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The Development and Validation of Radiopharmaceuticals Targeting Bacterial Infection

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The International Atomic Energy Agency organized a technical meeting at its headquarters in Vienna, Austria, in 2022 that included 17 experts representing 12 countries, whose research spanned the development and use of radiolabeled agents for imaging infection. The meeting focused largely on bacterial pathogens. The group discussed and evaluated the advantages and disadvantages of several radiopharmaceuticals, as well as the science driving various imaging approaches. The main objective was to understand why few infection-targeted radiotracers are used in clinical practice despite the urgent need to better characterize bacterial infections. This article summarizes the resulting consensus, at least among the included scientists and countries, on the current status of radiopharmaceutical development for infection imaging. Also included are opinions and recommendations regarding current research standards in this area. This and future International Atomic Energy Agency–sponsored collaborations will advance the goal of providing the medical community with innovative, practical tools for the specific image-based diagnosis of infection.

Key Words: infection; antibiotics; radiotracer; molecular imaging; development

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Infections remain a major threat to human health globally (*1*). The coronavirus disease 2019 pandemic has highlighted a pressing need to develop and translate innovative technologies to detect and treat infectious disease. Even before the onset of the pandemic, infections ranked third in mortality but first in morbidity among all human diseases in 2017, primarily affecting younger, healthier populations (*2*). There were an estimated 11 million sepsis-related deaths in 2017, accounting for about 20% of deaths globally, with the highest incidence reported in developing countries (*3*). By 2050, antimicrobial drug-resistant infections are expected to become the leading cause of death globally and surpass those due to cancer (*4*). The potential cost of drug-resistant infections has been estimated to be as high as $100 trillion worldwide (*5*). We have also observed a dramatic rise in hospital-acquired (nosocomial) infections affecting at-risk patients during the pandemic. Enterobacterales pathogens, especially *K. pneumoniae,* and fungi including *Aspergillus* spp. are an important cause of secondary pneumonias in hospitalized patients with coronavirus disease 2019 (*6*).

Current diagnostic approaches to detecting bacterial infections, such as microscopy, microbiology, and molecular techniques (nucleic acid amplification and mass spectrometry), require clinical samples (blood, urine, stool, or cerebrospinal fluid) for culturing and sensitivity testing and infection-relevant assays. However, it is increasingly recognized that many different infectious foci with distinct bacterial burdens, antimicrobial exposures, and local biology can coexist in the same host (*7–9*). Clinical samples may not accurately represent the local biology at infectious sites and thus are either not sensitive to or not representative of the bacterial infection (*10,11*). Surgical resection or biopsy is often the last

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resort for obtaining infected tissues, and because of the associated morbidity, these techniques are generally limited to the most accessible lesions identified at a single time point. Additionally, sampling methods fail to capture the heterogeneity of multiple lesions existing simultaneously in the same patient, as well as the temporal changes occurring over the course of the infection and its treatment.

Available imaging tools used in the clinic for detection of bacterial infections include radiography, ultrasonography, CT, and MRI, but these imaging tools are based on anatomic changes during disease, which are delayed compared with the biochemical events occurring within the affected tissues. Structural abnormalities are also nonspecific and reflect a combination of both the infectious agents and the host inflammatory response (*12*). Molecular hybrid imaging platforms have proven to efficiently localize pathology and assist in the clinical management of several diseases (*13,14*). These technologies, such as SPECT or PET, can measure molecular pathways in situ and are often used in combination with anatomic imaging (PET/CT, PET/MRI, or SPECT/CT). The use of radiolabeled leukocytes (white blood cells) is considered the gold standard technique for prosthetic (*15*), vascular graft (*16*), and diabetic foot (*17*) infections but requires skill and equipment for sterile blood manipulation. Radiolabeled antibodies against granulocyte antigens can induce human antimouse antibody production in approximately 4% of patients (*18*), limiting their use. The glucose analog [^{18}F]FDG has also been used to image infection but lacks specificity for pathogens (*19*).

Despite these advanced tools, there is no universally accepted approach to the specific detection of bacterial infections. Therefore, there is an urgent, unmet need for the development of radiopharmaceuticals that can demonstrate the presence of living pathogens in vivo. However, because of bacterial diversity and the frequency of polymicrobial infections, developmental efforts have focused on both radiopharmaceuticals for panbacterial imaging and radiopharmaceuticals for type- or species-specific imaging. For potential clinical applications, there are advantages and disadvantages to both concepts. Given the breadth of research interests, in general all approaches have been hindered by a relative lack of funding (compared with oncology, for example), lack of uniformly reported data for imaging agents, misconceptions regarding radiation risks, and hurdles in the clinical translation and dissemination of promising radiopharmaceuticals (*12*).

In March 2022, the International Atomic Energy Agency (IAEA) organized a technical meeting titled "The Status of Radiolabeled Molecules for Infection and Inflammation Imaging" in Vienna, Austria, to evaluate and address these challenges. This summary should be used as a road map for advancing research in this field, understanding the potential clinical use of radiopharmaceuticals and their role in clinical decision-making, and most importantly motivating funding agencies and industry to support and develop pathogen-specific imaging technologies. Although the focus of this meeting was bacterial infection, the conclusions rendered may be expanded and tailored to nonbacterial pathogens whose detection via nuclear imaging is increasingly reported in the peer-reviewed literature.

CLINICAL MOTIVATIONS

With the advance of medical imaging technologies, there has been a sustained interest in developing new tools to detect and monitor bacterial infections noninvasively—particularly in nuclear medicine. Ideally, nuclear imaging probes should have high sensitivity and specificity for a wide range of pathogens, with enough tissue penetration to reach infected areas despite poor vascular supply while providing quantitative signals proportionate to the bacterial burden. They should also be chemically stable in blood and tissues; safe, with acceptable radiation exposure; and manufacturable at a reasonable expense (*20,21*). However, this magic bullet is not feasible for all imaging applications since disease location, type of pathogen, presence of comorbidities, chronicity of infection, and therapeutic interaction may influence the diagnostic accuracy of a given technique.

