Reprogramming the Immunologic Microenvironment Through Radiation and Targeting Axl

Amato Giaccia PhD
Jack, Lulu and Sam Willson Professor of Cancer Biology
Stanford University School of Medicine

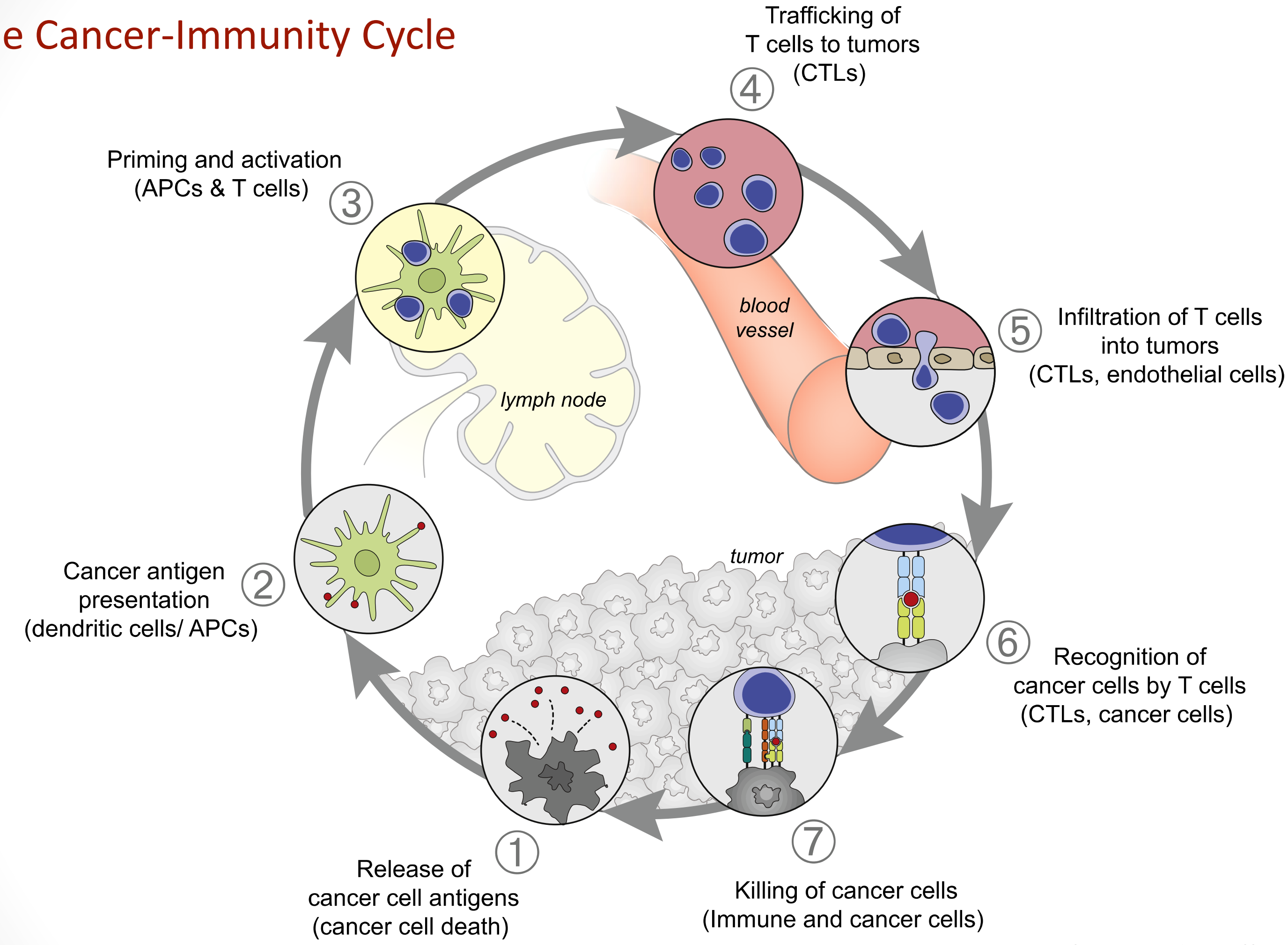
Transform Radiotherapy from a Localized Therapy to a Systemic Therapy

- Develop Normal Tissue Radioprotectors that do not Radioprotect Tumor Cells
- Develop New Targeted Therapies to Treat Metastatic Disease that could be combined with Radiotherapy
- Combine Radiotherapy with Immune Modulators, and understand the basis of Tumor Immune Heterogeneity

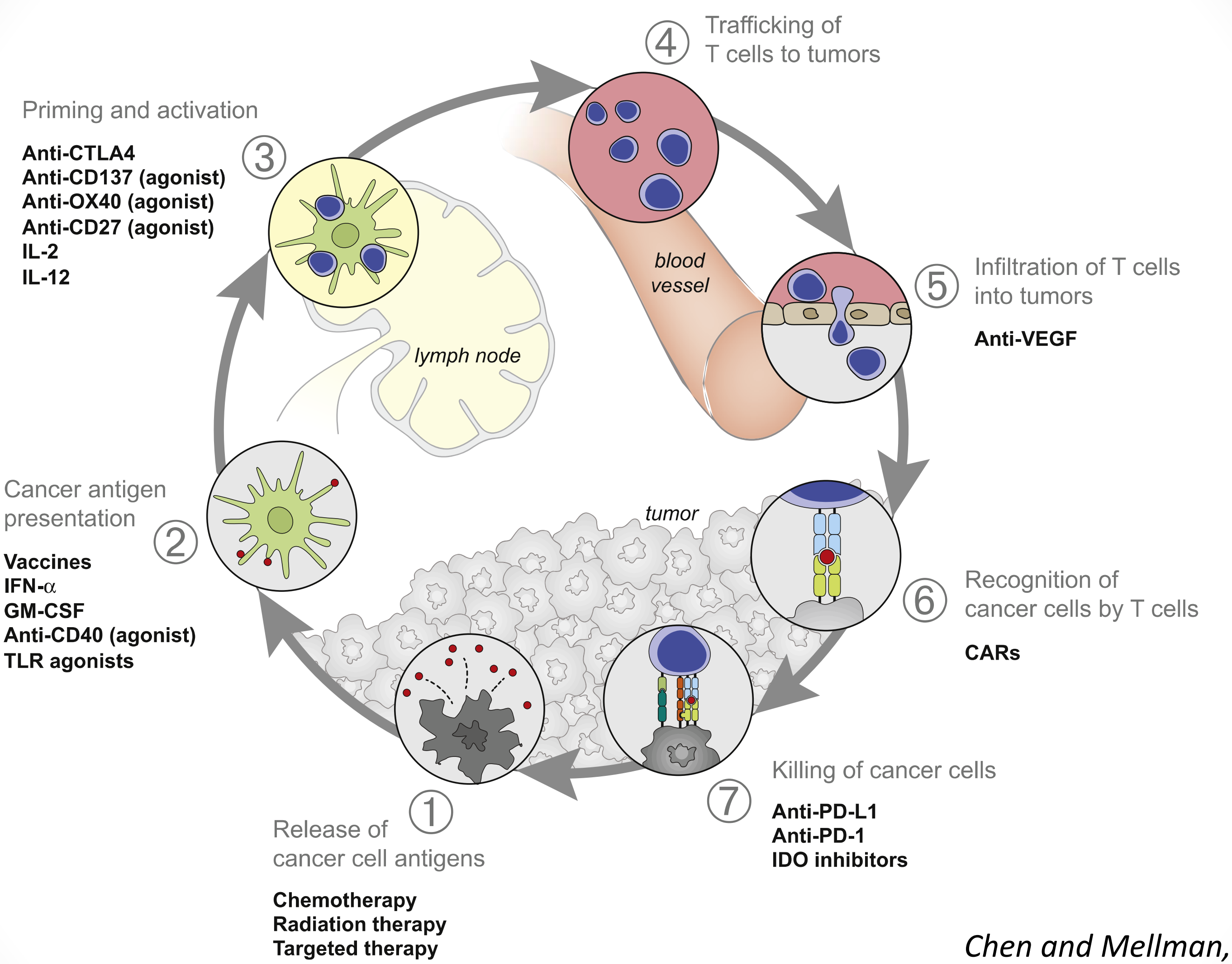
Outline

- The Cancer-Immunity Cycle
- A model of immunologic heterogeneity
- Activating T cells in a Suppressive Microenvironment

The Cancer-Immunity Cycle



Chen and Mellman, Immunity, 2013



Chen and Mellman, Immunity, 2013

Preclinical Models often Require Genetically Altered Cancers or Additional Immunotherapies

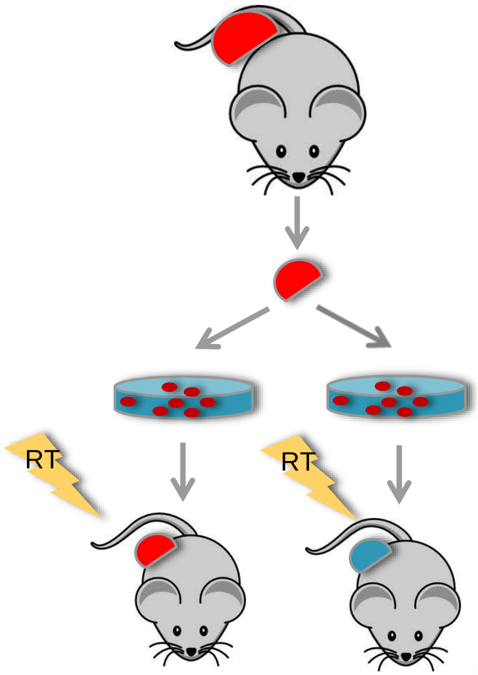
Study	Model	Type of immune activation	Response
RT			
Dewan, ClinCaRes, 2009	TSA BrCa MCA 38- Rat	RT 20x1, 8x3 RT 20x1, 8x3	aCTLA-4 synergy/abscopal RT
Demaria, ClinCaRes, 2005	4T1 BrCa	RT 12x1	aCTLA-4 synergy
Deng, JCI, 2014	TUBO BrCa- neu ag MC38 Colon- Rat neu ag	Ag, RT 12x1 Ag, RT 20x1	aPD-L1 synergy with RT
Liang, J Imm, 2013	TUBO BrCa- neu B16SIY- SIYpep	RT 15x1 RT 25x2.	aPD-L1 synergy and reached an equil
Verbrugge, CaRes, 2012	AT-3	RT 12Gy, PD-1 and CD137.	aPD-1 + CD 137, memory and synergy
Sharabi, Can Imm Res, 2014	B16-Ova 4T1-HA	RT 12 x1 or 7x3 +aPD-1 or T-reg	aPD-1 or T reg depletion synergistic
Bos, JEM, 2013	PyMT (Br)	TReg ablation w/ RT 12Gy	Synergy Treg deplete with RT
Lee, Bld, 2009	B16 B16 SIY	Adenovirus, 20x1, 25x1	Better effect in wt immune system
Chakraborty, J Imm, 2003	MC38-CEA	CTL tsf to CEA RT 8x1	Adoptive CTL to CEA and RT synergy Fas.

