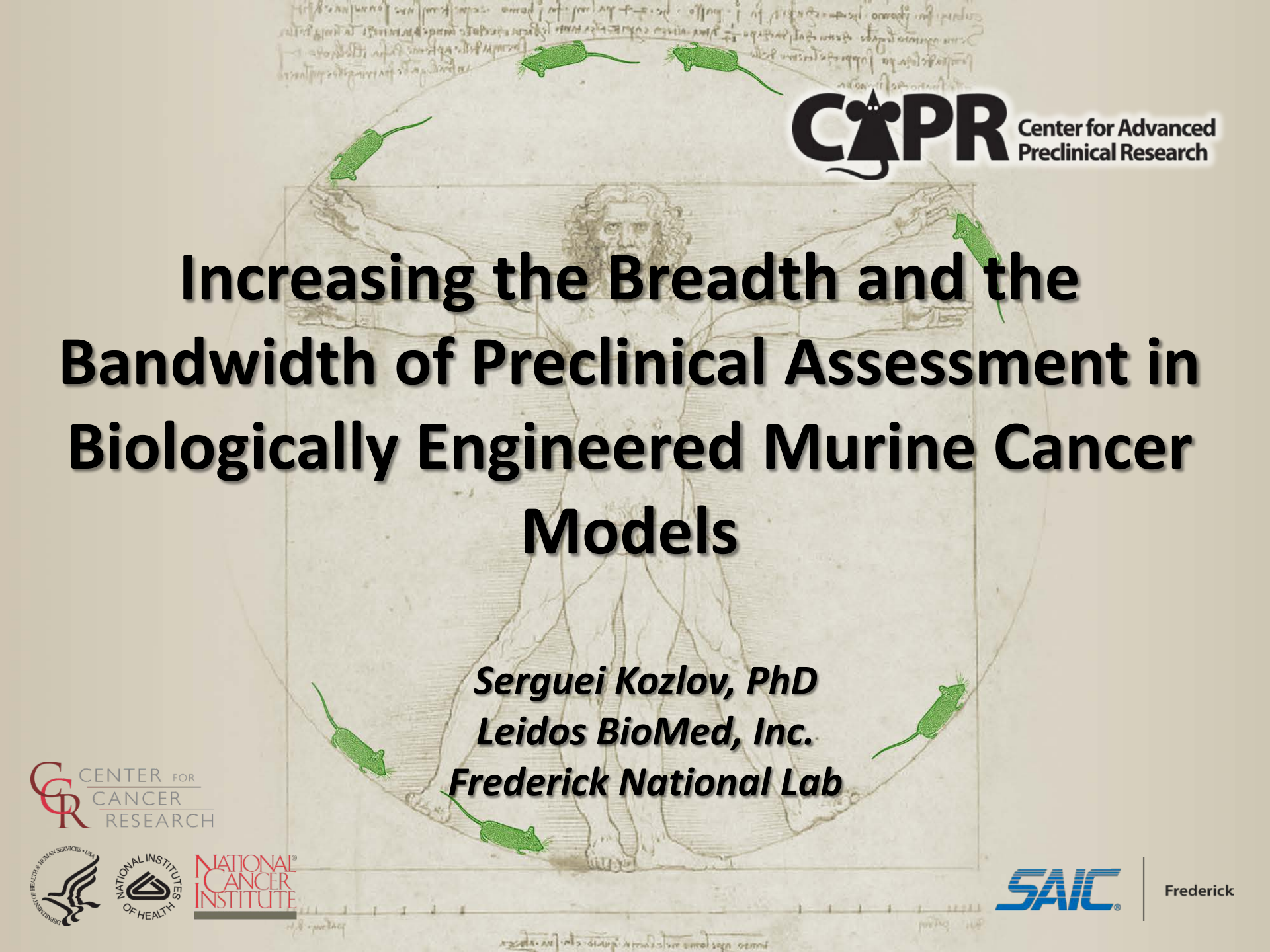

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Increasing the Breadth and the Bandwidth of Preclinical Assessment in Biologically Engineered Murine Cancer Models

Serguei Kozlov, PhD
Leidos BioMed, Inc.
Frederick National Lab

CCR CENTER FOR CANCER RESEARCH

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

SAIC Frederick

TALK OUTLINE:

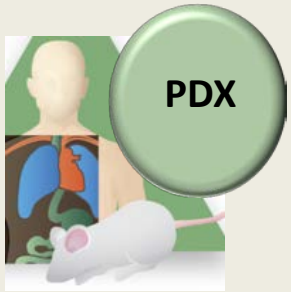
- **Non-germline GEMs: design overview and rationale for use in oncology translational research**
- **Application of ES technology to retool preclinical GEM model for astrocytoma/GBM**
- **Accelerating preclinical evaluation studies in orthotopic models for serous epithelium ovarian cancer**

I. Non-germline GEMs: design overview and rationale for use in oncology translational research

Clinical Needs Drive Cancer Model Evolution

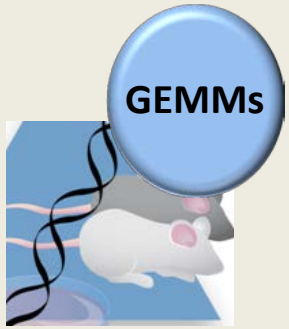
\+ PATIENTS are *THE* target for care

\- disease complexity
access to tissue
limited experimental variables
cost
limited patient resource



\+ patient-derived tumors
experimental replicates

\- lacks immunobiology
mouse stroma
bypasses initiation/progression
change possible



\+ initiation to progression
pathway-specific engineering
intact immune system
experimental replicates

\- not human
complex experimental systems
biology often not relevant to disease

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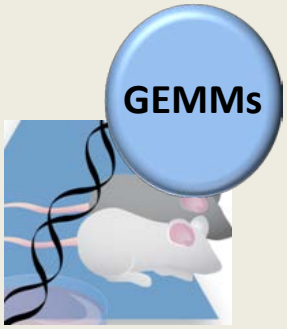
Challenges in Employing GEMM Models



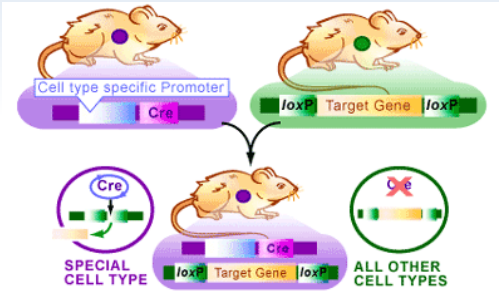
\+ initiation to progression
pathway-specific engineering
intact immune system
experimental replicates

\- not human
complex experimental systems
biology often not relevant to disease

- Require time for generation and modification
- Laborious and costly colony maintenance and genotyping
- Mendelian odds of breeding (particularly with 4+ alleles)
- Lethality of certain null mutations
- Low penetrability and long lead time for tumor to develop in some models
- Field effect and interactions with mutant stroma
- Rare progression to metastatic disease

Strategies in Designing the NG-GEMM Models

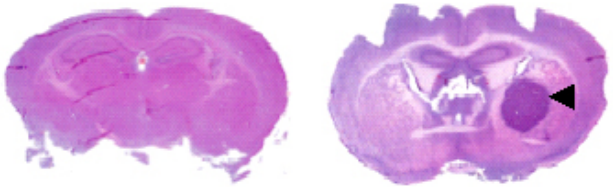
I. Conditional GEMMs



II. "Mouse in Mouse" NG-GEMMs:

- Chimeric models

- Orthotopic Allografts



III. "Human in Mouse" NG-GEMMs:

- Primary patient derived xenografts (pdx)

GEMM vs. NG-GEMM Models: Comparing Key Features

	Traditional GEMM	Conditional GEMM	Chimeric GEMM	Orthotopic GEMM	PDX
Timing/Penetrance	Depends/poor	Depends/poor	Usually high	High	Average
Synchronicity	Usually poor	Average-to-high	Average-to-high	High	Average
Host Immune System	Present	Present	Present	Present/Absent	Absent
Cost/speed in cohort generation	Usually no	Usually no	Yes	Yes	Model dependent
Relevant Stroma	Yes	Yes	Yes	Yes/partial	No/possible
Genome Instability	Generally no	Generally no	Likely	Generally no	Likely

Based on Heyer, et al., *Nat. Rev. Cancer*, 2010

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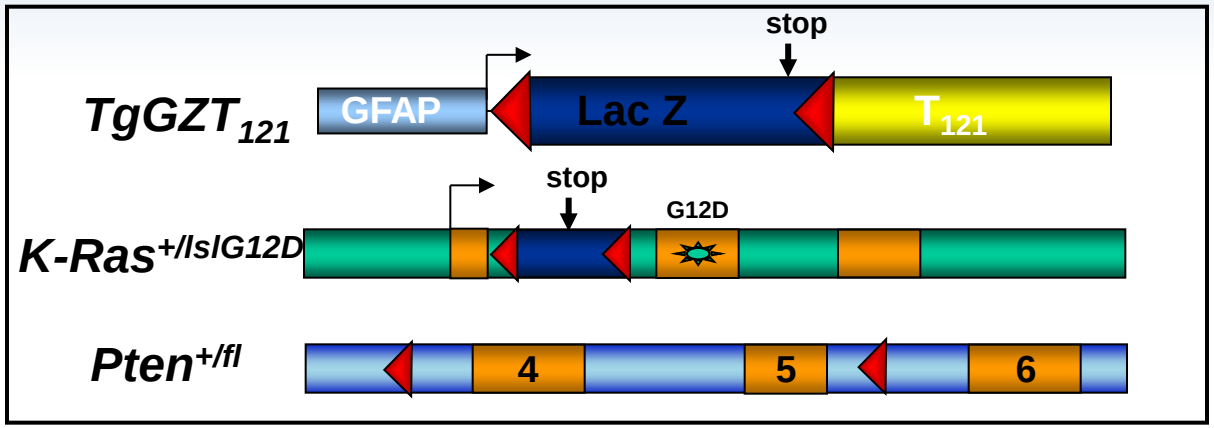
II. Application of stem cell technology to retool preclinical GEM model for astrocytoma/GBM

