New drugs and trials on the horizon:

Targeting the CDK 4/6 pathway

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Baylor College of Medicine, Huston (TX)
Disclosures

• Research support from Pfizer
Outline

• Introducing the CDK4/6 pathway
• Pre-clinical background of CDK4/6 in BC subtypes:
  ✓ Luminals
  ✓ HER2
  ✓ TN
• Available clinical data with CDK4/6 inhibitors
CDK 4/6 as a key regulator of cell cycle
CoA
Proliferation
Invasiveness

Cyclin D1/D2/D3
CDK 4/6

DNA replication, Cell cycle entry

E2F
RB
inactive

P

P

P

P

E2F

DNA replication, Cell cycle entry

Proliferation
Invasiveness
Integrins

GFRs

FAK

ERK1/2

p38

JNK

PI3K

HER 1/2

Cyclin D1/D2/D3

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DNA replication,
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CoA

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Integrins

GFRs

HER 1/2

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p16

p21

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D1/D2/D3

CDK 4/6

p16

p21

RB

E2F

inactive

CoA

DNA replication,
Cell cycle entry

Proliferation
Invasiveness
# Deregulation of CDK 4/6 pathway in BC subtypes

<table>
<thead>
<tr>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2 enriched</th>
<th>Basal-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1 amp (29%)</td>
<td>Cyclin D1 amp (58%)</td>
<td>Cyclin D1 amp (38%)</td>
<td>Cyclin E1 amp (9%)</td>
</tr>
<tr>
<td>CDK4 gain (14%)</td>
<td>CDK4 gain (25%)</td>
<td>CDK4 gain (24%)</td>
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</tr>
<tr>
<td>11q13.3 amp (24%)</td>
<td>11q13.3 amp (51%)</td>
<td></td>
<td>RB1 mut/loss (20%)</td>
</tr>
<tr>
<td>Low expression of p18/high expression of RB1</td>
<td>High FOXM1</td>
<td>High expression of p16/ low expression of RB1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luminal A</td>
<td>Luminal B</td>
<td>HER2</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td>RB1 mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB1 LOH</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RB1 copy</td>
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<td></td>
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<tr>
<td>CCND1 copy</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CCNE1 copy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB1 mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDKN2A mRNA</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CDKN2C mRNA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RB1 protein</td>
<td></td>
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<tr>
<td>CHICAS</td>
<td></td>
<td></td>
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<td>LARA</td>
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<tr>
<td>HERSCHKOWITZ</td>
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</table>
Outline

• Introducing the CDK4/6 pathway
• Pre-clinical background of CDK4/6 in BC subtypes:
  ✓ Luminals
  ✓ HER2
  ✓ TN
• Available clinical data with CDK4/6 inhibitors
Cross-talks of the CDK 4/6 and ER pathways

Integrins

GFRs

HER 1/2

PI3K

FAK

ERK1/2

p38

JNK

p16

p21

RB

E2F

RB

inactive

DNA replication, Cell cycle entry

Proliferation

Invasiveness

Cyclin D1, E2Fs, FOXM1

Cyclin D1/D2/D3

CDK 4/6

ER

AP-1

CoA

E2F

E2F

TRE

FOXM1

Proliferation

Invasiveness

Dahlman-Wright K. et al., Carcinogenesis 2012
• Estrogen modulation of E2F1 is critical for hormone regulation of the proliferative program of breast cancer cells (Stender J.D. et al, Mol. Endo. 2007)

• In long term estrogen deprived cells, ER retains genomic activity and drives a CDK4/E2F dependent transcriptional program despite estrogen deprivation therapy (Miller T.W. et al, Cancer Discovery 2011)

**A gene expression signature of E2F activation correlates with poor tumor response to AIs in patients.**

Miller T.W. et al, Cancer Discovery 2011
The CDK 4-6 inhibitor PD 0332991 has shown activity preferentially on ER+, luminal breast cancer cell lines with or without HER2 amplification.

