Advanced Imaging for Tuberculosis: Insights into Disease Pathogenesis

Unite to End TB by Making the Connections
Maryland Department of Health and Mental Hygiene
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SCHOOL of MEDICINE

Disclosures

- Grant funding: NIH
  Gilead Biosciences

- Patent: International patent PCT/US13/59897, 'Bacteria-specific labeled substrates as imaging biomarkers to diagnose, locate and monitor infections' filed

Sanjay K Jain

Objectives

- Describe a clinical vignette to demonstrate the power of imaging in monitoring drug-resistant TB
- Discuss limitations of current tools for diagnosis and monitoring of infections, and how imaging could overcome some of these limitations
- Discuss “perceived” and real risks of clinically available imaging modalities
- Describe two molecular imaging studies at Hopkins currently enrolling TB patients, and approved by the Maryland Health Department
- Describe some bacteria-specific imaging agents currently in development

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

A two year-old child from the United States developed pneumonia after a three-month visit to India:

- On arrival in India, she was immunized with BCG, stayed with her grandparents and attended a local day-care facility. During the last week of her visit, she developed fevers that continued after her return to the USA.
- Based on clinical presentation and CT imaging findings, she was started on empiric first-line TB treatment after obtaining sequential gastric-aspirates.
- Symptoms resolved with first-line TB treatment but extensively drug-resistant (XDR) *Mycobacterium tuberculosis* grew in culture.
- The child was started on an individualized drug regimen for XDR-TB consisting of intravenous streptomycin, linezolid, PAS, cycloserine and clofazimine.
- But treatment was complicated by uncertainties about drug selection, and lack of child-friendly formulations. ***So, we needed to know (quickly), whether the individualized drug regimen was working or not!!!***
- However, assessing response to treatment was challenging, as clinical response was noted with suboptimal regimens, and microbiology could also not be used in this child.

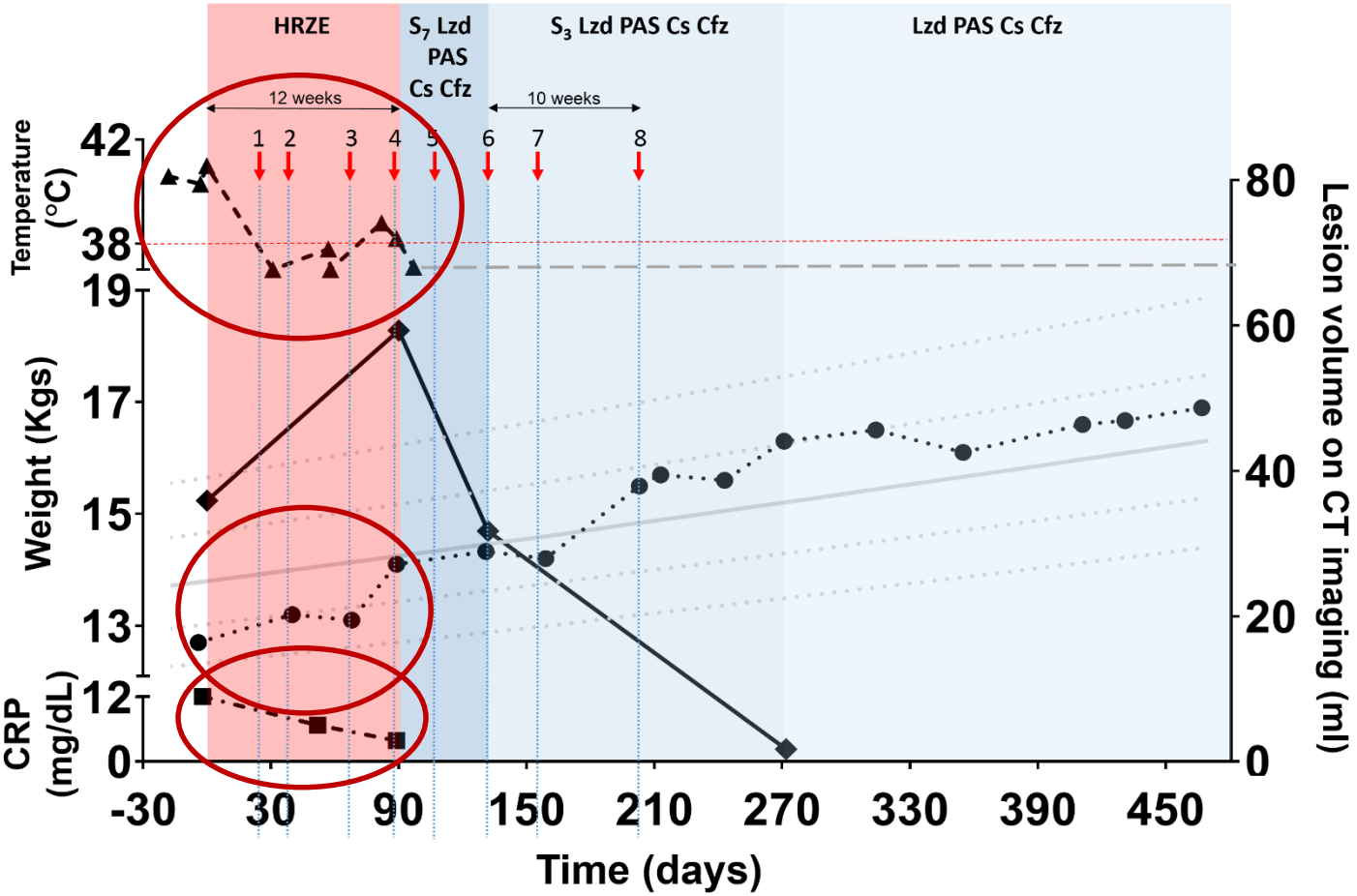
Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

- Clinical response was noted with a suboptimal regimen.
- Gastric aspirate smears were negative at the time of initiation of XDR-TB treatment, and cultures remained negative subsequently.
- Therefore, in the absence of clinical or microbiological markers, low-radiation exposure pulmonary CT imaging was used to monitor treatment response, and guide the individualized drug regimen for XDR-TB.

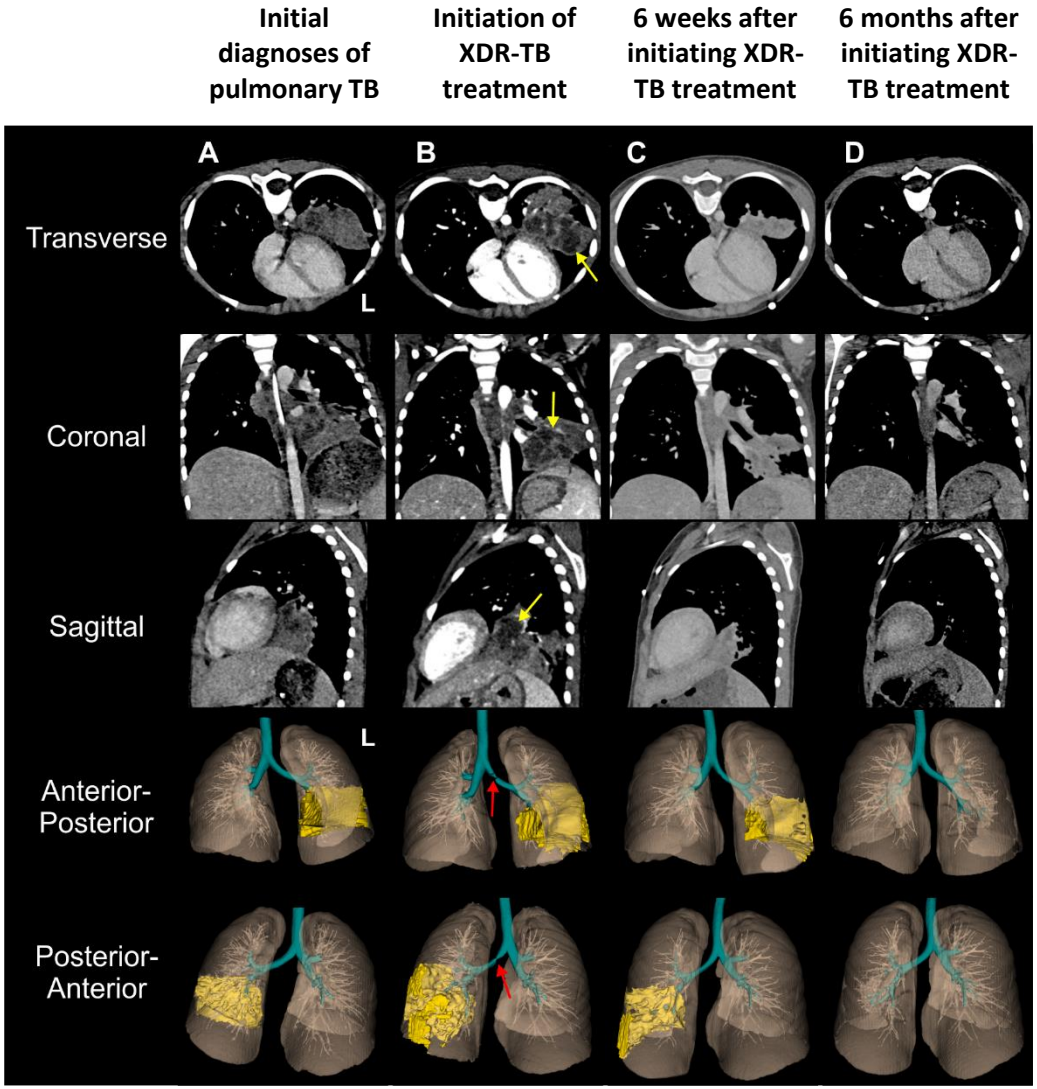
Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

