# Using Tandem Mass Spectrometry to Choose Appropriate Kinase Inhibitor Drugs in Cancers: A Personalized Medicine Approach Based on Protein-Protein Interactions (PPI)

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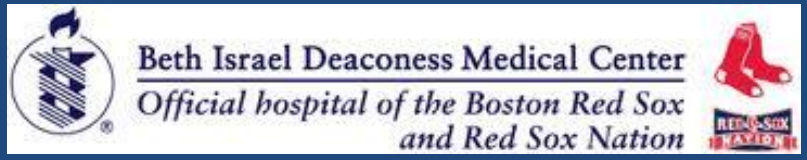
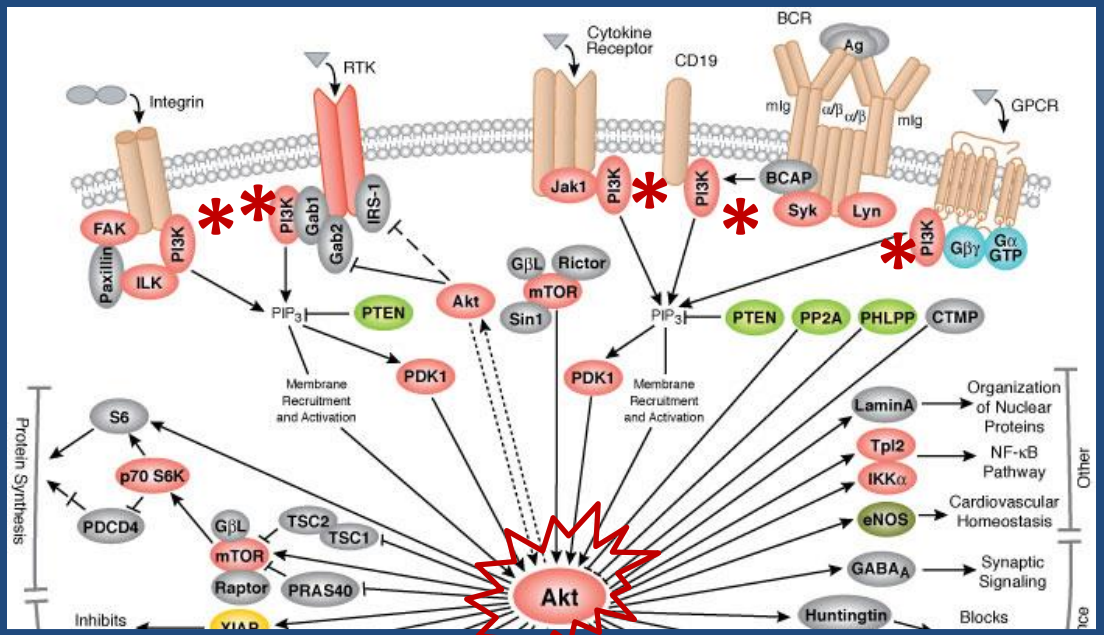


# Human PI3K / Akt Signaling

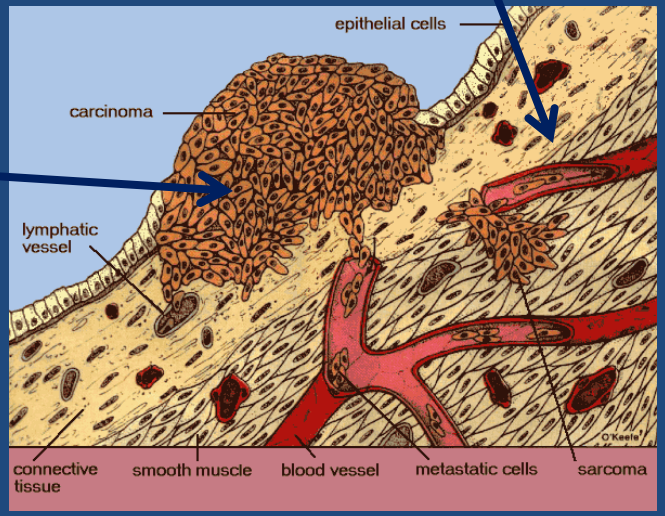



**Proliferation, protein synthesis, etc.**

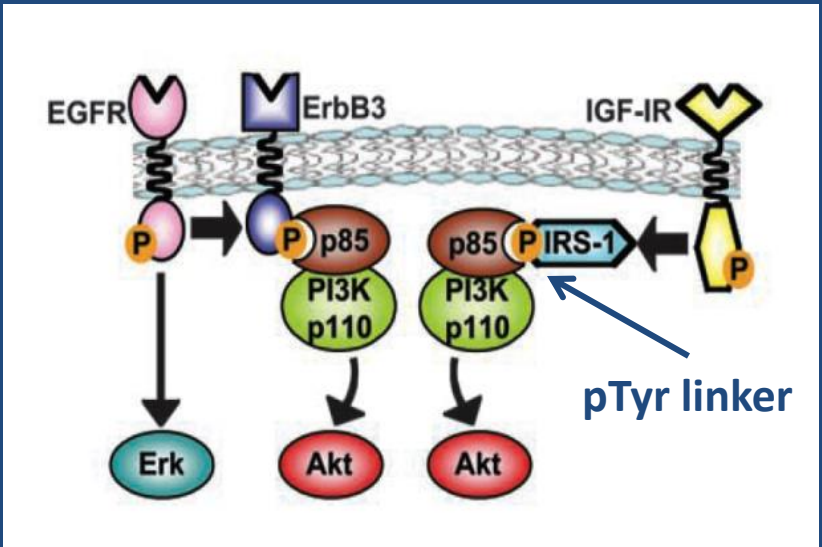
**Cancer growth**

Normal AKT signaling

*Uncontrolled*
AKT signaling

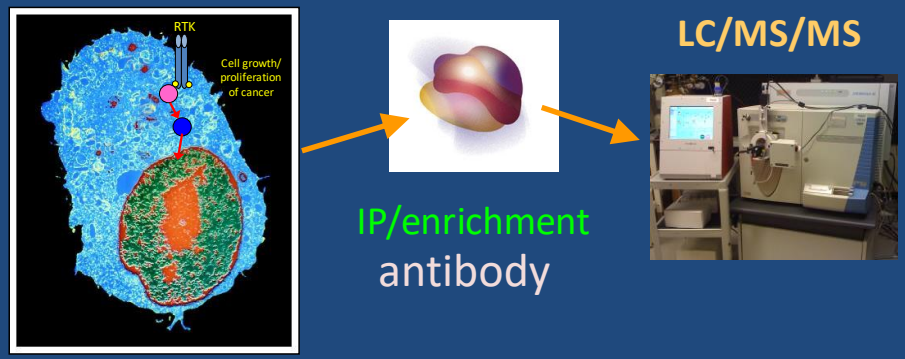


# Use of Mass Spectrometry to Identify and Quantify *Activating* Adaptors of PI3K

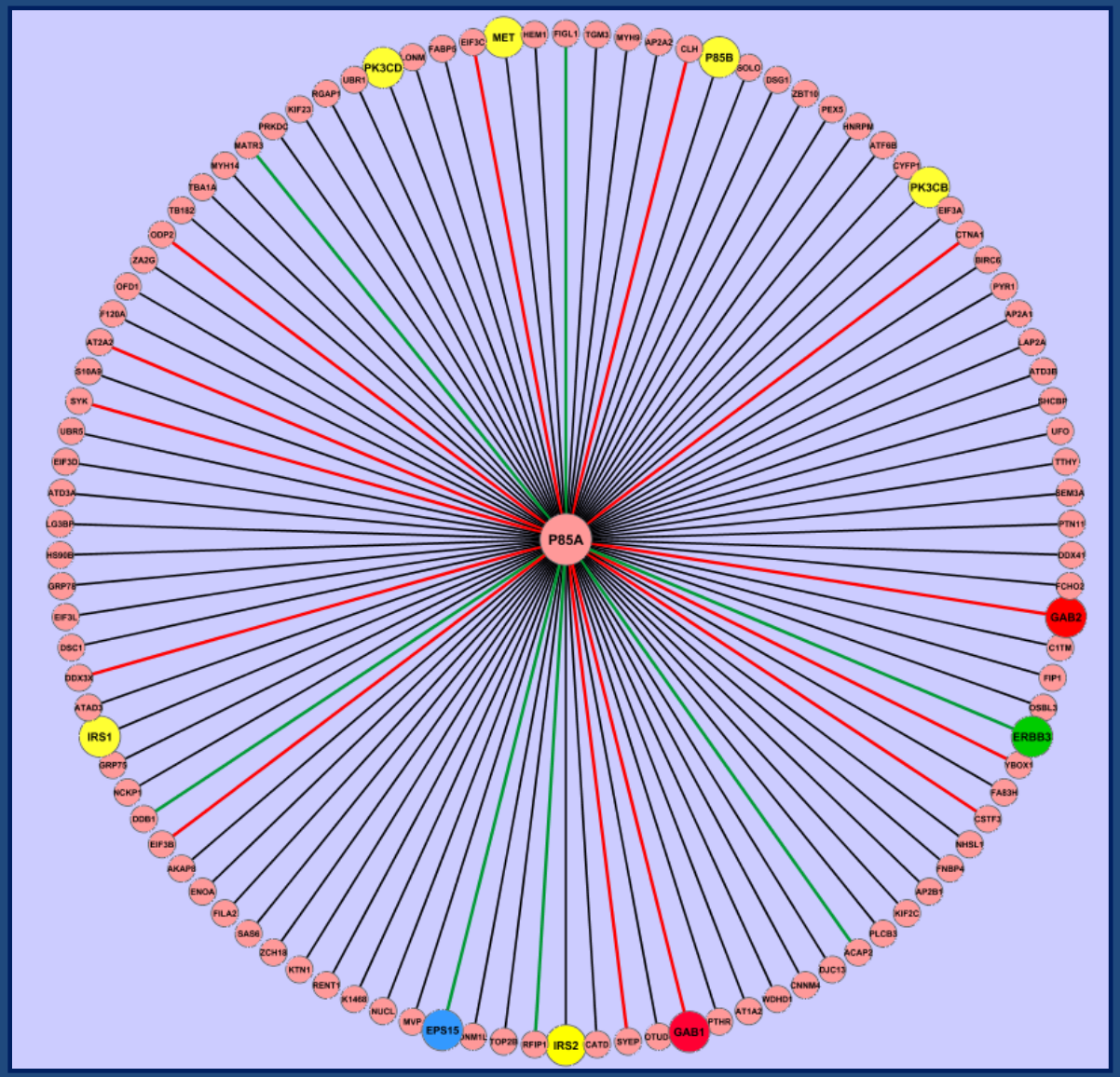



The protein-protein interaction (PPI) is what governs downstream signaling → tumor growth

