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The utility of microCT and MRI in the assessment of longitudinal growth of liver metastases in a preclinical model of colon carcinoma

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Abstract

Rationale and Objectives—Liver is a common site for distal metastases in colon and rectal cancer. Numerous *clinical* studies have analyzed the relative merits of different imaging modalities for detection of liver metastases. A number of exciting new therapies are being investigated in *preclinical* models. But, technical challenges in *preclinical* imaging make it difficult to translate conclusions from clinical studies to the preclinical environment. This study addresses the technical challenges of preclinical MR and micro-CT to enable comparison of state-of-the-art methods for following metastatic liver disease.

Materials and Methods—We optimized two promising preclinical protocols to enable a parallel longitudinal study tracking metastatic human colon carcinoma growth in a mouse model: T₂-weighted MRI using 2-shot PROPELLER (Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction), and contrast-enhanced micro-CT using a liposomal contrast agent. Both methods were tailored for high throughput with attention to animal support and anesthesia to limit biological stress.

Results and Conclusions—Each modality has its strengths. Micro-CT permitted more rapid acquisition (<10 minutes) with the highest spatial resolution (88-micron isotropic resolution). But

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detection of metastatic lesions requires the use of a blood pool contrast agent, which could introduce a confound in the evaluation of new therapies. MR imaging was slower (30 minutes) and had lower anisotropic spatial resolution. But MR eliminates the need for a contrast agent and the contrast-to-noise between tumor and normal parenchyma was higher, making earlier detection of small lesions possible. Both methods supported a relatively high-throughput, longitudinal study of the development of metastatic lesions.

Keywords

PROPELLER; multi-modality; MR; CT; mice; liver metastases

Introduction

Colorectal cancer is the third most common type of cancer in humans (1). It commonly metastasizes to the liver, at which point morbidity and mortality drastically increase. In a third of the patients who die of colorectal cancer, metastatic disease is found only in the liver. Liver metastases are also seen in other cancers such as pancreas, stomach, breast, and lung, making the liver one of the most common sites of distal metastases, second only to lymph nodes. Early detection and effective treatment of liver metastases would greatly improve prognosis for many patients.

Preclinical orthotopic disease models, which closely mimic human tumor conditions, are a tremendous resource for measuring the efficacy of many potential treatments now under study. Preclinical imaging is rapidly becoming one of the most critical methods for evaluating response to these therapies. But, extension of clinical methods/conclusions to the preclinical environment is fraught with challenges. The mouse, at 25g, is nearly 3000-times smaller than a human, so the spatial resolution in the preclinical system must be commensurately higher. Physiologic rates are also faster (heart rate is 10-times, and respiration is 5-times faster), so the temporal resolution of the preclinical system must be correspondingly faster. Small animal imaging usually requires anesthesia, and respiratory motion is a major technical challenge, particularly for imaging abdominal organs. While scan-synchronized respiration has become routine, it requires intubation, which induces stress in the animal and adds to the complexity of the study. The mouse is fragile. One must provide external thermal regulation and limit physical handling. Finally, for any protocol to be useful, it must be executed in a reasonable time. Thus, the criteria we have set in designing this comparative preclinical protocol are: a) stress on the animal must be minimized; b) setup and execution must be accomplished in <30 minutes; c) imaging must cover the entire liver; and d) images must be of the highest quality possible.

This last criterion of image quality imposes a particularly vexing conundrum. What are the most appropriate preclinical modalities and how does one optimize them for our given task—non-invasive study of liver metastases? Preclinical studies of mouse models of liver cancer have used several imaging modalities: positron emission tomography (PET) (2), bioluminescence imaging (3) (4), computed tomography (CT) (5), and magnetic resonance imaging (MRI) (6) (7) (8), albeit independently. PET provides excellent functional information regarding tumor metabolism. However, PET is costly, not widely available, and has resolution limits of $>1\text{mm}^3$ imposed by the physics of positron decay. While the spatial resolution limit is not a significant problem in the clinical domain, resolution at 1 mm or greater is particularly problematic in the mouse. Bioluminescence imaging, though highly sensitive, is also limited by spatial resolution, as well as the need for mouse models that genetically express luciferase. CT is more readily available, provides high spatial resolution, and is also preferred for clinical liver imaging, which makes translational studies more appealing. MRI has the best soft tissue contrast, and has been used frequently with an

extraordinary range of imaging sequences and contrast mechanisms. Thus, we chose to compare micro-CT and MR microscopy.

