European Journal of Nuclear Medicine and Molecular Imaging (2019) 46:1713–1722
https://doi.org/10.1007/s00259-019-04330-7

ORIGINAL ARTICLE

3′-Deoxy-3′-^{18}F-Fluorothymidine and ^{18}F-Fluorodeoxyglucose positron emission tomography for the early prediction of response to Regorafenib in patients with metastatic colorectal cancer refractory to all standard therapies

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Received: 1 December 2018 / Accepted: 2 April 2019 / Published online: 30 April 2019

Abstract

Purpose The purpose of this study was to evaluate the value of 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT) for early prediction of standard anatomic response and survival outcomes in patients with metastatic colorectal cancer (mCRC) receiving Regorafenib.

Methods Sixty-eight patients with mCRC refractory to standard cytotoxic chemotherapy were enrolled and received Regorafenib (160 mg/day on days 1–21, following a 7-day break). Standard anatomical response was evaluated every 8 weeks. Both scans were performed before and on day 21 of Regorafenib.

Results Of the 61 patients included in per-protocol analysis, complete response was not observed, but partial response was observed in 8.2% ($n = 5$), stable disease in 67.2% ($n = 41$), and progressive disease in 24.6% ($n = 15$). The objective response rate was 8.2% and disease control rate 75.4%. Five responders (8.2%) and 13 non-responders (21.3%) met the CT and ^{18}F-FLT PET/CT criteria (maximum standardized uptake value decrease ≥ 10.6% for responders). Forty-three (70.5%) exhibited discordant responses on CT and ^{18}F-FLT PET/CT (McNemar test, $P < 0.001$). At a median follow-up of 8.9 months, median progression-free survival (PFS) and median overall survival (OS) were 3.6 months (95% confidence interval [CI], 3.34–3.80 months) and 8.5 months (95% CI, 6.95–10.10 months), respectively. Comparison of PFS and OS according to ^{18}F-FLT PET/CT response revealed slightly longer PFS ($P = 0.015$) in responders, but the correlation with OS was not significant. The PET Response Criteria in Solid Tumours (PERCIST) of ^{18}F-FDG PET/CT revealed differences in PFS and OS between partial metabolic response (PMR) and non-PMR ($P = 0.048$ and $P = 0.014$, respectively), and between progressive metabolic disease (PMD) and non-PMD ($P = 0.189$ and $P = 0.007$, respectively).

Conclusions Survival outcome was significantly associated with PERCIST using ^{18}F-FDG PET/CT but the change of ^{18}F-FLT uptake was only slightly associated with PFS. ^{18}F-FDG PET/CT can be used as imaging biomarker to predict clinical outcomes early in patients with mCRC receiving Regorafenib.

Keywords ^{18}F-fluorothymidine · ^{18}F-fluorodeoxyglucose · Positron emission tomography · Regorafenib · Metastatic colorectal cancer

Jeong Eun Kim, Sun Young Chae, and Jwa Hoon Kim contributed equally to this study. The authors are not in training.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00259-019-04330-7) contains supplementary material, which is available to authorized users.

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Introduction

Recent advances have been made in the treatment of patients with metastatic colorectal cancer (mCRC) owing to the introduction of targeted agents, including bevacizumab, cetuximab, panitumumab, and aflibercept [1–7]. Regorafenib is an orally available multikinase inhibitor with activity against multiple targets, including factors involved in tumour angiogenesis (VEGFR-1, VEGFR-2, VEGFR-3, and TIE-2), oncogenesis (KIT, RET, RAF-1, BRAF, and $BRAF^{V600E}$), and the tumour microenvironment (PDGF and FGFR). Previous studies reported the anti-tumour effects of Regorafenib in various solid tumours [8, 9], with advanced gastrointestinal stromal tumours and colorectal cancers being representatives.

The CORRECT study, which compared the effects of Regorafenib vs placebo in patients with mCRC previously treated with all standard treatments, showed survival improvements with statistical significance [8]. The efficacy of Regorafenib vs placebo was confirmed in Asian patients with mCRC (median overall survival [OS] 8.8 vs 6.3 months; hazard ratio [HR], 0.55; 95% confidence interval [CI], 0.4–0.77; $P < 0.001$) [10]. Despite the widespread use of Regorafenib in patients with mCRC, no compelling biomarkers are available to date for predicting treatment responses to Regorafenib. The difficulties in identifying biomarkers for Regorafenib may be attributable to it being a multikinase inhibitor with many potential targets. Given these reasons, imaging modalities can be fascinating alternative candidates as predictive biomarkers of treatment response. Conventional anatomic imaging modalities such as computed tomography (CT) can hardly be used for the early prediction of treatment response, and the Response Evaluation Criteria in Solid Tumours (RECIST) of CT, which is widely used for measuring treatment response, might have several limitations in measuring the efficacy of targeted agents, such as the induction of cystic necrosis without tumour shrinkage [11–13]. In the CORRECT study, the overall response rate according to the RECIST was only 1%, even though the rate for disease stabilization was up to 40% [9]. This might be a good example for the limitations of the RECIST of conventional anatomic imaging for evaluating response to Regorafenib.

