Anti-HER2 Cationic Immunoemulsion as a Potential Targeted Drug Delivery System for the Treatment of Prostate Cancer

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Abstract

Present management of metastatic prostate cancer, which includes hormonal therapy, chemotherapy, and radiotherapy, are frequently palliative. Taxanes, and specifically docetaxel, are being extensively investigated to improve the survival of metastatic prostate cancer patients. Although paclitaxel exhibits a wide spectrum of antitumor activity, its therapeutic application is limited, in part, due to its low water solubility that necessitates the use of Cremophor EL, which is known to induce hypersensitivity reactions. Therefore, the objective of this present study was to assess the efficiency of paclitaxel palmitate–loaded anti-HER2 immunoemulsions, a targeted drug delivery system based on cationic emulsion covalently linked to anti-HER2 monoclonal antibody (Herceptin), in a well-established in vivo pharmacologic model of metastatic prostate cancer that overexpresses the HER2 receptor. It was clearly noted that the cationic emulsion and immunoemulsion did not activate the complement compared with the commercial and paclitaxel palmitate hydroalcoholic formulations. In addition, 10 mg/kg of paclitaxel palmitate–loaded immunoemulsion once weekly over 3 weeks inhibits the tumor growth in severe combined immunodeficient mice much more than the cationic emulsion (P < 0.05) and the paclitaxel palmitate formulation (P < 0.01). The histopathologic analysis suggested a therapeutic improvement trend in favor of the immunoemulsion. However, there was no significant difference in antitumor activity between the emulsion and the immunoemulsion despite the affinity of the immunoemulsion towards the HER2 receptor. Although the tumor growth was not fully inhibited, the actual results are encouraging and may lead to an improved therapeutic strategy of metastatic prostate cancer treatment. [Cancer Res 2007;67(1):269–75]

Introduction

Hormone-refractory prostate cancer is the second leading cause of cancer-related death for man in the United States (1). Present management of metastatic prostate cancer includes hormonal therapy, chemotherapy, and radiotherapy, which are frequently palliative. The failure of a variety of regimens to show significant responses and survival benefits in the hormone-refractory population led many researchers to believe that chemotherapy should not be considered standard for advanced prostate cancer patients (2, 3). Recently, taxanes, and more specifically docetaxel, are being extensively investigated to improve the survival of metastatic prostate patients (4, 5). Paclitaxel exhibits a wide spectrum of antitumor activity. However, its therapeutic application in cancer therapy is limited, in part, due to its low water solubility that necessitates the use of Cremophor EL, which is known to induce hypersensitivity reactions (6). Therefore, novel methods are needed that would allow for delivery of effective concentrations of paclitaxel over extended time intervals while minimizing systemic toxicity especially to advanced cancer patients. Targeting drugs to disseminated prostate metastases is one of the most challenging goals in prostate cancer therapy. Drug colloidal carriers (nanoemulsions, liposomes, and nanoparticles) have shown great potential as improved delivery systems for an increasing number of active molecules (7). Although capable of enhanced accumulation in the target tissue, these carriers cannot provide marked targeting unless specific ligands are attached to them (8–10). Preclinical and clinical data show that the activation of the HER-kinase axis is important for the progression of prostate cancer to androgen-independent disease. It has recently been shown that overexpression of HER-2/neu can allow androgen-independent growth in androgen-dependent xenograft models (11–13). Among HER family receptors, HER2 activation is particularly important in breast cancer, as HER2 gene amplification is associated with a distinct clinical course and response to treatment with a HER2-directed therapy using trastuzumab, a humanized anti-HER2 monoclonal antibody (mAb; ref. 14). Although HER2 is overexpressed in metastatic prostate cancer (42% of the tested patients were positive), it was found that trastuzumab is not effective as a single agent for the treatment of these patients (15). However, the coupling of trastuzumab molecules to the surface of oil droplets containing appropriate cytotoxic agent concentrations in oil/water nanoemulsions will open the possibility of targeting such droplets to specific disseminated cells overexpressing HER2 receptor, as is the case in metastatic prostate cancer.

