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A prospective feasibility study evaluating the role of multimodality imaging and liquid biopsy for response assessment in locally advanced rectal carcinoma

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Abstract

Purpose Colorectal cancer is a commonly encountered disease that poses several diagnostic and therapeutic challenges. The inherent heterogeneity of tumor biology and propensity to relapse despite "curative" resection pose significant challenges with regard to response assessment. Although MR imaging already plays a key role in primary staging of patients with rectal carcinoma, its reliability in restaging after neoadjuvant therapy is debatable (Van der broek et al. in Dis Colon Rectum 60(3):274–283, 2017). Therefore, there is significant interest in developing additional methods which may improve diagnostic accuracy. This study aims to evaluate the role of multimodality imaging and liquid biopsy in therapeutic response assessment.

Methods Seventeen patients were enrolled into the study over a span of 24 months. All underwent hybrid PET-MRI and CT-perfusion (CT-P), prior to and following neoadjuvant therapy for locally advanced rectal carcinoma. Twelve of the 17 patients also underwent liquid biopsy, which consisted of blood sampling and analysis of circulating tumor cells (CTCs) and extracellular vesicles (EVs), including cell fragments and microparticles (MPs), using the Cell Search System (Menarini Silicon Biosystems). SUV, DWI, and ADC were calculated during PET-MRI, and several parameters were evaluated during CT-perfusion, including average perfusion, blood flow (BF), blood volume (BV), mean transit time (MTT), permeability-surface area product (PS), contrast extraction efficiency (E), and K-trans (K). Changes observed pre- and post-neoadjuvant therapy in each modality were compared to tumor response at histopathology using a modified Ryan tumor regression grading system.

Results Of the 17 patients included in the study, 14 were classified as non-responders, and 3 were classified as responders as determined by the modified Ryan Tumor Regression Grade (TRG) scoring system (Van der broek et al. in Dis Colon Rectum 60(3):274–283, 2017). When combined, blood markers and CT-P parameters (mean transit time (MTT), K-trans, and permeability-surface area product (PS)) produced the strongest models ($p < 0.01$). PET (SUV measurement) combined with CT-P-derived K-trans produced a marginally significant ($p = 0.057$) model for predicting response. MRI-derived ADC value did not provide a significant model for response prediction.

Conclusion A model of CT-P parameters plus liquid biopsy more accurately predicts tumor response than PET-MRI, CT-P alone, or liquid biopsy alone. These results suggest that in the evaluation of treatment response, liquid biopsy could provide additional information to functional imaging modalities such as CT-P and should therefore be explored further in a trial with larger sample size.

Keywords MRI · PET-MRI · CT-perfusion · Extracellular vesicles · Rectal cancer

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide [1]. The incidence of rectal carcinoma is increasing, particularly in younger age groups and in the developing world [2]. Surgical management combined with adjuvant or neoadjuvant chemoradiation therapy (CRT) is the traditional cornerstone of

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therapy [3, 4], and recently, at some centers, non-operative management (consisting only of CRT) has gained acceptance as the sole therapy for specific indications [5]. Therefore, imaging methods are becoming increasingly important for evaluation of response to therapy.

Treatment of rectal carcinoma poses a unique challenge; currently, the only proven curative therapy is surgical resection with negative margins. Meticulous surgical technique and preoperative planning are required for a successful outcome, as a positive margin increases the risk of recurrence, and may lead to poor quality of life and reduced disease-free survival [6]. For locally advanced disease, adjuvant or neoadjuvant chemoradiation therapy (CRT) is the standard of care, consisting of several cycles of chemotherapy, coupled with either short course or long course radiation therapy. Post-CRT MRI is often performed for response assessment prior to surgical excision of the tumor.

Although patients diagnosed with early-stage CRC are usually treated surgically with curative intent, local recurrence or metastatic disease can occur in up to 30% of patients within five years of surgery. This suggests that early tumor cell dissemination or micrometastatic disease, presumably undetectable by conventional imaging methods, may already exist at the time of, or shortly following, surgery [7]. Therefore, exploring the role of imaging in tandem with biomarkers is of great interest in the early detection of residual tumor following neoadjuvant therapy.

High-resolution MRI is the modality of choice for preoperative (primary) tumor staging. In primary staging, functional sequences such as diffusion weighted imaging (DWI) are considered to provide even higher diagnostic performance than conventional MRI [8].

Positron emission tomography (PET) is capable of imaging tumor activity based on glucose metabolism, which directly correlates with metabolism. PET-MRI is an integrated hybrid system that provides simultaneous anatomic and functional information, which is advantageous compared to conventional PET-CT.

