



Research Paper

## Use of paclitaxel carried in lipid core nanoparticles in patients with late-stage solid cancers with bone metastases: Lack of toxicity and therapeutic benefits



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ABSTRACT

**Background:** Patients with heavily pretreated, late-stage cancer and bone metastasis are usually poor candidates for further chemotherapy. Previously, we showed that association to lipid nanoparticles (LDE) drastically decreases the toxicity of anti-cancer drugs. Here, we tested the hypothesis that paclitaxel (PTX) carried in LDE could benefit end-of-life patients with painful bone metastases that had been previously treated with conventional PTX. Methods: Eighteen consecutive patients with late-stage cancer, 8 with breast, 5 with prostate and 5 with lung carcinoma, aged  $59 \pm 9$  years, were included in this study. All were receiving opioid medication. LDE-PTX was administered at  $175 \text{ mg/m}^2$  every 3 weeks until disease progression. Clinical imaging examinations and serum biochemistry determinations were performed to monitor disease progression. Intensity of bone pain, use of opioid medications and occurrence of pathological bone fractures were also evaluated. Results: In total, 104 chemotherapy cycles were performed and none of the patients showed clinical and laboratorial toxicities or pathological bone fractures. In all patients, pain was reduced so as to allow substitution of non-opioid for opioid medication. Median progression-free survival (PFS) was four months (95% CI 2.4-5.5), but in five patients PFS was longer than 6 months. Conclusions: Absence of observable clinical and laboratorial toxicities from LDE-PTX treatment, improvement of bone pain and the possible effect on PFS in some patients, despite previous use of conventional PTX, suggest that LDEPTX merits further clinical investigation.

### 1. Introduction

Patients with late-stage cancers frequently lack other chemotherapy options or may become too debilitated to withstand the toxicity of further chemotherapy lines [1]. Bone metastases, that are present in 30–75% of patients with late-stage cancers [2], may pose an additional challenge in the management of such patients.

The lifetime risk of bone metastases has been estimated as ~70% for patients with breast and prostate carcinomas, and about 30–40% for lung cancer patients [3]. Bone metastases may bring increased risk of bone complications such as pathological skeletal fractures and spinal cord compressions [4]. Bone metastases are frequently accompanied by distressful bone pain that can only be relieved by the continuous use of analgesics, and the use of strong opioids is often necessary [5]. Pain and the other skeleton related events are associated with worsened quality of life and increased morbidity and mortality [6], as well as with sizeable treatment costs [7].

Chemotherapy and radiotherapy are the major treatments for bone metastases, but they may not be feasible because of intolerance to the chemotherapeutic agents or to bone marrow aplasia related with radiotherapy [8]. Bisphosphonates, that inhibit the osteoclast-mediated bone resorption, decrease the incidence of skeletal-related events [9] and may achieve pain reduction but they can cause side-effects such as jaw osteonecrosis [10] and become ineffective after some time of usage. Thus, novel therapeutic tools that could be both effective and devoid of toxicity are desirable to arrest tumor growth and to alleviate the disease symptoms. In this respect, nanomedicine-based strategies may offer the possibility of anticancer treatment to patients without clinical conditions of being submitted to conventional chemotherapy [11].

Our laboratory has introduced non-protein formulations of anti-cancer drugs carried in artificially made lipid nanoparticles, termed LDE, which mimics the structure of low-density lipoprotein (LDL), but without the protein moiety of LDL, apolipoprotein (apo) B [12]. When injected in the bloodstream, LDE acquires apo E from the plasma. Apo E

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is recognized by the LDL receptors on the surface of the plasma membrane and thereby LDE is internalized into cells by the LDL receptor mediated endocytic pathway [13–15]. LDL receptors are upregulated in neoplastic cells [16–18], which allows the concentration in the tumor tissues of chemotherapeutic agents incorporated to the LDE structure [19–24]. In experiments with animals with implanted tumors, drugs such as paclitaxel (PTX) [25,26], carmustine [27], daunorubicin [28] and etoposide [29] incorporated to LDE increased the anti-tumor action, whereas the drug toxicity was markedly decreased. The inherent ability of LDE to drastically reduce the toxicity of chemotherapeutic agents was also shown in clinical trials enrolling patients with advanced cancers [19–22,24,30–32].

