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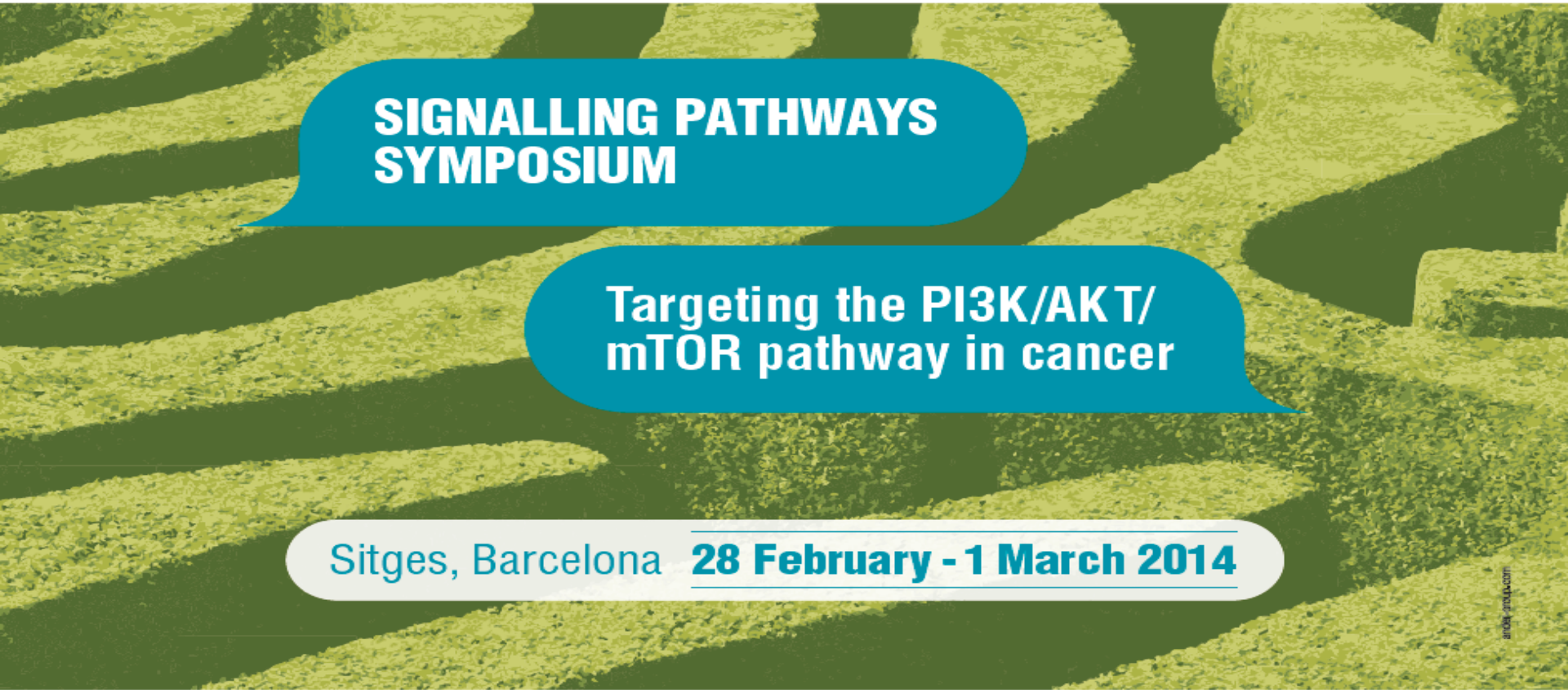

CLINICAL DEVELOPMENT OF PI3K INHIBITORS IN BREAST CANCER

Cristina Saura, MD

DISCLOSURE SLIDE

I have served in Advisory Boards for Puma Biotechnology

OUTLINE

- ✓PI3K/mTOR pathway as a target in breast cancer
- ✓Lessons learned from early drug development of PI3K inhibitors
- ✓Trials in progress
- ✓Outstanding questions and next steps

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- ✓**PI3K/mTOR pathway as a target in breast cancer**
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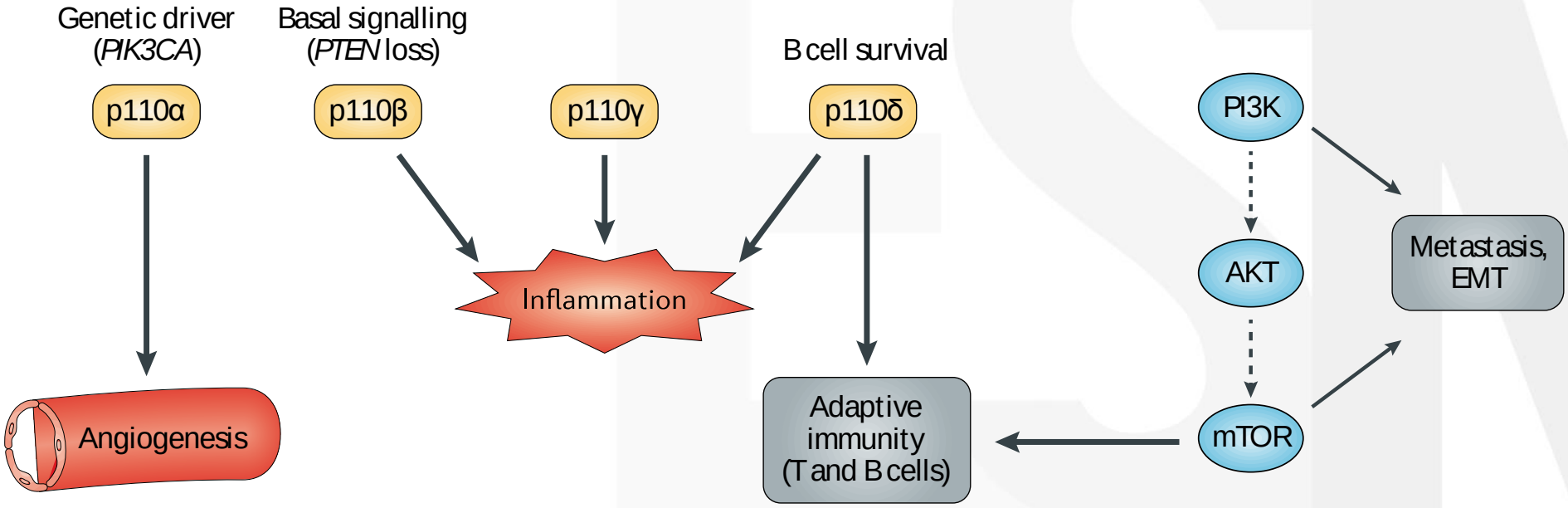
PI3K pathway and relevance in breast cancer

PI3K pathway has a prominent role in cancer cell metabolism, growth, migration, survival and angiogenesis

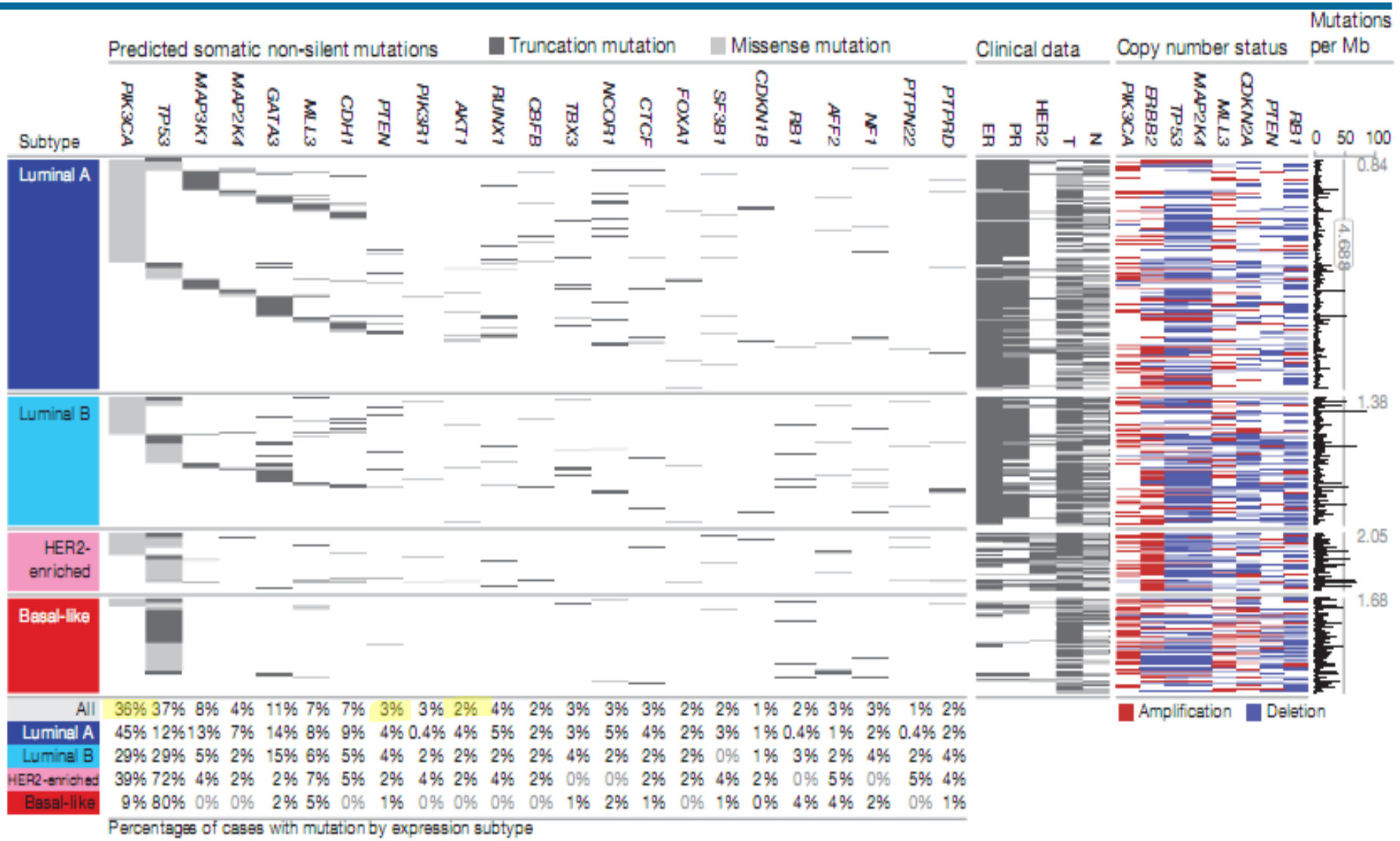


Fruman &Rommel, Nat Rev Drud Discov 2014: 140-156

PI3K pathway and relevance in breast cancer

- Activation of the PI3K pathway is commonly observed in human cancer and is critical for tumor progression and resistance to anti-neoplastic drugs
- PI3K pathway is the most frequently activated pathway in breast cancer