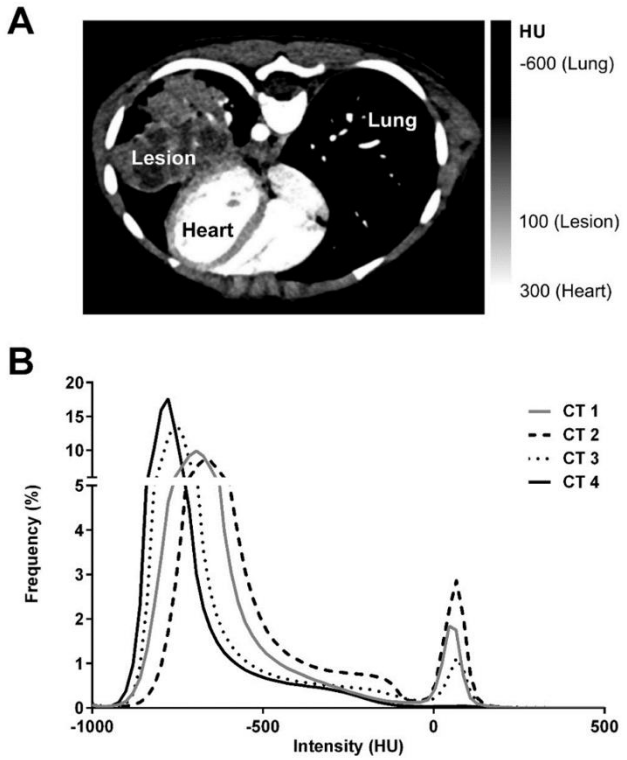
CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child



Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

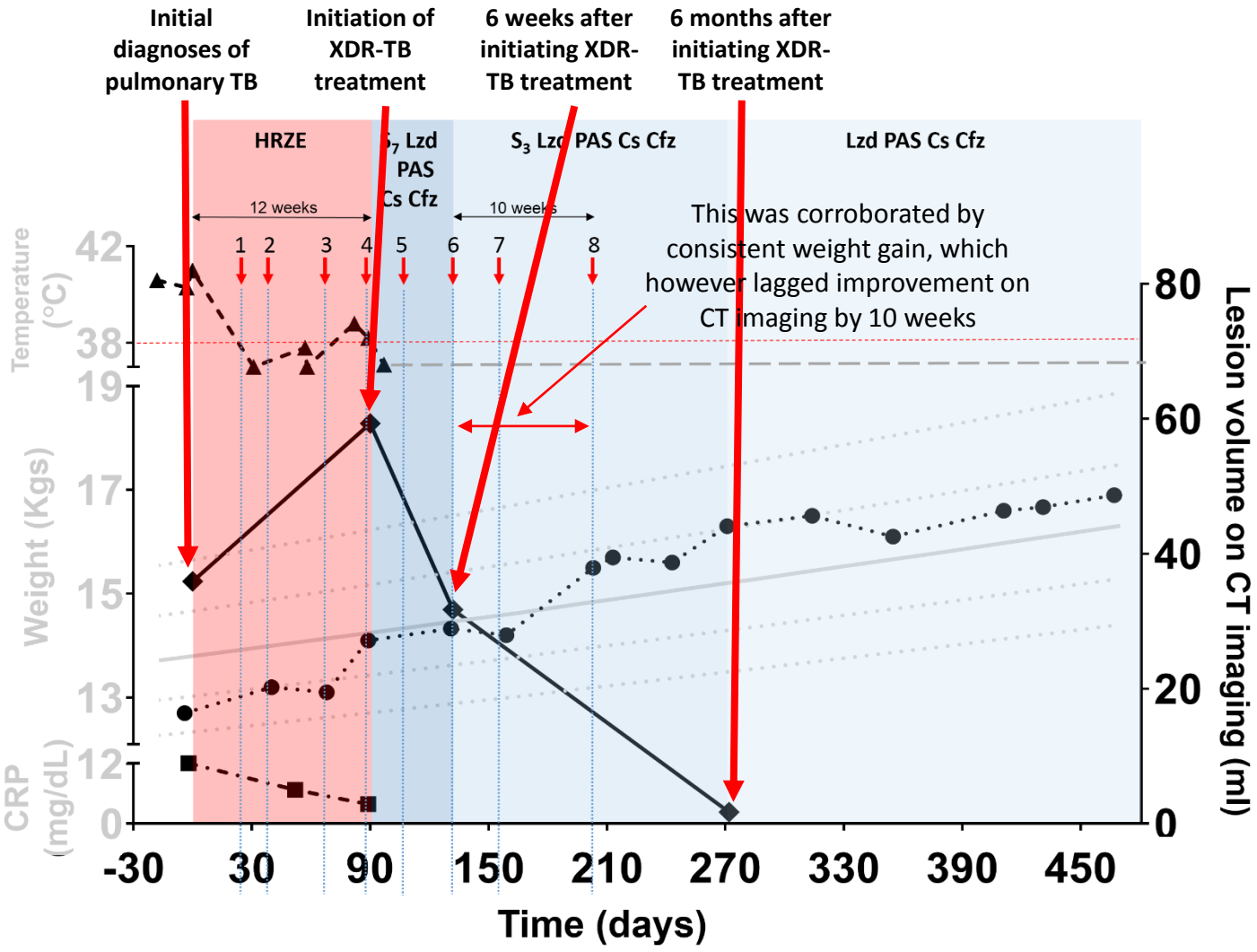
CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child





Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child



1, Growth of acid-fast bacilli in liquid broth;

2, Identification of *M. tuberculosis* complex by 16S rRNA sequencing;

3, Persistent left lower lobe infiltrate on chest radiography;

4, Sanger sequencing and initial TREK panel confirming XDR strain;

5, Agar proportion results (Maryland State);

6, CT demonstrating significant reduction in lesion volume;

7, Agar proportion results (National Jewish);

8, Consistent weight gain.

Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

CT Imaging to monitor Extensively drug-resistant (XDR) TB in a young child

- This report highlights the:
  - risks of acquiring drug-resistant (DR) TB overseas
  - *challenges of diagnosing DR-TB, especially in young children*
  - lack of dosing and child-friendly drug formulations
  - controversy regarding the infectious risk of young child and
  - *lack of tools to rapidly monitor DR-TB treatments*
- In this child, low-radiation exposure pulmonary CT demonstrated marked improvement as early as 6 weeks after initiation of treatment for XDR-TB, and corroborated by consistent weight gain, which however lagged improvement on CT imaging by 10 weeks.
- ***Treatments for drug resistant TB are often empiric, and the risks of treatment failure substantially outweigh the use of relatively advanced, but promising technologies that can provide rapid and reliable means to monitor treatments.***

Salazar-Austin *et al*. *Lancet Infect Dis.* 2015

Food for thought . . .

- Most experts agree that Infections will not be eradicated for decades, or maybe never . . .

- Are we planning for the future, and utilizing the advances in technology that may be applicable to Infectious Diseases?

- Does diagnosis and monitoring of infections in special situations (hard to get locations, difficult to grow bugs, e.g. *M. tuberculosis* or other fastidious bugs) merit the development and / or use of technologies, that may be different from those being developed currently?

The future is not what it used to be . . .