We needed to determine how to optimize these two modalities. Clinical systems have for the most part been standardized. But, preclinical systems vary widely. A micro-CT system with a 10W x-ray tube requires considerably longer scan time than a 50kW tube CT system, for constant noise. The contrast and noise in a MR image is dependent on a host of technical choices, such as field strength, imaging strategy (e.g. T₁-weighted, T₂-weighted, diffusion-weighted), use of contrast agents, and so on. Because of the much wider variation in technical specifications and approaches for preclinical imaging, we have optimized two imaging strategies that we believe represent the state-of-the-art for preclinical micro-CT and MR microscopy.

The three determinants of image quality in micro-CT are resolution, signal-to-noise ratio (dose), and contrast. We previously described a micro-CT system that supports 88- μ m isotropic spatial resolution with scan time <5 minutes. This system has a unique design that allows us to use multiple, high-power, rotating anode x-ray tubes that deliver radiation flux >250-times that of commercial systems using micro-focal tubes (9). Thus, the quantum noise is very low, resolution is high, and scan times are short. The Achilles heel of CT is contrast difference between metastatic and normal tissue. Conventional contrast agents are problematic in preclinical models, since they generally clear faster than the time required for completing the scans. We have addressed this by using a liposome contrast agent, which unlike conventional renal clearance has a slower clearance via the hepatic system.

Optimizing resolution and contrast in preclinical MRI is difficult, again because of the unique challenges of preclinical imaging. The highest-resolution preclinical MR systems operate at fields >7T. At these fields, T₁ values for all tissues are considerably longer than at clinical fields (<3T), and T₂ values are considerably shorter. T₂-weighted imaging, one of the most common methods for detecting liver metastases, requires a different approach for a preclinical system. We have previously demonstrated a technique for preclinical T₂-weighted imaging at high-fields using fast spin echo (FSE)-based multi-shot PROPELLER (Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction) (10) (11). The motion correction ability of PROPELLER acquisition and reconstruction (12) makes this technique ideal for imaging free-breathing mice. The FSE-based approach enables excellent T₂-weighted contrast. These characteristics led us to choose this T₂-weighted sequence as representative of the state-of-the-art in preclinical MR imaging of metastatic lesions. The sequence has been tuned to allow acquisition of free-breathing animals in 30 minutes. The goal of this study was to compare these two optimized (micro-CT and MR) imaging strategies to detect liver metastases in a mouse model. To the best of our knowledge, a rigorous preclinical study like this has not been previously undertaken.

Materials and Methods

Animal model

All animal studies were approved by the University Institutional Animal Care and Use Committee. Tumor inoculation procedures were performed at XXXX Research Center (XXXX, NC). Female athymic nude mice were implanted with 5×10^6 cells of HT29 colon carcinoma in 50 μ l volume of using a 25-gauge needle in the spleen. After a two-minute pause post-injection, a splenectomy was conducted under isoflurane anesthesia. Animals were allowed to recover for 9 days before imaging.

This model was unique because it enabled study of secondary tumor sites. The primary tumor site, which normally dominates the disease model, was removed by splenectomy,

which allowed sufficient time for development of secondary tumor sites—in this case, liver metastases. For colon carcinoma, the liver is the primary site of metastases. This was also a very aggressive disease model. After two weeks, the liver tumors showed exponential growth. Thus, the total study duration was restricted to approximately one month after implantation, at which point the mice had reached the humane end point for acceptable tumor burden.