Emulsions can incorporate significant doses of lipophilic and hydrophobic active ingredients. Cationic emulsions have been proven capable of escaping from the reticulum endothelial system uptake and have shown prolonged blood circulation. The lack of toxic effects of the cationic emulsions was reported showing the safety of the cationic emulsions following i.v. injection (16, 17). Furthermore, there is no unwanted opsonization due to steric hindrance induced by the poloxamer copolymer molecules comprised in the combined mixed emulsifier film at the oil/water interface of the oil droplets of the cationic emulsion and oriented towards the external aqueous phase. This was again confirmed in a recent study on in vitro adsorption of plasma proteins onto the surface (charges)–modified submicron emulsions for i.v. administration (18).

In a previous study (19), optimal experimental conditions for the coupling of AMB8LK mAb to cationic emulsions containing the...
lipophilic marker coumarin-6 were identified in an attempt to achieve efficient targeted drug delivery system. The objectives of this study were to assess the efficiency of anti-HER2 immunomolecules loaded with paclitaxel palmitate in a well-established in vivo pharmacologic model of prostate cancer that overexpresses the HER2 receptor and to examine whether or not in can activate the complement system.

Materials and Methods

Synthesis of paclitaxel palmitate. Paclitaxel palmitate was synthesized from paclitaxel (Farmachem, Lugano, Switzerland) and palmitoyl chloride (Sigma, St. Louis, MO) in a single step that coupled palmitate to paclitaxel at the 2'-hydroxyl position. To a solution of paclitaxel (35 mg, 41 μmol) in methylene chloride (2.5 mL) under argon were added 4-dimethylaminopyridine (5 mg, 41 μmol), 1,3-dicyclohexylcarbodiimide (16.9 mg, 82 μmol), and palmitoyl chloride (13.5 mg, 41 μmol). The reaction mixture was stirred at ambient temperature for 2 h. After dilution with diethyl ether, the reaction mixture was washed with 5% aqueous hydrochloric acid, water, and saturated aqueous sodium chloride. The mixture was dried (sodium sulfate) and concentrated. Radial chromatography (silica gel, ethyl acetate-hexane) of the residue gave 45 mg (94%) of solid paclitaxel palmitate. Product was analyzed using liquid chromatography mass spectrometry; TLC, and high-performance liquid chromatography (HPLC) methods. In all experiments presented here, both paclitaxel and paclitaxel–palmitate palmitate were formulated in 10% Cremophor EL/10% ethanol/80% normal saline.

Emulsion preparation and characterization. The cationic blank emulsion was prepared according to the method previously described (18). Briefly, the oil and aqueous phases were prepared separately and heated at 70°C and then mixed and stirred with a magnetic stirrer. The final emulsions were obtained after mixing and homogenization in Silent Crusher S (Heidelberg Instruments, Schwabach, Germany) under eight microfluidization cycles, each for 2 min. The formulations (w/w) consisted of medium chain triglycerides (5%), Lipoid E-80 (1%), o-capric semiperfused with ''specimen diluents'' (in triplicates), at an activator-to-serum volume ratio of 1:4. One aliquot of each formulation and component. Tumors were measured once weekly allowing tumors to grow, establish, and overexpress the HER2 receptor. Treatment began after 3 weeks from cell injection, DPBS were injected into the dorsolateral prostate lobes after surgical exposure of the prostate. Treatment began after 3 weeks from cell injection, allowing tumors to grow, establish, and overexpress the HER2 receptor. Mice were randomly assigned to different treatment groups (5–8 mice per group). Paclitaxel palmitate–loaded cationic emulsion and paclitaxel palmitate–loaded cationic immunoemulsions were incubated with undiluted human serum (taken from different donors) for 30 min at 37°C (in triplicates), at an activator-to-serum volume ratio of 1:4. The reaction was terminated by the dilution of serum with ''specimen diluents'' provided in the kit. Zymozan was used as a control to ensure the presence of functional complement system in human sera. In all experiments, 10 mmol/L PBS (pH 7.4) was used as a control.

