SITC CANCER IMMUNOTHERAPY WINTER SCHOOL

Preclinical Mouse Models for Oncology and Immuno-Oncology Applications

Maryland Franklin, PhD
Vice President and Enterprise Head, Cell and Gene Therapy

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Presentation Agenda

1. Understanding the Current Oncology Landscape
2. Common Preclinical Oncology and Immuno-Oncology Mouse Models
3. Advantages and Limitation of Preclinical Oncology Mouse Models
4. Strategies Around Selection of Preclinical Oncology Mouse Models
5. Case Studies and Applications of Selected Models

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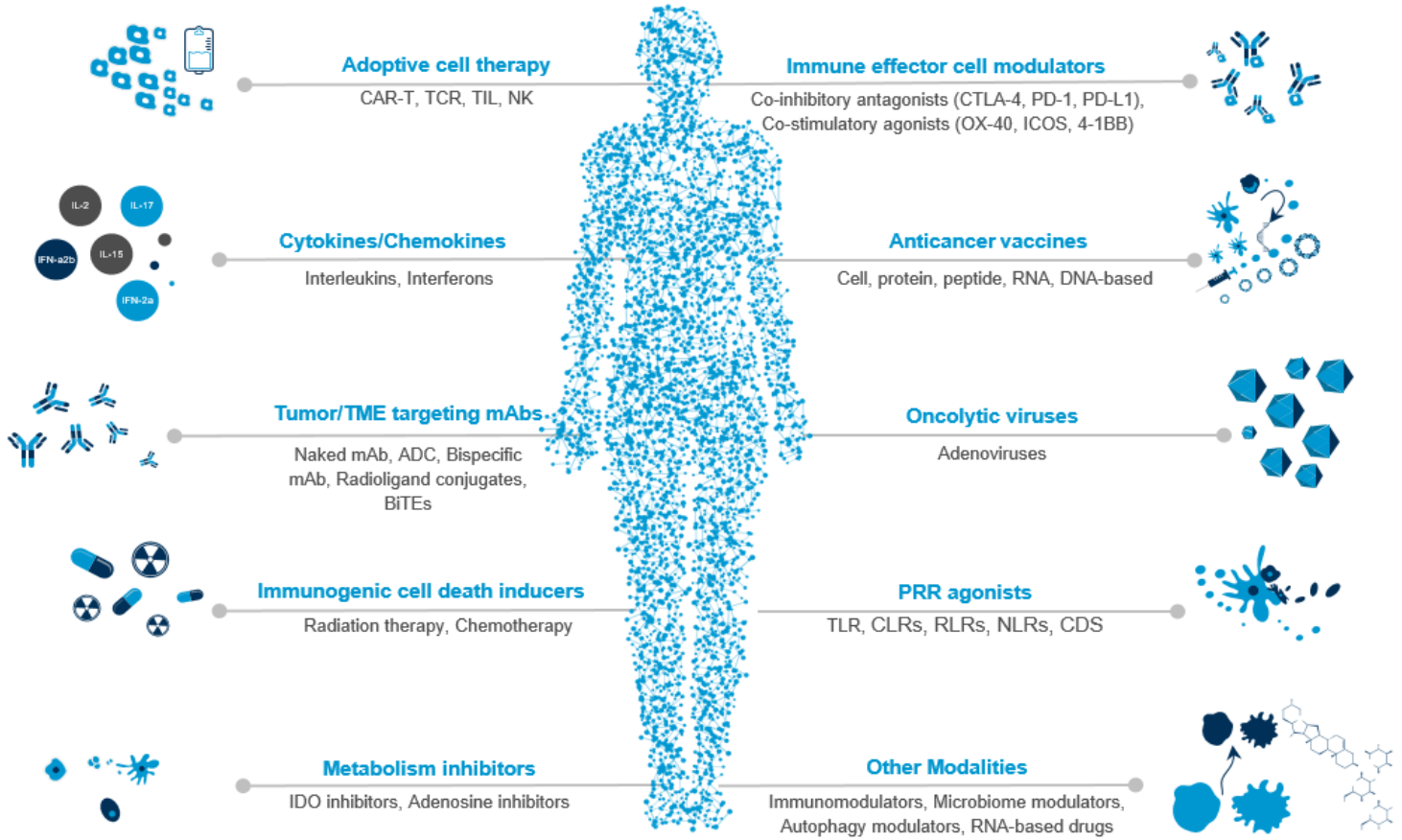
Understanding the Landscape

- Wide array of approaches undertaken
- Many approved drugs
- Extensive discovery and development continues in Pharma and Biotech
- No “one-size-fits-all” preclinical model exists
- Understanding the type of models and assays required is key as preclinical studies have become more and more complex



Abbreviations: *Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG- I -like receptors (RLRs), Nucleotide-binding Oligomerization Domain (NOD)-like receptors (NLRs), and cytosolic DNA sensors (CDS)*

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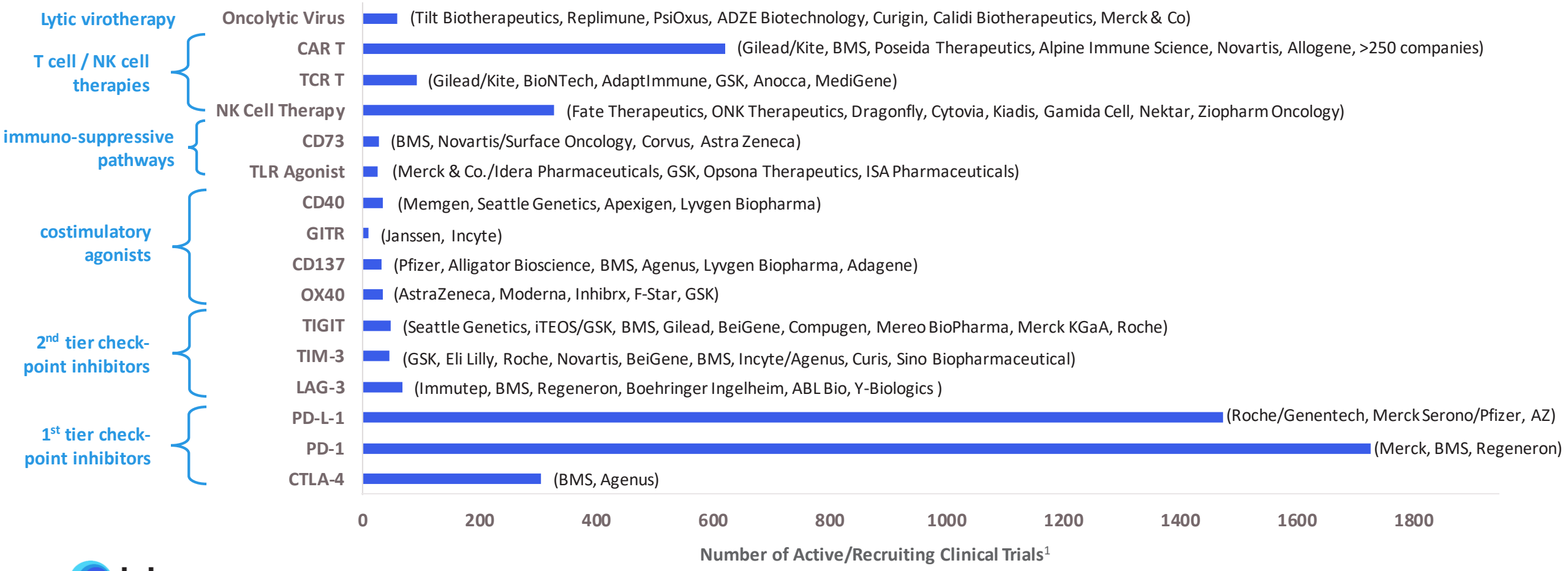
Understanding the Immuno-Oncology Landscape

Active or Recruiting Oncology Immunotherapy Clinical Trials by Target (examples of companies developing drugs in each area)



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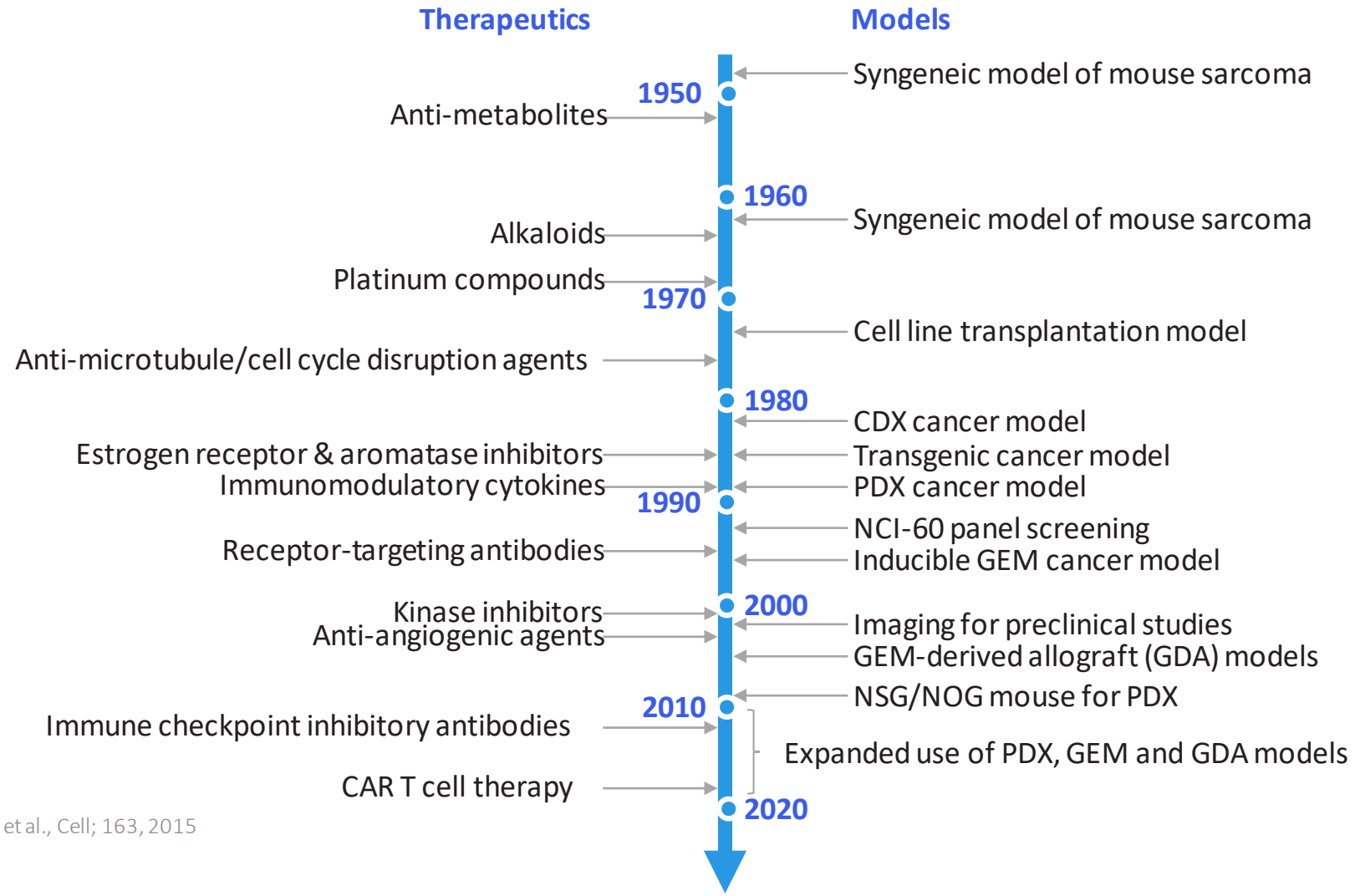
1. Data from: clinicaltrials.gov, January 2022

Timeline: Key Preclinical Milestones in Oncology



1.Modified from Day et al., Cell; 163, 2015

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Preclinical Oncology and Immuno-Oncology Models

Xenograft Models

- Species/strain of tumor line is different from host
- Generally implant of human tumor cells into immuno-deficient mouse strain
  - Cell line derived models (CDX)
  - Patient derived models (PDX)
- Subcutaneous (SC) is the most common
- Orthotopic (implant into clinically relevant location)
- Disseminated (IV) for hematologic malignancies
- Metastasis (limited models)

Syngeneic Models

- Species/strain of tumor line is similar to host
- Generally implant of mouse tumor cells into immuno-competent mouse strain
  - Cell line derived models (CDX)
  - Allograft derived models
- Subcutaneous (SC) is the most common
- Orthotopic (implant into clinically relevant location)
- Disseminated (IV) for hematologic malignancies
- Metastasis (limited models)

Transgenic Models

- Species/strain are alike with respect to tumor and host
- Generally mouse tumors in mice
  - Overexpression of oncogenes
  - Knockout of tumor suppressors

Humanized Models

- Species/strain of tumor line is different from host
- Implant of human tumor cells into immuno-deficient mouse strain
  - Co-injection of human immune cells
  - Delivery of human growth factors/cytokines
- Subcutaneous (SC) is the most common
- Disseminated being investigated

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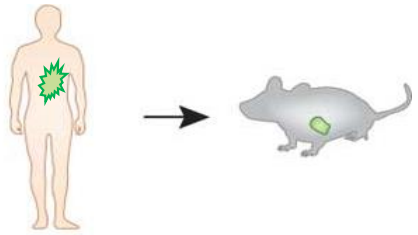
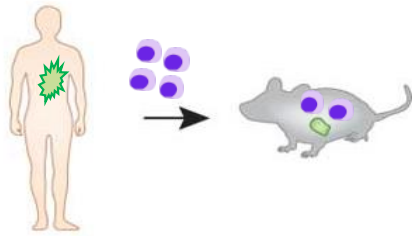
Preclinical Oncology and Immuno-Oncology Models

	Model Type	Advantages	Limitations
	Human cell line derived models (xenografts)	• Logistically easy • Great for screening • Readily available • Industry “standard” • Luciferase versions exist • Suitable for orthotopic or metastatic	• Can be poorly predictive • Established decades ago (genetic drift?) • Immune deficient mouse required
	Patient derived xenograft (PDX) models	• Histological “fidelity” to original patient tumor • Extensively characterized • Higher predictive value • Drug screening and resistance mechanism investigation	• Immune deficient mouse required • Challenging to establish • Some tumor types have limited availability • Slower growing (generally) vs xenografts • More predictive for clinical outcome
	Humanized immune system mice	• Can test human antibodies • Can use CDX or PDX lines	• Expensive studies • Sub-optimal immune system • Models allograft immunity • Graft vs. host disease

1.Modified from Singh & Ferrara, Nat. Biotechnology, 2012

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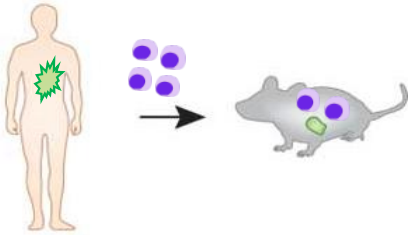
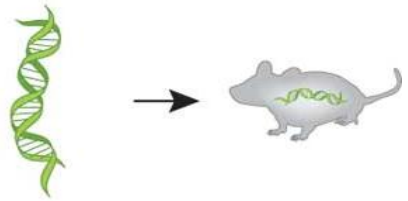
Preclinical Oncology and Immuno-Oncology Models

	Model Type	Advantages	Limitations
	PDX tumors in humanized immune system mice	• Same as above, plus: • Aspects of human immune system is present	• Highly dependent upon type of humanization • Models allograft immunity • Expensive (to very expensive) studies • Sub-optimal immune system • Graft vs host disease (hPBMC approach)
	Syngeneic cell line derived models	• Intact immune system • Logistically easy • Great for screening • Readily available • Industry “standard” for I/O • Luciferase versions exist • Suitable for orthotopic or metastatic	• Can be poorly predictive • Established decades ago (genetic drift?/variability) • Overall number of models is limited
	Genetically engineered mouse models (GEMM) & transplantable fragments	• Faithful stromal biology (TME) • Relevant genetic drivers • Many transplantable models show recapitulation of transgenic mouse disease	• Logistically challenging • Expensive licenses • Few neo-antigens

1.Modified from Singh & Ferrara, Nat. Biotechnology, 2012

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Preclinical Oncology and Immuno-Oncology Models

Subcutaneous Models

- CDX, PDX, syngeneic, allograft transplant
- Advantages:
  - Generally rapid studies
  - Relatively inexpensive
  - Good for drug screening
  - Good for PK/PD studies
- Disadvantages:
  - Implant location not clinically relevant
  - Growth kinetics can be unrealistically fast
  - Rarely metastasize

