Mouse 101:

Preclinical studies in genetically engineered mouse (GEM) models

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Targeted Drug Discovery

- Strategic drug discovery focused on the selection of models based on target expression or pathway genetics

- Development and selection of models that integrate tumor, host and microenvironment to better evaluate the next generation of anti-neoplastic agents

Examples of GEM models that recapitulate human solid cancers

Organ	Histopathology	Genetics
Lung	Adenocarcinoma	*Kras*[55]
	Squamous cell carcinoma	NA
	Large cell carcinoma	NA
	Small cell carcinoma	*Rb1;Trp53* (REF. 167)
Colon	Polypoid adenocarcinoma	*Kras;Apc*[168]
	Hereditary non-polyposis carcinoma	*Msh6*[169]
Breast	Ductal carcinoma	*Brca2;Trp53* (REF. 170)
	Lobular carcinoma	*Cdh1;Trp53* (REF. 171)
Pancreas	Ductal adenocarcinoma	*Kras; Cdkn2a*[172], *Kras;Trp53* (REF. 173)
	Mucinous cystic neoplasm	*Kras;Dpc4* (REF. 96)
	Intraductal papillary mucinous neoplasia	NA
Prostate	Prostate carcinoma	*Pten*[174], *Pten;Nkx.1* (REF. 175), *Rb1;Trp53* (REF. 176)
Liver	Hepatocellular carcinoma	*Apc*[177], *Myc;Trp53* (REF. 178), *Myc;TGFA*[179]
Ovary	Endometrioid carcinoma	*Kras;Pten*[180], *Apc;Pten*[181]
	Serous carcinoma	NA
	Mucinous carcinoma	NA
Oesophagus	Squamous cell carcinoma	*Pten;Dpc4* (REF. 182); *Ccnd1;Trp53* (REF. 183)
	Adenocarcinoma	NA
Bladder	Transitional cell carcinoma	*Hras*[184]
Kidney	Renal cell carcinoma	*Apc;Trp53* (REF. 185)
Brain	Astrocytoma	*Pten;Rb1* (REF. 186)
	Glioblastoma	*Nf1;Trp53* (REF. 198)
Stomach	Gastric carcinoma	*Wnt;Ptgs2;Ptges*[188]
Skin	Melanoma	*HRAS;Ink4a*[27]
	Squamous cell carcinoma	*Xpd**[189]

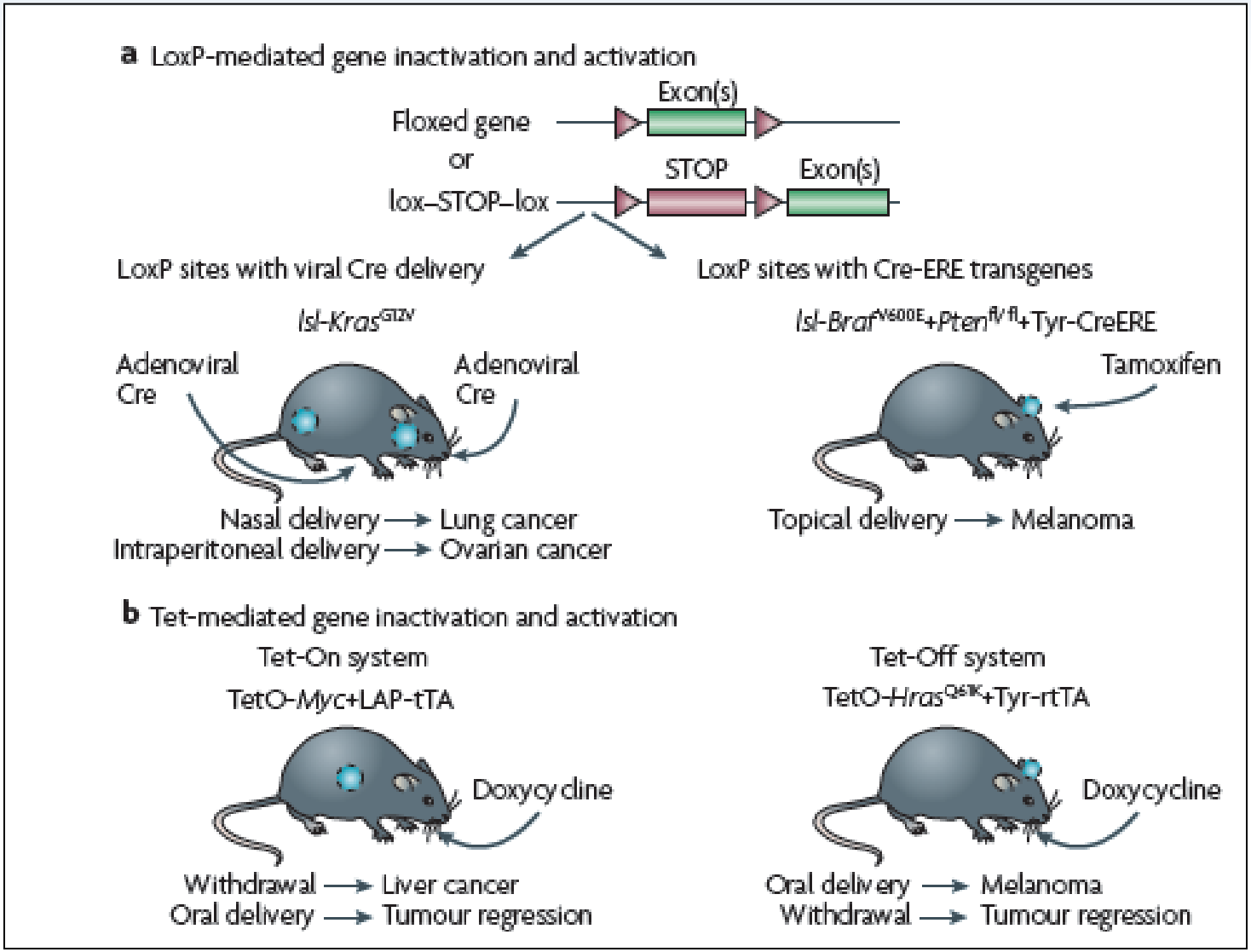
* Requires exposure to UVB light. NA, none available.

Kristopher K. Frese & David A. Tuveson, Nature Reviews Cancer 7, 654-658, 2007

What constitutes a good preclinical mouse model?

