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No financial relationships to disclose

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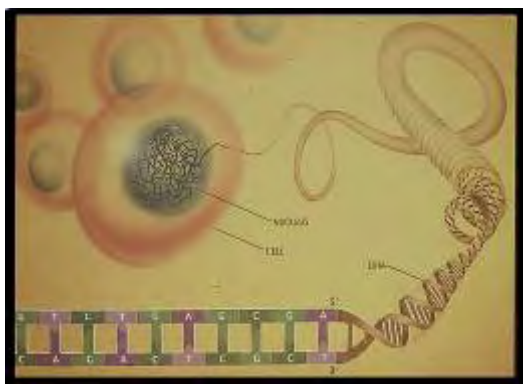
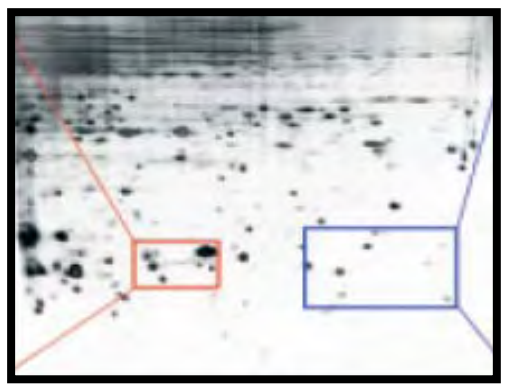
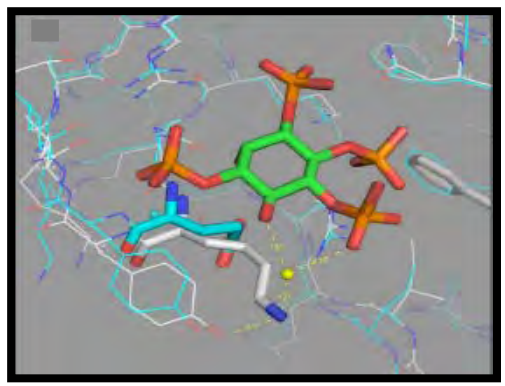
IMPaCT
DOD Prostate Cancer Research Program
Atlanta, GA

An Integrative "Omics" Approach to Molecularly Characterize Cancer: Towards Earlier Diagnostics and More Intelligent Therapy

John D. Carpten, Ph.D.
Director
Division of Integrative Cancer Genomics
Translational Genomics Research Institute







Integrative Cancer Genomics

- In this era of cancer genomics, where do we go from here?

- How do we best apply what we have (technologies) and what we know (knowledge mining) to best help patients?

- We're using Integrative Cancer Genomics to discover important genomic characteristics that can be exploited for potential clinical utility.

  - Biomarkers for clinical outcome (aggressiveness, survival, lethality)
  - Predictors of chemotherapeutic response
  - Novel targets for drug discovery
  - Patient stratification for clinical trial design

Targeted Chemotherapy

Tumor type	Genomic Alteration	Proposed Mechanism	Drug
Melanoma	BRAF mutation	Activating	Sorafenib
Breast	Her2-neu	Amplification	Herceptin
Breast/Colon	PI3KCA mutation	Activating	LY294002/Semiforin
CML	BCR-ABL (mutation)	Activating	Gleevec (dasatinib)
GIST	cKIT, PDGFR mutation	Activating	Gleevec
NSCLC	EGFR mutation	Activating	Iressa/Tarceva
Myeloma	Proteasome/NFkb mutation	Aberrant degradation/Activating/Inactivating	Velcade
Breast	Her2-neu/EGFR	Amplification	Tykerb (lapatinib)

- Most mutations are discovered after the fact, which sometimes make them more of a problem than a solution.

- Need to develop studies to identify novel alterations for targeted drug discovery.

Integrative Cancer Genomics

Acknowledgements

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James Thomas

Richard Gaynor

Kerry Blanchard

Lilly Singapore Center for Drug Discovery

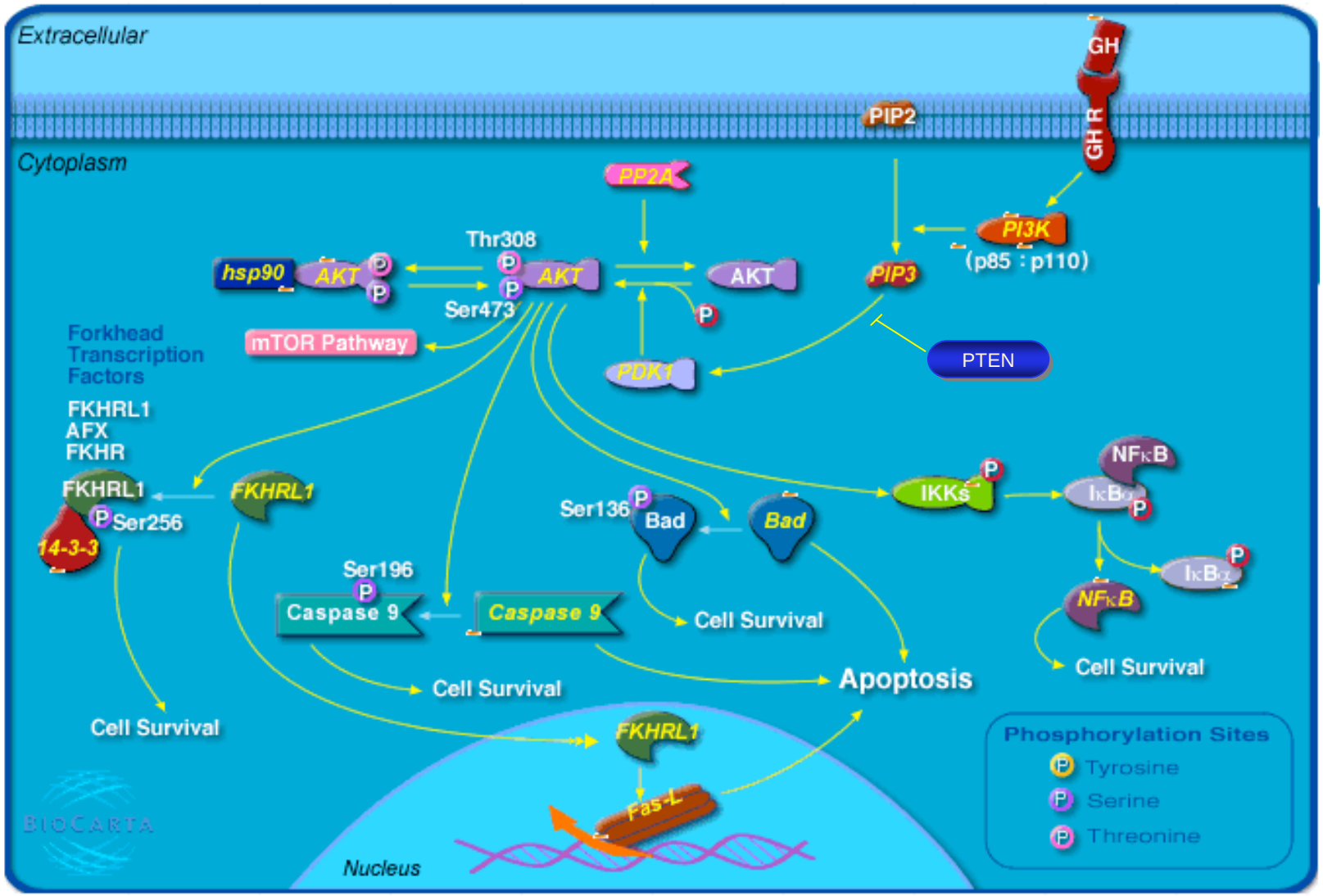
Greg Tucker-Kellogg

Ramneek Gupta

Ketan Patel

Integrative Cancer Genomics

- Integrated Cancer Genome Study
- 23 cancer cell lines
- 250 fresh frozen clinical specimens, some commercial and some from IU bank (Br, Co, Lu, Ov)
- Approaches:
  - Resequencing of ~200 exons in important cellular pathways
  - CGH arrays for global amplification/deletion
  - cDNA expression profiling (trial in one tumor type)
  - Immunohistochemistry to evaluate survival pathway activity
    - NFkB
    - PI3K/AKT
    - MAPK pathways
  - siRNA drug vulnerability screens

