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

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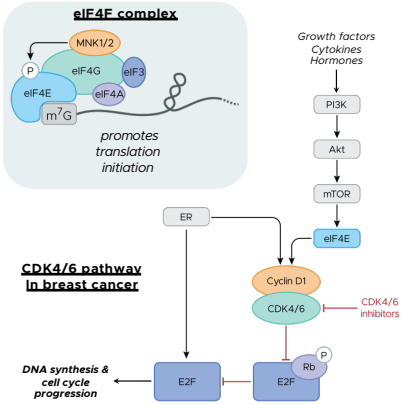
ABSTRACT

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 main regulator and rate limiting factor of protein synthesis, is the downstream integrator of several important oncogenic signaling pathways (PI3K/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 biochemical 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 (SOC), including palbociclib. Select analogs from the series demonstrate favorable ADMET/PK 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 SOC.

INTRODUCTION



- eIF4E binds the 5′ cap of mRNA and promotes translation initiation
- eIF4E is the rate-limiting factor for regulating protein synthesis
- eIF4E expression is elevated in many cancers, including breast cancer, and high levels are associated with poor prognosis
- Forced overexpression has been shown to drive tumors in animal models
- Genetic inhibition of eIF4E decreases viability in tumors but not in normal cells
- eIF4E inhibition complements CDK4/6 inhibitors such as palbociclib

RESULTS

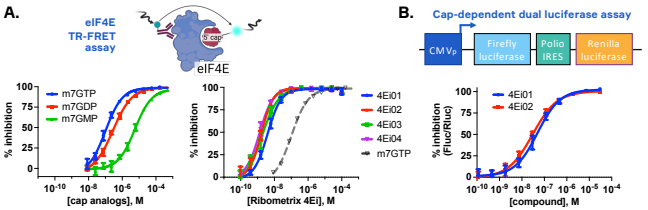
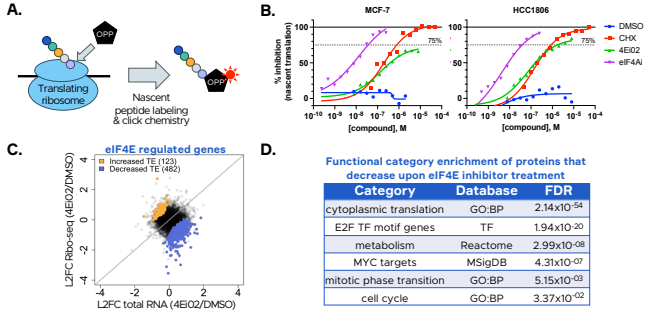


Figure 1. Development of novel potent Ribometrix (RBX) eIF4E inhibitors (eIF4Ei) that selectively inhibit cap-dependent translation. Multiple novel eIF4E inhibitors developed and biochemically validated at Ribometrix (RBX). **A)** TR-FRET assay using recombinant HIS-tagged human eIF4E and Europium-conjugated antibody incubated with fluorescently labeled 5′ mRNA cap analog. Titration of competitive cap analogs shows tight binding to 5′ mRNA cap (left), while RBX eIF4E inhibitors (right) show enhanced competition. **B)** HEK293-FlpIn cells stably transfected with bicistronic dual luciferase reporter and treated with compound for 24 hours. Ratio of cap-dependent (Fluc) to cap-independent (Rluc) translation demonstrates potent and selective inhibition of cap-dependent translation.

RESULTS



Category	Database	FDR
cytoplasmic translation	GO:BP	2.14x10^{-54}
E2F TF motif genes	TF	1.94x10^{-20}
metabolism	Reactome	2.99x10^{-08}
MYC targets	MSigDB	4.31x10^{-07}
mitotic phase transition	GO:BP	5.15x10^{-03}
cell cycle	GO:BP	3.37x10^{-02}

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 peptide chains which can be fluorescently labeled by click chemistry allowing for quantitative determination of total new protein synthesis. **B)** OPP assay in MCF-7 (ER+ BC) and HCC1806 (TNBC) cells incubated with OPP for 30 minutes. Cells were fixed, clicked and detected by in-cell western. Cycloheximide (CHX) control and eIF4Ai inhibit all nascent translation while eIF4Ei represses translation of ~75% of total protein. **C)** COLO 205 cells were treated for 1 hour 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 (TE). **D)** Functional enrichment analyses of translationally down-regulated genes yield multiple growth-associated categories.

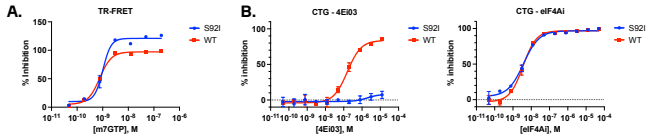


Figure 3. RBX eIF4E inhibitors inhibit cell growth through selective binding to eIF4E. eIF4E mutants that disrupt binding of RBX eIF4E inhibitors but maintain binding to m7GTP were generated **A)** TR-FRET assay with wild type or binding pocket mutant (S92I) eIF4E binding to m7GTP. Mutant does not disrupt binding to m7GTP. **B)** CellTiter-Glo (CTG) assay with eIF4A

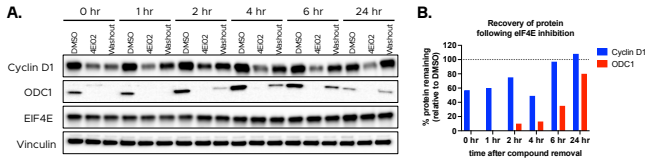


Figure 4. Inhibition with RBX eIF4E inhibitors is reversible. A) HCC1806 cells treated with compound for 24 hours, for samples labeled 'washout', media was then replaced with media containing DMSO only and collected at indicated time points to evaluate recovery of CCND1 and ODC1. Protein expression recovers after removal of eIF4E inhibitor. **B)** Densitometry quantification of Western blots in panel A. Cells start to show earliest signs of recovery by 2 hr and nearly complete recovery by 24 hr.