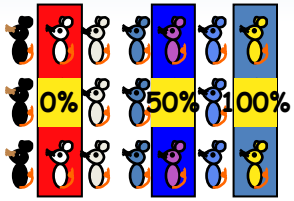
- Mimic sporadic cancer development which is triggered by initiating mutations in a single cell and subsequent acquisition of genetic alterations which result in tumor progression
- Somatic and tissue-specific induction providing the opportunity to direct a combination of oncogene and/or tumor suppressor mutations to the cell of origin of the cancer type
- Histopathologic and genetic resemblance of the human counterpart
- Balance of practical needs for short latency and high penetrance against the desire for the least biased model possible

Conditional GEM models allow tissue-specific and timed induction of genetic events



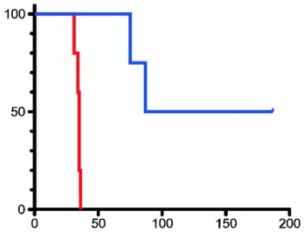
From Heyer, Kwong, Lowe & Chin, Nature Reviews Cancer, 2010

Challenges to up-scaling GEM Models for Therapeutic Evaluation

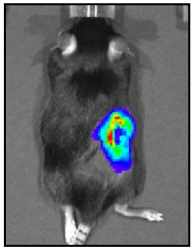


Ability to produce large synchronized cohorts (animal housing, genotyping)

Technical expertise for tumor induction

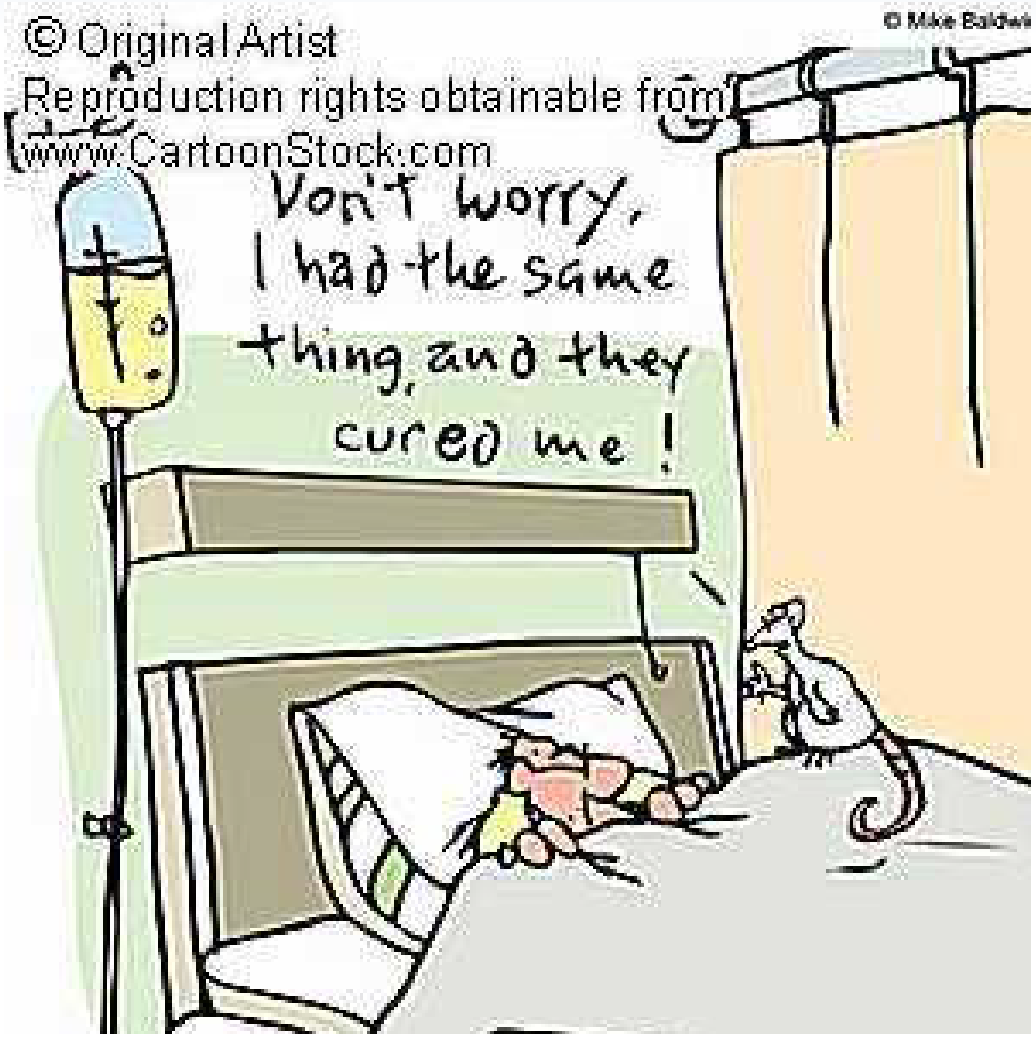


Timing until tumor onset and penetrance



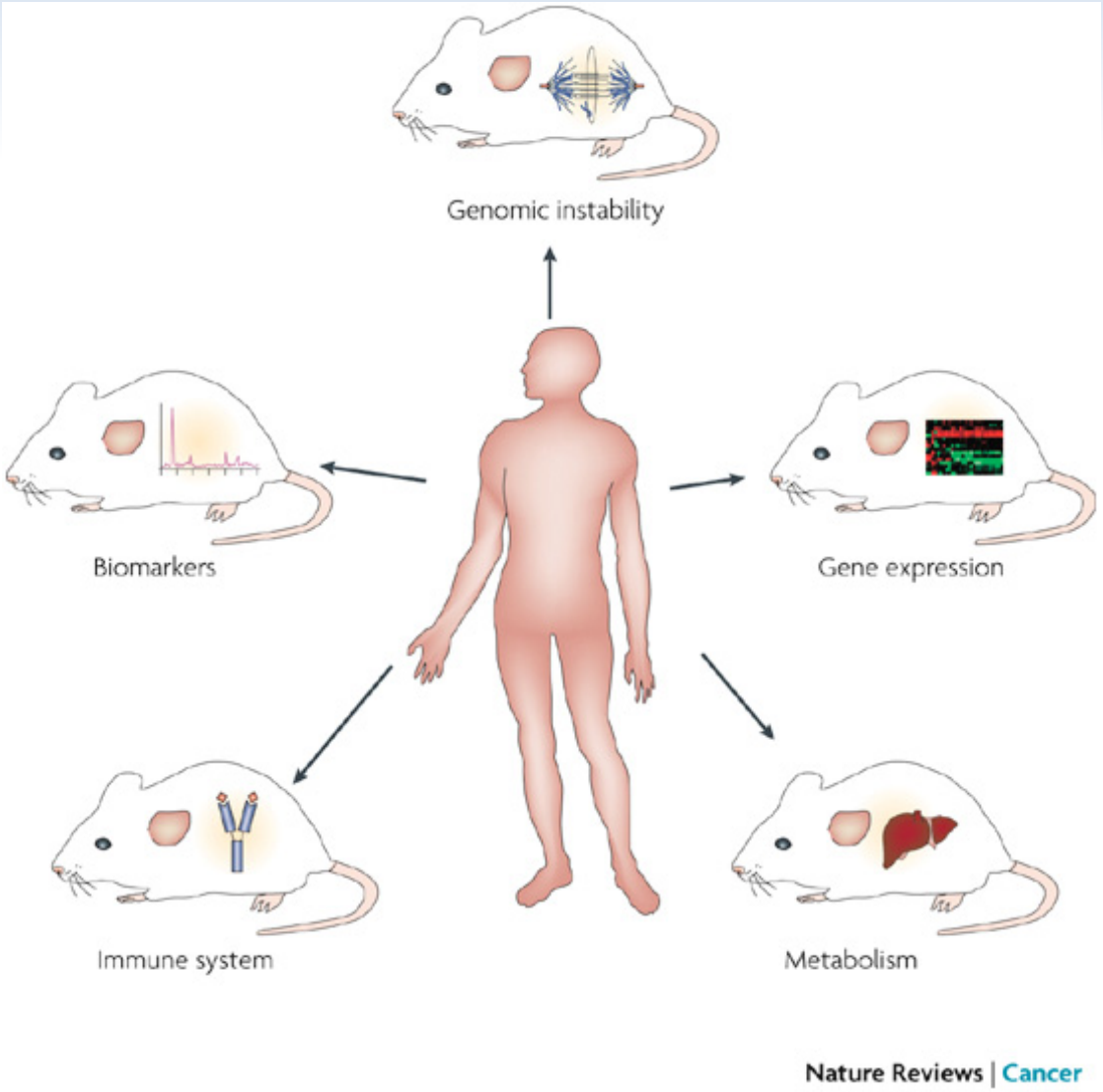
Serial imaging capabilities to monitor tumor growth

Goal: translation of preclinical findings for the benefit of patients…



…but mice are not humans!

