Imaging breast cancer using hyperpolarized carbon-13 MRI

Ferdia A. Gallagher\textsuperscript{a,b,c,1}, Ramona Woitek\textsuperscript{k,d,1,2}, Mary A. McLean\textsuperscript{c}, Andrew B. Gill\textsuperscript{a}, Raquel Manzano Garcia\textsuperscript{c,e}, Elena Provenzano\textsuperscript{c,f,g}, Frank Riemer\textsuperscript{a}, Joshua Kaggie\textsuperscript{d}, Anita Chhabra\textsuperscript{b,1}, Stephan Ursprun\textsuperscript{b,1}, James T. Grist\textsuperscript{c}, Jeremy C. Daniels\textsuperscript{a}, Fulvio Zaccagna\textsuperscript{a}, Marie-Christine Laurent\textsuperscript{b}, Matthew Locke\textsuperscript{e}, Sarah Hiliborne\textsuperscript{c}, Amy Frarry\textsuperscript{a}, Turid Torheim\textsuperscript{c,e}, Chris Boursnell\textsuperscript{c,e}, Amy Schiller\textsuperscript{b}, Ilse Patterson\textsuperscript{b}, Rhys Slough\textsuperscript{b}, Bruno Carмо\textsuperscript{b}, Justine Kane\textsuperscript{b}, Heather Biggs\textsuperscript{b}, Emma Harrison\textsuperscript{b}, Surrin S. Deen\textsuperscript{a}, Andrew Patterson\textsuperscript{b}, Titus Lanz\textsuperscript{g}, Zoya Kingsbury\textsuperscript{b}, Mark Ross\textsuperscript{b}, Bristi Basu\textsuperscript{c}, Richard Baird\textsuperscript{d}, David J. Lomas\textsuperscript{a}, Evis Sala\textsuperscript{b,c}, James Wason\textsuperscript{l,m}, Oscar M. Rueda\textsuperscript{e}, Suet-Feung Chinn\textsuperscript{c,e}, Ian B. Wilkinson\textsuperscript{n}, Martin J. Graves\textsuperscript{a,b}, Jean E. Abraham\textsuperscript{c,f,k}, Fiona J. Gilbert\textsuperscript{a,b,c}, Carlos Caldas\textsuperscript{c,e,f,k}, and Kevin M. Brindle\textsuperscript{c,e,o}

*Department of Radiology, University of Cambridge, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{b}Department of Radiology, Addenbrooke's Hospital, Cambridge University Hospitals National Health Service Foundation Trust, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{c}Cancer Research UK Cambridge Centre, University of Cambridge, Cambridge CB2 0RE, United Kingdom; \textsuperscript{d}Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, 1090 Vienna, Austria; \textsuperscript{e}Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge CB2 0RE, United Kingdom; \textsuperscript{f}Cambridge Breast Cancer Research Unit, Addenbrooke’s Hospital, Cambridge University Hospital National Health Service Foundation Trust, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{g}Department of Histopathology, Addenbrooke’s Hospital, Cambridge University Hospitals National Health Service Foundation Trust, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{h}Pharmacy Department, Cambridge University Hospitals National Health Service Foundation Trust, Cambridge, United Kingdom; \textsuperscript{i}RAPID Biomedical GmbH, 97222 Rimpard, Germany; \textsuperscript{j}Medical Genomics Research, Illumina, Great Abingdon, Cambridge CB21 6DF, United Kingdom; \textsuperscript{k}Department of Oncology, University of Cambridge, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{l}Medical Research Council Biostatistics Unit, University of Cambridge, Cambridge CB2 0QQ, United Kingdom; \textsuperscript{m}Institute of Health and Society, Newcastle University, Newcastle-upon-Tyne NE2 4AX, United Kingdom; \textsuperscript{n}Department of Experimental Medicine and Immunotherapeutics, University of Cambridge, Cambridge CB2 0QQ, United Kingdom; and \textsuperscript{o}Department of Biochemistry, University of Cambridge, Cambridge CB2 0QQ, United Kingdom

Edited by Matthew G. Vander Heiden, Charles Koch Institute, Massachusetts Institute of Technology, and accepted by Editorial Board Member Tak W. Mak November 26, 2019 (received for review August 12, 2019)