Orthotopic Models (solid tumors)

- CDX, PDX, syngeneic, allograft transplant
- Advantages:
  - Implant location more clinically relevant
  - Can use *in vivo* imaging to track disease burden and therapeutic benefit
  - Increased rate of metastatic disease reported
- Disadvantages:
  - Technically challenging
  - Typically more expensive and/or labor intensive

Disseminated Models

- CDX, syngeneic (some PDX exist)
- Advantages:
  - Evaluating disease in relevant "location"
  - Numerous luciferase-enabled lines exist; *in vivo* imaging to track disease and therapy
- Disadvantages:
  - May not fully mimic clinically disease
  - Growth kinetic can be unrealistically fast

Metastatic Models

- CDX, PDX, syngeneic, allograft transplant
- Spontaneous metastasis models
  - Limited number of models
- "Forced" metastasis models
  - IV injection to mimic lung mets
  - Intra-splenic to mimic liver mets
  - Intra-cardiac to mimic bone mets
  - Intra-cranial to mimic brain mets

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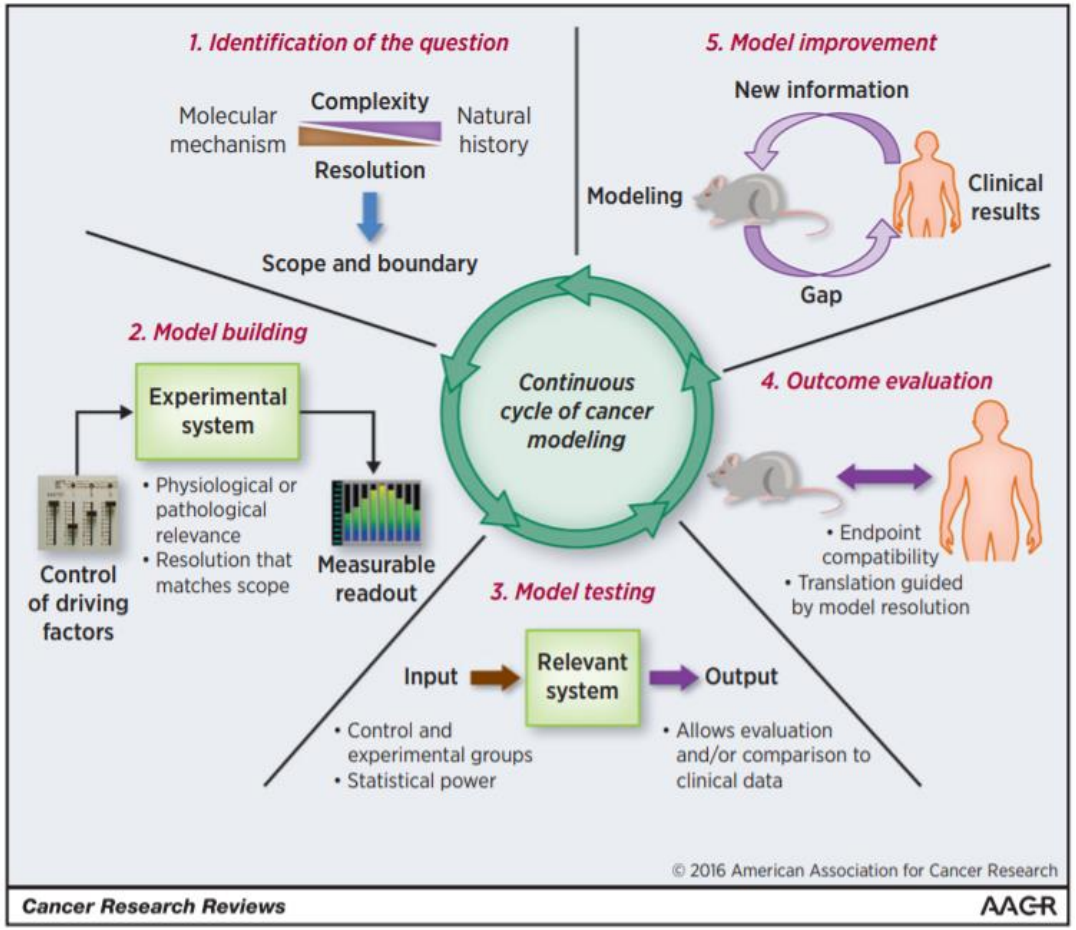
Choosing the Correct Model



1.Thomas et al., Cancer Res; 76(20), 2016

What's the main question?

1. Efficacy
2. Pharmacokinetics/pharmacodynamics
3. Mechanism of action
4. Tolerability
5. Immune cell engagement/involvement

What model is most appropriate?

1. Immune deficient mouse model
2. Immune competent mouse model
3. GEMM/HIS
4. SC, IV, orthotopic

What experimental design?

1. Appropriate controls
2. Appropriate statistical power

What endpoints should be used?

1. Dependent upon model and question
2. Needs to be appropriate for model selected

What improvements can be made?

1. What's still missing
2. What might work better

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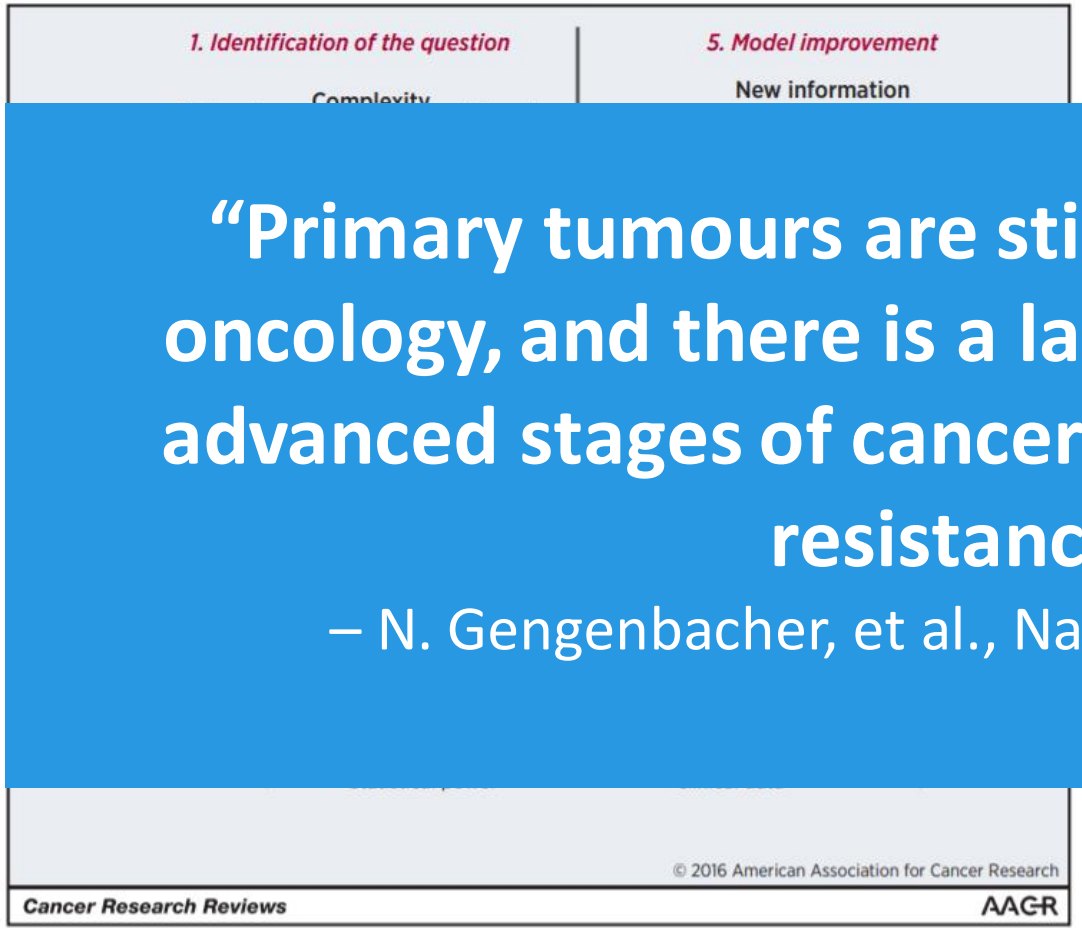
Choosing the Correct Model



What's the main question?

1. Efficacy
2. Pharmacokinetics/pharmacodynamics
3. Mechanism of action
4. Tolerability

"Primary tumours are still the major focus of preclinical oncology, and there is a lack of mouse models focusing on advanced stages of cancer progression such as metastasis, resistance and relapse."

– N. Gengenbacher, et al., Nature Reviews Cancer, December 2017

What improvements can be made?

1. What's still missing
2. What might work better

1.Thomas et al., Cancer Res; 76(20), 2016

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Presentation Agenda

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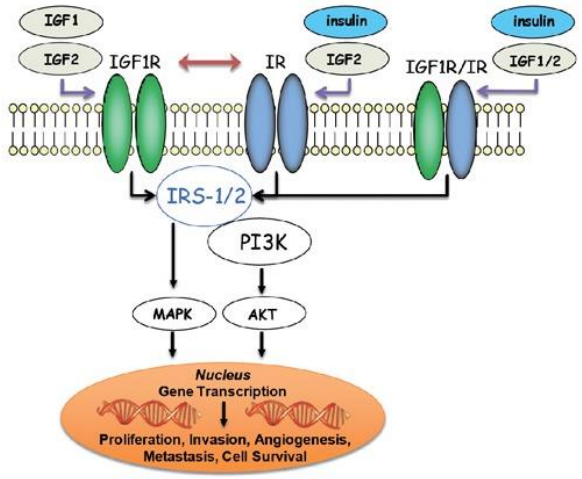
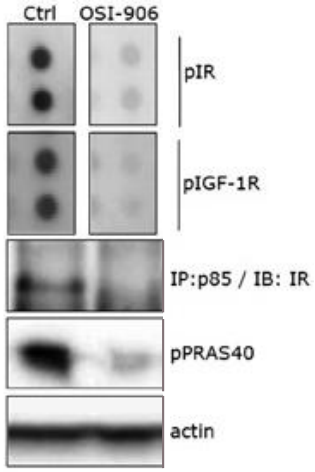
Case Study: Subcutaneous Xenografts

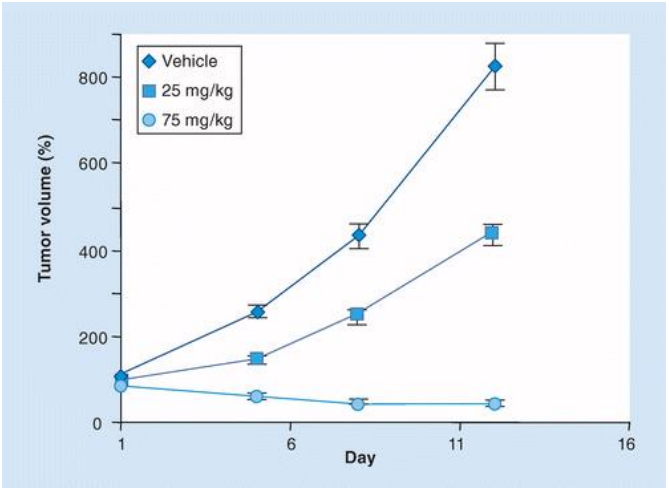
Understand Target Biology



Evaluate PD



Evaluate Efficacy



- Models of choice were SC xenografts
  - Data shown is from GEO and LISN human colorectal lines in nude mice
- Evaluated target inhibition in tumors
- Compared dose response anti-tumor activity

1.Jin, Buck and Mulvihill, Oncol Rev., 2013; Mulvihill, Cooke, Rosenfeld-Franklin, Buck, et al., Future Med Chem, 2009

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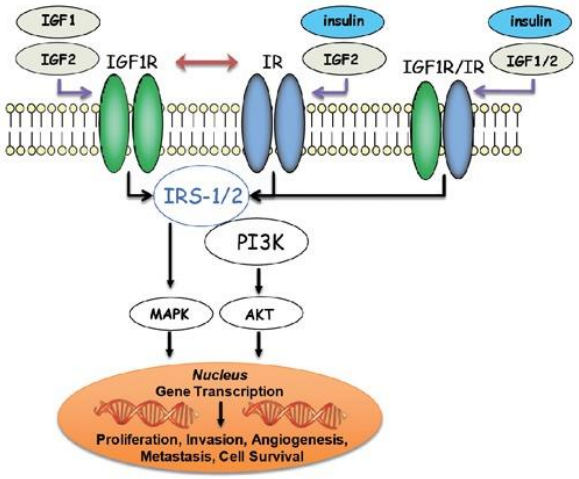
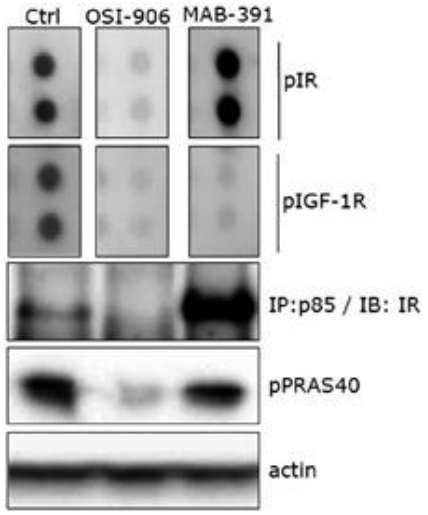
Case Study: Subcutaneous Xenografts

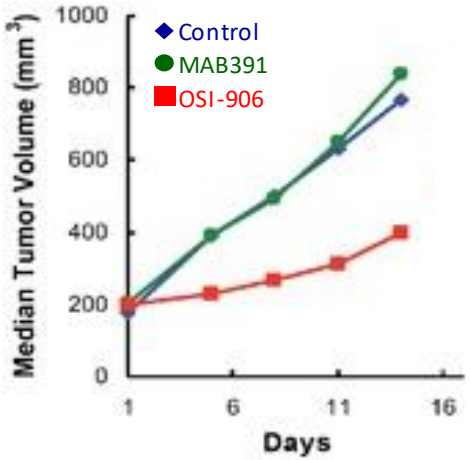
Understand Target Biology



Compare PD



Compare Efficacy



- Models of choice were SC xenografts
  - Data shown is from GEO human colorectal line in nude mice
- Compared target inhibition in tumors
- Compared small molecule dual pIGF-1R/pIR inhibitor to anti-pIGF-1R mAB

1.Jin, Buck and Mulvihill, Oncol Rev., 2013

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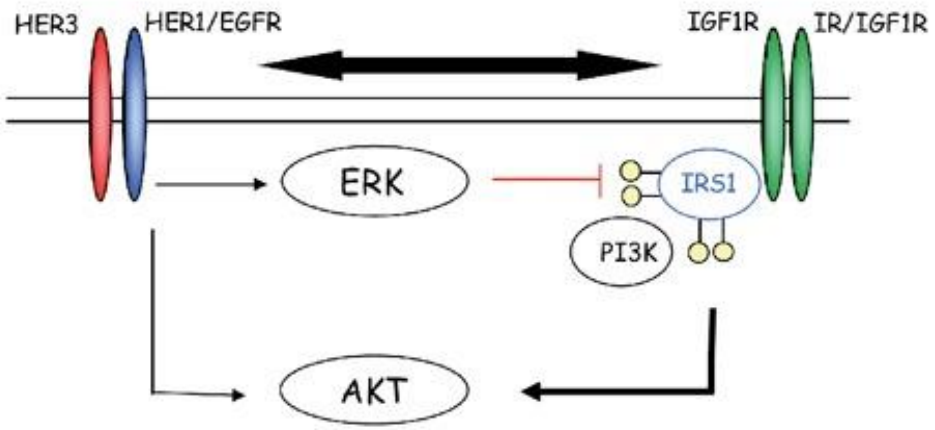
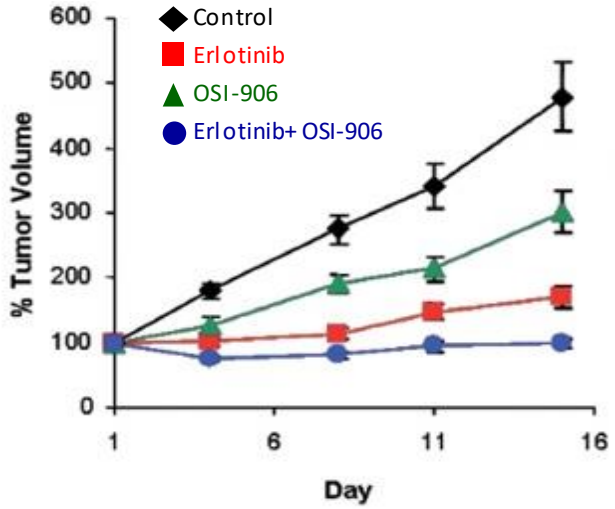
Case Study: Subcutaneous Xenografts