Humanized mouse models



Currently available technologies can be used to generate GEM that contain entire human genes. Such models can be used to humanize metabolic enzymes, glycosylation enzymes, telomere structure and the immune system, which will prove useful in biomarker discovery as well as preclinical testing of therapeutics.

From Frese & Tuveson, Nature Reviews Cancer, 2007

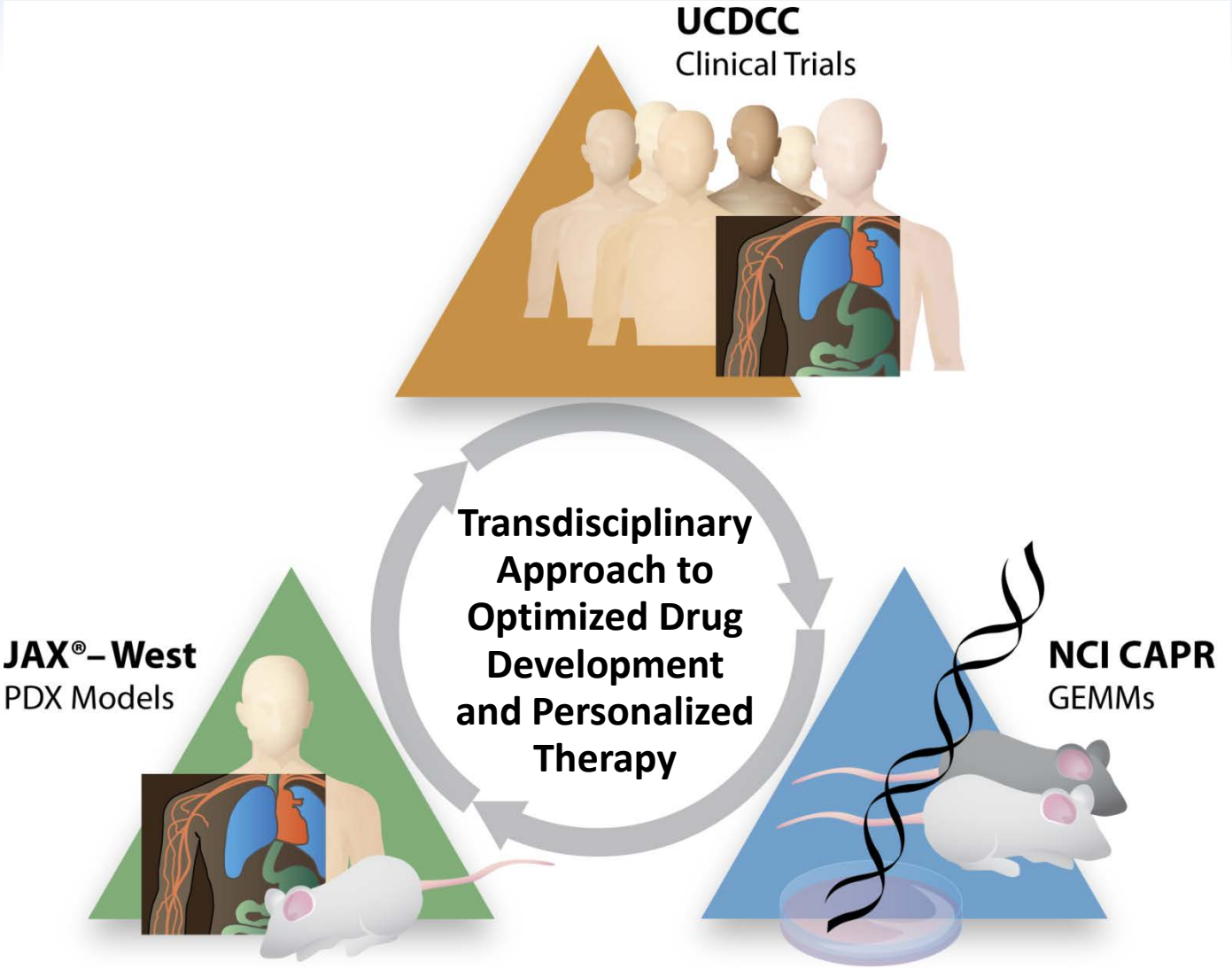
Comparison of key features of different preclinical model systems

Characteristics	Conditional GEMM	Mouse in mouse	Classic xenograft	Human in mouse
Penetrance*	Model-dependent	Usually high	Usually high	Low
Synchronicity‡	Model-dependent, can be high	High	High owing to cohorting	Poor
Replicability§	Model-dependent, can be high	High	Often depends on passaging conditions	Model-dependent
Immuno-competent host‖	Yes	Either	No	No
Rapid cohort generation¶	Model-dependent, usually no	Yes	Yes	Dependent on human tissue donations
Relevant genetic aberrations	Can be engineered	Can be engineered	Confounded by culturing artefacts	Can be engineered
Relevant microenvironment#	Yes	Yes or partial	Possible by addition of stroma	Possible by addition of stroma