Genetic modifications
Multiple combination therapies

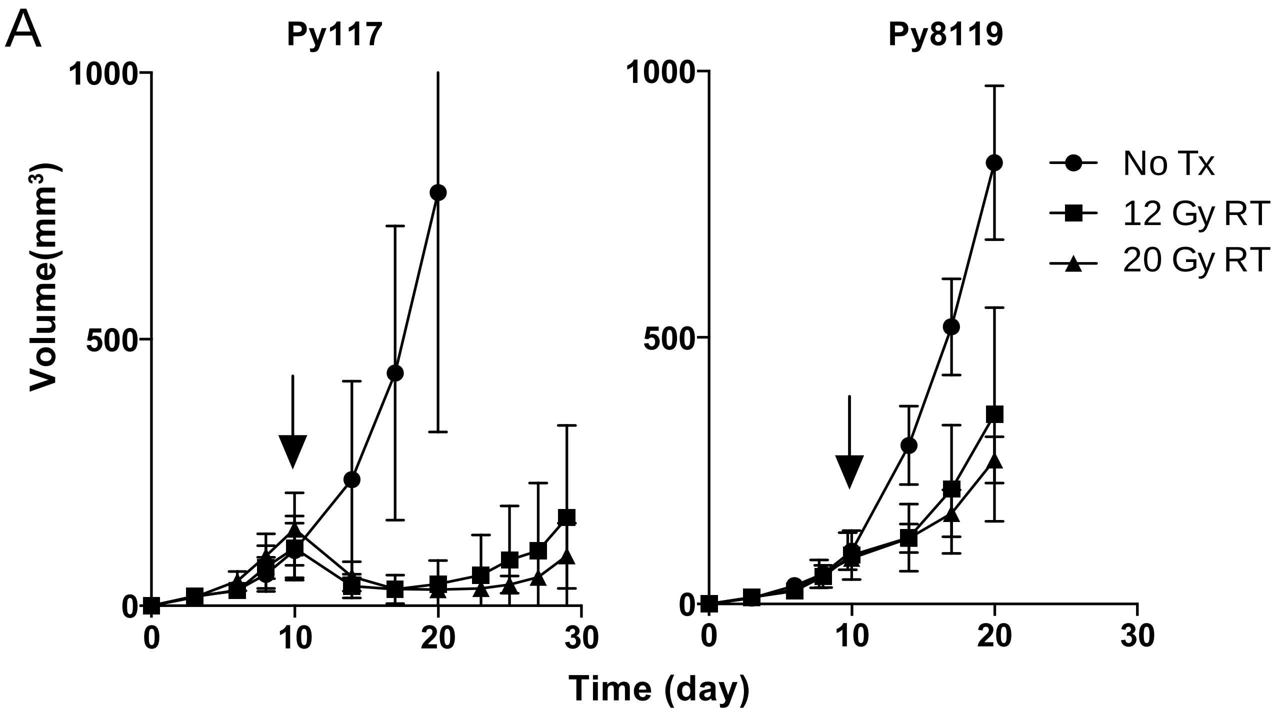
Can studying immunologically resistant tumor clones lead to immunotherapy approaches in the clinic?

- Transgenic mouse model
  - Polyoma Middle T antigen driven by Mouse mammary tumor virus promoter (C57Bl/6)
  - Selection of different tumor clones

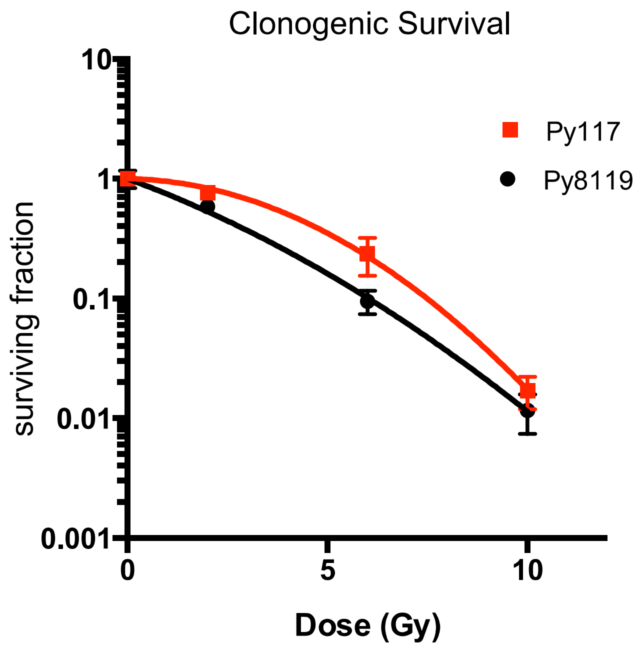
- Focus on 2 tumor clones Py117 (responsive) and Py8119 (unresponsive)
  - Evaluated in culture
  - Orthotopic implants +/- radiation



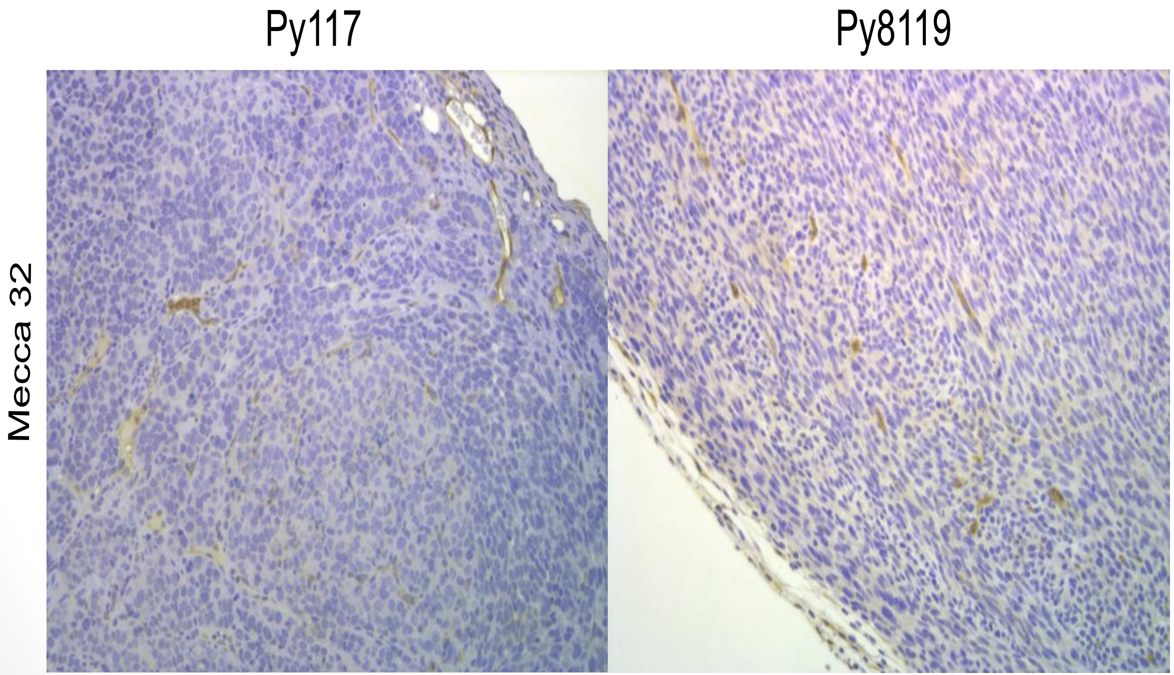
Py117 Tumors are Responsive and Py8119 Tumors are Unresponsive to Radiation

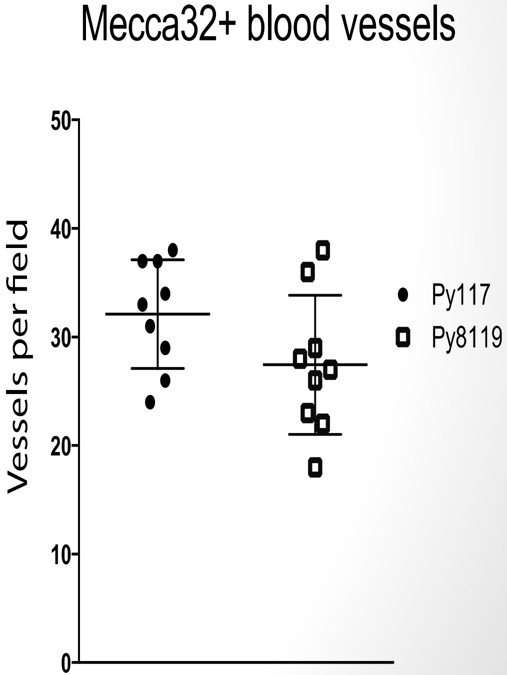


Radiation Sensitivity is not due to Cellular Factors in Culture



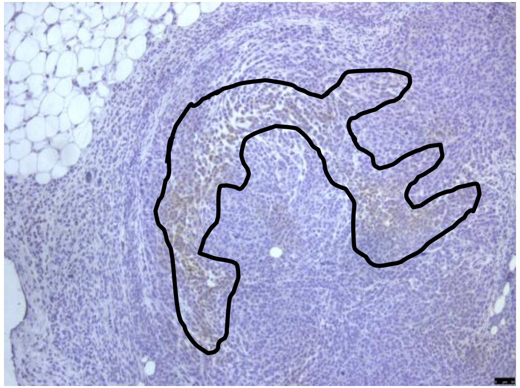
Radiation Sensitivity is Unlikely due to Differences in Angiogenesis



