AcidoCEST MRI was immediately administered IV and an infusion pump was completed before a 200 µL bolus of Iopamidol (370 mgI/mL) acidoCEST MRI scans (using the parameters above) in a total endogenous CEST signals after acquiring four pre-injection localize flank tumor. We then obtained a spectrum of ranging from -11 to 11 ppm with four ranges: -11 to -3 ppm (5 µM with no additional spoiling, fat saturation pulses or respiration 3.0 second continuous wave radio frequency (RF) block pulse Pharmaceuticals (Phoenix, AZ, USA) was measured using an time.

We performed a multi-slice, spin echo MRI acquisition to localize flank tumor. We then obtained a spectrum of endogenous CEST signals after acquiring four pre-injection acidoCEST MRI scans (using the parameters above) in a total scan time of 13 min. One minute of baseline scans were completed before a 200 µL bolus of iopamidol (370 mg/mL) was immediately administered IV and an infusion pump was connected to the catheter line to deliver the agent at 450 µL/h for the remainder of the scan. Six post-injection acidoCEST MRI scans were conducted in a total scan time of 23 min.

PET Acquisition: 18F-PDGF purchased from PetNet Pharmaceuticals (Phoenix, AZ, USA) was measured using an Atomlab 300 from Biodex Medical Systems (Shreves, NY USA). Activity was diluted with 0.9% saline and measured until 4-12 MBq (0.1-0.32 mCi) reached with 250 µL total volume. 18F-PDGF was intravenously (IV) injected into the mice, and the tracer uptake was allowed to circulate for 45 minutes before initiating simultaneous PET/MRI experiments. A 1 min localization PET scan was performed to center the mice within the detectors such that the activity spans the entire axial FOV of the PET scanner (v3.0) from Cubic Inc. (Winnipeg, MB, Canada) prior to initiation of simultaneous 1 h (3600 s) PET and MRI acquisition.

Data Processing: All PET/MRI analysis was performed with Bloch fitting to measure concentration of agent (uptake) and extracellular pH (pHe) using custom written programs in MATLAB (version 9.1, R2016b, Natluck, MA, USA). PET images were reconstructed using Ordered Subset Maximum A Posteriori One-Step-Lap (OSEMAP) iterative algorithm. Post-processing to overlay PET and MR images was performed using VivOQuant (v3.0) provided by INVICRO Imaging Services and Software (Boston, MA, USA).14

Simultaneous PET/MRI images were performed using VivoQuant (v3.0) provided by INVICRO Imaging Services and Software (Boston, MA, USA). Supports PET/MRI to evaluate drug response.

AcidoCEST MRI monitors tumor response to metformin. Bloch fitting estimation of tracer uptake (A) and tumor extracellular pH (pHe) (B) one day before initiating chemotherapy, one day after initiating therapy, and 7 days after starting therapy. An increase in agent uptake was observed for mice treated with metformin, with a statistically significant difference between Day -1 and Day 7 for metformin-treated mice (p=0.03), with no significant change in uptake for vehicle treated mice. A decrease in pHe (p=0.02) was observed for both groups of mice, with a greater decrease in average pHe observed for the metformin-treated mice. The decrease in tumor pHe in vehicle-treated mice suggested an increase in tumor metabolism as the tumor continued to grow, which is expected. However, the greater decrease in tumor pHe after treatment with metformin was unexpected, because treatment that reduces metabolism should decrease lactic acid production and increase tumor pH. Therefore, another test is needed to determine if metformin treatment was effective in altering tumor metabolism.

CONCLUSIONS
This study demonstrated that simultaneous PET/MRI improves interrogation of tumor glycolysis. The decrease in pH was surprising because metformin reduces overall tumor metabolism, thereby reducing lactate production.1 If we only performed acidoCEST MRI, we would have erroneously concluded that the drug enhanced metabolism. The addition of 18F-PDG avoided this interpretation. Similarly, if we had only performed 18F-PDG studies, we would not have been able to identify the role of metformin on the full glycolysis pathway.

REFERENCES

ACKNOWLEDGEMENTS
Support was provided by NIH Grants R01 CA169774-01 and P01 CA95080. We also thank the Contrast Agent Molecular Engineering Laboratory (CAMEL) for their collegiality.

CETR CONTRAST AGENT
CETR effects from iopamidol (Isovue™, Bracco Diagnostics, Inc.; FDA approved contrast agent) are used to measure tissue pH via MRI. Iopamidol has 2 types of hydrogen atoms (protons) that can be selectively saturated, which causes the MR signal from these protons to disappear. The saturated protons exchange with neighboring water molecules, transferring the proton saturation to water, reducing the MR signal from water.

CETR MRI ANALYSIS WITH BLOCH FITTING
Representative z-spectra are shown (A) to depict the change in contrast before and after injection of 0.5 M iopamidol. Bloch fitting is performed on the raw CETR data (B). The Bloch fitting process is modified to include pH as a fitting parameter.

CONCLUSIONS
This study demonstrated that simultaneous PET/MRI improves interrogation of tumor glycolysis. The decrease in pH was surprising because metformin reduces overall tumor metabolism, thereby reducing lactate production.1 If we only performed acidoCEST MRI, we would have erroneously concluded that the drug enhanced metabolism. The addition of 18F-PDG avoided this interpretation. Similarly, if we had only performed 18F-PDG studies, we would not have been able to identify the role of metformin on the full glycolysis pathway.

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