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Nuclear Imaging: A Powerful Novel Approach for Tuberculosis

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Abstract

Nearly 20 years after the World Health Organization declared tuberculosis (TB) a global public health emergency, TB still remains a major global threat with 8.6 million new cases and 1.3 million deaths annually. *Mycobacterium tuberculosis* adapts to a quiescent physiological state, and is notable for complex interaction with the host, producing poorly-understood disease states ranging from latent infection to fully active disease. Of the approximately 2.5 billion people latently infected with *M. tuberculosis*, many will develop reactivation disease (relapse), years after the initial infection. While progress has been made on some fronts, the alarming spread of multidrug-resistant, extensively drug-resistant, and more recently totally-drug resistant strains is of grave concern. New tools are urgently needed for rapidly diagnosing TB, monitoring TB treatments and to allow unique insights into disease pathogenesis. Nuclear bioimaging is a powerful, noninvasive tool that can rapidly provide three-dimensional views of disease processes deep within the body and conduct noninvasive longitudinal assessments of the same patient. In this review, we discuss the application of nuclear bioimaging to TB, including the current state of the field, considerations for radioprobe development, study of TB drug pharmacokinetics in

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infected tissues, and areas of research and clinical needs that could be addressed by nuclear bioimaging. These technologies are an emerging field of research, overcome several fundamental limitations of current tools, and will have a broad impact on both basic research and patient care. Beyond diagnosis and monitoring disease, these technologies will also allow unique insights into understanding disease pathogenesis; and expedite bench-to-bedside translation of new therapeutics. Finally, since molecular imaging is readily available for humans, validated tracers will become valuable tools for clinical applications.

Keywords

drug-resistance; PET; SPECT; probe-design; pharmacokinetics

Introduction

Mycobacterium tuberculosis has been a cause of human illness for over 9000 years [1]. It remains a serious pathogen, second only to HIV in annual mortality among infectious diseases worldwide. *M. tuberculosis* is estimated to infect 1/3 of the world's population (latent infection), and is responsible for 8.6 million new cases of active disease annually, resulting in 1.3 million deaths in 2012 alone. Drug resistance is increasing in prevalence and multidrug resistant (MDR) strains, resistant to two of the first-line TB drugs (isoniazid and rifampin), accounted for 450,000 new cases worldwide in 2012, with a 37% case-fatality rate [2]. Of these, approximately 10% were estimated to be extensively drug resistant (XDR), defined as MDR strains also resistant to second-line drugs including any fluoroquinolone and at least one of three injectable drugs (capreomycin, kanamycin, and amikacin). Despite the enormous burden of disease, current diagnostics are still woefully inadequate to meet clinical and research needs. Nuclear imaging approaches may offer novel approaches to the diagnosis, monitoring and delineation of tuberculosis (TB) infection and disease. In addition, nuclear imaging has some fundamental advantages over conventional methods, which include giving a holistic three-dimensional assessments of an organ or body, enabling a complete view of the disease process, and the ability to conduct noninvasive longitudinal assessments in the same individual.

M. tuberculosis is notable for its complex interaction with the host with multiple poorly understood disease states. Following inhalation, mycobacteria may be cleared by the host, develop asymptomatic latent infection, or infrequently, cause acute active pulmonary disease. While latent infection is asymptomatic, it has a 10% lifetime chance to reactivate causing active disease in the immunocompetent host and 5–8% chance annually in the HIV-infected host [3]. Latent infection exists on a continuum from clearance of organism with subsequent persistent adaptive immune response to containment of quiescent non-replicating organisms presumably within a granuloma, to subclinical disease with active bacillary replication [4, 5]. Radiological findings may be normal or overlap with active disease [6]. Active disease begins when host containment of the pathogen breaks down allowing *M. tuberculosis* to replicate rapidly. Multiple different pathological states may occur simultaneously in the lung with active disease, including cavitary disease, bronchiectasis, fibrosis, granuloma formation and liquefactive or caseous necrosis [7], each with distinct

bacillary burdens, immunological states, and drug concentrations. Moreover, disseminated disease, affecting various organs (brain, heart, kidneys, adrenals, genitourinary or gastrointestinal systems, bone, etc), with distinct lesional characteristics, may be present as isolated extrapulmonary disease or coexist with pulmonary disease.

