

3'-Deoxy-3'-¹⁸F-Fluorothymidine PET for the Early Prediction of Response to Leucovorin, 5-Fluorouracil, and Oxaliplatin Therapy in Patients with Metastatic Colorectal Cancer

Yong Sang Hong^{*1,2}, Hye Ok Kim^{*3}, Kyu-pyo Kim^{1,2}, Jae-Lyun Lee^{1,2}, Hwa Jung Kim⁴, Seung Jin Lee^{2,5}, Sang Ju Lee³, Seung Jun Oh³, Jae Seung Kim³, Jin-Sook Ryu^{2,3}, Dae Hyuk Moon^{2,3}, and Tae Won Kim^{1,2}

¹Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; ²Institute for Innovative Cancer Research, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; ³Department of Nuclear Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; ⁴Department of Clinical Epidemiology and Biostatistics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; and ⁵Institute for Biomedical Research Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

The aim of this study was to evaluate 3'-deoxy-3'-¹⁸F-fluorothymidine (¹⁸F-FLT) PET for early prediction of the standard anatomic response and survival outcomes in patients with metastatic colorectal cancer (mCRC) receiving leucovorin, 5-fluorouracil (5-FU), and oxaliplatin (FOLFOX). **Methods:** The main eligibility criteria included histologically confirmed mCRC, ≥ 1 extrahepatic measurable lesions, and no prior chemotherapy in a metastatic setting. Chemotherapy consisted of leucovorin on day 1, followed by the continuous infusion of 5-FU on days 1 and 2, and oxaliplatin on day 3. In the second and subsequent cycles of chemotherapy, oxaliplatin was administered simultaneously with leucovorin on day 1. ¹⁸F-FLT PET scans were obtained 3 times during the first cycle of chemotherapy: before chemotherapy, 24 h after infusion of 5-FU (day 2), and 48 h after completion of chemotherapy (day 5). The maximum standardized uptake value (SUV_{MAX}) of ¹⁸F-FLT was measured. Treatment responses were assessed by CT after 3 cycles of FOLFOX. **Results:** Eighteen patients were included in the study. The response rate after 3 cycles of FOLFOX was 27.8% (5/18). The SUV_{MAX} was increased in responders ($P = 0.043$) and nonresponders ($P < 0.001$) on day 2 and was decreased, compared with baseline values, on day 5 in responders only ($P = 0.043$). Receiver-operating-characteristic curve analysis indicated that the use of a threshold of an SUV_{MAX} increase on day 2 of $\leq 45.8\%$ resulted in a sensitivity of 100%, specificity of 69.2%, and relative risk of 2.250 ($P = 0.029$) for the diagnosis of responders. Use of a threshold of an SUV_{MAX} decrease on day 5 of $\geq 10.6\%$ resulted in a sensitivity of 100%, specificity of 76.9%, and relative risk of 2.667 ($P = 0.007$). Patients with low ¹⁸F-FLT flare tended to have longer survivals than patients with high flare (2-y overall survival rate, 77.8% vs. 44.4%; $P = 0.051$). **Conclusion:** The ¹⁸F-FLT flare observed during 5-FU infusion was associated with poor treatment response in patients with mCRC. The degree of ¹⁸F-FLT flare might be used to predict the outcome of patients who receive infusional 5-FU-based chemotherapy.

Key Words: ¹⁸F-fluorothymidine; positron emission tomography; 5-fluorouracil; oxaliplatin; metastatic colorectal cancer

J Nucl Med 2013; 54:1209–1216

DOI: 10.2967/jnumed.112.117010

Colorectal cancer (CRC) is a major cause of cancer-related mortality worldwide (1). Approximately half of all patients diagnosed with CRC develop metastasis and are potential candidates for systemic chemotherapy (1,2). The overall response rate of metastatic CRC (mCRC) to first-line palliative chemotherapy is limited (<50%). An early and accurate assessment of response may allow clinicians to avoid the risk of unfavorable adverse events and unnecessary cost in the case of treatment failure and to move on to the next available therapy in the case of disease progression.

PET with 3'-deoxy-3'-¹⁸F-fluorothymidine (¹⁸F-FLT) is potentially useful for the noninvasive measurement of cellular proliferation and may allow an early assessment of the response to chemotherapy (3). The FOLFOX regimen (oxaliplatin plus leucovorin and infusional 5-fluorouracil [5-FU]) is a reference doublet combination strategy widely used as a first-line chemotherapy. When ¹⁸F-FLT PET is used for treatment monitoring, drug-specific effects need to be considered. The best timing of therapy may also be variable, depending on the treatment (4).

The mechanism of ¹⁸F-FLT accumulation in the tumor involves nucleoside transporters and thymidine kinase 1. Adenosine triphosphate is an important cofactor for realizing the activity of thymidine kinase 1 (5,6). The upregulation and downregulation are controlled by distinct regulatory mechanisms. A negative feedback loop operates between the thymidine triphosphate pool and thymidine kinase 1, because maintenance of a balanced thymidine triphosphate pool is required for DNA replication and repair (7). By interfering with endogenous thymidine synthesis, 5-FU can cause rapid increases in the thymidine kinase levels (8,9), redistribution of nucleoside transporters (10), and marked retention of ¹⁸F-FLT. The temporary increase in retention of ¹⁸F-FLT, referred to as the flare effect, may be used in pharmacodynamic measurements of the effect of chemotherapeutic agents that

Received Nov. 9, 2012; revision accepted Feb. 26, 2013.

For correspondence contact: Tae Won Kim, Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, 88, Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, Republic of Korea.

E-mail: twkimmd@amc.seoul.kr

*Contributed equally to this work.

Published online Jun. 26, 2013.

COPYRIGHT © 2013 by the Society of Nuclear Medicine and Molecular Imaging, Inc.

block thymidylate synthase (11,12). On the other hand, by inhibiting the incorporation of thymidine triphosphate into DNA, oxaliplatin likely engenders an increase in the triphosphate nucleoside pool and reductions in thymidine kinase 1 levels and the trapping of ^{18}F -FLT (13–15).

We hypothesized that ^{18}F -FLT PET could be useful for identifying a subgroup of mCRC patients with clinical responsiveness to FOLFOX therapy. We conducted an exploratory study to assess the early prediction by ^{18}F -FLT PET of the standard anatomic response and survival outcomes in patients with mCRC receiving FOLFOX.

