Pharmacological eIF4E inhibition drives anti-tumor activity in ER+ breast cancer

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ABSTRACT
Elevated levels of eukaryotic initiation factor 4E (eIF4E) are found in a broad range of cancers, including breast, and have been associated with aggressive, drug resistant tumors. Furthermore, elevated eIF4E activity is sufficient to cause transformation in various cancers, while inhibition suppresses tumor growth.

eIF4E, the major regulator and rate limiting factor of protein synthesis, is the downstream integrator of several important oncogenic signaling pathways (PDG/akt/MTOR, Ras/Raf/MEK, and Myc). Activation of eIF4E results in increased protein levels of key proliferation and metabolism proteins such as Cyclin D1 (CCND1) and Ornithine Decarboxylase 1 (ODC1), as well as an overall increase in cellular synthesis machinery, causing cellular growth and proliferation.

Here, we describe the development and characterization of novel, potent, and selective eIF4E inhibitors. Compounds from this chemical series have nanomolar activity in multiple biochemical and biophysical m7G cap-competition assays as well as potent inhibition of translation in cellular and biosynthetic assays. In cells, these compounds rapidly and reversibly decrease protein levels of several oncogenes, including CCND1, leading to a G1 cell cycle arrest. We demonstrate that eIF4E inhibitors cause growth inhibition in a variety of breast cancer cell lines, including ER+ breast cancer. These inhibitors also inhibit growth of breast cancer lines with acquired resistance to palbociclib with the same potency as the parental cell lines. Additionally, eIF4E inhibition shows an increased sensitivity in resistant cell lines when combined with standard of care (SOI), including palbociclib. Select analogs from the series demonstrate favorable ADMET/properties with good oral bioavailability and low safety risk. Lastly, these compounds have demonstrated near complete tumor growth inhibition in non-breast cancer in vivo models. Ongoing experiments will address in vivo efficacy in ER+ breast cancer in both monotherapy and in combination with SOI.

RESULTS

Figure 2. RBX eIF4E inhibitors repress translation of a subset of mRNAs enriched for growth associated genes. A) Cells incorporate O-propargyl-puromycin (OPP) into actively translating polysomes, which can be fluoroscically labeled by click chemistry allowing for quantitative determination of total new protein synthesis. B) OPP assay in MCF-7 (ER+ BC) and HCC829 (TNBC) cells incubated with OPP for 30 min. Cells were fixed, clicked and detected in-cell western. Cycloheximide (CHX) control and eIF4Aii inhibit all nascent translation while eIF4E repression translates -70% of total protein. C) COL2A1 cells were treated for 1 hr with 4Ei02, both total RNA and ribosome associated RNA (Ribo-seq) were isolated and quantified by RNA-seq. Ribo-seq changes were normalized to total RNA identifying translationally regulated genes. (D) Functional enrichment analyses of translationally down-regulated genes yield multiple growth-associated categories.

Figure 3. RBX eIF4E inhibitors inhibit cell growth through selective binding to eIF4E. A) Mutants that disrupt binding of RBX eIF4E inhibitors but maintain binding to m7GTP were generated. TS-FRET assay with wild type or binding pocket mutant (SSD) eIF4E binding to m7GTP. Mutant does not disrupt binding to m7GTP. B) Cell-tier-02 (CTG) assay with 4Ei02.

Figure 4. Inhibition with RBX eIF4E inhibitors is reversible. A) HCC829 cells treated with compound for 24 hours, for samples plated with vehicle media was then replaced with media containing DMSO and collected at indicated time points to evaluate recovery of CCND1 and ODC1. Protein expression recovers after removal of RBX eIF4E inhibitors. B) Transient over-expression of eIF4E mutants pEIF4E and pEIF5 'max' was performed. Luciferase data demonstrates a 10-fold increase in luciferase activity in pEIF4E transfected cells compared to control.

Figure 5. RBX eIF4E inhibitors lead to cell cycle arrest in ER+ breast cancer. A) CTG assay for monitoring cell proliferation. B) Western blot of MCF-7 cells treated for 6 hr with 4Ei02. C) Resistant cell line MCF-7 with known G1 arrest induced by 4Ei02.

CONCLUSIONS
- RBX eIF4E inhibitors are novel, potent, selective inhibitors of cap-dependent translation initiation leading to growth arrest in many cancer types including breast cancer.
- Daily oral dosing of RBX eIF4E inhibitors shows anti-tumor efficacy in vivo with no signs of overt toxicity.
- Targeting breast cancer resistant to CDK4/6 inhibitors with an RBX eIF4E inhibitor has the potential to mitigate drug resistance and restore sensitivity to standard of care (SOIC) in 2L setting.
- eIF4E inhibition potentiates cancer cell sensitivity to CDK4/6 inhibitors supporting the combination of RBX eIF4E inhibitor and CDK4/6 inhibitors to improve SOIC response durability in ER+ breast cancer.
- IND-enabling studies are planned.