# Prioritizing Combination Immunotherapies and Combination Immune/Targeted Therapies: So Many Choices!

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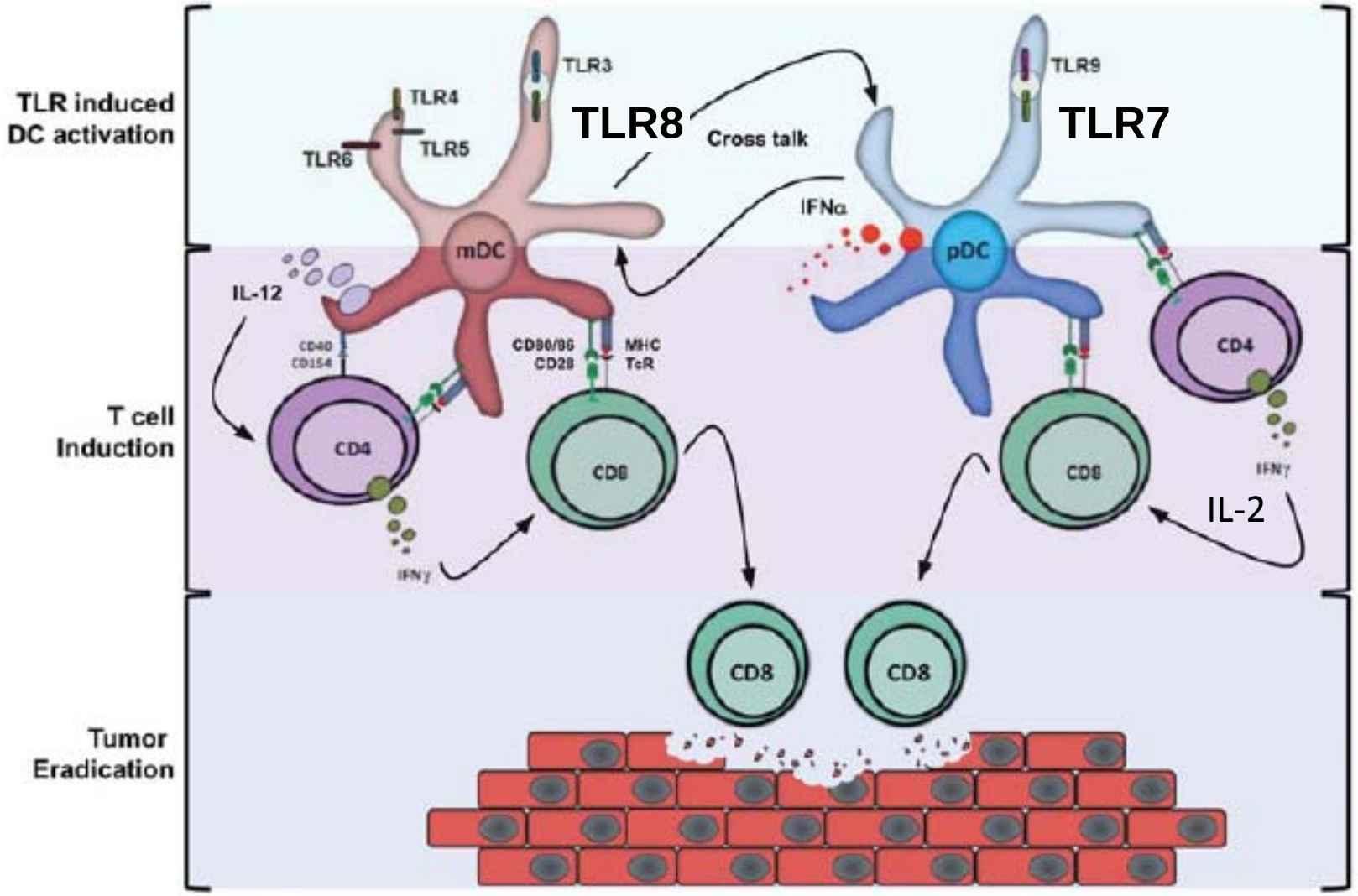
Department of Melanoma Medical Oncology
Co-Director Center for Cancer Immunology

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

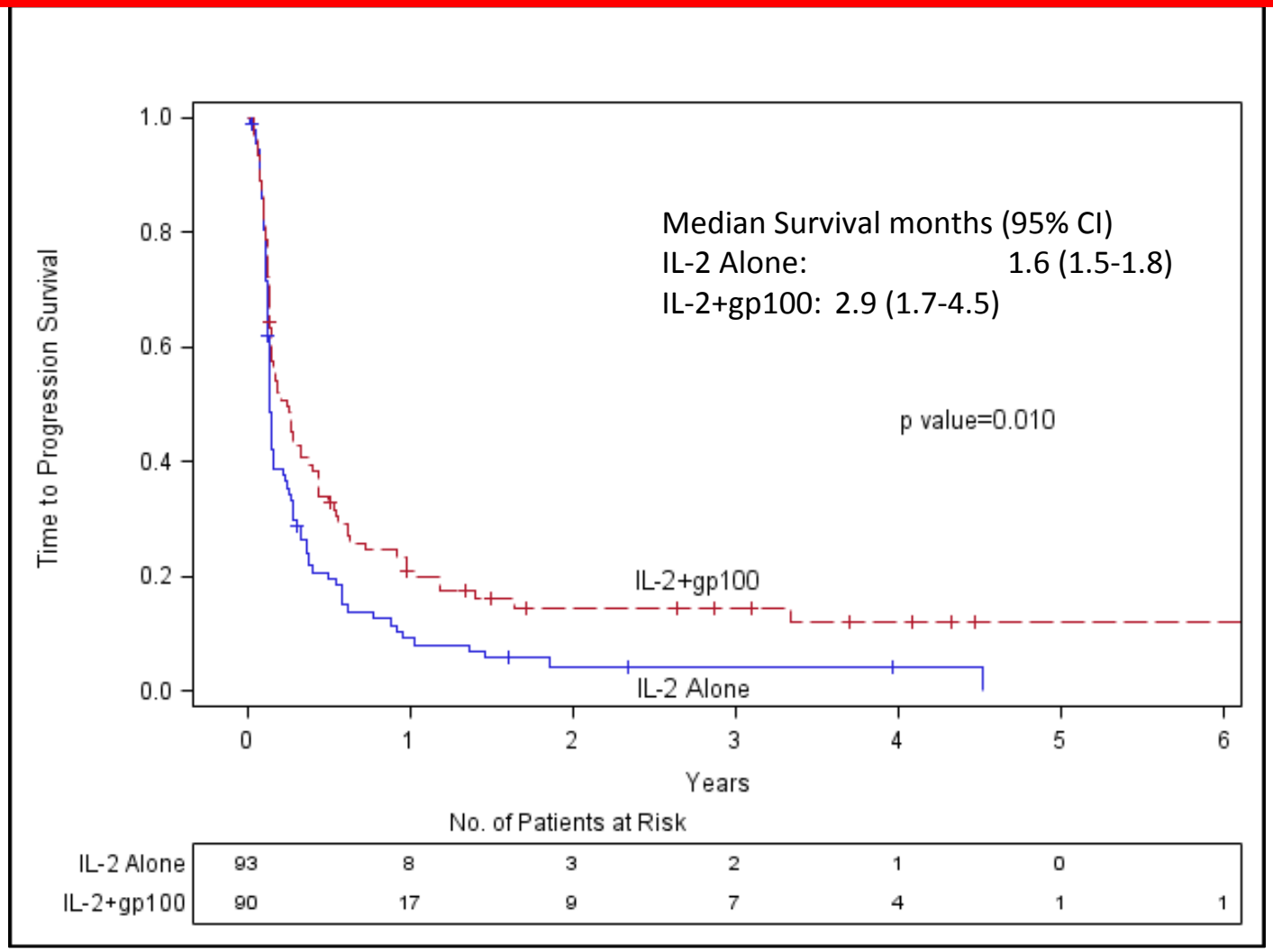
SITC, NIH Campus, April 2013

# The Immune Response Against Cancer is Complex




Schreibelt G. et al. Cancer Immunol Immunother (2010) 59:1573–1582

# Progression Free Survival in Melanoma Patients Treated with IL-2 vs Vaccine/IL-2



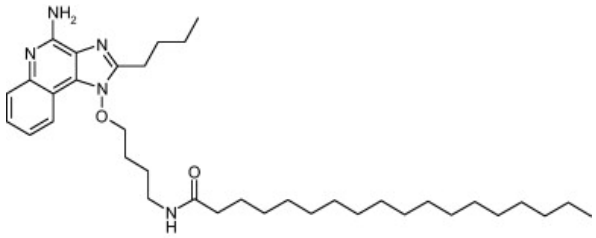

Schwartzentruber … Hwu. NEJM 364(22):2119-27, 2011.

# Responses Following Vaccination with Resiquimod

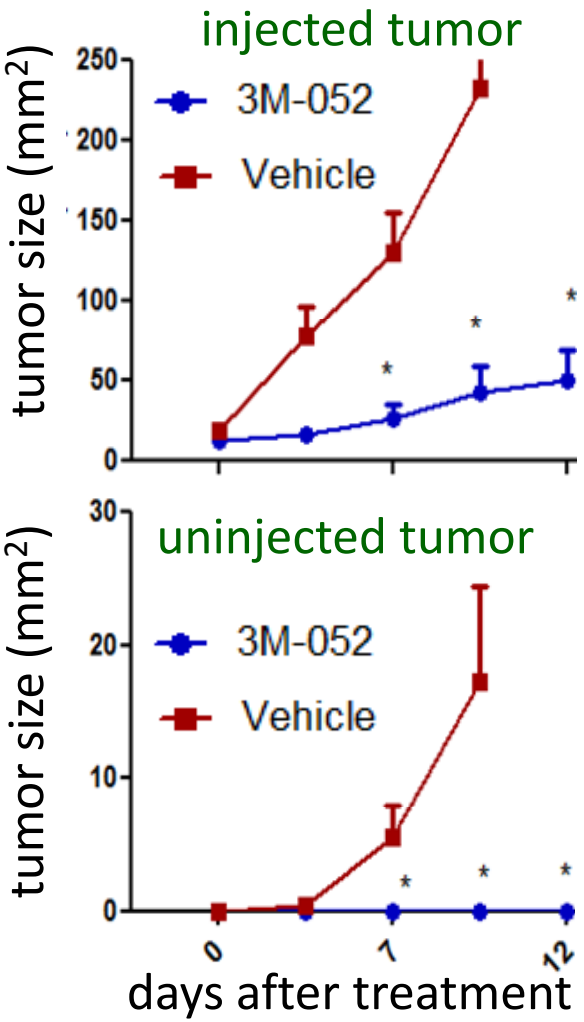
Baseline

After vaccination, Resiquimod

# Systemic Anti-tumor Activity after Local Treatment with 3M-052



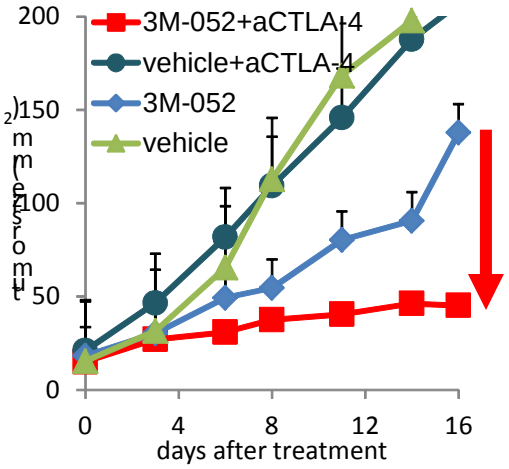
3M-052 (TLR7/8)



