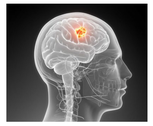

BTX-9341, a Bifunctional Degrader of CDK4 and CDK6 for Glioblastoma Multiforme

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Abstract # 5702

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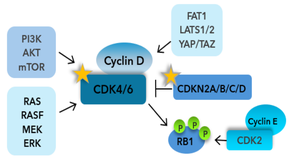
BACKGROUND



Glioblastoma multiforme (GBM) is the most common and aggressive malignancy making up 77% -81% of all malignant central nervous system tumors[1,2]. Over 12 thousand new patients are diagnosed every year in U.S. The median overall survival (OS) of GBM patients is only 15 months with only less than 5% of patients surviving 5 years[2,3].

GBM has a high frequency of dysregulation of the CDKN2A - cyclin D - CDK4 / CDK6 signaling node[4,5]. CDK4/6 inhibitors have been shown to synergize with TMZ in GBM models[5]. Efforts have also made to develop brain penetrant CDK4/6 inhibitors[6]. BTX-9341 is a Cereblon (CRBN) mediated CDK4/6 bifunctional degrader that we have developed for HR+/HER2- breast cancer. This degrader shows good exposure in brain tissues with a high brain to plasma ratio. Given the exposure in the brain, we explored the *in-vitro* and *in-vivo* efficacy of BTX-9341 in GBM cell line and xenograft models.

METHODS

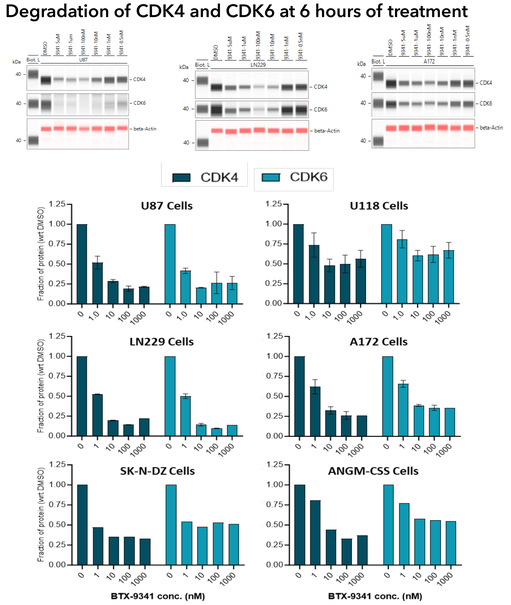
- PRODEGY platform was utilized to develop a series of Cereblon (CRBN) mediated CDK4/6 bifunctional degraders including development candidate BTX-9341.
- Knockout cell lines were generated by nucleofection of Cas9-gRNA complexes.
- Target degradation was analyzed by immunoblots of protein lysates from cells treated with BTX-9341 for 6 hours or as indicated.
- Phosphorylated Rb was analyzed by in cell western after 24 hours of treatment or by immunoblot where indicated.
- 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), and BTX-9341 were dosed orally in BALB/c nude mice xenograft subcutaneous or intracranial models.

RESULTS

- BTX-9341 is a potent, CRBN dependent degrader of CDK4 and CDK6 in multiple GBM cell lines.
- Kinome profiling indicates BTX-9341 is more selective than the CDK4/6i palbociclib at 100 nM, and proteomics indicates minimal off-target degradation.
- BTX-9341 is blood-brain barrier permeable and shows sustained intracranial exposure for more than 12 hours.
- BTX-9341 functionally inhibits cell proliferation in multiple GBM cell lines with IC_{50}s in the nanomolar range.
- BTX-9341 inhibits Rb phosphorylation in different GBM cell lines with pRb IC_{50}s below 10 nM.
- BTX-9341 induces cell cycle arrest at low nanomolar concentrations in GBM cell lines.
- BTX-9341 exhibits dose dependent tumor growth inhibition in U-87 intracranial xenograft model (MGMT-negative).
- BTX-9341 shows superior survival as compared to abemaciclib and Palbociclib in U-87 xenograft model (MGMT-negative).
- BTX-9341 inhibited tumor growth in U-118 xenograft model (MGMT-positive).

