L-[3-^{18}F]-α-Methyltyrosine Accumulation as a Definitive Chemoradiotherapy Response Predictor in Patients with Esophageal Cancer

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Abstract. *Aims: L-[3-^{18}F]-α-Methyltyrosine (^{18}F-FAMT) has high specificity for malignant tumors on positron emission tomography (PET), and its role and potential usefulness has been previously investigated in operable esophageal carcinoma. We aimed to assess the ability of ^{18}F-FAMT PET to predict the response of esophageal cancer to definitive chemoradiotherapy. Patients and Methods: We retrospectively reviewed 40 patients with esophageal cancer imaged with ^{18}F-FAMT PET. The relationship between ^{18}F-FAMT PET uptake before chemoradiotherapy and clinical outcomes was assessed. Results: The primary tumor was visualized in 95% patients. ^{18}F-FAMT uptake was significantly positively correlated with lymph node metastasis. The low-^{18}F-FAMT accumulation group had significantly higher complete response (CR) rates than did the high-accumulation group. The addition of a lymph node metastasis category with low ^{18}F-FAMT uptake provides a more precise predictor of CR. Conclusion: ^{18}F-FAMT uptake prior to treatment is a good predictor of CR rate after CRT for esophageal cancer.*

Esophageal cancer is a common malignant neoplasm. Despite recent improvements in surgical techniques and adjuvant therapies, prognosis for patients with advanced disease remains poor (1, 2). Moreover, the optimal management of esophageal cancer remains controversial. Although surgery is the mainstay of treatment, incorporation of chemotherapy with/without radiotherapy suggests that a combined approach is worthy of further investigation. Chemoradiotherapy (CRT) is effective for patients with stage II–III esophageal squamous cell carcinoma (SCC), with tolerable toxicities, making it a useful non-surgical treatment option (3). CRT is considered definitive when administered with curative intent for the treatment of locally advanced esophageal SCC. Definitive CRT is the standard management for nonsurgical cases of esophageal cancer, and its outcomes now approach that of surgery (4). However, conventional imaging cannot predict complete clinical response or provide assessment immediately after treatment. We have previously reported on the usefulness of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET for staging of esophageal SCC (5). ^{18}F-FDG-PET offers higher sensitivity, specificity and accuracy for detection of lymph node metastases compared with computed tomography (CT), particularly in the neck and upper thoracic region (5). Moreover, we suggested that the standardized uptake value (SUV) of ^{18}F-FDG-PET prior to definitive CRT is one of the most reliable predictors of response in esophageal cancer, in combination with tumor dimensions and classification (6).

We have also developed L-[3-^{18}F]-α-methyltyrosine (^{18}F-FAMT) as an amino acid tracer for PET imaging and confirmed its potential usefulness in the detection of neoplasms using experimental tumor models (7-9). ^{18}F-FAMT is accumulated in tumor cells solely via an amino acid transport system (10, 11). We originally reported ^{18}F-FAMT PET as being useful for the diagnosis of lymph node metastases in operable esophageal SCC, where its specificity was significantly higher than that of ^{18}F-FDG-PET and CT (12). Furthermore, we reported that ^{18}F-FAMT uptake was significantly positively correlated with depth of invasion, lymph node metastasis, pathological stage, and lymphatic invasion. In the current study, we retrospectively assessed the ability of ^{18}F-FAMT PET to predict the response of esophageal SCC to definitive CRT.

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Key Words: ^{18}F-FAMT PET, chemoradiotherapy, complete response, predictor, esophageal cancer.

Patients and Methods

Patients. We evaluated 40 patients with esophageal SCC who received definitive CRT at the Department of General Surgical Science, Graduate School of Medicine, Gunma University, Japan, between June 2008 and July 2012. Patients with histologically-confirmed primary esophageal SCC were eligible for inclusion. Clinical data from a consecutive series of patients was retrospectively reviewed. Patients were excluded from the study if they had any comorbid malignancies. After providing written informed consent, patients were enrolled in the study. The enrolled patients had the following characteristics: none had received prior treatment; the median age was 67.4 years (range, 52–82 years); and primary tumors were located in the cervical region in 9, upper region in 9, middle region in 19, and lower esophageal in 3 cases.

Tumor stage and disease grade were classified according to the sixth edition of the TNM classification of the International Union Against Cancer (13). Tumor stage was conventionally determined as follows: CT of the neck, chest, and abdomen; endoscopic ultrasound; endoscopy; esophagography; and FDG-PET/CT. Furthermore, none of the patients had diabetes and all blood sugar levels were <120 mg/dl when undergoing the PET scan.

