Development of novel eIF4E inhibitors to potently and selectively suppress tumor growth across multiple indications

Matthew B. Friedersdorf, Devon R. Blake, Sarah E.G. Thompson, Roheeth Pavana, Ramamurty V.S. Changalvala, Andrew Truong, Abha S. Bais, Krista Marran, Jessica A. Sorrentino
Ribometrix Inc., Durham, NC

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ABSTRACT

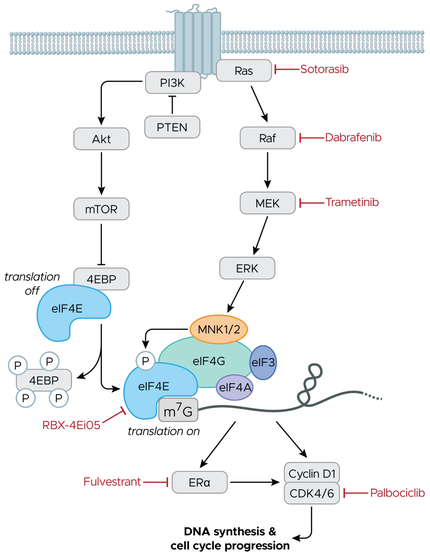
Despite considerable progress in treating cancer, drug resistance remains the primary hurdle to achieving cures in patients. One strategy for combating drug resistance is the combination of therapies, which with the right pairings can potentiate anti-tumor efficacy, as well as reducing the likelihood of developing resistance. Eukaryotic translation initiation factor 4E (eIF4E), the main regulator and rate limiting factor for protein synthesis, is a promising target to combine with other approved therapies due to its position as a critical regulatory node for multiple oncogenic signaling pathways. Furthermore, eIF4E is reactivated by many resistance mechanisms to promote translation of multiple pro-oncogenic factors including Cyclin D1/3, making it an attractive target to potentiate the anti-cancer activity of targeted therapies and to overcome drug resistance. Here, we present the development of novel, potent, and selective eIF4E inhibitors for use in both treatment-naïve and resistance settings across multiple tumor indications.

To capitalize on the potential of targeting eIF4E, we developed a series of compounds with unique properties that maintain anti-tumor efficacy while minimizing toxicity. Our novel, selective, and potent oral eIF4E inhibitor (RBX-4Ei05) elicits a reversible, dose-dependent cell cycle arrest. Unlike targeting eIF4A, eIF4E inhibition selectively regulates translation of cancer-dependent pathway proteins instead of global protein synthesis, thus reducing on-target toxicity and increasing tolerability. Cellular profiling demonstrates RBX-eIF4Ei are highly selective, nanomolar inhibitors across many tumor types including NSCLC, CRC, breast, and melanoma. Additionally, RBX-eIF4Ei demonstrate consistent efficacy across both sensitive and resistant cell lines, including intrinsic and acquired resistance models. Combining RBX-eIF4Ei with standard of care targeted therapies produces additive responses in both settings, suggesting its potential anti-neoplastic benefits post progression.

In vivo, daily oral monotherapy treatment with RBX-4Ei05 causes significant tumor growth inhibition across a variety of indications including BRAF^{V600E} CRC, BRAF^{V600E} melanoma, ER+ breast cancer, and KRAS^{G12C} NSCLC, with minimal signs of toxicity. Intratumoral concentration of RBX-4Ei05 correlates with significant tumor cell growth inhibition, as well as reductions in eIF4E target proteins, ODC1 and Cyclin D1.

Collectively, this data supports the addition of RBX-4Ei05 to standard of care in both the naïve and treatment resistance settings across a variety of indications including NSCLC, breast, CRC, and melanoma. IND enabling studies are planned, marking a significant step toward advancing these promising eIF4E inhibitors into clinical development.

INTRODUCTION



- eIF4E binds the 5′ cap of mRNA and promotes translation initiation
- eIF4E is the rate-limiting factor for regulating protein synthesis
- eIF4E selectively regulates the translation of oncogenic factors with highly structured 5′UTRs such as Cyclin D1, ODC1, and Myc
- eIF4E expression is elevated in many cancers, including breast cancer, and high levels are associated with poor prognosis
- Forced overexpression of eIF4E drives tumors in animal models, while genetic inhibition of eIF4E decreases tumor viability but not viability of normal cells
- eIF4E is a point of convergence for multiple pro-oncogenic signaling pathways, including Ras/Raf/MEK and PI3K/AKT/mTOR pathways
- Many resistance mechanisms for Ras/Raf/MEK pathway inhibitors result in hyper-activation of eIF4E