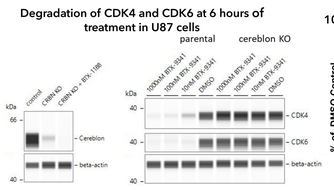
BTX-9341 exhibits potent CDK4 and CDK6 degradation

Degradation of CDK4 and CDK6 at 6 hours of treatment

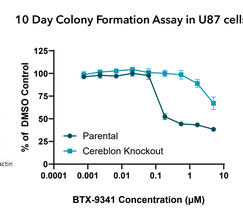


BTX-9341 mediated CDK4 and CDK6 degradation is CRBN dependent

Degradation of CDK4 and CDK6 at 6 hours of treatment in U87 cells



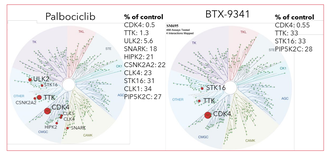
10 Day Colony Formation Assay in U87 cells



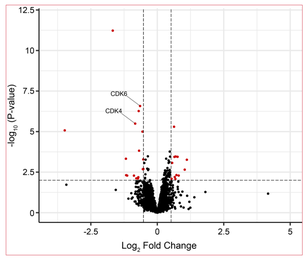
BTX-9341 exhibits selective binding and degradation

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.

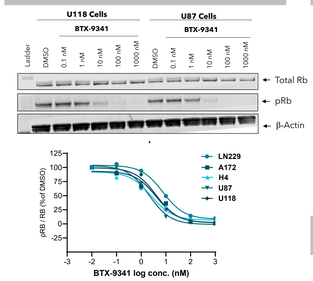


TMT mass spectrometry performed on samples generated in triplicate from MDA-MB-231 triple negative breast cancer cells treated with 100 nM of BTX-9341 or DMSO for 6 hours.

Volcano plot indicating the fold change comparing BTX-9341 treated and DMSO treated cells. CDK4 and CDK6 are labeled as some of the few proteins which were significantly altered after BTX-9341 treatment.

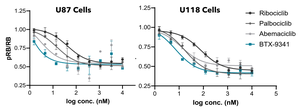
BTX-9341 potently inhibits downstream signaling and cell proliferation *in vitro* in Glioblastoma cells

BTX-9341 inhibits pRb (24 hr)



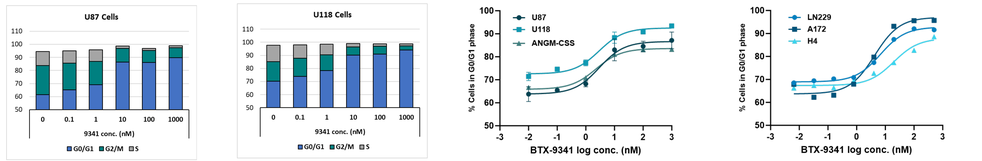
Data generated by western blot for total Rb and phosphorylated Rb (pSer807/811) taken as a ratio and normalized to DMSO treated control.

In-cell Western Assay (24 hr)



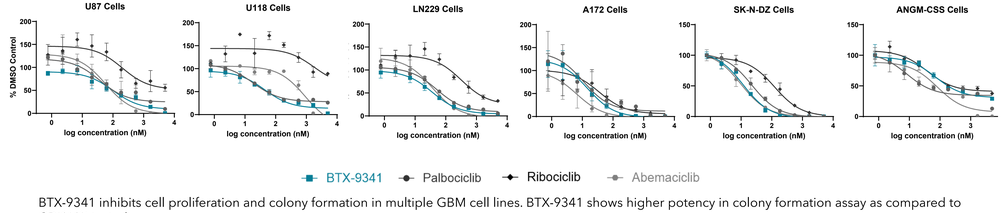
Data generated by in cell western for total Rb and phosphorylated Rb (pSer807/811) taken as a ratio and normalized to DMSO treated control.

BTX-9341 induces cell cycle arrest



BTX-9341 inhibits cell cycle progression after 24 hours of treatment in multiple GBM cell lines tested. Data generated by flow cytometry following propidium iodide staining and represented as % cells in different phases of cell cycle.

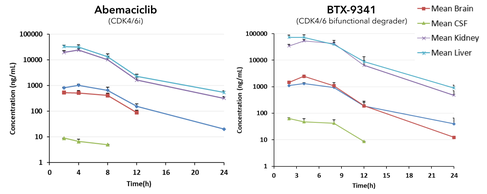
BTX-9341 inhibits cell proliferation in GBM cells



BTX-9341 inhibits cell proliferation and colony formation in multiple GBM cell lines. BTX-9341 shows higher potency in colony formation assay as compared to CDK4/6i tested.

