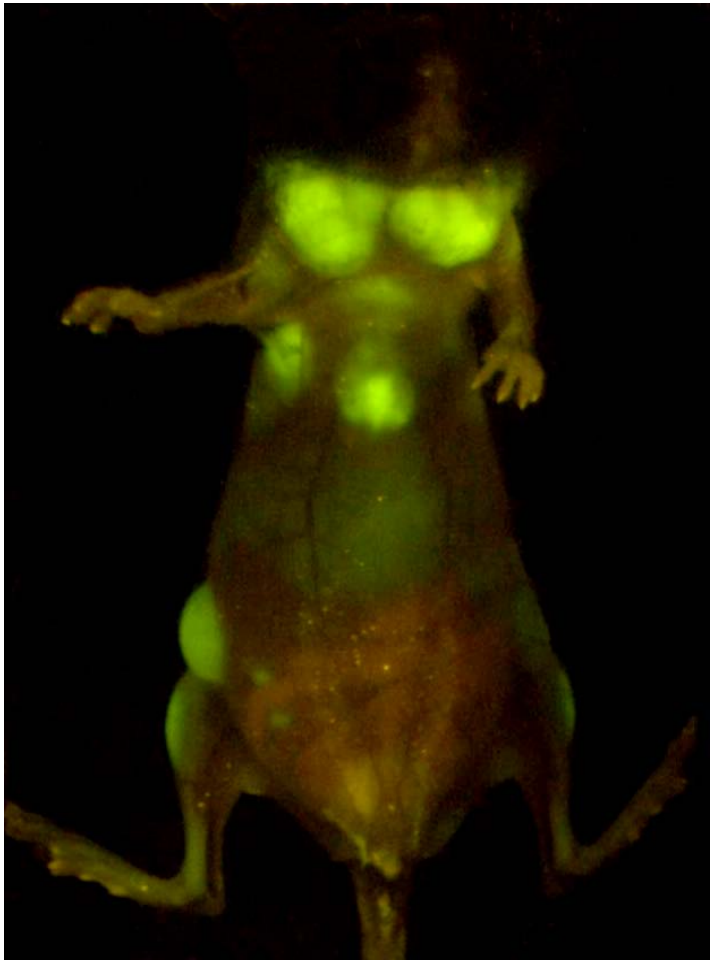
MSKCC Center for Precision Disease Modeling

Scott W. Lowe, Ph.D.

NIH Council of Councils Meeting
September 9, 2016

Precision Disease Models



Myc-induced B cell lymphoma tagged with GFP

- **Genomic information** is producing information about human disease at an extraordinary pace
- Genetic variation between individuals provides a rationale for **personalized medicine**
- **Functional studies** are needed to interpret the meaning of genetic variation
- **Predictive disease models** are needed for genetic information to be leveraged into improvements in patient care

MSKCC Center for Precision Disease Modeling

Goal: To facilitate an understanding of how genetic variation influences human disease and to develop preclinical models to test therapeutic strategies based on this understanding

- **Coordinate** institution wide efforts to develop next generation disease models using genetically engineering mice or from viable cells from genetically-annotated patients

- Provide **infrastructure** and support for the Center's Disease Modeling Units

- To **enable** investigators at MSKCC and other institutions to access center resources and expertise.

Precision Disease Models – Why MSKCC?

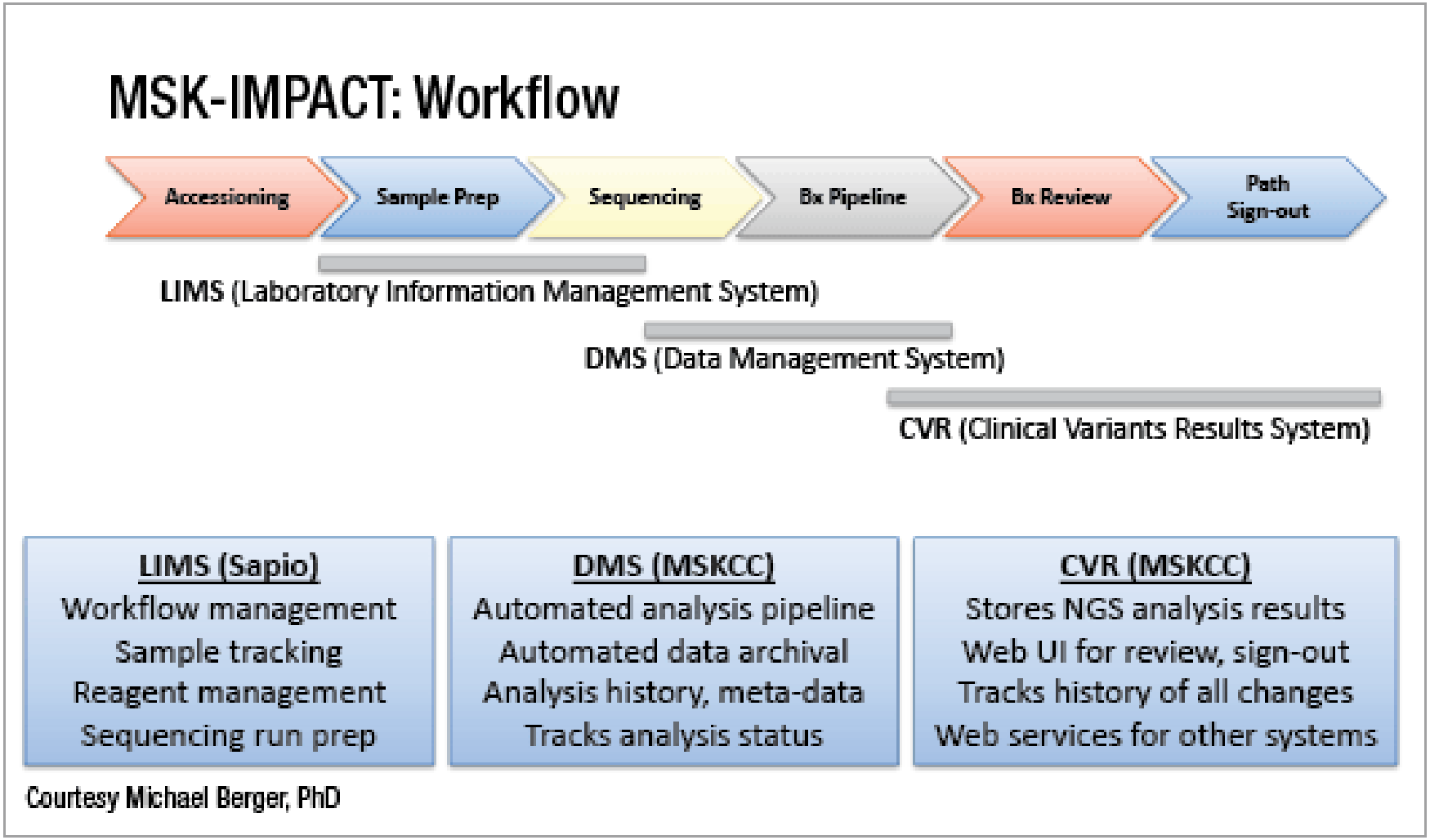
- MSKCC is a research institute and hospital with world-class **basic**, **translational**, and **clinical** researchers

- MSKCC is located in **New York City** and is surrounded by other world class research institutions

- MSKCC has **pre-existing infrastructure** to facilitate the development of a strong precision disease models program:
  - *History of producing animal models of human disease*
  - *"Mouse hospital" capable of performing preclinical studies*
  - *Viable tumor cell initiative to produce models using genetically annotated human tissue*
  - *Strong computational and genomics infrastructure to inform and interpret modeling efforts*
  - *Clinical structure primed to act on preclinical information*
  - *Sequence information obtained from >10,000 patients/year*

Precision Disease Models – Why MSKCC?

Genotyping of tumor and normal DNA from all cancer patients



Precision Disease Models – Why MSKCC?

cBioPortal for Cancer Genomics

Visualize, analyze, discover.

Memorial Sloan Kettering Cancer Center

You are logged in as solitd@mskcc

HOME | DATA SETS | TUTORIALS | FAQ | NEWS | TOOLS | ABOUT | VISUALIZE YOUR DATA

MSK-IMPACT Clinical Sequencing Cohort (MSKCC) Query this study

Targeted sequencing of clinical cases via MSK-IMPACT. Please follow the publication guidelines when using these data in abstracts or journal articles.

These data are available to MSK investigators only, are unpublished, and cannot be shared with anyone outside of MSK. **12079 samples from 11352 patients.**

Study Summary | Clinical Data | Mutated Genes

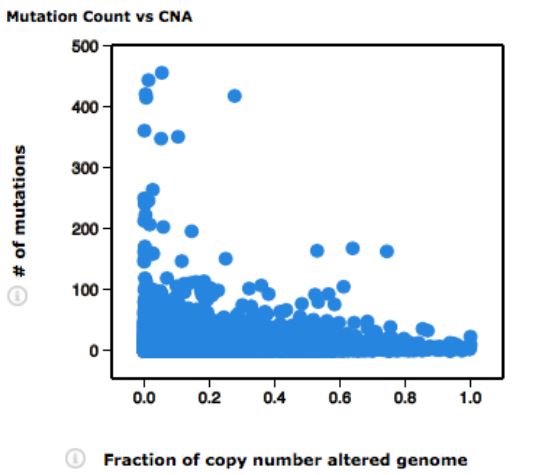
Samples selected: 12079 | query genes - click to expand | Query | Select cases by IDs | Add Chart

Cancer Type	#	Freq
Non-Small Cell Lung ...	1823	15.1%
Breast Cancer	1448	12.0%
Colorectal Cancer	1108	9.2%
Prostate Cancer	781	6.5%
Glioma	612	5.1%
Pancreatic Cancer	577	4.8%
Soft Tissue Sarcoma	492	4.1%
Bladder Cancer	438	3.6%
Melanoma	418	3.5%
Renal Cell Carcinoma	394	3.3%

Search...

Cancer Type Detailed	#	Freq
Lung Adenocarcinoma	1480	12.3%
Breast Invasive Ducta...	976	8.1%
Colon Adenocarcinoma	806	6.7%
Prostate Adenocarcinoma	762	6.3%
Pancreatic Adenocarci...	447	3.7%
Bladder Urothelial Ca...	324	2.7%
Glioblastoma Multiforme	315	2.6%
Renal Clear Cell Carc...	222	1.8%
Cutaneous Melanoma	216	1.8%
Breast Invasive Lobul...	198	1.6%

Search...

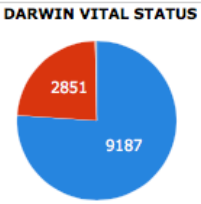


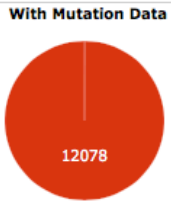
Mutated Genes (12078 profiled samples)

Gene	# Mut	#	Freq
TP53	5414	4946	41.0%
KRAS	1836	1805	14.9%
TERT	1713	1617	13.4%
PIK3CA	1653	1477	12.2%
APC	1856	1252	10.4%

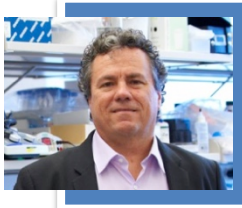
CNA Genes (12074 profiled samples)

Gene	Cytoband	CNA	#	Freq
CDKN2A	9p21	DEL	955	7.9%
CDKN...	9p21	DEL	947	7.8%
CDKN...	9p21	DEL	937	7.8%
CDKN2B	9p21	DEL	870	7.2%
CCND1	11q13	AMP	512	4.2%





Center organization



Leadership

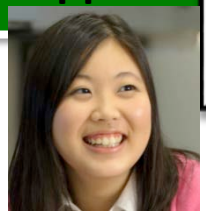
Scott Lowe
David Solit

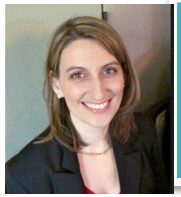
Steering Committee (FGI)

EAB

Administrative support

Eder Paraiso
Christy Park



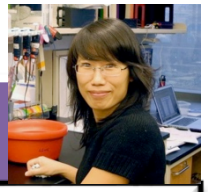


Coordination Core

Elisa de Stanchina

Genomics and Bioinformatics Core

David Solit
Barry Taylor



Pre-clinical and Co-Clinical Core

Elisa de Stanchina,
Zhen Zhao

Disease Modeling Units

Ross Levine, Scott Armstrong,
John Petrini, Scott Lowe

MSKCC Center for Precision Disease Modeling

Coordination Core – Data Integration

Genomics/Bioinformatics Core

Genomics

IMPACT
TCGA
GWAS

Bio-informatics

cBioportal
Genomics

Preclinical/co-Clinical Core

Mouse Modeling

GEMM-ESC
Xenografts
Organoids
PDXs

Mouse Hospital

PK/PD
Efficacy
Toxicology

Disease Modeling Units

Project 1

Sensitivity and Resistance to Molecularly Targeted Leukemia Therapies

Project 2

RTEL1 mutations in Hoyeraal Hreidarsson Syndrome

Project 3

Genetic determinants of CRC initiation and maintenance

MSKCC Center for Precision Disease Modeling

COORDINATION CORE

LEADERSHIP
Scott Lowe, Co-Director
David Solit, Co-Director
Steering Committee

NIH Program Officials

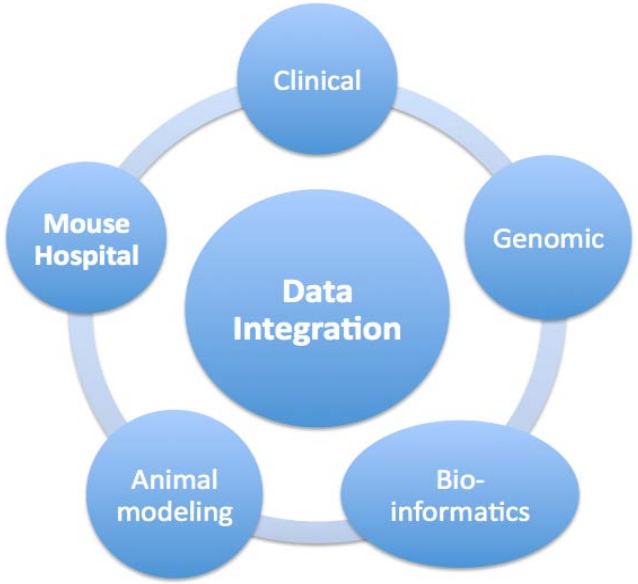
External Advisory Board

PROGRAM COORDINATION
Elisa de Stanchina

Resource Integration:

Administration:
Program meetings and symposia
Committee and Board meetings
Progress Reports

Regulatory:
Protocols (IRB, IACUC)
Compliance (GLP/FDA)
Intellectual Property



EAB Members

Lukas Dow, Ph.D. - Weill Cornell Medical College
Sohail Tavazoie, M.D., Ph.D.- Rockefeller University
Sergei Koralov, Ph.D., - NYU Langone Medical Center
Cory Abate-Shen, Ph.D., - Columbia University

MSKCC Center for Precision Disease Modeling

GENOMICS/BIOINFORMATICS CORE (B. Taylor)

Aim 1. **Analysis of human and murine genomic data**
- Sequence analysis and computing resources
- Special handling for sequencing PDXs

Aim 2. **Establish a minable cross-cancer/cross-species mutation compendium**

Aim 3. **Develop and implement knowledge management systems**
- Data and knowledge management in the cBioPortal
- Study, analysis, and workflow management

MSK-IMPACT^{Mouse} and Tumor Array

MSK-IMPACT^{Mouse}

- A hybridization **capture-based** next-generation sequencing assay
- For **targeted deep** sequencing of of **578 key cancer genes** in formalin-fixed, paraffin- embedded (FFPE) tumors

Tumor array

- High throughput **low pass whole genome** sequencing for inexpensive CNV analysis

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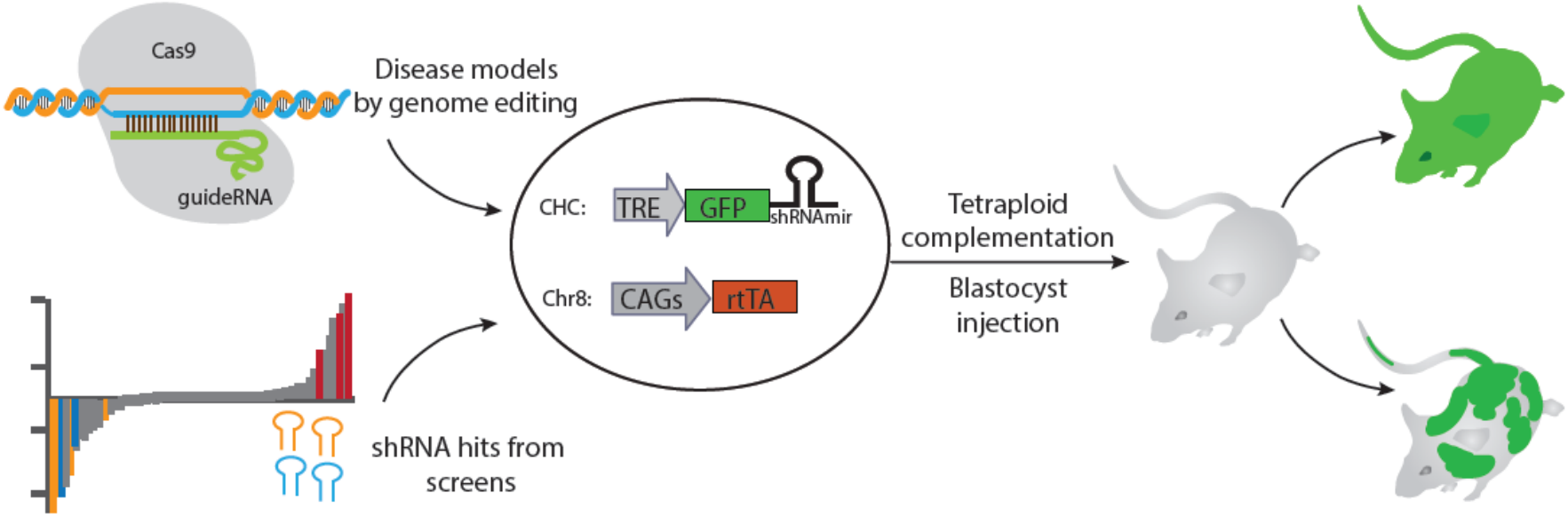
MOUSE MODELING UNIT (Z. Zhao)

The need for speed…



- Disease models generated by genome editing- shRNA to test disease alleles
- shRNA mice assess anti-tumor and potential toxicities of potential drug targets

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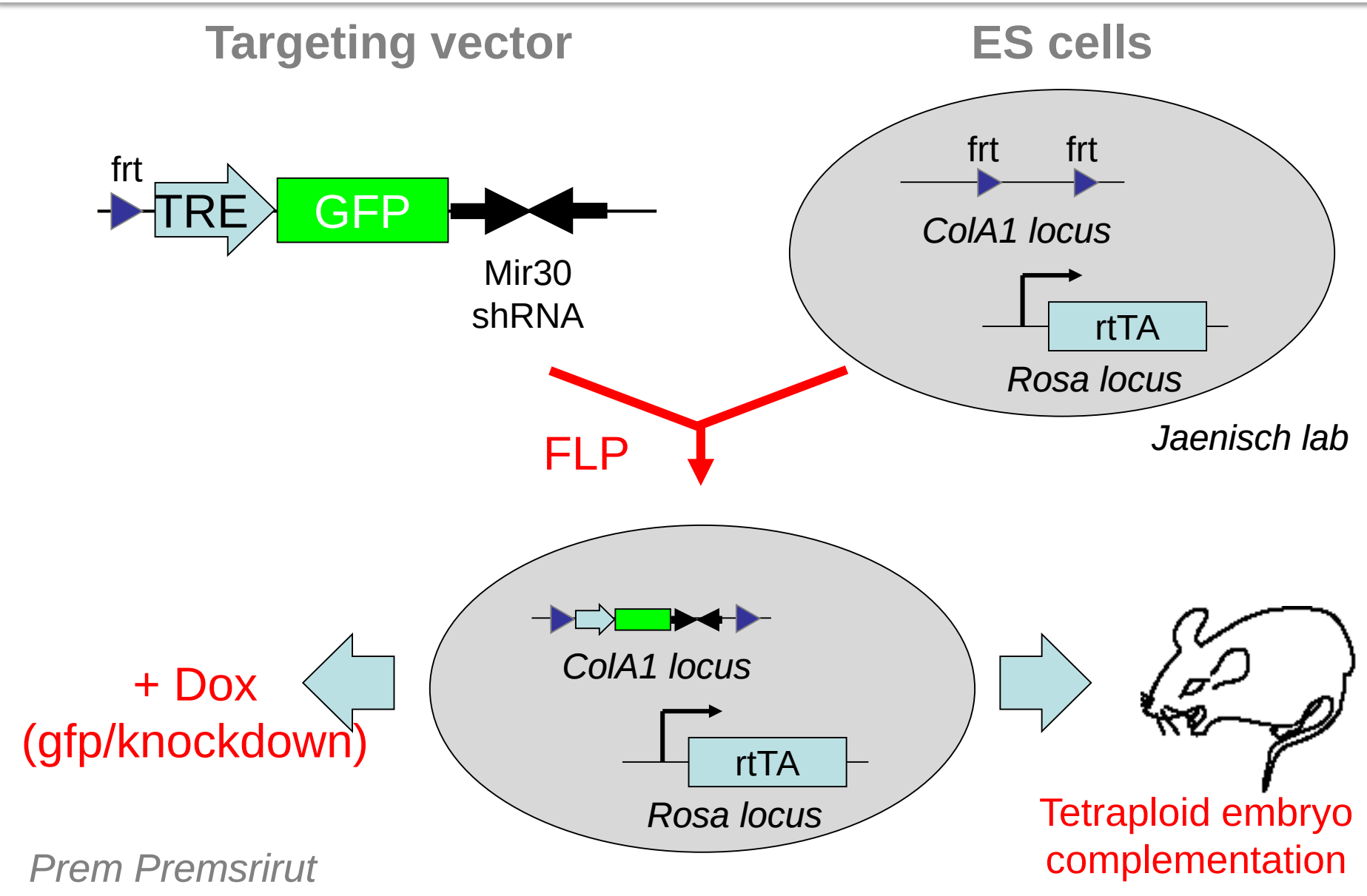
MOUSE MODELING UNIT (Z. Zhao)

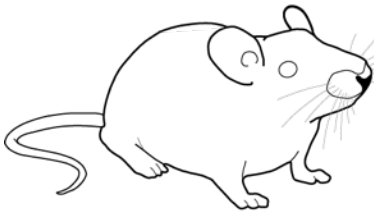


Prem Premsrirut

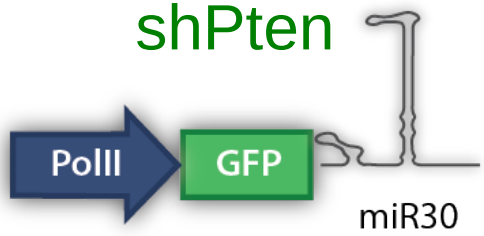
GEMM-ESCs to explore pancreas cancer genetics

MOUSE MODELING UNIT (Z. Zhao)

CAGs-LSL-rtTA3-Kate
Pdx1-Cre
LSL-Kras
Cola1 homing cassette



shPten



RMCE

12 new GEMM-ESC
models rederived

Dox

Tumor biology,
preclinical models

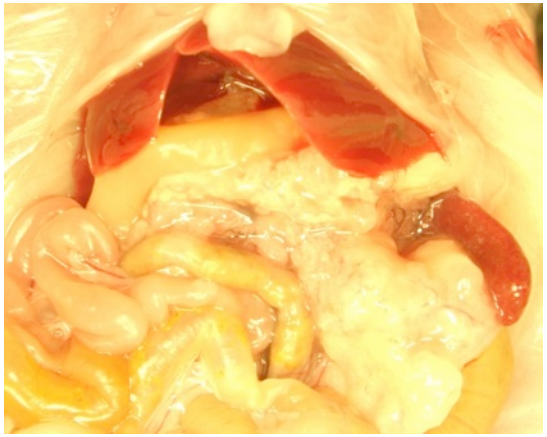
(>1200 animals produced)

GEMM-ESCs to explore pancreas cancer genetics

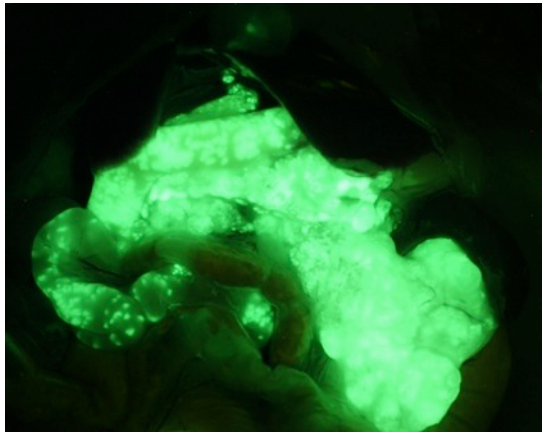
MOUSE MODELING UNIT (Z. Zhao)

LSL-Kras$^{G12D+/-}$	Cre-activatable oncogenic Kras
Pdx1-Cre$^{+/-}$	Pancreas specific cre
CAGs-LSL-RIK	Cre-activatable rtTA (linked to Kate)
TRE.GFP.shPten$^{+/-}$	Dox-inducible PTEN shRNA

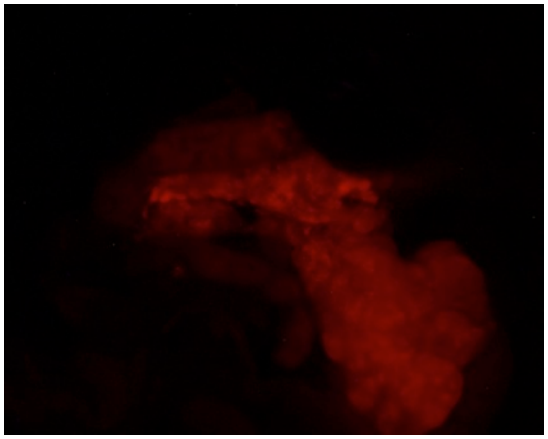
Murine pancreas (20d dox treatment)



Bright field



GFP fluorescence



Katushka fluorescence

Michael Saborowski

GEMM-ESCs to explore pancreas cancer genetics

MOUSE MODELING CORE (Z. Zhao/E. de Stanchina)