Experimental design

The experimental design was based on the constraints of the disease model. Table 1 shows the experiment time line. Day 0 (tumor inoculation) was followed by 9 days of recovery. Imaging was carried out from day 10 (after recovery from surgery) through day 31 (humane end point). Up to 8 mice were imaged with both MR and CT two times a week for a total of 7 time points. Mice lost due to excessive tumor penetration before the last time point were replaced with others from the same cohort. Four mice survived the entire duration of the study and their histological data was acquired after the last imaging day. Data for intermediate histology time points were acquired from a separate cohort of mice that also underwent a same-day *in vivo* MR imaging session.

Designing CT and MR protocols for fair comparison of the two modalities requires a number of choices. Clinical CT is typically performed with conventional iodinated contrast agents (e.g. Iovue). But, special consideration must be given to translation of clinical to preclinical protocols. Since the mouse heart beats considerably faster than humans (600 vs. 60 beats per minute), mice have much faster clearance of conventional contrast agent. Also, preclinical scans are typically longer than clinical scans (~5 minutes vs. 30 seconds). So, to fairly compare contrast-to-noise ratio (CNR) between CT and MR acquisitions, we used a blood pool contrast agent. In previous work, we compared the CNR achieved from a conventional iodinated contrast agent with that from a liposomal blood pool agent (13) described in (14), which consists of small (100 nm) liposomes that are synthesized with Iopamidol solution (Iovue® 370, Bracco Diagnostics, Princeton, NJ) encapsulated in the core of the liposome. The surface of the liposome is coated with polyethylene glycol to increase biological half-life. The agent used for this work had an effective iodine concentration of 123mg/mL. The mechanism to detect metastases relies on degradation of the liposome in normal liver, which releases the iodine to the interstitial space and yields enhancement of normal parenchyma. Metastatic lesions, which do not break down the liposome, are not enhanced. Consequently, the normal liver is hyperintense and the lesions are hypointense.

The contrast agent was administered via a tail vein injection at a dose of 0.4mL/25g. It was injected 24 hours before the imaging studies, based on a separate set of experiments, which ensured that the CT contrast agent was optimized and that it did not have appreciable effect on CNR of the MR experiments. In these separate experiments, CT scans were obtained from animals with the same metastatic disease model at multiple days after a single injection of the liposome.

Another initial concern was the potential effect of the CT contrast agent on the contrast in the MR experiment. Iodinated contrast agents have been reported to alter T_1 and T_2 (15). To address this concern, a separate set of experiments was conducted where tumor-bearing mice underwent MRI one day before administration of CT contrast agent. The same animals were also imaged up to 2 days post-contrast injection. CNR measurements between tumor and liver in the pre-contrast MR images were not statistically different from the post-contrast MR images.

In all CT and MR imaging, the mice were free-breathing and maintained under anesthesia by isoflurane administered by nose cone. The respiratory rate was monitored throughout the study (SA Instruments Inc., Edison, NJ). Surface body temperature was measured with a thermistor placed snugly under the neck. Temperature was maintained between 33–34°C, by blowing warm air into the magnet bore for MR, and by a heat lamp for CT. An integrated animal cradle that addresses anesthesia delivery, physiological signal monitoring, and animal-positioning needs was used for both CT and MRI (16). After the intermediate imaging sessions, the mice were administered 0.5 mL of saline and returned to their cages. After the final imaging time point, the mice were either euthanized with anesthesia overdose (n=2) or were perfusion-fixed for histology (n=4).

MR imaging protocol

All MR imaging was carried out on a 7T, 210mm bore, horizontal Magnex magnet with a GE EXCITE console on the EPIC Lx12.4 software platform (GE Healthcare, Milwaukee, WI), with shielded gradient coils (Resonance Research Inc., Billerica, MA) with a maximum gradient strength of 770mT/m and a rise time of 100 μ s. In this system, high-power amplifiers (Copley Controls, Canton, MA) drive the gradients. The strong gradients with high slew rate, high duty cycle, and high-power amplifiers were necessary to achieve short inter-echo spacing (ESP) that is critical at high fields to minimize susceptibility-induced losses (10). The RF coil used was a 35mm-diameter quadrature transmit/receive volume coil (m2m Imaging Corp., Cleveland, OH).