Cell culture. The human prostate cancer cell line PC3 was purchased from the American Type Culture Collection (Rockville, MD) and were maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD) with 10 mmol/L HEPES buffer. Medium was supplemented with 10% FCS (Life Technologies), penicillin/streptomycin solution (Biological Industries, Kibbutz Beit HaEmek, Israel), and fungizone (Life Technologies). Cells were cultured as monolayers in a humidified atmosphere of 95% air and 5% CO2 at 37°C.

Cell transfection. PC3.38 is a derivative of the PC3 cell line carrying the luc gene. PC3 cells were infected with a recombinant rLNC/Luc retrovirus and selected by G418 (270 μg/mL), generating stable PC3.38/Luc clone. The recombinant retrovirus rLNC/Luc was generated by packaging pLNC/Luc in HEK293T cells cotransfected with the packaging plasmid pCl (23).

Mouse strain and animal care. Strict animal care procedures set forth by The Authority for Animals Facilities of The Hebrew University of Jerusalem based on guidelines from the NIH guide for the Care and Use of Laboratory Animals were followed for all experiments (approval no. MD 105.03-4). All animals used in the study were male severe combined immunodeficient/beige mice, 8 weeks old (Harlan, Jerusalem, Israel).

Orthotopic prostate model and therapeutic studies. Male mice were anesthetized by i.m. injection of 120 mg/kg ketamine hydrochloride plus 6 mg/kg xylazine, and 5 × 107 PC3.38/Luc cells suspended in 50 μL sterile DPBS were injected into the dorsolateral prostate lobes after surgical exposure of the prostate. Treatment began after 3 weeks from cell injection, allowing tumors to grow, establish, and overexpress the HER2 receptor. Mice were randomly assigned to different treatment groups (5–8 mice per group). Paclitaxel palmitate–loaded cationic emulsion and paclitaxel palmitate–loaded cationic immunoemulsions were injected via tail vein once weekly for 3 weeks at dose of 10 mg/kg Paclitaxel palmitate solutionized in a vehicle similar to the marketed Taxol vehicle was also injected at the same dose as in the various emulsion formulations to evaluate the intrinsic effect of each formulation and component. Tumors were measured once weekly by bioluminescent imaging system in human.

In vivo imaging and light detection. For the continuous detection and quantification of tumor size in live mice, a cooled charged coupled device (CCCD) camera was employed as previously described (23). In brief, the CCCD system is comprised of a Roper Chemiluminescence Imaging System, which contains a CCCD model EN-CCD-1300EB equipped with an ST-133 controller and a 50-mm Nikon lens (Roper Scientific, Princeton Instrument,
Development was achieved using the HISTOFINE simple stain AEC Sol’ MAX PO for 30 min (Nichirei, Tokyo, Japan), washed again, and color wash buffer (BioGenex) for 5 min, incubated with HISTOFINE simple stain The HER2 antibody (1:1,000) was applied for 1 h. Slides were washed in incubations were conducted in a humidified box at room temperature. water and placed in wash buffer (BioGenex, San Ramon, CA). All slides were fixed in Ficin Sol’ (Zymed) for 10 min at 37 °C. The integration light was, in all cases, the result of a 2-min exposure and acquisition. Recorded measurements were the total sum of integrated signal, subtracted from the background area of equal size.

**Histopathology.** For toxicology safety evaluation of emulsions formulations and assessment of potential metastasis, the following organs were sectioned at (after 60 days): colon, duodenum, heart, kidneys, liver, lungs, spleen, stomach, and prostate. All organs listed were fixed and preserved in 10% neutral buffered formalin (~4% formaldehyde solution) for histopathologic examination. Organ/tissues are then trimmed, embedded in paraffin, sectioned at ~5 µm thickness, and stained with H&E. The histologic examination of all tissues includes a detailed examination of all tissue compartments, and histologic abnormalities were recorded and scored according to the compartment in which they are identified (24).

