Supplementary Information

A Peptide-Modified Solid Lipid Nanoparticle Formulation of Paclitaxel Modulates Immunity and Outperforms Dacarbazine in Murine Melanoma Model

Indranil Banerjee [a, b *], Manjarika De [a], Goutam Dey [b], Rashmi Bharti [b], Sankha Chattopadhyay [c], Nahid Ali [a], Partha Chakrabarti [d], Rui L Reis [e], Subhas C Kundu [e], and Mahitosh Mandal [b **]

[a] Division of Infectious Diseases and Immunology, Council of Scientific and Industrial Research (CSIR)–Indian Institute of Chemical Biology (CSIR-IICB), 4 Raja S C Mullick Road, Kolkata - 700032, West Bengal, India.

[b] School of Medical Science and Technology, Indian Institute of Technology Kharagpur, Kharagpur - 721302, West Bengal, India.

[c] Regional Centre, Radiopharmaceuticals Lab., Board of Radiation and Isotope Technology (BRIT), Variable Energy Cyclotron Centre, 1/AF Bidhan Nagar, Kolkata - 700064, West Bengal, India.

[d] Division of Cell Biology and Physiology, CSIR-IICB, 4 Raja S C Mullick Road, Kolkata - 700032, West Bengal, India.

[e] 3Bs Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark - 4805-017 Barco, Guimaraes, Portugal.

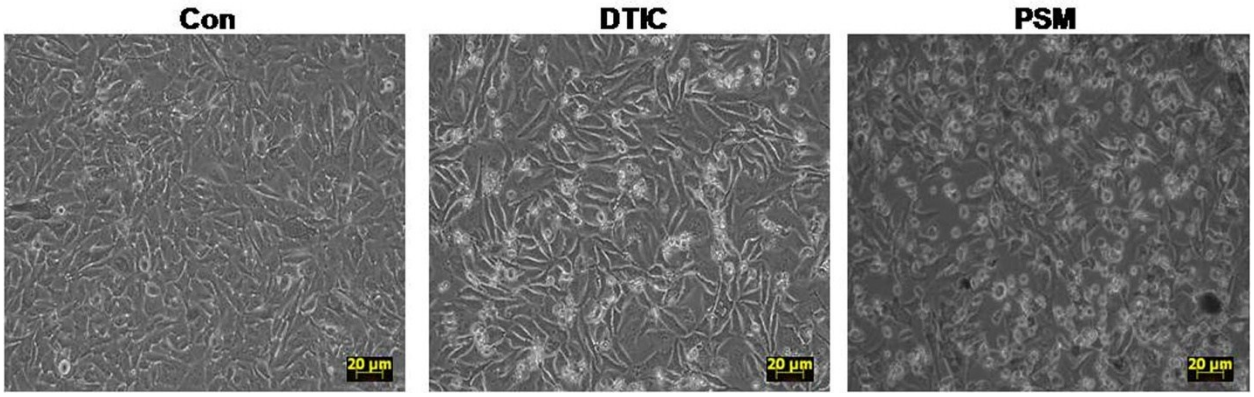
Corresponding authors:

* Indranil Banerjee. E-mail: samu.jubph@gmail.com, Phone No.: +913324730492.