PTX, the primordial drug of the taxane family, is used in the therapy of breast, prostate and pulmonary carcinomas, which are the most prevalent cancers in the population [33,34]. In pre-clinical studies, it was shown that PTX associated to LDE (LDE-PTX) had marked tolerability to mice: LD50 was nine-fold greater than of the conventional formulation of PTX, that uses Cremophor EL as vehicle. The therapeutic efficacy in mice bearing tumors of LDE-PTX was also pronouncedly greater than that of PTX-Cremophor EL, as indicated by the reduction in tumor growth, increase in survival rates and cure of the treated mice [26]. The pharmacokinetic studies performed in 5 patients with breast [24] and 5 with gynecologic cancers showed that LDE-PTX is stable while circulating in the bloodstream. The plasma half-lives of PTX were increased, which is considered a pharmacologic advantage for the treatment of oncologic diseases [23]. The safety and non-toxicity of the drug at the 175 mg/m<sup>2</sup> triweekly dose scheme was documented in 4 patients with advanced breast cancer [24].

Availability of chemotherapy agents without observable toxicity, such as LDE-PTX, can offer an attractive alternative to palliative therapy for the management of poly-treated, frail patients. Thus, the aim of this study was to investigate whether the use of LDE-PTX could benefit end-of-life patients with painful bone metastatic disease of breast, prostate and pulmonary carcinomas. Previously, all patients had received conventional PTX at first line chemotherapy schemes and had undergone radiotherapy directed at bone metastases. All had also received prior bisphosphonate treatment, that was subsequently discontinued for pain unresponsiveness. At the study commencement, all patients were being treated with strong opioids.

## 2. Materials and methods

### 2.1. Study patients and objectives

This was a one-arm, non-randomized, open-label phase II study. Eighteen consecutive volunteer patients aged  $\geq 18$  years with histological confirmation of breast (n = 8), prostate (n = 5), and of lung (n = 5) cancer in advanced stages and with diagnosed bone metastases were enrolled in the study at the Outpatient Clinics of the Arnaldo Vieira de Carvalho Cancer Institute, in São Paulo, Brazil. Patients with visceral metastases were also included. All patients had been previously submitted to polychemotherapy schemes, including hormonal therapy for prostate cancer, and palliative radiotherapy and had Eastern Cooperative Oncology Group (ECOG) performance status  $\leq 2$ . Their physical and clinical data and previous treatments are shown in Table 1. All 5 patients with prostate cancer were resistant to hormonal deprivation therapy at the moment they were enrolled.

The end-points were: 1) Tolerability and safety of LDE-PTX treatment. 2) Progression-free survival (PFS) period, based on the clinical observation and imaging exams. 3) Reduction of pain or of the use of analgesic medications.

This study was approved by Research Ethics Committee of the Arnaldo Vieira de Carvalho Cancer Institute (ref. number 216/09). Informed consent was obtained from all participants.

**Table 1**

Previous treatment schemes of 18 patients with primary breast (patients no. 1–8), prostate (9–13) and lung carcinoma (14–18) treated with LDE-PTX.