Fundamental diagnostic: 1882

Need to isolate the bug

Fundamental diagnostic: 2017

Need to isolate the bug

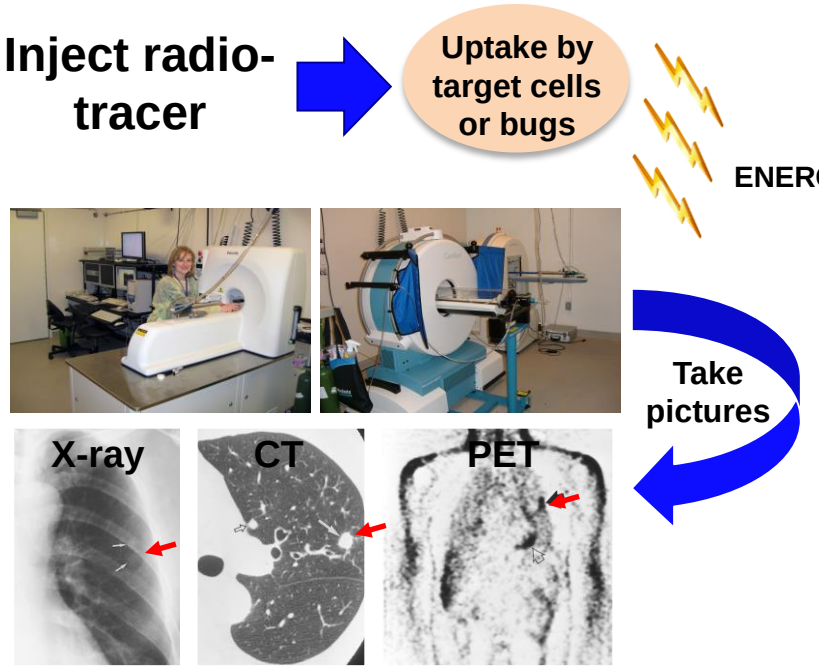
MGIT

GeneXpert

Problem(s) – Counting bugs

- Bugs: “need to bring them out, or go in and get them”
- Often impractical or dangerous
- Whole organ / body view of disease not available

Solution(s) – Take a picture instead



http://www.musc.edu/fnrd/primary.psych.images/page%2053.GIF
Goo JM et al. Radiology 2000

- Major investment by Oncologist to develop this technology for cancers
- Now used extensively for monitoring cancer patients during clinical trials and also for patient care
- ***Can these technologies be used in Infectious Diseases?***

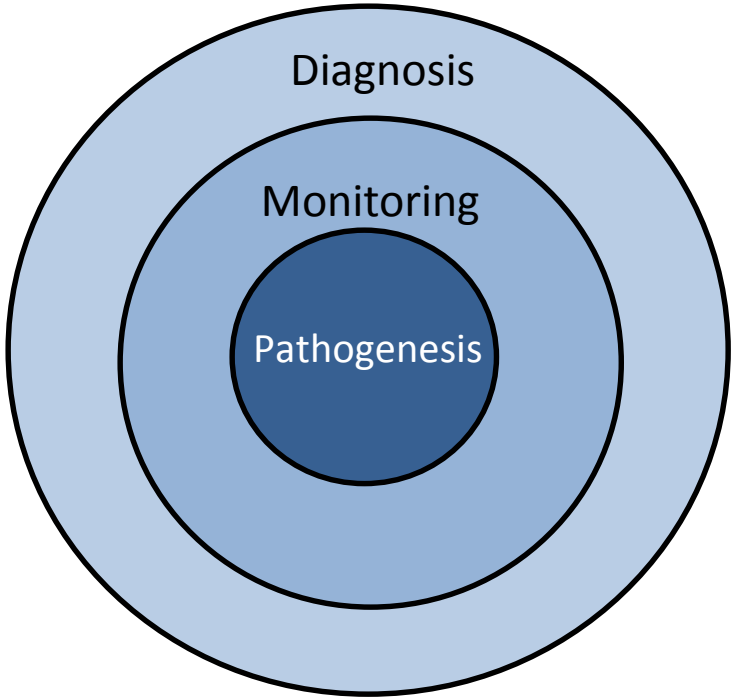
- Rapid, and *easily scalable to humans*

How can imaging help with TB trials and patients?

- Tomographic imaging can evaluate disease processes deep within the body, noninvasively and relatively rapidly.
- Longitudinal assessments can be conducted in the same individual, which is a fundamental advantage over the traditional invasive tools.
- Provides holistic, 3D views of the whole organ or body representative of the overall disease, and also less prone to sampling errors.

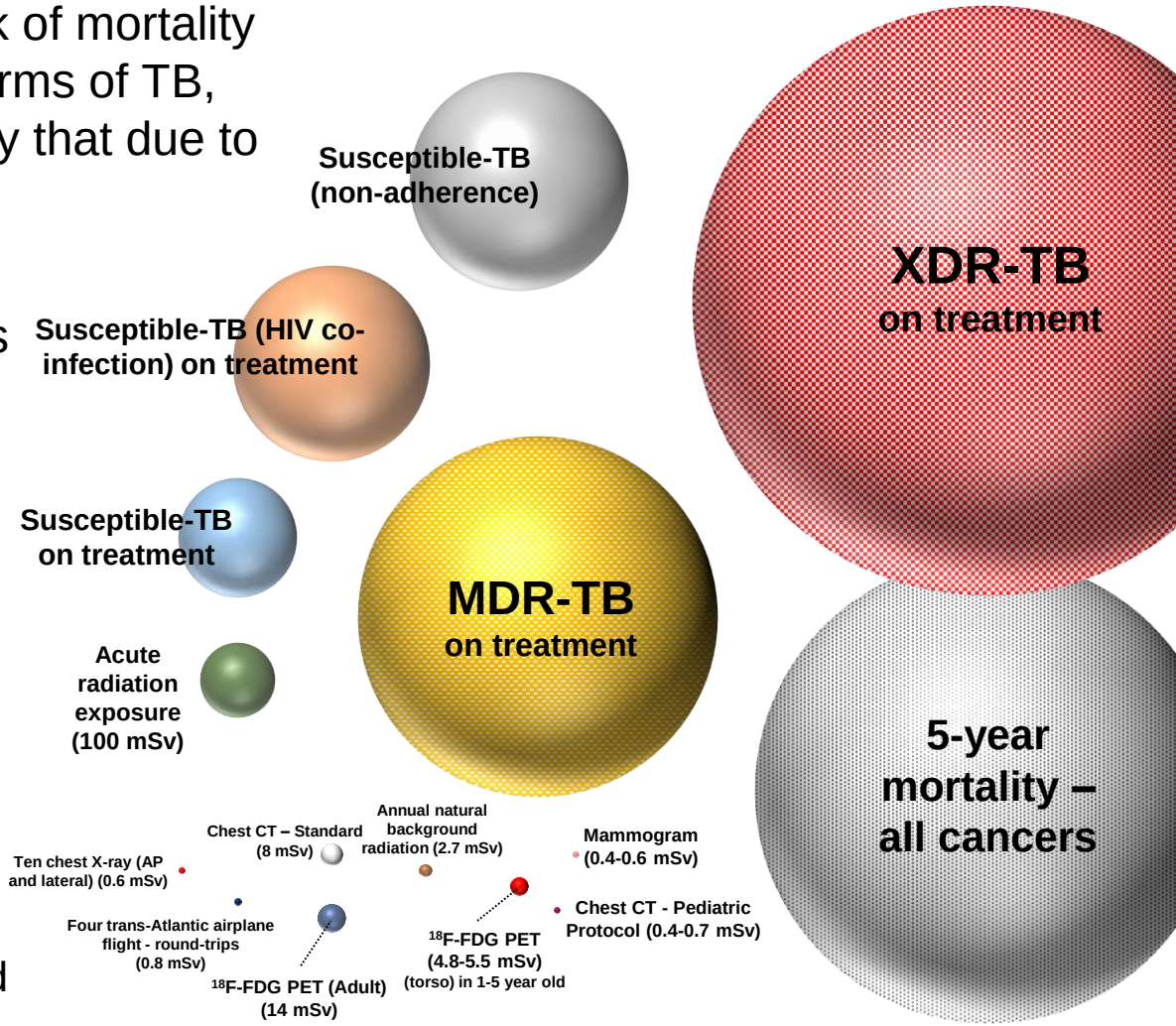
Purpose

- Diagnosis
- Monitoring and prognostication;
  end-points for treatment trials
  enable adaptive designs
- Understanding pathogenesis
  host-directed therapies
  multi-compartment PK



Risk of Imaging: Let's be real . . .

