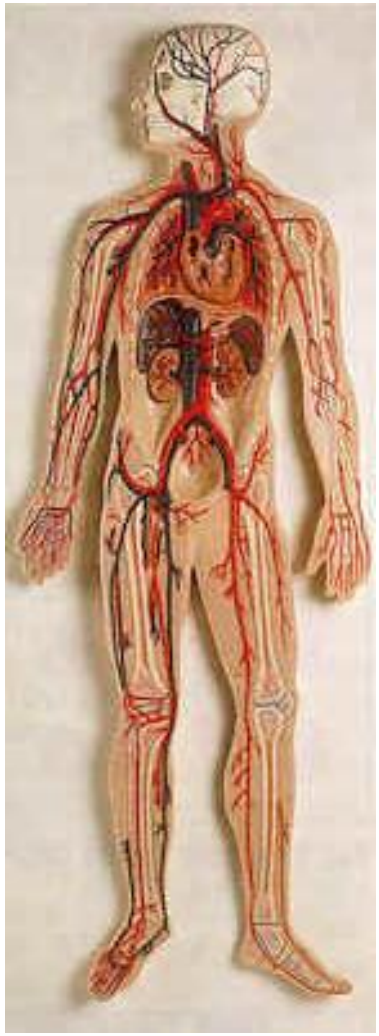
CAPR Center for Advanced Preclinical Research

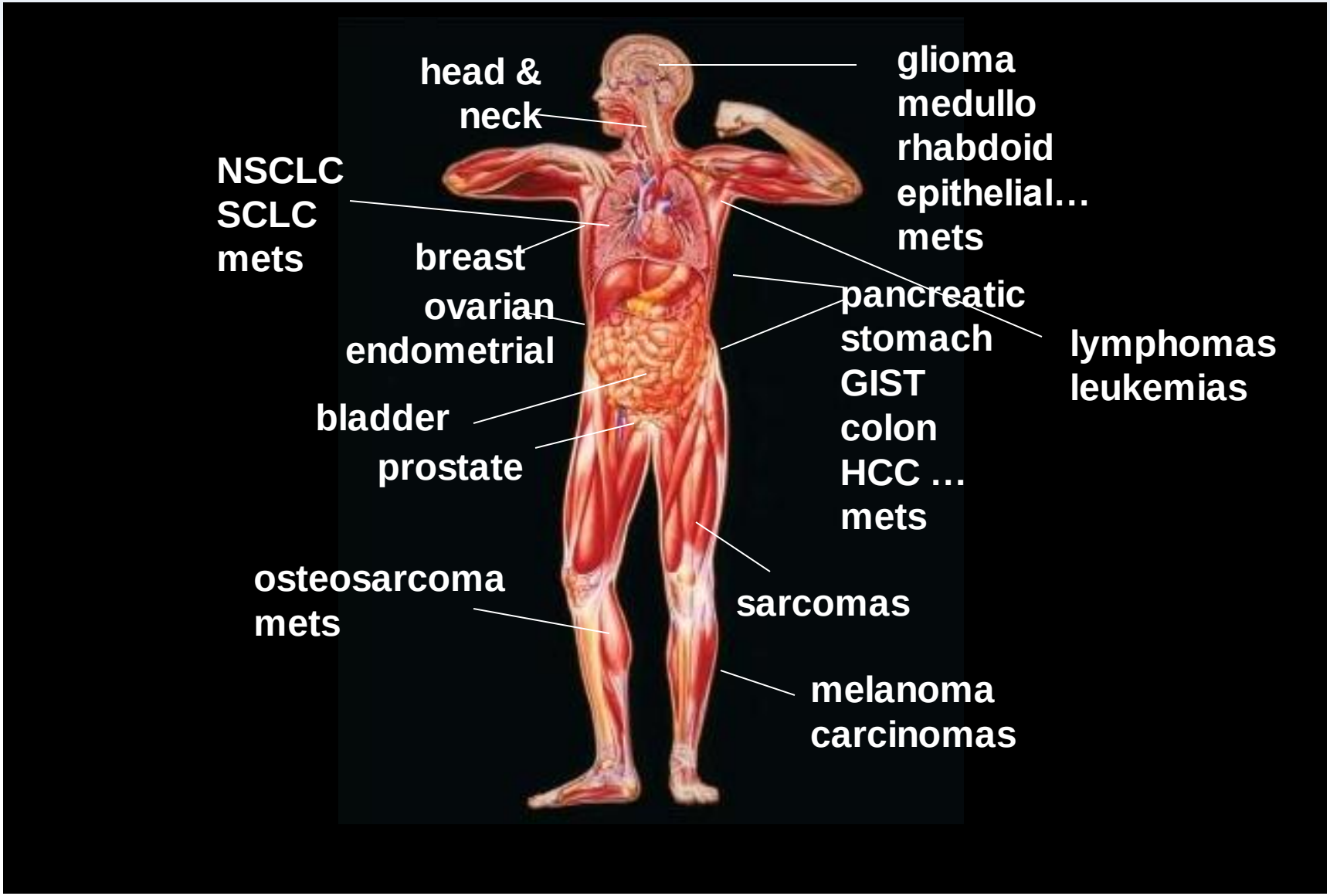


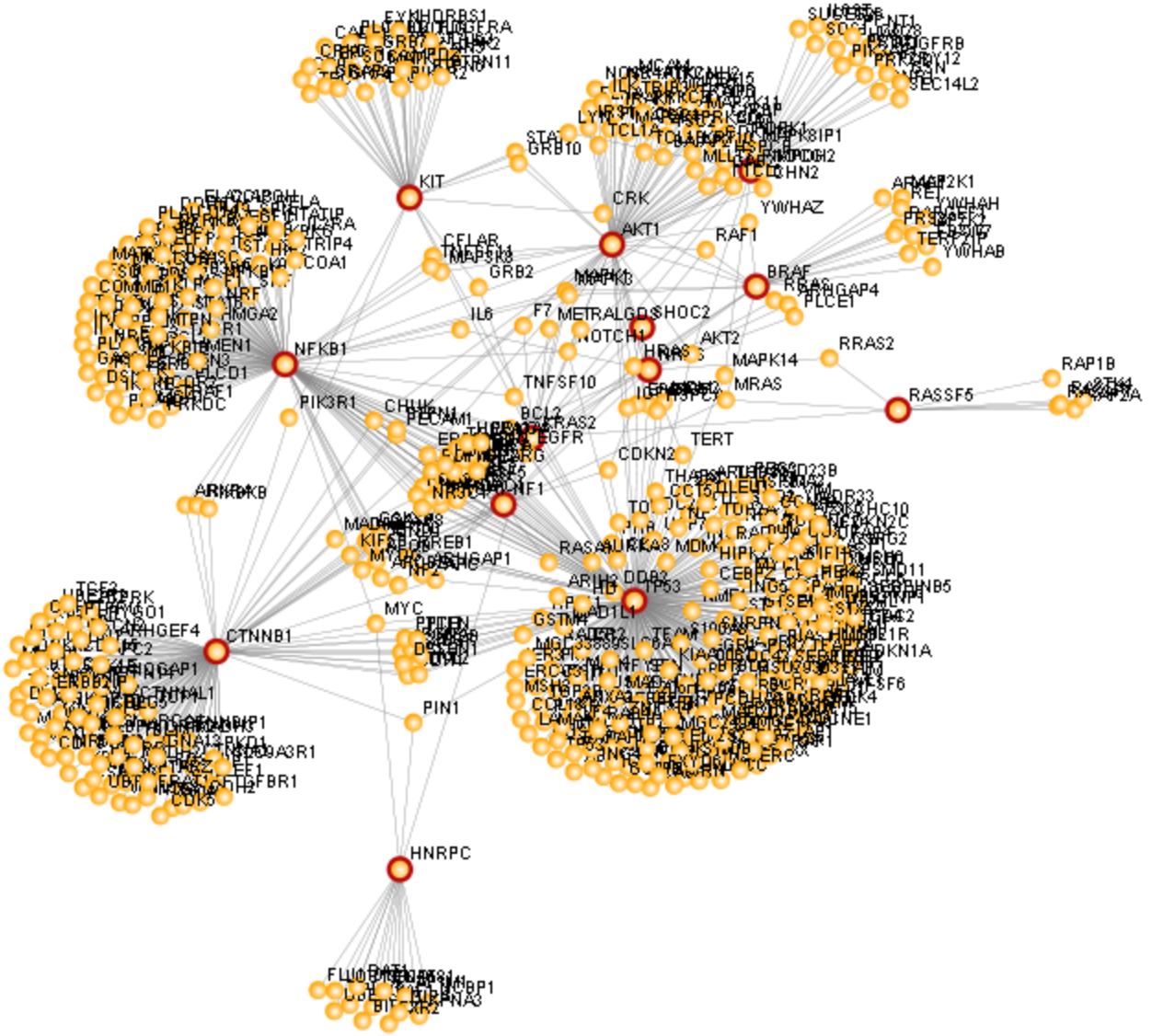
NCI CENTER FOR ADVANCED PRECLINICAL RESEARCH: A NOVEL PARADIGM IN TRANSLATIONAL SCIENCE AND EARLY STAGE ONCOLOGY DRUG DEVELOPMENT

