Supplementary Methods and Figures

Conjugation of anti-CD47-IR700

Water soluble, silica-phthalocyanine derivative, IRDye700DX NHS ester was obtained from LI-Cor Biosciences. Anti-CD47 monoclonal antibody (B6H12) was obtained from Bioxcell (Catalog Number BE0019-1). Conjugation of IRDye700DX to the monoclonal antibody was performed per the manufacture's protocol (13). In brief, anti-CD47 (1.0 mg/ml) was incubated with IRDye700DX NHS ester (8 µg in 0.1 M Na_2HPO_4 (pH 8.6)) at room temperature in the dark for 2 hours. Free dye was removed by purification with a Zeba® desalting column. Antibody protein concentration was determined with Coomassie Plus protein assay kit (Thermo Fisher Scientific Inc.) by measuring the absorption at 593nm with UV-Vis spectrophotometer (Thermo Fisher Scientific) as well as by mass spectrometry. Conjugated antibody was analyzed by SDS-PAGE on a 4-15% gradient gel (Life Technologies) with unlabeled anti-CD47 and protein molecular weight markers (Crystalgen Inc.). After electrophoresis, the gel was imaged with a Pearl Imager (LI-Cor Biosciences) using a 700-nm fluorescence channel. The gel was stained with Colloidal Blue (Bio-Rad, Hercules CA) to visualize the antibodies and standards.

The antibody-dye labeling ratio was determined by electrospray ionization mass spectrometry (ESI-MS) on a Agilent 1260 HPLC and Bruker MicroTOF-Q II as previously described (23). The column used was a Waters MassPREP 5x2.1mm diphenyl desalting column, at 50°C, with a flow rate of 0.3ml/min. The injection volume was 5µL. Spectra were collected in full scan MS mode with a mass range of 900-4000 Da and collision RF setting of 1200 Vpp.

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Western blot to confirm CD47 expression in cell lines

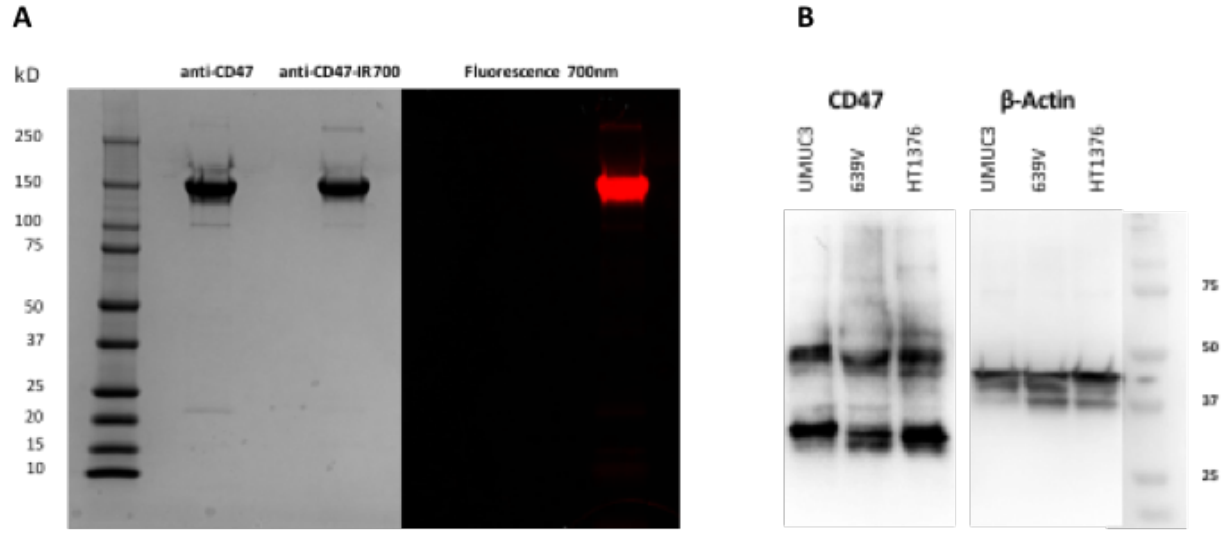
For western blot analysis, UMUC-3, HT1376 and 639V cells were cultured as described in the manuscript. Cells were lysed in RIPA buffer (Santa Cruz Biotechnology) and lysate protein concentration determined using the Bio-Rad DC protein assay. For each cell line 20 µg total protein was separated by SDS-PAGE on a 10% mini-protean TGX gel (Bio-Rad) and transferred

to a PVDF membrane. The blot was blocked in TBST, 3% BSA, then incubated overnight at 4ºC with 1/5000 anti-CD47-HRP (Abcam, ab199520) in TBST, 3% BSA. The antibody was detected using Clarity Western ECL substrate (Bio-Rad) and imaged on a ChemiDoc XRS+ molecular imager (Bio-Rad). After CD47 detection the blot was stripped of antibody and reprobed with anti-beta Actin (Abcam, ab20272) at 1/5000 in TBST, 3% BSA. Anti-beta Actin was detected as described for anti-CD47.