In addition to anatomic and metabolic information, evaluating tumor blood supply can be a useful indicator of tumor-associated angiogenesis. Rapidly growing tumors are supplied by newly developing, albeit structurally abnormal blood vessels. These vessels have an incomplete endothelium with fragile walls, and are typically larger in diameter than normal vessels, with blind-ending vessels in the center of the tumor [9]. CT-perfusion exploits the proliferation of these structurally aberrant vessels by evaluating changes in delivery of iodinated contrast agents to the tumor. These changes are measured by parameters such as blood flow (BF), blood volume (BV), and vascular permeability-surface area product (PS). These data can then be extrapolated to measure hypoxia [10] and angiogenesis [11].

Liquid biopsy is an emerging technique to evaluate the burden of disease in colorectal cancer, having shown promise as an alternative to traditional tumor markers such as carcinoembryonic antigen (CEA). Using blood or urine samples, liquid biopsy detects cancer in the form of circulating tumor DNA (ctDNA); circulating tumor cells (CTCs); and extracellular vesicles (EVs) such as cancer microparticles (MPs). Liquid biopsies have the potential to detect the following manifestations of disease: (1) residual tumor following therapy; (2) early tumor recurrence; or (3) micrometastatic disease. These forms of disease are often both clinically occult and below the resolution of available imaging modalities [12].

Previous studies have evaluated the roles of hybrid PET-MRI, CT-perfusion (CT-P), and liquid biopsy independently in the diagnosis of rectal cancer, and to some extent in the setting of therapeutic response assessment. This preliminary, hypothesis-generating study aims to discuss the potential role of combining imaging findings with blood-based biomarkers (via analysis of CTCs, extracellular vesicles/cancer microparticles, and cell fragments) in response assessment. Specifically, we question whether a model using a combination of PET-MRI, CT-P, and liquid biopsy can be developed to identify patients with and without a therapeutic response in locally advanced rectal cancer.

Methods

The study was approved by the institution's Research and Ethics Board. Informed consent was obtained from all patients in writing before the study.

Study population

As this is a preliminary, hypothesis-generating study, recruitment occurred sequentially and was limited by the practical nature of this pilot study, rather than the time needed to meet sample size requirement. Patients with locally advanced (T3 and above), histologically proven rectal adenocarcinoma were accrued over 24 months. Exclusion criteria were Stage IV disease, mucinous tumors, non-rectal primaries, prior history of radiotherapy, hip prosthesis (which would interfere with CT-perfusion measurements and MRI quality), contraindications to MRI, impaired renal function (eGFR < 30 mL/min), and allergy to iodinated contrast. The final cohort consisted of 17 patients, including 7 females and 10 males, with a median age of 57 years.

All patients who met the inclusion criteria for this study underwent pre- and post-neoadjuvant CT-P and PET-MR imaging prior to surgery. Twelve of the seventeen patients who received imaging also underwent liquid biopsy. Liquid biopsy data were not available for the remaining five

patients, each of whom declined repeat blood testing following neoadjuvant therapy.

PET-MRI

PET-MRI examination was performed on a hybrid system (Siemens Medical Biograph 3T mMR, Germany). All patients fasted for at least 6 h prior to the study. Blood sugar level was verified to be less than 11 mmol/L before F-18 FDG was administered at 4 MBq/kg, followed 10 min later by hydration with 250–500 mL saline. Sixty minutes post injection, hybrid PET-MR images of the pelvis only were obtained. The PET data were reconstructed with a FOV of 258 mm. Three-point DIXON VIBE sequence was acquired to generate a linear attenuation coefficient map for attenuation correction [13].

Rectal MRI sequences included axial T2 TSE of the entire pelvis (aortic bifurcation to pubic symphysis); small field of view (220–240 mm), high-resolution (3 mm) sagittal T2 TSE; axial oblique T2 TSE perpendicular to the rectal tumor; coronal T2 TSE; axial DWI (B values 0, 500, 1000) with ADC map. No IV contrast was administered. All patients received 20 mg Buscopan IV immediately prior to the start of the examination.

CT-perfusion (CT-P)

Following the PET-MR study, all patients underwent CT-perfusion on a 64-slice CT scanner (Discovery VCT, GE Healthcare, Waukesha, WI). Both the PET-MR and CT-perfusion (CT-P) studies were performed on the same day, except in one case, where the CT-P was done within 1 week of the PET-MR.

A non-enhanced scan was used to select a region for CT-P imaging by a trained abdominal radiologist based on the visible tumor volume. Dynamic imaging of the selected area was performed with intravenous injection of contrast. Scanner settings for the axial shuttle mode dynamic imaging were as follows: 120 kV, 125 mAs, 0.4-second gantry rotation, 5.0-mm-thick slices, 80 mm coverage centered at the primary tumor. Contrast (Isovue 300) was injected at a dosage of 0.7 mL/kg body weight via an antecubital vein at 2–3 mL/s. The CT-P images were acquired starting at 15 s into contrast injection at 2.8-s intervals for 65 s and then at 15-s intervals for the remaining 120 s to allow for tumor permeability-surface measurement. The scan dose-length-product for CT-P scan was 996.6 mGy-cm with a calculated effective dose of 15.6 mSv. CT-P images were transferred to an image processing workstation (Advantage Windows; GE Healthcare) for postprocessing.

