Peloruside A inhibits growth of human lung and breast tumor xenografts in an athymic
\textit{nu/nu} mouse model

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**Running title.** Peloruside A prevents tumor growth

**Key words.** antitumor, peloruside, paclitaxel, docetaxel, xenograft, doxorubicin

**Abbreviations.** MDR, multiple drug resistance; MSA, microtubule-stabilizing agent; MTD, maximum tolerated dose; NSCLC, non-small cell lung carcinoma; TGI, tumor growth inhibition

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Financial Support
This project was supported by funding from Reata Pharmaceuticals, Inc (C.J. Meyer, M. Krauth), Cancer Society of New Zealand (E1807) (J.H. Miller), Wellington Medical Research Foundation (E1707) (J.H. Miller, P.T. Northcote), Victoria University of Wellington (80824) (J.H. Miller). J.K. De Brabander thanks the Robert A. Welch Foundation for support (grant I-1422) (J.K. De Brabander) and holds the Julie and Luis Beecherl, Jr., Chair in Medical Science (J.K. De Brabander).

Conflict of Interest
Peter Northcote and John Miller are named on a US patent for development of peloruside A as a chemotherapeutic agent. University of Texas Southwestern Medical Center and Reata Pharmaceuticals, Inc had an agreement, now lapsed, with Victoria University of Wellington for development of peloruside A as an anticancer drug. Jef K. De Brabander and Jerry Shay are consulting for Reata Pharmaceuticals, Inc.
Abstract

Peloruside A is a microtubule-stabilizing agent isolated from a New Zealand marine sponge. Peloruside prevents growth of a panel of cancer cell lines at low nanomolar concentrations, including cell lines that are resistant to paclitaxel. Three xenograft studies in athymic nu/nu mice were performed to assess the efficacy of peloruside compared to standard anticancer agents such as paclitaxel, docetaxel, and doxorubicin. The first study examined the effect of 5 and 10 mg/kg peloruside (QDx5) on the growth of H460 non-small cell lung cancer xenografts. Peloruside caused tumor growth inhibition (%TGI) of 84% and 95%, respectively; whereas, standard treatments with paclitaxel (8 mg/kg, QDx5) and docetaxel (6.3 mg/kg, Q2Dx3) were much less effective (%TGI of 50% and 18%, respectively). In a second xenograft study using A549 lung cancer cells and varied schedules of dosing, activity of peloruside was again superior compared with the taxanes with inhibitions ranging from 51 to 74%, compared to 44% and 50% for the two taxanes. A third xenograft study in P-glycoprotein-overexpressing NCI/ADR-RES breast tumor model showed that peloruside was better tolerated than either doxorubicin or paclitaxel. We conclude that peloruside is highly effective in preventing the growth of lung and P-glycoprotein-overexpressing breast tumors in vivo and that further therapeutic development is warranted.
Introduction

