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oncology therapeutics

# Targeting RAS-Driven PI3Kα Activation in Human Tumors

**Pedro J. Beltran, Ph.D.**

**Chief Scientific Officer**

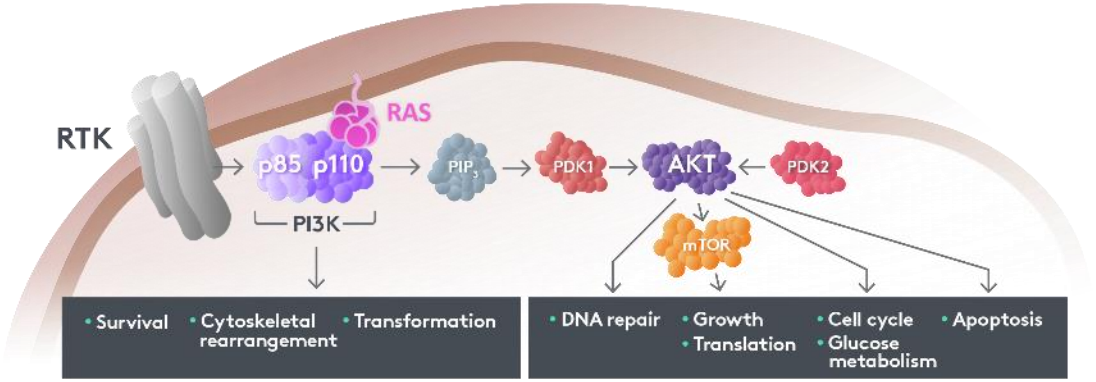
**September 2023**

RAS INITIATIVE

Lawrence Livermore National Laboratory

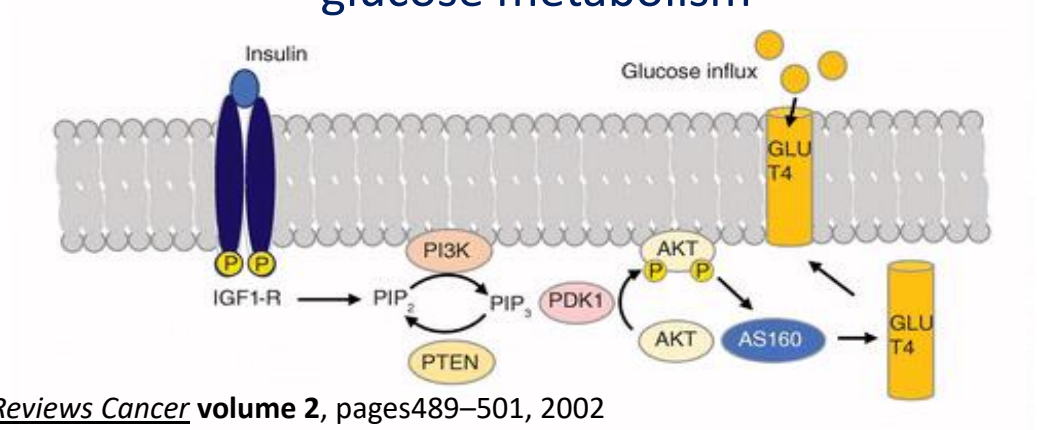

# PI3Kα is a key regulator of proliferation, survival and glucose metabolism

## **PI3Kα** is a key central node for growth and survival signaling



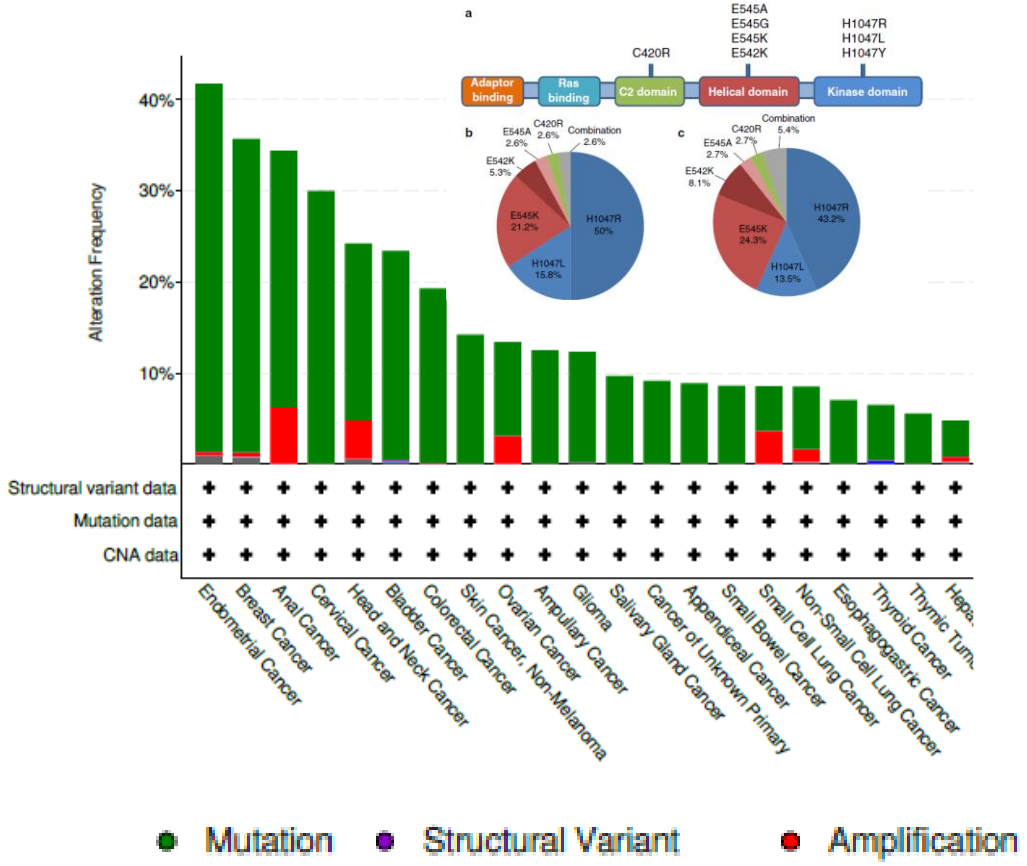

Adapted with permission from Vivanco & Sawyers, 2002.

## **PI3Kα** is also the main player in the control of glucose metabolism




*Nature Reviews Cancer* **volume 2**, pages489–501, 2002

## **PI3Kα** mutations are found at high prevalence in important indications

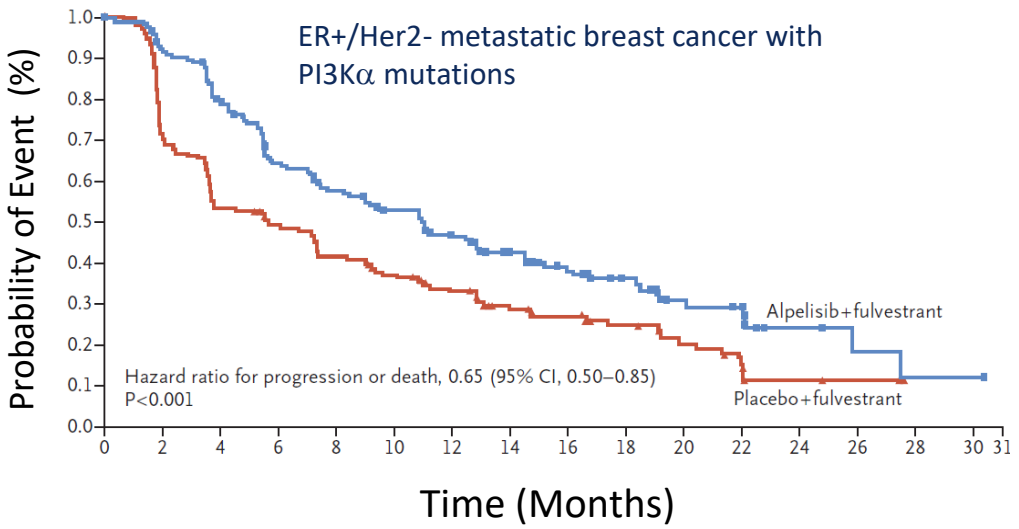



https://www.cbioportal.org/

# PI3Kα has been clinically validated as a target in human tumors but it's full potential has not been realized because of safety

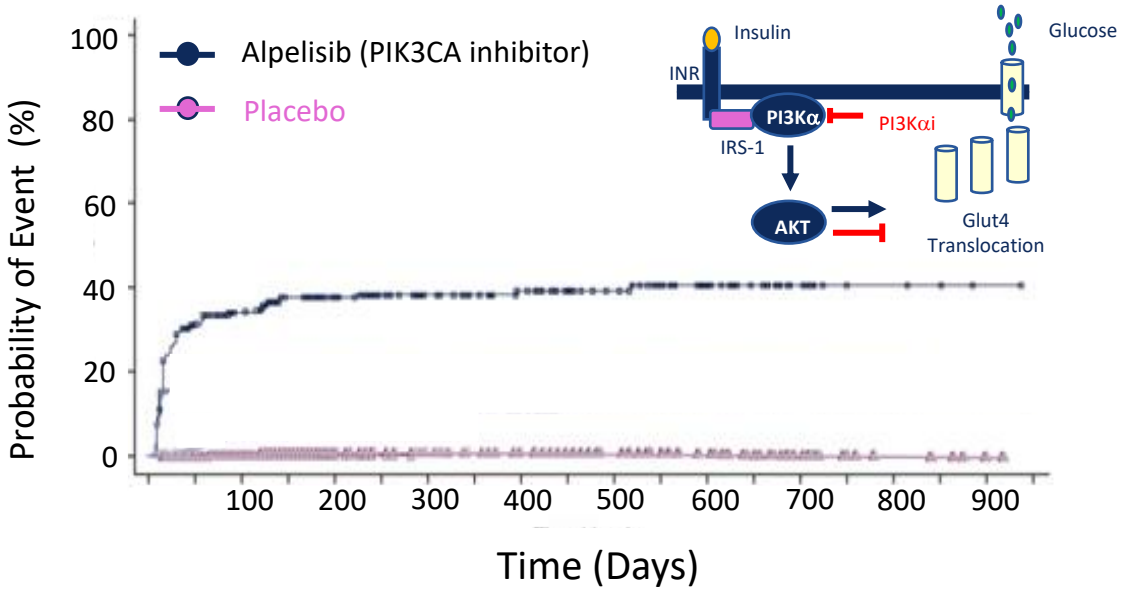
**Alpelisib is a PI3Kα "kinase" selective inhibitor approved for ER+ Breast cancer in combination with fulvestrant**

## Efficacy (PFS)




| | PFS (mo) | ORR (%) |
|---|---|---|
| Fulvestrant | 5.7 | 12.8 |
| Alpelisib + Fulvestrant | 11 | 26.6 |

## Safety



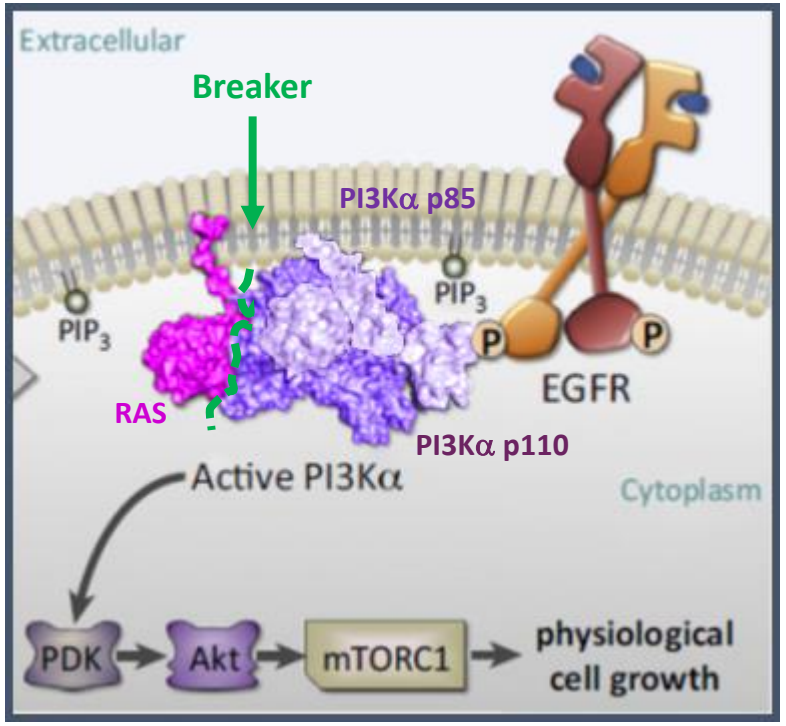

| | Hyperglycemia | Diarrhea | Rash |
|---|---|---|---|
| Fulvestrand | 9.8 | 15.7 | 6 |
| Alpelisib + Fulvestrand | 64 | 57.7 | 35.6 |

- Dose interruptions occurred in 66% versus 21% in placebo
- Dose reductions occurred in 55% versus 4.5% in placebo

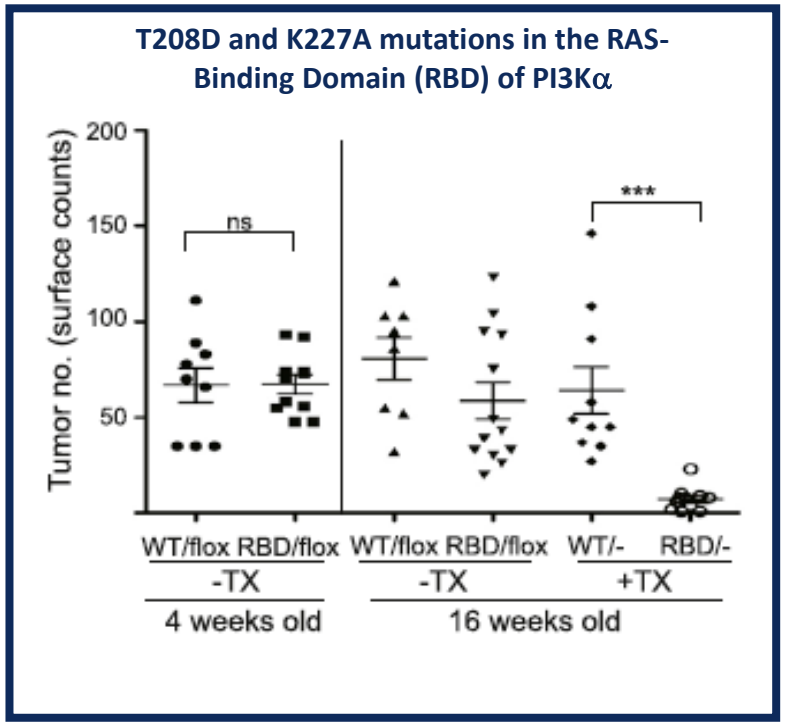
https://doi.org/10.1016/j.annonc.2020.05.001

