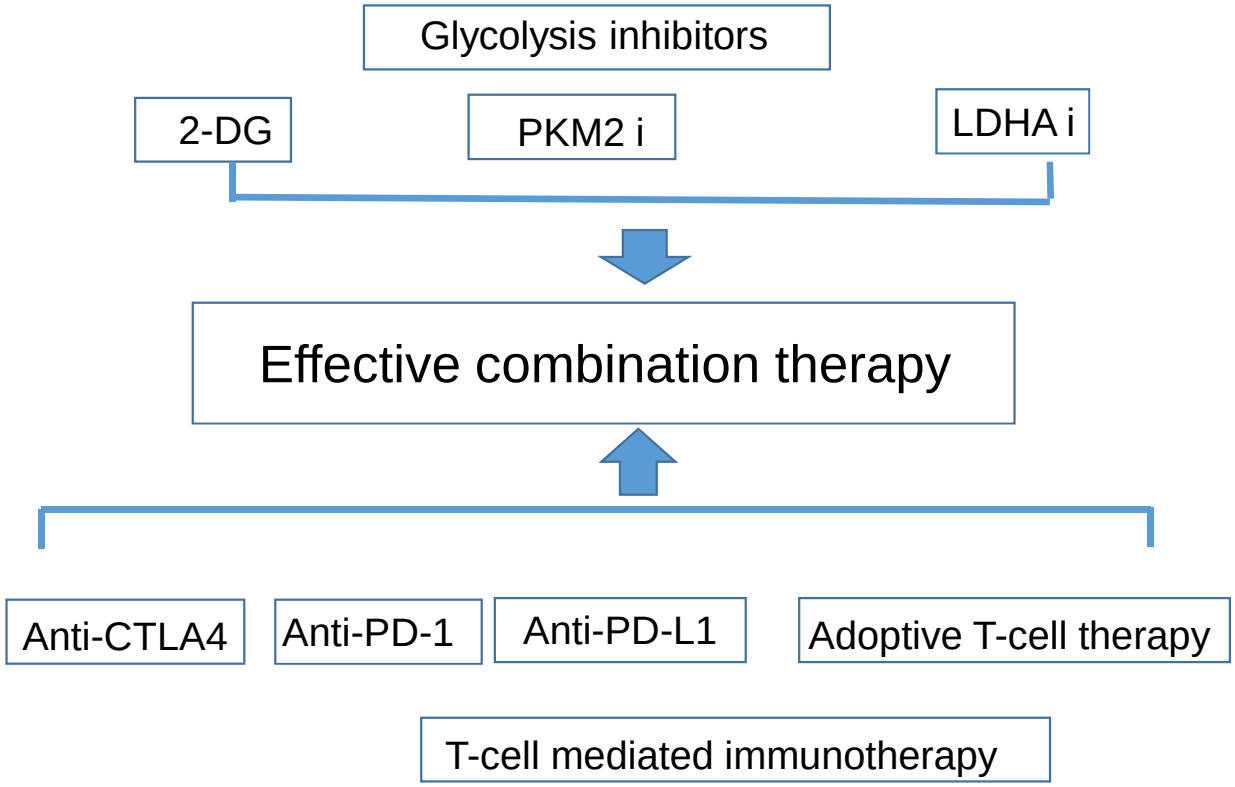
Implications



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Conclusions

We are beginning to understand pathways in the tumor which may modulate the antitumor immune response

- **BRAF**
- **Beta catenin**
- **PTEN/PI3K**
- **HSP90**
- **Aurora kinase**
- **Topo1**
- **MEX3B**
- **Glycolysis**

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