CAPR Technology and Optimization Team
Dr. Tomas Vilimas
Dr. Serguei Kozlov

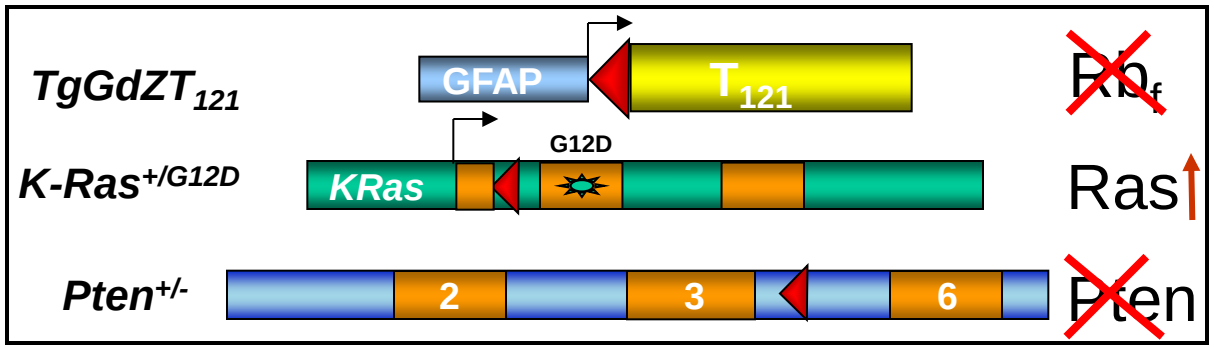
Engineering a Preclinical GBM Model

Drs. Qian, Song



mouse genotype

X *GFAP-CreER^{TAM}* (*K. McCarthy UNC*) ↓ *+ 4-OHTam* → **"TP/TRP" Model**



*adult astrocyte genotype

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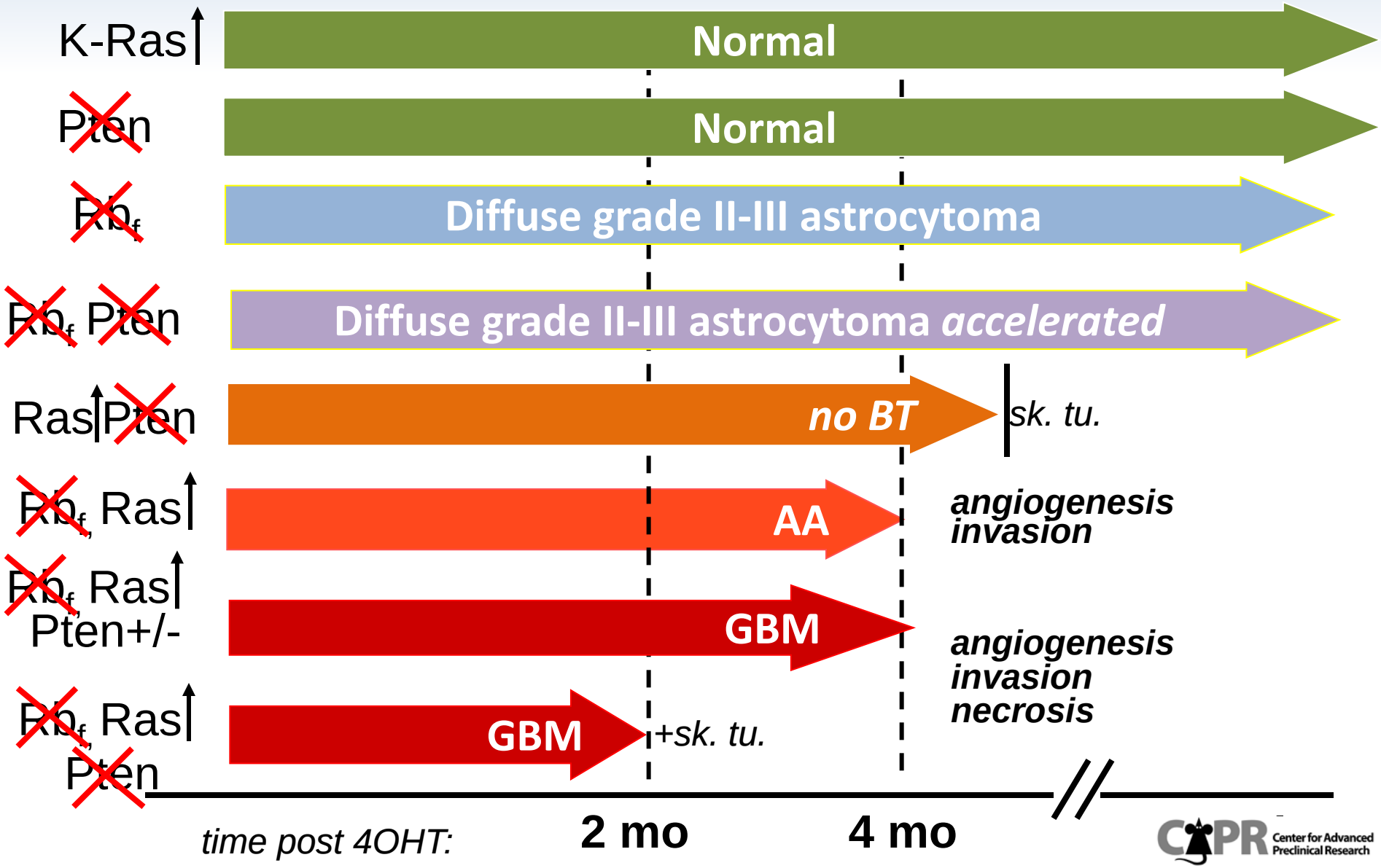
Engineering a Preclinical GBM Model



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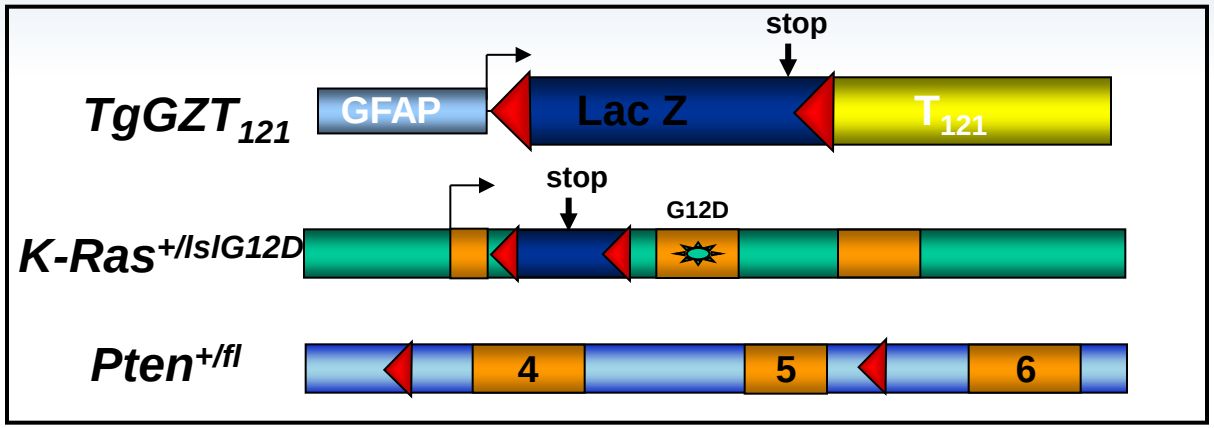
Engineering a Preclinical GBM Model

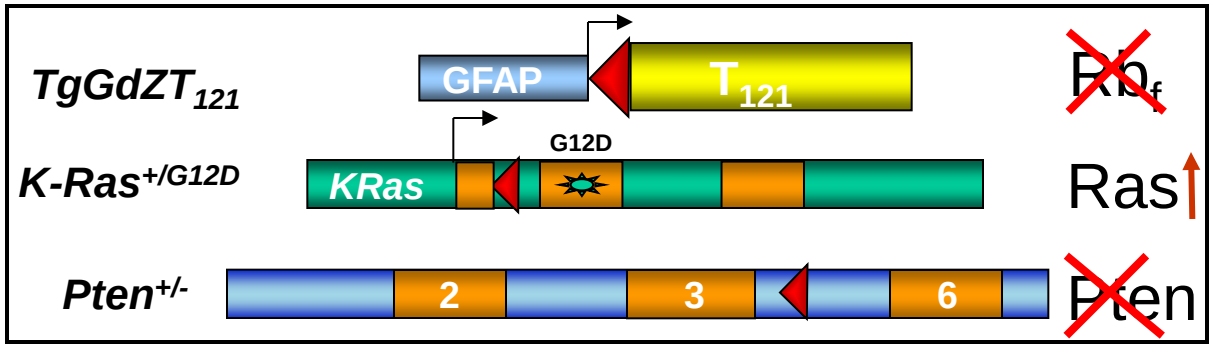


mouse genotype

X *GFAP-CreER^{TAM}*
(K. McCarthy UNC)

