Infection Dynamics and Response to Chemotherapy in a Rabbit Model of Tuberculosis using $[^{18}\text{F}]2$-Fluoro-deoxy-$d$-Glucose Positron Emission Tomography and Computed Tomography


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With a host of new antitubercular chemotherapeutics in development, methods to assess the activity of these agents beyond mouse efficacy are needed to prioritize combinations for clinical trials. Lesions in Mycobacterium tuberculosis-infected rabbits are hypoxic, with histopathologic features that closely resemble those of human tuberculous lesions. Using $[^{18}\text{F}]2$-fluoro-deoxy-$d$-glucose ($[^{18}\text{F}]\text{FDG}$) positron emission tomography—computed tomography (PET-CT) imaging, we studied the dynamics of tuberculosis infection in rabbits, revealing an initial inflammatory response followed by a consolidative chronic disease. Five weeks after infection, as much as 23% of total lung volume was abnormal, but this was contained and to some extent reversed naturally by 9 weeks. During development of this chronic state, individual lesions in the same animal had very different fates, ranging from complete resolution to significant progression. Lesions that remained through the initial stage showed an increase in volume and tissue density over time by CT. Initiation of chemotherapy using either isoniazid (INH) or rifampin (RIF) during chronic infection reduced bacterial load with quantitative changes in $[^{18}\text{F}]\text{FDG}$ uptake, lesion density and total lesion volume measured by CT. The $[^{18}\text{F}]\text{FDG}$ PET uptake in lesions was significantly reduced with as little as 1 week of treatment, while the volume and density of lesions changed more slowly. The results from this study suggest that rabbits may be a useful surrogate species for evaluating novel chemotherapies and understanding changes in both PET and CT scans in human clinical trials.

Curating drug-sensitive tuberculosis (TB) takes 6 to 9 months of combination therapy despite the availability of antibiotics with potent in vitro activity, yet other pulmonary infectious diseases can be cured with single drugs that have similar mechanisms of action with only 3 to 14 days of treatment. One hypothesis used to explain the extended duration required with TB therapy is that subpopulations of bacteria become phenotypically drug tolerant in response to specific local microenvironmental conditions determined by the pathology of individual lesions (37). Understanding the features of these microenvironments and the conditions that generate tolerance may allow a rational design of drug regimens capable of shortening the time required to achieve a durable TB cure, but the methods used to evaluate new regimens have changed little and rely heavily on murine models of tuberculosis that typically have less complex lung pathology than human lesions. Premature discontinuation of treatment in humans results in disease relapse and the presence of cavities, and advanced lung pathology is strongly correlated with relapse (7, 19, 23). Only the rabbit and nonhuman primate models of pulmonary tuberculosis develop similar heterogeneous pathology, including the formation of cavitary disease. Guinea pigs, and some newer mouse models, develop more highly organized lesions, but these do not progress to cavities (for a comprehensive review of the comparative pathology of tuberculosis animal models, see reference 2).

Nonterminal monitoring procedures, such as live imaging modalities, are increasingly being applied during TB drug efficacy experiments in animals and in human clinical trials (12, 32, 40, 52). Structural and/or functional features observed in imaging modalities such as computed tomography (CT) and positron emission tomography (PET) are particularly attractive because they can be measured serially in a single subject at many time points during treatment. Computed tomography (CT) can add highly detailed information to the characteristic features of pulmonary tuberculosis visualized using conventional chest X-rays (1). CT scanning is typically used to monitor patients, assist in diagnosis, and assess surgical options for drug-resistant cases of disease (26), but there have been few examinations of the rate of change in CT findings during chemotherapy. The most detailed study of TB chemotherapy in patients (25) examined high-resolution CT scans from patients undergoing TB chemotherapy for up to 20 months. Old fibrotic lesions could be distinguished from active lesions, and criteria for the state of metabolic activity of lesions were proposed. However, that study did not sequentially...
evaluate individual patients but rather imaged groups of patients at defined times. There is little literature on the evolution of chest CT changes during the course of antituberculosis drug treatment and no correlation with particular CT features in regard to the outcome of therapy or any clinical measurement of disease status (25). Such data are now being collected in several ongoing trials (NCT00425113, NCT00727844, and NCT01071603).

Positron emission tomography (PET) imaging has also seen little use in the evaluation of pulmonary TB. The few published reports employ [18F]fluoro-2-deoxy-D-glucose (FDG), a relatively nonspecific marker of inflammation, and have emphasized understanding the characteristics of lesions in asymptomatic patients to accurately distinguish tuberculomas from malignancies, driven by the diagnostic dilemma faced by oncologists (17, 21, 24).

A recent study evaluated the ability of maximum standardized uptake value (SUVmax) from [18F]FDG PET to retrospectively distinguish between biopsy-confirmed active and inactive tuberculomas (30). Serial [18F]FDG PET has also been used to monitor treatment in mice that developed caseating lesions in the lung using a small-animal PET-CT (12).

Most Mycobacterium tuberculosis strains are significantly less virulent in rabbits than Mycobacterium bovis and typically cause less progressive disease without cavities (9, 34). In aerosol infection of outbred New Zealand White (NZW) rabbits, the number of bacilli required to establish a visible pulmonary lesion is 3 for M. bovis Ravenel, whereas M. tuberculosis strains Erdman, H37Rv, and CDC1551 require several hundred to several thousand (5, 10, 35). While M. bovis strains often cause chronic or progressive disease, the M. tuberculosis strains are slowly cleared, with only strain Erdman establishing a chronic disease with coalescing or caseous lesions in 53% of rabbits (35, 36). M. bovis strains showed significant pathogenesis in a rabbit model of meningitis with more extensive pathogenesis and dissemination from the CNS to distal significant pathogenesis in a rabbit model of meningitis with more cavity production (9,11, 29).