Further Investigate Target Biology



Design & Test Rational Drug Combinations



- Models of choice were SC xenografts
  - Data shown is from BxPC-3 human pancreatic line in nude mice
- Used SC xenograft model to investigate potential mechanisms of resistance
- Designed and tested rational drug combination approaches

1.Jin, Buck and Mulvihill, Oncol Rev., 2013

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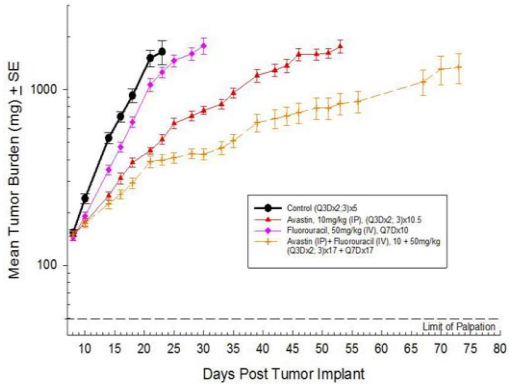
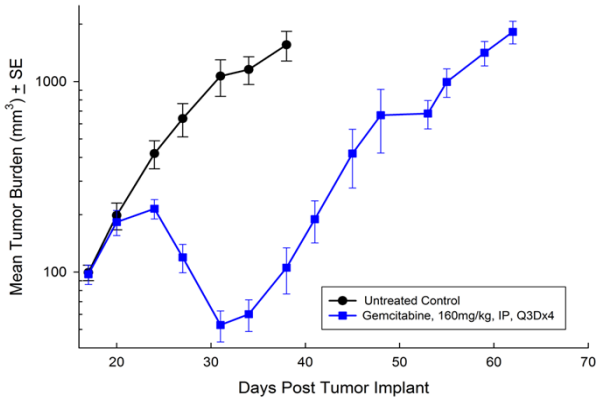
Subcutaneous Xenograft Model Examples

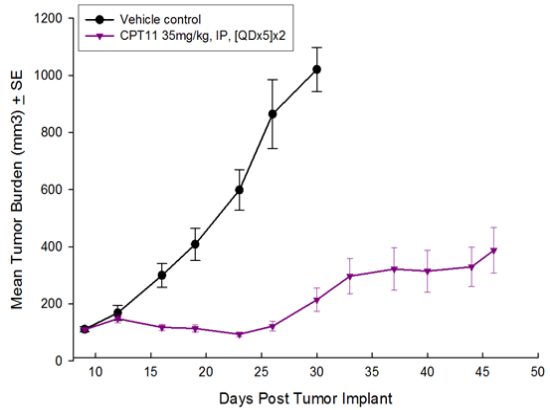
HCT-116 (colon)



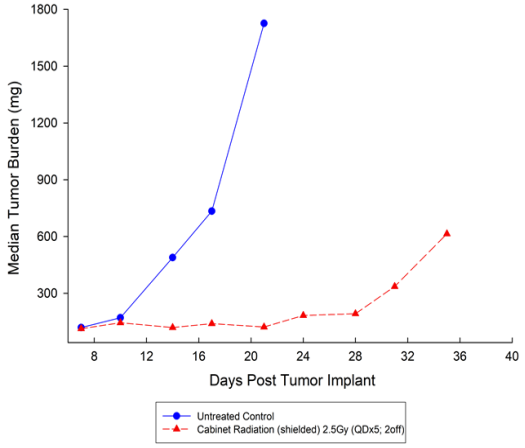
Panc-1 (pancreatic)



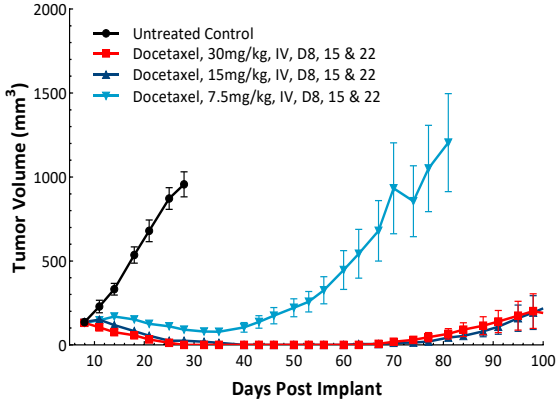
HT-29 (colon)



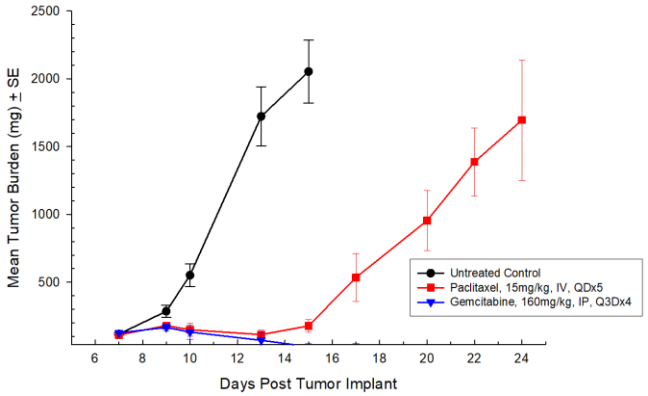
H460 (NSCLC)



PC-3 (prostate)



A2780 (ovarian)



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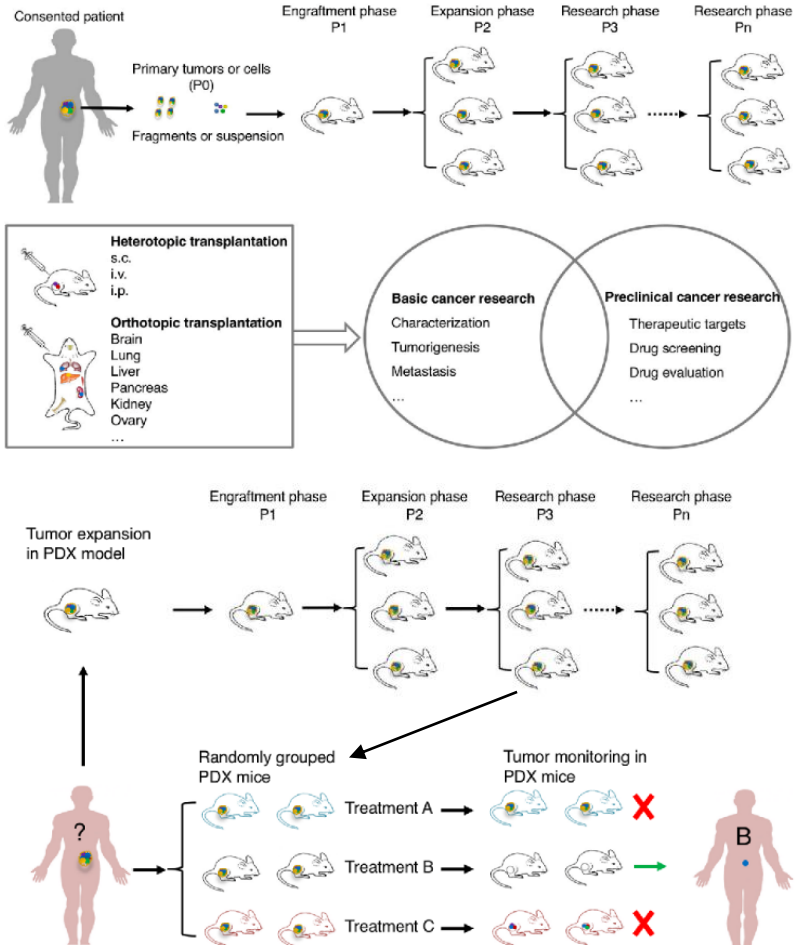
1. All animal work was approved by the site Institutional Animal Care and Use Committee and was performed in conformance with the Guide for the Care and Use of Laboratory Animals within an AAALAC-accredited program with humane euthanasia criteria predetermined on all studies.

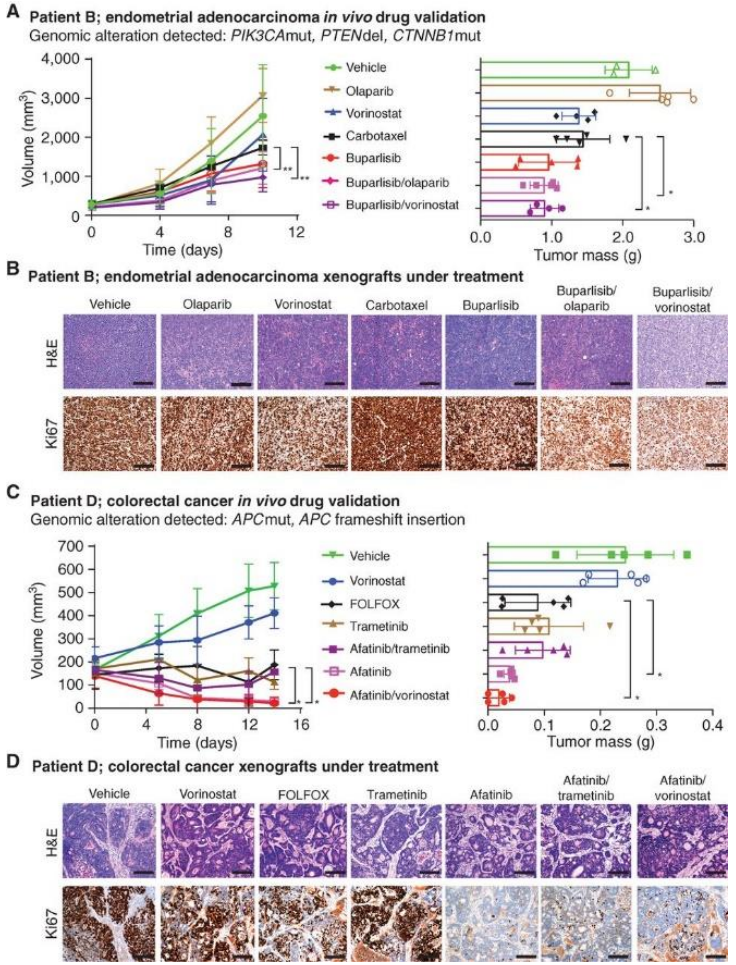
Case Study: PDX Models

Personalized models to guide precision medicine



Modified from: Roberto Vargas et al. Precision Oncology 2018, 2:14

In vivo validation of drug screens



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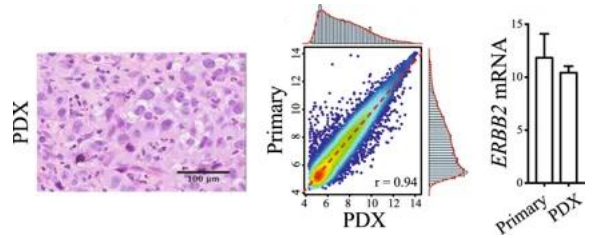
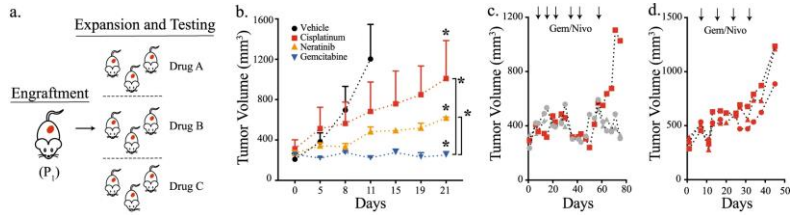
Case Study: PDX Models

Personalized models to guide precision medicine

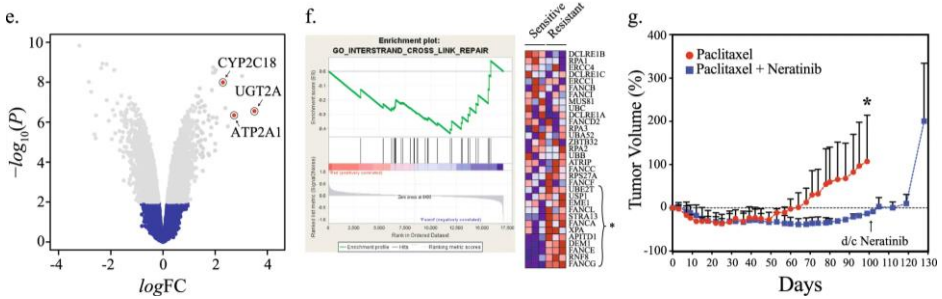


- Tumor material (liver biopsy) was capable of establishing PDX model
- Gene expression from PDX correlated well with primary tumor



Experimental design Initial treatment | Gem/Nivo until resistance | Gem/Nivo of resistant tumor

- Used 3 mouse x 1 drug approach with co-clinical trial design to try and longitudinally guide patient care
- Tumor also had increased PD-L1 expression
- Resistance demonstrated in mouse model prior to patient resistance



- Genome wide expression profiling of resistant tumors
  - Upregulation of genes critical for drug metabolism and detoxification
- 3 x 1 mouse trial again set up to evaluate other possible drug treatments
  - Paclitaxel + Neratinib showed greatest activity

Modified from: Roberto Vargas et al. Precision Oncology 2018, 2:14

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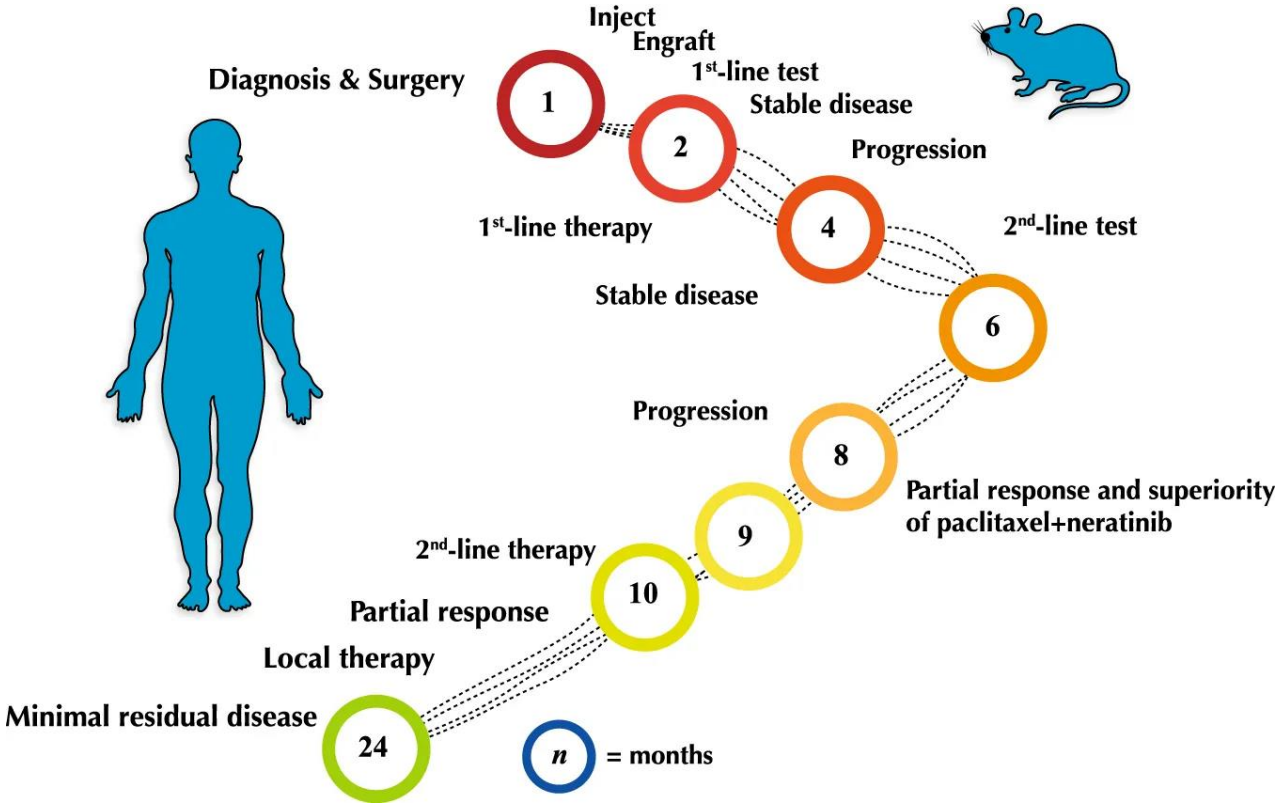
Case Study: PDX Models

