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Advances in Brief

ECG Triggering in Ultra-High Field Cardiovascular MRI
Daniel Stäb, Juergen Roessler, Kieran O’Brien, Christian Hamilton-Craig, and Markus Barth

Image Report

Computed Tomographic Characterization of Traumastem—A New Oxidized Cellulose Hemostatic Agent
Paul B. Lewis, Scott T. Wilson, Dustin R. Kentala, John Barry, and Kevin M. Lewis

Research Articles

Gd and Eu Co-Doped Nanoscale Metal–Organic Framework as a \( T_1-T_2 \) Dual-Modal Contrast Agent for Magnetic Resonance Imaging
Geoffrey D. Wang, Hongmin Chen, Wei Tang, Daye Lee, and Jin Xie

An Overdetermined System of Transform Equations in Support of Robust DCE-MRI Registration With Outlier Rejection
Adam Johansson, James Balter, Mary Feng, and Yue Cao

A Classification System for the Spread of Polymethyl Methacrylate in Vertebral Bodies Treated with Vertebral Augmentation
Joseph Frankl, Michael P. Sakata, Gagandeep Choudhary, Seung Hur, Andrew Peterson, and Charles T. Hennemeyer

Noninvasive Electric Current Induction for Low-Frequency Tissue Conductivity Reconstruction: Is It Feasible With a TMS-MRI Setup?
Stefano Mandija, Petar I. Petrov, Sebastian F. W. Neggers, Peter R. Luijten, and Cornelis A. T. van den Berg

Combination of an Integrin-Targeting NIR Tracer and an Ultrasensitive Spectroscopic Device for Intraoperative Detection of Head and Neck Tumor Margins and Metastatic Lymph Nodes
Younghyoun Yoon, Aaron M. Mohs, Michael C. Mancini, Shuming Nie, and Hyunsuk Shim

Magnetic Resonance Imaging-Based Radiomic Profiles Predict Patient Prognosis in Newly Diagnosed Glioblastoma Before Therapy
Sean D. McGarry, Sarah L. Hurrell, Amy L. Kaczmarowski, Elizabeth J. Cochran, Jennifer Connelly, Scott D. Rand, Kathleen M. Schmainda, and Peter S. LaViolette

Hypoxia Imaging With PET Correlates With Antitumor Activity of the Hypoxia-Activated Prodrug Evosfamide (TH-302) in Rodent Glioma Models
Ashley M. Stokes, Charles P. Hart, and C. Chad Quarles

Erratum

Erratum: Wangerin et al. (2015)
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ECG Triggering in Ultra-High Field Cardiovascular MRI

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Abbreviations: Magnetohydrodynamic (MHD), electrocardiogram (ECG), cardiovascular magnetic resonance (CMR), vectorcardiography (VCG)

ABSTRACT

Cardiac magnetic resonance imaging at ultra-high field (B0 ≥ 7 T) potentially provides improved resolution and new opportunities for tissue characterization. Although an accurate synchronization of the acquisition to the cardiac cycle is essential, electrocardiogram (ECG) triggering at ultra-high field can be significantly impacted by the magnetohydrodynamic (MHD) effect. Blood flow within a static magnetic field induces a voltage, which superimposes the ECG and often affects the recognition of the R-wave. The MHD effect scales with B0 and is particularly pronounced at ultra-high field creating triggering-related image artifacts. Here, we investigated the performance of a conventional 3-lead ECG trigger device and a state-of-the-art trigger algorithm for cardiac ECG synchronization at 7 T. We show that by appropriate subject preparation and by including a learning phase for the R-wave detection outside of the magnetic field, reliable ECG triggering is feasible in healthy subjects at 7 T without additional equipment. Ultra-high field cardiac imaging was performed with the ECG signal and the trigger events recorded in 8 healthy subjects. Despite severe ECG signal distortions, synchronized imaging was successfully performed. Recorded ECG signals, vectorcardiograms, and large consistency in trigger event spacing indicate high accuracy for R-wave detection.

INTRODUCTION

Cardiovascular magnetic resonance (CMR) is an important and well-established clinical tool for the diagnosis and management of cardiovascular diseases, and it is the standard of reference for the evaluation of cardiac morphology and function (1–3). CMR must overcome the challenges introduced because of cardiac and respiratory motion. In the clinic, CMR relies on accurate cardiac gating alongside parallel imaging (4, 5), simultaneous multi-slice imaging (6–8), or other acceleration methods (9, 10) to address limitations due to motion. However, the fact remains that CMR must always make a tradeoff between spatiotemporal resolution and signal-to-noise ratio.

The signal-to-noise ratio gain inherent at higher field strengths (11) has recently led to an increased use of high field systems with B0 = 3 T for clinical CMR (12), and moreover, it has encouraged investigations into ultra-high field (B0 ≥ 7 T) CMR (13–15). Apart from enabling spatial resolutions that exceed today’s limits (16), CMR at ultra-high field offers new opportunities for magnetic resonance-based tissue characterization (17, 18) or metabolic imaging (19).

Cardiac gating is usually performed using electrocardiogram (ECG) triggering. In general, vectorcardiography (VCG)-based QRS detection algorithms (20) are used, which aim to detect the R-wave in their peak by recognizing the R-wave’s rising edge. However, ECG signal distortions from several effects have been challenging at ultra-high field. The interaction of the conductive fluid blood with the static magnetic field B0, for instance, induces a voltage perpendicular to B0 and the direction of flow that superimposes on the ECG signal (21). This so-called magnetohydrodynamic (MHD) effect is particularly large during the early systolic phase, when the blood is ejected from the left ventricle into the aortic arch. Hence, it mainly affects the T-wave of the ECG signal (22–24). The probability that a rising edge of an MHD artifact is similar to the rising edge of the R-wave is generally nonzero. Consequently, deteriorated cardiac synchronization is likely in the presence of strong MHD artifacts that are similar to the R-wave’s rising edge. Problems have been observed at clinical field strengths such as 3 T (25, 26), and because the MHD effect scales with B0, distortions have been reported to be worse at ultra-high field (27–29). In addition, the time-varying magnetic gradient fields, which induce voltage perturbations in the ECG leads, also distort the ECG signal. To avoid motion artifacts, the lengthening of scan times and scan repetitions that result from poor ECG triggering, the establish-
ment of a stable cardiac synchronization technique is essential to advance ultra-high field CMR.

Pulse triggering is often used in cases were the conventional ECG approach fails (26). However, being derived from softly shaped peaks in the pulse wave signal, the trigger events are subject to immanent enhanced jittering (28, 30), which commonly introduces trigger-related image artifacts. Because the trigger events are also delayed with respect to the R-wave, pulse triggering is unsuitable for modalities like myocardial tagging that require an accurate detection of the R-wave.

Doppler ultrasound (30) and acoustic trigger devices (28, 31) as well as self-navigation (32–34) and pilot tone navigation (35) have recently been explored as alternative tools to conventional ECG triggering. In addition, advanced ECG detection algorithms (36–40) have been proposed, and promising results have been shown in initial studies.

Here, we explore the technical capabilities of a 7 T research MRI system and state-of-the-art 3-lead ECG equipment for cardiac synchronization at ultra-high field. Our initial study shows that by including an appropriate ECG learning phase outside of the magnetic field, existing ECG trigger technology in 7 T research systems allows for generating stable and reliable ECG trigger signals.

**METHODOLOGY**

All measurements were performed on a noncommercial 7 T whole-body research MRI scanner (Siemens Healthcare GmbH, Erlangen, Germany) under institutional review board permission. The gradient system provided a maximum gradient strength of 70 mT/m and a slew rate of 200 T/m/s. A dedicated 7 T cardiac Tx/Rx array with 8 transmit and 32 receive channels (MRI Tools GmbH, Berlin, Germany) was used for radiofrequency transmission and signal reception. The coil array was operated in single-channel transmit mode. To improve the B₀ field homogeneity, third-order shimming was used. For all human in vivo experiments, written informed consent was obtained before the examination as approved by the local ethics committee.

**Cardiac Synchronization**

For cardiac synchronization, a 3-lead ECG trigger device (Siemens Healthcare GmbH, Erlangen, Germany) using wireless signaling was used in conjunction with the standard trigger algorithm provided by the device manufacturer. The basic properties of this algorithm are briefly described in the following paragraph. For a detailed description, we refer the interested reader to work by Frank et al. (41).

To ensure an accurate detection of the peak of the R-wave, the trigger algorithm learns the shape of the rising edge of the R-wave during an initial learning phase in both ECG channels. Learning is performed while the subject is lying on the patient table outside of the magnet bore, where the MHD effect is typically negligible.

Once learning is completed, the trigger algorithm continuously compares different derived entities (ie, derivatives, filtered versions of derivatives) [for details refer Frank et al.’s study (41)] of the incoming ECG signal with the corresponding entities of the learned shape in real time. The comparisons are mainly based on 2 filter functions. The first is a matched filter, which is widely used in telecommunications (42) and mathematically corresponds to forming the correlation of the 2 signals. The filter function is given by the following equation:

\[ m_j(t) = a_j \cdot \sum_{t=0}^{\Delta t} s_j(t - \Delta t + t) \cdot r_j(t) \]

where \( a \) is a normalization factor, \( \Delta t \) depicts the period of comparison, and \( j \) refers to the signal entity for comparison. The incoming and the reference signal entities are depicted by \( s(t) \) and \( r(t) \), respectively. Both signal entities are complex with the real and imaginary components derived from the 2 ECG channels. The second filter function sums up the squared differences between the incoming ECG signal and the learned signal shape according to the following equation:

\[ q_j(t) = b_j \cdot \sum_{t=0}^{\Delta t} |s_j(t - \Delta t + t) \cdot r_j(t)|^2 \]

with \( b \) accounting for normalization. Trigger events are initiated by thresholding the filtered signals, \( m_j \) and \( q_j \). In addition to using those filters, the angle of the VCG vector, which is spanned by the signal in the 2 ECG channels at each time instant, is used for R-wave detection. This angle criterion is implemented as a necessary, but not sufficient, condition for a trigger generation. The trigger algorithm showed an overall high performance at 1.5 T (43).

**In Vivo Measurements**

To evaluate the performance of the underlying ECG trigger algorithm, cardiac cine imaging at ultra-high field was performed in 8 healthy volunteers. Before starting the imaging procedure, ECG electrodes were placed onto the chest of the subject, following the instructions of the trigger device manufacturer, and in conjunction with a senior electrophysiologist cardiac scientist. In 2 volunteers, the chest hair was removed in the target area before electrode placement to ensure good coupling at the body/electrode interface. After positioning the subject on the patient table, the ECG trigger device leads were connected to the electrodes, which automatically started the learning phase of the ECG algorithm. A pulse sensor (Siemens Healthcare GmbH, Erlangen, Germany) was attached to the subject’s index finger as a backup trigger device. After finishing all other preparatory steps, the learning phase of the ECG algorithm was stopped to initiate the R-wave detection mechanism. The signals detected in both available ECG channels and the generated trigger events were recorded throughout all examinations.

Cardiac cine imaging was performed using a high-resolution breath-held ECG retro-gated segmented 2-dimensional spoiled gradient echo (FLASH) sequence with the following acquisition parameters: field of view = 360 × 270 (360 × 338) mm², matrix = 256 × 192 (256 × 240), slice thickness = 4.0 mm, echo time = 3.13 milliseconds, repetition time = 6.1 milliseconds, receiver bandwidth = 592 Hz/px, flip angle = 60°, phases = 20, segments = 7, and temporal resolution = 43 milliseconds. Parallel imaging was used with an acceleration factor of R = 2. All images were reconstructed online using GRAPPA with 34 reference lines for weight set calculation.
Qualitative Evaluations
The performance of the underlying trigger algorithm and the impact of the MHD effect onto the performance was qualitatively evaluated based on the recorded ECG signals. Signal time curves for the individual ECG channels were visually assessed. VCG plots were generated and examined to identify mistriggering. Finally, the time intervals between succeeding trigger events were analyzed to obtain an estimate on the amount of false positive and false negative trigger events.

RESULTS
A general overview of the MHD effect at ultra-high field can be gained from Figure 1A. It shows the change of the detected signal in one of the 2 ECG channels over several RR-intervals during the transition from outside into the isocenter of the magnet. Outside of the magnet bore, the signal is generally smooth and undistorted, and the R-wave is easy to distinguish as the highest peak. With increasing magnetic flux density, the MHD-related alterations of the ECG become increasingly pronounced and result in a significant distortion of the ECG signal at the magnet’s isocenter. In the depicted case, the distorted T-wave clearly exceeds the R-wave.

Individual ECG channels can be affected in different ways, as shown in Figure 1B, which compares the MHD effect on the signals of the 2 different ECG channels. To reduce the influence of inter-RR signal fluctuations, the ECG signals were averaged over 40 consecutive RR-intervals. The signals detected in both channels experience distortions at the isocenter of the magnet. However, only in channel 2 does the overall shape of the signal considerably change, and the R-wave is exceeded by the distorted ECG segments. Despite the significant impact of the MHD effect, the QRS complex is clearly identifiable in both channels and—as can be seen from the accurate alignment of the individual RR-intervals—has been accurately detected by the trigger algorithm for each of the displayed cardiac cycles.

Exemplary VCG plots obtained in 3 volunteers outside and at the isocenter of the magnet are given in Figure 2. In each vectorcardiogram, the ECG signal recorded in channel 2 is plotted against the signal measured in channel 1 over several RR-intervals. Associated trigger events are superimposed (black circles). The data were collected outside of the magnet bore (first row, blue), at the isocenter in the absence of gradient activity during free breathing (second row, red) and during a breath-held cine scan (third row, yellow). The MHD effect-related increase of the ECG signal variations within each RR-interval is apparent when comparing the vectorcardiograms obtained outside and inside of the magnet. In addition, the large magnetic field introduces considerable changes in the shape of the vectorcardiograms. Alterations can also be observed, when comparing data collected during free breathing and breath-hold periods. The characteristic VCG curves including the trigger events tend to be dispersed along the vertical axis, when obtained during free breathing (second row). As depicted by the enlarged section in Figure 2C (third row), gradient activity seems to introduce only tiny additional deflections in the VCG signal. For each subject, the location of the trigger events in the VCG plots is preserved in the vast majority of cases when exposing the subject to the ultra-high static magnetic field and dynamic gradient fields.

The accuracy of the trigger algorithm is shown in more detail in Figure 3. For one of the volunteers, ECG signal curves obtained outside of the magnet bore (Figure 3A) and at the magnet’s isocenter (Figure 3, B and C) are compared with each other. The MHD effect-related distortions of the ECG signal are clearly recognizable. Despite these distortions, trigger events are typically placed accurately. Only on rare occasions, false nega-
True and misplaced trigger events were observed. False positive events were extremely rare. This is the case even in presence of severe additional distortions (Figure 3C), which, in this case, can be attributed to enhanced inhaling and exhaling in preparation of a scan-related breath-hold. As seen in the enlarged section, the gradient activity of the scan causes smaller variations of the ECG signal. The start of the scan is marked by the dashed line.

An impression of the high trigger accuracy can also be gained from Figure 4, which depicts histograms of the time intervals between consecutive trigger events in 3 healthy subjects. While the width of the histogram peak reflects the variation of the subject’s heart rate, outliers indicate false positive trigger events and undetected RR-intervals. The spacing between almost all trigger events is in the range of a single RR-interval. Only a few counts are spread out along the horizontal axis of the histogram.

ECG-triggered cardiac cine imaging worked generally well with the used synchronization setup. Representative examples of cardiac cine images obtained at 7 T are depicted in Figure 5. Shown are diastolic and systolic time frames of a short-axis (top row) and a 4-chamber long-axis view (bottom row) of a healthy subject’s heart (see online Supplemental Video 1 and Video 2). The myocardial walls are well delineated, and the images are free of visible artifacts that could be related to unsuccessful ECG triggering. The long-axis views show slight signal inhomogeneities introduced by

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**Figure 2.** Vectorcardiograms obtained in 3 healthy subjects outside of the magnet (first row, blue), at the isocenter of the magnet during free breathing (second row, red), and during a breath-held cine acquisition (third row, yellow), each within a time-frame of 20 seconds (A–C). The depicted scales reflect the actual relative signal amplitudes between the channels. The generally small high-frequency signal variations induced by the imaging gradients can be seen in the enlarged section in the third row for subject (C).
destructive B1 interferences (arrows). Moreover, flow effects are pronounced because of the high flip angle used.

**DISCUSSION**

For the medical application of CMR, the accurate synchronization of the imaging protocol to the cardiac cycle is essential to achieve high image quality and accurate results in functional evaluations. In this initial study, we explored the applicability of existing and state-of-the-art 3-lead ECG trigger technology for cardiac synchronization at the ultra-high field strength of 7 T. Without using additional hardware, the underlying trigger algorithm generated reliable ECG trigger signals and provided the

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**Figure 3.** ECG signal over time measured in one of the volunteers outside of the magnet (A) and at the isocenter of the magnet (B–C) in the first (red) and second (blue) channel of the ECG. Trigger events (green vertical lines) are typically placed accurately at the peak of the R-wave. The larger distortions in (C) can be attributed to deep breathing preceding a breath-held cine acquisition. The enlarged section visualizes the start of the sequence (dashed line) and the effect of the imaging gradients on the ECG signal (arrows).

**Figure 4.** Histograms analyzing the apparent time interval between succeeding trigger events in 3 healthy subjects (A–C) outside (blue) and at the isocenter (red) of the magnet. The bins along the horizontal axis are separated by 100 milliseconds. For almost all events, the time elapsed with respect to the previous event is in the range of 1 RR-interval. Counts at considerably larger or shorter intervals indicate false negative and false positive triggering.
basis for high-fidelity cardiac imaging. Despite severe ECG signal distortions due to the MHD effect and breathing motion, synchronized imaging was feasible without severe disruptions in all healthy volunteers. The attached pulse sensor was not required in any case as a substitute trigger device, and significant synchronization-related image artifacts were not observed.

In general, ECG signal distortions can be introduced by various motions and imaging gradients. In this initial study, the distorting effect of imaging gradients turned out to be small, although, as expected, considerable distortions were caused by the MHD effect. Particularly irregular and, in some cases, severe distortions could be attributed to breathing and subject motion. Even in presence of these adverse effects, the utilized trigger algorithm allows for an accurate R-wave detection, provided a learning phase in the absence of the magnetic field has been executed.

In the absence of the large magnetic field, ECG triggering was found to be highly accurate with the used setup. In the presence of the ultra-high field, the MHD effect led to significant distortion of the ECG signal time curves in all volunteers. Nevertheless, the overall synchronization accuracy remained high. As indicated by the histogram analysis, the length of almost all RR-intervals is within the range of a single RR-interval. The relatively high accuracy can be explained by the fact that the QRS complex of the ECG is typically only marginally impacted by the MHD effect, and the used trigger algorithm relies on real-time detection of the shape of the rising edge of the R-wave, rather than signal thresholding of the R-wave. In this way, the misplacement of trigger events and the generation of false positive events is minimized. Consequently, even in cases where the distorted T-wave of the ECG clearly exceeds the targeted R-wave, accurate cardiac synchronization is feasible.

The generally high trigger accuracy is also recognizable in the calculated vectorcardiograms. The location and the spread of the trigger events were not significantly affected by the exposure of the subject to the high magnetic field, despite the considerable increase in signal fluctuations. Inside and outside of the magnetic field, the VCG plots show different characteristic patterns. Although the pattern change itself is governed by the MHD effect, the additional dispersion of this pattern (Figure 2) can be attributed to the breathing motion and the associated movement of the chest with the electrodes through the static magnetic field. In this study, imaging gradients did not have a large influence on the VCG signal. As shown in Figures 2 and 3, they only caused tiny and high-frequency signal deflections.

To achieve high trigger accuracy at ultra-high field, careful subject preparation and electrode placement are essential to ensure good connection of the electrodes with the subject’s skin and to achieve high input signals in all ECG channels. As depicted in Figures 2 and 3, gradient-induced ECG signal distortions can thus be kept small. A good preparation is particularly important for applications such as retro-gated cine CMR, where imaging gradients interfere with the QRS complex. Given the additional effect of breathing motion corrupting the ECG signal, properly instructing subjects on breathing techniques might be helpful in achieving accurate triggering. This is particularly the case for free-breathing imaging applications.

In conclusion, we have shown that reliable cardiac ECG triggering was achieved by fully exploiting the technical capabilities of the ultra-high field scanner and trigger equipment. Apart from conducting an appropriate ECG learning phase, further hardware or software modifications were not required. Thus, the presented approach is widely available and ready to use. Recently, published studies indicate that using a larger number of ECG leads, the use of specifically tailored trigger algorithms or combinations of both can be advantageous in terms of trigger accuracy (36-40). Based on this, we assume that the use of more leads or further refinements of the algorithm could improve our results. However, the use of a large number of leads also adds to patient discomfort and preparation times.

Within the scope of this initial study, only a limited number of healthy subjects could be examined. Thus, it is important to note that the overall number of RR-intervals that could be analyzed outside of the magnet bore is rather low. Moreover, it is well-known that the success of ECG triggering can be highly subject-dependent, and certainly, a larger number of subjects need to be examined in future work to fully reveal the performance of the trigger technology at hand. Apart from that, ECG triggering can be particularly challenging in patient cohorts with cardiac arrhythmia, where the MHD effect can be more severe and variable.

In conclusion, we have shown that reliable cardiac ECG triggering is feasible in healthy volunteers at ultra-high...
field by using a state-of-the-art 3-lead trigger device. The used trigger algorithm provided sufficient accuracy for high-fidelity cardiac cine imaging, despite severe ECG signal distortions due to the MHD effect. Future work will need to further evaluate the algorithm in larger cohorts and patients with cardiac arrhythmia. Apart from CMR, other ultra-high field imaging applications such as human brain functional MRI with physiological noise correction may benefit from the easy instrumental setup and robust ECG triggering.

Supplemental Materials

Video 1: http://dx.doi.org/10.18383/j.tom.2016.00193.vid.01
Video 2: http://dx.doi.org/10.18383/j.tom.2016.00193.vid.02

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Disclosures: KO and JR disclose being employed by Siemens Healthcare. JR holds a patent related to the work presented.

REFERENCES

Computed Tomographic Characterization of Traumastem—A New Oxidized Cellulose Hemostatic Agent

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Key Words: oxidized cellulose, gossypiboma, diagnostic errors, computed tomography, Surgicel

Abbreviations: Oxidized regenerated cellulose (ORC), computed tomography (CT), oxidized nonregenerated cellulose (ONC), subcutaneous (SC), magnetic resonance imaging (MRI)

Oxidized regenerated cellulose (ORC) is a commonly used surgical hemostatic agent. When retained at the surgical site, it is frequently misdiagnosed on postoperative computed tomography (CT) images as an abscess or a recurrent tumor. Oxidized nonregenerated cellulose (ONC) is a new, more effective version of ORC. It is more effective because of its unorganized fiber structure and greater material density, which may also alter its appearance on CT images relative to ORC. This image report compares the CT characteristics of ONC and ORC. A rabbit’s bilateral femoral arteries were punctured to model peripheral vascular surgery. ORC was used to treat 1 of the femoral artery punctures and ONC to treat the contralateral puncture. Noncontrast CT imaging was performed immediately following surgery (day 0) and on postoperative day 14. On day 0, both ORC and ONC were isoattenuating relative to muscle and hyperattenuating to fat, although ONC appears more homogenous. On day 14, neither ORC nor ONC was clearly identifiable. Thus, postoperative retention of ONC can obscure immediate postoperative CT interpretation and, similar to ORC, lead to an erroneous diagnosis of an abscess. By day 14, ONC retention may not obscure CT interpretation. In noncontrast CT imaging, ONC appears more homogeneous than ORC, but is otherwise indistinguishable. The greater homogeneity of ONC may be caused by the unorganized fiber structure or greater material density. Intraoperative use of ONC should be clinically investigated before radiographically diagnosing a postoperative abscess or recurrent tumor.

INTRODUCTION
The interpretation and diagnostic utility of postoperative computed tomography (CT) imaging can be complicated by the presence of absorbable biomaterials (1-5). A commonly misdiagnosed biomaterial is oxidized regenerated cellulose (ORC) (1). ORC is a widely used hemostatic agent in nearly all surgical specialties. ORC induces hemostasis by means of a primary local hemostytic action and secondary activation of platelets to form a temporary platelet plug (6). Although ORC can be removed once hemostasis is achieved, it can also be left at the surgical site and degraded through normal wound healing (7). In a recent prospective, blinded study investigating the interpretation of CT images of patients treated with ORC, only 2 of 18 patients (11%) were accurately diagnosed as being treated with ORC (8). Interpretation of the remaining 16 patients was inconclusive, or ORC was misdiagnosed as an abscess, a hematoma, a lymphocele or a collection with hydroaeric levels.

Oxidized nonregenerated cellulose (ONC) is an alternate form of oxidized cellulose that was first used clinically in 2011 (9). ONC lacks organized cellulose fibers and has higher material density, which can give it a different appearance from ORC on CT images. Unorganized cellulose fibers of ONC have been shown to provide superior hemostasis relative to ORC (6). As the use of ONC becomes more widespread in surgical procedures, understanding the imaging characteristics of ONC on CT will aid in accurate interpretation and diagnosis.

