Loss of ASXL1 tumor suppressor promotes resistance to CDK4/6 inhibitors in ER+ breast cancer

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Disclosure statement

• AstraZeneca employee since October 2021

• All data were generated during my tenure at the Simmons Comprehensive Cancer Center, in the laboratory of Dr. Carlos Arteaga
Novel approaches to unravel the CDK4/6 inhibitor resistance landscape

Genomic profiling of MBCs progressing on CDK4/6i has identified several resistance-associated somatic alterations, albeit at low frequencies –

- **RB1** mutations (6/127; **4.7%**) (O'Leary et al, Cancer Discovery, 2018)
- **FGFR1** amplification (Formisano et al, Nature Communications, 2019)
- **FAT1** loss-of-function alterations (6/348; **1.7%**) (Li et al, Cancer Cell, 2018)
- **FGFR2** (3/41), **AKT1** (5/41), **AURKA** (11/41), **KRAS** (2/41), and **HRAS** (1/41) alterations (Wander et al, Cancer Discovery, 2020)

There is an unmet need to uncover the full spectrum of genomic drivers of resistance to CDK4/6 inhibitors

Accelerated mutagenesis approaches provide a robust and unbiased platform for the discovery of novel resistance mechanisms
Accelerated mutagenesis screen to discover a spectrum of novel mutations causally associated with resistance to CDK4/6 blockade

Impaired DNA repair accelerates the rate of nucleotide substitution leading to rapid accumulation of new mutations

Palbo Abema
Accelerated mutagenesis screen to discover a spectrum of novel mutations causally associated with resistance to CDK4/6 blockade

Number of missense and frameshift mutations accumulated by the MSH2<sup>-/-</sup> clones are higher compared to the parental cells.

Majority of alterations noted in these 10 candidate genes were frameshift or truncating mutations, suggesting loss of function.

*cross-resistant to fulvestrant
Top hits are associated with clinical resistance to CDK4/6 inhibitors

DFCI CCPM / MBC Project

<table>
<thead>
<tr>
<th>Hugo Symbol</th>
<th>Protein Change</th>
<th>Drug</th>
<th>Resistance Category</th>
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<td>ASXL1</td>
<td>p.RGGE65fs</td>
<td>Palbociclib</td>
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TEMPUS

<table>
<thead>
<tr>
<th>Hugo Symbol</th>
<th>Incidence</th>
<th>Alteration Frequency</th>
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<tbody>
<tr>
<td>ASXL1</td>
<td>37/1796</td>
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<tr>
<td>ATR</td>
<td>49/1796</td>
<td>2.72%</td>
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<tr>
<td>MIS18BP1</td>
<td>3/1796</td>
<td>0.17%</td>
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Heterozygous truncating mutations in the ASXL1 gene is a common event in myeloid malignancies.

ASXL1 inactivation induces epigenetic and transcriptional reprogramming through global loss of H3K27me3 chromatin repressive marks.

Abdel-Wahab et al. Cancer Cell, 2012
ASXL1 deficient ER+ breast cancer cells are resistant to CDK4/6 inhibition
ASXL1 deficiency leads to robust induction of E2F target genes

- E2F targets
- G2M checkpoint
- Myc targets
- Mitotic spindle
- WNT β-catenin signaling
- Hedgehog signaling
- Spermatogenesis
- EMT
- Hypoxia
- UV response
- mTORC1 signaling
- Apoptosis
- Glycolysis
- Complement response
- TNFα signaling via NFκB
- Cholesterol homeostasis
- Xenobiotic metabolism
- Bile acid metabolism
- p53 pathway
- INFγ response
- Oxidative phosphorylation
- Adipogenesis
- Protein secretion
- Estrogen response late
- Estrogen response early

shASXL1 vs. control - palbociclib

Normalized enrichment score (NES)
Loss of function ASXL1 mutations are acquired in CDK4/6 inhibitor resistant ER+ MBCs

ASXL1 loss gene signature queried in clinical datasets
ASXL1 deficiency leads to robust induction of cell cycle genes

Palbociclib (P); Ribociclib (R); Abemaciclib (A)

- P R A - P R A - P R A

pRB (780)
pRB (807) short exp
pRB (807) long exp
CDK4
CDK6
CDK2
Cyclin D1
Cyclin E1
Cyclin E2
Cyclin A2
Cyclin B1
p16
p21
p27
GAPDH

shRNA:
ctrl ASXL1#1 ASXL1#2

Palbociclib

shCTRL shASXL1#1 shASXL1#2

CDK2 #1
CDK2 #2
CDK6 #1
CDK6 #2
CCNE1 #1
CCNE1 #2
cyclin A2 #1
cyclin A2 #2
CDK1 #1
CDK1 #2
cyclin E1 #1
cyclin E1 #2
cyclin A2 #1
cyclin A2 #2

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ASXL1 deficient cells are sensitive to CDK1/CCNA2 targeting

RO3306 (CDK1 inhibitor)

CDK2 inhibitor: K03861
CDK1 inhibitor: RO3306
Summary

Accelerated mutagenesis screening platform discovered a spectrum of novel mutations associated with resistance to CDK4/6 blockade

ASXL1 deficiency leads to robust induction of several cell cycle genes thus bypassing G1 arrest induced by cell cycle inhibitors

Knockdown of cyclin A or CDK1, but not CDK2, completely inhibited growth of ASXL1 deficient cells

CDK1 may present a clinically actionable vulnerability of ASXL1-deficient, CDK4/6i resistant cells
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