Finn et al, BCR 2011
CDK 4/6 inhibitor + Endocrine therapy

PD-0332991 Acts Synergistically with Tamoxifen in ER+ Breast Cancer Cell Lines

Finn et al, BCR 2011

PD-0332991 improves efficacy of Fulvestrant and Letrozole in Luminal BC models

Koehler M. et al, IMPAKT meeting Poster walk 2014
Study design

To Reverse Endocrine Resistance

- **Study population**
  - Postmenopausal women with ER\(^+\) HER2 neg breast cancer progressing to 1\(^°\)/2\(^°\) line HT (AI or Fulvestrant)

- **Randomization**
  - 1:1

  - Palbociclib 125 mg QD x 3 weeks, 1 week off;

  - Palbociclib 125 mg QD x 3 weeks, 1 week off plus same HT as pre-progression

- **N** = 100

- **4-week treatment cycle**

**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
Summary: CDK4/6 inhibition in Her2+ breast cancer

Mechanisms of bypass of Her2-targeted agents are complex
* Aberrant cellular proliferation in the presence of agents
* Common deregulated signaling that feeds into CDK4/6

CDK4/6 inhibition has activity against Her2-positive models
* Cell Culture models, xenografts, GEMMS, tumor explants
* Markers of resistance (p16 and RB) can be identified in clinical specimens

CDK4/6 inhibitors cooperate with Her2-targeted agents
* Cooperation with multiple small molecule inhibitors (e.g. neratinib) in Her2-positive models
* Cooperation with T-DM1 to prevent growth of residual clones

Courtesy of E. Knudsen (summary of data presented at IMPAKT 2014)
Cyclin D1 or CDK 4/6 siRNA has opposite effects in ER+ vs ER neg BC cell lines

Cyclin D1/CDK4-6 siRNA

ER+ → Migration → ER neg

Mammosphere formation

Lamb R., Cell Cycle 2013
Interaction of CDK 4/6 inhibition with chemotherapy

MMTV-neu (Rb competent)

C3-TAg (Rb incompetent)
Molecular determinants of response to CDK4/6 inhibitors

- **Integrins**
- **GFRs**
- **HER 1/2**
- **p38**
- **JNK**
- **PI3K**
- **GFRs**
- **ERK1/2**
- **FAK**
- **HDACs**
- **CDK 4/6**
- **p16**
- **p21**
- **RB**
- **Cyclin D1/D2/D3**
- **CDK 4/6**
- **DNA replication, Cell cycle entry**
- **Proliferation, Invasiveness**

- **High p16 = CDK4/6i sens**
- **Low p16 = CDK4/6i res**
- **Intact RB = CDK4/6i sens**
- **Rb loss = CDK4/6i res**
- **High Cyclin D = CDK4/6i sens**

MORE COMPLEX?
**Rb loss signature in Luminal BC**

**A** ER-positive breast cancer

- **Low expression** vs. **High expression**
- **RB-loss signature**
- **Ave. RB-loss sig.**
- **ER+ subtype**
  - Luminal A
  - Luminal B

**Expression**

- **Low** to **High**

**Survival probability**

- **P = 5.55 x 10^{-16}**
- **P = 4.01 x 10^{-2}**

Thangavel C et al. Endocr Relat Cancer 2011
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  ✓ HER2
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• Available clinical data with CDK4/6 inhibitors

<table>
<thead>
<tr>
<th>CDK (Cyclin partner)</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK4/Cyclin D1</td>
<td>0.011</td>
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<tr>
<td>CDK4/Cyclin D3</td>
<td>0.009</td>
</tr>
<tr>
<td>CDK6/Cyclin D2</td>
<td>0.015</td>
</tr>
<tr>
<td>CDK2/Cyclin A</td>
<td>&gt;5</td>
</tr>
<tr>
<td>CDK1/Cyclin B</td>
<td>&gt;5</td>
</tr>
<tr>
<td>CDK5/p25</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CDK</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK4</td>
<td>0.002</td>
</tr>
<tr>
<td>CDK6</td>
<td>0.009</td>
</tr>
<tr>
<td>CDK1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CDK (Cyclin partner)</th>
<th>IC$_{50}$ (µM)</th>
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</thead>
<tbody>
<tr>
<td>CDK4/cyclin D1</td>
<td>0.010</td>
</tr>
<tr>
<td>CDK6/cyclin D3</td>
<td>0.039</td>
</tr>
<tr>
<td>CDK1/cyclin B</td>
<td>113</td>
</tr>
<tr>
<td>CDK2/cyclin A</td>
<td>76</td>
</tr>
<tr>
<td>CDK5/p25</td>
<td>45</td>
</tr>
<tr>
<td>CDK9/cyclin T1</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Paloma 1/TRIO 18 phase II study

