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TRANSLATIONAL RESEARCH IN ONCOLOGY
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Cell cycle checkpoints (CDK4/6) inhibitors

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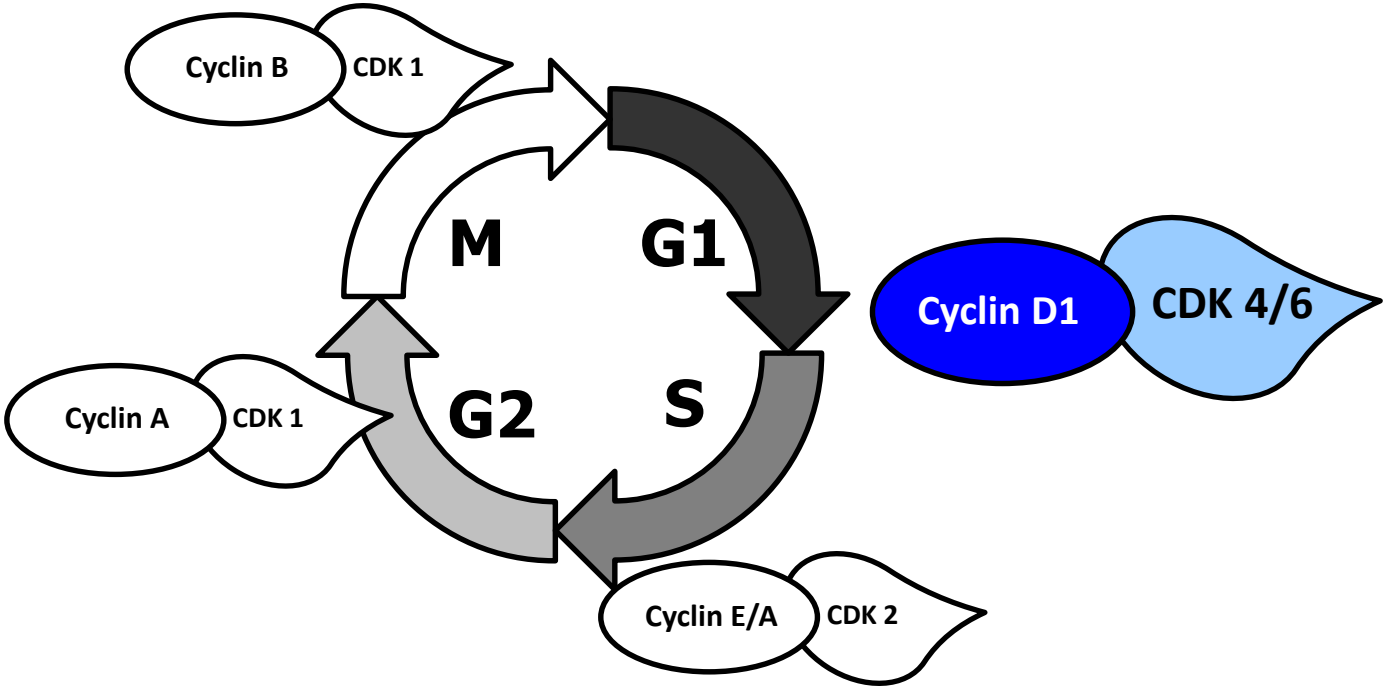
Fondazione Sandro Pitigliani
per la lotta contro i tumori - ONLUS

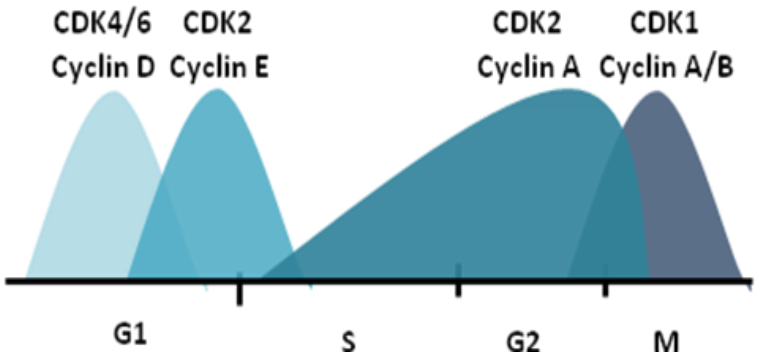
BCM Baylor College of Medicine

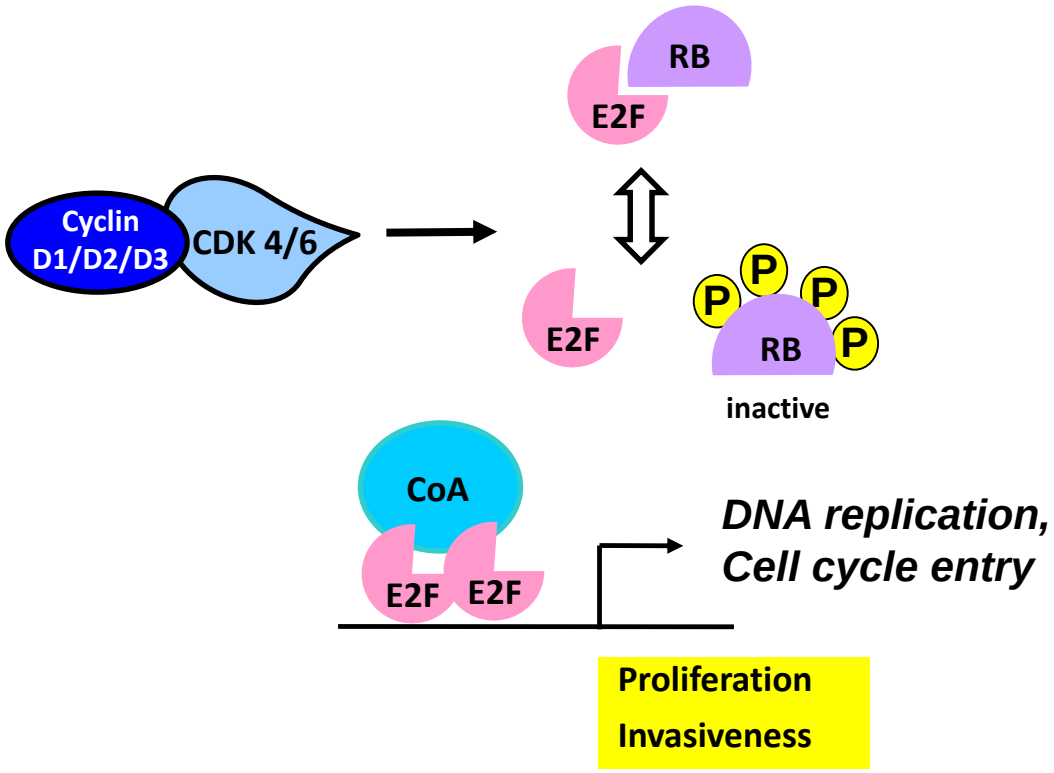
Outline

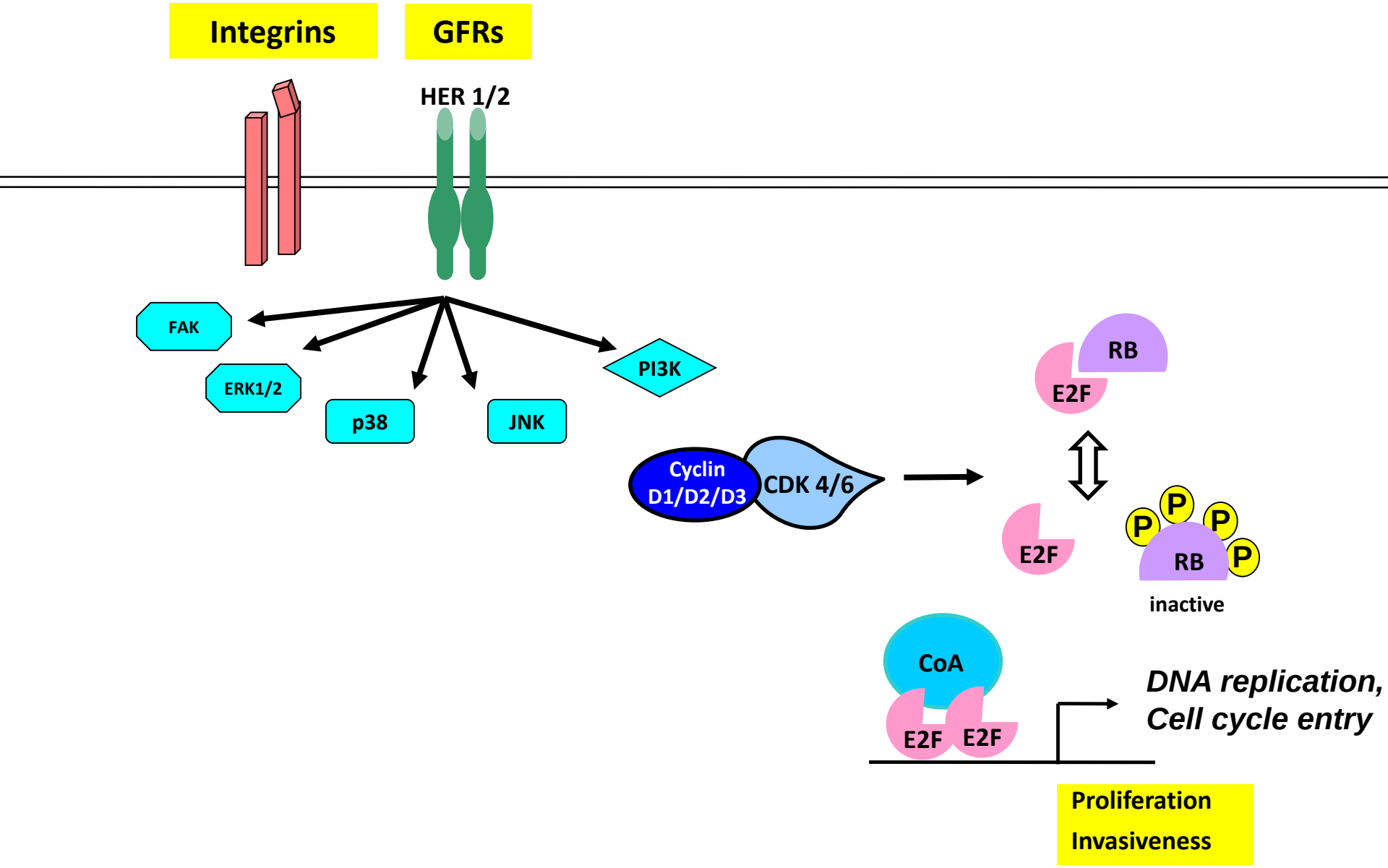
- **CDK 4/6 inhibitors: MoA and pre-clinical data**
- **Clinical data in ER+/HER2 neg metastatic breast cancer**
- **Biomarkers**

CDK 4/6 as a key regulator of cell cycle






RB
E2F
Cyclin
D1/D2/D3
CDK 4/6
E2F
P
P
P
RB
P
inactive
CoA
E2F
E2F
DNA replication,
Cell cycle entry
Proliferation
Invasiveness


Integrins
GFRs
HER 1/2
FAK
ERK1/2
p38
JNK
PI3K
Cyclin D1/D2/D3
CDK 4/6
RB
E2F
E2F
P
P
P
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CoA
E2F
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DNA replication,
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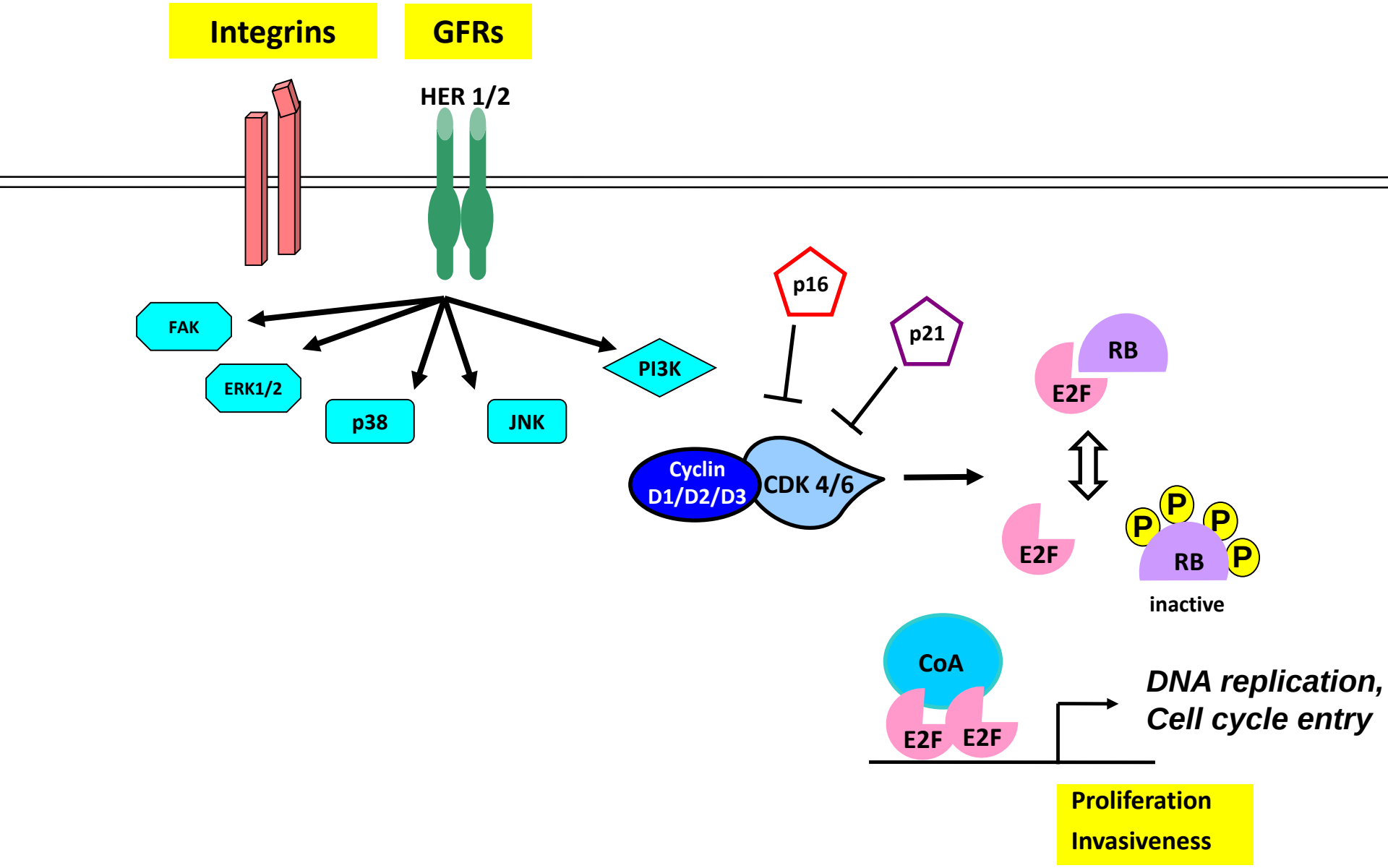
Invasiveness


Integrins
GFRs
HER 1/2
FAK
ERK1/2
p38
JNK
PI3K
p16
p21
Cyclin D1/D2/D3
CDK 4/6
RB
E2F
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E2F
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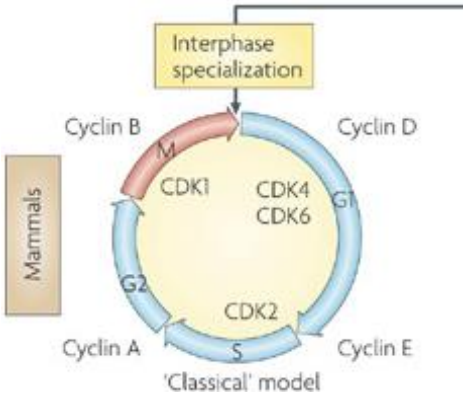
Invasiveness

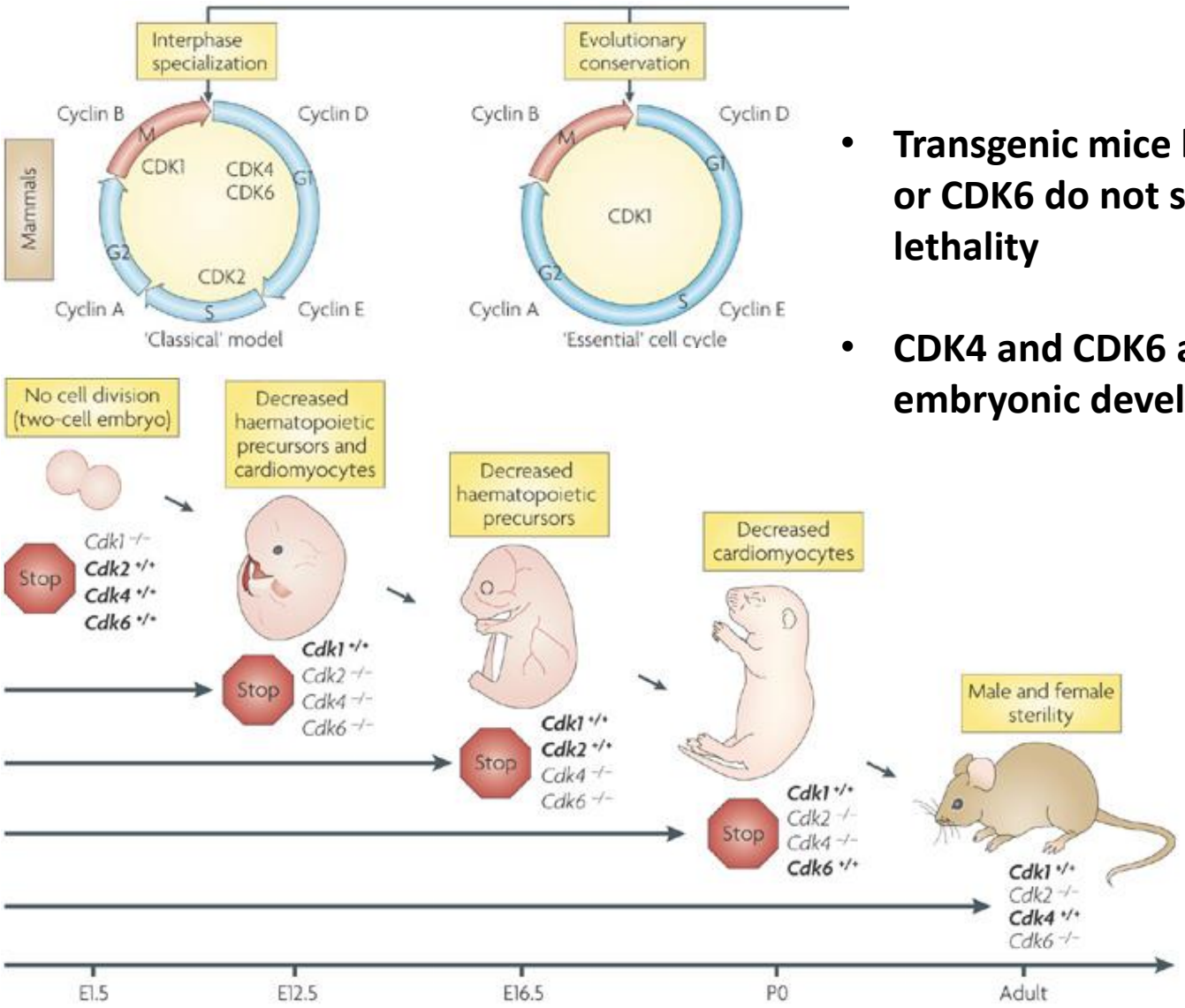
Cell cycle regulation: embryonic development



Adapted from Malumbres M. and Barbacid M.

NATURE REVIEWS | CANCER VOLUME 9 | MARCH 2009

Cell cycle regulation: embryonic development

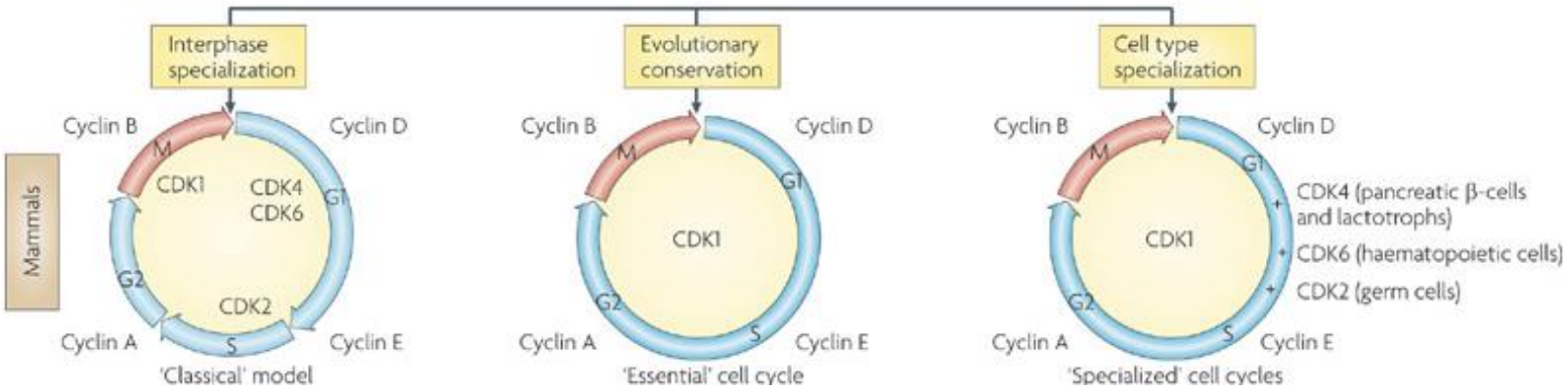


- **Transgenic mice lacking either CDK4 or CDK6 do not show embryonic lethality**
- **CDK4 and CDK6 are not essential for embryonic development**

Adapted from Malumbres M. and Barbacid M.