Towards Predictive *In vivo* Models for Informative Preclinical Evaluation

Serguei Kozlov, PhD, MBA
CAPR, SAIC-Frederick, NCI-Frederick

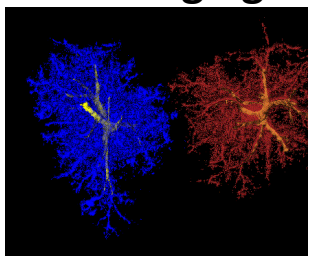
Over 100 Cell Types Susceptible to Cancer, Each with Multiple Molecular Etiologies

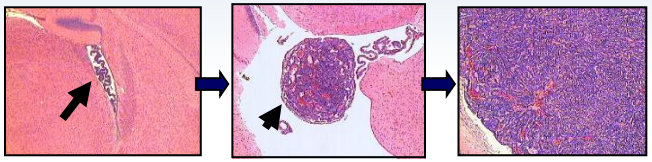



KIT
AKT1
CRK
RAF1
BRAF
MAPK14
AKT2
MRAS
RRAS2
RAP1B
RASSF5
SHOC2
NFKB1
PIK3R1
TNFSF10
TERT
CDKN2
MYC
CTNNB1
PIN1
HNRPC
TP53
GRB2
GRB10
YWHAZ
YWHAB
IL6
BCL2
EGFR
CDKN1A

Disease Models at CCR's Frontiers of Basic and Clinical Discovery

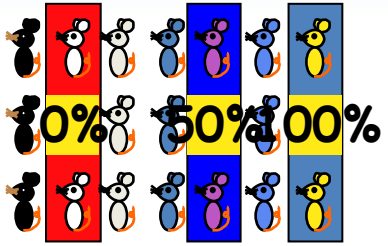
Cell Biology & Imaging

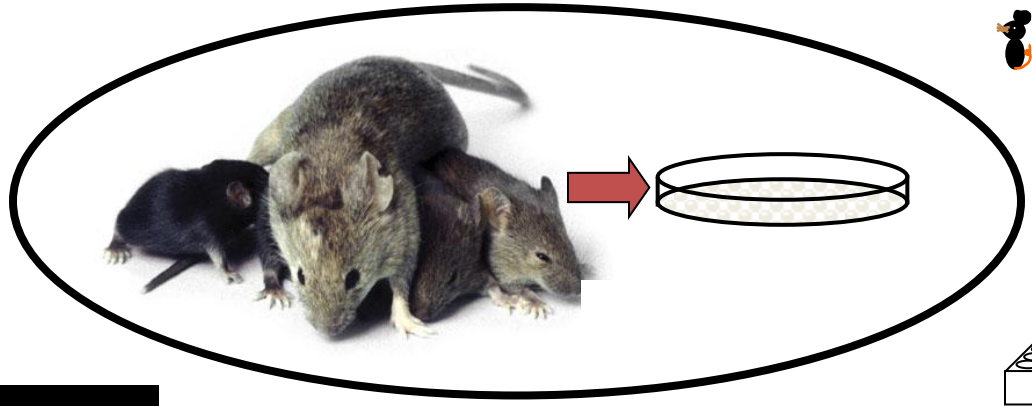


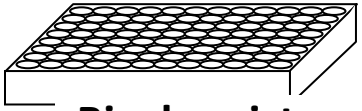


histopathology, physiology

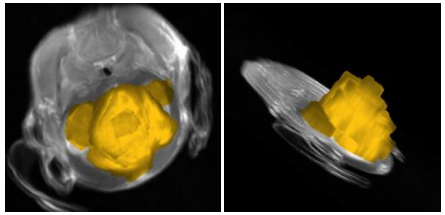
Complex Genetics





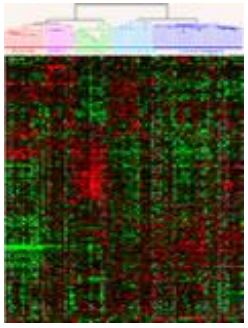


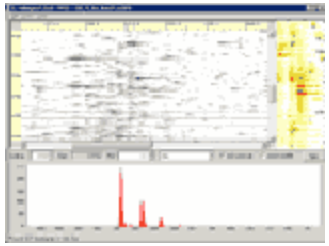
Biochemistry Genomics



Animal Imaging

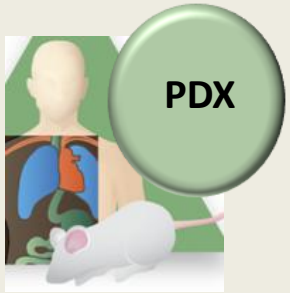
Genomics, Proteomics



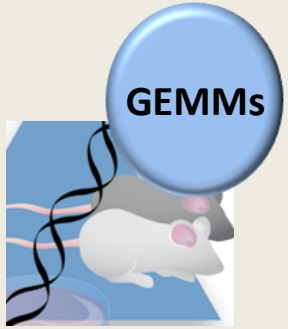


Clinical Research Challenges and Emerging Strategies

+	**PATIENTS are *THE* target for care**
-	**disease complexity** **access to tissue** **limited experimental variables** **cost** **limited patient resource**