2-shot PROPELLER (10) (11) with an echo train length of 10 was used to obtain ungated, heavily T₂-weighted images with TE=67ms, TR=3s. Multi-slice axial datasets covering the entire abdomen (125 μ m in-plane, 1mm slice) were acquired in 33 minutes. Image reconstruction used an offline MATLAB (MathWorks, Natick, MA) program described in (10) (11).

CT imaging protocol

All CT imaging was performed with a dual imaging chain micro-CT system, consisting of two x-ray tubes with a 0.8mm focal spot and two cooled CCD cameras (17). The animals were placed in an integrated animal cradle on the scanner's vertical rotating stage. This system is optimized to have a high photon flux, and thus provides what we believe to be the highest SNR currently available with micro-CT. Images of a 25mm water phantom using the same acquisition parameters as the animal studies yielded an average noise level of 68HU (18) at an estimated dose of 75mSv

Only one imaging chain was used for this experiment. Each scan consisted of 300 projections with a 0.65° step angle, acquired at 80kVp, 160mA, and 10ms exposure. A pressure-sensitive sensor in the cradle provided the signal for respiratory-triggered acquisitions. Volume images with 88- μ m isotropic resolution were acquired in ~6 minutes. Images were reconstructed in MATLAB with a 512 $\times$ 512 $\times$ 640 array covering a 45 $\times$ 45 $\times$ 56mm³ field of view. Comparing CNR between CT and MRI is complicated, since the baseline CT protocol has isotropic spatial resolution, whereas the MRI protocol has a lower anisotropic resolution. To enable comparison of CNR with MR images, two different CT datasets were constructed. In addition to the baseline CT that was reconstructed at 88- μ m isotropic resolution, a second CT dataset was constructed from the same projections with anisotropic spatial resolution identical to that in the MRI, i.e. 1mm-thick slices with 125 μ m in-plane resolution.

Image analysis

The CNR for both modalities was calculated according to Eq. [1], where SI represents signal intensity.

$$CNR = \left(\frac{|SI_{liver} - SI_{tumor}|}{\sigma_{noise}} \right) \quad [1]$$

Six slices with regions of both viable liver and tumors were chosen from each dataset, and three distinct regions of interest (ROIs) for liver and tumor were manually selected from each slice to determine the mean liver and tumor signal intensities. Noise was measured in the periphery of each image, i.e. outside the field occupied by the animal. CNR was calculated for each slice using the standard deviation of the noise for that slice.

Conventional histology

Conventional histology was performed to permit correlation between hematoxylin and eosin (H&E) slides and *in vivo* CT and MR images. The mice were transcardially perfused with 10% buffered formalin, so that the abdomen (from diaphragm and below) remained intact. The fixation procedure was performed under isoflurane anesthesia delivered via nose cone. An initial 4-minute saline flush at 8mL/minute was followed by 10% formalin for 5 minutes at the same rate. The fixed specimens were kept in a 10% formalin solution for at least a week and then transferred to 70% alcohol one day prior to histology. Based on the *in vivo* MR datasets, the specimens were sectioned into 4 slabs around regions that clearly showed both viable liver tissue and diseased tumor tissue, and 2–3 H&E slides with 5µm thickness were created from each slab.

Results

Figure 1 shows results of the preliminary experiment to study the evolution and clearance of the CT contrast agent. Three different time points after contrast injection are shown—day 1 (Fig. 1a), day 5 (Fig. 1b), and day 8 (Fig. 1c). As is evident, administering the contrast agent one day (16–18 hours) before imaging provided increased control of the contrast levels in the image. The viable liver was enhanced due to the contrast agent; tumors were dark (Fig. 1a black arrowheads) due to lack of contrast agent; and vasculature (Fig. 1a white arrowheads) remained bright, because the contrast agent had not yet washed out. The images acquired at day 5 post-injection showed similar low-intensity values for both tumors and vasculature, making them hard to differentiate. On day 8 post-injection, the images were virtually devoid of contrast (similar to pre-contrast).