Liquid biopsy

The total number of microparticles/cell fragments, consisting of both CTCs and GPA33-labeled colorectal cancer microparticles, was evaluated in 12 patients at baseline and at 4–6 weeks following completion of radiation therapy, similar to the protocol described by Cohen et al. [14]. CTCs included both intact and granular tumor cells. The analyzed cell fragments and microparticles (MP) included Large tumor cell fragments (L-TCF), Small tumor cell fragments (S-TCF), Large tumor microparticles (LTMP), and Small tumor microparticles (STMP). Tumor microparticles (large and small) in addition to tumor cell fragments (large and small) were enumerated using nanoscale flow cytometry after incubating blood samples with cell-surface antigen A33 (GPA33). Each patient provided 10 cc of blood for the analysis, both at initial consultation and prior to surgery.

CellSearch Imaging Flow Cytometry: The CellSearch System (Menarini Silicon Biosystems Inc., Hunting Valley, PA) was used for all imaging flow cytometry experiments. Whole blood was collected into CellSave vacutainer tubes (Menarini Silicon Biosystems Inc., Hunting Valley, PA). Protocol and analysis methodology for all CellSearch subclasses are as described by Biggs et al. [15].

Image analysis

PET-MRI

PET-MRI images were reviewed on a Siemens Syngovia workstation (SyngoVia) by a radiologist with seven years of experience in body imaging and PET interpretation. The reader was aware of clinical indications for the study, endoscopic findings, and biopsy results.

The MRI images were reviewed first, followed by the fused PET-MRI images. The findings were recorded in a routine synoptic report. The primary tumor was identified on DWI/ADC images and cross-referenced with the T2-weighted images. Maximum SUV, minimum, and mean ADC values of the primary tumor were determined using a manually drawn region of interest (ROI) that outlined the entire tumor margin. The ROI was initially drawn on the T2-weighted images, then transposed to the fused PET-MR and ADC images, respectively.

CT-perfusion

CT-P images first underwent non-rigid deformable registration (GE Healthcare) to minimize motion of the tumor. Registered images were then analyzed using CT-Perfusion software (GE Healthcare) by a research assistant with five years of experience. Arterial input ROI was selected in an external iliac artery. Functional maps generated by the software

included the average, blood flow (BF), blood volume (BV), mean transit time (MTT), permeability-surface area product (PS), contrast extraction efficiency (E), and K-trans (K).

The tumor volume in each slice was outlined by the radiologist using the average map and superimposed on the other functional maps to determine the average value for the whole tumor. In two of the 17 patients, the CT-P-derived permeability-related measures (PS, E, and K-trans) could not be estimated due to a short scan duration that did not capture the recirculation phase.

Surgical resection and pathologic analysis

Each patient underwent standard Total Mesorectal Excision (TME) following completion of neoadjuvant therapy. Average time between imaging and surgery was 61.9 days.

Each surgical specimen was evaluated using standard departmental TME protocol using H&E review by one pathologist with three years of experience in gastrointestinal pathology, who was blinded to the imaging analysis. The primary outcome was the pathological response of the tumor to neoadjuvant chemoradiotherapy (CRT). Tumor response was assessed histopathologically from the surgical specimen and dichotomized according to the degree of fibrosis vs. viable tumor following CRT. Patients were then classified as either "responders" or "non-responders" as per Table 1 below, using a modified Ryan score for tumor regression [16]:

Statistical analysis

In this preliminary, hypothesis-generating study, the relative change observed for each modality (CT, PET, MR, and liquid biopsy) was evaluated pre- and post-neoadjuvant therapy. Univariate logistic regression was initially performed to evaluate the effects of each parameter on the likelihood of tumor response, as determined by the pathologic tumor regression score. Only those parameters with a significance level of $p < 0.5$ in the univariate analysis of tumor response were retained for further analysis. Multivariate logistic regression was performed using two-parameter models to avoid overfitting of the model due to the limited size of the patient dataset. Model effects were considered significant at the level of $p < 0.05$. Statistical analysis was performed using SPSS statistics package, version 20 (Armonk, NY: IBM Corp.).

Results

Of the 17 patients analyzed, 14 were classified as non-responders and three were classified as responders as determined by the modified Ryan Tumor Regression Grade (TRG), see Table 1 above. A summary of patient characteristics is provided in Table 2.

A total of eleven parameters (BF, BV, MTT, PS, E, K, ADCmin, ADCmean, SUV, cell fragments/particles) from multimodality imaging and blood analysis were evaluated for each patient at baseline and follow-up, as indicated in Table 3. Of these eleven parameters, five had a $p < 0.5$ in univariate analysis of treatment response: (***1) CT-P: (MTT, PS); (2) K-trans; (3) PET: (SUV); (4) Blood Markers: (CTCs/MPs/cell fragments).

