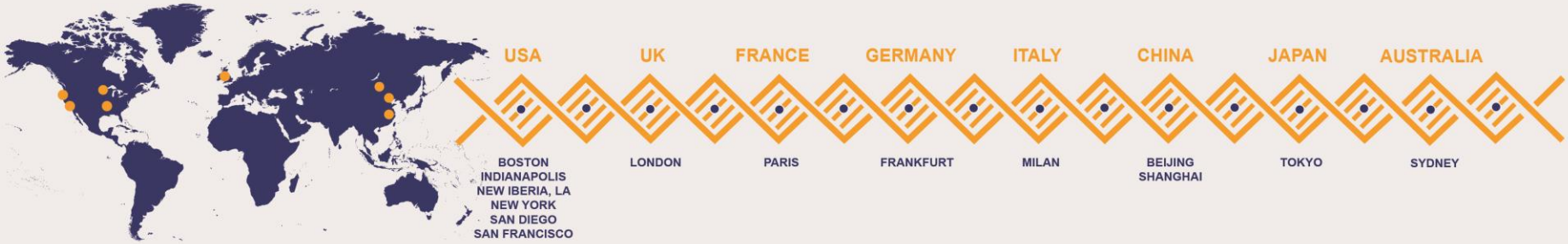
CrownBio
CONNECTING SCIENCE TO PATIENTS

Corporate Headquarters:
11011 Torreyana Road, Suite 200
San Diego, CA 92121
Tel: 855.827.6968
Fax: 888.882.4881
www.crownbio.com

Drug Discovery in the Immuno-Oncology Era: Applications for Humanized Mouse Models

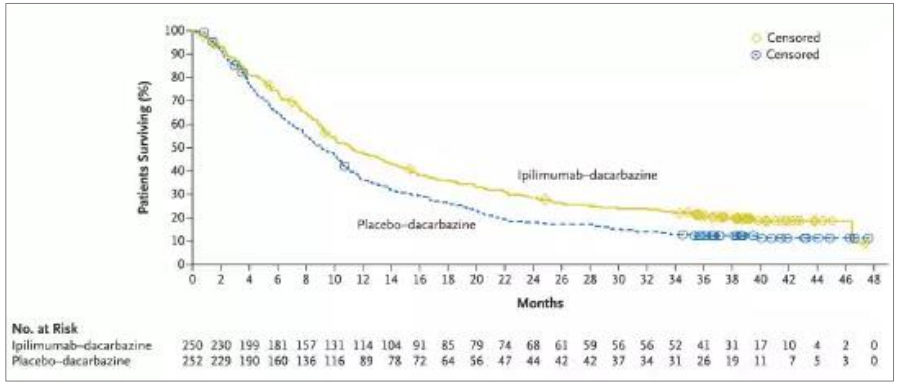
H. Toni Jun, PhD
Director of Scientific Engagement



Immunotherapy: Game Changer for Metastatic Melanoma

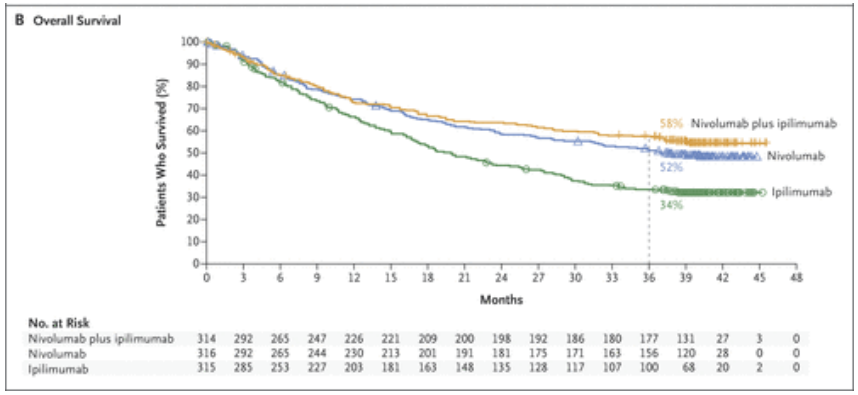
- 3 year overall survival (OS) before immune checkpoint inhibitors (ICI) ~ 15%
- 3 year OS after ICIs = 58% (anti-CTLA-4/anti-PD-1 combination)

Anti-CTLA-4 + Dacarbazine



Robert *et al*. *N Engl J Med* 2011;364:2517-26.

Anti-CTLA-4 + Anti-PD-1



Wolchok *et al. N Engl J Med* 2017;377:1345-56.

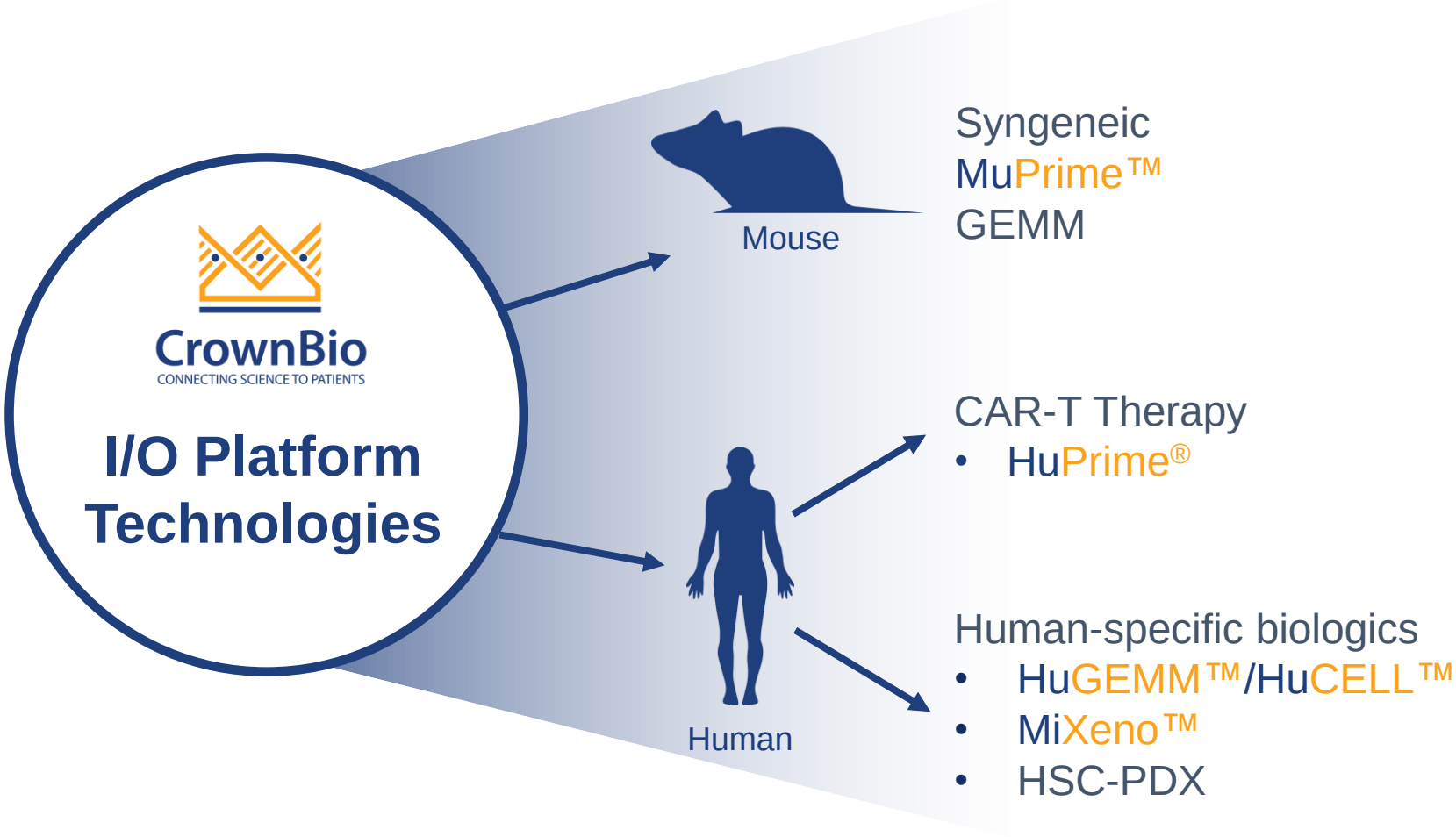
Types of Immunotherapy

- Immuno-oncology (I/O) agents activate a patient's immune system to fight the disease
  - **ICIs** – target immune regulators
  - **Adoptive T cell therapies** (CAR-T) – modify patient T cells to boost anticancer patient immune response
  - **Oncolytic virotherapy** selectively kills tumor cells while stimulating the patient's anti-tumor immune response
  - **Cancer vaccines** – use cancer neoantigen(s) to activate or boost the patient's immune system against the cancer

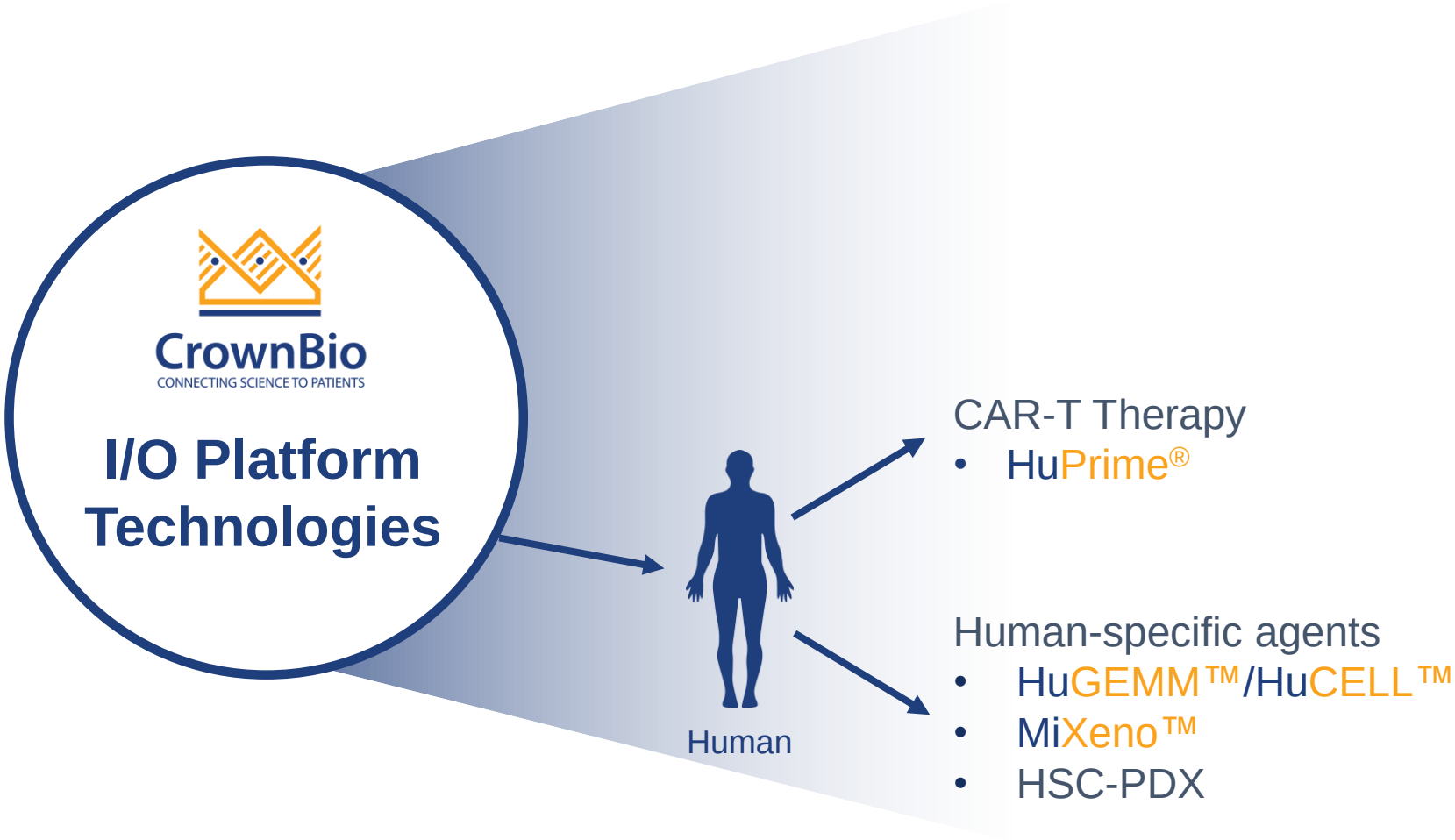
Challenges

- Limited availability of *in vitro* validation assays
  - Hampered by donor-dependent variability
  - Difficult to model interactions between multiple cell types
- *In vivo* testing requires models with a functional immune system
  - Traditional xenograft models use immunocompromised mice
  - Mouse and human immune systems are not completely analogous
- Immune response can be variable even under identical conditions

