Animal Model Workshop

A Novel In-Vitro CRISPR-Edited Syngeneic RCC Mouse Model: Establishment, Characterization, and Lessons Learned

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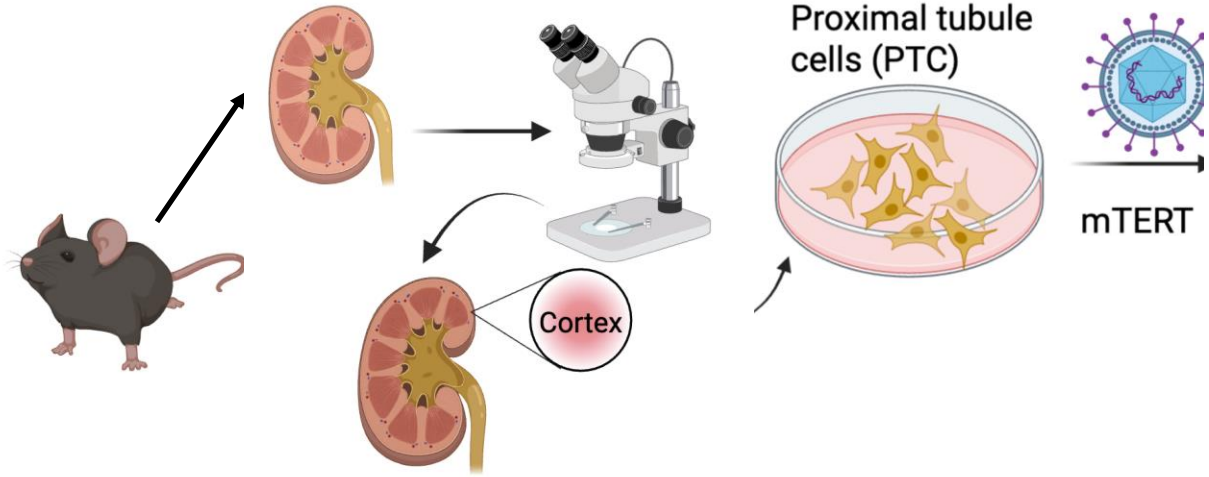
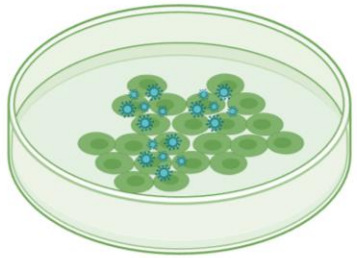
Establishing a Syngeneic Murine RCC Line via in vitro CRISPR/Cas9



Proximal tubule cells (PTC)

mTERT

TKPTS (Mouse renal epithelial-like cell immobilized by SV40(Immunogenic)



: Luca Gusella
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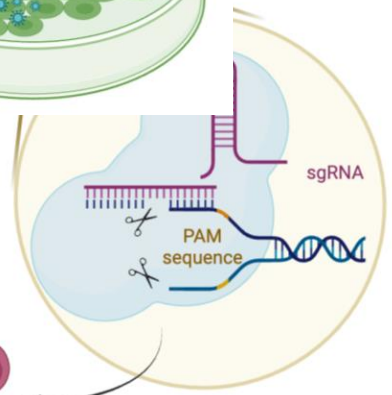
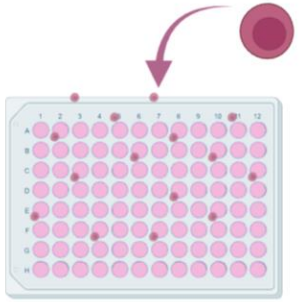
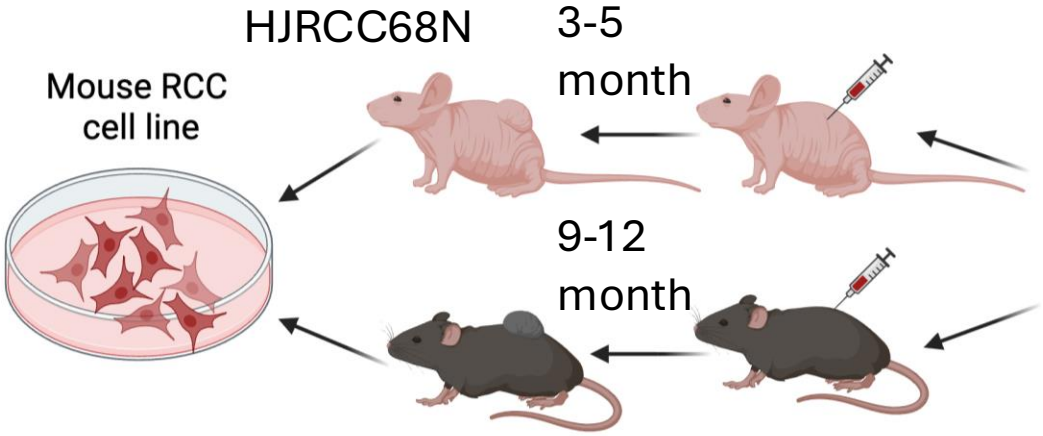