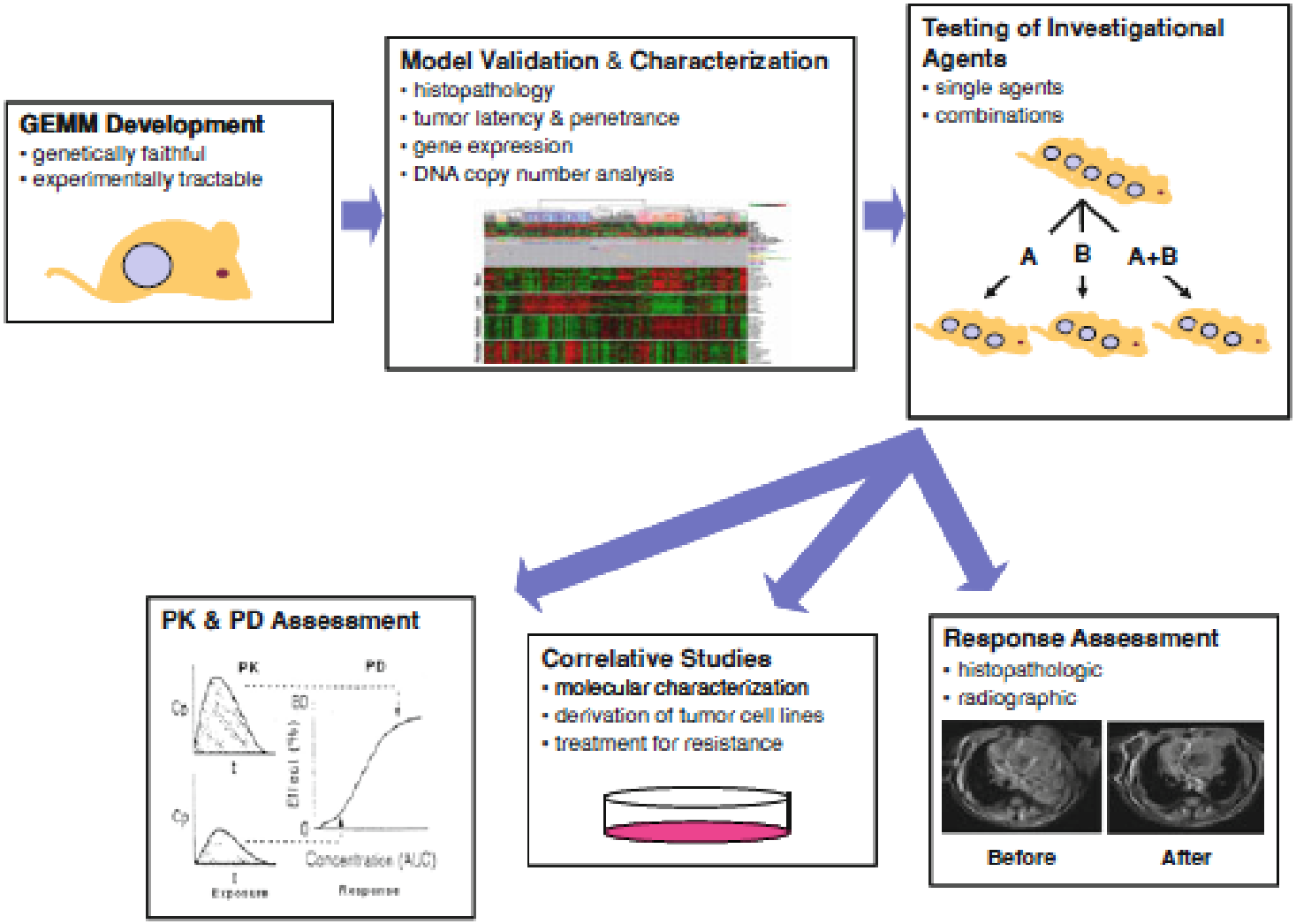
Modified from Heyer, Kwong, Lowe & Chin, Nature Reviews Cancer, 2010

*i*GXT Platform™

integrated GEMM • Patient-Derived Tumor Xenograft • Clinical Trials



Workflow and infrastructural needs for GEMM testing



William Kim & Norman Sharpless, Current Topics in Microbiology and Immunology, 2011

Example:

GEMM for serous epithelial ovarian cancer - from model development to preclinical application

Ludmila Szabova, Chaoying Yin, Sujata Bupp, Muhayimn Kamal, Theresa M. Guerin, Lidia Hernandez, Jerome J. Schlomer, Deborah B. Householder, Maureen L. Baran, Ming Yi, Yurong Song, Wenping Sun, Jonathan E. McDunn, Philip L. Martin, Christina Annunziata, Terry Van Dyke, Simone Difilippantonio

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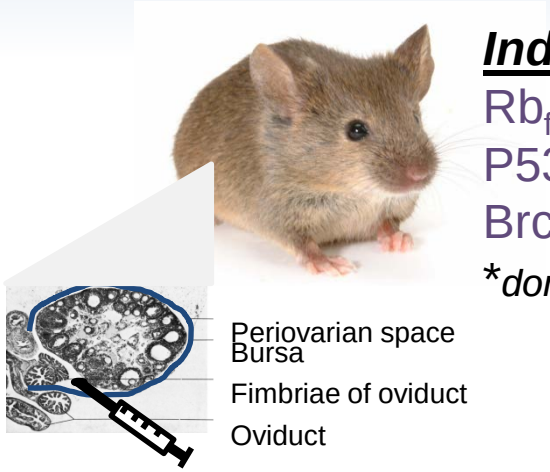
Generation of Mouse Models for Serous Epithelial Ovarian Cancer (SEOC)



Inducible events:

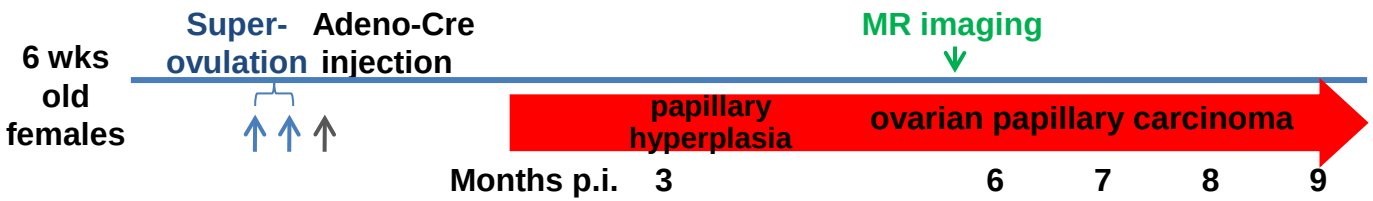
Rb_f inactivation (via K18-LSL-T121* BAC Tg)

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 Research, in revision

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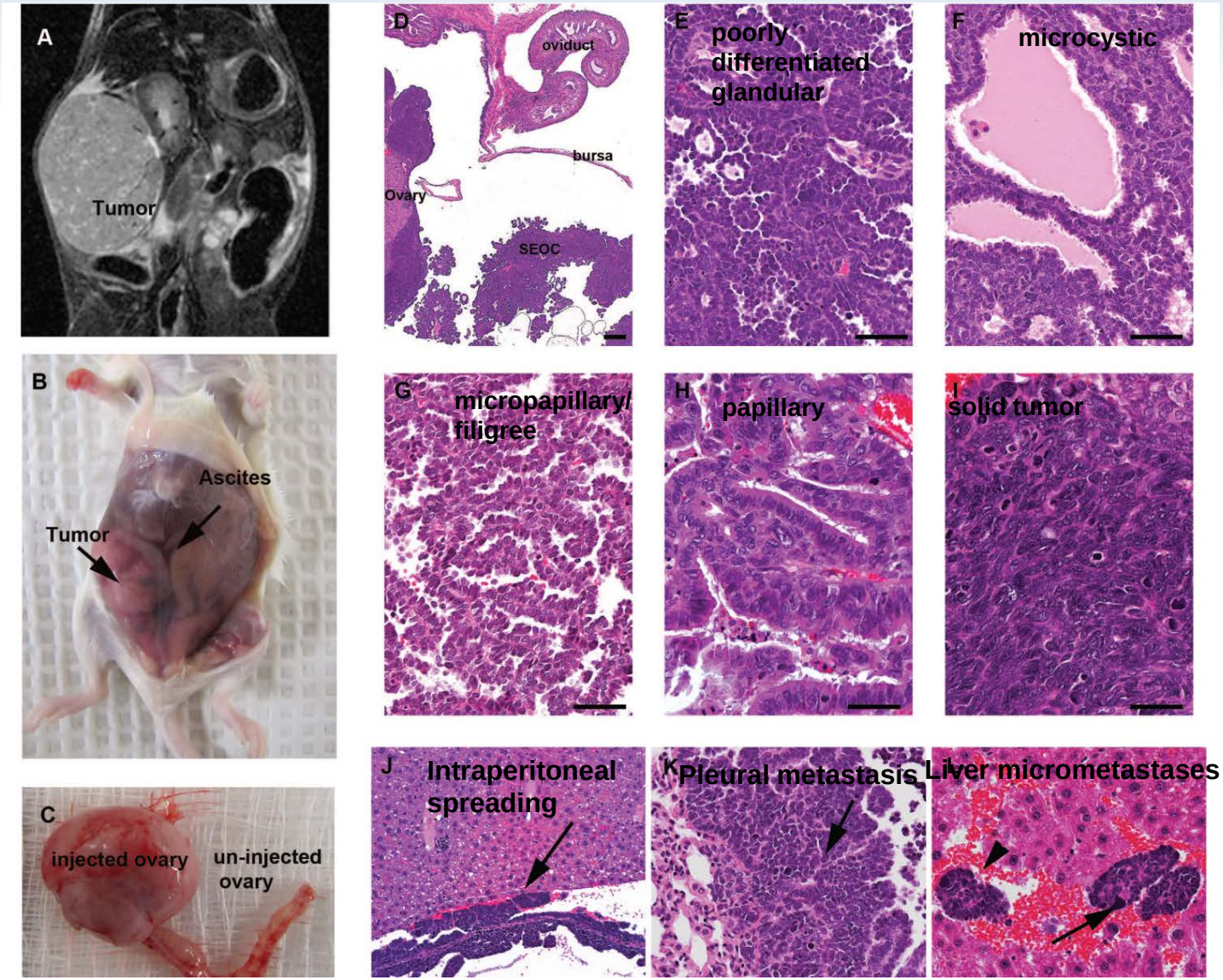
De novo mouse model of metastatic serous epithelial ovarian cancer (SEOC)