+ *4-OHTam*

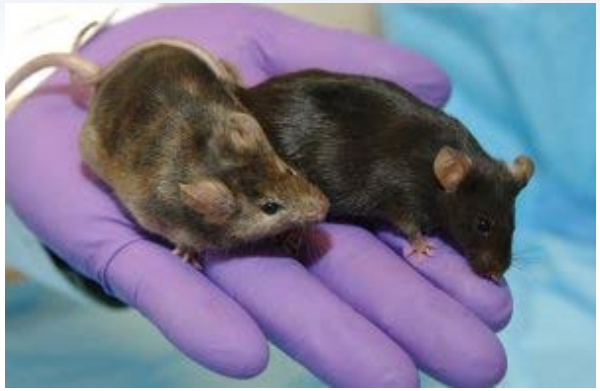
4/5 alleles

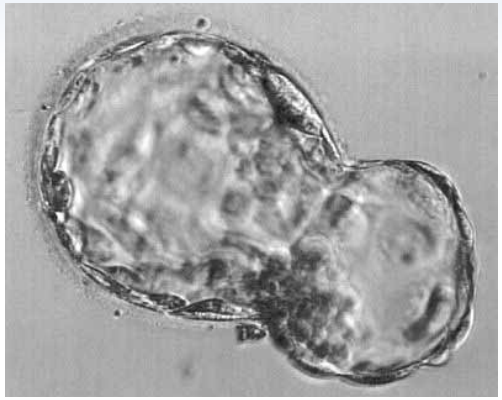


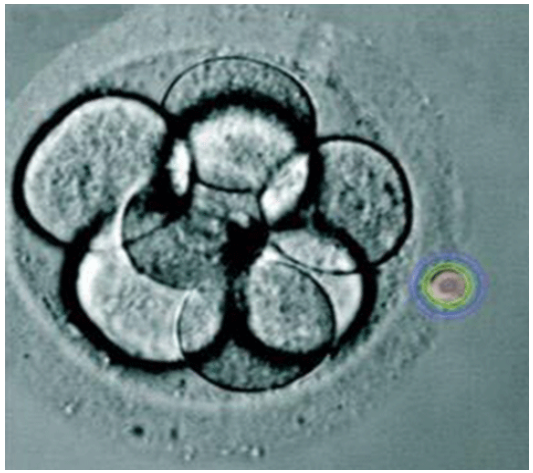
*adult astrocyte genotype

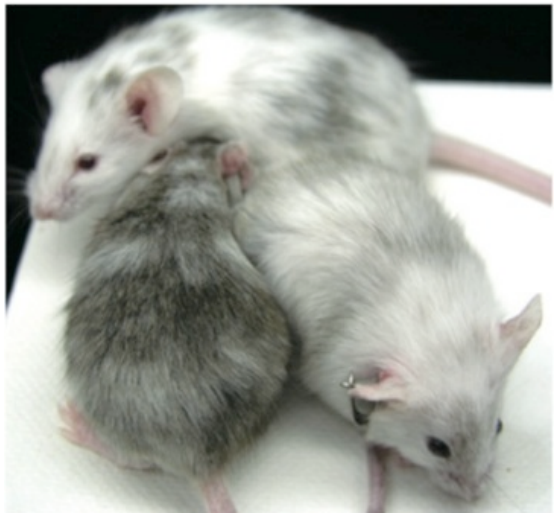
CANCER-CAUSING

ES Based Cohort Generation: The Workflow









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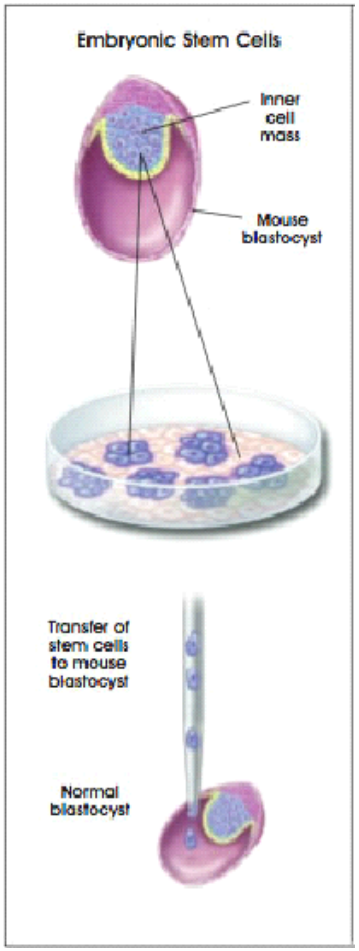
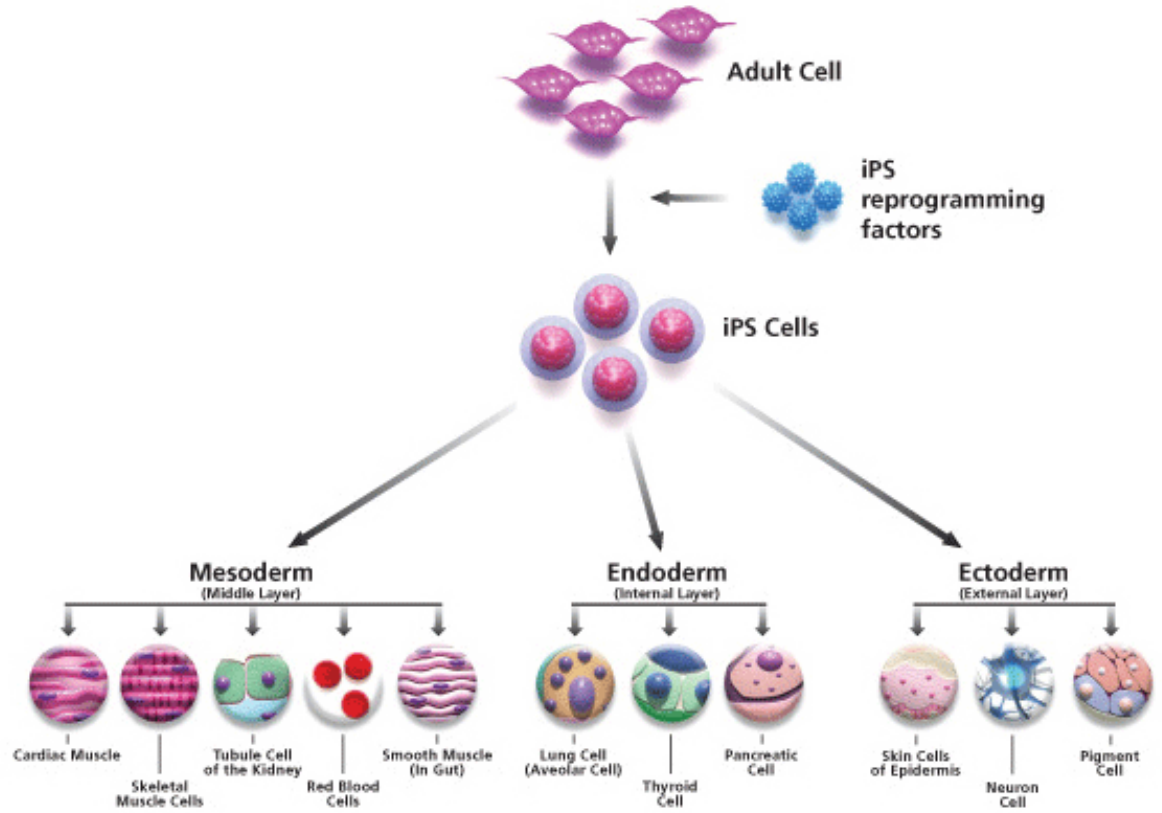
Approaches to Generate Pluripotent Cell Lines

A. Conventional generation of ES cells



B. Derivation of iPS cells via somatic cell reprogramming



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Features of Chimeric Non-Germline GEMs

Pros:

- cost-conscious upscaling potential;
- handling broad diverse portfolios of cancer GEMs becomes a feasible objective;
- consistent genetics among cohorts (e.g. comparative transcriptome studies);
- availability of multiple clonal cohorts may be exploited in gene discovery studies;

Cons:

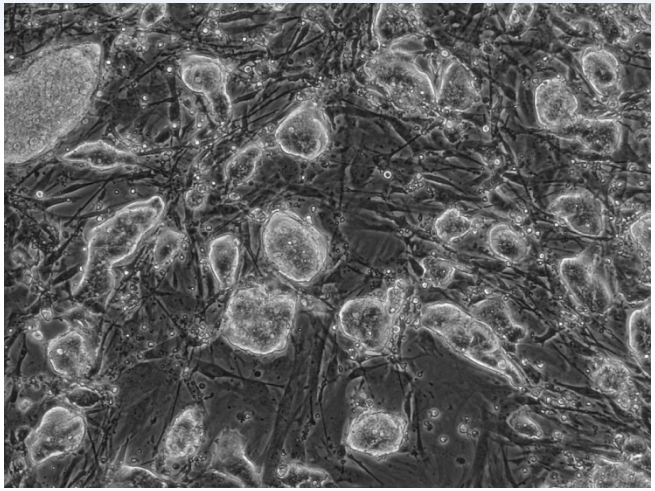
- requires specialized expertize and resource for embryo manipulations
- genetic diversity of experimental tumor sets is reduced
- epigenetic instability of ES (and iPS) cells

Features of ES Clones Established for TP/TRP Model

A

Genetic Background	# of Cultured E3.5 Embryos	# of Established ES Lines
C57/Black6N	73	26 (36%)
HGA model (TP/TRP)	153	17 (11%)
Prostate Cancer Model	29	4 (14%)

B



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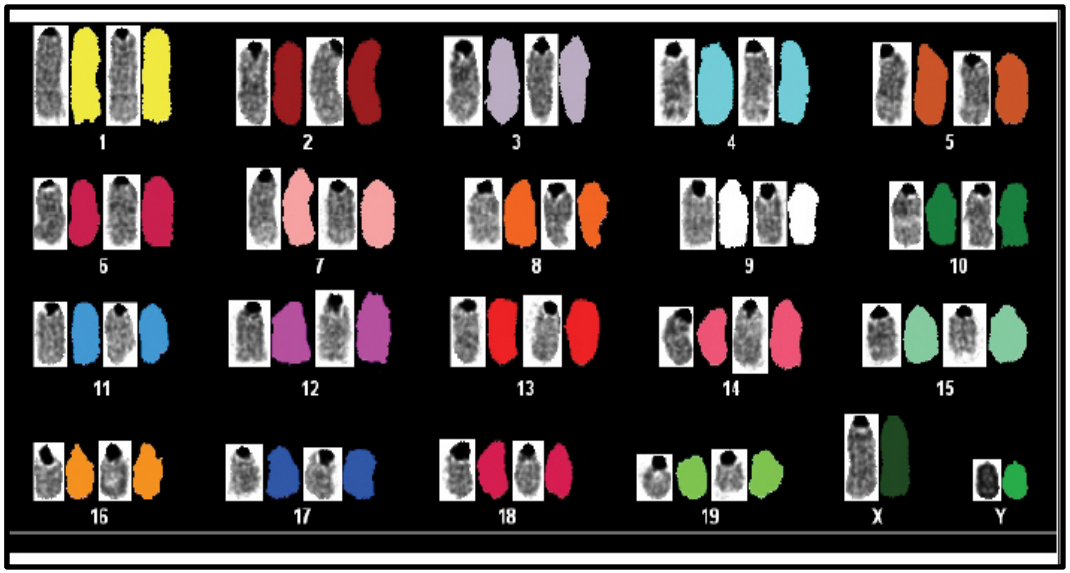
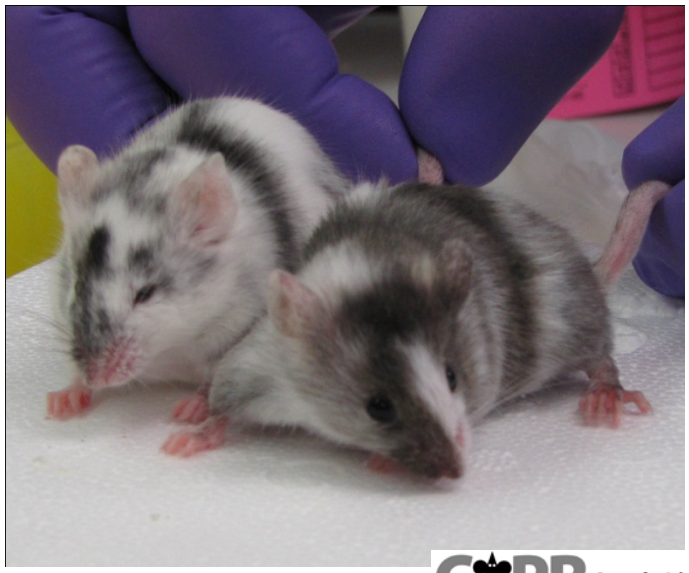


D



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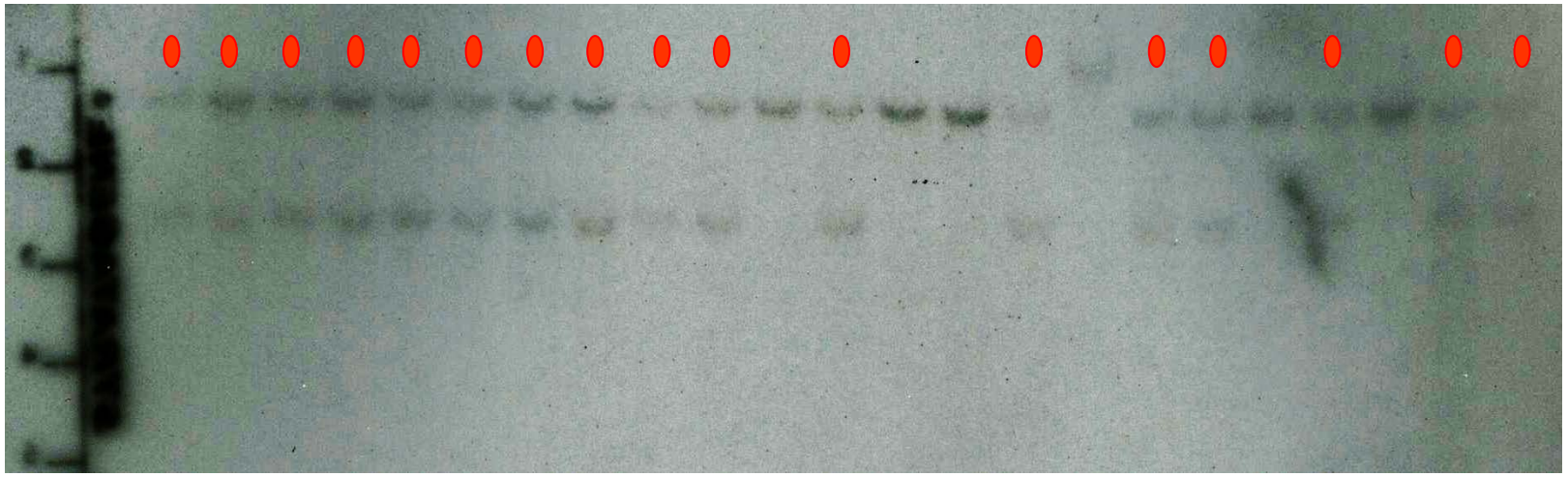
GEM-Derived ES Lines: Amenable to Gene Targeting

