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Enhancing fibroblast activation protein (FAP)-targeted radionuclide therapy with albumin binding, and beyond

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Fibroblast activation protein (FAP) and its inhibitors (FAPI) have attracted tremendous attention worldwide over the last several years [1-4]. A number of radiolabeled FAPI have been designed, synthesized, and evaluated in preclinical models, and many of which have entered clinical trials. Radiolabeled FAPI has been investigated in a variety of diseases such as cancer, cardiovascular disease, arthritis, fibrosis, and thyroiditis [1, 2]. In non-cancerous diseases, positron emission tomography (PET) and/or single-photon emission computed tomography (SPECT) imaging with radiolabeled FAPI is usually used for diagnosis and prognosis, as well as treatment monitoring of various targeted therapies. In solid tumors, in addition to these imaging-based applications, radiolabeled FAPI can potentially also be used for targeted radionuclide therapy (TRT) applications, similar to those radiopharmaceuticals that target the prostate specific membrane antigen (PSMA) [5, 6].

Although radiolabeled FAPI have been shown to provide good image contrast in a variety of cancer types, in many cases outperforming [^{18}F]FDG [1, 4], their absolute tumor uptake value is usually not high enough for therapeutic applications with TRT. In addition, there could also be significant clearance of radiolabeled FAPI from tumor tissues over time, which further diminishes their therapeutic potential. A few radiolabeled FAPI have been evaluated for therapeutic applications, mostly in small animal tumor models, and not surprisingly, their therapeutic efficacy is usually not ideal [1, 7, 8]. Therefore, many research groups around the world have devoted significant efforts towards improving tumor uptake and retention of radiolabeled FAPI, in order to enhance their therapeutic efficacy in solid tumors. Various approaches have been adopted and investigated. Over the last decade, a large number of studies have shown that prolonging the blood circulation of small molecules or

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radiopharmaceuticals via conjugation of various albumin binders, such as Evans blue (EB) and 4-(p-iodophenyl)butyric acid, can significantly improve their therapeutic dose delivery and enhance their anti-cancer efficacy [9]. Therefore, it is likely that albumin binding could also be a promising method for the enhancement of TRT with radiolabeled FAPI.

In this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, Zhang, Xu, Ding et al. reported a proof-of-concept study which suggests that conjugation of albumin binders may improve the cancer therapeutic efficacy of FAPI-based radiopharmaceuticals [10], which may become a general strategy to convert the diagnostic FAP-targeted radiopharmaceuticals into their therapeutic pairs and enable cancer TRT and/or theranostics. The albumin-binding moieties used in this study are simple fatty acid chains. Two fatty acids, lauric acid (C12) and palmitic acid (C16), were conjugated to FAPI-04 to give two albumin-binding FAPI: FAPI-C12 and FAPI-C16, respectively (Fig. 1). These two molecules were radiolabeled with either ^{68} Ga, ^{86}Y, or ^{177}Lu, and comprehensively studied in vitro and in vivo, not only for PET (^{68} Ga and ^{86}Y) and SPECT (^{177}Lu) imaging but also for TRT (^{177}Lu) in mouse tumor models. Importantly, radiolabeling was achieved with high yield and good radiochemical purity. In addition, the stability of these radiopharmaceuticals was also excellent in serum, which makes them suitable for in vivo studies. Based on cell binding assays using [^{68} Ga]Ga-FAPI-04 as the competitive radioligand, the half maximal inhibitory concentration (IC_{50}) values were measured to be 6.80 ± 0.58 nM and 5.06 ± 0.69 nM (n = 3) for FAPI-C12 and FAPI-C16, respectively, demonstrating good affinity towards FAP after fatty acid conjugation.