PI3K pathway and relevance in breast cancer



TCGA, Nature 2012: doi:10.1038/nature 11412

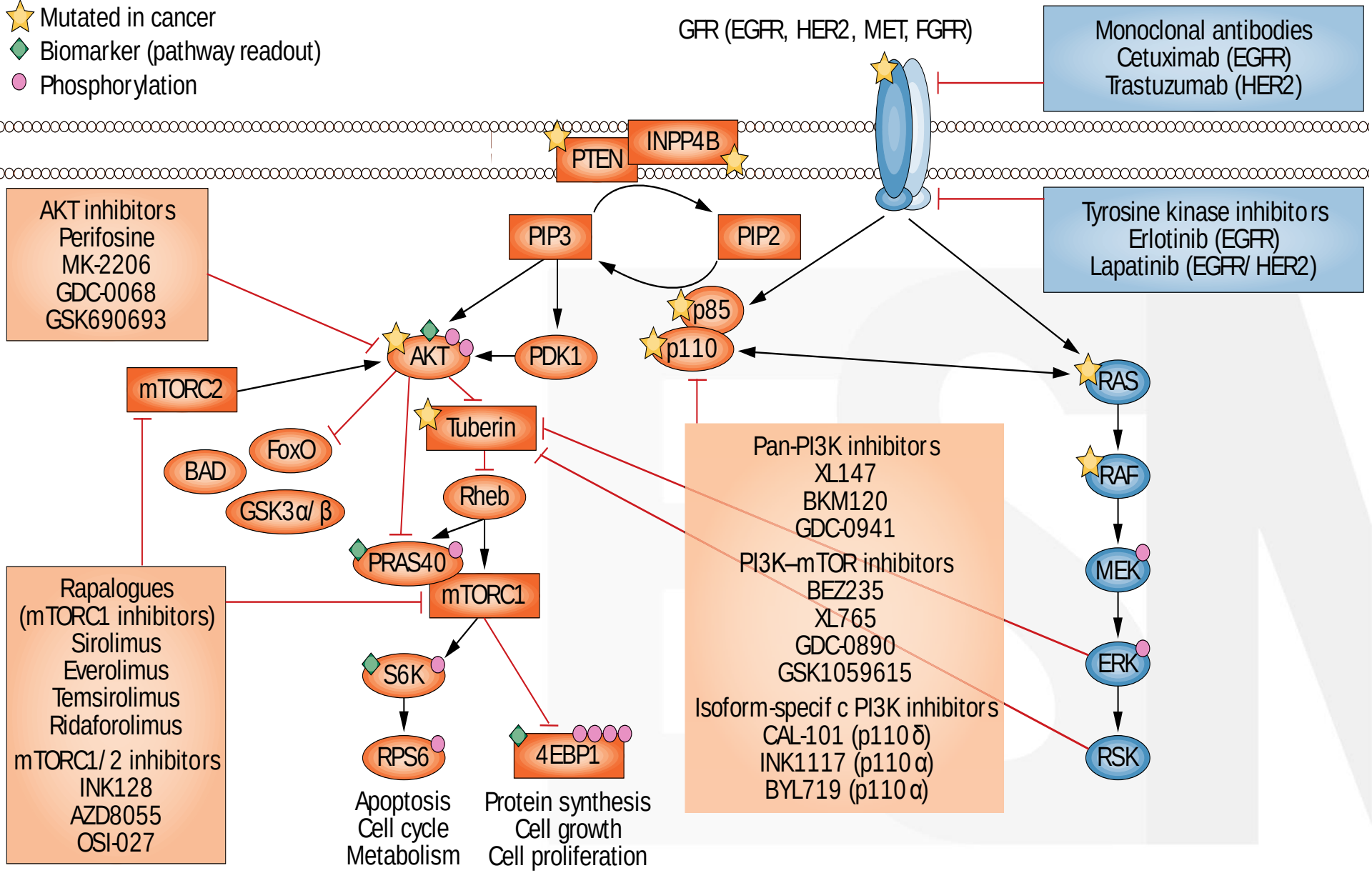
OUTLINE

✓PI3K/mTOR pathway as a target in breast cancer

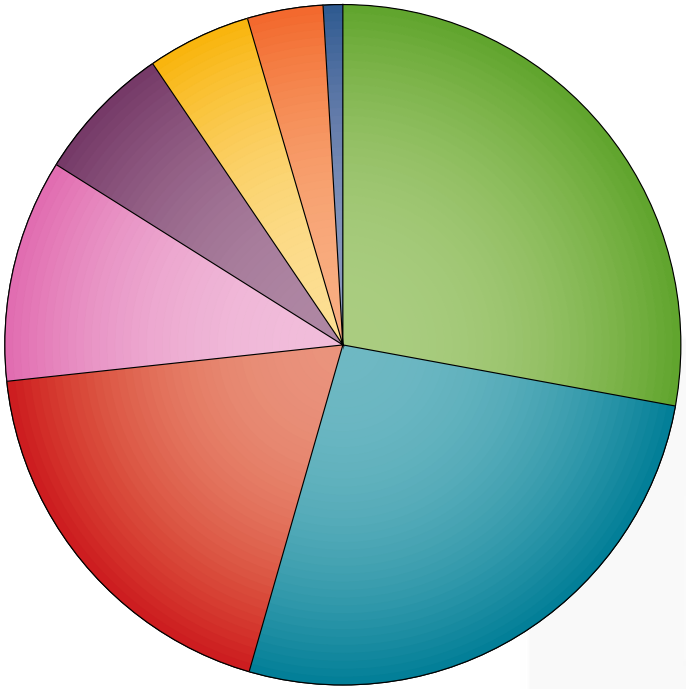
✓**Lessons learned from early drug development of PI3K inhibitors**

✓Trials in progress

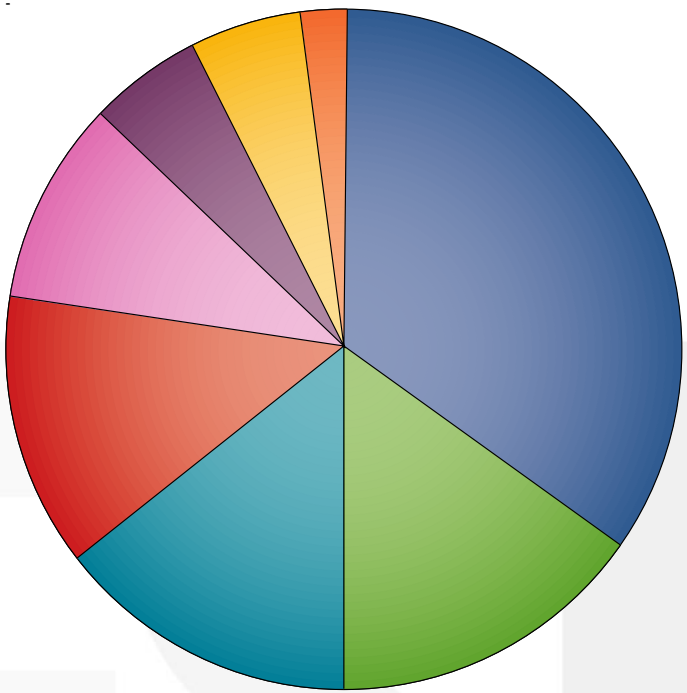
✓Outstanding questions and next steps



Rodon et al, Nat Rev Clinical Oncology 2013



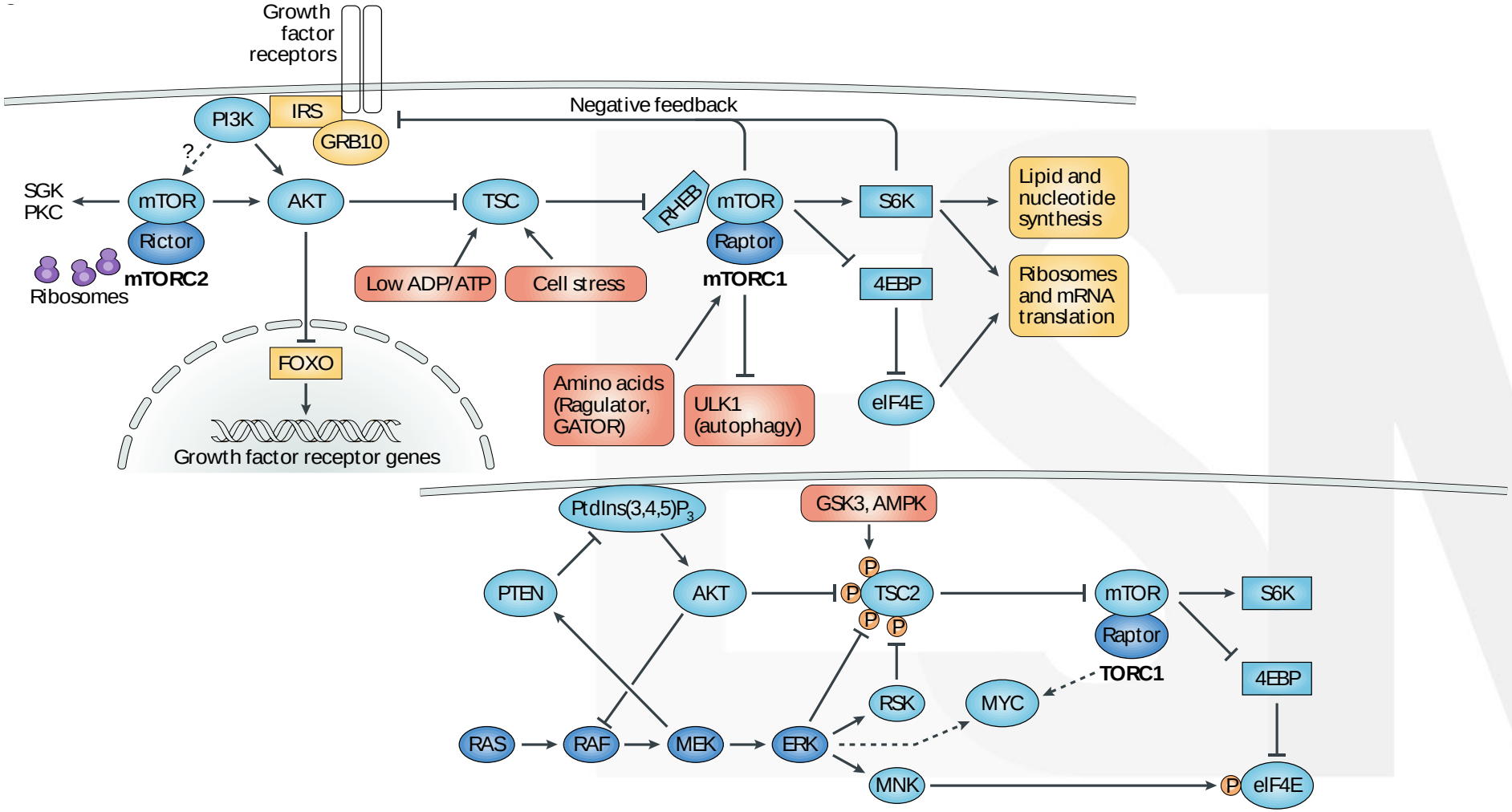
Class of agent
- Pan-PI3K inhibitor (27%)
- AKT inhibitor (27%)
- PI3K–mTOR inhibitor (19%)
- mTORC1–mTORC2 inhibitor (10%)
- PI3Kδ inhibitor (7%)
- Pan-PI3K or PI3K/ mTOR inhibitor (5%)
- PI3Kα inhibitor (4%)
- PI3Kβ inhibitor (1%)



Treatment strategy
- Monotherapy (35%)
- Combination with chemotherapy (15%)
- Combination with MEK inhibitors (14%)
- Combination with chemotherapy and mAb (13%)
- Combination with inhibitor against tyrosine kinase other than MEK (10%)
- Combination with mAb (6%)
- Combination with hormonal therapy (5%)
- Others (2%)

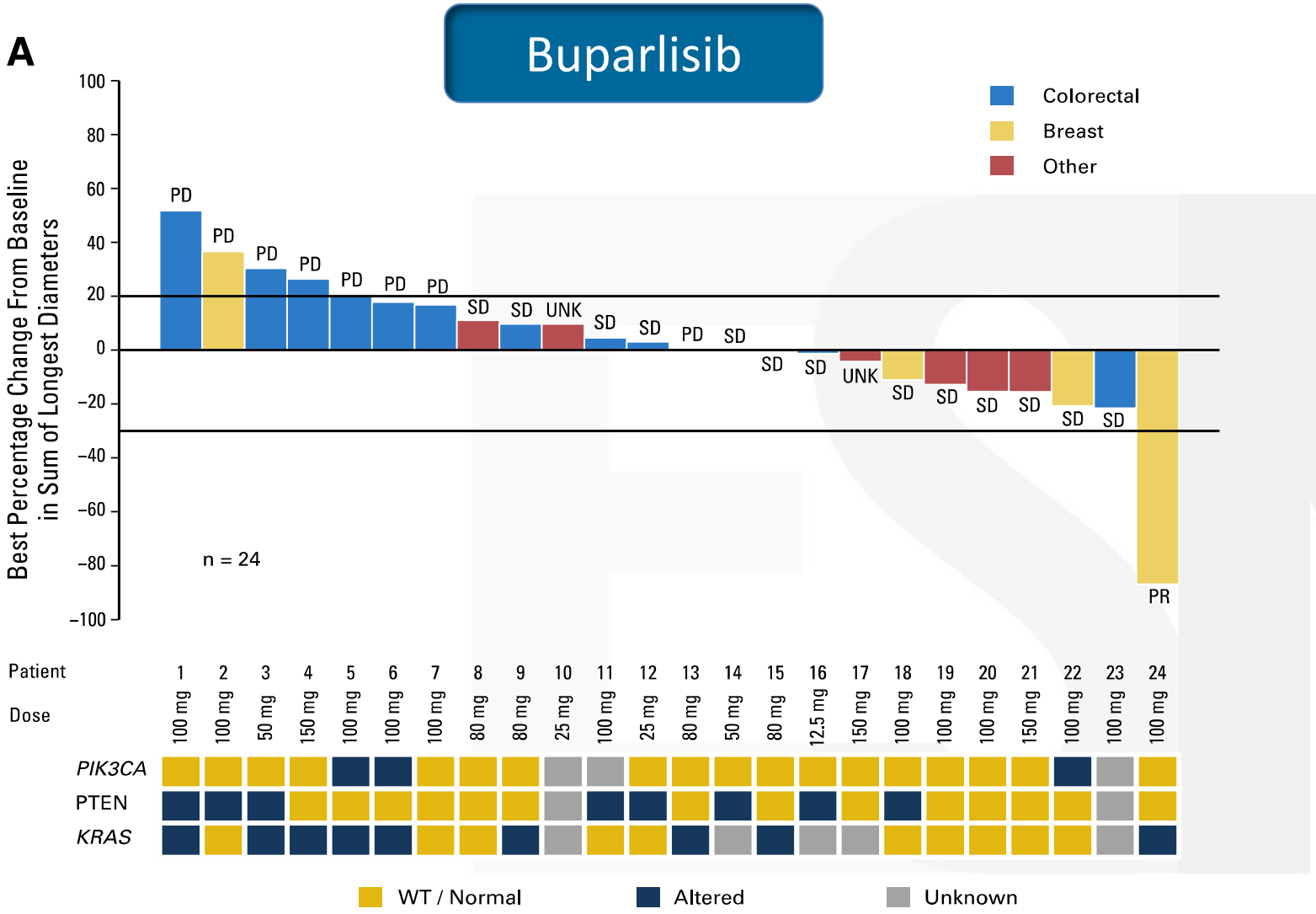
Rodon et al, Nat Rev Clinical Oncology 2013

Development of PI3Ki in breast cancer: alone or in combination?



