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

Hormonal Dependence

Surgery

Radiation/Chemotherapy

Cell Division

CDK/Cyclins

Analysis in Breast Cancer

Cell Division

CDK/Cyclins

Analysis in Breast Cancer

3,500 years ago

Hormonal Dependence

Surgery

Radiation/Chemotherapy

Cell Division

CDK/Cyclins

Analysis in Breast Cancer

1757

1882

1955

1895

1962

1967

3,500 years ago

Hormonal Dependence

Surgery

Radiation/Chemotherapy

Cell Division

CDK/Cyclins

Analysis in Breast Cancer

1757

1882

1955

1895

1962

1967

1250

1500

1750

1900

1925

1950

1975

1990

2000

Breast cancer

Endocrine therapy

Identification of Estrogen receptor

Breast Cancer and Cell Cycle

Robert Sutherland
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**
Many therapeutic agents act as “anti-proliferative signals” (Fulvestrant, Trametinib or Adriamycin, Taxanes)
Cyclin Dependent Kinases (CDK) drive the cell cycle

Mitogenic Signals

CycD
CDK4 or CDK6

Anti-proliferative signals

p16\(^{INK4A}\)

RB/E2F gene Expression program

Cell Cycle (e.g., CcnA2, CcnE1, CcnB1, Cdk2, Cdk1)

Replication (e.g., MCM2, 3, 5, 7, CDT1, CDC6)

Mitosis (e.g., CDC20, Plk1, Mad2 Cyclin B1)

Mitotic Machinery

DNA Replication Machinery

Anti-proliferative signals

CDK2
CycA
CycB

RB
E2F

Co-repressors

RB
Phosphatases

Cyclin Proteases

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

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

CDK4/RB-pathway is frequently disrupted in breast cancer

- Approximately 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB1</td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td></td>
</tr>
<tr>
<td>CCND1</td>
<td></td>
</tr>
</tbody>
</table>

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

CCND1 (29%) Amplification
RB1 (3%) Truncating Mutation

Luminal B: Cyclin D1 amplification

RB1 (42%) Deep Deletion
CCND1 (3%) Missense Mutation

TNBC: RB inactivation

Luminal
Her2
TNBC

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.

Many of the “drivers” in breast cancer impinge on cell cycle machinery:

*Her2
*Estrogen Receptor
*PIK3CA

Clinical Significance of Cell Cycle Deregulation
Long history of analyzing cell cycle regulatory proteins in ER+ breast cancer

<table>
<thead>
<tr>
<th>CELL CYCLE GENE</th>
<th>PROGNOSIS</th>
<th>ENDOCRINE THERAPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1 over expression</td>
<td>Controversial</td>
<td>Controversial</td>
</tr>
<tr>
<td>Cyclin D1 amplification</td>
<td>Poor Prognosis</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>Cyclin E1 over expression</td>
<td>Poor Prognosis</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>p27Kip1 loss</td>
<td>Poor prognosis</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>RB loss</td>
<td>Poor prognosis</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>Cyclin A2 over expression</td>
<td>Poor prognosis</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>Cyclin B1 over expression</td>
<td>Poor prognosis</td>
<td>Poor outcome</td>
</tr>
</tbody>
</table>

Musgrove & Sutherland. Nat Rev Cancer 2009
Millar E, et al. Oncogene 2010
Lenn et al. Cell Cycle 2011
Bindels E, et al. Oncogene 2002
AND MORE.....
Molecular profiling of the cell cycle

Mitogenic Signals

Anti-proliferative signals

(Cyclin D)

CDK4 or CDK6

RB

E2F

Co-repressors

RB loss

RB gain

CDK4/6 inhibition

p16INK4A

RB/E2F gene expression program

Cell Cycle (e.g., CcnA2, CcnE1, CcnB1, Cdk2, Cdk1)

Replication (e.g., MCM2, 3, 5, 7, CDT1, CDC6)

Mitosis (e.g., CDC20, Plk1, Mad2 Cyclin B1)

