Preclinical Testing: Animal Models & Clinical Models

Pasi A. Jänne, M.D.,Ph.D.

Lowe Center for Thoracic Oncology

Dana Farber Cancer Institute

DANA-FARBER CANCER INSTITUTE

Belfer Institute
FOR APPLIED CANCER SCIENCE

HARVARD MEDICAL SCHOOL

Disclosure Information

Pasi A. Jänne, MD, PhD

I have the following financial relationships to disclose:

Consultant for: Astra Zeneca, Boehringer Ingelheim, Pfizer, Genentech, Roche, Sanofi-Aventis, Clovis Oncology, Chugai Pharmaceuticals, Merrimack Pharmaceuticals

Stockholder in: Gatekeeper Pharmaceuticals

Other: LabCorp – post-marketing royalties from DFCI owned intellectual property on EGFR mutations

Preclinical model systems
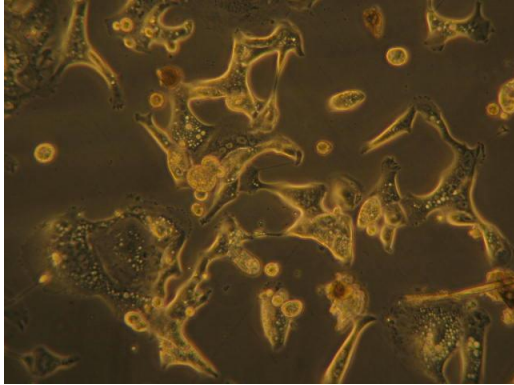
NSCLC patients

Guide clinical drug development →

← Inform preclinical studies

- Prioritize clinical therapies
- Identify resistance mechanisms
- Test novel combination therapies

- Evaluate targeted therapies
- Determine biomarker modulation
- Study clinical drug resistance

Preclinical Cell Line Models

Cell Line Model	Advantage	Disadvantage
Existing Cell Lines	-Characterized -Grow well -Endogenous context -Used to study efficacy and model resistance	-Limited number -Not all genotypes covered -May not completely be reflective
Ba/F3 or 3T3 Cells	-Uniform background -Compare genotypes -Good for drug screening	-Artificial model
Patient Derived Cell Lines	-Maybe more reflective of clinical scenario	-Resources needed to grow -Not always easy to establish

Existing NSCLC Cell Line Models

Genotype	Cell Lines	Comment
KRAS	~ 30	Multiple KRAS genotypes, LKB1 and P53 intact/deficient
EGFR	8 -10	Mostly Exon 19 deletion; 1 L858R, no exon 20 or rare EGFR genotypes
ALK	2	H2228 not sensitive in vitro
HER2	1	Rare HER2 mutation
ROS1	1	Not very sensitive in vitro
RET	1	
NTRK	0	
BRAF	5	1 V600E; 4 non-V600E

Need to develop additional cell lines to reflect diversity of clinical genotypes

Ba/F3 Cell Line Models
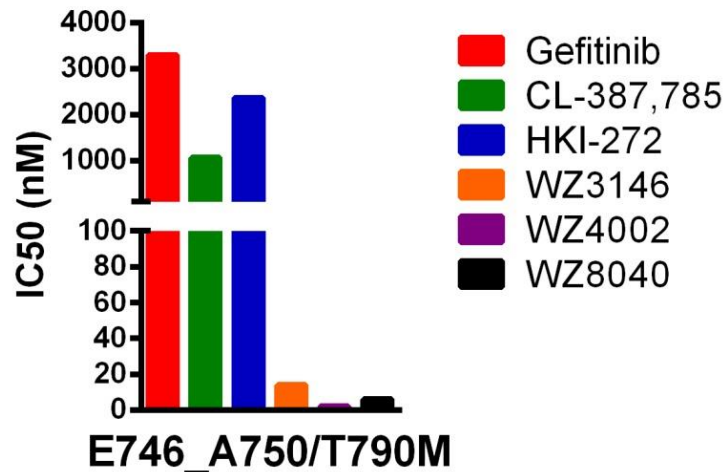
Comparison of drug efficacy
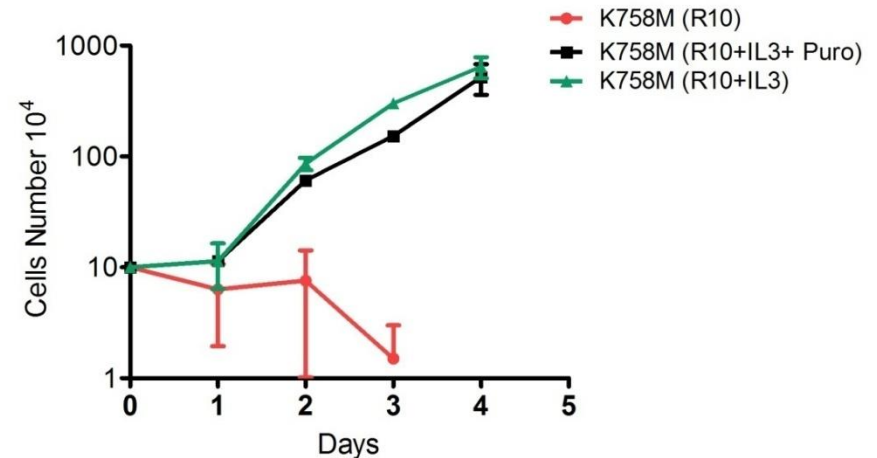
Requirement of RET kinase activity for IL-3 independent growth
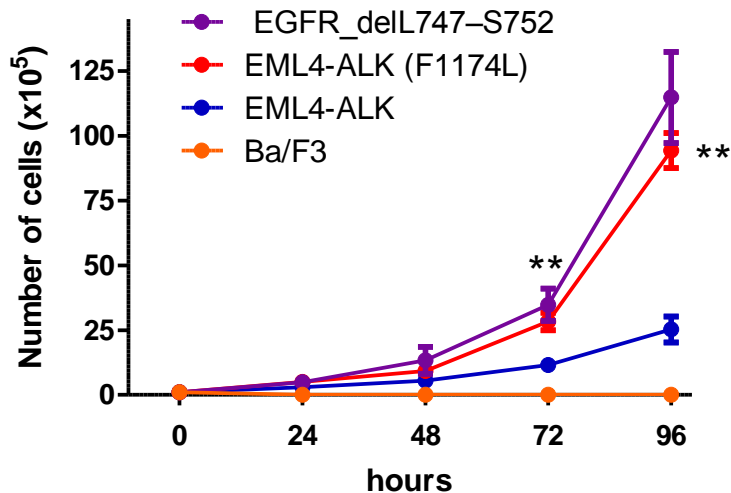

Enhanced growth rate for EML-ALK F1174L

Comparison of Animal Models

| Model	Advantage	Disadvantage
Xenograft	-Well Established -Can use many cell lines -Cheaper	-Somewhat artificial -Not always predictive of clinical outcome -Immunodeficient
Genetically engineered	-Endogenous model -More reflective of human disease -Intact immune system	-Cost (breeding & imaging) -Tumor latency - Driven by single genetic alteration
Patient derived xenograft	-Can study both tumor and stroma -Model may reflect clinical scenario -Ability to develop unique models	-Cost -Immunodeficient