This image report describes the postoperative noncontrast CT appearance of ONC and compares it with that of ORC when used to treat a femoral artery needle puncture in a rabbit at 0 and 14 days after implantation.

MATERIAL AND METHODS
Oxidized Cellulose Hemostatic Agents
Tabotamp (Ethicon, Inc., Somerville, New Jersey), also known as Surgicel® in North America, is the ORC used in this study. Traumastem (Baxter Healthcare SA, Zurich, Switzerland), also known as Celstat in North America, is the ONC used in this study.
Animal Model

All animal activities were performed according to the United States Animal Welfare Act and The Guide for the Care and Use of Laboratory Animals in an institution accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International following Institutional Animal Care and Use Committee Approval.

A rabbit femoral artery puncture model mimicking peripheral vascular surgery was performed in a 2.8-kg New Zealand White female rabbit (6). A fentanyl patch was placed 1 day before surgery. On the day of surgery, acepromazine (0.1 mg/kg, subcutaneously [SC]), glycopyrrolate (0.01 mg/kg, SC), and ketamine (25 mg/kg, SC) were administered to the animal. Following tracheal intubation, anesthesia was maintained using isoflurane.

The femoral arteries were exposed and isolated. An RB-1 curved tapered needle with 1-cm of 5-0 polyglactin 910 suture was passed through each artery in a proximal-to-distal direction. The needle entrance and exit holes were allowed to bleed freely for 6 seconds and then treated with a single application of 2 layers of 3-× 3-cm oxidized cellulose.

ORC was applied to the right femoral artery and ONC to the left femoral artery according to a randomization procedure using 30 seconds of digital pressure. Once hemostasis was achieved, the incisions were closed in an identical, routine fashion. The focus of this investigation was postoperative imaging, so hemostatic success was not measured.

Following postoperative imaging on the day of surgery (day 0), the animal was recovered and received meloxicam once daily for 3 days (0.2 mg/kg, intramuscularly). On day 14, the same anesthetic protocol was used for reimaging. After imaging, the animal was euthanized with pentobarbital (50 mg/kg, intravenously).

CT Imaging

With the rabbit in the dorsal positon, noncontrast CT imaging was performed after surgery and 14 days postoperatively (Figure 1). These time points were selected based on previous research by Pampal et al. (10). A preoperative CT was not conducted, as the interest of this report was the appearance of the implanted material. CT imaging was performed using a multiple-detector-row CereTom™ scanner (NeuroLogica Corporation, Danvers, Massachusetts).

No intravenous contrast was administered so that the retained oxidized cellulose material can be identified without obscuration (ie, beam hardening or scatter) by the intimately adjacent intravascular iodine contrast (11). Contiguous 160-mm (day 14) and 260-mm (day 0) step-and-shoot data acquisition of the pelvis was conducted from the iliac crests to the feet with 140-kVp, 4-mAs, and a field of view radius of 126.656-mm. The images were acquired to minimize subject radiation dose to as low as reasonably achievable while maintaining diagnostic imaging quality. Coronal images were generated with 0.625-mm thickness.

RESULTS

On day 0, the oxidized cellulose hemostatic agents are seen medial to the femoral arteries with symmetrical beam attenuation and with the same attenuation as observed in the thigh muscles (Figure 2). The appearance of ORC in the right groin is more heterogeneous than that of ONC in the left groin. Surgical sutures are seen in both groins. There is minimal fat stranding and foci of air in the regions of interest, but these findings are to be expected in the immediate postoperative period with or without the use of hemostatic agents.

On day 14, neither of the oxidized cellulose hemostatic agents is definitively identifiable (Figure 3). No femoral pseudoaneurysms are appreciated in either groin. In comparison with the immediate postoperative images, there are expected changes of normal wound healing, including some skin thickening and reduction in local fat stranding. There are no foci of air along either femoral artery on the 14-day postoperative image. There are again focal hyperattenuating surgical sutures in both groins. No adverse tissue reactions are seen.

DISCUSSION

Based on this investigation, ONC has a more homogeneous appearance compared with ORC in noncontrast CT imaging immediately following surgery that is not identifiable on postoperative CT images on day 14. The difference between the oxidized cellulose on day 0 is likely because of the different density structures. The different presentations of oxidized cel-
Lulose over time, however, are not uncommon and represent their 2-phase degradation process. In the first phase, polyuronic acid is solubilized within 18 hours, whereas in the second phase, fibrous material is phagocytized and hydrolyzed by local macrophage within 4 weeks (6).

During the first phase, ORC can be misdiagnosed as a bowel leakage (12), obstructive mass (13), hematoma (14), mediastinitis (15) or, most frequently, abscess (16-19). These misdiagnoses are attributed to the appearance of ORC as a mixed- or low-attenuation mass with focal collections of gas that is absent of air–fluid levels and contrast enhancement in or near the operative site on immediate postoperative CT scans (1), which is similar to their appearance in this investigation. Given the comparable appearance to ORC, ONC can be misdiagnosed during the acute postoperative period. As oxidized cellulose is degraded or encapsulated, its appearance may vary. An interesting follow-up study should investigate its appearance more frequently in the postoperative period and in different anatomical locations (eg, intracranial, intrathoracic, or retroperitoneal sites). Further, serial observations in multiple animals or clinical patients should be used to investigate its varying presentations.

During the second phase, oxidized cellulose degradation and wound healing can be delayed depending on the amount of material used, blood flow, and tissue bed. When delayed too long, ORC forms a fibrous-encapsulated sterile granuloma, that is, a gossypiboma or “Surgiceloma.” In this phase, ORC is misdiagnosed as a tumor (20-24). This misdiagnosis is attributed to the appearance of ORC as a heterogeneous soft tissue mass with or without rim enhancement and with variable to no contrast enhancement. In this study, the quantity of ORC and ONC used was insufficient to form a gossypiboma. Because a gossypiboma was not induced, this is a potential direction for further investigation. Similarly, the appearance of ONC in contrast-enhanced CT imaging or less conventional modalities for soft tissue postoperative imaging (eg, plain radiography, ultrasonography, and magnetic resonance imaging [MRI]) can be investigated.

Oxidized cellulose has lower attenuation than a hematoma and a simpler appearance. Abscesses generally have scattered,
variably sized, and patterned foci of gas with air–fluid levels and rim enhancement (25). Such distinguishing CT characteristics are suggestive but not confirmatory. Radiographic and sono- graphic examinations do not aid in the differentiation of oxid- ized cellulose from the above misdiagnoses (26, 27). Because of superior contrast between tissues, MRI may provide a more definitive diagnosis of oxidized cellulose postoperatively. Oto et al. described ORC to be hypointense in the early postoperative period on T2-weighted images, but variable thereafter (28).

Pampal et al. performed an exhaustive study comparing attenuation of ORC with normal tissue on CT and signal intensity on MRI within the intraperitoneal and retroperitoneal cav- ities of guinea pigs (10). Based on their CT findings, ORC is consistently hypoattenuating relative to paravertebral muscles and liver parenchyma when placed in the intraperitoneal space and hyperattenuating relative to fat when placed in the retro- peritoneal space. In comparison with Pampal et al., this inves- tigation shows that ORC is hyperattenuating to fat and overall isoattenuating to thigh musculature. The difference between the findings can be because of variation in the imaging technique, radiation dose, and anatomic location between the studies.

CONCLUSION

Although the CT imaging characteristics of ORC have been described over several years, this is the first report of the non- contrast CT imaging characteristics of ONC. This investigation characterized the appearance of ONC in noncontrast CT imaging as being similar to that of ORC. The only distinction between the two was that ONC appeared more homogenous. The homogeneity of ONC is likely because of the unorganized fiber structure or greater material density.

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Recently, a growing interest has been seen in the development of $T_1$–$T_2$ dual-mode probes that can simultaneously enhance contrast on $T_1$- and $T_2$-weighted images. A common strategy is to integrate $T_1$ and $T_2$ components in a decoupled manner into a nanoscale particle. This approach, however, often requires a multi-step synthesis and delicate nanengineering, which may potentially affect the production and wide application of the probes. We herein report the facile synthesis of a 50-nm nanoscale metal–organic framework (NMOF) comprising gadolinium ($\text{Gd}^{3+}$) and europium ($\text{Eu}^{3+}$) as metallic nodes. These nanoparticles can be prepared in large quantities and can be easily coated with a layer of silica. The yielded Eu,Gd-NMOF@SiO$_2$ nanoparticles are less toxic, highly fluorescent, and afford high longitudinal ($38 \text{ mM}^{-1}\text{s}^{-1}$) and transversal ($222 \text{ mM}^{-1}\text{s}^{-1}$) relaxivities on a 7 T magnet. The nanoparticles were conjugated with c(RGDyK), a tumor-targeting peptide sequence, which has a high binding affinity toward integrin $\alpha_v\beta_3$. Eu,Gd-NMOF@SiO$_2$ nanoparticles, when intratumorally or intravenously injected, induce simultaneous signal enhancement and signal attenuation on $T_1$- and $T_2$-weighted images, respectively. These results suggest great potential of the NMOFs as a novel $T_1$–$T_2$ dual-mode contrast agent.

INTRODUCTION

Magnetic resonance imaging (MRI) is one of the most widely used diagnostic tools in clinics. MRI affords a number of advantages such as noninvasiveness, high spatial and temporal resolutions, and good soft tissue contrast (1, 2). However, the intrinsic MRI signals are often suboptimal in delineating internal organs and diseased tissues. To improve imaging quality, contrast agents, often in the form of paramagnetic compounds or superparamagnetic nanoparticles, are administered before or during an MRI scan (3-5). These magnetic agents alter local magnetic environments, inducing shortened longitudinal relaxation times ($T_1$) and transverse relaxation times ($T_2$). Although most agents shorten both $T_1$ and $T_2$, the impact is often dominant on one side. So far in clinics, the most commonly used $T_1$ agents are gadolinium (Gd) complexes (6) and those for $T_2$ imaging are iron oxide nanoparticles (7). Recently, a growing interest has been seen in the development of $T_1$–$T_2$ dual-mode contrast agents that can simultaneously modulate $T_1$- and $T_2$-weighted contrasts. Such a technology is attractive because MRI has an intrinsic high background signal. Even with conventional $T_1$ and $T_2$ contrast agents, the diagnosis can often be affected by artifacts caused by truncation, motion, aliasing, or chemical shift (8). $T_1$–$T_2$ dual-mode imaging may minimize the risks of ambiguity and improve image conspicuity and diagnostic sensitivity (9-11). To this end, there have been some efforts of integrating $T_1$ and $T_2$ contrast components using nanoscale engineering. These include tethering Gd-complex onto the surface of iron oxide nanoparticles (12), doping Gd cations into the matrix of iron oxide nanoparticles (13, 14), and forming a core/shell nanostructure where the $T_1$ and $T_2$ components are magnetically decoupled (15, 16). However, these approaches often involve a multi-step synthesis and/or a delicate control over the interaction between the $T_1$ and

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**Key Words:** dual modal, fluorescence, MRI, dual mode, contrast agent, gadolinium, MOF

**Abbreviations:** Nanoscale metal–organic framework (NMOF), magnetic resonance imaging (MRI), europium (Eu), gadolinium (Gd), isophthalic acid (H$_2$IPA), polyvinylpyrrolidone (PVP), hexamethylenetetramine (HMTA), dimethyl formamide (DMF), tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), Fourier transform infrared (FTIR), phosphate-buffered saline (PBS), magnetic resonance (MR), fast-spin echo (FSE), repetition time (TR), field-of-view (FOV), spin-echo multi sections (SEMS), fast spin-echo multi-section sequence (FSEMS), arginylglycylaspartic acid (RGD), 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT)
$T_2$ components, which may potentially limit their production and applications.

Herein, we report the facile synthesis of a novel, nanoscale metal–organic framework (NMOF)-based $T_1$–$T_2$ dual-modal contrast agent. In particular, using isophthalic acid (H2IPA) as building blocks, Eu$^{3+}$ and Gd$^{3+}$ as metallic nodes, and polyvinylpyrrolidone (PVP) as a surfactant, as reaction precursors, we prepared ~50 nm of self-assembled Eu,Gd-NMOFs in large quantities. Unlike conventional NMOFs, which are rapidly degraded in an aqueous environment (17), our Eu,Gd-NMOFs are stable in water for up to 24 hours because of strong interactions between the lanthanides and H2IPA as well as between the lanthanides and the PVP coating. To improve the particle stability against transmetallation, the Eu,Gd-NMOFs were further coated with a layer of silica. The resulting Eu,Gd-NMOFs@SiO2 particles manifested both high $r_1$ and high $r_2$ relaxivities (38 mM$^{-1}$s$^{-1}$ and 222 mM$^{-1}$s$^{-1}$, respectively), suggesting their potential as a $T_1$–$T_2$ dual-modal contrast agent. Such a possibility was demonstrated first in vitro and then in vivo with either intratumorally or intravenously injected nanoparticles, resulting in simultaneous hyperintensities and hypointensities on $T_1$- and $T_2$-weighted images, respectively. Meanwhile, Eu,Gd-NMOFs@SiO2 nanoparticles also afford strong fluorescence that permits in vitro and potentially histological analysis of nanoparticle location within tissue specimens. Overall, the Eu,Gd-NMOFs can be synthesized in a straightforward and high-throughput fashion and afford excellent magnetic and optical properties, suggesting their great potential as a novel and versatile multimodal imaging probe.

**METHODOLOGY**

**Materials**

The following materials have been used in this study: Gd(NO$_3$)$_3$·6H$_2$O, Eu(NO$_3$)$_3$·6H$_2$O, H$_2$IPA, PVP40, hexamethylenetetramine (HMTA), dimethylformamide (DMF), tetrahydrofuran, tetraethylorthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), ammonia, and ethanol. All these materials were purchased from Aldrich (Sigma-Aldrich, St. Louis, Missouri) and used without further purification.

**Synthesis of Eu,Gd-NMOF**

In a typical synthesis, H$_2$IPA (1 mg), Gd(NO$_3$)$_3$·6H$_2$O (10 mg), Eu(NO$_3$)$_3$·6H$_2$O (0.5 mg), PVP (60 mg), and HMTA (16 mg) were first dissolved in a mixed solution containing 1.0 mL of DMF and 4.0 mL of water. Precursors of other ratios were also tested. The mixture was heated at 100°C for 4 minutes to induce Eu,Gd-NMOF growth. The resulting Eu,Gd-NMOFs were collected by centrifugation, washed with ethanol, and resuspended in ethanol for further characterization. For comparison, the synthesis was also performed without HMTA or H$_2$IPA.

**Synthesis of Silica-Coated Eu,Gd-NMOF (Eu,Gd-NMOF@SiO$_2$)**

Eu,Gd-NMOF@SiO$_2$ was prepared by mixing 10 mg of the as-synthesized Eu,Gd-NMOF with 100 µL of TEOS, 10 µL of APTES, and 0.5 mL of ammonia (28%) in 15 mL of ethanol at room temperature overnight. The Eu,Gd-NMOF@SiO$_2$ was isolated by centrifugation at 10 000 rpm for 10 minutes.

**Bio-Conjugation (Preparation of Arginylglycalspartic Acid [RGD]-NMOF@SiO$_2$)**

For bio-conjugation, 50 mg of Eu,Gd-NMOF@SiO$_2$ nanoparticles were dispersed in a borate buffer (50 mM, pH 8.3) with magnetic stirring. Into this solution, 0.5 mg of bis(sulfosuccinimidyl)suberate was added in 0.1 mL of dimethyl sulfoxide. After 0.5 hours, the conjugate intermediate was collected by centrifugation and redispersed in the borate buffer (50 mM, pH 8.3). (c(RGDyK)) in dimethyl sulfoxide was added to the solution, and the mixture was incubated at room temperature for 2 hours to form RGD-Eu,Gd-NMOF@SiO$_2$ nanoparticles.

**Characterizations**

All transmission electron microscopy images were obtained on an FEI Tecnai 20 transmission electron microscope operating at 200 kV (FEI, Hillsboro, Oregon). Optical measurements were performed at room temperature under ambient air conditions. Ultraviolet-visible absorption spectra were recorded on a Shimadzu 12450 UV-Vis spectrometer (Shimadzu Scientific, Columbia, Maryland). Fluorescence measurements were performed using an Hitachi F-7000 spectrofluorimeter (Hitachi America, Tarrytown, New York). Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet iS10 FT-IR Spectrometer (Thermo Scientific, Waltham, Massachusetts). Powder X-ray diffraction intensity data were collected on a PANalytical X’Pert PRO MRD powder diffractometer using Cu Kα radiation (ASD Inc., Boulder, Colorado).

**Stability of Eu,Gd-NMOF and Eu,Gd-NMOF@SiO$_2$ in Water and Phosphate-Buffered Saline**

Here, 5 mg of Eu,Gd-NMOFs or Eu,Gd-NMOF@SiO$_2$ were dispersed in 1 mL of aqueous solutions, with pH ranging from 3 to 11. Gentle agitation was applied. After 24 hours, aliquots of the solution were taken to measure the change in fluorescent intensity.

**MRI Phantom Study**

Eu,Gd-NMOF@SiO$_2$ with Gd concentrations ranging from 5 × 10$^{-5}$ to 0.08 mM were suspended in 1% agarose gel in 300 µL polymerase chain reaction tube. These tubes were then embedded in a homemade tank designed to fit the MRI coil. $T_1$- and $T_2$-weighted magnetic resonance (MR) images of the samples were acquired on a 7 T small animal MRI system (Varian Medical Systems, Inc., Palo Alto, California). For $T_2$-weighted images, a $T_1$ inversion recovery fast-spin echo (FSE) sequence was used with the following parameters: repetition time (TR) = 5000 milliseconds, echo time (TE) = 12 milliseconds, echo train length = 8, inversion times = 5, 10, 30, 50, 80, 200, 500, 700, 900, 1200, and 3000 milliseconds. For $T_2$-weighted images, an FSE sequence was used with the following parameters: TR = 3000 milliseconds, TE = 10–100 milliseconds, with the step size set at 10 milliseconds. For both imaging sets, the following section settings were applied: field-of-view (FOV) = 65 × 65 mm$^2$; matrix size = 256 × 256; coronal sections = 4 with section thickness = 1 mm.

**Cell Culture**

U87MG (human glioblastoma) cells (ATCC) were grown in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal...
bovine serum and 100 U/mL of penicillin/streptomycin (ATCC). The cells were maintained in a humidified incubator with 5% carbon dioxide (CO₂) atmosphere at 37°C.

**Toxicity of NMOF In Vitro**

U87MG cells were seeded into a 96-well culture plate at a density of 10,000 cells/well and were cultured overnight. The media were removed and replaced with fresh media containing different Eu,Gd-NMOF@SiO₂ concentrations (0–50 μM Gd³⁺). Plates were incubated for 24 hours at 37°C and 5% CO₂. Viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays (18).

**Cell Uptake**

U87MG cells were incubated with Eu,Gd-NMOF@SiO₂ or RGD-Eu,Gd-NMOF@SiO₂ (20 μg/mL) in a chamber slide for 1 hour. U87MG cells only served as a negative control. After the incubation, the cells were washed 3 times with phosphate-buffered saline (PBS) to remove unbound nanoparticles. The slides were then imaged on an Olympus (Olympus Co. of U.S.A., Center Valley, Pennsylvania) X71 fluorescence microscope.

**In Vitro MRI with Cell Pellets**

U87MG cells were cultured until ~70% confluency was reached. Cells were then washed with PBS, and incubated with 2 mL of media containing 100 μg of RGD-Eu,Gd-NMOF@SiO₂ or Eu,Gd-NMOF@SiO₂. After 1 hour, the media were removed and cells were collected as pellets in 200 μL tubes. These tubes were then embedded in a homemade tank designed to fit the MRI coil. T₁- and T₂-weighted MR images were acquired on a 7 T small animal MRI system (Varian) using an FSE sequence with the following parameters: TR/TE = 500/14 milliseconds (T₁), TR/TE = 3000/8 milliseconds (T₂), section thickness = 0.5 mm, FOV = 60 × 50 mm, echo train length = 8, matrices = 256 × 256, and repeated three times.

**In Vivo MRI with Subcutaneously Injected Nanoparticles**

Animal studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia. Before the in vivo experiments, the Eu,Gd-NMOF@SiO₂ nanospheres were filtered through sterilized membrane filters (pore size = 0.22 μm) and stored in sterilized vials. U87MG cancer cells were subcutaneously inoculated into the right flanks of a 6-week-old nude mouse.

**Figure 1.** Synthesis of Eu,Gd-NMOFs. Poor size control if hexamethylenetetramine (HMTA) and polyvinylpyrrolidone (PVP) are not used as reactants (A). Despite the ratio between the lanthanide cations and isophthalic acid (H₂IPA) (the amount of which increased from 10 to 200 mg), the nanoparticle products showed poor size distribution. Notably, the synthesis was conducted in a dimethylformamide (DMF)/tetrahydrofuran (THF) solvent, as the resulting nanoscale metal–organic frameworks (NMOFs) were not stable in water. The impact of HMTA and PVP on the nanoparticle formation (B). Left, when HMTA was added to the precursors, Eu,Gd-NMOFs were formed in a DMF/water solvent, but the particle showed a wide size distribution. Right, when both HMTA and PVP were used, uniform Eu,Gd-NMOFs were obtained.
mice. Imaging was performed ∼3 weeks later on a 7 T small animal MRI system (Varian). $T_1$- and $T_2$-weighted MR images were acquired using spin-echo multi sections sequence (SEMSs) and fast spin-echo multi-section sequence (FSEMS), respectively, with the following parameters: TR/TE = 500/14 milliseconds ($T_1$) and TR/TE = 3000/33 milliseconds ($T_2$), section thickness = 1.0 mm, FOV = 60 × 50 mm, matrices = 256 × 256, and repeated three times. Further, 0.8 mg/kg of Eu,Gd-NMOF nano-spheres were intratumorally injected. $T_1$- and $T_2$-weighted MR images before and 4 hours after the injection were acquired.

In Vivo Liver MRI with Systemically Injected Nanoparticles.
Six-week-old female BALB/c mice were imaged on a 7 T small animal MRI system (Varian). $T_1$- and $T_2$-weighted MR images were acquired using SEMSs and FSEMS with the following parameters: TR/TE = 500/16 milliseconds ($T_1$) and TR/TE = 2500/8.65 milliseconds ($T_2$), section thickness = 1.0 mm, FOV = 30 × 30 mm, and matrices = 256 × 256. Further, 0.8 mg/kg of Eu,Gd-NMOFs were intravenously injected. $T_1$- and $T_2$-weighted MR images of the liver before and 4 hours after the injection were acquired.

RESULTS AND DISCUSSION
Synthesis and Characterization of Eu,Gd-NMOFs
Eu,Gd-NMOFs were synthesized by mixing $H_2$IPA, Gd(NO$_3$)$_3$, Eu(NO$_3$)$_3$, HMTA and PVP in a mixed solution containing DMF and water, and the solution was heating at 100°C. Previously, Oh et al. reported NMOF synthesis with Gd$_3$, Eu$_3$, and $H_2$IPA in a mixed solvent containing polar aprotic DMF and tetrahydrofuran (19). However, the method has poor size controls over the NMOF products. As manifested in Figure 1A, when using different amounts of $H_2$IPA, Eu,Gd-NMOFs of varied morphologies were obtained, but all the products showed a wide size distribution (Figure 1A). Moreover, Eu,Gd-NMOFs synthesized using this method were immediately degraded in water (data not

Figure 2. Characterization of Eu,Gd-NMOF@SiO$_2$. Transmission electron microscopy (TEM) images of as-synthesized Eu,Gd-NMOF nanospheres on a large scale (A, B). TEM image of the core-shell structure of Eu,Gd-NMOF@SiO$_2$ (protection SiO$_2$ layer ∼30 nm) (C). X-ray diffraction (XRD) pattern of Eu,Gd-NMOF@SiO$_2$ nanospheres (D). Fourier transform infrared (FT-IR) spectra of $H_2$IPA, HMTA, PVP, Eu,Gd-NMOFs, and Eu,Gd-NMOF@SiO$_2$ nanospheres (E).
shown), which is a potential problem for bioapplications. To address the issue, we added HMTA to the reaction solution. HMTA increased the pH of the initial reaction solution from ~5.0 to ~8.15, and as such, promoted the ionization and coordination of H$_2$IPA with Gd$^{3+}$ and Eu$^{3+}$ (20). Furthermore, we also included PVP as part of the precursors, which was bound to the growing nanoparticle surface to improve the particle stability and control their growth. By adding HMTA and PVP to the reactants, Eu,Gd-NMOFs of narrow size distribution were obtained in a DMF/water mixed solvent (Figure 1B). As a comparison, without the 2 agents, no NMOF was formed under the same condition (data not shown).

