The Cell Cycle and Breast Cancer

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UT SOUTHWESTERN MEDICAL CENTER

1. **Cell cycle overview**
   *Simple concepts of cell cycle and CDK/cyclins
   *Features of deregulated cell cycle in breast cancer

2. **Prognostic/predictive significance of cell cycle deregulation**
   *Importance in commonly employed subtyping tools in ER+
   *Importance of context--treatment.

3. **Rationally targeting the cell cycle in ER+ breast cancer with CDK4/6 inhibitors**
   *Therapeutically targeting the cell cycle with CDK4/6 inhibitors
   *Concepts of combination approaches and trials update

4. **Emergent concepts**
   *Expanding the repertoire of CDK4/6 inhibitors
   *Selectively exploiting deregulated cell cycle control

Disclosures (2010-2015)

Permeon pharmaceuticals

- Consultant, research contract

Eli Lilly

- Honorarium, research contract

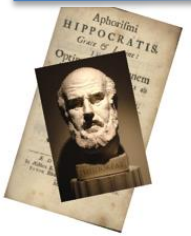
Pfizer

- Advisory activity, research contracts

Historical confluence – cell cycle and breast cancer

3,500 years ago

460 B.C.



Surgery

1757

1882

Hormonal Dependence

1895

Discovery of Tamoxifen

1962

Identification of Estrogen receptor

1967

Radiation/Chemotherapy

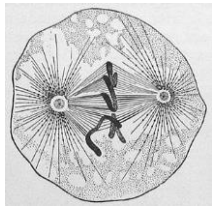
1955

HER2-positive
Breast cancer
Herceptin et al.

Surgery
Radiation
Chemotherapy

ER+
Breast cancer
Endocrine therapy

Cell Division



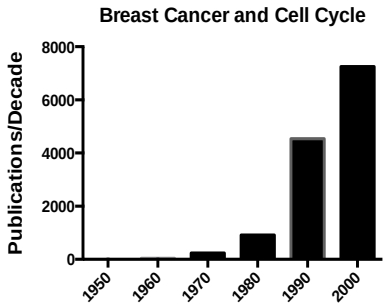
CDK/Cyclins

Leland H. Hartwell

Tim Hunt

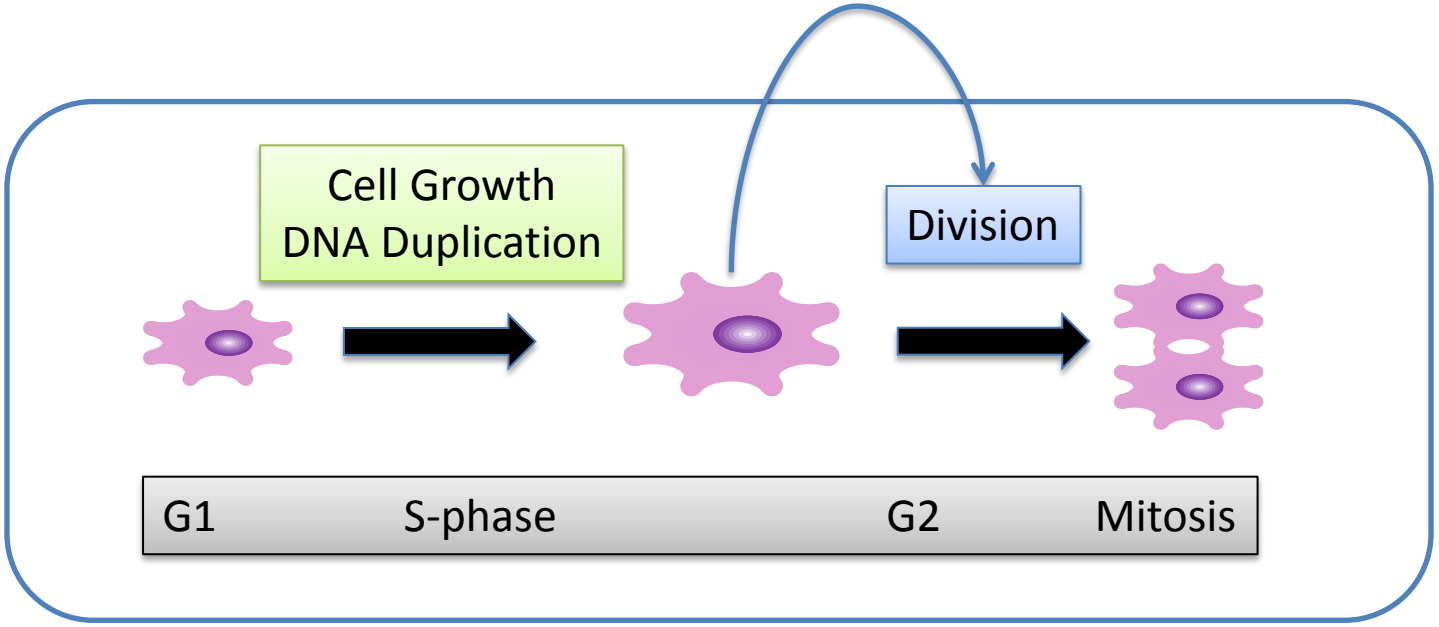
Sir Paul M. Nurse

Analysis in Breast Cancer



Robert Sutherland

The cell cycle-simple version



G1/S: Cell growth and DNA duplication is tightly controlled

*Positive signals (growth factors, hormones, nutrients, etc.)

*Negative signals (stress signals, lack of mitogen, lack of nutrients)

CANCER MUTATIONS IMPINGE HERE TO DEREGULATE PROLIFERATION

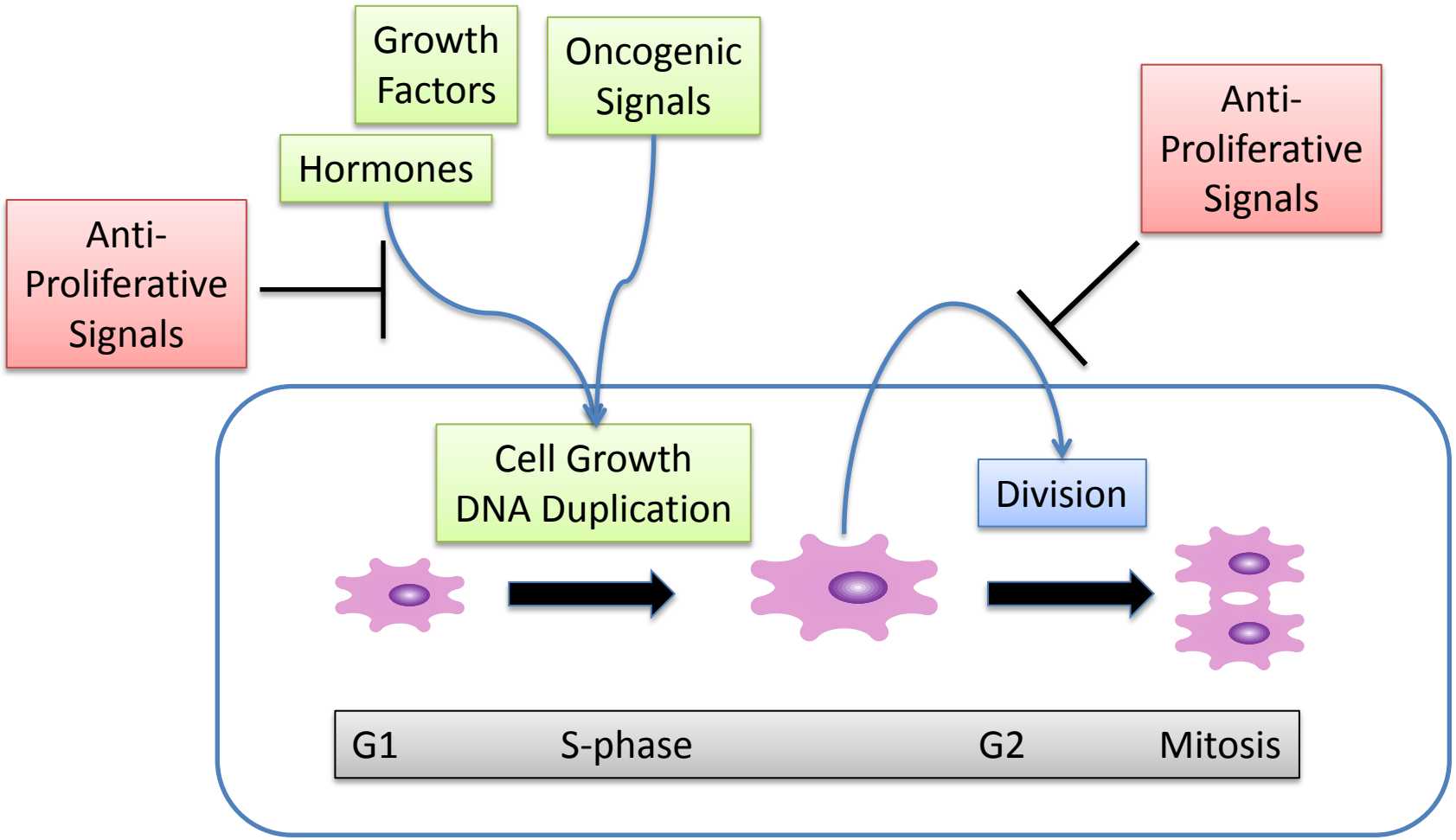
G2/M: Mitotic division will occur in the absence of stress

*Following G1/S, cells are committed to division

*Checkpoints will control mitosis to maintain genomic integrity

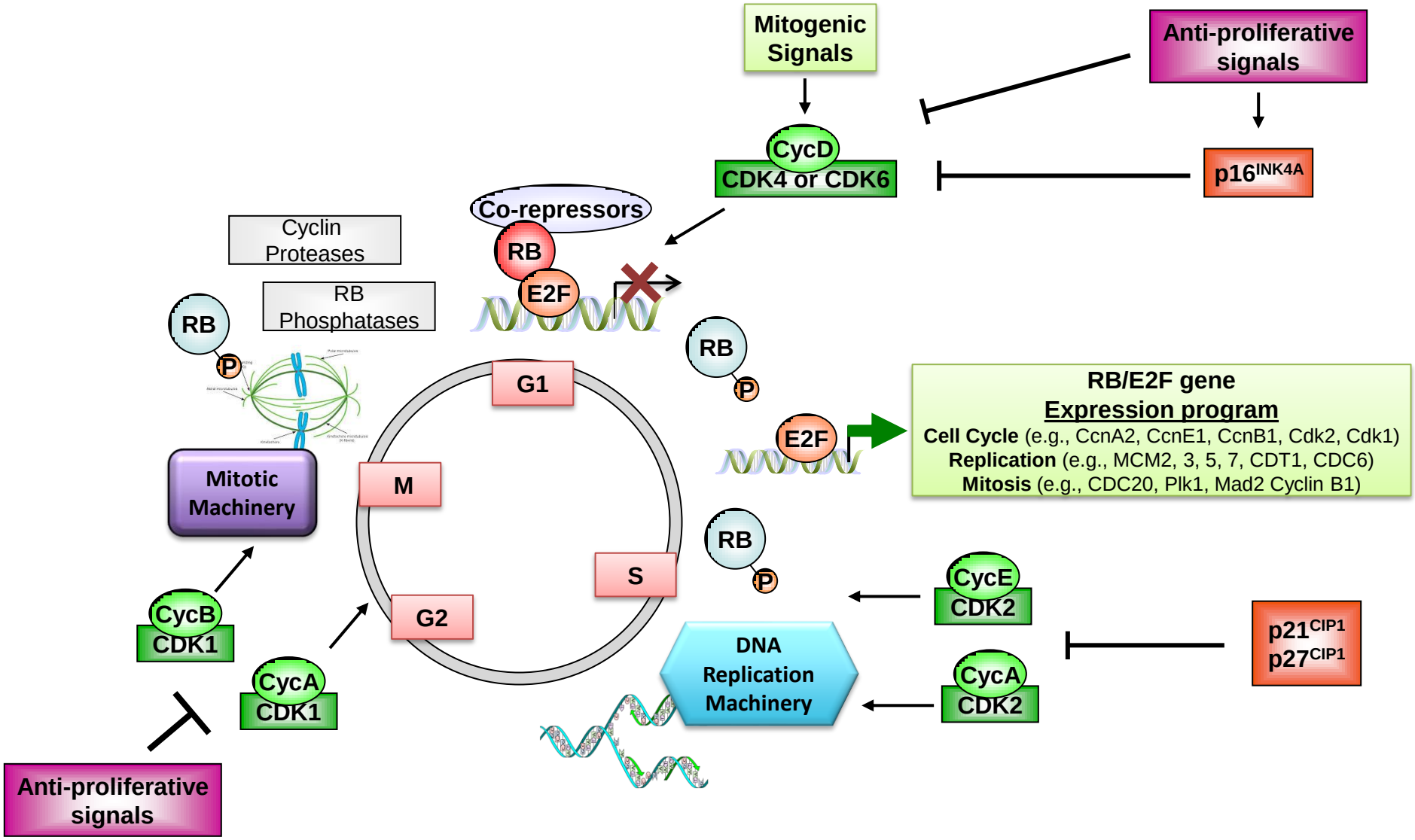
CANCER MUTATIONS HERE DEREGULATE CHROMOSOME STABILITY

The cell cycle: cancer and intervention



Many therapeutic agents act as "anti-proliferative signals" (Fulvestrant, Trametinib or Adriamycin, Taxanes)