Different Types of "Positive" Xenograft data

Turke et al. Cancer Cell 2011; Bulgar et al. Cell Death and Disease 2012
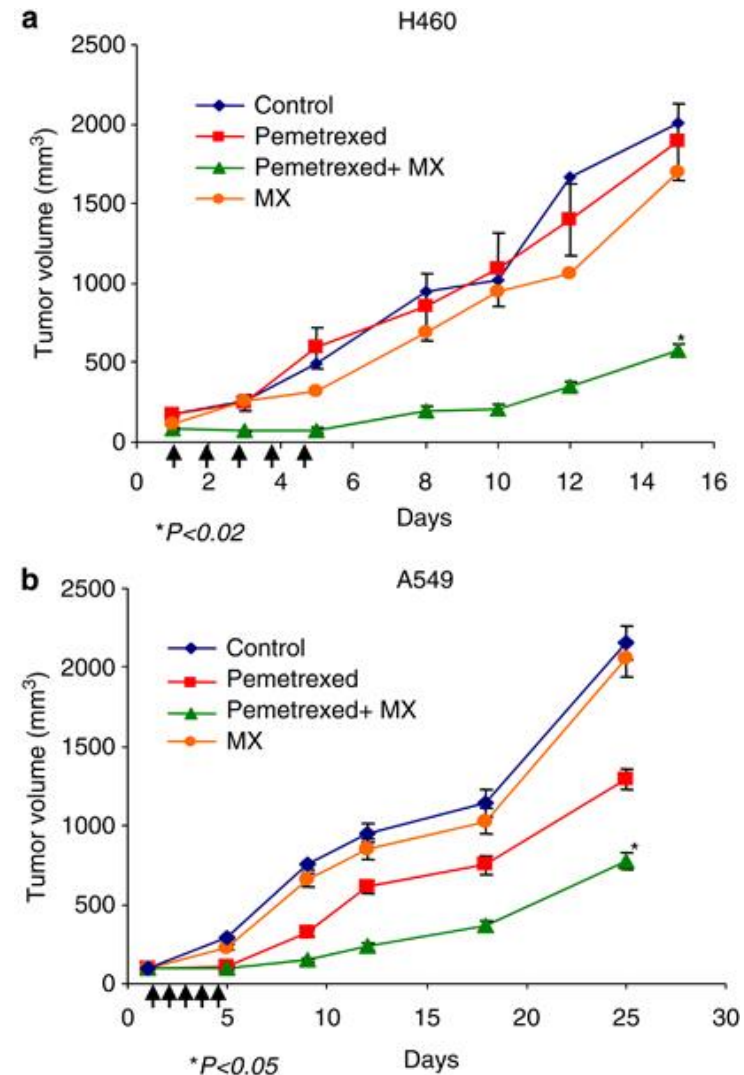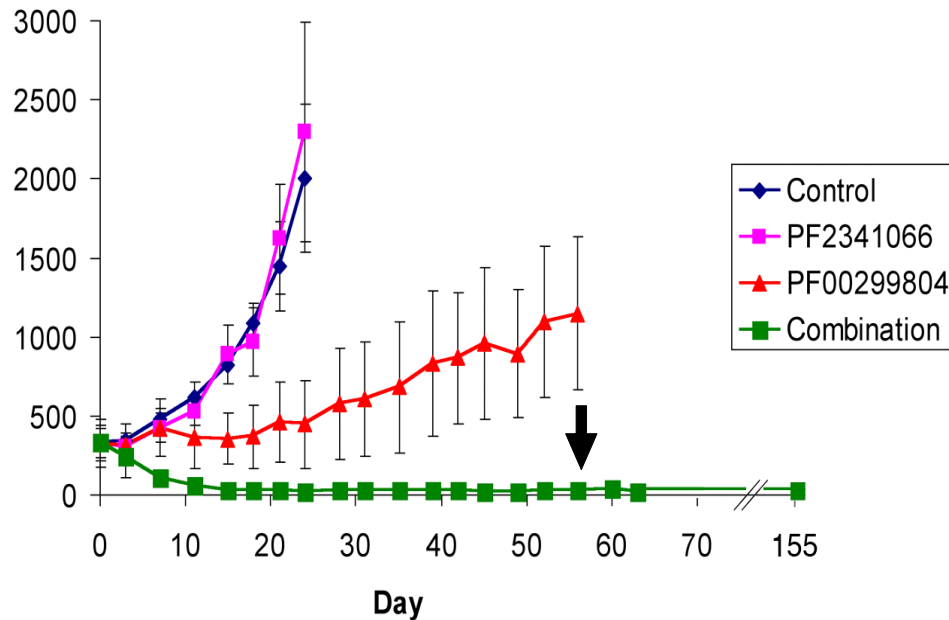
From Patient to In Vitro Model
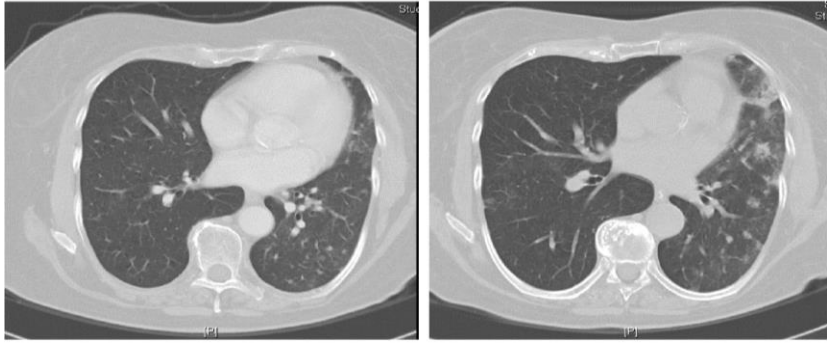
Drug Resistant Patients
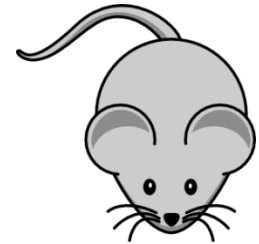
Primary Xenograft
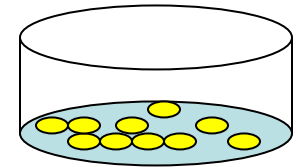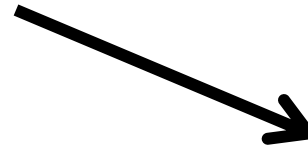
Design clinical trials
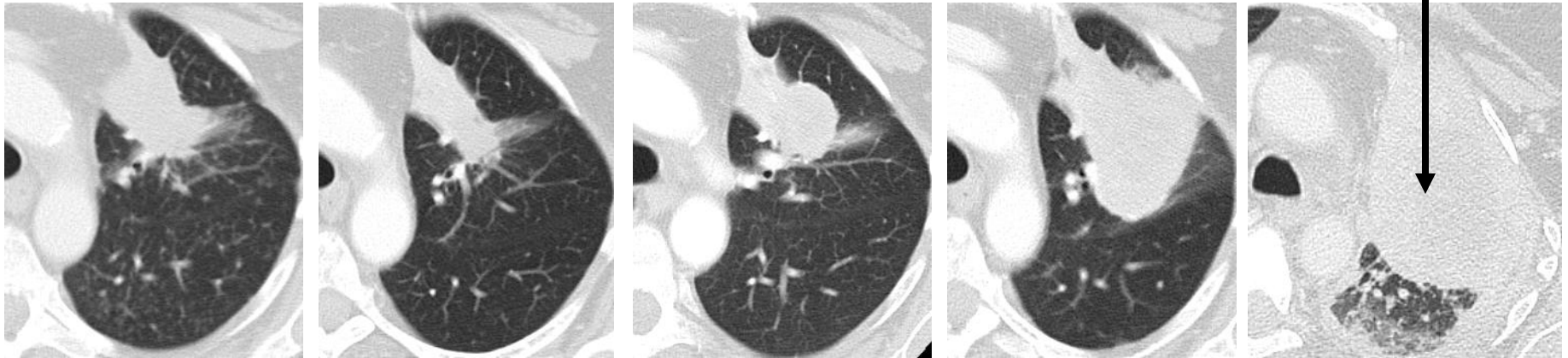
Primary Cell Line

1. Study heterogeneity of drug resistance

2. Identify novel resistance mechanisms

3. Test Novel Therapeutic Strategies

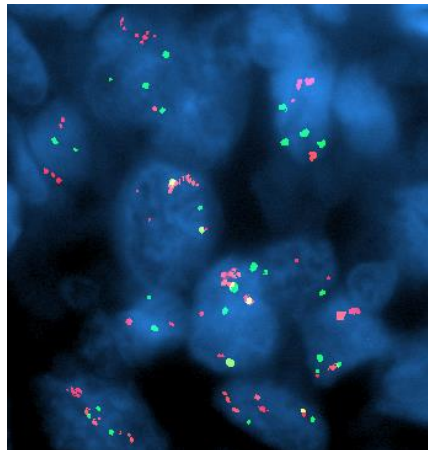# Generation of patient derived cell lines to study drug resistance mechanisms