Fig. 2a shows a representative T₂-weighted MR image. Multi-slice datasets with in-plane resolution of 125µm and 1mm-thick slices were acquired in 33 minutes. The entire thorax and abdomen of the mouse was imaged with a total of 31 slices. Figure 2a shows a number of anatomical features—kidneys (thick arrows), stomach (thin arrow on right side of figure), and intestines (thin arrow on left side of figure). In a T₂-weighted image, the liver tumors are hyperintense (white arrowheads) on the darker background, which is the viable liver.

The image also shows two markers used for assessing the measurement consistency in the MR datasets. Marker 1 has signal intensity (~10,000) comparable to the intermediate values in the live animal, while Marker 2 (~30,000) is representative of higher intensities. The markers showed minimal deviation (Marker 1: 6.3% and Marker 2: 5.3%) in measurements taken over multiple slices, different datasets, and all 7 imaging days. These shifts were considered to be within acceptable bounds imposed by systemic variations (19).

Figures 2b and 2c show the corresponding slice from the same mouse on the same day, as seen in Fig. 2a, with contrast-enhanced CT. These respiratory-gated CT images were acquired in 5–7 minutes, depending on respiratory rate. Both isotropic and anisotropic resolution (matching the MRI) images are shown. Figure 2b is the highest (88 μm isotropic) resolution that is the default reconstruction for the scanner. Figure 2c has been reconstructed at a lower resolution (125 μm in-plane with 1-mm slice thickness) to match the MR images and make CNR measurements more meaningful. In the CT images, the viable liver is hyperintense due to uptake of contrast agent. In comparison, the tumors, which do not take up any contrast agent, are hypointense (black arrowheads). The blood vessels, as well as the kidneys, have medium signal intensity, because the contrast agent has not yet completely washed out. The stomach (thin arrow on left side of figure) can be differentiated by the presence of small air pockets. The CNR was measured in the five separate lesions visible in all the images. The mean CNR for these five lesions in the two CT images scales according to the difference in their voxel volume (20). For Fig. 2b, the CNR is 11.54, but for Fig. 2c, where 8 consecutive slices (each 125 μm thick) have been averaged together to generate 1mm thick slabs, the CNR is 13.89. The mean CNR for the same lesions in the MRI is 13.49.

The baseline CT images have isotropic resolution, so it is possible to resection the data in any plane. Figure 3 shows coronal resections for both CT and MR. Compared, Fig. 3c and 3d clearly show the benefits of isotropic CT resolution. The figure also clearly demonstrates the inherent difficulty in comparing spatial and contrast resolution in these preclinical imaging modalities. The higher isotropic spatial resolution in the CT images allows one to see the tumor boundaries much more clearly in both the axial and coronal planes. But, the higher CNR in the MR allows one to appreciate the tumors in the axial MR images more readily than in the CT images.

The animal is placed horizontally (prone) for MR, while the animal is vertical for CT. The difference in gravitational effects of the two positions makes it difficult to obtain perfectly matched slices from the two modalities, though such an attempt was made in all images shown. The coronal sections (Fig. 3c and 3d) are at roughly the same level in the mouse. Both datasets clearly show the left kidney (thick arrow). Also seen is a section of the inferior vena cava (thin arrow). Tumors (arrowheads) are hypointense in the CT image, and hyperintense in the MR image. The region behind the tumors, with similar intensity levels as the tumors, is the intestine, which is easier to differentiate with MR by the presence of a brighter lumen.