Therefore, analogous to the development of imaging agents in oncology, pathogen-specific imaging will greatly benefit from having multiple complementary agents with applicability to different clinical conditions. Several radiopharmaceuticals should be developed targeting variable pathways, allowing differentiation between infection and sterile inflammation and characterization of individual or classes of pathogens (e.g., gram-positive vs. gram-negative bacteria). A collaborative multidisciplinary environment with expert perspectives is essential, as is sharing information regarding how to best conduct imaging studies, interpret data, and include appropriate controls. A central agency (e.g., the IAEA) with a global focus represents an ideal platform to share this information and conduct multicenter comparisons. To allow replication of experiments at different sites, transparency in experimental methods for both preclinical and clinical studies is required, as well as willingness by the researchers to distribute data and standardize reporting.

The bacterial imaging field, both for SPECT and for PET, has grown significantly from 2000 to 2019, followed by a reduction in publications during the coronavirus disease 2019 pandemic, based on articles found via PubMed from 2001 to 2022 by searching "bacteria AND imaging AND scintigraphy/PET AND [year]." This refocusing of research effort may reflect a new focus on coronavirus disease 2019–related diagnostics, a pandemic-related loss of resources needed to conduct this type of research, or most likely both. Therefore, it is essential to reinvigorate this field, particularly with our new knowledge of infections—their transmission, morbidity, and mortality.

With respect to pathogen-specific imaging, it is important to learn from our previous mistakes. One of the first agents to be clinically translated for infection imaging was [^{99m}Tc]ciprofloxacin, commercially known as Infecton (Draxis). Although it was rapidly evaluated in hundreds of infected patients with promising results, these initial hopes were dashed when subsequent clinical studies (*22–24*) showed poor specificity of [^{99m}Tc]ciprofloxacin for bacterial infections. The panel at the IAEA agreed that from the beginning [^{99m}Tc]ciprofloxacin was a poorly chosen and validated tracer for numerous reasons, including its limited affinity for bacteria (reflected by fast efflux rates from affected tissues) and binding to both bacteria and mammalian cells (*25,26*). This was a valuable lesson in the need to thoroughly characterize bacteria-specific imaging agents to confirm their mechanism of action and specificity before investing extensive resources in their clinical translation. In this case, in vitro tests were not satisfactory (*27–29*) and heterogeneity of clinical studies limited their credibility. These studies had variable imaging indications, divergent gold standards, and poor controls and proved challenging to interpret (*23,30–33*).

To attract industry investment in new imaging technologies, attention should be paid to their potential profitability at all stages of development. For example, the preservation of intellectual property via patents is essential and often overlooked. Indeed,

patented radiotracers can be more easily acquired and produced by pharmaceutical companies, reducing the risk of competing technologies. A lack of intellectual protection will likely discourage industry investment, even if the science itself is promising.

DEVELOPMENT OF PATHOGEN-SPECIFIC IMAGING METHODS

The discussion at the IAEA focused on the development of infection-targeted radiopharmaceuticals, including the basic in vitro and in vivo studies needed to validate their clinical relevance.

Infrastructure Requirements

An important consideration in developing any imaging agents is the required infrastructure, with the safe handling of infectious agents representing a special challenge. For any radiopharmaceutical, relevant technologies include a cyclotron or radionuclide generator, shielded fume hoods (i.e., hot cells), a radioactivity-counting instrument (e.g., a γ-counter), high-performance liquid chromatography, and preclinical scanning equipment (PET or SPECT). For clinical translation, quality control evaluation including identity testing, pyrogen evaluation, radiochemical evaluation (yield, purity), and chemical stability is required. Staff with radiochemistry and regulatory expertise imply potentially high costs, which can be a limitation for the development of new agents. Considerations for handling pathogens and biosafety training are critical for conducting these studies. However, groups focused on radiochemistry are not familiar with regulations surrounding pathogens, as they have traditionally focused on cancer and neurologic disorders. Additional infrastructure challenges are related to infections with drug-resistant pathogens requiring special regimes for patient care and cleaning of hospital spaces (waste management) and hospital beds (isolation areas).

Conception and Planning—Identifying the Target

Infectious diseases are widely heterogeneous illnesses associated with microorganisms that cause disease in humans. Although there is considerable overlap in the pathogenic mechanisms of these microorganisms and the host response to them, the intrinsic characteristics of these pathogens are highly variable. Bacteria, viruses, fungi, and parasites are genetically, biochemically, and metabolically different. Additionally, reference laboratory strains can be significantly different from pathogenic strains. For example, reference laboratory *E. coli* strains—the uropathogenic CFT073, enterohemorrhagic EDL933, and laboratory strain MG1655—all display a mosaic genome structure that can compose up to 40% of their genes (*34*). Therefore, it is fundamental to identify the target pathogen and its clinical presentation when planning to develop pathogen-specific imaging agents. The specific characteristics of the agent will vary depending on the target, and the use of clinical strains during the initial studies may be crucial.

Developing new infection-targeted agents depends both on the clinical need and on understanding of the mechanism the technology uses to generate image contrast. Bacteria (prokaryotes) are evolutionary and phylogenetically distinct from eukaryotic cells. These basic differences provide opportunities to leverage fundamental biochemical differences between bacteria and mammalian cells—that is, energetic pathways, nucleic acid use, and cell surface components for the discovery of novel molecules that could be developed into pathogen-specific agents. Although initial efforts to develop pathogen-specific radiopharmaceuticals were based on radiolabeled antibiotics, recent approaches have focused on radiopharmaceuticals that are incorporated by the cell wall or are metabolized by microbe-specific pathways. For example, D-methyl-[^{11}C]methionine and other positron-labeled D-amino acids have targeted bacterial peptidoglycan (Fig. 1) (*35–38*), whereas 2-deoxy-2-[^{18}F]fluoro-D-sorbitol detects bacteria via the unique metabolism of sorbitol by Enterobacteriaceae (Fig. 2) (*39,40*). For a more thorough understanding of the target, collaboration with microbiologists and infectious disease physicians is helpful. Generally, attention to the literature and careful screening (including in silico) can identify the targets most relevant to probe design (*41*). Using an artificial intelligence approach in the selection of potentially pathogen-specific radiopharmaceuticals can make radiopharmaceutical agent development more efficient (*42*).