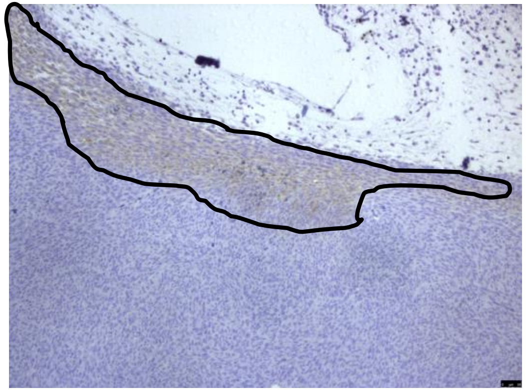


Differences are Unlikely due to Hypoxia

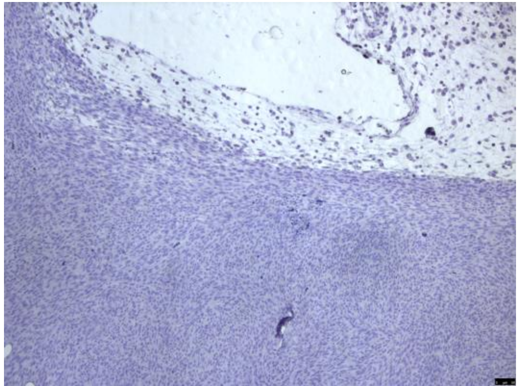
Py117

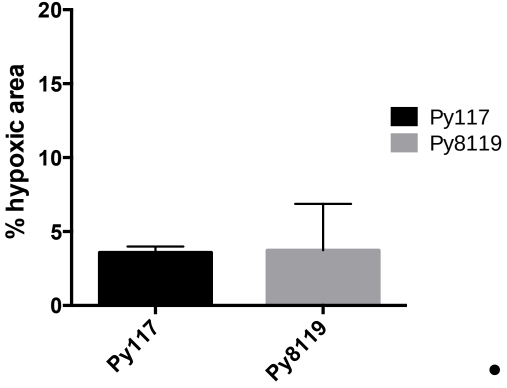


Py8119



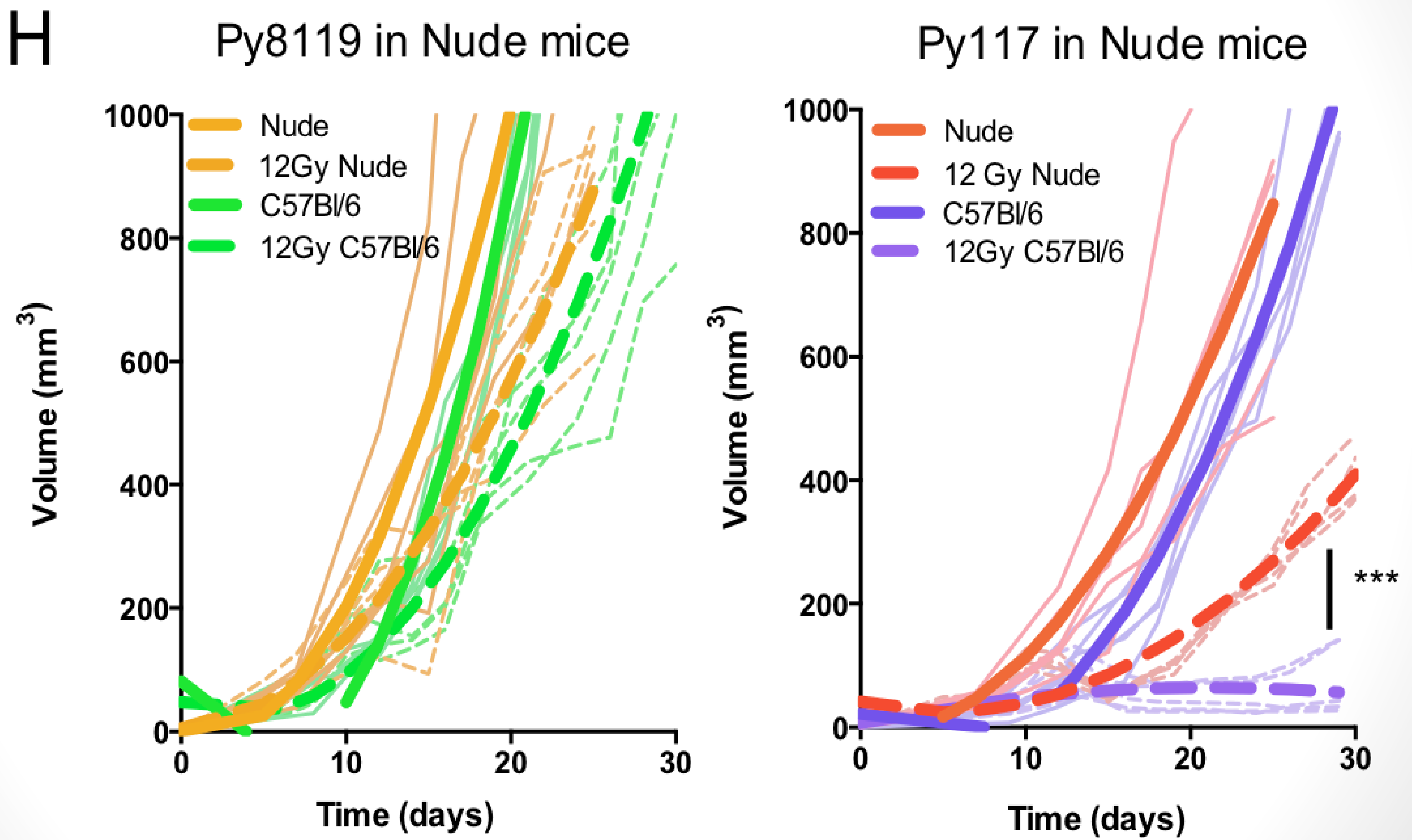
No primary Ab



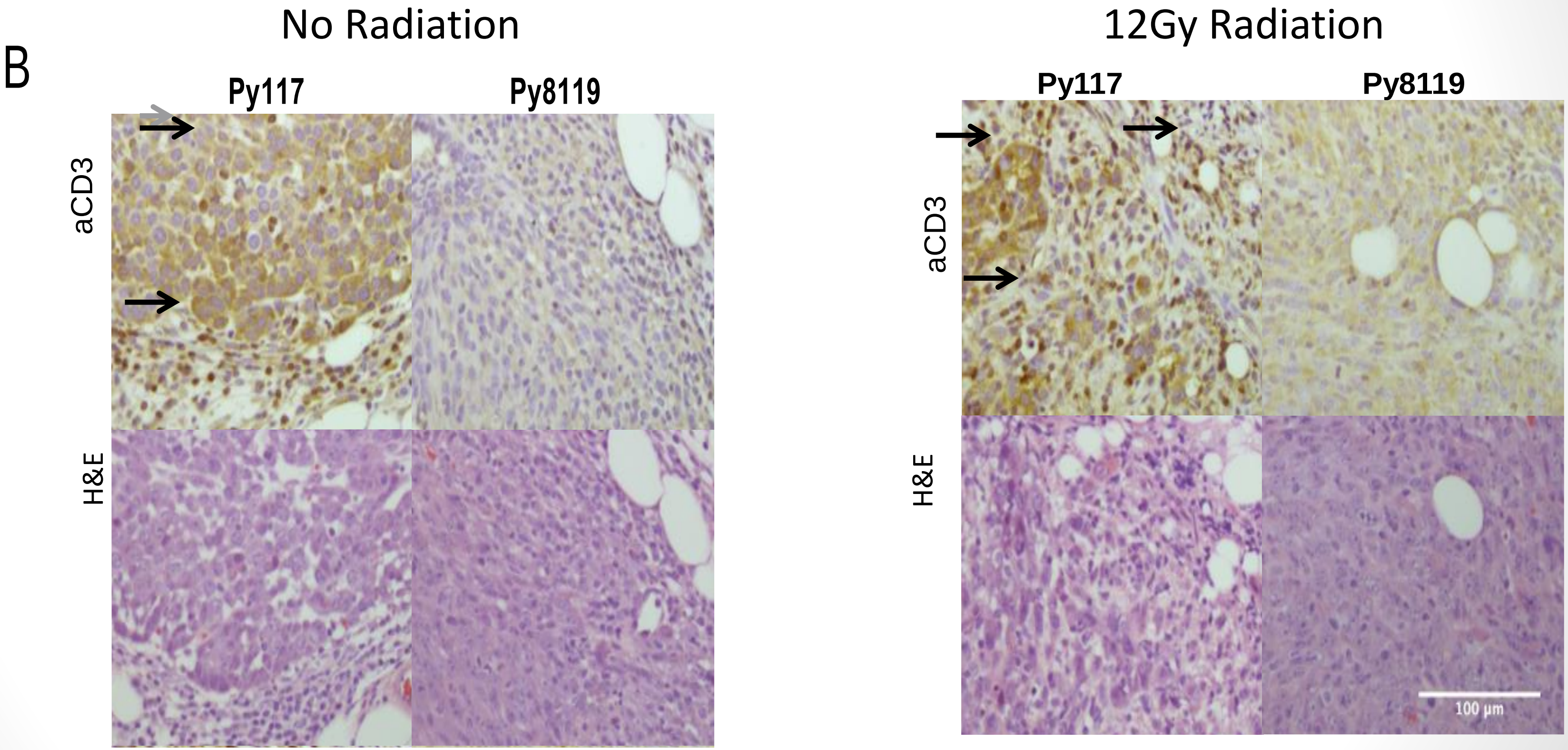


- Pimonidazole IHC

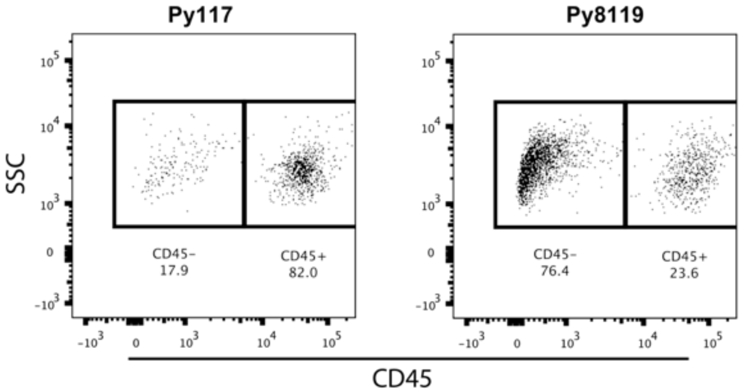
The Immune System Plays an Important Role