Transmission emission microscopy shows that the resulting Eu,Gd-NMOFs were spherical and had an average size of 50 ± 12 nm (Figure 2, A and B). The Eu,Gd-NMOFs were very stable in aqueous solutions, which is rare among NMOFs (17). However, the particles still decomposed when the aqueous solution had a relatively high ionic strength, for instance, PBS. This is presumably due to transmetallation and lanthanides binding with PO$_4^{3-}$. To further improve the particle stability, a silica coating was imparted to the surface of Eu,Gd-NMOFs. In particular, we followed the Stöber method (21, 22) and used both TEOS and APTES as silane precursors in the coating. The resulting Eu,Gd-NMOF@SiO$_2$ particles have a coating thickness of ~30 nm and an overall diameter of 100 ± 20 nm (Figure 2C). X-ray diffraction analysis found a broad peak at around 22.5° (2θ) (Figure 2D), which corresponds to the diffraction by Eu,Gd-NMOFs (JCPDS No. 01-086-1561). Similar results were observed by others in previous studies (23). FT-IR found absorption bands at 1609 cm$^{-1}$ and 1558 cm$^{-1}$ for Eu,Gd-NMOF and Eu,Gd-NMOF@SiO$_2$ respectively (Figure 2E). These absorption bands correspond to the C=O stretch, confirming successful H$_2$IPA coordination in the system. For the as-synthesized Eu,Gd-NMOFs, there was broad absorption band at around 3600 cm$^{-1}$, suggesting residual PVP coating on the nanoparticles (Figure 2E). Meanwhile, no characteristic HMTA absorption band at 1370 cm$^{-1}$ (attributed to the C-N stretch) was observed with

**Figure 3.** Optical and magnetic properties of Eu,Gd-NMOF@SiO$_2$. Ultraviolet-visible absorbance of Eu,Gd-NMOF@SiO$_2$ nanospheres (A). Fluorescent spectrum of Eu,Gd-NMOF@SiO$_2$. The inset is a photograph of (1) Eu,Gd-NMOF@SiO$_2$ powder, (2) water, and (3) aqueous solution of Eu,Gd-NMOF@SiO$_2$ (B). Relaxivity measurements of Eu,Gd-NMOF@SiO$_2$. Changes in R$_1$ (1/T$_1$) and R$_2$ (1/T$_2$) were plotted over various Gd concentration. r$_1$ and r$_2$ relaxivities were 38 mM$^{-1}$s$^{-1}$ and 222 mM$^{-1}$s$^{-1}$, respectively (C).
Eu,Gd-NMOF, suggesting minimal adsorption of HMTA on the particle surface (Figure 2E).

**Optical and Magnetic Properties of Eu,Gd-NMOF@SiO\textsubscript{2}**

Eu,Gd-NMOF@SiO\textsubscript{2} nanoparticles absorb at around 280 nm (Figure 3A) and have strong emission at 594 and 620 nm (Figure 3B). These 2 emission peaks are attributed to \( ^{5}D_{0} \rightarrow ^{7}F_{1} \) and \( ^{5}D_{0} \rightarrow ^{7}F_{2} \) transitions, respectively (24-26). Such fluorescence can be used to track the nanoparticles in vitro and in histological studies.

The MRI contrast ability of the Eu,Gd-NMOF@SiO\textsubscript{2} nanoparticles was evaluated by phantom studies on a 7 T magnet. In brief, Eu,Gd-NMOF@SiO\textsubscript{2} nanoparticles of increased concentrations were dispersed in 1% agarose gel, and the samples were scanned by MRI using SEMSs and FSEMSs. For both \( T_1 \)- and \( T_2 \)-weighted imaging, the signals were clearly concentration-dependent. In particular, significant signal enhancement was observed in \( T_1 \) images at elevated concentrations; in contrast, in \( T_2 \) images, signal reduction was observed at high particle concentrations. On the basis of the imaging results, it was deduced that \( r_1 \) was 38 mM\textsuperscript{-1}s\textsuperscript{-1} and \( r_2 \) was 222 mM\textsuperscript{-1}s\textsuperscript{-1} (Figure 3C). These relaxivity values are much higher than commonly used clinical contrast agents such as Gd–diethylenetriamine pentaacetic acid \( (r_1 \text{ of } 3.10 \text{ mM}\textsuperscript{-1}s\textsuperscript{-1}) \) and Feridex \( (r_2 \text{ of } 117 \text{ mM}\textsuperscript{-1}s\textsuperscript{-1}) \) (27). The exact mechanisms behind the high \( r_1 \) and \( r_2 \) values are unclear, but it may be attributed to the rigid confinement of Gd\textsuperscript{3+} in the nanosystem and slow interex-

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**Figure 4.** Stability and cytotoxicity. Fluorescence intensity (ex/em: 360/595 nm) changes when Eu,Gd-NMOF@SiO\textsubscript{2} nanospheres are incubated in aqueous solutions of different pH (A). Gd\textsuperscript{3+} release profiles of Eu,Gd-NMOFs and Eu,Gd-NMOF@SiO\textsubscript{2} nanospheres in phosphate-buffered saline (PBS) (pH = 6.5 and 7.4) (B). Cell viability is assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays with U87MG cells. Eu,Gd-NMOF@SiO\textsubscript{2} nanospheres with a Gd concentration ranging from 0 to 50 \( \mu \text{M} \) were incubated with cells (C).

**Figure 5.** Cell fluorescence microscopy and magnetic resonance imaging (MRI). Fluorescent images of U87MG cells that had been incubated for 1 hour with Eu,Gd-NMOF@SiO\textsubscript{2} or RGD-Eu,Gd-NMOF@SiO\textsubscript{2}. Scale bars: 50 \( \mu \text{m} \) (A). \( T_1 \)- and \( T_2 \)-weighted MRI of cells that had, or had not, been incubated with RGD-Eu,Gd-NMOF@SiO\textsubscript{2} (B).
change of Gd$^{3+}$ with water molecules (28). The $r_2/r_1$ ratio is 5.8, which is at the boundary between conventionally defined $T_1$ and $T_2$ agents (29).

**Nanoparticle Stability**

The stability of Eu,Gd-NMOF@SiO$_2$ nanoparticles was studied by monitoring fluorescence changes in different solutions. These included aqueous solutions, with pH ranging from 3 to 11, and PBS. It was observed that the Eu,Gd-NMOF nanoparticles were very stable when the pH was maintained between 4 and 9, and only degraded when the pH was above 9 or below 4 (Figure 4A), suggesting great resistance of the particles against pH changes. In contrast, Eu,Gd-NMOFs were much more labile in PBS, and were largely dissolved within 1 hour (Figure 4B). With the silica coating, however, Eu,Gd-NMOF@SiO$_2$ showed significantly enhanced stability, showing no fluorescence drop in PBS for at least 28 hours (Figure 4B).

**Cytotoxicity and Cell Uptake Studies**

Cytotoxicity of the nanoparticles was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays with U87MG cells, a human glioblastoma cell line. We found no detectable cytotoxicity with Eu,Gd-NMOF@SiO$_2$ even at very high concentration investigated (20 μM Gd$^{3+}$), indicating good biocompatibility (Figure 4C).

Next, we investigated whether Eu,Gd-NMOF@SiO$_2$ can be visualized by MRI when internalized by cells. To investigate, we conjugated c(RGDyK), a cyclic peptide with high binding affinity against integrin $\alpha_v\beta_3$ (30), to the surface of Eu,Gd-NMOF@SiO$_2$. This was achieved by covalently linking the primary amine of c(RGDyK) and the amine groups on Eu,Gd-NMOF@SiO$_2$ surface using bis(sulfo)succinimidyl)suberate as a homo-dimer crosslinker. U87MG cells were then incubated with RGD-Eu,Gd-NMOF@SiO$_2$ and Eu,Gd-NMOF@SiO$_2$ nanoparticles for 1 hour. Notably, U87MG cells are high in integrin $\alpha_v\beta_3$ expression (31).
Under a fluorescence microscope, we observed a significant increase in intracellular red fluorescence, suggesting efficient internalization of RGD-Eu,Gd-NMOF@SiO₂ (Figure 5A). As a comparison, Eu,Gd-NMOF@SiO₂ nanoparticles showed low cell uptake, indicating that the uptake was mainly mediated by RGD–integrin interaction.

Such RGD-Eu,Gd-NMOF@SiO₂-treated cells were also collected as cell pellets and scanned by MRI. On T₁-weighted images, significant signal enhancement was observed with cells that had been incubated with nanoparticles compared with those that had been not been incubated (Figure 5B). This is attributed to hyperintensities induced by RGD-Eu,Gd-NMOF@SiO₂ nanoparticles. Meanwhile, significant signal reduction was observed on T₂-weighted images (Figure 5B), which was attributed to hypointensities induced by the RGD-Eu,Gd-NMOF@SiO₂. These results confirm that Eu,Gd-NMOF@SiO₂-labeled cells can be visualized by both T₁- and T₂-weighted MRI and also by fluorescence microscopy.

**In Vivo MRI**

For a proof of concept, we investigated the dual-mode contrast capacity of Eu,Gd-NMOF@SiO₂ in two in vivo studies. In the first study, we intratumorally injected Eu,Gd-NMOF@SiO₂ (0.8 mg/kg in 100 µl PBS, n = 3) to U87MG models and scanned the animals on a 7 T magnet. Similar to the in vitro studies, relative to the prescans, there was significant signal enhancement on T₁-weighted images and signal reduction on T₂-weighted images (Figure 6, A and B). In particular, the average signals in tumors increased by 12% ± 6% on T₁-weighted images after injection and decreased by 89% ± 2% on T₂-weighted images. In the second study, Eu,Gd-NMOF@SiO₂ nanoparticles were intravenously injected (0.8 mg/kg) into BALB/c mice, and T₁- and T₂-weighted images of the liver area were acquired both before and 1 hour and 4 hours after the injections (Figure 6, C and D). It is well known that nanoparticles after systemic injection are efficiently accumulated in the liver, such as through uptake by Kupffer cells (32). Region of interest analysis showed that relative to the prescans, signals in the liver increased to 157% ± 9% on T₁-weighted images at 1 hour. Interestingly, the signal decreased to 105% ± 2% at 4 hours (relative to the prescans; Figure 6E). This is probably attributed to considerably high concentration of Eu,Gd-NMOF@SiO₂ in the liver at the time point, leading to signal saturation. Similar phenomenon has been observed by others (33, 34). Meanwhile, on T₂-weighted images, signals in the liver decreased to 57% ± 12% on T₂ images at 1 hour and to 38% ± 16% at 4 hours (Figure 6F). Overall, these results confirm the feasibility of using Eu,Gd-NMOF@SiO₂ nanoparticles as a T₁–T₂ dual-mode imaging probe.

**CONCLUSIONS**

We have developed a novel and facile procedure of synthesizing a highly hydrostable metal–organic framework, Eu,Gd-NMOFs. Silica-coated Eu,Gd-NMOFs exhibit high longitudinal (38 mM⁻¹ s⁻¹) and transversal (222 mM⁻¹ s⁻¹) relaxivities and strong fluorescence. In vitro and in vivo MRI studies confirm that Eu,Gd-NMOFs can induce both hyperintensities on T₁-weighted images and hypointensities on T₂-weighted images, suggesting great potential of the probe as a novel T₁–T₂ dual-mode imaging probe. The nanoparticle surface can be easily coupled with a variety of targeting moieties for different imaging purposes. It is also possible to impart onto the solid silica layer a mesoporous silica layer into which drug molecules can be loaded. These make the nanoparticles a modifiable platform technology that can find wide applications in modern imaging and theranostics.

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An Overdetermined System of Transform Equations in Support of Robust DCE-MRI Registration With Outlier Rejection

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Abbreviations: Dynamic contrast-enhanced (DCE), pharmacokinetic (PK), magnetic resonance (MR), principal component analysis (PCA), region of interest (ROI), right-hand side (RHS), left-hand side (LHS), time-normalized, intensity-normalized spike area (TISA)

Quantitative hepatic perfusion parameters derived by fitting dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) of liver to a pharmacokinetic model are prone to errors if the dynamic images are not corrected for respiratory motion by image registration. The contrast-induced intensity variations in pre- and postcontrast phases pose challenges for the accuracy of image registration. We propose an overdetermined system of transformation equations between the image volumes in the DCE-MRI series to achieve robust alignment. In this method, we register each volume to every other volume. From the transforms produced by all pairwise registrations, we constructed an overdetermined system of transform equations that was solved robustly by minimizing the \( L_{1/2} \) norm of the residuals. This method was evaluated on a set of 100 liver DCE-MRI examinations from 35 patients by examining the area under spikes appearing in the voxel time–intensity curves. The robust alignment procedure significantly reduced the area under intensity spikes compared with unregistered volumes \((P < .001)\) and volumes registered to a single reference phase \((P < .001)\). Our registration procedure provides a larger number of reliable time–intensity curve samples. The additional reliable samples in the precontrast baseline are important for calculating the postcontrast signal enhancement and thereby for converting intensity to contrast concentration. On the intensity ramp, retained samples help to better describe the uptake dynamics, providing a better foundation for parameter estimation. The presented method also simplifies the analysis of data sets with many patients by eliminating the need for manual intervention during registration.

INTRODUCTION

Dynamic contrast–enhanced magnetic resonance imaging (DCE-MRI) in the liver enables quantification of hepatic perfusion for assessment of tumor response to radiation therapy, as well as normal tissue damage (1-5). One of the challenges in liver DCE-MRI is respiratory motion during acquisition, which can cause blurring and artifacts in the images and intensity variations in the dynamic curves, and subsequently, errors in perfusion quantification using pharmacokinetic (PK) models. To reduce respiratory motion-induced degradation of liver images during DCE-MRI acquisition, a breathing control paradigm has been proposed, during which the patient is instructed to hold their breath multiple times (taking breaths in between) (5). The dynamic image volumes acquired during the 2–3 minutes of contrast uptake are still misaligned because of poor reproducibility of the liver position during multiple breath holds, loss of control during breath holding, and occasionally gross movement of the patient. Therefore, a voxel cannot be assumed to cover the same piece of tissue throughout the whole examination, but, instead, it covers different pieces at different points in time. Therefore, if not corrected for, breathing and gastrointestinal motions can induce artifacts, such as intensity spikes and wide bumps, in the contrast uptake curves. To estimate the arterial and portal venous perfusion in the liver, a sufficient number of reliable samples of the uptake curve must be collected to fully describe the contrast uptake dynamics. The shape of the precontrast plateau and the beginning of the intensity ramp following contrast agent administration can have a large effect on the estimated perfusion parameters, in particular on hepatic arterial perfusion. Unwanted artifacts introduced into the uptake curve by patient motion reduce the number of reliable samples, increasing the risk of inaccurate PK model parameter estimation.

To correct for image misalignment in the DCE series, image registration has been used to align the magnetic resonance (MR) images at different points in time or phases (1, 6-8). However, registration of the DCE-MRI phases has some unique challenges,
for example, a large number of dynamic image volumes, low spatial resolution, and low contrast-to-noise ratio that are tradeoffs for the high temporal resolution. In addition, the image contrast is changed completely over the whole course from precontrast to rapid contrast uptake and to full contrast enhancement. Combined with all these effects, the dynamic contrast agent-induced intensity changes pose a difficult problem even to registration methods using multimodality image-to-image metrics such as mutual information (9–11).

A number of methods have been developed to tackle the DCE-MRI registration problem. Some methods attempt to register each phase to a reference phase selected among all phases. Such methods use either region-limited rigid registration (12) or deformable registration (9, 10). Deformable registration may yield incorrect volume changes around contrast-enhanced lesions unless the method is adapted to explicitly counteract such changes (11). As an alternative to reference phase-based registration, image intensity changes can be modeled using a PK model and the model incorporated into the registration algorithm (13–16). Such PK-based methods can be slow because of the need for repeated PK model parameter estimation. For a faster procedure, principal component analysis (PCA) can be used to generate reference images for registration (17). However, PCA may not be able to adequately describe the spatially varying time–intensity curves. Therefore, the phases could alternatively be decomposed into a low-rank and a sparse image component in a procedure called robust PCA that can be combined with image registration (18, 19). Robust PCA can also be used to combine registration with reconstruction, but this requires access to raw data from the MRI scanner (20). However, when comparing the 3 representative registration methods (21), 1 PK model-based, 1 PCA-based, and 1 sequentially registering each phase to its preceding phase, the sequential registration method performed better (average error 14.7% in estimated parameters) than the more complex iterative, PCA-based and PK model-based methods (average errors: 39.5% and 39.2%, respectively). Nevertheless, all image registration methods reduced errors in the estimated parameters in the tumors in comparison with without image registration methods.

The performance of the sequential method can be understood by considering that the changes between neighboring phases are small. Therefore, registration has a greater chance of success. However, misregistration errors are accumulated and propagated from the earlier phases to the later phases. If registration at the early phase fails, all following phases will be incorrectly aligned with the first phase.

Inspired by the findings in Rajaraman S et al.’s study (21), and taking into account the aforementioned drawback of the sequential registration procedure, we introduce an overdetermined system for achieving robust registration for the DCE image series. In this procedure, rather than registering each phase to a reference phase or to a preceding phase, each phase is registered to every other phase. This produces an overdetermined system of transform equations that can be solved using robust statistical methods to reject outliers corresponding to failed registrations. This new method is evaluated on a set of 100 liver DCE-MRI examinations, and the results are compared with those of a conventional registration method where all phases are registered to 1 postcontrast reference phase. A robust registration method for liver DCE-MRI would greatly simplify the workflow of clinical liver perfusion studies with a large number of participating patients by eliminating the need for manual intervention following faulty registration.

### MATERIALS AND METHODS

#### Imaging

Under institutional review board approval, 100 DCE-MRI examinations of the liver from 35 patients were included in this study (women, 8; men, 27; age at examination, 51–83 years; number of examinations per patient, 1–4). The patients were imaged for about 3 minutes using a repeated breath-hold paradigm (5). For this paradigm, the patients were instructed to initially hold their breath for as long as they could and then hold their breath repeatedly with a single deep inhalation in between each breath hold. Images acquired during deep inhalations showed large liver movement and severe motion blur. Therefore, these were excluded from further analysis. Dynamic MR imaging was started at the beginning of the first breath hold and a gadobenate-dimeglumine-based contrast agent (MultiHance, Bracco S.p.A., Italy) was administered intravenously.

A 3 T MRI scanner (Magnetom Skyra, Siemens Medical Systems, Erlangen, Germany) was used for imaging. A 3-dimensional gradient echo sequence that speeds up acquisition using stochastic sampling with view sharing (time-resolved angiography with stochastic trajectories; TWIST) was used for 10 patients, and a sequence that enhances speed through partial Fourier reconstruction (volume interpolated breath hold examination; VIBE) was used for 25 patients. Sequence parameters varied among the patients (Table 1).

During a breath hold, the liver should ideally be still. Unfortunately, partial inhalation during a breath hold, imperfect reproducibility of the exhale state, and changes to patient posture result in liver movement between images. These movements, however, are smaller than those observed during free breathing. For the spatial resolution, signal-to-noise ratio, and

<table>
<thead>
<tr>
<th>TWIST</th>
<th>VIBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flip angle</td>
<td>10–20°</td>
</tr>
<tr>
<td>Repetition time</td>
<td>2.5–2.53 ms</td>
</tr>
<tr>
<td>Echo time</td>
<td>0.84–0.86 ms</td>
</tr>
<tr>
<td>Temporal resolution</td>
<td>2.3–4.3 s</td>
</tr>
<tr>
<td>Voxel volume</td>
<td>15.6–45.8 mm³</td>
</tr>
<tr>
<td>Number of phases</td>
<td>52–60</td>
</tr>
<tr>
<td>Acquisition time</td>
<td>2.0–3.7 min</td>
</tr>
<tr>
<td>Number of examinations</td>
<td>31</td>
</tr>
</tbody>
</table>

**Table 1.** DCE-MRI Sequence Settings for TWIST and VIBE Sequences

**Abbreviations:** DCE-MRI, Dynamic contrast-enhanced magnetic resonance imaging; TWIST, time-resolved angiography with stochastic trajectories; VIBE, volume interpolated breath hold examination.
artifact level seen in this study, we, therefore, chose to use rigid registration to correct for the observed range of motions.

**Preprocessing**

Before registration, a number of preprocessing steps were performed. These were the same for the robust registration procedure (section Robust Registration) and the conventional procedure (section Conventional Registration) to which it was compared.

**Reference Phase Selection.** The conventional procedure involved alignment of all phases to 1 reference phase. For each examination, this reference phase was selected among all phases as the phase with the smallest sum of mean absolute distances to the other phases in intensity, which is calculated as follows:

$$i_{\text{ref}} = \arg\min_i \sum_{j=1}^{n_{\text{phases}}} \frac{1}{n_{\text{voxels}}} \sum_{k=1}^{n_{\text{voxels}}} |I_r(r_k) - I_r(r_{k,i})|$$

where $i_{\text{ref}}$ is the index of the reference phase among the $n_{\text{phases}}$ phases, $n_{\text{voxels}}$ is the number of voxels in a phase, and $I_r(r_k)$ is the image intensity of the $i$th phase at a spatial position of the $k$th voxel, $r_k$. This step makes the selection of the reference phase deterministic and avoid manual intervention. The reference phase was also used for the robust registration procedure to define the coordinate system that the final transforms were given with respect to.

**Region-Limited Image Registration.** Because we wish to correct for the motion of the liver with a rigid transform and the rest of the body inside the field of view (FOV) does not move in the same way as the liver, region-limited registration was used. For this purpose, a region of interest (ROI) was drawn around the liver on the reference phase. This ROI was drawn snugly along the edge of the liver. For the region-limited registration, it is advantageous to include a margin outside the liver ROI. This margin covers the contrast-rich interface between the liver and the surrounding tissue, and it helps to drive the registration algorithm. The margin was set to 15 mm for all registrations.

**Bias Field Correction.** MR images commonly contain a spatial intensity variation that is not the result of intrinsic tissue properties but of the position of the tissue with respect to the transmission and receiver radiofrequency coils. This “bias field” can cause misregistration if it is the dominant feature in an image. Fortunately, the bias field typically varies slowly with respect to the spatial position. The effect of the bias field on the images used for registration was reduced by dividing each phase by a filtered version of itself. The modified phases used for registration were then given by the following equation:

$$I_{\text{reg}}(r) = I_r(r)/\langle I_r(r) * G(r;0, \sigma^2[I])\rangle$$

where $G(r, 0, \rho^2[I]$ is a 3-dimensional Gaussian filter kernel with standard deviation $\rho = 30$ mm in each dimension and * is the convolution operator. These bias-field-corrected images were only used for registration and not for the uptake curve extraction, as the uptake curves could be corrupted by the correction.

**Robust Registration**

The robust registration procedure for each DCE time series was divided into 5 stages—3 translational registration stages and 2 rigid registration stages with both translation and rotation. For the robust procedure, the workflow for each stage is illustrated in Figure 1. Either each possible pair of phases or each pair in a subset of all possible pairs was registered. Thus, each registered pair gave rise to a rigid transform describing the relative position of the 2 phases of the pair. For the registration of each pair, the first phase of the pair was used as a fixed image and the second as a moving image. The dilated liver ROI was applied as a mask to the fixed image to restrict the domain for which the registration metric was evaluated. Because of this asymmetry, the registration of pair $(i,j)$, where $i$ is the index of the fixed image and $j$ is the index of the moving image, did not produce the same transform as the registration of pair $(j,i)$.

After the registration of all pairs of 1 of the steps, the resulting transforms were used to produce a set of consistent transforms as described in the next section. These transforms were then applied to the phases before proceeding to the next stage.

The registration of the 2 phases of a pair was performed using the open source registration software Plastimatch, version 1.6.0-beta (http://plastimatch.org). The settings of the translational and rigid registration steps are shown in Table 2. For the robust procedure, the first 2 translational steps only registered a subset with $10n_{\text{phases}}$ pairs selected randomly among the $n_{\text{phases}}(n_{\text{phases}} - 1)$ possible pairs. These 2 steps helped roughly align the liver ROI for all the phases in preparation for the full registrations performed by the third through the fifth stage.

---

**Figure 1.** Registration of each possible pair of phases. The transforms produced by each registration transform any coordinate in the fixed image coordinate system into its corresponding point in the moving image coordinate system.
Consistent Solution

The registration of a pair \((i, j)\) of phases \(i\) and \(j\) results in a rigid transform described by a rotation matrix \(B_{i,j}\) and a translation vector \(b_{i,j}\). Thus, given a structure found in phase \(i\) at spatial coordinates \(r^{(i)} = (x, y, z)^T\), the corresponding structure, as indicated by the registration, can be found at position \(r^{(j)} = B_{i,j} r^{(i)} + b_{i,j}\) in phase \(j\).