NATURE REVIEWS | CANCER VOLUME 9 | MARCH 2009

Cell cycle regulation: embryonic development



Kinase	Genotype[§]	Phenotype	Refs
Loss-of-function strains			
CDK1	*Cdk1*$^{mut/mut}$	Deficiency in CDK1 results in embryonic lethality in the first cell divisions	19
CDK2	*Cdk2*$^{-/-}$	Sterility due to defective meiosis; no effect on mitotic cells	17,18
CDK4	*Cdk4*$^{-/-}$	Diabetes and defective postnatal proliferation of endocrine cells such as pancreatic β-cells or pituitary hormone-producing cells	14,15, 133–136
CDK6	*Cdk6*$^{-/-}$	Slight anaemia and defective proliferation of some haematopoietic cells	16
CDK11	*Cdk11*$^{-/-}$	Embryonic lethality in peri-implantation embryos accompanied by mitotic aberrations	109
CDK2; CDK4; CDK6	*Cdk2*$^{-/-}$; *Cdk4*$^{-/-}$; *Cdk6*$^{-/-}$	Deficiency in all these interphase CDKs provokes embryonic lethality by mid-gestation due to haematopoietic defects	19

- **However, CDK4 and CDK6 are important for "specialized" cell cycles such as those of hematopoietic and pancreatic beta-cells**

Adapted from Malumbres M. and Barbacid M.

NATURE REVIEWS | CANCER VOLUME 9 | MARCH 2009

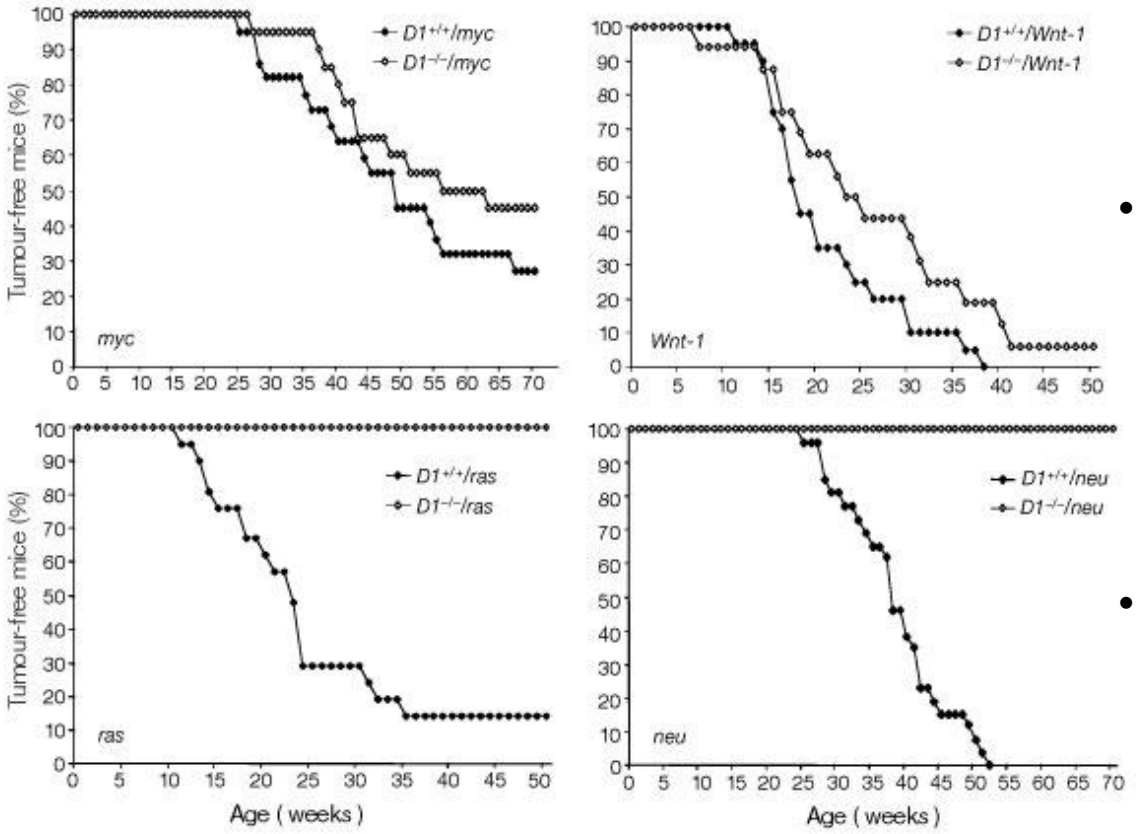
Cell cycle regulation: CANCER

NATURE | VOL 411 | 28 JUNE 2001 | www.nature.com

Specific protection against breast cancers by cyclin D1 ablation

Qunyan Yu, Yan Geng & Piotr Sicinski

Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA



- **Mice models of breast cancer induced by specific oncogenes are prevented by CyclinD1 ablation**
- **In particular, neu (HER2) and ras induced breast cancer models are completely dependent on CyclinD1**

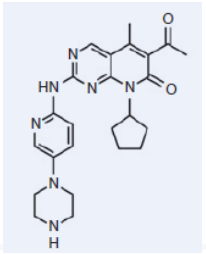
- **Although non essential in physiologic conditions, CDK4/6 and CyclinD1 may represent unique targets in cancer.**

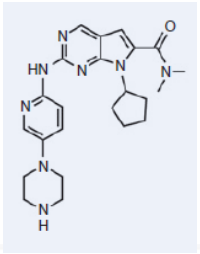
Deregulation of CDK 4/6 pathway in BC subtypes

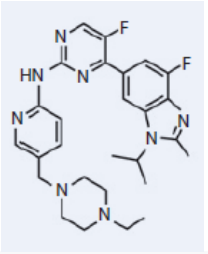
Luminal A	Luminal B	HER2 enriched	Basal-like
Cyclin D1 amp (29%)	Cyclin D1 amp (58%)	Cyclin D1 amp (38%)	Cyclin E1 amp (9%)
CDK4 gain (14%)	CDK4 gain (25%)	CDK4 gain (24%)	
11q13.3 amp (24%)	11q13.3 amp (51%)		
			RB1 mut/loss (20%)
Low expression of p18/high expression of RB1	High FOXM1		High expression of p16/ low expression of RB1

The Cancer Genome Atlas Network, Nature 2012

Modern CDK 4/6 inhibitors



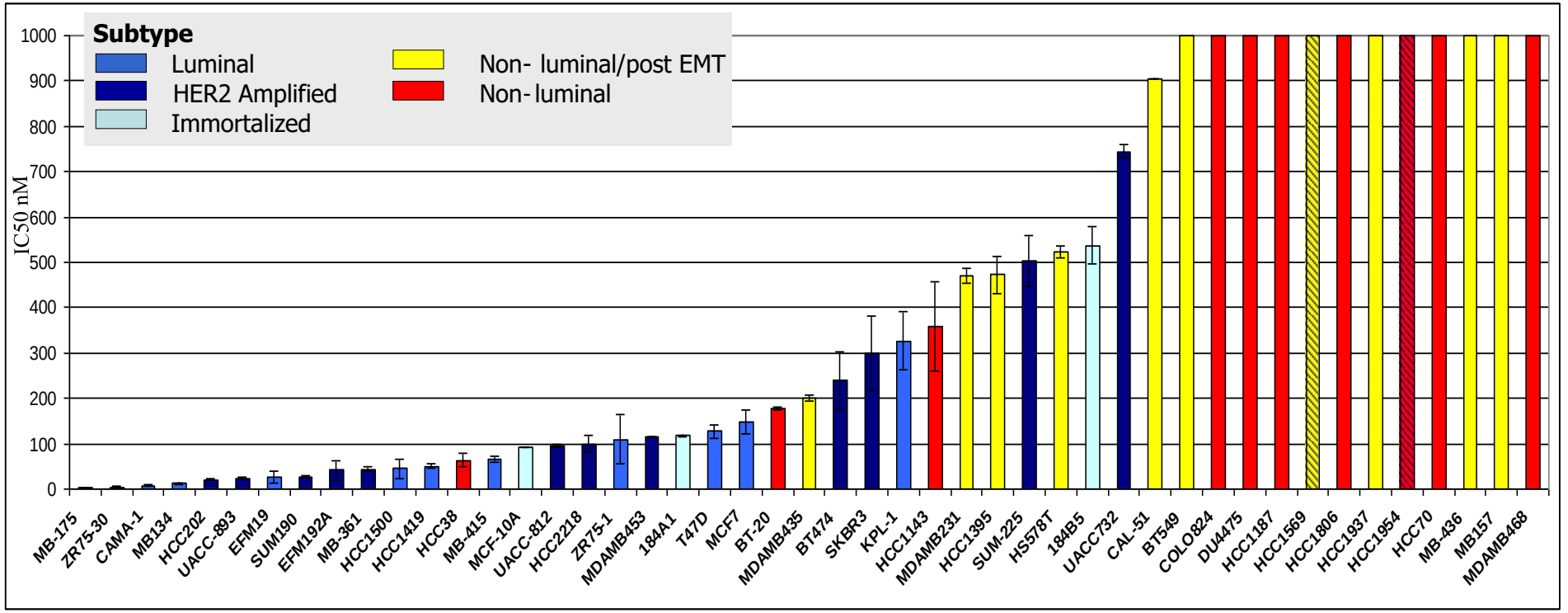




Drug	Palbociclib (Pfizer) (PD0332991, Ibrance)	Ribociclib (Novartis) (LEE011)	Abemaciclib (Eli Lilly) (LY2835219)
IC_{50} (*in vitro* kinase assay, recombinant proteins)	CDK4 (D1): 11 nmol/L CDK4 (D3): 9 nmol/L CDK6 (D2): 15 nmol/L CDK1: >10 µmol/L CDK2: >10 µmol/L (66, 67)	CDK4: 10 nmol/L CDK6: 39 nmol/L CDK1: >100 µmol/L CDK2: >50 µmol/L (1, 89)	CDK4 (D1): 0.6–2 nmol/L CDK6 (D1): 2.4–5 nmol/L CDK 9: 57 nmol/L CDK1: >1 µmol/L CDK2: >500 nmol/L (1, 88)
PK	T_{max} 4.2–5.5 hr $t_{1/2}$ 25.9–26.7 hr (69, 70)	T_{max} 4 hr $t_{1/2}$ 24–36 hr (90, 91)	T_{max} 4–6 h $t_{1/2}$ 17–38 h (crosses blood:brain barrier; refs. 92, 93)
Dosing	125 mg daily (3 weeks, 1-week drug holiday) or 200 mg daily (2 weeks, 1-week drug holiday; refs. 69, 70)	600 mg daily (3 weeks, 1-week drug holiday; ref. 90)	200 mg twice daily (continuous dosing; ref. 92)
Major dose-limiting toxicities	Neutropenia, thrombocytopenia	Neutropenia, thrombocytopenia	Fatigue
Other reported adverse events	Anemia, nausea, anorexia, fatigue, diarrhea (69, 70)	Mucositis Prolonged EKG QTc interval Elevated creatinine Nausea (90)	Diarrhea Neutropenia (92)

Sherr CJ, Cancer Discovery 2016

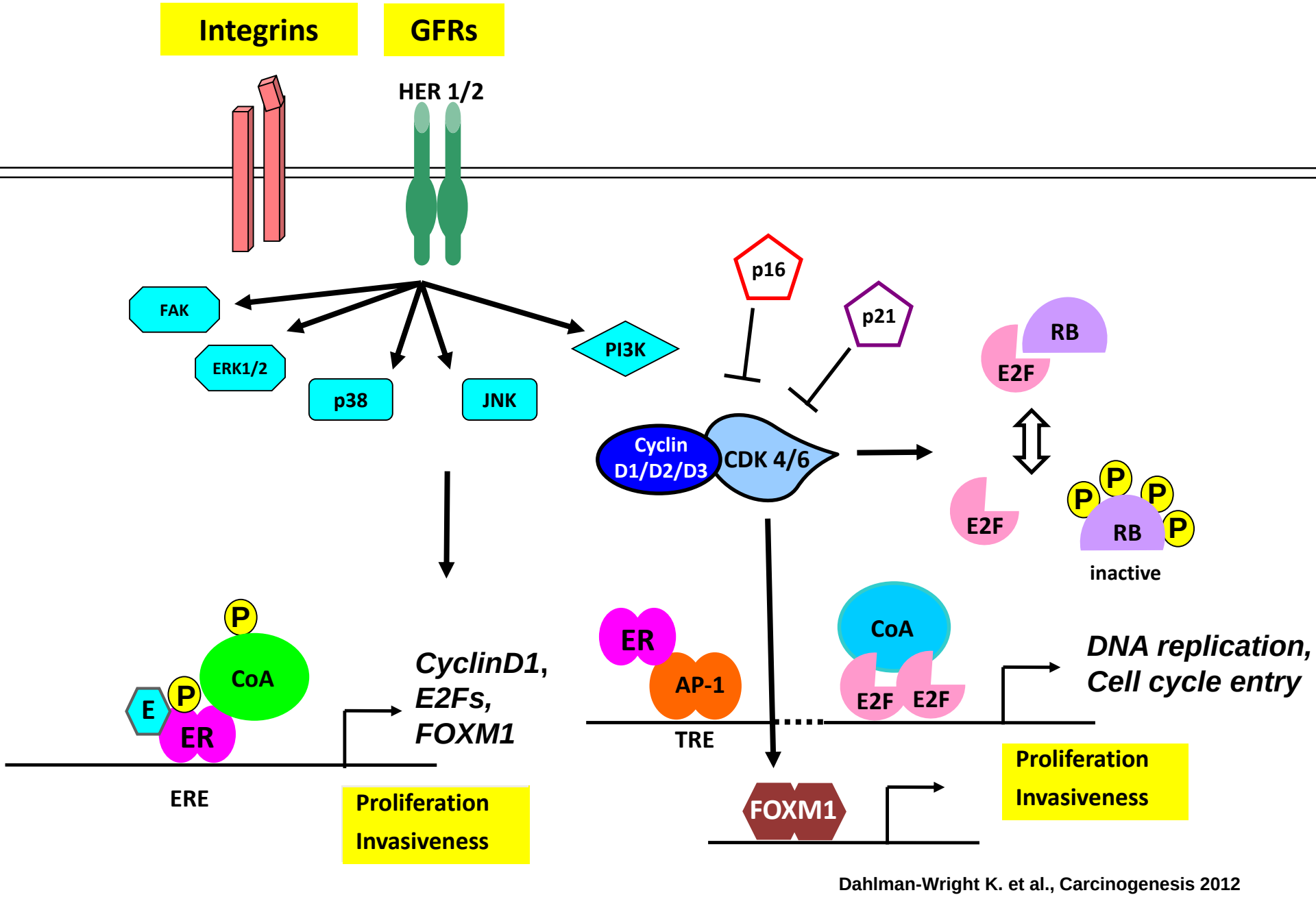
CDK4/6i are preferentially active in Luminal type BC cell lines



CDK 4-6 inhibitors have shown activity preferentially on ER+, luminal breast cancer cell lines with or without HER2 amplification.

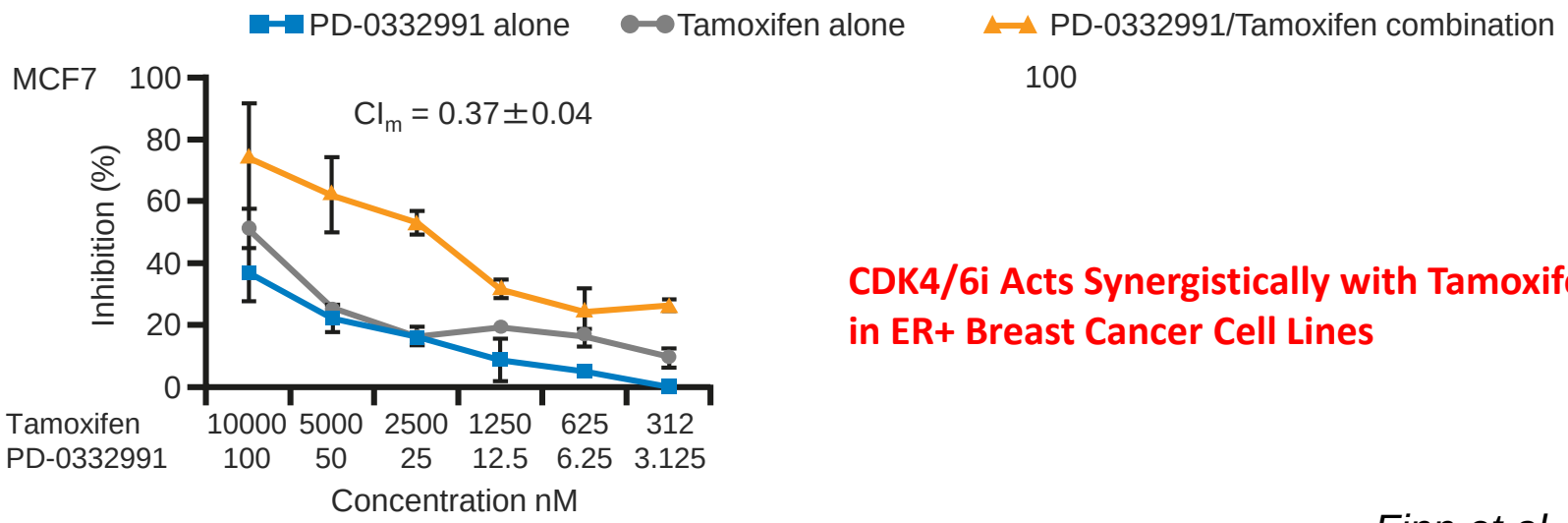
Finn et al, BCR 2011

Cross-talks of the CDK 4/6 and ER pathways



Dahlman-Wright K. et al., Carcinogenesis 2012

CDK 4/6 inhibitor + Endocrine therapy



CDK4/6i Acts Synergistically with Tamoxifen in ER+ Breast Cancer Cell Lines

Finn et al, BCR 2011

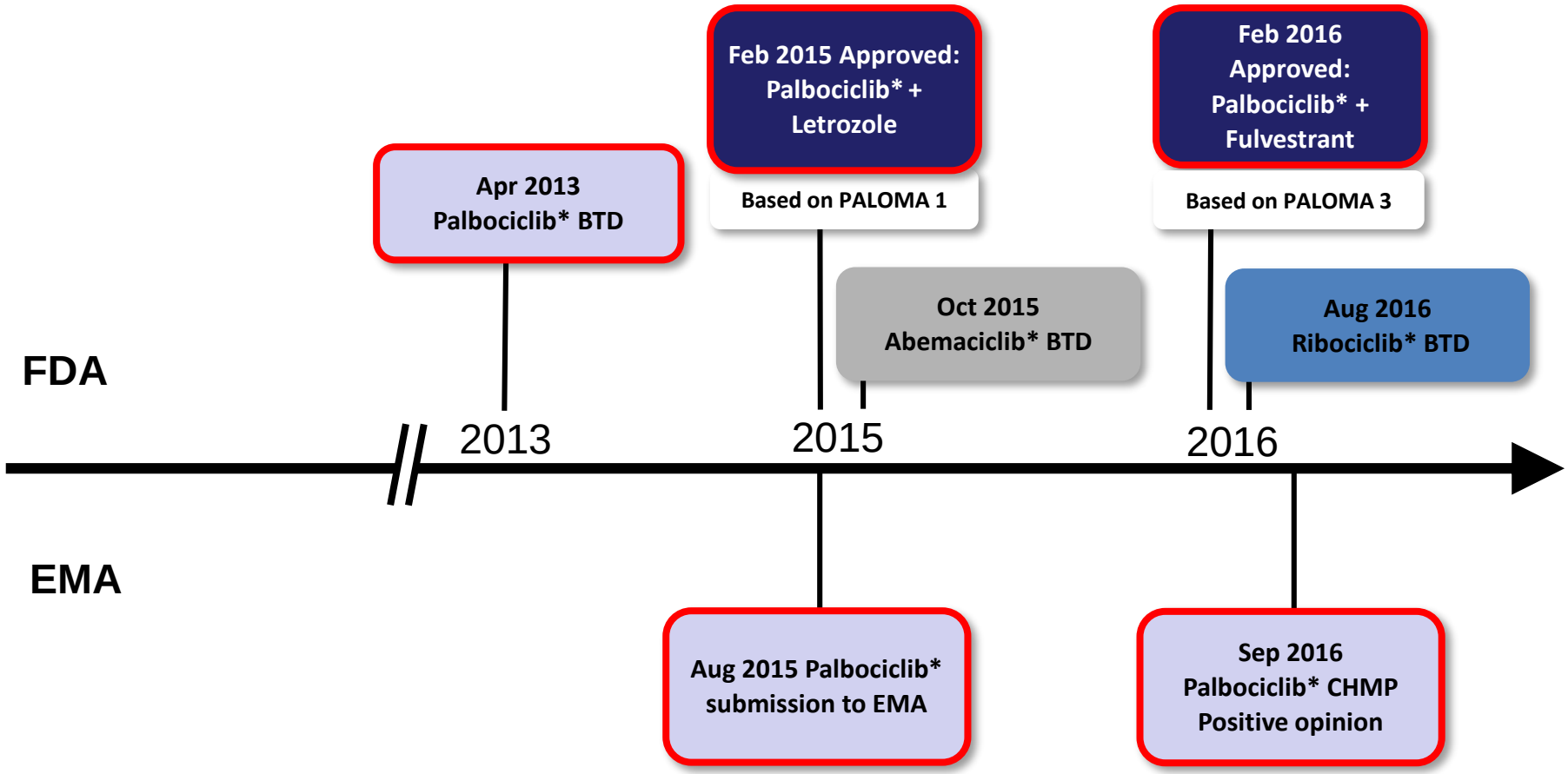
CDK4/6i improves efficacy of Fulvestrant and Letrozole in Luminal BC models

Koehler M. et al, IMPAKT meeting 2014

Outline

- CDK 4/6 inhibitors: MoA and pre-clinical data
- **Clinical data in ER+/HER2 neg metastatic breast cancer**
- Biomarkers

CDK 4/6 inhibitors in HR+/HER2– mBC



CDK 4/6 inhibitors in the first line MBC setting (ER+/HER2neg)

PALOMA-2

N=666

- Postmenopausal
- ER+, HER2– advanced breast cancer
- No prior treatment for advanced disease
- AI-resistant patients excluded

Finn RS, *et al.* Presented at the ASCO Annual Meeting 2016. Abstract 507.

