Discovery of CDK4/6 bifunctional degraders for ER+/HER2- breast cancer

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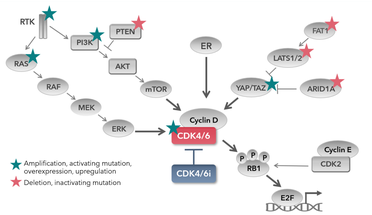
BACKGROUND



CDK4 and CDK6 are kinases which regulate cell cycle progression through the phosphorylation of retinoblastoma protein (RB) which releases the transcription factor E2F, driving the expression of cell cycle promoting genes. CDK4/6 are clinically validated targets in ER+/HER2- breast cancer, with multiple CDK4/6 inhibitors (CDK4/6i) approved for use in this indication, but resistance remains an issue with >20% of patients exhibiting intrinsic resistance and up to 70% of patients developing acquired resistance within 3 years.[1] Many resistance mechanisms converge on the upregulation of CDK6.[2-5] To address this we sought to generate CDK4/6 bifunctional degraders.

METHODS

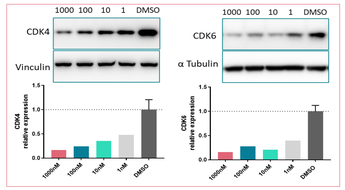
- PRODEGY platform was utilized to develop a series of cereblon (CRBN) mediated CDK4/6 bifunctional degraders including BTX-BD02-04 and development candidate BTX-9341.
- Knockout cell lines were generated by nucleofection of Cas9-gRNA complexes.
- Target degradation was analyzed by immunoblots of protein lysates from cells treated with BTX-9341 for 6 hours or as indicated.
- Phosphorylated RB was analyzed by in cell western after 24 hours of treatment.
- Cell cycle analysis was performed after 24 hours of treatment by flow cytometry following propidium iodide staining.
- Cell proliferation was measured by CellTiter-Glo 2.0 assay (Promega) after a 10-day colony formation assay.
- Vehicle, CDK4/6 inhibitor(s), BTX-BD04 and BTX-9341 were dosed orally in BALB/c nude mice MCF7 xenograft subcutaneous and/or intracranial models.

RESULTS

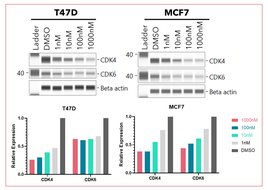
- BTX-9341 is a potent, CRBN and proteasome dependent degrader of CDK4 and CDK6 in multiple breast cancer cell lines. CDK4/6 degradation is rapid and sustained after compound washout.
- BTX-9341 exhibits a favorable safety profile in THLE2 cells and PBMCs with high μM IC_{50} values.
- Kinome profiling indicates BTX-9341 is more selective than the CDK4/6i palbociclib at 100 nM.
- BTX-9341 functionally inhibits cell proliferation more potently than CDK46i in multiple breast cancer cell lines with IC_{50}s in the low nanomolar range. This enhanced efficacy is CRBN dependent.
- BTX-9341 inhibits RB phosphorylation in breast cancer cells with pRB IC_{50}s below 50 nM.
- BTX-9341 induces cell cycle arrest at low nanomolar concentrations in breast cancer cells.
- BTX-9341 retains potency in a CDK4/6i resistant cell line with CDK6 upregulation and a similar CDK4/6 degrader (BTX-BD04) maintains potency in multiple PDX CDK4/6i resistant organoid models.
- BTX-9341 exhibits good tumor exposure when dosed orally, and induces a dose-dependent reduction in CDK4, CDK6, and pRB levels in MCF7 xenograft tumors.
- BTX-9341 exhibit dose dependent tumor growth inhibition and tumor regression at higher doses in an MCF7 xenograft model.
- BTD-BD04 is more efficacious than abemaciclib in an MCF7 intracranial model. BTX-BD04 had greater tumor growth inhibition than abemaciclib and this led to higher survival.