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

**Proliferation**
- Ki67
- STK15
- Survivin
  - CCNB1 (cyclin B1)
- MYBL2

**Her2**
- HER2
- GRB7
- HER2

**Estrogen**
- ER
- PGR
- BCL2
- SCUBE2

**Invasion**
- MMP11 (stromelysin 3)
- CTSL2 (cathepsin L2)

**Reference**
- ACTB (β-actin)
- GAPDH
- GUS
- IFRC

**Co-repressors**
- RB
- E2F

**RB/E2F gene Expression program**

**Mitogens Oncogenes**
- CycD
- CDK4 or CDK6

**Anti-proliferative signals**
- P16INK4A

**Cyclin E1**
- Cyclin A2
- Cyclin B1
- GMMN
- CDCA3
- RRM2
- MCM2

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

**GGI**
- ASPM
- AURKA
- AURKB
- BIRC5
- BBS1
- BLM
- BUB1B
- CENPA
- CENPF
- CEP55
- CMC2
- CXXC5
- CYBRD1
- DLGAP5
- DONSON
- DSCC1
- ESL1
- EXO1
- FAM64A
- FEN1
- FOXM1
- GMP5
- GTSE1
- H2AFZ
- HJURP
- HMGB3
- IFT46
- IFT88
- KIF11
- KIF14
- KIF15
- KIF20A
- KIF2C
- KIF4A
- KIF1
- KPN2A
- LAMB2
- LMNB1
- MARS
- MCM10
- MCM2
- MCM4
- MKI67
- MLPH
- MMP11
- MYBL2
- NUDT1
- NDC80
- NUSAP1
- ORMDL2
- PARBP
- PIGV
- PLK1
- POLQ
- PRC1
- PTTG1
- RACGAP1
- RNASEH2A
- RRM2
- SESN1
- SHMT2
- SIRT3
- SLC7A5
- SPAG5
- STARD13
- TIMELESS
- TPT1
- TPK2
- TTK
- UBE2C
- UBE2S
- UBE2N
- WDR19
- ZWINT
Cell cycle and treatment decisions in ER+ disease

Cell cycle deregulation as measured by these signatures is NOT invariably associated with poor outcome.
Cell cycle deregulation: favorable feature for response to neoadjuvant chemotherapy

Neoadjuvant treated cases

Proliferation associated genes

Ave. RB-loss sig. response

Residual disease

pCR

Complete Response

pCR

Residual Disease

Chemotherapy

Post-treatment

P = 3.57e-05

pCR

No pCR

Pathological outcome (%)

High

Low

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
   Oncoype, 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

[Graph showing expression levels of genes associated with pCR]
Endocrine therapy impinge on the CDK4/RB-pathway

- **Estrogen (ER)** inhibits ER-Signaling.
- CDK4 or CDK6
- RB phosphorylation inhibited
- RB/E2F gene Expression program

**Acquired resistance** disrupts ER signaling to cell cycle

**MCF-7**
- FBS
- ICI

**LCC9**
- FBS
- ICI

**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

**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.

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

%BrdU positive

Number of Cells ($\times 10^4$)

LCC9

DMSO  PD

Cooperative action of CDK4/6 inhibition with endocrine therapy

Gene Expression

- E2-withdrawal (CDT)
- CDK4/6 inhibition (PD-0332991)

Proliferation

- MCF7
- T47D

[Graph showing gene expression and proliferation]
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

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

References:
Dean J, et al. Oncogene 2010
Cox et al., Genes and Cancer 2014
Varo et al., Cancer Cell 2015
CDK4/6 inhibitor trials in ER+/Her2- breast cancer

### Paloma-1: Randomized Phase II

**Trial Name:** PENELOPE-B  
**Participants:** Women with ER+, HER-2 negative advanced breast cancer 
**Treatment Arms:** Palbociclib + endocrine treatment vs placebo + endocrine treatment  
**Endpoint:** Progression-free survival

“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

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

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...

Primary human tumors explants
* Frequency of response (33/35->90%)
  ER+: 100% (n=22)
  Her2+: 90%
  TNBC: 75%
* Biomarkers of response (RB/p16)

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

<table>
<thead>
<tr>
<th>Clinical Trial ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02296801</td>
<td>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)</td>
</tr>
<tr>
<td>NCT02400567</td>
<td>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)</td>
</tr>
<tr>
<td>NCT01723774</td>
<td>PD 0332991 and Anastrozole for Stage 2 or 3 Estrogen Receptor Positive and HER2 Negative Breast Cancer</td>
</tr>
<tr>
<td>NCT02008734</td>
<td>Randomized Phase II Study to Assess PD 0332991 in Breast Cancer (POP)</td>
</tr>
</tbody>
</table>
Additional combinations approaches ER+/Her2-

**Phosphorylated RB staining on biopsies**

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
50-125 mg PD-0332991, oral Days 5-18

PD-0332991 T1/2 ~1 day

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?

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

Using rational therapy to constrain tumor progression

Leveraging deregulation of cell cycle therapeutically....
* Selective cytotoxicity against tumors that fail prior therapies
* Chk, Plk1, Aurk, Wee1, etc.
* New approaches related to DNA and repair....