Through PET imaging studies, it was confirmed that the short half-life of ^{68} Ga (68 min) is not sufficient for monitoring the whole-body pharmacokinetics of FAPI-C12 or FAPI-C16, since the radioactivity signal was mainly in the blood pool even at 4-h post-injection (p.i.). Therefore, a long-lived PET isotope is necessary, and ^{86}Y was used, which has a half-life of 14.7 h. For [^{86}Y]Y-FAPI-C12, maximum tumor uptake in HT-1080-FAP tumor-bearing mice was observed at 1-h p.i. with gradual clearance over time. A similar trend was also observed for the blood pool and liver uptake of [^{86}Y]Y-FAPI-C12. In contrast, the tumor uptake of [^{86}Y] Y-FAPI-C16 increased gradually from 1-h to 12-h p.i., which then decreased slowly over time. Regarding in vivo stability of the radiotracers, [^{86}Y]Y-FAPI-C12 exhibited appreciable metabolism, evidenced by obvious radioactivity signal in the intestines until about 12-h p.i. Meanwhile, [^{86}Y]Y-FAPI-C16 appeared to be quite stable with much lower uptake in the intestines. FAP specificity of both tracers was confirmed by significantly higher tracer uptake in the HT-1080-FAP tumor than the HT-1080-vehicle tumor, in addition to blocking studies with unlabeled FAPI-C16. Overall, even though [^{86}Y]Y-FAPI-C16 has higher tumor uptake and retention, the tumor-to-blood and tumor-to-liver ratios were actually higher for [^{86}Y]Y-FAPI-C12, which may have significant ramifications in future TRT studies.

Subsequently, whole-body SPECT imaging of [^{177}Lu] Lu-FAPI-C12, [^{177}Lu]Lu-FAPI-C16, and [^{177}Lu]Lu-FAPI-04 was carried out to further investigate and compare their in vivo pharmacokinetics. Similar to what was observed with ^{86}Y-based PET, tumor retention of [^{177}Lu]Lu-FAPI-C16 was much better than [^{177}Lu]Lu-FAPI-C12. However, the liver uptake of [^{177}Lu]Lu-FAPI-C16 was also higher, which could potentially cause toxicity in high dose

therapeutic studies. For [^{177}Lu]Lu-FAPI-04, little radioactivity signal could be detected at 24-h p.i., indicating (as expected) much faster clearance and corroborating that FAPI-04 without an albumin-binding moiety is not suitable for TRT applications.

Next came the moment of truth, where TRT studies were carried out in HT-1080-FAP tumor-bearing mice, which were each injected with 29.6 MBq of [^{177}Lu]Lu-FAPI-C12, [^{177}Lu]Lu-FAPI-C16, or [^{177}Lu]Lu-FAPI-04. Significant tumor growth inhibition was observed in the [^{177}Lu]Lu-FAPI-C16 group, with a median survival of 28 days which was much longer than that of the [^{177}Lu]Lu-FAPI-04 and [^{177}Lu]Lu-FAPI-C12 treated groups (~ 10 days). When HT-1080-FAP tumor-bearing mice were treated with a lower activity (i.e., 18.5 MBq) of [^{177}Lu]Lu-FAPI-C16, the therapeutic efficacy was appreciably lower than 29.6 MBq. However, the median survival of 21 days was still much longer than that of [^{177}Lu]Lu-FAPI-C12 at 29.6 MBq (~ 12 days). Clearly, [^{177}Lu]Lu-FAPI-C16 is a more promising candidate for further optimization and investigation, which should be explored in more detail in future follow-up studies.

In summary, this is an intriguing study wherein the authors have demonstrated that conjugating FAPI-04 with a C16 chain could lead to significantly improved tumor uptake and retention, which led to much better therapeutic efficacy of TRT (after ^{177}Lu labeling) in small animal tumor models. This is certainly a strategy that holds promising potential for future clinical translation into TRT of cancer patients. A critical issue that one needs to bear in mind is that long circulation of radiopharmaceuticals could be a double-edged sword, where both higher tumor accumulation and higher non-specific uptake in normal tissues/organs (e.g., the liver, blood, bone marrow) could be observed. The latter can be quite harmful in some cases; hence, the optimal balance needs to be determined for better clinical cancer patient management. Personalized dosimetry investigations and treatment planning may be needed to find the right therapeutic window for effectively treating cancer patients. For instance, in this study, a single dose of ^{177}Lu-labeled FAPI-C12/C16 was used. Fractionation may need to be explored in future studies, which could provide better therapeutic outcome in some scenarios. Much future effort will be needed before FAPI-based TRT can be considered safely optimized for clinical translation and broad application.