- Need it to be <u>compatible with human tumor tissue</u>
- Ability to *quantify differences in PI3K binding* is essential
- p85 regulatory subunit of **PI3K** binds to **pYXXM** motifs of activating adaptor proteins



# Shotgun LC/MS/MS from a p85 (PI3K) IP of EBC Cancer Cells

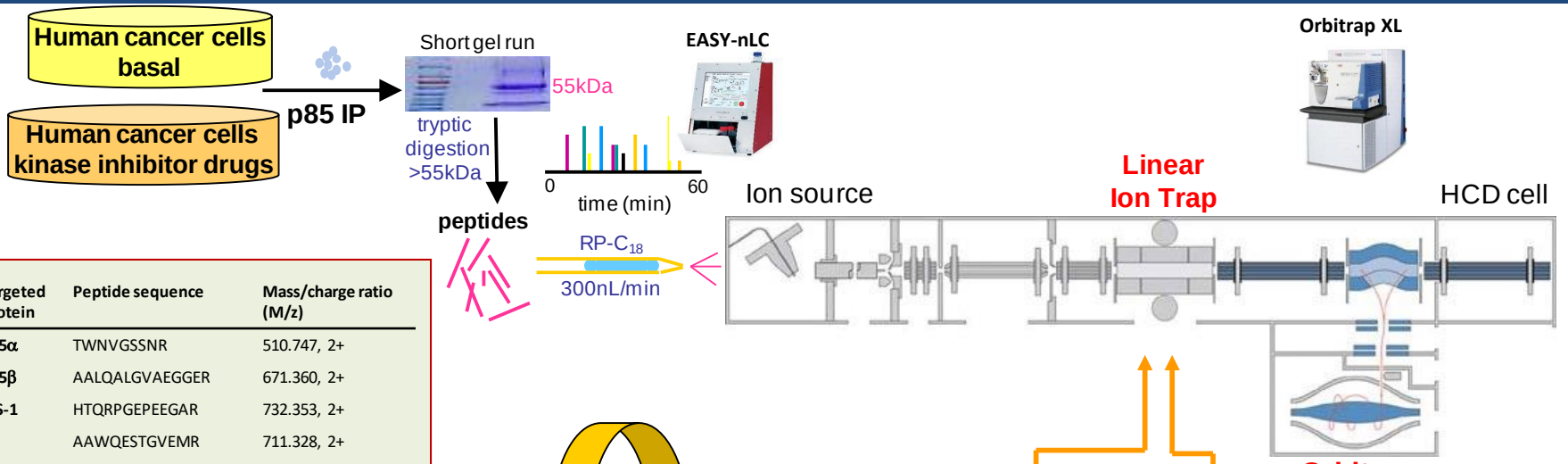



## Dirty IPs

Several known PI3K binders are detected but there are lots of non-specific binders

**Quantification was unreliable due to low spectral counts and high background**

# Targeted MS/MS Workflow Using Orbitrap XL



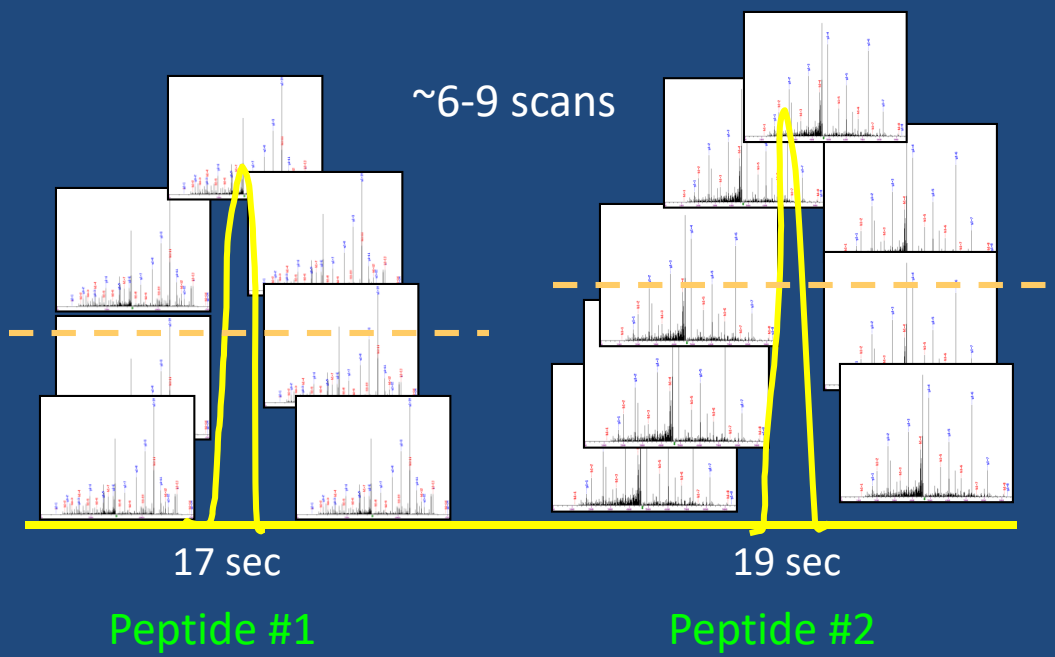

| Targeted protein | Peptide sequence | Mass/charge ratio (M/z) |
|---|---|---|
| p85α | TWNVGSSNR | 510.747, 2+ |
| p85β | AALQALGVAEGGER | 671.360, 2+ |
| IRS-1 | HTQRPGEPEEGAR | 732.353, 2+ |
| | AAWQESTGVEMR | 711.328, 2+ |
| IRS-2 | PVSVAGSPLSPGPVR | 710.401, 2+ |
| | SNTPESIAETPPAR | 735.365, 2+ |
| Gab1 | LTGPDVLEYYK | 706.851, 2+ |
| | APSASVDSSLYNLPR | 788.902, 2+ |
| Gab2 | SSPAELSSSSQHLLR | 799.910, 2+ |
| | SAESM$_{sx}$SDGVGSFLPGK | 792.864, 2+ |
| ERBB3 | ESGPGIAPGPEPHGLTNK | 879.444, 2+ |
| | GESIEPLDPSEK | 650.817, 2+ |
| | VLGSGVFGTVHK | 600.840, 2+ |
| | LAEVPDLLEK | 563.821, 2+ |
| PDGFR | VVEGTAYGLSR | 576.306, 2+ |
| | ATSELDLEM$_{sx}$EALK | 725.361, 2+ |

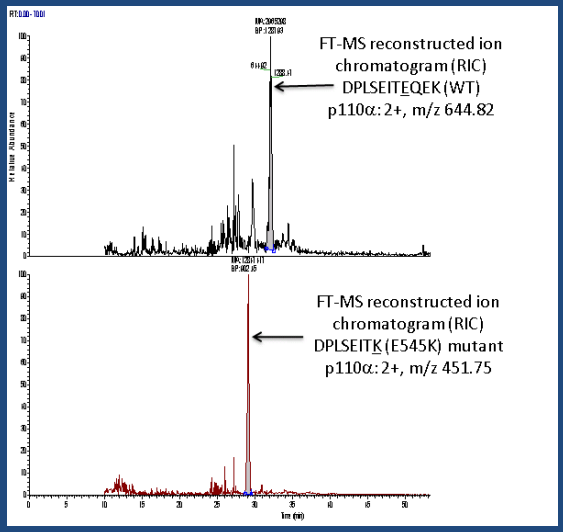
## Quantify using a Label-Free MS/MS approach

Calculate **average TIC ratio** per targeted protein =

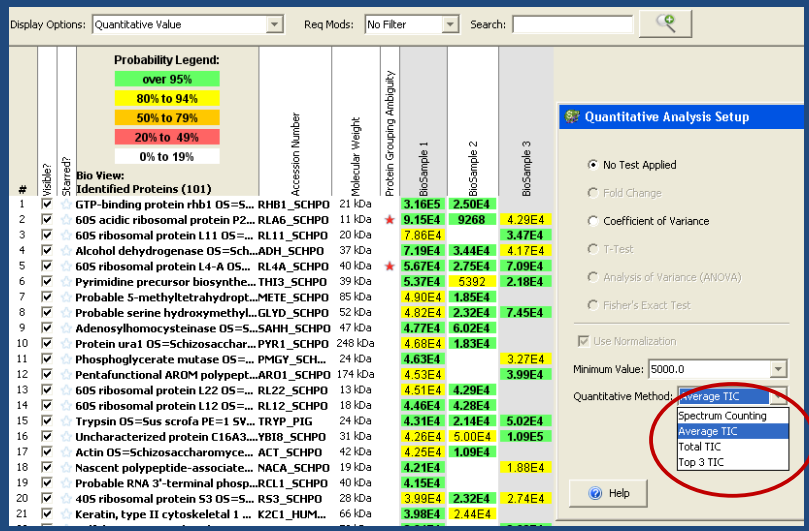
**Avg. TIC of all MS/MS spectra (Drug Treated vs. Untreated)**

