Intratumoral Spatial Distribution of Hypoxia and Angiogenesis Assessed by ^{18}F-FAZA and ^{125}I-Gluco-RGD Autoradiography

TO THE EDITOR: With great interest, we read the paper by Picchio et al. (*1*). The authors applied a double-tracer autoradiography technique for the identification of hypoxia and angiogenesis under ambient conditions and after carbogen breathing in EMT6 xenografts. For validation purposes, the endogenous hypoxia marker hypoxia-inducible factor-1α (HIF-1α) was stained immunohistochemically and quantified.

In this comment, we will discuss 2 aspects of the experimental design chosen by Picchio et al.: first, the timing and duration of carbogen breathing, and second, the limitations of using a single xenograft line as a tumor model.

In recent studies using direct partial pressure of oxygen measurement with the OxyLite (Oxford Optronics) system on xenograft tumor models, we have shown that hypoxia decreased within 5 min after the start of carbogen breathing (*2*). Important in this respect is that Kaanders et al. previously showed that the effect of carbogen breathing on hypoxia decreased after 60 min of breathing time and that prolonged carbogen breathing resulted in a return of hypoxia to baseline levels (*3*). In subsequent experiments, we analyzed the effect of 60 min of carbogen breathing on tumor hypoxia using ^{18}F-misonidazole (FMISO) autoradiography and immunohistochemical staining of the 2-nitroimidazole exogenous hypoxia marker pimonidazole. We found that the pimonidazole signal intensity decreased significantly after carbogen breathing (in 2 of 3 tumor lines) and that the ^{18}F-FMISO signal intensity decreased slightly, albeit not significantly (*4*).

Our experimental design and findings differ substantially from those of Picchio et al. (*1*). In their study, the authors chose HIF-1α as an endogenous marker for tumor cell hypoxia. Also, the carbogen breathing time was, at 4 h, relatively long compared with 60 min in our studies and may have subsequently led to a rehypoxygenation (*3*). Furthermore, van der Sanden et al. demonstrated that the CO_2 component of carbogen can lead to decreased blood perfusion in tumor vessels. This was explained by a steal effect of vessels surrounding the tumor tissue, which may affect tracer uptake and tracer washout (*5*). These phenomena might explain the discrepancy between the ^{18}F-azomycin arabinoside (FAZA) and HIF-1α results by Picchio et al., as their experimental design included a 4-h period of carbogen breathing. Through vascular shutdown, the ^{18}F-FAZA inflow and accumulation may have been hampered, whereas HIF-1α staining remained positive or returned to baseline levels in response to hypoxia as a consequence of the prolonged carbogen breathing time (4 h).

The use of a single tumor model may limit the generalizability, because recent studies from our laboratory show that the microregional distribution of ^{18}F-FMISO is dependent on tumor model (*6*). We have identified 3 distinct patterns of hypoxia distribution: ribbonlike, patchy, and mixed (*7*). By using ^{18}F-FMISO autoradiography and pimonidazole immunohistochemistry, we found that the correlation between the 2 imaging modalities was dependent on the tumor-model–specific microarchitecture. Of the 10 xenografted human head and neck cancer models studied, the 3 tumors with a ribbonlike distribution of hypoxia all showed a good correlation between ^{18}F-FMISO autoradiography and pimonidazole immunohistochemistry. In tumors with a patchy distribution of hypoxia, ^{18}F-FMISO accumulation was detected throughout the tumor section (*6*).

In conclusion, assessment of tumor hypoxia after carbogen breathing by means of PET depends on the timing and duration of carbogen breathing. Furthermore, the distinct microarchitecture of the tumor model studied affects the extent to which hypoxia can be reduced by carbogen breathing. Therefore, caution must be taken when using PET for hypoxia assessment and hypoxia monitoring during treatment.

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REPLY: We greatly appreciate the thoughtful comments of Troost et al. on the potential influence of timing and duration of carbogen breathing on tracer uptake in our study (*1*).

In their letter, Troost et al. stressed that the effect of carbogen breathing on hypoxia decreased after 60 min of breathing time and that prolonged carbogen breathing returned hypoxia to baseline measurements. In the cited article, tissue oxygenation measurements were performed on 2 mice bearing human tumor xenografts using a fiberoptic probe with a luminescence-based optical O_2 sensor (*2*). Typically, such probes are advanced into the tumor tissue and measurements are taken at the tip of the probe. Although direct oxygen probes are regarded as the gold-standard measurement of tissue oxygenation, they are limited because of their invasiveness

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.

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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