Diffusion-weighted and multiphase contrast-enhanced MRI as surrogate markers of response to neoadjuvant sunitinib in metastatic renal cell carcinoma

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Background: Current imaging criteria for categorising disease response in metastatic renal cell carcinoma (mRCC) correlate poorly with overall survival (OS) in patients on anti-angiogenic therapies. We prospectively assess diffusion-weighted and multiphase contrast-enhanced (MCE) MR imaging (MRI) as markers of outcome.

Methods: Treatment-naive mRCC patients on a phase II trial using sunitinib completed an MRI substudy. Whole-tumour apparent diffusion coefficient (ADC) maps and histograms were generated, and mean ADC and AUC low (proportion of the tumour with ADC values lying below the 25th percentile of the ADC histogram) recorded. On MCE-MRI, regions of interest were drawn around the most avidly enhancing components to analyse enhancement parameters. Baseline (n = 26) and treatment-related changes in surviving patients (n = 20) were correlated with OS. Imaged metastases were also analysed.

Results: Forty-seven per cent of the patients showed significant changes in whole-tumour mean ADC following therapy, but there was no correlation with outcome. Patients with a high baseline AUC low and greater-than-median AUC low increase had reduced OS (HR = 3.67 (95% confidence interval (CI) = 1.23–10.9), P = 0.012 and HR = 3.72 (95% CI = 0.98–14.21), P = 0.038, respectively). There was no correlation between MCE-MRI parameters and OS. Twenty-eight metastases were analysed and showed positive correlation with primary tumour mean ADC for individual patients (r = 0.607; P < 0.001).

Conclusion: Primary RCC ADC histogram analysis shows dynamic changes with sunitinib. Patients in whom the tumour ADC histogram demonstrated high baseline AUC low, or a greater-than-median increase in AUC low with treatment had reduced OS.

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Treatment of metastatic renal cell carcinoma (mRCC) has been revolutionised by targeted therapies such as sunitinib, a multi-targeted tyrosine kinase inhibitor with a broad spectrum of activity, which focuses mainly on vascular endothelial growth factor receptors which play a role in both tumour angiogenesis and tumour cell proliferation. These therapies have increased mean survival to over 2 years compared with less than 1 year previously and are now considered standard of care in mRCC (Motzer et al, 2007, 2009).

Current imaging criteria for categorising disease response is with RECIST (Response Evaluation Criteria in Solid Tumors) v1.1. However, response by RECIST, based on target lesion size, does not correlate with overall survival (OS) in mRCC patients treated with sunitinib (Kontovinis et al, 2009). Alternative imaging-based response parameters have been evaluated, including lesion texture, size and attenuation on contrast-enhanced computed tomography (CT) and glucose metabolism on positron emission tomography/CT, with variable success (Hahn et al, 2008; Han et al, 2010; Nathan et al, 2010; Thiam et al, 2010; Smith et al, 2010a,b; Goh et al, 2011; Krawiecki et al, 2011; Smith et al, 2011). There remains a lack of biological and radiological markers that reliably predict (i) response to sunitinib early in the course of treatment and (ii) which patients will develop early resistance to treatment.

Diffusion-weighted (DW) and dynamic contrast-enhanced (DCE) MR imaging (MRI) are imaging techniques that probe tumour physiology. Diffusion-weighted MRI explores the Brownian motion of water, which is restricted by interactions with cell membranes and macromolecules in tissues, and is quantified by the apparent diffusion coefficient (ADC) (Koh and Collins, 2007). Increased tissue cellularity, as seen in some tumours, restricts diffusion, resulting in low ADC values. Early change in DW-MRI/ADC following treatment has been proposed as a potential biomarker for assessment of response in other cancers (Harry et al, 2008; Jung et al, 2011; Kyriazi et al, 2011). Whole-tumour mean ADC histogram analysis can evaluate heterogeneity within a tumour by classifying regions of different cellularity and micro-environments (Kyriazi et al, 2011; Nowoselski et al, 2011; Pope et al, 2011). Previous studies have used quantitative DCE-MRI to assess contrast material kinetics within a tumour, reflecting the vascularity of the tissue. These studies have suggested that changes in the volume transfer constant of contrast agent ($K_t/\mu$) can be used as a pharmacodynamic biomarker in patients on anti-angiogenic therapy (Morgan et al, 2003; Stevenson et al, 2003; Liu et al, 2005; Mroz et al, 2005; Flaherty et al, 2008; Hahn et al, 2008; Notohamiprodjo et al, 2010). Multiple phase-enhanced (MCE) MRI allows semiquantitative parameters such as maximum relative enhancement ($S_{100}$) and relative wash-in rate ($WRI_{a}$) to be more readily measured in everyday clinical practice, but these have not previously been evaluated in the context of response to sunitinib therapy in mRCC.

