Dissection of cancer therapy combinations in RTK driven tumors using Zotatifin (eFT226), a potent and selective eIF4A inhibitor

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Abstract

Background: Oncoprotein expression is controlled at the level of mRNA translation and is regulated by the eukaryotic translation initiation factor 4F (eIF4F) complex. eIF4A, a component of eIF4F, catalyzes the unwinding of secondary structure in the 5′-untranslated region (5′-UTR) of mRNA facilitating ribosome scanning and translation initiation. Alterations in receptor tyrosine kinases (RTKs) lead to activation of the RAS/MAPK and PI3K/mTOR signaling pathways, enhance eIF4A activity, and promote the translation of select oncogenes that are required for tumor cell proliferation and survival.

Zotatifin (eFT226) is a selective eIF4A inhibitor that increases the affinity between eIF4A and sequence specific polypurine motifs in the 5′-UTR of zotatifin target genes, such as FGFR1/2 and HER2. Here we show that activation of eIF4A through RTK alterations along with downregulation of RTK protein expression by zotatifin creates a pathway dependency that drives selectivity to zotatifin treatment. Since many RTKs act as resistance mechanisms to current cancer therapies, regulation of RTKs by zotatifin also provides an effective drug combination strategy.

Results: Zotatifin inhibits the translation of FGFR1/2 and HER2 through the formation of a sequence dependent ternary complex with eIF4A1 and 5′-UTR mRNA polypurine motifs. In tumor cell lines driven by alterations in FGFR1/2 or HER2 and mTOR-dependent activation of eIF4A, downregulation of RTK expression by zotatifin results in decreased MAPK and AKT signaling, potent inhibition of cell proliferation and an induction of apoptosis. These same models tested *in vivo* demonstrate significant *in vivo* single agent activity. Using our mechanistic understanding of zotatifin targets and signaling pathways, combination strategies targeted vertical inhibition of the PI3K/mTOR/eIF4F pathway. Combination of zotatifin with HER2, PI3K, AKT or mTOR inhibitors was beneficial across RTK driven cancer models.

Conclusions: Downregulation of RTK expression by zotatifin coupled with RTK activation of eIF4A through mTOR signaling offers a unique pathway dependency for selective activity of zotatifin in cancers driven by RTK alterations. In addition, benefits achieved through vertical pathway inhibition, by combining zotatifin with PI3K/mTOR targeted agents, demonstrate the clinical potential of rationally designed combination strategies. A clinical trial in patients with solid tumor malignancies has been initiated.

Introduction

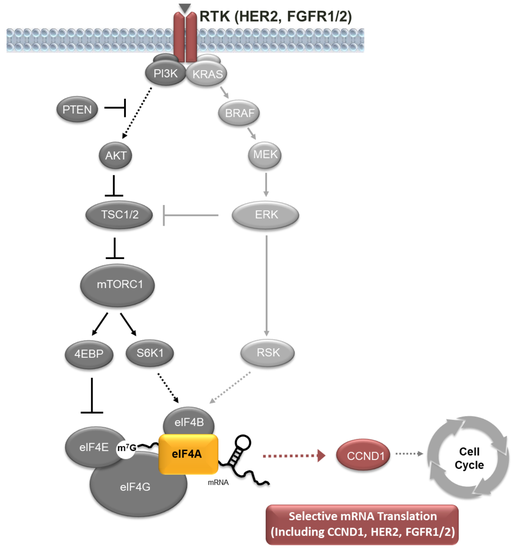


Figure 1. eIF4A is a central node controlling oncogenic gene expression. The RNA helicase eIF4A is an essential component of the translation initiation complex that regulates the translation of key oncogenes involved in tumor cell proliferation, survival and metastasis. Multiple oncogenic signaling pathways converge to modulate eIF4A activity. eFT226 is a potent and specific inhibitor of eIF4A that blocks the translation of select oncoproteins including several RTKs and Cyclin D1 (colored red).

Results

eFT226 Inhibits Target Gene Translation via Sequence Specific Regulatory Motifs in the 5′-UTR

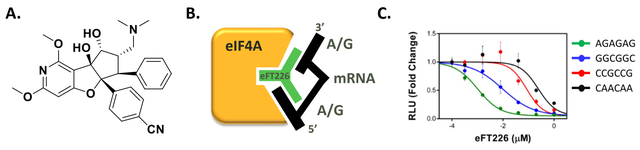
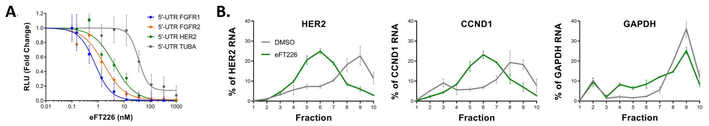