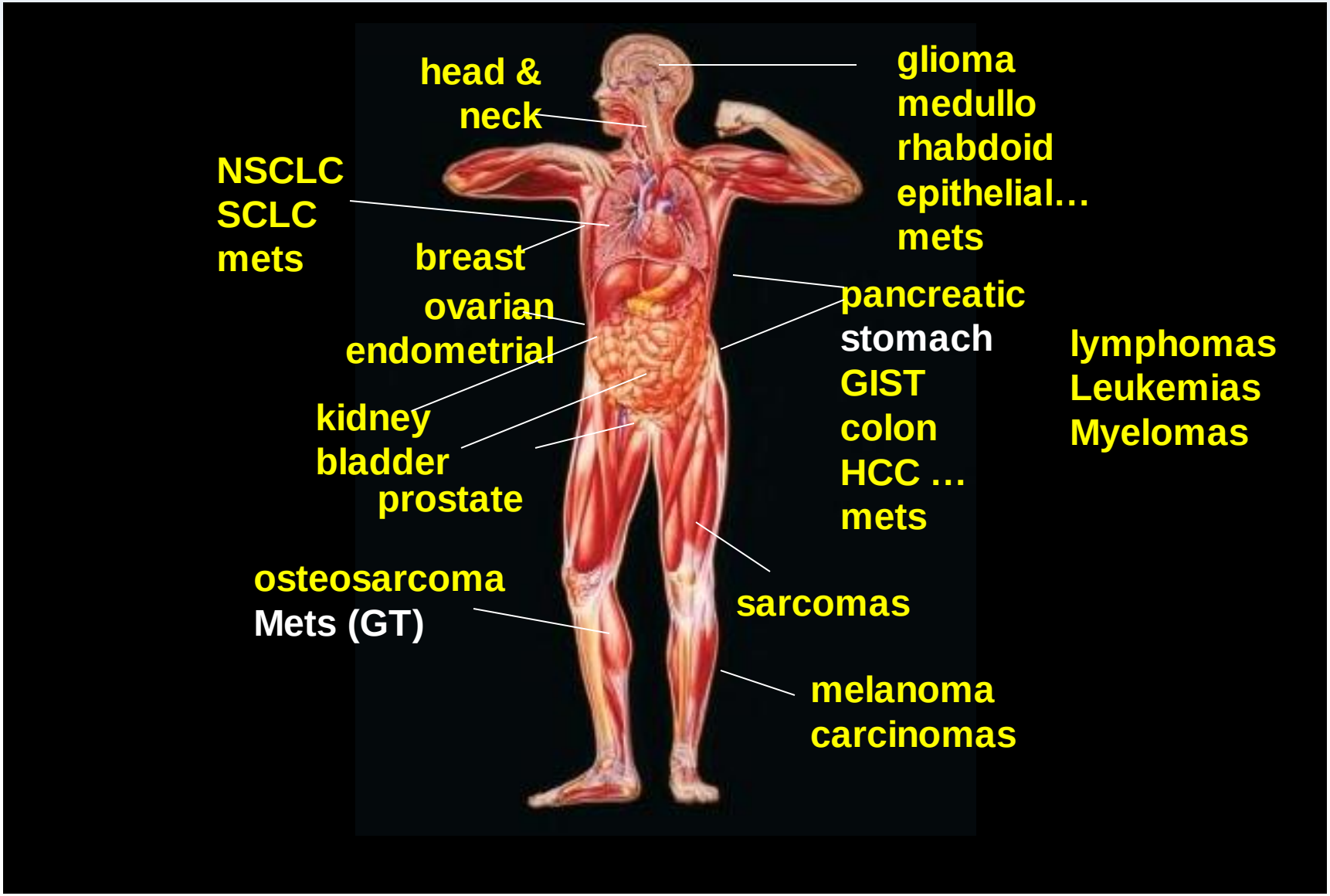


+	**patient-derived tumors** **experimental replicates**
-	**lacks immunobiology** **mouse stroma** **bypasses initiation/progression** **change possible**

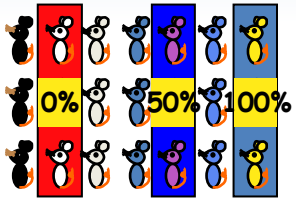


+	**initiation to progression** **pathway-specific engineering** **intact immune system** **experimental replicates**
-	**not human** **complex experimental systems** **biology often not relevant to disease**

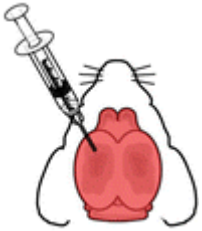
Multiple GEM Modeled on Human Cancer Genetics/Biology



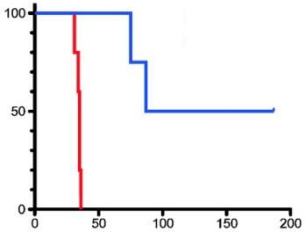
Challenges to the Use of GEM Models for Therapeutic Evaluation



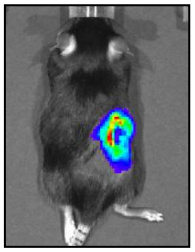
Ability to produce large synchronized cohorts



Technical expertise for tumor induction



Timing until tumor onset and penetrance



Ability to monitor tumor growth

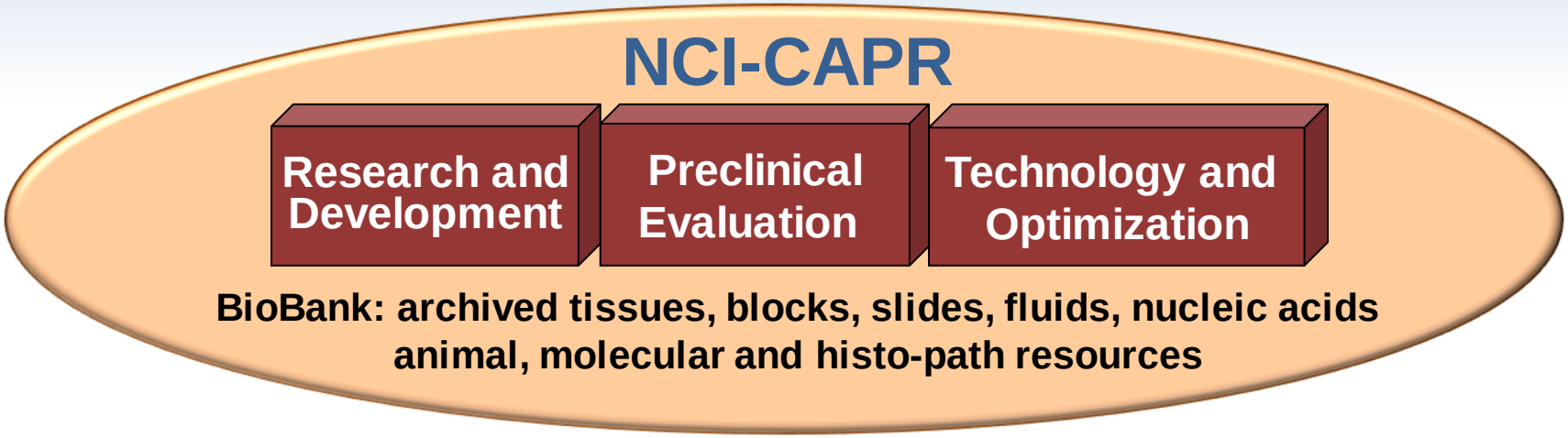
CAPR's Purpose

Develop strategies for predictive preclinical research in ***genetically and biologically engineered murine cancer models*** AND to facilitate routine application in clinical research for ***optimal*** outcomes in cancer patient management.

- Novel hybrid culture: integrated research rigor and project/goal management
- Business development (SAIC-Frederick)
- Internal staff of 22 (drawn from public and private sectors) in concert with NCI/NIH/SAIC service/research facilities

CAPR Center for Advanced Preclinical Research

CAPR's Operational Structure



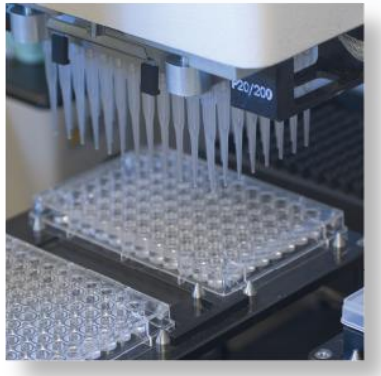
- Derivation, modification & validation of more predictive GEMMs
- Biomarkers/molecular signatures of tumorigenesis
- Breeding strategies for scale-up of mouse cohorts

- Efficacy studies on drug candidates
- Develop molecular and *in-vivo* imaging endpoints
- Biodistribution (PK/PD)
- Biomarkers/molecular signatures of treatment response

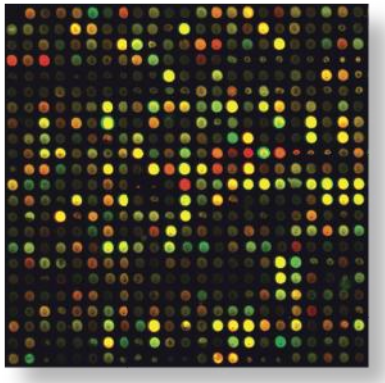
- New methodologies for expanding and scaling up preclinical study design
- ES and iPSC technologies for non-germline cohorts and preservation
- Optimization/retooling of GEMs