**Part 1**
- N=66
- Post-menopausal
- No prior treatment for advanced disease
- ER+, HER2– BC status

Palbociclib 125 mg QD + Letrozole 2.5 mg QD

**Part 2**
- N=99
- Same as part 1 but with CCND1 amplification and/or loss of p16

Letrozole 2.5 mg QD

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**Key Eligibility Criteria**
- Measurable disease (RECIST 1.0) or bone-only disease
- ECOG PS of 0 or 1
- Adequate blood counts and organ function
- No prior/current brain metastases

**Stratification Factors**
- Disease Site (Visceral vs Bone only vs Other)
- Disease-Free Interval (>12 vs ≤12 mo from end of adjuvant to recurrence or de novo advanced disease)

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)

- Palbociclib administered as single agent 125 mg/day g1-21 of a 28 day cycle

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

*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
Phase I Study Bemaciclib

- Open label phase I study: breast cancer expansion cohort
- Bemaciclib was administered at 150 mg or 200 mg orally b.i.d. days 1-28 of a 28-day cycle
- Patients (n=47) with heavily pretreated MBC (median nr of prior systemic tx= 7)

### Possibly Related Treatment-emergent Adverse Events in ≥15% of Patients

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade 1 (N = 47)</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>All Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>20 (42.6)</td>
<td>8 (17.0)</td>
<td>3 (6.4)</td>
<td>0 (0.0)</td>
<td>31 (66.0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>17 (36.2)</td>
<td>8 (17.0)</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
<td>27 (57.4)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>11 (23.4)</td>
<td>8 (17.0)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>20 (42.6)</td>
</tr>
<tr>
<td>Neutrophil count decreased</td>
<td>3 (6.4)</td>
<td>6 (12.8)</td>
<td>9 (19.1)</td>
<td>1 (2.1)</td>
<td>19 (40.4)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>14 (29.8)</td>
<td>4 (8.5)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>19 (40.4)</td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>9 (19.1)</td>
<td>1 (2.1)</td>
<td>5 (10.6)</td>
<td>0 (0.0)</td>
<td>15 (31.9)</td>
</tr>
<tr>
<td>White blood cell decreased</td>
<td>1 (2.1)</td>
<td>7 (14.9)</td>
<td>5 (10.6)</td>
<td>0 (0.0)</td>
<td>13 (27.7)</td>
</tr>
</tbody>
</table>

Patnaik A. AACR 2014
Response Rate (CR + PR) = 25.0%
Clinical Benefit Rate (CR + PR + SD ≥24 weeks) = 61.1%
Disease Control Rate (CR + PR + SD) = 80.6%
Median progression free survival (PFS) 9.1 months

Best Overall Response

<table>
<thead>
<tr>
<th>Response</th>
<th>N</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (CR)</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>9</td>
<td>25.0†</td>
</tr>
<tr>
<td>Stable disease (SD) ≥24 weeks</td>
<td>13</td>
<td>36.1</td>
</tr>
<tr>
<td>SD &lt;24 weeks</td>
<td>7</td>
<td>19.4</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>5</td>
<td>13.9</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>2</td>
<td>5.6</td>
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</table>
CLEE011X2101: Phase I Study LEE001

- Open label phase I study (dose escalation n=30), multiple cancers expansion cohort (n=40)
- Doses tested: 50–1200 mg; MTD: 900 mg; RP2D: 600 mg/day – 3 weeks on/1 week off
- All pts tumors RB+

### Treatment emergent AE

<table>
<thead>
<tr>
<th>Preferred terms</th>
<th>All Grades</th>
<th>Gr 3/4</th>
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<tr>
<td></td>
<td>N=78 n (%)</td>
<td>N=78 n (%)</td>
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<tr>
<td>Hematologic</td>
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</tr>
<tr>
<td>Neutropenia*</td>
<td>31 (40)</td>
<td>15(19)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>17 (22)</td>
<td>11(14)</td>
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<tr>
<td>Leukopenia</td>
<td>28 (36)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>19 (24)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Non hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>27 (35)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Prolonged QTcF**</td>
<td>10 (13)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>2 (3)</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

*Onset of neutropenia occurs by Day 15, reaching a nadir in the 3rd or 4th wk with recovery during the wk of drug holiday. Some patients require additional time for recovery (7–14 days).

