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

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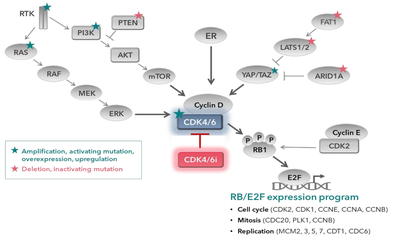
BACKGROUND



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

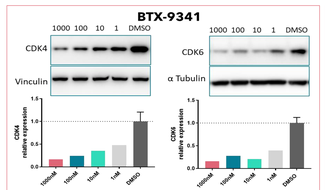
- PRODEGY platform was utilized to develop a series of cereblon (CRBN) mediated CDK4/6 bifunctional degraders including BTX-BD02-04 and development candidate BTX-9341.
- Knockout cell lines were generated by nucleofection of Cas9-gRNA complexes.
- Target degradation was analyzed by immunoblots of protein lysates from cells treated with CDK4/6 bifunctional degraders for 6 hours.
- 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

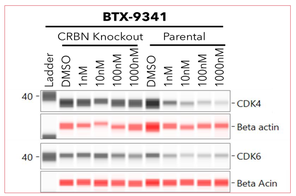
- BTX-9341 and BTX-BD02-BD04 are potent, CRBN dependent degraders of CDK4 and CDK6 in multiple breast cancer cell lines.
- Kinome profiling indicates that all CDK4/6 bifunctional degraders are more selective than the CDK4/6i palbociclib at 100nM.
- CDK4/6 degraders inhibit RB phosphorylation in breast cancer cells with pRB IC_{50}s below 50nM.
- CDK4/6 degraders functionally inhibit 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.
- CDK4/6 degraders are functional in a CDK4/6i resistant cell line with CDK6 upregulation and in multiple PDX CDK4/6i resistant organoid models.
- BTX-BD04 and BTX-9341 induce cell cycle arrest at low nanomolar concentrations in MDA-MB-231 cells.
- BTX-BD04 and BTX-9341 exhibit good tumor exposure when dosed orally, and induce a dose-dependent reduction in CDK4, CDK6, and pRB levels in MCF7 xenograft tumors.
- BTX-BD04 and 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.

CDK4/6 bifunctional degraders potently degrade CDK4/6 in a Cereblon-dependent manner

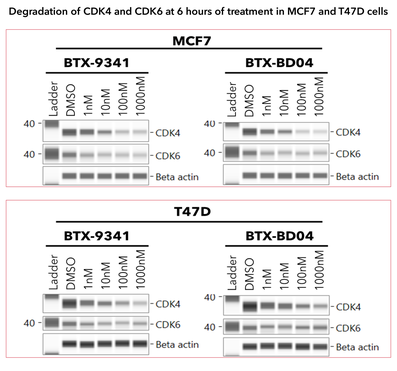
Degradation of CDK4 and CDK6 at 6 hours of treatment in MDA-MB-231 cells



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

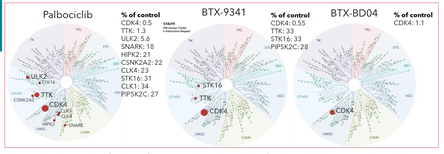


Degradation of CDK4 and CDK6 at 6 hours of treatment in MCF7 and T47D cells



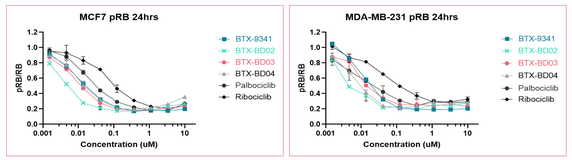
CDK4/6 bifunctional degraders exhibit selective binding

	Kinome S(35) score @100nM
BTX-9341	0.01
BTX-BD02	0.012
BTX-BD03	0.01
BTX-BD04	0.002
Palbociclib	0.027



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.

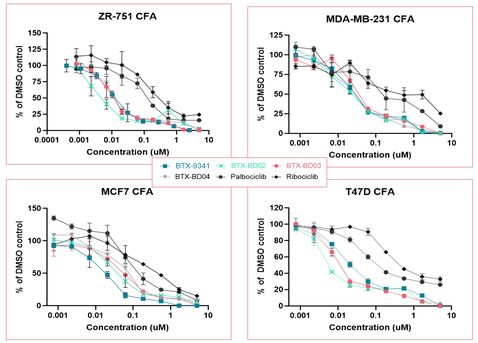
CDK4/6 bifunctional degraders potently inhibit pRB



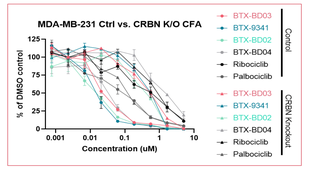
CDK4/6 bifunctional degraders inhibit RB phosphorylation after 24 hours of treatment. Data generated by in cell western for total RB and phosphorylated RB (pSer807/811) taken as a ratio and normalized to DMSO treated control.