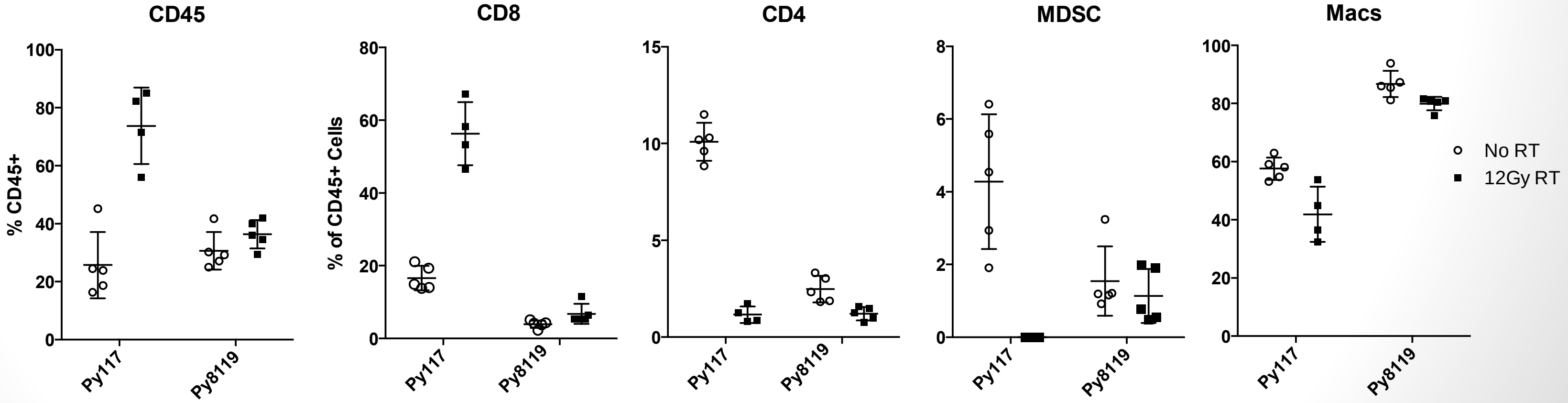
There are More Invading CD3+ T cells in Py117 Tumors



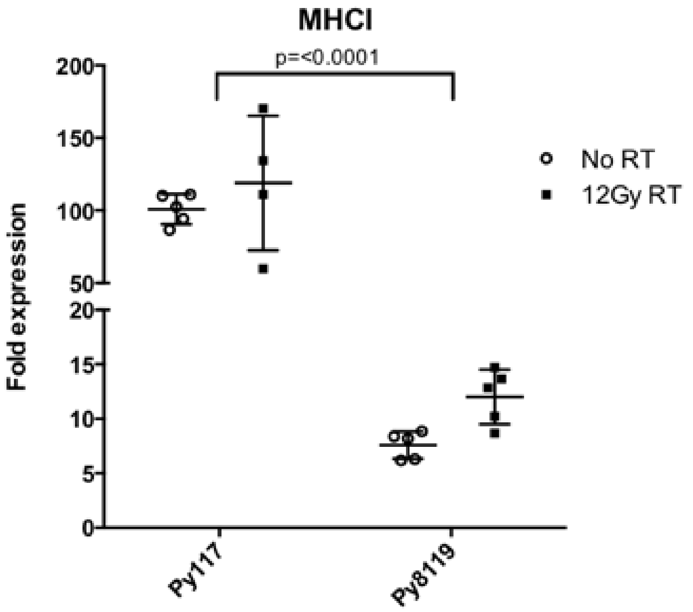
Infiltrating Leukocytes Suggest an Immune Mediated Antitumor Response

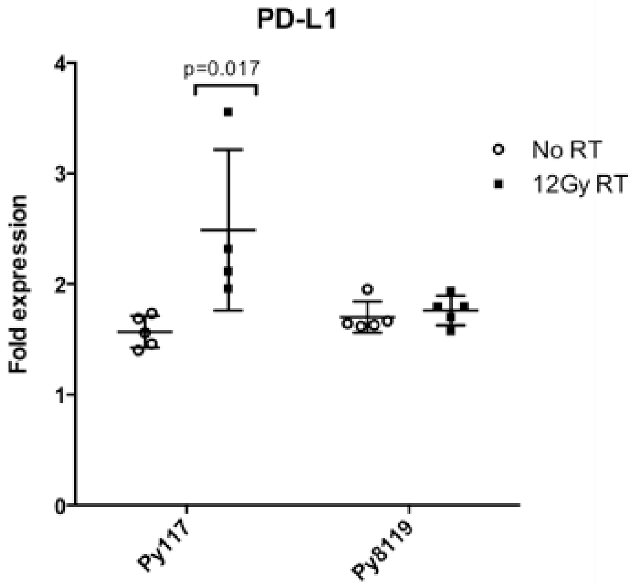


- 10 days after 12Gy



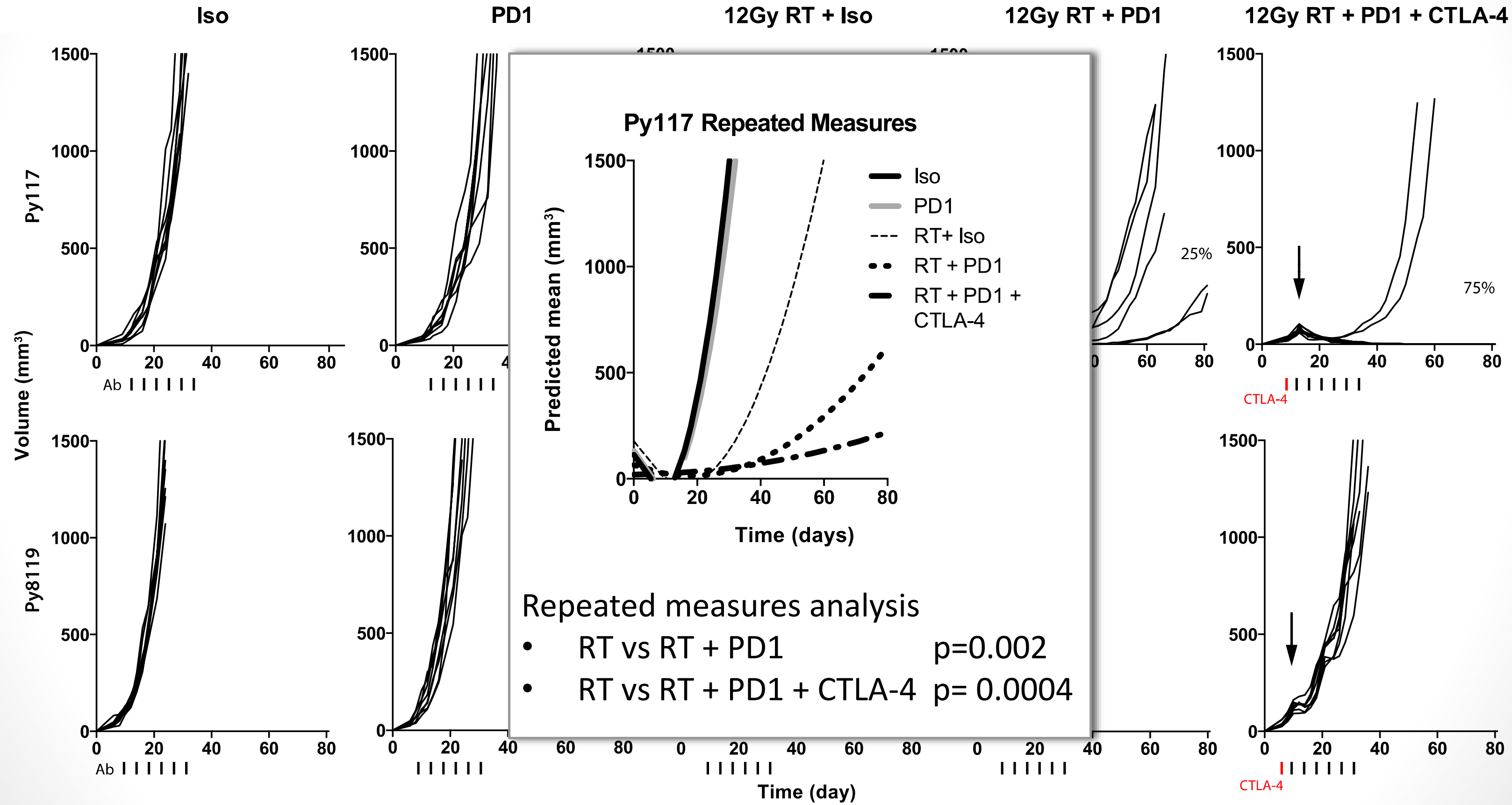
MHCI is Highly Expressed and PD-L1 is Induced after Radiation in Py117 Tumors





Hypothesis: Combination of Radiation and PD-1 Immunotherapy would lead to a Greater Response of Py117 Tumors

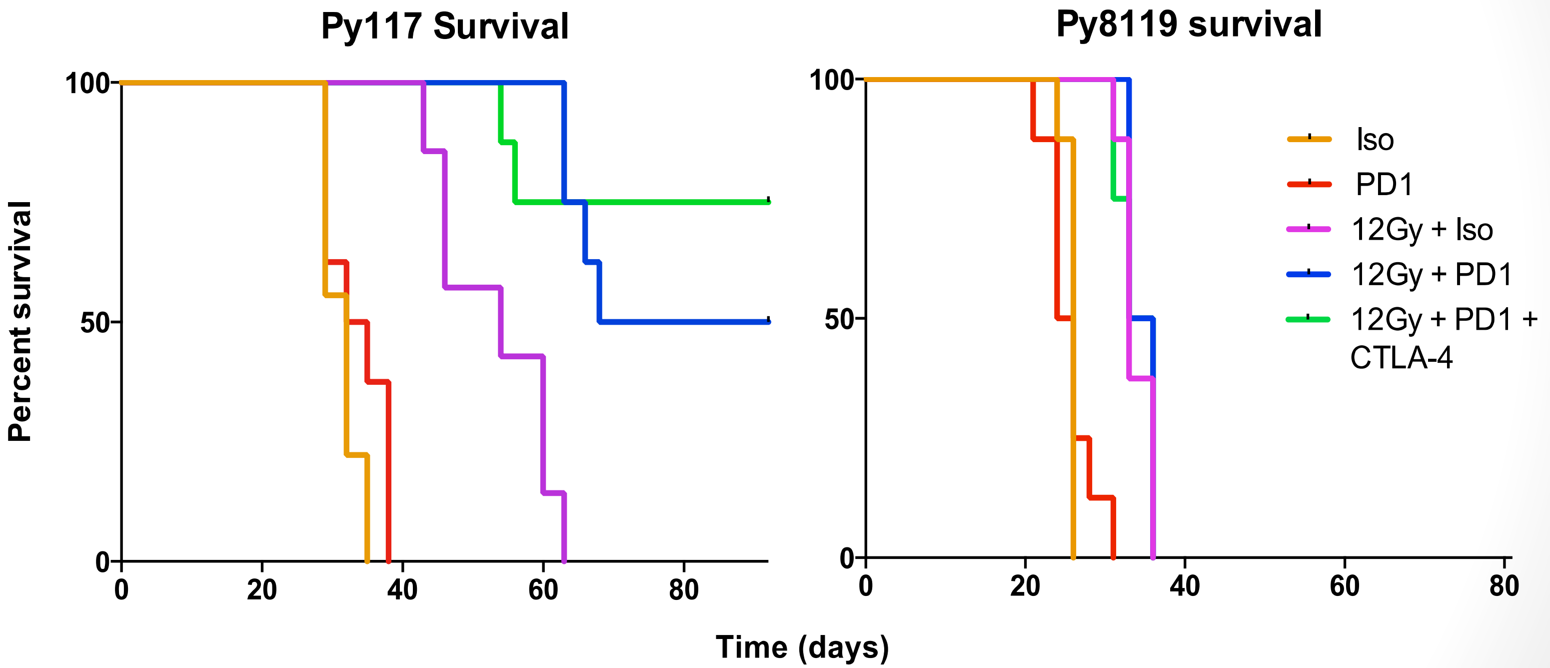
Py117 but not Py8119 Tumors Respond to Combination Immunotherapy