Baseline

EGFR Exon 19 del

Erlotinib

Biopsy

Chemotherapy

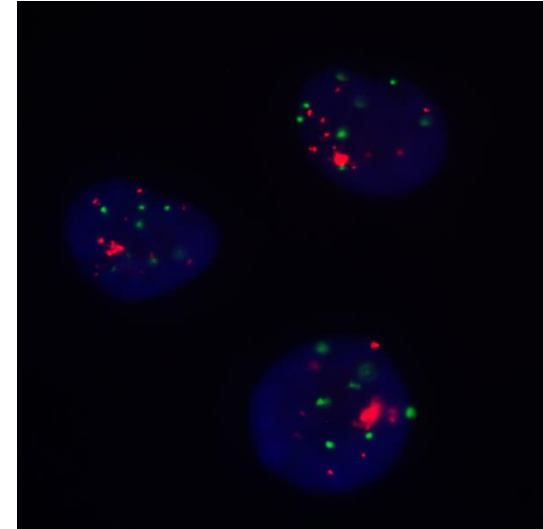Resistant tumor: EGFR Exon 19 del

Red : MET; Green: CEP 7

Atsuko Ogino

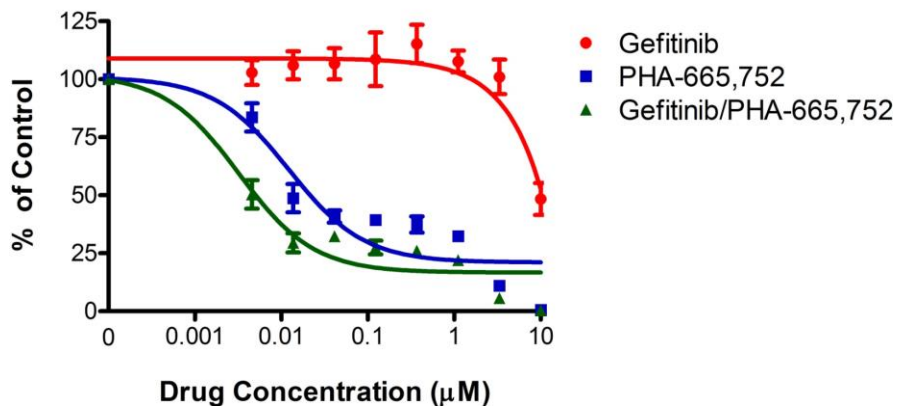# DFCI 81 cells retain MET amplification in vitro
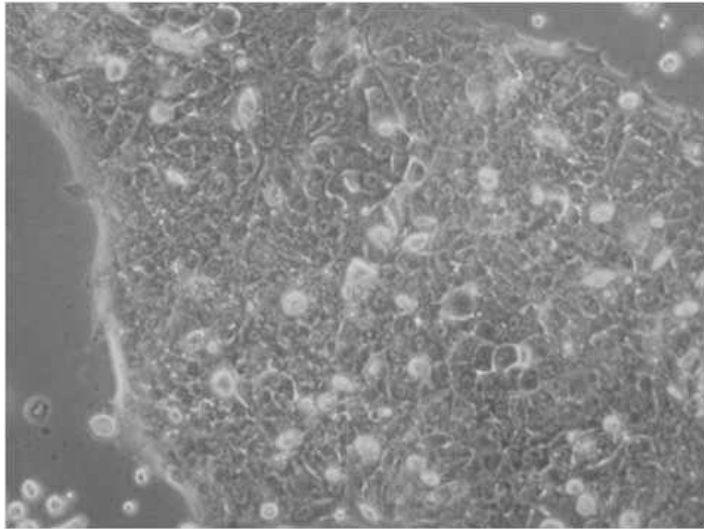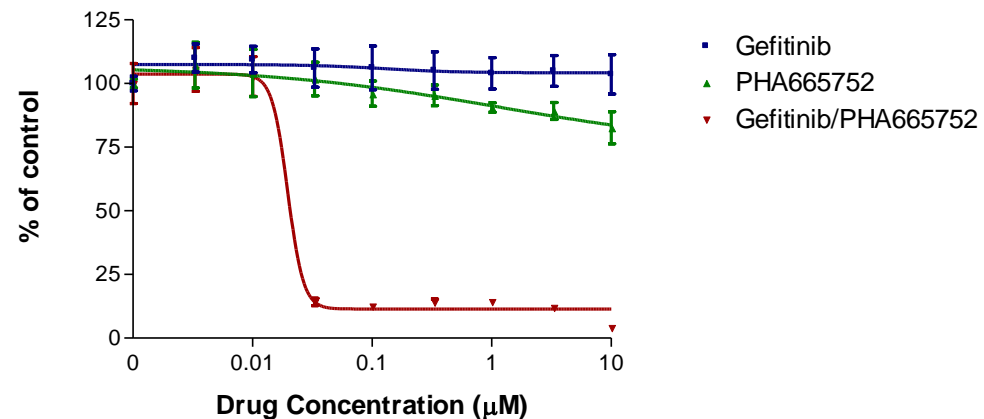
DFCI 81 Cells
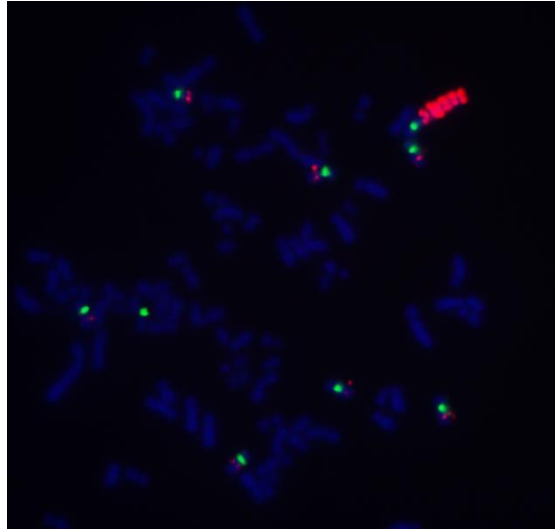
Metaphase

