First-In-Human Phase 1/2 Dose Escalation And Expansion Study Evaluating First-In-Class eIF4A Inhibitor Zotatifin In Patients with Solid Tumors

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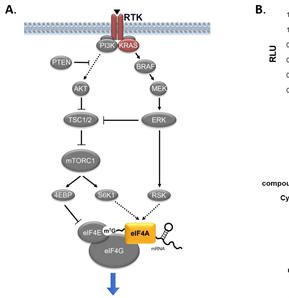
Background

Zotatifin (eFT226) is a selective inhibitor of eukaryotic translation initiation factor 4A (eIF4A)-mediated translation with a novel mechanism of action. Zotatifin binds selectively to defined nucleotide sequences present in the 5′ untranslated region (5′-UTR) of a subset of messenger ribonucleic acids (mRNAs) and concurrently to eIF4A. Zotatifin target sequences are found in the 5′-UTR of the estrogen receptor, Cyclin D1, CDK4, as well as several oncogenes and drivers of cell proliferation (HER2, ERBB3, FGFR1/2, EGFR, and KRAS). Zotatifin selectively blocks translation of these important mRNAs. Tumors with elevated protein levels, activating mutations or gene amplifications in zotatifin-sensitive proteins demonstrate selective downregulation of oncoprotein expression, inhibition of tumor cell proliferation and induction of apoptosis following zotatifin treatment. This mechanism of action results in significant in vivo tumor growth inhibition in multiple tumor models, including models of breast cancer (ER+, HER2 amplified, or FGFR1 overexpressed), non-small cell lung cancer (FGFR1 amplified or KRAS mutant), and colorectal cancer (KRAS mutant).

Collectively, these nonclinical data provided the foundation for initiation of Phase 1 clinical development of zotatifin. The initial development during dose escalation was in a mixed population of tumors with a molecular alteration (an activating mutation, amplification, or fusion) in HER2, ERBB3, FGFR1, FGFR2, EGFR, or KRAS and in cancers with very high prevalence of mutations in 1 of these genes, such as KRAS in pancreatic adenocarcinoma. Based on additional preclinical data and early clinical activity, Phase 2 expansion cohorts are now primarily focused on ER+ breast cancer.



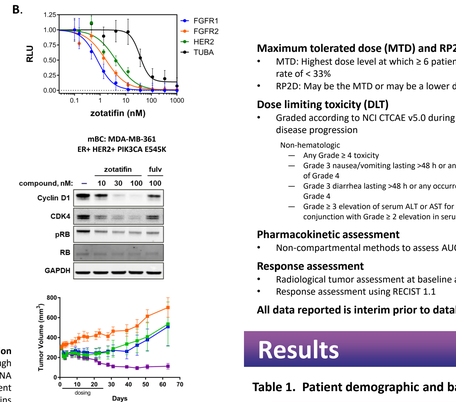


Figure 1. A. eIF4A is required for protein translation initiation and tumorigenesis. A. Oncogenic signaling through the RAS and PI3K pathways leads to activation of RNA helicase eIF4A. Activated eIF4A is required for efficient translation of mRNA encoding downstream effector proteins including ER, Cyclin D1, CDK4/6 as well as upstream pathway components including RTKs and KRAS. **B. Pre-clinical efficacy of zotatifin**. *Top panel*, zotatifin selectively inhibits the translation of eIF4A-dependent mRNAs with nanomolar potency in a cell-based reporter assay. *Middle panel,* zotatifin selectively inhibits production of cell-cycle proteins including Cyclin D1 and CDK4 in MDA-MB-361 (ER+ HER2+ PIK3CA E545K) cells. *Bottom panel*, zotatifin inhibits tumor growth in mice bearing MDA-MB-361 xenografts and leads to sustained tumor regression post dosing in combination with palbociclib.