Besides FAPI-04, there are many other FAPI molecules that could also be explored for albumin binding via various strategies [11-13]. For example, the same group of investigators recently reported two other molecules, TEFAPI-06 and TEFAPI-07 (Fig. 1, which were also derived from FAPI-04), with the inclusion of two types of well-studied albumin binders, a 4-(p-iodophenyl) butyric acid moiety (for TEFAPI-06) and a truncated EB moiety (for TEFAPI-07), respectively [13]. With comparable FAP-binding affinity to FAPI-04, both [^{177}Lu]Lu-TEFAPI-06 and [^{177}Lu]Lu-TEF-API-07 showed remarkable growth inhibition of patient-derived xenograft (PDX) tumors with negligible side effects, clearly demonstrating the potential for future clinical translation. In another recent study, a series of truncated EB-modified, FAP-targeted radiotracers were reported, which were derivatives of FAPI-02 (Fig. 1) [12]. When compared to the unmodified FAPI-02, it was found that these FAPI derivatives (after ^{177}Lu labeling) have remarkably enhanced tumor uptake and retention, which resulted in excellent tumor growth suppression in the U87MG tumor model. We

look forward to follow-up studies of these exciting FAPI-based radiopharmaceuticals, all of which hold great potential for clinical cancer therapy.

Besides albumin binding, another approach to improve tumor uptake and therapeutic efficacy of FAPI-based radiopharmaceuticals is multimerization, which is based on the multivalency effect [14]. Recently, a few studies have reported dimeric FAPI-based ligands [15-18]. In an elegant and comprehensive study that spans the entire translational spectrum of new tracer synthesis, in vitro characterization, preclinical investigation in PDX models, and pilot studies in healthy volunteers and cancer patients, a head-to-head comparison between a ^{68}Ga-labeled dimeric FAPI derivative (i.e., DOTA-2P(FAPI)$_2$; Fig. 1) and FAPI-46 was investigated in exquisite detail [18]. The encouraging results of this work (i.e., the dimeric FAPI derivative performed much better than FAPI-46 for PET imaging applications) strongly suggested that future investigation into the anti-cancer therapeutic applications of a ^{177}Lu-labeled FAPI dimer/multimer in PDX models is warranted, in order to evaluate whether multivalency can significantly enhance the therapeutic efficacy when compared to a ^{177}Lu-labeled monomeric FAPI (e.g. FAPI-46/FAPI-04). If this is proven to be true and successful, pilot clinical studies of a ^{177}Lu-labeled FAPI dimer or other optimized multimeric FAPI ligands can follow.

For such dimerization or multimerization, many parameters/aspects need to be considered altogether to achieve optimal results, such as linker length, flexibility, and hydrophilicity, to name just a few. On this note, it is important to mention that in another report, the advantage of dimerization over monomeric FAPI was not obvious [15], indicating that further optimization and/or a new design is needed. Another potential approach to enhance FAPI-based TRT efficacy could be dual targeting, which has been shown to work well for many other ligands in terms of increased affinity and/or specificity, which can in turn lead to enhanced tumor uptake, image contrast, and/or therapeutic efficacy [19-23]. We believe this research area deserves significant attention in the near future. Since the interactions of tumor cells and cancer-associated fibroblasts play diverse and critical roles in oncology, it is certainly logical to target both cell populations at the same time.

Very few other targets have attracted as much attention as FAP over the last several decades. FAPI-based imaging and therapeutic studies have just started and will surely remain a vibrant research topic in the foreseeable future. In terms of imaging, optimized FAPI-based PET tracers have become relatively mature and have seen frequent clinical use in many (academic) medical centers worldwide, for both cancer and non-cancerous diseases. New preclinical and clinical studies of FAPI-based agents appear in the literature virtually every week, and it is almost impossible to keep up with the state-of-the-art. Regarding FAPI-based TRT, there is still a very long way to go for the best FAP-targeted radiopharmaceuticals to make an impact in the clinic as significant as those for PSMA in prostate cancer (on March 23, 2022, the United States FDA granted approval to [^{177}Lu]Lu-PSMA-617 for the treatment of PSMA-positive metastatic castration-resistant prostate cancer patients who have previously been treated with androgen-receptor pathway and taxane-based chemotherapy). Development of therapeutic agents is much more complicated than that of diagnostic agents, since the potential toxicity and adverse effects are much more likely for the former; hence, more comprehensive safety studies will be needed to identify the best therapeutic

candidates with minimal adverse effects. Without any doubt, there is tremendous promise for FAP-targeted imaging and therapeutic agents, and we look forward to future studies and rapid translation of the most promising FAPI-based agents into the clinical arena to benefit (cancer) patients. With the increasing availability of long axial field-of-view and total-body PET/CT systems [24-27], the development of FAPI-based TRT agents can be facilitated and expedited, since total-body PET can enable unprecedented, facile evaluation of the whole-body distribution and pharmacokinetic profiles of novel PET tracers, which are usually critical initial steps for the development of targeted therapeutics (e.g., FAPI-based TRT agents).