Patient n°	Age (years)/ sex (M/F)	Previous treatment lines
1	54/F	1st: paclitaxel + carboplatin; 2nd: paclitaxel + gemcitabine; 3rd: docetaxel + fluorouracil
2	58/F	1st: paclitaxel + carboplatin; 2nd: paclitaxel + gemcitabine; 3rd: docetaxel + fluorouracil; 4th: paclitaxel + vinorelbine tartrate.
3	45/F	1st: fluorouracil + adriamycin + cyclophosphamide; 2nd: paclitaxel + carboplatin; 3rd: fulvestrant.
4	55/F	1st: fluorouracil + adriamycin + cyclophosphamide; 2nd: paclitaxel + carboplatin; 3rd: fulvestrant; 4th: paclitaxel.
5	63/F	1st: paclitaxel + vinorelbine tartrate + gemcitabine; 2nd: goserelin acetate; 3rd: vinorelbine tartrate + gemcitabine.
6	66/F	1st: fluorouracil + adriamycin + cyclophosphamide; 2nd: paclitaxel + carboplatin.
7	44/F	1st: fluorouracil + adriamycin + cyclophosphamide; 2nd: paclitaxel + carboplatin; 3rd: vinorelbine tartrate + gemcitabine; 4th: paclitaxel.
8	57/F	1st: fluorouracil + adriamycin + cyclophosphamide; 2nd: paclitaxel; 3rd: gemcitabine.
9	65/M	1st: goserelin acetate; 2nd: docetaxel.
10	67/M	1st: goserelin acetate; 2nd: docetaxel; 3rd: mitoxantrone.
11	66/M	1st: goserelin acetate; 2nd: docetaxel; 3rd: mitoxantrone.
12	67/M	1st: goserelin acetate; 2nd: docetaxel; 3rd: mitoxantrone.
13	76/M	1st: goserelin acetate; 2nd: docetaxel; 3rd: mitoxantrone.
14	59/M	1st: paclitaxel + carboplatin; 2nd: paclitaxel + gemcitabine + bevacizumab; 3rd: vinorelbine tartrate.
15	71/F	1st: paclitaxel + carboplatin; 2nd: paclitaxel + gemcitabine + bevacizumab; 3rd: vinorelbine tartrate.
16	45/F	1st: paclitaxel + carboplatin; 2nd: paclitaxel + gemcitabine + bevacizumab; 3rd: vinorelbine tartrate.
17	61/M	1st: paclitaxel + gemcitabine; 2nd: paclitaxel + carboplatin; 3rd: vinorelbine tartrate; 4th: docetaxel; 5th: gemcitabine.
18	46/M	1st: gemcitabine + cisplatin; 2nd: paclitaxel.

All patients had received zolendronic acid medication except for patient n° 15, who was treated with pamidronate.

## 3. LDE-PTX treatment and toxicity assessment

All patients were treated with LDE-PTX at 175 mg/m<sup>2</sup> body surface dose diluted in 200 mL saline solution, administered as I.V., infusion over 90 min, every 3 weeks. Treatment would be discontinued only upon disease progression or at patient request. Patients were submitted to clinical interview and physical examination before each chemotherapy cycle by one attending oncologist (S.R.G). Serum biochemistry examinations were also performed to evaluate toxicity and to monitor for the manifestation of any adverse events, based on the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) (National Cancer Institute, U. S. Department of Health and Human Services, Washington, DC, USA). Imaging examinations were performed every 3 months of treatment to monitor disease progression. It was established that the LDE-PTX treatment would be interrupted upon observation of disease progression.

## 4. Pain evaluation and skeletal-related events

Pain evaluation was performed using the Numerical Rating Scale (NRS) [35,36] (score zero means absence of pain while 10 means severe bone pain) before treatment with LDE-PTX and after each chemotherapy cycle. Skeletal-related events such as pathological bone fractures or spinal cord compression were evaluated by clinical and radiological exams.

## 5. Pain control assessment

The use of opioid or non-opioid analgesic medication was evaluated on the first day and at the end of treatment. The Analgesic Quantification Algorithm (AQA) was used to evaluate the use of analgesics.

## 6. Karnofsky performance status assessment

Karnofsky performance status score was assessed to evaluate the level of patient activity [37] at the beginning and at the end of the study.

## 7. Preparation of PTX oleate associated with LDE

To increase the stability and yield of the association with LDE, PTX oleate, a derivatized PTX compound (Pharmaceuticals, Shanghai, China), was synthesized as previously described [26].

The LDE-PTX formulation was prepared from a lipid mixture composed of cholestryloleate (Alfa Aesar, Haverhill, MA, USA), egg phosphatidylcholine (Lipoid GmbH, Ludwigshafen, Germany), medium-chain triglycerides or caprylic/capric triglycerides (Mygliol 812 N, Sasol, Hamburg, Germany), cholesterol (FabriChem, Milford, CT, USA) and PTX oleate, in the aqueous phase comprised of Tris-HCl buffer, pH 8.05. Emulsification of all lipids with the functionalized drug and the aqueous phase was obtained by high-pressure homogenization using an Emulsiflex C5 homogenizer (Avestin, Ottawa, Canada). After homogenization cycles, the formed nanoparticle was centrifuged and sterilized by passage through a 0.22 µm pore polycarbonate filter (EMD Millipore Corporation, Billerica, MA, USA) and kept at 4 °C until it was used. The incorporation of PTX to LDE was confirmed by using a high performance liquid chromatography Nexera 2 (Shimadzu, Columbia, MD, USA) developed in isocratic mode, mobile phase 100% methanol and UV-visible detector (227 nm).