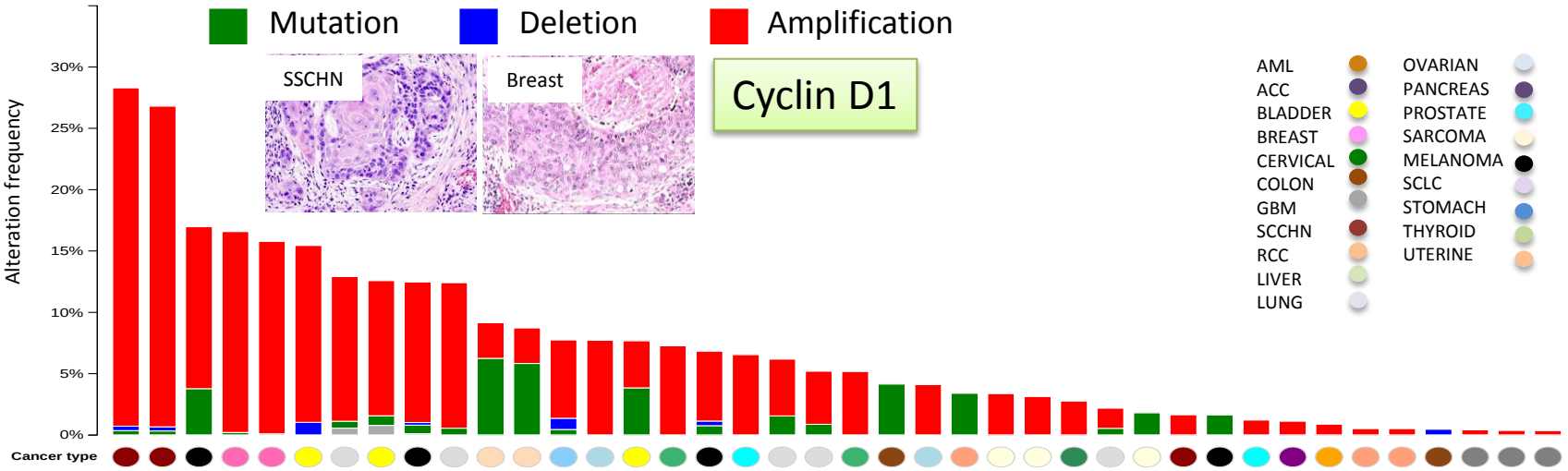
Cyclin Dependent Kinases (CDK) drive the cell cycle

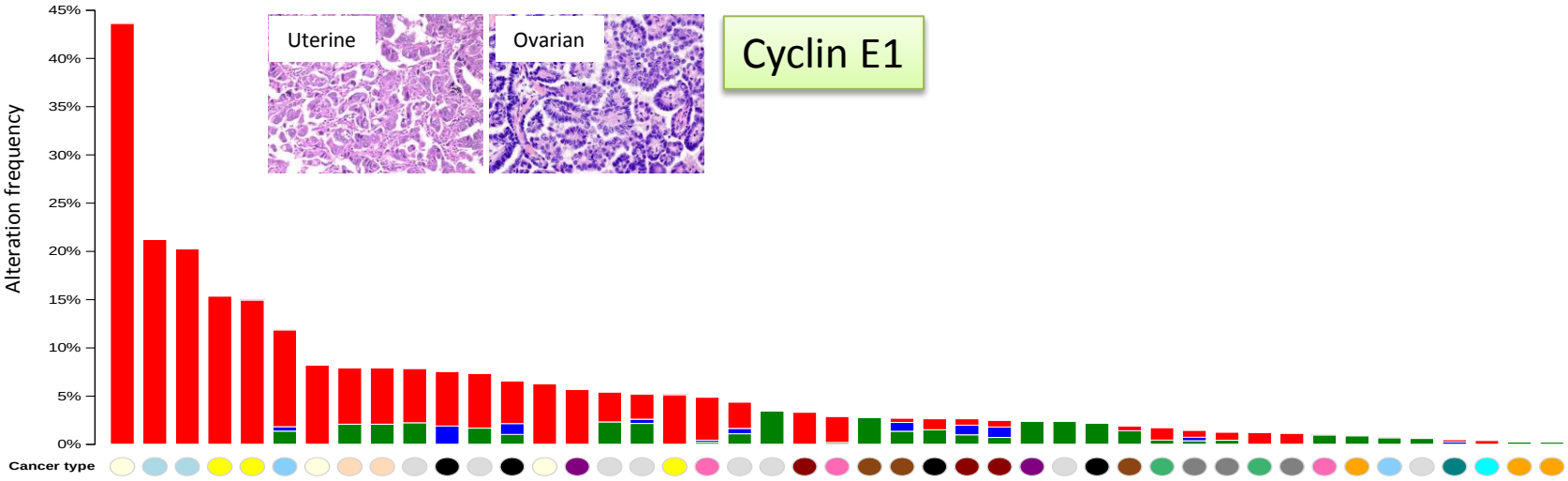


Adapted from Knudsen. *Nat Rev Cancer.* 2008;8:714-24.

CDK-deregulation in cancer

1. Cyclins/CDKs that act in control over G1/S and proliferation are oncogenes

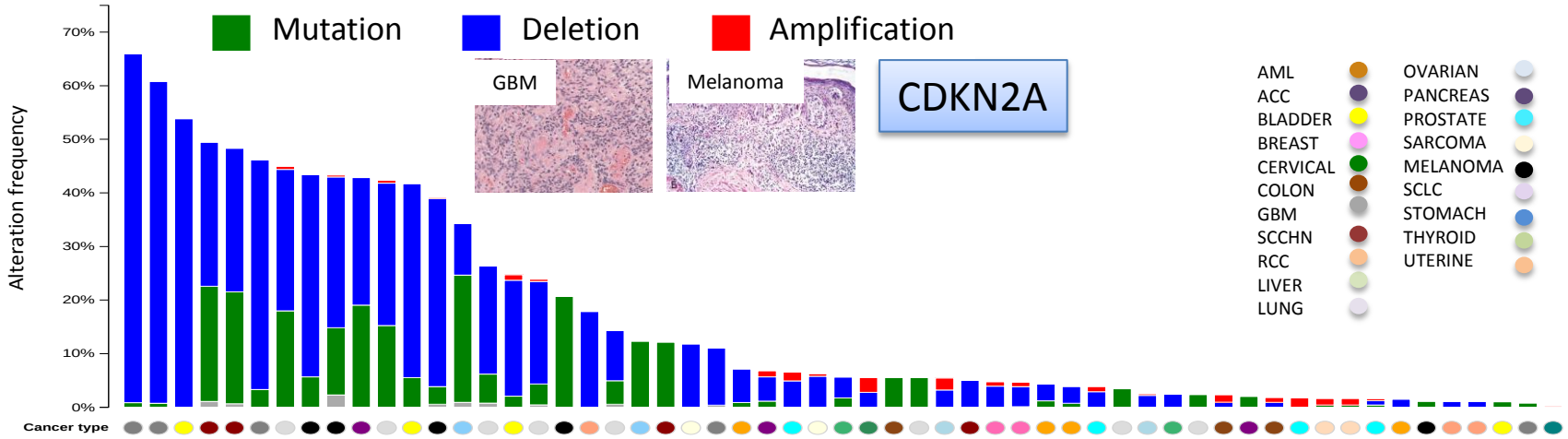


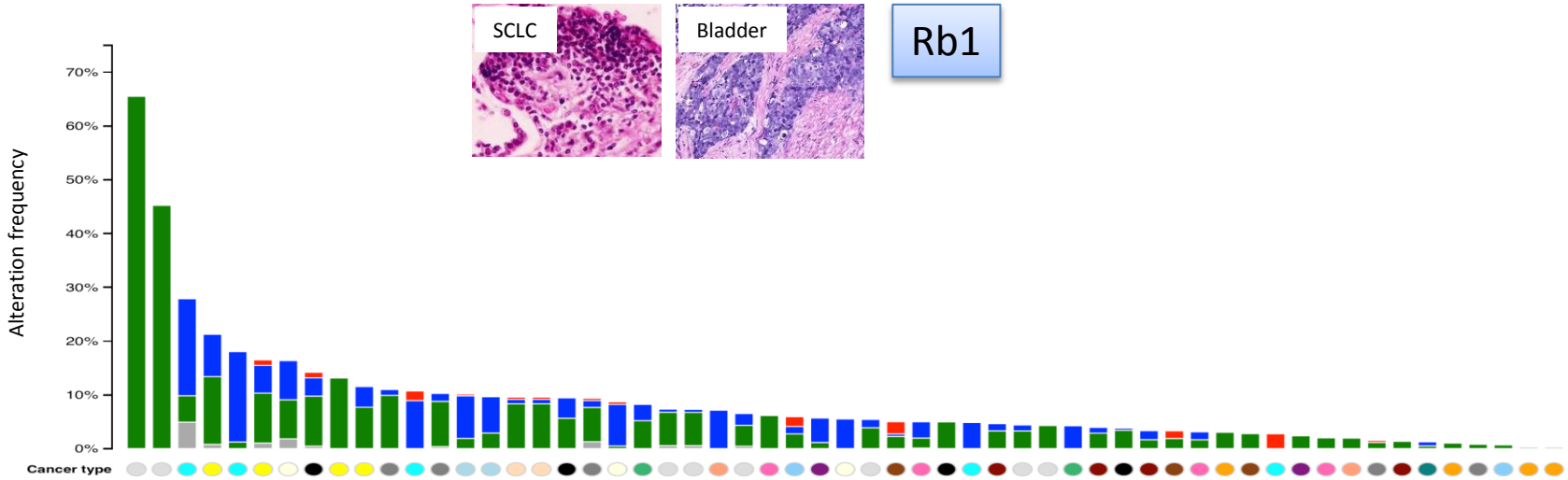


http://cbioportal.org. Cerami E, et al. *Cancer Discov.* 2012;2:401-4; Gao J, et al. *Sci Signal.* 2013;6:pl1.

CDK-deregulation in cancer

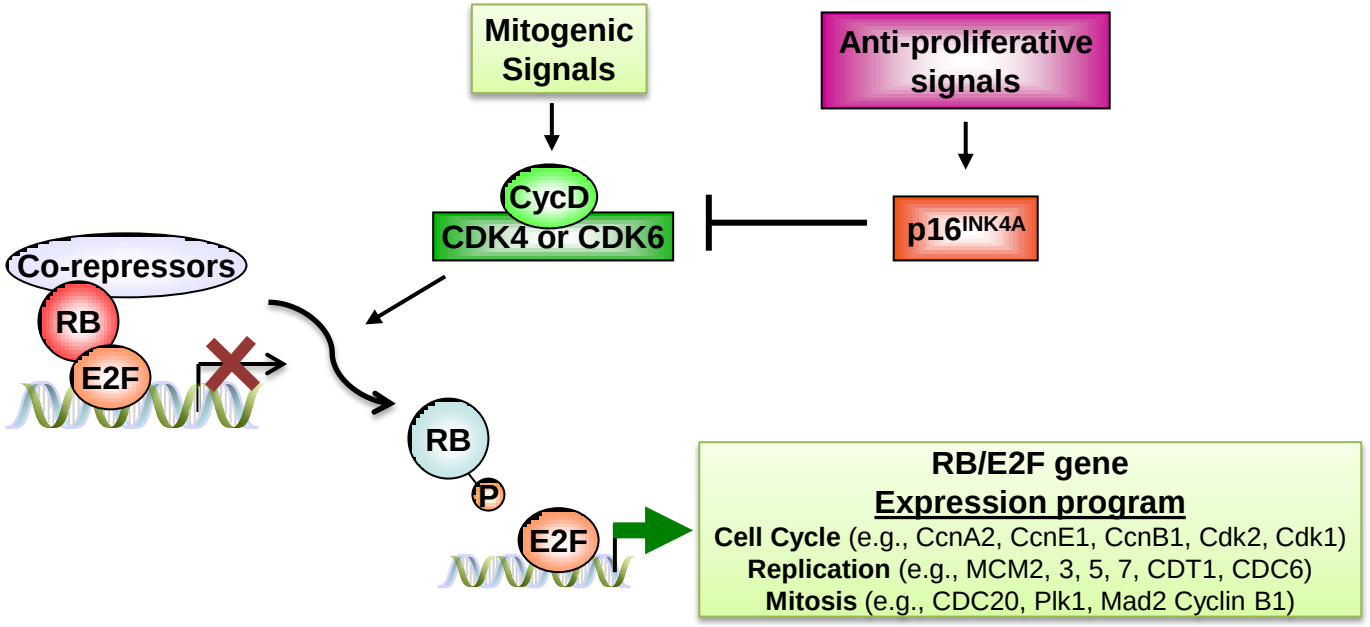
2. Proteins that antagonize these Cyclin/CDKs are tumor suppressors





http://cbioportal.org. Cerami E, et al. *Cancer Discov.* 2012;2:401-4; Gao J, et al. *Sci Signal.* 2013;6:pl1.

CDK4/RB-pathway is frequently disrupted in breast cancer



~50% of tumors exhibit some form of pathway deregulation

RB aka RB1: deletion/mutation/methylation

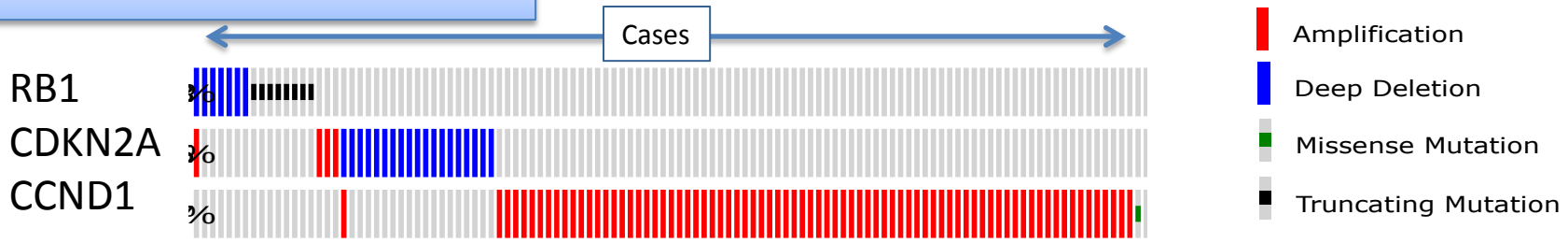
p16INK4A aka CDKN2A: deletion/methylation

Cyclin D1 aka CCND1: amplification/overexpression

Deregulation of "E2F-mediated transcription"

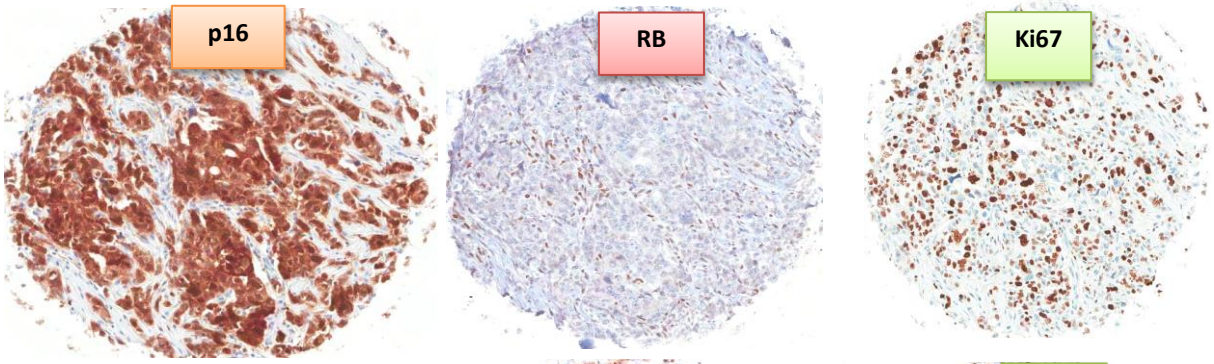
Mutually exclusive pathway relationship in cancer

