Photodynamic Therapy and Lung Cancer Stem Cells – The effects of $AlPcS_4Cl$ on Isolated Lung Cancer Stem Cells.

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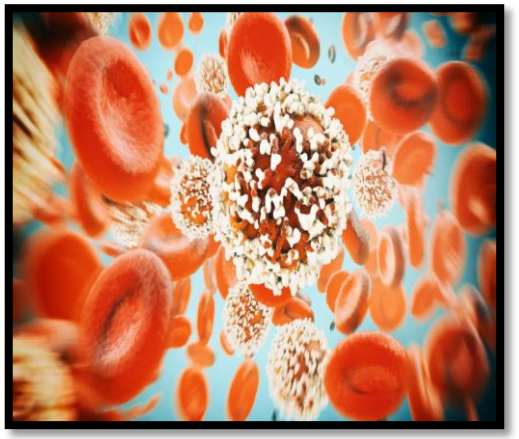
Introduction

CANCER

- **Malignancy** or **cancer** is a term for diseases in which atypical cells characteristically **evade cell death** through **rapid proliferation** and can **metastasize**, invading distant tissues by travelling through the blood and lymphatic system
- Cancer arises from progressive transformation of normal cells that encounter **genomic damages** leading to **mutations** in their DNA sequence
- Errors such as **inactivation of regulatory genes** maintaining genomic integrity facilitate additional mutations
- **Irrepressible** cell growth leads to **tumour** formation
- According to the Global Cancer Statistics of CANSA, this disease **kills more people every year** than AIDS, tuberculosis and malaria combined
- **More than 80%** of all cancer cases are made up of **carcinomas:** are cells that are **epithelial** in origin, this usually include breast, colon, prostate and lung



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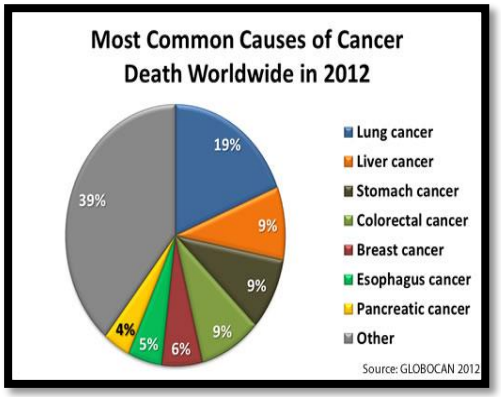
LUNG CARCINOMA

- Globocan stats indicate that the most commonly **diagnosed** cancer worldwide was:
  - **lung (1.8 million, 13.0% of the total)**
- The most common cancer-related **deaths** were from:
  - **lung (1.6 million, 19.4% of the total)**
- **Lung carcinoma** are **neoplastic** cells showing **unrestrained** development of **mutated lung cells** that are formed in the lung tissue lining the air passages
- The **mutated cells divide rapidly** leading to **tumour** formation
- As tumour formation progress, the numerous abnormal cells start **undermining** the **lungs primary function** preventing the lungs from **providing** the bloodstream with **oxygen**
- Subsequent to the primary cancer development, constant **addition of genetic and epigenetic abnormalities** follow during cancer proliferation, leading to tissue **invasion**, **metastasis**, and **resistance** to conventional therapies
- **Carcinogenic risk factors** for lung cancer is smoking, occupational exposures such as asbestos, long-term and accumulated exposure to air pollution and congenital inheritance



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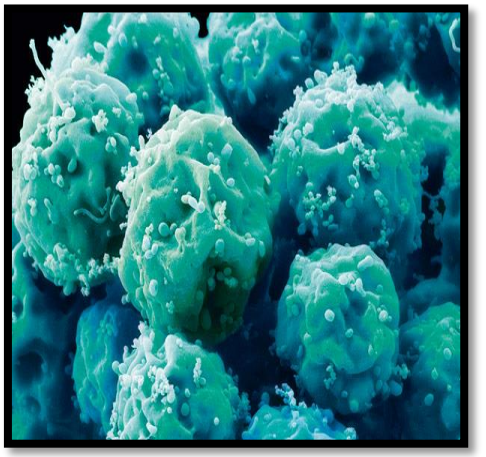
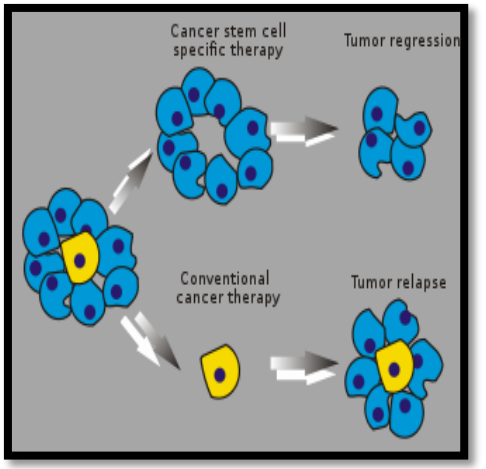
CANCER STEM CELLS

- It has been proven that tumour cells are **heterogeneous** comprising rare **tumour initiating cells/ cancer stem cells** and abundant non-tumour initiating cells
- Characteristically CSCs can **self-renew, proliferate**, are **resistant** to drugs and express typical **stem cell markers**
- CSCs have successfully been **identified** and **isolated**, and their existence proven in lung cancer, acute and chronic myeloid leukaemia, breast cancer, brain tumours and gastrointestinal tumours
- Although CSCs comprise of a small amount of the tumour bulk, it can **cause cancer relapse**, sometimes many years after the “successful” treatment of the primary tumour
- Hence the need for further characterization and understanding of CSCs in order to develop cancer therapies that not only manage malignant tumours but the CSCs residing within them





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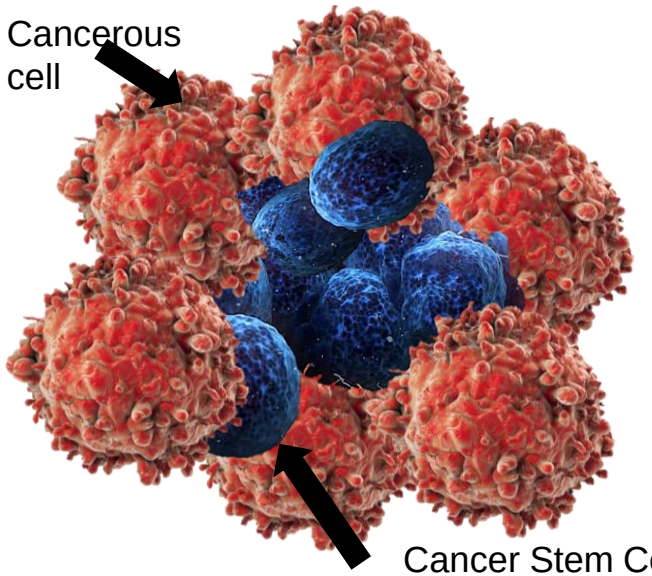
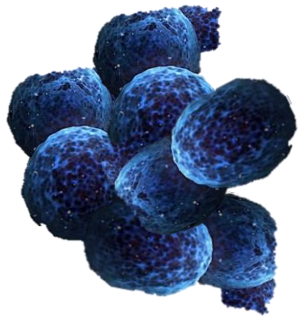
Cancer Stem Cell Characteristics

Tumour mass consisting of cancer and CSCs

Conventional cancer therapy: Chemo and radiation

CSCs evading conventional therapy leading to cancer relapse and metastasis





Cancer Initiation

Minor population < 1 %

Enhanced Resistance to conventional cancer therapy

Self renewing

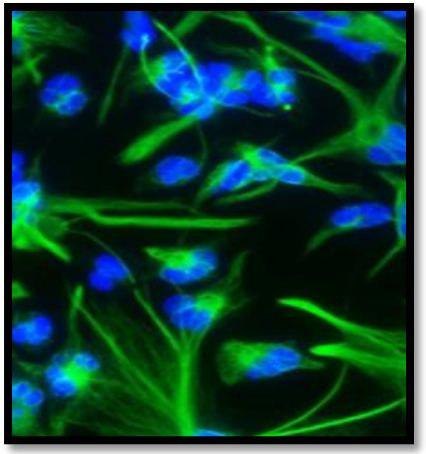
Relapse

Metastasis

Crous, A. Chizenga, E., Hodgkinson, N., and Abrahamse, H. (2018) Targeted Photodynamic Therapy: A Novel Approach to Abolition of Human Cancer Stem Cells. International Journal of Optics Volume 2018, Article ID 7317063, 9 pages https://doi.org/10.1155/2018/7317063