** QTcF changes become evident in the first cycle by Day 8.
CLEE011X2101: Preliminary Efficacy Data (dose escalation)

- 70 evaluable patients
- 1 confirmed PR at 600 mg in a ER+ breast cancer patient
- 1 unconfirmed PR response at 600 mg in a BRAF/NRAS wild-type melanoma patient
- 18/70 patients with SD for 4 cycles and more
- 10/70 patients with SD for 6 cycles and more

Infante JR et al. AACR-NCI-EORTC Meeting 2013
Ongoing Trials
<table>
<thead>
<tr>
<th>CDK4/6 inhibitor</th>
<th>Trial identifier</th>
<th>Trial status</th>
<th>Phase</th>
<th>Other drugs</th>
<th>Tumor type</th>
<th>Menopausal status</th>
<th>Biomarkers</th>
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</thead>
<tbody>
<tr>
<td>Palbociclib</td>
<td>NCT01684215</td>
<td>Active, not recruiting</td>
<td>Phase 1/2</td>
<td>Letrozole (phase 2)</td>
<td>ER+ HER2- ABC (phase 2)</td>
<td>Post</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>NCT01976169</td>
<td>Not yet recruiting</td>
<td>Phase 1b</td>
<td>T-DM1</td>
<td>HER2+ ABC</td>
<td>Pre and post</td>
<td>Rb, p16</td>
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<tr>
<td></td>
<td>NCT01723774</td>
<td>Recruiting</td>
<td>Phase 2</td>
<td>Anastrozole</td>
<td>ER+ HER2- EBC or LABD</td>
<td>Pre and post</td>
<td>No</td>
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<tr>
<td></td>
<td>NCT01864746</td>
<td>Recruiting</td>
<td>Phase 3</td>
<td>Endocrine</td>
<td>ER+ HER2- with residual after neoadjuvant</td>
<td>Pre and post</td>
<td>Rb, Cyclin D1</td>
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<td>NCT01740427</td>
<td>Recruiting</td>
<td>Phase 3</td>
<td>Letrozole</td>
<td>ER+ HER2- ABC</td>
<td>Post</td>
<td>No</td>
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<tr>
<td></td>
<td>NCT02028507</td>
<td>Recruiting</td>
<td>Phase 3</td>
<td>Exemestane</td>
<td>ER+ HER2- HT pretreated MBC</td>
<td>Post</td>
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<td></td>
<td>NCT00721409</td>
<td>Active, not recruiting</td>
<td>Phase 1/2</td>
<td>Letrozole</td>
<td>ER+ HER2- ABC</td>
<td>Post</td>
<td>CCND1 p16</td>
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<tr>
<td></td>
<td>NCT02040857</td>
<td>Recruiting</td>
<td>Phase 2</td>
<td>Endocrine</td>
<td>ER+ HER2- stage II/III (no T2N0)</td>
<td>Post</td>
<td>No</td>
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<td></td>
<td>NCT01942135</td>
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<td>Phase 3</td>
<td>Fulvestrant</td>
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<td>Phase 1b/2</td>
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<td></td>
<td>NCT01919229</td>
<td>Recruiting</td>
<td>Phase 2</td>
<td>Letrozole</td>
<td>ER+ HER2- EBC, presurgery</td>
<td>Post</td>
<td>No</td>
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<td>LY2835219</td>
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<td>Phase 2</td>
<td>No</td>
<td>ER+ HER2- MBC CT pretreated</td>
<td>Pre and post</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>NCT02107703</td>
<td>Not yet recruiting</td>
<td>Phase 3</td>
<td>Fulvestrant</td>
<td>ER+ HER2- LABC or MBC, including HT pretreated</td>
<td>Post</td>
<td>No</td>
</tr>
</tbody>
</table>
Acknowledgements
Thank you
Backup
Molecular determinants of response to CDK4/6 inhibitors

Integrins  GFRs

HER 1/2

PI3K

FAK  ERK1/2  p38  JNK

Cyclin D1/D2/D3  CDK 4/6

p16  p21

RB

E2F

Rb loss = CDK4/6i res

Rb functional inactivation = CDK4/6i sens?

Rb loss
GENETIC
= CDK4/6i res