Targeting the PI3K/AKT/mTOR pathway: Pre-clinical data II

Biomarkers in the pre-clinical setting

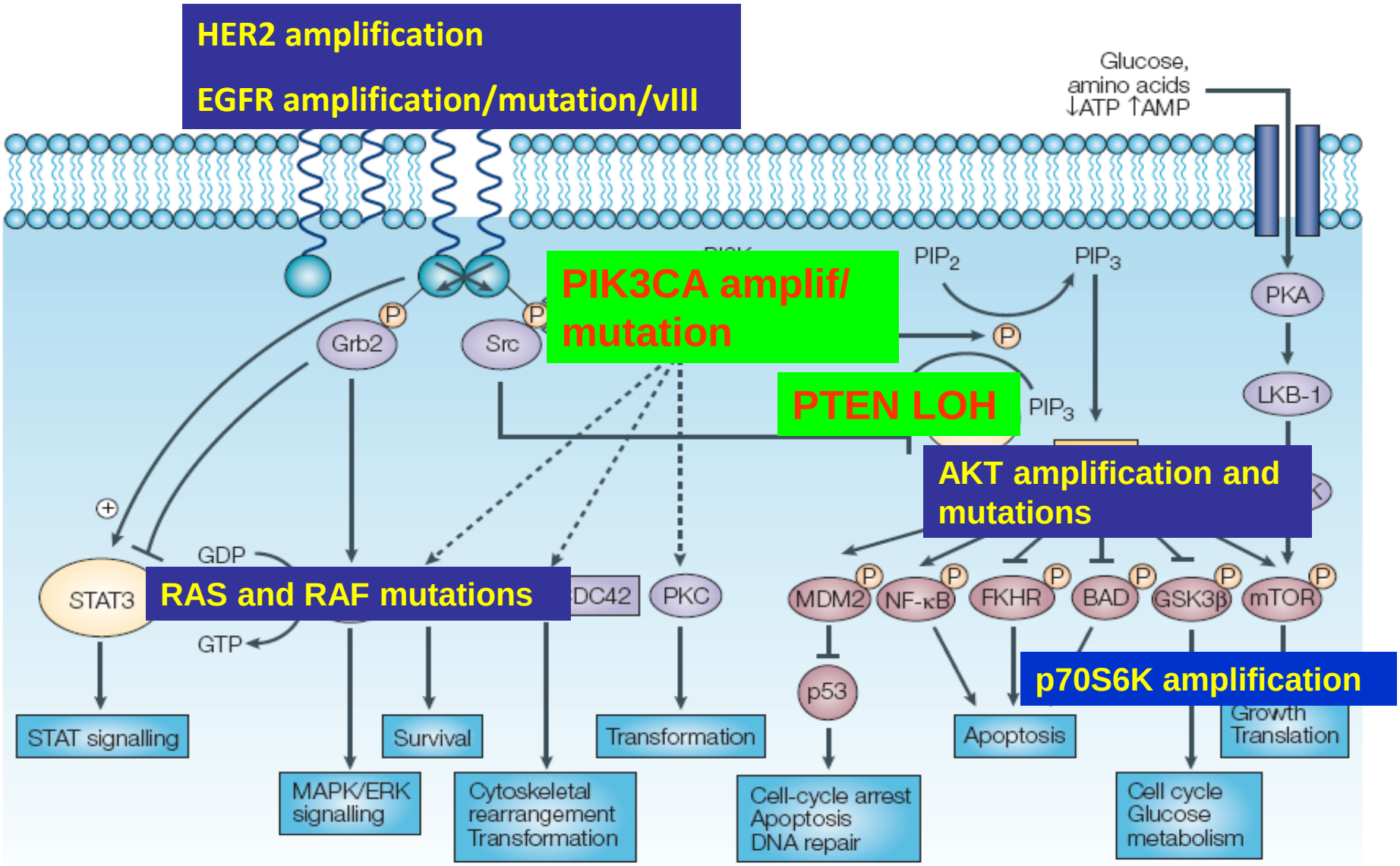
Bryan Hennessy, M.D. MRCPI

Consultant Medical Oncologist
Beaumont Hospital, Dublin, Ireland

Senior Lecturer
Royal College of Surgeons in Ireland (RCSI)

Adjunct Professor of Medicine
Department of Breast Medical Oncology
U. T. MD Anderson Cancer Center, Houston, Texas

Somatic Genomic Aberrations:



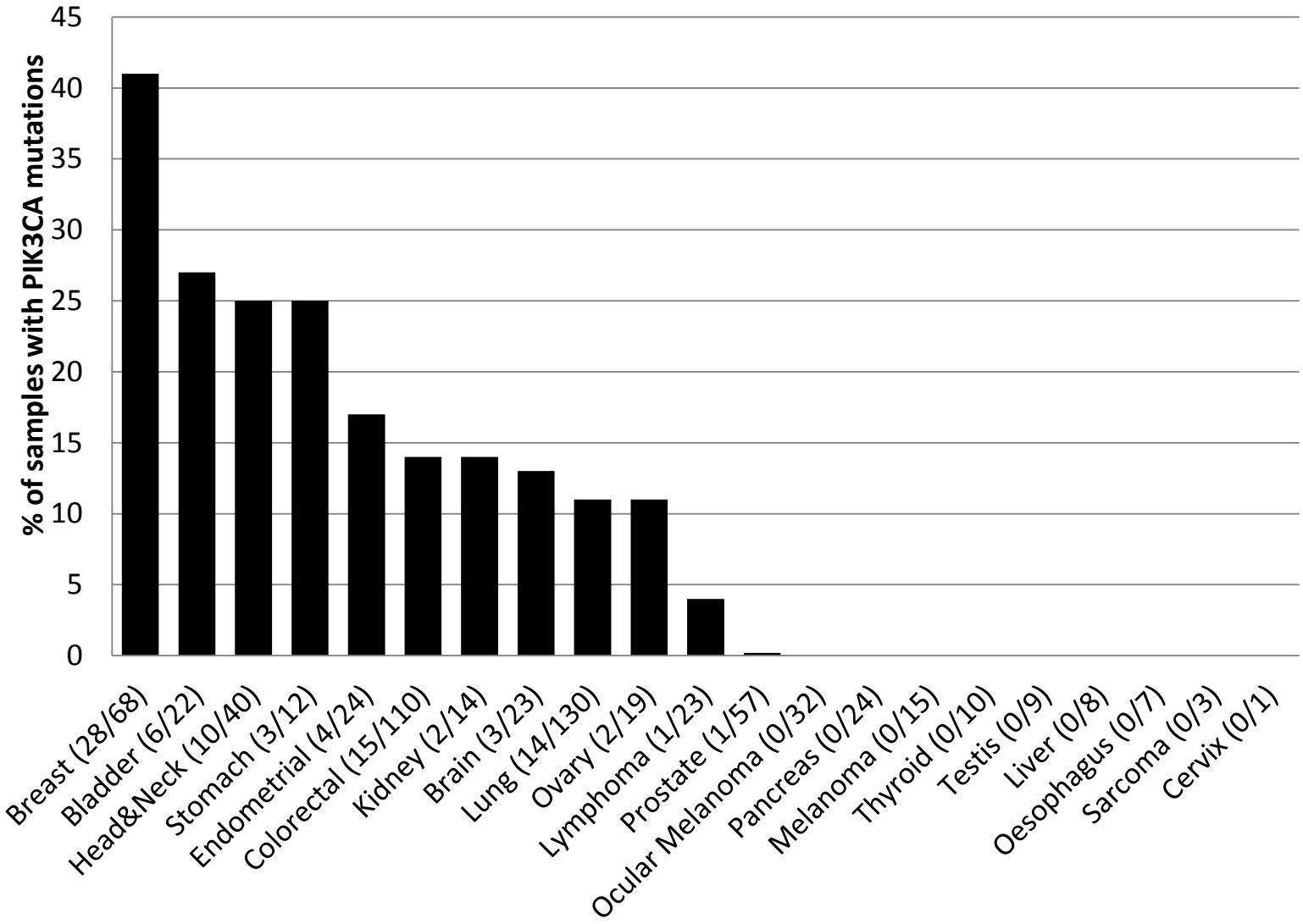
Hennessy BT et al *Nature Rev Drug Discovery* 2005

Mutations in ALL solid tumours (n=651)

Gene	No. of samples	Percentage
P53	152	23.3
PIK3CA	95	14.6
KRAS	80	12.3
IDH1	67	10.3
MET	56	8.6
PTPN11	27	4.1
BRAF	25	3.8
NRAS	21	3.2
STK11	18	2.8
GNA11	17	2.6
FBXW7	16	2.5
GNAQ	12	1.8
HRAS	12	1.8
CTNNB1	11	1.7
APC	10	1.5
CDKN2A	10	1.5

Gene	No. of samples	Percentage
EGFR	9	1.4
PTEN	5	0.8
KIT	5	0.8
MYC	4	0.6
FGFR1	4	0.6
GNAS	4	0.6
ERBB2	3	0.5
TBX3	3	0.5
FGFR2	2	0.3
PIK3R1	2	0.3
MAP3K13	2	0.3
NCOR1	1	0.2
MAP2K1	1	0.2
FGFR3	1	0.2
RB1	1	0.2
CDK4	1	0.2

Sinead Toomey, Aoife Carr, Yasir Elamin, John Crown



PIK3CA mutation frequency in different solid tumours

Sinead Toomey, Aoife Carr

Table. PI3K pathway Genetic Mutations and Other Aberrations in 1261 Breast, 332 Ovarian, 246 Endometrial Cancers (total 1839 cancers analyzed).