Lung Cancer Stem Cells

- Lung CSCs are **contributors** of lung cancer-related **mortality**, are **drug resistant** and lead to aggressive tumour **relapse**
- Due to their quiescence, lung CSCs are difficult to differentiate from normal lung epithelium, **characterisation** and **identification** is possible through **cancer stem cell markers**

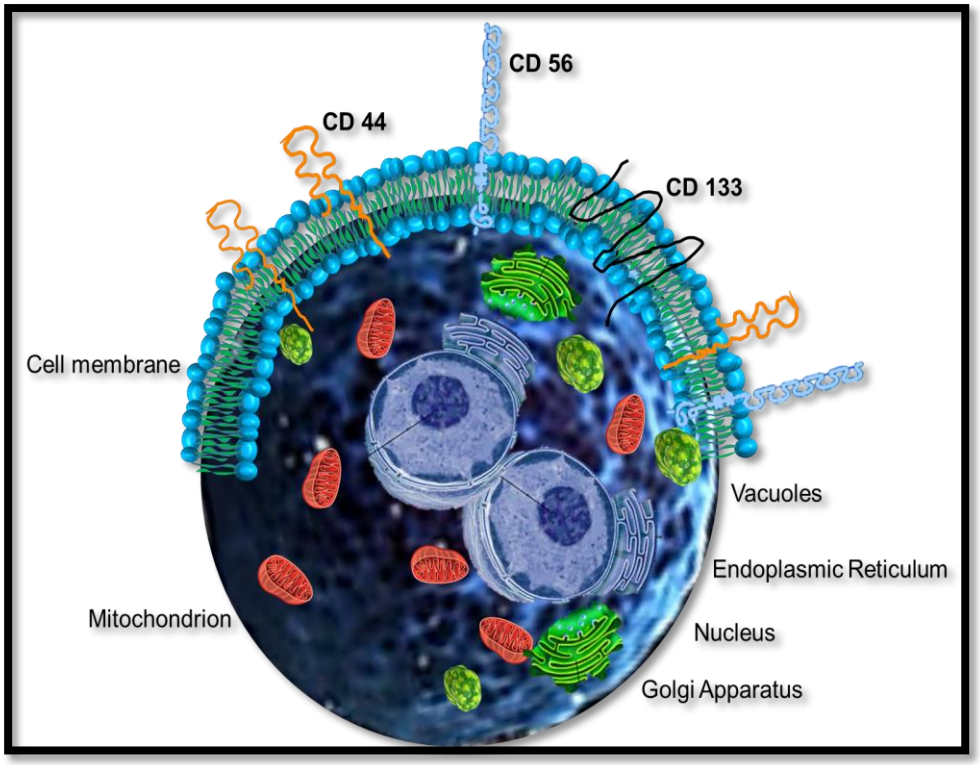


- **With the accepted notion that the CSCs are to blame for cancer relapse and drug resistance, targeting them can be an important aspect of lung cancer therapy**

MacDonagh, L., Gray, S. G., Breen, E., Cuffe, S., Finn, S. P., O'Byrne, K. J., & Barr, M. P. (2016). Lung cancer stem cells: The root of resistance. Cancer Lett, 372(2), 147-156. doi: https://doi.org/10.1016/j.canlet.2016.01.012.

Lung Cancer Stem Cell Markers

LUNG CSC MARKERS	
MARKER	**CELLULAR FUNCTION**
CD 133/ Promonin-1	Modulator of **intracellular accumulation of exogenous compounds** (transferrin-CD133-iron network), cell metabolism Neurotrophic receptor RET, tyrosine kinase expression
CD 44/ Pgp-1	**Hyaluronic acid receptor**
CD 56/ Neural cell adhesion molecule (NCAM)	Homophilic binding glycoprotein, cell-cell adhesion **or cell-matrix adhesion during embryonic development.**



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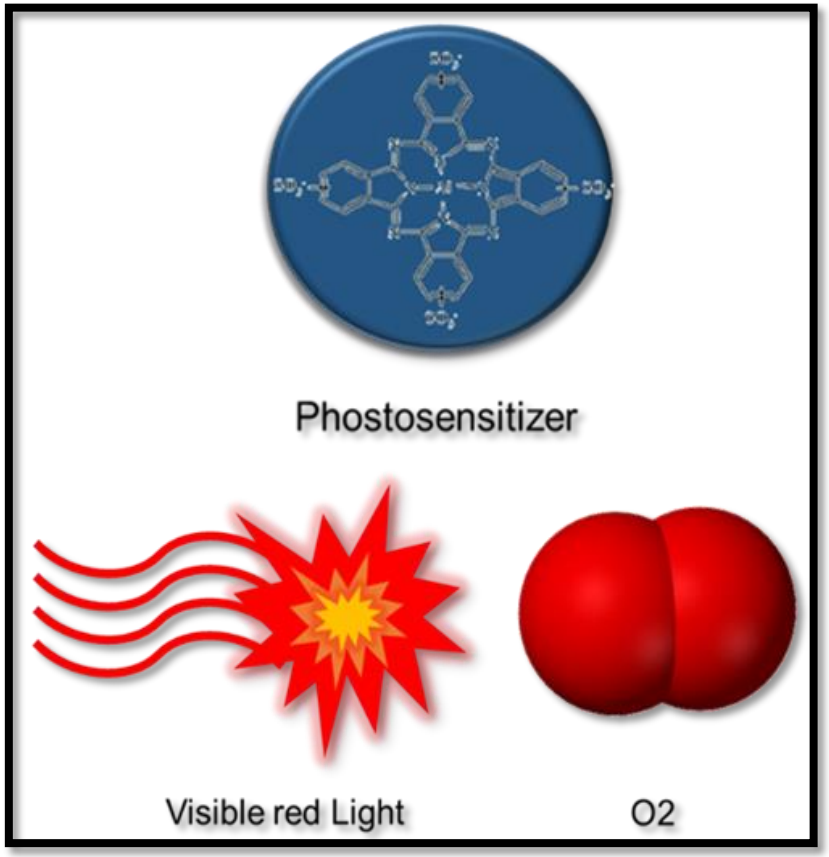
PHOTODYNAMIC THERAPY (PDT)

- Photodynamic therapy (PDT), sometimes called photochemotherapy, is a form of phototherapy involving **light** and a **photosensitizing chemical substance** (PS), used in conjunction with **molecular oxygen** to elicit cell death (**phototoxicity**)
- It is recognised as a treatment strategy that is both **minimally invasive** and **minimally toxic**
- PS: **naturally non toxic drug** which acquires its toxicity after illumination with light of the **appropriate wavelength**
- **Short term side effect**: skin photosensitivity after treatment
- PDT can further be improved by developing a targeted form of PDT that can enhance its selectivity and effectiveness