Fruman &Rommel, Nat Rev Drud Discov 2014: 140-156

There is still some role for monotherapy?



Bendell et al, J Clin Oncol, 2012, 282-290

There is still some role for monotherapy?

XL 147

Tumor type	Best response	Dose, mg	Time on study treatment, days	Number of prior regimens	Most recent prior regimen	Time on last prior regimen, days
NSCLC*	SD	30 21/7	224	4	Gemcitabine/Alvocidib	37
Basal cell†	SD	60 21/7	392	2	Docetaxel	64
NSCLC*	SD	60 21/7	216	5	Pemetrexed	99
NHL† ‡	SD	120 21/7	330	11	Rituximab	207
NSCLC*§	PR	225 21/7	616	4	Pemetrexed	43
Prostate **	SD	600 21/7	198	1	Biclutamide/Leuprorelin	764
NSCLC†	SD	600 21/7	132	4	Gemcitabine	72
Breast† (*HER2* amplified)	SD	600 21/7	160	10	Vinorelbine/Bevacizumab	127
Pancreas†	SD	100 CDD	177	6	Gemcitabine/Capecitabine/ Erlotinib	117
Adenoid cystic†	SD	100 CDD	721	1	Investigational	100
Colon*	SD	400 CDD	200	6	Investigational	55
NSCLC†	SD	600 CDD	168	4	Pemetrexed	366
Tongue (*PIK3CA E545K*)	SD	600 CDD	230	4	Cisplatin/Docetaxel/5FU	7
NSCLC	SD	600 CDD	175	4	Docetaxel	71

*No mutations affecting PI3K pathway detected; †tumor mutational analysis not performed; ‡enrollment of this patient occurred after a special allowance was granted by the sponsor; §Dose escalation to 400mg after 40 weeks; **PSA normalization >5 months. 21/7 = dose administered for the first 21 days of a 28-day cycle; CDD = continuous once-daily dosing; NA = not applicable; NHL = non-Hodgkin lymphoma; NSCLC = non-small cell lung cancer; PR = partial response; SD = stable disease.

ESMO

Shapiro et al, Clin Cancer Res, 2103

There is still some role for monotherapy?

GDC0941

Study GDC4254g: Clinical Activity

- Three patients were on study for ≥ 4 months (Table 2).
- One patient with metastatic melanoma had a confirmed partial response by RECIST.

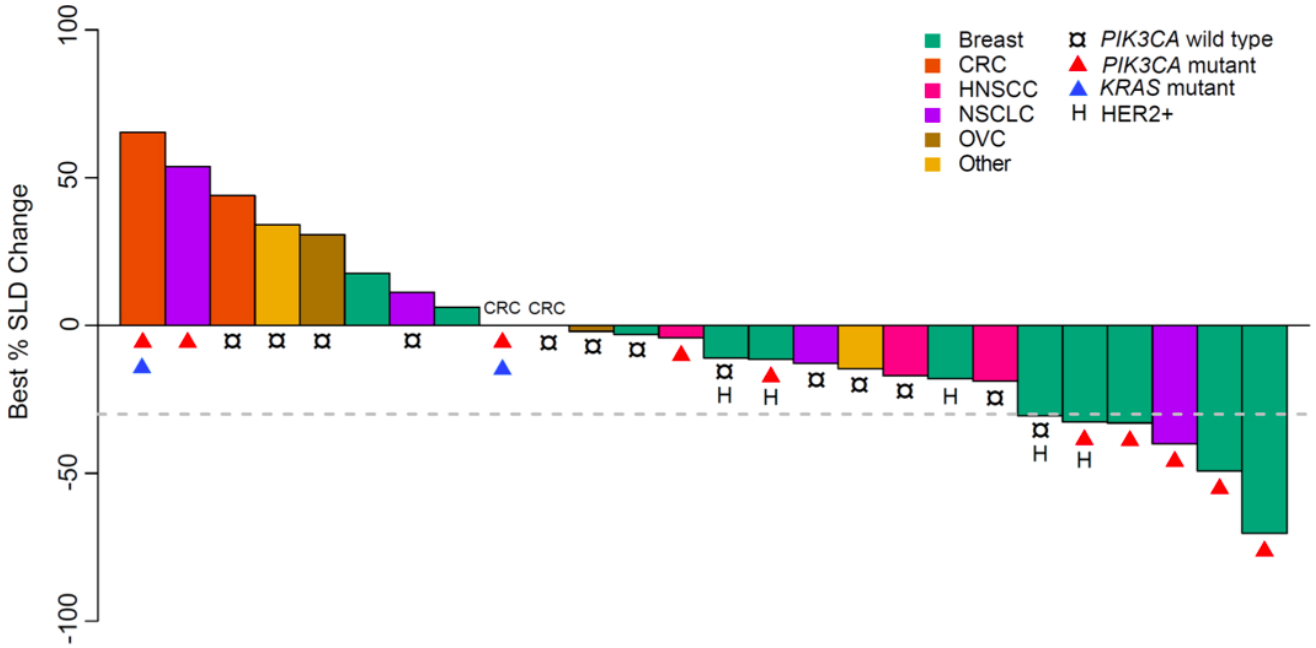
Diagnosis (Year) (Patient ID)	GDC-0941 QD Dose	Months	Day 15 AUC_{0-24h} (μM•hr)	Best RECIST Response[a]	Best FDG-PET Response[b]	Best CA-125 Response	pS6 Change[c]	pAKT Change[d]	Pathway Alteration
Melanoma (2005) (50033)	330 mg	9.6	15.4	- 39.2%	- 0.7%	---	ND	- 73%	BRAF V600E
GIST (2006) (50036)	450 mg	7.5	23.0	- 1.6%	- 49.4%	---	- 75%	Pend.	Pend.
Ovarian (2004) (50020)	100 mg	4.5	9.2	+ 4.4%	- 29.9%	- 86%[e]	- 56%	- 90%	PTEN Negative

ND = Not Done.
a) % change from baseline in sum of longest diameter of target lesions.
b) % change from baseline in mean SUV_{max} of regions of interest.
c) % change in pS6 levels in pretreatment and Cycle 1 tumor biopsies.
d) Maximum % change from baseline in pAKT measured on Day 1 after a single dose of GDC-0941
e) Represents change from highest to lowest value during treatment. CA-125 levels increased rapidly before treatment initiation. Change from screening value was -67%.

Moreno et al, ASCO 2011

There is still some role for monotherapy?

GDC0032



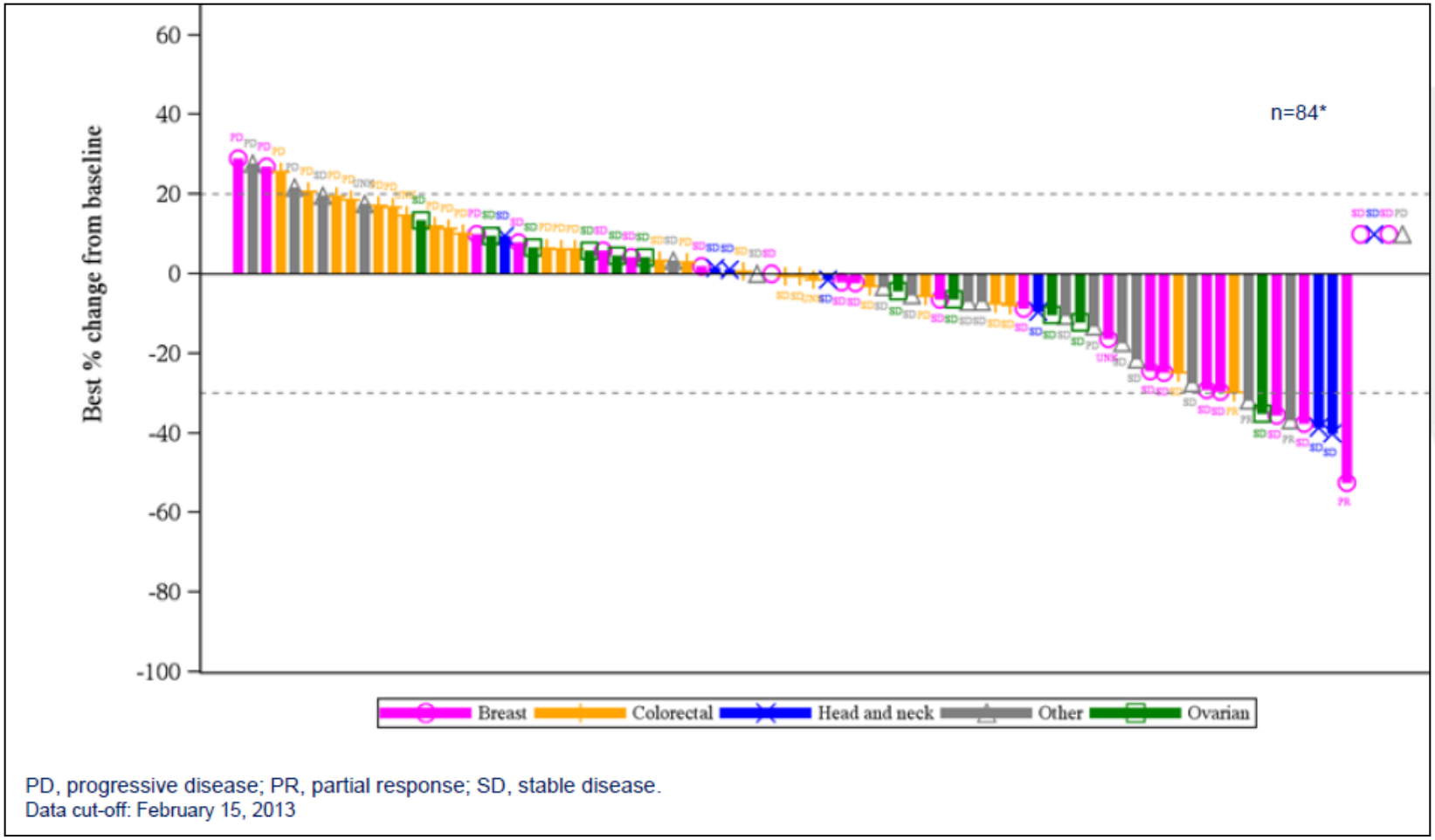
- Promising preliminary clinical activity in *PIK3CA* mutant cancers, especially breast cancer.
- *PIK3CA* mutant solid tumors: 5 cPR (at 3-12 mg QD) and 4 SD out of 12 pts
- *PIK3CA* mutant breast cancer: 4 cPR (RECIST -30 to -70%) and 2 SD out of 6 pts

Pts shown had measurable disease with pre-treatment and on-study tumor assessments as of 30 Nov 2012

Juric et al, AACR 2013

There is still some role for monotherapy?

