UC Irvine

Title

Immunophototherapy Using PDT Combined with Rapid Intratumoral Dendritic Cell Injection

Permalink

https://escholarship.org/uc/item/4wb9g03f

Journal

Photochemistry and Photobiology, 84(5)

ISSN

0031-8655

Authors

Sur, Brandon W
Nguyen, Phuong
Sun, Chung-Ho
et al.

Publication Date

2008-09-01

DOI

10.1111/j.1751-1097.2008.00356.x

Copyright Information

Peer reviewed

Immunophototherapy Using PDT Combined with Rapid Intratumoral Dendritic Cell Injection

Brandon W. Sur[1], **Phuong Nguyen**[1], **Chung-Ho Sun**[2], **Bruce J. Tromberg**[2,3,4], and **Edward L. Nelson**[1,3,5,*]

[1]Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of California, Irvine, CA

[2]Department of Surgery, Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA

[3]Chao Family Comprehensive Cancer Center, University of California, Irvine, CA

[4]Department of Biomedical Engineering, The Henry Samueli School of Engineering, Irvine, CA

[5]Department of Molecular Biology & Biochemistry, School of Biological Sciences, University of California, Irvine, CA

Abstract

The capacity of photodynamic therapy (PDT) to induce localized cell death and tissue damage suggests that when applied to tumors it could create a local depot of tumor-associated antigens, which would be available for uptake and presentation to the immune system, potentially leading to improved tumor control. Dendritic cells (DCs) are the most potent cells for antigen uptake, presentation, and stimulation of the immune system. However, it is unclear whether DCs would retain their viability and functional capacity for the requisite trafficking to draining lymph nodes when adoptively transferred in close temporal and anatomic proximity to the site of PDT-induced cytotoxicity. We conducted studies of combined PDT and adoptive DC therapy, "immunophototherapy," in a female, Fisher 344 rat orthotopic mammary tumor model. Using 5-aminolevulinic acid as a pro-drug, we demonstrated kinetically favorable biologic conversion to the photosensitive protoporphyrin IX, appropriate trafficking of syngeneic bone marrow-derived DCs injected into PDT-treated tumors within 15 min of completion of therapy, and improved survival over either modality alone. These data indicate that DCs rapidly administered into the site of PDT retain their viability and functional status, supporting the further evaluation of immunophototherapy strategies.

INTRODUCTION

Dendritic cells (DCs) are antigen-presenting cells that take up antigens and activate immune responses. In conventional DC-based immunotherapy, tumor-associated antigens (TAAs) in the form of peptides, tumor-derived protein extracts, or nucleic acid coding sequences, have served as the source of antigenic priming for the antitumor immune responses and have elicited robust anti-TAA immunity in laboratory and clinical experiments (1,2). Recently, DC-based immunotherapeutic strategies have shown promise, although for the majority of the DC immunotherapeutic strategies, clinical efficacy has been elusive (1,2). This may be due to the difficulties with appropriate trafficking of adoptively transferred DCs (3,4) or the

*Corresponding author enelson@uci.edu (Edward L. Nelson).

chosenDC phenotype (5). Additionally, the recognition of antigen-loss variants in patients undergoing monovalent antigen-specific immunotherapy (6–8) has driven the development of multivalent or polyvalent immunotherapy strategies (1,9–11). One strategic approach to generate multivalent therapies involves the use of allogeneic tumor lines that express a range of shared TAAs. However, the molecular heterogeneity of tumors with identical histology argues that an individual's own tumor may be the best source of the entire range of TAAs. Thus, methods that can safely and effectively provide the entire range of autologous TAAs, in the context of signals that stimulate appropriate activation and migration of a large number of DCs, would provide the opportunity to overcome this limitation and to fully realize the potential of DC-based immunotherapy.

Photodynamic therapy (PDT) is an approved cancer therapy for localized treatment of tumors and other diseases (12,13). In PDT, a photosensitizing drug is administered and the target site or cells are illuminated. Depending upon the light dose and dose rate, PDT has been reported to induce necrotic or apoptotic cell death *via* the induction of reactive oxygen species targeting both malignant cells and supporting mesenchymal structures, *i.e.* tumor vasculature and stroma (14–16). We and others have hypothesized that PDT could generate a depot of TAAs and, alone or in combination with other modalities, elicit antitumor immune responses (17–20). The efficiency of generation of antitumor immune responses by PDT alone is relatively low (17,21) providing a rationale for combining the most potent antigen-presenting, immunostimulatory cell for the immune system, DCs, with PDT. Yet, whether adoptively transferred DCs are able to survive in the microenvironment of PDT-treated tumor and retain their functional capacity remains an open question. This functional capacity includes the ability to traffic to draining lymph nodes in order to present the TAAs to the adaptive arm of the immune system and elicit antitumor immunity.

We examined a combined strategy of PDT and adoptive transfer of DCs, which we termed immunophototherapy (IPT), which was designed with considerations for ease of translation into the clinical arena. We selected a 5-aminolevulinic acid (5-ALA)-based PDT strategy that relies on the conversion of 5-ALA to protoporphyrin IX (PpIX), the photosensitive compound. PpIX has been reported to accumulate locally in the epithelium, both malignant and normal, although it has been thought to accumulate preferentially in tumor cells (22). The potential for preferential accumulation of PpIX in tumor cells, in conjunction with focal illumination of a tumor mass leads to tumor cell death with relative sparing of normal tissues. In this study, we report the pharmacokinetics of PpIX in orthotopic rat mammary tumors, trafficking of adoptively transferred DCs to draining lymph nodes after low light dose PDT, and evaluation of treatment efficacy.

MATERIALS AND METHODS

Animals and tumor models

An orthotopic mammary tumor model was used for these studies comprising Fisher 344 rats and the moderately immunogenic, syngeneic rat mammary tumor line, 13 762 MAT B III (CRL-1666; ATCC, Manassas, VA) (23). Tumor cells were inoculated into the proximal inguinal mammary fat pad immediately adjacent to one of the two more proximal nipples (24) at a dose of 1×10^5 viable cells. Tumor growth was followed with tumor volume calculated according to the equation $V = 0.4 (ab^2)$, where a is the larger diameter and b is the smaller of the perpendicular diameters (25). All experiments were initiated when animals were bearing tumors of 400–500 mm^3. Animals were killed when tumor exceeded 10 000 mm^3 or in distress. All studies were carried out under an IACUC approved protocol under conditions that meet the NIH principles of laboratory animal care (NIH publication no. 85–23).