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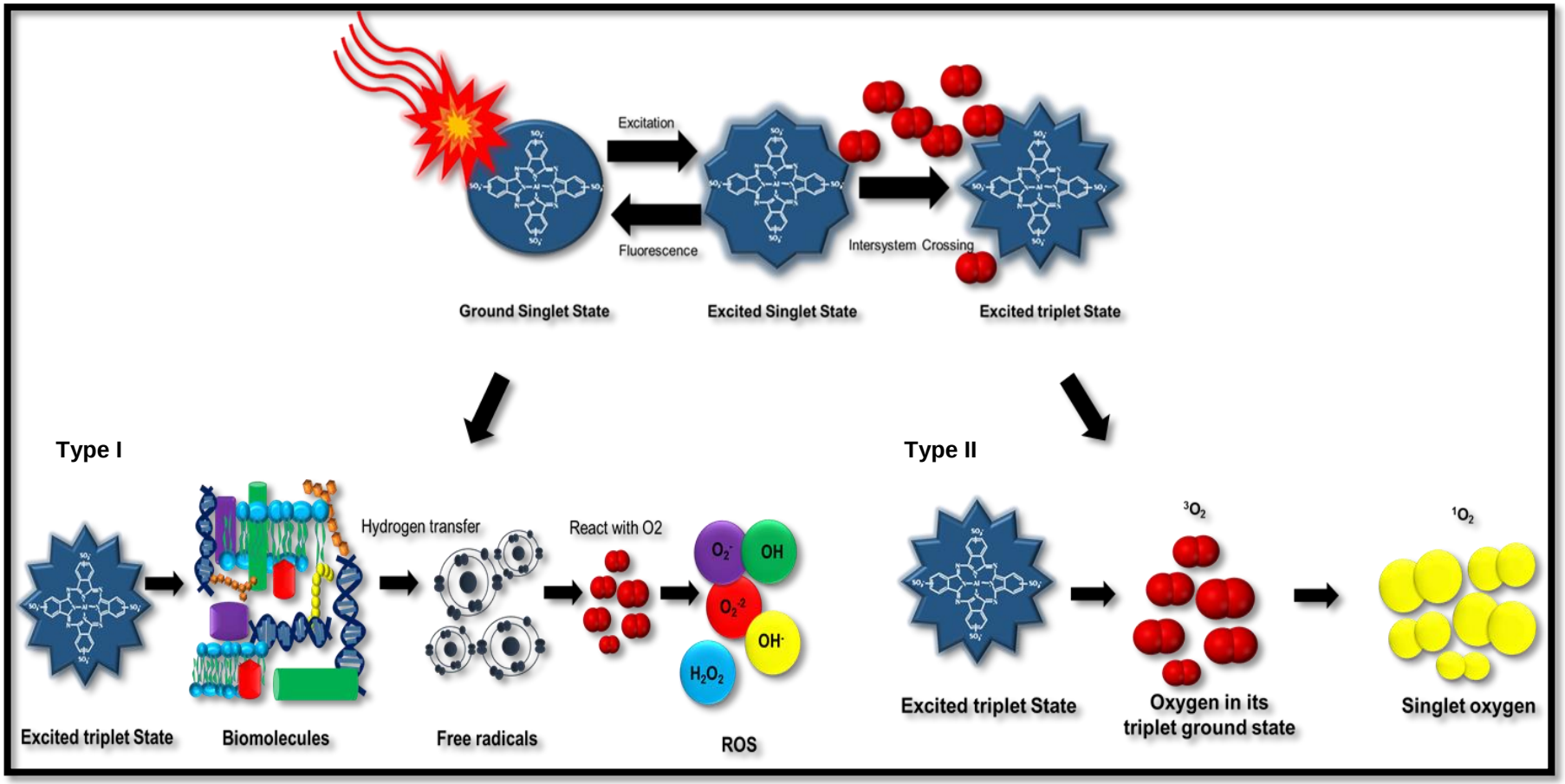
PDT MECHANISM



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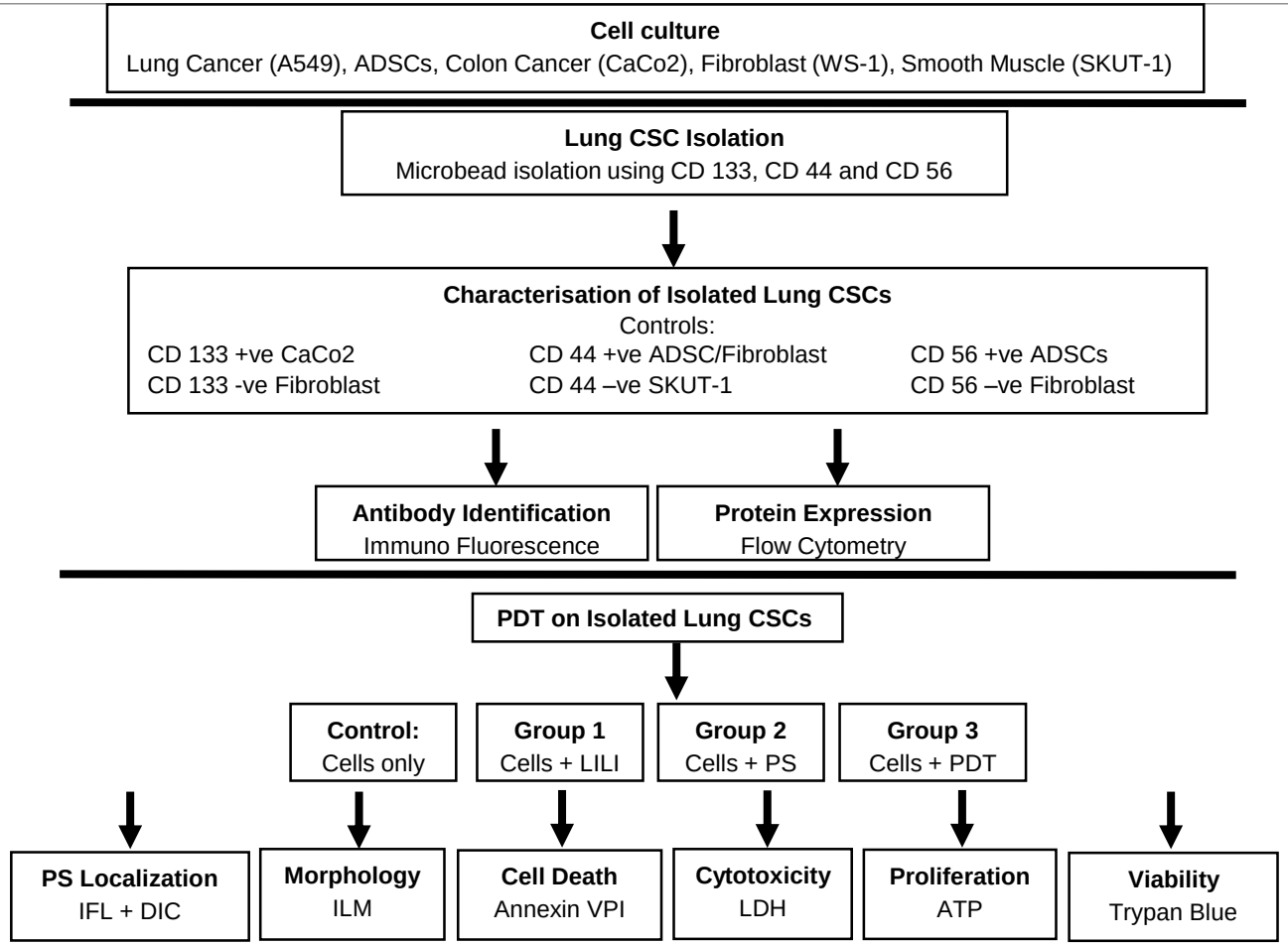
Methodology