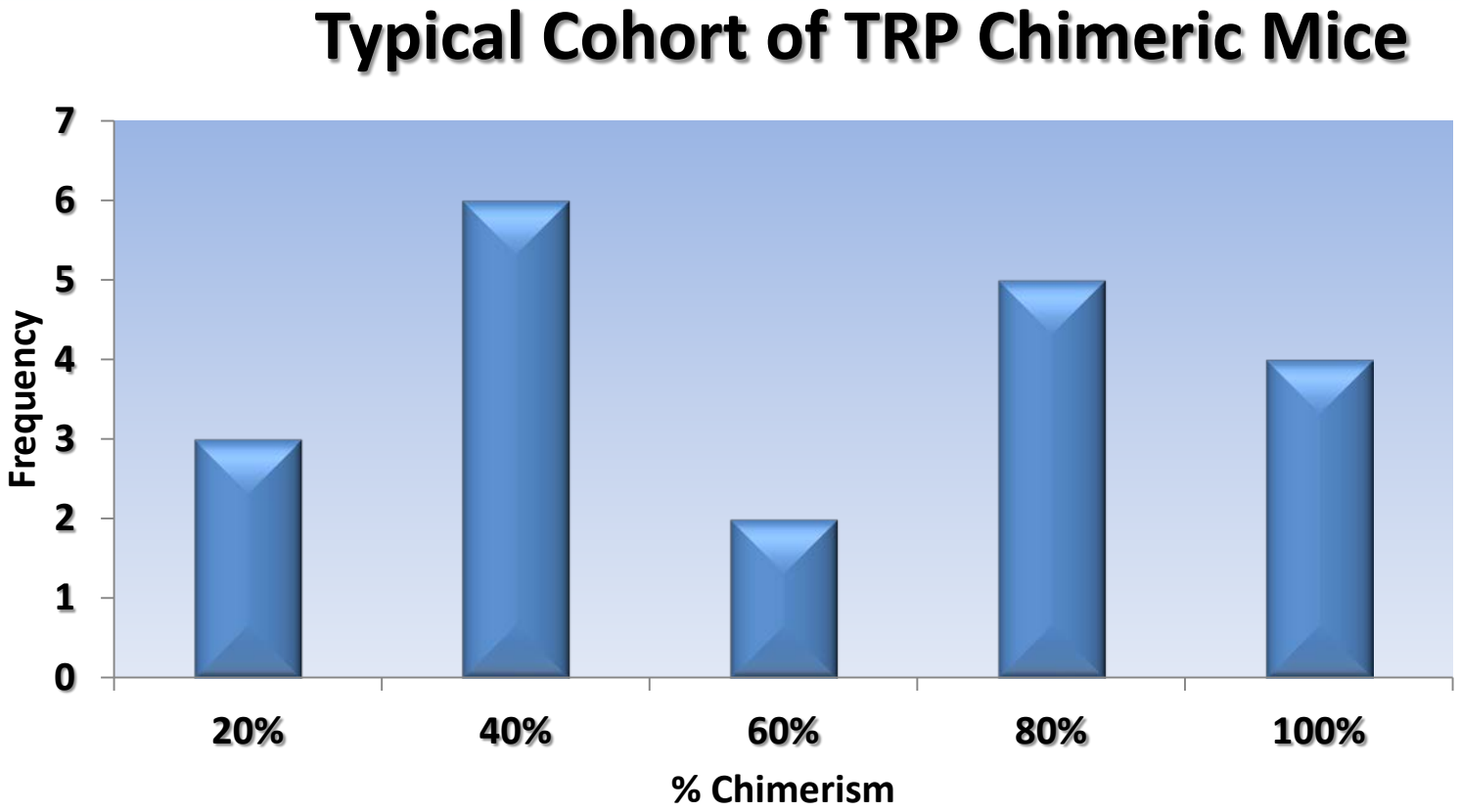
❖ **GFP-Luc4 Knock-In into ROSA26 locus in TRP-B4 ES cells**



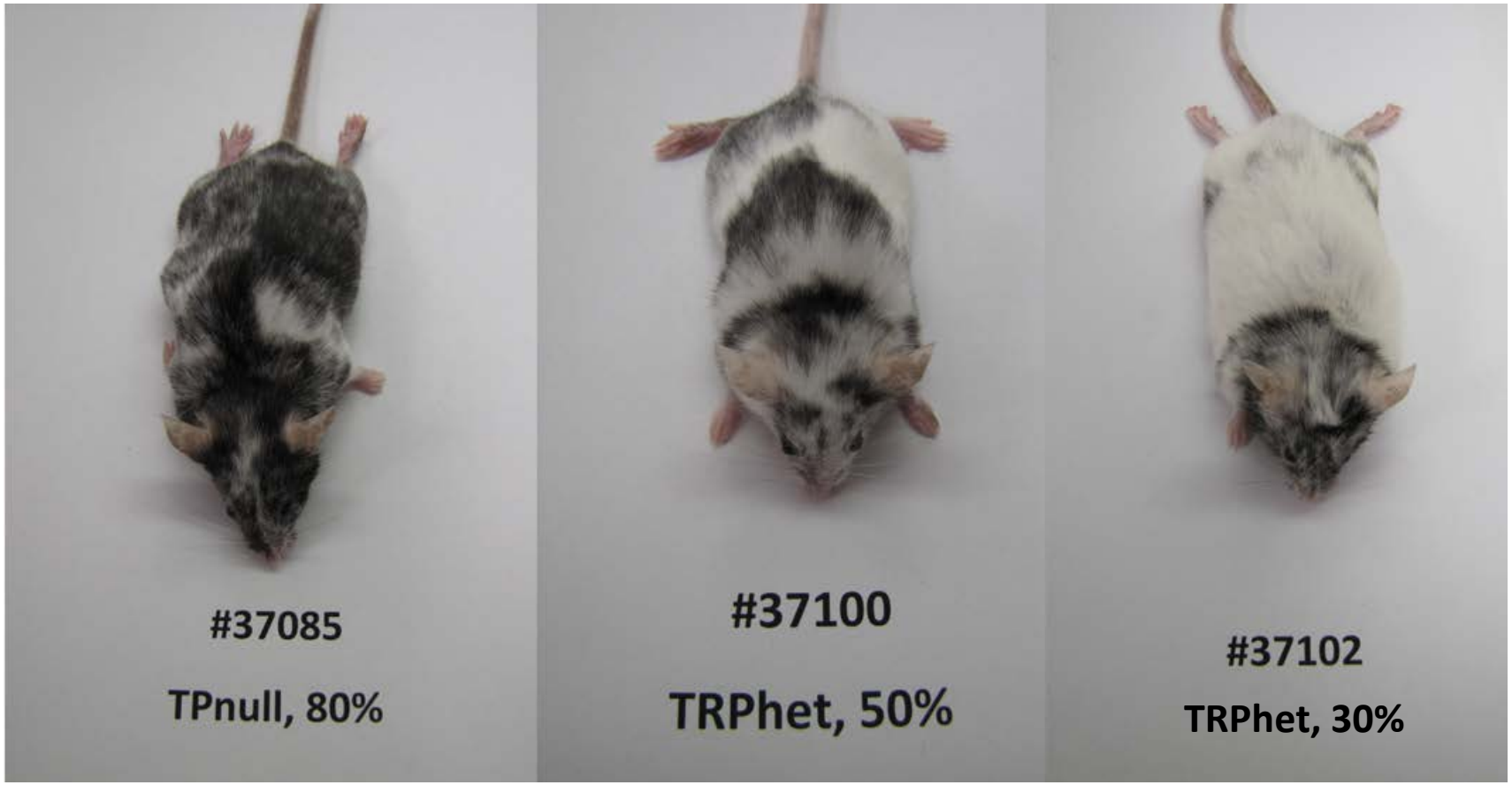
17 HR/24 total = 70%

- *immediate applications*: *in vivo* labeling/tagging; rapid screening of additional genetic events, e.g. detected by clinical tumor epidemiology

Example of a Non-Germline GEM Cohort (TRP)



A Representative Set of TRP Chimeric Animals



TP/TRP Chimeras: Excerpts of Pathology Assessment

Common findings:

- multifocal atypical gliosis (mild to severe), later – grade II progressing to grade III lesions
- cortex (frontal) and OB are mostly affected
- most neoplastic astrocytes are T121+
- Liver/Spleen/Kidney – no significant lesions

Chimera #37085 (TPnull, six weeks p/i):

- multifocal atypical gliosis, severe with grade II borderline lesions

Chimera #37100 (TPhet, six weeks p/i):

- multifocal atypical gliosis, mild

Chimera #37102 (TPnull, twelve weeks p/i):

- multifocal atypical gliosis, moderate

Chimera #37091 (TPhet, twelve weeks p/i):

- multifocal atypical gliosis, moderate to severe

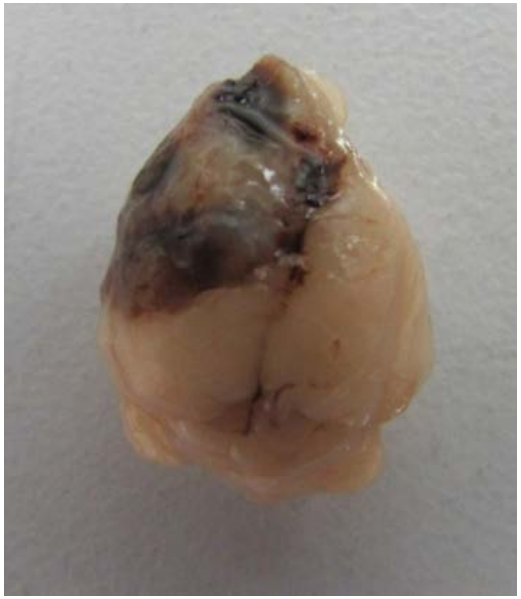
Chimera #37092 (TPnull, six months p/i):

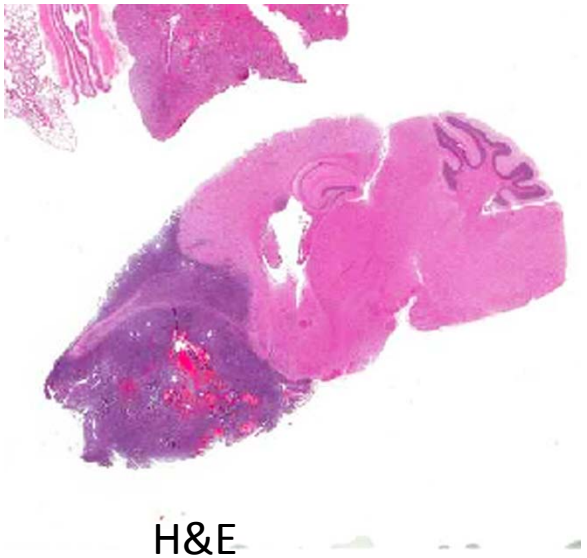
- multifocal atypical gliosis, severe, multiple grade II lesions

Chimera #37093 (TPhet, six months p/i):

- border line to moderate grade II lesions

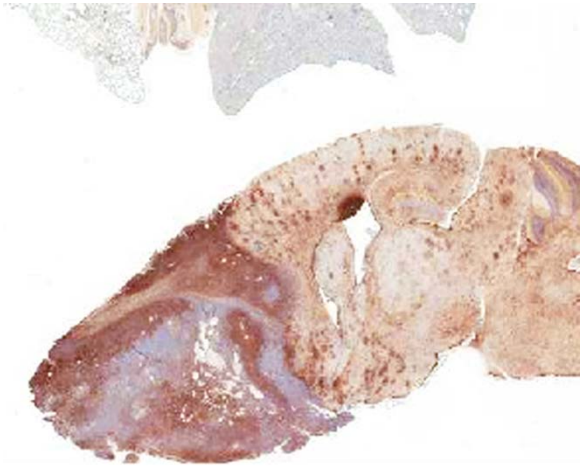
Histopathology of Grade IV GBM in Chimeric TRP Mice