TUMOR TYPES

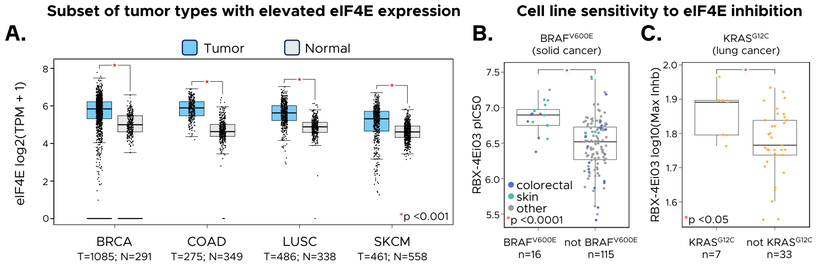
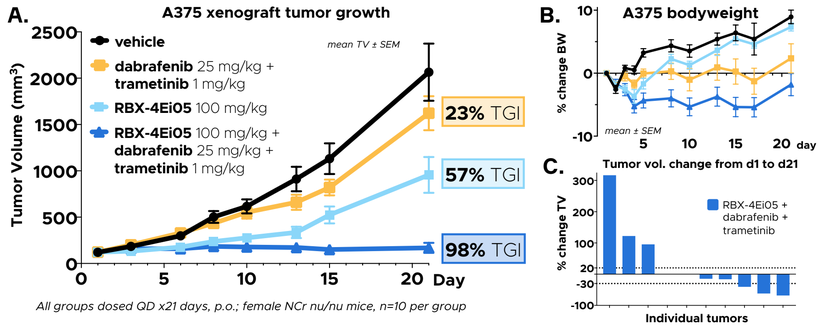


Figure 1. eIF4E expression is elevated in many tumor types. A) eIF4E RNA expression data from TCGA, made with GEPIA (Tang et al., *NAR* **45**, W98-102 (2017)), comparing tumor to normal tissues. Abbreviations: BRCA = breast invasive carcinoma, COAD = colon adenocarcinoma, LUSC = lung squamous cell carcinoma, SKCM = skin cutaneous melanoma, T = number of tumor samples, N = number of normal samples **B)** Comparing pIC50 values from a cancer cell line screen of an RBX eIF4E inhibitor against 131 cell lines from 8 different tissues of origin. Cells were treated with serial dilutions of eIF4E inhibitor for 72 hours and assayed for cell viability with CellTiter-Glo (CTG). **C)** Lung cancer cell line subset from cell line screen in **B)**.

BRAF^{V600E} MELANOMA & CRC



All groups dosed QD x21 days, p.o.; female NCr nu/nu mice, n=10 per group

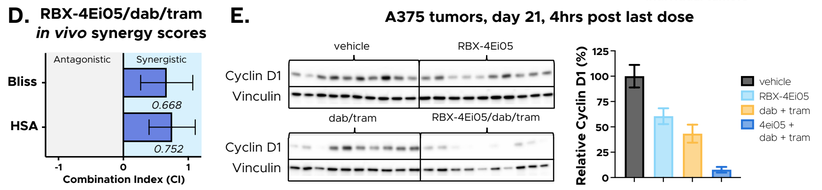
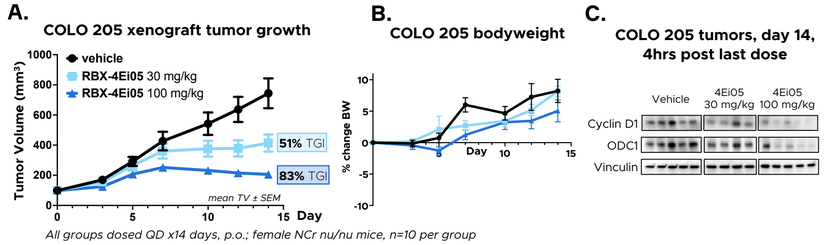