FLOW DIAGRAM



Results

CHARACTERIZATION OF ISOLATED LUNG CSCS

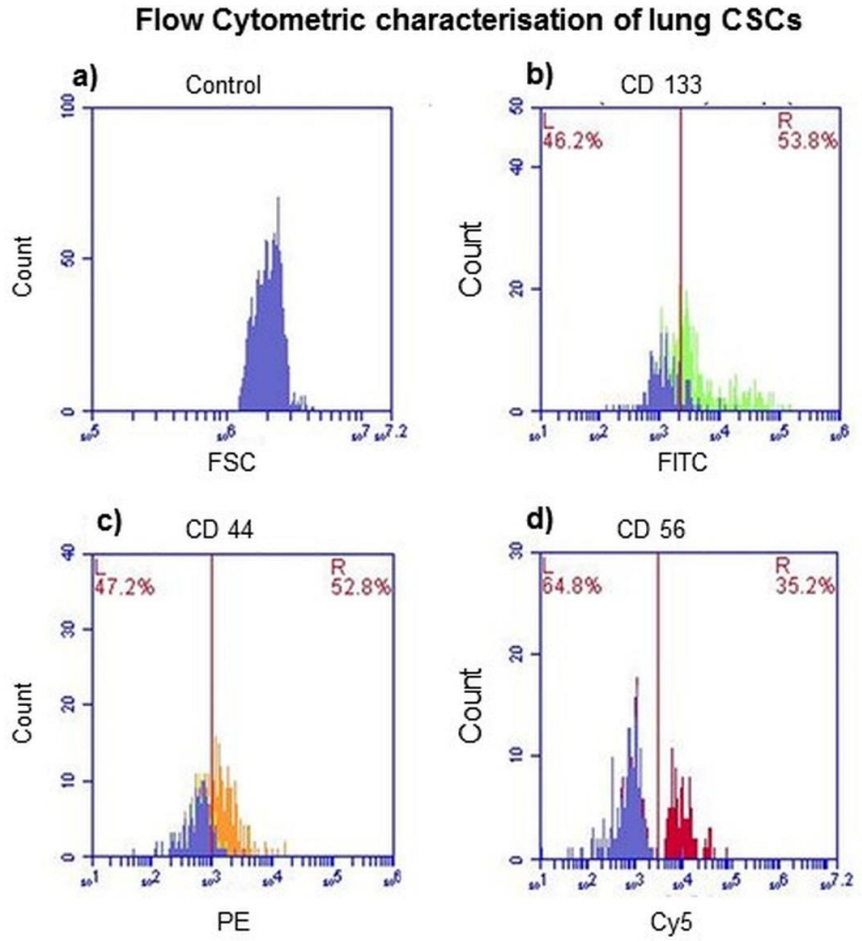


Figure 2. Fluorescence protein detection using flow cytometry. a) Unstained lung CSCs (control). b) Lung CSCs positive for CD 133 (FITC), c) Lung CSCs positive for CD 44 (PE), and Lung CSCs positive for CD 56 (Cy5). All positive samples are overlaid with the control to distinguish between the color shifts.

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Fluorescent antigenic detection of the surface marker CD 133

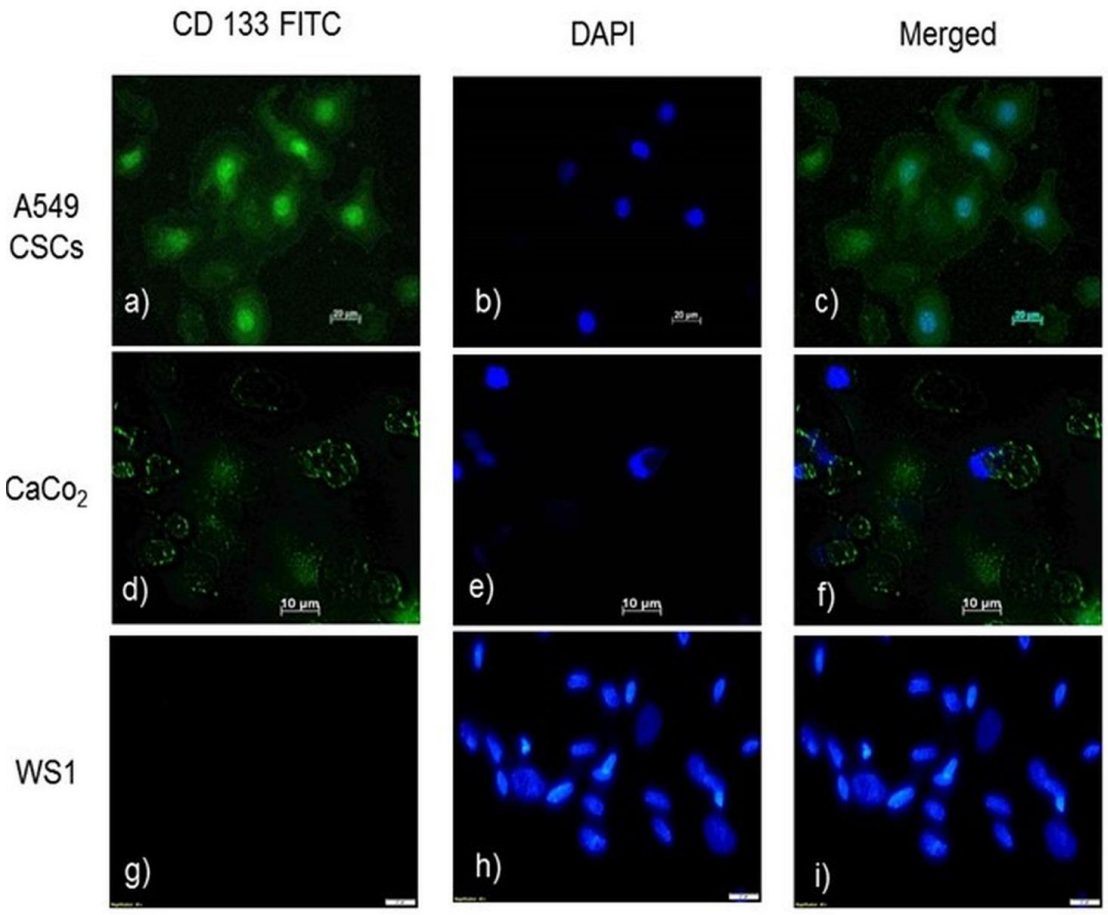

Figure 3. Fluorescent antigenic detection of the surface marker CD 133. Immunofluorescent staining of the isolated side population of Lung CSCs (a) and control cell CaCo2 (d) positive for the antigenic marker CD 133 indicated positive fluorescence with FITC (green). Negative control cell line WS1 (g) indicated no fluorescence. All cell lines were counter stained with DAPI indicated by blue fluorescence seen in the nuclei (b, e, and h). Merged fluorescent images of the labelled cell lines are indicated by image c, f and i.