The primary objective of this translational study was to assess whether sunitinib therapy was associated with sequential changes in the unresected treatment-naive primary renal tumour in patients recruited to an imaging substudy of a phase II trial (SUMR NCT01024205) and to evaluate whether these changes correlated with OS. We hypothesised that response to treatment, as defined by improved OS, would correlate with:

(i) an increase in ADC of the whole primary tumour volume due to apoptosis and
(ii) a reduction in primary tumour vascularity due to anti-angiogenic effects.

Our secondary objectives were:

(i) to evaluate novel methods of histogram analysis in the primary tumour and
(ii) to assess treatment-related diffusion and/or perfusion alterations in the imaged metastases.

**PATIENTS AND METHODS**

**Study design.** The study protocol was approved by the research ethics committee and all patients gave written consent.

All patients with newly diagnosed mRCC referred to our tertiary uro-oncology centre were considered for inclusion in the prospective phase II trial (SUMR NCT01024205). The primary endpoint of the SUMR trial was to assess the clinical benefit of upfront sunitinib in Memorial Sloan-Kettering Cancer Centre (MSKCC) intermediate- and poor-risk patients who had not had nephrectomy (Powles et al, 2011). Outcome data were available for OS with a follow-up period of 31.9 months (range 8.3–39.8 months).

Key inclusion criteria were histopathologically confirmed clear cell RCC with metastases, judged by the treating clinician to potentially derive benefit from sunitinib. Key exclusion criteria included previous treatment for mRCC and contra-indication to MRI.

**Patients.** Between January 2008 and February 2010, 30 consecutive patients (23 men, 7 women) with untreated mRCC were recruited to the MRI substudy. Inclusion in this substudy required the absence of contra-indications to MRI and patient consent for additional imaging. MR imaging results did not influence treatment decisions.

Four patients were excluded because they did not complete post-treatment imaging for reasons other than progressive disease leading to death (unable to schedule second MRI $n = 2$; declined second scan $n = 2$). Patient demographics are given in Table 1.

Baseline MRI was performed on all 26 patients. Six patients died from progressive disease before completion of three treatment cycles. Radiological response data (pre- and post-treatment imaging) was therefore available in 20 patients. Three patients recruited to the study had an incomplete set of $b$-values at baseline (only $b = 0$ and $b = 1000$ s mm$^{-2}$). To avoid possible data contamination by these differences in baseline DW-MRI acquisition, DW-MRI analysis has been performed following exclusion of the three patients with differing baseline DW-MRI acquisitions. Multiphase contrast-enhanced MRI was performed using the same protocol in all patients.

Figure 1 provides a flow diagram to illustrate the imaging performed in patients recruited to the substudy.

**Treatment schedule.** Patients were treated with three cycles of sunitinib until progression or withdrawal (50 mg daily for 4 weeks with a 2-week break between cycles). Doses were reduced to 37.5 mg and subsequently 25 mg in the face of toxicity (grade 3 or more). Interval debulking nephrectomy was offered to patients after the MRI study (following three treatment cycles). Following surgery, patients continued sunitinib treatment until progression by RECIST v1.1. The role of nephrectomy in metastatic disease is controversial and is under evaluation in studies such as SUMR.