- Even with treatment, the risk of mortality is high for XDR and MDR forms of TB, and similar to 5-year morality that due to cancers.
- Even drug-susceptible TB with adequate treatment has substantially higher risk of mortality (~1000 times) than radiation induced cancers with optimized imaging.
- Radiation due to:
  - Optimized PET (Pediatric torso), similar to annual background radiation
  - Chest CT – Pediatric protocol, similar to a mammogram or four round trip trans-Atlantic flights

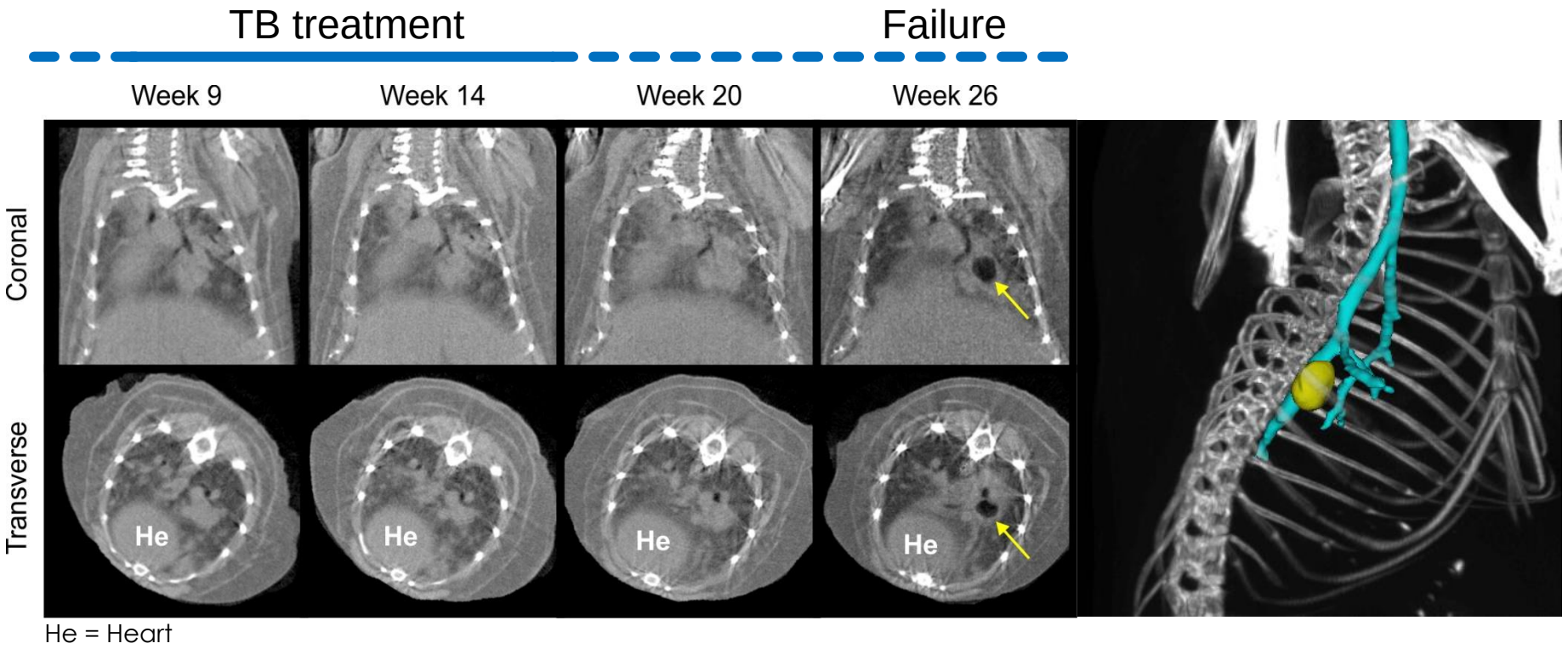


Only 52% and 28% of MDR- and XDR-TB treated successfully. WHO 2016
https://www.ncbi.nlm.nih.gov/books/NBK218704/
https://en.wikipedia.org/wiki/Orders_of_magnitude_(radiation)

Jain SK. *Mol Imaging Biol.* 2017

Following Cavity formation and Treatment Failure using High-Resolution CT Imaging

Development of a cavity during treatment failure, due to inadequate TB treatment



He = Heart

Ordonez and Tasneen *et al*. *Dis Model Mech.* 2016

TB granulomas (tumors) in mouse lungs

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2009, p. 4879–4884 Vol. 53, No. 11
0066-4804/09/$12.00 doi:10.1128/AAC.00789-09

Noninvasive Pulmonary [^{18}F]-2-Fluoro-Deoxy-D-Glucose Positron Emission Tomography Correlates with Bactericidal Activity of Tuberculosis Drug Treatment$^{\nabla}$†

Stephanie L. Davis,[1,2] Eric L. Nuermberger,[1,3] Peter K. Um,[1,2] Camille Vidal,[5,7] Bruno Jedynak,[6,7] Martin G. Pomper,[4] William R. Bishai,[1,3] and Sanjay K. Jain[1,2]*

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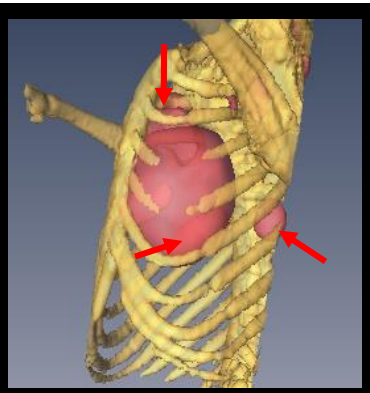
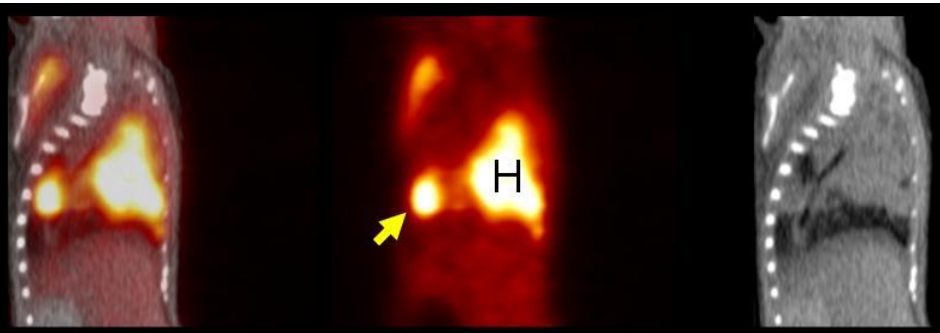
Received 12 June 2009/Returned for modification 15 July 2009/Accepted 26 August 2009



Sagittal

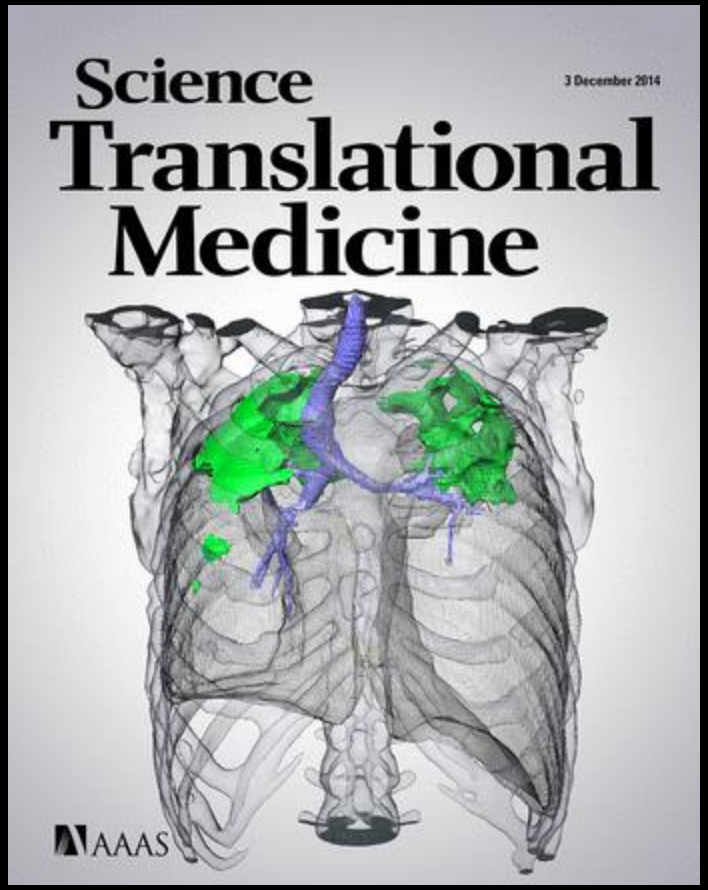
H = Heart



Davis *et al*. *Antimicrob Agents Chemother.* 2009

Human data: Sathekge *et al*. *J Nucl Med.* 2011; Chen *et al*. *Sci Transl Med.* 2014