Our purpose is to investigate the feasibility of imaging tumor metabolism in breast cancer patients using \textsuperscript{13}C magnetic resonance spectroscopic imaging (MRSI) of hyperpolarized \textsuperscript{13}C label exchange between injected [1-\textsuperscript{13}C]pyruvate and the endogenous tumor lactate pool. Treatment-naïve breast cancer patients were recruited: four triple-negative grade 3 cancers; two invasive ductal carcinoma mas that were estrogen and progesterone receptor-positive (ER/PR\textsuperscript{+}) and HER2/neu-negative (HER2\textsuperscript{−}); one grade 2 and one grade 3; and one grade 2 ER/PR\textsuperscript{+} HER2\textsuperscript{−} invasive lobular carcinoma (ILC). Dynamic \textsuperscript{13}C MRSI was performed following injection of hyperpolarized [1-\textsuperscript{13}C]pyruvate. Expression of lactate dehydrogenase A (LDHA), which catalyzes \textsuperscript{13}C label exchange between pyruvate and lactate, hypoxia-inducible factor 1 (HIF1\textsuperscript{α}), and the monocarboxylate transporters MCT1 and MCT4 were quantified using immunohistochemistry and RNA sequencing. We have demonstrated the feasibility and safety of hyperpolarized \textsuperscript{13}C MRI in early breast cancer. Both intratumoral and intratumoral heterogeneity of the hyperpolarized pyruvate and lactate signals were observed. The lactate-to-pyruvate signal ratio (LAC/PYR) ranged from 0.021 to 0.473 across the tumor subtypes (mean ± SD: 0.145 ± 0.164), and a lactate signal was observed in all of the grade 3 tumors. The LAC/PYR was significantly correlated with tumor volume (R = 0.903, P = 0.005) and MCT1 (R = 0.85, P = 0.032) and HIF1\textsuperscript{α} expression (R = 0.83, P = 0.043). Imaging of hyperpolarized [1-\textsuperscript{13}C]pyruvate metabolism in breast cancer is feasible and demonstrated significant intertumoral and intratumoral metabolic heterogeneity, where lactate labeling correlated with MCT1 expression and hypoxia.

Significance

Carbon-13 MRI was used to assess exchange of hyperpolarized \textsuperscript{13}C label between injected [1-\textsuperscript{13}C]pyruvate and the endogenous tumor lactate pool in breast cancer patients. Higher levels of \textsuperscript{13}C label exchange were observed in more-aggressive tumors, including all triple-negative cancers. The \textsuperscript{13}C label exchange correlated significantly with the expression of the transmembrane transporter mediating uptake of pyruvate into tumor cells and hypoxia inducible factor 1 (HIF1\textsuperscript{α}), but no significant correlation with the expression of lactate dehydrogenase, the enzyme that catalyzes the exchange. The study has shown that \textsuperscript{13}C MRI can be used for metabolic imaging of breast cancer patients in the clinic, creating possibilities for noninvasive cancer monitoring in this patient group.


Competing interest statement: A research agreement is in place between GE Healthcare and K.M.B. and F.A.G.

This article is a PNAS Direct Submission. M.G.V.H. is a guest editor invited by the Editorial Board.

This open access article is distributed under Creative Commons Attribution NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: RNA sequencing data are deposited at the European Phenome-genome Database (EGA ID EGAS000001044118) under a controlled license policy. The Data Access Committee can be contacted via radiology-13c-mri-breast@lists.cam.ac.uk. Imaging raw data and MATLAB scripts described in this manuscript can be obtained from radiology-13c-mri-breast@lists.cam.ac.uk.

1F.A.G. and R.W. contributed equally to this work.

To whom correspondence may be addressed. Email: nv585@cam.ac.uk.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1913841117/-/DCSupplemental.


Breast cancer accounts for ~25% of all cancer cases and is the leading cause of cancer death among women worldwide (1). Breast tumors show considerable heterogeneity, both within and between tumors, which partly accounts for the variable clinical course of the disease and response to treatment. Some of this heterogeneity is captured by hormone receptor expression and HER2 amplification/overexpression, which can be used to guide targeted treatment options. Genomic and transcriptional information can also indicate prognosis and may be used to select therapy pathways (2). Alterations in tumor metabolic pathways, which drive tumor growth, can influence treatment response but...
are not easily assessed in a routine clinical setting (3, 4). A clinical tool that measures spatial and temporal variations in tumor metabolism may further stratify patients in ways that are complementary to histological and molecular profiling.

