EIF4E inhibition drives anti-tumor activity in BRAF^{V600E} melanoma

Matthew B Friedersdorf, Devon R Blake, Abha Bais, Sarah EG Thompson, Manjunath Ramarao, Jessica A Sorrentino
Ribometrix Inc, Durham, NC

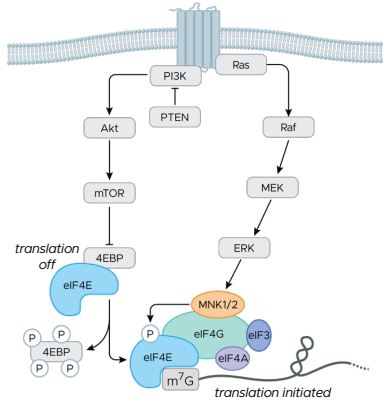
ABSTRACT

Aberrant activation of the BRAF/MEK pathway is a frequent driver of melanoma growth and progression. Although treatment with BRAF and MEK inhibitors is efficacious against BRAF mutant melanomas, complete and durable responses are uncommon with frequent emergence of drug resistance. A critical downstream effector of the BRAF/MEK signaling pathway is eukaryotic translation initiation factor 4E (eIF4E), which is the main regulator and rate limiting factor for cap-dependent mRNA translation and pro-oncogenic protein synthesis. Therefore, eIF4E is an attractive target to potentiate the anti-cancer activity of BRAF/MEK inhibition, and potentially overcome drug resistance to the combination.

eIF4E activation through the BRAF/MEK and/or PI3K/AKT pathways results in selective mRNA translation leading to increased levels of key oncogenic proteins including Cyclin D1 (CCND1), Ornithine Decarboxylase (ODC1), and MYC. Elevated eIF4E activity has been observed in many cancer types, including BRAF mutant melanomas; overexpression of eIF4E is sufficient to cause malignant transformation, whereas its inhibition suppresses tumor growth in diverse cancers. Notably, aberrant signaling of the BRAF/MAPK and PI3K/AKT pathways leading to activation of eIF4E is a common mechanism by which cancer cells develop resistance to BRAF/MEK inhibitors and other upstream targeted therapies, supporting eIF4E inhibition as a strategy to circumvent drug resistance.

Herein, we describe the development and characterization of novel, potent, and selective eIF4E inhibitors. Compounds in the series show 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 CCND1 and ODC1 protein leading to a G1 cell cycle arrest. Additionally, we demonstrate that eIF4E inhibitors cause growth inhibition in a variety of cancer cell lines, including BRAF mutant melanoma. Furthermore, combination of eIF4E inhibitors with BRAF or MEK inhibitors significantly enhances melanoma cell growth inhibition, indicating additive effects by combining eIF4E inhibitors with standards of care. Select analogs from the series demonstrate favorable ADMET/PK properties with good oral bioavailability and low safety/DDI risk. Ongoing experiments will address *in vivo* efficacy of our eIF4E inhibitors in BRAF mutant melanoma as well as in other cancer indications.

INTRODUCTION



- eIF4E binds the 5' cap of mRNA and promotes translation initiation
- eIF4E is the rate-limiting factor for regulating protein synthesis
- eIF4E is a point of convergence for many pro-oncogenic signaling pathways, including BRAF/MEK
- eIF4E expression is elevated in many cancers and forced overexpression has been shown to drive tumors in animal models
- Genetic inhibition of eIF4E decreases viability in tumors but not in normal cells

RESULTS

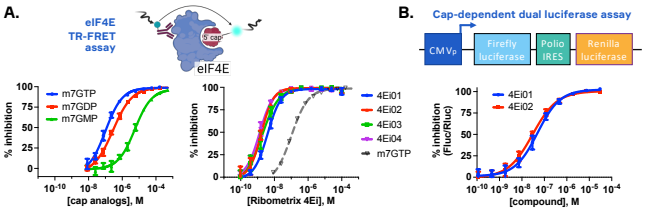
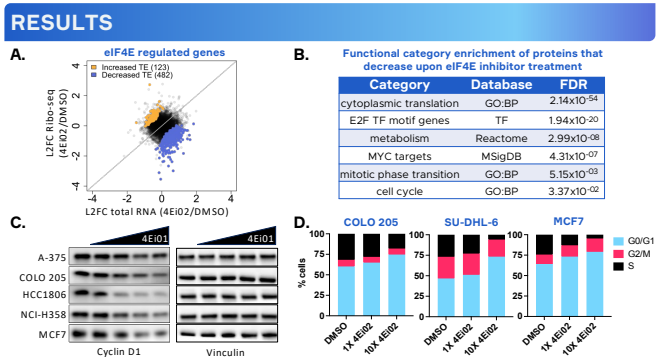