Photosensitizer

5-Aminolevulinic acid (Sigma-Aldrich, St. Louis, MO) was dissolved in 1× PBS (pH 7.4, at 20 mg mL^{-1}) and administered by oral gavage at a dose of 100 mg kg^{-1} per rat. The 5-ALA solution had a pH ≈ 3.5 immediately before administration.

Determination of PpIX conversion kinetics

Tumors and adjacent control skeletal muscle tissues were collected at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 h after administration of 5-ALA. The collected samples were frozen rapidly in OCT (Sakura Finetek U.S.A., Inc., Torrance, CA) at −180°C. Frozen sections at a thickness of 8 μm were cut and stored at −80°C until their fluorescence was checked by fluorescence microscopy.

Fluorescence microscopy

Fluorescence images were acquired with a low light level fluorescence microscopy system, consisting of a Zeiss Axiovert 10 inverted microscope (Zeiss, Oberkochen, Germany) with a 10× Zeiss Plan-NEOFLUAR objective (NA = 0.3) connected to a slow-scan cooled CCD camera (576× 384 pixels, model 57–180; Princeton Instruments, Trenton, NJ) with 16-bit per pixel dynamic range of data acquisition and a personal computer (Power Macintosh 8600/300; Apple Computer, Inc., Cupertino, CA). This system and the software (IPlab Ver 3.2; Signal Analytics Corp., Vienna, VA) were used to acquire bright-field, phase-contrast and fluorescent images and to determine location and intensity of fluorescence. Excitation was delivered by a 100 W Hg lamp. A long pass filter (excitation: 375–440 nm band pass and emission: 470 nm cutoff) was used to detect PpIX fluorescence.

Photodynamic therapy

Tumors were allowed to grow to 400–500 mm^3 (approximately 1 cm in diameter), usually taking about 10 days, before animals were used for experiments involving PDT. The site of illumination was subjected to depilation 30 min before PDT to insure effective light delivery. The location of the tumor was irradiated 2 h after the administration of 5-ALA using a 639 nm laser (Laser Diode IQ1C20, Mod #639-35; Power Technology, Alexander, AR) delivered *via* an optical fiber with a 0.06 inch diameter micro-lens Frontal Light Distributor Model CE 0120 (Medlight SA, Ecublens, Switzerland). A light dose rate of 25 mW cm^{-2} was delivered for 16 min for a total dose of 24 J cm^{-2} (area of illumination: 1 cm^2).

Assessment of apoptosis

Tumors from three groups of rats (control, 5-ALA alone and PDT; three rats for each group) were examined to determine whether the light dose of PDT (total output of 24 J cm^{-2}) induces apoptosis. Rats were euthanized 8 h after PDT and tumors were collected and frozen rapidly in OCT (Sakura Finetek U.S.A., Inc.). The frozen samples were frozen sectioned at 8 μm thickness and fixed with methanol. One hundred microliters of Anti-ACTIVE® Caspase-3 polyclonal antibody (Promega, Madison, WI) diluted 1:250 in 1× PBS (pH 7.4) was added to each of the frozen sectioned samples and was incubated in a humidified chamber overnight at 4°C. After incubation, 100 μL of goat antirabbit IgG (H + L) Cy3 conjugate (Caltag Laboratories, Burlingame, CA) diluted 1:500 in 1× PBS (pH 7.4) was added to the samples, protected from light and incubated for another 2 h at room temperature. The samples were checked for fluorescence with the low light imaging system with a fluorescent filter for Cy3 (excitation: 530–585 nm; emission: 610 nm).

Generation of DCs from bone marrow

Rat bone marrow–derived DCs were generated essentially as described for mouse DCs (26). Briefly, individual femurs from 6- to 8-week-old rats were harvested and the marrow flushed in a sterile fashion from the medullary cavity with PBS. Bone marrow was then subjected to erythrocyte lysis, using standard procedures and ACK lysis solution (ICN Biomedicals, Inc., Aurora, OH). The remaining nucleated cells were placed into culture at 10^6 cells mL^{-1} for 8 days in RPMI 1640 (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum, L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential amino acid mixture (1×), penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) (Gibco BRL Life Technologies, Carlsbad, CA), 2-mercaptoethanol (50 μM) (Sigma, St. Louis, MO), and recombinant rat GM-CSF (200 U mL^{-1}, 10 μg mL^{-1}; R&D Systems, Minneapolis, MN) and recombinant IL-4 (1000 U mL^{-1}, 25 μg mL^{-1}; R&D Systems). Cells were fed 50% media replacement containing replacement cytokines every 3 days. DCs were evaluated for morphology and cell surface phenotype by flow cytometry using appropriately fluorochrome labeled antibodies to confirm DC phenotype (27–29) prior to use. Our DC preparations routinely exhibit high level staining for CD54, and MHC Class II, positive staining for CD11c, CD80 and CD86, low level positive staining for CD40 and OX62 with other lymphocyte lineage markers being negative and other shared markers with macrophages being positive, *e.g.* CD11b & CD68 (ED1).

CFSE labeling of DC

Bone marrow–derived DCs were labeled with CFSE (CellTrace™ CFSE cell proliferation kit; Molecular Probes/Invitrogen, Carlsbad, CA) 1.5 h before injection by incubation with CFSE at a concentration of 5 μM in 1× PBS for 8 min, washed twice with 10 mL RPMI. Cells were then evaluated for efficiency of CSFE labeling and stability of cell surface phenotype by flow cytometry to confirm that labeling was adequate and that it did not modulate the cell surface phenotype.

Assessment of DCs trafficking to lymph nodes

Three groups of rats (control, CFSE-labeled DCs and PDT + CFSE-labeled DCs) were examined. 5×10^4 CFSE-labeled DCs in 0.1 mL of 1× PBS (pH 7.4) were injected into PDT-treated tumors for individual rats 15 min after each PDT session. At 0, 24 and 48 h after the injection, a minimum of three rats from each group were euthanized with immediate harvest of tumors, draining lymph nodes (lymphonodus subiliaci) (24) and contralateral draining lymph nodes, which were frozen rapidly in OCT (Sakura Finetek U.S.A., Inc.) at −180°C and then stored at −75°C. The frozen samples were sectioned at 8 μm thickness and evaluated for fluorescence with the low light imaging system and an appropriate filter set (excitation: 480 [21] nm bandpass and emission: 535 [29] nm bandpass) at a magnification of 40× for photomicroscopy and initial inspection. Sufficient nonserial sections were obtained to provide 10 high power (100×) microscopic fields for all examined tissues. Fluorescent images from equivalent visual field numbers were used across conditions and time for comparison of paired tissues.