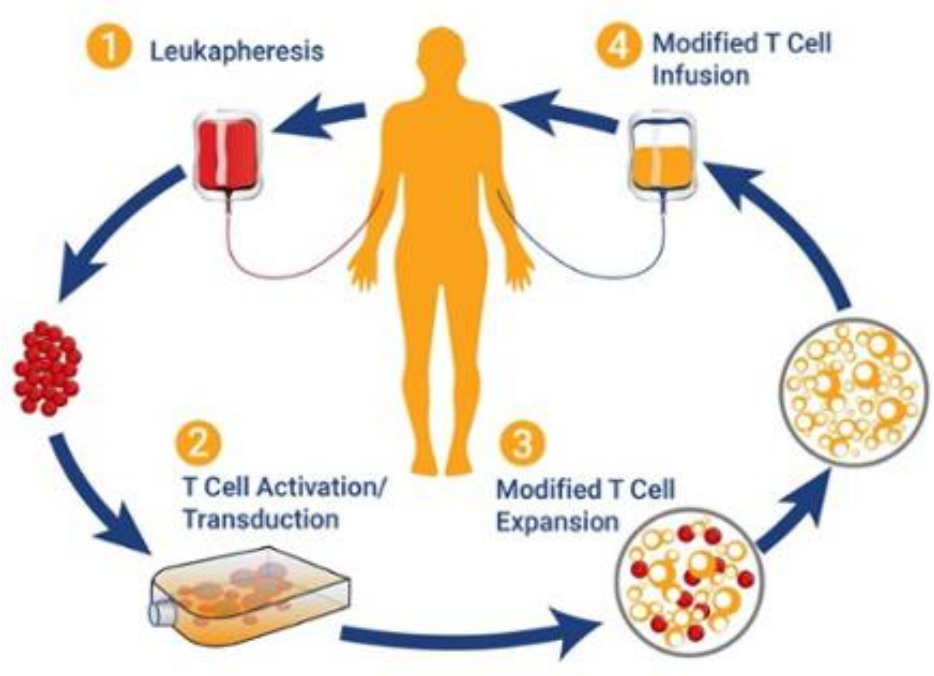
CrownBio's Integrated I/O Platform



CrownBio's Integrated I/O Platform



Introduction to CAR-T Therapy



- Highly personalized approach
- Neoantigen expression is key to a successful CAR-T therapy
- Two CAR-Ts currently approved with many more on the way

CrownBio CAR-T Therapy Platform

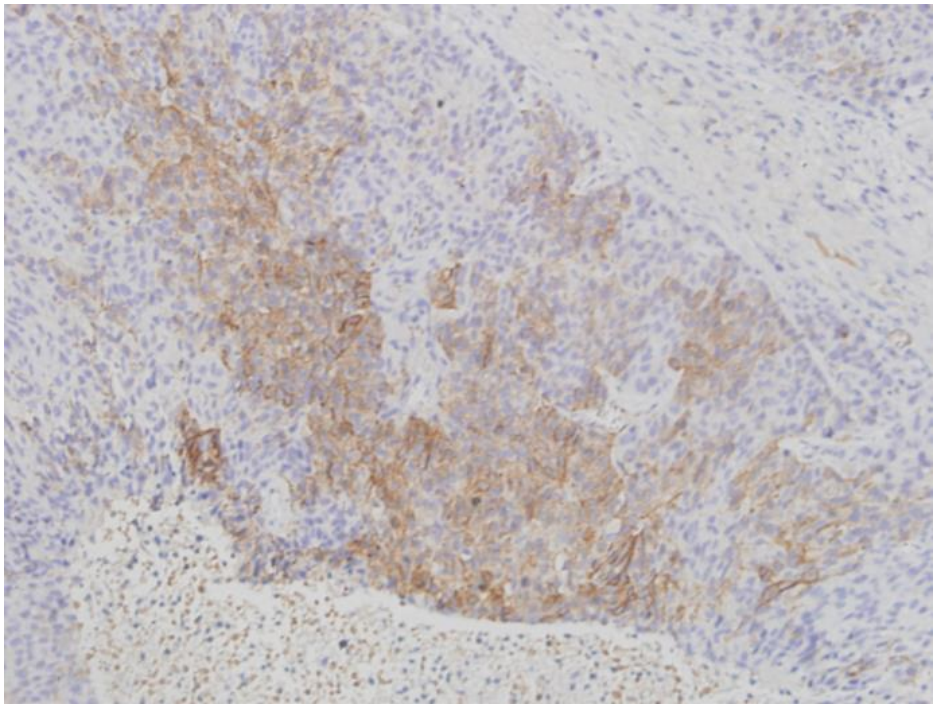
- HuPrime: well characterized collection of 2,500 patient-derived xenografts (PDXs)
  - Highly predictive models, preserving original patient tumor pathological features
  - Reflective of the genetic diversity from the patient population
- HuBase™: online searchable database to access
  - PDX phenotypic and genotypic data
  - Patient clinical information
  - Growth curves
  - Standard of care treatment data
- TMAs for additional biomarker analysis by IHC

GPC3-Targeted CAR-T Development

- PDX model selection via HuBase

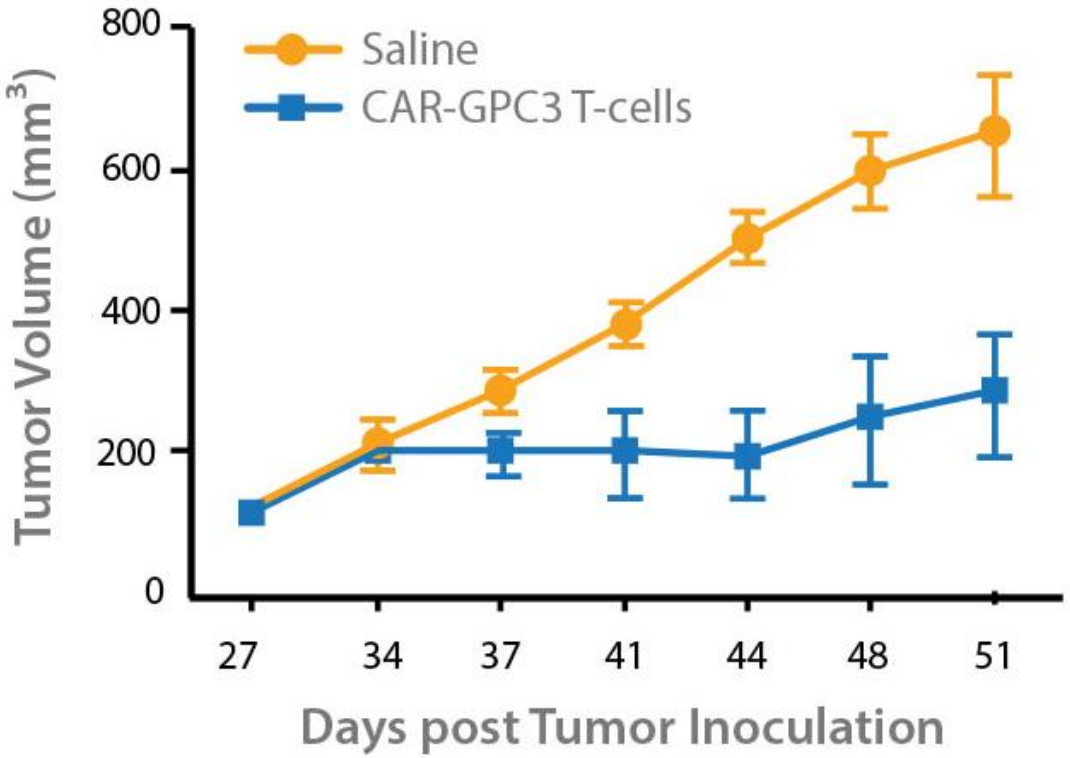
GPC3-Targeted CAR-T Development

- Validation of GPC3 expression in the NSCLC LU1542 PDX model via IHC



GPC3-Targeted CAR-T Development

- Evaluation of model LU1542 response to CAR-GPC3 T cell therapy

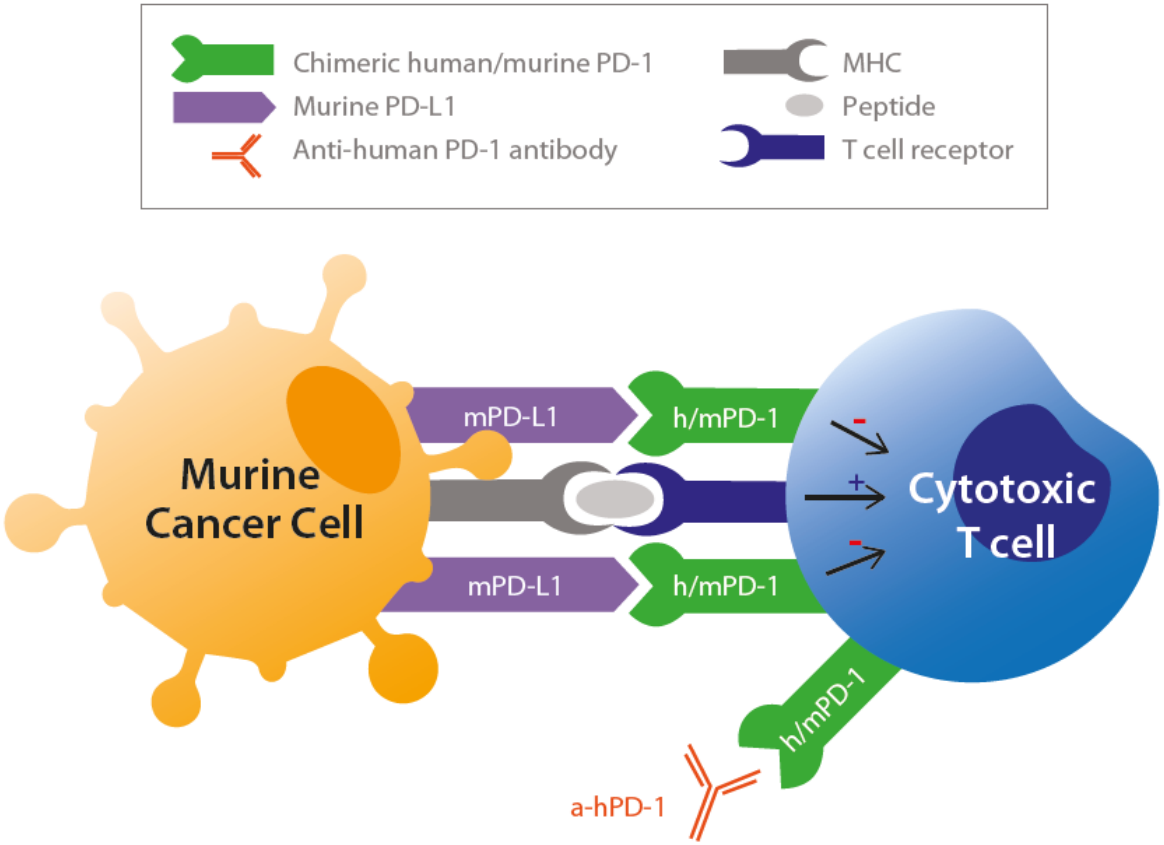


CrownBio Chimeric Models

- Syngeneic mouse tumor models widely used for testing I/O agents
  - Not suitable for testing human-specific therapeutics
- CrownBio has developed HuGEMM and HuCELL models to evaluate human specific agents
  - Chimeric knock-in mice that render syngeneic models effective in evaluating targeted human immunotherapies *in vivo*

