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Can localised ^{19}F magnetic resonance spectroscopy pharmacokinetics of 5FU in colorectal metastases predict clinical response?

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Abstract

Background 5-Fluorouracil remains widely used in colorectal cancer treatment more than 40 years after its development. ^{19}F magnetic resonance spectroscopy can be used in vivo to measure 5FU's half-life and metabolism to cytotoxic fluoronucleotides. Previous studies have shown better survival associated with longer 5FU tumour half-life. This work investigated 5FU pharmacokinetics in liver metastases of colorectal cancer.

Methods A total of 32 subjects with colorectal cancer undergoing 5FU treatment, 15 of whom had liver metastases, were examined in a 1.5T MRI scanner, using a large coil positioned over the liver. Non-localised spectra were acquired in 1-min blocks for 32 min after injection of a 5FU bolus. The 5FU half-life was measured in each subject, and averaged spectra were examined for the presence of fluoronucleotides. Associations with progression-free survival were assessed.

Results No association was observed between 5FU half-life, tumour burden and survival. Half-lives were all shorter than those associated with improved survival in the literature. Remarkably, in the group with liver metastases, high levels of fluoronucleotides were associated with poorer survival; this counterintuitive result may be due to the higher levels of fluoronucleotides (whose level is higher in tumour tissue than in normal liver) in patients with higher tumour burdens.

Conclusions It is recommended that future studies use chemical shift imaging at higher field strengths to better resolve tumour from normal liver. Non-localised spectroscopy retains prognostic potential by enabling straightforward detection of fluoronucleotides, which are present at very low concentrations distributed throughout the tissue.

Keywords ^{19}F · Magnetic resonance spectroscopy · 5FU · Liver · Metastasis · Colorectal

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Introduction

5-Fluorouracil (5FU), after several decades of use, is still one of the most widely used cytotoxic agents for the treatment of several cancers including colorectal cancer. 5FU-based chemotherapy improves overall and disease-free survival of patients with resected stage III colorectal cancer. Over the last 20 years, the regimes of treatment have been modified at intervals either by changing the way the dose is given (bolus or infusion) or by combining it with different modulators such as leucovorin to enhance effectiveness. In addition, combination of 5FU with newer chemotherapies (e.g. irinotecan or oxaloplatin) has significantly improved the response rates (see for instance, this recent review [1]). However, still only 40–50% of patients

respond to 5FU treatment, and the search for new therapeutic strategies is continuing.

After injection, 5FU is rapidly metabolised by two main pathways: the major end-product is fluoro- β -alanine (FBAL), which is non-toxic; a smaller proportion is metabolised to toxic 5-fluoronucleotides (FNuct), primarily in tumour cells but also to a lesser extent in normal liver cells, which express low levels of the required enzymes. 5FU treatment is uniquely suited to monitoring by ^{19}F magnetic resonance spectroscopy (MRS) for several reasons: because the MRS sensitivity of fluorine is very high, because there is no natural background signal, and because MRS can distinguish the parent drug from its active and inactive metabolites in vivo and with time resolution adequate for pharmacokinetic analysis of the drug metabolism.

In the search for predictive biomarkers of response to 5FU treatment, several clinical studies have been performed [2–6]. It has been reported [2] that in some patients with liver metastases (whom they termed “trappers”), 5FU appears to be retained longer ($t_{1/2} > 20$ min) in tumours than in normal tissues and that 5FU trapping is correlated with response; in other studies, however, no trapping or correlation between half-life and response was seen [7, 8].

In principle, the most attractive potential parameter for the prediction of response to 5FU would appear to be direct detection of the toxic fluoronucleotide species (FNucts) in the tumour. FNuct signals were detected in the first MRS studies of 5FU in mouse tumours [9], and subsequent studies in animal and human tumour xenografts have shown that FNuct predicts response to 5FU treatment [10–12]. Recent in vivo and ex vivo rodent studies have detected FNuct in both tumour tissue and normal liver [13]. Although there have been a number of reports of FNuct detection in tumours in patients [7, 14] it has proved to be extremely difficult to measure them routinely. To our knowledge, no clinical studies have addressed whether the toxic metabolites formed from 5FU in vivo are correlated with response to treatment.

