

Elesclomol (Formerly STA-4783) Induces Oxidative Stress Selectively in Cancer Cells and Inhibits Tumor Growth in Combination with Paclitaxel and Carboplatin in Mouse Tumor Models

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Abstract (#3300)

Introduction: Elesclomol (formerly STA-4783) is a novel, small molecule investigational drug candidate that stimulates production of reactive oxygen species (ROS) and preferentially induces apoptosis in cancer versus normal cells. In a double-blind, randomized and controlled multi-center Phase 2b trial in patients with stage IV metastatic melanoma, treatment with elesclomol plus the microtubule stabilizer paclitaxel doubled median progression-free survival (PFS) relative to treatment with paclitaxel alone, with a safety profile comparable to paclitaxel alone. This represents the first time in over three decades that a blinded trial in metastatic melanoma has met its primary endpoint of PFS with statistical significance. Elesclomol was granted Fast Track and Orphan Drug status, and a global pivotal Phase 3 trial in combination with paclitaxel in chemotherapy-naïve stage IV metastatic melanoma patients is currently enrolling under a Special Protocol Assessment with the FDA (SYMMETRY™ trial).

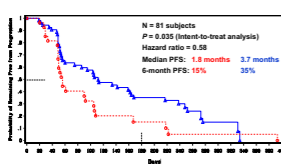
Results: *In vitro*, transcriptional profiling was performed on human Hs294T melanoma and Ramos B lymphoma cell lines treated with 100 nM elesclomol for 6 hours. Elesclomol induced a classic oxidative stress response characterized by increased expression of ROS-regulated genes such as *HSP70*, metallothionein, and antioxidants. ROS was induced beginning at 0.5 hr after treatment with elesclomol, followed by induction of *HSP70* beginning at 1 hr. Treatment with the anti-oxidant N-acetyl-L-cysteine (NAC) blocked this response, but had no effect on the induction of *HSP70* by a HSP90 inhibitor. Sustained ROS production resulted in activation of the mitochondrial apoptotic pathway as demonstrated by mitochondrial membrane depolarization and cytochrome c release at 3-6 hrs (data not shown), followed by cytochrome c release and caspase-3 activation at 6 hrs. Importantly, induction of oxidative stress by elesclomol preferentially occurred in tumor cells relative to normal cells such as primary human keratinocytes.

In vivo, elesclomol inhibited tumor growth as a single agent and synergized with paclitaxel in the human M14 melanoma xenograft tumor model conducted in T cell-deficient (*nu/nu*) mice. Elesclomol also synergized with a second microtubule stabilizer, docetaxel, in the human DU145 prostate carcinoma xenograft model. Since HSP70 protein has been reported to activate the immune system, we also examined synergy between elesclomol and paclitaxel in B/T cell-deficient (SCID or *Rag2* knockout) and B/T/NK cell-deficient (SCID-*Beige* or *Beige-nu-nu-Xid*) mouse strains implanted with the M14 melanoma cell line. In each case, elesclomol synergized with paclitaxel. Addition of elesclomol to the combination of paclitaxel plus carboplatin, which is commonly employed in the clinic, also demonstrated increased efficacy in the human HeLa cervical carcinoma xenograft model. Combination treatment did not alter the pharmacokinetics of elesclomol, paclitaxel or carboplatin (data not shown) or cause additional toxicity beyond that observed with the individual agents alone.

Conclusions: Our results demonstrate that elesclomol induced a classic oxidative stress response preferentially in tumor cells relative to normal cells, and that sustained ROS production led to activation of the mitochondrial apoptotic pathway. Furthermore, in mouse xenograft tumor models, synergy was observed between elesclomol and paclitaxel independent of immune status. Finally, the triple combination of elesclomol, paclitaxel and carboplatin demonstrated enhanced anti-tumor activity. Cancer cells produced increased ROS relative to normal cells, and hence exist in a state of elevated oxidative stress. Cancer cells are therefore susceptible to insults that further increase ROS levels, thus pushing the cancer cell beyond its tolerability limit for oxidative stress and leading to apoptosis. In this way, elesclomol takes advantage of a fundamental hallmark of cancer in order to chemosensitize and selectively kill cancer cells, with little effect on normal cells.

