# Perfusion Changes following EGFR Targeted Hollow Gold-Nanoshell Mediated Heating of Tumors

**A. Shetty[1], M. P. Melancon[1], A. Elliott[1], Q. Huang[1], X. Wen[1], C. Li[1], J. D. Hazle[1], and R. J. Stafford[1]**

[1]The University of Texas M.D.Anderson Cancer Center, Houston, Texas, United States

**Introduction** Drug delivery into tumors has been variable due to factors like increased interstitial pressure, poor vasculature and tumor heterogeneity. This has hampered the clinical translation of many drugs which were successful in initial *in vitro* experiments. To overcome this obstacle, researchers have used hyperthermia protocols to improve tumor drug uptake with good results. But lack of reliable focal heating and monitoring capabilities have limited its widespread use. The goal of this study was to investigate the effects of EGFR targeted hollow-gold nanoshell ( HGNSs ) heating as monitored with MR temperature imaging on vascular permeability and perfusion as observed by dynamic contrast MRI and Doppler microultrasound as well as accumulation of a drug surrogate (PG-Gd-NIR813) in the tumor after heating. It utilizes MR temperature imaging for heat monitoring, EGFR targeted nanoparticles for tumor specific heating, dynamic contrast enhanced MR (DCE-MRI) and Doppler ultrasound (dUS) to image perfusion changes and fluorescence imaging for tracking drug uptake in tumors.

**Materials and Methods**: EGFR (+) A431 tumors were grown subcutaneously in both thighs of nude mice (n=25,20-25 g; Harlan Sprague Dawley, Indianapolis, IN) by injecting $1 \times 10^6$ viable tumor cells suspended in PBS. HGNSs (~40 nm in diameter, peak absorbance ~810 nm) were conjugated with C225, a monoclonal antibody directed against EGF receptors. The nanoparticles were injected intravenously when the tumors were ~ 8mm in diameter. Twenty four hours after the injection of C225-HGNSs, mice were irradiated with NIR range laser light (808 nm at 4W/cm$^2$ for 3 min). Magnetic resonance temperature imaging (MRTI) was performed by a complex phase-difference technique with a fast 2-D RF-spoiled gradient-recalled echo sequence (TR/TE = 49.5 ms/ 20 ms, flip angle = 30, bandwidth = 9.62 kHz). DCE-MRI and dUS were used to measure changes in tumor perfusion and vascularity (n = 5/group) after heating. For dynamic DCE studies, an axial 2D fast RF-spoiled gradient-echo acquisition with gadolinium-DTPA (Gd-DTPA Magnevist, Schering, 1:5 dilution in saline, dose: 2 l/gram body wt.) was used. Doppler studies were performed on a small animal system, Vevo 770 (Visualsonics Inc. Toronto, Canada). PG-Gd-NIR813, a dual MR/optical imaging agent used as a model polymeric drug, was injected intravenously at 5 min and 24 h after laser treatment to evaluate modulation of drug delivery. Uptake of PG-Gd-NIR813 in the tumor was measured by near-infrared fluorescent optical imaging.

**Results:** MRTI of mice treated with C225-HGNSs plus laser showed an average increase in temperature of 30.6ºC; while mice treated with saline plus laser (control) had an increase of 15.5ºC. DCE-MRI of mice injected with C225-HGNSs demonstrated a 3-fold increase in vascular perfusion compared with the control. Ultrasound studies revealed a 4-6 fold perfusion increase in C225-HGNSs + laser group as compared to the control. A significant increase in tumor uptake of PG-Gd-NIR813 was observed in the group of mice injected with PG-Gd-NIR813 at 5 min after laser treatment as compared with the group received PG-Gd-NIR813 at 24 h after laser treatment. No perfusion difference (before and after heating) was noted in control group. At 24 hrs, extensive necrosis was noted in C225-HGNSs + laser group.