^{18}F-FDG PET/CT correlates with treatment outcome in patients with MDR-TB

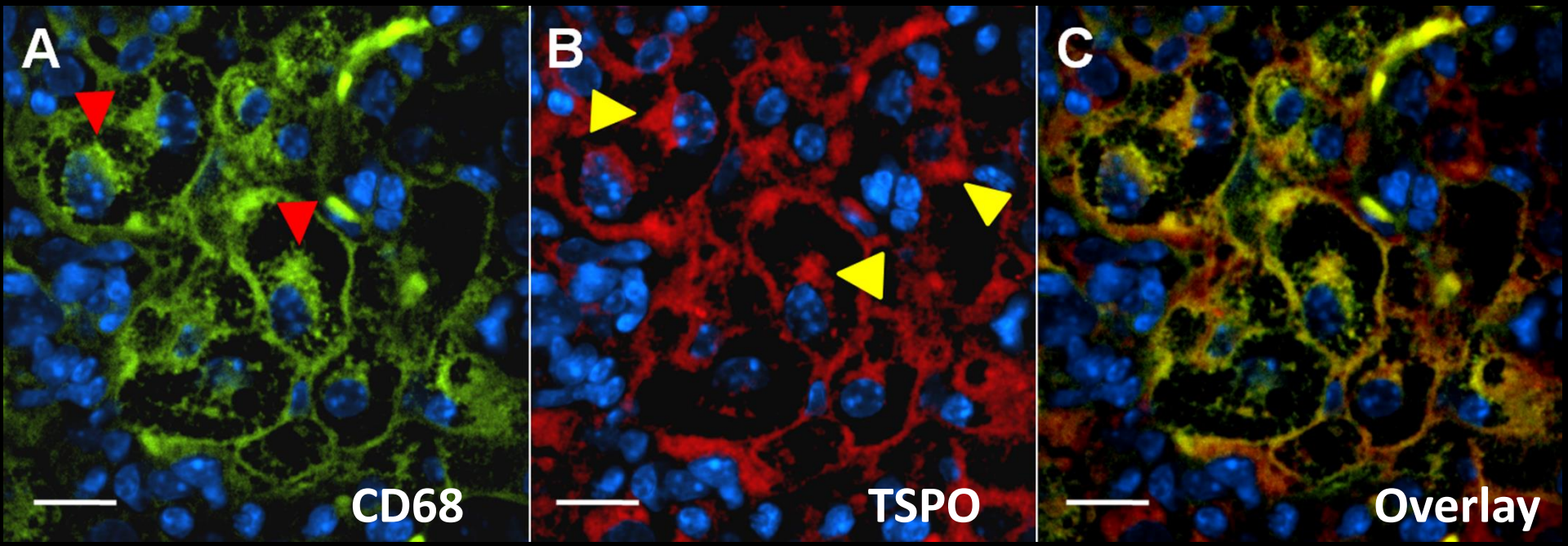


- Prospective imaged 35 adults (median age 37 years) with MDR-TB, on second-line TB treatment, using ^{18}F-FDG PET and CT at 2 and 6 months after starting treatment .
- Imaging assessed by radiologists or automated analyses.
- ^{18}F-FDG PET at 2 months and automated CT at 6 months were more sensitive than sputum smear or solid culture conversion at 2 months, these differences were not statistically significant, possibly because of the small sample size in our study.
- Automated methods were more reliable than radiologists.

Chen *et al*. *Sci Transl Med*. 2014

Imaging TB-associated inflammation with iodo-DPA-713

- Iodo-DPA-713 is a ligand for translocator protein (TSPO)
- Up-regulated in inflamed microglia and macrophages
- TB lesions full of activated macrophages



TSPO expression in macrophages within TB lesions

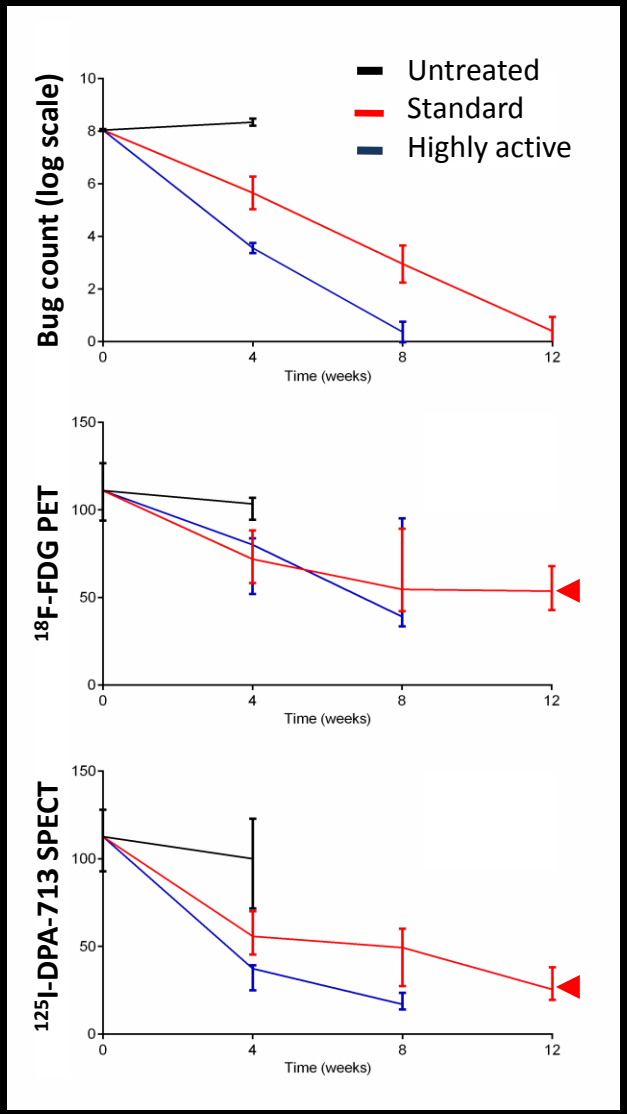
Foss *et al*. *J Infect Dis* 2013 (Cover article)

Imaging TB-inflammation to monitor treatments: ^{125}I-DPA-713-SPECT versus ^{18}F-FDG-PET

^{125}I-DPA-713-SPECT (blue-green) 4.06 ± 0.52

^{18}F-FDG-PET (orange) 2.00 ± 0.28

skeletal outline (grey)



Pulmonary ^{125}I-DPA-713 SPECT, *but not ^{18}F-FDG PET*, correctly identified the bactericidal activities of the TB treatments as early as 4 weeks after starting treatment ($P < 0.03$)

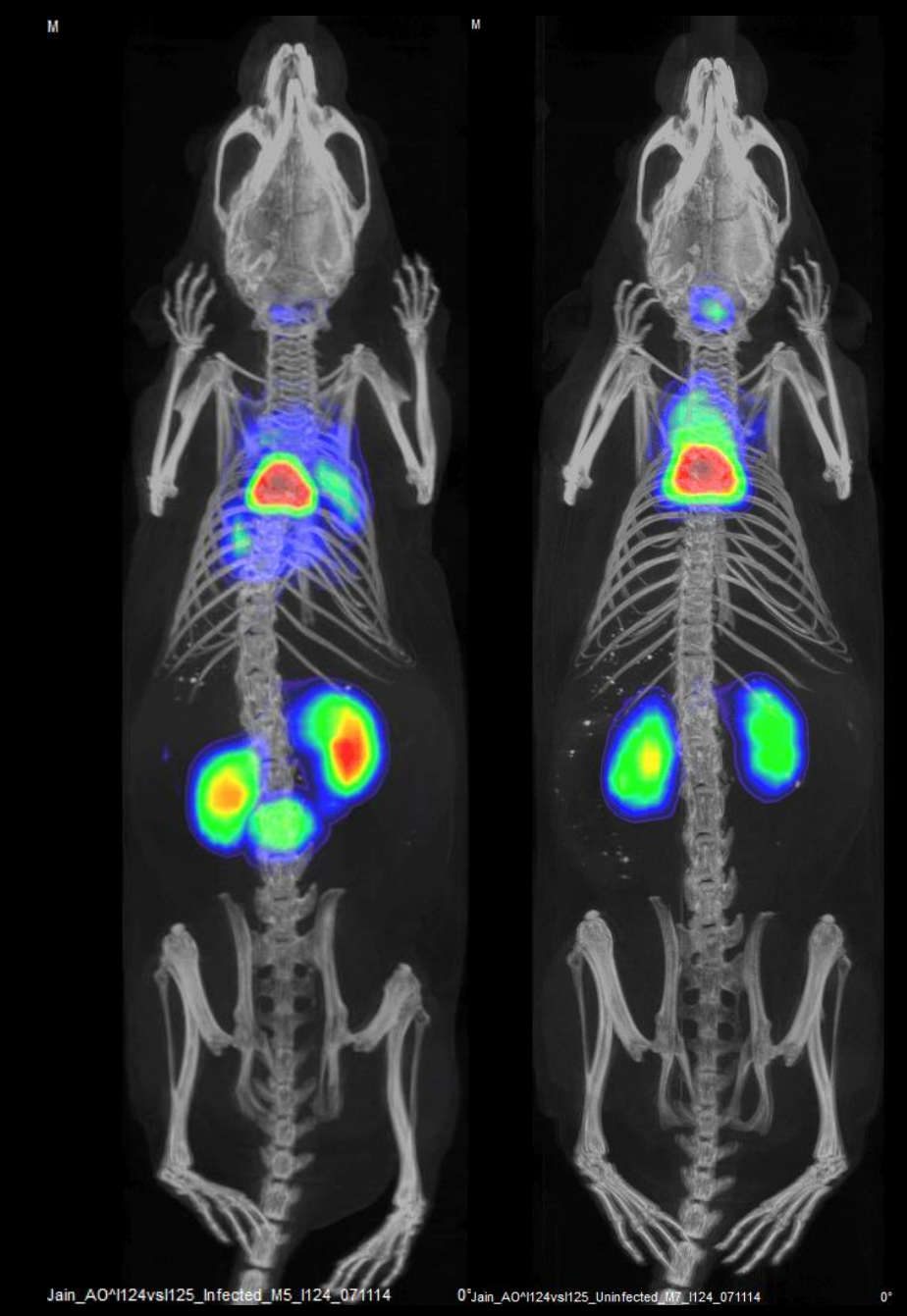
Iodo-DPA-713 bound activated (CD68^{+}) antigen presenting cells and imaging correlated with tissue TNF-α (Spearman's $\rho = 0.94$; $P < 0.01$)

