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Functionalized silica nanoparticles in the detection and treatment of Her2-positive breast cancer

Belloli Sara[1,2], Riva Benedetta[3], Rainone Paolo[1,2], Sudati Francesco[2], Ripamonti Marilena[1], Gianolli Luigi[2], Gilardi Maria Carla[1], Prosperi Davide[3] and Moresco Rosa Maria[2,4].

1. Institute of Molecular Bioimaging and Physiology (IBFM), CNR, Milan (Italy) ;
2. Experimental Imaging Center, IRCCS San Raffaele Scientific Institute, Milan, Italy
3. Biotechnologies and Biosciences Department, University of Milan-Bicocca, Milan (Italy);
4. Medicine and Surgery Department, University of Milan-Bicocca, Monza (Italy).

INTRODUCTION

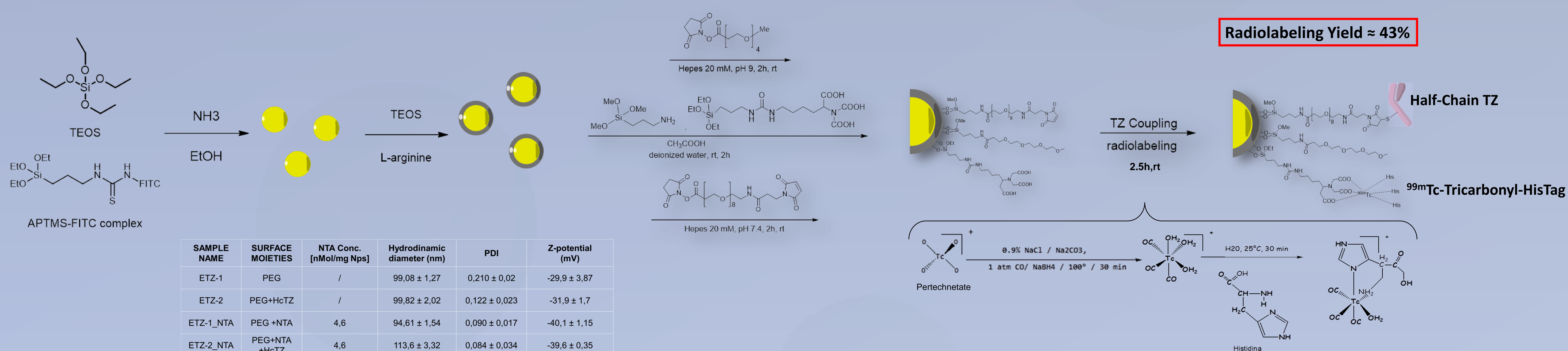
Breast cancer (BC) is the most common tumour in women, being the first cause of all deaths due to cancer. Human epidermal growth factor receptor 2 (HER2) is a strong phenotype determinant present in approximately 20-30% of all BCs. Specific therapy against this receptor with Trastuzumab, a humanized monoclonal antibody, decisively contributed to a prognostic improvement. Despite its efficacy in prolonging progression-free-survival, 70% of patients with HER2-positive BC demonstrates resistance to Trastuzumab, stressing the importance of developing new therapeutic strategies. Recent advances in Nanobiotechnology have led to development of nanoparticles for the diagnosis and treatment of human cancer. Aim of this work was to develop and validate a nanoparticles-based system for early detection and treatment of HER2 positive breast cancer lesions. Spherical silica nanoparticles (SiNPs) were engineered with anti-HER2 antibody fragment and functionalized with both radioactive and fluorescence probe for respectively PET/SPET and microscopy detection. In addition, the antibody-receptor interaction will be used to specifically target therapy by transporting treatment into cancer cells.

METHODS

SiNPs of 50 nM were filled with FITC for fluorescence imaging and conjugated with the half-chain of a monoclonal antibody Trastuzumab (ETZ-2) or not (ETZ-1, control) and with nitril-triacetic acid (NTA).

