UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Petitioner, GE Healthcare Ltd.,
Petitioner

v.

Johns Hopkins University,
Patent Owner.

Case No. IPR2025-00808
U.S. Patent No. 11,938,201

Declaration of Brian Zeglis, Ph.D.

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I. Introduction

A. Background and Qualifications

1. My educational background, career history, and other relevant qualifications are summarized below. I attach to this Declaration my curriculum vitae, which provides a full and accurate description of my educational background, professional experience, and qualifications (Appendix A).

2. I received my Ph.D. in Chemistry from the California Institute of Technology in 2009, where I studied the bioinorganic chemistry of DNA mismatch-binding metal complexes under the guidance of Professor Jacqueline K. Barton. I received a Bachelor of Science in Chemistry from Yale University in 2004, where I studied organometallic *N*-heterocyclic carbene complexes of ruthenium and iridium under the tutelage of Professor Robert H. Crabtree. I completed my postdoctoral work at Memorial Sloan Kettering Cancer Center in 2015, where I studied the design, synthesis, and *in vivo* validation of radiopharmaceuticals for the nuclear imaging, theranostic imaging, and targeted therapy of cancer under the auspices of Professor Jason S. Lewis.

3. I currently serve as a Professor of Chemistry at Hunter College, City University of New York. I have been a professor at Hunter College since 2015. I began as an Assistant Professor in the Department of Chemistry from January 2015 through September 2019. I served as an Associate Professor in the same

department from September 2019 to August 2022. Since August 2022, I have been employed as a Full Professor in the Department of Chemistry.

4. I have taught courses in Inorganic Chemistry and Inorganic Chemistry Laboratory at Hunter College as recently as the 2023-2024 term. I also previously taught an Introduction to Radiochemistry course as recently as Spring 2017.

5. I have authored or co-authored 118 publications, largely in the field of radiochemistry. I have published two textbooks on the subject of radiopharmaceutical design and development, entitled "Radiopharmaceutical Chemistry" (1st edition in 2019; 2nd edition coming in 2025) and "Radiopharmaceutical Therapy" (published in 2023). I have also published several book chapters in the field of radiopharmaceuticals for oncology applications. To date, I have been invited to deliver 54 lectures at conferences, universities, hospitals, and other institutions across the world.

6. I am also a named inventor on three patents: U.S. Patent Nos. 7,786,298, 11,000,604, and 11,135,320. U.S. Patent Nos. 11,000,604 (entitled "Reagent for Site-Selective Bioconjugation of Proteins or Antibodies") and 11,135,320 (entitled "Radioligands for Pretargeted PET imaging and Methods of their Therapeutic Use") describe compounds and methods of radiolabeling compounds for use in radiochemistry applications.

7. I have received a number of awards in the field of nuclear medicine. For example, in October 2022, I received the Roger Tsien Award for Excellence in Chemical Biology from the World Molecular Imaging Society for my contributions to the use of bioorthogonal chemistry to molecular imaging and nuclear medicine.

8. From 2020-2021 and since 2022, I have served as a Standing Member of the National Institutes of Health Imaging Probes and Contrast Agents (IPCA) Study Section.

9. I currently serve as the Deputy Editor-in-Chief for the *Molecular Imaging and Biology* scientific journal. I have served in this role since 2024. Before that, I was an Associate Editor for the same journal from 2020-2024. Since 2016, I have also served on the Editorial Board of the *Journal of Nuclear Medicine*. In addition, I have served as a reviewer for several journals in the past, including *Cancer Research*, *Clinical Cancer Research*, *Cancer Discovery*, *Proceedings of the National Academy of Sciences*, *Chemical Communications*, *Journal of the American Chemical Society*, *Journal of Nuclear Medicine*, and *European Journal of Nuclear Medicine*.

10. Since 2015, I have supervised 6 post-doctoral researchers, 16 graduate students, and 15 undergraduate students in my laboratory.

B. Compensation

11. I am being compensated for my time at the rate of $900 per hour for my work in connection with this matter. I am being reimbursed for reasonable and customary expenses associated with my work in this investigation. This compensation is not dependent in any way on the contents of this Declaration, the substance of any further opinions or testimony that I may provide, or the ultimate outcome of this matter.

C. Person of Ordinary Skill in the Art

12. I understand that my analysis and opinions are to be provided using the perspective of a person of ordinary skill in the art up to the date of October 23, 2017. I will refer to this period as the "2017 timeframe" in this Declaration.

13. The scientific field of the patent concerns radiopharmaceuticals, and more particularly, radiopharmaceuticals designed for the nuclear imaging of cancer cells. I am very familiar with this field, and the individuals who work within it, including in the 2017 timeframe.

14. I have been informed by counsel that a person of ordinary skill in the art is a hypothetical person who is presumed to have the typical skills and knowledge of someone working in the field of the invention. Based on my review of the patent and my experience, I believe a person of ordinary skill in the art (who I may refer to as "a skilled artisan") would have had an undergraduate degree and a

Ph.D. in chemistry, biochemistry, medicinal chemistry or a comparable field. The person would have had experience with and/or knowledge of chemical synthesis methods (*e.g.*, organic synthesis, radiometal chelation and radiohalogen labeling of prosthetic groups), assessment of cellular targets for radiopharmaceuticals, and other techniques used in the design, development, testing and/or evaluation of radiopharmaceuticals. The person would also be familiar with how radiopharmaceuticals are distributed and used in patients to perform therapy or nuclear imaging of diseases, including cancer.

15. In the 2017 timeframe, I had at least the qualifications I outline above for a person of ordinary skill in the art. The opinions I provide in this Declaration are provided from the perspective of a person of ordinary skill in the art in the 2017 timeframe as I have described above.

16. I understand that the disclosure of a patent consists of a narrative section called the specification, which often includes drawings. I understand that a patent ends with claims that define the invention.

D. Materials Considered

17. My opinions are based on my years of education, research, and experience, as well as my investigation and study of relevant materials. I reviewed a number of publications in the course of my assessment, including those listed in

Appendix A. I also relied on my extensive familiarity with the scientific literature in this field.

E. Legal Principles

18. I am not a lawyer and am not offering opinions on the law. However, I have been provided a general explanation of some of the legal requirements for obtaining a patent.

19. I have been informed that one requirement for patentability is that an invention must not have been obvious to a person of ordinary skill in the art in view of what was known in the prior art before the filling date of the patent. I also have been informed that if a patent claim encompasses a compound that would have been obvious in light of the prior art, that claim is unpatentable.

20. I have been informed that for a claimed compound to be found obvious, a person of ordinary skill in the art must have found a reason in the prior art to make that compound and must have had a reasonable expectation of success in achieving the claimed invention. I have been informed this does not require the skilled artisan to have absolute certainty about achieving a desired result and that an invention can be found obvious if a result is expected but still requires some experimentation to confirm.

21. I have been informed that if there is evidence that a particular compound exhibits unexpected properties, enjoys significant commercial success,

or meets a long-felt need, that evidence can support a finding that the compound is not obvious. I have also been informed that for a claim defining a large class of compounds, all of the members of the class must share the property or characteristic to support a finding that the class of compounds is not obvious. I have been informed that a claim defining a large class of compounds cannot benefit from evidence showing only one or a few of the compounds within it exhibits the particular unexpected property or characteristic associated with the evidence. I also have been informed that to credit such evidence as supporting non-obviousness, it must not be associated with a prior art feature of the claimed invention.

II. Scientific Principles Relevant to Radiopharmaceuticals

22. The scientific field of the ’201 Patent concerns compounds used in nuclear imaging or therapy, particularly those targeting FAP. The development of radiopharmaceuticals, including those targeting FAP, was well-established in the 2017 timeframe. The explanations and observations I provide below reflect what a person of ordinary skill in the art would have known as of October of 2017, and are consistent with what that person would have believed as of that date.

A. Terminology Used in this Declaration

23. I will use the following abbreviations and terminology in this declaration:

(a) "FAP" refers to the Fibroblast Activation Protein-α. FAP is also referred to as "seprase."[1]

(b) "PSMA" refers to prostate-specific membrane antigen.

(c) "PET" refers to Positron Emission Tomography.

(d) "SPECT" refers to Single-Photon Emission Computed Tomography.

(e) "Radiopharmaceutical" is a chemical compound that contains a radionuclide that is used for therapeutic or diagnostic purposes.

(f) "Radiotracer" is a radiopharmaceutical that is used for nuclear imaging purposes.

(g) "Targeting moiety" refers to the portion of a radiopharmaceutical that binds selectively to a cellular target (e.g., FAP). This is also referred to as the "pharmacophore," the "warhead", or comparable terms.

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[1] EX1026 (Jia), 1 ("Fibroblast Activation Protein alpha (FAP-α) or seprase is an integral membrane serine peptidase."); EX1004 (US-633), [0003] ("Seprase, also known as fibroblast activation protein alpha (FAP-α), is a transmembrane serine peptidase that belongs to the prolyl peptidase family.").

(h) "Radiolabeling moiety" refers to the portion of a radiopharmaceutical that is covalently linked to or non-covalently complexed with a radionuclide. It may also be referred to as an "imaging moiety."

(i) "Linker" refers to a portion of the radiopharmaceutical that links the radiolabeling moiety to the targeting moiety. It may also be referred to as a "tether," "spacer," or similar term.[2]

B. Radiopharmaceuticals

24. Radiopharmaceuticals are specialized chemical compounds that incorporate a radioactive form of an element (a radionuclide). When a dose of a radiopharmaceutical is administered to a patient, the radiopharmaceutical bearing the radionuclide migrates to and accumulates in targeted tissues.[3] In therapeutic applications, the radionuclide exerts a cytotoxic effect on the cells in which it has accumulated. In diagnostic applications, the radionuclides emit radiation at the location in the body where they have accumulated, which are then detected by a

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[2] EX1009 (Jamous), 3881. See also EX1017 (Sarko), 2669.

[3] EX1005 (US-121), [0175], [0183]-[0184]. See also EX1009 (Jamous), 3379-3380; EX1011 (Zeglis 2013), 1891; EX1018 (Fichna), 8-9.

device, such as a PET or SPECT scanner,[4] to generate an image that can be evaluated by a clinician.[5]

25. Radiopharmaceuticals are of particular interest in the clinical diagnosis and treatment of cancers because tumors contain cells that have unique characteristics that can be differentiated from cells in normal tissue and therefore can be targeted for diagnostic and therapeutic purposes.[6] As Jamous (EX1009) explains:

> Many tumors overexpress specific targets on the surface of their cells. The target ligands are used with radiolabels in cancer diagnosis and therapy in accordance with the key-lock principle.[7]

This "key-lock" principle ensures that the carrier molecule (the key) fits precisely into the target receptor (the lock), allowing for high specificity in targeting tumor cells.[8]

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4 EX1004 (US-633), [0005]. See also EX1010 (Zeglis 2011), 2; EX1009 (Jamous), 3380-3381.

5 EX1004 (US-633), [0002], [0005], [0049]. See also EX1010 (Zeglis 2011), 2; EX1009 (Jamous), 3380-81.

6 EX1004 (US-633), [0005]. See also EX1010 (Zeglis 2011), 2.

7 EX1009 (Jamous), 3380.

8 EX1009 (Jamous), 3380.

26. Radiopharmaceuticals used in nuclear imaging are called "radiotracers" or "tracers." Radiotracers are given to patients at much lower doses than radiopharmaceuticals given for therapeutic purposes. Due to the risks associated with radiation exposure;[9] the dose of a radiotracer is limited to the minimum amount needed to produce emissions sufficient for imaging.[10] As Agdeppa (EX1027) explains:

> Radiotracers are given at doses that do not elicit a pharmacologic event (orders of magnitude below therapeutic doses), are infrequently administered, and are designed to measure molecular processes, not modify the disease (97,98). These factors reduce the safety risks associated with radiotracers compared to therapeutics, yet they are regulated as though they carry the same risks. The

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[9] EX1037 (Karakatsanis), 528 ("However, radiation exposure can be a serious concern for adult and particularly children patients, especially in the case of PET/CT hybrid systems, due to the ionizing nature of both PET and CT radiation, with the latter contributing to relatively higher absorbed doses than the former modality.").

[10] EX1037 (Karakatsanis), 528 ("By systematically and quantitatively analyzing…a range of dose levels, an accurate NECR-dosage response model can be designed allowing for the prediction of the minimum possible amount of dosage required to sufficiently maintain NECR, or statistically useful counts, at a quantitatively acceptable level.").

reduced risks and different usage of imaging tracers support development of alternatives to the current regulatory process.[11]

C. Common Components of Radiopharmaceuticals

27. Radiopharmaceuticals often employ a "modular" design with three primary components:

(a) a ***targeting moiety*** that enables the radiopharmaceutical to selectively bind to a biological target of interest;

(b) a ***radiolabeling moiety*** that includes (i) a radionuclide and (ii) a chemical moiety such as a chelator or prosthetic group that forms a non-covalent complex with (chelator) or covalent bond to (prosthetic group) the radionuclide; and

(c) a ***linker*** (also called a "spacer" or a "tether"), which is a chemical moiety that connects and positions the targeting and radiolabeling moieties relative to each other to ensure that each can carry out its respective function (i.e., binding to target or delivery of radionuclide).

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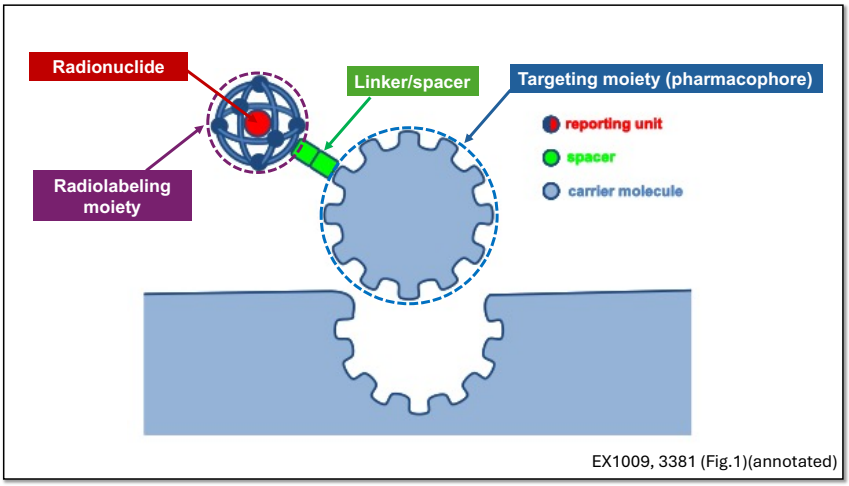
[11] EX1027 (Agdeppa), 293.

28. An illustration of this modular, three-component radiopharmaceutical design is shown below (with annotations).[12] One benefit of the modular three-component design is that it is flexible—it allows one to replace individual components when designing, synthesizing and evaluating a new radiopharmaceutical. I briefly discuss these common components below.



1. Targeting Moiety

29. The "targeting moiety" in a radiopharmaceutical facilitates delivery of the radiopharmaceutical to a specific biological target within the body (*e.g.*, a tumor, particular tissues, a particular organ).[13] The targeting moiety is a

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12 EX1009 (Jamous), 3381 (Figure 1) (annotated).

13 EX1009 (Jamous), 3381 ("There are numerous different carriers that have been designed and developed for the targeting of tumors. Several radiolabeled small molecules have been applied *in vivo* for PET imaging [].").

pharmacophore that binds selectively to a particular chemical structure that is present on cells within the biological target. The selective presence of the chemical structure on the targeted cells but not on other cells in the body is important for ensuring that the radionuclide is delivered precisely to the biological target of interest.[14] Selectivity can be achieved by the unique expression of the structure on target cells, or by a relatively higher level of expression of the structure on target cells as compared to normal cells. The ability of the radiopharmaceutical to bind selectively to target cells enhances the accuracy of delivery of the radionuclide for both diagnostic and therapeutic radiopharmaceuticals while minimizing damage to normal tissues.

30. Targeting moieties in a radiopharmaceutical can be small molecules, peptides, small proteins, and antibodies.[15] As I wrote in 2011, small molecule PET radiotracers have dominated the field of molecular imaging, as they can penetrate tissues quickly and have short half-lives in circulation.[16] Small molecule

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[14] EX1009 (Jamous), 3381.

[15] EX1009 (Jamous), 3380 ("They can be classified into three major categories…(a) radiolabeled monoclonal antibodies (b) receptor specific small proteins and peptides and (c) small molecules.").

[16] EX1010 (Zeglis 2011), 1-3; EX1012 (Wadas), 2859. See also EX1020 (Saha), 161.

radiotracers are also particularly useful in PET imaging because they are quickly cleared from the patient's body due to their rapid pharmacokinetic profiles.[17] A skilled artisan considering options for the targeting moiety of a radiopharmaceutical intended for imaging of tumors would have certainly considered small molecule candidates, given that many had been used previously in radiopharmaceuticals and due to the person's extensive familiarity with the design and production of such compounds.

2. Radiolabeling Moiety

31. A radiopharmaceutical must be capable of delivering the radionuclide to the target cells or tissues to enable non-invasive imaging or treatment of pathological conditions.[18] To do that, the radionuclide must be stably attached to the radiopharmaceutical so that it does not dissociate before it reaches and accumulates within tissues containing the targeted cells.[19]

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[17] EX1009 (Jamous), 3381.

[18] See, e.g., EX1004 (US-633), [0005], [0049]; EX1009 (Jamous), 3380-81 (the "reporting unit"); EX1010 (Zeglis 2011), 1-3; EX1013 (Price), 265-66 ("kinetic inertness *in vivo* is ultimately the most crucial consideration.").

[19] See, e.g., EX1009 (Jamous), 3385; EX1017 (Sarko), 2668; EX1018 (Fichna), 5; EX1010 (Zeglis 2011), 3, 5-6.

32. Radionuclides are incorporated in radiopharmaceuticals either by stably coordinating the radionuclide within a chelator moiety or by covalently linking the radionuclide to the radiopharmaceutical; both approaches have proven effective in ensuring that the radionuclide does not dissociate from the radiopharmaceutical.[20] Chelators are used with radiometals (*e.g.*, ^{68}Ga, ^{99m}Tc) and form stable, coordination complexes with the radiometal. Covalent bonds are used to attach non-metallic radionuclides, particularly radiohalogens (e.g., ^{18}F, ^{123}I) to a prosthetic group. Chelators and prosthetic groups also must be capable of being covalently attached to the linker moiety of the radiopharmaceutical.

33. Chelators are not "one-size-fits-all" propositions. Metallic cations can have dramatically different chemical properties, and thus a chelator that coordinates one cation with high thermodynamic and kinetic stability may not be adequate for the sequestration of another. Put differently, the choice of radiometal largely dictates the choice of chelator.[21] For example, smaller cationic radiometals, such as ^{68}Ga and ^{64}Cu, prefer to be coordinated by a mix of oxygen and nitrogen

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[20] See, e.g., EX1017 (Sarko), 2668; EX1018 (Fichna), 5. See also EX1004 (US-633), [0048]; EX1010 (Zeglis 2011), 3.

[21] EX1011 (Zeglis 2013), 1884.

donor atoms.[22] Many widely used chelators for binding radiometals are structurally related to DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which is known to bind tightly to cationic radiometals.[23] There are many known relatives of DOTA used for binding radiometals as part of a radiolabeling moiety, such as NOTA, DOTAGA, and TETA.[24] A skilled artisan would have been familiar with chelators to use with different radiometals, as that topic has been extensively addressed in the scientific literature.[25]

34. Radiohalogens (*e.g.,* ^{18}F, ^{123}I, ^{125}I) are ordinarily attached to a radiopharmaceutical via a covalent bond.[26] The radiohalogen can be incorporated

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22 EX1011 (Zeglis 2013), 1882 (surveying common PET radiometals and their compatible chelators). See also EX1012 (Wadas), 2865, 2869.

23 EX1013 (Price), 266 ("DOTA is one of the primary workhorse chelators for radiometal chemistry and is one of the current 'gold standards' for a number of isotopes, including ^{111}In, ^{177}Lu, $^{86/90}Y$, ^{225}Ac, and $^{44/47}Sc$.").

24 EX1013 (Price), 266-269 (identifying derivatives and analogs of DOTA). See also EX1018 (Fichna), 6-7; EX1009 (Jamous), 3398-3399.

25 EX1011 (Zeglis 2013), 1884, 1888, 1893-1894; EX1010 (Zeglis 2011), 5-9; EX1012 (Wadas), 2863-2869; EX1013 (Price), 267-268, 270-272, 274-276.

26 EX1014 (Riondato), 44-45.

directly into the targeting moiety structure or attached using a "prosthetic group."[27] In the latter approach, the prosthetic group is covalently attached to the radiohalogen, and then the radiolabeled prosthetic group is attached to the radiopharmaceutical precursor.[28] One benefit of using prosthetic groups is that they facilitate reproducibility and modularity because standard production methods can be developed for a single prosthetic group, eliminating the need to develop and optimize a unique radiosynthesis for each novel radiopharmaceutical.

3. Linkers

35. A linker (also called a "spacer" or "tether") is a molecular component that connects the targeting moiety to the radiolabeling moiety in the radiopharmaceutical. Generally speaking, linkers can serve three purposes in a radiopharmaceutical. First, they can spatially separate the radiolabeling moiety from the targeting moiety to prevent the radiolabeling moiety from sterically interfering with the binding of the targeting moiety to its target or altering the

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[27] EX1014 (Riondato), 44 (discussing direct versus indirect radiolabeling of radiopharmaceuticals using ^{18}F). See also EX1033 (Mach), 137.

[28] See EX1014 (Riondato), 44; EX1019 (Schirrmacher), 475 (observing that "a PG [prosthetic group] in radiopharmaceutical chemistry is a serviceable auxiliary that can easily be attached to a precursor molecule" to perform radiolabeling).

targeting moiety's specificity or affinity for its target.[29] Second, they can facilitate the efficient assembly of the radiopharmaceutical, including both during the synthesis of the non-radiolabeled precursor molecule of the radiopharmaceutical and during the assembly of the final radiolabeled form of the radiopharmaceutical.[30] Third, linkers can enhance the stability, solubility, or other characteristics of the radiopharmaceutical when it is in a formulation prior to administration, as well as when the radiopharmaceutical is exposed to physiological conditions in the patient (including in plasma).[31]

36. Linkers can be selected or designed to influence the biochemical and pharmacokinetic characteristics of the radiopharmaceutical. For example, a radiopharmaceutical chemist can introduce hydrophobic character into a

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[29] See EX1011 (Zeglis 2013), 1884.

[30] EX1010 (Zeglis 2011), 9 ("This link must be stable under physiological conditions and must not significantly compromise the binding strength and specificity of the biomolecule."). See also EX1017 (Sarko), 2669.

[31] EX1010 (Zeglis 2011), 9. See also EX1013 (Price), 282 ("In order to optimally tune the properties of radiometal-based pharmaceuticals, a large variety of different tools (*e.g.* chelators, linkers, vectors) are crucial…so that physical properties of an agent can be easily modified."); EX1017 (Sarko), 2668.

radiopharmaceutical by incorporating one or more benzyl groups into the linker moiety.[32] Changing the hydrophobicity of radiotracers in this way is a well-established approach to altering their pharmacokinetic profiles. More specifically, more hydrophobic probes tend to bind better to serum proteins, thereby increasing their residence time in the blood.[33] The reason that increased residence times in the blood are desirable is that they — generally — lead to increased uptake in target tissues, a desirable outcome for both nuclear imaging and radiopharmaceutical therapy. Similarly, a linker that is modified to be more lipophilic can increase the lipophilicity of a radiopharmaceutical to enhance its penetration of the tissue of interest, which can improve the compound's half-life in blood.[34]

37. Linkers in small molecule radiopharmaceuticals use covalent bonds to link the two functional domains of the compound. Common types of covalent bonds used to attach linkers to other parts of the radiopharmaceutical include ether,

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[32] EX1005 (US-121), [0187].

[33] EX1040 (Meyer 2017), 8204-8205 (exemplifying the impact of linker characteristics on plasma half-life by examining tetrazine-based radiotracers with poly(ethylene glycol) ("PEG") linkers).

[34] EX1005 (US-121), [0187].

amide, and thioether bonds. This requires each functional domain (*i.e.* the targeting moiety and radiolabeling moiety) to contain a moiety that can be exploited to form these covalent bonds to the linker.[35] Linkers are typically designed for the radiopharmaceutical and then integrated into the radiopharmaceutical via a stepwise synthesis.

D. Radionuclides

38. Every radiopharmaceutical contains a radionuclide, an atom with an unstable nucleus that emits radiation as it decays.[36] Different types of radiation may be desired depending on the purpose of the radiopharmaceutical (*i.e.*, diagnostic or therapeutic), as Jamous explains:

> These agents can be labeled with radionuclides that accumulate in the tissue of interest. Depending on the purpose, gamma or positron emitters are used for diagnosis and beta, alpha or Auger electron emitters are used for therapeutic applications in cancer treatment.[37]

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35 EX1013 (Price), 261.

36 EX1004 (US-633), [0049]; EX1018 (Fichna), 8; EX1017 (Sarko), 2667 ("The radioisotopes comprised in radiopharmaceuticals emit either gamma rays for diagnostic use or alpha or beta particles for therapeutic use.").

37 EX1009 (Jamous), 3380.

39. In diagnostic applications, the choice of a radionuclide will first depend on whether the nuclear imaging agent will be used with a PET or a SPECT scanner. For PET scanners, a positron-emitting radionuclide is required. As I explained in my 2011 publication, "[a] wide array of small molecule PET radiotracers have been developed that employ the short half-life radionuclides ^{11}C, ^{13}N, ^{15}O, and ^{18}F."[38] PET imaging may also be performed using radioactive isotopes of metals, called radiometals. "The principal radiometals employed for the labeling of biomolecular tracers are ^{64}Cu, ^{68}Ga, ^{86}Y, and ^{89}Zr."[39] All four radiometals emit positrons and favorably complement the biological half-lives of known radiopharmaceuticals.[40] If the agent in question will be used with SPECT, a gamma-emitting radionuclide must be chosen. Examples of gamma-emitting radionuclides capable of use with SPECT include ^{99m}Tc, ^{188}Re, ^{123}I, ^{111}In, and

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38 EX1010 (Zeglis 2011), Abstract. See also; EX1012 (Wadas), 2859.

39 EX1010 (Zeglis 2011), 4.

40 EX1010 (Zeglis 2011), 3-4 (surveying commonly used radionuclides and their characteristics).

^{67}Ga.[41] A table of radionuclides used in PET and SPECT imaging is shown below, along with the half-life of each radionuclide.[42]

	Isotope	Type	Emission	Half-Life
SPECT	^{99m}Tc	Metal	Gamma	6.0 h
	^{188}Re	Metal	Gamma	16.9 h
	^{123}I	Halogen	Gamma	13.2 h
	^{111}In	Metal	Gamma	67.2 h
	^{67}Ga	Metal	Gamma	78.3 h
PET	^{68}Ga	Metal	Positron	1.1 h
	^{18}F	Halogen	Positron	109 min
	^{64}Cu	Metal	Positron	12.7 h
	^{86}Y	Metal	Positron	14.7 h
	^{89}Zr	Metal	Positron	78.4 h
	^{124}I	Halogen	Positron	100.3 h

40. For radiotracers, after selecting the appropriate emission type based on the technology in use, it is crucial to consider the half-life of the radionuclide to ensure it will be compatible with the type of imaging to be performed. In practice, this means that slow-moving vectors like monoclonal antibodies are typically labeled with radionuclides like ^{89}Zr ($t_{1/2}$ ~ 78 h) and ^{124}I ($t_{1/2}$ ~ 100 h), while faster-moving antibody fragments, peptides, and small molecules are usually labeled with

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41 EX1017 (Sarko), 2668, Table 1.

42 *See, e.g.*, EX1010 (Zeglis 2011), 3, Table 2 (^{68}Ga and ^{89}Zr), Table 1 (^{18}F and ^{124}I); EX1034 (Meyer 2016), 2795 (^{99m}Tc); EX1012 (Wadas), 2860, Table 2 (^{64}Cu), Table 1 (^{67}Ga); EX1013 (Price), 262, Table 1 (^{111}In).

radionuclides like ^{86}Y ($t_{1/2}$ ~ 14.7 h), ^{64}Cu ($t_{1/2}$ ~ 12.7 h), ^{18}F ($t_{1/2}$ ~ 109 min), ^{68}Ga ($t_{1/2}$ ~ 68 min), ^{99m}Tc (~6 h) or ^{188}Re (~ 16.9 h). As Fichna explains:

> The half-life is a critical factor. For diagnostic imaging the half-life of a radionuclide must be long enough to enable the synthesis of the labeled compound and to facilitate the accumulation in the target tissue, while allowing clearance through the nontarget organs. Ideally, the half-life should be as short as possible to reach these two goals.[43]

41. A typical rule-of-thumb in the design of radiopharmaceuticals—particularly those used for imaging—is that the physical half-life of the radionuclide should match the pharmacological half-life of the vector that delivers the radiopharmaceutical to its target.[44] For radiopharmaceuticals with slow pharmacokinetic profiles, using a radionuclide with a longer half-life ensures that there is still radioactivity present after it reaches the target tissue. Some examples of publications where this rule of thumb is mentioned include the following:

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[43] EX1018 (Fichna), 8-9.

[44] EX1010 (Zeglis 2011), 4-5 ("In this regard, one of the most important considerations is matching the radioactive half-life of the isotope to the biological half-life of the biomolecule.").

(a) EX1011 (Zeglis 2013), 1884 ("Once an imaging modality has been chosen, matching the radioactive half-life of the isotope to the biological half-life of the biomolecule is critical.");

(b) EX1012 (Wadas), 2893 ("researchers now have the ability to match the physical characteristics of a specific radiometal with the biokinetics of a particular targeting molecule leading to the development of diagnostic and therapeutic radiopharmaceuticals that can be tailored to individual disease processes.");

(c) EX1014 (Riondato), 46-48 ("The half-life of the radionuclide in a radiotracer should correlate with the kinetic of the process to investigate. In other words, the radiotracer, after injection, should cross the [Blood-Brain Barrier] and interact quantitatively with the target, in a time frame that has to be consistent with the radionuclide half-life.");

(d) EX1018 (Fichna), 8-9 ("for diagnostic imaging the half-life of a radionuclide must be long enough…to facilitate the accumulation in target tissue, while allowing clearance through the non-target organs.");

(e) See EX1017 (Sarko), 2674 (flagging importance of matching "pharmacokinetic properties and the physical half-life of ^{99m}Tc" in developing ^{99m}Tc-based radiopharmaceuticals.); and

(f) See EX1013 (Price), 280 (observation that half-life of ^{89}Zr made it ideally paired for antibody vectors which have a half-life of 2-3 weeks).

42. Before 2017, ^{18}F and ^{68}Ga were seen as popular choices for radionuclides to use in radiotracers for PET-based nuclear imaging of tumors.[45]

(a) For example, Riondato et al. (EX1014) describe ^{18}F as "the most important radionuclide for PET imaging" because "[^{18}F's] exceptional employment for *in vivo* imaging is primarily due to the minimal perturbation caused by its incorporation (similar to a hydroxyl substituent) into the final molecule, combined with advantageous physical half-life of 109.7 min, which permits

[45] EX1028 (Mankoff), 150S (Table 1 shows the target, imaging probe, and imaging modality of a number of tumor receptor imaging agents developed around 2008).

multistep syntheses as well as a major flexibility in postproduction procedures."[46]

(b) Similarly, Wadas et al. (EX1012) describe ^{68}Ga as "an important positron-emitting radiometal," because "[^{68}Ga] can be produced from a compact generator system that contains the parent radionuclide. The ^{68}Ge/^{68}Ga generator system provides a continuous source of Ga-based PET radiopharmaceuticals for approximately 1 year; it has been extensively reviewed, and numerous commercial systems are available."[47]

43. Before 2017, ^{99m}Tc was the most commonly used radionuclide in radiotracers used for SPECT. As Jamous explains, "^{99m}Tc is still the most frequently used radionuclide in diagnostic applications of nuclear medicine, due to its ideal nuclear physical properties, the availability through a commercial ^{99}Mo-^{99m}Tc generator, the low production cost and easy and rich labeling chemistry."[48]

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46 EX1014 (Riondato), 44.

47 EX1012 (Wadas), 2879.

48 EX1009 (Jamous), 3387.

E. Considerations Influencing the Development of Radiopharmaceuticals

44. A number of functional and practical considerations influence the design of a radiopharmaceutical, particularly one intended for use in nuclear imaging. I summarize these considerations below.

1. Selectivity and Affinity of the Targeting Moiety

45. When choosing the pharmacophore that will act as the targeting moiety of a radiopharmaceutical, two issues are paramount: (i) the selectivity of the pharmacophore for the molecular target (*i.e*. how much better it binds the desired target compared to related biomolecules)[49] and (ii) the affinity of the pharmacophore for the molecular target (*i.e*. how tightly it binds the molecular target). While both are necessary, binding selectivity is ultimately the most important factor.[50] For example, a radiotracer that has high affinity but low selectivity for a desired molecular target may bind to both the desired target and other targets *in vivo*, thereby potentially visualizing non-target tissues in the body and producing "false positive" imaging results. Conversely, a radiotracer that has high selectivity but low affinity for a desired molecular target may not bind the

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[49] EX1010 (Zeglis 2011), 4.

[50] EX1010 (Zeglis 2011), 4 ("Of course, the most important facet of the biomolecule moiety is its specificity for its biomarker target.").

desired molecular target well but will at least not bind other targets *in vivo*. Such a radiotracer could be effective if the desired target was highly abundant but could produce "false negative" imaging results if the desired target was scarce.[51]

46. A skilled artisan would have evaluated the affinity of a FAP inhibitor candidate compound by considering its IC_{50} value for FAP [*i.e.*, what amount of the compound is needed to bind (or "inhibit") at least 50% of the target]. The IC_{50} value for a compound conveys a sense of the compound's potency in inhibiting the enzyme's activity (*i.e.*, a compound that requires a lower concentration to inhibit 50% of FAP's activity signals it has greater potency and tighter binding to the enzyme than a compound that requires a higher concentration to do so).

47. A skilled artisan would evaluate the selectivity of a FAP inhibitor candidate compound by comparing the IC_{50} value of that compound for FAP relative to its IC_{50} values for other members of the prolyl oligopeptidase family, including DPPIV, DPP8, and DPP9, and — particularly — prolyl oligopeptidase (PREP). A straightforward way of doing this is to calculate a selectivity index of each compound for FAP relative to PREP by dividing the measured IC_{50} value for

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[51] EX1051 (Harris), 308.

a compound for PREP by the measured IC_{50} value of that compound for FAP.[52] This yields a numeric grade or index of relative selectivity of each compound for FAP relative to PREP (higher value = more selective). For example, Jansen (EX1006) used a "selectivity index" ("SI") that was determined as follows:[53]

> [b]SI stands for "selectivity Index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]).

48. Calculating a selectivity index for each candidate compound allows one to classify the selectivity of each compound for FAP relative to PREP using an objective, quantitative metric. A skilled artisan will typically determine what values would be appropriate to use to classify a set of compounds for a particular cellular target (*e.g.*, values for high, medium, or low selectivity).

2. Stability, Bioavailability and Other Factors Influence Design of Radiopharmaceuticals

49. Several characteristics of the radiopharmaceutical also will influence its design, particularly one intended for use in diagnostic tumor imaging.

(a) *Stability in vivo*. A radiopharmaceutical must remain intact in the body for a long enough time to accumulate in its biological

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[52] See, e.g., EX1006 (Jansen), 3065 (bridging columns) (describing assay used to measure IC_{50} values for FAP, PREP, DPPIV, DPPII, DPP8, DPP9).

[53] EX1006 (Jansen), 3054 (legend to Table 1).

target. Even a compound with very high selectivity and affinity will not be effective *in vivo* if it is metabolized rapidly or the radionuclide dissociates or decays before binding the target. Sources of instability can be identified and mitigated; for example, if there is a problem with protease degradation of the radiopharmaceutical, the compound can be modified to replace peptide bonds susceptible to cleavage with bonds that are not susceptible to cleavage. Thus, targeting moieties and the radiopharmaceutical as a whole must be resistant to metabolism or decomposition for a period long enough to perform imaging or therapy.

(b) *Amenability to Radiolabeling*. To become a viable radiopharmaceutical, the targeting moiety must be modified to append a chelator or prosthetic group for radiolabeling. This can often be a tricky endeavor, as slight modifications to the structure of a targeting moiety can alter its selectivity and affinity for its target. Thus, it is desirable to have several possible sites on the targeting moiety that can serve as a point of attachment for the linker and radiolabeling moiety that are

far enough away from the structural motif of the targeting moiety that interacts with the target on the targeted cells.

(c) *Pharmacokinetic Profile*. As I explained above (¶ 41), a general principle of radiopharmaceutical design is that the physical half-life of the radionuclide should match the pharmacological half-life of the radiopharmaceutical precursor to ensure that there is sufficient distribution in the target tissue that can be imaged before the compound is cleared or the radionuclide decays.

(d) *Bioavailability*. Bioavailability refers to the relative amount of an administered radiopharmaceutical that enters systemic circulation *in vivo* in an unchanged state and is largely dictated by the compound's pharmacokinetic profile. The hydrophobicity/hydrophilicity of a radiopharmaceutical is a crucial determinant of its bioavailability. Radiopharmaceuticals that are too hydrophobic can have poor bioavailability.

3. Requirements for Distributing Radiopharmaceuticals Can Influence Their Design

50. Practical requirements associated with the production, handling and use of radiopharmaceuticals can influence their design. For example, the short half-lives of radionuclides, particularly those used for PET, mean that

radiopharmaceuticals must often be prepared close in time to their use in patients (almost always the same day they are prepared).[54] For example, a ^{68}Ga-based radiopharmaceutical must typically be used within 4 hours of its elution from a generator and its formulation into a finished product.[55] Radionuclides are also hazardous materials that require the use of specialized facilities and equipment and specially trained technicians.

