Combination of ZEN-3694 with CDK4/6 inhibitors reverses resistance in ER-positive breast cancer

***Olesya A. Kharenko**, Reena G. Patel, Cyrus Calosing, Edward van der Horst*
Zenith Epigenetics, Suite 300, 4820 Richard Road SW, Calgary AB, Canada and Suite 4010, 44 Montgomery St. San Francisco CA, USA

#1750 Abstract #2346

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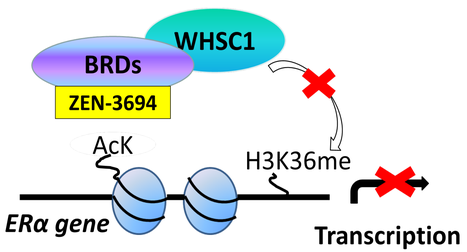
Abstract

CDK4/6 inhibitors have been shown to significantly prolong progression-free survival in patients with advanced hormone receptor (HR)-positive, HER2-negative breast cancer. In spite of the great successes with the currents lines of therapies, patients still acquire resistance over time and, the development of additional novel therapeutic strategies is needed.

The bromodomain and extra-terminal domain (BET) proteins are key epigenetic regulators that interact with acetylated lysine (AcLys) residues of histones or transcription factors, leading to the regulation of gene transcription. BET proteins have also been shown to be directly involved in the transcription of the estrogen receptor (ER) mRNA as well as cell cycle and therefore, BET inhibitors can potentially offer new strategies in the treatment of CDK4/6i- resistant and endocrine resistant ER+ breast cancer.

ZEN-3694 is an orally bioavailable small molecule inhibitor of BET proteins currently being evaluated in Phase 1/2 clinical trials in metastatic castration-resistant prostate cancer (NCT02711956) and triple negative breast cancer (NCT03901469). We have shown previously that ZEN-3694 inhibits proliferation and induces apoptosis in ER+ cell lines. To assess the effects of ZEN-3694 in the combination with CDK4/6 inhibitors as a potential therapy in a CDK4/6i resistant population, we have developed a panel of ER+ cell lines resistant to palbociclib or abemaciclib by continuous stepwise exposure to increasing concentrations of CDK4/6 inhibitors. These cell lines have demonstrated cross-resistance to all three CDK4/6 inhibitors. Here, we describe that the combination treatment of ZEN-3694 with CDK4/6 inhibitors potently inhibits proliferation and induces apoptosis in all CDK4/6i resistant cell lines. The resistance to both palbociclib and abemaciclib was associated with strong upregulation of CDK6 and CCND1protein levels in MCF7 which is consistent with their clinical mechanism of resistance. ZEN-3694 showed efficacy in all CDK4/6i–resistant models leading to downregulation of ER and CDK6 protein levels. Furthermore, our RNAseq data revealed several potential pathways involved in CDK4/6i resistance, including upregulation of cell cycle pathway and decrease of interferon signaling and demonstrated that the mechanisms of resistance to abemaciclib and palbociclib are similar but not identical. Additionally, we elucidated the mechanism of action of ZEN-3694 and in combinations with CDK4/6 inhibitors alleviating these resistance mechanisms. The pathway analysis demonstrated that the combination of ZEN-3694 with CDK4/6 inhibitors led to the strong downregulation of multiple pathways including cell cycle regulation, signaling by Rho family GTPases, STAT3, IL6 and other pathways. We conclude that ZEN-3694 has therapeutic potential in a combination with CDK4/6 inhibitors in ER+ breast cancer patients that developed resistance to endocrine therapies and/or CDK4/6 inhibitors.