Figure 2 provides a study schema to illustrate the relationship between neoadjuvant therapy, imaging and surgery in the patient cohort.

**MR imaging protocol.** MR imaging was acquired pre-treatment and following three treatment cycles on a 1.5-Tesla Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) with a four-element phased array coil. The second MRI study was performed off treatment during the second week, following the completion of cycle 3 and before nephrectomy (day 10±2 off treatment; see Figure 2). Hyoscine butylbromide (Buscopan; Boehringer, Ingelheim, Germany; 20 mg intravenously) was
administered before image acquisition to reduce artefact related to bowel movement. For all sequences, the field of view was optimised to include the entire primary renal tumour. The following morphological sequences were obtained: axial $T_2$-weighted turbo spin-echo MRI (repetition time (TR), range 1500–1750 ms; echo time (TE), 100 ms; turbo factor, 28; slice thickness, 6 mm; slice gap, 1 mm; field of view (FOV), 375 mm; rectangular FOV (RFOV), 75%; 3 signal averages; 400 × 512 matrix) and axial $T_2$-weighted fast field-echo MRI (TR, 150–225 ms; TE, 5 ms; flip angle, 80°; slice thickness, 6 mm; slice gap, 1 mm; FOV, 375 mm; RFOV, 75%; 2 signal averages; 256 × 256 matrix).

Diffusion-weighted-MR images were obtained using a free-breathing multislice spin-echo-planar imaging sequence (TR, range 6000–7000 ms; TE, 67 ms; EPI factor, 60; slice thickness, 6 mm; slice gap, 1 mm; FOV, 400–450 mm; RFOV, 75%; 3 signal averages; 256 × 256 matrix). Six motion-probing gradients with $b$-values of 0, 100, 200, 500, 750 and 1000 s mm$^{-2}$ were applied in three orthogonal directions and trace images were synthesised for each $b$-value using the mean of three orthogonal directions. Apparent diffusion coefficient maps were calculated on a pixel-by-pixel basis using a mono-exponential fit excluding $b = 0$ from the calculation to eliminate perfusion effects (Padhani et al, 2009). Average DW-MRI acquisition time was 8 min.

The variability of the free-breathing multislice DW-MRI sequence was previously measured in a phantom and volunteer study at our centre (Miquel et al, 2012). In vivo repeatability for renal ADC measurements demonstrated a coefficient of reproducibility of 7.9% for three-dimensional volumes of interest, but up to 24% for single regions of interest (ROI) in the abdomen.

Multiphase contrast-enhanced MRI was performed with volumetric fat-suppressed, spoiled gradient-echo $T_1$-weighted acquisitions in the coronal plane (TR, 4 ms; TE, 1.9 ms; flip angle, 10°; slice thickness, 6 mm; slice gap, 1 mm; FOV, 400–450 mm; RFOV, 75%; 2 signal averages; 256 × 256 matrix). Six motion-probing gradients with $b$-values of 0, 100, 200, 500, 750 and 1000 s mm$^{-2}$ were applied in three orthogonal directions and trace images were synthesised for each $b$-value using the mean of three orthogonal directions. Apparent diffusion coefficient maps were calculated on a pixel-by-pixel basis using a mono-exponential fit excluding $b = 0$ from the calculation to eliminate perfusion effects (Padhani et al, 2009). Average DW-MRI acquisition time was 8 min.