BTX-9341 is blood-brain barrier penetrant

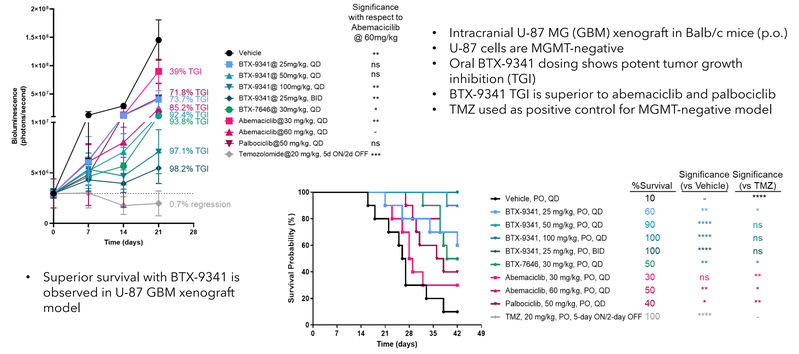
BTX-9341 PK in mouse / 30 mg/kg (p.o.)



	Brain:Plasma AUC ratio
BTX-9341	1.36
Abemaciclib	0.523
Ribociclib	0.216
Palbociclib	0.227

- BTX-9341 is Blood-brain barrier permeable
- Sustained exposure in the brain for > 12 hours
- BBB permeability makes BTX-9341 an ideal candidate for GBM

BTX-9341 shows tumor growth inhibition and a survival benefit in intracranial GBM xenograft model

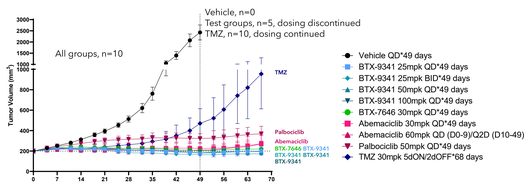


	%Survival	Significance (vs Vehicle)	Significance (vs TMZ)
Vehicle, PO, QD	10	-	****
BTX-9341, 25 mg/kg, PO, QD	60	**	*
BTX-9341, 50 mg/kg, PO, QD	90	****	ns
BTX-9341, 100 mg/kg, PO, QD	100	****	ns
BTX-9341, 25 mg/kg, PO, BID	100	****	ns
BTX-7646, 30 mg/kg, PO, QD	50	**	*
Abemaciclib, 30 mg/kg, PO, QD	30	ns	**
Abemaciclib, 60 mg/kg, PO, QD	50	**	*
Palbociclib, 50 mg/kg, PO, QD	40	*	**
TMZ, 20 mg/kg, PO, 5-day ON/2-day OFF	100	****	-

- Intracranial U-87 MG (GBM) xenograft in Balb/c mice (p.o.)
- U-87 cells are MGMT-negative
- Oral BTX-9341 dosing shows potent tumor growth inhibition (TGI)
- BTX-9341 TGI is superior to abemaciclib and palbociclib
- TMZ used as positive control for MGMT-negative model

- Superior survival with BTX-9341 is observed in U-87 GBM xenograft model

BTX-9341 shows tumor growth inhibition in U-118 GBM sub-cutaneous xenograft model



- U-118 MG (GBM) sub-cutaneoous xenograft in Balb/c mice (p.o.)
- U-118 cells are MGMT-positive
- Potent tumor growth inhibition with oral dosing of BTX-9341
- BTX-9341 TGI comparable to abemaciclib and superior to palbociclib and Temozolomide (TMZ, chemotherapy)

CONCLUSIONS

These preclinical data show that BTX-9341 promotes CRBN dependent degradation of CDK4 and CDK6 in multiple Glioblastoma (GBM) cell lines. This degradation is specific, with limited off-target binding and degradation. This degradation leads to a more potent phenotype in *in vitro* compared to CDK4/6i with a deeper and more sustained inhibition of cell cycle progression and phospho-Rb. BTX-9341 exhibited more potent tumor growth inhibition in multiple GBM xenograft models compared to CDK4/6i. BTX-9341 exhibited efficacy in a both MGMT-negative and MGMT-positive cell lines indicating that a degrader approach may work well in larger GBM patient groups. Considering these properties, we are testing BTX-9341 as a drug candidate for GBM patients.

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