***In vitro* kinetic and toxicity:** to study *in vitro* kinetic in breast cancer cells, ETZ-1_NTA and ETZ-2_NTA were incubated at different times (20 min, 1h, 4h, 24h) with HER2 positive (SK-BR-3) and negative (MDA-MB-468) human cell lines and analyzed by flow cytometry (FACS) and optical fluorescence microscopy. For cytotoxicity assessment, viability test and MTT assay were performed incubating SK-BR-3 (mammary adenocarcinoma, HER2 positive) and MCF-10A (non-transformed epithelial cell line derived from human fibrocystic mammary tissue, HER2 negative) with ETZ-1_NTA or ETZ-2_NTA for 20 min, 1h, 4h and 24h. **Radiolabeling:** ETZ-1 and ETZ-2 derivatized with NTA, were reacted with His-Tag, previously labeled with the ^{99m}Tc-Tricarbonyl complex. ***In vitro* binding:** to study *in vitro* kinetic uptake of radiolabeled SiNPs, ^{99}Tc-NTA_ETZ-1 and ^{99}Tc-NTA_ETZ-2 were incubated at different times (20 min, 1h, 4h, 24h) with SK-BR-3 and MDA-MB-468 breast cancer cell lines. Cells and medium were counted using a γ-counter and cell uptake expressed as % of total radioactivity. ***Ex vivo* biodistribution:** the same radiolabeled SiNPs were used for *ex vivo* biodistribution in an animal model of breast cancer obtained after the implantation of SK-BR-3 or MDA-MB-468 cells under the right shoulder. Animals were injected in a tail vein with 100-120 µCi of radiolabeled ETZ-1 or ETZ-2 and sacrificed at 4h. Tumour and peripheral organs were dissected and counted in a γ-counter to obtain the percentage of injected dose per gram of tissue (%ID/g). Subsequently, *ex vivo* distribution was achieved for labeled ETZ-2 and ETZ-1 also at 6h, 24h in the HER2+ model. In parallel, a tumour sample were also frozen in liquid N_2 and sectioned into 10µm slices for fluorescence microscopy and a sample of tumour and peripheral organs were treated for proteogenomic analysis.

SYNTHETIC PATHWAY AND RADIOLABELING



SAMPLE NAME	SURFACE MOIETIES	NTA Conc. [nMol/mg Nps]	Hydrodinamic diameter (nm)	PDI	Z-potential (mV)
ETZ-1	PEG	/	99,08 ± 1,27	0,210 ± 0,02	-29,9 ± 3,87
ETZ-2	PEG+HcTZ	/	99,82 ± 2,02	0,122 ± 0,023	-31,9 ± 1,7
ETZ-1_NTA	PEG +NTA	4,6	94,61 ± 1,54	0,090 ± 0,017	-40,1 ± 1,15
ETZ-2_NTA	PEG+NTA +HcTZ	4,6	113,6 ± 3,32	0,084 ± 0,034	-39,6 ± 0,35