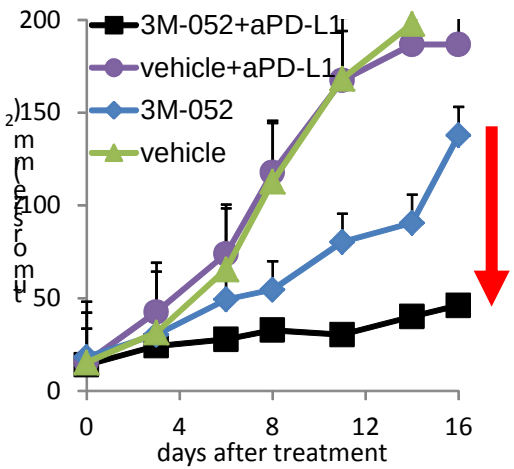

Manisha Singh/Willem Overwijk

# 3M-052-based Combination Therapy

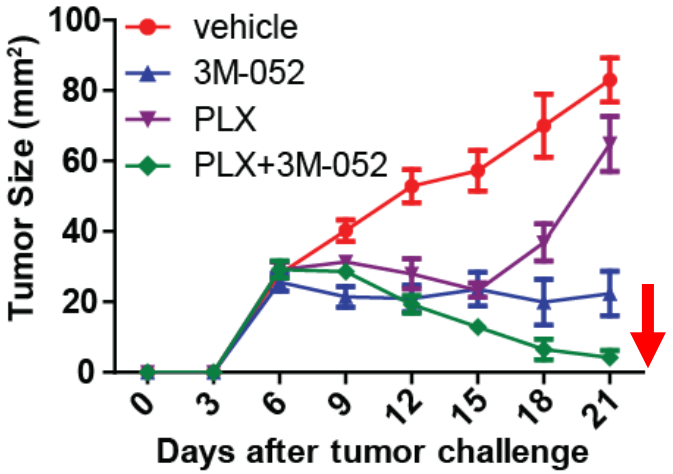
3M-052
+ αCTLA-4




3M-052
+ αPD-L1

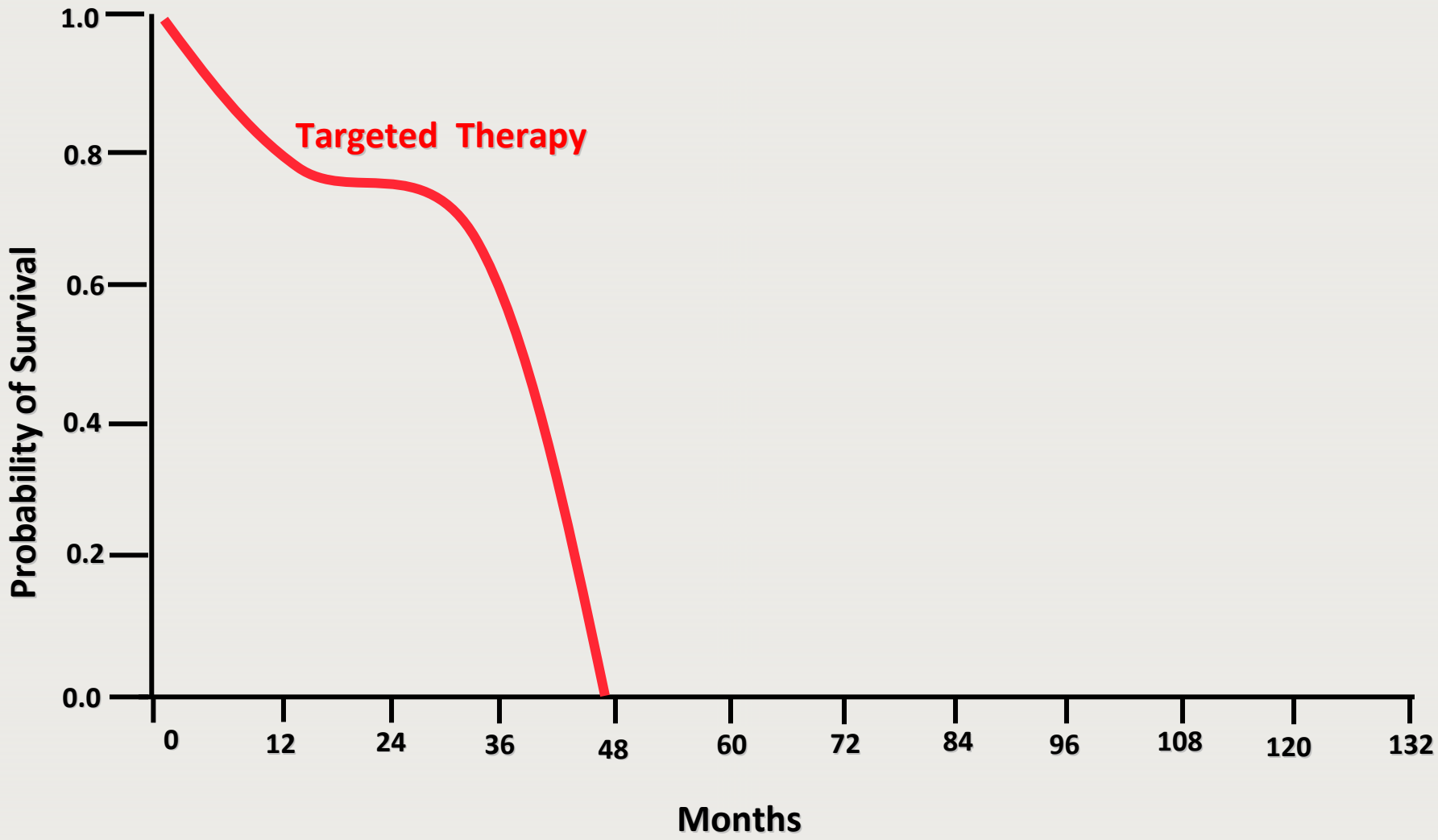



3M-052
+ mutBRAF inh.

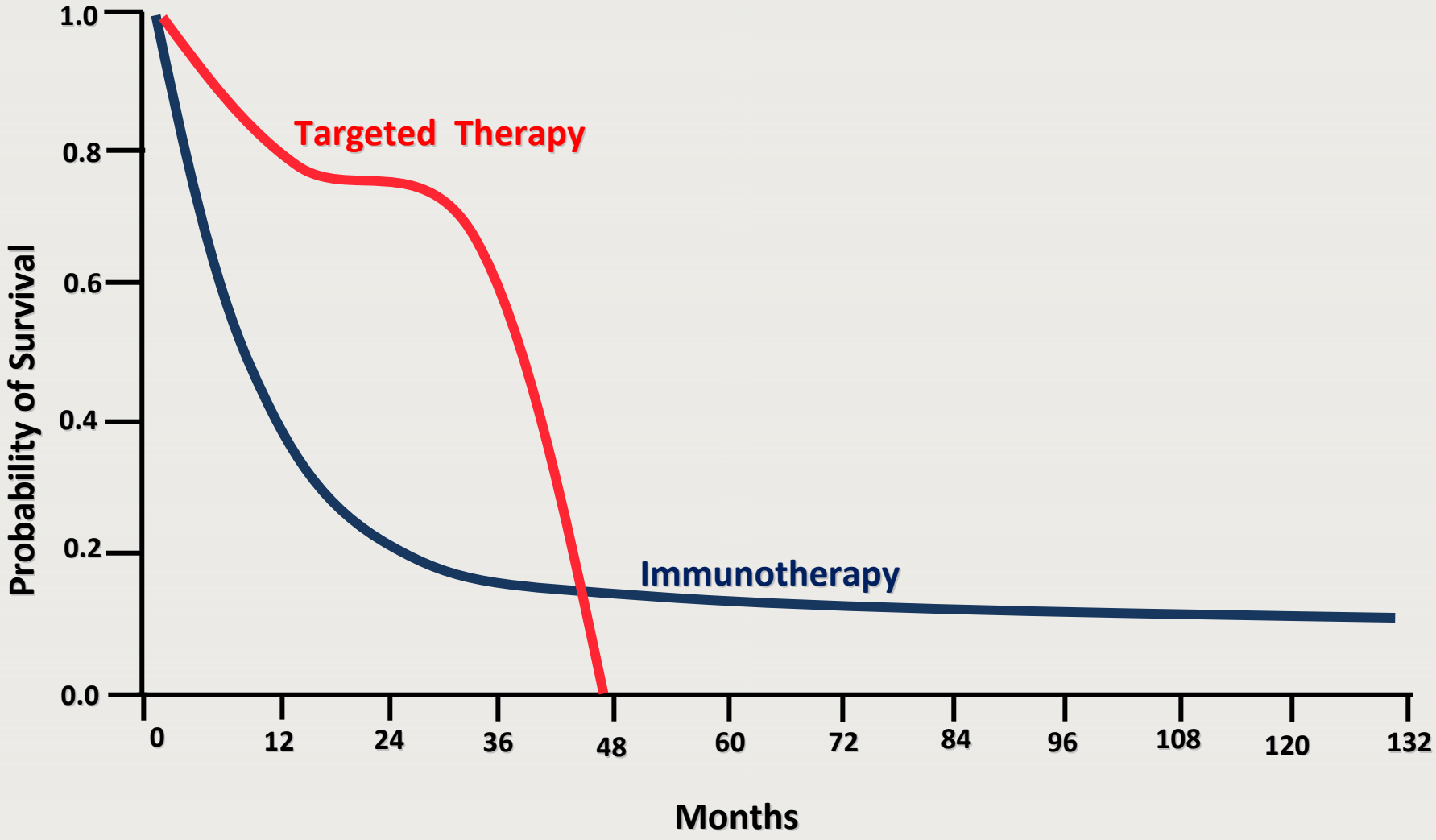



Manisha Singh, Weiyi Peng, Chengwen Liu, Willem Overwijk

# The Goal: Increase the Tail of the Curve



# The Goal: Increase the Tail of the Curve



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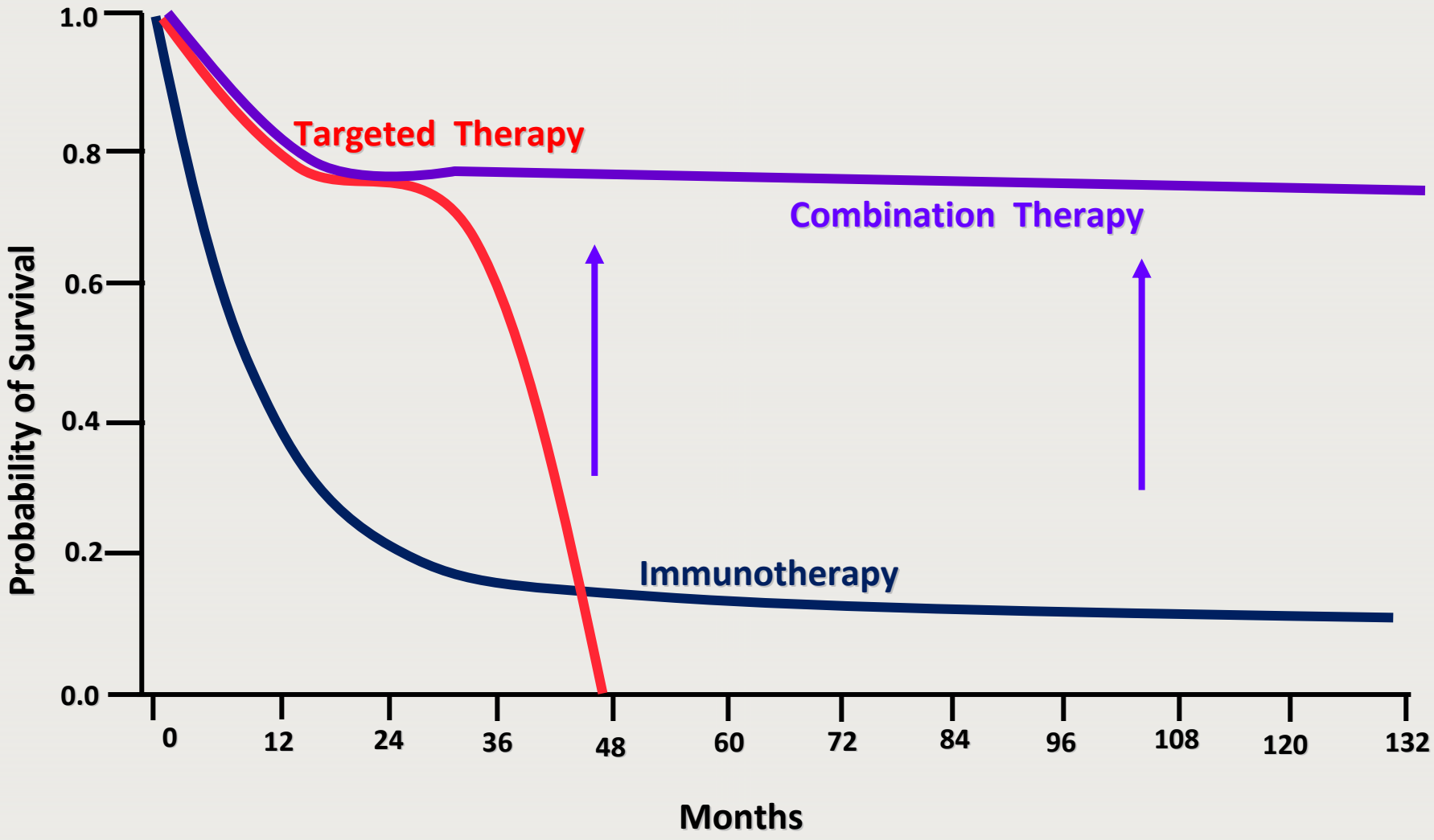


# There is Great Potential for Targeted and Immune Therapy Combinations: However, there are too many possibilities.

| Promising Targeted Agents | Immune Agents | Treatment Schedules |
|---|---|---|
| BRAFi | anti-PD-1 | Targeted then Immune Rx |
| MEKi | anti-CTLA4 | |
| cKITi | anti-PDL1 | |
| CDK4i | anti-OX40 | Immune then Targeted Rx |
| PI3Ki | anti-CD40 | |
| AKTi | IL-2 | |
| mTORi | IFN | Targeted and Immune Rx together at same time |
| ERKi | T-cells | |
| IGF1i | IL-21 | |
| EGFi | Vaccines | |
| | TLR Agonists | |

