and technical challenges. Even if great care is taken to maintain the position of the head of the probe, movements are difficult to monitor and usually cannot be excluded, potentially influencing measurement results. Longitudinal oxygen-probe measurements have also been criticized because the continued presence of the head of the probe in tissue may decrease or disrupt tissue perfusion because of local tissue injury, causing edema and microhematomata that might interfere with longitudinal measurements (even if the oxygen sensor system itself does not consume oxygen during measurements). Also, animals breathing carbogen for more than 3 h must have been anesthetized, and the observed effects on tumor oxygenation may have been in part related to an overall effect of prolonged anesthesia. In addition, because of the heterogeneity of tumor tissue, oxygenation readings may not have been representative of the tumor as a whole. Therefore, some researchers favor motorized probes that allow the investigation of a certain fraction of the tumor by advancing and retracting the tip of the probe in defined ways. Using such an oxygen sensor system, we were able to show that the uptake of ^{18}F-misonidazole (FMISO) is inversely correlated with tissue oxygenation in a dedicated hypoxia model in porcine liver (*3,4*). Even if prolonged carbogen breathing resulted in a return of hypoxia in the tumors investigated by Kaanders et al., such a behavior does not necessarily have to occur in the EMT6 tumor xenografts used in our study. We have now repeatedly shown that prolonged (4 h) carbogen breathing generally decreases tumor tissue hypoxia as measured by ^{18}F-azomycin arabinoside (FAZA) in EMT6 tumors using biodistribution studies, autoradiography, and small-animal PET (*1,5*).

Troost et al. argued that the signal intensity of pimonidazole decreased significantly after carbogen breathing (in 2 of 3 tumor lines) and that the ^{18}F-FMISO signal intensity decreased slightly, although not significantly (*6*). Because pimonidazole is generally regarded as a suitable immunohistochemical marker of tissue hypoxia, a strong and stable correlation of pimonidazole staining and ^{18}F-FMISO uptake would be expected if the retention of ^{18}F-FMISO in tissue is in fact (mostly) oxygenation-dependent. We would like to point out that the in vivo kinetics of ^{18}F-FMISO and ^{18}F-FAZA are quite different. Compared with ^{18}F-FAZA, ^{18}F-FMISO displays a significantly slower clearance from normal (normoxic) tissues (*5*). Imaging at relatively early time points (1 h after tracer injection) may therefore not be sufficient to detect an oxygenation-specific signal from ^{18}F-FMISO and could have contributed to a more variable spatial correlation between pimonidazole and ^{18}F-FMISO in their study (*6*).

Troost et al. further reasoned that the CO_2 component of the carbogen can lead to a decreased tumor perfusion mediated by a steal effect of vessels surrounding the tumor tissue (*7*) and that this effect may have caused the discrepancy found between the results for ^{18}F-FAZA and for hypoxia-inducible factor-1α. Our results indicated that after 4 h of carbogen breathing, the HIF-1α expression was not influenced whereas the hypoxic tumor surface as depicted by ^{18}F-FAZA was significantly decreased, compared with ambient (control) conditions. We dispute that this effect would explain our results. ^{18}F-FAZA was coinjected with ^{125}I-gluco-RGD peptide. Although the mean ^{18}F-FAZA uptake decreased, the mean ^{125}I-gluco-RGD uptake was unaffected; therefore, any reduction in tumor perfusion (steal effect) would have caused a reduction in tracer delivery and, thus, a reduction of uptake for both tracers. However, our data indicated that the overall ^{125}I-gluco-RGD uptake was not modified by carbogen, making a steal phenomenon highly unlikely.

