Human Cerenkov Imaging Using ^{18}F-FDG

TO THE EDITOR: We read with great interest the ahead-of-print article of Thorek et al. (*1*) recently published online in *The Journal of Nuclear Medicine* on human Cerenkov imaging using ^{18}F-FDG. Having obtained a similar finding (*2*) using ^{131}I, we would like to share our knowledge on this topic and comment on some specific claims.

The first issue we would like to underline is related to the set-up of the imaging apparatus. More precisely, it is well known that the Cerenkov light spectrum has an inverse square dependence on the wavelength (*3*) and, thus, is more intense in the ultraviolet region than in the visible region. However, if one considers the strong tissue absorption of light below 620 nm caused by hemoglobin, the spectrum of the Cerenkov radiation escaping from the tissues contains mainly wavelengths above 630 nm. In a recent paper (*4*), cited also by Thorek et al., we showed that to improve the in vivo detection of Cerenkov sources it is useful to optimize the optical imaging system in the red, near-infrared region (650–850 nm). It is thus not clear to us why the authors decided to use a short-pass filter with a cutoff at 605 nm since in this way they rejected most of the Cerenkov light reaching the body surface. We also are interested in knowing the characteristics and the manufacturer of the objective used to acquire axilla images (estimated field of view, at least 10 × 10 cm) at a very short distance.

Looking at Figure 2, we noticed the absence of any direct charge-coupled-device detection of γ rays. This is a bit surprising considering also the small working distance from the patient (8 cm). It would thus be interesting to know if the authors applied any γ-rejection algorithm.

Thorek et al. (*1*) claimed that our human Cerenkography image was obtained with a much higher dose of ^{131}I. This is not entirely true, since the difference between the injected doses is only 14%, or more precisely, 550 MBq of ^{131}I with respect to 470 MBq of ^{18}F-FDG for the representative patient shown in Figure 2. Second, for a fair comparison of the results in terms of Cerenkov light production, it is useful to remember that the emission of Cerenkov radiation is closely related to the decay scheme of the radioisotope; in this case, ^{18}F emits about 2.5 times more Cerenkov light for each decay than does ^{131}I (*5*). We do agree that ^{131}I thyroid uptake can be typically up to 50%, resulting in an equivalent ^{18}F-FDG uptake of 110 MBq. Considering a spheric lymph node 1.5–2 cm in radius and an uptake value of 0.05 MBq/mL, the corresponding ^{18}F-FDG activity is approximately 0.7–1.7 MBq—that is, 2, not 4 (!), orders of magnitude less than the value claimed in the "Discussion" section by Thorek et al.

Figure 3 of the article by Thorek et al. (*1*) plots a correlation between the Cerenkov signal and the ^{18}F-FDG concentration measured by PET. This correlation measured in vivo is somewhat surprising since, in this case, the different tissue attenuation (e.g., >different source depth) and not the source strength (MBq/mL) should dominate in determining the average value of the detected Cerenkov signal. Also, the plotted data show that the magnitude of the Cerenkov signal is almost comparable to the contralateral side (except for a single patient). In particular, by considering the point corresponding to the patient in Figure 2 (maximum ^{18}F-FDG concentration. 0.05 MBq/mL), one finds a small difference with respect to the contralateral side points.

Thorek et al. (*1*) provides a set of system linearity measurements by performing in vitro imaging of a 24-well polycarbonate plate filled with ^{18}F-FDG at different time points and, thus, of different concentrations. As one can see by looking at Figure 1A of the article, the detected Cerenkov signal is quite noisy at a concentration of 0.1 MBq/mL even without any attenuating material. We were thus a bit surprised that the authors were able to detect, at a tissue depth greater than 1 cm, a Cerenkov signal corresponding to an ^{18}F-FDG concentration of 0.03–0.05 MBq/mL. Figure 2 also seems to show that the patient was not shaved, thus making it even more surprising that the authors could detect Cerenkov light crossing the axillary hair.

To summarize, the paper of Thorek et al. (*1*) contains some puzzling imaging methods and results that in our opinion need to be better explained or justified.

Note: The ahead-of-print article (*1*) of Thorek et al. was modified after the submission of our letter to the editor. Our criticisms related to the optical filter and the correlation shown in Figure 3 no longer apply to the final version (*6*).

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REPLY: We appreciate the opportunity to reply to the letter by Spinelli and Boschi about our recent publication (*1*). In that pilot work, a high-sensitivity intensified charge-coupled-device camera was used to collect Cerenkov luminescence emitted from lymph node uptake of ^{18}F-FDG in patients after their diagnostic PET/CT scan. The work represented an early result of an ongoing pilot clinical trial of the feasibility of Cerenkov luminescence imaging of ^{18}F-FDG in patients. As noted in the article, this work followed the demonstration by Spinelli et al. (*2*) of acquisition of Cerenkov luminescence images in a patient after injection of therapeutic ^{131}I for hyperthyroidism.

Several questions were raised about the characteristics of the image-capture device. Further elaboration will help to enable replication of these measurements. A 50-mm Schneider wide-

aperture (f/0.95) lens was used with the camera running at a frame rate of 120 frames/s, similar to a list-mode acquisition in nuclear medicine. This method enables software-based real-time removal of frames with γ strikes from the radiopharmaceutical or from extraterrestrial cosmic rays (*3*), as well as correction for patient movement. The individual frames are then summed to a final image.

