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

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Abstract: 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 regulating 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. Many resistance mechanisms converge on the upregulation of CDK6. To address this we sought to generate CDK4/6 bifunctional degraders.

Biology of CDK4/6/BTX: CDK4 and CDK6 are key drivers of cell cycle progression and cellular proliferation. CDK4/6 inhibitors (CDK4/6i) have been extensively studied in ER+/HER2- breast cancer in the clinic. CDK4/6i exhibit potent anti-proliferative effects in vitro and in vivo.

METHODS
- PRODEEP platform was utilized to develop a series of cecolixins (CRBNi) as CDK4/6 bifunctional degraders including BTX-BD04
- Knockout cell lines were generated by nucleofection of Cas9/gRNA complexes. Target degradation was analyzed by immunoblot 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 IC50 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 CDK4/6 in multiple breast cancer cell lines with IC50 in the low nanomolar range. This enhanced efficacy is CRBN dependent.
- BTX-9341 inhibits RB phosphorylation in breast cancer cells with pRB IC50 below 50 nM.
- BTX-9341 induces cell cycle arrest at low nanomolar concentrations in breast cancer cell lines.
- BTX-9341 retains potency in a CDK4/6 resistant cell line with CDK6 upregulation and a similar CDK4/6 degrader (BTX-BD04) maintains activity in multiple PDX CDK4/6 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.
- BTX-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.

CONCLUSIONS
- These preclinical data show that BTX-9341 is more potent 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 CDK4/6-dependent PDX organoid models indicating that a degrader approach may work well in patients resistant to CDK4/6 inhibitors. CDK4/6 degraders had good exposure in the brain in mice, and PDX degrader BTX-BD04 showed enhanced tumor growth inhibition and increased survival in an MCF7 intracranial model compared to brain penetrated 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 resistant models. BTX-9341 also exhibited potent in vivo tumor growth inhibition and tumor regression. Considering these properties, we have recently progressed BTX-9341 into IND enabling studies.

REFERENCES

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