# ICCI CANCER RESEARCH SYMPOSIUM

# Immunogenic cell death in triple negative mammary cancer cells treated with 5-aminolevulinic acid photodynamic therapy

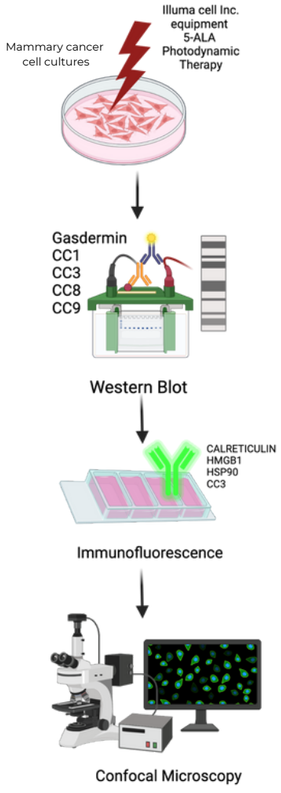
Ana Valeria Vilchis Celis,[1,3] Jeff Hummel,[2] Eva Ramón-Gallegos[1]*, Alicia Viloria-Petit [3]*

1. Environmental Cytopathology Laboratory, Department of Morphology, Instituto Politécnico Nacional, Ciudad de México, Mexico. 2. Illumacell Inc, Hamilton, ON, Canada
3. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.
*Principal Investigator

## INTRODUCTION

Immunogenic cell death (ICD) is a type of cell death that triggers an adaptive immune response against cancer cells. Photodynamic Therapy (PDT) can stimulate ICD by promoting the release of danger signals and activating the immune system. During 5-ALA (5-aminolevulinic acid) photodynamic therapy (PDT), a photosensitizer agent (5-ALA) is administered, which selectively accumulates in target cells. Upon activation by specific wavelengths of light, the PS generates reactive oxygen species (ROS), leading to cellular damage and death, as the release of damage-associated molecular patterns (DAMPs) from dying cancer cells. DAMPs act as danger signals that alert the immune system to the presence of abnormal cells. The release of DAMPs, such as high-mobility group box 1 (HMGB1), calreticulin, and HSP90, from dying cells triggers a series of immunological events that lead to the activation of an adaptive immune response against cancer cells that results on the recognition and elimination of not only the treated tumor but also distant tumor sites and micrometastases. This aproach is extremely useful for immunosupressive cancers such as Triple Negative Mammary Cancer (TNMC).

## METHODS




## RESULTS

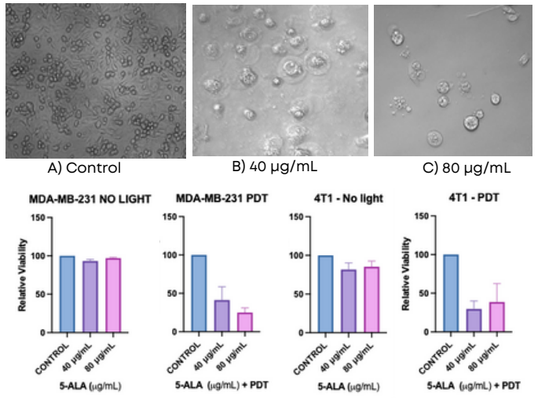



**Fig.1 CELL VIABILITY OF MAMMARY CANCER CELLS AFTER 5-ALA PDT TREATMENT** using a light dose of 50 J/cm2 at a fluence rate of 100 mW for 12 mins in cycles of 3 min on 3 mins off.

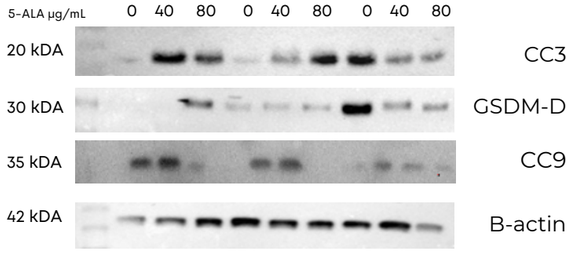



**Fig.2 ICD/DEATH BIOMARKERS EXPRESSION BY IMMUNOBLOT AFTER 8 HOURS OF 5-ALA PDT TREATMENT** of the mammary cancer cell lines 4T1 and MDA-MB-231, three independent repetitions. Expression of this proteins is indicative of apoptosis and the release of GSDM-D is a sign of pyroptosis, a form of immunogenic cell death.