Treatment and clinical outcomes. After the diagnostic procedures, all 40 patients underwent CRT without pretreatment. All patients were considered inoperable because of the presence of one of the following: distant organ metastasis, distant lymph node metastasis, severe organ dysfunction, or patient preference (rejection of surgery). CRT was administered to four patients with cervical esophageal cancer for functional preservation. External radiotherapy was delivered by a two-field technique using a 10-15 MV photon beam at 2 Gy per fraction/day, 5 fractions/week, to a total of 60-66 Gy. Concurrent chemotherapy consisted of docetaxel (60 mg/m^2), cisplatin (50 mg/m^2) administered intravenously over one hour on days 1 and 29 and 5-fluorouracil (5-FU; 600 mg/m^2) administered as a continuous intravenous infusion on days 1-4, and days 29-32.

Clinical evaluation of the primary tumor included repeat endoscopy, esophagography, and CT. All patients underwent a CT scan of the neck, chest, and abdomen with continuous scans of 5-mm slices obtained from the neck to the bottom of the liver after intravenous injection of contrast medium. The clinical response of each primary tumor was evaluated within three months of treatment completion. Treatment evaluations were classified as follows: complete response (CR: complete disappearance of all clinical evidence of existing lesions beyond four weeks) and non-complete response (non-CR: all states except CR such as partial response, stable disease, and progressive disease). Treatment evaluation by ^{18}F-FAMT was performed before CRT at approximately one month before CRT.

PET–CT Studies. ^{18}F-FAMT was produced at our cyclotron facility using the method developed by Tomiyoshi *et al*. (7) and modified based on the method described by Hamacher *et al*. (14). PET–CT studies were performed after injection with 5-6 MBq/kg of ^{18}F-FAMT after fasting for more than 6 h. Sixty min after the administration of the tracer, whole-body images were obtained using PET–CT scanners (Discovery STE; GE Healthcare, USA; Biograph 16; Siemens Medical Solutions Inc., USA). Patients were scanned from the thigh to the head in the arms-down position. X-Ray CT was acquired to perform transmission correction for the PET using the following parameters: 140 kV and 120-240 mAs (varied according to somatometry). No intravenous contrast material was used for CT scanning. Limited breath-holding at normal expiration was employed to avoid motion-induced artifacts and match co-registration of CT and PET images in the area of the diaphragm. On completion of the CT, the PET data (3 min/bed position) were acquired in a three-dimensional mode. CT images were reconstructed using a conventional filtered back-projection method. Attenuation-corrected PET images were reconstructed using an ordered subsets expectation-maximization algorithm into 128 × 128 matrices.

Our Institutional Review Board approved the imaging protocols (3), and all patients gave informed consent before undergoing the examination. Two experienced nuclear medicine physicians qualitatively evaluated all PET images. For semiquantitative analysis, functional images of the standardized uptake value (SUV) were produced using attenuation-corrected transaxial image, injected dose of ^{18}F-FAMT, patient's body weight, and the cross-calibration factor between PET and dose calibrator. SUV was defined as the concentration of radioactivity in the tissue or lesion (MBq/ml) × patient body weight (g)/injected dose (MBq). Maximal SUV was used to represent the uptake of ^{18}F-FAMT in the tumor. Regional lymph nodes evaluated by PET scans were assigned specific numbers to indicate localization in accordance with the Japanese Society for Esophageal Diseases classification guidelines (15). Slight ^{18}F-FAMT uptake was considered a positive result, and no visualized uptake was considered a negative result (SUV=0).

Statistical analysis. The relationships between ^{18}F-FAMT SUVs and both clinical features and the efficacy of treatment were assessed by analysis of variance. Probability values of $p<0.05$ indicated a statistically significant difference.

Results

Primary tumor. In all patients, ^{18}F-FAMT uptake before CRT, determined by the maximal SUV, ranged between 0 and 8.5 g/ml (median, 2.9 g/ml). The mean SUV±standard error of the mean for ^{18}F-FAMT was 3.16±0.31 g/ml. The primary tumor was visualized by ^{18}F-FAMT PET imaging in 38 patients (95%). Using PET, ^{18}F-FAMT uptake was detected in the following tumors (based on TNM classification): 2 of 3 patients at T1 (67%), 4 of 5 patients at T2 (80%), 7 of 7 patients at T3 (100%), and 25 of 25 patients at T4 (100%).