Human features of tuberculosis in terms of granuloma formation disease, the tested in the meningitis model, caseous lesions in 53% of rabbits (35,36). While M. tuberculosis strains that have been virulent in rabbits than M. bovis, and typically cause less progressive disease without cavities (9, 34). In aerosol infection of outbred New Zealand White (NZW) rabbits, the number of bacilli required to establish a visible pulmonary lesion is 3 for M. bovis Ravenel, whereas M. tuberculosis strains Erdman, H37Rv, and CDC1551 require several hundred to several thousand (5, 10, 35). While M. bovis strains often cause chronic or progressive disease, the M. tuberculosis strains are slowly cleared, with only strain Erdman establishing a chronic disease with coalescing or caseous lesions in 53% of rabbits (35, 36). M. bovis strains showed significant pathogenesis in a rabbit model of meningitis with more extensive pathogenesis and dissemination from the CNS to distal organs (46–48). Among M. tuberculosis strains that have been tested in the meningitis model, M. tuberculosis HN878 and W4 persisted in the rabbit CNS until at least 8 weeks, whereas H37Rv and CDC1551 were cleared completely. Aerosol infection of rabbits with M. tuberculosis strain HN878 recapitulates many of the human features of tuberculous in terms of granuloma formation and cavity production (9, 11, 29).

In this study, we used [18F]FDG PET-CT to monitor disease development and chemotherapy in M. tuberculosis HN878 aerosol-infected rabbits. We used two front-line antituberculosis drugs, isoniazid (INH) and rifampin (RIF), to treat M. tuberculosis-infected rabbits and monitored their response to treatment by PET-CT followed by necropsy and lesion characterization.

**MATERIALS AND METHODS**

Additional details of the experimental methods are available in the supplemental material.

**Animals and ethics assurance.** This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Committee on the Ethics of Animal Experiments of the National Institute of Allergy and Infectious Disease approved the experiments described herein under protocol LCID-3 (permit issued to NIH Intramural Research Program as A-4149-01), and all efforts were made to minimize suffering of these animals. Female New Zealand White (NZW) rabbits were individually housed in cages in a biological level 3 animal facility approved for the containment of M. tuberculosis. Females were used to reduce fighting while the animals were acclimatized prior to infection and eliminate the need for birth control during the experiments.

**PET-CT scanning and data analysis.** [18F]fluorodeoxyglucose ([18F]FDG) was injected intravenously (IV) (1 mCi/kg), and scans were performed to 60 min later. The uptake periods and doses were matched within 5 min and 10%, respectively, to facilitate quantitative assessment of [18F]FDG PET uptake parameters as recommended in the PERCIST criteria (51). Once the animal was stably anesthetized, a 250-mm-long CT scan through the lung and into the abdominal cavity was obtained (~25 s, with a breath hold accomplished using mechanical ventilation at about 70% inflation). The animal bed was then repositioned in the micro-PET gantry, and a series of three 10-min emission scans with 75-mm-thick slices (windows) with a 20-mm overlap were acquired in the caudal-to-cranial orientation. CT scans were exported from a Ceretom workstation (NeuroLogica Corporation, Danvers, MA) into an Inveon research workstation (IRW; Siemens Preclinical Solutions, Knoxville, TN) equipped with a Focus 220 PET scanner and coregistered to four radioactive, CT–detectable fiduciary markers. Alignment of the fiduciary markers was not always sufficient for accurate thoracic alignment; final registration was best achieved by aligning blood vessels within the thoracic cavity with the FDG signal. Upon registration, the aligned scans were DICOM converted. Further analysis used both IRW software and Osirix (version 3.8, 64 bit; Pixmeo SARL, Bernex, Switzerland) (43).

Regions of interest (ROI) for accessing rabbit lung density and volume were defined by isolating the lungs using the CT. The top of the lung was defined as 10 slices above the tracheal bifurcation, 6.25 mm to the caudal-most region. Parenchymal lesions were identifiable as high-density areas surrounded by low-density lung tissue; lesions that were adjacent to the chest wall, heart, and large vessels were isolated by tracing the natural contour of the chest wall, heart, large vessels, or lesions. The remaining areas of the lungs were then segmented based upon the observed density measured in Hounsfield units (HU), a quantitative radiodensity scale in which air is −1,000, water is 0 and bone ranges from +700 to +3,000. Lesions partially overlap soft tissue and have densities that range from −300 to −100 HU but can be higher for calcified areas. Therefore, the lung volumes were divided into three ranges: high density (−125 to 175), medium density (−625 to −225) and low density (−1,024 to −725). Changes in lesion volume were measured from the CT scans using IRW software (Siemens Preclinical). Three-dimensional images were reconstructed using Amira software (Visage Imaging, Inc., San Diego, CA).

In order to determine the densities of individual lesions in serial scans, the axial slice with the greatest area of the lesion was identified in the pretreatment scan, and the brush tool was used to select the entire lesion; the mean, maximum, and minimum HU (the standard deviation [SD]) across each ROI were automatically extracted and exported. This process was repeated for the subsequent scans using the corresponding axial slice. The growing region tool was used to define the upper and lower threshold density units for the lesion, and the ROI was propagated in three dimensions. The accuracy of the propagation was confirmed by examining each axial slice, the thresholds were adjusted if necessary, and the volume was recorded.

In order to examine the [18F]FDG PET characteristics, the axial ROI was propagated in three dimensions using the grow-region tool and the neighbor algorithm as for CT, and each ROI was confirmed by examining the corresponding axial slices. Thresholds were adjusted if necessary, and the mean, maximum, and minimum standardized uptake value (SUV) for the three-dimensional (3D) ROI was recorded. SUV is a dose-, weight-, and decay-corrected estimate of tissue radioactivity concentration obtained by taking the measured radioactivity and dividing by the injected dose and the body weight of the animal. In addition, a background mean SUV in the local region was recorded, since the background varied in the rabbit lung. Lesions whose FDG uptake or CT density was obscured by the heart were not used for analysis. A partial volume correction factor was applied from a curve constructed from determining signal loss from hollow lesion microspheres (2- to 20-mm diameters) suspended...
in a 5-in. diameter phantom from Data Spectrum with nominal volumes of 31, 63, 125, and 250 µl in comparison to a large chamber containing the same decay-corrected $[^{18}F]$ FDG concentration. The recovery coefficients ($R$) were calculated as $A_{\text{sphere}}/A_{\text{chamber}}$, where $A_{\text{sphere}}$ and $A_{\text{chamber}}$ are the measured SUVs using the maximum pixel value method in the sphere and the chamber as described by Degirmenci et al. (13) and Vesselle et al. (49).