Image analysis. Diffusion-weighted-MR images were analysed using OsiriX (Pixmeo SARL, Bernex, Switzerland) software by a single reader (2 years of body MRI fellowship experience), blinded to clinical outcome. The whole primary tumour was segmented on the ADC map, in conjunction with $b = 1000$ s mm$^{-2}$ and $T_2$-weighted sequences. The largest possible ROI was drawn on each slice containing primary tumour, without contamination from adjacent tissues (Figure 3). OsiriX software calculated the tumour volume and pixel-by-pixel mean ADC values for the entire volume. Mean ADC values were exported into Microsoft Excel software for whole-tumour mean ADC calculation and generation of per patient volume-corrected ADC histograms (bin width $50 \times 10^{-6}$ mm$^{-2}$ s$^{-1}$). The histogram-derived parameter was the AUC$_{low}$, the proportion of the tumour with ADC values lying below the 25th percentile point of the ADC histogram (the pixel ADC value below which 25% of all tumour ADC values lie) after the highest and lowest 1% of ADC values were discarded to remove artefact. AUC$_{low}$ represents the most restricted, and probably the most cellular, components of the tumour (see Figure 4 for AUC$_{low}$ derivation).

Multiphase contrast-enhanced-MR images were analysed using the MR Breast Imaging package of Philips Extended Workspace (R 2.6.3.2, 2009, Philips Healthcare, Best, The Netherlands) by two readers (each with 1 year of body MRI experience) in consensus following training by an experienced (10 years) MRI reader, all blinded to clinical outcome. The most avidly enhancing solid tumour component was identified on the baseline study using the coloured parametric map and a circular ROI (diameter $>3$ mm) was manually drawn. This ROI was then copied to the same anatomical position on the post-treatment study, visually matching the same tumour region. Computer software automatically calculated the following parameters:

$$S_{rel} = \frac{S_{max} - S_{0}}{S_{0}} \times 100$$

total maximal tissue enhancement from baseline.

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**Table 1. Patient demographics and characteristics at diagnosis**

| Parameter | Value |
|-----------|-------|
| Number of patients | 26 |
| Age (years) | 61.5 ± 10.3 (range 38–78) |
| Gender | |
| Male | 21 |
| Female | 5 |
| MSKCC prognostic risk | |
| Intermediate | 12 |
| Poor | 14 |
| Metastatic sites | |
| Lung | 16 |
| Liver | 5 |
| Bone | 8 |
| Lymph nodes | 14 |
| Adrenal | 4 |
| Other | 8 |
| Clear cell tumour grade | |
| 1–2 | 14 |
| 3–4 | 12 |
| Median OS, months | 13.6 |
| Median PFS, months | 6.4 |
| Median follow-up, months | 31.9 (range 8.3–39.8) |

Abbreviations: MSKCC – Memorial Sloan-Kettering Cancer Centre; OS – overall survival; PFS – progression-free survival.
lower ADC values than the primary tumour. LK = demonstrates involved retroperitoneal nodes (white stars), which return magnetic performance.

Figure 2. Magenetic resonance imaging substudy schema illustrating the relationship between neoadjuvant therapy, MR imaging and surgery in patients recruited to the MRI substudy. The second MRI study was performed in the second week off treatment following cycle 3 of sunitinib and before nephrectomy.

Figure 3. Region of interest placement. Axial section through a right-sided renal cell carcinoma (white arrow) showing the placement of a ROI within the low ADC tumour. Care is taken to sample only tumour tissue without contamination from adjacent normal tissues. Workstation-generated analysis gives a mean ADC value of tumour tissue without contamination from adjacent normal tissues. The volume of necrosis in each primary tumour (defined as the volume of non-enhancing tissue with corresponding high signal intensity) was calculated using OsiriX software (Rosset et al, 2004). Because of the heterogeneity of the primary tumour on $T_2$ and the presence of internal high signal intensity on $T_1$, ROIs were defined on consecutive axial $T_2$-weighted images by matching areas of high $T_2$ signal intensity with regions of non-enhancement on the subtracted MCE-MR images. Separate volumes of necrosis were summed to give a total necrotic volume that was expressed as a percentage of the primary tumour volume.

Metastatic deposits. The imaging volume was optimised to include the entire primary renal tumour. Whole-body imaging was not performed and, therefore, the majority of metastases identified on the patient’s staging CT (e.g., pulmonary deposits) were not assessed on MRI.