Relationships between ^{18}F-FAMT uptake and clinical features. Relationships between ^{18}F-FAMT uptakes before CRT with the clinical features are shown in Table I. ^{18}F-FAMT uptake was significantly positively correlated with the longitudinal dimension of the tumor, which was measured by pre-treatment esophagography ($p=0.003$), and lymph node metastasis (cN; $p=0.019$) but not with other clinical features. ^{18}F-FDG significantly correlated with depth of invasion (cT; $p=0.007$) but not with other clinical features.

The relationship between ^{18}F-FAMT uptake before CRT and clinical CR is shown in Figure 1. ^{18}F-FAMT uptakes were divided into high-(>3.0 g/ml average of SUV_{max}) and low-accumulation (≤3.0 g/ml) groups. The mean SUV±standard error of the mean for the high- and low-

Table I. *Correlation of L-[3-^{18}F]-a-Methyltyrosine (^{18}F-FAMT) and clinical characteristics in 40 patients with esophageal squamous cell carcinoma.*

Parameter	^{18}F-FAMT uptake (SUV) g/ml		
	No of cases	Mean±SEM	*p*-Value
Gender			
Male	35	3.31± (0.33)	0.213
Female	5	2.13±(0.87)	
Location			
Cervix	9	2.37± (0.48)	
Upper thoracic	9	2.96± (0.44)	0.307
Middle	19	3.39± (0.48)	
Lower	3	4.68± (2.05)	
Longitudinal dimension of tumor			
<Median (51 mm)	20	4.03± (0.50)	0.003
≥Median (51 mm)	20	2.28± (0.25)	
Tumor type			
0	4	1.93± (0.71)	
1	8	2.49± (0.49)	
2	8	2.73± (0.57)	0.092
3	17	4.13± (0.54)	
4	3	2.23± (0.46)	
TNM clinical classification			
cT			
Tl	3	1.56± (0.84)	
T2	5	1.65± (0.50)	0.081
T3	7	3.19± (0.61)	
T4	25	3.64± (0.41)	
cN			
N0	8	1.87± (0.53)	0.019
Nl	32	3.48± (0.34)	
cM			
M0	36	3.22± (0.33)	0.560
Ml	4	2.61± (0.78)	
cStage			
I	2	0.89± (0.89)	
II	4	1.70± (0.64)	0.110
III	5	3.14± (0.81)	
IV	29	3.52± (0.36)	

SEM: Standard error of the mean, SUV: standardized uptake value.

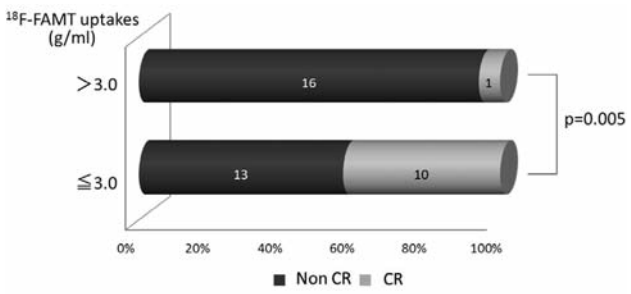

Figure 1. *Relationship between L-[3-^{18}F]-α-Methyltyrosine (^{18}F-FAMT) uptake before chemoradioterapy and clinical complete response (CR). The group with uptake ≤3.0 g/ml had significantly higher CR rates than the group with uptake >3.0 g/ml.*

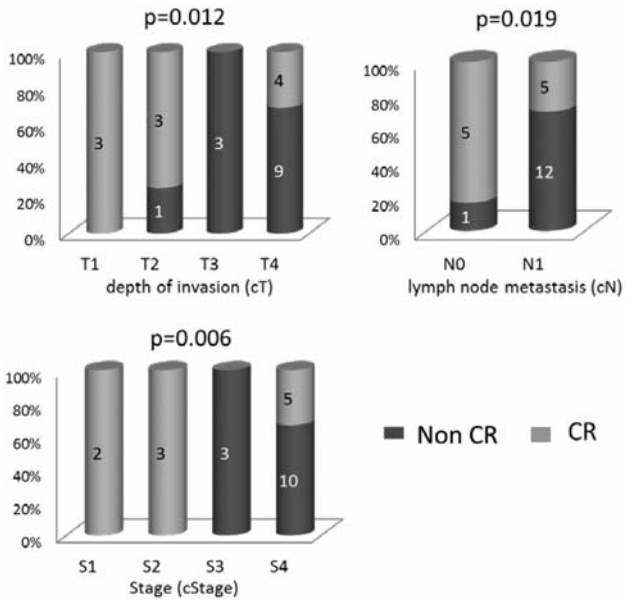

Figure 2. *Relationships between complete response (CR) rate and clinical features in the low-^{18}F-FAMT accumulation group. The CR rate was significantly negatively correlated to depth of invasion, lymph node metastasis, and stage.*