## 8. Immunohistochemistry assay

Five-micron-thick sections of formalin-fixed paraffin-embedded breast, prostate and lung cancer tissue, from before surgery and previous chemotherapy treatment, were routinely processed. For immunohistochemical analysis, the anti-LDL receptor rabbit anti-mouse polyclonal antibody (LifeSpan Biosciences, Seattle, WA, USA) was used in this study. For immunostaining for LDLR, antigen retrieval was not necessary.

Briefly, endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide. Each tissue section was incubated in 10% fetal calf serum for 1 h at 42 °C. The slides were then incubated overnight at 4 °C with a 1:50 dilution of anti-LDLR antibody. Next, the sections were incubated for 30 min at room temperature with a SuperPicTure Polymer Detection System (Invitrogen, Camarillo, CA, USA). The sections were incubated with a 3,3'-diaminobenzidine-tetrahydrochloride (DAB) chromogen system (Agilent Dako, Santa Clara, CA, USA) for 1.5 min at room temperature and then counterstained with hematoxylin. Fields from each section were captured at 200x magnification, by the Image Analysis System Quantimet 500+ (Leica Microsystems, Wetzlar, Germany).

## 9. Results

The mean age of the patients was 59 ± 9 years. All had multiple painful bone metastases. Four patients with breast and one with prostate cancer also had visceral metastases in the liver. As shown in Table 1, the enrolled patients had been previously treated with at least 3 conventional chemotherapy schemes and bisphosphonates, with disease progression. All patients had been treated with conventional taxanes, either PTX or docetaxel, as part of first or second line chemotherapy regimens.

None of the patients manifested clinical or laboratorial toxicities that could be ascribed to the treatment with LDE-PTX. Thus, in all 104

performed LDE-PTX chemotherapy cycles, hematological adverse events, which are frequent under treatment with taxanes were absent: leukopenia, thrombocytopenia and anemia were all grade zero. Hepatic and renal toxicities, as assessed by AST, ALP, bilirubin, urea and creatinine values were also grade zero, as well as nausea, vomiting, fever, arterial hypertension, dyspnea or alopecia. Hypersensitivity reactions and peripheral neuropathy which are typical of the commercial PTX formulations, including vasomotor changes, were also grade zero in all treatment cycles.

Following LDE-PTX treatment, one patient (n° 18) with lung cancer showed partial response to treatment, according to imaging exams. Fig. 1 illustrates the PFS data of the eighteen study subjects treated with LDE-PTX. The median PFS was 4.0 months (95% CI 2.4 – 5.5). Among them, 9 patients showed PFS equal or higher than 6 months, and 2 showed >1 year PFS. Patient n° 13 had PFS 15 months, but he requested treatment interruption to move residence to another region of the country. In the study protocol, it was not established that the patients would be systematically followed after tumor progression to acquire data on total survival period.