# A novel approach is needed to inhibit PI3Kα activity in human tumors

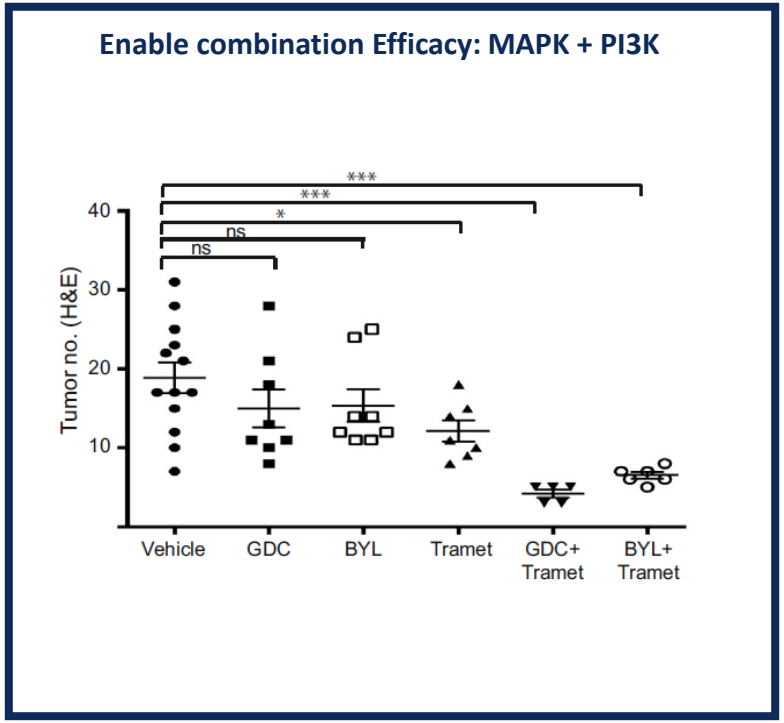
## Breaker: Inhibiting the physical interaction between PI3Kα and RAS




PI3Kα can be activated by RAS at the plasma membrane




Genetic disruption of the interaction results in efficacy




Combination of MAPK and PI3Kα inhibition drives strong efficacy……but also toxicity

**Requirement for Interaction of PI3-Kinase p110α with RAS in Lung Tumor Maintenance**

Esther Castellano,[1,7] Clare Sheridan,[1,7] May Zaw Thin,[2] Emma Nye,[3] Bradley Spencer-Dene,[3] Markus E. Diefenbacher,[4] Christopher Moore,[1] Madhu S. Kumar,[1] Miguel M. Murillo,[1,6] Eva Grönroos,[2] Francois Lassailly,[2] Gordon Stamp,[3] and Julian Downward[1,6,*]

**NUSSINOV ET. AL. TRENDS IN CANCER, REVIEW**| VOLUME 3, ISSUE 3, P214-224, MARCH 2017

# Targeting the physical interaction between RAS and PI3Kα opens a new therapeutic avenue

R.Baserga

J.Downward

R. Williams

**The interaction between RAS and PI3Kα presents an opportunity for novel drug development efforts to target mutant RAS**

**1994** **1996** **2000** **2007** **2013**

**Phosphatidylinositol-3-OH kinase as a direct target of Ras**

Pablo Rodriguez-Viciana*, Patricia H. Warne*, Ritu Dhand†, Bart Vanhaesebroeck†, Ivan Gout†, Michael J. Fry†, Michael D. Waterfield†‡ & Julian Downward*§

The EMBO Journal vol.15 no.10 pp.2442–2451, 1996

Cell, Vol. 103, 931–943, December 8, 2000, Copyright ©2000 by Cell Press

Crystal Structure and Functional Analysis of Ras Binding to Its Effector Phosphoinositide 3-Kinase γ

Requirement for Interaction of PI3-Kinase p110α with RAS in Lung Tumor Maintenance

Esther Castellano,[1,7] Clare Sheridan,[1,7] May Zaw Thin,[2] Emma Nye,[3] Bradley Spencer-Dene,[3] Markus E. Diefenbacher,[4] Christopher Moore,[1] Madhu S. Kumar,[1] Miguel M. Murillo,[1,6] Eva Grönroos,[5] Francois Lassailly,[2] Gordon Stamp,[3] and Julian Downward[1,6,*]

**Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation**

Binding of Ras to Phosphoinositide 3-Kinase p110α Is Required for Ras-Driven Tumorigenesis in Mice

Surbhi Gupta,[1,4] Antoine R. Ramjaun,[1,4] Paula Haiko,[3] Yihua Wang,[1] Patricia H. Warne,[1] Barbara Nicke,[1] Emma Nye,[2] Gordon Stamp,[2] Kari Alitalo,[3] and Julian Downward[1,*]

Effect of a Null Mutation of the Insulin-Like Growth Factor I Receptor Gene on Growth and Transformation of Mouse Embryo Fibroblasts

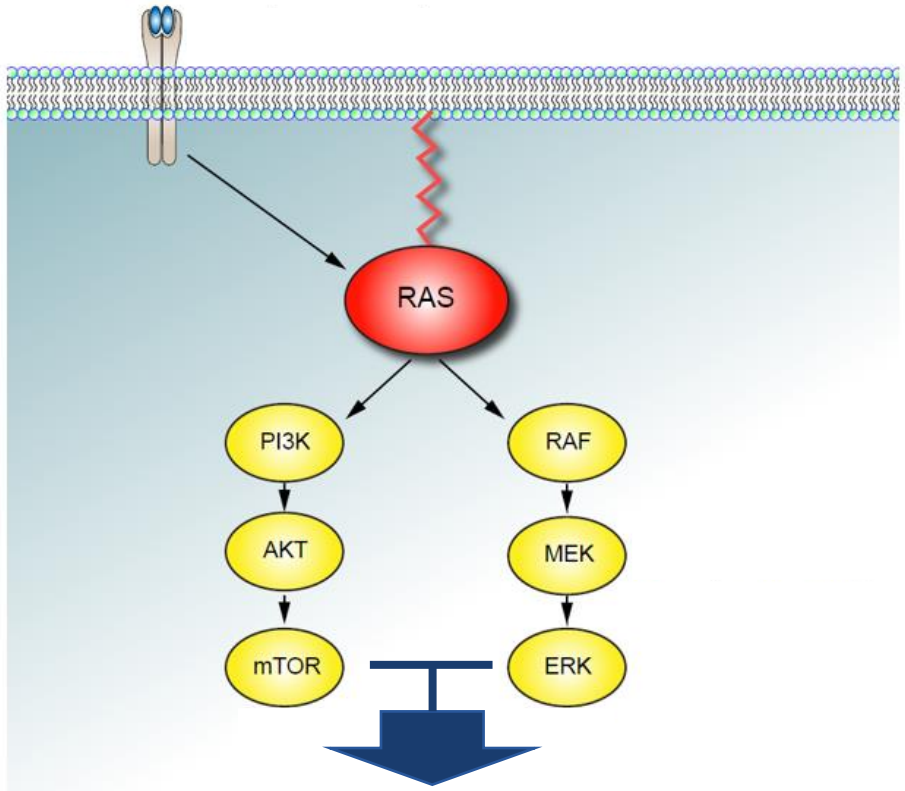
CHRISTIAN SELL,[1] GUILLAUME DUMENIL,[1] CATHERINE DEVEAUD,[1] MASAHIKO MIURA,[1] DOMENICO COPPOLA,[1] TIZIANA DEANGELIS,[1] RAPHAEL RUBIN,[1] ARGIRIS EFSTRATIADIS,[2] AND RENATO BASERGA[1]*

**Structural insights into phosphoinositide 3-kinase catalysis and signalling**

Edward H. Walker*, Olga Perisic*, Christian Ried*, Len Stephens† & Roger L. Williams*

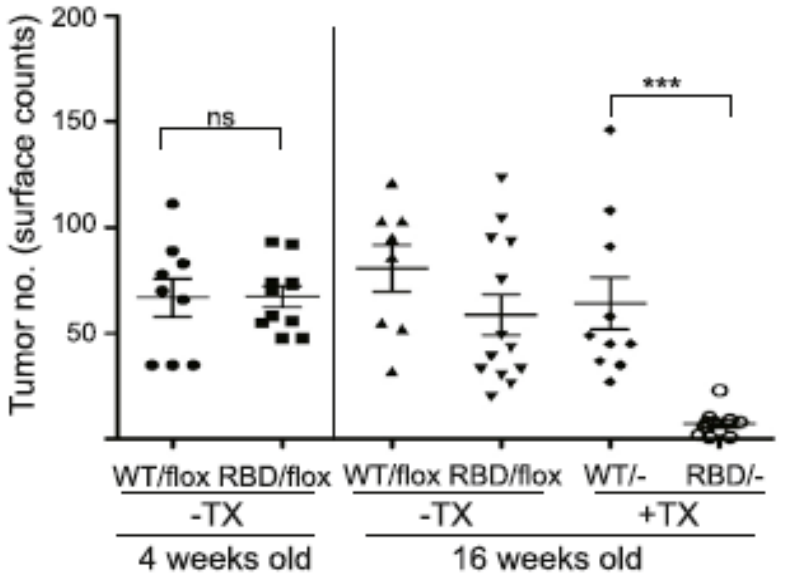
# Genetic data suggests that "breaking" RAS and PI3Kα should lead to efficacy (monotherapy and/or combination with KRASi)

**In malignant cells, RAS likely plays a pivotal role in coordinating the signal for both pathways**




**Tumor Growth & Survival**

**$KRAS^{G12D}$-driven tumor growth is inhibited in mice with T208D and K227A mutations in the RAS-Binding Domain (RBD) of PI3Kα**




**Efficacy and tolerability**

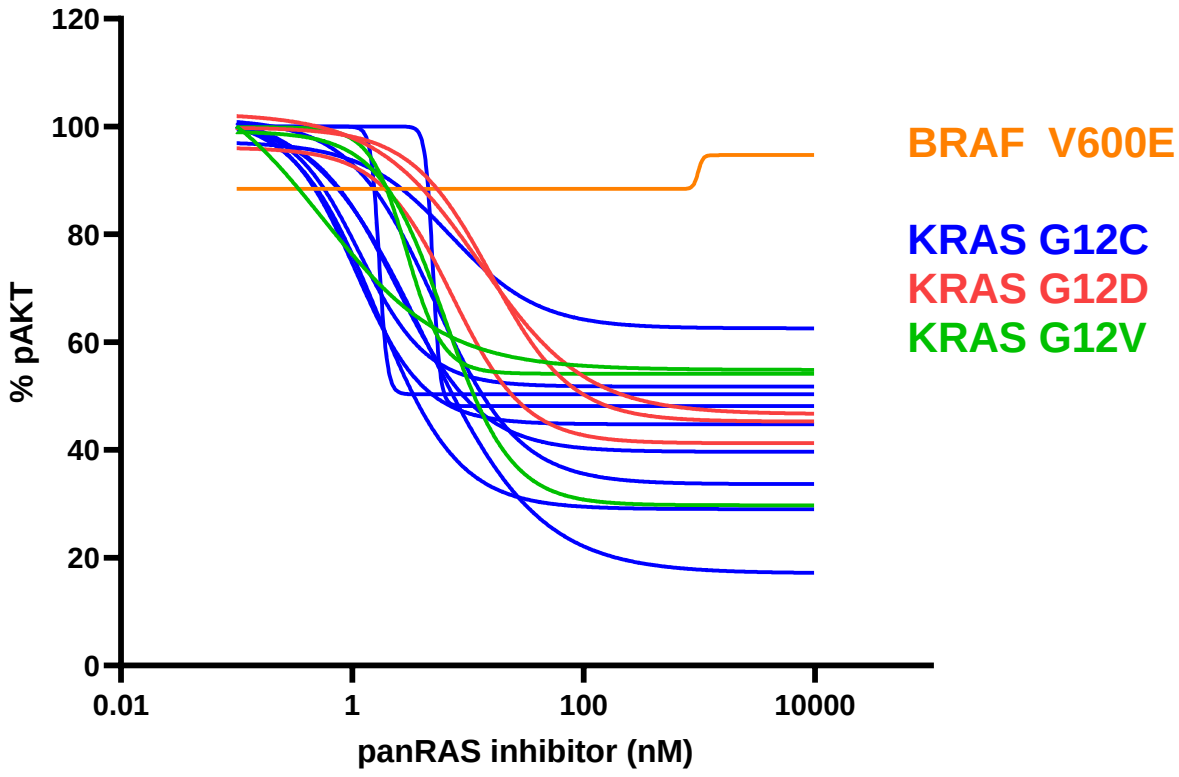


Modified from: National Cancer Institute/Marielle Yohe, M.D., Ph.D.

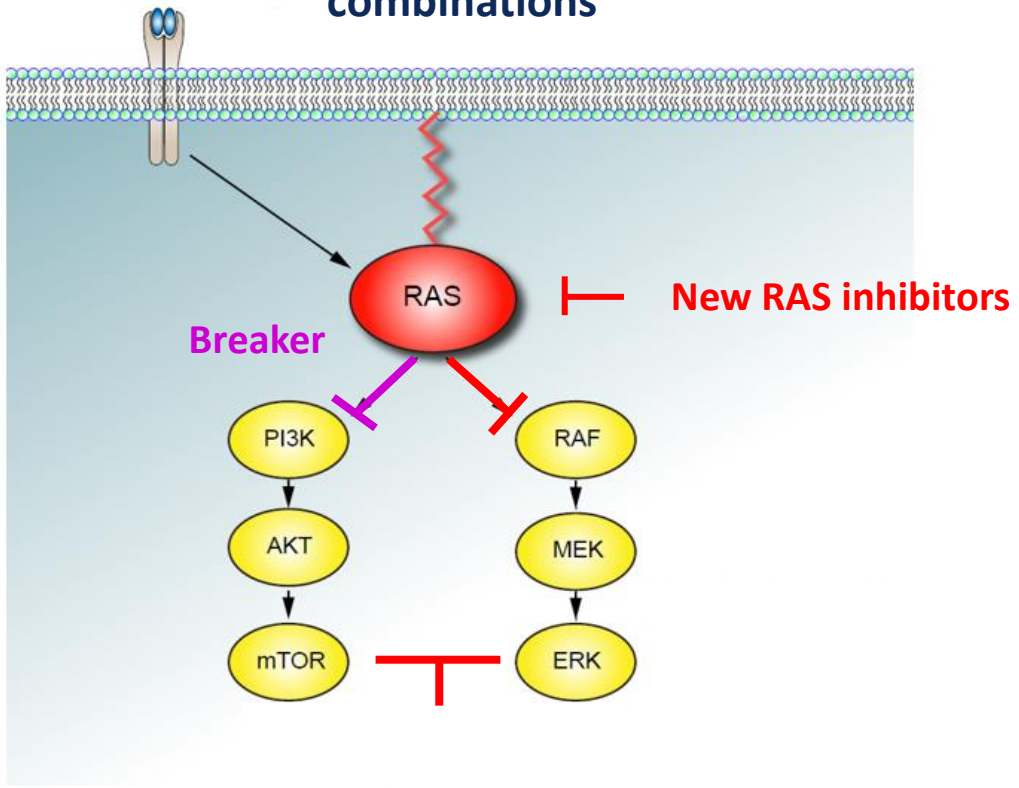
Cancer Cell 24, 617–630, November 11, 2013

# Can PI3Kα signaling be inhibited by disrupting its RAS interaction? Or would conventional (IGF1R/INR/IRS) signaling be able to overcome this approach?