Peloruside A (peloruside) (Fig. 1), a marine sponge secondary metabolite originally isolated by West et al. (1) from *Mycale henscheli* collected in Pelorus Sound off the coast of New Zealand, is a potent microtubule stabilizing agent (MSA) (2) similar to paclitaxel, docetaxel, and ixabepilone (aza-epothilone B). Peloruside shows activity in the low nanomolar range against proliferation of cultured cancer cell lines (Table 1). Although standard chemotherapeutic agents such as paclitaxel (Taxol®) and docetaxel (Taxotere®) have been extremely effective in treating solid tumors of the lung, breast, and ovary, they have significant off-target toxic effects in addition to a low aqueous solubility, making it necessary to use a solubilizing agent such as Cremophor EL for paclitaxel and polysorbate 80 (Tween 80) for docetaxel for clinical use (3-6). Allergic and anaphylactic reactions to the vehicles used with the taxanes can cause patient discomfort and even death (6). The taxanes are also highly susceptible to development of multiple drug resistance (MDR) by overexpression of drug efflux pumps, in particular the P-glycoprotein (P-gp) pump (7, 8). Thus, new generation MSAs are being sought that have similar anticancer cell activity, reduced side effects, improved tolerability, and are not overly sensitive to drug efflux pump activity. A semisynthetic taxane derivative, cabazitaxel, has been developed that shows activity in a range of taxane-resistant tumor models and has been effective in clinical trials against docetaxel-resistant breast and prostate cancer (9). Cabazitaxel possesses similar microtubule-stabilizing activity as paclitaxel and docetaxel. The nature of its activity in docetaxel-resistant tumors is unknown since it is active in some Pgp overexpressing cell lines but ineffective in others, an example of the latter being the breast cancer cell line Calc18TXT that developed resistant to docetaxel in vitro as a result of overexpression of Pgp. Two other MSAs that bind to the same site on β-tubulin as the taxanes (10), epothilone and discodermolide, have entered
clinical trials, with the epothilone B derivative, ixabepilone (Ixempra<sup>®</sup>), reaching the market in 2007 as a therapeutic agent against metastatic breast cancer (11). Ixabepilone, however, similar to the taxanes, has significant side effects, including peripheral neuropathy, nausea, muscle pain, joint pain, and leucopenia and has recently been withdrawn from the New Zealand market due to lack of interest. Discodermolide entered phase 1 clinical trials with Novartis in 2004, but was later discontinued in development due to pneumotoxicity. It was then picked up by Kosan Biosciences in conjunction with Amos Smith’s research laboratory at the University of Pennsylvania where its binding domain is being characterized and its structure-activity relationships are being used to design and synthesize novel analogues with improved activity compared with the natural product (12).

Peloruside, another MSA under development as an anticancer agent, is particularly interesting because unlike the other new generation MSAs discussed above, peloruside binds to a unique, non-taxoid site on β-tubulin that it shares with another marine sponge natural product, laulimalide (10, 13-15). In addition, peloruside and laulimalide, similar to the epothilones and discodermolide, retain their activity in cells that overexpress the P-gp efflux pump (13, 14), thus remaining active in cells that have acquired resistance to the taxanes by Pgp overexpression. Although peloruside inhibits proliferation of various cancer cell lines (1, 14, 16-18), as well as activated T cells in a murine model of multiple sclerosis (19), in non-replicating cells such as bone marrow-derived macrophages and unstimulated T cells (19), peloruside shows limited to no cytotoxic activity in these non-mitotic cells. Although cancer-specific targeting has also been reported in which ras-transformed murine cancer cells (32D-ras) were more sensitive to peloruside compared to their parental cell line (32D), the mechanism of the cancer cell-selective action may not be antimitotic since the generation times of the two cell lines were similar (20). Non-mitotic actions of microtubule targeting
agents have been recently reviewed (21, 22). A preclinical study using xenografts in mice was carried out with laulimalide that gave discouraging results because of significant toxicity and poor effectiveness in inhibiting tumor growth (23), although results were more encouraging in a second *in vivo* study on laulimalide (24).

The aim of the present study was to test peloruside for its effectiveness against tumor growth *in vivo* using a nude, immunocompromised mouse model and to determine how well the compound was tolerated relative to the taxane drugs, paclitaxel and docetaxel, and doxorubicin, another standard chemotherapeutic agent that causes DNA damage. Flank injections of cancer cells were used to establish xenografts of lung and breast tumor cells in an athymic *nu/nu* mouse model.

**Materials and Methods**

**Materials**

Peloruside A was prepared from marine sponge extracts as previously described (1) and synthesized according to the procedure of Liao et al. (25). Purity of the natural product and the synthesized sample exceeded 98%. Paclitaxel, docetaxel, and doxorubicin were purchased from commercial sources.

**Cell culture**

*In vitro* cell culture was carried out in appropriate culture media supplemented with 10% fetal calf serum and 100 units/mL penicillin/streptomycin. Cells were grown at 37°C in a humidified 5% CO₂-air atmosphere. All cultured cells retained the characteristic phenotype as shown on the ATCC website or the NCI repository; however, no independent authentication was carried out to check the identification. In the present study, cells tested in cell culture but...
not *in vivo* included human metastatic breast cancer cells stably transfected with a luciferase transporter (MDA-MB-231/Luc) and human prostate cancer cells (PC-3), both obtained from the American Type Culture Collection (ATCC, Manassas, VA). The luciferase transfection had no effect on the generation time of the MDA-MB-231 cells. All cells were obtained prior to 2005.