$Vhl^{-/-}Bap1^{-/-}Cdkn2b^{-/-}$
PTC68 & PTC69

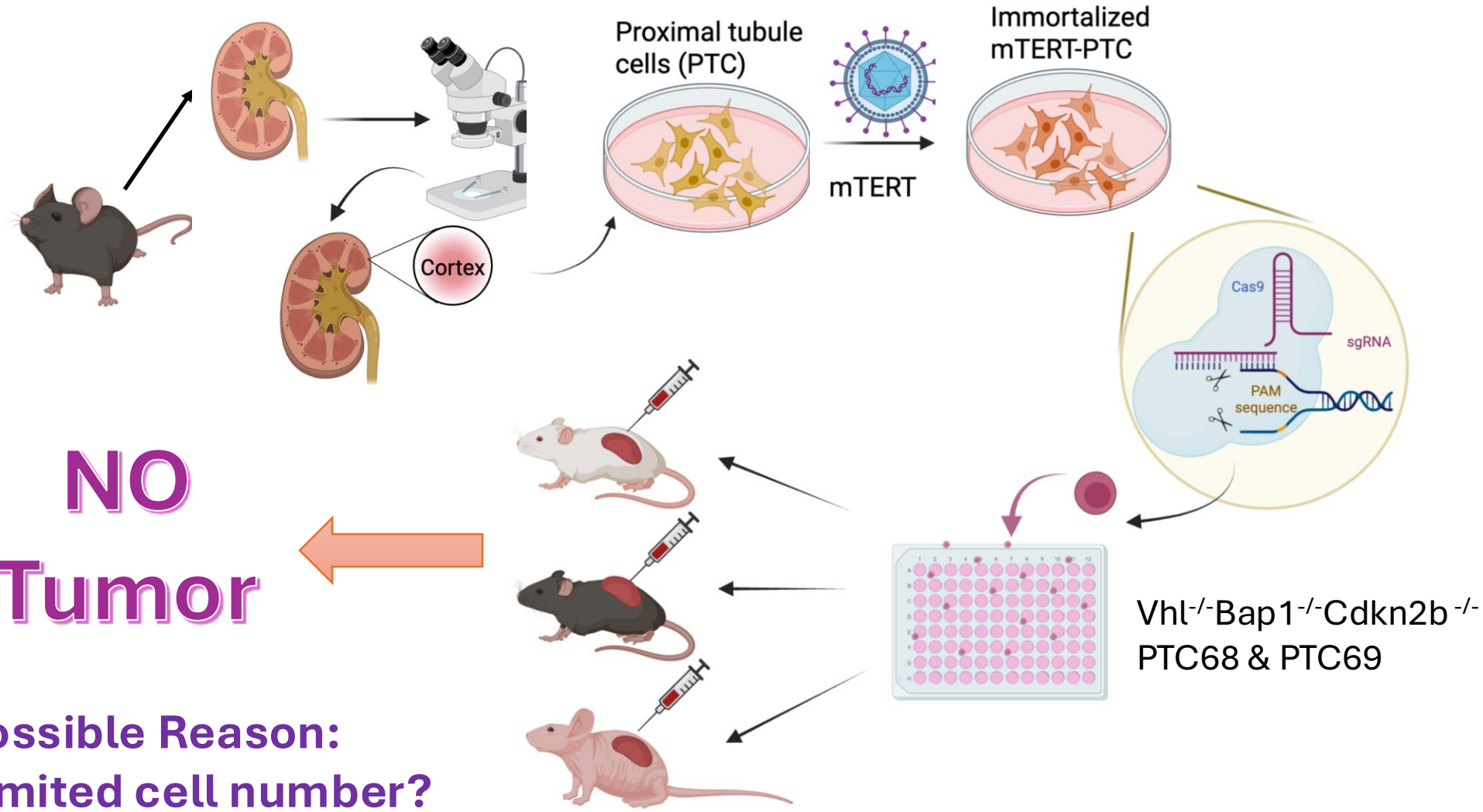


- $Vhl^{-/-}$ ✗
- $Vhl^{-/-}Bap1^{-/-}$ ✗
- $Vhl^{-/-}Bap1^{-/-}Cdkn2a/2b^{-/-}$ ✓
- $Vhl^{-/-}Pbrm1^{-/-}$ ✗
- $Vhl^{-/-}Pbrm1^{-/-}Setd2^{-/-}$ ✗

- **Only rare single-cell clones expanded**
- **No biallelic Vhl-KO clones recovered**
- **Vhl loss appears cell-lethal (or severely growth-limiting) in vitro**

Unpublished

Establishing a Syngeneic Murine RCC Line via in vitro CRISPR/Cas9

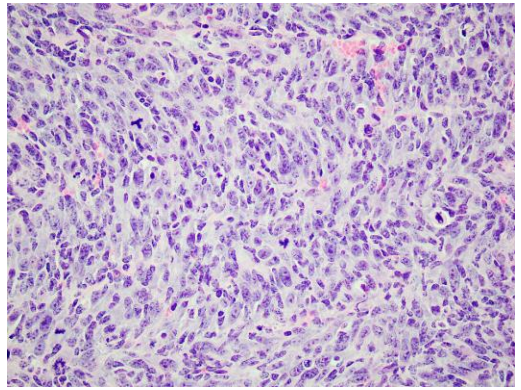


Possible Reason:
Limited cell number?

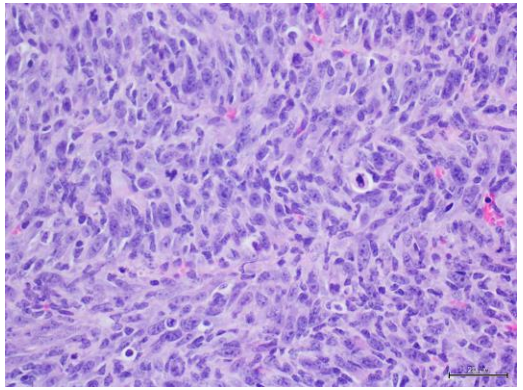
Unpublished

CRISPR-Edited Proximal Tubule Cell (PTC) Lines Form Sarcomatoid RCC In Vivo

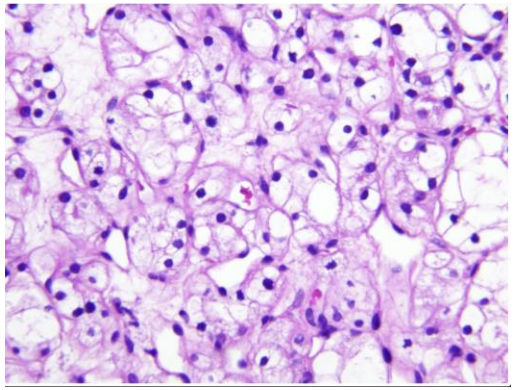
HJRCC68N



HJRCC68B



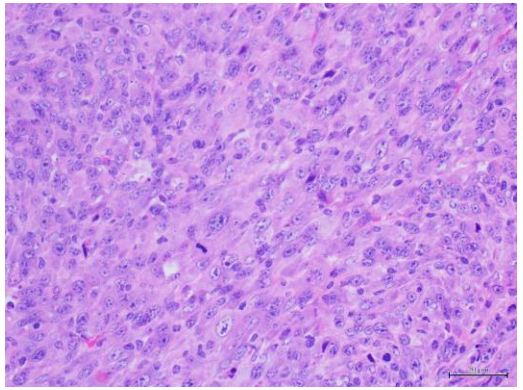
Human ccRCC (low grade)



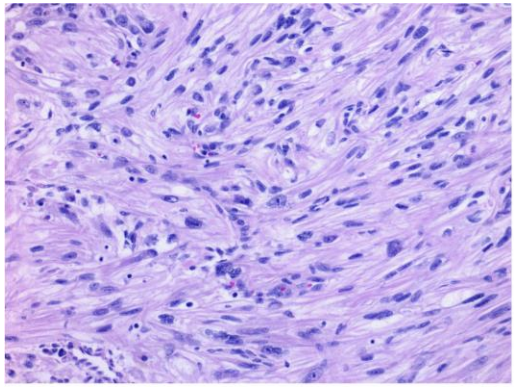
HJRCC68N
HJRCC68B
HJRCC69B

Sarcomatoid tumor “atypical spindle cells”