BYL719



PD, progressive disease; PR, partial response; SD, stable disease.
Data cut-off: February 15, 2013

Rodón et al , AACR 2013

PI3K pathway inhibitors against breast cancer

Agent	Trial	Description	Patients (*n*)
mTORC1/2 inhibitors			
INK128 (Intellikine)	Phase I (NCT01351350)	Dose escalation in combination with paclitaxel ± trastuzumab	Advanced or metastatic solid tumours (95)
AZD2014 (AstraZeneca)	Phase I (NCT01597388)	Safety and tolerability in combination with fulvestrant	ER+ advanced MBC (30)
Dual PI3K–mTOR inhibitors			
XL765 (Sanofi)	Phase I–II (NCT01082068)	Dose escalation in combination with letrozole	HR+, HER2– recurrent or MBC (99)
BEZ235 (Novartis)	Phase I (NCT01248494) Phase I–II (NCT01471847) Phase I (NCT01285466)	Safety and tolerability in combination with endocrine therapy Dose escalation in combination with trastuzumab Dose escalation in combination with paclitaxel ± trastuzumab	HR+ MBC (72) HER2+ locally advanced MBC (5) HER2+ MBC (72)
GDC-0980 (Genentech)	Phase II (NCT01437566)	Safety and efficacy in combination with fulvestrant versus fulvestrant	ER+ locally advanced or MBC (270)
GSK2126458 (GlaxoSmithKline)	Phase I (NCT00972686)	Dose escalation, first in human	Solid tumours or lymphoma (150)
Pan-PI3K inhibitors			
XL147 (Sanofi)	Phase I–II (NCT01042925) Phase I–II (NCT01082068)	Study in combination with trastuzumab ± paclitaxel Dose escalation in combination with letrozole	HER2+ MBC with progression on trastuzumab (42) HR+, HER2– recurrent or MBC (99)
BKM120 (Novartis)	Phase II (NCT01572727) Phase III (NCT01633060) Phase III (NCT01610284)	Study in combination with paclitaxel Study in combination with fulvestrant Study in combination with fulvestrant	HER2– locally advanced or MBC with or without PI3K activation (200) HR+, HER2–, AI treated, locally advanced or MBC that progressed on or after mTOR inhibitor therapy (615) HR+, HER2– locally advanced or MBC refractory to AI (842)
GDC-0941 (Genentech)	Phase II (NCT01437566)	Safety and efficacy in combination with fulvestrant versus fulvestrant	ER+ locally advanced or MBC (270)
PI3Kα inhibitors			
BYL719 (Novartis)	Phase I (NCT01219699)	Dose escalation ± fulvestrant	Advanced solid malignancies (140)
GDC-0032 (Genentech)	Phase I (NCT01296555)	Dose escalation ± fulvestrant and letrozole	Locally advanced or metastatic solid tumours (122)
PI3Kβ inhibitor			
GSK2636771 (GlaxoSmithKline)	Phase I–IIa (NCT01458067)	Dose escalation	Advanced solid tumours with PTEN deficiency (150)
AKT inhibitors			
MK-2206 (Merck)	Phase I (NCT01344031) Phase II (NCT01277757)	Dose escalation + anastrozole, letrozole, exemestane, or fulvestrant Efficacy	ER+ MBC (54) Advanced BC with a *PIK3CA* mutation and/or PTEN loss (40)
AZD5363 (AstraZeneca)	Phase I (NCT01625286)	Safety, tolerability and efficacy in combination with paclitaxel	Advanced ER+ BC (110)

Zardavas & Baselga & Piccart, Nat Rev Clin Oncol 2013: 191-210

What limits or enhances the development of a PI3Ki?

- ✓**Toxicity profile: manageable?**
  - ✓Hyperglycemia
  - ✓Rash
  - ✓Gastrointestinal tolerance: anorexia, nausea, vomiting, dyspepsia, diarrhea
  - ✓Stomatitis
- ✓**Preliminary signs of activity**
- ✓**Strategic decisions of the company that owns the drug**

OUTLINE

- ✓PI3K/mTOR pathway as a target in breast cancer
- ✓Lessons learned from early drug development of PI3K inhibitors
- ✓**Trials in progress**
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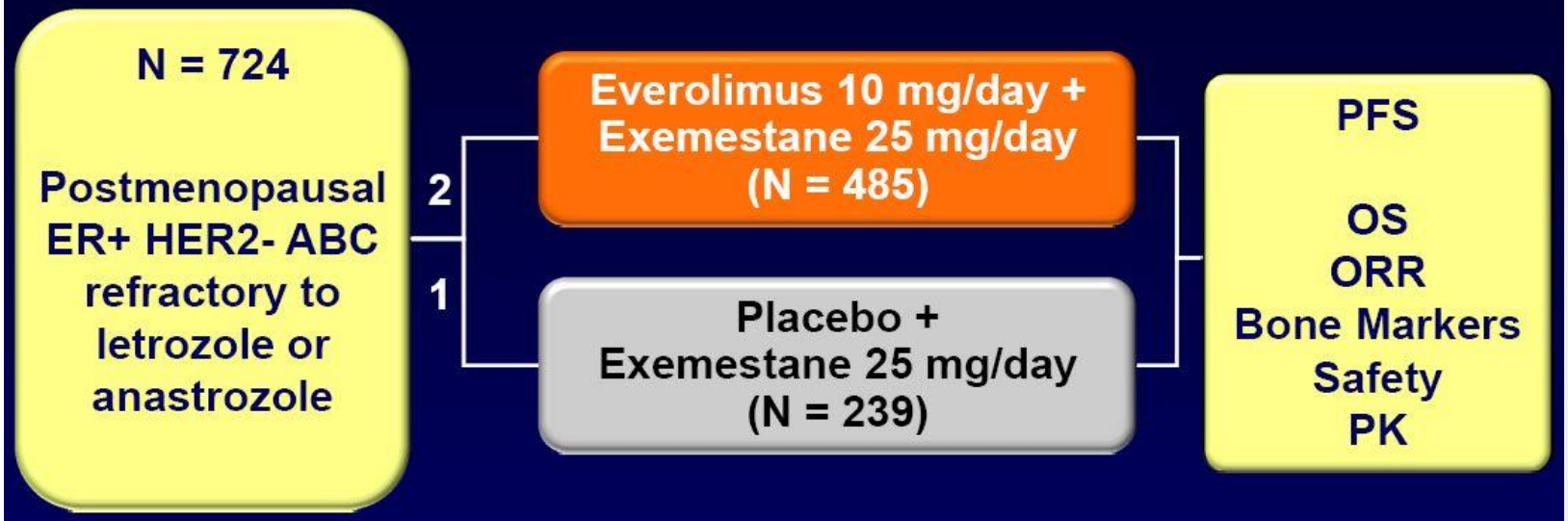
BOLERO-2: Trial Design



- **Stratification:**
  1. **Sensitivity to prior hormonal therapy**
  2. **Presence of visceral disease**
- **No crossover**

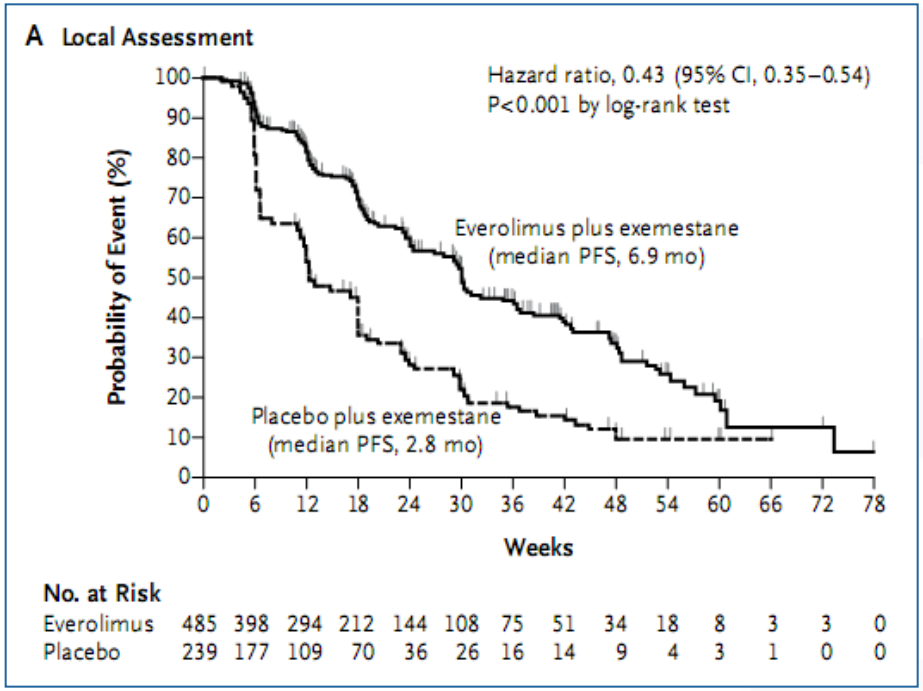
ABC: advanced breast cancer, NSAI: non steroidal aromatase inhibitors, HER2-: human epidermal growth factor receptor 2 – negative; PFS: progression-free survival; PK: pharmacokinetics

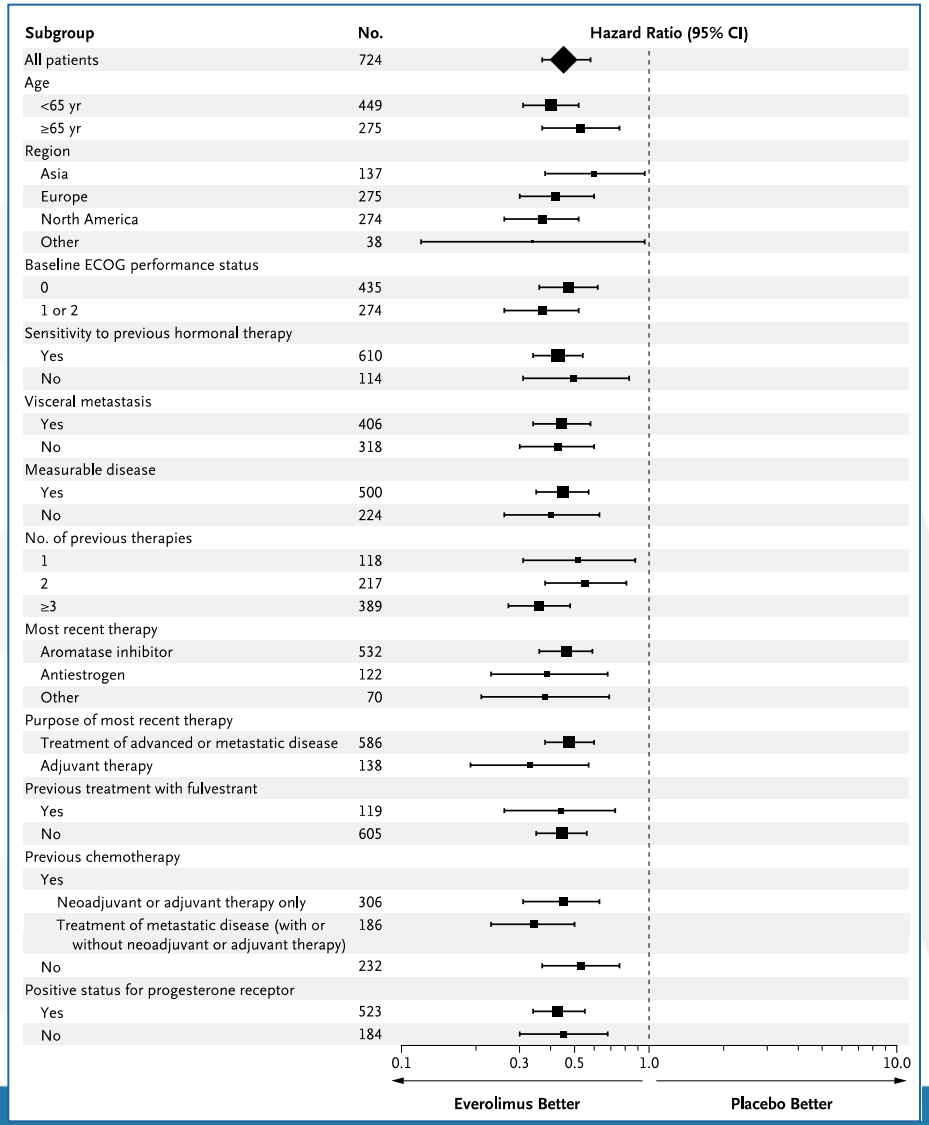
Baselga J, et al. *Ann Oncol.* 2011;47(Suppl 2): Abstract: 9LBA.