Genetic Alterations from TCGA

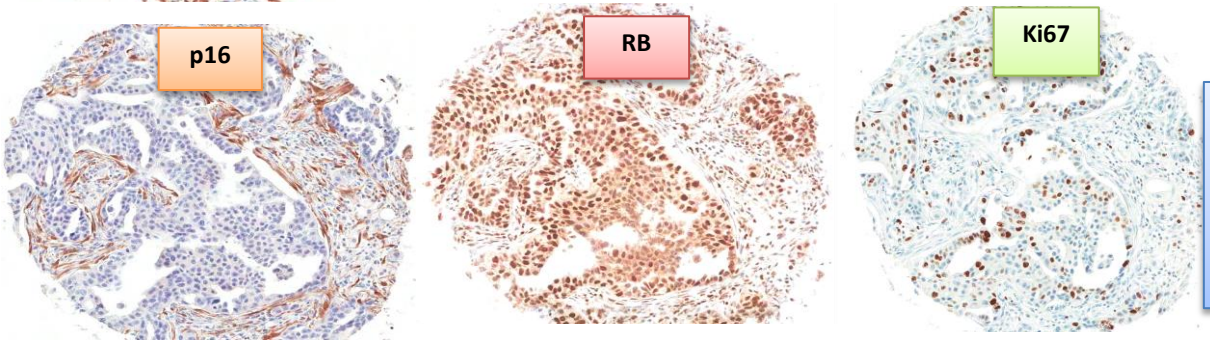


Reciprocal protein expression

Case with RB Loss

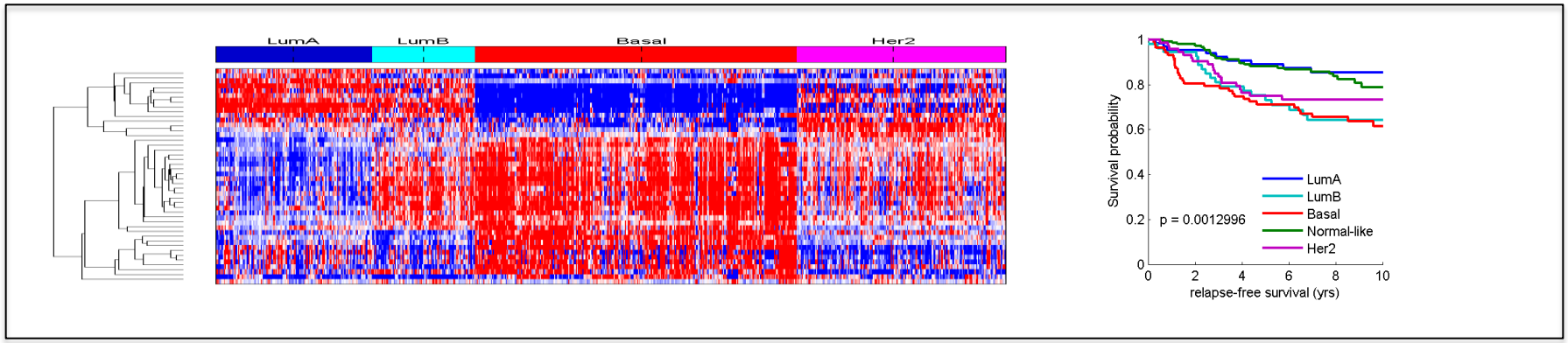


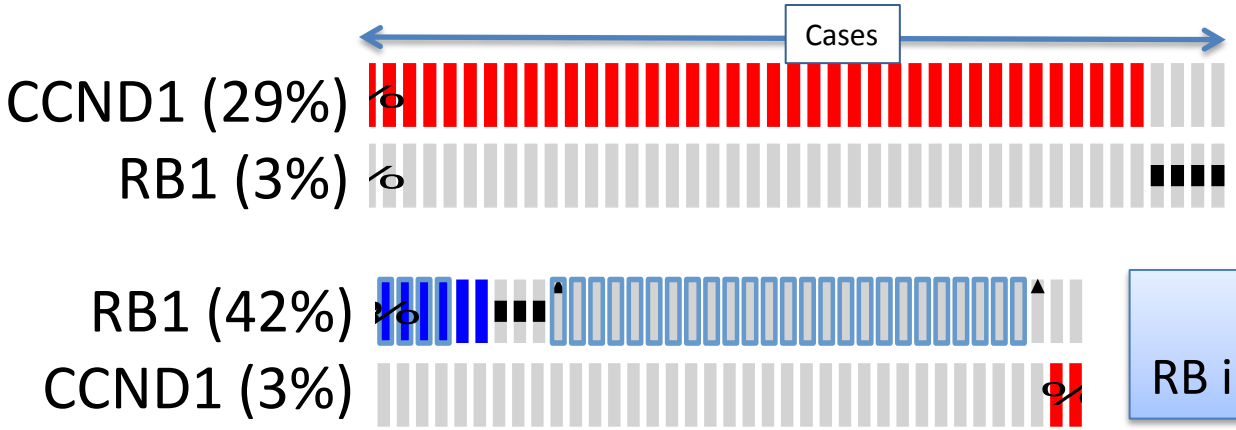
Case with p16 Loss



"Supra-physiological" p16 in a proliferative tumor is a surrogate RB functional loss

Subtype selective features of cell cycle deregulation

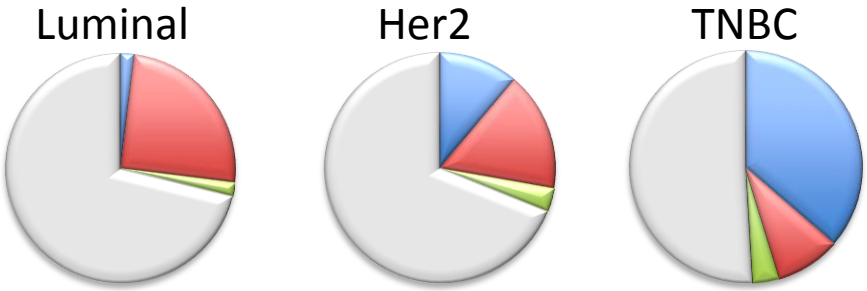




Luminal B:
Cyclin D1 amplification

TNBC:
RB inactivation

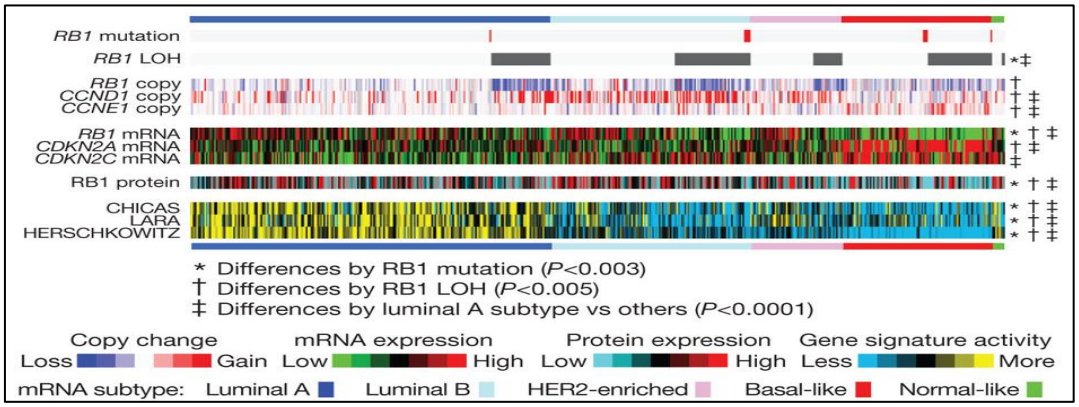
Amplification
Deep Deletion
Missense Mutation
Truncating Mutation



RB deletion/mutation/reduced expression
Cyclin D1 amplification/overexpression
CDKN2A deletion/mutation

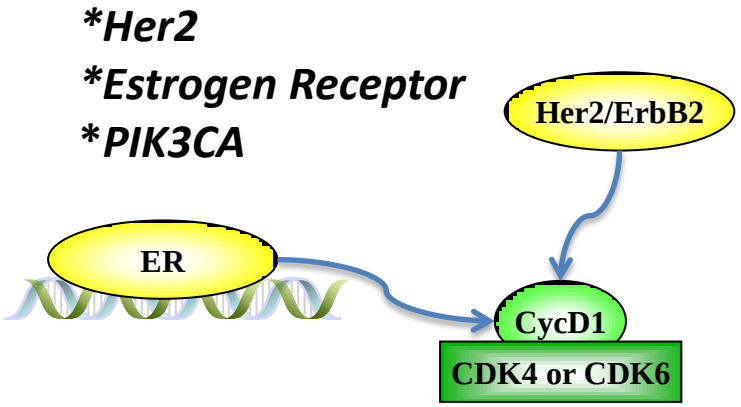
Summary 1: Cell cycle deregulation in breast cancer

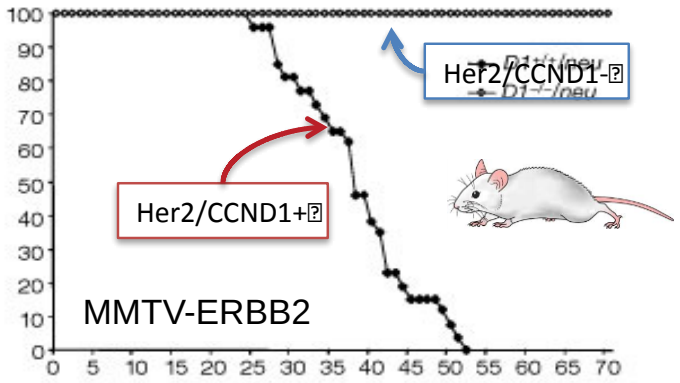
Genetic alterations that impinge on cell cycle control are common in breast cancer



Breast Cancer TCGA, Nature 2012

Many of the "drivers" in breast cancer impinge on cell cycle machinery





Geng & Sicinski 2001 Nature

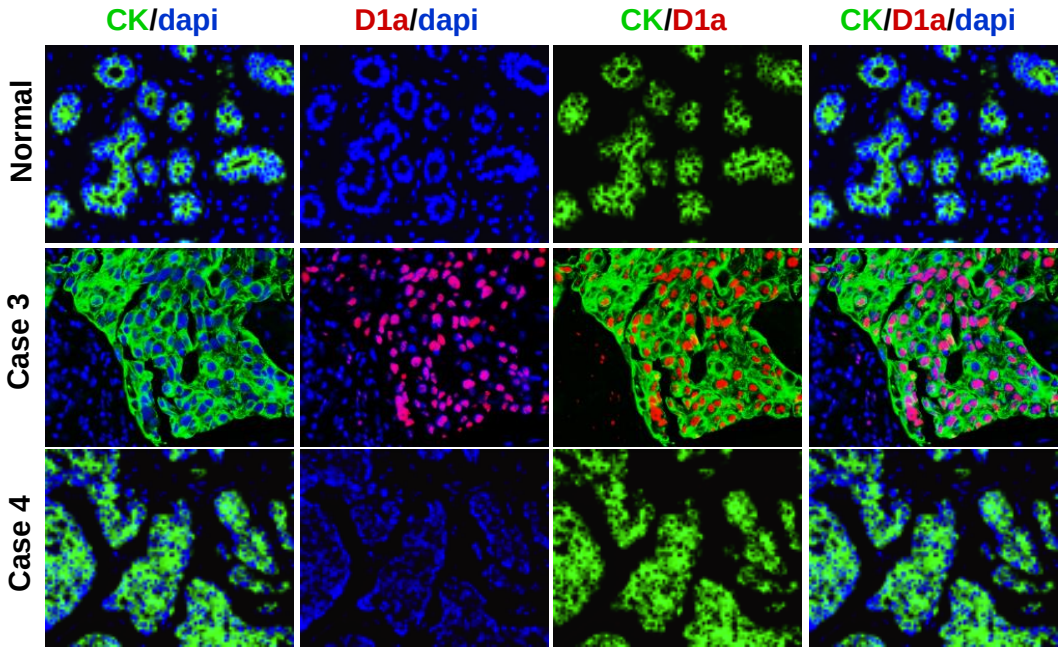
CLINICAL SIGNIFICANCE OF CELL CYCLE DEREGULATION

