Identification of early predictive imaging biomarkers and their relationship to serological angiogenic markers in patients with ovarian cancer with residual disease following cytotoxic therapy

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Background: Patients with recurrent ovarian cancer often achieve partial response following chemotherapy, resulting in persistent small volume disease. After completion of treatment, the dilemma of when to initiate subsequent chemotherapy arises. Identification of biomarkers that could be used to predict when subsequent treatment is needed would be of significant benefit.

Design: Twenty-three patients with advanced ovarian cancer and residual asymptomatic disease following chemotherapy underwent dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) at study entry, 4, 8, 12, 18 and 26 weeks or disease progression. A subgroup of patients provided plasma samples within which a panel of angiogenic biomarkers was quantified.

Results: By 4 weeks, significant differences in whole tumour volume, enhancing fraction and Ca125 were observed between patients whose disease progressed by 26 weeks and those who remained stable. Significant correlations between plasma soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and sVEGFR-2 concentrations, and blood volume and tumour endothelial permeability surface area product measured by DCE-MRI were observed.

Conclusions: Imaging markers have a potential role in early prediction of disease progression in patients with residual ovarian cancer and may supplement current measures of progression. The correlation of DCE-MRI and serological biomarkers suggests that tumour angiogenesis affects these markers through common biological means and warrants further investigation.

Key words: angiogenesis, biomarkers, DCE-MRI, ovarian

introduction

Ovarian cancer accounts for ~6% of all female cancer deaths in the UK [1]. In 2005, there were 6806 newly diagnosed cases of ovarian cancer in the UK, equating to a lifetime risk of developing ovarian cancer of 1 : 48 for women living in England and Wales.

Of newly diagnosed patients, more than 70% present with advanced disease (stage III/IV). Despite advances in surgical and cytotoxic chemotherapy management leading to first-line response rates of 75%–80%, 5-year survival for patients with advanced disease (stage IV) remains low (16%). Even when complete remission is achieved following optimum first-line therapy ~80% of patients will eventually develop recurrent disease.

One of the main dilemmas in the management of patients with low volume recurrent disease or who attain a partial response following initial therapy is deciding at what point to re-treat patients who are asymptomatic from their disease. Predictors of response to subsequent chemotherapy are largely based on the interval following exposure to previous platinum-based agents [2–4]. Standard practice in the UK has been to wait until the disease reaches ~4–5 cm in the asymptomatic patient following data which suggested that tumour size (largest lesion <5 cm), the number of sites of disease and histological subtype [5] were associated with response to subsequent chemotherapy. However, at present, there are no data to suggest that the early treatment of patients’ asymptomatic, low volume disease improves survival.
In view of the incurable nature of recurrent or residual disease, new treatments have been developed for ovarian cancer. Recent data have demonstrated that the disease responds to single-agent vascular endothelial growth factor (VEGF) inhibitors; the prototypic class of anti-angiogenic agents [6]. As both imaging and circulating candidate biomarkers for VEGF inhibitors are being explored in the clinic [7, 8], we hypothesised that an integrated analysis of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and circulating biomarkers could be used to monitor disease in patients with recurrent or residual disease and that these biomarkers would inform on the rate of progression and when to intervene with subsequent treatments. Ovarian cancer offers a unique opportunity to monitor tumour behaviour with biomarkers in patients who remain under observation until signs of tumour progression occur. In this exploratory study, we report the biomarker changes in these patients; the first angiogenesis-related biomarker study of the natural history of ovarian cancer.

**methods**

**patients**

Patients were recruited at Christie Hospital (Manchester), through the Gynaecological Medical Oncology clinics; before initiation of the study, it received favourable ethical committee approval. Patients who had achieved a partial response following any line of chemotherapy and were asymptomatic from their residual disease were eligible to participate in the study. They were required to have histologically or cytologically proven ovarian or primary peritoneal carcinoma and to have disease that secreted CA125. The target lesion identified for DCE-MRI analysis was required to be at least 0.2 cm in diameter and deemed assessable by the investigators. Standard MRI exclusion criteria were employed within the study including, a creatinine clearance of ≥50 ml/min calculated via the Cockcroft Gault formula.

