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Evaluation of Anti-Tumor Efficacy of EC1456 in Low-Passage and Pretreated Patient-Derived Xenograft Models of Triple-Negative Breast Cancer

ENDOCYTE

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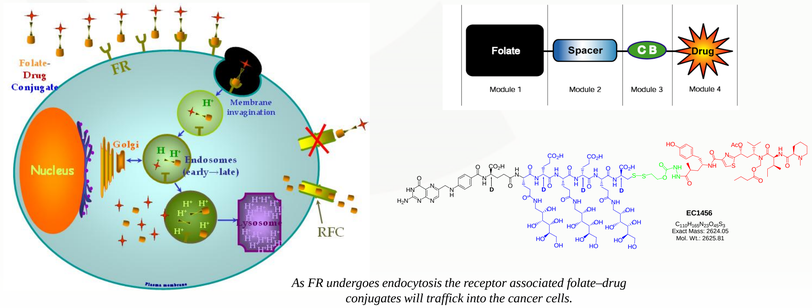
Abstract

Triple negative breast cancer (TNBC) patients are insensitive to hormonal or anti-HER2 therapy and have a higher recurrence rate among all breast cancer subtypes. There is a lack of common therapeutic targets in TNBC due to its six distinct molecular characteristics. Recently, ~50% of TNBC cases were found to express the folate receptor alpha (FRa) on tumor cells. FRa is a GPI-anchored membrane glycoprotein capable of bringing folate-targeted small-molecule drug conjugates (SMDCs) inside the cell. EC1456 is a folic acid-tubulysin B hydrazide (TubBH) SMDC that specifically binds to the membrane FRa and is internalized by endocytosis. While encapsulated within the early endosome, EC1456 releases TubBH into the cytosol where it inhibits the polymerization of tubulin into microtubules, thus blocking spindle formation to arrest cells in metaphase which ultimately induces apoptosis. EC1456 is currently under Phase 1 clinical investigation in patients with common solid tumors.

The purpose of this study is to evaluate EC1456 activity in Champions TumorGraft™ TNBC patient-derived xenograft (PDX) models to help guide our drug development strategies. These PDX models were derived from patients who were treated with multiple lines of standard-of-care agents. A total of six low-passage, FR-positive TNBC models were tested against two different treatment regimens of EC1456 (once or twice a week for 2 weeks only). Plasma and tumor drug concentrations were quantified by LC-MS/MS using satellite study animals. The tumor-bearing animals were monitored for up to 60 days to assess both short- term (i.e. % TGI) and long-term (%PR, CR, TFS) anti-tumor responses. Using a stringent efficacy criteria (≥60% CR/TFS), 3 of the 6 TNBC models were found highly sensitive to EC1456 and 3 were found resistant. To identify potential gene signatures of EC1456 response, bioinformatics analysis was performed using existing RNA-seq data and compared across a broad panel of TumorGraftTM TNBC models, regardless of FR expression status. Specific biomarkers of interest were further analyzed by qRT-PCR using control tumors from the current study. Together, our analysis revealed potential resistance mechanisms associated with microtubule dynamics as well as a cancer cell's ability to undergo apoptosis.

EC1456 is a Antimitotic Folate Receptor Targeted Chemotherapy



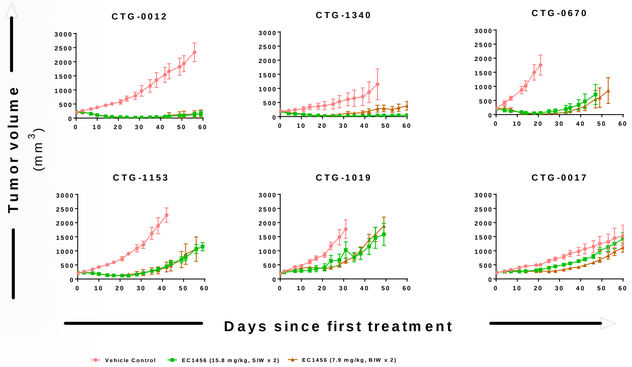
As FR undergoes endocytosis the receptor associated folate–drug conjugates will traffick into the cancer cells.

Evaluation of EC1456 Pharmacokinetics and Single-Agent Activity in Six TumorGraft™ TNBC PDX Mouse Models

EC1456 and its active metabolites detected in plasma and tumor (4 h post i.v.)