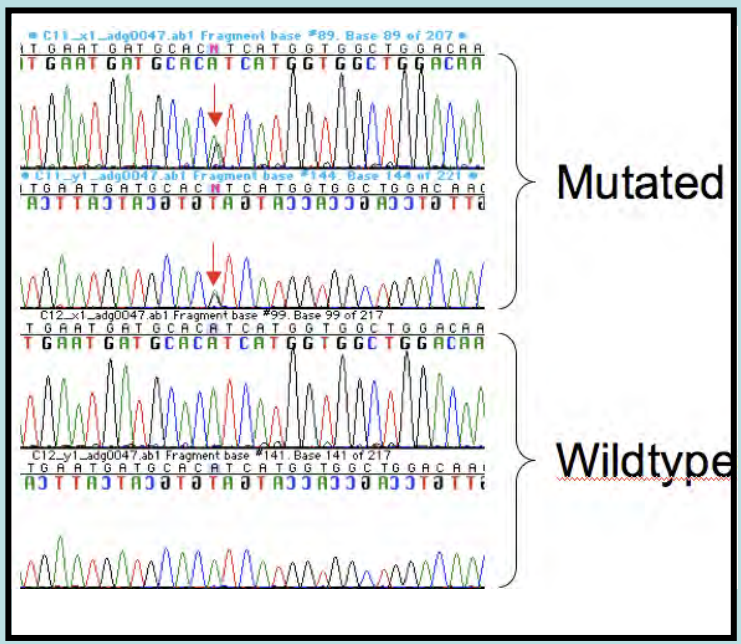


www.biocarta.com

Predictive Pharmacogenomics Study

Resequencing Project

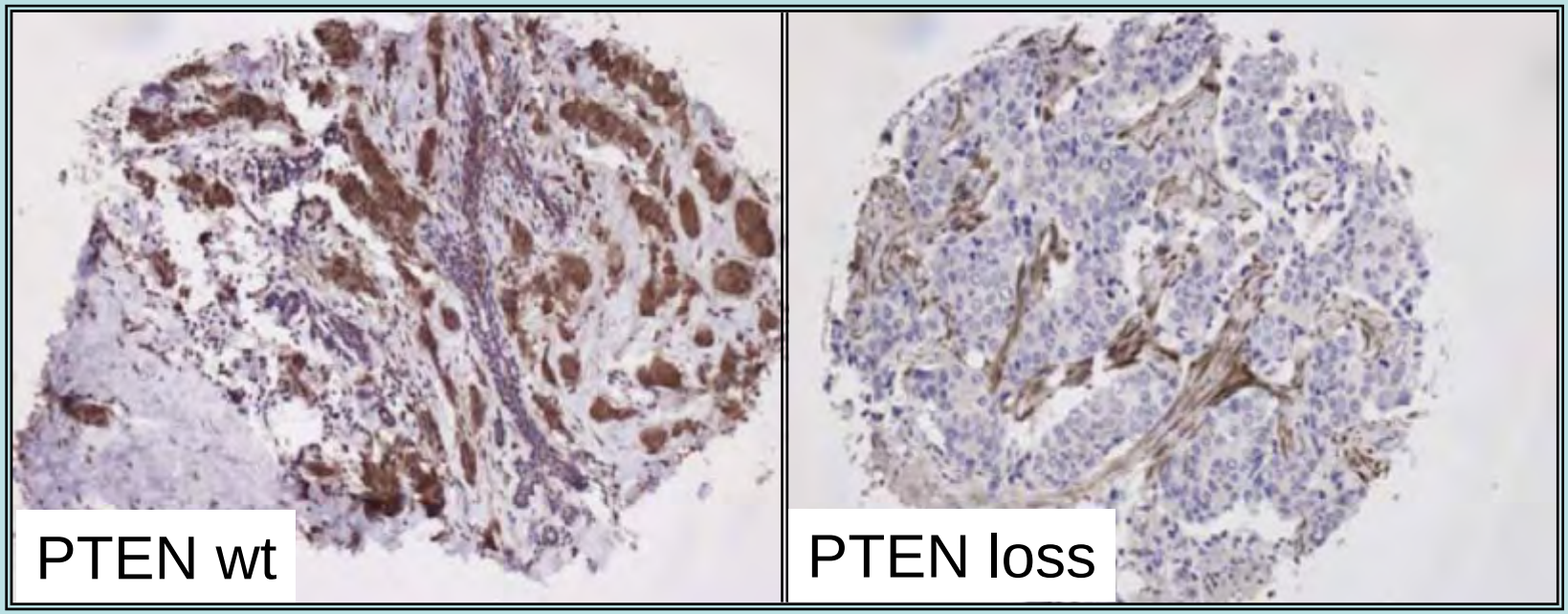
- Somatic mutations in PIK3/AKT pathway
- Discovered somatic mutations in PIK3/AKT pathway



PI3K kinase domain mutations		
Tumor type	Exon 9 mutations	Exon 20 mutations
Breast (n=61)	6%	10%
Colorectal (n=51)	18%	2%
Lung (n=59)	3%	0%
Ovarian (n=50)	5%	0%

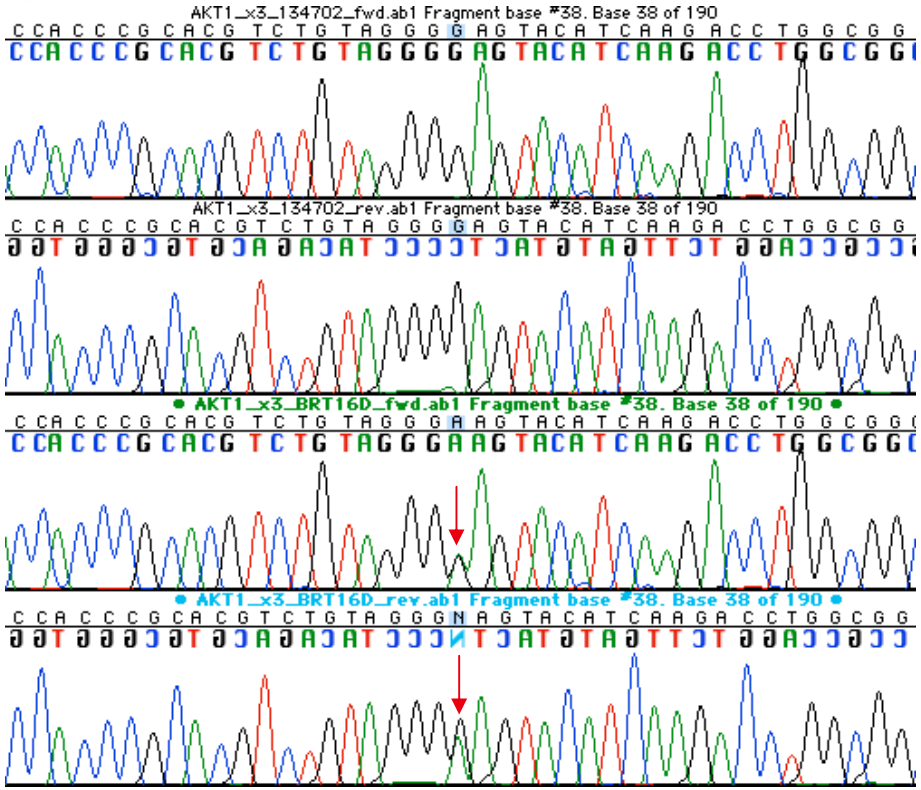
Predictive Pharmacogenomics Study

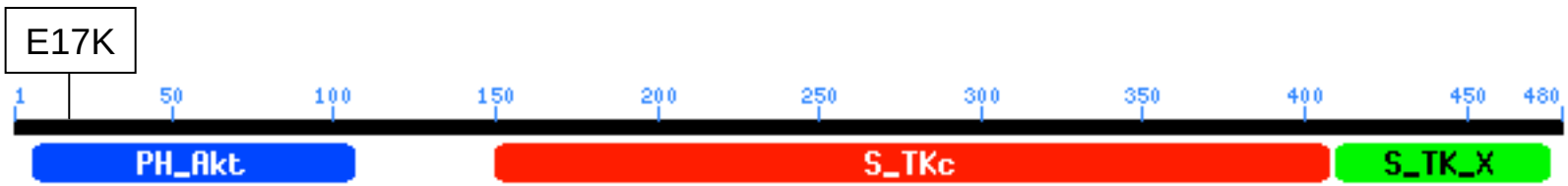
- Somatic mutations in PIK3/AKT pathway
  - PTEN mutation in ~2%
  - PTEN deletion by aCGH and IHC