# Quantitative Targeted runs using an Ion Trap portion of LTQ Orbitrap XL

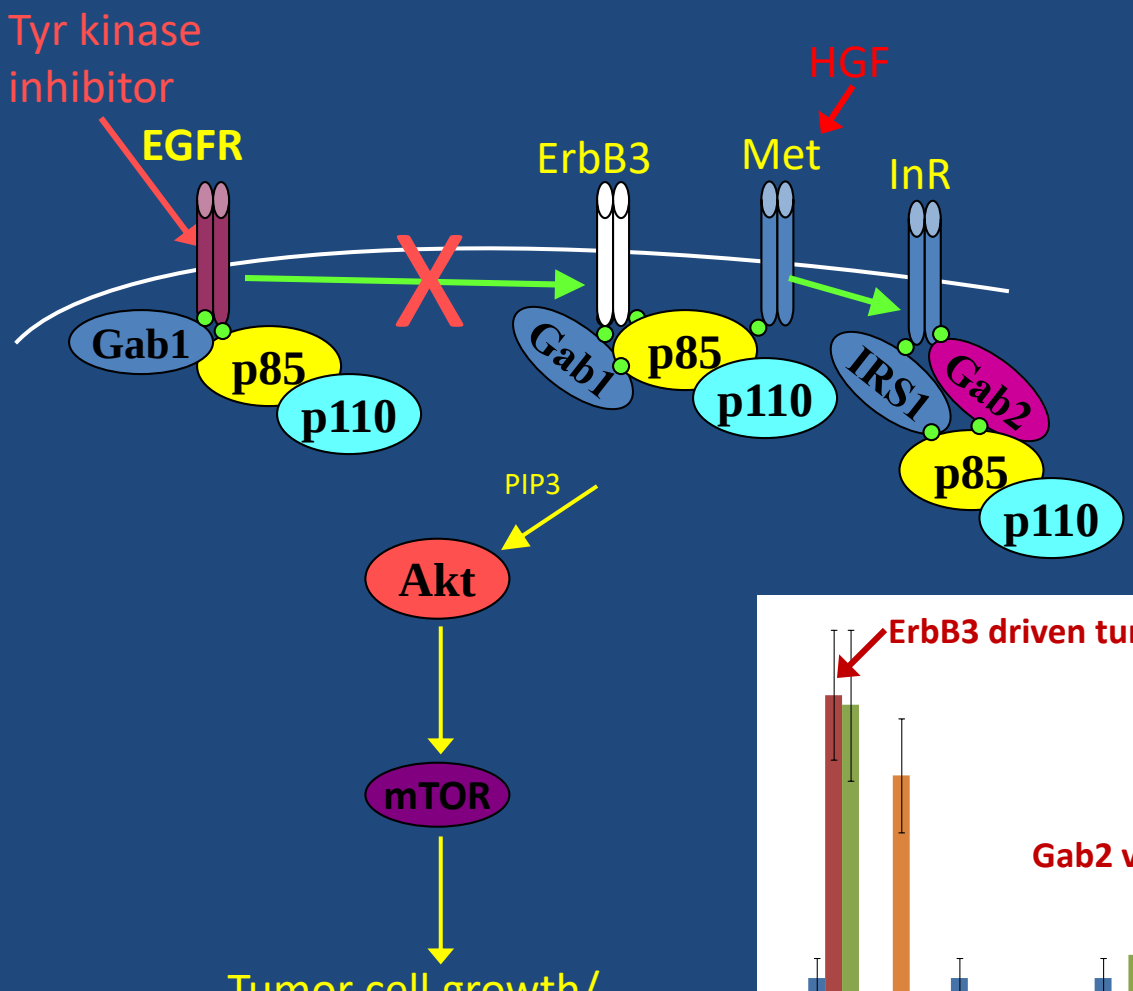


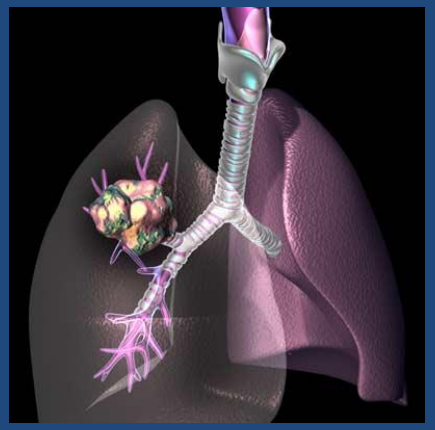

- Target ~ 2 peptides per protein in MS/MS mode
- Average the MS/MS TIC for all peptides across each protein in Scaffold 3.1
- Quantitation is *only relative* to a reference sample