Aerosol infection of rabbits. For rabbit infection, the aerosol inocula of $M. \text{tuberculosis}$ strain HN878 were prepared by diluting titrated frozen stocks to $1 \times 10^5$ CFU/ml in phosphate-buffered saline (PBS). The aerosol was generated using a Bang nebulizer delivering 18 liters/min of filtered air and 6.4 liters/min of aerosol to the CH Technologies inhalation system (Westwood, NJ) housed and operated in a dedicated class 2A biological safety cabinet. Awake rabbits (2.4 to 3 kg) were restrained in veterinary bags with hoods and placed in nose-only exposure tubes, and 8 rabbits/run were exposed to the aerosol for 10 min followed by clean air for 5 min, removed from the tubes, and returned to their cages. This procedure delivers approximately 100 CFU/liter of infectious aerosol and generates ~50 granulomas per rabbit lung.

Drug treatment. Animals were habituated to a raspberry syrup vehicle compounded by the NIH veterinary pharmacy in a 50:49:1 ratio of Oraplus suspending vehicle, Orasweet flavored syrup vehicle (Paddock Laboratories, Minneapolis, MN), and raspberry flavor (LorAnn Oils Inc., Lansing, MI) as described previously (50). Isoniazid was dissolved directly in the syrup to a final concentration of 30 mg/ml. IV rifampin (600 mg) was dissolved in 12.5 ml of sterile water for injection and mixed with 12.5 ml of raspberry syrup to prepare a 24-mg/ml solution, and the solution was administered within 45 min of mixing with syrup. Animals were randomized to receive one of these drugs and received 1 ml/kg body weight/day dropwise via a syringe positioned at the back of the throat. Pharmacokinetic analysis of drug levels was performed as previously described (for details, see the supplemental materials) (31).

Statistical analysis. The statistical analyses were performed using an unpaired t test or the Mann-Whitney test for comparisons between two groups and one-way analysis of variance (ANOVA) with Dunnett’s multiple comparison test for three or more groups in Prism 5.0 (GraphPad), and $P$ values were calculated. Repeated-measures ANOVA or a paired t test was used to compare serial measurements of the same lesions or animals. Values are expressed as means ± standard deviations (SD). $P$ values less than 0.05 were considered significant, and confidence intervals (CI) represent the 95% intervals unless otherwise noted.

RESULTS

Rabbits infected by aerosol with $M. \text{tuberculosis}$ strain HN878 develop slowly progressing chronic disease. Thirty female rabbits (3.0 to 3.4 kg) were infected with $M. \text{tuberculosis}$ strain HN878 (a recent clinical isolate of the Beijing clade virulent in rabbits [29, 35]) by nose-only aerosol exposure in groups of 12 animals; 5 animals each were sacrificed at 0, 2, 6, 10, and 18 weeks. Aerosol infection delivered an average of 2.2 ± 0.13 (mean ± standard error of the mean [SEM]) log CFU/g lung at week 0 (2 h postinfection [p.i.]). By 2 weeks p.i., although lesions were invisible by gross inspection of the lung parenchyma, the number of bacteria had risen to 4.4 ± 0.13 log CFU/g (Fig. 1A). By 4 to 5 weeks p.i., visible granulomas had formed, with diameters ranging from 1 mm to 3 mm, and the bacterial load reached its maximum at 5.2 ± 0.19 log CFU/g, for approximately 6.5 log CFU/lung. By 6 weeks p.i. the bacterial load had dropped to 4.0 ± 0.15 log CFU/g. At subsequent time points, the bacterial burden was stable at 4.1 ± 0.30 (8 to 10 weeks) and 3.9 ± 0.20 log CFU/g (17 to 18 weeks). At the later time points of this experiment, the rabbits weighed about 4 kg and the infection had generated an average of 58 ± 16 (mean ± SD) granulomas per animal, demonstrating that $M. \text{tuberculosis}$ HN878 was able to establish a chronic infection in the rabbits.

During the inflammatory stage at peak bacterial load (weeks 4 to 5), HN878 lesions were typically solid cellular granulomas widely distributed in single focal lesions or in multifocal clusters (Fig. 1B) often located adjacent to the pleural surface. The small (1- to 3-mm) lesions typically consisted of central histocytes and neutrophils, with a thin layer of lymphocytes making up the periphery, but lacked central necrosis, which is often observed by 5 weeks in $M. \text{bovis}$-infected rabbits (9, 50). By 8 to 10 weeks p.i., most granulomas were surrounded by a thicker lymphocytic cuff, and within this cuff were more centrally located epithelioid macrophages mixed with rare multinucleated giant cells and a necrotizing center with eosinophilic staining material, typically with punctate nuclear debris (Fig. 1C). Cavities were occasionally observed among the necrotizing lesions, and these often still contained an interior partially filled with necrotic material. Cavities were identified either during CT scans as lesions with centers approaching −1,000 HU (the density of air in Hounsfield units) or during dissection as lesions that contained air. The cavity structure included an extensive central necrotic zone surrounded by a layer of intact macrophages, lymphocytes, and fibroblasts contained within an outer fibrotic wall. The chronic stage of progressive pulmonary infection with $M. \text{tuberculosis}$ HN878 was characterized by the development of heterogeneous lesions within the same animal, similar to those described in the human lung: solid granulomas that were exclusively cellular, tuberculomas containing a caseous necrotic center, and fibrotic lesions undergoing liquefaction.