ESMO

Baselga et al, NEJM 2012 366, 36: 520-529

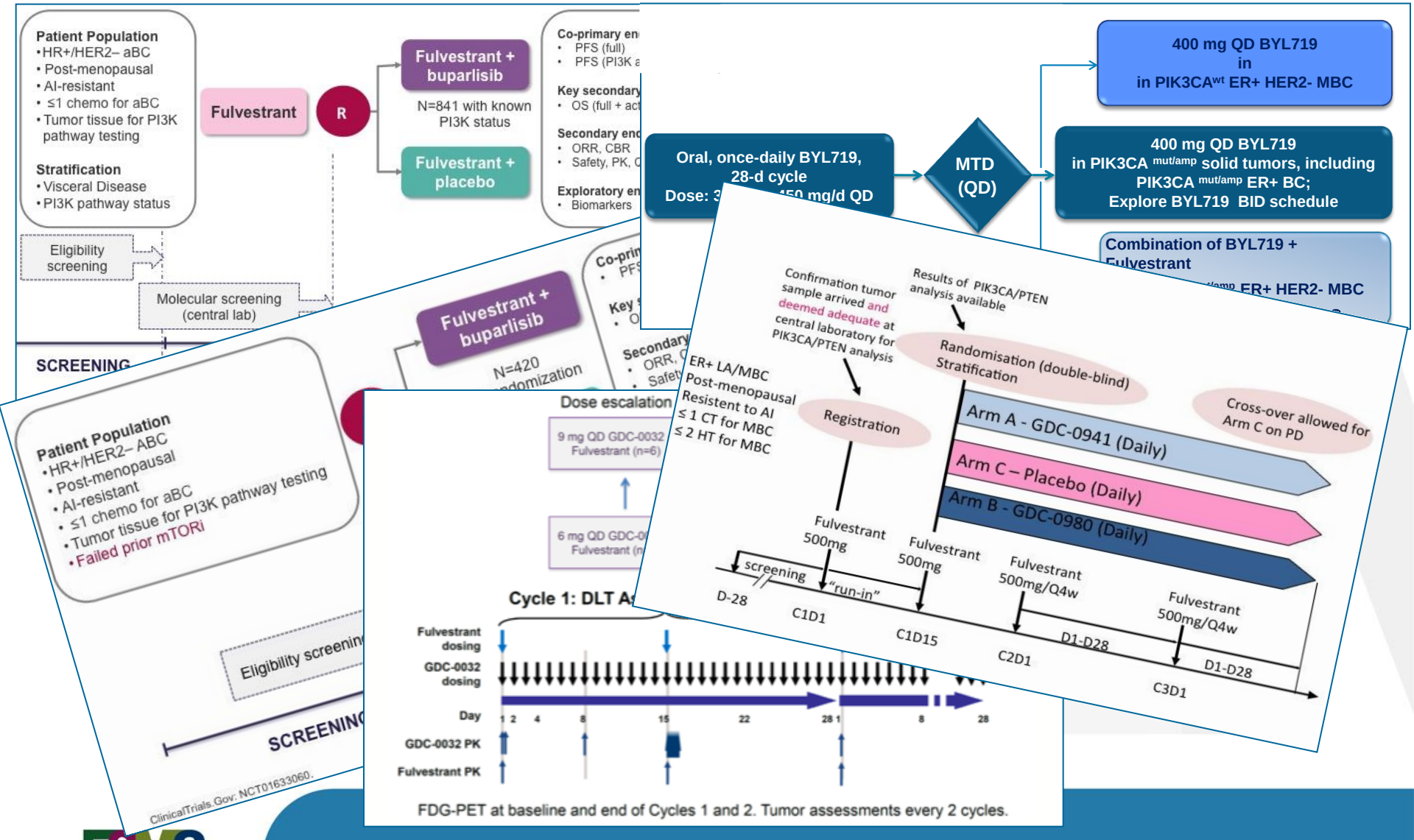
Bolero 2





Baselga et al, NEJM 2012 366, 36: 520-529

Ongoing Clinical Trials ….HR+ disease



Ongoing Clinical Trials ….HER+ disease

BOLERO-3: Study Design

N = 572*

- Locally advanced or metastatic HER2+ breast cancer
- Prior taxane required
- TRAS resistance
  - Adjuvant: progression on or within 12 months of TRAS
  - Metastatic: progression within 4 weeks of TRAS
- Measurab…

Randomize 1:1

CBKM120X2107: Ph II analysis sets

Enrolled in Phase II N=45

Treatment (100 mg/day P…

BEZ235A2101: Study design

Dose-escalation part

Oral, once-daily BEZ235 + weekly trastuzumab (2 mg/kg)

Dose levels*

400-mg capsule

600-mg sachet

800-mg sachet

HER2+ metastatic breast cancer with *PIK3CA/PTEN*† alterations and progression on trastuzumab (N≥3 per dose cohort)

Declara… of MT…

Dose-expansion part

Oral, once-daily BEZ235 (SDS sachet) + weekly …ab (2 mg/kg)

P5 18-04 — Tolerability and efficacy of targeting both mTOR and HER2 signaling in trastuzumab-refractory HER2+ metastatic breast cancer

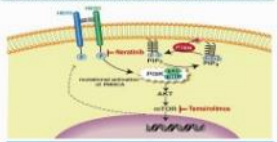
Details

Gajria D, King T, Pannu H, Sakr R, Modi S, Drullinsky P, Syldor A, Patil P, Seidman A, Norton L, Rosen N, Hudis C, Chandarlapaty S.
Memorial Sloan-Kettering Cancer Center, New York, NY

Background

Aberrant PI3K-AKT-mTOR pathway activation is a common mechanism of resistance to anti-HER2 therapy and suggests mTOR as a therapeutic target.[1-4] Temsirolimus inhibits mTORC1 but has limited single agent activity in metastatic breast cancer.[7] This may be due to loss of feedback inhibition resulting in induction of HER3 and AKT activity in response to mTORC1 inhibition.[5,6] Blockage of the induction of HER3/AKT can be achieved with HER1/2 inhibitors in tumors expressing activated HER1/2 kinases. Neratinib, an HER1/2 kinase inhibitor, dosed at 240 mg showed clinical activity with a 26% overall response rate in trastuzumab-refractory HER2-amplified breast cancer.[8]

Figure 1: PI3K pathway activation and inhibition with neratinib + temsirolimus



Hypothesis

Combined inhibition of mTORC1 and HER1/2 with temsirolimus and neratinib will be tolerable and overcome induction of HER3 and AKT signaling that results from loss of feedback inhibition, resulting in clinical responses for trastuzumab-refractory HER2-amplified breast cancer.

Objectives

Primary Endpoints
- Determine the maximum tolerated dose (MTD) of weekly temsirolimus with a fixed dose of daily neratinib (phase I).
- Estimate overall response rate at the established MTD (phase II).

Secondary Endpoints
- Safety and tolerability
- Response rate and progression free survival at 6 months
- Correlate prevalence of expression or mutational changes of PTEN or PI3K with response

Study Design & Schema

Eligibility criteria
- Metastatic HER2+ (IHC 3+ or FISH>2) breast cancer with progression on trastuzumab
- Measurable disease by RECIST
- Any number of prior chemotherapy regimens for phase I. No more than 4 regimens for phase II
- LVEF ≥50% by MUGA scan
- Biopsy of metastatic disease prior to therapy

Phase I: Dose Escalation Schema to Determine MTD

Cohort	Temsirolimus	Neratinib
1	8 mg IV weekly	240 mg oral daily
2	15 mg IV weekly	240 mg oral daily
3	25 mg IV weekly	240 mg oral daily

- Cycle length = 4 weeks
- Response assessment q8 wk
- Toxicity assessment q2 wk
- MUGA scan q12 wk
- Prophylactic anti-diarrheal with loperamide 4 mg oral daily

Phase I Biostatistics
- Standard 3+3 dose-escalation design
- DLT= clinically significant grade 3 or 4 toxicity
- MTD= dose of temsirolimus and neratinib at which <2/6 pts experienced a DLT

Phase II: Simon Two-stage to Determine Efficacy
- HER2-amplified cohort will enroll 9 pts and expand to 34

Tumor analysis
- HER2 by immunohistochemistry and FISH
- PIK3CA hotspot mutation by Sequenom MassARRAY
- PTEN expression by immunohistochemistry

Results

Table 1. Baseline Patient Characteristics For Phase I and II
N=22; (Phase I n=6, Phase II n=21)

Median age, y (range)	48 (31-73)
Median ECOG performance status (range)	0 (0-2)
ER(+) or PR(+)	14
Site of metastases	
Visceral	20
Brain	3
Median # of prior cytotoxic regimens (range)	3 (1-12)
Median # of trastuzumab-containing regimens (range) (n=27)	2 (1-9)
Median # of temsirolimus-neratinib cycles	4 (1-24)

Table 2. Possible Drug-related Toxicity – Maximal grade (N=27)

Toxicity	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Diarrhea	9 (33%)	6 (22%)	-
Mucositis	9 (33%)	4 (15%)	-
Leukopenia	8 (30%)	1 (4%)	-
Hyperglycemia	6 (22%)	1 (4%)	-
Fatigue	6 (22%)	1 (4%)	-
Rash-acneiform	5 (19%)	0	-

Table 3. Best Response by RECIST for patients treated at MTD
HER2-Amplified (N=27; Phase I n=6, Phase II n=21)

Complete response	0
Partial response	12*
Stable disease >6 months	1
Progression of disease	2
Off trial due to toxicity	3

* 9 pts have confirmed PRs, 3 pts had progression of disease on confirmation scan

Figure 2. Kaplan-Meier curve of progression-free survival (N=27)

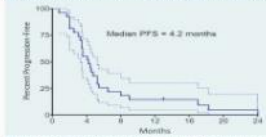
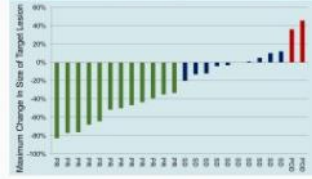


Table 4. Biomarkers of resistance to anti-HER2 therapy: analysis of pre-treatment tumor biopsy and relation to response observed

PIK3CA mutation status		Clinical benefit (PR or SD)
Wild type	7/9	4/7
Mutant	2/9	2/2
PTEN status		**Clinical benefit (PR or SD)**
Wild type	2/17	2/2
Reduced	8/17	7/8
Absent	7/17	6/7

Figure 3. Waterfall plot of evaluable patients treated at MTD (N=2…



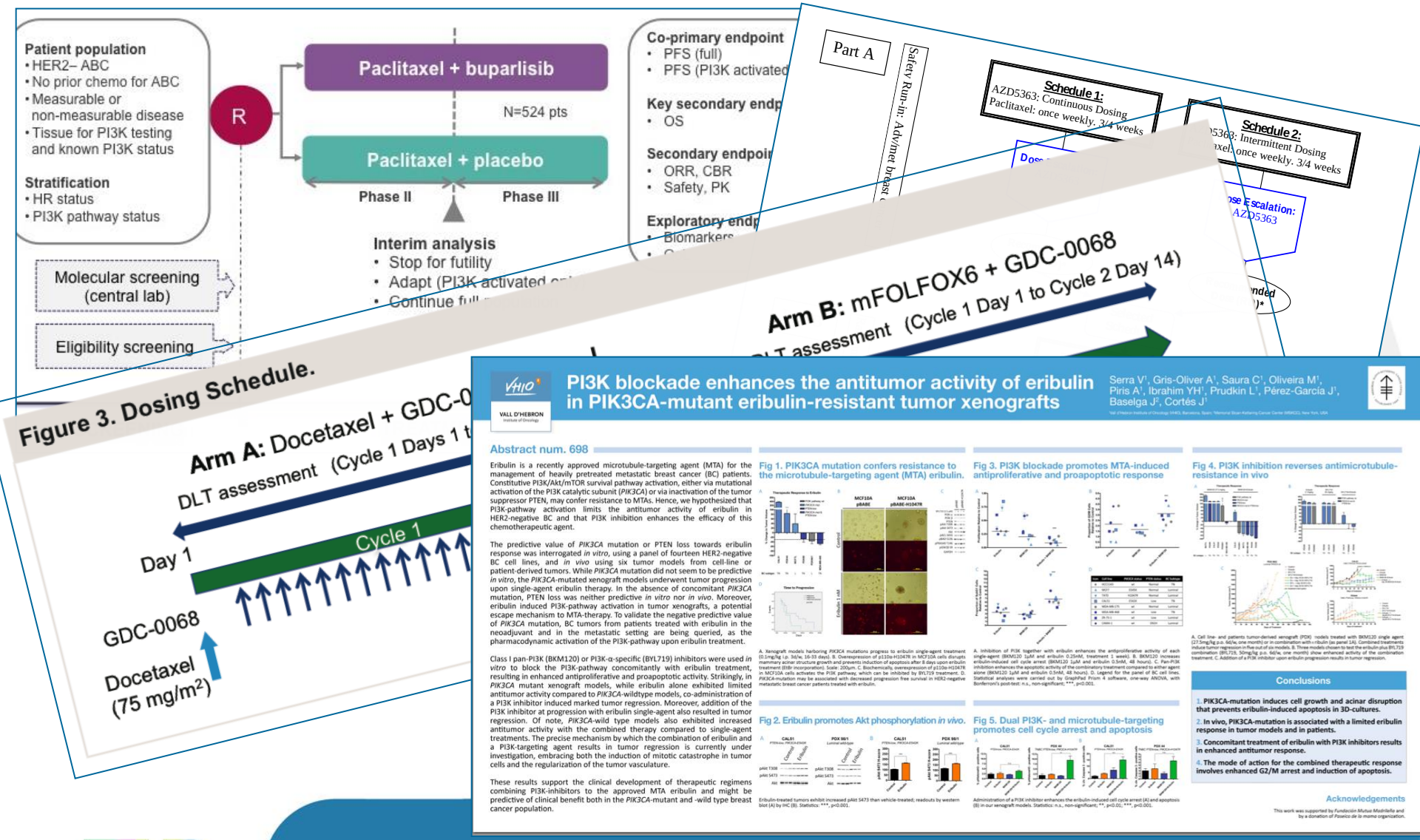
Conclusions

- The phase I trial identified temsirolimus 8 mg IV weekly with neratinib 240 mg oral daily to be the recommended phase II dose
- The dose-limiting toxicity of the regimen was grade 3 diarrhea.
- The combination has demonstrated activity and feasibility in HE… positive, trastuzumab-refractory, metastatic breast cancer with prolonged response durations.
- Patients with prior progression on HER2-targeted therapy with trastuzumab/chemotherapy, lapatinib, T-DM1, and pertuzumab ha… clinical benefit with this combination.
- Though preliminary, aberrant PI3K pathway activation, through PIK3CA mutational activation or PTEN loss, did not preclude response to temsirolimus and neratinib.
- Accrual to the phase II trial and tumor biopsy analysis is ongoing.

Supported by National Comprehensive Cancer Network, ASCO Young Investigator Award, Susan G. Komen For the Cure, and Terri Brodeur Breast Cancer Foundation.