Tumor treatment

Individual cohorts of 8–10 rats each with established tumors received one of the following treatments—PDT alone, DC alone, combined PDT and DC (IPT) or no additional treatment. Animals receiving PDT were administered 5-ALA (100 mg kg^{-1}) 2 h before PDT, as above. Animals receiving adoptive DC transfer received one aliquot of 1×10^5 syngeneic bone marrow–derived DCs in PBS injected into the middle of the tumor 15 min after completion of illumination, PDT. Survival of the rats and tumor growth were monitored for 60 days.

RESULTS

5-ALA conversion kinetics

We investigated the kinetics of conversion of 5-ALA to PpIX as well as the distribution of PpIX in tumors in order to determine the optimal relationship between 5-ALA administration and PDT administration. We observed that maximum fluorescence, due to PpIX, both in terms of intensity and selective distribution, occurred 2 h after administration of 5-ALA (Fig. 1), even though we observed fluorescence as early as 1 h after administration of 5-ALA indicating fairly rapid metabolism of 5-ALA by tumor cells. Tumor fluorescence decreased after 2 h relative to background normal tissues, but persisted up to 12 h with oral gavage administration. Intratumoral injection of 5-ALA failed to provide any significant benefit over oral administration (data not shown). Very limited fluorescence from PpIX was detected in control tissues during the first 2 h indicating high specificity of 5-ALA accumulation and its conversion to PpIX in tumor cells. In subsequent experiments, all PDT was performed 2 h after 5-ALA administration.

Apoptosis induced by PDT

As PDT can induce either apoptotic or necrotic cell death, we examined tumors treated with PDT at a light dose of $24 \, J \, cm^{-2}$ for induction of apoptosis. In our experiment, fluorescence from Cy3-labeled antiactive capase-3 antibody was detected only in tumors treated with PDT and not observed in tumors from control or tumors from rats administered 5-ALA only (Fig. 2). Moreover, the location of maximal fluorescence was confined to the center portion of the tumors coinciding with the area of laser irradiation during PDT. This demonstrated that the PDT dose we selected induced apoptosis, specifically in the irradiated area of tumors, and that 5-ALA alone did not induce tumor cell apoptosis.

Trafficking of DCs to draining lymph nodes

It is unknown as to whether the process of PDT affects migration of DCs to draining lymph nodes when DCs are administered in close temporal proximity to the delivery of PDT at the treatment site. Thus we evaluated the trafficking of CFSE-labeled bone marrow–derived DCs. We characterized the kinetics and magnitude of DC trafficking using low light imaging of sections from ipsilateral draining lymph nodes at given periods after injection of labeled DCs into tumors. We observed no fluorescent cells in the draining lymph nodes immediately after injection of the CFSE-labeled DC (Fig. 3), but a high density of labeled cells in tumors right after the injection. The number of fluorescent cells in the draining lymph nodes from PDT-treated rats increased dramatically 24 h after the injection peaking between 24 and 48 h after injection, supporting the persistence of migrated DCs in the lymph nodes long enough for interaction with T lymphocytes and the induction of immune responses. In contrast, the number of fluorescent cells in the lymph nodes from non–PDT-treated rats showed only a minimal increase 24 h after the injection that was comparable to that seen after 48 h, indicating the limited migration of DCs administered locally into the tumor bed in the absence of PDT. These data are consistent with that reported in other settings of adoptive transfer of DCs (3). In addition to the ipsilateral draining lymph nodes, contralateral draining lymph nodes from both PDT-treated and non–PDT-treated rats were examined and no significant numbers of CFSE-labeled DCs were observed (data not shown).

DC injection after PDT (IPT) enhances survival

Based upon our above data indicating feasibility of IPT, we investigated the potential for enhanced survival with IPT over that of PDT or DC administration alone. The tumor model that we employed consisted of an established, rapidly growing syngeneic mammary tumor.

In the control animals, tumors grew rapidly requiring euthanization within 22 days from tumor inoculation. The administration of IPT significantly slowed tumor growth and enhanced survival (Fig. 4). Moreover, no recurrence of tumor was observed once the tumor was eliminated. In contrast, administration of DC or PDT alone had limited treatment efficacy (Fig. 4), with the majority of rats in the groups requiring euthanization within 10 days from the PDT treatment. This demonstrates the induction of an enhanced antitumor activity by the IPT combination.

DISCUSSION

These studies have demonstrated the feasibility and antitumor efficacy of combined PDT and rapid adoptive transfer of immature DCs to the site of PDT tumor treatment. This therapeutic strategy was designed taking into consideration the potential for translation into the clinical arena, specifically by minimizing potential toxicities and simplifying the therapeutic interventions. In an orthotopic tumor model, using an oral pro-drug, we have demonstrated the rapid accumulation and conversion to the photosensitizing agent within tumor cells. The use of a nontoxic oral pro-drug has distinct advantages for human use. Additionally, a relatively low light dose, 24 J cm^{-2}, induced tumor cell apoptosis and when combined with the immediate one-time adoptive transfer of syngeneic immature DCs resulted in improved survival. These data corroborate observations from other groups using heterotopic tumor models, more toxic photosensitizing agents, higher light doses and delayed or repeated administrations of DCs (18,20). The retention of DC functional capacity and antitumor efficacy in this more streamlined therapeutic strategy supports the investigation of this or similar strategies in human clinical studies.

Numerous photosensitizing agents have been employed for PDT studies. However, currently there is a limited range of approved systemic agents for human use, which include 5-ALA, temoporfin (Foscan®) and porfimer sodium (Photo-frin ®). Of these, 5-ALA has the advantage of being an oral pro-drug that appears to have favorable pharmacokinetic parameters (30–32) with more rapid accumulation and metabolic conversion to PpIX (the photoactive compound) in tumors along with more rapid clearance. Although the preferential uptake and conversion to PpIX varies between tumor cell lines, the use of iron chelators has shown promise for minimizing this variability (31,33) and the recent approval of an oral iron chelating agent, deferasirox (Exjade®), provides further support for translational investigations of 5-ALAbased PDT strategies.

Because activated lymphocytes, macrophages and DCs have been reported to rapidly accumulate 5-ALA and convert it to PpIX (34), it was critical to address whether DCs would retain their functional capacity when administered in close temporal proximity to both 5-ALA administration and PDT treatment. Our data support the capacity of DCs to both survive and acquire the "mature" phenotype required for migration to draining lymph nodes. This suggests that the functional capacity of DCs is not significantly compromised by their adoptive transfer into a tumor site immediately after 5-ALA-based PDT treatment.