Tumor Type	PIK3CA	PIK3CA	PIK3CB	AKT1	AKT1/2	PTEN	PTEN	INPP4B	PDK1	p70S6	RAS/RAF	HER2	p53	BRCA1	BRCA2
	Mutations	Amplification	Amplification	Mutation	Amplification	Mutation	Protein loss	deletion	Amplification	Amplification	Mutation	Amplification	Mutation	Mutation	Mutation
Breast Total	339/1261 **(26.9%)**	5%	5%	27/1008 (2.6%)	5%	6/209 (2.3%)	25/110 **(22.7%)**	**~20%**	27/129 **(20.9%)**	**30%**	2/406 (0.5%)	15%	46/121 **(38%)**	8%	2%
Breast HR+	101/305 **(33.1%)**			6/232 (2.6%)		4/131 (3.4%)	10/69 (14.5%)	rare	16/79 **(23.2%)**			0	18/73 **(24.6%)**		
Breast HER2+	24/98 **(24.5%)**			0/75		0/33	2/18 (11%)	rare	5/19 **(26.3%)**			**100%**	14/23 **(60.9%)**		
Breast TN	21/262 (8.0%)			0/111		2/41 (4.9%)	11/21 **(52%)**	**60%**	2/15 (13.3%)			0	14/22 **(63.6%)**		
Ovarian	2/332 (0.6%)	**45%**	5%	2/332 (0.6%)	5%	4/132 (3%)	**40%**	**~20%**	rare	rare	12/428 (2.8%)	8%	90/132 (**68%**)	31/235 (13.2%)	12/178 (6.7%)
Endometrial*	**73/246 (30%)**	low	low	3/150 (2.0%)	low	**20/76 (26%)**	**>50%**	8.00%	rate	rare	**44/206 (21%)**	rare	9/96 (9%)	1/199 (0.5%)	2/199 (1%)

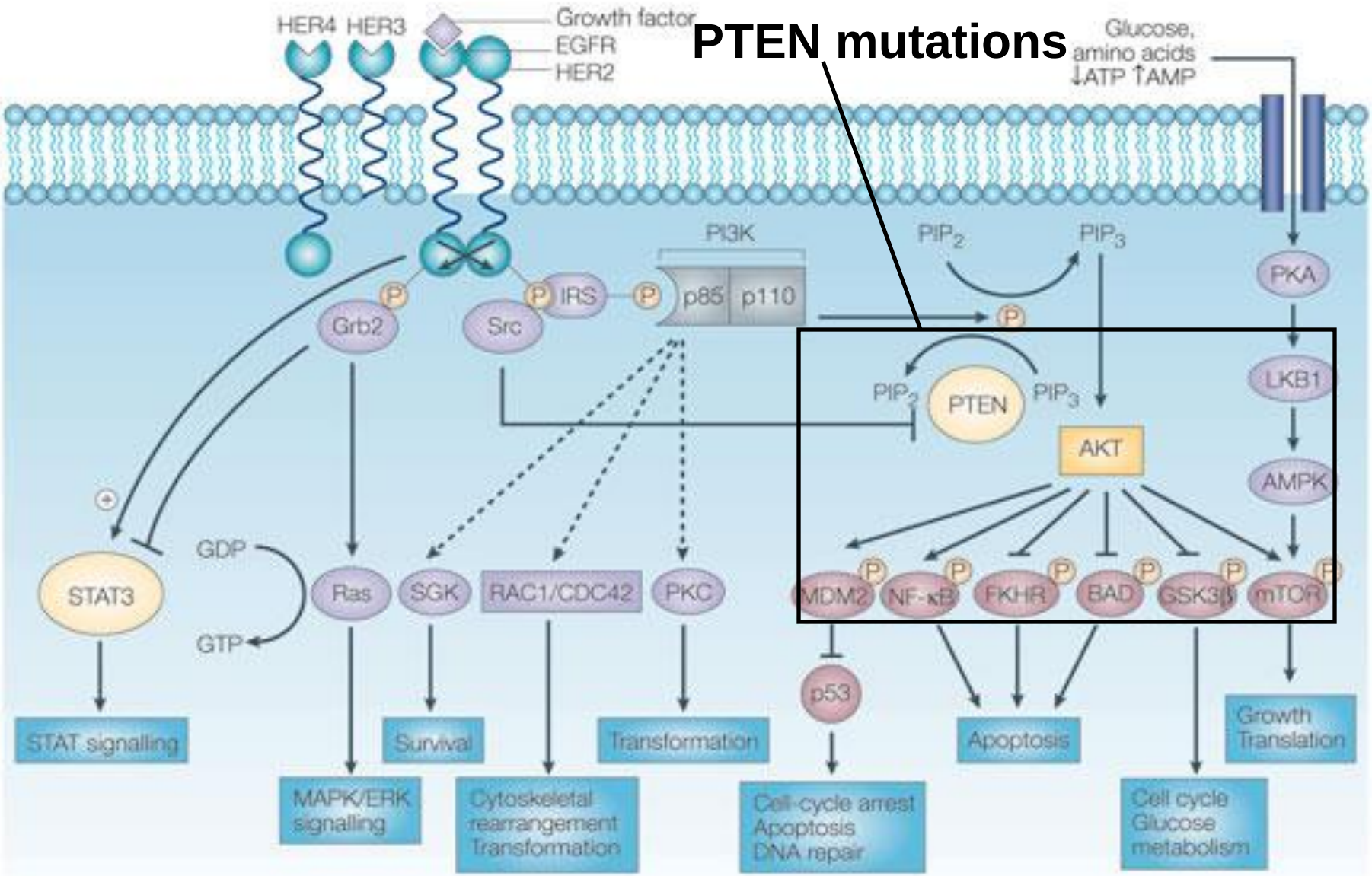
Events that occur in 20% or more of patients are shown in bold.

1. Hennessy BT, et al. *J Clin Oncol* 2005
2. Hale K……..., Mills GB, Hennessy BT. *Cancer Research* 2008
3. Hennessy BT,……Mills GB. *Cancer Research* 2009
4. Gonzalez AM…Hennessy BT. *CCR* 2009

The frequency of activating PI3K pathway genetic aberrations in cancer suggests that the pathway will be a useful target in many human tumors

Defining the functional effects of PI3K pathway mutations and other aberrations in cancer:

What specific isoforms and downstream effectors mediate effects?