SAIC-Frederick Core Services

Laboratory of Molecular Technology
Genotyping



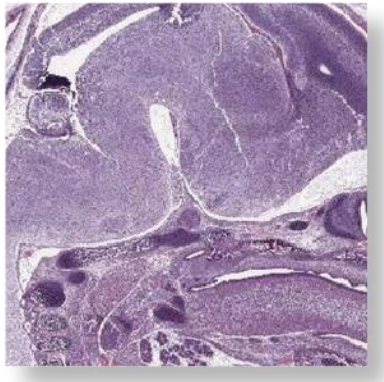
Laboratory of Molecular Technology
Microarray Analysis



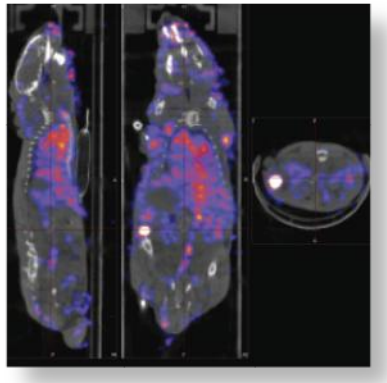
Laboratory Animal Sciences Program
Animal Resources

CAPR

Laboratory Animal Sciences Program
Pathology/Histotechnology

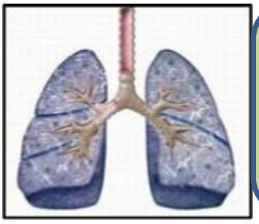
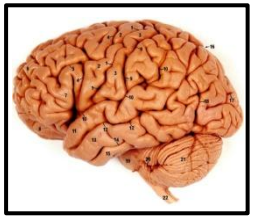
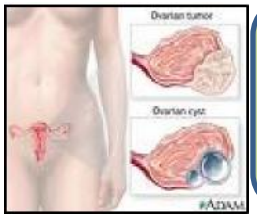


Laboratory Animal Sciences Program
Small Animal Imaging

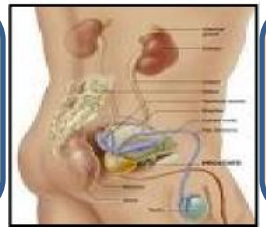
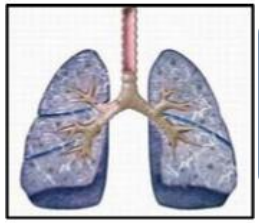
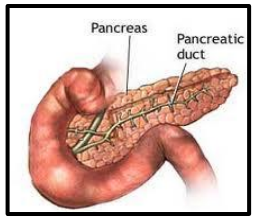
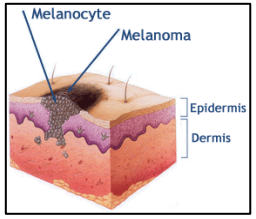


CAPR Mouse Cancer Model Portfolio

Models in Preclinical Evaluation

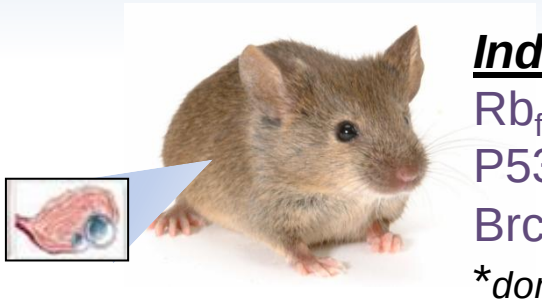
Model Type	Genetic Events Induction Mode
 Non-Small Cell Lung Cancer: ➢ Lung Adernocarcinoma ➢ Squamous Cell Carcinoma	➢ EGFR-L858R ➢ EGFR-L858R/T790M ➢ Lkb1/Kras ➢ Doxycycline ➢ Adeno-Cre/Lenti-Cre
 ➢ Anaplastic Astrocytoma III ➢ Glioblastoma	➢ pRb/Kras/PTEN ➢ Tamoxifen/Adeno-Cre ➢ Orthotopic
 ➢ Serous Ovarian Carcinoma	➢ pRb/p53/Brca1/Brca2 ➢ Tamoxifen/Adeno-Cre ➢ Orthotopic

Models in Characterization

Model Type	Genetic Events Induction Mode
 ➢ Prostate Carcinoma	➢ pRb/PTEN ➢ Tamoxifen
 ➢ Small Cell Lung Cancer	➢ pRb/p53 ➢ Lenti-Cre
 ➢ Pancreatic Adenocarcinoma	➢ p53/Kras ➢ Spontaneous *de novo*
 ➢ Melanoma	➢ BRAF-V600E ➢ HGF/SF ➢ Tamoxifen

EXAMPLE 1: DEVELOPMENT OF SEROUS EPITHELIUM OVARIAN CANCER MODEL

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



Inducible events:

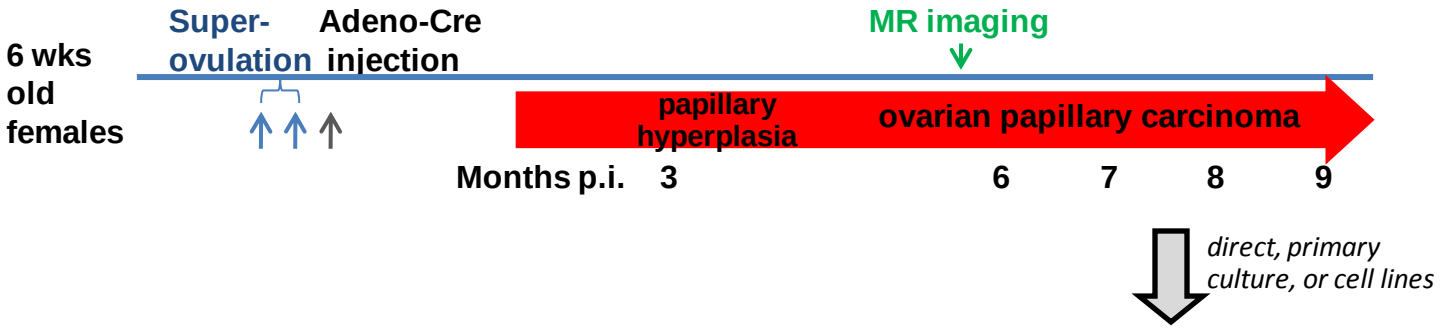
Rb_f inactivation (via K18-lsl-T121* BAC Tg)

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*

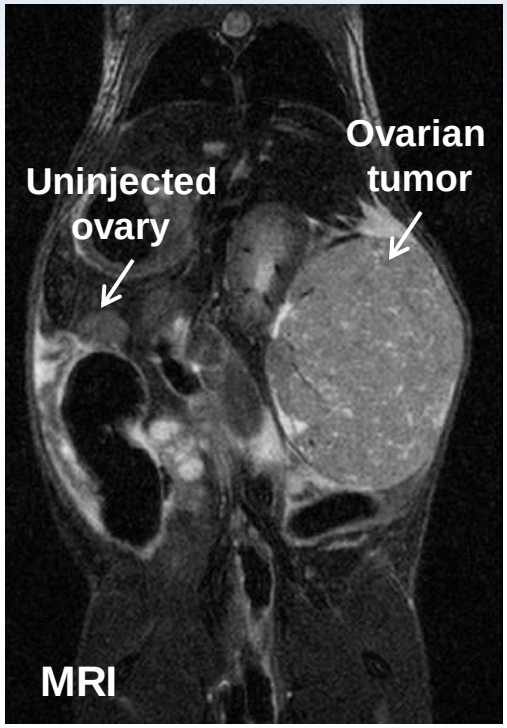
De novo model: Intra-bursal injection of adeno-Cre