Limitations and Challenges of Current TB Diagnostics

Robert Koch first used sputum microscopy to identify TB over 130 years ago [8], and diagnosis of active TB in many parts of the world has not changed since. Although inexpensive and accessible, the technique is operator dependent and poorly sensitive (45–80%) [9]. Sputum culture, the gold standard for diagnosis, has specificity as high as 98%, but may take from 2–8 weeks for results, depending on culture media and bacillary burden, limiting its clinical utility [10]. Additionally, sputum is difficult to collect from infants and children, and culture sensitivity is low, often requiring multiple samples from an individual [11, 12]. Plain chest radiography and computed tomography (CT), are mainstays for diagnosing pulmonary TB, but are often non-specific and unable to provide definitive diagnosis due to the highly heterogeneous presentation of TB [13, 14], particularly in HIV/TB coinfecting patients with low CD4 counts [15].

In response to the limitations of traditional diagnostics, new approaches are being developed. The interferon gamma release assay (IGRA), a blood test measuring cellular immune response to *M. tuberculosis* antigens, assesses infection in a manner similar to the traditional tuberculin skin test (TST), but with higher sensitivity and specificity (against other mycobacterial infections) than TST. However, like TST, IGRAs are limited by their inability to differentiate between infection and disease and are dependent on host-responses, which may be compromised in immune-deficient patients [16, 17]. Other new diagnostics include urine lipoarabinomannan, a point-of-care test indicating active TB in patients with AIDS with high specificity, but with modest sensitivity [18]. The Xpert MTB/RIF assay rapidly detects *M. tuberculosis* nucleic acid in sputum (and more recently in other clinical samples) and rifampin resistance. It is approved by the US Food and Drug Administration (FDA), endorsed by the World Health Organization (WHO) and being rolled out in high-incidence settings. Xpert MTB/RIF has high (98%) specificity, but sensitivity is variable depending on setting and sputum smear positivity [19, 20]. While useful for rapid initial diagnosis, it has been shown to be inferior to culture for monitoring changes in bacillary burden on treatment [21].

Benefits of Imaging for TB

Despite recent progress, currently available techniques to diagnose and monitor TB provide limited data about the underlying processes and do not fully capture the complexity (location, extent of disease and dissemination) of TB disease, all of which are critical in making clinical decisions for the patient. Moreover, tests that directly detect mycobacteria (culture and nucleic acid tests) require infected clinical tissues or fluids, which are not easily available due to location (e.g. deep seated, or extrapulmonary disease) or population (e.g. children). Therefore, tomographic imaging modalities such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and functional magnetic resonance imaging (MRI) have enormous potential to associate the pharmacological,

immunological and microbiological aspects of TB lesions with anatomical information (generally available through CT or MRI), allowing a holistic approach to understanding the disease. While nuclear imaging is a comparatively expensive technology requiring advanced infrastructure it is available in most large cities in many developing countries such as India, South Africa, South Korea and China, which are epicenters of TB disease and transmission. This makes nuclear imaging a useful research tool that can be applied to clinical studies and trials. In addition, it could also serve as a regional clinical tool in settings where it will have the largest impact. Additionally, new PET radioligands face lower hurdles from various regulatory agencies such as the FDA due to microdosing of the parent drug [22], facilitating the rapid application of novel nuclear medicine approaches to human subjects.

General Considerations for Targeted Probe Design for TB

An ideal radioprobe should have a number of biochemical, pharmacokinetic (PK) and pharmacodynamic (PD) features for maximum performance and usability (Table 1). Biochemical properties for probe selection include moderate lipophilicity, low plasma protein binding, metabolic stability and target saturation behavior [23, 24]. Radioprobe concentrations in the picomolar range can be visualized with high specific activity agents, producing signals of very high sensitivity [25, 26]. However to achieve this, the probe design must minimize background noise. The approach to maximize the signal-to-noise ratio is dependent on rapidly localizing high affinity probes with little non-specific binding [27]. For example, positively charged molecules may cause higher background noise due to non-specific binding to negatively charged eukaryotic cell membranes [28]. An optimal clearance rate allows sufficient time for a probe to reach its target but is rapid enough to excrete unbound probe [29–31]. Signal can be increased via strategies to promote cellular internalization of the probe, such as selective modification and trapping by intracellular enzymes [32].