Indirect enzymatic immunohistochemical peroxidase method was used for HER2 receptor detection. Formalin-fixed, paraffin-embedded tissue blocks were cut (4 µm) and attached to the slides. The sections were stained using antibodies to HER2 (28-8003; Zymed, San Francisco, CA). Positive control was human breast carcinoma graded +3 (strong complete membrane staining of >10% of the tumor cells), according to the HercepTest from DAKO (Carpinteria, CA). Following dehydration through a series of graded alcohols, slides were blocked for endogenous peroxidase activity with 3% H2O2 for 10 min. Epitope retrieval was conducted by incubating the slides in Ficin Sol’ (Zymed) for 10 min at 37°C. Slides were rinsed in distilled water and placed in wash buffer (BioGenex, San Ramon, CA). All incubations were conducted in a humidified box at room temperature. The HER2 antibody (1:1000) was applied for 1 h. Slides were washed in wash buffer (BioGenex) for 5 min, incubated with HISTOFINE simple stain MAX PO for 30 min (Nichirei, Tokyo, Japan), washed again, and color development was achieved using the HISTOFINE simple stain AEC SoF (Nichirei) for 10 min. Slides were rinsed in water, counterstained with Mayer’s hematoxylin (Pioneer Research Chemicals, Colchester, United Kingdom), rehydrated through a series of graded alcohols, and coverslipped with GVA mounting solution (Zymed).

**Statistical analysis.** Treatments effects were compared using one-way ANOVA followed by Tukey post-test for tumor volume at each time point using Instat GraphPad Software. Differences were considered significant if $P < 0.05$.

**Results**

**Emulsion and immunoemulsion characterization.** The well-established emulsion manufacturing method yielded cationic nanoemulsions containing 4% paclitaxel palmitate exhibiting an average droplet size of 160 ± 30 nm and a positive zeta potential value of 50 ± 10 mV. The concentration of paclitaxel palmitate was monitored using HPLC, and 93% recovery was observed (i.e., final observed concentration of 3.72% w/w). The experimental conditions were controlled because high reproducibility was achieved upon preparation of three replicate batches with a range variation of ±12.5%.

Paclitaxel palmitate immunoemulsions exhibited an average mean droplet diameter of 160 to 180 nm and a positive zeta potential value of +50 mV identical to the zeta potential value of the unconjugated drug-loaded cationic emulsion. The palmitaxel palmitate incorporation yield was similar to the yield achieved with the unconjugated emulsion formulation.

The total amount of mAb conjugated to emulsions was evaluated with the BCA protein assay kit, and conjugation efficiency ranging from 60% to 70% was observed. Conjugated antibodies were visualized by TEM following incubation of anti-HER2 immunoemulsion with a conjugate of a 12-nm gold goat anti-human secondary antibody. The dark spots detected on the surface of the oil droplets (Fig. 1) suggest that trastuzumab molecules were covalently linked to the maleimide anchor present at the oil/water interface of preformed emulsions. An in vitro binding study to SK-BR3 cells (breast cancer cells), which are well known to overexpress the HER2 receptor, elicited good binding results for these immunoemulsions compared with unconjugated cationic emulsions and showed the qualitative mAb affinity to the surface of the oil droplets. The results for these immunoemulsions compared with unconjugated cationic emulsions and showed the qualitative mAb affinity to the

![Figure 1.](image1.png) TEM of anti-HER2 immunoemulsion with observed density of 50 antibodies per droplet negatively stained with phosphotungstic acid solution, following incubation with a goat anti-human IgG secondary antibody conjugated with 12-nm gold particle.

![Figure 2.](image2.png) Complement activation analysis by measuring SC5b-9 concentration in human sera following incubation with PBS, zymozan, Taxol, paclitaxel palmitate (PP), paclitaxel palmitate–loaded cationic emulsion, and immunoemulsion.
HER2 receptor despite the chemical modification and coupling process (data not shown).