Among imaging modalities, positron emission tomography (PET)/CT is a useful tool for the non-invasive measurement of functional changes after treatment with targeted agents, and the most commonly used PET tracer is ^{18}F-fluorodeoxyglucose (^{18}F-FDG) because the degree of ^{18}F-FDG uptake on PET reflects the level of tumour glucose metabolism [14, 15]. However, ^{18}F-FDG PET/CT has some limitations in the presence of ^{18}F-FDG-avid treatment-induced inflammation around tumours [16]. 3′-Deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) PET/CT is a potentially useful tool for the earlier prediction of treatment responses because it can detect earlier changes in cellular proliferation by using ^{18}F-FLT, a radio-traceable substitute for thymidine which is essential for DNA synthesis. Several studies have reported that ^{18}F-FLT PET/CT may allow an early assessment of the response to chemotherapy including targeted agents [17–23]. Our previous study showed that ^{18}F-FLT PET/CT could act as an earlier predictive marker of treatment response in patients with mCRC treated using cytotoxic chemotherapy (oxaliplatin, 5-fluorouracil, and leucovorin [FOLFOX]) [24], thereby demonstrating that the earlier prediction of response using ^{18}F-FLT PET/CT is also feasible in patients with mCRC.

We hypothesised that ^{18}F-FLT PET/CT and/or ^{18}F-FDG PET/CT could be useful for identifying a subgroup of patients with mCRC with clinical responsiveness to Regorafenib. Therefore, we conducted an exploratory study to assess the value of ^{18}F-FLT PET/CT and ^{18}F-FDG PET/CT for the early prediction of standard anatomic response and survival outcomes in patients with mCRC receiving Regorafenib.

Materials and methods

Study design and patients

This was a prospective, single-centre, open-labelled phase II, imaging biomarker study to evaluate the feasibility of ^{18}F-FLT and ^{18}F-FDG PET/CT as potential candidates for use as predictive imaging biomarkers of Regorafenib treatment in patients with mCRC who had experienced progression after receiving standard therapy. The primary objective was to investigate the earlier prediction of treatment response by using ^{18}F-FLT PET/CT at baseline and day 21 following Regorafenib treatment and to demonstrate whether the maximum standardized uptake value (SUVmax) changes on ^{18}F-FLT PET/CT could be predictive of disease control rates at 8 weeks by using the RECIST version 1.1. The secondary objectives were to compare survival outcomes between responders and non-responders by using ^{18}F-FLT PET/CT and ^{18}F-FDG PET/CT and the safety of ^{18}F-FLT. This study was conducted in accordance with the Helsinki Declaration. All patients provided written informed consent prior to participation. The Institutional Review Board of Asan Medical Center approved the study protocol. This trial was registered on http://www.clinicaltrials.gov with the identifier NCT02175095.

Patients with mCRC who failed to respond to cytotoxic chemotherapy with three active agents (fluoropyrimidines, oxaliplatin, and irinotecan) with or without targeted agents (bevacizumab or cetuximab) were enrolled. Patients were included if they had histologically or cytologically confirmed adenocarcinoma of the colon and rectum that was not

amenable to surgery or radiation therapy of curative intent, with ≥1 measurable extrahepatic lesion(s) according to the RECIST, an Eastern Cooperative Oncology Group performance status of 0–1, life expectancy ≥3 months, no prior chemotherapy in a metastatic setting, and adequate haematologic, hepatic, and renal functions. Patients with liver-limited metastasis were excluded. Prior radiotherapy was permitted if it was not administered to the target lesions selected for this study and had been completed ≥4 weeks before registration. Patients were recruited via referral by the investigators.

Study treatment and radiologic response evaluation

Regorafenib was administered orally at a dose of 160 mg/day on days 1 to 21 following a 7-day break, with each cycle lasting 4 weeks. Treatment was repeated every 4 weeks and continued until disease progression, unacceptable toxicity, or patient refusal. Standard anatomic response evaluation was performed on CT scans according to the RECIST version 1.1 [25] every 8 weeks (irrespective of the cycles or schedules of chemotherapy). ^{18}F-FLT and ^{18}F-FDG PET/CT were performed before and on the 21st day of Regorafenib administration.

Procedures for ^{18}F-FLT and ^{18}F-FDG PET/CT

^{18}F-FLT was synthesized as previously described [26]. Prior to ^{18}F-FDG injection, patients were asked to avoid strenuous exercise for 24 h to minimize muscle uptake, and fasted for at least 6 h. The injected dose for each patient scan was 2.6 MBq/kg of ^{18}F-FLT and 5.2 MBq/kg of ^{18}F-FDG. ^{18}F-FLT PET/CT and ^{18}F-FDG PET/CT were performed before and on the 21st day of Regorafenib administration. Each patient underwent both ^{18}F-FLT and ^{18}F-FDG PET/CT scans on two separate days (1 day apart). ^{18}F-FLT and ^{18}F-FDG PET/CT images were acquired for 2 min per each bed 60 min after the intravenous injection of the radiotracer by using a PET/CT scanner (Discovery PET/CT 690, Discovery PET/CT 690 Elite, or Discovery PET/CT 710; GE Healthcare, Milwaukee, WI, USA). For attenuation correction and lesion localization, a low-dose CT (100 kVp; 35 mA) from the skull base to the mid-thigh was performed. PET images were reconstructed using VPFXS reconstruction with four iterations and 18 subsets. No correction for partial volume effects was performed.

^{18}F-FLT and ^{18}F-FDG PET/CT studies were assessed by the consensus of two experienced nuclear medicine physicians who were blinded to the clinical outcome results. For quantitative image analysis, a volume of interest was drawn on all lesions by using the vendor's software (Advantage Workstation 4.6 software; GE Healthcare, Milwaukee, WI, USA).

PET response of ^{18}F-FLT was assessed using the SUVmax. The SUV values were normalized to the injected dose and the patient's body weight. The single hottest SUVmax from the extrahepatic target lesions was measured on each ^{18}F-FLT PET/CT scan. The percentage of change of SUVmax in the target lesion was calculated as follows: 100 ×(SUVmax day 21−SUVmax baseline)/SUVmax baseline. The non-responders on ^{18}F-FLT PET/CT were defined as those with decreased SUVmax <10.6% or new lesions on a follow-up scan.