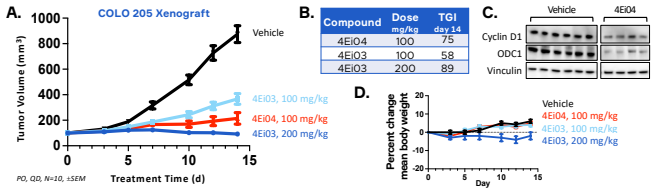
Figure 1. Development of novel potent Ribometrix (RBX) eIF4E inhibitors (eIF4Ei) that selectively inhibit cap-dependent translation. Multiple novel eIF4E inhibitors developed and biochemically validated at Ribometrix (RBX). **A)** TR-FRET assay using recombinant HIS-tagged human eIF4E and Europium-conjugated 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 regulate cell cycle progression through preferential repression of growth associated genes. A) 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). **B)** Functional enrichment analyses of translationally down-regulated genes yield multiple growth-associated categories. **C)** Western blot analysis of Cyclin D1 in cells treated with 4Ei01 shows selective decrease of Cyclin D1 across cancer cells lines from diverse tissues of origin. **D)** Cells treated with 4Ei02 (1x or 10x IC$_{50}$) and analyzed by DNA content flow cytometry shows concentration dependent increase in G0/G1 population.



Compound	Dose mg/kg	TGI day 14
4Ei04	100	75
4Ei03	100	58
4Ei03	200	89

Figure 3. eIF4E inhibition with RBX compounds shows strong anti-tumor efficacy in BRAF^{V600E} colorectal cancer model. A) COLO 205 CDX tumor growth curves after daily oral treatment of RBX eIF4E inhibitors. **B)** Tumor growth inhibition (% TGI) at day 14. **C)** Western blots from day 14 tumors 4 hours post-dosing. Data representative of other tumors and treatments. **D)** Mean percent change in body weight. No treatment group showed significant body weight loss. No observed signs of toxicity in any treatment group.

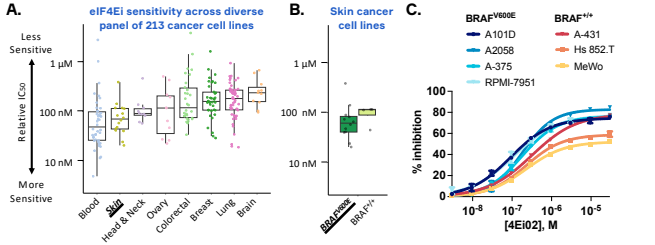


Figure 4. BRAF^{V600E} melanoma are particularly sensitive to RBX eIF4E inhibitors. A) 213 cancer cells lines from 8 different tissues of origin were treated with serial dilutions of eIF4E inhibitor for 72 hours and assayed for cell viability with CellTiter-Glo (CTG). Relative IC$_{50}$ values were calculated from DMSO normalized values for each cell line. Blood and skin cancer are the most sensitive types. **B)** Skin cancer cell lines from panel "A" subdivided by BRAF mutational status. Cell lines with BRAF^{V600E} are more responsive. **C)** CTG viability assay on select skin cancer lines treated for 72 hours with 4Ei02 shows BRAF^{V600E} cell lines (blues) are more responsive than wild type (reds).