A major metabolic change in most cancer types is a switch to aerobic glycolysis, known as the Warburg effect, which results in increased lactate formation (5, 6). Hyperpolarized 13C MRI (HP 13C MRI), which increases the MRI signal acquired from 13C-labeled substrates by more than 10,000-fold (7), is an emerging clinical tool that can be used to probe this altered metabolism. The most widely used hyperpolarized 13C-labeled substrate is [1,13C]pyruvate, which is the product of glycolysis, and lies at the intersection of several key metabolic pathways (8). Pyruvate is reduced to lactate in the reaction catalyzed by lactate dehydrogenase (LDH), which, in tumors, is predominantly the A isoform (LDHA) (9). The massive gain in sensitivity afforded by hyperpolarization means that the spatial distribution of intravenous (i.v.) injected hyperpolarized 13C-pyruvate and the hyperpolarized 13C-lactate formed from it, can be imaged in real time (10).

Preclinical studies have shown that the tumor metabolic phenotype revealed by hyperpolarized 13C-lactate labeling can reflect disease aggressiveness (11) and provide rapid assessment of treatment response, with multiple studies demonstrating an early reduction in 13C-lactate labeling following therapy (12, 13). MRI of hyperpolarized 13C-pyruvate metabolism has translated into the clinic with lactate labeling demonstrated in prostate cancer and brain tumors, and preliminary data show 13C-lactate MRI could be used for treatment response assessment in prostate cancer (7, 14, 15). The latter studies suggest that the technique could also be used to monitor the response of breast cancer patients to neoadjuvant treatment (NAT), which is used increasingly in routine patient management. NAT can be used to downstage the tumor, permitting breast-conserving surgery, improving operability, and allowing direct response assessment in situ. A complete pathological response (pCR) at surgery indicates a better prognosis, especially in estrogen receptor-negative breast cancer (16–19). Identifying effective treatments on an individual patient basis is essential to reduce both morbidity and cost, as ineffective NAT increases the time during which patients harbor chemoresistant tumor cells, thus increasing the risk of metastatic spread (20). Despite the increasing use of advanced multiparametric MRI in breast cancer patients undergoing NAT, there is an unmet need to develop more-specific and sensitive treatment response biomarkers to differentiate responders and nonresponders at an early time point. The aims of this study were, firstly, to demonstrate the feasibility of translating this imaging technique into the clinical breast cancer field; secondly, to metabolically phenotype breast cancers and evaluate which subtypes are most suitable for response assessment using HP 13C MRI; and, thirdly, to correlate lactate labeling with biological factors that may determine this labeling, such as expression of the monocarboxylate transporters, LDH, and markers of hypoxia and vascularity.

**Methods**

**Patient Recruitment.** Local research ethics committee approval was obtained for this prospective study (National Research Ethics Service Committee East of England, Cambridge South, Research Ethics Committee number 15/EE/0378; National Institute for Health Research [NIHR] portfolio number 30388). Seven women diagnosed with invasive carcinoma of the breast measuring at least 1.5 cm in maximum diameter on ultrasound or mammogram were consented between November 2016 and June 2018.

**Proton MRI.** Patients were imaged in a clinical 3T scanner (MR750; GE Healthcare). The MRI system inbuilt 1H body coil was used to acquire three-dimensional (3D) fast gradient echo scout images and, subsequently, T1-weighted axial and coronal fast spoiled gradient echo images, which were used to plan the 13C MRI. Following 13C MRI, diagnostic quality proton breast imaging was undertaken in the prone position in a dedicated eight-channel phased array receive-only breast coil (SI Appendix, Methods). For dynamic contrast-enhanced (DCE) MRI, a 3D fast spoiled gradient echo sequence with k-space data sharing was used (volume image breast assessment–time-resolved imaging of contrast kinetics [VIBRANT-TRICKS]) as described previously (21, 22) and reconstructed using an in-plane voxel-size of 0.68 × 0.68 mm (slice thickness = 1.4 mm). Gadobutrol (Gadovist; Bayer, Schering) was injected at 0.1 mmol/kg body weight and a flow rate of 3.0 mL/s followed by a 25-mL saline flush. In total, 48 VIBRANT-TRICKS volumes were acquired, over 8 min with a temporal resolution of 9.4 s. Contrast agent injection was started between phases 2 and 3.