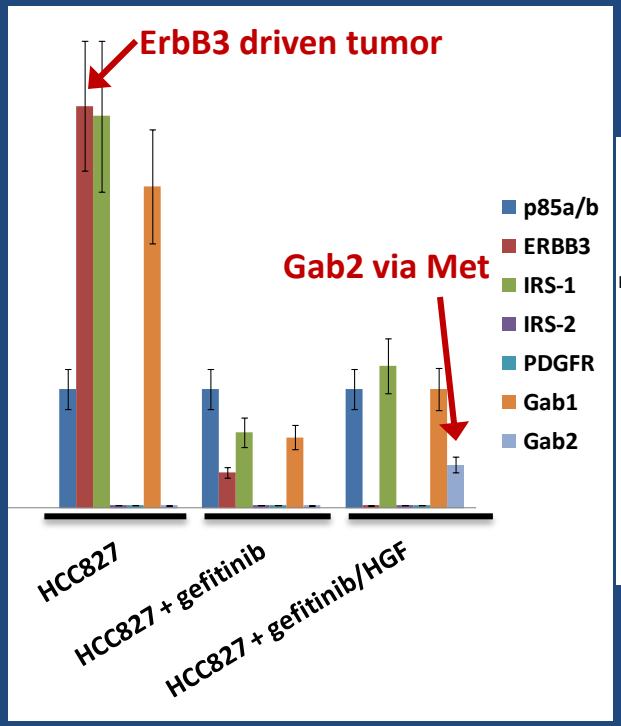


# Functional Role of PI3K in NSCL Cancer

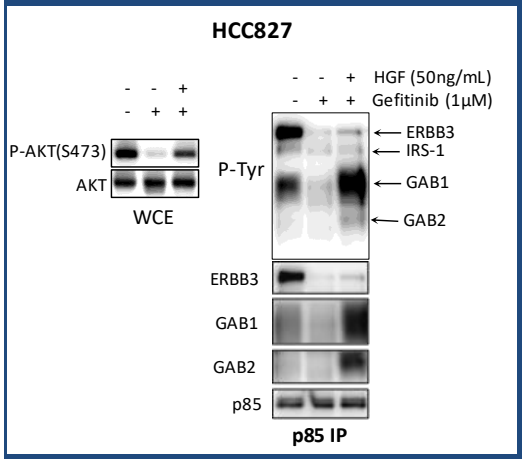



Lung Cancer

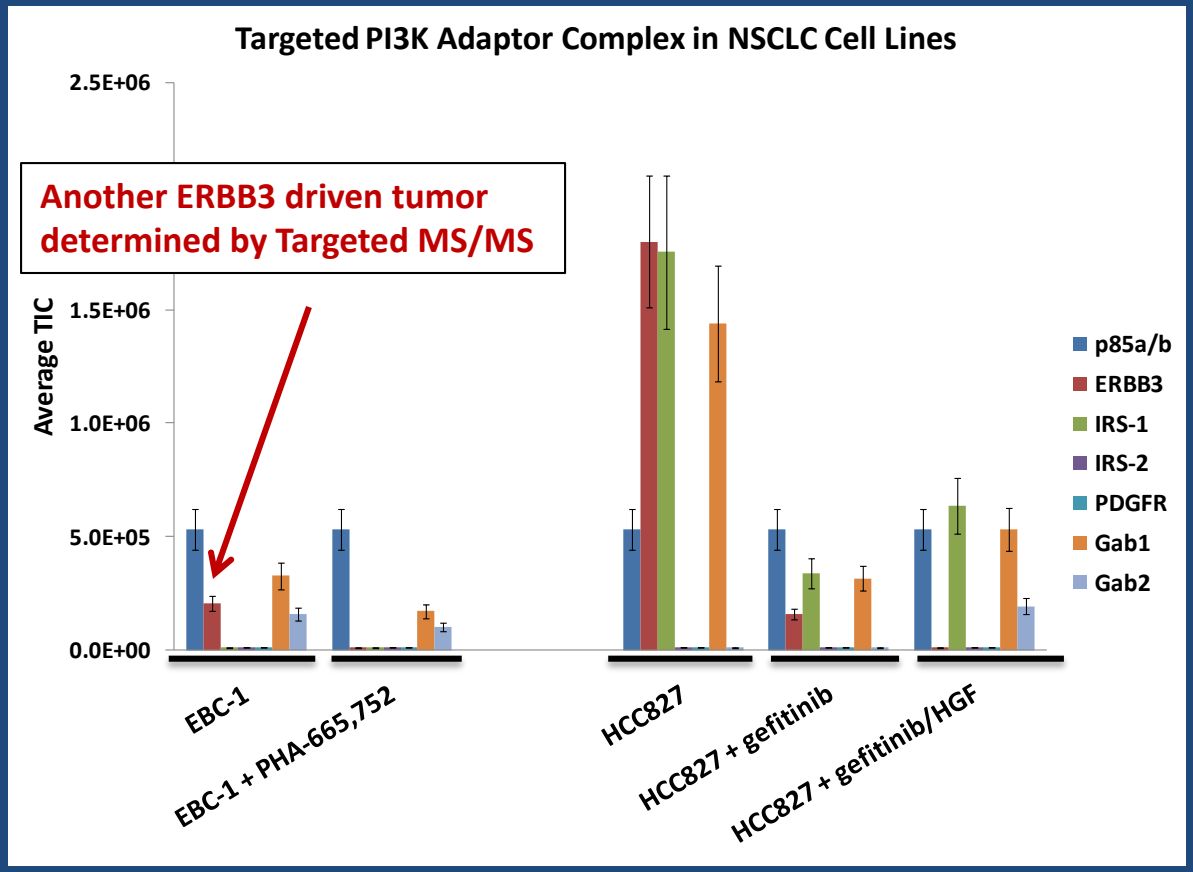


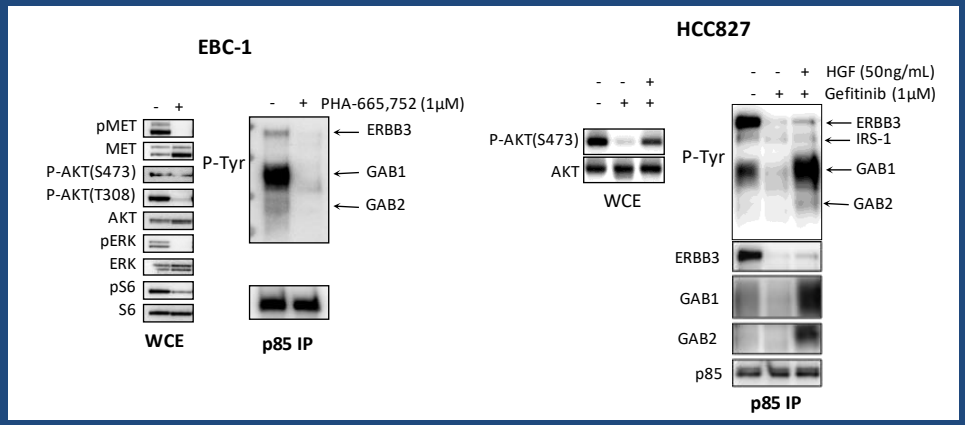


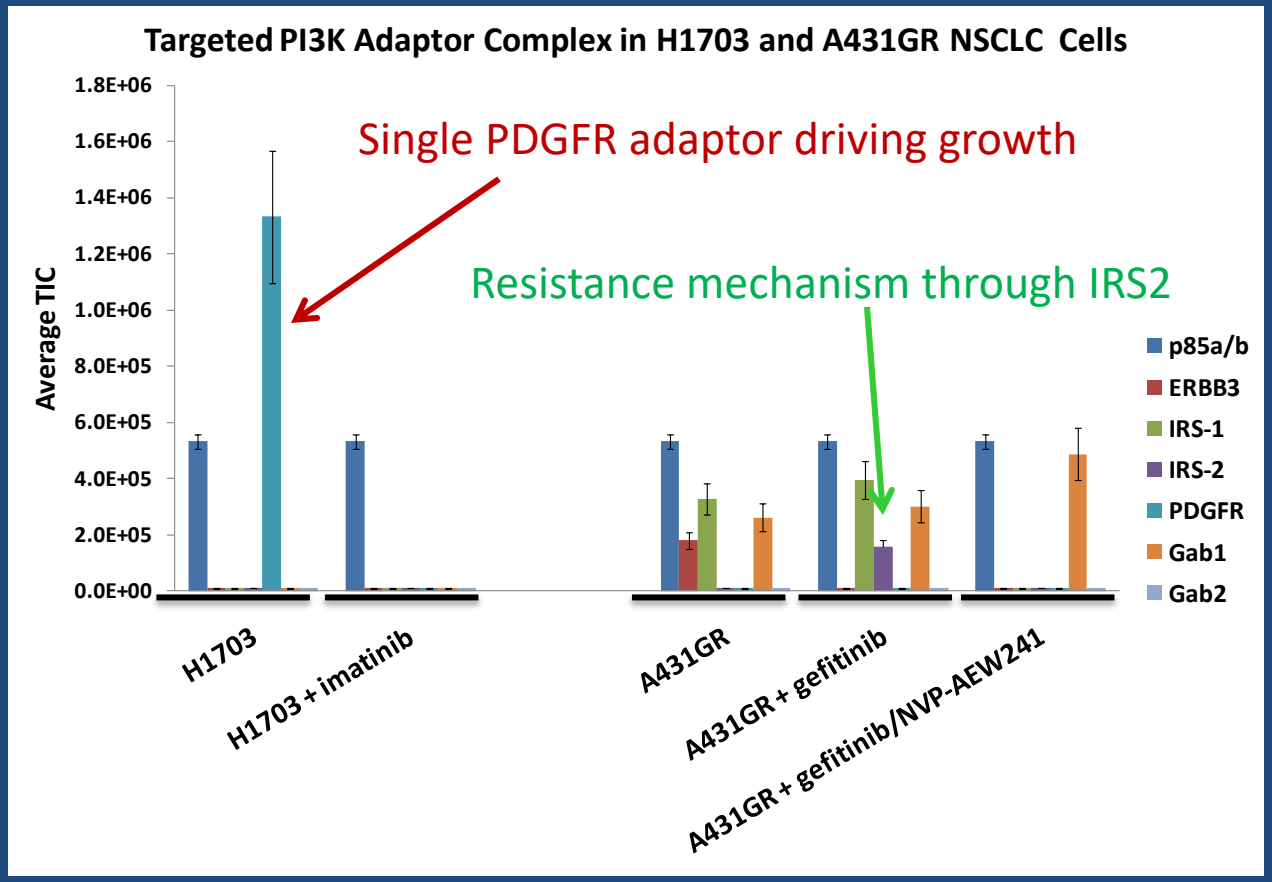

Western blot verification

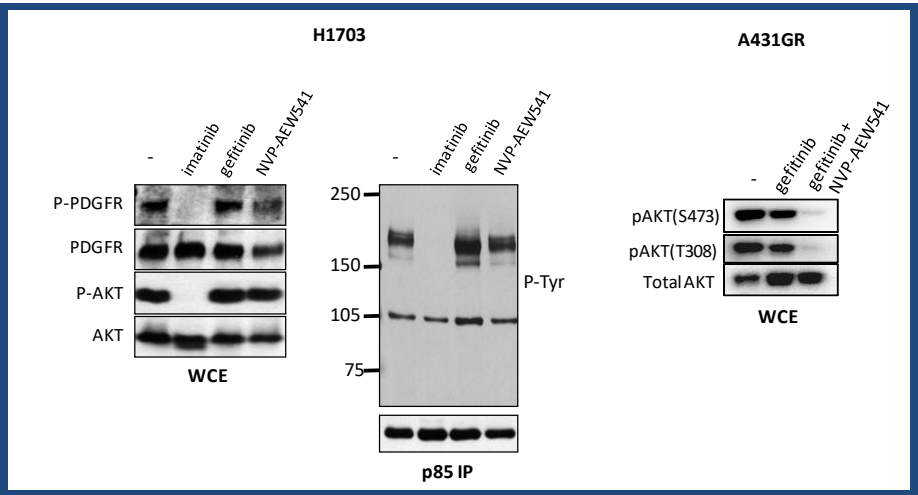


# The Activating Adaptors to PI3K/AKT in Lung Cancer Cell Lines

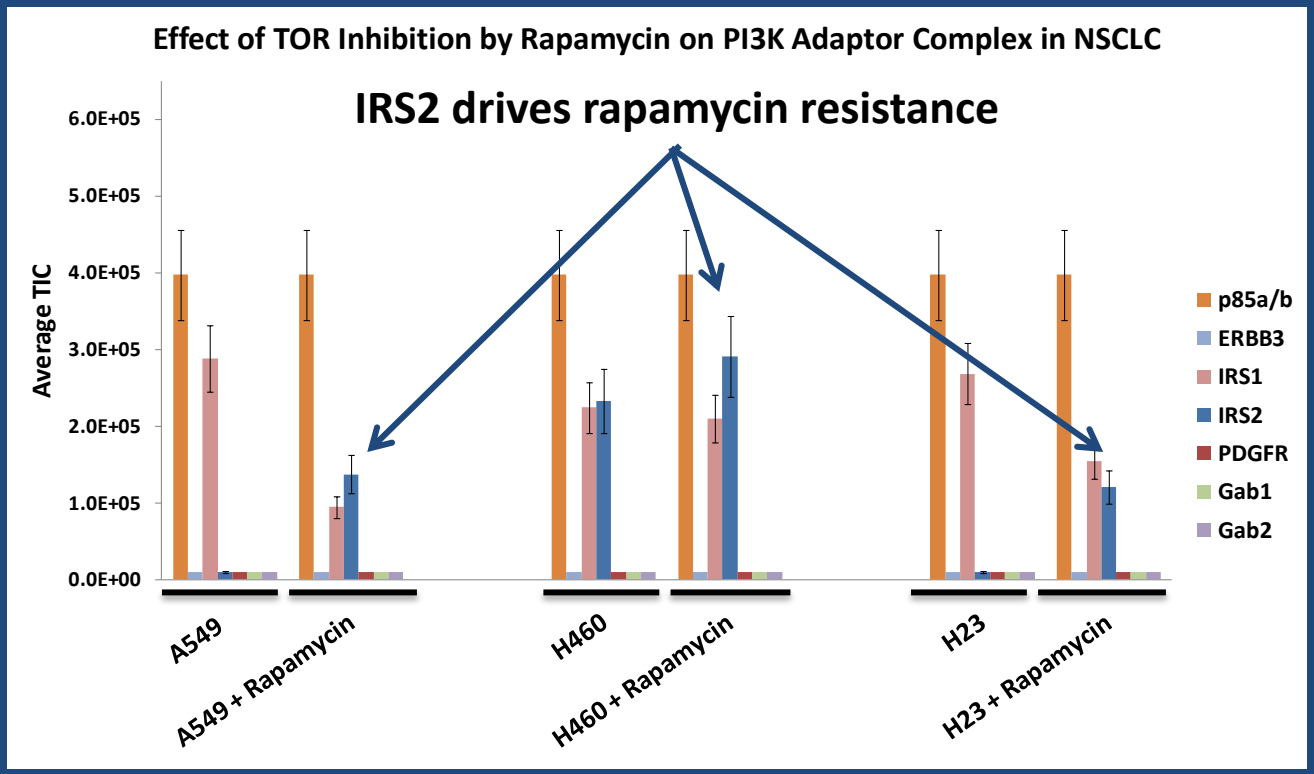


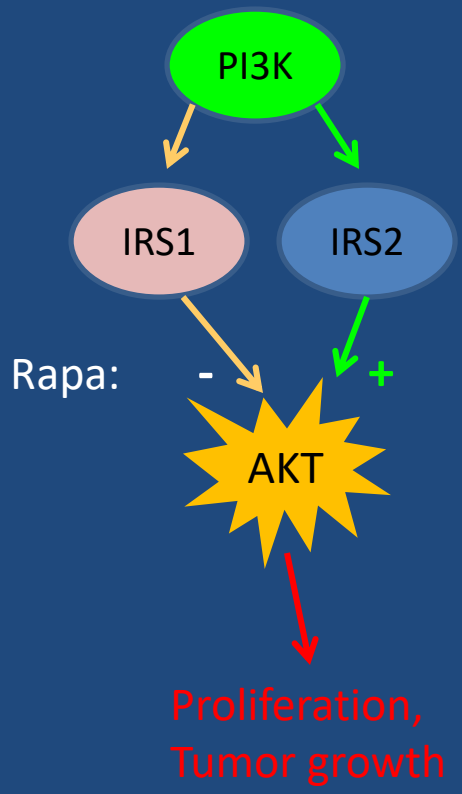




Targeted PI3K Adaptor Complex in H1703 and A431GR NSCLC Cells
Single PDGFR adaptor driving growth
Resistance mechanism through IRS2
Average TIC
1.8E+06
1.6E+06
1.4E+06
1.2E+06
1.0E+06
8.0E+05
6.0E+05
4.0E+05
2.0E+05
0.0E+00
p85a/b
ERBB3
IRS-1
IRS-2
PDGFR
Gab1
Gab2
H1703
H1703 + imatinib