10 X 11 X 3

**330 trials X $3-million/trial = ~$1-billion**

# Solution: “De-Risk” Clinical Trials with Focused Modeling

- **In Vitro Models**
- **Mouse In Vivo Models**
- **Clinical Trial Monitoring**

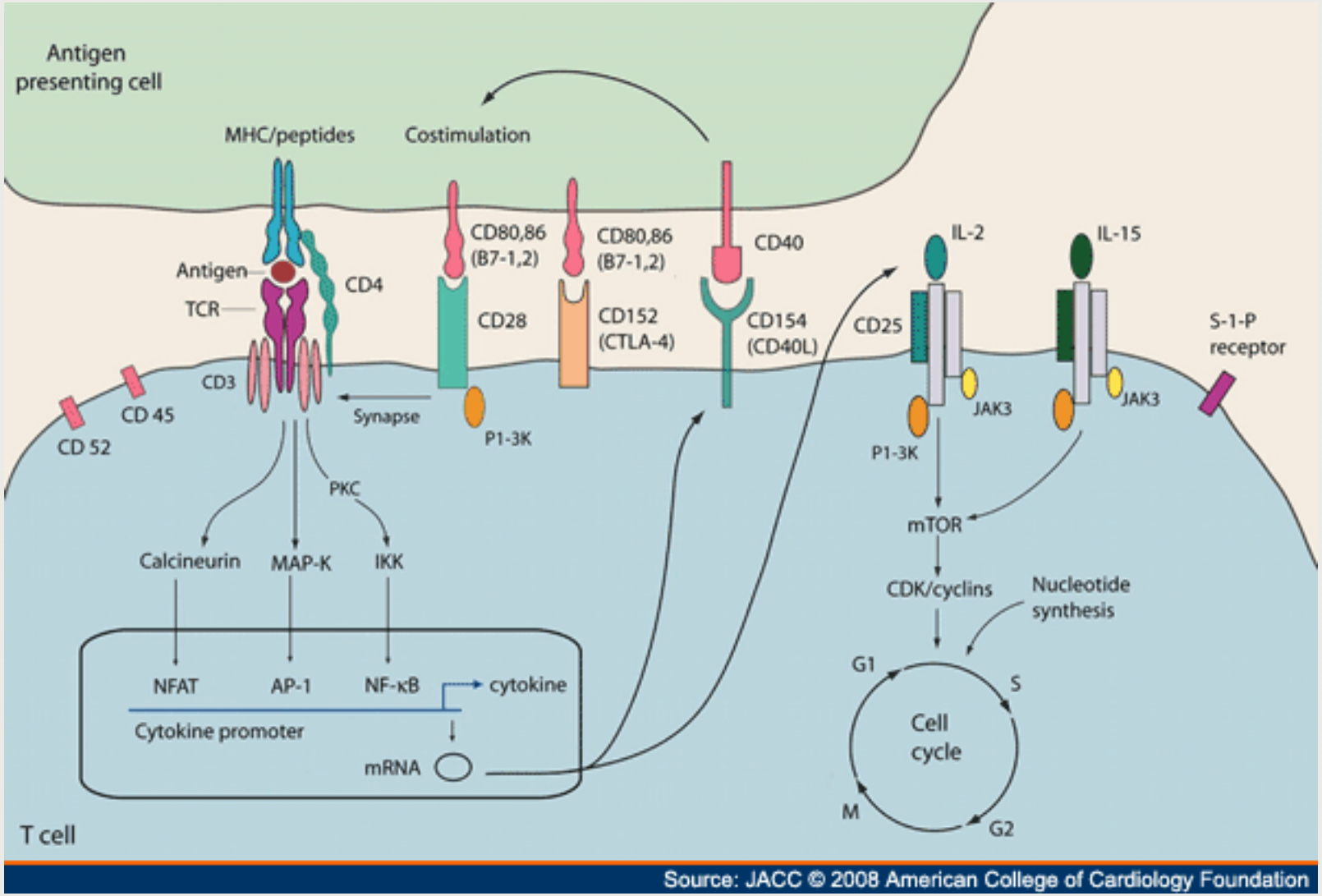
# Goal is to Determine…

**...Optimal Combination(s) of Agents**

**…Optimal Schedules**

**…the Effects of Targeted Agents on the Immune Response**

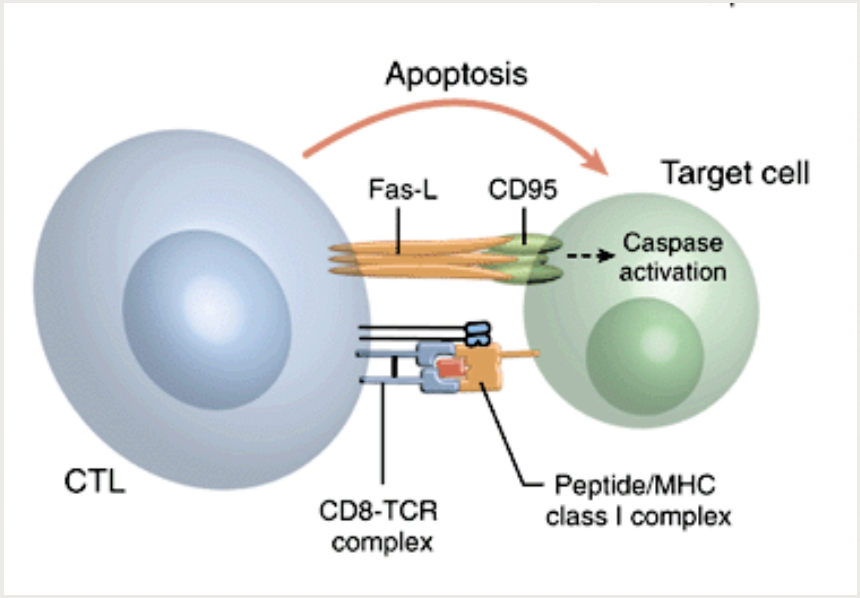
# T-cells and Tumors Share Common Signaling Pathways

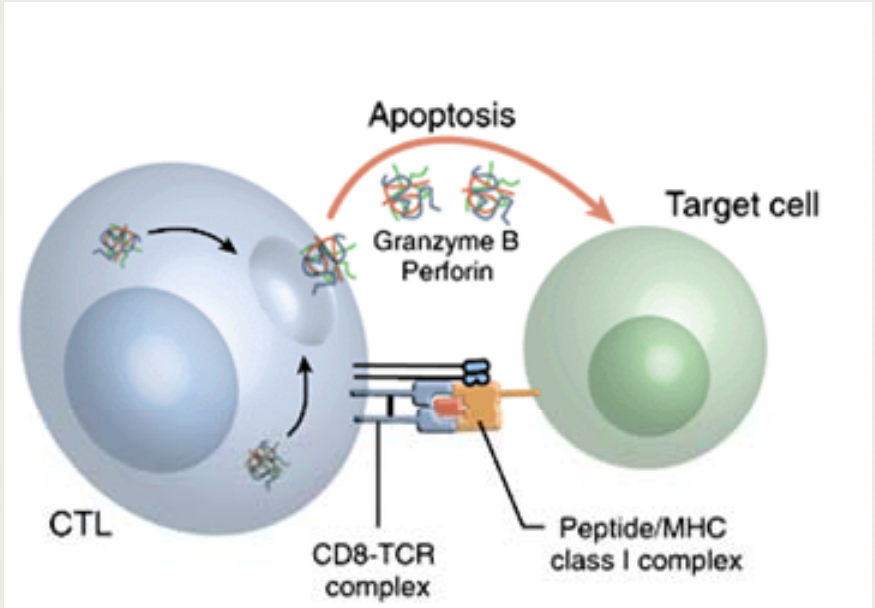


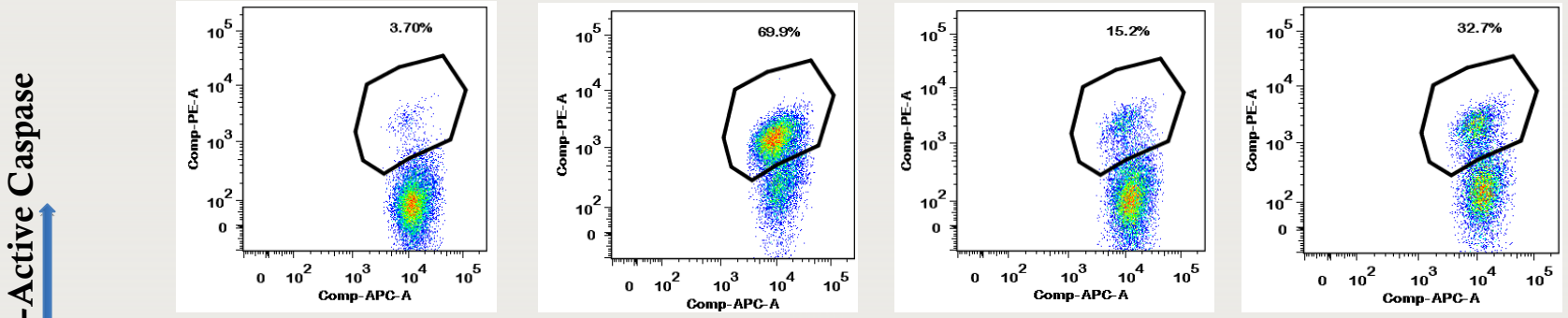
# Solution: “De-Risk” Clinical Trials with Focused Modeling

- **In Vitro Models**
- **Mouse In Vivo Models**
- **Clinical Trial Monitoring**

# Mechanisms of T-cell Mediated Cytotoxicity: Active caspase-3 based Assay




Melanoma cells alone

Melanoma cells + Staurosporine

TILs + Tum (3:1) (T-cell mediated)

TILs + TUM (10:1) (T-cell mediated)

# Screen for Candidates to Combine with Immunotherapy

**Treatment**

- Treat 50,000 melanoma tumor cells with a chemical compound at a concentration of 1μM for 24 hours at 37°C.
- Treat tumor reactive TILs (Tumor infiltrating lymphocytes) with a chemical compound at a concentration of 1μM for 24 hours at 37°C.

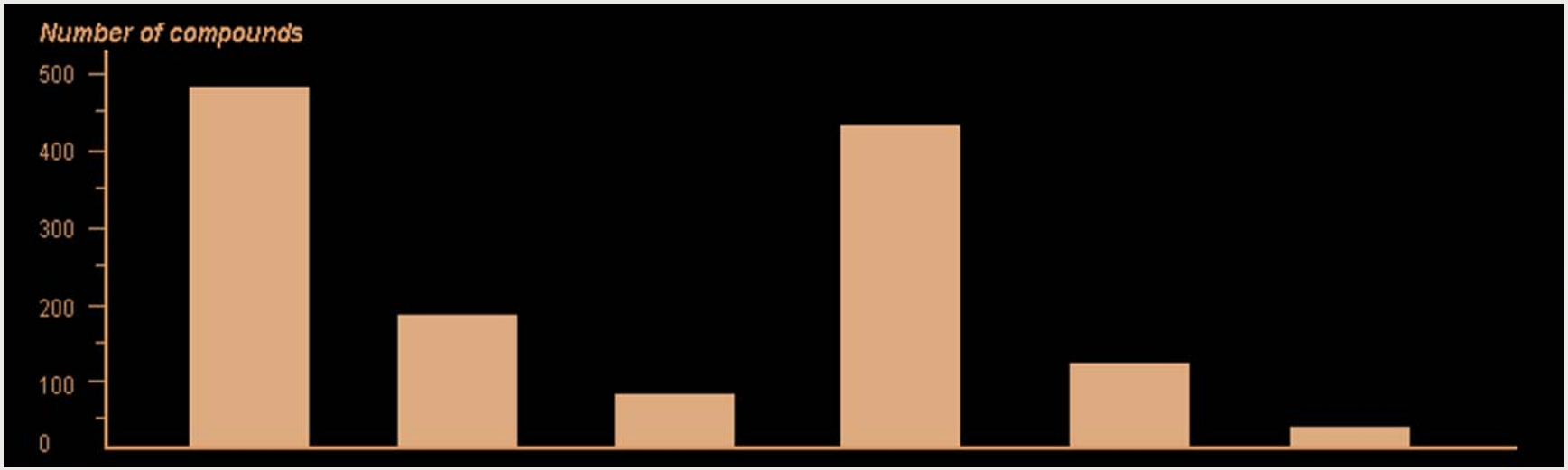
**T-cell cytotoxicity assay**

- At 24 hours, wash 1X and add tumor reactive TILs at a ratio of 3:1 (TIL:Tumor) to *treated tumor cells* and incubate for 3 hours at 37°C.
- At 24 hours, wash 1X and add tumor reactive *treated TILs* at a ratio of 3:1 (TIL:Tumor) to tumor cells and incubate for 3 hours at 37°C.

**Staining with anti–Active Caspase Antibody**

- Perform intracellular staining with anti-Active Caspase 3 Antibody
- Run FACS in a 96 well, high throughput fashion

# Screen for Candidates to Combine with Immunotherapy



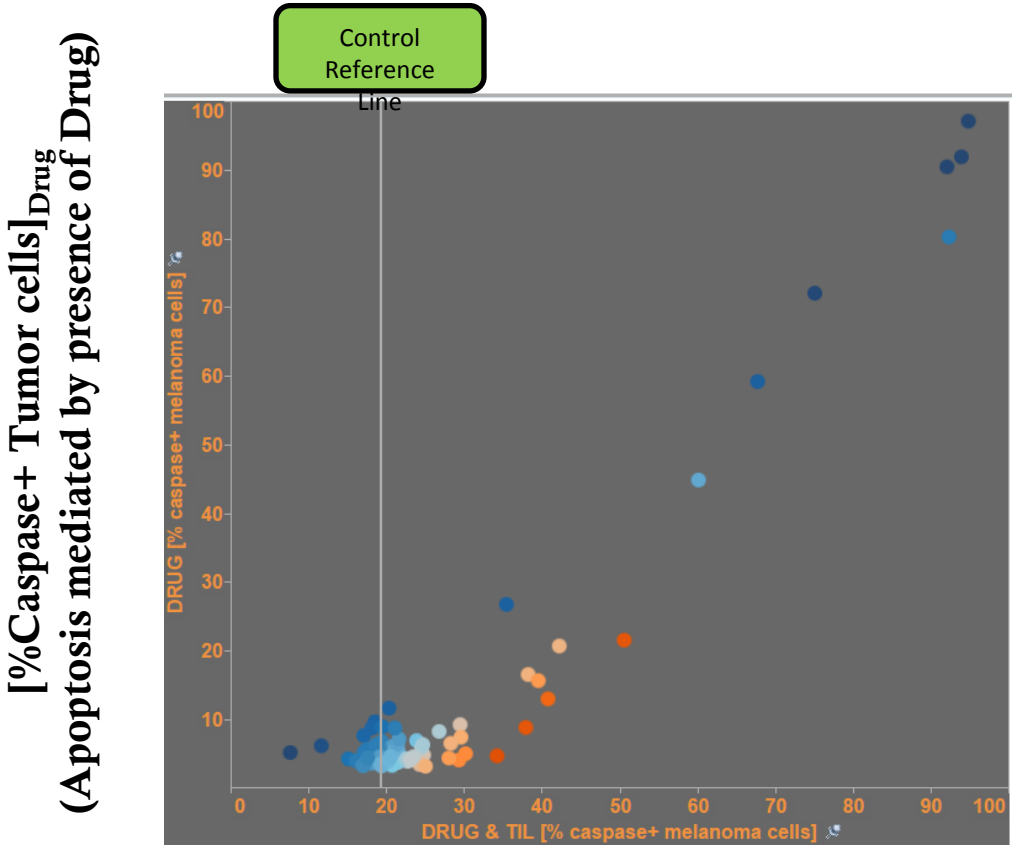

*~ 850 bioactive compounds from Selleckchem*

# Screen for Candidates to Combine with Immunotherapy

Melanoma tumor line 2549

BRAF, NRAS, c-Kit Wildtype

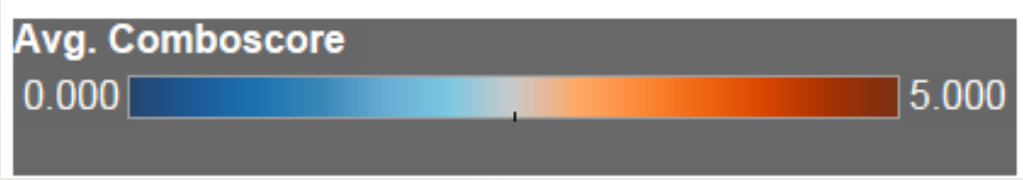
Undergoing exome analysis




Drugs Plate 10: representative plate

# Treatment of Tumor with Compounds

$$\textit{Melanoma Exposure COMBOSCORE} = \left\{ \frac{[\%\text{Caspase+ Tumor cells}]_{\text{Drug+Tcells}} - [\%\text{Caspase+ Tumor cells}]_{\text{Drug}}}{[\%\text{Caspase+ Tumor cells}]_{\text{Control+ T cells}} - [\%\text{Caspase+ Tumor cells}]_{\text{Control}}} \right\}^{2}$$