HJRCC69B



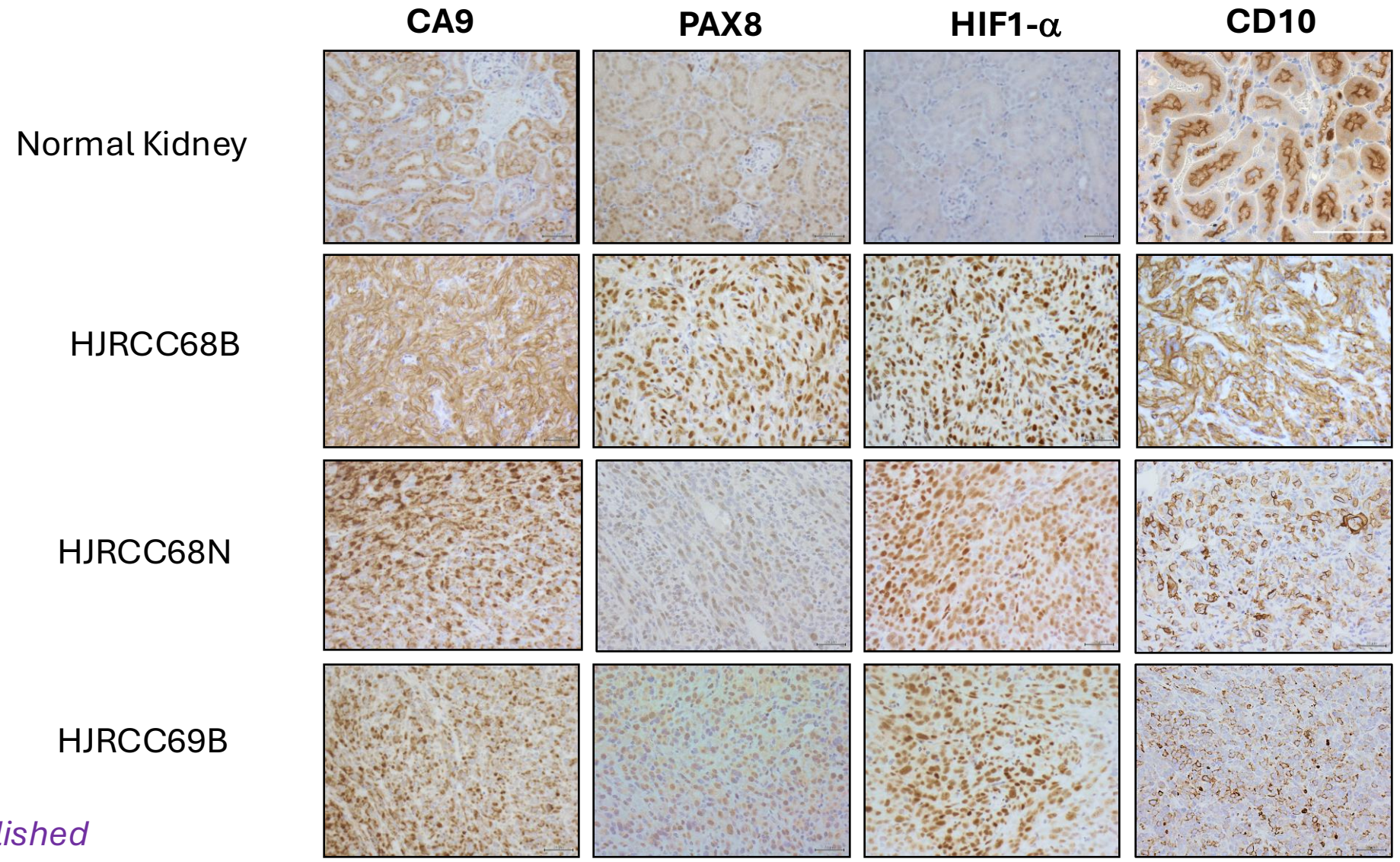
Human Sarcomatoid ccRCC



Reviewed by Yingbei Chen

Unpublished

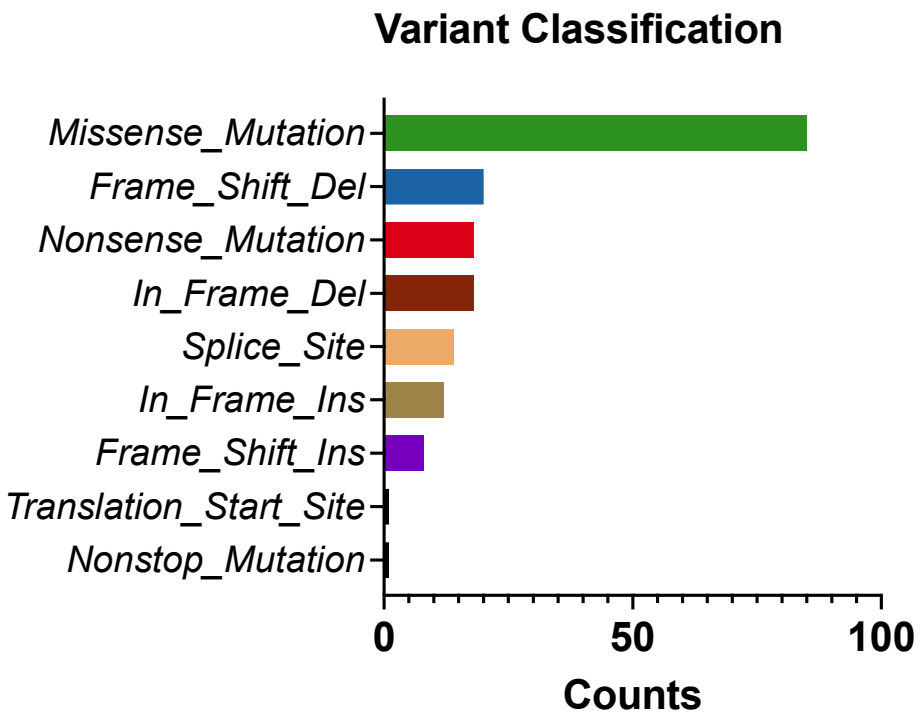
CRISPR-Edited Proximal Tubule Cell (PTC) Lines Form Sarcomatoid RCC In Vivo