51. Radiopharmaceuticals that are based on radionuclides with short half-lives are often produced and distributed in a precursor form that does not contain the (radioactive) radionuclide. Then, at a time and at a location that is proximate to the use of the radiopharmaceutical in patients, this precursor form of the radiopharmaceutical is converted into the final radiolabeled form of the radiopharmaceutical containing the radionuclide.

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54 EX1020 (Saha), 161; See also EX1012 (Wadas), 2859.

55 EX1039 (Nelson), 21 ("A shelf life of 4 h is usually sufficient for generator supplied ^{68}Ga radiopharmaceuticals due to the limited radioactivity that can be obtained. However, since cyclotron produced ^{68}Ga offers a significant increase in radiopharmaceutical [*sic*] yields, additional measurements may be warranted if producing larger product activities for use at extended timepoints.").

52. The synthesis of the radiolabeled form of the radiopharmaceutical is typically performed at a specialized facility. In those facilities, automated devices are typically used that are "controlled by microprocessors and software programs to carry out the sequential physical and chemical steps to produce the radiolabeled product."[56] Such devices are commercially available and are designed to help avoid the risk of contamination or radiation exposure, while facilitating the rapid preparation of the radiolabeled form of the radiopharmaceutical.[57]

53. Procedures used to prepare a radiolabeled form of a radiopharmaceutical from its non-radiolabeled precursor need to be straightforward, high-yielding, rapid, and capable of being carried out under mild conditions. To achieve that goal, radiohalogens like ^{18}F are often incorporated into a so-called "prosthetic group" instead of being linked directly to the targeting moiety of the radiopharmaceutical. The ^{18}F-radiolabeled prosthetic group can then be covalently linked to the radiopharmaceutical under mild conditions. See ¶ 34 (above). Techniques for efficiently generating ^{18}F-labeled prosthetic groups were

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56 EX1020 (Saha), 161-162.

57 EX1020 (Saha), 161-162 (describing known automated synthesis modules for known PET radiotracers such as ^{18}F-FDG, ^{13}N-NH3, ^{11}C-CH3I, ^{11}C-HCN, ^{11}C-acetate).

well known by 2017, including those using so-called "click chemistry" techniques.[58] As Ross (EX1015) explains:

> The major concern of fluorine-18 chemists of unsuitably long reaction times proved unfounded as most ^{18}F-conjugations needed short periods of time. Moreover, often quantitative incorporation of the ^{18}F prosthetic group into the desired biomolecule was achieved under very mild conditions. These very mild conditions and the extraordinary orthogonality of the Cu(I)-catalysed 1,2,3-triazole formation to other functionalities make this reaction particularly suitable for the ^{18}F-labelling of sensitive biomolecules such as peptides, proteins, antibodies, nucleotides, etc. In addition, the click reaction is highly efficient over a broad range of conditions, including the unbalanced stoichiometry used in n.c.a. fluorine-18 chemistry.[59]

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58 EX1015 (Ross), 202-203 (describing click chemistry reactions using Cu(I)-catalyzed cycloadditions). See also EX1034 (Meyer 2016), 2792 ("Put simply, peptides, proteins, and antibodies should be radiolabeled under aqueous conditions at room temperature…This is especially true for ^{18}F-radiofluorination reactions which often require organic solvents and high temperatures. Radiolabeled prosthetic groups provide an efficient way to circumvent these issues.").

59 EX1015 (Ross), 219; see also EX1021 (Kettenbach), 12 ("The field of click cycloadditions had and still has a major impact in ^{18}F-labeling chemistry. The

54. I co-authored a review of click-chemistry techniques known before 2017 that could be used to prepare ^{18}F prosthetic groups and covalently link them to radiotracers in 2016. As I explained, one of the main benefits of click-chemistry techniques is that they can be used to radiolabel a prosthetic group compound without having to resort to harsh conditions that could degrade the radiotracer or decrease the yield of the radiolabeled form of it.[60]

55. A second common approach to the efficient, high-yield preparation of radiolabeled forms of radiopharmaceuticals uses precursors that contain chelator moieties. The radionuclide is then added into the chelator moiety to yield the radionuclide-bearing radiopharmaceutical. Several review articles authored before 2017 addressed the construction of radiotracers that incorporated chelator moieties

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very mild reaction conditions mostly applicable and the excellent efficiency of all types of these reactions are particularly suitable for ^{18}F-labeling. Especially, complex and sensitive biomolecules benefit from this methodology. No protection group chemistry is needed and the ^{18}F-click cycloaddition step provides the final radiotracer.").

[60] EX1034 (Meyer 2016), 2792 (Prosthetic groups are radiolabeled reactive small molecules that can be appended to biomolecules under benign conditions.")

that can form stable coordination complexes with a metal radionuclide.[61] Before 2017, it was straightforward for skilled artisans to prepare the radiolabeled form of radiotracer having a chelator moiety selected to work with a particular radiometal (*e.g.*, ^{68}Ga, ^{99m}Tc).

F. Before 2017, There Was Significant Interest in Developing Radiopharmaceuticals that Selectively Targeted FAP for Therapy and Diagnosis of Cancer

1. FAP Is Selectively Expressed by Many Types of Tumors

56. Fibroblast Activation Protein (also called "FAP", "FAP-α", and "seprase") is a serine peptidase within a family of enzymes including DPPIV, DPP7, DPP8, DPP9 and POP/PREP.[62] The enzymatic capability that distinguishes this family of proteins is their ability "to cleave the Pro-Xaa peptide bond (where Xaa represents any amino acid)…"[63] As Jansen explains:

> Fibroblast activation protein (FAP, FAP-α, seprase) belongs to the prolyl oligopeptidase family S9, which consists of serine proteases that cleave peptide substrates preferentially after proline residues.

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61 See, e.g., EX1010 (Zeglis 2011), 5-9, Figs. 3, 4; EX1011 (Zeglis 2013), 1884, 1886-1888, Fig. 6; EX1012 (Wadas), 2860-2862, 2869-2872, 2879, 2893, Fig. 1; EX1013 (Price), 260-266; EX1009 (Jamous), 3385; EX1018 (Fichna), 5.

62 EX1006 (Jansen), 3053. See also EX1016 (Brennen 2012), 259 (Table 1).

63 EX1016 (Brennen 2012), 259.

> Other members of this family include the dipeptidyl peptidases (DPPs: DPPIV, DPP8, DPP9) and prolyl oligopeptidase (PREP, POP).[64]

57. The family of enzymes that includes FAP is referred to using a variety of names in the literature.[65] For example, Brennen 2012 (EX1016) refers to this family of enzymes as the "post-prolyl peptidase" family in a 2012 review article (below). Jansen (EX1006) refers to it as the "prolyl oligopeptidase family S9." Wilson (EX1046) refers to it as "serine protease S9b DP4-like gene family." Hamson (EX1024) refers to the family as "dipeptidyl proteases." For simplicity, I will refer to this family in this declaration using the term "prolyl oligopeptidase" as used by Jansen.

Table 1. Characteristics of known post-prolyl peptidases

Prolyl peptidase	Enzymatic activity	Cellular localization	References
DPPIV	Dipeptidase	Membrane	(25, 36–38)
FAP	Dipeptidase/endopeptidase	Membrane	(25, 36, 38)
DPP6	Inactive	Membrane (K_v^+ channel)	(25, 37)
DPP8	Dipeptidase	Cytoplasm	(25, 37, 38)
DPP9	Dipeptidase	Cytoplasm	(25, 37, 38)
DPP10	Inactive	Membrane (K_v^+ channel)	(25, 37)
AAP	Acylpeptide hydrolase	Cytoplasm	(38, 39)
POP	Prolyl oligopeptidase	Cytoplasm	(38)
DPPII (DPP7)	Dipeptidase	Intracellular vesicles	(37, 38)
PCP	Prolyl carboxypeptidase	Lysosome	(38)

64 EX1006 (Jansen), 3053.

65 EX1016 (Brennen 2012), 259, Table 1; EX1046 (Wilson), 1; EX1024, 454.

58. Unlike other prolyl oligopeptidases, the expression of FAP is limited in normal tissues, but it is overexpressed in diseases associated with activated stroma, including wound healing, rheumatoid arthritis, osteoarthritis, cirrhosis, and pulmonary fibrosis.[66] More significantly, "FAP is expressed selectively by cancer-associated fibroblasts (CAFs) and pericytes rather than tumor cells in more than 90% of human epithelial malignancies, including colorectal, ovarian, breast, bladder and lung."[67] FAP expression also has been linked to breast, colorectal,

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66 EX1022 (Liu), 124; EX1024 (Hamson), 456 ("FAP expression has been observed during wound healing [24], at sites of inflammation including arthritis [] and in atherosclerotic plaques []."); EX1023 (DiMagno), 288 (FAP expression is "essentially absent in normal adult tissues and in nonmalignant tumors."); EX1041 (Gorrell), 2 ("DPIV is expressed in all organs, by capillary endothelial cells and activated lymphocytes and on apical surfaces of epithelial, including acinar, cells. In humans, DPIV is present in the gastrointestinal tract, biliary tract, exocrine pancreas, kidney, thymus, lymph node, uterus, placenta, prostate, adrenal, parotid, the sweat, salivary and mammary glands and endothelia of all organs examined, including liver, spleen, lungs and brain …").

67 EX1022 (Liu), 124; EX1006 (Jansen), 3053 ("FAP is also highly expressed on activated fibroblasts in over 90% of common human epithelial tumors."); EX1024 (Hamson), 456 ("FAP is also strongly expressed by stromal fibroblasts in over 90% of epithelial carcinomas [].").

skin, pancreatic, hepatocellular, ovarian, gastrointestinal, and prostate cancers, as well as in some soft tissue and bone sarcomas.[68]

2. Skilled Artisans Were Actively Looking in the 2010's for Small Molecules that Selectively Bound FAP to Use in Radiopharmaceuticals

59. By the early 2010's, FAP was recognized as a compelling diagnostic and therapeutic target for radiopharmaceuticals given its selective expression on the stroma of a large number of epithelial tumors.[69] Some observations on FAP's potential as a tumor-specific biomarker (including as a target for nuclear imaging agents) before 2017 include the following:

(a) "Due to the highly restricted and specific expression of FAP, particularly within tumor stroma, FAP is clinically interesting as a target for immunotherapy in the treatment of cancer and non-invasive bio-imaging. … It appears that FAP shows more promise as a biomarker and target for non-invasive imaging of

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68 EX1016 (Brennen 2012), 260.

69 EX1022 (Liu), 127; EX1023 (DiMagno), 288-289; EX1006 (Jansen), 3053; EX1004 (US-633), [0003], [0005]-[0007].

tumours or for use as site-directed targeted cancer radiotherapy…"[70]

(b) "Due to its restricted expression pattern and dual enzymatic activities, FAP is emerging as a unique therapeutic target."[71]

(c) "Based on the highly regulated expression and restricted distribution of FAP, it has been identified as a marker of reactive tumor stromal fibroblasts."[72]

(d) "Although the biological function of FAP-α still needs further study, the specific expression and the unique enzymatic activity of FAP-α make it a potentially attractive therapeutic and diagnostic target in the tumor microenvironment."[73]

(e) "FAP is a post-prolyl protease with distinct substrate requirements, and its expression is restricted to the surface of

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70 EX1046 (Wilson), 26.

71 EX1024 (Hamson), 454; also 455 ("…FAP is attracting attention in cancer, cardiology and fibrosis research because its expression is greatly upregulated in disease.")

72 EX1022 (Liu), 124.

73 EX1045 (Ji), 2427.

> reactive fibroblasts localized to the tumor microenvironment in normal, healthy adults. At present, these 2 characteristics make FAP uniquely suited for therapeutic strategies aimed at targeting CAFs."[74]

60. Because a number of prolyl oligopeptidases act on substrates similar to FAP, many compounds that inhibit (and thus bind with high affinity to) FAP can also have bind to those other enzymes. Finding a compound that can selectively inhibit FAP but not those other prolyl oligopeptidases was thus seen as a major challenge to developing a viable FAP-targeting radiopharmaceutical. For example, if a FAP-targeting radiopharmaceutical binds to any of the other prolyl oligopeptidases to a significant degree, it could accumulate in tissues in which the radiotracer is not meant to accumulate and cause off-target effects in therapy or false positives in imaging. As Hamson explained in a 2014 review article:

> A major hurdle in the study of FAP enzyme activity has been the lack of selective inhibitors against this protease. FAP shares DPP specificity with the enzyme members of the DPP4 family, DPP4, DPP8 and DPP9, as well as endopeptidase specificity with prolyl

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[74] EX1016 (Brennen 2012), 264.

> endopeptidase (PREP). Thus, designing inhibitors that are selective for FAP over other DPPs and PREP is challenging.[75]

61. A significant amount of research during the early-to-mid 2010's focused on the discovery and characterization of small molecules that bind with high selectively to FAP, inhibit its proteolytic activity, and potentially reduce tumor growth and metastasis.[76] Many of these early FAP inhibitors incorporated a boronic acid pyrrolidine structure, such as PT-100 (Val-boro-Pro), which showed promise but lacked sufficient selectivity as they also inhibited other dipeptidyl peptidases (DPPs).[77] More selective inhibitors based on an Ac-Gly-boro-Pro scaffold were discovered that exhibited greater selectivity for FAP over other DPP family members.[78] As noted by O'Brien:

> *N*-acyl-Gly-Pro dipeptides were identified as Seprase selective substrate motifs and a second boronic acid inhibitor was designed, Ac-Gly-BoroPro. It inhibited these prolyl peptidases with K_i values

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75 EX1024 (Hamson), 457.

76 EX1023 (DiMagno), 288. DiMagno provides a useful historical survey of these activities.

77 EX1006 (Jansen), 3054.

78 EX1016 (Brennen 2012), 262.

ranging from ~9- to 5400-fold higher than that for Seprase inhibition.[79]

62. Subsequent work in the field led to the development of FAP inhibitors based on a pyrrolidine *without* a boronic acid that exhibited high affinity for FAP. These studies also underscored the importance of developing compounds that are selective for FAP over other DPP enzymes or PREP. For example, Ryabtsova et al. (EX1029) reported in 2012 that pyrrolidine-based FAP inhibitors showed favorable affinity for FAP but also showed high affinity for PREP, highlighting the need to measure the selectivity of FAP over PREP when evaluating inhibitors intended to specifically target FAP.[80]

III. US-633, Jansen and Meletta Would Lead a Skilled Artisan to Develop Radiopharmaceuticals Based on Compound 60 of Jansen

63. Before 2017, many groups had published reports concerning radiopharmaceuticals specifically targeting FAP or other tumor antigen targets. The following publications are examples of such reports, and would have

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79 EX1025 (O'Brien), 1140-1141.

80 EX1029 (Ryabtsova), 3413-3417 (reporting some compounds showing more affinity for PREP than FAP). *Also* EX1006 (Jansen), 3053 ("… numerous reported FAP inhibitors have limited or no selectivity with respect to PREP."); EX1004 (US-633), [0006]; EX1024 (Hamson), 457.

influenced the thinking of a skilled artisan developing a FAP-targeting radiopharmaceutical:

(a) Published U.S. patent application number US2010/0098633, first inventor Craig Zimmerman ("US-633") (EX1004),

(b) Published U.S. patent application number US 20121/0009121 ("US-121"), first inventor Martin Pomper (EX1005), and

(c) Jansen et al., J. Med. Chem., 57:3053-74 (2014) ("Jansen") (EX1006).

64. I note that while a skilled artisan would have recognized the value of FAP-targeting radiopharmaceuticals in both therapeutic and nuclear imaging applications, I will focus in this declaration on how a skilled artisan would have approached the design and construction of a FAP-targeting radiopharmaceutical used in nuclear imaging (referred to as a "radiotracer").

A. US-633 (EX1004)[81]

1. US-633 Describes Low Molecular Weight Radiopharmaceuticals that Selectively Target FAP

65. US-633 describes radiopharmaceuticals based on small molecule targeting moieties that selectively inhibit "seprase" (another name for FAP).[82] The ability of these small molecules to inhibit FAP protease activity indicates that they selectively bind to a portion of the FAP protein that is involved in the enzymatic hydrolysis of peptides.

66. US-633 explains that its FAP-targeting radiopharmaceuticals are useful in both nuclear imaging and therapy. As it states:[83]

> **[0002]** This invention relates in general to small molecule inhibitors of seprase that can be used as therapeutic agents through inhibition of seprase's enzymatic activity, or as radiopharmaceuticals that bind to seprase and therefore enable imaging of tissues that express seprase or for delivering radiotherapy to tumor tissues that express seprase.

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81 EX1004 (US-633).

82 EX1004 (US-633), [0003] ("Seprase, also known as fibroblast activation protein alpha (FAP-α), is a transmembrane serine peptidase that belongs to the prolyl peptidase family.").

83 EX1004 (US-633), [0002].

67. US-633 identifies FAP as an attractive target for radiopharmaceuticals, both for therapy and for nuclear imaging, stating: "expression of seprase on tumors makes it an attractive target to exploit for noninvasive imaging as well as targeted radiotherapy."[84]

68. US-633 explains that the FAP protein is expressed on the surface of cells (*i.e.*, "it is a transmembrane serine protease"), which is what enables FAP-targeting radiopharmaceuticals to bind to tumors containing cells expressing the FAP protein.[85] As it states: "[t]he expression of distinct proteins on the surface of tumor cells offers the opportunity to diagnose and characterize disease by probing the phenotypic identity and biochemical composition and activity of the tumor."[86] Radiopharmaceuticals, and particularly radiotracers, must be able to access the target structures on cells that their targeting moieties bind to in order to perform their imaging or therapeutic function.

69. US-633 explains that FAP is selectively expressed on tumors. It notes that FAP "is expressed in epithelial cancers and has been implicated in

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84 EX1004 (US-633), [0005].

85 EX1004 (US-633), [0005].

86 EX1004 (US-633), [0005].

extracellular matrix remodeling, tumor growth, and metastasis."[87] It then points out that "[r]adioactive molecules that ***selectively*** bind to specific tumor cell surface proteins allow for the use of noninvasive imaging techniques, such as molecular imaging or nuclear medicine, for detecting the presence and quantity of tumor associated proteins." (below)[88] And it then states that "'[t]he expression of seprase on tumors makes it an attractive target to exploit for noninvasive imaging as well as targeted radiotherapy."[89]

70. US-633 thus captures why FAP was seen even in the early 2010's as a compelling target for nuclear imaging of tumors: its selective expression on cells within tumors but not on normal tissue means that a FAP-selective radiotracer will primarily accumulate in tumors rather than normal tissues in the patient and enable those tissues to be identified with nuclear imaging techniques.

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87 EX1004 (US-633), [0003].

88 EX1004 (US-633), [0005].

89 EX1004 (US-633), [0005].

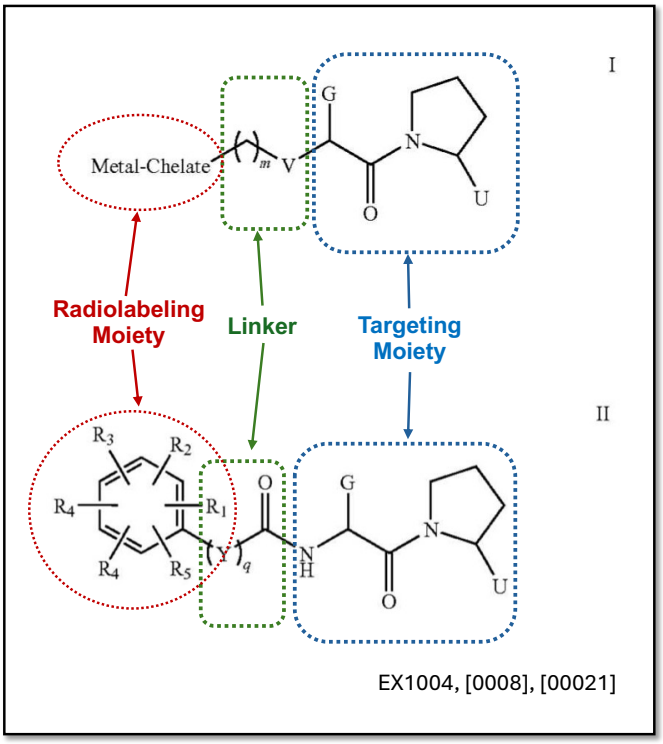
[0005] The expression of distinct proteins on the surface of tumor cells offers the opportunity to diagnose and characterize disease by probing the phenotypic identity and biochemical composition and activity of the tumor. Radioactive molecules that selectively bind to specific tumor cell surface proteins allow for the use of noninvasive imaging techniques, such as molecular imaging or nuclear medicine, for detecting the presence and quantity of tumor associated proteins. Such methods may provide vital information related to the diagnosis and extent of disease, prognosis and therapeutic management options. For example, therapy may be realized through the use of radiopharmaceuticals that are not only capable of imaging disease, but also are capable of delivering a therapeutic radionuclide to the diseased tissue. The expression of seprase on tumors makes it an attractive target to exploit for noninvasive imaging as well as targeted radiotherapy.

2. US-633 Describes Construction of FAP-Targeting Radiopharmaceuticals

71. US-633 describes two classes of FAP-targeting radiopharmaceuticals that are based on targeting moieties containing a modified pyrrolidine structure (labeled "Formula I" and "Formula II") (below).[90] The radiopharmaceuticals being described in US-633 employ a modular three-component design, in which the FAP targeting moiety is connected via a "tether" (a linker) to a radiolabeling moiety (below). As I explained in ¶ 28, a skilled artisan would have recognized this

[90] EX1004 (US-633), [0008], [0021].

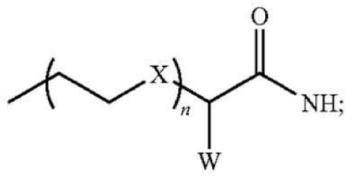
modular design enables any of the three components to be changed to a different moiety.



72. In the annotation of Formula I above, I have included the "V" variable as being within the linker component. That is because the options specified for V (below) include structures that are part of linker ("tether") components of examples of compounds based on Formula I.[91] For example, one of the options for "V" is a polyethylene glycol (PEG) structure ("$(CH_2—CH_2—X)_n$, with "X" being "O").

[91] EX1004 (US-633), [0012]-[0018].

[0012] V is a bond, O, S, NH, $(CH_2—CH_2—X)_n$, or a group of formula



[0013] X is O, S, CH_2, or NR;
[0014] R is H, Me or CH_2CO_2H;
[0015] W is H or NHR';
[0016] R' is hydrogen, acetyl, t-butyloxycarbonyl (Boc), 9H-fluoren-9-ylmethoxycarbonyl (Fmoc), trifluoro-acetyl, benzoyl, benzyloxycarbonyl (Cbz) or substituted benzoyl;
[0017] n is an integer ranging from 0 to 6;
[0018] m is an integer ranging from 0 to 6;

73. A radiopharmaceutical based on the small molecule targeting moieties being described in US-633 would have a molecular weight that is orders of magnitude lower than a radiopharmaceutical that uses an antibody or equivalently sized-protein as the targeting moiety (*i.e.*, <2 kDa vs. >150 kDa, respectively). Based on my experience, a skilled artisan would generally classify the radiopharmaceuticals being described in US-633 as "low molecular weight" radiopharmaceuticals because of their general size and because they are based on a small molecule targeting moiety covalently linked to linkers and radiolabeling groups.

74. US-633 provides step-by-step guidance for constructing FAP-targeting radiopharmaceuticals based on the boronic acid pyrrolidine FAP-

targeting moieties discussed in the document.[92] The guidance is consistent with what would be generally known by a skilled artisan in this field prior to 2017.

3. US-633 Describes Using Known Radionuclides with FAP-Targeting Radiopharmaceuticals

75. US-633 lists examples of radionuclides that can be used in the radiopharmaceuticals intended for use with PET and SPECT imaging platforms. These include radiohalogens (*e.g.* ^{18}F, ^{76}Br, ^{123}I, ^{125}I) as well as radiometals (*e.g.* ^{67}Ga, ^{68}Ga, ^{67}Cu, ^{64}Cu, ^{99m}Tc, ^{186}Re, ^{188}Re). A skilled artisan would have known which radionuclides are suitable for use with different imaging platforms. For example, ^{68}Ga and ^{18}F can be used with PET imaging, while ^{99m}Tc and ^{188}Re can be used with SPECT imaging.[93]

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92 See generally EX1004 (US-633), [0124]-[0180].

93 EX1004 (US-633), [0049].

[0049] "Radionuclide" refers to molecule that is capable of generating a detectable image that can be detected either by the naked eye or using an appropriate instrument, e.g. positron emission tomography (PET) and single photon emission computed tomography (SPECT). Radionuclides useful within the present disclosure include penetrating photon emitters including gamma emitters and X-ray emitters. These rays accompany nuclear transformation such as electron capture, beta emission and isomeric transition. Radionuclides useful include those with photons between 80 and 400 keV and positron producers, 511 keV annihilation photons and acceptable radiation doses due to absorbed photons, particles and half life. Radionuclides include radioactive isotopes of an element. Examples of radionuclides include ^{123}I, ^{125}I, ^{99m}TC, ^{18}F, ^{62}Cu, ^{111}In, ^{131}I, ^{186}Re, ^{90}Y, ^{212}Bi, ^{211}At, ^{89}Sr, ^{166}Ho, ^{153}Sm, ^{67}Cu, ^{64}Cu, ^{100}Pd, ^{212}Pb, ^{109}Pd, ^{67}Ga, ^{68}Ga, ^{94}Tc, ^{105}Rh, ^{95}Ru, ^{177}Lu, ^{170}Lu, ^{11}C, and ^{76}Br. "Radiohalogen," as used herein, refers to those radionuclides that are also halogens (i.e. F, Br, I, or At).

4. US-633 Describes Chelators and Prosthetic Groups to Use in Radiopharmaceuticals

76. US-633 indicates that known chelating complexes ("chelators") and prosthetic groups can be used to associate a radionuclide with the remainder of the radiopharmaceutical.[94] It describes two general classes of radiopharmaceuticals: one (Formula I) that uses a chelator-radiometal group, and a second (Formula II) that uses a radiolabeled prosthetic-group (annotated below).

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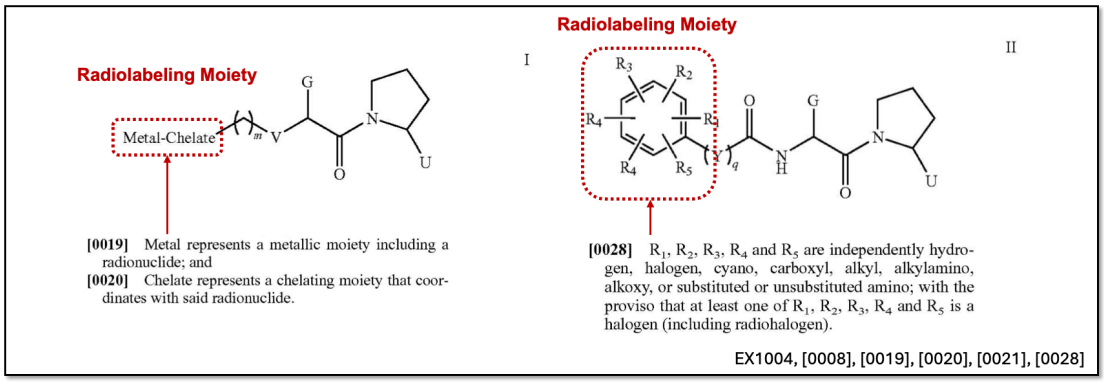
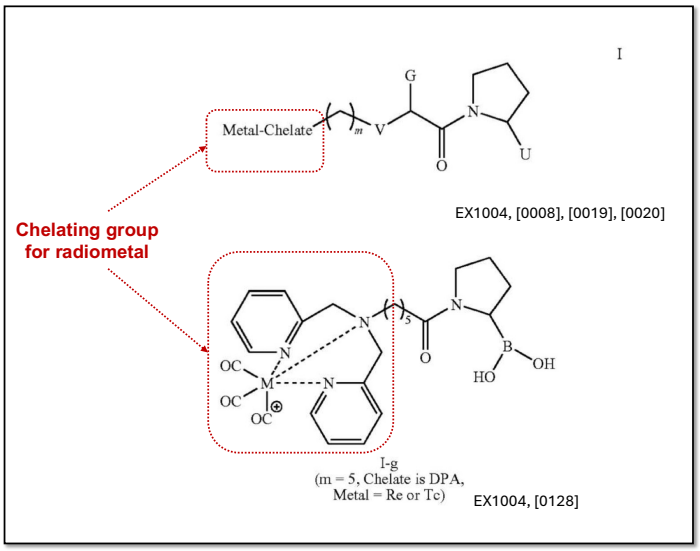
94 EX1004 (US-633), [0029].



a. *Chelator Moieties*

77. Chelators are illustrated within Formula I and in several examples of radiopharmaceutical compounds in US-633 (one of which is compared to Formula I below).[95]



95 EX1004 (US-633), [0008], [0019], [0020], [0128]. Also EX1004 (US-633), [0087], [0097], [0098], [0101]-[0110].

78. US-633 lists different classes and examples of chelators that can be used in its radiopharmaceuticals ([0100] (below)) and provides two tables listing specific chelators (Tables 3 and 4).[96] The chelators being described were well-known before 2017 (*e.g.*, DOTA, DPA, DTPA, PAMA, etc.). I discussed several of these chelators in my 2011 review article.[97]

[0100] Any suitable chelating moiety may be used to provide a covalent or other association with a radionuclide. Examples of chelating agents include but not limited to a substituted or unsubstituted N_2S_2 structure, a N_4 structure, an isonitrile, a hydrazine, a triaminothiol, a chelating agent with a hydrazinonicotinic acid group, a phosphorus group, phosphinothiols, thioesters, thioethers, a picolineamine monoacetic acid, a pyridine or bipyridyl based compound, and a substituted or unsubstituted cyclopentadienyl. By way of example, suitable chelating agents include tetra-azacyclododecanetetra-acetic acid (DOTA), diethylenetriaminepentaacetic acid (DTPA), bis(pyri din-2-ylmethyl)amine (DPA), quinolinemethylamino acetic acid, 2,2'-azanediyldiacetic acid, 2,2'-azanediylbis(methylene)diphenol, 2-((1H-imidazol-2-yl)methylamino)acetic acid, bis(isoquinolinemethyl)amine, bis(quinolinemethyl)amine, pyridine-2-ylmethylamino acetic acid (PAMA), 2-(isoquinolin-3-ylmethylamino)acetic acid, bis((1H-imidazol-2-yl)methyl) amine, bis(thiazol-2-ylmethyl)amine, 2-(thiazol-2-ylmethylamino)acetic acid, and their derivatives, e.g. bis(5-dimethylamino pyridine-2-ylmethyl)amine, bis((1-methyl-/H-imidazol-2-yl)methyl)amine, 2,2'-(2,2'-azanediylbis (methylene)bis(1H-imidazole-2,1-diyl))diacetic acid, 2-((1-(carboxymethyl)-/H-imidazol-2-yl)methylamino)acetic acid, 2,2'-(2-(2-(azanediylbis(methylene)bis(1H-imidazol-1-yl)acetylazanediyl)diacetic acid, and the like. Other chelating groups that may be incorporated into the compound of Formula I, include, but are not limited, to those groups as illustrated in Tables 3 and 4, below.

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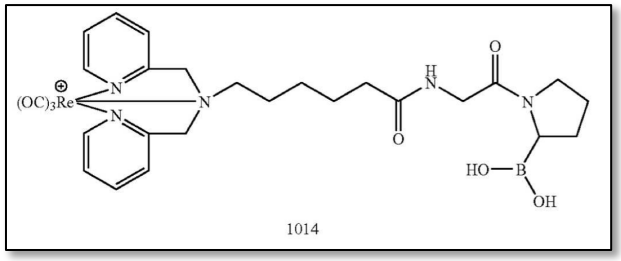
96 EX1004 (US-633), [0100], Table 3, Table 4.

97 EX1010 (Zeglis 2011), 5-9.

79. US-633 illustrates use of chelators with two radionuclides that are used in SPECT imaging: ^{99m}Tc (half-life of ~ 6 hours) and ^{188}Re (half-life of ~ 17 hours). For example, compound 1014 (below) in US-633 was illustrated being used with both of these radionuclides coordinated by the DPA chelator.[98]



1014

80. Other examples of chelators are identified in US-633 that a skilled artisan would understand would be suitable for ^{99m}Tc. These include the structures designated I-a (DPA) to I-f in the patent.[99] A skilled artisan considering chelator options for a radiotracer that uÅses ^{99m}Tc would consider using these chelators as well.

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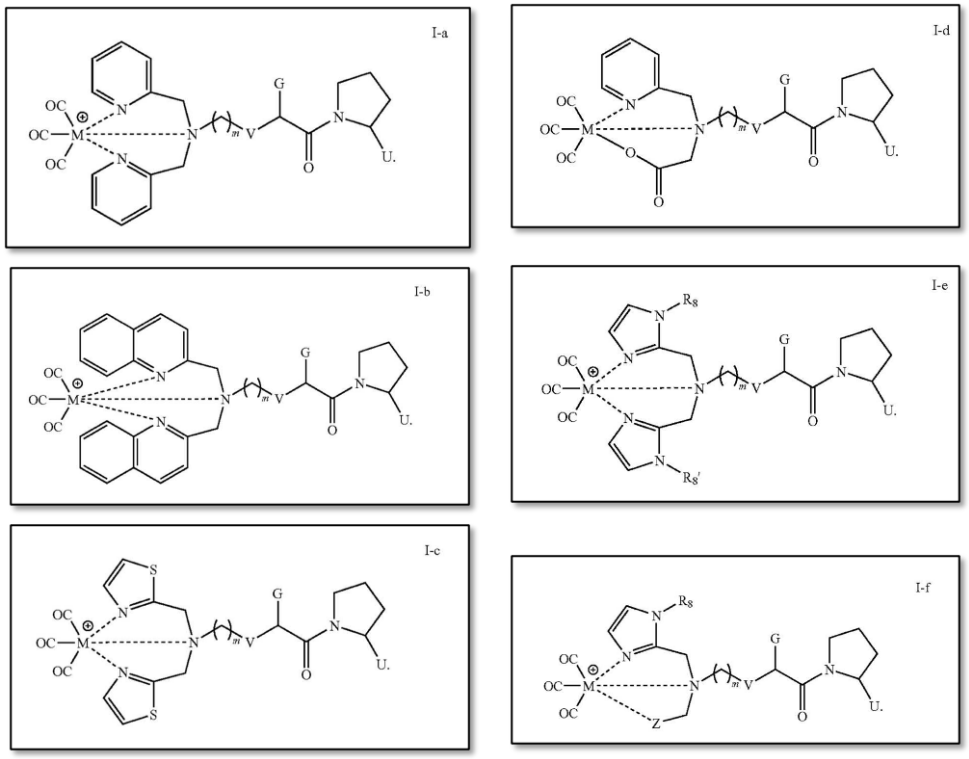
98 EX1004 (US-633), [0160] (illustrated with Re), [0189] (referencing compound 1014/1109), Figure 4 (showing compound 1109 using ^{99m}Tc).

99 EX1004 (US-633), [0102]-[0107].



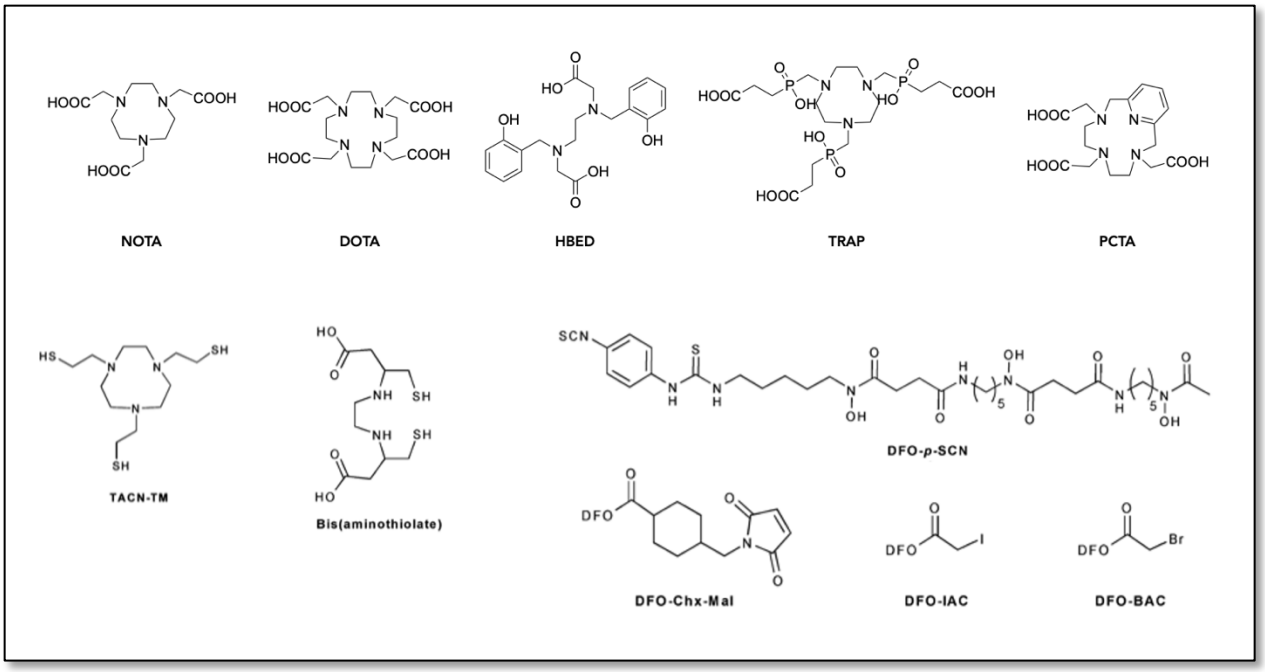
81. US-633 makes clear that the examples of chelators listed in it are not the only chelators it is proposing to use in the radiopharmaceuticals being described. Instead, it explains that "[a]ny suitable chelating moiety may be used to provide a covalent or other association with a radionuclide."[100] A skilled artisan would have read this sentence as indicating that she should consider as an option any chelator known to be suitable for use with the particular radionuclide being considered. So, for example, if a skilled artisan in early 2017 was designing a

[100] EX1004 (US-633), [0100].