**Preparation and Injection of 13C-Pyruvate.** Hyperpolarization of samples containing 1.47 g of [1-13C]pyruvic acid (Sigma Aldrich) and 15 mM electron paramagnetic agent (EPA; Synom) was performed in a clinical hyperpolarizer (SPINlab; ST Research Circle Technology) by microwave irradiation at 139 GHz at −0.8 K for ∼3 h followed by rapid dissolution in 38 mL of superheated sterile water and filtration to remove EPA to a concentration below ≤3 μM. The filtered formulation was neutralized with a buffer solution (SI Appendix, Methods). Sample pH, temperature, pyruvate and EPA concentrations, polarization, and volume were measured by the SPINlab quality control (QC) module to ensure appropriate QC criteria were obtained. Then 0.4 mL/kg of ~250 mM hyperpolarized pyruvate solution was injected at 5 mL/s using a power injector (Medrad) followed by a saline flush of 25 mL.

**Carbon-13 MRI.** A dedicated eight-channel 13C breast coil (Rapid Biomedical) was used. A 13C-labeled 8 M urea sample (Sigma-Aldrich), positioned adjacent to the tumor-containing breast, was used to set the 13C transmit gain and center frequency. Images were acquired using a dynamic coronal iterative Decomposition with Echo Asymmetry and Least squares estimation (IDEAL) spiral chemical shift imaging (CSI) sequence (SI Appendix, Methods and Table S1) (23). IDEAL spiral imaging data were reconstructed in MATLAB R2018b (The MathWorks, Inc.). Complex imaging data from the eight breast coil channels were combined as the square root of the sum of squares, with complex data were summed over all time points prior to coil combination to minimize noise propagation. Images at the pyruvate, pyruvate hydrate, lactate, alanine, and bicarbonate resonance frequencies were reconstructed with an in-plane voxel size of 1.6 × 1.6 × 1.9 mm2 and a slice thickness of 30 mm.

Tumor regions of interest (ROIs) were generated by thresholding the sum of the summed lactate and pyruvate signals using a MATLAB program developed in-house. The threshold was chosen for each tumor individually so that, when comparing thresholded 13C images and the DCE images, the diameter of the ROI on the 13C images matched the maximum diameter of the tumor on the coronal DCE images at peak enhancement. This semi-automated approach was validated by comparing the results with those obtained using ROIs drawn manually. There was a good correlation between the lactate-to-pyruvate signal ratio (LAC/PYR) obtained using ROIs drawn manually and those drawn using a semiautomated fashion and those drawn manually for LAC/PYR (P = 0.711), as shown by comparing the means of the two approaches. A potential bias introduced by drawing ROIs manually, the semi-automated thresholding approach was used for further analyses in this study. Estimation of noise (mean and SD) was performed using entire bicarbonate images, summed over the time course (or pyruvate hydrate, if there were visible artifacts in the bicarbonate image). The noise distribution in images of the different individual metabolites was expected to be the same, since all of the images were calculated from the same underlying set of multi-echo time data, which was confirmed empirically. Noise was best characterized from an entire image where spiral acquisition artifacts were absent. To generate the SNR for pyruvate and lactate (SNRmetabolite) within each tumor, the following formula was used:

$$\text{SNR}_{\text{metabolite}} = \frac{\text{mean } S_{\text{ROI tumor}} - \text{mean } S_{\text{noise}}}{\sqrt{2 SD\left(S_{\text{noise}}\right)}}$$

Mean $S_{\text{ROI tumor}}$ is the mean signal intensity in the tumor ROI; mean and SD of $S_{\text{noise}}$ were computed from the entire noise image series as described above. The factor of $\sqrt{2}$ accounts for the narrowed Rayleigh distribution of magnitude noise, with an approximate adjustment for the use of multiple receivers (24, 25). The summed metabolite SNR over the entire image time course is referred to as summed SNR_{PYR} and summed SNR_{LAC}. By dividing the latter by the former, the [1-13C]lactate/[1-13C]pyruvate SNR ratio (LAC/PYR) was calculated. As an alternative metric for assessing tumor metabolism of
that had low summed SNRLAC, the LAC/PYR was chosen as a more robust system (Leica Biosystems Newcastle Ltd) (fine Detection System (DS9800) in combination with their Bond automated RNA Sequencing.

percentage of positive tissue for MCT1 and MCT4 expression. staining were summed and divided by the total tissue area to obtain the provided in tissue was available for analysis in one patient.