Compound Screening and Radiochemistry

Once the bacterial target has been identified, the next step is to obtain lead molecules, which may require a conventional compound screen, structure-based design, modification of molecular probes developed for other imaging techniques, or radiosynthesis of metabolite analogs (*43,44*). Using an unbiased screening approach is essential to the discovery of candidates for pathogen-specific imaging. Screening of candidate compounds should be performed in whole bacterial cell cultures since working with an isolated target ignores critical determinants of clinical performance, such as cell wall penetration.

Multiple comprehensive reviews have been published on the radiochemistry of pathogen-specific imaging radiopharmaceuticals (*43,45,46*). However, many publications in this field lack the minimal requirements to allow validation and reproducibility of the described radiochemistry methodology. When a new agent is described, information regarding its radiochemical purity, radiochemical yield, molar activity, stability, and metabolism is necessary to allow other researchers to evaluate the presented approach and potentially reproduce it. Molar activity is particularly important to report and evaluate to address the presence of competing cold materials in a radiopharmaceutical sample. Numerous other considerations are relevant to the chemical specifics of the probe.

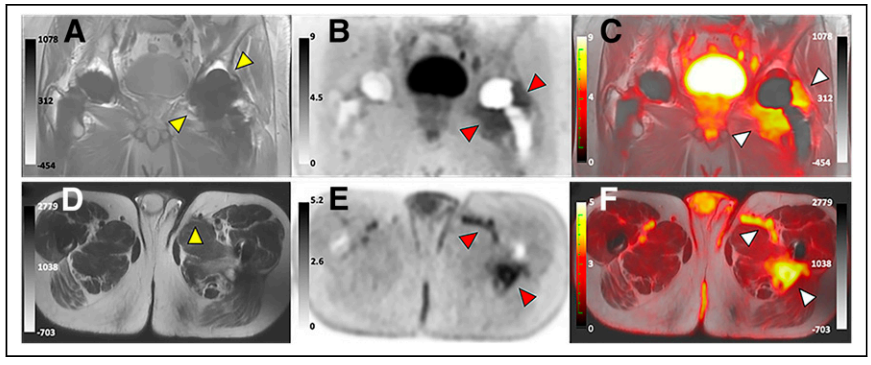

FIGURE 1. D-methyl-[^{11}C]methionine PET/MR images of 61-y-old man with bilateral hip prostheses and confirmed *C. acnes* infection of left hip. Left bar represents SUV color scale, and right bar represents MRI color scale. (A, B, and C) Coronal MR, PET, and PET/MR images, respectively. Arrows indicate infected joint in A and area of radiotracer uptake surrounding joint in B and C. (D–F) Axial MR, PET, and PET/MR images. Arrows depict sinus tract communicating with skin in D and regions of radiotracer uptake in E and F. (Adapted from (*35*).)

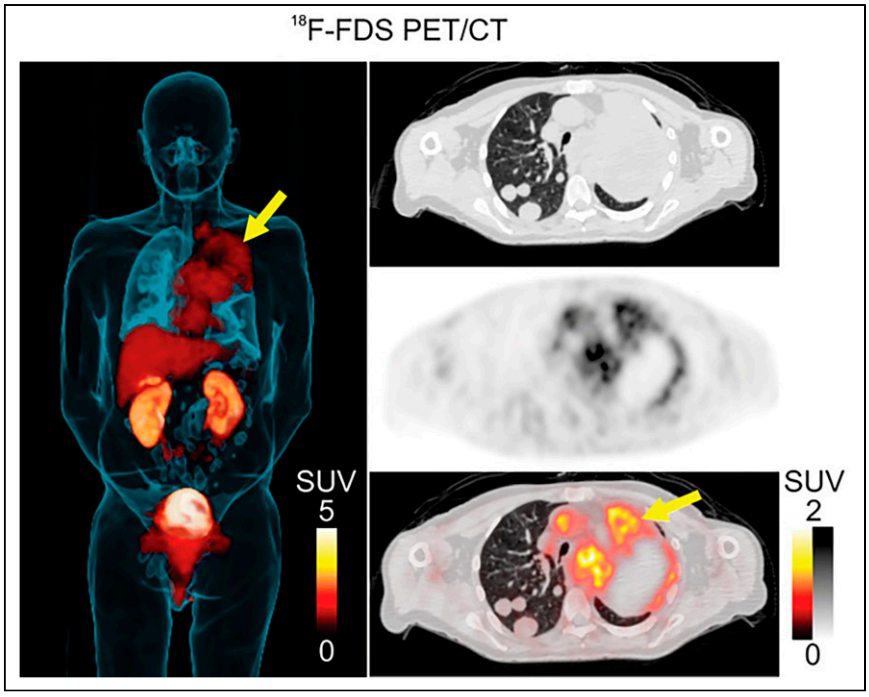

FIGURE 2. [^{18}F]FDS PET study of 67-y-old man with squamous cell carcinoma of lung and *K. pneumoniae* pneumonia. On left, 3-dimensional minimum-intensity projection is shown, with arrow indicating [^{18}F]FDS signal in infected tissues. On right, transverse CT, PET, and PET/CT images (from top to bottom) indicate minimal [^{18}F]FDS signal in right-sided cancerous lesions (arrow). (Adapted from (*40*).)