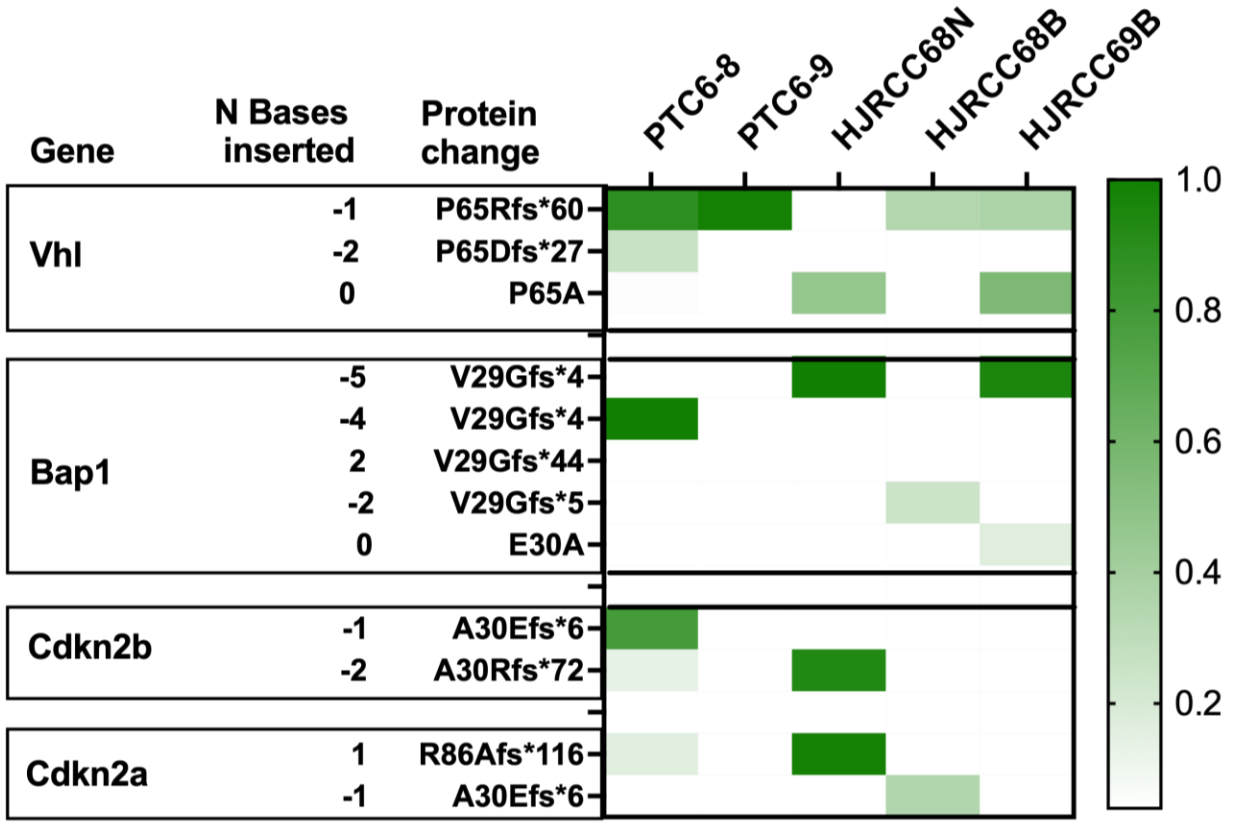


Unpublished

Yingbei Chen
Lyn Vuong
Ankur Mascareno

Whole-Exome Sequencing Validates Edits in Three Novel Murine sRCC Lines





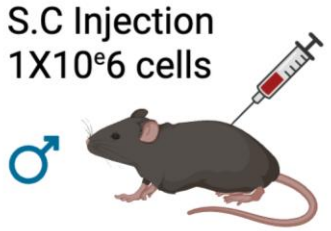
HJRCC68N:
$Vhl^{mut}Bap1^{del}Cdkn2a/Cdkn2b^{del}$

Unpublished

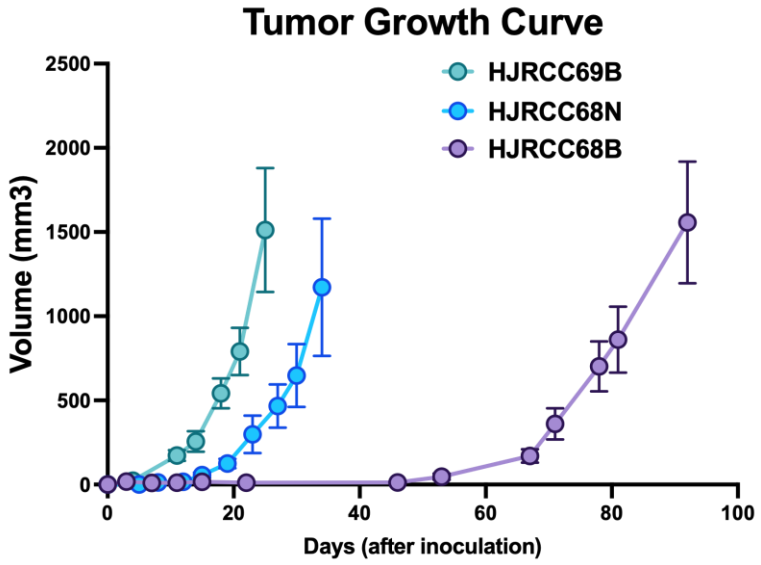
Alex Penson

Sex-Dependent Subcutaneous Growth of Murine sRCC in C57BL/6J Mice

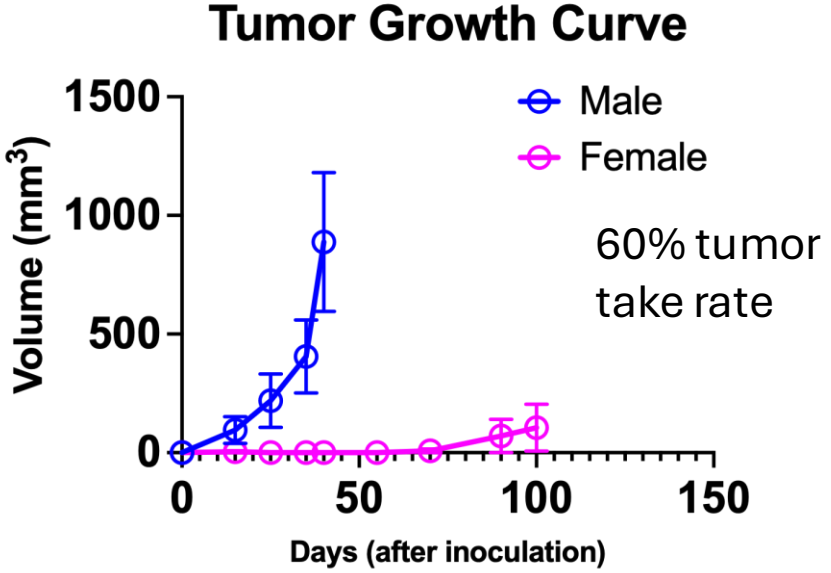
Male C57BL/6J: robust subcutaneous engraftment and growth



95% tumor take rate



Female C57BL/6J: delayed and attenuated subcutaneous growth

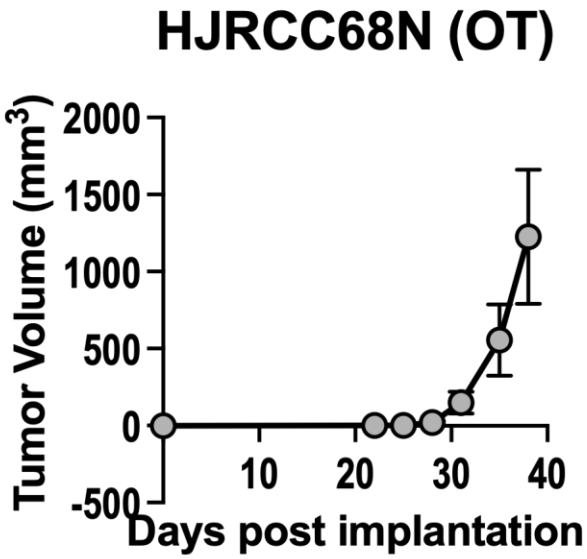


60% tumor take rate

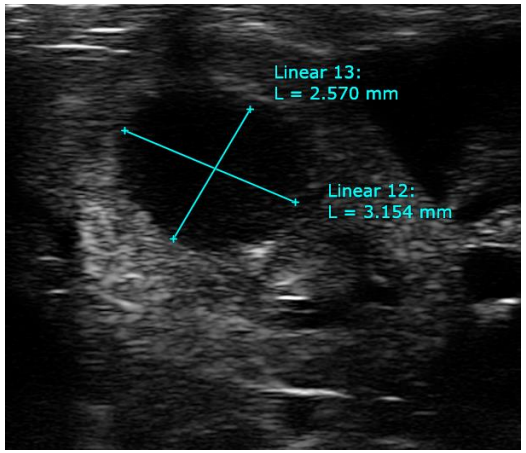
Unpublished

Robust Orthotopic Growth of Murine sRCC in Male C57BL/6J

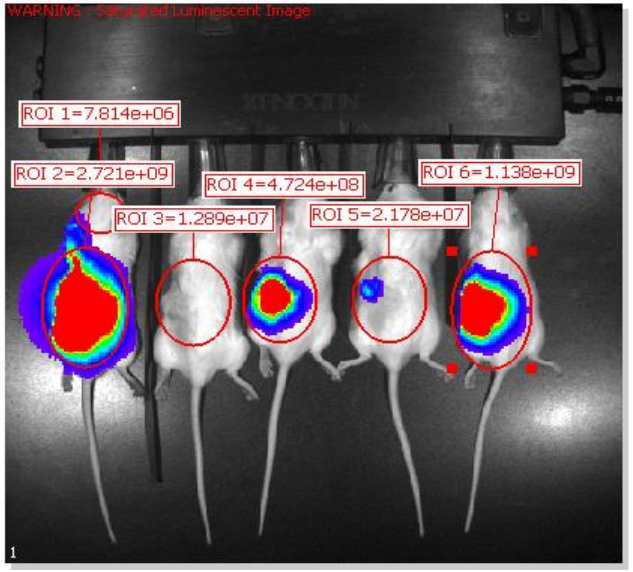
C57BL/6J orthotopic model:
robust, reproducible tumor growth