We have found that by summation of a time series of ^{19}F MRS spectra, it is possible to detect fluoronucleotide signals in the livers of many patients treated with 5FU. In the present study, we have used this method to monitor 5FU metabolism in 32 patients with colorectal cancer to see whether the formation of fluoronucleotides correlated with clinical response. A novel spatial analysis was then developed to estimate the proportion of the detected signal due to metastases. The hepatic fluoronucleotide levels detected in each individual patient were then correlated with progression-free survival and with the volume of liver metastasis. A small cohort of patients underwent two scans, one breathing room air and one breathing carbogen (95% O_2 /5% CO_2) for 5 min to investigate any alteration of

the 5FU kinetics resulting from carbogen breathing, a gas breathing challenge that could potentially improve drug delivery to tumours [10].

Patients and methods

This research was carried out with informed consent from the patients and with full local ethics committee approval (Wandsworth Health Authority). Patients with histologically proven colorectal carcinoma, for whom chemotherapy was planned, were eligible for referral by the consulting clinician. Of the 32 patients enrolled, 18 were men and 14 were women, mean age 66 years, range 37–85 years. The primary focus was on patients with metastatic liver disease, but patients free from metastatic disease receiving 5FU in an adjuvant role were also studied to characterise 5FU metabolism in normal liver. Fifteen of 32 subjects had liver metastases. The scans were taken between 1999 and 2004.

Chemotherapy

Patients were prescribed 5FU-containing regimens as a single agent with modulation by leucovorin. The 5FU was administered either as a single bolus each day for 5 days (MAYO regimen), as a weekly bolus, or on the de Gramont regimen that involves a loading bolus dose of 5FU followed by a 48-h infusion. Whichever the regimen, the patients received a bolus of 5FU (400–425 mg/m²) during the MR examination. Chemotherapy was prescribed either for the treatment of existing metastatic disease or as adjuvant therapy immediately after initial colorectal surgery to prevent the development of metastases.

Carbogen breathing

A small cohort of patients ($n = 9$) underwent two scans at 3–7 days apart. On the first scan, the bolus was administered while the patient was breathing room air freely. On the second scan, the patient was asked to breathe carbogen (95% O_2 /5% CO_2) delivered at 30 l min⁻¹ through a mask for 5 min, starting 3 min before the 5FU bolus delivery was initiated, and then subsequently air. This component of the study was designed to investigate any alteration of the 5FU kinetics resulting from carbogen breathing and thus the potential of carbogen for enhancing 5FU potency.

Response

Clinical response was assessed as progression-free survival (PFS) by the attending Medical Oncologist; this was defined as the time until the patient showed disease

progression or died. At this point, the treatment of surviving patients was either stopped or switched to another regimen.

^{19}F MRS

^{19}F MRS and MR imaging were performed on a clinical 1.5T MR system (Signa: GE, Milwaukee, USA) with a dual-tuned $^1\text{H}/^{19}\text{F}$ surface coil (20 by 16 cm). The coil was semi-flexible and allowed conformation to the curvature of the chest for optimal coverage of the liver. Coronal and axial gradient echo images were first acquired to confirm correct positioning of the coil over the patient's liver. For patients found to have liver metastases, a respiratory-gated T_2 -weighted axial fast spin-echo sequence was used to localise the metastases. A fluorinated silicone oil reference sample situated at the coil centre was used to calibrate the ^{19}F pulse power for each patient and to provide a reference signal for quantitative comparison of data between different patients. ^{19}F spectra were acquired using a 90° pulse at the coil centre with a 500-ms repetition time, 16-kHz bandwidth and 1024 or 2048 data points. Thirty-two averages with the reference signal on resonance were first acquired. The spectrometer frequency was then offset to observe the 5FU signal on resonance. As the bolus i.v administration of 5FU was started, spectral acquisitions were begun and acquired continuously for 32 min in 1-min blocks. Administration of 5FU lasted typically 1 min, followed by a bolus of leucovorin (20 mg/m^2). 5FU was administered by a nurse who remained in the magnet room during the examination; for the cohort breathing carbogen during 5FU administration, the nurse also removed the patient's mask to allow free breathing of room air once the carbogen breathing was complete.