accumulation group were 4.81±0.43 and 1.93±0.18 g/ml, respectively. The low-accumulation group had significantly higher CR rate than the high-accumulation group (p=0.005). In the low-accumulation group, the CR rate was significantly negatively correlated with the depth of invasion (cT; p=0.012), lymph node metastasis (cN; p=0.019), and Stage (cStage; p=0.006) (Figure 2). In the high-^{18}F-FAMT accumulation group, there was no significant correlation with CR rate or clinical features.

The lymph node metastasis category (N0/N1), diagnosed by PET, was added as a precise predictor of treatment effect. The low-^{18}F-FAMT accumulation group with N0 had a significantly higher CR rate than those with N1 (Figure 3, p=0.016). Moreover, addition of cM0 to the low ^{18}F-FAMT accumulation group with N0 revealed a higher CR rate than that the group of cM1 (p=0.021).

Discussion

The role and potential value of PET as a non-invasive imaging modality has been widely investigated in recent years (16-19). ^{18}F-FDG PET provides physiological information that enables the diagnosis of cancer based on altered tissue glucose metabolism (20), and it may be of value in assessing the

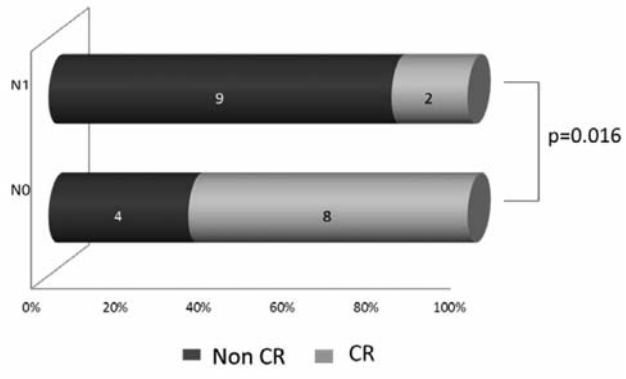

Figure 3. *The effect of the addition of lymph node metastasis category (N0/N1) to the low ^{18}F-FAMT accumulation group. The group with uptake ≤3.0 g/ml with N0 disease had a significantly higher CR rate than those with N1 disease.*

pathological response to neoadjuvant therapy. In particular, low ^{18}F-FDG uptake after therapy may provide a reliable assessment of response to therapy (21). Moreover, multivariate analysis revealed that uptake (SUV) before CRT was an independent predictor of clinical response to definitive CRT for esophageal cancer (6). ^{18}F-FAMT-PET has also been shown to have a high specificity for malignant tumors; we had previously reported on the usefulness of ^{18}F-FAMT PET for the diagnosis of lymph node metastasis in operable esophageal SCC (12). In the study, the specificity of ^{18}F-FAMT PET was significantly higher than that of ^{18}F-FDG PET and CT in the evaluation of individual lymph node groups. Therefore, as a diagnostic procedure, ^{18}F-FAMT PET has a higher specificity and positive predictive value compared to ^{18}F-FDG-PET. This point is important for the preoperative workup.

In addition, we investigated the usefulness of ^{18}F-FAMT PET as a predictor of definitive CRT response in patients with esophageal cancer. This study included more advanced esophageal cancer stages (cT4=25) than previous reports. In patients with esophageal cancer, disease was widely spread to lymph nodes of the neck, mediastinal, and abdominal regions. Unfortunately, it is difficult to diagnose the spread of advanced esophageal cancer. There is no reported correlation between ^{18}F-FAMT PET and treatment efficacy in patients with esophageal cancer, although several reports have demonstrated the utility of ^{18}F-FDG PET in predicting treatment outcomes (22-24).