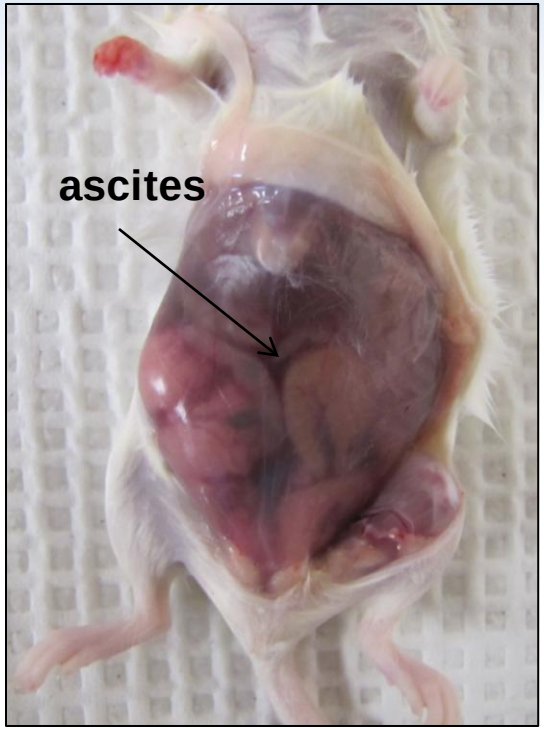


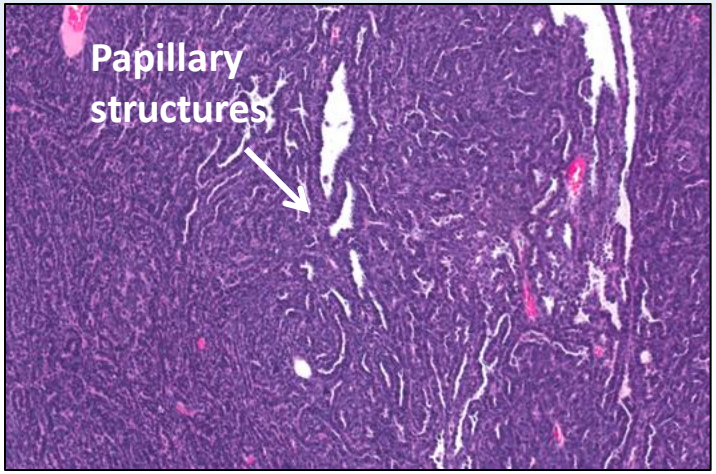
Transplantation models: Generation of preclinical models via s.c./i.p. and intra-bursa injection of murine carcinoma cells

CAPR Center for Advanced Preclinical Research

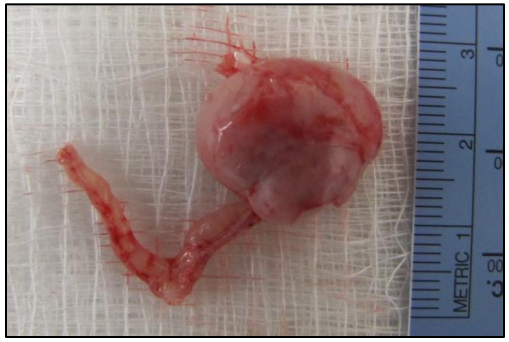
De novo Mouse Model of Serous Epithelial Ovarian Cancer (SEOC)

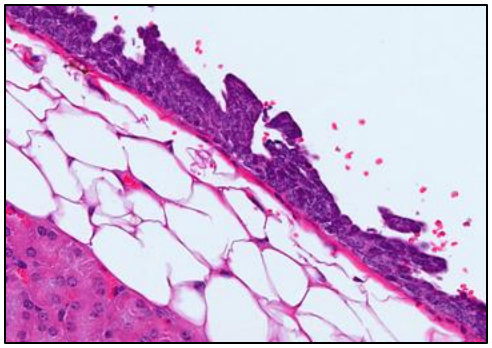




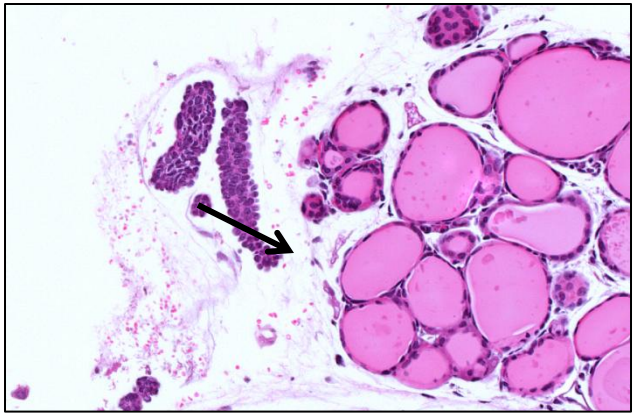


Ovarian serous carcinoma with papillary structure





Peritoneal carcinomatosis: carcinoma on surface of renal capsule.



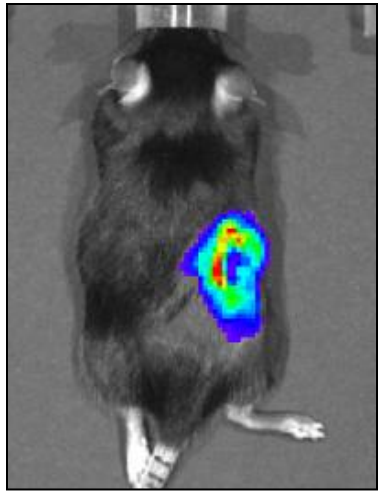
Mediastinal metastasis next to the thyroid gland

CAPR Center for Advanced Preclinical Research

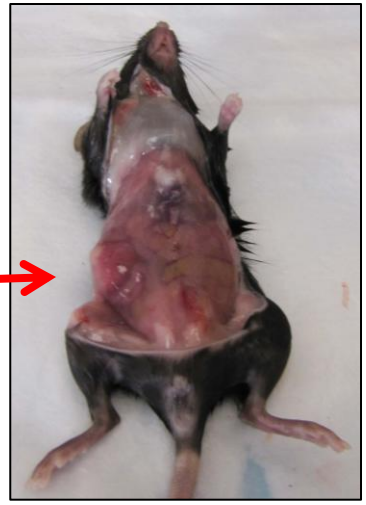
Orthotopic ovarian mouse models

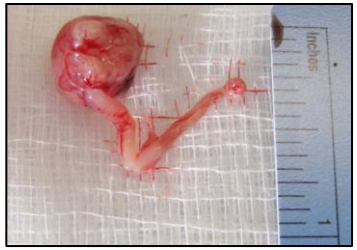
Genotype	Tumor Histology	# of primary tumor cell cultures	# of ascites cell cultures
Brca1$^{fl/fl}$, K18-T121^{tg}, p53$^{fl/fl}$	SEOC	5	1
Brca1$^{fl/fl}$, K18-T121^{tg}, p53 $^{LSL\ R172H/fl}$	SEOC	2	1
Brca2$^{fl/fl}$, K18-T121^{tg}, p53$^{LSL\ R172H/fl}$	SEOC	1	1