Fluorescent antigenic detection of the surface marker CD 44

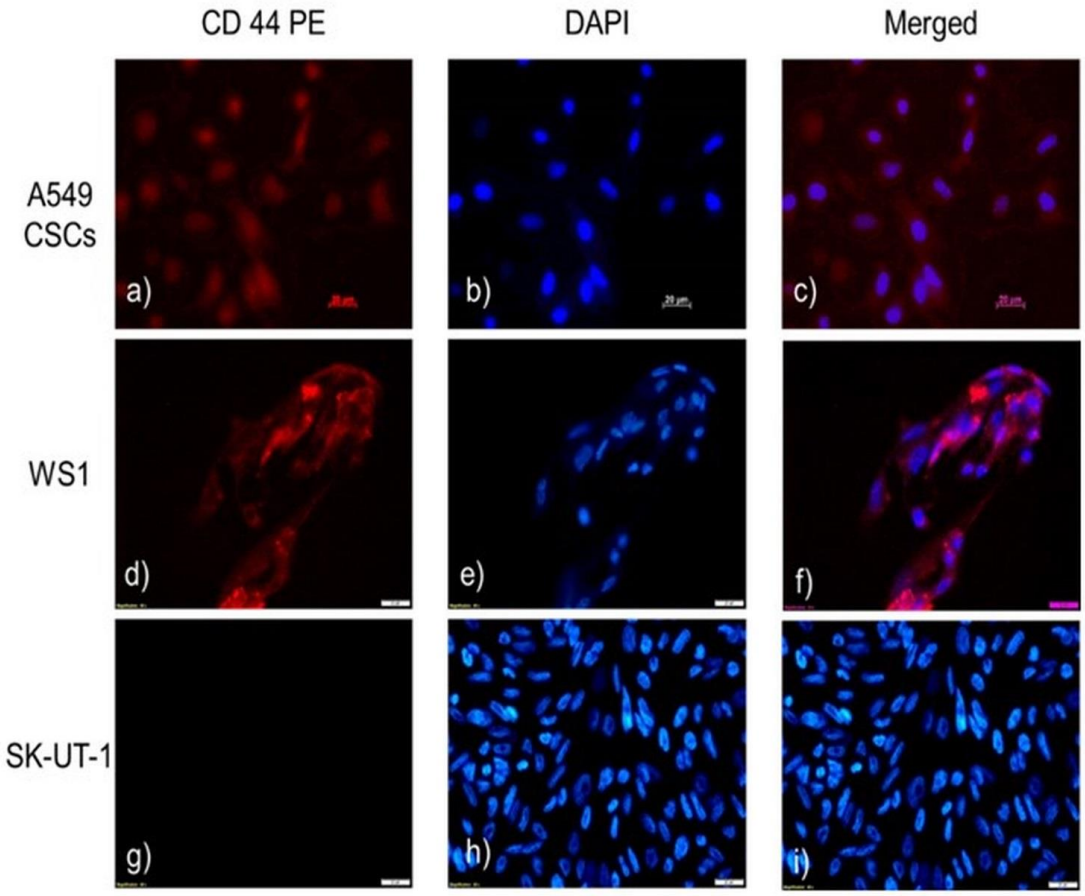

Figure 4. Fluorescent antigenic detection of the surface marker CD 44. Immunofluorescent staining of the isolated side population of Lung CSCs (a) and control cell WS1 (d) positive for the antigenic marker CD 44 indicated positive fluorescence with PE (red). Negative control cell line SK-UT-1 (g) indicated no fluorescence. All cell lines were counter stained with DAPI indicated by blue fluorescence seen in the nuclei (b, e, and h). Merged fluorescent images of the labelled cell lines are indicated by image c, f and i.

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Fluorescent antigenic detection of the surface marker CD 56

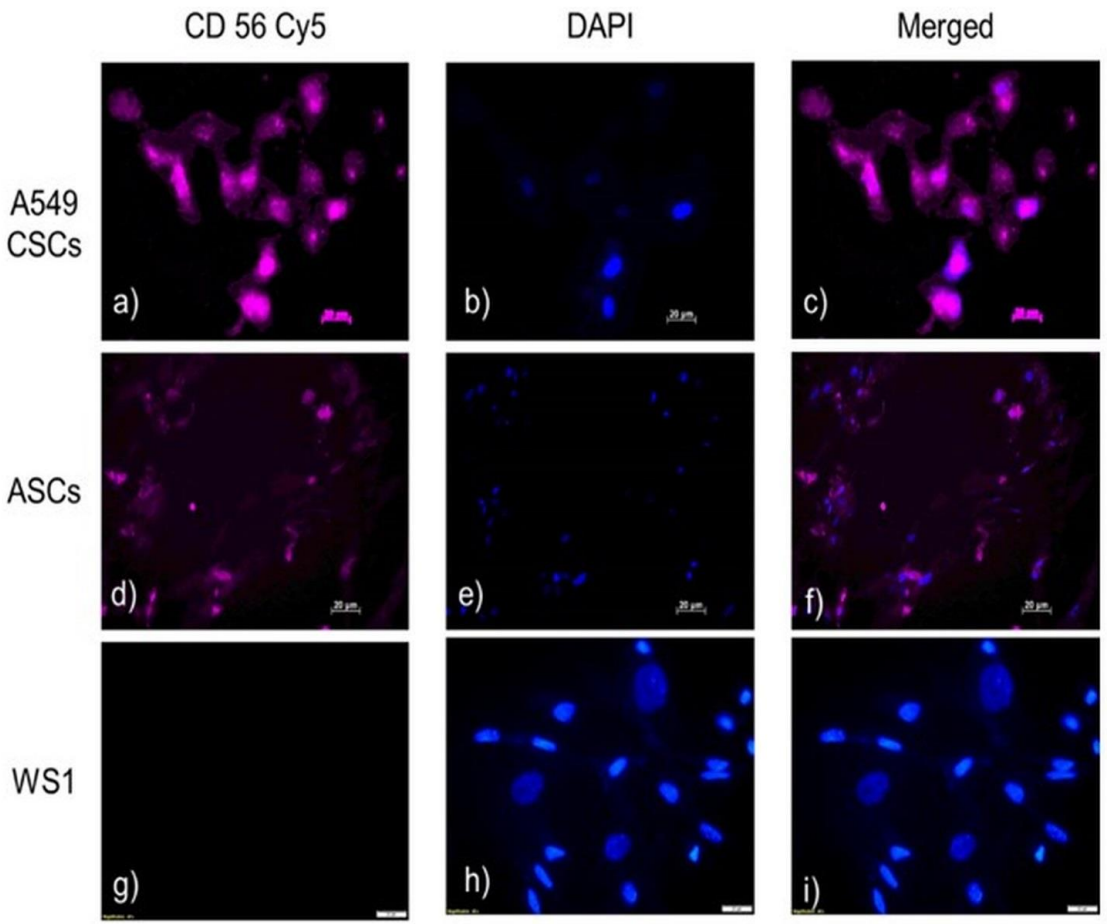

Figure 5. Fluorescent antigenic detection of the surface marker CD 56. Immunofluorescent staining of the isolated side population of Lung CSCs (a) and control cell ASC (d) positive for the antigenic marker CD 56 indicated positive fluorescence with Cy5 (magenta). Negative control cell line WS1 (g) indicated no fluorescence. All cell lines were counter stained with DAPI indicated by blue fluorescence seen in the nuclei (b, e, and h). Merged fluorescent images of the labelled cell lines are indicated by image c, f and i.

LOCALIZATION OF PS IN LUNG CSCS

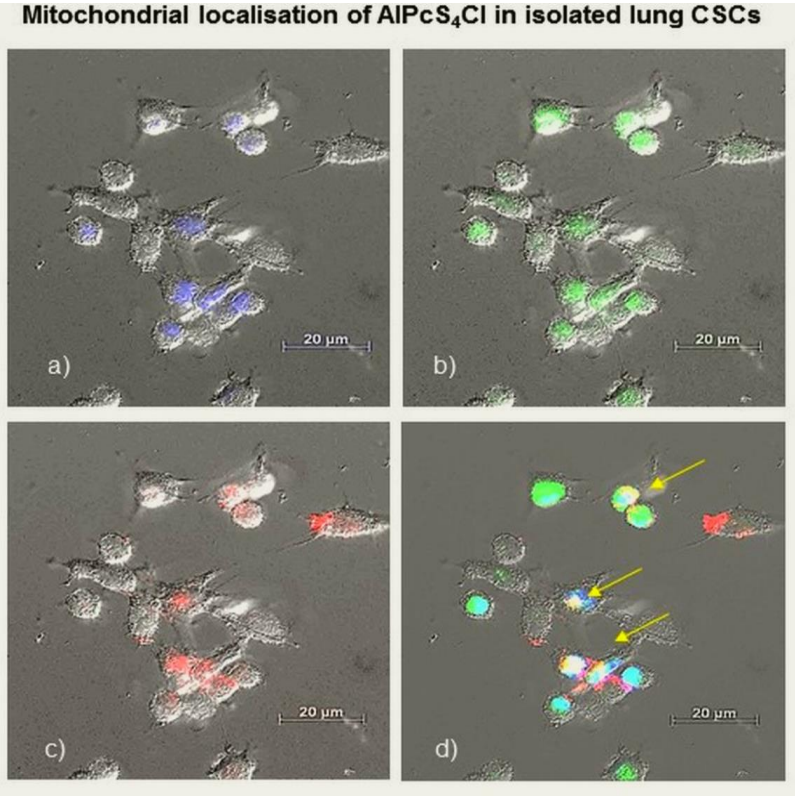

Figure 6. Mitochondrial localization of $AlPcS_4Cl$ in isolated lung CSCs. a) Nuclei are stained blue using DAPI, b) Mitochondria fluoresces green (FITC), c) $AlPcS_4Cl$ auto fluoresces red (Cy5), d) Intermediate yellow is seen in the superimposed images where the green and red channels are merged and fluorescence from the mitochondrion and PS are overlapping.