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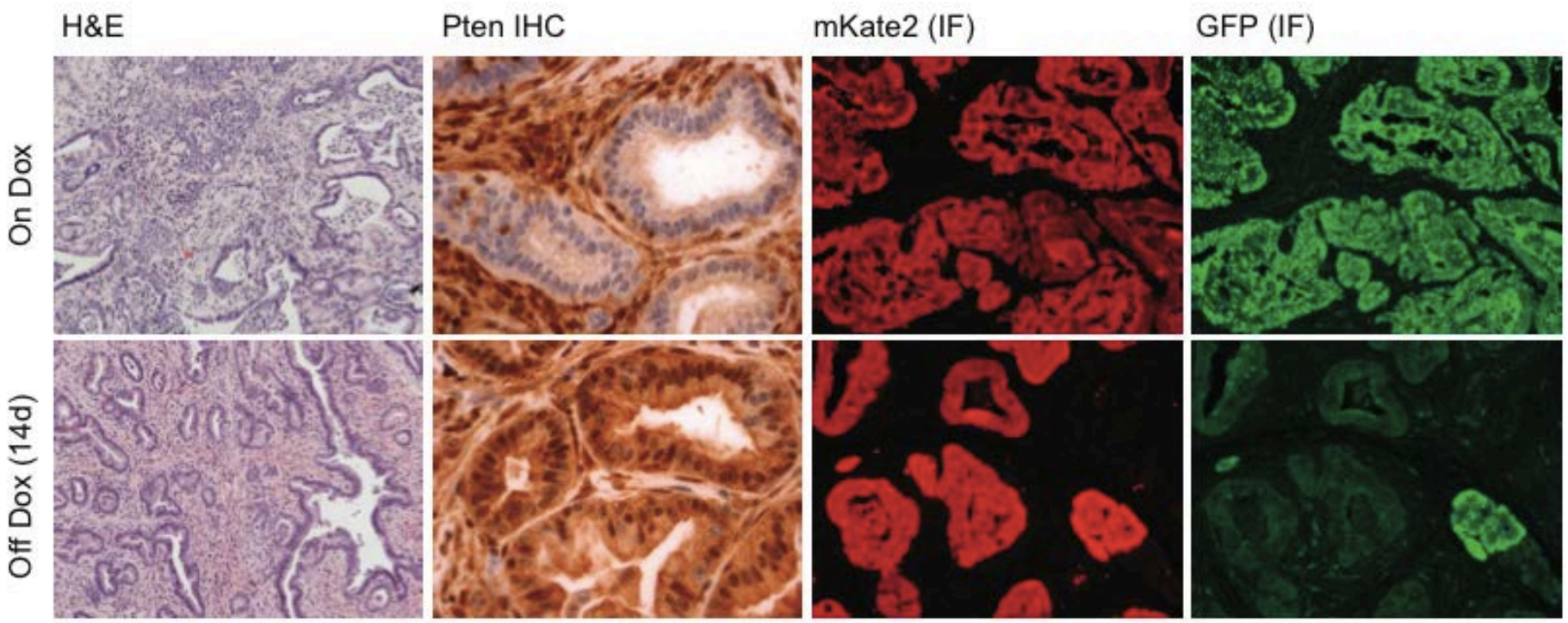

Michael Saborowski

GEMM-ESCs to explore pancreas cancer genetics

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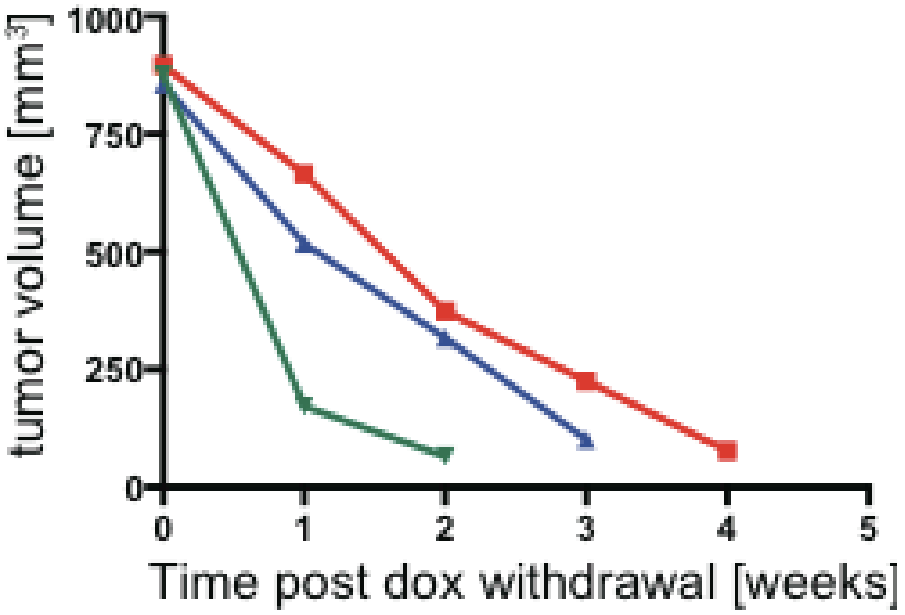
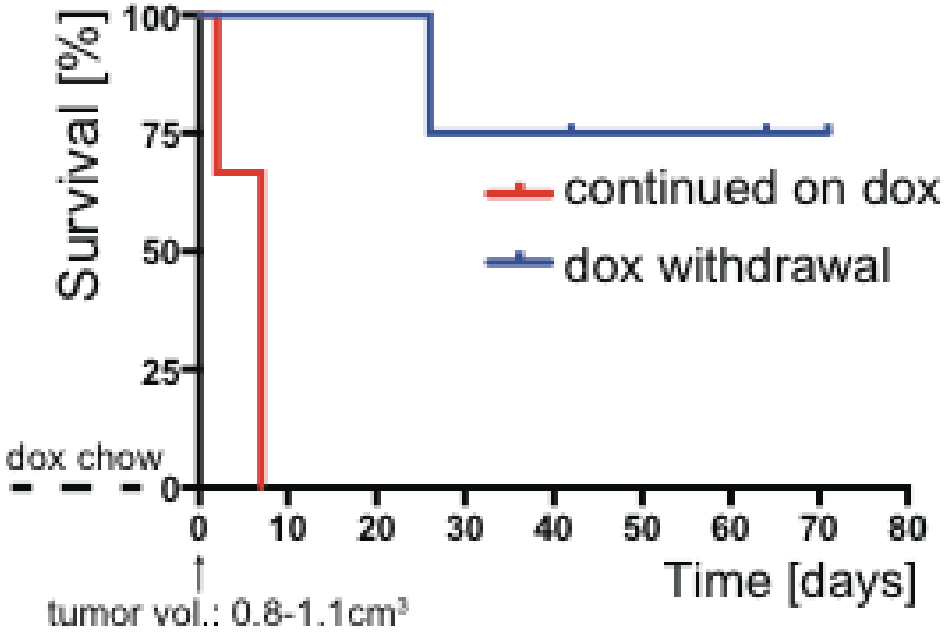
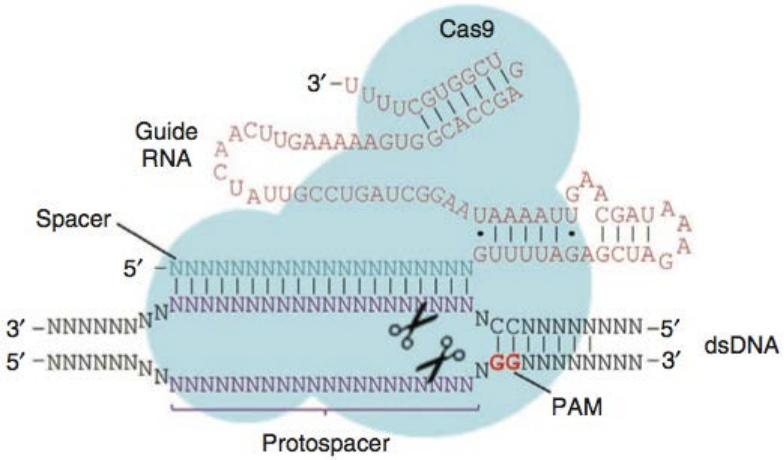




Michael Saborowski

Genome editing using CRISPR/Cas9

MOUSE MODELING UNIT (Z. Zhao)



Mali et al, Nature Methods, 2013

- CRISPR/Cas9 for germline engineering
- Inducible CRISPR and CRISPRa for temporal gene manipulation in vivo
- CRISPR for mosaic modeling of somatic mutation (SCNV, SNP, rearrangements)

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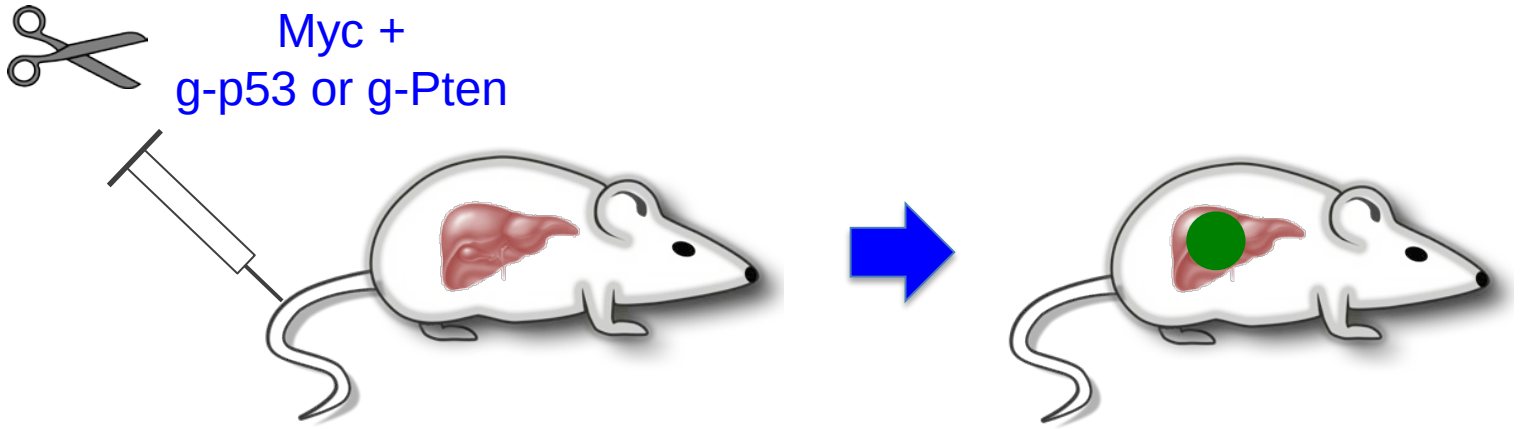
MOUSE MODELING UNIT (Z. Zhao)



Targeting construct | Strain production | Mating strains/ Colony/colony expansion | Tumor assessment

Conventional Method

Liver DNA editing

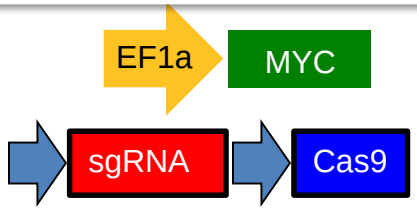
Multiplexed

0 1 2 3

Time (years)

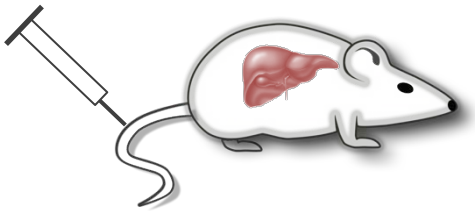
Mosaic mouse models for studying cancer genetics

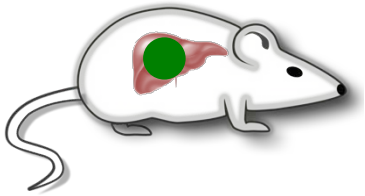
MOUSE MODELING UNIT (Z. Zhao)



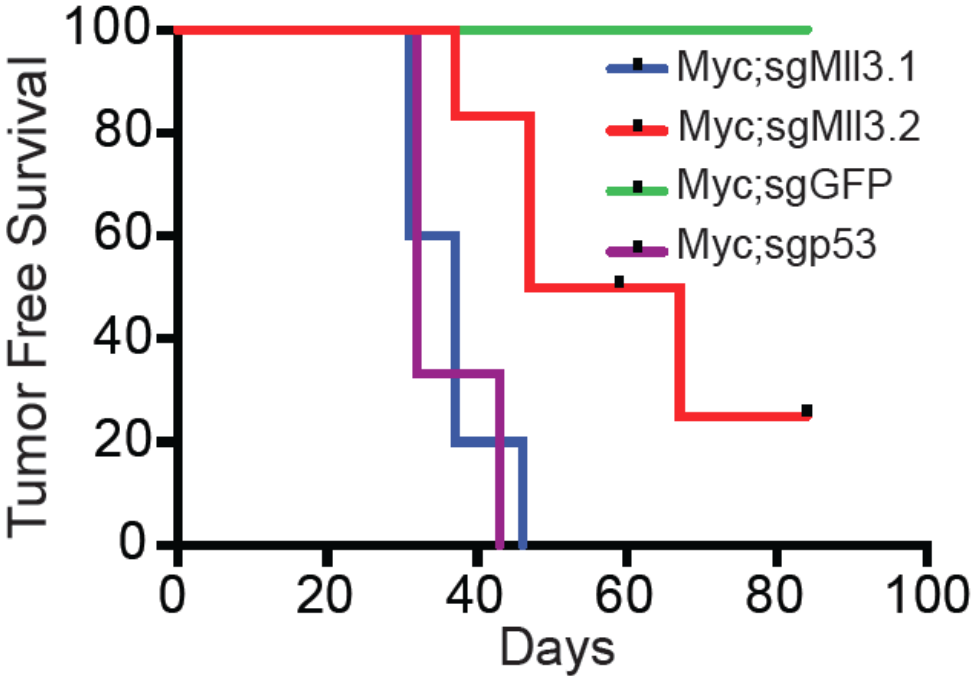
Transposon Vector (+transposase)