^{68}Ga-based radiotracer, that person would have considered chelators that were known at that time to be suitable for use with ^{68}Ga.

82. In early 2017, a skilled person would have known that $^{68}Ga^{3+}$ radiometal is smaller than other metal cations and prefers to be coordinated by a mix of oxygen and nitrogen donor atoms, and that, in practice, the chelators that had proven most effective for $^{68}Ga^{3+}$ included: NOTA, DOTA, HBED, TRAP, PCTA, TACN-TM, and DFO (structures below). A skilled artisan would have believed that any of these chelators, thus, would be a "suitable chelating moiety" for ^{68}Ga.[101]

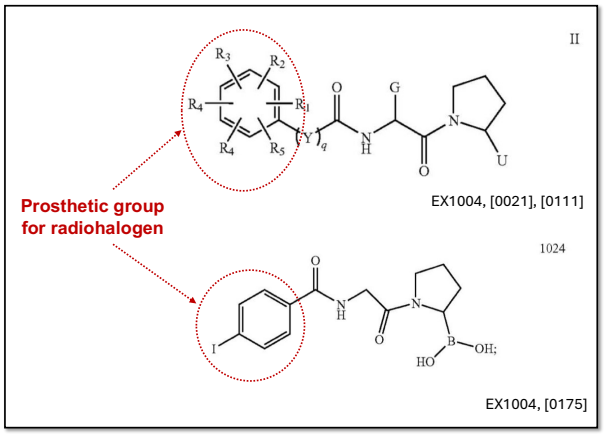
[101] EX1011 (Zeglis 2013), 1882-1883, 1887-1888 (describing effective chelators aside from DOTA for ^{68}Ga). See also EX1013 (Price), 267, 272, 275, 276.



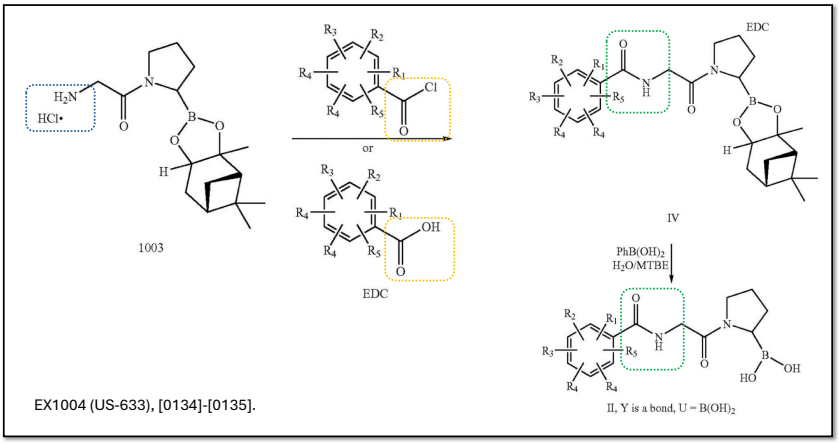
b. *Prosthetic Groups*

83. US-633 describes examples of "prosthetic group" structures that incorporate radiohalogens (*e.g.*, ^{18}F, ^{123}I, ^{131}I). For example, it illustrates a number of structures featuring substituted phenyl groups that can be used to carry the radiohalogen, which is covalently attached to a position on the benzene ring of the phenyl group.[102]

[102] EX1004 (US-633), [0111]-[0122] ("In some embodiments, the radiohalogen is selected radioiodine or radiofluorine.").



84. Prosthetic groups can be covalently linked to a radiopharmaceutical compound using straightforward chemical synthesis steps. For example, US-633 illustrates attachment of a prosthetic group to a linker-targeting moiety structure through formation of an amide bond. In it, the linker-targeting moiety has a free amine, while the prosthetic group has a free carboxylic or acyl chloride (illustrated below):[103]



103 EX1004 (US-633), [0134]-[0135].

85. By early 2017, a skilled artisan would have been familiar with a variety of prosthetic groups that had been used to associate a radiohalogen (*e.g.*, ^{18}F) to a radiopharmaceutical. Examples of prosthetic groups known before 2017 that would be suitable to use in an ^{18}F-labeled radiotracer are described in review articles from that period.[104] A skilled artisan would have considered any of these prosthetic groups to be options to use in radiotracers being discussed in US-633.

5. US-633 Describes Conjugating FAP-Binding Moieties to Imaging Agents Using Bi-Functionalized Linkers

86. US-633 describes use of a "linker" or "tether" to connect the proline-derived boronic acid FAP targeting moieties to radiolabeling moieties. It describes these "tethers" as being "a chemical linking moiety between a metal ion center and another chemical moiety.[105] US-633 also illustrates this with examples of synthetic procedures for constructing radiopharmaceuticals. For example, it provides examples of linking a radiometal chelator to proline-derived boronic acid FAP targeting moieties (Example 13) as well as examples in which a radioiodinated prosthetic group is linked to its FAP targeting moiety (Example 14).[106] Both use

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[104] EX1042 (Kiesewetter), 410-411; EX1043 (Chansaenpak), 12439-51; EX1034 (Meyer 2016), 2792-93, Figure 2.

[105] EX1004 (US-633), [0051].

[106] EX1004 (US-633), [0181] (Example 13); [0182] (Example 14).

conventional chemical synthesis techniques well-known to skilled artisans before 2017.

87. Several passages in US-633 discuss ways to alter the characteristics or size of the tether/linker can (below). One is to incorporate within the linker additional bonds to yield the desired separation between the radiolabeling and targeting moieties. Another is to omit or incorporate heteroatoms to alter the overall chemical character of the linker (*e.g.*, to increase hydrophilicity). Also, while US-633 illustrates how different length and types of "tether" (linker) structures are used with radiotracers having a metal chelator group, a skilled artisan would have viewed these illustrations of linker options to be equally relevant to radiotracers with radiohalogenated prosthetic groups. The relevant design principles are the same for both classes of radiotracers (*i.e.*, modulate the distance between the radionuclide bearing moiety and the targeting moiety, and influence the character of the compound). As US-633 explains (below): [107]

[107] EX1004 (US-633), [0101]. *Also* EX1004 (US-633), [0130], [0133].

[0101] The distance between the Metal-Chelate moiety and the pyrrolidine moiety of the complex represented by Formula I can be varied by altering the tether and/or expanding the length of the tether between them to modify the affinity and selectivity of the complex for seprase. The pharmacokinetic properties of the complex may also be modified by incorporating heteroatoms into the tethers. The following structures represented by Formulas I-a to I-k are some exemplary embodiments with different tethers and/or the length of tethers. To facilitate description, the complexes are described

[0130] Scheme 3 can be utilized to synthesize functionalized proline-$M^{+}(CO)_3$ complexes to explore the effect of more significant variations of the distance of the metal chelator from the proline moiety by incorporating a tether into these structures. The tether may comprise a simple alkyl chain as shown, a PEG $(CH_2CH_2O)_n$, a polyethylene amine $((CH_2CH_2NH)_n)$, or the like. Terminal aminoalkanoic acids (such as (β-alanine, 4-aminobutanoic acid, 5-aminopentanoic acid, 6-aminohexanoic acid and the 8-aminooctanoic acid) or amino-PEG-acids $(NH_2—(CH_2CH_2O)_n—CH_2—COOH$, e.g. 2-(2-(3-aminopropoxy)ethoxy)acetic acid) may be utilized as tethers, according to some embodiments.

[0131] According to some embodiments, glycine and/or other appropriate amino acid can be incorporated as a linker, as well as an additional binding moiety to afford seprase inhibitors. Scheme 4 illustrates synthesis of boronoproline-$Re^{+}(CO)_3$ or $Tc^{+}(CO)_3$ complexes having the structure as represented by Formula I-b and I-c. Lysine is used to prepare compounds of Formula I with additional amine moiety in the linkers.

88. US-633 also describes a variety of examples of linkers that can be used to connect a FAP targeting moiety and a radiolabeling moiety. It points out that linkers are often simple alkyl chains while other linkers can be polyethylene glycol or polyethylamine oligomers, amino acids or other simple bifunctional

compounds capable of forming amide, ether or thioether bonds at both ends.[108] In one passage (below), US-633 points out the practical benefits of using PEG-based linkers, given that they are commercially available in a variety of lengths and are functionalized in various ways to enable them to be readily incorporated into radiotracers (*e.g.*, PEG diamine compounds that have amines at both termini).[109] A skilled artisan would be very familiar with the use of PEG-based linkers before 2017.[110]

> **[0133]** The above reaction scheme is applicable to any modification of the tether by incorporation of heteroatoms into the tether chain. This may have additional benefits on the affinity as well as the selectivity for seprase. Incorporation of heteroatoms into the tether such as oxygen can take advantage of the commercially availability of a variety of short polyethylene glycol (PEG) diamines that can be readily incorporated into the complexes. One of ordinary skilled in the art would also readily apply other chelates to prepare functionalized proline-$M^{+}(CO)_3$ complexes.

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[108] EX1004 (US-633), [0130], [0131], [0133].

[109] EX1004 (US-633), [0133].

[110] See, e.g., EX1018 (Fichna), ("The most popular linkers are long poly(ethylene glycol) (PEG) or hydrocarbon chains to increase the lipophilicity and polyamino acid sequences, such as polyglycine, to increase the hydrophilicity, as well as esters and disulfides capable of rapid metabolism.").

89. A skilled artisan, based on the guidance in US-633 and their own training and experience, would have known how to select an appropriate linker to use in a radiopharmaceutical to connect the targeting moiety to either a chelator (for a radiometal) or a prosthetic group (for a radiohalogen).

6. US-633 Describes Examples of FAP-Targeting Radiopharmaceutical Compounds

90. US-633 includes examples of compounds based on either a boronic acid pyrrolidine or a cyano-pyrrolidine targeting moiety based on the radiotracer designs of Formulas I and II. Some employ a chelator-radiometal as the radiolabeling moiety, while others use a prosthetic group with a radiohalogen. The examples link the two moieties with "tethers" (a linker) of varying length and having varying characteristics. These examples illustrate how skilled artisans use the modular three-component design concept to devise variants of a radiotracer by combining particular linkers and particular radiolabeling moieties with a chosen targeting moiety.

91. US-633 reports that a number of the radiotracer compounds were tested and exhibited strong binding to FAP (*i.e.*, low nanomolar IC_{50} FAP inhibition). The test results for the compounds are compiled in Table 2.[111]

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[111] EX1004 (US-633), [0185].

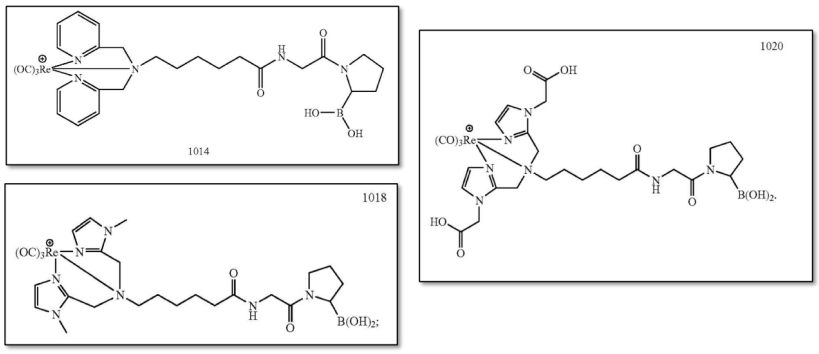
Compounds 1014, 1020, 1023-1030, and 1032 were among the most potent inhibitors of FAP (*i.e.*, <10 nM IC_{50} values). US-633, however, did not provide data on the affinities of these compounds for other prolyl oligopeptidases, so it does not enable one to assess the selectivity of these compounds for FAP. A skilled artisan would have wanted to review such data to differentiate compounds that exhibit high affinity for FAP from those that exhibit high selectivity for FAP relative to other prolyl oligopeptidases.

TABLE 2

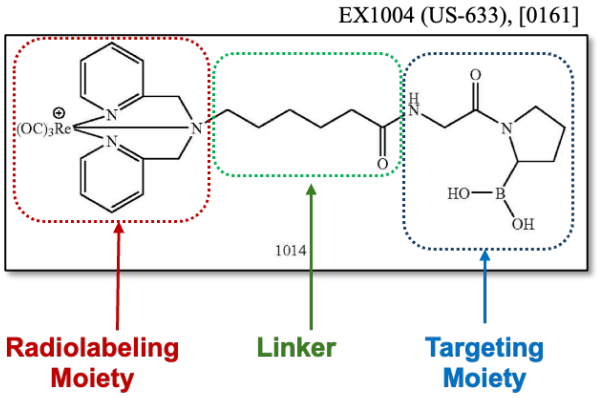
Summary of in vitro data against seprase

Compound No.	FAP (IC_{50} nM)
1010	2,540
1014	21
1016	3,533
1018	20
1020	4
1022	236
1023	7
1024	3
1025	2
1026	3
1027	5
1028	5
1029	8
1030	2
1031	262
1032	11
1033	500
1034	11
1035	340
1036	37
1039	511
1040	35
1041	692
1042	785
1043	115
1044	23,680
1045	1,437
1046	970
1048	7,414
1049	953
1050	1152
1051	67
1060	41,300
1061	24,540

92. Compounds 1014, 1018, and 1020 are examples of FAP-targeting radiopharmaceuticals that use chelators in the radiolabeling moiety. The structures for each are shown below.[112]



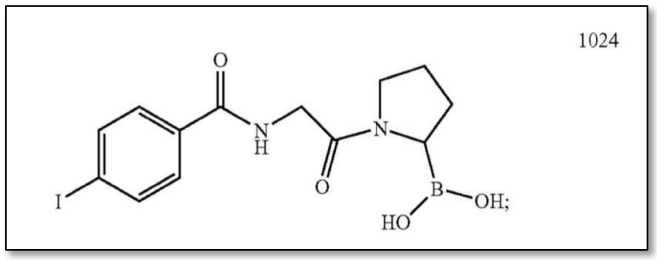
93. Below is a version of compound 1014 marked to identify the radiolabeling moiety, the linker, and the targeting moiety within the radiopharmaceutical compound.[113]

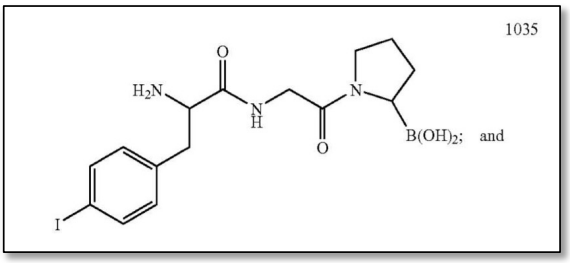


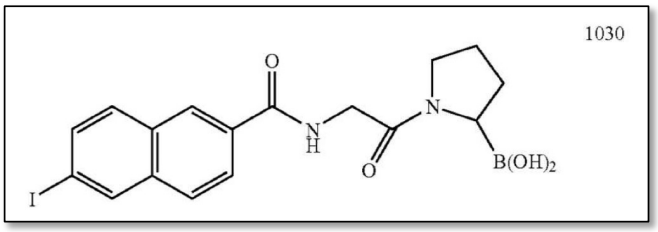
112 EX1004 (US-633), [0160], [0163], [0164].

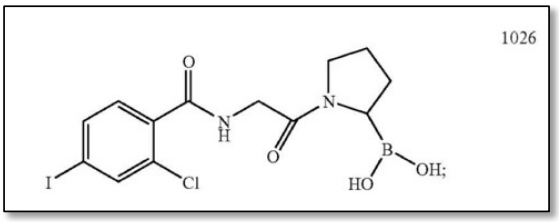
113 EX1004 (US-633), [0161].

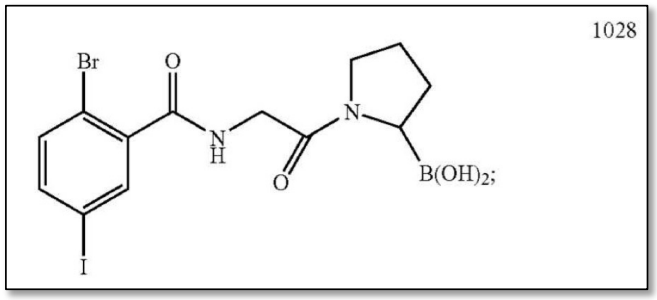
94. Compounds 1024, 1026, 1028, 1030, and 1040 are examples of potent FAP-targeting radiopharmaceuticals that use a prosthetic group in the radiolabeling moiety. Compound 1040 is one of the examples that uses a cyano-pyrrolidine targeting moiety instead of a boronic acid-bearing pyrrolidine. The structures for each are shown below.[114]

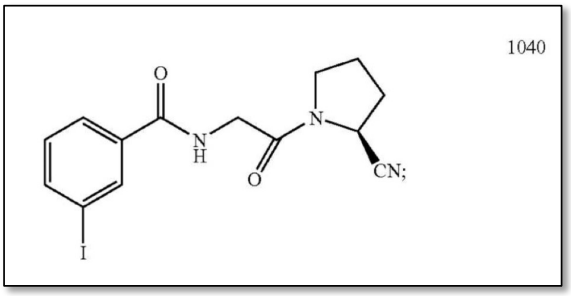








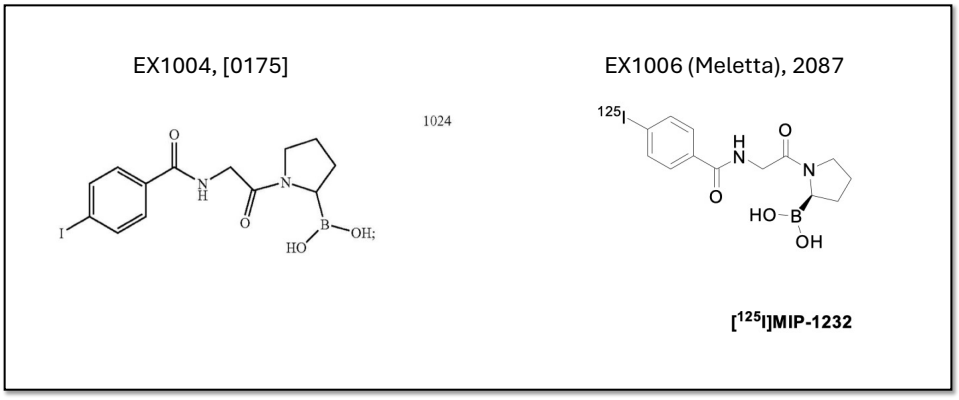




114 EX1004 (US-633), [0175] (compounds 1024, 1026, 1028, 1030); [0180] (compound 1040).

B. Meletta (EX1008)

95. Meletta reported results from investigations into the performance of a compound designated ^{125}I-MIP-1232 as FAP-targeting a radiotracer for atherosclerotic plaques.[115] ^{125}I-MIP-1232 is an ^{125}I radiolabeled form of compound 1024 from US-633.[116]



96. Meletta reported that "[m]ost FAP inhibitors share the pyrrolidine-2-boronic acid moiety as a common structural motif."[117] It also reported that the first such FAP inhibitor to enter clinical testing for treatment of cancer, Val-boro-Pro (PT-100), was ultimately unsuccessful "due to missing selectivity." A skilled

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115 EX1008 (Meletta), 2083 ("The goal of this study was to evaluate FAP as a target for atherosclerosis imaging with a small molecule.").

116 EX1008 (Meletta), 2087 (Scheme 2); EX1004 (US-633), [0174]. Meletta radiolabeled MIP-1232 with ^{125}I instead of ^{123}I used in US-633).

117 EX1008 (Meletta), 2083.

artisan would understand this to be indicating that the compound exhibited insufficient selectivity for FAP.[118]

97. Meletta indicated that MIP-1232 exhibited a 32-fold greater potency in inhibiting FAP than PREP, as well as high affinity (30 nM IC_{50}) for FAP, citing a 2009 abstract as the source of the IC_{50} values for compound 1024/MIP-1232 for PREP and other prolyl oligopeptidases (below).[119] Meletta thus provided data that allowed for the selectivity of the boronic acid pyrrolidine moiety for FAP to be assessed, which was not provided in US-633.

> The high binding affinity to FAP and the selectivity profile in combination with the possibility to radioiodinate MIP-1232 without altering its structure make this compound a promising molecule to assess the potential of FAP as an imaging target for the staging of plaque vulnerability and to detect FAP-positive tumors that may respond to FAP-targeted therapy.[120]

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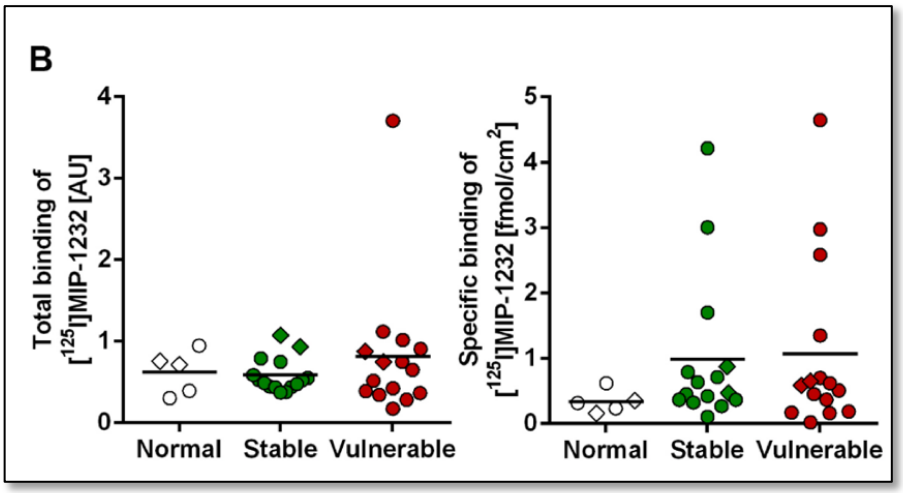
[118] EX1008 (Meletta), 2083.

[119] EX1008 (Meletta), 2083. See also EX1035 (Marquis), 1 ("The IC_{50} values of … MIP-1232 … for POP were … 19 …nM, respectively, with POP/FAP ratios of …32…, respectively."). POP is another acronym for PREP.

[120] EX1008 (Meletta), 2083.

98. Meletta reported that [^{125}I]MIP-1232 lacked sufficient selectivity for FAP when tested in three forms of tissue (*i.e.*, normal arteries, stable plaques, atherosclerotic plaques) to warrant further investigation.[121] As Meletta explained:

> [^{125}I]MIP-1232 binding was higher in atherosclerotic plaques than normal arteries. Vulnerable plaques showed a slightly higher radioactivity signal integrated over the tissue slice than stable plaques. However, after correction for the size of the tissue samples, average total binding was similar for the three categories (Figure 3A,B). Radiotracer binding was reduced under blockade conditions with an excess of unlabeled MIP-1232 indicating displaceable (specific) binding of [^{125}I]MIP-1232 (Figure 3A). No significant difference was detected comparing the specific binding of the three groups (Figure 3B).



[121] EX1008 (Meletta), 2087 ("No significant difference was detected comparing the specific binding of the three groups.").

99. Despite having several favorable attributes for a FAP-targeting radiotracer (*e.g.*, 3 nm FAP IC_{50}), Meletta reported that [^{125}I]MIP-1232 did not exhibit sufficient selectivity to make it a viable radiotracer for the imaging of atherosclerotic plaques. The basis for that conclusion was that [^{125}I]MIP-1232 exhibited low affinity, non-specific accumulation and had "a low FAP/PREP affinity ratio" (*i.e.*, insufficient selectivity for FAP over PREP). As it explained:

> Based on our data we cannot conclude on the selectivity of [^{125}I]MIP-1232 for FAP. In the absence of a known selective inhibitor, we investigated specificity by blocking with the unlabeled compound itself. The relatively high amount of remaining radiotracer after blocking must, therefore, accumulate with low affinity. Lipophilicity is most probably not involved as logP of MIP-1232 is about 0.5. The non-specific accumulation may result from interactions with highly abundant hydrolases or other proteins with affinities in the high micromolar range, considering that our blocker concentration was 100 µM. Specificity analysis of MIP-1232 was performed exclusively with FAP and PREP [29]. A conclusive evaluation of the binding affinity to dipeptidyl peptidases such as DPP-2, DPP-4, DPP-8 and DPP-9 would be required, irrespective of the fact that DPPs display in general low affinities for N blocked peptides [43,44]. As [125 I]MIP-1232 did not selectively accumulate in the atherosclerotic tissue and ***as its low FAP/PREP affinity ratio*** is already known we did not further investigate its selectivity profile. For future studies more selective inhibitors are needed to reduce non-specific tissue accumulation. To

> overcome limitations in specificity, novel lead structures and the use of antibodies and fusion proteins was proposed to minimize off-target effects [24,34,44].[122]

100. A skilled artisan would have found the non-specific binding observed for [^{125}I]MIP-1232 to be incompatible with using MIP-1232 as a targeting moiety in a radiopharmaceutical used for treatment or imaging. The Meletta authors effectively make this point: after pointing to the non-selective accumulation of [^{125}I]MIP-1232 in atherosclerotic tissue and the known "low FAP/PREP affinity ratio" for that molecule, they indicated that they "did not further investigate its selectivity profile" and that "more selective inhibitors are needed to reduce non-specific tissue accumulation."[123]

101. A skilled artisan would not have read Meletta as suggesting that FAP was a poor target for radiotherapy or nuclear imaging. Meletta instead indicates the problem was the specificity of MIP-1232. For example, Meletta proposed "novel lead structures and the use of antibodies and fusion proteins" to overcome the limitations in specificity of MIP-1232. Meletta also concludes by stating that "FAP imaging with a selective ligand would enable the identification of FAP-

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[122] EX1008 (Meletta), 2089-2090.

[123] EX1008 (Meletta), 2089-2090.

positive tumors sensitive to a FAP-targeted radiotherapy with existing antibodies [].”[124]

102. I reviewed statements about the results reported in Meletta that were provided by Dr. Martin Pomper in a declaration he submitted to the Patent & Trademark Office (PTO) during the examination of the ’201 Patent (EX1001). Dr. Pomper (like I did) interpreted the results reported in Meletta as indicating that the MIP-1232 molecule was a failure as a radiopharmaceutical candidate because of its insufficient selectivity for FAP. Dr. Pomper’s statements are reproduced below:[125]

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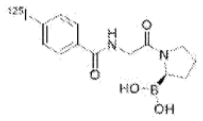
[124] EX1008 (Meletta), 2090.

[125] EX1002 (’201 FW), 363-365 (¶¶ 32-37).

32. The unpredictability of the art is clearly manifested by the documented failure of a compound disclosed in Zimmerman.

33. In particular, a representative compound disclosed in Zimmerman, namely MIP-1232, was extensively investigated as a potential medical imaging agent for atherosclerotic



[^{125}I]MIP-1232

plaque, and was determined as not suitable for the application. *See* Meletta et al., Molecules. 2015 Jan 27;20(2):2081-99 (hereinafter "Meletta").

34. As provided on page 2089 of Meletta, while pronounced binding of [^{125}I]MIP-1232 to carotid plaques was observed, Meletta did not observe, however, any difference in average specific binding between stable and vulnerable plaques and between plaques and normal arteries. Based on these data, Maletta could not conclude on the selectivity of [^{125}I]MIP-1232 for FAP.

35. Accordingly, because of the low specificity MIP-1232 displayed in the testing, Meletta concluded that MIP-1232 was not suitable for the medical imaging application for the particular medical condition being tested in Meletta. *Id*. at 2095.

36. Based on my review, I am not aware of any other compounds disclosed in Zimmerman that fared better than MIP-1232 and that was developed successfully as a medical imaging agent.

37. The failure of investigations with MIP-1232, which is a compound disclosed in the cited prior art, is especially telling as it demonstrates the high unpredictability of the art and lack of reasonable expectation of success by combining the prior art to arrive at the compounds of the pending claims for medical imaging applications.

EX1002, 363-365 (¶¶ 32-37).

103. I do not believe a skilled artisan would have read the observations in Meletta as suggesting that FAP was not a viable target for a small molecule radiotracer. Instead, Meletta made clear that the problem with MIP-1232 was the low selectivity of the targeting moiety in compound. Meletta's observations actually reinforce that FAP was still as a compelling target for imaging tumors (below).

> The high binding of [^{125}I]MIP-1232 to a FAP-positive SK-Mel-187 xenograft but low binding to a xenograft with low FAP levels is

promising towards the imaging of FAP to support FAP-targeted therapy in oncology.[126]

104. I believe the findings reported in Meletta would have encouraged a skilled artisan to continue efforts to find new small molecule FAP-inhibitors with greater selectivity for FAP over PREP that could function as a targeting moiety in a FAP-targeting radiotracer. Doing that would solve the problem of the boronic acid pyrrolidine FAP inhibitors described in US-633 and Meletta (*i.e.*, that they exhibited insufficient FAP selectivity relative to PREP and other prolyl oligopeptidases).

C. Jansen (EX1006)

105. Jansen is a 2014 publication that reports the synthesis and characterization of around 60 novel FAP-binding compounds. [127]

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[126] EX1008 (Meletta), 2095.

[127] EX1006 (Jansen), 3054 ("A total of around 60 novel inhibitors were synthesized for this study. All compounds were prepared following the general strategies in Schemes 1 and 2, in which target compounds are clustered according to the modification type they contain, relative to reference compound **4**.").

1. Jansen Identifies the Need for Improved FAP-Targeting Moieties for Radiopharmaceuticals

106. The introduction of the Jansen paper identifies attributes of FAP that I discussed above (¶ 59) that made FAP an appealing target for radiopharmaceuticals before 2017, particularly radiotracers for the nuclear imaging of tumors. As it explains:

> … FAP is also highly expressed on activated fibroblasts in over 90% of common human epithelial tumors. [] It has been demonstrated in syngeneic mouse models that FAP activity promotes tumorigenesis and that FAP inhibition attenuates tumor growth.[] The enzyme is furthermore expressed only transiently during wound healing and is essentially absent in normal adult tissues and in nonmalignant tumors. [] ***These appealing characteristics of FAP account for its ongoing evaluation as a drug target***. Both immunotherapy and small-molecule based approaches have so far been reported, most of them focusing on applications in the oncology domain (vide infra).[128]

107. Jansen summarized experiences of others attempting to develop FAP targeting chemical compounds as of 2014. It points out, for example, that the similarity of FAP to other prolyl oligopeptidase enzymes, particularly PREP, had made it difficult to develop targeting moieties that would selectively bind FAP but

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[128] EX1006 (Jansen), 3053 (citations omitted, emphasis added).

not PREP (which did not share FAP's limited expression pattern in tumors). As it explains:

> FAP possesses both dipeptidyl peptidase and endopeptidase activity, catalyzed by the same active center. This is in contrast with the DPPs, possessing only the former activity type, and PREP, which is an enzyme of strict endopeptidase capability. [] ***While designing out DPP affinity in FAP inhibitors is relatively straightforward, obtaining inhibitors possessing selectivity for FAP over PREP is considered to be far more challenging***. This is, among others, illustrated by the significant overlap between in vitro processable substrate sequences for FAP and PREP and the fact that numerous reported FAP inhibitors have limited or no selectivity with respect to PREP.[][129]

108. Jansen surveyed six FAP inhibitor molecules that had been reported in the literature prior to 2014 (Figure 1, reproduced below).[130] All but one of these compounds are based on a 2-substituted pyrrolidine structure.

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[129] EX1006 (Jansen), 3053 (citations omitted, emphasis added).

[130] EX1006 (Jansen), 3054.

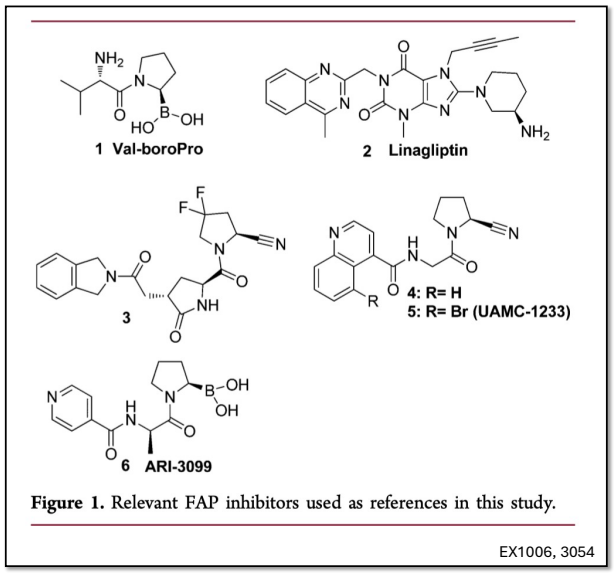

Figure 1. Relevant FAP inhibitors used as references in this study.

EX1006, 3054

109. The comments in Jansen about the six FAP inhibitor molecules in Figure 1 would have led a skilled artisan to conclude that none of molecules would be a viable targeting moiety for a FAP-targeting radiopharmaceutical.

(a) Compound 1 (ValboroPro, talabostat, PT-100) was investigated clinically in phase II clinical trials, but, as Jansen points out, it "was withdrawn, apparently because of both safety and efficacy reasons."[131]

(b) Compound 2 (linagliptin) "received approval as a DPPIV inhibitor but also displays substantial FAP affinity."[132] The

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131 EX1006 (Jansen), 3054.

132 EX1006 (Jansen), 3054.

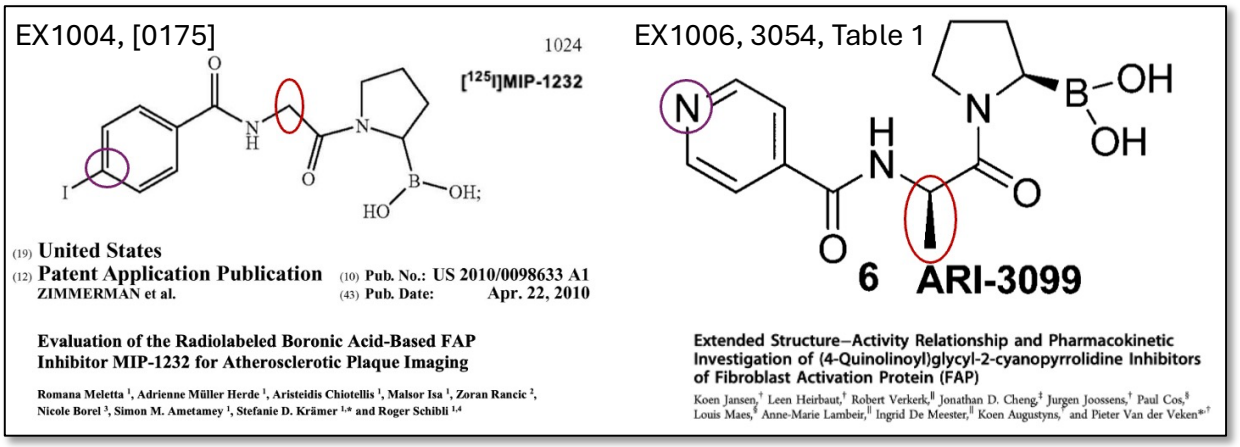
affinity of linagliptin for both enzymes would be undesirable for a FAP-selective targeting moiety.

(c) Compounds 3, 4 and 5 were from publications by Tsai (3) and the Jansen group (4 and 5). All are based on a 2-cyanopyrrolidine scaffold. Jansen reported mixed results for these compounds. For example, it indicated that while compound 3 had "highly satisfactory FAP over PREP selectivity" it had "poor pharmacokinetic (PK) behavior in mice."[133] Compounds 4 and 5 were found to not be as favorable as compound 60, as I discuss below (¶¶ 114, 120-122, 125-126).

(d) Compound 6 is a boronic acid inhibitor that shares a pyrrolidine-2-boronic acid moiety motif with the compound 1024/MIP-1232 molecule described in US-633 (EX1004) and Meletta (EX1008) (below). The two molecules differ at the two positions noted in the illustration below. Jansen observed that compound 6 had a selectivity index of 39.6 for FAP over PREP,

[133] EX1006 (Jansen), 3054. Also EX1047 (Tsai), 6576 (compound 19), 6577-78.

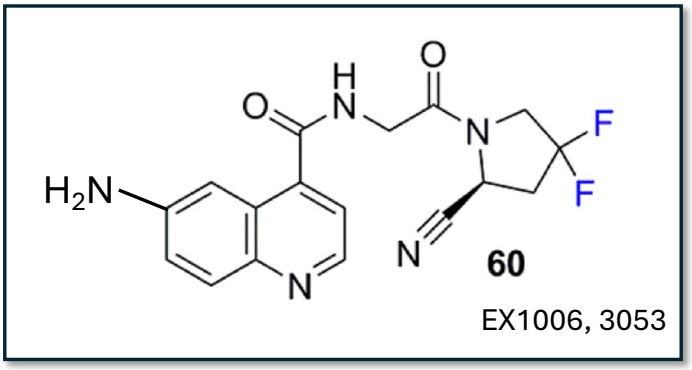
and that "no in vivo PK data have so far been published for 6."[134]



2. Jansen Showcases Compound 60 as a Promising FAP Targeting Moiety

110. Jansen's introduction would have led a skilled artisan to believe that, at least as of 2014, there was a pronounced need for new FAP inhibitors that were highly selective for FAP relative to other prolyl oligopeptidases, particularly PREP, and that had other characteristics that make them suitable to use in a FAP-targeting radiopharmaceutical. The skilled artisan logically would focus on the FAP inhibitors described in Jansen, and particularly compound 60 (below).