Data analysis

5FU data were analysed in the time-domain MRS fitting package jMRUI [15, 16]. Data were averaged in 2-min block to give 16 time points. 5FU half-lives were evaluated by non-linear fitting of data points starting from the highest point reached, typically in the second block of data. Additionally, all spectra for a given patient were added together to give an average spectrum for the full time course. This average spectrum was assessed for the presence of FNuct (which is rarely present in concentrations high enough to be detected in any single 2-min block and often not detectable even in the average spectrum). Average spectra in which the SNR for FNuct from the jMRUI analysis was greater than 2.5 were defined to be FNuct positive. An example is shown in Fig. 1a, together with an example time course stack plot in Fig. 1b. Additionally, FNuct was assessed semi-quantitatively by calculating the

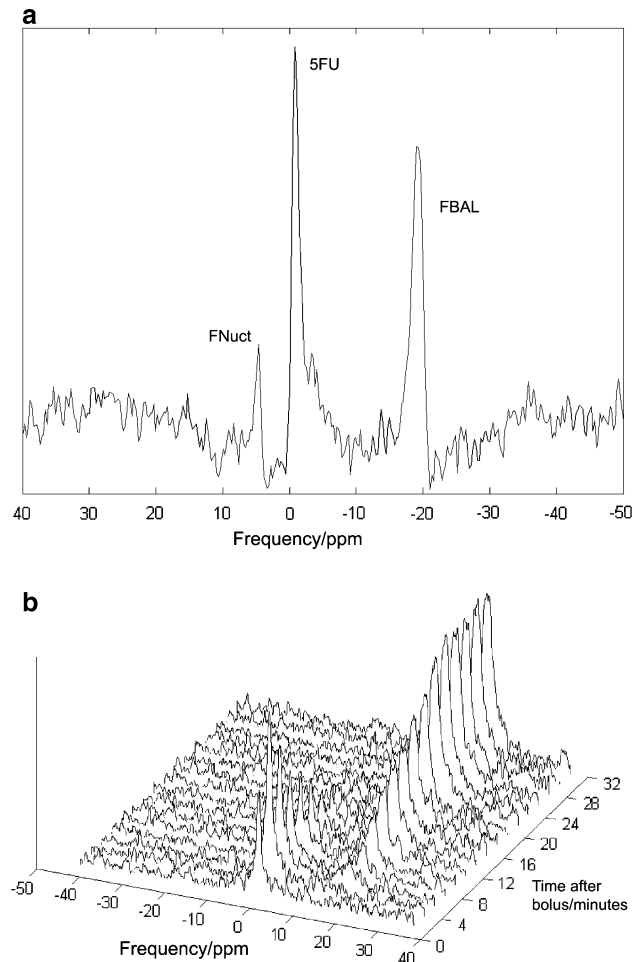


Fig. 1 **a** Example of summed time course data from subject A, showing 5FU, the main catabolite FBAL, and an easily detectable peak from the cytotoxic FNuct. Frequencies are referenced to 5FU at 0 ppm. **b** ^{19}F MRS time course stack plot from subject P. Data averaged in 2-min blocks. FNuct is not visible in individual spectra, but the 5FU bolus and metabolism to FBAL are clear

ratio of the FNuct peak area to the largest 5FU peak area observed from the individual spectra, thus normalising the FNuct peak area independently of the 5FU half-life. This semi-quantitative parameter represents the FNuct AUC normalised to the peak 5FU and depends on the peak FNuct concentration as well as the time constants of FNuct synthesis and breakdown.

MRI tumour volume and MRS sensitivity analysis

Image data were analysed using a program custom-written in MATLAB 7.4 (The Mathworks, Inc, Natick, MA) that numerically simulated the sensitivity of the ^{19}F spectroscopy coil. The coil was located on the images by its internal position marker consisting of a tube of water. ROIs were drawn delineating any visible metastases and all of the liver tissue visible within the field of view of the

coil, from which ROI volumes were calculated. The coil sensitivity was computed at every voxel within each ROI. We then calculated the ratio of the metastasis volume (summed over all the metastasis ROIs) to the total liver volume, weighted voxelwise according to the ^{19}F MRS coil sensitivity. We refer to this ratio within this paper as the fractional metastasis signal (FMS); this measure represents the fraction of detected ^{19}F signal from metastases, assuming the metabolites to be uniformly distributed. The higher the FMS, the more likely that the ^{19}F measurements and kinetics are representative of the tumour (see Table 2).