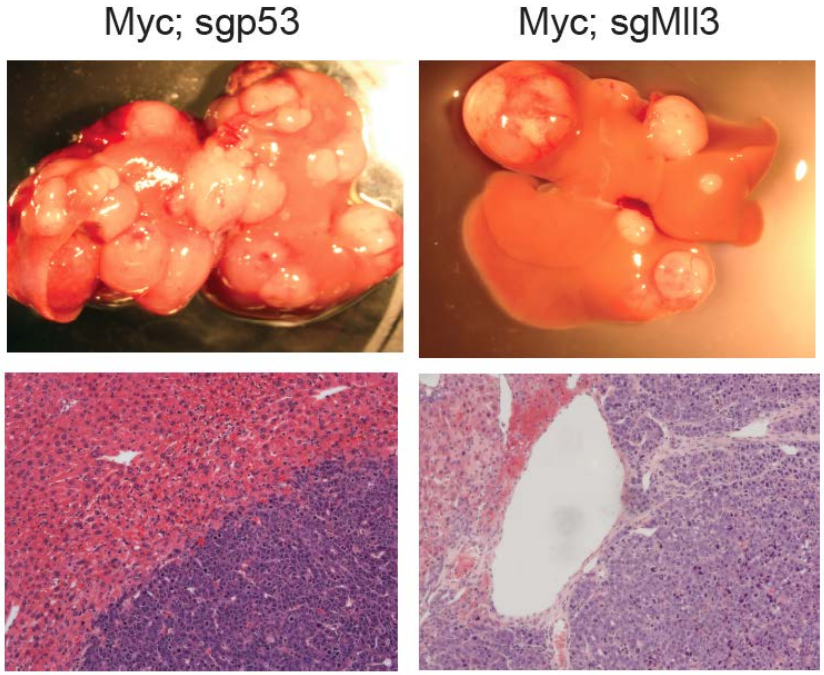
Hydrodynamic Injection





Disease monitoring (GFP+ cells)





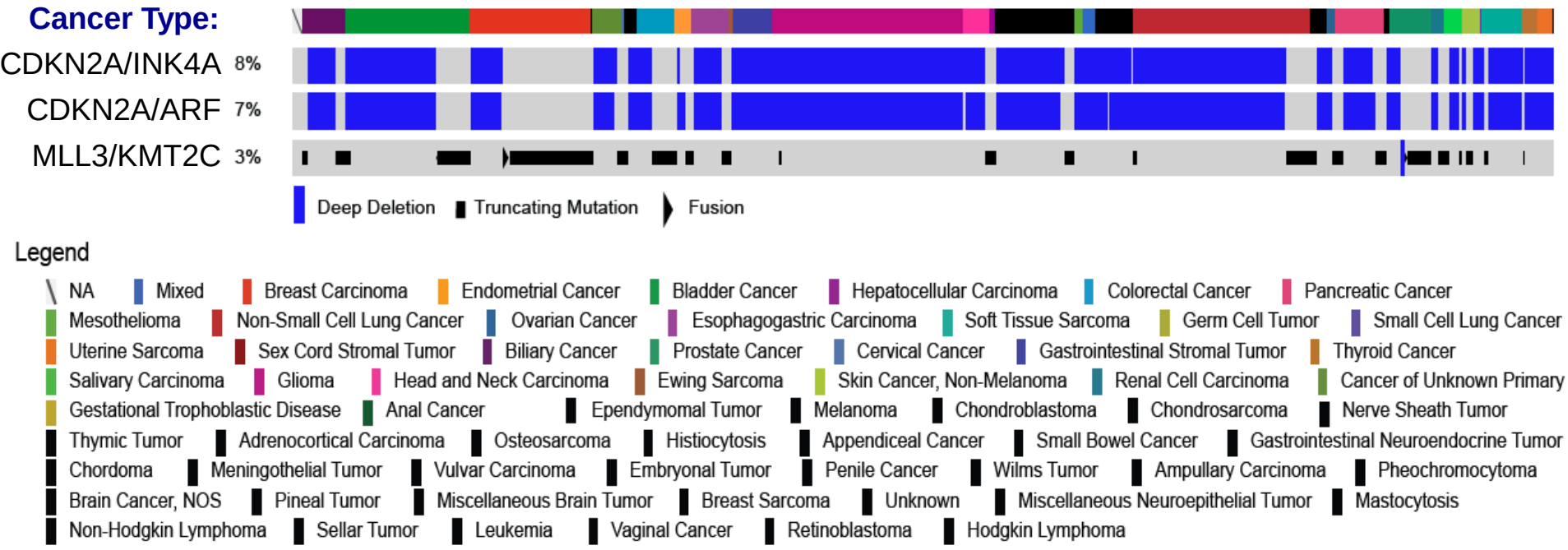
Darjus Tschaharganeh, Chun-Hao Huang, John Morris IV

Establishing human relevance of functional studies

BIOINFORMATICS UNIT

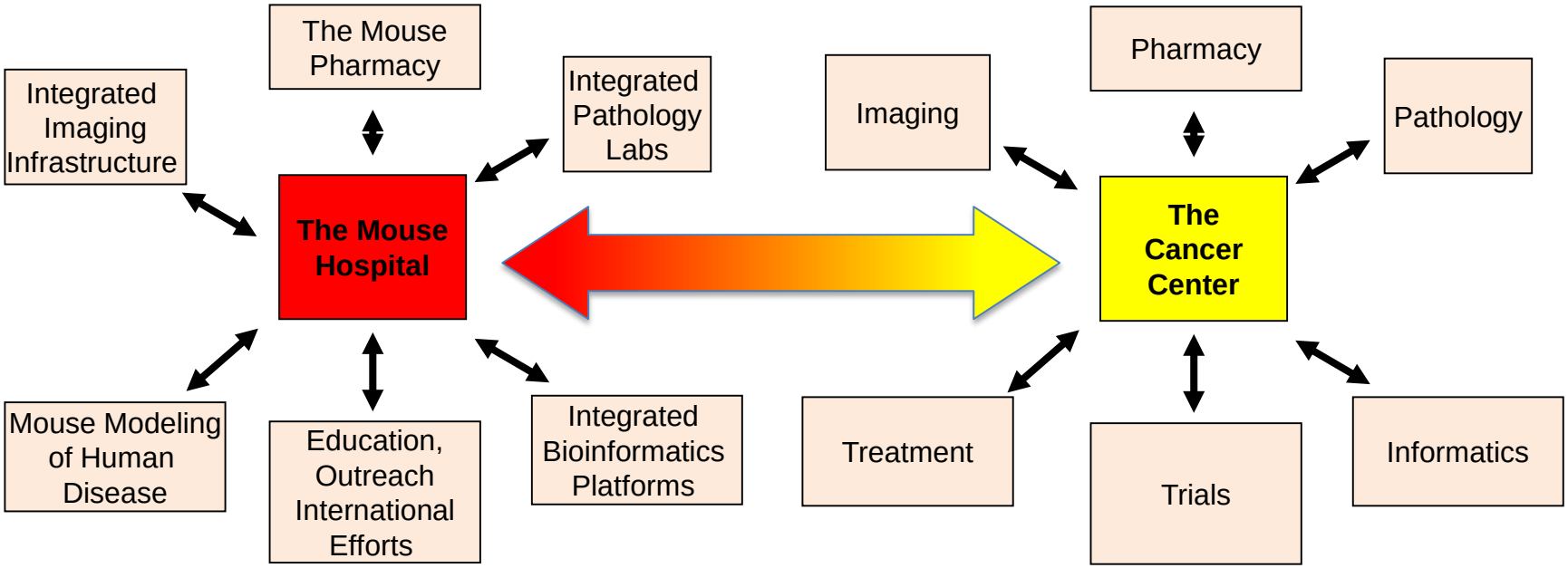
MYC ⇨ MLL3 ⇨ CDKN2A ⇨ Apoptosis

MSK IMPACT (~8200 samples)



MSKCC Center for Precision Disease Modeling

MOUSE HOSPITAL UNIT (E. de Stanchina)



- Phenotyping of models to evaluate consequences of mutations and establish relevance to human disease
- Preclinical studies to mirror treatment plans used in patients

Efficacy and Toxicology

MOUSE HOSPITAL UNIT (E. de Stanchina)

Efficacy

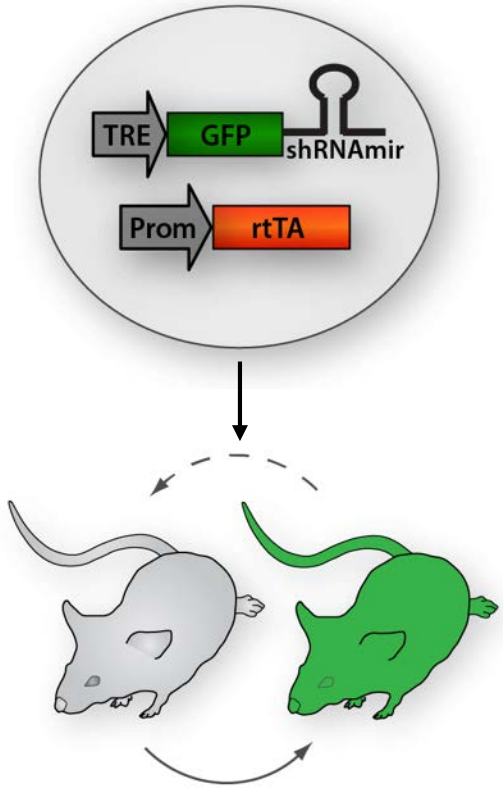
- >100 efficacy studies since U54 funding began
- Conventional, molecular targeted and immunotherapy for cancer
- Cell therapy for Hirschprung Disease (delivery of enteric neuronal stem cells to rescue mortality)

Toxicity

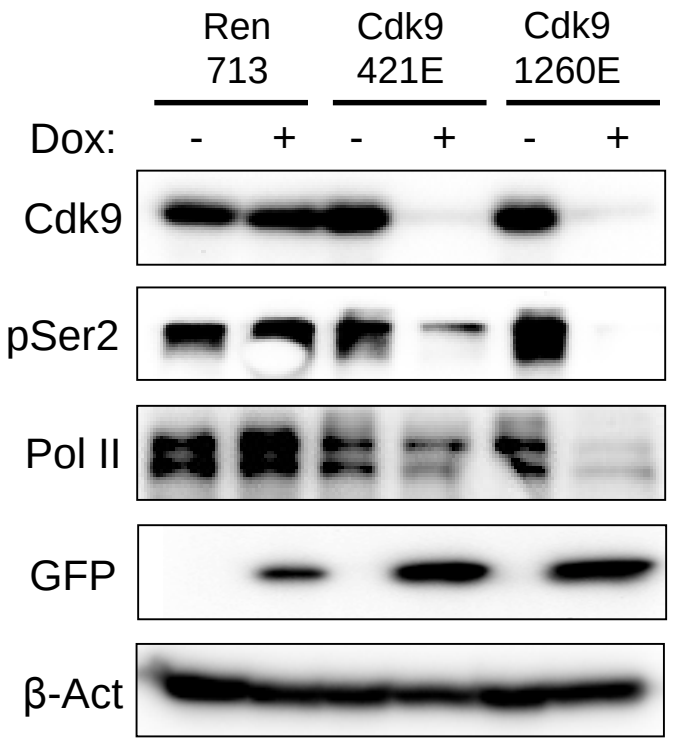
- Support to develop fully GLP compliant facility
- First FULLY GLP compliant study (Ab for Neuroblastoma (pediatric) - IND submission is expected by end of year
- 7 more GLP studies already in queue for 2017