Several studies have shown that PDT can induce either apoptosis or necrosis depending on light dose and dose rate, with low output short duration of light exposure causing cell death primarily by apoptosis (35,36). The low dose (24 J cm^{-2}) was chosen to insure that PDT alone does not lead to complete regression of tumor, to minimize potential toxicity of the PDT itself, and to create an effective microenvironment for antigen uptake and activation of DCs. High light dose PDT has been shown to lead to the release of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1 and interleukin-6 (37) but has been associated with less antitumor efficacy in some models (38). Our findings indicate that low light dose PDT tumor treatment can function effectively to generate an activating or

maturation signal for DCs, which may be able to overcome the "locking in" of DCs at the tumor site and the suppressed antigen uptake and activation of DCs observed in cancer patients (39).

Two other groups have reported combined PDT and DC adoptive transfer studies. The first of these (18) employed heterotopic footpad injections of the murine colon carcinoma cell line, CT26, intraperitoneal administration of Photofrin 24 h before PDT and 90 J cm^{-2} light dose. This was combined with administration of DCs at the treated tumor site on day 1 (1 h after PDT), 24 h after PDT and in some experiments on day 8. Although they showed DC trafficking with a single DC administration, their treatment effect was dependent upon at least two DC administrations, in contrast to our findings using a single administration of DCs in close temporal proximity to the performance of PDT. This temporal sequence poses several hurdles to clinical translation; the requirement for an additional DC dose administered after 24 h requires a second procedure, which heightens patient risk for adverse events, results in a greater likelihood of disparate anatomic locations for PDT and instillation of DCs, and finally diminishes patient tolerance. Additionally, in their trafficking experiment, this group reported finding fewer labeled DCs in regional lymph nodes from PDT-treated tumors relative to untreated tumors, 24 h after DC injection. These findings reinforce the concern that PDT results in a local environment with detrimental effects on administered DCs. The fact that we examined lymph node frozen sections whereas Jalili *et al.* evaluated the DC population by flow cytometry of single cell suspensions from lymph nodes may account, in part, for the discrepancy between our respective results. Nevertheless, taken together our trafficking results and the treatment efficacy suggest that DCs administered in close temporal and anatomic proximity to PDT retain their functional capacity.

A second group (20) also employed heterotopic (subcutaneous, flank inoculation of either CT26 or B16 melanoma cell lines) tumor models. This group used intravenous administration of a photosensitizer that is not approved for clinical use, ATX-S10 Na(II), followed by PDT at a light dose of 150 J cm^{-2} 5 h after drug administration. Separate doses of DCs were injected 24, 48, 72 and 120 h after PDT treatment. Although *in vitro* antitumor immunity was demonstrated supporting an intact functional capacity of the administered DCs, the contribution of the various doses of DCs to this immunity, DC survival and trafficking to draining lymph nodes were not reported. Nevertheless, both of these groups have also reported increased clinical efficacy of the combination of PDT and adoptive transfer of DCs, albeit in more involved strategies. Although *in vitro* antitumor immune assays are significantly more challenging in rat models, the use of an orthotopic rat mammary tumor model permitted treatment of larger tumors in their native anatomic compartment and provides demonstration of clinical efficacy in a distinctly separate tumor model and species. This tumor model has been reported to produce high levels of transforming growth factor-β that compromise both *in vivo* and *in vitro* antitumor immune responses (23,40). Nevertheless, taken together, these three sets of studies provide substantial support for this strategy of combined PDT and adoptive DC transfer.

The strength of our IPT design lies not only in its potentially streamlined TAA uptake process by DCs, but also in ease of preparation and its wide applicability. Available pharmacokinetic data suggest that patients could consume an oral dose of 5-ALA approximately 2 h before administration of PDT. This is comparable to the consumption of oral contrast agents for computed tomography. This advantageous timing, in addition to being able to administer DCs as a single dose within minutes of completing the PDT treatment, results in a therapeutic strategy that could easily be administered in a single patient visit lasting only a few hours. Additionally, we focused on low light dose PDT and demonstrated enhanced trafficking of DCs along with clinical efficacy. Although it remains

to be determined whether higher light doses will result in improved or diminished DC trafficking, toxicity and/or therapeutic outcome, the fact that low light dose PDT has this level of activity suggests that clinical application of low light dose PDT should be investigated. As a result of tumor cell death due to PDT, the injected DCs are likely to have access to and ultimately present the entire *in situ* TAA repertoire to the immune system. Thus, there is less of a need to identify or isolate specific TAAs in order to perform DC immunotherapy and the DCs are likely to elicit a polyvalent antitumor immune response that is fully matched to the patient's tumor. Moreover, this strategy does not rely on harvest or culture of autologous tumor, which is simply not practical for many patients.

The rapidly advancing minimally invasive technologies and procedures will significantly enhance the capacity to deliver low light dose PDT and adoptive DC therapy to tumor masses previously inaccessible. These technologies can be employed without subjecting patients to prolonged duration treatments (due to the use of low light PDT) or to high-risk surgical procedures. The role for IPT is likely to be dependent upon tumor type and may require modifications for different tumors in order to maximize therapeutic potential of autologous DCs in the setting of IPT. Nevertheless, this or similar IPT strategies could be readily adapted for human clinical applications and should be investigated expediently.

Acknowledgments

REFERENCES

1. Osada T, Clay TM, Woo CY, Morse MA, Lyerly HK. Dendritic cell-based immunotherapy. Int. Rev. Immunol. 2006; 25:377–413. [PubMed: 17169781]

2. Ridgway D. The first 1000 dendritic cell vaccinees. Cancer Invest. 2003; 21:873–886. [PubMed: 14735692]

3. Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. Cancer Res. 1999; 59:56–58. [PubMed: 9892184]

4. Soruri A, Zwirner J. Dendritic cells: Limited potential in immunotherapy. Int. J. Biochem. Cell Biol. 2005; 37:241–245. [PubMed: 15474968]

5. Turtle CJ, Hart DN. Dendritic cells in tumor immunology and immunotherapy. Curr. Drug Targets. 2004; 5:17–39. [PubMed: 14738216]

6. Igney FH, Krammer PH. Immune escape of tumors: Apoptosis resistance and tumor counterattack. J. Leukoc. Biol. 2002; 71:907–920. [PubMed: 12050175]

7. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat. Immunol. 2002; 3:999–1005. [PubMed: 12407407]

8. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: Molecular mechanisms and functional significance. Adv. Immunol. 2000; 74:181–273. [PubMed: 10605607]

9. Bystryn JC, Zeleniuch-Jacquotte A, Oratz R, Shapiro RL, Harris MN, Roses DF. Double-blind trial of a polyvalent, shed-antigen, melanoma vaccine. Clin. Cancer Res. 2001; 7:1882–1887. [PubMed: 11448900]