Background

Figure 1. Elesclomol plus paclitaxel doubled PFS in a controlled Phase 2b clinical trial in metastatic melanoma



Elesclomol was examined in a double-blind, randomized and controlled, Phase 2b trial in stage IV metastatic melanoma patients at 21 different centers in the US. The primary endpoint of the trial was met, with the combination of elesclomol plus paclitaxel doubling both median and 6-month PFS relative to paclitaxel alone (S. O'Day *et al.*, J Clin Oncol, 2007 ASCO Annual Meeting Proceedings Part 1, 25(18):5226, 2007). There was also a trend toward an overall survival advantage with the combination therapy (median overall survival was 11.9 months for the elesclomol plus paclitaxel group vs. 7.8 months for the paclitaxel alone group). Elesclomol was generally well-tolerated, with the most common adverse events in the elesclomol plus paclitaxel group including: fatigue, alopecia, constipation, nausea, hypoaesthesia, arthralgia, insomnia, diarrhea and anemia.

Results

Table 1. Elesclomol induced a transcriptional profile characteristic of an oxidative stress response

Gene	Expression	Annotation	Pathway
MTF1	↑	MTF1	Transcription
MTF2	↑	MTF2	Transcription
MTF3	↑	MTF3	Transcription
MTF4	↑	MTF4	Transcription
MTF5	↑	MTF5	Transcription
MTF6	↑	MTF6	Transcription
MTF7	↑	MTF7	Transcription
MTF8	↑	MTF8	Transcription
MTF9	↑	MTF9	Transcription
MTF10	↑	MTF10	Transcription
MTF11	↑	MTF11	Transcription
MTF12	↑	MTF12	Transcription
MTF13	↑	MTF13	Transcription
MTF14	↑	MTF14	Transcription
MTF15	↑	MTF15	Transcription
MTF16	↑	MTF16	Transcription
MTF17	↑	MTF17	Transcription
MTF18	↑	MTF18	Transcription
MTF19	↑	MTF19	Transcription
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MTF97	↑	MTF97	Transcription
MTF98	↑	MTF98	Transcription
MTF99	↑	MTF99	Transcription
MTF100	↑	MTF100	Transcription

The human Hs294T melanoma cell line was treated *in vitro* with 100 nM elesclomol for 6 hrs, and the expression of 47,000 transcripts was monitored using an Affymetrix GeneChip Human Genome U133A 2.0 array. Elesclomol induced the expression of 266 transcripts by >1.5 fold (relative to vehicle) and with a hybridization signal intensity of greater than 500 units. The top 30 most highly induced genes are shown (duplicate genes result from different probe sets interrogating the same transcript). In particular, elesclomol induced the expression of many genes known to be regulated by the heat shock stress response, including those encoding: heat shock 70kDa (HSPA6); heat shock 70kDa (HSPA1B); DNAJ (Hsp40) homolog member 1 (DNAJB1); BCL2-associated anthogone (BAG3); heat shock 70kDa (HSPA1A); spermidine/spermine N1 (SAT); heat shock 105/110kDa (HSPH1); crystalline alpha B (CRYAB); interleukin 8 (IL-8); and DNAJ (Hsp40) homolog member 9 (DNAJB9). Elesclomol also induced the metallothionein genes, which encode a family of metal-binding proteins with antioxidant activity, including: MTF1; MTF2; MTF3; MTF4; MTF5; MTF6; MTF7; MTF8; MTF9; MTF10; MTF11; MTF12; MTF13; MTF14; MTF15; MTF16; MTF17; MTF18; MTF19; MTF20; MTF21; MTF22; MTF23; MTF24; MTF25; MTF26; MTF27; MTF28; MTF29; MTF30; MTF31; MTF32; MTF33; MTF34; MTF35; MTF36; MTF37; MTF38; MTF39; MTF40; MTF41; MTF42; MTF43; MTF44; MTF45; MTF46; MTF47; MTF48; MTF49; MTF50; MTF51; MTF52; MTF53; MTF54; MTF55; MTF56; MTF57; MTF58; MTF59; MTF60; MTF61; MTF62; MTF63; MTF64; MTF65; MTF66; MTF67; MTF68; MTF69; MTF70; MTF71; MTF72; MTF73; MTF74; MTF75; MTF76; MTF77; MTF78; MTF79; MTF80; MTF81; MTF82; MTF83; MTF84; MTF85; MTF86; MTF87; MTF88; MTF89; MTF90; MTF91; MTF92; MTF93; MTF94; MTF95; MTF96; MTF97; MTF98; MTF99; MTF100.

