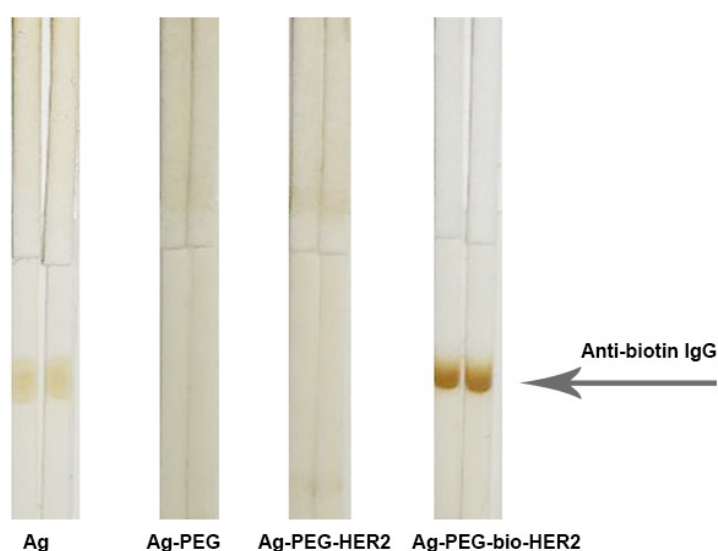


# Supplementary Materials: Photothermal Therapy with HER2-Targeted Silver Nanoparticles Leading to Cancer Remission

Victoria O. Shipunova, Mariia M. Belova, Polina A. Kotelnikova, Olga N. Shilova, Aziz B. Mirkasymov, Natalia V. Danilova, Elena N. Komedchikova, Rachela Popovtzer, Sergey M. Deyev, Maxim P. Nikitin



**Figure S1. Immunochromatography assay on the conjugation efficiency.** The anti-biotin IgG was applied to the nitrocellulose test strip. The strips were placed into the tube with: unmodified Ag NPs, PEG-modified AgNPs, AG-PEG-HER2 particles, and Ag-PEG-bio-HER2 particles (modified with biotin-labeled affibody).

## Supporting Note 1

### Methods

Affibody labeled with biotin was prepared as follows. 130  $\mu$ L of affibody  $Z_{HER2:342}$  in PBS at 1 g/L was mixed with 10  $\mu$ L of biotin-NHS ester (Thermo Fisher, USA, EZ-Link NHS-Biotin) in DMSO at 8 g/L and incubated for 2 h at room temperature. The excess of unreacted molecules was removed with Zeba Spin Desalting Columns 7 kDa MWCO (Thermo Fisher, USA) according to the manufacturer's recommendations. The concentration of protein was determined with the BCA protein assay. The conjugation of biotin-labeled affibody with Ag-PEG particles was performed absolutely in the same way as for pristine affibody.

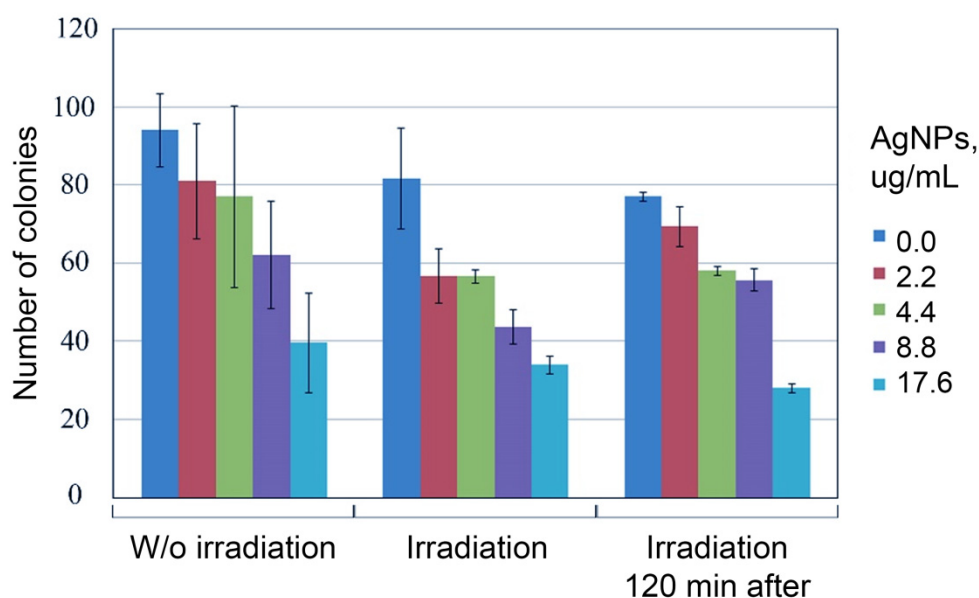
For immunochromatography test, the 2 mm test strip (MDI, # LKDFXXX060X260X 90CNPH-N-SS40-L2-P25) was applied with 1  $\mu$ L of anti-biotin IgG (IgG Fraction Monoclonal Mouse Anti-Biotin, Jackson ImmunoResearch) at 1.2 g/L and air-dried. Next, the strip was placed into the tube with 40  $\mu$ L Tris, 1% BSA, 0.05% Tween-20 and 2  $\mu$ g of test particles. The particles were allowed to migrate until the all liquid was absorbed on absorbent pad. The strips were imaged with a smartphone camera.