**design**

Patients were eligible to enter in the study at any time following completion of their chemotherapy. DCE-MRI scans were then performed at baseline (study entry), 4 weeks, 8 weeks, 12 weeks, 18 weeks and 26 weeks. If disease progression was suspected either clinically, biochemically or through the DCE-MRI scan, a conventional anatomical computed tomography (CT) scan was performed. If this confirmed progressive disease (RECIST criteria), patients were withdrawn from the imaging study.

**DCE-MRI protocol**

The DCE-MRI scanning protocol employed in this study was the standard dynamic scanning acquisition protocol has been reported in previous studies [9, 10]. First, localiser acquisitions were performed to identify the target lesion position. Axial precontrast T₁-weighted fast field echo (FFE, gradient echo) and T₁-weighted fast spin echo volumetric acquisitions provided anatomical detail for the identified target lesion.

The dynamic scan sequence consisted of a series of three-dimensional (3D) T₁-FFE (spoiled gradient echo) measurements performed over a 6-min time frame following an initial 3D T₁ measurement performed using the variable flip angle spoiled gradient echo method that provided baseline tissue T₁ measurements [11]. Intravenous gadolinium-based contrast agent (Omniscan™, GEHC, Amersham, UK) was administered (0.1 mmol/kg) at the sixth time frame after the commencement of the T₁-FFE sequence. Upon completion of the dynamic sequence, a final post-contrast T₁-weighted FFE acquisition was performed to assist with lesion identification. The T₁ measurements from the dynamic sequence permitted the calculation of rate of change of T₁ in the tissue over time and thereby determination of the change in contrast agent concentration within the tissue over time.

Tumour regions of interest were defined from the anatomical scans to provide 3D whole tumour volume (WTV). Volumes within the WTV with an IAUC₆₀ (integrated area under the contrast agent-concentration time curve at 60 s; a measure of contrast agent delivery and retention within the tumour) value of >0 was defined as enhancing. This enabled calculation of the enhancing fraction (EnF) of the tumour (the number of enhancing voxels divided by the total number of voxels in the WTV), representing the proportion of the tumour that demonstrated detectable perfusion on DCE-MRI [10, 12, 13].

The extended Tofts tracer kinetic model employing contrast agent concentration kinetics [14–17] was then applied to the enhancing voxels providing quantifiable parameters of $K_{trans}$ (volume transfer coefficient of contrast agent across the capillary wall); a composite parameter reflecting both blood flow and capillary permeability of the tumour, $v_p$, the fractional blood plasma volume within the tumour, and $v_c$, the fractional volume of the extravascular, extracellular space within the tumour accessible by the contrast agent after delivery to the tumour. Arterial input functions were extracted from the imaging data using a previously described automated method [18]. Where a suitable artery was not visible within the field of view or if image artefacts obscured the vessel, a previously defined population-derived arterial input function was employed [19]. In this case, the same arterial input function was used for each visit of a given patient.

Figure 1 presents an example of a parameter map for $K_{trans}$ in a patient with a pelvic tumour. The tumour region of interest has been defined on each slice allowing calculation of the 3D volume of the tumour. The number of enhancing voxels within the tumour can be determined to provide the EnF (%) of the tumour, with non-enhancing regions, i.e. cystic regions being excluded from subsequent analysis. Application of a kinetic model to the enhancing voxels provides estimates of flow/permeability for the tumour through calculation of $K_{trans}$. In this example, the brighter rim of the tumour corresponds to areas of increased flow/permeability at the tumour periphery.

One- and two-dimensional (1D and 2D) tumour measurements were obtained using Image J software (National Institute of Health). The longest
unidimensional diameter of the target lesion was measured as per RECIST criteria [20] and followed by the longest perpendicular diameter for the calculation of the 2D measurement per World Health Organisation (WHO) criteria [21]. If the sum of the long diameter of tumours within the DCE-MRI field increased by more than 20%, patients underwent a conventional anatomical CT scan. As previously described, 3D volumes were also obtained from the DCE-MRI scans to provide a 3D measure of WTV.

serological markers
Plasma samples were obtained from a subset of patients participating in the study. Samples were obtained immediately before the DCE-MRI scan, 5 ml blood was collected in EDTA, centrifuged at 3000 g at room temperature for 10 min and aliquots of supernatant were stored at −80°C.