Interphase

DFCI 81 Cell line

HCC827 GR Cells

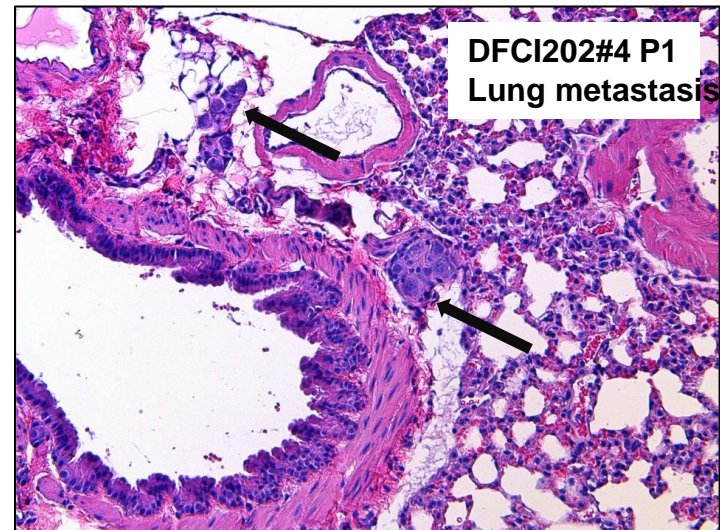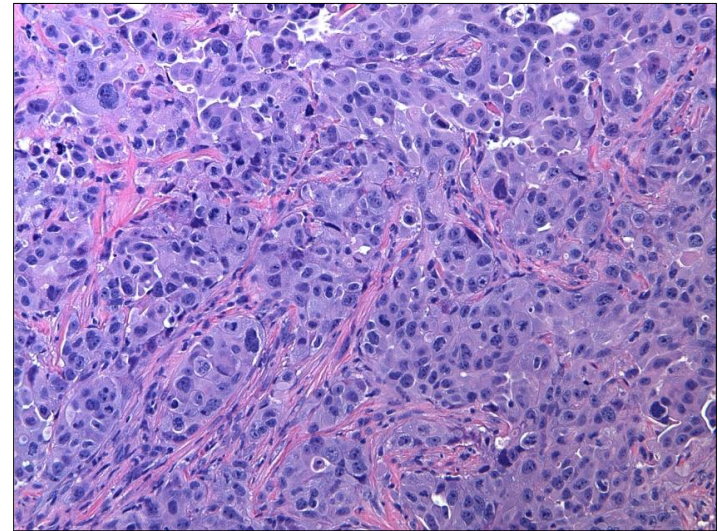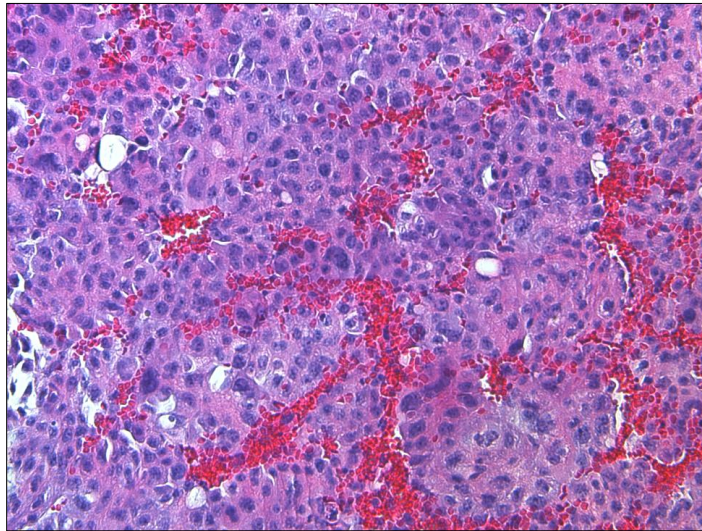# DFCI202 – Erlotinib resistant EGFR mutant NSCLC patient derived xenograft – no T790M

DFCI202	DFCI202 NSG
Patient	P1

H&E

**DFCI202#4 P1
Lung metastasis**

Antonio Calles, Parfulla Gokhale, Sangeetha Palakurthi

DFCI 193 – Erlotinib resistant patient derived xenograft – del 19/T790M

DFCI193	DFCI193Nx
Patient	P1

H&E

DFCI-193#271 P1 xenograft

Antonio Calles, Parfulla Gokhale, Sangeetha Palakurthi
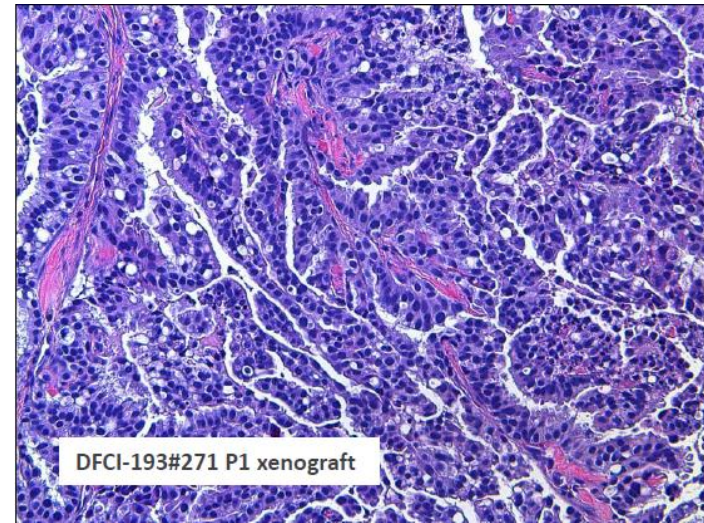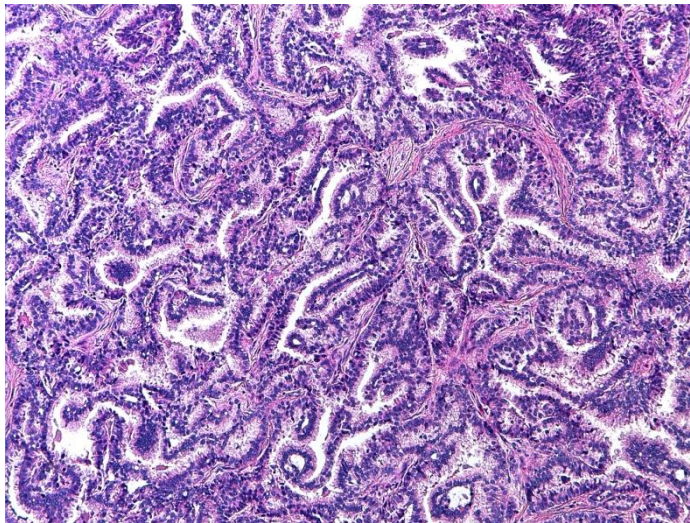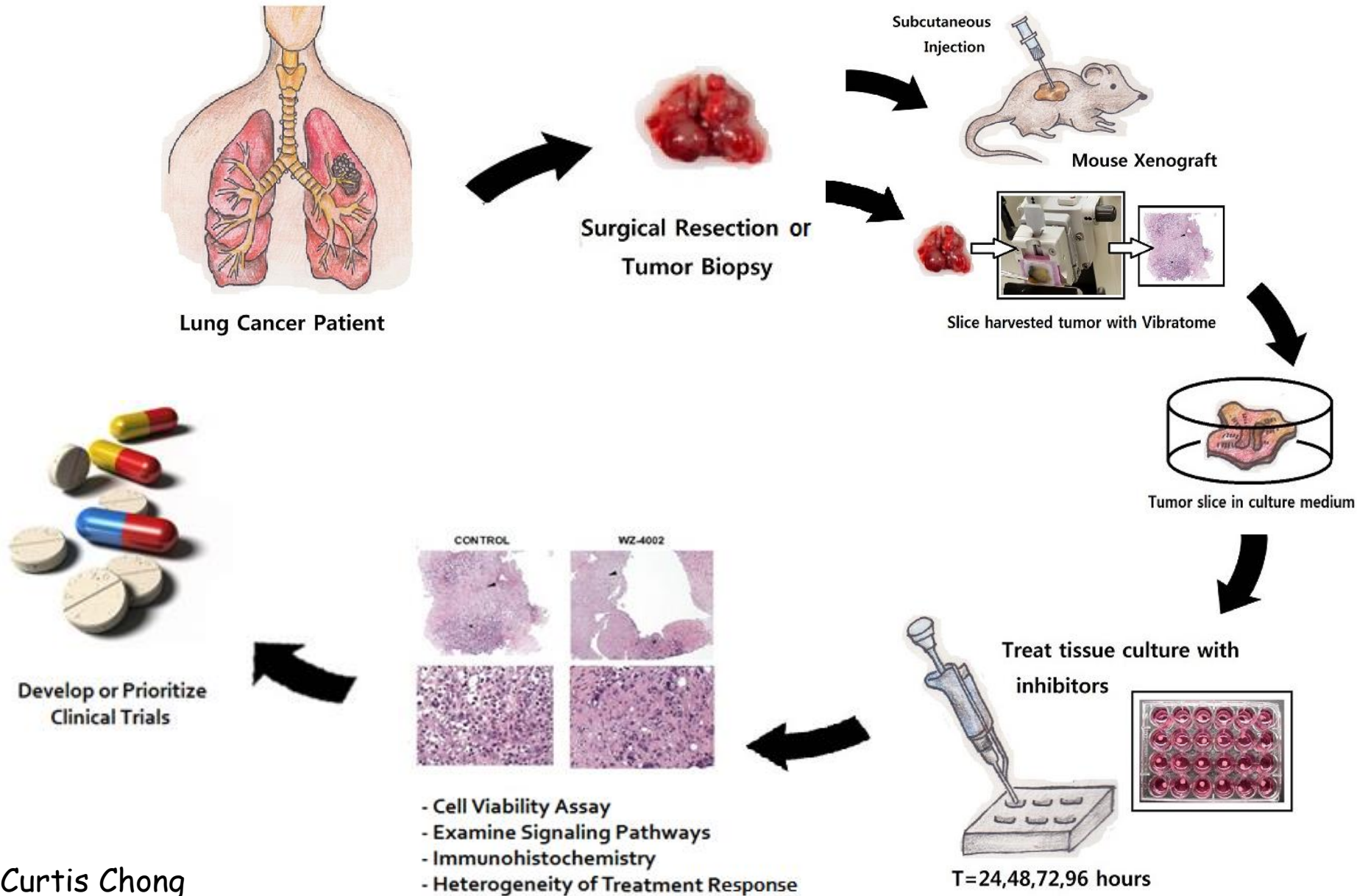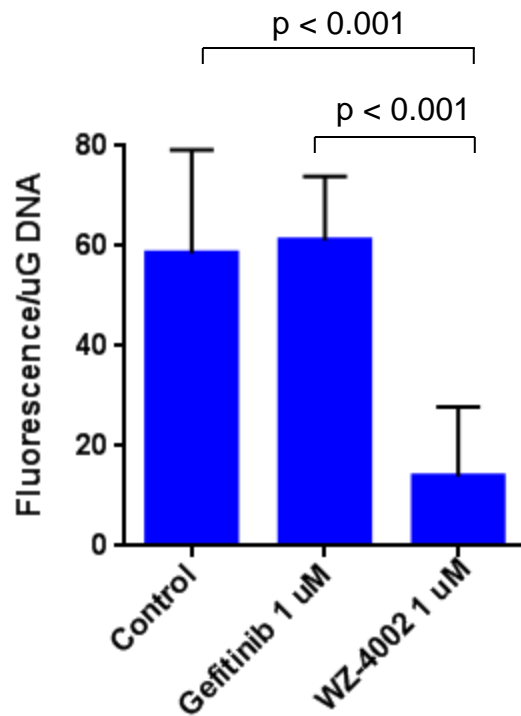
Tumor derived "slice" cultures to study drug resistance mechanisms

