Pre-clinical imaging of transgenic mouse models of neuroblastoma using a dedicated 3-element solenoid coil on a clinical 3T platform

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Background: The use of clinical MRI scanners to conduct pre-clinical research facilitates comparisons with clinical studies. Here the utility and sensitivity of anatomical and functional MRI data/biomarkers acquired from transgenic mouse models of neuroblastoma using a dedicated radiofrequency (RF) coil on a clinical 3T scanner was evaluated.

Methods: Multiparametric MRI of transgenic mice bearing abdominal neuroblastomas was performed at 3T, and data cross-referenced to that acquired from the same mice on a pre-clinical 7T MRI system. T²-weighted imaging, quantitation of the native longitudinal relaxation time (T¹) and the transverse relaxation rate (R²*) and dynamic contrast-enhanced (DCE)-MRI, was used to assess tumour volume, phenotype and response to cyclophosphamide or cabozantinib.

Results: Excellent T²-weighted image contrast enabled clear tumour delineation at 3T. Significant correlations of tumour volume (R=0.98, P<0.0001) and R²* (R=0.87, P<0.002) measured at 3 and 7T were established. Mice with neuroblastomas harbouring the anaplastic lymphoma kinase mutation exhibited a significantly slower R²* (P<0.001), consistent with impaired tumour perfusion. DCE-MRI was performed simultaneously on three transgenic mice, yielding estimates of Ktrans for each tumour (median Ktrans values of 0.202, 0.168 and 0.114 min⁻¹). Cyclophosphamide elicited a significant reduction in both tumour burden (P<0.002) and native T¹ (P<0.01), whereas cabozantinib induced significant (P<0.01) tumour growth delay.

Conclusions: Simultaneous multiparametric MRI of multiple tumour-bearing animals using this coil arrangement at 3T can provide high efficiency/throughput for both phenotypic characterisation and evaluation of novel therapeutics, and facilitate the introduction of functional MRI biomarkers into aligned imaging-embedded clinical trials.

High field (>4.7T) small animal dedicated MRI systems that provide high resolution and signal to noise ratios (SNR) have and continue to be the preferred platform for pre-clinical imaging investigations. The physical contrast characteristics and imaging performance of such pre-clinical scanners differs substantially from clinical MRI systems. The use of clinical scanners to conduct pre-clinical research is attractive, as it facilitates a more direct comparison with clinical studies, the application and development of clinically applicable imaging protocols, matching of field strength-related mechanisms such as relaxation, and provides evidence to support the clinical relevance of functional MRI data/biomarkers (Brockmann et al, 2007; Chen et al, 2007; Inderbitzin *Correspondence: Dr SP Robinson; E-mail: simon.robinson@icr.ac.uk

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et al., 2007; Linn et al., 2007; Bilgen, 2013). This approach also benefits from the hardware and software advances continuously being made on modern clinical MRI systems, such as parallel transmit and receive technology, motion and contrast tracking, k-space under-sampling and view-sharing strategies (Ullman et al., 2004; Wech et al., 2012; Jerome et al., 2016). Additionally, the homogeneous, non-bespoke high resolution RF coil for use on a clinical 3T scanner for the acquisition of anatomical and functional sensitivity of a non-bespoke high resolution RF coil for use on a typically arises within deep-seated anatomical locations. To assess the progression and treatment response of tumours that demand non-invasive methods to longitudinally and accurately static potential and therapeutic response in vivo growth, tumour-host stromal interactions and vasculature, meta-

et al., 2014) (Scientific Procedures) Act 1986, the United Kingdom National Cancer Research Institute guidelines for the welfare of animals in pre-clinical cancer research performed in vivo, transgenic mouse models, in which tumours are driven by expression of the target gene of interest and arise spontaneously within the native tissue of origin, are being increasingly exploited (de Jong et al., 2014). These models more faithfully emulate human tumour growth, tumour-host stromal interactions and vasculature, metastatic potential and therapeutic response in vivo. Such models demand non-invasive methods to longitudinally and accurately assess the progression and treatment response of tumours that typically arise within deep-seated anatomical locations. The purpose of this study was to evaluate the utility and sensitivity of a non-bespoke high resolution RF coil for use on a clinical 3T scanner for the acquisition of anatomical and functional MRI data/biomarkers from transgenic mouse models of neuroblastoma in vivo.