10. Tanaka M, Kaneda Y, Fujii S, Yamano T, Hashimoto K, Huang SK, Hoon DS. Induction of a systemic immune response by a polyvalent melanoma-associated antigen DNA vaccine for prevention and treatment of malignant melanoma. Mol. Ther. 2002; 5:291–299. [PubMed: 11863419]

11. Vilella R, Benitez D, Mila J, Vilalta A, Rull R, Cuellar F, Conill C, Vidal-Sicart S, Costa J, Yachi E, Palou J, Malvehy J, Puig S, Marti R, Mellado B, Castel T. Treatment of patients with progressive unresectable metastatic melanoma with a heterologous polyvalent melanoma whole cell vaccine. Int. J. Cancer. 2003; 106:626–631. [PubMed: 12845663]

12. Taub AF. Photodynamic therapy: Other uses. Dermatol. Clin. 2007; 25:101–109. [PubMed: 17126748]

13. Triesscheijn M, Baas P, Schellens JH, Stewart FA. Photodynamic therapy in oncology. Oncologist. 2006; 11:1034–1044. [PubMed: 17030646]

14. Harrod-Kim P. Tumor ablation with photodynamic therapy: Introduction to mechanism and clinical applications. J. Vasc. Interv. Radiol. 2006; 17:1441–1448. [PubMed: 16990463]

15. Henderson BW, Busch TM, Snyder JW. Fluence rate as a modulator of PDT mechanisms. Lasers Surg. Med. 2006; 38:489–493. [PubMed: 16615136]

16. Peng Q, Nesland JM. Effects of photodynamic therapy on tumor stroma. Ultrastruct. Pathol. 2004; 28:333–340. [PubMed: 15764581]

17. Castano AP, Mroz P, Hamblin MR. Photodynamic therapy and anti-tumour immunity. Nat. Rev. Cancer. 2006; 6:535–545. [PubMed: 16794636]

18. Jalili A, Makowski M, Switaj T, Nowis D, Wilczynski GM, Wilczek E, Chorazy-Massalska M, Radzikowska A, Maslinski W, Bialy L, Sienko J, Sieron A, Adamek M, Basak G, Mroz P, Krasnodebski IW, Jakobisiak M, Golab J. Effective photoimmunotherapy of murine colon carcinoma induced by the combination of photodynamic therapy and dendritic cells. Clin. Cancer Res. 2004; 10:4498–4508. [PubMed: 15240542]

19. Le NT, Chen BJ, Chao NJ. Selective elimination of alloreactivity from immunotherapeutic T cells by photodynamic cell purging and memory T-cell sorting. Cytotherapy. 2005; 7:126–133. [PubMed: 16040391]

20. Saji H, Song W, Furumoto K, Kato H, Engleman EG. Systemic antitumor effect of intratumoral injection of dendritic cells in combination with local photodynamic therapy. Clin. Cancer Res. 2006; 12:2568–2574. [PubMed: 16638867]

21. Skivka LM, Gorobets OB, Kutsenok VV, Lozinsky MO, Borisevich AN, Fedorchuk AG, Kholin VV, Gamaleya NF. 5-aminolevulinic acid mediated photodynamic therapy of Lewis lung carcinoma: A role of tumor infiltration with different cells of immune system. Exp. Oncol. 2004; 26:312–315. [PubMed: 15627065]

22. Tunstall RG, Barnett AA, Schofield J, Griffiths J, Vernon DI, Brown SB, Roberts DJ. Porphyrin accumulation induced by 5-aminolaevulinic acid esters in tumour cells growing in vitro and in vivo. Br. J. Cancer. 2002; 87:246–250. [PubMed: 12107850]

23. Frey AB, Appleman LJ. Rat adenocarcinoma 13762 expresses tumor rejection antigens but tumor-bearing animals exhibit tumor-specific immunosuppression. Clin. Immunol. Immunopathol. 1993; 69:223–233. [PubMed: 8403560]

24. Krinke, G. The Laboratory Rat. San Diego: Academic Press; 2000.

25. Attia MA, DeOme KB, Weiss DW. Immunology of spontaneous mammary carcinomas in mice II. Resistance to a rapidly and a slowly developing tumor. Cancer Res. 1965; 25:451–457. [PubMed: 14297481]

26. Talmor M, Mirza A, Turley S, Mellman I, Hoffman LA, Steinman RM. Generation or large numbers of immature and mature dendritic cells from rat bone marrow cultures. Eur. J. Immunol. 1998; 28:811–817. [PubMed: 9541575]

27. Jung S, Siglienti I, Grauer O, Magnus T, Scarlato G, Toyka K. Induction of IL-10 in rat peritoneal macrophages and dendritic cells by glatiramer acetate. J. Neuroimmunol. 2004; 148:63–73. [PubMed: 14975587]

28. Kawashima M, Ide M, Nakanishi M, Kuwamura M, Kumagai D, Yamate J. Immunophenotypical changes of neoplastic cells and tumor-associated macrophages in a rat dendritic cell sarcoma-derived transplantable tumor line (KB-D8). Virchows Arch. 2003; 442:141–150. [PubMed: 12596065]

29. Yrlid U, Macpherson G. Phenotype and function of rat dendritic cell subsets. Apmis. 2003; 111:756–765. [PubMed: 12974777]

30. Bellnier DA, Greco WR, Loewen GM, Nava H, Oseroff AR, Dougherty TJ. Clinical pharmacokinetics of the PDT photosensitizers porfimer sodium (Photofrin), 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (Photochlor) and 5-ALA-induced protoporphyrin IX. Lasers Surg. Med. 2006; 38:439–444. [PubMed: 16634075]

31. Gibbs SL, Chen B, O'Hara JA, Hoopes PJ, Hasan T, Pogue BW. Protoporphyrin IX level correlates with number of mitochondria, but increase in production correlates with tumor cell size. Photochem. Photobiol. 2006; 82:1334–1341. [PubMed: 16771607]

32. Hautmann H, Pichler JP, Stepp H, Baumgartner R, Gamarra F, Huber RM. In-vivo kinetics of inhaled 5-aminolevulinic acid-induced protoporphyrin IX fluorescence in bronchial tissue. Respir. Res. 2007; 8:33. [PubMed: 17445266]

33. Uehlinger P, Ballini JP, van den Bergh H, Wagnieres G. On the role of iron and one of its chelating agents in the production of protoporphyrin IX generated by 5-aminolevulinic acid and its hexyl ester derivative tested on an epidermal equivalent of human skin. Photochem. Photobiol. 2006; 82:1069–1076. [PubMed: 17205631]