Repeated measures analysis

- RT vs RT + PD1 p=0.002
- RT vs RT + PD1 + CTLA-4 p= 0.0004

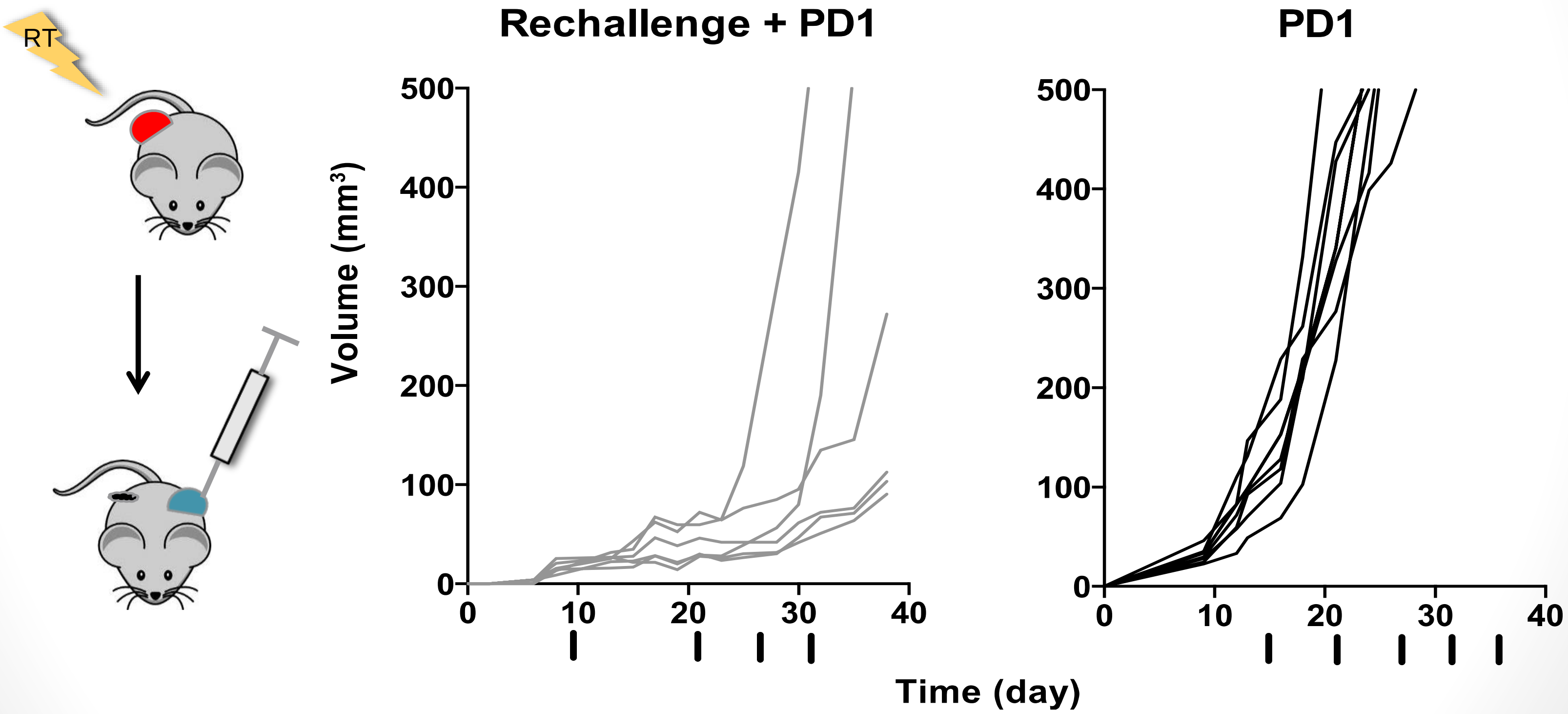
Combination Therapy Translates to a Survival Benefit in the Py117 Tumors



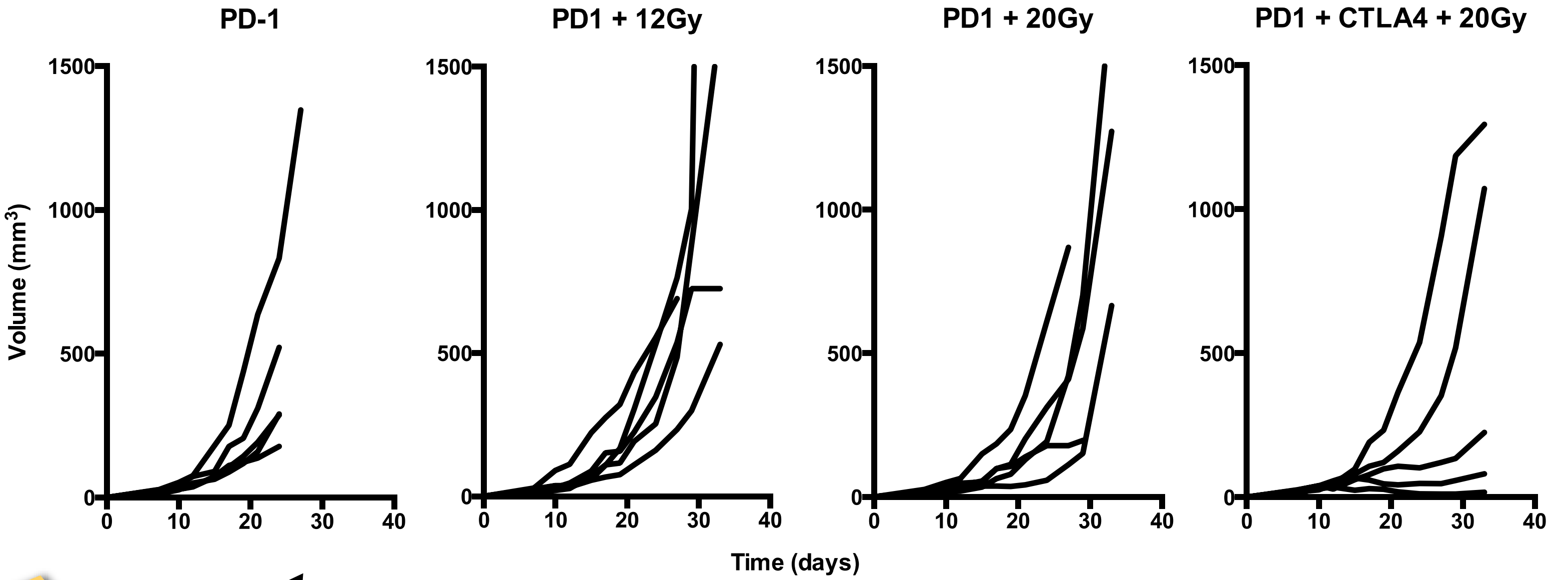
Survival

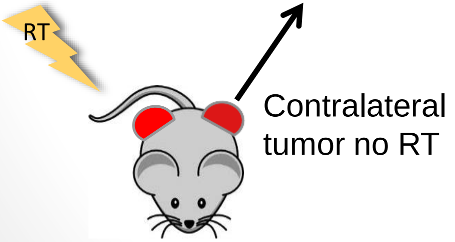
- P<0.0001 on Log-Rank

There is a Greater Tumor Latency Upon Rechallenge in Cured Mice



CTLA-4 and PD-1 Enables Antitumor Response to Contralateral Unirradiated Tumors (Abscopal Effect)

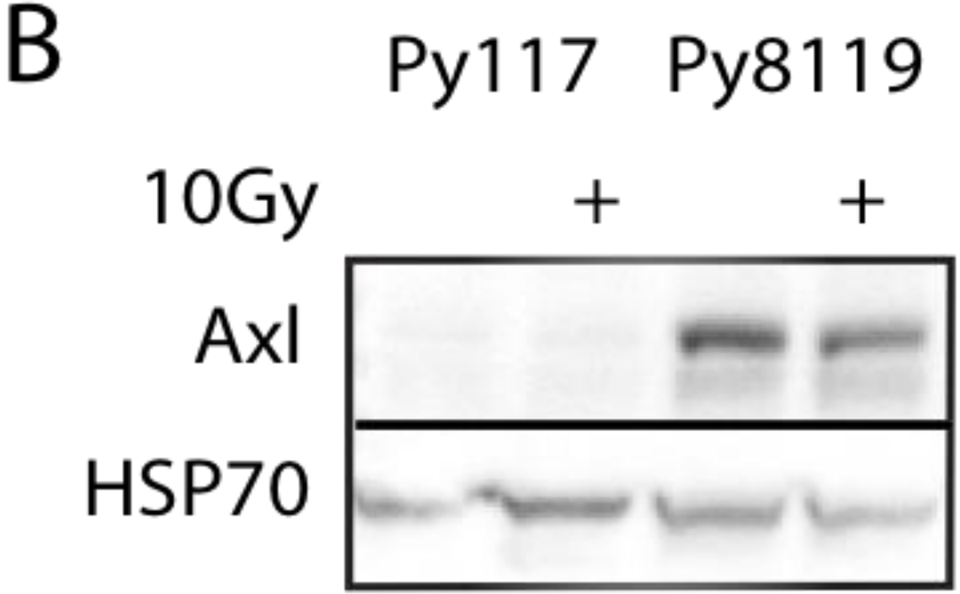




How Can We Overcome Py8119 Therapeutic Resistance?