RANDOMISATION 2:1

- Palbociclib (125 mg QD, 3/1 schedule) + letrozole (2.5 mg QD)
- Placebo (3/1 schedule) + letrozole (2.5 mg QD)

Primary endpoint
Investigator-assessed PFS

Secondary endpoints
Response, OS, safety, biomarkers, patient-reported outcomes

Stratification factors
- Disease site (visceral, non-visceral)
- Disease-free interval (de novo metastatic; ≤12 mo, >12 mo)
- Prior (neo)adjuvant hormonal therapy (yes, no)

MONALEESA-2

N=668

- Postmenopausal women with HR+/HER2– advanced breast cancer
- No prior therapy for advanced disease

Hortobagyi GN, *et al.* NEJM 2016- Presented at 2016 ESMO

RANDOMISATION 1:1

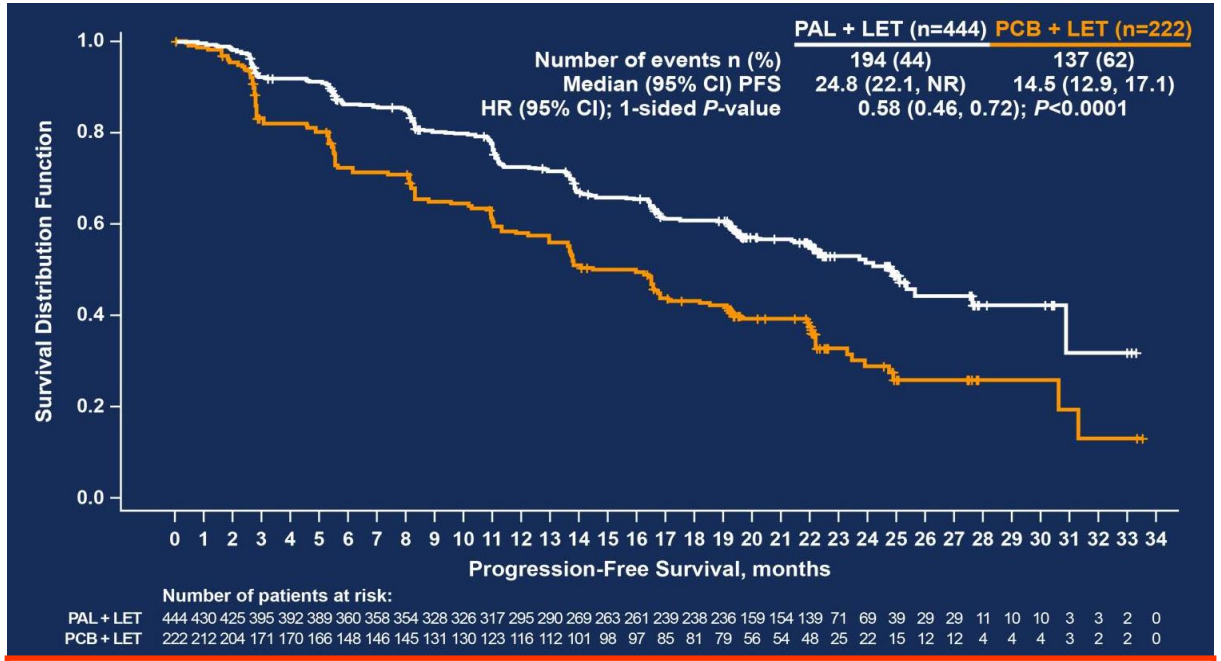
- Ribociclib (600 mg/day) 3/1 schedule + Letrozole (2.5 mg/day)
- Placebo + Letrozole (2.5 mg/day)

Primary endpoint
- PFS (locally assessed)

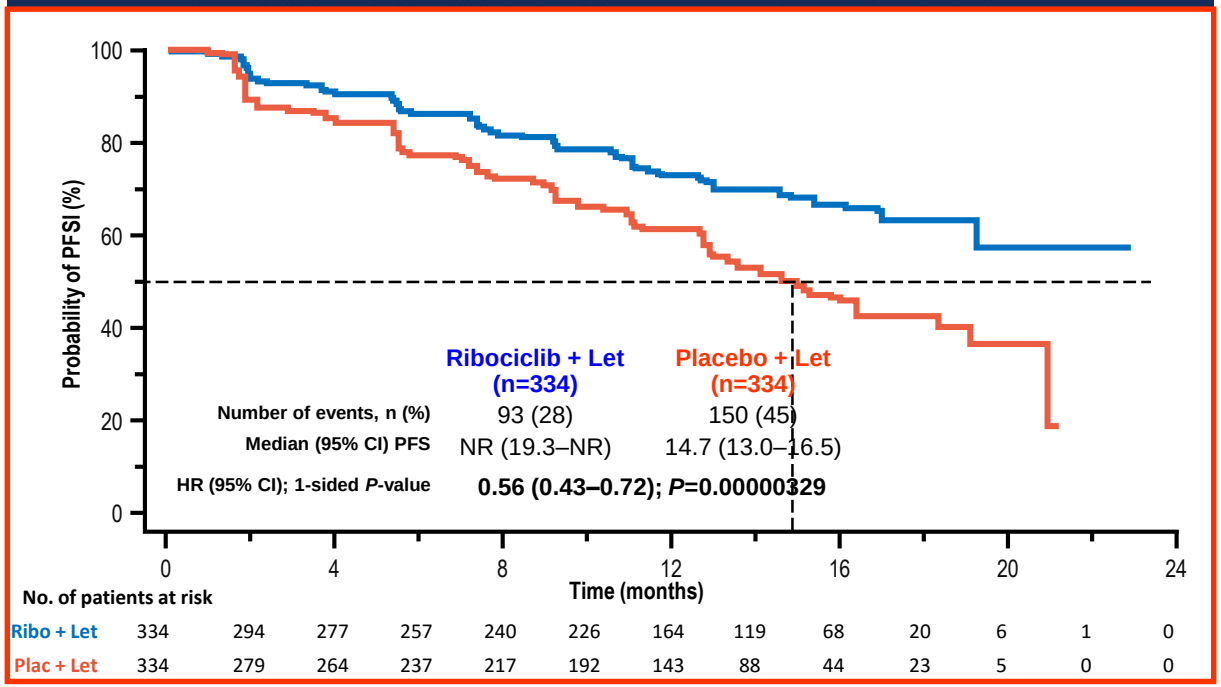
Secondary endpoints
- Overall survival (key)
- Overall response rate
- Clinical benefit rate
- Safety

Stratified by the presence/absence of liver and/or lung metastases

PFS (Investigators assessed) in PALOMA-2 and MONALEESA-2

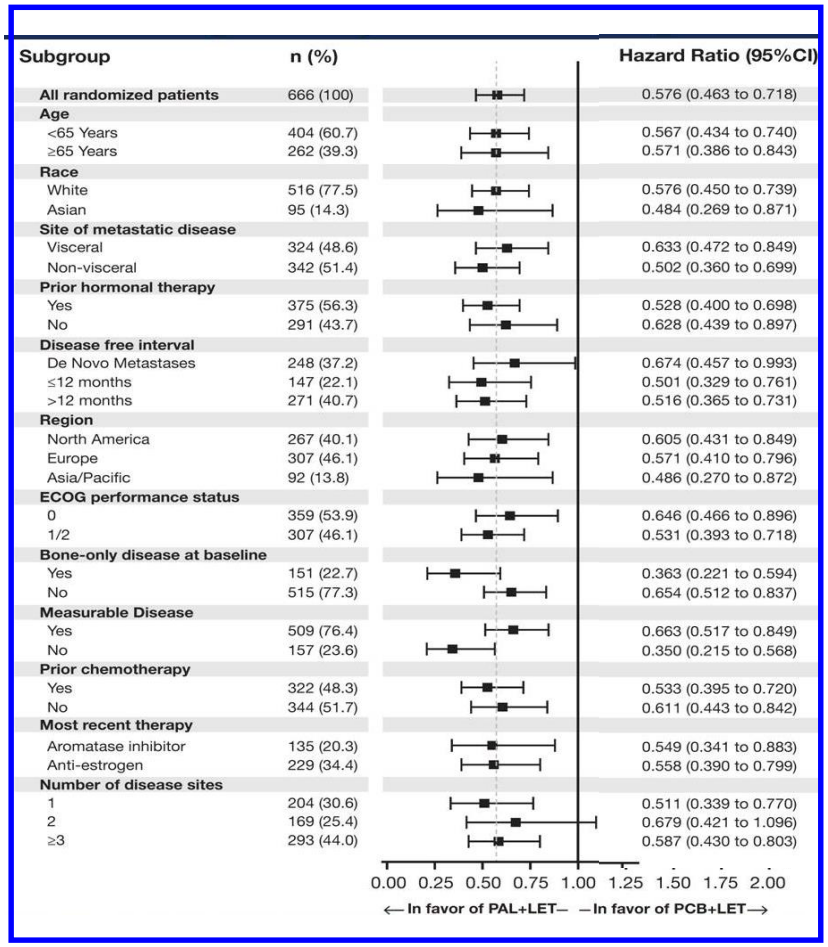


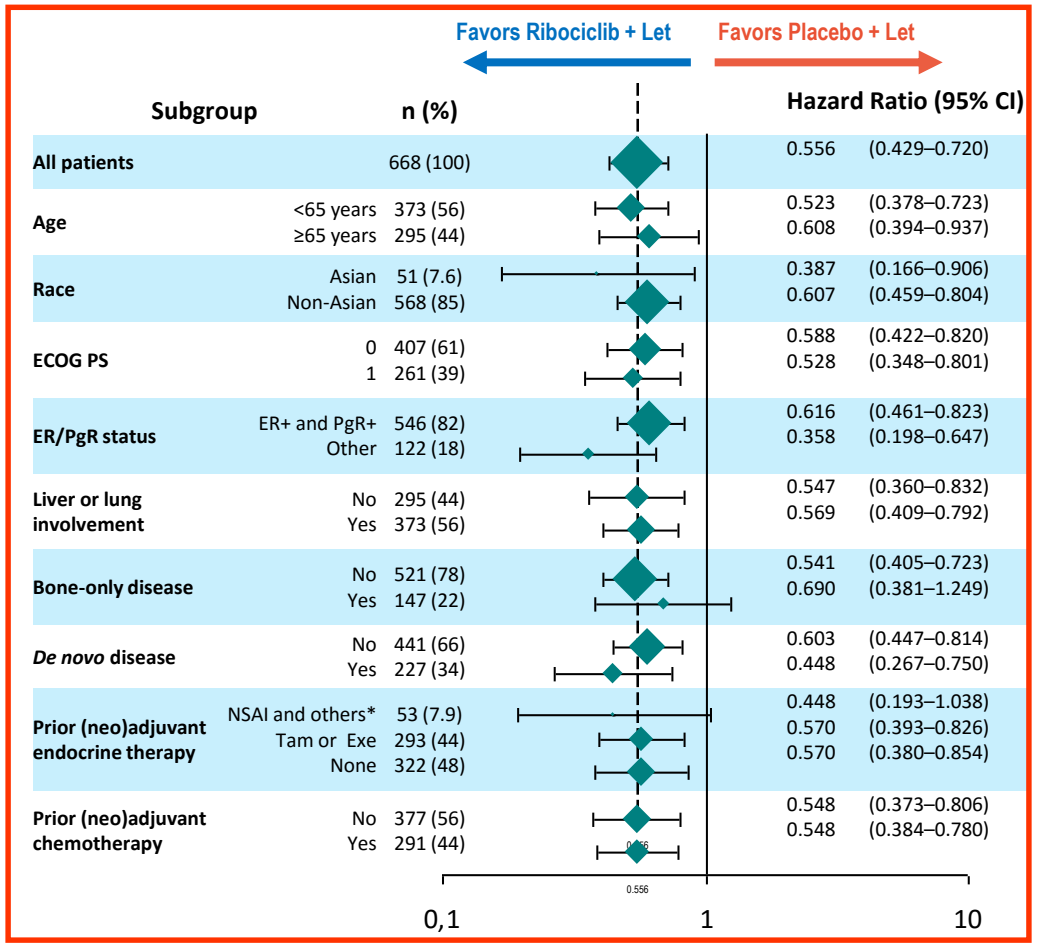
Finn RS, *et al.* Presented at the ASCO Annual Meeting 2016. Abstract 507.



Hortobagyi GN, *et al.* NEJM 2016-Presented at 2016 ESMO

Subgroup analyses in PALOMA-2 and MONALEESA-2





Finn RS, *et al.* Presented at the ASCO Annual Meeting 2016. Abstract 507.

Hortobagyi GN, *et al.* NEJM 2016- Presented at 2016 ESMO

Hematological AE in PALOMA-2 and MONALEESA-2

	Palbociclib + Letrozole (N=444)			Placebo + Letrozole (N=222)		
	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
Any AE, %	99	62	14	95	22	2
Neutropenia[a]	80	56	10	6	1	<1
Leukopenia[a]	39	24	1	2	0	0
Anemia[a]	24	5	<1	9	2	0
Thrombocytopenia[a]	16	1	<1	1	0	0

Finn RS, *et al.* Presented at the ASCO Annual Meeting 2016. Abstract 507.

Adverse Event ≥5% in Either Arm, %	Ribociclib + Letrozole n=334			Placebo + Letrozole n=330		
	All	Grade 3	Grade 4	All	Grade 3	Grade 4
Neutropenia	74	50	9.6	5.2	0.9	0
Leukopenia	33	20	1.2	3.9	0.6	0
Anemia	19	0.9	0.3	4.5	1.2	0
Lymphopenia	11	5.7	1.2	2.1	0.9	0
Thrombocytopenia	9.0	0.6	0	0.6	0	0

– Febrile neutropenia occurred in 5 (1.5%)* patients in the ribociclib arm vs. none in the placebo arm

Hortobagyi GN, *et al.* NEJM 2016- Presented at 2016 ESMO

Non-hematological AE in PALOMA-2 and MONALEESA-2

	Palbociclib + Letrozole (n=444)			Placebo + Letrozole (n=222)		
	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
Any adverse event, n (%)	439 (99)	276 (62)	60 (14)	212 (95)	49 (22)	5 (2)
Neutropenia[a]	353 (80)	249 (56)	46 (10)	14 (6)	2 (1)	1 (<1)
Leukopenia[a]	173 (39)	107 (24)	3 (1)	5 (2)	0	0
Fatigue	166 (37)	8 (2)	0	61 (28)	1 (<1)	0
Nausea	156 (35)	1 (<1)	0	58 (26)	4 (2)	0
Arthralgia	148 (33)	3 (1)	0	75 (34)	0	0
Alopecia	146 (33)	0	0	35 (16)	0	0
Diarrhea	116 (26)	6 (1)	0	43 (19)	3 (1)	0
Cough	111 (25)	0	0	42 (19)	0	0
Anemia[a]	107 (24)	23 (5)	1 (<1)	20 (9)	4 (2)	0
Back pain	96 (22)	6 (1)	0	48 (22)	0	0
Headache	95 (21)	1 (<1)	0	58 (26)	4 (2)	0
Hot flush	93 (21)	0	0	68 (31)	0	0
Constipation	86 (19)	2 (<1)	0	34 (15)	1 (<1)	0
Rash[a]	79 (18)	4 (1)	0	26 (12)	1 (<1)	0
Asthenia	75 (17)	10 (2)	0	26 (12)	0	0
Thrombocytopenia[a]	69 (16)	6 (1)	1 (<1)	3 (1)	0	0
Vomiting	69 (16)	2 (<1)	0	37 (17)	3 (1)	0
Pain in extremity	68 (15)	1 (<1)	0	39 (18)	3 (1)	0
Stomatitis	68 (15)	1 (<1)	0	13 (6)	0	0
Decreased appetite	66 (15)	3 (1)	0	20 (9)	0	0
Dyspnea	66 (15)	5 (1)	0	30 (14)	3 (1)	0
Insomnia	66 (15)	0	0	26 (12)	0	0

Finn RS, *et al.* Presented at the ASCO Annual Meeting 2016. Abstract 507.

Adverse Event ≥15% in Either Arm, %	Ribociclib + Letrozole n=334			Placebo + Letrozole n=330		
	All	Grade 3	Grade 4	All	Grade 3	Grade 4
Nausea	52	2.4	0	29	0.6	0
Infections	50	3.6	0.6	42	2.1	0.3
Fatigue	37	2.1	0.3	30	0.9	0
Diarrhea	35	1.2	0	22	0.9	0
Alopecia	33	–	–	16	–	–
Vomiting	29	3.6	0	16	0.9	0
Arthralgia	27	0.6	0.3	29	0.9	0
Constipation	25	1.2	0	19	0	0
Headache	22	0.3	0	19	0.3	0
Hot flush	21	0.3	0	24	0	0
Back pain	20	2.1	0	18	0.3	0
Cough	20	0	–	18	0	–
Decreased appetite	19	1.5	0	15	0.3	0
Rash	17	0.6	0	7.9	0	0
ALT increased	16	7.5	1.8	3.9	1.2	0
AST increased	15	4.8	0.9	3.6	1.2	0

Hortobagyi GN, *et al.* NEJM 2016- Presented at 2016 ESMO

- In the ribociclib arm 10 (3.0%) patients experienced Grade 2 QTcF (481–500 ms) and 1 (0.3%) patient experienced Grade 3 QTcF (>500 ms); no dose reductions were required

CDK 4/6 inhibitors in endocrine pre-treated MBC (ER+/HER2neg) PALOMA-3

HR+ HER2– MBC

Pre-/peri- or postmenopausal
Progressed on prior endocrine therapy:
–On or within 12 mo of completion of adjuvant treatment
–On or within 1 mo of treatment for MBC
≤1 prior chemotherapy regimen for advanced cancer

2:1 randomisation

N=521

Stratification:

- Visceral metastases
- Sensitivity to prior hormonal therapy
- Pre-/peri- vs. postmenopausal

n=347

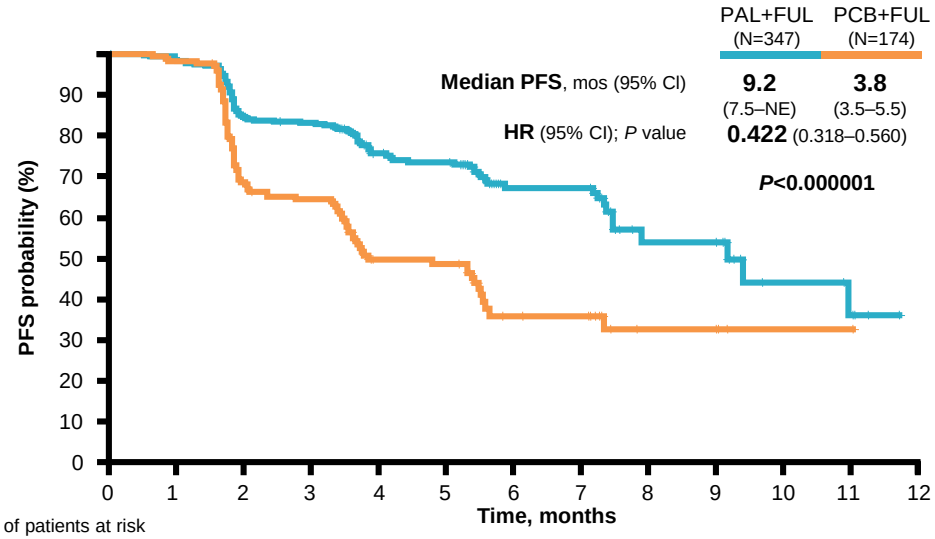
Palbociclib (125 mg QD; 3 wks on/1 wk off) + Fulvestrant (500 mg IM q4w)

n=174

Placebo (3 wks on/1 wk off) + Fulvestrant (500 mg IM q4w)

Turner NC, *et al. N Engl J Med*. 2015;373(3):209–219.
Cristofanilli M, *et al. Lancet Oncol*. 2016;17(4):425–439.