A complex manufacturing scheme or administration plan can limit the practical use of a probe. Short half-life radiopharmaceuticals that must be synthesized immediately before use require a cyclotron or nuclear reactor-derived generator on site and may not be practical outside of a research setting. An extensive production and use chain can quickly become restrictive to the point of rendering a probe impossible to use clinically. Radiation risks during production and patient use are an inherent concern, including disposal of radioactive waste. Cost is a concern in resource limited areas where TB is endemic, but may be overcome with an economy of scale if a probe is widely adopted. It should also be noted that there are unrecognized cost savings if the use of the probe provides information that decreases use of other medical resources, for example identification and modification of a failing TB drug regimen before drug resistance emerges.

Target Selection

M. tuberculosis pathophysiology presents a number of unique challenges to probe design. First, under conditions of active replication *M. tuberculosis* divides roughly once every 18 hours *in vivo* [33], making it challenging to use probes that target (and therefore accumulate based on) the metabolic machinery required for cell division. Second, the bacteria reside in

complex, heterogeneous structures with necrosis, fibrin deposition, and a combination of bacilli both residing extracellularly and within macrophages[34]. Adequate probe penetration into these lesions may be problematic, and further complicated if imaging of intracellular bacteria is desired. Third, *M. tuberculosis* is capable of prolonged periods of metabolically quiescent non-replicative persistence conferring resistance to many antibiotics [35]. Consequently, metabolic and antibiotic based probes developed against rapidly dividing bacilli *in vitro* may be less effective when tested *in vivo*.

Diagnostic radiopharmaceuticals can be divided into three general classes: 1) biomimetics, 2) labeled antibodies, peptides and their fragments, and 3) labeled drugs and drug-like compounds [36]. Biomimetics are natural compounds or closely related analogs that use native transporter and enzymatic machinery to target the probe. Although they often have high sensitivity, they may engage transporters and/or metabolic pathways shared among different cell types resulting in lower specificity. For example 2-[¹⁸F]-fluorodeoxyglucose ([¹⁸F]-FDG), a glucose mimetic, is avidly taken up by metabolically active cells in a manner proportional to the cellular metabolic rate [37–39]. However, [¹⁸F]-FDG is limited by a lack of discrimination among metabolically active tissues, including myocardium, brain, neoplasm and sites of inflammation.

A biomimetic approach unique to infectious diseases involves rational radioprobe design targeting pathogen enzymes or transporters not found in the host as a control point, thus conferring specificity. The nucleotide analog 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodouracil (FIAU) is a substrate of bacterial, but not mammalian, thymidine kinase (TK) [40]. Intracellular bacterial TK phosphorylates [^{124/5}I]-FIAU thereby trapping the probe and allowing it to accumulate in the bacteria. This approach has been successfully used to image musculoskeletal bacterial infections[41]. While wild-type *M. tuberculosis* does not express TK, genetically modified *M. tuberculosis* expressing TK has been imaged using [¹²⁵I]-FIAU in experimentally infected animals [42]. Beyond these examples, sugars [43], amino acids [44], and other natural compounds have been proposed for the detection of *M. tuberculosis*.

One promising approach to radioprobe development is akin to rational drug design. Ligands are developed to exploit disease associated control points (e.g. processes mediated by host microenvironment, or enzymes associated with the pathogen or host response), allowing amplification of signal with high specificity [22]. Biomimetic substrates of pathogen transporters or enzymes are possible applications of this approach, but other approaches appropriate for TB include the targeting of pH, hypoxia or inflammation.

In contrast to biomimetics, radiolabeled antibodies and their fragments bind with high specificity and affinity. While this approach has been applied to cell surface proteins in imaging cancer [45, 46], large molecules such as antibodies may have limited penetration into necrotic or fibrosed TB lesions, and systemic clearance of the labeled antibody may take days or weeks, resulting in high background signal, requiring patients to return for imaging days after administration of the radioprobe. Following this strategy, fluorescent anti-ESAT-6 monoclonal antibodies have been developed to detect *M. tuberculosis* in a murine model of infection[47].