MATERIALS AND METHODS

Study Design

This study was an open-label, nonrandomized, exploratory trial to assess whether ^{18}F -FLT PET could be used to predict the clinical responsiveness of mCRC patients to FOLFOX as a first-line chemotherapy. The primary outcome measure was the standard anatomic objective response, as determined by the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0) (16) at 6 wk after first-line FOLFOX. The secondary outcome measure was survival outcome. This study was conducted in accordance with the Helsinki Declaration. All patients provided written informed consent before participation. The local Institutional Review Board approved the study protocol.

Patient Population

Patients were included if they had histologically confirmed adenocarcinoma that was not amenable to surgery or radiation therapy of curative intent, with ≥ 1 extrahepatic measurable lesions according to RECIST; an Eastern Cooperative Oncology Group performance status of 0–1; a life expectancy of ≥ 3 mo; no prior chemotherapy in a metastatic setting; and adequate hematologic, hepatic, and renal functions. Patients with liver-limited metastasis were excluded. Adjuvant chemotherapy with fluoropyrimidines alone was allowed if it

was completed ≥ 6 mo before enrollment, but no prior adjuvant chemotherapy with oxaliplatin was allowed. Prior radiotherapy was permitted if it was not administered to the target lesions selected for this study and had been completed ≥ 4 wk before registration. Patients were recruited by referral from the investigators.

Study Treatment and Radiologic Response Evaluation

Patients were treated with a modified FOLFOX regimen, consisting of leucovorin (200 mg/m²) on day 1, subsequent continuous infusion of 5-FU on days 1 and 2 (2,400 mg/m² for 46 h), and oxaliplatin (85 mg/m²) on day 3 (Fig. 1). Oxaliplatin was administered simultaneously with leucovorin on day 1 from the second cycle of chemotherapy. Study treatments were planned to be continued until disease progression, patient refusal, or unacceptable toxicity. CT scans were obtained within 2 wk before chemotherapy and every 3 cycles of chemotherapy (6 wk) or when disease progression was suspected. Morphologic response was evaluated by CT every 6 wk according to RECIST 1.0, without knowledge of the results of ^{18}F -FLT PET.

^{18}F -FLT PET Imaging

^{18}F -FLT was synthesized as previously described (17). The amount of ^{18}F -FLT injected was 159.1 ± 22.2 MBq (range, 111–185 MBq). The specific activity was ≥ 223 TBq/mmol. The radiochemical purity was 100.0% (range, 98.7%–100.0%).

^{18}F -FLT PET imaging was performed 3 times before, during, and after FOLFOX chemotherapy with a PET/CT scanner (Biograph True Point 40; Siemens). Baseline ^{18}F -FLT PET was performed within 2 wk before chemotherapy. Subsequent ^{18}F -FLT PET studies were performed 24 h after the continuous infusion of 5-FU (day 2) and 48 h after completion of chemotherapy (day 5). The ^{18}F -FLT PET images were obtained at 1 h after the intravenous injection of ^{18}F -FLT. Non-contrast CT imaging (120 kV, 50 mAs, CARE Dose 4D; Siemens) from the skull base to the mid thigh was performed in a spiral mode for attenuation correction. A 3-dimensional emission scan of the same area was obtained for 2 min per bed position. For the third ^{18}F -FLT PET study, limited-area tumor imaging with ≥ 2 bed positions was

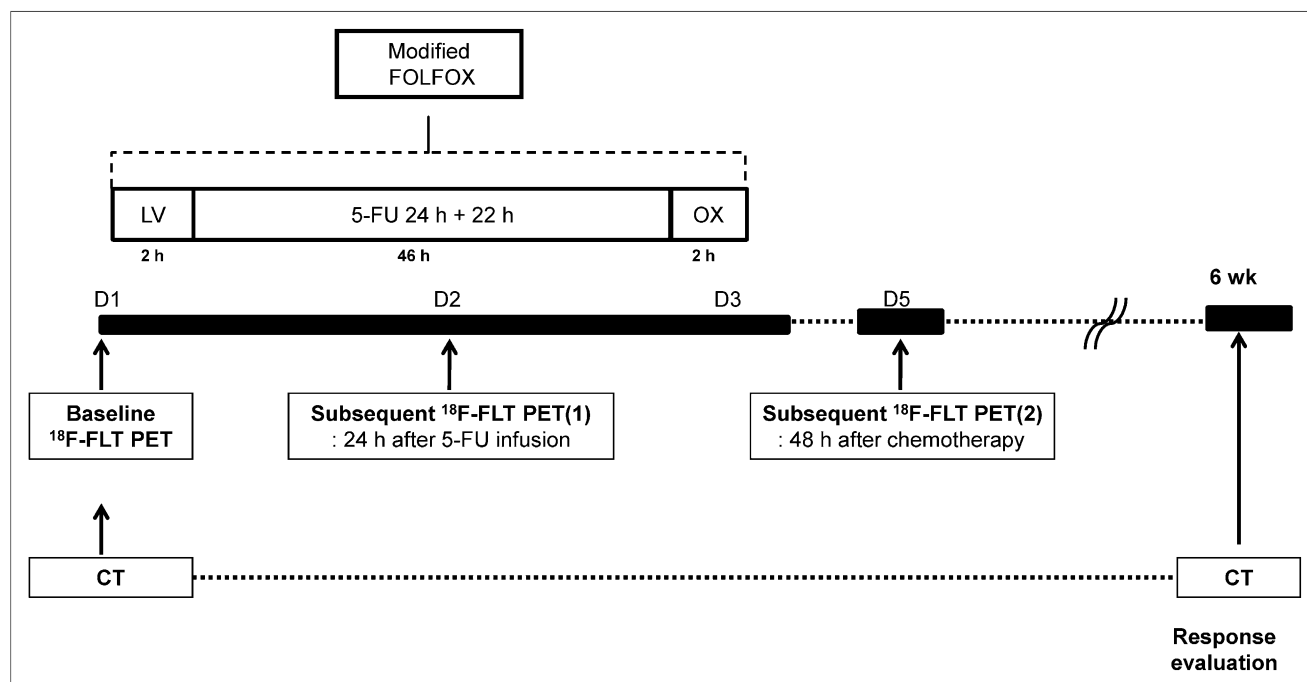


FIGURE 1. Schematic design of FOLFOX (oxaliplatin [OX] plus leucovorin [LV] and infusional 5-FU) chemotherapy and ^{18}F -FLT PET imaging procedures.

performed with the same acquisition parameters as were applied for the previous scan.