MATERIALS AND METHODS

All procedures involving animals were performed in accordance with the local ethical review panel, the UK Home Office Animals (Scientific Procedures) Act 1986, the United Kingdom National Cancer Research Institute guidelines for the welfare of animals in cancer research and the ARRIVE guidelines (Workman et al., 2010; Kilkenny et al., 2010).

$T_1$ phantom. To assess the linearity of $T_1$ measurements on both the 3 and 7T platforms, phantoms with increasing concentrations of gadolinium (from 30 to 500 nM) were imaged in both scanners at ambient temperature. These solutions were transferred into 5 mm diameter solenoids, with the long axis oriented perpendicular to the magnet $B_0$ field. This coil arrangement enables the simultaneous acquisition of MRI data from up to three animals using independent receiver channels. The 3T imaging protocol, which included $T_2$-weighted, $T_1$ and $R_s$ measurements, was tested and optimised using phantoms prior to the in vivo study (Yakes et al., 2011).

$T_1$ data acquisition and analysis. MRI data was acquired on a clinical scanner (Philips Achieva, Best, Netherlands) using a dedicated high-resolution RF coil ('Mouse Hotel', Philips, Best, Netherlands) (Figure 1). The coil consists of three 40 mm inner diameter solenoids, with the long axis oriented perpendicular to the magnet $B_0$ field. In the in vivo study. A compromise between SNR, spatial and temporal resolution was prioritised over a direct parameter match to pre-clinical 7T protocols. Anaesthesia was induced by intraperitoneal injection of midazolam (5 mg kg$^{-1}$), fentanyl (0.05 mg kg$^{-1}$), and medetomidine (0.5 mg kg$^{-1}$) in sterile water. For 3T data acquisition, up to three anaesthetised mice were positioned supine with their abdomens in the centre of each individual element of the coil. Mouse core body temperature was maintained by a built-in heating system controlled within the 'Mouse Hotel' coil.

Initially, whole body anatomical $T_1$-weighted images were acquired at 3T using a coronal orientation with full anatomical coverage of all three animals (TSE, 20 slices, slice thickness: 1 mm, FOV = 250 × 200 mm, resolution = 0.2 × 0.2 mm,ETL = 14, TE/TR = 80/3000 ms, NSA = 1, TA = 3 min 48 s). $T_2$-weighted images were used to assess the extent of the disease and aid axial sequence planning, which was performed across the central part of the tumour. Proton density and $T_1$-weighted images were then overexpression of MYCN to the neural crest under the control of the tyrosine hydroxylase promoter resulted in the Th-MYCN transgenic mouse model in which abdominal tumours spontaneously develop that faithfully replicate the disease biology of high-risk neuroblastoma (Weiss et al., 1997). More recently, the identification of mutations in the anaplastic lymphoma kinase (ALK) gene in neuroblastoma, and its close association with MYCN amplification, stimulated the development of a novel double transgenic mouse model for ALK and MYCN (Berry et al., 2012). In both the Th-MYCN and Th-ALK$^F1174L$/Th-MYCN transgenic mice, solid tumours spontaneously arise within the retroperitoneum in peri-renal and para-spinal abdominal regions, consistent with the typical clinical distribution and radiological presentation of human neuroblastoma.

In this study, male and female tumour-bearing Th-MYCN and Th-ALK$^F1174L$/Th-MYCN mice, initially identified by palpation, underwent MRI at around 50 and 30 days old, respectively (Berry et al., 2012; Rasmussen et al., 2012). Their genotype was confirmed by analysing DNA from the tail using real-time quantitative reverse transcription polymerase chain reaction.