RESULTS

In vitro uptake of non-radiolabeled SiNPs

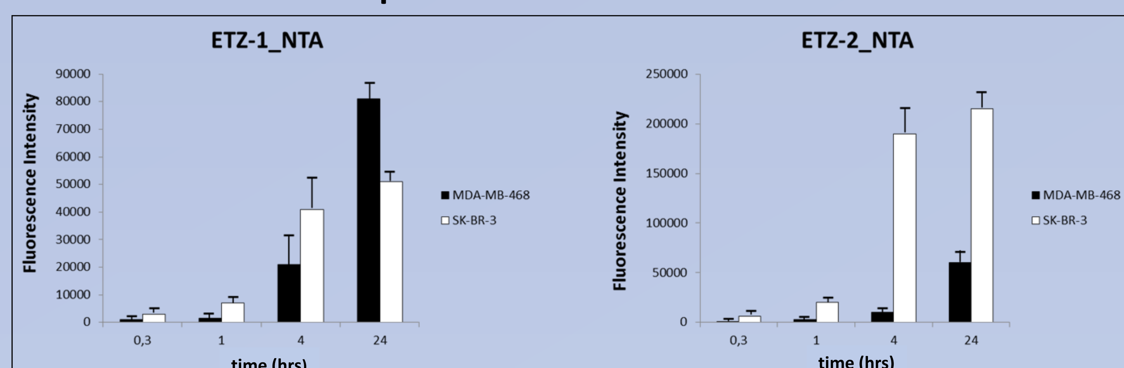


Figure 1. *In vitro* kinetic of SiNPs in HER2 positive and negative breast cancer cell lines by FACS analysis.
HER2 positive cells (SK-BR-3) showed a strong increase over time of ETZ-2_NTA nanoparticles uptake (right) compared to HER2 negative cells (MDA-MB-468, right). ETZ-1_NTA uptake followed a nonspecific trend over time, resulting at 24h higher in HER2- compared to HER2+ cells.

In vitro toxicity assay of non-radiolabeled SiNPs

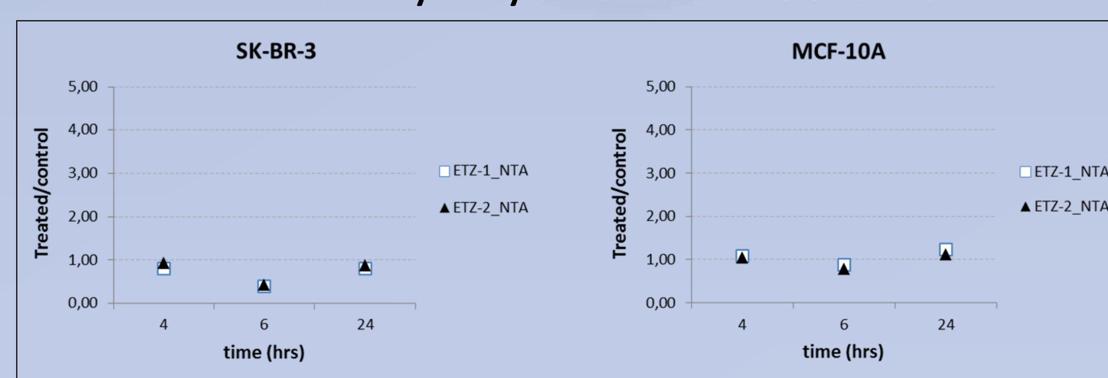


Figure 2. *In vitro* toxicity evaluation of SiNPs in breast cancer cell line SK-BR-3 (HER2+) and in non-transformed mammary epithelial cell line MCF-10A (HER2-) by UV/VIS spectrophotometry.
The analysis revealed no significant modification over time of succinate dehydrogenase activity (MTT test) in both cell lines (MCF-10A and SK-BR-3), after incubation with ETZ-1_NTA and ETZ-2_NTA nanoparticles.

