Innovative targeted therapy to HER3 for drug delivery against breast cancer cells

Context: Breast cancer is a heterogeneous disease characterized by distinct molecular subtypes such as hormone receptor-positive (HR+), human epidermal growth factor receptor 2-positive (HER2+), and triple-negative breast cancer (TNBC) with varied prognoses and differential treatment responses. While progresses have been made in breast cancer identification, its resistance and escape mechanisms remain a major issue. Understanding the molecular landscape and identifying therapeutic targets is crucial.

Human epidermal growth factor receptor 3 (HER3/ErbB3) is a tyrosine kinase receptor belonging to the HER family alongside epidermal growth factor receptor. Nonetheless, HER3 is able to form heterodimers, preferentially with HER2 and/or EGFR, which dramatically enhances transphosphorylation and the consequent activation of mitogenic downstream pathways[1]. Preclinical data shows that HER3 expression is associated with worse overall survival and contributes in resistance to tamoxifen, HER2 and EGFR-targeted therapy and chemotherapy. Consequently, reduction of HER3 expression can reverse this resistance in breast cancer cell lines[2]. Overall, there is a strong rationale behind the therapeutic targeting of HER3.

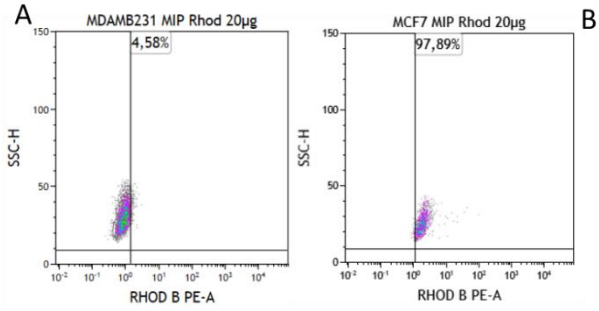
Over the last few decades, nanoparticles have drawn remarkable attention as a promising drug delivery agent due to their low cytotoxicity, excellent biocompatibility, high solubility, photostability, and outstanding selectivity towards target. However, nanoparticles, requires targeting ligands for this. Generally, antibodies are widely used as the targeting agent[3]. However, these antibodies have low stability, high risk of degradation, have poor cell membrane permeability and are highly expensive. Treatment with anti-HER3 monoclonal antibodies or bispecific antibodies, both as single agents and in combination with other compounds, unfortunately led to unsatisfactory results across several tumor types. The HER3-directed delivery of cytotoxic payloads through antibody-drug conjugates has recently demonstrated encouraging activity in several tumor types, however, suggesting a potential role for the therapeutic targeting of HER3 in cancer treatment.

Scientific objective: In this context, the objective of the thesis is to develop theranostic nanoparticles composed of i) molecularly imprinted polymers as a substitute for antibodies, ii) carbon nanodots for cells imaging and iii) a drug for cancer therapy. Molecularly imprinted polymer nanoparticles (nanoMIPs) are artificial receptors which can specifically identify targeted molecules according to the corresponding binding site in terms of shape, size and chemical functionalities. Compared with biomolecules, molecularly imprinted nanoparticles have the superiority of good stability, low cost and simple synthesis, and were even increasingly used as “plastic antibodies” for cell targeting and disease diagnostics. Carbon nanodots are interesting fluorescent nanoparticles suitable for imaging.

This project aims to evaluate the efficacy of nanoMIPs as a substitute for antibodies in targeting HER3 overexpressing breast cancer cells. The dual imprinted MIP is designed to specifically detect the HER3 marker molecules on the surface of breast cancer cells and emit fluorescence. Additionally, these nanoparticles are capable of delivering a drug molecule to inhibit the growth of to kill cancer cells. This approach not only tackles the limitations of traditional treatment methods but also offers a highly specific and targeted treatment option.

Preliminary results

As we ever obtained a PhD funding from Sorbonne University/IIT Delhi for three months started in February 2024, we ever started to develop this ambitious project. Preetha Ganguly developed HER3-imprinted fluorescent polymer nanoparticles. The developed materials are small, stable and fluorescent and able to efficiently selectively target breast cancer cells that overexpress HER3 (MCF7 cells) as presented in panel A using flow cytometry.