The comments on the spectral distribution of the Cerenkov emission from tissue are certainly valid, and we thank the authors for bringing this to our attention. There was an error in the ahead-of-print article (*4*) that was corrected in the final version of the article (*1*). At the time of acquiring the data, we found it necessary to suppress a contaminating dim background light and improve the signal-to-noise ratio by using a 605-nm long-pass filter. In fact, in several patients, imaging without the filter was inferior to imaging with the filter because of the additionally captured background signal. We agree that optimization of the imaging system toward the exiting spectrum of the Cerenkov emission will be helpful, and such optimization is currently part of ongoing studies.

Optical signal through tissue from the Cerenkov spectral domain is strongly attenuated, thus making Cerenkov imaging challenging. We did not, however, experience an issue with the sparse axial hair in this patient, and we were able to detect the low-intensity Cerenkov light. We furthermore tested this issue in a mouse bearing 2 LNCaP tumors on the flanks. Cerenkov imaging was possible without removing the hair (Fig. 1). Although in this case the fur was white, it was certainly much denser than the sparse axillary hair in our example. The linear measurement in our Figure 1, which was criticized by the authors, was performed in a black box at camera and acquisition settings that were different from those of the clinical image, thus accounting for the different appearance of the images. The comparison of the signal-to-noise ratios and imaging performance of different camera types is complex. In contrast to the study of Spinelli et al. (*2*), we did not use an electron-multiplying charge-coupled-device camera but an intensified charge-coupled-device camera with different performance characteristics.

It is difficult to comment on the comparison of ^{18}F-FDG and ^{131}I uptake in patients. We were not comparing administered doses but the doses present at the imaged sites. Neither iodine uptake data nor thyroid volume is available for the ^{131}I patient who underwent the Cerenkov scan in the study of Spinelli et al. (*2*). Assuming a 50% uptake in a hyperthyroid gland, 225 MBq would have been imaged in the thyroid gland. In our axillary lymph node case (Fig. 2 in our article (*1*)), for example, the node was 7 cm^3 in volume (determined from the CT scan) and had an average uptake of 0.03 MBq, resulting in a total of 0.21 MBq. This is a 4-log difference in activity imaged and the order of magnitude we provided. If we account for the difference in β-particle energy introducing a factor of 2.5, we have a 3-log difference (log [110 MBq/0.21 MBq]). We disagree, however, with the statement that "the magnitude of the Cerenkov signal is almost comparable to the contralateral side" since despite the low signal intensity, we find a significant difference ($P = 0.02$) between the Cerenkov emissions from the PET-positive lymph nodes and the contralateral side (Fig. 2).

We firmly believe that both reports of these early findings—the first Cerenkography report (*2*) and our study on clinical Cerenkov luminescence imaging (*1*)—complement each other and demonstrate the overall feasibility of clinical Cerenkov imaging with different clinically approved radiotracers, which is the important conclusion of these studies. Larger trials are certainly required to establish the value of this new method in the clinic and to optimize imaging parameters such as the wavelength window, the best radiotracer, and suitable clinical settings.

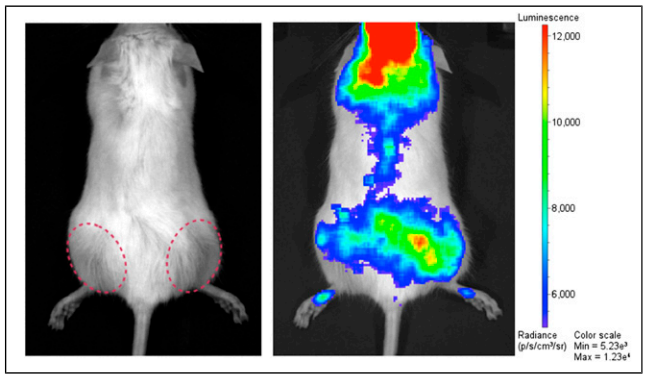

FIGURE 1. Cerenkov imaging through hair. Male white SCID mouse was implanted with LNCaP tumors in its flank (dotted red circles). After injection of 18.5 MBq and uptake time of 1 h, 5-min Cerenkov acquisition was performed on IVIS 200 optical in vivo imaging system (PerkinElmer). Cerenkov light is seen originating from tumors (more from larger tumor on right flank of animal; additional signal is originating from retroorbital injection side and bladder between tumors).

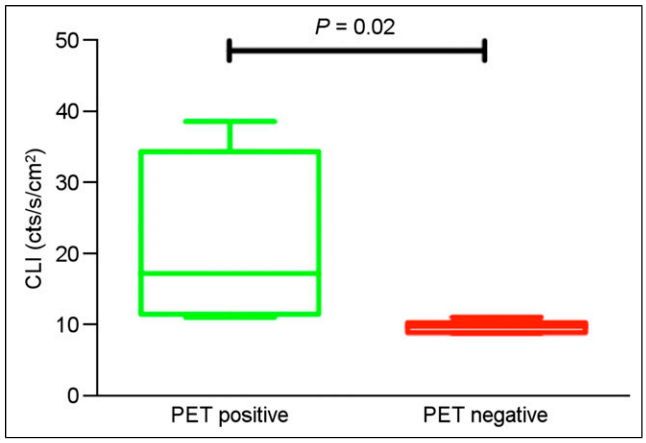

FIGURE 2. Box plot comparing Cerenkov emission (CLI) from pathologic, PET-positive lymph nodes vs. contralateral PET-negative control, demonstrating significantly higher signal from PET-positive side. This graph was included as Figure 3B in the final article.

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