Figure 2. Rapid induction of ROS by elesclomol was blocked by the antioxidant NAC

(Left) Human Ramos B lymphoma cells were treated with 100 nM elesclomol for the indicated times and then assayed for ROS levels by flow cytometry using 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), which is activated by intracellular esterases and then fluoresces when oxidized by ROS. Induction of ROS was observed 0.5 hr after treatment with elesclomol. (Middle) Ramos cells were treated with 100 nM elesclomol for the indicated times and then assayed for *HSP70* RNA levels by quantitative PCR analysis. Induction of *HSP70* RNA was observed by 1 hr after treatment with elesclomol. (Right) Ramos cells were treated with 100 nM elesclomol for 6 hrs, with 10 mM N-acetyl-L-cysteine (NAC), an antioxidant, added 30 min prior to the addition of elesclomol. NAC blocked *HSP70* RNA induction by elesclomol, but not by the unrelated HSP90 inhibitor STA-9090 (HSP90). The antioxidant Tron also blocked *HSP70* RNA induction by elesclomol in Ramos cells (data not shown).

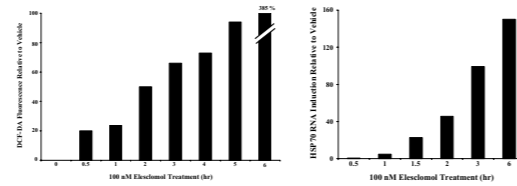


Figure 3. The entire transcriptional oxidative stress response induced by elesclomol is blocked by the antioxidant NAC

To assess whether oxidative stress mediated the entire transcriptional signature induced by elesclomol, a transcription profiling experiment was performed on Ramos cells treated with 100 nM elesclomol for 6 hrs. 984 transcripts were induced by elesclomol, and the top 35 most highly induced genes are shown. As in Table 1, elesclomol treatment alone induced a transcription profile characterized by increased expression of the heat shock responsive and metallothionein gene families. The addition of 5 mM NAC 30 min prior to elesclomol treatment potently inhibited the induction of the elesclomol-responsive gene profile. This result suggests that oxidative stress is responsible for the induction of most, if not all, genes by elesclomol and highlights the central importance of oxidative stress in the mechanism of action of elesclomol.

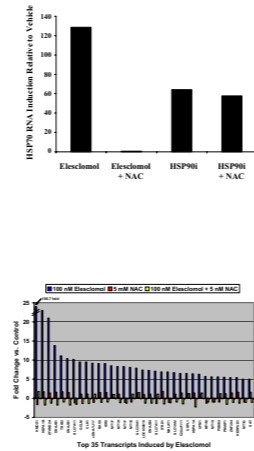


Figure 4. Elesclomol induced the mitochondrial apoptotic cascade

(Top) To examine induction of apoptosis by elesclomol, the human HSB2 T-cell leukemia cell line was treated for 18 hrs with 200 nM elesclomol in the presence or absence of 5 mM NAC (added 30 minutes prior to elesclomol), and then cultures were double-stained with annexin-V/PI (for apoptosis) and PI (for nuclear DNA content) and analyzed by flow cytometry. The subpopulations of live (annexin-V/PI negative), early apoptotic (annexin-V positive/PI negative), late apoptotic (annexin-V/PI positive) and dead (annexin-V negative/PI positive) cells are indicated. Induction of apoptosis by elesclomol was blocked by pretreatment with NAC. (Bottom) Ramos cells were treated with 100 nM elesclomol for the indicated times and then assayed for the amounts of cytosolic versus total mitochondrial cytochrome C and processed versus total caspase-3 by immunoblot analysis. Elesclomol induced cytochrome C release and caspase-3 activation by 6 hrs. Additionally, Ramos cells were treated with 100 nM elesclomol for the indicated times, labeled with the JC-1 probe for mitochondrial membrane potential and analyzed by flow cytometry. Elesclomol induced the loss of mitochondrial membrane potential by 6 hrs (data not shown).