We agree that our results are specific to the tumor model used. However, we were limited to infrequent tumor cell lines that regularly result in tumor hypoxia and at the same time lack any $\alpha_v\beta_3$ expression on the tumor cell surface (*8*), allowing us to use ^{125}I-gluco-RGD uptake on activated endothelial cells as a measure of $\alpha_v\beta_3$-mediated angiogenesis. Although our tumor model resulted in an unpredictable (random) pattern of hypoxia and angiogenesis within the tumor core, other tumor cell lines may produce different patterns of hypoxia, which—by the way—will also likely undergo changes over time, especially when treatment is applied. Because of the extensive tissue heterogeneity observed in many malignancies, molecular imaging of the tumor microvasculature of individual cancers is crucial. We should not be discouraged by technical difficulties but continue to translate these observations into the clinic by evaluating tumor perfusion, angiogenesis, and tissue oxygenation to improve our understanding of treatment response to chemotherapy and radiation on an individual basis.

REFERENCES

1. Picchio M, Beck R, Haubner R, et al. Intratumoral spatial distribution of hypoxia and angiogenesis assessed by ^{18}F-FAZA and ^{125}I-gluco-RGD autoradiography. *J Nucl Med.* 2008;49:597–605.
2. Kaanders JH, Bussink J, van der Kogel AJ. ARCON: a novel biology-based approach in radiotherapy. *Lancet Oncol.* 2002;3:728–737.
3. Piert M, Machulla H, Becker G, et al. Introducing fluorine-18 fluoromisonidazole positron emission tomography for the localisation and quantification of pig liver hypoxia. *Eur J Nucl Med.* 1999;26:95–109.
4. Piert M, Machulla HJ, Becker G, Aldinger P, Winter E, Bares R. Dependency of the [^{18}F]fluoromisonidazole uptake on oxygen delivery and tissue oxygenation in the porcine liver. *Nucl Med Biol.* 2000;27:693–700.
5. Piert M, Machulla H-J, Picchio M, et al. Hypoxia-specific tumor imaging with ^{18}F-fluoroazomycin arabinoside. *J Nucl Med.* 2005;46:106–113.
6. Troost EG, Laverman P, Kaanders JH, et al. Imaging hypoxia after oxygenation-modification: comparing [^{18}F]FMISO autoradiography with pimonidazole immunohistochemistry in human xenograft tumors. *Radiother Oncol.* 2006;80:157–164.
7. van der Sanden BP, Heerschap A, Hoofd L, et al. Effect of carbogen breathing on the physiological profile of human glioma xenografts. *Magn Reson Med.* 1999;42:490–499.
8. Frochot C, Di Stasio B, Vanderesse R, et al. Interest of RGD-containing linear or cyclic peptide targeted tetraphenylchlorin as novel photosensitizers for selective photodynamic activity. *Bioorg Chem.* 2007;35:205–220.

Morand Piert
University of Michigan
Ann Arbor, Michigan

DOI: 10.2967/jnumed.108.053835

Brain SPECT by ^{99m}Tc-Tetrofosmin for the Differentiation of Tumor Recurrence from Radiation Injury

TO THE EDITOR: We read with great interest the article by Terakawa et al. (*1*) concerning the discrimination between tumor recurrence and radiation necrosis by PET with L-methyl-^{11}C-methionine (^{11}C-MET). The authors studied 77 brain tumor patients after surgical excision and radiotherapy; all cases presented with an indication of recurrent tumor (metastasis or glioma) or radiation necrosis on MRI follow-up. The results showed that the mean lesion-to-normal ratio was the most valuable index for differentiating recurrence from radiation necrosis. A mean lesion-to-normal ratio greater than 1.41 provided the best sensitivity and specificity for metastatic brain tumor, and a mean ratio greater than 1.58 provided the best sensitivity and specificity for glioma (*1*).

Radiation necrosis is a potential long-term complication of radiotherapy or radiosurgery and is usually indistinguishable from true tumor recurrence by means of CT and MRI. Several advanced

MRI techniques such as diffusion- and perfusion-weighted imaging and MR spectroscopy have been suggested to have increased sensitivity and accuracy, compared with conventional imaging (*2*); however, these techniques have certain limitations in cases of mixed necrosis and recurrence, and overlap between tumor recurrence and radiation necrosis groups has also been noted (*3*).