In vitro fluorescence microscopy for non-radiolabeled SiNPs

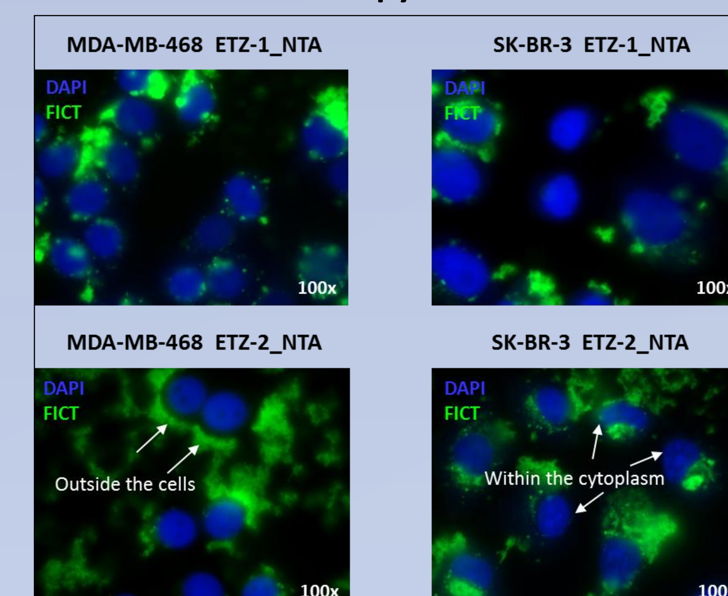


Figure 3. *In vitro* binding specificity evaluation of SiNPs in HER2 positive and negative breast cancer cell lines by fluorescence microscopy.
At 4h after incubation, we observed exclusively for ETZ-2 nanoparticles a specific binding to SK-BR-3 (HER2+) cells, as showed by intracytoplasmic fluorescence, compared to the MDA-MB-468 cells (HER2-). ETZ-1 binding resulted not specific in both HER2+ and HER2- cell lines.

In vitro uptake for radiolabeled SiNPs

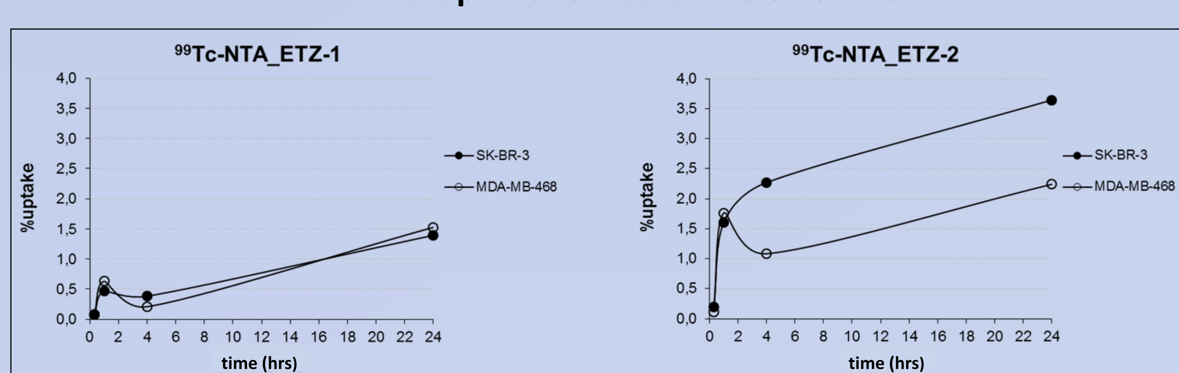


Figure 4. *In vitro* uptake of radiolabeled SiNPs in HER2 positive and negative breast cancer cell lines.
ETZ-1 nanoparticles bound in nonspecific way both to MDA-MB-468 (HER2-) and SK-BR-3 (HER2+) cells. ETZ-2 showed a specific binding only to SK-BR-3 cells. This binding is 2,3 times higher than that observed for MDA-MB-468 cells, after 4h of incubation.