Significant correlation was found between an increase in ^{125}I-DPA-713 SPECT activity (*but not with ^{18}F-FDG PET*) with bacterial burden at relapse (Spearman's $\rho = 0.79$; $P < 0.01$)

Foss *et al. J Infect Dis.* 2013 (Cover Article)

Ordonez *et al. Antimicrob Agents Chemother.* 2015

Imaging TB-associated inflammation with ^{124}I-DPA-713-PET



Infected Uninfected

- ^{124}I-DPA-713 synthesized under a research contract
- Mouse studies demonstrate (^{124}I-DPA-713 PET in blue-green-red; and skeletal outline in grey) excellent signal-to-noise ratio: 4.0-fold higher in TB lesions versus uninfected controls (P = 0.03)
- Toxicology studies completed and first-in-human studies have started under an FDA Exploratory Investigational New Drug application (#121615)
- ***Half life of I-124 is 4.2 days and so this can be shipped worldwide.***

Ordonez *et al*. *Antimicrob Agents Chemother.* 2015

Proposed ^{124}I-DPA-713 PET human studies to specifically localize sites of TB infections

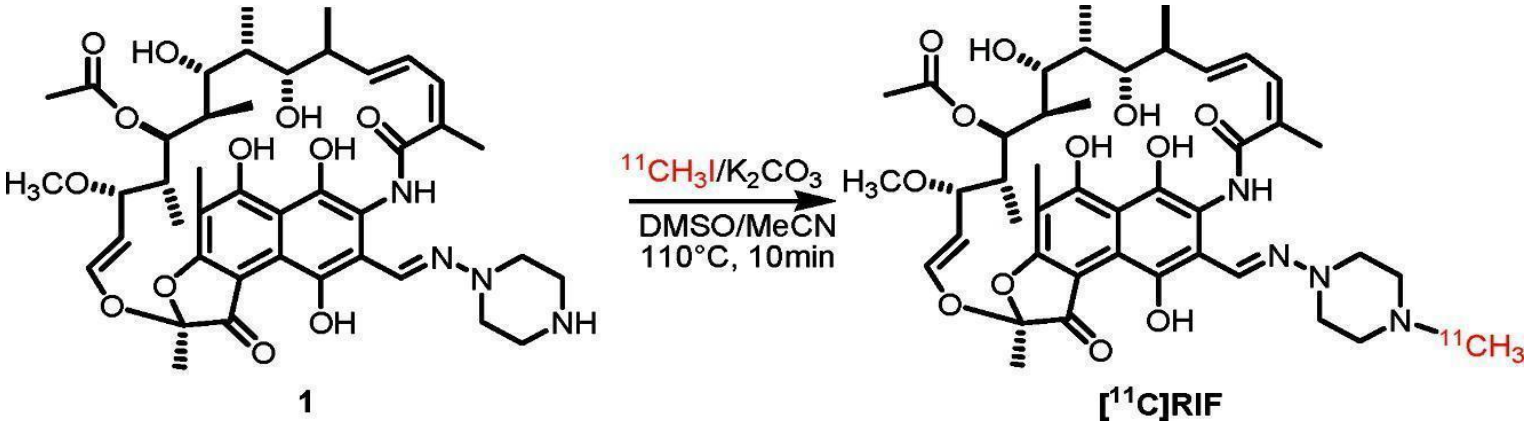
- Age: 18-65 years old
- Culture or molecular (GeneXpert, etc.) confirmation of TB
- On TB treatment for ≤ 4 weeks by the time of study
- Can understand English

If so, fill the screening script and refer to the study staff (JHU). The study, funded by the U.S. National Institutes of Health, requires three visits:

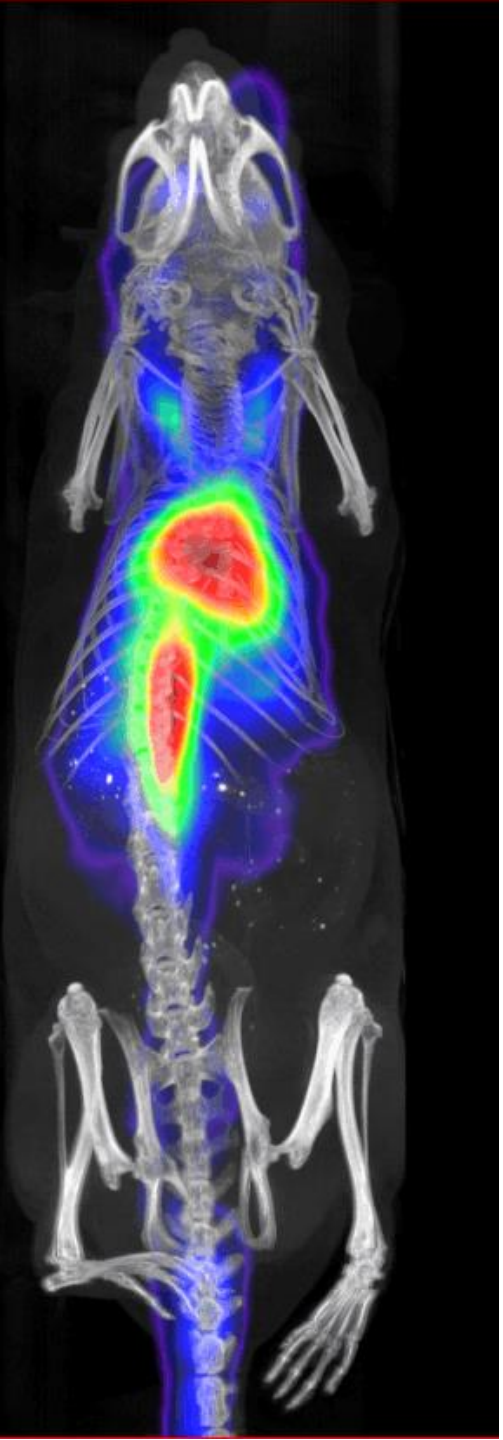
- Screening visit: history, written consent, screening labs
- Imaging visit day 1 (~half a day); injection of tracer
- Imaging (day 2 or 3) may last up to 3 hours
- During the imaging visit, we will use a new investigational PET/CT imaging technique
- Paid a total of $200 for participation in this study

Radiosynthesis and Bioimaging of Rifampin

- First-line drug essential for shortening therapy against *M. tuberculosis*
- Dosing based on serum / plasma concentrations (confirmed by post mortem resection)
- Drug concentration within necrotic pulmonary lesions (post-mortem) lower than blood concentrations*
- Labeled rifampin with ^{11}C using methods descrtibed by Liu *et al*



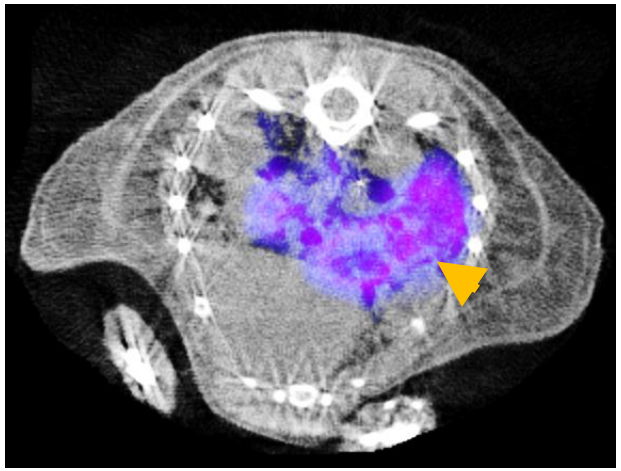
*Kjellsson *et al*. *Antimicrob Agents Chemother.* 2012
Liu *et al*. *J Med Chem.* 2010