PFS (Investigators assessed) in PALOMA-3

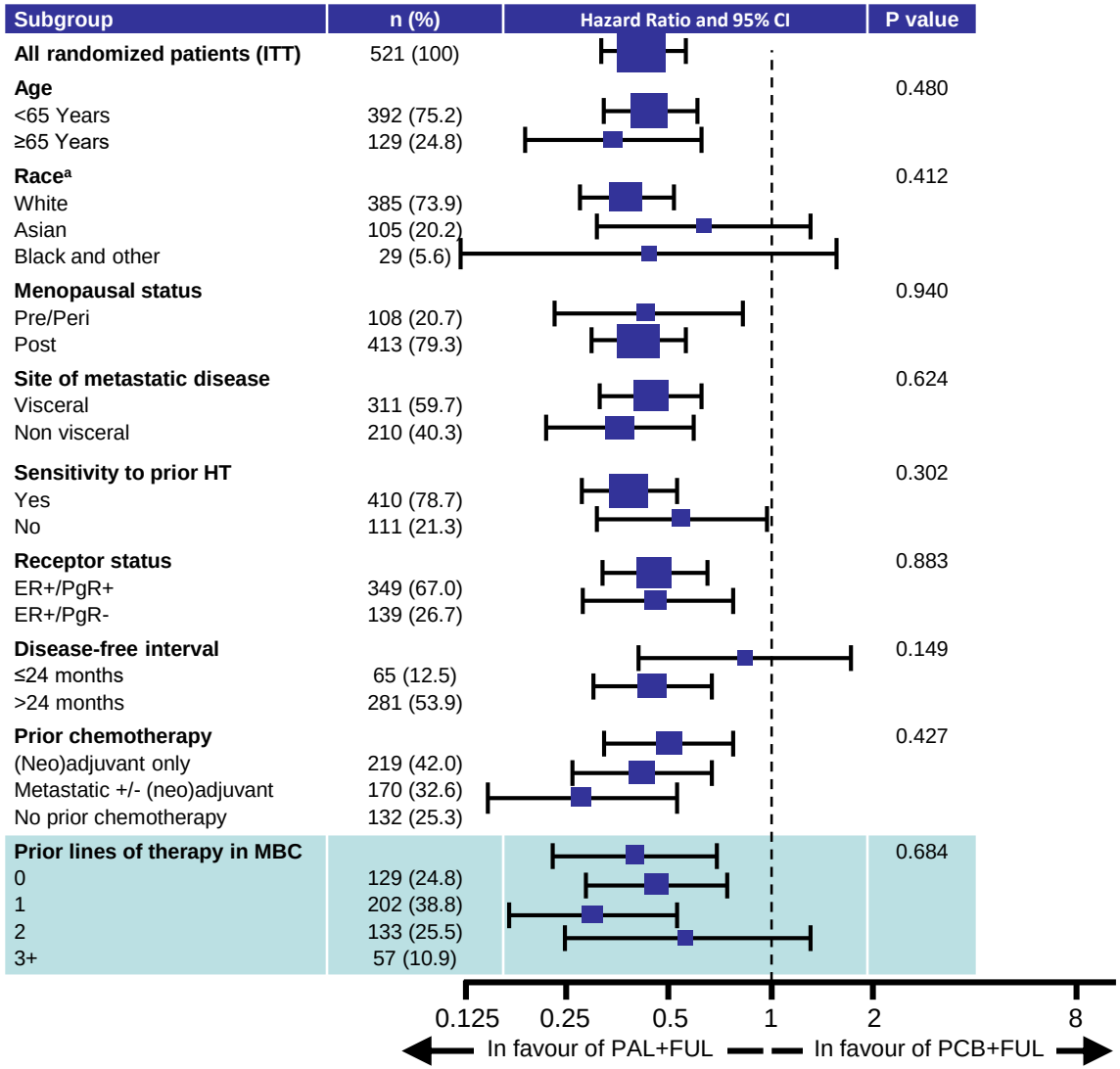


Number of patients at risk

PAL+FUL	347	279	132	59	16	6
PCB+FUL	174	109	42	16	6	1

Turner NC, *et al. N Engl J Med*. 2015;373(3):209–219.

Subgroup analysis in PALOMA-3



Turner NC, *et al. N Engl J Med*. 2015;373(3):209–219.

Palbociclib monotherapy in later treatment lines MBC

- **Phase II study. Breast cancer cohort comprised patients with histologically confirmed, RB-positive, stage IV, pretreated breast cancer (median nr of prior HT for MBC=2; median nr of prior CT for MBC=3) (NCT01037790)**

Group	n	Complete response n (%)	Partial response n (%)	Stable disease <6 mo n (%)	Stable disease ≥6 mo n (%)	Progressive disease n (%)	Clinical benefit* n (%)
HR+	30	0	2 (7)	14 (47)	3 (10)	11 (36)	5 (16)
HR-/HER2-	6	0	0	0	1 (17)	5 (83)	1 (17)
Total	**36**	**0**	**2 (6)**	**14 (39)**	**4 (11)**	**16 (44)**	**6 (17)**

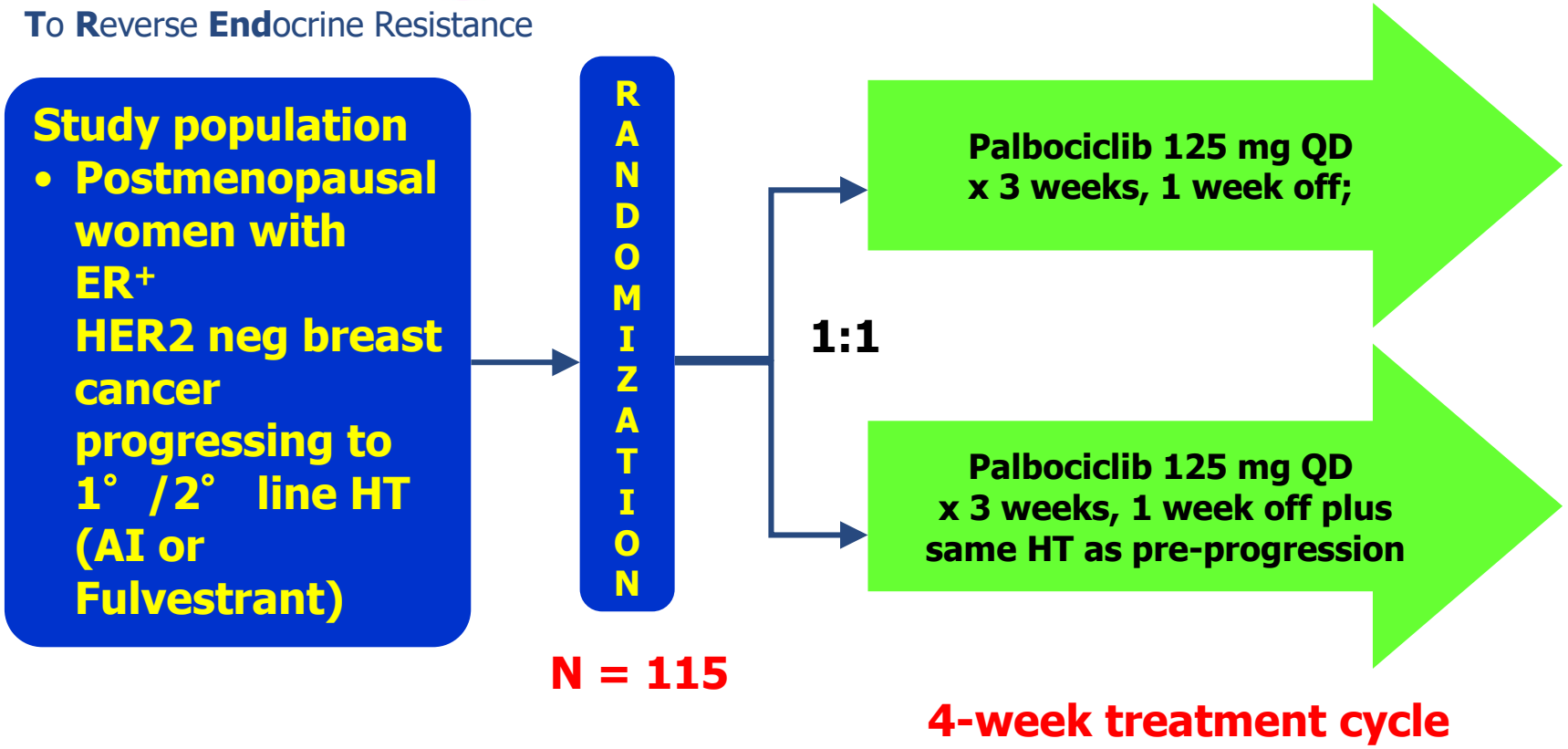
*Partial response or stable disease ≥6 months

- **Modest single-agent activity in this heavily pretreated population**
- **Well tolerated. Only grade 3/4 toxicity observed was neutropenia and thrombocytopenia, mostly uncomplicated**

DeMichele A, et al. ASCO 2013. Abstract 519.

TREnd Study design

To **R**everse **End**ocrine Resistance



Stratification Factors

1. Disease site (visceral vs bone only vs other)
2. number or prior lines of endocrine treatment (1 vs. 2)
3. duration of prior line of endocrine treatment (>6 vs. ≤6 months);
4. treating center

Abemaciclib in later treatment lines MBC (JPBA)

A Phase 1 Study of a CDK 4 and CDK 6 Dual Inhibitor in Participants With Advanced Cancer

Dose Escalation (3+3)
abemaciclib orally Q12H or Q24H
Days 1-28 of a 28-day cycle

Cohort A: Advanced cancer
Q24H (n=13)
Q12H (n=20)

Tumor Expansions
abemaciclib 150 mg or 200 mg orally Q12H
Days 1-28 of a 28-day cycle

Cohort B: Non-small cell lung cancer (N=68)

Cohort C: Glioblastoma multiforme (N=17)

Cohort D: Breast cancer (N=47)

Cohort E: Melanoma (N=26)

Cohort F: Colorectal cancer (N=15)

Cohort G: HR+ Breast cancer (N=19)
(Abemaciclib + Fulvestrant)

	Cohort D: Breast Cancer Abemaciclib (N=47)	Cohort G: HR+ Breast Cancer Abemaciclib + Fulvestrant (N=19)
Prior systemic therapies	47 (100%)	19 (100%)
≤3 regimens	11 (23%)	7 (37%)
≥4 regimens	36 (77%)	12 (63%)

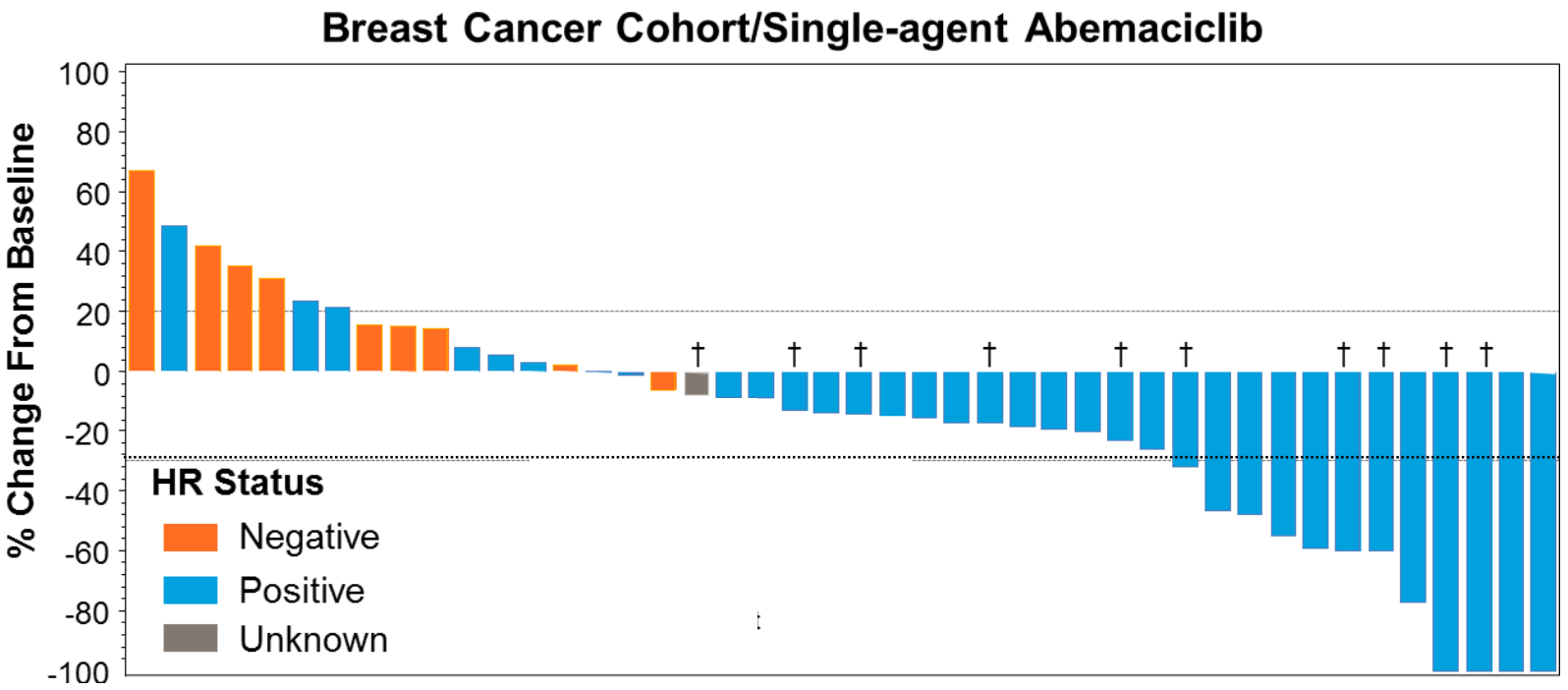
Patnaik A et al. *Cancer Discovery* 2016;(Ahead of print)

Abemaciclib (JPBA) clinical outcome

	Breast Cancer Cohort/Single-agent Abemaciclib (N=47)[a]				
			HR+		
Best Overall Response (%)	**All (N=47)**	**HR- (n=9)**	**HR+ (n=36)**	**HER2+ (n=11)**	**HER2- (n=25)**
Clinical benefit rate (CR + PR + SD ≥24 weeks)	49	11	**61**	55	64

	HR+ Breast Cancer Cohort/Abemaciclib + Fulvestrant (N=19)
Clinical benefit rate (CR + PR + SD ≥24 weeks)	**63**

Change in Tumor Size at Best Response in Patients With Breast Cancer



†Received concomitant hormonal therapy

1. Patnaik A et al. *Cancer Discovery* 2016;(Ahead of print)
2. Tolaney SM et al. San Antonio Breast Cancer Symposium 2014. Abstract 763

Adverse events in JPBA (Phase I)

JPBA: Possibly Related TEAEs in >10% of Patients in Tumor-specific Cohorts (B-F)

TEAE, n (%)	Grade 1	Grade 2	Grade 3	Grade 4	All Grades (N=173)[a]
Diarrhea	75 (43)	25 (15)	9 (5)	0	109 (63)
Nausea	59 (34)	15 (9)	4 (2)	0	78 (45)
Fatigue	38 (22)	27 (16)	5 (3)	0	70 (41)
Vomiting	31 (18)	10 (6)	2 (1)	0	43 (25)
Leukopenia	9 (5)	17 (10)	17 (10)	0	43 (25)
Thrombocytopenia	21 (12)	7 (4)	12 (7)	0	40 (23)
Neutropenia	6 (4)	15 (9)	16 (9)	2 (1)	39 (23)
Anemia	13 (8)	14 (8)	7 (4)	0	34 (20)
Anorexia	22 (13)	8 (5)	0	0	30 (17)
Creatinine increased[b]	12 (7)	7 (4)	0	0	19 (11)
Weight loss	14 (8)	4 (2)	0	0	18 (10)

- No Grade 5 adverse events reported

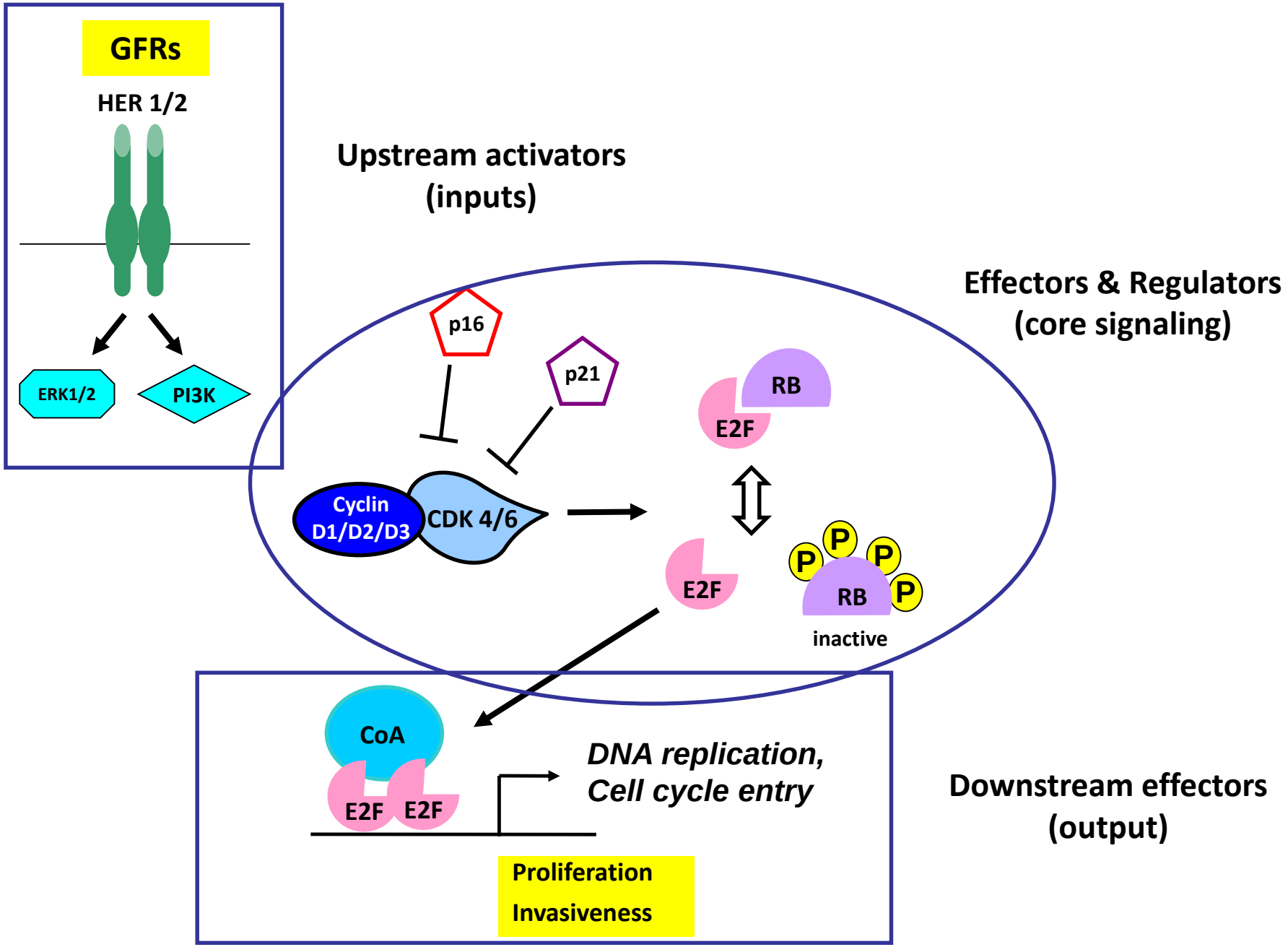
[a]Includes all tumor-specific cohorts receiving single-agent abemaciclib for NSCLC, glioblastoma, breast cancer, melanoma, or colorectal cancer.
[b]Abemaciclib inhibits renal transporters that mediate tubular secretion of creatinine, so serum creatinine may not accurately reflect renal function in patients receiving abemaciclib