## PanRAS inhibitor shows how much pAKT is driven by RAS




## A small molecule protein:protein inhibitor (PPI) should recapitulate this activity and allow for combinations




**No Tumor Growth ?**



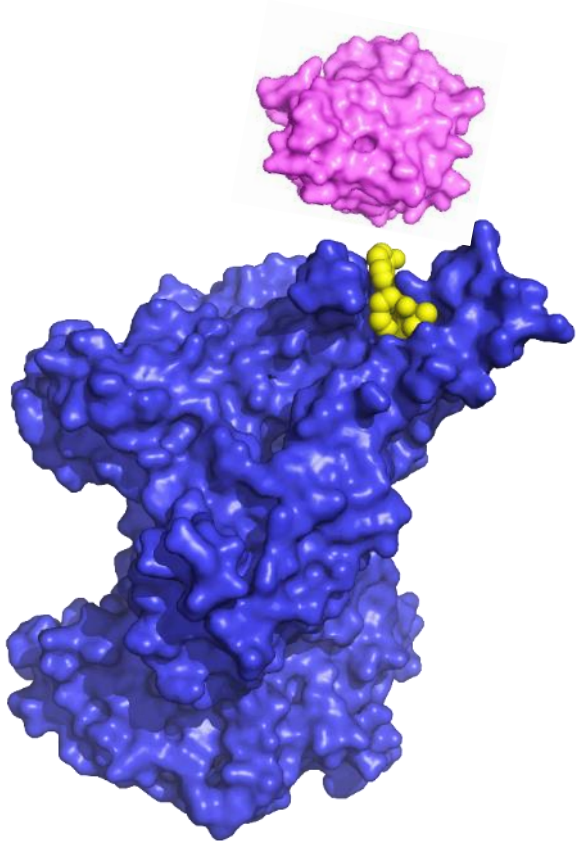
Modified from: National Cancer Institute/Marielle Yohe, M.D., Ph.D.

# BridgeBio has designed first-in-class, potent, and selective PI3Kα:RAS breakers

RAS

PI3Kα

Breaker



- Structural insights provide a novel approach to develop PI3Kα:RAS breakers
- Small molecules covalently bind to a new induced pocket in PI3Kα
- PI3Kα:RAS breakers selectively bind to PI3Kα
  - PI3Kα binding pocket is unique among isoforms
  - Breakers exhibit no binding affinity to KRAS
- PI3Kα:RAS breakers do not affect kinase activity

**Multiple series of potent PI3Kα:RAS covalent inhibitors have been identified**

# PI3Kα:RAS Breaker “must have” characteristics

| | Status |
|---|---|
| • Selective inhibition of the physical interaction between PI3Kα and Ras | ● |
| • Blockade of K-, H, and N-RAS isoforms | ● |
| • Dose-dependent target (PI3Kα) engagement in multiple cell types | ● |
| • Significant inhibition of RAS-driven pAKT signal | ● |
| • No pAKT inhibition in adipocytes and no hyperglycemia *in vivo* | ● |
| • PK/PD and efficacy relationship in human cancer models | ● |
| • Monotherapy and combination benefit with KRAS inhibitors | ● |

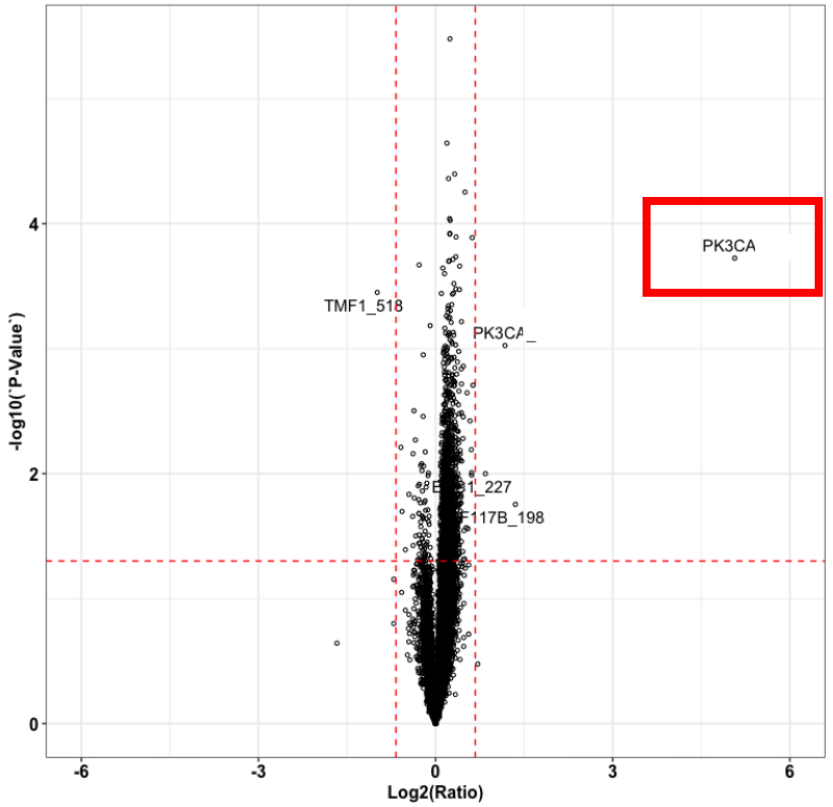
# Covalent binding mechanism drives cellular potency

## MALDI-TOF MS correlates with pAKT $IC_{50}$

| | Time | Cmpd1 | Cmpd2 | Cmpd3 | Cmpd4 |
|---|---|---|---|---|---|
| % Modified | 15’ | 0 | 28 | 89 | 100 |
| | 30’ | 0 | 45 | 98 | 100 |
| | 120’ | 6 | 91 | 100 | 100 |
| | 240’ | 11 | 97 | 100 | 100 |
| pAKT $IC_{50}$ (nM) | - | 650 | 130 | 14 | 1 |

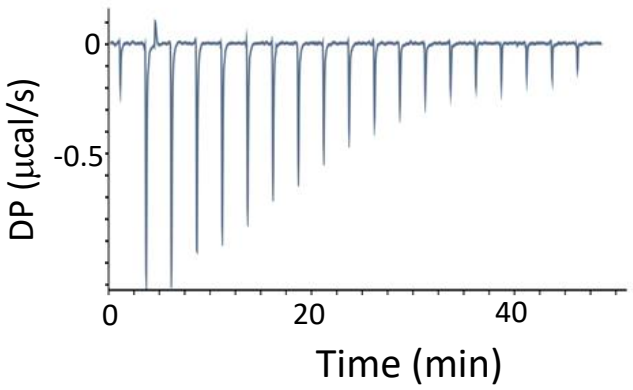
## Cysteine Proteome shows high selectivity for PI3Kα

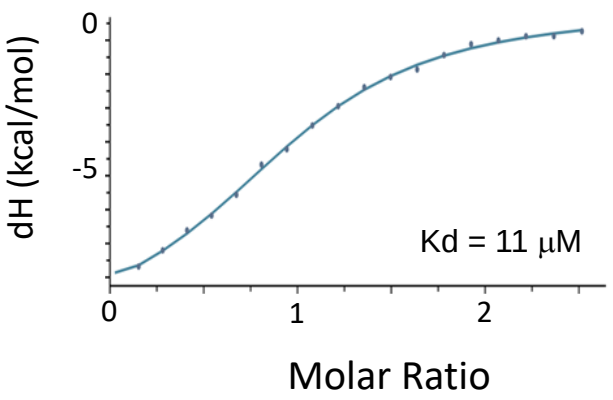
DMSO v Breaker



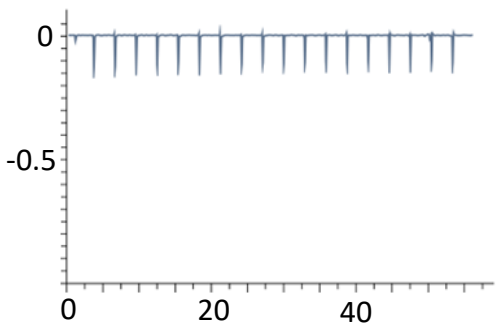
# Breakers effectively and completely block the PI3Kα:RAS (K/H/N) interaction

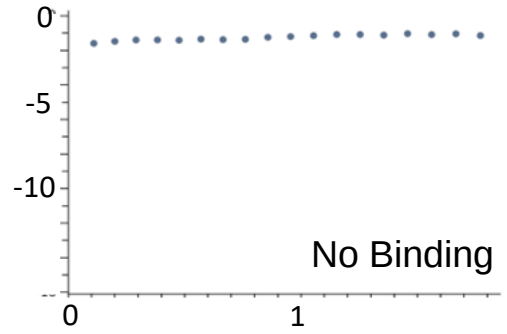
KRAS +
PI3Kα (no compound)



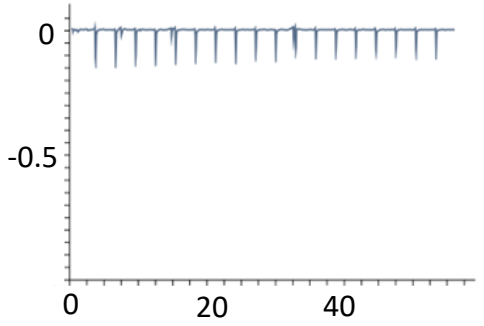



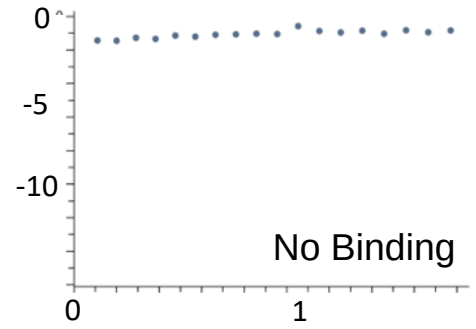

KRAS +
Breaker tethered PI3Kα



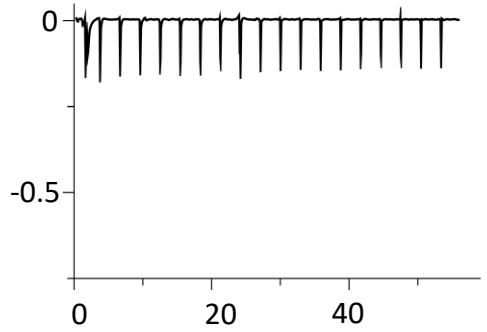



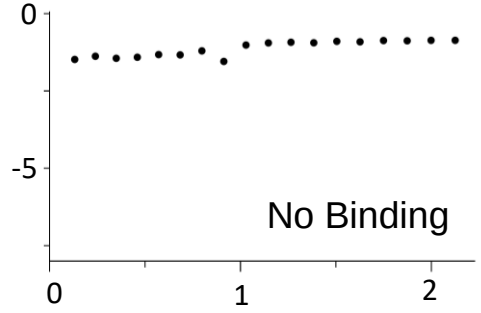

NRAS +
Breaker tethered PI3Kα




HRAS +
Breaker tethered PI3Kα

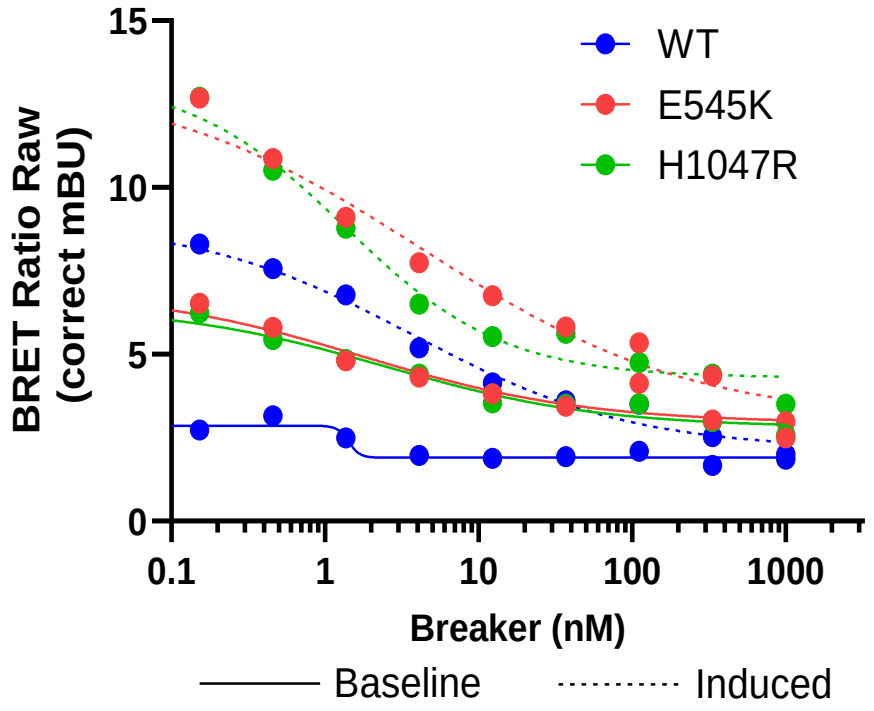



**Novel, small molecule covalent inhibitors prevent the interaction of Pi3Kα with K/H/N RAS in the ITC assay**

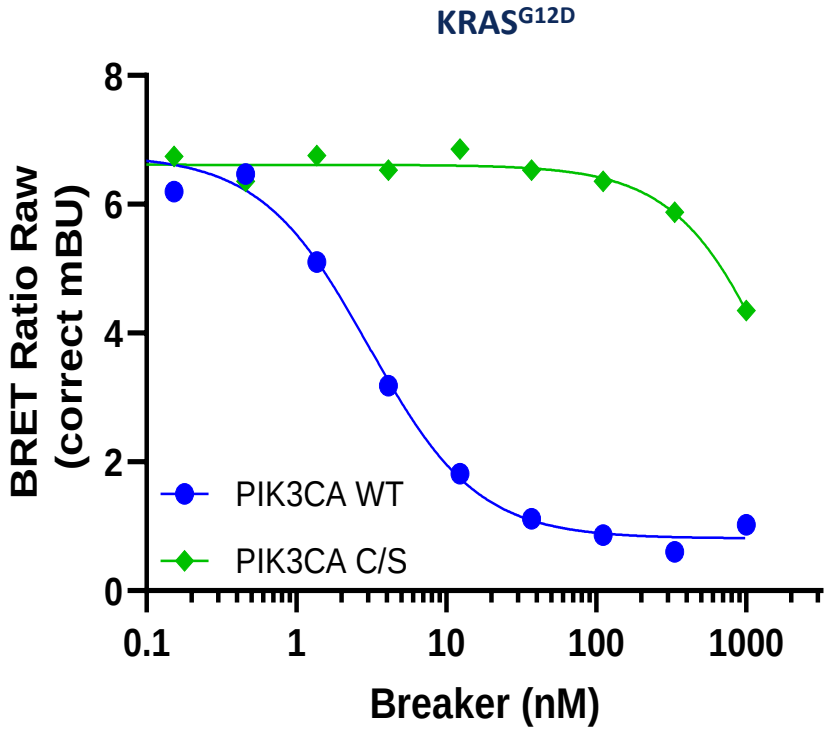
# Breaker shows an equipotent effect on wild-type and mutant PI3Kα; covalent interaction is the key to potency