Metastatic deposits were defined as lesions >1 cm in diameter (or >1.5 cm in short axis for nodes) that had typical CT and MRI appearances for RCC metastases (Griffin et al, 2009). Inferior vena cava (IVC) thrombus was included in analysis where enhancement on MCE-MRI indicated tumour rather than bland thrombus.

Mean ADC values were obtained from a single ROI on the central slice through each lesion (the majority were too small for volume assessment) at baseline and following three treatment cycles.

Standard response assessment. Standard response was classified according to RECIST v1.1, as specified in the clinical phase II trial protocol. Contrast-enhanced CT of the chest, abdomen and pelvis (100 ml Omnipaque350 (GE Healthcare, Milwaukee, WI, USA) administered at 3.5 ml s$^{-1}$) was performed at baseline and after every three cycles of treatment. Progression-free survival (PFS) was not used as an endpoint in this study, as 36% of patients have been shown to progress during the surgery-related break when treated with upfront sunitinib before planned nephrectomy (as in the SUMR protocol) and, therefore, PFS does not act as a good surrogate marker (Powles et al, 2011). Because of the lack of second-line therapy in the United Kingdom at the time of the study, OS was considered the most robust endpoint.

Statistical analysis. Statistical analysis was conducted using STATA software (Statacorp LP, College Station, TX, USA). Descriptive statistics were used to analyse parameters. Overall survival was analysed using the Kaplan–Meier (KM) method with patients being separated into two groups, those above and those below the median, for each parameter. Comparison of groups was conducted using the log-rank test. Correlation coefficients (Pearson’s product–moment correlation coefficient) were used to compare the relationship between groups.

A level of $P<0.05$ was used to assess for the significance of the results obtained and there has been no correction for multiple comparisons.

RESULTS

Best-response evaluation by RECIST v1.1 in this MSKCC intermediate- and poor-risk population showed partial response in 2 patients (8%), stable disease in 16 patients (61%) and progressive disease in 8 patients (31%). Overall survival for all patients was 13.6 months (95% confidence interval (CI): 4–22.7).

Primary tumour

Morphological analysis. Characteristics of the primary tumour at baseline and after three cycles of treatment in patients who had sequential scans were analysed (Table 2). Interval changes in primary tumour volume ranged from $-70.7$ to $+57.8\%$ (mean, $-18.3 \pm 35.1$) and changes in the percentage of primary tumour necrosis ranged from $-46.2\%$ (mean, $14.0 \pm 15.8$). Patients who had a tumour volume below median at baseline had a prolonged survival compared with those with a baseline tumour volume above median (OS 28.3 months compared with 4 months; $P=0.017$). The percentage change in tumour volume with treatment did not correlate with survival ($P=0.087$). Those patients in whom the change in the percentage necrosis was below
The proportion of the tumour with ADC values lying below this point was the point of the histogram range, the pixel ADC below which 25% of discarded to remove artefact (blacked out regions). The 25th percentile lowest 1% of ADC values (the tails of the bell-shaped curve) were ADC measurements from the entire tumour volume. The highest and treatment ADC histograms were generated from pixel-by-pixel mean related changes in the histogram and AUC low.

Following three treatment cycles (Figure 5B, and in purple baseline and post three treatment cycles AUC low (Figure 5B, 0.80 and 2.1/C2 with treatment. Whole-tumour mean ADC values varied between significant change in skewness or kurtosis of the ADC histogram heterogeneity (skewness 0.56, kurtosis 3.63). There was no in the 23 patients at baseline demonstrated marked tumour Diffusion-weighted MRI parameters. Analysis of ADC histograms in the 23 patients at baseline demonstrated marked tumour heterogeneity (skewness 0.56, kurtosis 3.63). There was no significant change in skewness or kurtosis of the ADC histogram with treatment. Whole-tumour mean ADC values varied between 0.80 and 2.1 × 10⁻³ mm² s⁻¹. We evaluated whether primary tumour DW-MRI parameters correlated with OS. A high (above median) or low (below median) mean ADC result at baseline did not correlate with OS (HR = 0.61 (95% CI: 0.23–1.60), P = 0.31). However, patients with a high (above median) AUCₜₗₒₜ at baseline had reduced OS (HR = 3.67 (95% CI: 1.23–10.9), P = 0.012; Figure 6A).