Three tumorigenic cancer cell lines were used to generate xenografts in mice. The non-small cell lung carcinoma (NSCLC) line NCI-H460 (H460) was obtained from the NCI Division of Cancer Treatment and Diagnosis (DCTD) Tumor Repository, (Frederick, MD), a second NSCLC line A549 was purchased from ATCC, and a P-gp-overexpressing MDR phenotype of OVCAR-8 ovarian carcinoma cells, designated NCI/ADR-RES, were obtained from the NCI DCTD Tumor Repository. No independent authentication of these tumorigenic cell lines was carried out. These cell lines were obtained prior to 2005.

Animals

Female athymic *nu/nu* mice between 5 and 6 weeks of age were obtained from Harlan, Inc. (Madison, WI or Indianapolis, IN) and maintained in pathogen-free conditions at the Institute for Drug Development (IDD) in San Antonio, TX or the University of Texas Southwestern Medical Center (UTSWMC) in Dallas, TX. All animal experiments were carried out following IACUC guidelines with ethical approval obtained from the appropriate institutional animal ethics committee.

Cell proliferation Assay
In addition to direct cell counting with a Coulter Counter, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric cell proliferation assay was used to monitor growth of cells in culture, as previously described (16).

In vivo xenograft studies

Three cell lines were used for mouse xenograft studies: two NSCLC cell lines H460 and A549, and one ovarian carcinoma cell line with an overexpressed P-gp phenotype NCI/ADR-RES. All cell lines were implanted subcutaneously (s.c.) by trocar into the right flank of female nude mice (nu/nu). Tumor dimensions were measured by vernier caliper. When the tumors had grown to approximately 58 mm$^3$ in size, animals were paired by tumor size (day 1) into control and treatment groups (n = 7 animals per group). Pleroside and paclitaxel were administered as single agents via intraperitoneal (i.p.) injection once per day for five days (QDx5) for H460 NSCLC cell xenografts. Other dosage schedules were also used for Pleroside and paclitaxel in A549 and NCI/ADR-RES cells. Docetaxel was administered intravenously (i.v.) every other day for a total of three doses (Q2Dx3) for H460 and A549 cell xenografts. Weight loss and tumor growth were monitored periodically through the treatment schedule. The percent tumor growth inhibition (TGI) was calculated from the formula: TGI = \[\frac{[(DVc − DVe) / DVc] × 100}{\text{in which } DVc \text{ is the difference between the final and the initial tumor volume of the non-drug-treated group and } DVe \text{ is the change in tumor volume for the drug-treated group. Any animals that showed partial or complete tumor regression were excluded from the TGI calculation but were included in the statistical analysis calculation.}

Statistical Analysis

Statistical analyses were carried out using GraphPad Prism v 5.0 (GraphPad Software, Inc., San Diego, CA). $P$ values of $\leq 0.05$ were accepted as significant. Given that multiple doses
were compared, a one-way Anova with Dunnett’s multiple comparison post-hoc test was used.

## Results

### Peloruside effects on proliferation of cultured cancer cells in vitro

Peloruside is known to be effective in the low nanomolar range at inhibiting cell proliferation of a number of cancer and non-cancer cell lines (Table 1). To extend this range, growth of MDA-MB-231/Luc and PC-3 cells was monitored after treatment in culture with peloruside for up to 14 days (Fig. 2). Significant growth inhibition was observed at concentrations of 1-10 nM in both cell lines, with nearly 100% inhibition at 10 nM after 7 days exposure to peloruside. A 7-day IC$_{50}$ value was estimated for peloruside in both cell lines and added to the data of Table 1.