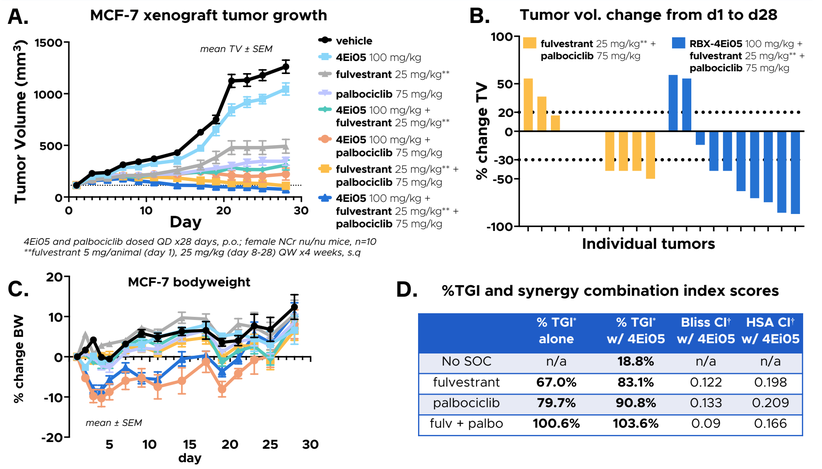
Figure 2. RBX-4Ei05 synergizes with BRAF/MEK inhibitors to cause tumor regression in a BRAF^{V600E} melanoma model. A) A375 xenograft tumor growth curves with daily oral treatment of dabrafenib (BRAF inhibitor) + trametinib (MEK inhibitor), RBX-4Ei05, and RBX-4Ei05 plus dabrafenib/trametinib. Tumor growth inhibition (TGI) is the percent change in tumor volume from day 1 to day 21 relative to vehicle change. **B)** Mean percent change in bodyweight. **C)** Percent change in tumor volume for individual mice in RBX-4Ei05 combo. Dashed lines indicate cutoffs for: progressive disease >+20%, stable disease <+20% and >-30%, and partial response <-30%. **D)** Bliss and HSA (highest single agent) combination index scores where >0 is synergistic, =0 is additive, and <0 is antagonistic. Method from Huang et al., *Sci Rep* **12**, 12984 (2022). **E)** Left, Western blots of eIF4E responsive gene, Cyclin D1, and loading control, Vinculin, from A375 tumor lysates collected 4 hours after the final dosing on day 21. Right, densitometry quantification of Western blot.



All groups dosed QD x14 days, p.o.; female NCr nu/nu mice, n=10 per group

Figure 3. RBX-4Ei05 shows anti-tumor efficacy against BRAF^{V600E} colon cancer *in vivo*. A) COLO 205 xenograft tumor growth curves with daily oral treatment of RBX-4Ei05 at two concentrations. Tumor growth inhibition (TGI) is the percent change in tumor volume from day 1 to day 14 relative to vehicle change. B) Mean percent change in body weight. C) Western blots of two eIF4E responsive genes: Cyclin D1 and ODC1, plus a loading control, Vinculin, from COLO 205 lysates collected 4 hours after the final dosing on day 14.

ER+ BREAST CANCER



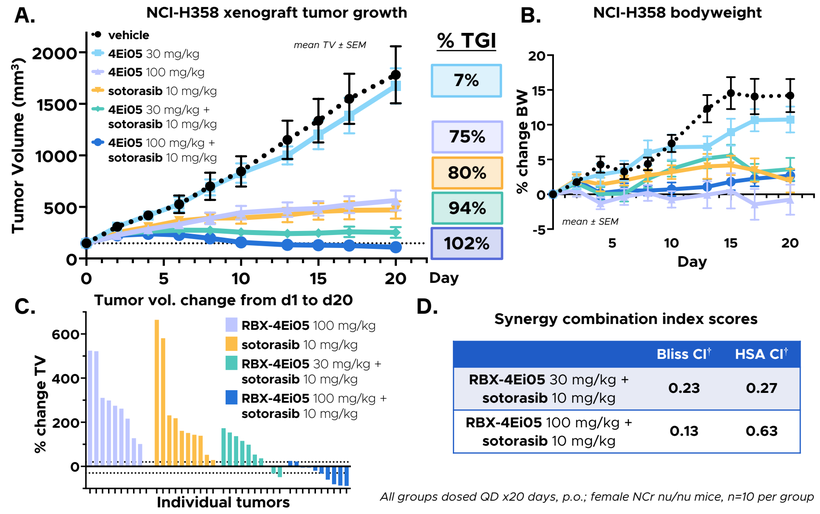
4Ei05 and palbociclib dosed QD x28 days, p.o.; female NCr nu/nu mice, n=10
***fulvestrant 5 mg/animal (day 1), 25 mg/kg (day 8-28) QW x4 weeks, s.q*

D. %TGI and synergy combination index scores

	% TGI alone	% TGI w/ 4Ei05	Bliss CI w/ 4Ei05	HSA CI w/ 4Ei05
No SOC	n/a	**18.8%**	n/a	n/a
fulvestrant	**67.0%**	**83.1%**	0.122	0.198
palbociclib	**79.7%**	**90.8%**	0.133	0.209
fulv + palbo	**100.6%**	**103.6%**	0.09	0.166

Figure 4. RBX-4Ei05 synergizes with CDK4/6 inhibitors and SERDs to cause tumor regression in an ER+ breast cancer model. A) MCF-7 xenograft tumor growth curves with daily oral treatment of SOC (palbociclib, fulvestrant, or palbociclib + fulvestrant), RBX-4Ei05, and combinations of RBX-4Ei05 with SOC. **B)** Percent change in tumor volume for individual mice in RBX-4Ei05 + SOC group. Dashed lines indicate cutoffs for: progressive disease >+20%, stable disease <+20% and >-30%, and partial response <-30%. **C)** Mean percent change in bodyweight. **D)** Tumor growth inhibition (TGI) from day 1 to day 28 relative to vehicle change. Bliss and HSA (highest single agent) combination index scores where >0 is synergistic, =0 is additive, and <0 is antagonistic. Method from Huang et al., *Sci Rep* **12**, 12984 (2022).