Novel AKT1 Mutation

49G>A





Pleckstrin homology

Serine/Threonine kinase active domain

Kinase extension domain

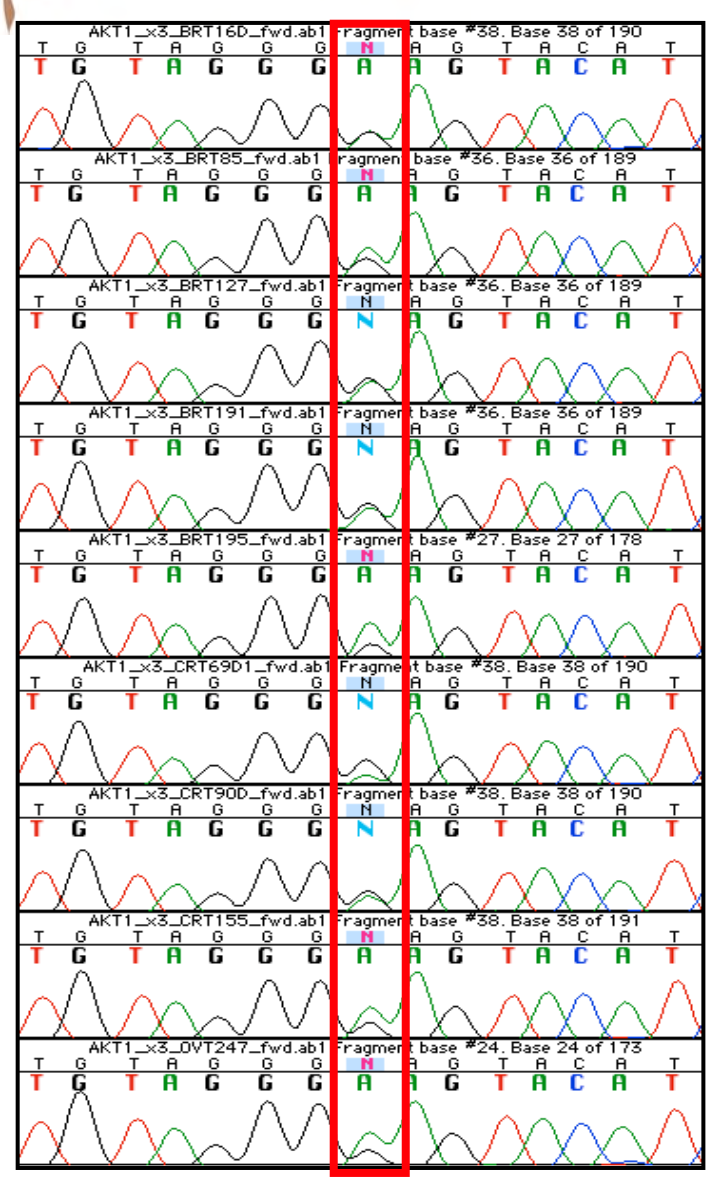
ARTICLES

A transforming mutation in the pleckstrin homology domain of AKT1 in cancer

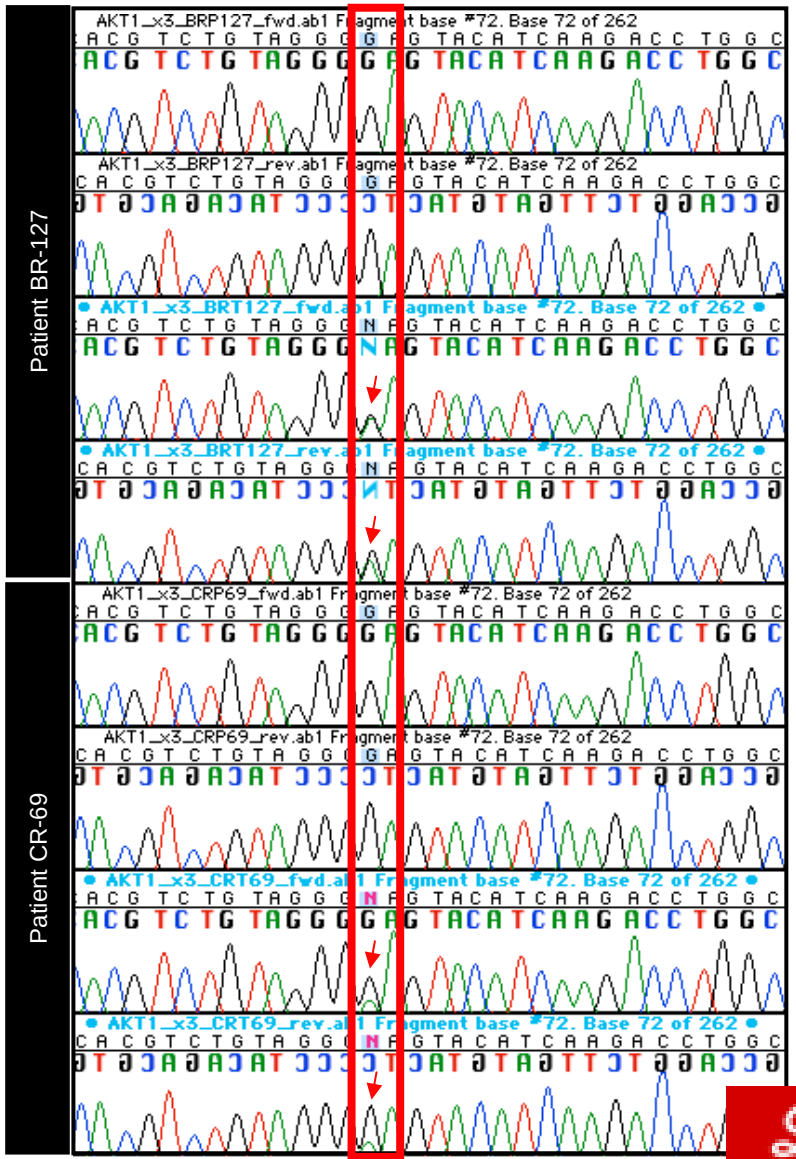
John Carpten[1], Andrew L Faber[2], Candice Horn[2], Greg Donoho [2], Stephen L Briggs[3] , Christiane M Robbins[1], Galen Hostetter[1], Sophie Boguslawski[2], Tracy Y Moses[1], Stephanie Savage[1], Mark Uhlik[2], Aimin Lin[4], Jian Du[2], Yue-Wei Qian[4], Doug Zeckner[2], Greg Tucker-Kellogg[5], Jeffrey Touchman[1], Ketan Patel[5], Spyro Mousses[6], Michael Bittner[1], Richard Schevitz[3], Mei-Huei T Lai[2], Kerry L Blanchard[2*], James E Thomas[2*]

AKT1 49G>A (E17K)

49G>A



Somatic



Germline Forward

Germline Reverse

Tumor Forward

Tumor Reverse

Germline Forward

Germline Reverse

Tumor Forward

Tumor Reverse

AKT1 49G>A (E17K)

Novel PIK3/AKT pathway oncogenic mutation	
Tumor type	Frequency
Breast (n=74)	8%
Colorectal (n=51)	6%
Lung (n=110)	0%
Ovarian (n=50)	2%

- Present in tumor types with high frequencies of PIK3CA mutations
  - Other tumors of interest (Prostate, Endometrial)

AKT1 49G>A (E17K)

Table 1. Characteristics of tumors harboring the AKT1 E17K mutation.

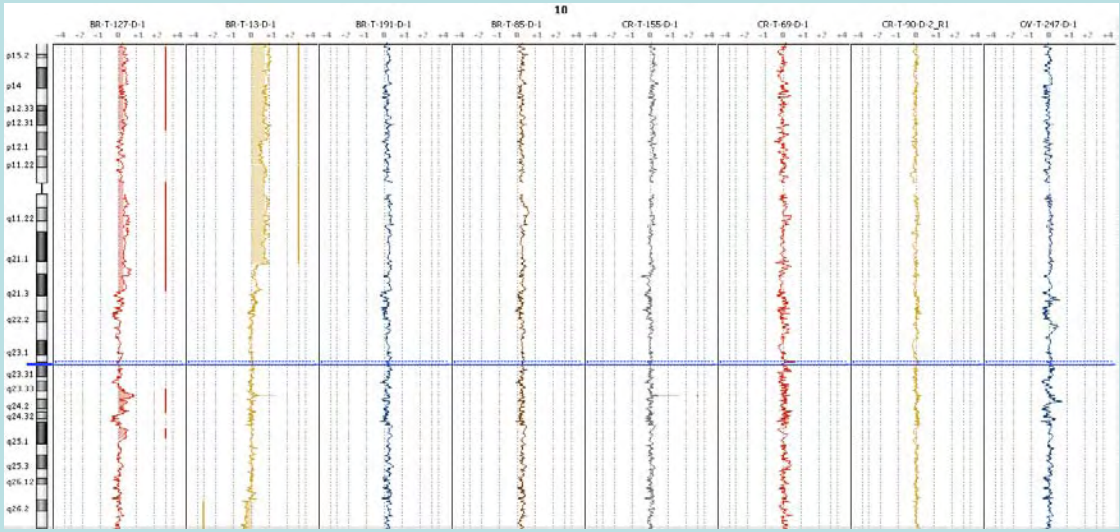
Sample ID	Tumor Type	Tumor Stage	PTEN	ER	PR	Her2	MSI
BR-T-16	Breast	IIB	NA	pos	neg	neg	neg
BR-T-85	Breast	IIA	pos	pos	pos	neg	neg
BR-T-127	Breast	IIA	partial	pos	pos	neg	neg
BR-T-191	Breast	IIA	pos	neg	pos	neg	neg
BR-T-195	Breast	IIB	pos	pos	neg	neg	NA
CR-T-69	Colon	II	pos	NA	NA	NA	pos
CR-T-90	Colon	III	pos	NA	NA	NA	pos
CR-T-155	Colon	III	partial	NA	NA	NA	neg
OV-T-247	Ovarian	IIIC	NA	NA	NA	NA	neg

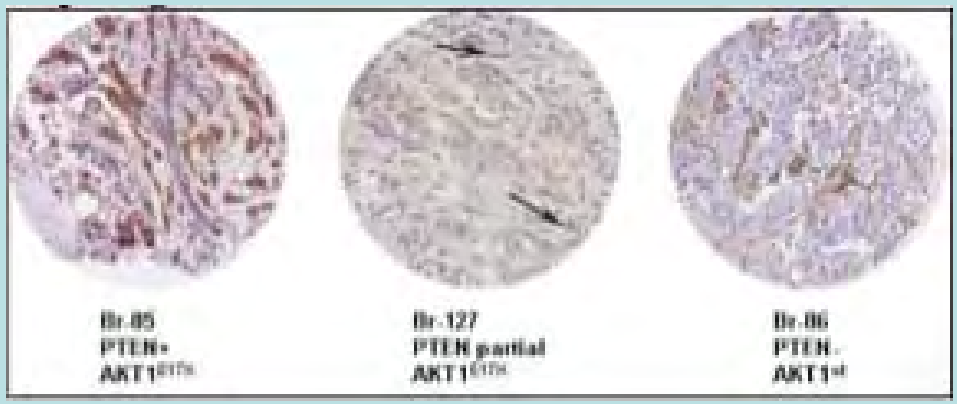
ER – Estrogen Receptor, PR – Progesterone Receptor, MSI – microsatellite instability

Neg – negative staining, Pos – positive staining, NA – data not available

AKT1 49G>A (E17K)