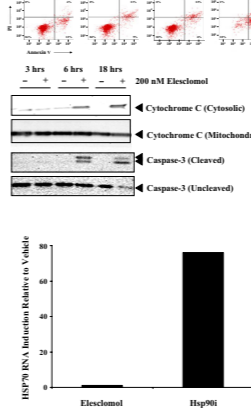


Figure 5. Elesclomol failed to induce HSP70 RNA in normal human cells

Normal human keratinocyte (NHK) were treated for 6 hrs with either 100 nM elesclomol or 100 nM of the HSP90 inhibitor STA-9090 (HSP90; Synta Pharmaceuticals Corp.) and assayed by qPCR. In contrast to the robust induction of *HSP70* RNA observed in tumor cell lines (Figure 2, bottom panel), elesclomol was unable to induce *HSP70* RNA in NHK cells. Similar results have also been observed with other normal human cell types, such as mammary epithelial cells and skin fibroblasts (data not shown).

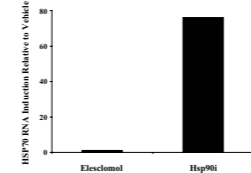


Figure 6. Elesclomol rapidly induces ROS and selectively activates the mitochondrial apoptotic cascade in cancer cells

Treatment of tumor cells with elesclomol, but not normal cells, rapidly induces ROS. This is followed by a classic oxidative stress response that is characterized by the induction of heat shock protein 70 (HSP70), other HSPs, metallothioneins and antioxidant proteins. However, this protective stress response is ultimately futile, and sustained oxidative stress leads to activation of the mitochondrial apoptotic cascade and tumor cell death.

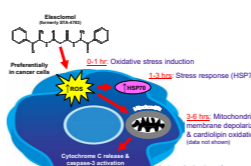


Figure 7. Elesclomol was active as a single-agent and synergized with paclitaxel without increased toxicity in a melanoma xenograft model

(Left) Intravenous (i.v.) treatment 5X/week with high-dose elesclomol alone significantly inhibited tumor growth in the M14 human melanoma xenograft model conducted in T cell-deficient *nu/nu* mice. (Middle) A low dosage of elesclomol that was not efficacious as a single agent synergized with the microtubule stabilizing agent paclitaxel and caused significant tumor regressions in 14/14 animals in the M14 model. No pharmacokinetic interactions between these dosages of elesclomol and paclitaxel were observed in mice (data not shown). (Right) Combination treatment with elesclomol and paclitaxel was well tolerated in the above study and did not significantly affect the cumulative average body weight change relative to treatment with either compound alone. (Methods) For all *in vivo* studies presented in this poster, tumor cells were subcutaneously implanted (with or without 50% Matrigel Basement Membrane Matrix) in the flanks of female mice, with the exception that the M14 cell line was implanted into the corpus adiposum (a fat body located at the juncture of the femur and pelvic bone). Unless otherwise noted, anti-cancer agents were formulated in 10/18 DRD (10% DMSO, 18% Cremophor RH40, 3.6% dextrose and 68.4% water) and were i.v. bolus dosed via the tail vein at 10 mL/kg. Combination dosing was performed by mixing both agents together in 10/18 DRD. %T/C values as a measure of efficacy are shown to the right of each tumor growth curve (T. Corbett *et al.*, 1997). Error bars represent +/- SEM. P values were generated by a one-way analysis of variance (ANOVA).

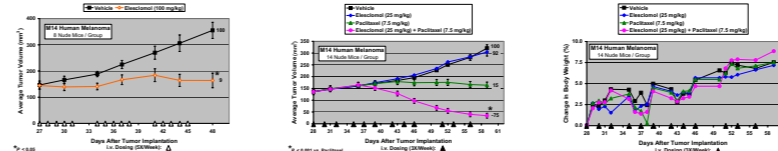


Figure 8. Elesclomol synergized with docetaxel in a prostate carcinoma xenograft model

A low dosage of elesclomol that was not efficacious as a single agent synergized with the microtubule stabilizing agent docetaxel in the DU145 human prostate carcinoma xenograft model conducted in *nu/nu* mice. No pharmacokinetic interactions between these dosages of elesclomol and paclitaxel were observed in mice (data not shown).