1. Patnaik A et al. *Cancer Discovery* 2016;(Ahead of print)

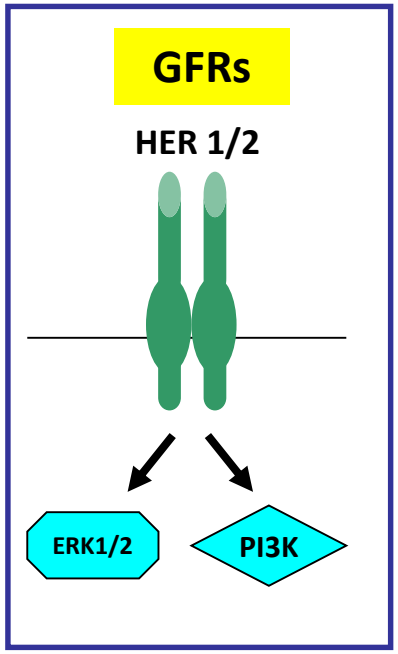
Outline

- CDK 4/6 inhibitors: MoA and pre-clinical data
- Clinical data in ER+/HER2 neg metastatic breast cancer
- **Biomarkers**

Molecular determinants of response to CDK4/6 inhibition



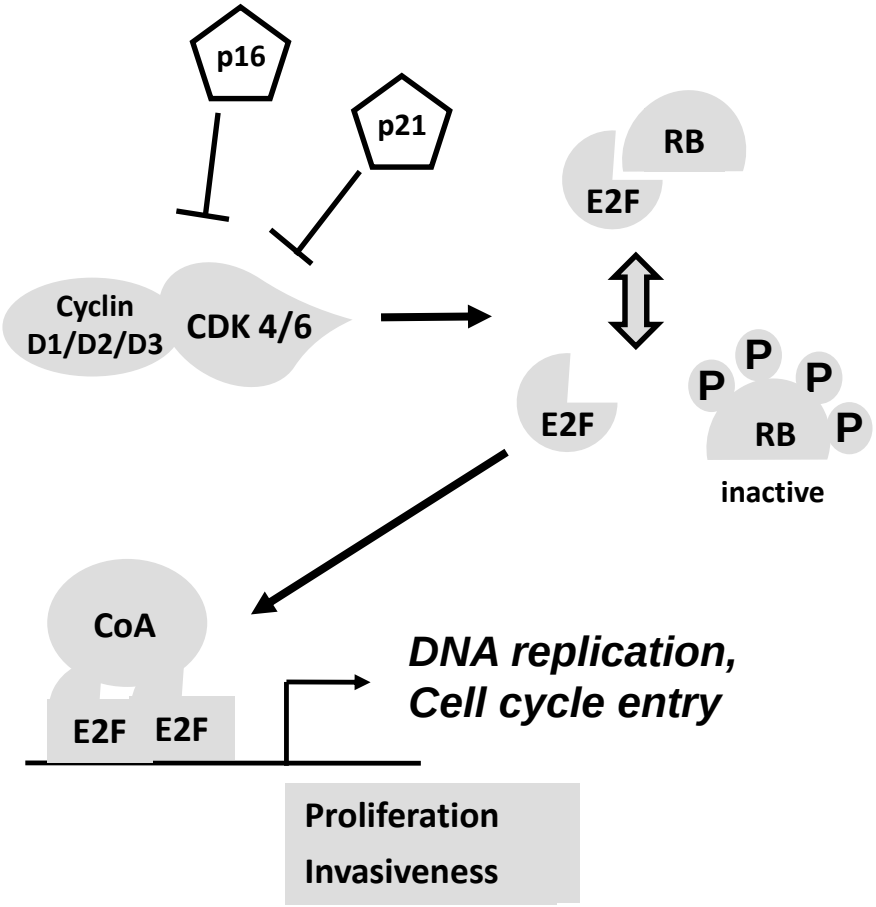
Molecular determinants of response to CDK4/6 inhibition



Upstream activators (inputs)

PROs: may have some sensitivity for CDK4/6i (correctly identifies responders)

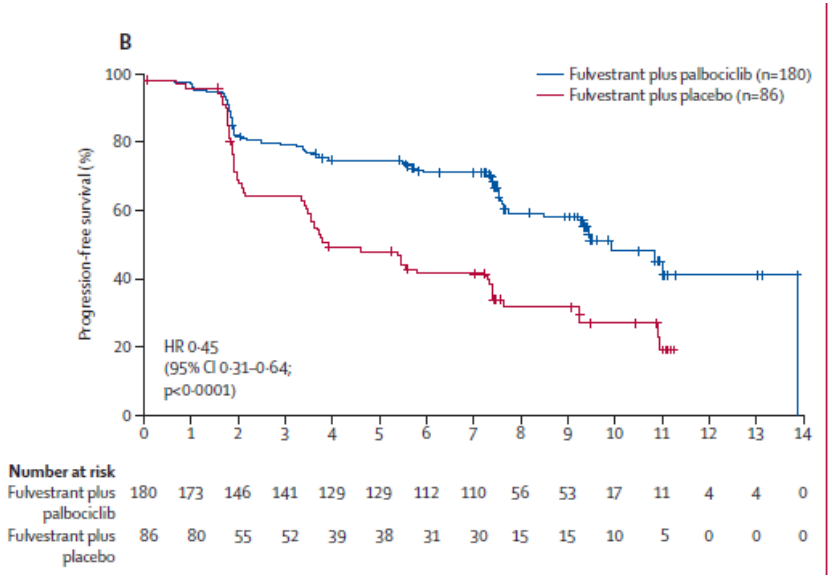
CONs: certainly non specific for CDK4/6i (will fail to identify all non-responders)



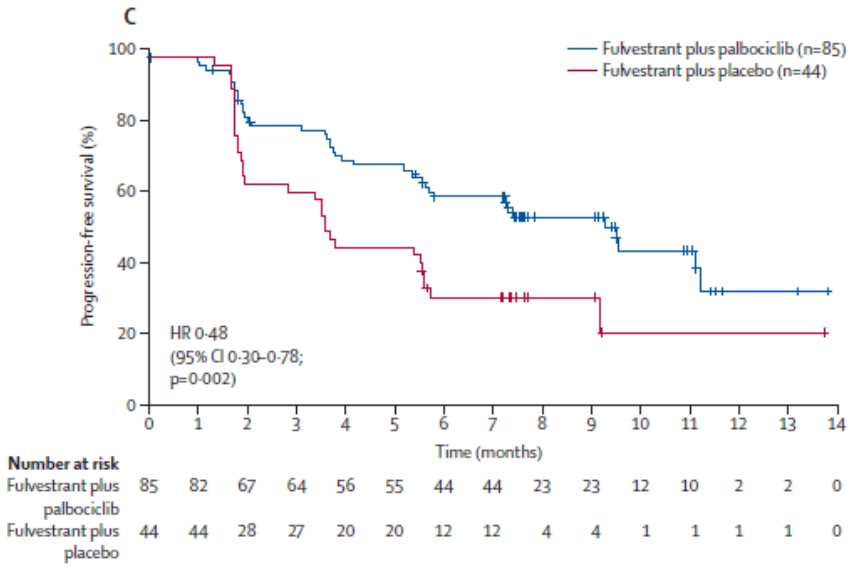
PIK3CA mutation status- PALOMA-3

PIK3CA status (exon 9 and 20 hotspots) was determined by BEAMING assay on circulating DNA in 395 pts in PALOMA 3

PIK3CA WT



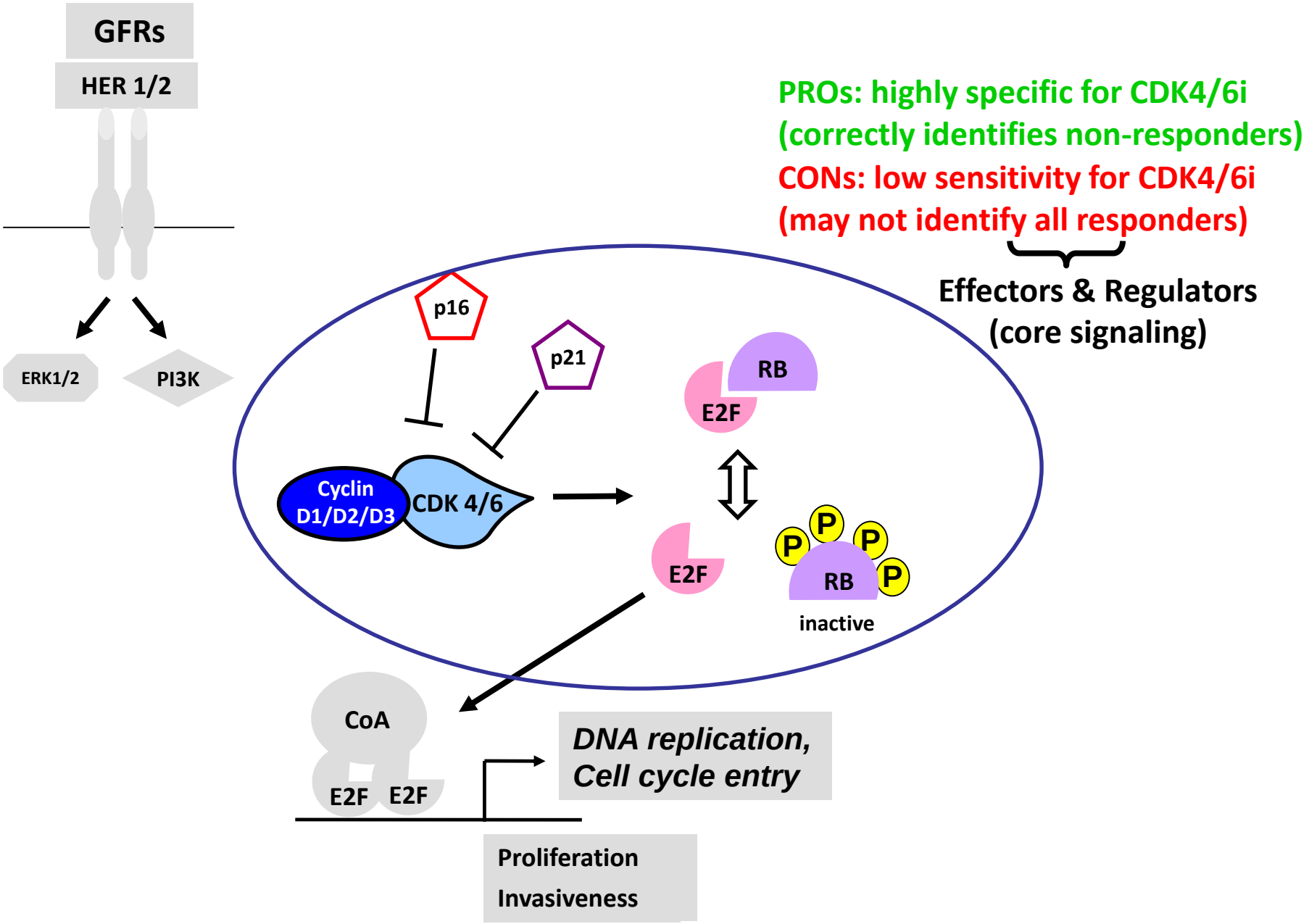
PIK3CA Mut



PIK3CA status does not impact the magnitude of benefit from palbociclib

Cristofanilli M, *et al. Lancet Oncol*. 2016;17(4):425–439.

Molecular determinants of response to CDK4/6 inhibition



PALOMA 1- role of CCD1 and p16

- Phase II, 1° line
- ER+, HER2– BC status
- Same as part 1 but with CCND1 amplification and/or loss of p16

Part 1 (N=66)

Part 2 (N=99)

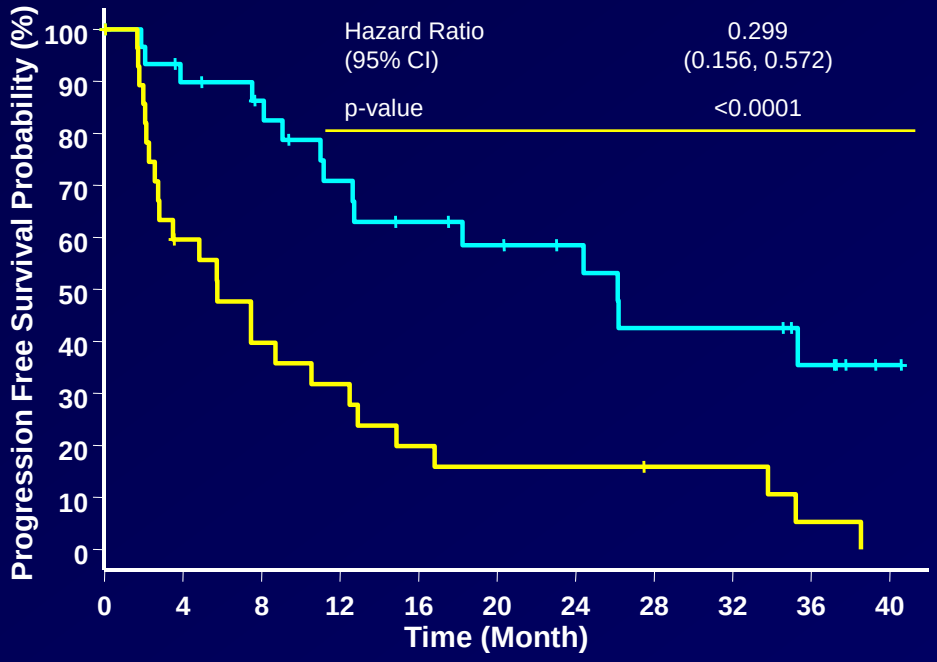
R

Palbociclib 125 mg QD + Letrozole 2.5 mg QD

Letrozole 2.5 mg QD

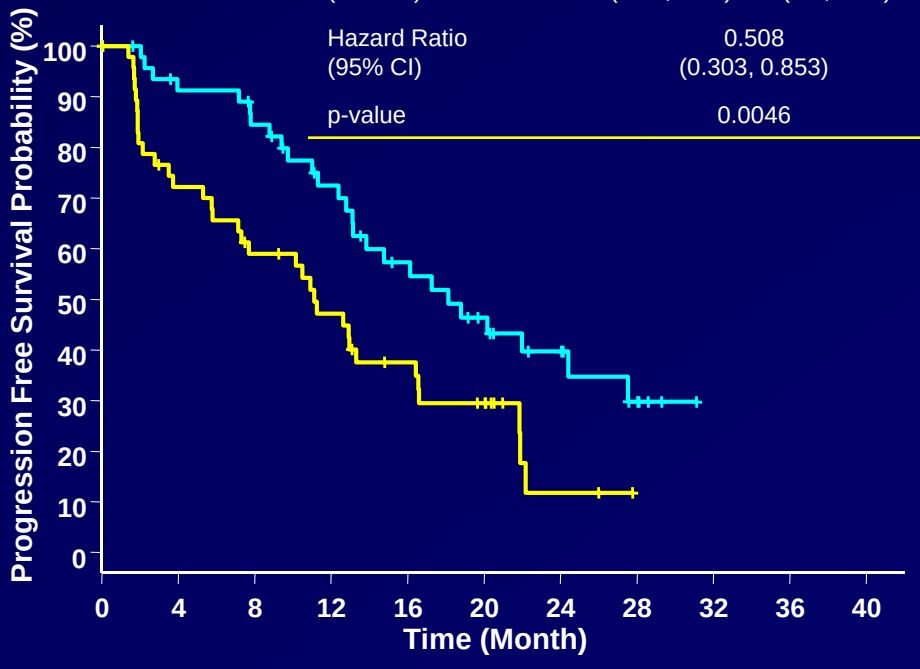
UNSELECTED (ER+/HER2 neg)

Part 1	*PAL + LET (N=34)*	*LET (N=32)*
Number of Events (%)	15 (44)	25 (78)
Median PFS, months (95% CI)	26.1 (11.2, NR)	5.7 (2.6, 10.5)
Hazard Ratio (95% CI)	0.299 (0.156, 0.572)	
p-value	<0.0001	



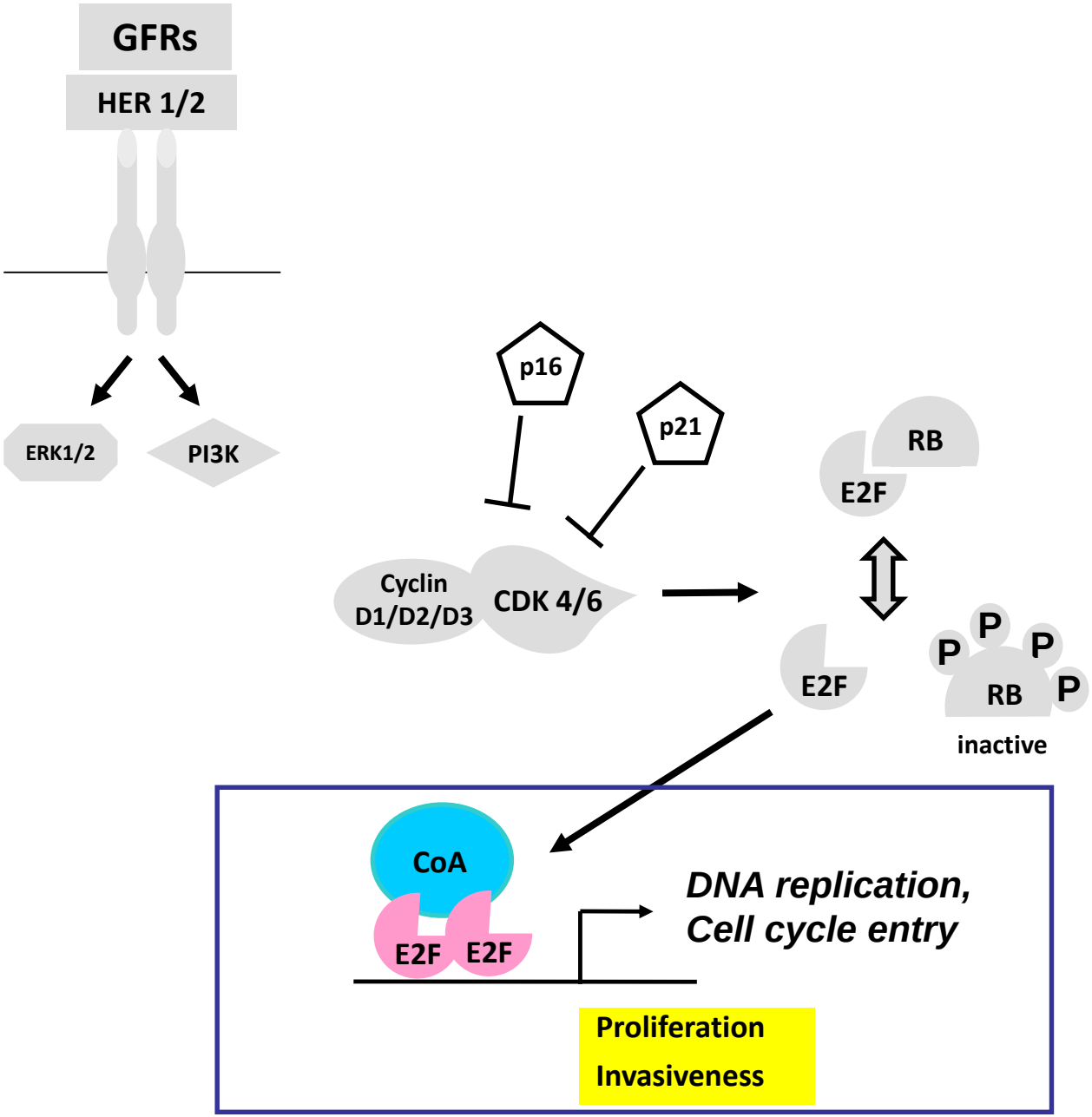
CCD1 amplif. and/or p16 loss

Part 2	*PAL + LET (N=50)*	*LET (N=49)*
Number of Events (%)	26 (52)	34 (69)
Median PFS, months (95% CI)	18.1 (13.1, 27.5)	11.1 (7.1, 16.4)
Hazard Ratio (95% CI)	0.508 (0.303, 0.853)	
p-value	0.0046	