Characterization of anti-CD47–IR700

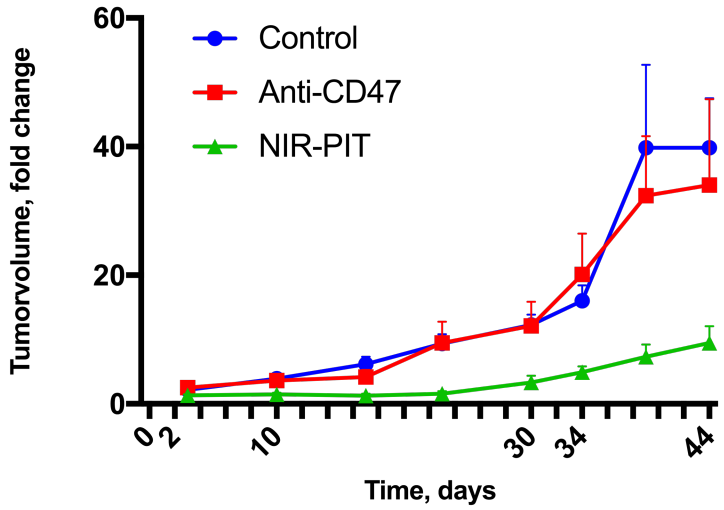
Antibody labeling efficiency for conjugation of near-infrared dye, IRDye700 (LI-Cor Biosciences) to the anti-CD47 monoclonal antibody (B6H12) was determined. Previously, B6H12 was validated in in vitro phagocytosis assays (40) and has previously been labeled with a quantum dots for a molecular fluorescence imaging of human bladder cancer (21). SDS-PAGE of the anti-CD47-IR700 demonstrated similar molecular weight and electrophoretic mobility as the unlabeled anti-CD47 (Supplementary Figure 1A). Near-infrared fluorescence imaging of the gel confirmed the conjugation of the IR700 dye to anti-CD47 (Supplementary Figure 1B). To determine the ratio between the antibody and dye, electrospray ionization mass spectrometry (ESI-MS) was applied to the labeled and unlabeled antibody. The comparison of deconvoluted labeled versus deconvoluted unlabeled anti-CD47 showed an antibody-dye ratio of 1:1 in 50%, 1:2 in 35%, and 1:3 in 15% and a labeling efficiency of 70% (data not shown).

Supplementary Figure 1



Supplementary Figure 1. Conjugation of near-infrared dye IR700 to anti-CD47. (A) SDS-page using Colloidal blue verified the molecular weight of the labeled antibody at 155kD. This molecular weight perfectly corresponds to the reported molecular weight of unlabeled anti-CD47 which also was used as unlabeled control. Conjugation of IR700 to the anti-CD47 was verified using the 700nm fluorescence channel of the Pearl imager. The anti-CD47/IR700 ratio was verified using mass spectrometry. **(B)** Western blot of three human bladder cancer cell lines. The CD47 protein was apparent as both monomer (lower band) and dimer (upper band).

Supplementary Figure 2



Supplementary Figure 2. Decrease in tumor volume following repeated rounds of anti-CD47-IR700-mediated NIR-PIT in xenograft mouse model of human bladder cancer. Data derived from the same *in vivo* experiments using 639V mouse xenograft model shown in Figure 6. Tumor volumes were calculated by the formula: *length (mm) x width (mm)*^{2} *x 0.5*. The animals were divided into 3 groups: 1) No treatment controls (n=7); 2) Anti-CD47 only (n=7); and 3) NIR-PIT receiving weekly tail vein injection of anti-CD47-IR700 followed by NIR-irradiation (n=7). On Day 34, there were 1 death in the control group and 2 in the anti-CD47 group. On Day 39, there were 3 deaths in the control group and 4 in the anti-CD47 group. On Day 44, there were 5 deaths in the control group and 5 in the anti-CD47 group. Quantitative measurement of tumor volume showed significantly larger tumor growth in control and anti-CD47 groups, compared to the NIR-PIT group.