MRI data acquisition

Study protocol. Twenty-three transgenic mice (Th-MYCN $n = 14$, Th-ALK$^F1174L$/Th-MYCN $n = 9$) underwent imaging on the 3T platform. Of these, 10 mice (Th-MYCN $n = 7$, Th-ALK$^F1174L$/Th-MYCN $n = 3$) were scanned at both 3 and 7T within 24 h of each other in order to compare tumour volume determination and quantification of tumour R$s$ at 3 and 7T. Dynamic contrast-enhanced (DCE)-MRI was performed simultaneously on three Th-MYCN mice at 3T. To evaluate the 3T platform for imaging-embedded intervention trials, anatomical and functional (native $T_1$ and $R_s$) MRI measurements were acquired from Th-MYCN tumours prior to and 24 h post-treatment with either saline (control, $n = 4$) or a single 25 mg kg$^{-1}$ i.p. dose of the DNA alkylating agent cyclophosphamide (CPM, $n = 5$), or prior to and 48 h post treatment with a daily oral dose of either water (control, $n = 4$) or 30 mg kg$^{-1}$ of the multi-kinase inhibitor cabozantinib (CBZ, $n = 5$) (Yakes et al., 2011).
acquired using a 3D spoiled gradient echo sequence with variable flip angle (axial orientation, 7 slices, slice thickness: 1.5 mm, resolution = 0.25 × 0.25 mm, FOV = 200 × 85 mm, FA = 3° and 16°, 10 dummy scans, TE/TR = 2.3/7 ms, NSA = 1, TA = 27 s). Multigradient echo (MGE) images were acquired using a 2D gradient echo sequence with multiple echo times (axial orientation, 3 slices, slice thickness: 1.5 mm, resolution = 0.3 × 0.3 mm, FOV = 200 × 50 mm, FA = 24°, TE = 4.6, 11.5, 18.4, 25.3, 32.2 and 39.1 ms, TR = 500 ms, NSA = 1, TA = 30 s). No fat suppression was employed.

For the acquisition of DCE-MRI data, a lateral tail vein of each mouse was cannulated with a 27G butterfly catheter connected to a 10 m long line of polyethylene tubing (BPTE-T10, 0.23 mm.o.d.), and attached through the available wave-guides to syringe pumps located in the scanner control room, thereby enabling the simultaneous remote intravenous administration of contrast agent to each mouse. DCE-MRI data were acquired using the 3D spoiled gradient echo sequence with 200 time points, a temporal resolution of 2.5 s and a total acquisition time of 8 mins 33 s. A bolus injection of 0.1 mmol kg⁻¹ gadopentate dimeglumine (Gd-DTPA) was simultaneously administered to all eight mice, enabling the simultaneous remote intravenous administration of contrast agent to each mouse. DCE-MRI data were acquired using dedicated software (MRIW, working under IDL (Doran et al, 2006), and the ratio between the proton density (FA = 3°) and T₁-weighted (FA = 16°) 3D spoiled gradient echo used to provide estimates of the native tumour spin-lattice relaxation time T₁ (ms) (Fram et al, 1987). The MGE images were acquired using dedicated software (MRIW, working under IDL (d’Arcy et al, 2006)), and the ratio between the proton density (FA = 3°) and T₁-weighted (FA = 16°) 3D spoiled gradient echo used to provide estimates of the native tumour spin-lattice relaxation time T₁ (ms) (Fram et al, 1987). The MGE imaging was employed.

RESULTS

Measurements from the T₁ phantom for all Gd-DTPA concentrations at both field strengths are shown in Table 1. A highly significant correlation (R = 0.99, P < 0.0001) was found between the T₁ values obtained from the 3T clinical scanner and the 7T pre-clinical scanner, with the median T₁ values being higher at 7T.

Representative anatomical T₂-weighted MR images of tumour-bearing mice acquired at 3 and 7T are displayed in Figure 2A and B, revealing large peri-renal masses within each mouse abdomen, and occasionally an additional thoracic tumour, consistent with the radiological presentation of neuroblastoma. The excellent image quality of the 7T data acquisition and analysis. Volumetric analysis and quantification of T₁ and R₂* of tumours arising within Th-MYCN and Th-ALKK717L4I/MYCN transgenic mice was performed on a 7T horizontal bore microimaging system (Bruker Instruments, Ettlingen, Germany) using a 3-cm birdcage volume coil, as previously described (Jamin et al, 2013, 2014).

