Bioresponsive hydrogel embedded dark-gold nanoswitch to sense and overcome cancer multidrug resistance

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Statement of Purpose: Multidrug resistance (MDR) in cancer cells is a substantial limitation to the success of chemotherapy. Here, we describe facile means to overcome resistance by silencing the multidrug resistance protein 1 (MRP1), prior to chemotherapeutic drug delivery *in vivo*. Our platform contains hydrogel embedded with dark-gold nanoparticles modified with 5-Fluorouracil (5FU)-intercalated nanobeacons that serve as an ON/OFF molecular nanoswitch triggered by the increased MRP1 expression within the tumor tissue microenvironment. This nanoswitch can sense and overcome MDR prior to local drug release. These nanobeacons comprise a 5-FU intercalated DNA-hairpin, which is labeled with a NIR dye and a dark-quencher. The nanobeacons are designed to open and release the intercalated drug only upon hybridization of the DNA hairpin to a complementary target, an event that restores fluorescence emission due to nanobeacons conformational reorganization. Despite the cross-resistance to 5-FU, over 90% tumor reduction is achieved *in vivo* in a triple negative breast cancer model following 80% MRP-1 silencing, compared to the continuous tumor growth following only drug or nanobeacons administration. Our approach can be applied to reverse cross-resistance to many available drugs and restore treatment efficacy.

Methods: Synthesis of gold nanoparticles- 10 nM of the bare-gold nanoparticles were mixed with 0 PEG solution in an aqueous solution of SDS. Synthesis of Dark-Gold Nanobeacons- we prepared three different sequences of Gold Nanobeacons: a nanobeacon anti-MRP1 (detects and inhibits MRP1 mRNA), a nanobeacon anti-Luc (internal control, hybridizes with luciferase mRNA and releases the drug, but does not target MRP1) and nanobeacon nonsense (does not hybridize). The platform contains quenching molecules that are designed to restore emission upon conjugation to the target and drug release. Hydrogel platform- Dendrimer:dextran hydrogel was dopped with the nanobeacons-conjugated gold nanoparticles and was used to coat the tumor to enable local and sustained release of 5FU to tumor cells that have cross-resistance to this drug. Triple negative breast cancer model was developed to examine whether silencing MRP1 reverses the resistance to the drug, thus restoring drug efficacy.

Results: Fluorescently-labeled scaffolds loaded with dark-gold nanobeacons (Figure 1a) were implanted adjacent to the mammary fat pad of tumor-bearing mice. Hydrogel (Figure 1b, green) was embedded with nanobeacons (Figure 1c, red), and xenografts followed in vivo (Figure 1**d-e**) using IVIS for tumors implanted with hydrogels containing nanobeacon anti-MRP1 with and without 5-FU, nanobeacon anti-Luc with 5-FU and nanobeacon nonsense with 5-FU. Monitoring the change in tumor size as a function of time following treatment revealed a significant reduction in tumor growth ($n = 5$, $P < 0.005$) at day 14 after NP treatment (**Figure 1f**) with an almost complete tumor regression for nanobeacons anti-MRP1 loaded with 5-FU only. An approximately 90% decrease in luciferase activity (**Figure 4f**) and in tumor size were observed exclusively for the nanobeacons anti-MRP1 loaded with 5-FU treated tumors compared to anti-MRP1 only, anti-Luc loaded with 5-FU and nanobeacons nonsense with 5-FU ($n = 5$, $P < 0.005$). Nanobeacon fluorescence signal was OFF at day 0 (2 hours after surgery) and is turned ON at day 1 (24h), reaching a maximum intensity for MRP1 and luciferase detection at day 2 (**Figure 4g**).

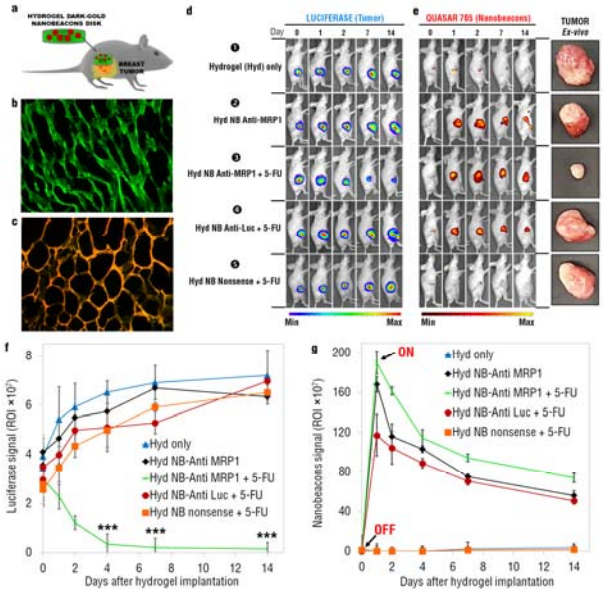

Figure 1. Hydrogel implanted in tumor-bearing mice (a) where hydrogel is labeled in green (b) and nanobeacons in red (c) show significant tumor regression when drug was released following silencing of MRP1 (d). Tumor size was followed (f) and nanobeacons release followed (g) to optimize the system.

Conclusions: This is the first proof-of-concept showing that hydrogels doped with dark-gold nanobeacons can be used to reverse the resistance of tumor cells to a chemotherapeutic drug. The system provides on-demand presentation of an inhibitor based on local MDR activity. This approach can be used to reverse the resistance to many other chemotherapeutic drugs to treat other types of cancer.