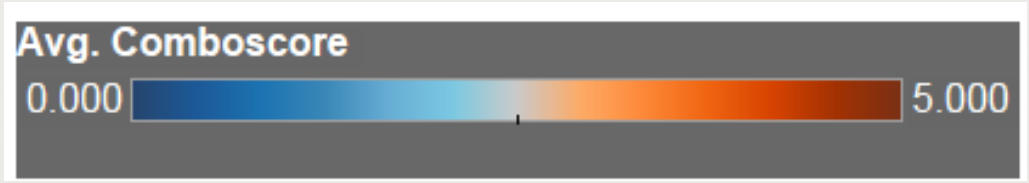



*Resistance to T-cell Mediated Killing*

*Improved T-cell Mediated Killing*

# Treatment of TILs with Compounds

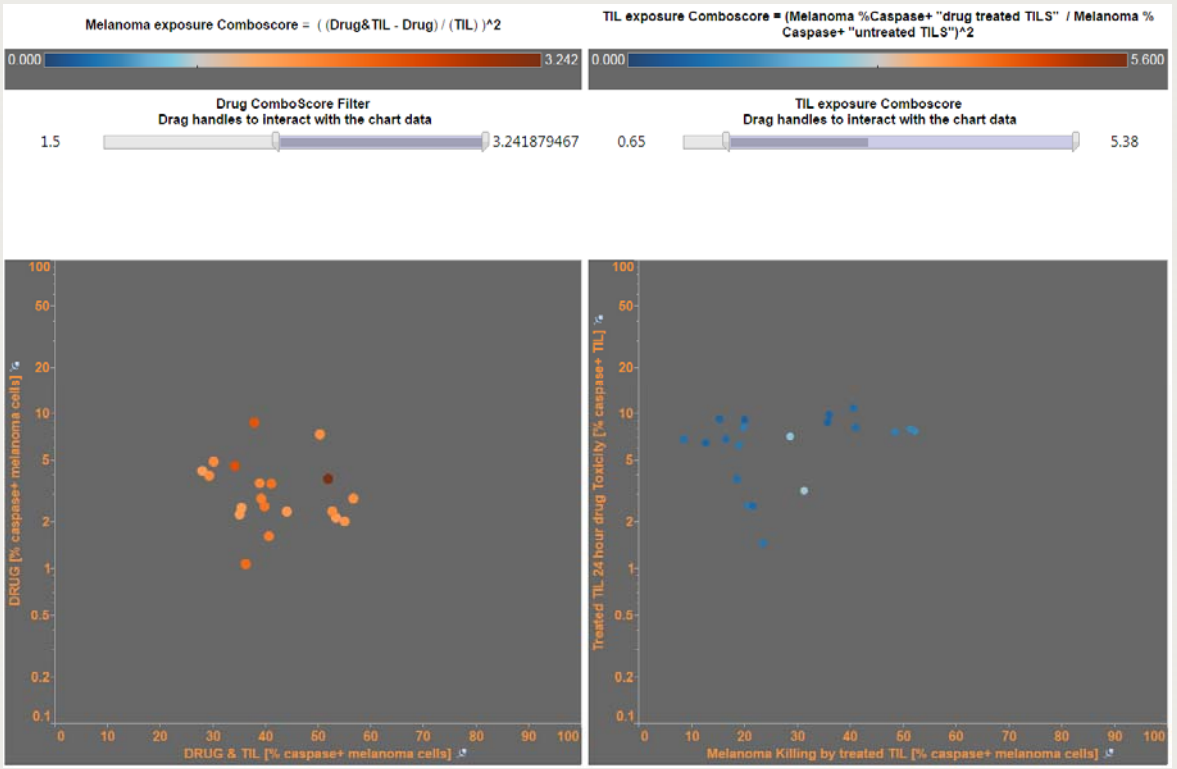
$$\text{TIL exposure COMBOSCORE} = \left\{ \frac{[\%\text{Caspase+ Tumor cells}]_{\text{Treated Tcells}}}{[\%\text{Caspase+ Tumor cells}]_{\text{Untreated Tcells}}} \right\}^{2}$$




*Not Toxic to T-cells* *Toxic to T-cells*

# Top 20: Combination of Melanoma Exposure and TIL Exposure Comboscores

- WAY-600
- Cinacalcet hydrochloride
- VX-680
- U0126-EtOH
- Sunitinib Malate
- Rivaroxaban (Xarelto)
- RAF265
- PD0325901
- Irinotecan HCl Trihydrate (Campto)
- CI-1040 (PD184352)
- Capecitabine (Xeloda)
- Bumetanide
- BMS-708163
- Bleomycin sulfate
- AZD6244 (Selumetinib)
- Amuvatinib
- AMG900
- ADX-47273
- Abiraterone Acetate (CB7630)
- 17-AAG




*Compounds at concentration tested are immunosparing and have high synergistic potential*

# Murine Melanoma Models

- **Transplantation models**
  - **Xenograft models**
  - **Syngeneic models**
- **Genetically engineered mouse model (GEM)**

# Xenograft Model

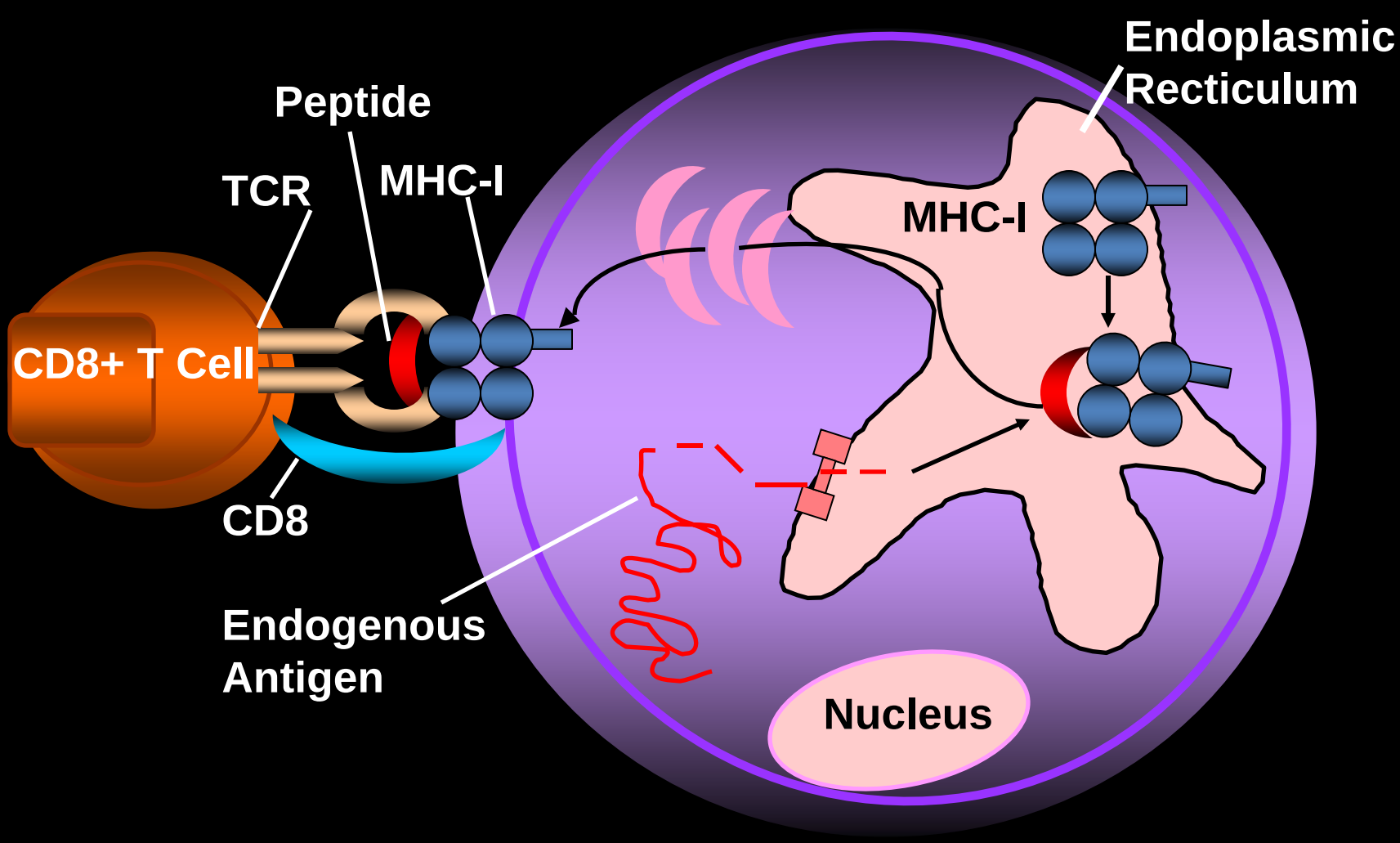
## Advantage

- Ease of implantation and performance of therapeutic studies
- Rapidity of results
- Can be used to study targeted therapy

## Drawback

- Requires immune-deficient mice
- Cannot  fully replicate the interaction between tumor cells and host stromal cells

# T-cells Can Recognize Intracellular Peptides
## Presented by MHC Molecules



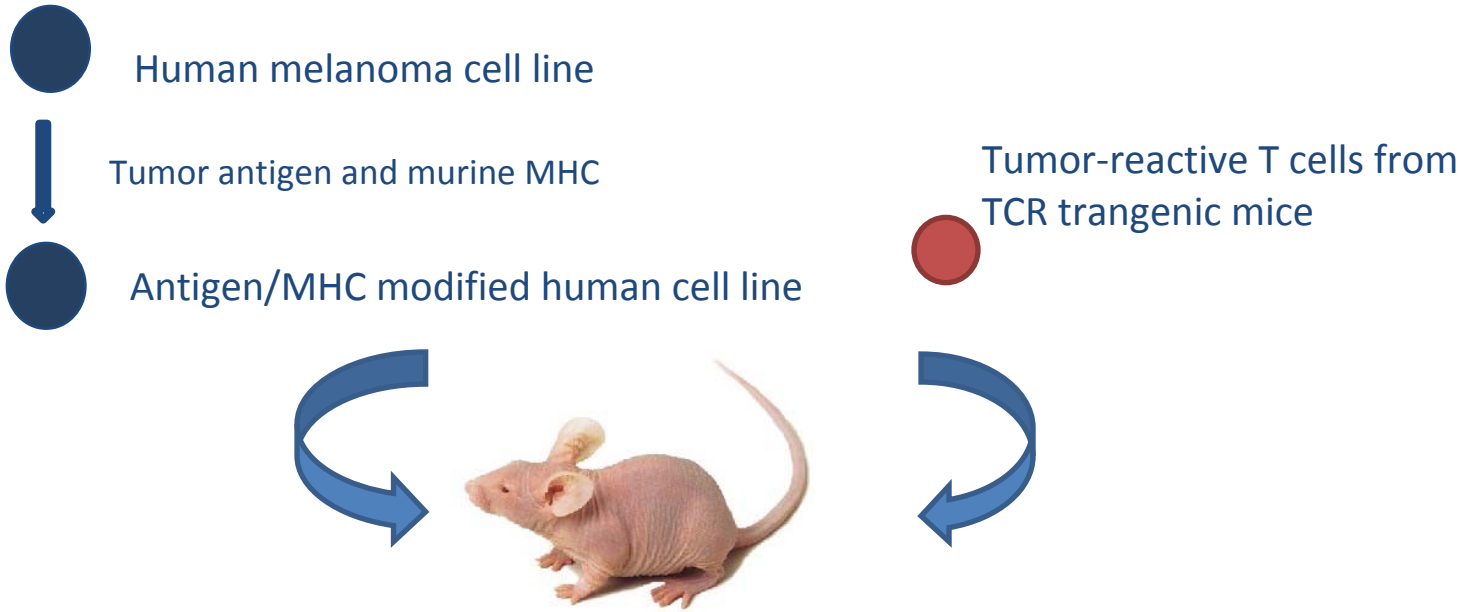
# Antigen/MHC Modified Xenograft Model

## Advantage

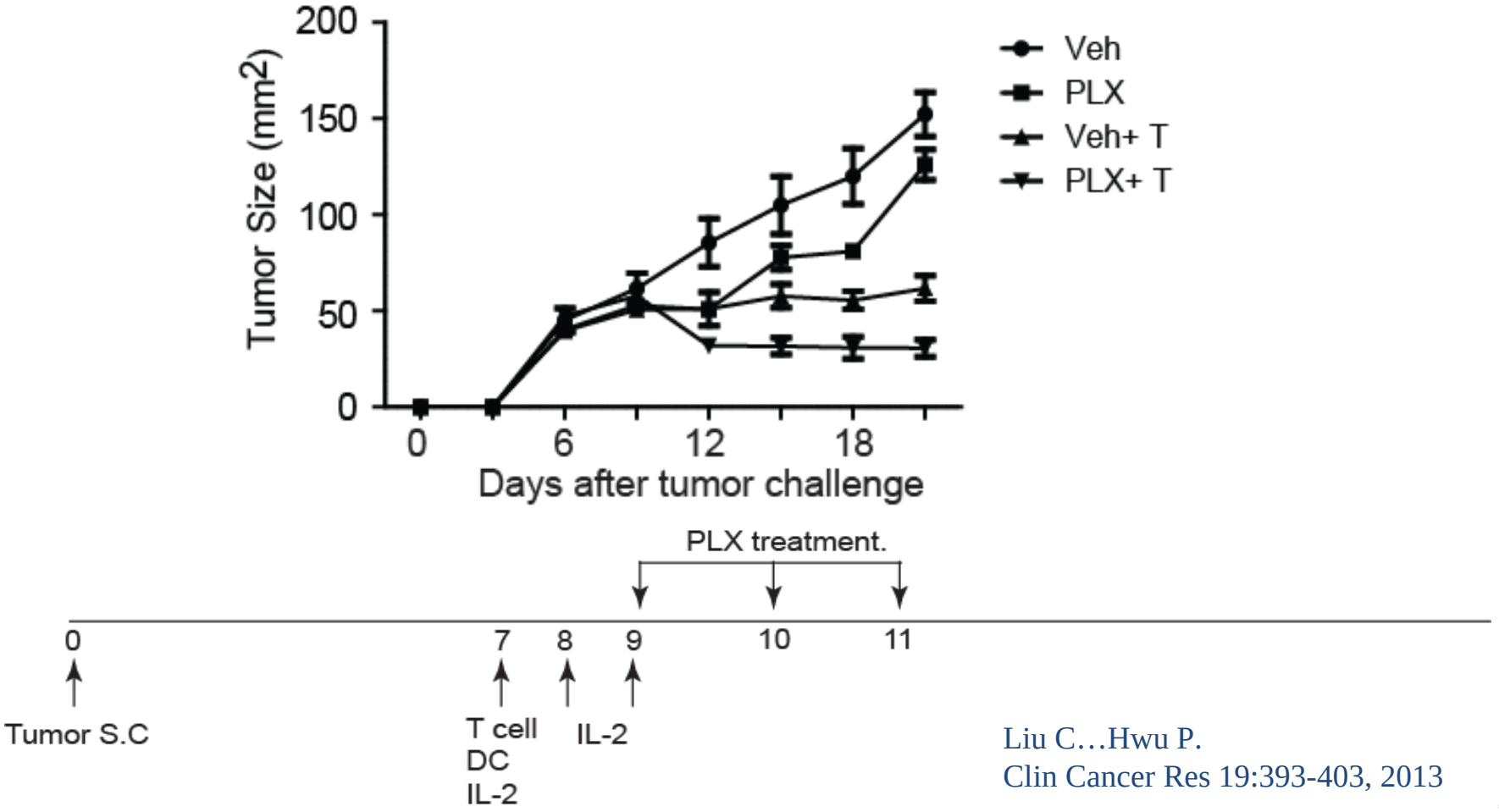
**Provides a useful platform to evaluate the interactions between targeted agents and T-cell mediated immune response**