PET-CT imaging reveals that $M. \text{tuberculosis}$ HN878 induced granulomatous inflammation peaks in the rabbit lung at 4 to 5 weeks p.i. Six age- and weight-matched rabbits with negative preinfection scans were serially scanned after infection with HN878. These animals were imaged every 2 to 3 weeks until 10 weeks p.i. and then once every 4 to 5 weeks until 20 weeks p.i. using a standardized imaging protocol where the feeding status, dose (mg/kg), tracer uptake period, and calibration of the scanner were carefully controlled (39). Three animals were scanned and necropsied at 4 weeks, and three more animals were scanned and necropsied at 9 weeks in order to match the PET-CT observations with histopathological observations and obtain data on bacterial burden in lesions.

Preliminary PET-CT studies had indicated that uninfected, commercially bred NZW rabbits lacked significant $[^{18}F]$ FDG uptake above background in the lung and thoracic lymph nodes. Scanning at 2 weeks p.i. produced CT and $[^{18}F]$ FDG PET images similar to baseline scans. Slight increases in lung density as measured by CT and diffuse uptake of $[^{18}F] $ FDG in the lung was detectable beginning at 3 weeks p.i. (Fig. 2). Figure 2 shows the progression of disease by CT and PET over 20 weeks of infection in one representative animal from this group. Each panel of three images depicts (from left to right) the CT, fused PET–CT, and PET images from 3 merged axial slices (3.75 mm of lung) carefully matched in absolute position throughout the infection. Although the lung appeared superficially unaffected in the 3-week scans, the background mean SUV of the lung increased to ~2 at 50 min after $[^{18}F] $ FDG injection from a baseline of 1 preinfection. By 4 weeks, a pronounced granulomatous inflammation was present with many small, apparently homogeneous lesions. PET-CT revealed a surprising variability in the FDG avidity of lesions that appear
highly similar by CT. The fate of individual lesions was likewise highly variable. For example, two adjacent lesions with similar volumes and densities also had a similar SUV\textsubscript{max} at 4 weeks (SUV\textsubscript{max} = 23 and 22) and at 6 weeks (SUV\textsubscript{max} = 24 and 21), yet the more FDG-avid lesion maintained FDG avidity and eventually formed a cavity that continued to increase in size from 9 to 20 weeks, while the slightly less avid lesion had almost completely resolved by 9 weeks (SUV\textsubscript{max} = 10) before coalescing with the growing cavity around 14 weeks. Across all of these animals, the formation and fate of individual lesions appeared to be largely independent of each other, and the factors that controlled the outcome of individual lesions were not clear.

By 9 weeks, many of the original lesions had resolved, and those that remained appeared to be stable or to slightly increase in volume over the remaining 2 months. Notably, we also observed that many of the transient lesions formed during the inflammatory phase of the infection showed quantitative decreases in the \([^{18}\text{F}]\)FDG PET uptake prior to a slow disappearance from the CT scans, indicating a decrease in metabolic activity prior to the loss of volume. After this time, both lesion volume and lesion SUV

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**FIG 1** NZW rabbits infected with *M. tuberculosis* strain HN878 experience a transient replicative phase, followed by chronic disease with lesions slowly increasing in density. (A) Time course of *M. tuberculosis* HN878 infection in the NZW rabbit lung compiled from data for untreated animals, depicting the mean bacterial burden (error bars show SD). (B) Image of H&E stained section of rabbit lung showing solid cellular lesions typical of lesions observed 4-6 weeks p.i. including peribronchial lymphoid aggregates (arrow). (C) Necrotizing tuberculomas were the predominant lesion type after 9 weeks p.i. (D) Graphic representation of the changes in lung density (HU) during the course of infection in a representative rabbit (Fig. 2). Each bar represents the total volume of the lung divided into three density ranges, with the HD lesion percentage indicated on the top of each bar. (E) HD and MD tissue volumes (mean ± SD) for 4 or 5 rabbits during 20 weeks of infection. (F) Densities of individual lesions with increasing time PI (n = 5 rabbits). Data are means and 95% confidence intervals. As the time PI increased, the mean density of the lesions increased (P < 0.0001, ANOVA and posttest for linear trend).
FDG uptake at 14 weeks but remained detectable in the parenchyma. (left arrow in week 4 panel) shrank to 1.5 mm in diameter and decreased in lost FDG avidity and resolved (white arrowhead). The 3-mm-diameter lesion served (arrows in CT panel). Some lesions increased in density (as expressed in HU) and size slowly and cavitated (weeks 6 to 20, downward arrowhead) or resolved (arrows in CT panel). A pronounced inflammatory response was observed from 4 to 6 weeks p.i. This experiment suggests the existence in the rabbit of a transient stage of early inflammation where small, mostly cellular lesions form and regress in the rabbit lung while other apparently indistinguishable lesions progress, cavitate, and develop a supplicative, semifluidic core similar to that shown in Fig. 1C. The mediastinal and hilar lymph nodes occasionally showed [18F]FDG PET activity, but few bacteria (<3 log/g) were recovered (data not shown).