Rationale for BET inhibition



Adapted from Feng et al, Cell Res., 2014

- ERα is still recruited to chromatin in drug-resistant breast cancer cells
- BRD3/4 recruit WHSC1 to the ERα promoter resulting in H3K36 methylation and transcription elongation in tamoxifen-resistant cells

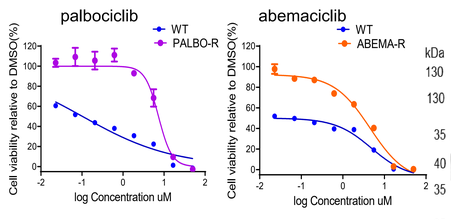
PALBO-R –palbociclib resistant, ABEMA-R-abemaciclib resistant, WT-wild type

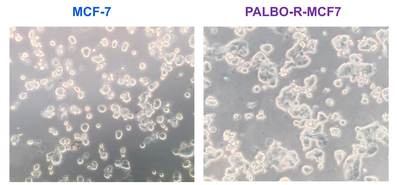
Results

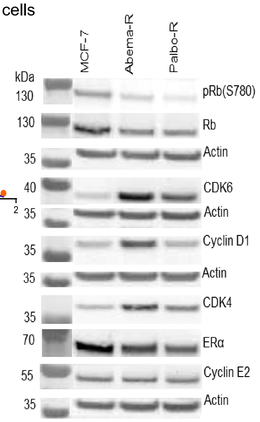
ZEN-3694 inhibits proliferation of ER+ breast cancer cell lines resistant to CDK4/6 inhibitors

Development of CDK4/6i-resistant ER+ cell lines

7 day viability of CDK4/6i-resistant vs. parental MCF7 cells







Resistance to CDK4/6 inhibitors is associated with upregulation of CDK4, CDK6, CCND1 and downregulation of ERα protein levels.

Pathway analysis of RNAseq for PALBO-R and ABEMA-R MCF7

Canonical Pathways (Z-score)*	ABEMA-R DMSO vs. WT DMSO	PALBO-R DMSO vs. WT DMSO
Gα12/13 Signaling	0.89	2.71
Ovarian Cancer Signaling	2.83	2.45
Dendritic Cell Maturation	-1.57	2.00
eNOS Signaling	1.63	1.94
Cyclins and Cell Cycle Regulation	2.67	1.63
Tec Kinase Signaling	1.53	1.39
Endothelin-1 Signaling	-2.14	0.73
Phospholipases	-2.67	0.71
Signaling by Rho Family GTPases	2.69	0.50
Antioxidant Action of Vitamin C	2.98	-0.30
Interferon Signaling	-2.31	-1.63

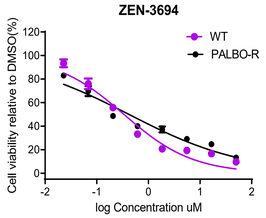
Pathway analysis of RNAseq results demonstrates that the mechanisms of resistance to abemaciclib and palbociclib are similar but not identical.

Resistance to CDK4/6 inhibitors in MCF7 cells is associated with up-regulation of several signaling pathways including cell cycle regulation, estrogen-mediated S-phase entry, and eNOS pathways.

Data were analyzed through the use of IPA® (QIAGEN Inc.)

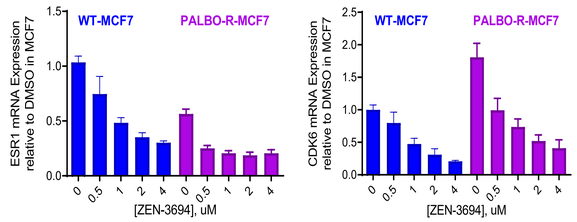
ZEN-3694 inhibits proliferation of both CDK4/6i sensitive and resistant ER+ cell lines

Cell line	7 Day Viability IC50 uM
ZR-75-1	1.7
Palbociclib-R ZR-75-1	1.8
CAMA1	0.3
Abemaciclib-R CAMA1	0.4
MCF7	2.4
Palbociclib-R MCF7	2.6
Abemaciclib-R MCF7	0.3

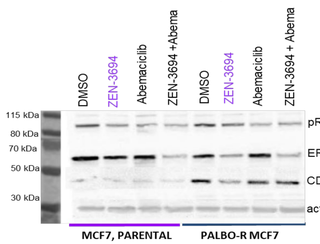


ZEN-3694 downregulates key markers of CDK4/6i resistance

ESR1 and CDK6 mRNA downregulation by ZEN-3694



ERα and CDK6 protein downregulation by ZEN-3694



ZEN-3694 leads to downregulation of ER and CDK6 mRNA and protein levels

Pathway analysis of RNAseq for PALBO-R MCF7 treated with ZEN-3694 alone or in combination with abemaciclib