Let us assume that there is a transform given by rotation matrix \(A_i\) and translation vector \(a_i\), which transforms the coordinates of any structure in the reference phase \(i_{\text{ref}}\) into the coordinates of the same structure in phase \(i\). If all of the coordinates for the voxels inside the liver ROI are inserted into the columns of a matrix \(R\), the corresponding points in phase \(j\) are given by \(A_j R + a_j\), where \(1 = [1, \ldots, 1]^T \times n_{\text{voxels}}\). However, if the registration of phase pair \((i, j)\) is successful, the same coordinates are also given by the application of the true transform from the reference phase to phase \(i\) followed by the registration transform from \(i\) to \(j\) such that: such that:

\[
B_{i,j}(A_i R + a_i, 1) + b_{i,j} = (A_j R + a_j, 1) .
\] (1)

We then have \(n_{\text{pair}}\) equations of the form of equation (1), 1 for each registered phase pair, and up to \(n_{\text{phases}}(n_{\text{phases}} - 1)\) are registered for a given stage. However, there are only \(n_{\text{phases}} - 1\) true transforms given by \(A_j\) and \(a_j\). Therefore, the system in equation (1) is overdetermined if \(n_{\text{pair}} > n_{\text{phases}} - 1\). Because the registration of a phase pair may not produce the true transform but only an estimate, equation (1) is not valid for all registered pairs \((i, j)\) in general. Therefore, we must choose in what sense to solve the system in equation (1). If we define equation (2) as follows:

\[
E_{i,j} = B_{i,j}(A_i R + a_i, 1) + b_{i,j} - (A_j R + a_j, 1)
\] (2)

we can observe that \(\|E_{i,j}\|^2_F\) is the sum of the squared distances between the liver voxel coordinates given by \(B_{i,j}(A_i R + a_i, 1) + b_{i,j}\) and \(A_j R + a_j, 1\). Finding the true transforms given by \(A_i\) and \(a_i\) by minimizing the following equation:

\[
\sum_{(i,j)} \|E_{i,j}\|^2_F
\] (3)

where \(\|\cdot\|_F\) is the Frobenius norm and would produce the least squares solution. If the error in the mapping of the liver voxel coordinates could be described by a normally distributed random variable, then minimizing equation (3) would produce a good solution for \(A_i\) and \(a_i\). However, because some of the phase pair registrations could fail and produce severely erroneous transforms, \(B_{i,j}\) and \(b_{i,j}\), the sum in equation (3) could be dominated by a few large terms that would thwart the accurate estimation of \(A_i\) and \(a_i\). Therefore, to lessen the impact of outlier registration transforms, \(A_i\) and \(a_i\) can be robustly estimated by minimizing the following equation:

\[
\sum_{(i,j)} \|E_{i,j}\|^p_F
\] (4)

with \(p < 2\) (\(p = 1/2\) for this work). This can be done by using the iteratively reweighted least squares algorithm. For iteratively reweighted least squares, the following equation is solved repeatedly:

\[
\beta^{(k+1)} = \arg \min_\beta \sum_{(i,j)} w_{i,j}(\beta^{(k)}) \|E_{i,j}(\beta)\|^2_F
\] (5)

where

\[
\begin{align*}
E_{i,j}(\beta) &= (E_0 + \|E_{i,j}(\beta)\|_F) P^{-2} \quad (6)
\end{align*}
\]

and \(\beta\) is the set of all transform parameters \(\{A_i\}\) and \(\{a_i\}\), and \(\beta^{(k)}\) is the \(k\)th iteration of \(\beta\). Equation (5) is iterated until convergence. To avoid division by 0 in equation (6), a nonzero regularization term \(E_0\) is needed. As \(E_0\) is selected as \(0.01\) mm.

Each iteration of equation (5) amounts to solving a constrained, weighted-least-squares problem, where the constraint is that all \(A_i\) must be rotation matrices. Because the minimization problem in equation (5) is a rigid-body problem, it can be broken up into 2 independent problems, 1 problem for the translation of the liver center of mass and 1 problem for the rotation of the liver about its center of mass. This can be seen by noting that:

\[
\|E_{i,j}\|^2_F = \text{Tr}(E_{i,j} F_{i,j}) = n_{\text{pairs}} \text{Tr}(F_i F_i^T) + G_{i,j} G_{i,j}^T
\] (7)

where

\[
F_{i,j} = (B_{i,j} A_i - A_j)L
\] (8)

\[
G_{i,j} = B_{i,j} \tilde{a}_i + \tilde{b}_{i,j} - \tilde{a}_j
\] (9)

\[
\tilde{a}_i = a_i - (1 - A_i) \mu
\] (10)

\[
\tilde{b}_{i,j} = b_{i,j} - (1 - B_{i,j}) \mu
\] (11)

\[
\Sigma = (R - \mu 1) (R - \mu 1)^T / n_{\text{pairs}}
\] (13)

and \(L\) is the lower triangular matrix in the Cholesky decomposition of \(\Sigma = LL^T\). Thus, the problem in equation (5) can be written as the following 2 problems:
\[
\beta_{i}^{(k+1)} = \arg\min_{\beta_i} \sum_{(i,j)} \beta_i^* W_{i,j}(\beta_i) ||F_{i,j}(\beta_i)||^2_F \quad (14)
\]
\[
\beta_1^{(k+1)} = \arg\min_{\beta_1} \sum_{(i,j)} \beta_1^* W_{i,j}(\beta_1^*) ||G_{i,j}(\beta_1^*)||^2_F \quad (15)
\]

where equation (14) is a problem to find the rotation matrices \(\beta_1 = \{\alpha_i\}\) only, whereas equation (15) is a problem to find the transformed translation vectors \(\beta_2 = \{\alpha_i\}\) only and \(\beta = \{\beta_1, \beta_2\}\).

The problem in equation (15) is a system of linear equations of the following form:
\[
w_{i,j}^{1/2}(B_{i,j}\alpha_i + \tilde{b}_{i,j} - \bar{\alpha}_i) = w_{i,j}^{1/2}G_{i,j}
\]
that can be solved in a least squares sense for \(\{\bar{\alpha}_i\}\) using the Moore–Penrose pseudoinverse. When solving this system, the solution from equation (14) is used to fix \(\bar{\alpha}_i\) to a constant by setting \(a_{i,ref} = 0\). The problem in equation (14) can similarly be written as a system of matrix equations as follows:
\[
w_{i,j}^{1/2}(B_{i,j}A_i - A_j)L = w_{i,j}^{1/2}F_{i,j}
\]
(17)

To find a solution for equation (17), a heuristic algorithm was used as described below. By summing over \(i\), equation (17) can be rewritten as follows:
\[
A_j + \sum_i w_{i,j}^{1/2}F_{i,j}L^{-1} = \sum_i w_{i,j}^{1/2}B_{i,j}A_i
\]
where the RHS is an approximation of the first term in the LHS, with an error given by the second term of the LHS. This formula can be solved recursively. However, \(\{A_i\}\) is a rotation matrix and needs to be orthogonal with \(\det(A_i) = 1\). To ensure this, the RHS of equation (18) can be decomposed into its singular value decomposition to produce a set of the following iteration equations:
\[
U_j^{(l+1)}V_j^{(l+1)}(V_j^{(l+1)})^T = \sum_i w_{i,j}^{1/2}B_{i,j}A_i^0
\]
\[
C_j^{(l+1)} = U_j^{(l+1)} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & \pm 1 \end{pmatrix} (V_j^{(l+1)})^T
\]
\[
A_j^{(l+1)} = C_j^{(l+1)}(C_j^{(l+1)})^{-1}
\]
(21)

where the sign of the last diagonal element in equation (20) is selected to make the determinant of \(C_j^{(l+1)}\) positive and equation (21) is needed to ensure that \(A_j^{(l+1)} = I\). The stopping criteria for equations (19) to (21) are as follows:
\[
\max_i ||A_j^{(l+1)} - A_j^0|| < 10^{-12}
\]
(22)

Empirically, the outer recursive sequence given by equation (5) was observed to converge within 20 iterations for a few test cases. Therefore, 20 iterations of equation (5) were used for all examinations.

**Conventional Registration**

A conventional registration procedure was used as a benchmark for the robust registration. This conventional procedure regis-
tered each phase to the reference phase using the same 5 registration stages as for the robust procedure. However, phases were not registered to anything but the reference phase, and no processing of the resulting transforms was performed except the combination of the transforms from the 5 stages for each phase. The same ROI was used to limit the registration region, and the settings in Table 2 were used for alignment.

Evaluation
The effect of registration on the mean signal intensity versus time for a 1-cm³ cubic ROI placed 1 cm below the dome of the liver for 1 patient is illustrated in Figure 2. The spikes seen in the intensity for the nonregistered time series are caused by tissue moving in and out of the ROI due to breathing.

Given the appearance of the signal variations seen in Figure 2, a suitable metric for the quality of the DCE-MRI time series should reflect how smooth the time–intensity curves are for the voxels inside the liver ROI. The temporal resolution of the time series as compared with the rise time of the time–intensity curve should also be reflected by the alignment quality metric. If only a few samples are collected during the rise time of a curve, the impact of 1 erroneous sample will be greater than that if a larger number of samples are collected. The variation of temporal sampling density across examinations can be taken into account by transforming the time of a given phase \( t \) into a normalized time \( \tau \) as follows:

\[
\tau = \frac{t - t_0}{T}
\]

where \( t_0 \) is the time at the center of the intensity ramp and \( T \) is half of the rise time. Thus, the rise time of the mean liver intensity for each examination is similar after transformation. The values of \( t_0 \) and \( T \) were determined by fitting the time–

---

**Figure 4.** The intensity along a 40-cm-long column extending in the inferosuperior direction is seen for the phases before registration (A), after conventional registration (C), and after robust registration (E). The mean intensity of a 1-cm³ cubic ROI placed 1 cm below the liver dome is seen as a solid black curve for the phases before registration (B), after conventional registration (D), and after robust registration (F). The black circles indicate the intensities of phases excluded because of deep inhalations during examination. For each column of (A), (C), and (E), the intensity profile was divided by its mean to improve the visual contrast of the figure.
intensity curve for the whole liver for each examination to the phenomenological function as follows:

\[ s(t) = s_0 \left( 1 + \frac{\Delta_{\text{max}}}{2} \left( 1 + \text{erf} \left( \frac{t-t_0}{T} \right) \right) e^{-\frac{\alpha(t-t_0)}{\tau}} \right) \]  

where \( s_0 \) is the baseline intensity, \( \Delta_{\text{max}} \) is the maximum signal enhancement, and \( \alpha \) is the decay rate of the plateau. The function was fitted to the data using minimization of the \( L_1 \)-norm of the signal residuals.

Furthermore, errors in the baseline will be more troublesome for parameter estimation than had they appeared in the postcontrast plateau. In light of these considerations, we have chosen to use an approximation of the local spike area divided by the mean temporally local signal intensity to calculate the metric (see right panel of Figure 2 for illustration).

If the normalized temporal spacing between phases is \( \Delta r \), an approximation of the time-normalized, intensity-normalized spike area (TISA) metric, \( m(r, i) \), at phase \( i \) and position \( r \) is then given by the following equation:

\[ m(r, i) = \frac{|I_{i-1}(r) + 2I_i(r) - I_{i+1}(r)| \Delta r/2}{|I_{i-1}(r) + I_i(r) + I_{i+1}(r)|/3} \]  

where the numerator is the spike area and the denominator is the mean signal intensity of the voxel for the phase and its 2 temporal neighbors. Because the preceding and the following phases are needed to evaluate equation (25) for a phase, equation (25) cannot be evaluated for phases adjacent to others omitted because of deep inhalations during the examination.

**RESULTS**

After the consistent registration solution has been found from the overdetermined system of transform equations by minimizing equation (4), the individual norms \( \|E_i\|_F \) show how well a particular transform \( \{B_{i_0}, b_{i_0}\} \) agrees with the overall solution. This is illustrated in Figure 3 for the last registration stage of 1 examination. The small dark square in the lower left corner of the figure represents the registration errors among the precontrast phases. The larger dark square in the upper right corner represent the errors among the postcontrast phases. These 2 groups have relatively small deviations from the consistent solution compared with the errors found for registrations between pre- and postcontrast phases as represented by the brighter rectangles in the upper left and lower right corners. This pattern was seen for a majority of the examinations registered and indicates that registrations between pre- and postcontrast phases are more likely to produce transforms that do not agree with the consistent solution.

By sampling the intensity along a 1×1-cm-thick column extending 20 cm above and 20 cm below the liver dome, the effect of registration on the position of the liver can be illustrated. Figure 4 shows the intensity along such a column for the phases of 1 examination along with the time–intensity curves for a 1-cm³ cubic ROI placed 1 cm below the liver dome. The inferosuperior intensity profile exhibits motion before registration. After conventional registration, the motion is reduced for most phases, but it fails for a few precontrast phases. The intensity profile produced by the robust registration method has no such obviously failed registrations. Failed registrations like the one seen in Figure 4C–D were not found among any of the registrations produced by the robust method.

The mean TISA, \( m \), for all examinations is shown with respect to the normalized time in Figure 5B. As a reference to help interpret the normalized time, \( \tau \), the mean of all liver time–intensity curves is shown in Figure 5A. This figure further illustrates where a particular value of \( \tau \) corresponds to the precontrast region (\( \tau < -1.5 \)), the intensity ramp (\( -1.5 < \tau < 1.5 \)), or the postcontrast region (\( \tau > 1.5 \)). The TISA curves are consistently higher in the precontrast region compared with those in the postcontrast region. This is a result of the lower mean intensity of the phases in the precontrast region. In addition, at the beginning of the intensity ramp (around \( \tau = -1 \)), the conventional registration procedure performs almost as poorly as no registration, as seen by the close proximity of their TISA curves (green and red). Confidence intervals for the paired TISA differences between the robust method, the conventional
method, and no registration are given in Table 3. The robust method is significantly better than no registration for the whole time interval (paired t test, \( P < .001 \)). It is also significantly better than the conventional method for the precontrast and early contrast regions (\( P < .001 \)).

### DISCUSSION
In this study, we have implemented an overdetermined registration procedure for liver DCE-MRI examinations. We found that our procedure significantly reduces the image registration errors in the DCE time curves, particularly in the precontrast and early contrast uptake phases, compared with a conventional reference-phase-based registration method. Registration errors in the precontrast baseline could profoundly affect the calculation of signal intensity changes and the conversion to contrast concentration, whereas errors in the early contrast uptake curve propagate into derived parameters that are sensitive to the early curve dynamics, for example, hepatic arterial perfusion in liver DCE-MRI. The improved DCE curves describe the contrast agent uptake dynamics more correctly, and thereby, provide a better foundation for PK model parameter estimation.

DCE images acquired at different parts of the dynamic curves have different sensitivities to image registration errors. We found that registering precontrast phases to postcontrast phases is more likely to result in registration errors than registering within the precontrast phases or within the postcontrast phases. The conventional reference-based registration method likely results in a higher misregistration rate for the precontrast phases, as well as the early contrast uptake phases, if the postcontrast phase is chosen as reference. As an effect of this phenomenon, spikes can appear in or around the baseline plateau in the time–intensity curves. The parameters of a PK model may still be possible to estimate with such spikes present by using robust regression methods. However, each registration failure reduces the number of reliable samples. Even worse, if sample points are lost on the intensity ramp by removing the misregistration points, the remaining samples may not be able to correctly describe the dynamics of the curve, resulting in faulty PK parameter estimates. Some parameters are highly sensitive to the shape of the initial ramp of the uptake curve, for instance, hepatic arterial perfusion in the liver. The robust registration procedure presented reduces the number of failed registrations in the precontrast region by rejecting them as outliers in an overdetermined system of transform equations and thereby provides a greater number of reliable samples for parameter estimation.

For the postcontrast plateau, the robust registration method and the conventional registration method perform almost equally well. This is a result of the higher signal-to-noise ratio and more stable contrast for the phases of this region. Both registration methods produce smoother curves than no registration. The robust method is seen to have a slightly lower TISA than the conventional method. This is because the robust method takes the mutual information of all pairs of phases into account when estimating the transform. Therefore, the robust method acts as a more efficient estimator for the true transforms than does the conventional method.

In this work, we performed each registration separately and then combined the resulting transforms using a method that rejects outliers. Another possible approach would be to maximize the sum of the mutual information metrics of all pairs of phases using strategies similar to those reported by others (22-24). Such an approach might improve the registration of DCE-MRI further. In addition, although the method presented in this paper is only applicable to rigid registration, a method based on the maximization of pairwise mutual information could aid in improving the robustness of deformable registration for DCE-MRI. Robust deformable registration could allow free breathing examinations, which would relieve subjects from the burden of repeatedly holding their breath.

The robust method produced a consistent registration quality across all examinations, without failed alignments such as those observed for the conventional method. It therefore reduces the need for manual intervention following registration and helps to simplify the workflow of clinical studies with many subjects.

### CONCLUSIONS
A method has been presented that registers every possible pair of phases from a DCE-MRI examination and derives a final set of transforms from them using \( L_{1/2} \) minimization. The method is robust, insofar as to be able to reject such failed registrations that can appear when registering precontrast to postcontrast phases. The robust registration method improves the smoothness of the resulting time–intensity curves of voxels in the liver by eliminating spurious intensity spikes induced by motion or failed registration. Therefore, a baseline with reduced bias and a larger number of reliable samples in the intensity ramp are made available for the PK model parameter estimation. The consistent registration quality produced for all examinations studied shows that the method could simplify the workflow of clinical studies with many patients by eliminating the need for manual intervention following registration.
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REFERENCES
A Classification System for the Spread of Polymethyl Methacrylate in Vertebral Bodies Treated with Vertebral Augmentation

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Key Words: kyphoplasty, polymethyl methacrylate, vertebral compression fracture
Abbreviations: Polymethyl methacrylate (PMMA), vertebral compression fractures (VCFs), balloon kyphoplasty (BKP), percutaneous vertebroplasty (PVP)

In this study, we develop a classification system for describing polymethyl methacrylate (PMMA) spread in vertebral bodies after kyphoplasty or vertebroplasty for vertebral compression fractures (VCFs) and for assessing whether PMMA spread varies between operators, VCF etiology, or vertebral level. Intraoperative fluoroscopic images of 198 vertebral levels were reviewed in 137 patients (women, 84; men, 53; mean age, 75.8 ± 12.5; and those with a diagnosis of osteoporosis, 63%) treated with kyphoplasty between January 01, 2015 and May 31, 2015 at a single center to create a 5-class descriptive system. PMMA spread patterns in the same images were then classified by 2 board-certified radiologists, and a third board-certified radiologist resolved conflicts. A total of 2 primary PMMA spread patterns were identified, namely, acinar and globular, with subtypes of localized acinar, diffuse globular, and mixed, to describe an equal combination of patterns. Interrater reliability using the system was moderate (κ = 0.47). After resolving conflicts, the most common spread class was globular (n = 63), followed by mixed (n = 58), diffuse globular (n = 30), acinar (n = 27), and localized acinar (n = 20). The spread class after treatment by the 2 most frequent operators differed significantly (n1 = 63, n2 = 70; P < .0001). There was no difference in the spread class between VCF etiologies or vertebral levels. PMMA spread may, therefore, be a modifiable parameter that affects kyphoplasty and vertebroplasty efficacy and adverse events.

INTRODUCTION
Vertebral compression fractures (VCFs) can be asymptomatic or present with signs and symptoms such as height loss, kyphosis, and functional disability (1, 2). Balloon kyphoplasty (BKP) and percutaneous vertebroplasty (PVP) are commonly used procedures performed under fluoroscopic guidance to treat painful VCFs refractory to medical management or bracing, which remain the subject of investigation (3). Large open-label trials have shown earlier decreased pain, decreased pain at 1 year, more pain-free days over 1 year, and decreased analgesic use among patients treated with vertebral augmentation compared with conservative therapy for painful acute VCFs (4, 5). Serious adverse events are rarely caused by either BKP or PVP, and a considerable proportion of those that occur are due to cement embolism (6, 7). We also reported a case of vertebral refracture after asymmetric polymethyl methacrylate (PMMA) spread (8). Biomechanical studies have shown that distribution of PMMA in vertebral bodies is correlated with strength and stiffness parameters that may impact treatment efficacy (9, 10). Variability in how the procedures are performed presents an obstacle to effective analysis of adverse event frequency and pain-reducing efficacy (11). The absolute volume of injected PMMA has been shown to have a large variability (12). Cement viscosity has been shown to significantly impact PMMA spread; yet, there is no routine quantitative measurement of viscosity before injection (13). Finally, properties of vertebrae itself are associated with PMMA spread (14).

Multiple known and unknown factors result in an ultimate PMMA spread pattern that is visible on radiographs. Currently, there is no standardized language to describe the imaging appearance of PMMA spread within a vertebral body. This prevents retrospective and prospective analyses of a possible association between PMMA spread and either BKP or PVP outcomes. The purpose of this study was to develop a classification system to describe PMMA spread in vertebral bodies and assess whether PMMA spread varied between
operators or because of the properties of the vertebrae that were injected.

**METHODOLOGY**

Approval for retrieval and analysis of electronic medical records (EpicCare EMR; Epic, Verona, Wisconsin) was obtained from the local institutional review board, and informed consent was waived. Accessed records included demographic and clinical data and intraoperative fluoroscopic images.

**Patient Population**

BKP was recommended for patients with acute VCFs refractory to conservative therapy, who exhibited edema on spinal magnetic resonance images (MAGNETOM Aera; Siemens, Munich, Germany) or active technetium-99m radiotracer (GE Healthcare, Little Chalfont, United Kingdom) uptake on single-photon emission computed tomography/computed tomography (Optima NM/CT 640; GE Healthcare) bone scans, and had localized tenderness over the fractured level. Conservative therapy included thoracolumbosacral orthosis bracing and/or pain medications.

A total of 198 VCFs (women, 84; men, 53; mean age, 75.8 ± 12.5; and those with a diagnosis of osteoporosis, 63%) were treated with BKP between January 01, 2015 and May 31, 2015 at a single center. Patient characteristics are described in Table 1. Radiofrequency ablation (SpineSTAR; DFine, San Jose, California) was performed in addition to BKP in 6 patients with either primary or metastatic osteolytic cancers at 9 total vertebral levels. A bone biopsy was collected in addition to BKP from 1 patient at 1 vertebral level.

**Procedure**

BKP, which has been previously described (15), was performed at all treated vertebral levels. In brief, a bone tamp was inserted into the vertebral body under fluoroscopic guidance, the inner stylet removed leaving the trocar, and a kyphoplasty balloon inserted through the trocar (Kyphon; Medtronic, Dublin, Ireland). The balloon was inflated with radiocontrast medium, which allows for visualization, compacts cancellous bone, and re-expands the vertebral body. Last, the balloon was deflated and removed, and PMMA from the Kyphon kit was injected through the trocar under fluoroscopic guidance. The method of vertebral body access was recorded for BKP at 160 levels. A unipedicular approach was used at 111 (69.4%) vertebral levels, and a bipediculat approach at 49 (39.6%) vertebral levels. Fluoroscopy time was recorded during 96 procedures for treatment of 137 vertebral levels, and mean time was 10.2 ± 5.9 minutes per procedure or 8.3 ± 4.1 minutes per vertebral level.

**PMMA Spread Classification**

We developed a 5-class system to describe PMMA spread after a review of anterior–posterior and lateral intraoperative fluoroscopic images from all procedures. A preliminary classification system was developed while viewing the complete image set for the first time. The system was refined during a subsequent review of the images by the same viewers. The same intraoperative fluoroscopic images were then reviewed by 2 additional board-certified radiologists who classified PMMA spread at each level according to the system. A third board-certified radiologist resolved conflicts.

**Statistics**

Continuous variables were expressed as mean ± standard deviation. Interrater reliability was assessed using Cohen’s kappa coefficient. Associations between PMMA spread characteristics and categorical variables were assessed using Pearson’s χ² tests. The 5th–8th and 9th–11th thoracic vertebrae, 12th thoracic vertebra through the 2nd lumbar vertebra, and the 3rd–5th lumbar vertebrae were binned together to meet expected cell-count assumptions of the χ² tests. All analyses were performed using SAS 9.3 and SAS Enterprise Guide 5.1.

**RESULTS**

**Description of the 5-Class System**

We identified 2 primary patterns of PMMA spread that were visible on the intraoperative fluoroscopic images. Subclassification of these patterns considering the number of the vertebral bodies infiltrated with PMMA, and a pattern admixture yielded a total of 5 PMMA spread classes: acinar and globular, with subtypes of localized acinar, diffuse globular, and mixed, to describe an equal combination of patterns within the vertebral body. Prototypes are shown in Figure 1.

The acinar pattern of spread was defined as expected when filling complex cortical bone with a low-viscosity fluid. Characteristics commonly associated with the acinar pattern spread included numerous small dot-like collections of PMMA showing lobular smooth margins and homogeneous texture. The prototypical globular pattern was a circular cannonball without extension across the vertebral body midline. However, globular pattern variants also included more lobulated amorphous shapes. Vertebral bodies showing a mixture of at least 40% of both acinar and globular spread pattern components on at least 1 imaging angle were classified as having a mixed spread.

A substantial proportion of treated vertebral bodies showed near-complete PMMA filling in at least 1 viewing angle. Diffuse spread was defined by >90% of the anterior–posterior or medial–lateral axis of the vertebral body making up the border of PMMA spread on at least 1 viewing angle with spread height and pattern homogeneity throughout. Globular pattern spread was most often localized and not diffuse. Therefore, diffuse

| Table 1. Patient Characteristics |
|-----------------|----------------|----------------|
| Number of treated patients | 53 | 84 | 137 |
| Number of treated levels | 75 | 123 | 198 |
| Age (years) | 74.4 ± 13.6 | 76.6 ± 11.7 | 75.8 ± 12.5 |
| Etiology by patienta | 21/11/7/2 | 49/7/14/0 | 70/18/21/2 |
| Etiology by levela | 35/19/7/2 | 73/14/17/0 | 108/33/24/2 |

a Fracture etiology was recorded for 111 patients treated at 167 levels. Counts are listed as osteoporosis/cancer/trauma/other.
globular spread was considered as a subtype. The acinar pattern spread was most often diffuse. Similarly, the localized acinar spread was considered as another subtype. In theory, a diffuse mixed spread would appear as layers of globular and acinar spread extending across the vertebral body, although this was not observed in our sample.