BTX-9341 exhibits rapid, potent and sustained CDK4 and CDK6 degradation that is CRBN and proteasome dependent

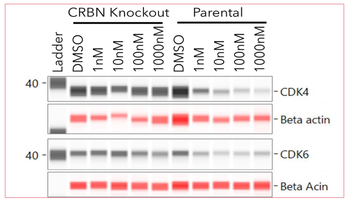
Degradation of CDK4 and CDK6 at 6 hours of treatment in MDA-MB-231 cells



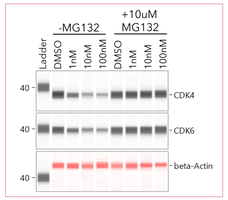
Degradation of CDK4 and CDK6 at 6 hours of treatment in MCF7 and T47D cells



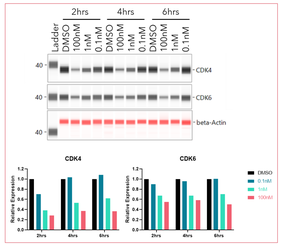
Degradation of CDK4 and CDK6 at 6 hours of treatment in MDA-MB-231 control and CRBN knockout cells



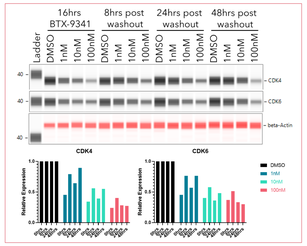
Degradation of CDK4 and CDK6 at 6 hours of treatment in MDA-MB-231 cells with proteasomal inhibitor MG132



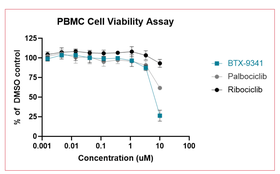
Degradation of CDK4 and CDK6 at 2,4, and 6 hours of treatment in MDA-MB-231 cells



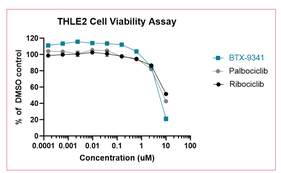
CDK4 and CDK6 protein recovery 8, 24 and 48 hours after washout of 16 hours treatment in MDA-MB-231 cells



BTX-9341 exhibits favorable safety profile in PBMCs and THLE2



PBMCs treated with compound for 24hrs

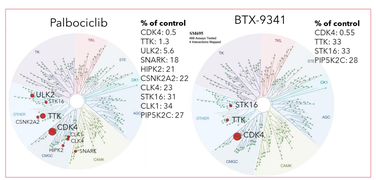


THLE2 cells treated with compound for 72hrs

BTX-9341 exhibits selective binding

Compound	Kinome S(35) score @100nM
BTX-9341	0.01
Palbociclib	0.027

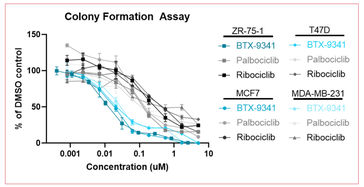
Compound	CDK6 Kd
BTX-9341	31 nM
Palbociclib	5.1 nM



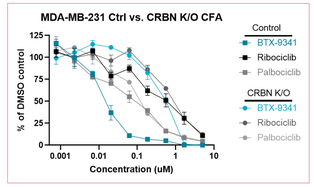
Kinome S(35) score: the fraction of kinases with less than 35% of control at 100nM among the 468 kinases tested with the KINOMEscan™ platform from Eurofins.

BTX-9341 potently inhibits downstream signaling and cell proliferation *in vitro* in HR+/HER2- BC cells, CDK4/6i resistant cells and TNBC

BTX-9341 inhibits cell proliferation

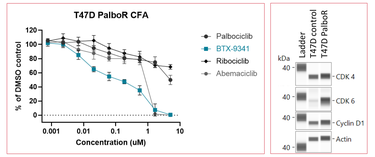


BTX-9341 proliferation inhibition enhancement is CRBN dependent

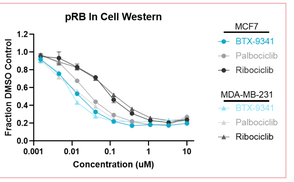


CDK4/6 bifunctional degraders inhibit proliferation in CDK4/6i resistant models

BTX-9341 inhibits cell proliferation in a Palbociclib resistant cell line with CDK6 upregulation

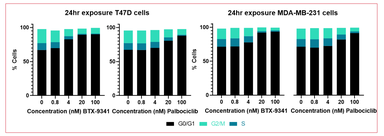


BTX-9341 exhibits cell cycle arrest



BTX-9341 inhibits RB phosphorylation after 24 hours of treatment. Data generated by in cell western for total RB and phosphorylated RB (pSer807/811) taken as a ratio and normalized to DMSO treated control.