Personalized models to guide precision medicine



Roberto Vargas et al. Precision Oncology 2018, 2:14

- Time line of events in the mouse and in the human patient
- Rapid growth in the mouse setting allowed clinical intervention in this particular case
- Mouse studies were able to predict both the development of resistance and the response to 2nd line therapy BEFORE these events were observed in the patient

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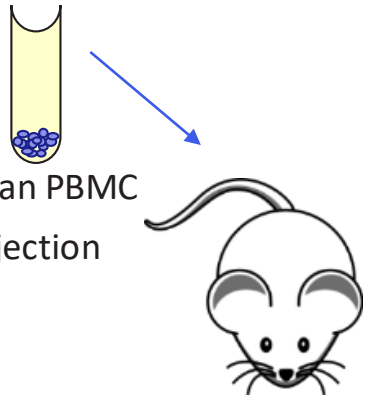
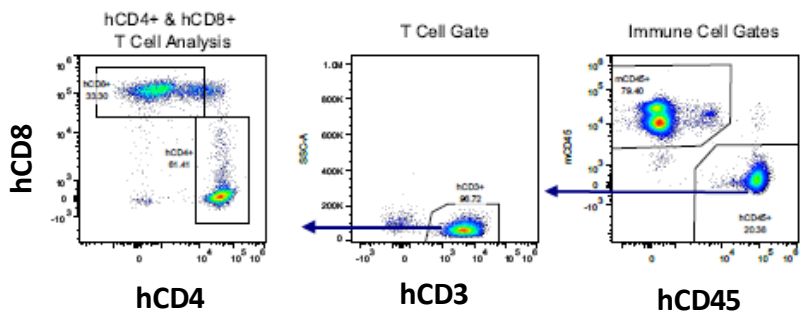
Case Study: Humanized Mouse Models

Two primary methods: human PBMC; human CD34+ stem cells

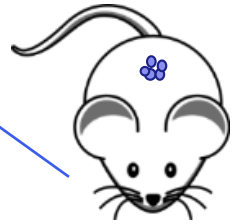
- **PBMC h-NSG:**
  - Short-term studies with hT cells
  - Strong effector/memory T cell function
  - T cell driven GvHD
  - Short lifespan (<3 months)
  - Donor-to-donor variability



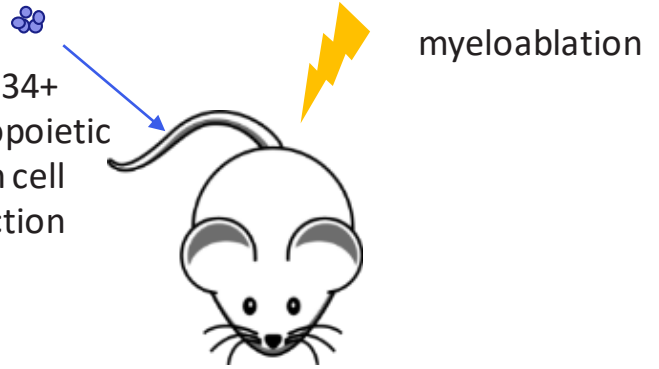
Human PBMC injection



7-14+ days



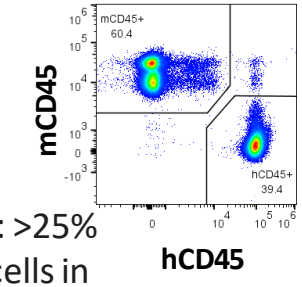
- **CD34+ h-NSG:**
  - Stably engraft bone marrow
  - Multi-lineage hematopoiesis occurs within 12 weeks
  - Stable engraftment (> 1 year) without GvHD
  - Donor-to-donor variability



hCD34+ hematopoietic stem cell injection

myeloablation

~12 weeks



Validated: >25% hCD45+ cells in peripheral blood

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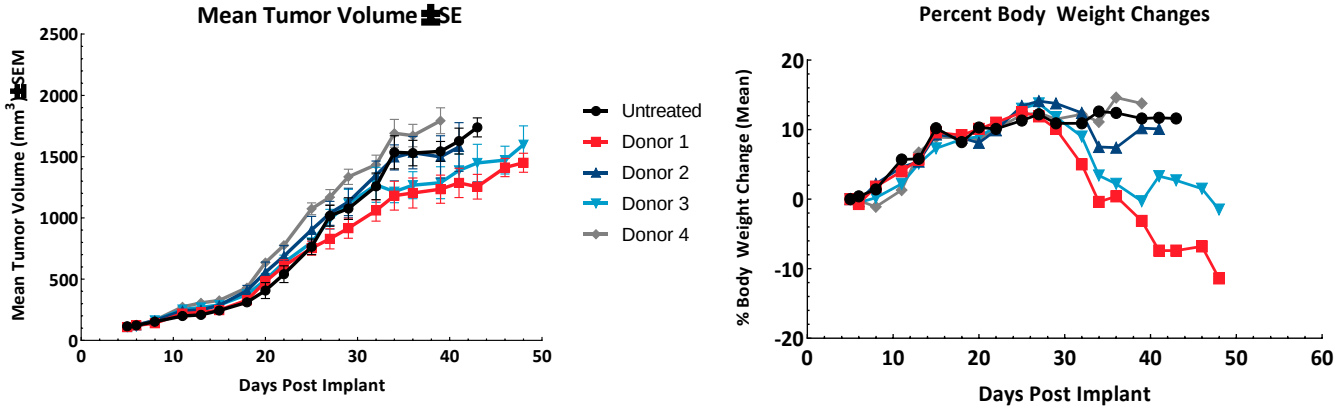
Case Study: Humanized Mouse Models

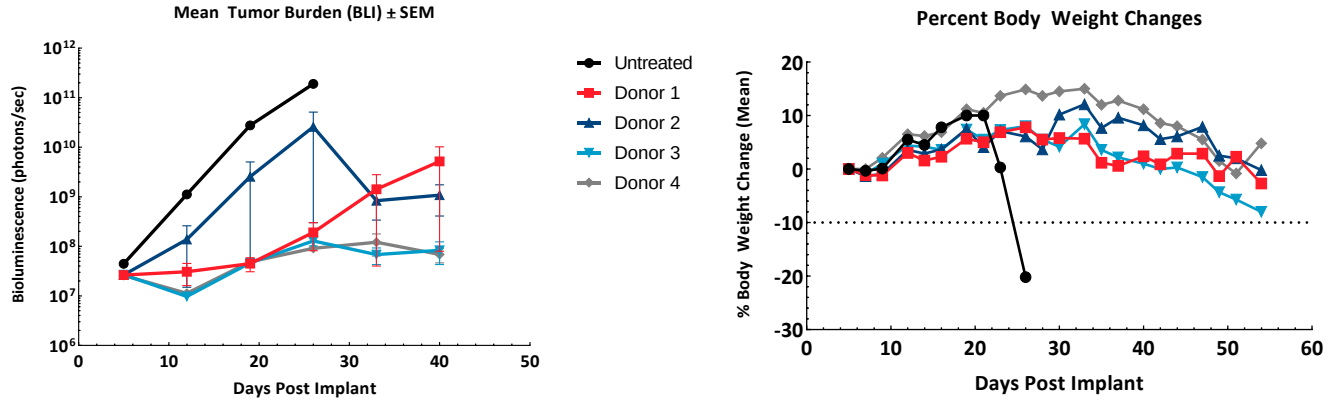
Considerations for the hPBMC Model:

- Onset of characteristics of GvHD
  - Progression variable between donors
  - Clinical signs scored: BWL >10% of baseline, rough pelage, hunched posture, skin lesions/integrity and diarrhea
- Engraftment variability
  - And response to therapy is variable between donors
- Optimized conditions ensure viability of the model, sufficient engraftment and therapeutic window for treatment

SC MiaPaCa-2



IV MM1.S-Luc



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Case Study: Humanized Mouse Models

PBMC humanization – CDX model

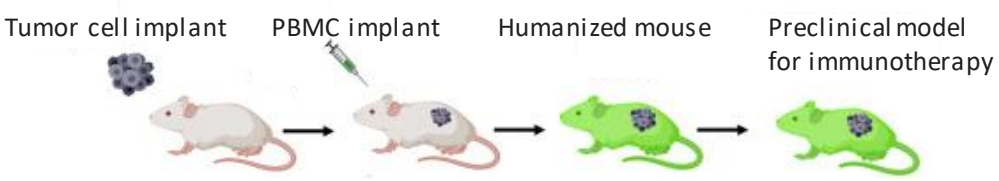
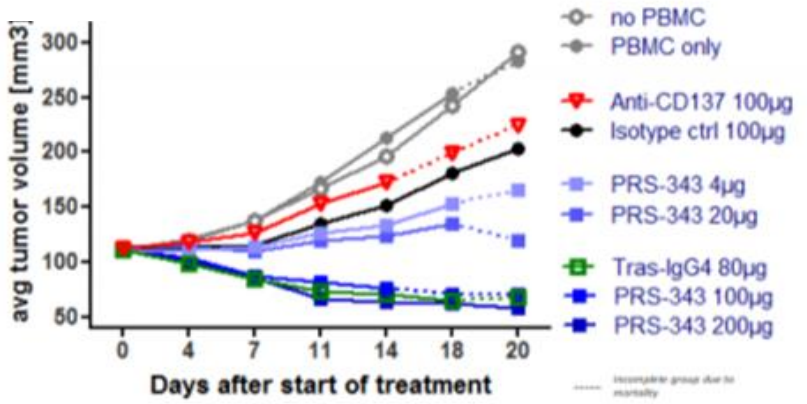


Image modified from: Pawel Sobczuk et al. Translational Oncology 2020

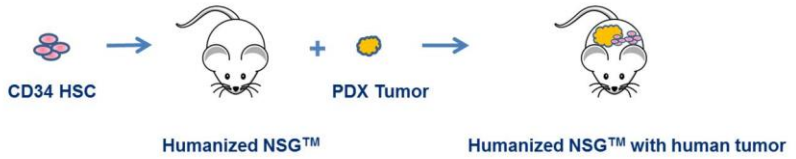
SC SKOV-3 HER2+ model in PBMC-hNOG
Treated with 4-1BB/HER2 bispecific Ab

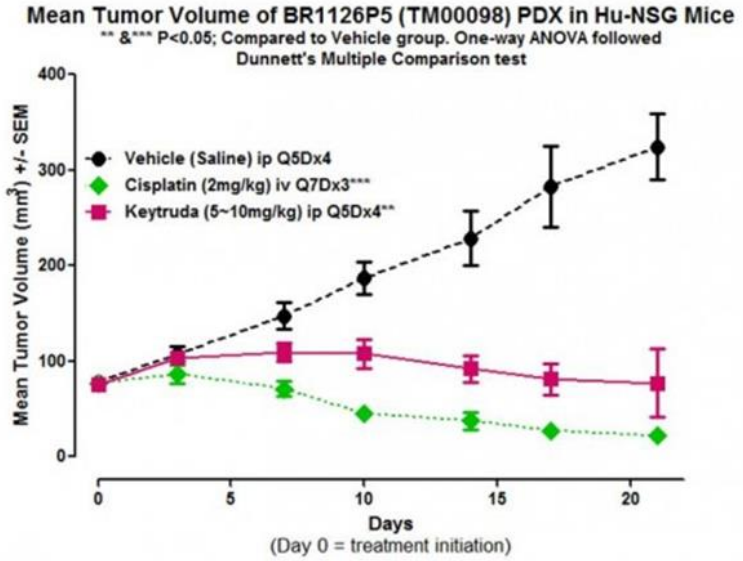


https://d1io3yog0oux5.cloudfront.net/pierisag/files/170401+AACR+Poster+April+2017+final.pdf

Pieris Pharma, AACR 2017

CD34⁺ humanization – PDX model





https://www.jax.org/news-and-insights/jax-blog/2015/april/the-next-big-thing-in-cancer-modeling-patient-derived-xenografts-in-humaniz

Jackson Labs

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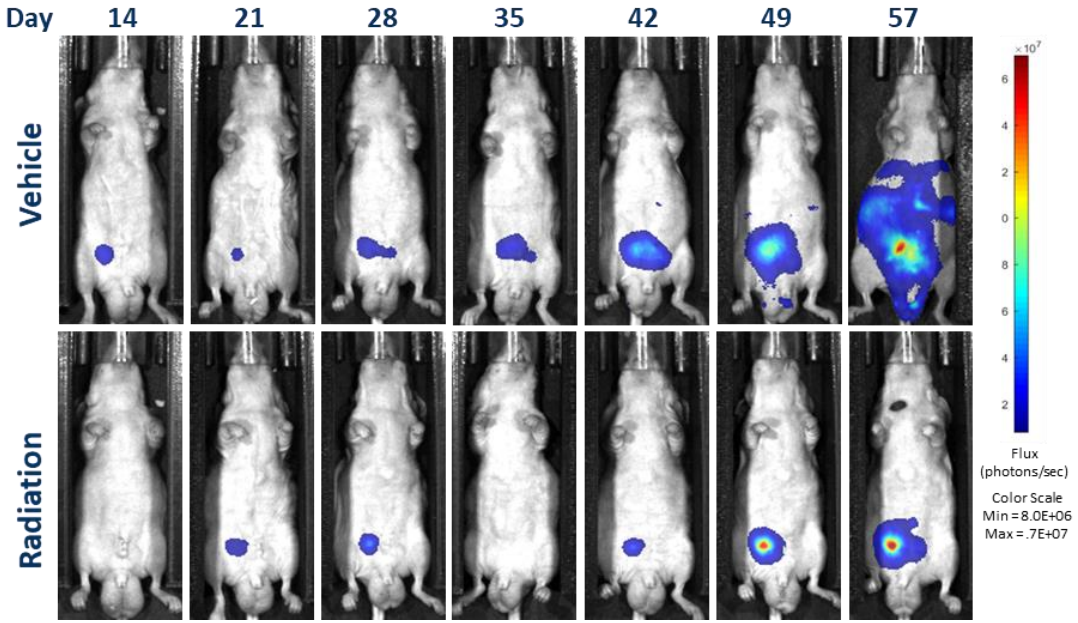
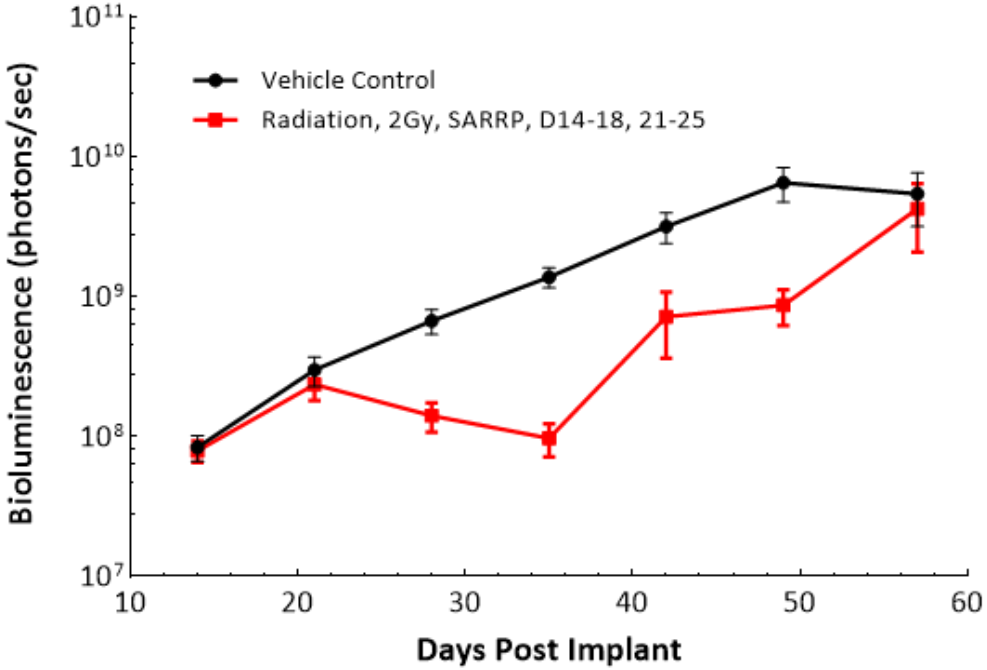
Case Study: Orthotopic Models – Xenografts

