PET with a ^{68}Ga-Labeled FAPI Dimer: Moving Toward Theranostics

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In recent years, quinoline-based fibroblast activation protein (FAP) inhibitors (FAPIs; e.g., FAPI-04 and FAPI-46) have shown promising results in the diagnosis of cancer and various other diseases, making them the hot spot of much productive research (*1*). However, one major issue is that these FAPI molecules have a relatively short tumor retention time, which may hamper the use of FAPI molecules for targeted radionuclide therapy applications. In this issue of *The Journal of Nuclear Medicine,* a novel dimeric FAPI molecule, ^{68}Ga-DOTA-2P(FAPI)$_2$, was designed and synthesized by Zhao et al. (*2*). This intriguing work moves the field forward by addressing an important issue in the development and optimization of FAPI-based tracers, that is, how to increase uptake and tracer retention in tumors for potential therapeutic or theranostic applications. Taking advantage of the multivalency effect, the dimeric FAPI tracer ^{68}Ga-DOTA-2P(FAPI)$_2$ demonstrated significantly higher tumor uptake in mouse tumor models than did ^{68}Ga-FAPI-46. More importantly, results from patient-derived xenograft models, healthy volunteers, and cancer patients also indicated that ^{68}Ga-DOTA-2P(FAPI)$_2$ has better tumor uptake and longer tumor retention time than ^{68}Ga-FAPI-46. Therefore, ^{68}Ga-DOTA-2P(FAPI)$_2$ could be a promising tracer for both diagnostic imaging and targeted radionuclide therapy (when ^{68}Ga is replaced by therapeutic isotopes such as ^{177}Lu, ^{90}Y, or ^{225}Ac) in malignant tumors with high FAP expression.

In general, FAPI-based radiotracers are a promising avenue for research in nuclear medicine. On the basis of the currently available data for lesion detection, the sensitivity of most FAPI-based PET/CT for all lesions falls in the range of 85%–100%, which is comparable to or even superior to that of ^{18}F-FDG PET/CT (*3*,*4*). FAPI-based tracers are especially superior for detecting gastrointestinal cancer, nasopharyngeal cancer, liver cancer, peritoneal carcinomatosis, and brain tumors. As part of this study (*2*), ^{68}Ga-DOTA-2P(FAPI)$_2$ PET/CT imaging in 3 cancer patients (1 with thyroid cancer, 1 with nasopharyngeal cancer, and 1 with hepatocellular carcinoma) showed a rapid and stable accumulation of the tracer in tumorous lesions. Tumor uptake of ^{68}Ga-DOTA-2P(FAPI)$_2$ in most lesions was significantly higher than that of ^{68}Ga-FAPI-46, leading to clearer visualization of primary lesions and metastases.

However, the relatively high level of physiologic uptake of ^{68}Ga-DOTA-2P(FAPI)$_2$ that was observed in the blood pool, thyroid, liver, and pancreas was not the case in previous FAPI-based PET/CT imaging. The high background uptake of ^{68}Ga-DOTA-2P(FAPI)$_2$ in these normal organs may result in relatively low tumor-to-background ratios, which may affect the lesion detection rate of ^{68}Ga-DOTA-2P(FAPI)$_2$ PET/CT in these organs. Therefore, from our perspective, monomeric FAPI-based tracers (e.g., ^{68}Ga-FAPI-04 and FAPI-46) are still recommended for diagnostic imaging purposes because of the rapid blood clearance and low background uptake, whereas DOTA-2P(FAPI)$_2$ may be more suitable for labeling with ^{177}Lu, ^{90}Y, or ^{225}Ac for future therapeutic applications. For the latter, the delayed blood pool radioactivity of radiolabeled DOTA-2P(FAPI)$_2$ may contribute to a relatively high bone marrow toxicity. As such, dosimetry estimation for major organs should be carefully investigated for safety dose limitation, when DOTA-2P(FAPI)$_2$ is labeled with ^{177}Lu or ^{90}Y or, potentially, α-emitters (e.g., ^{225}Ac or ^{213}Bi).