BTX-9341induces cell cycle arrest



CDK4/6 bifunctional degrader inhibits CDK4/6i resistant breast cancer patients derived organoids *ex vivo*

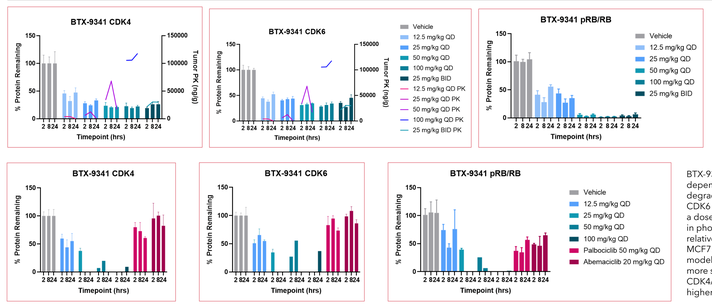
IC_{50} (uM) in models	CTG-3533	CTG-1727	*CTG-3302	CTG-1207	CTG-1365
Type/Treatment Response	ER+/PR+/HER2+ R/R	ER+/PR+/HER2- Unavailable	ER+/PR+/HER2- Refractory	ER+/PR+/HER2- Unavailable	ER+/PR+/HER2- Unavailable
Palbociclib (CDK4/6i)	7.17	>10	>10	>10	5.78
Ribociclib (CDK4/6i)	>10	>10	5.13	6.05	>10
Abemaciclib (CDK4/6i)	1.76	0.53	0.8	1.47	0.53
BTX-BD04	0.95	0.93	0.3	0.14	3

- Patients derived PDX models showed *in vivo* resistance to palbociclib with CRO.
- PDX samples were treated *ex vivo* with compounds for 6 days.

* Refractory to Fulvestrant+palbociclib

BTX-9341 induces tumor regression in MCF7 xenograft model

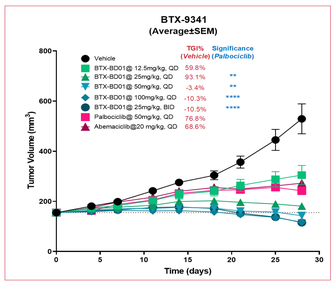
BTX-9341 degrades CDK4, and CDK6 and inhibits pRB in MCF7subcutaneous tumors



BTX-9341 exhibits dose-dependent target degradation of CDK4 and CDK6 which correlates with a dose-dependent decrease in phosphorylated RB relative to total RB that is rapid and sustained.

BTX-9341 exhibits dose-dependent target degradation of CDK4 and CDK6 which correlates with a dose-dependent decrease in phosphorylated RB relative to total RB in and MCF7 xenograft efficacy model. Decreases in pRB more significant than CDK4/6i at dose levels higher than 25mpk.

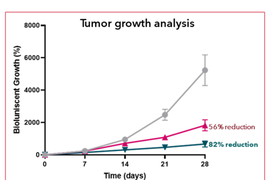
BTX-9341 induces more potent tumor growth inhibition that CDK4/6i in an MCF7 xenograft model



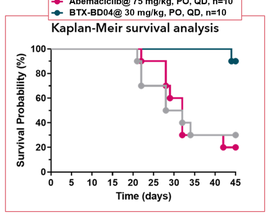
BTX-9341 exhibits dose-dependent tumor growth inhibition with tumor regression at 100 mpk QD and 25 mpk BID for BTX-9341

BTX-BD04 inhibits tumor growth and promotes survival in an intracranial MCF7 xenograft model

Compound	AUC Brain/Plasma ratio	Kp,uu Brain
Palbociclib	0.227	--
Ribociclib	0.216	--
Abemaciclib	0.579	--
BTX-BD04	1.25	--
BTX-9341	1.36	2.17



Comparison	p-value	Hazard Ratio (logrank)
Vehicle vs. Abemaciclib	ns	1.007
Vehicle vs. BTX-BD04	**	8.853
Abemaciclib vs. BTX-BD04	***	13.29



CONCLUSIONS

These preclinical data show that BTX-9341 is more potent in *in vitro* and *in vivo* compared to CDK4/6 inhibitors and induced tumor regression at some doses in an MCF7 xenograft model. BTX-9341 exhibited efficacy in a Palbociclib-resistant cell line and a CDK4/6 degrader showed efficacy in several CDK4/6i-resistant PDX organoid models indicating that a degrader approach may work well in patients who are resistant to CDK4/6 inhibitors. CDK4/6 degraders had good exposure in the brain in mice, and POC degrader BTX-BD04 showed enhanced tumor growth inhibition and increased survival in an MCF7 intracranial model compared to brain penetrant CDK4/6i abemaciclib, indicating that a degrader could have enhanced efficacy in patients with brain metastases. BTX-9341 displayed rapid, potent and sustained degradation of its targets, which led to excellent potency *in vitro* including in resistant models. BTX-9341 also exhibited potent *in vivo* degradation and tumor growth inhibition. Considering these properties, we have recently progressed BTX-9341 into IND enabling studies.

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