Statistical analysis was carried out in GraphPad Prism 5.01 (GraphPad Software, San Diego, CA).

Results and discussion

^5FU half-life

The half-life of 5FU in the full patient cohort ($n = 32$) was 8.0 ± 0.48 min (mean \pm SEM), and the range was 4.0–15.5 min (see Table 1). None of the patients we studied had a 5FU half-life of longer than 20 min, and therefore none would be defined as a 5FU “trapper” in the terminology of Presant et al [2]. No statistical correlation was observed between PFS and 5FU half-life, either in the whole population or in the population with detectable hepatic metastases ($P = 0.31$, $R^2 = 0.04$ and $P = 0.18$, $R^2 = 0.13$, respectively).

Paired survival curves were analysed for the relationship between progression-free survival and the presence of liver metastases. As would be expected, the PFS was significantly longer in the group of patients without liver metastases ($P = 0.001$, Fig. 2a). Patients with and without liver metastases have been considered separately for the rest of the analysis to avoid any confounding effects from this variation in PFS and to investigate the significance of spectroscopic data in understanding the therapy of metastases. Retrospectively, we decided to investigate the proportion of tumour in the liver in a subset of patients where the volumes of the metastases were assessed from the images taken prior to the 5FU bolus. The purpose of this was twofold: to investigate whether the signal from the metastasis was overwhelmed by the signal from the liver (thus masking the half-life of 5FU in the tumour tissue) and to investigate correlations between tumour volume and pharmacokinetic parameters.

MRI volume data (see “Methods” for details) were available for 16 subjects, 11 of whom had been clinically reported to have liver metastases and one to have liver cysts. Data were uninterpretable due to motion in 2 cases (1 reported to have metastases). Data were analysed for the

remaining 14 subjects, and metastasis detection was consistent with the clinical report in all cases. For the 10 subjects with metastases, the FMS varied from 0.05 to 0.52 (mean 0.21, standard deviation 0.17). It should be noted that this group did not include the two patients with metastases who survived for longer than 5 months.

Both quantifiable ^{19}F MRS and quantifiable MRI were available for all these 14 subjects. Although none of them (like the other patients we studied) had a 5FU half-life long enough (>20 min) to be defined as a 5FU “trapper” [2], we still investigated whether there was a correlation between the relatively short half-lives that we measured and the hepatic metastatic load. If the metastases had trapped 5FU and hence had a longer 5FU half-life than normal liver, this should have given a correlation between FMS and the half-life measured by MRS. Notably, the two subjects with the highest FMS values of 0.49 and 0.52 had relatively long half-life values of 15.5 and 9.4 min (Table 2), with the other subjects showing half-life values ranging from 4.9 to 11.8 min. However, there was no significant correlation between FMS and the half-life of 5FU ($R^2 = 0.40$, $P = 0.25$). The other 8 subjects with metastases had values ranging from 0.05 to 0.27 (mean 0.14, standard deviation 0.09).

Interpretable MRI data were not available for all patients with liver metastases, including the patients with the longest PFS; nevertheless, some useful inferences can be drawn from the available data. In the majority of the 14 subjects whose data could be analysed, the metastases represented a small proportion of the sensitive volume of the coil (FMS <0.3 in 8/10 patients with metastases, see Table 2), and therefore it is likely that the MRS data will be dominated by the kinetics of the metabolites in normal liver if their concentrations approach those in the tumours. There is no statistical relationship between metastatic load and 5FU half-life in this group, which is perhaps to be expected, since the patients where the metastases are the source of even half the signal are suffering a very high disease burden and are not responding to treatment, thus would not be expected to fall into the group of patients showing long 5FU half-lives. Conversely, the group with smaller metastases may have tumour kinetics that were undetectable against the background normal liver metabolism.