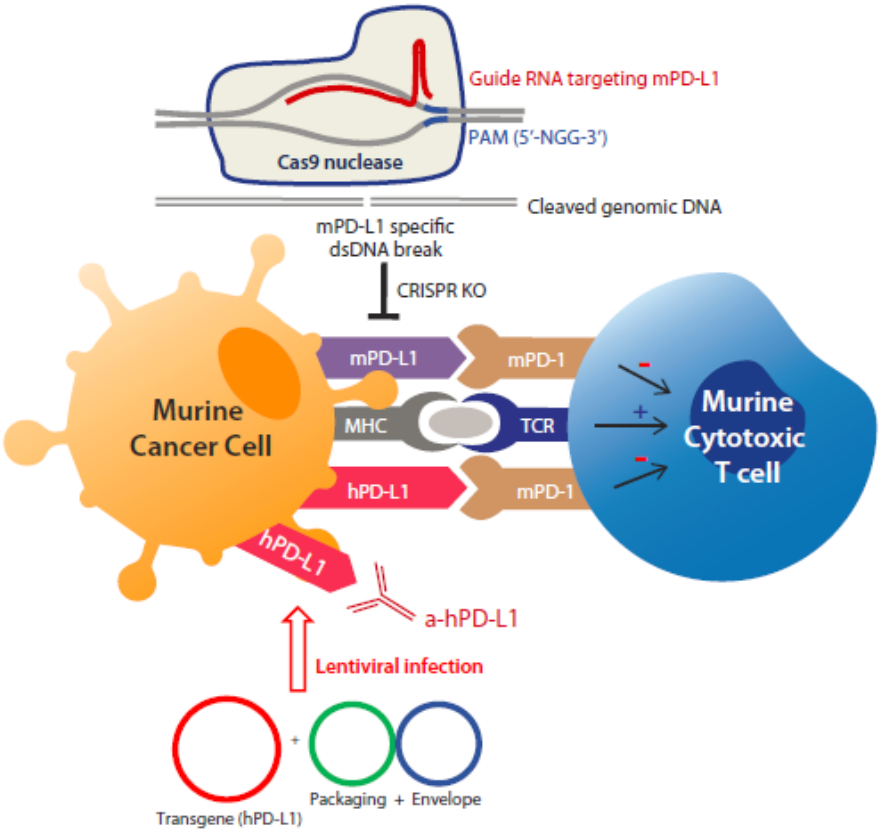
HuGEMM Concept

- A chimeric mouse tumor model with fully functional murine immune system but a humanized drug target



HuCELL Concept

- Mouse tumor cells that have been engineered to express humanized ligands

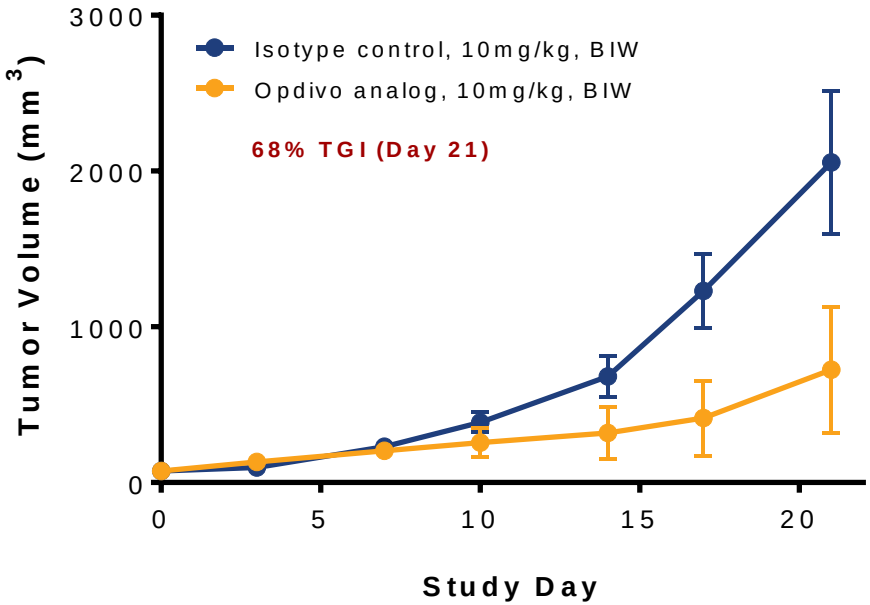


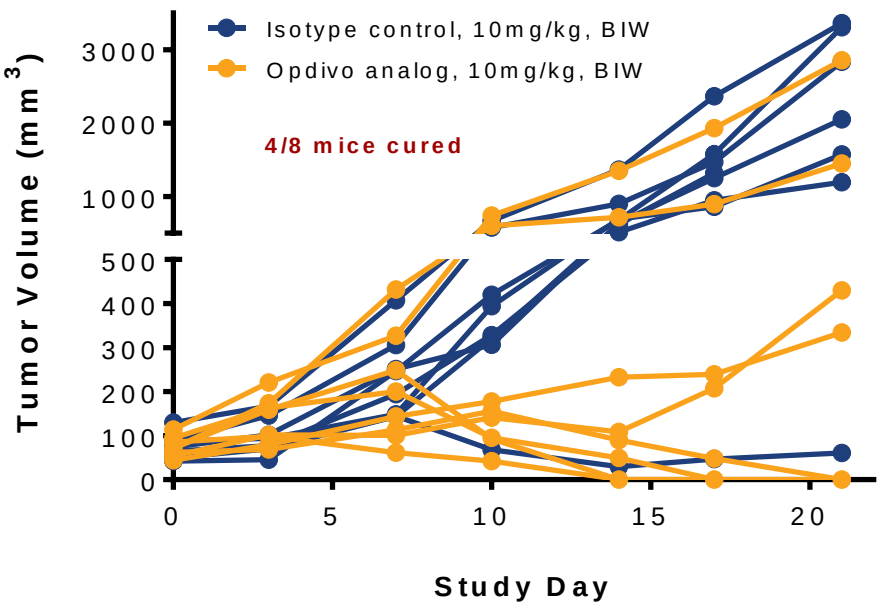
Validated PD-1 HuGEMM Models

- Validated models:
  - huPD-1 HuGEMM host with MC38 murine colon adenocarcinoma model expressing mouse PD-L1
  - huPD-1 HuGEMM host with HuCELL MC38 model expressing human PD-L1 (exon 3 human PD-L1 knock-in)
- Model response to anti-hPD-1 mAb treatment (Keytruda® and Opdivo®) evaluated

PD-1 HuGEMM Efficacy Study: Opdivo Treatment

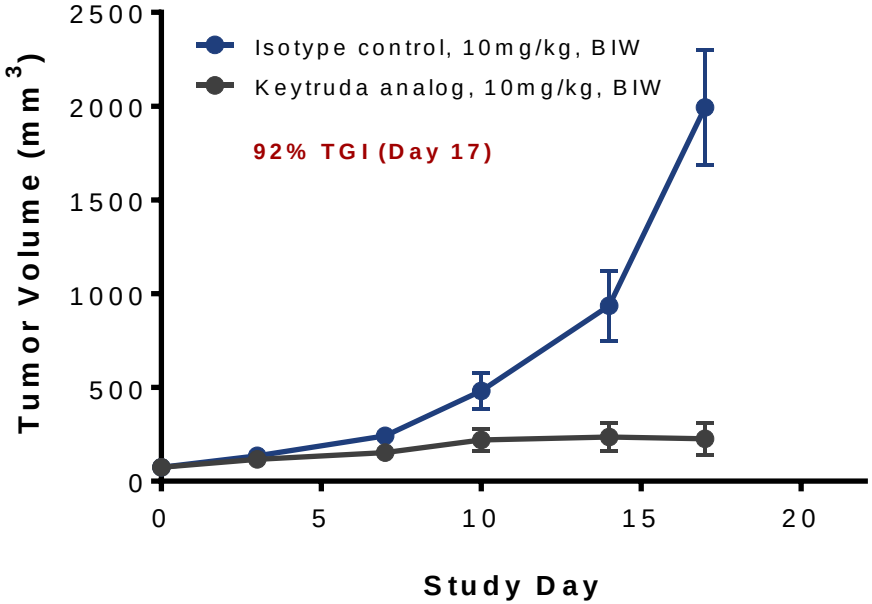
- Anti-PD-1 (Opdivo) treatment effectively reduced tumor burden with 4/8 mice cured

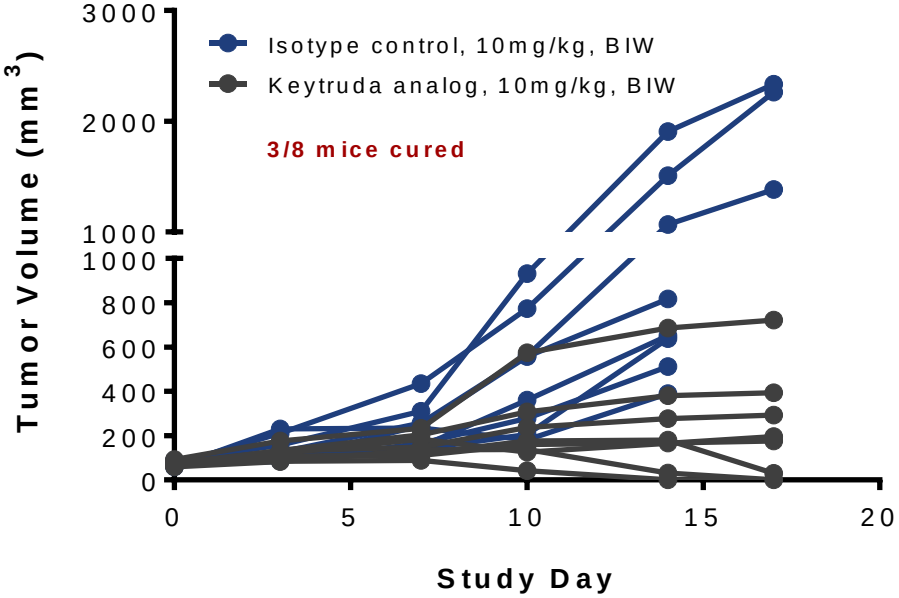




PD-1 HuGEMM Efficacy Study: Keytruda Treatment

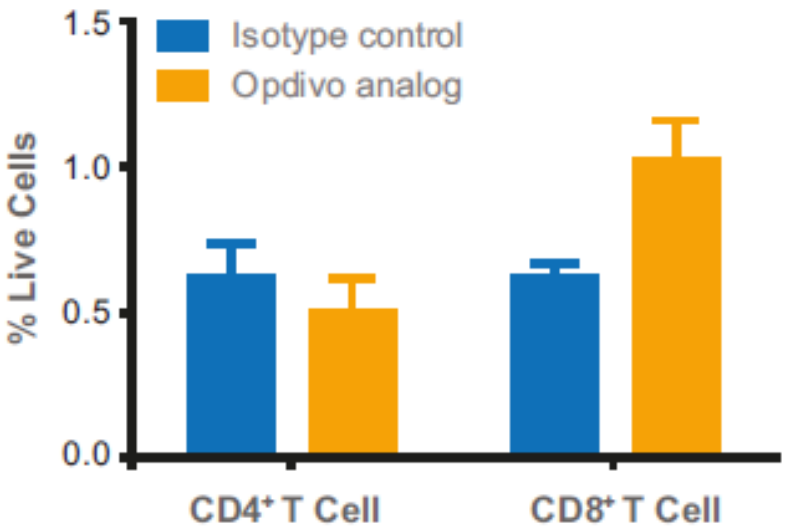
- Anti-PD-1 (Keytruda) treatment effectively reduced tumor burden with 3/8 mice cured