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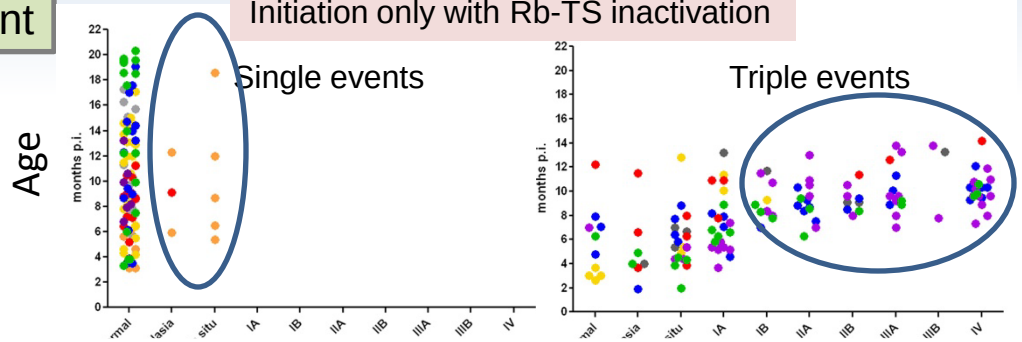
SEOC GEMM: Human Similarity in Molecular Attributes

Etiology Assessment

Initiation only with Rb-TS inactivation

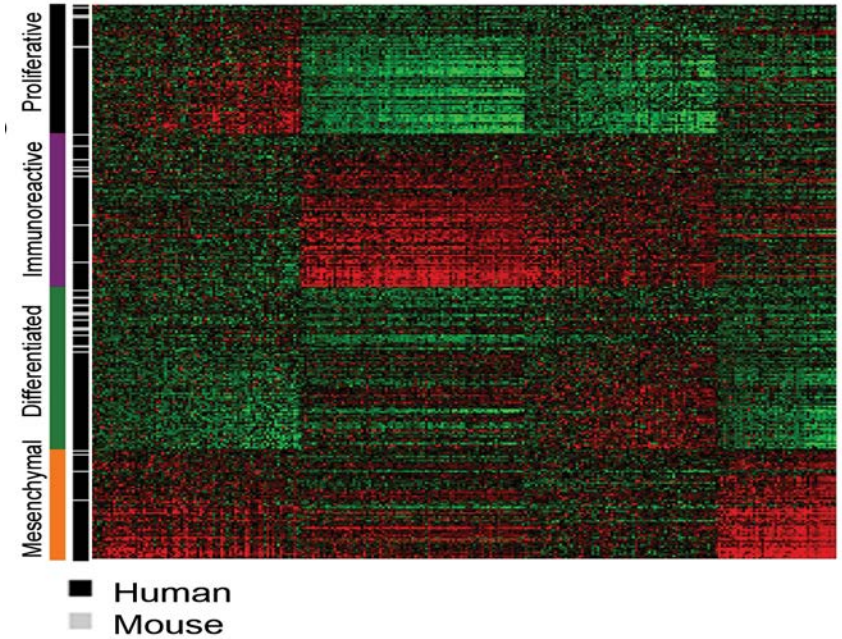
Progression requires p53 missense/loss; Brca1 or 2 loss subtypes



Severity/grade: →

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 Research, in revision

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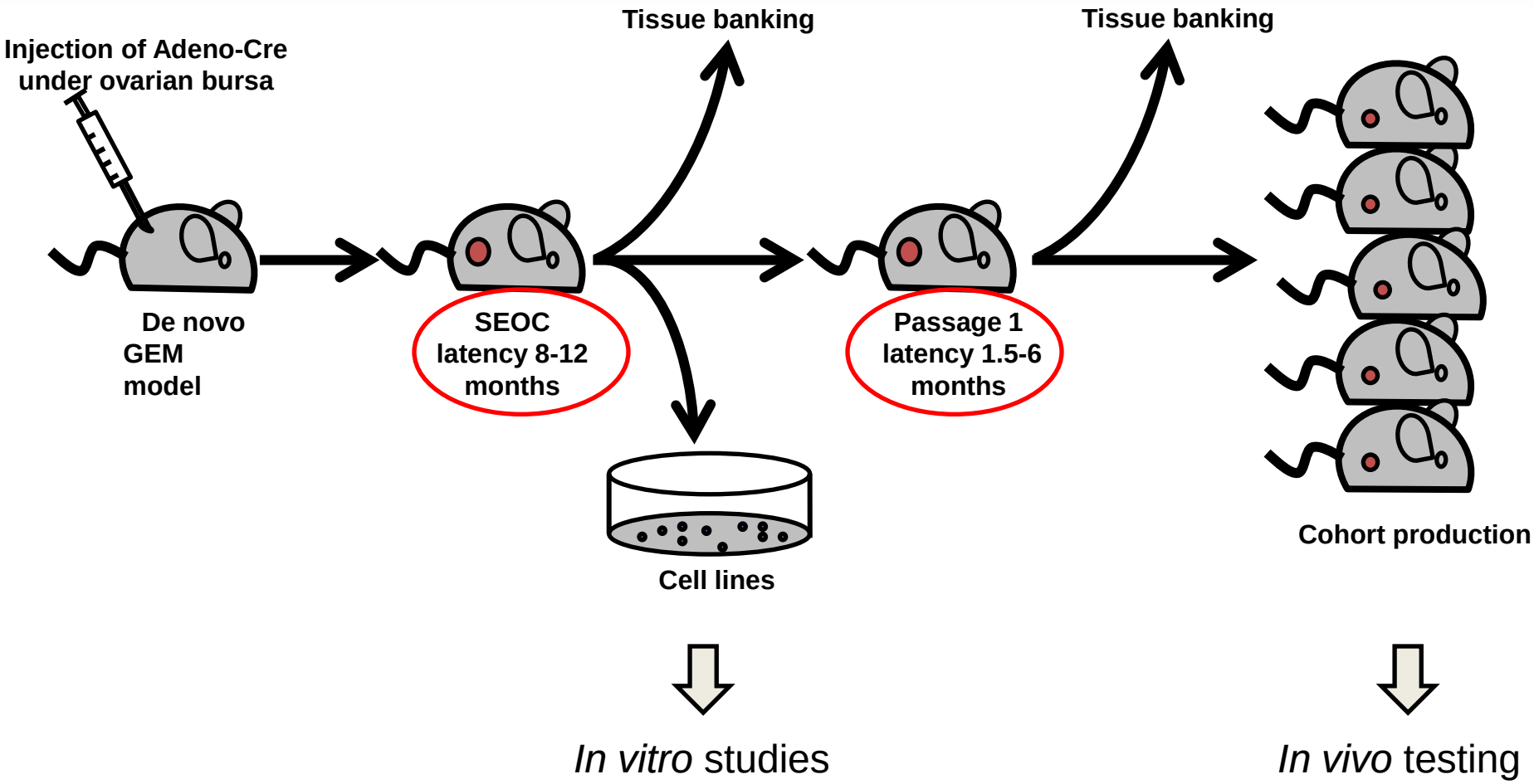
Second generation ovarian models