Since there was evidence from animal studies (for example, McSheehy et al [10]; Kamm et al [17]) that carbogen (95% O_2 /5% CO_2) breathing could increase the uptake of 5FU into the liver metastases, we also invited several of the patients ($n = 9$) to attend scanning twice, once breathing air during 5FU infusion and once breathing carbogen (see also Table 1). However, the mean 5FU half-life was found to be not significantly different ($P = 0.75$, paired t -test, mean \pm SEM = 8.9 ± 0.7 breathing

Table 1 Clinical details, 5FU half-life values and progression-free survival (PFS) of patients

Patient	Age	Sex	FU half-life (min) (*with carbogen)	Liver mets	FNuct	PFS Months
A	73	M	8.9, 10.3*	+	+	2
B	60	M	11.9, 8.9*	+	+	1
C	62	M	15.5, 12.0*	+	+	1
D	64	M	6.9	– (liver cysts)	+	30+
E	79	M	9.4, 11.7*	+	–	2
F	71	F	7.5, 8.08*	+	+	4
G	51	F	7.4	+	–	4
H	66	M	6.9	–	–	Adjuvant treatment. Discharged at 5 years in full health
I	59	F	5.9, 6.5*	+	–	3
J	71	F	11.8	+	–	3
K	67	M	5.5	–	–	10
L	85	M	10.3	+	–	3
M	76	F	9.3	+	–	3
N	78	M	5.3	–	–	5
O	67	M	6.3	–	–	10
P	70	F	4.9	+	+	3
Q	71	M	8.6	–	–	2
R	67	F	6.8, 6.1*	–	+	3
S	69	M	7.4, 6.8*	+	–	5
T	47	M	5.3	+	–	5
U	74	M	11.4	–	+	6
V	79	F	5.9	+	–	17
W	67	F	4.8	–	+	9
X	59	M	8.0	+	–	14
Y	74	M	5.4	–	+	14
Z	54	F	9.5, 7.0*	–	+	20
AA	68	M	9.6	–	+	72
BB	64	M	11.9	–	–	7.5
CC	37	F	9.5	–	–	Adjuvant treatment. Discharged at 8 years in full health
DD	69	F	4.0	–	–	Adjuvant treatment. Discharged at 6 years in full health
EE	44	F	5.1	–	–	Adjuvant treatment. Discharged at 5 years in full health
FF	74	F	7.7	–	–	17
Mean +/- sem	66+/-1.9	–	8.0+/-0.48			

carbogen, 8.7 +/-0.9 breathing air), and this approach was not continued (see also [18] for two detailed case studies). There was also no significant difference between the levels of FNuct detected on the two scans (paired *t*-tests on semi-quantitative values, contingency table test on positive/negative classification). It has been proposed on the basis of preclinical studies that the enhanced effect of 5FU in combination with carbogen is a combination of two processes: acidification by the CO₂ component causing increased intracellular/extracellular ΔpH (which has been

shown to increase 5FU uptake in isolated cells [19] and experimental tumours [20]) and a trapping effect, where tumour blood vessels open to allow increased blood flow and then reclose, slowing the outflow of 5FU [10]. Such a mechanism appears not to apply to normal liver, and thus changes in tumour 5FU kinetics may be hidden by the signal from normal liver. Howe et al [21] used ⁵¹Cr radioactivity measurements to show that carbogen breathing increased tumour blood volume in the GH3 prolactinoma grown in rats, but liver blood volume did not