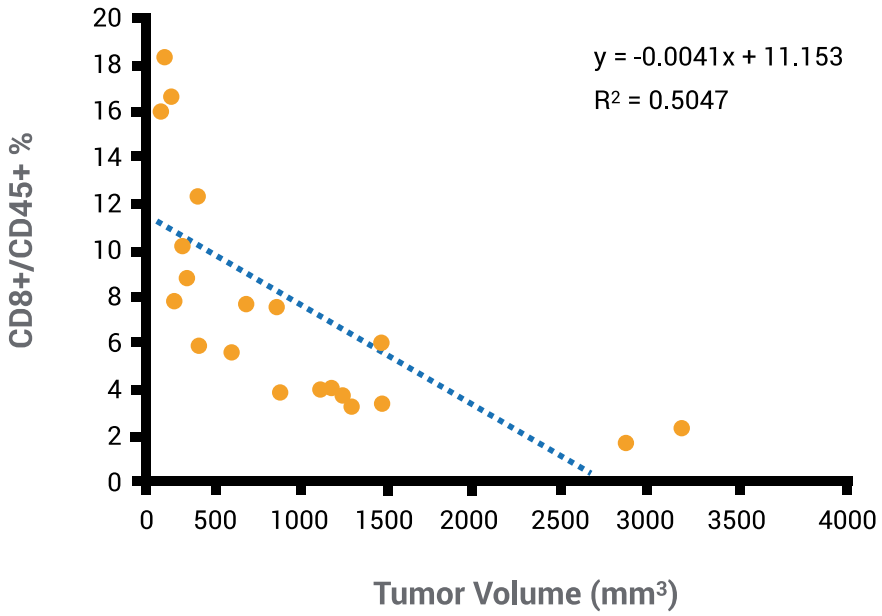


PD-1 HuGEMM TIL Analysis

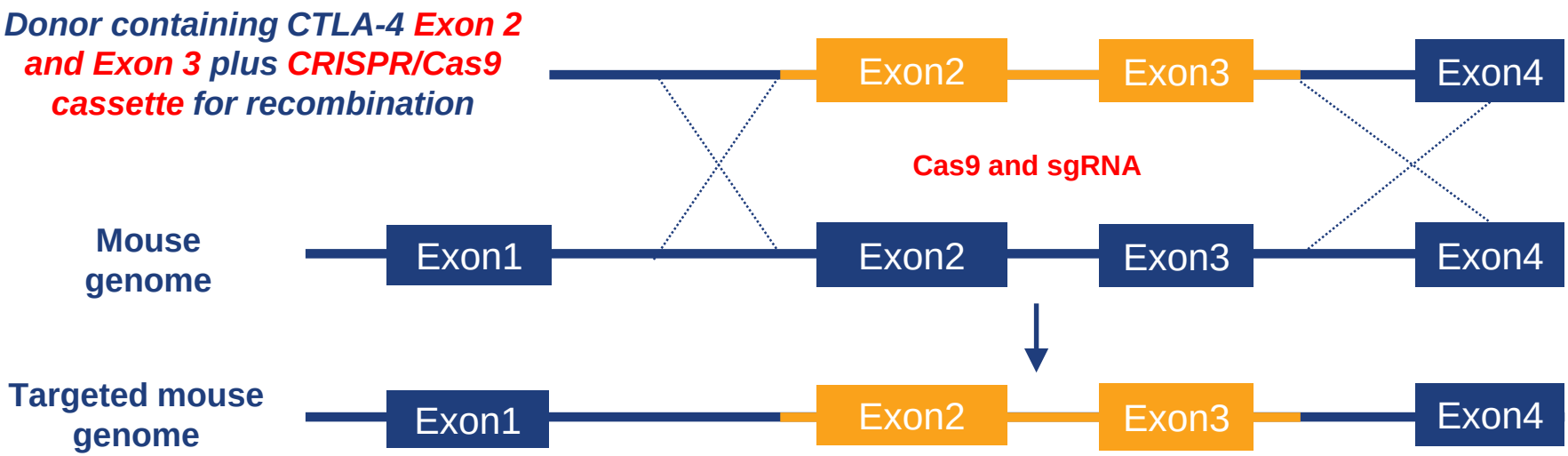
TIL analysis 48hrs following 2 doses of Opdivo



Correlation of tumor volume and CD8+ T cells following Opdivo treatment

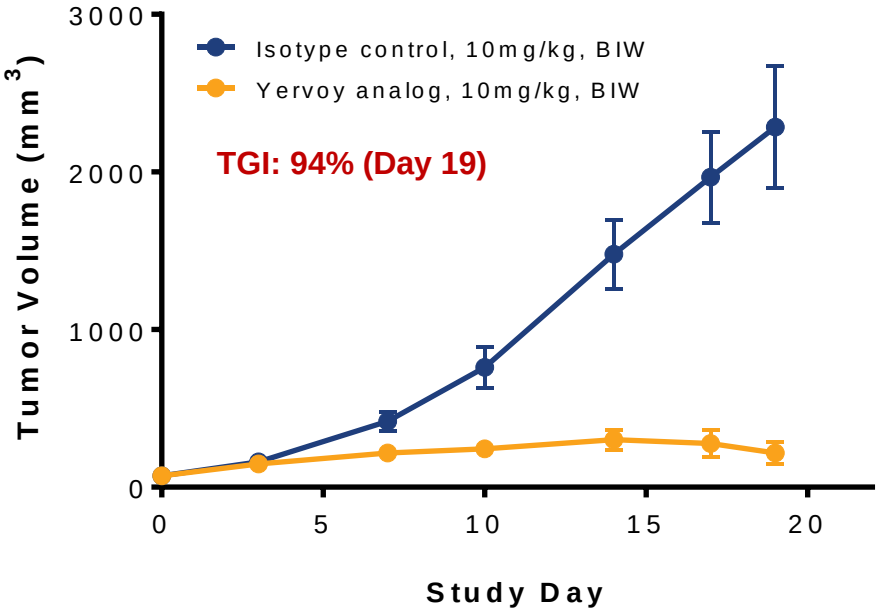


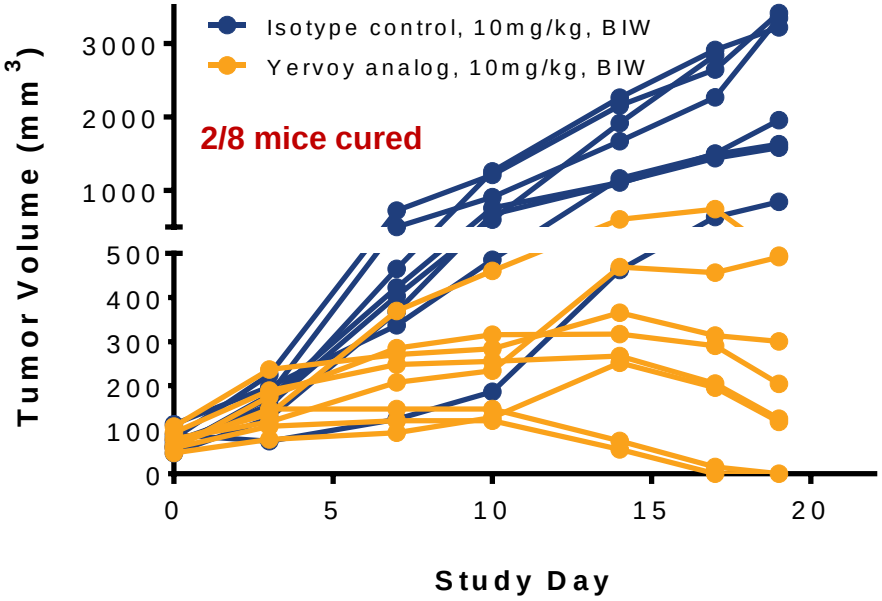
CTLA-4 HuGEMM KI Strategy



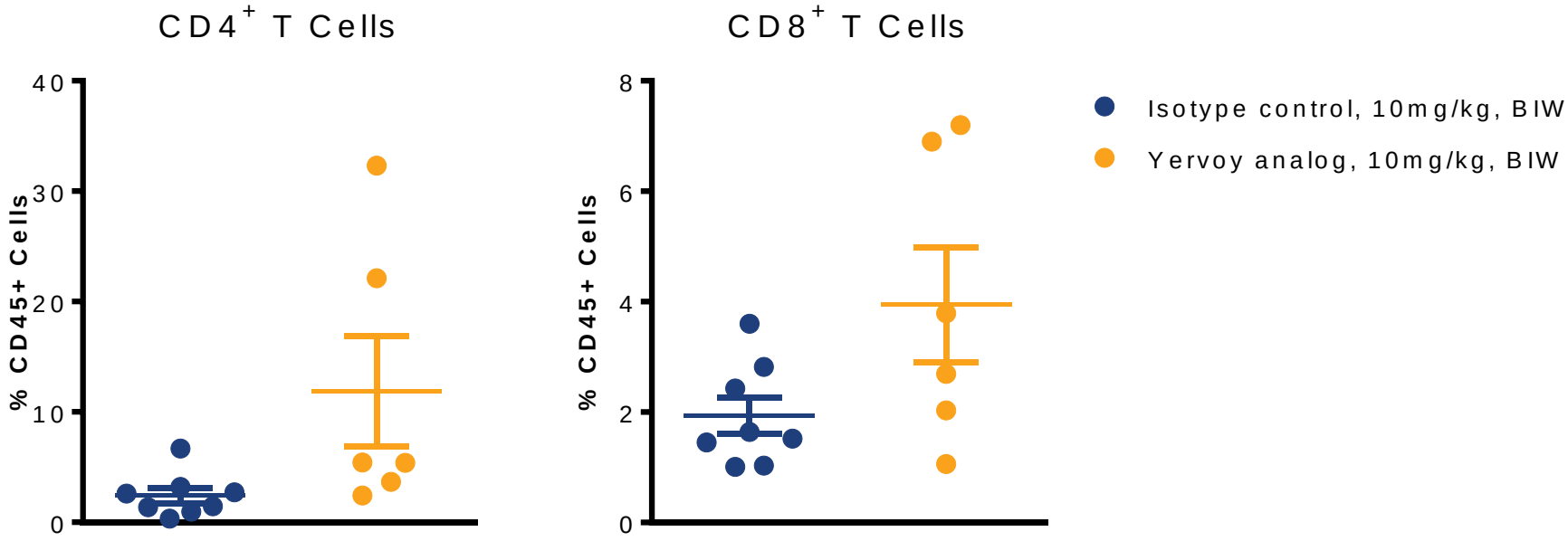
CTLA-4 HuGEMM Efficacy Study: Yervoy® Treatment

- Anti-CTLA-4 (Yervoy) treatment effectively reduced tumor burden with 2/8 mice cured