### Results

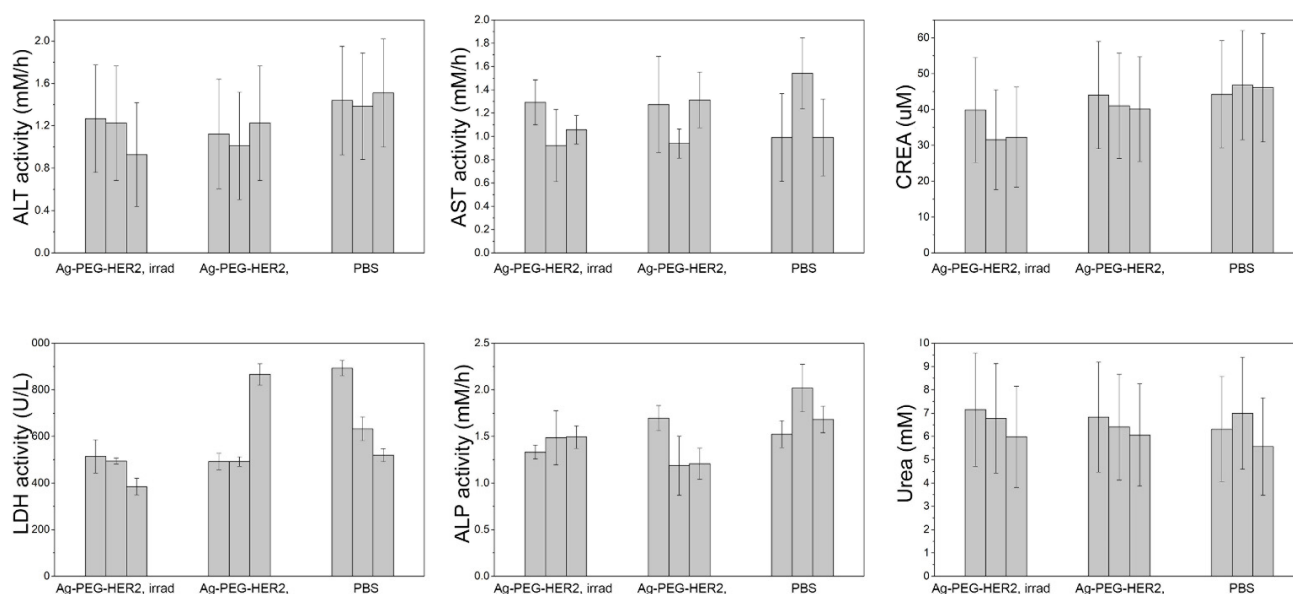
To validate the presence of protein – affibody  $Z_{HER2:342}$  on the nanoparticle surface, we performed a lateral flow assay with immunochromatography strips coated with anti-biotin IgG and particles conjugated to the biotinylated affibody, namely, Ag-PEG-bio-HER2.