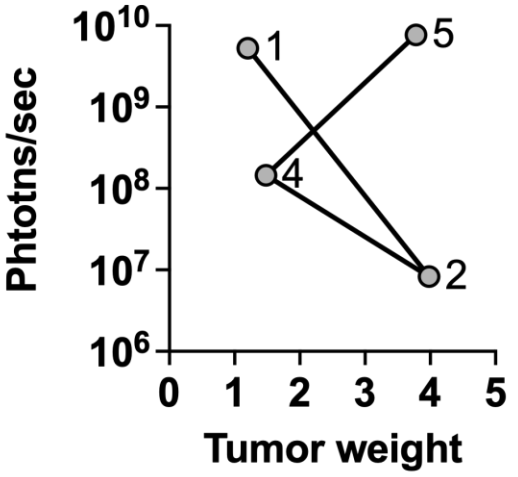


Ultrasound



BLI caveat: bioluminescent signal does **not** reflect tumor burden in vivo





Poor correlation of BLI vs. volume

‼ Luciferase Is Immunogenic:
BLI Decouples from Growth

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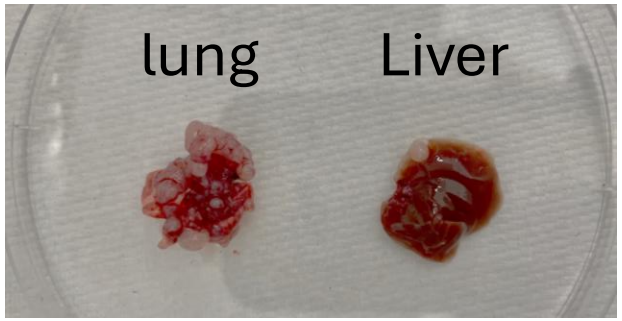
Orthotopic HJRCC68N-Derived Metastatic Line Produces Spontaneous Liver and Lung Metastases

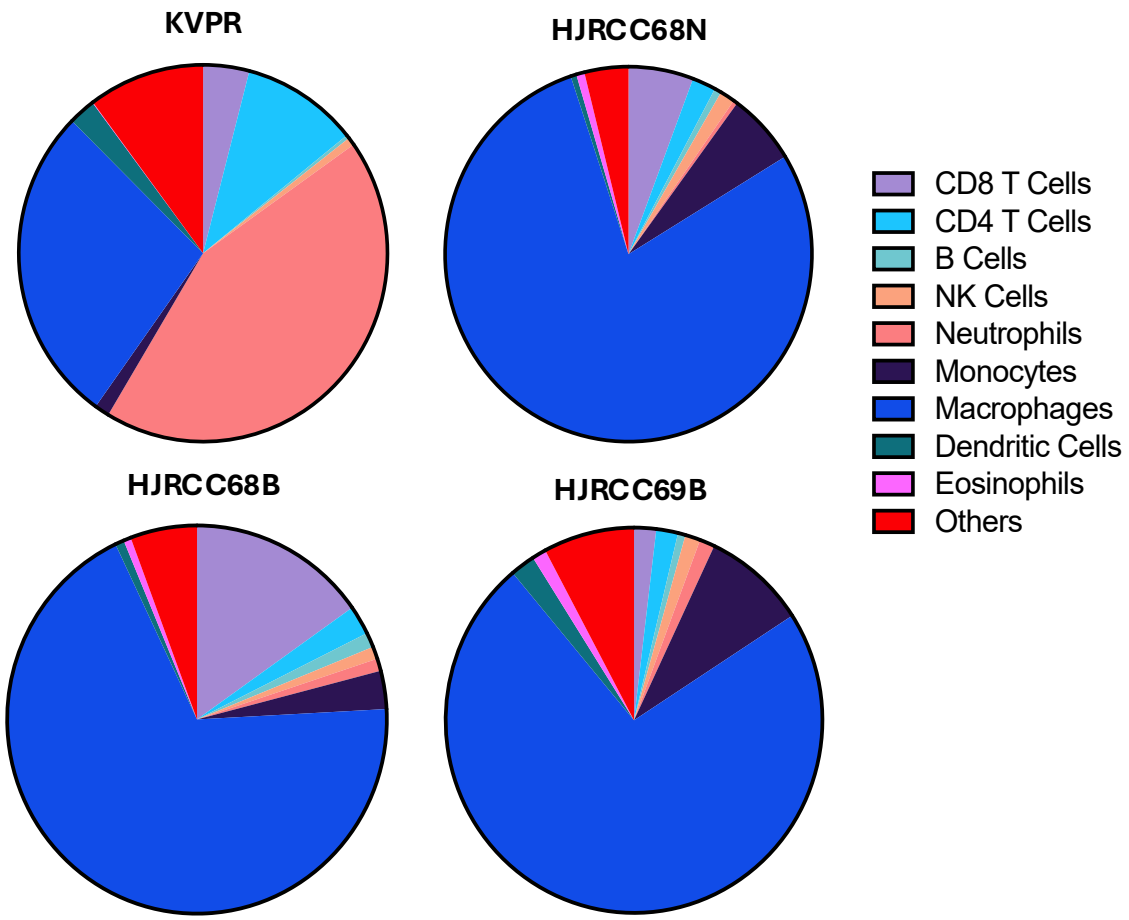
Cell line	Metastases
HJRCC68N	NO
HJRCC68B	NO
HJRCC69B	NO
HJRCC68NF2	O.T.: liver and lung (60%) IV: liver and lung (100%) S.C.: NO

Metastatic HJRCC68N cell line development

Metastases



Subcutaneous HJRCC Tumors: Myeloid-Dominant, Treg-Enriched TME



HJRCC cell line subcutaneous tumor:

- **Immune enriched**

  ~60% of viable cells are immune cells ($CD45^+$ of live singlets)

- **Macrophage predominant**

  macrophages comprise ~70% of immune cells ($CD11b^+F4/80^+$)

- **T-cell presence**

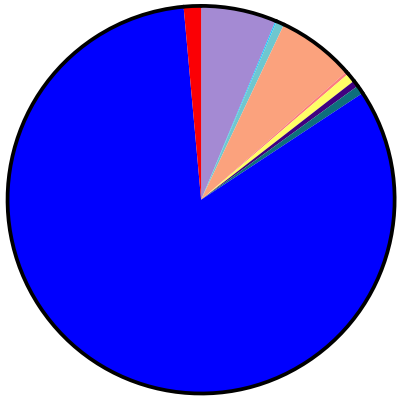
  T cells are 5–10% of immune cells ($CD3^+$ of $CD45^+$)