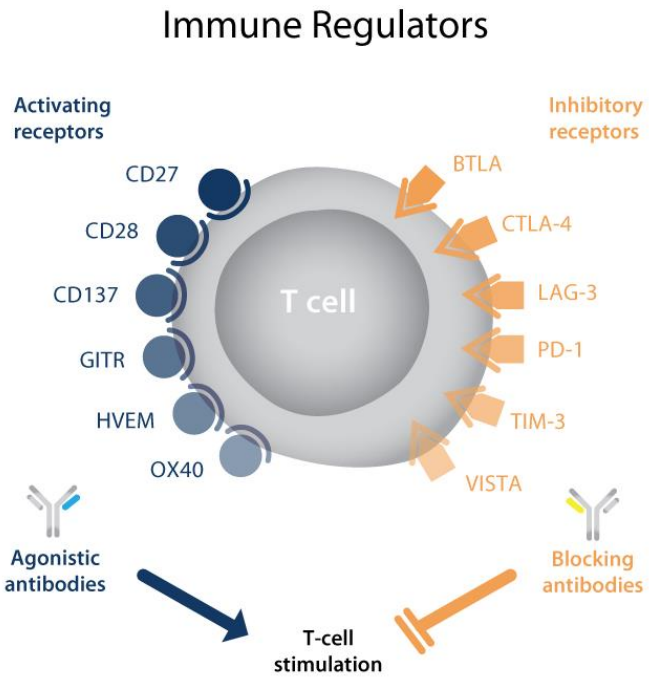


CTLA-4 HuGEMM TIL Analysis



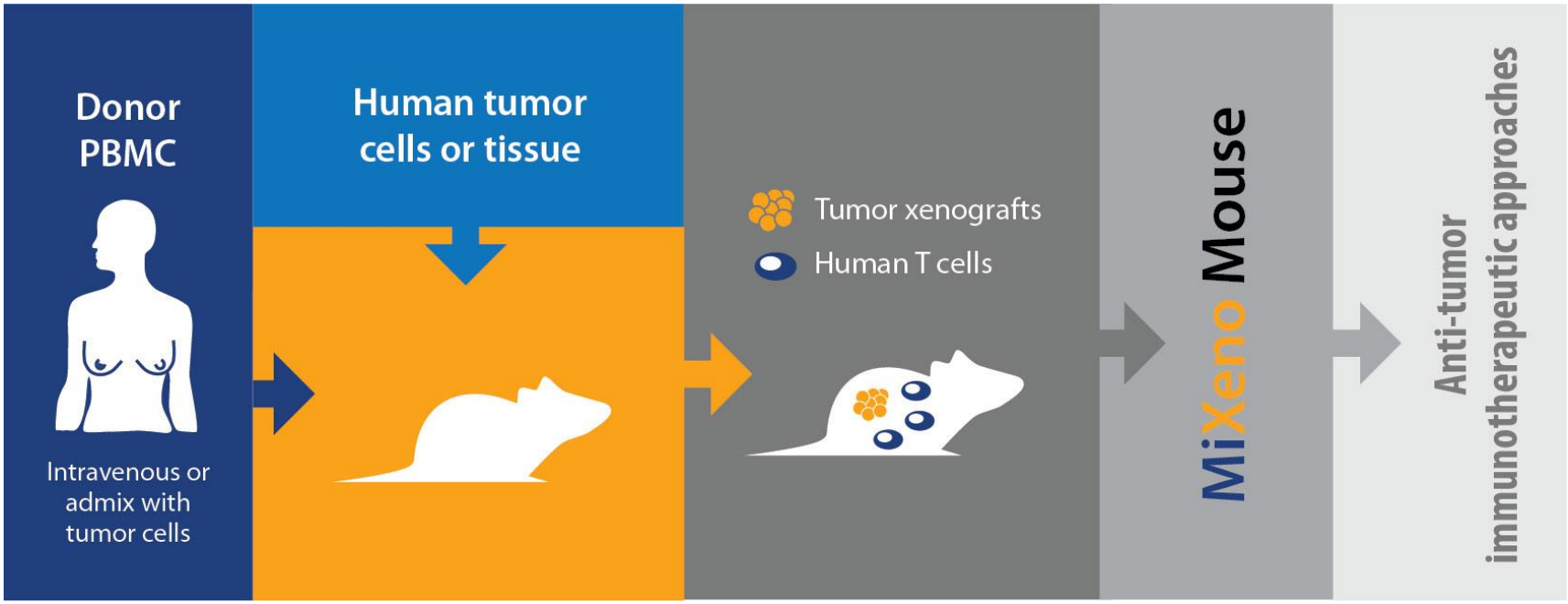
HuGEMM Pipeline

Single KI	Status	Double KI	Status
PD-1	Available	PD-1/PD-L1	Available
CTLA-4	Available	PD-1/TIM-3	Homozygous breeding
CD137	Available	PD-1/LAG3	Heterozygous breeding
TIM-3	Available	PD-L1/ CTLA-4	Heterozygous breeding
OX40	Available		
PD-L1	Available		
LAG3	Validating		
GITR	Homozygous breeding, validating in 2 months		
CD40	Homozygous breeding		
ICOS	Homozygous breeding, validating in 2 months		
TIGIT	Validating		



CrownBio Humanized Model

MiXeno

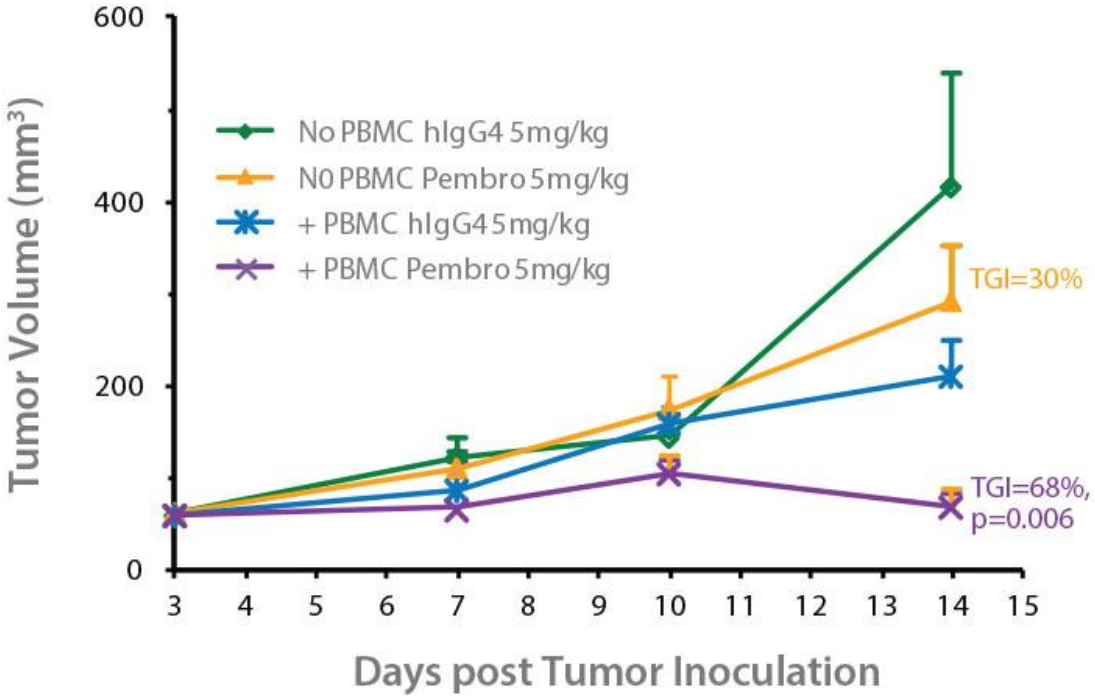


MiXeno Immuno-Oncology Applications

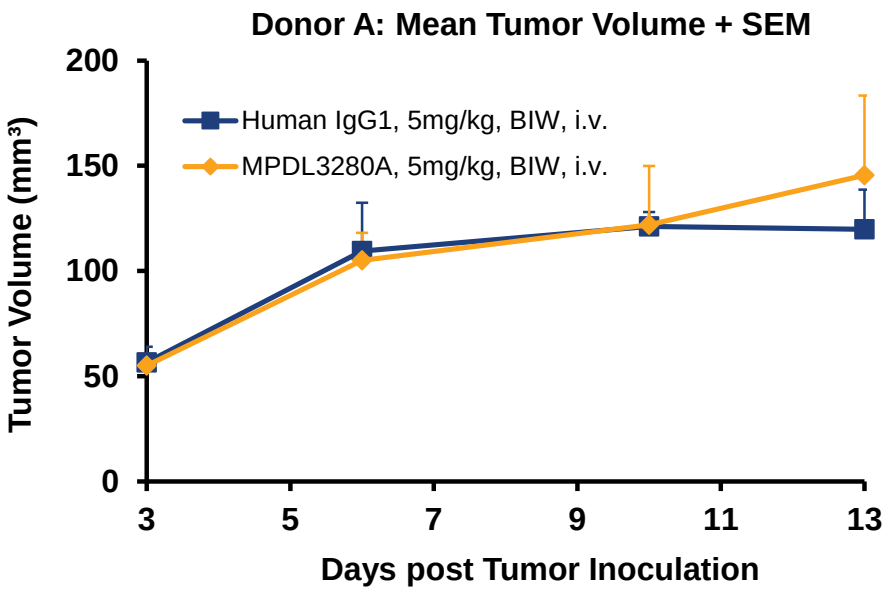
Immune Function Involved	Test Substance	CrownBio Experience
T cell function	BiTE®-like Ab	CD19, HER2, EGFR BiTEs
	Immune checkpoint inhibitors/agonists	PD-1, PD-L1,CTLA-4 inhibitors
NK cell function	ADCC	Cetuximab
	NK modulating agents	N/A

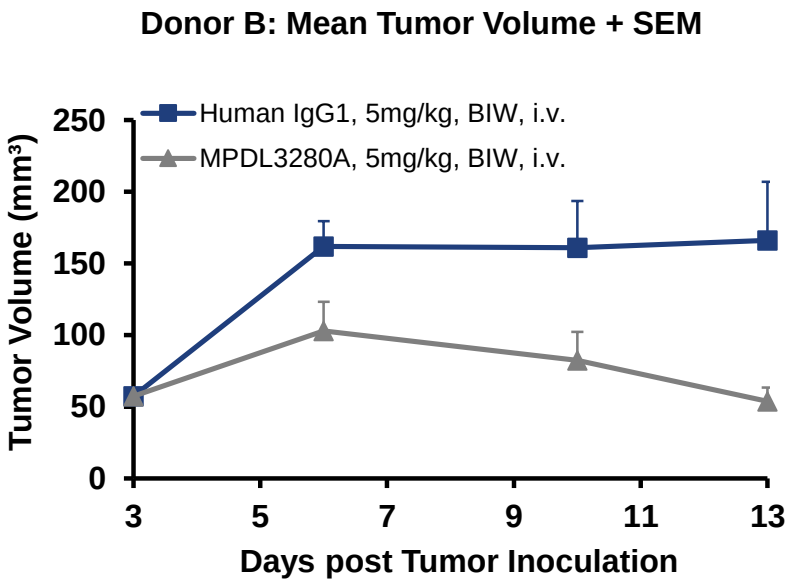
MiXeno HCC827 Model Response to Anti-PD-1 Antibody