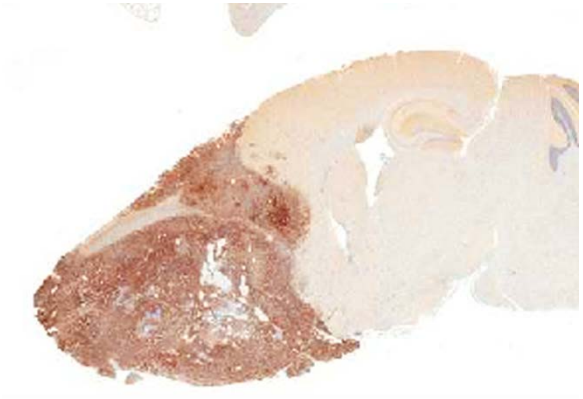
H&E

Nestin

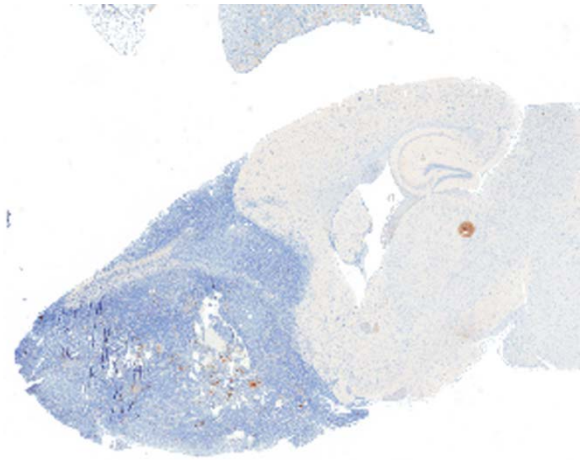


GFAP

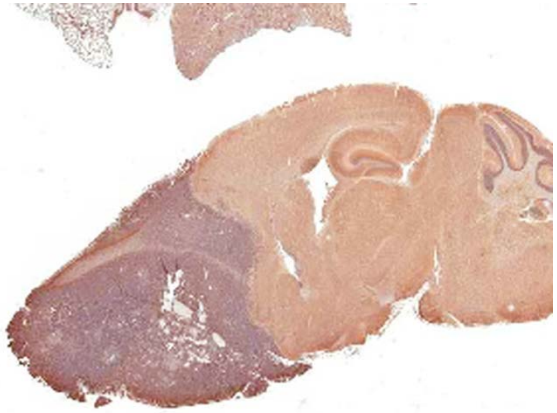
Histopathology of Grade IV GBM in Chimeric TRP Mice, cont'd



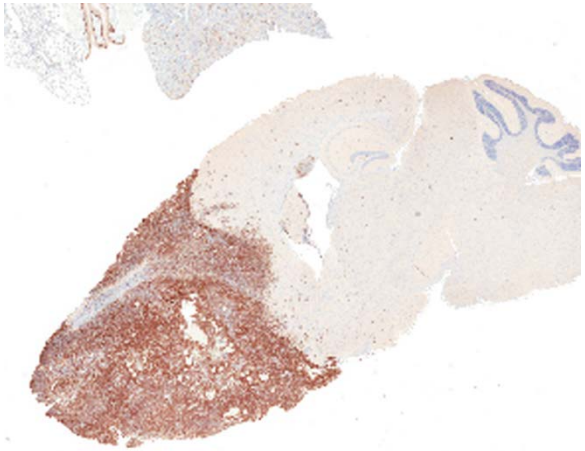
p53



CLC3

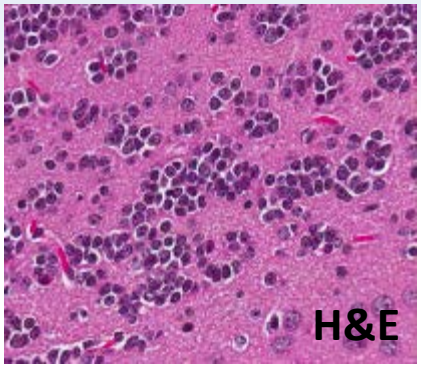


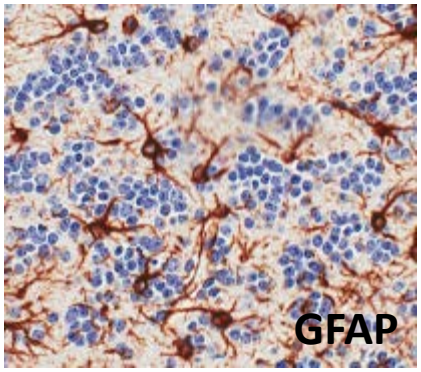
Olig2

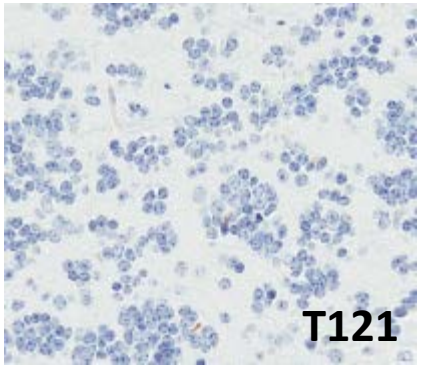


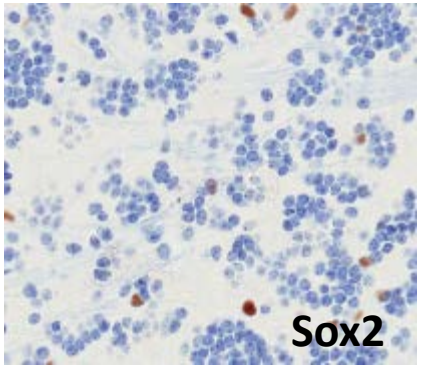
Ki67

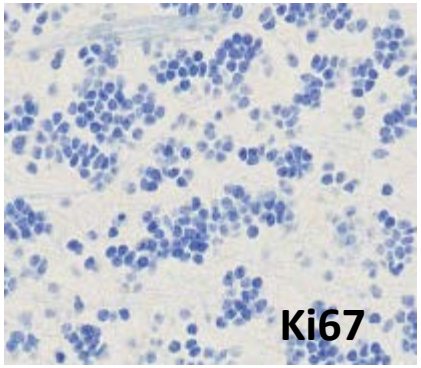
Non-Induced TRPhet Brain (Olfactory Bulb, 10X)
No lesions, normal GFAP+ astrocytes, rare Sox2, no T121/Ki67











Courtesy of Dr. Martin, DVM

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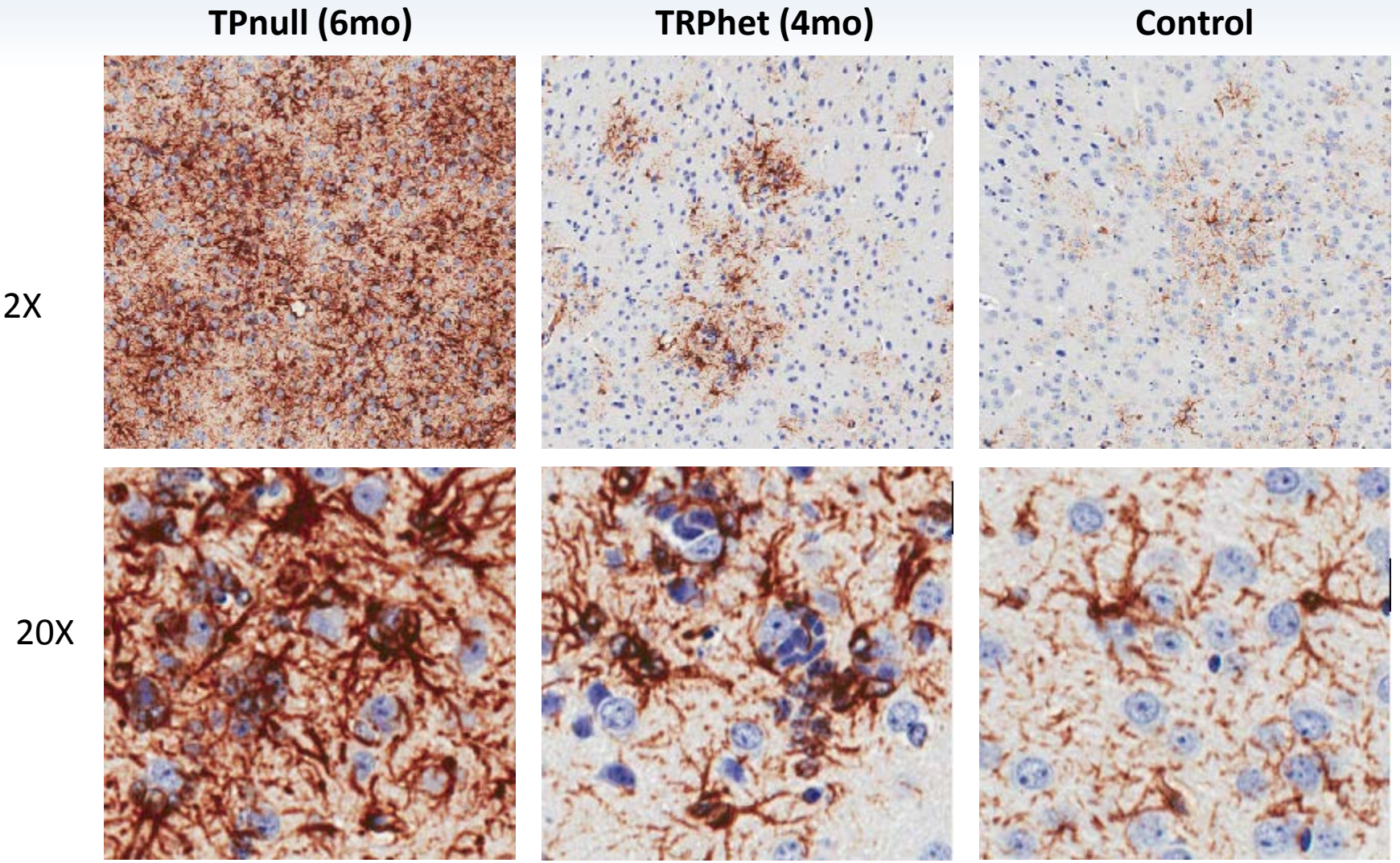
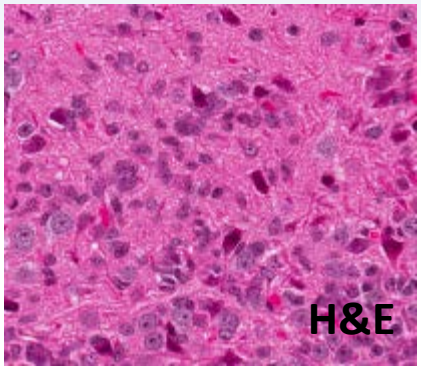
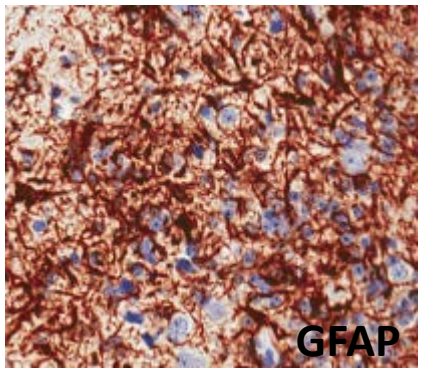
Induced chimeras develop foci of neoplastic (GFAP+) astrocytes similar to TRPhet GEMs however the lesions are multifocal vs. diffuse in the GEM

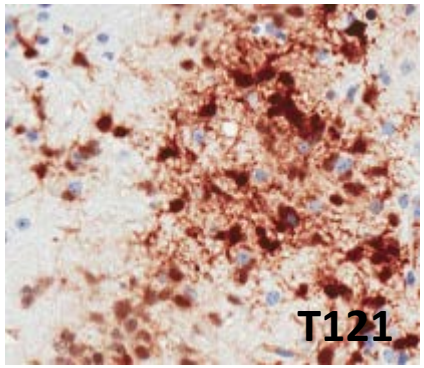


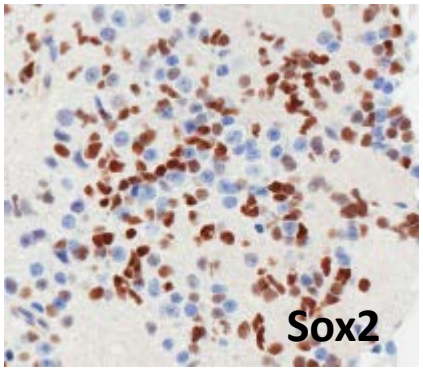
Chimeras with grade II lesions neoplastic T121/GFAP/Ki67/Sox2 expressing astrocytes

