



PRECLINICAL EVALUATION OF TL-1892, A NOVEL ORALLY ACTIVE TAXANE WITH SUPERIOR *IN VITRO* AND *IN VIVO* EFFICACY IN PACLITAXEL AND DOCETAXEL RESISTANT MODELS

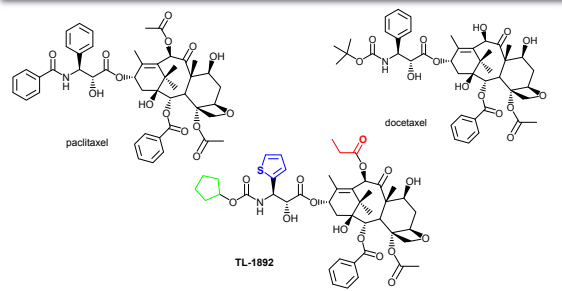
Robert Holton², Lewis Metts¹, Robert Abramowitz¹, Heather Arrington¹, Michael Edler¹, Everett Wilcox¹, Andy Williams¹, and Diego Zorio¹

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

Abstract

Paclitaxel and docetaxel have demonstrated parenteral, but not oral, activity in some *in vitro* and *in vivo* preclinical tumor models. However, neither paclitaxel nor docetaxel are effective in resistant models such as those tumors that over express p-glycoprotein (MDR1). The comparative cytotoxic effects of TL-1892, paclitaxel and docetaxel were tested in a panel of 14 different cell lines using the *in vitro* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell lines tested included a wide variety of human cancer cell lines: renal, melanoma, ovarian, lung, brain, colon, pancreatic and mesothelioma. TL-1892 was more potent than paclitaxel and docetaxel in all fourteen human cancer cell lines. In the taxane resistant cell lines, such as TK10 (renal), OVCAR-5 (ovarian), DLD-1 (colon), MSTO-211H (mesothelioma), 786-0 (renal) and A375 (melanoma), TL-1892 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-1892 was tested in athymic nude mice in four different xenograft models. TL-1892 dosed intravenously (i.v.) at 30 mg/kg was completely curative in the MX1 human breast cancer model. TL-1892 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 when dosed on its optimum schedule of every 7 days for three doses (Q7Dx3). In the taxane resistant MSTO-211H human mesothelioma model, TL-1892 when dosed at 25 mg/kg Q4Dx4 showed approximately 90% tumor regression. Docetaxel was not effective in this model. In the HT29 human colon model, TL-1892 was dosed orally at 50 mg/kg Q4Dx4 and i.v. at 25 mg/kg Q4Dx4. Both dosing regimens showed similar activity with greater than 90% tumor regression indicating a high bioavailability of TL-1892 when dosed orally. The toxicity of TL-1892 and docetaxel in rats was compared. The docetaxel treated rats showed high amounts of axonal degeneration while the TL-1892 treated animals showed almost no axonal degeneration indicating that TL-1892 may have advantages in the clinic with lessened neurotoxicity compared to paclitaxel and docetaxel. In summary, the oral activity of TL-1892 along with its *in vivo* and *in vitro* activity in resistant tumors and its neurotoxicity profile in rats distinguishes it from the clinically useful taxanes, paclitaxel and docetaxel.

Structures



Materials and Methods

CYTOTOXICITY AND IC₅₀ DETERMINATION

TL-1892, 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 10⁶ cells/ml) were added to each well of a 96-well plate and incubated for 24 h at 37°C in 5% CO₂ in 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/0.1% DMSO). Plates were incubated for 72 h and cell viability was determined by adding 50 µL of warm TCM containing 5 mg/mL MTT to each well and incubating for 1 h at 37°C. Plates were processed and the absorbancy of the resulting solutions were measured by a plate reader at 570 nm. 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)

Materials and Methods (cont'd)

XENOGRAFT 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 fed *ad libitum* water (reverse osmosis, 1 ppm Cl) and NIH 31 Modified 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-dm[®] 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 groups mean tumor sizes 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-1892 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-1892 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 and 95% Liposyn[™] II (5%K 95% L-H). 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-1892 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-1892 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 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

Rat toxicity studies were conducted with female Charles River Sprague-Dawley rats approximately 225 to 250g split into groups of three with similar body weights (BW) per group. The formulation consisted of 10% ethanol, 10% Cremophor[®] and 80% saline. Dosing was done either on a single dose schedule or a weekly schedule for three doses. Body weights were taken on day 1, 8 and 10 on the single dose schedule. Additional body weight measurements were taken when dosed weekly for three weeks (dosed on day 1, 8 and 15) for up to 24 days. Animals were observed daily for evidence of toxicity or mortality. Blood was collected on day 4 and 10 and also on days 18 and 24 for the groups with weekly administration for Complete Blood Count and Differential (CBC/Diff.). Clinical chemistry was done on day 4 and 10. At the end of the study, the sciatic nerve was analyzed for axonal degeneration by a scoring system from 0 to 4. A score of 0 indicated no observed degeneration in the specified viewing area, with 1 indicating 1-3 axons degenerated, up to greater than 50+ for a score of 4 which indicates very high neurotoxicity. A score of 1 is not significantly different than background from vehicle control.