Response assessment using ^{18}F-FDG PET/CT was based on the PERCIST 1.0 [27, 28]. The peak SUV value corrected for lean body mass (SULpeak) was measured from the single hottest tumour at each time point. For a tumour to be measurable at baseline, the SULpeak in the target lesion was greater than or equal to 1.5 times the mean SUL in the 3-cm-diameter spherical volume of interest plus two times its standard deviation of the liver. The percentage of change in SULpeak in the measurable target lesion was computed as follows: 100×(SULpeak day 21−SULpeak baseline)/SULpeak baseline. When a non-target lesion showed different responses from the measurable target lesion, we used the overall response considering both the target and non-target responses as previously described [28].

Statistical analysis

In our previous study [24], which demonstrated the predictive role of ^{18}F-FLT PET/CT in patents with mCRC treated with FOLFOX, the SUVmax decreased from baseline with statistical significance in the responders on CT. However, the SUVmax was unchanged in the non-responders on CT, when a threshold of SUVmax decrease ≥10.6% resulted in a sensitivity of 100%, specificity of 76.9%, and relative risk of 2.667. We hypothesized that the percentage of responders on ^{18}F-FLT PET/CT but non-responders on CT was less than 17%; inversely, the percentage of non-responders on ^{18}F-FLT PET/CT but responders on CT was less than 1%. Accordingly, a sample size of 55 would achieve 81% power to detect an odds ratio of 17 by using a two-sided McNemar test with a significance level of 0.05 [29]. Assuming 10% rates for ineligibility (to the study treatment) and additional 10% rates for unavailability in terms of comparisons between the results of ^{18}F-FLT PET/CT and CT scans, a total of 68 patients were planned for inclusion in this study.

Descriptive statistical data were reported as median values with ranges or numbers (proportions) unless otherwise specified. Comparison of quantitative parameters was conducted using the t-test. The McNemar test was used to determine the agreement between the responses to CT and ^{18}F-FLT PET/CT. We estimated the proportion of patients with ^{18}F-FDG and ^{18}F-FLT PET/CT responses after 21 days of Regorafenib treatment and compared the response rates of

PET with the response rate of CT RECIST at 8 weeks by per-protocol analysis. The objective response rate was defined as the proportion of patients with complete response (CR) or partial response (PR). Disease control rate was defined as the proportion of patients evaluated as having a CR, PR, or stable disease (SD).

Overall survival (OS; time to death), progression-free survival (PFS; time to progression or death), and 95% CIs were assessed using the Kaplan-Meier method. A P value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) and Prism version 4.00 (GraphPad, San Diego, CA, USA).

Results

Patients, treatment, and survival

Between July 2014 and June 2016, we enrolled 68 patients with mCRC who showed progression after all standard therapies, and initiated Regorafenib administration (160 mg/day) at Asan Medical Center in Seoul, Republic of Korea. No patients declined participation after receiving the ^{18}F-FLT injection. The study scheme is shown in Fig. 1, and patient characteristics are listed in Table 1. The median number of cycles of Regorafenib administered was 4 (range, 1–28 cycles), and the mean relative dose intensity was 90.1% (95% CI, 86.9–93.3). The most common reason for treatment discontinuation was disease progression (60/68, 88.2%).

The objective response rate and disease control rate with Regorafenib were 8.2% and 75.4%, respectively, in 61 patients by per-protocol analysis. CR was not achieved in this study, but PR was observed in 8.2% (n = 5), SD in 67.2% (n = 41), and progressive disease (PD) in 24.6% (n = 15) (Table 2). At a median follow-up of 8.9 months, the median PFS and median OS were 3.6 months (95% CI, 3.34–3.80 months) and 8.5 months (95% CI, 6.95–10.10 months), respectively (Online Resource 1).

Assessment of early response by using ^{18}F-FLT and ^{18}F-FDG PET/CT on day 21 of Regorafenib

Both ^{18}F-FLT and ^{18}F-FDG PET/CT scans were performed in 64 patients; among the remaining four patients, one withdrew consent and three discontinued Regorafenib treatment during the first cycle because of toxicities. With the exception of three patients who had no follow-up CT (n = 2) or no uptake of ^{18}F-FLT (n = 1), 61 were evaluable for the treatment response on both CT and PET/CT in final. There were no violations of the planned protocol. The ^{18}F-FLT PET/CT studies were well tolerated by all patients, completed without any problems, and performed as planned, without any adverse events.

The median baseline value of SUVmax for the representative metastatic lesion was 5.2 (range, 1.2–18.6), and ^{18}F-FLT uptake decreased on day 21 (3.6; range, 0.6–12.3; P < 0.001) in 61 patients. The decreased percent change of SUVmax on ^{18}F-FLT PET/CT is shown in Fig. 2a. The percent changes on