The effective radiation dose from three ^{18}F -FLT administrations was estimated to be 15.5 and 18.2 mSv for male and female subjects, respectively (18). The total radiation exposure from the 3 CT examinations was ≤ 10 mSv. Emission images were reconstructed by True X reconstruction with 3 iterations and 21 subsets. No correction for partial-volume effects was performed.

The ^{18}F -FLT PET results were analyzed quantitatively by the consensus of 2 board-certified nuclear medicine physicians who were masked to the clinical outcome results. Quantitative image analysis of ^{18}F -FLT uptake included calculation of the maximum standardized uptake value (SUV_{MAX} , single maximum pixel count within the volumes of interest) of all target lesions, which was obtained with the vendor's software (Syngo TrueD; Siemens). The SUV_{MAX} of 1 extrahepatic target lesion with the most intense ^{18}F -FLT uptake was selected for the comparative analysis between treatment responses by CT scan and survival outcomes. The percentage change in SUV_{MAX} between baseline and subsequent PET images was calculated. The standardized uptake value (SUV) of the blood pool was calculated to assess changes in ^{18}F -FLT availability in blood to the tumor after FOLFOX by drawing a circular region of interest of 1 cm in diameter on the descending aorta and extended 2 cm over the z -axis. The SUV was defined as activity (Bq/g)/[injected activity (Bq)/body weight (g)].

Statistical Analysis

A sample size of 16 patients achieves 82% power to detect a difference of 0.1 between the null hypothesis mean of 0.30 and the alternative hypothesis mean of 0.20 for SUV_{MAX} , with an estimated SD of 0.15 and significance level of 0.05 by 1-sided 1-sample t test. We planned for the enrollment of 18 patients, because a dropout rate of 10% was assumed.

Quantitative measurements of ^{18}F -FLT PET are reported as median values with ranges. Other descriptive statistical data are presented as proportions and medians with ranges. The Wilcoxon signed-rank test was used to assess the significance of the change in SUV during FOLFOX treatment. Differences between 2 groups of patients were tested by the Mann-Whitney U test. Receiver-operating-characteristic curves were used to identify the optimum cutoff value of the percentage change in SUV_{MAX} for differentiating responding and nonresponding patients. The Fisher exact test was used to correlate changes in ^{18}F -FLT SUV_{MAX} with treatment response after 3 cycles of FOLFOX. Overall survival (OS, time to death), progression-free survival (PFS, time to progression or death), and 95% confidence intervals (CIs) were assessed with the Kaplan-Meier method. A P value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed with SPSS (version 19.0; SPSS Inc.) for Windows (IBM Co.) and GraphPad Prism (version 4.00; GraphPad Software).

RESULTS

Patient Characteristics, Treatment Response, and Survival

Between September 2009 and October 2010, 18 patients with mCRC were enrolled in this study and received FOLFOX as their first-line treatment at Asan Medical Center in Seoul, Republic of Korea. No patient declined participation after receiving the ^{18}F -FLT injection. The patient characteristics are listed in Table 1. A total of 262 cycles of FOLFOX were administered (median, 15 cycles; range, 3–20 cycles). The most common reason for treatment cessation was disease progression (16/18 [89%]). All patients completed the ^{18}F -FLT PET studies and were evaluable for treatment response after 3 cycles of FOLFOX. There were no deviations from the planned protocol. The response rate after 3 cycles of FOLFOX was 27.8% (95% CI, 9.8%–53.5%), with 5 partial

responses, 12 stable diseases, and 1 progressive disease (Table 2). Median follow-up time was 24.5 mo (range, 7.1–34.6 mo). During this interval, 16 patients developed progressive disease, 11 of whom died. Median PFS was 8.6 mo (95% CI, 5.1–12.1 mo), and median OS was 27.8 mo (95% CI, 21.0–34.6 mo). The 2-y OS rate was 61.1%.

Early Response Assessment by ^{18}F -FLT PET

The ^{18}F -FLT PET studies were well tolerated by all patients, completed without any problems, and performed as planned, without any adverse events related to ^{18}F -FLT PET. The mean time interval between ^{18}F -FLT injection and PET imaging was 60.7 ± 3.3 min (range, 55–77 min). The mean time interval between baseline ^{18}F -FLT PET imaging and FOLFOX chemotherapy was 2 h 30 min (range, 1 h 15 min to 3 h 42 min). There was no significant difference in the blood-pool SUVs between baseline (0.7 ± 0.1 ; range, 0.5–0.8) and day 2 (0.7 ± 0.1 ; range, 0.5–0.8; $P > 0.05$) or day 5 (0.7 ± 0.1 ; range, 0.5–0.9; $P > 0.05$).

Baseline SUV_{MAX} before treatment and percentage changes of SUV_{MAX} on days 2 and 5 during the first cycle of FOLFOX are listed in Table 2. The median baseline value of SUV_{MAX} for the representative metastatic lesion was 4.9 (range, 1.2–11.3). ^{18}F -FLT uptake increased on day 2 (6.9; range, 1.7–24.9; $P < 0.001$), but there was no significant difference in the SUV_{MAX} between baseline and day 5 (3.5; range, 1.0–15.4). Figure 2 shows the changes in ^{18}F -FLT uptake (SUV_{MAX}) during and after 1 cycle of FOLFOX. The baseline SUV_{MAX} of the representative metastatic lesion did

TABLE 1
Patient Characteristics

Characteristic	No. of patients ($n = 18$)	Percentage or median
Sex		
Male	10	55.6
Female	8	44.4
Performance status		
0–1	18	100
Primary tumor		
Colon	13	72.2
Rectum	5	27.8
Prior neoadjuvant treatment		
No prior treatment	7	38.9
Prior radiotherapy	3	16.7
Prior chemotherapy	8	44.4
Sites of metastasis		
Liver	9	50.0
Lung	8	44.4
Lymph node, abdomen	9	50.0
Peritoneum/omentum	4	22.2
Bone	1	5.6
Ovary	1	5.6
No. of involved organs		
1	8	44.4
2	8	44.4
≥ 3	2	11.2
Pretreatment carcinoembryonic antigen (ng/mL)		9.2 (range, 1.3–1010)
Median age was 64.5 y, and age range was 36–77 y.		

