COMBINATION OF ZEN-3694 WITH TALAZOPARIB IS A NOVEL THERAPEUTIC APPROACH IN ER POSITIVE BREAST CANCER RESISTANT TO CDK4/6 INHIBITORS, INDEPENDENT OF BRCA STATUS

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#1287 Abstract #1129

Abstract

Estrogen receptor positive breast cancer (ER+) remains a very prevalent disease with a high mortality rate despite recent successes with new therapies such as CDK4/6, PARP, and PI3K inhibitors. These therapies have shown to significantly prolong progression free survival of metastatic patients; however, resistance develops over time in all of the patients and the need to develop new therapeutic strategies is pressing.

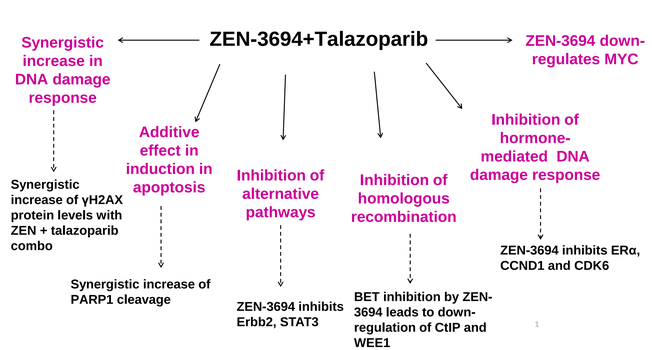
The bromodomain and extra-terminal domain (BET) proteins play an important role in the regulation of transcription through interaction with acetylated lysine (AcLys) residues of histones or transcription factors. Moreover, BET proteins directly regulate transcription of the estrogen receptor (ER) as well as cell cycle pathway genes. ZEN-3694 is an orally bioavailable BET inhibitor currently being evaluated in triple negative breast cancer patients with WT BRCA genotype in combination with PARP inhibitor talazoparib (NCT03901469) and the data to date suggest that the combination is active.

Here, we report a novel combination strategy in ER+ wildtype BRCA1/2 breast cancer resistant cell lines to either palbociclib or abemaciclib combining ZEN-3694 with talazoparib. We demonstrate that this combination has a potent and synergistic effect on inhibition of proliferation of palbociclib and abemaciclib-resistant ER+ cell lines with a WT BRCA phenotype. We also report that ZEN-3694 + talazoparib induced DNA damage response reflected in the synergistic increase of γH2AX levels, resulting in the increase of apoptosis of the CDK4/6i sensitive and resistant models. Moreover, this combination led to the impairment of the homologous recombination pathway through downregulation of CtIP and WEE1 expression.

Furthermore, our data demonstrate that ZEN-3694 targets several mechanisms of endocrine and CDK4/6i resistance including inhibition of estrogen receptor expression and ER signaling, a significant downregulation of several drivers of CDK4/6i resistance such as CDK6, CDK4 and CCND1, as well as inhibition of alternative pathways such as STAT3 and Erbb2. Our RNAseq analysis revealed that ZEN-3694 leads to a strong downregulation of key genes involved in the BRCA1-mediated DNA damage response pathway, as well as double-strand break repair by non-homologous end joining.

We conclude that ZEN-3694 in combination with PARP inhibitors is a novel therapeutic strategy in ER+ breast cancer patients progressing on CDK4/6 inhibitors and a promising approach in HR proficient breast cancer.

Mechanisms of action of ZEN-3694+talazoparib combination in CDK4/6i resistant models of ER+ breast cancer

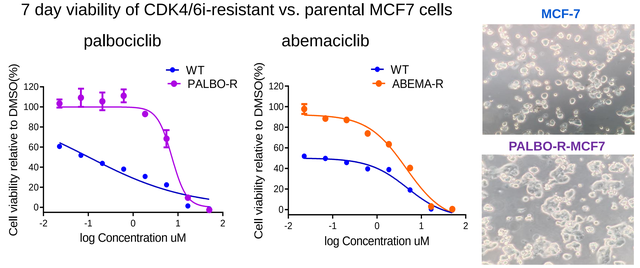


Results

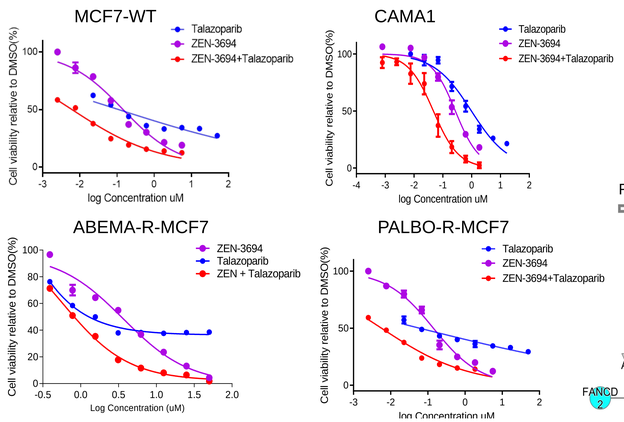
ZEN-3694 inhibits proliferation of ER+ breast cancer cell lines resistant to CDK4/6 inhibitors