Results

Comparative Cytotoxic Effects of TL-1892, Paclitaxel and Docetaxel

The *in vitro* cytotoxic activity of TL-1892 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-1892 exhibited potent cytotoxicity against the DLD-1 colon carcinoma which over expresses p-glycoprotein (MDR1) and is resistant to both paclitaxel and docetaxel. TL-1892 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-1892 is at least 10 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-1892 is approximately 5 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-1892 is greater than 10 times more potent than paclitaxel. In every model tested, as shown in Tables 1 and 2, TL-1892 has superior cell cytotoxicity when compared to paclitaxel and docetaxel. In those models that over express MDR1, MRP2, MRP9 or BCRP1, TL-1892 retains its activity while the activity of docetaxel and paclitaxel are reduced.

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

	Panc1	HT29	A549	TK10	MALME3	OVCAR2	OVCAR4	HOP18	MALME3	OVCAR4
Resistance	Colon	Lung	Renal	Skin	Ovarian	Ovarian	Lung	Skin	Skin	Ovarian
Paclitaxel	4.1	2.8	3.8	4.8	6.5	5.6	2.9	7.2	8.4	6.1
Docetaxel	2.0	1.3	1.3	1.4	1.8	1.1	1.4	1.4	2.1	1.9
TL-1892	6.5	0.4	0.4	0.3	1.8	0.2	1.0	1.4	0.6	1.0

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

	DLD1	MSTO	786-0	A375
Colon	Mesothelioma	Renal	Melanoma	
Resistance	MDR1+	MRP2/BCRP1	MDR1+	MRP9
Paclitaxel	41	6.2	>10	8.0
Docetaxel	20	4.0	6.2	2.4
TL-1892	0.8	0.4	0.7	0.5

Figure 1: TL-1892 shows a wide therapeutic window with a single dose in the MX1 human breast cancer xenograft model. At a single dose of 30 mg/kg TL-1892 is curative with 5 out of 5 complete responses.

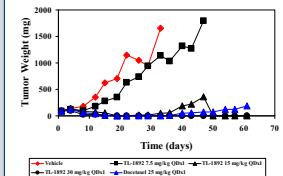


Figure 2: TL-1892 shows high activity with tumor regression in the resistant human mesothelioma model, MSTO-211H. In this model, docetaxel is not active even at its optimum dosing schedule.

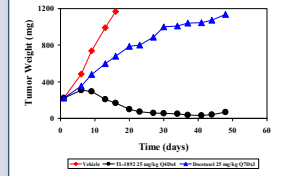


Figure 3: TL-1892 shows high activity in the resistant human melanoma model A375. At a multiple dose of 30 mg/kg TL-1892 is curative with 6 out of 6 complete responses. Docetaxel shows little activity in this model even when dosed at its optimum dosing schedule.

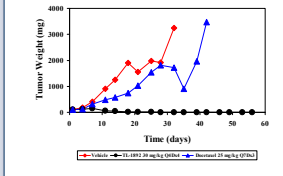
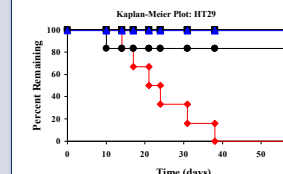
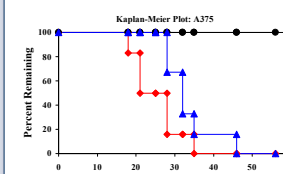
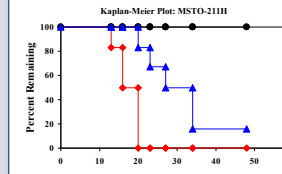
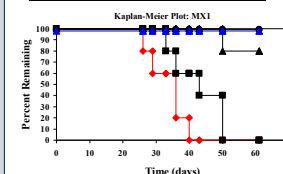
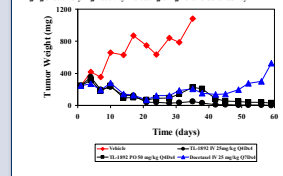


Figure 4: TL-1892 shows high activity in the HT29 human colon adenocarcinoma xenograft model with 4 out of 4 complete responses at an IV dose of 25 mg/kg on a multiple dose schedule. TL-1892 dosed orally on the same dosing schedule at 50 mg/kg shows very high activity indicating a high oral bioavailability.