TABLE 2
Treatment Responses to FOLFOX and Changes in ¹⁸F-FLT Uptake

Patient no.	No. of target lesions	RECIST 1.0 response achieved			Assessment with ¹⁸ F-FLT PET				
		Percentage change in tumor size	After 3 cycles of FOLFOX	Best overall response	Lesion	Baseline SUV _{MAX}	Percentage change of SUV _{MAX} on day 2	Percentage change of SUV _{MAX} on day 5	
1	5	-25.7	Stable disease	Partial response	Lymph node	5.6	69.6	5.4	
2	2	-2.3	Stable disease	Stable disease	Peritoneum	5.3	107.5	-3.8	
3	2	-12.8	Stable disease	Partial response	Peritoneum	2.5	380.0	24.0	
4	3	-25.0	Stable disease	Partial response	Lymph node	3.1	48.4	80.6	
5	1	-16.7	Stable disease	Partial response	Lung	1.7	5.9	0.0	
6	1	-18.8	Stable disease	Partial response	Lymph node	5.4	131.5	181.5	
7	4	-48.3	Partial response	Partial response	Lung	1.2	41.7	-16.7	
8	4	8.7	Progressive disease*	Progressive disease	Lung	2.6	69.2	-34.6	
9	5	-51.9	Partial response	Complete response	Lymph node	5.2	36.5	-67.3	
10	5	-23.2	Stable disease	Stable disease	Lymph node	8.7	73.6	-21.8	
11	3	-46.0	Partial response	Partial response	Lymph node	7.2	45.8	-76.4	
12	6	-23.3	Stable disease	Partial response	Lung	1.7	52.9	5.9	
13	4	-30.7	Partial response	Partial response	Lymph node	5.1	19.6	-23.5	
14	2	-8.3	Stable disease	Stable disease	Peritoneum	10.3	141.7	36.9	
15	3	-31.4	Partial response	Partial response	Lung	4.7	42.6	-10.6	
16	2	-8.3	Stable disease	Stable disease	Lymph node	3.7	2.7	-16.2	
17	2	-27.3	Stable disease	Partial response	Lung	2.0	10.0	15.0	
18	9	-13.3	Stable disease	Partial response	Lymph node	11.3	17.7	36.3	

*Patient showed unequivocal progression of nontarget lesions after 3 cycles of FOLFOX.

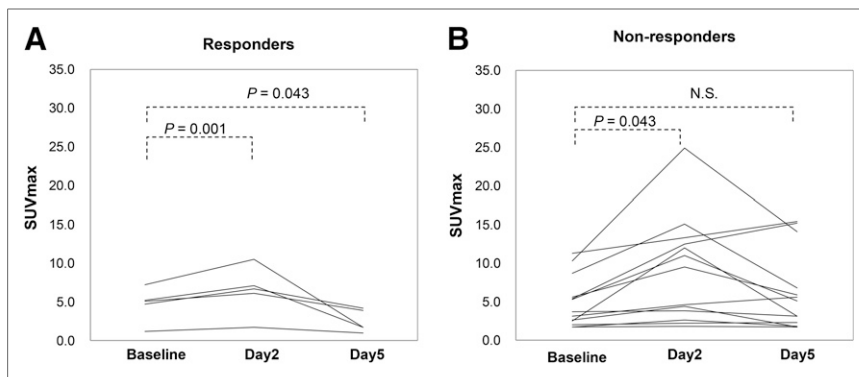


FIGURE 2. Changes in ^{18}F -FLT uptake (SUV_{MAX}) during (day 2) and after (day 5) 1 cycle of FOLFOX. On day 2, SUV_{MAX} was increased significantly in responders and nonresponders. On day 5, SUV_{MAX} was decreased, compared with baseline values, in responders ($P < 0.05$), but nonresponders did not show significant change. N.S. = not significant.

not differ between responders (5.1; range, 1.2–7.2) and non-responders (3.7; range, 1.7–11.3). On day 2, the SUV_{MAX} was increased in responders (6.7; range, 1.7–10.5; $P = 0.043$) and nonresponders (9.5; range, 1.8–24.9; $P < 0.001$). Percentage changes tended to be higher in nonresponders than in responders, but the difference was not significant (Fig. 2). After 1 cycle of treatment on day 5, the SUV_{MAX} was decreased, compared with baseline, in responders (1.7; range, 1.0–4.2; $P < 0.05$), but nonresponders did not show a significant change (5.1; range, 1.7–15.4; Fig. 2).

The receiver-operating-characteristic curve analysis showed that a threshold of an SUV_{MAX} increase on day 2 of $\leq 45.8\%$ (low ^{18}F -FLT flare) was optimal for differentiating responders from nonresponders, with a sensitivity of 100% (95% CI, 48.0%–100.0%), specificity of 69.2% (95% CI, 38.6%–90.7%), and relative risk of 2.250 (95% CI, 1.084–4.671; $P = 0.029$; Table 3). Use of a threshold of an SUV_{MAX} decrease on day 5 of $\geq 10.6\%$ provided a sensitivity of 100% (95% CI, 48.0%–100.0%), specificity of 76.9% (95% CI, 46.2%–94.7%), and relative risk of 2.667 (95% CI, 1.090–6.524; $P = 0.007$; Table 3). The CT and ^{18}F -FLT images of a representative nonresponder (patient 2) with high ^{18}F -FLT flare on day 2 and a small reduction in ^{18}F -FLT on day 5 are shown in Figure 3. Results for patient 11, who showed a partial response, low ^{18}F -FLT flare on day 2, and large reduction in ^{18}F -FLT SUV_{MAX} on day 5, are illustrated in Supplemental Figure 1 (supplemental materials are available online only at <http://jnm.snmjournals.org>).

Interestingly, 3 patients (patients 4, 6, and 18) showed persistently increasing SUV_{MAX} over time (Fig. 2). All these patients were nonresponders. Even after excluding these patients, SUV_{MAX} increase on day 2 and decrease on day 5 differentiated responders and nonresponders significantly ($P = 0.026$).