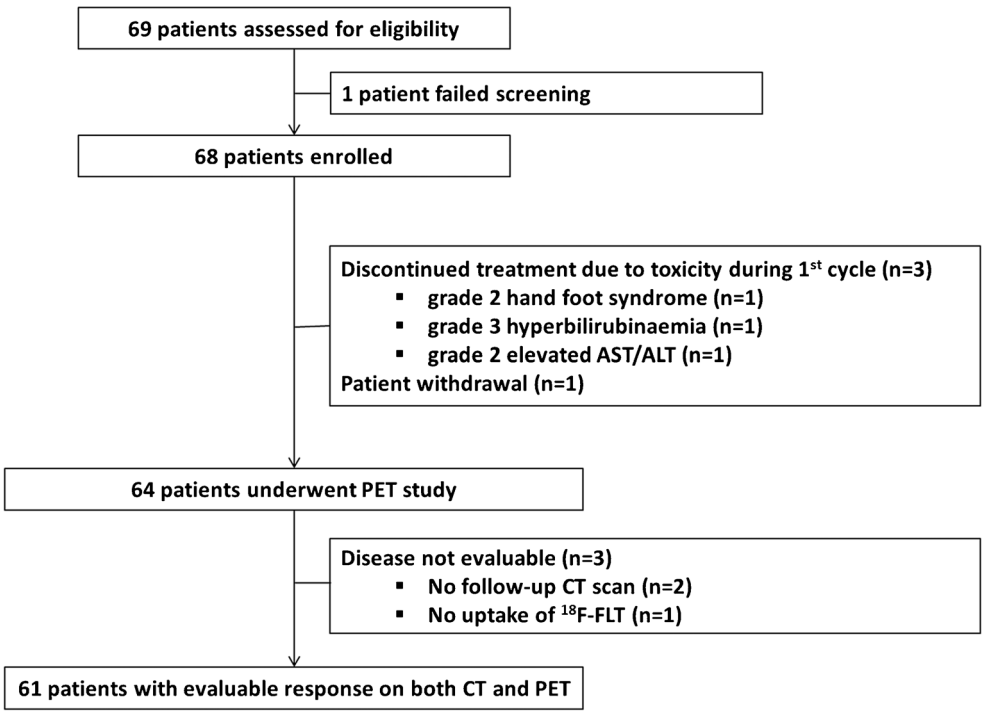

Fig. 1 Scheme for this study

Table 1 Baseline characteristics of patients with metastatic colorectal cancer receiving Regorafenib

Variable		Number, *n* (%)
Age	Median (range)	58 (26–72)
	<65	59 (86.7)
	≥65	9 (13.3)
Sex	Male	42 (61.8)
	Female	26 (38.2)
ECOG PS	0–1	68 (100.0)
Primary tumour	Right colon	11 (16.2)
	Left colon	25 (36.8)
	Rectum	32 (47.1)
Histology	Well differentiated	7 (10.3)
	Moderately differentiated	52 (76.5)
	Poorly differentiated	6 (8.8)
	Mucinous differentiated	3 (4.4)
Status	Initially metastatic	39 (57.4)
	Recurrence with metastasis	29 (42.6)
Sites of metastasis	Liver	28 (41.2)
	Lung	57 (83.8)
	Lymph node, abdomen	29 (42.6)
	Peritoneum/omentum	11 (16.2)
	Bone	7 (10.3)
	Ovary	2 (16.2)
RAS status	Wild	34 (50.0)
	Mutant	31 (45.6)
	Unknown	3 (4.4)
BRAF status	Wild	55 (80.9)
	Mutant	4 (5.9)
	Unknown	9 (13.2)
Previous targeted agents	None	21 (30.9)
	Any	47 (69.1)
	Anti-VEGF but not anti-EGFR	28 (41.2)
	Anti-EGFR but not anti-VEGF	9 (13.2)
	Anti-VEGF and anti-EGFR	10 (14.7)
Previous chemotherapy	2	24 (35.3)
	3	21 (30.9)
	≥4	23 (33.8)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor

day 21 were significantly higher in the responders than in the non-responders ($P = 0.023$). The percent change of SULpeak on ^{18}F-FDG PET/CT is shown in Fig. 2b. The SULpeak on ^{18}F-FDG PET/CT showed a greater decreasing trend in the responders than in the non-responders ($P = 0.057$).

All the non-responders on ^{18}F-FLT PET/CT ($n = 13$, 21.3%) met the criteria for non-responders on CT, indicating a negative predictive value of 100% (Table 3; Fig. 3). However, there were two discordant pairs of responses on CT and ^{18}F-FLT PET/CT. Responders on ^{18}F-FLT PET/CT but determined as non-responders on CT were 43 (70.5%). Response assessment by using ^{18}F-FLT PET/CT on day 21 was significantly different compared with that by using CT at 8 weeks ($P < 0.001$).

According to the PERCIST using ^{18}F-FDG PET/CT, 45.9% ($n = 28$) showed a partial metabolic response (PMR), 39.3% ($n = 24$) stable metabolic disease, and 14.8% ($n = 9$) progressive metabolic disease (PMD) (Table 2).

Survival outcomes according to ^{18}F-FLT and ^{18}F-FDG PET/CT response on day 21 of Regorafenib

The PFS and OS were compared according to the percent change of SUVmax of ^{18}F-FLT PET/CT on day 21. The median PFS was 3.9 months (95% CI, 1.93–5.87 months) and 3.4 months (95% CI, 2.72–4.16 months) for patients showing a decrease of SUVmax ≥10.6% (responders on ^{18}F-FLT PET/CT) and non-responders on ^{18}F-FLT PET/CT, respectively ($P = 0.015$) (Figs. 3 and 4a). According to concordant pairs of responses on day 21 ^{18}F-FLT PET/CT and 8 weeks CT (Table 3), the median PFS was 9.0 months (95% CI, 2.44–15.53 months, $n = 5$) for responders and 3.4 months (95% CI, 2.72–4.16, $n = 13$) for non-responders, respectively ($P = 0.007$). The median OS was 10.6 months (95% CI, 7.33–13.85 months) for the responders on ^{18}F-FLT PET/CT and 7.0 months (95% CI, 4.45–9.45) for the non-responders ($P = 0.205$) (Figs. 3 and 4b).