Labeled antibiotics and antibiotic-like compounds have inherent specificity by targeting bacterial processes, and many have been developed [48–50]. However the targets of these drugs may not be highly expressed, producing a signal of low intensity. Additionally, desirable pharmacologic characteristics of antibiotics may not match those for radioligands. Examples include engineering a long half-life for convenient daily dosing or compensating for low target affinity with higher drug concentrations. Labeled antibiotics and their derivatives are susceptible to the same mechanisms of resistance that affect the parent antibiotic, such as efflux pumps, cell permeability changes, decreased target affinity or degradative metabolism. Moreover, the host may form antibodies against the exogenous antibody following repeated administration. This may potentially interfere with the sensitivity and specificity, or lead to adverse side effects.

Interpretation of SPECT and PET imaging

Approach to Interpretation

Radioisotopes with relatively short half-lives that emit either gamma rays or positrons are used in nuclear imaging. Typically, following administration of a radioactive probe into a subject it distributes within the body and accumulates or clears from the different tissues based on the nature of the probe. The distribution of the probe is determined by detecting either gamma ray emissions by a multi-head gamma camera (SPECT isotopes) or paired coincident gamma photons emitted when a positron annihilates (PET isotopes). The emission data are collected at one or more specific time ranges post injection and reconstructed using computer algorithms into three-dimensional datasets reflecting local probe concentrations. These data may be aligned with CT or MRI scan to provide an anatomical reference for the radioactivity distribution [51]. Structures or regions of the body that have accumulated the probe are said to have a positive signal.

The two dimensional unit of the reconstructed image is referred to as the pixel, and the three-dimensional unit is the voxel. The resolution of these units depends on the imaging cameras used and distance traveled by the emitted gamma ray. The standard uptake value (SUV) is a measurement of the radioactive accumulation per voxel defined as the tissue concentration of the probe divided by the probe activity injected, divided by the body weight. When an area of the image is examined by drawing a region of interest (ROI) around it, additional parameters that can be evaluated include maximum uptake of any voxel in the ROI (SUV_{max}), average uptake of all voxels (SUV_{mean}), and total activity of all voxels (SUV_{total}). When data from multiple scans are compared, a SUV_{index} may be calculated to assist in normalizing variability in tracer uptake from exam to exam or from individual to individual. This is defined as the SUV_{mean} of the ROI divided by the SUV_{mean} of a reference organ, often the liver, brain, or a muscle. When two or more imaging scans are collected after a single probe administration, the retention index (RI) of the probe may be determined and has been reported to be useful in distinguishing types of disease as was reported with [^{18}F]-FDG[52]. Conducting serial imaging exams makes it possible to compare changes in the SUV of a ROI that may indicate progression of disease or response to treatment. While SUV measurements are practical, they may not be universally applicable, as imaging probes have diverse chemical and subsequent PK and PD metrics.

SUV ratios (SUVR) or SUV_{index} should be considered in settings where assumptions regarding PK and PD metrics cannot be validated [53].

Study design considerations

Designing studies to assess the utility of imaging in diagnosis or assessing treatment response requires definable endpoints. Defining clinical endpoints when developing new TB diagnostics can be difficult since disease occurs even with negative sputum culture, the gold standard for diagnosis. Similarly, participant loss to follow-up or difficulties in obtaining relevant samples also hinders collection of study endpoints. One approach to assess the relationship between imaging and treatment response may be inclusion of novel imaging approaches within TB treatment trials, allowing regular imaging during the treatment phase and follow up. The ideal frequency of imaging within a trial is unclear, since the number of scans must be limited in order to reduce the radioactive exposure to the subject, though new scanners and protocols that deliver much lower radiation and could allow for more frequent scans[54]. Key time-points to capture may include pretreatment, time of median expected sputum conversion, the end of treatment and 1–2 years after completion of treatment.

Once the issues of probe synthesis, *in vivo* stability, target, and quality control have been addressed, the calibration of the scanning equipment must be assured and the scanning protocol harmonized if multiple systems will be used [55]. The protocol for imaging exams should be strictly defined so that the probe dose, the uptake period, and fasting state of the subject are standardized to reduce the intra-subject and inter-subject variability [56]. The dwell time of the probe that gives the greatest signal to background ratio must be determined in humans as well as the most predictive time points for the imaging exam after therapy initiation. Finally, host genetics can alter the binding or distribution of the probes. For example, translocator protein (TSPO) host genotype (A147T) alters binding by PBR28 significantly more than other TSPO probes (DPA-713 and PK11195) [57].

