



AACR Abstract # 1401

TL-1836, A NEW ORALLY ACTIVE TAXANE WITH CURATIVE ANTITUMOR ACTIVITY AGAINST HUMAN TUMOR XENOGRAPTS

Robert Holton², Lewis Metts¹, Robert Abramowitz¹, Heather Arrington¹, Michael Edler¹, Everett Wilcox¹, Andy Williams¹, and Diego Zorio¹

¹Taxolog, Inc. Fairfield, NJ, Tallahassee, FL; ²Department of Chemistry, Florida State University, Tallahassee, FL

Abstract

Paclitaxel and docetaxel are the two taxanes currently used for the treatment of patients with a wide variety of tumors such as breast cancer and non-small cell lung cancer (NSCLC). However, both paclitaxel and docetaxel are not effective in resistant models such as those tumors that over express p-glycoprotein (MDR1). There is a strong need for a better taxane in order to achieve long term survival and objective responses. The antitumor activity of TL-1836 was evaluated *in vivo* in numerous human tumor cell lines and *in vivo* in athymic nude mice bearing human tumor xenografts derived from several different histological origins. The *in vitro* cell lines tested included a wide variety of human cancer cell lines: renal (2); melanoma (3); ovarian (2); lung (2); brain (1); colon (2); and mesothelioma (1). TL-1836 was more potent than paclitaxel in all cell lines and more potent than docetaxel in all but one cell line. In the taxane resistant cell lines such as OVCAR5 (ovarian), DLD-1 (colon), MSTO-211H (mesothelioma), 786-0 (renal) and A375 (melanoma), TL-1836 was at least 10 times more potent than paclitaxel. These cell lines included MDR1 resistant models and some models with other mechanisms of resistance. The *in vivo* activity of TL-1836 was tested in athymic nude mice in four different xenograft models. TL-1836 when administered intravenously (i.v.) at a single dose (QDx1) of 30 mg/kg was completely curative in the MX1 human breast cancer model. TL-1836 when dosed i.v. at 30 mg/kg on an every four day schedule for four doses (Q4Dx4) was completely curative in the A375 human melanoma model. Docetaxel was not effective in this model. In the taxane resistant MSTO-211H human mesothelioma model, TL-1836 when dosed i.v. at 30 mg/kg Q4Dx4 showed greater than 50% tumor regression, while docetaxel was not effective in this model. In the HT29 human colon model, TL-1836 was dosed orally at 50 mg/kg Q4Dx4 and i.v. at 25 mg/kg Q4Dx4. On the oral dosing schedule TL-1836 showed 3 partial regressions and 3 complete regressions with 3 long term tumor free survivors (TFS). The oral bioavailability is estimated to be greater than 50%. The toxicity of TL-1836 in rats was compared to docetaxel at 12 mg/kg at both a QDx1 and Q7Dx3 schedule. The overall toxicity of the two compounds was similar except the docetaxel treated rats showed high amounts of axonal degeneration on the Q7Dx3 schedule, while the TL-1836 treated animals showed almost no axonal degeneration. The lack of axonal degeneration in rats with TL-1836 treatment may provide advantages in the clinic with lessened neurotoxicity compared to paclitaxel and docetaxel. In summary, the curative and oral activity of TL-1836 in human xenografts, activity against resistant tumor lines, and its neurotoxicity profile in rats distinguishes it from the clinically useful taxanes, paclitaxel and docetaxel.

Materials and Methods (cont'd)

XENOGRFT STUDIES

MSTO-211H mesothelioma xenograft studies were conducted at Taxolog Inc., Tallahassee, Florida. HT29 human colon, MX1 human breast and A375 human melanoma xenograft studies were conducted at Piedmont Research, North Carolina.

Mice

Female athymic nude mice (Harlan) were 13-14 weeks old on Day 1 of the study. The animals were given *ad libitum* access to water (reverse osmosis, 1 ppm Cl) and irradiated Lab Diet consisting of 18.0% crude protein, 5.0% crude fat, and 5.0% crude fiber. The mice were housed on ALPHA-dri[®] bed-o-cobs[®] Laboratory Animal Bedding in static microisolators on a 12 hour light cycle at 21-22 °C (70-72 °F) and 40-60% relative humidity

Tumor Implantation

All tumor lines used for this study were maintained in athymic nude mice. A tumor fragment (~1 mm³) was implanted subcutaneously (s.c.) into the right flank of each test mouse. Tumors were monitored twice weekly and then daily as their volumes approached 200-400 mm³. On day 1 of the study, the animals were sorted into treatment groups and each group's mean tumor size determined.

$$\text{Tumor Volume (mm}^3\text{)} = \frac{w^2 \times l}{2}$$

Where w = width and l = length in mm of the tumor. Tumor weight was estimated with the assumption that 1 mg is equivalent to 1 mm³ of tumor volume.