Genetic and pharmacologic toxicity assessment
MOUSE MODELING AND HOSPITAL UNITS

CDK9 is a candidate cancer drug target



Premsrirut, Dow et al. Cell 2011



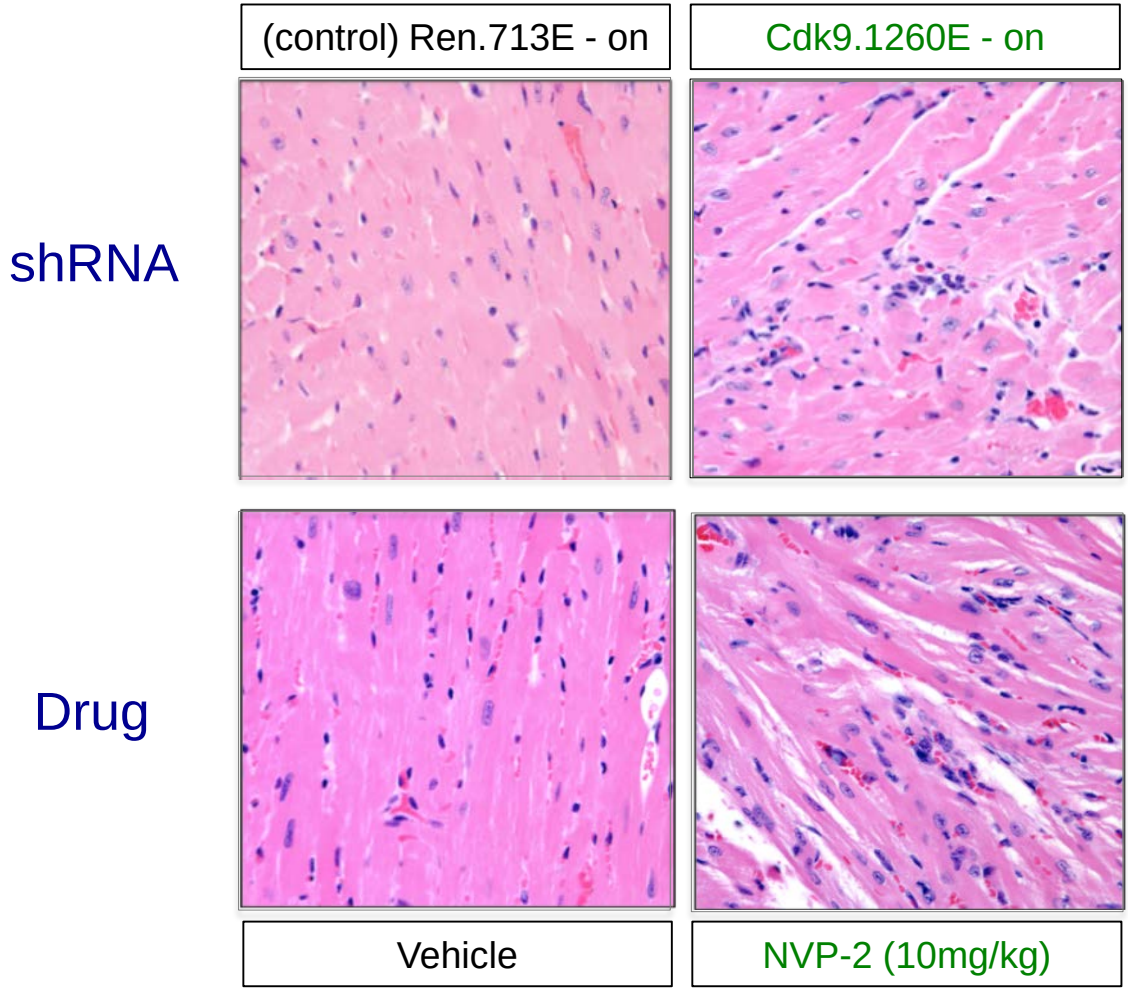
KH2 ES cells

Chun Hao Huang, Luke Dow

Genetic and pharmacologic toxicity assessment

MOUSE MODELING AND HOSPITAL UNITS

Heart histology following Cdk9 inhibition



A pipeline for patient derived xenograft production

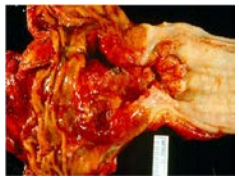
MOUSE HOSPITAL UNIT (E. de Stanchina)

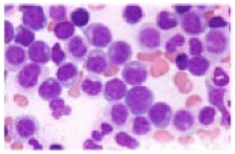
- Streamlined workflow for acquisition of clinical samples and establishment of PDX models

Patient selection

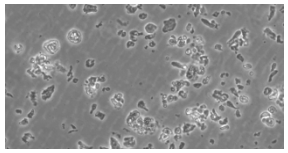
Patient consent

Biopsy or resection



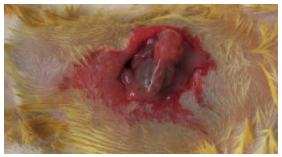


Blood



Cell lines

s.c. PDX



o.t. PDX

	Pre-PDX Core	Post-PDX Core
# Established PDXs	95	215
Take rate (%)	26%	60%
# Different tumor types	6	30

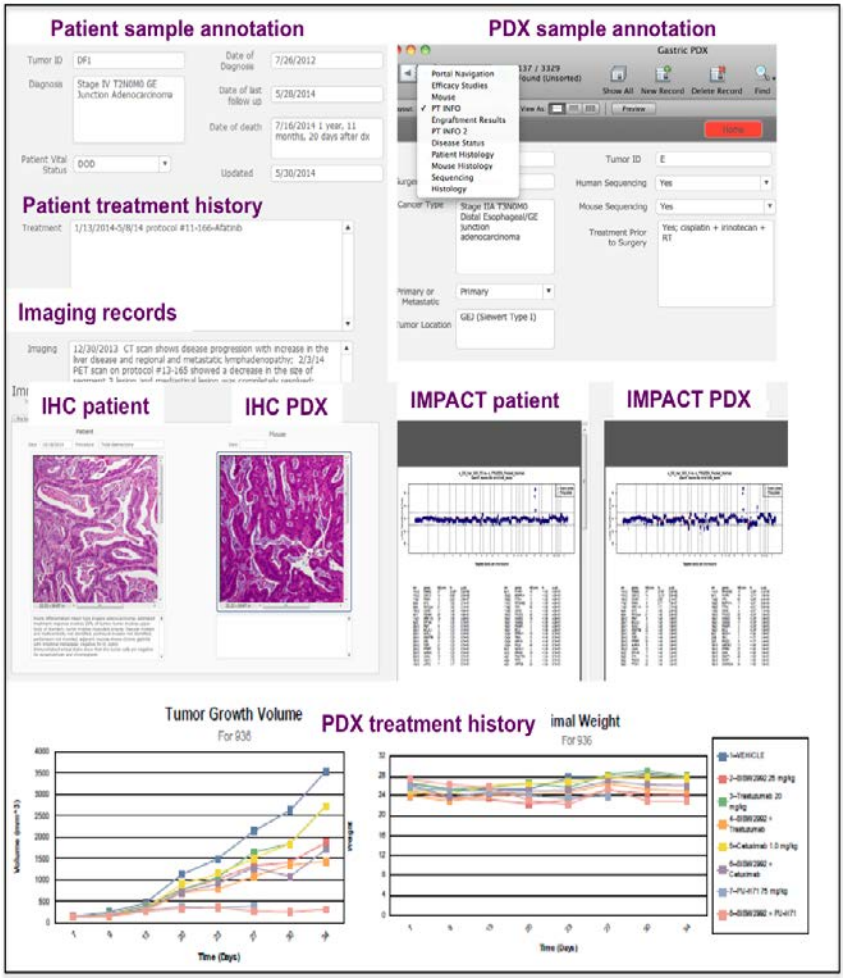
Patient screening/consent and sample collection coordinated by the Core under approved IRB protocol

Sample implantation and propagation carried out by the Core under approved IACUC protocol

Streamlined workflow led to improved take rate for all tumor types and larger spectrum of tumor subtypes

A pipeline for patient derived xenograft production

MOUSE HOSPITAL UNIT (E. de Stanchina)



SAMPLE ANNOTATION

Patient data	PDX data
Diagnosis	Tumor Type
Histology	Histology
Sequencing	Sequencing
Medical History	Treatment history
Treatment history	Sample banking history

PDX DATABASE

Searchable database for available PDX models based on:

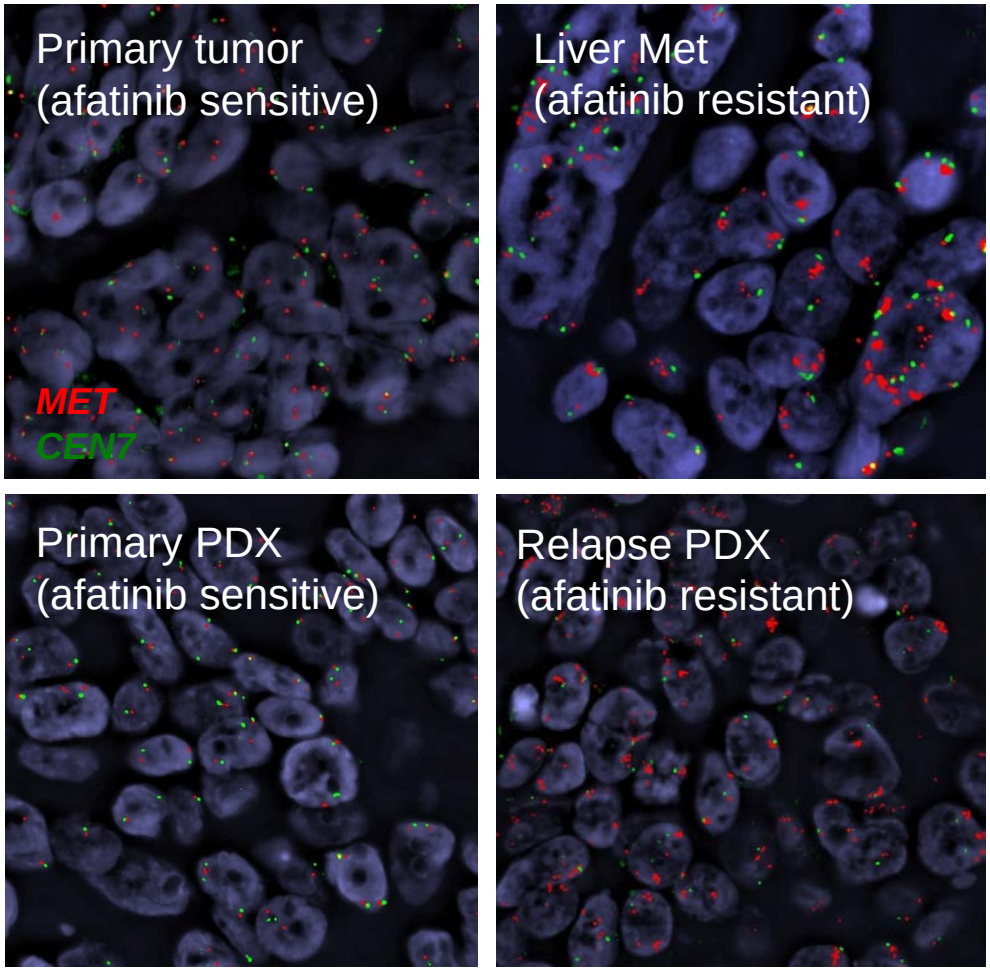
- tumor type/subtype
- genomic profile
- treatment history

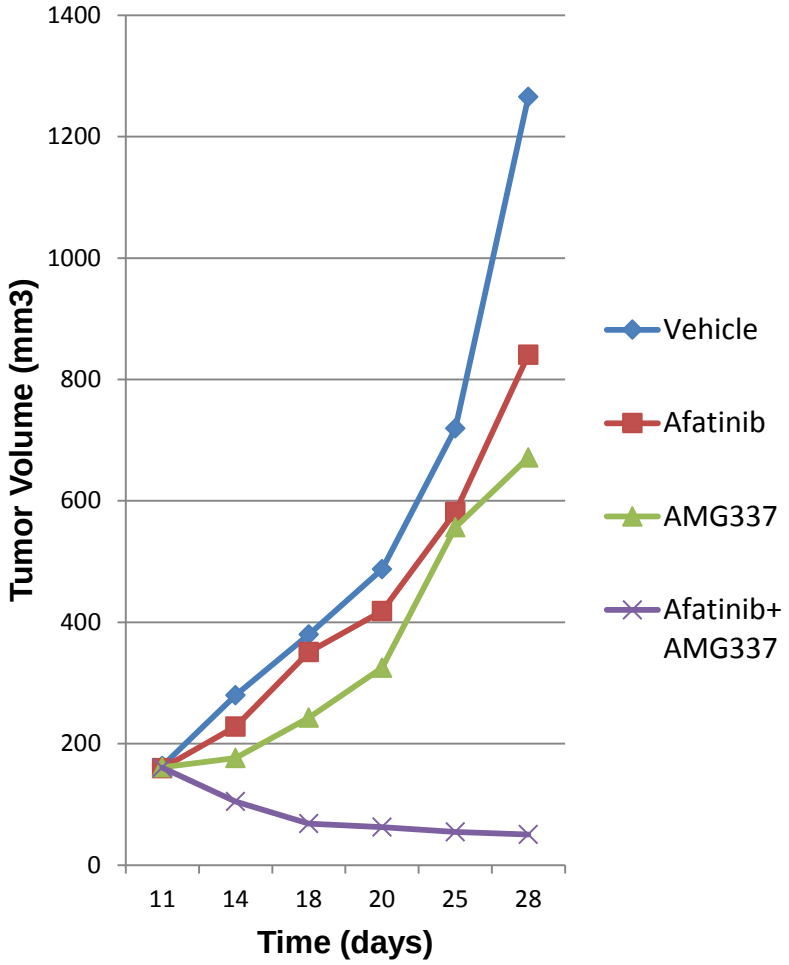
Our experience:
PDX models frequently retain genotype of primary tumor

Efficacy and Toxicology

MOUSE HOSPITAL UNIT (E. de Stanchina)

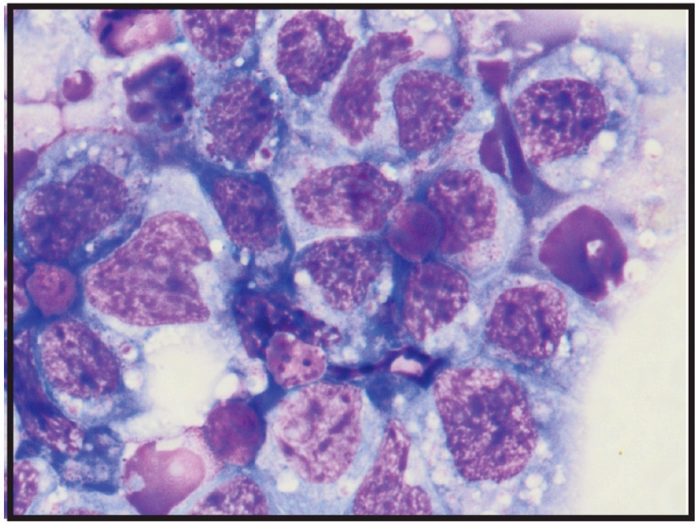
Establishing new treatment strategies using PDX models





HER2+ Gastric Cancer

Pilot project 1: Role of Mutations in Epigenetic Modifiers in AML (Levine)



Aim 1. Evaluate the impact of concurrent mutations on sensitivity to targeted leukemia therapies

Aim 2. Investigate mechanisms of sensitivity and resistance to FLT3, IDH, and DOT1L inhibition in preclinical models and therapeutic trials