Szabova, Bupp et al, unpublished

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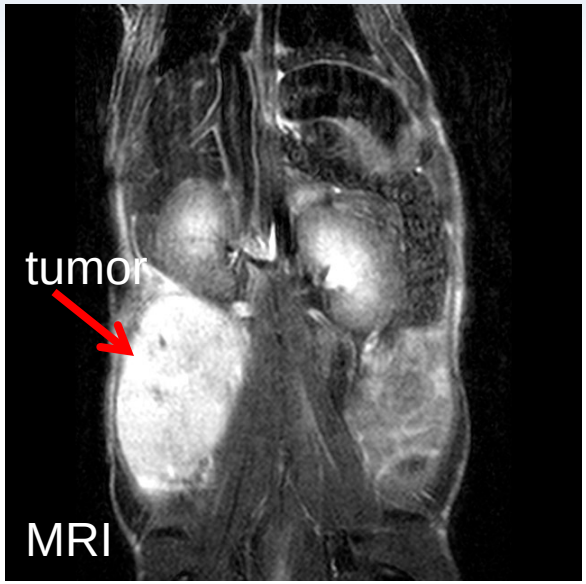
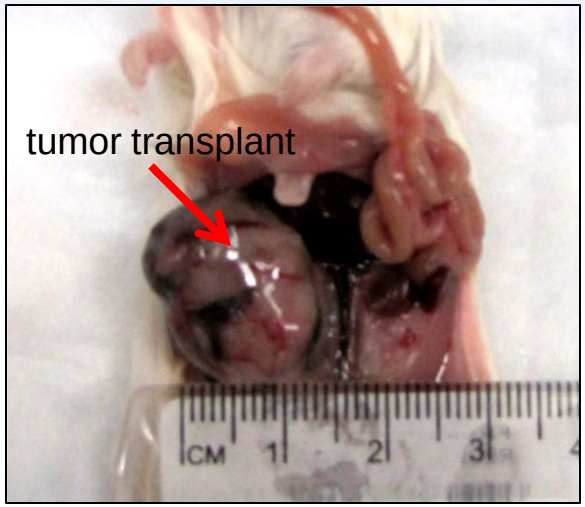
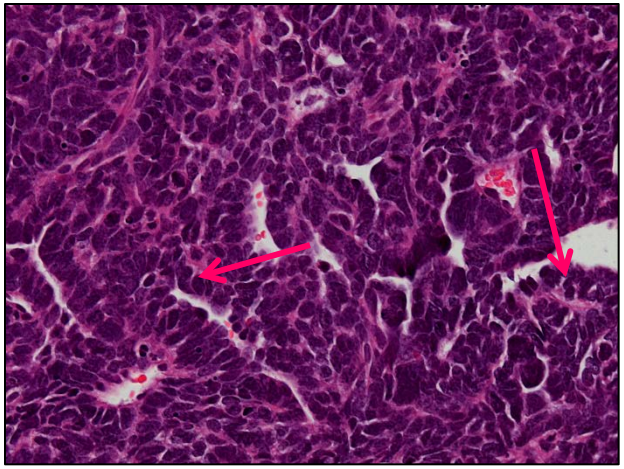
Serial transplantation models (i.b., Fvb)

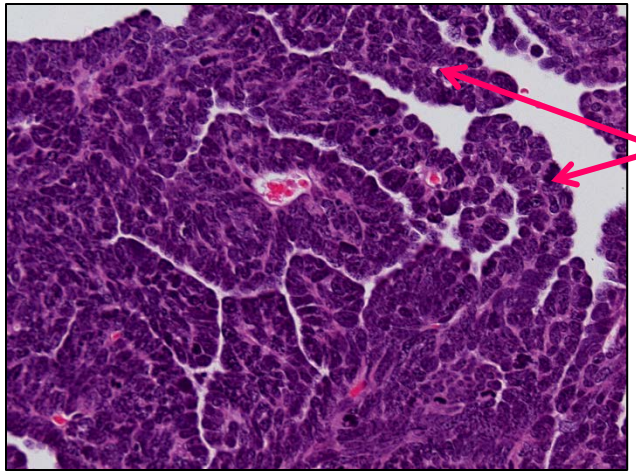




Donor tumor



P1 tumor



Papillary structures of SEOC

Szabova, Bupp et al, unpublished

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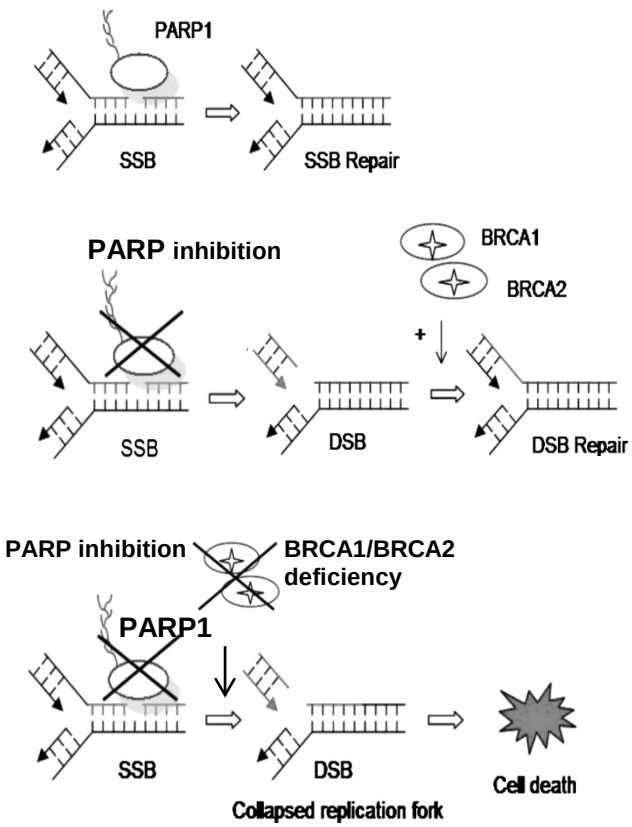
Use of Orthotopic Models for Preclinical Applications – Proof of Concept