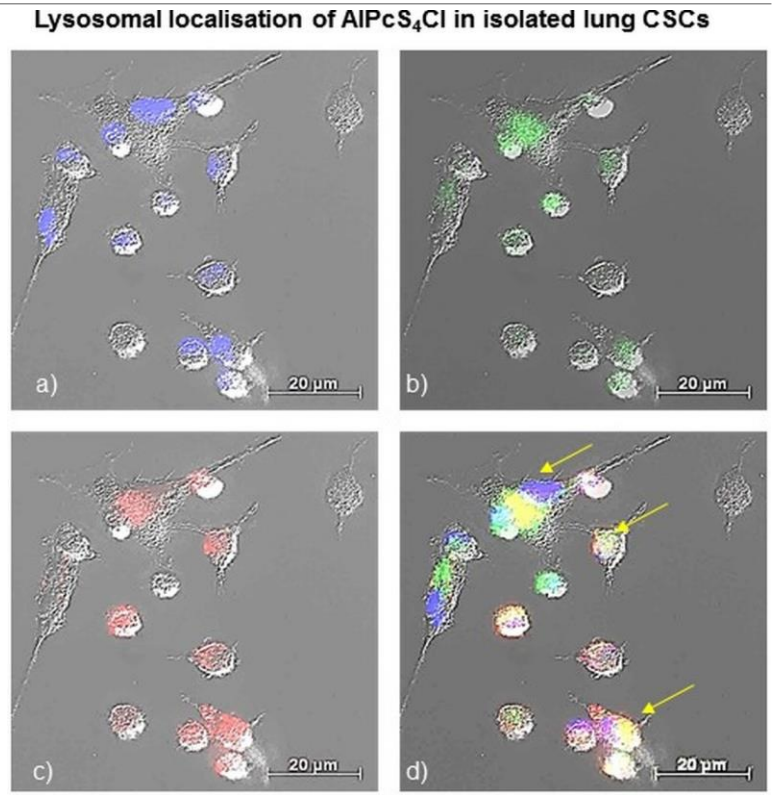

Figure 7. Lysosomal localization of $AlPcS_4Cl$ in isolated lung CSCs. a) Nuclei are stained blue using DAPI, b) Mitochondria fluoresces green (FITC), c) $AlPcS_4Cl$ auto fluoresces red (Cy5), d) Intermediate yellow is seen in the superimposed images where the green and red channels are merged and fluorescence from the lysosomes and PS are overlapping.

PHOTODYNAMIC THERAPY

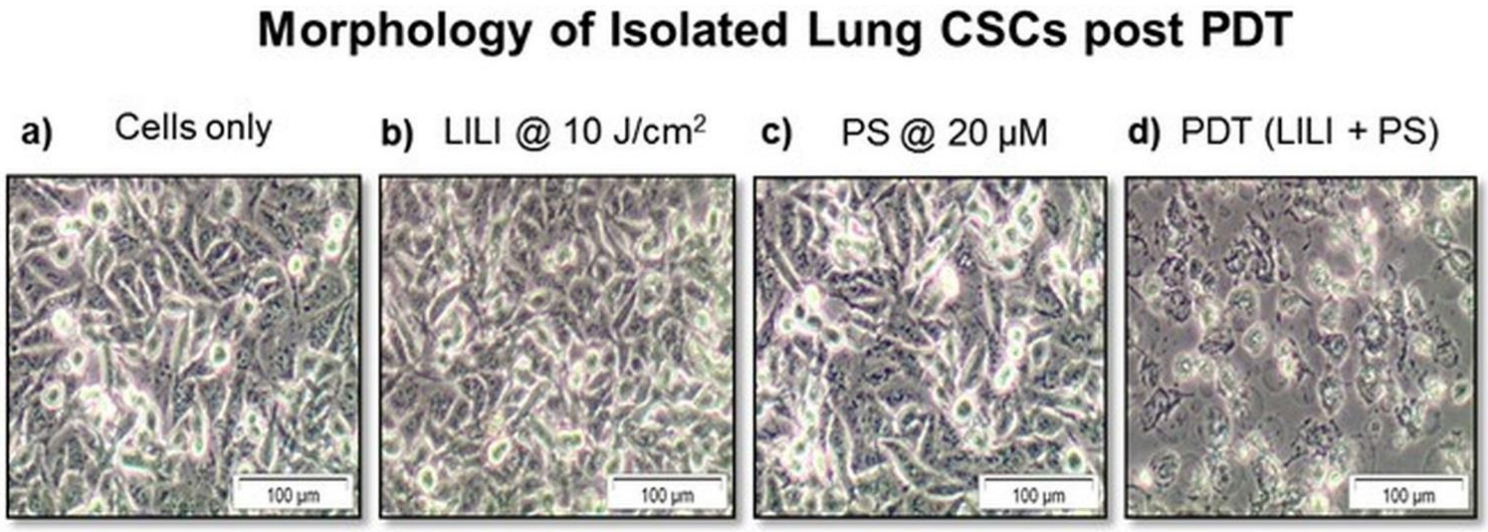

Figure 8. Morphology of Isolated Lung CSCs post PDT. Cells only represent healthy viable lung CSCs. CSCs receiving LILI with an energy of 10 J/cm^2 indicated an increase in cell proliferation, seen by an increase in the cell monolayer density when compared to the density of the control sample. No cytotoxic morphological changes are seen in lung CSCs after receiving $AlPcS_4Cl$ of 20 µM without photo activation. Cellular morphology resemble that of the control sample receiving no PS. Lung CSCs that received PDT with a 20 µM $AlPcS_4Cl$ concentration activated using 10J/cm^2 irradiation, show indications of cell death, following an apoptotic and necrotic pathway.