Lung Cancer Patient

Surgical Resection or Tumor Biopsy

Subcutaneous Injection

Mouse Xenograft

Slice harvested tumor with Vibratome

Tumor slice in culture medium

Treat tissue culture with inhibitors

T=24,48,72,96 hours

CONTROL   WZ-4002

- Cell Viability Assay
- Examine Signaling Pathways
- Immunohistochemistry
- Heterogeneity of Treatment Response

Develop or Prioritize Clinical Trials

Curtis Chong

"Slice" culture using PC9 GR (EGFR del 19/T790M) xenografts

Cell Viability

Histology

Curtis Chong
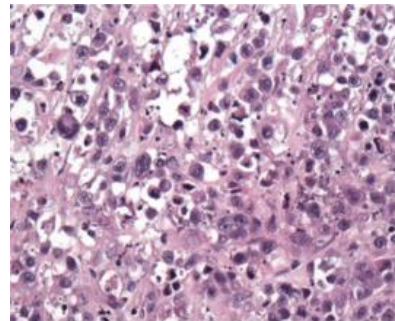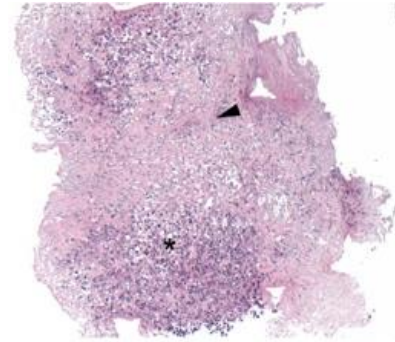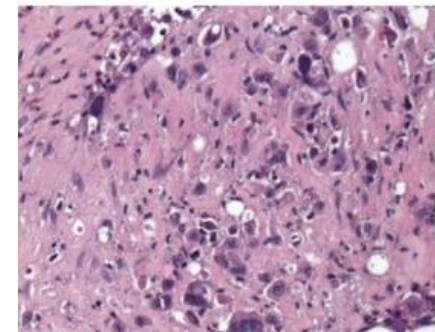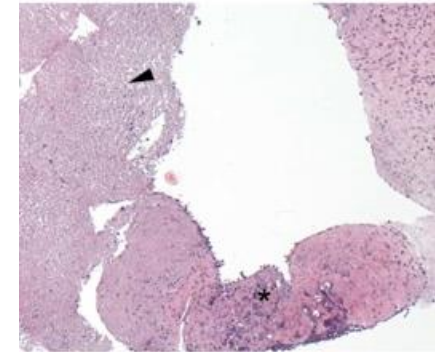