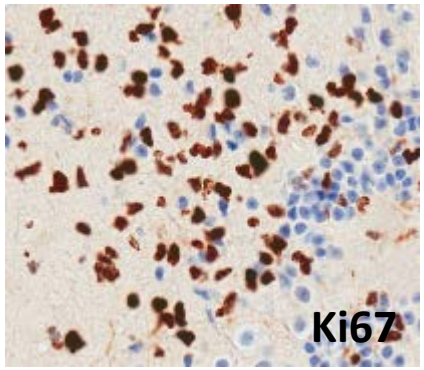
TRPhet Chimera



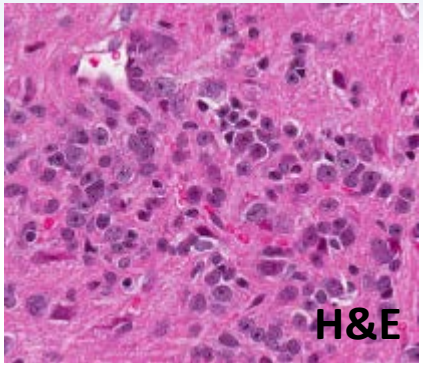


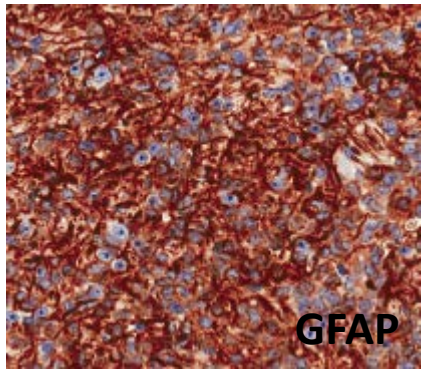


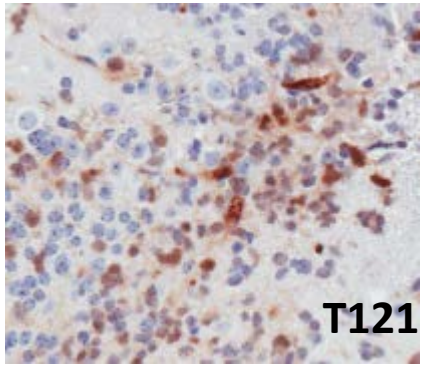


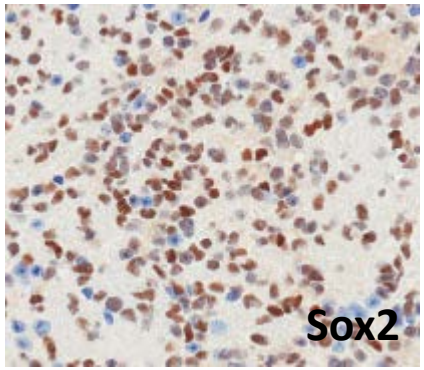


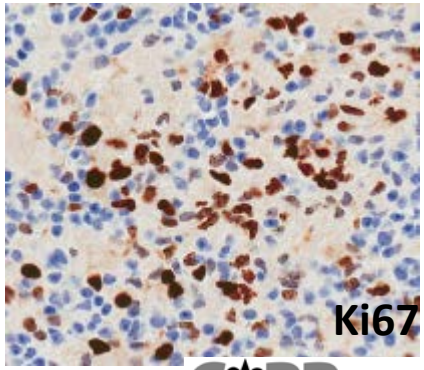
TPnull Chimera











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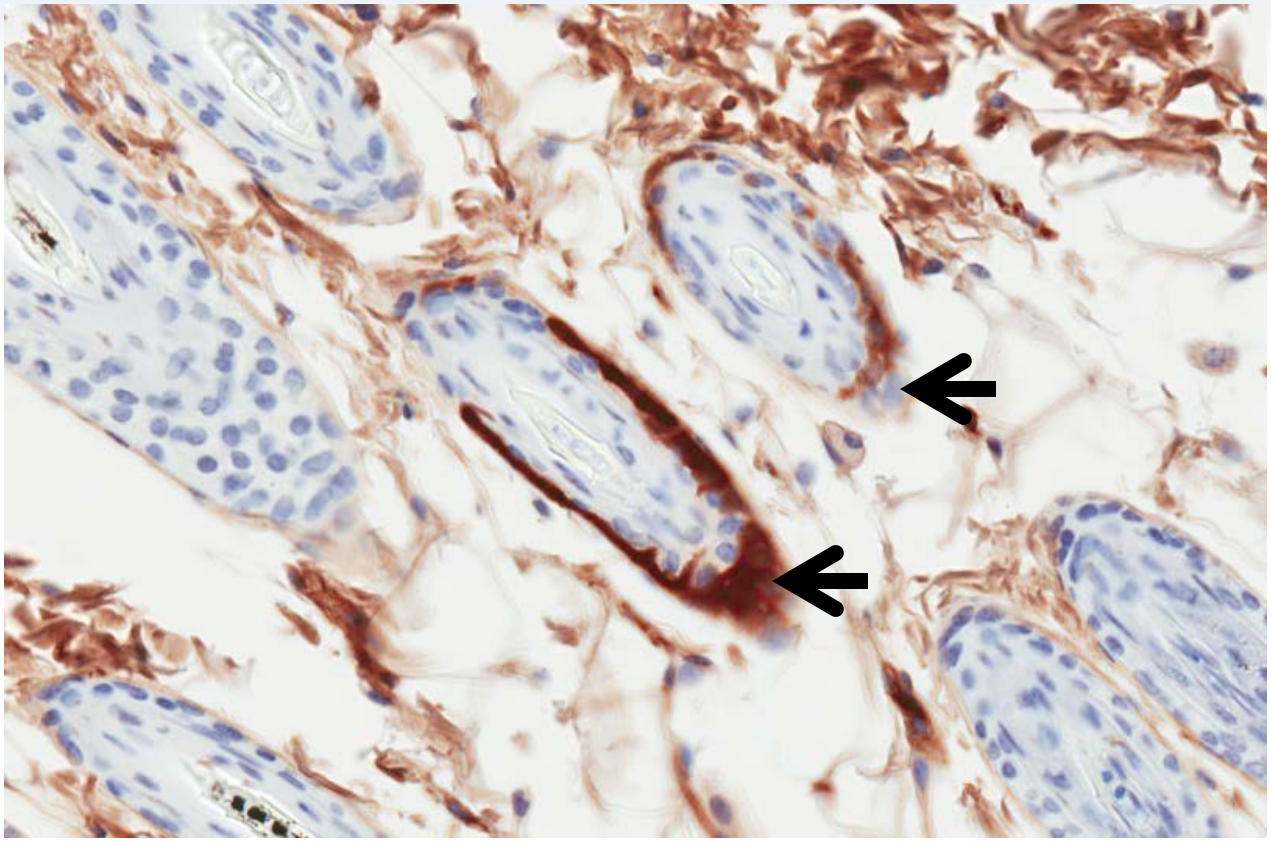
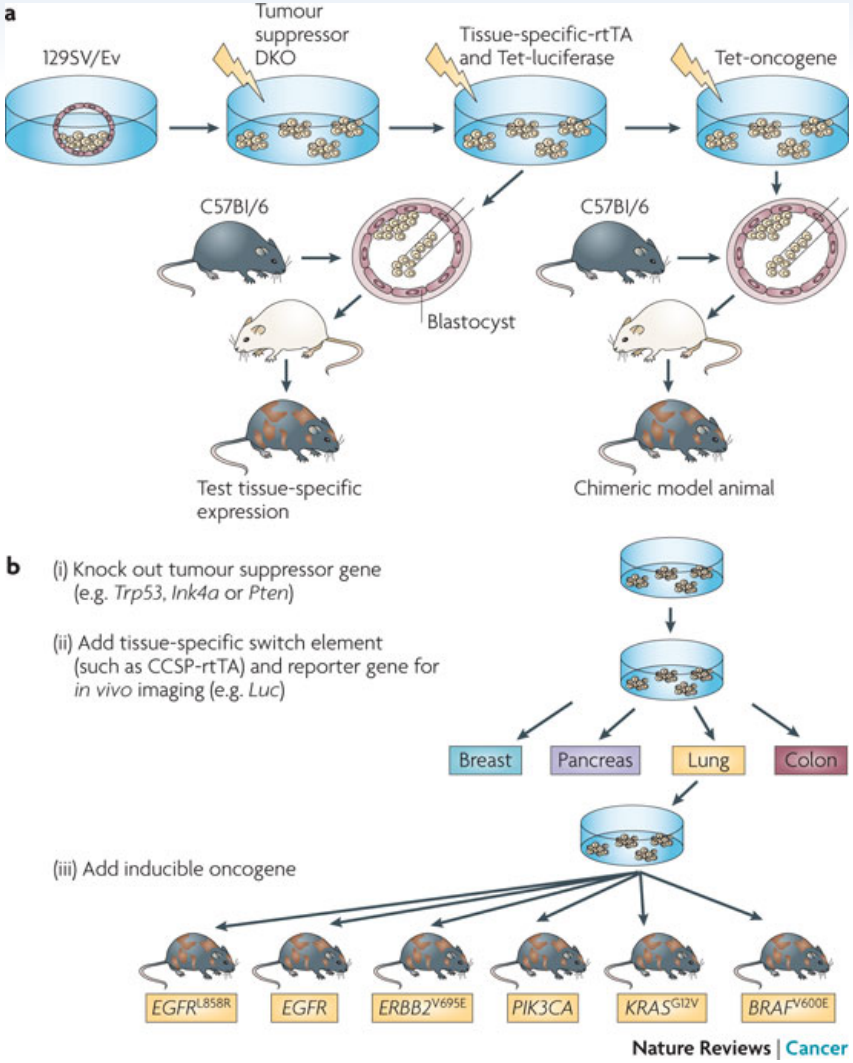
Prominent T121 Expression in occasional hair follicles



<u>"Off-target carcinogenesis":</u>

- **Can not be resolved in *germline* GEMs**
- **May be alleviated in *non-germline* GEMs (chimeric models)**

Summary of Chimeric NG-GEMs: industry approach to preclinical drug development



AVEO Pharmaceuticals

Nat. Biotech, Jan 2010

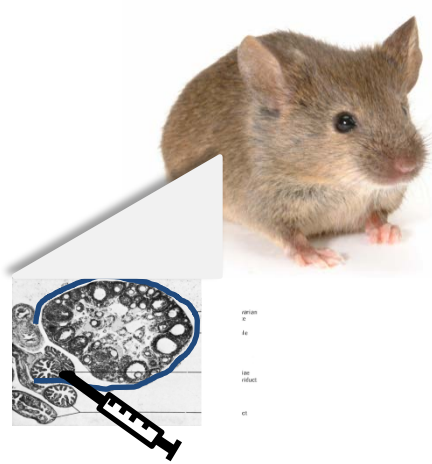
Listed benefits:

- **Allelic series with similar molecular lesions positioned at different sites**
- **Elimination of a "field effect"**
- **Amenable timeline of cancer progression (even in aggressive models) to afford good therapeutic windows**
- **Synchronous onset of carcinogenesis**
- **Speed/costs in generating cohorts**

III. Derivation and validation of an orthotopic model for serous epithelium ovarian carcinoma

CAPR Research and Development Team
Dr. Simone Difilippantonio, team leader

Generation of Mouse Models for Serous Epithelial Ovarian Cancer (SEOC)