Functional metabolic imaging by PET and SPECT has been also evaluated. Nonetheless, PET studies are often cost-prohibitive and not widely available. Therefore, there has been much interest in using SPECT as a feasible alternative imaging technique. The major tumor-seeking radiotracers that have been extensively evaluated are ^{201}Tl and ^{99m}Tc-sestamibi. Furthermore, several in vitro studies on glioma cell lines have substantiated a potential superiority of ^{99m}Tc-tetrofosmin (^{99m}Tc-TF) over ^{99m}Tc-sestamibi for brain tumor imaging, since ^{99m}Tc-TF accumulation is independent of the multidrug-resistance phenotype of the cell (*4,5*). On the basis of these reports, we investigated the in vivo imaging properties of ^{99m}Tc-TF in tumors of the central nervous system. ^{99m}Tc-TF is a tumor-seeking diphosphine that does not cross the intact blood–brain barrier, and uptake of ^{99m}Tc-TF depends on regional blood flow and cell membrane integrity, thus reflecting cellular metabolic status and viability. We fount that ^{99m}Tc-TF could successfully differentiate tumor recurrence from radiation injury (*6*). We also evaluated the relationship between glioma and meningioma proliferation (as assessed by the immunohistologic index Ki-67 and flow cytometry) and ^{99m}Tc-TF uptake. In both tumor types, we verified a strong positive linear correlation between tracer uptake and tumor proliferative potential and aggressiveness (*7–9*). Furthermore, we reported that ^{99m}Tc-TF SPECT could hold a role in differentiating neoplastic from nonneoplastic intracerebral hemorrhage (*10*). Thus, we propose that metabolic brain imaging by ^{99m}Tc-TF SPECT can contribute considerably to the management of patients who undergo radiotherapy and develop new lesions or symptoms. Comparative studies with other metabolic imaging techniques such as ^{11}C-MET would be valuable to evaluate this critical issue.

REFERENCES

1. Terakawa Y, Tsuyuguchi N, Iwai Y, et al. Diagnostic accuracy of ^{11}C-methionine PET for differentiation of recurrent brain tumors from radiation necrosis after radiotherapy. *J Nucl Med.* 2008;49:694–699.
2. Hollingworth W, Medina LS, Lenkinski RE, et al. A systematic literature review of magnetic resonance spectroscopy for the characterization of brain tumors. *AJNR.* 2006;27:1404–1411.
3. Rock JP, Scarpace L, Hearshen D, et al. Associations among magnetic resonance spectroscopy, apparent diffusion coefficients, and image-guided histopathology with special attention to radiation necrosis. *Neurosurgery.* 2004;54:1111–1117.
4. Le Jeune N, Perek N, Denoyer D, Dubois F. Study of monoglutathionyl conjugates TC-99M-sestamibi and TC-99M-tetrofosmin transport mediated by the multidrug resistance-associated protein isoform 1 in glioma cells. *Cancer Biother Radiopharm.* 2005;20:249–259.
5. Le Jeune N, Perek N, Denoyer D, Dubois F. Influence of glutathione depletion on plasma membrane cholesterol esterification and on Tc-99m-sestamibi and Tc-99m-tetrofosmin uptakes: a comparative study in sensitive U-87-MG and multidrug-resistant MRP1 human glioma cells. *Cancer Biother Radiopharm.* 2004;19:411–421.
6. Alexiou GA, Fotopoulos AD, Papadopoulos A, Kyritsis AP, Polyzoidis KS, Tsiouris S. Evaluation of brain tumor recurrence by ^{99m}Tc-tetrofosmin SPECT: a prospective pilot study. *Ann Nucl Med.* 2007;21:293–298.
7. Alexiou GA, Tsiouris S, Goussia A, et al. Evaluation of glioma proliferation by ^{99m}Tc-tetrofosmin. *Neuro Oncol.* 2008;10:104–105.
8. Alexiou GA, Vartholomatos G, Tsiouris S, et al. Evaluation of meningioma aggressiveness by ^{99m}Tc-tetrofosmin SPECT. *Clin Neurol Neurosurg.* May 7, 2008 [Epub ahead of print].
9. Fotopoulos AD, Alexiou GA, Goussia A, et al. ^{99m}Tc-tetrofosmin brain SPECT in the assessment of meningiomas: correlation with histological grade and proliferation index. *J Neurooncol.* May 6, 2008 [Epub ahead of print].
10. Alexiou GA, Bokharhii JA, Kyritsis AP, Polyzoidis KS, Fotopoulos AD. Tc-99m tetrofosmin SPECT for the differentiation of a cerebellar hemorrhage mimicking a brain metastasis from a renal cell carcinoma. *J Neurooncol.* 2006;78:207–208.