Radiosynthesis and Bioimaging of Rifampin

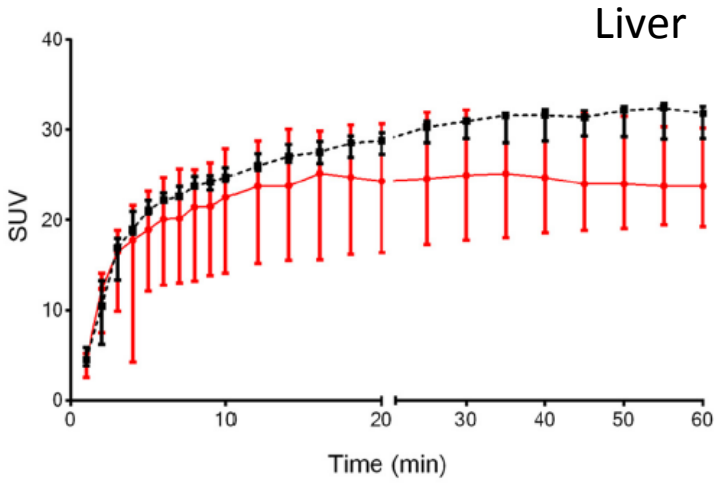
- Dynamic ^{11}C-Rifampin PET/CT of a *M. tuberculosis* infected mouse
- Granulomatous tissue depicted by yellow circle
- Purple represents concentration of rifampin (highlighted by orange arrow)

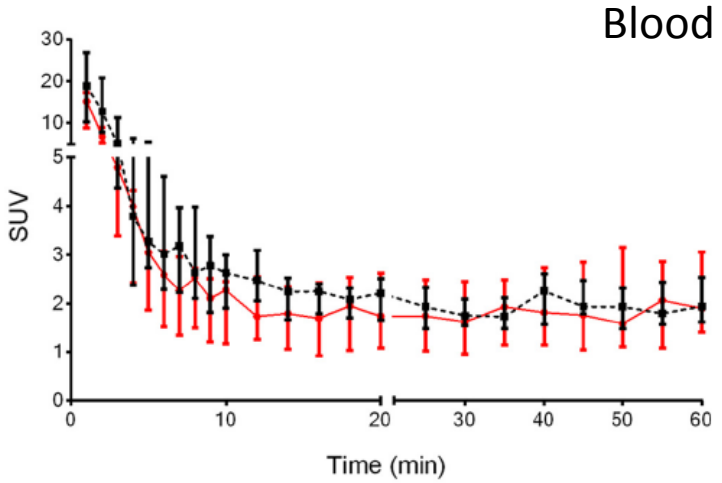
^{11}C-Rifampin PET/CT

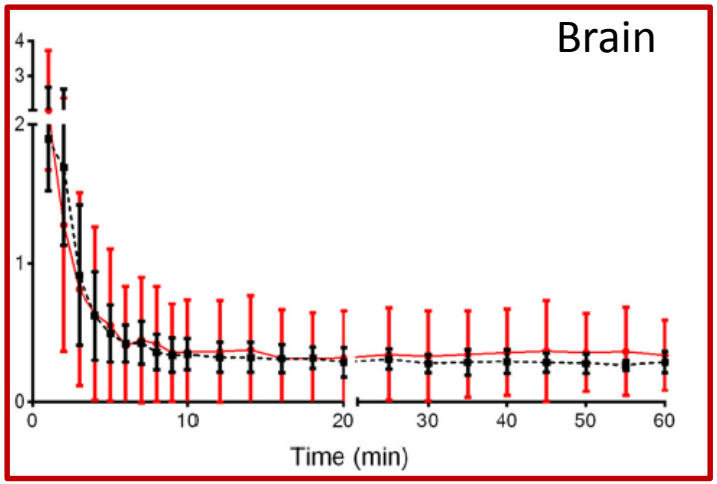


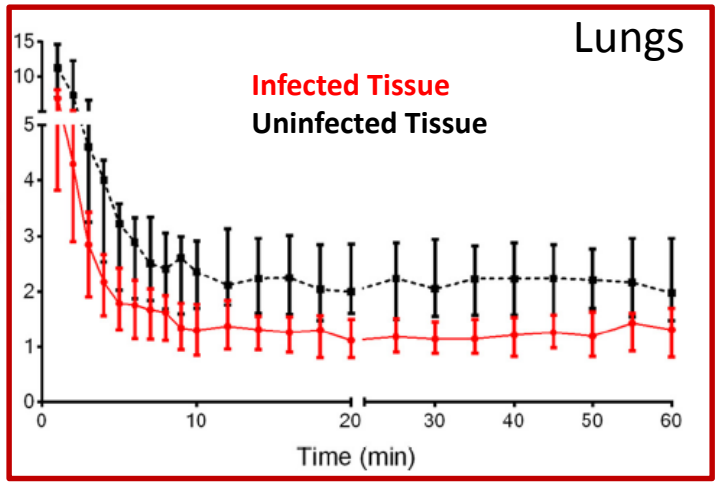
DeMarco and Ordonez *et al*. *Antimicrob Agents Chemother.* 2015

Radiosynthesis and Bioimaging of Rifampin Lower concentrations in infected lung tissues









^{11}C-rifampin concentrations in the brain ~10-20% of blood levels

^{11}C-rifampin concentrations were significantly lower in infected (~50%) versus uninfected lung tissues

Data are represented as medians and interquartile ranges. 5 mice were used for each group.

DeMarco and Ordonez *et al*. *Antimicrob Agents Chemother.* 2015

Radiosynthesis and Bioimaging of Rifampin
Lower concentrations in infected lung tissues

TABLE 1 Characteristics of study animals and results of noncompartmental pharmacokinetic analyses[a]

Parameter	Mean ± SD for animal type:		
	Mycobacterium tuberculosis infected	Uninfected	*P* value
Weight (g)	30.54 ± 1.90	34.18 ± 2.50	0.07
Injected dose (ng)	0.07 ± 0.02	0.07 ± 0.02	0.80
Injected dose (MBq)	8.61 ± 2.09	8.26 ± 2.51	0.80
Blood			
C_{max} (ng/ml)	0.0622 ± 0.0289	0.0591 ± 0.0447	0.90
AUC_{0-60} (ng · h/ml)	0.0056 ± 0.0015	0.0061 ± 0.0021	0.68
Brain			
C_{max} (ng/ml)	0.0086 ± 0.0085	0.0050 ± 0.0022	0.39
AUC_{0-60} (ng · h/ml)	0.0012 ± 0.0013	0.0008 ± 0.0025	0.53
Liver			
C_{max} (ng/ml)	0.0608 ± 0.0167	0.0656 ± 0.0156	0.65
AUC_{0-60} (ng · h/ml)	0.0583 ± 0.01406	0.0538 ± 0.0168	0.65
Lung			
C_{max} (ng/ml)	0.0221 ± 0.0095	0.0326 ± 0.0205	0.33
AUC_{0-60} (ng · h/ml)	0.0034 ± 0.0008	0.0054 ± 0.0015	0.03

[a] Rifampin concentrations (nanograms per milliliter) derived from PET imaging of *Mycobacterium tuberculosis*-infected and uninfected mice were analyzed using a noncompartmental intravenous bolus model (WinNonlin Standard). Peak concentrations (C_{max}) and areas under the concentration-time curve for the first 60 min (AUC_{0-60}) for different compartments are shown as means ± standard deviations. *P* values were calculated using a two-tailed Student *t* test. Five animals were used for each group.