Interrater reliability using the entire system was moderate ($\kappa = 0.47$). Similarly, interrater reliability was moderate for assessing PMMA infiltration ($\kappa = 0.49$) and pattern ($\kappa = 0.48$). A total of 51.2% of the levels were classified as having the same spread class by the first 2 raters. Of the vertebral levels not assigned the same spread class by the first 2 raters, 68.8% were assigned spread classes that were considered to be the most similar (Figure 2). Raters assigned 78.3% of the levels as having the same degree of PMMA infiltration (localized vs diffuse) and 60.1% of the levels as having the same spread pattern (acinar vs globular vs mixed). Examples of difficult-to-classify images are in Figure 3.

The most common spread class after resolving conflicts was globular ($n = 63$), followed by mixed ($n = 58$), diffuse globular ($n = 30$), acinar ($n = 27$), and localized acinar ($n = 20$). Therefore, 80.1% of levels were considered as localized and 19.9% as diffuse. The distribution of levels that were assigned various spread patterns was 23.7% acinar, 47.0% globular, and 29.3% mixed.

### Spread Pattern Varies by Operator

There was a significant difference in the spread class of vertebral bodies treated by the 2 most frequent operators ($n_1 = 63$, $n_2 = 70$; $P < .0001$). PMMA infiltration also differed by operator ($P < .0001$), and there was a marginal difference in the spread pattern between operators ($P = .07$). The 2 most common spread patterns for operator 1 were mixed and globular, whereas the 2 most common spread patterns for operator 2 were diffuse globular and acinar.
PMMA spread was provided despite an analysis of the radiographic outcomes such as height and kyphotic angle restoration (16). Baroud et al. stated that an appearance similar to a “single, uniformly expanding cloud” was preferable to that like the “fingers of a glove,” which again correspond with our globular and acinar types without distinguishing localized from diffuse spread or describing mixed spread (17). A case report attributed lateral wedging after BKP to “abnormal spatial distribution of PMMA cement . . . [with] insufficient filling of PMMA cement . . . on the right side” (18). Radiopacity was highlighted as a major imaging difference between 2 cements used in an ex vivo biomechanical study (19). In another ex vivo study, Loeffel et al. made the sole quantification of PMMA spread by calculating circularity (a ratio of the actual distribution perimeter to the perimeter of a circle with equal area) and mean cement-spread-distance (14). However, these measurements did not assess the pattern within the filled area, which may reflect the PMMA concentration. Our classification system required an assessment of the spread pattern within the filled area, as well as an assessment of the degree of PMMA infiltration into the vertebral body. Interrater agreement was considered moderate on the basis of a frequently cited scale (20, 21), which is similar to the agreement found using the current protocols for prostate cancer (22), pancreatic cancer (23), and pulmonary nodule (24). Our system’s categories are related, and image interpretation disagreement was most often found between closely related categories (Figure 2). This gives the classification system utility in outcomes’ trials beyond what its moderate interrater reliability suggests because the effect of related classes on outcomes is likely similar and the effect of less-related classes is divergent. Raters agreed more frequently on the spread pattern (acinar vs globular vs mixed) and PMMA infiltration (localized vs diffuse) than on the spread class. However, interrater reliability was similar between all 3 measures, indicating that reducing the number of spread classes would not make the system more robust.

We used the newly created classification system to first assess differences in PMMA spread between operators. It is paramount to understand interoperator variability in BKP and PVP, as the largest clinical trials, to date, have been conducted at multiple sites and have not adjusted for potential heterogeneity between operators (4, 5, 25, 26). McDonald et al. showed, in a sample of 2 operators experienced in PVP and 5 novice operators, that several procedural measures and short-term clinical outcomes significantly change as novice operators gain experience (27). Other studies have found that the volume of PMMA injected is an important operator-dependent variable in BKP and PVP (28, 29). In addition, the time between PMMA mixing and delivery modifies viscosity, which may impact the risk of adverse events (13, 17). We showed that the 2 experienced operators achieved significantly different PMMA spreads. The first most often created a localized PMMA infiltration with a trend toward a globular pattern, whereas the other most often created a diffuse spread without a trend toward either an acinar or a globular pattern. This indicates that there is a significant heterogeneity even between experienced operators. Clinical outcomes could differ between approaches.

There are several mechanisms through which PMMA spread could affect either BKP or PVP outcomes. Liu et al. found that
PMMA distribution in the inferior portion of the vertebral body or along the endplate increases the risk of adjacent-level refracture (30). Biomechanical and finite-element analysis studies have shown that limited distribution of PMMA after unipedicular BKP or PVP potentially result in biomechanical imbalance, which could lead to lateral wedge deformities or painful tugging (9, 10). Intrathecal nerve damage has been hypothesized as a potential mechanism for pain relief after BKP or PVP (31). PMMA spread could therefore impact which nerves and how many nerves are damaged within the vertebral body. Operators may use the classification system presented in this study to quickly evaluate cement spread after procedures. Although clear recommendations do not currently exist, previous reports suggest that globular diffuse spread provides maximal biomechanical support to treated vertebral bodies with minimal risk of cement extravasation (9, 10, 17).

The next step in our study was to assess whether VCF etiology or vertebral level were associated with PMMA spread class. Loeffel et al. found that artificial media with smaller pores aided in creating denser, more circular PMMA spread (14). They interpreted this to indicate that PMMA will spread widely and unevenly in osteoporotic bone. There are few in vivo comparisons of spread between VCFs of different etiologies. In 1 study without a systematic approach for assessing spread, PMMA spread appearance was interpreted as different in VCFs because of osteoporosis compared with metastatic lesions (13). However, other studies that included patients with VCFs secondary to osteoporosis and osteolytic lesions did not compare appearance (4, 32–34). There have been no in vivo comparisons of PMMA spread within the vertebrae from different levels. In contrast to previous reports, we found no difference in PMMA spread class between fracture etiologies. We also did not observe a difference between the vertebral levels.

The limitations of our study must be acknowledged. First, the sample was drawn from a single center and only 2 operators performed a sufficient number of procedures to be compared. However, 198 total vertebral levels were treated, and finding differences in a small sample of operators may indicate that heterogeneity is prevalent. Second, VCF etiology was not uniformly reported. Despite this, an adequate number were available for comparison. Our analysis was limited to fluoroscopic images during BKP, but the classification system encompasses PMMA distributions expected after PVP. Last, a standardized follow-up was not obtained from patients, which prevents the analysis of a potential association between PMMA spread class with adverse events and pain reduction in this sample. However, the classification scheme we developed will enable future assessment of this.

We created a standardized language with which to describe PMMA spread after either BKP or PVP. PMMA spread is primarily operator-dependent, and therefore, may be a modifiable parameter that affects BKP and PVP outcomes. Future research is needed to determine if PMMA spread classes are associated with clinical outcomes after either BKP or PVP.

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REFERENCES


Noninvasive Electric Current Induction for Low-Frequency Tissue Conductivity Reconstruction: Is It Feasible With a TMS-MRI Setup?

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Key Words: low frequency, conductivity, TMS-MRI, MR phase maps

Abbreviations: Magnetic resonance (MR), transcranial magnetic stimulation (TMS), magnetic resonance imaging (MRI), low frequency (LF), electromagnetic (EM), MR-electrical impedance tomography (MR-EIT), radiofrequency (RF), signal-to-noise ratio (SNR)

Noninvasive quantification of subject-specific low-frequency brain tissue conductivity ($\sigma_{LF}$) will be valuable in different fields, for example, neuroscience. Magnetic resonance (MR)-electrical impedance tomography allows measurements of $\sigma_{LF}$. However, the required high level of direct current injection leads to an undesirable pain sensation. Following the same principles, but avoiding pain sensation, we evaluate the feasibility of inductively inducing currents using a transcranial magnetic stimulation (TMS) device and recording the magnetic field variations arising from the induced tissue eddy currents using a standard 3 T MR scanner. Using simulations, we characterize the strength of the incident TMS magnetic field arising from the current running in the TMS coil, the strength of the induced magnetic field arising from the induced currents in tissues by TMS pulses, and the MR phase accuracy required to measure this latter magnetic field containing information about $\sigma_{LF}$. Then, using TMS-MRI measurements, we evaluate the achievable phase accuracy for a typical TMS-MRI setup. From measurements and simulations, it is crucial to discriminate the incident from the induced magnetic field. The incident TMS magnetic field range is $-10^{-4}$ T, measurable with standard MR scanners. In contrast, the induced TMS magnetic field is much weaker ($\pm 10^{-8}$ T), leading to an MR phase contribution of $\sim 10^{-4}$ rad. This phase range is too small to be measured, as the phase accuracy for TMS-MRI experiments is $\sim 10^{-2}$ rads. Thus, although highly attractive, noninvasive measurements of the induced TMS magnetic field, and therefore estimations of $\sigma_{LF}$, are experimentally not feasible.

INTRODUCTION

Noninvasive mapping of tissue electrical properties in the megahertz range has recently become feasible with the development of magnetic resonance imaging (MRI)-based electrical property tomography (1-5). However, precise knowledge on tissue electrical conductivity at low frequency (LF: Hz–100 kHz) and the relation between electrical conduction and tissue composition in this frequency range is still limited. In the kilohertz range, the human body is electrically very heterogeneous (6-8) as cellular fraction, water-ionic content, and cell membranes modulate electrical conductivity. Unfortunately, pathologies change these factors, causing differences in tissue conductivity values ($\sigma_{LF}$) between healthy and nonhealthy subjects (9, 10). The ability to measure these subject-specific $\sigma_{LF}$ values of brain tissues is particularly a desired competence in neuroscience, as various diagnostic techniques and neurostimulation modalities like transcranial magnetic stimulation (TMS) operate in this frequency range (11-14).

TMS is an emerging technique that allows noninvasive modulation of cortical neurophysiology to diagnose and treat neurological disorders (15-20). Based on the Faraday induction principle, TMS uses a strong, time-varying magnetic field to inductively induce an electric field in the brain that can cause neuroactivation (Figure 1A) (21-24). Practically, TMS dosimetry is performed in a highly empirical fashion by using the “motor threshold” method (20, 23), where the motor cortex serves as...
reference area. However, because the electric field induced in the brain is modulated by the varying dielectric properties and the gyrification of the cortex (25, 26), the TMS dose varies for brain regions that are different from the motor cortex (27-30). Therefore, the motor threshold method is unreliable for most TMS purposes (31-33).

To precisely guide TMS administration and to better understand the behavioral consequences of the deployed TMS electric field, different research groups are focusing their investigations on how stimulation parameters (number of TMS pulses, pulses’ strength, coil models, and orientation) affect the induced TMS electric field by means of electromagnetic (EM) simulations (34-39). Although these valuable studies correctly adopt heterogeneous conductive brain models in the computation of the induced TMS electric field, the adopted conductivity values are simply derived from healthy group averages (40-42). Unfortunately, as argued in other studies (24, 30, 43-46), healthy group averages of \( \sigma_{LF} \) cannot ensure optimal subject-specific dosimetry, as various factors such as ageing (47) and pathologies (10) induce variations in \( \sigma_{LF} \) values. Moreover, because the induced electric field is also modulated by the tissue geometry, having subject-specific brain models would be valuable (32, 44, 48). Although this latter requirement can be satisfied by segmenting magnetic resonance (MR) images acquired before TMS administration, being able to noninvasively and nonpainfully determine subject-specific tissue \( \sigma_{LF} \) values is still an unresolved issue.

LF tissue conductivity can be mapped using MR-electrical impedance tomography (MR-EIT) (49-51). In this technique, strong direct current (10 mA) is injected into the brain via skin surface electrodes (Figure 2A) while the subject is positioned in an MR scanner. The spatial pattern of these currents is modulated by the underlying tissue \( \sigma_{LF} \) distribution. In turn, these injected currents lead to an induced magnetic field, in which information on \( \sigma_{LF} \) is thus imprinted. By measuring this induced magnetic field using MR phase measurements, \( \sigma_{LF} \) maps can be reconstructed (52, 53). However, strong currents and long injection times (10 milliseconds) are needed to achieve adequate MR phase accuracy in MR-EIT experiments. These requirements result in a sensation of pain that limits the in vivo applicability of MR-EIT.

To map tissue \( \sigma_{LF} \) by avoiding direct current injection, using time-varying magnetic fields created by external coils to inductively induce currents has been suggested (Figure 1B and Figure 2B) (54). Subsequently, by following this inductive fashion, directly using the MR gradient coils to induce currents (55-57) has been suggested. Thus, high current density at injection points and, thus, pain sensation are avoided, making this approach very attractive and applicable to standard clinical MR scanners. However, tissue \( \sigma_{LF} \) reconstructions were not feasible. In fact, the phase contribution arising from the induced magnetic field is too small to be accurately measured with standard MR systems (58, 59). In addition, it has also been shown that subtle, unavoidable imaging distortions hamper measurements of this phase contribution by creating a pseudo-LF conductivity contrast (60).

By following the appealing idea of inductively inducing currents in tissues, in this study, we use a TMS setup to induce much stronger currents (Figure 1A) in combination with an MR scanner used to measure the arising induced magnetic field (Figure 1B). Moreover, while standard MR gradient coils allow slew rates of 20 T/s at 10 cm from the gradients’ isocenter, a TMS device can generate slew rates of up to 20 000 T/s. Thus, the reported 3 orders of magnitude increase that are needed to measure the induced magnetic field could be theoretically achieved (59). We have divided this study in 2 parts. First, using simulations, we characterize the strength of the induced magnetic field carrying information on the induced currents in the

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**Figure 1.** A time-varying current (I_{coil}) running in a figure-8-shaped TMS coil creates a time-varying magnetic field (B_{coil}) which, in turn, induces an electric field (E_{tissue}) in brain tissues (A). Because of the conductive nature (\( \sigma_{LF} \)) of brain tissues, induced currents (J_{tissue}) arise from E_{tissue} (B). These induced currents create an induced magnetic field (B_{tissue}). Information on low-frequency (LF) tissue conductivity \( \sigma_{LF} \) is, therefore, imprinted only in B_{tissue} and not in B_{coil}.
tissue and, thus, the LF conductivity and compare with that of the incident TMS magnetic field. This was evaluated for different conductive cylindrical models and realistic human brain models. Thus, we characterize the required phase accuracy to detect these induced magnetic fields with MRI. Moreover, we study the impact of different TMS waveforms. Second, MR measurements on phantoms are presented to investigate the achievable phase accuracy for a typical TMS-MRI setup. With this study, we investigate whether inductively inducing currents in tissues by using a TMS-MRI setup is a feasible methodology for performing noninvasive LF tissue conductivity reconstructions.

THEORY

In TMS, the presence of conductive tissues such as the brain underneath the TMS coil leads to correction terms in the computation of the TMS EM field, which are a function of the tissue conductivity distribution (Figure 2B). In this kilohertz range, where displacement currents are negligible, these corrections can be modeled by the so-called quasi-static approximation (35). For this purpose, Maxwell equations are expanded in power series in the frequency domain (61): 

$$\nabla \times E^{[k]} = -\frac{\partial B^{[k-1]}}{\partial t},$$

$$J^{[k]} = \sigma_{LF} E^{[k]},$$

$$\nabla \times B^{[k]} = \mu_0 J^{[k]},$$

where a quasi-static condition is assumed in equation (2) (35, 62). For readability purposes, we do not explicitly write the spatial dependency ($r$) of the vector fields. In addition, the conductivity $\sigma_{LF}$ is also a tensor because of tissue anisotropy, but we can consider it as a scalar value for simplicity of derivation. From equation (3), the Biot–Savart law can be derived as follows:

$$B^{[k]} = \frac{\mu_0}{4\pi} \int \frac{J^{[k]} \times (r - r_0)}{|r - r_0|^3} \, dV_0. \quad (4)$$

In TMS, for $k = 0$, the zero-order vectors in the brain/object satisfy the static field equations $\nabla \times E^{[0]} = 0$, $E^{[0]} = 0$, $\nabla \times B^{[0]} = 0$, $\nabla \cdot B^{[0]} = 0$. $B^{[0]}$ is the incident TMS magnetic field arising from the current running in the TMS coil, thus not carrying any information about tissue conductivity. Throughout the paper, we will refer to this magnetic field as $B_{coil}$ as follows:

$$B^{[0]} = B_{coil} \quad (5)$$

which gives an MR phase contributions defined as $\Phi_{B_{coil}}$ (63).

Higher-order field corrections of order $k$ can be computed using the vectors of order $k - 1$ as sources (Figure 2B). For $k = 1$, the following equations are computed:

$$\nabla \times E^{[1]} = \frac{\partial B^{[0]}}{\partial t} = -\frac{\partial B_{coil}}{\partial t},$$

$$J^{[1]} = \sigma_{LF} E^{[1]},$$

$$\nabla \times B^{[1]} = \mu_0 J^{[1]},$$

where $E^{[1]}$ and $J^{[1]}$ are, respectively, the first-order electric field and current density induced in a conductive domain such as the brain, and $B^{[1]}$ is the first-order induced magnetic field arising from $J^{[1]}$. Therefore, information on tissue conductivity is imprinted in $B^{[1]}$.

The total induced electric field in brain tissues, called as $E_{tissue}$ throughout the paper, is:

$$E_{tissue} = \sum_{k=1}^{\infty} E^{[k]}.$$

$$
In principle, \( E_{\text{tissue}} \) is a solenoidal electric field induced by the time-varying incident TMS magnetic field \( B_{\text{coil}} \) [equation (6)]. However, because of the nonhomogeneous conductivity distribution of brain tissues, charge is accumulated at the boundaries between different conductive structures, leading to a conservative electric field that affects the incident, solenoidal electric field \((25, 35, 48)\) as follows:

\[
E_{\text{tissue}} = E_{\text{solenoidal}} + E_{\text{conservative}} \tag{11}
\]

\( E_{\text{solenoidal}} \) is proportional to the time-varying incident vector potential \( A_{\text{coil}} \), which depends solely on the TMS coil configuration and level of current running into it \((E_{\text{solenoidal}} = \frac{\partial A_{\text{coil}}}{\partial t})\). Thus, \( E_{\text{solenoidal}} \) is always present, independently from the conductor underneath the TMS coil. Instead, \( E_{\text{conservative}} \), which arises from the charge accumulation at tissue boundaries between different conductive tissues \((E_{\text{conservative}} = -\nabla \Phi, \text{with } \Phi \text{ electrical potential})\), is directly modulated by the underlying tissue geometry and conductivity distribution \( \sigma_{\text{LF}} \) \((25, 35, 48)\). From equation (2), the total induced current density in tissue, called as \( J_{\text{tissue}} \), is, therefore, \( J_{\text{tissue}} = \sigma_{\text{LF}} E_{\text{tissue}} \).

Analogous to equation (10), the total induced TMS magnetic field, called as \( B_{\text{tissue}} \), is:

\[
B_{\text{tissue}} = \sum_{k=1}^{\infty} B_{k}^{[k]} \tag{12}
\]

which gives an MR phase contributions defined as \( \Phi_{B_{\text{tissue}}} \).

By combining equations (5) and (12), the total TMS magnetic field is, therefore, defined as follows:

\[
B_{\text{total}} = \sum_{k=0}^{\infty} B_{k}^{[k]} = B_{0}^{[0]} + \sum_{k=1}^{\infty} B_{k}^{[k]} = B_{\text{coil}} + B_{\text{tissue}} \tag{13}
\]

**MATERIALS AND METHODS**

**Simulations**

EM simulations aimed to characterize the strength of the incident and the induced TMS magnetic fields \( B_{\text{coil}} \) and \( B_{\text{tissue}} \), respectively) by using the quasi-static approximation described in the theory section. We then assessed the phase accuracy needed to detect \( \Phi_{B_{\text{tissue}}} \) in concurrent TMS-MRI experiments. In addition, we characterized the impact of different conductivity distributions of \( \sigma_{\text{LF}} \) on \( B_{\text{tissue}} \).

Three simulations were performed in SCIRun (64), namely, 2 on conductive cylinders and 1 on a realistic human brain model. For the performed simulations, the TMS coil was modeled using 2 single-plane spiral wings (65), reflecting the geometry of the TMS coil used in the measurements. These wings were placed at 5 cm from the cylinders to mimic the actual position used in the measurements and in contact with the scalp to mimic the position in realistic TMS treatments.

In the first simulation, we characterized the strength of the \( z \)-component of the net (time average over the TMS pulse) incident TMS magnetic field \( B_{\text{coil}} \), the only 1 component (parallel to the MR static magnetic field \( B_{0} \)) measurable in an MR experiment. We also characterized the range of the net phase contribution \( \Phi_{B_{\text{coil}}} \) that would arise from \( B_{\text{coil}} \) in an MR experiment. In an MR experiment, the phase contribution \( \Phi_{B_{\text{coil}}} \) is proportional to the area underneath the TMS current waveform (63). The same phase contribution can be obtained by using the time average value of the TMS current waveform (see online supplemental Appendix A, \( I_{\text{coil}} \)) computed from independent oscilloscope measurements. For this simulation, a typical bipolar TMS pulse that lasts for a full period was used (Figure 3A). The TMS output was set to 1%, leading to \( I_{\text{coil}} = 3.5 \text{ A} \). By applying the Biot–Savart law, the net \( B_{\text{coil}} \) was computed. This simulation was performed using a homogeneous conductive cylinder (Figure 4A) with the same geometry and electric conductivity as that of the phantom used in the measurements (Figure 4D). Thus, consistent comparison with measurements could be performed. However, for a bipolar TMS pulse that lasts for a full period, the net induced current in tissue \( J_{\text{tissue}} \) is zero (see online supplemental Appendix A and Figure 3B) (66). Thus, obviously, the induced magnetic field \( B_{\text{tissue}} \) and its related phase contribution \( \Phi_{B_{\text{tissue}}} \) are zero.

Because information on \( \sigma_{\text{LF}} \) is imprinted solely in \( B_{\text{tissue}} \) to induce a non-zero net \( B_{\text{tissue}} \), a truncated TMS waveform should be used (see online supplemental Appendix A and Figure 3, case 2). Consequently, in the second simulation, we used the same waveform adopted in the first simulation but truncated at the first quarter (63), TMS output 1%, and \( t_{1} = 0.1 \text{ ms} \), leading to \( I_{\text{coil}} = 35 \text{ A} \) and a rate of change of the coil current of \( 0.55 \times 10^{6} \text{ A} \), in line with other studies (36, 42). We characterized the strength of the net \( B_{\text{coil}} \) and \( B_{\text{coil}} \) for such a truncated TMS pulse. Then, the 3-dimensional mesh model, the conductivity distribution, and the vector potential (computed using the rate of change of the coil current) (35) were given as input to the finite element method (FEM) solver to compute \( E_{\text{tissue}} \) and \( J_{\text{tissue}} \). From \( J_{\text{tissue}} \), the strength of \( B_{\text{tissue}} \) and the range of \( \Phi_{B_{\text{tissue}}} \) were characterized. By performing this simulation on 2 different conductive cylinders (one homogeneous and one consisting of 2 different conducting compartments, (Figure 4, A and B) (67), the impact of different conductive compartments was evaluated.

Then, in the third simulation, we defined the strength of \( B_{\text{coil}} \) and \( B_{\text{tissue}} \) for realistic in vivo situations by using a realistic human brain model (68) and the truncated TMS waveform adopted in the second simulation. We, therefore, explored the feasibility of measuring \( \Phi_{B_{\text{tissue}}} \) in vivo by characterizing the required phase accuracy for concurrent TMS-MRI experiments. In addition, we evaluated the impact of different \( \sigma_{\text{LF}} \) distributions on \( E_{\text{tissue}} \) (relevant quantity for TMS dosimetry) and \( B_{\text{tissue}} \). Finally, we characterized the phase accuracy needed to detect subtle variations in \( B_{\text{tissue}} \) arising from these variations in \( \sigma_{\text{LF}} \). The phase accuracy determines the feasibility of this technique. The adopted \( \sigma_{\text{LF}} \) values reflect the conductivity variations reported in other studies (Figure 4C) (35, 38, 42).

**Measurements**

Concurrent TMS-MRI measurements were conducted in a clinical 3 T MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) with elliptical surface MR receive coils (flex-M) and using a standard TMS stimulator (Magstim Rapid2, Whitland, UK) with an MR-compatible figure-8-shaped TMS coil (28, 69). Using a typical TMS-MRI setup, the phase accuracy characterized by these measurements is representative.
The MR sequence adopted was a spin-echo sequence with the following parameters: relaxation time/echo time = 1000/50 milliseconds, field of view = 160 x 160 x 25 mm³, resolution = 2.5 x 2.5 x 2.5 mm³, voxel/bandwidth = 0.15/3 kHz, and number of signal averages = 2. The TMS device was synchronized with the MR sequence by using the MR-transistor–transistor logic signal delivered at every radiofrequency (RF) excitation as a reference time point. TMS pulses were delivered before each readout gradient (69). The surface of the TMS coil was placed at 4.5 cm from the phantoms. For each experiment, 4 measurements were performed to correctly isolate the phase contributions $B_{coil}$ and $B_{tissue}$ (see online supplemental Appendix B and Figure 4D, measurement numbers M1–4). For the measurements with TMS-on, the TMS outputs were 1% and 4%, for the first and the second experiments, respectively. Because a standard TMS stimulator was used, only bipolar pulses that lasted for a full period could be used (Figure 3, case 1). For these measurements, we prepared 2 agar phantoms sturdy enough to prevent motion artifacts (60) as follows: 1 conductive (1.6 S/m) and 1 nonconductive, as a reference to compensate for $B_{coil}$ (see online supplemental Appendix B, Figure 4D). The 2 phantoms were carefully placed at the same position in the scanner by using a dedicated phantom holder designed for this purpose.