Of note, all six patients who progressed and died before completion of three cycles of sunitinib therapy (and therefore did not have the second MRI study) also had high AUCₜₗₒₜ values at baseline.

The mean ADC and AUCₜₗₒₜ after three treatment cycles were also analysed in this way and did not predict OS (HR = 0.49 (95% CI: 0.14–1.67), P = 0.24 and HR = 2.28 (95% CI: 0.66–7.84), P = 0.18, respectively).

Treatment-related changes in mean ADC ranged from −13.8 to +32.9% (mean, +7.8 ± 15.4). Treatment-related changes in AUCₜₗₒₜ ranged from −54.3 to +56.4% (mean, −7.2 ± 30.8). Applying the 7.9% coefficient of reproducibility for our MRI system attained from an earlier repeatability study, 8 of the 17 treated patients had changes in primary tumour mean ADC that were greater than the coefficient of reproducibility (Miquel et al, 2012). However, there was no significant difference in the whole-tumour mean ADC at baseline and following three treatment cycles (Figure 5A, P = 0.23) and no correlation between the percentage change in whole-tumour mean ADC with treatment and OS (HR = 1.50 (95% CI: 0.44–5.16), P = 0.51). After three cycles of sunitinib, there was no correlation between the percentage change in primary tumour volume and the percentage change in whole-tumour mean ADC (correlation coefficient, −0.102, P = 0.93).

There was no statistically significant difference between the baseline and post three treatment cycles AUCₜₗₒₜ (Figure 5B, the median (−14.1 to +4.6% change) had a trend towards prolonged OS compared with those above median (+8.5 to +46.2% change; P = 0.058).

**Diffusion-weighted MRI parameters.** Analysis of ADC histograms in the 23 patients at baseline demonstrated marked tumour heterogeneity (skewness 0.56, kurtosis 3.63). There was no significant change in skewness or kurtosis of the ADC histogram with treatment. Whole-tumour mean ADC values varied between 0.80 and 2.1 × 10⁻³ mm² s⁻¹. We evaluated whether primary tumour DW-MRI parameters correlated with OS. A high (above median) or low (below median) mean ADC result at baseline did not correlate with OS (HR = 0.61 (95% CI: 0.23–1.60), P = 0.31). However, patients with a high (above median) AUCₜₗₒₜ at baseline had reduced OS (HR = 3.67 (95% CI: 1.23–10.9), P = 0.012; Figure 6A).

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There was no statistically significant difference between the baseline and post three treatment cycles AUCₜₗₒₜ (Figure 5B,
There is an urgent need for surrogate markers to reliably predict the probable response early in the course of targeted therapy in mRCC. To date, attempts with other imaging techniques have shown variable success (Hahn et al, 2008; Nathan et al, 2010; Thiam et al, 2010; Smith et al, 2010a,b; Goh et al, 2011; Han et al, 2010; Kayani et al, 2011; Krajewski et al, 2011; Smith et al, 2011). Size and CT attenuation criteria have been reported to predict outcome better than RECIST criteria; however, this
requires intravenous contrast material, which may not be given in up to a third of patients with mRCC (Nathan et al, 2011). Our work evaluates the role of sequential DW- and MCE-MRI as potential surrogate markers of outcome in untreated mRCC patients at baseline and following three cycles of sunitinib. Although biomarkers obtained following a shorter treatment period, for example, 2 weeks, may provide very early response data, they would not identify those patients who develop early resistance to sunitinib therapy. By performing imaging after approximately 4 months of treatment, we hoped to identify this early resistance sooner than it is detected on CT (typically following 10 months of treatment). Diffusion-weighted MRI may evaluate the tissues of initially low ADC (the proportion of the whole-tumour ADC histogram, that is, a greater proportion of tumour lying below the 25th percentile point of the whole-tumour histogram) in patients treated with sunitinib whereas other groups have shown no significant correlation between \( K_{\text{trans}} \) and ADC values, but also histogram analysis of the mean ADC within each voxel occupied by tumour. This provides greater detail regarding the tumour microenvironment and is potentially more sensitive to treatment-related changes. It is also important to note that the study performed by Desar et al (2011) evaluated patients while on treatment with sunitinib, whereas our study evaluates changes in the primary tumour 10±2 days after completion of three cycles of sunitinib therapy (i.e., off therapy). There is a very labile relationship between the timing of imaging relative to the treatment patients are receiving; as Desar et al (2011) showed, mean ADC changes at different time points within the same treatment cycle and it is likely to be that further changes occur when patients come off treatment. Indeed, we know that patients treated with neoadjuvant sunitinib have been shown to progress during the treatment break before surgery (Powles et al, 2011).