- Keytruda demonstrates significant antitumor activity



Donor Variability Affects Efficacy in the HCC827 MiXeno Model



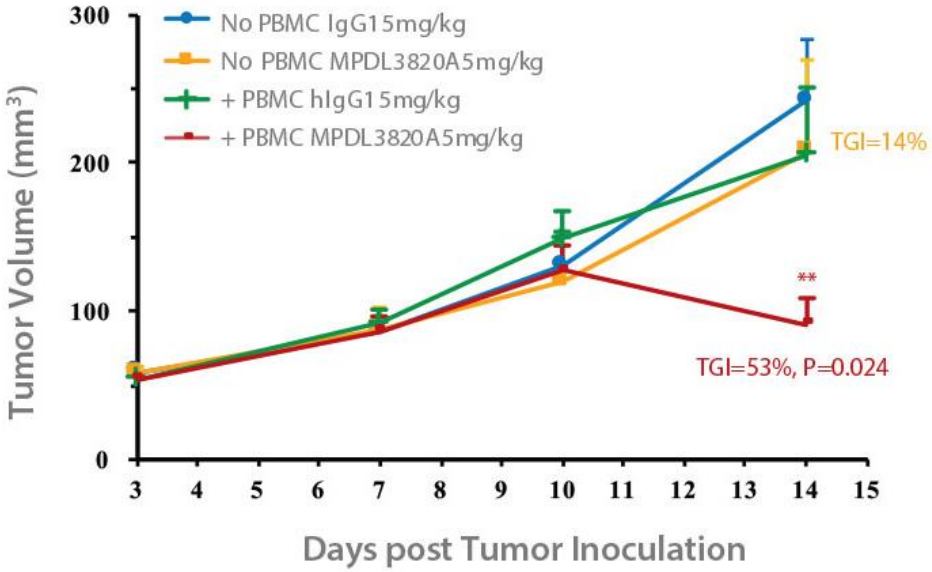


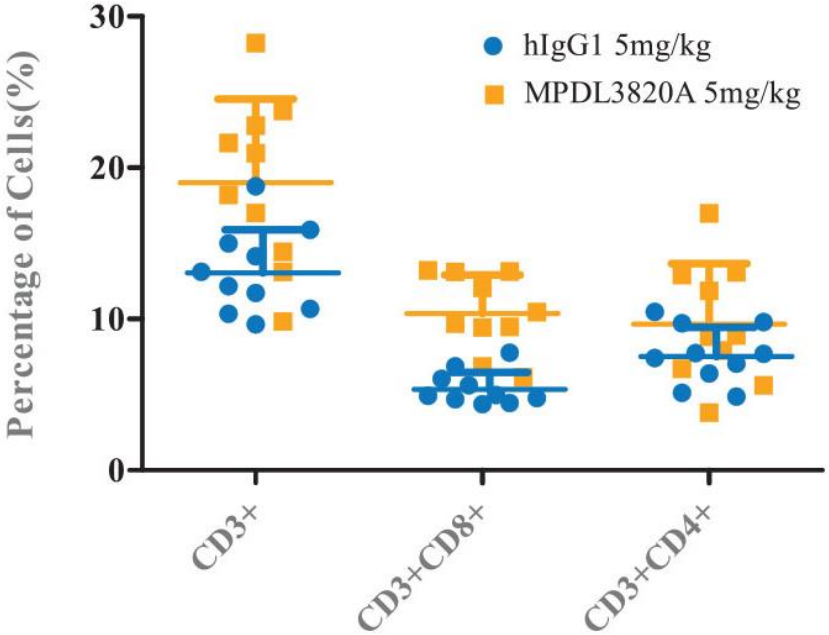
NOG mice

Treatment	Tumor Volume (mm^3)	T/C Value (%) on Day 13	p Value
Human IgG4 (Donor A)	120 ± 19	--	--
MPDL3280A (Donor A)	145 ± 38	131	0.567
Human IgG4 (Donor B)	166 ± 41	--	--
MPDL3280A (Donor B)	54 ± 10	32	0.067

MiXeno HCC827 Model Response to Anti-PD-L1 Antibody

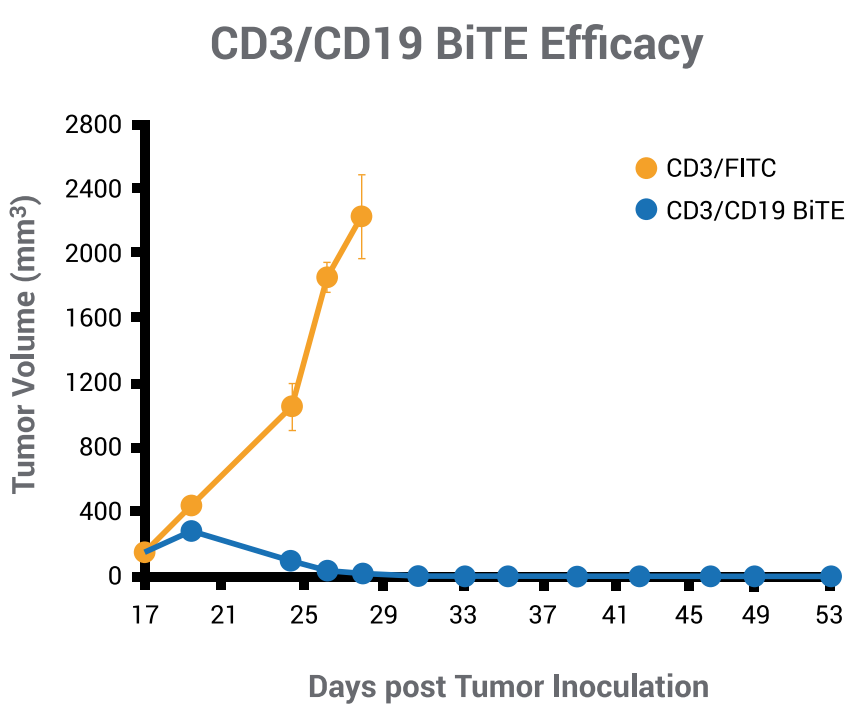
- MPDL3820A demonstrates significant antitumor activity
- The number of lymphocytes increases in treated animals

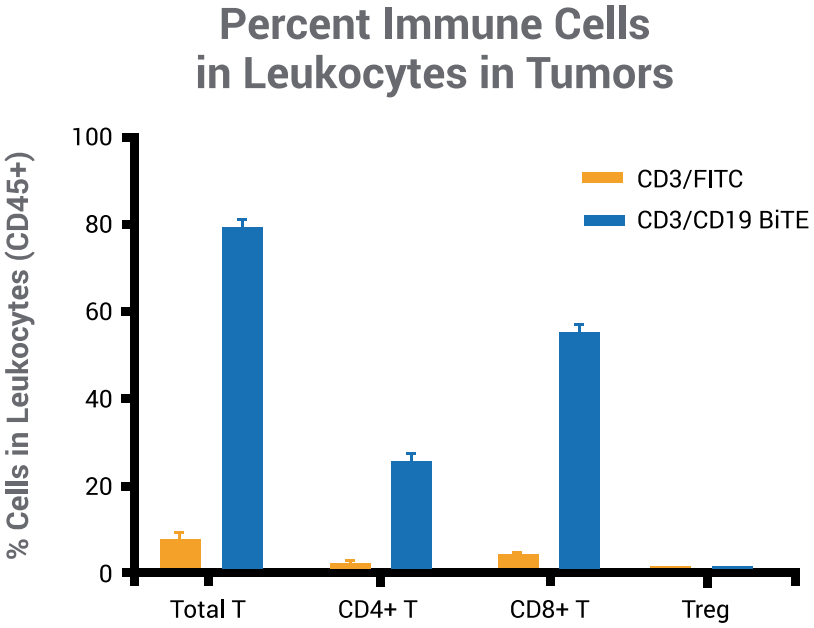




MiXeno to Test BiTE Efficacy: Jeko-1 Model

- A CD3/CD19 BiTE molecule effectively redirects T cells to the tumor in the Jeko-1 MiXeno model





CrownBio Humanized Model Platform: Value of HSC-PDX

Relevance To Human-Specific Antibodies
Essential when no mouse surrogate exists or there is no cross-reactivity

Combination: IO+Targeted
Access full diversity of cancer genetics from PDX collection (e.g. PD-1/EGFR, CTLA-4/BRAF)

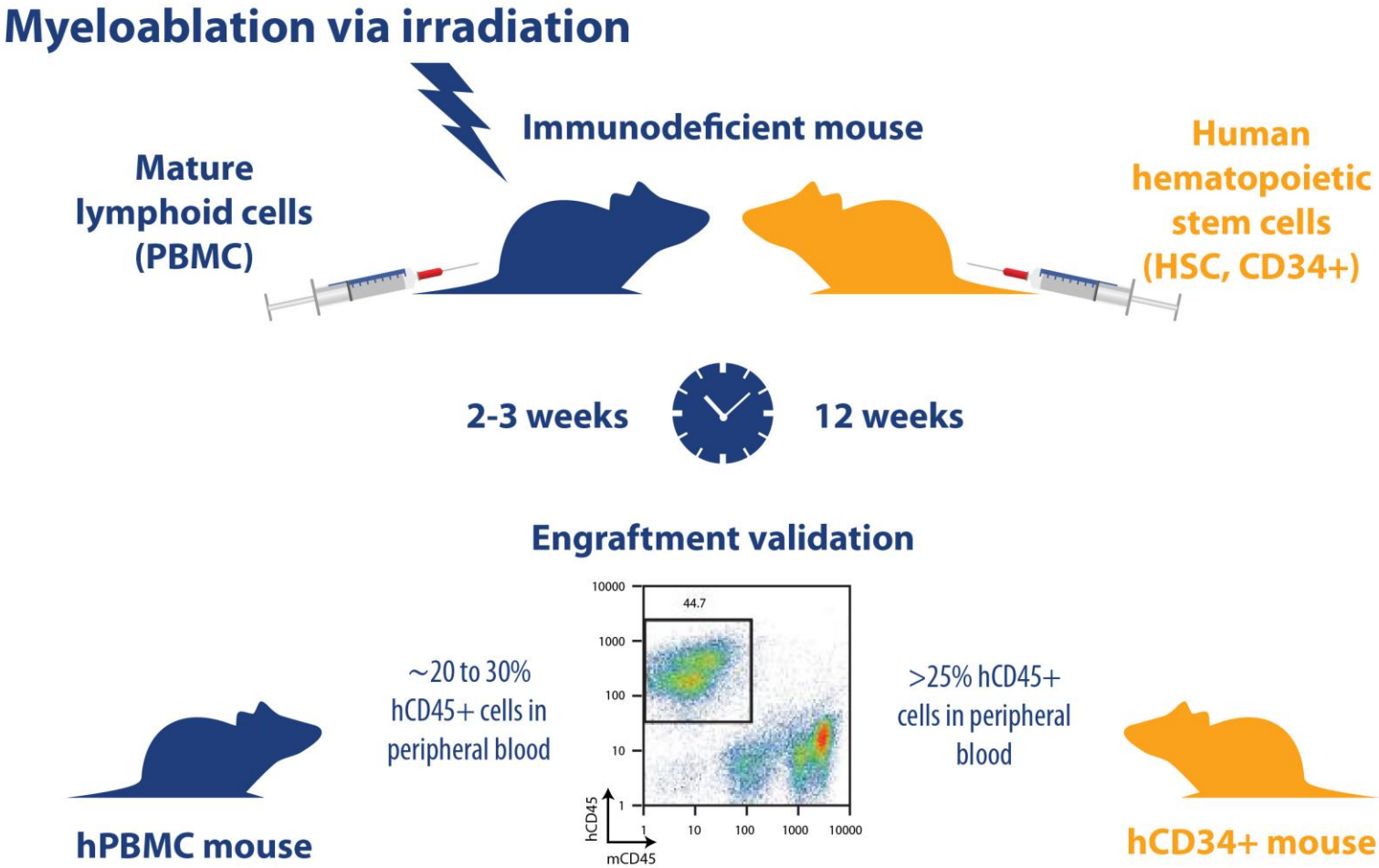
Humanized PDX Model

Relevance To Known And Novel IO Targets
Ability to evaluate multiple IO targets
Model captures diversity of human lymphocyte targets (e.g. model is not humanized for just PD-1)