**CT analysis reveals that persisting lesions increase in density during progression to chronic infection.** Radiodensity in the lung parenchyma is often an indication of active pathology, so we examined changes in lung CT density during infection. The total three-dimensional lung volume of the 6 rabbits was extracted from serial CT images, the volumes of tissue at specific densities (expressed in Hounsfield units [HU]) were calculated, and these volumes were grouped into three categories: high density (HD) (175 to −224 HU), medium density (MD) (−225 to −624 HU), and low density (LD) (−625 to −1,000 HU). In the naïve uninfected rabbit lung, the HD tissue, including arterial blood vessels and bronchi, makes up less than 2% of the volume of the lung, and the MD tissue, including thinner blood vessels, bronchioles, and lymphatic lung tissue, makes up about 15% of the volume, with the remaining LD volume being mostly parenchyma and air. Figure 1D shows the progression of density changes in the animal represented in Fig. 2. While no granulomatous nodules were visible on the CT at 3 weeks, a clear increase in the volume of HD and MD tissue in the whole lung was observed. This abnormal lung volume continued to increase and had more than doubled by 4 weeks p.i., when visible lesions could be detected. Although the percentage of affected lung decreased by 6 weeks, as did the number of lesions observed on the CT scans (Fig. 2), the percentage of affected lung did not return to baseline levels and in fact began to increase at later time points as the rabbits developed expanding cavitary disease (compare Fig. 2 at weeks 14 and 20 and with the evolution of MD and HD lung volume in Fig. 1D). This pattern was common among the 6 rabbits that were followed for the full 20 weeks (Fig. 1E) and the increase in HD and MD lung volume as a function of time PI was significant in 4 of 5 time points compared to naïve lung (P < 0.001).

The images were also examined to identify individual CT-dense nodules and other CT abnormalities located in the lung parenchyma. In order to determine the density of a lesion, an ROI was drawn using the brush tool in the axial slice with the greatest area of the lesion, and the mean HU ± SD were recorded for the ROI. As the time postinfection increased, the mean density of individual lesions increased, as shown in Fig. 1F (P < 0.0001, ANOVA and posttest for linear trend). Each symbol on the graph represents a single lesion (>20/time point), and each time point included lesion measurements from at least five of six rabbits. Once the rabbits reached 10 to 11 weeks p.i., the increasing mean HU of the lesions began to level off, and the difference in mean density at 11, 14, and 20 weeks p.i. was not significant. This observation was not simply a by-product of disappearance of the less dense lesions in the animals over time, as tracking of individual lesions showed the same density changes in successive scans. Lesions that were in the process of disappearing from the CT scan often decreased in density (as expressed in HU) prior to disappearing. This analysis revealed an overall hardening of persisting lesions over time.

**Comparison of bacteriologic response of 1 and 8 weeks of treatment with INH or RIF.** To assess the effects of chemotherapy on the radiologic features of this chronic disease, a group of 24 rabbits were infected with *M. tuberculosis* HN878 and treated with either INH or RIF. Daily doses of either INH or RIF were selected based on pharmacokinetic parameters determined in uninfected

FIG 2 FDG PET-CT scanning of *M. tuberculosis*-induced lung disease reveals an early inflammatory phase of disease that partially resolves, followed by slow progression. A group of 6 rabbits was infected and monitored by scanning 6 to 8 times over 20 weeks of infection. Images are presented with the sternum toward the top and vertebrae toward the bottom of each image and are composed of three merged axial slices (3.75-mm region of lung) from a single representative animal scanned 7 times (labeled [in weeks] on the left of the figure). A pronounced inflammatory response was observed from 4 to 6 weeks with many relatively homogeneous, 2- to 4-mm-diameter, density lesions observed (arrows in CT panel). Some lesions increased in density (as expressed in HU) and size slowly and cavitated (weeks 6 to 20, downward arrowhead) or lost FDG avidity and resolved (white arrowhead). The 3-mm-diameter lesion (left arrow in week 4 panel) shrank to 1.5 mm in diameter and decreased in FDG uptake at 14 weeks but remained detectable in the parenchyma.
rabbits (Table 1) and human exposure observed at doses in clinical use. Since the tolerability in rabbits of these two drugs for 2 months was unknown, the lower range of the calculated dose (30 mg/kg/day INH or 24 mg/kg/day RIF) was selected. The infected rabbits (8 to 9 weeks p.i.) were arranged randomly into six groups of 4 rabbits each. One group was euthanized after scanning at rabbits (8 to 9 weeks p.i.) were arranged randomly into six groups of 4 rabbits each. One group was euthanized after scanning at ~9 weeks; two groups were scanned, given 1 week of orally administered treatment, scanned again, and euthanized. The final three groups received 2 months of daily therapy with INH, RIF, or vehicle only and were then scanned a final time and euthanized.

After treatment, two types of lung samples, the entire right middle lobe and individual small granulomas from other areas of the lungs, were assessed for bacterial load. We included additional control data from animals in the disease progression experiment whose sacrifice times aligned with those of the treatment experiment. Animals treated for 1 week showed only a small, nonsignificant change in the mean CFU in the whole right middle lobe (Fig. 3A) and in individual lesions (INH, RIF) and in individual lesions (Fig. 3B) compared with the control animals. After 2 months of single drug treatment, even by gross pathology it was apparent that the number and size of lesions in the lung were reduced. Although lesions were less obvious after 2 months of treatment, fibrotic scars on the pleural surface of the lung were still evident, and these as well as caseous lesions were collected for CFU assessment. Both the INH and RIF treatment groups had a significant reduction in CFU in the whole right middle lobe (−1.9 log [P < 0.001] and −1.4 log [P < 0.01], respectively) (Fig. 3A) and in individual lesions (INH, −3.6 log/g, and RIF, −2.7 log/g, both P < 0.001) (Fig. 3B) compared with the vehicle control animals.