134 EX1006 (Jansen), 3054, Table 1; EX1004 (US-633), [0175].

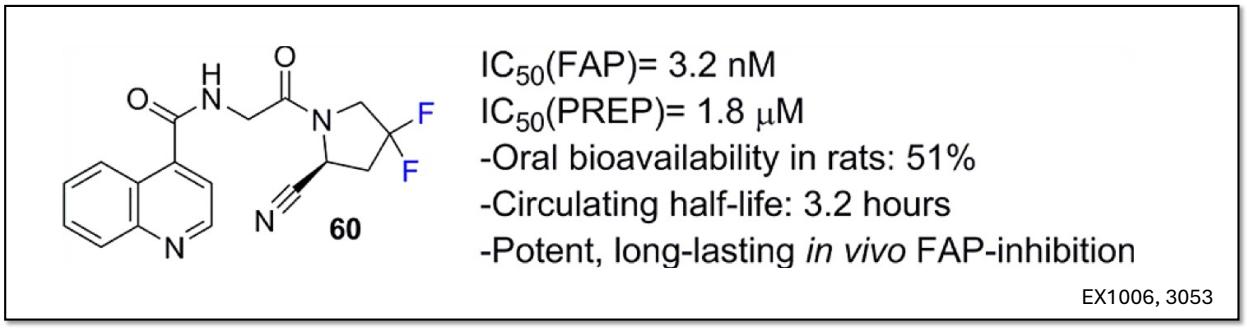


111. A skilled artisan would have read the Jansen paper as portraying compound 60 as the most promising FAP inhibitor and as a compound that had potential for being developed into a radiopharmaceutical that could be used in nuclear imaging or therapy. Several sections of the paper support this conclusion.

112. First, and most significantly, the Jansen paper showcases compound 60 as a particularly appealing FAP inhibitor. Jansen presents compound 60 in its abstract and lists a set of its properties that a skilled artisan would recognize make compound 60 an excellent candidate for the targeting moiety of a FAP-targeting radiopharmaceutical (below).[135] These properties include its high affinity for FAP (i.e., 3 nanomolar IC_{50} for FAP inhibition), its high relative specificity (*i.e.*, selectivity) for FAP over PREP (562.5:1), its favorable stability, toxicity, and

[135] EX1006 (Jansen), 3053, Abstract.

pharmacokinetic characteristics, and its ability to cause potent, long-lasting *in vivo* FAP inhibition.



113. Jansen portrayed compound 60 was one of the four "most promising FAP inhibitors" based on the N-4-quinolinoyl-Gly-(2S)-cyanoPro scaffold that had been tested to date.[136] These four molecules were the ones that Jansen reported were tested in rats (*i.e.*, compounds 4, 5, 60 and 61).[137]

> A selection was made among ***the most promising FAP inhibitors discovered so far in the studied series***. In vitro PK and toxicity parameters were determined for these, generally predicting potential for satisfactory in vivo behavior. Four of these compounds were then submitted to in vivo PK analysis in rats.[138]

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136 EX1006 (Jansen), 3063.

137 EX1006 (Jansen), 3064 ("The in vivo PK parameters were then determined for inhibitors 4, 5, 60, and 61 in rats.").

138 EX1006 (Jansen), 3064.

114. While Jansen reported that four compounds were tested in rats, one of them (compound 4) killed all the rats it was administered to, while the other three compounds (5, 60 and 61) showed no such toxicity.[139] Without considering other factors, a skilled artisan would have deprioritized compound 4 relative to other compounds that did not exhibit toxic effects in rats.[140] Consistent with this point, Jansen did not test compound 4 in additional *in vivo* experiments and omitted compound 4 from its comparative assessment of the pharmacokinetic characteristics of molecules tested in rats (Table 8 below), which was limited to compounds 5, 60 and 61.[141]

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[139] EX1006 (Jansen), 3064 ("Notably, all rats subjected to 4 were found to die within 6 h. No signs of toxicity were observed during the observation period or upon autopsy in any of the other animals treated with compounds 5, 60, and 61, and parameters for these molecules are summarized in Table 8. … Taking into account the high degree of structural similarity between the four compounds evaluated, we do not have a clear view on possible factors that could explain the singular toxicity to rats of 4.").

[140] EX1006 (Jansen), 3064 ("However, some questions remain with respect to compound 4.").

[141] EX1006 (Jansen), 3063.

Table 8. In Vivo PK Properties of Selected FAP Inhibitors in Rats

	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC (μg·h/mL)	Cl (mL/min)	V_d (L)	relative bioavailability (%)
5 (iv)[a]		6.5	1.73	6.1	11.7	1.75	52
5 (po)[b]	0.33	5.6	1.7	13.4	23.0	3.5	
60 (iv)[a]		11.8	1.74	23.4	2.83	0.43	74
60 (po)[b]	0.33	14.6	3.4	76.7	1.55	0.34	
61 (iv)[a]		8.5	1.40	11.1	6.5	0.77	79
61 (po)[b]	0.75	14.7	1.22	39.39	8.2	0.86	

[a]Compound was formulated in PEG_{200} and administered via single intravenous injection at 5 mg/kg. [b]Compound was formulated in PEG_{200} and administered per os (gavage) at 20 mg/kg.

EX1006, 3063

115. Based on what Jansen itself says, a skilled artisan would have viewed compound 60 as one of the most promising FAP inhibitors that had been discovered to date, and that it was an appealing candidate for the targeting moiety of a FAP-targeting radiopharmaceutical (below). A skilled artisan would certainly have considered compound 60 to be a promising candidate as a FAP-targeting moiety in a radiotracer for imaging tumors.

> Summarizing, we have identified a series of highly potent FAP inhibitors with promising pharmacokinetic behavior. We believe that our selective, in vivo active inhibitors are ***currently best placed among all published compounds*** to help elucidate the function of FAP in different animal models of disease and to allow its continuing validation as a drug target.[142]

[142] EX1006 (Jansen), 3065 (emphasis added).

3. The Reported Properties of Compound 60 Make It an Appealing FAP-Targeting Moiety

116. In addition to being showcased by Jansen's discussion, compound 60 would have been considered one of the most promising FAP inhibitors that could become the targeting moiety of a FAP-targeting radiotracer. This is the conclusion that a skilled artisan would have reached based on an analysis of the data reported in Jansen from testing compound 60 and the other ~60 compounds it describes.

117. As I explained previously (¶¶ 45, 60, 107, 110), the most important characteristic of the compounds is their selectivity for FAP relative to PREP. If a radiotracer intended to visualize FAP-expressing tissues was not sufficiently selective, it could inadvertently bind to one (or more) of these other four enzymes (DPPIV, DPP8, DPP9, or PREP) and accumulate in tissues that express those proteins rather than FAP. For example, a radiotracer that binds to PREP could accumulate in potentially healthy tissues that express PREP but not FAP, leading to false positives in the tissue distribution image.

118. To identify the FAP inhibitor compounds described in Jansen that would be expected to be the best choice to use as a targeting moiety in a FAP-targeting radiopharmaceutical (particularly one for nuclear imaging), a skilled artisan would have classified the candidate molecules using a series of classification steps.

(a) FAP selectivity: Jansen reported data from testing the ability of the compounds to inhibit FAP and three other prolyl oligopeptidases: DPPIV, DPP9, and PREP.[143] It also reported a selectivity index rating which compared the IC_{50} of each compound for FAP to the IC_{50} for PREP. A skilled artisan would first rank the compounds based on their selectivity index and select those exhibiting the highest selectivity values.

(b) After prioritizing compounds based on their selectivity for FAP, a skilled artisan would prioritize the compounds based on their affinity for FAP. High affinity enables the compound to stay bound to FAP on target cells long enough to generate sufficient data for imaging.

(c) After selecting compounds that exhibited the highest selectivity and highest affinity, a skilled artisan would assess the structures of the compounds to identify any concerns with respect to stability, hydrophobicity, or ease of modification. A skilled artisan would proceed through this sequence of classification

[143] EX1006 (Jansen), 3055.

steps to yield a set of highest priority candidates that scored highest in each of the successive metrics.

119. I reviewed the data reported in the Jansen paper (EX1006) for the various FAP inhibitor compounds described in the paper. I first classified the molecules using their selectivity index, then I classified the molecules with high selectivity based on their affinity, and finally, I classified the high selectivity, high affinity compounds based an assessment of their stability, hydrophobicity, and ease of modification. I used the following scales for classifying the compounds on the basis of selectivity[144] and affinity (potency of inhibition):

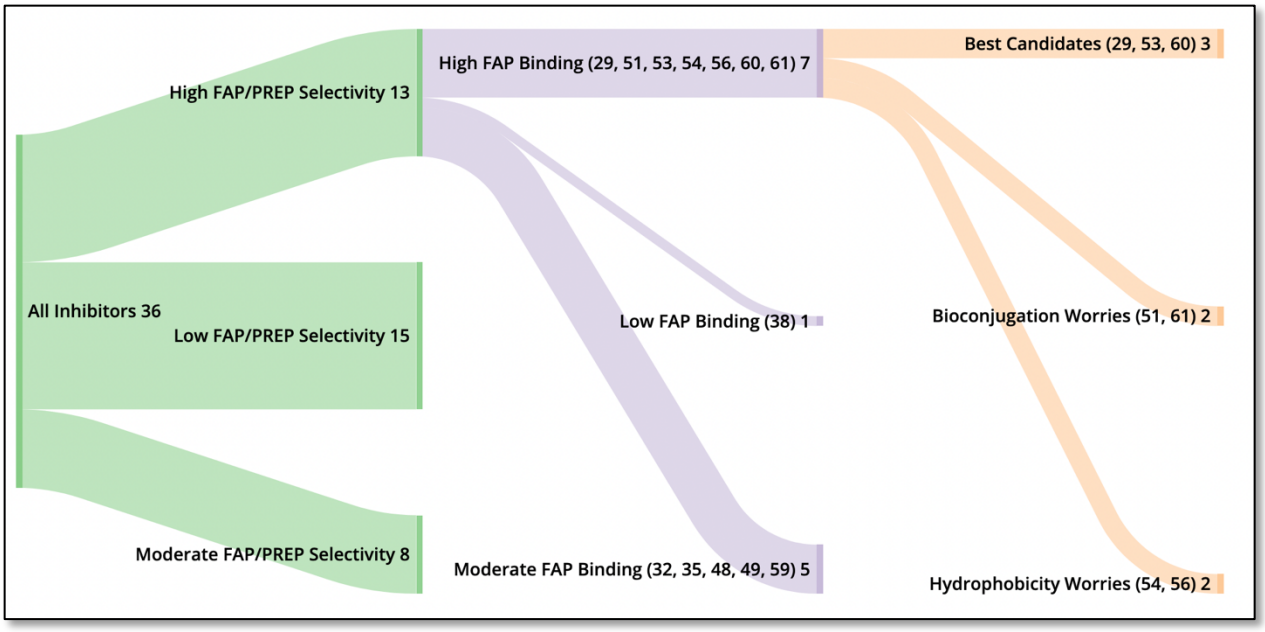
High selectivity	$IC_{50}^{PREP}/IC_{50}^{FAP} > 100$
Moderate Selectivity	$10 < IC_{50}^{PREP}/IC_{50}^{FAP} < 100$
Low Selectivity	$IC_{50}^{PREP}/IC_{50}^{FAP} < 10$

High affinity	$IC_{50}^{FAP} < 0.1\ \mu M$
Moderate affinity	$0.1\mu M < IC_{50}^{FAP} < 1.0\ \mu M$
Low affinity	$IC_{50}^{FAP} > 1.0\ \mu M$

[144] EX1033 (Mach), 140 ("Ideally, a PET probe should possess a high (i.e., nanomolar) affinity and high selectivity (>100-fold) for the target protein versus other proteins in the CNS [Central Nervous System].").

120. I first divided the original 36 inhibitors into categories of compounds with high (13), moderate (8), or low (15) selectivity for FAP over PREP. Then, I further categorized the 13 compounds with high selectivity into groups with high (7), moderate (5), and low (1) affinity for FAP. Finally, I examined the characteristics of the compounds exhibiting high selectivity and affinity for FAP more carefully. Of the seven compounds with high selectivity and affinity for FAP, two (51 and 61) would pose bioconjugation issues because of their 7 and 6 position substitutions, respectively, on the quinolinyl ring would be occupied. Another two (54 and 56) raised hydrophobicity concerns due to pendant phenyl groups. That yielded what I believe a skilled artisan would have viewed as the three most promising candidates in Jansen: compounds 29, 53 and 60.

121. I prepared a visual representation of classification process using a so-called Sankey diagram, which shows the sequential classification of the compounds pursuant to the three-tiered approach (below). Compounds 29, 53 and 60 showed the best overall profile using these criteria.



122. Below is a compilation of the measured selectivity and affinity characteristics of compounds 4, 5, 29, 53, 60 and 61.[145] Compounds 5, 60 and 61 showed the highest selectivity for FAP over PREP (*i.e.*, >4,500, 562, and 976, respectively). Compound 60, however, had the highest affinity of this group (*i.e.*, 0.0032 μM IC_{50} vs. 0.11 μM for compound 5).

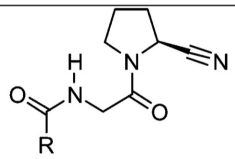
[145] EX1006 (Jansen), 3054 (Table 1) (4, 5), 3059-60 (Table 3)(29, 53), 3062 (Table 5)(61, 62).

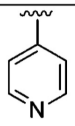
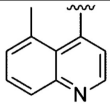
Table 1. IC_{50} of Reference FAP Inhibitors

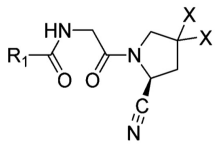
compd	IC_{50} (μM)[a] FAP	PREP	DPPIV	DPP9	DPP2	SI (FAP/PREP)[b]
1	0.066 ± 0.011	0.98 ± 0.06	0.022 ± 0.001	ND[c]	0.086 ± 0.007	14.8
2	0.37 ± 0.002	>100	0.0020 ± 0.0002	>100	>100	>250
3	0.017 ± 0.001	>100	>100	>100	>100	5882.4
4	0.0103 ± 0.0004	0.86 ± 0.07	>100	>100	>100	83.5
5	0.011 ± 0.0004	>50	>100	>100	>100	>4500
6	0.025 ± 0.001	0.99 ± 0.04	>100	>50	>100	39.6

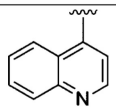
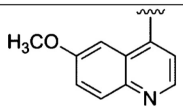
[a]Determined under our own assay conditions. [b]SI stands for "selectivity Index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]). [c]ND stands for "not determined".

EX1006, 3054



Nr	R =	IC_{50} (µM) FAP	PREP	DPPIV	DPP9	DPPII	SI (FAP/PREP)[a]
29		0.063± 0.003	11.3 ± 1.2	>100	>100	>100	179.4
53		0.0043± 0.0001	9.1 ± 0.6	>100	>50	>100	2116.3



Nr	R_1	X	IC_{50} (µM) FAP	PREP	DPPIV	DPP9	DPP2	SI (FAP/PREP)[a]
60		F,F	0.0032± 0.0004	>1.8 ± 0.2	>100	>12.5	>100	562.5
61		F,F	0.0085± 0.0009	8.3 ± 0.7	19±1.3	27.2±0.8	>100	976.4

EX1006, 3059-60 (29,53), 3062 (60, 61)

123. Jansen reported that compounds 4, 5, 60, and 61 were subjected to additional testing *in vitro* and *in vivo*. Jansen did not report comparable data for compounds 29 and 53, so it was not possible to compare the *in vitro* stability, PK, bioavailability and *in vivo* characteristics of compound 60 to compounds 29 and 53. All of those characteristics for compound 60, however, are favorable.

124. With regard to the *in vitro* experiments (compiled in Table 7 below), Jansen reported that "[i]n general, no large differences between individual inhibitors were observed for the evaluated parameters."[146] Consistent with Jansen's observation, with some exceptions, the results do not significantly differentiate the compounds relative to each other.

Table 7. In Vitro Pharmacokinetic Properties of Selected FAP Inhibitors[a]

parameter	4	5	68	60	61
kinetic solubility (μM)	>200	>200	>200	>200	>200
log *D*	0.5	0.7	0.8	1	nd
plasma stability (% unchanged at 6 h) (mouse/rat/human)	100/90/nd	90/nd/100	nd/100/80	85/95/nd	nd
microsomal stability (% unchanged at 6 h) (mouse/rat)	nd	75/nd	90/nd	90/94/nd	nd
cytotoxicity (MRC-5 cells) (μM)	>64	>64	>64	>64	>64

[a]nd stands for "not determined".

EX1006, 3063

125. Compounds 4, 5, 60, and 61 were subjected to *in vivo* testing in rats to determine certain pharmacokinetic characteristics of the compounds. As I noted

146 EX1006 (Jansen), 3063. Jansen does not report data for compound "68." This appears to be a typo, and it is unclear what compound the data concerns.

earlier (¶ 114), compound 4 was toxic in all tested animals, and Jansen did not test it in other *in vivo* experiments. The pharmacokinetic profiles for each of compounds 4, 60, and 61 are compiled in Table 8 (below). As was the case for the *in vitro* testing, all three compounds showed good bioavailability. However, compound 60 exhibited the slowest clearance rate and the highest overall exposure (AUC) of the three compounds. Jansen similarly observed that "[a]ll inhibitors evaluated displayed significant, roughly comparable oral bioavailability (50−79%) and reasonable elimination half-lives (1.5−3 h).[147]

Table 8. In Vivo PK Properties of Selected FAP Inhibitors in Rats

	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC (μg·h/mL)	Cl (mL/min)	V_d (L)	relative bioavailability (%)
5 (iv)[a]		6.5	1.73	6.1	11.7	1.75	52
5 (po)[b]	0.33	5.6	1.7	13.4	23.0	3.5	
60 (iv)[a]		11.8	1.74	23.4	2.83	0.43	74
60 (po)[b]	0.33	14.6	3.4	76.7	1.55	0.34	
61 (iv)[a]		8.5	1.40	11.1	6.5	0.77	79
61 (po)[b]	0.75	14.7	1.22	39.39	8.2	0.86	

[a]Compound was formulated in PEG_{200} and administered via single intravenous injection at 5 mg/kg. [b]Compound was formulated in PEG_{200} and administered per os (gavage) at 20 mg/kg.

EX1006, 3063

126. Jansen reported results of additional *in vivo* experiments with compounds 5, 60, and 61, including the overall degree of inhibition of FAP activity *in vivo* at time points over a 24-hour period. Jansen reported that when the compounds were administered orally, there was no significant deviations in the

147 EX1006 (Jansen), 3064.

sustained suppression of FAP activity. However, when the compounds were administered intravenously (*i.e.*, how radiopharmaceuticals and radiotracers are administered), compound 60 separated from the other two compounds—it caused "the most extensive and prolonged inhibition of FAP in the PK studies."[148] The *in vivo* characterization of the compounds, particularly the longer and more pronounced *in vivo* FAP inhibition results observed for compound 60 relative to compounds 5 and 61 when the compounds are administered intravenously would have provided significant additional insights into the potential of compound 60 to function as a targeting moiety in a FAP-targeting radiotracer. This additional data in Jansen would have influenced how a skilled artisan would have assessed the compounds being described, and it differentiated compound 60 as being the best FAP-targeting moiety candidate of all the reported compounds.

127. Based on the entirety of the data provided in the Jansen paper, and the structures of the various compounds tested in Jansen, I believe a skilled artisan would have identified compound 60 as the most promising overall candidate compound among the compounds described in Jansen. That person would have found from the data characterizing compound 60 in Jansen a compelling reason to use compound 60 as the basis of radiopharmaceuticals, and particularly as the

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[148] EX1006 (Jansen), 3064.

targeting moiety of a FAP-targeting radiotracer to be used in the nuclear imaging of tumors.

D. A Skilled Artisan Would Have Considered the Collective Guidance in US-633, Meletta and Jansen to Design Novel FAP-Targeting Radiotracers

128. In early 2017, I believe a skilled artisan would have considered the information and observations reported in US-633, Meletta and Jansen publications together to gain insights into and guidance concerning the design of FAP-targeting radiotracers for tumor imaging. Several reasons support my conclusion.

(a) All three references report investigations in the same scientific field—compounds that selectively target FAP—and highlight the benefits of FAP-targeting radiopharmaceuticals in the diagnosis and treatment of cancer.[149] As I explained earlier

[149] EX1004 (US-633), [0005] ("The expression of seprase on tumors makes it an attractive target to exploit for noninvasive imaging as well as targeted radiotherapy.") [0002]-[0003], [0007]; EX1006 (Jansen), 3053 (explaining FAP is expressed "90% of common human epithelial tumors" but "is essentially absent in normal adult tissues and in non-malignant tumors" which are "appealing characteristics for its ongoing evaluation as a drug target."); EX1008 (Meletta), 2082-2083 ("FAP was initially identified as a pivotal component of the tumor microenvironment expressed by reactive stromal

(¶¶ 59, 61-62), there was substantial interest in developing FAP-targeting radiopharmaceuticals by 2017, and each of the three publications contributed to that growing interest.[150]

(b) The three references provide reports from investigations into structurally similar FAP-targeting compounds containing a substituted pyrrolidine core structure. For example, a number of compounds in US-633 contain a glycyl-2-boronic acid pyrrolidine structure (*e.g.*, compound 1024), while Jansen describes compounds incorporating a glycyl-2-cyano pyrrolidine structure (below). All three publications provide data on the affinity and/or selectivity of these molecules for FAP relative to other prolyl oligopeptidases.[151]

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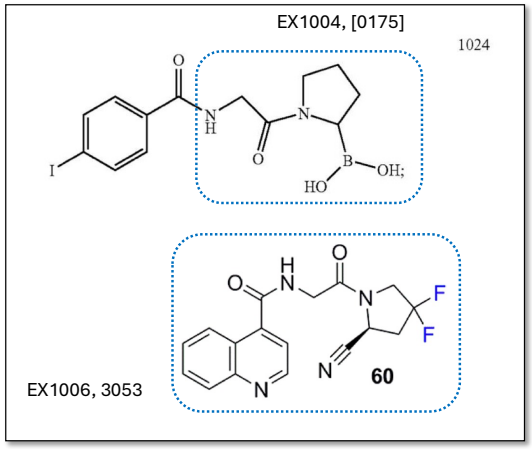
fibroblasts in over 90% of common human epithelial carcinomas and may serve as a therapy target in oncology[].").

150 EX1016 (Brennen 2012), 260-262 ("The unique enzymatic activity and highly restricted expression of FAP in the reactive stroma associated with >90% of epithelial cancers examined thus far make it a very attractive candidate for tumor-specific therapies.").

151 EX1004 (US-633), [0185]; EX1008 (Meletta), 2083; EX1006 (Jansen), 3063.



(c) The Meletta and Jansen papers both address earlier work with boronic acid pyrrolidine FAP inhibitors that were the primary focus of US-633. Meletta directly cites the US-633 publication,[152] and discusses the testing of one of the molecules described in US-633 (compound 1024).[153] Jansen introduced its findings by providing an overview of experiences with boronic acid pyrrolidine FAP inhibitors.[154]

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[152] EX1008 (Meletta), 2098 (Footnote 31) ("Zimmerman, C.; Babich, J.W.; Joyal, J.; Marquis, J.; Wang, J. Selective Seprase Inhibitors. Patent US 2010/0098633 A1, 22 April 2010.")

[153] EX1008 (Meletta), 2086.

[154] EX1006 (Jansen), 3054, Figure 1.

E. A Skilled Artisan Would Have Selected a Radionuclide Based on the Imaging Platform to be Used

129. An important decision that must be made early in the design of a radiotracer is which radionuclide to use in it. That decision is guided by a number of considerations. The first is the imaging platform that will be used with the radiotracer (*i.e.*, PET or SPECT). A skilled artisan in 2017 would have considered either PET or SPECT to be appropriate imaging platforms to use for a new radiotracer intended for use in nuclear imaging of tumors.

130. The next consideration is whether the radionuclide should have a long half-life ($t_{1/2}$ > 24 h), a medium half-life (6 h < $t_{1/2}$ < 24 h), or a short half-life ($t_{1/2}$ < 6 h). Based on my experience, a general rule-of-thumb is that the physical half-life of the radionuclide should match the pharmacological half-life of the vector (*i.e.*, the targeting moiety).[155] See ¶ 41 (above). From the pharmacokinetic data reported in Jansen (and a general knowledge of the pharmacokinetic profiles of small molecules), a skilled artisan would conclude that a radiotracer with a short half-life

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[155] See, e.g., EX1011 (Zeglis 2013), 1884; EX1012 (Wadas), 2893; EX1018 (Fichna), 8-9; EX1017 (Sarko), 2674; EX1013 (Price), 280.

would be appropriate given that a radiotracer based on compound 60 would be a low molecular weight compound with a relatively short half-life in patients.[156]

131. Once the half-life of the radionuclide has been chosen, the last step is to decide whether a radiometal (*e.g.*, ^{68}Ga) or a radiohalogen (*e.g.*, ^{18}F) is preferable. Both have advantages and disadvantages. As I discussed above (¶¶ 32-34, 53-55), radiohalogens can be attached via a covalent bond to a prosthetic group, which is then covalently attached to the targeting moiety, but their radiochemistry tends to be more cumbersome; radiometals require bulky chelators for labeling, but their radiochemistry tends to be quite simple. A skilled artisan would consider either type of radionuclide to be a viable option in 2017.

132. I prepared a Sankey diagram showing how a skilled artisan might go about selecting a radionuclide to use in a radiopharmaceutical that takes into account the initial choice of the imaging platform (PET vs. SPECT), the half-life of the radionuclide, and finally the class of radionuclide. As it illustrates, ^{68}Ga and ^{18}F would be choices for a radiotracer based on compound 60 that was to be used

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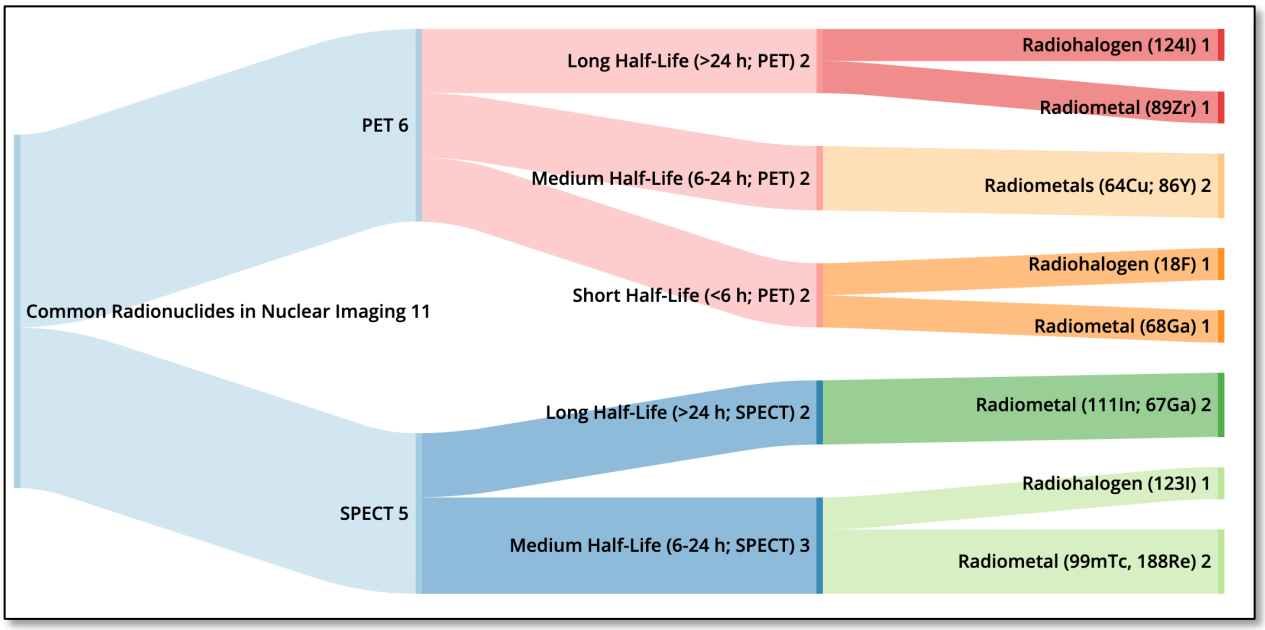
[156] EX1006 (Jansen), 3063 (Table 8), 3064 ("We therefore conclude that the longer in vivo half-life of 60 (compared to 5 and 61) is the main contributor to the prolonged ex vivo inhibition of FAP activity observed for this compound.").

in PET-based nuclear imaging, while ^{99m}Tc and ^{123}I would be options for a radiotracer used in SPECT-based nuclear imaging.



F. A Skilled Artisan Would Have Improved the FAP Radiotracers Illustrated in US-633 by Using Compound 60 as the Targeting Moiety

133. As I explained above (¶¶ 97-101), the failure of MIP-1232 due to the insufficient selectivity of its targeting moiety would have prompted a skilled artisan to search for new, more selective small molecule targeting moieties that could be used in FAP-targeting radiotracers. A skilled artisan also would have recognized that the boronic acid pyrrolidine targeting moiety in compound 1024/MIP-1232 is shared by many of the examples of the more promising radiotracers reported in US-633 (*e.g.*, compounds 1014, 1018, 1020, 1023-1030, 1032, and 1034). A skilled artisan would thus have recognized that the entire class

of radiotracers described in US-633 could be improved by using a new, more selective small molecule FAP inhibitor as the targeting moiety.

134. With this motivation to find new, more selective FAP inhibitors, a skilled artisan would have invariably discovered the compounds characterized in Jansen. That person would have identified compound 60 in particular as the most promising candidate for a new, small molecule FAP-targeting moiety and superior to the boronic acid pyrrolidine targeting moieties used in US-633. For example, while compound 60 has a comparable FAP IC_{50} value as compound 1024/MIP-1232 (3.2 nM vs. 3 nM), it has a much higher selectivity for FAP (*i.e.*, 562-fold selectivity for FAP over PREP (Table 5, below) compared to 32 fold selectivity for compound 1024/MIP-1232.[157] In other words, compound 60 is ***17 times*** more selective for FAP than compound 1024. The much higher selectivity of compound 60 for FAP over PREP reported in Jansen would have provided a strong motivation for a skilled artisan to use compound 60 as the targeting moiety in the FAP-targeting radiotracers described in US-633.

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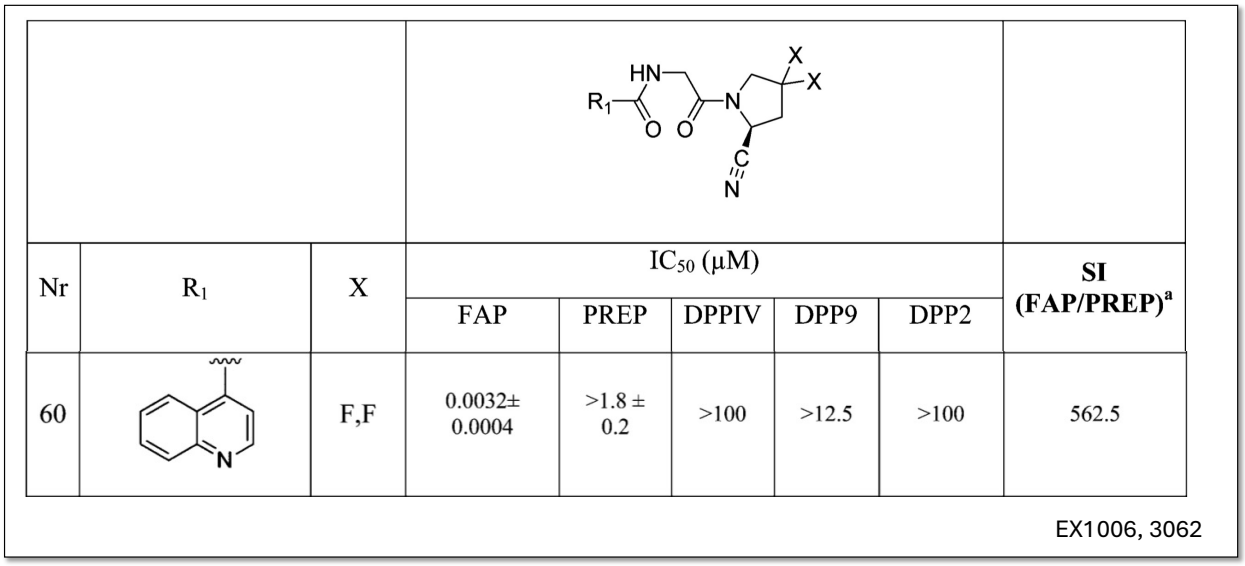
[157] EX1006 (Jansen), 3062 (excerpted to show affinity/selectivity data for compound 60); EX1004 (US-633), [0185] (Table 2) (showing the IC_{50} value for compound 1024).



Nr	R_1	X	IC_{50} (μM)					**SI (FAP/PREP)[a]**
			FAP	PREP	DPPIV	DPP9	DPP2	
60		F,F	0.0032± 0.0004	>1.8 ± 0.2	>100	>12.5	>100	562.5

EX1006, 3062

135. As I explained earlier (see ¶¶ 110-115, 120-127), Jansen also reports that compound 60 exhibits a number of additional favorable characteristics for a targeting moiety: (i) stability in rat, mouse, and human plasma, (ii) a favorable pharmacokinetic profile and (iii) sustained suppression of FAP activity *in vivo*. These superior characteristics of compound 60 would have provided even more reasons for a skilled artisan to improve the radiotracers described in US-633 by using compound 60 as the targeting moiety instead of the boronic acid pyrrolidine moiety.

G. Specific Radiotracers Containing Compound 60 and that Use a ^{68}Ga or ^{18}F Radionuclide Would Have Been Obvious

136. By 2017, skilled artisans were very familiar with the design and construction of radiopharmaceuticals, particularly radiotracers. Once the targeting moiety and radionuclide are determined for a radiotracer, it is straightforward for a

skilled artisan to select radiolabeling moieties and linkers to use with the targeting moiety, and to then synthesize the radiotracer.

137. To design an appropriate chemical structure for a radiotracer, a skilled artisan would need to make three key decisions: (i) where on the targeting moiety to attach the linker and radiolabeling moiety, (ii) which chemical structure(s) to use as the radiolabeling moiety, (iii) which linker(s) to use to attach the targeting moiety to the radiolabeling moiety. The skilled artisan would also consider synthetic pathways to construct the radiotracer. All of these choices would have been guided by the skilled artisan's knowledge, training and experience in 2017.

1. A Skilled Artisan Would Have Attached a Linker-Radiolabeling Moiety at C^6 or C^7 of the Quinolinyl Ring of Compound 60

138. The most important principle a skilled artisan would have followed in selecting where on compound 60 to attach a linker would be to avoid modifications that interfere with the affinity or selectivity of the compound. This design rule had been articulated many times in the literature before 2017. As I explained in a 2011 review:

> "The final piece of the anatomy of a radiometal PET bioconjugate is the covalent attachment of the chelator to the biomolecule. This link must be stable under physiological conditions and must not

> significantly compromise the binding strength and specificity of the biomolecule."[158]

Others had published similar observations. Some examples are shown below:

> "In this case, the radiolabel must be introduced into the lead compound in a manner that does not reduce the affinity of the ligand for the target macromolecule."[159]

> "For smaller biomolecules, such as peptides the radioisotope label may significantly affect binding to the receptor and in vivo metabolism. In this situation, the choices of radionuclide, labeling position or location of the radiolabel can be critical[]. Radiolabeling at a specific-site (chelation-site) remote from the binding region is important to prevent the loss of binding affinity and biological activity of the radio-labeled peptides []."[160]

> "For smaller molecules, the isotope label may significantly affect binding to the receptor, binding to transport proteins, and in vivo metabolism."[161]

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158 EX1010 (Zeglis 2011), 9. See also EX1011 (Zeglis 2013), 1884 ("For the linkage between the chelator and targeting vector, the only requirements are that the link must be stable under physiological conditions and must not significantly compromise the binding strength or specificity of the vector.").

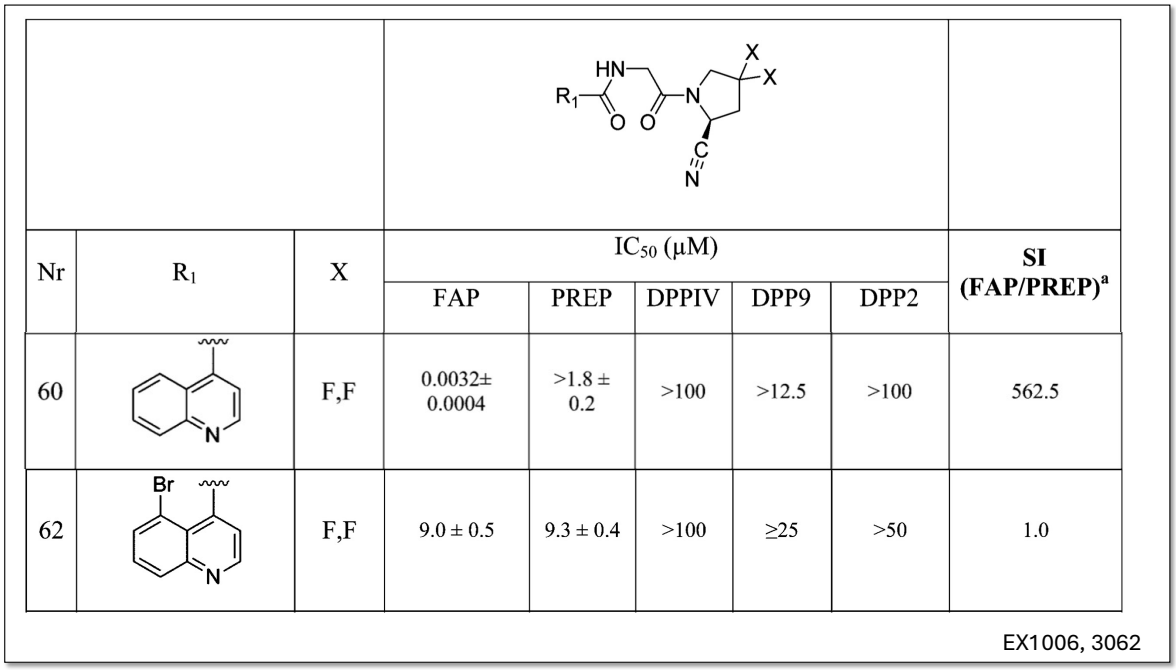
159 EX1033 (Mach), 143.