For example, with radiometal modification of antibodies or related protein formats (i.e., single-domain antibodies), stoichiometry should be evaluated and reported. Both retention of activity and stability of conjugated antibodies should be determined. To rule out in vivo transchelation of ^{99m}Tc, a cysteine challenge study may be considered. Basic radiopharmaceutical design criteria are beyond the scope of this discussion, but a summary has been provided in a previous review (*47*).

In Vitro Testing

Evaluating uptake of radiopharmaceuticals by bacteria in vitro is a critical aspect of their validation. Since certain radionuclides both are costly and have short half-lives (e.g., ^{11}C for PET), the in vitro study of radiopharmaceutical analogs can begin with β-emitting nuclei (^{14}C, ^{3}H) and scintillation counting (*41*), stable isotope MR spectroscopy (*48*) (e.g., ^{13}C, ^{1}H, ^{2}H, and ^{19}F), or mass spectroscopy (*49*). The essential validation of a new radiotracer concept is when it has been successfully labeled and incubated with bacterial cultures to detect specific incorporation (*37*). These studies are usually conducted with bacterial cultures in the growth or exponential phase, although of course other assessments are possible. After bacterial washing and detection of retained radioactive signals, a gross assessment of tracer retention can be made by the percentage of tracer retained, that is, the percentage uptake. However, these data should be normalized to bacterial count, which is not obtained via an estimate (e.g., an *E. coli* culture with an optical density of 1 measured at a wavelength of 600 nm represents 8×10^8 organisms) but by serial dilutions and plating to determine the number of colony-forming units to be reported. The most relevant controls include the use of heat-killed organisms and blocking using a nonradiolabeled version of the radiopharmaceutical. These blocking, or competition, studies can be used to explore the effect of molar activity on radiotracer performance. Finally, several pathogenic species, as well as multiple strains of the same species, should be included in these analyses. In addition to clinical isolates, commercially available bacteria should be used to allow reproduction of results by other groups.

Several in vitro studies are infrequently performed or use variable experimental conditions. For example, some investigators perform efflux studies whereby after bacterial radioactivity retention, the cells are washed and the subsequent loss of radioactivity over time is determined (*50*). There is also variability in the medium used, and some components may compete with exogenous radiopharmaceuticals for bacterial incorporation. At this point, there is no standard medium used although investigators should consider appropriate mimicry of the nutrient makeup of the human body.

In Vivo Validation

Once the in vitro characterization of a probe has been completed, subsequent validations in animal models are frequently performed. Regulatory requirements for the development of animal models of infections vary considerably across different countries and institutions. If excessive, these can be an additional burden to researchers (*51*). Animal models and relevant controls are well summarized in the consensus report by Signore et al. (*52*). When choosing an animal model, it is important to thoroughly understand the human infection that is being studied, as well as the strengths and limitations of a given model. The European Association of Nuclear Medicine recently published useful guidelines for choosing the appropriate animal model for preclinical experiments (*53*). In most cases, the models used should recapitulate human pathologies. When tracer sensitivity to different pathogens is being compared, a dual infection model (e.g., a mouse infected with 2 pathogens) (*38*) or separate carefully generated cohorts (e.g., in comparing [^{99m}Tc]hydrazinonicotinamide polymyxin B accumulation in *Pseudomonas aeruginosa* and *Staphylococcus aureus*) may be used (Fig. 3) (*54*). The volume of distribution, metabolism, excretion, vascular leakage, etc., are also key variables that should be considered before choosing a specific model.

Determining and standardizing the readouts used to quantify signals from pathogen-specific agents in animal models are also key to comparing different agents and reproducing the reported findings. For example, for PET imaging of bacterial infections, the agent should be injected at a time point when the infection has been allowed to incubate for a sufficient time (e.g., 8–24 h) to resemble human pathology when inflammatory response peaks and bacteria are in different metabolic states. Determination of the stability of the agent in blood (or tissues if applicable) at the time of imaging should also be reported. Because of replication, the bacterial burden injected at a given site is much lower than found hours later. Therefore, the bacterial burden at the site evaluated should be determined immediately after imaging has been performed.

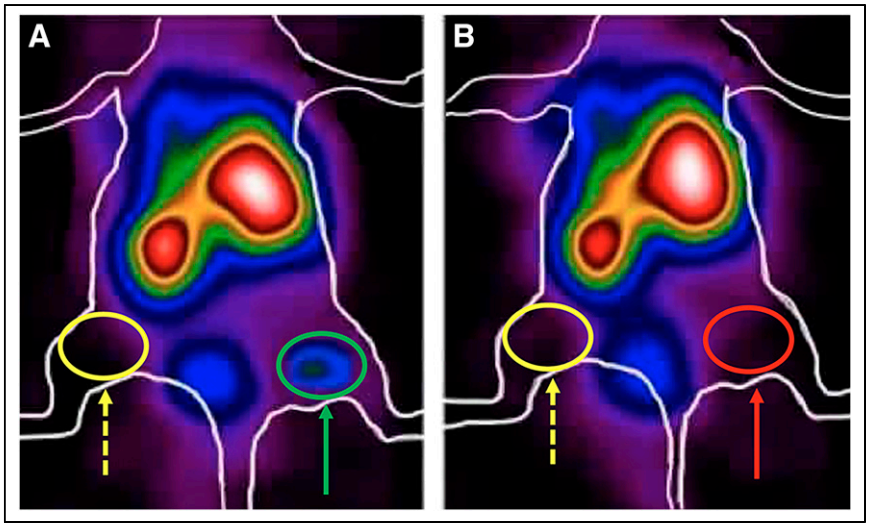

FIGURE 3. Representative planar γ-camera images of 2 mice infected with 10^9 colony-forming units of *P. aeruginosa* (A) (green arrow and circle) and *S. aureus* (B) (red arrow and circle) vs. contralateral thigh, injected with only hydrogel (yellow arrows and circles) as control. Images were acquired 6 h after injection of 3.7 MBq of [^{99m}Tc]-hydrazinonicotinamide polymyxin B. Radiolabeled antibiotic binds only to gram-negative bacteria, thus highlighting presence of *P. aeruginosa* but not of gram-positive *S. aureus*. (Adapted from (*54*).)