formalin-fixed, paraffin-embedded tumor blocks using Leica carboxylate transporters 1 and 4 (MCT1 and MCT4) was performed on six patients, immunohistochemical (IHC) staining for the mono-

Immunohistochemistry and Quantification of Monocarboxylate Transporters 1 and 4. For six patients, immunohistochemical (IHC) staining for the monocarboxylate transporters 1 and 4 (MCT1 and MCT4) was performed on formalin-fixed, paraffin-embedded tumor blocks using Leica’s Polymer Re-

developed MATLAB software was used to generate B0-maps. MISTar (Apollo Medical imaging) was used to generate E-$\text{r}^{-}$ corrected T1 maps, to perform motion correction of the DCE-MRI data using a 3D affine model, and for pharmacokinetic modeling using the standard Tofts model (26). Tumor ROIs were drawn on the DCE-MRI data by a con-
sultant radiologist specialized in breast imaging with 10 y of experience (V8.5.2 Pixmeo SARL; OsiriX). These ROIs were used to calculate tumor vol-

In-house −developed MATLAB software was used to generate B0-maps. MISTar (Apollo Medical imaging) was used to generate E-$\text{r}^{-}$ corrected T1 maps, to perform motion correction of the DCE-MRI data using a 3D affine model, and for pharmacokinetic modeling using the standard Tofts model (26). Tumor ROIs were drawn on the DCE-MRI data by a con-
sultant radiologist specialized in breast imaging with 10 y of experience (V8.5.2 Pixmeo SARL; OsiriX). These ROIs were used to calculate tumor vol-

RNA Sequencing. RNA from frozen tumor tissue sections from six patients was extracted using the QIAGEN miRNeasy Mini Kit (catalog no. 217004; QIAGEN; details in SI Appendix, Methods). RNA quantification was performed using Nanodrop technology (ThermoFisher Scientific). Assessment of the RNA in-

RNA sequencing libraries were constructed using the TruSeq Stranded Total RNA Gold library preparation kit (Illumina). The libraries were se-

DNA methylation, smoking, and SUNAC were used as confounding variables, and Cox regression analyses were performed to estimate the hazard ratio

Statistical Analysis. The lactate signal is likely to be dominated by the intracellular compartment, particularly at early time points, whereas experi-

Results

The feasibility of using HP $^{13}$C MRI in breast cancer was demon-

Fig. 1. Spectra and images from the patient cohort. (Top) Spectra from a coronal dynamic IDEAL spiral CSI slice covering each tumor; the spectra have been summed over all 15 time points. (Bottom) Axial slices from the equivalent DCE-MRI data at the time point of maximum tumor enhancement. Abbreviations: ppm, parts per million; ER, estrogen receptor; PR, progesterone receptor; HER2, HER2/neu.
HER2− (one G2 and one G3); and four IC NST ER/PR− HER2− G3 (triple-negative breast cancer [TNBC]; ER and PR negativity defined as Allred score 0 to 3) (Fig. 1). Patient characteristics (age, body mass index, breast parenchymal density) are shown in SI Appendix, Table S2. No adverse effects were observed when the patients were monitored for 1.5 h after injection of the hyperpolarized agent. In all patients, HP^{13}C-lactate signal was observed exclusively in the tumors but not in other areas of the breast. Fibroglandular breast tissue demonstrated low HP^{13}C-pyruvate signal in some patients, whereas adipose breast tissue showed no signal. No other metabolite signals were observed in breast tissue.

**Intratumoral and Intertumoral Metabolic Heterogeneity.** Intratumoral metabolic heterogeneity was observed with variation in the summed LAC/PYR, summed SNRPYR, and summed SNRLAC. The LAC/PYR ranged from 0.021 to 0.473 (mean ± SD, 0.145 ± 0.164), summed SNRPYR ranged from 6.2 to 74.3 (43.8 ± 25.8), and summed SNRLAC ranged from −0.1 to 22.3 (6.5 ± 7.8) (Fig. 2). Hyperpolarized lactate signal was observed in all of the G3 tumors (TNBC and IC NST). There was no discernable lactate signal in the two G2 tumors despite detectable pyruvate in all seven tumors (Fig. 1). In addition, there was significant variation in the LAC/PYR and summed SNRLAC within the TNBC subgroup (ranges 0.031 to 0.473 and 6.5 to 22.3, respectively).

