Two component system for MR/optical image-guided delivery and cell surface targeting of HER2(+) cancer cells.

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Target audience: Scientists who are interested in image-guided drug delivery.

Purpose: The HER2/*neu* (ErbB-2) is one of four classes of epidermal growth factor receptors (EGFRs) which regulate the cell proliferation and differentiation. The receptor HER2/*neu* is overexpressed in 25-30% of breast cancer cases as a result of gene amplification, and consequently shows poor prognosis, cancer growth, and progression [1]. The humanized monoclonal antibody, trastuzumab (Herceptin®) is a well-known biotherapeutic used clinically for HER2/*neu* positive cancers. However recent clinical observations reveal that some patients have no beneficial effect from trastuzumab even though HER2/*neu* is overexpressed in tumors [2]. The combination therapy based on antibody-drug conjugates is a promising strategy to overcome the multidrug resistance of tumors. In this study we followed a two-component delivery system for the controlled and enhanced internalization to increase the efficacy of therapeutics. Due to the small size, the two-component delivery system overcomes the barriers of extravasation from vessels and diffusion to the target site. The first component, modified azido-trastuzumab specifically binds with the extracellular domain on HER2/*neu* receptors. The second component albumin modified with dibenzocyclooctyne (DIBO) cross links on the first components (trastuzumab) via multiple bioorthogonal click reactions (Figure 1). Eventually cell surface network of antibody-albumin conjugate undergoes macro-scale internalization and leads to the efficient delivery of high dose of therapeutics to the cytoplasm (Figure 2.). This strategy exhibits the low effect on non-specific binding sites and on normal cells expressing basal levels of HER2/*neu* receptor.

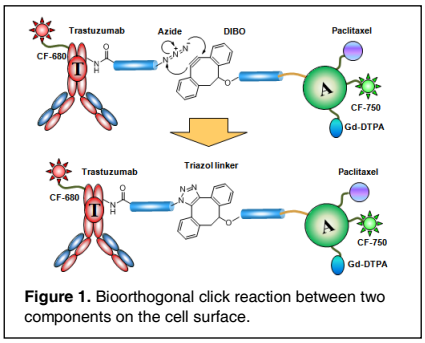

Figure 1. Bioorthogonal click reaction between two components on the cell surface.

Methods: Trastuzumab was functionalized with azide groups (NHS-PEG_4-Azide) and labeled with rhodamine or NIR CF-680 to obtain $T(PEG_4\text{-}Az)_8(Rhod/CF\text{-}680)_2$. Albumin (BSA) was substituted with Gd-DTPA, functionalized with dibenzocyclooctyne (DIBO) and labeled with AlexaFluor488 or NIR CF-750 to obtain, $A(DIBO)_{10}(Gd\text{-}DTPA)_6(Alexa\text{-}488/CF\text{-}750)_2$. For cytotoxicity study $A(DIBO)_{10}(Pac)_{2/4}(Gd\text{-}DTPA)_6(Alexa\text{-}488/CF\text{-}750)_2$ was prepared following a modified procedure including paclitaxel substitution after Gd-DTPA labeling. The two-component delivery system was tested by *in vitro* confocal microscopy and MRI of human breast cancer BT-474 cells. *In vivo* studies with BT-474 xenografted athymic female nude mice were carried out using Kodak optical and MRI imaging at 9.4T. Quantitative T_1 maps were reconstructed from 3D T_1-weighted RARE sequence acquired pre-injection and at 1, 3, 6, and 12 h post-injection of modified albumin with TR = 250 ms, 500 ms, 1s, 2s, and 4s using a 9.4T Bruker MRI spectrometer. The cytotoxicity of two-component delivery and conjugation was examined *in situ* following a standard protocol (Dojindo WST-8 IC_{50} cytotoxicity assay). Briefly, BT-474 cells were grown in 46X media for 24h (96-well plate, 5000 cells per well) and treated with $T(PEG_4\text{-}Az)_8(Rhod)_2$ (10μg/mL for 20 min at 4 ºC, unmodified trastuzumab was used in controls) followed by functionalized albumin with two or four paclitaxel substitutions.