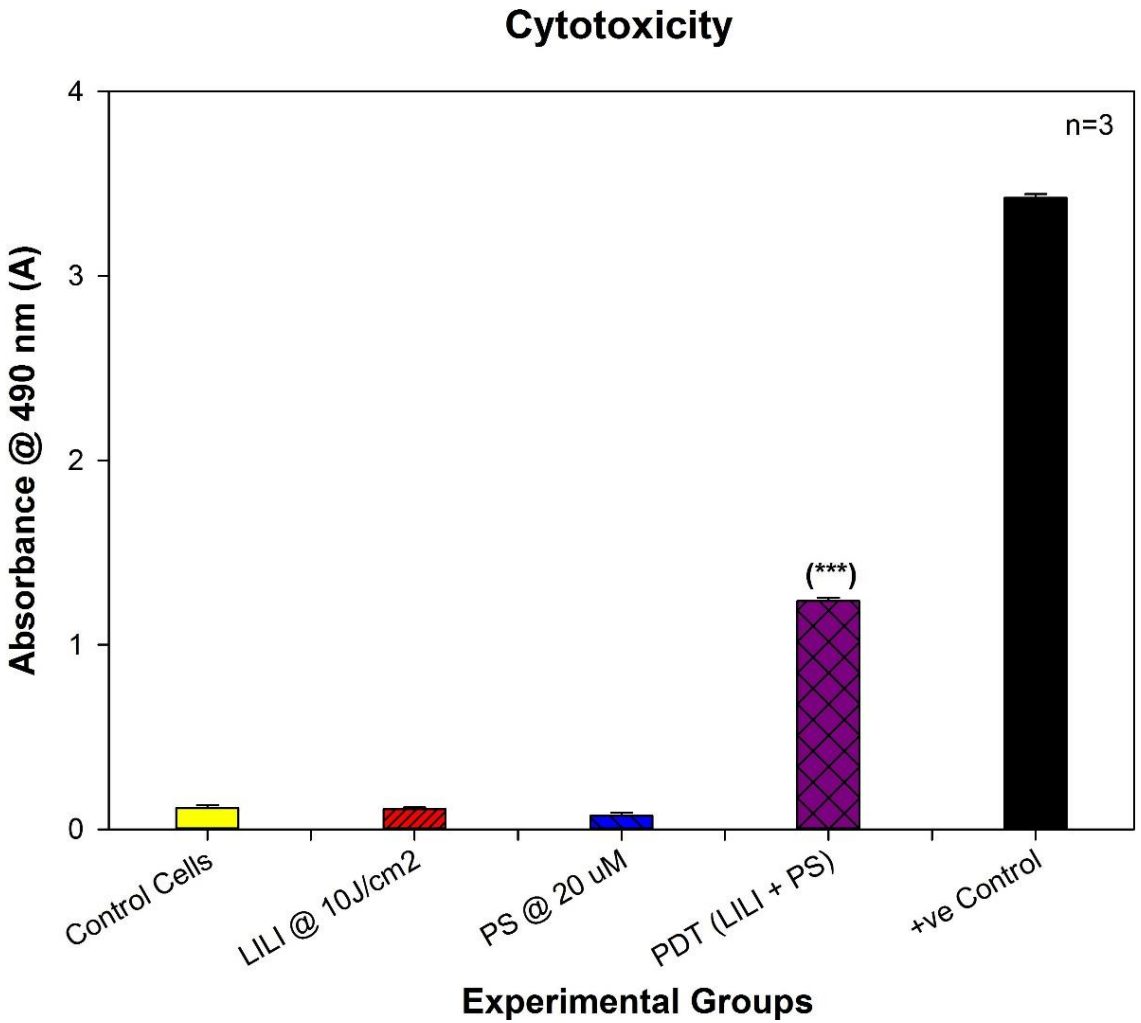

Figure 9. LDH Cytotoxicity of Isolated Lung CSCs post PDT. Cytotoxicity was measured as an absorbance value @ 490 nm. All test samples were compared to their respective control cells. No statistical significance was seen when exposing the cells to PS or LILI alone. A statistical significance of $p<0.001$ in LDH cytotoxicity was observed when treating the cells with PDT using 20 µM $AlPcS_4Cl$ and 10 J/cm^2 LILI, which was calculated as a percentage value in comparison to the positive control sample representing 100 %. The percentage cytotoxicity achieved in isolated lung CSCs was 36.13 %.

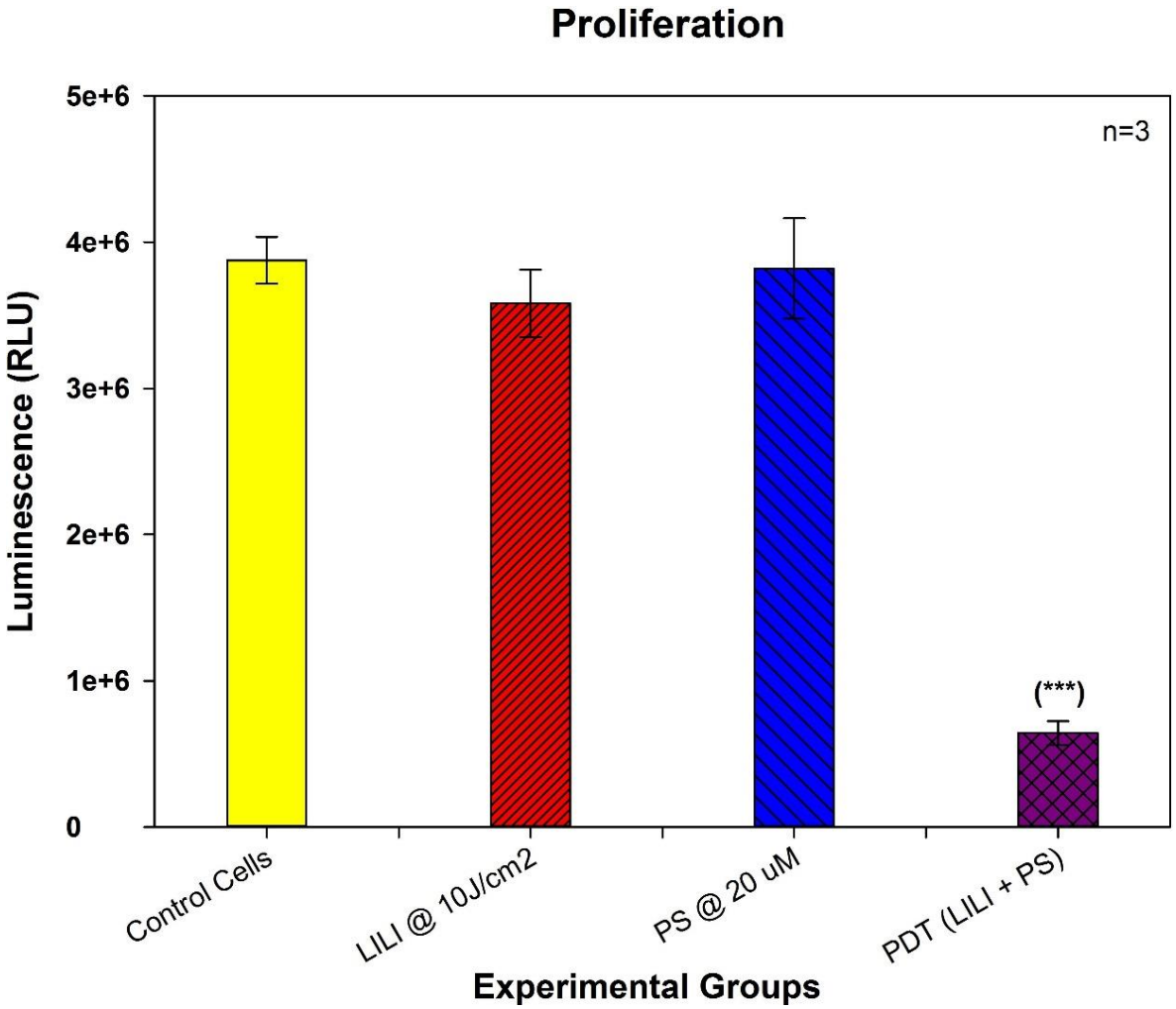

Figure 10. ATP Proliferation of Isolated Lung CSCs post PDT. ATP was measured as a luminescent value in relative light units (RLU). All test samples were compared to their respective control cells. No statistical significance was seen when exposing the cells to PS or LILI alone. A statistical significant decrease in ATP proliferation of $p<0.001$ was observed when treating the cells with PDT using 20 µM $AlPcS_4Cl$ and 10 J/cm^2 LILI.