Intra-bursa injection of ovarian tumor cells

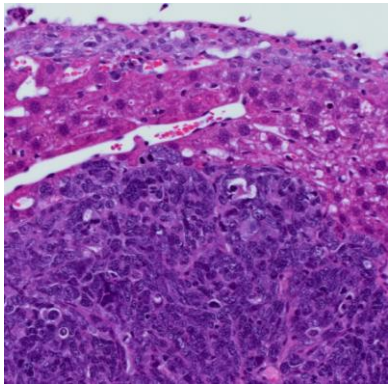


Ascites

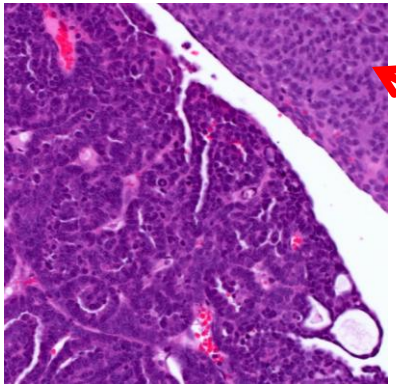




Orthotopic carcinoma



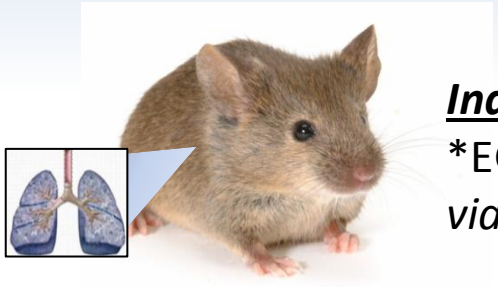
Liver Metastasis



Recipient Ovary

EXAMPLE 2: REPRESENTATIVE TREATMENT SYSTEMS EVALUATION IN RESISTANT EGFR-T790M-L858R-DRIVEN NSCLC

Erlotinib-Resistant huEGFR T790M-L858R Driven NSCLC

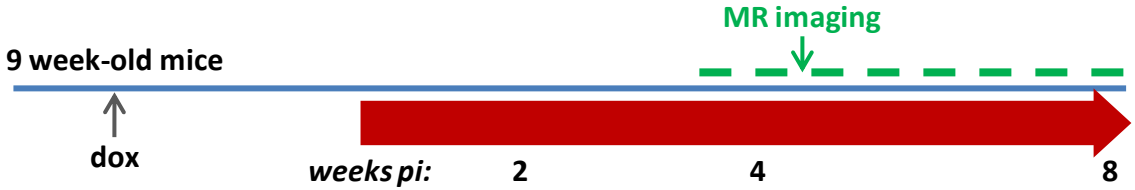


Inducible event:

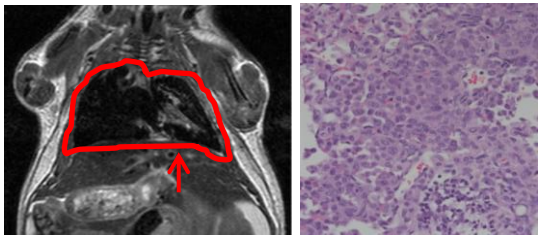
*EGFR T790M-L858R

*via Tg*CCSP-rtTA and Tet-op (EGFR TL)

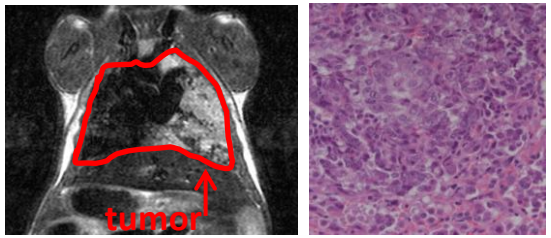
De novo model: induction by dox feed

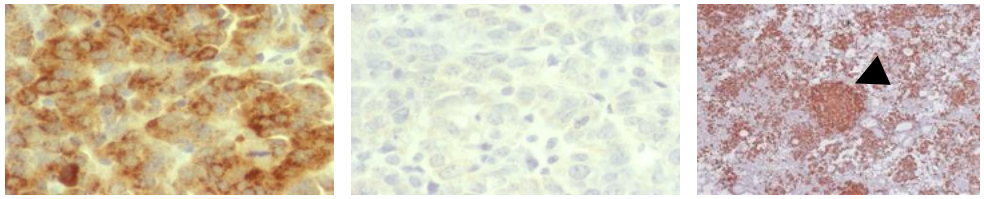


2 wks post-induction: adenoma



6 to 8 wks post-induction: adenocarcinoma



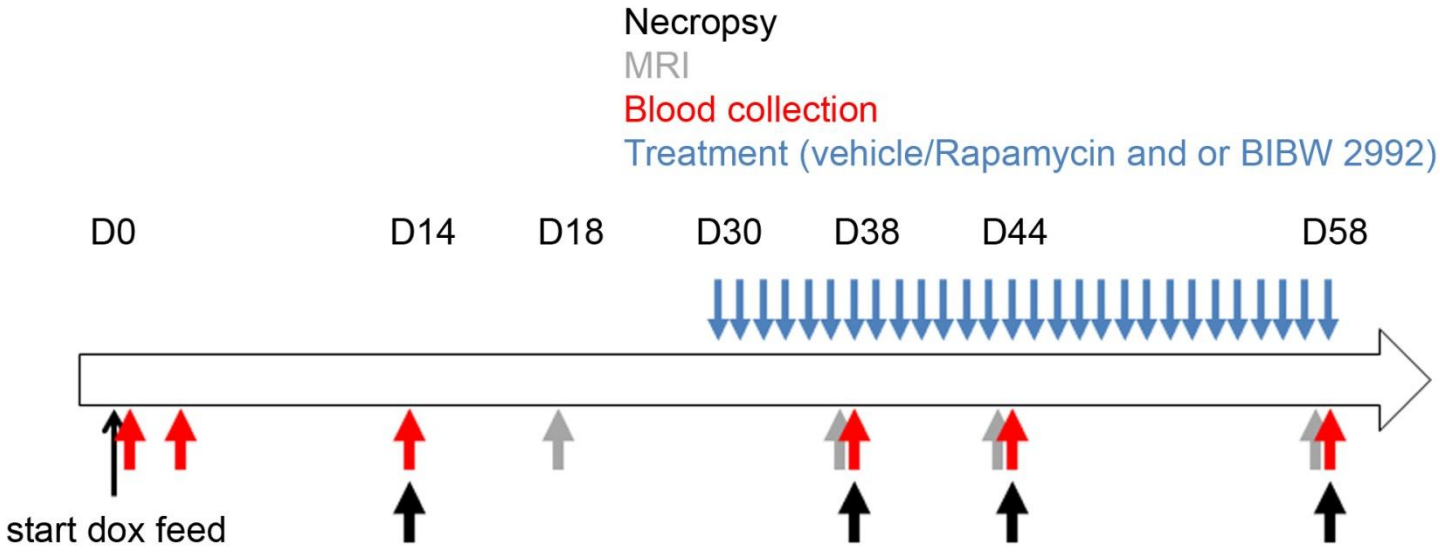


SPC **C10** **EGFR**

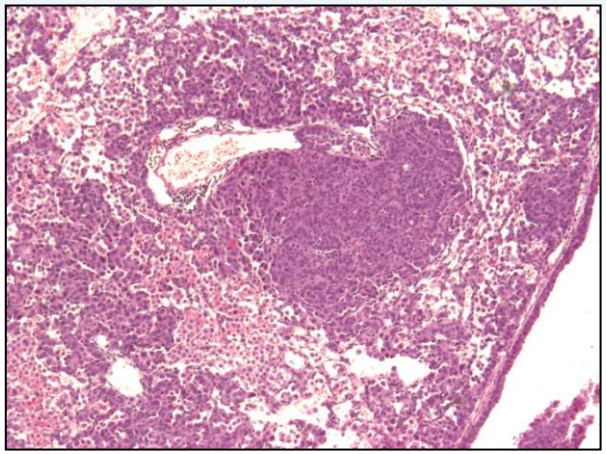
**Collaborator: Kwok-Kin Wong, DFCI*

Drug Treatment and Response: Scale-Up and Systems Biology Analysis

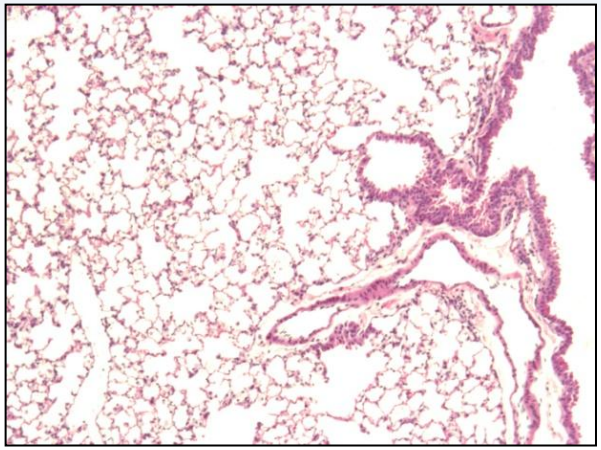
- Systems biology study on drug combination expected to be effective
- Treatment for 1 week, 2 weeks or 4 weeks/concurrent blood and tissue biomarker study
- Rapamycin is an mTOR inhibitor and BIBW 2992 (Afatinib) is an irreversible inhibitor of EGFR and HER2 tyrosine kinases