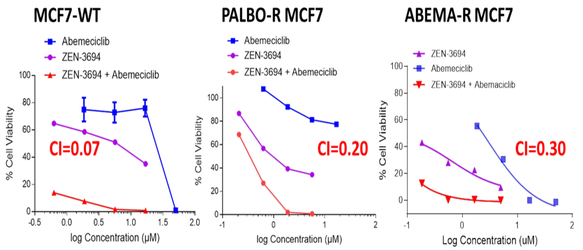
Canonical Pathways (Z-score)*	ZEN -3694	ABEMA+ ZEN	PALBO-R DMSO baseline
Signaling by Rho Family GTPases	-1.8	-2.0	3.6
IL-8 Signaling	-1.9	-1.9	3.5
Phospholipase C Signaling	-2.0	-2.3	2.7
Estrogen-mediated S-phase Entry	-2.9	-3.1	2.5
Gαq Signaling	-2.2	-2.3	2.1
HMGB1 Signaling	-3.2	-3.1	2.0
Cyclins and Cell Cycle Regulation	-1.5	-2.0	2.0
STAT3 Pathway	-2.2	-2.2	1.6
IL-7 Signaling Pathway	-1.6	-2.3	1.4
Estrogen-Dependent Breast Cancer Signaling	-1.8	-2.6	1.1
ILK Signaling	-3.7	-4.0	1.1

(https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis)

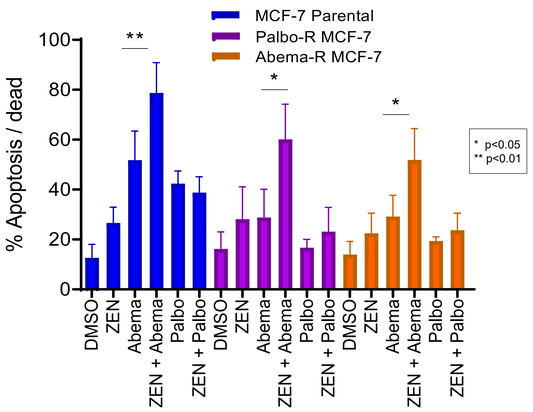
The upregulated pathways contributing to CDK4/6i resistance are significantly downregulated by ZEN-3694 alone or in combination with abemaciclib.

ZEN-3694 and abemaciclib synergistically inhibit proliferation and induce apoptosis

ZEN-3694 synergizes with abemaciclib by inhibiting proliferation in ER+ breast cancer cell lines



ZEN-3694 synergizes with abemaciclib by inducing apoptosis in CDK4/6i-resistant cell lines



% Apoptosis: red – strong effect; green – less effect

Cell line / Treatment	MCF7-WT	Palbo-R MCF7	Abema-R MCF7	CAMA1	ABEMA-R CAMA-1	ZR-75-1	Palbo-R ZR-75-1
DMSO	14.4	16.2	14.0	13.6	15.8	9.4	6.9
ZEN-3694	28.5	34.5	22.5	43.3	31.4	27.2	16.7
Abemaciclib	51.9	28.8	29.2	39.7	18.7	25.9	19.3
ZEN+Abemaciclib	78.7	60.2	51.8	75.0	65.0	89.3	43.6
Palbociclib	42.4	16.7	19.4	33.8	28.0	16.3	9.5
ZEN+Palbociclib	38.8	23.1	23.7	55.5	42.6	38.6	20.0

Conclusions

ZEN-3694 in combination with abemaciclib has potential as a clinical strategy for patients developing resistance to CDK4/6 inhibitors:

1. ZEN-3694 downregulates key players and pathways of CDK4/6 inhibitor resistance
2. ZEN-3694 synergizes with abemaciclib by inhibiting proliferation and inducing apoptosis of ER+ breast cancer cell lines resistant to CDK4/6 inhibitors

*Data were analyzed through the use of IPA® (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis)

Acknowledgements: We thank Laura Tsujikawa for her help with IPA® data upload and analysis