Samples were then analysed in duplicate using a validated, multiplex ELISA method (Searchlight multiplex ELISAs; Aushon Biosystems Inc., Billerica, MA) [22]. The two multiplex assays used consisted of the following nine angiogenic markers: soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), sVEGFR-2, interleukin-8 (IL-8), placental growth factor (PIGF), keratinocyte growth factor (KGF), platelet derived growth factor-bb (PDGF-bb), hepatocyte growth factor (HGF), fibroblast growth factor basic (FGFb), and VEGF. All patients also had plasma assessed for Ca125 analysis at the time of DCE-MRI scanning.

statistical analysis
As this was an exploratory pilot study, no formal power calculations were performed before initiation of the study. Variables for the linear multiple regression models were determined by categorising the patients into two groups according to their status at their last DCE-MRI scan while in the study. Patients who remained in study for the total duration (26 weeks) were deemed to have stable disease; those who came off study early were categorised as having progressive disease.

Following categorisation, imaging parameters and serological markers (both absolute values and percentage change from baseline) were assessed for statistically significant differences using the Mann–Whitney test. Those parameters that were found to be significantly different between the groups (P ≤ 0.05) at 4 weeks and 8 weeks were identified as variables for the linear multiple regression models. These were entered into a simultaneous linear multiple regression model to produce a model for prediction of progression-free interval (PFI). Limited patient numbers restricted the analysis at the later time points. Identification of significant correlations between variables and outcome measures was determined using appropriate scatter plots and formally assessed by Spearman’s bivariate correlation.

To assess the behaviour patterns of different-sized tumours, target lesions were categorised according to 1D (six groups), 2D (seven groups) and WTV (five groups) measures of tumour size. Tumours were also classified according to whether the tumour diameter was more or less than 5 cm [5]. Each of these tumour classifications was then assessed using non-parametric tests (Mann–Whitney and Kruskal–Wallis).

A subset of patients had blood taken for plasma biomarker analysis at the time of each scan to assess the relationship of serological markers of angiogenesis to DCE-MRI imaging markers of angiogenesis. To increase the quantity of paired data values, the results from all the time points were pooled on the basis that there was no therapeutic intervention in these patients. This strategy generated 30 paired plasma biomarker and imaging study datasets. These data were then examined using non-parametric techniques.

results
patient characteristics
Twenty-five patients were recruited. Two patient datasets were excluded from analysis because of missing data and patient withdrawal, leaving 23 datasets for analysis. Of these, all had disease that secreted Ca125 and eight underwent analysis of angiogenic biomarkers at the time of scanning.

The patient group was typical of patients with recurrent ovarian cancer. The majority had relapsed advanced disease (International Federation of Gynecology and Obstetrics stage III, 52.2% and stage IV, 26.1%) and had received a median of two lines of previous therapy. The interval between the final cycle of previous chemotherapy and entry to the study was a median 15 weeks (mean: 45 weeks, minimum: 3 weeks, maximum: 212 weeks).

The target lesion(s) imaged using DCE-MRI varied within the group of patients depending upon the site of their residual disease. Full patient characteristics are provided in Table 1.

early predictive markers of progressive disease
No baseline DCE-MRI parameters (IAUC60, $K_{\text{trans}}$, $v_g$ and $v_p$) showed a significant difference between patients who went on to develop progressive disease and those with stable disease for the duration of the study period.

Analysis of data from scans taken at 4 weeks revealed that the percentage change from baseline in WTV, EnF, and Ca125 levels differed significantly between patients whose disease remained stable while in the study and those who developed progressive disease ($P \leq 0.05$). The differences within these three parameters remained statistically significantly at 8 weeks. Patients with progressive disease had a greater percentage increase observed in the parameters WTV, EnF and Ca125 at both time points (Figure 2).