Drugs and Formulation

TL-1836 for oral dosing in the HT29 xenograft study and in the MX1 i.v. xenograft study was first dissolved in 50% ethanol and 50% Cremophor[®] EL to prepare a stock solution. The stock solutions were diluted with D5W (5% dextrose in water) or normal saline immediately prior to dosing to yield dosing solutions in a vehicle consisting of 10% ethanol, 10% Cremophor[®] EL, and 80% D5W for i.v. administration or 5% ethanol, 5% Cremophor[®] EL and 90% normal saline for oral administration. For i.v. administration in the remaining xenograft models, TL-1836 was dissolved in 100% ethanol to prepare a 20X stock solution. The stock solutions were diluted with Liposyn[®] II 20% on each day of dosing to yield dosing solutions in a vehicle consisting of 5% ethanol, 95% Liposyn[®] II (5% 95% L-I). Docetaxel was dissolved in 50% ethanol and 50% Tween[®] 80 to prepare a 6.67X stock solution. The docetaxel stock solution was diluted with D5W immediately prior to dosing to yield a dosing solution in a vehicle consisting of 7.5% ethanol, 7.5% Tween[®] 80, and 85% D5W (7.5%E 7.5%T in D5W).

Treatment

Mice were sorted into appropriate groups with five or six mice per group, and treated in accordance with the protocol for each study. Some studies included docetaxel as a control. Docetaxel was always administered at its optimum dose (25 or 30 mg/kg), route (intravenously, i.v.), and schedule (weekly for three cycles, Q7Dx3). Administration of TL-1836 was either i.v. or oral (po) in the case of the HT29 xenograft study. Control group mice received vehicle. Treatment schedules tested for TL-1836 included once daily (QDx1) and an every four days times four cycles (Q4Dx4).

Mean Days of Survival

The mean days of survival (MDS) values were calculated for all groups. MDS values were the mean number of days required for the tumor to reach a specified weight (either 1.2 g or 2.0 g), depending on the study.

Tumor Regressions

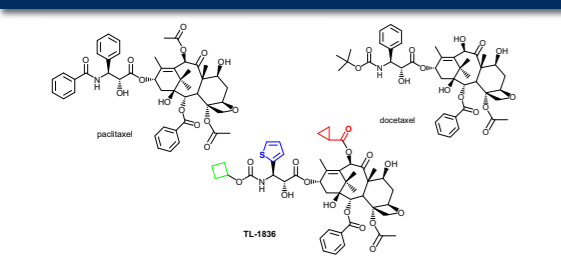
Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume is 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm³ for one or more of these three measurements. In a CR response, the tumor volume is less than 13.5 mm³ for three consecutive measurements during the course of the study. An animal with a CR response at the termination of a study is additionally classified as a long-term tumor free survivor (TFS).

Statistical and Graphical Analyses

The log rank test was employed to analyze the significance of the difference between the time to endpoint (TTE) values of a drug-treated group and the vehicle-treated control group. The log rank test analyzes the data for all animals except the non-treatment related (NTR) deaths. The two-tailed statistical analyses were conducted at P = 0.05, using Prism 3.03 (GraphPad) for Windows.