In a subgroup of patients with only extrahepatic metastasis ($n = 37$), similar trends were observed in the PFS and OS according to the percent change of SUVmax of ^{18}F-FLT PET/CT on day 21. The PFS was significantly longer in responders on ^{18}F-FLT PET/CT than in non-responders (5.9 months, 95% CI, 3.30–8.57 months vs 3.4 months, 95% CI, 2.94–3.94 months; $P < 0.001$) (Online Resource 2a). The OS did not show a significant difference based on the response on ^{18}F-FLT PET/CT (14 months, 95% CI, 6.34–21.66 months vs 8.0 months, 95% CI, 0.00–16.70 months; $P = 0.150$) (Online Resource 2b).

The PFS and OS were compared according to the PERCIST by using ^{18}F-FDG PET/CT scans. The PFS was 9.2 months (95% CI, 1.60–6.27 months) for the PMR group and 5.7 months (95% CI, 3.26–3.76 months) for the non-PMR group ($P = 0.048$) (Fig. 4c). The OS was 11.8 months (95% CI, 7.54–16.13 months) and 7.9 months (95% CI, 5.25–10.62 months) for the PMR and non-PMR groups, respectively ($P = 0.014$) (Fig. 4d). The PFS was 3.6 months (95% CI, 3.18–4.02 months) for the non-PMD groups and 1.8 months (95% CI, 0.00–3.79 months) for the PMD groups ($P = 0.189$) (Figs. 3 and 4e). The OS was 10.5 months (95% CI, 8.07–12.83 months) and 3.9 months (95% CI, 2.22–6.18 months) for the non-PMD and PMD groups, respectively ($P = 0.007$) (Figs. 3 and 4f).

Table 2 Clinical response to Regorafenib on CT and ^{18}F-FDG PET/CT

RECIST[a]		PERCIST[b]	
Partial response	5 (8.2%)	Partial metabolic response	28 (45.9%)
Stable disease	41 (67.2%)	Stable metabolic disease	24 (39.3%)
Progressive disease	15 (24.6%)	Progressive metabolic disease	9 (14.8%)

[a] Response Evaluation Criteria in Solid Tumours

[b] PET Response Criteria in Solid Tumours

Survival outcomes according to RECIST on CT at 8 weeks of Regorafenib

The PFS and OS were compared according to the disease response (PR vs. non-PR) or disease control (PD vs. non-PD) on RECIST using CT. The PFS was 8.9 months (95% CI, 2.43–15.53 months) for the PR group and 3.5 months (95% CI, 3.45–3.63 months) for the non-PR group ($P = 0.033$). The OS was 22.5 months (95% CI, 0.00–45.64 months) for the PR group and 9.2 months (95% CI, 7.47–11.01 months) for the non-PR group ($P = 0.245$). The PFS was 5.3 months (95% CI, 3.36–7.33 months) for the non-PD group and 1.6 months (95% CI, 1.33–1.88 months) for the PD group ($P < 0.001$). The OS was 10.7 months (95% CI, 8.00–13.37 months) for the non-PD group and 4.6 months (95% CI, 1.83–7.48 months) for the PD group ($P < 0.001$).

Discussion

This study evaluated the predictive role of ^{18}F-FLT and ^{18}F-FDG PET/CT for assessing clinical responsiveness to Regorafenib in patients with mCRC. The results showed that the decrease of SUVmax of ^{18}F-FLT uptake by ≥10.6% on day 21 of Regorafenib administration was related to a slightly longer PFS ($P = 0.015$) with no significant association in OS ($P = 0.205$). Non-responders on day 21 ^{18}F-FLT PET/CT all fell into the category of non-responders on CT at 8 weeks, indicating a high negative predictive value, and showed a shorter PFS than did the responders who met the criteria of both ^{18}F-FLT PET/CT and CT. In the PERCIST of ^{18}F-FDG PET/CT, patients with PMR had longer median PFS ($P = 0.048$) and median OS ($P = 0.014$) and there were differences between non-PMD and PMD groups ($P = 0.189$ and $P = 0.007$, respectively). Taken together, our findings confirmed