Hennessy et al. Nature Reviews Drug Discovery 2005

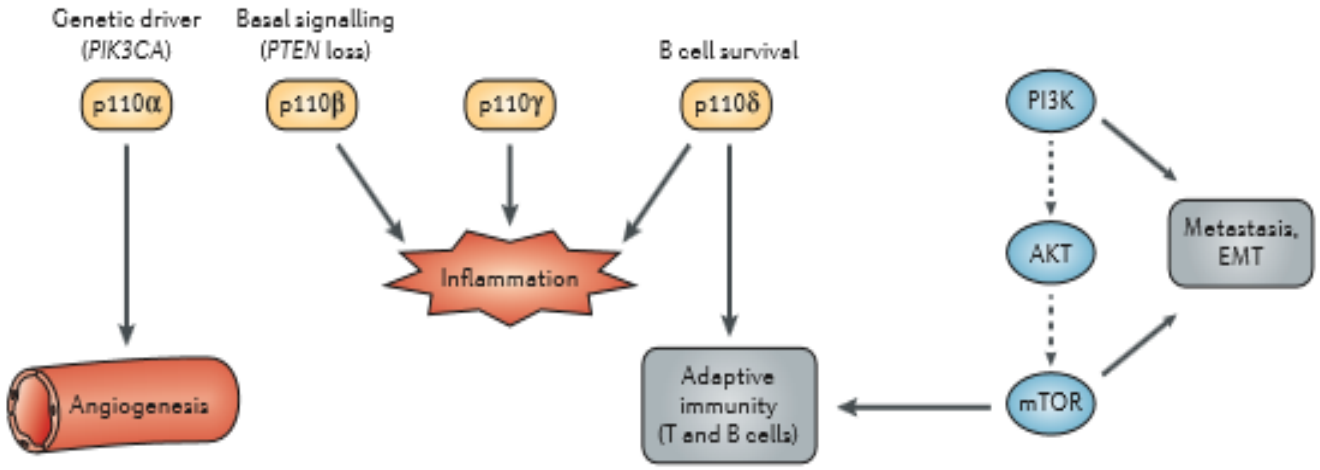
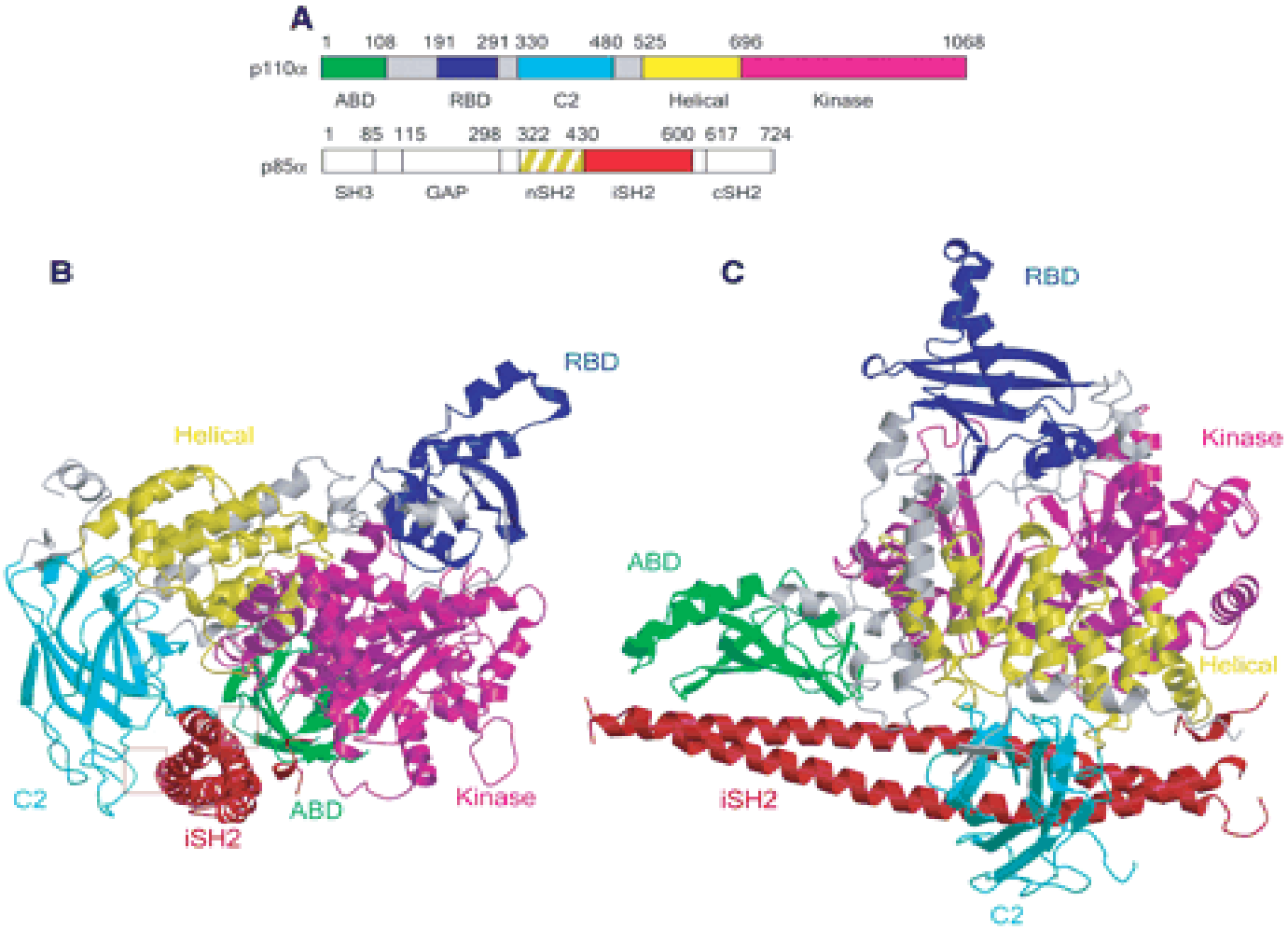


Figure 1 | **Targets in the signalling network and their role in tumour biology.** This diagram shows a highly simplified scheme of the signalling pathway leading from phosphoinositide 3-kinase (PI3K), to AKT, to mammalian target of rapamycin (mTOR). The four isoforms of class I PI3K are shown in orange boxes. The cancer-cell-intrinsic functions of the isoforms are illustrated above: the PI3K catalytic isoform p110α (encoded by *PIK3CA*) is a frequent genetic driver (*PIK3CA* mutations); basal activity of p110β is implicated in tumours with loss of phosphatase and tensin homolog (*PTEN*); and p110δ has a fundamental role in the survival of normal B cells and is implicated in malignancies of this lineage. PI3K and mTOR drive tumour metastasis by promoting cell motility and epithelial–mesenchymal transition (EMT). The bold arrows represent cell-extrinsic functions of various components in the network. p110α drives angiogenesis; p110γ, p110δ and p110β have important functions in inflammatory cells; and p110δ and mTOR control key aspects of adaptive immunity, including lymphocyte activation, differentiation and tolerance. Drugs in clinical development that target the nodes in this network are listed in Supplementary information S1 (table).

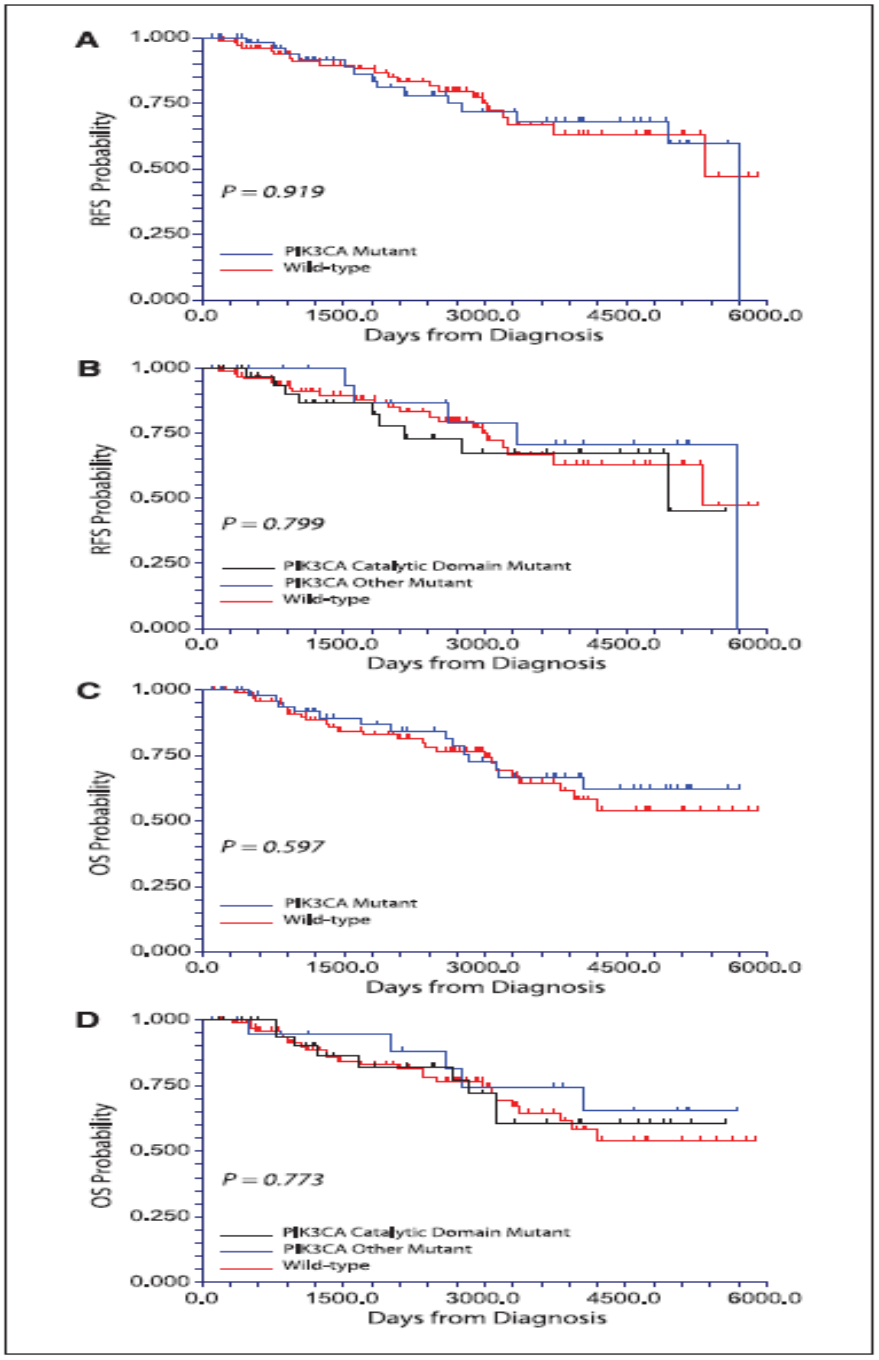
Fruman DA, Rommel C. Nat Rev 2014

Structure of the p110 alpha subunit of PI3K (PIK3CA)