**In vitro complement activation studies.** The results of the complement activation determination that measure the concentration of SC5b-9 in human serum are depicted in Fig. 2. It can be clearly noted that the unconjugated cationic emulsion and the anti-HER2 immunoemulsion did not activate the complement and even yielded significantly lower value than the market free drug Taxol and the paclitaxel palmitate solution in two different sera. Zymozan serves as a positive control and elicited a significantly higher value than the cationic emulsions and immunoemulsions. The substantial individual variation of SC5b-9 response was already observed and discussed by other authors when investigating the complement activation of various Taxol solutions (22).

**Orthotopic animal model validation.** Before the initiation of the animal experimentation, the PC3.38/Luc cells were tested for the expression of HER2 receptor by flow cytometry following incubation with trastuzumab. It was clearly noted that the cells did express the receptor (data not shown). In addition, the HER2 expression pattern in the animal prostate implemented with the cells was examined. It can be deduced from the results presented in Fig. 3 that the in vivo model is valid because the cancerous cells did overexpress HER2 receptors.

**Pharmacologic evaluation.** The inoculation of PC3.38/Luc tumor cells induced human prostate cancer, the growth of which was monitored noninvasively as noted in the saline control group (Fig. 4). Furthermore, the response of tumors and metastasis before, during, and after treatment was assessed. The longitudinal evaluation of bioluminescent tumor and metastatic development within the same groups of animals permitted sensitive and quantitative assessment of both primary and metastatic prostate tumor response in vivo. It can clearly be deduced from the data depicted in Fig. 4 that the paclitaxel palmitate–loaded immunoeulsion inhibits the tumor growth much more than the cationic emulsion and the paclitaxel palmitate formulation. In addition, it was qualitatively noted during the image analysis at day 56 from the cell injection that numerous metastases are visible in the saline group, whereas few are visible in the cationic emulsion group, and no metastases were detected in the immunoemulsion group. However, the presence of metastases in the various organs cannot be excluded based on the image analysis only. Thus, histopathologic analysis of the various organs in the different groups was carried out. The data indicated the presence of metastases in the lungs and kidneys of all the groups tested, and some sporadic metastases were observed in the stomach of the saline and emulsion groups (Table 1). It is interesting to note that no metastases were detected in the heart of the cationic emulsion and immunoemulsion groups, whereas metastases were observed in the saline and paclitaxel palmitate groups (Fig. 5). Most importantly, no compound-related toxicity lesions were seen in any of the organs examined.

**Discussion**

We have recently reported the results of the conjugation of an anti-ferritin mAb (AMB8LK) to oil droplets of cationic immunoemulsions (19). It was shown that the processes used for the preparation of these immunoemulsions did not affect the physicochemical properties of the emulsions (average droplet size of ~120 nm with a zeta potential of +50 mV) and did not alter the immunoreactivity of the mAb. This was again confirmed in the present study with respect to the conjugation of trastuzumab to cationic emulsions because the trastuzumab immunoemulsion was significantly bound to the well-known breast cancer cell line SKBR-3 overexpressing the HER2 receptor. In addition, the stability over storage time in room temperature was followed up during all the period of the animal experimentation. Before any injection, the size, potential zeta, pH, and drug content were determined and found unchanged. Paclitaxel is a highly promising drug against advanced and refractory cancers, such as breast and ovarian carcinomas. However, there is a need for development of an i.v formulation of the drug, which is safer and better tolerated than
the present Cremophor EL–based preparation (Taxol). Reformulation could also provide a possibility to improve the efficacy of paclitaxel-based anticancer therapy. Paclitaxel is difficult to formulate for i.v administration because of its poor aqueous solubility and general hydrophobicity. Indeed, paclitaxel can be incorporated into the internal oil phase of an emulsion (7). However, upon i.v. administration, the emulsion formulation is diluted infinitely, and the paclitaxel partitioned in favor of the serum owing to its log $P$ of 4.7. Thus, the biofate of paclitaxel in the emulsion is similar to the biofate of paclitaxel in the commercial product (25). It has already been reported by other authors that for a drug to be retained in an emulsion following i.v. administration, the log $P$ should be >7 (26); otherwise, the drug will be released rapidly in the serum, loosing the advantage of long blood circulation and possibility of organ-passive targeting. To allow paclitaxel to remain entrapped in the internal oil phase of an emulsion, paclitaxel lipophilicity should be markedly increased by esterification with a fatty acid as recently reported by Lundberg et al. (7) who entrapped in an anionic emulsion paclitaxel oleate. In the present study, paclitaxel palmitate (calculated log $P$ of 9) was synthesized and incorporated into the cationic emulsion before antibody conjugation. It should be emphasized that in preliminary animal studies using the orthotopic prostate cancer model, paclitaxel palmitate was compared with paclitaxel using an identical vehicle consisting of Cremophor EL and ethanol (1:1). Each drug formulation was injected to a group of four animals 24 h following inoculation with PC3.38/Luc cancer cells. Both drugs behaved similarly and prevented the growth of the prostate tumors over a testing period of 30 days at a dose of 10 mg/kg following three consecutive weekly injections. It could then be deduced that the esterification of paclitaxel did not affect the antitumor activity.