Objectives

Primary objectives:

Part 1 (Dose escalation)

- To define the safety and tolerability of zotatifin as monotherapy in patients with defined, advanced solid tumors
- To determine the MTD or RP2D for zotatifin as monotherapy
- To evaluate the PK profile of zotatifin

Part 2 (Expansion cohorts)

- To evaluate the preliminary antitumor activity of zotatifin as monotherapy and as combination therapy in patients with defined, advanced solid tumors
- To determine the MTD or RP2D for zotatifin as combination therapy

Secondary objectives:

Part 1 (Dose escalation)

- To evaluate the preliminary antitumor activity of zotatifin as monotherapy in patients with defined, advanced solid tumors

Part 2 (Expansion cohorts)

- To assess the safety of zotatifin as monotherapy and as combination therapy
- To assess progression free survival (PFS)
- To evaluate the PK profile of zotatifin in combination therapy

Exploratory objectives:

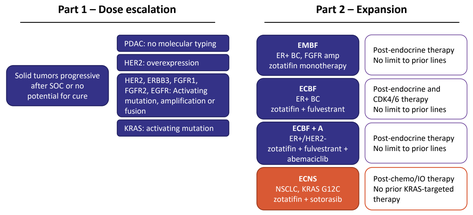
- To explore the effects of zotatifin on pharmacodynamic markers relating to drug mechanism
- To explore additional biomarkers to further elucidate mechanism of action, predict response to therapy, and understand potential resistance mechanisms

Methods

Study design and treatment

- Open label study in adult patients with advanced solid tumors
- **Part 1**: 3+3 dose escalation scheme with weekly intravenous administration of zotatifin in a 21 day cycle
- **Part 2**: Expansion at MTD or RP2D as monotherapy or in combination with SOC therapy in tumor types harboring mutational changes that may make them more sensitive to zotatifin therapy as a Simon 2-stage design, with seven patients in stage 1 of each cohort

Patient eligibility - key inclusion criteria



Maximum tolerated dose (MTD) and RP2D

- MTD: Highest dose level at which ≥ 6 patients have been treated and associated with a first cycle DLT rate of < 33%
- RP2D: May be the MTD or may be a lower dose within the tolerable dose range

Dose limiting toxicity (DLT)

- Graded according to NCI CTCAE v5.0 during the 21-day DLT evaluation period and not clearly related to disease progression

Non-hematologic
- Any Grade ≥ 4 toxicity
- Grade 3 nausea/vomiting lasting >48 h or any occurrence of Grade 4
- Grade 3 diarrhea lasting >48 h or any occurrence of Grade 4
- Grade ≥ 3 elevation of serum ALT or AST for >7 days or in conjunction with Grade ≥ 2 elevation in serum bilirubin

Hematological
- Any Grade 5 toxicity
- Grade 4 neutropenia lasting >5 days or any febrile neutropenia
- Grade 4 anemia unexplained by underlying disease
- Grade 3 thrombocytopenia with bleeding or any Grade 4

Pharmacokinetic assessment

- Non-compartmental methods to assess AUC, CL, Vss, T1/2

Response assessment

- Radiological tumor assessment at baseline and every 8 weeks
- Response assessment using RECIST 1.1

All data reported is interim prior to database lock (data cut-off 3/04/2022)

Results

Table 1. Patient demographic and baseline characteristics

Characteristic		Part 1 (N=37)	Part 2 (N=17)
Age, median (range), years		62 (36-80)	55 (37-81)
Gender	• Male	18	0
	• Female	19	17
Race	• White	30	13
	• Black or African descent	1	4
	• Asian	2	0
	• Other	4	
Median number of prior metastatic therapies (range)		3 (0-9)	4 (1-11)
Primary diagnosis/cancer type, n (%)	• Colorectal	11 (29.7)	
	• Pancreatic	6 (16.2)	
	• NSCLC	6 (16.2)	1 (5.9)
	• Breast	4 (10.8)	16 (94.1)
	• Cholangiocarcinoma	2 (5.4)	
	• Melanoma	2 (5.4)	
	• Other (1 patient/tumor type*)	6 (16.2)	

*appendiceal, ovarian, sarcoma, small bowel, testicular, unknown

Table 2. Patient disposition

	Part 1 , N=37 (%)	Part 2, N=17 (%)
Continuing on study	0 (100)	8 (47.1)
Discontinued therapy		
• Disease progression	31 (83.8)	8 (47.1)
• Adverse event	2 (5.4)	1 (5.9)
• Withdrawal of consent	1 (2.7)	0 (0.0)
• Investigator or sponsor decision	2 (5.4)	0 (0.0)
• Other	1 (2.7)	0 (0.0)

Results (continued)