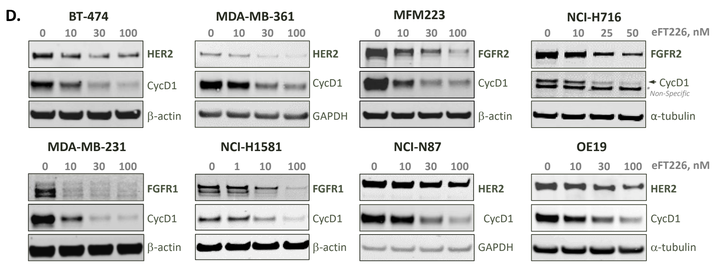
Figure 2. Translational regulation by eFT226 is mediated by sequence motifs in the 5′-UTR. A) Chemical structure of eFT226. **B)** Diagram of the ternary complex interactions between eIF4A, eFT226, and mRNA. **C)** Inhibition of reporter gene expression by eFT226 is dependent on the 5′ UTR sequence. Luciferase reporter gene constructs containing 5′-UTRs encoding different 6-mer sequence motifs were transiently transfected into MDAMB231 cells and treated with eFT226 for 4 h.

eFT226 Downregulates the Translation of Specific Oncogenic Driver Genes Resulting in Anti-Tumor Activity



C.

Cell Line	BT474	MDAMB361	MFM223	NCIH716	MDAMB231	NCIH1581	NCIN87	OE19	Calu3
Tumor Tissue	Breast	Breast	Breast	Colorectal	Breast	NSCLC	Gastric	Esophageal	NSCLC
RTK Amplified	HER2	HER2	FGFR2	FGFR2	FGFR1	FGFR1	HER2	HER2	HER2



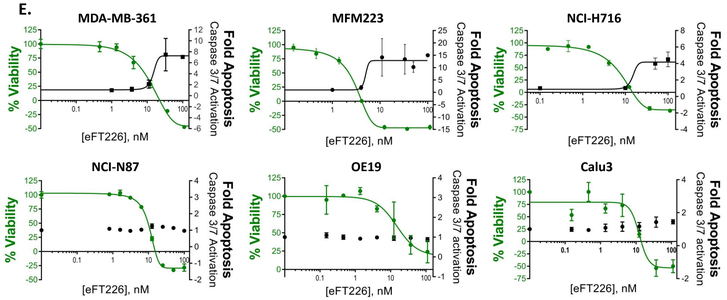
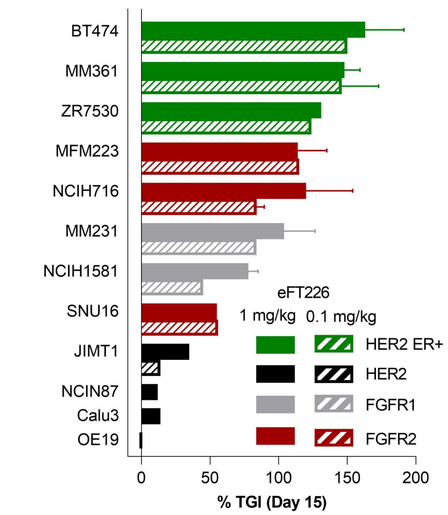
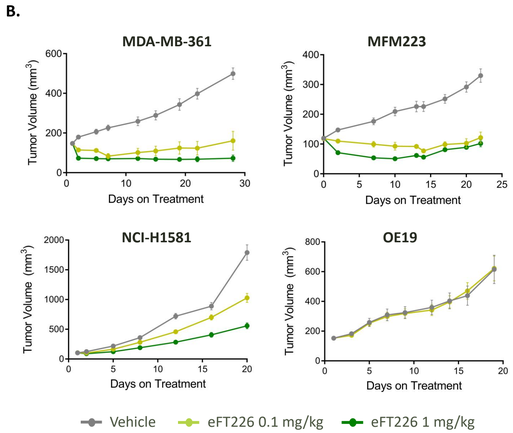


Figure 3. Translational downregulation of driver oncoproteins by eFT226 results in anti-tumor activity. A) Luciferase reporter gene constructs containing 5′-UTRs of FGFR1/2, HER2 or TUBA were transiently transfected into HEK293 cells and treated with eFT226 for 4 h. **B)** mRNA distribution using polysome profiling: HER2 amplified MDAMB361 breast cancer cells were treated with eFT226 prior to analysis by polysome fractionation and qPCR. eFT226 inhibits translation of HER2 and CCND1, but not of control gene, GAPDH. **C)** RTK driven cell lines used and their associated drivers. **D)** Representative western blots, 24 h treatment with eFT226. eFT226 induces a dose-dependent reduction in HER2, FGFR1/2, Cyclin D1 (CycD1) expression, but not control genes. **E)** eFT226 inhibits proliferation and induces apoptosis *in vitro*. 48 h treatment with eFT226. Green curves (Left Y axis), % viability. Black curves (Right Y axis), fold induction of apoptosis (Caspase 3/7 activation) relative to DMSO.