With these experiments, we characterized the phase range of $B_{coil}$, which, in principle, includes both the contributions $B_{coil}$ and $B_{tissue}$ by using the conductive phantom (see online supplemental Appendix B). We also characterized the phase range of only $B_{coil}$ by using the nonconductive phantom. This allowed direct comparison with the first simulation. We finally characterized the achievable MR phase accuracy (inverse of the signal-to-noise ratio [SNR]) (59) to enable $B_{tissue}$ measurements in concurrent TMS-MRI experiments for a realistic TMS-MRI setup.

**RESULTS**

The impact of a realistic TMS pulse shape that lasts for a full period (Figure 3, case 1) on the TMS-related phase contribution, $B_{coil}$, is characterized by using the homogeneous cylinder (Figure 5A). The results of this first simulation are shown on the same plane where measurements were performed, thus mimick-
ing the experimental setup and allowing direct comparison with the measurements. From simulations, $B_{coil,z}$ is on the order of $10^{-5}$ T (Figure 5B), leading to a $\Phi_{B_{coil}}$ in the order of radians (Figure 5C). This result suggests that $\Phi_{B_{coil}}$ and thus $B_{coil,z}$ can be measured in an MR experiment.

Figure 6 shows the results from the second simulations performed on 2 conductive cylinders (one homogeneous and one with 2 different conductive compartments, Figure 6A and Figure 6F, respectively) and using the truncated TMS pulse waveform to induce a nonzero $B_{tissue,z}$ (Figure 3, case 2). From these simulations, we observe that the use of a truncated TMS waveform leads to an increase in $B_{coil,z}$ ($10^{-4}$ T) and, consequently, in $\Phi_{B_{coil}}$ ($10^2$ rads), with respect to the use of a full TMS waveform (Figure 5). In addition, by comparing the results obtained from the 2 different conductive cylinders, we observe that the incident magnetic field $B_{coil,z}$ (Figure 6, B and G) and its related phase contribution $\Phi_{B_{coil}}$ (Figure 6, C and H) are not affected by the presence of different conductive compartments. This is because the incident magnetic field does not depend on the conductivity of the structure underneath the TMS coil. Instead, as shown by these simulations, the conductivity distribution $\sigma_{LF}$ modulates the induced magnetic field $B_{tissue,z}$ (Figure 6, D and I) and thus its related phase contribution $\Phi_{B_{tissue}}$ (Figure 6, E and J). The impact of $\sigma_{LF}$ variations is clearly visible from the discrepancy between the histograms of the 2 $B_{tissue,z}$ maps (Figure 6K). However, it is important to note that the induced magnetic field $B_{tissue,z}$ ($10^{-5}$ T) is about 4 orders of magnitude lower than the incident magnetic field $B_{coil,z}$ ($10^{-4}$ T). As shown in Figure 6, E and J, $\Phi_{B_{tissue}}$ is in the range of $\sim 10^{-4}$ rads. This result characterizes the phase accuracy needed
to measure $B_{tissue,z}$ in concurrent TMS-MRI experiments. Moreover, these results highlight the challenge of correctly disentangling the phase contributions arising from $B_{coil,z}$ and $B_{tissue,z}$, as the latter field constitutes a very small fraction of the former.

In the third simulations, the impact of inter-subject variations of $\sigma_{LF}$ on $E_{tissue}$ and $B_{tissue,z}$ and the range of $B_{coil,z}$ and $B_{tissue,z}$ are characterized for a realistic human brain model (Figure 7 and Figure 8, respectively). For the adopted 3 different conductive brain models, the norm of $E_{tissue}$ is shown on the gray matter and white matter surfaces (Figure 7). Our results correspond with the results presented in a previous valuable study (35). By comparing the mean and standard deviation of the top 30% values of $|E_{tissue}|$ for each brain model in the gray matter and the white matter, we observe that different $\sigma_{LF}$ values induce significant variations in the deployed $E_{tissue}$ (Figure 7, bar-plots). This highlights the importance of accurately predicting subject-specific tissue conductivity values to correctly guide TMS dosimetry.

For each brain model (Figure 8A), $B_{tissue,z}$ and the related phase contribution $\Phi_{B_{tissue}}$ are computed (Figure 8C). The induced magnetic field $B_{tissue,z} (10^{-8} \text{T})$ is about 4 orders of magnitude lower than the incident magnetic field $B_{coil,z} (10^{-4} \text{T})$ (Figure 8B), in line with the value observed for the cylindrical structure (Figure 6). In addition, $B_{tissue,z}$ maps show slightly different patterns between the 3 different brain models because of the different conductivity distributions. This is a direct consequence of the previously observed variations in the $E_{tissue}$ maps. As shown in Figure 8D, variations in $\sigma_{LF}$ lead to magnetic field variations in the range of nanotesla. From these results (Figure 8C), we conclude that the necessary MR phase accuracy needed to measure $\Phi_{B_{tissue}}$ for in vivo TMS-MRI experiments is about $10^{-4}$ rads. However, an even higher accuracy will be needed to actually detect variations in tissue conductivity distributions.

**Figure 6.** Phantom simulations (truncated TMS waveform). Homogeneous (A) and 2-compartment cylindrical models (F). z-Component of $B_{coil}$ (B, G) and $\Phi_{B_{coil}}$ maps (C, H), independent from the sample conductivity. z-Component of $B_{tissue}$ (D, I) and $\Phi_{B_{tissue}}$ maps for the 2 cylindrical models (E, J). Histograms of $B_{tissue}$ for the 2 models (K). Clearly visible variation in $B_{tissue}$ patterns between the 2 models.

**Figure 7.** Norms of $E_{tissue}$ in the gray matter (GM—top row) and white matter (WM—bottom row) for the 3 brain models. Mean and standard deviation of the top 30% values of the norm of $E_{tissue}$ for each brain model in GM and WM. It is visible how different $\sigma_{LF}$ distributions lead to significant variations in the induced electric field.
In Figures 9 and 10, the results of the 2 experiments performed by using a realistic TMS-MRI setup and a full TMS waveform are proposed. With these experiments, we assess the attainable MR phase accuracy for concurrent TMS-MRI experiments. In both experiments, 2 phase maps were acquired for each phantom (one conductive and one nonconductive): one with TMS-on (Figure 9 and Figure 10, A and C) and one with TMS-off (Figure 9 and Figure 10, B and D). The significant

**Figure 8.** Realistic head model and cut plane used for visualization purposes (A). z-Component of $B_{\text{coil}}$ and $\Phi_{B_{\text{coil}}}$ maps, both independent from $\sigma_f$ (B). For each model, the z-component of $B_{\text{tissue}}$ and $\Phi_{B_{\text{tissue}}}$ is computed (C). Variations in the z-component of $B_{\text{tissue}}$ because of different conductivity distributions (D). These maps were computed by subtracting $B_{\text{tissue}}$ of model 3 and $B_{\text{tissue}}$ of model 1 (upper part), and by subtracting $B_{\text{tissue}}$ of model 3 and $B_{\text{tissue}}$ of model 2 (bottom part).

**Figure 9.** Experiment 1: TMS $\sigma = 1\%$. Phase maps with TMS-on for the conductive (A) and nonconductive (C) phantoms. Phase maps with TMS-off for the conductive (B) and nonconductive (D) phantoms. Reconstructed $\Phi_{\text{TMS}}$ map for the conductive phantom (E). Reconstructed $\Phi_{B_{\text{coil}}}$ map for the reference phantom (F). Comparison between $\Phi_{\text{TMS}}$ and $\Phi_{B_{\text{coil}}}$ profiles (G). Reconstructed $B_{\text{coil}_z}$ map (H). $\Phi_{B_{\text{tissue}}}$ map (subtraction between $\Phi_{\text{TMS}}$ and $\Phi_{B_{\text{coil}}}$) (I).
while performing the subtraction between maps, the z-component (RF phase contribution, and T) quantities can be, in principle, obtained by subtracting is zero, as the net induced (radians) and B from the phase contribution and B would be valuable for is reconstructed (Figure 9H and Figure 10H). The profiles (G). Reconstructed B (Figure 9F and Figure 10F) are maps, we can observe a certain pattern in the maps [see online supplemental Appendix B, subtraction | VOLUME 2 NUMBER 3 SEPTEMBER 2016 211 | TOMOGRAPHY.ORG]. By subtracting the phase maps measured with TMS-on and TMS-off [subtraction inside parentheses in equation (B.2); see online supplemental Appendix B], \( \Phi_{\text{TMS}} \) (Figure 9E and Figure 10E) and \( \Phi_{B_{\text{coil}}} \) (Figure 9F and Figure 10F) are computed, respectively, for the conductive and the nonconductive phantoms. As shown in the plots (Figure 9G and Figure 10G), \( \Phi_{\text{TMS}} \) coincides with \( \Phi_{B_{\text{coil}}} \). From \( \Phi_{B_{\text{coil}}} \) maps, the z-component of B is reconstructed (Figure 9H and Figure 10H). The range of the measured \( \Phi_{B_{\text{coil}}} \) (radians) and \( B_{\text{coil},z} \) \( \left(10^{-3} \, \text{T}\right) \) quantitatively reflects the values previously observed in simulations. Finally, \( \Phi_{B_{\text{tissue}}} \) maps can be, in principle, obtained by subtracting \( \Phi_{\text{TMS}} \) and \( \Phi_{B_{\text{coil}}} \) maps [see online supplemental Appendix B, subtraction between parentheses in equation (B.2)] (Figure 9I and Figure 10I).

For the performed measurements, the actual MR phase accuracy for \( \Phi_{B_{\text{tissue}}} \) detection is estimated to be in the order of \( 10^{-2} \) rad, which is 2 orders higher than what is required from simulations. In addition, for the adopted full TMS waveform, we should observe that \( \Phi_{B_{\text{tissue}}} \) is zero, as the net induced \( J_{\text{tissue}} \) is zero. However, in \( \Phi_{B_{\text{tissue}}} \) maps, we can observe a certain pattern in the range of 0.1 rad. This pattern is caused by an imperfect compensation of \( \Phi_{B_{\text{coil}}} \) while performing the subtraction between \( \Phi_{\text{TMS}} \) and \( \Phi_{B_{\text{coil}}} \) (relative error \( \sim 1\% \)). Therefore, this result highlights that very high precision is required to correctly compensate for \( \Phi_{B_{\text{coil}}} \).

Finally, to evaluate whether a stronger net incident TMS magnetic field could be of benefit, we performed a second experiment with a TMS output of 4% from our measurements (Figure 10), significant image corruption can be observed in the region underneath the TMS coil. This corruption arises from the intra-voxel dephasing created by the stronger incident, highly nonuniform TMS magnetic field \( B_{\text{coil}} \).

**DISCUSSION**

Being able to measure subject-specific \( \sigma_{\text{LF}} \) would be valuable for different fields of research such as oncology and neuroscience (11-14, 70). In MR-EIT, in vivo conductivity measurements require direct injection of eddy currents in tissue and measurements of their impact on the MR phase (49-51). In this study, we explored whether inductive generation of currents using an MR-compatible TMS setup could be a less painful alternative to MR-EIT. Such a setup is able to generate much stronger time-varying magnetic fields than switching MR gradient coils previously proposed in other studies (58-60). However, as shown by our analysis, 3 main challenges hamper measurements of the induced magnetic field arising from inductively induced currents in tissues.

First, for such an inductive technique to work, it is crucial to correctly disentangle the incident magnetic field from the induced magnetic field. This is because, only this latter field contains information on tissue \( \sigma_{\text{LF}} \). For this purpose, subtractions between different phase images are needed (see online supplemental Appendix B). The fundamental problem is that the induced magnetic field \( B_{\text{tissue}} \) is about \( 10^{-4} \) lower than the incident magnetic field \( B_{\text{coil}} \). Therefore, very high precision and reproducibility is required to correctly disentangle the phase contribution \( \Phi_{B_{\text{tissue}}} \) arising from \( B_{\text{tissue}} \) from the phase contribution \( \Phi_{B_{\text{coil}}} \) arising from \( B_{\text{coil}} \).

Second, as demonstrated, information on \( \sigma_{\text{LF}} \) is only imprinted in \( B_{\text{tissue}} \). Therefore, to reconstruct \( \sigma_{\text{LF}} \), the net \( B_{\text{tissue}} \) has to be nonzero. As discussed in this work, this requirement is satisfied if a truncated TMS pulse is used. However, for standard TMS setups, only TMS pulses that last for a full period can be used; thus, the net \( B_{\text{tissue}} \) is zero (see online supplemental Appendix A, Figure 3, case 1) (66).
Therefore, an additional setup (pulse modulator) should be used to comply with this requirement.

Third, supposing that it would be possible to correctly isolate $B_{tissue}$ from all the other phase contributions, from our simulations, the phase range of $B_{tissue}$ is in the order of $10^{-4}$ rads. This phase range is about 2 orders of magnitude lower than the detectable phase in concurrent TMS-MRI experiments. In addition, to distinguish small variations in tissue conductivity, an even higher phase accuracy would be needed.

To bring $B_{tissue}$ into a measurable phase range, one should increase $B_{tissue}$ of at least 2 orders of magnitude. To strengthen $B_{tissue}$, one can increase the strength of the induced current $J_{tissue}$ by increasing the TMS pulse frequency and pulse strength.

By increasing the pulse frequency, that is, reducing the induction time $t_i$, stronger $J_{tissue}$ and, therefore, $B_{tissue}$ can be achieved. In contrast, attention has to be paid to not enter into a different dispersion band. Brain tissues exhibit the beta dispersion centered between $10^5$–$10^7$ Hz. Above this dispersion band (100 MHz), the cell membranes exhibit a negligible impedance, so currents are capable of passing through both the extracellular and intracellular media (71). To avoid conductivity reconstructions in a different frequency dispersion band, and thus not directly translatable to LF tissue conductivity, the TMS-pulse frequency (1–10 kHz) can be increased by an additional factor of $10^7$ Hz. However, in an MRI experiment, the measured net $B_{tissue}$ is proportional to $B_{tissue_z}$ and the induction time $t_i$. Hence, in the computation of $B_{tissue_z}$, the increase in $B_{tissue_z}$ is cancelled by the reduction in the induction time $t_i$, leading to an unchanged $B_{tissue_z}$ range. For this reason, the increase of the TMS-pulse frequency would not be a beneficial solution.

As aforementioned, another strategy to increase the induced $J_{tissue}$ can be to strengthen the TMS output. $B_{tissue}$ increases with a stronger TMS output, but $B_{coil}$ also increases. Unfortunately, as already observed in Figure 10, this leads to considerable signal dephasing. From our results, a truncated TMS pulse and much stronger TMS outputs should be used to bring $B_{tissue}$ above the noise level. To comply with such a requirement, one should measure a much stronger, highly nonuniform $B_{coil}$. Consequently, to avoid signal dephasing, one should therefore considerably reduce the voxel size (in the range of micrometers).

This latter observation brings us to a final consideration. A smaller voxel size comes quickly at the cost of SNR loss. Instead, to detect very small magnetic field fluctuations such as the one produced by $B_{tissue}$, the SNR should be considerably increased. Thus, only the number of scan repetitions can be increased. However, in practice, unfeasible scan time would be required to achieve enough SNR for $B_{tissue}$ measurements.

As discussed, the unsuccessful ability to measure $B_{tissue}$ by inductively inducing currents in the brain using a combined TMS-MRI setup arises from the physical limitations behind the physics of the induction principle. On the contrary, by injecting currents in tissues (MR-EIT), direct measurements of $B_{tissue}$ and, consequently, $\sigma_\text{LF}$ reconstructions are feasible. The first macroscopic difference between the 2 techniques is that in MR-EIT, images subtractions between different conductive phantoms are not needed. This is because currents are directly injected into the brain, thus there is no incident magnetic field $B_{coil}$. Second, for both techniques, $B_{tissue}$ is proportional to $B_{tissue_z}$ and to $t_i$, time of injection/induction. Despite the comparable $B_{tissue_z}$ range ($\pm 10^{-8}$ T), the relevant difference in the time of injection/induction (10 milliseconds in MR-EIT and 0.1 milliseconds in TMS-MRI) leads to a measurable/nonmeasurable $B_{tissue}$ in MR-EIT and TMS-MRI, respectively (72).

These observations define the physical limitations hampering the feasibility of noninvasively measuring subject-specific $\sigma_\text{LF}$. Hence, future studies should focus on alternative methodologies to noninvasively and nonpainfully measure $B_{tissue}$ for subject-specific $\sigma_\text{LF}$ reconstructions.

CONCLUSIONS

LF tissue conductivity $\sigma_\text{LF}$ reconstructions can only be performed by measuring the phase contribution arising from the induced magnetic field, in which information on $\sigma_\text{LF}$ is imprinted. However, despite stronger currents being inductively induced using a TMS stimulator compared with MR gradient coils, these measurements are not feasible with a standard TMS-MRI setup. This is because, the induced magnetic field is very weak; thus, very high SNR is required to correctly measure it. If a higher level of current running through the TMS coil is used to strengthen the induced currents in tissues and to increase the induced magnetic field, considerable image dephasing would be observed because of the strong, highly nonuniform incident TMS magnetic field. In light of our observations, we believe that direct $\sigma_\text{LF}$ reconstructions performed by inductively inducing currents in the brain are not feasible even if a TMS-MRI setup is used.

Supplemental Materials

Supplemental Appendix A–B: http://dx.doi.org/10.18383/j.tom.2016.00232.s01

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Combination of an Integrin-Targeting NIR Tracer and an Ultrasensitive Spectroscopic Device for Intraoperative Detection of Head and Neck Tumor Margins and Metastatic Lymph Nodes

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Abbreviations: Squamous cell carcinoma (SCC), SCC head and neck (SCCHN), hematoxylin and eosin (H&E), Food and Drug Administration (FDA), positron emission tomography (PET), phosphate buffered saline (PBS), near-infrared (NIR), signal-to-noise ratio (SNR), epidermal growth factor receptor (EGFR)

INTRODUCTION

Squamous cell carcinoma (SCC) is a malignant tumor of epithelial origin. More than 90% of all head and neck cancers are SCC (1). About 60% of patients with SCC head and neck (SCCHN) cancer have lymph node metastases, whereas ~20% to 25% of patients with SCCHN have developed distant metastases primarily in the lungs (2). Oral SCC comprises 2%–3% of all new malignancies diagnosed in the USA, and it is the 10th most common malignancy. However, during the last a few decades, the average 5-year survival rate of 50% has not changed despite advances in surgical and medical therapies (3).

Kavasz et al. examined the relation of local recurrence with the presence or absence of tumors at the surgical margin, which was combined with other indications of postoperative radiotherapy (4). Local recurrence was observed in 20% of patients and was correlated with tumor thickness over 5 mm, spidery growth, and tumor-positive margins. Standardized reporting of head and neck cancer resections, according to guidelines issued by the UK Royal College of Pathologists, was introduced as a routine procedure in 1998 (5). They reported the frequency, type, and morphological features of involved margins and assessed the influence of tumor site and pathological tumor and nodal stages from 301 radical resection specimens for oral/oropharyngeal SCC (cases from 1998 to 2005). Seventy resections (23%) had involved margins, and the frequency was related to the primary tumor site and pathological tumor and node stages. It is reasonable to assume that large tumors are more difficult to resect, given the anatomical constraints at many sites within the...
oral cavity and oropharynx. An involved, deep, soft tissue margin was present in 61 (87%) of the 70 cases. It was also noted that it is difficult to "visualize" particular growth patterns and other features of the deep advancing tumor front, both pre- and intraoperatively, as those particularities are only evident after microscopy (5).

Positive tumor margins are strongly associated with tumor recurrence and poor patient survival. To achieve complete resection, surgeons attempt, on average, 3–4 rounds of intraoperative pathological consultations, which significantly lengthen the operative procedure. The pathological consultations require surgeons to send resected tumor margins to a histology laboratory to be processed via frozen sections. A few selected hematoxylin and eosin (H&E)-stained slides have to be evaluated quickly by a pathologist for the presence of malignant tumor cells, which requires an extra 20–30 minutes per round. Three additional potential problems that are associated with the procedure include sampling error (where the tissue is collected), false negative reading of frozen sections, and significant delay of the intraoperative surgery (average of 3 rounds per head and neck cancer surgery). Intraoperative tumor imaging has the potential of allowing surgeons to clearly delineate tumor margins and assess residual disease. Transoral surgical laser resections of head and neck cancers are associated with improved postoperative function (6). If tumor margins could be accurately intraoperatively assessed, unnecessary removal of healthy tissue would be minimized, complete removal of tumors would be ensured, and neck dissection would be limited to only those cases with tumor-positive lymph nodes. An accurate assessment of tumor margins would provide the opportunity to increase survival and improve the quality of life for patients with head and neck cancer. Therefore, an improved, real-time imaging method to intraoperatively guide malignant tumor margin detection at the point-of-care is urgently needed.

Successful application in SCCHN may allow expansion of the technique to other cancers. By definition, carcinomas begin “in situ” on the epithelial side of the basement membrane and are considered to be benign as long as the cells forming them remain on this side. Eventually, however, carcinomas acquire the ability to breach the basement membrane, and individual cancer cells or groups of cancer cells begin to invade the nearby stroma (7). At the leading invasive edge, the melanoma cells display beta integrins that enable the cancer cells to attach to the extracellular matrix in front of them (8). The cyclic peptide, cyclopentapeptide cyclolys-Arg-Gly-Asp-phe [c(KRGDl)], is known to target αvβ3 integrin. Integrins are a family of heterodimeric glycoproteins consisting of α and β subunits that noncovalently interact with form cell surface adhesion receptors. This receptor class is involved in invasive tumor cells and tumor-associated neovascularature. The cell adhesion molecule integrin αvβ3 is highly expressed on tumor neovasculature and invasive tumor cells, but not on quiescent vessels or normal cells (9, 10). Thus, integrin αvβ3 may be a specific biomarker of tumor neovasculature and infiltrating malignant tumor cells. In recent years, several research groups have developed a series of Arg-Gly-Asp (RGD) peptide-based positron emission tomography (PET) radiotracers for noninvasive imaging of integrin αvβ3 expression to detect tumor neovasculature (11-13). Among these, F18-labeled RGD peptides are under clinical investigation in patients with cancer (14). However, RGD peptides have never been used for the detection of infiltrating tumor cells in intraoperative settings. IRDye800CW (LI-COR Biosciences, Lincoln, Nebraska) has an emission spectrum centered around 800 nm. In 2007, this dye passed animal toxicity studies using a protocol reviewed by the Food and Drug Administration (FDA). RGD peptides have already been used as PET imaging radiotracer in humans. Therefore, RGD-conjugated IRDye800CW could be readily translated to the clinical setting.

METHODOLOGY

Cell Culture

We established metastatic SCCHN tumor cell E3 from a poorly metastatic 686LN parental cell line by 4 rounds of in vivo selection using an orthotopic SCCHN xenograft mouse model (15). The metastatic SCCHN cell E3 was cultured in 5% CO2 at 37°C in Dulbecco’s Modified Eagle’s Medium/F-12 (Invitrogen, Carlsbad, California) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, Missouri), 200 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin (Invitrogen).

Orthotopic SCCHN Xenograft Mouse Model

Orthotopic animal experiments were performed on 6-week-old nude female mice (Harlan, Indianapolis, Indiana). We established highly metastatic SCCHN tumor cell E3 from a poorly metastatic 686LN parental cell line by 4 rounds of in vivo selection using an orthotopic SCCHN xenograft mouse model (15). The orthotopic metastatic SCCHN mouse model was described in our previous publication. In brief, we injected metastatic E3 cells (1 × 106 cells in 50 μL phosphate buffered saline [PBS] with 10% matrigel) into the submandibular subcutaneous tissue to the mylohyoid muscle of the nude mice to create an orthotopic SCCHN xenograft. These metastatic cells not only formed large primary tumors but also spread to lymph nodes and lungs within 30 days.

Near-Infrared Tracer

IRDye 800CW (untargeted dye, control) optical probe and IRDye 800CW RGD optical probe (LI-COR Biosciences) were used for detecting tumor margins of the infiltrating (invasive) tumors. These dyes were diluted to the concentration of 5 μM with PBS, and 100 μL was injected into each nude mouse.