Table 1. Longitudinal relaxation times quantified from the gadolinium phantoms at 3 and 7T

| Concentration | 7T (ms) | 3T (ms) |
|---------------|--------|--------|
| Saline        | 2358 ± 21 | 1608 ± 223 |
| 100 nm        | 178 ± 34 | 1238 ± 84 |
| 500 nm        | 34 1238 | 21 1608 |
| 5000 nm       | 400 ± 4 | 241 ± 3 |

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Statistical analysis. Statistical analysis was performed using GraphPad Prism 6. The absolute values for tumour volume, and median values for T₁ and R₂* were used. Any significant difference within the same group was identified using Student’s two-tailed paired t-test, with a 5% level of significance. Any significant difference between groups after treatment was tested using an unpaired t-test. The linear correlation coefficient R was used to test correlation strength, direction and linear association between volumes and relaxation times measured at both field strengths.

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contrast yielded clear tumour delineation across contiguous slices that could be used for accurate volumetric analysis in vivo. No significant difference was found between volumetric measurements acquired at the different field strengths (mean tumour volume 819 ± 153 mm³ at 3T vs 889 ± 191 mm³ at 7T, n = 10, P > 0.05), and which were significantly correlated (R = 0.98, P < 0.0001, Figure 2C).

Parametric $R_2^*$ maps from a tumour arising in a Th-MYCN mouse acquired at both 3 and 7T are shown in Figure 3A. While anatomical $T_2$-weighted images revealed some anticipated differences in animal positioning, it was possible to identify similar tumour positions at both field strengths. A heterogeneous distribution of $R_2^*$ values was apparent across all tumours imaged at both field strengths. A statistically significant positive correlation of $R_2^*$ values quantified at 3 and 7T was determined (n = 10, R = 0.87, P = 0.0012, Figure 3B). Unlike the phantom data, no significant correlation in tumour $T_1$ relaxation times measured at 3 and 7T in vivo was found. This may be a consequence of different

![Figure 2. Anatomical imaging at 3 and 7T. (A) Anatomical $T_2$-weighted coronal images acquired simultaneously from three Th-MYCN mice with abdominal neuroblastoma using the high resolution 3-channel/3-animal MR RF coil on the 3T clinical scanner, with a scale bar in blue. Each tumour is indicated by yellow arrows, with kidney (K) and lungs (L) also annotated. An additional thoracic lesion identified in one of the mice is indicated with a red arrow. (B) Expanded $T_2$-weighted image of the abdomen of the first mouse in (A) acquired at 3T, and the corresponding $T_2$-weighted image acquired from the same mouse at 7T, with corresponding scale bars in blue. (C) Linear regression analysis of the volumetric measurements determined from $T_2$-weighted MRI of tumour-bearing transgenic mice imaged at 3 and 7T revealed a positive and highly significant correlation (R = 0.98, P < 0.0001).](image)
methods of $T_1$ quantification (multiple slice variable flip angle approach at 3T vs single slice inversion-recovery at 7T), impossibility of achieving the exact same positioning and acquiring data from the same central slice on the two imaging platforms, and the absence of any $B_1$ correction for the data acquired at 3T.

Parametric maps of native $T_1$ and $R_2^*$ obtained at 3T from representative Th-MYCN and Th-ALK$^{F1174L}$/Th-MYCN mice are shown in Figure 3C and D. A more homogeneous distribution of both $T_1$ and $R_2^*$ values was apparent in tumours arising in the Th-ALK$^{F1174L}$/Th-MYCN mice. The quantitative data are summarised in Figure 3E and F. While there was no significant difference in native tumour $T_1$ between the Th-MYCN and the Th-ALK$^{F1174L}$/Th-MYCN mice (1098 ± 71 ms and 1149 ± 118 ms; $P > 0.1$), $R_2^*$ was significantly faster in tumours in the Th-MYCN mice when compared to the Th-ALK$^{F1174L}$/Th-MYCN cohort (49.7 ± 4 s$^{-1}$ and 27.7 ± 3 s$^{-1}$; $P = 0.0007$).