### Peloruside, paclitaxel, and docetaxel effects on H460 xenografts in nu/nu mice

Using H460 NSCLC cell xenografts, peloruside was tested at three different doses for effects on tumor growth over a 12-day period. The inhibition of proliferation was compared to inhibition by the standard anticancer drugs paclitaxel and docetaxel at close to their maximum tolerated doses (MTD) (Fig. 3A). Peloruside and paclitaxel were given i.p. and docetaxel was administered i.v. Control animals received i.p. vehicle consisting of 0.9% saline supplemented with 10% DMSO and 20% PEG 400. This is a standard formulation for lipophilic compounds. Because of time considerations and a shortage of peloruside stocks, it was not possible to experiment with different formulations. It was also important for comparison between drugs that the same vehicle was used for all the compounds in the vivo
tests, since different formulations can affect the antitumor responses. Docetaxel was given i.v. as it is the recommended method by Sanofi-Aventis, using a stock solution in polysorbate 80 diluted in 13% w/w ethanol for i.v. injection (Sanofi product information). Tumor volume was measured by caliper every 4 days. Peloruside caused a dose-dependent decrease in tumor growth over the treatment period, with TGI values of 88% at 5 mg/kg and 99% at 10 mg/kg ($P<0.01$, one-way Anova followed by a Dunnett’s post-hoc test) (Table 2). Any drug resulting in a TGI of ≥58% (the NCI standard criterion for antitumor activity) was considered as being a valid anticancer agent (26). One mouse showed a partial tumor regression of 53% at the 10 mg/kg peloruside dose. No tumor necrosis was seen in any of the mice, and no deaths occurred for the 5 and 10 mg/kg peloruside single agent treatments. The 5 and 10 mg/kg peloruside treatments caused significantly greater TGI than that seen with either paclitaxel or docetaxel (Fig. 3, Table 2). In the H460 xenograft model, the changes in tumor volume with docetaxel were not significantly different from the control. The difference in TGI between the 5 and 10 mg/kg peloruside treatments was not significant. There was a significant loss in body weight (Fig. 3C) ranging from 20% to 26% by 12 days for all treated animals except the docetaxel treatment group. Weight loss in the peloruside group was similar to that seen with paclitaxel.

Two combination treatments (QDx5) were also tested in the H460 model, combining peloruside at 5 mg/kg i.p. with either paclitaxel (8 mg/kg i.p.) or docetaxel (6.3 mg/kg i.v.) (Fig. 3B). The peloruside-paclitaxel combination led to the death of all animals on day 8 (Table 2). In the peloruside-docetaxel combination group, two animals underwent drug-related deaths. Of the five remaining individuals, the TGI was 99% ($P < 0.0001$). Weight loss in the peloruside-docetaxel combination was similar to that in the single agent study. Overall, peloruside showed an impressive dose-dependent single agent antitumor activity.
in the H460 xenograft model. Combination therapy between peloruside and either paclitaxel or docetaxel was less encouraging due to significant mortality in both groups, two mice in the docetaxel group and all seven mice in the paclitaxel group.

Peloruside, paclitaxel, and docetaxel effects on A549 xenografts in \textit{nu/nu} mice

Because of the significant body weight loss in the 12-day treatment with H460 cells, a second xenograft study was carried out with A549 cells for a longer duration of 30 days. Different doses of peloruside and different schedules of administration were compared to paclitaxel and docetaxel (Fig. 4A). Peloruside was administered at 5 or 10 mg/kg i.p. QDx5, or at 10 or 15 mg/kg Q2Dx3. Paclitaxel was administered at 16 mg/kg i.p. QDx5, and docetaxel was administered at 13 mg/kg i.v. Q2Dx3. Peloruside, with TGI values ranging from 51% to 74%, again out-performed docetaxel and paclitaxel. Although the 10 mg/kg, Q2Dx3 peloruside treatment had a TGI of only 51%, the decrease in tumor volume compared to control was significant ($P<0.01$, one-way Anova with Dunnett’s post-hoc test). The TGI values for paclitaxel (44%) and docetaxel (50%) were also significant ($P<0.05$ and $P<0.01$, respectively) although below the NCI criterion for antitumor activity. All treatments resulted in moderate to significant dose- and schedule-dependent weight loss that was regained after stopping administration of the drugs (Fig. 4B). The greatest weight loss (23.5%) was seen in the 10 mg/kg QDx5 peloruside group; however, all weight lost was regained two-weeks after dosing had finished, and positive weight gain was seen after this recovery. Mild to severe tumor necrosis was observed in a number of the animals (Table 2), and although there were no deaths, there were no partial or complete tumor regressions either.