Mutation is mutually exclusive of PTEN and PIK3 mutation

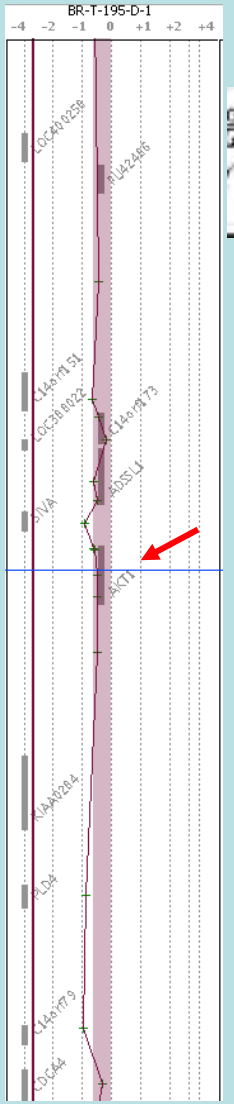


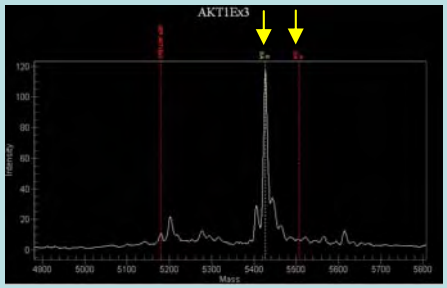


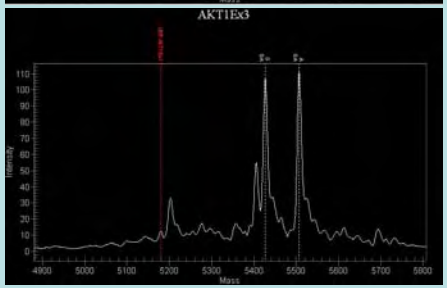
AKT1 49G>A (E17K)

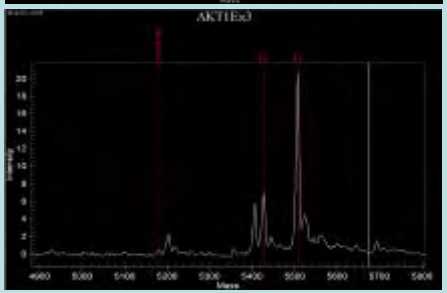
Evidence of genomic deletion of the wildtype allele

BR-T-195

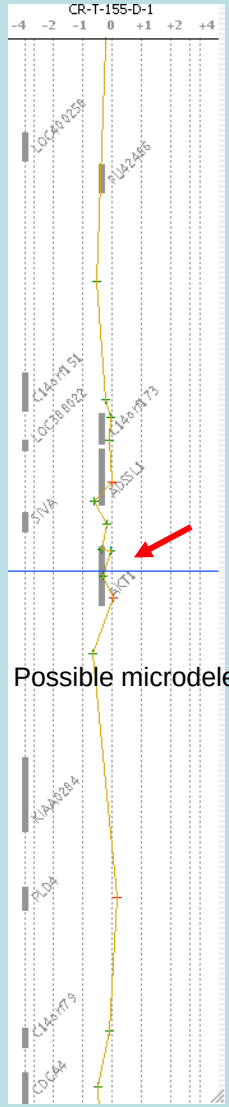




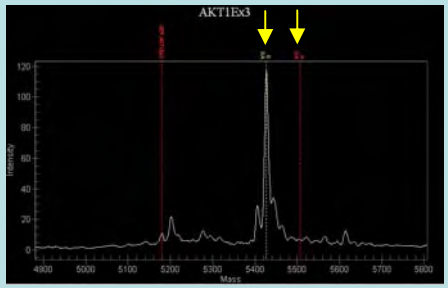


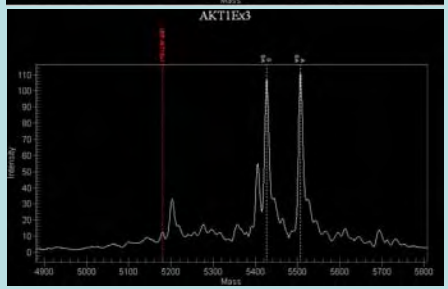


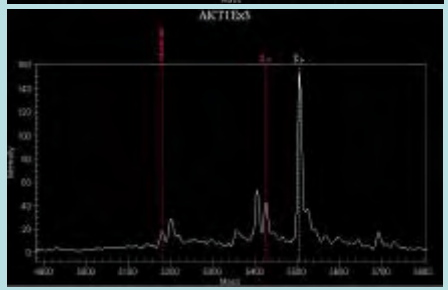
CR-T-155



Possible microdeletion

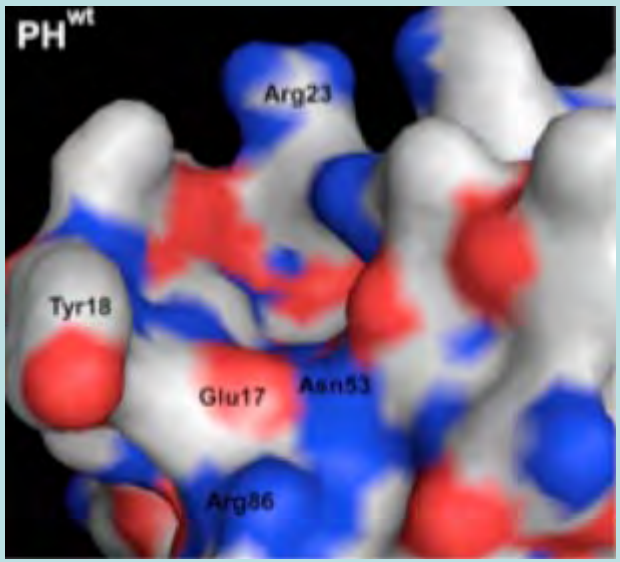


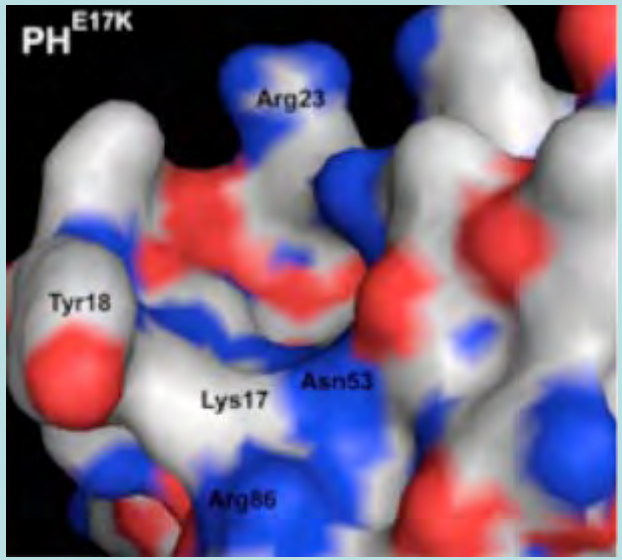




AKT1 49G>A (E17K)

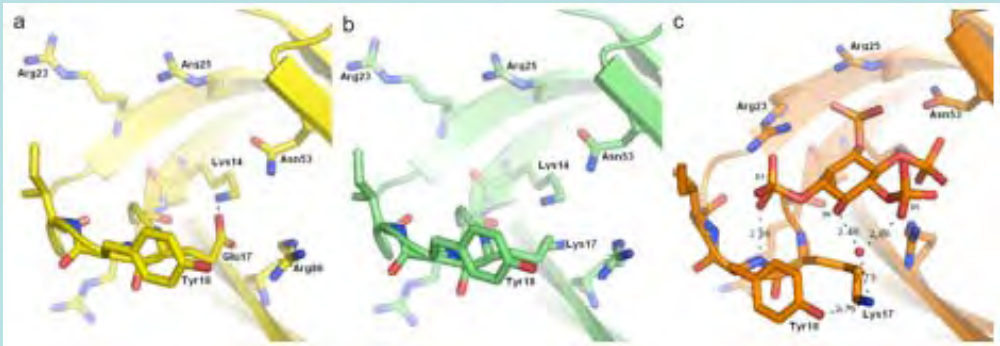
Solved the crystal structure of wt and E17K PHDs from human AKT1 to1.1 and 1.9 angstoms (Á) and E17K PHD-Ins(1,3,4,5)P4 (PIP4) complex to 2.6 Á resolution.

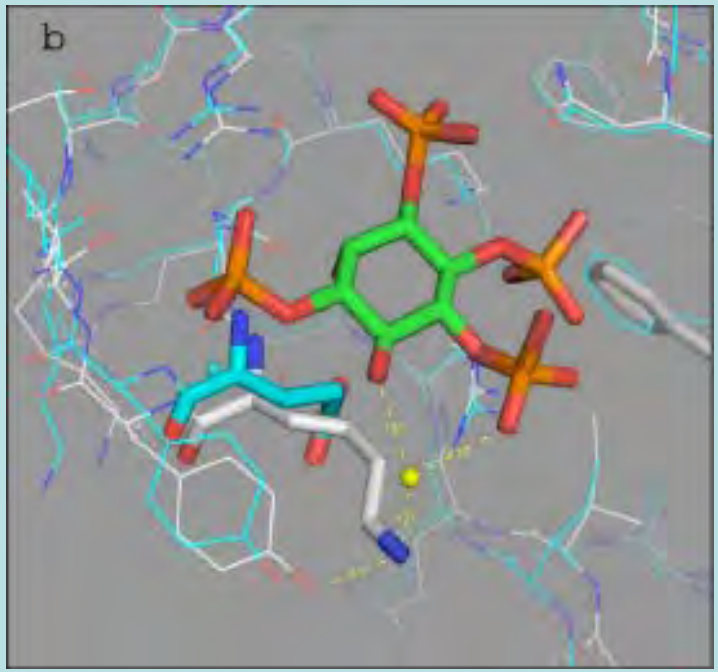




The Lys17 substitution results in a shift in the surface charge around the pocket from negative with Glu17 to effectively neutral in the mutant.