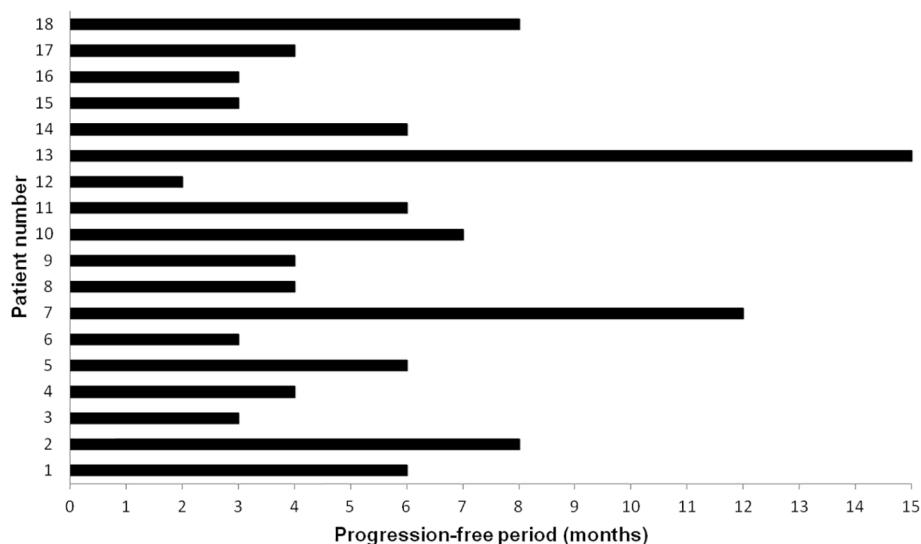
Before treatment with LDE-PTX, the median pain score was 8, which stands for severe pain. After the treatment the median pain score decreased to 5, or moderate pain. As shown in Table 2, consistent reduction of the pain grading score was observed in 13 patients (number 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 17 and 18); in two patients there was transient pain reduction followed by pain increase (number 5 and 16) and in three there was clearly no reduction of the pain grade (numbers 1, 10 and 15). The mean AQA score was 4 (use of strong opioids, equivalent to > 75 mg oral morphine per day) in the beginning of the treatment and was 2 (use of non-opioids only) at the end. In all study patients, there were no signs or symptoms of occurrence of pathological fractures in the bones or of spinal cord compression, and none of the patients required radiotherapy for pain relief. None of the patients underwent orthopaedic surgery during the study.

All patients showed improvement of performance status that by the end of the follow-up periods was > 80% in all, based on the Karnofsky performance status scale.

Fig. 2 shows representative images of immunohistochemistry staining for LDL receptors in breast, prostate and lung cancer, and adjacent tumor infiltration areas from tissue fragments excised in the former debulking surgery patients were submitted, which show abundant presence of LDL receptors in the primary malignant tissues.

## 10. Discussion

Of chief importance in our findings was the absence in all study patients of detectable toxicities from the LDE-PTX treatment, as evaluated by assessment of patient complaints, physical examination by the attending oncologist and laboratory exams. The 175 mg/m<sup>2</sup> triweekly dose scheme adopted here was equal to that commonly used for conventional PTX [38–40] in the Oncology practice. In this respect, the conventional PTX formulation, that uses Cremophor EL as vehicle, may often elicit high toxicity levels, manifested as neutropenia [41], nausea and vomiting, alopecia and hypersensitivity reactions, weakness, arthralgias and myalgias, peripheral neuropathy and other toxicities [42]. In our previous reports, LDE-PTX had also showed lack of observable toxicity at the 175 mg/m<sup>2</sup> triweekly dose in patients with breast [24], epithelial ovarian carcinoma [30] and patients with cardiovascular disease [43]. Since the patients studied here comprised only frail, poly-treated, end-of-life, patients, they by no means could tolerate additional conventional chemotherapy schemes and would be otherwise scheduled for palliative measures [1]. Fractionation of the triweekly dose into weekly infusions is also used in conventional PTX treatment, mainly to decrease toxicity but, as shown in our previous studies and confirmed here, it was not necessary for LDE-PTX treatment at the 175 mg/m<sup>2</sup> dose level. At any rate, in the outpatient clinical setting, more frequent visits to the day-hospital facility for drug administration are inconvenient for



**Fig. 1.** Progression-free period of 18 end-of-life patients with primary breast (patients no. 1–8), prostate (patients no. 9–13) and lung carcinoma (patients no. 14–18) and bone metastases treated with LDE-PTX.

**Table 2**

Bone pain score evaluated before and after each LDE-PTX treatment cycle (175 mg/m<sup>2</sup> I.V. infusion, 3/3 weeks). Treatment was discontinued when disease progression was diagnosed.