Tumor	Animal #	Concentrations (nM)					
		EC1456		EC0347		EC1009	
		Plasma	Tumor	Blood	Tumor	Blood	Tumor
CTG-0012	142682	2.24	0.618	120	86.5	3.54	4.65
	142733	3.92	<LLOQ	148	78.0	5.26	4.68
CTG-1340	147807	5.29	1.24	61.1	42.7	3.42	0.852
	147811	2.24	3.66	65.6	95.8	5.71	2.71
CTG-0670	165913	3.21	0.75	22.5	17.0	4.7	1.87
	165526	2.26	<LLOQ	29.3	43.6	4.11	1.69
CTG-1153	142650	1.47	2.22	161	49.7	5.65	3.08
	142642	1.24	1.83	105	44.2	3.59	1.66
CTG-1019	147293	8.07	1.46	42.8	71.4	3.54	2.10
	147266	3.56	0.837	45.6	86.2	3.70	2.76
CTG-0017	155259	7.30	0.621	33.1	25.5	4.99	2.52
	155220	9.47	1.58	42.5	44.1	8.24	2.5

EC1456 activity as a single agent

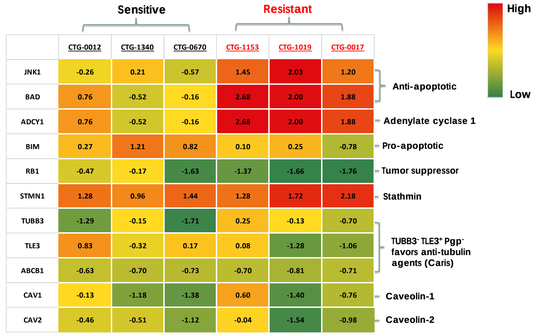


Characteristics of TNBC PDX Models and EC1456 Response Summary

Models	TNBC subtype	Age	Race	Mutations (TP53, RB1, BRCA, PTEN, PI3KCA)	Prior chemotherapy		Tumor FR levels (pmol/mg)	Best EC1456 response		
					#	Responded to Rx containing microtubule binding agents		%TGI	%PR/CR/TFS	Overall impression
CTG-0012	UNS	36	Caucasian	TP53; BRCA1 p.Y978*; PTEN	3	Yes / / No	6.25 ± 1.43	104	20/0/80	Sensitive
CTG-1340	Basal-like	57	Asian	TP53	3	/ /Yes	6.65 ± 1.05	124	40/20/40	Sensitive
CTG-0670	UNS	48	Caucasian	TP53; BRCA1 p.X1777X	3	/Yes/	3.24 ± 0.29	112	20/40/20	Sensitive
CTG-1153	BL1	36	Caucasian	TP53; RB1	1	No	4.30 ± 1.04	89	20/0/20	Resistant (partial)
CTG-1019	BL1	53	Caucasian	TP53; RB1	5	Yes/Yes//No/	2.83 ± 0.43	48	0/0/0	Resistant
CTG-0017	Basal-like	57	Caucasian	TP53	2	N/A	2.43 ± 0.66	59	0/0/0	Resistant

Differential Gene Expression in EC1456 Sensitive Vs. Resistant Models

Heat map showing Z-scores of RPKM values of 11 genes normalized across ~350 PDX tumor models and 30+ different cancer types. RNA-Seq analysis was performed at a minimum depth of 50 million reads with 50/76/101*2 paired-end on the HiSeq-2500 Illumina platform. Mouse-derived sequences were filtered from the data set after alignment of the reads to concatenated mouse-human genomes (Hg19/mm10). Any samples with greater than 40% mouse content were excluded. Genes expression counting was conducted using RSEM (RNA-Seq by Expectation Maximization) with default parameter on Genecode GRch37.p13 coordinates. Results were normalized for sequencing depths in R using the TMM (trimmed mean of M-values) algorithm from the edgeR package. RPKM (Read Per Kilobase Million) values were calculated and transformed to a log2 scale.



Conclusions

1. In general, EC1456-sensitive models (~50%) showed a favorable molecular profile and EC1456-resistant models showed a trend towards (a) low apoptosis potential (higher JNK/BAD & lower BIM expression), (b) RB1 loss, and (d) activation of ADCY1 (adenylate cyclase 1), an enzyme responsible for cAMP synthesis.
2. EC1456 responses in TNBC PDX models did not correlate to functional FR levels measured using a ^{3}H-folic acid assay of the membrane preparation of whole tumor homogenates.
3. Neither patients' prior Rx history nor sensitivity to anti-tubulin agents predicted PDX tumor responses to EC1456.
4. The presence of mutations in TP53, BRCA1, and PTEN also did not predict PDX responses to EC1456.

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