Finn R, *et al* Lancet Oncology 2015; 16: 25–35

Molecular determinants of response to CDK4/6 inhibition

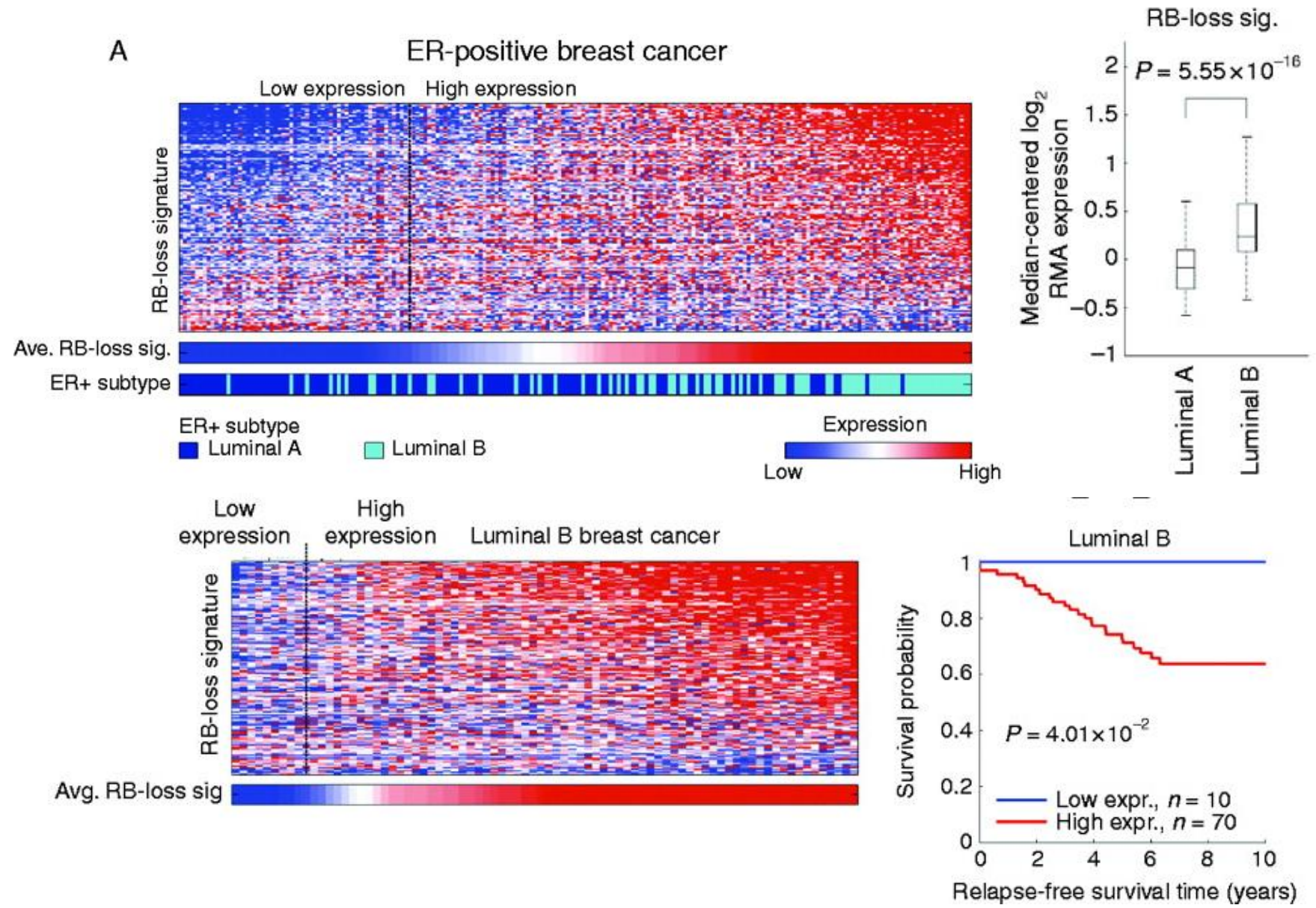


PROs: highly specific and sensitive for CDK4/6i

CONs: more complex

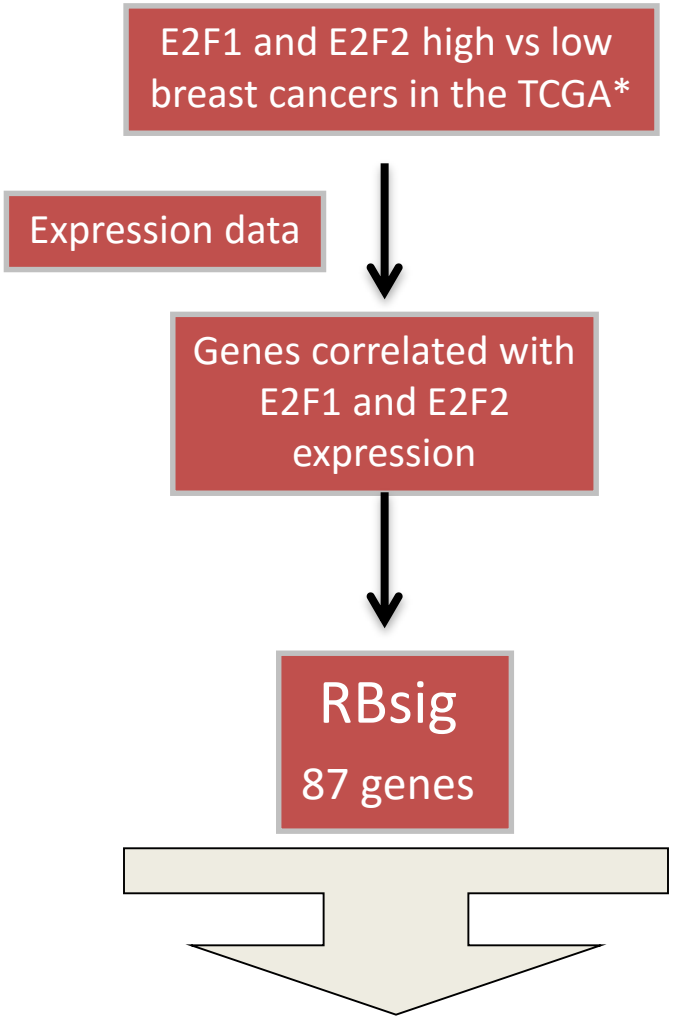
Downstream effectors (output)

Rb loss signature in Luminal BC



Thangavel C et al. Endocr Relat Cancer 2011

Construction of our RBsig

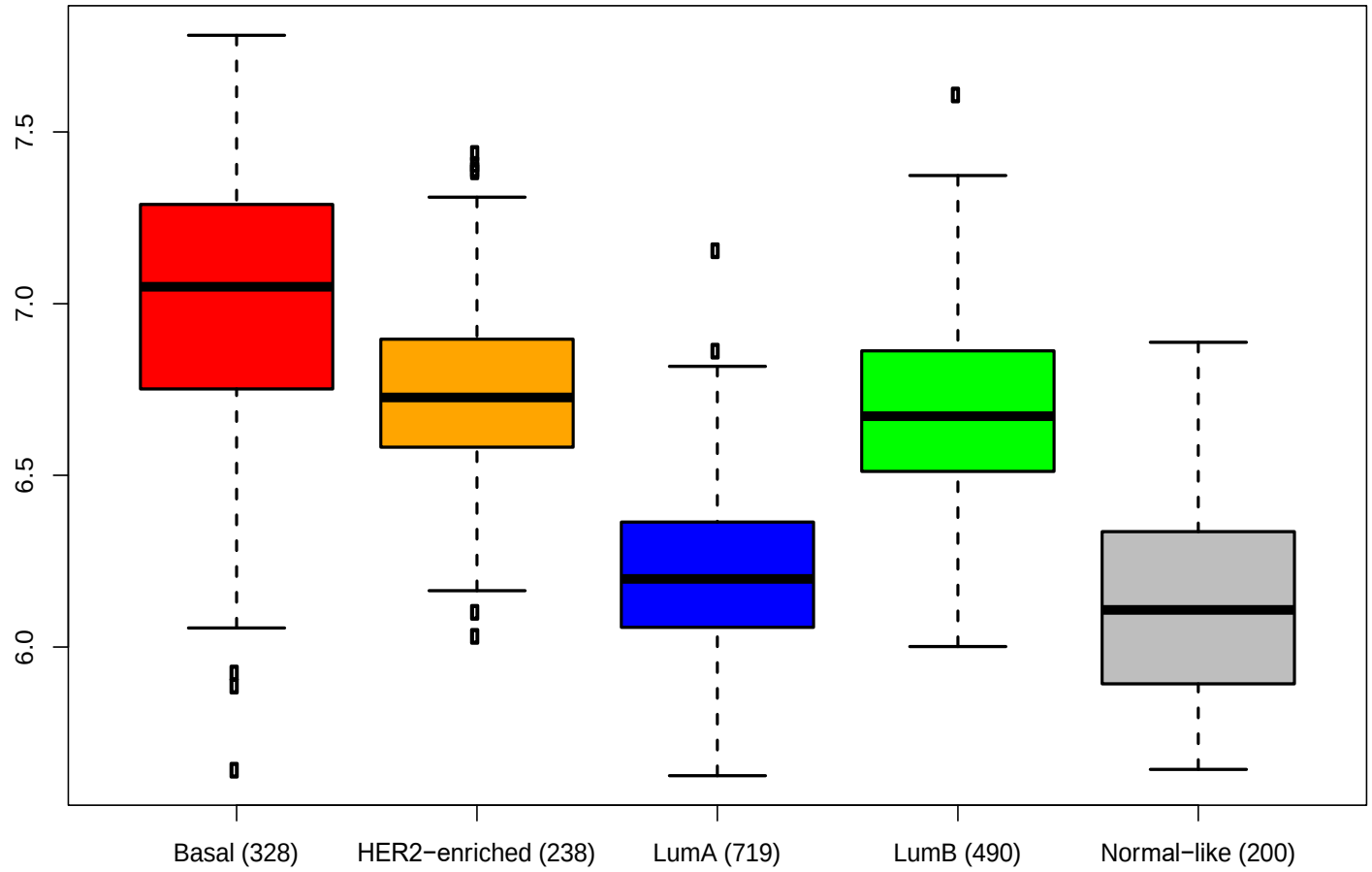


Functional analysis

Association with molecular subtypes

*TCGA: The Cancer Genome Atlas, CCLE: Cancer Cell Line Encyclopedia

RBsig expression in BC subtypes



RBsig levels are higher basal BC and, among Luminal BC, are higher in LumB

Malorni L. et al; Oncotarget 2016

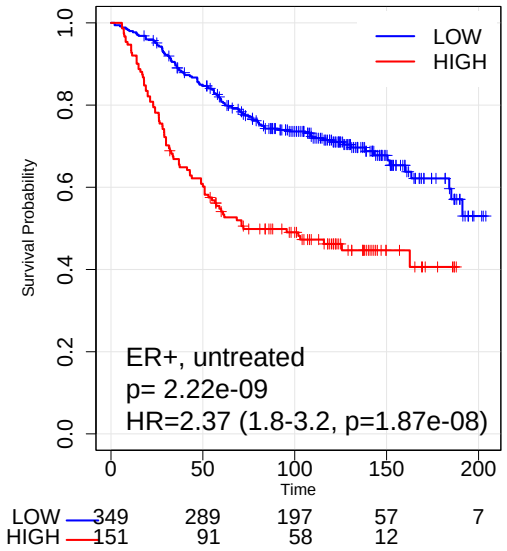
Does RBsig hold prognostic information in ER+ BC?

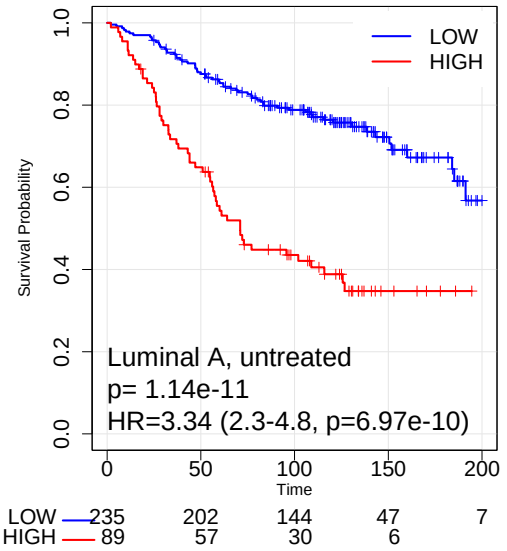
RBsig

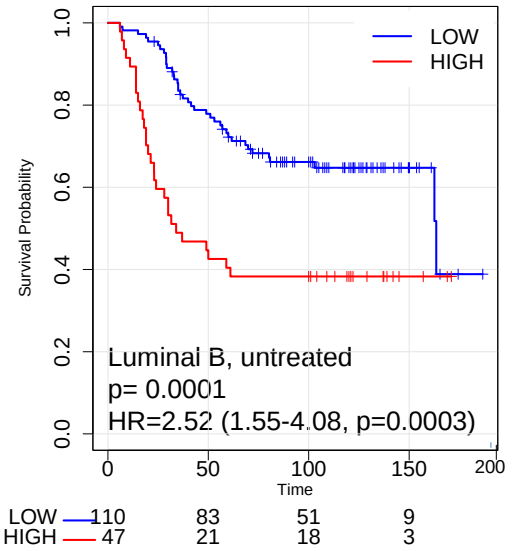
BC gene expression meta-dataset (N=3458)

Recurrence free survival (RFS) of pts with ER+ tumors, untreated or endocrine treated only

Prognostic value in BC patients

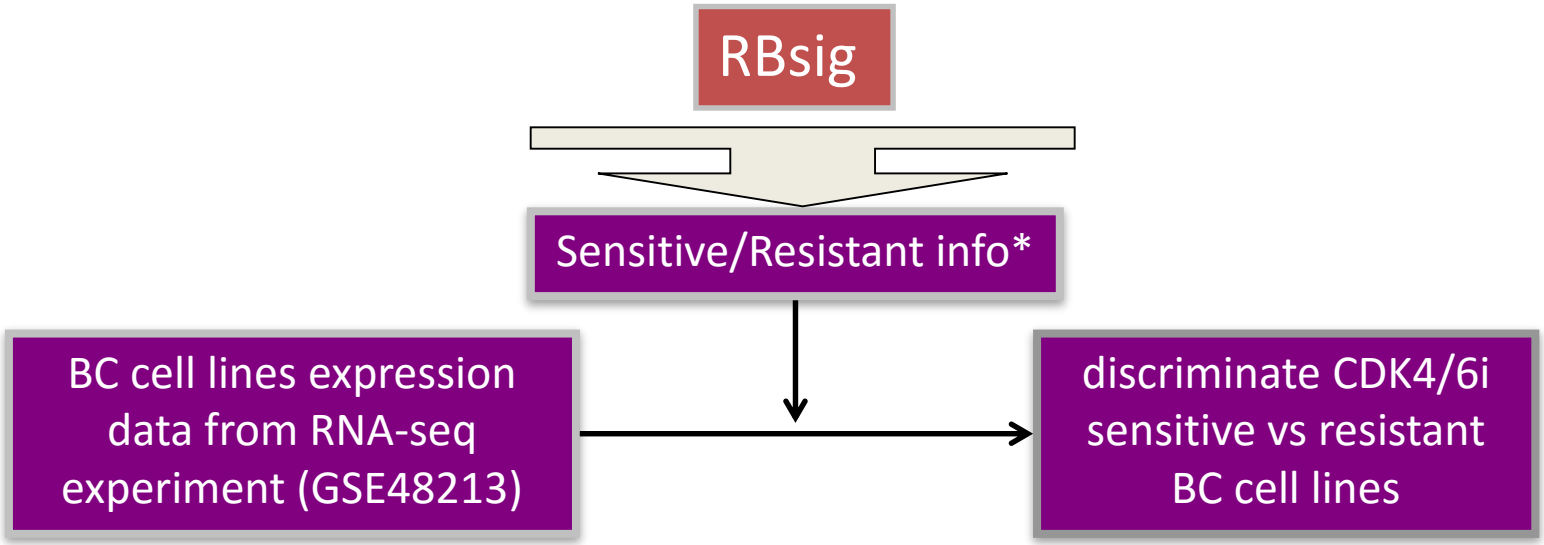


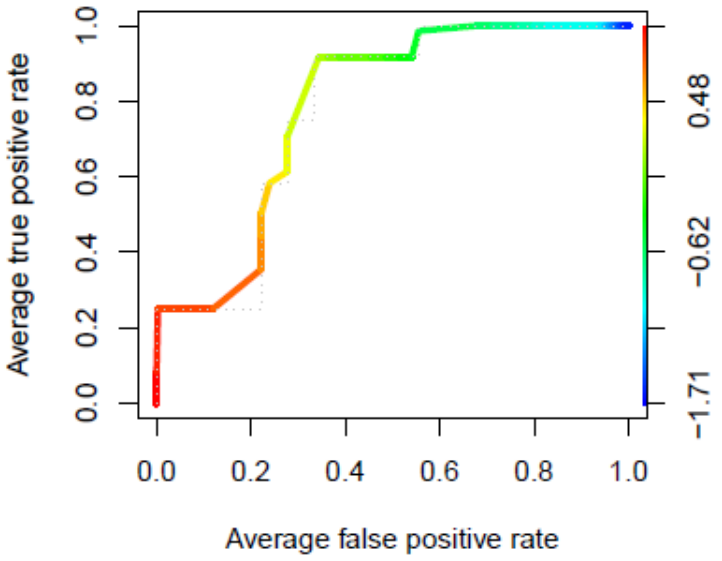




Malorni L. et al; Oncotarget 2016

Does RBsig predict response to CDK4/6 inhibitors?





RBsig identifies CDK4/6i resistant vs sensitive cell lines with and Area Under the Curve (AUC) of 0,7778

*Finn RS et al. BCR 2009; 11:R77

Malorni L. et al; Oncotarget 2016

Conclusions

- **CDK4/6 inhibitors represent a new standard of care for the treatment of ER+/HER2neg MBC**
- **Clinical data are very convincing but… biomarkers are lacking**
- **Given the high activity and good tolerability of single agent hormonal therapy, biomarkers for selecting patients more likely to benefit from CDK4/6 inhibition would be of great clinical utility to maximize benefit and containing costs.**

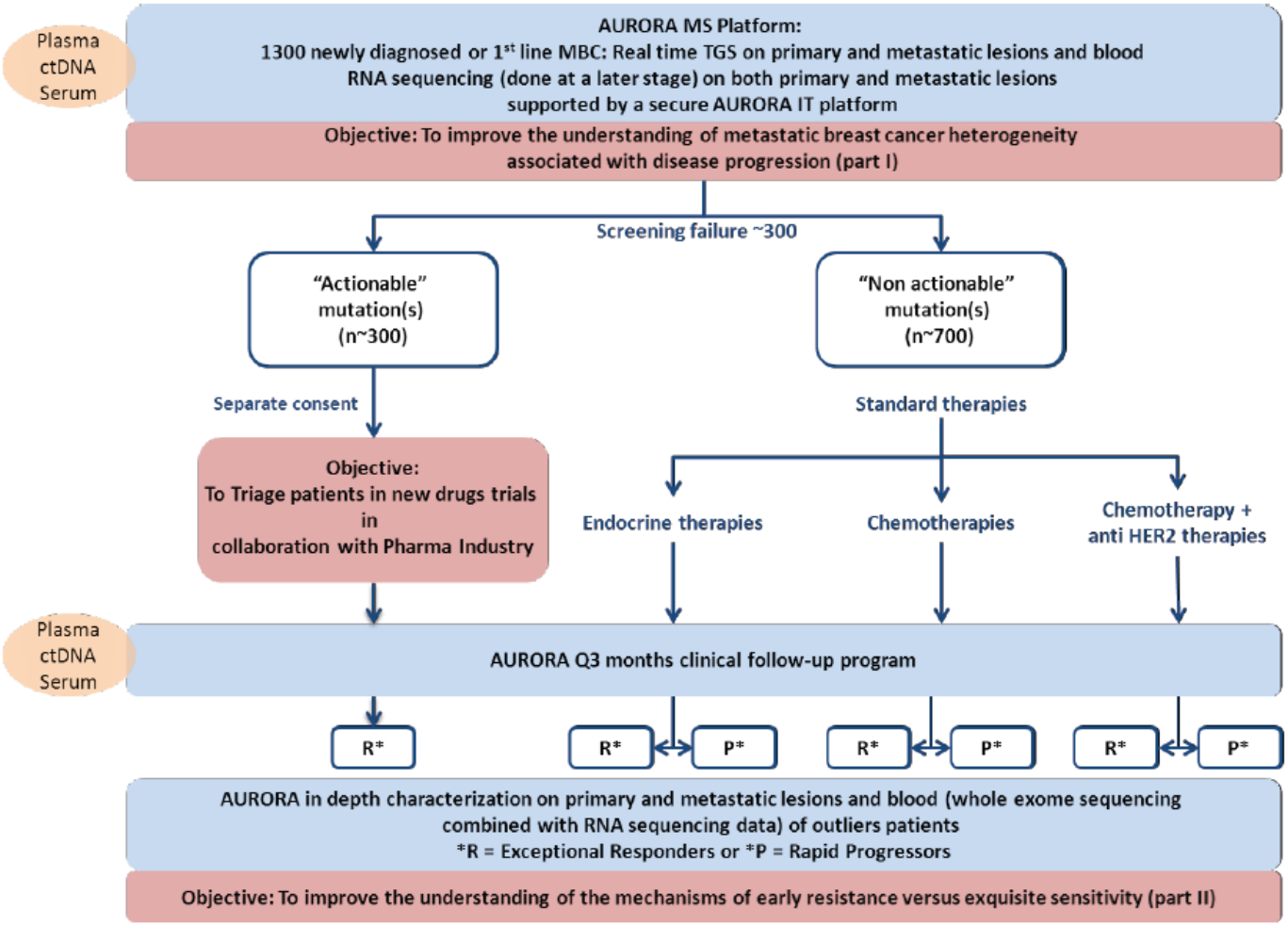
Perspectives

- **A more detailed knowledge of the biology of metastatic breast cancer is needed to ensure that our fight to this disease will finally be successful (AURORA program)**
- **Comprehensive assessment of molecular pathways functional status vs. single marker status**