AKT1 49G>A (E17K)

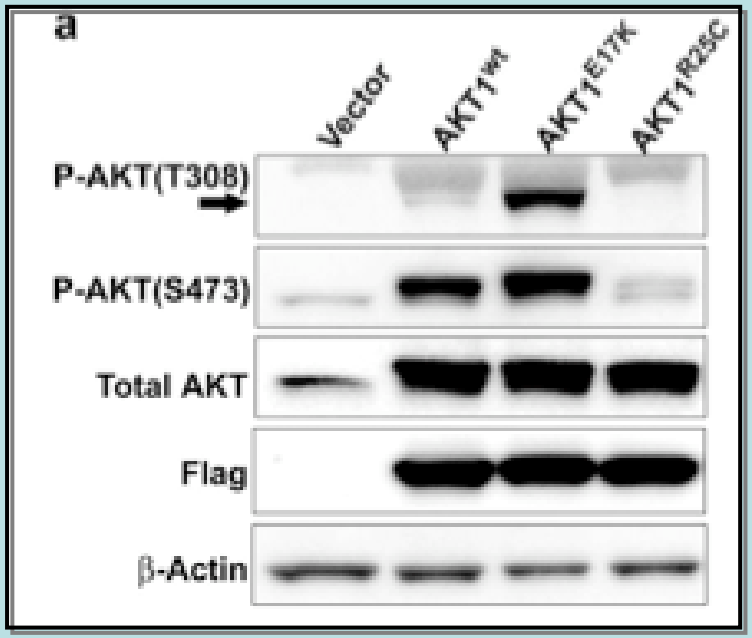


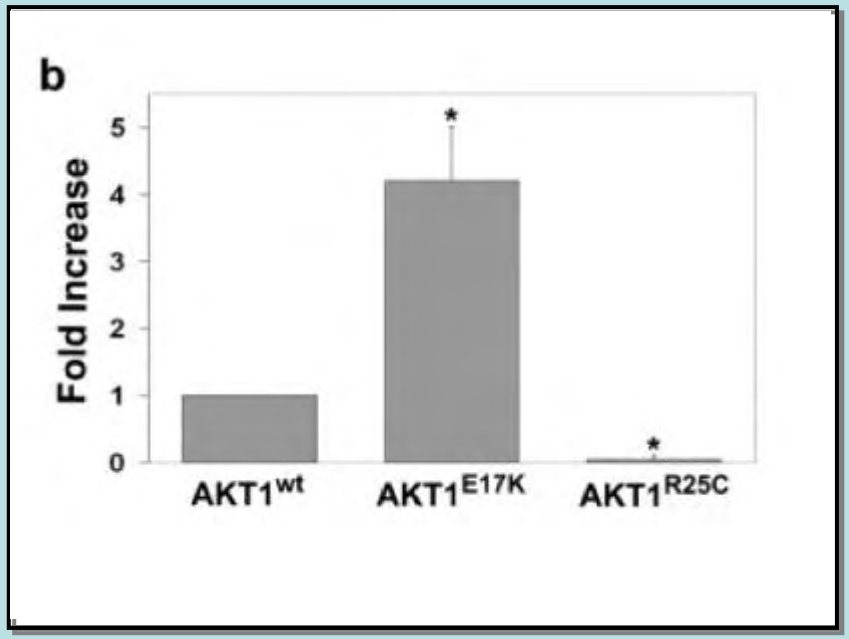


In the E17K PHD Tyr18 moves 7.5 Å out of the pocket and forms a hydrogen bond with Lys17, and additional hydrogen bonds form between Lys17, a conserved water molecule and the 5-phosphate and 6-hydroxyl of Ins(1,3,4,5)P4

AKT1 49G>A (E17K)

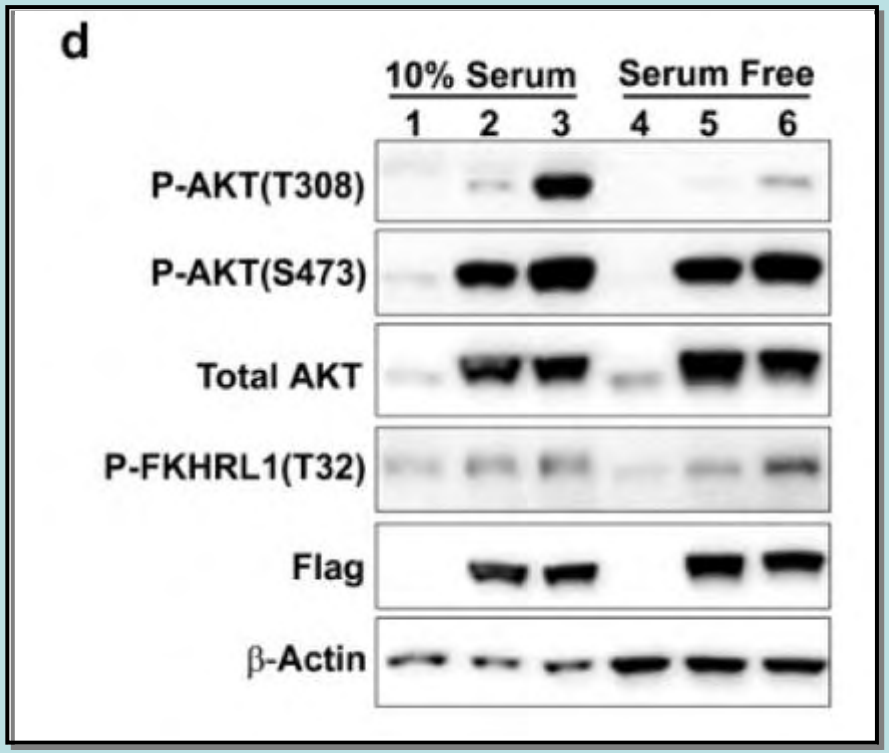
The E17K mutation increases AKT1 activation





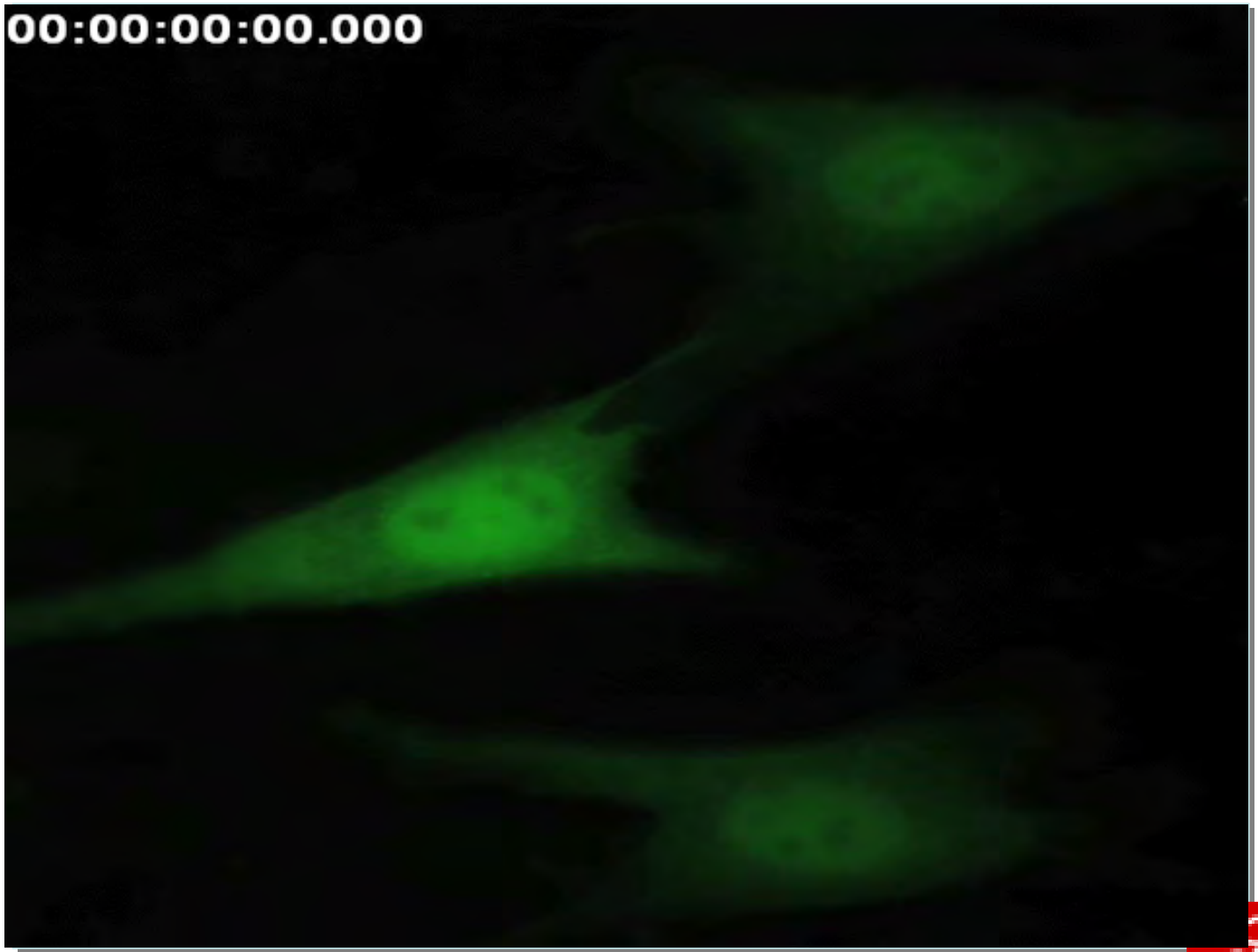
AKT1 49G>A (E17K)