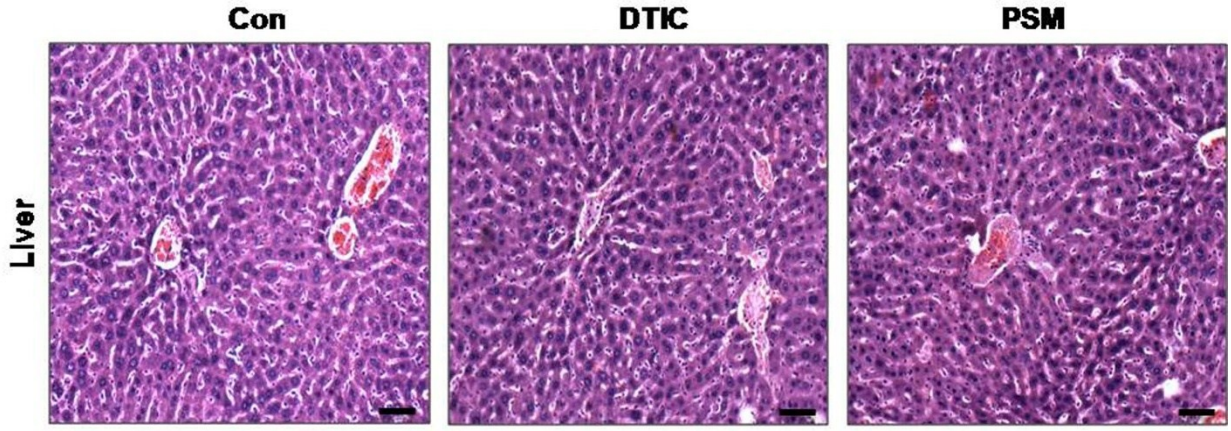
(Present address: School of Medical Science & Technology, Indian Institute of Technology Kharagpur, Kharagpur - 721302, West Bengal, India. Phone No.: +91-3222-283578).

** Mahitosh Mandal. E-mail: mahitosh@smst.iitkgp.ernet.in, Phone No.: +91-3222-283578.

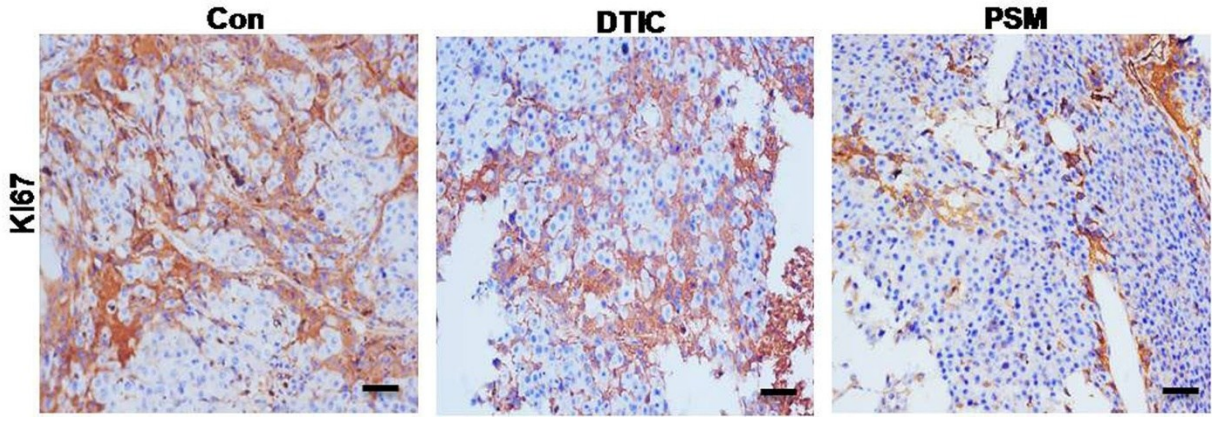
Supplementary Figures



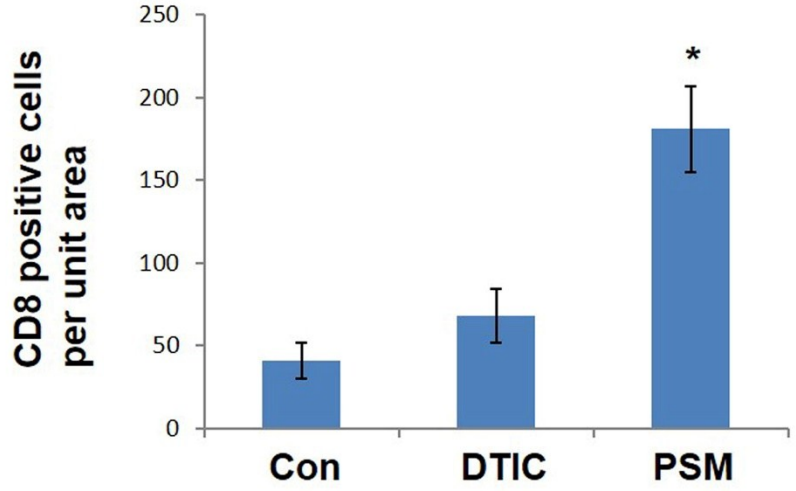
Supplementary Information, Figure 1: Phase-contrast microscopic observations of differently treated B16F10 cells. Treatment of DTIC or PSM to B16F10 cells for 6 h followed by 24 h incubation in drug-free medium. Prominent morphological changes of apoptosis were observed in PSM treated group compared to DTIC and the untreated control group. PSM treated group showed the highest apoptotic effect as per the characteristic of morphological changes. Representative microphotographs are presented.