## Drawback

**Cannot replicate the interaction between tumor-reactive T-cells and other immune effector cells**



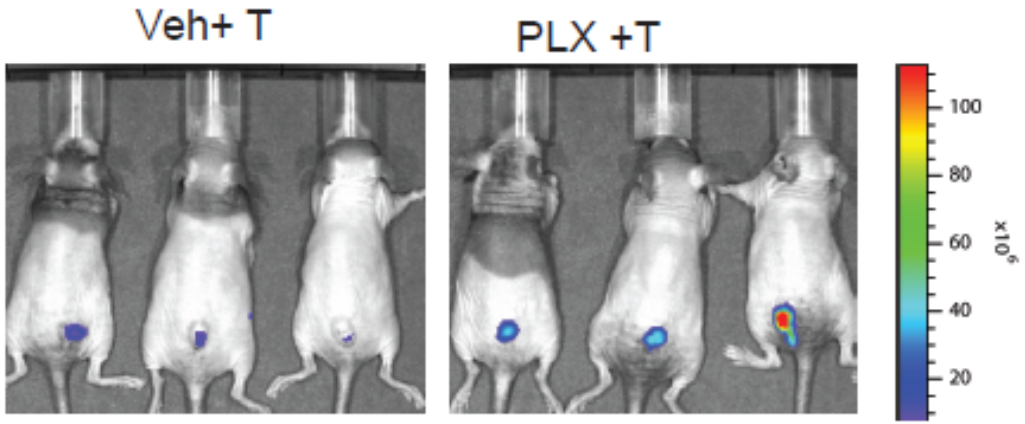
# Combination of PLX4720 with Adoptive T-cell Therapy Leads to Enhanced Anti-tumor Activity (B6 nude mice)



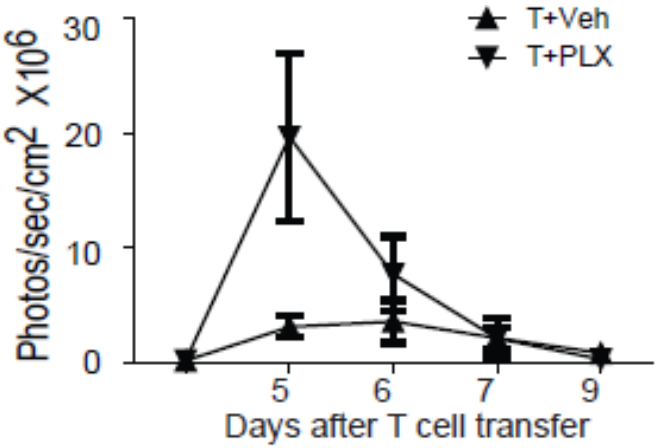

Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

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

**A**

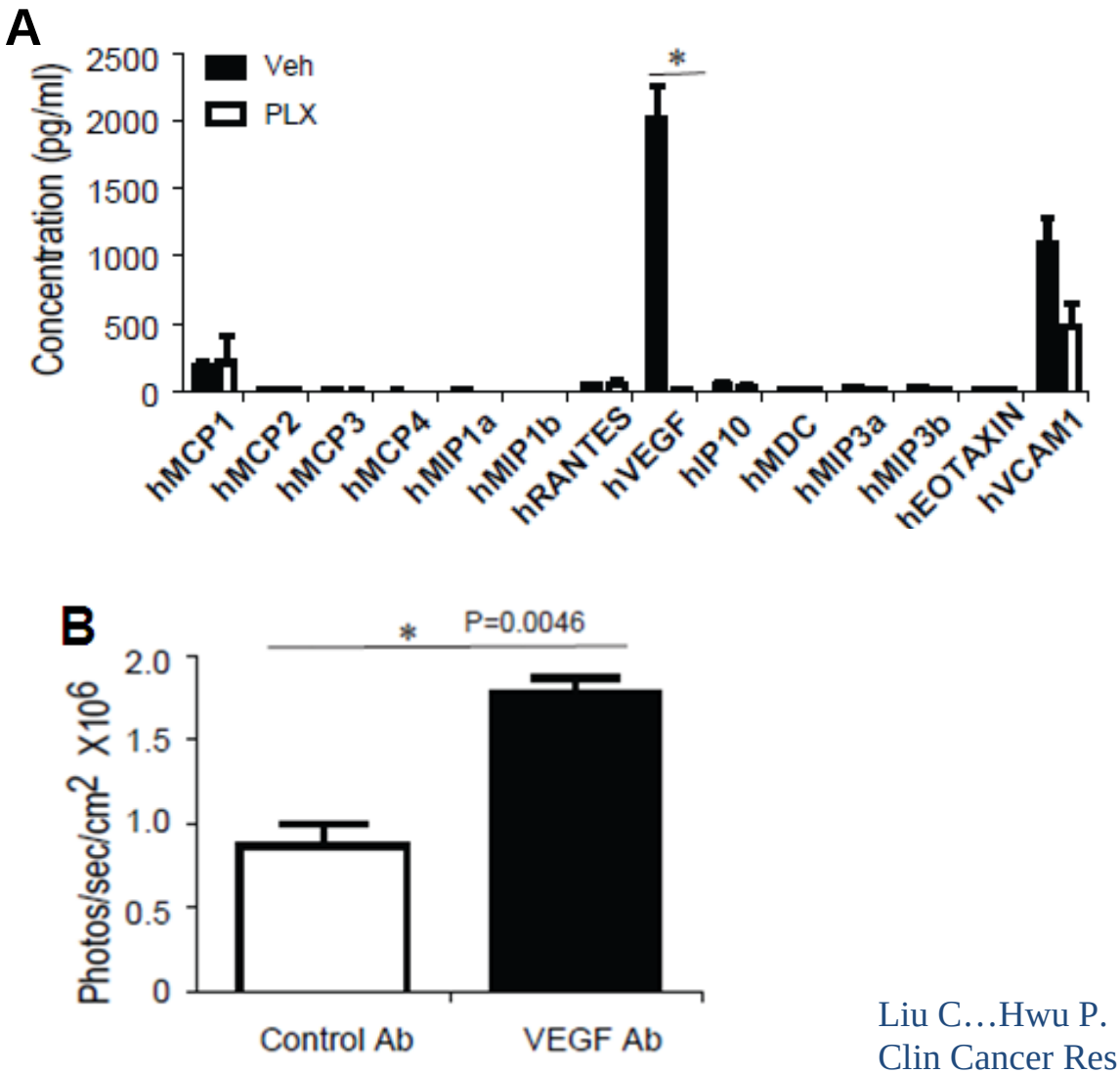



**B**



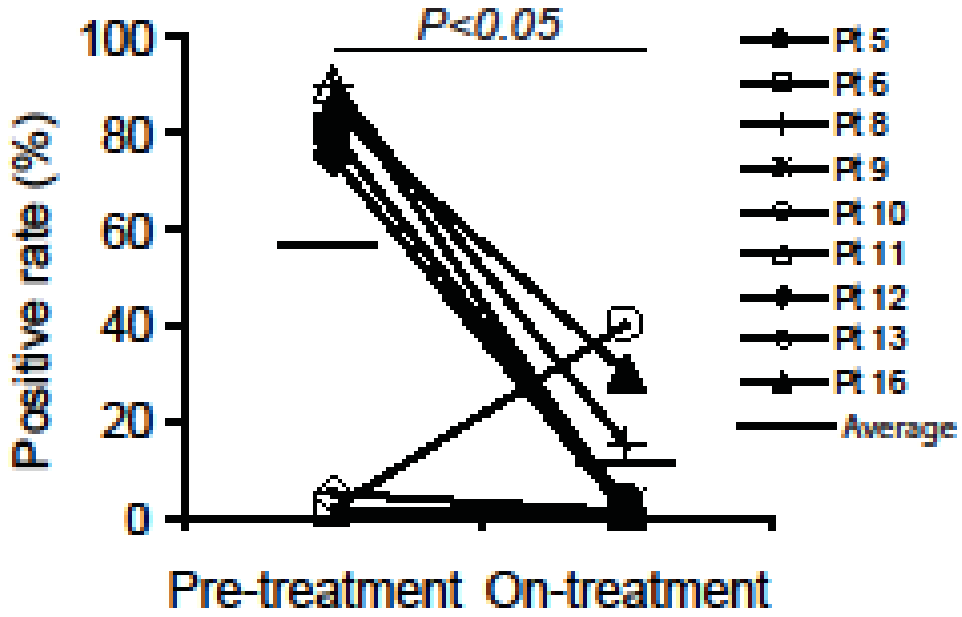

Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

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




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

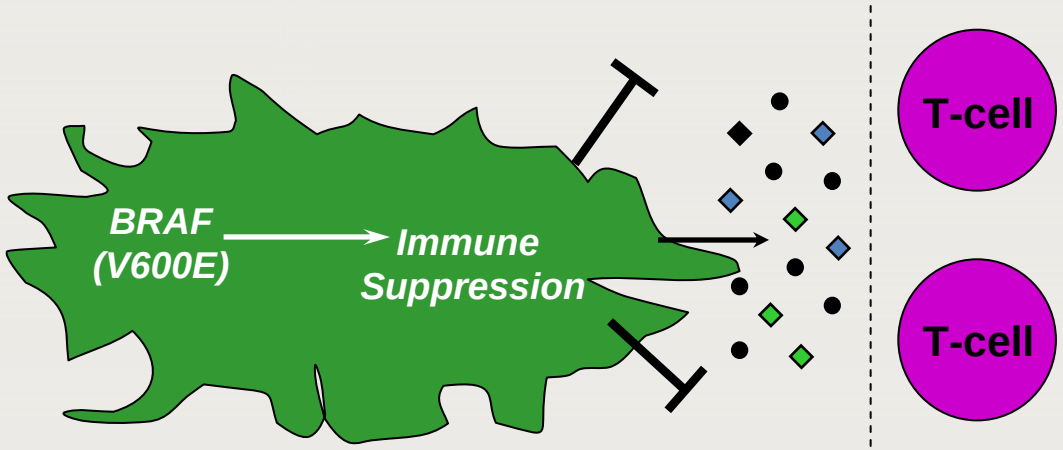
# BRAF Inhibition Downregulates VEGF at the Tumor Site in Melanoma Patients



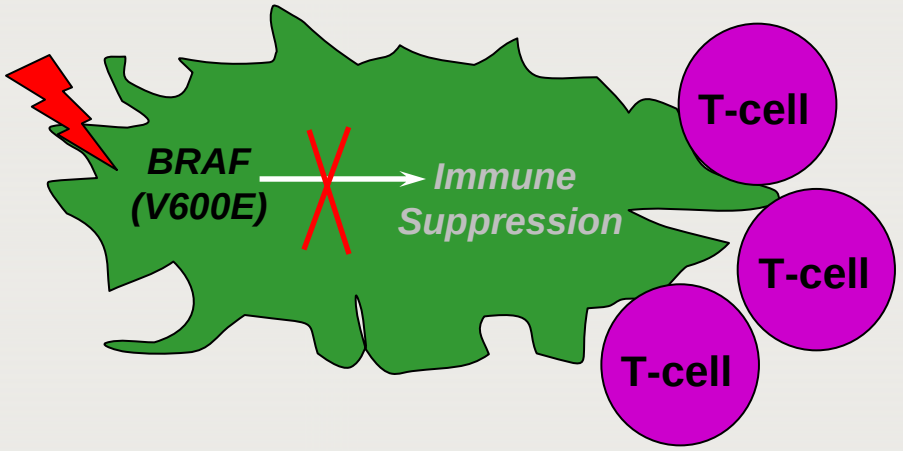

Liu C…Hwu P.
Clin Cancer Res 19:393-403, 2013

# Combining BRAF(V600E) Inhibition and Immunotherapy

**Immunotherapy Alone**




**Immunotherapy Plus BRAF(V600E) Inhibition**



# Syngeneic Model

## Advantage

- Useful for experiments that study immune responses to melanoma which require an intact immune system
- Useful to evaluate the therapeutic efficacy of targeted therapy

