PHYSICIAN SCIENTIST
LA MEDICINA INCONTRA LA RICERCA
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Principal Investigator	Mazzucchelli Serena
Hosting institution	Università degli Studi di Milano
Proposal title	Exploitation of Indocyanine green loaded engineered protein nanocages for photodynamic therapy of Breast Cancer
Keywords	Breast ca.; Nanotechnology/Nanoparticles; In vivo imaging; Photodynamic therapy and photodetection; Fluorescence imaging system
PhD project description	Photodynamic therapy (PDT) has emerged as a minimally invasive cancer treatment that combines localized irradiation of photothermal agents with the generation of heat and reactive oxygen species (ROS) to induce immunogenic cell death and recruit T lymphocytes. This dual action makes PDT especially attractive for tumors unresponsive to standard therapies, such as triple-negative breast cancer (TNBC), and for converting "cold" tumors into immunologically active sites. Indocyanine green (ICG), a fluorescent dye long used in diagnostics, has recently shown promise as a photothermal agent, but its rapid degradation and poor tumor selectivity limit its clinical potential. To overcome these hurdles, here we propose to encapsulated ICG within human H-ferritin nanocages (HFn-ICG), leveraging HFn's high affinity for the transferrin receptor 1 (TfR1), which is ubiquitously overexpressed on cancer cells. In breast cancer cell lines, HFn-ICG demonstrated significantly enhanced PDT efficacy compared with free ICG, and in vivo studies confirmed rapid, tumor-specific accumulation of the nanoconstruct. Building on these preliminary successes, during this PhD project the candidate will engineer HFn's outer surface to extend circulation time and further sharpen tumor targeting. Three objectives will be achieved: (i) optimize production of engineered HFn (HP) and efficient ICG loading (HP-ICG); (ii) assess HP-ICG's targeting ability, tissue penetration, PDT effectiveness, and immunostimulatory impact in breast cancer cell monolayers and patient-derived organoids; and (iii) characterize HP-ICG's biodistribution and tumor tropism in an in vivo TNBC model. By unveiling HP's tumor-homing capacity and validating HP-ICG's boosted immunogenic PDT in highly translational, patient-derived systems, this project paves the way for broad application across diverse cancer types.
Main topics of the lab	Development of nanodelivery systems for cancer application
Short description of the lab activity	Our laboratory workflow centers on developing and validating an protein-based nanodelivery systems for theranostic applications in breast cancer. We will produce protein-based nanodelivery systems in E. coli as recombinant proteins and we will set up purification methods to ensure high yield, purity, and colloidal stability. Protein-based nanodelivery systems will be loaded with dyes, tracers and/or drugs and the resulting product will be characterized by dynamic light

	scattering and transmission electron microscopy. Then we will test the nanoformulation stability in different storage conditions and physiologic media. Next, nanoformulation interaction with a panel of breast cancer cell lines is used to evaluate uptake by flow cytometry and confocal microscopy. Also nanoformulation toxicity and efficacy will be assayed. In this specific project we will test PDT efficacy, measuring cell viability, apoptosis, reactive oxygen species, and immunogenic cell death indicators such as calreticulin exposure and cytokine release. In parallel, breast cancer patient-derived organoids will serve as 3D models for assessing nanoformulation's tissue penetration and PDT response within a native tumor microenvironment, using fluorescence imaging and immunostaining. Finally, an orthotopic triple-negative breast cancer mouse model evaluates nanoformulation biodistribution and tumor tropism by in vivo and ex vivo fluorescence imaging, establishing optimal tumor-to-background ratios for future therapeutic studies.
Main research area	Molecular Therapy
Group composition	8 people of whish: 1 PI and head of the lab 1 clinician2 post-docs 3 post-graduate fellows 1 technician
Institutional page link	https://dibic.unimi.it/it/ricerca/gruppi-e-risorse-della-ricerca/gruppi-di-ricerca/laboratorio-di-nanomedicina
Lab website link	nan
Social media link	https://www.instagram.com/namedicalab?igsh=MWdqeHNrbnBma2lsZA==
Lab bibliography	In Vitro Immunoreactivity Evaluation of H-Ferritin-Based Nanodrugs. Sitia L, Galbiati V, Bonizzi A, Sevieri M, Truffi M, Pinori M, Corsini E, Marinovich M, Corsi F, Mazzucchelli S BIOCONJUGATE CHEM 2023 May; 34: 845