Table 3. Safety Summary

Category	zotatifin, mg/kg								
	Part 1, N (%)								Part 2, N (%)
	0.005[a] (N=3)	0.01[a] (N=3)	0.02[a] (N=3)	0.035[a] (N=7)	0.035[b] (N=3)	0.05[b] (N=3)	0.07[b] (N=8)	0.1[b] (N=7)	0.07[b] (N=17)
Subjects with TEAEs	3 (100)	3 (100)	2 (66.7)	7 (100)	3 (100)	3 (100)	8 (100)	7 (100)	15 (88.1)
Subjects with TEAEs related to zotatifin[c]	1 (33.3)	3 (100)	2 (66.7)	7 (100)	2 (66.7)	2 (66.7)	7 (87.5)	6 (85.7)	9 (52.9)
Subjects with DLTs	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)	1 (5.9)
Subjects with TESAEs	1 (33.3)	0 (0.0)	0 (0.0)	3 (42.9)	0 (0.0)	0 (0.0)	1 (12.5)	3 (42.9)	3 (17.6)
Subjects with TESAEs related to zotatifin[c]	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)
Subjects with CTCAE Gr 3/4 TEAEs	2 (66.7)	0 (0.0)	0 (0.0)	5 (71.4)	0 (0.0)	1 (33.3)	2 (25.0)	2 (28.6)	4 (23.5)
Subjects with CTCAE Gr 5 TEAEs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with CTCAE Gr 3/4 TEAEs related to zotatifin[c]	0 (0.0)	0 (0.0)	0 (0.0)	5 (71.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)
Subjects with CTCAE Gr 5 TEAEs related to zotatifin[c]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with TEAEs leading to dose interruption or reduction of zotatifin	1 (33.3)	0 (0.0)	0 (0.0)	4 (57.1)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	1 (5.9)
Subjects with TEAEs leading to discontinuation of zotatifin	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)

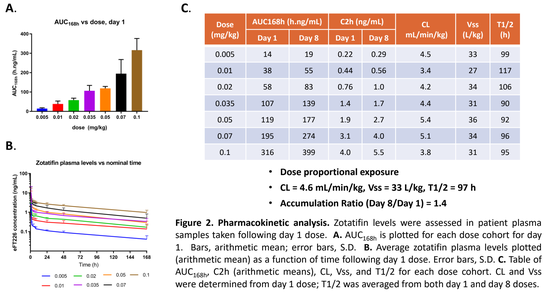
After 7 patients had been dosed at 0.035 mg/kg weekly, dosing frequency was reduced to days 1 and 8 of a 21 day cycle to mitigate anemia
TEAE, Treatment-Emergent Adverse Event, DLT, Dose Limiting Toxicity; CTCAE, Common Terminology Criteria for Adverse Events (version 5.0). Treatment-emergent adverse events (TEAEs) are AEs that start during or after zotatifin therapy, or AEs with an onset prior to initiating eFT226 therapy that worsen after zotatifin initiation.
[a] Dosed on days 1, 8, and 15 of a 21 day cycle; [b] Dosed on days 1 and 8 of a 21 day cycle; [c] As assessed by the Investigator as possibly related.

Table 4. Treatment emergent adverse events (TEAEs) related to zotatifin

MedDRA term	Grade	zotatifin, mg/kg								
		Part 1, N (%)								Part 2, N (%)
		0.005[a] (N=3)	0.01[a] (N=3)	0.02[a] (N=3)	0.035[a] (N=7)	0.035[b] (N=3)	0.05[b] (N=3)	0.07[b] (N=8)	0.1[b] (N=7)	0.07[b] (N=17)
Fatigue	1-2	1 (33.3)	1 (33.3)	0 (0.0)	4 (57.1)	1 (33.3)	1 (33.3)	1 (12.5)	2 (28.6)	1 (5.9)
	3-4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia	1-2	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (14.3)	3 (17.6)
	3-4	0 (0.0)	0 (0.0)	0 (0.0)	3 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)
Diarrhea	1-2	1 (33.3)	0 (0.0)	2 (66.7)	1 (14.3)	0 (0.0)	0 (0.0)	3 (37.5)	0 (0.0)	3 (17.6)
	3-4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vomiting	1-2	0 (0.0)	1 (33.3)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	2 (25.0)	2 (28.6)	3 (17.6)
	3-4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	1-2	0 (0.0)	1 (33.3)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (14.3)	2 (11.7)
	3-4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Events listed are >10% incidence. Coded with Medical Dictionary for Regulatory Activities (MedDRA) Version 25.0. Percentage is calculated using the number of treated subjects as the denominator. Treatment-emergent adverse events (TEAEs) are AEs that start during or after zotatifin therapy, or AEs with an onset prior to initiating eFT226 therapy that worsen after zotatifin initiation.
[a] Dosed on days 1, 8, and 15 of a 21 day cycle; [b] Dosed on days 1 and 8 of a 21 day cycle

Pharmacokinetic analysis



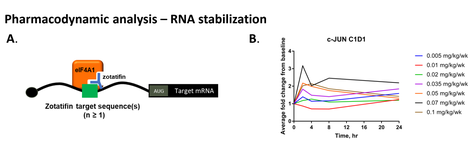
C.