Following therapy, the combination of relative changes in the total number of CTCs, MPs, and cell fragments (CTCs/MPs/cell fragments) together with CT-P-derived MTT was the most statistically significant model ($p = 0.004$) for classifying patients as either "responders" or "non-responders," with a classification accuracy of 100% (Table 4). The total number of CTCs and microparticles when combined with CT-P-derived MTT, K-trans, or PS produced the strongest statistical models ($p < 0.01$). No paired combination of CT-P specific parameters was found to have an effect on tumor response type. PET (SUV) was found to a produce a marginally significant model ($p = 0.057$) only when combined with CT-P derived K-trans. No combination of either imaging or blood marker parameters with the MR-derived ADCmin or ADCmean were identified that could produce a statistically significant model.

Table 1 Classification of "Responders" and "Non-responders" as per the Modified Ryan Score

Modified Ryan Score	Pathologic description
Responders	
No residual cancer cells (TRG-1); complete regression	No viable cancer cells or single cells, or small groups of cancer cells
Rare residual cancer cells; near complete regression (TRG-2)	Single cells or rare small groups of cancer cells
Non-responders	
Predominant fibrosis with increased number of residual cancer cells (TRG-3)	Residual cancer with evident tumor regression/fibrosis but more than single cells or rare small groups of cancer cells
Significant fibrosis outgrown by cancer, or no fibrosis with extensive residual cancer (TRG-4)	Extensive residual cancer with no evident tumor regression

Table 2 Patient characteristics ($n = 17$)

Characteristic	Number of patients
Sex	
Male	10
Female	7
Median age (range)	57 (40–78) years
Average length of time between completion of neoadjuvant therapy and surgery	61.9 days
Clinical stage (American Joint Committee on Cancer, 8th edition)	
I	2
II/IIA	5
IIIA/IIIB/IIIC	10
Pathologic stage	
I	4
IIA	7
IIIA/IIIB/IIIC	5
IVA	1
Responders	3
Non-responders	14

Table 3 Summary of patient dataset and results of univariate logistic regression for each parameter

Modality parameter	*N*	Model Sig. (*p*)	Classification accuracy (%)
CT-perfusion			
Blood flow (BF)	17	0.958	82.4
Blood volume (BV)	17	0.694	82.4
Mean transit time (MTT)	17	0.182	76.5
Permeability-surface area product (PS)	15	0.222	86.7
Extraction efficiency (E)	15	0.581	80.0
Endothelial transfer constant (K-trans)	15	0.358	80.0
MR			
Minimum apparent diffusion coefficient (ADC_{min})	17	0.687	82.4
Mean apparent diffusion coefficient (ADC_{mean})	17	0.539	82.4
PET			
Standardized uptake value (SUV)	17	0.084	82.4
Liquid biopsy			
Total CTCs, cell fragments and MPs	12	0.113	75.0

Table 4 Multi-parameter model results and classification accuracy using paired combinations of those parameters identified in the multivariate analysis

Model parameters	*N*	Model Sig. (*p*)	Classification accuracy (%)	Sensitivity	Specificity	Non-responders (*N*)
CTCs/MPs/cell fragments; MTT	12	0.004	100	1.00	1.00	10
CTCs/MPs/cell fragments; PS	10	0.007	80	0.00	1.00	8
CTCs/MPs/cell fragments; K-trans	10	0.007	100	1.00	1.00	8
SUV, K-trans	15	0.057	86.7	0.67	0.92	13

Only those models with $p < 0.05$ are shown

Discussion

In patients with locally advanced rectal cancer, neoadjuvant therapy has become the standard of care for downstaging prior to surgery. It is also employed in select cases of "watchful waiting," whereby disease status is monitored with close clinical imaging and endoscopic follow-up, rather than being surgically resected. As a result, response assessment has become an increasingly important question for surgeons and medical oncologists and is often evaluated with imaging. It is therefore important for practicing radiologists to have a thorough understanding of the utility and limitations of imaging modalities in the assessment of treatment response.

Rectal MRI is widely utilized in most centers because of its relatively widespread availability and proven effectiveness in primary staging, as evidenced by the MERCURY Study, which reported 88% accuracy in predicting a clear surgical margin [17]. In the setting of post-CRT response assessment, however, the accuracy of MRI is debatable. In a study of 215 patients, Van den Broek et al. reported frequent interobserver variability and overstaging at post-CRT MRI; furthermore, MRI could not differentiate between good responders and poor responders [18].