**BRET - KRAS$^{G12D}$**




| | WT | E545K | H1047R |
|---|---|---|---|
| $IC_{50}$ (nM) | 1.4 | 1.8 | 2.5 |

**BRET - WT v. C/S mut**

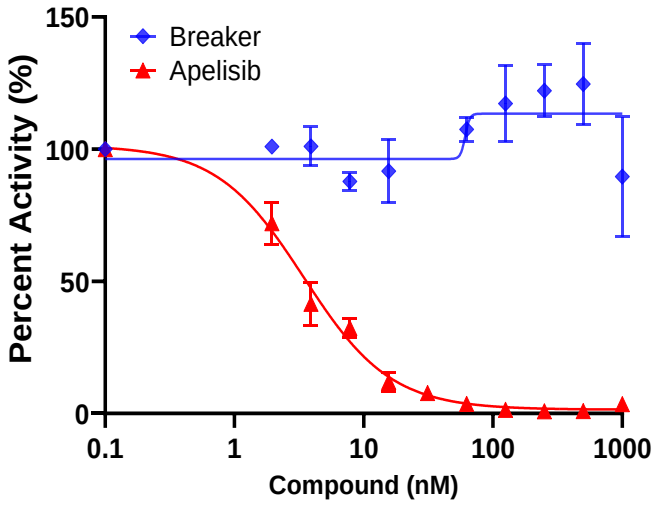



**Covalent interaction indispensable for potency**

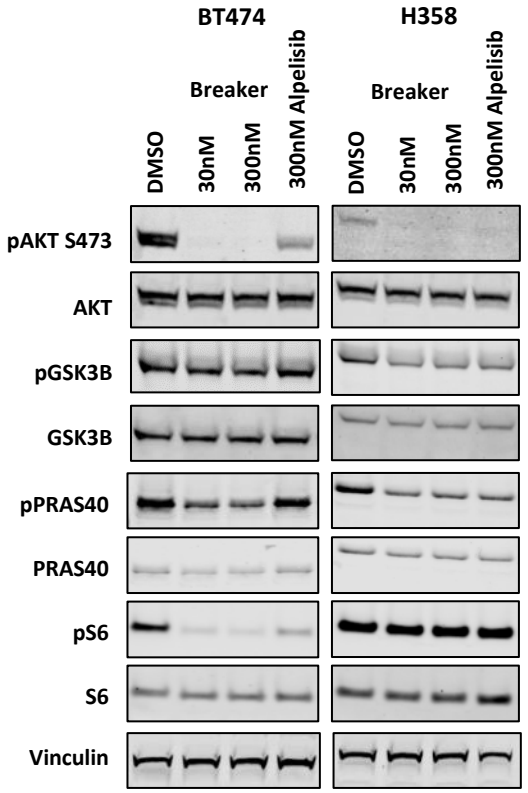
# Breaker effects transcriptional regulation and signaling inhibition similar to alpelisib, without inhibiting kinase activity

## Kinase inhibition

**Breaker MOA does NOT inhibit the kinase activity of PI3Kα**

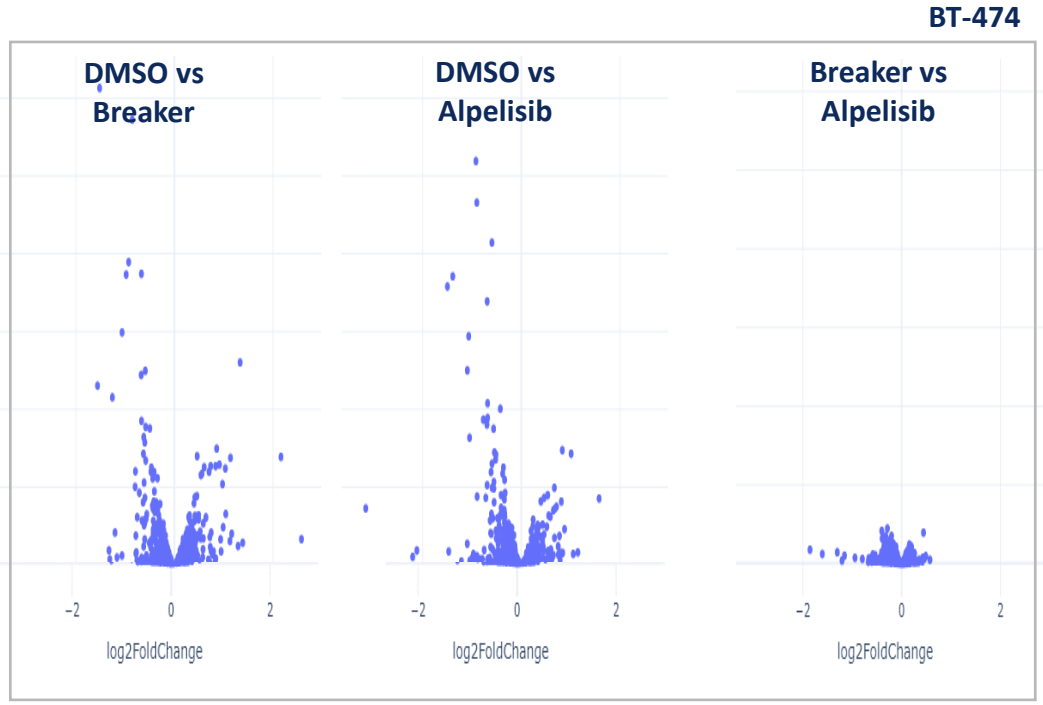



## Akt Signaling




**Signaling inhibition identical to alpelisib**

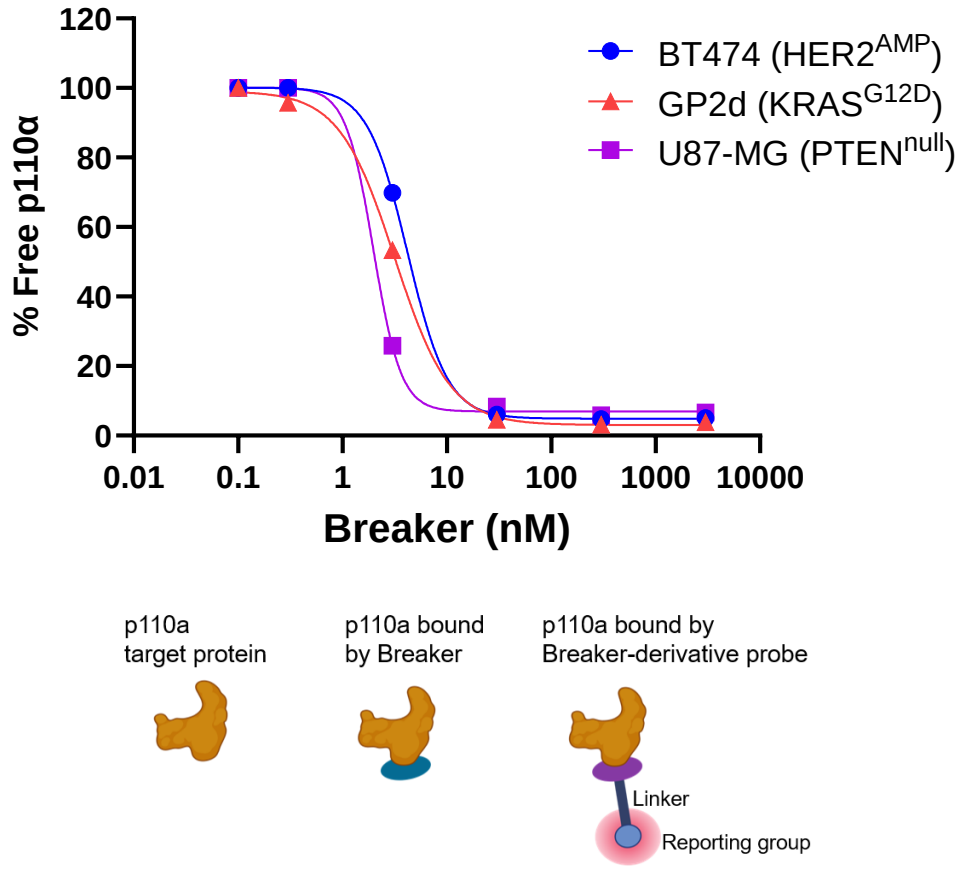
## Transcriptional Regulation



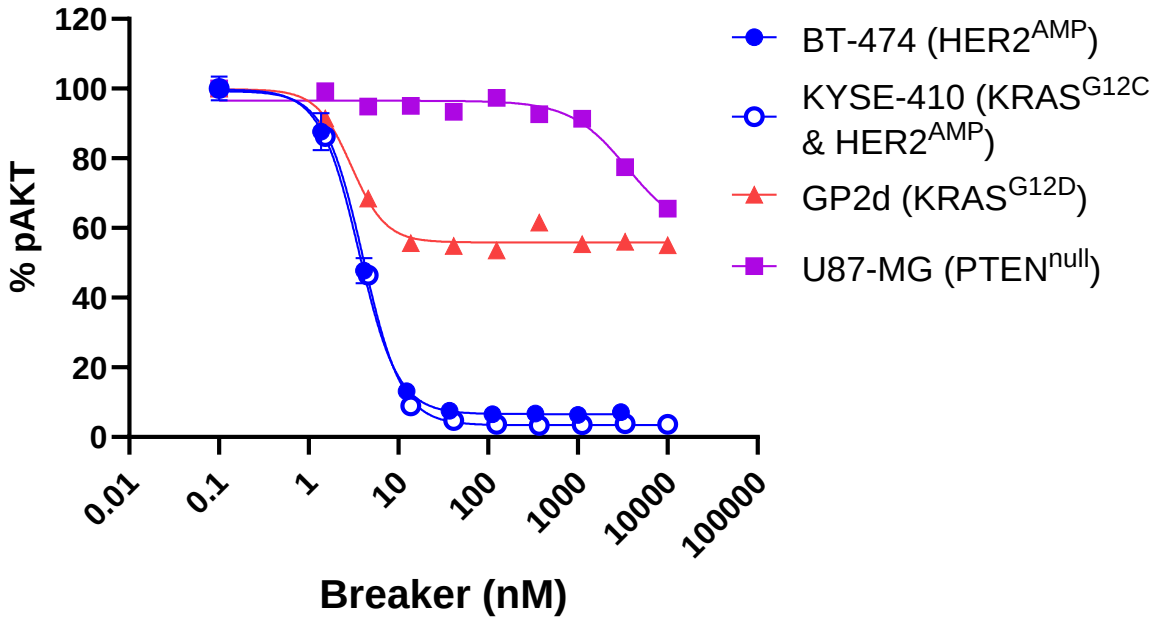

- No genes are significantly differentially regulated between breaker and alpelisib
- Data strongly suggest “on mechanism” effects of breaker

# Target engagement does not always result in pAKT inhibition – only if RAS-driven

## Target Engagement




## pAKT

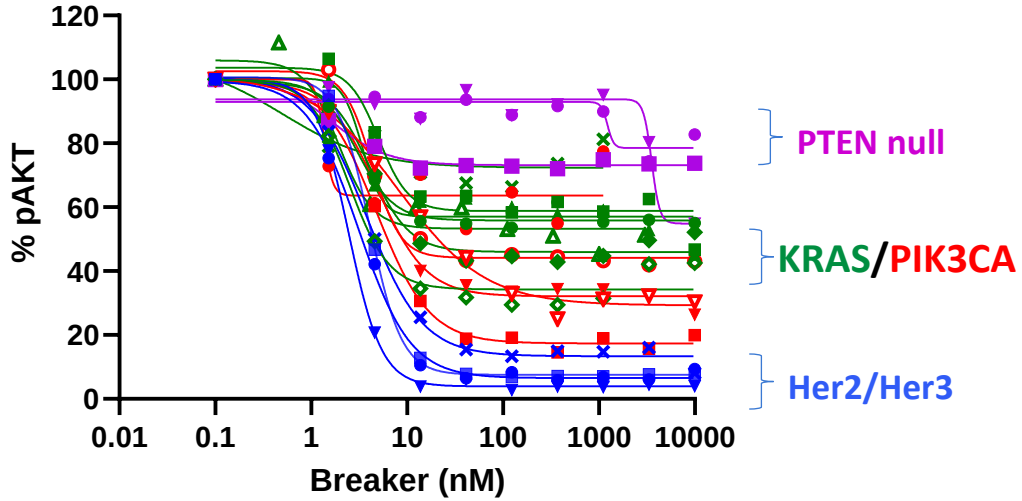



**Downstream signaling inhibition is driven by biology**

# Breaker inhibits RAS-driven pAKT in tumor cells

Data suggests Her2/Her3 tumor cells are highly dependent on PI3Kα:RAS interaction

## pAKT inhibition by genotype




**Her2/Her3**

- KYSE410
- BT474
- N87
- SKBR3

**ER+ Her2-/PIK3CA**

- MCF7
- MDA-MB-361
- MDA-MB-453
- SK_OV-3
- SKUT1

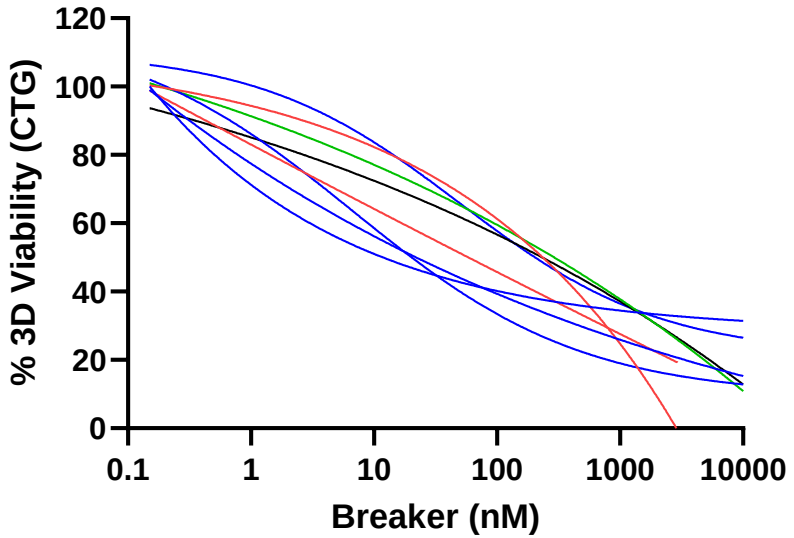
**KRAS**

- Gp2D
- LU-65
- H358
- SW1463
- Capan-2
- LS513
- AGS

**PTEN null**

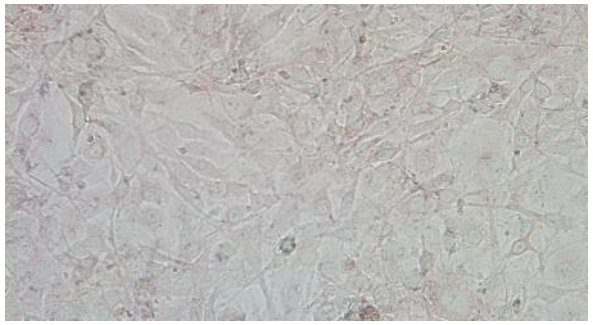
- U87MG
- UM-UC-3
- PC-3

## 3D viability

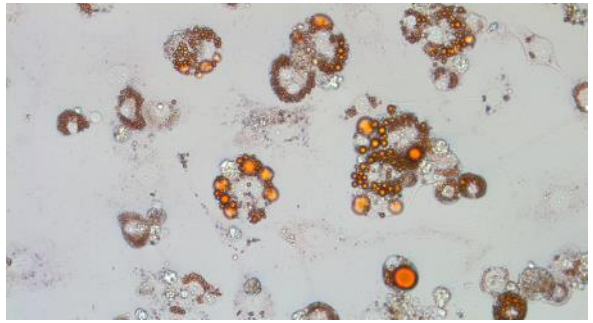



- NCI-N87
- SKBR3
- MDA-MB-361
- MDA-MB-453
- BT474
- GP2d
- NCI-H1975
- KYSE-410

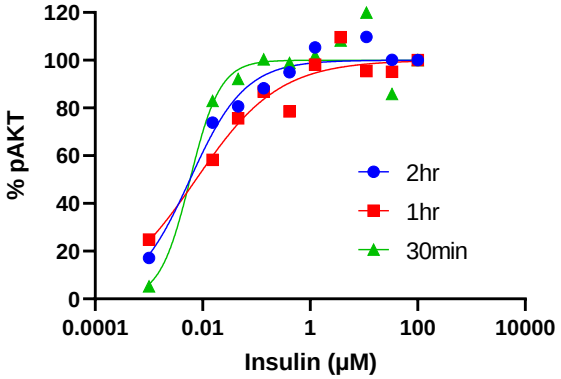
# Breaker does not inhibit insulin-mediated pAKT activation in an adipocyte model