A431GR
A431GR + gefitinib
A431GR + gefitinib/NVP-AEW241

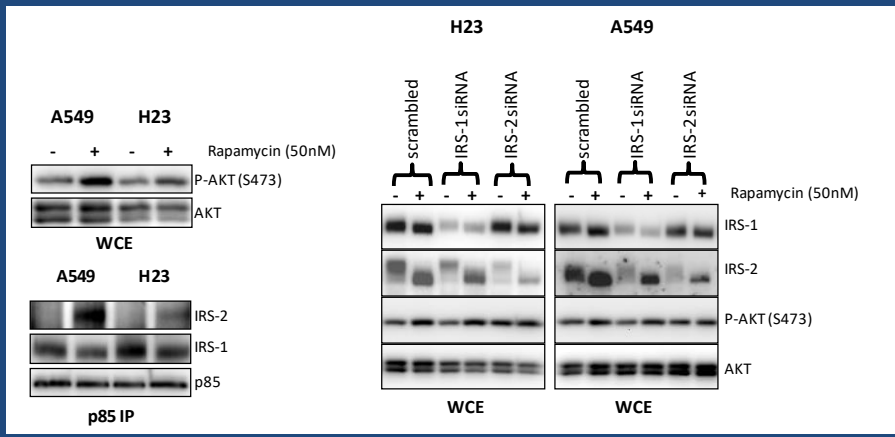

H1703
A431GR
-
imatinib
gefitinib
NVP-AEW541
P-PDGFR
PDGFR
P-AKT
AKT
WCE
250
150
105
75
P-Tyr
p85 IP
gefitinib
gefitinib +
pAKT(S473)
pAKT(T308)
Total AKT

# Discovering drug resistance mechanisms…



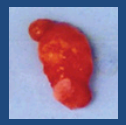

- Rapamycin (TOR inhibitor) activates AKT at long exposures
- Targeted IP-MS discovers a PI3K switch from IRS1 to IRS2



# PI3K Proteomic Assay Now Working with In Vivo Tumor Tissue

## Mouse Tumor

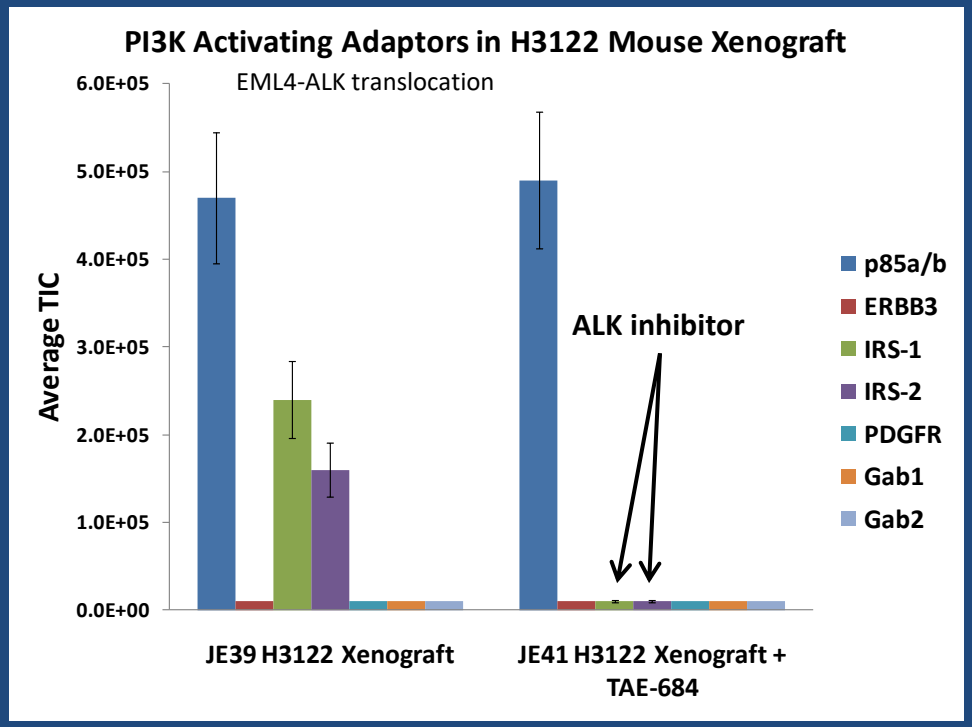

extraction



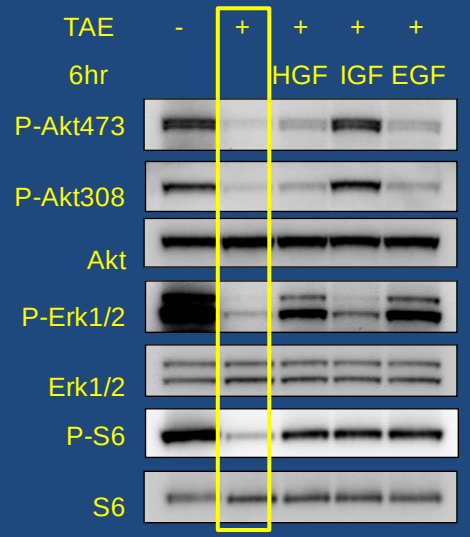
p85 IP

targeted LC/MS/MS

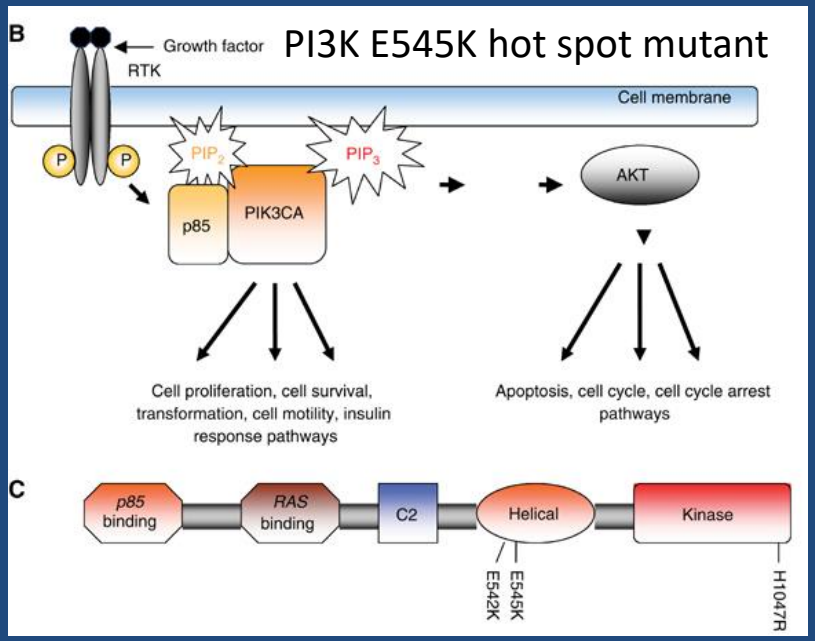
**Colorectal Cancer**
**Chronic Lymphocytic Leukemia**
**Breast Cancer**