34. Hryhorenko EA, Oseroff AR, Morgan J, Rittenhouse-Diakun K. Antigen specific and nonspecific modulation of the immune response by aminolevulinic acid based photodynamic therapy. Immunopharmacology. 1998; 40:231–240. [PubMed: 9858066]

35. Madsen SJ, Sun CH, Tromberg BJ, Yeh AT, Sanchez R, Hirschberg H. Effects of combined photodynamic therapy and ionizing radiation on human glioma spheroids. Photochem. Photobiol. 2002; 76:411–416. [PubMed: 12405149]

36. Oleinick NL, Morris RL, Belichenko I. The role of apoptosis in response to photodynamic therapy: What, where, why, and how. Photochem. Photobiol. Sci. 2002; 1:1–21. [PubMed: 12659143]

37. Gollnick SO, Liu X, Owczarczak B, Musser DA, Henderson BW. Altered expression of interleukin 6 and interleukin-10 as a result of photodynamic therapy in vivo. Cancer Res. 1997; 57:3904–3909. [PubMed: 9307269]

38. Henderson BW, Gollnick SO, Snyder JW, Busch TM, Kousis PC, Cheney RT, Morgan J. Choice of oxygen-conserving treatment regimen determines the inflammatory response and outcome of photodynamic therapy of tumors. Cancer Res. 2004; 64:2120–2126. [PubMed: 15026352]

39. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: Mapping the way. Nat. Med. 2004; 10:475–480. [PubMed: 15122249]