References: 1 Gajria D, et al. Exp Rev Anticancer Therapy 2011. 2 Nagata Y, et al. Cancer Cell 2004. 3 Berns K, et al. Cancer Cell 2007. 4 Stepniski PL, et al. Mol Path 2001. 5 Chan S, et al. JCO 2005. 6 O'Reilly KE, et al. Cancer Res 2006. 7 Chandarlapaty S, et al. Cancer Cell 2011. 8 Burstein HJ, et al. JCO 2010.

Ongoing Clinical Trials ….chemo+PI3Ki



PI3K blockade enhances the antitumor activity of eribulin in PIK3CA-mutant eribulin-resistant tumor xenografts

Serra V[1], Gris-Oliver A[1], Saura C[1], Oliveira M[1], Piris A[1], Ibrahim YH[1], Prudkin L[1], Pérez-García J[1], Baselga J[2], Cortés J[1]

Abstract num. 698

Eribulin is a recently approved microtubule-targeting agent (MTA) for the management of heavily pretreated metastatic breast cancer (BC) patients. Constitutive PI3K/Akt/mTOR survival pathway activation, either via mutational activation of the PI3K catalytic subunit (*PIK3CA*) or via inactivation of the tumor suppressor PTEN, may confer resistance to MTAs. Hence, we hypothesized that PI3K-pathway activation limits the antitumor activity of eribulin in HER2-negative BC and that PI3K inhibition enhances the efficacy of this chemotherapeutic agent.

The predictive value of *PIK3CA* mutation or PTEN loss towards eribulin response was interrogated *in vitro*, using a panel of fourteen HER2-negative BC cell lines, and *in vivo* using six tumor models from cell-line or patient-derived tumors. While *PIK3CA* mutation did not seem to be predictive *in vitro*, the *PIK3CA*-mutated xenograft models underwent tumor progression upon single-agent eribulin therapy. In the absence of concomitant *PIK3CA* mutation, PTEN loss was neither predictive *in vitro* nor *in vivo*. Moreover, eribulin induced PI3K-pathway activation in tumor xenografts, a potential escape mechanism to MTA-therapy. To validate the negative predictive value of *PIK3CA* mutation, BC tumors from patients treated with eribulin in the neoadjuvant and in the metastatic setting are being queried, as the pharmacodynamic activation of the PI3K-pathway upon eribulin treatment.

Class I pan-PI3K (BKM120) or PI3K-α-specific (BYL719) inhibitors were used *in vitro* to block the PI3K-pathway concomitantly with eribulin treatment, resulting in enhanced antiproliferative and proapoptotic activity. Strikingly, in *PIK3CA* mutant xenograft models, while eribulin alone exhibited limited antitumor activity compared to *PIK3CA*-wildtype models, co-administration of a PI3K inhibitor induced marked tumor regression. Moreover, addition of the PI3K inhibitor at progression with eribulin single-agent also resulted in tumor regression. Of note, *PIK3CA*-wild type models also exhibited increased antitumor activity with the combined therapy compared to single-agent treatments. The precise mechanism by which the combination of eribulin and a PI3K-targeting agent results in tumor regression is currently under investigation, embracing both the induction of mitotic catastrophe in tumor cells and the regularization of the tumor vasculature.

These results support the clinical development of therapeutic regimens combining PI3K-inhibitors to the approved MTA eribulin and might be predictive of clinical benefit both in the *PIK3CA*-mutant and -wild type breast cancer population.

Fig 1. PIK3CA mutation confers resistance to the microtubule-targeting agent (MTA) eribulin.

A. Xenograft models harboring *PIK3CA* mutations progress to eribulin single-agent treatment (0.1mg/kg i.p. 3d/w, 16-33 days). B. Overexpression of p110α-H1047R in MCF10A cells disrupts mammary acinar structure growth and prevents induction of apoptosis after 8 days upon eribulin treatment (EdBr incorporation). Scale: 200µm. C. Biochemically, overexpression of p110α-H1047R in MCF10A cells activates the PI3K pathway, which can be inhibited by BYL719 treatment. D. *PIK3CA*-mutation may be associated with decreased progression free survival in HER2-negative metastatic breast cancer patients treated with eribulin.

Fig 2. Eribulin promotes Akt phosphorylation *in vivo*.

Eribulin-treated tumors exhibit increased pAkt S473 than vehicle-treated; readouts by western blot (A) by IHC (B). Statistics: ***, p<0.001.

Fig 3. PI3K blockade promotes MTA-induced antiproliferative and proapoptotic response

A. Inhibition of PI3K together with eribulin enhances the antiproliferative activity of each single-agent (BKM120 1µM and eribulin 0.25nM, treatment 1 week). B. BKM120 increases eribulin-induced cell cycle arrest (BKM120 1µM and eribulin 0.5nM, 48 hours). C. Pan-PI3K inhibition enhances the apoptotic activity of the combinatory treatment compared to either agent alone (BKM120 1µM and eribulin 0.5nM, 48 hours). D. Legend for the panel of BC cell lines. Statistical analyses were carried out by GraphPad Prism 4 software, one-way ANOVA, with Bonferroni's post-test: n.s., non-significant; ***, p<0.001.

Fig 5. Dual PI3K- and microtubule-targeting promotes cell cycle arrest and apoptosis

Administration of a PI3K inhibitor enhances the eribulin-induced cell cycle arrest (A) and apoptosis (B) in our xenograft models. Statistics: n.s., non-significant; **, p<0.01; ***, p<0.001.

Fig 4. PI3K inhibition reverses antimicrotubule-resistance in vivo

A. Cell line- and patients tumor-derived xenograft (PDX) models treated with BKM120 single agent (27.5mg/kg p.o. 6d/w, one month) or in combination with r-bulin (as panel 1A). Combined treatments induce tumor regression in five out of six models. B. Three models chosen to test the eribulin plus BYL719 combination (BYL719, 50mg/kg p.o. 6d/w, one month) show enhanced activity of the combination treatment. C. Addition of a PI3K inhibitor upon eribulin progression results in tumor regression.

Conclusions

1. **PIK3CA-mutation induces cell growth and acinar disruption that prevents eribulin-induced apoptosis in 3D-cultures.**
2. **In vivo, PIK3CA-mutation is associated with a limited eribulin response in tumor models and in patients.**
3. **Concomitant treatment of eribulin with PI3K inhibitors results in enhanced antitumor response.**
4. **The mode of action for the combined therapeutic response involves enhanced G2/M arrest and induction of apoptosis.**

Acknowledgements

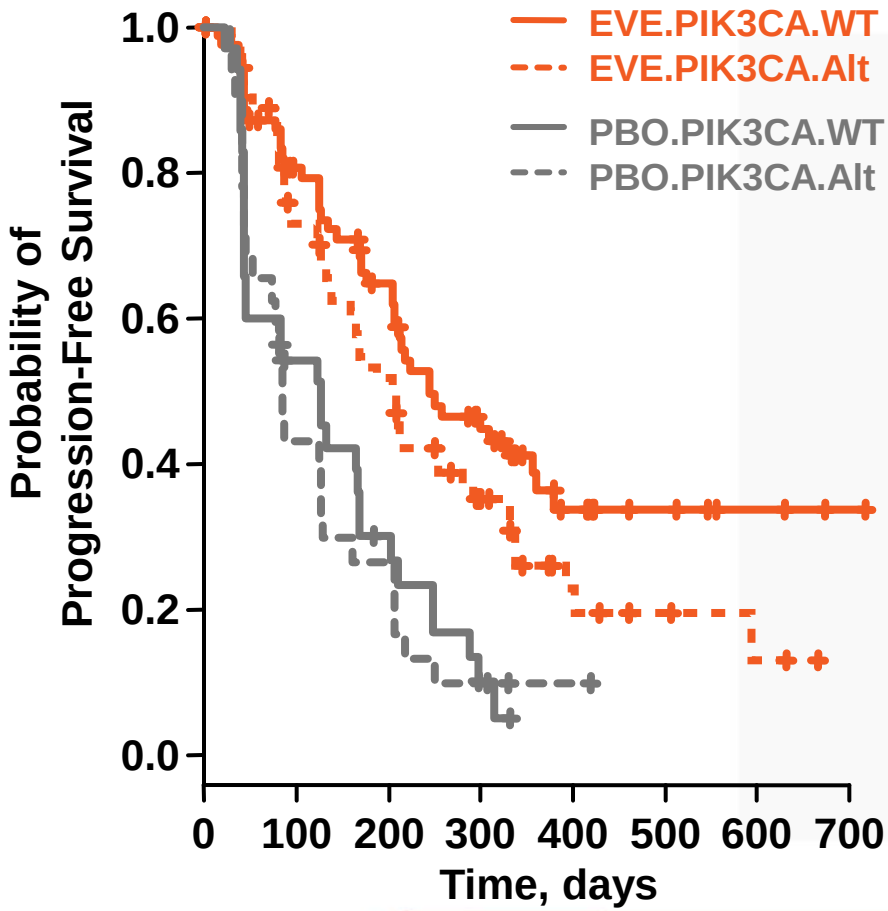
This work was supported by *Fundación Mutua Madrileña* and by a donation of *Paseico de la mama* organization.

OUTLINE

- ✓PI3K/mTOR pathway as a target in breast cancer
- ✓Lessons learned from early drug development of PI3K inhibitors
- ✓Trials in progress
- ✓**Outstanding questions and next steps**

Patient selection

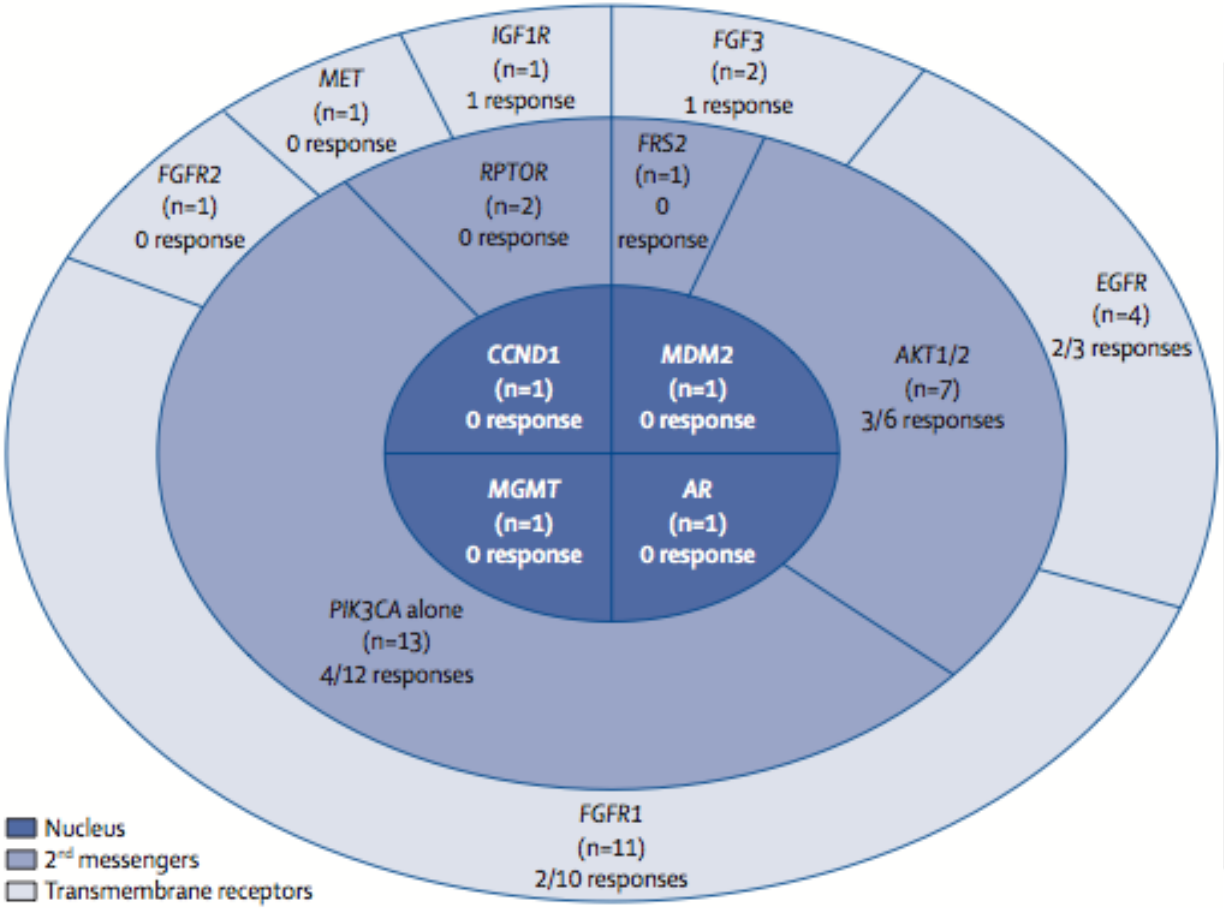
EVE Benefit Maintained in Patients Regardless of Gene Alterations in PIK3CA