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

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

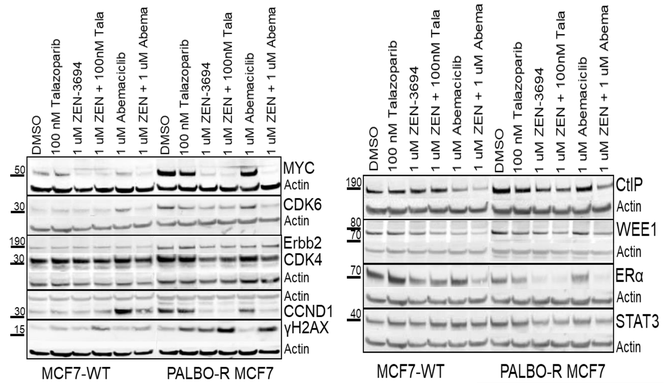


ZEN-3694 in combination with talazoparib inhibits proliferation of both CDK4/6i sensitive and resistant ER+ cell lines



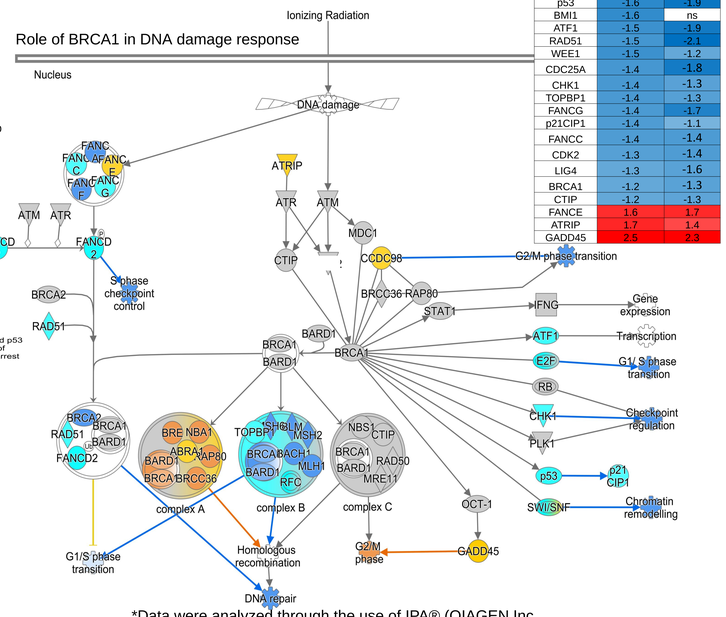
ZEN-3694 and talazoparib induce DNA damage and inhibit homologous recombination

ZEN-3694 inhibits CtIP, WEE1, ERα and induces γH2AX



GENE	Expr Fold Change	
	PALBO-R	ABEMA-R
c-MYC	-3.1	-1.7
FANCD2	-1.9	-2.3
CDKN2A	-1.7	ns
XRCC4	-1.7	-2.1
p53	-1.6	-1.9
BMI1	-1.6	ns
ATF1	-1.5	-1.9
RAD51	-1.5	-2.1
WEE1	-1.5	-1.2
CDC25A	-1.4	-1.8
CHK1	-1.4	-1.3
TOPBP1	-1.4	-1.3
FANCG	-1.4	-1.7
p21CIP1	-1.4	-1.1
FANCC	-1.4	-1.4
CDK2	-1.3	-1.4
LIG4	-1.3	-1.6
BRCA1	-1.2	-1.3
CTIP	-1.2	-1.3
FANCE	1.6	1.7
ATRIP	1.7	1.4
GADD45	2.5	2.3

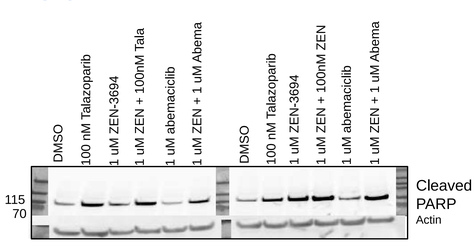
ZEN-3694 treatment affects DNA damage pathways in CDK4/6i- resistant models



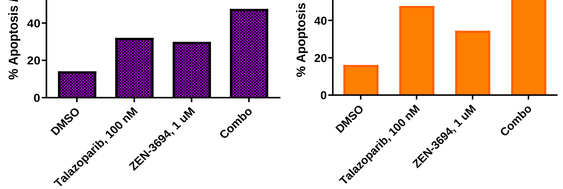
*Data were analyzed through the use of IPA® (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis)

ZEN-3694 and talazoparib synergistically induce apoptosis in CDK4/6i-resistant cells

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



Apoptosis assay at 7 day (Flow cytometry)



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

Cell line /Treatment	MCF7-WT	Palbo-R MCF7
DMSO	14.18	16.18
ZEN-3694, 1 uM	29.96	34.5
Abemaciclib, 1 uM	46	28.8
ZEN + Abemaciclib	74.53	60.15
Palbociclib, 1 uM	42.38	16.7
ZEN + Palbociclib	38.8	23.13
TALA 0.01	21.1	20.6
TALA 0.01+ZEN 1uM	**32.25**	**52.7**
TALA 0.1 uM	32.1	47.75
TALA 0.1+ZEN 1uM	**47.63**	**67.75**

Conclusions

ZEN-3694 in combination with talazoparib 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 talazoporib by inhibiting proliferation and inducing apoptosis of ER+ breast cancer cell lines resistant to CDK4/6 inhibitors.

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