eFT226 Inhibits Tumor Growth Across Diverse RTK Driven Solid Tumors





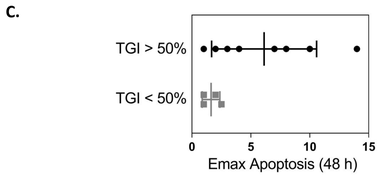
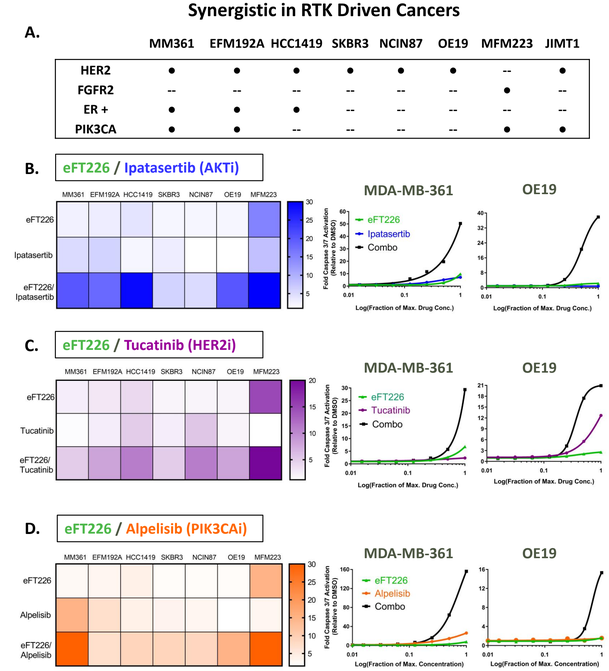


Figure 4. eFT226 inhibits tumor growth *in vivo* in many, but not all, RTK driven cancers. A-B) Athymic nude or NOD/SCID mice were implanted with subcutaneous xenograft models of FGFR1/2 or HER2 driven tumors and treated with eFT226 administered Q4D IV. Tumor volumes were measured over time, and percent tumor growth inhibition (% TGI) was calculated. **C)** *In vitro* Emax Apoptosis values of cancer lines (48 h treatment with eFT226, see Fig. 3E) are grouped based on their sensitivity to eFT226 *in vivo*. Data indicates that induction of apoptosis *in vitro* is a strong predictor of response to eFT226 *in vivo*.

Vertical Inhibition of the PI3K-AKT-mTOR-eIF4F Pathway is Synergistic in RTK Driven Cancers



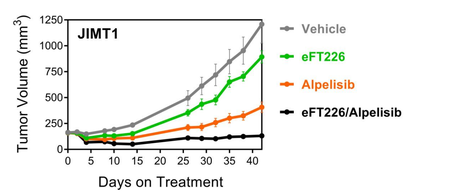


Figure 5. Combination treatments with eFT226 that induce vertical Inhibition of the PI3K-AKT-mTOR-eIF4F are beneficial in RTK-driven cancers *in vitro* and *in vivo* A) Cell lines used and their relevant genetic background. **B-D)** Left, Heat map representations of fold induction of apoptosis relative to DMSO, measured by Caspase 3/7 activation. Right, representative plots for OE19 and MDA-MB-361 of fold Caspase 3/7 activation relative to DMSO (per treatment condition). **B) Ipatasertib,** AKT inhibitor **C) Tucatinib,** HER2 inhibitor **D) Alpelisib,** PI3K inhibitor. Data in heat maps refers to the following concentrations: [eFT226] = 25 nM; [Ipatasertib] = 1.25 uM; [Tucatinib] = 0.25 uM; [Alpelisib] = 2.5 uM. **E)** SCID Beige mice bearing JIMT1 xenografts were treated with either vehicle, eFT226 (1 mg/kg), Alpelisib (50 mg/kg), or combination therapy for 40 days. Combination treatment yielded optimal growth inhibition.

Summary

- eFT226 is a potent and selective inhibitor of eIF4A dependent translation
- eFT226 inhibits the translation of key oncoproteins via 5′-UTR regulatory motifs and blocks the expression of specific oncogenic drivers and cell cycle targets
- eFT226 shows significant tumor growth inhibition and regression as a single agent across diverse RTK driven tumor types
- Vertical inhibition of the PI3K-AKT-mTOR-eIF4F pathway is synergistic in RTK driven cancers
- eFT226 is being evaluated in clinical trials for patients with solid tumor malignancies harboring activating RTK alterations (NCT04092673)