DCE-MRI was successfully performed on three transgenic mice simultaneously using the 3T platform, yielding data with sufficient temporal and spatial resolution to evaluate vascular biomarkers of each imaged tumour in vivo. Pharmacokinetic modelling was employed to derive and spatially map DCE-MRI parameters. Parametric $K_{trans}$ maps obtained from each tumour-bearing mouse are shown in Figure 4, along with representative contrast agent uptake curves obtained from ROIs positioned over the tumour, kidney and paraspinal muscle. The mean of median and range values of $K_{trans}$, $V_e$ and IAUGC$_{60}$ acquired from the three simultaneously imaged animals were 0.161 min$^{-1}$ (0.124–0.202 min$^{-1}$), 0.396 (0.374–0.415) and 3.28 (2.38–4.27) mM.s, respectively.

Anatomical images and parametric maps acquired from representative Th-MYCN mice prior to and 24 h after treatment with saline or CPM are shown alongside individual tumour volume, $T_1$ and $R_2^*$ values in Figure 5A–C, and summarised in Figure 5D. Treatment with CPM elicited a significant ($P = 0.0015$) reduction in tumour burden, and was associated with a significant decrease in native $T_1$ ($P = 0.0085$). Collectively this translated to a significant difference in both relative tumour volume ($P = 0.0004$) and native $T_1$ ($P = 0.0035$) between the control and CPM groups (Figure 5D). There was no significant treatment-induced change in any parameter over 24 h in the control cohort. Treatment with CBZ elicited marked tumour growth delay compared to control, resulting in a significant difference in the relative tumour volume ($P = 0.0076$). There was however no associated significant change in relative native $T_1$ or $R_2^*$ with CBZ, and no significant treatment-induced change in any parameter over 48 h in the control group (summarised in Figure 5E).
DISCUSSION

Pre-existing and new clinical MRI scanners operating between 1 and 3T, available to clinically orientated research groups, are being increasingly used for pre-clinical imaging studies in rodents (Dazai et al., 2004; Brockmann et al., 2007; Chen et al., 2007; Inderbitzin et al., 2007; Linn et al., 2007; Nieman et al., 2007; Jeremie et al., 2016). The potential and advantages of simultaneous MRI of multiple tumour-bearing animals has been highlighted (Dazai et al., 2004; Li et al., 2005; Beuf et al., 2006; Wilmes et al., 2007). Such pre-clinical applications require the use of appropriately adapted or dedicated RF coils for small fields of view, especially when the acquisition of functional MRI data is considered. For dedicated clinical MRI research centres, the use of such a coil arrangement for performing pre-clinical studies is however more cost-effective compared to low-field benchtop MRI systems that are becoming increasingly available for routine pre-clinical investigations. The necessity to generate linear magnetic field gradients over greater volume on clinical systems limits the geometrical design and hence gradient system performance in comparison to dedicated pre-clinical systems. As a consequence, maximum gradient strengths and slew rates are typically lower, which is also often desirable in order to reduce patient peripheral nerve stimulation. It can however limit EPI-based acquisitions such as DWI, rapid or ultra-high resolution imaging.

In this study, the utility and sensitivity of anatomical and functional MRI data, acquired using a high resolution RF coil on a clinical 3T scanner, from two transgenic mouse models of neuroblastoma was evaluated. In addition, the data were cross-referenced to multiparametric data acquired from the same transgenic mice on a dedicated pre-clinical 7T system, with a particular focus on quantification of native longitudinal relaxation time $T_1$, and transverse relaxation rate $R_2^*$, previously shown to be sensitive imaging biomarkers of treatment response and geno/phenotype respectively (Jamin et al., 2013, 2014).