40. Alvarez E, Westmore M, Galvin RJ, Clapp CL, Considine EL, Smith SJ, Keyes K, Iversen PW, Delafuente DM, Sulaimon S, Zambrano C, Ma L, Sato M, Martin TJ, Teicher BA, Galbreath EJ. Properties of bisphosphonates in the 13762 rat mammary carcinoma model of tumor-induced bone resorption. Clin. Cancer Res. 2003; 9:5705–5713. [PubMed: 14654555]
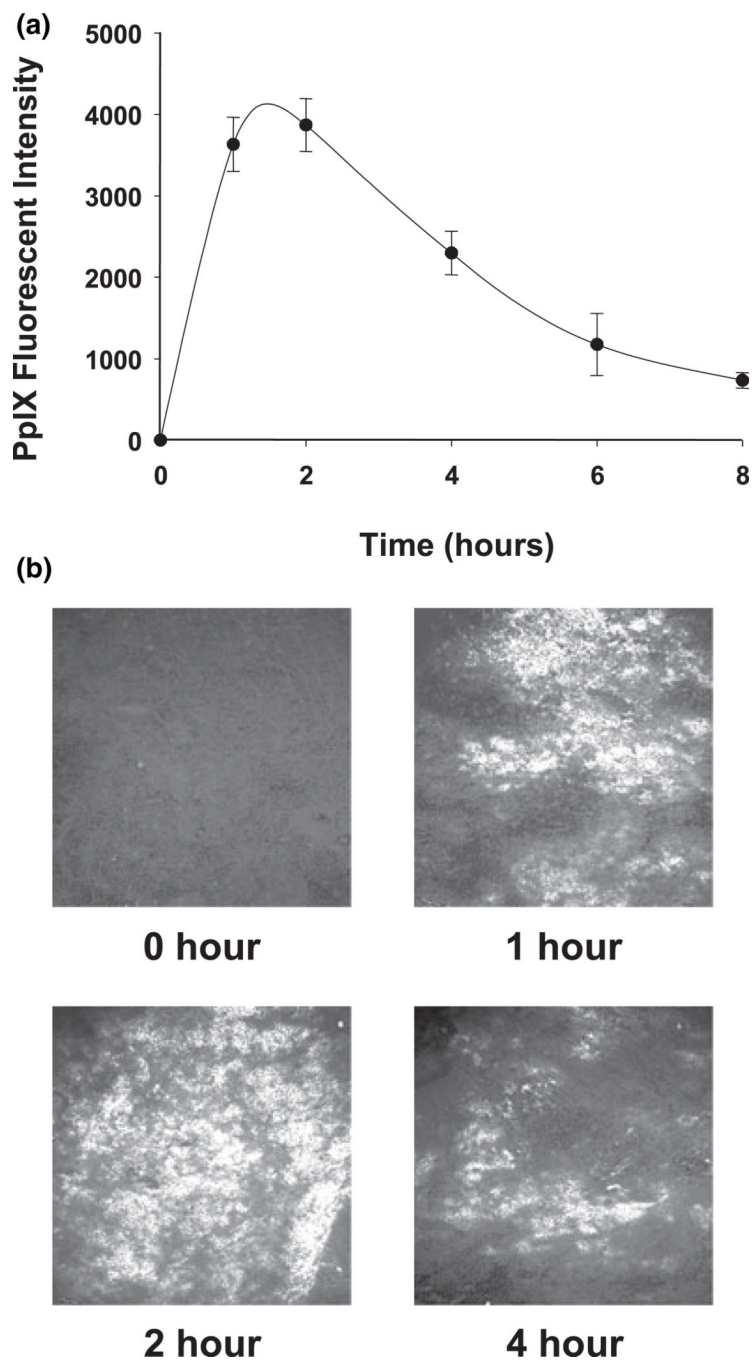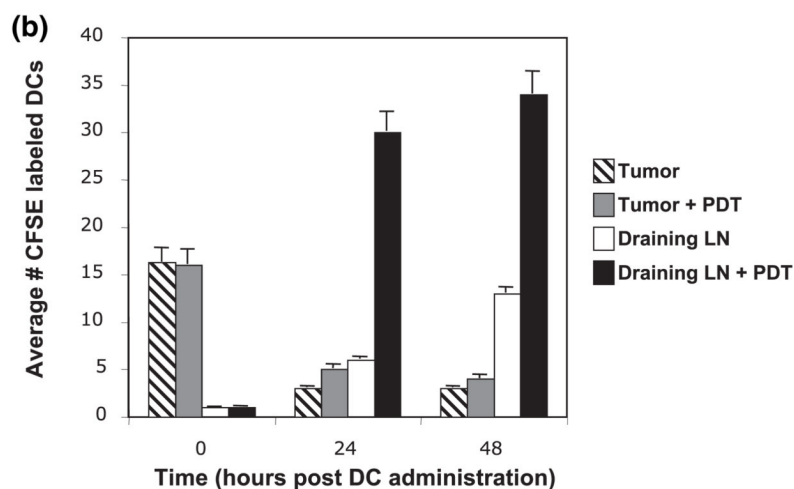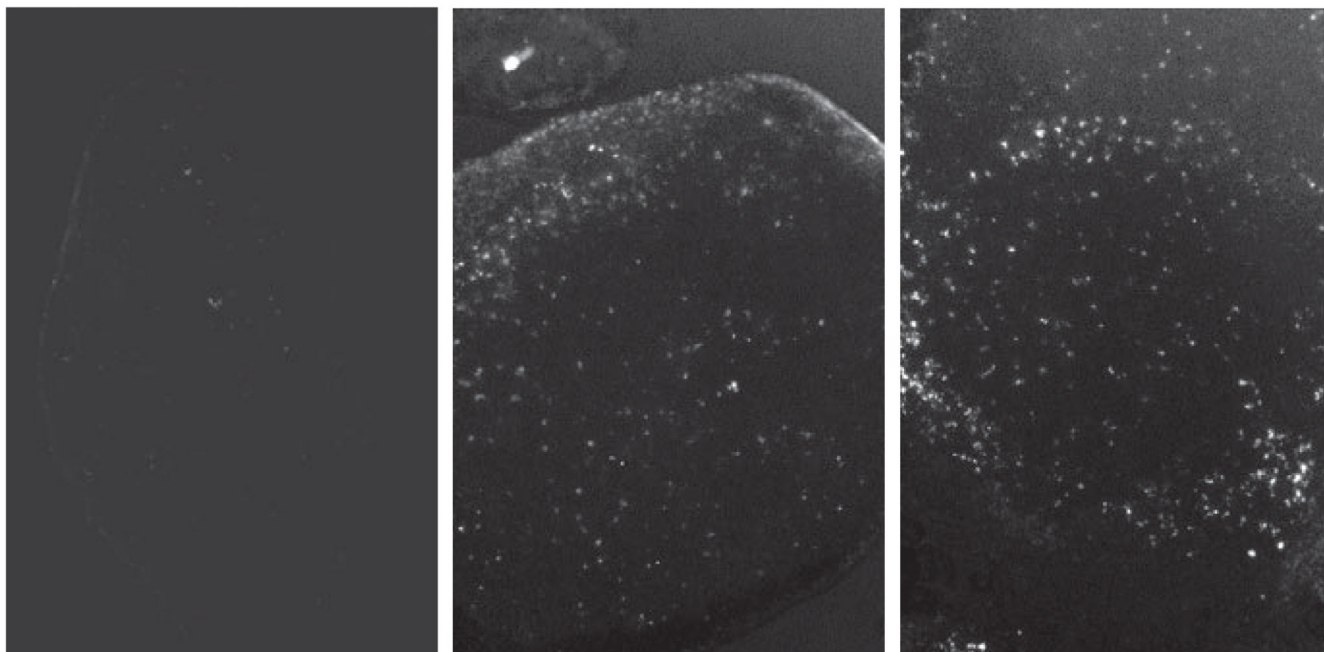
Figure 1.
Tumor protoporphyrin IX (PpIX) fluorescence. 5-Aminolevulinic acid (5-ALA) was administered PO, by oral gavage to animals with preestablished tumors. The data in (a) and (b) depict that obtained from animals with tumors measuring approximately 0.5 cm× 0.5 cm. Individual data points in (a) represent mean of data obtained from three animals per time point. Error bars represent SD. (b) PpIX fluorescence present in tumors at different time points after 5-ALA administration.
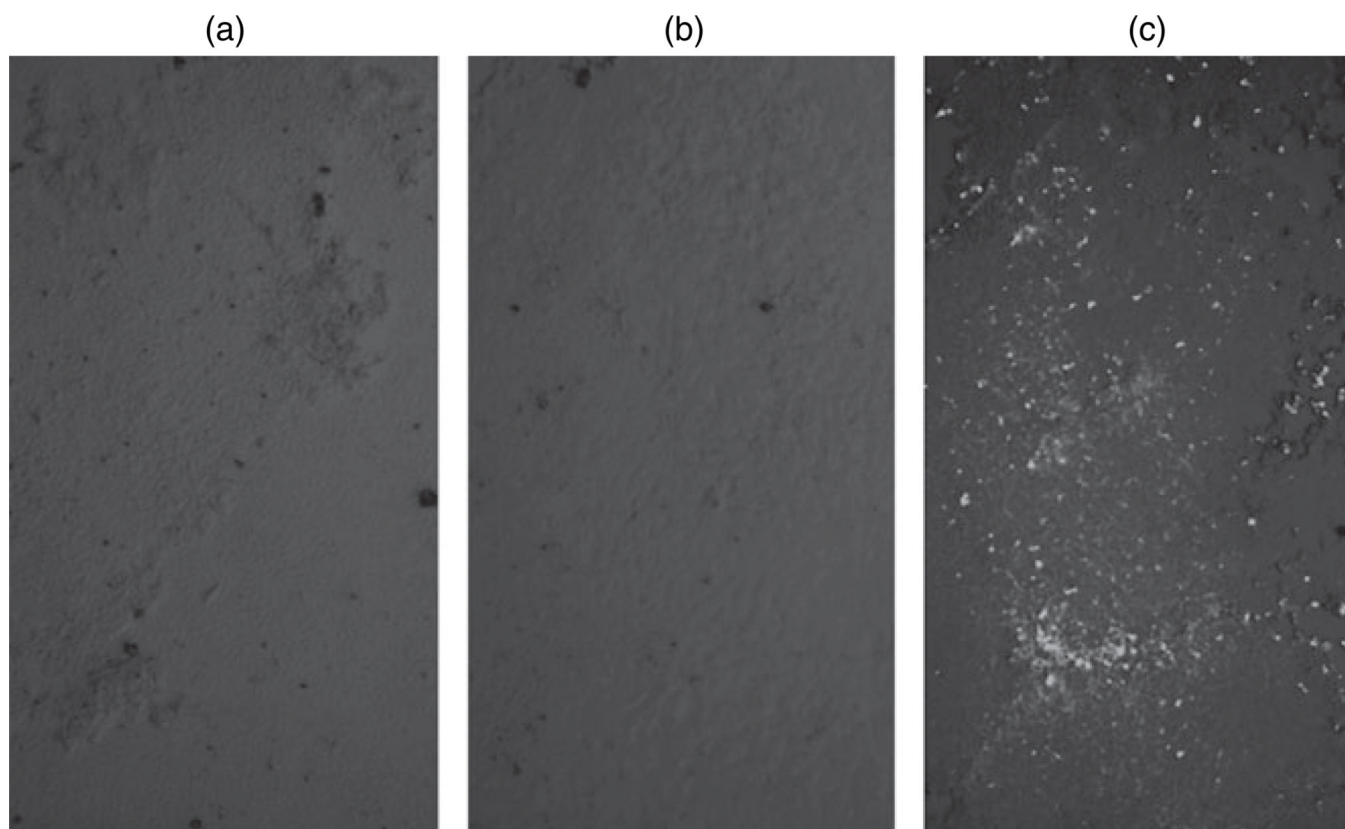
Figure 2.
Photodynamic therapy (PDT) induction of apoptotic tumor cell death. Individual panels depict representative images from frozen sections of established tumors. (a) An untreated tumor. (b) Tumor from an animal receiving 5-aminolevulinic acid, but no laser light illumination. (c) Tumor from an animal receiving PDT 8 h prior to tumor harvest. Fluorescence due to caspase activation was observed only in PDT-treated tumors. Original magnification 40×.