Near-Infrared Fluorescence

Before the imaging experiment, the mice bearing orthotopic head and neck tumors were intravenously injected with 0.5 nmol (per 25 g of mouse) of RGD-IRDye800CW or control-IRDye800 CW (without RGD). Further, 22 hours later, these mice were transported to the imaging suite. This room was equipped with a spectroscopic device (called SpectroPen) and illuminated by a light-emitting diode light (16, 17). The mouse was sacrificed by cervical dislocation, and we opened the skin of the anterior neck region to expose the primary tumor for visualization. Near-infrared (NIR) signals from primary tumor, lung, lymph node, and mylohyoid muscle tissue without primary tumor were recorded on a color video and displayed on a monitor. Collected
organ specimens were fixed in formalin and were paraffin-embedded. The paraffin-embedded specimens were sectioned into 6-μm sections and placed on glass slides and stained using H&E. All protocols for animal studies were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

**Handheld Spectroscopic Device (SpectroPen) for Intraoperative Use**

To translate RGD-IRDye800CW as an intraoperative imaging agent, we developed a pen-sized, fiber-optic probe device for spectroscopic measurements at NIR wavelengths (16). The pen is a compact 1 × 10-cm probe connected to a 5-m fiber-optic cable for both laser excitation and efficient light collection. The excitation source is a 785-nm diode laser with a focal spot of 1–2 mm on the tissue sample. It covers the spectral range of 800–930 nm with a resolution of 0.6 nm. An attached spectrometer and laptop computer instantly acquire and analyze the spectral data at a speed of 1–2 seconds. For the surgeon to monitor the entire surgical field, we have integrated the SpectroPen device with an integrated, wide-field, color/NIR imaging system (Figure 1). There are 3 optical channels that are separated by using dichroic beam splitters, which are as follows:

1. An NIR channel for the probe.
2. A second NIR channel for detecting the laser focal area.
3. A color monitor channel for spatial reference.

The signals in these 3 channels are processed by a computer and are co-displayed and recorded on a color video. During a surgical procedure, the SpectroPen channel provides spectroscopic information about tumor presence or absence.

**RESULTS**

Detection of NIR Signals from a Mouse Bearing Head and Neck Tumors that Infiltrated to the Mylohyoid (Neck) Muscle and Metastasized to the Lungs

The NIR signals (color coded blue) from the bulk of the primary tumor were very strong and oversaturated the detector (Figure 2A). The NIR signal from the ear was measured to show the background from skin. This NIR signal was minimal and appeared dim blue (Figure 2B) with the same image intensity display scale. We resected the entire primary tumor, but there were residual tumors that infiltrated the mylohyoid neck muscle beyond the tumor boundaries. These residual infiltrating tumors were clearly detected by the SpectroPen device (Figure 2C). We removed the lungs from the same mouse and scanned them using the SpectroPen. Then, the NIR signals were found from the spot indicating lung metastasis (Figure 2D). Specimen of the mouse neck region without the neck bone was collected from the mouse as shown in Figure 2C and was paraffin-embedded. The paraffin block was sectioned by 6 μm and stained using H&E. The H&E-stained specimens were found to contain infil-
trating tumor cells (Figure 2E). We collected specimens of the NIR-positive lung lesions and paraffin-embedded these in a certain orientation, so that we could easily identify the location of micrometastases with positive NIR signals (Figure 2F). We admit that the image–histology correlation was not performed in a fully user-unbiased manner. We also collected specimens of the NIR-positive lungs lesions and fixed these in formalin and then paraffin-embedded. The whole lungs were sectioned into 6-μm sections and placed on glass slides. Every fifth slice was scanned with the SpectroPen for NIR signals. We found that several sections were positive of the NIR signal at 805 nm (Supplemental Figure 1). NIR signal was also observed at 880 nm from the glass slide. Then, we H&E-stained the NIR-positive slides and analyzed for the presence of metastases. All the lung sections that were positive for NIR signal contained metastatic cell clusters (Supplemental Figure 1). In these samples, the average size of the metastatic cluster of tumor cells was 50–100 cells. These results indicate that it might be feasible to detect small clusters of 50–100 tumor cells in intraoperative settings.

Detection of NIR Signals from Lymph Nodes Containing Metastatic Tumor Cells

We completely removed primary tumors from 3 mice bearing head and neck tumors, and searched NIR-positive lymph nodes by using the SpectroPen device (Supplemental Figure 2). Once the NIR signal was detected, we resected the suspicious lymph nodes, which were reconfirmed for NIR signal ex vivo. Then, positive lymph nodes were formalin-fixed and paraffin-embed-
ded for histological validation, and we found that 8 out of 8 lymph nodes contained infiltrating tumor cells.

**Spectra from the SpectroPen**

Figure 2 shows the representative NIR spectra of the primary tumor, infiltrating clusters of tumor cells in mylohyoid tissues at tumor margin, tumor-involved lymph node, and lung with micrometastases. The upper spectra in black traces were obtained from RGD-IRDye800CW-injected mice, whereas the bottom spectra in red traces were obtained from control IRDy800CW-injected mice. RGD-IRDye800CW-injected mice exhibited strong, saturating NIR signals from the bulk primary tumors, whereas IRDye800CW-injected mice exhibited weak NIR signals from the bulk primary tumors (Figure 3A). Once the bulk primary tumors were removed, we could easily detect the NIR signals from the invasive clusters of tumor cells in the mylohyoid muscle tissues of RGD-IRDye-injected mice (Figure 3B). But when the IRDye800CW was used, we were unable to detect the NIR signals. We also detected NIR signals from the lymph nodes and lungs of the RGD-IRDye800CW-injected mouse (Figure 3, C and D, upper panel), but could not detect the NIR signal from lymph nodes and lungs of IRDye800CW-injected mice (Figure 3, C and D, bottom panel).

**Detection of NIR Signals from a Nonmetastatic (Encapsulated) Tumor**

A mouse bearing a medium-sized tumor was intravenously injected with 0.5 nmol of RGD-IRDye800CW, and 22 hours later, this mouse was sacrificed by CO₂ asphyxiation followed by cervical dislocation; the skin on the anterior neck region was opened to expose the tumor for visualization. The tumor was encapsulated within a boundary (Figure 4A), and had not yet infiltrated beyond the boundary of the tumor into the neck muscle. Once the tumor was completely removed, we could not detect any NIR signals from the neck area and the lung as shown in Figure 4, B and C. The collected lung specimens were formalin-fixed and paraffin-embedded. In addition, we could not find tumor cells in H&E-stained lung specimens (data not shown). From another mouse bearing a similar-size tumor, we partially removed the tumor and collected specimens from the neck region without the neck bone for histological evaluation. From H&E-stained specimens, we could not find infiltrating clusters of tumor cells beyond the tumor boundary into the neck muscle (Figure 4D), consistent with the lack of NIR signals in Figure 4B.

**Detection of NIR Signals from Control IRDye**

For the control experiment, we injected the control IRDye800CW (without RGD conjugation) in a mouse bearing a large tumor. The primary tumor had a weak NIR signal (Supplemental Figure 3A). After removal of the primary tumors, there were residual infiltrating tumors; however, we could not detect NIR signals from these cells (Supplemental Figure 3B). In addition, we could not detect any NIR signal from the lungs (Supplemental Figure 3C) despite the presence of H&E-stained specimen-confirmed lung metastases (Supplemental Figure 3D).
DISCUSSION

In contrast to radionuclides used in radioimmunoscintigraphy, fluorophores have no finite half-life and can be excited with light to produce multiple emissions. This is advantageous for the following 2 reasons:

(1) The optical imaging probe can be synthesized and stored nearly indefinitely, unlike radionuclides with finite half-lives.

(2) Imaging of infiltrating clusters of tumor cells can be carried out in 24–48 hours following systemic administration of the optical imaging probes because infiltrating clusters of 50–100 cells have not developed (or associated with) leaky neovascularature.

Therefore, the optical probe may require 24–48 hours to reach the target cells in human. Thus, radionuclides with a half-life of 2–6 hours will be inappropriate for the purpose.

The NIR wavelength range between 650 and 950 nm is considered a “clear window” for in vivo imaging because of reduced blood absorption, tissue scattering, and autofluorescence [18]. IRDye800CW (LI-COR Biosciences) with fluorescence emission centered around 800 nm is a commercially available NIR dye with functional groups for conjugation, and it has passed animal toxicity studies using a protocol reviewed by the FDA in 2007 (https://www.licor.com/clinical_translation/index.html).

Houston et al. have compared optical imaging with nuclear imaging by using cyclic RGD conjugated with a dual-labeled probe with a radiotracer ($^{111}$indium) for gamma scintigraphy and with RDye800CW [19]. Image acquisition time was 15 minutes for the gamma scintigraphy images and 800 milliseconds for the optical images acquired using an intensified charge-coupled device-equipped whole-body animal imager. Their results show that the signal-to-noise ratio (SNR) was significantly higher for optical than nuclear imaging. Furthermore, an anal-

![Figure 4. Video still of color/NIR merged images of a mouse bearing head and neck tumors that is well circumscribed as a negative control. When the tumor size is not large enough, tumor cells are encapsulated (confined), and there were no infiltrating tumor cells unlike the mouse shown in Figure 2 (A). With the tumor removal, there remained no residual tumors and no NIR signal was detected (B). For this stage of tumor development, lung metastasis is yet to occur (C). As anticipated, its lungs did not have any NIR signal detectable by the SpectroPen. H&E staining of the neck region from the same mouse: tumor cells have not infiltrated the mylohyoid neck muscle beyond the tumor boundary (D). Specimens from their neck region, including partial tumors (most of the bulk tumors were removed) without the neck bone, were collected, formalin-fixed, and paraffin-embedded, which were then sectioned by 6 μm for H&E staining. No infiltrating cohort of tumor cells was found.](image-url)
ysis of signal-to-noise versus contrast showed greater sensitivity of optical over nuclear imaging for subcutaneous tumor targets. Their work strongly supports that IRDye800CW offers a potentially better tracer than radioisotopes for intraoperative guidance. Furthermore, Sampath et al. used a similar dual-label approach using trastuzumab (Herceptin®, Genentech, Inc.) (20) in subcutaneous xenograft models to show enhanced SNRs in NIR fluorescence compared with nuclear imaging. Because subcutaneous xenograft models offer shallow penetration depths, the results may be as expected and similar to what might be encountered for intraoperative guidance where the surgical fields are exposed.

Characterization of RGD-IRDye800CW, including in vitro and in vivo binding to a variety of tumor cell lines (ie, U87, A431, PC3M-LN4, and 22Rv1), was reported by Kovar et al. in collaboration with LI-COR Bioscience at the American Association for Cancer Research Annual Meeting (http://biosupport.licor.com/docs/IntegrinSpecNIRImagingv2_AACR09_JK.pdf). Specificity of the conjugate for the integrin receptor was confirmed by competition with either an unlabeled RGD peptide or unlabeled RAD (Arg-Ala-Asp), nonspecific peptide in U87 cells. In addition, whole-body animal images confirmed that RGD-IRDye800CW maintains specificity to the target tissue when tested for in vivo applications. Images of nude mice bearing 2 subcutaneously implanted tumors on the rear flank show that RGD-IRDye800CW localized to tumor tissues. A dose of unlabeled RGD (given intravenously via the tail vein) before RGD-IRDye800CW resulted in reduced signals in both tumors, confirming specificity.

We established metastatic SCCHN cells that not only form large primary tumors but also spread to lymph nodes and lungs within 30 days. RGD conjugated with IRDyeCW800 or IRDyeCW800 without RGD conjugation was intravenously injected 22 hours before the imaging experiment. From our preliminary studies, we found that 22 hours following the injection of 0.5 nmol of RGD-IRDye800CW provided us with the best tumor-to-muscle uptake ratio of RGD-IRDye800CW. In addition, data from LI-COR Biosciences (http://biosupport.licor.com/docs/IntegrinSpecNIRImagingv2_AACR09_JK.pdf) also support a 22-hour waiting period before imaging, as the best SNR with a reasonably good background clearance was obtained at 22 hours post injection of RGD-IRDye800CW. It is worth noting that another important biomarker for head and neck tumors is the epidermal growth factor receptor (EGFR). Head and neck cancer has the highest rate of EGFR overexpression compared with other cancers (21, 22). Cetuximab, a monoclonal antibody directed against the EGFR, is effective in treating head and neck cancer when used alone or in combination with radiotherapy and has recently been approved by the FDA for head and neck cancer (23). An EGFR-targeted NIR dye may also have excellent potential as an intraoperative imaging probe, which can be evaluated in future studies to expand the available number of dyes (24). This would further improve the possible clinical applications along with additional molecular diagnostic specificity for using this point-of-care technology in the surgical suite.

To conclude, we have reported the combination of a targeted NIR tracer, a handheld spectroscopic pen device, and a multichannel wide-field imaging system for highly sensitive detection of the integrin αvβ3, which is overexpressed by tumor neovascularization and invading tumor cells, but not by quiescent vessels or normal cells. By using a metastatic orthotopic head and neck cancer animal model, we have shown that this combination allows intraoperative detection of both invasive tumor margins and metastatic lymph nodes and lungs. Correlated histological data further indicate that microscopic clusters of 50–100 tumor cells can be detected intraoperatively, raising new possibilities in guiding surgical resection of microscopic tumors and metastatic lymph nodes. In comparison with other imaging modalities such as computed tomography, PET, or magnetic resonance imaging, this optical imaging technology is highly sensitive and molecularly specific, but its tissue penetration depth is currently limited to about 3–5 mm, so deeply buried or hidden tumor nodules cannot be detected under in vivo or intraoperative conditions. This problem can be overcome or alleviated by recent advances in photoacoustic imaging and NIR fluorescence in the second NIR window (between 1000 and 1700 nm in wavelength), which have shown that the tissue penetration depth could be considerably (by 5–10-fold) improved in comparison with optical imaging in the first NIR window (650–950 nm) (25–27).

Supplemental Materials
Supplemental Figures 1–3: http://dx.doi.org/10.18383/j.tom.2016.00253.sup.01

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Magnetic resonance imaging (MRI) is used to diagnose and monitor brain tumors. Extracting additional information from medical imaging and relating it to a clinical variable of interest is broadly defined as radiomics. Here, multiparametric MRI radiomic profiles (RPs) of de novo glioblastoma (GBM) brain tumors is related with patient prognosis. Clinical imaging from 81 patients with GBM before surgery was analyzed. Four MRI contrasts were aligned, masked by margins defined by gadolinium contrast enhancement and T2/fluid attenuated inversion recovery hyperintensity, and contoured based on image intensity. These segmentations were combined for visualization and quantification by assigning a 4-digit numerical code to each voxel to indicate the segmented RP. Each RP volume was then compared with overall survival. A combined classifier was then generated on the basis of significant RPs and optimized volume thresholds. Five RPs were predictive of overall survival before therapy. Combining the RP classifiers into a single prognostic score predicted patient survival better than each alone (P < .005). Voxels coded with 1 RP associated with poor prognosis were pathologically confirmed to contain hypercellular tumor. This study applies radiomic profiling to de novo patients with GBM to determine imaging signatures associated with poor prognosis at tumor diagnosis. This tool may be useful for planning surgical resection or radiation treatment margins.

INTRODUCTION
Glioblastoma (GBM) is a diffuse and highly invasive brain tumor of astrocytic origin. Despite recent advances in medical imaging diagnostics, radiation treatment, and chemotherapy, gliomas remain the most lethal cancer of the central nervous system (1). The median survival time is 14.6 months with a 5-year survival rate of 5.1% (2, 3).

GBMs are pathologically defined by pseudopalisading necrosis (4) and endothelial proliferation (5) and are highly heterogeneous with areas of differing cellularity, vascularity, and necrosis. This heterogeneity is further influenced by differing cellular microenvironments (6-8). Recent studies have hypothesized that these microenvironments create unique radiographic signatures on magnetic resonance (MR) imaging that may indicate information of clinical importance (8-10).

Magnetic resonance imaging (MRI) is commonly used to diagnose GBM, monitor tumor progression, and assess response to therapy (11, 12). Multiparametric MRI (MP-MRI) combines different MR contrast sequences to provide additional complementary structural information, potentially illuminating hidden characteristics and offering insight into both tumor and normal tissues. Recently, techniques that use advanced image processing to extract and analyze quantitative imaging features have been used in combination with clinical variables. These techniques are broadly defined as radiomics (9, 10, 13-15). Features quantified include metrics such as intensity distribution, spatial relationships, textural heterogeneity, and shape descriptors, among many other characteristics (16-18). The resulting matrices of feature data can then be mined along with a clinical variable of interest, such as a genetic profile, to determine previously hidden patterns (9, 19, 20).

The goal of this study is to develop an intuitive methodology for extracting voxel-wise radiomic profiles (RPs) from clinical imaging to describe distinct intensity characteristics from a given combination of MRI contrasts. We hypothesized that heterogeneous RPs represent underlying tumor characteristics, and thereby contain prognostic significance. This retrospective study looks at imaging from patients with GBM before treatment and derives a model of risk stratification for patient prognosis based on volumetrically thresholded RPs.
METHODOLOGY

Patient Population and Image Acquisition
In total, 81 patients with pathologically confirmed primary GBM with T1-weighted pre- and postcontrast images, diffusion-weighted imaging, and T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging before therapy were included in this study. Overall survival (OS) was corrected to account for contrast-enhancing tumor size, which is known to be associated with OS. Imaging was gathered before surgery using one of our institutional MRI scanners, including 1.5 T and 3 T GE (General Electric Health, Waukesha, Wisconsin) and Siemens magnets (Siemens Healthcare, Erlangen, Germany). The following are example scan parameters (all given in the format repetition time/echo time) at 1.5 T: T1 spin-echo sequence, 666/14 milliseconds; contrast-enhanced T1 acquired with gadolinium, 666/14 milliseconds; apparent diffusion coefficient, calculated from diffusion-weighted images acquired with an echo planar/spin echo sequence, 10 000/90.7 milliseconds; and FLAIR, acquired with an inversion recovery sequence, 10 002/151.8 milliseconds and TI of 2200 milliseconds. All images were acquired with submillimeter in-plane resolution.

Preprocessing
Images were coregistered to the T1 image using FSL’s FLIRT command (FMRIB Software Library, Oxford) (21–23). The FLAIR hyperintensity and contrast-enhancing lesion were semiautomatically segmented using a thresholding technique followed by manual correction of misclassified voxels (24). To correct for subtle intensity variance, images were smoothed with a 2-mm full width at half maximum Gaussian filter. Each image was then intensity-normalized by dividing voxel intensity by the standard deviation of the whole brain (25, 26). A total region of interest (ROI) mask was created using the union of the contrast-enhancing lesion and the FLAIR hyperintensity ROIs. Each contrast was then masked to only include abnormal regions. FSL’s FAST command (FMRIB Software Library, Oxford) (27) was used to create a 3 tissue-class segmentation of each of the 4 images, ranking voxel intensities by low, medium, and high. Each was then coded with a value of 1, 2, and 3, respectively.

RP Generation
Images were coded based on the RP on a voxel-wise basis; for ease of interpretation, a 4-digit code was assigned to each voxel representing the intensity-based segmentation from each of the 4 images. The digit order chosen was T1, ADC, T1+C, and FLAIR. Codes ranged from 1111, representing dark voxels on all images, to 3333, representing all bright voxels. The left panel of Figure 1 shows the 4 clinical images used to generate RPs. The ROI used for all 4 images is the T1 enhancement combined with the FLAIR hyperintensity. These images are segmented individually within the ROI and given values of 1–3 based on intensity and neighboring pixel information. These 4 images are combined into one, with each voxel containing the segmentation value from all 4 images.

An RP is defined as all voxels within an image that contain the same 4-digit code. The resulting map contains 81 (3^4) potential RPs. Profiles were evaluated within the following 4 ROIs:

1. Within the contrast-enhancing lesion only (T1E).
2. Within the FLAIR hyperintensity only (FLAIR).
3. Within the union of the FLAIR hyperintensity and contrast-enhancing lesion (FTU).
4. Within the FLAIR hyperintensity excluding contrast enhancement (FEC).

Statistical Approach
We performed an exploratory analysis to first determine which RPs were correlated with OS. The optimal volumetric threshold was then calculated for each significant profile using a log-rank Kaplan–Meier survival analysis. High- and low-volume groups were required to have at least 10 patients. Because of the high number of statistical tests performed, a strict P value of <.0005 was considered significant.

To generate a combined indicator score, each patient was given a score from 0 to 5, indicating how many RP thresholds were exceeded. A Cox regression approach was used to calculate the hazard ratio associated with the number of profiles above threshold. A final Kaplan–Meier analysis was performed between patients scoring 0–2 and patients scoring 3–5.

Histological Validation
The imaging data from the final time point before death were processed as described above in 2 additional patients undergoing autopsy. These patients underwent treatment. The patients’ brains were sectioned in the same orientation and thickness as their last MRI. Tissue specimens were acquired from areas suspicious of tumor, including regions highlighted by RPs, then paraffin-embedded and stained using hematoxylin and eosin. The histology was then coregistered to the T1 image using methods previously established (24, 28). To assess the underlying pathology, the RP was overlaid on the histology.

RESULTS
Five profiles were identified as highly correlated with survival independent of the tumor volume, where higher volumes of each RP were associated with poorer prognosis. These included 2133...
within FTU ROIs and 1133, 1213, 1233, and 3133 within T1E ROIs ($P < .0005$). Figure 2 shows the profiles significantly correlated with survival overlaid on FLAIR (2133 in FTU) or T1/T1+C (all others).

Volume thresholds associated with each RP are shown in Table 1. The number of RPs above threshold predicted patient survival, where patients above threshold in 3–5 of the RPs showed decreased OS compared with those with 2 or fewer RPs above threshold ($P < .001$), and survival curves associated with prognostic score and risk group can be seen in Figure 3. The addition of each RP above volume threshold was associated with a hazard ratio of 1.44 ($P < .001$). Profile 1133 was histologically confirmed to contain dense hypercellularity and necrosis in 2 patients. Figure 4 shows the histological validation of profile 1133.

### DISCUSSION

This study presents a method of combining information from a variety of MRIs to create profiles that quantify heterogeneity in tumor appearance. We found that 5 RPs significantly correlated with survival when thresholded by volume. When combined, these profiles created a prognostic indicator that may be used to evaluate prognosis noninvasively before treatment. Figure 3 shows 2 Kaplan–Meier plots, and it represents the risk-stratification model these profiles generate. In the left panel, all 6 prognostic scores have individual Kaplan–Meier curves. Although the sample size is smaller, the survival times stratify on the basis of the prognostic score. The right panel shows a combined prognostic score, where all patients with 0–2 score are grouped together in a low-risk group, and patients scoring 3–5 are considered high risk. There is a statistically significant difference in survival time between low- and high-risk patients ($P < .001$). At autopsy, hypercellular tumor and necrosis was found in voxels indicated by one of the RPs in 2 patients.

Previous studies have found that the visual appearance of brain tumors on imaging such as mass effect, brain tumor contrast enhancement, degree of T2 edema, and contrast to T2 ratio is related to the genetic phenotype of brain tumors (19). Previous radiomic analysis of MP-MRI has found that imaging features such as standard deviation of energy and gray level run emphasis are significantly correlated with patient prognosis (29). Radiomic analysis typically produces a larger number of agnostic features, features that are mathematically extracted descriptors of tumor properties (14). Image features used in radiomic analysis have been shown to capture distinct phenotypic differences in cancers such as those of the lungs (30–33) and the head and neck (9, 33–36).

One of the goals of this study was to bridge the gap between what radiologists interpret with MP-MRI and the field of radiomics. Agnostic radiomic features such as image entropy or

### Table 1. Profiles Correlated With Survival

<table>
<thead>
<tr>
<th>Profile</th>
<th>Condition</th>
<th>Cutoff (mm$^3$)</th>
<th>N Above/Below Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>2133**</td>
<td>FTU</td>
<td>236</td>
<td>52/29</td>
</tr>
<tr>
<td>1133***</td>
<td>T1E</td>
<td>30</td>
<td>53/28</td>
</tr>
<tr>
<td>1213***</td>
<td>T1E</td>
<td>178</td>
<td>17/64</td>
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<td>1233**</td>
<td>T1E</td>
<td>451</td>
<td>33/48</td>
</tr>
<tr>
<td>3133**</td>
<td>T1E</td>
<td>125</td>
<td>57/24</td>
</tr>
</tbody>
</table>

**$P < .0001$; ***$P < .00001$. 

---

**Figure 2.** Profiles correlated with survival isolated and overlaid on FLAIR (2133 in FTU) or T1+C (all others).

**Figure 3.** Survival vs prognostic score for scores of 0–6 (Left). Low (0–2) and high (3–5) risk group vs survival (Right).
image energy are often found to be associated with a clinical metric of interest. Because these features are typically calculated within an ROI, radiologists are limited to interpreting a score rather than a heterogeneous image. The radiomic profiling method presented here results in a simple-to-interpret 4-digit code that maps tumor heterogeneity in a visually meaningful way. Figure 5 compares 2 patients, one with a small tumor and a high-risk score and the other with a larger tumor and a lower prognostic score. The raw FLAIR and T1+ C images, as well as the combined ROI, are shown. The bottom panel groups all profiles into one of 3 categories such that the map is easier to read. Blue indicates profiles not significantly correlated with survival, green represents profiles correlated with survival below threshold, and yellow indicates profiles correlated with survival that are above threshold. Despite the smaller tumor in the patient on the right, 5 profiles above threshold result in a much smaller survival time. Additional analysis of the RP volumetrics provides risk stratification, predicting patient prognosis in a manner that is intuitively understandable to a radiologist. The resulting profile maps may be useful for defining resection margins or targeted radiotherapy.