Recent developments in imaging of TB

Host response probes

As oncology applications of diagnostic nuclear imaging became commonplace, radiologists realized that while tumors and metabolically active organs accumulated many radiotracers, so did TB and other infectious diseases, complicating the diagnosis of cancer in TB endemic areas [52, 58–62]. Early approaches to this problem included dual-phase ^{201}Tl chloride (a SPECT probe) imaging to determine if probe RI might assist in distinguishing malignancies from TB. A negative ^{201}Tl RI was associated with benign lesions including TB lesions in several studies [60, 63]. $^{99\text{m}}\text{Tc}$ Technetium tetrofosmin and sestamibi, used for perfusion analysis of the myocardium and tumor evaluation, were found to accumulate in regions of active TB and showed delayed wash out, or a positive RI, in comparison to inactive TB lesions. However, in a study of solitary pulmonary nodules, uptake was somewhat predictive of malignancy rather than TB [63–65]. ^{18}F -FDG uptake is proportional to the cellular metabolic rate [37–39] and is known to accumulate in inflammatory cells such as neutrophils and activated macrophages at the site of inflammation[66, 67]. ^{18}F -FDG-PET has been utilized in imaging TB lesions in humans[6,

68, 69]. Several studies in animal models and humans suggests that [^{18}F]-FDG may be useful to gauge disease progression or response to treatment[70–76]. A study by Sathekge *et al* in TB-HIV coinfecting patients demonstrated that the number of [^{18}F]-FDG -PET active TB lesions (rather than SUV_{max}) were predictive of successful TB treatments[77]. In mice, [^{18}F]-FDG uptake was monitored and could correctly identify the bactericidal activity of various drug regimens and risk of relapse[74]. In *M. tuberculosis*-infected rabbits treated with effective anti-tubercular agents, uptake in lesions was significantly reduced within a week of treatment and detectable prior to changes in CT lesion volume and density[71]. Similar experiments were also performed in cynomolgus macaques which also demonstrated marked reduction in [^{18}F]-FDG activity with effective treatment, as well as the dynamic nature of individual TB lesions [70]. In *M. tuberculosis*-infected marmosets, the $\text{SUV}_{\text{total}}$ of whole lung increased over time commiserate with the bacterial burden and disease volume measured from the co-registered CT [72]. However, [^{18}F]-FDG is plagued with non-specific accumulation into tissues with high metabolic rates, including both tumors and TB lesions to varying extents [78–81].

In contrast, new probes with greater specificity for *M. tuberculosis* or the host cells that harbor bacteria may provide more diagnostic capability and deserve more study [82]. Recently, Foss *et al* described radioiodo-DPA-713, a TSPO ligand, as an imaging modality for macrophage-associated pulmonary inflammation in mouse models of TB [82]. They demonstrated that DPA-713 accumulated specifically in TB-associated inflammatory lesions by selective retention within macrophages and phagocytic cells and that [^{125}I]DPA-713-SPECT imaging provided higher lesion-specific signal-to-noise ratios than [^{18}F]-FDG -PET. While [^{18}F]-FDG-PET and [^{125}I]DPA-713-SPECT are good imaging modalities to assess inflammation *in situ*, PET imaging can also be used to measure other host microenvironments. Harper *et al* utilized noninvasive [^{64}Cu]ATSM-PET imaging and demonstrated that necrotic TB lesions in “Kramnik” mice were hypoxic [83]. More recently, CT was utilized to monitor the development of cavitary disease in the same mouse model [84, 85]. Using another PET hypoxia tracer ([^{18}F]FMISO), a recent study demonstrated that tissues surrounding pulmonary cavities in patients with TB were hypoxic, and that hypoxia potentially up-regulated the expression of host matrix metalloproteinases (MMP) [86].

Bacterial probes

While several studies have evaluated the role of host-inflammation in TB, there are few technologies to directly image the bacteria *in situ*. Kong *et al* demonstrated that *M. tuberculosis* could be tracked in the lungs of live mice with fluorescence imaging probes that targeted an endogenous β -lactamase in the bacterial cell wall [87]. Similarly, optical imaging with genetically-modified fluorescent bacteria has been utilized to serially monitor TB treatments in mice[88, 89]. While optical imaging has excellent sensitivity, detection is restricted by the depth of the origination of the signal, which generally limits its use to small animals. However, optical imaging is relatively inexpensive and remains an important tool for preclinical studies. Using a recombinant strain of *M. tuberculosis* expressing bacterial thymidine kinase, Davis *et al*, have also demonstrated *in vivo* imaging of *M. tuberculosis* using SPECT [42]. Though SPECT is not limited by the depth of the signal and is clinically translatable, the need for a recombinant bacterial strain limits this technology to

experimentally infected animals. Therefore, there is an urgent need for developing bacteria-specific imaging tracers that can be used in large animals and with potential for clinical translation.