AKT1E17K up regulates survival signaling under adverse conditions



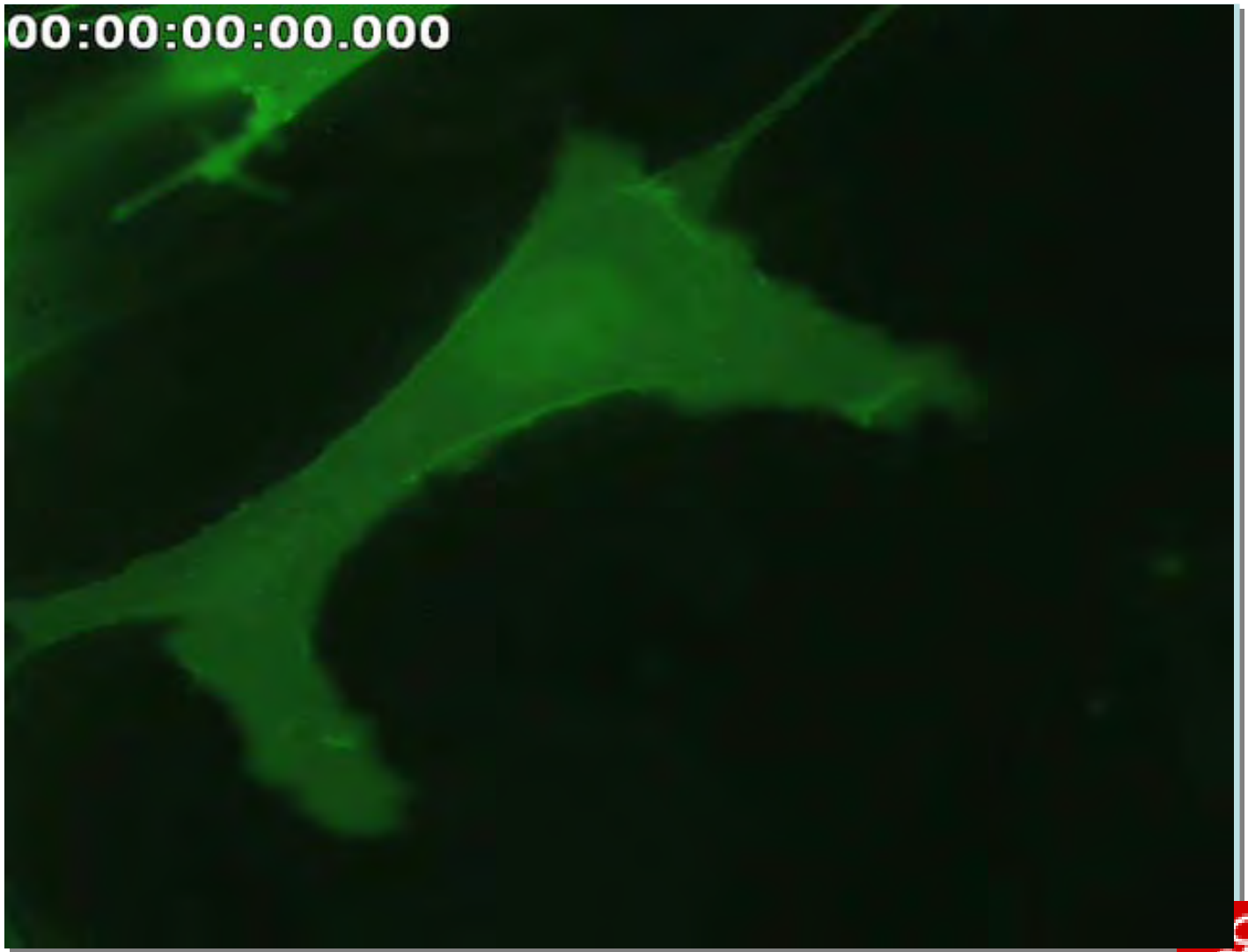
AKT1 49G>A (E17K)

AKT WT with PDGF



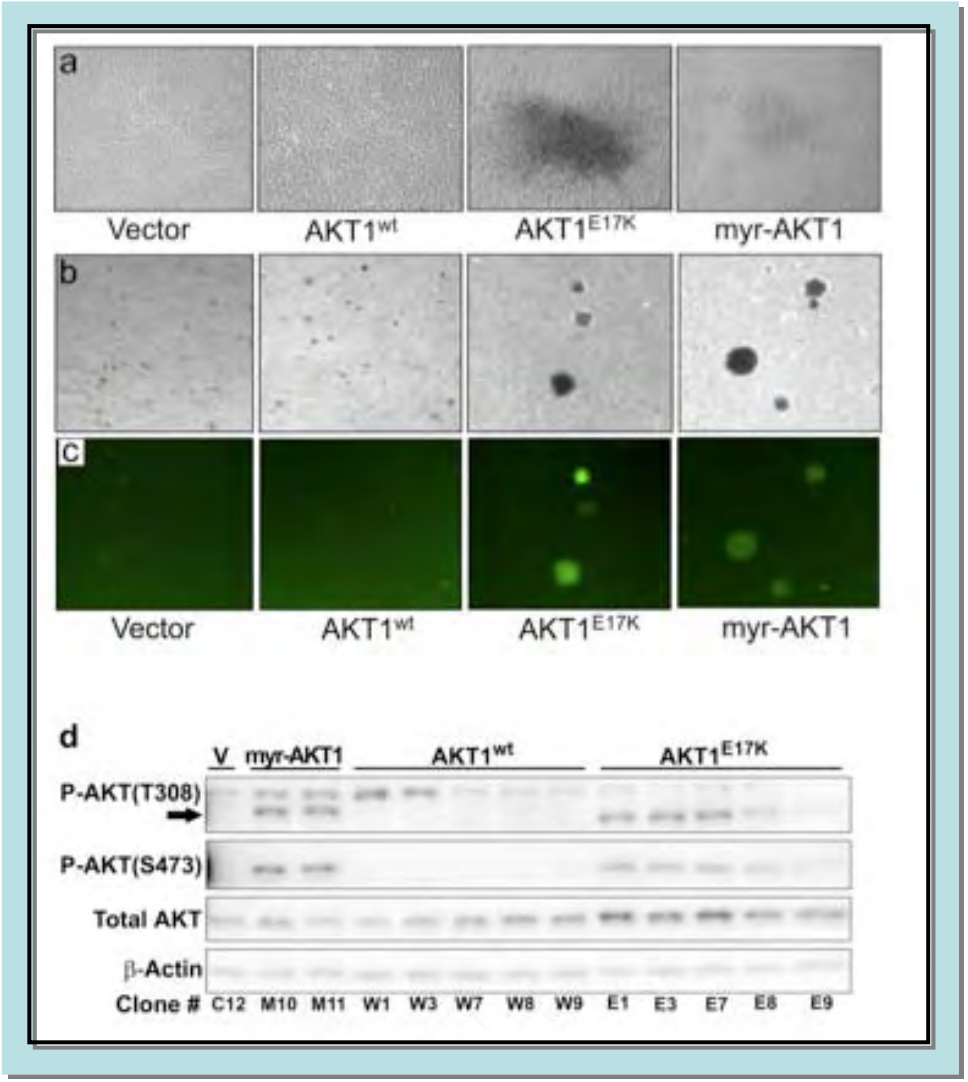
AKT1 49G>A (E17K)