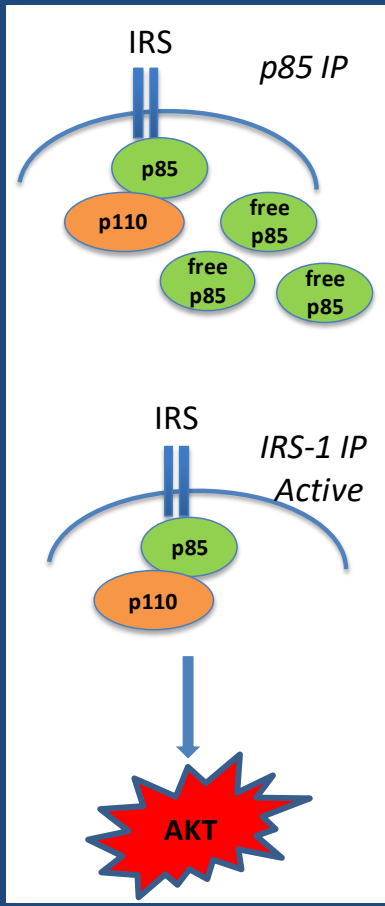


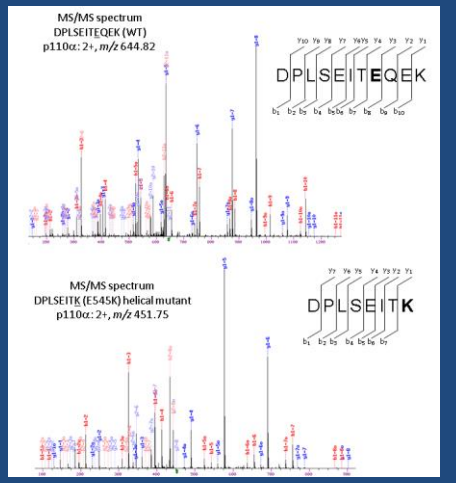

## Biochemical Validation of Mass Spec Assay

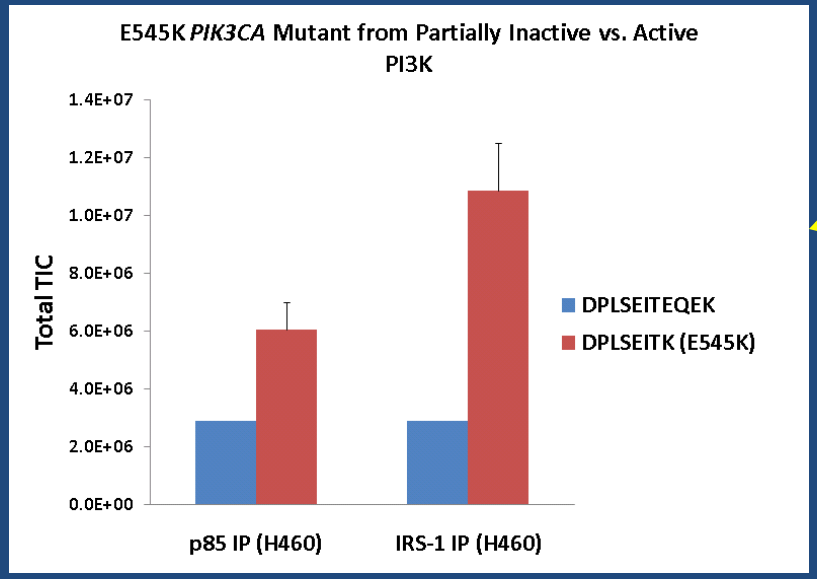


# Quantifying a Mutation's Role in Proliferation (PI3K Activation)



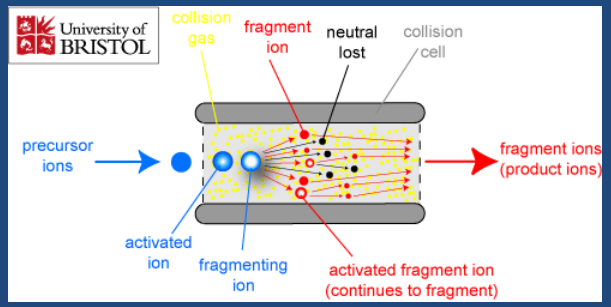



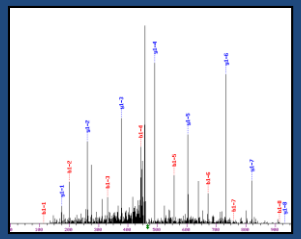

**Prove the mutation plays a direct role in activation**

# 5500 QTRAP has allowed us to expand our targeted complex list to accommodate both **direct** and *secondary* binding proteins

**Orbitrap XL** (Ion Trap CID)

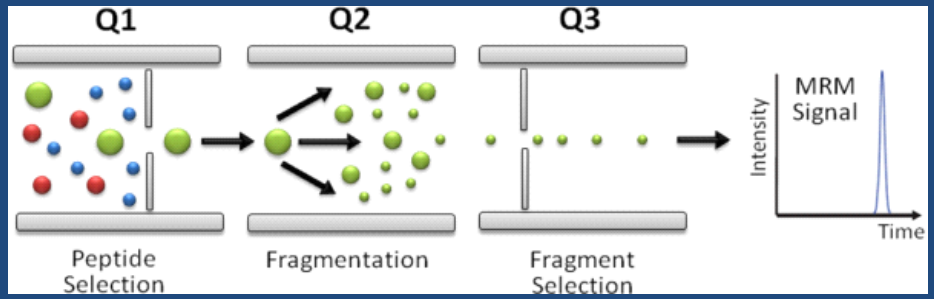
8 total proteins in p85 complex represented by 2 peptides
(16 CID scans per cycle) ~2.4 sec



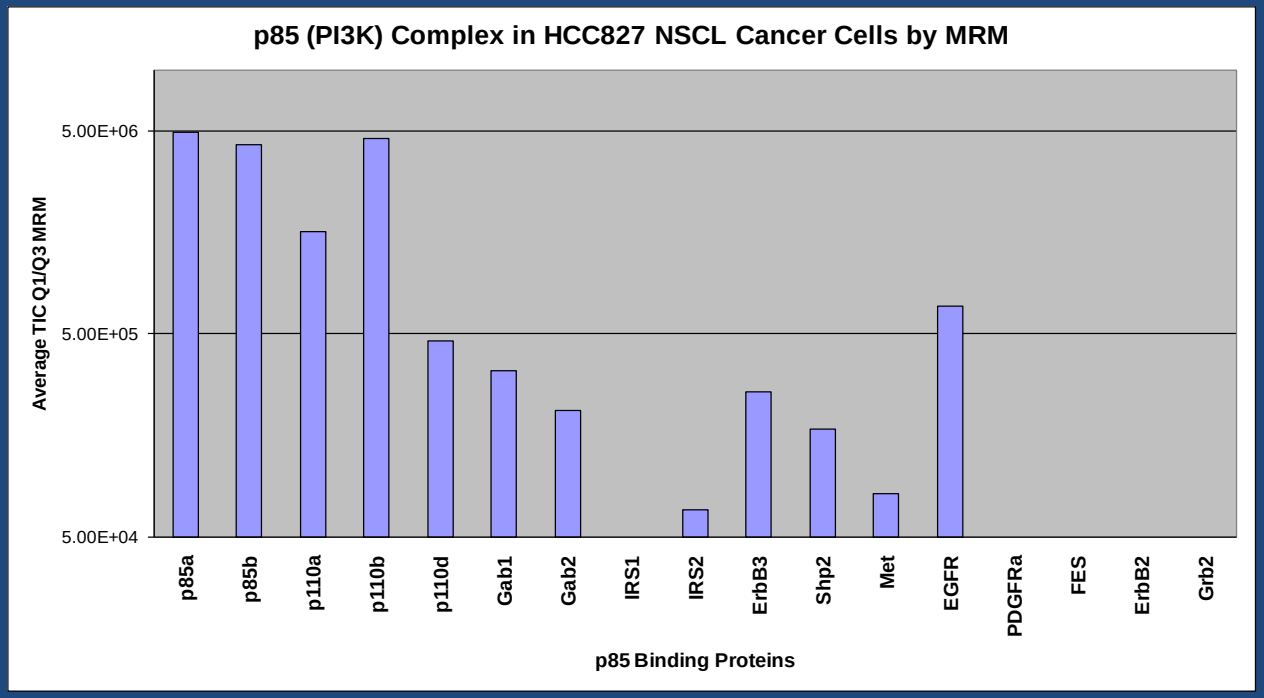



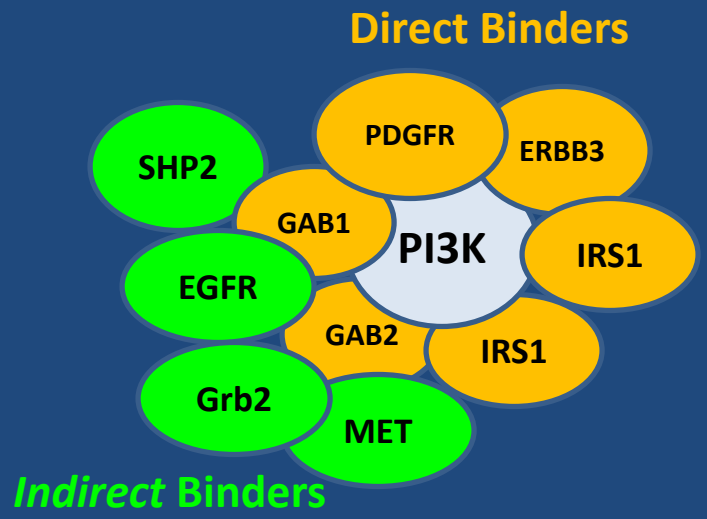
**5500 QTRAP** (MRM + CID)

16 total proteins in p85 complex represented by 3 peptides and 3 transitions per peptide
(144 Q1/Q3 scans and 48 CID scans per cycle**)** ~ 1.8 sec