The use of animal models beyond rodents in development of imaging research has also been suggested (*53,55*). The rabbit stands out among nonrodent mammals used in research because of its relatively small size, short gestation period (29–31 d), and potential for timed mating and superovulation (*56*). Myriad imaging studies have reported the successful use of rabbit infection models (*57–59*). Given ethical concerns, using nonhuman primates (macaques, baboons, marmosets, and African green monkeys) in biomedical research is usually allowed only in research areas for which no alternative is available (*60,61*); however, research on nonhuman primates regularly necessitates special facilities and expertise. Although expensive, nonhuman primates are invaluable tools to study complex infection pathogenesis (simian HIV or tuberculosis) (*62*) and are suitable for preclinical imaging studies (*63,64*), in particular for tracer biodistribution or radiation dosimetry (*65–67*), translational research on human respiratory infections, and pharmaceutical drug development (*68–70*). Beyond these common animals, the study of species-specific diseases might involve pigs, cats, dogs, cattle, horses, fish, and birds (*71,72*).

Distinguishing infection from inflammation depends on the typical host response via initiation of the innate immune system followed by an adaptive response targeting the pathogen (*19,73*). Frequently, inflammation may persist despite infection control (*74*). Inclusion of appropriate controls is an important determinant when developing specific infectious imaging agents. Whenever possible, contralateral limb or skin site controls, as demonstrated by the tissue cage model (*75*), can be used to test the specificity of the agent and further distinguish between infectious and sterile inflammation. Therefore, a rational preclinical screen should be designed to, first, understand the kinetics of the test molecular imaging agent in sterile (*76–78*) as well as infectious (*39,79*) animal inflammation models and, second, understand target organ function to compare the sensitivity of the imaging agent in animal models of infection and sterile inflammation. The imaging agents should be validated with the capability for dual or hybrid imaging platforms such as SPECT/CT, PET/CT, and PET/MRI (*80*).

An accurate analysis of the preclinical images is fundamental to determine the viability of the candidate agent for pathogen-specific imaging. The most used approach to determining the region (or volume) of interest via images is intrinsically operator-dependent. Therefore, efforts should be made to minimize bias (e.g., using the CT instead of the PET images to determine the region of interest). A frequently used unit to represent imaging results in PET/SPECT is SUV_{mean}, which considers average signals in a region of interest, corrected for the dose-decay–adjusted injected dose and the weight of the animal. Other methods of data quantification are available, and researchers should explain the methodology used for the analysis. Careful dissection and ex vivo analyses of all tissues should be performed via a radiation-detecting instrument (i.e., γ-counter). Accurate identification of infected and noninfected tissues can be used to generate an uptake value, normalized to mass (i.e., percentage injected dose per gram). The tissues can be subsequently homogenized and plated to normalize the data for the number of viable bacteria (colony-forming units); this type of analysis is essential for evaluating the sensitivity of a radiopharmaceutical or comparing the sensitivity of different tracers.

TRANSLATION AND THE FUTURE

The general process of translating new nuclear medicine technologies has been explored in numerous reviews (*81,82*) and in the context of dedicated workshops, such as that organized by the National Institute of Biomedical Imaging and Bioengineering (National Institutes of Health) (*83*). The basic approach involves the approval of both government and institutional regulatory bodies, toxicology studies as required, radiochemical optimization, and first-in-humans studies usually initiated for dosimetry evaluation. For any tracer, a major challenge is securing the funding to accomplish this work, given administrative expenses and the high cost of radiopharmaceutical production. Many researchers at the meeting felt these costs diminished the number of patients who could reasonably be scanned using a new tracer—thus limiting the conclusions obtained. A second challenge is the difficulty of proving the utility of infection-targeted radiopharmaceuticals in rigorous, multicenter studies. Even for researchers who wish to share and collaborate, securing the funding required for this effort is difficult. Finally, infection-targeted tracers face particular barriers to widespread clinical adoption, described below.

There Is Currently Limited Engagement of Stakeholders

To be successful, physicians in numerous disciplines need to consider infection imaging essential to clinical practice. The collaboration of radiologists, nuclear medicine physicians, infectious disease doctors, surgeons (especially orthopedic surgeons and neurosurgeons), and other specialists will be essential in driving this field forward. In addition, partnerships with industry, including commercial radiopharmacies, are crucial to rendering these technologies profitable and sustainable.

Current Patient Studies Are Not Sufficiently Convincing

The latest generation of microbe-specific tracers is highly compelling, but few carefully conducted patient studies support their use. Infectious disease is a broad topic, requiring time for researchers to produce relevant data in patients.

Infection-Targeted Nuclear Medicine Tools Do Not Fit into an Existing Clinical Workflow

Access to nuclear medicine tools may be limited for the diagnosis of infection in the acute care setting. Most radiotracers cannot be synthesized on demand even during the regular operating hours of a radiopharmaceutical facility and, as a result, can often be used only when the patient is already undergoing antimicrobial therapy due to the urgency of treatment in acute infections. This is a significant limitation to first-in-humans studies, as imaging results can be confounded by the effects of the therapeutic regimen.