RAT TOXICITY STUDIES

Structures



Materials and Methods

CYTOTOXICITY AND IC₅₀ DETERMINATION

TL-1836, paclitaxel and docetaxel were analyzed for their effects on proliferation of the following cell lines from American Type Tissue Culture: HT29 (human colon adenocarcinoma), DLD-1 (human colon adenocarcinoma), PANC-1 (human pancreatic adenocarcinoma), A549 (human lung carcinoma), A375 (human skin melanoma), MALME-3 (human skin melanoma), MSTO-211H (human pleural mesothelioma), 786-0 (human renal cell adenocarcinoma), SK-MEL-28 (human skin melanoma) and cell lines from NCI DCTD Tumor/Cell Line Repository: SNB-19 (human brain glioblastoma), HOP-18 (human lung NSCLC), OVCAR4 (human ovarian carcinoma), OVCAR5 (human ovarian carcinoma), TK-10 (renal cell carcinoma). All cell lines were maintained in RPMI-1640 tissue culture medium (TCM), supplemented with antibiotics and 10% fetal bovine serum, and cultured at 37°C in humidified air containing 5% CO₂. To assess the antiproliferative effects of the test compounds, 172 µL of tumor cell suspension (1.45 x 10⁵ cells/ml) were added to each well of a 96-well plate and incubated for 24 h at 37°C in 5% CO₂ in humidified air to allow cells to adhere. Seven 2-fold drug dilutions in TCM/Dimethyl sulfoxide (DMSO) were performed in triplicate in separate 96-well plates and 28 µL was transferred to the wells containing tumor cells (200 µL final volume/1% DMSO). Plates were incubated for 72 h and cell viability was determined by adding 50 µL of warm TCM containing 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to each well and incubating for 1 h at 37°C. Plates were processed and the absorbance at 570 nm was measured by a plate reader. The absorbance of the test wells were divided by the absorbance of drug-free wells and the concentration of the agent that resulted in 50% of the absorbance of untreated cultures (IC₅₀) was determined by analyses of best fit curve of the data. (GraphPad Prism version 4.00 for Windows)

Results

Comparative Cytotoxic Effects of TL-1836, Paclitaxel and Docetaxel

The *in vitro* cytotoxic activity of TL-1836 compared to that of paclitaxel and docetaxel in taxane sensitive and taxane resistant/refractory human tumor cell lines is shown in Table 1 and Table 2. Table 2 shows cell lines with reported mechanisms of resistance such as MDR, MRP or BCRP. The results in Table 2 show that TL-1836 exhibited potent cytotoxicity against the DLD-1 colon carcinoma which over expresses p-glycoprotein (MDR1) and is resistant to both paclitaxel and docetaxel. TL-1836 was at least 20 to 40 fold more potent compared to both paclitaxel and docetaxel in killing DLD-1 tumor cells *in vitro*. In the mesothelioma cancer cell line, MSTO-211H which over expresses MRP2 and BCRP1 and is resistant to both paclitaxel and docetaxel *in vivo*, TL-1836 is at least 6 times more potent. In the A375 melanoma model which has been reported to have high levels of MRP9 (Taxolog, Inc. data not shown) and is resistant to both paclitaxel and docetaxel *in vivo*, TL-1836 is approximately 4 times more potent than docetaxel and greater than 10 times more potent than paclitaxel. In the 786-0 renal model that also over expresses p-glycoprotein but to a lesser extent than DLD-1, TL-1836 is at least 10 times more potent than paclitaxel. In every model tested as shown in Table 1 and Table 2, TL-1836 has superior *in vitro* cell cytotoxicity when compared to paclitaxel and docetaxel (Except Malme3). In those models that over express MDR1, MRP2, MRP9 or BCRP1, TL-1836 retains its activity while the activity of docetaxel and paclitaxel are reduced.