Long history of analyzing cell cycle regulatory proteins in ER+ breast cancer

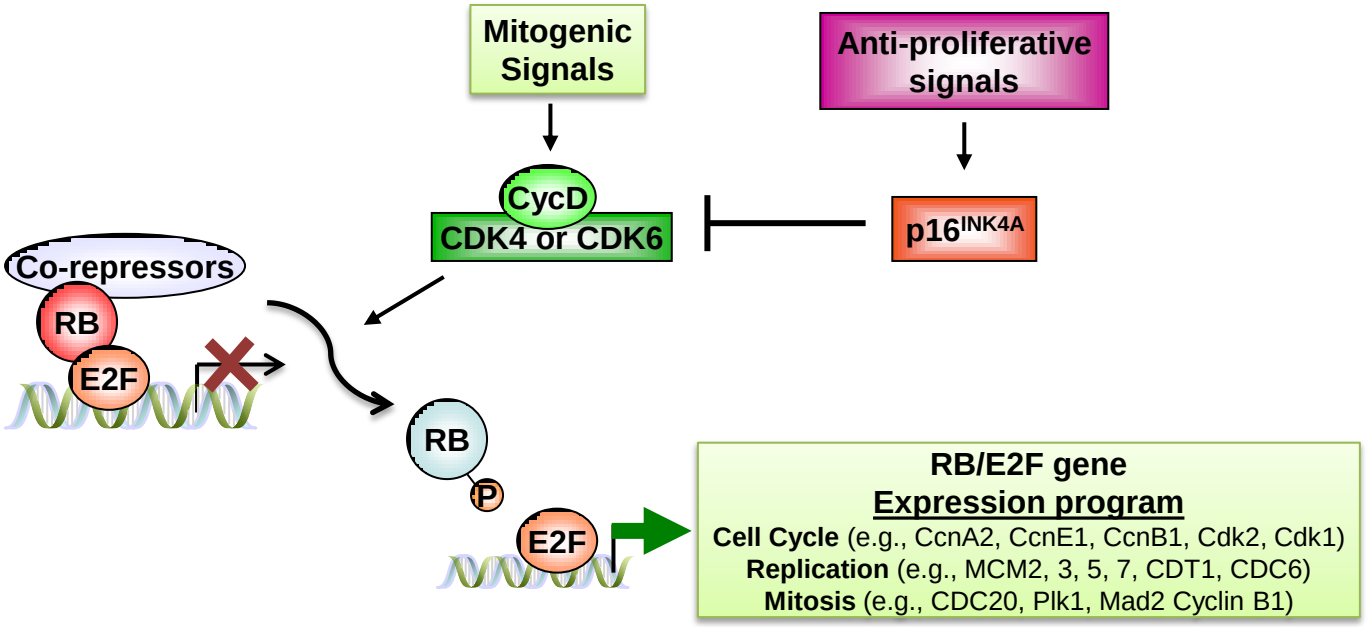
CELL CYCLE GENE	PROGNOSIS	ENDOCRINE THERAPY
Cyclin D1 over expression	Controversial	Controversial
Cyclin D1 amplification	Poor Prognosis	Poor outcome
Cyclin E1 over expression	Poor Prognosis	Poor outcome
p27Kip1 loss	Poor prognosis	Poor outcome
RB loss	Poor prognosis	Poor outcome
Cyclin A2 over expression	Poor prognosis	Poor outcome
Cyclin B1 over expression	Poor prognosis	Poor outcome

Musgrove & Sutherland. *Nat Rev Cancer* 2009
Aagarwal R, et al. *Clin Cancer Res* 2009
Lundgren K, et al. *Breast Cancer Res* 2012
Millar E, et al. *Oncogene* 2010
Lenn et al. *Cell Cycle* 2011
Lodén M, et al. *Oncogene* 2002
Aaltonen K, et al. *Br J Cancer* 2009
Bindels E, et al. *Oncogene* 2002
Aaltonen K, et al. *Breast Cancer Res Treat* 2009
Michalides R, et al. *Br J Cancer* 2002
van Diest P, et al. *Am J Pathol* 1997
Barnes & Gillet. *Breast Cancer Res Treat* 1998
Porter P, et al. *Nat Med* 1997

AND MORE…..



Molecular profiling of the cell cycle



Pathway-Functional Signature (RB or E2F signatures)

Multiple Functional models To perturb pathway

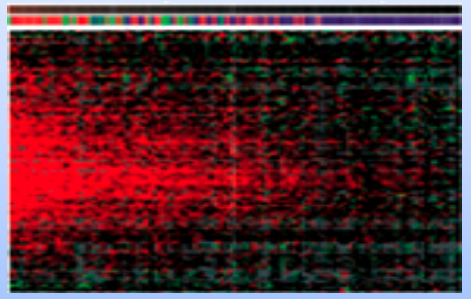
E2F overexpression
RB loss
RB gain
CDK4/6 inhibition

Gene Expression Profiling

Select for common genes in multiple contexts

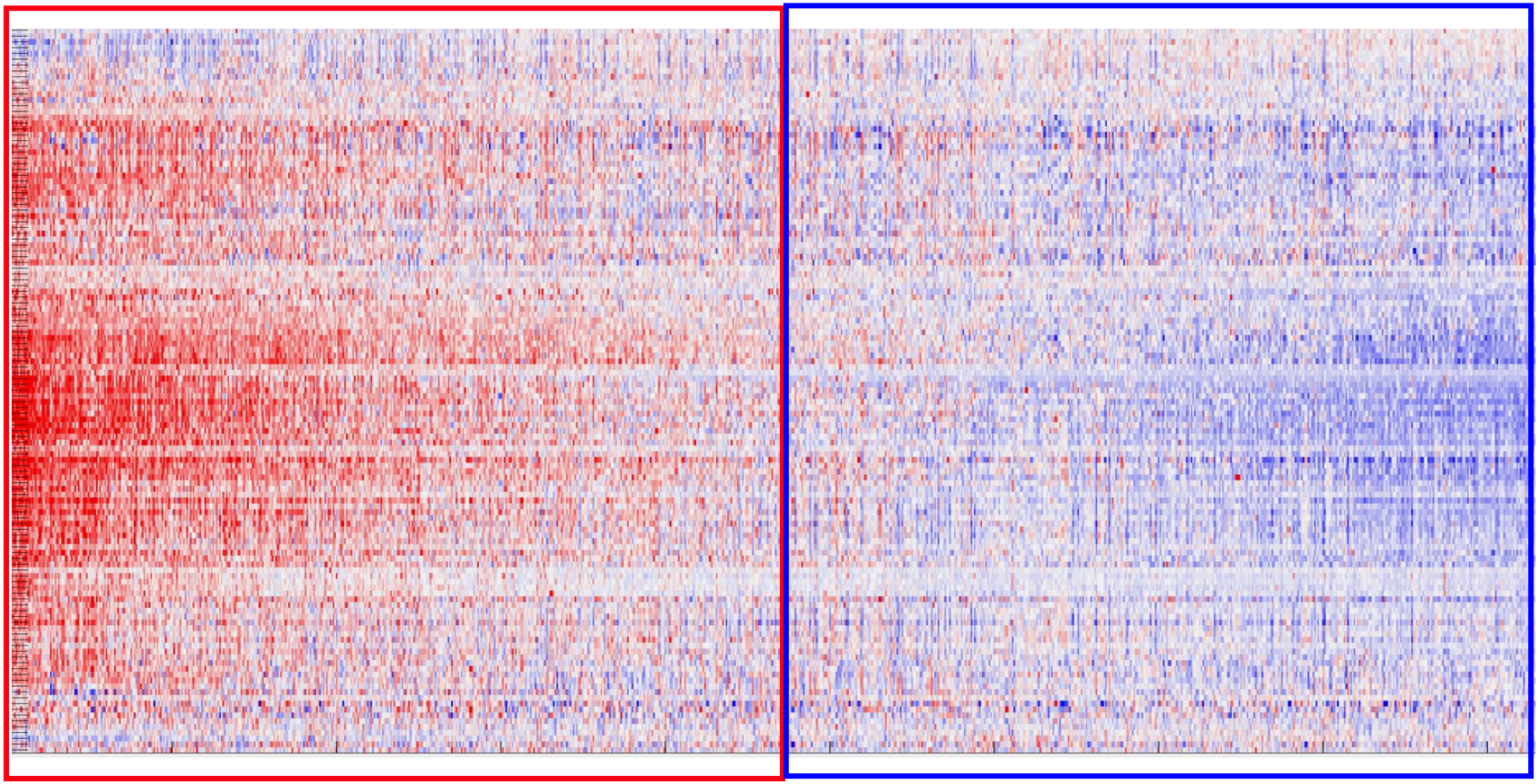
pathway signature

Apply to patient specimens

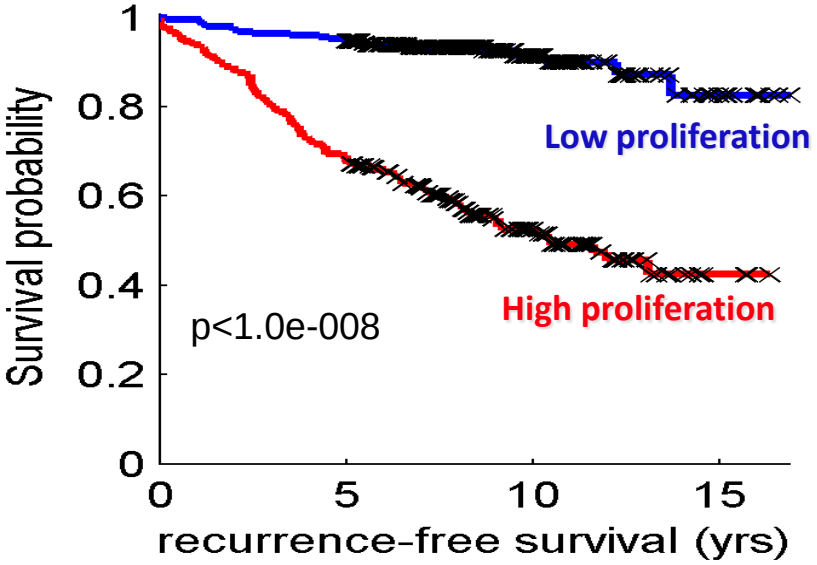


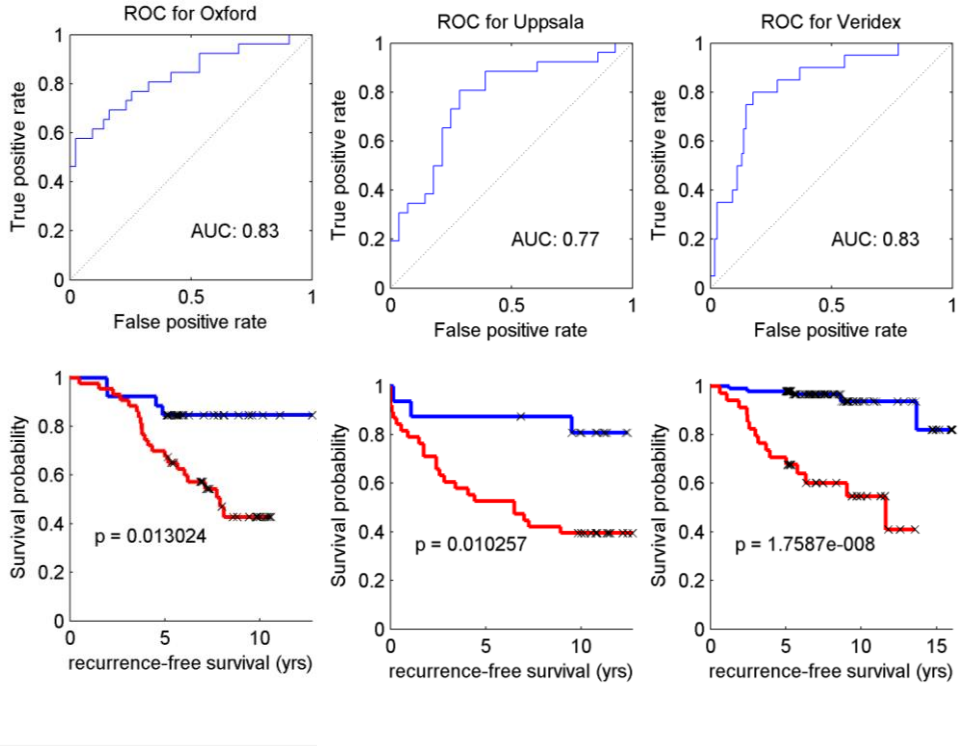
Proliferative heterogeneity in ER+ breast cancer

ER+/Her2– Breast cancer cases (~600 cases treated with tamoxifen)



Proliferative-signatures associate with recurrence in ER+ breast cancer

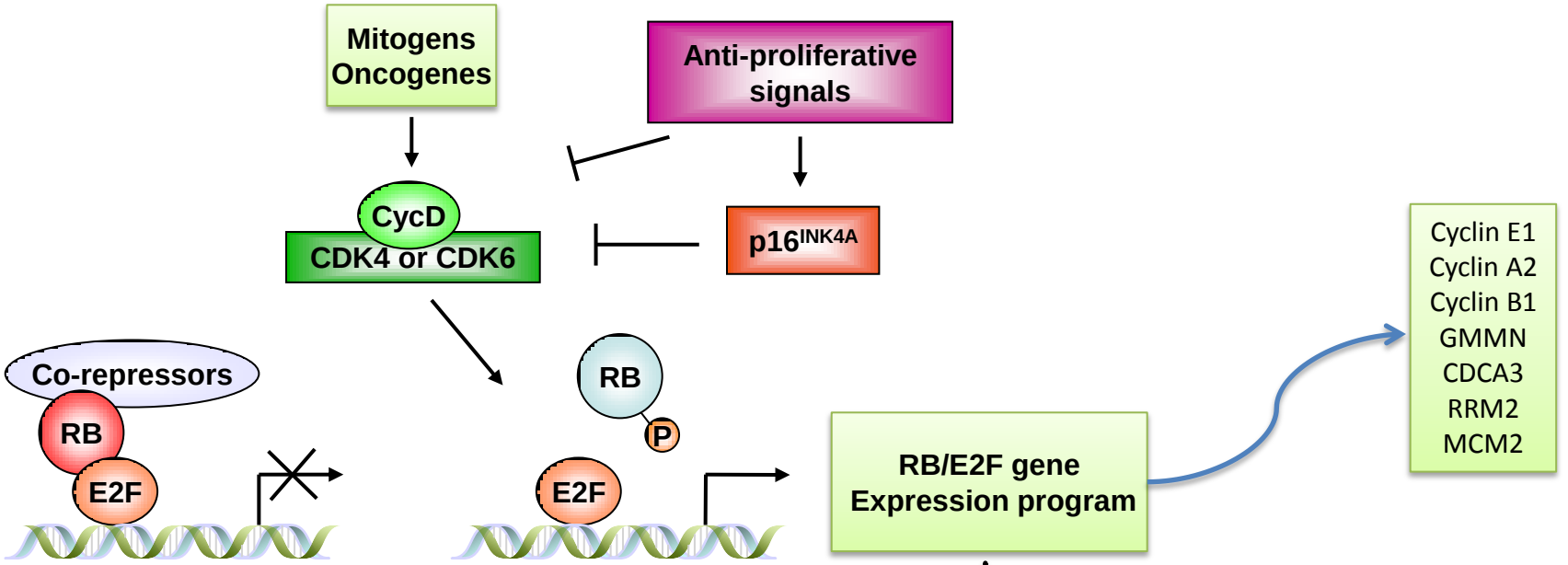




PROLIFERATION: THE MOST PROMINENT PREDICTOR OF CLINICAL OUTCOME IN BREAST CANCER

Desmedt & Sotiriou. *Cell Cycle.* 2006;5:2198-202.

Cell cycle is “embedded” in multiple signatures (PAM50, GGI, OncotypeDx, etc.)