Peloruside, paclitaxel, and doxorubicin effects on NCI/ADR-RES xenografts in \textit{nu/nu} mice

A third xenograft study was performed with a P-gp overexpressing NCI/ADR-RES breast
tumor model with treatment extended over 92 days (Fig. 5). Peloruside TGI values ranged from 19% to 73%, depending on the dose and schedule of delivery (Table 2). With the Q2Dx4x3, drug was given as four doses, one every other day for three cycles, with cycles starting at days 1, 19, and 61; however, the last cycle was dosed on a Q4Dx3 schedule. The QDx5x3 schedule involved once daily injections for five days for a total of three cycles. Doxorubicin was administered at 2.5 mg/kg i.p., and 3 of the 7 animals in this group died during the treatments. Although TGI values for peloruside were more variable in this study, with only two of the four peloruside schedules giving TGI values above the NCI 58% cut-off for anticancer activity, treatment with peloruside was better tolerated compared to doxorubicin, thus allowing administration of multiple cycles. Peloruside at 10 mg/kg showed a greater maximum weight loss of 20% compared with doxorubicin at 10%; however, weight gain after cessation of dosing was less in the doxorubicin group than in the peloruside groups. Paclitaxel gave the best overall response in the NCI/ADR-RES xenograft model, but at a 43% mortality (Table 2). Only one animal died in the peloruside treatments, and that occurred at the highest dose schedule: 15 mg/kg i.p. Q2Dx4x3. It was unexpected that paclitaxel and doxorubicin would be as effective as they were, given that the NCI/ADR-RES cell line is reported to be resistant to these drugs which are good Pgp substrates. It is possible that Pgp expression in the tumour cells was less than normal during the study.

Discussion

Properties of peloruside.

Peloruside is effective against a wide variety of cancer cell types and non-cancer cells (Table 1). The original interest in peloruside as a potential lead compound for therapeutic development was stimulated by the discovery that it had a similar mode of action to paclitaxel
and docetaxel, that of stabilization of the microtubule (2). It was later shown, similar to other
drugs of its class, to also inhibit microtubule dynamics (17) and be active against paclitaxel-
resistant cells (14).

The future of cancer therapy is likely to reside in therapies that target specific cancer gene
products or oncogene networks (27, 28), but similar to standard chemotherapy, targeted
therapies also run into problems of secondary off-target effects and resistance. Some of these
problems stem from the genetic complexity and heterogeneity of individual cancers,
redundancy in cancer networks, the lack of unique oncogene-specific drivers and signaling
pathways, and the formation of resistant clones (27). The mapping of the cancer genome is an
exciting development in anticancer research, and it is estimated that only about 5% of the
cancer genome has been drugged. Paclitaxel and docetaxel as chemotherapeutic drugs,
although directed at a target found in both cancer cells and normal cells, have been hugely
successful in treating solid tumors (4) but have several drawbacks. These include dose-
limiting toxicities (3-5) and problems with vehicle reactivity (6). A major potential advantage
of peloruside over the taxanes is that it would be effective in taxane-resistant tumors that have
inherent or acquired drug resistance (mechanisms of resistance have been reviewed by Orr et
al. (29) and Kavallaris et al. (30)). These mechanisms include P-gp overexpression, changes
in β-tubulin isotype expression, and mutation of amino acids at the tubulin binding site for the
taxane drugs (14). Thus, peloruside might be able to expand on the options available in long-
term taxane treatment, in particular since it is better tolerated than the taxanes (this study and
Crume et al. (19)) and can be used in vivo in combination with the taxanes to give additive or
synergistic responses (31). Synergistic interactions between peloruside and the taxane-site
drugs have been previously described in vitro in cultured cancer cell lines (32, 33).