Study Arm	PIK3CA	Subgroup, n	PFS events, n (%)	HR (95%CI)
EVE	WT	83	44 (53%)	0.36 (0.22 - 0.57)
PBO	WT	36	31 (86 %)	
EVE	Alt	74	50 (68%)	0.44 (0.27 - 0.70)
PBO	Alt	34	28 (82%)	

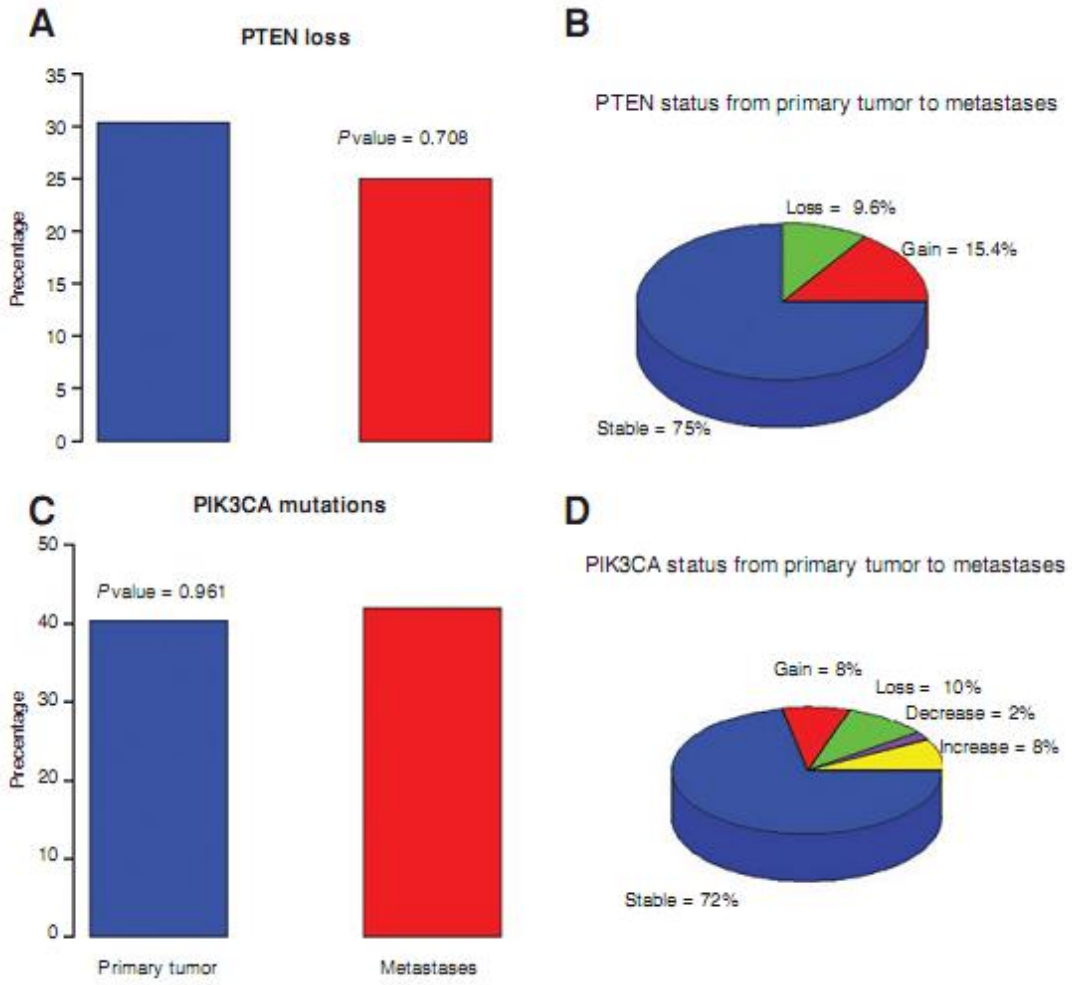
Hortobagy G et al, ASCO 2013, Oral Abs 509

Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER)



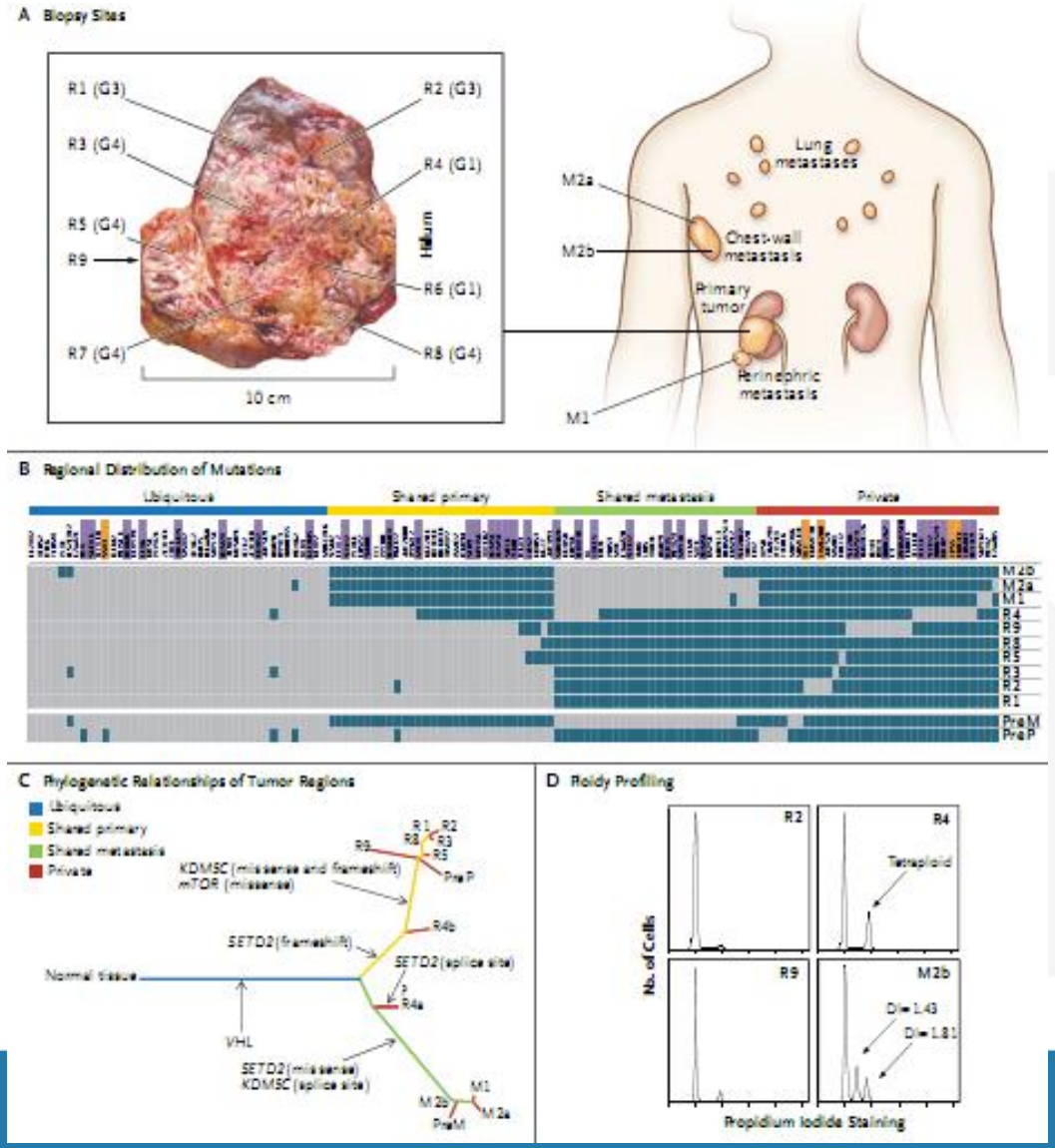
Andre F et al, Lancet Oncol 2014

Changes in PI3K status from primary to metastasis



Gonzalez-Angulo et al, Mol Cancer Ther 2011:10, 1093-1101

Intratumoral Heterogeneity



Gerlinger et al, NEJM 2012, 366:10: 883-892

Liquid biopsies

Detection of Tumor *PIK3CA* Status in Metastatic Breast Cancer Using Peripheral Blood

Michaela J. Higgins, Danijela Jelovac, Evan Barnathan, et al.

Longitudinal Massively Parallel Sequencing Analysis of Circulating Cell-Free Tumor DNA: A Feasibility Study

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Background

Massively parallel sequencing studies have revealed that cancers harbor intra-tumor genetic heterogeneity. In addition, differences in the mutational repertoire between primary tumors and their metastases have been observed. Biomarker assessment using single tumor biopsies of the primary or metastatic lesions therefore may not be representative of the entire mutational repertoire. Plasma-derived cell-free tumor DNA (ctDNA) has been shown to constitute a potential surrogate for tumor DNA obtained from tissue biopsies for the assessment of tumor markers[1,2].

We hypothesize that:

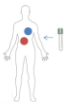
1. Genetic data obtained from massively parallel sequencing analysis of ctDNA of breast cancer patients may be more informative than those of single tumor tissue biopsies.
2. ctDNA would constitute a tool to identify the presence of potentially actionable driver somatic genomic alterations, and monitor changes in the genetic landscape during the course of therapy.

Patients and Methods

- One patient with estrogen receptor (ER)-positive/ HER2-negative, highly proliferative breast cancer (BC) and synchronous distant metastases was included in this study.

Patient:
BC and synchronous bone and liver metastases

Multiple plasma samples were collected during the 4th line of treatment with an AKT inhibitor.



- DNA was extracted from archival tumor, plasma and peripheral blood leucocytes.
- 22-250ng of DNA were subjected to targeted capture massively parallel sequencing on a Illumina HiSeq2000 using the IMPACT platform[3], which comprises 300 cancer genes known to harbor actionable mutations.
- Paired-end sequencing reads were aligned to the reference human genome hg19 using the Burrows-Wheeler Aligner (BWA).
- Somatic mutations were called using muTect for single base substitutions, and Somatic Indel Detector for insertions and deletions (indels).
- Targeted capture massively parallel sequencing yielded average read depths ranging from 25x to 139x in the archival primary and metastatic tumors, from 282x and 918x in the ctDNA samples, and from 29-76x in the normal samples.

Results

1. Mutant allele frequencies are distinct between primary tumor and metastasis

Gene	Mutation	Class Mutation	Primary tumor	Liver metastasis
AKT1	p.E17K	Missense	80%	72%
CDH1	p.159_171PPISCPENEKGPF>L	In Frame Deletion	62%	50%
CDKN2A	p.S12*	Nonsense	85%	52%
TP53	p.K132N	Missense	65%	42%
NF1	p.V2420fs	FrameShift Deletion	53%	49%
TSC1	p.S1046C	Missense	39%	41%
JAK3	p.T21M	Missense	38%	40%
MLL3	p.Q292E	Missense	21%	18%
EPHB1	p.I332M	Missense	6%	26%
PIK3C2G	p.K978N	Missense	5%	45%
ESR1	p.E380Q	Missense	5%	68%
MAP2K2	p.E207Q	Missense	4%	35%
CTNNB1	p.A522G	Missense	3%	39%
GATA1	p.K315N	Missense	3%	32%
FLT4	p.R262Q	Missense	0%	26%
PAK7	p.E494*	Nonsense	0%	38%

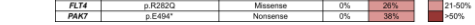


Table 1. Mutant allele frequencies of somatic mutations identified in the primary breast tumor and liver metastasis. Low allele frequency mutations in the primary tumor, such as *ESR1*, were found to be enriched in the liver metastasis. The nonsense mutation in *PAK7* and the missense mutation *FLT4* in the metastasis could not be identified in the primary tumor.

2. ctDNA analysis captures the heterogeneity of primary tumor and metastasis

Figure 1. Venn diagram of somatic mutations identified in the primary tumor, liver metastasis and ctDNA using targeted capture massively parallel sequencing. Not all mutations identified in the metastasis were found in the primary tumor. Analysis of ctDNA of this patient, however, captured all mutations present in the primary tumor and liver metastasis.

3. ctDNA for disease monitoring

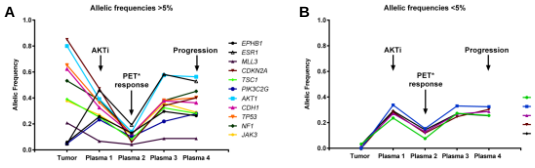


Figure 2. Longitudinal monitoring of the mutant alleles of the primary tumor and four plasma DNA samples. **A**, genes whose high confidence mutations were detected in ≥5% of the alleles of the primary tumor; **B**, genes whose high confidence mutations were detected in the plasma-derived ctDNA , but either absent or present in <5% of the alleles of the primary tumor. Arrow, initiation of AKT inhibitor treatment. *PET, pharmacodynamic response.

4. Clinical assessment of response to AKT inhbition

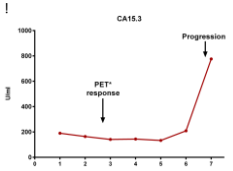


Figure 3. Longitudinal monitoring of the CA15.3 levels throughout the 4th line of systemic treatment with single agent AKT inhibitor. *PET, pharmacodynamic response.

