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

Hannah Majekis, Akinori Okano, Angela Pasis, Casey Carlson, Qiao Liu, Arvind Shyka, Shelin Huang, Aparajita Hoskote Chourasia and Leah Fung

Biotheryx, Inc., San Diego, CA

Abstract ID: 1553

DISCOVERY OF CDK4/6 BIFUNCTIONAL DEGRADERS FOR ER+/HER2- AND TRIPLE NEGATIVE BREAST CANCER

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.

METHODS

- PreOCEDF platform was utilized to develop a series of cereblon (CRBN) meditated CDK4/6 bifunctional degraders including BTX-BD02-04 and development candidate BTX-9341.
- Knockdown cell lines were generated by nucleofection of Cas9-gRNA complexes.
- Target degradation was analyzed by immunoblot 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 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 100μM.
- CDK4/6 degraders inhibit RB phosphorylation in breast cancer cells with pRBCPs below 50%.
- CDK4/6 degraders functionally inhibit cell proliferation more potently than CDK4/6i in multiple breast cancer cell lines with pRBCPs in the low nanomolar range. This enhanced efficacy is CRBN dependent.
- CDK4/6 degraders are functional in a CDK4/6 resistant cell line with CDK6 upregulation and in multiple PDx CDK4/6 resistant organoids models.
- BTX-BD04 and BTX-9341 induce cell cycle arrest at low nanomolar concentrations in MDA-MB-231 cells.
- BTX-BD04 and BTX-9341 inhibit good tumor exposure when dosed orally, and induce a dose-dependent reduction in CDK4, CDK6, and pRBCPs 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.
- BTX-BD04 is more efficacious than abemaciclib in an MCF7 intracranial model. BTX-9340 had greater tumor growth inhibition than abemaciclib and this led to higher survival.

CONCLUSIONS

These preclinical data show that CDK4/6 degraders are more potent in vitro and in vivo compared to CDK4/6 inhibitors with BTX-BD04 and BTX-9341 showing 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 CDK4/6i inhibitors. POC degrader BTX-BD04 also showed enhanced tumor growth inhibition and increased survival in an intracranial model compared to brain penetrant CDK4/6 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.

REFERENCES


http://www.biotheryx.com