Cores to engage

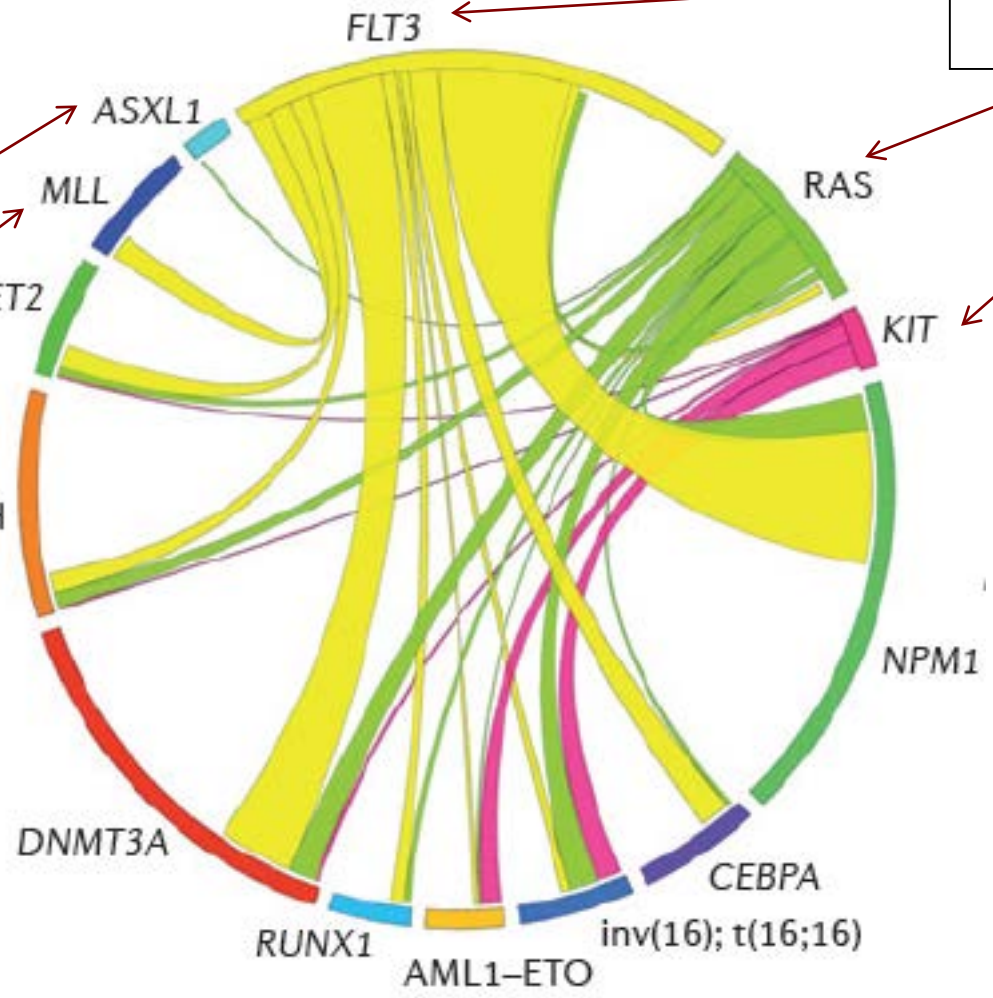
Preclinical/co-Clinical Core- mouse modeling and mouse hospital

Genomics/Bioinformatics Core- genomic analysis

Pilot project 1: Role of Mutations in Epigenetic Modifiers in AML (Levine)

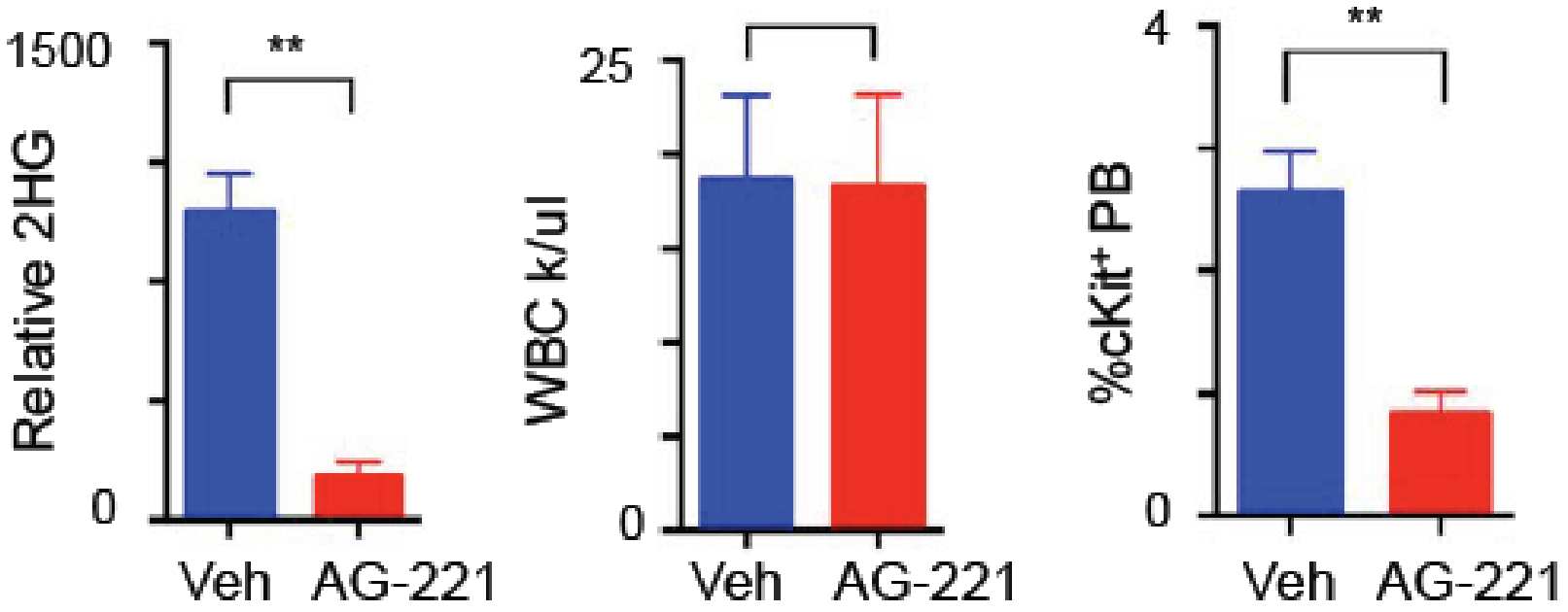
Signaling Mutations

Mutations in Epigenetic Regulators



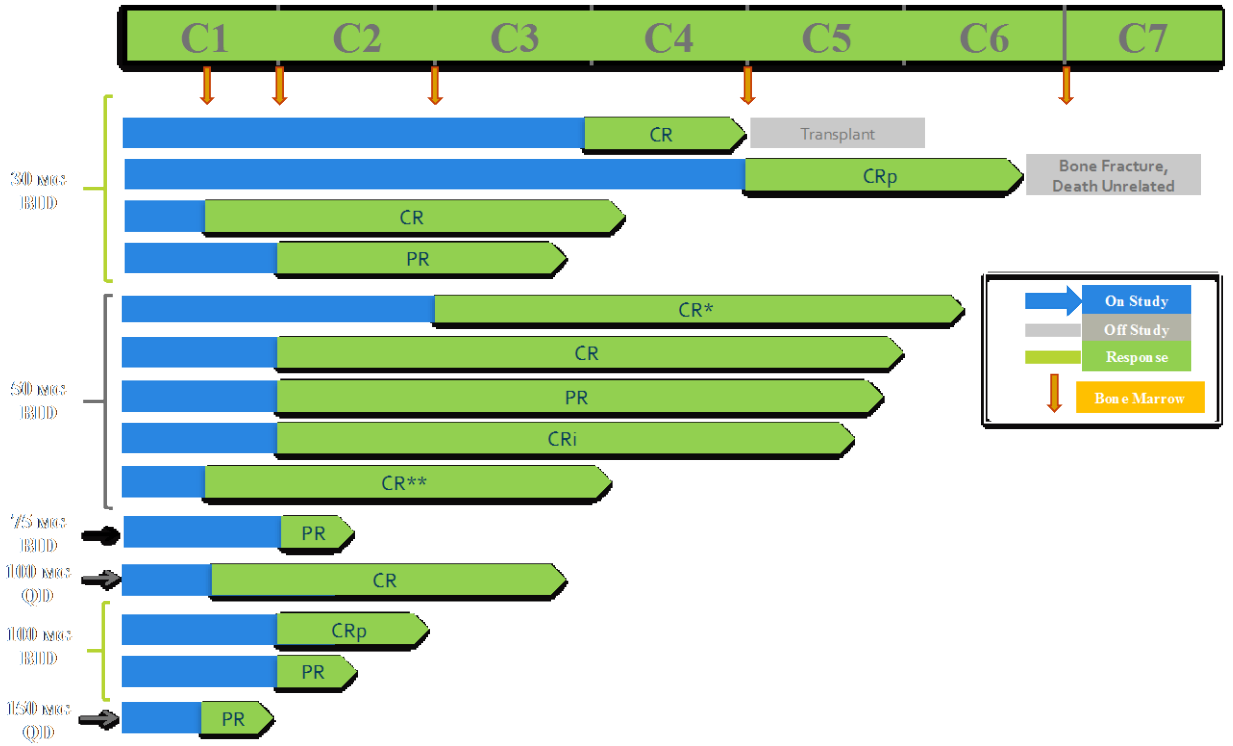
Ross Levine

Treatment of murine mutant IDH2 leukemia with AG221



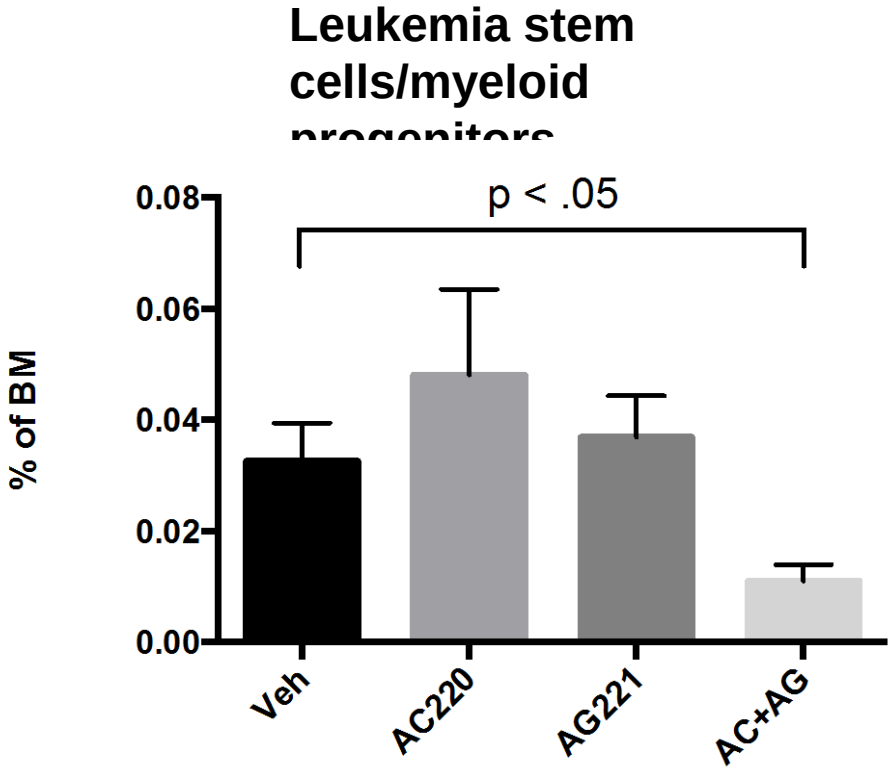
- In vitro and in vivo assays show significant efficacy
- Drug reduces leukemic blasts and promotes myeloid differentiation

Treatment of relapse/refractor human mutant IDH2 leukemia with AG221



- AG221 has significant clinical activity in AML patients with IDH2 mutations
- As in mice, AG221 appears to promote myeloid differentiation

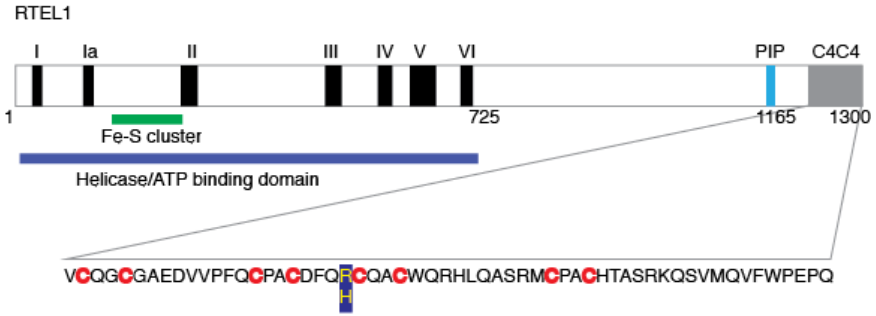
Combined targeting of epigenetic and signaling pathways in AML



AC220: Flt 3 inhibitor
AG221: mutant IDH2 inhibitor

- FLT3 and IDH2 mutations co-occur in AML patients
- FLT3 and IDH2 mutations cooperate in mice
- FLT3 and IDH2 inhibitors show combined activity in mouse models
- Clinical trials involving drug combination in development

Project 2: RTEL1 mutations in Hoyeraal Hreidarsson Syndrome (Petrini)



Telomere and genome wide replication
Genome stability and repair
Antirecombinase
RNA Trafficking

Aim 1. To define the biochemical activities of the RTEL1 C4C4 domain.