- **Treg enrichment**

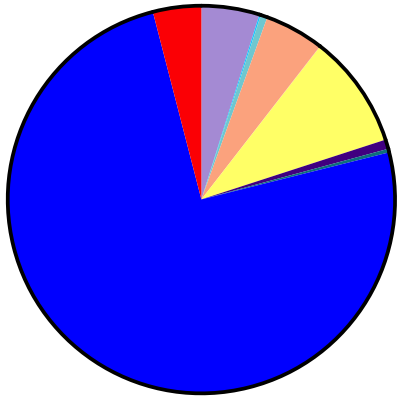
  Tregs are 40–60% of $CD4^+$ T cells

Orthotopic vs Subcutaneous HJRCC: Similar Immune Load, Shifted TME

SC Tumor



OT Tumor

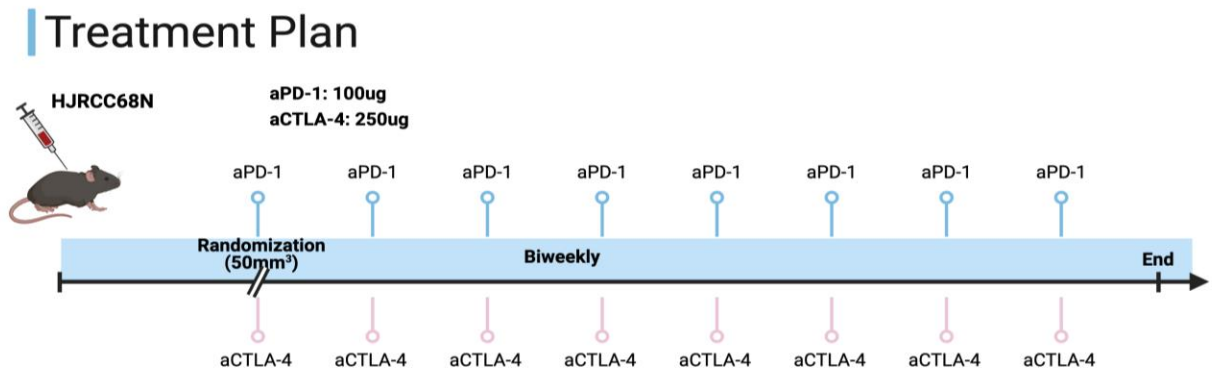


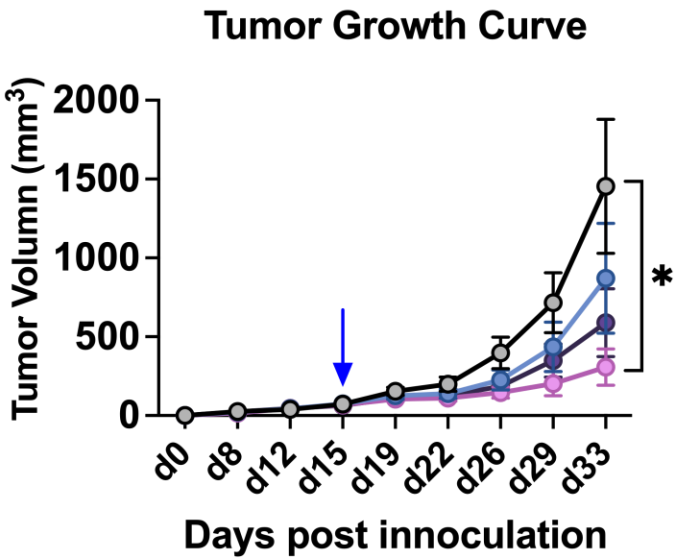
T cells
TCRgd%
NK cell
Monocytes
Eosinophils
Neutrophils
B cells
DCs
macrophage
others

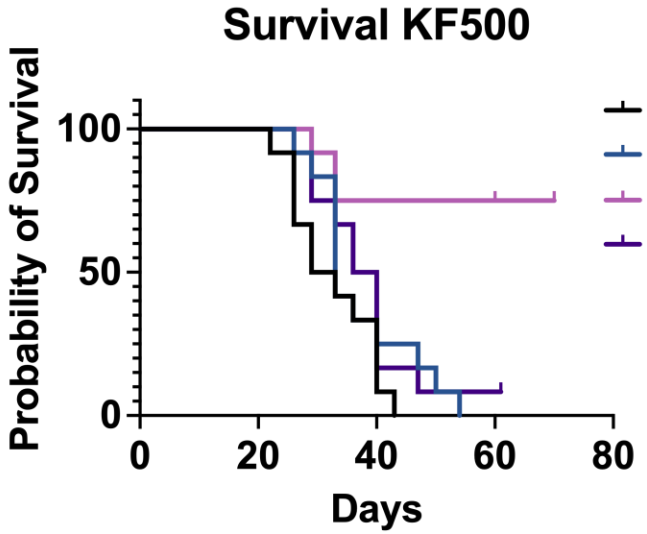
	Subcutaneous	Orthotopic
Immune cell (of live cells)	60%	60%
Macrophages (of CD45+)	80%	60-70%
T cells (of CD45+)	5-10%	3-7%
Tregs (of CD4 T cells)	50%	15%
Neutrophils (of CD45+)	1%	10%