CT-P is a robust functional imaging technique that may be easily incorporated into existing imaging protocols, as is the current status for stroke imaging and other indications. CT-P allows clinicians to combine both anatomical and vasculature assessments [19]. Similar to the role of MRI in rectal cancer response assessment, however, CT-P-derived parameters for CRT response assessment are still disputed, and in recent studies, have been unable to definitively classify responders and non-responders to CRT [20, 21]. Although the integration of anatomic and functional imaging available in modern PET-CT scanners would logically be a powerful tool for evaluating the physiological tumor response to therapy, several studies have shown mixed results when correlating SUV to TRG in the setting of response assessment[22–24].

To our knowledge, this is the first prospective study investigating the combined role of multimodality imaging and liquid biopsy (via total number of tumor-specific blood markers including CTCs, MPs, and cell fragments) in predicting treatment response in patients with rectal cancer following neoadjuvant therapy.

Liquid biopsy is a relatively novel concept in colorectal carcinoma. Both CTCs and MPs have been used as indicators of prognostic features and treatment monitoring in patients with early-stage and metastatic cancers [25, 26]. In addition to CTCs, which were employed in our study, isolation of circulating tumor DNA and circulating free DNA (ctDNA/cfDNA) in the peripheral blood has also been shown as a promising alternative for therapeutic monitoring in colorectal carcinoma [27]. In our preliminary, hypothesis-generating study, the use of CTCs/MPs was preferred over ctDNA, as substantial tumor bulk and robust samples are required for sequencing of ctDNA in the peripheral blood, in order to accurately detect genetic mutations. These requirements were not considered to be within the scope of this study, and therefore preference was given to the FDA-approved CellSearch system for CTC enumeration.

Our study found that the use of CT-P-derived parameters alone was not sufficient to predict tumor response in either univariate or multivariate analyses. Previous studies [20, 21] evaluating CT-P-derived parameters in the neoadjuvant setting observed decreases in BF, BV, and PS following neoadjuvant CRT, while MTT increased. Concordant with these findings, we observed a decline in PS by 20% and an increase in tumor MTT by 25% in at least one responder following treatment. In the other responders, however, increases in both MTT and PS were observed, as indicated in Fig. 1. Although this is a relative contradiction which could be attributed to the relatively small number of responders in this hypothesis-generating study, neither of the previous two quoted studies were able to accurately differentiate responders from non-responders to therapy. In our study, the observed increase in tumor PS for both responders might also be indicative of a hyperemic and endothelial response to radiation therapy as reported by Harvey et al. [28]. Of the non-responders in our study, a significant increase in tumor MTT was observed following treatment ($p = 0.001$), similar to those findings reported by Curvo-Semedo et al. [20]. No appreciable change in tumor PS ($p > 0.05$) was observed in non-responders following treatment, as has previously been observed by Bellomi et al. [29]. Similar to both of these studies, neither tumor MTT nor PS were able to accurately distinguish responders from non-responders. These findings highlight the limitations of using a univariate approach to predicting tumor response using CT-P-derived parameters alone.

Our results also demonstrated that imaging biomarkers including PET (SUV) and MR (mean and min ADC) did not provide additional information to improve assessment of treatment response. In a study of patients with locally advanced rectal cancer, Aiba et al. examined the value of MRI and FDG-PET/CT in response assessment [30]. Interestingly, they also found that although the relative change in SUV was informative, the addition of FDG-PET/CT to MRI did not improve overall accuracy in the assessment of pathological response.

In our study, blood markers (CTCs, MPs, cell fragments) added useful information in the setting of response assessment, specifically when combined with CT-P-derived measures of permeability (PS and K-trans) and MTT. When