CONCLUSION

Meetings such as that recently sponsored by the IAEA are essential in identifying ways for researchers and physicians to better diagnose and treat bacterial infections. The remarkable progress made over the last decade indicates that the successful application of new molecular imaging tools in the clinic will profoundly impact patient care.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* 2022;400:2221–2248.
2. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2018;392:1736–1788.
3. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet.* 2020;395:200–211.
4. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance website. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Published May 2016. Accessed August 30, 2023.
5. Dadgostar P. Antimicrobial resistance: implications and costs. *Infect Drug Resist.* 2019;12:3903–3910.
6. Dhesi Z, Enne VI, Brealey D, et al. Organisms causing secondary pneumonias in COVID-19 patients at 5 UK ICUs as detected with the FilmArray test. medRxiv website. https://www.medrxiv.org/content/10.1101/2020.06.22.20131573v1. Published June 23, 2020. Accessed August 31, 2023.
7. Cassat JE, Moore JL, Wilson KJ, et al. Integrated molecular imaging reveals tissue heterogeneity driving host-pathogen interactions. *Sci Transl Med.* 2018;10:eaan6361.
8. Ordonez AA, Tucker EW, Anderson CJ, et al. Visualizing the dynamics of tuberculosis pathology using molecular imaging. *J Clin Invest.* 2021;131:e145107.
9. Ordonez AA, Wang H, Magombedze G, et al. Dynamic imaging in patients with tuberculosis reveals heterogeneous drug exposures in pulmonary lesions. *Nat Med.* 2020;26:529–534.
10. Tucker EW, Guglieri-Lopez B, Ordonez AA, et al. Noninvasive ^{11}C-rifampin positron emission tomography reveals drug biodistribution in tuberculous meningitis. *Sci Transl Med.* 2018;10:eaau0965.
11. Ruiz-Bedoya CA, Mota F, Tucker EW, et al. High-dose rifampin improves bactericidal activity without increased intracerebral inflammation in animal models of tuberculous meningitis. *J Clin Invest.* 2022;132:e155851.
12. Jain SK, Andronikou S, Goussard P, et al. Advanced imaging tools for childhood tuberculosis: potential applications and research needs. *Lancet Infect Dis.* 2020;20:e289–e297.
13. Strosberg J, El-Haddad G, Wolin E, et al. Phase 3 trial of ^{177}Lu-dotatate for midgut neuroendocrine tumors. *N Engl J Med.* 2017;376:125–135.
14. Rowe SP, Gorin MA, Pomper MG. Imaging of prostate-specific membrane antigen with small-molecule PET radiotracers: from the bench to advanced clinical applications. *Annu Rev Med.* 2019;70:461–477.
15. Romanò CL, Petrosillo N, Argento G, et al. The role of imaging techniques to define a peri-prosthetic hip and knee joint infection: multidisciplinary consensus statements. *J Clin Med.* 2020;9:2548.
16. Lauri C, Signore A, Glaudemans AWJM, et al. Evidence-based guideline of the European Association of Nuclear Medicine (EANM) on imaging infection in vascular grafts. *Eur J Nucl Med Mol Imaging.* 2022;49:3430–3451.
17. Lauri C, Tamminga M, Glaudemans AWJM, et al. Detection of osteomyelitis in the diabetic foot by imaging techniques: a systematic review and meta-analysis comparing MRI, white blood cell scintigraphy, and FDG-PET. *Diabetes Care.* 2017;40:1111–1120.
18. Steinsträsser A, Oberhausen E. Granulocyte labelling kit BW 250/183: results of the European multicenter trial. *Nuklearmedizin.* 1996;35:1–11.
19. Jamar F, Buscombe J, Chiti A, et al. EANM/SNMMI guideline for ^{18}F-FDG use in inflammation and infection. *J Nucl Med.* 2013;54:647–658.
20. Welling M, Stokkel M, Balter J, Sarda-Mantel L, Meulemans A, Le Guludec D. The many roads to infection imaging. *Eur J Nucl Med Mol Imaging.* 2008;35:848–849.
21. Polvoy I, Flavell RR, Rosenberg OS, Ohliger MA, Wilson DM. Nuclear imaging of bacterial infection: the state of the art and future directions. *J Nucl Med.* 2020;61:1708–1716.
22. Langer O, Brunner M, Zeitlinger M, et al. In vitro and in vivo evaluation of [^{18}F]ciprofloxacin for the imaging of bacterial infections with PET. *Eur J Nucl Med Mol Imaging.* 2005;32:143–150.
23. Sarda L, Crémieux A-C, Lebellec Y, et al. Inability of ^{99m}Tc-ciprofloxacin scintigraphy to discriminate between septic and sterile osteoarticular diseases. *J Nucl Med.* 2003;44:920–926.
24. Dumarey N, Blocklet D, Appelboom T, Tant L, Schoutens A. Infecton is not specific for bacterial osteo-articular infective pathology. *Eur J Nucl Med Mol Imaging.* 2002;29:530–535.
25. Pauwels EK, Welling MM, Lupetti A, Paulusma-Annema A, Nibbering PH, Balter HS. Concerns about ^{99m}Tc-labelled ciprofloxacin for infection detection. *Eur J Nucl Med.* 2000;27:1866.
26. Welling MM, Nibbering PH, Paulusma-Annema A, Hiemstra PS, Pauwels EK, Calame W. Imaging of bacterial infections with ^{99m}Tc-labeled human neutrophil peptide-1. *J Nucl Med.* 1999;40:2073–2080.
27. Auletta S, Baldoni D, Varani M, et al. Comparison of ^{99m}Tc-UBI 29-41, ^{99m}Tc-ciprofloxacin, ^{99m}Tc-ciprofloxacin dithiocarbamate and ^{111}In-biotin for targeting experimental *Staphylococcus aureus* and *Escherichia coli* foreign-body infections: an ex-vivo study. *Q J Nucl Med Mol Imaging.* 2019;63:37–47.
28. Larikka MJ, Ahonen AK, Niemelä O, Junila JA, Hämäläinen MM, Syrjälä HP. Specificity of ^{99m}Tc-ciprofloxacin imaging [letter]. *J Nucl Med.* 2003;44:1368.
29. Alexander K, Drost WT, Mattoon JS, Kowalski JJ, Funk JA, Crabtree AC. Binding of ciprofloxacin labelled with technetium Tc 99m versus ^{99m}Tc-pertechnetate to a live and killed equine isolate of *Escherichia coli*. *Can J Vet Res.* 2005;69:272–277.
30. Dutta P, Bhansali A, Mittal BR, Singh B, Masoodi SR. Instant ^{99m}Tc-ciprofloxacin scintigraphy for the diagnosis of osteomyelitis in the diabetic foot. *Foot Ankle Int.* 2006;27:716–722.
31. De Winter F, Gemmel F, Van Laere K, et al. ^{99m}Tc-ciprofloxacin planar and tomographic imaging for the diagnosis of infection in the postoperative spine: experience in 48 patients. *Eur J Nucl Med Mol Imaging.* 2004;31:233–239.
32. Fuster D, Soriano A, Garcia S, et al. Usefulness of ^{99m}Tc-ciprofloxacin scintigraphy in the diagnosis of prosthetic joint infections. *Nucl Med Commun.* 2011;32:44–51.
33. Gemmel F, De Winter F, Van Laere K, Vogelaers D, Uyttendaele D, Dierckx RA. ^{99m}Tc ciprofloxacin imaging for the diagnosis of infection in the postoperative spine. *Nucl Med Commun.* 2004;25:277–283.
34. Welch RA, Burland V, Plunkett G, et al. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA.* 2002;99:17020–17024.
35. Polvoy I, Seo Y, Parker M, et al. Imaging joint infections using D-methyl-^{11}C-methionine PET/MRI: initial experience in humans. *Eur J Nucl Med Mol Imaging.* 2022;49:3761–3771.
36. Neumann KD, Villanueva-Meyer JE, Mutch CA, et al. Imaging active infection in vivo using D-amino acid derived PET radiotracers. *Sci Rep.* 2017;7:7903.
37. Stewart MN, Parker MFL, Jivan S, et al. High enantiomeric excess in-loop synthesis of D-[methyl-^{11}C]methionine for use as a diagnostic positron emission tomography radiotracer in bacterial infection. *ACS Infect Dis.* 2020;6:43–49.
38. Parker MFL, Luu JM, Schulte B, et al. Sensing living bacteria in vivo using D-alanine-derived ^{11}C radiotracers. *ACS Cent Sci.* 2020;6:155–165.