Huang CH et al. Science 2007

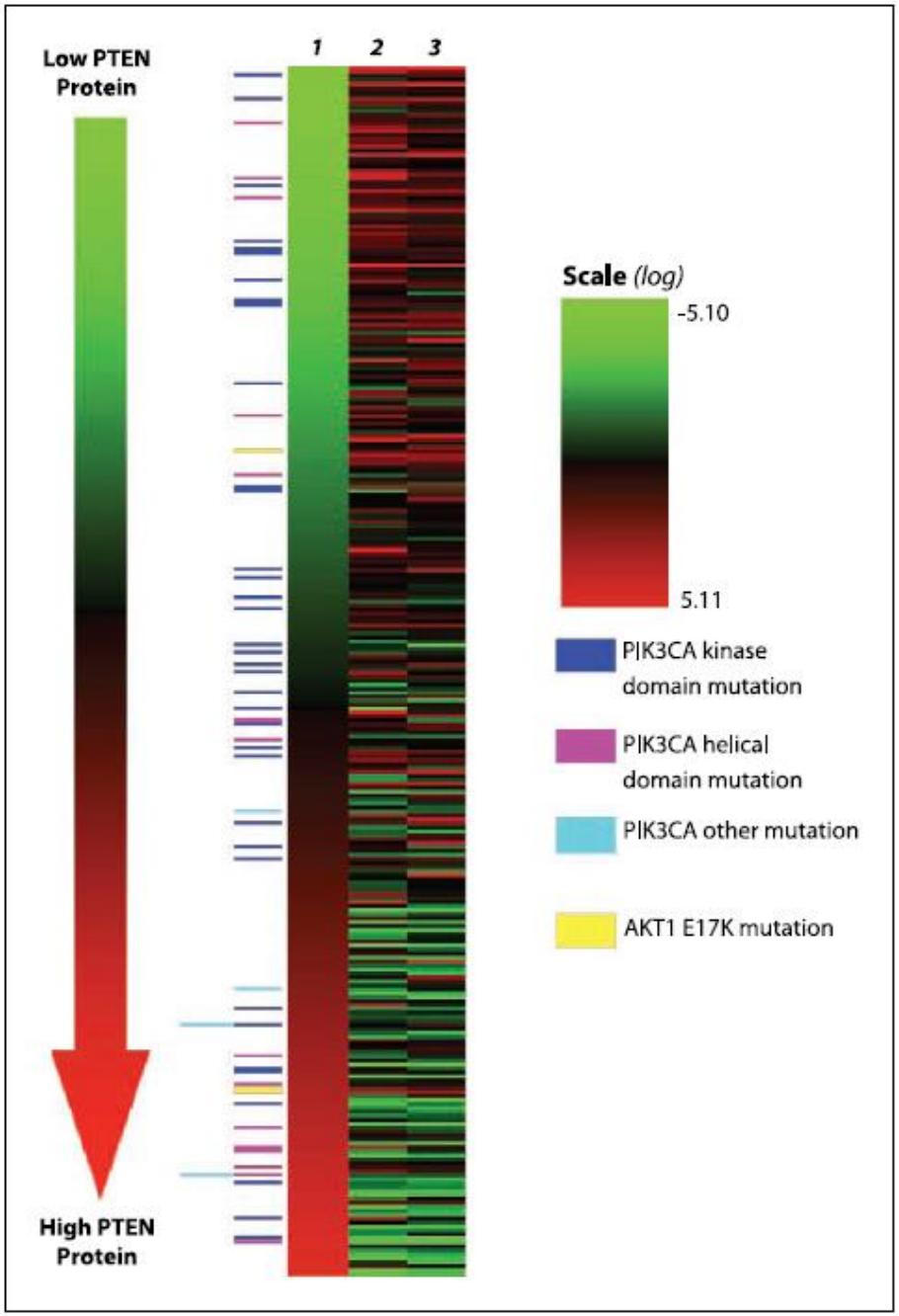
PIK3CA mutations: outcome implications



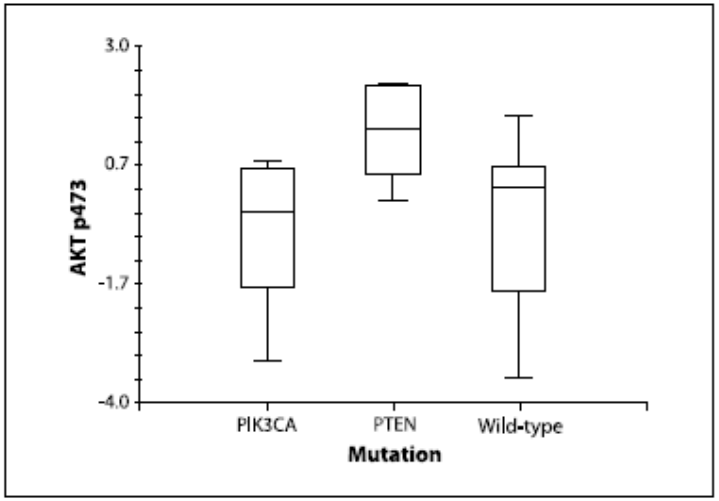
PIK3CA mutations do not impact HR-positive breast cancer patient outcomes after treatment with adjuvant tamoxifen

Hale……..Hennessy BT. Cancer Research 2008

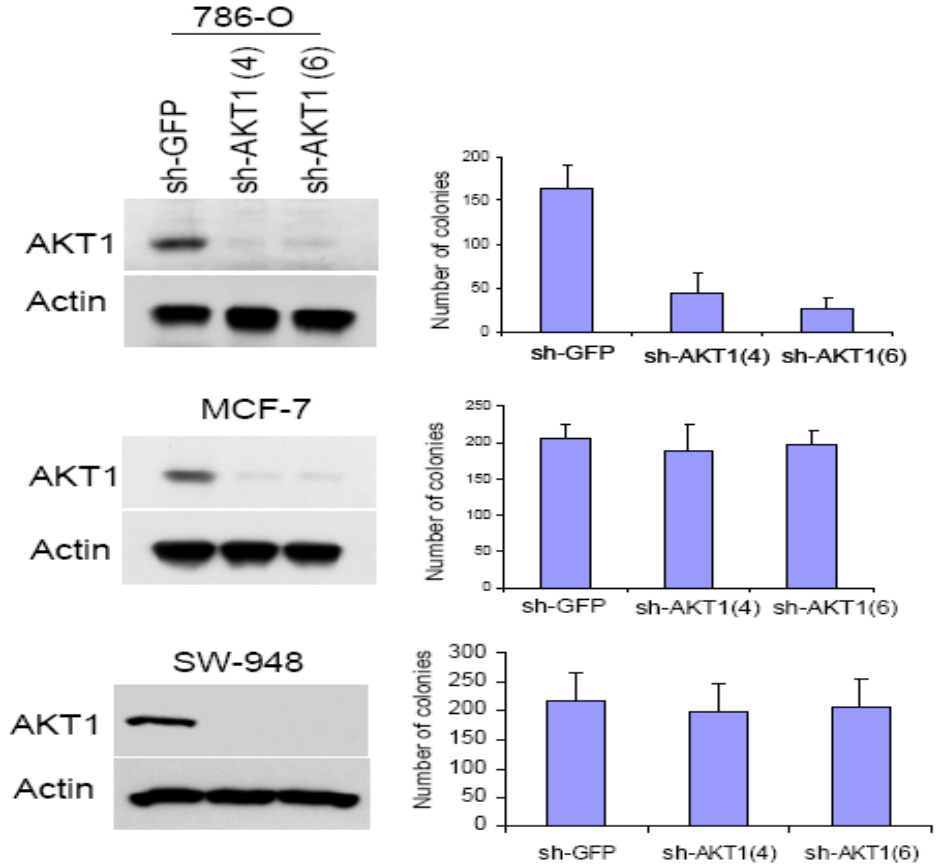
PIK3CA mutations: signaling implications