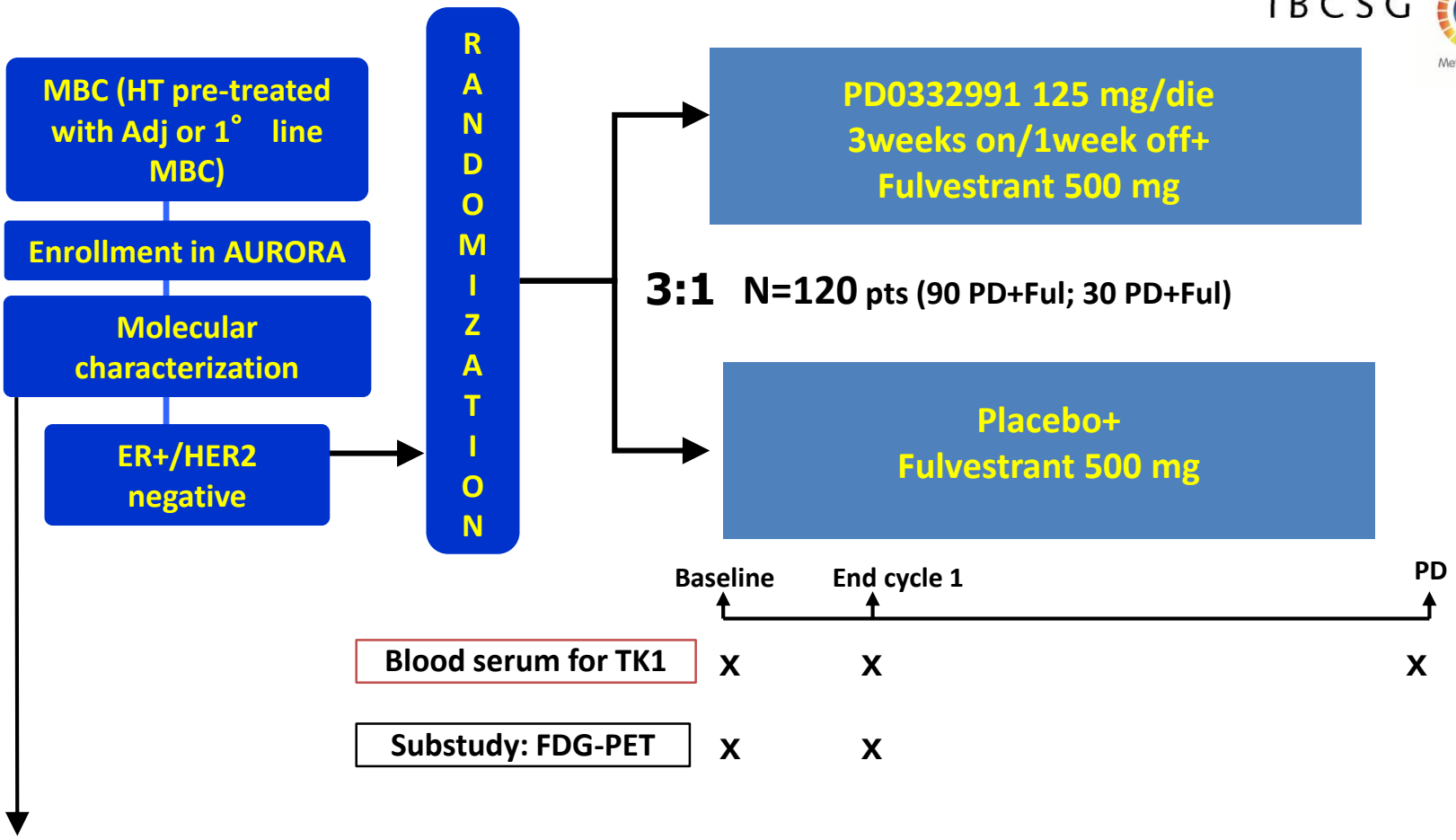
BIG
Breast International Group

AURORA

Metastatic Breast Cancer - molecular aberrations



PYTHIA trial (NCT02536742)



Biological samples for translational research through AURORA:

- FFPE blocks from primary tumor and metastatic lesion
- Whole blood sample for pharmacogenomics
- Serial plasma & serum samples for biomarker analysis: at baseline, every 6 months and at progression

Acknowledgements

AIRC
(MFAG 14371)

Fondazione Sandro Pitigliani
per la lotta contro i tumori - ONLUS

ITT Istituto Toscano Tumori

SST Azienda USL 4 Prato
Servizio Sanitario della Toscana

Backup

Acknowledgements

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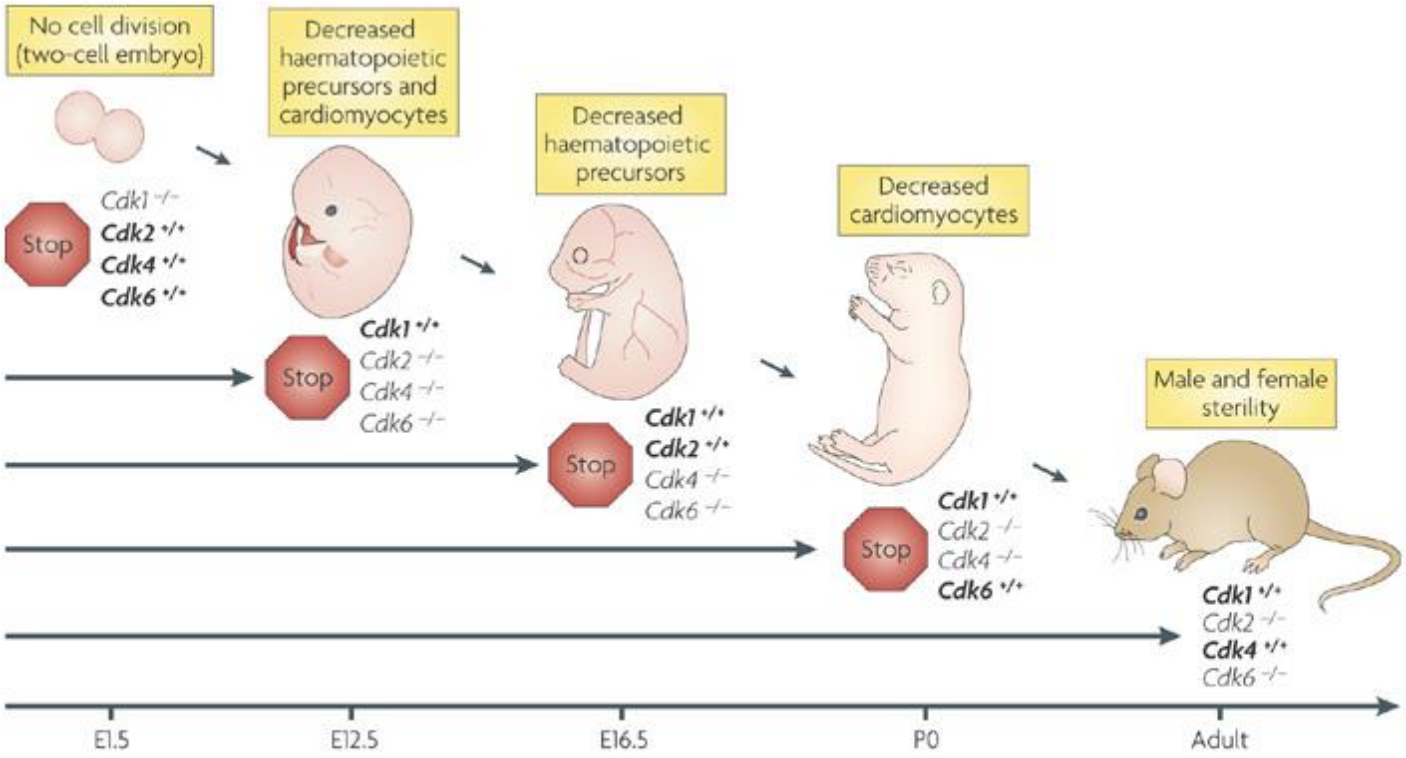
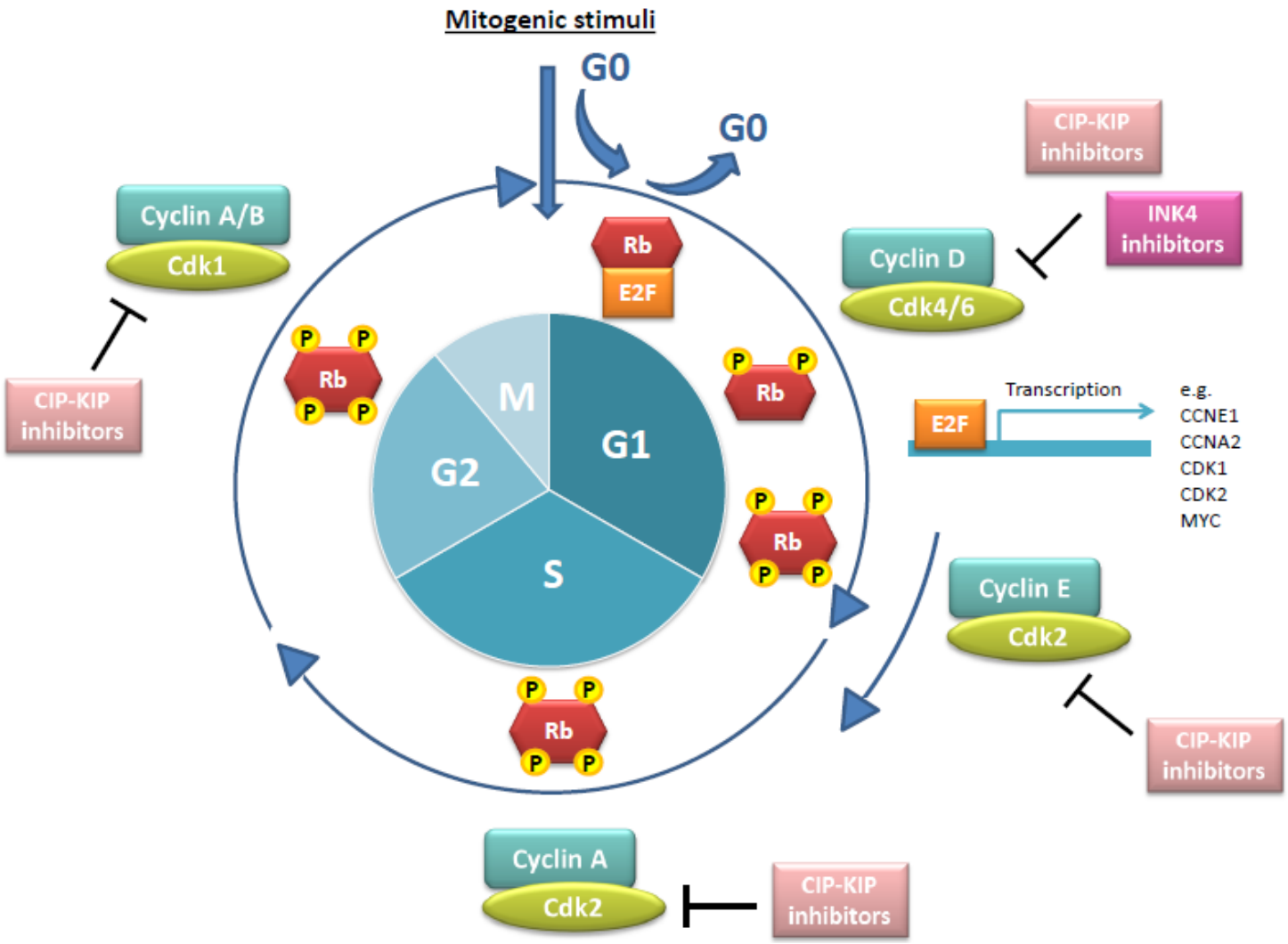
“Sandro Pitigliani” Medical Oncology Unit, Hospital of Prato

Functional Genomics & Bioinformatics Units, Proxenia S.r.l

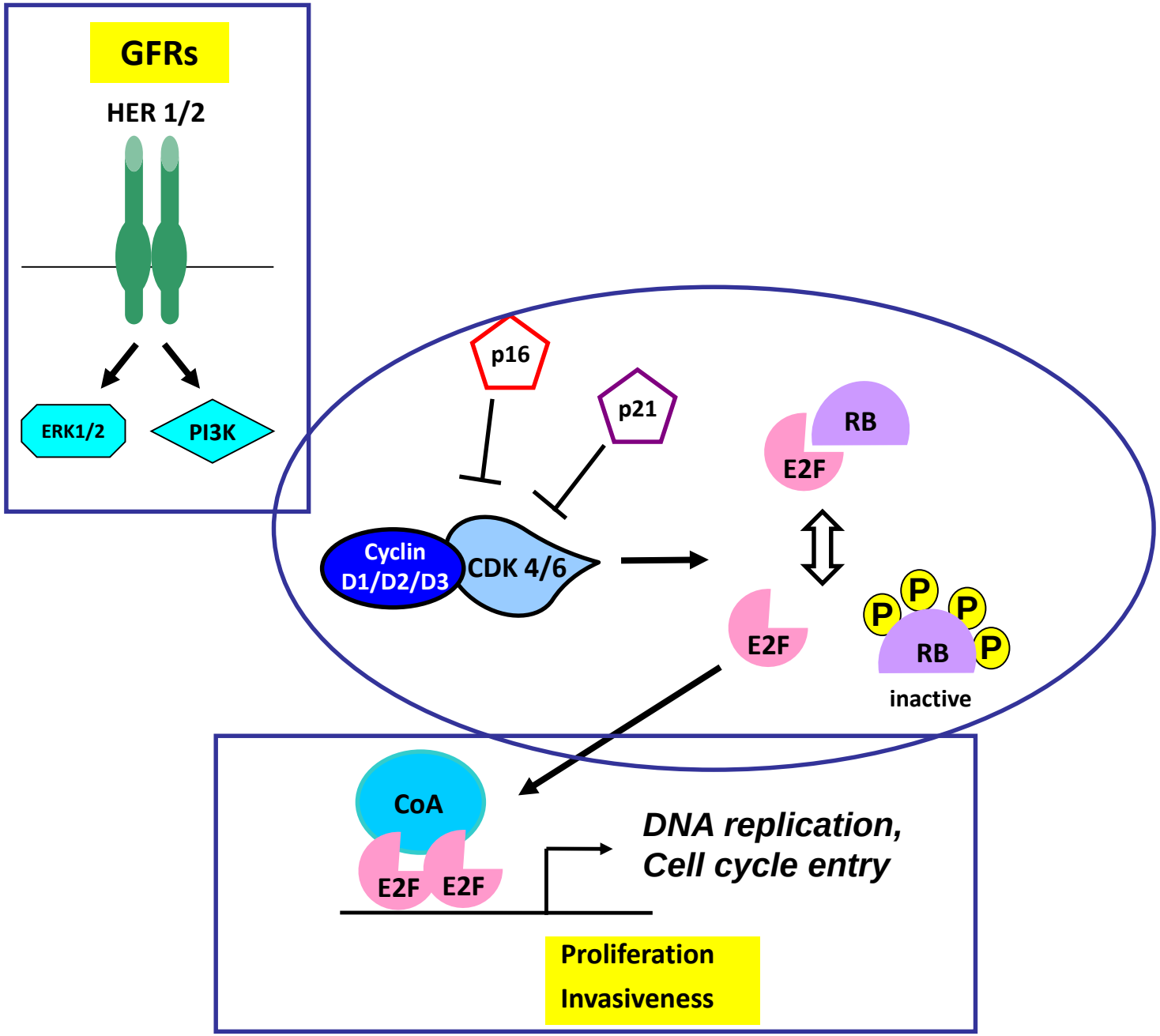
AIRC (MFAG 14371)

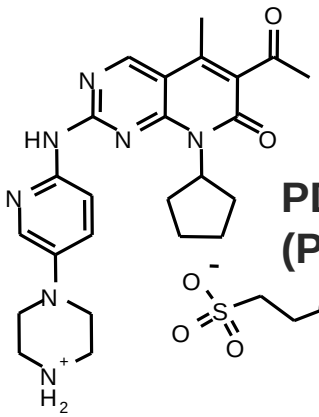
Fondazione Sandro Pitigliani
per la lotta contro i tumori - ONLUS


No cell division (two-cell embryo)
Stop
Cdk1 −/−
Cdk2 +/+
Cdk4 +/+
Cdk6 +/+
Decreased haematopoietic precursors and cardiomyocytes
Stop
Cdk1 +/+
Cdk2 −/−
Cdk4 −/−
Cdk6 −/−
Decreased haematopoietic precursors
Stop
Cdk1 +/+
Cdk2 +/+
Cdk4 −/−
Cdk6 −/−
Decreased cardiomyocytes
Stop
Cdk1 +/+
Cdk2 −/−
Cdk4 −/−
Cdk6 +/+
Male and female sterility
Cdk1 +/+
Cdk2 −/−
Cdk4 +/+
Cdk6 −/−
E1.5
E12.5
E16.5
P0
Adult


Mitogenic stimuli
G0
G0
CIP-KIP inhibitors
INK4 inhibitors
Cyclin A/B
Cdk1
Cyclin D
Cdk4/6
Rb
E2F
CIP-KIP inhibitors
M
G1
G2
S
Transcription
E2F
e.g.
CCNE1
CCNA2
CDK1
CDK2
MYC
Cyclin E
Cdk2
CIP-KIP inhibitors
Cyclin A
Cdk2
CIP-KIP inhibitors

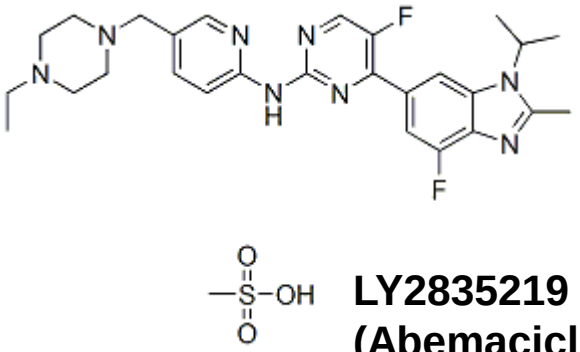
Molecular determinants of response to CDK4/6 inhibition





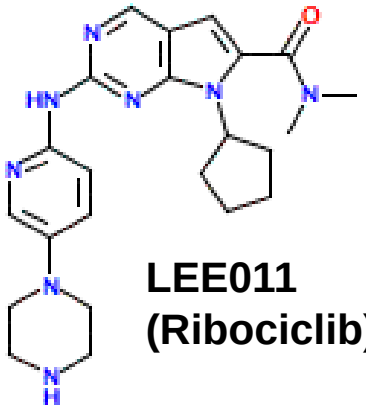
PD-0332991 (Palbociclib)

CDK (Cyclin partner)	IC_{50} (μM)
CDK4/Cyclin D1	0.011
CDK4/Cyclin D3	0.009
CDK6/Cyclin D2	0.015
CDK2/Cyclin A	>5
CDK1/Cyclin B	>5
CDK5/p25	>5



LY2835219 (Abemaciclib)

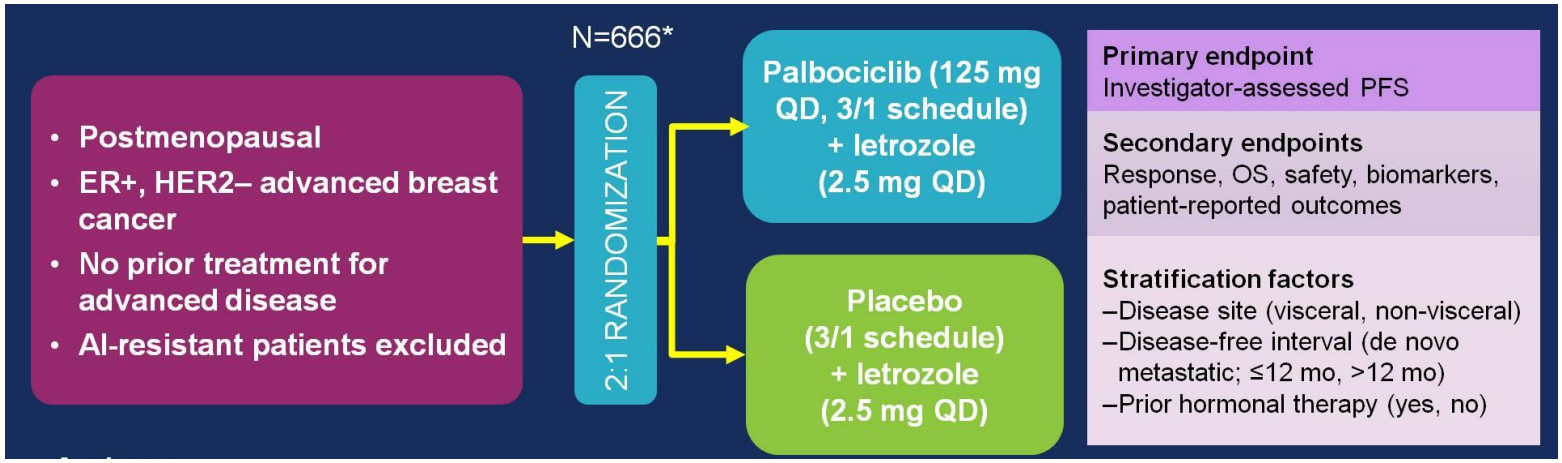
CDK	IC_{50} (μM)
CDK4	0.002
CDK6	0.009
CDK1	1.6