160 EX1032 (Fani), 484.

161 EX1028 (Mankoff), 153S.

139. Based on my review of compound 60's structure, I believe a skilled artisan would have viewed use of a linker in a radiotracer based on compound 60 to be preferable to direct attachment of a radiohalogen to the quinolinyl ring of compound 60. One reason is that it would allow use of a radiometal as the radionuclide. A second is that some adverse effects on selectivity/affinity were observed when a halogen (e.g., bromine) was attached to C^5 position of the quinolinyl ring of compound 60 (forming compound 62) (*i.e.*, FAP to PREP selectivity was eliminated (SI = 1), and the IC_{50} values for FAP inhibition were reduced ~1000-fold (compare compounds 60, 61 and 62).[162] A linker would diminish adverse effects by separating the warhead of compound 60 from the rest of the radiotracer molecule.

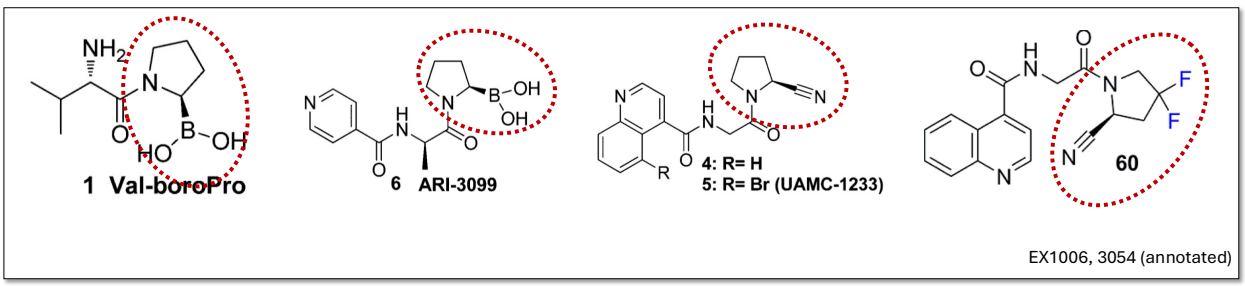
[162] EX1006 (Jansen), 3062 (Table 5).



Nr	R_1	X	IC_{50} (μM)					**SI (FAP/PREP)[a]**
			FAP	PREP	DPPIV	DPP9	DPP2	
60		F,F	0.0032± 0.0004	>1.8 ± 0.2	>100	>12.5	>100	562.5
62		F,F	9.0 ± 0.5	9.3 ± 0.4	>100	≥25	>50	1.0

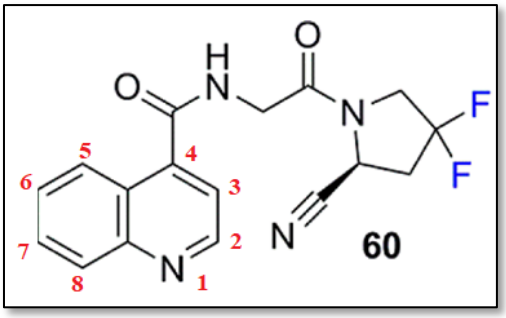
EX1006, 3062

140. To answer the question where on compound 60 to attach the linker and radiolabeling moiety, a skilled artisan must first determine what part of compound 60 controls its binding to the target. Thankfully, the answer to that question is readily apparent for quinolinoylglycyl(2-cyanopyrrolidine) FAP inhibitors based on information in Jansen and other publications in the scientific literature before 2017: it is the modified glycyl-pyrrolidine ring in compound 60. For example, FAP inhibitors listed in Jansen's Figure 1 all have a substituted pyrrolidine ring.



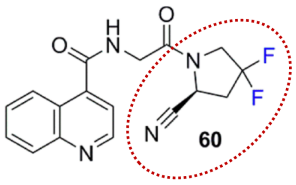
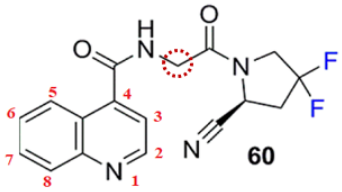
EX1006, 3054 (annotated)

141. A depiction of compound 60 with numbering of the quinolinyl ring is provided below. I will use this numbering scheme in my comments below. As shown, the *N*-acyl-glycyl(2-cyano)-pyrrolidine warhead of compound 60 is conjugated to position 4 of the quinolinyl ring.

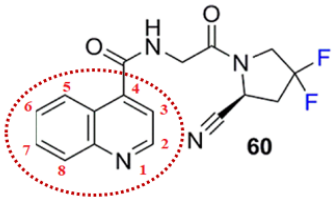
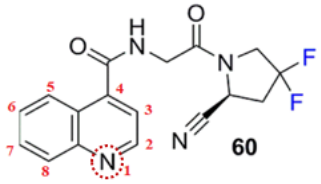


142. A skilled artisan would have concluded that positions C^6 and C^7 of the quinolinyl ring structure offer the best chances for modifying the quinolinyl ring to attach a linker without interfering with the FAP affinity or FAP/PREP selectivity of compound 60. That conclusion flows from analyzing the structure of compound 60 and evaluating data characterizing other compounds based on the *N*-4-quinolinoyl-Gly-(2S)-cyano-Pro scaffold having modifications at various positions. Data on such compounds was published before 2017, including in Jansen (EX1006), Jansen-2013 (EX1040), US-650 (EX1044), and Ryabtsova (EX1029). Most notably, compounds with modifications at positions C^6 and C^7 tolerated the changes without significant changes to FAP affinity or FAP/PREP selectivity (e.g., compounds 52 (C^7-Me), 54 (C^7-Ph), 55 (C^7-NH-Ph), and 61 (C^6-OMe) all exhibit

good FAP affinity and FAP/PREP selectivity.[163] Conversely, the data shows adverse effects from modifications at positions other than C^6 or C^7 on the compound's selectivity, affinity or stability (discussed below). A skilled artisan, thus, would have chosen to attach a linker to positions C^6 or C^7 on the quinolinyl ring of compound 60. The table below compiles my observations on why modifications to positions other than C^6 and C^7 would be disfavored.

Compound 60 Position	Reasons Not to Modify
	• The difluoro-cyano-pyrrolidine is instrumental in the superior FAP affinity and selectivity of compound 60. Attachment to the difluoro-cyano-pyrrolidine ring risks disrupting binding to FAP due to steric hindrance.
	• Substitution at the glycine α-carbon position risks disrupting binding due to steric hindrance with the 2-cyano-pyrrolidine ring.

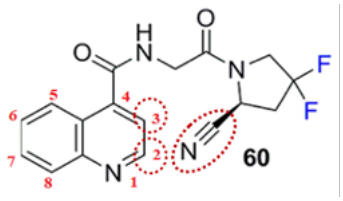
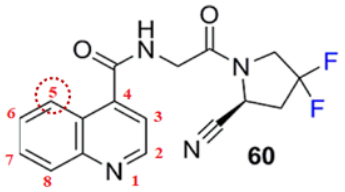
[163] EX1006 (Jansen), 3061 (Table 4), 3062 (Table 5) (e.g., compound 61 showing substitution at position 6 is tolerated, exhibiting good selectivity (*i.e.*, > 976 SI (FAP/PREP)).

	• Modifying the glycine α-carbon of compound 4 in Jansen significantly reduced FAP affinity or abolished inhibition.[164]
	• The 4-quinolinyl group significantly improved the selectivity of these structures for FAP, while other ring structures did not.[165]
	• Compounds with quinolinyl rings have 60-fold greater affinity for FAP than identical compounds in which the nitrogen is replaced with a carbon.[166] • Moving the nitrogen in the quinolinyl ring away from position 1 decreases FAP binding affinity

[164] EX1006 (Jansen), 3056 ("When [D-Ala was] introduced as a replacement for compound 4's glycine residue however (11 and 12), the FAP affinities observed dropped 600- and 300-fold, respectively. FAP inhibition was completely abolished by introducing the closely related amino acids L-Ala and 1-amino-1-carboxycyclopropane at P2.").

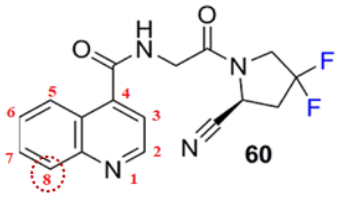
[165] EX1044 (US-650), [0633] ("Of all positional isomers synthesized, the 4-quinolinyl ring clearly displays the best results and takes in a singular position within this series.").

[166] EX1007 (Jansen 2013), 492 ("The results summarized in Table 2 show that the *N*-(4-quinolinoyl) substituted compound 7 has about 60 times more FAP-affinity than the initial *N*-(1-naphthoyl) based 'hit' 3."), Table 2.

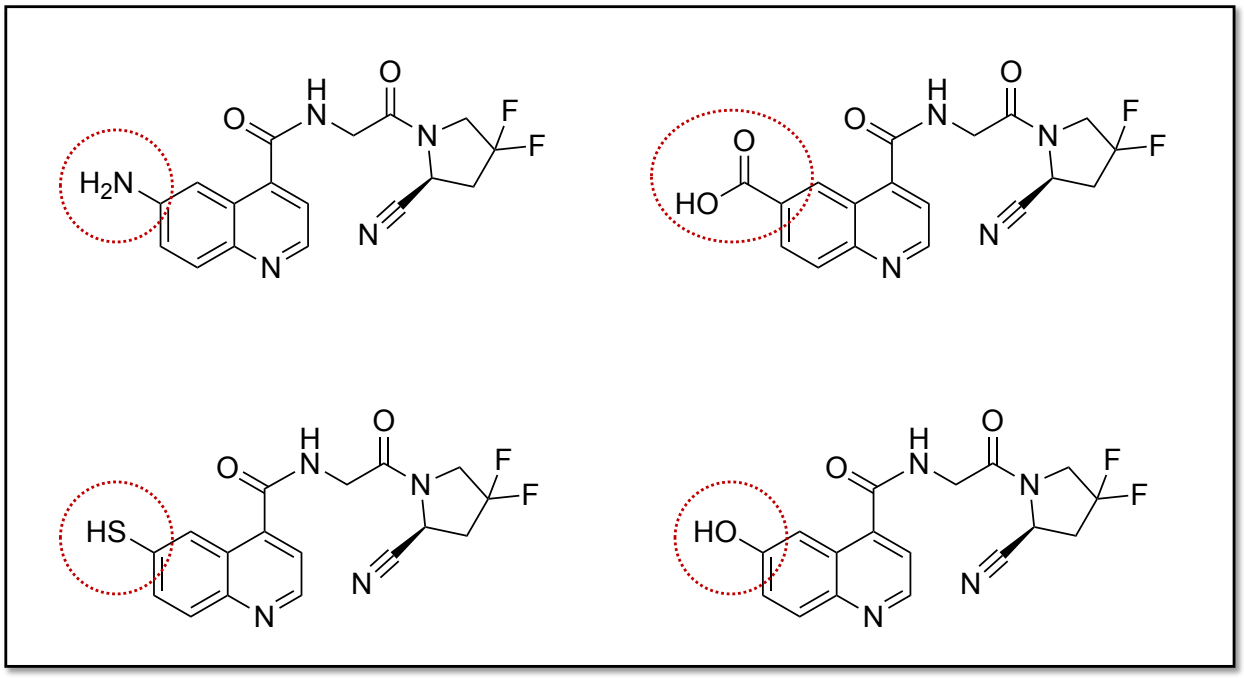
	by at least one order of magnitude.[167] This is "indicative for a specific interaction with the enzyme," suggesting that the 1-position nitrogen plays a key role in FAP binding.
	• Modifications to positions C^2 and C^3 of the quinolinyl ring risk disrupting FAP binding due to steric hindrance with the 2-cyano-pyrrolidine ring.
	• There were variable effects at position C^5. Compound 53 had the highest selectivity, while the affinity and selectivity of compounds 57 (C^5-OMe), 62 (C^5-Cl), and 63 (C^5-Br) significantly decreased relative to compound 60, suggesting that not all modifications at C^5 are tolerated.[168]

167 EX1007 (Jansen 2013), 494 ("With FAP-affinities spanning almost 3 orders of magnitude, evaluation results of compounds 35−39 nonetheless reveal a pivotal importance of the nitrogen's position. Of all the positional isomers synthesized, the 4-quinolinoyl ring of 'lead' 7 clearly displays the best results and takes in a singular position within this series.").

168 EX1006 (Jansen), 3081-3082 (the affinity and selectivity of compounds 57 (C^5-OMe), 62 (C^5-Br), and 63 (C^5-CN) significantly decrease relative to compound 60, suggesting that not all modifications at C^5 are tolerated).

	• There is little data in Jansen (EX1006) or US-633 that characterizes the effects of modifying the quinolinyl structure at position C^8. This absence of evidence makes it hard to justify selecting position 8 instead of positions 6 and 7, which tolerate modifications.

143. To attach compound 60 to the rest of the radiotracer, the C^6 or C^7 position could be functionalized. Common functional groups for this purpose include amine, carboxyl, hydroxyl and thiol groups. Examples of compound 60 functionalized with these types of functional groups are shown below, with the C^6 position functionalized.



2. A Skilled Artisan Would Have Used Well-Known Chelators Compatible with ^{68}Ga or ^{99m}Tc

144. Several of the examples of chelators or types of chelators that were identified in US-633 would have been recognized by a skilled artisan as being suitable for a FAP-targeting radiotracer that was using ^{68}Ga or ^{99m}Tc as the radionuclide. See ¶¶ 76-82.

145. For example, US-633 identifies DOTA as a chelator option, which a skilled artisan would understand to be a suitable chelator to use in a radiotracer using ^{68}Ga as the radionuclide. I also explained that US-633 explains that any chelator that was known to be suitable for ^{68}Ga would be an option. A skilled artisan, considering that point and from their training and experience, would have considered using any of the chelators that was known in 2017 to be suitable for ^{68}Ga even if that particular chelator was not listed as one of the examples in US-633 itself. *See* ¶ 79. A skilled artisan would have considered any of the ^{68}Ga-suitable chelators I listed in ¶ 82 to be a "suitable chelating moiety" in the meaning of US-633 in early 2017.[169] Similarly, a skilled artisan would have considered any of the examples of chelators in US-633 that are suitable for ^{99m}Tc to be an option for use in a SPECT radiotracer that used ^{99m}Tc as the radionuclide. A skilled

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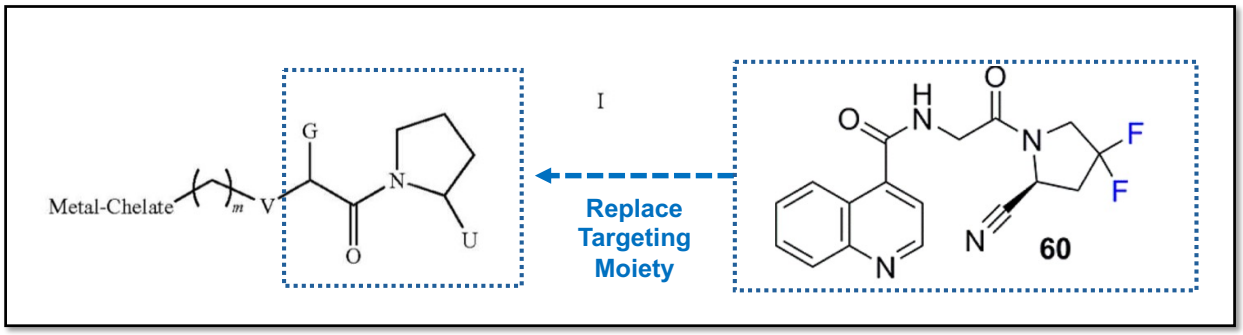
[169] EX1004 (US-633), [0100].

artisan also would have known how to select other chelating moieties suitable for ^{99m}Tc in 2017.

3. Examples of Radiotracers that Combine Compound 60 with Chelators and Radiometals

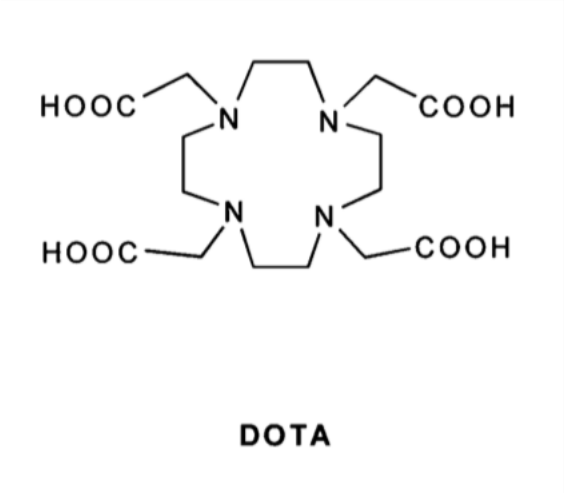
146. As I explained in III.F, a skilled artisan would have found it obvious to replace the boronic acid pyrrolidine targeting moiety in the FAP-targeting radiotracers described in US-633 with compound 60 from Jansen. In particular, in Formula I of US-633, the skilled artisan would have replaced the boronic acid pyrrolidine structure within Formula I with compound 60 (illustrated below):



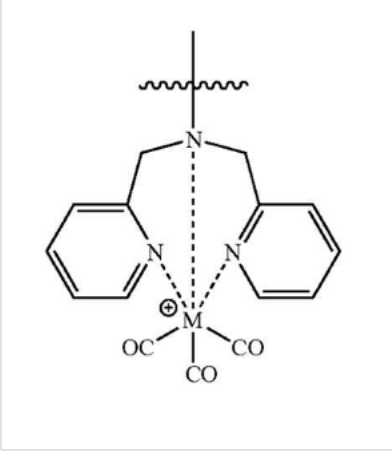
147. In 2017, a skilled person also would have selected a radionuclide suitable for either PET or SPECT (*e.g.*, ^{68}Ga for PET, ^{99m}Tc for SPECT). That person would then have selected "suitable chelating moieties" for each radionuclide, drawing both from the examples in US-633 and their knowledge of chelating moieties known to be suitable with ^{68}Ga or ^{99m}Tc in 2017. Having determined the particular chelator to combine with compound 60 in the radiotracer, the skilled artisan would finally select a linker design that would provide sufficient

separation between the radiolabeling and targeting moieties and attach it to the C^6 or C^7 positions on the quinolinyl ring of compound 60.

148. I illustrate this process with two examples, one using ^{68}Ga for a PET radiotracer, and the other ^{99m}Tc for a SPECT tracer. A suitable chelator for ^{68}Ga that was well known and identified in US-633 is DOTA (below left). A number of suitable chelators for ^{99m}Tc are listed in US-633, including DPA (below right).



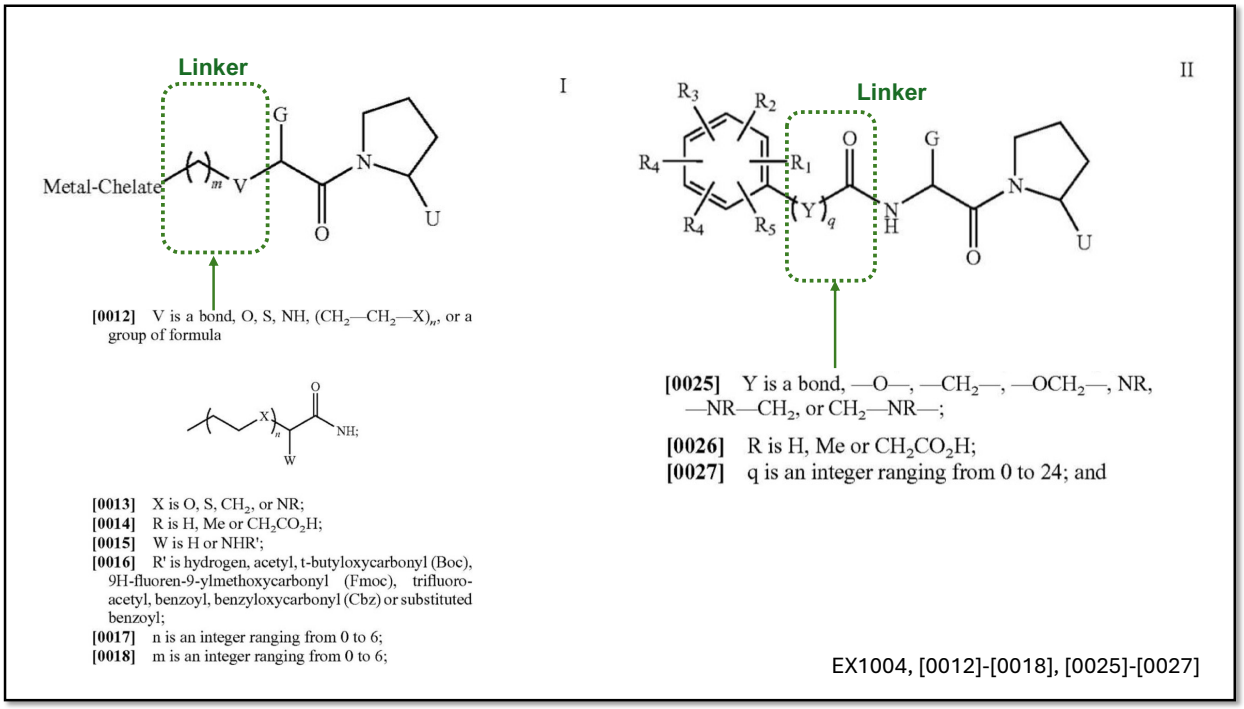
DOTA

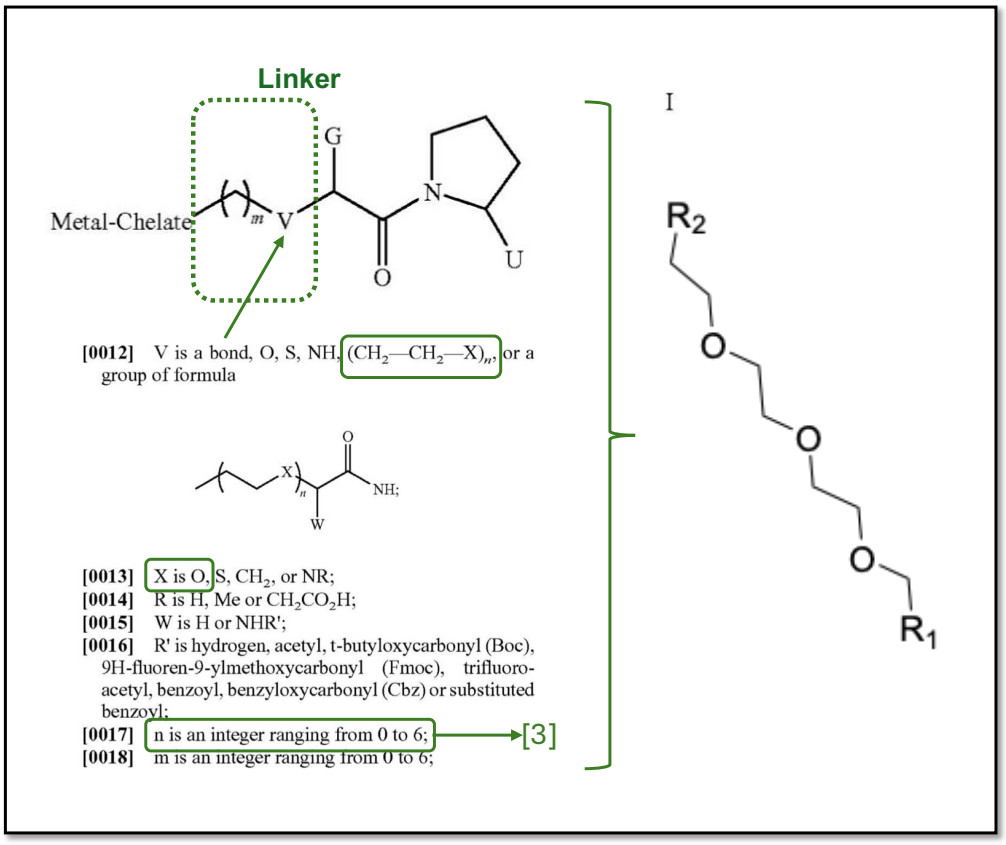


DPA

149. As I explained above (§ III.A.5), US-633 illustrates use of a variety of linkers in its radiotracers. US-633 also provides guidance regarding linkers via the parameters of the positions in the two chemical formulae used to define the common structures of its radiopharmaceuticals (Formula I and Formula II) (below, top). For example, a 3 monomer unit PEG unit would result from selecting in Formula I the options (i) "$(CH2\text{-}CH2\text{-}X)_n$" for V, (ii) oxygen "O" for X, and (iii) 3 for "n" (below, bottom). A skilled artisan also would have known that the termini

of the 3-monomer PEG would need to be appropriately functionalized to enable the linker to be covalently attached to the radiolabeling and targeting moieties.





150. US-633 also refers to certain practical benefits of using PEG-based linkers, including that they are commercially available in a variety of lengths and in functionalized forms (*e.g.*, diamines) that fit the needs one has based on the design of the radiotracer being produced.[170] A skilled artisan thus could have obtained a PEG linker with R_1 and R_2 functional groups that enabled the formation of covalent bonds with compound 60 and the chelator, respectively.

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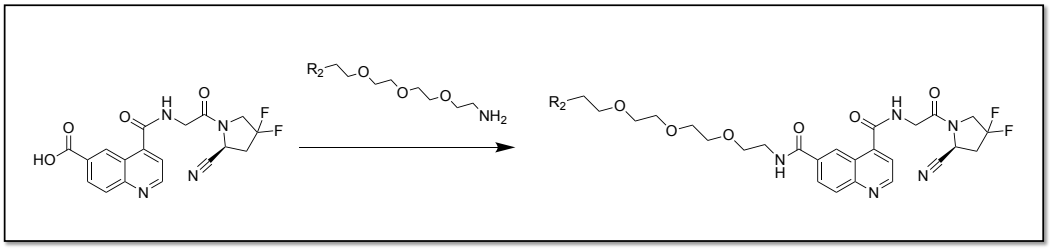
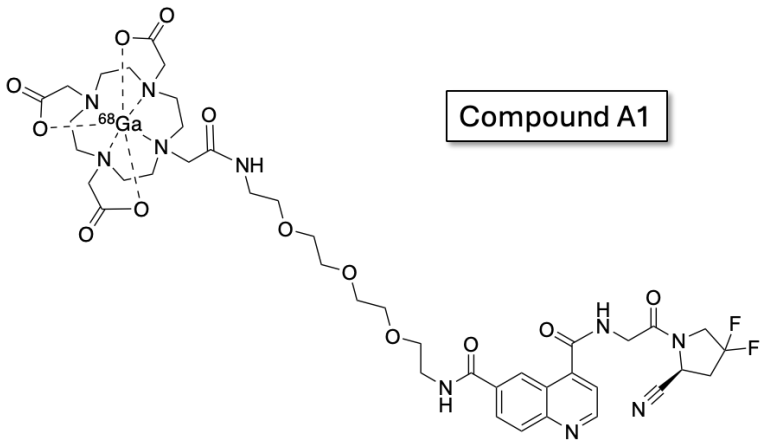
[170] EX1004 (US-633), [0133]. See also ¶¶ 87-88 (above).

151. Generally, a skilled artisan would have wanted to separate the labeling moiety from the targeting moiety by about 10 to 20 bond lengths to avoid interactions between the two parts of the radiotracer. The 3-monomer diamine PEG compound I discussed in ¶ 149 would introduce about 12 bonds between the radiolabeling and targeting moieties and would be one option a skilled artisan would consider appropriate. This 3-monomer diamine PEG linker could be linked via an amide bond to a functionalized form of compound 60 having a carboxylic acid at position C^6 (below).



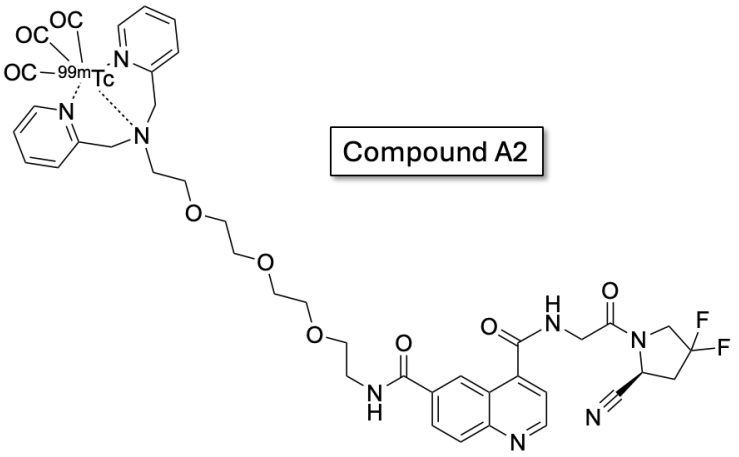
152. A diamine-bearing PEG-based linker also would enable the skilled person to covalently link the linker-bearing variant of compound 60 to DOTA by forming a second amide bond between one of the carboxylic groups of DOTA and the other amine group of the PEG linker.

153. The resulting compound, which I designate Compound A1, is shown below.



Chemical Formula: $C_{42}H_{55}F_2{}^{68}GaN_{10}O_{13}$
Molecular Weight: 1013.88

154. In a second example suitable for a SPECT imaging radiotracer based on ^{99m}Tc, one could use a DPA chelator. Here, one would start by alkylating the central nitrogen of the DPA with an appropriate functional group that could then be reacted with a correspondingly R1-functionalized 3-monomer PEG. The PEG would need to be protected at the R_1 end during its attachment to the DPA (e.g., with a common Boc protecting group), while the other end would have a reactive chloride (*i.e.*, Cl-PEG_3-NH_2-Boc). Once the DPA-PEG intermediate was produced, it could then be attached to the carboxy-functionalized compound 60 using straightforward chemical synthetic techniques. That would yield a compound I designate Compound A2 (below).

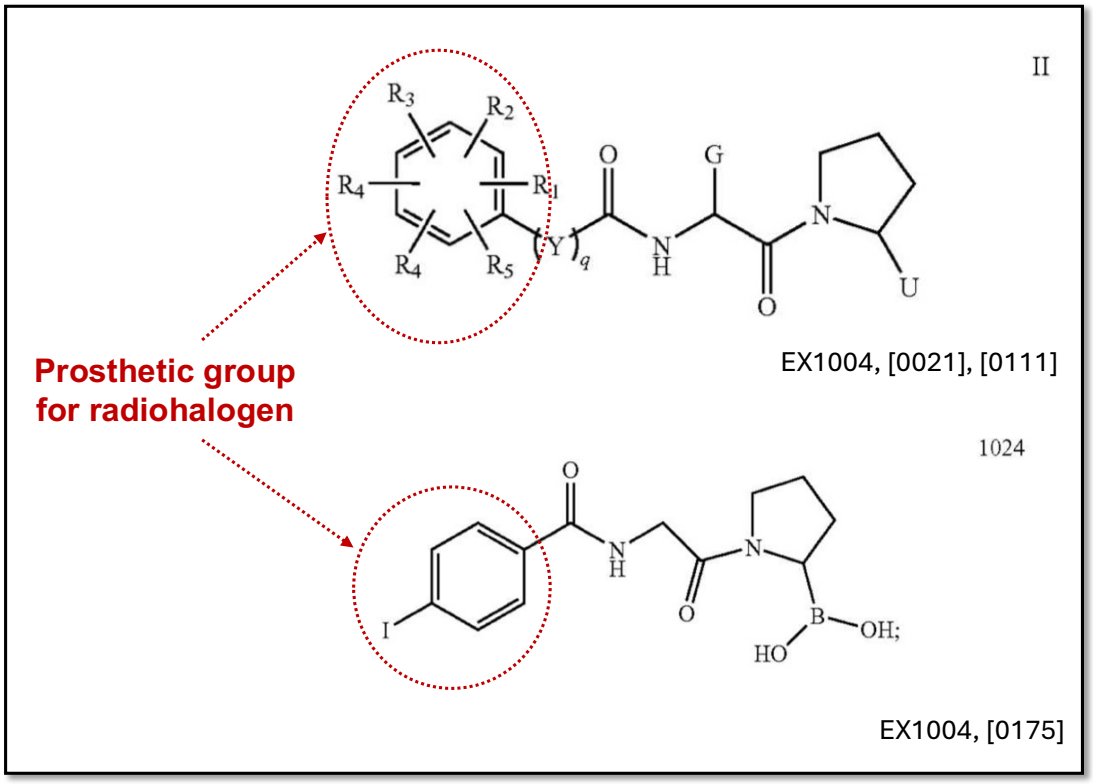


Chemical Formula: $C_{41}H_{42}F_2N_8O_9{}^{99}Tc$
Molecular Weight: 927.74

155. The steps I describe above involve straightforward chemical synthetic methods that would have been known to a skilled artisan before 2017.

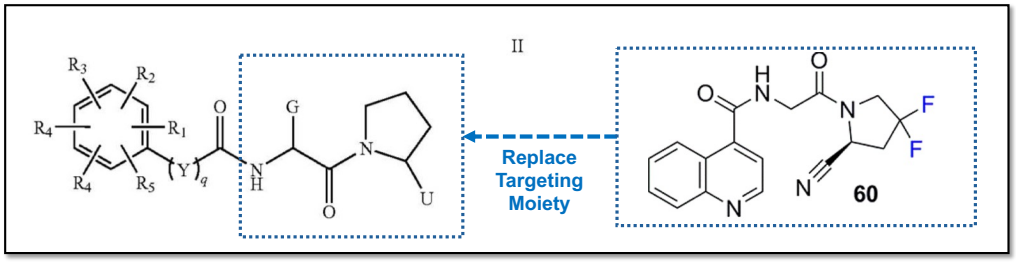
4. Examples of Radiotracers that Combine Compound 60 with Prosthetic Groups and Radiohalogens

156. As I explained above (¶ 83), US-633 illustrates radiotracers with radiohalogenated prosthetic groups connected via a linker to a boronic acid pyrrolidine targeting moiety (Formula II). US-633 illustrates several examples of compounds based on this Formula II (*e.g.*, compound 1024) (below).



157. The radiohalogens a skilled artisan would have chosen to include in radiopharmaceuticals for PET and SPECT are ^{18}F (half-life of 109 minutes) and ^{123}I (half-life of 16.9 hours), respectively. Both radiohalogens can use the same array of prosthetic groups illustrated in US-633, as each will be covalently linked to the prosthetic group, which is then attached to the remainder of the radiotracer. I will illustrate examples of compounds that incorporate a radiofluorinated phenyl group based on the prosthetic groups illustrated in US-633.

158. For the reasons I provided in § III.F, a skilled artisan would have replaced the boronic acid pyrrolidine targeting moiety in Formula II of US-633 (below) with compound 60 (illustrated below).



159. Also, for the reasons I provided in § III.G.1, a skilled artisan would have functionalized compound 60 at position C^6 or C^7 of the quinolinyl ring to enable the attachment of a diamine functionalized PEG linker, for example, with a carboxylic acid group.

160. A skilled artisan would have considered the 3-monomer PEG linker I described above (¶¶ 149-150) as being appropriate to use in a radiotracer containing compound 60 and one of the examples of prosthetic groups in US-633. For example, using that 3-monomer PEG would introduce a sufficient distance (*e.g.*, an additional 10+ bonds) between the radionuclide-prosthetic group and the compound 60 component of the radiotracer.[171]

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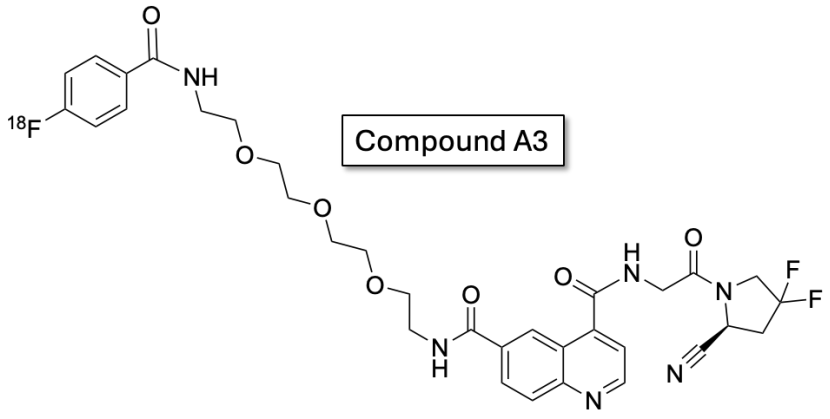
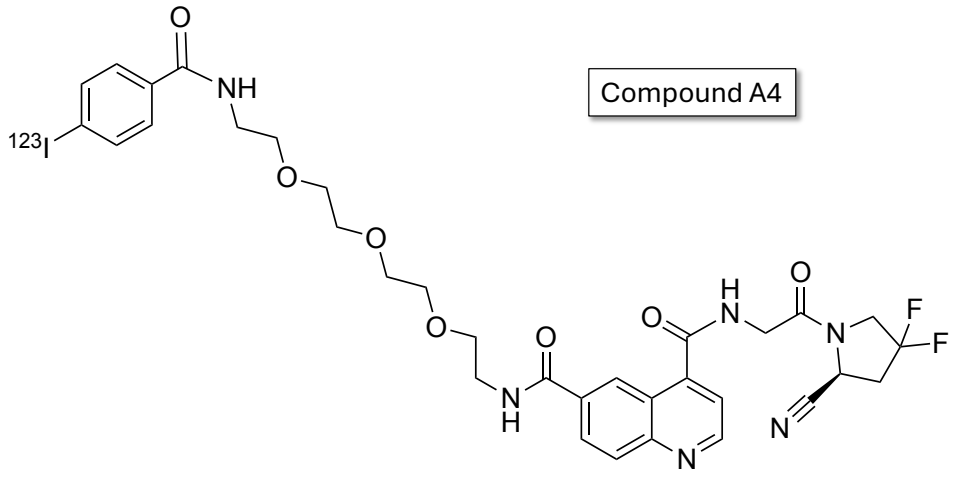
[171] EX1004 (US-633), [0025]-[0027], *also* [0101] ("The distance between the Metal-Chelate moiety and the pyrrolidine moiety of the complex represented by Formula I can be varied by altering the tether and/or expanding the length of the tether between them to modify the affinity and selectivity of the complex for seprase"). See also ¶¶ 86-88, 160 (above).