Orthotopic PC3-M-Luc (male nude mice)



Mean Tumor Burden ± SE



- Parental cell line transduced with luciferase construct
- Tumors implanted into clinically relevant organ
- Bioluminescence imaging utilized to track disease burden and therapeutic response

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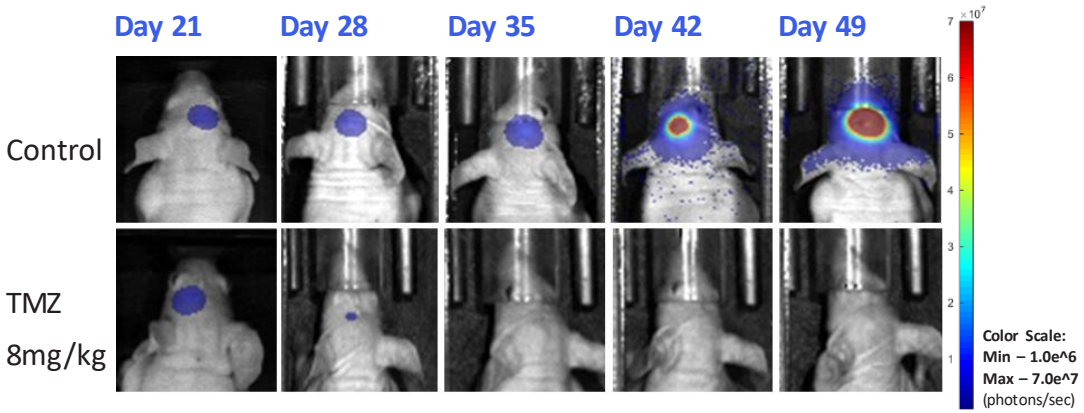
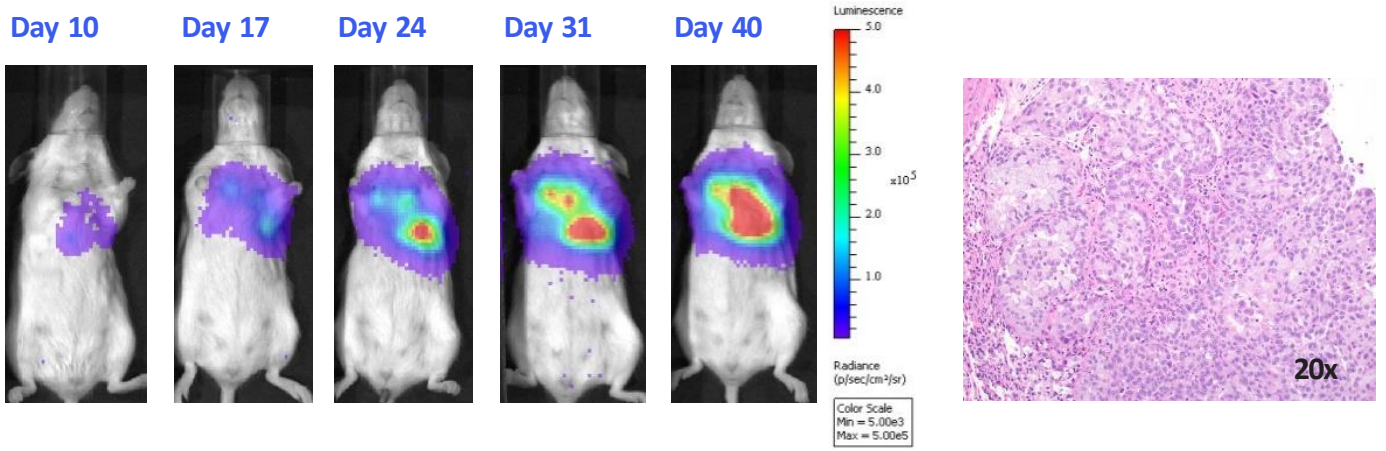
Case Study: Orthotopic Models – Xenografts

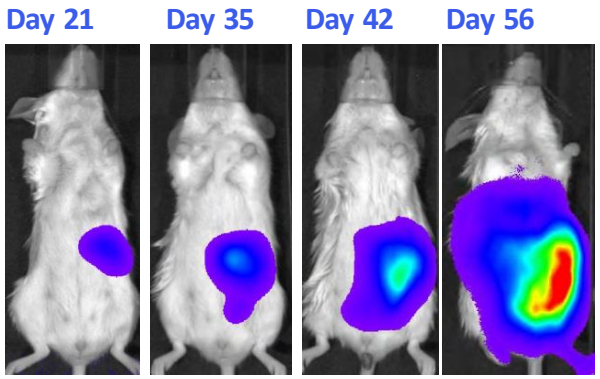
Brain OT: U87MG-Luc



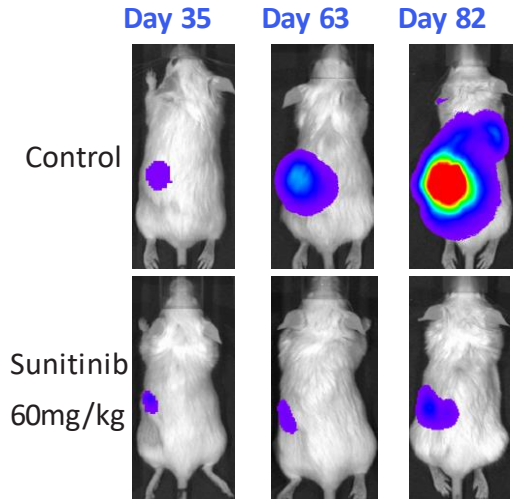
Lung OT: A549-Luc

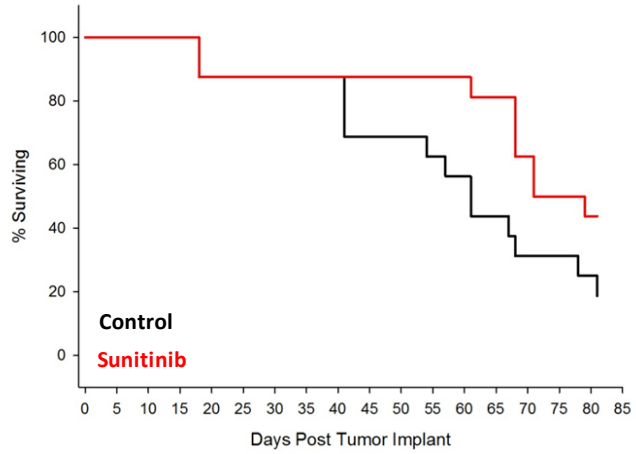


IP SK-OV-3-Luc



Renal OT: 786-O-Luc



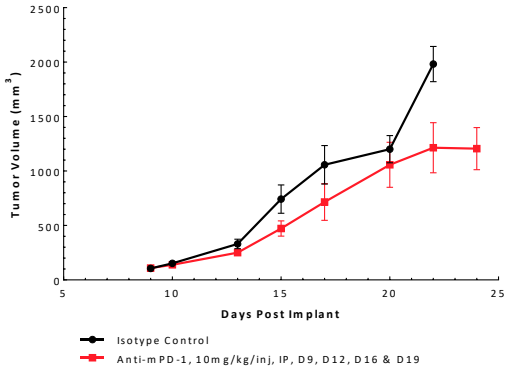


Labcorp Internal Data; all work performed in an AAALAC-accredited facility following methods & procedures as outlined in the Guide for the Care and Use of Laboratory Animals

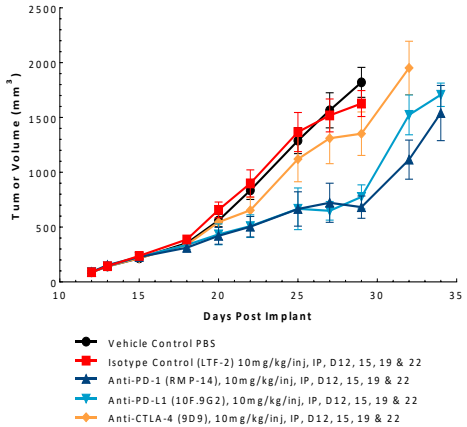
Case Study: Syngeneic Mouse Models

Commonly used murine tumor models – evaluating response to checkpoint inhibitors

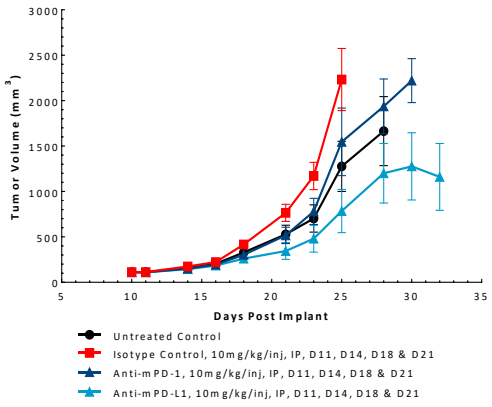
CT26



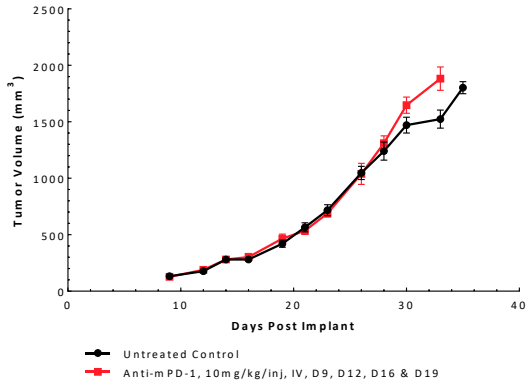
MC38



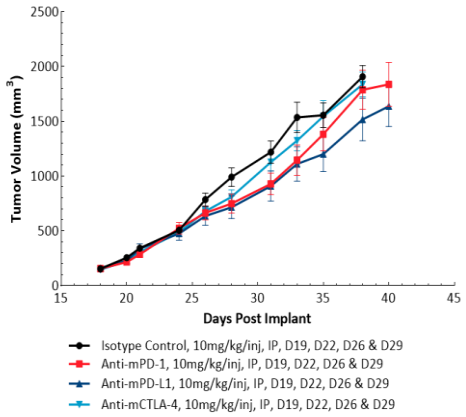
A20



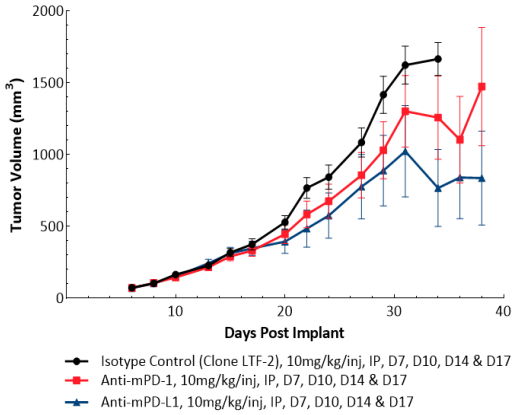
4T1-Luc



E0771

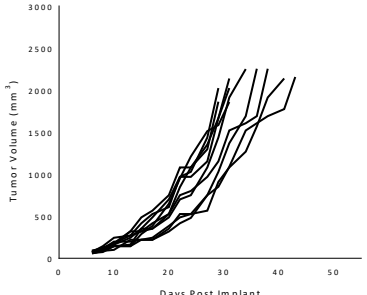


EMT-6

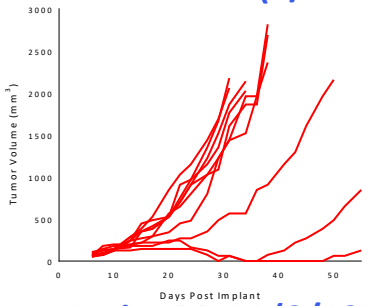


EMT-6 individual mice

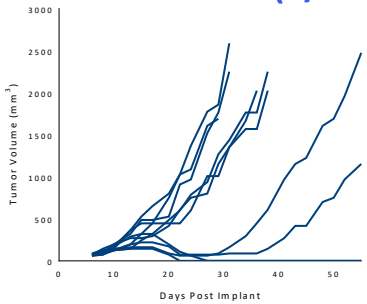
Isotype Control



Anti-mPD-1 (0/10 TFS)



Anti-mPD-L1 (2/10 TFS)



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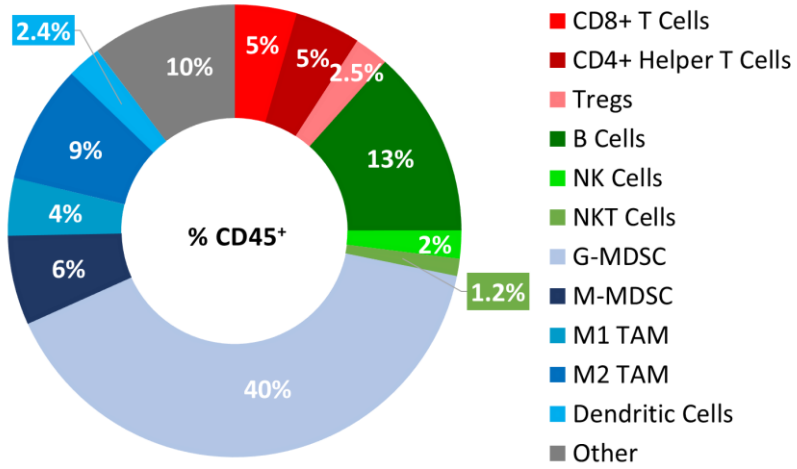
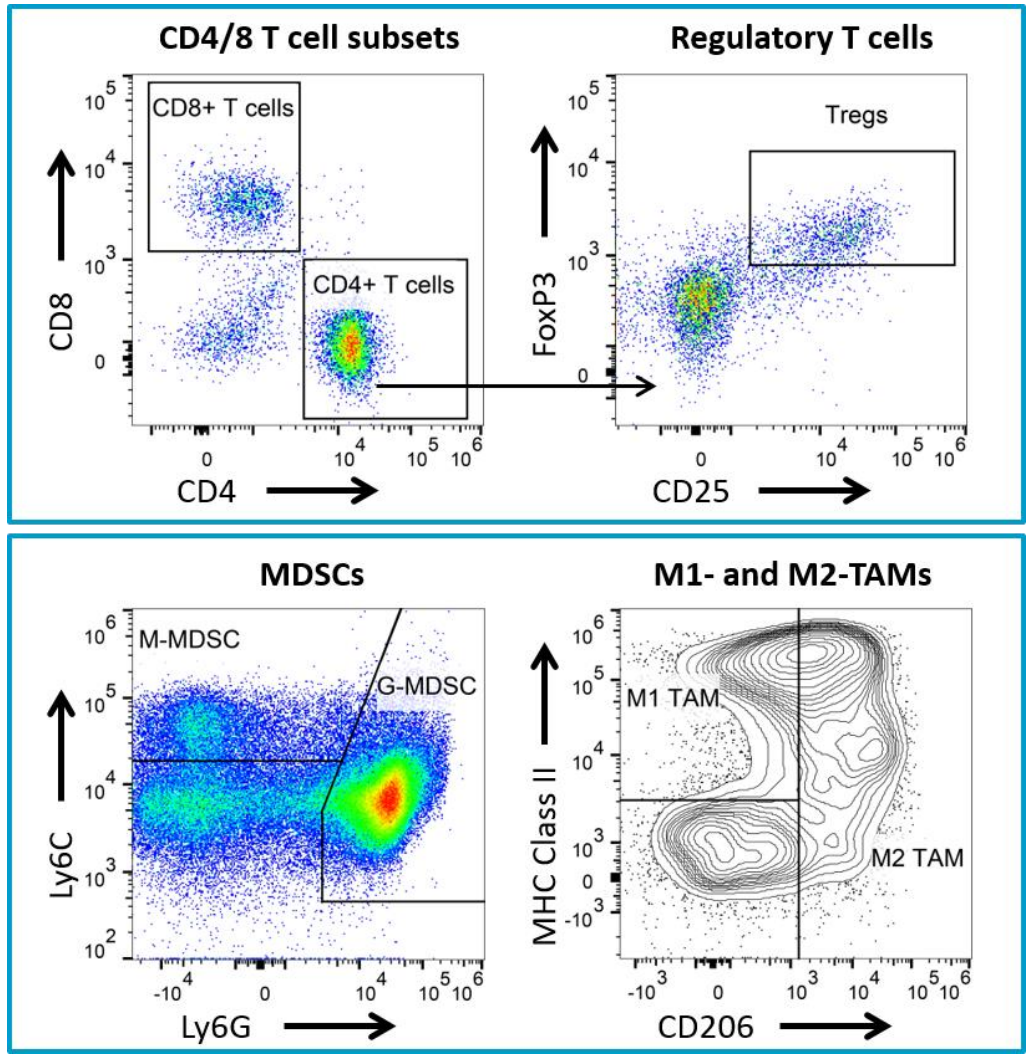
Case Study: 4T1-Luc Baseline TIL Immune Profile