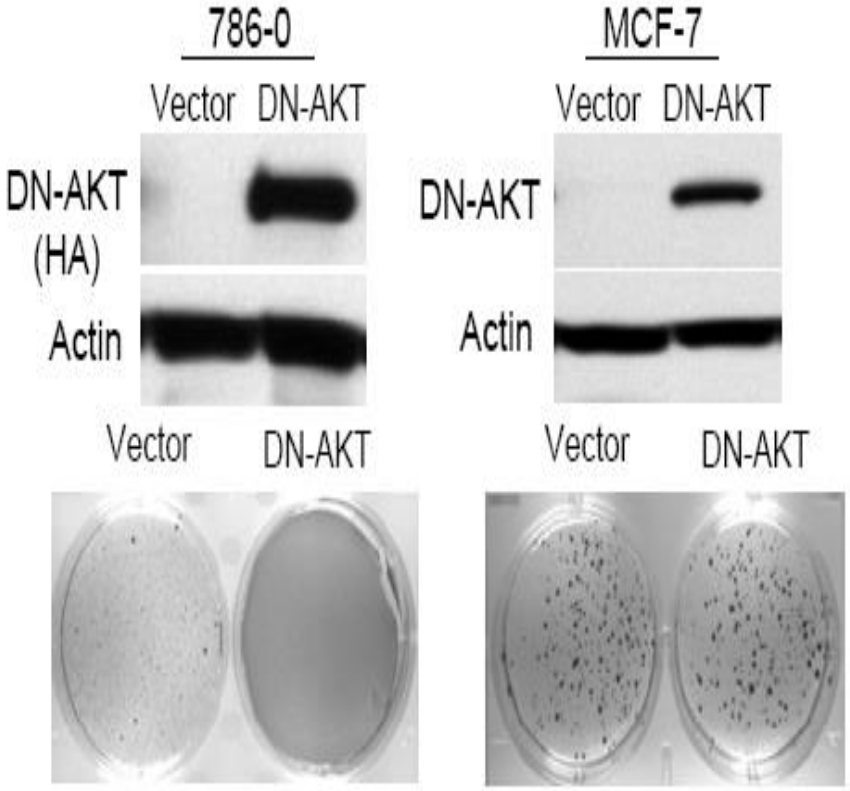


In contrast to PTEN loss, PIK3CA mutations do not lead to consistent activation of AKT in HR-positive breast cancers or cell lines

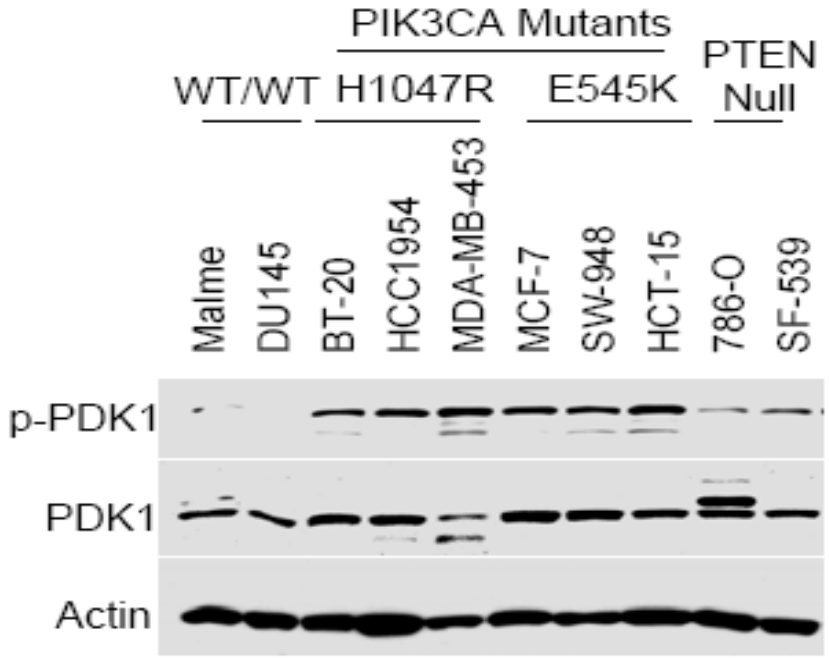


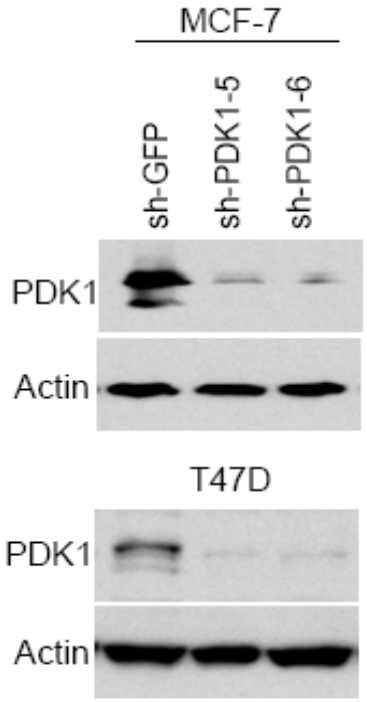
Hale…….....Hennessy BT. Cancer Research 2008

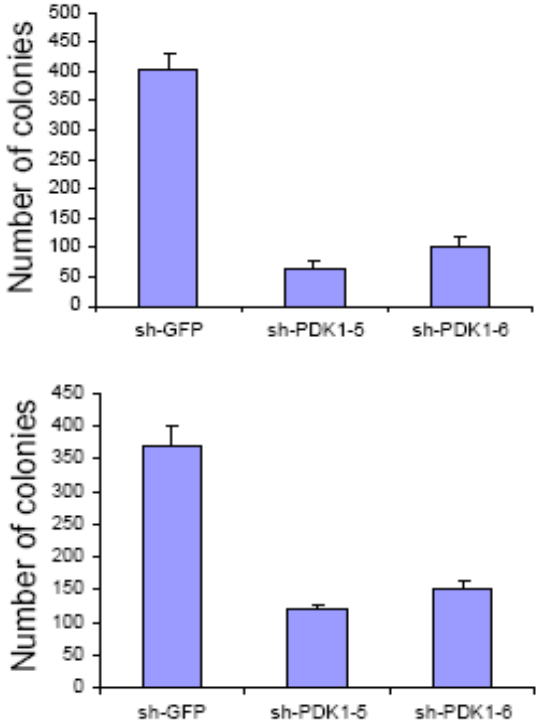




Vasudevan, K.M., et al. Cancer Cell 2009



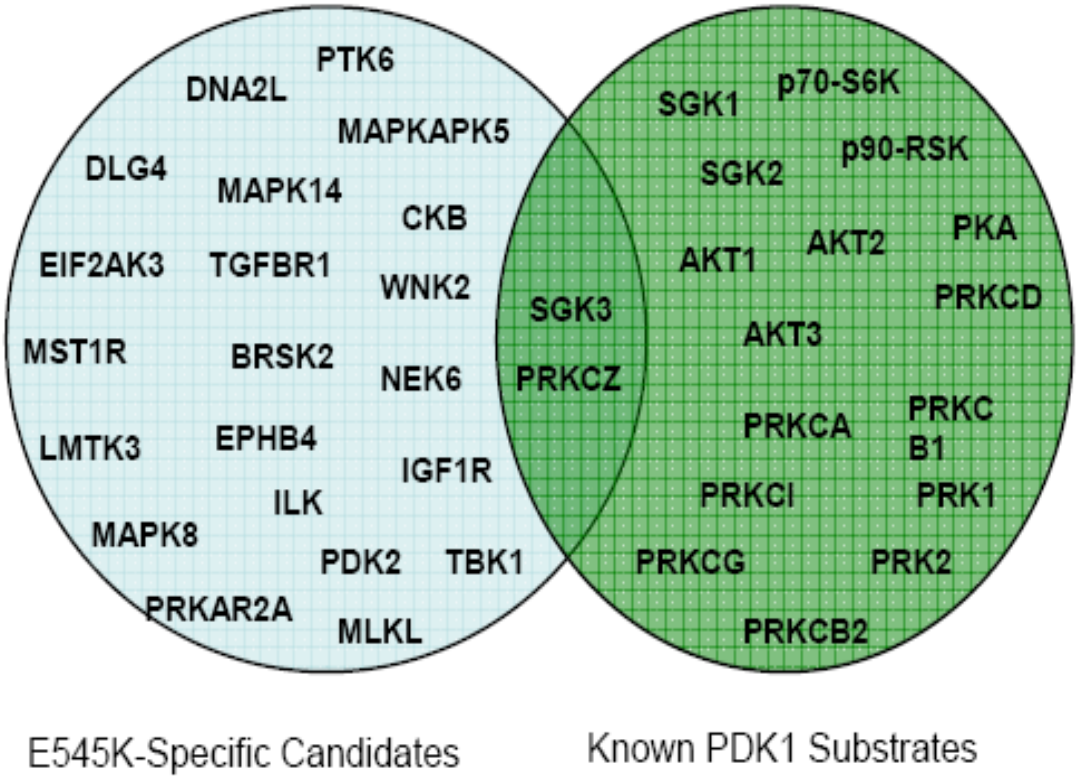




implicate a novel AKT-independent and PDK1-dependent oncogenic signal that may help us to refine new targeted therapeutic opportunities in tumors defined by *PIK3CA* mutations