CDK4/6 bifunctional degraders potently inhibit cell proliferation *in vitro* in HR+/HER2- BC cells, CDK4/6i resistant cells and TNBC

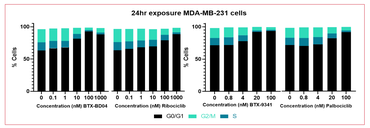
CDK4/6 bifunctional degraders inhibit cell proliferation



CDK4/6 bifunctional degraders proliferation inhibition enhancement is CRBN dependent

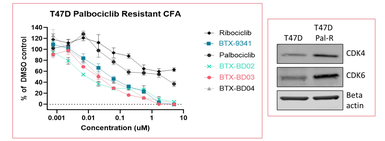


CDK4/6 bifunctional degraders induce cell cycle arrest



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

CDK4/6 bifunctional degraders inhibit cell proliferation in a Palbociclib resistant cell line with CDK6 upregulation



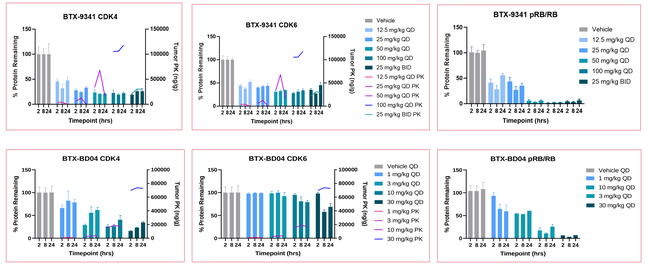
CDK4/6 bifunctional degrader inhibits CDK4/6i resistant breast cancer patients derived organoids *ex vivo*

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	340	79.79	>10	5.78
Ribociclib (CDK4/6i)	>10	11.02	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

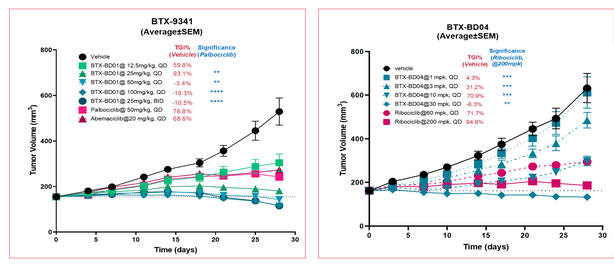
CDK4/6 bifunctional degraders induce tumor regression in MCF7 xenograft model

BTX-9341 and BTX-BD04 degrade CDK4, and CDK6 and inhibit pRB in MCF7subcutaneous tumors



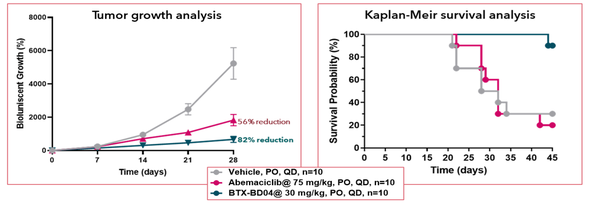
BTX-9341 and BTX-BD-04 exhibit dose-dependent target degradation of CDK4 and CDK6 which correlates with a dose-dependent decrease in phosphorylated RB relative to total RB that is rapid and sustained.

BTX-9341 and BTX-BD04 are highly efficacious in MCF7 xenograft model compared to CDK4/6i and exhibit tumor regression at some dose levels



BTX-9341 and BTX-BD-04 exhibit dose-dependent tumor growth inhibition, with tumor regression at 100mpk QD and 25mpk BID for BTX-9341 and 30mpk QD for BTX-BD04

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



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 CDK4/6 degraders are more potent in *in vitro* and *in vivo* compared to CDK4/6 inhibitors with BTX-BD04 and BTX-9341 exhibiting tumor regression at some doses. These degraders also show efficacy in cell lines and PDX organoid models which are resistant to CDK4/6i, indicating that a degrader approach may work well in patients who are resistant to CDK4/6 inhibitors. POC degrader BTX-BD04 also showed enhanced tumor growth inhibition and increased survival in an intracranial model compared to brain penetrant CDK4/6i abemaciclib indicating that a degrader could have enhanced efficacy in patients with brain metastases. Considering BTX-9341 showed excellent potency *in vitro* and exhibited potent *in vivo* degradation and tumor growth inhibition, we have recently progressed BTX-9341 into IND enabling studies.

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