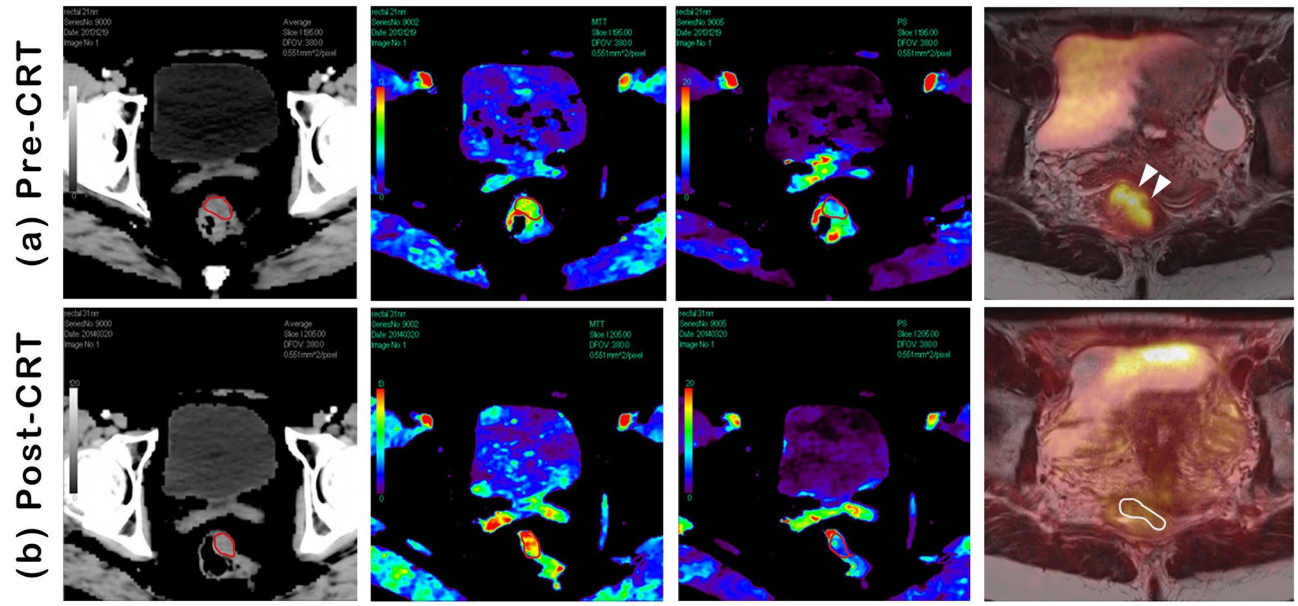

Fig. 1 Multimodality assessment of tumor response at CT-P and PET-MRI, in a partial responder with mrT3bN3 rectal carcinoma. The tumor is outlined or indicated with arrows, both prior to (**a**) and following (**b**) neoadjuvant CRT, as follows (from left to right): CT-perfusion average, MTT, PS, and PET-MR fusion image. Following CRT, marginal increases in both MTT and PS are observed at the tumor bed. SUV decreases from 15.9 to 3.0 following CRT, and there is reduced FDG uptake in the tumor bed on post-CRT PET-MR. Final stage was ypT2N0 (partial response)

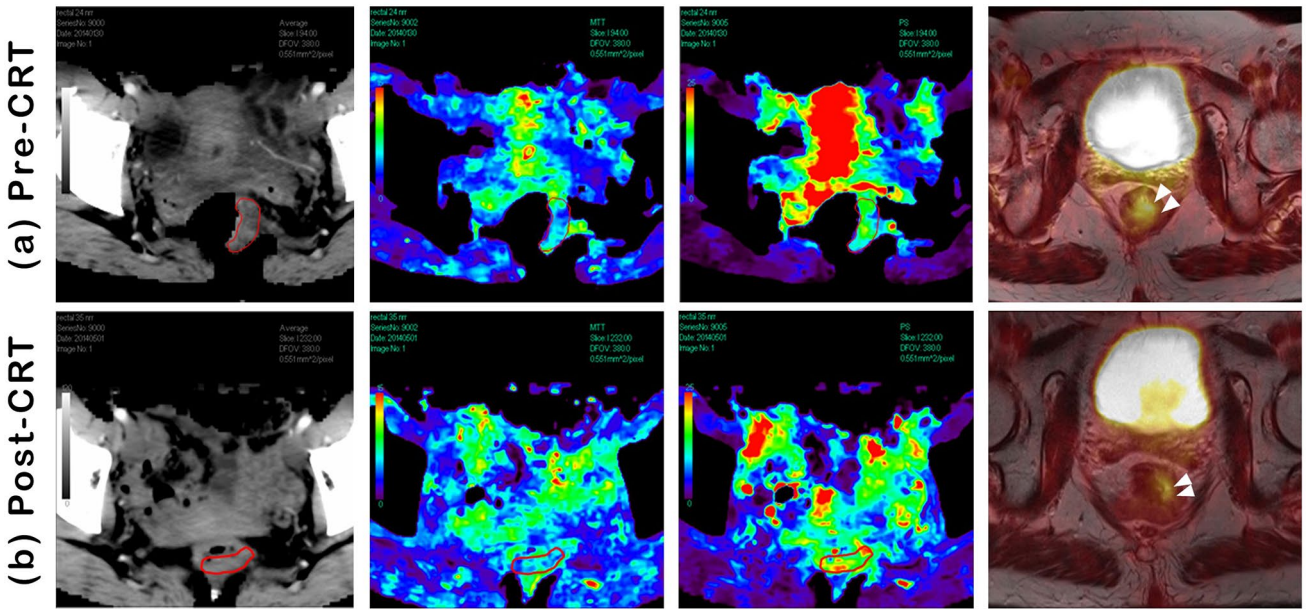

Fig. 2 Multimodality assessment of tumor response at CT-P and PET-MRI, in a non-responder with mrT2N1 rectal carcinoma. The tumor is outlined or indicated with arrows, prior to (**a**) and following (**b**) neoadjuvant CRT, as follows (from left to right): CT-perfusion average, MTT, PS, and PET-MR fusion image. Following neoadjuvant CRT, evaluation of the tumor bed reveals marked increase in MTT relative to baseline, and reduced PS. There is a mild decrease in SUV from 6.1 pre-CRT to 4.7 post CRT. Final stage was ypT2N0 (no response)