The applications of FAPI-based PET tracers are certainly not limited to lesion detection. Many investigators and stakeholders in the field would agree that the two other most advisable uses are the selection of cancer patients for treatments involving FAP-targeted radionuclide therapy and the quantitative and noninvasive monitoring of patients receiving such therapies. Considering that the next logical step is to explore the therapeutic efficacy of ^{177}Lu-DOTA-2P(FAPI)$_2$, ^{68}Ga-DOTA-2P(FAPI)$_2$ or ^{68}Ga-FAPI-46 PET/CT should certainly be investigated for precisely selecting the patients who will most likely benefit from FAP-targeted radionuclide therapy. Regarding therapeutic response monitoring, several studies have reported that ^{68}Ga-FAPI-04 PET/CT may be useful for evaluating the treatment response to chemotherapy (*5*,*6*). However, another study revealed that fibrosis induced by radiation exhibited high uptake of ^{68}Ga-FAPI-04 (*7*). Thus, FAPI-based PET/CT might be problematic in differentiating between residual or recurrent disease and postradiation inflammatory reactions. Consequently, the real potential of FAPI-based PET/CT in therapeutic response monitoring needs to be studied and confirmed with well-designed clinical investigations.

In the radioligand binding study, the half-maximal inhibitory concentration (IC_{50}) values for the monomeric and dimeric FAPI were

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comparable (2.06 ± 1.84 nM vs. 3.68 ± 1.82 nM) (*2*). To our knowledge, if the dimerization can allow for simultaneous binding to 2 FAPs, then it should be observed (i.e., reflected by significantly different IC_{50} values) in this in vitro assay. However, increased avidity was not observed, meaning that only 1 FAPI is actually binding to FAP at any given time. We speculate that the distance between the 2 FAPI molecules in DOTA-2P$(FAPI)_2$ may not be enough to enable simultaneous binding. Nonetheless, the binding of 1 FAPI motif to FAP will increase the local concentration of a second FAPI motif in the vicinity. The locally enhanced FAPI concentration may explain the higher tumor uptake and retention of ^{68}Ga-DOTA-2P$(FAPI)_2$ than that of ^{68}Ga-FAPI-46. To further improve FAP-targeting capability, the distance between 2 (or more) FAPI molecules needs to be determined, optimized, and leveraged so that they can enable simultaneous binding of multiple FAPs. To the best of our knowledge, no dimer or multimer of FAPI has been reported to date, making this the first example. However, we predict that increasingly more such studies will soon appear in the literature.

This elegant and comprehensive study (*2*), which spans the entire translational spectrum of new tracer synthesis, in vitro characterization, preclinical investigation in patient-derived xenograft models, and pilot studies in healthy volunteers and cancer patients, is a prime example of translational research in the modern era. The head-to-head comparison between ^{68}Ga-DOTA-2P$(FAPI)_2$ and ^{68}Ga-FAPI-46 was also thorough, including blocking and histology studies to confirm FAP specificity in vivo, which provided invaluable information. The encouraging results of this work strongly suggest that future investigation into the anticancer therapeutic applications of a ^{177}Lu-labeled FAPI dimer or multimer such as ^{177}Lu-DOTA-2P$(FAPI)_2$ in patient-derived xenograft models is warranted, to explore whether multivalency could enhance the therapeutic efficacy when compared with a ^{177}Lu-labeled FAPI monomer (e.g., FAPI-46 or FAPI-04). If this investigation is proven to be successful, pilot clinical studies of ^{177}Lu-DOTA-2P$(FAPI)_2$ or other optimized multimeric FAPI ligands can follow.