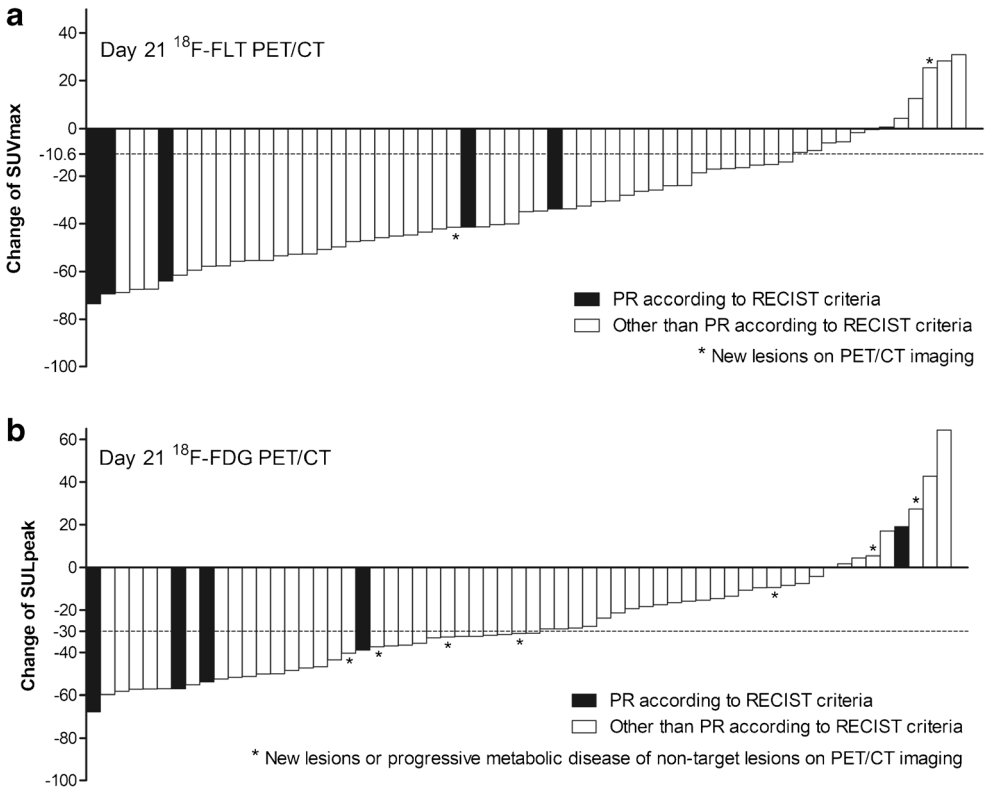

Fig. 2 Changes in (**a**) ^{18}F-FLT uptake (SUVmax) and (**b**) ^{18}F-FDG uptake (SULpeak) between baseline and on day 21 of Regorafenib administration in patients with metastatic colorectal cancer. (**a**) Five responders (8.2%) and 13 non-responders (21.3%) met the CT and ^{18}F-FLT PET/CT criteria. Forty-three patients (70.5%) exhibited discordant responses on CT and ^{18}F-FLT PET/CT (below *horizontal dotted line*; responders with decrease of SUVmax on day 21 ^{18}F-FLT PET/CT ≥ 10.6%, *black plot*; PR according to RECIST criteria). (b) Twenty-eight (45.9%) showed partial metabolic response (PMR), and non-progressive metabolic disease was observed in 52 (85.2%) (below *horizontal dotted line*; PMR with decrease of SULpeak on day 21 ^{18}F-FDG PET/CT ≥ 30%, *black plot*; PR according to RECIST criteria)

Table 3 Responders and non-responders on CT and ^{18}F-FLT PET/CT

61 evaluable patients	Responders according to RECIST	Non-responders according to RECIST
Responders on ^{18}F-FLT PET/CT	5 (8.2%)	43 (70.5%)
Non-responders on ^{18}F-FLT PET/CT	0 (0%)	13 (21.3%)

McNemar test, $P < 0.001$

the value of ^{18}F-FDG PET/CT in predicting survival in patients with mCRC receiving Regorafenib but showed the limited feasibility of ^{18}F-FLT PET/CT in predicting early response to Regorafenib.

Accumulating evidence suggests that ^{18}F-FLT PET/CT seems a good predictor of early response to systemic chemotherapy in various cancers [16]. Although the correlation with the OS is less consistent, the PFS correlates with ^{18}F-FLT uptake [16]. ^{18}F-FLT crosses the cell membrane and is phosphorylated by thymidine kinase 1 [30]. Thymidine kinase 1 is involved in the salvage pathway of thymidine synthesis, which is a part of DNA synthesis. ^{18}F-FLT uptake is related to tumour cell proliferation, and ^{18}F-FLT PET/CT could be used to evaluate early treatment response [30]. To date, the potential role of ^{18}F-FLT PET/CT has not been adequately investigated for the assessment of treatment response in patients with CRC. In a few clinical studies [24, 31–33], most patients were administered cytotoxic chemotherapy without currently preferred targeted agents. The ^{18}F-FLT uptake may also be associated with survival in other cancers treated using anti-EGFR tyrosine kinase inhibitors and anti-VEGF agents [16], and ^{18}F-FLT PET/CT predicted the response to BRAF inhibitors in a preclinical study [21]. In line with these investigations, we believe the present study is the first to evaluate the association of ^{18}F-FLT PET/CT with survival in mCRC treated using Regorafenib, although its clinical utility was not enough in terms of PFS and OS in this setting.

We found that ^{18}F-FDG PET/CT was an informative tool in predicting survival outcomes early in patients with mCRC receiving Regorafenib, and PERCIST using ^{18}F-FDG PET/CT on day 21 of Regorafenib was comparable to RECIST using CT at 8 weeks of Regorafenib. Considering the fact that most patients may be particularly vulnerable to further treatment due to prolonged exposure to chemotherapies and that issues regarding dose reduction and dose schedule have been continuously raised due to low tolerance for Regorafenib, early prediction of response to Regorafenib is essential to provide the precise treatment. Our results suggest that ^{18}F-FDG PET/CT at day 21 of Regorafenib, which may not be enough time to observe morphological change of tumours by anatomical image, could detect early metabolic response. Metabolic response assessment by ^{18}F-FDG PET/CT on day 21 may