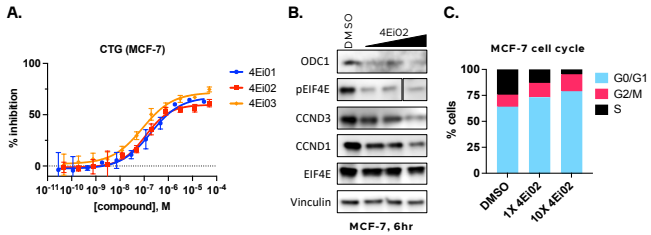


Figure 5. RBX eIF4E inhibitors lead to cell cycle arrest in ER+ breast cancer. A) CTG assay for MCF-7 cells treated for 72 hr with compound. RBX eIF4E inhibitors decrease viable cells in a concentration responsive manner. **B)** Western blot of MCF-7 cells treated for 6 hr with 4Ei02. Concentration dependent selective decrease of eIF4E target genes (ODC1, CCND1, and CCND3). **C)** Cells treated with two concentrations of 4Ei02 analyzed by DNA content flow cytometry. 4Ei02 treated MCF-7 cells show concentration dependent increase in G0/G1 population.

RESULTS

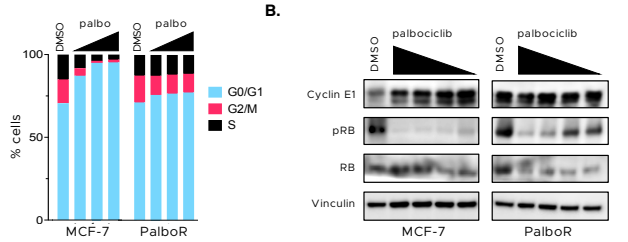


Figure 6. Generation and validation of MCF-7 cells with acquired resistance to CDK4/6 inhibitor palbociclib. MCF-7 cells were selected for resistance to palbociclib by culturing in the presence of 1 μM palbociclib. **A)** DNA content flow cytometry of parental MCF-7 (MCF-7) and Palbo resistant MCF-7 (PalboR) cells with increasing concentrations of palbociclib show that palbociclib induces G1 arrest in MCF-7 but is severely reduced in palbociclib resistant cells. **B)** Western blots of MCF-7 and PalboR cells validating that the CDK4/6 pathway is refractory to palbociclib in PalboR cells.

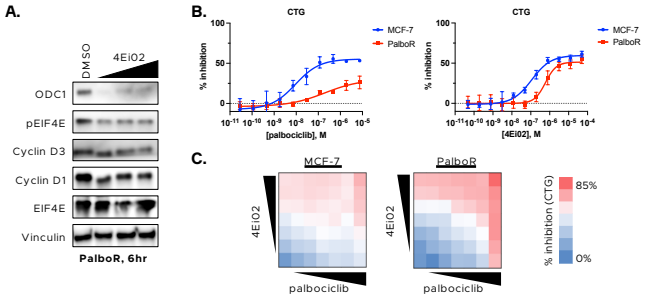


Figure 7. Palbociclib resistant cells are sensitive to eIF4E inhibition and RBX eIF4E inhibitors sensitize resistant cells to palbociclib. A) Western blot of PalboR cells treated for 6hr with 4Ei02. eIF4E pathway markers are similarly responsive in PalboR cells as parental MCF-7 (Figure 5B) **B)** CTG assay showing sensitivities of MCF-7 and PalboR cells to palbociclib (left) and 4Ei02 (right). PalboR cells have reduced sensitivity to eIF4E inhibition but are many times more responsive to eIF4E inhibition than to palbociclib. **C)** Combination concentration response matrices of increasing concentrations of RBX eIF4E inhibitor and palbociclib in CTG assay. Heat map represents max inhibition. Slight additive effect of palbociclib and 4Ei02 observed in MCF-7, effect much more pronounced in PalboR cells. 4Ei02 re-sensitizes resistant cells to palbociclib.

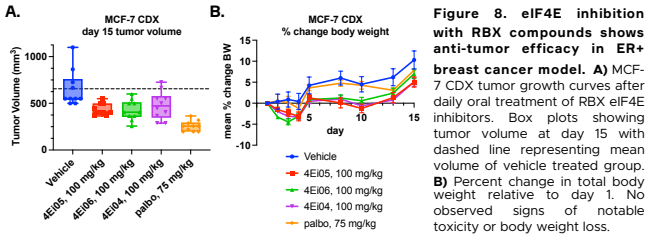


Figure 8. eIF4E inhibition with RBX compounds shows anti-tumor efficacy in ER+ breast cancer model. A) MCF-7 CDX tumor growth curves after daily oral treatment of RBX eIF4E inhibitors. Box plots showing tumor volume at day 15 with dashed line representing mean volume of vehicle treated group. **B)** Percent change in total body weight relative to day 1. No observed signs of notable toxicity or body weight loss.

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 (SoC) 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 SoC response durability in ER+ breast cancer**
- **IND-enabling studies are planned**

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