Radiolabeled drugs

TB treatment regimens were developed using serum PK values and historic measures of efficacy[90, 91]. Nevertheless, a growing number of studies support the importance of monitoring drug concentration in infected tissues[92, 93] and current FDA guidelines require tissue drug distribution studies at infected and uninfected sites [92]. Serious consequences of inadequate drug concentration in target tissues include treatment failure and selection pressure for antibiotic resistant organisms [94]. Conversely, in severely ill patients, normal physiology may be compromised, thus elevating the risk of renal and hepatic toxicity [95]. Therefore, methods that can report on drug concentration at the site of infection are needed. New techniques, such as matrix-assisted laser desorption ionization (MALDI) mass spectrometry have the power to detect drugs and their metabolites within TB granulomas *ex vivo* [96], but are invasive and rely on accurate resection of tissue. To overcome these limitations, PET imaging has been developed as a noninvasive and real-time alternative, to quantify drug absorption, distribution and elimination, and thus yield *in situ* PK data in live animals [97]. Moreover, since PET imaging is extensively used in humans, this technology has significant potential for rapid bench-to-bedside translation, and use in humans[98]. Li *et al* labeled first-line TB drugs - isoniazid, rifampin, and pyrazinamide with carbon-11 and studied their biodistribution in healthy baboons using PET [99]. Unless radionuclides can be substituted in a drug without altering the chemical structure (e.g. fluoroquinolones, linezolid have fluorine), PET radiotracers are generally developed using carbon-11. However, carbon-11 has a short half-life, and chemically modified radioprobe with longer half-life isotopes (e.g. F-18) would need prior validation. For example, Weinstein *et al* reported the bioimaging of 2-[¹⁸F]-fluoroisonicotinic acid hydrazide (2-[¹⁸F]-INH), a fluorinated analog of isoniazid (also a prodrug), as a PET probe for imaging *M. tuberculosis*-infected mice [100]. Validating 2-[¹⁸F]-INH as a marker of INH distribution required verifying both that it accumulated intracellularly like INH and that it matched INH in its mechanism of conversion from prodrug to active drug. Dynamic PET imaging demonstrated that 2-[¹⁸F]-INH was extensively distributed, and rapidly accumulated at the sites of infection, including necrotic pulmonary TB granulomas.

Future directions / knowledge gaps

Clinical needs and applications

Effective diagnostics and biomarkers for TB are essential for improving patient care and advancing clinical research, but currently available diagnostics and biomarkers for TB are insensitive, provide modest information about disease status, and generally have significant time delays (several weeks) between sample collection and results. Nuclear imaging potentially offers an excellent match with these unmet clinical diagnostic needs, but further development of new radioligands and image analysis approaches are needed. Nuclear imaging is inherently rapid, noninvasive and offers anatomical detail of underlying processes, potentially providing a diagnostic gold standard for research and patient care.

This may allow clinical decision making to proceed in real-time for cases of smear-negative pulmonary disease and diagnosis of sputum culture-negative and extrapulmonary TB without biopsy. Additionally, nuclear bioimaging could provide an accurate means of diagnosing extrapulmonary and pediatric TB, which is more frequently culture negative and extrapulmonary than in adult cases [11, 12, 101]. Other desired qualities of a novel nuclear imaging approach to TB would include detection of paucibacillary or culture negative disease, discrimination between TB and other mycobacterial infections, and applicability regardless of immune or HIV status.

A majority of patients can be successfully treated for TB without relapse with shorter than standard courses of therapy [102]. However, current technologies cannot prospectively predict those who will relapse, requiring additional months of treatment for all patients. A nuclear imaging biomarker that could determine risk of relapse would allow selecting patients who may be cured with shortened length of treatments. This is of particular benefit in MDR-TB, where treatments have considerable toxicity and involve injectable agents. Additionally, this would be extremely useful in a clinical research setting as monitoring for relapse for 1–2 years following is time consuming and expensive.