XENOGRFT STUDIES

Summary: Effects of TL-1836 on MX1 human breast murine xenografts

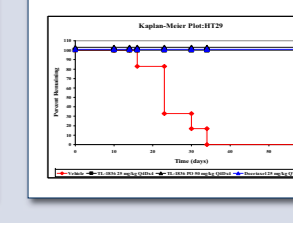
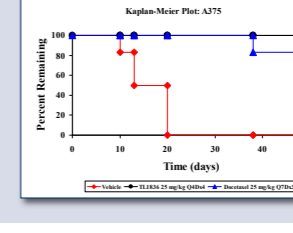
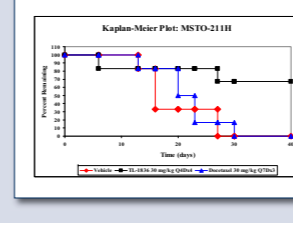
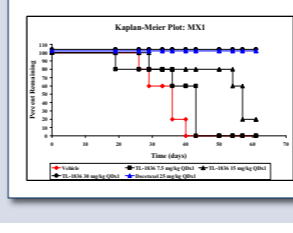
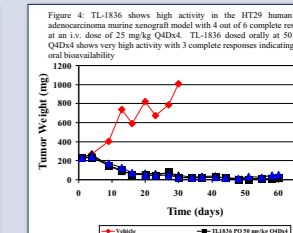
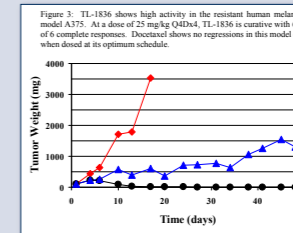
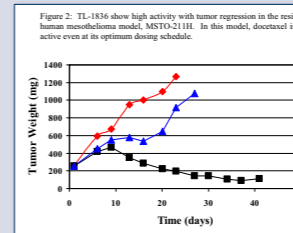
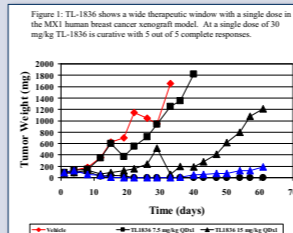
TL-1836 was dosed i.v. at 7.5, 15, and 30 mg/kg QDx1, respectively. At 7.5 mg/kg, TL-1836 produced a 13% tumor growth delay (TGD) and non-significant activity. At 15 mg/kg TL-1836 produced a 76% TGD, significant activity (P < 0.05), one day 61 survivor with a 109g tumor, one PR, and two transient CRs. At 30 mg/kg TL-1836 produced a 90% TGD, significant activity (P < 0.01), and 5 out of 5 TFS. TL-1836 at 15 and 30 mg/kg caused 6.8% and 16% maximum group mean BW losses respectively on day 5. Docetaxel at 25 mg/kg QDx1 yielded 1 transient CR and 4 out of 5 TFS, with a 10.2% group mean BW loss. The mean tumor growth and Kaplan-Meier curves for these groups are shown in Figure 1.

Table 1: Cytotoxicity (IC₅₀ nM) in a panel of cancer cells

	Paclitaxel	Docetaxel	TL-1836
Human: Colon	4.1	2.8	3.8
Human: Lung	4.8	3.8	4.8
Human: Breast	4.8	6.5	5.8
Human: Ovary	2.9	7.2	5.4
Human: Skin	5.4	6.1	6.1
Human: Bladder	2.9	1.8	1.4
Human: Stomach	1.4	3.4	2.1
Human: Pancreas	1.2	8.5	8.5
Human: Ovary	4.1	6.3	5.1
Human: Skin	8.7	8.8	8.8

Table 2: Cytotoxicity (IC₅₀ nM) in a panel of cancer cells with known mechanisms of resistance

	DLD1 Colon	MSTO Mesothelioma	786-0 Renal	A375 Melanoma
Resistance	MDR1+	MRP2/BCRP1	MDR1+	A375 MRP9
Paclitaxel	41	6.2	>10	8.0
Docetaxel	20	4.0	6.2	2.4
TL-1836	1.1	0.7	1.1	0.6



Conclusions

TL-1836 is a highly potent taxane analog with high *in vitro* activity in a wide variety of human cancer cells. TL-1836 shows remarkable activity in cell lines that over express MDR1, MRP and BCRP that are typically resistant to paclitaxel and docetaxel. TL-1836 is highly efficacious when administered i.v. at both single dose and multidose schedules in a number of taxane sensitive and taxane resistant tumor models. In particular, TL-1836 was curative in the A375 human melanoma murine xenograft model where docetaxel showed no tumor regression. In addition, contrary to paclitaxel and docetaxel, TL-1836 possesses remarkable oral activity as demonstrated

in the HT29 human colon cancer murine xenograft model. Based on its low axonal degeneration in rats, TL-1836 may have decreased neurotoxicity when compared to docetaxel. Less neurotoxicity would provide TL-1836 with a clinical advantage over docetaxel. TL-1836's superior efficacy, both *in vitro* and *in vivo*, combined with its oral bioavailability and with a favorable toxicity profile in i.v. dosed rats warrants its advancement to Phase I clinical trials.

Acknowledgements

Beth Hollister, Shih-Fong Chen, Robert Mullin and Chuck Harrison, Piedmont Research Center, Morrisville, NC.