- Standard of care: platinum drugs (cisplatin, carboplatin)
- PARP inhibitors (in clinical trials): 110022 11-C-0022, NCT01237067; 080092 08-C-0092, NCT01445418

- Analysis of cytotoxicity in primary cell lines derived from SEOC GEM models
- validation of target inhibition in vivo (PD)
- *In vivo* efficacy testing of experimental therapeutic compounds in orthotopic models



Modified from Underhill C et al. Ann Oncol 2011;22:268-279

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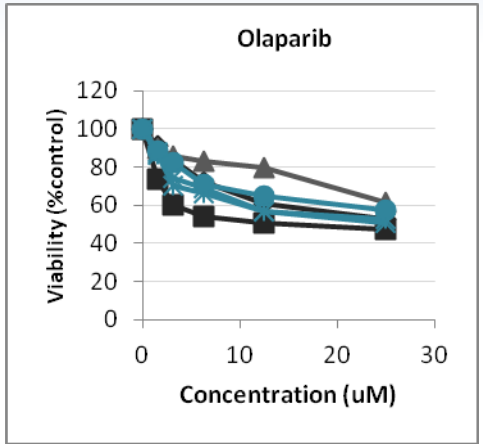
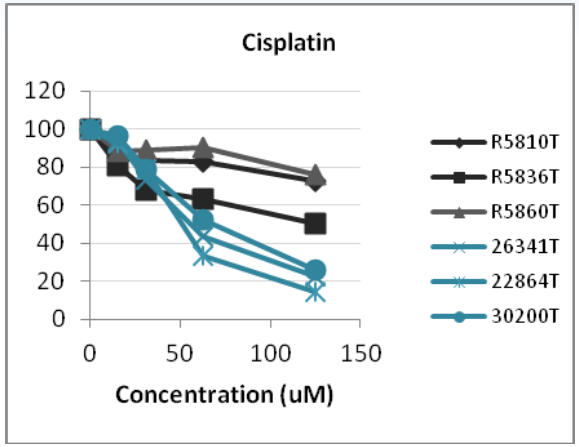
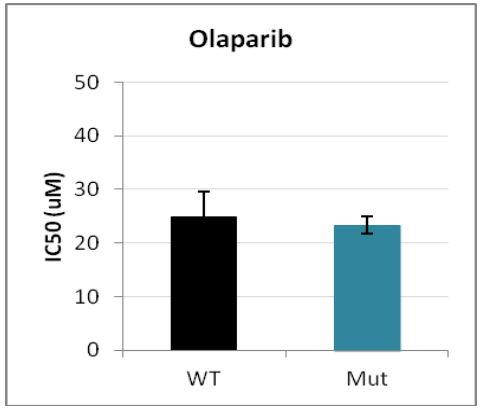
Cytotoxicity assays on primary ovarian cancer cell lines

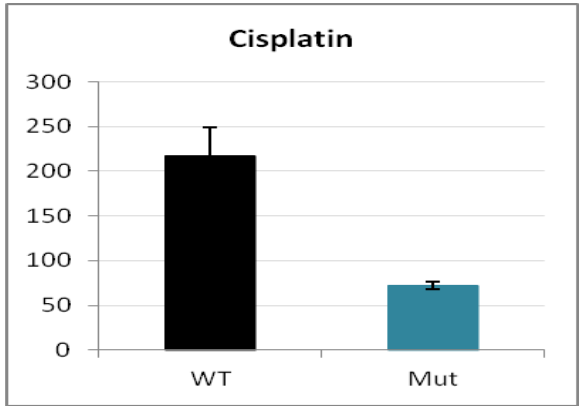
Cytotoxicity assays are used to determine if a therapeutic targets rapidly dividing cancer cells and inhibits viability.

The **half maximal inhibitory concentration (IC_{50})** represents the concentration of a drug that is required for 50% inhibition *in vitro*.









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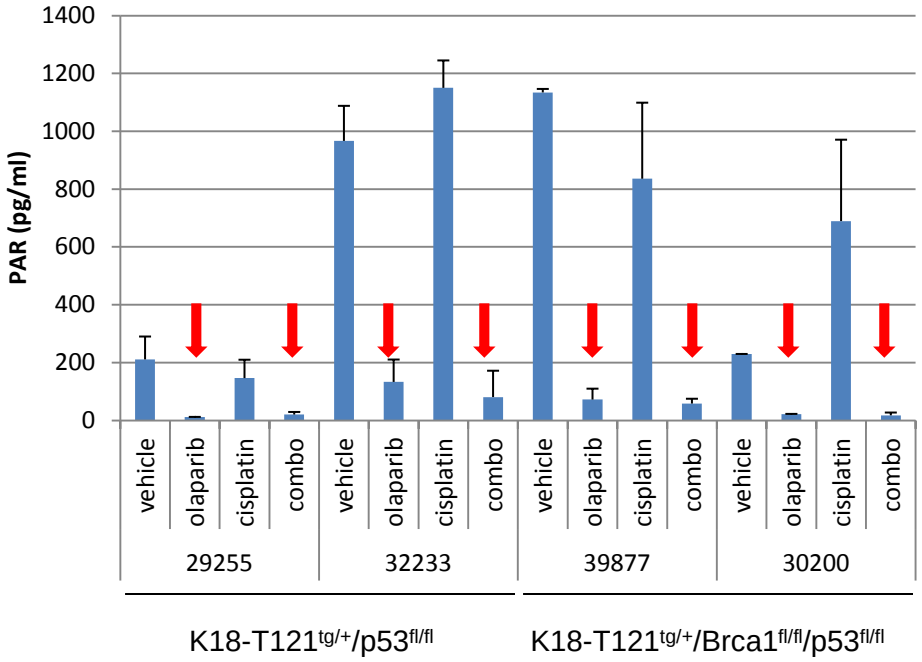
Validation of in vivo target inhibition

-tumor bearing mice treated 2 days:

Cisplatin i.p. (1 dose, 5mg/kg)

Olaparib (PARP inhibitor) orally (2 doses, 50 mg/kg each)



effect of PARP inhibitor on tumor lysates evaluated by PAR in vivo assay (Trevigen)