- Immune cell populations are shown as % CD45+ cells
- Profiling shows data from n=6 untreated tumors ~500mm^3 in size
- The right panel shows representative images of flow cytometry gating strategy
- The lymphocyte population is mostly represented by B cells with minimal T cell infiltration into the tumor microenvironment while the myeloid population is predominantly G-MDSC cells



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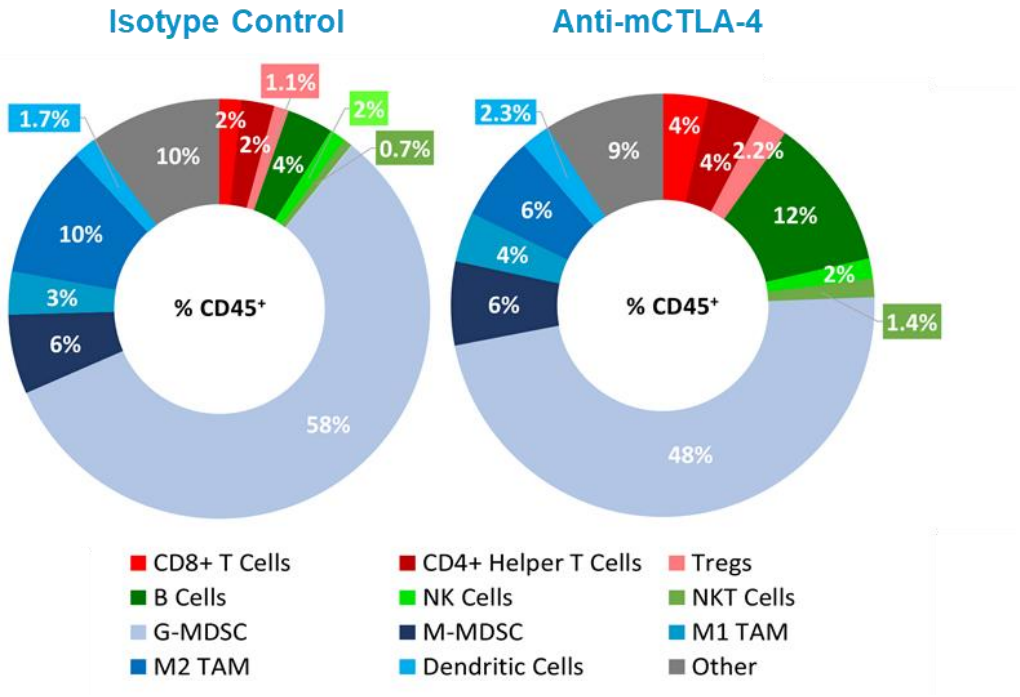
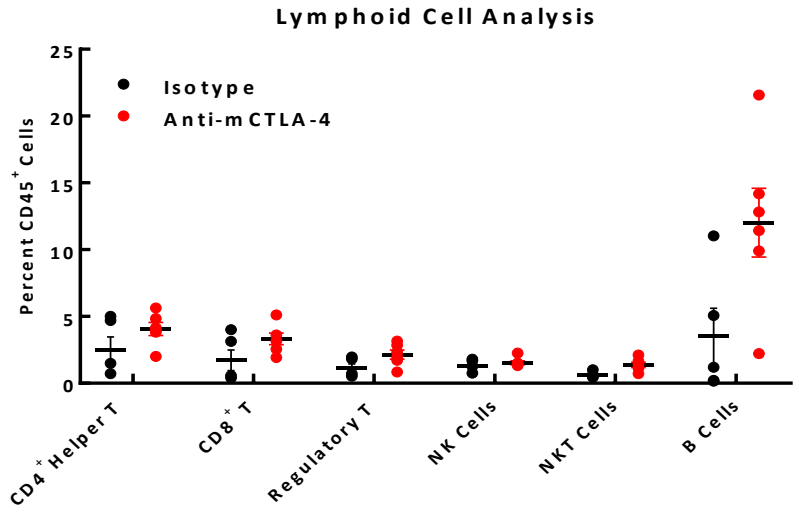
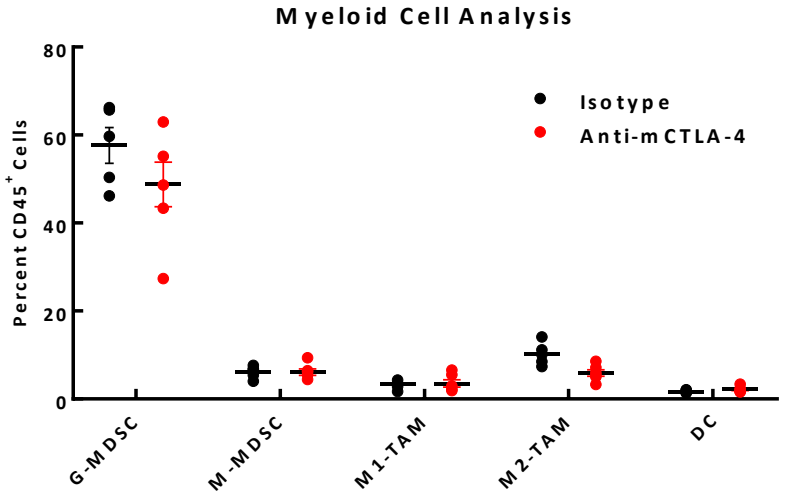
Case Study: 4T1-Luc TIL Profile Following Treatment







- TIL Profiling of n=5 tumors ~500mm^3 in size on d21 post-implant
- Anti-mCTLA-4 treatment shows trends toward increased T & B cells, decreased G-MDSCs & M2 TAMs compared to isotype control

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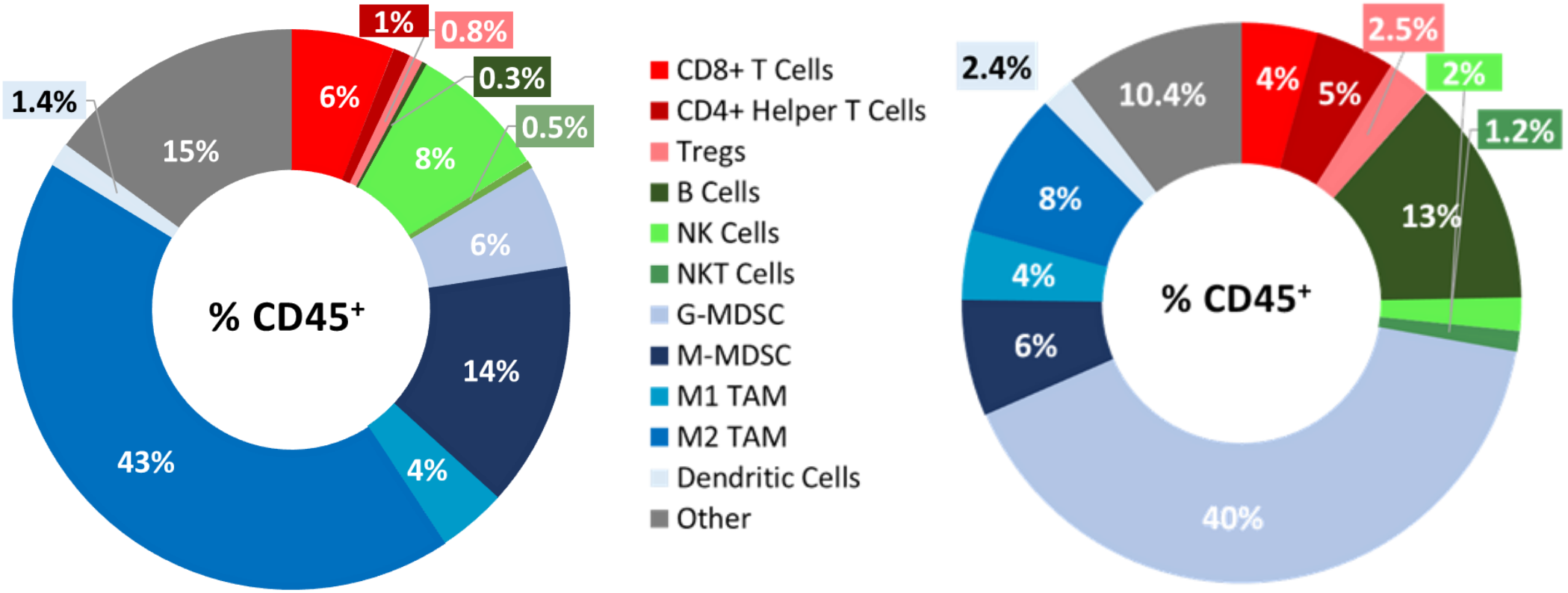
Case Study: Baseline TIL Profile Comparisons

CT26—Immunologically Warm



4T1-Luc—Immunologically Cold

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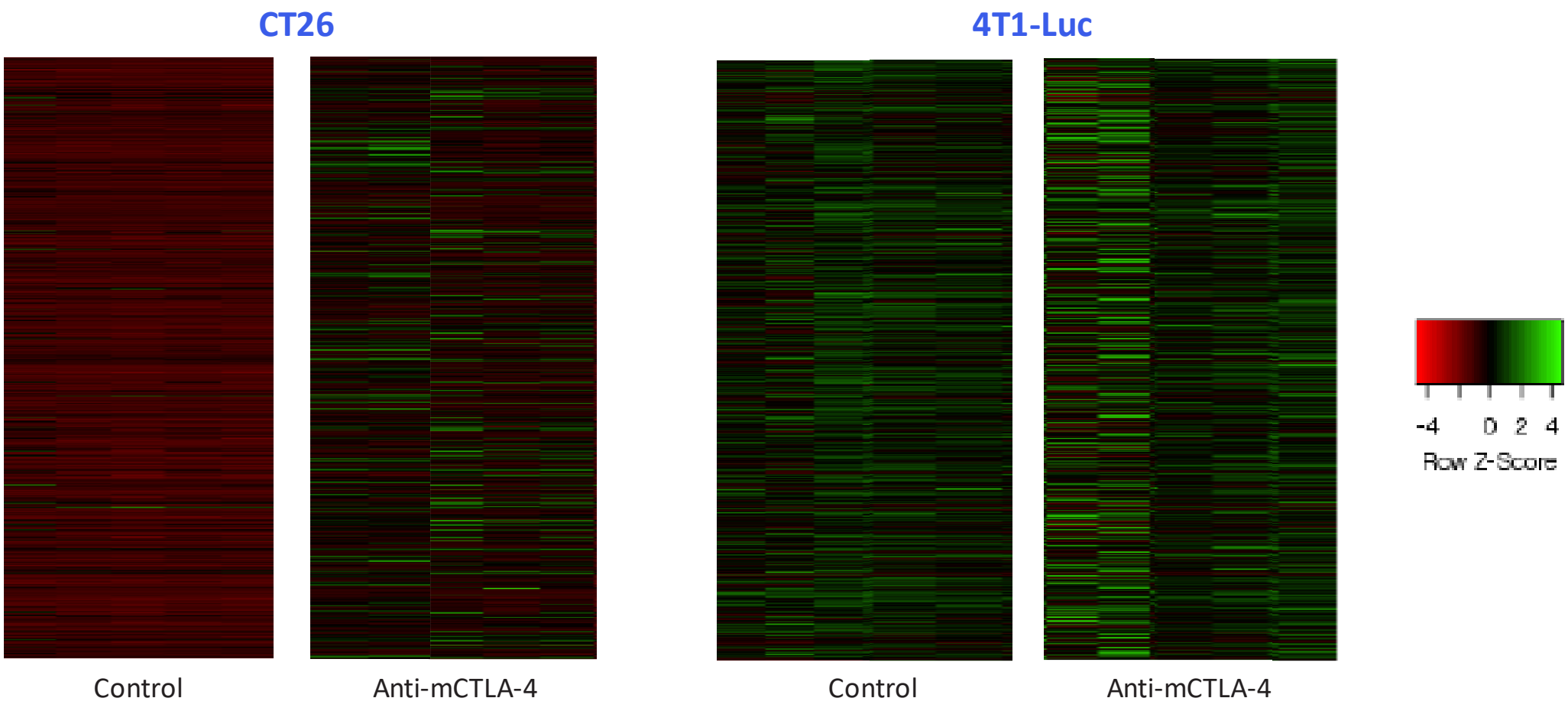
Case Study: Tumor Expression – Model Selection

NanoString Mouse PanCancer IO 360 Panel



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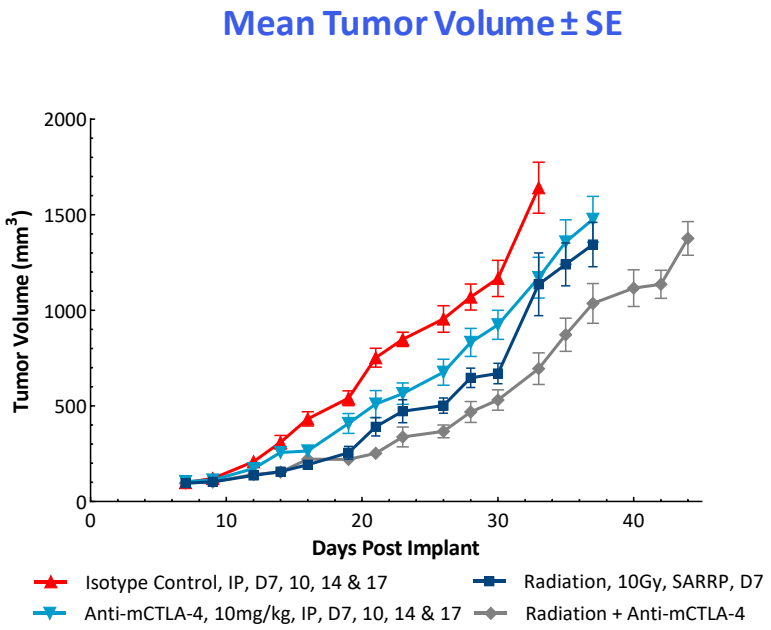
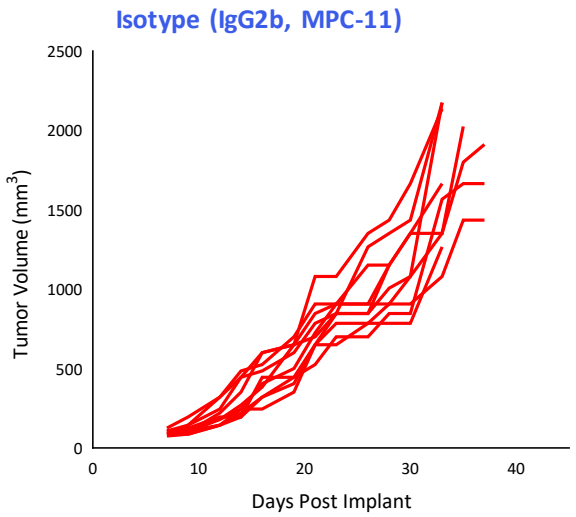
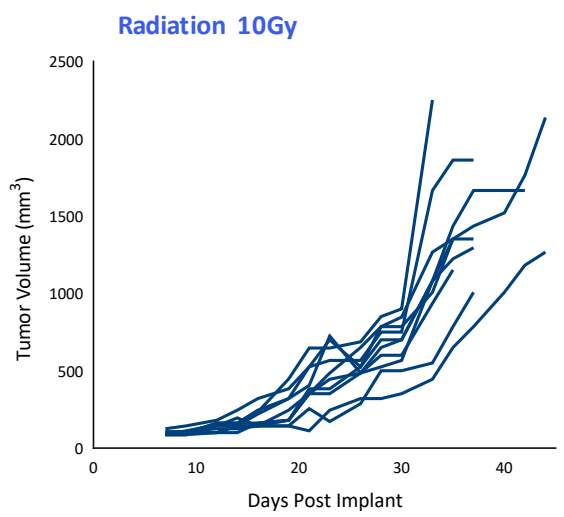
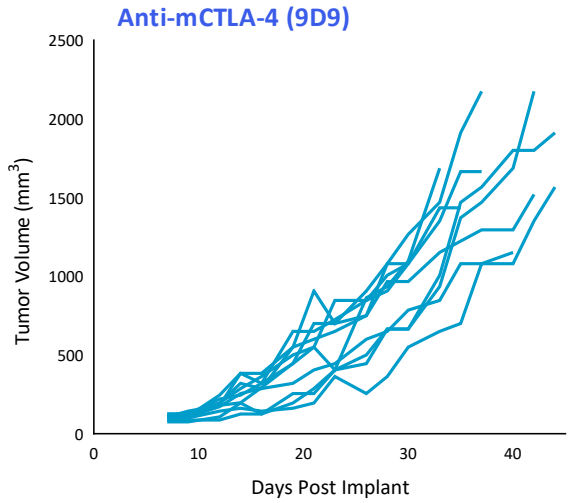
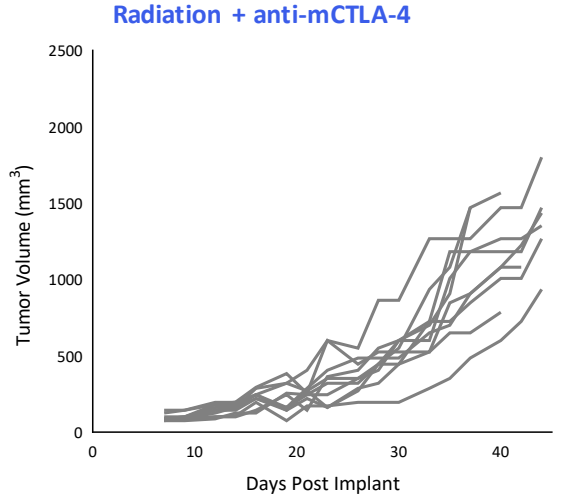
Case Study: Use of Syngeneic Model – 4T1-Luc Drug Combination