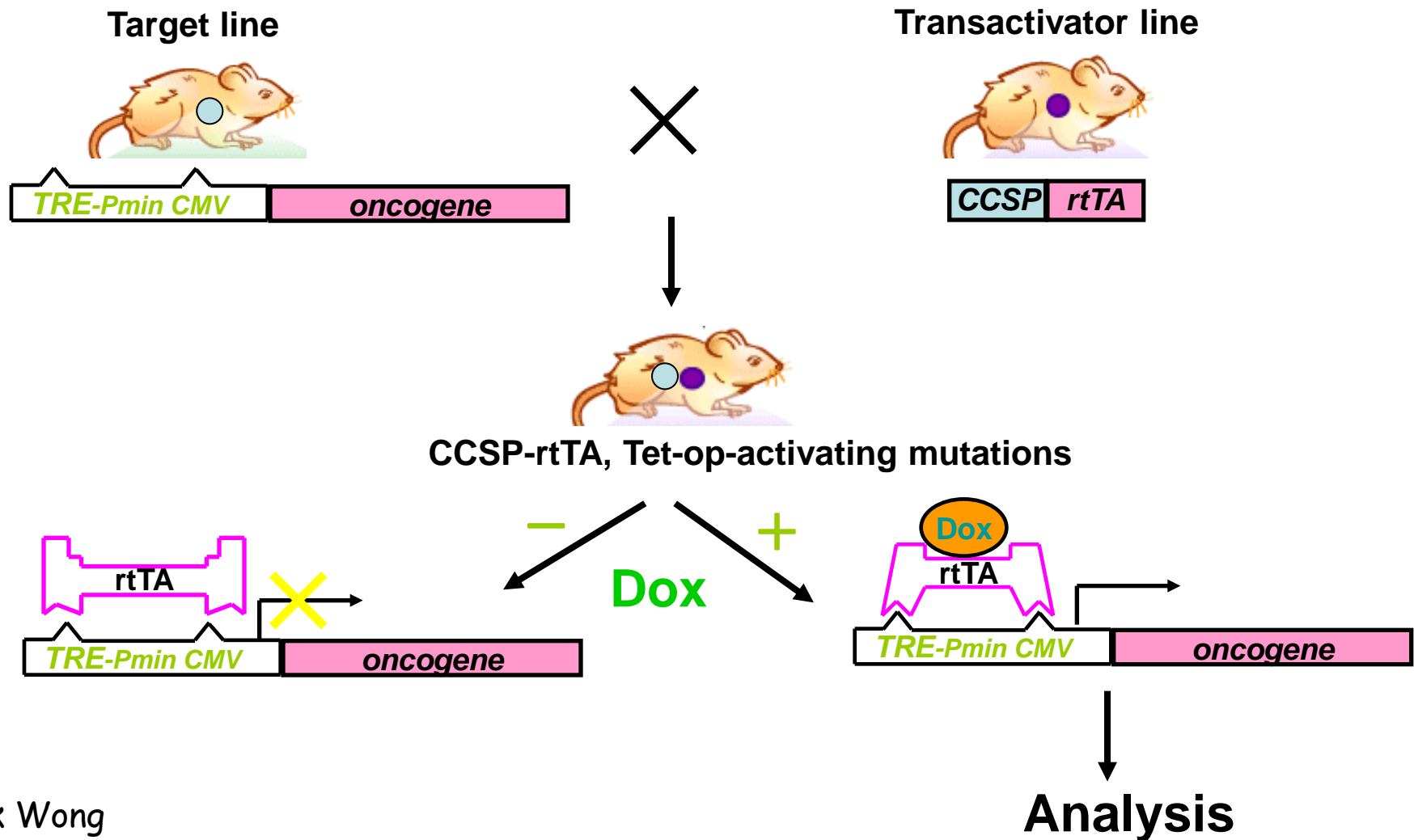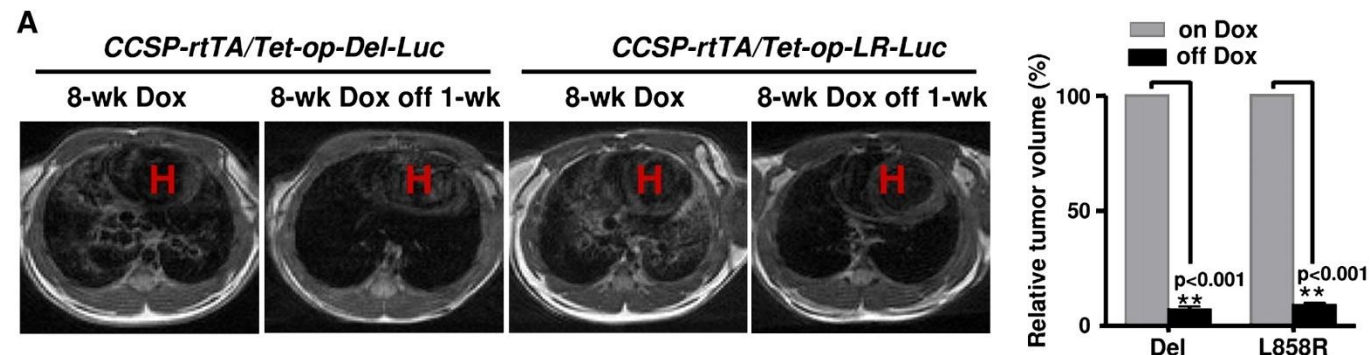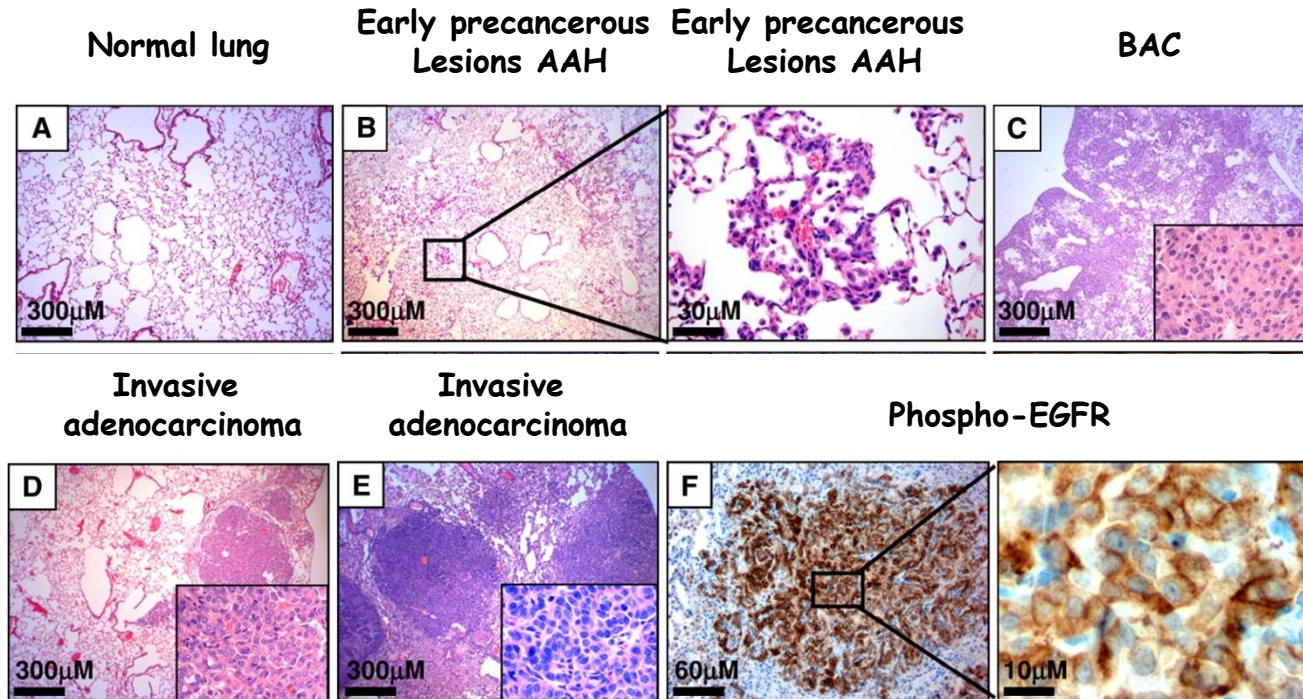
The use of inducible bitransgenic mouse modeling to examine the *in vivo* role of activating oncogenic mutations in lung tumorigenesis
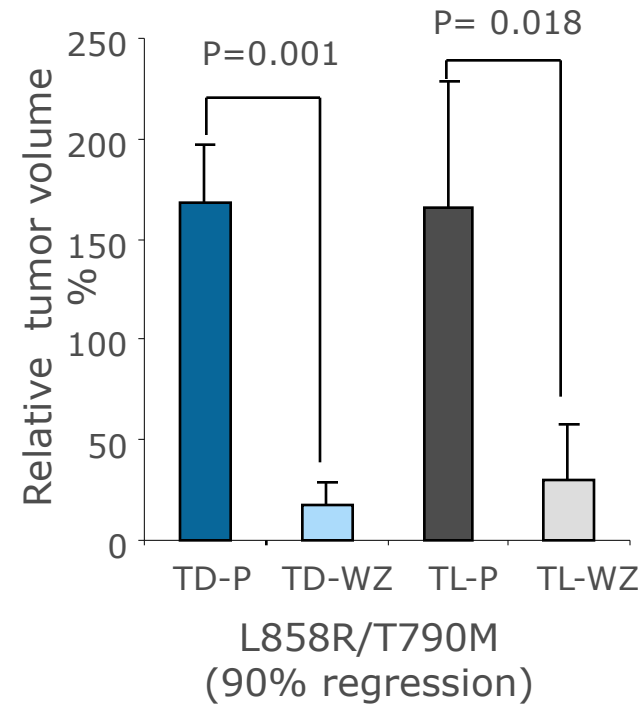

Target line

TRE-Pmin CMV | *oncogene*

Transactivator line

CCSP | *rtTA*

CCSP-rtTA, Tet-op-activating mutations

– Dox +

rtTA
TRE-Pmin CMV | *oncogene*

Dox
rtTA
TRE-Pmin CMV | *oncogene*

Analysis

Kwok Wong

EGFR mutations are oncogenic in vivo and cause lung cancer in mouse models

Normal lung | Early precancerous Lesions AAH | Early precancerous Lesions AAH | BAC