The data presented in Fig. S1 demonstrate that uncoated Ag NPs possess slight non-specific binding to anti-biotin IgG. However, this non-specific binding is successfully shielded by modification of nanoparticle surface with PEG (ag-PEG). The affibody-conjugated Ag-PEG-HER2 particles do not exhibit any binding to anti-biotin

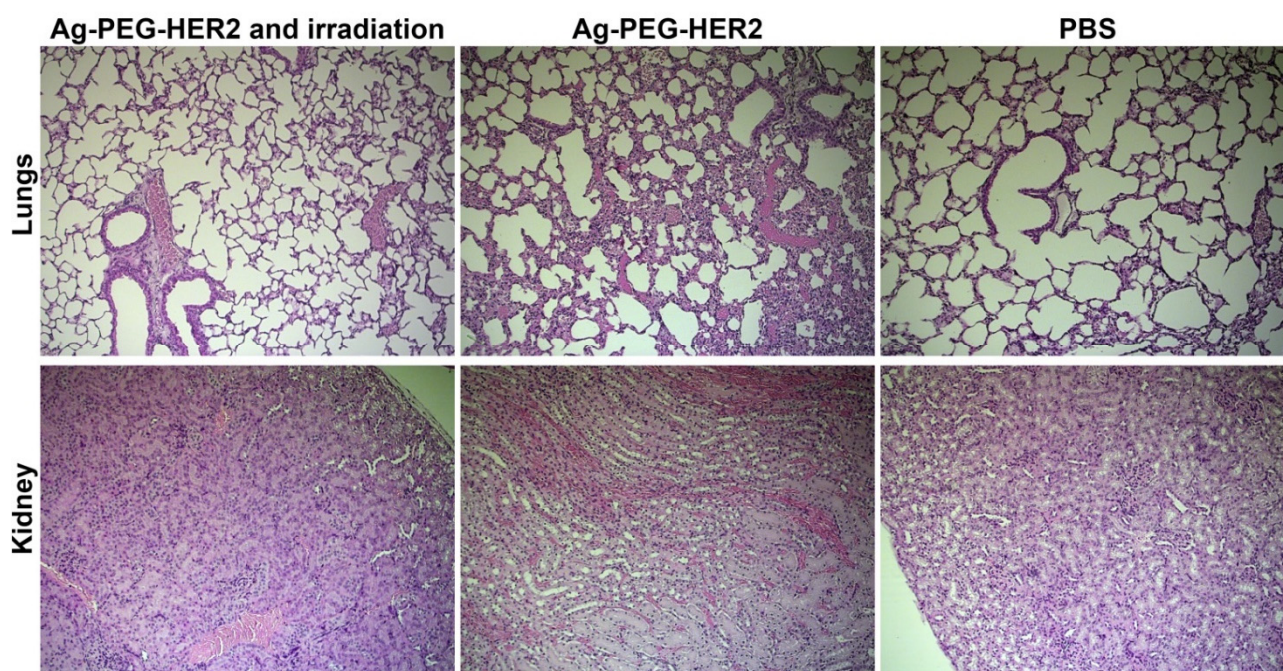
IgG. And only the biotin-affibody conjugated Ag-PEG-bio-HER2 particles interact specifically with the anti-biotin IgG thus forming a strong absorbing line on the test strip thus confirming the efficiency of conjugation.



**Figure S2. Clonogenic assay.** The number of SKOV3-lip cell colonies depending on the Ag-PEG-HER2 concentration and irradiation options with an LED matrix at a power of 95 mW/cm<sup>2</sup>.



**Figure S3. Safety aspects of Ag-based PTT.** The levels of various blood biochemical parameters after the Ag-PEG-HER2 treatment in three experimental groups that received the injections of i) group 1 – Ag-PEG-HER2 nanoparticles followed with light irradiation, ii) group 2 – Ag-PEG-HER2 nanoparticles, iii) group 3 – PBS (control group). Liver function was determined by serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Nephrotoxicity was assessed by creatinine (CREA) and urea levels. Cardiac damage was assessed by lactate dehydrogenase (LDH) activity. Alkaline phosphatase (ALP) activity is an indicator of hepatobiliary or bone damage.



**Figure S4. Histology study.** Hematoxylin and eosin staining of lungs and kidneys of mice from three experimental groups that received the injections of i) group 1 – Ag-PEG-HER2 nanoparticles followed with light irradiation, ii) group 2 – Ag-PEG-HER2 nanoparticles, iii) group 3 – PBS (control group). Magnification, 100x.