Survival Outcomes According to ^{18}F -FLT PET Response

The PFS and OS were compared according to the degree of ^{18}F -FLT flare on day 2 and ^{18}F -FLT reduction on day 5 (Fig. 4). The median PFS was 10.0 mo (95% CI, 5.0–15.0 mo) and 8.6 mo (95% CI, 6.8–10.4 mo) for the low- and high-flare group, respectively (Fig. 4A, $P = 0.422$). Patients with low ^{18}F -FLT flare tended to have a longer survival than patients with high flare. OS did not reach the median value for the low-flare group, and the median OS was 18.7 mo for the high-flare group. The 2-y OS rate was 77.8% in those with low flare and 44.4% in those with high flare ($P =$

0.051, Fig. 4B). Six of the 9 patients with low ^{18}F -FLT flare was still alive, whereas only 1 of 9 with high ^{18}F -FLT flare was alive at the time of this writing. When PFS and OS were compared according to the presence of low flare on day 2 and a large reduction on day 5, 6 patients who met both criteria tended to have a higher median PFS (10.0 mo; 95% CI, 2.7–17.3 mo) than those who did not (8.3 mo; 95% CI, 7.3–9.3 mo), without statistical significance ($P = 0.139$, Fig. 4C). At the time this article was being written, 2 patients had no evidence of disease progression. Both of these patients showed low ^{18}F -FLT flares on day 2 and large reductions in ^{18}F -FLT uptake on day 5. OS showed the same tendency, without

significant difference (not reached the median value vs. 18.7 mo; 95% CI, 1.4–36.0 mo; $P = 0.116$; Fig. 4D). Four of 6 patients with both low ^{18}F -FLT flare and a large reduction and 3 of 12 without these criteria were alive at the time of this writing. Two-year OS rates for these groups were 83.3% and 50.0%, respectively. The PFS and OS did not show significant differences with respect to the degree of ^{18}F -FLT reduction on day 5 (data not shown).

DISCUSSION

In this exploratory study, the ^{18}F -FLT flare, which reflects an increased uptake of ^{18}F -FLT at 24 h during continuous 5-FU infusion, was related to poor treatment response as assessed by CT and a tendency for poor survival in mCRC patients who received FOLFOX. Decreased ^{18}F -FLT uptake after oxaliplatin was also related to treatment response. The 2 patients without disease progression showed low ^{18}F -FLT flares after 5-FU and large reductions after oxaliplatin. Our findings suggest the feasibility of using ^{18}F -FLT PET imaging for the earlier prediction of treatment response in patients with mCRC receiving FOLFOX.

The combined use of 5-FU and leucovorin inhibits thymidylate synthase and depletes deoxythymidine triphosphate, which is the major effector of 5-FU cytotoxicity (19,20). Resistance to 5-FU may develop through various mechanisms. One mechanism that is related to ^{18}F -FLT flare is the activity of the salvage pathway in circumventing the inhibition of pathways of de novo synthesis (21). The contribution of the salvage pathway to clinical resistance to 5-FU is supported by the increased accumulation of substrates of the salvage pathway after the inhibition of de novo synthesis (7,22), reduced activity of thymidylate synthase inhibitors in the presence of extracellular thymidine (23–26), enhancement of the anticancer activity of 5-FU after thymidine transport is inhibited (27–29), and increased activity of thymidylate synthase inhibition in thymidine kinase-deficient tumors (25,30). Increased thymidine is available through the flare-related redistribution of transporters and increased thymidine kinase 1 activity. The observed association between ^{18}F -FLT flare and poor treatment responses may be explained by the fact that the salvage of de novo synthesis inhibition contributes to resistance to 5-FU. In a previous in vitro study, even subtherapeutic doses of thymidylate synthase inhibitors were able to induce an increase in ^3H -thymidine uptake (15). This finding also suggests that ^{18}F -FLT flare may not be related to treatment response. The relative

TABLE 3
Treatment Response After 3 Cycles of FOLFOX According to Percentage Changes in ^{18}F -FLT SUV_{MAX}

Percentage changes in...	CT response after 3 cycles				Overall response rate (%)	Relative risk	<i>P</i>
	Responder (<i>n</i> = 5)		Nonresponder (<i>n</i> = 13)				
	CR	PR	Stable disease	PD			
¹⁸ F-FLT uptake on day 2*						2.250 (95% CI, 1.084–4.671)	0.029†
≤45.8	0	5	4	0	55.6		
>45.8	0	0	8	1	0		
¹⁸ F-FLT reduction on day 5*						2.667 (95% CI, 1.090–6.524)	0.007†
>10.6	0	0	10	0	0		
≤10.6	0	5	2	1	62.5		

*Receiver-operating-characteristic curve analysis was used to identify optimum cutoff of percentage change in SUV_{MAX} from baseline value for differentiating responding and nonresponding patients.

†P value by Fisher exact test.

use of the exogenous and endogenous pathways are potentially confounding factors that must be considered when interpreting ^{18}F -FLT PET results (31). ^{18}F -FLT flare may indicate the activity of the salvage pathway for cellular proliferation when therapy is intended to suppress the de novo pathway. It is important to consider that ^{18}F -FLT PET in this study assessed only the pharmacodynamic effect of 5-FU, whereas treatment responses were assessed after FOLFOX. It is known that patients who have failed therapy with 5-FU alone respond better to oxaliplatin if it is combined with 5-FU. It is probable that the low ^{18}F -FLT flare associated with favorable outcome in this study

may also be linked to the synergistic effect of oxaliplatin and 5-FU.