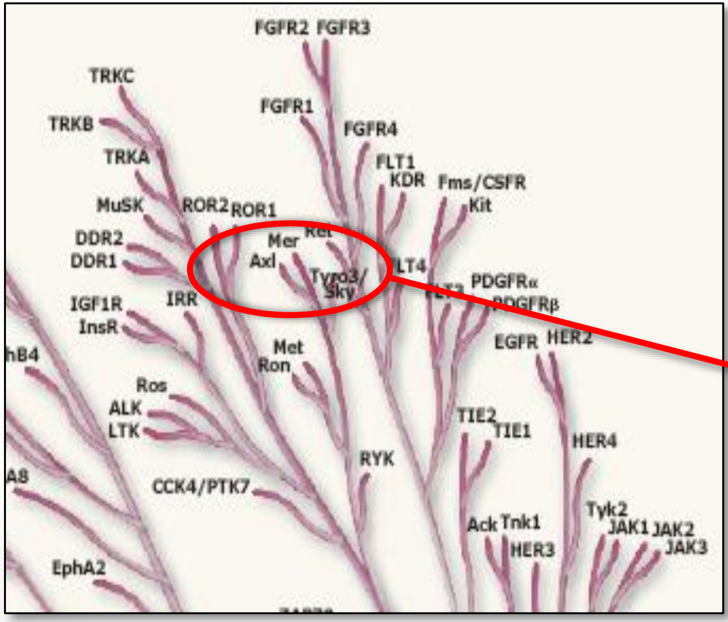
- Uncloaking MHCI Expression
- Increasing T cell Recruitment and Activity

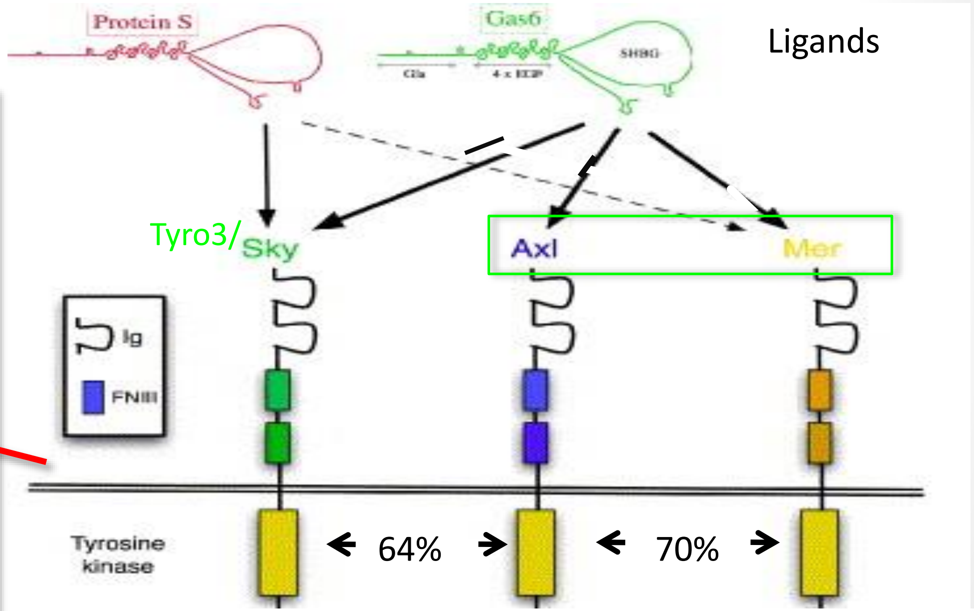
Receptor Tyrosine Kinase Axl is Overexpressed in Resistant Cells



AXL is a Member of the TAM Receptor Tyrosine Kinase Family (Tyro3/Sky, AXL and Mer)