In comparison to ^{177}Lu-FAPI-46, which has been clinically tested (*8*), a ^{177}Lu-labeled dimeric FAPI will likely be more efficacious, because of its higher uptake and longer retention time in the tumor tissue. Additionally, it could even compare with ^{177}Lu-FAP-2286, a novel FAP-targeting molecule with a cyclic peptide binding motif, which was reported to have a longer tumor retention time by Baum et al. (*9*) and hence could be useful to treat diverse adenocarcinomas. In addition, shortening the interval between treatments, increasing the radioactivity dose administered, or using α-emitters (e.g., ^{225}Ac, ^{213}Bi, or ^{211}At) may further enhance the therapeutic efficacy of FAP-targeted radionuclide therapy. Recently, Xu et al. (*10*) reported 2 albumin binder-conjugated FAPI molecules derived from FAPI-04, which is another strategy to improve tumor uptake and retention time for therapeutic or diagnostic applications. Named as TEFAPI-06 and TEFAPI-07, both molecules have been successfully labeled with ^{68}Ga, ^{86}Y, and ^{177}Lu (*10*). Comparison of dimeric FAPI ligands to these molecules, and perhaps the combination of albumin binders and multimerization of FAPI ligands, are both possible promising avenues for future research.

Without any doubt, FAPI-based imaging and therapy of cancer and various other diseases has been a highly vibrant research field over the last several years. New preclinical and clinical studies appear in the literature virtually every week. We look forward to future studies and rapid translation of the most promising FAPI ligands into the clinical arena to benefit (cancer) patients. The recent development and commercial availability of PET/CT systems with a long axial field of view and total-body imaging capability can also play an important role in the development and translation of novel FAPI-based PET tracers, since it can enable unprecedented, facile evaluation of the whole-body distribution and pharmacokinetic profiles of radiotracers.

DISCLOSURE

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REFERENCES

1. Altmann A, Haberkorn U, Siveke J. The latest developments in imaging of fibroblast activation protein. *J Nucl Med.* 2021;62:160–167.
2. Zhao L, Niu B, Fang J, et al. Synthesis, preclinical evaluation, and a pilot clinical PET imaging study of ^{68}Ga-labeled FAPI dimer. *J Nucl Med.* 2022;63:862–868.
3. Chen H, Pang Y, Wu J, et al. Comparison of [^{68}Ga]Ga-DOTA-FAPI-04 and [^{18}F]FDG PET/CT for the diagnosis of primary and metastatic lesions in patients with various types of cancer. *Eur J Nucl Med Mol Imaging.* 2020;47:1820–1832.
4. Sollini M, Kirienko M, Gelardi F, et al. State-of-the-art of FAPI-PET imaging: a systematic review and meta-analysis. *Eur J Nucl Med Mol Imaging.* 2021;48:4396–4414.
5. Zhao L, Pang Y, Zheng H, et al. Clinical utility of [^{68}Ga]Ga-labeled fibroblast activation protein inhibitor (FAPI) positron emission tomography/computed tomography for primary staging and recurrence detection in nasopharyngeal carcinoma. *Eur J Nucl Med Mol Imaging.* 2021;48:3606–3617.
6. Zhao L, Chen S, Lin L, et al. [^{68}Ga]Ga-DOTA-FAPI-04 improves tumor staging and monitors early response to chemoradiotherapy in a patient with esophageal cancer. *Eur J Nucl Med Mol Imaging.* 2020;47:3188–3189.
7. Chen H, Zhao L, Ruan D, et al. Usefulness of [^{68}Ga]Ga-DOTA-FAPI-04 PET/CT in patients presenting with inconclusive [^{18}F]FDG PET/CT findings. *Eur J Nucl Med Mol Imaging.* 2021;48:73–86.
8. Assadi M, Rekabpour SJ, Jafari E, et al. Feasibility and therapeutic potential of ^{177}Lu–fibroblast activation protein inhibitor–46 for patients with relapsed or refractory cancers. *Clin Nucl Med.* 2021;46:e523–e530.
9. Baum RP, Schuchardt C, Singh A, et al. Feasibility, biodistribution and preliminary dosimetry in peptide-targeted radionuclide therapy (PTRT) of diverse adenocarcinomas using ^{177}Lu-FAP-2286: first-in-human results. *J Nucl Med.* June 24, 2021 [Epub ahead of print].
10. Xu M, Zhang P, Ding J, et al. Albumin binder–conjugated fibroblast activation protein inhibitor radiopharmaceuticals for cancer therapy. *J Nucl Med.* September 30, 2021 [Epub ahead of print].