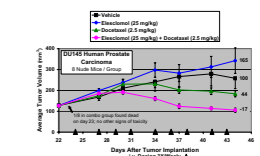


Figure 9. Elesclomol synergized with paclitaxel independent of immune status in a melanoma xenograft model

Since HSP70 protein has been reported to activate the immune system (Vega *et al.*, J Immunol 180:4299-307; 2008), synergy between elesclomol and paclitaxel were examined in xenograft models conducted on a variety of different immuno-deficient mouse backgrounds. A low dosage of elesclomol that was not efficacious as a single agent synergized with paclitaxel in the M14 human melanoma xenograft model conducted in B/T/NK cell-deficient *Beige-nu-nu-Xid* mice. Similar results were also observed in other mouse strains that were B/T cell-deficient (SCID or *Rag2* knockout) or B/T/NK cell-deficient (SCID-*Beige*). Additional studies are underway to examine the effect of deficiencies in other blood cell types, such as macrophages.

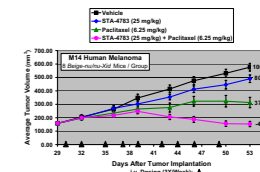


Figure 10. Elesclomol enhanced the combination of paclitaxel plus carboplatin in a cervical carcinoma xenograft model

Since paclitaxel plus carboplatin is commonly employed in the clinic, we examine the addition of elesclomol to this combination. Elesclomol and paclitaxel were dosed 3X/week together and carboplatin was dosed 2X/week on the off days. The triple combination of elesclomol/paclitaxel/carboplatin demonstrated superior efficacy to elesclomol/paclitaxel in the HeLa human cervical carcinoma xenograft model conducted in *nu/nu* mice. Similar results were observed when dosing 25 mg/kg carboplatin 1X/week (%T/C values of 63 and 42 for elesclomol/paclitaxel and elesclomol/paclitaxel/carboplatin, respectively). For clarity, the elesclomol alone, paclitaxel alone and carboplatin alone groups are not shown (%T/C values of 100, 89, 82, respectively). It is interesting to note that this model appears to be largely resistant to both paclitaxel and carboplatin as single agents. Combination treatment with elesclomol/paclitaxel/carboplatin was well tolerated in this study and did not significantly affect the cumulative average body weight change relative to the other treatment groups. No pharmacokinetic interactions between these dosages of elesclomol, paclitaxel and carboplatin were observed in mice (data not shown).

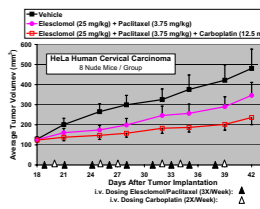
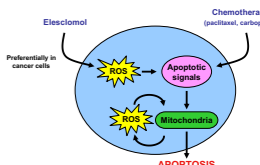


Figure 11. Elesclomol induced oxidative stress preferentially in cancer cells and enhances the *in vivo* efficacy of chemotherapy



Conclusions

- We provide evidence that elesclomol is a first-in-class anti-tumor agent that preferentially induced apoptosis in cancer cells relative to normal cells by elevating ROS levels beyond the tolerability limit for oxidative stress.
- Elesclomol displayed single-agent activity and enhanced the *in vivo* efficacy of multiple anti-cancer therapies in mouse tumor models: paclitaxel, docetaxel and the combination of paclitaxel plus carboplatin.
- Clinical proof-of-concept has been demonstrated in a double-blind, randomized and controlled multi-center Phase 2b trial in Stage IV metastatic melanoma patients in which a doubling of PFS was observed.
- The mechanism of action of elesclomol suggests broad potential for treating many additional tumor types beyond melanoma, particularly those with elevated oxidative stress, such as breast, hematological, ovarian, pancreatic and prostate cancers.
- Induction of oxidative stress by elesclomol represents a novel and promising approach to the treatment of cancer.