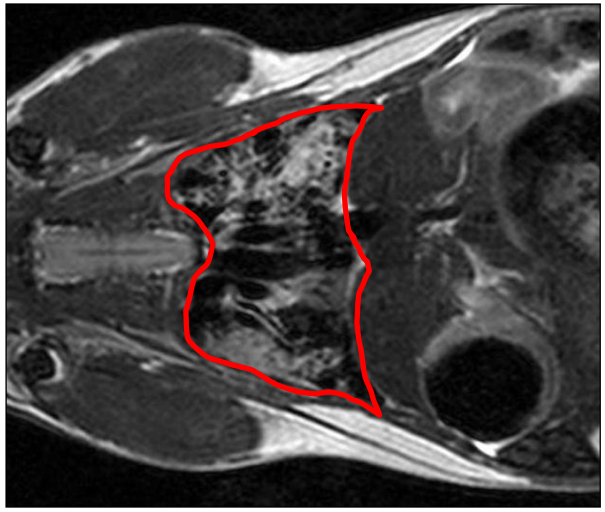
MRI and Pathology Confirm Drug Treatment Response



Post-treatment histology: minimal to no remaining lesions

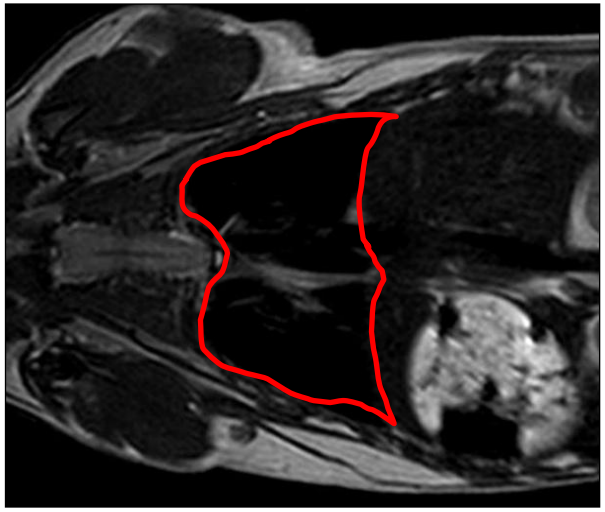


Pre-treatment



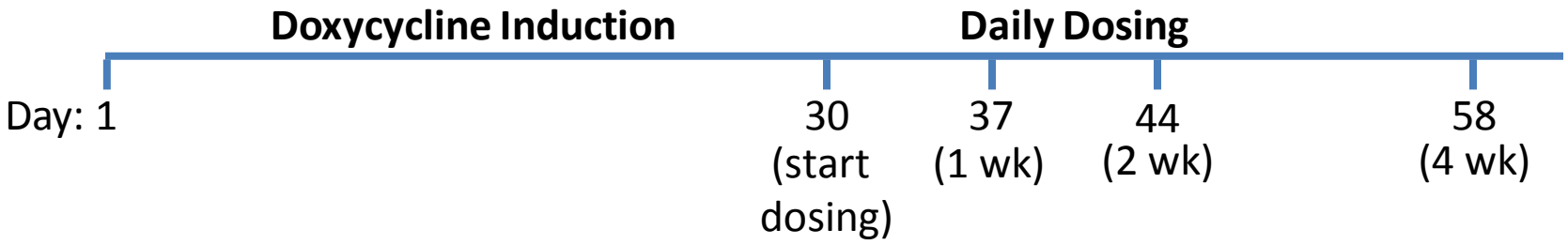
MRI scans: Regression of diffuse masses

BIBW2992+Rapamycin (4 weeks treatment)

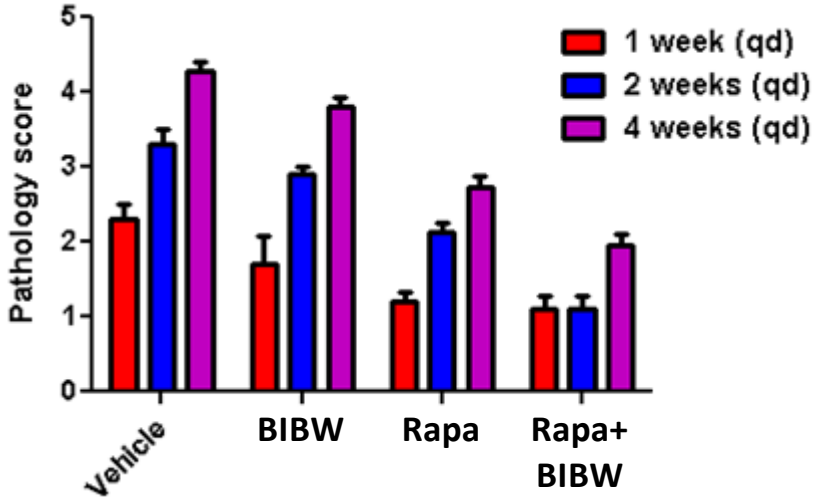


**Top panels are different mice; bottom: the same mouse imaged before and after treatment*

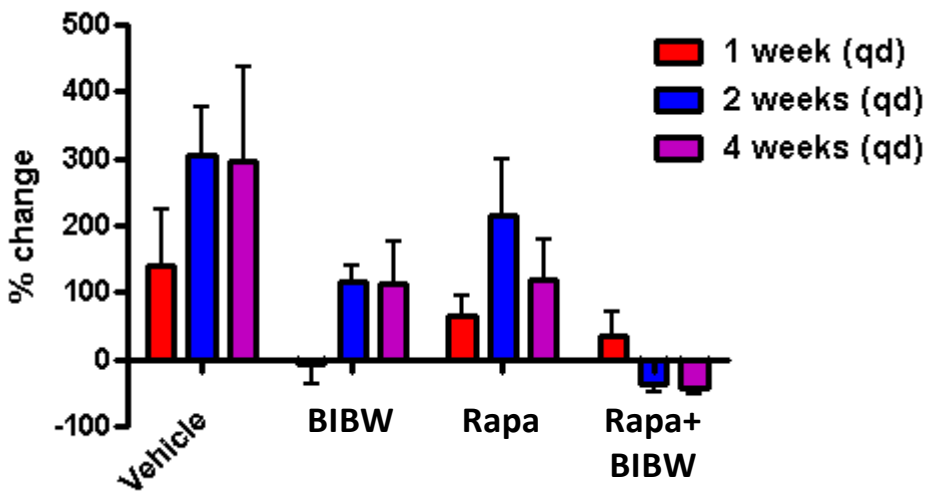
Temporal Assessment: Changes in Response Degree with Time