161. US-633 illustrates attachment of prosthetic groups having a para-radiohalogenated benzoic acid. Using a 3-monomer PEG linker terminating in a free amine (i.e., R_2=NH_2) would allow for the straightforward attachment of the prosthetic group to the PEG-compound 60 intermediate via an amide bond to yield the assembled radiotracer. Again, ^{18}F would be used in the prosthetic group for a PET tracer, while ^{123}I would be used in the prosthetic group for a SPECT tracer. Compounds that I designate A3 and A4 correspond to those two radionuclide choices, respectively, and are shown below.



Chemical Formula: $C_{33}H_{35}F_2{}^{18}FN_6O_7$
Molecular Weight: 683.68



Chemical Formula: $C_{33}H_{35}F_2{}^{123}IN_6O_7$
Molecular Weight: 788.58

H. Compounds A1 to A4 Meet the Requirements of the Claims of the '201 Patent

162. The text of claims 1 to 3 of the '201 Patent is shown below. The '201 Patent (EX1001) has three claims in it.[172] All the claims define "low molecular weight compounds" having a general formula "**B**-**L**-**A**".

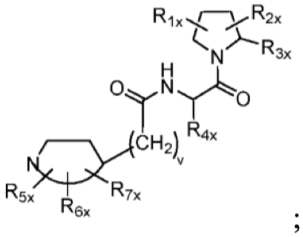
[172] EX1001 (US-201), 64:45-67:5 (unchanged in claims amended by certificate of correction).

1. A low molecular weight compound of Formula (I):

B-L-A (I)

wherein:

A is a targeting moiety for FAP-α, wherein A has the structure of:



;

wherein:

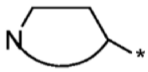
R_{1x} and R_{2x} are each independently selected from the group consisting of H, OH, halogen, $C_{1\text{-}6}$alkyl, -O-$C_{1\text{-}6}$alkyl, and -S-$C_{1\text{-}6}$alkyl;

R_{3x} is selected from the group consisting of H, -CN, -$B(OH)_2$ -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-$S(O)_2$aryl, -CO_2H, -SO_3H, -SO_2NH_2, -PO_3H_2, and 5-tetrazolyl;

R_{4x} is H;

R_{5x}, R_{6x} and R_{7x} are each H;

v is 0;



represents a quinolinyl ring;

B is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and L is a linker having bi-functionalization adapted to form a chemical bond with B and A; or

a stereoisomer, tautomer, racemate, salt, hydrate, or solvate thereof.

1. Compounds A1 to A4 Contain the "B" (Radiolabel) and "L" (Linker) Components of the Claims

163. The first two components of the claimed compound, **B** and **L**, are defined as follows:

> **B** is any optical or radiolabeled functional group suitable for optical imaging, positron-emission tomography (PET) imaging, single-photon emission computed tomography (SPECT) imaging, or radiotherapy; and

> **L** is a linker having bi-functionalization adapted to form a chemical bond with B and A[173]

164. When the chelators and prosthetic groups I discuss in §§ III.A.4, III.G.3 and III.G. are radiolabeled with their corresponding radionuclides, they meet the broad definition of "B" components in the claims as they are "radiolabeled functional groups suitable for … positron-emission tomography (PET) imaging" or for "single-photon emission computed tomography (SPECT) imaging." These include the ^{68}Ga-DOTA complex in Compound A1 and the ^{99m}Tc-DPA complex in Compound A2. Similarly, the ^{18}F-prosthetic group in Compound A3 and the ^{123}I-radiolabeled prosthetic group in Compound A4. Each of compounds A1, A2, A3 and A4 thus contain a "radiolabeled functional group" that meets the "B" component requirement of claims 1-3.

165. The linkers I discuss in § II.C.3 and ¶¶ 149-150 are examples of compounds that fall within the broad definition of the "L" component in the claims. Compounds A1, A2, A3, and A4 each incorporate a 3-monomer PEG linker with terminal amines that enabled it to be attached via amide bonds to the functionalized compound 60, as well as functionalization that allowed it to be covalently attached to the radiolabeling moiety in each compound. Each of

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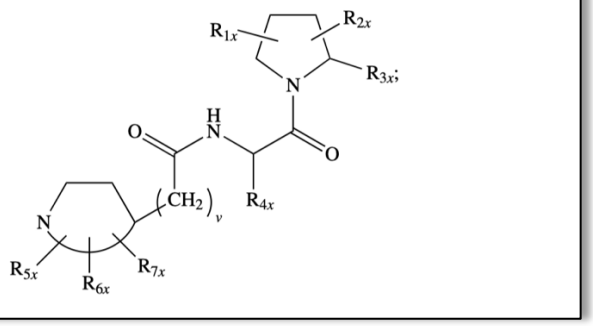
[173] EX1001 (US-201), 65:54-60, 66:64-67:2.

compounds A1, A2, A3 and A4 contains a linker "having bi-functionalization adapted to form a chemical bond." The diamine-bearing 3-monomer PEG in compounds A1, A2, A3 and A4 thus meets the definition of the "L" component of claims 1 to 3.

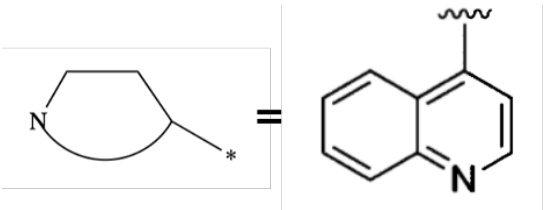
2. Compound 60 Meets the Requirements of the "A" (Targeting Moiety) Component of the Claims

166. The "A" component is a targeting moiety for FAP-α defined using a chemical formula, with lists of options at different positions of the structure for substituents that may be incorporated at that position (below):

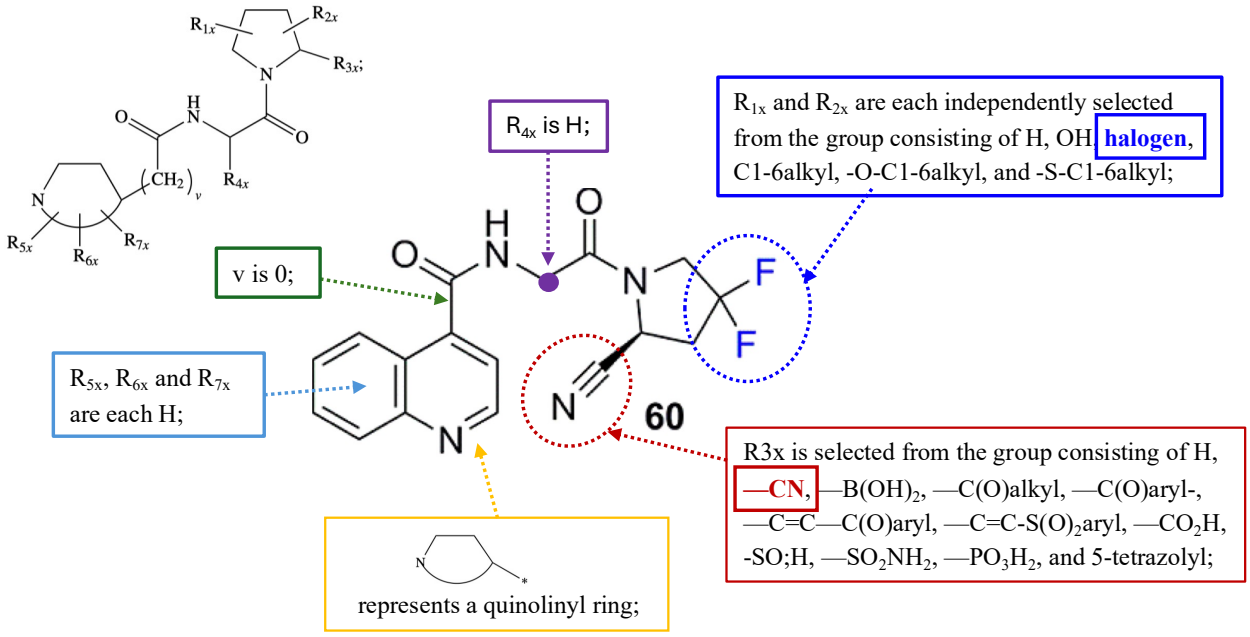
A is a targeting moiety for FAP-α, wherein A has the structure of:



167. The claims also require the nitrogen containing ring structure at the bottom of the structure to be a quinolinyl group.

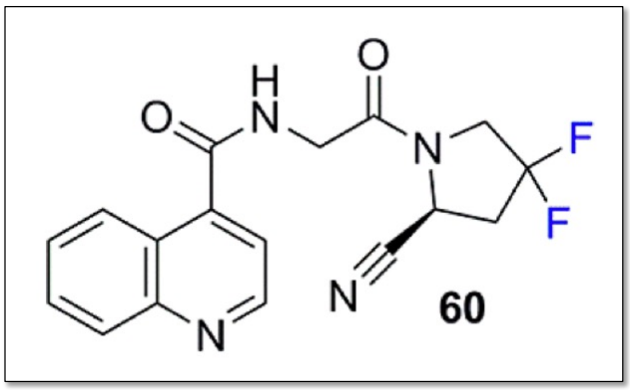


168. Compound 60 in Jansen (EX1006) falls within the definition of the "A" part of the molecule. Each of the "R" groups in the claims has an option at a particular position in the structure that is met by the substituent or atom at that position in compound 60. The figure below maps the claim's definition of "A" to compound 60. Because compound 60 is in each of Compounds A1, A2, A3 and A4, each of those compounds meets the requirements of the "A" component of claims 1-3.



169. Compound 60 is also identical to a FAP inhibitor described in the '201 Patent (below). This also makes it clear that the definition of "A" includes compound 60.

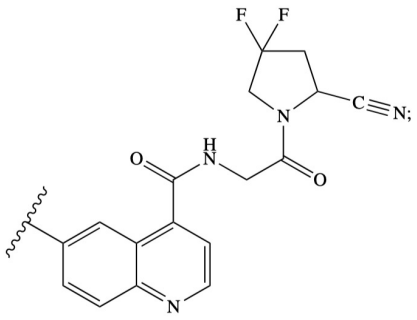
Jansen (EX1006)



EX1006, 3053

(10) Patent No.: US 11,938,201 B2
(45) Date of Patent: *Mar. 26, 2024

In more particular embodiments, A is selected from the group consisting of:



EX1001, 13:34-14:15

3. Compounds A1 to A4 Meet the "Low Molecular Weight Compound" Requirement of the Claims

170. The claims also require "a low molecular weight compound of Formula (I)" which is "B-L-A." The '201 Patent does not provide a definition of a "low molecular weight" compound.

171. I reviewed the '201 Patent to determine what it means when it uses the phrase "low molecular weight" compound. There are two places in the patent where the "low molecular weight" phrase is used. The first passage uses the phrase "low molecular weight" to distinguish "low molecular weight agents" from antibody-based molecular imaging agents. It points to drawbacks of antibody-based molecular imaging agents and then explains (by contrast) low molecular weight radiotracers have faster pharmacokinetics, higher signals, and can be synthesized more easily (below).

> The use of ***antibodies as molecular imaging agents,*** however, suffers from pharmacokinetic limitations, including slow blood and non-target tissue clearance (normally 2-5 days or longer) and non-specific organ uptake. ***Low molecular weight (LMW) agents demonstrate faster pharmacokinetics*** and a higher specific signal within clinically convenient times after administration. They also can be ***synthesized in radiolabeled form more easily*** and may offer a shorter path to regulatory approval. (Coenen, et al., 2010; Coenen, et al., 2012; Reilly, et al., 2015). To date, however, no LMW ligand has been reported with ideal properties for nuclear imaging of FAP-α.[174]

172. A skilled artisan would have recognized that the description of "low molecular weight" radiotracers cited in ¶ 171 corresponds to the characteristics of radiotracers based on small molecules, peptides and small proteins. See ¶ 30. For example, a 2011 review (Zhou) observed that "small peptides with less than 30 amino acids or molecular weight less than 3500 Daltons" have characteristics similar to those the '201 Patent attributes to "low molecular weight" radiotracers (*e.g.,* faster pharmacokinetics, more easily synthesized in radiolabeled form). As it explains:

> … small peptides with less than 30 amino acids or molecular weight less than 3500 Daltons are of particular interest. Compared

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[174] EX1001 (US-201), 1:55-67 (emphasis added).

> to monoclonal antibodies and antibody fragments, small peptides offer several advantages. Peptides are necessary elements in more fundamental biological processes than any other class of molecule. They can also tolerate harsher conditions for chemical modification or radiolabeling. Small peptides are easy to synthesize and modify, less likely to be immunogenic, and can have rapid blood clearance. The faster blood clearance results in adequate T/B ratios earlier so that it is practical to use ^{99m}Tc, which is the preferred radionuclide for diagnostic nuclear medicine.[175]

173. The second instance of "low molecular weight" in the '201 patent uses the phrase to refer "ligands of FAP-α targeting moieties" rather than an entire assembled radiopharmaceutical (below).[176] I also note that this passage is referring to "low molecular weight (LMW) ligands of FAP-α" that are described in the '201 Patent, one of which is identical to compound 60 (below).

> Accordingly, in some embodiments, the presently disclosed subject matter provides potent and selective low molecular-weight (LMW) ligands of FAP-α, i.e., an FAP-α selective inhibitor, conjugated with a targeting moiety feasible for modification with optical dyes and radiolabeling groups, including metal chelators and metal complexes, which enable in vivo optical imaging, nuclear imaging

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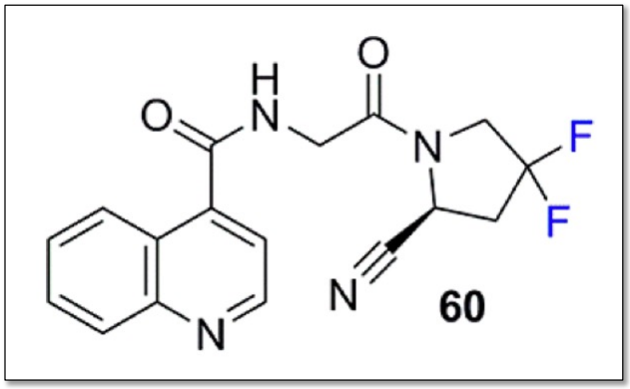
[175] EX1052 (Zhou), 59-60.

[176] EX1001 (US-201), 7:45-64.

(optical, PET and SPECT), and radiotherapy targeting FAPα. Importantly, the presently disclosed compounds can be modified, e.g., conjugated with, labeling groups without significantly losing their potency.

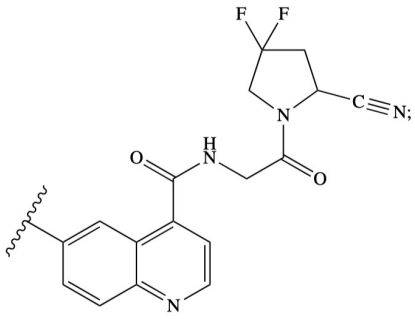
Jansen (EX1006)



EX1006, 3053

(10) **Patent No.: US 11,938,201 B2**
(45) **Date of Patent: *Mar. 26, 2024**

In more particular embodiments, A is selected from the group consisting of:



EX1001, 13:34-14:15

174. The ’201 patent does not set an upper limit for the size of a “low molecular weight” compound. Based on how the phrase is being used in the patent, a skilled artisan would understand “low molecular weight” compounds to be radiopharmaceuticals that do not include a large protein like an antibody as the targeting moiety. Antibody-based radiopharmaceuticals have a high molecular weight on the order of 150,000 Da (150 kDa). They also have very different pharmacokinetic profiles (e.g., slow clearance). Low molecular weight radiopharmaceuticals, by contrast, are radiopharmaceuticals that use a small molecule or peptide as the targeting moiety and have molecular weights at least an

order of magnitude (and often two orders of magnitude) below the molecular weight of an antibody (*e.g.*, from a few hundred to a few thousand Daltons). Compounds in this size range have comparable pharmacokinetics (e.g., rapid uptake, rapidly clearance).

175. The ’201 Patent also indicates that various prosthetic groups and chelators such as DOTA and NOTA meet the definition of the “B” component in the claims.[177] It also describes L similarly to how US-633 describes its “tethers” (e.g., it includes PEG oligomers having up to 8 PEG monomers in them), and lists examples of linkers.[178] It also describes examples of targeting moieties having the structure for the “A” component in the claims. These moieties have sizes on the order of several hundred Daltons, as do the linker and radiolabeling moieties illustrated in the patent that can be combined with these targeting moieties to yield complete radiopharmaceuticals. In other words, if a compound includes any combination of one of these examples of linkers and one of these examples of radiolabeling moieties in the patent along with Compound 60, it would have to be a

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[177] EX1001 (US-201), 17:55-25:18.

[178] EX1001 (US-201), 14:56-17:54, 15:14-16 (“and —$(CH_2—CH_2—O)_q$, wherein q is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8…”).

"low molecular weight compound" as the '201 Patent uses that phrase to refer to equivalently-sized compounds.

176. Each of the Compounds A1, A2, A3 and A4 has a molecular weight below 1,500 Da, which is orders of magnitude less than the size of an antibody (~150 kDa). Each is also based on a small molecule targeting moiety (compound 60) that is linked to a conventional linker and radiolabeling moiety. I also understand that Dr. Pomper has indicated that a compound having a molecular weight below 1500 Da would be a "low molecular weight compound."[179] I believe a skilled artisan would consider each of Compounds A1, A2, A3 and A4 to be "low molecular weight" compounds as that phrase is used in claims 1 to 3 of the '201 Patent.

I. A Skilled Artisan Would Have had a Reasonable Expectation of Successfully Developing a FAP-targeting Radiopharmaceutical Based on the Guidance of US-633, Jansen and Meletta

177. I believe a skilled artisan would have reasonably expected that the examples I describe above (see ¶¶ 146 and 158) would be viable FAP-targeting radiotracers that could be used in the imaging of tumors.

(a) First, a skilled artisan would expect that the warhead shared by these examples of radiotracers (compound 60 of Jansen) would

[179] EX1002 ('201 FW), 363-364 (¶¶ 32-37).

effectively deliver the radiopharmaceutical to FAP-expressing tissues in the patient, based on the favorable balance of characteristics it exhibited. As I explained above (¶¶ 110-115, 120-127), compound 60 exhibited high selectivity and affinity for FAP, is amenable to conjugation into a radiotracer, and exhibited favorable pharmacokinetic, stability, and bioavailability characteristics.

(b) Second, each example of a radiotracer I identified has a structure that follows well-established design principles for the construction of radiotracers and other radiopharmaceuticals. The warhead in each example is sterically separated from the radiolabeling moiety and the radionuclide. The point of attachment of the linker on compound 60 is also at a location that indicates it will not disrupt the affinity and selectivity characteristics of the warhead.

(c) Third, the linkers, chelators, and prosthetic groups used in the examples have an established track record of being successfully used in and compatible with radiopharmaceuticals and radiotracers based on ^{18}F and ^{68}Ga.

178. A skilled artisan also would have known how to synthesize these compounds based on the guidance provided in US-633 and Jansen, and the knowledge, experience and training the person would have had.[180] The skilled artisan also would have been familiar with efficient compound synthesis methods and automated synthesis devices, as I explained above (¶¶ 52-55).

IV. US-121 and Jansen Would Lead a Skilled Artisan to Develop FAP-Based Radiopharmaceuticals Using Compound 60 of Jansen

179. Before 2017, biomarkers of cancer other than FAP had been investigated as targets for radiopharmaceuticals, particularly those used in nuclear imaging. One such biomarker is prostate-specific membrane antigen (PSMA), a cancer antigen expressed by prostate cancer cells. A 2016 review surveyed work in this area and observed that "several small molecule compounds for labeling PSMA have been developed and are currently being investigated as imaging

[180] See, e.g., EX1004 (US-633), [0171]-[0175] (describing the synthesis process for US-633 compound 1024), [0181]-[0182] (describing radiolabeling processes); EX1006 (Jansen), 3056, 3071 (describing synthesis methods and characteristics of compound 60); EX1008 (Meletta), 2086 (describing synthesis method for the MIP-1232 radiopharmaceutical).

probes for PET…"[181] It also reported that a variety of those radiotracers ("imaging probes") used as their targeting moieties small molecule inhibitors of PSMA.[182]

180. A skilled artisan working in the field of nuclear imaging of cancer would have been aware of work being done to develop PSMA-targeting radiotracers before 2017. One example was the work reported in U.S. patent application number US2012/0009121 ("US-121") (EX1005) by Dr. Martin Pomper and his colleagues at the Johns Hopkins University.

A. US-121 (EX1005)[183]

181. US-121 is a published U.S. patent application that lists as the inventors Martin Pomper, Ronnie Charles Mease, Ray Sangeeta, and Ying Chen. It was published on January 12, 2012.

1. US-121 Describes Low Molecular Weight Radiotracers for Targeting PSMA

182. US-121 describes "new imaging and therapeutic compounds for targeting prostate cancer and cancer angiogenesis."[184] It explains these compounds

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[181] EX1036 (Maurer), Abstract, 226-227.

[182] EX1036 (Maurer), 228.

[183] EX1005 (US-121).

[184] EX1005 (US-121), [0012].

target PSMA, a membrane-associated enzyme that is expressed on the surface of prostate tumors.[185] PSMA and FAP have a number of similarities: (i) they are both cell-surface enzymes that can be targeted with small molecule enzyme inhibitors, (ii) they are both selectively expressed on epithelial cells within tumors, and (iii) they were both known to be attractive targets for tumor imaging using radiotracers.[186]

183. US-121 describes examples of radiotracers based on compounds that show high target selectivity for PSMA-expressing tumors.[187] The PSMA-specific compounds used as the targeting moieties of the radiotracers described in US-121 are a class of urea-based inhibitors of PSMA. In US-121, they are illustrated using

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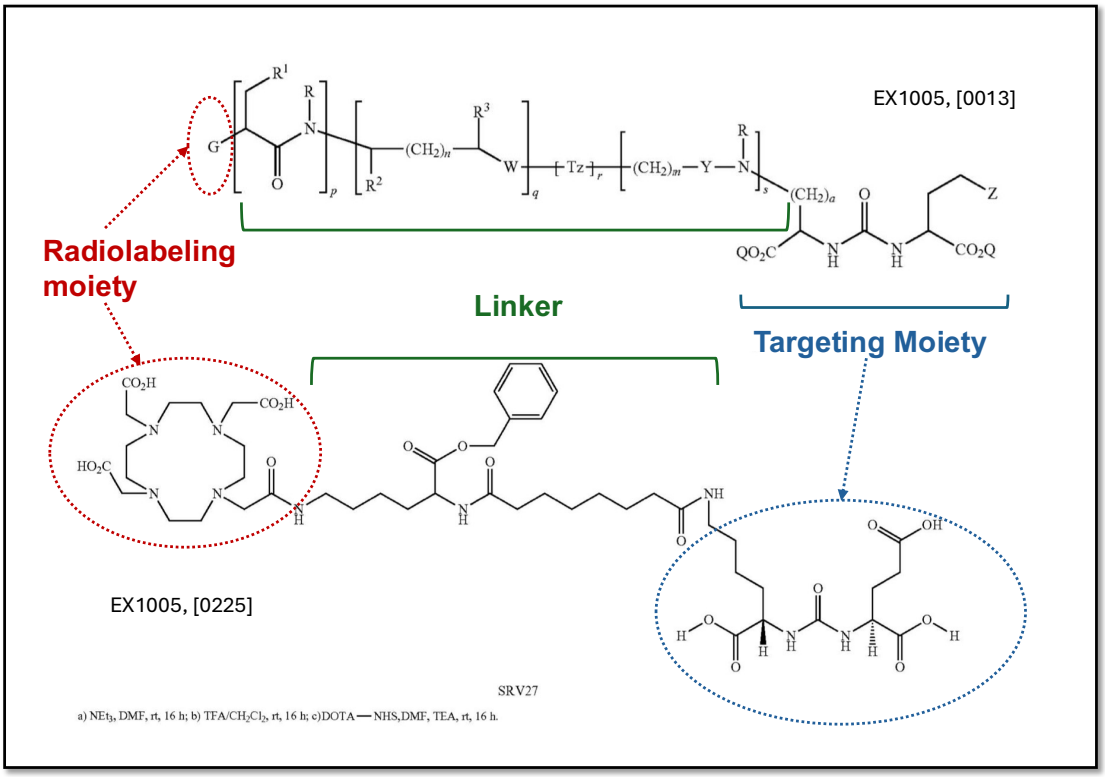
[185] EX1005 (US-121), [0007], [0012]; See also EX1030 (Eder), 688.

[186] EX1005, [0007] ("As an enzyme with an extracellular active site, PSMA represents an excellent target for imaging and therapy directed toward solid tumor neovasculature in addition to prostate cancer itself."); EX1004, [0003] (Seprase, also known as fibroblast activation protein alpha (FAP-α), is a transmembrane serine peptidase …"), [0005] ("The expression of seprase on tumors makes it an attractive target to exploit for noninvasive imaging as well as targeted radiotherapy.")

‘[187] EX1005 (US-121), [0240] ("FIGS. 2 and 3 demonstrate the high target selectivity of [^{68}Ga]SRV27 and [^{68}Ga]SRV100 by delineating the PSMA+ tumors.").

a general radiotracer design (below, top). I have added annotations to identify the components of these radiotracers. I also show one of the examples of radiotracers ("SRV27"), which attaches DOTA to one of the urea-based targeting moieties via a particular linker.[188]



184. US-121 explains that one advantage of using a modular structure (*i.e.*, chelator-linker-targeting moiety) is that it allows one to easily optimize the pharmacokinetics of the radiopharmaceutical by altering individual components in the radiotracer (*e.g.*, switching a linker to one that is more hydrophobic while

188 EX1005 (US-121), [0225].

retaining the same radiolabeling and targeting moieties).[189] US-121 also suggests certain radiotracers can be designed to support both PET and SPECT imaging with ^{68}Ga and ^{99m}Tc, respectively.[190] While DTPA has been used with both ^{68}Ga and ^{99m}Tc, the different coordination chemistries of ^{68}Ga and ^{99m}Tc typically require different chelators. A skilled artisan would have read these different observations in US-121 as simply reflecting the general understanding that once a viable ^{68}Ga-based radiotracer has been developed for one imaging platform (*i.e.*, PET), it can usually be adapted to support the other one (*i.e.*, SPECT), most commonly by replacing the chelator component to one compatible with ^{99m}Tc, or (less commonly) by using a single chelator that supports both ^{68}Ga and ^{99m}Tc (or another SPECT-compatible radionuclides compatible with the ^{68}Ga-compatible chelator).

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189 EX1005 (US-121), [0279] ("We arrived at YC-27 based on structure-activity relationships developed for PSMA-binding ureas, which were focused on improving pharmacokinetics for use in vivo by optimization of the linker-chelate complex."). See also EX1005 (US-121), [0187] ("[c]ompounds with increased hydrophobicity, such as compounds having hydrophobic linkers, may have longer circulation times, thereby providing more prolonged supply of tracer to bind to cells.").

190 EX1005 (US-121), [0242] ("Gallium-68 provides a link between PET and single photon emission computed tomography (SPECT) since metal chelating methodology needed for ^{99m}Tc can also be applied to ^{68}Ga.")

185. A skilled artisan would have recognized that the radiotracers being described in US-121 are "low molecular weight" compounds as they combine a small molecule targeting moiety, typically below 1,500 Da, with conventional linkers and chelator structures. US-121 also describes its radiotracer compounds as being "low molecular weight" compounds.[191]

186. US-121 identifies some of the reasons why a skilled artisan would have used low molecular weight radiotracers in nuclear imaging. I note these are very similar to the reasons in the '201 patent for using low-molecular-weight radiotracers instead of antibody-based radiotracers (below).

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[191] EX1005 (US-121), [0012] ("The invention also provides ***compounds with*** greater cellular retention and ***low molecular weight***.") (emphasis added); [0279] ("A wide variety of low molecular weight PSMA-based imaging agents have been synthesized, including those using the urea scaffold …").

US 2012/0009121 A1

[0008] ProstaScint™ is an ^{111}In-labeled monoclonal antibody against PSMA that is clinically available for imaging PCa. Radioimmunotherapy based on ProstaScint™ and radiolabeled variations of this antibody are fraught with similar difficulties to the use of radiolabeled antibodies for imaging, including prolonged circulation times, poor target to nontarget tissue contrast, unpredictable biological effects and the occasional need for pre-targeting strategies, limiting the utility of these agents (Lange, P. H., *Urology, vol.* 57, pp. 402-406, 2001; Haseman et al., *Cancer Biother Radiopharm, vol.* 15, pp. 131-140, 2000; Rosenthal et al., *Tech Urol, vol.* 7, pp. 27-37, 2001). Furthermore, antibodies may have less access to tumor than low molecular weight agents, which can be manipulated pharmacologically.

[0009] The development of low molecular weight radiotherapeutic agents is much different from developing radiopharmaceuticals for imaging in that longer tumor residence times can often be important for the former.

EX1005, [0008]-[0009]

US 11,938,201 B2

Because FAP-α is expressed in tumor stroma, anti-FAP antibodies have been investigated for radioimmunotargeting of malignancies, including murine F19, sibrotuzumab (a humanized version of the F19 antibody), ESC11, ESC14, and others. (Welt, et al., 1994; Scott, et al., 2003; Fischer, et al., 2012). Antibodies also demonstrated the feasibility of imaging inflammation, such as rheumatoid arthritis. (Laverman, et al., 2015). The use of antibodies as molecular imaging agents, however, suffers from pharmacokinetic limitations, including slow blood and non-target tissue clearance (normally 2-5 days or longer) and non-specific organ uptake. Low molecular weight (LMW) agents demonstrate faster pharmacokinetics and a higher specific signal within clinically convenient times after administration. They also can be synthesized in radiolabeled form more easily and may offer a shorter path to regulatory approval. (Coenen, et al., 2010; Coenen, et al., 2012; Reilly, et al., 2015). To date, however, no LMW ligand has been reported with ideal properties for nuclear imaging of FAP-α.

EX1001, 1:49-67

2. US-121 Highlights Benefits of PET Scanning with Radiotracers That Use ^{68}Ga

187. US-121 lists radionuclides commonly used in nuclear imaging and explains that a skilled artisan would know how to select one that would be appropriate to use in a radiotracer to be used with a particular scanner and for a particular clinical purpose (*e.g.*, therapy or imaging).[192] As it indicates, "the suitability of a particular radioisotope for a particular purpose (i.e. imaging or therapeutic) is well understood in the art."[193] This is consistent with the general knowledge in the field as I explained in ¶¶ 38-39 above. US-121 also explains that its radiotracers can be used with well-known imaging technologies used for tumor

[192] EX1005 (US-121), [0104].

[193] EX1005 (US-121), [0175].

imaging, such as "a gamma camera, a PET apparatus, a SPECT apparatus, a fluorescence camera and the like."[194]

188. US-121 identifies several advantages of using ^{68}Ga as the radionuclide and using PET as the imaging platform.[195] This is consistent with what a skilled artisan would have known. *See* ¶¶ 42, 132 (above). US-121 identifies four reasons why ^{68}Ga was a good option for radiotracers.

(a) *Ease of Use*: Their ease of use, as ^{68}Ga can be easily generated using ^{68}Ga/^{68}Ge generators that can be kept in-house, and which "provide readily available isotope, with no need for an in-house cyclotron."[196] US-121 even provides step-by-step guidance for

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194 EX1005 (US-121), [0184].

195 EX1005 (US-121), [0011] ("Recently, the application of ^{68}Ga-labeled peptides has attracted considerable interest for cancer imaging because of the physical characteristics of Ga-68.").

196 EX1005 (US-121), [0011] ("^{68}Ga-based PET agents possess significant commercial potential and serve as a convenient alternative to cyclotron-based isotopes for positron emission tomography (PET) such as ^{18}F or ^{124}I."), [0242].

generating and radiolabeling a compound with ^{68}Ga based on a protocol provided in the literature.[197]

(b) *High Resolution Imaging:* US-121 explains that radiotracers based on ^{68}Ga generate high resolution images on par with the high-quality images generated when using ^{18}F-based radiotracers, which are generally recognized as generating some of the highest resolution PET images. [198]

(c) *Half-Life Well Matched to Small Molecule Radiotracers*: US-121 explains that because ^{68}Ga has a physical half-life of 68 min, it "is also matched nicely to the pharmacokinetics of many peptides used for imaging."[199] That is consistent with my explanation above (¶ 40) that a skilled artisan would pick a radionuclide for a new radiotracer based on the alignment of the

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197 EX1005 (US-121), [0218]-[0222] ("^{68}Ga labeling protocol for compound SRV27 was done following a literature procedure (Zhernosekov et al., J Nucl Med, vol. 48, pp. 1741-1748, 2007). A detailed description is given below.").

198 EX1005 (US-121), [0011] (internal citations omitted).

199 EX1005 (US-121), [0011].

physical half-life of the radionuclide and the pharmacokinetic half-life of the radiotracer.

(d) *Many Chelator Options:* US-121 portrays ^{68}Ga as a versatile radionuclide due to its compatibility with different chelator complexes, which gives a skilled artisan flexibility in designing the structure of the radiopharmaceutical compound.[200]

3. US-121 Describes Conventional Chelators to Use in Radiotracers

189. US-121 identifies a number of well-known chelating complexes that can be used in the radiotracers it is describing. A list of these structures is compiled in paragraph [0102] of US-121. This list of examples includes chelators that a skilled artisan would have recognized could be used with ^{68}Ga, such as DOTA and DTPA.[201] See ¶¶ 78, 82. It also describes chelators that can be used

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200 EX1005 (US-121), [0011] ("^{68}Ga is introduced to biomolecules through macrocyclic chelators, which allows possible kit formulation and wide availability of the corresponding imaging agents.").

201 EX1005 (US-121), [0102].

with ^{99m}Tc (*e.g.*, DPA), and provides examples that use those chelator compounds.[202]

190. US-121 explains that the examples being described in it are just some of the chelators that would be suitable to use in its urea-based radiotracers. It points out that "[n]umerous metal chelating moieties are known in the art" and then explains that "[a]ny acceptable chelator can be used with the present invention as long as compatible and capable of chelating a desired metal."[203] A skilled artisan would have read the guidance in US-121 as indicating that the chelators being illustrated could be used, as well as other known chelators that are not listed within the US-121 document. As I explained above (¶¶ 33, 81-82), a skilled artisan would have been familiar with a number of other chelators that are particularly well-suited for use with ^{68}Ga or ^{99m}Tc as the radionuclide. *See* ¶¶ 33, 80-82.

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[202] EX1005 (US-121), [0214], [0249]-[0250], [0102], [0103](pages 12-17).

[203] EX1005 (US-121), [0102].

4. US-121 Describes Linker Moieties Used to Assemble Radiotracers

191. US-121 describes conjugating the radiolabeling moiety to its urea-based targeting moieties using a linker.[204] It indicates that the linkers can include "alkyl, aryl, combination of alkyl and aryl, or alkyl and aryl groups having heteroatoms may be present in the chelating moiety."[205] It also explains that the metal chelating moiety "includes any additional atoms or linkers necessary to attach the metal chelating moiety to the rest of the compound."[206]

192. US-121 illustrates structures that covalently attach the urea-based targeting moiety via linkers functionalized with a terminal amine group to facilitate amide bond linkages to chelator groups.[207] For example, it describes a synthetic

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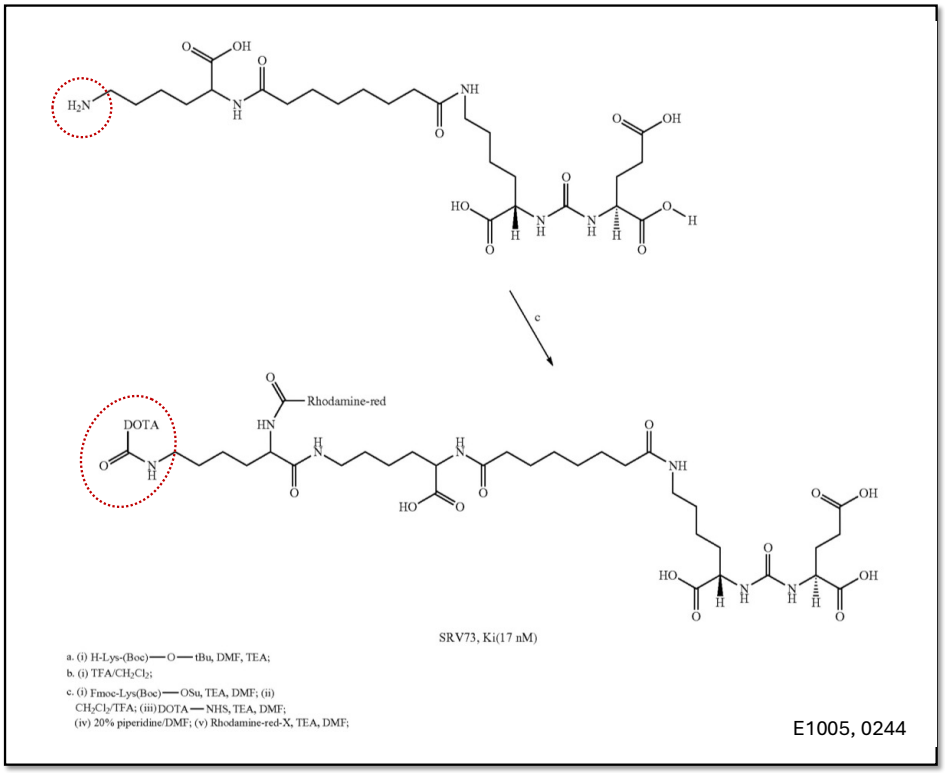
[204] EX1005 (US-121), [0102] ("For instance, linking groups having alkyl, aryl, combination of alkyl and aryl, or alkyl and aryl groups having heteroatoms may be present in the chelating moiety.").

[205] EX1005 (US-121), [0102].

[206] EX1005 (US-121), [0102] ("Numerous metal chelating moieties are known in the art. Any acceptable chelator can be used with the present invention as long as compatible and capable of chelating the desired metal.").