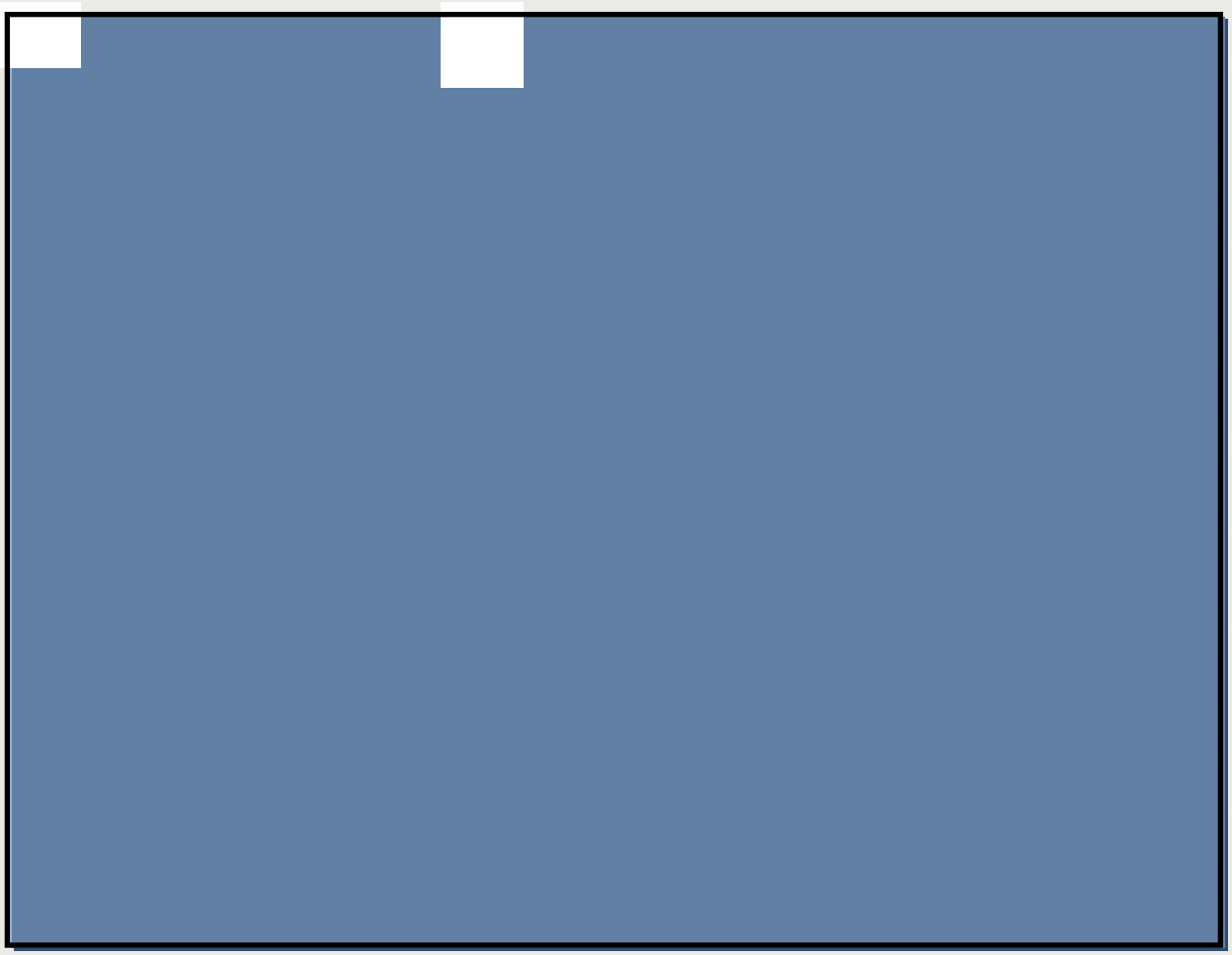
## Drawback

- Unclear mutation status of most tumor cell lines
- Lack of information regarding the alterations that drive tumor formation and progression

### Examples of murine tumor cell lines

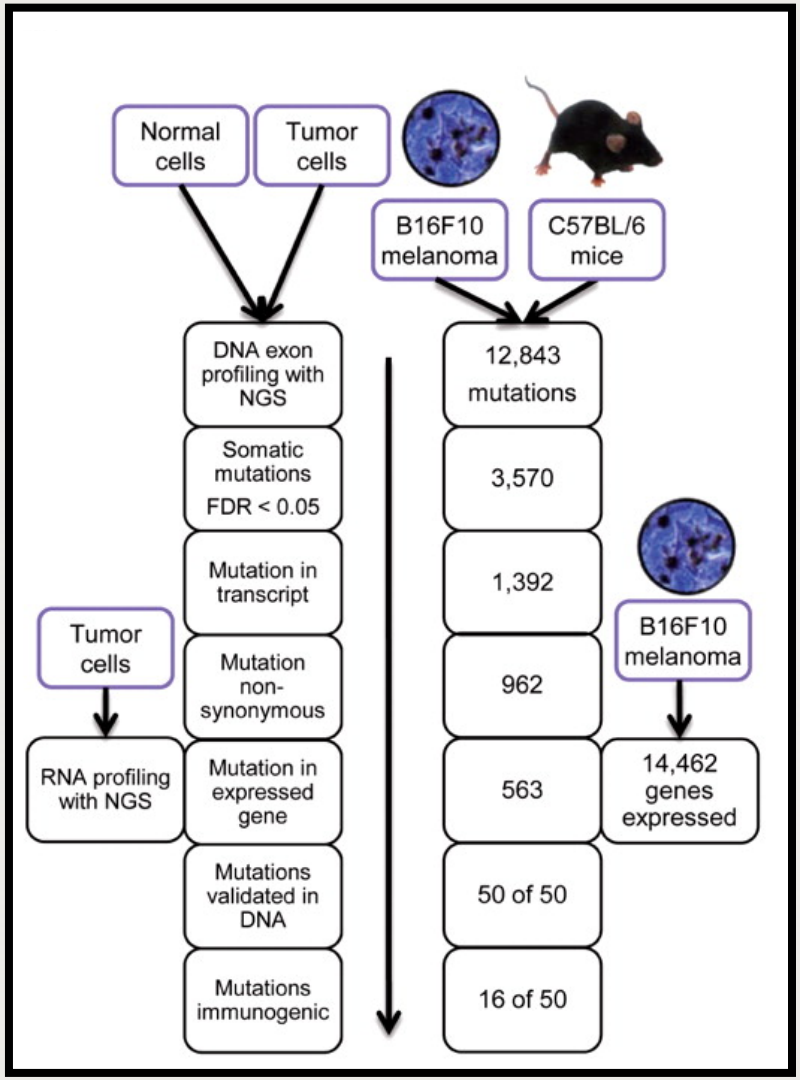
| Name | Tumor type | MHC class I | Tumor Antigen | Mutation | PD-L1 |
|---|---|---|---|---|---|
| BP | Melanoma | low | no gp100( with overexprssion cell line) | Pten $^{-/-}$; Braf $^{V600E}$ | + |
| MC38 | Colon Cancer | High | no gp100( with overexprssion cell line) | Unknown | + |
| B16 | Melanoma | - | Express gp100 | Unknown | + |

# Mutation Rates for Human Cancers and 2 Methylcholanthrene-induced Sarcomas



Matsushita H…Schreiber RD.
Nature 482:400-404, 2012

# Immunogenic Mutated Peptides in B16 Melanoma




Castel JC, et al
Cancer Res 72:1081-1091, 2012

# pAKT Expression in Murine and Human Melanomas

A375

hgp100/H2-Db

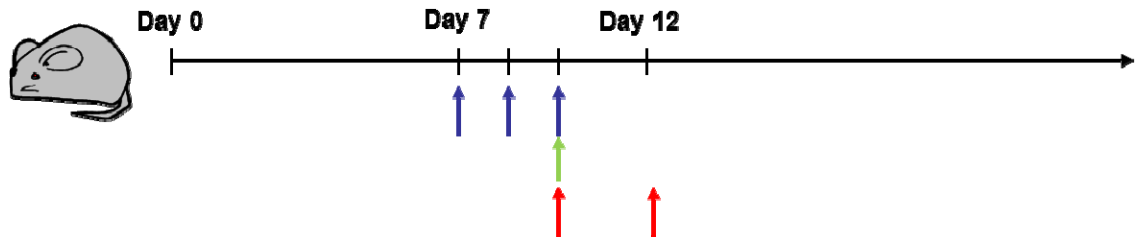
A375/gp

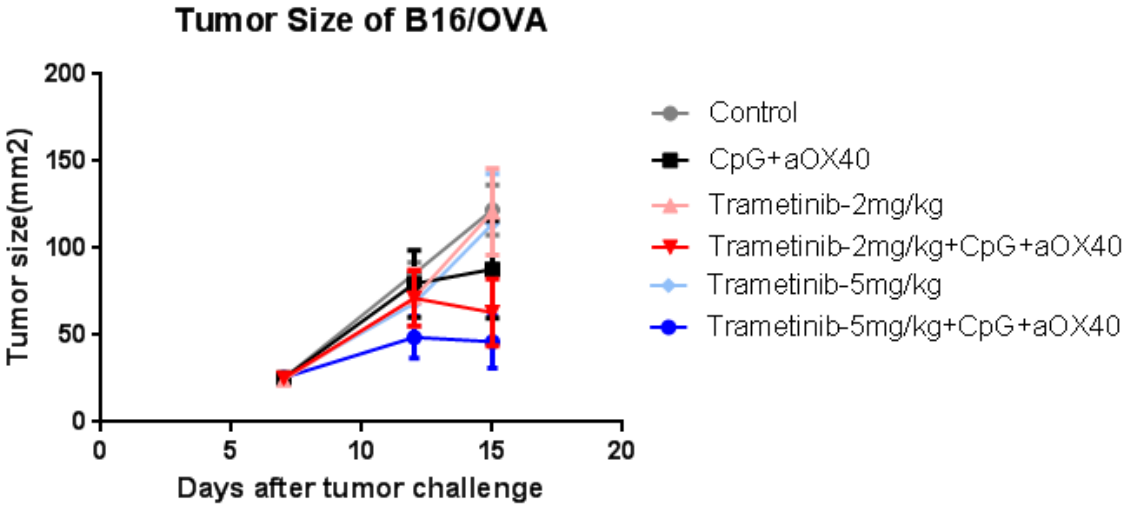
Pten specific shRNA

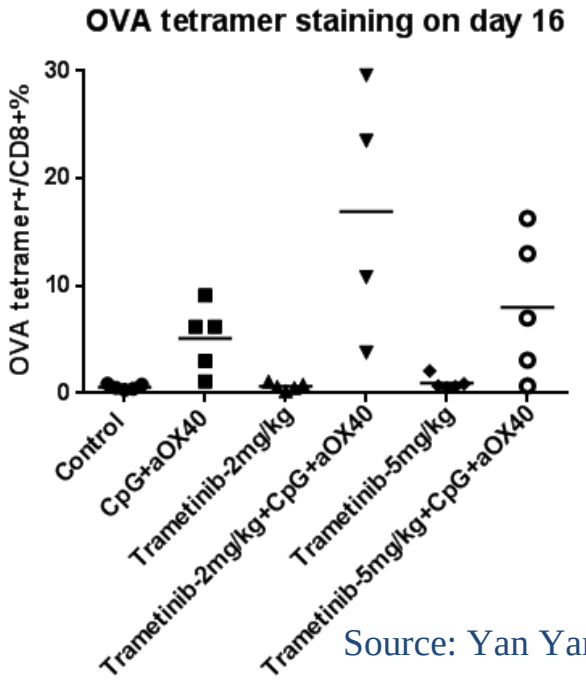
A375/shPten-17 or A375/shPten-60

# Combination of Trametinib and CpG+ α OX40 has a Synergistic Therapeutic Effect on B16/OVA

**Trametinib:**
**2mg/kg/day or**
**5mg/kg/day**
**CpG:50ug/day**
**aOX40: 200ug/day**



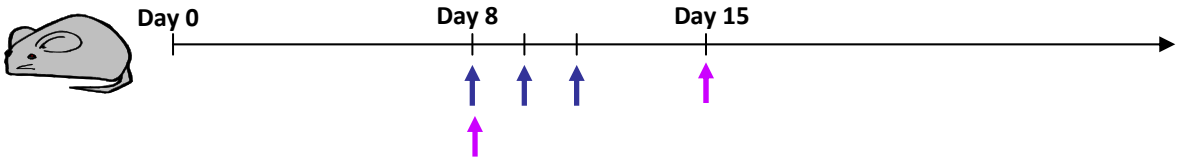



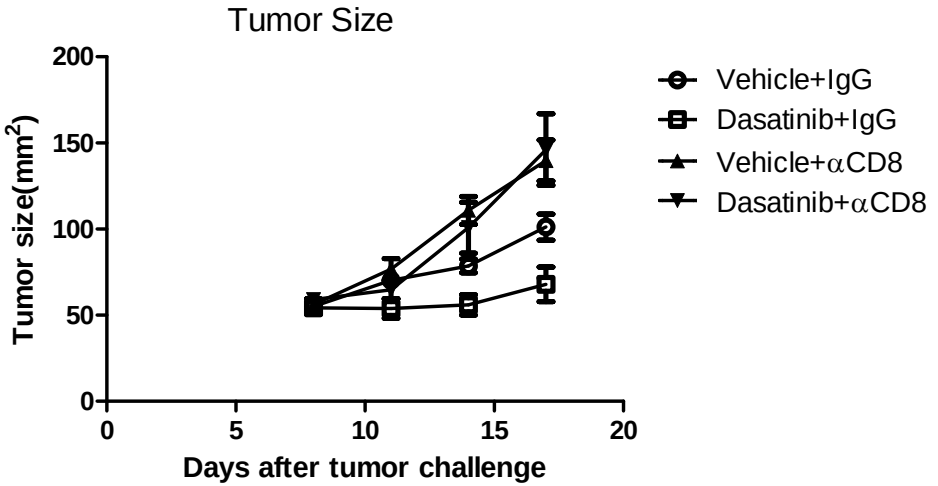



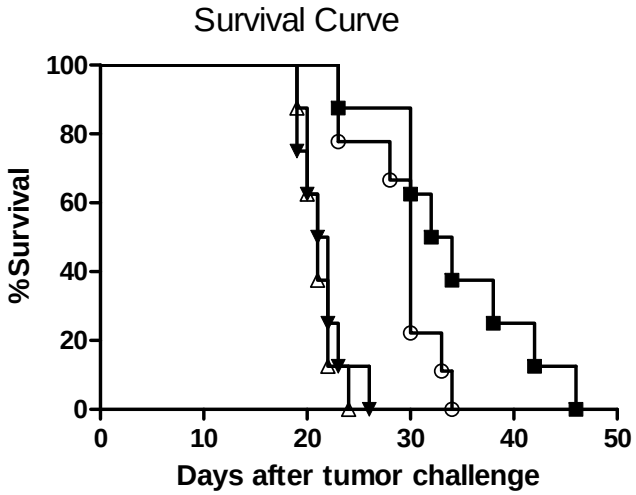

Source: Yan Yang

# The Therapeutic Effect of Dasatinib is Dependent on CD8+ T-cells

**P815: 2×10$^6$/mouse**
**Dasatinib: 3mg/day**
**aCD8: 200ug/day**




P815 Mastocytoma cells have a constitutively activated mutated c-kit receptor (D814Y)