Patient n°	Pain score at each LDE-PTX treatment cycle														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	8														
2	6	7	7	6	5	5	5	5	5	5					
3	10	10	8	7											
4	8	6	5	5	5										
5	8	7	5	5	7	7	8								
6	10	9	8	8											
7	7	6	5	5	4	4	3	3	3	3	4	3	4		
8	8	3	2	0	0										
9	5	5	5	4	3										
10	3	7	7	2	2	2	7	2							
11	9	5	2	2	2	2	2								
12	10	8	8												
13	7	7	6	7	5	4	4	4	3	3	3	4	4	3	4
14	9	7	7	5	5	3	4								
15	8	8	8	5											
16	9	9	8	7											
17	8	7	8	7	4										
18	8	8	8	7	6	6	6	4	4						

patients with limited mobility.

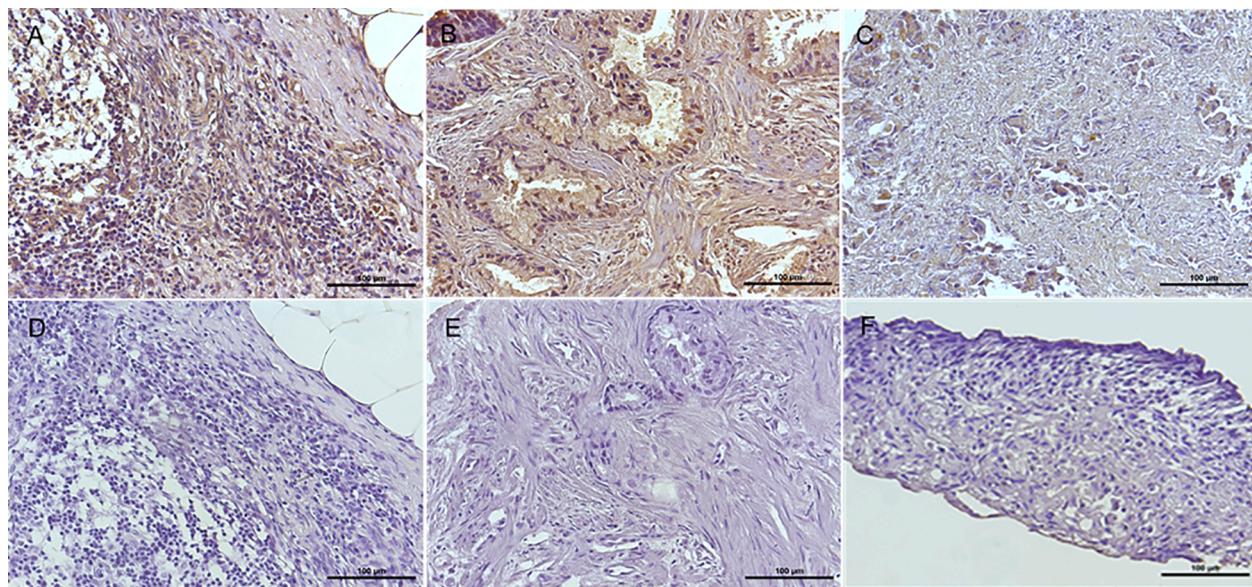
In view of the small number of cases in each one of the three cancer types, comparison of the PFS data of our patients with data from the literature is not possible. However, 9 out of 18 patients with metastatic bone disease had PFS equal or >6 months, and 2 had PFS greater than one year, which is noteworthy. PFS ≥ 6 months was observed in all three cancer types: 2 out of 5 patients with lung, 3 out of 5 patients with prostate and 4 out of 8 with breast carcinoma. Finally, patient number 18, with lung carcinoma, had disease regression documented by the imaging exams. Taken together, these data suggest that LDE-PTX treatment might have had some arresting effects on tumor growth.

Taxanes are extensively used in the combined chemotherapy of prostate [44,45], breast [46–48] and pulmonary [40] carcinomas, and PTX or docetaxel were previously used in all patients enrolled in this study. Recently, we showed that LDE-PTX, as administered at third or fourth line of chemotherapy, apparently extended the PFS of patients with metastatic ovarian carcinomas, who had been already treated with standard PTX at first-line chemotherapy [30]. Since association with LDE profoundly changes the biodistribution and the uptake and intracellular compartmentalization of PTX, it is tempting to hypothesize

whether the use of LDE as vehicle may somewhat diminish drug resistance to PTX [26]. In this respect, the phenomenon of LDL receptor overexpression that endows LDE with drug-targeting properties, illustrated in Fig. 2, was consistently documented in breast [49,50], prostate [51] and lung carcinomas [52].