^{18}F -FLT uptake in the tumor, as viewed by in vivo imaging, reflects the viable cell density and the avidity of ^{18}F -FLT incorporation on a per-cell basis (32). Previous in vitro and animal studies showed that a transient increase in the cellular ^{18}F -FLT uptake followed by a dose-dependent decrease in ^{18}F -FLT uptake was correlated with a decrease in cell viability or inhibition of DNA synthesis after 24 h (10,13–15). ^{18}F -FLT flare was reported as early as 1 h after the administration of drugs inhibiting thymidylate synthase (10–12), until up to 24 h after 5-FU treatment

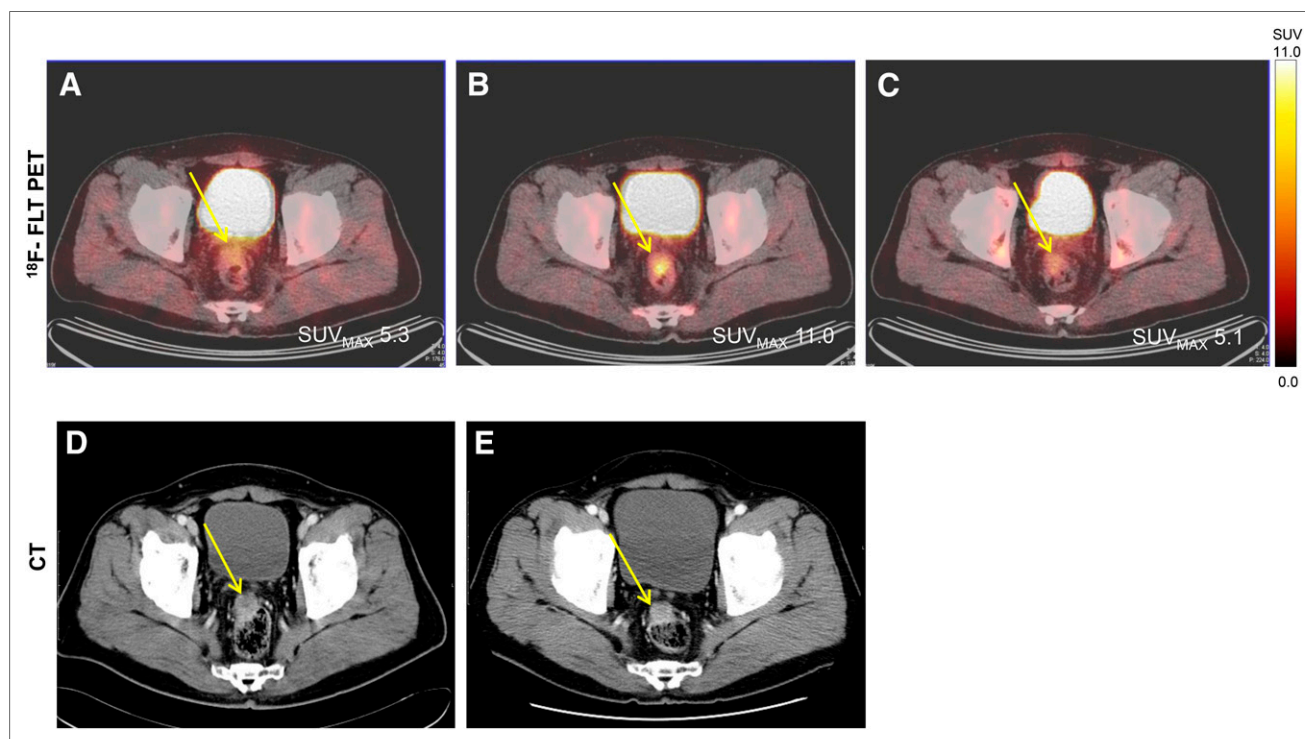


FIGURE 3. A 60-y-old man with stable disease after 3 cycles of FOLFOX (patient 2). Representative baseline (A) and day 2 ^{18}F -FLT PET (B) images showed increase in SUV_{MAX} of peritoneal seeding nodule from 5.3 to 11.0 during 5-FU infusion (107.5% change, high ^{18}F -FLT flare). SUV_{MAX} after completion of first cycle (5.1) showed only –3.8% change, compared with baseline value (C). Transverse CT images before (D) and after FOLFOX (E) show 2.3% reduction in tumor size.

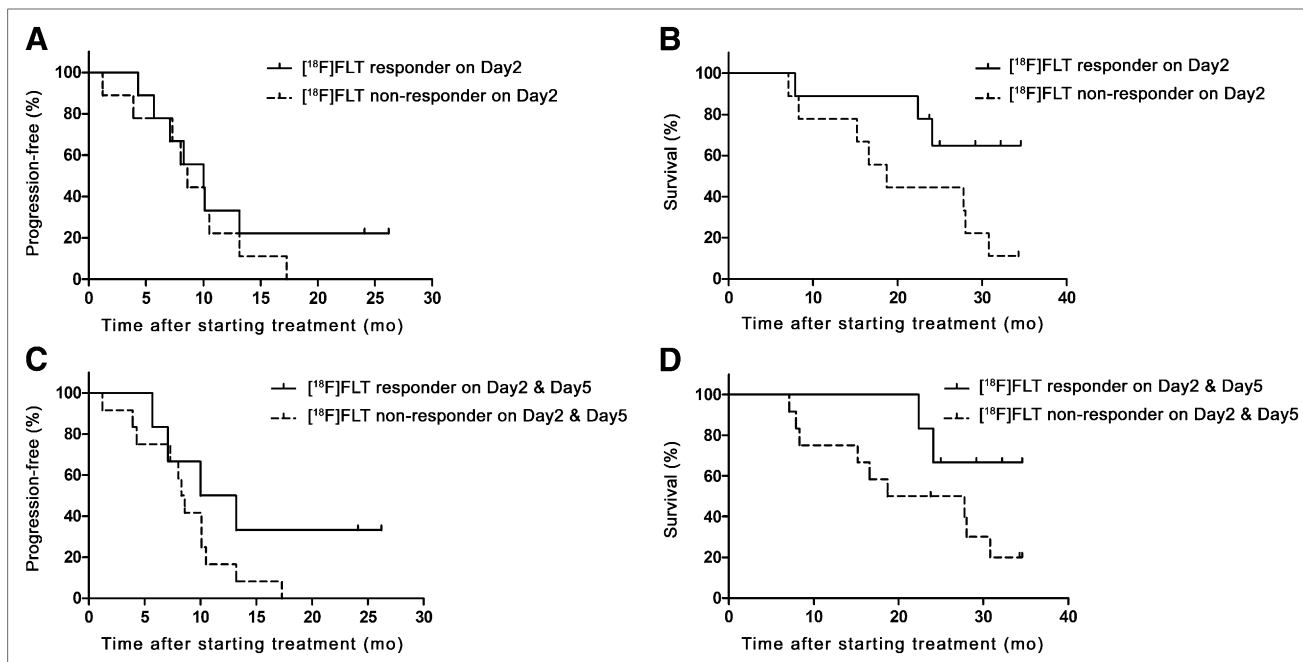


FIGURE 4. Survival outcomes according to changes in ^{18}F -FLT uptake during and after first cycle of FOLFOX. PFS (A) and OS (B) of patients by ^{18}F -FLT uptake response during 5-FU infusion (^{18}F -FLT flare) were not significantly different, although there was tendency for longer survival in patients in low-flare group (2-y OS rate, 77.8% vs. 44.4%). PFS (C) and OS (D) according to presence of low flare on day 2 and large reduction on day 5 showed same tendency without significant difference.

