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PET with a ^{68}Ga-labeled FAPI dimer: moving towards theranostics

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In recent years, quinoline-based fibroblast activation protein (FAP) inhibitors (FAPI; e.g. FAPI-04 and FAPI-46) have shown promising results in the diagnosis of cancer and various other diseases, making them the hotspot 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/optimization of FAPI-based tracers, i.e. how to increase the uptake and tracer retention in tumors for potential therapeutic/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 when compared to ^{68}Ga-FAPI-46. More importantly, results from patient-derived xenograft (PDX) models, healthy volunteers, and cancer patients also indicated that ^{68}Ga-DOTA-2P(FAPI)$_2$ has improved 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/^{225}Ac) in malignant tumors with high FAP expression.

In general, FAPI-based radiotracers are a promising avenue for research in nuclear medicine. Based on 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 than 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 three cancer patients (one thyroid cancer, one nasopharyngeal cancer, and one 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 ^{68}Ga-FAPI-46, leading to clearer visualization of primary lesions and metastases.

However, relatively high level of physiological uptake of ^{68}Ga-DOTA-2P(FAPI)$_2$ in the blood pool, thyroid, liver, and pancreas was also observed, which 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/FAPI-46) are still recommended for diagnostic imaging purposes due to the rapid blood clearance and low background uptake, while DOTA-2P(FAPI)$_2$ may be more suitable for labeling with ^{177}Lu/^{90}Y/^{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/^{90}Y or potentially alpha-emitters (e.g. ^{225}Ac/^{213}Bi).

The applications of FAPI-based PET tracers are certainly not limited to lesion detection. Many investigators/stakeholders in the field would agree that 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)$_2$ and/or ^{68}Ga-FAPI-46 PET/CT should certainly be investigated for precisely selecting 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 and/or recurrent disease and post-radiation inflammatory reactions. Consequently, the real potential of FAPI-based PET/CT in therapeutic response monitoring needs to be studied/confirmed with well-designed clinical investigations.

In the radioligand binding study, the IC_{50} values for the monomeric and dimeric FAPI were 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 two 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 one FAPI is actually

binding to FAP at any given time. We speculate that the distance between the two FAPI molecules in DOTA-2P(FAPI)$_2$ may not be long enough to enable simultaneous binding. Nonetheless, the binding of one 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)$_2$ when compared to ^{68}Ga-FAPI-46. To further improve FAP-targeting capability, the distance between two (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 more and more such studies will appear in the literature in the near future.

This elegant and comprehensive study (2), which spans the entire translational spectrum of new tracer synthesis, in vitro characterization, preclinical investigation in PDX models, 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 very thorough, including blocking and histology studies to confirm FAP specificity in vivo, which provided invaluable information. The encouraging results of this work strongly suggested that future investigation into the anti-cancer therapeutic applications of a ^{177}Lu-labeled FAPI dimer/multimer such as ^{177}Lu-DOTA-2P(FAPI)$_2$ in PDX models in warranted, in order to explore whether multivalency could enhance the therapeutic efficacy when compared to a ^{177}Lu-labeled FAPI monomer (e.g. FAPI-46/FAPI-04). If this 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, owing to its higher uptake and longer retention time in the tumor tissue. Additionally, it could even compare to ^{177}Lu-FAP-2286, a novel FAP-targeting molecule with a cyclic peptide binding motif which was reported to have longer tumor retention time by Baum et al. (9), hence could be useful to treat diverse adenocarcinomas. In addition, shortening the time interval between treatments, increasing the radioactivity dose administered, and/or using α-

emitters (e.g. ^{225}Ac/^{213}Bi/^{211}At) may further enhance the therapeutic efficacy of FAP-targeted radionuclide therapy. Recently, Xu et al. (10) have reported two albumin binder-conjugated FAPI molecules derived from FAPI-04, which is another strategy to improve tumor uptake and retention time for therapeutic and/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, or 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 have 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 long axial field-of-view and total-body PET/CT systems 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

Weibo Cai is a scientific advisor, stockholder, and grantee of Focus-X Therapeutics, Inc. All other authors declare no conflict of interest.

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