Pathology score changes in response to 1 or 8 weeks of treatment with INH or RIF. The gross findings at necropsy were tabulated into a pathology score (PS) that allowed comparisons between groups of animals exposed to different treatments or times of infection. It enumerated the number and size of pathological lesions in the lung and associated lymph nodes within the thorax and extrapulmonary organs (6). Additional factors, such as the presence of focal parietal pleural adhesions, thickening of the parenchyma, and cavitation, were also included. The PS was a composite number per rabbit; the data for the groups of rabbits given chemotherapy are summarized in Fig. 3C. Uninfected rabbits housed in same facility routinely had a PS of 0. The scores for untreated animals 9 to 10 weeks p.i. ranged from 16 to 35 (mean ± SD, 25 ± 6.9). Animals sacrificed 2 months later after having been administered only vehicle control had a similar PS of 20 ± 7.3 (Fig. 3C). Animals treated for 1 week had lower but not significantly different mean scores: 15 ± 7 and 16 ± 8 for INH and RIF, respectively. Animals treated with INH for 8 weeks had a significantly lower mean PS (7.6 ± 5.2; CI, 1.61 to 23.59; P < 0.05 in Bonferroni’s multiple comparison test for difference) than the

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### Table 1: Pharmacokinetic parameters of INH and RIF in uninfected rabbits and human equivalent doses based on clinical exposure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INH (mg/kg)</th>
<th>RIF (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Cmax (µg/ml)</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Tmax (h)</td>
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<td>0.5</td>
</tr>
<tr>
<td>AUC (µg · h/ml)</td>
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<td>18</td>
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<tr>
<td>t½ (h)</td>
<td>1.1</td>
<td>1.2</td>
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**INH**

- Human daily dose (mg): 300
- Human AUC_{0–24} (µg · h/ml): 17–48
- Equivalent rabbit dose (mg/kg): 30–60

**RIF**

- Human daily dose (mg): 600
- Human AUC_{0–24} (µg · h/ml): 20–60
- Equivalent rabbit dose (mg/kg): 20–30

**NOTES:**

- For more details, see the supplemental material and reference 30.
- Data are from references 41, 42, and 53 (for INH) and references 18, 41, and 54 (for RIF).

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### Figure 3: Comparison of *M. tuberculosis* bacterial burden after 1 week or 8 weeks of INH or RIF treatment.

(A) Mean bacterial burden (log CFU/g) (error bars show SD) of the right middle lobe of rabbits treated with either INH (30 mg/kg) or RIF (24 mg/kg) for 1 or 8 weeks in comparison with control rabbits (8 to 10 weeks p.i.) or rabbits treated with vehicle alone for 8 weeks (vehicle control). The ANOVA for the 6 groups did not show significant differences, but the difference in the means of the control and the INH 8 and RIF 8 CFU were each significant (*, P = 0.0061, nonpaired t test). (B) Mean bacterial burden of excised lung lesions (1- to 3-mm diameter) of rabbits treated with either INH or RIF as described for panel A (error bars show SD). Lesions that had become fibrotic but were still identifiable as abnormal lung were included but had few remaining cultivable bacilli, and many of these samples were at or below the limit of detection. The median CFU/g lesion of the control rabbits and those treated for 1 week were not significantly different, but values were significantly different when the control was compared with INH 8-week and RIF 8-week medians (* and **, P = 0.0002 and 0.0001, respectively, by ANOVA). (C) Box-and-whisker plot (whiskers represent minimum to maximum) of pathology scores of rabbits treated with INH or RIF for 1 or 8 weeks compared to untreated controls (9 to 10 weeks p.i.) or vehicle for 8 weeks (P = 0.025 ANOVA) with only a significant difference in control compared to INH for 8 weeks (*, P < 0.05 by Bonferroni’s multiple comparison).
vehicle controls. Treatment with RIF did not achieve a high level of statistical significance using this scoring system despite the highly significant reduction in bacterial burden. While this scoring system has proved to be useful in macaque studies, where the animals typically have more varied lesion types in more organs and drug treatment is begun with the onset of disease symptoms (6), the usefulness of this score in rabbits, where treatment is initiated in asymptomatic animals, is less clear.

**Histopathological and morphometric changes in response to treatment with INH or RIF.** For each animal, at least one cross-sectional slice of each lung lobe (other than the right middle lobe, which was used for CFU enumeration) was prepared by paraffin embedding and sectioning, and hematoxylin-and-eosin (H&E)- and Ziehl-Nielsen-stained sections were prepared. The H&E sections were scanned, and the resulting files were examined using ImageScope version 10 (Aperio Technologies). The percentage lesion area for each section was calculated by morphometric image analysis by using Matlab, as described previously (20). Figure 4 shows a composite of representative low-magnification images of rabbit lungs. Well-organized solid and necrotizing granulomas existed (Fig. 4A to H) at 9 to 10 weeks p.i. Most granulomas were surrounded by a lymphocytic cuff, and within this cuff were more centrally located epithelioid macrophages mixed with occasional plasma cells and multinucleated giant cells; in addition, necrotizing granulomas contained eosinophilic staining material (best seen in Fig. 4D, G, and H). There was no apparent difference in the sections collected at 9 to 10 weeks (Fig. 4A and B), which contained 3.4% ± 2.3% (mean ± SD) lesion tissue by area, and those collected after 1 week of treatment (INH, Fig. 4C and D; RIF, Fig. 4E and F), which contained 2.1% ± 1.6% and 1.9% ± 1.5% lesion areas, respectively. These sections contained frequent small (1- to 2-mm) lesions located adjacent to the blood vessels (Fig. 4A and C) and the bronchioles (Fig. 4E and F) along with regions of bronchiolar thickening (A and E), as well as lesions on the pleural surface (B, D, and H). Lesions near the vessels along with bronchiolar thickening were visible by microscopic examination but were more difficult to measure with CT scanning, although [18F]FDG activity colocalizing with vessels was often detected in the lung regions (Fig. 4C to F). In the animals treated with INH (Fig. 4I and J) and RIF (Fig. 4K and L) for 2 months, these small lesions were infrequently observed compared with the vehicle control animals (Fig. 4G and H) and the majority of lesions remaining after treatment were either small aggregates of histocytes (Fig. 4I), occasional necrotizing granulomas, or a cavity showing

![Image of rabbit lungs with granulomas and lesions](http://aac.asm.org/)