Two simultaneous linear multiple regression analyses using progression-free survival as the target variable were performed. The first model used percentage change in the variables of WTV, EnF and Ca125 at 4 weeks, while the second model was on the basis of the percentage change in the same parameters at 8 weeks. The independent correlations of these variables to PFI were significant for Ca125 at weeks 4 and 8, and WTV at week 8 ($P \leq 0.05$).

The proportion of variance of PFI explained by the variables in the first regression model was 15% ($R^2 = 0.15$ $P = 0.417$), and for the second model it was 28.5% ($R^2 = 0.285$ $P = 0.0278$). Of the regressor variables, percentage change in Ca125 contributed the most to the prediction of PFI for both models ($P = 0.278$ and 0.474 for the 4-week and 8-week model, respectively). Application of stepwise multiple regression models showed that the additional variables did not significantly contribute to the model. Scatter plots of predicted PFI against actual PFI from 4-week and 8-week percentage change in variables are shown (Figure 3).

relationship of imaging markers to PFI
Baseline tumour vascularity represented by the dynamic parameters (IAUC60, $K_{\text{trans}}$, $v_g$ and $v_p$), and volumes (1D, 2D and WTV) or percentage change in dynamic parameters showed no significant correlations with PFI.

The percentage change in WTV correlated with PFI ($P \leq 0.05$) and reached significance at week 8 ($P \leq 0.05$); however, the percentage change in both 1D and 2D measures did not significantly correlate with PFI, highlighting the potential importance of WTV as a more sensitive imaging biomarker than RECIST or WHO reporting criteria.
The range of Ca125 upon entry in the study was from within normal range (minimum value 12) to 8893 µg/ml. The average percentage changes of Ca125 observed for the patients with stable disease compared with those who developed progressive disease were 58% versus 96%. The percentage change in Ca125 strongly correlated with PFI ($P \leq 0.005$), an observation that was detected at 4 weeks and which preceded the development of a significant correlation of percentage change in WTV to PFI.

**tumour growth patterns**

To investigate whether different-sized tumours grew in a heterogeneous fashion over the period of observation and to determine if tumour burden was a negative prognostic factor, we categorised the patients according to their 1D, 2D and WTV tumour dimensions. Analysis of the data showed no significant difference in the percentage change of DCE-MRI parameters, or Ca125, according to size-based categorisation; demonstrating no difference in tumour vascular characteristics or Ca125 evaluation according to tumour size.

Previous publications have highlighted the prognostic significance of tumour diameter with regard to subsequent treatment with cytotoxic agents. When grouped according to such criteria [5], half the patients had tumours measuring <5 cm and the remainder had disease >5 cm diameter. There was no difference in the PFI between these groups, and at each time point no differences were observed in the percentage changes in either the DCE-MRI parameters or Ca125.

When response to subsequent chemotherapy following the scanning period was assessed, tumours >5 cm showed no significant difference in response to chemotherapy than tumours that were <5 cm in diameter.

**serological biomarkers**

Of the nine analytes assessed, seven were detectable (VEGFR-1, VEGFR-2, VEGF-A, IL-8, PlGF, PDGF-bb and HGF). Basic FGFb and KGF were below the lower limit of detection. Due to the small number of patients within the cohort, it was not possible to determine significant dynamic changes over time. However, although there is a paucity of robust literature on baseline variation of these biomarkers in cancer patients, our previous studies suggest that the fold changes noted over the extended time-course of the study are greater than expected for baseline variation.

Assessment of the relationship of DCE-MRI parameters at each visit and serological angiogenic markers at the same visits identified some significant correlations. $v_p$, the fractional blood volume, was significantly negatively correlated with soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and sVEGFR-2; $P = 0.011$ and 0.001, respectively. Conversely, a strong positive correlation was observed between sVEGFR-1 and sVEGFR-2 to $K^{trans}$ ($P = 0.001$ and 0.026, respectively; Figure 4).

Assessment of baseline values and percentage change in the angiogenic biomarkers in this small group of patients showed no relationship of the serological markers to PFI; a larger study would be required to formally address this question.