Results from murine xenografts.
Preclinical trials with peloruside in three mouse xenograft studies provided very strong in vivo support for peloruside to undergo further development as a potential chemotherapeutic agent to inhibit tumor growth and even, in some cases, cause tumor regression (Fig. 3-5, Table 2). TGI values exceeded those of the taxanes in the H460 and A549 models, although in the NCI/ADR-RES model, paclitaxel displayed the highest TGI but at a high cost with 3 of 7 animals dying from drug-related causes. In this same NCI/ADR-RES model, peloruside was more effective than doxorubicin and better tolerated (3 deaths with doxorubicin; 1 at the highest dosing schedule with peloruside). As with the taxanes, peloruside treatment caused significant weight loss over the first 10-12 days, but this loss was regained in longer treatment schedules, indicating a transient, but significant toxicity for all the MSAs tested. Recovery from doxorubicin treatment, however, was much reduced compared to peloruside and paclitaxel. The MTD for peloruside in a mouse was determined to be between 15 mg/kg and 20 mg/kg, since mortality was 100% at 20 mg/kg after 8 days of treatment. A number of different dosing schedules were attempted, and with more trials and different schedules, even 20 mg/kg may be possible without significant mortality. Co-administration of peloruside with paclitaxel and docetaxel caused an unacceptable number of animal deaths in the H460 model. Peloruside at 5 mg/kg in combination with docetaxel at 6.3 mg/kg gave a TGI of 99%, a value greater than the single agent values for peloruside of 88% and docetaxel of 19%, suggesting possible synergistic interactions between the two MSAs. Because different concentrations of peloruside and taxanes were not tested on tumor growth in vivo, a true synergy calculation was not able to be carried out (32, 33).

Comparing peloruside effects in xenograft models with cabazitaxel (9), durable tumor responses were seen in cabazitaxel in which no tumor growth was seen for 30-40 days after cessation of dosing compared with about 20 days for peloruside. In the cabazitaxel study the
highest non-toxic dose was based on a weight loss of no more than 20%; whereas, some peloruside treatments reached 23.5% weight loss maximum.

Conclusions and future directions.

Based on the overall anti-tumor activity of peloruside, an intensive single agent and combination treatment in a panel of xenograft models would be a logical next step in the preclinical development of peloruside. A dose-finding study is needed to determine the MTD for peloruside and to try to minimize its toxicity that leads to high weight loss during and immediately after administration. It would also be important to begin a pharmacokinetic/pharmacodynamic study on peloruside in both human cell lines and mouse models of cancer, prior to moving into Phase I clinical trials. Advancement of peloruside into clinical trials is currently restricted by limited access to the natural product (34, 35) and the lack of availability of a scaled-up synthesis to make clinically relevant amounts of peloruside. Efforts are currently underway to address this problem of supply.

Acknowledgements

This project was supported by funding from Reata Pharmaceuticals, Inc, the Cancer Society of New Zealand, Wellington Medical Research Foundation, and Victoria University of Wellington (to JHM and PTN). Jef K. De Brabander thanks the Robert A. Welch Foundation for support (grant I-1422) and holds the Julie and Luis Beecherl, Jr., Chair in Medical Science. The authors would also like to thank Dr Deborah Ferguson of Reata Pharmaceuticals for critically reading the manuscript.
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Table 1. IC$_{50}$ values (nM) for peloruside A in different cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Tissue Type</th>
<th>IC$_{50}$ (nM)</th>
<th>Reference</th>
</tr>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>HL-60</td>
<td>Promyelocytic leukemia</td>
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<td>(16)</td>
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<tr>
<td>H441</td>
<td>Lung adenocarcinoma</td>
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<td>(16)</td>
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<td>(14)</td>
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<td>A2780</td>
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<td>(14)</td>
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<td>Breast cancer</td>
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<td>(17)</td>
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<tr>
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<td>50</td>
<td>Present study</td>
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<td>SH-SY5Y</td>
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<td>(16)</td>
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<tr>
<td>PC-3</td>
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<td>10</td>
<td>Present study</td>
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<td><strong>Mouse cancer cell lines</strong></td>
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<td>Leukemia</td>
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<td>(16)</td>
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<td>Mouse Splenocytes</td>
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<td>LLC-PK$_{1}$</td>
<td>Pig kidney</td>
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Growth inhibition was calculated from an MTT cell proliferation assay after 3-4 days culture in different concentrations of peloruside A. Seven-day IC$_{50}$ values for peloruside in MDA-MB-231/Luc and PC-3 cells were estimated from Fig. 2 of the present study. Other values were taken from the literature. BMMφ = bone marrow macrophages.
Table 2. Mouse xenograft result summary.