---

**Fig. 2.** Correlation of hyperpolarized {superscript}13C MRI data with tumor volume and expression of the MCT1 and HIF1α. (A) Each patient is represented by an individual point, with the size of each circle proportional to the respective tumor size. (B and C) Correlation of LAC/PYR and summed SNRLAC with tumor volume. (D–G) Correlation of LAC/PYR and summed SNRLAC with expression of MCT1, determined by both (D and E) IHC and (F and G) RNA sequencing. (H and I) Correlation of LAC/PYR and summed SNRLAC with expression of HIF1α determined by RNA sequencing. Abbreviations: IHC [% pos tissue], percentage of formalin fixed paraffin embedded tissue positive for IHC staining; RNAseq, normalized expression based on RNA sequencing.
Intratumoral metabolic heterogeneity was observed in the largest TNBC. The summed SNR_LAC and summed SNR_PYR were higher in the tumor periphery than in the tumor core, which was similar to the pattern of rim-like contrast enhancement on DCE-MRI, suggesting that the rate of pyruvate delivery to the tumor has a significant influence on lactate labeling. (Fig. 3).

Correlation of Lactate Labeling with Tumor Volume and MCT1 and HIF1α Expression. The summed SNR_LAC and LAC/PYR showed significant correlations with tumor volume (R = 0.974, P < 0.001 and R = 0.903, P = 0.005, respectively; Fig. 2 A–C). The LAC/ PYR was also significantly correlated with the expression of MCT1 on IHC (R = 0.85, P = 0.032), and the summed SNR_LAC was significantly correlated with MCT1 on RNA sequencing (R = 0.907, P = 0.013; Fig. 2 D–G). HIF1α expression determined by RNA sequencing was significantly correlated with the LAC/PYR (R = 0.83, P = 0.043; Fig. 2 H and I). However, a 42-gene RNA-based hypoxia signature that had been developed in breast cancer (30) did not correlate significantly with the LAC/ PYR (R = 0.39, P = 0.442) or summed SNR_LAC (rho = 0.23, P = 0.658; Fig. 2 F–I). There were no significant correlations between the LAC/PYR or summed SNR_LAC and MCT4 expression, where this was determined by IHC (rho = 0.54, P = 0.297 and rho = 0.14, P = 0.803, respectively) or by RNA sequencing (R = 0.41, P = 0.420 and rho = −0.54, P = 0.297, respectively; SI Appendix, Fig. S4 A–D), nor with the expression of LDHA determined by RNA sequencing (R = 0.439, P = 0.383 and rho = 0.257, P = 0.658, respectively; SI Appendix, Fig. S4 E and F).

Correlation of 13C MRI with DCE-MRI. A significant correlation was observed between ve and the LAC/PYR (R = 0.84, P = 0.035). However, this correlation was driven mainly by one tumor showing low ve and high LAC/PYR (SI Appendix, Fig. S3) and is thus unlikely to be representative of the entire cohort. No other significant correlations were observed between DCE parameters (Kt, kpe, ve, or IAUC90) and the LAC/PYR, summed SNR_PYR, and summed SNR_LAC.

Discussion

Previous clinical studies have demonstrated 13C MRI with hyperpolarized [1-13C]pyruvate in human prostate cancer and in a range of brain tumors (7, 14, 15). Here we investigated the metabolism of hyperpolarized [1-13C]pyruvate in breast cancer patients and demonstrated the feasibility and safety of the technique, as well as significant intertumoral metabolic heterogeneity. Hyperpolarized 13C-lactate signal was observed in the summed spectra from all of the TNBCs and in all of the higher-grade (G3) tumors, whereas there was no discernable hyperpolarized 13C-lactate signal in the two lower-grade (G2) tumors. This is consistent with previous preclinical studies in prostate cancer, which have shown higher lactate signal in more-aggressive tumors, and suggests that increased lactate labeling can be used as a biomarker for aggressive disease (11). In addition, we observed intratumoral heterogeneity in lactate labeling in a single large triple-negative breast tumor, where the level of lactate labeling was correlated with delivery of a gadolinium-based contrast agent. Extensive preclinical studies have shown that hyperpolarized 13C-pyruvate metabolism is frequently modulated following treatment (31). The high levels of lactate labeling in TNBC, which commonly undergo NAT, would make them suitable for response assessment using this technique.