PAM50: ACTR3B, ANLN, BAG1, BCL2, BIRC5, BLVRA, CCNB1, CCNE1, CDC20, CDC6, CDH3, CENPF, CEP55, CXXC5, EGFR, ERBB2, ESR1, EXO1, FGFR4, FOXA1, FOXC1, GPR160, GRB7, KIF2C, KRT14, KRT17, KRT5, MAPT, MDM2, MELK, MIA, MKI67, MLPH, MMP11, MYBL2, MYC, NAT1, NDC80, NUF2, ORC6L, PGR, PHGDH, PTTG1, RRM2, SFRP1, SLC39A6, TMEM45B, TYMS, UBE2C, UBE2T

Proliferation
Ki67
STK15
Survivin
CCNB1 (cyclin B1)
MYBL2

Invasion
MMP11 (stromolysin 3)
CTSL2 (cathepsin L2)

HER2
GRB7
HER2

GSTM1

CD68

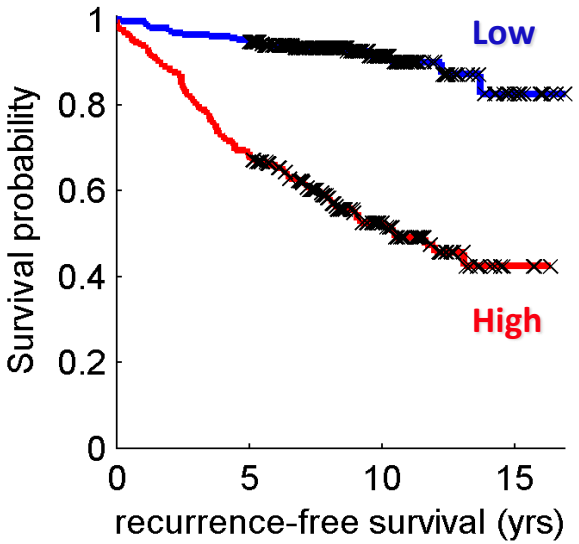
BAG1

Estrogen
ER
PGR
BCL2
SCUBE2

Reference
ACTB (β-actin)
GAPDH
RPLPO
GUS
IFRC

GGI: ASPM, AURKA, AURKB, BIRC5, BBS1, BLM, BUB1, BUB1B, CCNA2, CCNB1, CCNB2, CCNE2, CCTC5, CDC20, CDC25A, CDCA3, CDCA8, CDK1, CDK2, CDKN3, CENPA, CENPF, CENPN, CEP55, CMC2, CXC3R1, CYBRD1, DLGAP5, DONSON, DSCC1, ESPL1, EXO1 FAM64A, FEN1, FOXM1 FRY, GMPS, GTSE1, H2AFZ, HJURP, HMGB,3 HMMR, IFT46, IFT88, KIF11, KIF14, KIF15, KIF20A, KIF2C, KIF4A, KIFC1, KPNA2, LAMB2, LMNB1 MAD2L1, MARS, MCM10, MCM2, MCM4, MKI67, MLPH, MMP11, MYBL2, NCAPH, NUDT1, NDC80, NUSAP1, ORMDL2, PARPBP, PIGV, PLK1, POLQ, PRC1, PTTG1, RACGAP1, RNASEH2A, RRM2, SESN1, SHMT2, SIRT3, SLC7A5, SPAG5, STARD13, TIMELESS, TPT1, TPX2, TTK, UBE2C, UBE2S, UBE2N WDR19, ZWINT

Cell cycle and treatment decisions in ER+ disease



Luminal A
Low OncotypeDx RS
Low PAM50 ROR

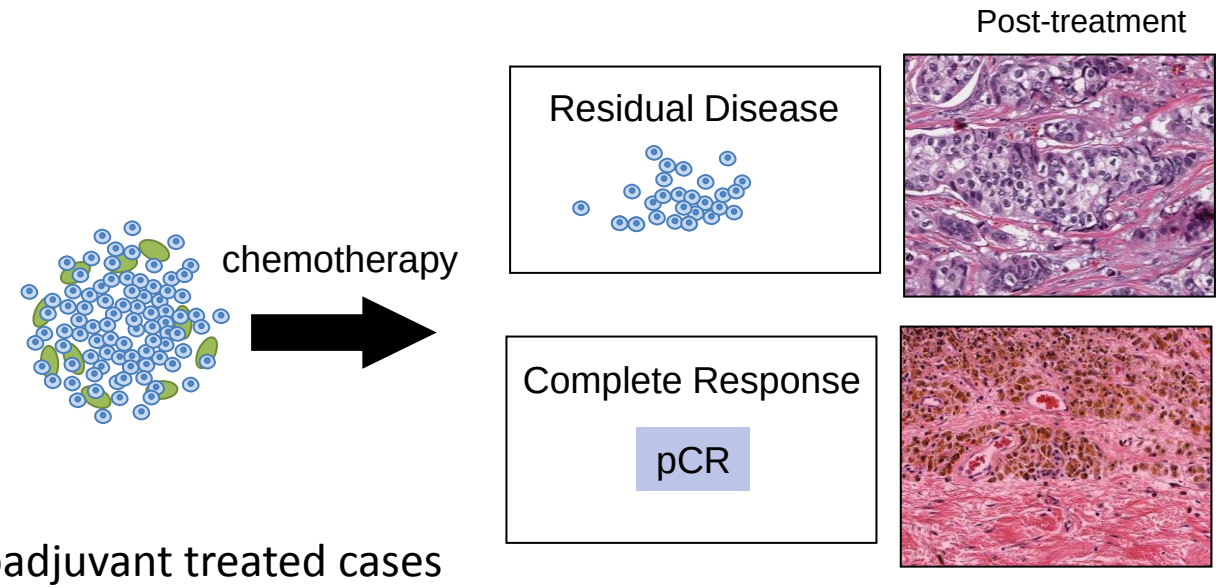
→ **LOW RISK**
Endocrine Therapy
(NO chemotherapy)

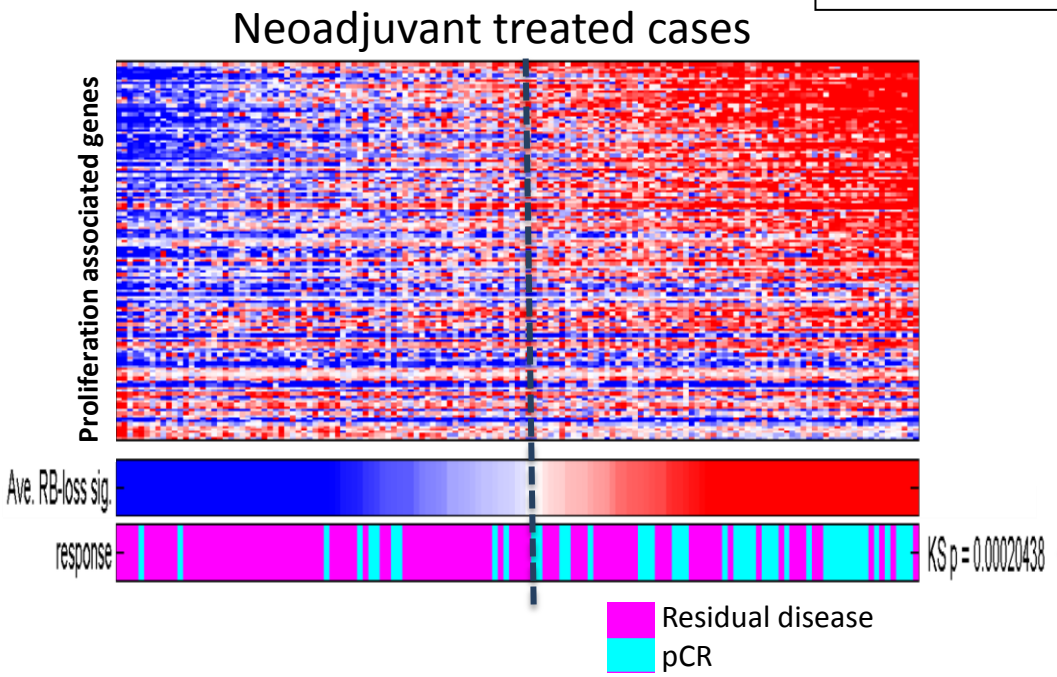
Luminal B
High OncotypeDx RFS
High PAM50 ROR

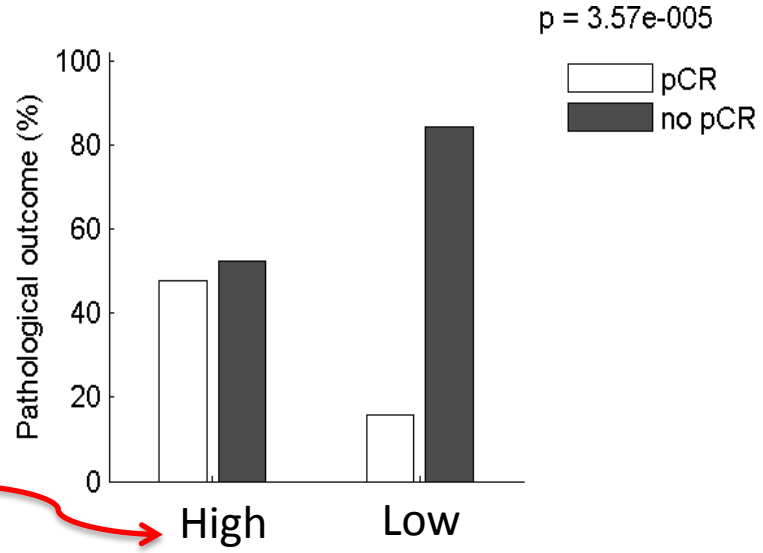
→ **HIGH RISK:**
Endocrine Therapy
+
Chemotherapy

Cell cycle deregulation as measured by these signatures is
<u>NOT</u>
invariably associated with poor outcome

Cell cycle deregulation: favorable feature for response to neoadjuvant chemotherapy







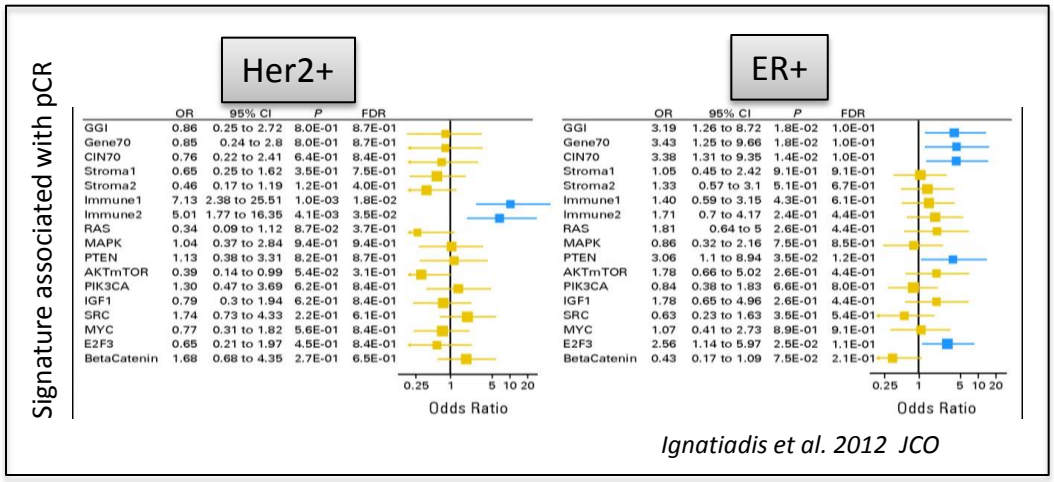
Witkiewicz et al., Clin Can Res. 2014

Summary 2: Cell cycle and prognosis/therapy response

1. **Deregulation of proliferative/cell cycle genes are associated with poor outcome**
   - *ER+/Her2–
   - *Risk of early recurrence
     - *Oncoytpe, PAM50, GGI, CIN, etc.*

2. **Cell cycle "gene information" is incorporated in prognostic tools to inform treatment decision for ER+/Her2- breast cancer**
   - *Risk of recurrence
   - *Benefit from chemotherapy

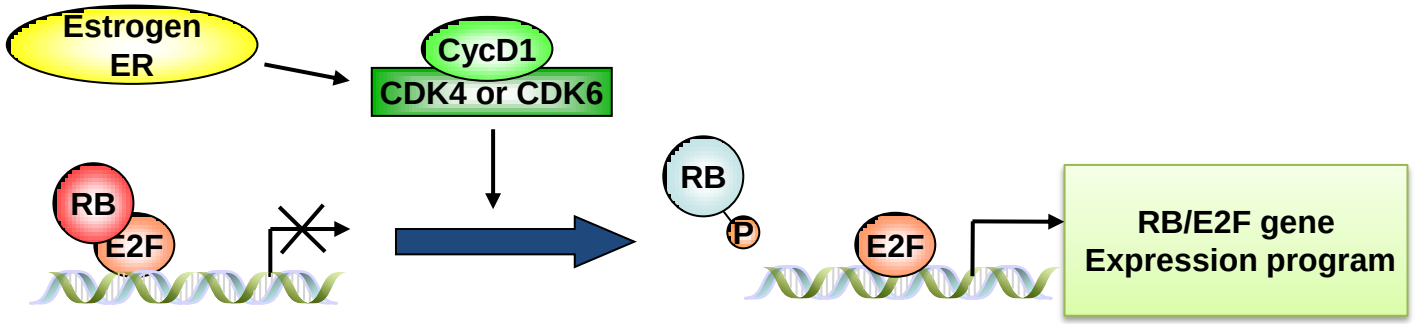
3. **Cell gene signature associated with improved response to neoadjuvant chemotherapy**
   - *ER+/Her2-
   - *TNBC
   - *Not significant in Her2+ cancer



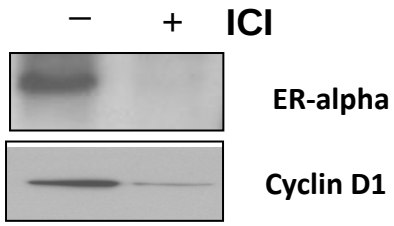
Ignatiadis et al. 2012 JCO

THERAPEUTICALLY TARGETING THE CELL CYCLE: ER+/HER2-

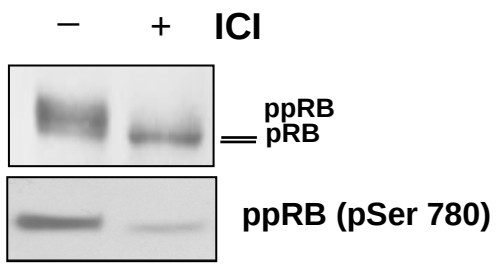
Endocrine therapy impinge on the CDK4/RB-pathway