39. Weinstein EA, Ordonez AA, DeMarco VP, et al. Imaging Enterobacteriaceae infection in vivo with ^{18}F-fluorodeoxysorbitol positron emission tomography. *Sci Transl Med.* 2014;6:259ra146.
40. Ordonez AA, Wintaco LM, Mota F, et al. Imaging *Enterobacterales* infections in patients using pathogen-specific positron emission tomography. *Sci Transl Med.* 2021;13:eabe9805.
41. Ordonez AA, Weinstein EA, Bambarger LE, et al. A systematic approach for developing bacteria-specific imaging tracers. *J Nucl Med.* 2017;58:144–150.
42. Webb EW, Scott PJH. Potential applications of artificial intelligence and machine learning in radiochemistry and radiochemical engineering. *PET Clin.* 2021;16: 525–532.
43. Mota F, Ordonez AA, Firth G, Ruiz-Bedoya CA, Ma MT, Jain SK. Radiotracer development for bacterial imaging. *J Med Chem.* 2020;63:1964–1977.
44. Parker MFL, Flavell RR, Luu JM, Rosenberg OS, Ohliger MA, Wilson DM. Small molecule sensors targeting the bacterial cell wall. *ACS Infect Dis.* 2020;6: 1587–1598.
45. Gouws AC, Kruger HG, Gheysens O, et al. Antibiotic-derived radiotracers for positron emission tomography: nuclear or "unclear" infection imaging? *Angew Chem Int Ed.* 2022;61:e202204955.
46. Lawal I, Zeevaart J, Ebenhan T, et al. Metabolic imaging of infection. *J Nucl Med.* 2017;58:1727–1732.
47. Vermeulen K, Vandamme M, Bormans G, Cleeren F. Design and challenges of radiopharmaceuticals. *Semin Nucl Med.* 2019;49:339–356.
48. Halouska S, Zhang B, Gaupp R, et al. Revisiting protocols for the NMR analysis of bacterial metabolomes. *J Integr OMICS.* 2013;3:120–137.
49. Bauermeister A, Mannochio-Russo H, Costa-Lotufo LV, Jarmusch AK, Dorrestein PC. Mass spectrometry-based metabolomics in microbiome investigations. *Nat Rev Microbiol.* 2022;20:143–160.
50. Namavari M, Gowrishankar G, Srinivasan A, Gambhir SS, Haywood T, Beinat C. A novel synthesis of 6″-[^{18}F]-fluoromaltotriose as a PET tracer for imaging bacterial infection. *J Labelled Comp Radiopharm.* 2018;61:408–414.
51. Everitt JI, Berridge BR. The role of the IACUC in the design and conduct of animal experiments that contribute to translational success. *ILAR J.* 2017;58: 129–134.
52. Signore A, Artiko V, Conserva M, et al. Imaging bacteria with radiolabelled probes: is it feasible? *J Clin Med.* 2020;9:2372.
53. Aarntzen EHJG, Noriega-Álvarez E, Artiko V, et al. EANM recommendations based on systematic analysis of small animal radionuclide imaging in inflammatory musculoskeletal diseases. *EJNMMI Res.* 2021;11:85.
54. Auletta S, Galli F, Varani M, et al. In vitro and in vivo evaluation of ^{99m}Tc-polymyxin B for specific targeting of gram-bacteria. *Biomolecules.* 2021;11:232.
55. Madeja ZE, Pawlak P, Piliszek A. Beyond the mouse: non-rodent animal models for study of early mammalian development and biomedical research. *Int J Dev Biol.* 2019;63:187–201.
56. Esteves PJ, Abrantes J, Baldauf H-M, et al. The wide utility of rabbits as models of human diseases. *Exp Mol Med.* 2018;50:1–10.
57. Via LE, Schimel D, Weiner DM, et al. Infection dynamics and response to chemotherapy in a rabbit model of tuberculosis using [^{18}F]2-fluoro-deoxy-D-glucose positron emission tomography and computed tomography. *Antimicrob Agents Chemother.* 2012;56:4391–4402.
58. Ordonez AA, Parker MF, Miller RJ, et al. ^{11}C-para-aminobenzoic acid PET imaging of *S. aureus* and MRSA infection in preclinical models and humans. *JCI Insight.* 2022;7:e154117.
59. Elgazzar AH, Dannoon S, Sarikaya I, Farghali M, Junaid TA. Scintigraphic patterns of indium-111 oxine-labeled white blood cell imaging of gram-negative versus gram-positive vertebral osteomyelitis. *Med Princ Pract.* 2017;26:415–420.
60. Walker RL, Eggel M. From mice to monkeys? Beyond orthodox approaches to the ethics of animal model choice. *Animals (Basel).* 2020;10:77.
61. 't Hart BA, Abbott DH, Nakamura K, Fuchs E. The marmoset monkey: a multipurpose preclinical and translational model of human biology and disease. *Drug Discov Today.* 2012;17:1160–1165.
62. Hatziioannou T, Evans DT. Animal models for HIV/AIDS research. *Nat Rev Microbiol.* 2012;10:852–867.