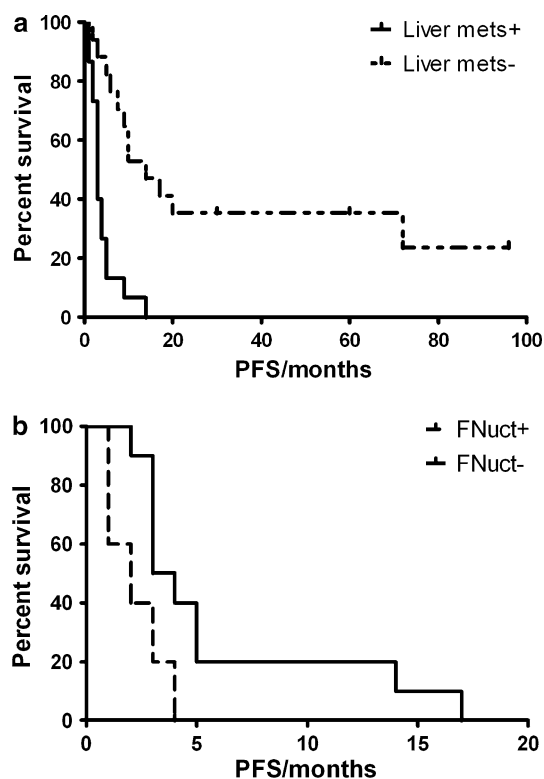


Fig. 2 **a** Progression-free survival for patients with and without metastatic liver disease. **b** Progression-free survival for patients with metastatic disease, divided by the presence or absence of FNucl in their summed ^{19}F spectra. Detection of FNucl is associated with poorer survival

Table 2 The fractional metastatic signal in the subset of patients with metastatic disease for whom interpretable T_2 -weighted MRI data were available ($n=10$)

Patient	FU Half-life (min)	FMS	Mets
A	8.9	0.05	+
B	11.9	0.05	+
C	15.5	0.49	+
E	9.4	0.52	+
F	7.5	0.19	+
G	7.4	0.10	+
I	5.9	0.21	+
J	11.8	0.05	+
M	9.3	0.27	+
P	4.9	0.19	+

The FMS is the fraction of detected ^{19}F signal which would result from metastases if 5FU and its metabolites were distributed uniformly throughout the liver and metastases

increase. Similarly, Honess and Bleehen [22] demonstrated that carbogen breathing resulted in 50–70% increases in perfusion in RIF-1 tumours and showed a smaller but significant reduction of up to 20% in liver perfusion,

depending on the carbogen delivery rate; thus, an increased 5FU uptake in liver metastases could be masked by simultaneous decreases in liver uptake. A recent PET study with carbogen breathing of liver metastases in man [23] demonstrated increased perfusion and specific uptake value in metastases, accompanied by reduction in both parameters in normal liver, although the total area under the curve was not altered. van Laarhoven et al [24] demonstrated that different colorectal tumour lines grown in mice respond differently to carbogen; compared to the well-differentiated C38 line, the poorly differentiated C26a showed smaller changes in 5FU kinetics, but larger changes in response to drug treatment, which the authors ascribe primarily to vascular differences. Thus, the absence in our study of a significant change of the 5FU half-life with carbogen breathing, as measured over the whole liver, may not necessarily mean that there was no change in the 5FU kinetics in the metastases.

The previous observations of Wolf, Presant and co-workers have demonstrated improved survival in a substantial subgroup of patients [2, 5], showing 5FU half-lives longer than 20 min (5FU “trappers”); 51 of their 99 evaluable subjects were classified as trappers, and 70% of these responded to chemotherapy, while none of the non-trappers responded to chemotherapy. None of the 32 patients in the present study had a half-life longer than 15.5 min, and thus, none of them would have fallen into the “trapper” category. It is possible that this is in part due to differences between the coil designs used in the work of Wolf et al. and that used in the present study. The present study used a large coil (16 × 20 cm) sensitive to the entire liver volume; the work of Wolf et al. used surface coils of 3, 7 or 15 cm in diameter, and the “surface coil most suitable to each patient’s study was selected, the choice being dictated by the size of the tumour and its distance from the skin” [5]. It is possible that a smaller probe more tightly coupled to a superficial liver metastasis would be more revealing of the tumour-specific pharmacokinetics than the larger coil used in the study presented here. Another explanation for the difference could be a different patient population; it may be that 5FU was administered to a group of patients with more advanced and/or less chemosensitive cancer in the present study, so that none of them achieved the responses found by Wolf et al. In addition, Wolf et al. studied some patients with primary breast cancer and other unspecified cancers as well as those with liver metastases; the responses are not broken down by tumour type.

Fluoronucleotide signals

There was no significant difference in the mean FNucl levels between the patients with liver metastases

(mets + ($n = 15$) 0.49 ± 0.12 ,) and those without (mets– ($n = 17$), 0.59 ± 0.14 , $P = 0.61$). Overall, these data suggest that patients vary greatly in FNuct level, but that substantial amounts of FNuct are generated in normal liver in some patients; detailed illustrative case studies of hepatic FNuct signals in two early patients from this study group in whom we verified the absence of liver metastases were previously presented in [16].