KRAS^{G12C} NSCLC



	Bliss CI†	HSA CI†
RBX-4Ei05 30 mg/kg + **sotorasib** 10 mg/kg	**0.23**	**0.27**
RBX-4Ei05 100 mg/kg + **sotorasib** 10 mg/kg	**0.13**	**0.63**

All groups dosed QD x20 days, p.o.; female NCr nu/nu mice, n=10 per group

Figure 5. RBX-4Ei05 synergizes with KRAS inhibitors to cause tumor regression in a KRAS^{G12C} NSCLC model. A) NCI-H358 xenograft tumor growth curves with daily oral treatment of sotorasib, RBX-4Ei05, and RBX-4Ei05 plus sotorasib. Tumor growth inhibition (TGI) is the percent change in tumor volume from day 1 to day 21 relative to vehicle change. **B)** Percent change in bodyweight relative to start of dosing. **C)** Percent change in tumor volume for individual mice. Dashed lines indicate cutoffs for: progressive disease >+20%, stable disease <+20% and >-30%, and partial response <-30% **D)** Synergy combination index scores for combination of sotorasib with two RBX-4Ei05 concentrations. Bliss and HSA (highest single agent) combination index scores where >0 is synergistic, =0 is additive, and <0 is antagonistic. Method from Huang et al., *Sci Rep* **12**, 12984 (2022).

SOC RESISTANT CANCER CELLS

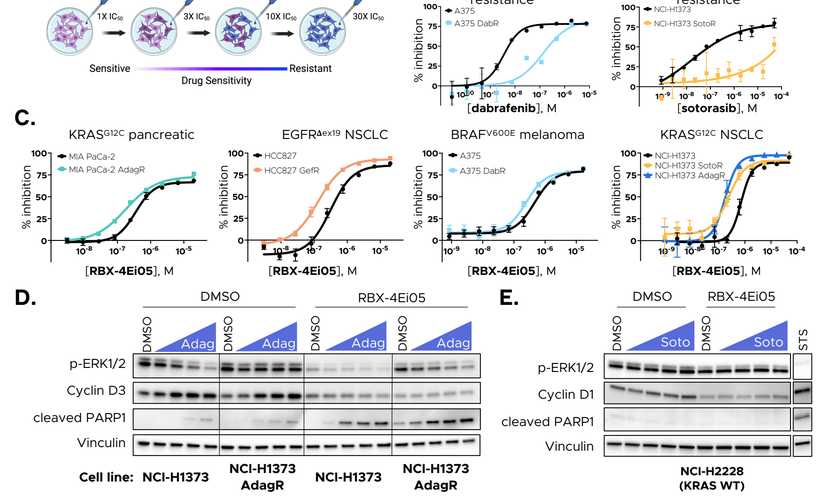


Figure 6. SOC resistant cancers are more dependent on eIF4E for growth and RBX-4Ei05 re-sensitizes resistant lines to SOC treatment. A) Serial passaging strategy to generate acquired resistance cell lines. **B)** Resistance validation of dabrafenib resistant A375 cells (DabR, left) and sotorasib resistant NCI-H1373 cells (SotoR, right) using CTG assay. **C)** Comparison of RBX-4Ei05 sensitivity in CTG between parental and acquired resistance cells. MIA PaCa-2 adagrasib resistant (AdagR) and HCC827 gefitinib resistant (GefR) lines generated and validated by WuXi AppTec. **D)** Western blots of combination treatment with adagrasib (Adag) and RBX-4Ei05 in parental and resistant cells. **E)** Western blots of combination treatment with sotorasib (Soto) and RBX-4Ei05 in KRAS WT cells, NCI-H2228. Staurosporine (STS) used as cleaved PARP1 control.

CONCLUSIONS

- **Daily oral dosing of RBX-4Ei05 shows *in vivo* anti-tumor efficacy against multiple cancer types, including melanoma, colon, breast, and lung with no overt signs of toxicity**
- **RBX-4Ei05 synergistically combines with standard-of-care (SOC) Ras/Raf/MEK and cell cycle pathway inhibitors for enhanced anti-tumor efficacy**
- **Combination of RBX-4Ei05 with Ras/Raf/MEK pathway inhibitors cause tumor regression**
- **Cell lines with acquired resistance to Ras/Raf/MEK pathway inhibitors are more sensitive to RBX eIF4E inhibitors**
- **Combination with RBX eIF4E inhibitors re-sensitizes resistant cells to Ras/Raf/MEK pathway inhibitors**
- **IND-enabling studies are ongoing**

Contact email address: jsorrentino@ribometrix.com