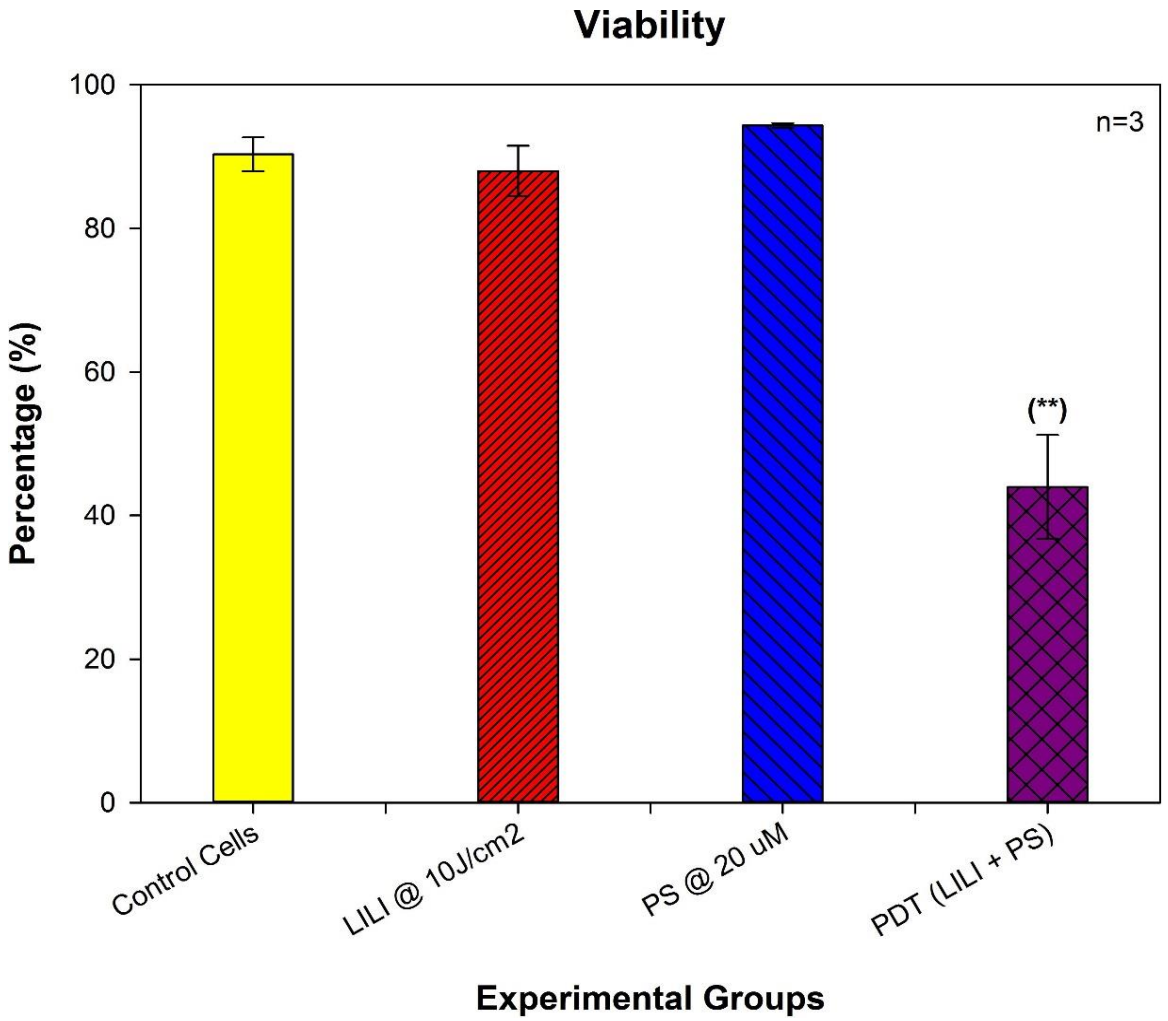

Figure 11. Trypan Blue Viability of Isolated Lung CSCs post PDT. Trypan Blue was used as a dye exclusion assay, where viable cells excluding the dye was recorded as a percentage value. All test samples were compared to their respective control cells. No statistical significance was seen when exposing the cells to PS or LILI alone. A statistical significant decrease ($p<0.01$) in viability of 46 % was seen when treating the cells with PDT using 20 µM $AlPcS_4Cl$ and 10 J/cm^2 LILI, when compared to the respective control samples.

Cell Death – Annexin V PI

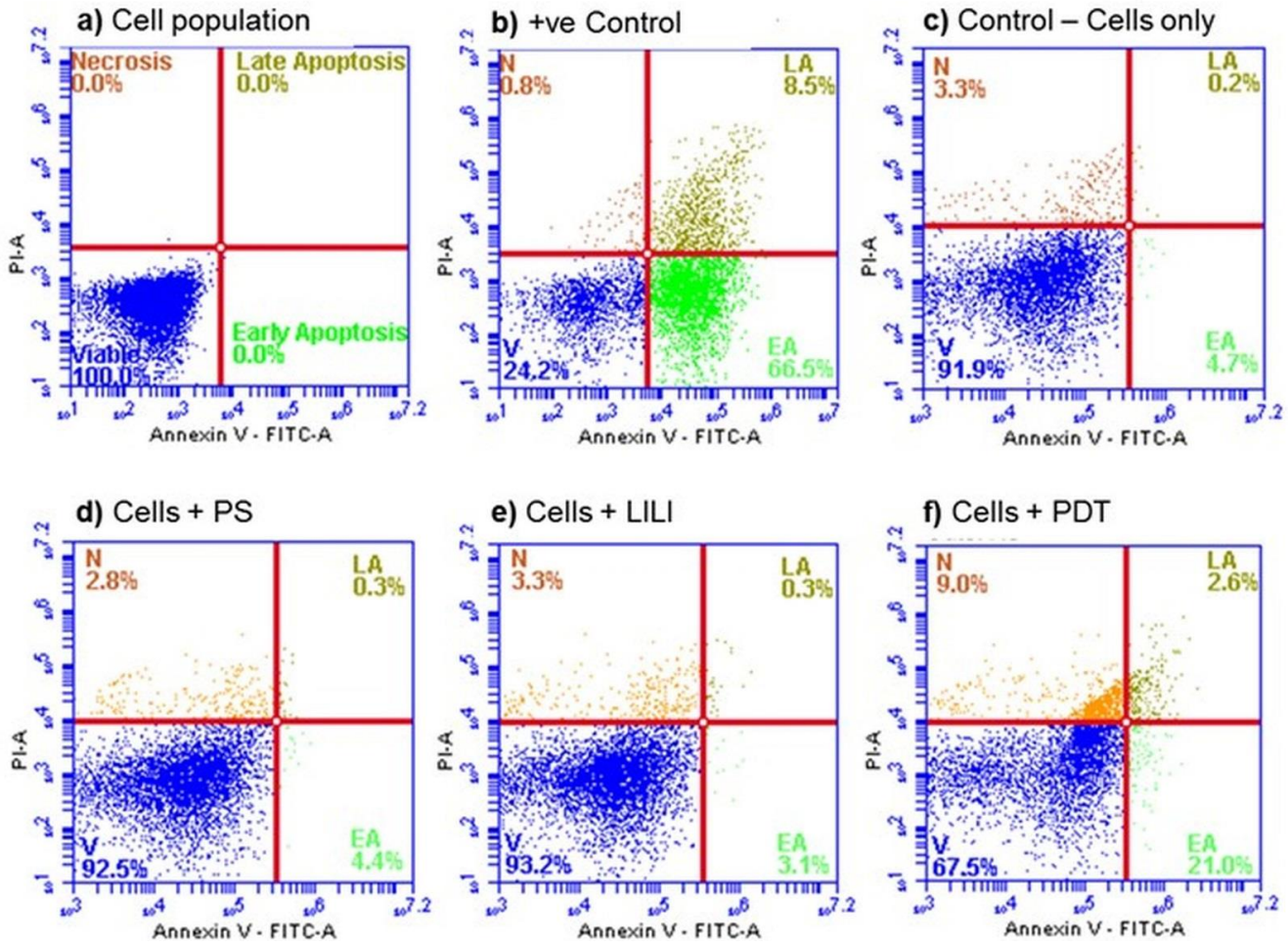

Figure 12. Cell Death – Annexin VPI of Isolated Lung CSCs post PDT. Unstained cells were used to establish the cell population (a). A positive control sample was used where apoptosis was induced using hydrogen peroxide (b). Cells receiving no treatment was used as the control (c). Cells receiving PS or LILI alone had no significant cell death with viabilities of 92.5 % and 93.2 % respectively (d – e). Cells receiving PDT showed a statistical significant increase ($p<0.001$) in early apoptosis (21 %), late apoptosis (2.6 %) and necrosis (9 %).

CONCLUSION

- CSCs have been **identified** in numerous tumors and have been proposed to explain **metastatic capacity, recurrence, and resistance** to radiotherapy and chemotherapy
- CSCs can **self-renew, proliferate infrequently,** express several **pluripotency genes** and are responsible for **tumor initiation and metastasis**
- CSCs that **evade cancer therapy** will be responsible for tumor drug resistance and relapse.
- CSCs can be **identified and isolated** using different approaches including flow cytometry and **magnetic-associated cell sorting**.
- This study proves that **PDT using $AlPcS_4Cl$** has the desired effects of **killing lung CSCs**. This is seen in **morphological** features of **apoptosis and necrosis**; results on **cell toxicity, proliferation, viability and cell death** corroborated these morphological findings, where $AlPcS_4Cl$-PDT caused a **significant increase** in **cytotoxicity**, and **significant decreases** in cell **proliferation and viability**, as well as cell death analysis presenting significant increases in **early-, late-apoptosis and necrosis**.
- PDT can be considered as a palliative treatment along with established lung cancer therapies, which can enhance the prognostic outcome of the treatments by killing of CSCs.
- Photodynamic anticancer therapy is aimed at destroying cancerous cells alone, preserving normal cells. Therefore the effects of the particular PS need to be explored on normal lung cells.

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ACKNOWLEDGEMENTS