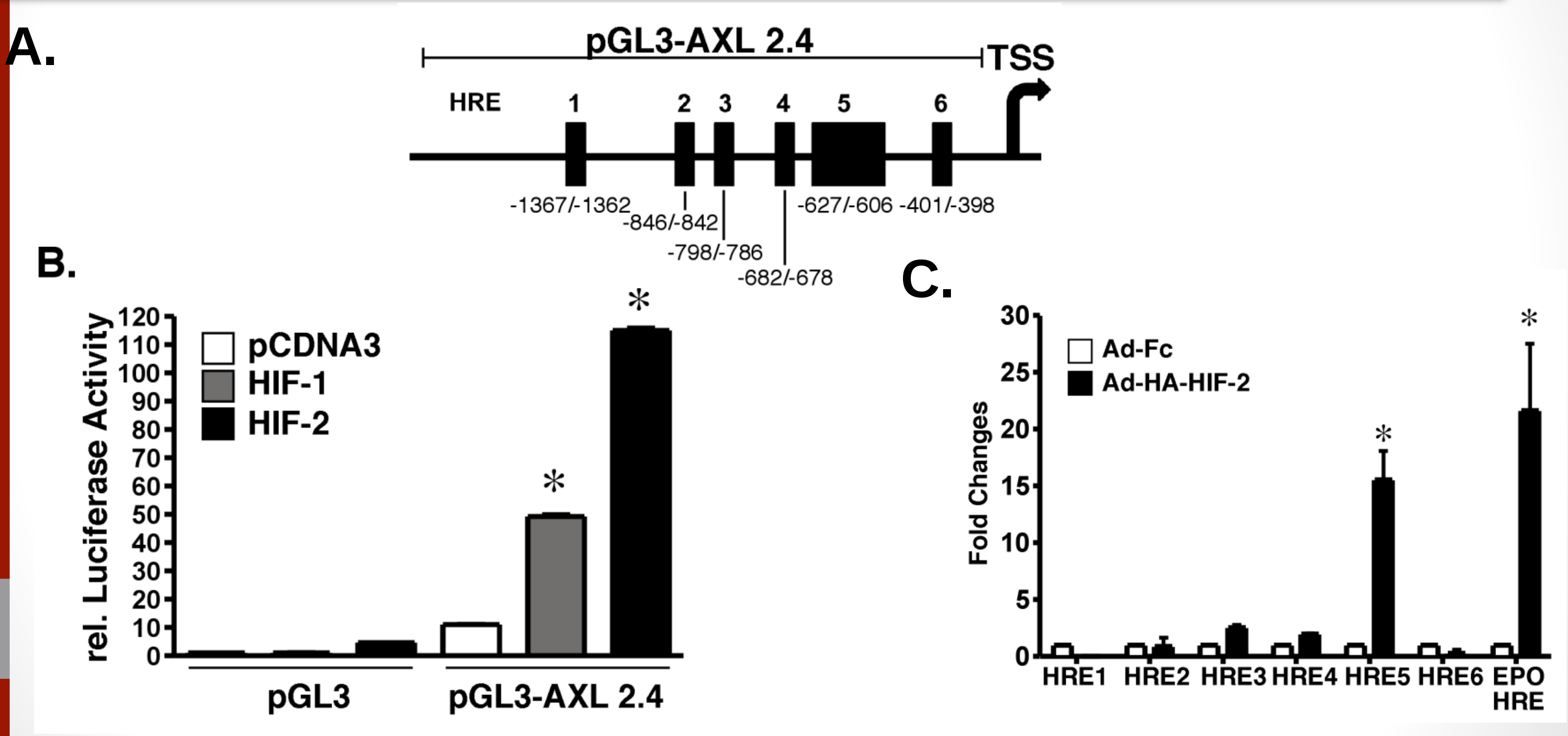
Human Kinome: Tyrosine Kinases





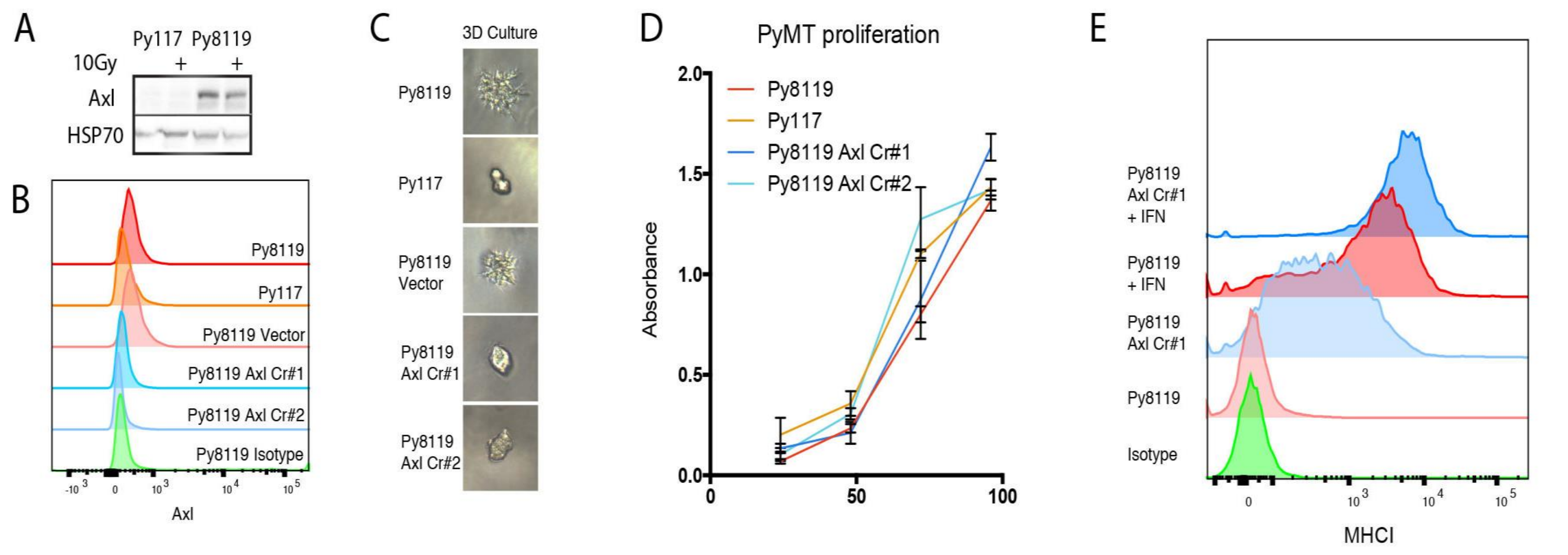
Edited from Hafizi et al., 2006

AXL is a Direct HIF Target

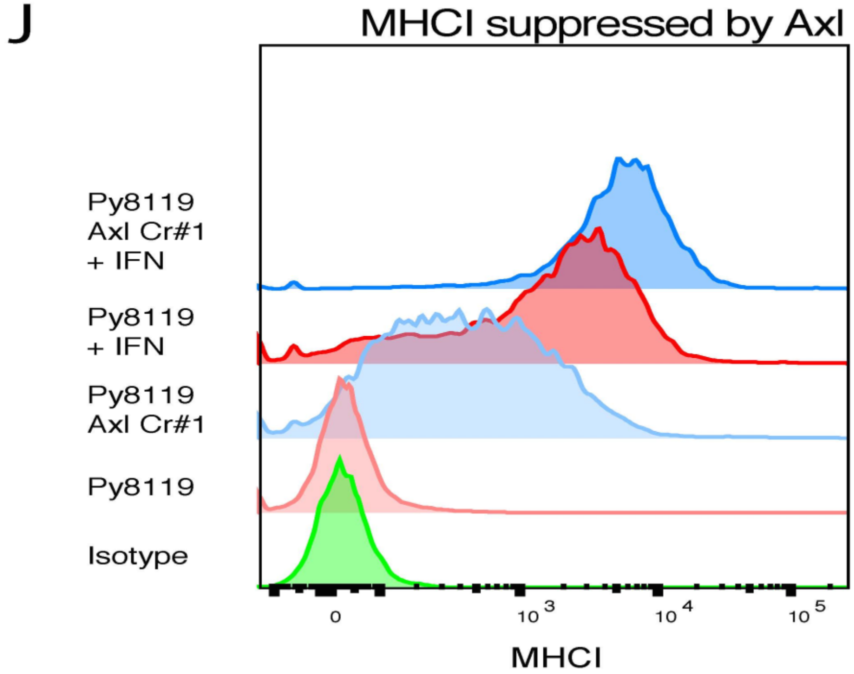


Rankin et al. PNAS 2014

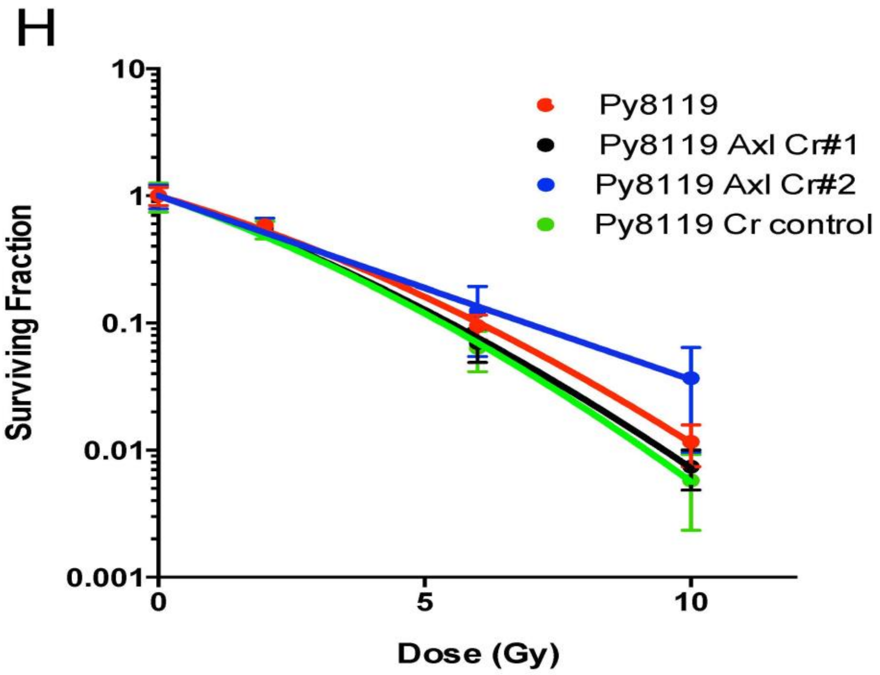
Effect of AXL on Tumor Cell Proliferation and MHC1 Expression



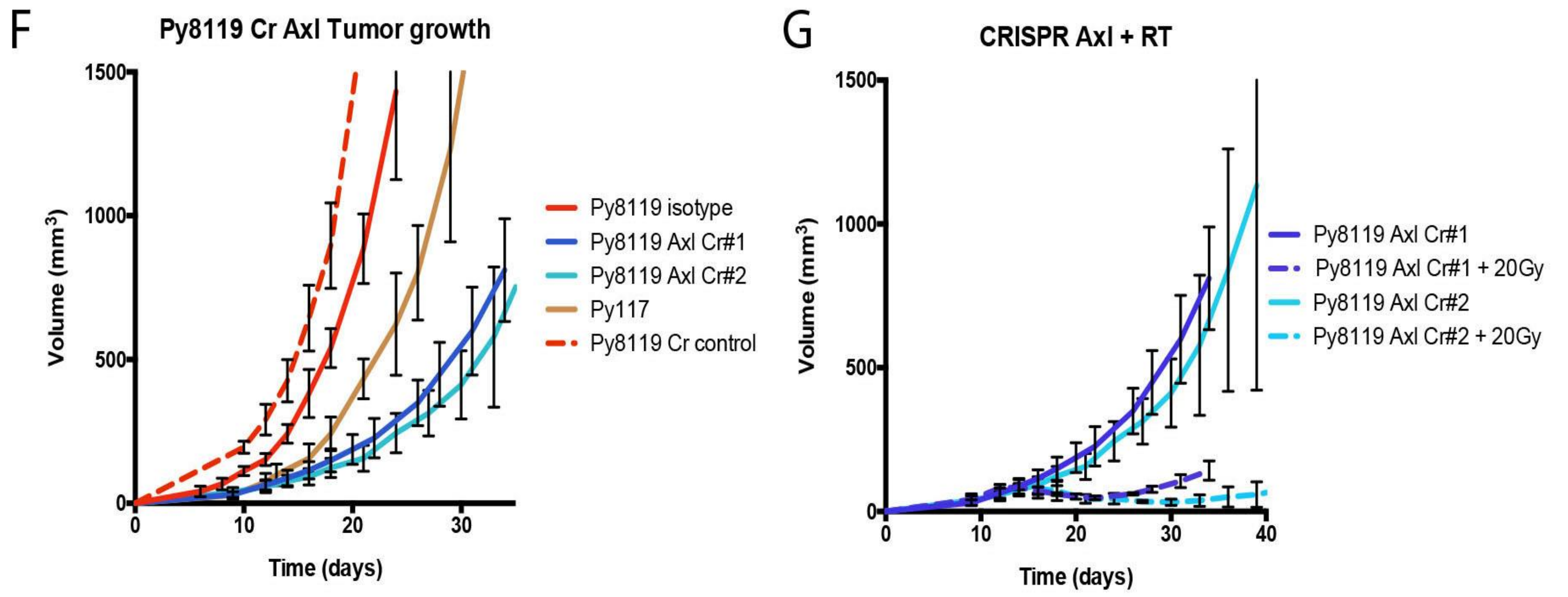
MHCI is Suppressed by Axl



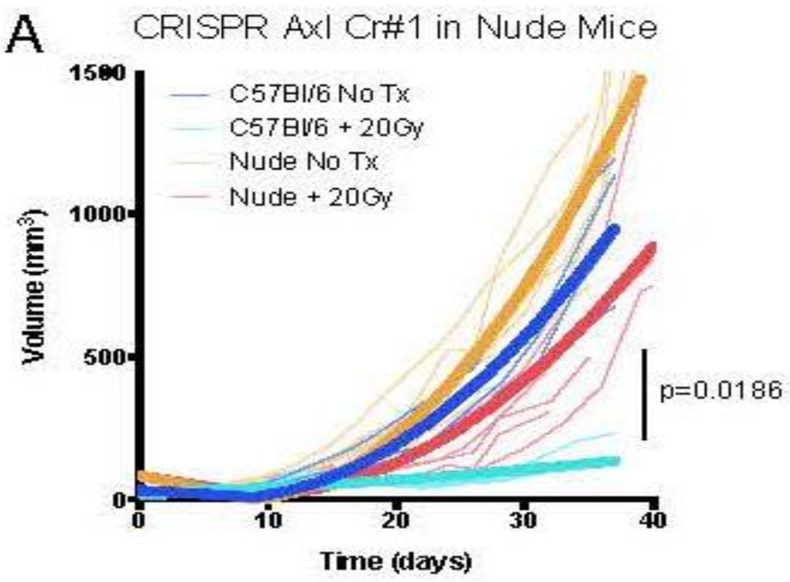
Little Difference in In Vitro Radiosensitivity with Axl



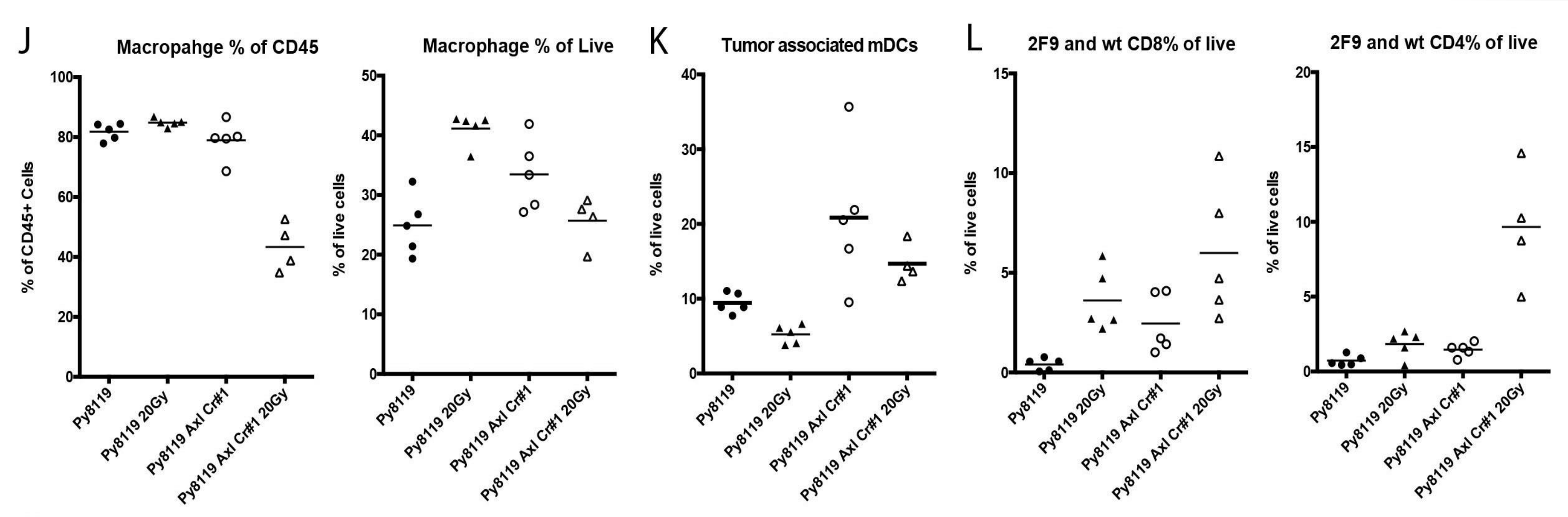
Effect of AXL on Tumor Growth Alone or in Combination with a Single Radiation Dose