Our work evaluates the role of sequential DW- and MCE-MRI as potential surrogate markers of outcome in untreated mRCC patients at baseline and following three cycles of sunitinib. Our study differs from that of Desar et al (2011) in evaluating the whole-tumour volume, considering both whole-tumour mean ADC values, but also histogram analysis of the mean ADC within each voxel occupied by tumour. This provides greater detail regarding the tumour microenvironment and is potentially more sensitive to treatment-related changes. It is also important to note that the study performed by Desar et al (2011) evaluated patients while on treatment with sunitinib, whereas our study evaluates changes in the primary tumour 10±2 days after completion of three cycles of sunitinib therapy (i.e., off therapy). There is a very labile relationship between the timing of imaging relative to the treatment patients are receiving; as Desar et al (2011) showed, mean ADC changes at different time points within the same treatment cycle and it is likely to be that further changes occur when patients come off treatment. Indeed, we know that patients treated with neoadjuvant sunitinib have been shown to progress during the treatment break before surgery (Powles et al, 2011).

Our study found a positive correlation between the mean ADC in the metastatic deposits and primary renal tumour in individual patients before and after sunitinib treatment. This suggests that there is a similarity in treatment response between the primary sites and metastatic deposits. This is an important finding for biomarker research in mRCC and supports the hypothesis that molecular markers taken from primary renal tissue may be representative of the metastatic sites.

Previous studies using DCE-MRI have suggested a correlation between high pre-treatment \( K_{\text{trans}} \) and PFS (Flaherty et al, 2008; Hahn et al, 2008), and treatment-related reduction in \( K_{\text{trans}} \) and PFS (Flaherty et al, 2008), whereas other groups have shown no significant correlation between \( K_{\text{trans}} \) and ADC values, but also histogram analysis of the mean ADC within each voxel occupied by tumour. This provides greater detail regarding the tumour microenvironment and is potentially more sensitive to treatment-related changes. It is also important to note that the study performed by Desar et al (2011) evaluated patients while on treatment with sunitinib, whereas our study evaluates changes in the primary tumour 10±2 days after completion of three cycles of sunitinib therapy (i.e., off therapy). There is a very labile relationship between the timing of imaging relative to the treatment patients are receiving; as Desar et al (2011) showed, mean ADC changes at different time points within the same treatment cycle and it is likely to be that further changes occur when patients come off treatment. Indeed, we know that patients treated with neoadjuvant sunitinib have been shown to progress during the treatment break before surgery (Powles et al, 2011).

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Our study found a positive correlation between the mean ADC in the metastatic deposits and primary renal tumour in individual patients before and after sunitinib treatment. This suggests that there is a similarity in treatment response between the primary sites and metastatic deposits. This is an important finding for biomarker research in mRCC and supports the hypothesis that molecular markers taken from primary renal tissue may be representative of the metastatic sites.

