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# Development of an immunogenic integrin-targeted photodynamic therapy in head and neck tumoroids to re-sensitize patients to immunotherapy

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## Résumé

Cancers of the upper aerodigestive tract, including head and neck squamous cell carcinoma (HNSCC), represent a major public health challenge due to their high morbidity and mortality rates. Immune checkpoint inhibitors are currently used as first-line treatments in recurrent or metastatic HNSCC. However, only about 20% of patients respond, highlighting the need for strategies to re-sensitize tumors to immunotherapy. In this context, stimulating anti-tumor immune responses is of particular interest. Photodynamic therapy (PDT), which relies on the activation of photosensitizers (PS) by light to generate reactive oxygen species (ROS) and singlet oxygen (<sup>1</sup>O), induces oxidative damage and cell death including immunogenic cell death known to stimulate anti-tumor immune responses.

The aim of this project is to develop and validate, in collaboration with Dr. Figliola's team (ICPEES), an optimized PDT strategy for HNSCC by integrating innovative, tumor-targeted photosensitizers with 3D tumor models. Specifically, we seek to (i) optimize key PDT parameters such as PS concentration, light dose, and oxygenation conditions; (ii) characterize the diffusion and localization of PS within different cellular compartments of tumor organoids; and (iii) investigate post-treatment cellular responses, including immunogenic cell death.

We use patient-derived tumor organoids (PDTOs), which faithfully recapitulate the histological, molecular, and functional features of native tumors. In this study, we compared four BODIPY-based PS designed to assess how structural modifications affect phototoxic potency under both normoxic and hypoxic conditions, intracellular trafficking, and cell death mechanisms. The series includes: a BODIPY core (BOD), a BODIPY conjugated with a RGD peptide for integrin targeting (BOD-RGD), a BODIPY bearing a pH-responsive group activated in acidic organelles (BOD-pH), and a dual-functional construct combining RGD targeting with pH sensitivity (BOD-pH-RGD). Cell viability was assessed using standard colorimetric assays, while confocal microscopy, flow cytometry, western blotting, and immunofluorescence were used to evaluate PS uptake, subcellular distribution, and the expression of hallmarks of immunogenic cell death.

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\*Intervenant

Across the series, BOD showed the highest phototoxicity at low concentrations, whereas the addition of targeting or pH-responsive groups modestly reduced potency, likely due to increased specificity. Regarding localization, BOD and BOD-pH showed a rather diffuse distribution, whereas RGD functionalization promoted a plasma membrane localization. For all PS, IC values decreased with increasing light dose, and phototoxic effects were maintained under hypoxic conditions, highlighting their potential to efficiently target hypoxic tumors. Post-illumination analysis revealed the activation of damage-associated molecular patterns, together with the activation of apoptotic and autophagic pathways, indicating an immunogenic cell death.

Future work will further investigate cell death mechanisms in more detail and explore the induction of anti-tumor immune responses, in order to determine whether this strategy can re-sensitize HNSCC tumors to immunotherapy.

**Mots-Clés:** tumoroids, photodynamic therapy, head and neck cancer