**FIG 4** Histopathologic changes in response to 1 or 8 weeks of INH or RIF treatment. (A and B) Granulomas 1 to 3 mm in diameter (both necrotizing and cellular) were observed in animals euthanized just prior to experimental treatment initiation at 9 to 10 weeks p.i. (C to F) Similar lesions with central acellular necrotic regions often located adjacent to blood vessels or airways were present after 1 week of treatment with vehicle (G and H), INH (I and J), or RIF (K and L), the drug-treated lungs contained fewer lesions than lungs from rabbits given vehicle alone (P = 0.0306 by ANOVA). Small lesions proximal to the airways were less frequent in the drug-treated animals, but some fibrotic scars (I, arrow) and cavities (L, arrow) with mineralized necrotic interiors (L) remained. Bars, 1 mm. (M) Percent area of lesions was determined for each animal using custom image analysis on 3 to 5 lung sections per animal. The median lesion area was significantly reduced in the groups treated with either RIF or INH compared to those receiving vehicle alone (P = 0.016 and P = 0.016, respectively, by Mann-Whitney test with 95% confidence intervals). Bars show the medians.
some mineralization (Fig. 4L). These measurements represent a significant response to treatment ($P = 0.015$) with either INH (lesion area [mean ± SD], 0.3% ± 0.2%) or RIF (mean lesion area, 0.5% with the cavity and 0.3% ± 0.2% [SEM] without the cavity) compared to the vehicle control (lesion area [mean ± SD], 6.6% ± 5.7%) (Fig. 4M). Both mean lesion number and mean lesion size calculated by the morphometry analysis were reduced in the 8-week drug treatment groups, but only mean lesion area reached significance (data not shown). Cavities were observed in both untreated animals (Fig. 2; 9, 14 and 20 weeks) and in the RIF-treated group (Fig. 4L; also, see Fig. 5B).

PET-CT changes in lesion volume and [$^{18}$F]FDG avidity quantified the efficacy of 2 months of treatment with INH and RIF in vivo. Studies of human lungs using serial imaging by CT show that there is a relatively slow response to treatment (25), so it was hypothesized that rabbits receiving only 1 week of treatment would show little or no change in lesion structure (assessed by CT) but greater changes in metabolic activity (assessed by [$^{18}$F]FDG PET uptake). The rabbit groups receiving 2 months of treatment were expected to show changes in both imaging modalities compared to control animals. The [$^{18}$F]FDG PET-CT images clearly documented changes in lesion volume and number after 4 and 8 weeks of treatment with INH or RIF (Fig. 5). Examples of this response can be seen in Fig. 5A, which shows two CT-dense lesions and a larger consolidation that shrank as treatment was administered (4 and 8 weeks). The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively. The middle lesion of the three indicated with arrows had an initial volume of 130 mm$^3$, which was reduced to 49 and 14 mm$^3$ after 4 and 8 weeks of therapy, respectively.
PET-CT Response to Chemotherapy in TB-Infected Rabbits

Assessing quantitative measures of drug efficacy from PET-CT images. Using manual analysis tools, the total volume of pulmonary lesions in each animal was measured from the series of CT scans collected during treatment; these totals included all abnormal findings in the lungs, including granulomas, consolidations, and obvious thickening of the airways or vessels (see Fig. S2 in the supplemental material). A repeated-measures ANOVA was used to assess the change in volume over time. The total volume of the animals given vehicle was not significantly reduced from 8 to 16 weeks p.i., as expected (see Fig. S2A). Although the total volume of lesions was reduced in 6 of 8 animals receiving INH treatment (see Fig. S2B), the change for the group as a whole was not significant. This is despite reduction of the bacterial burden and reduction in the volume of lesions calculated from histological morphometry. One possible explanation for this observation was an increase in lung consolidations in one animal completing treatment (two consolidations each with a volume of at least 700 mm$^3$ but relatively low SUV$_{\text{max}}$s (5 to 5.7) formed late in treatment, and this tissue had low bacterial numbers (1.9 to 2.2 log$_{10}$ CFU/g lung) that were not INH resistant. A second animal in the 8-week INH group experienced weight loss and symptoms consistent with INH-induced hepatic encephalopathy and had to be sacrificed prior to the final scan. The change in lesion volume in RIF-treated animals was significant for the repeated-measures ANOVA after 4 weeks ($P < 0.01$; CI, 111.2 to 593.1) and 8 weeks ($P < 0.01$; CI, 136 to 618) of therapy but not after only 1 week of treatment (see Fig. S2C).

We also analyzed three independent lesion features with respect to time receiving treatment and drug: volume, density, and corrected SUV$_{\text{max}}$ (Fig. 7). The CT volumes of individual lesions in each rabbit scan were determined by manually tracking lesions in sequential scans during therapy. There were nonsignificant reductions in CT volume as early as 1 week posttreatment (Fig. 7A). These became statistically significant after 4 weeks and 8 weeks of treatment, but the development of consolidations in one animal confounded the analysis at 8 weeks (Fig. 7B). We also investigated if treatment was associated with a change in density of the lesions due to reduced bacterial burden resulting in more fibrosis or calcification. One rabbit treated with RIF (Fig. 5B and in Fig. 4L) did have lesions that increased in density as treatment continued, but the majority of animals had lesions that decreased in density during treatment. Although the changes were not significant at 1 week (Fig. 7D), the reduction in lesion density was highly significant.
and correlated with treatment response after 4 and 8 weeks of treatment. Finally we looked at changes in $[^{18}\text{F}]$FDG PET avidity for individual lesions as measured by SUV_{max}. After 1 week of therapy, SUV_{max} corrected values were significantly decreased in both the INH and RIF therapy groups (Fig. 7E) (for INH, $P = 0.003$; CI, 0.9456 to 4.035; for RIF, $P = 0.007$; CI, 0.862 to 4.596). FGD uptake of most lesions decreased with increasing treatment time for both INH ($P < 0.0001$ by one-way ANOVA and $P < 0.0001$ for linear trend, negative slope) and RIF ($P < 0.0001$ by one-way ANOVA and $P < 0.0001$ for linear trend, negative slope) as shown in 7E. In animals not receiving treatment, the changes in $[^{18}\text{F}]$FDG PET avidity and lesion volume over time were not significant, but lesion density ($P = 0.02$) increased over time and had a positive linear slope.