[207] EX1005 (US-121), [0225], [0242], [0249] [0166].

scheme by which a DOTA chelator is covalently attached via an amide bond to a linker terminating in an amine group (final step below).[208]



193. US-121 identifies benefits of using particular linkers in its examples of radiotracers. It explains, for example, that it arrived at one of its example compounds (YC-27) "…based on structure-activity relationships developed for PSMA-binding ureas, which were focused on improving pharmacokinetics for use in vivo by optimization of the linker-chelate complex."[209] It also indicates that

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208 EX1005 (US-121), [0244].

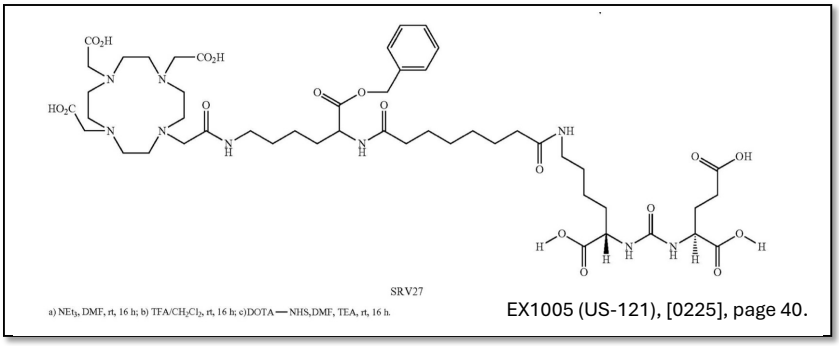
209 EX1005 (US-121), [0279].

using hydrophobic linkers increased the overall hydrophobicity of the radiotracer compounds, and provided "a more prolonged supply of tracer to bind to cells." [210]

5. Examples of Radiotracers Illustrated in US-121

194. The US-121 publication lists a number of examples of radiotracers that combine its urea scaffold-based targeting moiety with different chelators and linkers.[211] Four examples are shown below that I will use as a basis for producing compound 60-based radiotracers.

195. For example, US-121 describes a method of synthesizing a DOTA-based PSMA-targeting radiotracer compound suitable for use with ^{68}Ga designated "SRV27" (below).[212]



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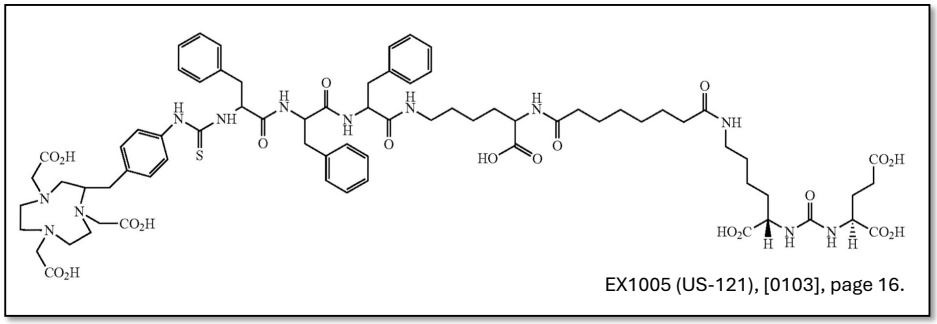
[210] EX1005 (US-121), [0187].

[211] EX1005 (US-121), [0103].

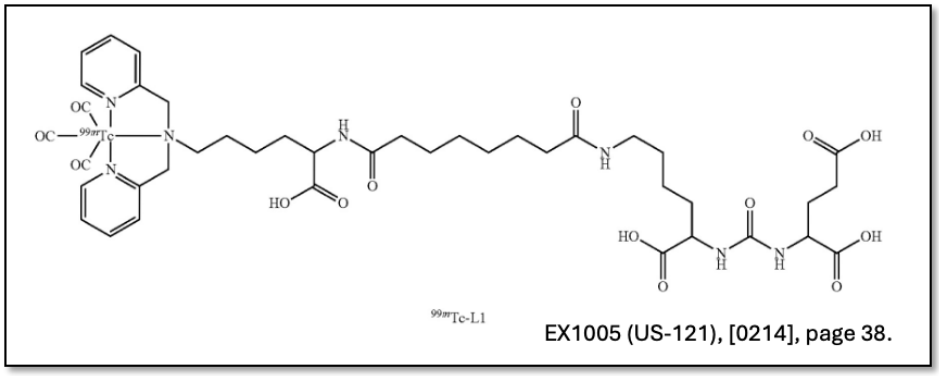
[212] EX1005 (US-121), [0225], page 40.

196. US-121 describes a NOTA-based PSMA-targeting radiotracer compound suitable for use with ^{68}Ga (below).[213]



EX1005 (US-121), [0103], page 16.

197. US-121 also describes radiotracer compounds using the ^{99m}Tc radionuclide. For example, it displays the structure of a DPA-based PSMA-targeting radiotracer compound designated "^{99m}Tc-L1" (below) and provides data on the compound's tumor uptake *in vivo* retention.[214]



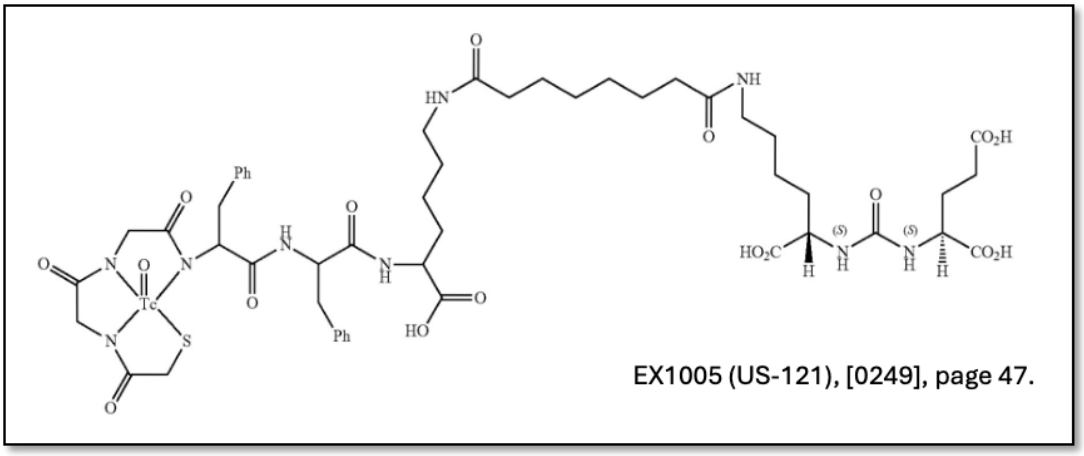
^{99m}Tc-L1

EX1005 (US-121), [0214], page 38.

213 EX1005 (US-121), [0103], page 16.

214 EX1005 (US-121), [0214].

198. US-121 describes the structure and method of synthesis for the PSMA-targeting radiotracer compound suitable for use with ^{99m}Tc designated "SRV134" (below).[215]



B. A Skilled Artisan Would Have Considered US-121 and Jansen Together Given their Common Focus on Targeting Cancer-Specific Biomarkers

199. In early 2017, a skilled artisan would have considered the radiotracer designs illustrated in US-121 when evaluating how to create other low molecular weight radiotracers for tumor imaging based on small molecule targeting moieties. The examples shown in US-121 reflect the result of using common design principles followed in the field of radiopharmaceutical development. A skilled artisan looking for guidance on constructing new, small-molecule based

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[215] EX1005 (US-121), [0249], page 16.

radiopharmaceuticals (particularly radiotracers) would have found the examples and observations in US-121 instructive and helpful.

200. I previously discussed the Jansen publication that described compound 60 and other 2-cyano-pyrrolidine FAP inhibitors. See § III.C. As I indicated, a skilled artisan would have found compound 60 to be a compelling candidate as a small molecule targeting moiety for a radiotracer due to its high selectivity and high affinity for FAP, as well as its stability, pharmacokinetic profile, bioavailability, and *in vivo* inhibitory effects on FAP activity. See ¶¶ 110-115, 120-127. Compound 60 also has a structure that can be functionalized (*i.e.*, at C^6 or C^7) to facilitate its attachment to linker-chelate structures used in radiotracers. See ¶¶ 140-143.

201. Both US-121 and Jansen report investigations in the same scientific field—the development of small molecule compounds that selectively target cell-surface enzymes that are biomarkers of tumors and that can be targeted by radiopharmaceuticals for the diagnosis and treatment of cancer.[216]

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[216] EX1005 (US-121), [0012] ("The present invention satisfies the long standing and unmet need for new imaging and therapeutic compounds for targeting prostate cancer and cancer angiogenesis."), [0007] ("As an enzyme with an extracellular active site, PSMA represents an excellent target for imaging and

C. A Skilled Artisan Would Have Designed a Low Molecular Weight FAP-Targeting Radiotracer Using the Particular Linker-Chelator Combinations in the Examples in US-121 with Jansen's Compound 60 as the Targeting Moiety

1. A Skilled Artisan Would Have Selected Jansen Compound 60 as the Targeting Moiety for a FAP-based Radiotracer

202. As I explained earlier (¶¶ 59, 62-68), there was substantial interest in developing FAP-targeting radiopharmaceuticals before 2017. The selective expression of FAP in the stroma of over 90% of common human epithelial tumors combined with the lack of expression of FAP in normal tissue fits a desirable profile for a cancer biomarker that can be exploited for use in the diagnosis or treatment of cancers.[217] A skilled artisan interested in developing radiopharmaceuticals to be used in the diagnosis and treatment of cancers would

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therapy directed to solid tumor neovasculature in addition to prostate cancer itself."]; EX1006 (Jansen), Abstract (noting that FAP "has been convincingly linked to multiple diseases states" and "FAP inhibition is investigated as a therapeutic option for several of these diseases, with most attention so far devoted to oncology applications.").

[217] EX1006 (Jansen), 3053 ("FAP is also highly expressed on activated fibroblasts in over 90% of common human epithelial tumors."); EX1038 (LeBeau), 1384 ("Given its restricted expression in the reactive stroma of potentially >90% of epithelial cancers studied (7), FAP represents an attractive target for tumor-directed therapies.").

have had a strong motivation to use targeting moieties with high selectivity for FAP.

203. Before 2017, a skilled artisan would have recognized the importance of identifying a selective and specific targeting moiety when developing a radiotracer, particularly one that is used in tumor imaging.[218] For the reasons I explained in paragraphs (¶¶ 133-135) above, such a person would have viewed Jansen's compound 60 to be a very promising targeting moiety that could be incorporated into a FAP-targeting radiotracer for use in imaging tumors. For example, a skilled artisan would have been motivated to design radiotracers based on compound 60 not only because FAP was seen as an excellent cancer biomarker to use for imaging and therapeutic purposes but because compound 60 is a highly selective small molecule inhibitor of FAP. See §§ III.C, III.D, III.F (above).

2. A Skilled Artisan Would Have Viewed ^{68}Ga or ^{99m}Tc as Appropriate Radionuclides to Use in the Radiotracers Being Described in US-121

204. A skilled artisan following the guidance in US-121 would have focused on radiometals compatible with PET or SPECT imaging that could be

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[218] See EX1005 (US-121), [0239]-[0240] ("FIGS. 2 and 3 demonstrate the high target selectivity of [^{68}Ga]SRV27 and [^{68}Ga]SRV100 by delineating the PSMA+ tumors.").

delivered by the small molecule-based radiotracers US-121 is describing, particularly ^{68}Ga or ^{99m}Tc. See ¶¶ 39-42, 187-188 (above). As I explained in § IV.A.2 above, US-121 identifies certain benefits of using ^{68}Ga in PET-based imaging, including its ease of synthesis using generators, its favorable pharmacokinetic profile, the high resolution of images generated from using it, and its versatility from having a number of options for chelating complexes.[219] However, it also provides examples of its urea-based radiotracers that are designed to use ^{99m}Tc as the radionuclide, reflecting the commercial benefit of the large installed base of SPECT imaging equipment in the United States.

D. A Skilled Artisan Would Have Replaced the Targeting Moiety in the Examples of Radiotracers Illustrated in US-121 with Jansen's Compound 60

205. A skilled artisan would have found it obvious to alter the examples of PSMA-targeting radiotracers described in US-121 by replacing the urea-based targeting moiety in them with compound 60 described in Jansen. For example, the skilled artisan would have recognized that the urea-based targeting moiety in the US-121 examples (below left) is comparable in size to compound 60 (below right) (*i.e.*, 319 Da vs. 344 Da). That would suggest the radiotracer designs could be suitable for use with compound 60. The high FAP selectivity and favorable *in*

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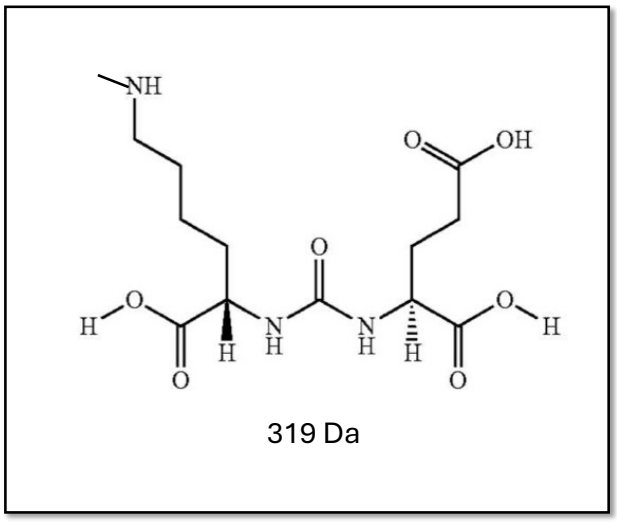
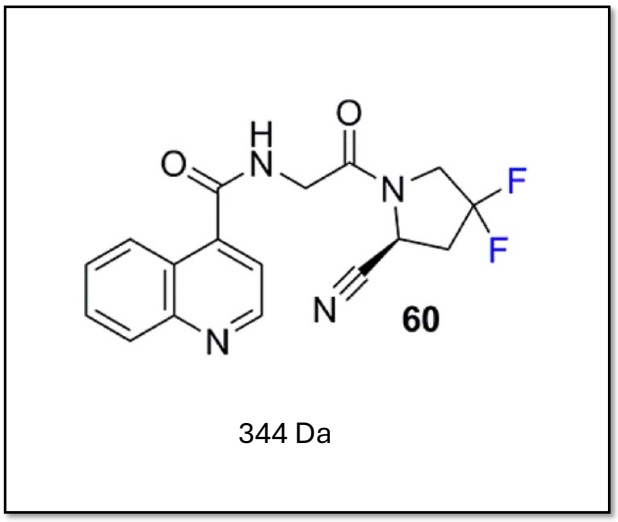
[219] EX1005 (US-121), [0011].

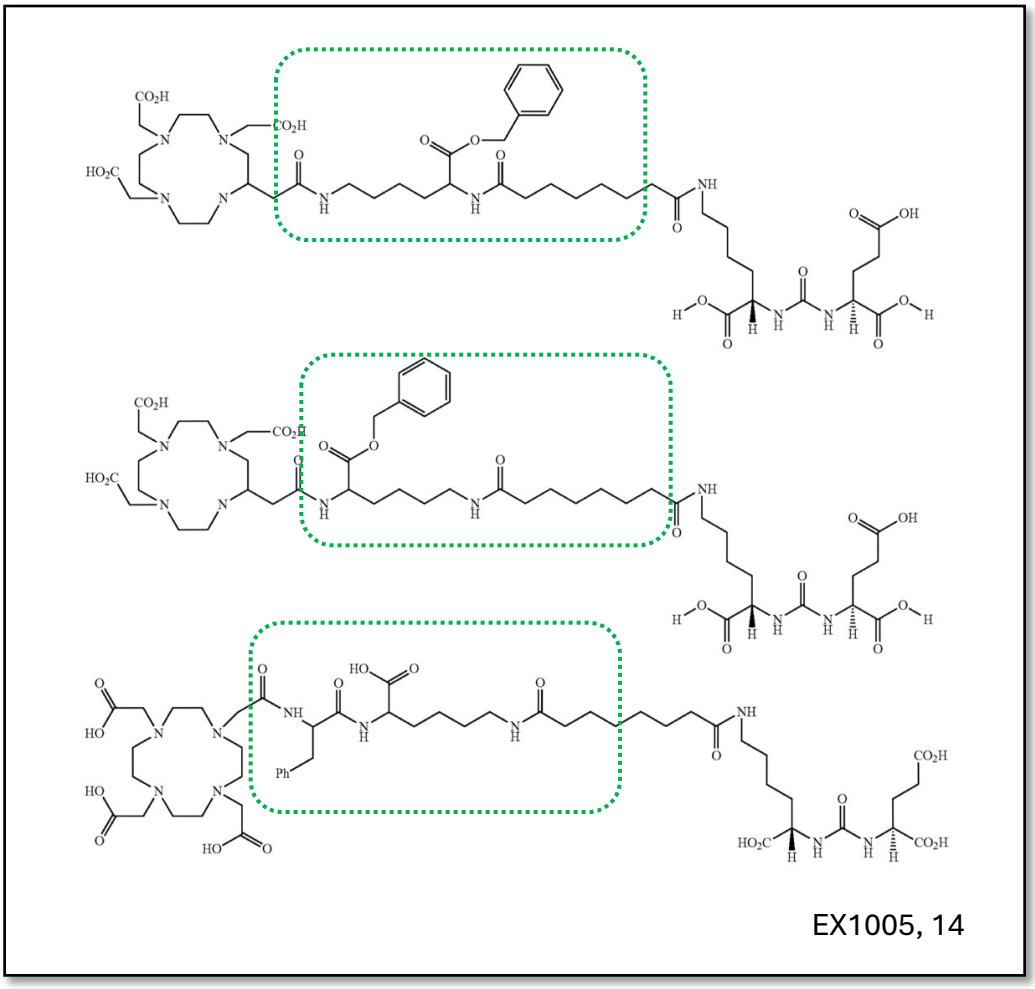
vitro and *in vivo* properties of compound 60 that Jansen reported, combined with and attractiveness of FAP as a target for nuclear imaging would have motivated the skilled artisan to create radiotracers based on compound 60 and led them to consider use of the radiotracer designs shown in US-121.



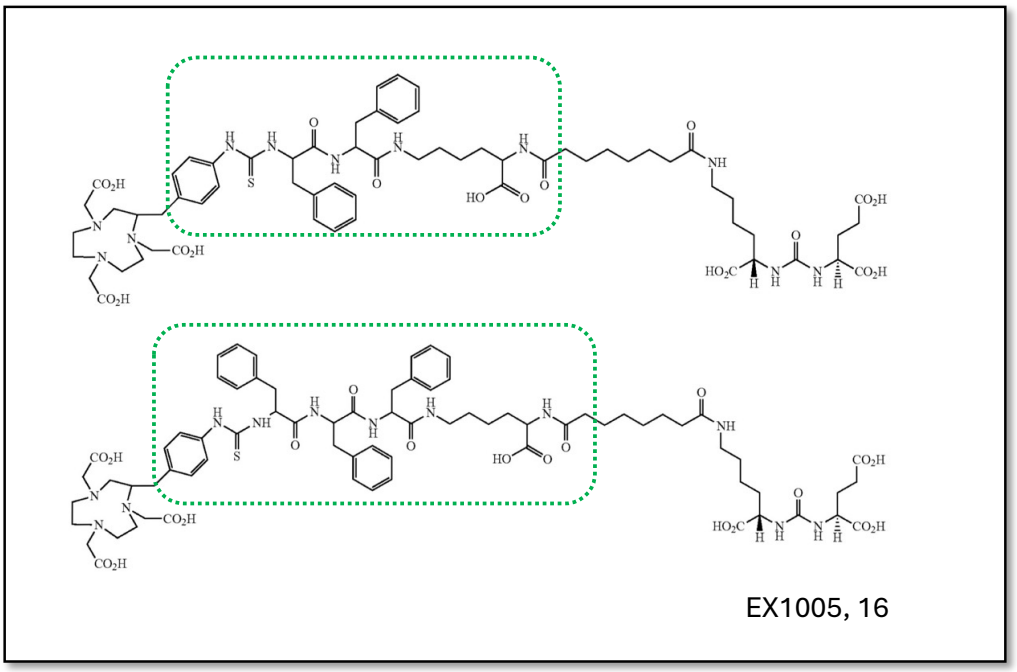


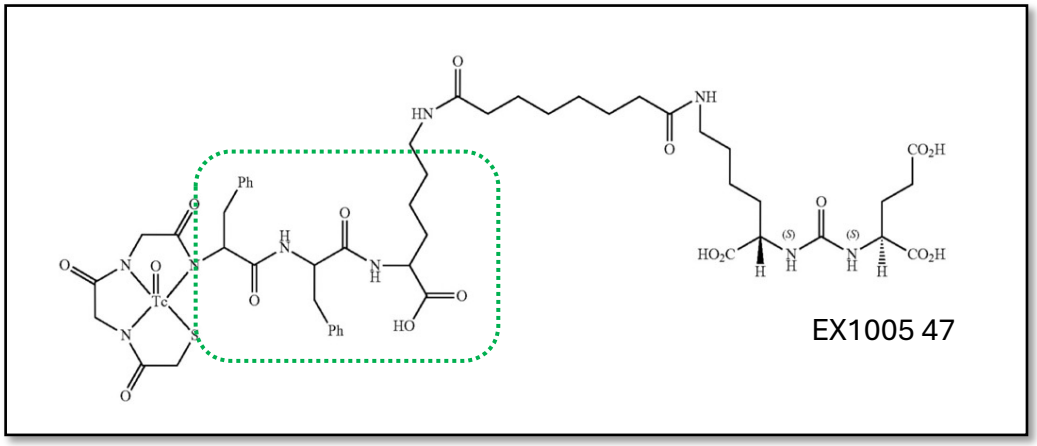
206. The skilled artisan would have elected to retain the remainder of the radiotracer structure when adapting the radiotracer examples in US-121 to incorporate compound 60 as the targeting moiety because that person would have recognized that one of the important design strategies evident in the array of examples of DO3A-, DOTA-, and NOTA-bearing radiotracers in US-121 is the deliberate and systematic alteration of the linkers in them to modulate the hydrophobicity of the radiotracers. For example, the PSMA-targeting radiotracers shown on pages 14-16 and 47 of US-121 have linkers with varying numbers of pendant benzyl groups (below). A skilled artisan would recognize that the

hydrophobicity of these radiopharmaceuticals would likely increase with the addition of each successive aromatic moiety.



EX1005, 14





207. US-121 also explains why the linkers were altered to increase their hydrophobic character: "highly hydrophilic compounds may be excreted quickly, while "[c]ompounds with increased hydrophobicity, such as compounds having hydrophobic linkers, may have longer circulation times, thereby providing more

prolonged supply of tracer to bind to cells."[220] US-121 also identifies benefits of using more hydrophobic linkers in its examples incorporating a chelator (*e.g.* the increased hydrophobicity provided a more prolonged supply of the tracer to bind cells).[221] These observations in US-121 would have motivated a skilled artisan interested in developing new radiotracers for use in nuclear imaging of tumors to avoid "recreating the wheel" by starting with one or more of the examples of radiotracers in US-121 and using the chelator-linker portion of these examples with a new targeting moiety (*i.e.*, compound 60).

208. The skilled artisan would have selected examples that use chelators compatible with ^{68}Ga, given the benefits that US-121 identifies with using ^{68}Ga-based radiotracers and PET-based imaging.[222] See § IV.A.2 (above). A skilled artisan also would have recognized that compound 60 would need to be functionalized to support an amide linkage with the terminus of the linker in the example radiotracer compounds in the US-121. For example, the quinolinyl group of compound 60 would need to be functionalized with an amine group at positions

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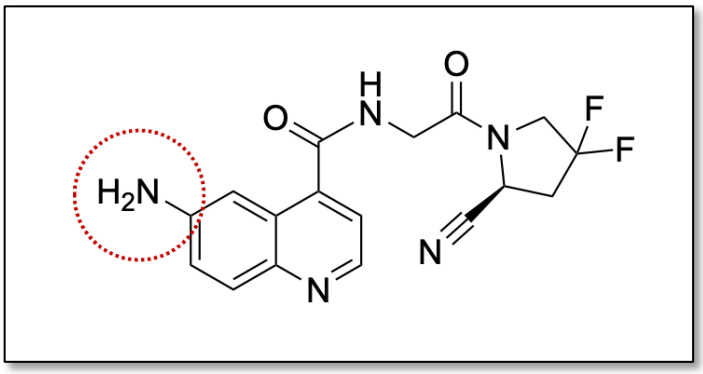
220 EX1005 (US-121), [0187].

221 EX1005 (US-121), [0187].

222 EX1005 (US-121), [0011].

6 (shown below) or 7 to enable its covalent attachment to the linker via a simple amide bond.[223]



209. A skilled artisan would have started with examples of PSMA-based radiotracers that incorporate a chelator compatible with ^{68}Ga (*e.g.*, DOTA, NOTA) or with ^{99m}Tc (e.g., DPA). The skilled artisan would then replace the urea-based targeting moiety using compound 60 of Jansen which had been functionalized to incorporate an amine group at position C^6 to facilitate formation of the amide bond that links it to the chelator-linker remainder of the starting compound.

210. For example, starting with compound SRV27 that is shown on page 40 of US-121, and replacing the targeting moiety with Compound 60, yields a radiotracer I designated Compound B1, which has a molecular weight of ~1185 Da (below).

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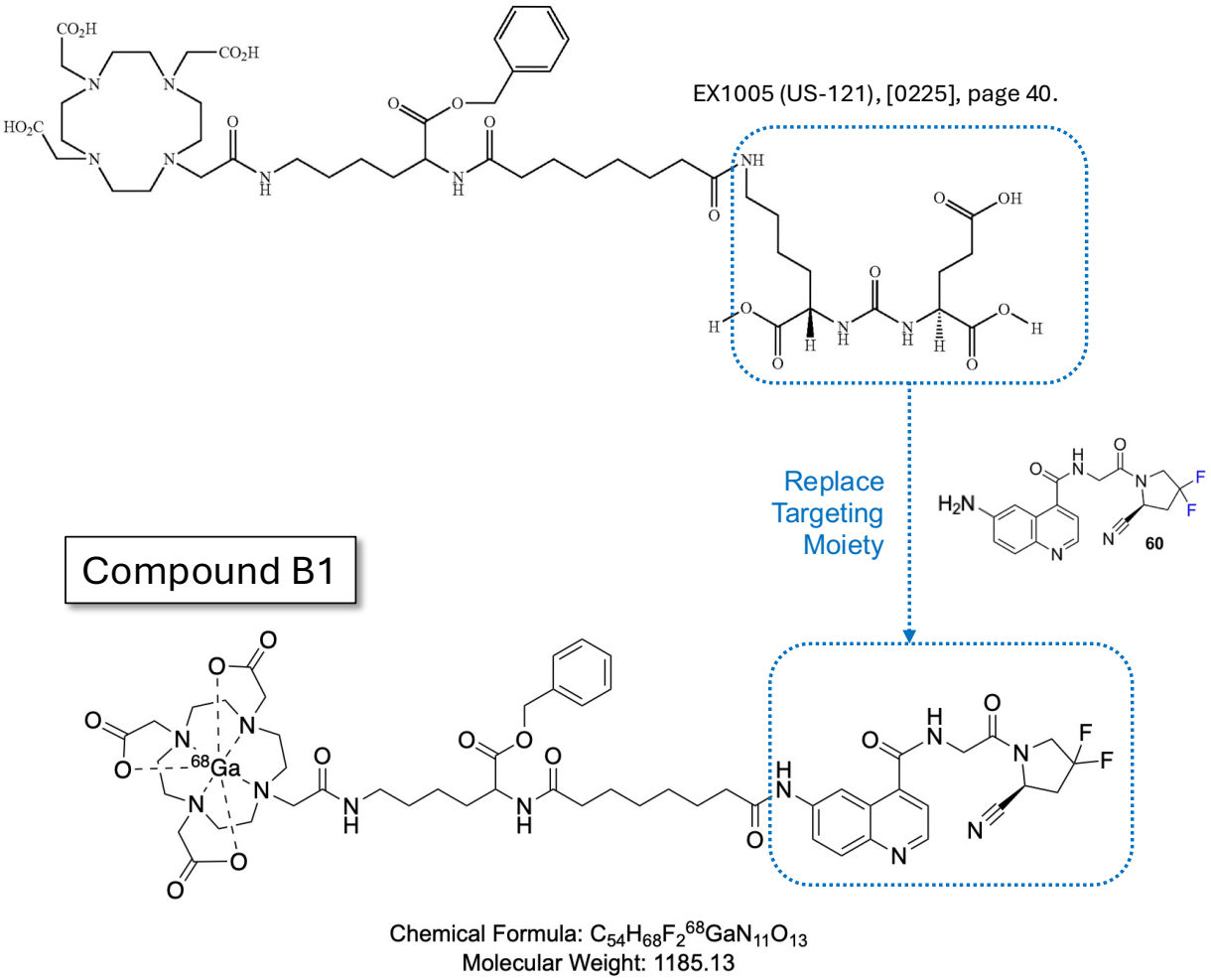
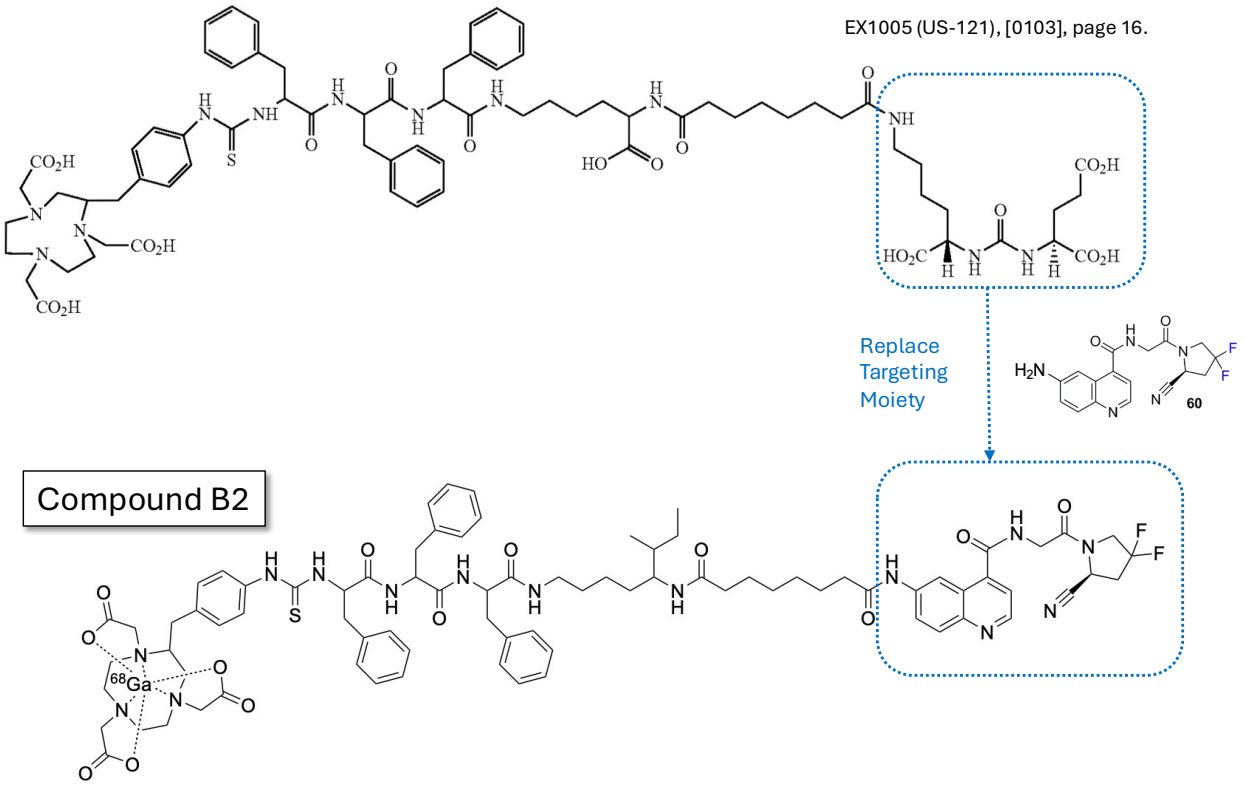
[223] See § III.G.1.



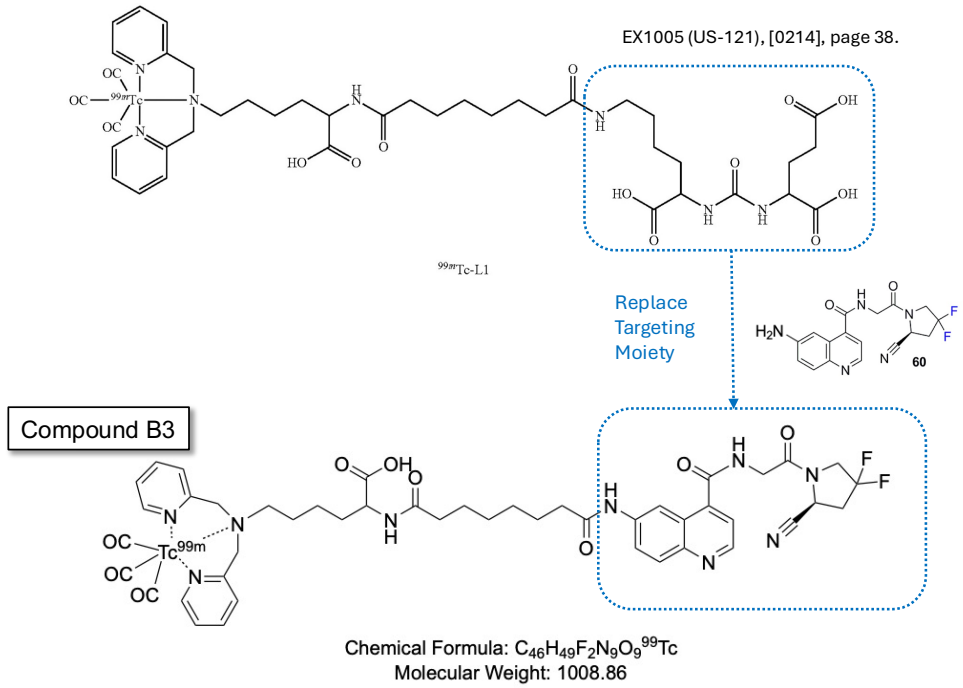
211. Other examples of compounds that would result from replacing the targeting moiety of compounds in US-121 with compound 60 include Examples B2, B3 and B4 (below). The starting examples for compounds B2, B3 and B4 are described in US-121 on the noted pages in each of the four examples below.

EX1005 (US-121), [0103], page 16.

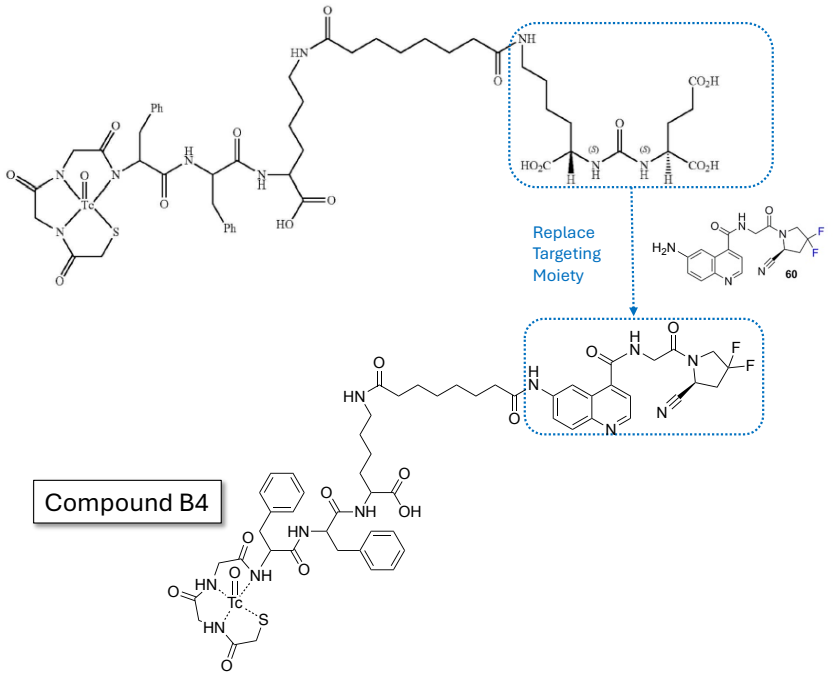


Chemical Formula: $C_{81}H_{97}F_2{}^{68}GaN_{14}O_{13}S$
Molecular Weight: 1612.74

EX1005 (US-121), [0214], page 38.



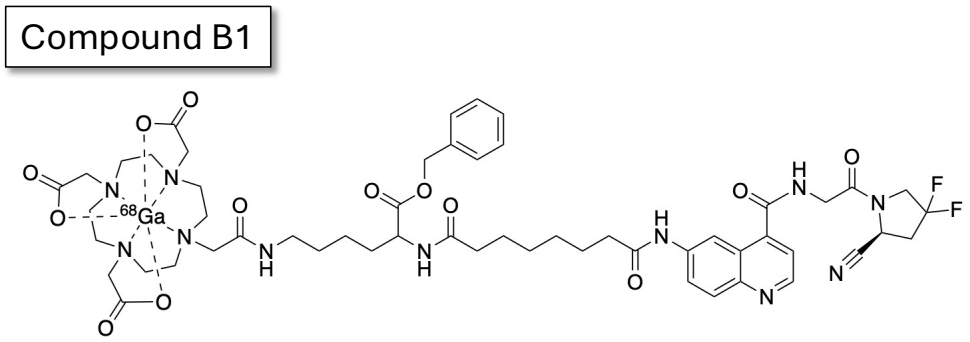
Chemical Formula: $C_{46}H_{49}F_2N_9O_9{}^{99}Tc$
Molecular Weight: 1008.86



Chemical Formula: $C_{55}H_{64}F_2N_{11}O_{12}STc$
Molecular Weight: 1238.24

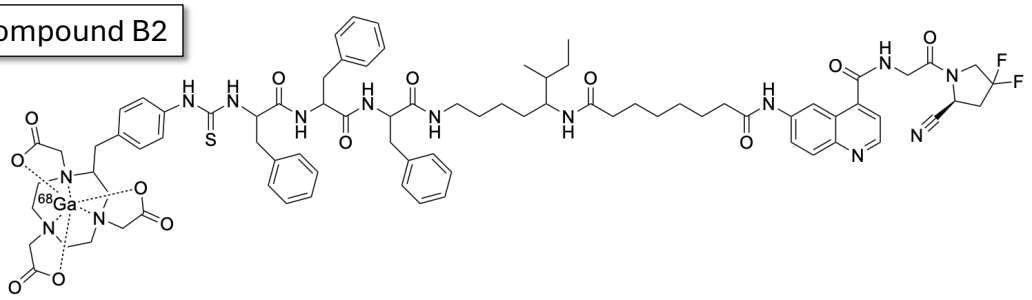
E. Compounds B1 to B4 Meet the Requirements of the Claims of the '201 Patent

212. The modified examples of compounds B1 to B4 (below) that incorporate compound 60 from Jansen as the targeting moiety in a radiotracer having linker and radiolabeling moiety components described in US-121 would meet all of the requirements of the '201 Patent claims.