Invasive adenocarcinoma | Invasive adenocarcinoma | Phospho-EGFR
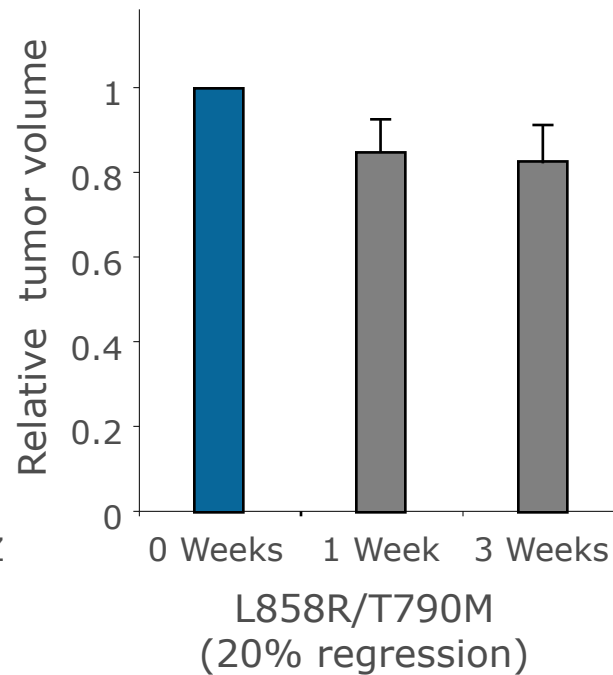
Ji et al. Cancer Cell 2006

Superior to Efficacy Relative to Clinical Agents in Mouse Models

Kwok-Kin Wong, Liang Chen
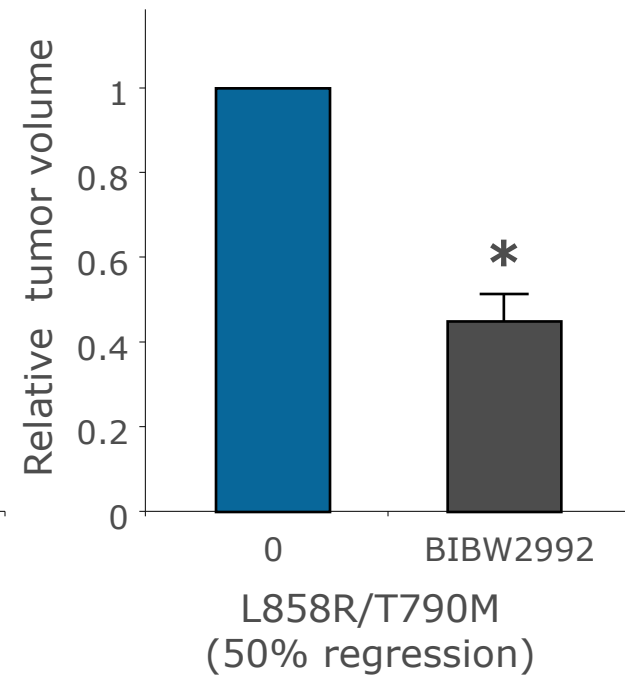
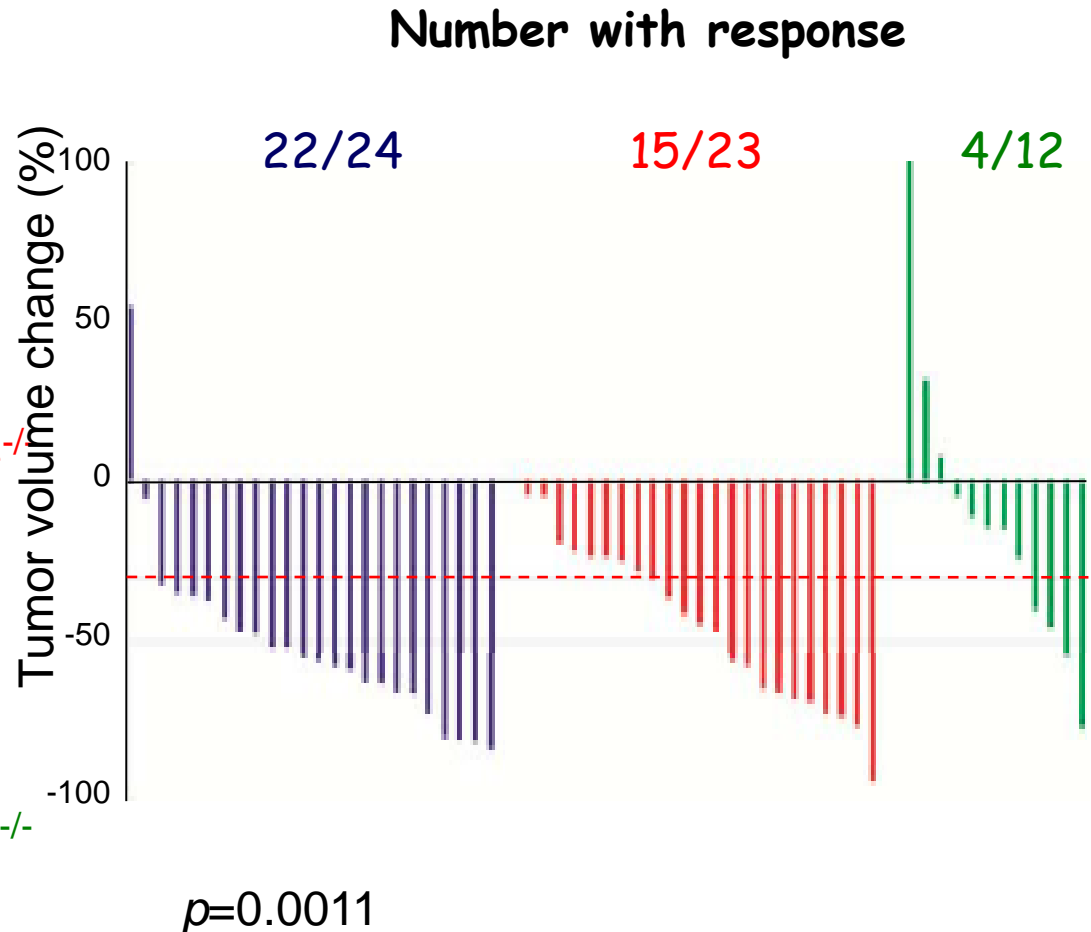# Impact of genotype on treatment with selumetinib/docetaxel

Chen et al. Nature 2012

Improved PFS with Docetaxel/Selumetinib compared with docetaxel in *Kras* G12D murine model of NSCLC
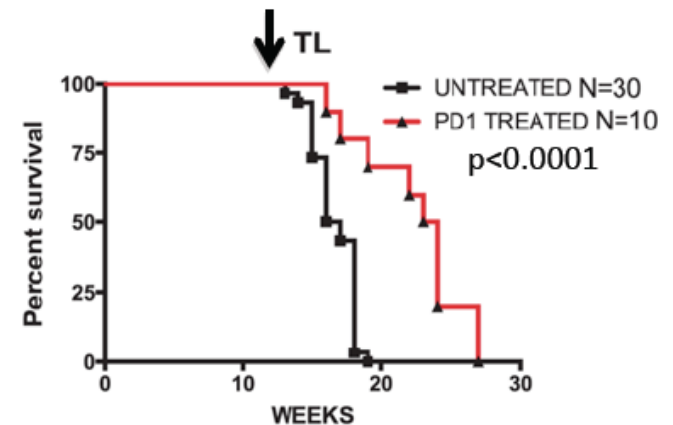