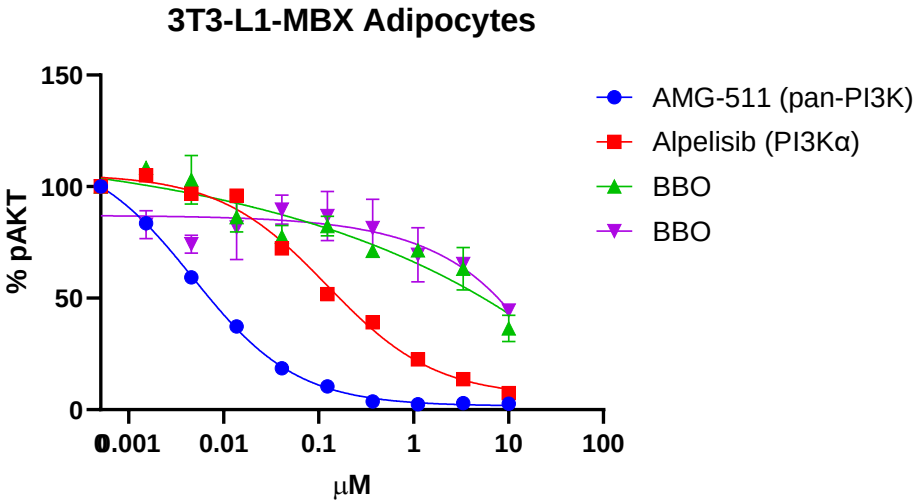


Pre-differentiation
(fibroblast)



Post-differentiation
(adipocyte)

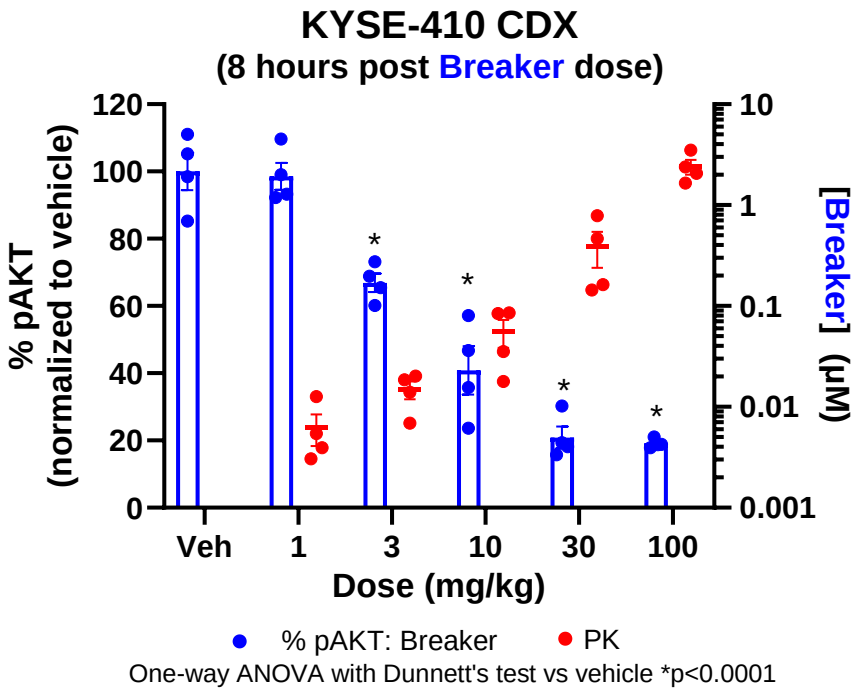


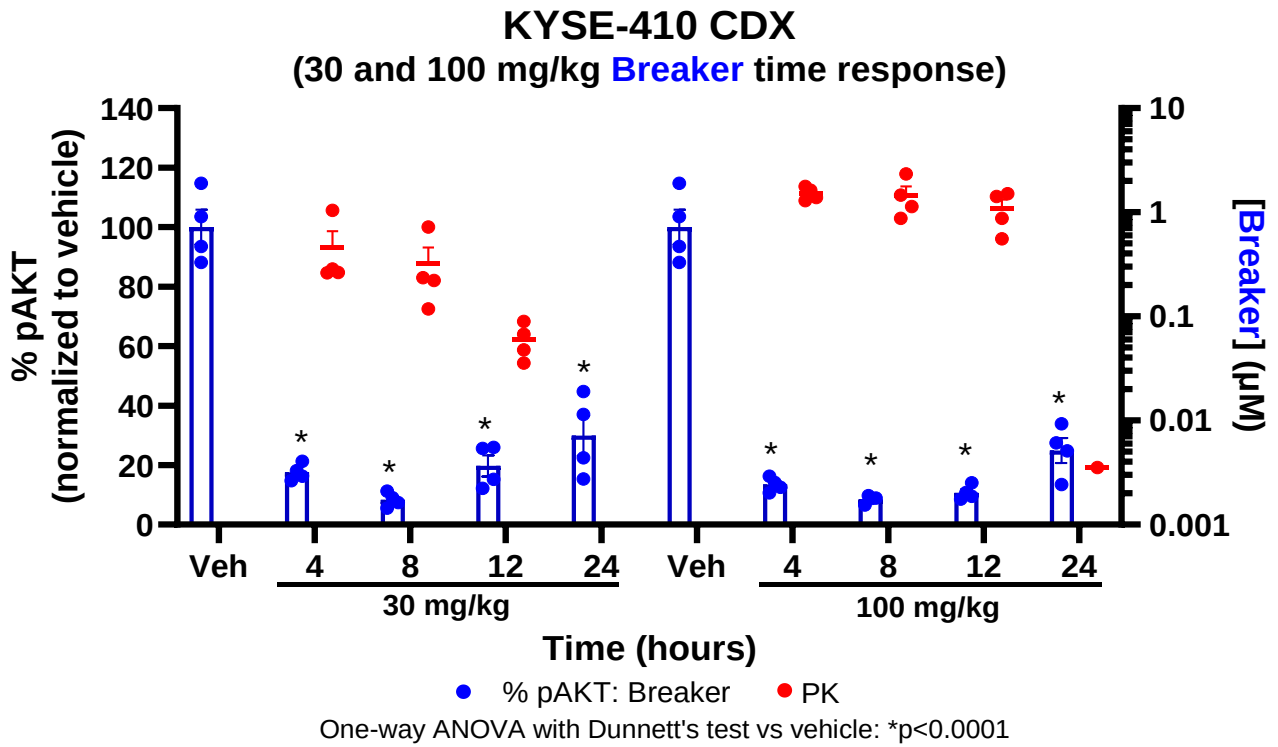

| 3T3-L1-MBX, pAKT $IC_{50}$ (µM) | | | |
|---|---|---|---|
| **AMG511** | **Alpelisib** | **BBO** | **BBO** |
| 0.005 | 0.123 | >5 | >5 |

**Effect should lead to no hyperglycemia *in vivo***

# Breaker shows dose- and time-dependent pAKT inhibition in the KYSE-410 (Her2$^{amp}$/KRAS$^{G12C}$) CDX model




One-way ANOVA with Dunnett's test vs vehicle *p<0.0001




One-way ANOVA with Dunnett's test vs vehicle: *p<0.0001

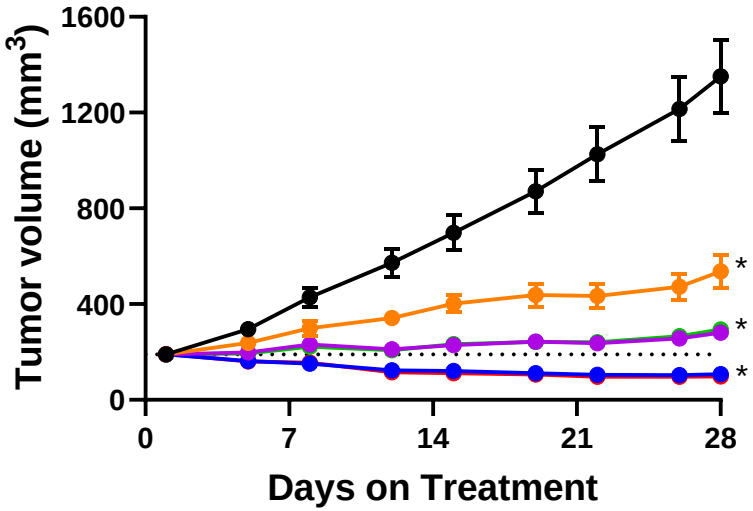
| Dose (QDx1, po) | pAKT | | Plasma [compound] |
|---|---|---|---|
| | Inhibition | p value vs vehicle | |
| 1 mg/kg | 1% | 0.9996 | 6 nM |
| 3 mg/kg | 33% | p<0.0001 | 15 nM |
| 10 mg/kg | 59% | p<0.0001 | 56 nM |
| 30 mg/kg | 79% | p<0.0001 | 390 nM |
| 100 mg/kg | 81% | p<0.0001 | 2408 nM |

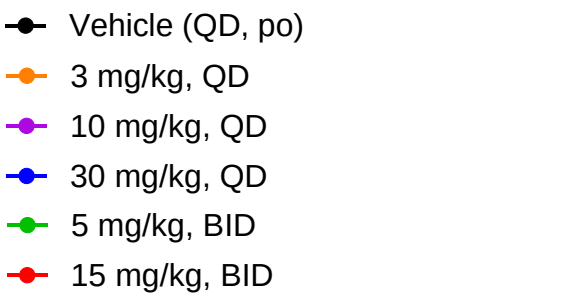
| pAKT $IC_{50}$ | KYSE-410 In Vitro Data | KYSE-410 In Vivo Data |
|---|---|---|
| | Cmpd bound in 10% FBS | CDX PD Study |
| | pAKT FF adj $IC_{50}$ | $EC_{50}$ [95% CI] |
| 3.6 nM | 160 nM | 45 nM [29 – 75] |

*In vivo* $EC_{50}$ are consistent with *in vitro* data

# Breaker drives strong efficacy in the KYSE-410 (HER2$^{amp}$ / KRAS$^{G12C}$) CDX model

## Tumor Volume

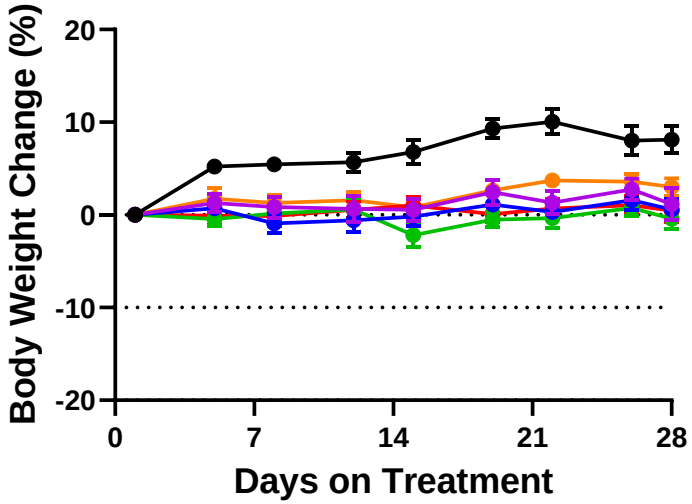



All treatment groups *p<0.0001 compared to vehicle group

| $ED_{50}$ (mg/kg) [95% CI] | $ED_{90}$ (mg/kg) [95% CI] |
|---|---|
| 2.5 [1.5 - 4.2] | 3.9 [3.4 – 4.5] |

## Body Weight % Change

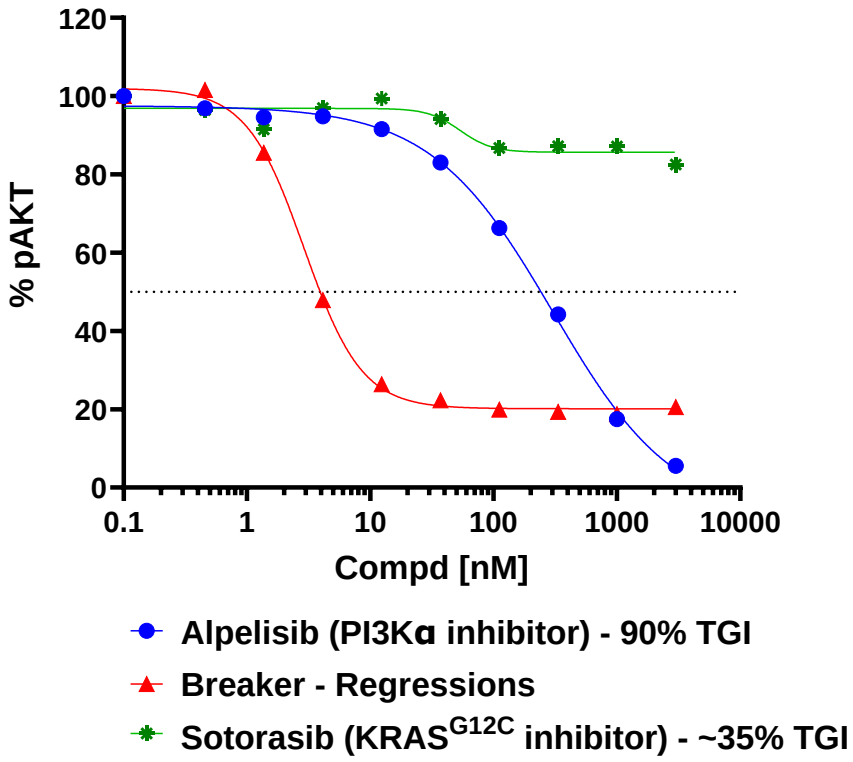



| Group (n=9) | Day 28 | | | | | | |
|---|---|---|---|---|---|---|---|
| | TGI | Mean tumor regression | Number of regressions | p value vs vehicle | p value vs 5 mg/kg BID | p value vs 15 mg/kg BID | Mean body weight change |
| Vehicle | - | - | 0/10 | - | - | - | +8.1% |
| 3 mg/kg, QD | 70% | - | 0/10 | <0.0001 | - | - | +3.0% |
| 10 mg/kg, QD | 92% | - | 1/10 | <0.0001 | 0.9403 | - | +1.1% |
| 30 mg/kg, QD | - | 44% | 10/10 | <0.0001 | - | 0.7373 | +0.6% |
| 5 mg/kg, BID | 91% | - | 1/10 | <0.0001 | - | - | -0.4% |
| 15 mg/kg, BID | - | 49% | 10/10 | <0.0001 | - | - | +0.5% |

Two-way repeated measuress ANOVA followed by Dunnett's multiple comparisons test was performed for statistical analyses for vehicle group comparisons (day 5 to 28)
Two-way repeated measures ANOVA of the indicated QD versus BID group means were performed for the statistical analyses (day 5 to 28)