Use of the high resolution 3-channel/3-animal RF coil on a clinical 3T platform yielded high quality $T_2$-weighted anatomical images of up to three mice simultaneously, with sufficient resolution to accurately define and quantify the volume of neuroblastomas arising within the abdomen of the transgenic mice in vivo. The limited region of homogeneous field on dedicated high field pre-clinical systems usually limits the acquisition of functional imaging data from just a few representative slices across the centre of a tumour. This is not the case for clinical systems which allow functional measurements to be made across the whole tumour. In addition, the multiple coil arrangement enabling simultaneous scanning of several subjects enables high throughput ideally suited for efficient cohort screening.

In addition, acquisition of whole mouse body data enables the detection of any distant metastasis in the same imaging session, a clear advantage when compared to the imaging performed on high-field pre-clinical scanners with a more limited region of magnetic field homogeneity. In cancer research, the increasing development and use of more sophisticated orthotopic and transgenic mouse models of primary and secondary disease demands accurate non-invasive imaging methods to confirm successful engraftment/propagation and longitudinal monitoring of deep-seated tumours in vivo (Bielen et al., 2012; Jamin et al., 2013; Graham et al., 2014). The coil arrangement used herein can clearly be used to facilitate such tumour model development, provides a potential screening tool to confirm tumourigenesis, and may alleviate demand at research establishments where the availability/capacity for high-field imaging is limited.

Figure 4. Simultaneous DCE-MRI at 3T. Parametric $K^{trans}$ maps obtained from three tumour-bearing transgenic mice from which DCE-MRI data were simultaneously acquired using the high resolution 3-channel/3-animal MR RF coil on the 3T clinical scanner, showing a heterogeneous distribution of vascular permeability/perfusion across all three neuroblastomas. Gadolinium uptake curves obtained from one mouse for ROIs positioned over the tumour, kidney and paraspinal muscle are shown. Median $K^{trans}$ values of 0.202, 0.168 and 0.114 min$^{-1}$ were estimated from the upper, middle and lower tumour ROIs, respectively.
The ability to quantify functional MRI data using the 3-channel/3-animal RF coil at 3T was also explored. The primary objective was quantification of tumour native $T_1$ and $R_2^*$, imaging biomarkers previously shown to be sensitive to successful treatment response and haemodynamic vasculature within the Th-MYCN and Th-ALK$^{F1174L}$/Th-MYCN mice, measured at 7T (Jamin et al., 2013, 2014). A key aim was to achieve a good compromise between sufficient resolution, SNR and scan time for quantitation of native $T_1$ and $R_2^*$, rather than replicating sequences and parameters routinely used on the 7T system. The excellent linearity and positive correlation determined using the $T_1$ phantom, and the strong significant correlation of tumour $R_2^*$ values in vivo, determined across both scanners, suggests that the coil has sufficient sensitivity to acquire and accurately measure native $T_1$ and $R_2^*$ in these transgenic mouse models of neuroblastoma. As expected, the native $T_1$ relaxation times in tumours arising within the Th-MYCN mice were lower at 3T than those previously reported at 7T (Sasaki et al., 2005; Jamin et al., 2013).

Figure 5. Assessment of tumour response to CPM or CBZ treatment using the 3T platform. (A) $T_2$-weighted coronal images acquired at 3T using the high resolution 3-channel/3-animal MR RF coil from Th-MYCN transgenic mice bearing abdominal neuroblastomas prior to and 24 h after treatment with either saline (control) or CPM. Each tumour is delineated in yellow. Treatment with CPM resulted in a consistent and significant (**$P<0.01$) reduction in tumour volume. Parametric maps of tumour (B) native $T_1$ and (C) $R_2^*$ acquired at 3T from Th-MYCN mice prior to and 24 h after treatment with either saline (control) or CPM. While treatment with CPM elicited a significant reduction in native $T_1$ (**$P<0.01$), there was no change in tumour $R_2^*$. Relative changes in tumour volume, native $T_1$ and $R_2^*$ determined from Th-MYCN mice treated with vehicle, or either CPM or CBZ, are shown in (D) and (E) respectively. Data shown are the individual tumour median values and the mean ± 1 s.e.m. (**$P<0.001$, **$P<0.01$).
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The authors declare no conflict of interest.

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