<table>
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<tr>
<th>Treatment</th>
<th>Schedule</th>
<th>TGI (%)</th>
<th>Tumor Regression</th>
<th>Tumor Necrosis</th>
<th>Deaths</th>
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<td>QDx5</td>
<td>53%&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>99%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
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</tr>
<tr>
<td>PLA 20 mg/kg</td>
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<tr>
<td>PLA &amp; DTX as above</td>
<td>QDx5</td>
<td>99%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>1 (d8), 1 (d12)</td>
</tr>
<tr>
<td>PLA &amp; PTX as above</td>
<td>QDx5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7 (d8)</td>
</tr>
</tbody>
</table>

| **A549 Cell Xenografts** |          |         |                  |                |        |
| Control              | QDx5     | -       | mod (2), sev (2) | 0              | 0      |
| PTX 16 mg/kg         | QDx5     | 44%<sup>a</sup> | 0               | mild (2), mod (2), sev (2) | 0 |
| DTX 13 mg/kg         | Q2Dx3    | 50%<sup>a</sup> | 0               | mild (1), mod (3), sev (2) | 0 |
| PLA 5 mg/kg          | QDx5     | 74%<sup>b</sup> | 0               | mild (1), mod (1) | 0 |
| PLA 10 mg/kg         | QDx5     | 71%<sup>b</sup> | 0               | 0              | 0      |
| PLA 10 mg/kg         | Q2Dx3    | 51%<sup>b</sup> | 0               | mild (3), mod (1), sev (1) | 0 |
| PLA 15 mg/kg         | Q2Dx3    | 70%<sup>b</sup> | 0               | mild (2), mod (3) | 0 |

| **ADR-RES Cell Xenografts** |          |         |                  |                |        |
| Control              | QDx5     | -       | (1)              | mild (1), mod (1), sev (4) | 0      |
| PTX 16 mg/kg         | QDx5x2   | 86%<sup>b</sup> | 20%<sup>c</sup> (1), 100%<sup>c</sup> (1) | sev (1) | 2 (d11), 1 (d26) |
| DOXO 2.5 mg/kg       | QDx5     | 67%<sup>a</sup> | 100%<sup>a</sup> (1) | mod (1), sev (1) | 1 (d22), 1 (d26), 1 (d36) |
| PLA 5 mg/kg          | QDx5x3   | 68%<sup>b</sup> | 100%<sup>b</sup> (1) | sev (5) | 0 |
| PLA 10 mg/kg         | QDx5     | 19%     | 0                | sev (6) | 0 |
| PLA 15 mg/kg         | Q2Dx3    | 56%<sup>a</sup> | 100%<sup>a</sup> (1) | mod (1), sev (3) | 0 |
| PLA 15 mg/kg         | Q2Dx4x3  | 73%<sup>b</sup> | 100%<sup>b</sup> (1) | mod (1), sev (4) | 1 (d26) |

Cancer cell xenografts in *nu/nu* mice were treated with peloruside A (PLA), docetaxel (DTX), paclitaxel (PTX), or doxorubicin (DOXO). There were 7 animals per group, and the number of animals in each group that showed tumor regression or tumor necrosis (mild, moderate (mod), or severe (sev)) are indicated in parentheses. TGI = average percent tumor growth inhibition. <sup>a</sup> P<0.05; <sup>b</sup> P<0.01; <sup>c</sup> P<0.0001, one-way Anova with Dunnett’s post-hoc test.
**Figure Legends**

Figure 1. Structure of peloruside A.

Figure 2. Peloruside effects on growth of MDA-MB-231-Luc and PC-3 cells in vitro. Metastatic lung carcinoma cells MDA-MB-231-Luc and prostate cancer cells PC-3 were cultured in the presence of different concentrations of peloruside for up to 2 weeks. Proliferation was assessed by counting the cells in a Coulter Counter.