Szabova, Bupp et al, unpublished

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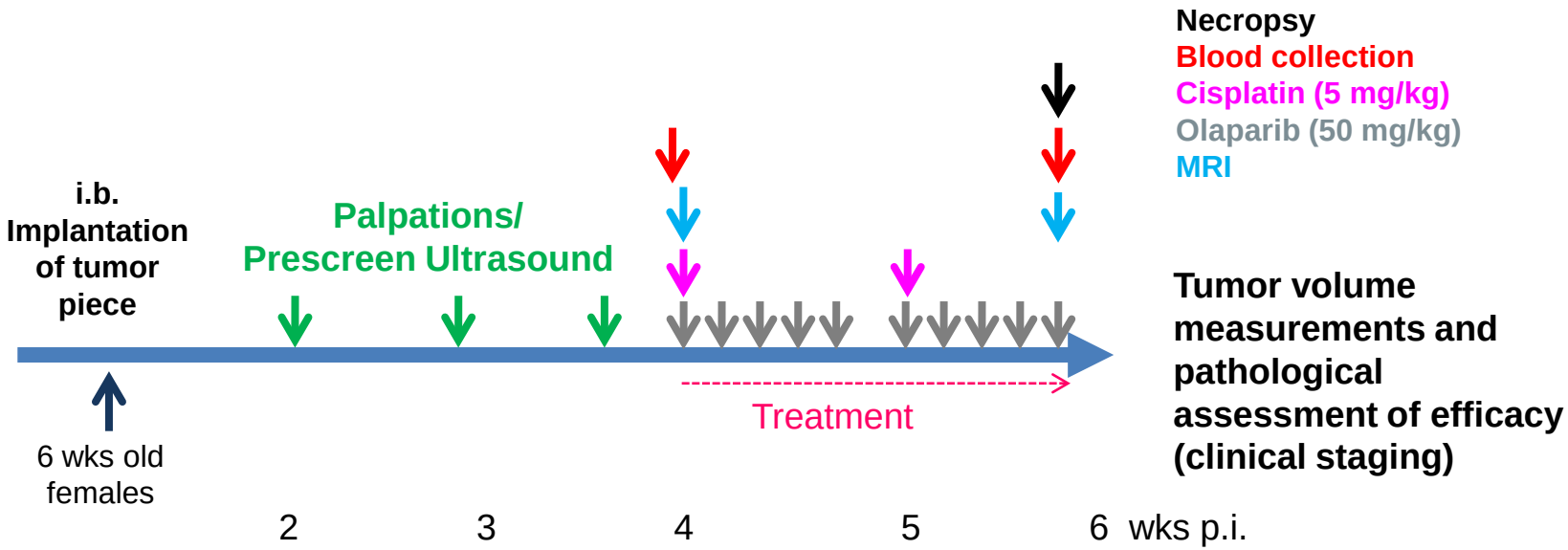
Preclinical efficacy study design using orthotopic models

Efficacy indicates the capacity for a beneficial change (or therapeutic effect) of a given intervention.



Szabova, Bupp, Kamal et al, unpublished

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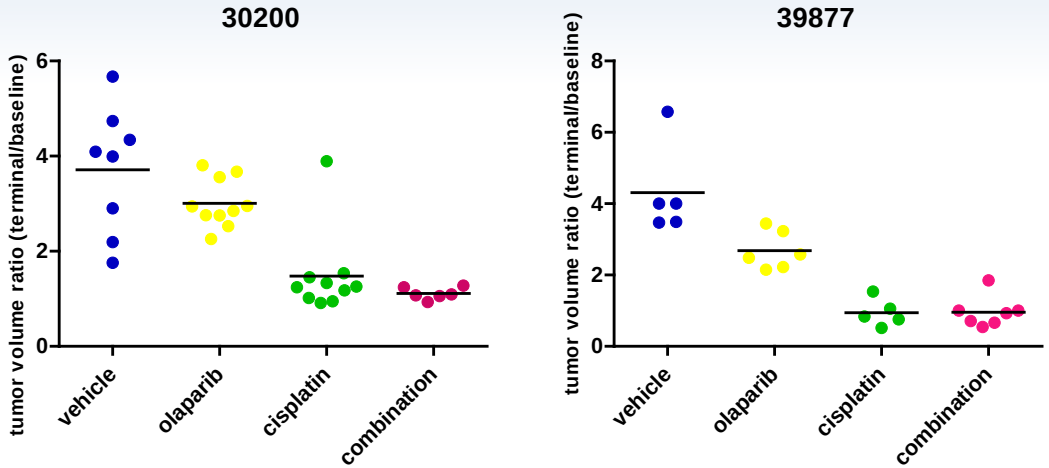
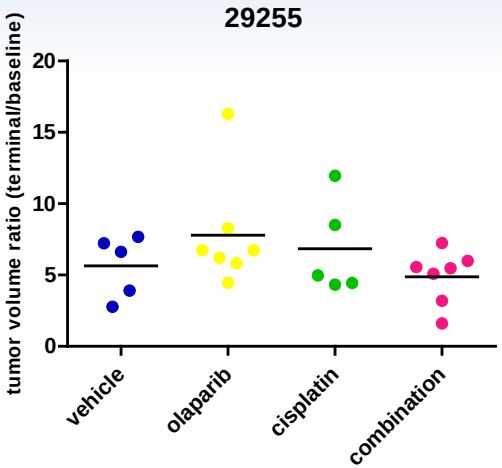
Efficacy study using orthotopic models

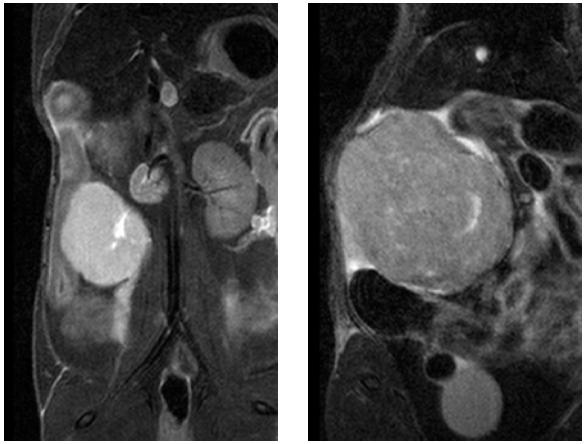
K18-T121$^{tg/+}$/Brca1$^{fl/fl}$/p53$^{fl/fl}$



K18-T121$^{tg/+}$/p53$^{fl/fl}$

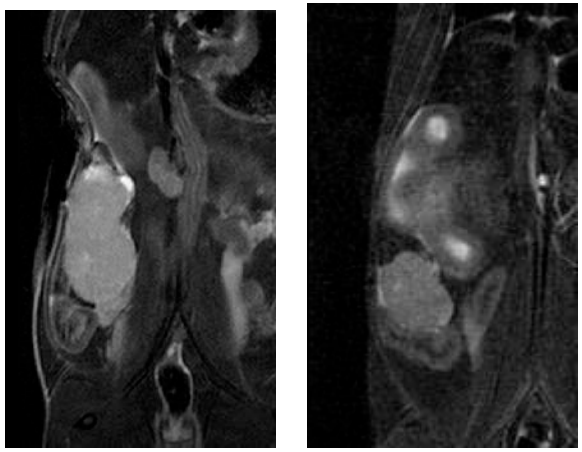


vehicle



baseline terminal

cisplatin



baseline terminal

Szabova, Bupp, Kamal et al, unpublished

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Additional applications for preclinical models

- Survival study to determine long-term combinatorial effect of combination vs. single drug therapy
- Drug resistance studies
- Molecular analysis of archived tissue and blood to identify new biomarkers for monitoring of treatment response and resistance

Selected references

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- Norman Sharpless & Ronald DePinho: The mighty mouse: genetically engineered mouse models in cancer drug development. Nature Reviews Drug Discovery 10, 1-14, 2006

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- William Kim & Norman Sharpless: Drug efficacy testing in mice, Current Topics in Microbiology and Immunology, 2011