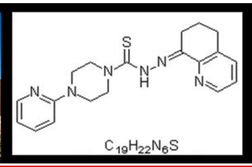

COTI-2, a potent orally available small molecule targeting mutant p53, with promising efficacy as monotherapy and combination treatment in preclinical tumor models

#6040

Richard T. Ho[1], K. Y. Salim[1], Antje Lindemann[2], Ameeta A. Patel[2], Hideaki Takashi[2], Li Wang[3], Mei Zhao[2], Steven J. Frank[3], Abdullah A. Osman[2], Jeffrey N. Myers[2], C. Lynam[1], Alison D. Silva[1].
[1]Cotinga Pharmaceuticals, [2]Dept Head and Neck Surgery, UT MD Anderson Cancer Center, [3]Dept Radiation Oncology, UT MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background

- Mutated p53 tumor suppressor protein is involved in >50% of all cancers.
- COTI-2, an orally available 3rd-gen thiosemicarbazone, has been shown to restore the structure and function of mutated p53 proteins in vitro.
- COTI-2 monotherapy has preclinical *in vitro* and *in vivo* efficacy in Head and Neck Squamous Cell Carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal, ovarian, and other cancer cell lines.

Objective

Explore possible synergy of COTI-2 reactivating normal p53 function with DNA damage-inducing therapies including chemotherapy and radiation.

Methods

1. Cell viability was measured with crystal violet or indicator dye to assess IC_{50} *in vitro*.
2. Xenograft studies used cancer cell lines with specified p53 mutations.
3. Drug synergy was determined by isobolograms and combination-indices calculated using Chou-Talalay method.

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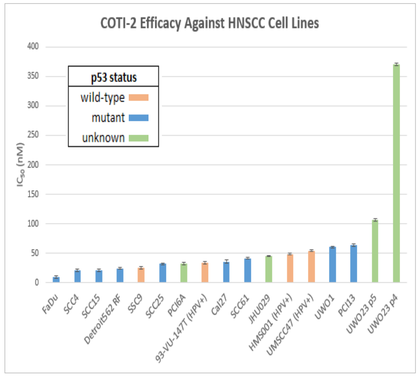
Results

Figure 1. COTI-2 is highly effective *in vitro* against a range of HNSCC lines bearing a variety of TP53 mutations. Sixteen HNSCC cell lines were treated with COTI-2 for 72 hours to test the ability of COTI-2 to inhibit HNSCC cell line growth and proliferation. All HNSCC cell lines responded in the nanomolar range, with most of the IC_{50} values within the 20 to 60 nM range.



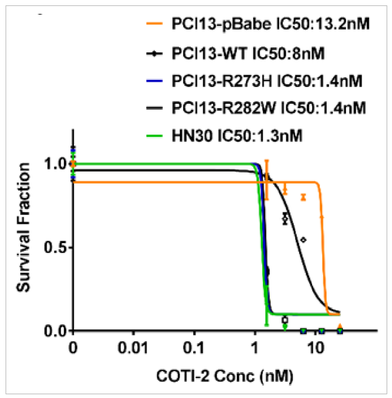


Figure 2. COTI-2 displays single-agent activity and inhibits *in vitro* growth of HNSCC cell lines harboring various *TP53* mutations. Isogenic R273H, R282W, and the null *TP53* cell line (PCI13-pBabe) were used to explore the degree of sensitivity of these cells to COTI-2 in clonogenic survival assays. Dose-response curves were calculated, and all cell lines tested were sensitive to COTI-2 with IC_{50} values ranging between 1.4 to 13.2 nM. Cell lines with mutant p53 had increased sensitivity to COTI-2.

Figure 3. COTI-2 as monotherapy inhibits tumor growth *in vivo* in an orthotopic mouse model of HNSCC with *TP53* mutations. PCI13-R273H cells were injected into the tongues of nude mice followed by treatment with COTI-2 administered by oral gavage 5 days per week (75 mg/kg) for 4 weeks. COTI-2 induced significant tumor growth inhibition from day 12 of treatment onwards compared to untreated control ($P < 0.001$).

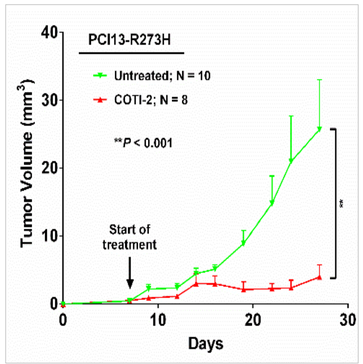
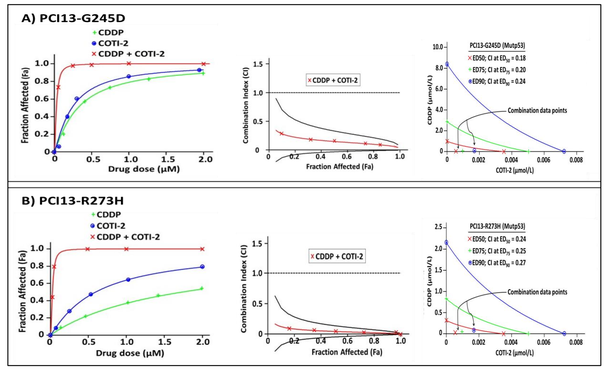


Figure 4. COTI-2 synergizes with cisplatin (CDDP) *in vitro* in HNSCC cells expressing different p53 mutations. Clonogenic survival assays were done with G245D and R273H p53 mutant PCI13 cell lines. Isobolograms show that COTI-2 at the median combination index (CI) which equals an effective dose (ED) at the IC_{50} (ED_{50}) = 0.18, is highly synergistic with cisplatin *in vitro* in two p53 mutants.