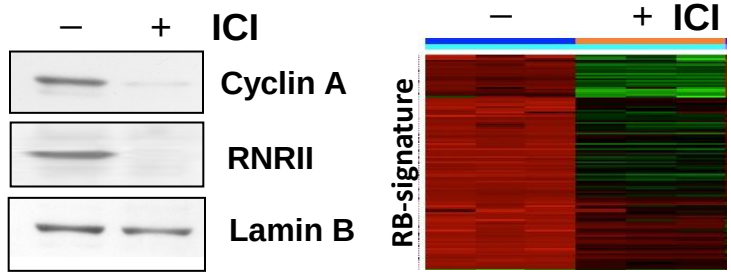
ER Signaling inhibited



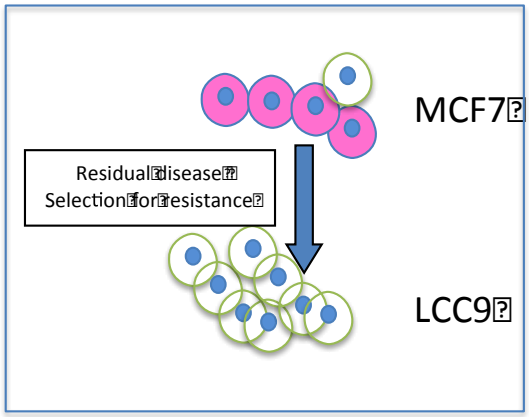
RB phosphorylation inhibited

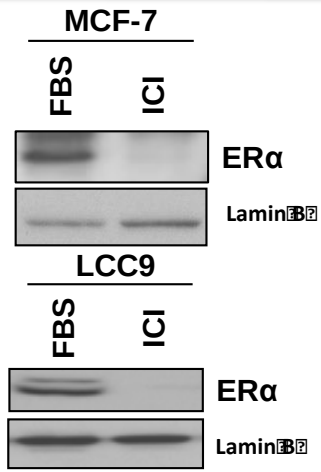


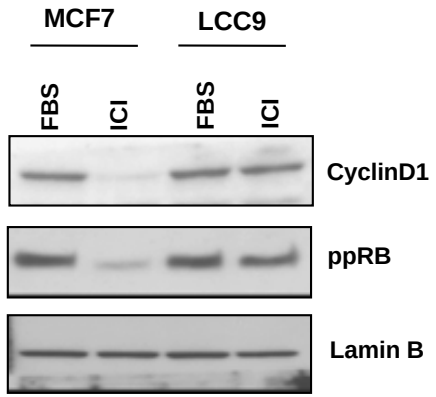
RB/E2F targets down-regulated



Acquired resistance disrupts ER signaling to cell cycle







Thangavel C, et al. Endocr Relat Cancer.

Cell cycle disruption in resistance to endocrine therapy deregulation of CDK4/6 signaling

Deregulated CDK4/6 downstream from ER associates with resistance to endocrine therapy

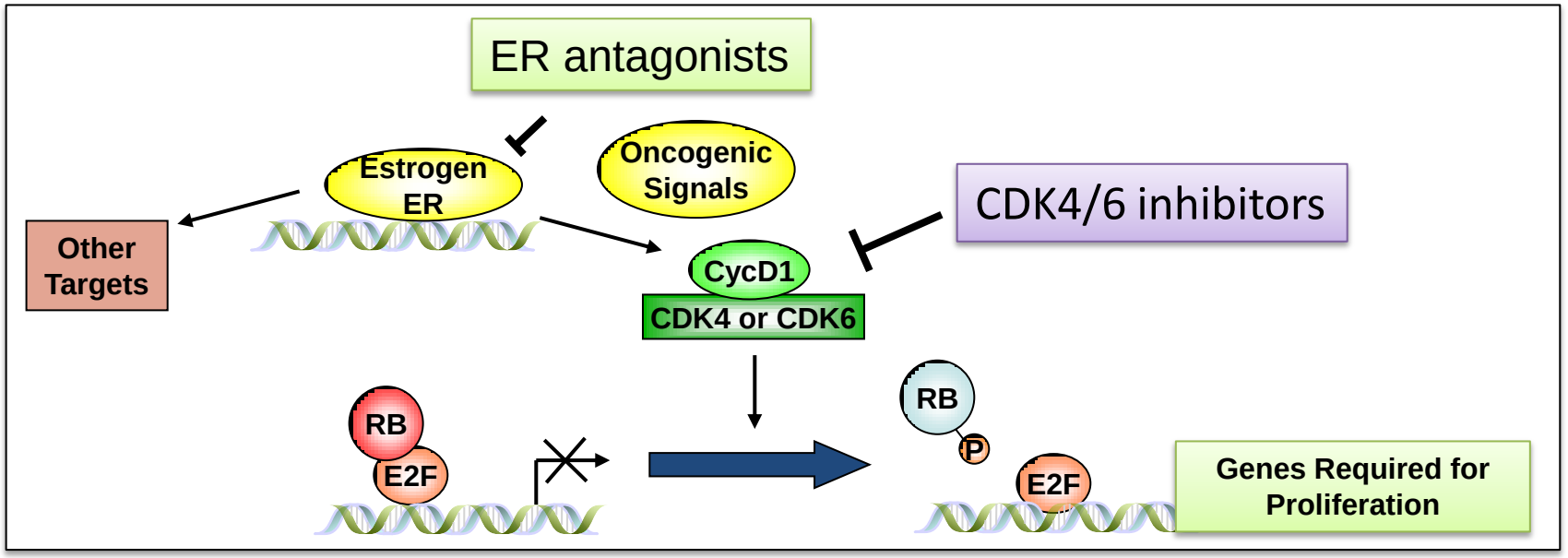
*CDK4/6 is a key downstream target of ER

*Deregulated CDK4/6 activity bypasses therapeutic response

RB protein/gene is maintained in majority of ER+ breast cancer

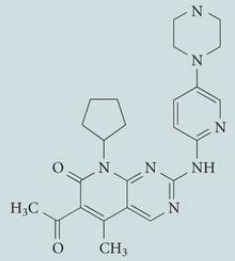
*Is lost in a small subset that have very poor response to endocrine therapy

CDK4/6 as a potential actionable target in ER+ breast cancer



CDK4/6 Selective inhibitors

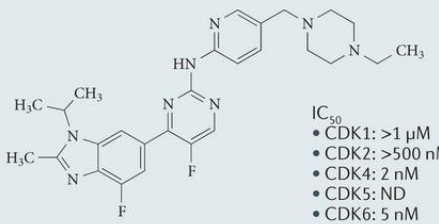
Palbociclib



PD-0332991

IC_{50}
- CDK1: >10 μM
- CDK2: >10 μM
- CDK4 : 9–11 nM
- CDK5: >10 μM
- CDK6 : 15 nM
- CDK7: ND
- CDK9: ND

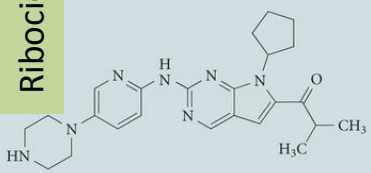
Abemaciclib



LY-2835219

IC_{50}
- CDK1: >1 μM
- CDK2: >500 nM
- CDK4: 2 nM
- CDK5: ND
- CDK6: 5 nM
- CDK7: 300 nM
- CDK9: 57 nM

Ribociclib



LEE011

IC_{50}
- CDK1: >100 μM
- CDK2: >50 μM
- CDK4: 10 nM
- CDK5: ND
- CDK6: 39 nM
- CDK7: ND
- CDK9: ND

Asghar et al., 2015 Nature Rev Drug Dis.

Selectivity:

*Active in the low nanomolar range against CDK4 and 6

*Minimal activity vs. other CDK proteins

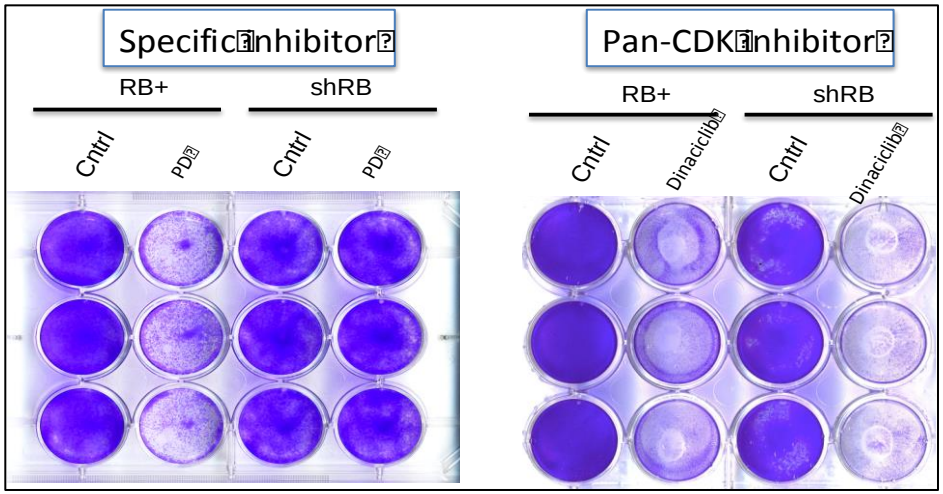
Primary mode of action cytostatic:

*Arrest cell cycle in G1

*Can elicit durable proliferative suppression

Selective for RB-positive tumors:

*RB required for drug response



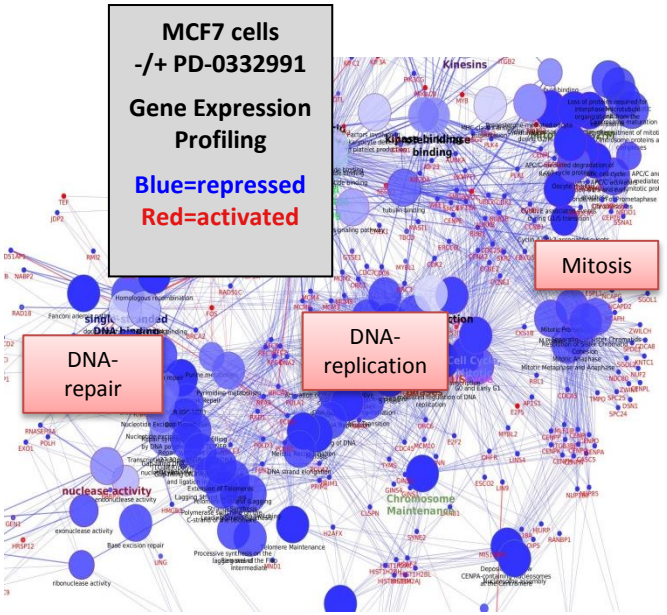
Cooperative with select therapeutics:

*Additive/synergistic with endocrine therapy

*Additive/synergistic with other targeted therapy

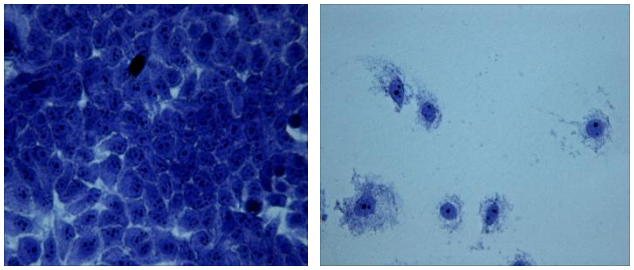
*Can antagonize chemotherapy

CDK4/6 inhibition suppresses proliferation

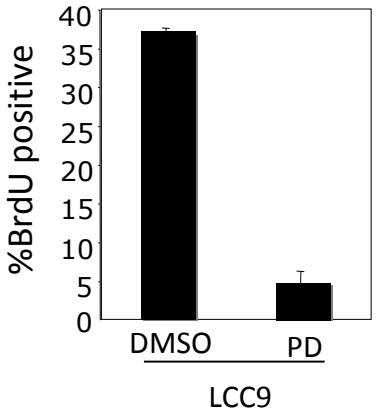


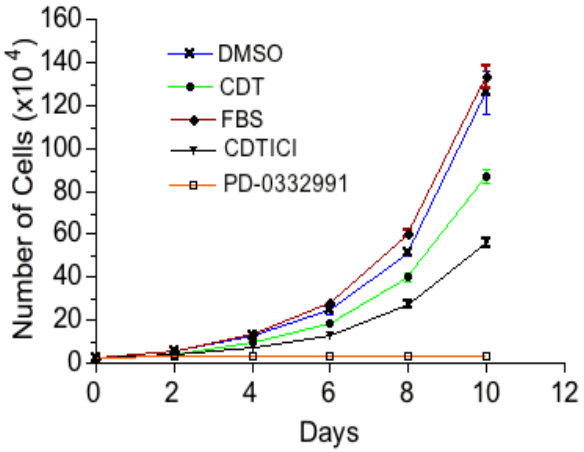
Targets:
CDK2, CDK1
CCNA2, CCNB1
MCM7, CDC45
DHFR, THMS
PLK1, AURK, etc.