Fig. 4 shows a detailed time course of disease progression in the same animal, with both MR (left column) and CT (right column). All images were reconstructed with an in-plane resolution of 125 μm and 1mm slice thickness. The first time point is day 17, as datasets from earlier time points do not provide much information with either modality. The slice depicted in Fig. 4 shows three distinct tumor nodes in the ventral liver lobe that can be tracked with both modalities. The tumors are easier to distinguish in the early MR images (4a, 4b) than in the early CT images (4f, 4g). Additionally, the MR images appear to have increased intensity in regions (thin arrows) that at later time points are clearly tumors. Such information is not apparent from the CT images. MR images also show greater heterogeneity within the tumor. Note for example, the darker central tumor region (thick arrow) in Fig. 4d and 4e, which likely represents central necrosis. Both modalities show good contrast between viable liver and tumors. The contrast increases for later time points, as demonstrated in Fig. 5.

Figure 5 shows CNR with both modalities averaged over 3 different mice. CNR for 6 different slices for each of the 3 mice are included (total of 18 measurements). Because

variations exist in the tumor growth rate between animals, there is considerable variability in CNR. Nevertheless, the following can be determined from the graph in Figure 5—CNR in both modalities shows a gradual increase at later time points, and CNR in the T₂-weighted MR images is higher than that in the contrast-enhanced CT images.

The final set of images show the correlation between *in vivo* MR and CT, and conventional histology. Figure 6 shows an anatomically-matched slice from the same mouse 31 days post-inoculation; top row shows *in vivo* T₂-weighted MRI and *in vivo* contrast-enhanced CT, and bottom-left shows the H&E section. The perfusion fixation followed by sectioning with the microtome caused separation of the hepatic lobes. However, correspondence between the three modalities is clear. The basophilic regions (tumors) in the histology section match the hyperintense tumors in T₂-weighted MR and hypointense tumors in contrast-enhanced CT. The heterogeneous region marked in the MR image by the large arrow correlates with histology, where the tumor node has central necrosis (and stroma) and is surrounded by viable liver. This demarcation is not as apparent in the CT image. Arrowheads point out a small tumor node (0.33mm³), which is visible with histology and MRI, but not with CT.

The higher-resolution H&E section in Fig. 6d shows tumor, as well as viable liver. The white space is a larger blood vessel in the liver. The pale pink cells are Kupffer cells. The liposomal contrast agent is phagocytosed by these cells and hence, they have considerably more swollen cytoplasm than is usually observed. Also, the Kupffer cells seem to be proliferating in an unnatural manner, particularly around blood vessels. This, in turn, is causing compression of the normal hepatocytes, though no unusual cell death is observed. The contrast agent also appears to be accumulating in the stroma supporting the liver tumors, though not in the actual tumor cells.

Discussion

We have executed a side-by-side comparison of MRI and CT for detection and characterization of liver metastases in a preclinical model. Our protocols were implemented for the real-world scenario required for routine high-throughput evaluation in a longitudinal study. The requirements included minimal stress to the animal, fast setup with scan times of ~30 minutes, and full liver coverage with the *highest image quality possible*. Numerous studies have optimized and compared clinical protocols. But, the unique requirements for preclinical imaging, as well as the lack of standardization have limited such studies for small animals. We have systematically optimized protocols in this study for both MRI and CT to allow comparison with what we believe to be the state-of-the-art for both modalities.

We have shown previously that the 2-shot PROPELLER technique (10) is particularly appropriate for small animal applications at high magnetic fields. The PROPELLER trajectory is inherently motion-insensitive and is self-navigated, allowing for motion correction. Thus, it was possible to carry out ungated acquisitions in free-breathing mice with minimal stress to the animal. The 2-shot modification, along with the high-field correction (11), provided what we believe are representative of the state-of-the-art for MR scanning with the time constraints (30 minutes) set at the outset of the study. Multi-slice datasets covering the entire abdomen with an in-plane spatial resolution of 125μm and 1mm-thick slices were acquired in 30 minutes each, making the protocol highly conducive for high-throughput studies.