Dose (mg/kg)	AUC168h (h.ng/mL)		C2h (ng/mL)		CL mL/min/kg	Vss (L/kg)	T1/2 (h)
	Day 1	Day 8	Day 1	Day 8			
0.005	14	19	0.22	0.29	4.5	33	99
0.01	38	55	0.44	0.56	3.4	27	117
0.02	58	83	0.76	1.0	4.2	34	106
0.035	107	139	1.4	1.7	4.4	31	90
0.05	119	177	1.9	2.7	5.4	36	92
0.07	195	274	3.1	4.0	5.1	34	96
0.1	316	399	4.0	5.5	3.8	31	95

- **Dose proportional exposure**
- **CL = 4.6 mL/min/kg, Vss = 33 L/kg, T1/2 = 97 h**
- **Accumulation Ratio (Day 8/Day 1) = 1.4**

Figure 2. Pharmacokinetic analysis. Zotatifin levels were assessed in patient plasma samples taken following day 1 dose. **A.** AUC_{168h} is plotted for each dose cohort for day 1. Bars, arithmetic mean; error bars, S.D. **B.** Average zotatifin plasma levels plotted (arithmetic mean) as a function of time following day 1 dose. Error bars, S.D. **C.** Table of AUC_{168h}, C2h (arithmetic means), CL, Vss, and T1/2 for each dose cohort. CL and Vss were determined from day 1 dose; T1/2 was averaged from both day 1 and day 8 doses.

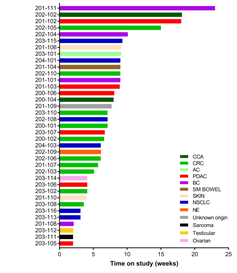
Pharmacodynamic analysis – RNA stabilization



- **Zotatifin forms a ternary complex with eIFA and sequence elements in the 5′ UTR of specific mRNA**
  - Key target genes involved in cell signaling, proliferation, and survival
- **Ternary complex formation stabilizes mRNA but blocks translation initiation**

Figure 3. Pharmacodynamic analysis A. Schematic of zotatifin mechanism of action inferred from eIF4A1/rocaglamide structure (Iwasaki, et al Mol Cell 2019)**. B.** RNA isolated from patient blood at time points post-dose on day 1 was analyzed by Nanostring. Average fold-change from baseline for c-JUN plotted for each time point is shown.

Efficacy analysis – Part 1, dose escalation in mixed tumors

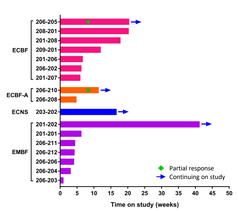


zotatifin mg/kg (N)	Part 1, Best Overall Response, N (%)			
	CR/PR	SD	PD	Not evaluable
0.005[a] (N=3)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)
0.01[a] (N=3)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)
0.02[a] (N=3)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)
0.035[a] (N=7)	0 (0.0)	0 (0)	7 (100)	0 (0.0)
0.035[b] (N=3)	0 (0.0)	0 (0)	3 (100)	0 (0.0)
0.05[b] (N=3)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)
0.07[b] (N=7)	0 (0.0)	2 (25.0)	4 (50.0)	2 (25.0)
0.1[b] (N=7)	0 (0.0)	0 (0.0)	6 (85.7)	1 (14.3)

[a] Dosed on days 1, 8, and 21 of a 21 day cycle
[b] Dosed on days 1 and 8 of a 21 day cycle

- **During Part 1 dose escalation, no PRs or CRs were observed**
- **The longest stable disease (23 weeks) was observed in a subject with FGFR1-amplified breast cancer treated at the RP2D who had received three prior lines of treatment for metastatic disease, including palbociclib/anastrozole followed by fulvestrant**

Interim efficacy analysis – Part 2, defined expansion cohorts



zotatifin 0.07 mg/kg[a] (N)	Part 2, Best Overall Response, N (%)			
	CR/PR	SD	PD	Not evaluable
ECBF (N=7)	1 (14.3)	3 (42.9)	3 (42.9)	0 (0.0)
ECBF+ A (N=2)	1 (50.0)[b]	0 (0.0)	1 (50.0)	0 (0.0)
ECNS (N=1)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
EMBF (N=7)	0 (0.0)	1 (14.3)	3 (42.9)	3 (42.9)