AKT E17K with PDGF

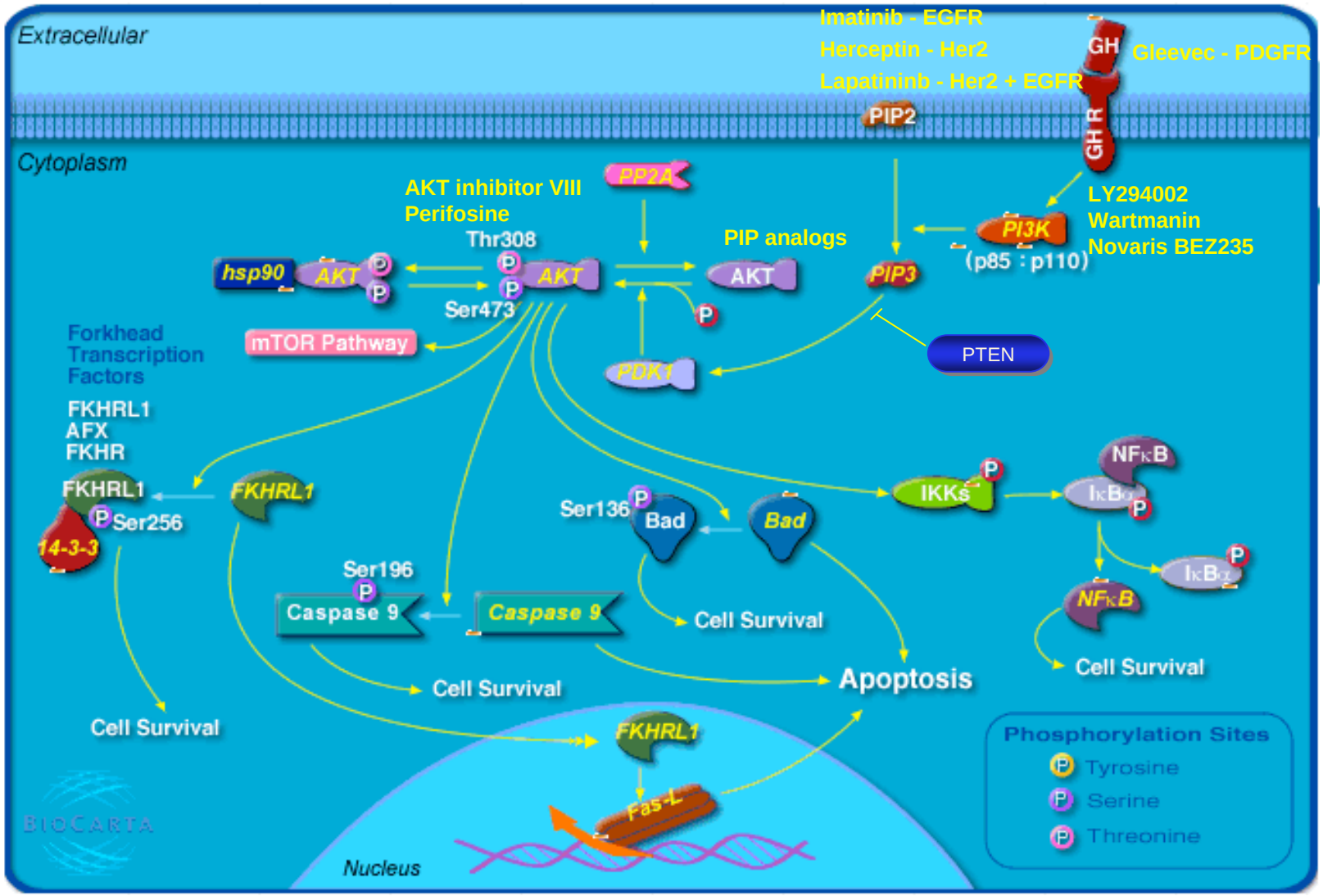


AKT1 49G>A (E17K)

AKT E17K Transforms Rat1 cells *in vitro*

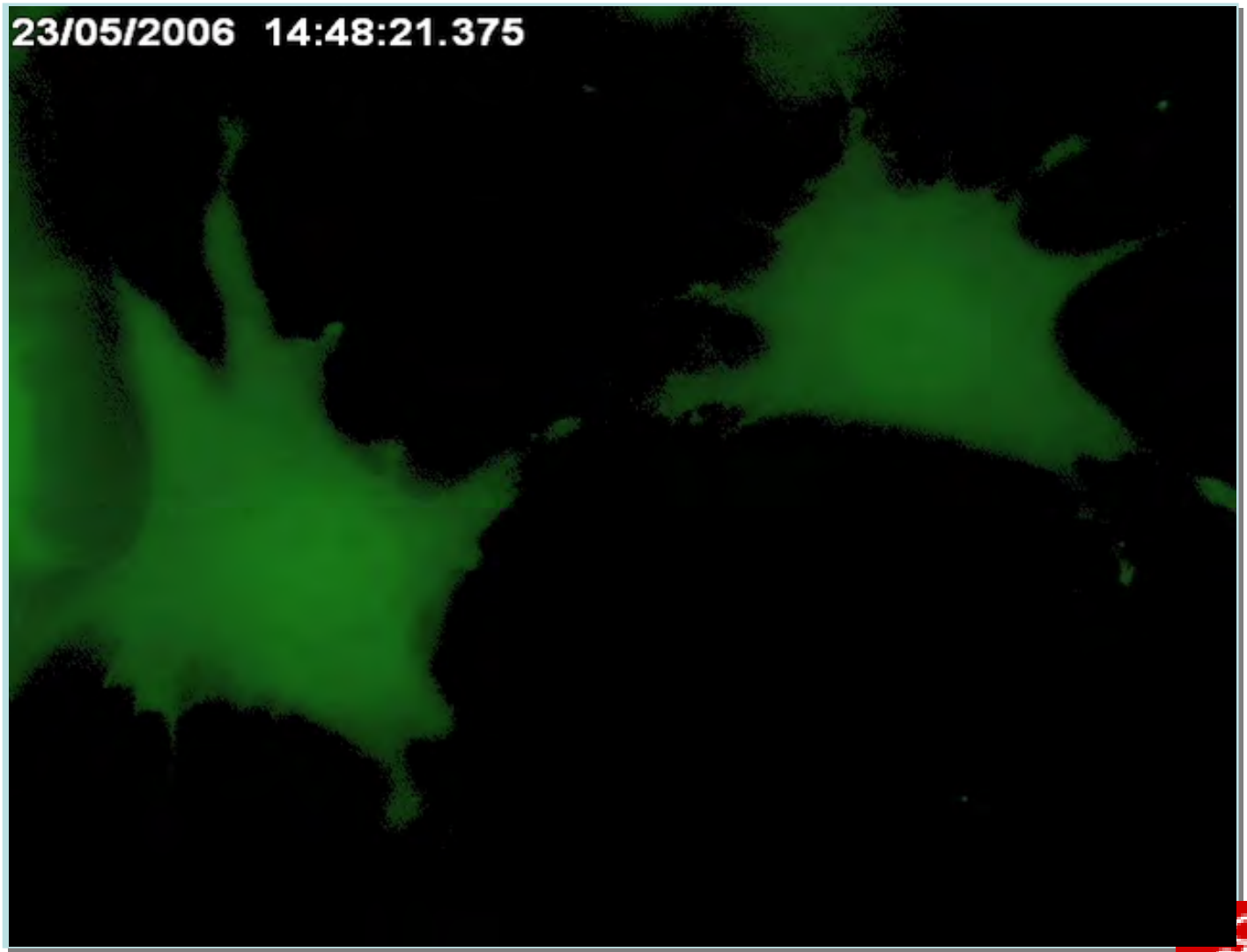


Predictive Pharmacogenomics Study



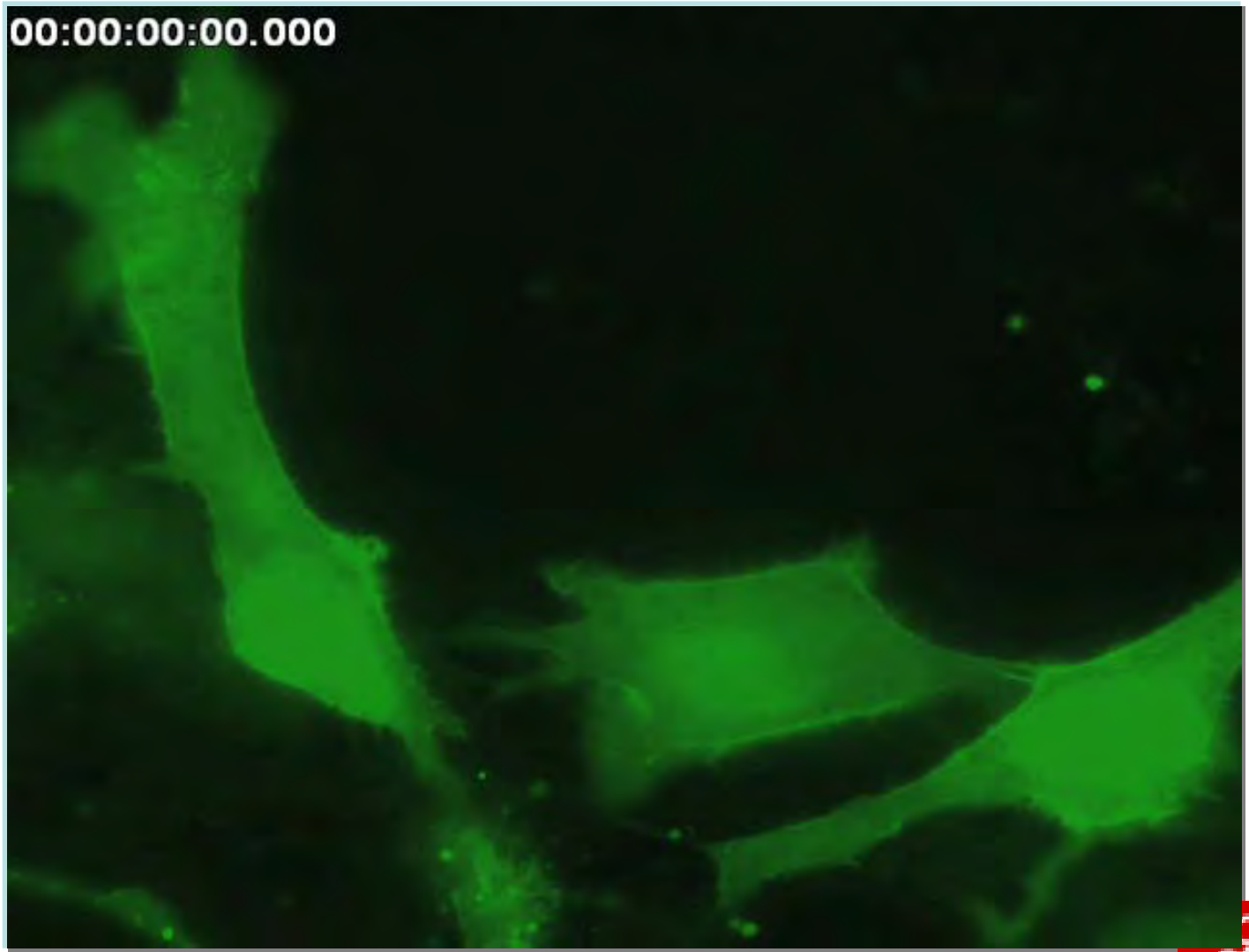
AKT1 49G>A (E17K)