The challenges of scaling CT imaging from the clinical (human) to the preclinical (small animal) domain include many of the same challenges as MRI, most notably those arising from the tradeoff between signal-to-noise ratio (SNR), scan time, spatial resolution, and x-ray dose (20). The scan time is highly dependent on the SNR one requires. Since

commercial systems employ x-ray tubes with small focal spots, i.e. low power, the only alternatives for high SNR acquisitions are to reduce the spatial resolution or to increase the signal averages. The system developed at our Center has addressed this barrier through a unique geometry that allows using high-power rotating anode tubes simultaneously, supporting higher spatial resolution, higher SNR, and short scan times (9). We believe this protocol represents the highest spatial resolution (88 μ m isotropic) with the highest SNR (noise=68 HU) that has yet been reported for a scan time <10 minutes.

The use of contrast agents is essential to make CT a competitive modality. But again, the challenges of working with the small animal require a different approach than in the clinical setting. Traditional iodinated contrast agents (such as Isovue, Omniview) are designed for rapid renal clearance. The transit time, i.e. time for blood to make one complete cycle through the vascular system, is ~10 seconds in humans. The time to acquire a full volume CT image of a live human is also ~10 seconds. The transit time for the mouse is ~5 seconds. Even with our unique scanning system, the scan times (~6 minutes) are considerably longer, particularly in the context of the relevant transit time (scan time/transit time is ~1 for humans; ~70 for mice). The result is that routine contrast agents in the clinical arena are not effective for small-animal CT, particular of the liver. This problem was addressed by using a contrast agent with a significantly longer biological half-life—a blood pool contrast agent (liposomal iodine)—that was injected one day before each imaging session. The liposome is degraded by viable liver, which releases iodine to the interstitial space and causes enhancement of normal parenchyma. Thus with this contrast regime, it was possible to differentiate viable liver, liver vasculature, and liver tumors with contrast differences that were comparable to what is obtained clinically.

For animals with already limited liver function, twice-weekly contrast injections were a concern. Conventional H&E sections revealed that the contrast agent accumulated in the liver's Kupffer cells (Fig. 6d), as well as in other macrophages throughout the body. The rate of contrast clearance was slower than the rate of injection, which caused swelling and vacuolation of the macrophages, along with excessive proliferation. This, in turn, caused compression of the healthy hepatocytes, and it is likely that liver perfusion was compromised. Even so, no unusual cell deaths were observed and no toxicity was reported. In addition to contrast injections, the CT also exposed mice to repeated radiation, which again is a potential limiting factor in longitudinal studies. But MR, being completely non-invasive and non-ionizing, had no such limitations.

The sensitivity of the exogenous CT contrast agent was lower than the pathology-dependent T₂ contrast in the MR images, particularly at early time points (Fig. 4) and for very small tumors (Fig. 6). The initial MR images provide early indication of tumor information that is not evident in the CT images. A possible explanation for this could be the contrast uptake in the stroma, which is the connective tissue supporting the tumors. Thus, at earlier time points and when tumors are very small, the signal from the stroma might be more dominant. Additionally, CT images have a negative contrast, i.e. though we are imaging tumors, we are trying to detect the lack of signal. In comparison, MR images have a positive contrast, i.e. the objects (tumors) being imaged have increased signal intensity, and this is typically easier to interpret. Also, due to superior soft-tissue contrast, anatomical landmarks were much easier to discern in the MR than in the CT images.

At later time points and for larger tumors, both modalities could detect liver metastases, but MR had higher CNR than CT. These results confirm those previously reported (21), though both modalities, and in particular CT, have significantly higher values in our study because the protocols were optimized. The CNR measurements from MR showed a larger variability (Fig. 5) than CT. Since T₂ is tissue-dependent, the MR images showed increased

heterogeneity even within the tumors (Fig. 4) as the disease progressed. Also, for both modalities, CNR progressively increased at later time points. In MR, this can be attributed to the nature of the tumor growth, which at later time points was solid and clearly demarcated. In CT, this increase was most likely due to accumulation of the contrast agent in the liver arising from repeated injections. This was reaffirmed with data acquired with a double-dose of contrast agent (not shown), which caused a 10% increase in the CT CNR. The effect of the CT contrast agent on the MR data was minimal.