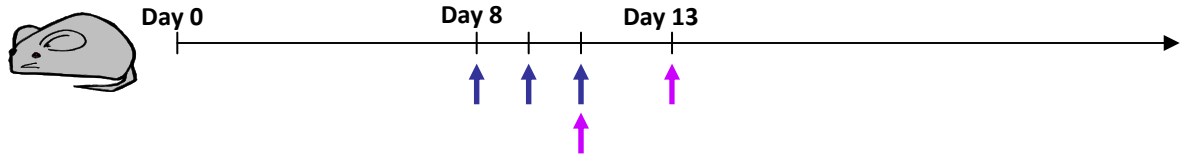
Yang Y…Hwu P.
Blood 120(23):4533-43, 2012

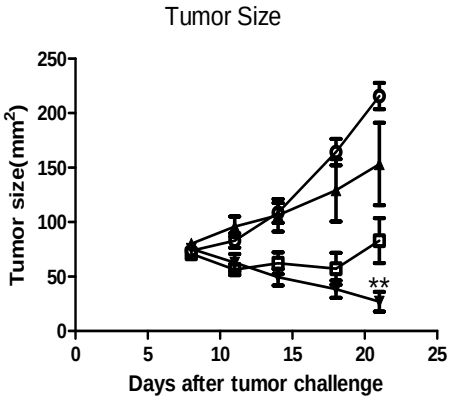
# Dasatinib Combined with anti-OX40 Improves the Antitumor Response

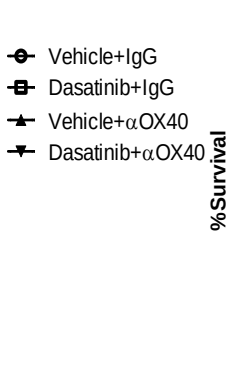
**P815: 2×10$^6$/mouse**

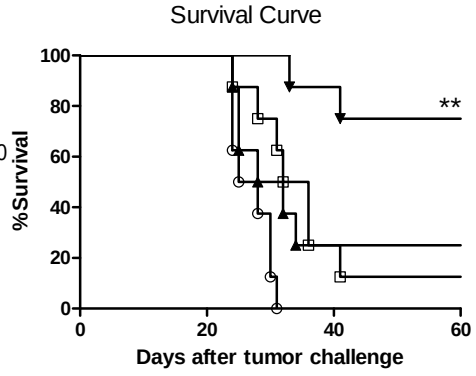
**Dasatinib: 3mg/day**

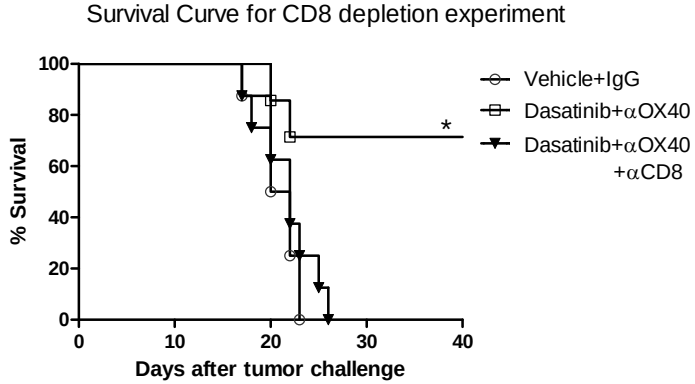
**aOx40: 200ug/day**

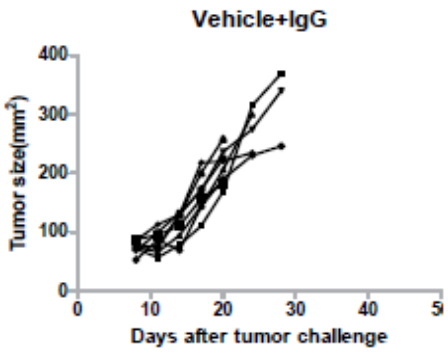


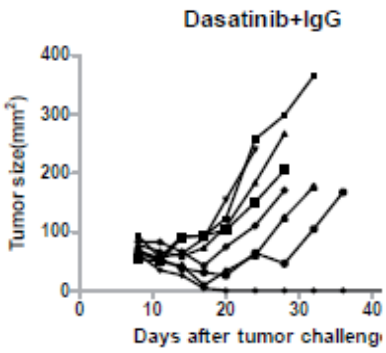



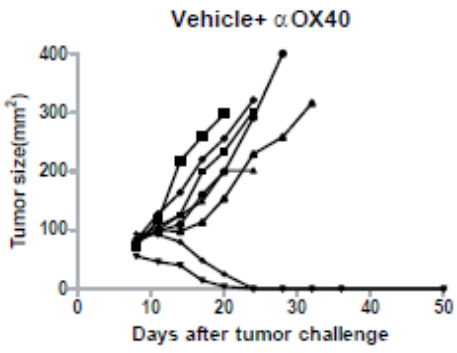



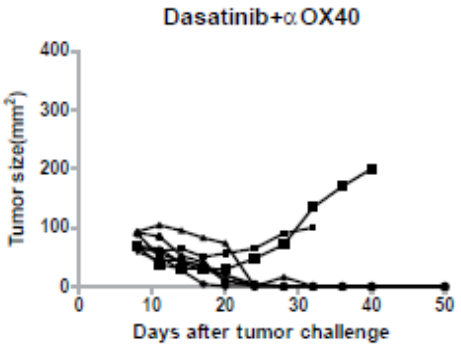



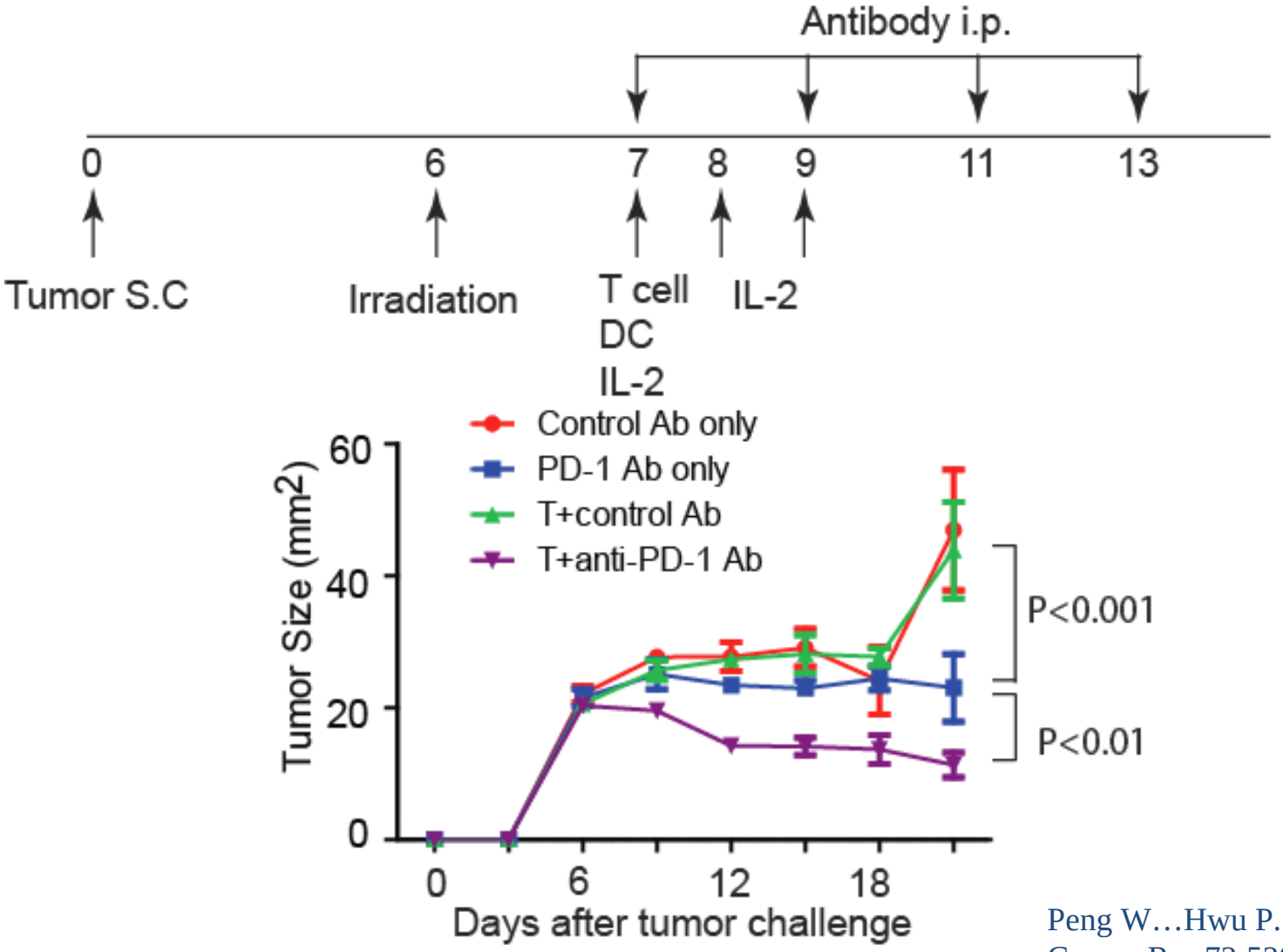



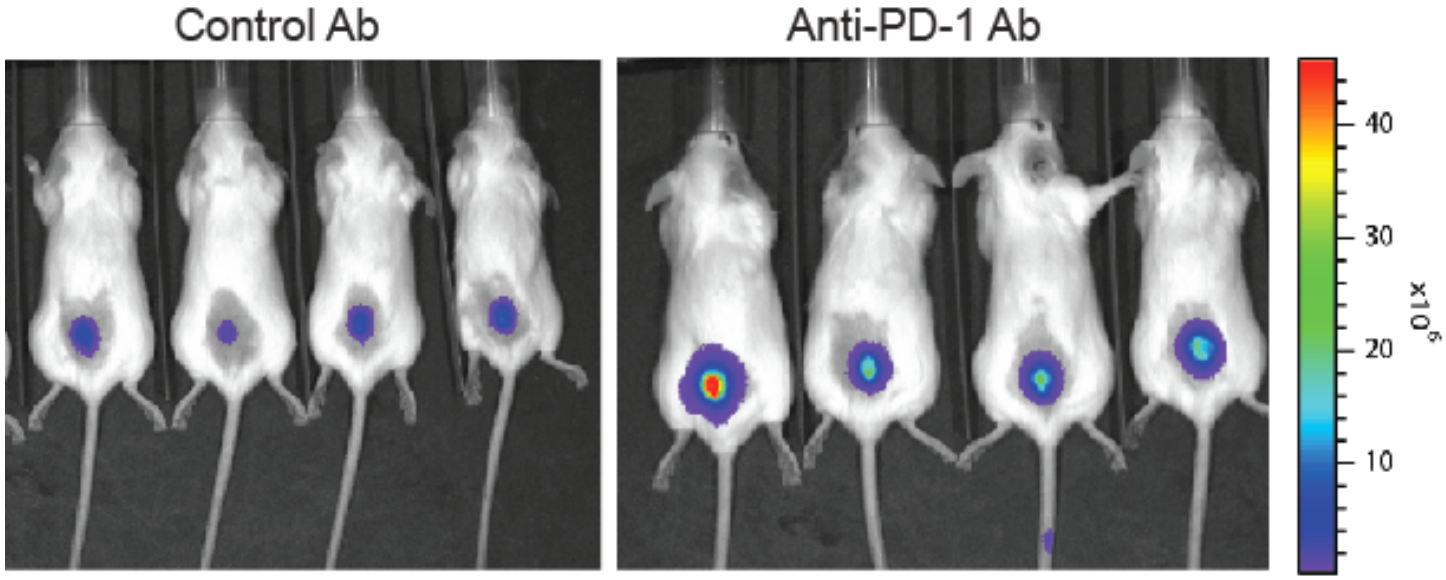

Yang Y…Hwu P.
Blood 120(23):4533-43, 2012

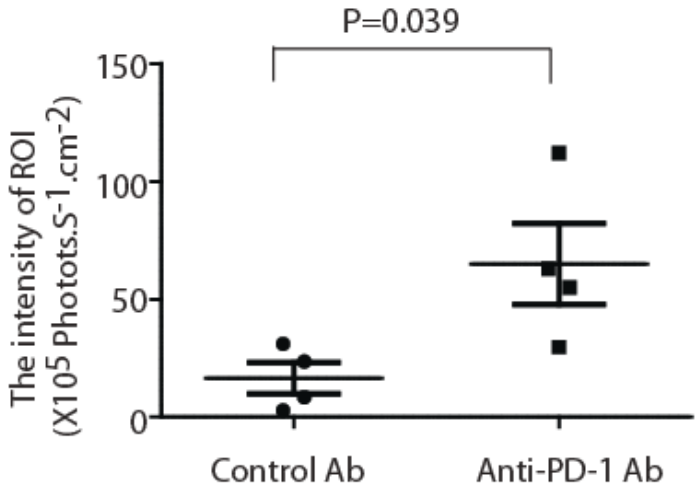
# Delayed Tumor Progression in Tumor-bearing Mice Receiving anti-PD-1 and ACT Treatment