Supplementary Information, Figure 2: Representative pictures of H & E stained liver tissues procured from differently treated subcutaneous melanoma-bearing mice that received therapy for 10 days. Bar = 20 µm.

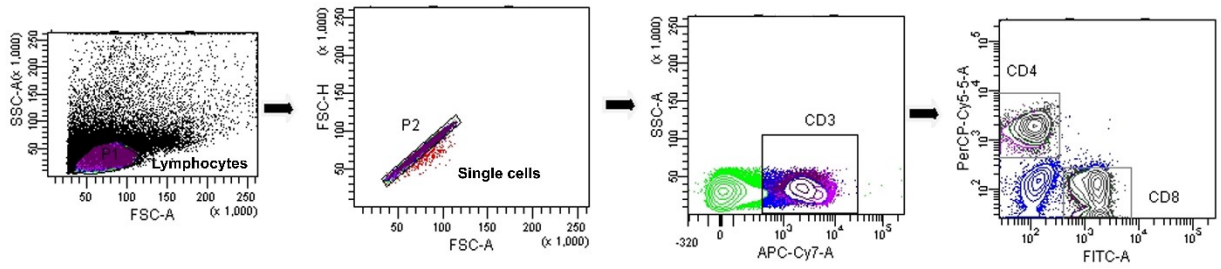


Supplementary Information, Figure 3: IHC staining of Ki67 positive cells of differently treated subcutaneous melanoma. Melanoma-bearing mice received therapy for 10 days. Representative images are shown. Bar = 20 µm.

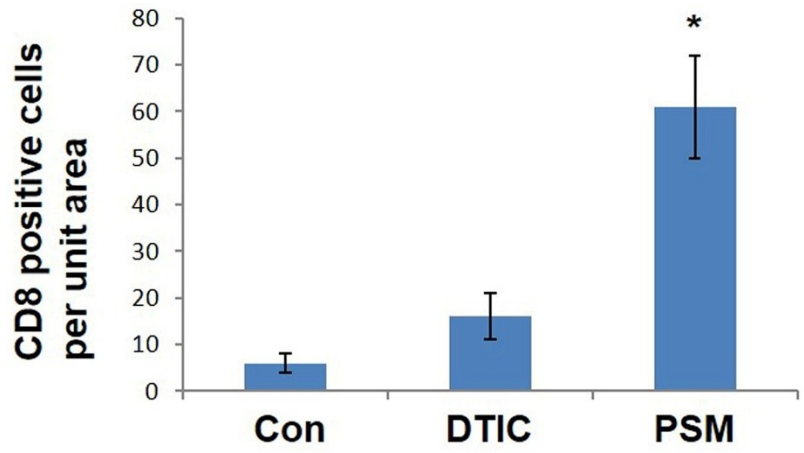


Supplementary Information, Figure 4: The quantitative data for the CD8 staining (represented as CD8 positive cells per unit area) of tissue sections of subcutaneous melanoma-developed mice

that receive different treatments. Data represent average value ± SD (n = 3). * $p < 0.05$, significantly distinct from Con and DTIC group.