Vasudevan, K.M., et al. Cancer Cell 2009

PIK3CA mutations: Potential substrates:

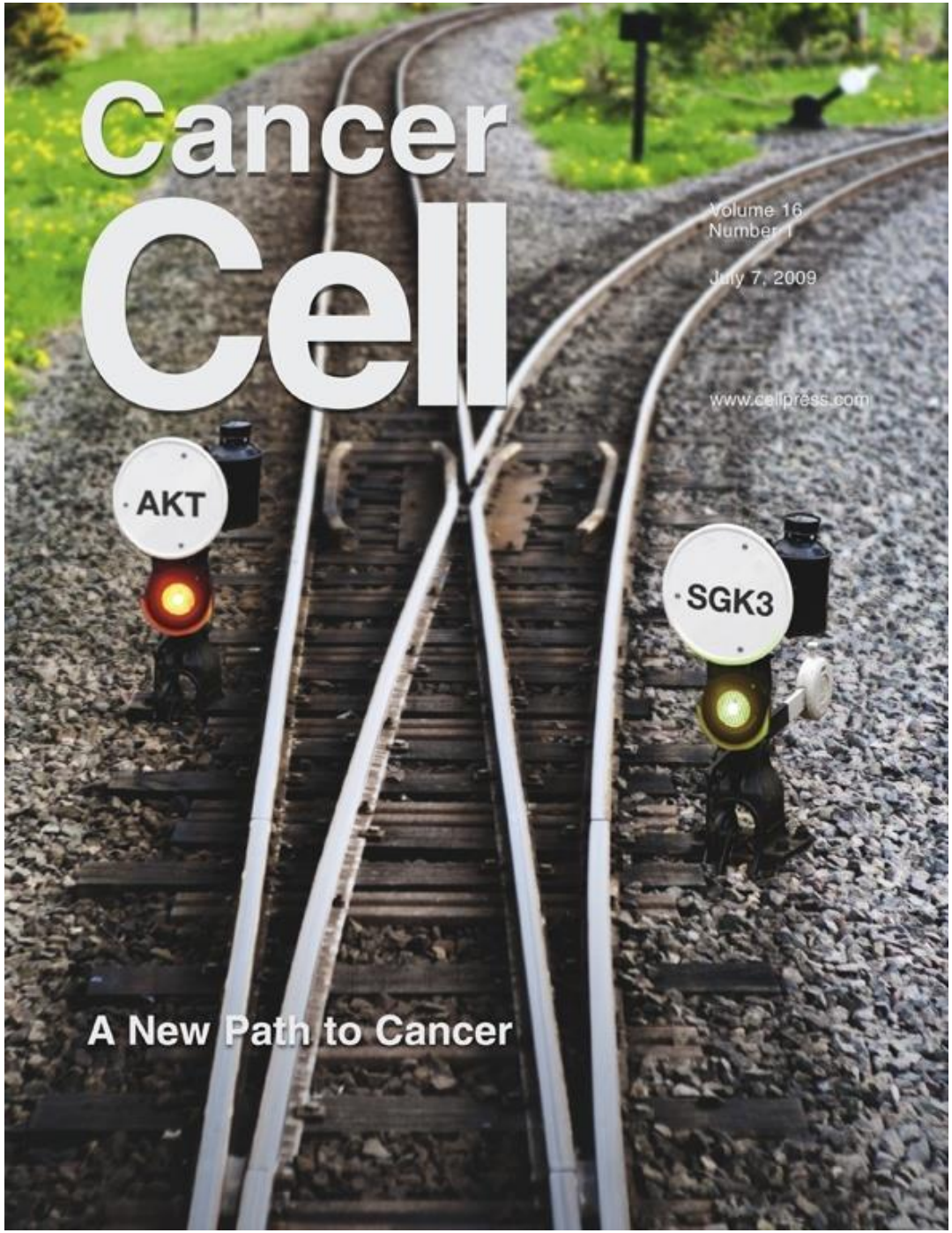


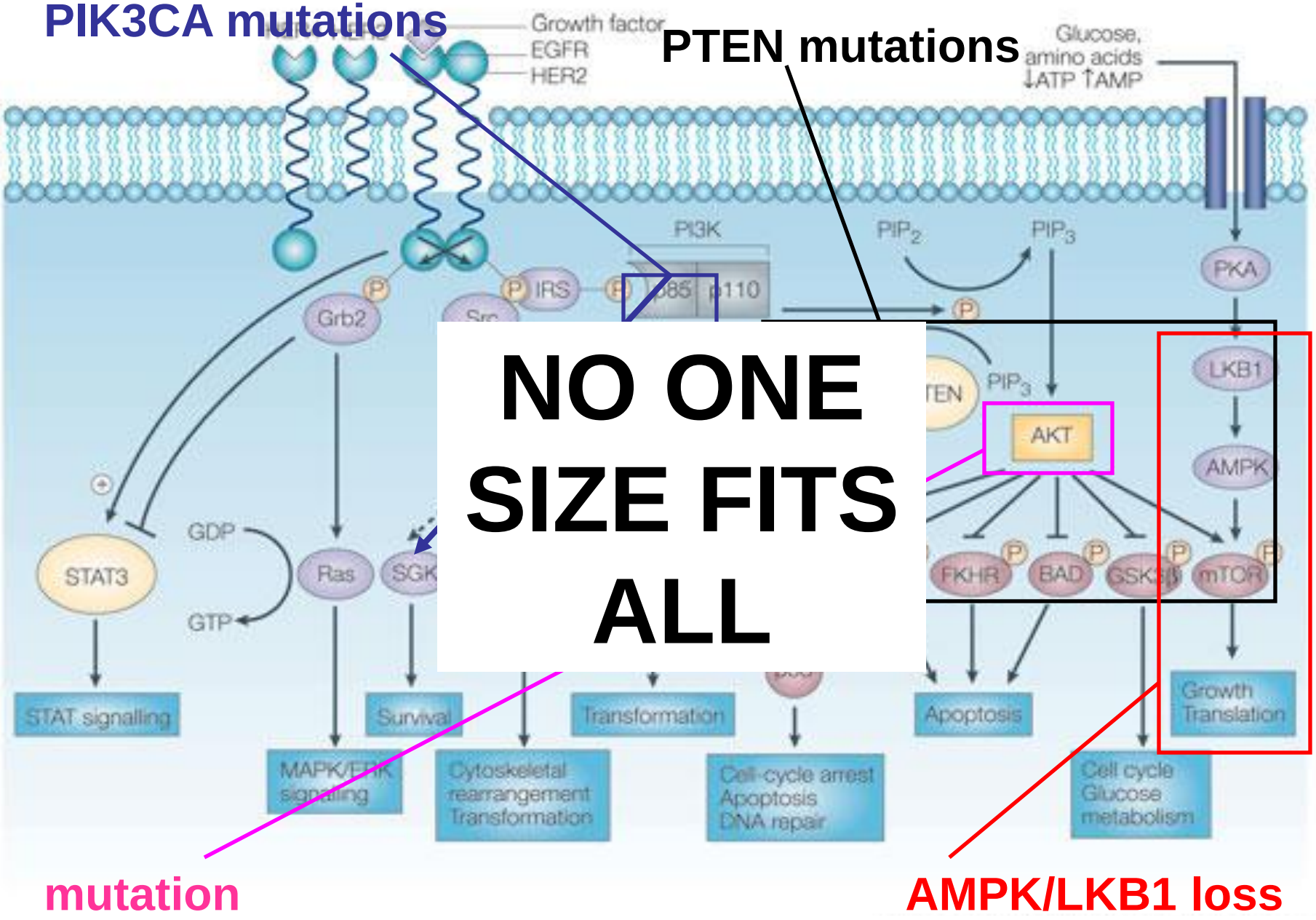
Overlap between:

-synthetic lethality candidates identified in a shRNA screen in a PIK3CA mutant cell line