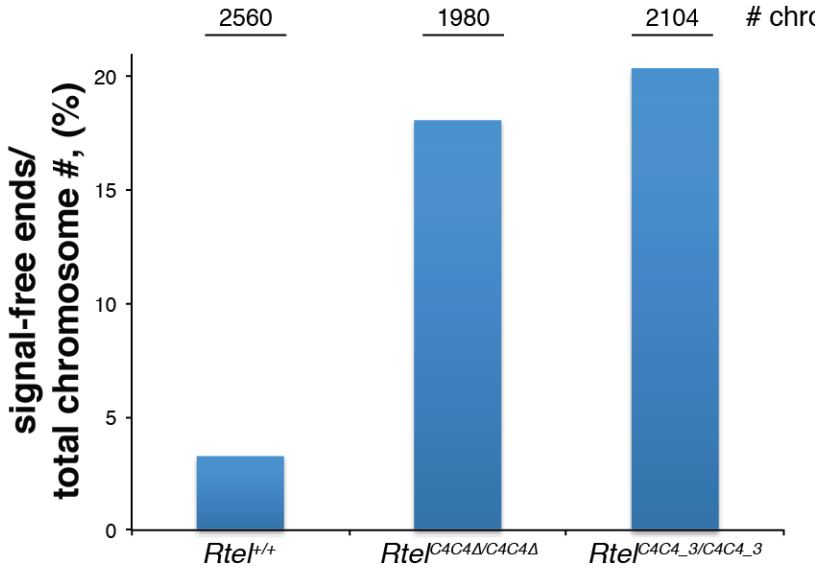
Aim 2. To examine the functions of RTEL1 at the cellular level.

Aim 3. To examine the functions of RTEL1 *in vivo*.

Cores to engage

Preclinical/co-Clinical Core- mouse modeling
Genomics/Bioinformatics Core- genomic analysis of mouse models

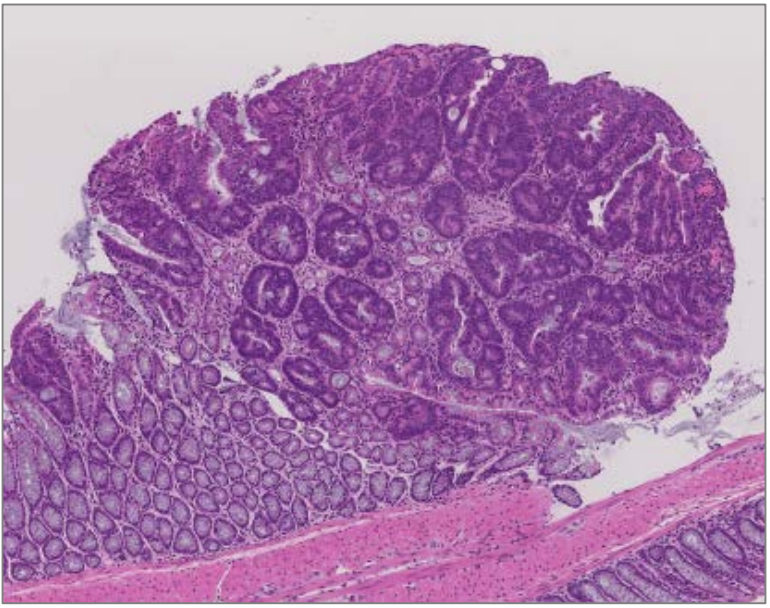
Cells with RTEL1 mutations show phenotypes consistent with telomere replication defects



- RTEL1 is a helicase with 5’-3’ directionality
- Mutations identified in MSKCC patients lie within a C4C4 RING finger domain
- C34C4 domain mutations disrupt ability of to bind TERRA G-quadruplex RNA
- RTEL1 mutation in MEFs phenocopies the global telomere loss and genomic instability observed in cells from patients

John Petrini

Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)



Aim 1. The Generation of an orthotopic model to study metastatic CRC.

Aim 2. To determine whether SWI/SNF disruption contributes to CRC progression and maintenance.

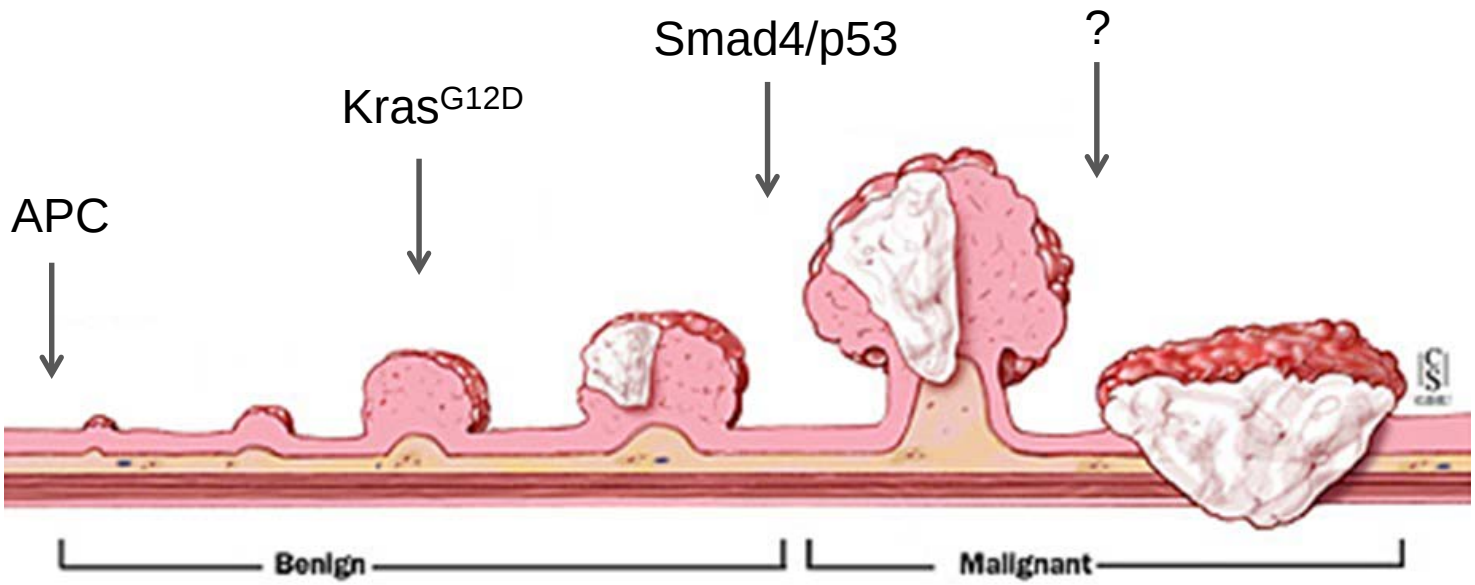
Aim 3: Assessing the impact of secondary mutations on WNT-pathway dependence in CRC

<u>Cores to engage</u>

Preclinical/co-Clinical Core- mouse modeling and mouse hospital

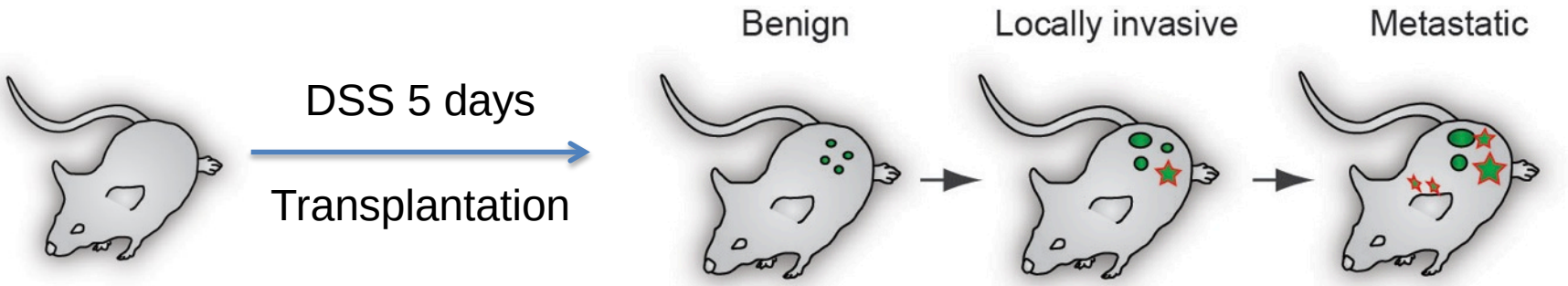
Genomics/Bioinformatics Core- genomic analysis

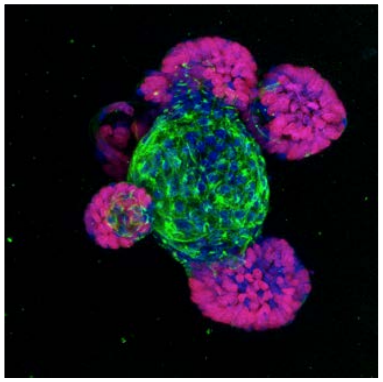
Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)



- Rationale: Metastatic CRC is second leading cause of cancer deaths in the US
- Challenge: Mouse models develop small intestinal polyps that do not progress to advanced disease
- Solution: Orthotopic transplantation of genetically engineered colon organoids

Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)



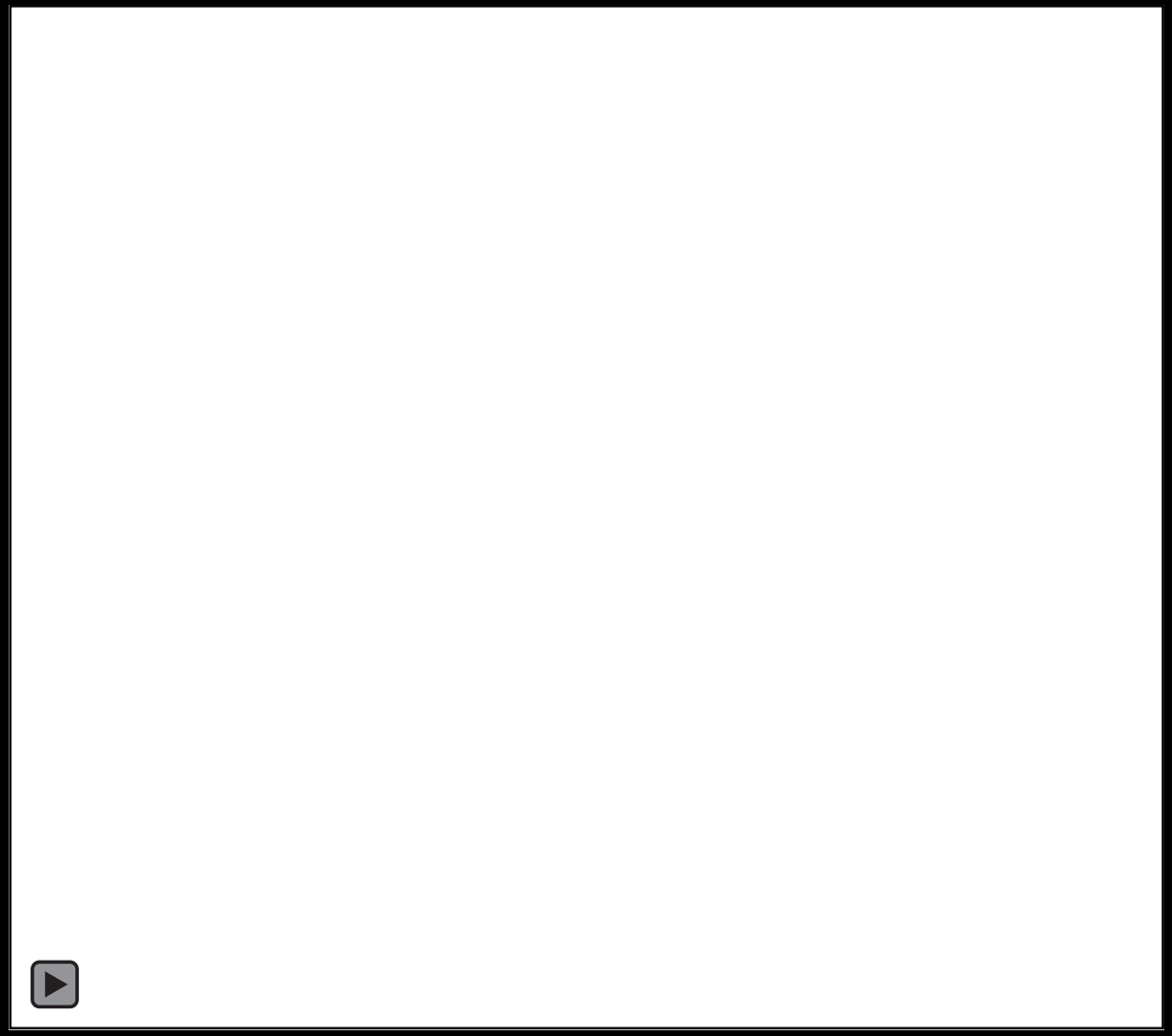


- Apc-/- or shAPC
- Apc-/-/Kras^{G12D}
- Apc-/-/Kras^{G12D}/p53$^{R172H/-}$

Genetically modified organoid cultures (GEMM/genome editing)

Kevin O'Rourke

Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)