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**Table 1.** Patient characteristics

<table>
<thead>
<tr>
<th>FIGO stage at diagnosis</th>
<th>Histological subtype</th>
<th>No. lines of chemotherapy</th>
<th>Time from chemotherapy to first scan (weeks)</th>
<th>Target lesion</th>
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<tbody>
<tr>
<td>Ia</td>
<td>Serous adenocarcinoma</td>
<td>1 (surgery for initial relapse)</td>
<td>156</td>
<td>Mesenteric mass</td>
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<td>IIc</td>
<td>Transitional cell carcinoma</td>
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<td>5</td>
<td>Pelvic mass</td>
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<td>Primary peritoneal carcinoma</td>
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<td>Omental</td>
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<td>Liver</td>
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<td>6</td>
<td>Para-renal mass</td>
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<tr>
<td>IV</td>
<td>Carcinosarcoma</td>
<td>1</td>
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<tr>
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<tr>
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<td>2</td>
<td>3</td>
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<td>13</td>
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</tr>
<tr>
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<td>Endometrioid/clear-cell carcinoma</td>
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<td>30</td>
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<tr>
<td>IIib</td>
<td>Serous adenocarcinoma</td>
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<td>15</td>
<td>Lymph node</td>
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<tr>
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<td>3</td>
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<tr>
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<td>Carcinosarcoma</td>
<td>6</td>
<td>14</td>
<td>Liver</td>
</tr>
</tbody>
</table>

FIGO, International Federation of Gynecology and Obstetrics.
discussion

We have tested for the first time the clinical significance of current vascular imaging protocols in untreated patients with asymptomatic, residual volume ovarian cancer to define the natural history. The principal clinical dilemma in this setting is at what time point to re-introduce cytotoxic therapy. Biomarkers may be useful in this situation, allowing clinical decisions to be taken early, thus reducing treatment-related morbidity and potentially improving outcome. The biomarkers utilised within this study represented characteristics of tumour vascularity and angiogenic potential that have been identified as poor prognostic factors in ovarian cancer.

The most striking findings are the relationships between the circulating angiogenic biomarkers and DCE-MRI imaging biomarkers. In tumours where the blood supply is reduced, we would expect to see reduced $v_p$, the fractional blood plasma volume. Tumours with reduced blood supply will be under hypoxic stress and respond to their hypoxic environment through activation of various biological pathways. Included within these will be activation of the hypoxia inducible factor-1α pathway, which leads to the increased expression of hypoxia response genes including those of VEGF and its receptors VEGFR-1 and VEGFR-2 [23]. This hypothesis would

Figure 2. Box plots to illustrate the percentage change in whole tumour volume, enhancing fraction (EnF), and Ca125 at (A) 4 weeks and (B) 8 weeks in patients who attained stable or progressive disease at the end of the study. Boxes represent 25th to 75th percentile range with median value shown by the bold line, whiskers show minimum and maximum range.

Figure 3. Scatter plot of predicted progression-free interval (PFI) from multivariate regression analysis (dependent variable) against actual PFI calculated from (A) variable regressors of percentage change in whole tumour volume (WTV), enhancing fraction (EnF), and Ca125 at 4 weeks and (B) variable regressors of percentage change in WTV, EnF, and Ca125 at 8 weeks.
therefore be consistent with our observations of a negative correlation between \( v_p \) and sVEGFR-1 and sVEGFR-2, with tumours with low blood volume up-regulating and increasing their expression of pro-angiogenic receptors. The question remains, however, of whether enhanced cell membrane-based expression of VEGFR is associated with increased concentrations of plasma VEGFRs.

The second relationship involving circulating and radiological factors was the positive correlation between \( K_{\text{trans}} \) and sVEGFR-1 and sVEGFR-2. As with the first relationship, hypoxia may be responsible. \( K_{\text{trans}} \) is a composite function of blood flow and permeability. In our studies, fractional blood plasma volume (\( v_p \)) was not correlated to \( K_{\text{trans}} \) \((p = -0.152, \ P = 4.78)\), as previously reported [24]. This suggests that \( K_{\text{trans}} \) is more heavily regulated by permeability than flow (which is likely to be coupled to blood volume). Vessel permeability is a phenotype controlled by several biological pathways including those related to VEGF, which is in turn regulated by hypoxia. Thus, this positive correlation may reflect the relationship between hypoxia-induced VEGF activity, resulting in tumours with increased vascular permeability (\( K_{\text{trans}} \)), and increased endothelial cell turnover, generating increased amounts of soluble VEGFR.