To continue this long work, we extended

Preetha's contract for one additional month. She synthesized fluorescent HER3-particles containing curcumin and HER3-particles containing carbon nanodots as presented on TEM pictures in panel B. The carbon nanodots seem to be encapsulated in the MIP nanoparticles.

Research plan/Methodology: Four interconnected research work packages WP1, WP2, WP3, WP4 will be pursued:

WP1: Synthesis of the hybrid nanomaterial:

The synthesis will be inspired from the well-established glass beads method. In this method, the epitope is fixed on the glass beads, then the polymer synthesis is performed at room temperature during 1 h with a redox initiation. After washing at 60°C, the MIP nanoparticles are recovered and analyzed using TEM, fluorescence's, DLS. Carbon dots nanoparticles and the drug are encapsulated during the polymerization to obtain the final MIP nanoparticles. As a control, a scrambled epitope is used.

WP2: Targeting and drug release in cancer cells *in vitro.*

Breast cancer cell lines will be incubated at 37°C with MIPs containing the drugs (1 to 6 hours). The cytotoxic potential of the drug released will be determined using a standard viability test and apoptosis using annexin V assay. TEM and confocal microscopy analysis will provide extremely high-resolution images for observing the fine structure and shape of MIPs and cells integrity.

WP3: Pharmacokinetics.

To assess drug concentrations in MIP and released in breast cancer cells, an ultra-performance Liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS) will be used to quantify simultaneously multiple drugs.

WP4: Targeting and drug release in cancer cells *in vivo*

Effect of MIP will be evaluated i) on the primary tumor by local and ii) on distant metastasis, after intravenous injection of cells to form metastasis, MIP will be injected in the blood. We will determine i) tumor and metastasis growth by fluorescence imaging system ii) Pharmacokinetic parameters such as half-life (t1/2), clearance (CI/F), and volume of distribution (Vd/F) will be measured. The nonlinear mixed effect modelling program Monolix (version 2019R2) will be used to develop a model to describe the pharmacokinetics of each drug used. Finally, iii) A specific method will also be specifically developed to quantify drugs in organs. All samples (plasma and organs) will be analyzed using ultra-performance liquid chromatography (UPLC) system coupled to mass spectrometry (LC-MS/ MS). The calibration range concentrations for all cytotoxic drugs is 0.1 -500 ng/mL and 0.01 -100 ng/g in plasma and organs respectively.

Justification of the scientific approach: Outcomes of these studies will increase our understanding of the interaction between the MIP and HER3 receptor. The use of biomass derived carbon nanodots and MIP as a therapeutic agent for cancer treatment will potentially reduce the cost of production, minimize toxicity and side effects, and improve the delivery of drugs and bio-imaging. Improvement of such technology could potentially launch cheaper, more accessible, and well-regulated breast cancer treatment in both the developed and developing countries.

This project is the proof of concept of this innovative imaging and therapy method and is the beginning of a collaboration between Sorbonne university (PHENIX and CRSA) and IIT Delhi. The next step is to deposit a project to CEFIPRA concerning the development of hybrid MIP nanomaterials for theranostic. It will be the opportunity to go to an in vivo study.

The profile required for the application will be a student with skills in chemistry and biology, and the ability to adapt, as he or she will be working with different researchers on several sites. He/she must be fluent in English and willing to spend time in India at IIT Delhi.

1. Alimandi M, Romano A, Curia MC, et al. Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. Oncogene. 1995; 10(9):1813-1821.
2. Liu B, Ordonez-Ercan D, Fan Z, Edgerton SM, Yang X, Thor AD. Downregulation of erbB3 abrogates erbB2-mediated tamoxifen resistance in breast cancer cells. Int J Cancer. 2007;120(9):1874-1882.
3. Zahavi D., et al. (2020). Monoclonal Antibodies in Cancer Therapy. Antibodies (Basel) 20;9(3):34