Research needs and applications

Imaging and Preclinical Research

Animal models employed in TB research include the mouse, guinea pig, rabbit and nonhuman primate (NHP). Each has their own advantages and disadvantages, which range from similarity to human disease (summarized in Table 2) to the availability of reagents and genetically modified organisms to understand the immunology and pathology associated with TB. The mouse, of course, is the least expensive of these. The use of inbred strains, genetic knockouts, full genome sequences and a wealth of immunologic reagents allow for extensive investigation of disease mechanisms. However, the major disadvantage of the standard mouse model is the lack of necrotic (caseous) granulomas - the hallmark of human TB. Unlike TB granulomas in humans, guinea pigs, rabbits, and non-human primates (NHP), granulomas in the standard mouse model are not hypoxic [103].

A more recent development in the field, however, is the characterization by Pan *et al* of the C3HeB/FeJ “Kramnik” mouse. These mice have been shown to develop well-defined pulmonary granulomas with central caseous necrosis in response to *M. tuberculosis* infection due to the lack of expression of *Ipr1* within the *sst1* locus in this strain [104]. It has also been demonstrated that the necrotic pulmonary TB granulomas in “Kramnik” mice are also hypoxic [83, 105] and develop cavitory lesions [84, 85].

Still, the guinea pig and rabbit are both highly valuable models in TB research. Each exhibits a similar array of pathologies as seen in human disease. They can also serve to confirm that observations discovered in the mouse are not restricted to the one species. Rabbits may also be an excellent model to study cavitory disease and latent infections due to *M. tuberculosis*. However, similar to the mouse, current tools for assessing disease in these larger and more expensive animals are not real-time and are generally limited to analyses of serial post-mortem samples. Moreover, conventional tools used to evaluate TB drugs and

vaccines utilize microbiologic methods where whole organs are generally homogenized and plated for assessing the bacterial burden at several time-points during the experiment. Artifacts introduced during animal sacrifice and subsequent tissue processing make them less reliable. In addition, characteristics of TB lesions can generally not be assessed separate from the whole organ. Therefore, comprehensive analysis of bacteria and the host-microenvironment at several time-points during the course of infection is cumbersome, if not impossible. Moreover, since temporal assessment of lesions in the same animal cannot be made, assessment of different lesions in the different animals sacrificed during the course of the experiment can be misleading. Therefore, noninvasive imaging biomarkers with the ability to assess disease at several time-points in the same animal are better suited to study these processes. Use of imaging requires fewer animals, is available in real-time (as opposed to the 4-week culture lag for standard microbiological assessment) and can also detect relapse. As there are few immunological reagents for the guinea pig or the rabbit, the development of *M. tuberculosis*-specific probes, in combination with currently available species-independent probes targeting inflammation or mechanisms shared across species (e.g., FDG, TPSO ligands) could significantly increase the utility of these models. And, given the advantages described above, the “Kramnik” model may be ideal for the initial development of such imaging biomarkers.

Nonhuman primates occupy a critical space in understanding TB disease. Similar to humans, following a low-dose aerosol infection, only a fraction (albeit higher, at 50–60%) of cynomolgus macaques develop active disease [106]. However, in contrast to mice, guinea pigs and rabbits, studies involving nonhuman primates under BSL-3 conditions are significantly limited due to cost and logistical considerations. The heterogeneity in genetic background of the NHP makes their response to infection and treatment more variable than in inbred species, requiring larger groups to assess the efficacy of potential interventions. It is essentially impossible to power studies to allow for serial sacrifices and the understanding of disease progression, with the primary readouts, including bacterial burden and pathology, captured at necropsy. The ability of *in vivo* imaging to provide longitudinal information from the same animal has the potential to significantly increase the power of studies of a relatively small number of animals. Serial CT scans are already being implemented in a significant portion of nonhuman primate vaccine evaluation studies for TB, allowing for the generation of a significantly more comprehensive data set from a limited number of animals beyond that typically captured from a single necropsy. [¹⁸F]-FDG PET is also being used in some laboratories to monitor both disease progression and response to treatment. The inclusion of nuclear imaging has the potential to allow for an even greater understanding of disease progression in general and, more specifically, the ability of a vaccine or therapeutic to interrupt this progression.