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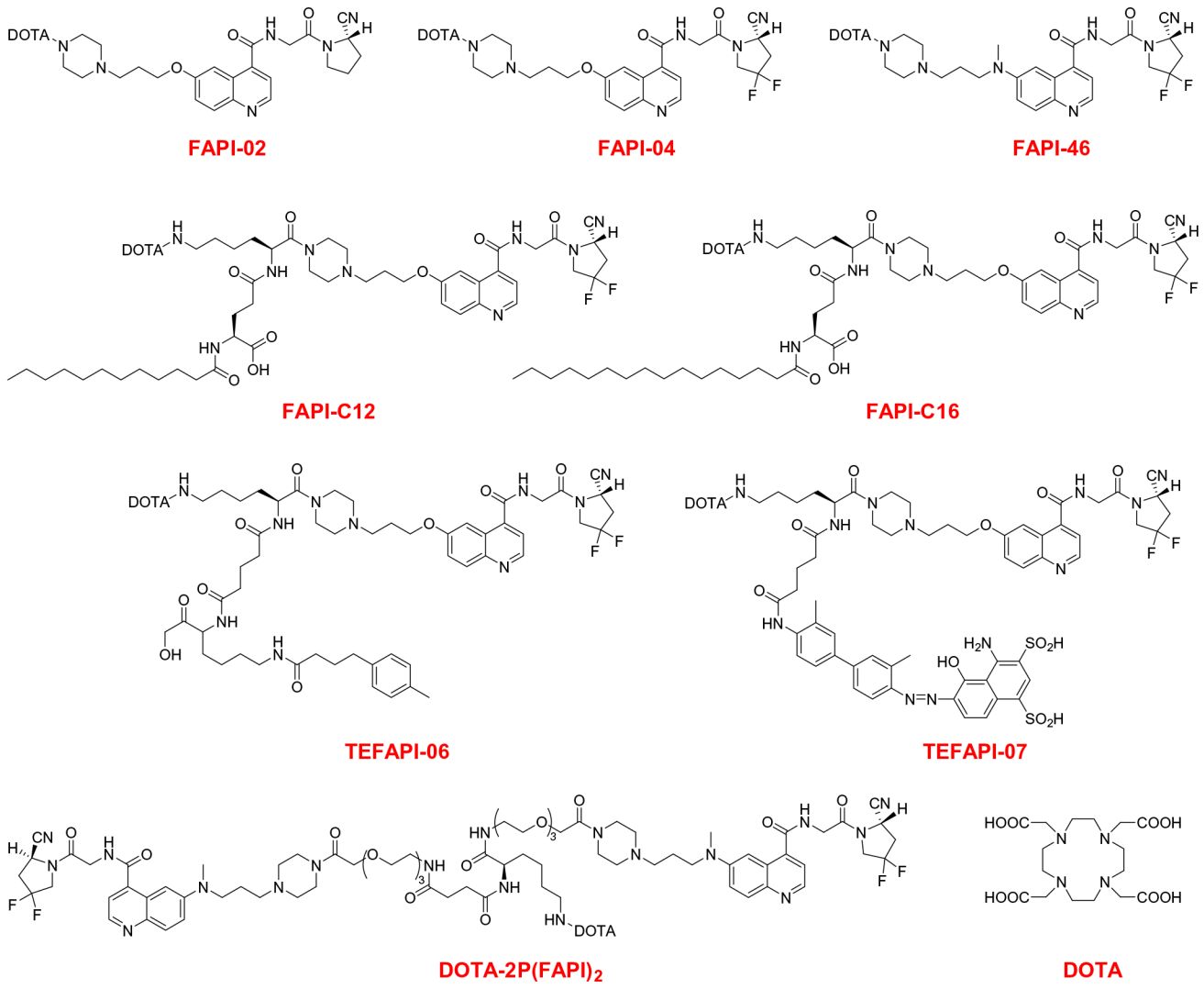

Fig. 1.
Chemical structures of several FAPI-based agents described in this editorial