(9,13). However, successful therapy will eventually reduce ^{18}F -FLT uptake in the tumor as early as 24 h after 5-FU treatment, and afterward the reduction may be even more pronounced (10,13–15). This notion implicates the importance of the time point when ^{18}F -FLT PET is used to assess the tumor response after treatment. In this study, ^{18}F -FLT flare was assessed at 24 h after 5-FU infusion. The time point of day 2 was chosen on the basis of our previous observation that high ^{18}F -FLT flare occurred at 24 h after 5-FU treatment in most of the cell lines tested (9). It is possible that the 5-FU-induced ^{18}F -FLT flare may indicate resistance to 5-FU. Compromised cell viability or inhibition of DNA synthesis may predominate and lead to decreased rather than increased ^{18}F -FLT uptake at 24 h after 5-FU treatment in tumor cells with responders. In the clinical setting, ^{18}F -FLT uptake in the tumor after 5-FU treatment is highly complex and variable. More information on the short- and intermediate-term effects of 5-FU on the enzyme and transporter activities and ^{18}F -FLT uptake is clearly needed. It will also be essential to determine what the clinically appropriate time point for ^{18}F -FLT PET testing should be.

We showed a correlation between the radiologic response and SUV_{MAX} changes on day 5 after oxaliplatin administration. The timing of the third ^{18}F -FLT PET study on day 5 was based on the results from preclinical studies that showed the effect of cisplatin on ^{18}F -FLT or thymidine uptake (13–15). Modern treatment regimens usually use combinations of drugs and radiotherapy. The various components of these therapies may affect the thymidine triphosphate pool size in opposite directions, making the effects on ^{18}F -FLT uptake difficult to predict. Because 5-FU and oxaliplatin may increase or decrease the thymidine kinase 1 activity, it may not be possible to investigate the predictive role of ^{18}F -FLT PET on day 5 relative to the oxaliplatin efficacy.

Further investigations on time-induced changes in the expression of nucleoside transporter proteins and key enzymes of thymidine synthesis and metabolism are necessary to confirm our observation. A later time point in the treatment cycle may be better to measure the overall antiproliferation effect of the combination. In this study, we modified FOLFOX for only the first cycle, delaying the administration of oxaliplatin until day 3 after completion of 5-FU infusion. This modification was used to isolate the effects of 5-FU on ^{18}F -FLT flare. The timing of oxaliplatin administration might not have affected the entire efficacy of FOLFOX in this study (33).

This study has several limitations, including small sample size and the exclusion of patients with liver-limited metastases. ^{18}F -FLT is actively taken up into the liver and metabolized to ^{18}F -FLT glucuronide. Increased background activity in liver tissue can affect tumor uptake by partial-volume effects and respiratory motion (34). We may overcome this limitation by including large-sized metastatic lesions (34) or using a dynamic imaging analysis (35). Another limitation of this study is that we included target lesions in the liver and other organs for the evaluation of morphologic treatment response, whereas the measurements of ^{18}F -FLT were performed with hepatic metastases excluded. However, morphologic responses of hepatic and extrahepatic target lesions were in the same direction in each patient. We have no patients who appeared to have a discrepant response, where target lesions in and outside the liver behaved differently (data not shown). Finally, only static images were acquired and later time points analyzed. However, we analyzed the ^{18}F -FLT flare in which a high fraction of ^{18}F -FLT might be phosphorylated. Our SUV analysis at later time points was simple and well tolerated in all patients. No significant difference in the blood-pool SUVs after FOLFOX may indicate no changes in ^{18}F -FLT availability in blood to the

tumor. Our measurements might not introduce a significant bias associated with the ^{18}F -FLT metabolite and perfusion.

CONCLUSION

^{18}F -FLT flare observed during continuous infusion of 5-FU and decreased ^{18}F -FLT uptake seen after oxaliplatin administration were related to poor treatment response as assessed by delayed anatomic imaging and were also associated with a tendency for poor survival outcomes. Further investigations are needed to assess whether ^{18}F -FLT uptake has potential as a predictive imaging biomarker of treatment response to FOLFOX. More studies are needed to establish the clinical value of ^{18}F -FLT flare in predicting chemoresistance after thymidylate inhibition in a larger cohort of patients.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A070001 and A062254). No other potential conflict of interest relevant to this article was reported.

REFERENCES

- Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. 2011;61:212–236.
- Van Cutsem E, Nordlinger B, Adam R, et al. Towards a pan-European consensus on the treatment of patients with colorectal liver metastases. *Eur J Cancer*. 2006;42:2212–2221.
- Sohn HJ, Yang YJ, Ryu JS, et al. ^{18}F fluorothymidine positron emission tomography before and 7 days after gefitinib treatment predicts response in patients with advanced adenocarcinoma of the lung. *Clin Cancer Res*. 2008;14:7423–7429.
- Shields AF. Positron emission tomography measurement of tumor metabolism and growth: its expanding role in oncology. *Mol Imaging Biol*. 2006;8:141–150.
- Barthel H, Cleij MC, Collingridge DR, et al. 3'-deoxy-3'- ^{18}F fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy in vivo with positron emission tomography. *Cancer Res*. 2003;63:3791–3798.
- Barthel H, Perumal M, Latigo J, et al. The uptake of 3'-deoxy-3'- ^{18}F fluorothymidine into L5178Y tumours in vivo is dependent on thymidine kinase 1 protein levels. *Eur J Nucl Med Mol Imaging*. 2005;32:257–263.
- Bianchi V, Pontis E, Reichard P. Interrelations between substrate cycles and de novo synthesis of pyrimidine deoxyribonucleoside triphosphates in 3T6 cells. *Proc Natl Acad Sci USA*. 1986;83:986–990.
- Pressacco J, Mitrovski B, Erlichman C, Hedley DW. Effects of thymidylate synthase inhibition on thymidine kinase activity and nucleoside transporter expression. *Cancer Res*. 1995;55:1505–1508.
- Lee SJ, Kim SY, Chung JH, et al. Induction of thymidine kinase 1 after 5-fluorouracil as a mechanism for 3'-deoxy-3'- ^{18}F fluorothymidine flare. *Biochem Pharmacol*. 2010;80:1528–1536.
- Perumal M, Pillai RG, Barthel H, et al. Redistribution of nucleoside transporters to the cell membrane provides a novel approach for imaging thymidylate synthase inhibition by positron emission tomography. *Cancer Res*. 2006;66:8558–8564.
- Wells P, Aboagye E, Gunn RN, et al. 2-[^{11}C]thymidine positron emission tomography as an indicator of thymidylate synthase inhibition in patients treated with AG337. *J Natl Cancer Inst*. 2003;95:675–682.
- Kenny LM, Contractor KB, Stebbing J, et al. Altered tissue 3'-deoxy-3'- ^{18}F fluorothymidine pharmacokinetics in human breast cancer following capecitabine treatment detected by positron emission tomography. *Clin Cancer Res*. 2009;15:6649–6657.