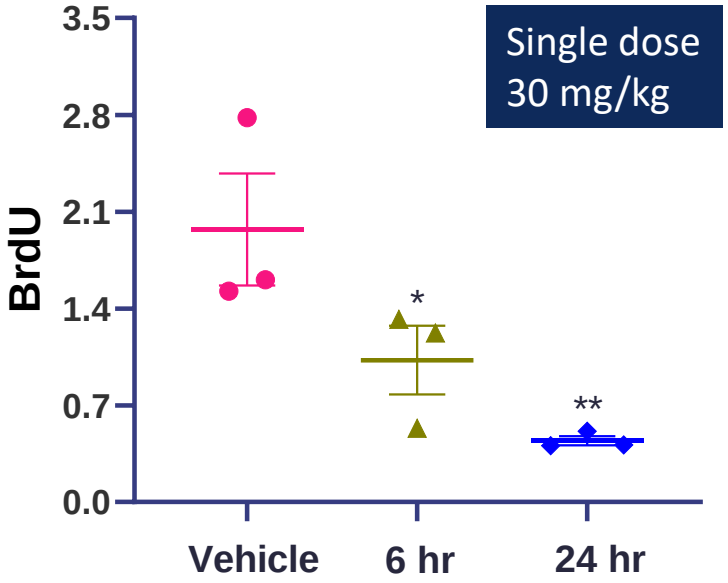
# Anti-tumor activity in the KYSE-410 CDX model is driven by strong decrease in proliferative fraction

## Data supports, specific, on-target efficacy of breaker MOA KRAS$^{G12C}$ does not drive pAKT




**Breaker and alpelisib achieve regressions, sotorasib is NOT efficacious**

## Reduction in proliferative fraction is observed after single dose of Breaker

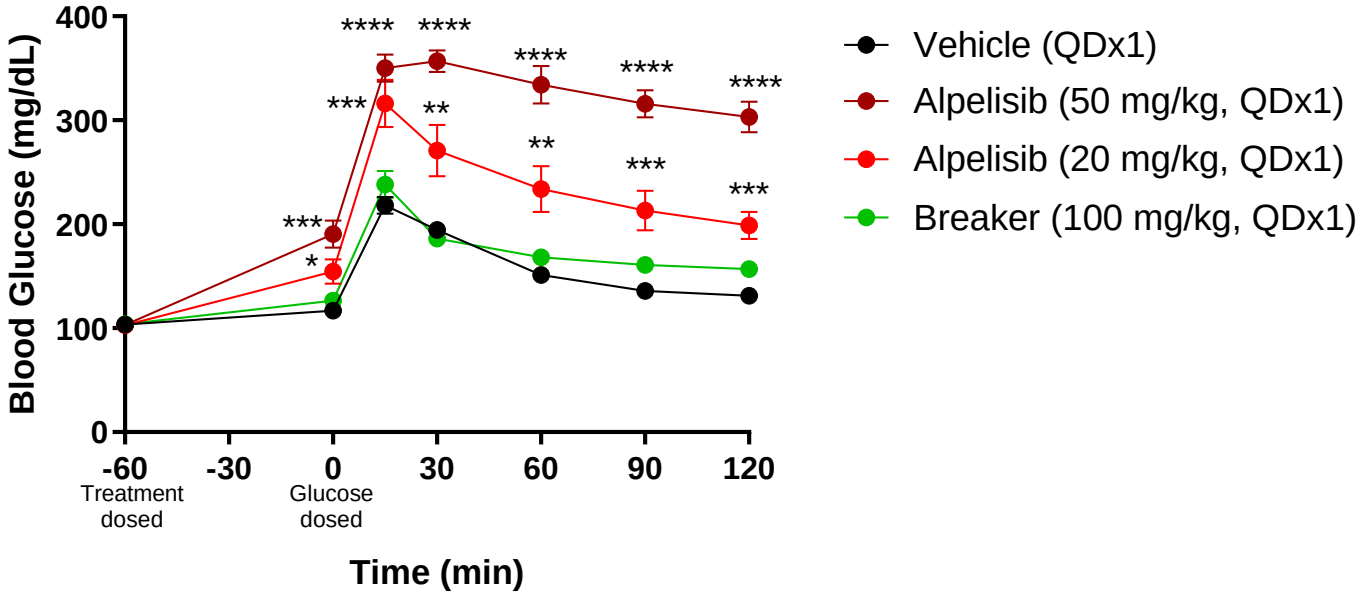



**G1 arrest is the most common effect observed following treatment with a PI3Kα kinase inhibitor**

BrdU positive area / solid tumor area (µm^2/ µm^2), *p<0.05, p<0.01 vs vehicle

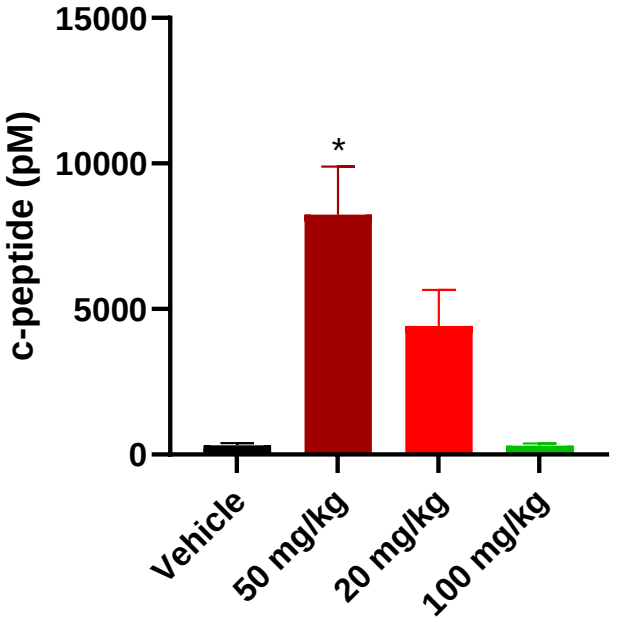
# Lack of insulin-driven pAkt inhibition in adipocytes translates *in vivo*

## oGTT Results: Blood Glucose Levels




One-way ANOVA with Dunnett's multiple comparisons test vs vehicle: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

## C-peptide



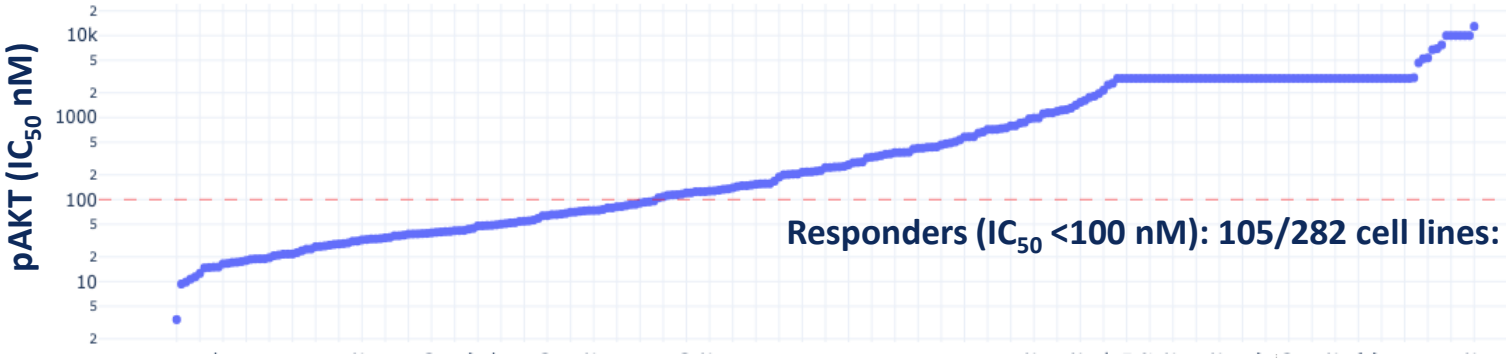

One-way ANOVA with Dunnett's test vs vehicle: *p<0.001
(Note: vehicle vs 20 mg/kg alpelisib: p=0.052)

**No changes in blood glucose observed at 100 mg/kg (>3x regression dose)**

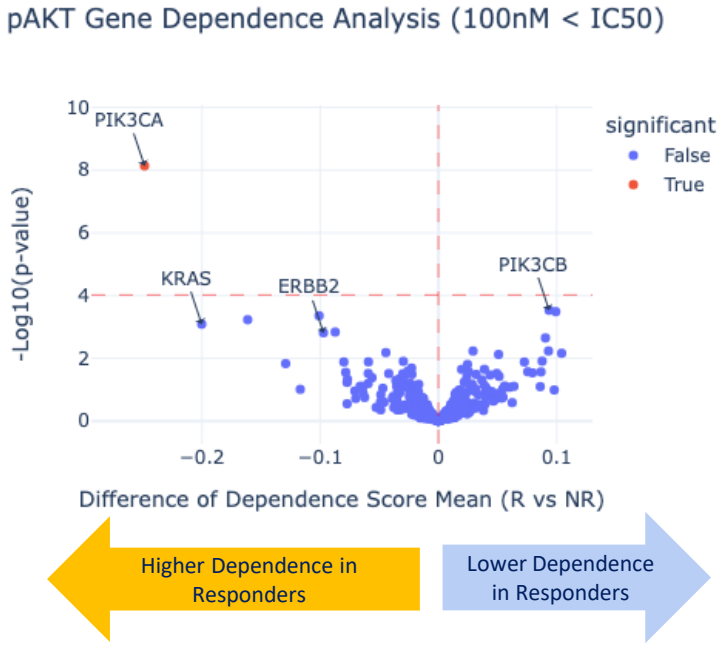
# Identifying genotypes most dependent on the PI3Kα:RAS interaction

## One third of all cancer cell lines depend on PI3Kα:RAS interaction for activation of AKT signaling

### Crown Biosciences pAKT cell line screen (250+ cell lines)




| | Her2$^{amp}$ | EGFR$^{amp}$ | KRAS G12 | KRAS G13 | KRAS Other | Helical mutPI3Kα | PTEN-null |
|---|---|---|---|---|---|---|---|
| % genotype (n=105) | **15.2% (16/105)** | 0.9% (1/105) | **27.6% (29/105)** | 1.9% (2/105) | 3.8% (4/105) | **10.5% (11/105)** | 2.8% (3/105) |
| % responders | **76.2% (16/21)** | 14.3% (1/7) | **58% (29/50)** | 28.5% (2/7) | 44.4% (4/9) | **61%* (11/18)** | 11.11% (3/27) |