Drug modulation, AKT WT with pretreated with LY294002



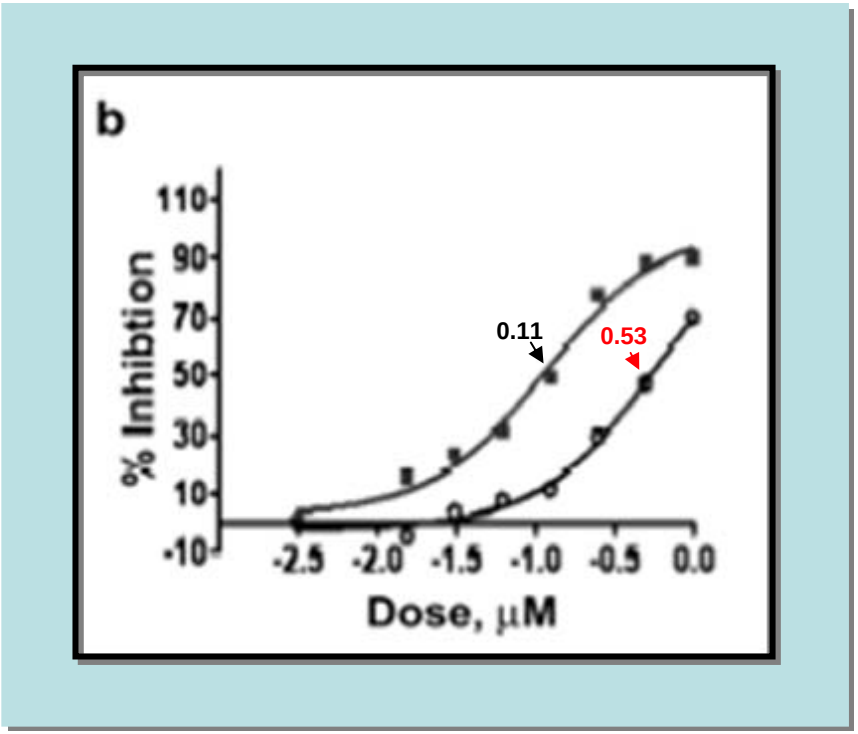
AKT1 49G>A (E17K)

Drug modulation, AKT E17K with pretreated with LY294002



AKT1 49G>A (E17K)

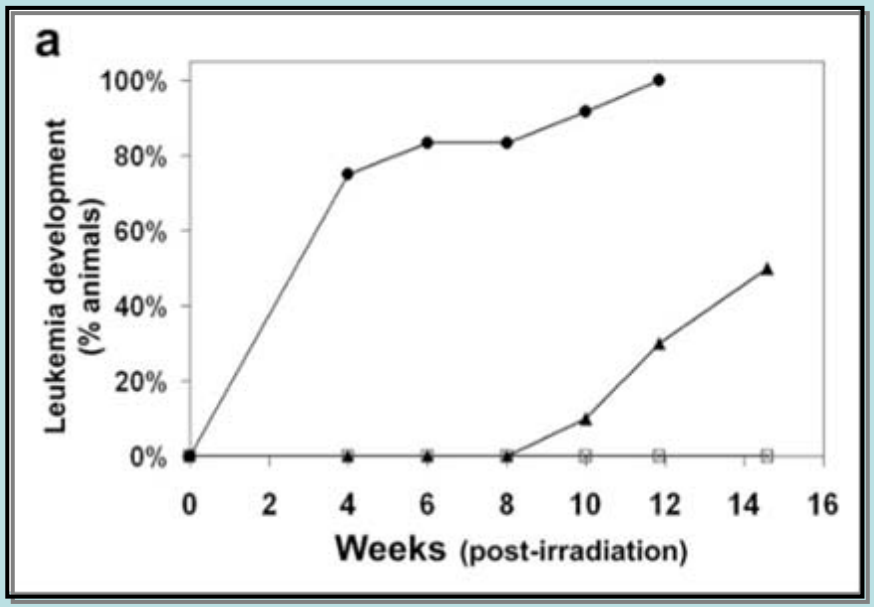
Drug modulation, AKT E17K increases IC50 for an known allosteric AKT inhibitor

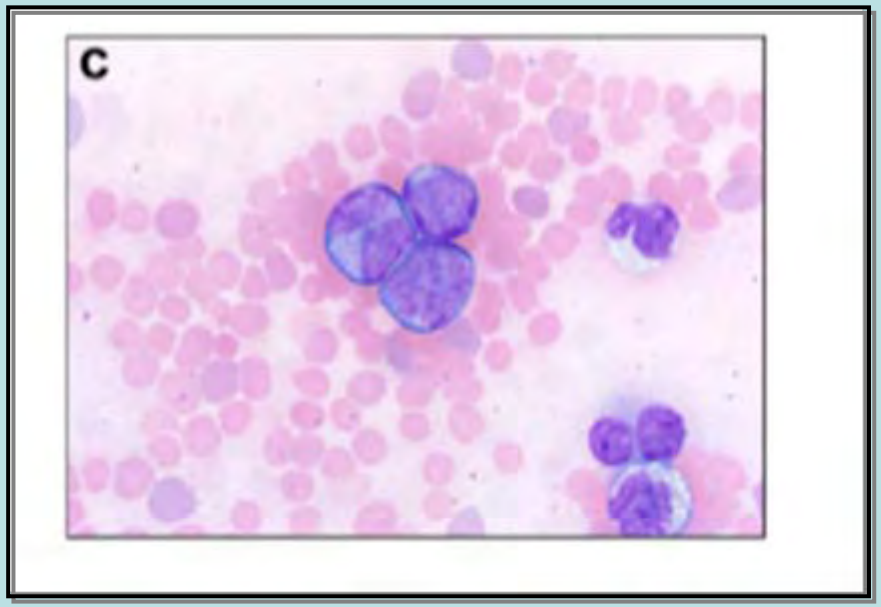


AKT1 49G>A (E17K)

E17K causes leukemia in the μMyc mouse model (confirmed)

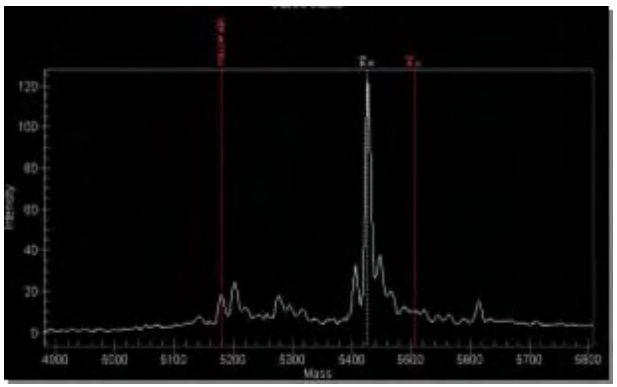
- *In vivo* model to test for inhibitors (current and under-development) ability to abolish tumor growth *in vivo*?

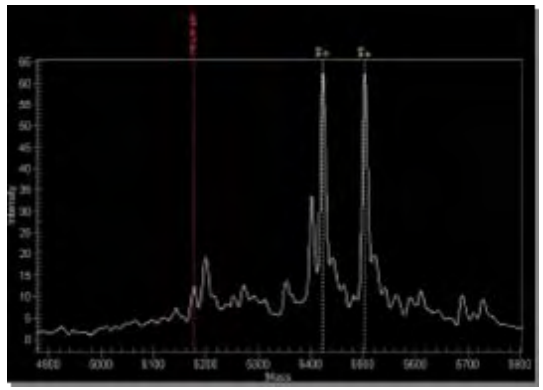




AKT1 49G>A (E17K)

- Recurring mutation and we have developed a Sequenom assay for easy, cost effective screening of tumors for this mutation (FFPE too)





- Target for novel drug discovery

- Identify patients that might respond to a therapy specifically targeting the mutated version of the protein

AKT1 49G>A (E17K)

- Prevalence seems to be lower than in our original sample set
- Our breast tumors harboring the mutation were mainly hormone receptor positive
- Other tumors studied to date
  - Prostate ~2%
  - Endometrial ~2%
  - Head and Neck 0%
  - Ovarian (serous) 1%
  - Gastric - ND
  - Colon - 1%-2%
  - Breast - ~2%
- May be ethnicity/geography specific
  - Varying frequency of EGFR mutations (5%-57%).

Summary

- We have used Integrative Cancer Genomics studies to discover important cancer genes associated with cancer, including prostate cancer.
- These genes may someday have clinical impact as markers of diagnosis or prognosis.
- These discoveries can have an immediate impact on patient care through the discovery of markers which predict response to chemotherapies.
- These studies should further our knowledge of the molecular characteristics of cancer to help develop earlier diagnostics and smarter treatments.

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