Pharmacodynamic Transcriptional Markers of Carboxyamidotriazole Orotate (CTO) Exposure in Anagen Hair

#367

Rashida Karmali[1] & Gino Miele[2]

[1] Tactical Therapeutics 99 Wall St., 23th Floor, New York, NY 10005

[2]Epistem Ltd, 48 Grafton Street, Manchester, M13 9XX, UK

Background

Carboxyamidotriazole Orotate (CTO) is the orotic acid salt of carboxyamodotriazole (CAI) (Figure 1) which is an inhibitor of calcium - dependent intracellular and extracellular signal transduction pathways, also possessing antiproliferative, antiangiogenic and anti-invasive properties. The activity of CTO alone and in combination with temozolomide or 5-fluorouracil was demonstrated in human glioblastoma, melanoma and colon tumor xenografts. In Phase I (NCT01107522), nine patients pretreated with 2 to 8 targeted and non-targeted drugs and having refractory tumors responded to CTO at doses ranging 75mg/m^2/day through 427mg/m^2/day, and achieved stable disease for different periods (3 to 14 months, Figure 2). Four pretreated tumors were found to have different genomic mutations (PI3KCA, EGFR multiple, BRAFV600 and NRAS) consistent with CTO's suggested mechanism of action (MOA) to inhibit multiple tyrosine kinases in addition to modulating calcium signaling pathways and calcium-dependent signal transduction pathways (as yet uncharacterized).

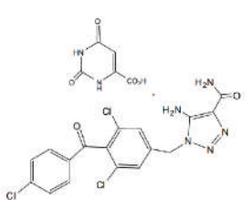
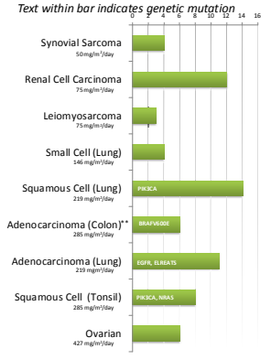


Figure 1: Structural Formula of CTO (CAI + Orotic Acid)

Figure 2: Patients Demonstrating Stable Disease

Text within bar indicates genetic mutation



Methods

Using Epistem's *ex vivo* anagen hair culture assay platform, global gene expression assessment by microarray analysis was performed to assess transcriptional response to varying doses of CTO (2, 5 and 10 µM). Plucked anagen scalp hairs from five healthy donors were exposed to varying doses of CTO over a 24 hr period, and also to Erlotinib and BEZ235 as positive control inhibitors for EGFR and PI3K/mTOR respectively. Total RNA was isolated from the hair bulbs post culture and used to assess global transcriptional alterations by Affymetrix GeneChip microarray.

Results

Significant biologically relevant alteration of the anagen hair bulb transcriptome, ranging from -100 fold to +25 fold differential expression of some mRNAs was observed at CTO levels shown to be clinically relevant (Figure 3), with a high degree of overlap between doses and times.

3(A)

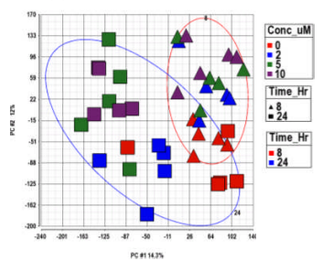


Figure 3. (A) PCA visualisation of microarray data. (all probes). Clear separation of samples based on time in culture and CTO treatment is evident. (B) Summary of ANCOVA model detailing number of probes passing each contrast condition (with multiple test correction & fold change thresholding). Range of fold changes noted are detailed.

3(B)

	CTO						BEZ235	Tarceva
	8 Hours			24 Hours				
	2µM	5µM	10µM	2µM	5µM	10µM	1µM	1µM
FDR 0.05 & 1.5 FC	1669	3321	4419	5090	5407	7846	1551	27
Fold Change Range	-17 To 15	-23 To 23	-23 To 29	-41 To 14	-102 To 24	-75 To 32	-8 To 25	-7 To 3