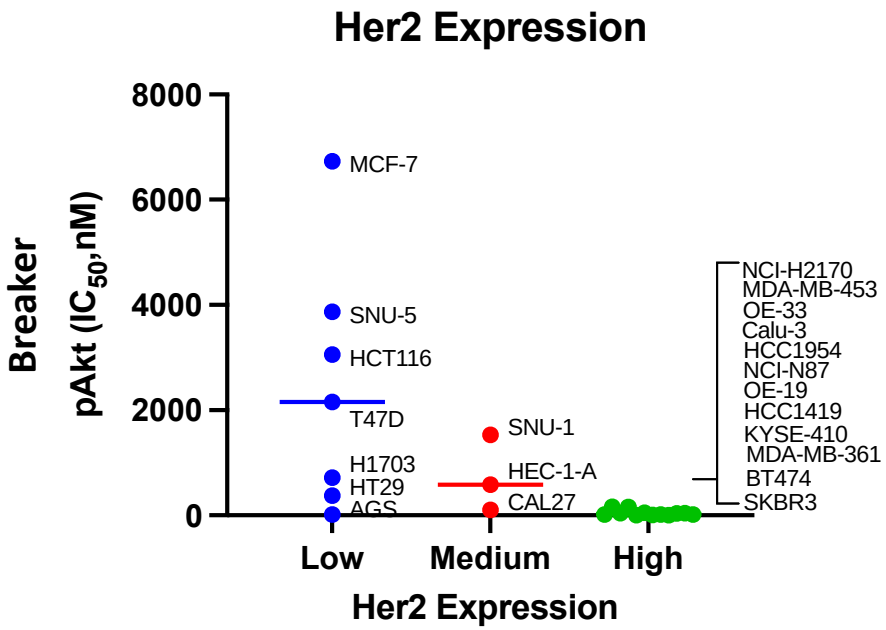



**Good correlation between "positive genotypes" and gene dependency**

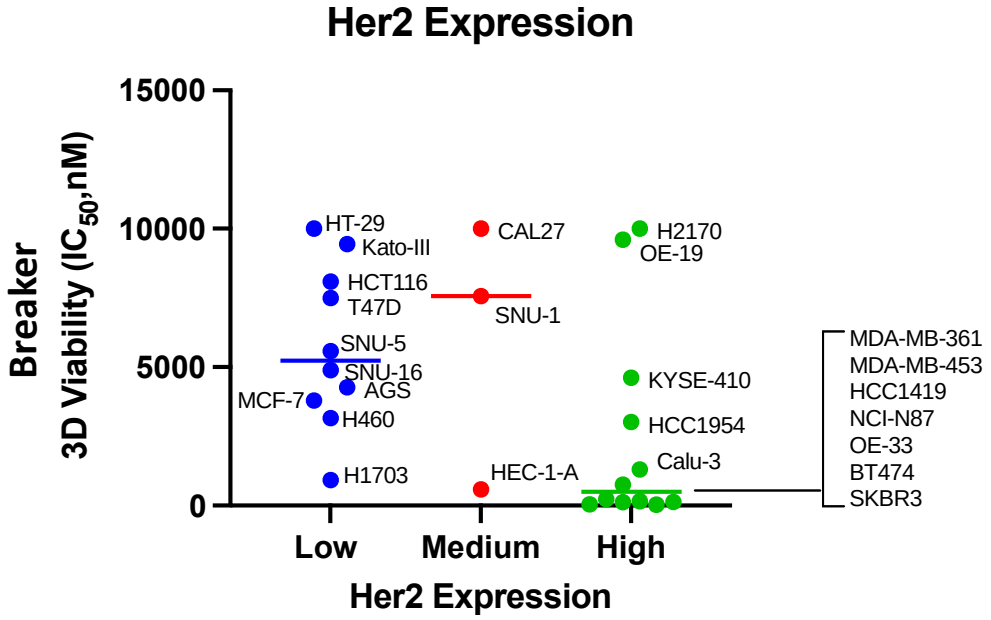
*17/18 (94%) if 200 nM used

# Her2-expressing cells demonstrate strong sensitivity to Breaker activity

## pAKT in Her2+ cell lines

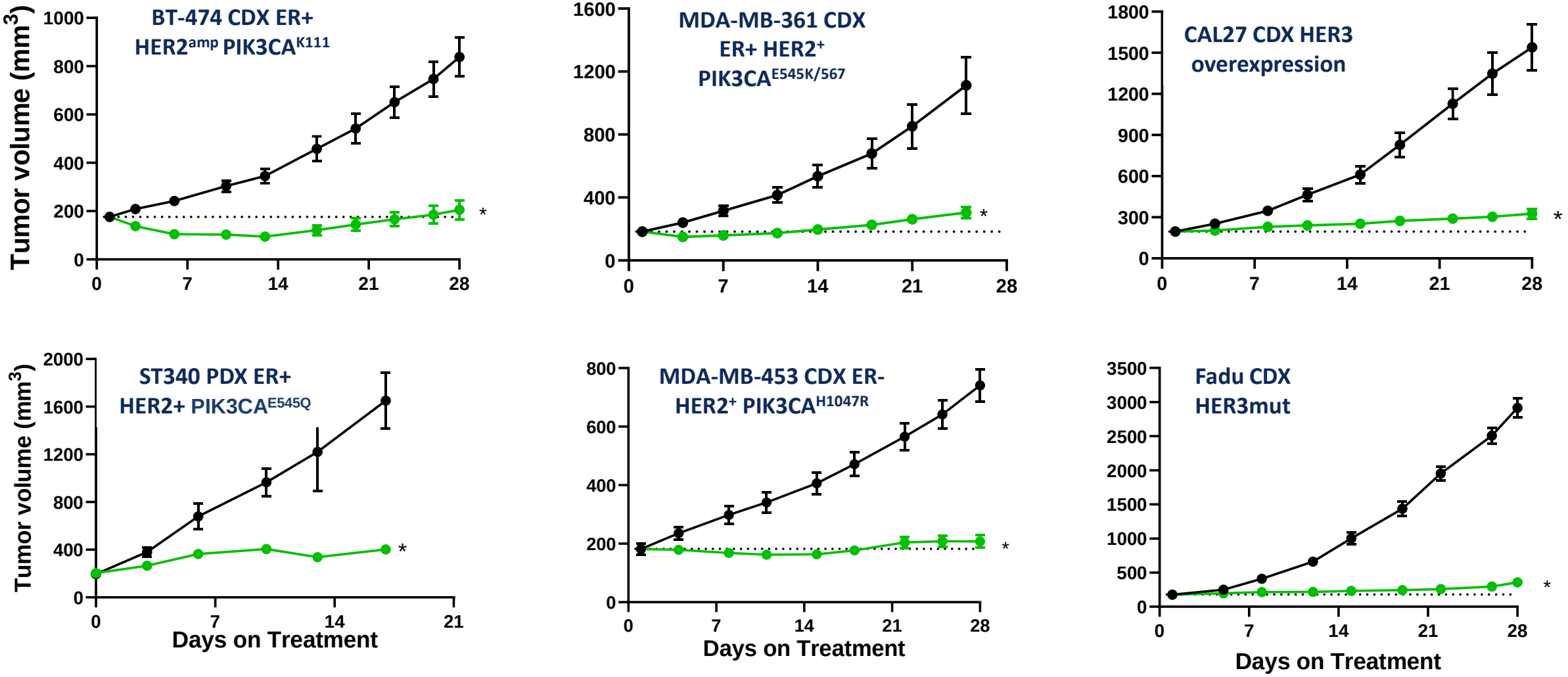



## Viability in Her2+ cell lines




**Cell lines with high Her2-expression demonstrate sensitivity to both pAKT and 3D viability inhibition**

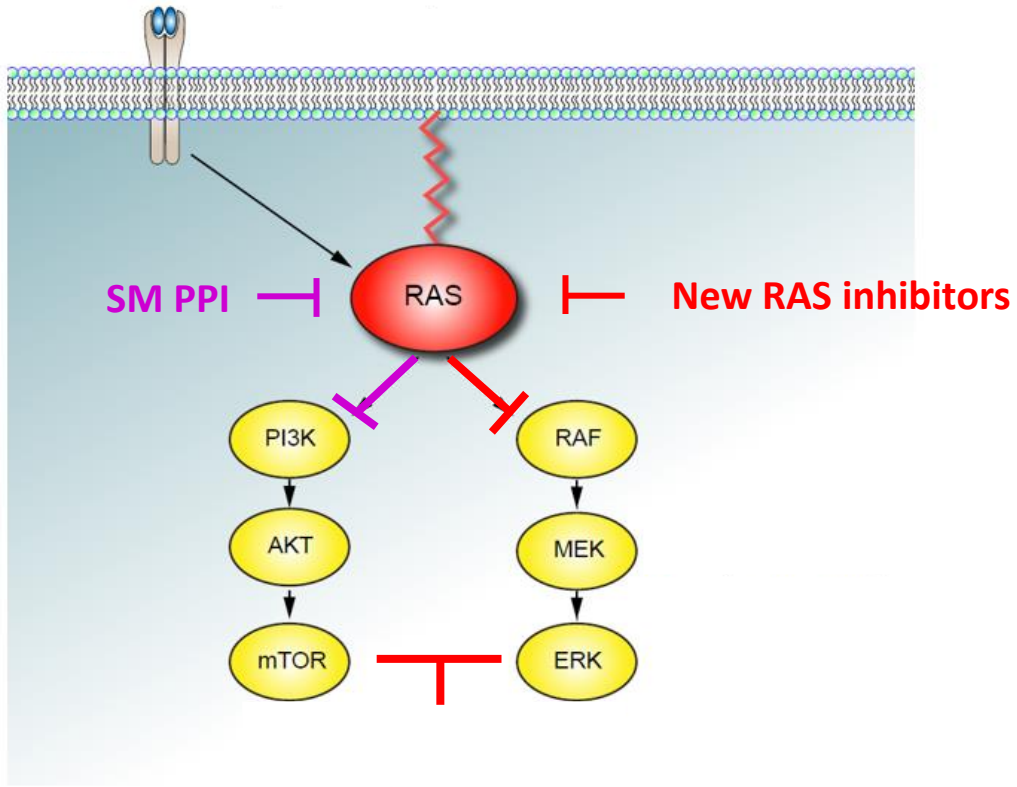
# Strong monotherapy efficacy observed in breast cancer models with Her2 expression, with or without PIK3CA mutations




*RMANOVA

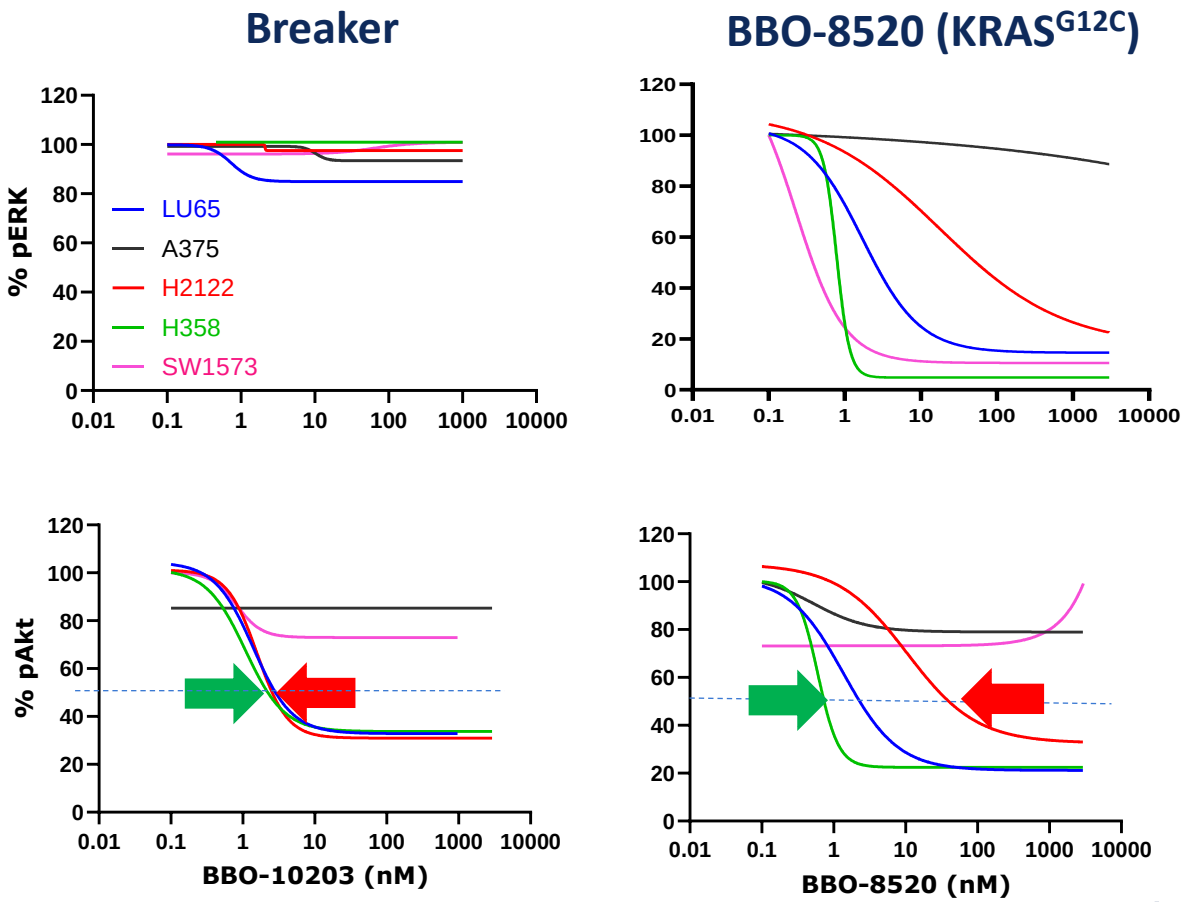
# Breaker activity can optimize target coverage of KRAS inhibitors

## Combination of Breaker and RASi should optimize target coverage for AKT pathway




**No Tumor Growth ?**

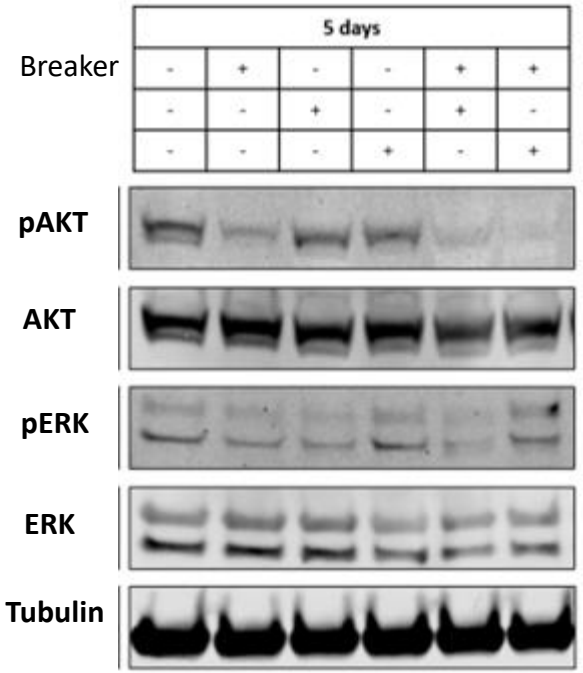
## Homogenous inhibition of pAKT amongst NSCLC KRAS$^{G12C}$ cell lines




Modified from: National Cancer Institute/Marielle Yohe, M.D., Ph.D.

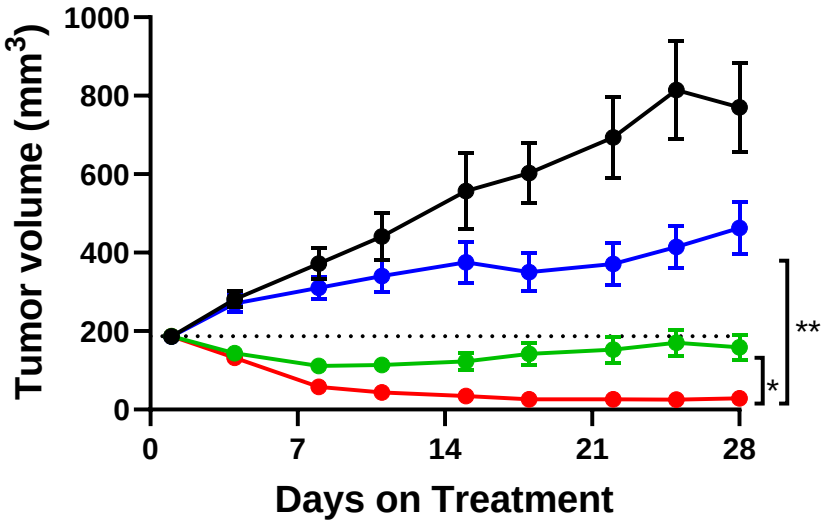
# Strong combination benefit seen in the KRAS$^{G12Ci}$ sensitive NSCLC H358 Model

## Signaling 5 days (*in vitro*)




**Combination benefit seen even in "very sensitive" model**

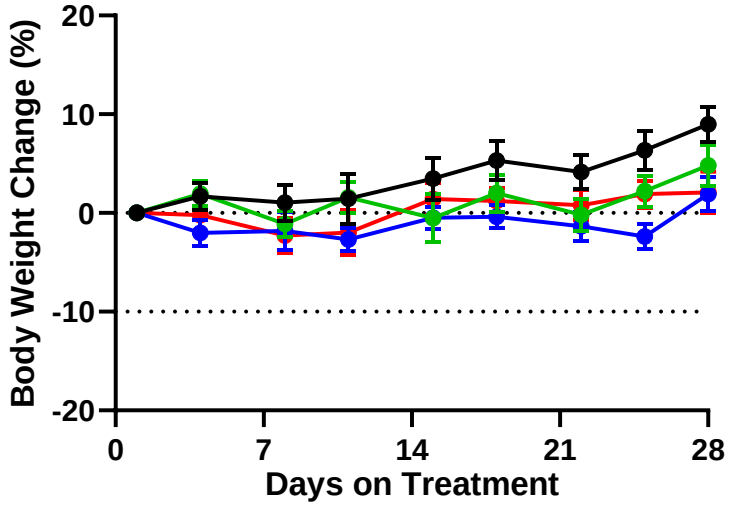
## Efficacy model (*in vivo*)




*p<0.01, **p<0.0001 compared to monotherapy group

Vehicle (QD, po)
BBO-8520 (3 mg/kg)
BBO-10203 (100 mg/kg)
BBO-8520 + BBO-10203

## Body Weight (*in vivo*)

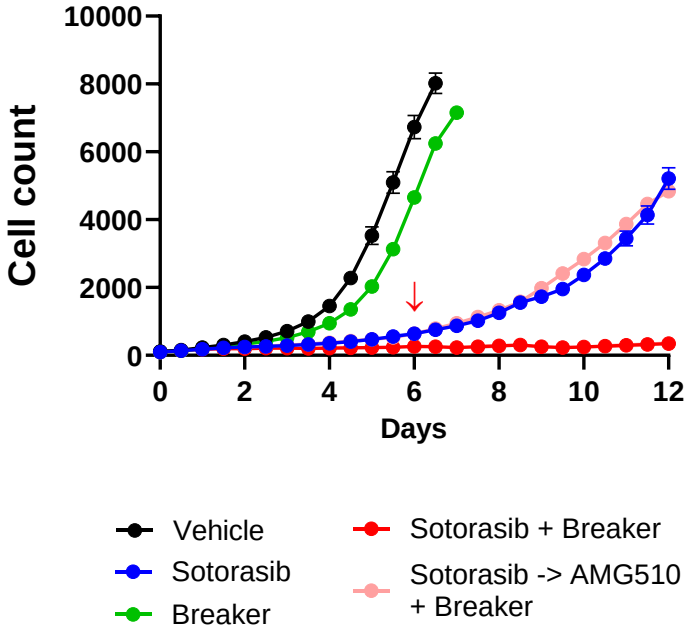



**Combination is very well tolerated**

BBO-8520: Direct KRAS$^{G12C}$ (ON) inhibitor, *RMANOVA

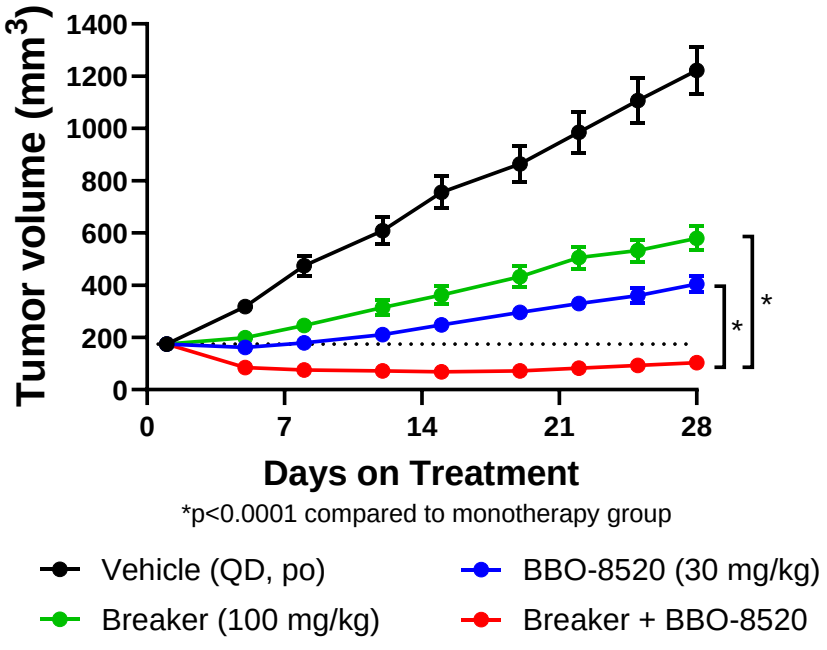
# Strong combination benefit is also observed in the KRAS$^{G12Ci}$ resistant H2122 NSCLC model