combined, these bivariate models each produced significant models ($p < 0.01$) for classifying tumor response to therapy as assessed by TRG (Table 4). This is particularly notable given the observed variability in tumor PS observed in our study's responders. Our results suggest that blood markers such as extracellular vesicles (EVs), including cancer microparticles and cell fragments, may play an important role in stratifying the spatial and functional heterogeneity of tumor vasculature noted in CT-perfusion, as observed in Figs. 1 and 2. When combined with MRI features (mean and min ADC), or PET (SUV), EVs did not produce accurate models ($p > 0.05$) for predicting tumor response similar to results reported by Aiba et al. [30]. In our study, a statistically significant model for classifying responders and non-responders could only be produced with blood-sampled EVs such as CTCs/MPs/cell fragments in combination with CT-P-derived tumor permeability and MTT, as shown in Fig. 3. These results suggest that the use of a liquid biopsy could possibly provide a more robust measure of treatment response and should be explored further.

Of the imaging and blood markers investigated independently in this study for response assessment to CRT, tumor SUV ($p = 0.084$), combined blood markers ($p = 0.113$), and tumor MTT ($p = 0.182$) were the most accurate, albeit not

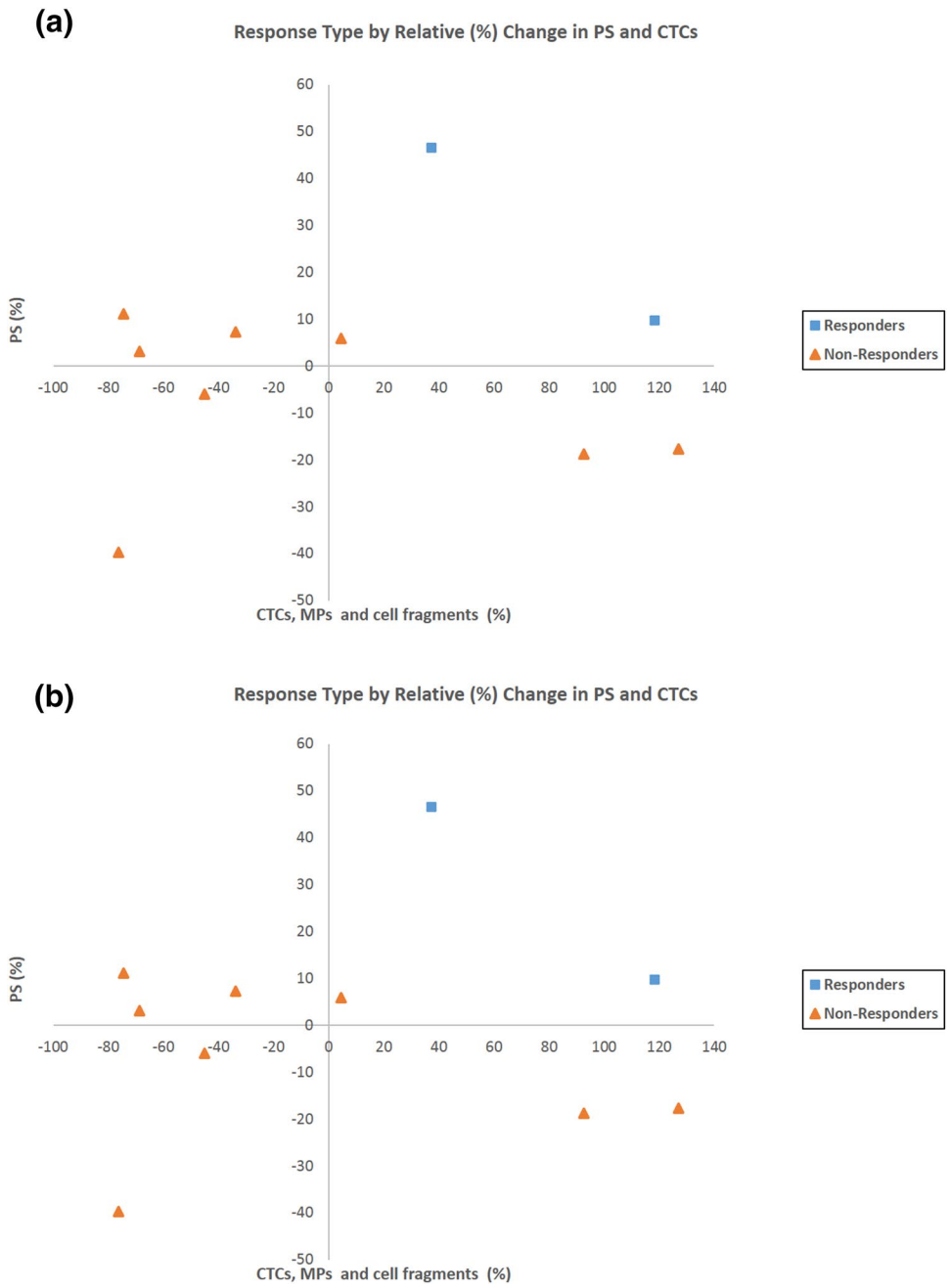

Fig. 3 Plots of the relative change from baseline for both MTT (**a**) and PS (**b**) together with the relative change in total CTCs, microparticles, and cell fragments for both responders and non-responders