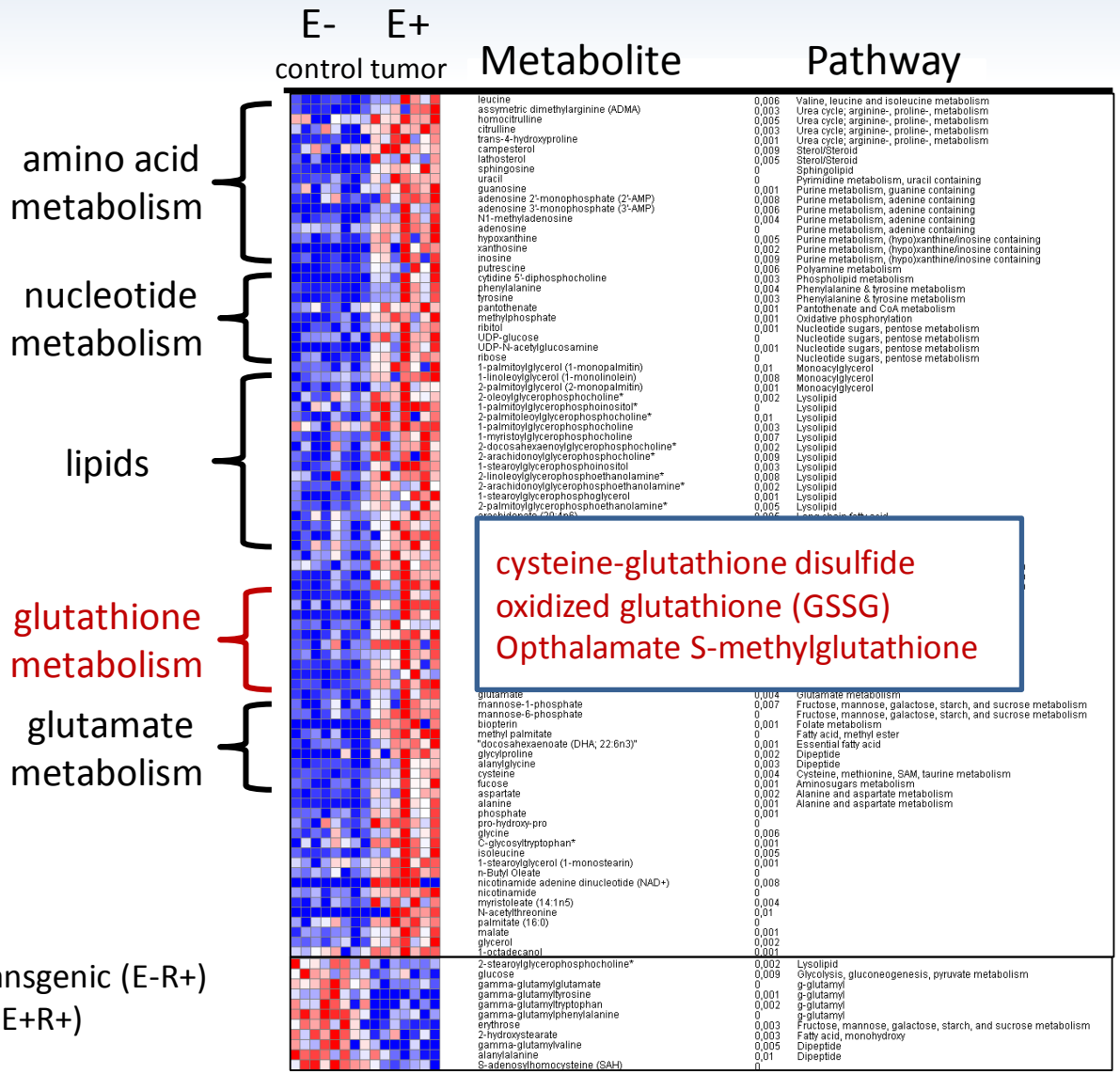
Histological Disease Severity



% Change in Tumor Volume (MRI)

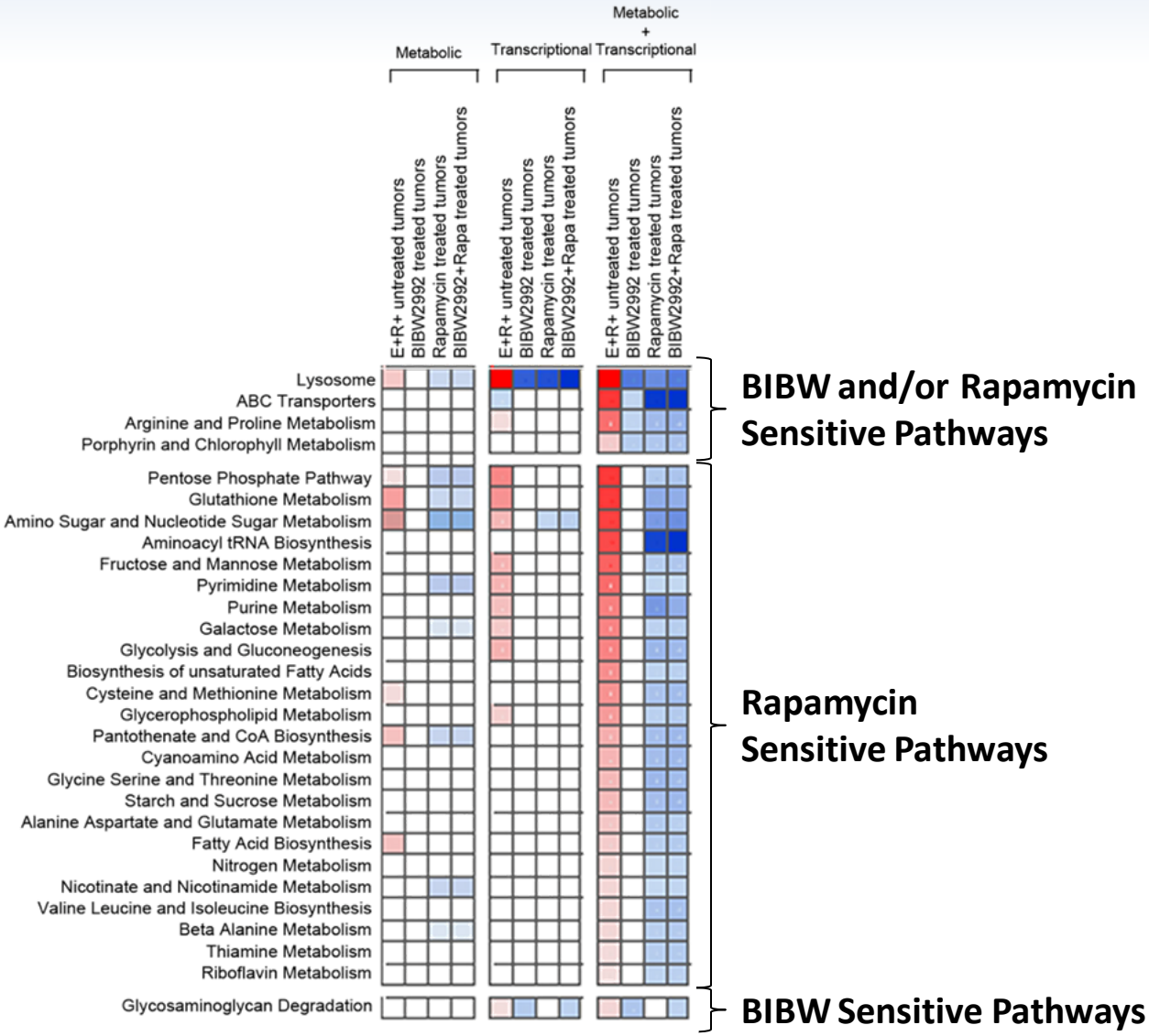


Metabolomic Results Reveal Set of Pathways Up in Tumor-Bearing vs. Tumor-Free Mice



T-test analysis of single transgenic (E-R+) versus double transgenic (E+R+)
P<0.01, 98 metabolites

Enrichment Analysis of Metabolomic and Transcriptomic Signatures in KEGG Pathways



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