Current practice in GBM imaging involves regular imaging sessions. Radiomic profiling in its current state best functions at the first scans after diagnosis, potentially directing, radiotherapy or surgical resection. Future studies will validate this method over time, accounting for treatment effects and monitoring if changes in RPs continue to correlate with prognosis. The current method is semiautomatic, involving human input only in checking the ROIs after they are initially identified by thresholding. With a more advanced ROI-detection method, this approach could become a fully automated tool at the physicians’ disposal. No additional scans would be required to implement this method. The time to generate the RP maps is under 30 seconds assuming the ROIs are already available.

The 4 MRI contrasts included in this analysis are standard in most clinical brain tumor protocols. ADC, which measures the diffusion of water within tissue, has been shown to be inversely correlated with tissue cellularity (37). An increase in ADC has also been hypothesized to indicate cell death (38). FLAIR is a
heavily T2-weighted fluid inversion sequence, which suppresses cerebrospinal fluid, so that excess fluid within the brain becomes hyperintense (39). FLAIR hyperintensity is generally interpreted as a combination of infiltrative tumor cells and vasogenic edema.

The code 1133 was hypothesized to be the most significant profile before analysis because it best describes the appearance of invasive tumor on all the following 4 contrasts evaluated: dark on T1, diffusion restricted on ADC, enhancing on T1+C, and within the region of edema on FLAIR. The environments 1113 and 1123 (the same profile with different T1+C values) are not significant in any condition, suggesting that contrast enhancement was the important characteristic in this profile. Four of the 5 significant profiles take the form XX33, indicating that they are contrast-enhancing and hyperintense in the FLAIR. Interestingly, no profiles outside of the contrast-enhancing lesion were found to be indicative of OS.

The volume threshold for each profile was unexpectedly variable, where some profiles, such as 1133, have a near-zero threshold, whereas others, such as 1233, have a threshold over 450 mm³. Future studies should assess phenotypic differences in these RPs.

There were several sources of potential error in this study. Segmentation of both the T1E and FLAIR hyperintensity were performed semiautomatically, with manual correction of misclassified voxels, and thus, are prone to human error. Variable imaging parameters, magnification strength, and vendor may also contribute as sources of error. Because of the retrospective nature of the study, images were acquired over the course of several years under similar but slightly varying imaging parameters. The data set would likely produce more robust profiles if the parameters and imaging systems had been identical. We controlled for this by intensity-normalizing each image.

These profiles will have greater clinical importance if they can be linked to a distinct histological pattern. Each profile may describe a specific phenotype or microenvironment. Further histological validation with more patients is necessary. Treatment effects, however, may change the profile volume, threshold, or which profiles are most useful in predicting prognosis. Monitoring profile growth over time will allow us to better control for both treatment effects and time dependence. A variation in prognostic score over time may provide useful clinical information.

In conclusion, this study presents an easily interpretable method for creating radiographic profiles by combining intensity information from multiple MRI scans. When thresholded by volume, 5 RPs significantly correlated with survival. A prognostic indicator that combined the 5 may potentially be used to evaluate prognosis noninvasively before treatment. We also pathologically validated that voxels indicated by one of the RPs contained hypercellular tumor and necrosis in 2 patients. The method presented in this paper may prove clinically useful in providing a risk-stratification model for clinicians treating newly diagnosed GBM.

ACKNOWLEDGMENTS

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REFERENCES


Hypoxia Imaging With PET Correlates With Antitumor Activity of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) in Rodent Glioma Models

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Key Words: hypoxia imaging, 18F-FMISO PET, glioma, TH-302, evofosfamide, hypoxia-activated prodrugs

Abbreviations: 18–fluoromisonidozole positron emission tomography (18F-FMISO-PET), computed tomography (CT), magnetic resonance (MR), regions of interest (ROIs), diffusion weighted imaging (DWI)

High-grade gliomas are often characterized by hypoxia, which is associated with both poor long-term prognosis and therapy resistance. The adverse role hypoxia plays in treatment resistance and disease progression has led to the development of hypoxia imaging methods and hypoxia-targeted treatments. Here, we determined the tumor hypoxia and vascular perfusion characteristics of 2 rat orthotopic glioma models using 18-fluoromisonidozole positron emission tomography. In addition, we determined tumor response to the hypoxia-activated prodrug evofosfamide (TH-302) in these rat glioma models. C6 tumors exhibited more hypoxia and were less perfused than 9L tumors. On the basis of these differences in their tumor hypoxic burden, treatment with evofosfamide resulted in 4- and 2-fold decreases in tumor growth rates of C6 and 9L tumors, respectively. This work shows that imaging methods sensitive to tumor hypoxia and perfusion are able to predict response to hypoxia-targeted agents. This has implications for improved patient selection, particularly in clinical trials, for treatment with hypoxia-activated cytotoxic prodrugs, such as evofosfamide.

INTRODUCTION
Glioblastoma is the most common type of primary malignant brain tumor. Despite advances in detection and treatment, the prognosis remains poor for patients with glioma with median survival time of only 12–15 months (1). One possible factor contributing to this poor prognosis is hypoxia, as high-grade gliomas can have considerable regions of hypoxia (2–4) with extremely low regional tissue partial pressure of oxygen (pO2) levels of <10 mmHg (4, 5). Hypoxic, but viable, cells can be noninvasively probed with 18-fluoromisonidozole positron emission tomography (18F-FMISO-PET; chemical structure in Figure 1A) (6–8), and the uptake of 18F-FMISO has been found to inversely correlate with overall survival in patients with glioblastoma (7, 9). Using a separate marker for perfusion, Bruehlmeier showed that hypoxia occurs in regions of both hypo- and hyperperfusion (6), suggesting that an independent process leads to a hypoxic phenotype. In addition to hypoxia, 18F-FMISO-PET imaging can also provide a relative measure of perfusion (tracer delivery) using a dynamic scan (6, 10, 11). Hypoxic tumors are known to have poor response to chemotherapy (12). Insufficient perfusion in hypoxic tumors could reduce the delivery of chemotherapeutic agents and further reduce their effectiveness (13).

Tumor hypoxia is known to lead to both poor long-term prognosis and poor therapeutic response to conventional treatments (3, 14, 15). The adverse role hypoxia plays in treatment resistance and disease progression has led to the development of hypoxia-targeted treatments (16–18). Evofosfamide (also known as TH-302; chemical structure shown in Figure 1B) is a hypoxia-activated prodrug designed to provide significant cytotoxicity in and around extreme hypoxic regions while remaining relatively nontoxic in well-oxygenated regions (19). Using the same hypoxia-targeting moiety as 18F-FMISO, evofosfamide is composed of a hypoxia-sensitive nitroimidazole trigger covalently linked to a cytotoxic bromo-isophosphoramide mustard (18). Evofosfamide is reduced at the nitroimidazole group, leading, under hypoxic conditions, to release of the mustard toxin that acts by alkylating and cross-linking DNA (18–21). Numerous
studies have shown broad in vivo activity and efficacy of evofosfamide both as monotherapy or in combination with other chemotherapeutics in preclinical (18, 20–24) and clinical (19, 25, 26) studies.

Because there is substantial heterogeneity in the development and extent of tumor hypoxia, combined with differences in tumor perfusion, 18F-FMISO PET imaging could help select potential patients who would benefit from hypoxia-targeted treatments. Preclinically, 2 commonly used rat glioma models—C6 glioblastomas and 9L gliosarcomas—have markedly different levels of hypoxia and perfusion. In particular, C6 tumors tend to be less vascular and more hypoxic, whereas 9L tumors are more vascular and less hypoxic (27–31). On this basis, C6 tumors, compared with 9L tumors, are expected to be more responsive to evofosfamide. In this study, the perfusion and hypoxia levels in C6 and 9L tumors were determined using 18F-FMISO PET. In addition, C6 and 9L tumor response to evofosfamide treatment was assessed.

**MATERIALS AND METHODS**

**Animals Methods**

All animal studies were performed in accordance with National Institutes of Health Institutional Animal Care and Use Committee protocols. For all procedures and imaging, the animals were immobilized in a stereotactic head holder. Anesthesia was induced using 3%–5% isoflurane in air and maintained with 1%–2.5% isoflurane in air. Body temperature was maintained at 38°C using forced warm air. Two rat tumor models were used to provide a range of tumor hypoxia (C6 glioblastomas are relatively hypoxic and 9L gliosarcomas are more normoxic) (29). Male Fischer and Wistar rats (Harlan Laboratories, Indianapolis, Indiana) were inoculated with 1 × 10⁵ 9L and C6 glioma cells (American Type Culture Collection, Manassas, Commonwealth of Virginia), respectively, at 1 mm anterior and 3 mm lateral to the bregma, with a depth of 4 mm from the dural surface. Imaging was performed after 14 days. During imaging, the rats were anesthetized with isoflurane in air. The animals were separated into 2 cohorts—one to assess tumor perfusion and hypoxia with 18F-FMISO (cohort 1) and one to assess treatment response to evofosfamide (cohort 2).

**Hypoxia Imaging (Cohort 1)**

A day before PET imaging, intravenous jugular and arterial catheters were inserted for PET contrast administration and PET blood sampling, respectively. PET data were collected with a microPET Focus 220 system (Concorde Microsystems Inc., Knoxville, Tennessee). Dynamic 18F-FMISO PET (42 time frames/2 h) began simultaneously with bolus injection of ~1.2 mCi (range 1.0–1.4) 18F-FMISO. The reconstructed PET time frames (frames × seconds) were 12 × 10, 8 × 15, 4 × 30, 3 × 60, 3 × 120, 5 × 300, 5 × 600, and 2 × 900 seconds (for a total of 2 hours). To determine the arterial input function, 19 blood samples of 80 μL each were drawn from the arterial catheter into heparinized tubes, with the first 7 blood samples drawn as quickly as possible in the first 90 seconds of PET imaging and the last 12 blood samples drawn at 2, 4, 6, 8, 12, 20, 30, 45, 60, 75, 90, and 120 minutes after injection. Plasma radioactivity was measured using a well counter and then decay-corrected to the time of injection.

**Drug Treatment (Cohort 2)**

Evofosfamide (Threshold Pharmaceuticals, Inc., South San Francisco, California) dosing solution was prepared immediately before treatment at a concentration of 10 mg/mL in sterile saline. In particular, the evofosfamide solution was vortexed for 1 minute, followed by sonication at 45°C for 30 minutes, and then vortexed every 10 minutes until the solution was clear. This solution was filtered through a 0.2-μm filter before intraperitoneal injection. Following pretreatment imaging, treatment with evofosfamide (50 mg/kg; treated group: C6, n = 8; 9L, n = 6) or sterile saline (control group: C6, n = 8; 9L, n = 6) was performed once daily for 4 days. Post-treatment imaging was performed 1 day after the final treatment (4 days after pretreatment).

**Magnetic Resonance Imaging Methods (Cohorts 1 and 2)**

Magnetic resonance imaging (MRI) was performed at 4.7 T (Agilent, Santa Clara, California). Anatomical imaging was performed using a T2-weighted fast-spin echo MRI imaging with the following parameters: relaxation time = 2 seconds, echo time = 80 milliseconds, averages = 12, scan time = 3 minutes 16 seconds, field of view = 36 × 36 mm², section thickness = 1 mm, and acquisition matrix = 128 × 128 with at least 8 sections (up to 16 sections, as needed). For cohort 1, anatomical images were acquired on the same day as PET imaging. For cohort 2, anatomical images were acquired on the same day as the initial treatment time point (day 0) and 1 day after the final treatment (day 4).
Postprocessing and Analysis (Cohorts 1 and 2)

Image registration was performed using an automated rigid registration algorithm built in-house (Matlab, MathWorks Inc., Natick, Massachusetts), and all registrations were manually verified following registration. For cohort 1, the PET images were initially registered to computed tomography (CT) images, as PET and CT were acquired consecutively using the same animal bed. The CT image was then registered to the magnetic resonance (MR) images (acquired on a separate animal bed), and the resulting transformation matrix was used to register the PET images to the MR images.

The PET data were converted to percentage injected dose per gram of tissue (%ID/g). Perfusion can be determined from the time–activity curves immediately after 18F-FMISO injection. Perfusion was calculated by linear fitting the time–activity curves to obtain the early slope (0–60 seconds) of 18F-FMISO uptake (6, 10). As 18F-FMISO uptake in hypoxic tissues increases over time, a positive late slope (eg, between 1 and 2 hours after injection) can serve as a marker for tumor hypoxia (10, 32, 33). In contrast, normal tissue exhibits decreasing or constant signal at later time points, resulting in zero or negative late slope. The late slope was calculated by linear fitting the time–activity curves from 1 to 2 hours after injection.

As C6 tumors exhibited a wide range of tumor sizes, the effects of tumor size on hypoxia and treatment response were further explored. C6 tumors of cohort 1 were split into groups with tumors sized <25 mm³ (n = 3) and between 25 and 100 mm³ (n = 5). Similarly, C6 tumors of cohort 2 were split into groups with tumors sized <25 mm³ (n = 2), tumors sized between 25 and 100 mm³ (n = 3), and tumors sized >100 mm³ (n = 3). The size distribution of 9L tumors was much smaller.

Statistical Analysis

Data analysis was performed on regions of interest (ROIs) that were initially drawn from anatomical MRI images. For cohort 1, ROIs were drawn in the tumor core and in normal-appearing contralateral rat brain, and these MR-based ROIs were then transferred to the registered PET images for analysis. For cohort 2, tumor ROIs were drawn on the blinded pre- and post-treatment anatomical images. Tumor volume was measured using these MR-based ROIs. Linear tumor growth rates were also calculated from the pre- and post-treatment tumor sizes. Results are presented as means ± standard deviation for rats in each group. Individual groups were statistically compared using paired and unpaired Student t test. Results were considered significant at P < .05.

RESULTS

Analysis of Perfusion and Hypoxia in C6 and 9L Tumors

Representative dynamic 18F-FMISO curves are shown in Figure 2 for C6 and 9L tumor models and contralateral normal brain. The perfusion component taken from the first minute following injection was only slightly higher in the C6 tumor compared with that in the contralateral normal tissue, whereas the 9L tumor showed substantially higher perfusion than the normal tissue. At later time points, between 1 and 2 hours after injection, the time–activity curve in C6 tumor ROI steadily increased, whereas the time–activity curves in the 9L tumor and both contralateral normal tissue ROIs remained flat or was decreasing.

The corresponding anatomical MRI, PET perfusion, and PET hypoxia images in Figure 2 show clear differences in perfusion and hypoxia between C6 and 9L tumors (indicated by the arrows). C6 tumors had slightly higher perfusion than the contralateral normal tissue, whereas 9L tumors were characterized by much higher perfusion compared with both C6 tumors and the contralateral normal tissue. The C6 tumor exhibited regions of hypoxia, as identified by a highly positive late slope, whereas 9L tumor appeared normoxic. Two disparate regions in the C6 tumor are evident—one showing higher perfusion and substantial hypoxia (indicated by the arrow) and one showing limited perfusion and hypoxia (indicated by the arrowhead). The 9L tumor exhibited perfusion “hotspots” and hypoxia image contrast consistent with the normal tissue.

C6 and 9L tumor voxels from each rat were combined to determine the regional heterogeneity of perfusion and hypoxia within each tumor type. The boxplots in Figure 3 show the voxelwise perfusion (A) and hypoxia (B) for C6 and 9L tumors (combining all voxels from 8 C6 and 6 9L tumors, respectively). 9L tumors showed a wider range of perfusion values, with higher median perfusion, compared with C6 tumors (0.59 vs 0.36%ID/(g · min)) (Figure 3A). The blue markers indicate the median values for each tumor (C6, n = 8; 9L, n = 6). In addition to a wider voxelwise range for 9L tumors, the median perfusion for each tumor showed greater heterogeneity (C6: median range, 0.29–0.42%ID/(g · min); 9L: 0.33–0.84%ID/(g · min)). Conversely, C6 tumors showed a slightly wider range of hypoxia values (Figure 3B), with both tumor types spanning hypoxic and normoxic late-slope values. For C6 tumors, late slopes between −1.2e−3 and 2.8e−3%ID/(g · min) comprise the 25th to 75th percentile, whereas for the 9L tumor, late slopes between −2.7e−3 and 0.9e−3%ID/(g · min) comprise the 25th to 75th percentile. C6 tumors tend to have higher median hypoxia compared with 9L tumors (0.74e−3 vs −0.96e−3%ID/(g · min)), with most tumors showing positive median late slopes (5 of 8 C6 tumors).

The hypoxia and perfusion values for each rat are shown in the plot in Figure 4A. C6 tumors (black “x”, n = 8) exhibited considerably more hypoxia (higher late slope, y-axis) than the contralateral normal tissue (gray “x”), with 6 of the 8 tumors having mean positive late slope values. 9L tumors (black circles, n = 6) had similar late slope values to contralateral normal tissue (gray circles). Perfusion in C6 and 9L tumors was higher than that in the normal tissue. There was no significant correlation between perfusion and hypoxia in either tumor type. Overall, both C6 and 9L tumors had significantly higher perfusion than normal tissue (P = .0005 and 0.02, respectively) (Figure 4B). C6 and 9L tumors did not have significantly different perfusion (P = .07). The normal tissue perfusion was not significantly different between C6 and 9L groups (P = .18). C6 tumors were significantly more hypoxic than the contralateral normal tissue (P = .0003) and 9L tumors (P = .00005) (Figure 4C). Late slopes in 9L tumors were not significantly different from those in normal tissue.
The normal tissue late slopes were not significantly different between the groups ($P = .93$).

**Determination of Treatment Response to Evofosfamide**

Figure 5A shows the pre- and post-treatment tumor volumes for C6 ($n = 8$) and 9L ($n = 6$) tumors. The tumor volumes were not significantly different before treatment between the treated and control groups for either tumor type, although C6 tumors had substantially wider range of pretreatment tumor volumes in both the treated (range, 9.0–204 mm$^3$) and control groups (range, 11.5–131.4 mm$^3$). 9L tumors had a much smaller pre-treatment size range (8.6–21.7 and 4.6–32.3 for treated and untreated groups, respectively). At the post-treatment time point, the control groups had larger tumors than the treated groups, with significant differences for 9L tumors ($P = .002$). Because of the wide range of pretreatment tumor sizes in C6 groups, tumor volume was not significant at the post-treatment time point. Post treatment, C6 tumor volumes ranged from 15.9 to 245 mm$^3$ for the treated group and from 38.2 to 328.3 mm$^3$ for the untreated group. 9L post-treatment tumor volumes ranged from 34.6 to 77.5 for the treated group and 82.2 to 141.4 mm$^3$ for the untreated group.

To account for differences in pretreatment tumor size, linear tumor growth rates were calculated for each group (Figure 5B). The mean ($\pm$ standard deviation) growth rates were 6.5 ($\pm$ 4.2) mm$^3$/d and 25.8 ($\pm$ 16.6) mm$^3$/d for C6 treated and control rats, respectively, and 10.5 ($\pm$ 3.7) mm$^3$/d and 23.1 ($\pm$ 4.7) mm$^3$/d for 9L treated and control rats, respectively. Growth rates in the treated group were significantly smaller than those in the control group ($P = .007$).
and $P = .0005$ for C6 and 9L, respectively). Accordingly, treatment with evofosfamide resulted in 4- and 2-fold decreases in the tumor growth rates of C6 and 9L tumors, respectively.

As hypoxia levels in C6 tumors are expected to be proportional to the tumor size (27), we further investigated the role of tumor size on both perfusion and hypoxia in cohort 1 and on treatment efficacy in cohort 2. Figure 6A shows that both size groups had significantly different perfusion relative to the normal tissue ($P = .03$ and 0.01 for the $<25$ mm$^3$ tumors and $25–100$ mm$^3$ tumors, respectively), although the level of perfusion did not depend on size ($P = .8$). Both size groups also had significant levels of hypoxia relative to the normal tissue ($P = .04$ and 0.001 for the $<25$ mm$^3$ tumors and $25–100$ mm$^3$ tumors, respectively) (Figure 6B). In addition, tumors sized $25–100$ mm$^3$ had showed significantly more hypoxia than tumors sized $<25$ mm$^3$ ($P = .0007$). Given the dependence of hypoxia on tumor size, Figure 6C shows the different responses to hypoxia-activated treatment based on initial tumor size. The smallest tumors ($<25$ mm$^3$) had slightly smaller tumor growth rates for the treated group compared with those in the control group, but these differences were not statistically significant. Conversely, the larger tumors ($>25$ mm$^3$) showed significantly smaller tumor growth rates in the treated group compared with those in the control group ($P = .005$ and 0.04 for the $25–100$ mm$^3$ and $>100$ mm$^3$ groups, respectively).

**Figure 3.** Boxplots showing the voxelwise tumor perfusion (A) and hypoxia (B) for C6 and 9L tumors (voxels from 8 C6 tumors and 6 9L tumors). Blue marker indicates the median values for each tumor (B) (C6, n = 8; 9L, n = 6).

**Figure 4.** Scatter plot showing mean hypoxia and perfusion for each rat in both tumor and contralateral normal brain (C6 tumor and normal, n = 8; 9L tumor; normal, n = 6) (A). Boxplots for perfusion (B) and hypoxia (C) in C6 and 9L tumors and contralateral normal brain, with diamond markers indicating the mean (B–C). ** $P < .01$ and * $P < .05$. 
DISCUSSION

Hypoxia develops from an imbalance between the supply and consumption of oxygen. Two notable causes of hypoxia are perfusion deficits (acute hypoxia) and oxygen diffusion deficits (chronic hypoxia). Acute hypoxia is associated with abnormal tissue microvasculature and is highly unpredictable. Under chronic hypoxia, tumor cells adapt to the hypoxic microenvironment by upregulating prosurvival proteins; these proteins are associated with increased angiogenesis, proliferation, invasion, and metastases. Although \( ^{18}\text{F}-\text{FMISO} \) could potentially report on either acute or chronic hypoxia (34), it appears likely that the major source of \( ^{18}\text{F}-\text{FMISO} \) signal results from chronic hypoxia (35). Similarly, evofosfamide could be sensitive to either acute or chronic hypoxia, and recent work has proposed inducing acute hypoxia to improve the efficacy of evofosfamide (36, 37). Another promising alternative would be to combine evofosfamide and a general chemotherapeutic agent to provide more uniform tumor cell death across hypoxic and normoxic tumor regions (22).

Perfusion can have a profound effect on drug efficacy, as access to tumor cells for chemotherapeutic drugs may be limited in the presence of perfusion deficits (13). Previous studies have...

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**Figure 5.** Mean tumor volume pretreatment and 4 days after treatment initiation (mm\(^3\)), with error bars indicating standard error of the mean (SEM) (A). Boxplots for linear tumor growth rate (mm\(^3\)/d, right) for C6 and 9L treated and control groups (B). **P < .01.

**Figure 6.** Bar plots showing perfusion (A) and hypoxia (B) in C6 tumors separated by tumor size and the corresponding contralateral normal brain (n = 3 for tumors sized <25 mm\(^3\) and n = 5 for tumors sized between 25 and 100 mm\(^3\)). Bar plot showing linear tumor growth rate (mm\(^3\)/day) in C6 tumors separated by tumor size for evofosfamide-treated and vehicle-control groups (n = 2 for tumors sized <25 mm\(^3\), n = 3 for tumors sized between 25 and 100 mm\(^3\), and n = 3 for tumors sized >100 mm\(^3\)) (C). **P < .01 and *P < .05.
Hypoxia Imaging With PET Correlates With Antitumor Activity of TH-302

In conclusion, hypoxia is an important factor in the progression of brain tumors and is associated with poor treatment response. Hypoxia can be imaged noninvasively using dynamic 18F-FMISO-PET, with the added benefit of complementary perfusion information. C6 tumors were found to be more hypoxic overall than 9L tumors, whereas 9L tumors exhibited microregional hypoxia and higher overall perfusion than C6 tumors. Evofosfamide is a promising hypoxia-activated prodrug and showed a significant effect on tumor growth rates in the 2 glioma tumor models mentioned in this study. The less hypoxic 9L tumors showed favorable response to evofosfamide, likely because of regional hypoxia and increased perfusion, although the reduction in tumor
growth rates was less than that in the growth rates of C6 tumors. In C6 tumors, both hypoxia characteristics and treatment response were modulated by tumor size, with a higher severity of hypoxia and greater treatment response in larger tumors. Evofosfamide is expected to predominantly affect hypoxic tumor regions—which likely vary spatially—highlighting the need for spatially sensitive measures of hypoxia and treatment response.

ACKNOWLEDGMENTS
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REFERENCES


Erratum: Wangerin et al. (2015)

In the article “Effect of 18F-FDG Uptake Time on Lesion Detectability in PET Imaging of Early-Stage Breast Cancer,” by Kristen A. Wangerin, Mark Muzi, Lanell M. Peterson, Hannah M. Linden, Alena Novakova, Finbarr O’Sullivan, Brenda F. Kurland, David A. Mankoff, and Paul E. Kinahan (Tomography, Vol. 1, No. 1, pp. 53–60. http://dx.doi.org/10.18383/j.tom.2015.00151), errors were discovered in Table 2. These errors have been corrected and the revised table is shown below. These corrections do not affect the main conclusions and interpretation of the original paper.

Table 2. Estimated Kinetic Parameters for All Patients for Nonreversible Model ($k_4 = 0$).

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<td>$k_2$ (min⁻¹)</td>
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<tr>
<td>$k_3$ (min⁻¹)</td>
<td>0.015 ± 0.013</td>
<td>0.001–0.045</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.
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