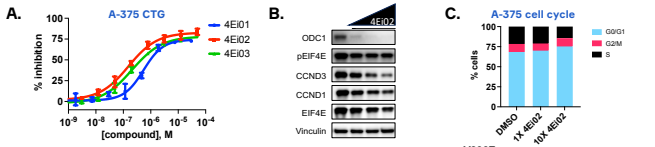
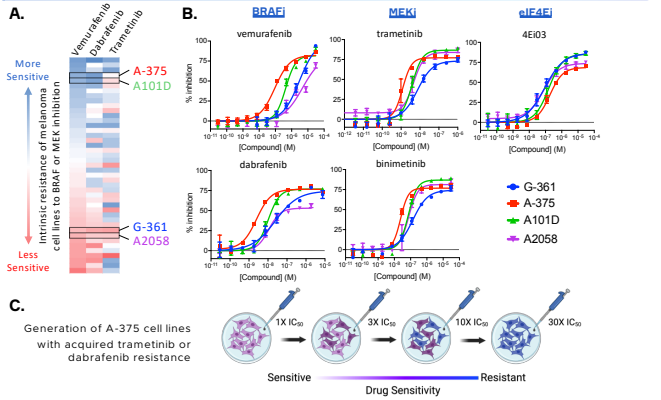


Figure 5. RBX eIF4E inhibitors lead to cell cycle arrest in BRAF^{V600E} melanoma. A-375 cells treated with RBX eIF4E inhibitors for 72 hours. **A)** CTG assay shows concentration dependent inhibition of cell viability. **B)** Western blots show decrease in eIF4E growth-associated targets. **C)** DNA content cell cycle analysis shows accumulation in G0/G1 population.

RESULTS



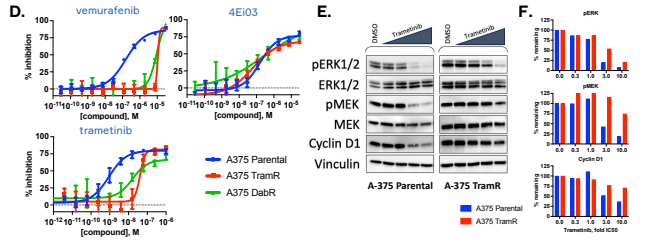


Figure 6. BRAF and MEK inhibitor resistant melanoma lines are sensitive to eIF4E inhibition. A) Reported sensitivity of melanoma cell lines to vemurafenib, dabrafenib, or trametinib from DepMap. **B)** CTG inhibition curves from melanoma cell lines treated with compound for 72 hours demonstrates similar eIF4E dependency despite different sensitivities to BRAF and MEK inhibitors. **C)** Serial passaging strategy to generate A-375 lines with acquired resistant to trametinib or dabrafenib. **D)** CTG curves in A-375 parental and acquired resistance lines confirms eIF4E dependency despite different sensitivities to BRAF and MEK inhibitors. **E)** Western blots of A-375 parental or trametinib resistant lines treated with serial dilutions of trametinib confirms decreased response of ERK/MEK pathway, including decreased inhibition of eIF4E target, Cyclin D1. **F)** Densitometry quantification of Western blots.

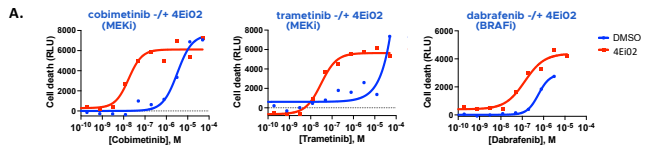


Figure 7. eIF4E inhibition sensitizes BRAF^{V600E} melanoma to MEK or BRAF inhibitors. A) A-375 cells treated with serial dilutions of BRAF or MEK inhibitors in the presence (red) or absence (blue) of 4Ei02. After 72 hours cells were assayed for cell death with Cell Tox Green reagent, leftward shifts show increased sensitization.

CONCLUSIONS

- **RBX eIF4E inhibitors are novel, potent, selective inhibitors of cap-dependent translation initiation, leading to growth arrest in many cancer types including melanoma**
- **Daily oral dosing of RBX eIF4E inhibitors show strong anti-tumor efficacy *in vivo* with no signs of toxicity**
- **BRAF^{V600E} melanoma cells are particularly sensitive to RBX eIF4E inhibitors**
- **Targeting melanoma resistant to BRAF/MEK inhibitors with an RBX eIF4E inhibitor has the potential to mitigate drug resistance and restore sensitivity to SoC in 2L setting**
- **eIF4E inhibition potentiates cancer cell sensitivity to BRAF and MEK inhibitors supporting the combination of RBX eIF4E inhibitor with BRAF/MEK inhibitors to improve SoC response durability in metastatic melanoma**
- **IND-enabling studies are planned**

Contact email address: jsorrentino@ribometrix.com