-known PDK1 substrates

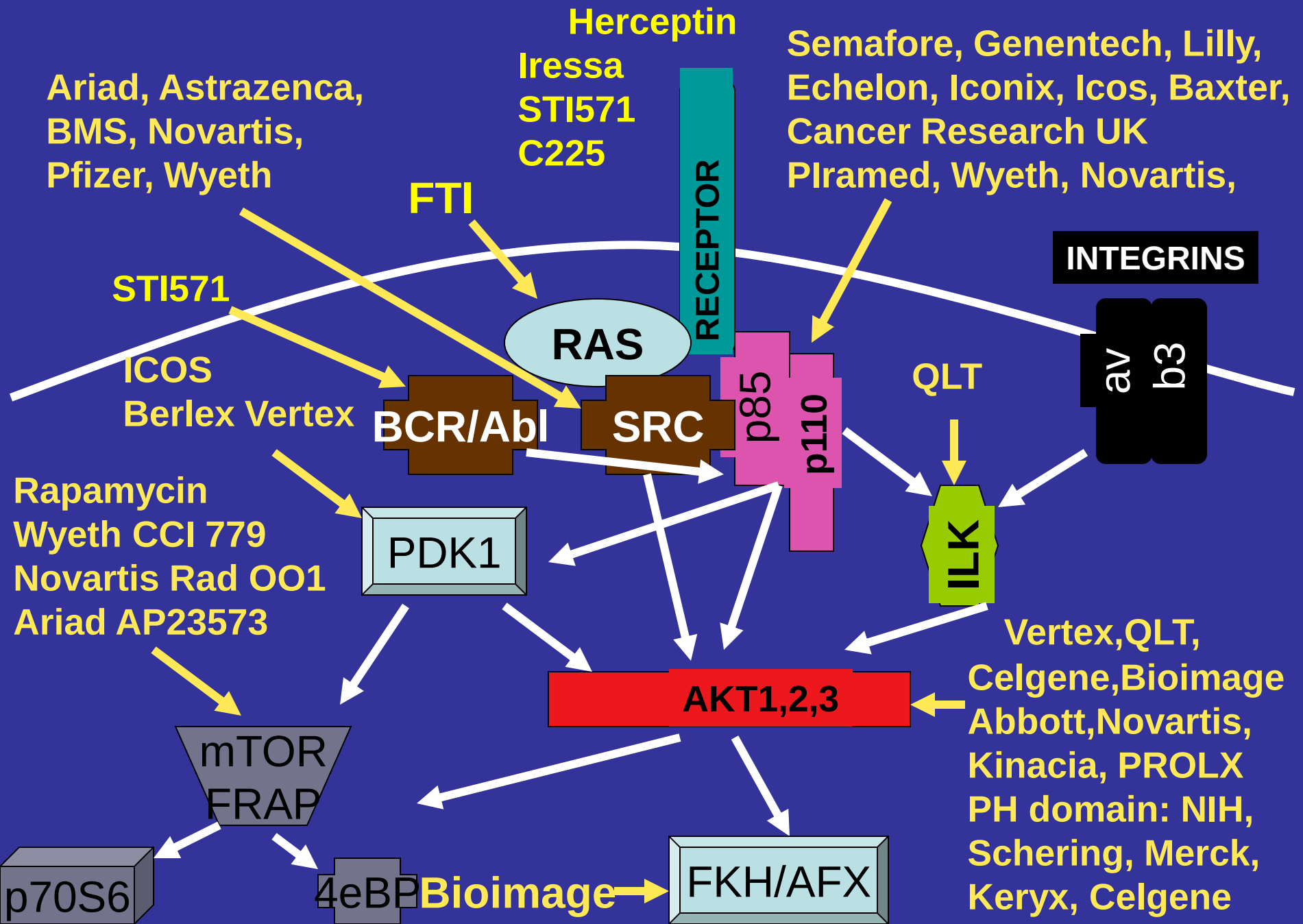
Vasudevan, K.M., et al. Cancer Cell 2009


Cancer
Cell
Volume 16
Number 1
July 7, 2009
www.cellpress.com
AKT
SGK3
A New Path to Cancer