George A. Alexiou
Spyridon Tsiouris
Athanasios P. Kyritsis
Konstantinos S. Polyzoidis
Andreas D. Fotopoulos
University of Ioannina School of Medicine
Ioannina, Greece

DOI: 10.2967/jnumed.108.054494

REPLY: We thank Dr. Alexiou and his colleagues for their thoughtful comments regarding our article evaluating the diagnostic accuracy of PET with L-methyl-^{11}C-methionine (^{11}C-MET) in differentiating recurrent brain tumors from radiation necrosis after radiotherapy (*1*).

Although several studies show the utility of SPECT in distinguishing recurrent brain tumors from radiation necrosis, PET is known to be superior to SPECT in spatial resolution and ability to be quantified. Furthermore, our previous study evaluating brain tumors using ^{11}C-MET and ^{201}Tl-chloride suggested that ^{11}C-MET PET is more useful than ^{201}Tl SPECT for that purpose (*2*). On the basis of these findings, we have recently preferred ^{11}C-MET PET to ^{201}Tl SPECT, and our paper demonstrated that quantitative values determined from ^{11}C-MET PET data can differentiate recurrent brain tumors from radiation necrosis with acceptable diagnostic accuracy (*1*).

However, the use of ^{11}C-MET is often limited to facilities equipped with a cyclotron because the half-life of ^{11}C-MET is relatively short, thus making it costly. In contrast, SPECT is a less costly imaging technique and widely available. Nevertheless, a definitive SPECT radiotracer for the differentiation of recurrent brain tumors from radiation necrosis has not yet been established. We have examined patients with brain tumors using both ^{99m}Tc-tetrofosmin and ^{99m}Tc-sestamibi SPECT in clinical settings but have not yet obtained sufficient data to draw any conclusions. In the field of SPECT, ^{99m}Tc-tetrofosmin may be a promising radiotracer for the differentiation of recurrent brain tumors from radiation necrosis (*3*) and is the area in which we are most interested. We agree with the statement by Dr. Alexiou and his colleagues that comparative studies between these 2 imaging techniques with ^{11}C-MET PET and ^{99m}Tc-tetrofosmin SPECT are most interesting and helpful.

REFERENCES

1. Terakawa Y, Tsuyuguchi N, Iwai Y, et al. Diagnostic accuracy of ^{11}C-methionine PET for differentiation of recurrent brain tumors from radiation necrosis after radiotherapy. *J Nucl Med.* 2008;49:694–699.
2. Tsuyuguchi N, Sunada I, Iwai Y, et al. Methionine positron emission tomography of recurrent metastatic brain tumor and radiation necrosis after stereotactic radiosurgery: is a differential diagnosis possible? *J Neurosurg.* 2003;98:1056–1064.
3. Alexiou GA, Fotopoulos AD, Papadopoulos A, Kyritsis AP, Polyzoidis KS, Tsiouris S. Evaluation of brain tumor recurrence by ^{99m}Tc-tetrofosmin SPECT: a prospective pilot study. *Ann Nucl Med.* 2007;21:293–298.

Yuzo Terakawa
Naohiro Tsuyuguchi
Osaka City University Graduate School of Medicine
Osaka, Japan

DOI: 10.2967/jnumed.108.054783