In conclusion, using the optimized protocols reported here, both high-field T₂-weighted MRI and contrast-enhanced micro-CT were sufficiently simple and fast for rapid acquisitions required for high-throughput studies, and were also sufficiently non-invasive to allow multiple scans in a preclinical model of metastatic liver disease. Both modalities overcome the effects of respiratory motion at the imaging site to yield artifact-free images. Up to 8 mice/day were imaged with both MR and CT, for a total of 7 imaging time points. Each modality has its strengths (and weaknesses). Micro-CT has higher spatial resolution and isotropic datasets that enable resections in any plane, allowing more accurate measures of tumor volume—particularly at later stages in the tumor development. With a respiratory-triggered acquisition of ~6 minutes, micro-CT is more conducive for high-throughput studies than MRI (33 minutes). T₂-weighted MRI has higher CNR than contrast-enhanced micro-CT (between viable liver and metastatic tumors). Additionally, heterogeneity within the tumors can also be seen in the MR images, which we speculate represent different stages of tumor growth. We believe that MRI could be used in the future to quantify additional tumor properties in this model, compared to tumor detection alone. Finally, MRI is less invasive than micro-CT, which requires both contrast injections as well as ionizing radiation, and thus MRI is more suitable for longitudinal studies.

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Figure 1. Contrast-enhanced CT images (isotropic resolution) acquired (a) day 1, (b) day 5, and (c) day 8 post-contrast injection. Arrowheads in (a) indicate tumors (black) and blood vessels (white).

Figure 2.

Representative slice of a mouse with liver metastases showing (a) T₂-weighted MR image (1mm slice, 125μm in-plane), and contrast-enhanced micro-CT reconstructed with (b) 88μm isotropic resolution, and (c) 1mm slice, 125μm in-plane resolution. Smaller arrowheads show liver metastases. Arrows point to kidneys (solid arrows) and stomach (dashed arrow on right side of figure). MR image (a) also shows intestines (dashed arrow on left side of figure), and Marker 1 (3% Agarose) and Marker 2 (CuSO₄), which were used for quality control. The mean contrast to noise for the five tumors marked was 13.49 for MRI (a); b) 11.89 for isotropic CT (b); and 13.89 for anisotropic CT (c).

Figure 3.

Axial datasets (a,b), along with coronal resection (c,d) with both CT (a,c) and MR (b,d). The coronal section shows left kidney (thick arrow), inferior vena cava (dashed arrow), and multiple tumor nodules (arrowheads).

Figure 4.

T₂-weighted MR images (left) and contrast-enhanced CT images (right) from similar anatomic locations of the same mouse showing liver metastases from HT29 colon carcinoma at day 17 (a,f), day 21 (b,g), day 24 (c,h), day 28 (d,i), and day 31 (e,j) post-inoculation. Growth of three metastatic nodes in the ventral liver lobe can be followed throughout the study. Dashed arrows (a,b) point to regions of increased intensity in MR images that later convert to tumors, and solid arrows (d,e) point to central necrosis in the lower node.

Figure 5.
CNR between viable liver and tumors as a function of imaging day for T₂-weighted MR (gray) and contrast-enhanced CT (black).

Figure 6.

Correlation of *in vivo* MR (a) and CT (b) with conventional histology (c). Arrow points to a tumor with central necrosis surrounded by viable liver. Arrowhead shows a small tumor nodule visible in MR, but not in CT. (d) Higher-resolution histology section showing tumor (purple-blue), viable liver (red), and contrast accumulation in Kupffer cells (pale pink).

Table 1

Experiment timeline.

	Day of study														
	0	9	10	13	14	16	17	20	21	23	24	27	28	30	31
Tumor inoculation (number of mice)	X 24														
CT contrast injection (number of mice)		X 8		X 8		X 8		X 8		X 8		X 7		X 6	
CT imaging (number of mice)			X 8		X 8		X 8		X 8		X 8		X 7		X 6
MR imaging (number of mice)			X 8		X 8		X 8		X 8		X 8		X 6		X 6
Histology (number of mice)							X 2				X 2				X 2