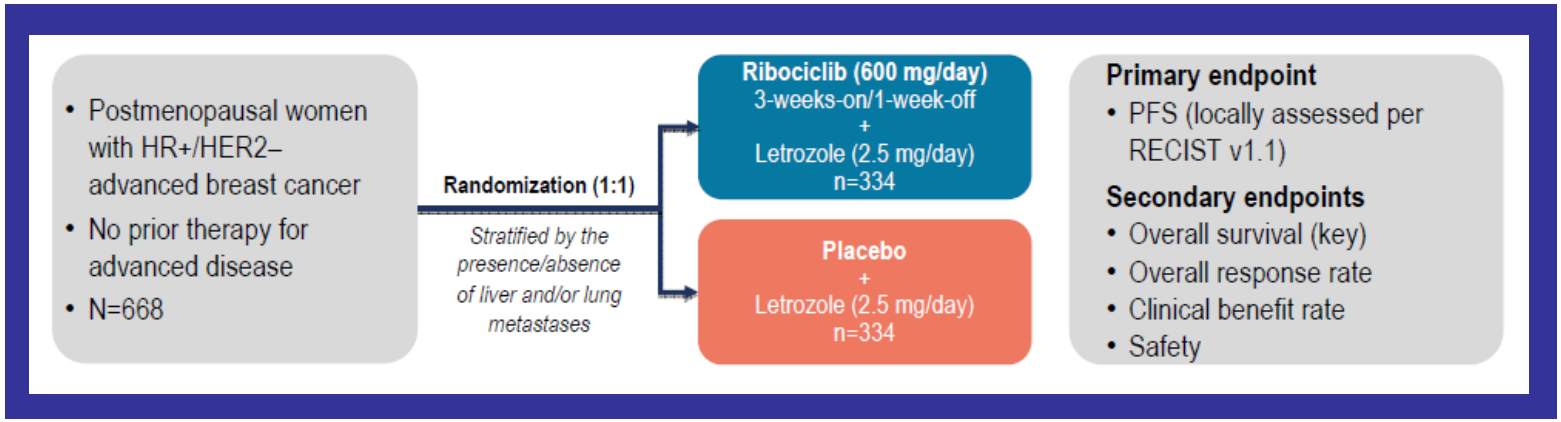


LEE011 (Ribociclib)

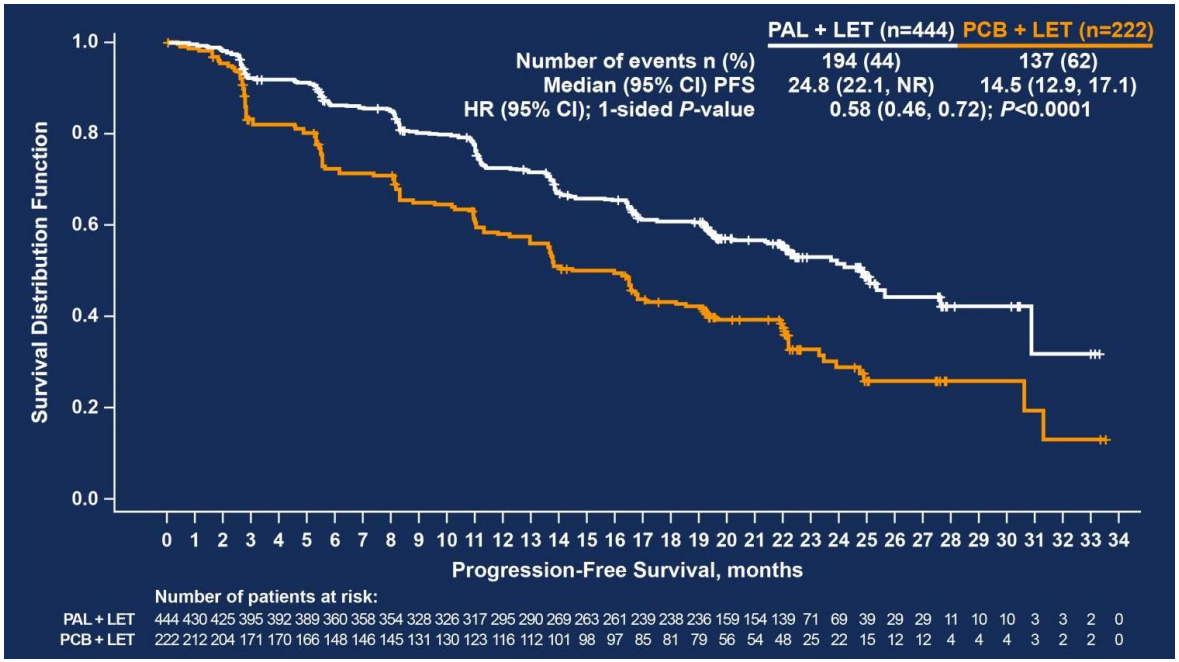
CDK (Cyclin partner)	IC_{50} (μM)
CDK4/cyclin D1	0.010
CDK6/cyclin D3	0.039
CDK1/cyclin B	113
CDK2/cyclin A	76

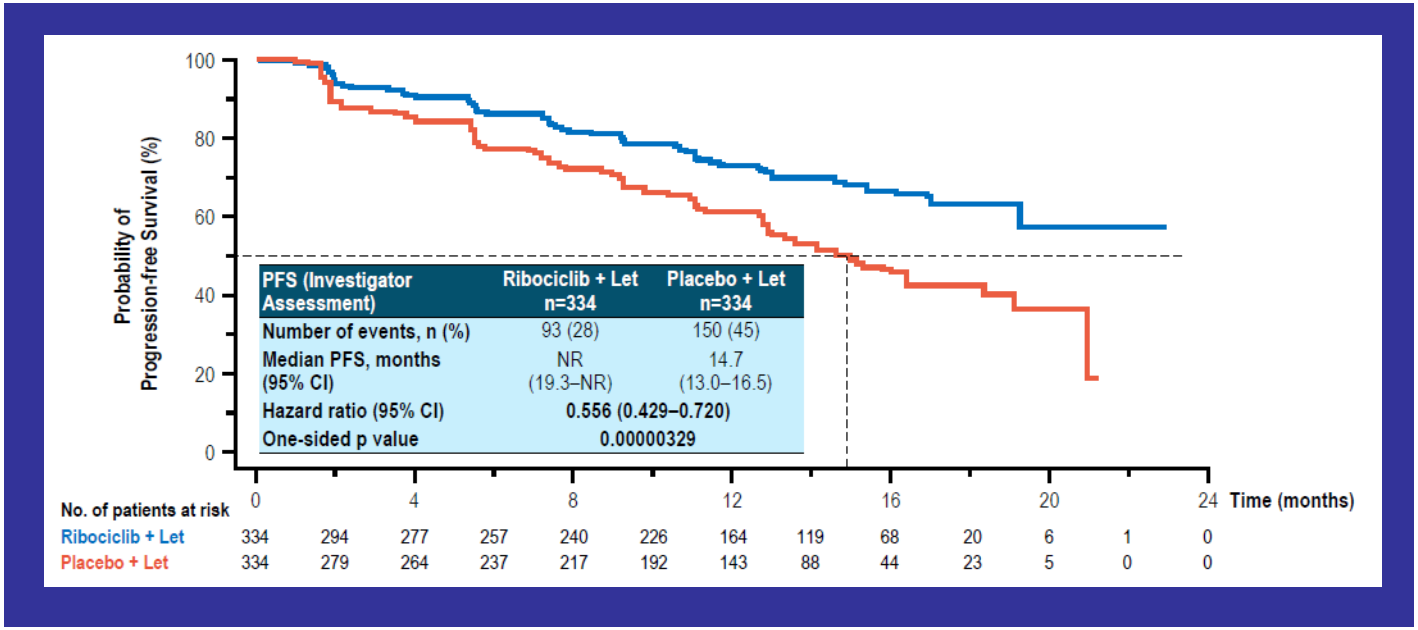
Fry DW, et al. *Mol Cancer Ther* 2004;


N=666*
Postmenopausal
ER+, HER2– advanced breast cancer
No prior treatment for advanced disease
AI-resistant patients excluded
2:1 RANDOMIZATION
Palbociclib (125 mg QD, 3/1 schedule) + letrozole (2.5 mg QD)
Placebo (3/1 schedule) + letrozole (2.5 mg QD)
Primary endpoint
Investigator-assessed PFS
Secondary endpoints
Response, OS, safety, biomarkers, patient-reported outcomes
Stratification factors
–Disease site (visceral, non-visceral)
–Disease-free interval (de novo metastatic; ≤12 mo, >12 mo)
–Prior hormonal therapy (yes, no)


Postmenopausal women with HR+/HER2– advanced breast cancer
No prior therapy for advanced disease
N=668
Randomization (1:1)
Stratified by the presence/absence of liver and/or lung metastases
Ribociclib (600 mg/day)
3-weeks-on/1-week-off
+
Letrozole (2.5 mg/day)
n=334
Placebo
+
Letrozole (2.5 mg/day)
n=334
Primary endpoint
PFS (locally assessed per RECIST v1.1)
Secondary endpoints
Overall survival (key)
Overall response rate
Clinical benefit rate
Safety

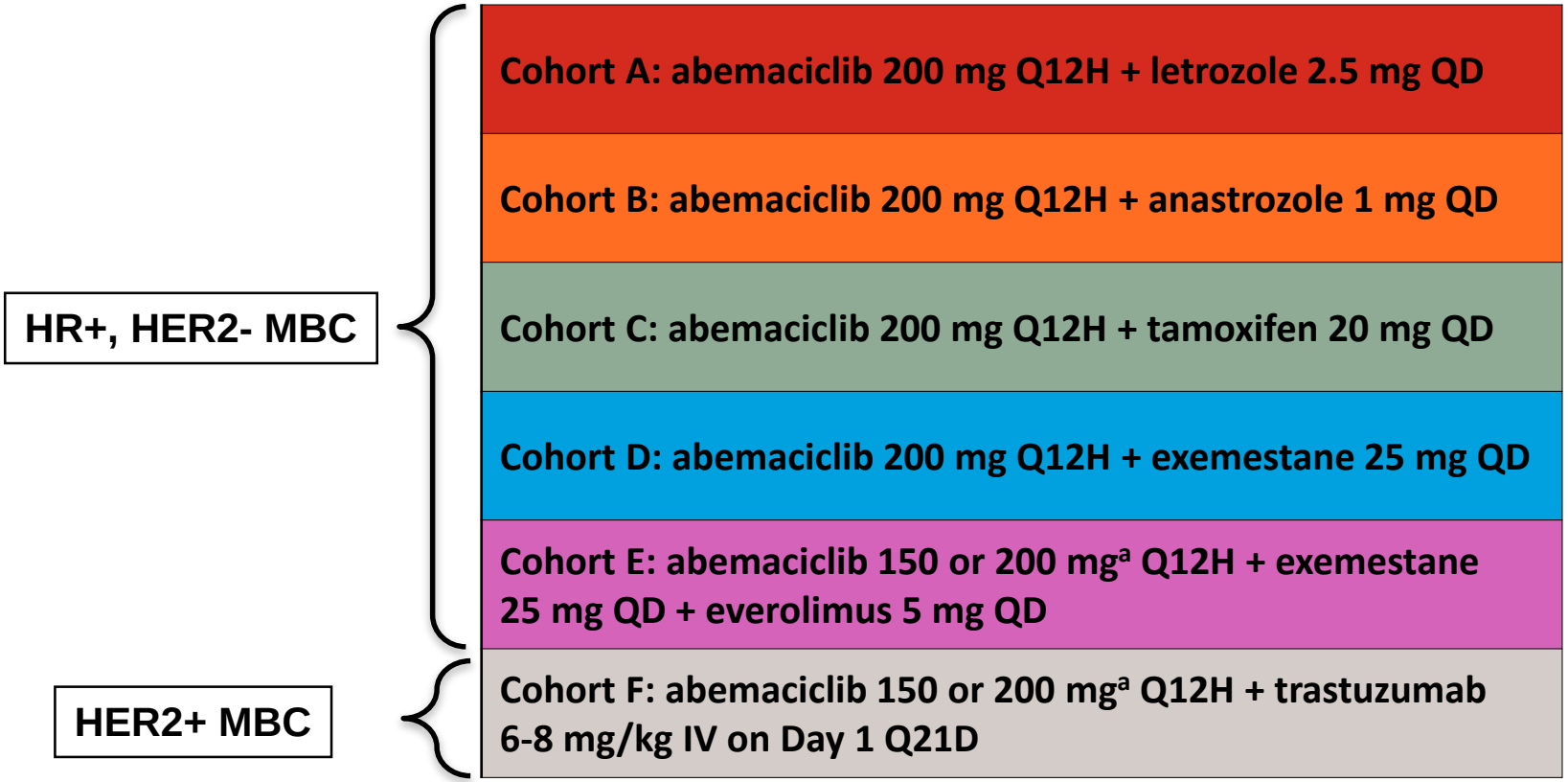
ITT LOCALLY ASSESSED





Abemaciclib in later treatment lines MBC (JPBH)

A Phase 1b Study of Abemaciclib in Combination With Therapies for Patients With MBC



Median number of regimens received prior to study entry:

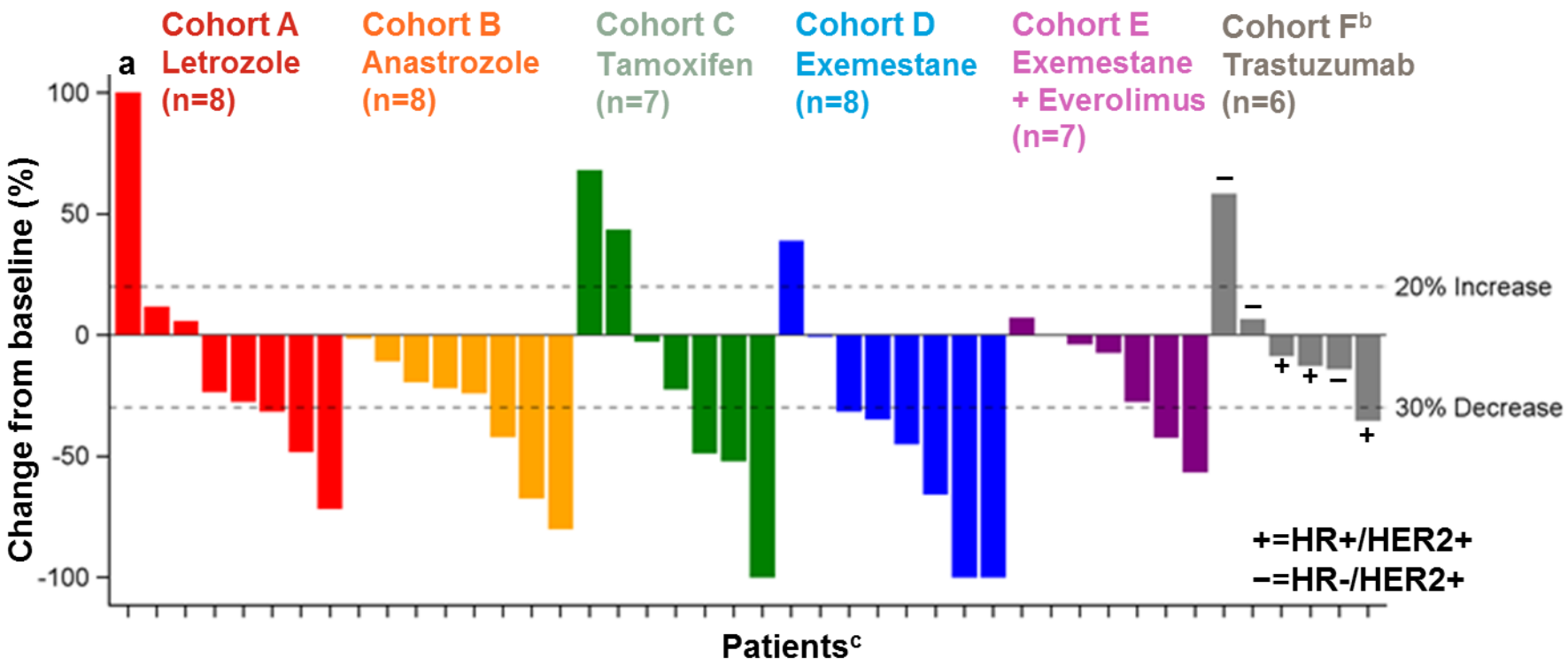
- **2/4 (Cohorts A-E)**
- **10.5 (Cohort F)**

1. Tolaney SM et al. Poster presented at ASCO 2015. Abstract 522
2. Goetz MP et al. Presented at SABCS 2015. P4-13-25

Abemaciclib (JPBH) clinical outcome

	Cohort A Letrozole (N=20)	Cohort B Anastrozole (N=16)	Cohort C Tamoxifen (N=16)	Cohort D Exemestane (N=15)	Cohort E EXE + EVE (N=17)	
					150 mg (n=13)	200 mg (n=4)
Clinical Benefit Rate (CR+PR+SD ≥24 wks), %	40	81	75	60	Data not mature	

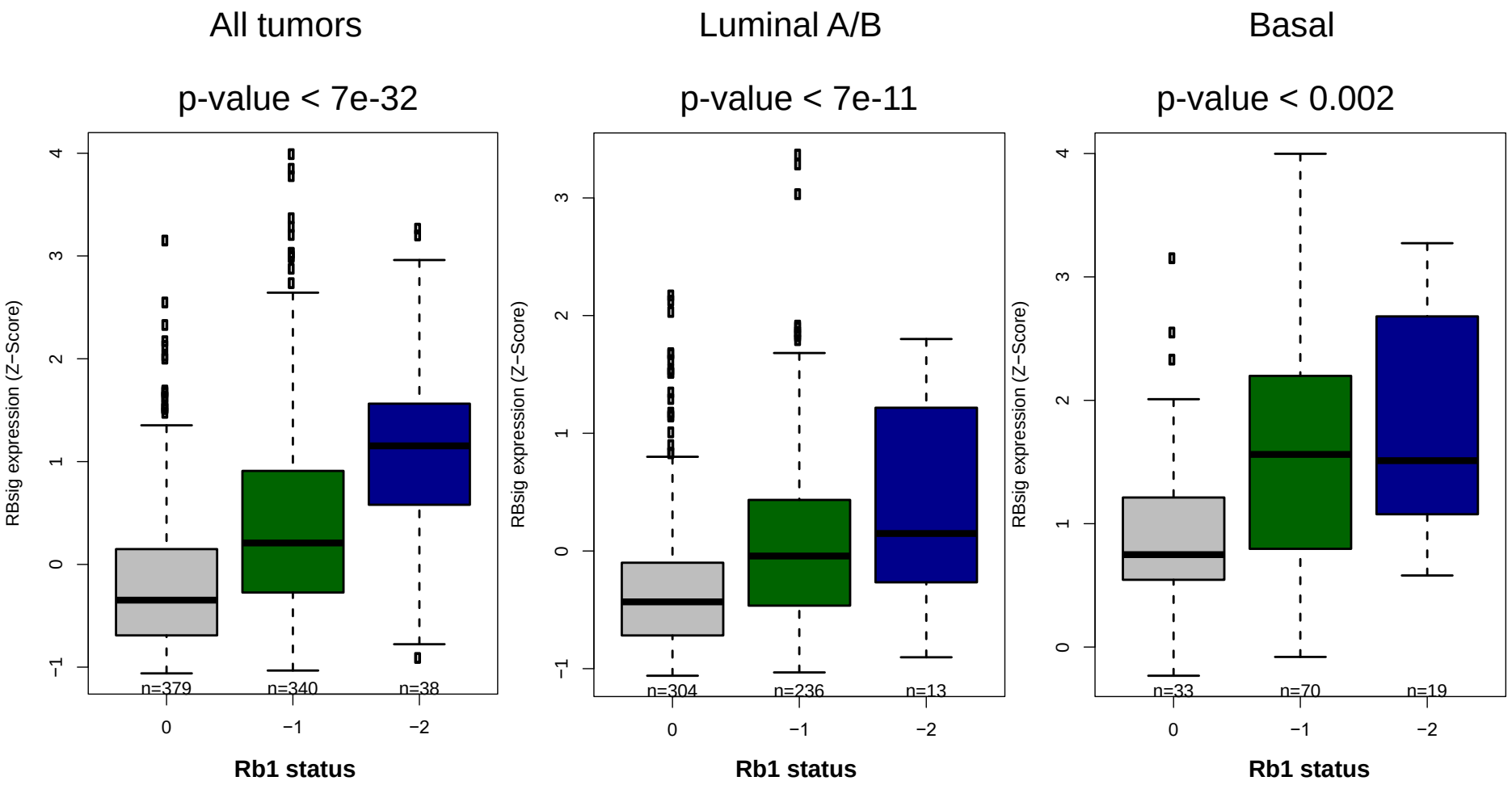
Best Change in Tumor Size From Baseline for Patients With Measurable Diseasease



[a]For this patient, change in tumor size greater than 100% [b]For Cohort F, data not mature due to short duration of enrollment [c]Graph includes only patients with available pre- and post-treatment lesion measurements

Goetz MP et al. Presented at SABCS 2015. P4-13-25

RBsig correlates with RB1 status in BC subtypes



RBsig levels are higher in BC samples with loss of Rb, across multiple BC subtypes

Malorni L. et al; Oncotarget 2016