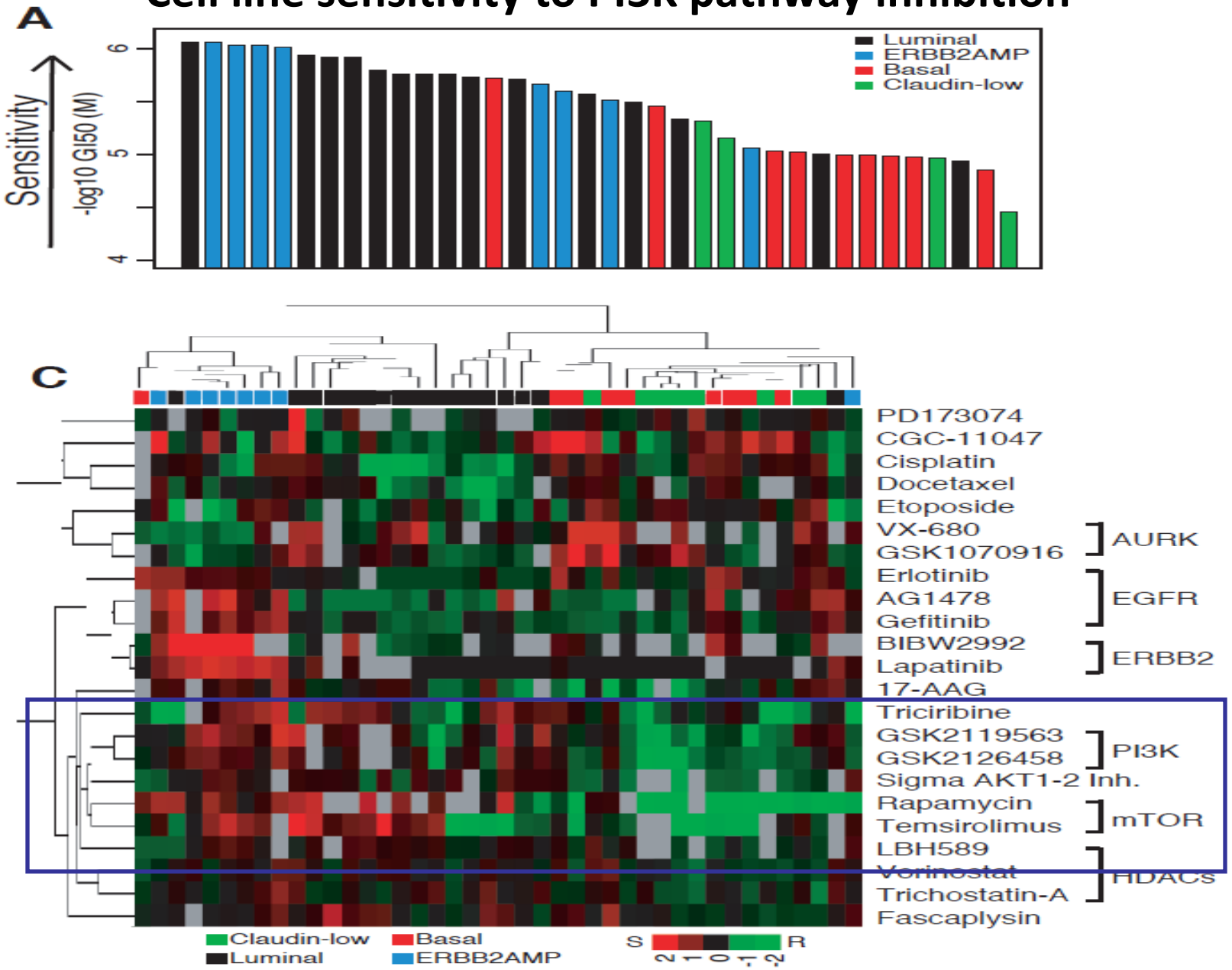


Hennessy et al. Nature Reviews Drug Discovery 2005

PI3K PATHWAY DRUGS IN DEVELOPMENT



Cell line sensitivity to PI3K pathway inhibition

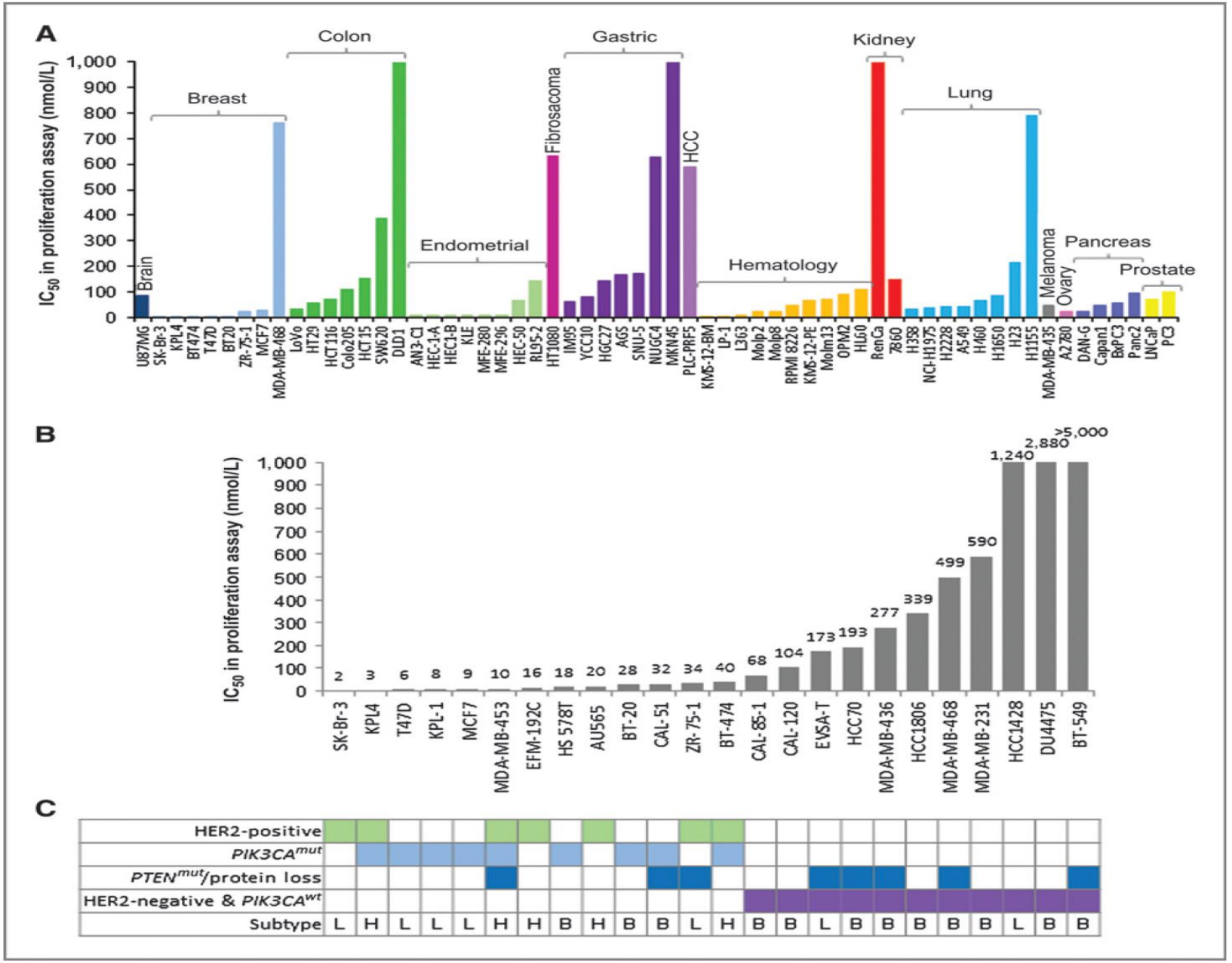


Heiser LM et al. PNAS 2009

PIK3CA-mutated cancers

- p110 alpha-specific inhibitors demonstrate particular preclinical antitumour activity
  - including tumor growth arrest and significant regression in xenograft models

Olivero AG; Genentech team. AACR 2008 (GDC0032)
Liu N et al. Mol Cancer Ther 2013 (BAY80-6946)



AKT phosphorylation by PI3Kα compared with PI3Kβ in cells. BAY 80-6946 showed superior antitumor activity (>40-fold) in *PIK3CA* mutant and/or HER2 overexpression as compared with HER2-negative and wild-type *PIK3CA* breast cancer cell lines. In addition, BAY 80-6946 revealed potent activity to induce apoptosis in a subset of tumor cells with aberrant activation of PI3K as a single agent. *In vivo*, single intravenous

PIK3CA-mutated cancers

- p110 alpha-specific inhibitors demonstrate particular preclinical antitumour activity
  - including tumor growth arrest and significant regression in xenograft models

Olivero AG; Genentech team. AACR 2008 (GDC0032)
Liu N et al. Mol Cancer Ther 2013 (BAY80-6946)

- However, persistent mTORC1 signaling a/w preclinical resistance to p110 alpha inhibitors

Elkabets M et al. Sci Transl Med 2013

PTEN-deficient cancers

- Some studies suggest that p110 beta activity is essential in cancer cells lacking PTEN (particularly in prostate and breast cancers)

- Thus, p110 beta inhibitors may be more effective than p110 alpha inhibitors in PTEN-deficient tumours

- However, this may be tumor type- and context-dependent

Wee S et al. PNAS 2008

Berenjeno IM et al. Biochem J 2012

Role for PI3K pathway aberrations in treatment resistance:

For example, PIK3CA mutations and PTEN loss are associated with trastuzumab resistance in HER2-amplified breast cancer:

Nagata, Y et al. Cancer Cell 2004

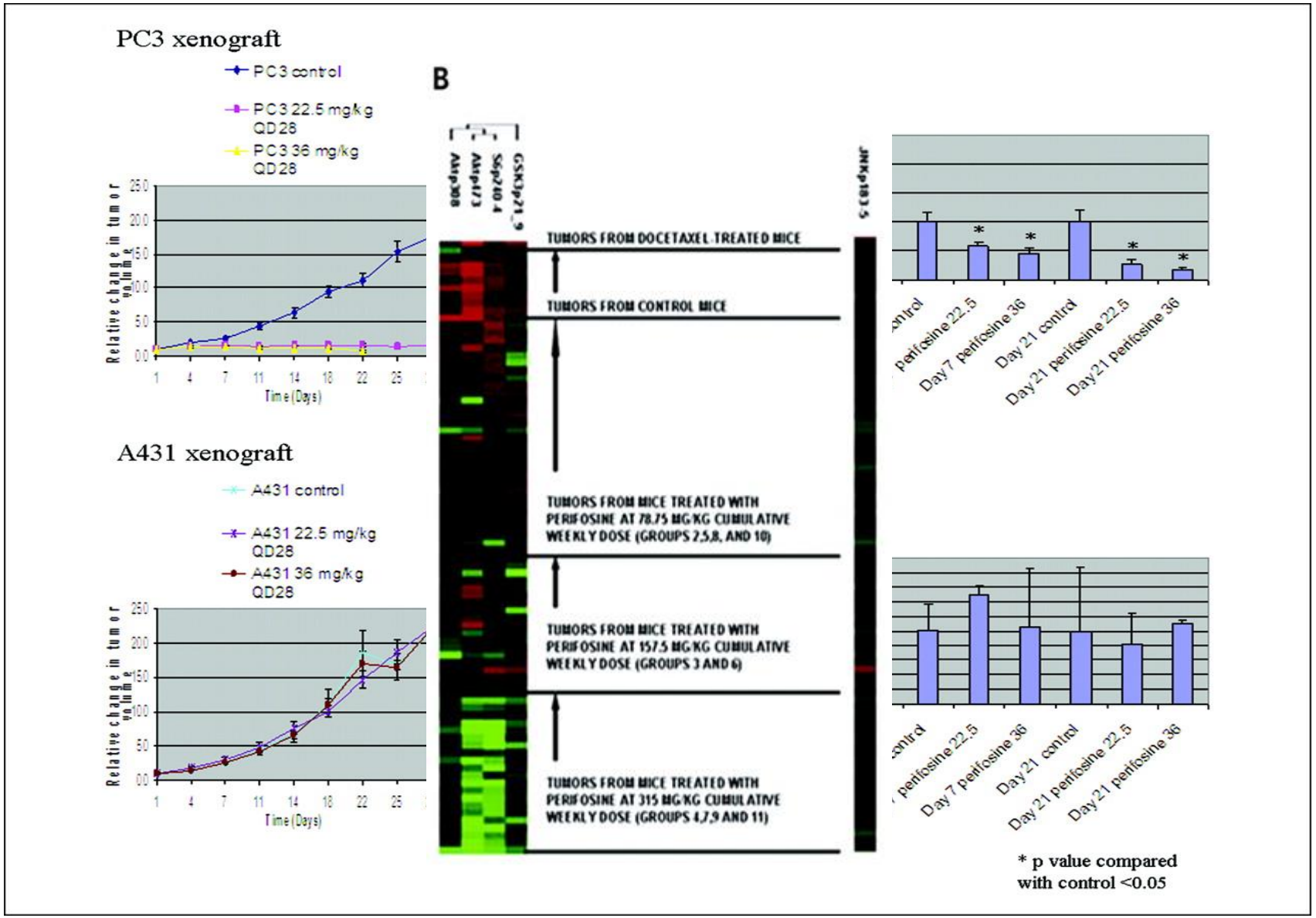
Berns (1st), Horlings (1st), Hennessy et al. Cancer Cell 2007

We are also working on novel methods for identification of PD biomarkers indicating benefit from PI3K pathway inhibitors

- *PD biomarkers:* quantitative protein assays such as high throughput reverse phase protein arrays (RPPA) can objectively and reproducibly quantitate the activity of several proteins and pathways simultaneously in tumors

Hennessy BT, et al. Clin Can Res 2007

RPPA: Pharmacodynamic biomarkers for antitumor efficacy of perifosine, a PH domain inhibitor of AKT



Hennessy, B. T. et al. Clin Cancer Res 2007;13:7421-7431

In summary

- PI3K pathway aberrations are very common in solid cancers
- PI3K pathway aberrations are associated with targeted and cytotoxic therapy resistance
- PI3K pathway inhibitors have potential clinical utility but the optimal inhibitors and combinations in different settings still requires clear definition
- Careful attention to clinical trial design and correlative studies required to define those PI3K pathway inhibitors that result in optimal pathway modulation in human tumors along with the specific biomarkers a/w benefit from treatment

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