We found that uptake of ^{18}F-FAMT in primary tumors was higher than previously reported, consistent with the inclusion of more advanced esophageal cancer. ^{18}F-FAMT uptake was significantly positively correlated with lymph node metastasis, consistent with findings in the previous study on patients with operable esophageal cancer. Uptake of ^{18}F-FAMT by the primary tumor was a good predictor of lymph node metastasis in both operable cases and those requiring definitive CRT.

We assessed whether ^{18}F-FAMT uptake prior to CRT was a predictor of clinical response to definitive CRT; significant correlations were found between clinical response and uptake. In addition, the low ^{18}F-FAMT accumulation group had significantly higher CR rates than the high accumulation group, indicating that ^{18}F-FAMT uptake prior to CRT is useful in predicting the rate of CR. Furthermore, the addition of a lymph node metastasis category (diagnosed by CT) to the low ^{18}F-FAMT accumulation group resulted better prediction of the CR rate for CRT. Thus, it was possible to identify groups where treatment is less effective, allowing for resources to be focused where treatment is likely to be most beneficial. Furthermore, CR was found to be significantly correlated with depth of invasion (cT), lymph node metastasis (cN), and stage (cStage) in the low ^{18}F-FAMT accumulation group suggesting that ^{18}F-FAMT is a significant predictor of esophageal cancer progression in this group. When ^{18}F-FAMT uptake by the primary tumor is low (<3.0 g/ml), the tumor has a higher possibility of CR.

We report on the effectiveness of ^{18}F-FAMT-PET for inoperable cases of esophageal cancer. Significant correlations were identified between clinical response and ^{18}F-FAMT uptake before CRT, particular in cases with low ^{18}F-FAMT accumulation and those without lymph node metastases. An important limitation of the present study is that it included a small number of patients; further clinical research with more patients will be required to confirm the results and demonstrate reliability. In addition, we were unable to show the prognostic value of ^{18}F-FAMT PET due to the short observation period, which is a factor that future research will need to resolve. We anticipate that diagnostic imaging with ^{18}F-FAMT PET can be implemented in the near future, in order to facilitate individualized therapy for patients with esophageal cancer.

References

1 Daly JM, Karnell LH and Menck HR: National Cancer Data Base report on esophageal carcinoma. Cancer *78*: 1820-1828,1996.

2 Wobst A, Audisio RA, Colleoni M and Geraghty JG: Oesophageal cancer treatment: Studies, strategies and facts. Ann Oncol *9*: 951-962, 1998.

3 Kato K, Muro K, Minashi K, Ohtsu A, Ishikura S, Boku N, Takiuchi H, Komatsu Y, Miyata Y and Fukuda H: Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group (JCOG): Phase II study of chemoradiotherapy with 5-fluorouracil and cisplatin for stage II-III esophageal squamous cell carcinoma: JCOG trial (JCOG 9906). Int J Radiat Oncol Biol Phys *81*: 684-690, 2011.

4 Ohtsu A: Chemoradiotherapy for esophageal cancer: current status and perspectives. Int J Clin Oncol *9*: 444-450, 2004.

5 Kato H, Miyazaki T, Nakajima M, Takita J, Kimura H, Faried A, Sohda M, Fukai Y, Masuda N, Fukuchi M, Manda R, Ojima H, Tsukada K, Kuwano H, Oriuchi N and Endo K: Comparison

between whole-body positron-emission tomography and bone scintigraphy in evaluating bony metastases of esophageal carcinomas. Anticancer Res *25*: 4439-4444, 2005.

6 Kato H, Fukuchi M, Miyazaki T, Nakajima M, Tanaka N, Inose T, Kimura H, Faried A, Saito K, Sohda M, Fukai Y, Masuda N, Manda R, Ojima H, Tsukada K, Oriuchi N, Endo K, Nonaka T, Shioya M, Ishikawa H, Sakurai H, Nakano T and Kuwano H: Prediction of response to definitive chemoradiotherapy in esophageal cancer using positron-emission tomography. Anticancer Res *27*: 2627-2633, 2007.

7 Tomiyoshi K, Amed K, Muhammad S, Higuchi T, Inoue T, Endo K and Yang D: Synthesis of isomers of ^{18}F-labelled amino acid radiopharmaceutical: Position 2- and 3-L-^{18}F-α-methyltyrosine using a separation and purification system. Nucl Med Commun *18*: 169-175, 1997.

8 Inoue T, Tomiyoshi K, Higuichi T, Ahmed K, Sarwar M, Aoyagi K, Amano S, Alyafei S, Zhang H, Endo K: Biodistribution studies on L-3-[fluorine-18]fluoro-α-methyl tyrosine: A potential tumor-detecting agent. J Nucl Med *39*: 663-667, 1998.