Peng W…Hwu P.
Cancer Res 72:5209-18, 2012

# Increased Number of Transferred T-cells at the Tumor Site in Tumor-bearing Mice Receiving anti-PD-1 and ACT treatment




Peng W…Hwu P.
Cancer Res 72:5209-18, 2012

# Genetically Engineered Mouse Model (GEM)

## Advantage

- Has intact immune system
- Useful to assess tumor development and treatment

## Drawback

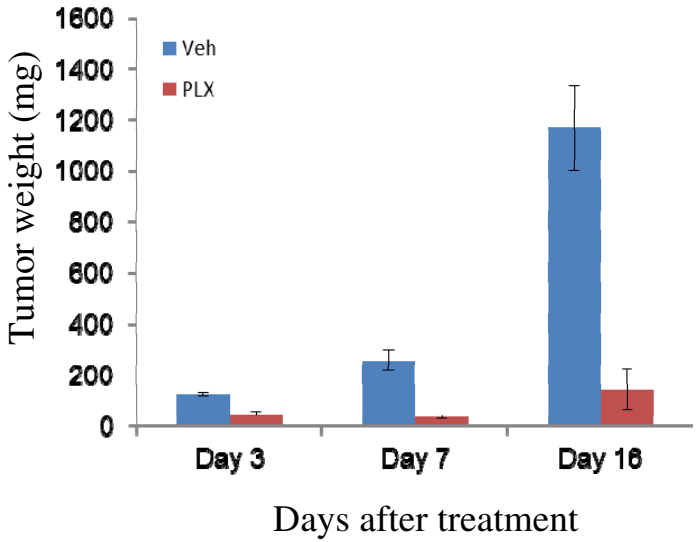
- Few mutations which may cause tumors to be less immunogenic
- Difficult to obtain mice (costly and labor intensively)
- Challenging to perform therapy studies

Examples of GEMs

| Author (year) | Genetic Modification | Latency/Penetrance |
|---|---|---|
| Dankort et al. (2009) | TYR::CreERT2Pten$^{f/f}$Braf $^{ca/wt}$ | 10 weeks/100% |
| Chin et al. (1999) | TYR::rtTAtetO::HRas$^{G12V}$ | 60 days/25% |
| Held et al. (2010) | TYR::CreERT2Pten$^{f/f}$Cdkn2a $^{f/f}$±β-catenin $^{Loxex3/wt}$ | 40 weeks/100% |

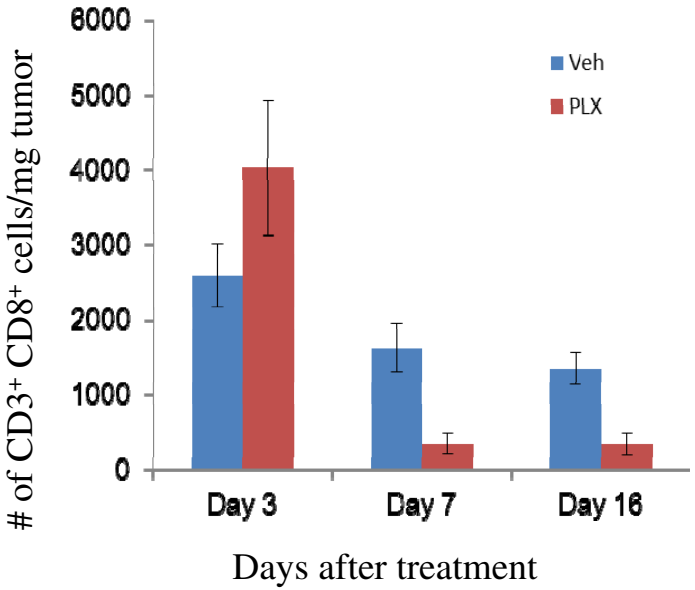
# BP (mutant BRAF/PTEN$^{-/-}$)Conflicting Results

A

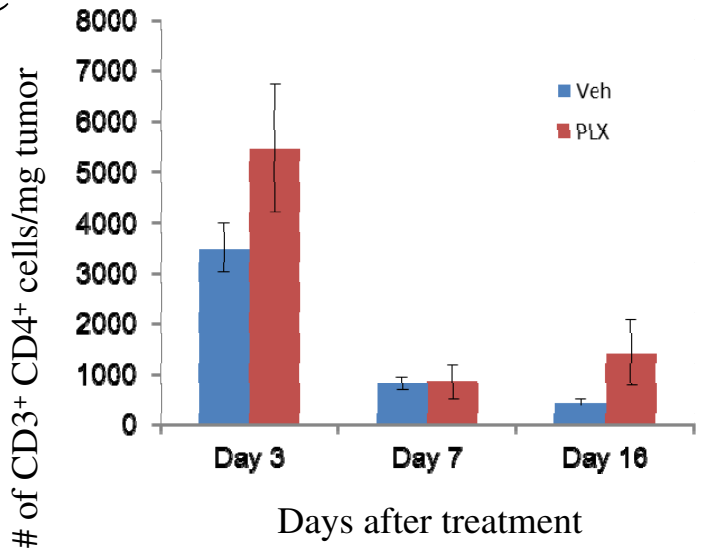



BP fish cells (1e6/mouse) were inoculated on day -7. Tumor-bearing mice were fed with a diet containing PLX4720 (417mg/kg) or control diet from day 0. Mice were sacrificed on day 3, 7 and 16. Tumors and spleens were harvested, single-cell suspensions were stained with anti-CD45, anti-CD3, anti-CD8 and anti-CD4 for flow cytometry assay.

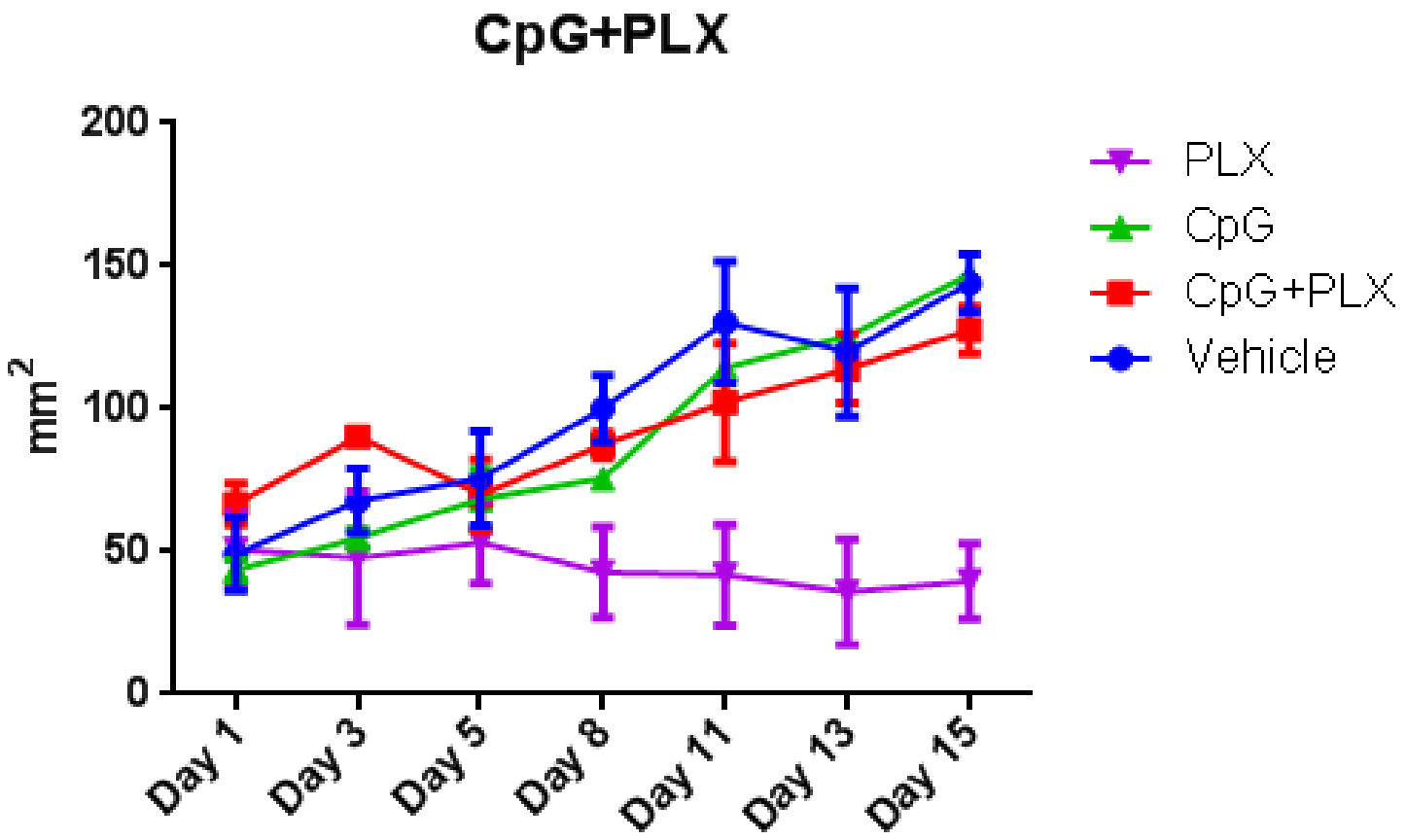
B




C



# PLX (BRAFi) +/- CpG in GEM Model



# Solution: “De-Risk” Clinical Trials with Focused Modeling

- **In Vitro Models**
- **Mouse In Vivo Models**
- **Clinical Trial Monitoring**

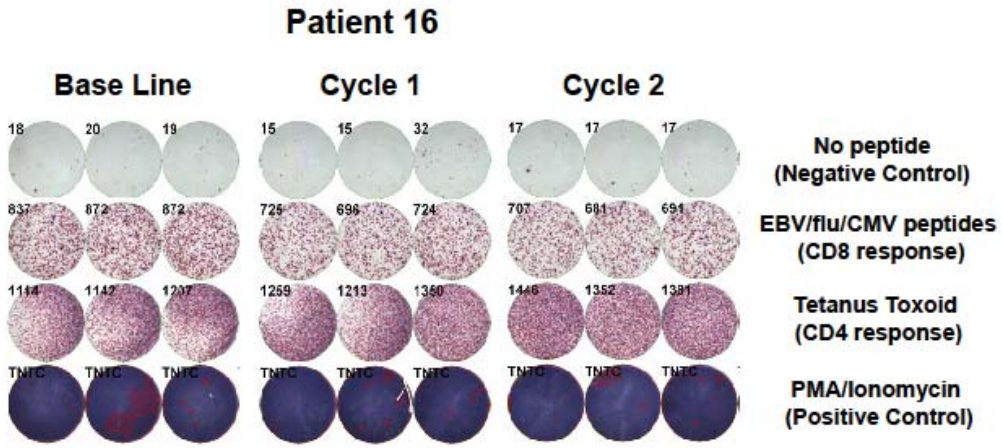
# Evaluation of Immune Cells in Patients Receiving a BRAF inhibitor (GSK)

- **PBMC and serum were collected from 13 patients before and after a 28 day cycle of BRAF inhibition.**

- **No changes were found in serum cytokines, peripheral blood cell counts, T-cell subsets, or CD4 or CD8 recall responses.**

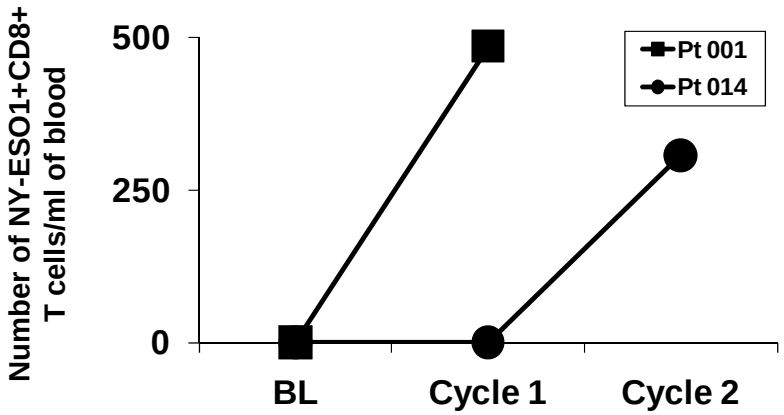
Hong DS…Hwu P.
CCR 18(8):2326-35, 2012

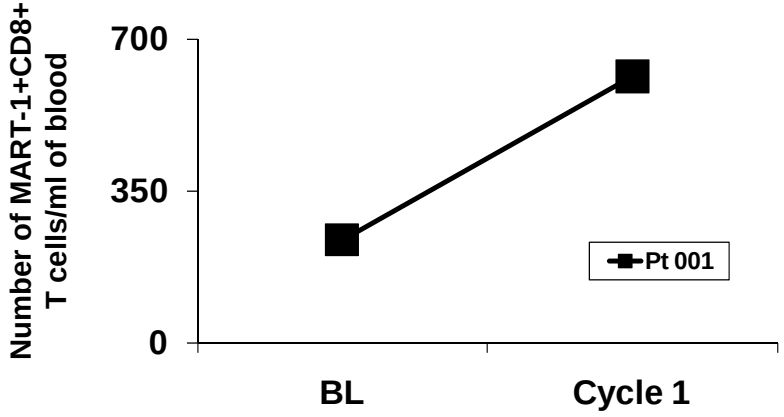
# The V600 Mutant BRAF Inhibitor Treatment Does Not Affect CD4+ and CD8+ Memory T-cell Responses to Recall Antigens

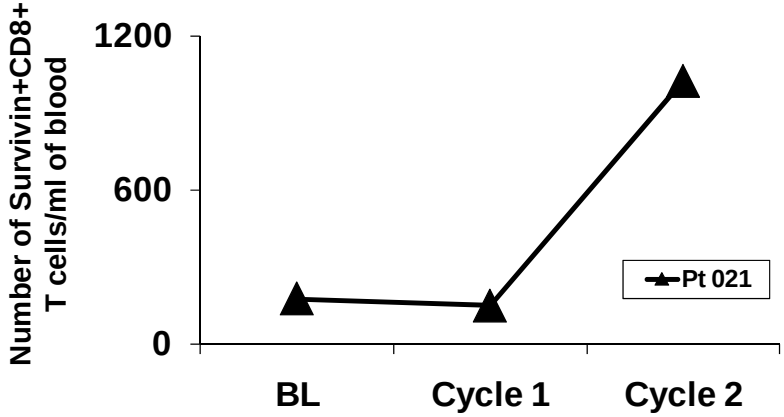



Hong DS…Hwu P.
CCR 18(8):2326-35, 2012

# Increase in Melanoma Antigen Specific T-cells Alter BRAF Inhibition




Hong DS…Hwu P.
CCR 18(8):2326-35, 2012

# Future Studies

**Understand the *in vivo* effects on the immune system of:**

- **PI3K inhibitors**
- **AKT inhibitors**
- **MEK and BRAF/MEK inhibitors**

# Solution: “De-Risk” Clinical Trials with Focused Modeling

- **In Vitro Models**
- **Mouse In Vivo Models**
- **Clinical Trial Monitoring**

# Acknowledgements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GSK

3M

NCI