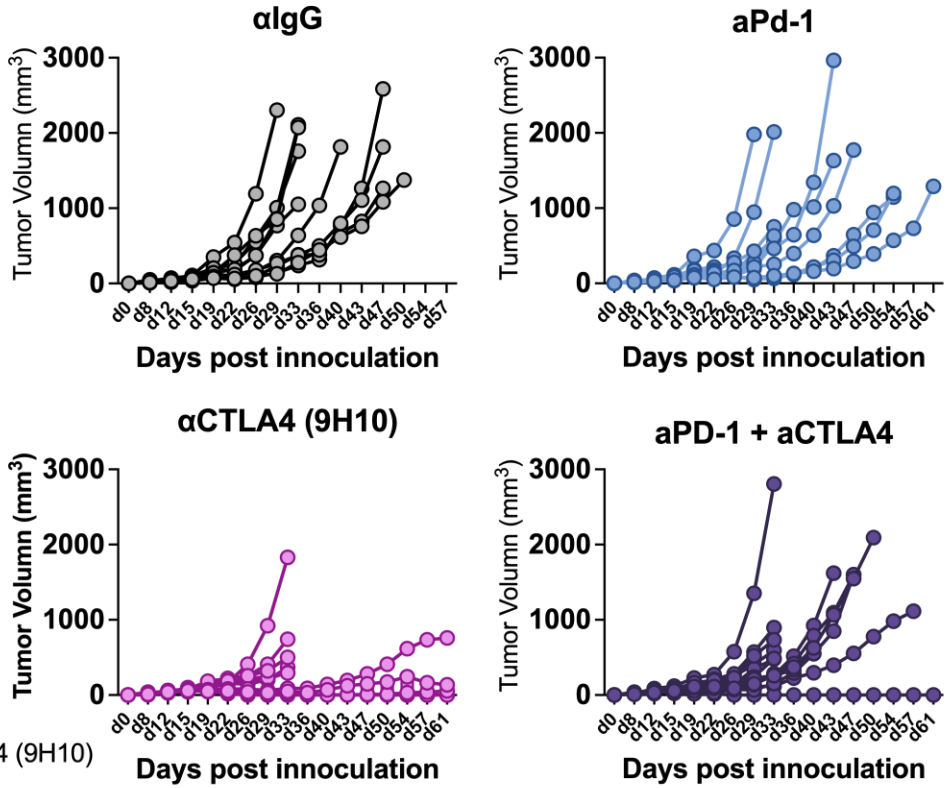
Unpublished

Syngeneic Murine sRCC Is Sensitive to Anti–CTLA-4 Therapy









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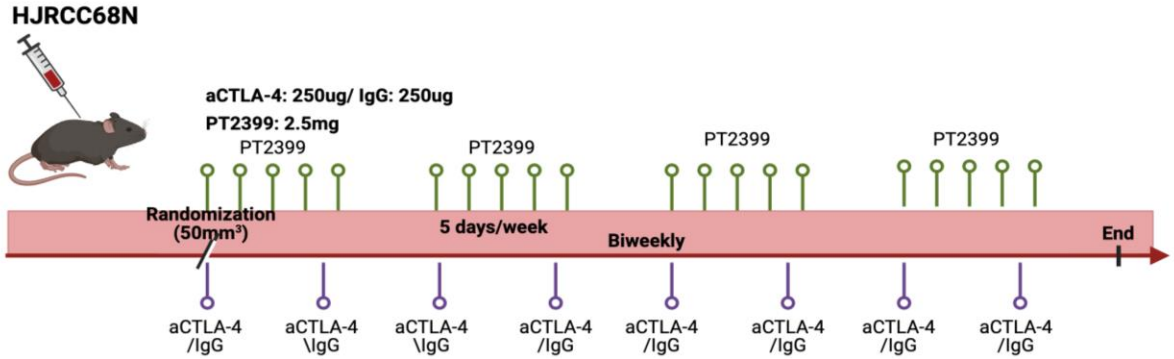
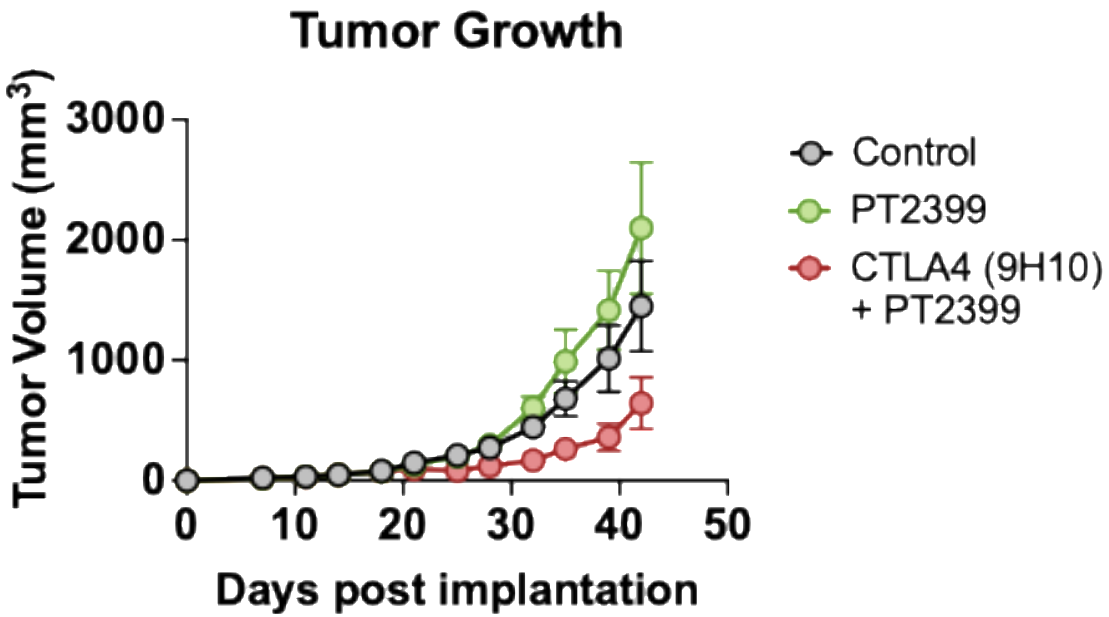
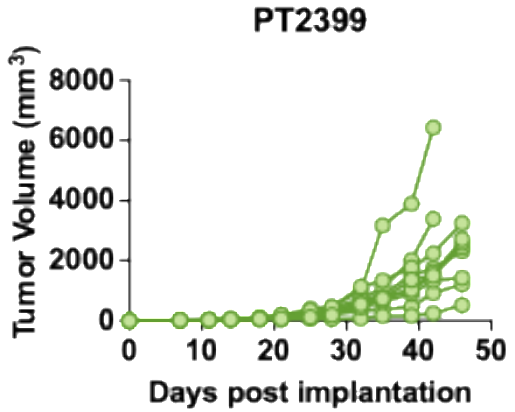
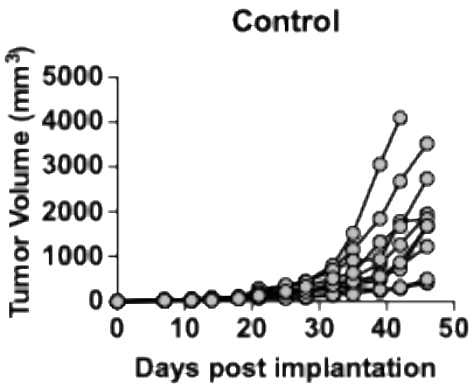
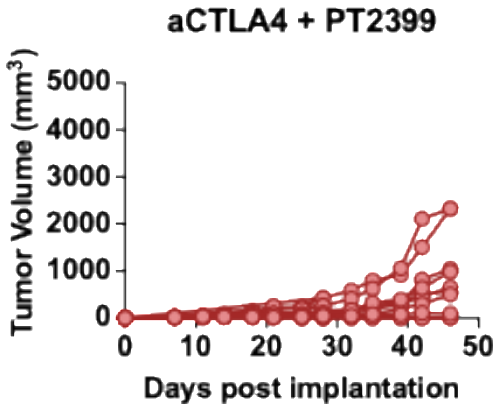
PT2399 (HIF-2α inhibitor) Does Not Inhibit Growth in Murine sRCC











Summary of the HJRCC68N Syngeneic sRCC Model

- **Model established:** novel murine sRCC line (HJRCC) in an immunocompetent C57BL/6J background
- **Histology:** features consistent with **sarcomatoid RCC** (pathology/IHC)
- **Growth: robust** subcutaneous and orthotopic growth in **males**; **attenuated/delayed** in females
- **TME (s.c.): Treg-enriched** within the $CD4^+$ compartment
- **Immunotherapy:** both **subcutaneous** and **orthotopic** tumors **respond to anti-CTLA-4**

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Take-Home Points

- **Vhl knockout:** biallelic Vhl loss is incompatible with in-vitro proliferation
- **Inoculum matters:** tumor establishment depends on initial viable cell number
- **Sex matters:** tumor growth can be sex-dependent
- **BLI caveat:** in immunocompetent mice, bioluminescence can under-report tumor burden—cross-validate with volumetrics (US/MRI)
- **Site matters:** the TME shifts between subcutaneous and orthotopic models

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Sherry Gao
Peng Li

David Solit (MSK)