- Focal radiation (RT) was delivered by SARRP (Xstrahl)
- Single agent anti-mCTLA-4 or RT showed expected responses
- Combination treatment showed improved response with increased tumor growth delay
- Model spontaneously metastasizes to thoracic region
  - Evaluate through *in vivo* BLI imaging

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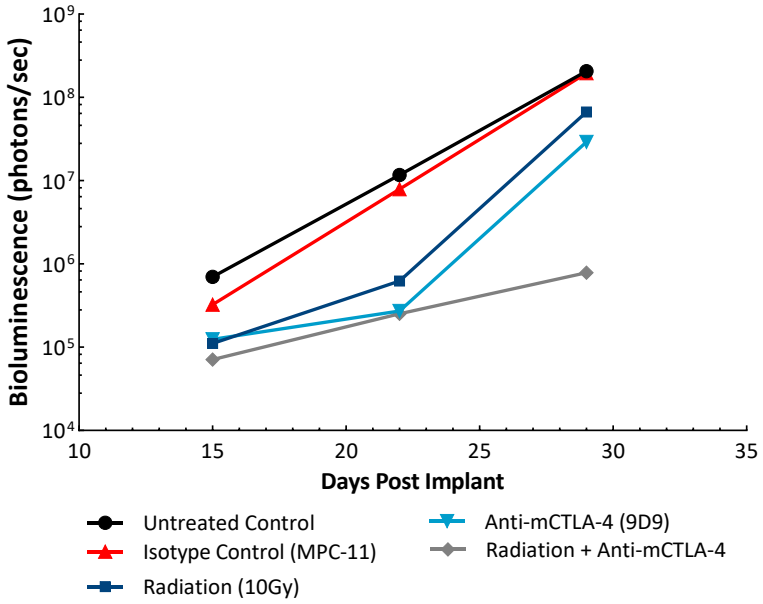
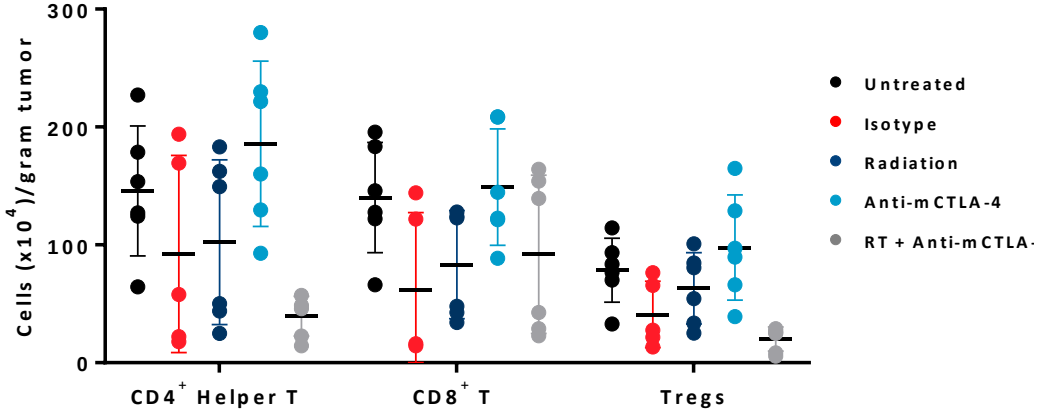
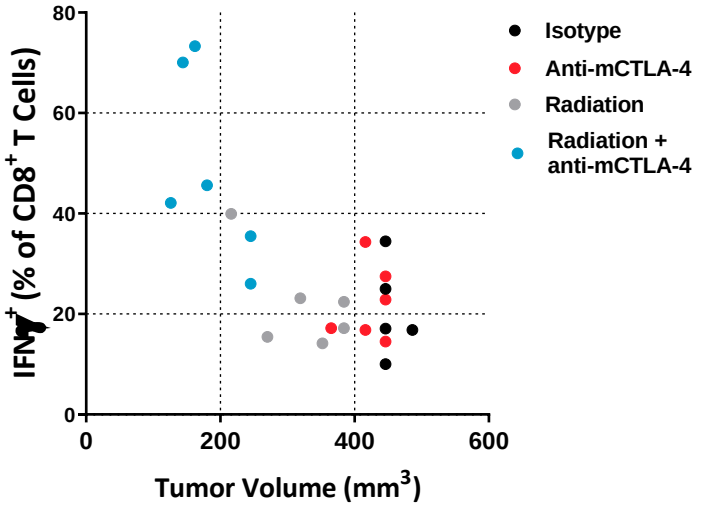
Case Study: Use of Syngeneic Model – 4T1-Luc Drug Combination

Thoracic Region Metastasis by *in vivo* BLI







- Reduced thoracic metastasis in combination group (through *in vivo* imaging)
- Can evaluate phenotypic changes by flow cytometry
- Can evaluate changes in activation state of CD8+ T cells by flow cytometry
- Can evaluate functional changes through intracellular cytokine signaling (flow)

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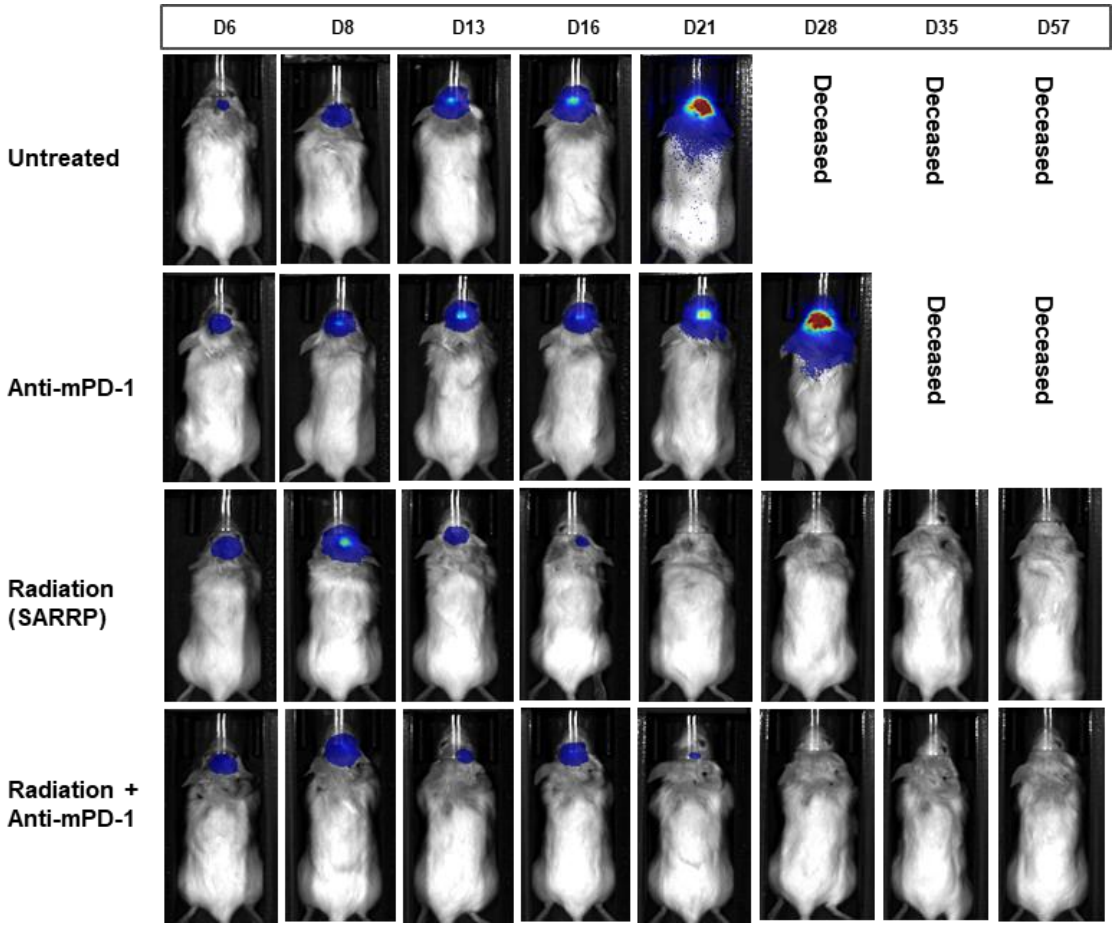
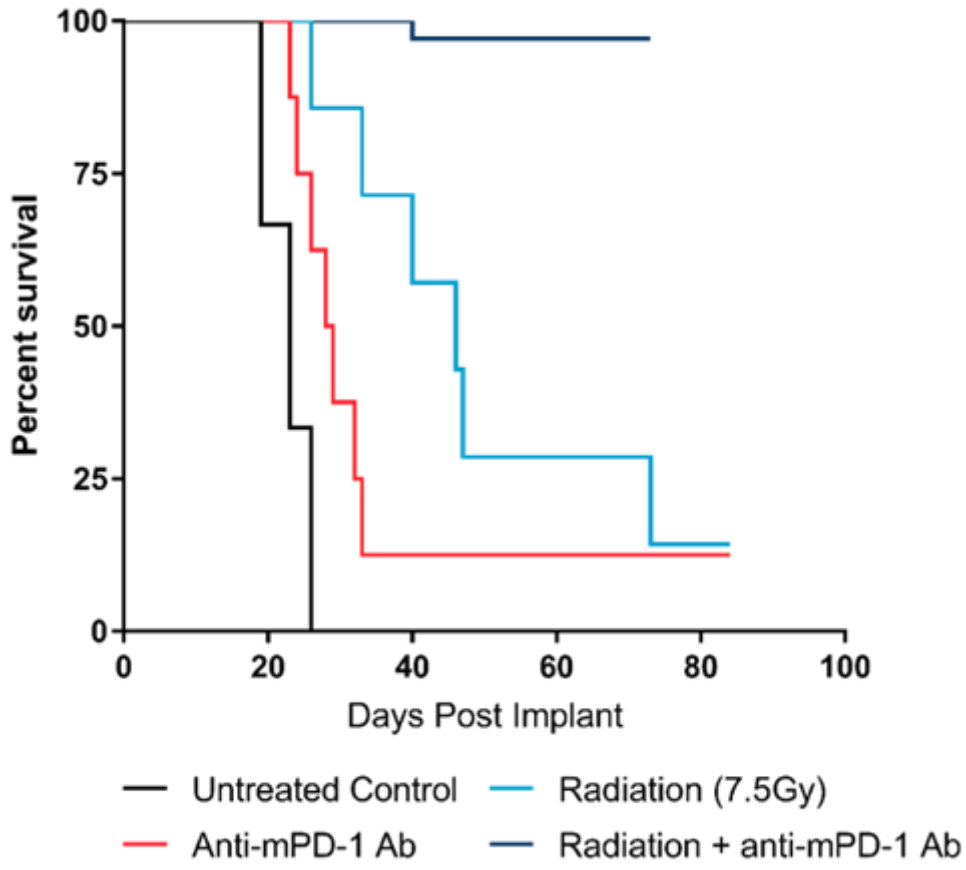
Case Study: Orthotopic Syngeneic Models

Murine GL261-Luc intracranial implant (albino C57BL/6)





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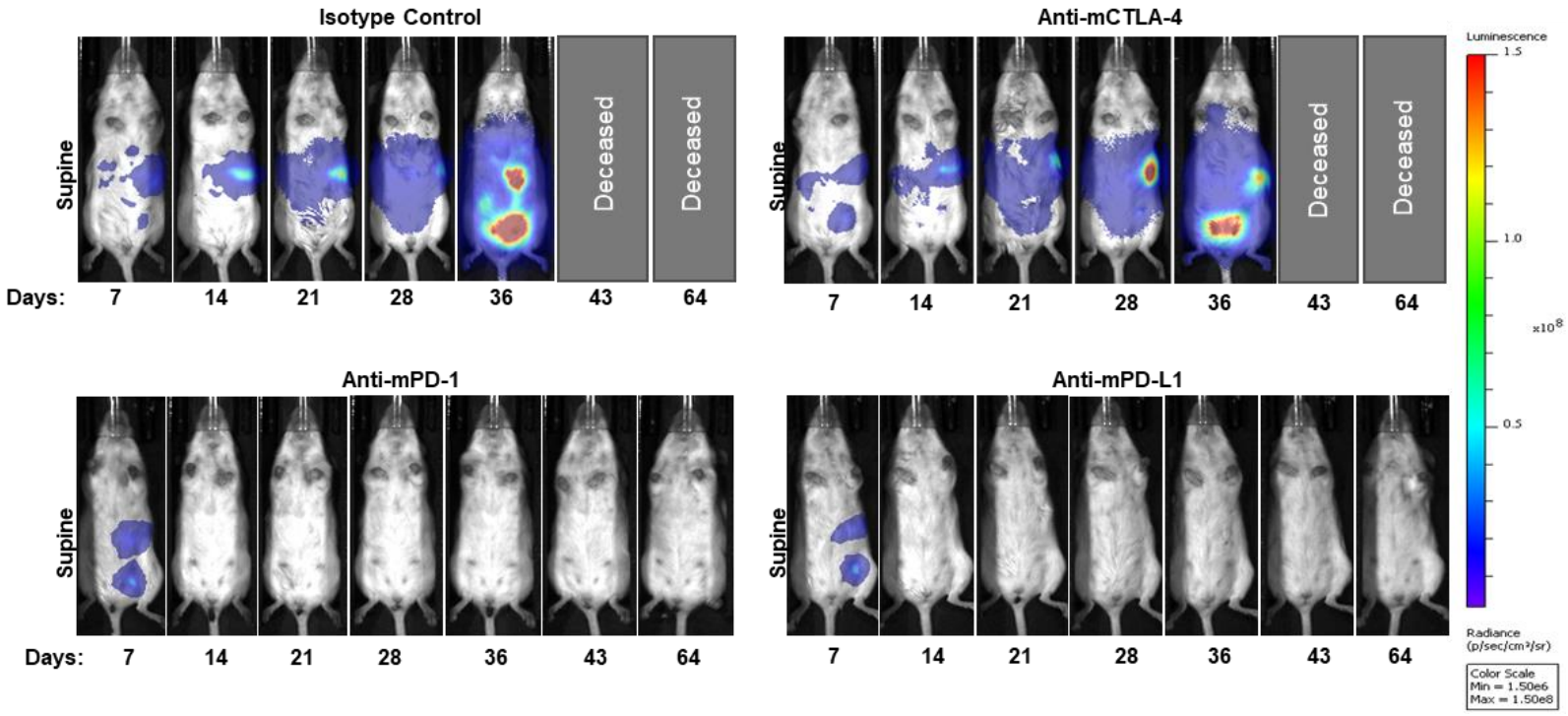
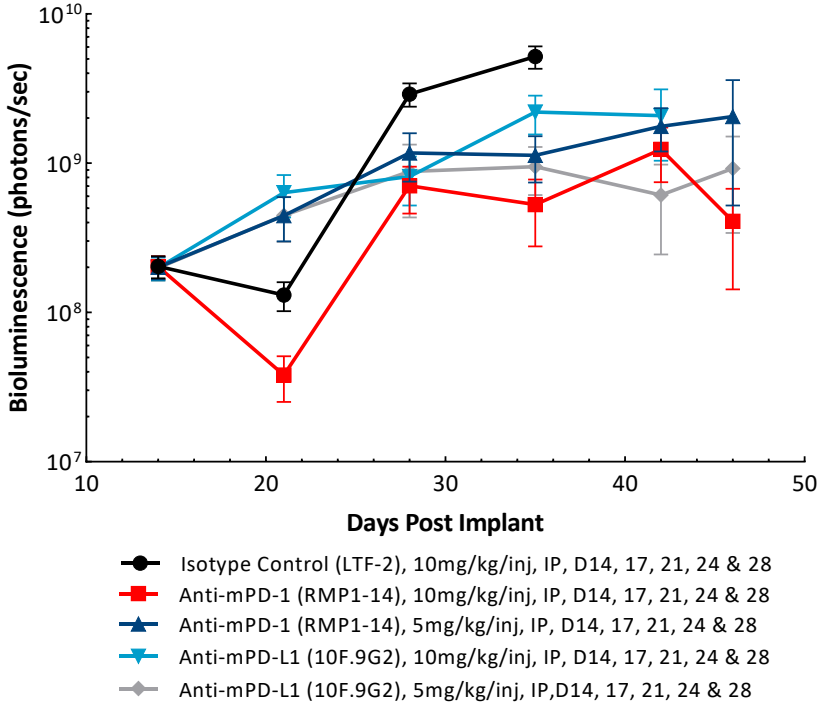
Case Study: Orthotopic Syngeneic Models