Conclusions

TL-1892 is a highly potent taxane analog with impressive *in vitro* activity in a wide variety of human cancer cells. TL-1892 shows remarkable activity in cell lines which over express MDR1, MRP and BCRP that are typically resistant to paclitaxel and docetaxel. TL-1892 is highly efficacious when administered i.v. at both single dose and multiple dose schedules, in a number of taxane sensitive and taxane resistant tumor models. In particular, TL-1892 was curative in the A375 human melanoma murine xenograft model where docetaxel showed no tumor regression. In addition, contrary to paclitaxel and docetaxel, TL-1892 possesses remarkable oral activity

XENOGRAFT STUDIES

Summary: Effects of TL-1892 on MX1 human breast murine xenografts

TL-1892 was dosed i.v. at 7.5, 15, and 30 mg/kg, QDx1 respectively. At 7.5 mg/kg TL-1892 produced a 17% TGD (tumor growth delay), and non-significant activity. Activity increased sharply at the 15 mg/kg dose, which generated 4 out of 5 TFS, with 6.6% maximum group mean BW loss. The 30 mg/kg treatment yielded 5 out of 5 TFS and caused 15.5% maximum group mean BW loss. Docetaxel at 25 mg/kg QDx1 yielded 1 transient CR and 4 TFS, with 10.2% maximum group mean BW loss. The mean tumor growth and Kaplan-Meier curves for these groups are shown in Figure 1.

Summary: Effect of TL-1892 on MSTO-211H human mesothelioma murine xenografts

TL-1892 was dosed i.v. at 25 mg/kg Q4Dx4 and docetaxel was dosed i.v. at 25 mg/kg Q7Dx3. TL-1892 showed a marked 86% tumor regression with a nadir at day 41 and a mean tumor weight of 32 mg compared to the initial tumor weight of 221 mg. Docetaxel showed no tumor regression in this model. On day 48, when the study was terminated, all of the TL-1892 animals were still alive while none of the docetaxel animals remained. Docetaxel showed high weight loss in this model with a 37.9% maximum group mean BW loss on day 37 compared to a 23.9% maximum mean BW loss on day 23 for the TL-1892 treated group. The mean tumor growth and Kaplan-Meier curves for these groups are shown in Figure 2.

Summary: Effect of TL-1892 on A375 human melanoma murine xenografts

TL-1892 had curative activity in A375 melanoma when dosed i.v. at 25 mg/kg, Q4Dx4. TL-1892 produced CR in all 6 treated mice and all of these were TFS. The regressions were initially evident on days 11-18. The TGD was the maximum possible 37.9 days (p=0.0005). Docetaxel dosed i.v. at 25 mg/kg Q7Dx3 produced a modest TGD of 8.9 days. This TGD was statistically significant compared to the vehicle control (p = 0.049). Docetaxel produced no regressions of A375 melanoma. The i.v. dosed TL-1892 caused a 13.3% maximum group mean BW loss on day 18 while the i.v. dosed docetaxel caused a 4% maximum group mean BW loss on day 25. The mean tumor growth and Kaplan-Meier curves for these groups are shown in Figure 3.

Summary: Effects of TL-1892 on HT-29 human colon murine xenografts

Oral and i.v. TL-1892 dosed Q4Dx4 at 50 and 25 mg/kg respectively each produced a 237% TGD. Oral activity was strong and highly significant (P < 0.001). The i.v. activity was stronger but had one treatment related death. In the orally treated group, the mean tumor weight (n=6) was 21 mg and five PRs and one TFS were recorded. In the i.v. treated group, five mice survived to day 59 with 0 mg tumors, and each mouse classified as TFS. Docetaxel i.v. at 25 mg/kg, QD7x3 produced highly significant antitumor activity (P < 0.001), and a median TTE of 59.0 days. This median TTE corresponds to a 41.5-day T-C and 237% TGD. The mean tumor weight (n=6) was 600 mg, and three PRs were recorded. Intravenous TL-1892 caused a 13.5% maximum group mean BW loss on day 17 while oral TL-1892 caused a 10.4% maximum group mean BW loss on day 17. Docetaxel caused a 5.6% maximum group mean BW loss on day 24. The mean tumor growth and Kaplan-Meier curves for these groups are shown in Figure 4.

RAT TOXICITY STUDIES

TL-1892 was tested side by side with docetaxel at 12 mg/kg (72 mg/m²) in a rat toxicity study. Both compounds were dosed QDx1 and Q7Dx3. The QDx1 groups were followed for 10 days after dosing and the Q7Dx3 groups were followed for 24 days after dosing. There was no mortality observed in any group. The results of the study indicate that TL-1892 may have an advantage over docetaxel in neurotoxicity based on the analysis of axonal degeneration. The results of the study are contained in Table 3.

Table 3: Toxicity of TL-1892 compared to docetaxel in rats when dosed as a single dose at 12 mg/kg (72 mg/m²) and on a weekly schedule for three weeks

Compound	Dose mg/kg	Schedule	Weight Loss %	Axonal Degeneration Animal 1	Axonal Degeneration Animal 2	Axonal Degeneration Animal 3
Docetaxel	12	QDx1	8.7			
TL-1892	12	QDx1	8.8			
Docetaxel	12	Q7Dx3	18.5	+++	+++	+++
TL-1892	12	Q7Dx3	26.0	++	++	+

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