9 Amano S, Inoue T, Tomiyoshi K, Ando T and Endo K: *In vivo* comparison of PET and SPECT radiopharmaceuticals in detecting breast cancer. J Nucl Med *39*: 1424-1427, 1998.

10 Uchino H, Kanai Y, Kim DK, Wempe MF, Chairoungdua A, Morimoto E, Anders MW and Endou H: Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1): Insights into the mechanisms of substrate recognition. Mol Pharmacol *61*: 729-737, 2002.

11 Kim DK, Kanai Y, Choi HW, Tangtrongsup S, Chairoungdua A, Babu E, Tachampa K, Anzai N, Iribe Y, Endou H: Characterization of the system L amino acid transporter in T24 human bladder carcinoma cells. Biochim Biophys Acta *1565*: 112-121, 2002.

12 Sohda M, Kato H, Suzuki S, Tanaka N, Sano A, Sakai M, Inose T, Nakajima M, Miyazaki T, Fukuchi M, Oriuchi N, Endo K, Kuwano H: ^{18}F-FAMT-PET is useful for the diagnosis of lymph node metastasis in operable esophageal squamous cell carcinoma. Ann Surg Oncol *17*: 3181-3186, 2010.

13 Sobin LH and Wittekind C: TNM Classification of Malignant Tumors. 6th ed. New York: John Wiley & Sons. 2002.

14 Hamacher K, Coenen HH and Stöcklin G: Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med *27*: 235-238, 1986.

15 Japanese Society for Esophageal Disease. Guidelines for the clinical and pathological studies on carcinoma of the esophagus (10th edition). Tokyo: Kanehara, 2008.

16 Bares R, Klever P, Hauptmann S, Hellwig D, Fass J, Cremerius U, Schumpelick V, Mittermayer C and Bull U: F-18 Fluorodeoxyglucose PET *in vivo* evaluation of pancreatic glucose metabolism for detection of pancreatic cancer. Radiology *192*: 79-86, 1994.

17 Inoue T, Kim EE, Komaki R, Wong FC, Bassa P, Wong WH, Yang DJ, Endo K and Podoloff DA: Detecting recurrent or residual lung cancer with FDG-PET. J Nucl Med *36*: 788-793, 1995.

18 Ahuja V, Coleman RE, Herndon J, Patz EF Jr.: The prognostic significance of fluorodeoxyglucose positron emission tomography imaging for patients with non-small cell lung carcinoma. Cancer *83*: 918-924, 1998.

19 Coleman RE: PET in lung cancer. J Nucl Med *40*: 814-820, 1999.

20 Bar-Shalom R, Valdivia AY and Blaufox MD: PET imaging in oncology. Semin Nucl Med *30*: 150-185, 2000.

21 Kato H, Kuwano H, Nakajima M, Miyazaki T, Yoshikawa M, Masuda N, Fukuchi M, Manda R, Tsukada K, Oriuchi N, Endo K: Usefulness of positron-emission tomography for assessing the response of neoadjuvant chemoradiotherapy in patients with esophageal cancer. Am J Surg *184*: 279-283, 2002.

22 Swisher SG, Maish M, Erasmus JJ, Correa AM, Ajani JA, Bresalier R, Komaki R, Macapinlac H, Munden RF, Putnam JB, Rice D, Smythe WR, Vaporciyan AA, Walsh GL, Wu TT and Roth JA: Utility of PET, CT, and EUS to identify pathologic responders in esophageal cancer. Ann Thorac Surg *78*: 1152-1160, 2004.

23 Westerterp M, van Westreenen HL, Reitsma JB, Hoekstra OS, Stoker J, Fockens P, Jager PL, Van Eck-Smit BL, Plukker JT, van Lanschot JJ and Sloof GW: Esophageal cancer: CT, endoscopic US, and FDG PET for assessment of response to neoadjuvant therapy – systematic review. Radiology *236*: 841-851, 2005.

24 Flamen P, Van Cutsem E, Lerut A, Cambier JP, Haustermans K, Bormans G, De Leyn P, Van Raemdonck D, De Wever W, Ectors N, Maes A and Mortelmans L: Positron emission tomography for assessment of the response to induction radiochemotherapy in locally advanced oesophageal cancer. Ann Oncol *13*: 361-368, 2002.

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