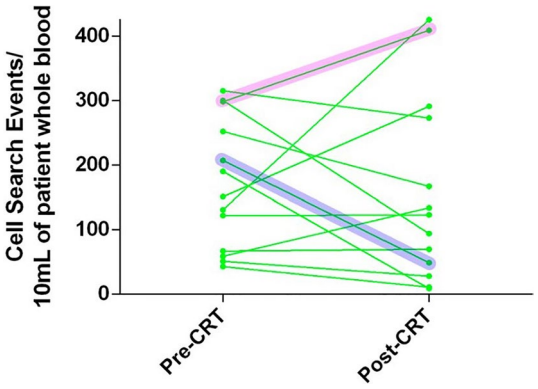

Fig. 4 CellSearch imaging flow cytometry results for whole blood samples from patients prior and post CRT. One of the three classified responders did not receive a liquid biopsy. A partial responder is indicated in pink; non-responder events are highlighted in green and violet. CT-perfusion and PET-MR imaging for the non-responder (violet) and partial responder (pink) are provided in Figs. 2, 3 for comparison. In isolation, the role of CTCs may be limited. In our cohort, the addition of CT-perfusion increased accuracy in prediction of a therapeutic response (see Figs. 2, 3)

statistically significant. These results align with recently reported findings of the strong prognostic value of both CTCs [31] and PET SUV [32] in CRC. In previous studies, significant changes in MTT have been observed following treatment [27], and CTCs have shown utility as potential indicators for resistance to neoadjuvant therapy [25]. A prospective study of 97 patients with rectal carcinoma found that the addition of PET-CT to the primary staging algorithm altered the treatment regimen in 14.4% of patients [33].

In isolation, the persistence of post-therapeutic CTCs and microparticles could be considered an indicator for resistance to the treatment regimen [34]. Interestingly, however, in our study, the total number of CTCs/MPs/cell fragments in the responders with liquid biopsy data was found to increase by as much as 37% and 118%. In a group of 48 patients with advanced CRC, Krebs et al. showed similar findings, with clinically relevant improvement in median overall survival in patients with high CTCs [35]. Furthermore, decreases in the total blood markers were observed for some non-responders, as shown in Fig. 4, opposite to that of the responders. These findings suggest that the relationship between CTCs/MPs/cell fragments and disease status may be more complex than a simple linear correlation. To our knowledge, there are no studies that demonstrate a positive and linear relationship between cancer EV counts in the blood and tumor burden. In our study, statistically significant models of tumor response assessment were obtained only when liquid biopsy findings (observed changes in total blood CTCs/MPs/cell fragments) were combined with CT-P derived measures of whole-tumor MTT, PS, or K-trans. This suggests that further investigation with larger sample sizes may provide additional information regarding the use of these parameters for the purposes of tumor response assessments by TRG.

The primary purpose of conducting univariate analysis was to identify and exclude those parameters with little predictive power ($p > 0.5$) from the multivariate models, to reduce the likelihood of overfitting the patient dataset. The number of predictors was also restricted to two for each model, to maintain a conservative ratio of predictors to outcomes.

Major limitations of our study include the small number of patients that completed all the imaging studies and blood sampling prior to and following CRT, and the low number of responders compared to non-responders. As a result, no correction was made for multiple comparisons in the regression models. This underscores the challenges associated with the retention of patients in multimodality, temporal studies, and limits the scope and interpretation of these results solely for the purposes of hypothesis generation.

Even with these limitations, it is significant that two-parameter models consisting of both blood markers and CT-P-derived measures of tumor MTT, PS, or K-trans were able to accurately separate responders from non-responders. Previous studies involving the use of MRI, CT-perfusion, and PET-CT have so far shown mixed results in accurately assessing therapeutic response in locally advanced rectal cancer.

Methods of response assessment in this group of patients will continue to play a pivotal role in refining treatment strategies that may become more personalized, ranging from "watchful waiting" to surgical resection. Our results indicate that liquid biopsy may contribute to improved accuracy of therapeutic response assessment, when combined with CT-P-derived measures of whole-tumor permeability (K-trans, PS) and MTT. Due to the limited sample size of this hypothesis-generating study, additional study with a larger sample size is warranted, to further evaluate the role these blood markers may have in conjunction with diagnostic imaging studies.

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