Figure 3.
CFSE-labeled dendritic cell (DC) trafficking to draining lymph nodes (LN), post-PDT. (a) Representative images from draining lymph nodes harvested at the designated time following adoptive transfer of 5×10^4 CFSE labeled rat bone marrow–derived DCs into tumor masses, 15 min after PDT treatment. Only fluorescent signals with appropriate cellular morphology were included, typical signal artifact is noted in upper left of the 24 h image. Original magnification 40×. (b) Average number of CFSE-labeled DCs observed for tumors and draining lymph nodes at the designated time points with and without administration of PDT. These results are based upon evaluation of a minimum of 10 separate random high power fields, from nonserial tissue sections, from two separate experiments. Although tumor sections contained greater tissue than lymph node sections, there was essentially no difference in size of the tumor sections or lymph nodes across these

conditions and experiments. Error bars represent SD derived from tissues from different animals.

(a)

(b)
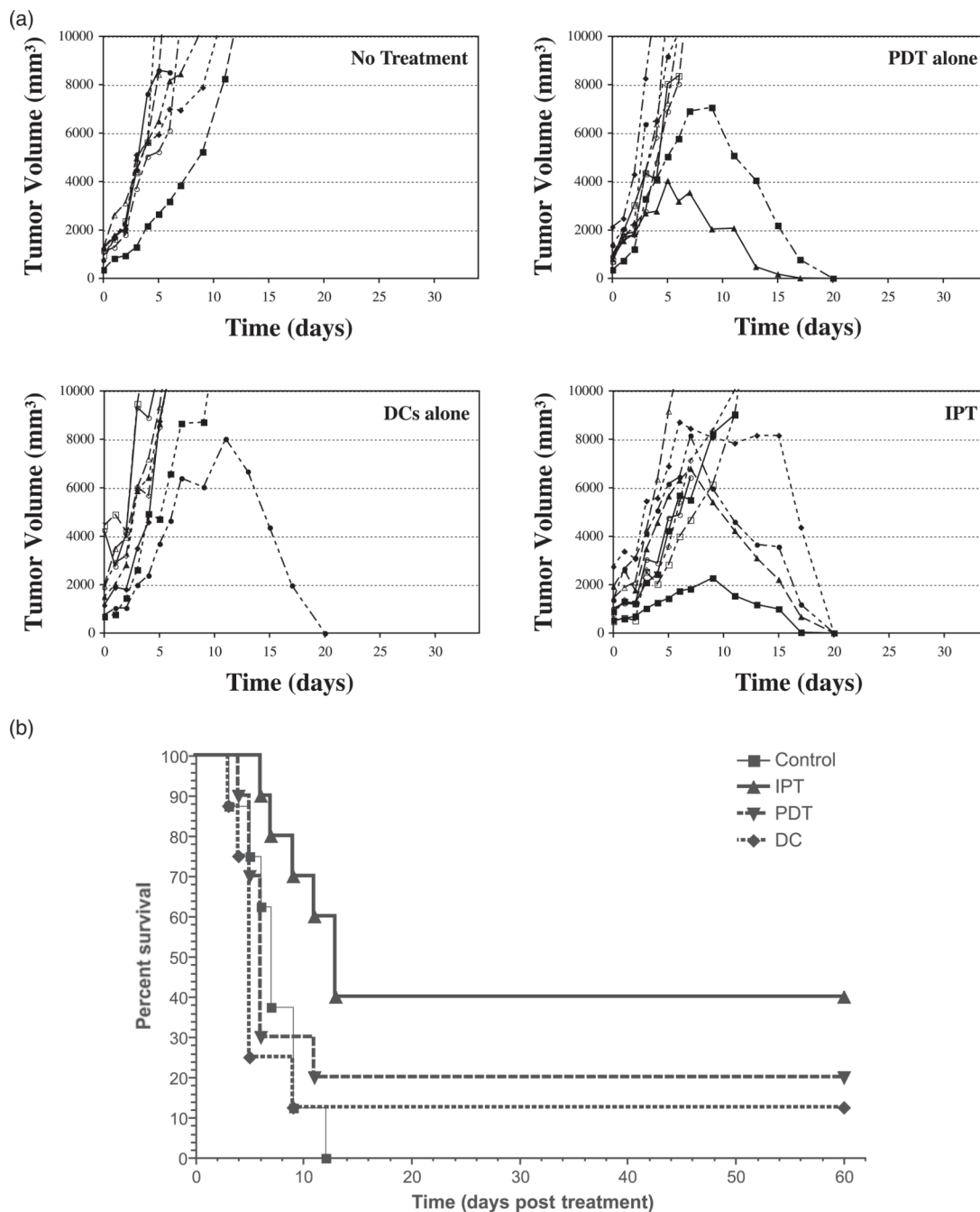
Figure 4.
Tumor volume changes. A total of 13 762 MAT B III mammary tumors were established to approximately 1000 mm^3 in cohorts of 8–10 animals per condition. (a) Graphs depict tumor growth kinetics in animals receiving the designated treatments, as indicated by the label in the upper right-hand corner of each graph. All animals received 5-aminolevulinic acid (5-ALA) by gavage, PDT animals were exposed to laser light 2 h after 5-ALA administration, immunophototherapy (IPT) animals underwent photodynamic therapy (PDT) and then received 1×10^5 bone marrow–derived DCs. Animals received only one treatment. Day 0 indicates the day that animals received 5-ALA by gavage, PDT or IPT. (b) Survival curves for all conditions. Significant differences in survival were observed for IPT relative to the

PDT alone (P = 0.0234), DC alone (P = 0.016) and control (5-ALA alone) (P = 0.004)-treated animals by log rank test.