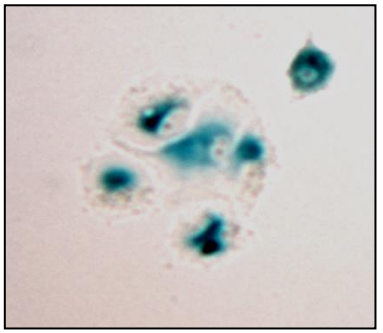
End Point Growth Stained With Crystal Violet (Day 10)



CDK4/6 inhibition is effective in models of endocrine therapy refractory ER+ breast cancer

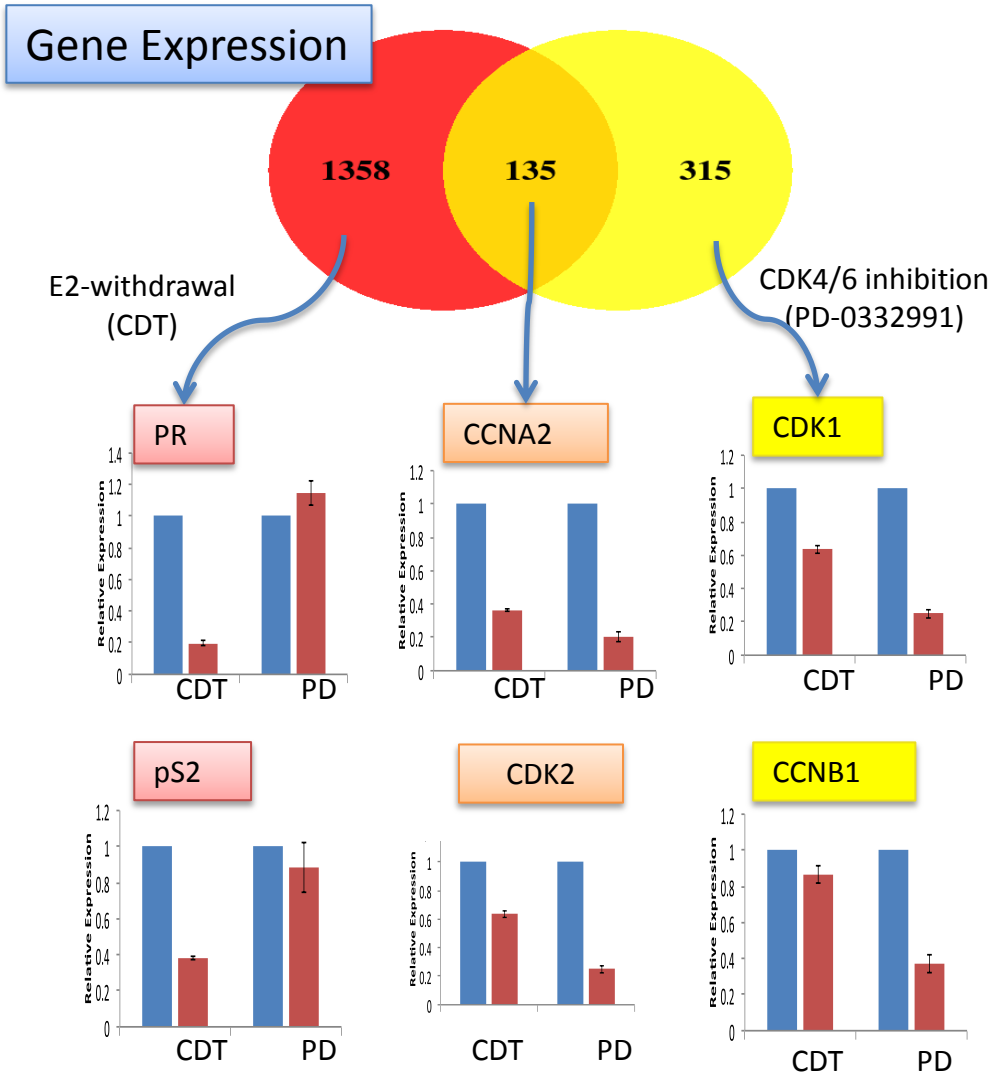


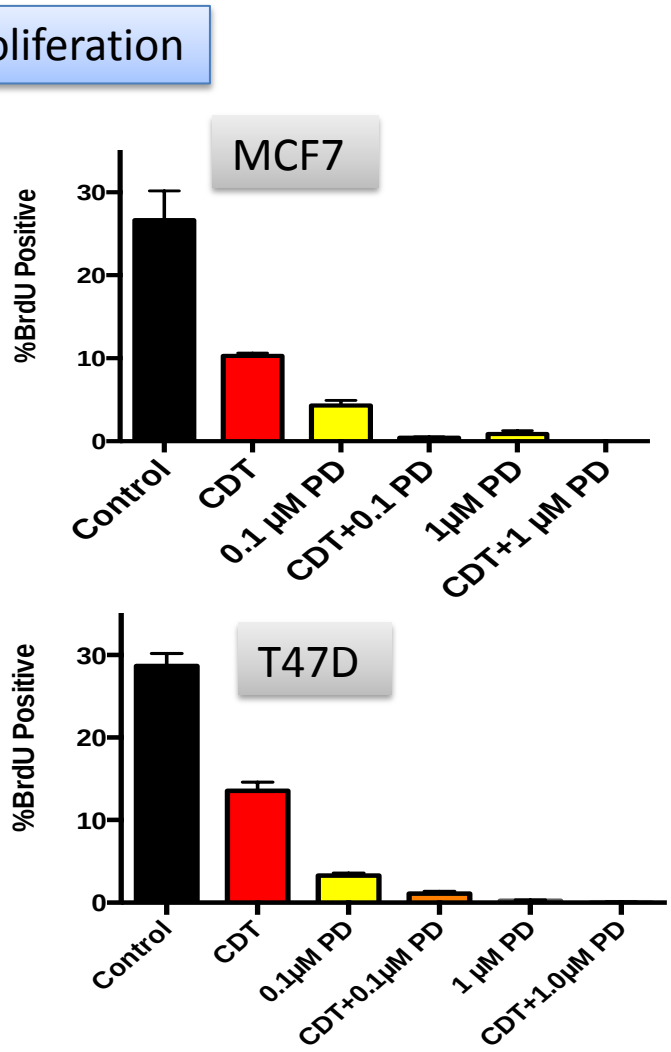




Thangavel C, et al. Endocr Relat Cancer.

Cooperative action of CDK4/6 inhibition with endocrine therapy





Targeting CDK4/6 in Breast Cancer

CDK4/6 inhibitors are effective cytostatic agents in models that retain RB

*tumor cells, xenografts, GEMMs, explants

Biomarkers for intrinsic resistance to CDK4/6 inhibitors in breast cancer

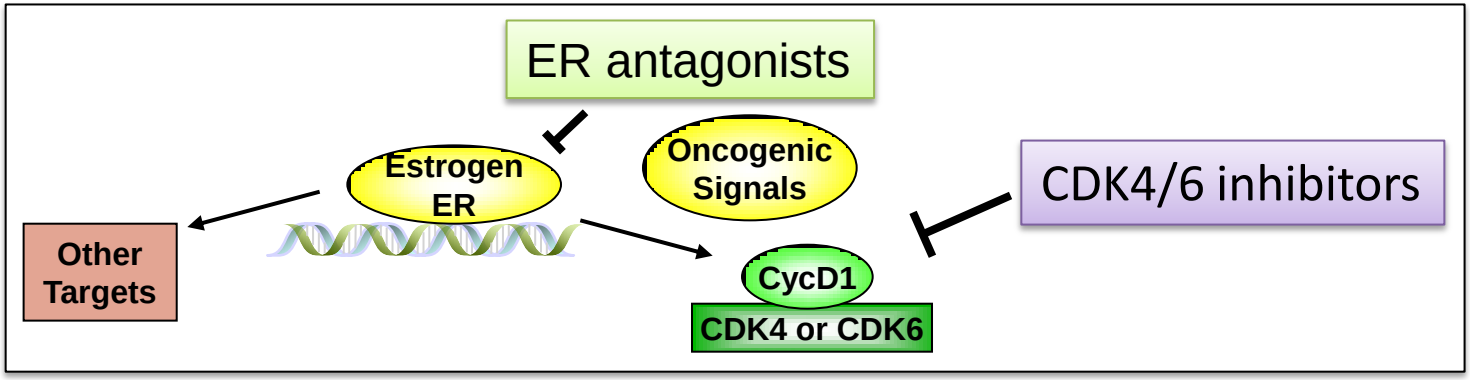
*RB loss

*Supraphysiological p16ink4a levels

CDK4/6 inhibitors and endocrine therapy-complementary mechanism of action

*Complementary effects on gene expression/biology

*Combinatorial drug effects

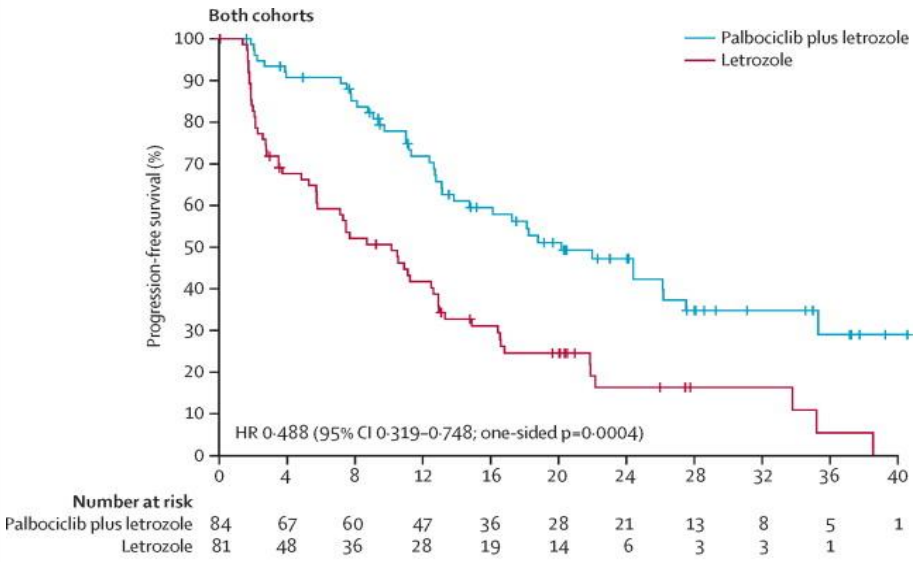


Finn R, et al. *Breast Cancer Res* 2009
Thangavel, et al. *Endocr Relat Cancer* 2011
Dean J, et al. *Oncogene* 2010
Miller T, et al. *Cancer Discov* 2011
Cox et al., *Genes and Cancer* 2014
Varo et al., *Cancer Cell* 2015

Clinical evidence of efficacy for CDK4/6 inhibition in ER+/Her2– Breast Cancer
Multiple ongoing clinical studies to delineate efficacy

CDK4/6 inhibitor trials in ER+/Her2- breast cancer

Paloma-1: Randomized Phase II



"Our results show that palbociclib, a novel, oral, inhibitor of CDK4/6, used in combination with letrozole resulted in **longer progression-free survival** than letrozole alone in postmenopausal women with advanced oestrogen receptor-positive, HER2-negative breast cancer."

Finn et al. Lancet Oncology 2015

Active Phase III CDK4/6 Trials in ER+/Her2- Breast Cancer

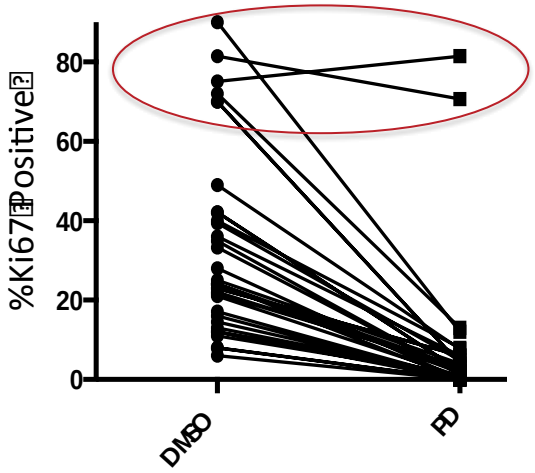
CDK 4/6 Inhibitor	Participants	Treatment Arms	Endpoint
Palbociclib **Trial Name: PENELOPE-B**	Women with ER+, HER-2 negative breast cancer with residual disease after neoadjuvant chemotherapy and surgery	Palbociclib + endocrine treatment vs placebo + endocrine treatment	Invasive disease-free survival
Palbociclib **Trial Name: PALOMA-2**	Postmenopausal women with ER+ HER-2 negative advanced breast cancer, no prior systemic therapy	Palbociclib + letrozole vs letrozole + placebo	Progression free survival
Palbociclib **Trial Name: PALOMA-3**	Women with ER+ HER-2 negative metastatic breast cancer with progression after prior endocrine therapy	Palbociclib + fulvestrant vs fulvestrant + placebo	Progression free survival
Palbociclib **Trial Name: PEARL**	Postmenopausal women with ER+ HER-2 negative metastatic breast cancer with resistance to nonsteroidal aromatase inhibitors	Palbociclib + exemestane vs chemotherapy (capecitabine)	Progression free survival
LEE011 **Trial Name: MONALEESA-2**	Postmenopausal women with ER+ HER-2 negative advanced breast cancer, no prior systemic therapy	LEE011 + letrozole vs letrozole + placebo	Progression free survival
LY2835219 **Trial Name: MONARCH2**	Postmenopausal women with ER+ HER-2 negative advanced breast cancer	Abemaciclib + fulvestrant vs fulvestrant + placebo	Progression free survival

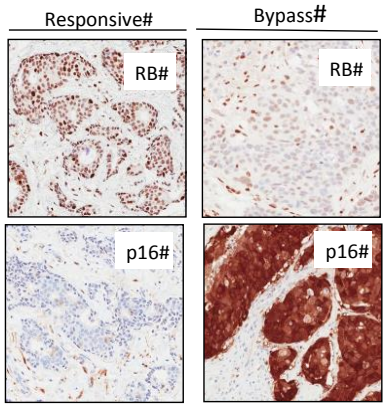
Summary 3: Targeting CDK4/6 in ER+ breast cancer

Potent activity in multiple preclinical models

*Mechanism of action is understood in models

*Determinants of response in preclinical models





Primary human tumors explants

*Frequency of response (33/35->90%)

ER+: 100% (n=22)

Her2+: 90%

TNBC: 75%

*Biomarkers of response (RB/p16)

Positive signal in clinical trial with endocrine therapy

*Significant improvement in PFS over endocrine therapy alone

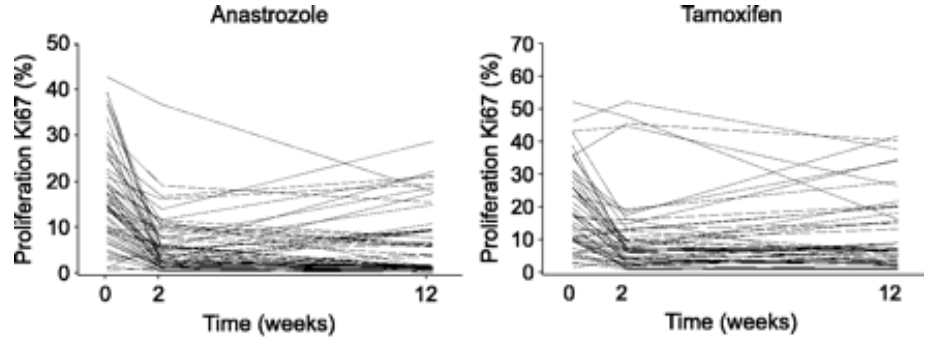
*FDA approval for Palbociclib+Letrozole (Feb 2015)

*Paloma-3 data pending (ASCO, 2015)

*Multiple ongoing trials…

OTHER IMPORTANT INDICATIONS, COMBINATIONS, AND CONCEPTS

CDK4/6 inhibition in neoadjuvant studies



Who would have thought a single Ki67 measurement would predict long-term outcome?