Alexis Jones
Paul Gao
Aphrothiti Hanrahan

Mount Sinai

Luca Gusella

MSK Medical Oncology

Robert Motzer
Martin Voss

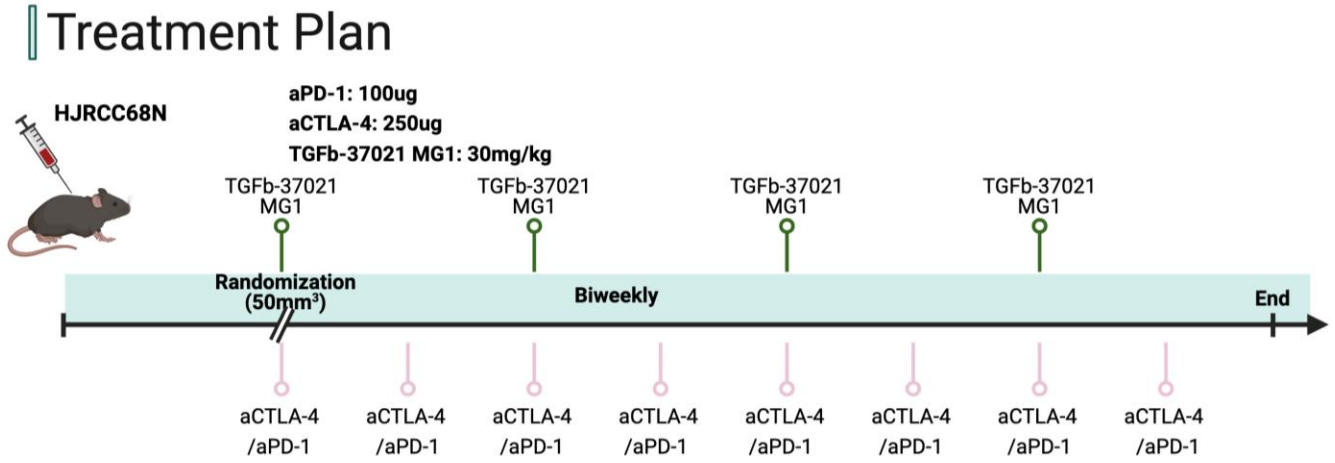
Urology (MSK)

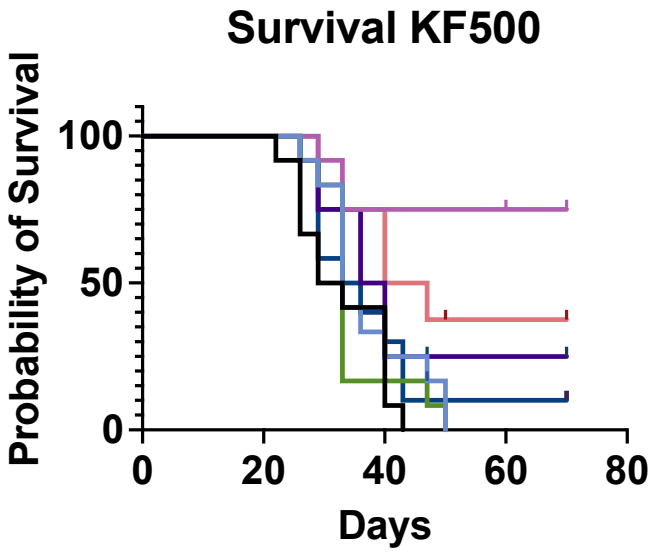
Paul Russo
Jonathan Coleman

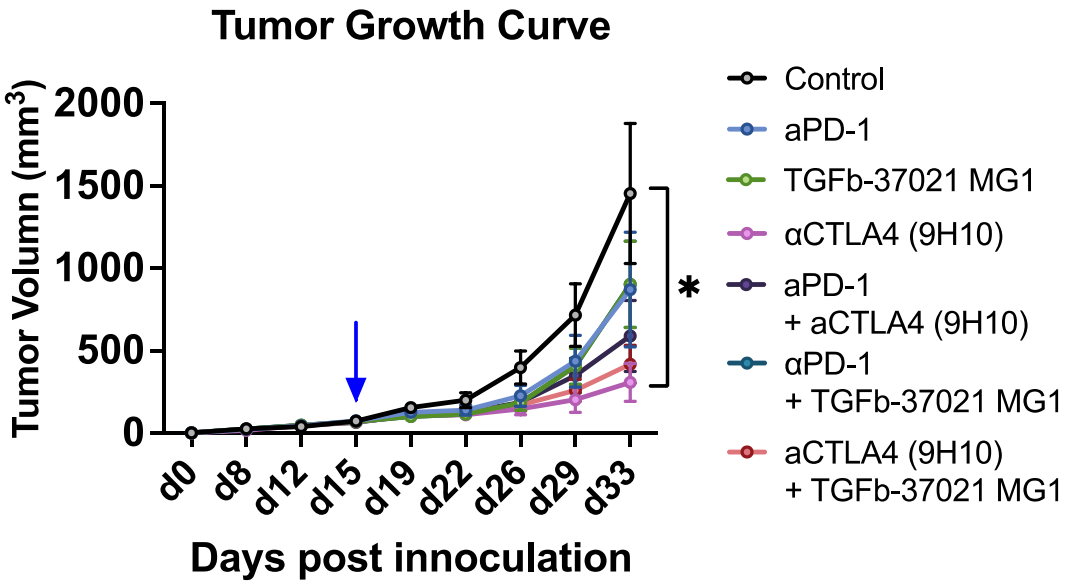
Pathology (MSK)

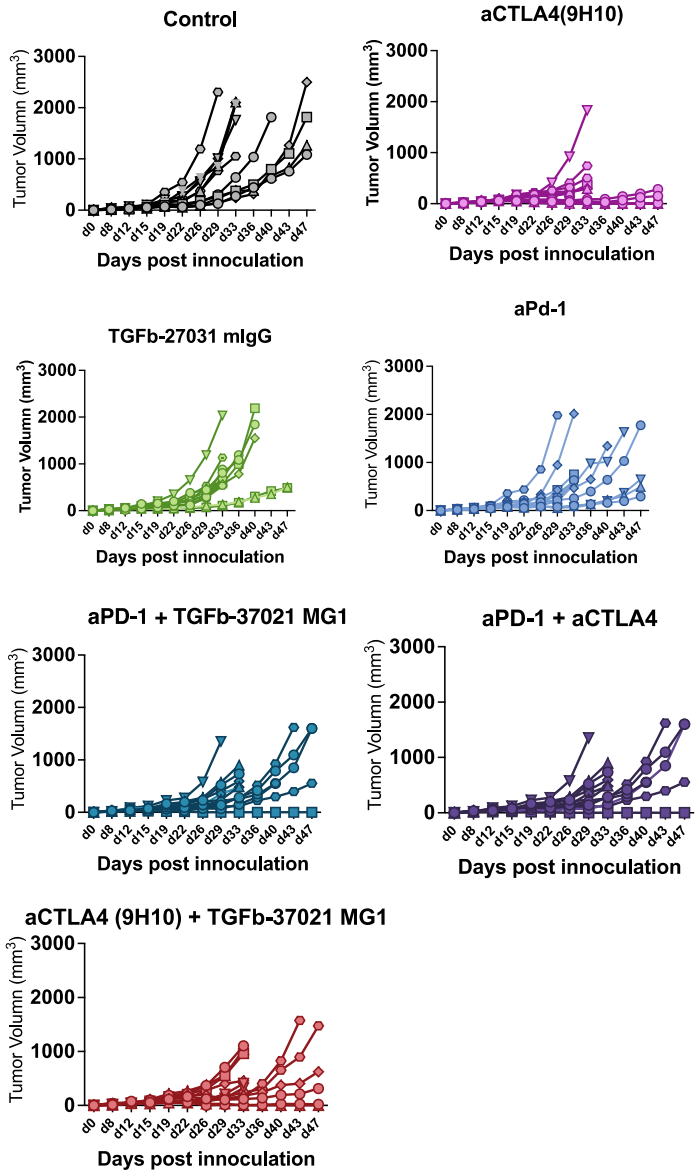
Yingbei Chen

TGF-β1 Inhibitor Fails to Suppress sRCC Tumor Growth









No Growth Inhibition with Anti-CCR8 in This sRCC Model