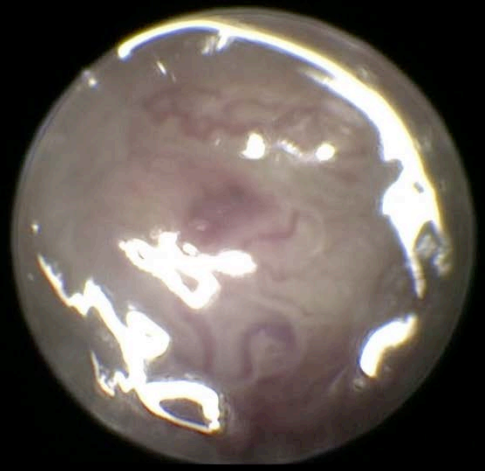


Kevin O'Rourke

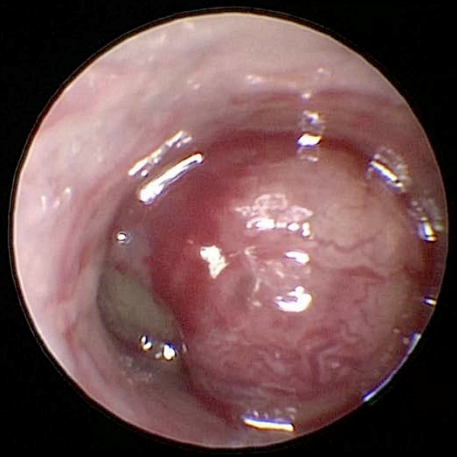
Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)

APC OFF (3 wk)

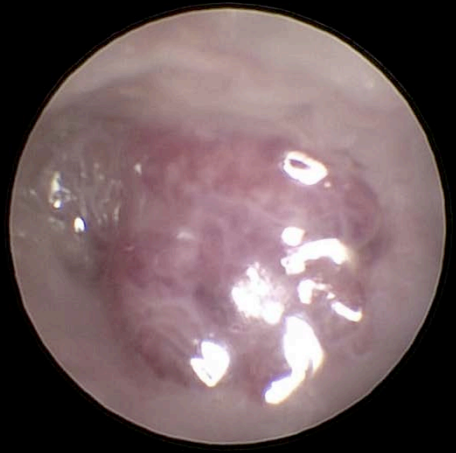
APC OFF (5 wk)



APC OFF (6 wk)



APC ON (1 wk)



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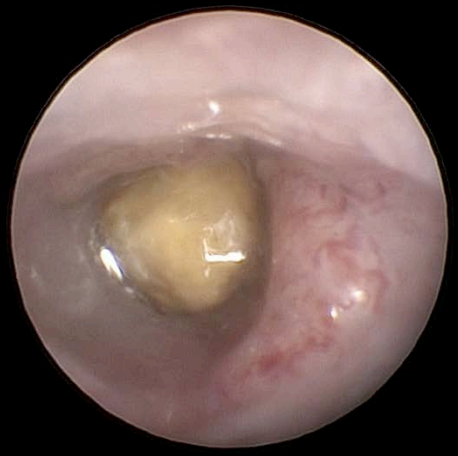
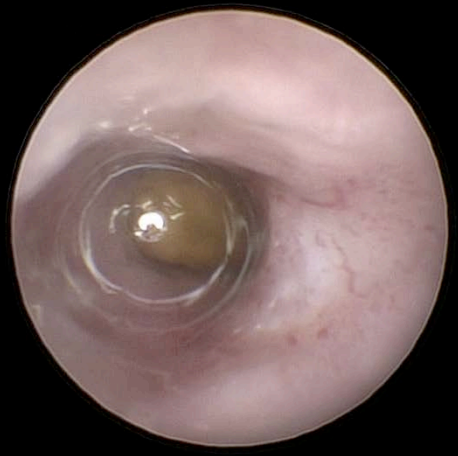
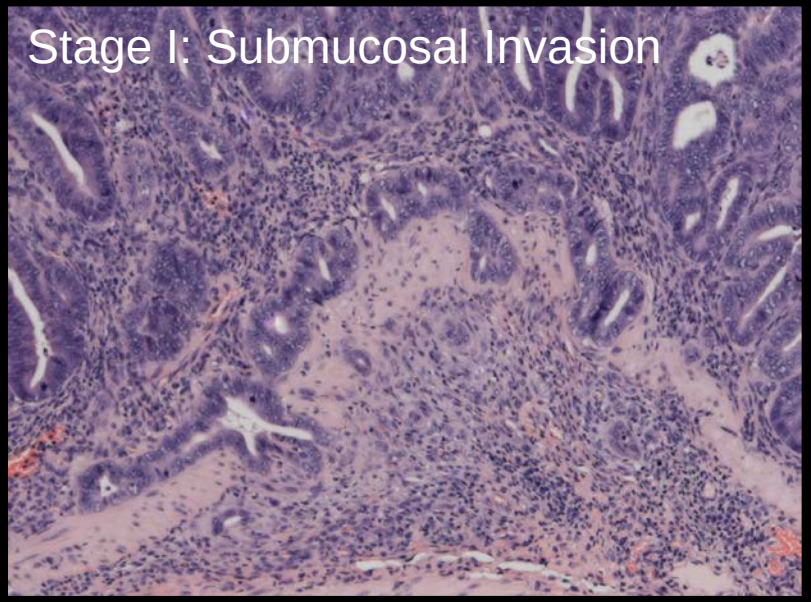


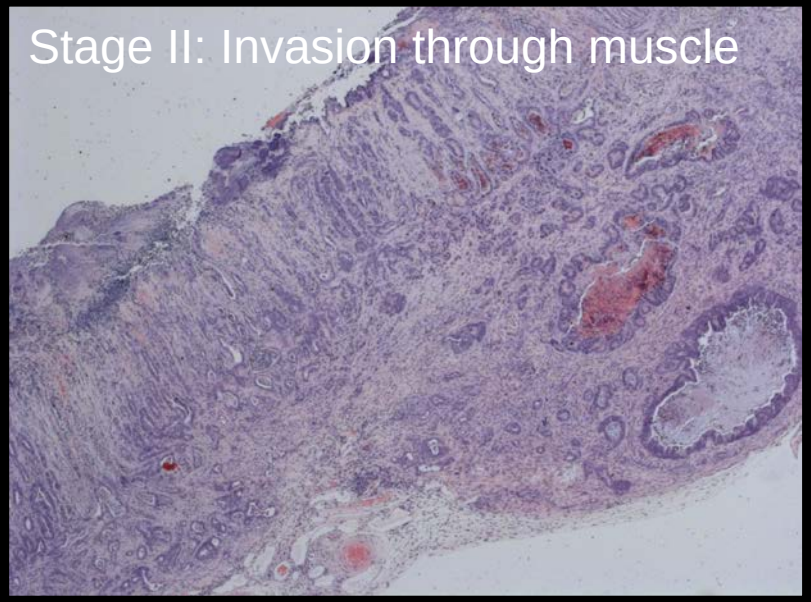
APC ON (4 wk)

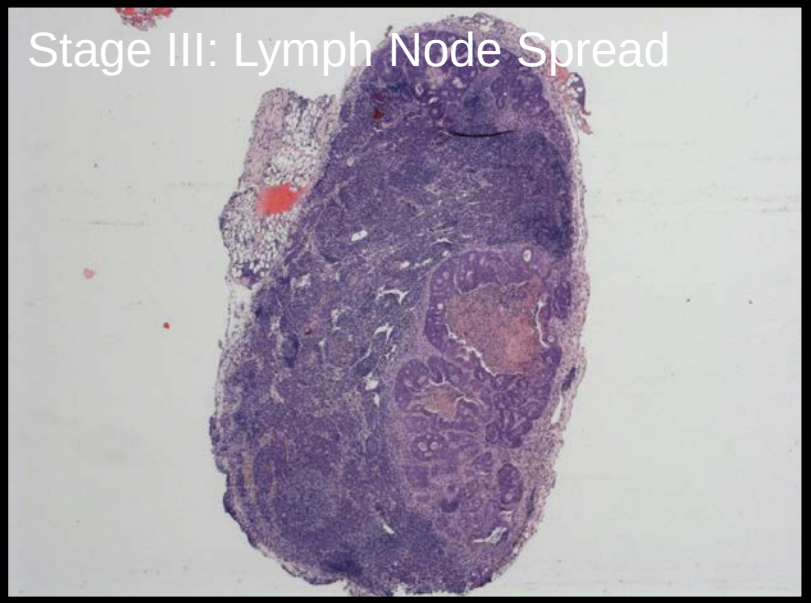


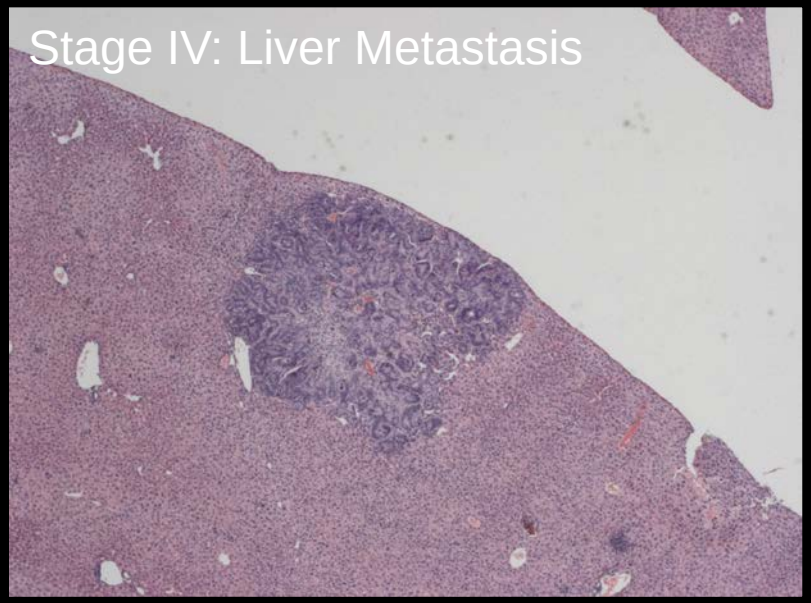
Kevin O'Rourke, Luke Dow

Project 3: Modeling advanced colorectal cancer *in vivo* (Lowe)

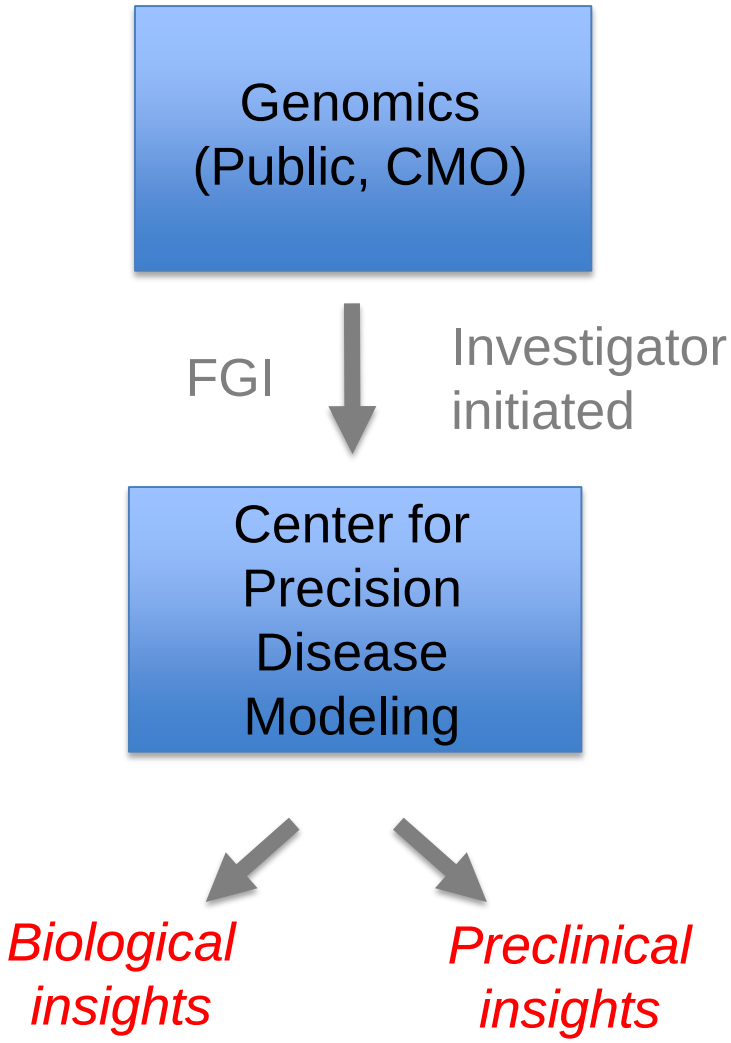






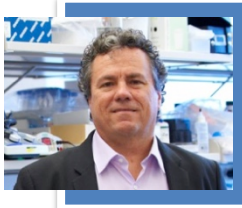


MSKCC Center for Precision Disease Modeling



- **Funding strategy**: support salaries in key positions to subsidize user costs – modular
- **Leveraging resources:** additional funds from Geoffrey Beene Cancer Research Center and NCI CCSG bolster U54 support
- **New projects:** Functional Genomics Initiative at MSKCC and community outreach

Acknowledgments



Leadership

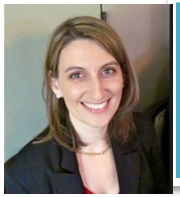
Scott Lowe
David Solit

Steering Committee (FGI)

EAB

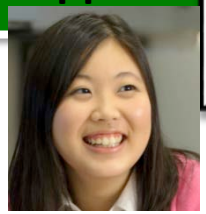
Administrative support

Eder Paraiso
Christy Park



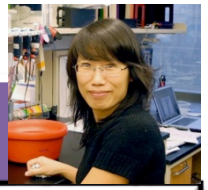
Coordination Core

Elisa de Stanchina



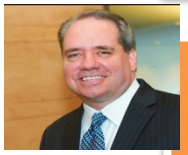
Genomics and Bioinformatics Core

David Solit
Barry Taylor



Pre-clinical and Co-Clinical Core

Elisa de Stanchina,
Zhen Zhao



Disease Modeling Units

Ross Levine, Scott Armst
John Petrini, Scott Lowe