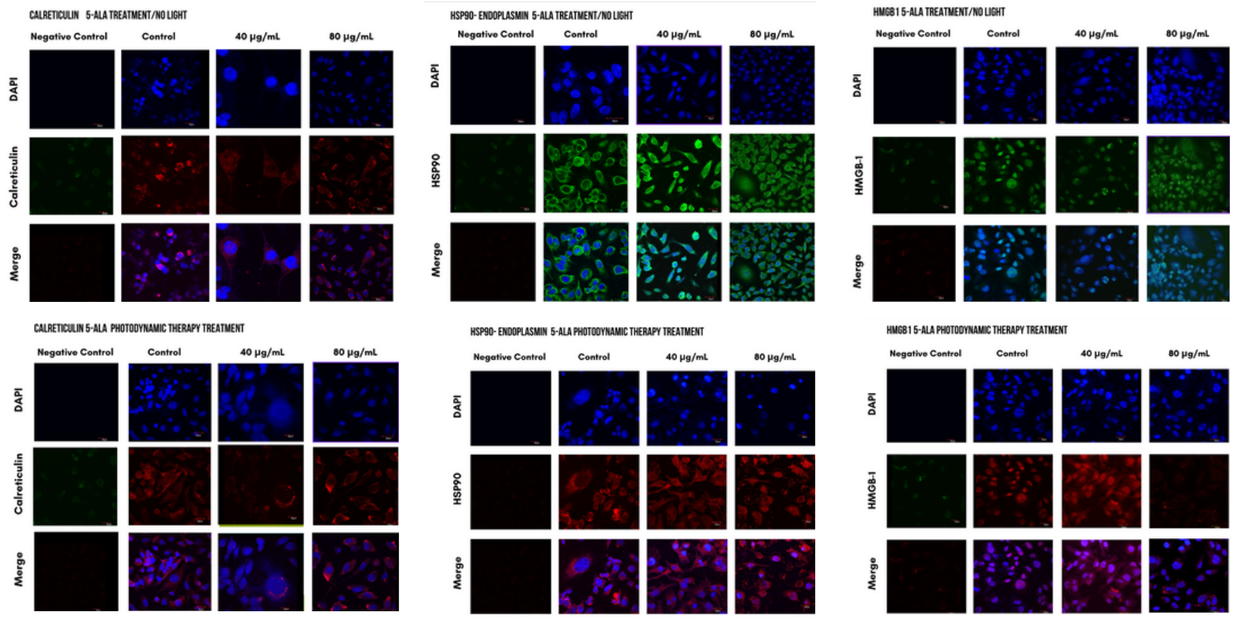



**Fig. 3 IMMUNOFLUORESCENCE BY CONFOCAL MICROSCOPY OF MDA-MB-231 CELL LINE TREATED BY 5-ALA PHOTODYNAMIC THERAPY (60x OIL) .**
In order for Calreticulin, HMGB1, and HSP90 to be considered as DAMPs, they need to be released from their normal cellular location and appear in the cell membrane or extracellular space. This phenomenon can be observed through immunofluorescence analysis. In the control and non-light-treated conditions, these biomarkers remain in their usual cellular locations. However, after light treatment, they translocate to signal the immune system that the cell is undergoing stress or approaching death.

## CONCLUSION

When used in conjunction with the light-sensitive precursor 5-ALA, photodynamic therapy (PDT) demonstrates a significant reduction in viability in triple-negative breast cancer cells. This treatment also leads to the release of calreticulin, HMGB1, and HSP90 from their regular positions in the endoplasmic reticulum and nuclei to the cell membrane and cytoplasm. The therapy induces cell death through both apoptosis and pyroptosis, primarily mediated by the expression of Gasdermin-D. By combining PDT-induced tumor cell death with subsequent immune activation, PDT-mediated immunogenic cell death (ICD) emerges as a promising approach for cancer treatment. This strategy strengthens the body's own immune system to target and eliminate cancer cells, offering the potential for long-lasting protection against tumor recurrence and metastasis.

## FUTURE DIRECTIONS

Our ongoing research aims to optimize this 5-ALA PDT protocol to maximize the immunogenicity of treated cells, reduce the damage of normal tissue and enhance the therapeutic efficacy of this approach to advance it into a clinical trial.

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**REFERENCES:** Galluzzi, L., Vitale, I., Warren, S., Adjemian, S., Agostinis, P., Martinez, A. B., Chan, T. A., Coukos, G., Demaria, S., Deutsch, E., Draganov, D., Edelson, R. L., Formenti, S. C., Fucikova, J., Gabriele, L., Gaipl, U. S., Gameiro, S. R., Garg, A. D., y Golden, E. (2020). Consensus guidelines for the definition , detection and interpretation of immunogenic cell death. 7, 1–21. https://doi.org/10.1136/jitc-2019-000337 .