Previous studies using DCE-MRI have suggested a correlation between high pre-treatment \( K_{\text{trans}} \) and PFS (Flaherty et al, 2008; Hahn et al, 2008), and treatment-related reduction in \( K_{\text{trans}} \) and PFS (Flaherty et al, 2008), whereas other groups have shown no significant correlation between \( K_{\text{trans}} \) and ADC values, but also histogram analysis of the mean ADC within each voxel occupied by tumour. This provides greater detail regarding the tumour microenvironment and is potentially more sensitive to treatment-related changes. It is also important to note that the study performed by Desar et al (2011) evaluated patients while on treatment with sunitinib, whereas our study evaluates changes in the primary tumour 10±2 days after completion of three cycles of sunitinib therapy (i.e., off therapy). There is a very labile relationship between the timing of imaging relative to the treatment patients are receiving; as Desar et al (2011) showed, mean ADC changes at different time points within the same treatment cycle and it is likely to be that further changes occur when patients come off treatment. Indeed, we know that patients treated with neoadjuvant sunitinib have been shown to progress during the treatment break before surgery (Powles et al, 2011).

Our work evaluates the role of sequential DW- and MCE-MRI as potential surrogate markers of outcome in untreated mRCC patients at baseline and following three cycles of sunitinib. Although biomarkers obtained following a shorter treatment period, for example, 2 weeks, may provide very early response data, they would not identify those patients who develop early resistance to sunitinib therapy. By performing imaging after approximately 4 months of treatment, we hoped to identify this early resistance sooner than it is detected on CT (typically following 10 months of treatment). Diffusion-weighted MRI may evaluate the tissues of initially low ADC (the proportion of the whole-tumour histogram, that is, a greater proportion of tumour lying below the 25th percentile point of the whole-tumour ADC histogram) in patients treated with sunitinib whereas other groups have shown no significant correlation between \( K_{\text{trans}} \) and ADC values, but also histogram analysis of the mean ADC within each voxel occupied by tumour. This provides greater detail regarding the tumour microenvironment and is potentially more sensitive to treatment-related changes. It is also important to note that the study performed by Desar et al (2011) evaluated patients while on treatment with sunitinib, whereas our study evaluates changes in the primary tumour 10±2 days after completion of three cycles of sunitinib therapy (i.e., off therapy). There is a very labile relationship between the timing of imaging relative to the treatment patients are receiving; as Desar et al (2011) showed, mean ADC changes at different time points within the same treatment cycle and it is likely to be that further changes occur when patients come off treatment. Indeed, we know that patients treated with neoadjuvant sunitinib have been shown to progress during the treatment break before surgery (Powles et al, 2011).

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significance. Sequential imaging was not possible in a proportion of patients (six patients; 26%) owing to disease progression resulting in death. The cohort therefore under represents patients with primary refractory disease. Imaging was performed at specific time points to fit in with events in the clinical trial. However, results may have been different if the MRI scan were performed at an earlier time point and more patients may have achieved the post-treatment MRI scan if this had been done following cycle 1. Results may have also been different if the second imaging study had been performed on treatment at the end of a cycle rather than off treatment. Progression-free survival was not considered to be a reliable endpoint for this study, as a high proportion of patients have radiological progression during the treatment break for surgery. Intra- and interobserver variability have not been evaluated in this study.

CONCLUSION

In conclusion, DW-MRI provides a potential biomarker, AUClow, for OS in mRCC treated with sunitinib. A high baseline AUCmed and a greater-than-median increase in AUClow with treatment show a statistically significant correlation with reduced OS. Although our cohort is too small to suggest cut-off values for these parameters, with further validation in a larger study AUClow could act as a threshold for treatment change in this patient group. Furthermore, a correlation between ADC change in the primary tumour and metastases exists in individual patients, an important finding for future biomarker research.

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CONFLICT OF INTEREST

TP – education grant, advisory role from Pfizer, AZ, GSK and Novartis; advisory role for GE Roche and Genetech. AGR – education grant, advisory role from Pfizer, AZ, GSK and Novartis; advisory role for GE Roche and Genetech. KJ – honoraria and travel support for educational role, Guerbet. All other authors declare no conflict of interest.

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