Response to Interventions

There are several stages after initial infection culminating in disease at which candidate vaccines for TB are being targeted. Ideally, lung-resident cellular or humoral immune responses would lead to elimination of the pathogen shortly after infection. However, as very few bacilli are required to establish a productive infection, the minimal inflammation induced by these few bacteria, together with a potentially limited presence of antigen-

specific T-cells, make this a difficult challenge. Failing that, a cellular immune response could halt disease progression through the formation of a protective granuloma. Finally, a vaccine could also act to prevent the reactivation of existing latent infections. Moreover, serial monitoring capabilities of noninvasive imaging could also shed light on the stage of disease where the bacterium is most susceptible to such an immunologic intervention. The ability to conduct longitudinal studies in the same animal, especially after challenge with one to a handful of organisms lends itself to answering these types of questions.

Central to answering these questions, is being able to evaluate the very early immunological events following infection. Indeed, little is known about the early stages of infection or the kinetics of host-pathogen interactions. Further insight into these events could significantly aid vaccine development. For example, understanding which immune cells (e.g. CD4 T-cells, CD8 T-cells, natural killer T-cell (NKT), mucosal-associated invariant T-cells (MAIT), etc) are the first to engage an infection could guide the development of vectors specifically designed to induce these responses. Again, probes to detect lower levels of bacterium, along with targeting specific immune cells, would be required. This technology could also be employed in human challenge studies, which for safety reasons could not be allowed to progress to the point of significant disease.

Pharmacology and drug development research

A major advantage of radioligand-based bioimaging is its ability to measure *in situ* PK in real-time, and simultaneously in multiple organ system or compartments with relatively unaltered physiology. As plasma levels may not reflect tissue PK, nuclear imaging with radiolabeled drugs could provide detailed preclinical data for appropriate dosing of new TB drugs, both to ensure sufficient drug in target tissue and evaluate for its presence in sites of potential toxicity. Additionally, competition studies with radiolabeled probes allow assessment of target avidity *in vivo* [107]. Since imaging with these probes would generally be species-independent, it will provide a uniform cross-species platform for animal studies, expediting bench-to-bedside translation of new TB drugs. Such tracers would also enable the first-in-human clinical (Phase 0) trials that are strongly encouraged by the FDA to provide PK data for IND applications [108]. Finally, since PET imaging is readily available for humans, validated PET-tracers could also be used to study PK in clinical studies.

Conclusion

Tuberculosis is a 21st century global health threat reliant on 19th century diagnostic and research tools. Nuclear imaging of tuberculosis is an emerging technology with significant advantages over existing tools, giving detailed views of the microbiological, immunological and physiological states of the disease in living hosts. By noninvasively revealing links between pathology and anatomy, nuclear bioimaging will aid clinical management and accelerate the development of new therapeutics, vaccines and diagnostics. However, much work must be done to fully realize the potential of this technology. New probes targeting the pathology of the disease need to be developed, and new approaches to interpreting the images are needed as well. Due to the complex pathology of the disease significant challenges remain and close partnerships between medicinal chemists, cell biologists,

animal scientists, pharmacologists, infectious disease clinicians and nuclear medicine researchers are key to developing effective, novel applications of nuclear imaging for TB.

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Table 1

Desirable properties of an ideal PET probe (adapted from Gemmel et al [48])

Sensitive	High target to background signal ratio. Low limit of detection.
Specific	Probe targets infectious, not inflammatory lesions.
Quantitative	Signal proportionate to infectious burden.
Rapid	Fast localization to the site of infection.
Stable	Probe retained at the site of infection. No degradation of the probe by the host.
Safe	Acceptable radiation dose. Repeat injection feasible, without pharmacologic or immunologic effect.
Manufacturable	Uncomplicated synthesis with reasonable expense.

Table 2

Pathological characteristics of animal models of TB

Species	Susceptibility to <i>M. tuberculosis</i>	Caseous Necrosis	Hypoxic Lesions	Cavity Formation
Humans	+	+	+	+
Standard mice	++	–	–	–
“Kramnik” mice	+++	+	+	+
Guinea Pig	++++	+	+	–
Rabbits	+	+	+	+
Non-human primates	+	+	+	+

“+” indicates the presence of the given characteristic.