It is important to evaluate the emulsion formulations for complement activation as part of potential toxicity and side effect induction. In addition, complement activation decreases the plasma circulation time of colloidal systems. Complement activation determination was done using the Quidel SC5b-9 (TCC) Enzyme Immunoassay kit, which measures the concentration of SC5b-9 in human serum and plasma, thereby giving an indication of the status of the terminal complement pathway in the specimen. It can clearly be noted that the cationic emulsion and immunoemulsion did not activate the complement compared with the commercial and paclitaxel palmitate formulations that contained Cremophor EL, known to activate the complement (6). It should also be noted that trastuzumab injected at the doses of the present study did not activate the complement in a separate independent study. The substantial individual variation noted in the present study (Fig. 2) is not different from the variation reported by other authors for Taxol formulations who stated that the wide variation of SC5b-9 response to Taxol was probably multifactorial and due to individual differences in C3 conversion, C3b inactivation, and terminal complex formation (22).

The selection of the appropriate in vivo test system is crucial to establishing the worth of active molecules. A major drawback of animal models that use s.c. tumor implants is that they clearly do not reproduce the primary site of the common human cancer nor do they represent the common sites of metastasis (27, 28). Thus, it is now well accepted that s.c. growing human tumors in immunodeficient mice do not represent clinical cancer, especially with regard to metastasis and drug sensitivity (29). In an attempt to address these issues, orthotopic transplantation of tumor material to the appropriate site has been established. In fact, some authors have already reported about the noninvasive imaging technique for

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### Table 1. Individual metastases detection and histopathologic findings 56 days after injection of three different paclitaxel palmitate formulations in severe combined immunodeficient/beige mice bearing orthotopic prostate cancer

<table>
<thead>
<tr>
<th>Organ*</th>
<th>Saline</th>
<th>Paclitaxel palmitate formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>Heart</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Metastatic prostate carcinoma</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prostate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Metastatic prostate carcinoma</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Very large nodules</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Papilla, necrosis</td>
<td>−</td>
<td>3</td>
</tr>
<tr>
<td>Stomach</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Metastatic prostate carcinoma</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Spleen</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>White pulp lymphoid cell-reduced cellularity</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

NOTE: +, presence of metastases; −, no metastases. Histopathologic changes were described and scored using semiquantitative grading of five grades (0–4), taking into consideration the severity of the changes (0, unremarkable; 1, minimal; 2, mild; 3, moderate; 4, marked). Three mice from each group were examined (M1, M2, and M3).

*No metastases or histopathologic findings were detected in liver, duodenum, and colon.