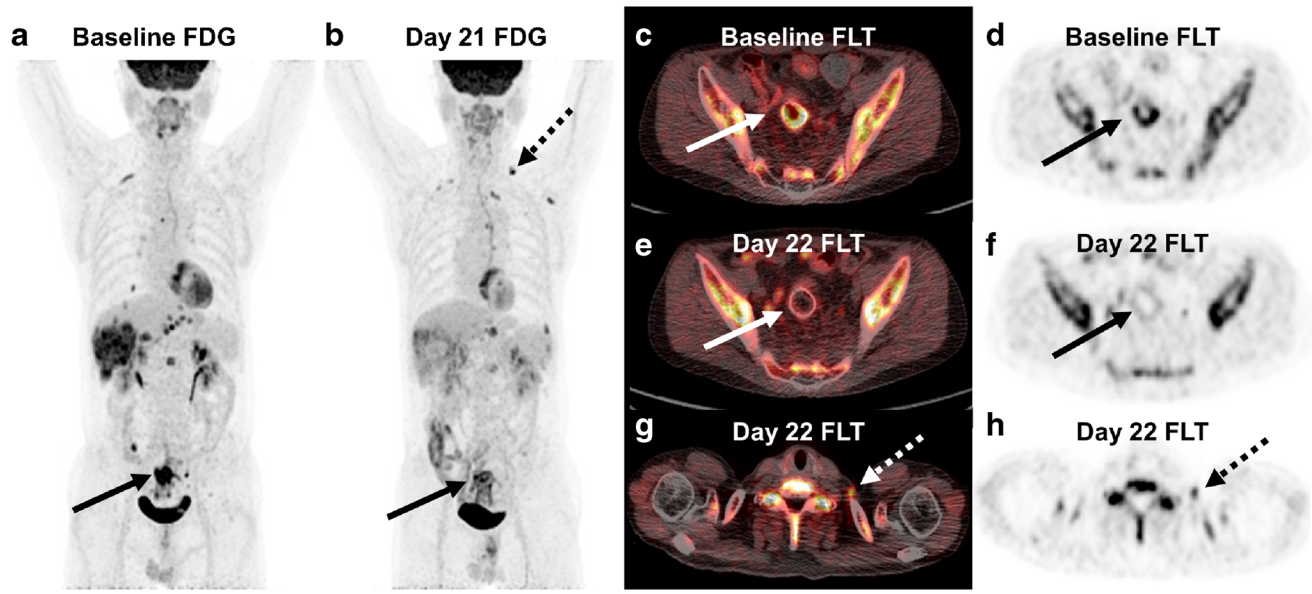

Fig. 3 ^{18}F-FDG and ^{18}F-FLT PET/CT images of a 53-year-old man with rectal cancer and multiple metastases. Maximum-intensity projection of ^{18}F-FDG at baseline (**a**) and day 21 (**b**) shows a decrease in the SULpeak of rectal cancer from 9.0 to 5.4 after receiving Regorafenib (40% change, *arrow*). Trans-axial ^{18}F-FLT PET/CT images acquired at baseline and day 22 show a decrease in the SUVmax of rectal cancer from 8.1 (**c**, **d**, *arrow*) to 4.0 (50.6%, e, f, *arrow*). A new left supraclavicular lymph node metastasis is seen in the maximum-intensity projection of ^{18}F-FDG (b, *dotted arrow*) on day 21 and in the trans-axial ^{18}F-FLT PET/CT image acquired on day 22 (g, h, *dotted arrow*). Overall response was considered as PMD on ^{18}F-FDG PET/CT and as no response on ^{18}F-FLT PET/CT. PFS and OS in the patient were 1.6 months and 3.6 months, respectively