Inducible events:

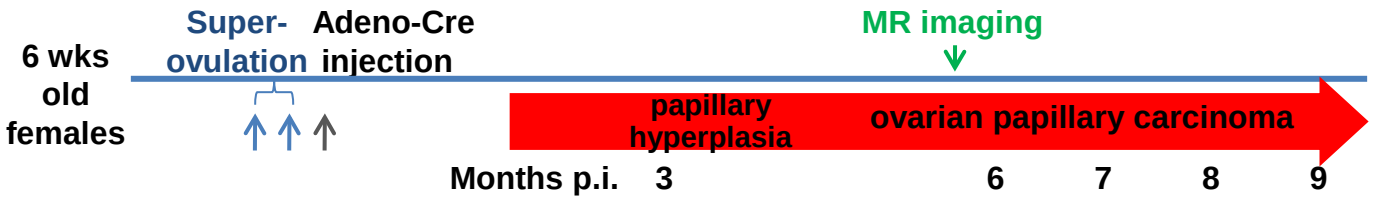
Rb_f inactivation (via K18-LSL-T121* BAC Tg)

P53 mutation/loss (via p53 mutation or conditional null)

Brca1 or Brca2 loss (via Brca1/Brca2 conditional null)

**dominant negative inactivates pRb, p107, p130, thus removing redundancy*

De novo model: Intra-bursal injection of adeno-Cre



Szabova, et al., *Cancer Res.*, 2012

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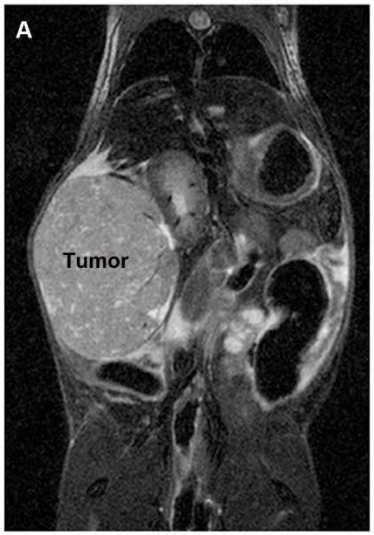
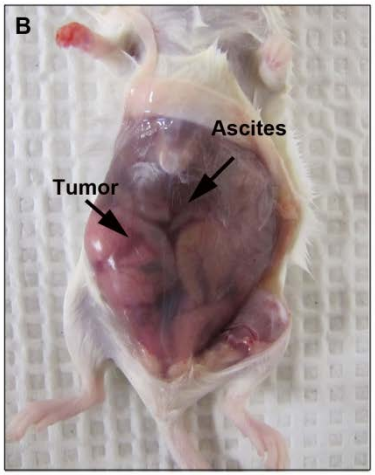
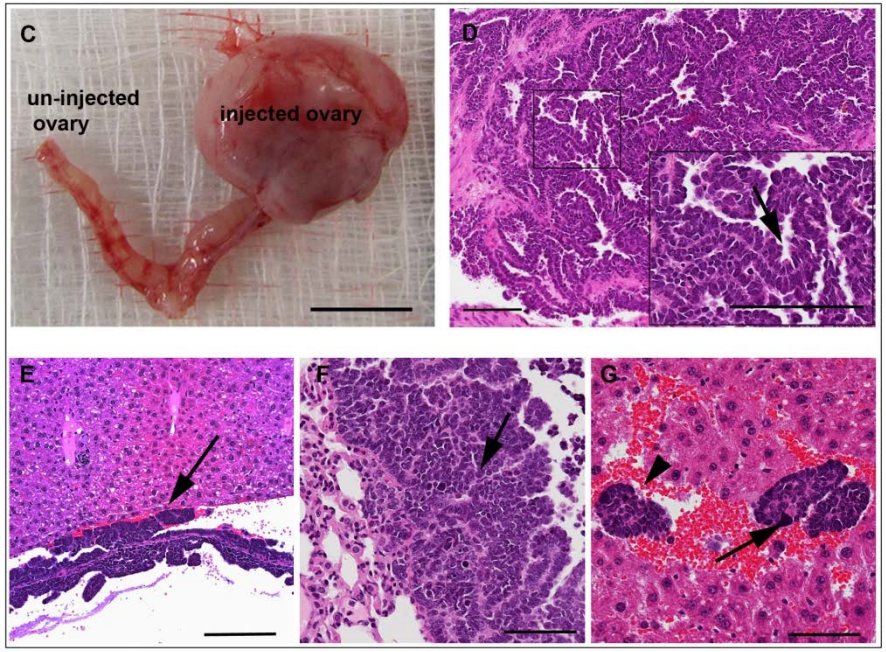
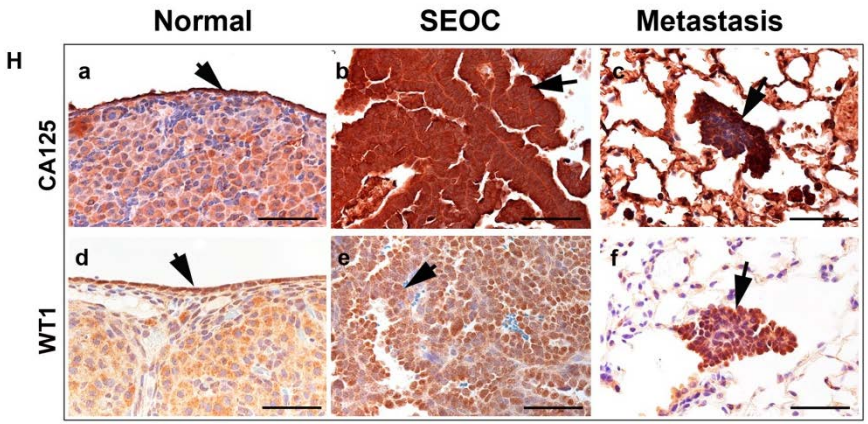
De novo mouse model of serous epithelial ovarian cancer (SEOC)









Szabova, et al., *Cancer Res.*, 2012

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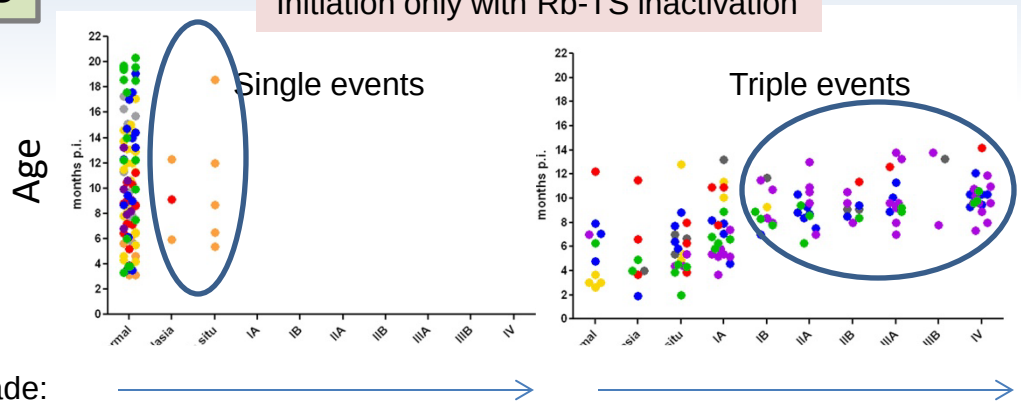
SEOC GEMM: Human Similarity in Molecular Attributes

Disease Outcomes

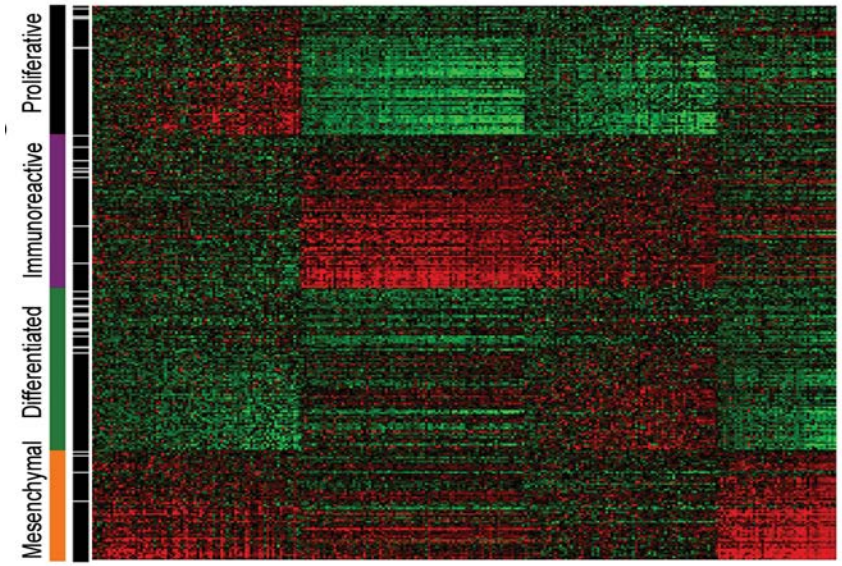
Initiation only with Rb-TS inactivation

Progression requires p53 missense/loss; Brca1 or 2 loss subtypes



Human vs. mouse SEOC

transcriptome



metabolites

	Human ovarian cancer (Fong et al.) TISSUE	Mouse ovarian cancer (our study) SERUM
α-hydroxybutyrate	↑	↑
α-tocopherol	↑	↑
β-hydroxybutyrate	↑	↑
citrate	↑	↑
short chain acyl carnitines	↑	↑
taurine	↓	↓

Szabova, et al., *Cancer Res.*, 2012

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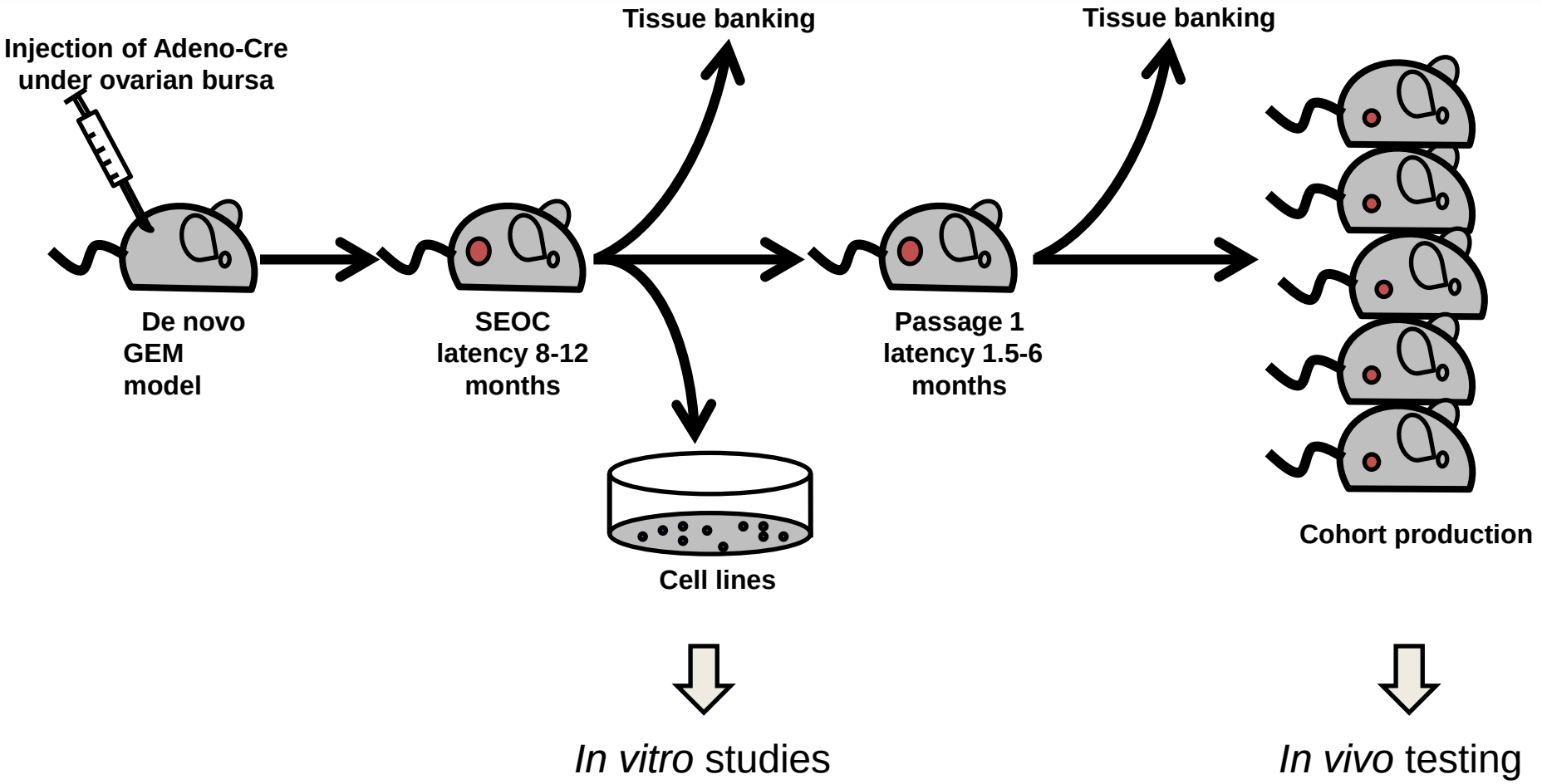
“Second generation” ovarian models