Previous studies showing that intertumoral metabolic heterogeneity is more pronounced than intratumoral metabolic heterogeneity (32) and that core biopsy samples can be used to reliably assess intertumoral differences (33–37) provided us with a rationale for comparing global imaging-based metrics (mean LAC/PYR and mean summed SNR_LAC) with the results from IHC and RNA sequencing of single tumor biopsies. The strong correlation between the LAC/PYR with tumor volume, which is known to correlate with hypoxia (38), led us to investigate the contribution of hypoxia to the measured lactate signal. In tumors with a high LAC/PYR, there was a significant increase in HIF1α expression on RNA sequencing (R = 0.83, P = 0.043) (SI Appendix, Fig. S4), suggesting that hypoxia may account for the correlation between LAC/PYR and tumor volume.

Metabolic reprogramming of cancer cells is often the downstream effect of oncogene activation or deletion of tumor suppressor genes (39–41). MCT1, which imports pyruvate into cells, and LDHA, which catalyzes hyperpolarized 13C label exchange between the injected pyruvate and endogenous lactate pool, can be up-regulated by the transcription factors HIF-1α and c-Myc, either constitutively, such as following activation of the
The lack of correlation between the other parameters tissue perfusion, the correlation with summed SNRPYR is to be expected. A number of studies have demonstrated that the tumor cell pyruvate transport can have a significant influence on 13C label exchange and the apparent exchange rate, including experiments on the effects of pyruvate concentration (44), cell lysis (45), modulations of LDH activity (45), and MCT1 inhibition (29), and a recent study in prostate cancer patients which analyzed MCT1 expression (46). Hyperpolarized 13C MRI was complementary to the DCE-MRI measurements, with a correlation between $k_{np}$ and the summed SNRPYR but no correlation between the other parameters derived from the DCE-MRI and the hyperpolarized 13C magnetic resonance spectroscopic imaging data. Since $k_{np}$ is related to tissue perfusion, the correlation with summed SNRPYR is to be expected. The lack of correlation between the other parameters suggests that the hyperpolarized [1-13C]pyruvate experiment reflects other aspects of tumor biology that are not captured by DCE-MRI. The combination of the two imaging strategies could be exploited for early response assessment, where quantitative DCE-MRI has demonstrated the potential to increase accuracy in the prediction of pCR compared to standard clinical MRI, but the results still depend on the molecular tumor subtype (47, 48). Response assessment using proton magnetic resonance spectroscopy (1H-MRS) has proven challenging in the breast, due to overlap of the lactate resonance with the intense lipid signals from adipose tissue (49). A recent multicenter study on the assessment of early treatment response in breast cancer using 1H-MRS showed very limited feasibility (50). Although uptake of the glucose analog 18-F-FDG imaged with positron emission tomography is widely used in oncological imaging for cancer detection, staging, and response assessment, ionizing radiation should ideally be minimized in women of reproductive age, and it is not used routinely in the assessment of primary breast cancer. In addition, it reflects cellular uptake via the glucose transporters and phosphorylation by hexokinase, but it does not allow the assessment of the downstream metabolism that can be probed with hyperpolarized [1-13C]pyruvate.

This study, although in a relatively small cohort, demonstrates the feasibility and safety of hyperpolarized 13C MRI in patients with early breast cancer and that metrics obtained from 13C MRI measurements of hyperpolarized [1-13C]pyruvate metabolism are correlated with the molecular characteristics of the tumors. Increased hyperpolarized [1-13C]lactate signal in larger and more-aggressive tumors appears to be driven by hypoxia, through increased MCT1 expression.

ACKNOWLEDGMENTS. This work was supported by a Wellcome Trust Strategic Award, Cancer Research UK (CRUK: Grants C8742/A18097, C19212/A16628, C19212/A211376, and C19/1A16465), the Austrian Science Fund (Grant J4025-B26), the CRUK Cambridge Centre, the CRUK & Engineering and Physical Sciences Research Council Cancer Imaging Centre in Cambridge and Manchester, the Mark Foundation for Cancer Research and Cancer Research UK Cambridge Centre (Grant C9685/A25177), Addenbrooke’s Charitable Trust, the National Institute for Health Research Cambridge Biomedical Research Centre, Cambridge Experimental Cancer Medicine Centre, and Cambridge University Hospitals National Health Service Foundation Trust.