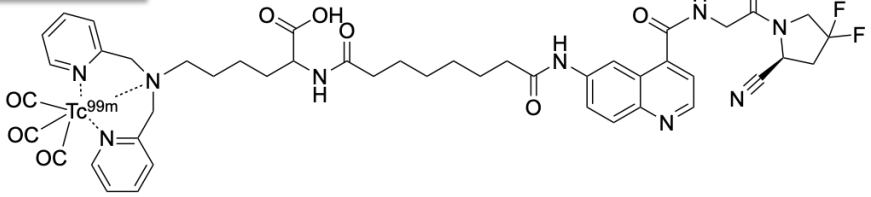
Chemical Formula: $C_{54}H_{68}F_2{}^{68}GaN_{11}O_{13}$
Molecular Weight: 1185.13

Compound B2



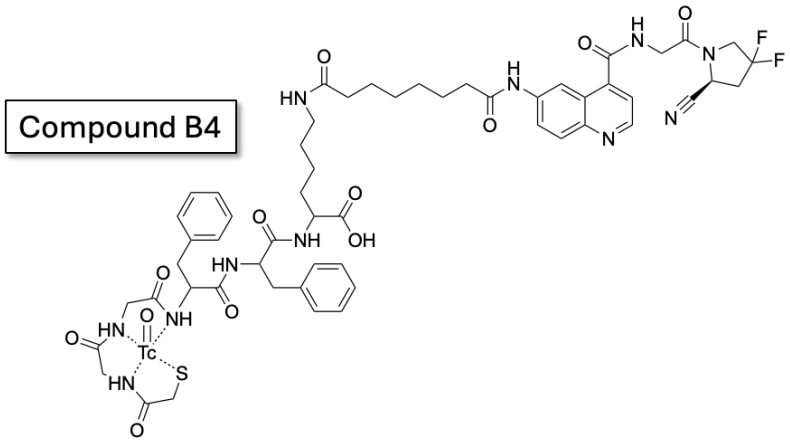
Chemical Formula: $C_{81}H_{97}F_2{}^{68}GaN_{14}O_{13}S$
Molecular Weight: 1612.74

Compound B3



Chemical Formula: $C_{46}H_{49}F_2N_9O_9{}^{99}Tc$
Molecular Weight: 1008.86

Compound B4



Chemical Formula: $C_{55}H_{64}F_2N_{11}O_{12}STc$
Molecular Weight: 1238.24

213. As I explained in (¶¶ 166-168) above, compound 60 of Jansen is a structure that falls within the definition of the "A" moiety of claims 1 and 3.

214. When the chelators I discuss in § IV.A.3 and those described in US-121 are radiolabeled with either ^{68}Ga and ^{99m}Tc, they meet the broad definition of "B" units of the claims, as they are "radiolabeled functional groups suitable for … positron-emission tomography (PET) imaging" or for "single-photon emission computed tomography (SPECT) imaging." These include the ^{68}Ga-DOTA complex of Compound B1, the ^{68}Ga-NOTA complex of Compound B2, the ^{99m}Tc-DPA complex of Compound B3, and the ^{99m}Tc-DPA complex of Compound B4. Each of these compounds thus contains a "radiolabeled functional group" that meets the "B" unit requirement of claims 1-3.

215. As I explained in (§§ IV.A.4, III.H) above, the linkers used in Compounds B1-B4 above, taken directly from US-121, would fall within the broad definition of the "L" unit.

216. The '201 Patent identifies these linkers in US-121 as being suitable for the radiotracers in that patent. As it states:

> Suitable linkers are disclosed in … U.S. Patent Application Publication No. ***US2012/0009121*** Al, for "PSMA-Targeting Compounds and Uses Thereof," published Jan. 12, 2012, to Pomper et al., each of which is incorporated by reference in its entirety.[224]

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[224] EX1001 (US-201), 17:45-53.

217. As I explained in (§§ IV.A.1, III.H) above, a skilled artisan would recognize that a "low molecular weight" compound is one that has a molecular weight an order of magnitude below that of an antibody (<15 kDa) and incorporates a small molecule as the targeting moiety. Each of the Compounds B1, B2, B3, and B4 has a molecular weight below or near 1,500 Da and is based on a small molecule targeting moiety (compound 60) that is linked to a conventional linker and radiolabeling moiety.[225] Each has a molecular weight more than an order of magnitude smaller than that of an antibody. A skilled artisan would consider all of these examples of compounds to be "low molecular weight" compounds as that phrase is used in the '201 Patent claims.

F. A Skilled Artisan Would Have Reasonably Expected the Modified Compounds Based on the US-121 Examples that Incorporate Compound 60 Would be Viable Radiotracers

218. A skilled artisan would have reasonably expected that the examples of radiotracers described in US-121, when modified to incorporate compound 60 of Jansen as the targeting moiety (*i.e.*, compounds B1 to B4) would preserve the favorable selectivity and affinity exhibited by Jansen compound 60. For example,

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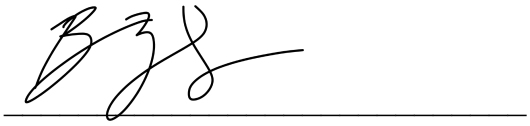
[225] As I noted in ¶ 127, Dr. Pomper stated that a compound with a molecular weight below 1500 Da would be a "low molecular compound." EX1002 ('201 FW), 360 (¶ 17).

a skilled artisan would have expected compounds B1 to B4 to sufficiently separate the FAP-targeting moiety (compound 60) from the radiolabeling moiety to avoid interactions between the two moieties that could affect the selectivity and affinity of compound 60 for FAP. A skilled artisan thus would have expected compounds B1 to B4 to be capable of being used successfully as radiotracers. Also, as I illustrated above, a skilled artisan would have known how to synthesize each of the example compounds (B1 to B4) using routine synthetic chemistry principles.

I, Brian Zeglis, do hereby declare and state, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, under Section 1001 of Title 18 of the United States Code.



Brian Zeglis, Ph.D.

Executed on: 3/28/25

APPENDIX A

C.V. of Brian Zeglis, Ph.D

Curriculum Vitae

Brian M. Zeglis, Ph.D.

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E-mail: bz102@hunter.cuny.edu
Lab website: www.zeglislab.com
Office phone: 212.896.0443

Office: 413 East 69th Street, Room BB430
New York, New York, 10021

Scientific Positions

Hunter College, City University of New York — New York, NY
- Full Professor, Department of Chemistry (Aug. 2022 – present)
- Associate Professor, Department of Chemistry (Sept. 2019 – Aug. 2022)
- Assistant Professor, Department of Chemistry (Jan. 2015 – Sept. 2019)

Memorial Sloan Kettering Cancer Center (Oct. 2015 – present) — New York, NY
- Assistant Attending Radiochemist (Affiliate), Department of Radiology

Weill Cornell Medical College (Mar. 2015 – present) — New York, NY
- Assistant Professor (Adjunct), Department of Radiology

Memorial Sloan Kettering Cancer Center (Sept. 2009 – Jan. 2015) — New York, NY
- Postdoctoral Research Fellow

California Institute of Technology (2004-2009) — Pasadena, CA
- Ph.D., Chemistry — June 2010

Yale University (2000-2004) — New Haven, CT
- B.S., summa cum laude, Chemistry — May 2004

Awards and Honors

Roger Tsien Award for Excellence in Chemical Biology, World Molecular Imaging Society	October 2022
PSC-CUNY Research Award, City University of New York	June 2019
William Stewart Travel Award	June 2019
President's Award for Excellence in Scholarly and Creative Achievement, Hunter College	June 2017
William Stewart Travel Award, City University of New York	March 2017
PSC-CUNY Research Award, City University of New York	May 2016
Feliks Gross Award, City University of New York	April 2016
Junior Faculty Research Award, City University of New York	Jan. 2016
William Stewart Travel Award, City University of New York	Oct. 2015
Chief Radiology Laboratory Research Fellow, MSKCC	Sept. 2013 – Jan. 2015
CMIIT Young Investigator Award, Society of Nuclear Medicine	June 2014
Alavi Mandell Award, Society of Nuclear Medicine	June 2014
Editor's Choice Award, Society of Nuclear Medicine	June 2014
Berson-Yalow Award, Society of Nuclear Medicine	June 2013
World Molecular Imaging Society Travel Award	Sept. 2012
Finalist, World Molecular Imaging Society Young Investigator Award	Sept. 2012
Society of Radiopharmaceutical Sciences Travel Award	Aug. 2011
Arthur Fleischer Award for Excellence in Chemistry, Yale University	May 2004
Saybrook College Marshall, Yale University	May 2004
Phi Beta Kappa, early induction, Yale University	May 2002

Funding

Active Support

National Institutes of Health R01 Award — September 2023 – August 2028
"ImmunoPET Probes for the Imaging of Lyme Disease" (Contact PI)
Hunter College/CUNY/University of Tennessee Health Sciences Campus

National Institutes of Health R01 Award — July 2023 – June 2028
"Antibodies to Tumor-Derived Neoepitopes as Biomarkers and ImmunoPET agents For the Early Detection of Small Cell Lung Cancer" (MPI)
Fred Hutchinson Cancer Research Center/Hunter College/CUNY

National Institutes of Health R21 Award — Sept. 2023 – Aug. 2025
"Targeting DNA Mismatches for Auger Electron Radiotherapy" (Contact PI)
Hunter College/CUNY/MSKCC

Clinical and Translational Science Center Pilot Award — Sept. 2023 – Aug. 2025
"PET as a Diagnostic Tool for Endometriosis" (Contact PI)
Hunter College/CUNY/WCMC

National Institutes of Health R01 Award — July 2020 – June 2025
"Novel Transgenic Mouse Models Addressing Outstanding Translational Barriers in Antibody-Based Therapeutics " (MPI)
Rockefeller University/Hunter College/CUNY

Completed Support

National Institutes of Health R01 Award — July 2019 – June 2024
"Novel Reagents for Rapid and Stable Thiol-Based Bioconjugations" (Contact PI)
Hunter College/CUNY

Memorial Sloan Kettering Cancer Center Imaging and Radiation Sciences Award — Sept. 2021 – Aug. 2023
"Ovarian Cancer Theranostics: A MUC16-targeted Antibody for ImmunoPET Imaging and Radioimmunotherapy" (MPI)
Memorial Sloan Kettering Cancer Center/Hunter College

National Institutes of Health U01 Award — Dec. 2018 – Nov. 2023
"Pretargeted Clinical Imaging of CA19.9 in Pancreatic Cancer" (MPI)
Memorial Sloan Kettering Cancer Center/Hunter College/CUNY

National Institutes of Health R21 Award — June 2020 – May 2022
"A PET Radiotracer for the Diagnostic and Theranostic Imaging of Lyme Disease" (Contact PI)
Hunter College/CUNY

National Institutes of Health R01 Award — April 2016 – March 2021
"The Clinical PET Imaging of Metastatic Breast Cancer with Site-Specifically Labeled ^{89}Zr-Trastuzumab" (MPI)
Memorial Sloan Kettering Cancer Center/Hunter College/CUNY

Memorial Sloan Kettering Cancer Center Department of Surgery Award — Sept. 2018 – Aug. 2020
"Intraoperative Imaging of High Grade Serous Ovarian Cancer During Cytoreductive Surgery" (MPI)
Memorial Sloan Kettering Cancer Center/Hunter College/CUNY

Cookies for Kids Cancer Research Project Award — Jan. 2016 – Dec. 2018
"The Development of Preclinical Validation of Site-Specifically Radiolabeled hu3F8 for the PET Imaging and Radioimmunotherapy of Neuroblastoma" (MPI)
Memorial Sloan Kettering Cancer Center

TeamConnor Childhood Cancer Foundation Research Project Award — Jan. 2016 – Dec. 2017
"Pretargeted Radioimmunotherapy of Pediatric Neuroblastoma" (PI)
Hunter College/CUNY

Hunter College Center for Translational and Biological Research Pilot Project Award — Jan. 2016 – Dec. 2017
"Pretargeted PET Imaging of Pancreatic Cancer" (PI)
Hunter College/CUNY

Weill Cornell Medical Center Clinical and Translational Science Center Pilot Award — Oct. 2015 – Sept. 2017
"Discovery of Targeted Teretoxin Imaging Agents" (MPI)
Hunter College/Memorial Sloan Kettering Cancer Center

National Institutes of Health K99/R00 Career Transition Award — July 2014 – June 2018
"Pretargeted Radioimmunotherapy Based on Bioorthogonal Click Chemistry" (PI)
Memorial Sloan Kettering Cancer Center/Hunter College/CUNY

Translational and Integrative Medicine Research Fund Grant — May 2014 – May 2015
"The First-in-Human Clinical Trial of a Pretargeted Methodology for the PET Imaging of Colorectal Cancer" (MPI)
Memorial Sloan Kettering Cancer Center

Clinical and Translational Science Center Seed Funding Grant — Mar. 2014 – May 2014
"Assessing the Pharmacology and Toxicity of the Molecular Components of a Pretargeted Methodology for the PET Imaging of Colorectal Cancer" (MPI)
Memorial Sloan Kettering Cancer Center

MSKCC Imaging and Radiation Sciences Research Award — Sept. 2012 – Sept. 2014
"PET Imaging of Highly Reactive Oxygen Species" (PI)
Memorial Sloan Kettering Cancer Center

Department of Defense PCRP Hypothesis Development Award — Sept. 2012 – Sept. 2013
"Imaging of Oxidative Stress in Prostate Cancer" (PI)
Memorial Sloan Kettering Cancer Center

NIH F32 Postdoctoral National Research Service Award — Sept. 2009 – Sept. 2012
"PET Imaging of Topoisomerase Expression in Breast Cancer" (PI)
Memorial Sloan Kettering Cancer Center

National Science Foundation GRFP Pre-Doctoral Fellowship — Sept. 2004 – Sept. 2007
California Institute of Technology

Clinical Trials

NCT03109977 (Role: Investigator) Memorial Sloan Kettering Cancer Center
"Imaging With a New Agent That Finds a Cancer Protein Called HER2"

NCT02286843 (Role: Investigator) Memorial Sloan Kettering Cancer Center
"^{89}Zr-DFO-Pertuzumab for the ImmunoPET Imaging of Patients with HER2-Positive Metastatic Breast Cancer"

NCT04692831 (Role: Investigator) Memorial Sloan Kettering Cancer Center
"HER2-Targeted ImmunoPET Imaging with a Site-Specifically Labeled Radioimmunoconjugate"

NCT05737615 (Role: Investigator) Memorial Sloan Kettering Cancer Center
"Pretargeted PET Imaging in Patients with CA19-9-Expressing Pancreatic, Gastric, and Bladder Adenocarcinoma"

Publications

Citations = 8,207; h-index = 51; i10-index = 89; *co-corresponding author

1. Rodriguez, C., Sarrett, S. M., Sebastiano, J., Delaney, S., McGlone, S. A., Hosny, M. M., Thau, S., Bournazos, S., Zeglis, B. M. "Exploring the Interplay between Radioimmunoconjugates and Fcγ Receptors in Genetically Engineered Mouse Models of Cancer" *ACS Pharmacology and Translational Science* 7(11), 3452 (2024)

2. Delaney, S., Keinänen, O., Lam, D., Wolfe, A. L., Hamakubo, T., Zeglis, B. M. "Cadherin-17 as a Target for the ImmunoPET of Adenocarcinoma" *European Journal of Nuclear Medicine and Molecular Imaging* 51(9), 2547 (2024)

3. Sebastiano, J., Rodriguez, C., Samuels, Z. V., Pepin, K., Zeglis, B. M. "Molecular Imaging and Gynecology: Beyond Cancer" *Journal of Nuclear Medicine* 65(7), 998 (2024)

4. Sebastiano, J., Samuels, Z. V., Kao, W. -S., Zeglis, B. M. "Site-Specific Bioconjugation and Molecular Imaging" *Current Opinion in Chemical Biology* 81, 102471 (2024)

5. Delaney, S., Grimaldi, C., Houghton, J. K., Zeglis, B. M. "MIB Guides: Measuring the Immunoreactivity of Radioimmunoconjugates" *Molecular Imaging and Biology* 26(2), 213 (2024)

6. MacPherson, D. S., Dave, D., Kassem, S., Doganata, S., Zeglis, B. M., Ulijn, R. V. "Tuning Supramolecular Chirality in Iodinated Amphiphilic Peptides Through Tripeptide Linker Editing" *Biomacromolecules* 25(4), 2277 (2024)

7. Sarrett, S. M., Rodriguez, C., Delaney, S., Hosny, M. M., Sebastiano, J., Santos Coquillat, A., Keinänen, O. M., Carter, L. M., Lastwika, K. J., Lampe, P. J., Zeglis, B. M. "Evaluating CD133 as a Radiotheranostics Target in Small Cell Lung Cancer" *Molecular Pharmaceutics* 21(3), 1402 (2024)

8. Scott, A. M., Zeglis, B. M., Lapi, S. E., Scott, P. J. H., Windhorst, A. D., Abdel-Wahab, M., Giammarile, F., Paez, D., Jalilian, A., Knoll, P., Korde, A., Vichare, S., Ayati, N., Lee, S. T., Lyashchenko, S. K., Zhang, J., Urbain, J.-L., Lewis, J. S. "Trends in Nuclear Medicine and the Radiopharmaceutical Sciences in Oncology: Workforce Challenges and Training in the Age of Theranostics" *The Lancet Oncology* 25(6), e250 (2024).

9. Lapi, S. E., Scott, P. J. H., Scott, A. M., Windhorst, A. D., Zeglis, B. M., Abdel-Wahab, M., Baum, R. P., Buatti, J. M., Giammarile, F., Kiess, A. P., Jalilian, A., Knoll, P., Korde, A., Kunikowska, J., Lee, S. T., Paez, D., Urbain, J.-L., Zhang, J., Lewis, J. S. "Recent Advances and Impending Challenges for the Radiopharmaceutical Sciences in Oncology" *The Lancet Oncology* 25(6), e236 (2024).

10. Yeh, R., O'Donoghue, J. A., Jayaprakasam, V. S., Mauguen, A., Min, R., Park, S., Brockway, J. P.,

Bromberg, J. F., Zhi, W. I., Robson, M. E., Sanford, R., Modi, S., Agnew, B. J., Lyashchenko, S. K., Lewis, J. S., Ulaner, G. A., Zeglis, B. M. "First-in-Human Evaluation of Site-Specifically Labeled ^{89}Zr-Pertuzumab in Patients with HER2-Positive Breast Cancer" *Journal of Nuclear Medicine* 65(3), 386 (2024)

11. Bauer, D., Cornejo, M. A., Hoang, T. T., Lewis, J. S., Zeglis, B. M. "Click Chemistry and Radiochemistry: An Update" *Bioconjugate Chemistry* 34(11), 1925 (2023)

12. Delaney, S., Rodriguez, C., Sarrett, S. M., Dayts, E. J., Zeglis, B. M., Keinänen, O. "Unraveling the In Vivo Fate of Inhaled Micro- and Nanoplastics with PET Imaging" *Science of the Total Environment* 904, 166320 (2023)

13. Delaney, S., Sebastiano, J., Zeglis, B. M., Keinänen, O. "Molecular Imaging, Radiochemistry, and Environmental Pollutants" *Journal of Nuclear Medicine* 64, 1179 (2023)

14. Rodriguez, C., Delaney, S., Sebastiano, J., Sarrett, S. M., Cornejo, M. A., Thau, S., Hosny, M. M., Zeglis, B. M. Zeglis, B. M. "Site-Selective Radiolabeling Using Mushroom Tyrosinase and the Strain-Promoted Oxidation-Controlled 1,2-Quinone Cycloaddition" *RSC Advances* 13, 17705 (2023)

15. Keinanen, O., Sarrett, S. M., Delaney, S., Rodriguez, C., Dayts, E. J., Capone, E., Sauniere, F., Ippoliti, R., Sala, G., Iacobelli, S., Zeglis, B. M. "Visualizing Galectin-3 Binding Protein Expression with ImmunoPET" *Molecular Pharmaceutics* 20(6), 3241 (2023)

16. Delaney, S., Nagy, A., Karlström, A. E.*, Zeglis, B. M.* "Site-Specific Photoaffinity Bioconjugation for the Synthesis of ^{89}Zr-Labeled Radioimmunoconjugates" *Molecular Imaging and Biology* 25, 1104 (2023)

17. Bauer, D., Sarrett, S. M., Lewis, J. S., Zeglis, B. M. "Click Chemistry: A Transformative Technology in Nuclear Medicine" *Nature Protocols* 18(6), 1659 (2023)

18. MacPherson, D., Hwang, D., Sarrett, S. M., Keinanen, O., Rodriguez, C., Rader, CC., Zeglis, B. M. "Leveraging a Dual Variable Domain Antibody to Create a Site-Specifically Modified Radioimmunoconjugate." *Molecular Pharmaceutics* 20(1), 775 (2023)

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20. Feng, Y., Sarrett, S. M., Meshaw, R. L., Vaidyanathan, G., Cornejo, M. A., Zeglis, B. M., Zalutsky, M. R. "Site-Specific Radiohalogenation of a HER2-Targeted Single Domain Antibody Fragment Using a Novel Residualizing Prosthetic Agent." *Journal of Medicinal Chemistry* 65(22), 15358 (2022).

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22. Sarrett, S. M., Rodriguez, C., Rymarczyk, G., Hosny, M., Keinänen, O., Delaney, S., Thau, S., Krantz, B. A.*, Zeglis, B. M. "Lysine-Directed Site-Specific Bioconjugation for the Creation of Radioimmunoconjugates" *Bioconjugate Chemistry* 33(9), 1750 (2022).

23. Rodriguez, C., Delaney, S., Sarrett, S. M., Keinänen, O., Zeglis B. M. "Antibody Engineering for Nuclear Imaging and Radioimmunotherapy" *Journal of Nuclear Medicine* 63, 1316 (2022).

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27. Sharma, S. K., Mack, K. N., Piersigilli, A., Pourat, J., Edwards, K. J., Keinänen, O., Jiao, M. S., Zhao, H., White, B., Brooks, C. L., de Stanchina, E., Madiyalakan, M. R., Hollingsworth, M. A., Radhakrishnan, P., Lewis, J. S., Zeglis, B. M. "ImmunoPET of Ovarian and Pancreatic Cancer with AR9.6, a Novel MUC16-Targeted Therapeutic Antibody" *Clinical Cancer Research.* 28, 948 (2022).

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Zeglis, B. M., Holland, J. P., Lebedev, A. Y., Cantorias, M. V., Lewis, J. S. "Radiopharmaceuticals for Imaging in Oncology with Special Emphasis on Positron-Emitting Agents" in *Nuclear Oncology: Pathophysiology and Clinical Applications.* Strauss W., Mariani G., Volterrani, D., and Larson, S. M., eds. Springer: New York, USA, 2012.

Books

Radiopharmaceutical Chemistry, 2nd Edition. Lewis, J. S., Windhorst, A. D., and Zeglis, B. M., Eds. Springer: New York, 2024.

Radiopharmaceutical Therapy. Bodei, L., Lewis, J. S., and Zeglis, B. M., Eds. Springer: New York, 2023.

Radiopharmaceutical Chemistry. Lewis, J. S., Windhorst, A. D., and Zeglis, B. M., Eds. Springer: New York, 2019.

Patents

Barton, J. K., Zeglis, B. M., Lau, I. H., Hart, J. R., and Lim, M. H. "Compounds and Methods for Nucleic Acid Mismatch Detection." U. S. Patent #7,786,298 (Issued August 31, 2010)

Zeglis, B. M., Adumeau, P., and Davydova, M. "Reagent for Sile-Selective Bioconjugation of Proteins or Antibodies." U. S. Patent #11,000,604 (Issued May 11th, 2021)

Zeglis, B. M., Lewis, J. S. Reiner, T., Houghton, J. H., Meyer, J-. P., and Brand, C. "Radioligands for Pretargeted PET Imaging and Methods of their Therapeutic Use" U. S. Patent #11,135,320 (Issued October 5th, 2021)

Entrepreneurship

Co-founder, Sharp RTx., Inc. (2021-2023)

Teaching

Introduction to Radiochemistry – Spring 2016, Spring 2017
Inorganic Chemistry – Fall 2016, Fall 2017, Fall 2018, Fall 2019, Fall 2020, Fall 2021, Fall 2022, Fall 2023
Inorganic Chemistry Laboratory – Spring 2019, Spring 2022, Spring 2024

Mentoring

Current Students and Fellows:

Dr. Mark Kao (Postdoctoral Fellow)
Mr. Mike Cornejo (Graduate Student; anticipated graduation – Winter 2026)
Ms. Joni Sebastiano (Graduate Student; anticipated graduation – Winter 2026)
Mr. Zach Samuels (Graduate Student; anticipated graduation – Winter 2027)
Ms. Camilla Grimaldi (Graduate Student; anticipated graduation – Winter 2027)
Dr. Mayuresh Mane (Graduate Student; anticipated graduation – Winter 2028)
Ms. Gina Dehlavi (Graduate Student; anticipated graduation – Winter 2028)
Mx. Ava Stoddard (Graduate Student; anticipated graduation – Winter 2028)

Former Postdoctoral Fellows

Dr. Outi Keinänen (2018-2023; K99/R00; Asst. Prof. at the University of Alabama at Birmingham)
Dr. Aaron Nash (2020-2022; Scientific Advisor; Amster Rothstein & Ebenstein, LLP.)
Dr. Sai Kiran Sharma (2015-2019, Lead *In Vivo* Imaging Scientist at Regeneron Pharmaceuticals, Inc.)
Dr. Pierre Adumeau (2015-2018; Study Director at Oncodesign, Inc.)
Dr. Delphine Vivier (2016-2018; Research Fellow at the University of Burgundy, France)

Former Graduate Students:

Dr. Cindy Rodriguez (2019-2024, Postdoctoral Fellow, Laboratory of Jason Lewis, MSKCC)
Dr. Samantha Delaney (2019-2022; Postdoctoral Fellow, Laboratory of Matthias Herth, Univ. of Copenhagen)
Dr. Samantha Sarrett (2018-2023, Staff Scientist at Novartis, Inc.)
Dr. Douglas McPherson (2018-2022; Equity Research Associate; H. C. Wainwright & Co.)
Dr. Guillaume Dewaele Le Roi (2018-2022; Staff Scientist at Evergreen Theragnostics, Inc.)
Dr. Stephen Jannetti (2015-2019; Principal Scientist at Ionetix, Inc.)
Dr. Kimberly Fung (2015-2019; Medical Director at IMPRINT Science
Dr. Rosemery Cook (2015-2018, Director of Communications, World Molecular Imaging Society)s
Dr. Brendon Cook (2015-2018; Senior Scientist at Biogen, Inc.)

Service to the University

Member (2021-Present), Hunter College Senate
Member (2019-Present), Hunter Chemistry Department Personnel and Budget Committee
Member (2021-2023), Hunter College Research Strategic Planning Committee
Committee Member (2020), Committee on Developing a Framework for the Undergraduate Honors Thesis
Committee Member (2017), Search Committee for Radiochemistry Faculty Member (Prof. Jennifer Shusterman)
Co-Chair (2017), Hunter College Symposium on Radiometals
Committee Member (2016), Search Committee for Radiochemistry Research Associate (Dr. Ali Younes)

Service to the Scientific Community

NIH Proposal Review

Standing Member, National Institutes of Health, Imaging Probes and Contrast Agents (IPCA) Study Section (2020-2021; 2022-present)
Ad Hoc Member, National Institutes of Health, Center for Molecular Imaging Probe Development (CMIP) Study Section (2017-2020)
Ad Hoc Member, National Institutes of Health, Imaging Guided Interventions and Surgery (IGIS) Study Section (2017-2019)

Editorial Work

Associate Editor (2020-2023), *Molecular Imaging and Biology*
Deputy Editor-in-Chief (2023-present), *Molecular Imaging and Biology*
Editorial Board (2016-present), *Journal of Nuclear Medicine*

Other Service

Secretary and Treasurer (2024 – present), Center for Molecular Imaging Innovation and Translation, Society of Nuclear Medicine and Molecular Imaging
Founding Member (2016) and Chair (2017-2019), Early-Stage Investigators in Molecular Imaging Sciences (ESPMIS) Interest Group, *World Molecular Imaging Society*
Reviewer for several journals, including *Cancer Research*, *Clinical Cancer Research, Cancer Discovery, Proceedings of the National Academy of Sciences, Chemical Communications, Journal of the American Chemical Society, Journal of Nuclear Medicine, and European Journal of Nuclear Medicine*

Invited Lectures

1. *European Association of Nuclear Medicine.* "Pretargeting: A Clinical Perspective on Strengths and Challenges" Hamburg, Germany: October 22nd, 2024.

2. *European Society for Molecular Imaging and Technology.* Educational Presentation on "The Promise and Pitfalls of In Vivo Pretargeting" Porto, Portugal: September 1st, 2024.

3. *Gordon Research Conference on Radiotheranostics. "In Vivo Pretargeting for the Radiopharmaceutical Therapy of Cancer"* Newry, Maine: July 2nd, 2024.

4. *International Atomic Energy Agency.* "Exploiting Innovative Animal Models for the Exploration of Radiopharmaceutical Delivery System" Vienna, Austria: May 20th, 2024.

5. *Olivet Nazarene University. "*The Inverse Electron-Demand Diels-Alder Reaction in Radiochemistry" Boubonnais, Illinois: April 30th, 2024.

6. *University of Alabama Birmingham.* "Leveraging Bioorthogonal Chemistry to Improve Radiopharmaceuticals" Birmingham, Alabama: April 10th, 2024.

7. *City College of New York.* "Harnessing Bioorthogonal Chemistry to Improve Nuclear Medicine*"* New York, New York: March 27th, 2024.

8. *Annual Meeting of the American Chemical Society.* "Harnessing Copper-Free Click Chemistry for Site-Specific Bioconjugation" Indianapolis, Indiana: March 28th, 2023.

9. *University of Virginia.* "Harnessing Selective Chemistries to Improve Radiopharmaceuticals" Invited Speaker. Charlottesville, Virginia: December 7th, 2022.

10. *Oak Ridge National Laboratory Meeting on Evolving Targeted Therapies for Cancer.* "Leveraging Bioorthogonal Chemistry to Improve Radiopharmaceuticals" Oak Ridge, Tennessee: November 2nd, 2022.

11. *World Molecular Imaging Congress.* "Harnessing Selective Chemistries to Improve Radiopharmaceuticals" Roger Tsien Award Lecture. Miami, Florida: September 30th, 2022 .

12. *North Carolina State University.* "Harnessing Bioorthogonal Chemistry for Nuclear Imaging and Endoradiotherapy" Virtual Presentation: February 28th, 2022.

13. *Newcastle University.* "Harnessing Bioorthogonal Chemistry for Nuclear Imaging and Endoradiotherapy" Virtual Presentation: December 14th, 2021.

14. *Wayne State University and Karmanos Cancer Center.* "Harnessing Bioorthogonal Chemistry for Nuclear Imaging and Endoradiotherapy" Detroit, Michigan: December 7th, 2021.

15. *Annual Meeting of the European Society of Nuclear Medicine.* "Antibodies as Radiopharmaceutical Vectors: Do the Benefits Outweigh the Costs" Virtual Meeting: October 5th, 2021.

16. *Department of Energy Nuclear Chemistry Summer School.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Virtual Meeting: July 23rd, 2021.

17. *Weill Cornell Medical College.* "Harnessing Click Chemistry for Pretargeted PET Imaging and Radioimmunotherapy" New York, New York: May 21st, 2021.

18. *Annual Meeting of the International Society for Radiopharmaceutical Sciences.* "Robin Hood and the Merry Pre-Targeters: On the Utility and Promise (or Lack of) Pretargeting Methods" Virtual Meeting: May 19th, 2021.

19. *Annual Meeting of the Australia and New Zealand Society of Nuclear Medicine.* "Harnessing the Heavy Chain Glycans for the Creation of Site-Specifically Modified Radioimmunoconjugates" Virtual Meeting: August 6th, 2020.

20. *Annual Meeting of the Australia and New Zealand Society of Nuclear Medicine.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Virtual Meeting: July 23rd, 2020.

21. *Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging.* "Highlights Lecture for Radiopharmacy and Radiopharmaceutical Chemistry" Virtual Meeting: June 27th, 2020.

22. *Annual Meeting of the American Association of Physicists in Medicine.* "ImmunoPET: Leveraging Antibodies for Diagnostic and Theranostic Nuclear Imaging" San Antonio, Texas: July 16th, 2019.

23. *Northeast Regional Meeting of the American Chemical Society.* "Pretargeted Radioimmunotherapy with Metallic Radionuclides" Saratoga Springs, New York: June 24th, 2019.

24. *Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging.* "Highlights Lecture for Radiopharmacy and Radiopharmaceutical Chemistry" Anaheim, California: June 23rd, 2019.

25. *Iona College Nanoscience Symposium.* "The Emergence of ^{89}Zr-ImmunoPET: Harnessing Antibodies for Nuclear Imaging" New Rochelle, New York: April 9th, 2019.

26. *University of Copenhagen.* "Pretargeted Radioimmunotherapy Based on Bioorthogonal Click Chemistry" Copenhagen, Denmark: December 6th, 2018.

27. *Stony Brook University.* "In Vivo Pretargeting: Performing Radiochemistry Within the Body" Stony Brook, New York: September 18th, 2018.

28. *Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging.* "Highlights Lecture for Radiopharmacy and Radiopharmaceutical Chemistry" Philadelphia, Pennsylvania: June 15th, 2018.

29. *Vrije Universiteit Brussel.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Workshop in Immuno-Imaging and Molecular Therapy. Brussels, Belgium: April 27th, 2018.

30. *VU University Medical Center Amsterdam.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Amsterdam, the Netherlands: April 26th, 2018.

31. *University of California, Los Angeles.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Crump

Institute of Molecular Imaging. Los Angeles, California: March 26th, 2018.

32. *Annual Meeting of the Radiology Society of North America.* "A Primer in ^{89}Zr-ImmunoPET" Chicago, Illinois: December 1st, 2017.

33. *Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface." Denver, Colorado: June 12th, 2017.

34. *St Jude Children's Research Hospital.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Memphis, Tennessee: April 28th, 2017.

35. *St Jude Children's Research Hospital.* "Bioorthogonal Chemistry for Better Radiopharmaceuticals" Memphis, Tennessee: April 27th, 2017.

36. *City of Hope Hospital.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Duarte, California: April 4th, 2017.

37. *California Institute of Technology.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Pasadena, California: April 3rd, 2017.

38. *University of Missouri Columbia.* "In Vivo Pretargeting: Radiosynthesis at the Tumor Surface" Columbia, Missouri: March 10th, 2017.

39. *Annual Meeting of the Radiology Society of North America.* "A Primer in ^{89}Zr-ImmunoPET" Chicago, Illinois: December 2nd, 2016.

40. *European Association of Nuclear Medicine Congress.* "Strategies for the Site-Specific Bioconjugation of Antibodies" Barcelona, Spain: October 14th, 2016.

41. *World Molecular Imaging Congress.* "The Anatomy of a Radioimmunoconjugate" New York, New York: September 7th, 2016.

42. *Annual Symposium of the Memorial Sloan Kettering Cancer Center Imaging and Radiation Sciences Program.* "Harnessing Bioorthogonal Chemistry for Pretargeted Imaging and Therapy" New York, New York: May 31st, 2016.

43. *The University of the West Indies.* "PET Imaging with ^{89}Zr" Kingston, Jamaica: March 21st, 2016.

44. *International Workshop on Molecular Imaging.* "Harnessing Bioorthogonal Chemistry for Pretargeted PET Imaging" San Sebastien, Spain: November 11th, 2015.

45. *European Association of Nuclear Medicine Congress.* "Advances in ^{89}Zr PET Imaging" Hamburg, Germany: October 10th, 2015.

46. *International Symposium on Technetium and Radiometals in Chemistry and Medicine (TERACHEM).* "The Site-Specific Radiometallation of Antibodies on the Heavy Chain Glycans" Bressanone, Italy: September 11th, 2014.

47. *International Conference and Expo on Isotopes. "The Site-Specific Labeling of Antibodies on the Heavy Chain Glycans"* Chicago, Illinois: August 28th, 2014.

48. *Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging.* "Labeling Peptides and Proteins Using Click Chemistry" Vancouver, Canada: June 8th, 2013.

49. *Revolutionaries for Global Health Summit.* "^{89}Zr-ImmunoPET: Emergent Targets and Clinical Translation." Boston, Massachusetts: May 8th, 2013.

50. *Annual Meeting of the Society of Nuclear Medicine.* "Radiometal Chelates and Click Chemistry: The Development of Modular Systems" Miami, Florida: June 9th, 2012.

51. *Congress of the World Federation of Nuclear Medicine and Biology.* "New Radiopharmaceuticals: Availability, Development, and Challenges" Cape Town, South Africa: September 20th, 2010.