- Dittmann H, Dohmen BM, Kehlbach R, et al. Early changes in ^{18}F FLT uptake after chemotherapy: an experimental study. *Eur J Nucl Med Mol Imaging*. 2002;29:1462–1469.
- Leyton J, Latigo JR, Perumal M, Dhaliwal H, He Q, Aboagye EO. Early detection of tumor response to chemotherapy by 3'-deoxy-3'- ^{18}F fluorothymidine positron emission tomography: the effect of cisplatin on a fibrosarcoma tumor model in vivo. *Cancer Res*. 2005;65:4202–4210.
- Yau K, Price P, Pillai RG, Aboagye E. Elevation of radiolabelled thymidine uptake in RIF-1 fibrosarcoma and HT29 colon adenocarcinoma cells after treatment with thymidylate synthase inhibitors. *Eur J Nucl Med Mol Imaging*. 2006;33:981–987.
- Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*. 2000;92:205–216.
- Lee SJ, Oh SJ, Chi DY, Lee BS, Ryu JS, Moon DH. Comparison of synthesis yields of 3'-deoxy-3'- ^{18}F fluorothymidine by nucleophilic fluorination in various alcohol solvents. *J Labelled Comp Radiopharm*. 2008;51:80–82.
- Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, Krohn KA. ^{18}F -Fluorothymidine radiation dosimetry in human PET imaging studies. *J Nucl Med*. 2003;44:1482–1488.
- Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer*. 2003;3:330–338.
- Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. Meta-analysis Group In Cancer. *J Clin Oncol*. 1998;16:301–308.
- Weber G. Biochemical strategy of cancer cells and the design of chemotherapy: G. H. A. Clowes Memorial Lecture. *Cancer Res*. 1983;43:3466–3492.
- Plagemann PG, Marz R, Wohlhueter RM. Transport and metabolism of deoxycytidine and 1-beta-D-arabinofuranosylcytosine into cultured Novikoff rat hepatoma cells, relationship to phosphorylation, and regulation of triphosphate synthesis. *Cancer Res*. 1978;38:978–989.
- Jackman AL, Taylor GA, Gibson W, et al. ICI D1694, a quinazoline antifolate thymidylate synthase inhibitor that is a potent inhibitor of L1210 tumor cell growth in vitro and in vivo: a new agent for clinical study. *Cancer Res*. 1991;51:5579–5586.
- Yin MB, Guimaraes MA, Zhang ZG, Arredondo MA, Rustum YM. Time dependence of DNA lesions and growth inhibition by ICI D1694, a new quinazoline antifolate thymidylate synthase inhibitor. *Cancer Res*. 1992;52:5900–5905.
- Jackman AL, Kimbell R, Aherne GW, et al. Cellular pharmacology and in vivo activity of a new anticancer agent, ZD9331: a water-soluble, nonpolyglutamate, quinazoline-based inhibitor of thymidylate synthase. *Clin Cancer Res*. 1997;3:911–921.
- Cole PD, Smith AK, Kamen BA. Osteosarcoma cells, resistant to methotrexate due to nucleoside and nucleobase salvage, are sensitive to nucleoside analogs. *Cancer Chemother Pharmacol*. 2002;50:111–116.
- Grem JL, Fischer PH. Alteration of fluorouracil metabolism in human colon cancer cells by dipyrindamole with a selective increase in fluorodeoxyuridine monophosphate levels. *Cancer Res*. 1986;46:6191–6199.
- Curtin NJ, Harris AL. Potentiation of quinazoline antifolate (CB3717) toxicity by dipyrindamole in human lung carcinoma, A549, cells. *Biochem Pharmacol*. 1988;37:2113–2120.
- Lehman NL, Danenberg PV. Modulation of RTX cytotoxicity by thymidine and dipyrindamole in vitro: implications for chemotherapy. *Cancer Chemother Pharmacol*. 2000;45:142–148.
- Duch DS, Banks S, Dev IK, et al. Biochemical and cellular pharmacology of 1843U89, a novel benzoquinazoline inhibitor of thymidylate synthase. *Cancer Res*. 1993;53:810–818.
- Mankoff DA, Shields AF, Krohn KA. PET imaging of cellular proliferation. *Radiol Clin North Am*. 2005;43:153–167.
- Bading JR, Shields AF. Imaging of cell proliferation: status and prospects. *J Nucl Med*. 2008;49(suppl 2):64S–80S.
- Fischel JL, Etienne MC, Formento P, Milano G. Search for the optimal schedule for the oxaliplatin/5-fluorouracil association modulated or not by folinic acid: preclinical data. *Clin Cancer Res*. 1998;4:2529–2535.
- Eckel F, Herrmann K, Schmidt S, et al. Imaging of proliferation in hepatocellular carcinoma with the in vivo marker ^{18}F -fluorothymidine. *J Nucl Med*. 2009;50:1441–1447.
- Gray KR, Contractor KB, Kenny LM, et al. Kinetic filtering of ^{18}F fluorothymidine in positron emission tomography studies. *Phys Med Biol*. 2010;55:695–709.