To see if there was a direct relationship between SUV_{max} and bacterial burden in individual lesions, we isolated lesions at necropsy and plated them for assessment of bacterial burden. The number of lesions available for this analysis was limited, as many lesions, especially in the treated animals, were no longer visible by CT and showed only background SUV. The data for small (1- to 4-mm-diameter) nodules presented in Fig. S3 in the supplemental material are suggestive but not conclusive that a positive linear relationship exists between SUV and bacterial burden in untreated animals.

**DISCUSSION**

In this study, we employed both structural (CT) and functional (FDG PET) imaging to serially follow the progression or resolution of disease in rabbits over time. The use of these imaging modalities allowed us to observe an inflammatory stage of disease characterized by increases in lung density and $[^{18}\text{F}]$FDG uptake as early as 18 days postinfection, continuing to the point where formation of CT dense lesions with a distinct margin occurs, by 4 to 5 weeks. The peak of this period of active inflammation involved a total of nearly 20% of the entire lung volume of the animal and corresponded to the peak bacterial burden of the infection. This inflammatory stage was followed by a resolving period during which approximately half of the disease volume disappeared and the bacterial burden declined by almost 10-fold. As the inflammatory stage waned, individual lesions that survived increased in mean tissue density and became much more diverse in pathological presentation. This second stable phase of the infection was
characterized by dynamic, local changes in individual lesions that waxed and waned on a time scale of weeks, occasionally liquefying to form cavitary disease.

Whether such changes occur during the course of human infection with *M. tuberculosis* is not known, but the poor correlation between peripheral markers of immune activation and extent of disease suggests that the local immune environment in different parts of the lung may vary significantly (22, 45). Most reports divide the spectrum of pulmonary tubercular disease into latent and active disease for the examination of peripheral blood markers correlated with active disease, so that a large range of disease burden is lumped together. It hardly seems surprising then that markers like chemokine ligand 2 (CCL2), associated with monocyte chemotaxis, have variable results in different studies (8, 22). At this point, no one factor seems to be able to predict the progression to active disease or the extent of disease once disease is established (15). In humans, 75% of disease occurs within the first year following infection, although such estimates are always confounded by uncertainty as to the exact time of infection (38). In mice, the chronic phase of infection follows activation of the innate immune response and typically occurs 4 to 6 weeks following infection (44). In our rabbit model, the chronic stage of disease develops about 2 months after infection, consistent with activation of innate immunity, and the resulting lesions suffer highly local fates, again consistent with the heterogeneity observed in the human host.

Both whole-lung-level and individual-lesion-level PET-CT data were useful for monitoring the course of disease. *In silico* isolation of the whole-lung volume and construction of a tissue density distribution (−1,025 to 175 HU) made it possible to follow the changes in the lung tissue density and estimate the volume of diseased lung in the scan. This type of quantitative analysis was not previously possible, and it allows a comparison of features with the subtypes of pathologically defined lesions that are regularly observed in human disease (33). Although most lesions were visible by histology as infiltrated alveoli, small granulomas, or lymphoid aggregates, some lesions appeared only as increasing density of lung parenchyma or as *[18F]FDG*-avid areas associated with airways or blood vessels in the rabbit. Human tuberculosis is also characterized by thickening of both bronchi and vasculature. Interestingly, the density of the majority of lesions in the inflammatory stage (mean, −235 HU) of the rabbits in this study corresponds to lesions identified by histological methods as containing solid cellular lesions lacking a large eosinophilic center. These lesions have almost identical counterparts in human tuberculosis as nonnecrotizing granulomas usually thought to arise from hematicogenous seeding of the infection upon secondary spread. Both rabbit and human tuberculosis share the occurrence of necrotizing granulomas with central necrosis and a lower central CT density. While these features were not delineated in this study, rabbit and human lesions also share a similar organization and cell composition, including the propensity to undergo liquefaction and form cavities (29, 33).

This chronic stage of rabbit infection offers the potential to obtain additional insights into chemotherapeutic response rates of specific types of tubercular lesions. To provide quantitative comparisons, we developed a method for measuring a variety of parameters from both CT and PET findings. Each of these parameters showed a statistically significant change with treatment, suggesting that they may be useful in assessing the activity of a new agent or regimen. In general, lesion density and volume, both measures of structural changes, required longer treatment times to provide robust measurements than functional imaging using PET. The change in *[18F]FDG* avidity, as measured by SUV<sub>max</sub> of lesions, could be assessed in as little as 1 week and provided robust, quantifiable changes that correlate with decreases in both pathology and bacterial numbers. These more rapid changes in *[18F]FDG* uptake could be particularly useful, for example, in dose-finding experiments or other situations where a rapid assessment of efficacy is desirable.

Autoradiography of FDG distribution in soft tissue infections have shown that uptake coincides with areas rich in inflammatory cells, typically neutrophils and macrophages (28). Pulmonary *[18F]FDG* uptake in inflammatory processes has been largely attributed to neutrophil uptake, since these cells have a large ability to modulate their glucose uptake (14, 27). Interestingly, we and others recently established the importance of neutrophils in human tuberculosis (4, 16).

In summary, the rabbit model of experimental tubercular chemotherapy provides a means to perform quantitative comparisons among candidates (or candidate regimens) in an animal model that accurately recapitulates human pathology. The model performed well with two of the most highly active constituents of front-line antituberculosis chemotherapy used in initial treatment of nearly all TB patients. In addition, we have developed numerous candidate endpoints that provide data to benchmark the ability of this animal model to predict response rates observed in prospective human clinical trials. PET-CT imaging methodologies that reveal changes in host inflammatory response have the potential to reduce the number of animals needed for intermediate time points during evaluation of new agents, and particularly in studies with relapse endpoints (12). Validation of this model against on-going clinical trials and development of a relapse endpoint are ongoing, emphasizing the possibility that this may become a truly predictive animal model for testing treatment strategies for human chemotherapy.

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REFERENCES