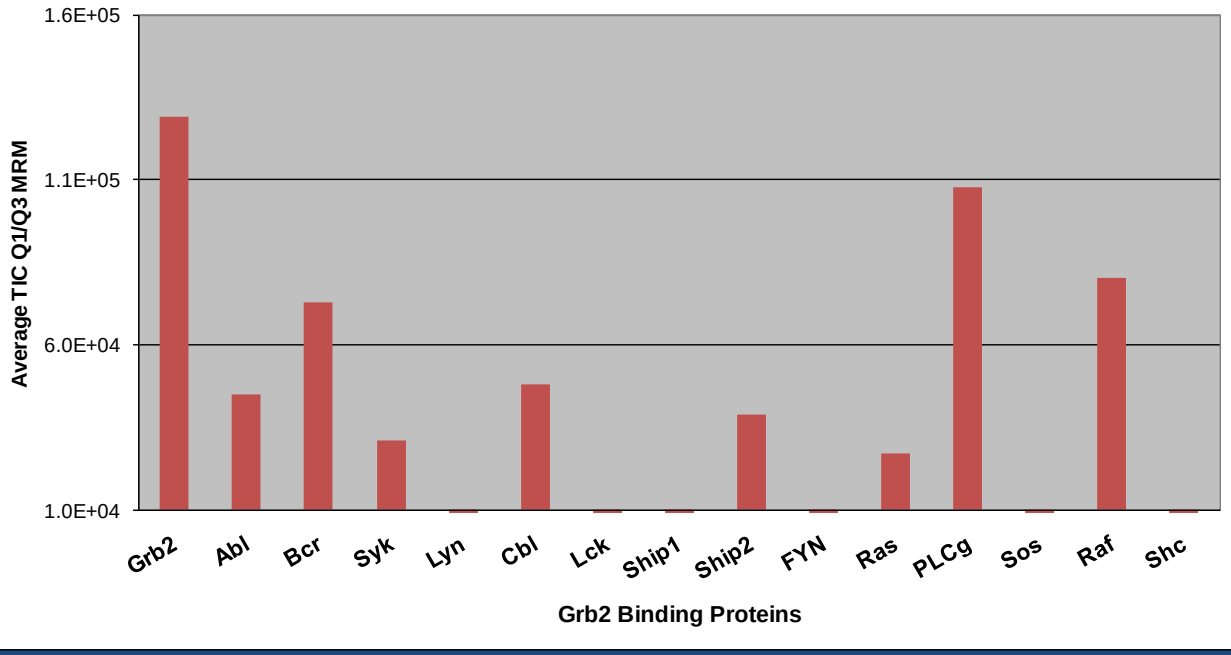
# The ability to target secondary (indirect ) protein-protein interactions allows us to dig deeper into functional mechanisms with more MRM targets

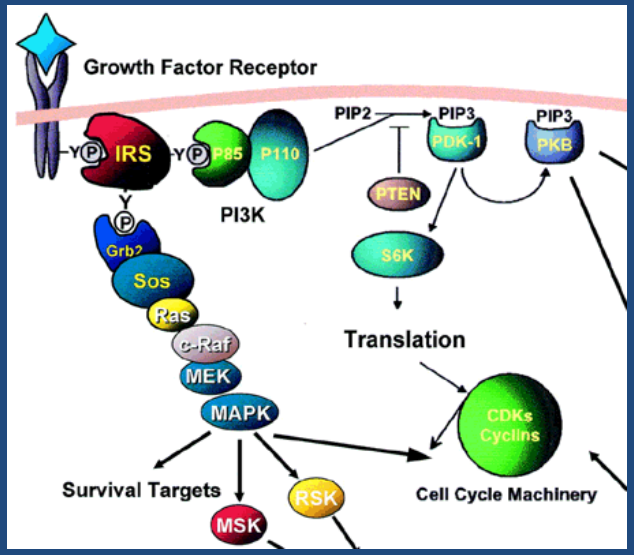


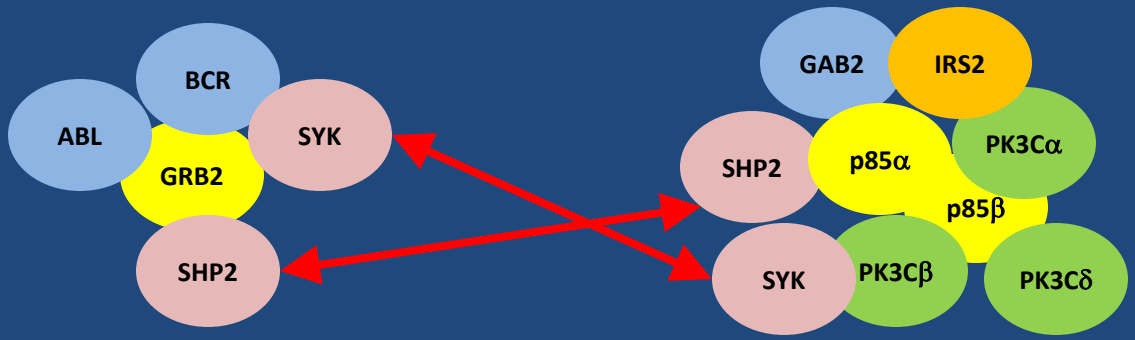
# Grb2 IP for MAPK Signaling

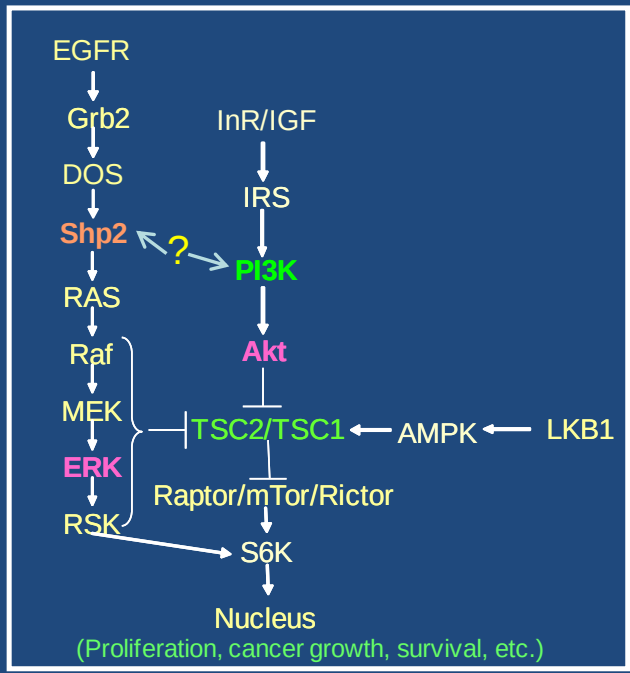
## Grb2 Targeted Protein Complex via MRM in Cancer Cells




## Cross-Talk of Signaling Pathways by IP-MS



# How do we choose the appropriate kinase inhibitor therapies ?

## FDA Approved Tyrosine Kinase Inhibitors

| Drug | Key targets for therapeutic activity | US FDA-approved indication |
|---|---|---|
| Imatinib | BCR–ABL, PDGFR and KIT | CML and GIST |
| Dasatinib | BCR–ABL | CML |
| Nilotinib | BCR–ABL | CML |
| Gefitinib | EGFR | Lung cancer |
| Erlotinib | EGFR | Lung and pancreatic cancers |
| Lapatinib | EGFR and ERBB2 | Breast cancer |
| Sunitinib | VEGFR2, PDGFR and KIT | Kidney cancer and GIST |
| Sorafenib | VEGFR2 and PDGFR | Kidney and liver cancers |
| Pazopanib | VEGFR2, PDGFR and KIT | Kidney cancer |
| Everolimus | mTOR | Kidney cancer |
| *Antibody* | | |
| Trastuzumab | ERBB2 | Breast cancer |
| Cetuximab | EGFR | Colorectal, and head and neck cancers |
| Panitumumab | EGFR | Colorectal cancer |
| Bevacizumab | VEGF | Colorectal, lung and breast cancers |

CML, chronic myeloid leukaemia; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; GIST, gastrointestinal stromal tumour; PDGFR, platelet-derived growth factor receptor; VEGFR2, vascular endothelial growth factor receptor 2.

## Lots More Kinase Inhibitors in Clinical Trials

| Inhibitor | Company | Phase of clinical trial | Refs |
|---|---|---|---|
| *Dual PI3K and mTOR inhibitors* | | | |
| BEZ235 | Novartis | Phase I/II | 37,92,96,103,149 |
| BGT226 | Novartis | Phase I/II | NS |
| XL765 | Exelixis | Phase I | NS |
| SF1126 | Semafore | Phase I/II | NS |
| GSK1059615 | GSK | Preclinical | 150 |
| *PI3K inhibitors* | | | |
| XL147 | Exelixis | Phase I | NS |
| PX866 | Oncothyreon | Phase I | 100,151,152 |
| GDC0941 | Genentech/Piramed/Roche | Phase I | NS |
| BKM120 | Novartis | Phase I | NS |
| CAL101 (targets p110δ) | Calistoga Pharmaceuticals | Phase I | NS |
| *Akt inhibitors* | | | |
| Perifosine | Keryx | Phase I/II | 153–156 |
| GSK690693 | GSK | Phase I | 157,158 |
| VQD002 | Vioquest | Phase I | NS |
| MK2206 | Merck | Phase I | NS |
| *mTOR inhibitors (catalytic site)* | | | |
| OSI027 | OSI Pharmaceuticals | Phase I | NS |
| AZD8055 | AstraZeneca | Phase I/II | NS |

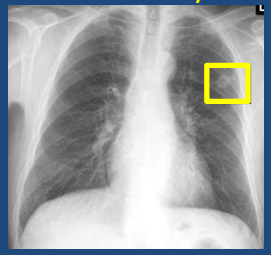
NS, not stated.

