

## COMBINATORY THERAPEUTIC STRATEGY TO METASTATIC COLORECTAL CANCER TREATMENT USING FUNCTIONALIZED NANOPARTICLES

Maria José Silveira<sup>1,2</sup>, Flávia Sousa<sup>1,3</sup>, Sérgia Velho<sup>4</sup>, Maria José Oliveira<sup>1,5</sup>, Bruno Sarmiento<sup>1,3</sup>

<sup>1</sup> I3S/INEB- Instituto de Investigação e Inovação em Saúde/Instituto de Engenharia Biomédica, Universidade do Porto, Portugal

<sup>2</sup> ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

<sup>3</sup> CESPU-IUCS – Instituto Universitário de Ciências da Saúde, Portugal

<sup>4</sup> IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal

<sup>5</sup> FMUP – Faculdade de Medicina da Universidade do Porto, Portugal

**Background:** Colorectal cancer (CRC) is one of the deadliest cancers in the world, mainly due to distant metastasis events. Despite the improvements on the chemotherapy, 5-fluorouracil (5-FU), remains one of the most effective drugs commonly used to treat CRC, but is also associated with several limitations due to its high toxicity. To overcome those drawbacks, nanomedicine might be used as a promising strategy to treat those cases providing an effective, controlled and targeted therapy which be useful to decrease the side effects and improve treatments. Carcinoembryonic antigen (CEA) is one of the most interesting candidates to target CRC cells, due to its overexpression at most CRC tumors. Besides, the treatment outcome, could be widely associated with the immunosuppressive tumor microenvironment (TME) which promotes the cancer cell immune escape. In our project, the major aim is to develop an innovative combinatory therapy based on immunochemotherapy targeted NPs. Two different targeted NPs will be developed in this work. The first nanosystem is planned with tropism to CRC cells expressing the CEA, carrying the chemotherapeutic agent 5-FU. The other one will target a molecule in TME highly expressed in CRC tumors, carrying one cytokine.

**Methods:** Polymeric NPs were produced by double emulsion and loaded with 5-FU followed by functionalization with an engineered antibody (scFv) targeting CEA. Physical-chemical properties were assessed by Dynamic Light Scattering (DLS) and Laser Doppler Anemometry (LDA). Morphology, drug loading (DL) and conjugation efficiency were evaluated by TEM and HPLC, respectively.

**Results:** NPs with  $121.41 \pm 5.35$  nm were achieved, with about PDI 0.1, confirming the monodisperse population and around of 6% of DL. The surface charge was close of the neutrality ( $-3.51 \pm 0.46$  mV) and the spherical shape was confirmed. Conjugation Efficiency is still being evaluated. *In vitro* studies to assess drug release, binding efficiency, cytotoxicity and targeting ability of the NPs against CEA-expressing and non-expressing cells will be further performed.

**Conclusions:** Through this work, we developed and characterized 5-FU-loaded NPs functionalized with a scfv targeting CEA to improve chemotherapy delivery, decreasing its side effects and enhancing its efficacy. *In vitro* studies will be complemented evaluating the impact of these NPs on immune cell profile and function. Finally, biodistribution and ability to modulate the immune response, impairing tumor progression will be evaluated *in vivo* using the AOM-CRC mouse model. As future work, we intend to develop the functionalized NP carrying a relevant cytokine to target the TME. After performing both functionalized NPs *in vitro* and *in vivo* assays will be performed to assess the distribution and internalization of the NPs, as well as the impact on cancer and immune cell profile. Therefore, this combinatory strategy will have a dual role acting on cancer cells and recruiting immune cells, changing the TME activity, improving the outcome of chemotherapy and modulating the tumor immunosuppressive environment.