Primary and ascites-derived cell lines from adeno-Cre induced mice with ovarian carcinomas available for *in vitro* testing

ASCITES LINES

K18-T121$^{tg/+}$/Brca2$^{fl/fl}$/p53$^{R172H/fl}$
15825 ASC

K18-T121$^{tg/+}$/Brca1$^{fl/fl}$/p53$^{R172H/fl}$
21981 ASC
23172 ASC
23615 ASC
24661 ASC
26341 ASC
27719 ASC

K18-T121$^{tg/+}$/Brca1$^{fl/fl}$/p53$^{fl/fl}$
R5817 ASC
R5830 ASC
R5848 ASC
R5854 ASC

PRIMARY TUMOR CELL LINES

K18-T121$^{tg/+}$/Brca1$^{fl/fl}$/p53$^{R172H/fl}$
21981 TUM
22084 TUM
22864 TUM
23158 TUM
23172 TUM
23185 TUM
23615 TUM
25604 TUM
26341 TUM
15825 TUM

K18-T121$^{tg/+}$/Brca2$^{fl/fl}$/p53$^{R172H/fl}$
15825 TUM LUC
21975 TUM
22064 TUM
22101 TUM
27719 TUM
29410 TUM

PRIMARY TUMOR CELL LINES

K18-T121$^{tg/+}$/Brca1$^{fl/fl}$/p53$^{fl/fl}$
R5826 TUM
R5831 TUM
30200 TUM
39647 TUM
56217 TUM
58033 TUM
58033 TUM
59241 TUM
60577 TUM
60580 TUM
60651 TUM
61345 TUM
61348 TUM
R5814 TUM
R5817 TUM
R5828 TUM
R5830 TUM
R5843 TUM
R5848 TUM
R5854 TUM
R5860 TUM

PRIMARY TUMOR CELL LINES

K18-T121$^{tg/+}$/p53 $^{fl/fl}$
34706 TUM
39022 TUM
56229 TUM
58025 TUM
60510 TUM
R5810 TUM
R5836 TUM
R5838 TUM

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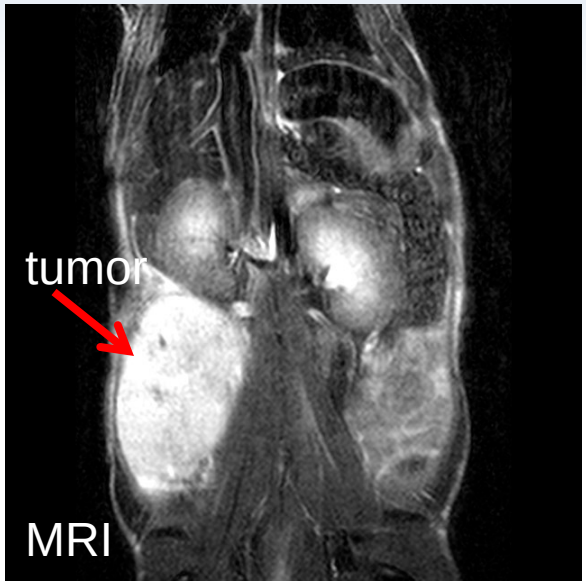
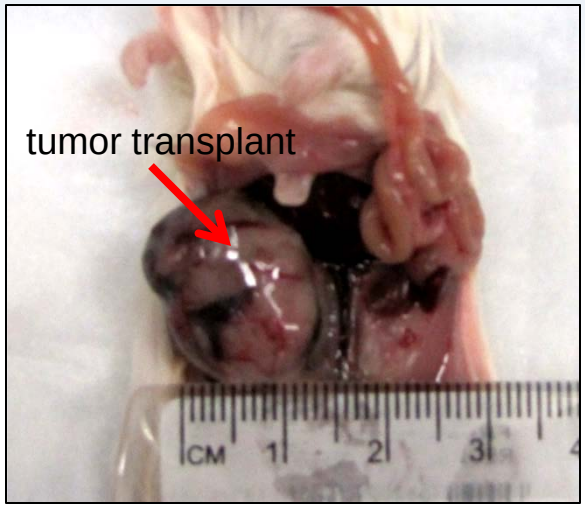
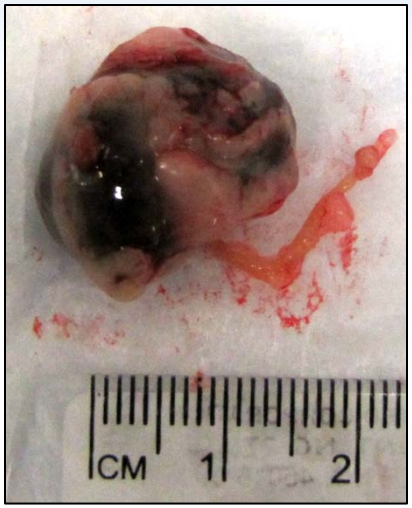
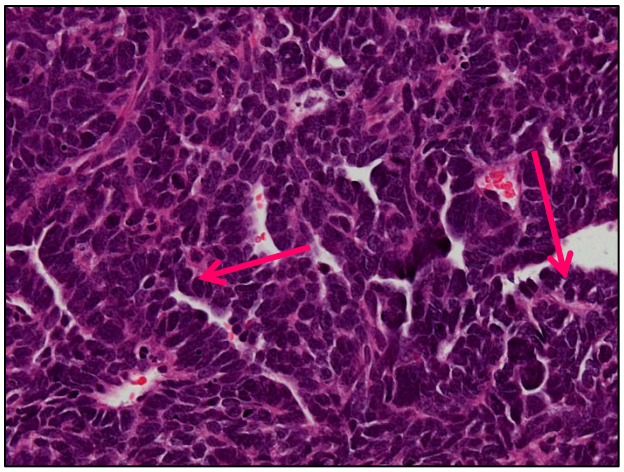
Serial transplantation models (i.b., Fvb)



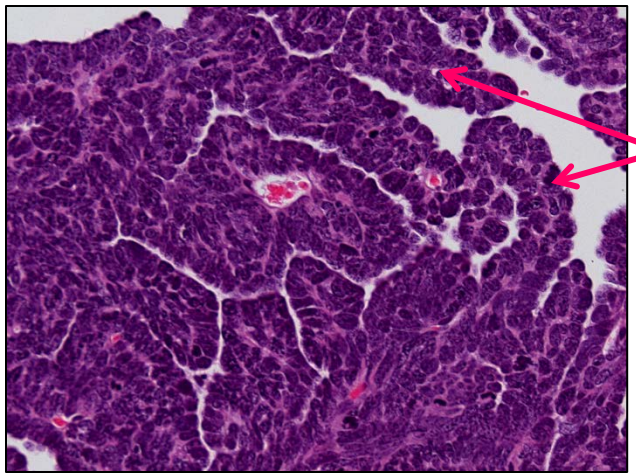




Donor tumor

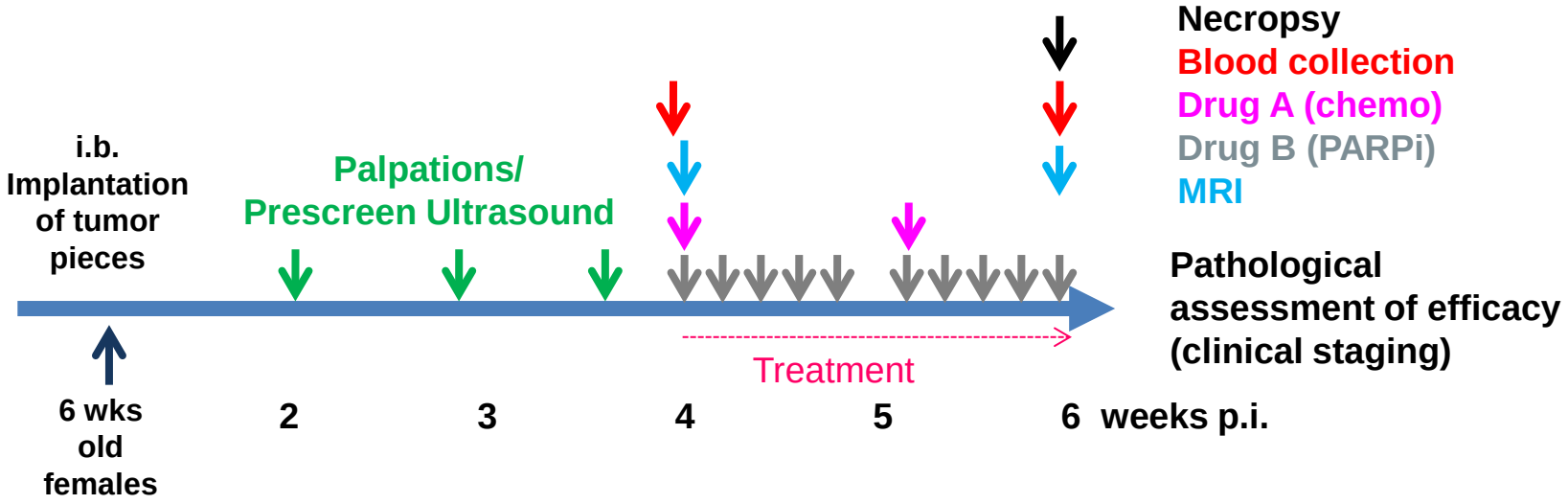


P1 tumor



Papillary structures of SEOC

Preclinical study workflow using SEOC orthotopic model



- **Take rate : ~100%**
- **Cohort size: at least 10 mice per treatment arm**

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S U M M A R Y:

- Non-germline genetically engineered models provide another promising direction in cancer disease modeling for preclinical purposes
- In some cases, retooling of conventional models by applying the NG-GEMs technology allows to accelerate and/or rationalize preclinical study resulting in both higher quality data and significant cost savings
- Two examples of applying NG-GEMs in translational and preclinical workflows illustrate benefits and challenges associated with such models
- Widespread adoption of non-germline GEMs will be driven by technology development, but also by growing demand for more complex and better predictive models

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Q&A

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