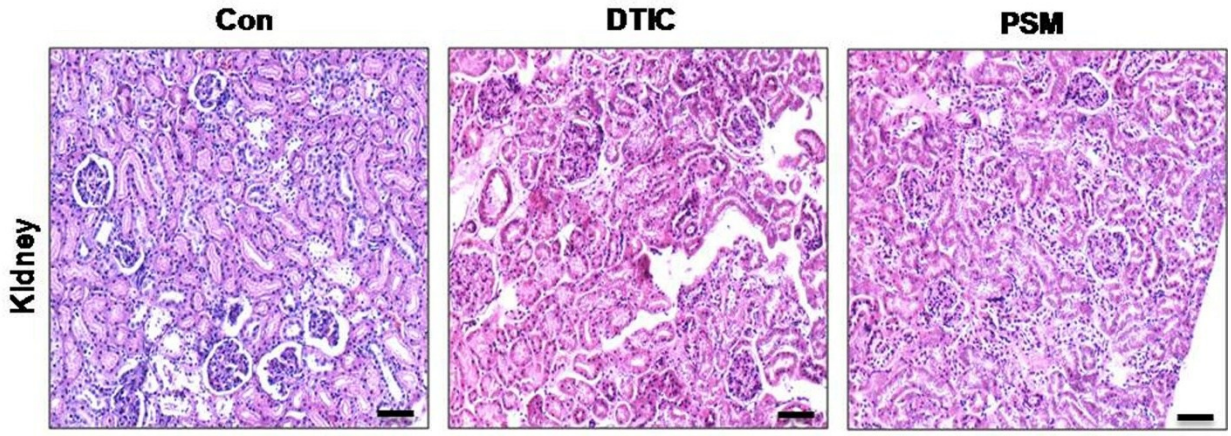


Supplementary Information, Figure 5: Representative figure of gating strategy for the intracellular cytokine analysis. Lymphocytes were gated on FSC-A versus SSC-A followed by $CD3^+$ events and subsequently on $CD4^+$ and $CD8^+$ events. Then, stringent gating for each cytokine-positive (IFN γ, TNF α, and IL 2) cells in the $CD4^+$ and $CD8^+$ compartments was done, and analysis was performed using FACSDiva™ software.

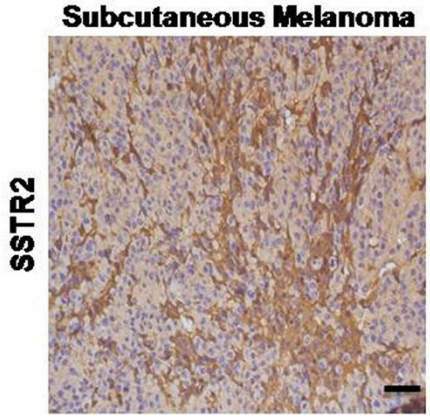


Supplementary Information, Figure 6: The quantitative data for the CD8 staining (represented as CD8 positive cells per unit area) of tissue sections of experimental lung metastasis-developed

mice that receive different treatments. Data represent average value ± SD (n = 3). * $p < 0.05$, significantly distinct from Con and DTIC group.



Supplementary Information, Figure 7: Representative pictures of H & E stained kidney tissues procured from differently treated experimental lung metastasis-developed mice that received therapy for 10 days. Bar = 20 μm.



Supplementary Information, Figure 8: IHC staining of SSTR2 positive cells of a tissue section of subcutaneous melanoma tumor developed in C57BL/6 mice. A representative picture is exhibited. Bar = 20 μm.

Parameters	B16F10 cells treated with DTIC	B16F10 cells treated with PSM
Q3 quadrant, viable cells (%)	89.2 ± 1.8	81.9 ± 1.8
Q4 quadrant, early apoptotic cells (%)	6.9 ± 0.8	14.7 ± 0.7
Q2 quadrant, apoptotic plus necrotic cells (%)	1.4 ± 0.3	2.7 ± 1.1
Apoptosis proportion (AP)	2.1 ± 0.1	

Supplementary Tables

Supplementary Information, Table 1: Results of B16F10 cell apoptosis assay. Data are presented as average value ± standard deviation (SD) (n = 3).

Supplementary Information, Table 2: Calreticulin (CRT) exposure assessed by flow cytometry. Mean fluorescent intensity was estimated after treating B16F10 cells with different drugs. Data are presented as average value ± standard deviation (SD) (n = 3).

Group	Mean Fluorescent Intensity

Control	3713 ± 154
DTIC	2864 ± 106
PSM	4850 ± 64