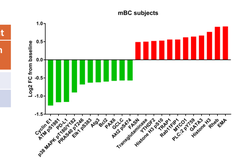
[a] Dosed on days 1 and 8 of a 21 day cycle; [b] Awaiting confirmatory scan
Reflects data through 5/02/2022

ECBF: Expansion, Combination, Breast, Fulvestrant
ECBF+ A: Expansion, Combination, Breast, Fulvestrant + Abemaciclib
ECNS: Expansion, Combination, NSCLC, Sotorasib
EMBF: Expansion, Monotherapy, Breast, FGFR

Pharmacodynamic analysis – Proteins/phosphoproteins

A.

Average fold-change between pre- and on-treatment	Proteins (~389)	Phospho-protein markers (~95)
10 subjects	0.99	0.95
7 BC subjects	0.99	0.91



B.

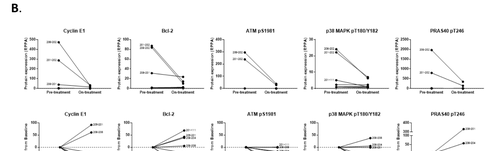
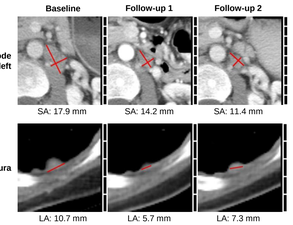


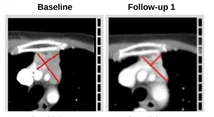
Figure 3. RPPA analysis. Paired frozen biopsies from pre-treatment and on-treatment (N=10, including 7 breast cancer patients) were analyzed by reverse phase protein array (RPPA core, MD Anderson) for ~488 protein markers (total or phospho-proteins). **A.** The fold-change between pre- and on-treatment was calculated. Proteins that deviated > 2 standard deviations above or below the mean in breast cancer (BC) subjects are represented as averaged log2 fold change on the waterfall plot. **B.** Before-after plots of selected protein and phospho-protein markers represented as RPPA score (top panels) and % change from baseline (bottom panels).

Interim efficacy analysis – Part 2, expansion cohorts (continued)

Patient with amplified Cyclin D1 and ESR1 L536H mutation treated with zotatifin combined with fulvestrant experienced a confirmed PR. Prior to study entry, patient had received seven prior lines of therapy for metastatic disease, including endocrine therapy alone and combined with multiple CDK4/6 inhibitors, fulvestrant combined with everolimus, and three chemotherapies.



Patient with PIK3CA Q546H and PIK3CA N1044K mutations treated with zotatifin combined with fulvestrant and abemaciclib experienced a PR on their first post-treatment scan and is awaiting confirmatory scan. Prior to study entry, patient had received three prior lines of treatment for metastatic disease, including a CDK4/6 inhibitor combined with endocrine therapy, fulvestrant combined with alpelisib, and chemotherapy.



Summary

Demonstration of relevant pharmacodynamic effects in humans

- **Target engagement was demonstrated via stabilization of c-JUN mRNA circulating in blood**
- **Modulation of translation was shown to be highly selective with less than 1% of protein expression altered**
- **Reductions in key oncogenic drivers such as Cyclin E and Bcl-2 were observed with the most dramatic effects in patients with the highest pre-treatment signals**

Demonstration of a safely tolerated dose/schedule and initial efficacy signals in targeted patients

- **Zotatifin was well tolerated at the RP2D with no related Gr 3/4/5 TEAEs in 25 patients**
- **A confirmed PR was observed in combination with fulvestrant in a patient with amplified Cyclin D1 and an ESR1 mutation whose disease had previously progressed on fulvestrant combined with everolimus**
- **A not yet confirmed PR was observed in combination with fulvestrant and abemaciclib in a patient with PIK3CA mutations whose disease had progressed on fulvestrant combined with alpelisib and a CDK4/6 inhibitor combined with endocrine therapy**

Future trial expansion

- **Cohort ECBF, zotatifin combined with fulvestrant in patients whose disease has progressed on endocrine therapy and a CDK4/6 inhibitor, has been expanded to enroll up to 18 patients**
- **Additional phase 2 cohorts in defined subsets of breast cancer patients are planned**

Acknowledgements

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