Nature Reviews Cancer 9, 550-562 (2009)

# Hypothetical 'Personalized Treatment Plan' for Cancer Based on PI3K Mass Spec Assay…

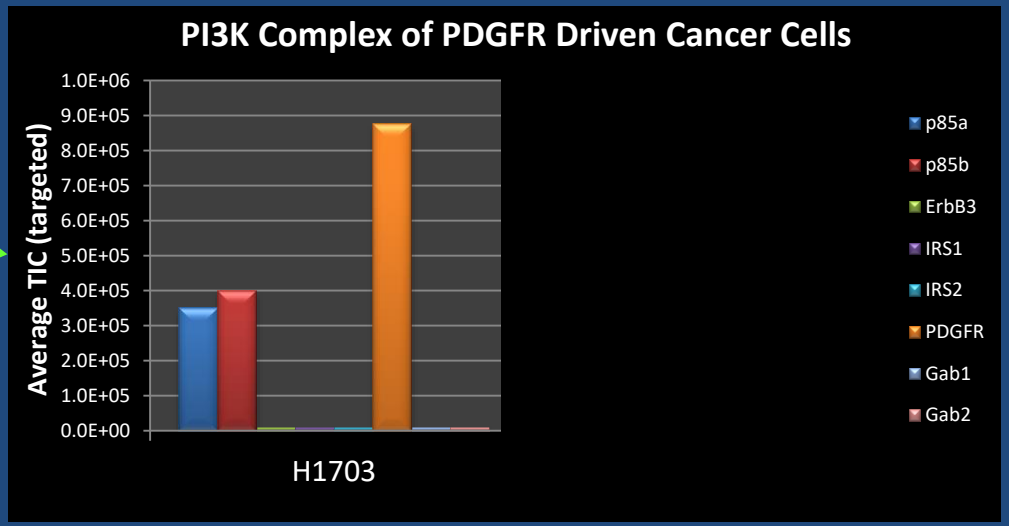
Shapiro Clinical Center

Chest X-ray



Excise/biopsy

Targeted Mass Spec

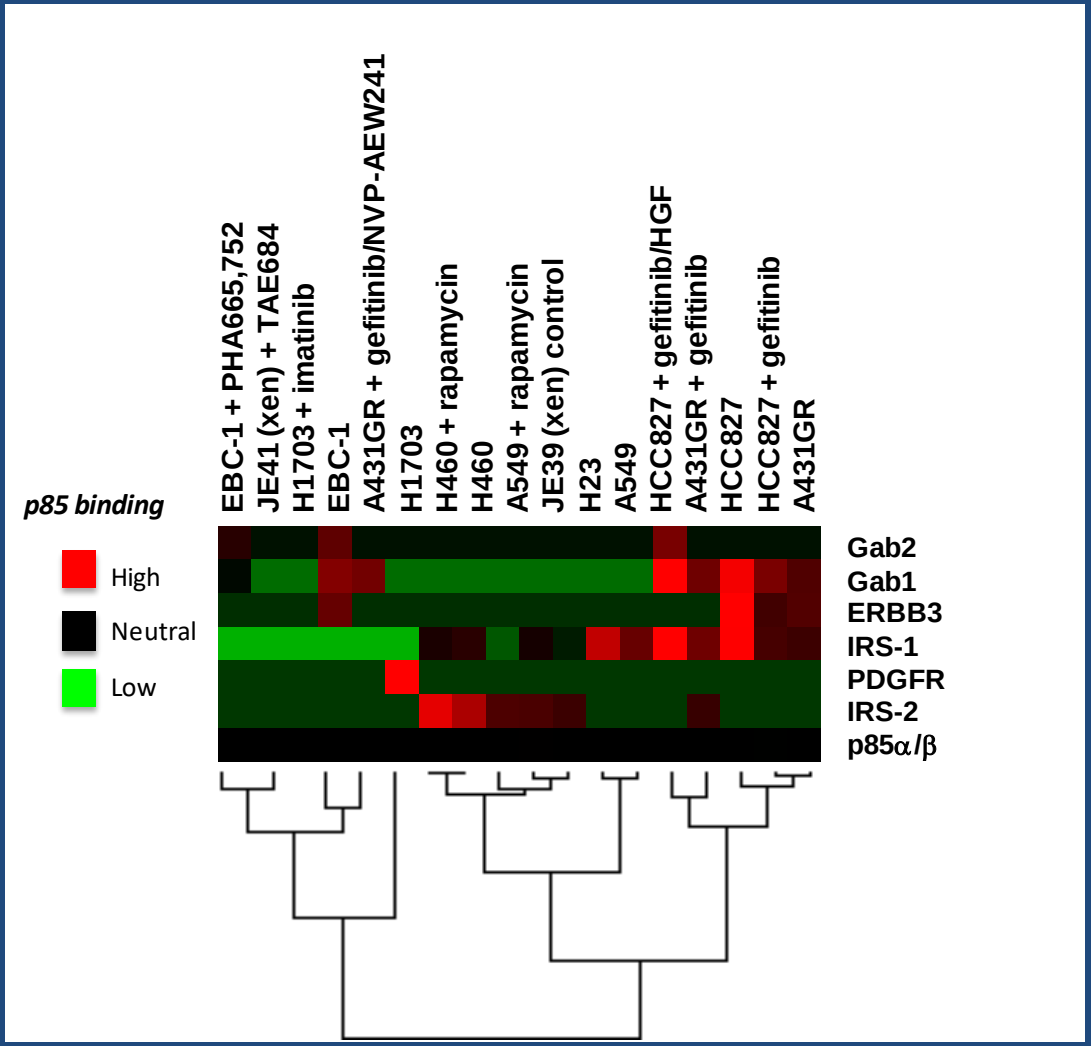



Treatment decision

**Treat with PDGFR inhibitor (Imatinib/Gleevec)**

Chest X-ray

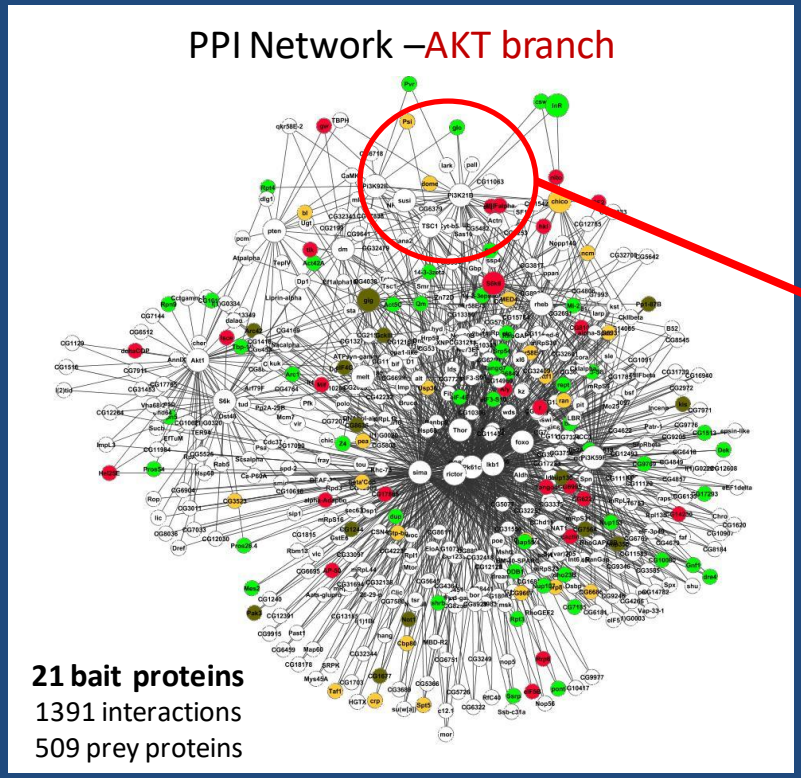

# Unsupervised Hierarchical Heat Map for p85 Complex across Cell Lines and Xenograft Tumors

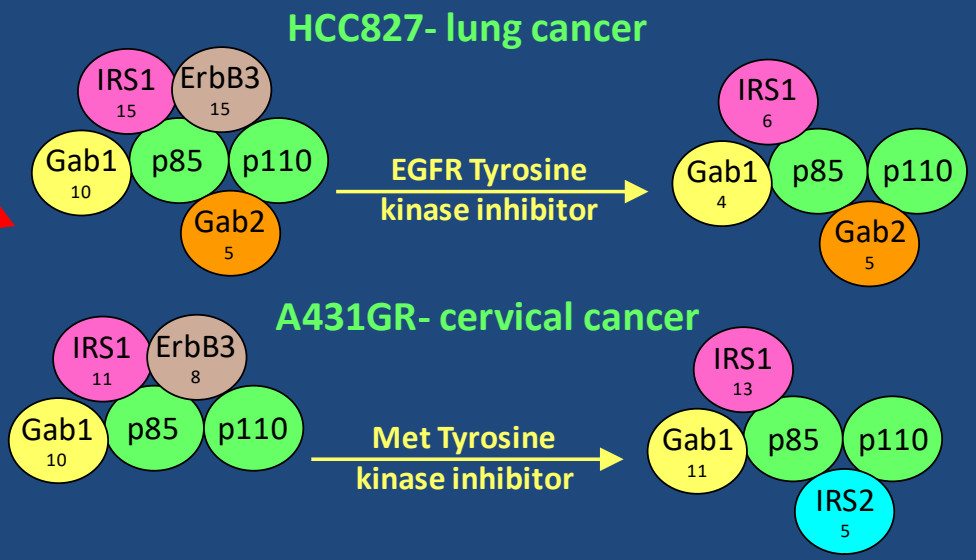



**This can ultimately be used as a reference to make therapeutic decisions in a cancer according to the PI3K signature**

# Summary

•Cancer cells typically involve a small set of oncogene addictions that govern their uncontrolled proliferation




•We can use IPs and targeted MS to predict response to drug therapies for a particular cancer through quantitative protein-protein interactions (PPI)

•Multiple MS technologies can be used (orbitrap, ion trap, QqQ, qExactive, etc.)

# Acknowledgements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Vanderbilt University

Todd Miller
Brent Rexer
Carlos Arteaga

Dana Farber Cancer Institute

Pasi Janne

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