It is important to note that we were able to find these relationships only by using a DCE-MRI model that is more sophisticated than the generally applied ‘Tofts model’, which does not include a term accounting for blood volume. The fact that the ‘extended Tofts model’ is sensitive to vascular changes in tumours related to the expression of sVEGFR-1 and sVEGFR-2 provides a strong incentive for the routine use of this more informative model.

Interestingly and consistent with our data, the same relationship is seen in patients treated with VEGF inhibitors. During the phase I evaluation of cediranib, a pan-VEGFR tyrosine kinase inhibitor, reductions in DCE-MRI parameters were associated with a reduction in soluble VEGFR2 [7]. This study used the DCE-MRI measure IAUC_{60} to assess the effects of therapy upon tumour vascularity, whereas our study applied the extended Tofts model providing the additional measure of \( v_p \).

Figure 4. Scatter plots demonstrating the negative correlation between \( v_p \) and (A) soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and (B) sVEGFR-2 \((R^2 = 0.459 \ P = 0.002 \text{ and } R^2 = 0.398 \ P = 0.026, \text{ respectively})\), with the hashed line showing the linear regression line of best fit.
The study identified early parameters that could potentially be utilised as tools for clinical decision making. Measurements at 4 weeks of WTV, EnF and Ca125 showed a significant difference between patients who attained stable disease and progressive disease in patients categorised according to the final scan. We investigated whether we could construct a statistical model to combine the parameters to improve the predictive value of the measurements. However, the model was not statistically superior to any of the three parameters used alone, and combination of the variables did not improve the predictive value of the model that showed a tendency to overestimate the PFI at lower mean values of PFI and underestimate at higher. This highlights that the variability seen in PFI (the dependent variable) is not solely attributable to the independent variables used in the regression model. This may be due to the number and heterogeneity of patients recruited to the study or, perhaps more likely, to unmeasured factors. Extension of this study with increased numbers of patients with a more defined treatment-free interval might increase the probability of developing such a model.

The observation that the percentage change in Ca125 at 4 weeks showed the earliest relationship to PFI that was statistically significant suggests that this may be the most sensitive measure we have in assessing early disease relapse in patients with Ca125-secreting tumours. The recently reported MRC Ov05 study has shown that early treatment on the basis of raised Ca125 gave no survival benefit to patients over those where treatment was commenced when clinical or symptomatic occurrence occurred [25]. The identification of early markers of progression either serological or imaging may therefore not provide any additional survival benefit in this population of patients.

This study did not identify tumour size as a predictive marker of response to subsequent cytotoxic therapy in contradiction to evidence from previous studies. Percentage change in WTV did show a correlation to PFI but statistical significance was not achieved until 8 weeks. However, in comparison with 1D and 2D measures of tumour size, WTV was a more sensitive tool. Thus, quantification of WTV in clinical trials of mechanism-based therapeutics might offer much greater opportunity to detect statistically significant changes in tumour growth, particularly in the short duration investigations that typify phase I trials.

In conclusion, we have evaluated circulating and radiological biomarkers of angiogenesis in untreated patients with residual or recurrent ovarian cancer. For the first time, we demonstrate a negative relationship between soluble VEGF receptors and tumour blood plasma volume and a positive relationship between the receptors and \( K^{trans} \), which in this situation largely reflects vascular permeability. To what extent these observations are dictated by tumour hypoxia remains to be established. We also tracked the natural history of untreated ovarian tumours and found that by dichotomising the patients according to whether they had stable disease or progressive disease at the time of leaving the study, we could detect statistically significant differences in WTV, Ca-125 and EnF, 4 weeks after enrolment in the study. Further evaluation of these biomarkers as predictors of PFI is warranted.

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### references