Ex vivo biodistribution of radiolabeled SiNPs at 4h

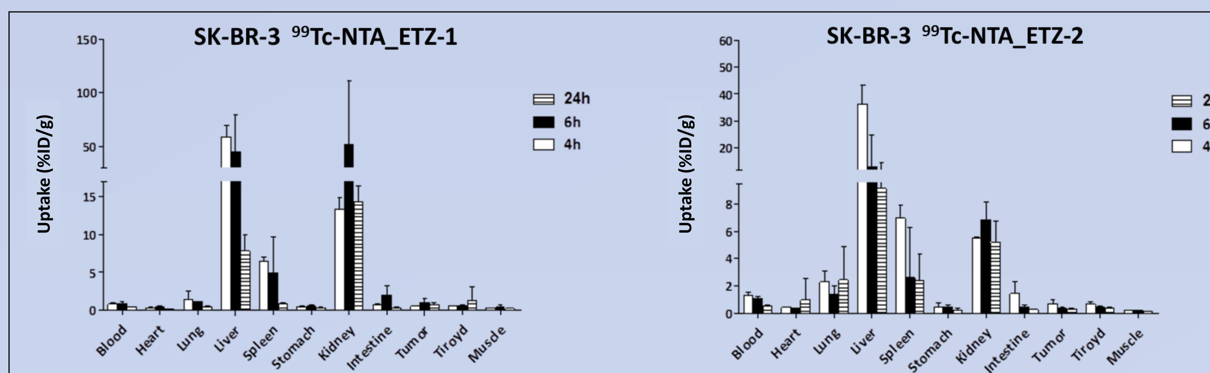


Figure 5. *Ex vivo* biodistribution of radiolabeled SiNPs in HER2 positive and negative breast cancer model at 4h.
ETZ-1 and ETZ-2 showed different kinetic of distribution in SK-BR-3 tumour and eliminating organs as liver and kidney. ETZ-2 uptake in tumour was maximum at 4h and decreased thereafter; ETZ-1 uptake increased slowly over time. Same results were obtained from biodistribution study performed at 4h after ETZ-2 injection in both HER2+ and HER2- models (data not shown). Tumour to muscle ratio of ETZ-2 nanoparticles was 3,53 in the HER2+ (SK-BR-3) tumour at 4h p.i., while the ratio for ETZ-1resulted 1,69 in the same tumour model.

Ex vivo fluorescence microscopy for radiolabeled SiNPs

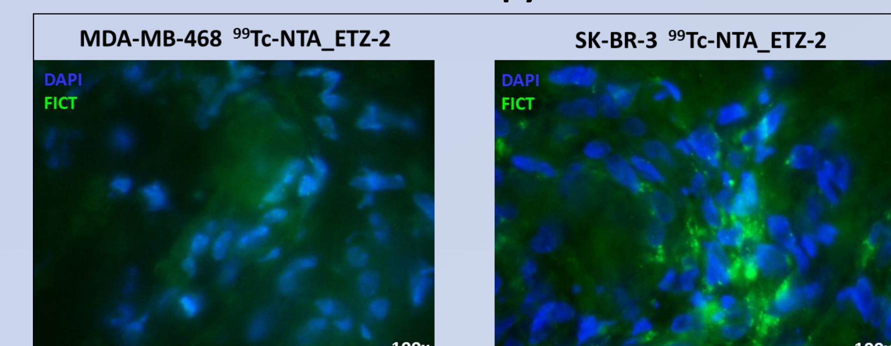


Figure 6. *Ex vivo* binding specificity evaluation of SiNPs in HER2 positive and negative breast cancer animal models by fluorescence microscopy.
Imagines obtained from tumour slices, at 4h after injection, showed a great and deep signaling localization for ETZ-2 SiNPs in HER2+ (SK-BR-3) breast cancer tissue, respect to that HER2- (MDA-MD-468).

CONCLUSIONS AND PERSPECTIVES

Preliminary results showed that ^{99m}Tc-NTA_ETZ-2 SiNPs could be a useful system for HER2 positive breast cancer detection and treatment. Proteomic and genomic characterization of tumour and tissues deriving from animals treated or not with SiNPs are ongoing. Next step will be the evaluation of SiNPs efficacy in the targeting of chemotherapy, by filling nanoparticles with doxorubicin. Treatment efficacy will be evaluated *in vitro* and *in vivo* in SK-BR-3 (HER2+) and MDA-MB-468 (HER2-) cells, in comparison with free and liposomal doxorubicin administration. Moreover efficacy, toxicity and safety of DOXO-SiNPs will be evaluated *in vitro* in MCF-10A and SK-BR-3 cell lines and *in vivo* by monitoring tumour growth and proteogenomic analysis.

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Contact author: moresco.rosamaria@hsr.it

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