References


Figure 5. A, histologic section of the heart: paclitaxel palmitate cationic emulsion (no abnormality detected in the heart). Arrow, intact pericardium. Magnification, ×4. B, magnification, ×40. C, histologic section of the heart: paclitaxel palmitate solution. Arrow, presence of metastatic prostatic carcinoma growing on the pericardium. Magnification, ×4. D, magnification, ×40. E, histologic section of the heart: paclitaxel palmitate immunoemulsion (no abnormality detected in the heart). Magnification, ×4. F, magnification, ×40.

monitoring luciferase-expressing human prostate tumors and metastasis in nude mice after i.p. inoculation of luciferin (30), as also done in the present study.

The longitudinal evaluation of bioluminescent tumor and metastatic development within the same groups of animals permitted sensitive and quantitative assessment of both primary and metastatic prostate tumor response in vivo. It can clearly be deduced from the data depicted in Fig. 4 that the paclitaxel palmitate–loaded immunoemulsion inhibits the tumor growth much more than the cationic emulsion (P < 0.05) and the paclitaxel palmitate formulation (P < 0.01) at day 56. It was not possible to carry a thorough statistical analysis of the data before day 56. The limited number of tumor bearing animals in each treatment group and the greater-than-anticipated variability in control tumor growth prevented the apparent increase in the cationic immunoemulsion efficacy from reaching statistical significance before day 56. In addition, it was qualitatively noted during the image analysis at day 56 from the cell injection that numerous metastases are limited in number of tumor bearing animals in each treatment group and the greater-than-anticipated variability in control tumor growth prevented the apparent increase in the cationic immunoemulsion efficacy from reaching statistical significance before day 56. In addition, it was qualitatively noted during the image analysis at day 56 from the cell injection that numerous metastases are detected in the immunoemulsion animal group. However, histopathologic analysis clearly showed the presence of metastases in all the organs tested, with the exception of the heart in cationic emulsion and immunoemulsion as shown in Fig. 5. There was no difference in antimetastatic activity between the emulsion and the immunoemulsion despite the affinity of the immunoemulsion towards the HER2 receptor. This can be attributed to the low efficiency of paclitaxel palmitate in treating prostate metastases generated from PC3 cell as reported by El Hillali et al. (31). These authors also reported that paclitaxel is effective in reducing prostate tumor growth but cannot eradicate prostate metastases as a single agent. A similar behavior for paclitaxel palmitate can be envisioned in the present study. Furthermore, the results of clinical trials in prostate cancer using trastuzumab alone or in combination with docetaxel or paclitaxel have been disappointing (15, 32). Although the tumor growth was not fully inhibited, these results are encouraging and need to be further investigated. It has been shown (33, 34) that vascular endothelial growth factor (VEGF) induction and its release from cells are stimulated immediately in breast cancer cells and prostate cancer cells when exposed to estrogens and androgens, respectively. Thus, when the prostate cancer becomes hormone independent, VEGF and its receptors play a crucial role in the neovascularization and progression of cancer. The abnormal features (leakage, hemorrhage, and tortuosity) of the tumor vasculature decrease markedly the cytotoxic drug delivery within the tumor tissue. Indeed, an antiangiogenic mAb should be aimed to normalize these tumor vessels, resulting in an increase of cytotoxic drug delivery within the tumor tissue. This hypothesis is supported by the findings showing that antibodies to VEGF slow tumor growth in androgen-independent prostate cancer xenograft models, an effect that was augmented with the addition of chemotherapy (35, 36). Apparently, a more improved specific drug delivery system to prostate tumor and disseminated prostate metastases is needed in terms of targeting and intracellular uptake. It is intended to conjugate an additional mAb to the actual immunoemulsion that exhibits antiangiogenic activity. Thus, the novel bifunctional immunoemulsions will elicit a more efficient effect than the actual encouraging targeted drug delivery system, allowing for specificity to the prostate cancer cells, whereas the antiangiogenic mAb will diminish the resistance of the cancer cells and should enhance the cell uptake of paclitaxel palmitate.

Nevertheless, the actual results are encouraging and may lead to an improved therapeutic strategy of metastatic prostate cancer treatment.

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