Table 1 shows the PFS and FNuct data for each patient, and Fig. 2a shows survival plots of the PFS for the groups with and without liver metastases. Figure 2b shows survival plots for PFS for patients with metastatic disease grouped by the presence or absence of FNuct in their averaged spectra. Remarkably, in this group of patients, the presence of FNuct in the spectra was associated with poorer outcome, measured as PFS ($P = 0.027$). This is unexpected, as the presence of high levels of cytotoxic FNuct would intuitively be expected to result in higher cell kill and better outcome.

To analyse this further, we calculated correlations between PFS, FNuct level, FMS and 5FU half-life in the metastasis-positive group (Table 3). FMS values are available for only half of the cases. Intriguingly, the correlations between FNuct and the other three parameters are large, though only the positive correlation between FNuct and 5FU half-life is significant ($P = 0.016$). The trend towards a negative correlation between FNuct and PFS is consistent with the poorer survival in the FNuct-positive group of patients. A plausible hypothesis is that in the metastasis-positive group, the level of FNuct is dominated by the volume of metastasis, and larger volumes of metastasis are correlated with poorer survival. In the metastasis-free group, in contrast, the level of FNuct might relate to the levels of relevant enzymes in normal liver cells.

Table 3 (a) P -values (b) Pearson r -values for correlations between half-life, progression-free survival, FNuct levels (measured as FNuct/peak 5FU) and fractional metastasis signal in the patient group with liver metastatic disease

	Half-life	PFS	FNuct	FMS
a				
Half-life		0.17	0.016	0.25
PFS	0.17		0.14	0.38
FNuct	0.016	0.14		0.09
FMS	0.25	0.38	0.09	
b				
Half-life		−0.38	0.61	0.40
PFS	−0.38		−0.40	−0.32
FNuct	0.61	−0.40		0.56
FMS	0.40	−0.32	0.56	

In conclusion, with the large coil used in this study, it is possible to detect FNuct in liver and tumour tissue. The similarity of 5FU half-life in patients with and without liver metastases suggests that the observed signal may be dominated by normal liver metabolism except when the metastatic load is very high; notably, the two subjects with the highest FMS values had long 5FU half-lives. Within the patient group with liver metastases, FNuct signal is positively correlated with 5FU half-life. There is no clear association between 5FU half-life and outcome, and the 5FU half-lives observed in this study are shorter than those associated with improved outcome in the literature. Overall, these results suggest that metabolism of 5FU in normal liver is confounding any association between half-life and treatment response in the data. High FNuct levels appear to be associated with poorer outcome as measured by PFS; this is likely to reflect higher metastatic load since FNuct is more likely to be produced in tumour cells.

The use of 3T or higher power magnets should in future facilitate such studies by reducing the minimum volume of interest that can be studied and by permitting the use of localised MRS methods. A localised ^{19}F MRS study at both 1.5T and 3T on the metabolism of capecitabine (a pro-drug of 5FU) in liver metastases from colorectal cancer has been reported [25]. Magnetic resonance spectroscopic imaging was used at both field strengths to acquire a 3D grid of $8 \times 8 \times 8$ voxels, with each voxel of $4 \times 4 \times 4$ cm. No FNuct signals were reported, but the 3T spectra had clearly improved signal/noise compared to the 1.5T spectra. The same group subsequently published a capecitabine study at 3T with absolute quantitation of ^{19}F CSI data, using water ^1H CSI data acquired from the same coil tuned to ^1H [26], demonstrating considerable heterogeneity of capecitabine and its metabolites even within normal liver. An approach of this kind should make it possible to assess tumour 5FU kinetics with minimal contamination from normal liver; this would allow, for example, accurate assessment of the effect of carbogen breathing on 5FU kinetics in the tumour, and aggregating signals from multiple voxels may allow comparison of FNuct levels in tumour and normal liver. However, our current results suggest that there may still be a useful role for non-localised ^{19}F spectroscopy as a prognostic marker, by enabling the straightforward detection of FNuct, which is present at very low concentrations distributed throughout the tissue.

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