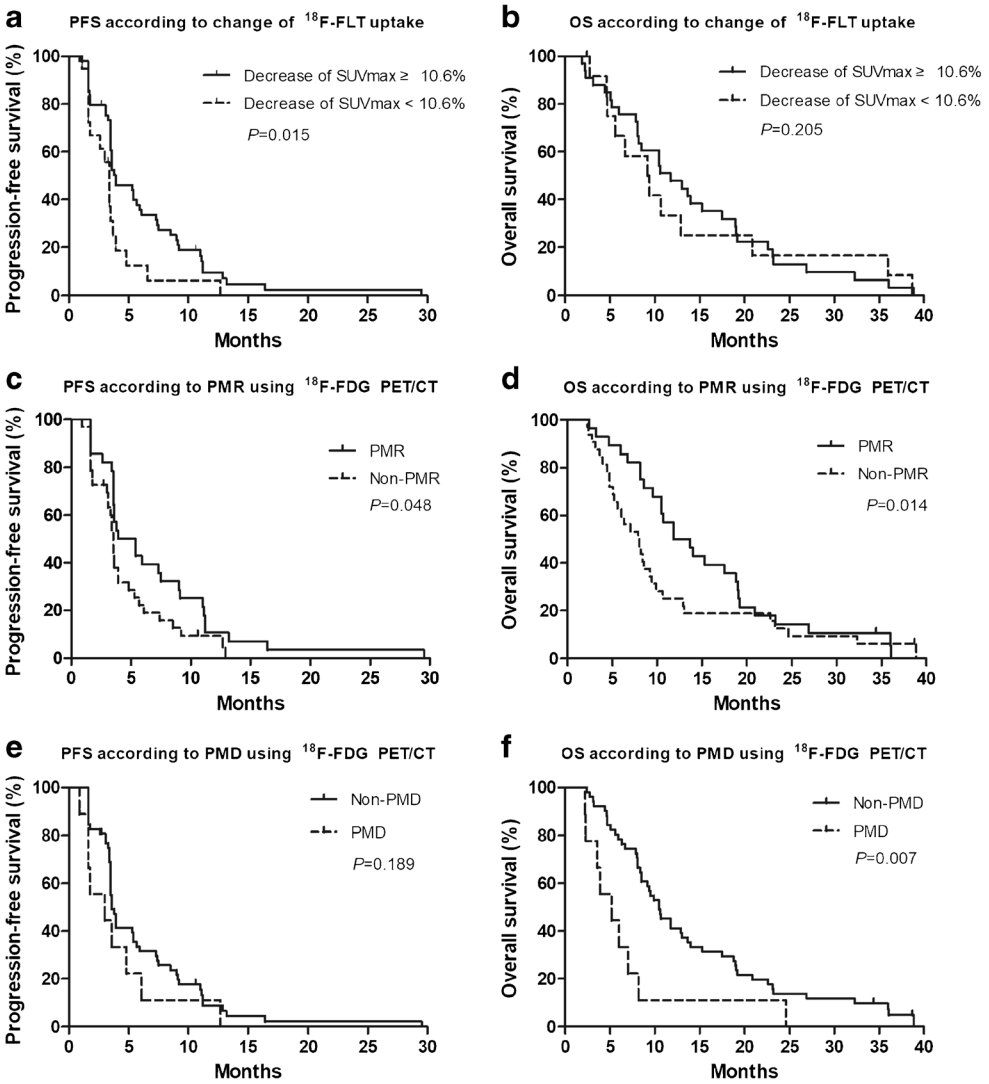

Fig. 4 Kaplan-Meier curves showing the PFS and OS according to the change of ^{18}F-FLT uptake and PET Response Criteria in Solid Tumours (PERCIST) using ^{18}F-FDG PET/CT in patients with metastatic colorectal cancer receiving Regorafenib. (**a**) The median PFS was 3.9 months (95% CI, 1.93–5.87) for patients with a decrease of SUVmax ≥10.6% on day 21 of Regorafenib (responders on ^{18}F-FLT PET/CT) and 3.4 months (95% CI, 2.72–4.16) for non-responders ($P = 0.015$). (**b**) The median OS was 11.6 months (95% CI, 7.33–13.85) for the responders and 7.0 months (95% CI, 4.45–9.45) for the non-responders ($P = 0.205$). According to the PERCIST using ^{18}F-FDG PET/CT on day 21 of Regorafenib, (**c**) the PFS was 9.2 months (95% CI, 1.60–6.27 months) for the partial metabolic response (PMR) groups and 5.7 months (95% CI, 3.26–3.76 months) for the non-PMR groups ($P = 0.048$). (**d**) The OS was 11.8 months (95% CI, 7.54–16.13 months) and 7.9 months (95% CI, 5.25–10.62 months) for the PMR and non-PMR groups, respectively ($P = 0.014$). (e) The PFS was 3.6 months (95% CI, 3.19–4.03) for the non-progressive metabolic disease (PMD) groups and 1.8 months (95% CI, 0.00–3.84) for the PMD groups ($P = 0.189$). (f) The OS was 10.5 months (95% CI, 8.07–12.92) and 3.9 months (95% CI, 2.22–6.18) for the non-PMD and PMD groups ($P = 0.007$), respectively

contribute to making a decision for early discontinuation of Regorafenib in vulnerable patients to avoid unnecessary toxicities.

The predictive role of ^{18}F-FDG PET/CT for response evaluation has been reported in various cancers including CRC [34, 35]. As tyrosine kinase inhibitors re-programme the glycolysis and other glucose-related pathways in tumours [36], ^{18}F-FDG PET/CT, which reflects glucose metabolism, may be considered an effective imaging method particularly with targeted agents. ^{18}F-FDG PET/CT has already shown its efficacy in assessing responses to bevacizumab and Regorafenib [37, 38]. Cytostatic targeted agents often improve survival without decreasing tumour size; likewise, the overall response rate with Regorafenib was only 1% according to the RECIST in patients with mCRC in the CORRECT study [8], despite significant improvements in survival. This finding suggested that assessing not only morphologic changes but also biological changes in tumour is valuable in assessing the efficacy of Regorafenib.

This study has some limitations. Patients with liver-limited metastasis were excluded because ^{18}F-FLT is actively taken up into the liver and metabolised to ^{18}F-FLT glucuronide.

Increased background activity in the liver tissue can affect tumour uptake via the partial volume effects [39]. A second limitation of this study is that we included target lesions in the liver and other organs to evaluate morphological treatment response, while the measurements of ^{18}F-FLT were performed after excluding hepatic metastases. However, morphological responses of hepatic and extrahepatic target lesions were concordant in each patient. Third, although significant differences in PFS and OS using ^{18}F-FDG PET/CT on day 21 were observed, the results require careful interpretations when directly comparing with predictive values of CT at 8 weeks. When considering that CT was not performed at the same time point with ^{18}F-FDG PET/CT, it was difficult to evaluate the advantage of ^{18}F-FDG PET/CT over CT. It would be helpful to compare ^{18}F-FDG PET/CT with CT at the same time point in further studies. Fourth, patients with non-PMD of PERCIST using ^{18}F-FDG PET/CT did not have significantly longer PFS than did those with PMD. This may be explained by the small number of patients with PMD. Finally, we performed PET/CT images at only two time points of baseline and day 21 of Regorafenib. ^{18}F-FDG PET/CT on day 21 may not be the most optimal time point to predict therapeutic effect of Regorafenib. Further larger studies need to evaluate more optimal time points of ^{18}F-FDG PET/CT and validate the response assessment using PERCIST by ^{18}F-FDG PET/CT in patients receiving Regorafenib.

The present study clearly showed that survival outcome was significantly associated with PERCIST using ^{18}F-FDG PET/CT but the change of ^{18}F-FLT uptake on day 21 was only slightly associated with PFS. Therefore, our results suggest that ^{18}F-FDG PET/CT can be used as an imaging biomarker to predict clinical outcomes early in patients with mCRC receiving Regorafenib. Further studies are necessary to validate their clinical implication based on solid evidence.

Acknowledgements This study was supported by a grant (2014-9079) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea, and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI18C2383). Regorafenib was kindly provided by Bayer.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Asan Medical Center and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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