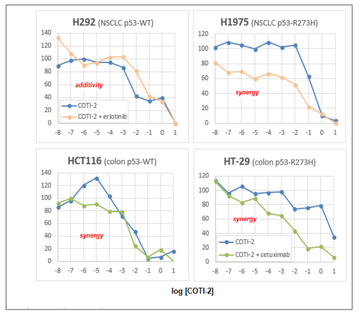


Figure 5. COTI-2 is also synergistic or additive *in vitro* with standard anticancer agents against colorectal and Non-Small Cell Lung Cancer (NSCLC) lines. Cell lines were treated with COTI-2 for 72 hours to test the ability of COTI-2 to inhibit HNSCC cell line growth and proliferation.

[erlotinib] = 0.4nM; 50uM
[cetuximab]= 0.7nM; 0.7nM

Combination indices were calculated by Chou-Talalay method.

CI for H292=1; H1975<0.1
CI for HCT116<0.1; HT-29<0.1

Table 1. *In vivo* results show that COTI-2 is additive to liposomal doxorubicin (Doxil) against A2780 ovarian cancer xenografts.
COTI-2 alone: 22%, 20% Tumor Growth Inhibition (TGI)
Doxil alone: 25% TGI
Combinations of COTI-2 + Doxil: 47%, 54% TGI are consistent with additive effects

	Group	n	Dose	Route	Schedule	Day 17 Tumor Weight (mg)	Day 17 TGI (%)	Day 17 CS*	Day 17 Deaths
1	Vehicle Control	10	---	IV	Day 1,3,5,7,8,10,12,15,16,17	2150.7 ± 352.5	---	0	0
2	COTI-2	10	12.5 mg/kg	IV	Day 1,3,5,7,8,10,12,15,16,17	1699.2 ± 241.4	22.04	0	0
3	COTI-2	10	25 mg/kg	IV	Day 1,3,5,7,8,10,12,15,16,17	1738.4 ± 216.0	20.12	0	0
4	COTI-2	10	50 mg/kg	IV	Day 1,3,5,7,8,10,12,15,17	1622.8 ± 403.0	25.76	0	2
5	Doxil	10	2 mg/kg	IV	Day 1	1607.1 ± 343.4	25.35	0	0
6	COTI-2 Doxil	10	12.5 mg/kg 2 mg/kg	IV IV	Day 1,3,5,8,10,12,15,17 Day 1	1045.3 ± 305.7	47.44	1/10	1
7	COTI-2 Doxil	10	25 mg/kg 2 mg/kg	IV IV	Day 1,3,5,8,10,12,15,17 Day 1	1046.0 ± 261.3	53.80	0	0

* CS = Complete Tumor Shrinkage

Figure 6. COTI-2 displays single agent activity and synergizes with cisplatin to inhibit HNSCC tumor growth *in vivo*. In an orthotopic model of oral cancer using wild type or G245D mutant p53 PCI13 cell line, mice were treated with COTI-2 75mpk PO 5x/wk, cisplatin IV 4mg/kg on day 1, or the combination. Compared to untreated controls or either drug alone, the combination showed significant suppressive effects on tumor growth.

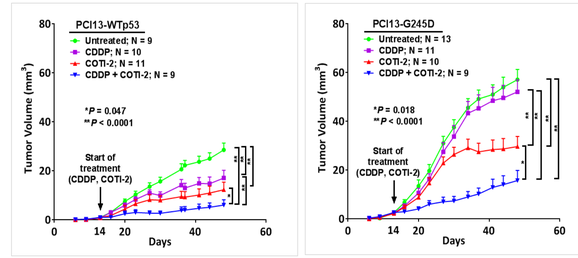
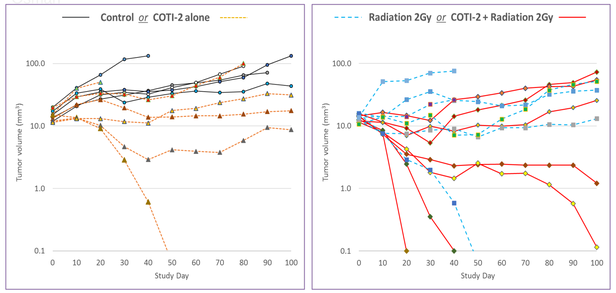


Figure 7. COTI-2 synergizes with radiation therapy to inhibit HNSCC tumor growth *in vivo*. In an orthotopic model of oral cancer using a G245D mutant p53 PCI13 cell line, mice were treated with COTI-2 75mpk PO 5x/wk, radiation 2 Gray/fraction 5x/wk x1wk, or the combination. Compared to untreated controls or either treatment alone, the combination showed significantly more tumors cured.



Conclusions

- COTI-2 is a highly potent small molecule efficacious against many HNSCC and other cancer cell lines.
- By reactivating normal p53 function, COTI-2 synergizes with radiation and some chemotherapy in HNSCC and other cancer cell lines.
- COTI-2 has strong potential for synergy with clinically relevant anticancer therapies.