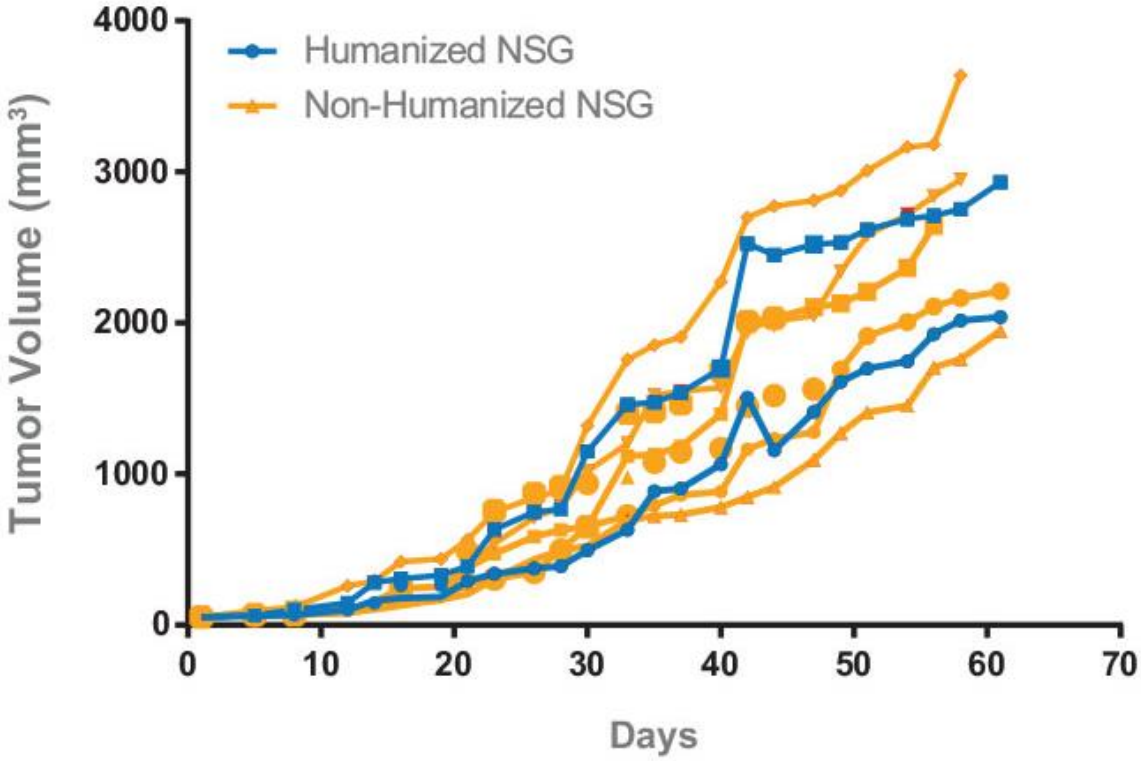
Combination: IO/IO
Access full diversity of human lymphocyte targets (e.g. PD-1/CTLA-4)

HuTrial™: Assess Diversity Across Population
Both immune system diversity (multiple stem cell donors) & tumor diversity (multiple PDX models)

CD34+ Humanized Mice

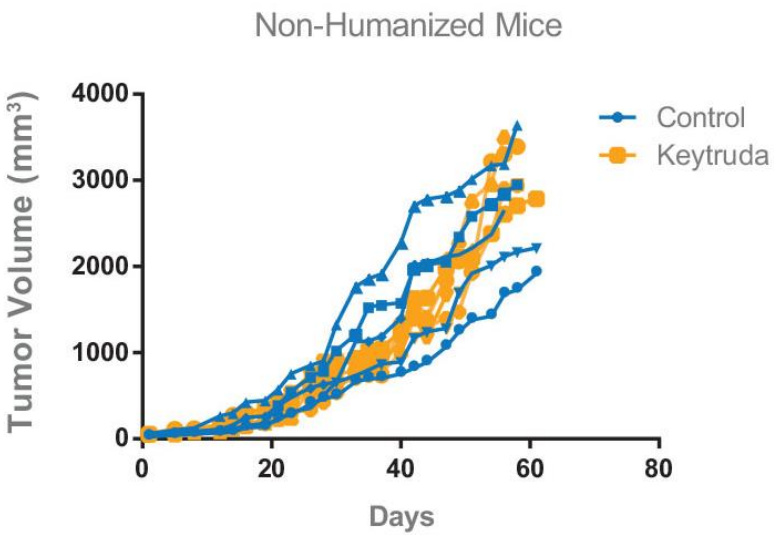


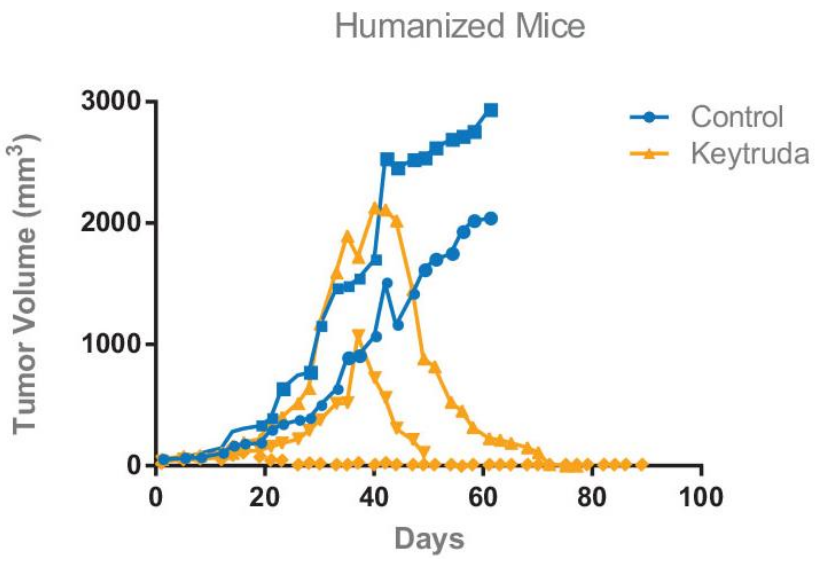
Humanization of NSG Mouse Does Not Effect MDA-MB-231 Growth



Humanization of NSG Mouse Required for Keytruda Effect: MDA-MB-231

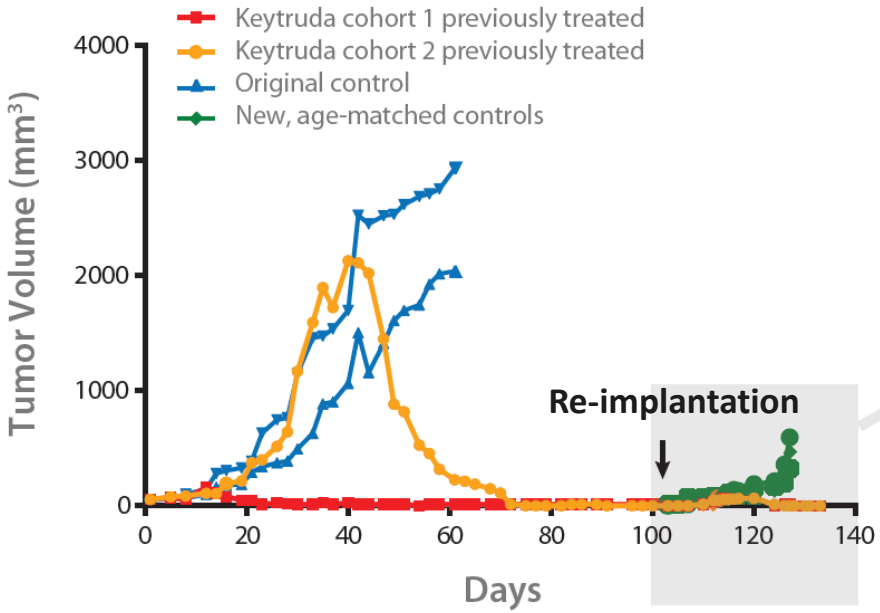
- Humanized mice respond to an anti-PD-1 mAb

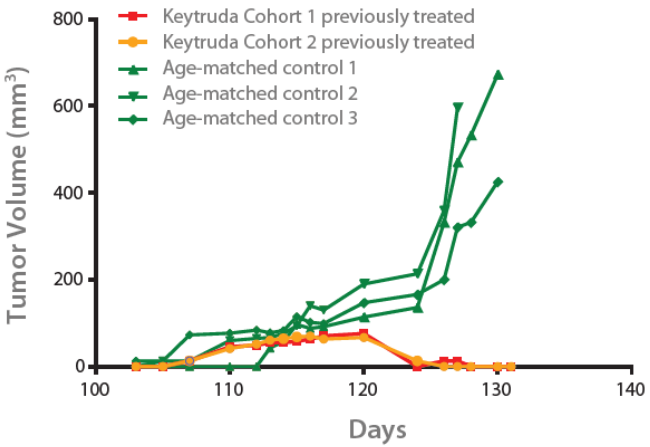




Humanized Mice Display a Memory Response

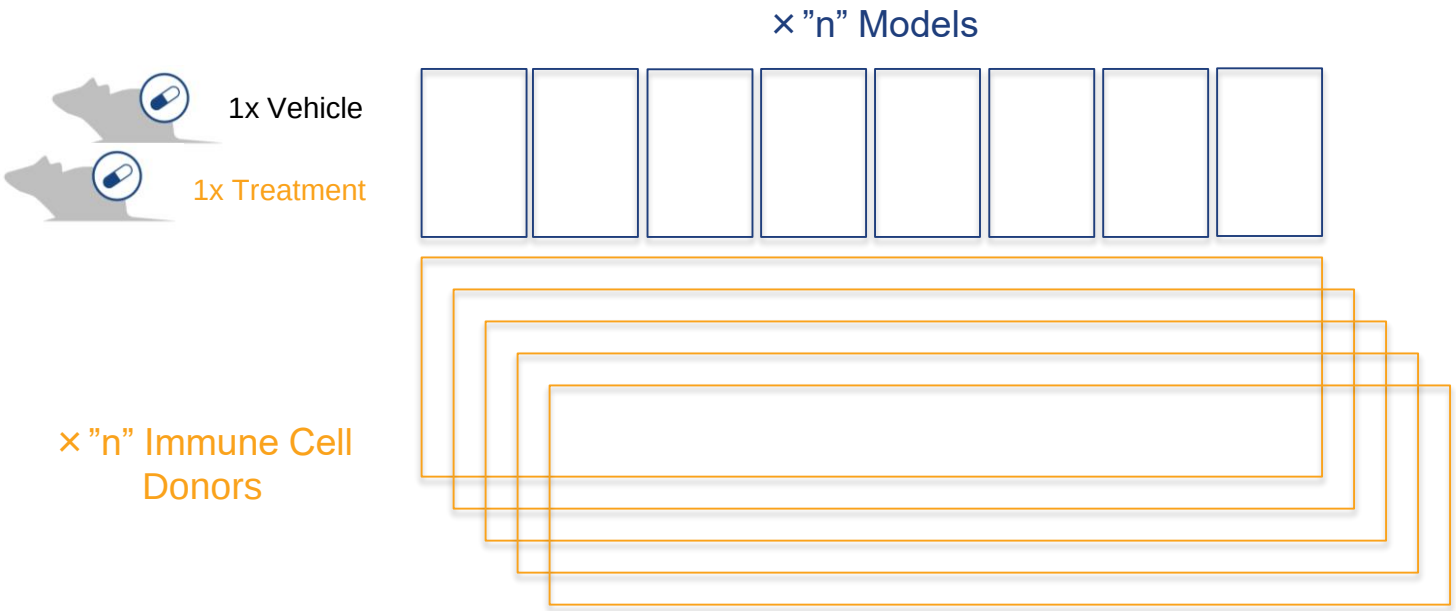
- Previously treated humanized NSG mice are rechallenged with tumor cells to evaluate memory response
  - Re-implanted tumors fail to grow in pretreated animals, indicating a memory response