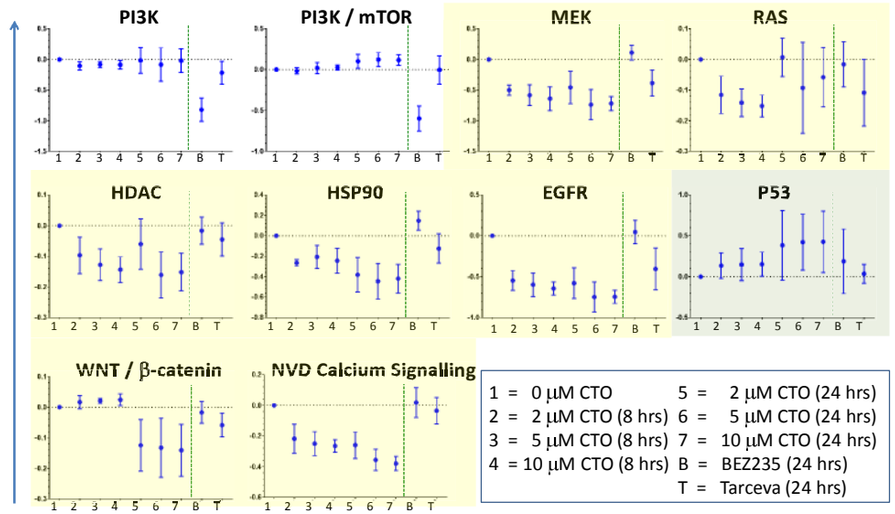
Multivariate transcriptional "signatures" of onco-pathway modulation (derived both from the literature and proprietary to Epistem or Tactical Therapeutics), were applied to gain insights into the molecular MOA of CTO. Generally, alteration of 25% of genes in a putative signature set by 2-fold will result in a reduction in "score" by 0.15 units.

Transcriptional signatures associated with inhibition of EGFR, MEK, HDAC and HSP90 were strongly suppressed at all doses and times of CTO exposure (Figure 4 & 5). In addition, signatures associated with non-voltage dependent calcium signaling were strongly suppressed, together with those associated with RAS and Growth Factor signaling. Modest suppression of transcriptional signatures of WNT signaling was evident at longer CTO exposure.

	µM CTO					
	2	5	10	2	5	10
	8 hours			24 hours		
EGFR inhibition	-0.6	-0.6	-0.6	-0.8	-0.8	-0.8
MEK inhibition	-0.5	-0.5	-0.5	-0.5	-0.7	-0.7
HSP90 inhibition	-0.2	-0.2	-0.2	-0.4	-0.4	-0.4
Non voltage dependent calcium signalling	-0.2	-0.2	-0.2	-0.2	-0.4	-0.4
P53 stabilisation	0.15	0.15	0.15	0.4	0.4	0.4
WNT / β-catenin inhibition	0	0	0	-0.14	-0.14	-0.14
HDAC inhibition	-0.1	-0.12	-0.12	-0.08	-0.16	-0.16
Growth Factor signature	-0.1	-0.1	-0.16	-0.07	-0.01	-0.07
RAS signature	-0.12	-0.14	-0.14	0	0	0
Pan-PI3K inhibition	0	0	0	0	0	0
Pan-PI3K/mTOR dual inhibition	0	0	0	0	0	0
Gamma secretase inhibition	0	0	0	0	0	0

Figure 4. Summary of assessments of transcriptional signature behavior in CTO treated anagen hair.

Figure 5. Graphical representation of transcriptional behaviour of signatures on CTO treatment (expressed relative to 0 mM baseline). BEZ235 and Tarceva data are also included.



1 = 0 µM CTO
2 = 2 µM CTO (8 hrs)
3 = 5 µM CTO (8 hrs)
4 = 10 µM CTO (8 hrs)
5 = 2 µM CTO (24 hrs)
6 = 5 µM CTO (24 hrs)
7 = 10 µM CTO (24 hrs)
B = BEZ235 (24 hrs)
T = Tarceva (24 hrs)

Conclusion

- These data provide multiple pharmacodynamic markers associated with the suggested mechanism of action of CTO e.g., inhibition of genes of non-voltage dependent calcium signaling
- Inhibition of multiple oncopathways:
  - EGFR
  - MEK
  - HDAC
  - HSP90
  - RAS
  - Growth Factor Signature
  - WNT, and
  - Activation of signatures associated with tumor suppressor P53
- These results may provide the molecular MOA of CTO's observed clinical benefit in a broad spectrum of malignant tumors with different genomic types and a tool to design customized combination therapies of CTO with other agents.