Dowsett et al., Clin Can Res 2005

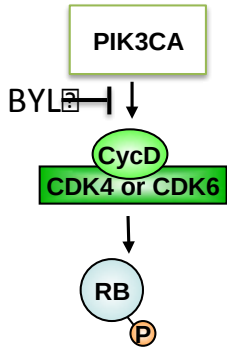
Dowsett et al., Breast Cancer Res. 2007

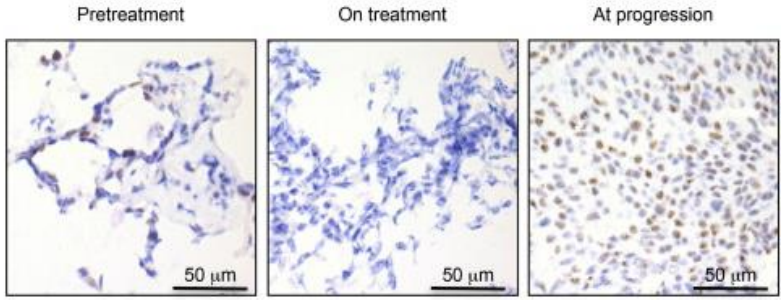
Mechanism of action, surrogate markers of response, and biomarker discovery

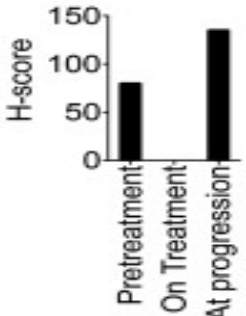
NCT02296801: **A Phase II Randomized Study Evaluating the Biological and Clinical Effects of the Combination of Palbociclib With Letrozole as Neoadjuvant Therapy in Post-Menopausal Women With Estrogen-Receptor Positive Primary Breast Cancer (PALLET)**	**Suppression of Ki67** **cCR**
NCT02400567: **Efficacy of Letrozole + Palbociclib Combination as Neoadjuvant Treatment of Stage II-IIIA PAM 50 ROR-defined Low or Intermediate Risk Luminal Breast Cancer, in Postmenopausal Women (NeoPAL)**	**Residual Cancer Burden (0-1)**
NCT01723774: **PD 0332991 and Anastrozole for Stage 2 or 3 Estrogen Receptor Positive and HER2 Negative Breast Cancer**	**pCR** **Suppression of Ki67**
NCT02008734: **Randomized Phase II Study to Assess PD 0332991 in Breast Cancer (POP)**	**Suppression of Ki67** **Senescence**

Additional combinations approaches ER+/Her2-

Phosphorylated RB staining on biopsies

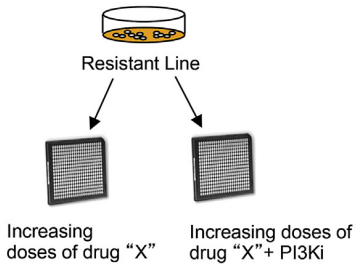


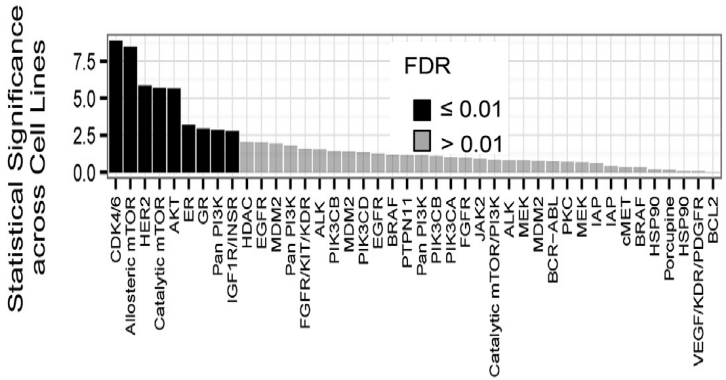


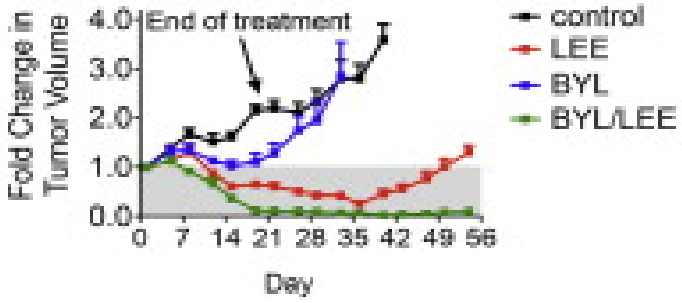


Varo et al. Cancer Cell, 2015.

Potent cooperation between CDK4/6 inhibition and PI3K inhibitors





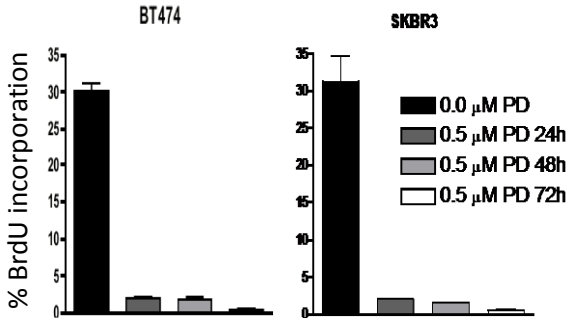


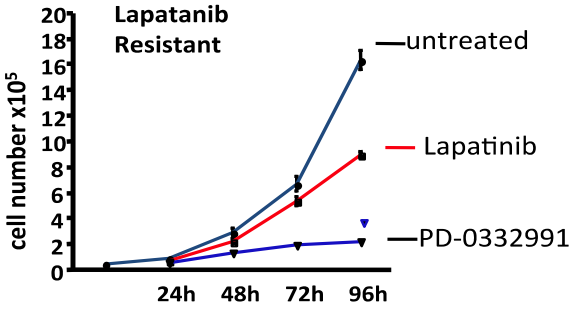
NCT02088684: **Study of LEE011 With Fulvestrant and BYL719 or BKM120 in Advanced Breast Cancer**

NCT02389842: **PIPA: Combination of PI3 Kinase Inhibitors and Palbociclib**

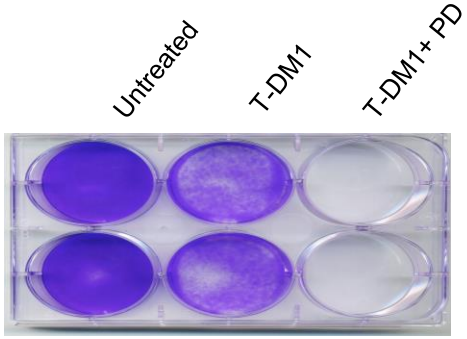
Her2+ Breast cancer and CDK4/6 inhibitors

Potent Activity in parental and “recurrent” models

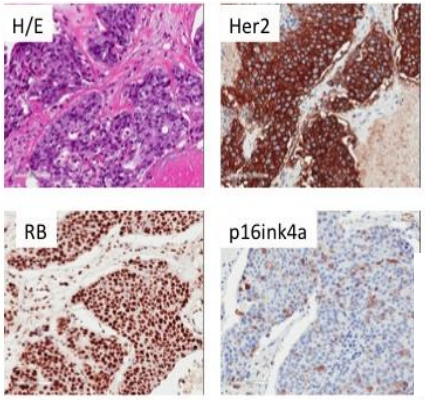




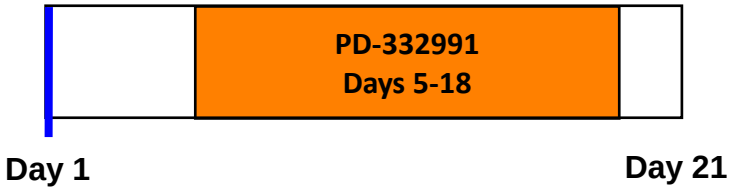
Suppress residual growth



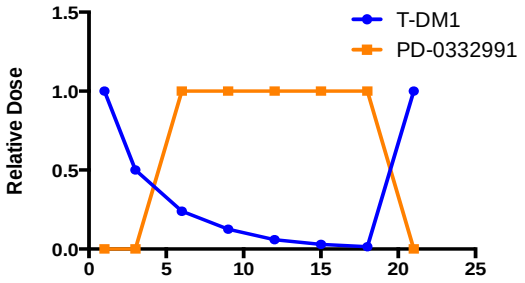
T-DM1+PD-0332991
Adv. Her2+ Disease
Failed prior therapy
RB-proficient Tumor



3.6 mg/kg T-DM1, IV every 21 days
50-125mg PD-03322991 , oral Days 5-18



PD-0332991 T1/2 ~1day



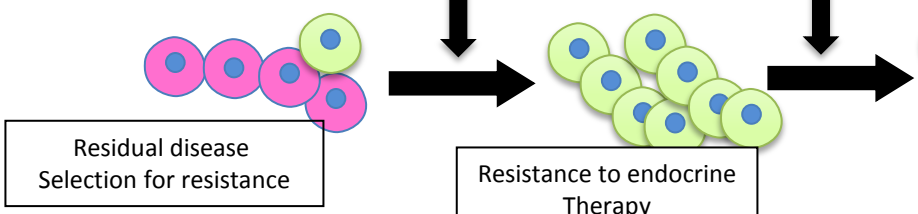
NCT01976169: **Phase 1b Study of PD-0332991 in Combination With T-DM1(Trastuzumab-DM1)**

NCT02308020: **A Study of Abemaciclib (LY2835219) in Participants With Breast Cancer That Has Spread to the Brain**

Overall concept and alternative approaches

Block proliferation:
with CDK4/6 inhibition
&
Active combinations

Target cell cycle deregulation?
Chromosome Damage-DNA Repair?



Residual disease
Selection for resistance

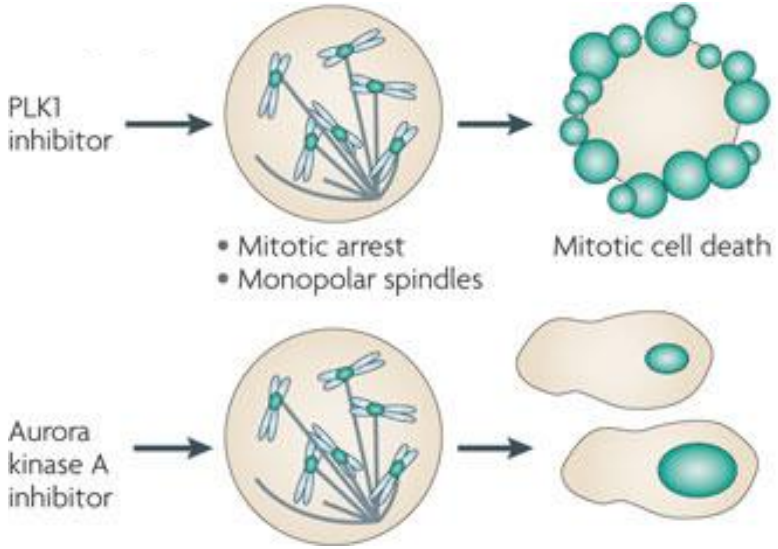
Resistance to endocrine Therapy

Escape from dual Therapy—Cell Cycle

Study to Compare Alisertib With Paclitaxel vs. Paclitaxel Alone in Metastatic or Locally Recurrent Breast Cancer

Alisertib and Fulvestrant in Treating Patients With Hormone Receptor Positive Breast Cancer That is Metastatic or Locally Advanced and Cannot Be Removed by , Surgery

Chk1/2 Inhibitor (LY2606368) in Women With BRCA1/2 Mutation Associated Breast or Ovarian Cancer, Non-High Risk Triple Negative Breast Cancer



Medema et al., Nat Rev Can 2013

Summary 4: Targeting the cell cycle across breast cancer

Neoadjuvant studies in ER+

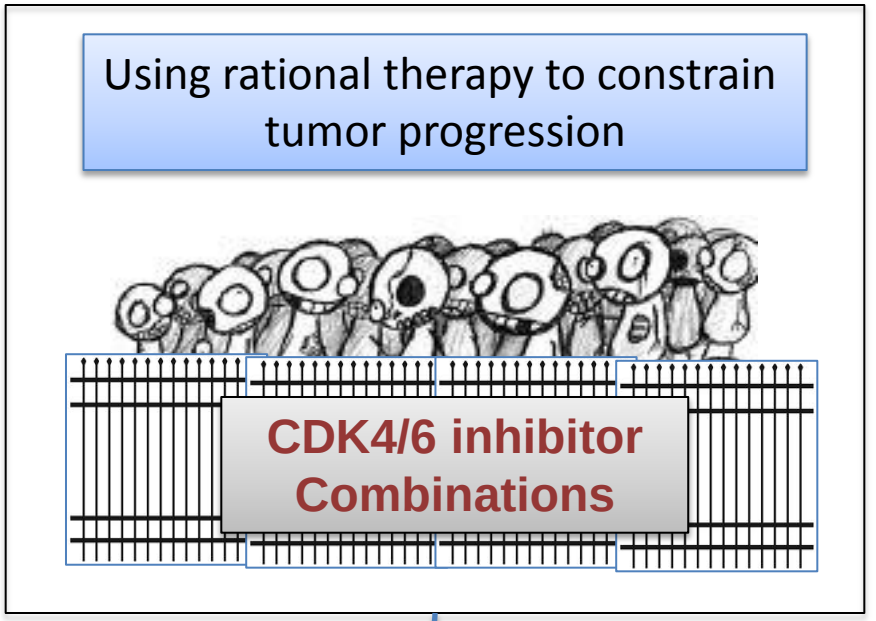
- *Mechanism of action
- *Surrogate markers
- *Biomarkers

Combination studies with active targeted agents

- *PI3K inhibitors
- *MTOR inhibitors
- *Therapeutic "triplets"

CDK4/6 inhibitors in distinct disease settings

- *Her2+ disease
- *TNBC will likely be a challenge due to frequent RB loss



Leveraging deregulation of cell cycle therapeutically….

- *Selective cytoxicity against tumors that fail prior therapies
- *Chk, Plk1, Aurk, Wee1, etc.
- *New approaches related to DNA and repair….