63. Coleman MT, Maiello P, Tomko J, et al. Early changes by ^{18}fluorodeoxyglucose positron emission tomography coregistered with computed tomography predict outcome after *Mycobacterium tuberculosis* infection in cynomolgus macaques. *Infect Immun.* 2014;82:2400–2404.
64. Mattila JT, Maiello P, Sun T, Via LE, Flynn JL. Granzyme B-expressing neutrophils correlate with bacterial load in granulomas from *Mycobacterium tuberculosis*-infected cynomolgus macaques. *Cell Microbiol.* 2015;17:1085–1097.
65. Toyohara J, Sakata M, Tago T, Colabufo NA, Luurtsema G. Automated synthesis, preclinical toxicity, and radiation dosimetry of [^{18}F]MC225 for clinical use: a tracer for measuring P-glycoprotein function at the blood-brain barrier. *EJNMMI Res.* 2020;10:84.
66. Prado C, Kazi A, Bennett A, MacVittie T, Prado K. Mean organ doses resulting from non-human primate whole thorax lung irradiation prescribed to mid-line tissue. *Health Phys.* 2015;109:367–373.
67. Ebenhan T, Sathekge MM, Lengana T, et al. ^{68}Ga-NOTA-functionalized ubiquicidin: cytotoxicity, biodistribution, radiation dosimetry, and first-in-human PET/CT imaging of infections. *J Nucl Med.* 2018;59:334–339.
68. Tanner R, White AD, Boot C, et al. A non-human primate in vitro functional assay for the early evaluation of TB vaccine candidates. *NPJ Vaccines.* 2021;6:3.
69. Uno Y, Uehara S, Yamazaki H. Utility of non-human primates in drug development: comparison of non-human primate and human drug-metabolizing cytochrome P450 enzymes. *Biochem Pharmacol.* 2016;121:1–7.
70. Orsi A, Rees D, Andreini I, Venturella S, Cinelli S, Oberto G. Overview of the marmoset as a model in nonclinical development of pharmaceutical products. *Regul Toxicol Pharmacol.* 2011;59:19–27.
71. Seyedmousavi S, Bosco S de MG, de Hoog S, et al. Fungal infections in animals: a patchwork of different situations. *Med Mycol.* 2018;56(suppl 1):165–187.
72. Vamathevan JJ, Hall MD, Hasan S, et al. Minipig and beagle animal model genomes aid species selection in pharmaceutical discovery and development. *Toxicol Appl Pharmacol.* 2013;270:149–157.
73. Signore A, Casali M, Lauri C. An easy and practical guide for imaging infection/inflammation by [^{18}F]FDG PET/CT. *Clin Transl Imaging.* 2021;9:283–297.
74. Corrales-Medina VF, deKemp RA, Chirinos JA, et al. Persistent lung inflammation after clinical resolution of community-acquired pneumonia as measured by ^{18}FDG-PET/CT imaging. *Chest.* 2021;160:446–453.
75. Baldoni D, Waibel R, Bläuenstein P, et al. Evaluation of a novel Tc-99m labelled vitamin B12 derivative for targeting *Escherichia coli* and *Staphylococcus aureus* in vitro and in an experimental foreign-body infection model. *Mol Imaging Biol.* 2015;17:829–837.
76. Aulakh GK, Brocos Duda JA, Guerrero Soler CM, Snead E, Singh J. Characterization of low-dose ozone-induced murine acute lung injury. *Physiol Rep.* 2020;8:e14463.
77. Aulakh GK, Kaur M, Brown V, Ekanayake S, Khan B, Fonge H. Quantification of regional murine ozone-induced lung inflammation using [^{18}F]F-FDG microPET/CT imaging. *Sci Rep.* 2020;10:15699.
78. Duda JAB, Kaur M, Aulakh GK. Visualizing lung cellular adaptations during combined ozone and LPS induced murine acute lung injury. *J Vis Exp.* 2021;(169).
79. Henneberg S, Hasenberg A, Maurer A, et al. Antibody-guided in vivo imaging of *Aspergillus fumigatus* lung infections during antifungal azole treatment. *Nat Commun.* 2021;12:1707.
80. Welling MM, Duszenko N, van Willigen DM, et al. Cyclodextrin/adamantane-mediated targeting of inoculated bacteria in mice. *Bioconjug Chem.* 2021;32:607–614.
81. Cho SY, Pomper MG. Clinical translation of molecular imaging probes. In: Chen A, ed. *Molecular Imaging Probes for Cancer Research.* World Scientific; 2012: 1041–1065.
82. Wester H-J. Nuclear imaging probes: from bench to bedside. *Clin Cancer Res.* 2007;13:3470–3481.
83. Liu CH, Sastre A, Conroy R, Seto B, Pettigrew RI. NIH workshop on clinical translation of molecular imaging probes and technology: meeting report. *Mol Imaging Biol.* 2014;16:595–604.