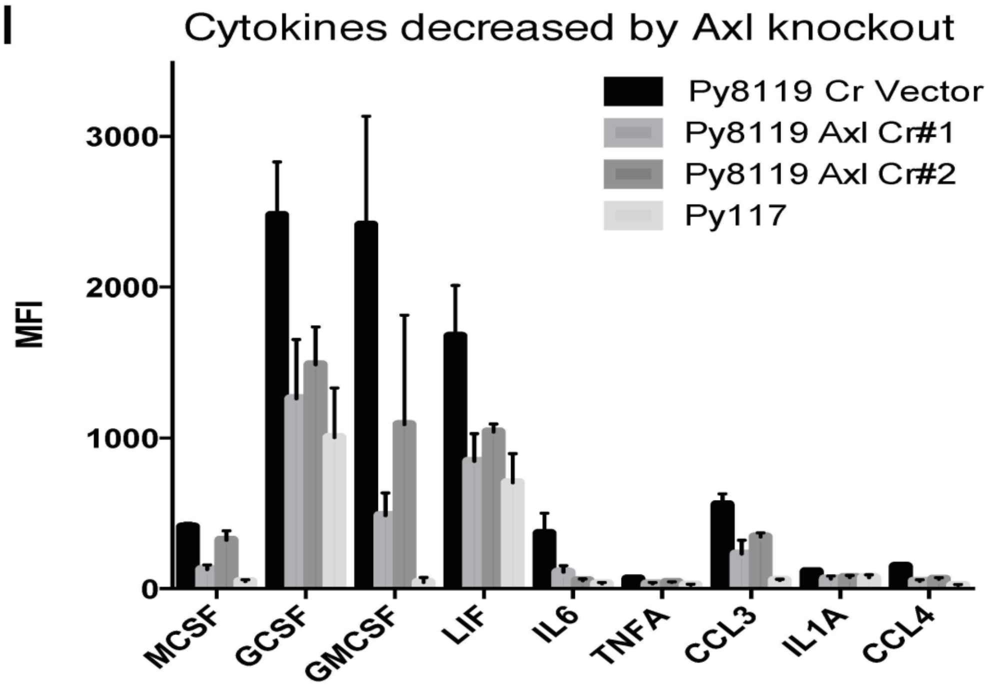
AXL Increases in Radiosensitivity Requires an Intact Immune System



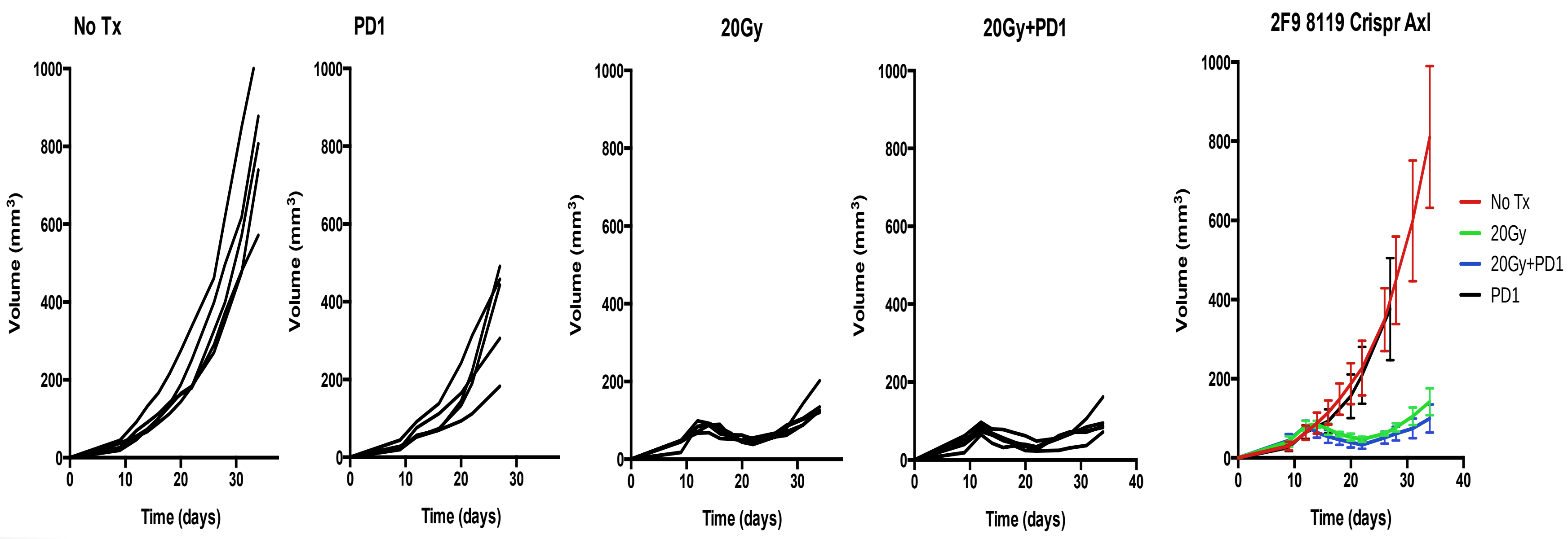
Loss of AXL Results in Decreased CD11b+F4/80+ macrophages



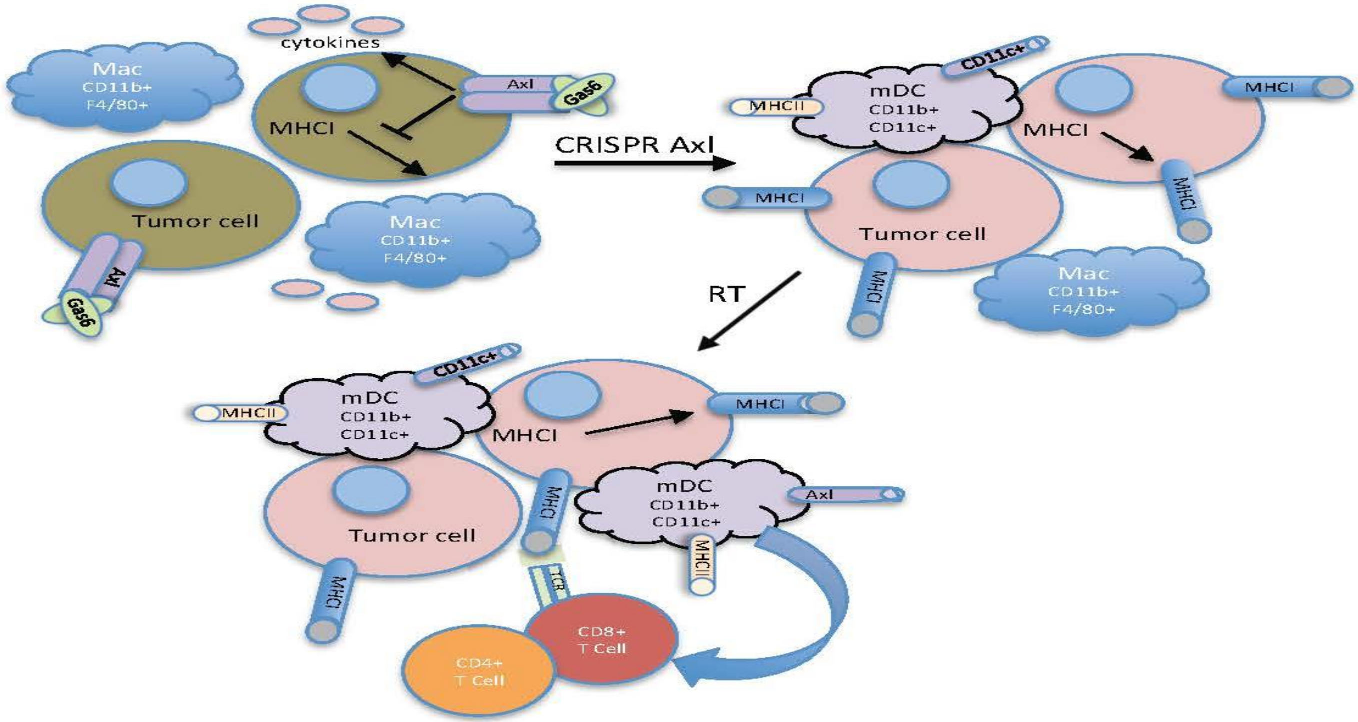
Cytokine and Chemokine Regulation by Axl



Axl Deficient Tumors are Radiosensitive with No Apparent Anti-PD1 Synergy



Loss of Axl Enables Licensing an Antitumor Immune Response after RT



Summary

- Model of immunologic heterogeneity
  - Many tumors are resistant to immunotherapy and radiation
  - Radiation and PD-1 therapy is an attractive approach
    - Additional targets needed
      - improve responses in resistant tumors
  - Anti-AXL therapies are promising additions to target macrophages and enhance antigen presentation
    - Good combination effect in poorly immunogenic cancers


Spirit of Gold

Acknowledgements

Stanford Radiation Oncology

- **Todd Aguilera**
- Mihalis Kariolis
- Marjan Rafat

- Rie von Eyben

Engleman Lab

- Ed Engleman
- Yaron Carmi

UCSD

- Lesley Ellies