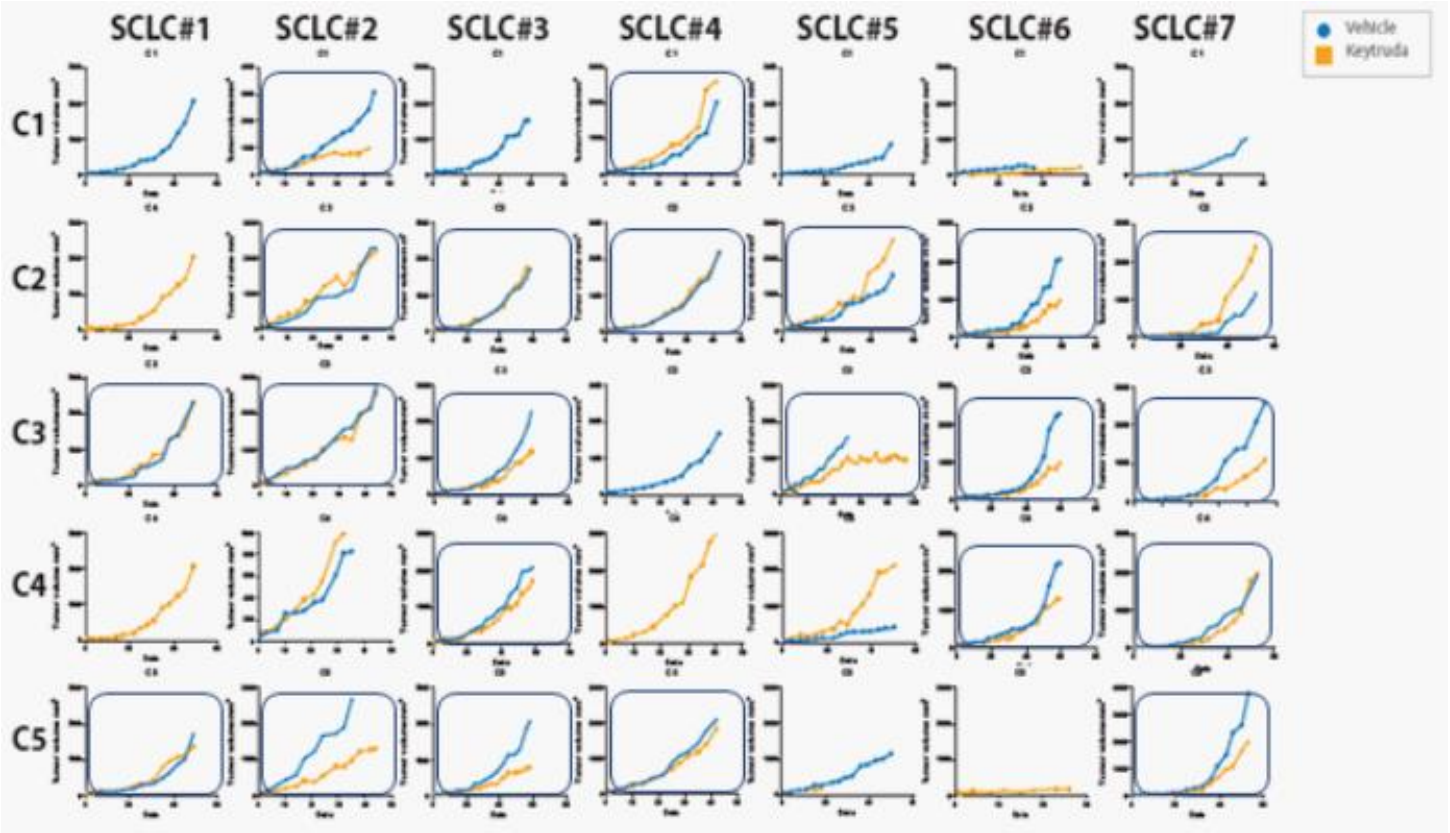
Case Study: Humanized PDX Help Predict Response

- Checkerboard study design
  - Reduces the number of humanized animals required per each arm by increasing the number of models tested
  - Allows assessment of donor-to-donor variability



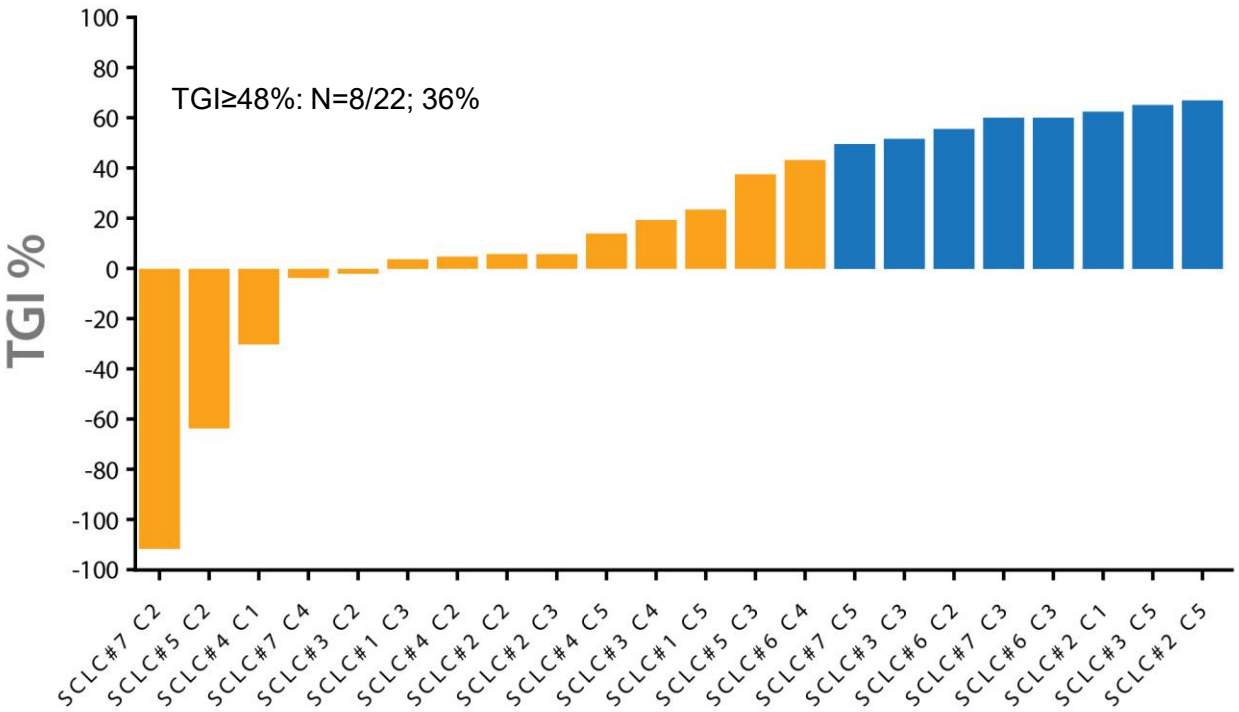
Case Study: Humanized Tumor Bearing Models Help Predict Response

- Objective: evaluate response to Keytruda in SCLC PDX models
- Study design: 7 SCLC PDX models, 5 immune donors, N=1



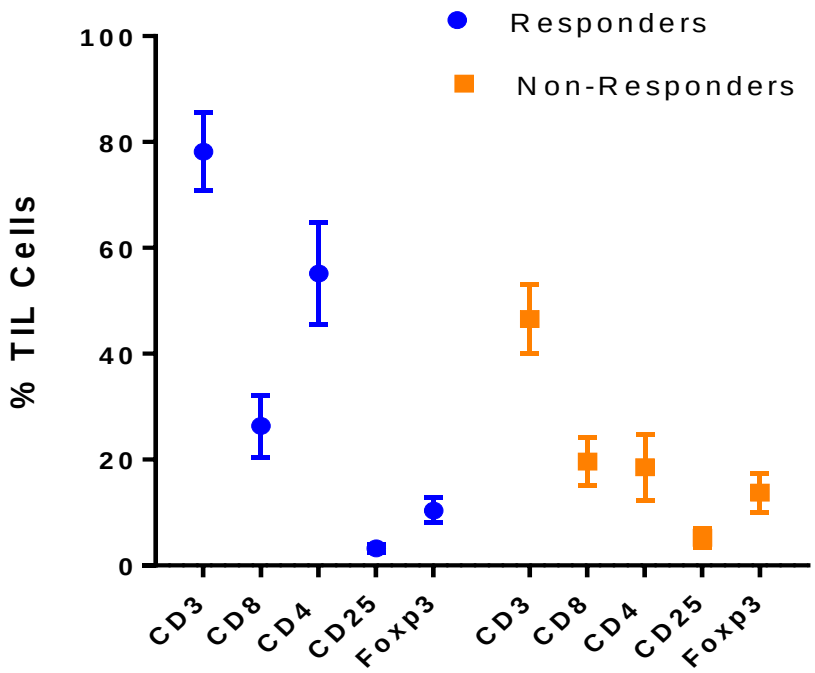
Case Study: Humanized Tumor Bearing Models Help Predict Response

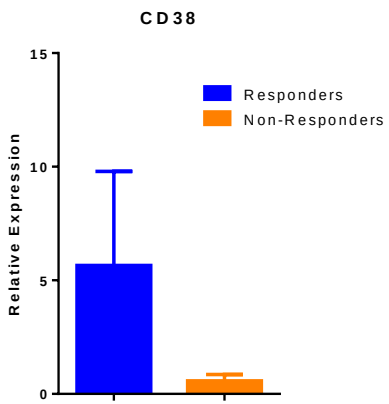
- 36% of SCLC PDX in the study respond to treatment (TGI≥48%)
- Translatable study design with outcomes comparable to the clinic

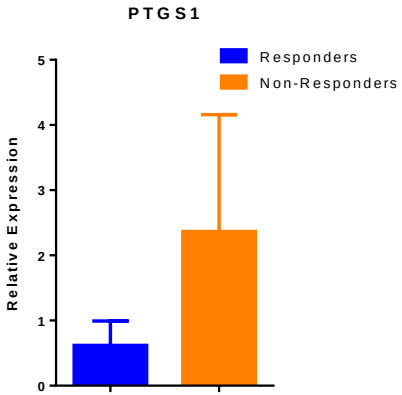


Case Study: Humanized Tumor Bearing Models Help Predict Response

- Increased % of TILs in tumors from responder PDXs
- M1 (CD38) macrophage polarization in responders vs M2 (PTGS1) in non-responders







Summary of CrownBio Humanized Models

CrownBio Platform	Model Type	Immune System	Applications
HuPrime	PDX	Immunocompromised animals	CAR-T or other cell therapies with immune cells supplied by infusion
HuGEMM	Syngeneic mouse tumor	Competent mouse immunity	Known ICI targeting agents, combinations of ICIs with novel mouse cross reactive test articles
MiXeno	Cell line derived xenografts or fast growing PDX	PBMC reconstituted human immunity. GvHD limits experimental window. Only compatible tumor models	BiTEs, ICIs, other immunomodulatory reagents with a fast response (21 days or less)
HSC-PDX	PDX	CD34+ reconstituted human immunity	All immunomodulatory reagents

Get in Touch

Contact us for more information

busdev@crownbio.com

Or visit

www.crownbio.com