Chen et al. Nature 2012
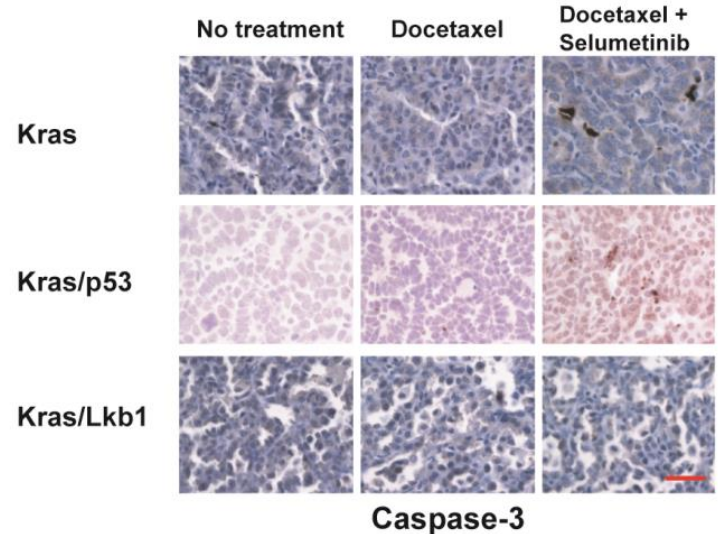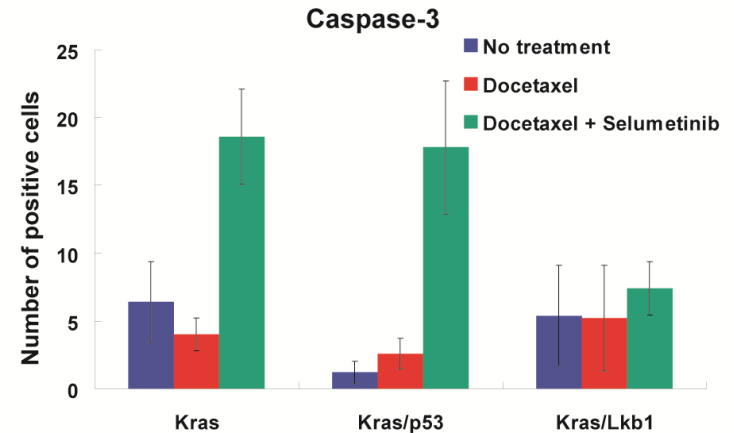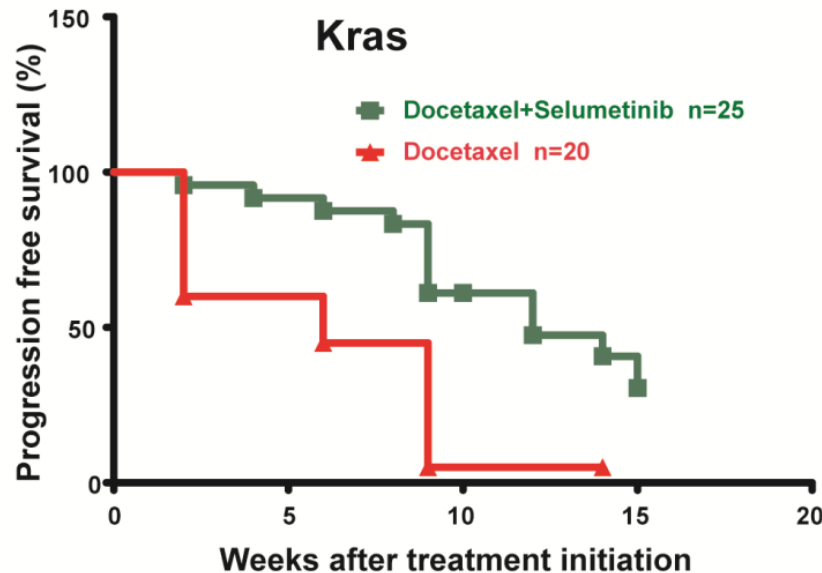
Activation of PD1 pathway in EGFR mutant NSCLC

H&E PDL1
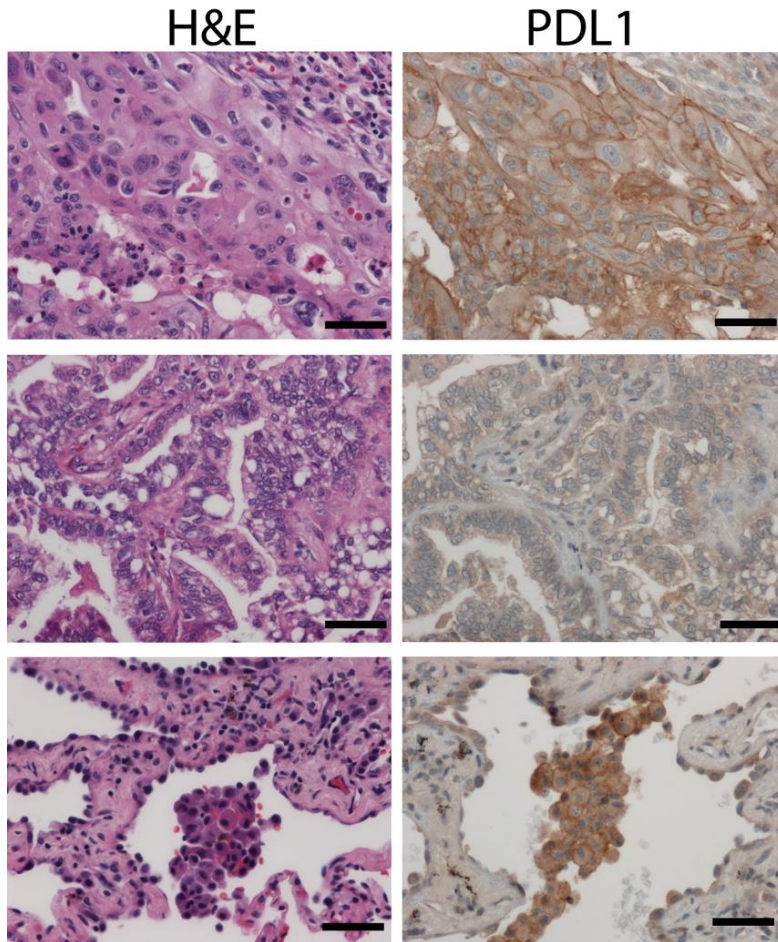
PDL1 expression in human
EGFR mutant tumors
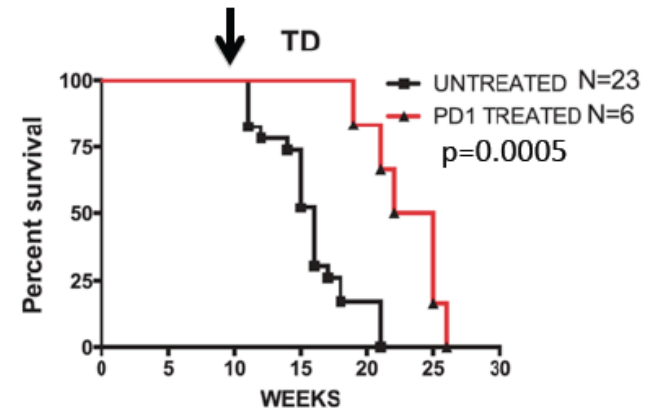
EGFR Exon 19 del/T790M

EGFR L858R/T790M

Akbay EA et al. Cancer Discovery 2013

Preclinical model systems

NSCLC patients
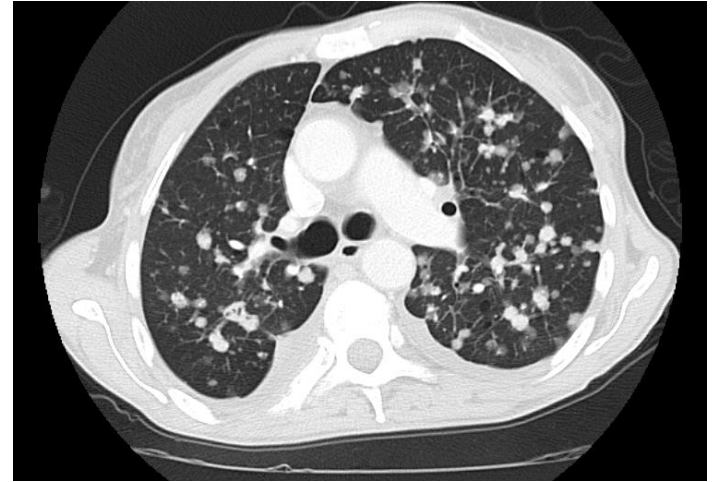

Guide clinical drug development →

← Inform preclinical studies

•Prioritize clinical therapies
•Identify resistance mechanisms
•Test novel combination therapies

•Evaluate targeted therapies
•Determine biomarker modulation
•Study clinical drug resistance