Clinical assessment:
CA15.3 levels: longitudinal monitoring and modulation.

Best response: Stable disease as per RECIST1.1 (8 months)

Conclusions

- Analysis of the mutational repertoire of a single diagnostic biopsy of a primary tumor may not be representative of that of the metastases.
- Targeted capture massively parallel sequencing analysis of plasma-derived ctDNA captures the mutations present in both, the primary tumor and distant metastasis, providing evidence to suggest that ctDNA may be a useful source of biological material for biomarker assessment in patients with advanced breast cancer.
- Targeted capture massively parallel sequencing of plasma-derived ctDNA may be used as a quantitative marker for longitudinal follow-up and disease monitoring of genetic somatic alterations during the course of targeted therapy.

References

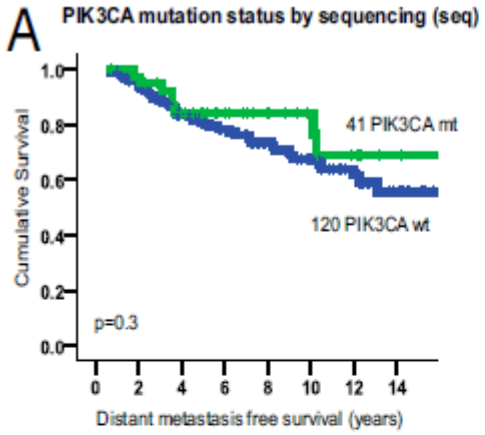
1. De Mattos-Arruda L, Cortes J, Santarpia L, *et al.* Circulating tumour cells and cell-free DNA as tools for managing breast cancer. *Nature Reviews Clinical Oncology* 2013; **10**: 377-389.
2. Bidard FC, Weigelt B, Reis-Filho JS. Going with the flow: from circulating tumor cells to DNA. *Sci Transl Med* 2013; **5**: 207ps214.
3. Wagle N, Berger MF, Davis MJ, *et al.* High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov* 2012; **2**: 82-93.

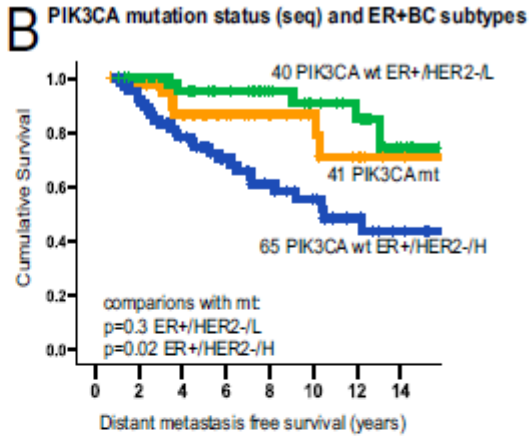
Email to: ldmattos@ir.vhebron.net

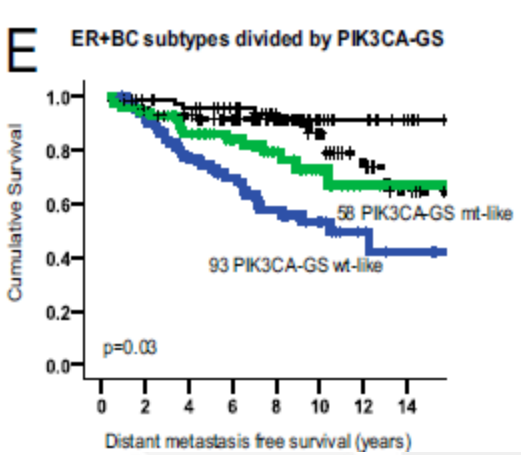
Higgins et al, Clin Cancer Res 2012: 3462-69
De Mattos et at, SABCS 2013, PD4-5

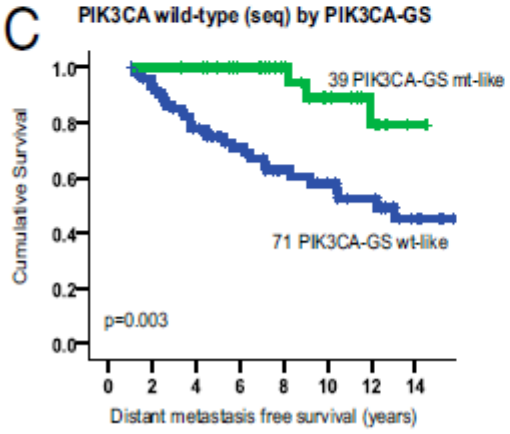
Defining better biomarkers of response

PIK3CA mut associated with gene signature of low mTORC1 signaling and better outcome s in ER+ BC









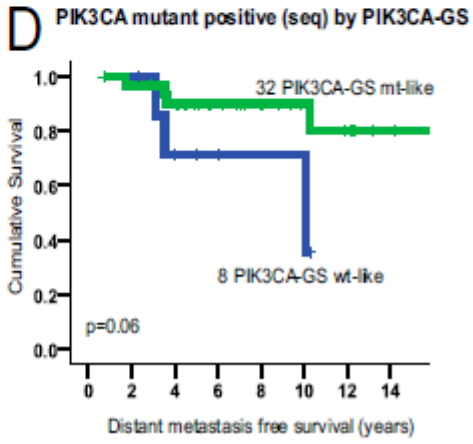
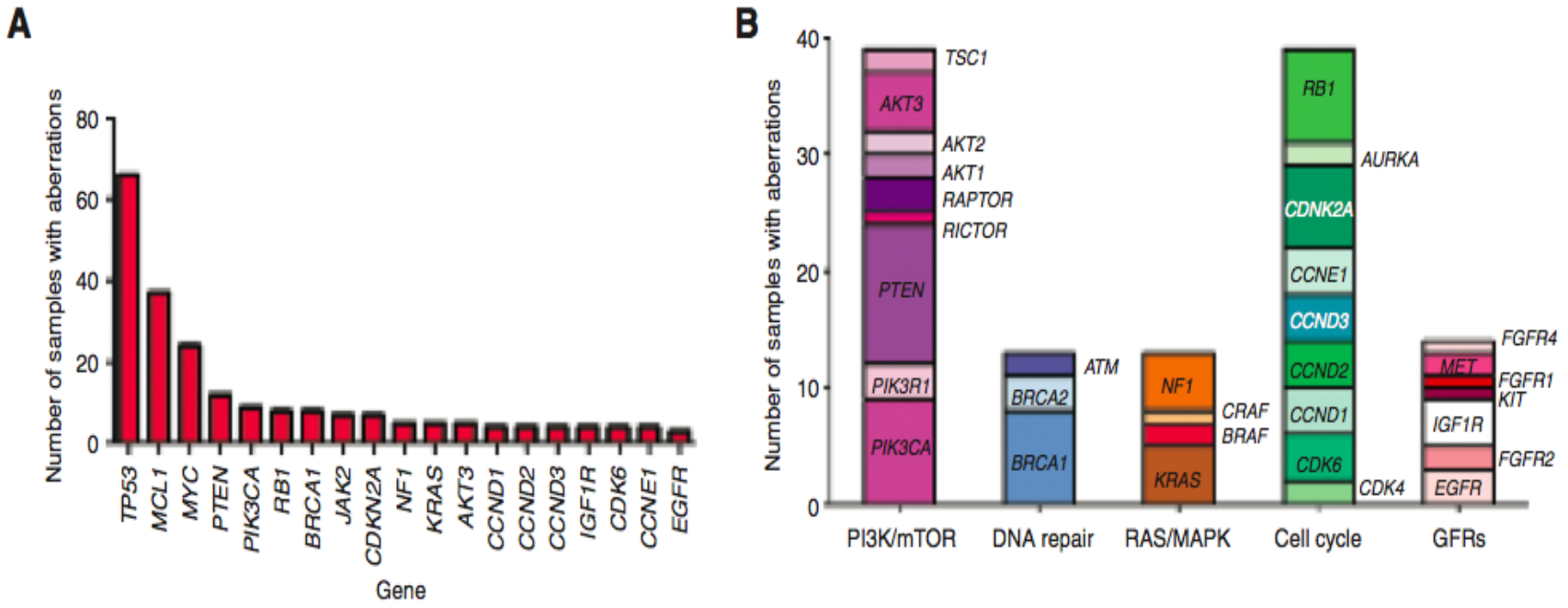


Table 3. Multivariate analysis in patients with ER+ BC who had received adjuvant tamoxifen (TAM dataset, *n* = 302)

Factor	HR (95% CI)	*P* Value
Age (binary)	1.9 (0.4–8.1)	0.4
Nodal status	1.6 (0.8–3.1)	0.2
Tumor size (binary)	1.9 (1.1–3.3)	0.03
Histologic grade	2.4 (0.9–6.2)	0.07
PIK3CA-GS	0.5 (0.3–0.8)	0.01

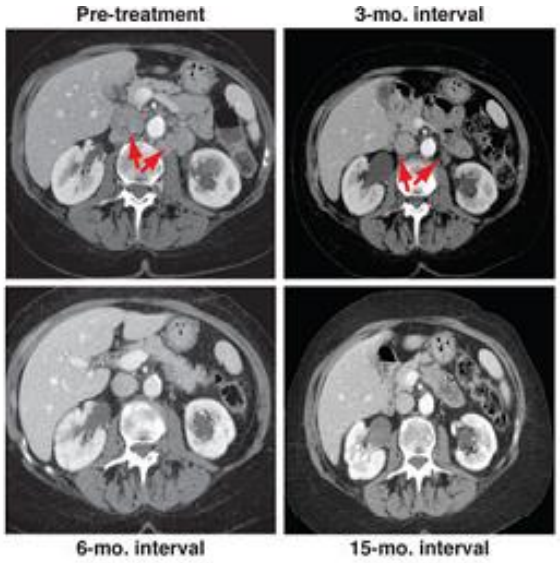
Loi S. *et al.* PNAS 2010;107(22):10208-10213

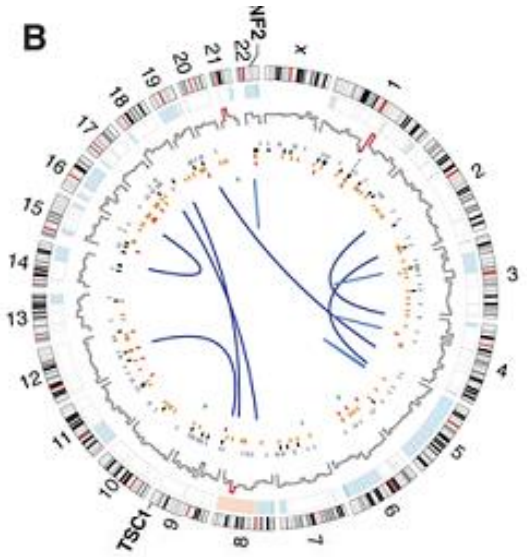
Neoadjuvant Model

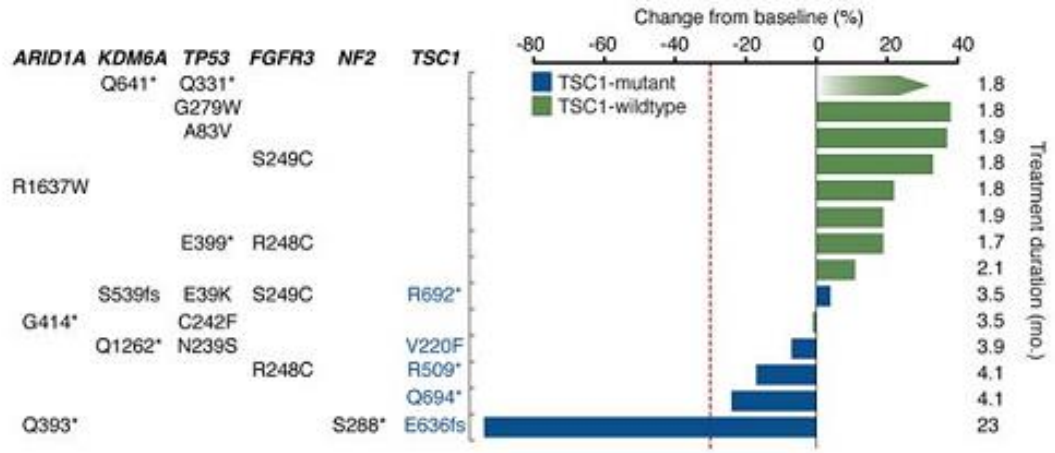


Balko et al, Cancer Discovery 2014: 232-245

From bedside to bench







CONCLUSIONS

- **PI3K is an important pathway to target to overcome resistances to different agents in the clinic**
- **The combination strategy seems the most appropriate to develop in the clinic**
- **PI3K mutation status alone does not seem to select patients who derive more benefit from treatments**
- **Prospective well designed clinical trials will hopely define how to better select population to maximize the benefit of PI3Ki**
  - Biopsies in metastatic disease / circulating DNA
  - Neoadjuvant clinical trials
  - Accessibility to gene signatures and deep sequencing techniques