**Conclusion:** Our data indicates that the early increase in vascular perfusion mediated by C255-HGNSs through laser treatment enhances the delivery of surrogate agent to the tumors. Synergistic utilization of MRTI, DCE-MRI and dUS enabled effective spatiotemporal monitoring of temperature and perfusion changes. Use of C225-HGNS was important for controlled heating of tumors and PG-Gd-NIR813 allowed clear visualization of surrogate agent uptake. Vascular shutdown at 24 hrs indicated that this heating protocol could be devised to improve drug uptake and enhance its tumor retention time. Future studies with an actual polymeric drug PG-TXL (paclitaxel) will evaluate the effectiveness of the hyperthermia protocol on tumor regression.

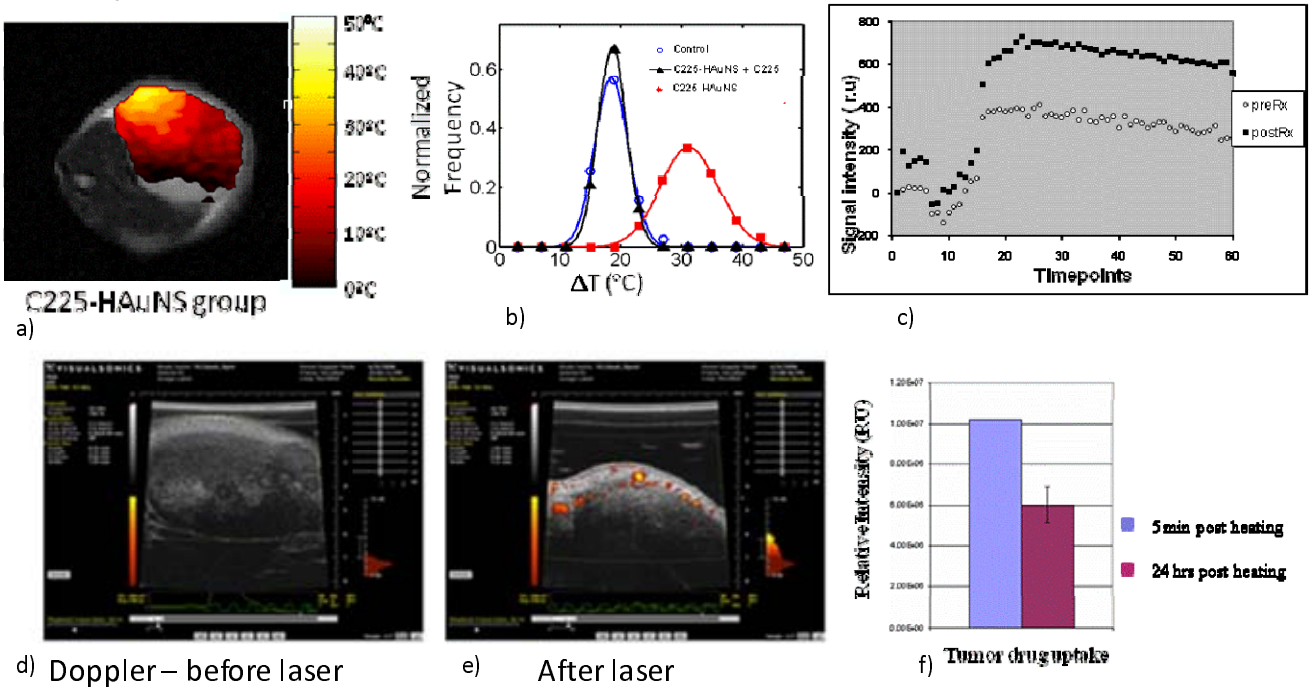


**FIGURE:** a) Maximum temperate image of a tumor in the C225-HAuNS group b) Temperature histograms of C225-HAuNS , C225 blocked and control groups with C225-HAuNS showing ~30 C rise c) Contrast uptake plots showing increased contrast after heating d) & e) Pre and post heating doppler images showing increased perfusion after laser heating f) Bar diagram demonstrating the higher drug uptake in the group , where the drug was injected within 5 min of heating