## Clonogenic Assay (*in vitro*)

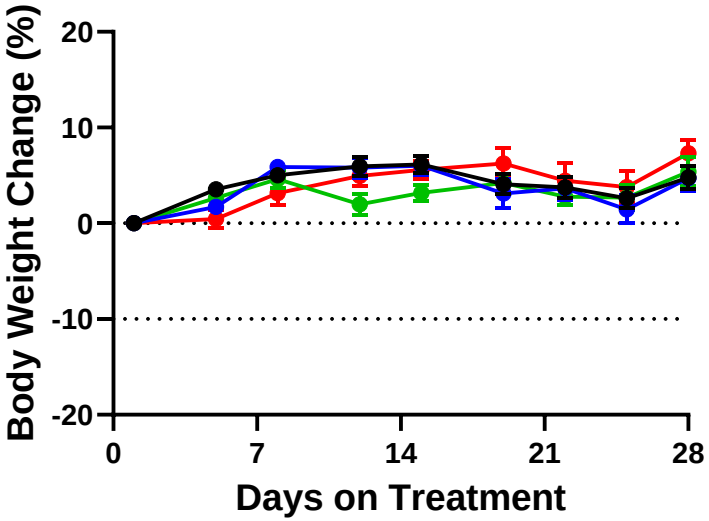



**Combination benefit seen even in "Resistant" model**

## Efficacy model (*in vivo*)




## Body Weight (*in vivo*)



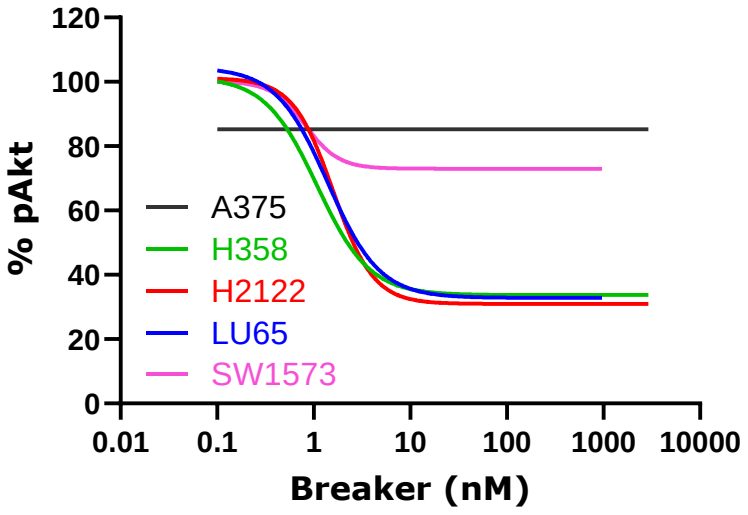

**Combination is very well tolerated**

**H2122** KRASG12C / KEAP1mut / STK11mut

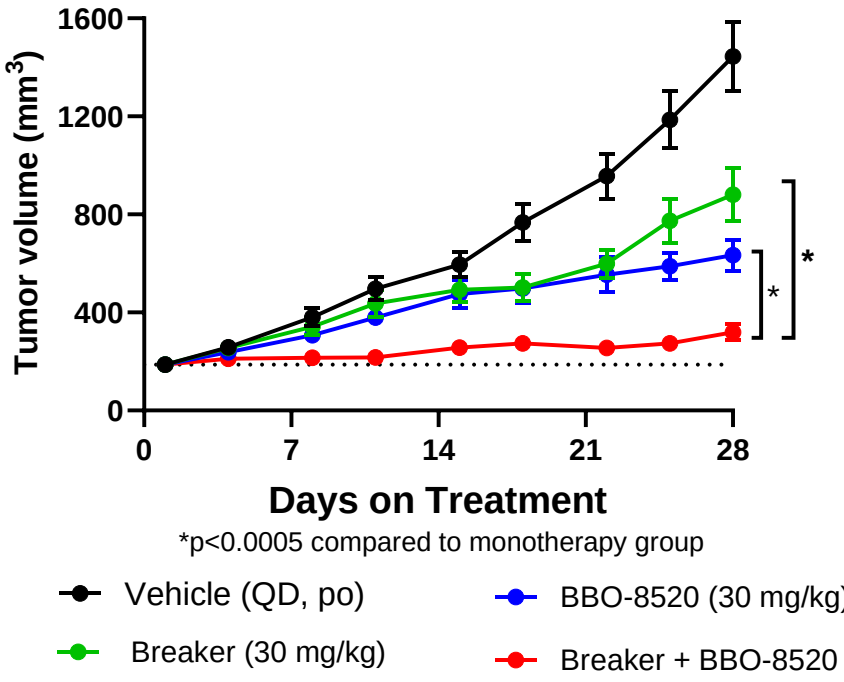
BBO-8520: Direct KRAS$^{G12C}$ (ON) inhibitor, *RMANOVA

# Effect of breaker combination is similar to a pan-PI3K inhibitor

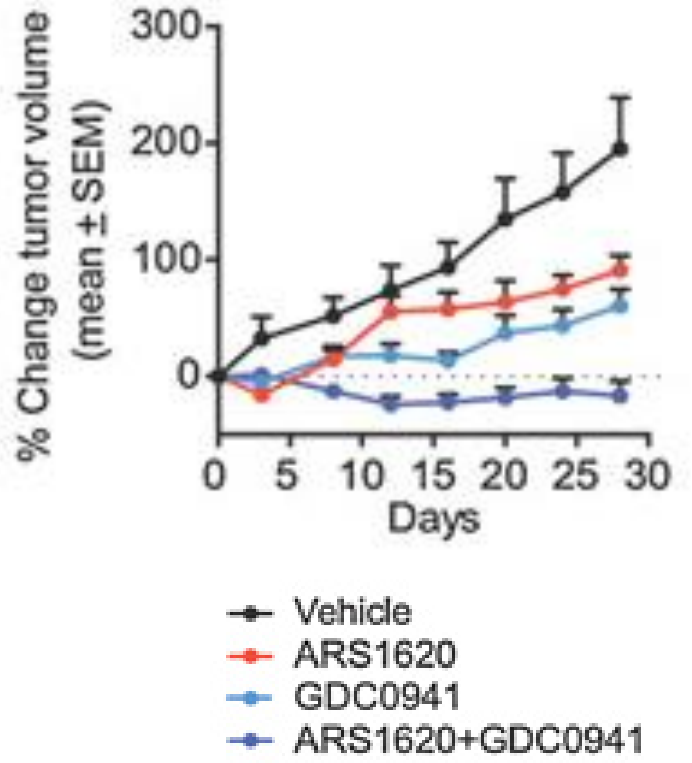
## Inhibition of pAKT

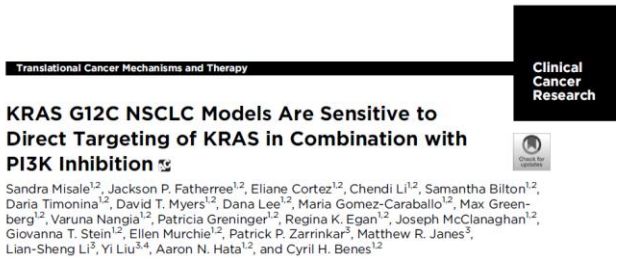



## Efficacy model (*in vivo*)




## Efficacy very similar to that achieved with a panPi3K inhibitor (Misale et.al.)

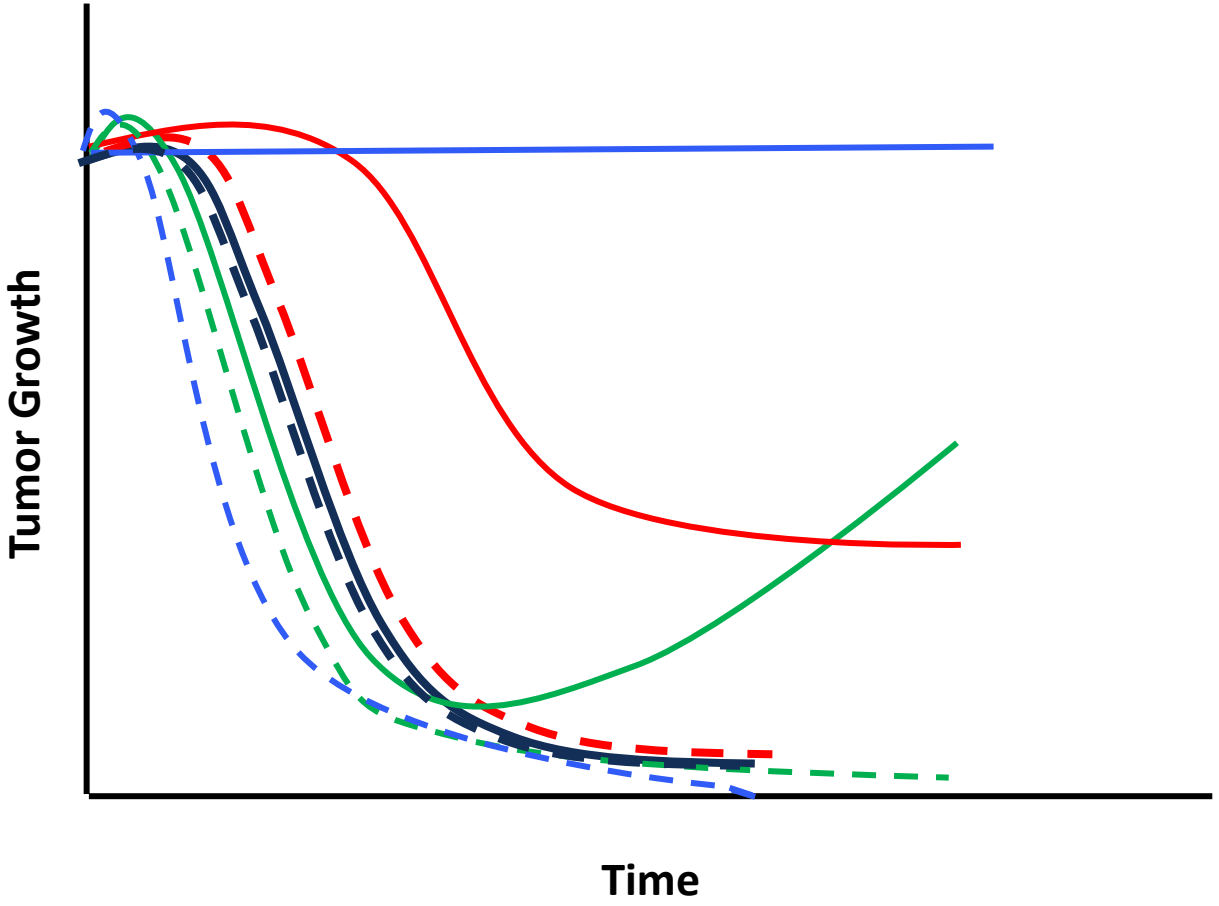



BBO-8520: Direct KRAS$^{G12C}$ (ON) inhibitor; Binimetinib: Mek inhibitor, *RMANOVA

# Diverse roles of RAS in driving PI3Kα activity can be addressed with Breaker

## PI3Kα inhibition is key in optimizing the anti-tumor activity of mutant KRAS inhibitors




| Legend | Phenotype | Model |
|---|---|---|
| ——— / - - - - | Mutant RAS drives PI3Kα | GP2D |
| ——— / - - - - | Mutant RAS and other RAS drives PI3Kα | H2122/SW1573 |
| ——— / - - - - | Mutant RAS drives PI3Kα other RAS emerges under pressure | H358 |
| ——— / - - - - | Other RAS drives PI3Kα | KYSE-410 |

——— KRASi

- - - - KRASi + Breaker

# Summary: PI3Kα:RAS Breaker

- BridgeBio has identified first-in-class, potent (~10 nM), small molecule PI3Kα:RAS breakers that validate the importance of the physical interaction between PI3Kα and RAS in human tumor biology
- Breakers present a new therapeutic avenue to inhibit PI3Kα signaling in a tumor selective manner w/o hyperglycemia
- Pharmacology experiments show that this interaction is important in $Her2^{amp}$, KRASG12x, and PI3Kα mutant tumors
- Breakers may enable the execution of clinical combinations of MAPK inhibitors (KRAS inhibitors) with PI3Kα inhibition
- We have selected a development candidate that is progressing towards the clinic

# Team Effort

bridgebio

| | | |
|---|---|---|
| Olga Botvinnik | Christina Liang | Kyle Sullivan |
| Howard Chang | Ken Lin | Bin Wang |
| Tony Chen | Frank McCormick | Keshi Wang |
| Nathan Collett | Sadaf Mehdizadeh | Paul Wehn |
| Sofia Donovan | Mike Monteith | James Winter |
| Ferdie Evangelista | Rick Panicucci | Maggie Yandell-Zhao |
| Cindy Feng | Erin Riegler | Cathy Zhang |
| Siyu Feng | Saman Setoodeh | Zuhui Zhang |
| Lijuan Fu | Jin Shu | James Rizzi |
| Jennifer Gansert | Devansh Singh | Dana Minnick |
| Foster Gonsalves | Kanchan Singh | Robert Czerwinski |
| Victoria Hodson | Kerstin Sinkevicius | Eli Wallace |
| Jin Ju | Carlos Stahlhut | Pedro Beltran |
| Sunyoung Lee | James Stice | Rui Xu |

RAS INITIATIVE

| | |
|---|---|
| Frank McCormick | Erik Larsen |
| Dwight Nissley | Tao Liao |
| Dhirendra Simanshu | Roger Ma |
| Patrick Alexander | Anna Maciag |
| Bill Bocik | Dana Rabara |
| Albert Chan | Megan Rigby |
| Daniel Czyzyk | Alok Sharma |
| Caroline DeHart | Swapnil Singh |
| John-Paul Denson | Brian Smith |
| Sathiya Dharmaiah | Thomas Sova |
| Robert D'Ippolito | Andy Stephen |
| Marcin Dyba | Monalisa Swain |
| Dominic Esposito | David Turner |
| William Gillette | Jayasudhan Yerabolu |
| Claudia Haywood | RAS Reagent Research Team |

Lawrence Livermore National Laboratory

| |
|---|
| Felice Lightstone |
| Yue Yang |

……and all the work that came before this effort, by many in this room, that set the basis for this project to start