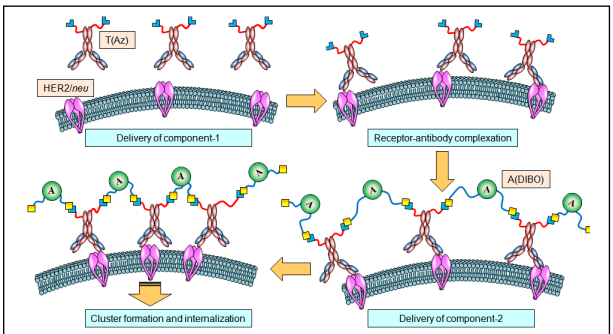

Figure 2. Cell surface assembling and enhanced internalization of two-component delivery system.

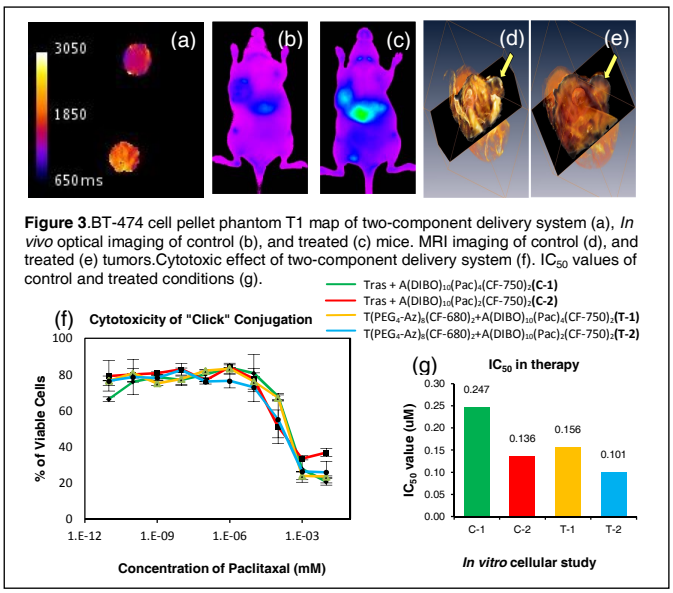

Figure 3.BT-474 cell pellet phantom T1 map of two-component delivery system (a), *In vivo* optical imaging of control (b), and treated (c) mice. MRI imaging of control (d), and treated (e) tumors.Cytotoxic effect of two-component delivery system (f). IC_{50} values of control and treated conditions (g).

Results and Discussion: *In vitro* cell labeling fluorescence images showed the colocalization of two delivery components in the cytoplasm proving the enhanced-internalization. Cell pellet phantom MRI study revealed a significant drop of T_1 contrast (Figure 3, a). *In vivo* Kodak multispectral images (Figure 3. b and c) exhibits 33% enhanced tumor uptake of second delivery component. After 24 h, 8.5% drop of T_1 contrast was observed in the tumor site. Cytotoxic study showed IC_{50} values (with respect to paclitaxel concentration) of 0.247 μM for control 1, 0.136 μM for control 2, 0.156 μM for treated 1, and 0.101 μM for treated 2. Comparing two controls and two treated samples, we observed the enhanced cytotoxic effects in two-component delivery system. Albumin conjugated with four drug molecules exhibited less cytotoxic effect than albumin with two drugs. We suggest this observation is due to the steric effect of large paclitaxel molecules for bioorthogonal click reactions or solubility issues of hydrophobic paclitaxel molecules. Hence, two component delivery strategy based on $T(PEG_4\text{-}Az)_8(CF\text{-}680)_2$ and $A(DIBO)_{10}(Pac)_2(Gd\text{-}DTPA)_6(CF\text{-}750)_2$ is a promising system for image-guided drug delivery to treat HER2/*neu* positive cancers.

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References: (1) Zhu W. et al, Cancer Biol Ther 2007;6:1960-6. (2) Musolino, A. et al, J Clin Oncol 2008;26(11):1789-96.