Pain relief and improvement of physiological function of the skeletal system are dominant purposes of treatments directed to bone metastases [53]. All participants of the present study had been submitted to previous radiotherapy for bone metastases and had been under bisphosphonate therapy that was discontinued for unresponsiveness. In this setting, it is indeed noteworthy that pain relief was obtained in 13 out of 18 participants of the study by the LDE-PTX administration, with diminution of dose of analgesics or shift to weaker analgesics. It could be a matter of future investigation whether pain relief was consequent to the anticancer or to the anti-inflammatory action of PTX [54] on the bone metastatic sites. In rabbits with atherosclerosis, we had shown that treatment with taxanes brought marked reduction of pro-inflammatory cytokines [55].

Pathological fractures and skeletal-related events that may appear in bone metastatic disease [6,56,57] occurred in none of the 18 patients



**Fig. 2.** Representative photomicrographs of immunohistochemical staining for LDL receptors (LDLR) (A-C) or negative control (D-F) in primary tumor tissues: breast cancer (A and D), prostate cancer (B and E) and lung cancer (C and F). Brown staining indicates the expression of lipoprotein receptors immunostained by 3,3-diaminobenzidine chromogen. Negative control was performed which the primary antibody was replaced by bovine serum albumin. x200.

during the total 104 treatment cycles performed in the study. Likewise, nerve-related complications resulting from progression of bone metastases [58,59] did not occur during the study.

Finally, LDE-PTX treatment promoted the improvement of performance status in all the participants, reaching levels superior to 80% in all of them. Taxanes, together with platin and doxorubicin [60], rank among the most widely used chemotherapeutic agents, which adds-up to the therapeutic importance of our results.

Taken together with our previous studies [24,30,43], the toxicology data have also important implications for Geriatric Oncology since, by using LDE as drug vehicle, aged patients with expected low tolerability to conventional chemotherapy could be eligible for chemotherapy [61]. LDE-PTX was recently tested patients with ovarian cancer as third-fourth line chemotherapy [30] and the tolerability and safety was shown at the standard 175 mg/m<sup>2</sup> body surface PTX dose level [38–40]. Furthermore, the capacity of LDE to buffer drug toxicity in cancer patients had been also shown with the formulations of LDE with carbustine [21] and etoposide [20,31]. The use of LDE-PTX, in addition to provide chemotherapy without the toxicity burden to the patients, has potential of greater cost effectiveness by avoiding the use of medications to treat drug side effects and of patient hospitalizations. This is relevant in view of the mounting costs of cancer treatments especially in developing countries [62].

In this exploratory study, the lack of observable toxicity of LDE-PTX in all participant patients, together with consistent rates of pain improvement justify the design of large studies on the use of this formulation in patients with carcinomas of lung, breast or of prostate carcinoma after failure of testosterone deprivation therapy. Low toxicity makes room for dose-escalation protocols to determine the maximum tolerated dose (MTD), starting with doses substantially higher than the 175 mg/m<sup>2</sup> every three weeks tested here. Fractionation to weekly doses often used for conventional PTX could also be tested to further increase MTD for LDE-PTX.

In conclusion, our results show that LDE-PTX is well-tolerated and safe for use in debilitated, end-of-life patients and may decrease pain from bone metastasis, with improvement of well-being. Thus, this formulation may become an interesting alternative for the treatment of end-of-life patients without therapeutic options, especially those with bone metastatic disease. These results also entitle LDE-PTX for future trials for use in second or first-line schemes for solid cancers that are

currently treated with the conventional PTX formulation.

## 11. Declarations

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## 12. Availability of data and material

Upon request.

## 13. Ethics approval

This study was approved by the Ethics Committee of Arnaldo Vieira de Carvalho Cancer Institute Research, São Paulo, reference number 216/09. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

## 14. Consent to participate

All participants gave written informed consent.

## 15. Consent for publication

All authors read and approved the final manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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