Figure 3. Tumor volume and body weight changes in H460 xenografts. Female nude mice (nu/nu) were injected with 5 x 10^6 H460 NSCLC cells s.c. by trocar with tumor fragments harvested from s.c. growing tumors in host nude mice. When tumors reached approximately 58 mm³ in size, about 7 days following implantation, animals were pair-matched by tumor size into one control group given vehicle (n=7 mice) and five treatment groups (n=7 mice/group). One treatment group, peloruside at 20 mg/kg, QDx5 is not presented in the graph because of high mortality (Table 2). Two combination treatment groups peloruside/paclitaxel and peloruside/docetaxel were also set up. Again, because of a high mortality in the peloruside/paclitaxel group (Table 2), only the peloruside/docetaxel group is graphed. The average weight of the control group was 21.7 g, and the range of the average weights for all the groups was 21.7 g to 22.9 g. Drug treatments were begun on day one following pair-matching. Single agent (A) and combination treatment (B) effects on tumor volume (average mm³ ± SEM) are presented over a 12-day period. Body weight changes (C) are also graphed as the average percent change ± SEM. Abbreviations are PLA, peloruside A; PTX, paclitaxel; and DTX, docetaxel.

Figure 4. Tumor volume and body weight changes in A549 xenografts.
Subcutaneous flank xenografts in nude mice were established with A549 NSCLC cells as described in the legend of Figure 4. There were 7 mice/group, and all data are presented as the mean ± SEM. The average weight of the control group was 22.9 g, and the range of the mean weights for all the groups was 21.8 g to 23.2 g. The effect of single agent treatments on tumor volume (A) and percent change in body weight (B) are presented. Abbreviations are PLA, peloruside A; PTX, paclitaxel; DTX, docetaxel.

Figure 5. Tumor volume and body weight changes in NCI/ADR-RES xenografts.

Subcutaneous flank xenografts in nude mice were established with A549 NSCLC cells as described in the legend of Figure 3. There were 7 mice/group, and all data are presented as the mean ± SEM. The average weight of the control group was 22.6 g, and the range of the mean weights for all the groups was 20.6 g to 22.6 g. The effect of single agent treatments on tumor volume (A) and percent change in body weight (B) are presented. Abbreviations are PLA, peloruside A; PTX, paclitaxel; and DOXO, doxorubicin.
Fig. 1

Peloruside A
Fig. 2

MDA-MB-231/Luc

3 Days Growth
7 Days Growth
14 Days Growth

Concentration (nM)
Growth Inhibition (%)

PC-3

3 Days Growth
7 Days Growth
14 Days Growth

Concentration (nM)
Growth Inhibition (%)

Author Manuscript Published OnlineFirst on June 8, 2015; DOI: 10.1158/1535-7163.MCT-15-0167
Fig. 3

A. H460 Tumor Growth

B. H460 Tumor Growth - PLA+DTX Combined

C. H460 % Change in BW
A549 Tumor Growth

A.

- Control QDx5
- PTX (16 mg/kg) QDx5
- DXT (13 mg/kg) Q2Dx3
- PLA (5 mg/kg) QDx5
- PLA (10 mg/kg) QDx5
- PLA (10 mg/kg) Q2Dx3
- PLA (15 mg/kg) Q2Dx3

B.

A549 % Change in BW

Change in BW (%)
Fig. 5

A. NCI/ADR-RES Tumor Volume

- ▲ Control QDx5
- ▲ PTX (16 mg/kg) QDx5x2
- ○ DOXO (2.5 mg/kg) QDx5
- ● PLA (5 mg/kg) QDx5x3
- ○ PLA (10 mg/kg) QDx5
- □ PLA (15 mg/kg) Q2Dx3
- △ PLA (15 mg/kg) Q2Dx4x3

B. NCI/ADR-RES % Change in BW

- ▲ Control QDx5
- ▲ PTX (16 mg/kg) QDx5x2
- ○ DOXO (2.5 mg/kg) QDx5
- ● PLA (5 mg/kg) QDx5x3
- ○ PLA (10 mg/kg) QDx5
- □ PLA (15 mg/kg) Q2Dx3
- △ PLA (15 mg/kg) Q2Dx4x3
Molecular Cancer Therapeutics

Peloruside A inhibits growth of human lung and breast tumor xenografts in an athymic nu/nu mouse model


Mol Cancer Ther  Published OnlineFirst June 8, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-15-0167

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