Murine ID8-Luc ovarian model (IP)





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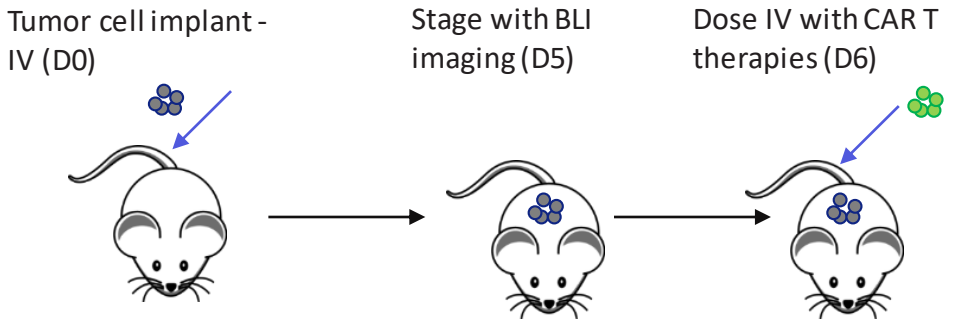
Case Study: Models for Adoptive Cell Therapy

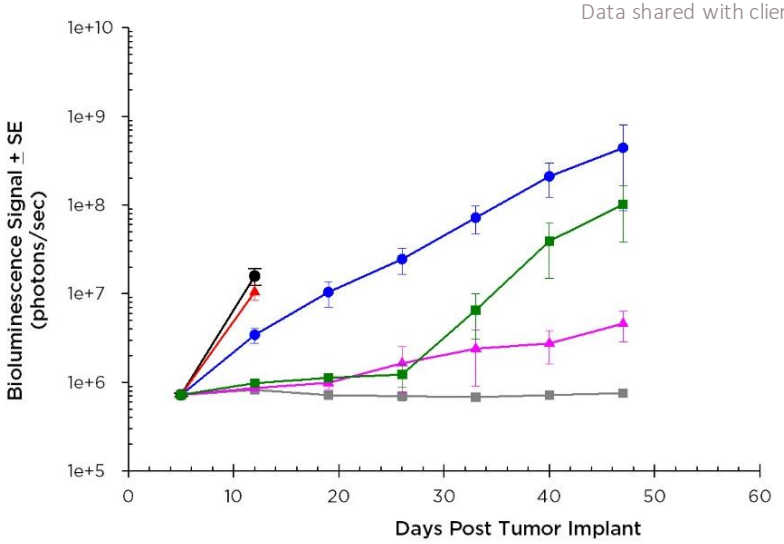
Human tumor cell line (Raji-Luc) implanted into NSG mice

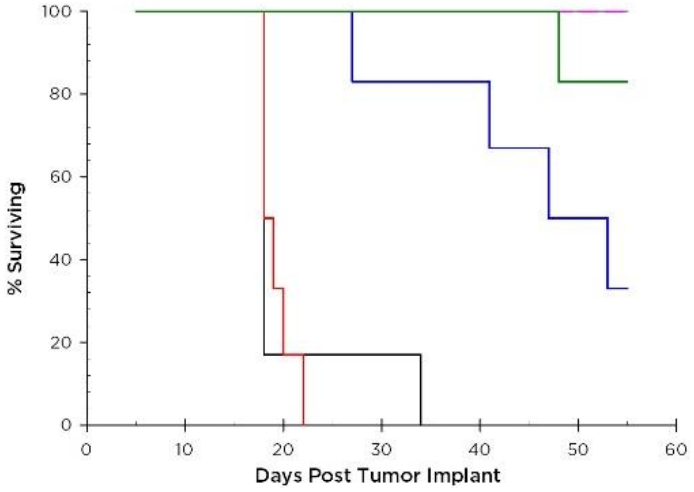


- Understand growth of human xenograft in NSG mice
- Experimental design should include vehicle treated group
- Experimental design should include non-transduced T cell group
- Monitor disseminated disease progression through BLI imaging
- Monitor overall survival (morbidity/mortality)
- T cell persistence can be tracked via flow cytometry (not shown)
- Studies can also be done in the humanized mouse setting

Efficacy of CAR T therapies in disseminated Raji-Luc model

Data shared with client permission





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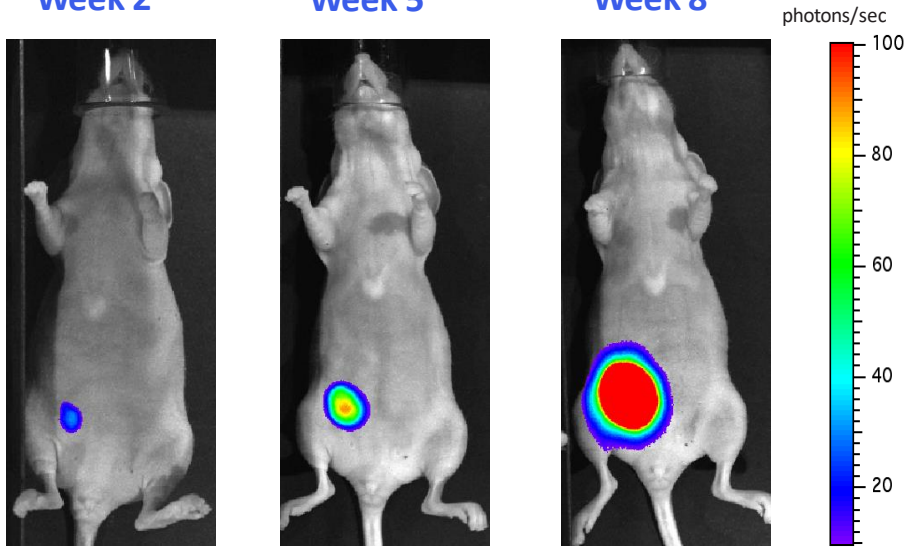
Case Study: Human Xenograft Metastatic Models

Human Breast Models – MDA-MB-231-D3H1-Luc & MDA-MB-231-D3H2LN-Luc

Primary Tumor

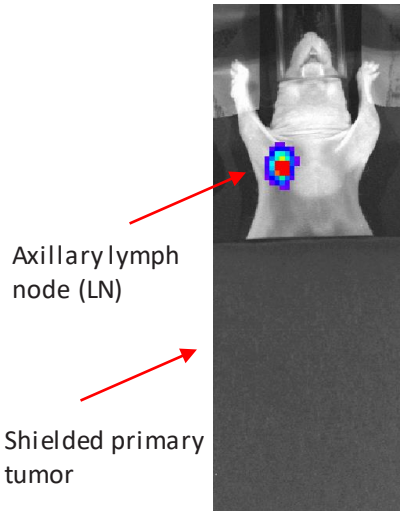
Week 2 | Week 5 | Week 8



MDA-MB-231-D3H1-Luc

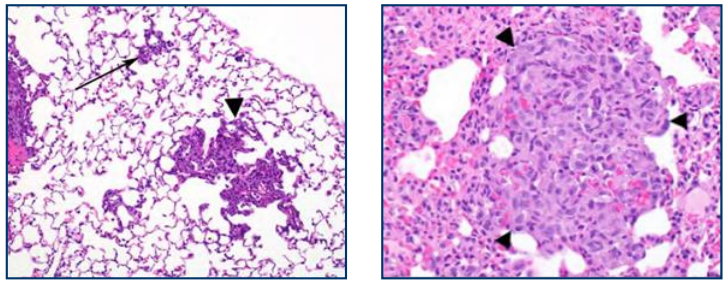
Shielded Image (LN)

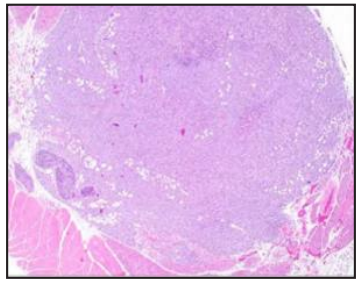
Week 7



MDA-MB-231-D3H2LN-Luc

Lung lesion





LN lesion

- Some modes spontaneously metastasize; GEMM models more readily than CDX
- Primary tumor size generally rate limiting step in life-span of animal
- Number of CDX models is relatively low

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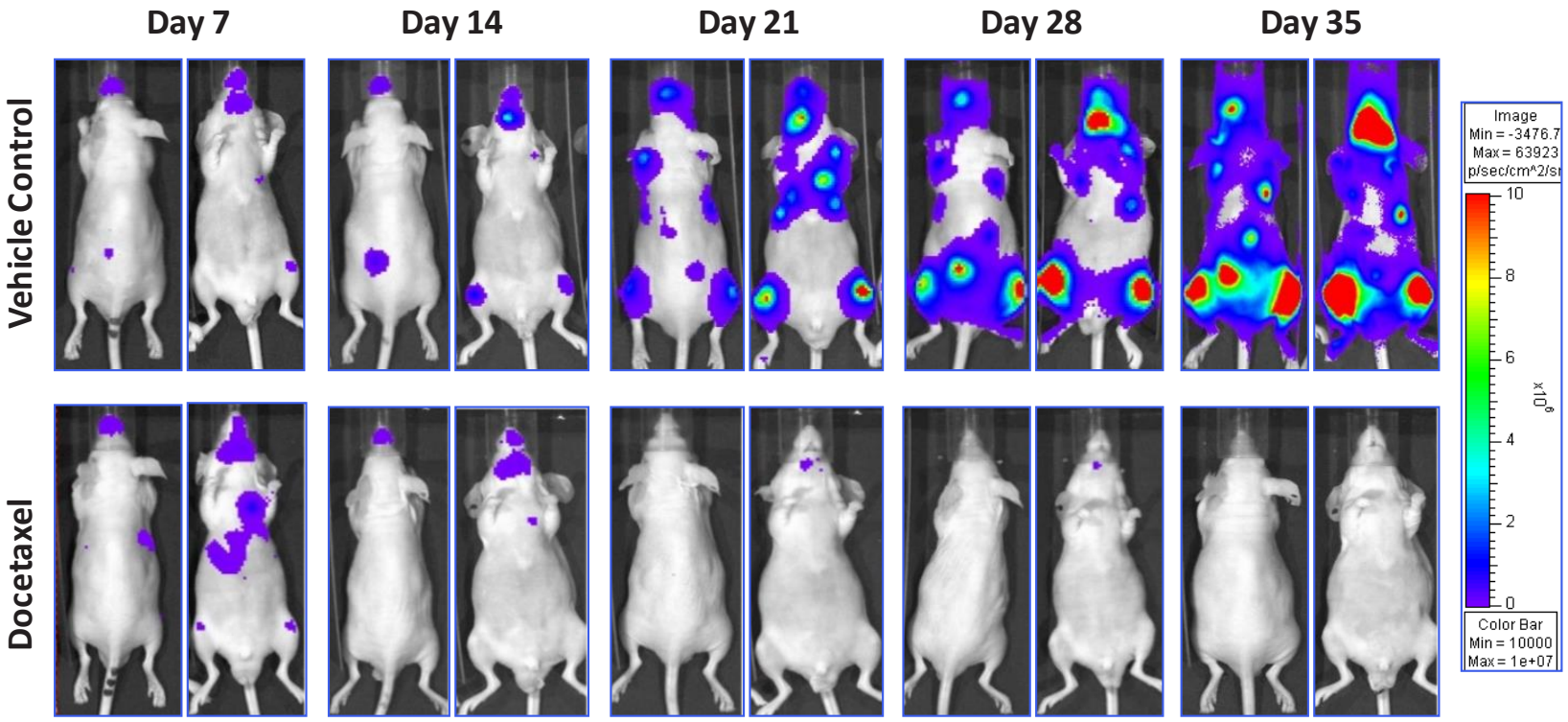
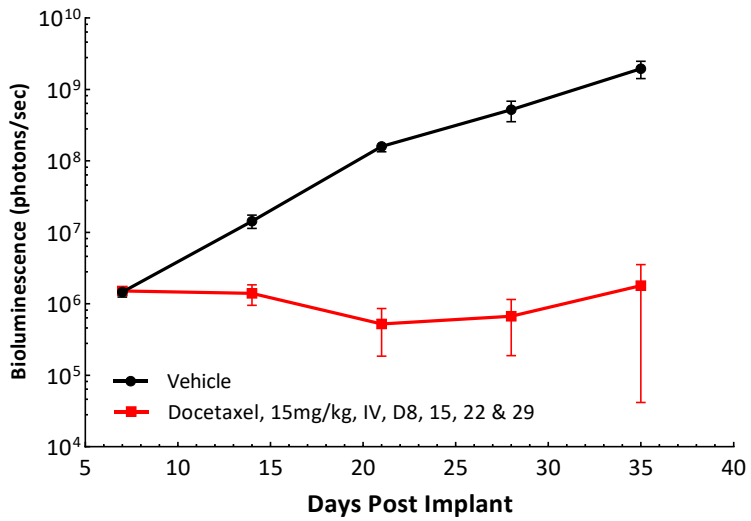
Case Study: Human Xenograft Metastatic Models

Human Prostate Model – PC-3M-Luc, intracardiac injection – bone metastasis model



Mean Tumor Burden ± SE



- Injection models of metastasis exist
- IV injection for lung mets
- Intracardiac for bone mets
- Intrasplenic for liver mets
- Intracranial for brain mets
- Relatively easy to perform
- Quantitative readouts
- No primary tumor
- No metastatic progression

labcorp Drug Development

1. All animal work was approved by the site Institutional Animal Care and Use Committee and was performed in conformance with the Guide for the Care and Use of Laboratory Animals within an AAALAC-accredited program with humane euthanasia criteria predetermined on all studies.

Summary

- The oncology landscape is populated by a large number of approaches in drug development with growing numbers of clinical trials.
- The clinical validation of immunotherapies has spurred additional research into mouse models with competent immune systems.
- Each model type has advantages and disadvantages that should be thoroughly considered in the context of the questions being investigated.
- The use of patient derived xenografts, with or without a humanized mouse model background, is important for addressing questions related to precision medicine. However, human xenograft (CDX) models are still the most utilized.
- CDX and PDX models are being used to test cell-based therapies. These types of studies are rapidly moving into the humanized mouse model setting.
- Syngeneic mouse models are considered the standard for immuno-oncology approaches. These studies are run with supportive *ex vivo* analysis to provide phenotypic and functional endpoints.
- Orthotopic mouse models can play important roles in understanding the interplay between tumors and the tumor microenvironment and the advent of *in vivo* imaging makes these types of studies easier, quantitative and more accessible.
- Metastatic disease remains a clinical challenge with limited mouse models. However, many questions can be answered with the models at hand but care should be taken in understanding the model limitations.
- A large number of preclinical oncology models exist but there is not a “one-size-fits-all” approach.

Thank You!