***Human studies:** Dynamic ^{11}C-rifampin PET imaging studies in TB patients have begun to evaluate the penetration of rifampin in target, infected tissues – necrotic, cavitary and CNS lesions. **cGMP syntheses complete and RDRC / IRB approvals obtained.**

DeMarco and Ordonez *et al*. *Antimicrob Agents Chemother.* 2015

Proposed ^{11}C-rifampin PET human studies in patients with Rifampin-susceptible TB

- Age: ≥18 years old
- Culture or molecular (GeneXpert, etc.) confirmation of TB susceptible to rifampin
- On TB treatment for ≤ 6 weeks by the time of study
- Can understand English

If so, fill the screening script and refer to the study staff (JHU). The study, funded by the U.S. National Institutes of Health, requires two visits:

- Screening visit: history, written consent, screening labs
- Imaging visit day 1 (~half a day)
- During the imaging visit, we will use a new investigational PET/CT imaging technique
- Paid a total of $150 for participation in this study

Current Imaging Agents: Lack of Infection-specific tracers

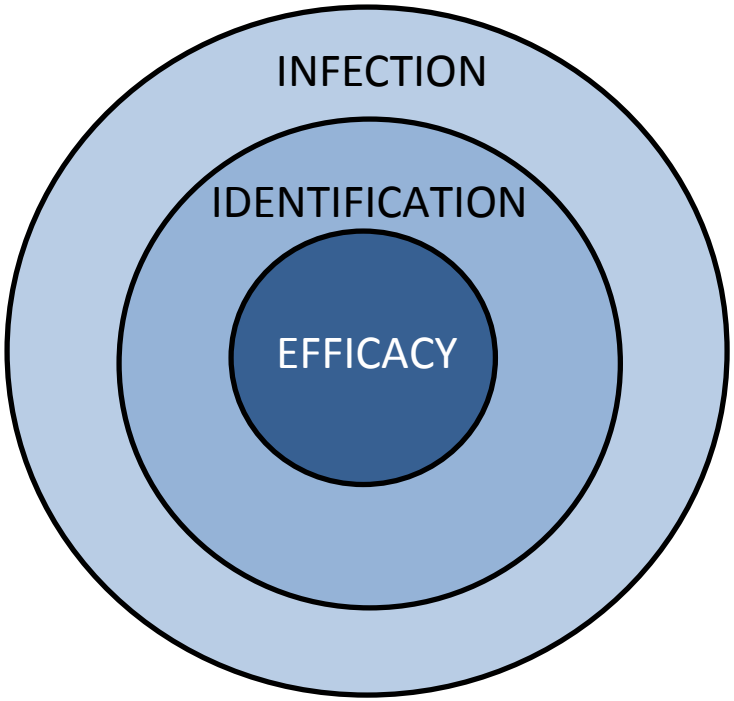
Pros		Cons
Readily available	CT / MRI / US	Non-specific for bacteria or infection
Excellent sensitivity	^{18}F-FDG PET/ WBC scan	Non-specific; host-dependent

Developing Bacteria-specific Imaging Tracers

AIM: To develop a pipeline of class specific bacterial imaging probes that would provide a platform to identify, localize and monitor a wide range of pathogenic bacteria

HYPOTHESIS: Small molecules metabolized by prokaryotic-specific pathways could be utilized as bacteria-specific imaging tracers to:

a) discriminate infection from non-infectious processes
b) categorize the causative bacterial species and
c) provide information on antibiotic efficacy



Ordonez and Weinstein *et al*. *J Nucl Med.* 2017

Candidate Bacteria-specific Imaging Tracers

	In vitro % uptake (mean ± standard deviation)				
Name	*S. aureus* (Gram-positive)	*E. coli* (Gram-negative)	*P. aeruginosa*	Mycobacteria*	Macrophages (J774)
L-Arabinose [1-^{14}C]	0.41 ± 0.03	41.61 ± 9.91	0.21 ± 0.02	0.28 ± 0.01 (Mtb)	0.18 ± 0.01
Cellobiose [^{3}H]	1.81 ± 0.10	0.80 ± 0.05	--	0.13 ± 0.02 (Ms)	--
D-Lyxose [1-^{14}C]	0.03 ± 0.01	1.86 ± 0.14	0.12 ± 0.04	0.35 ± 0.08 (Mtb)	0.04 ± 0.01
D-Mannitol [1-^{14}C]	68.40 ± 7.39	81.80 ± 1.96	0.69 ± 0.05	0.29 ± 0.13 (Mtb)	0.12 ± 0.01
Methyl-α-D-glucopyranoside [methyl-^{14}C]	11.01 ± 0.71	26.78 ± 0.59	--	0.11 ± 0.01 (Ms)	--
para-Aminobenzoic acid [3,5-^{3}H]	16.82 ± 1.03	18.99 ± 5.80	4.02 ± 1.11	32.93 ± 4.73 (Mtb)	0.11 ± 0.0
L-Rhamnose [^{3}H]	4.96 ± 0.13	4.73 ± 0.07	0.24 ± 0.04	3.82 ± 0.84 (Mtb)	0.60 ± 0.0
Shikimic acid [3-^{3}H]	7.54 ± 0.01	1.52 ± 0.02	1.31 ± 0.02	0.17 ± 0.01 (Ms)	--
D-Sorbitol [^{14}C] (^{18}F-FDS)†	0.47 ± 0.09	72.20 ± 9.09	0.52 ± 0.46	--	0.21 ± 0.0
D-Xylose [1-^{14}C]	0.31 ± 0.01	73.94 ± 2.06	0.53 ± 0.08	0.18 ± 0.02 (Mtb)	0.19 ± 0.0

Detect the presence of bacterial infection

Identify the "type" of bacteria

FDS or Fluorodeoxysorbitol is a fluoro analog of Sorbitol – FDA approved sugar-free sweetener

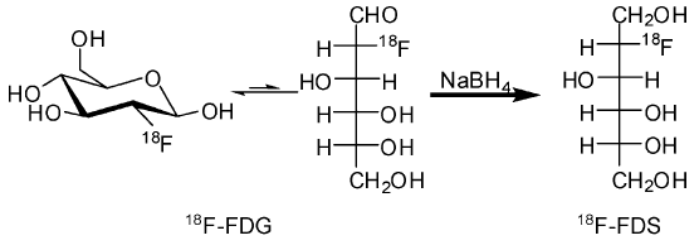
Mycobacterium smegmatis = Ms or *Mycobacterium tuberculosis* = Mtb

† 2-[^{18}F]-fluorodeoxysorbitol (^{18}F-FDS) used for uptake assays

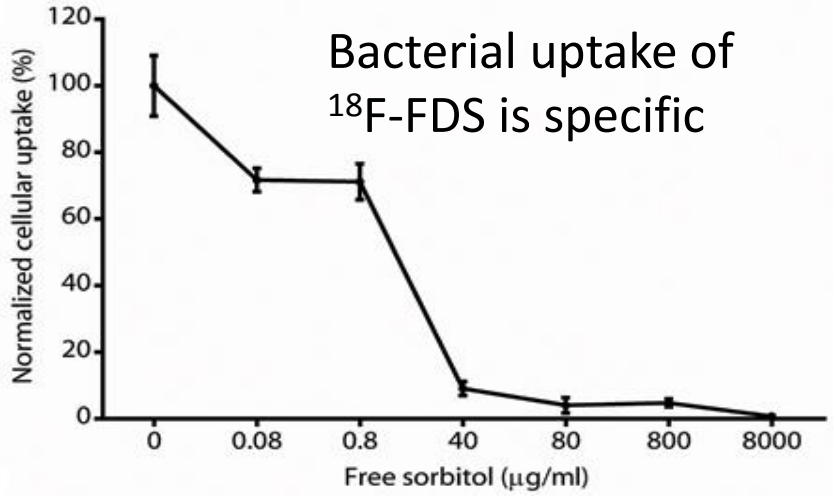
No uptake by heat killed bacteria for any of the tracers demonstrating specificity

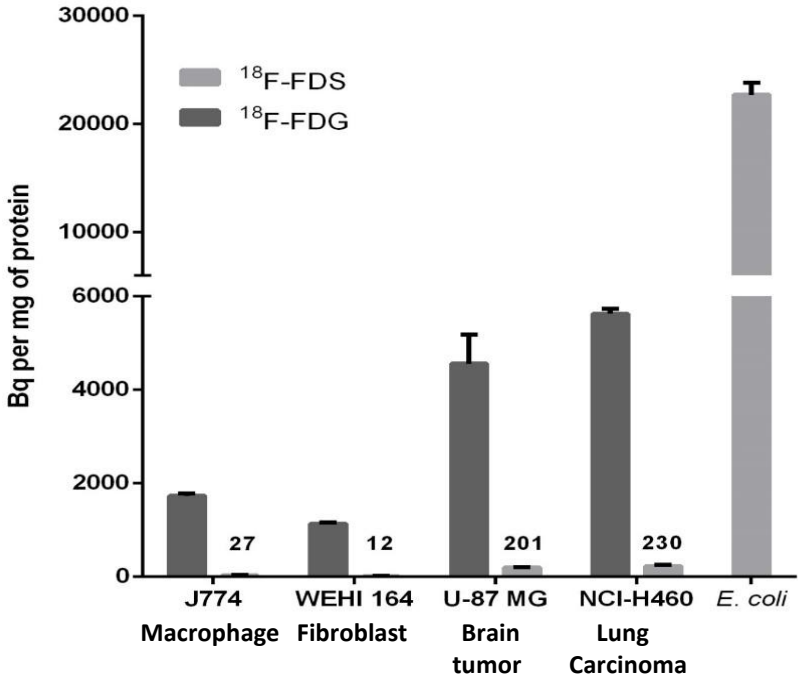
Ordonez and Weinstein *et al*. *J Nucl Med.* 2017

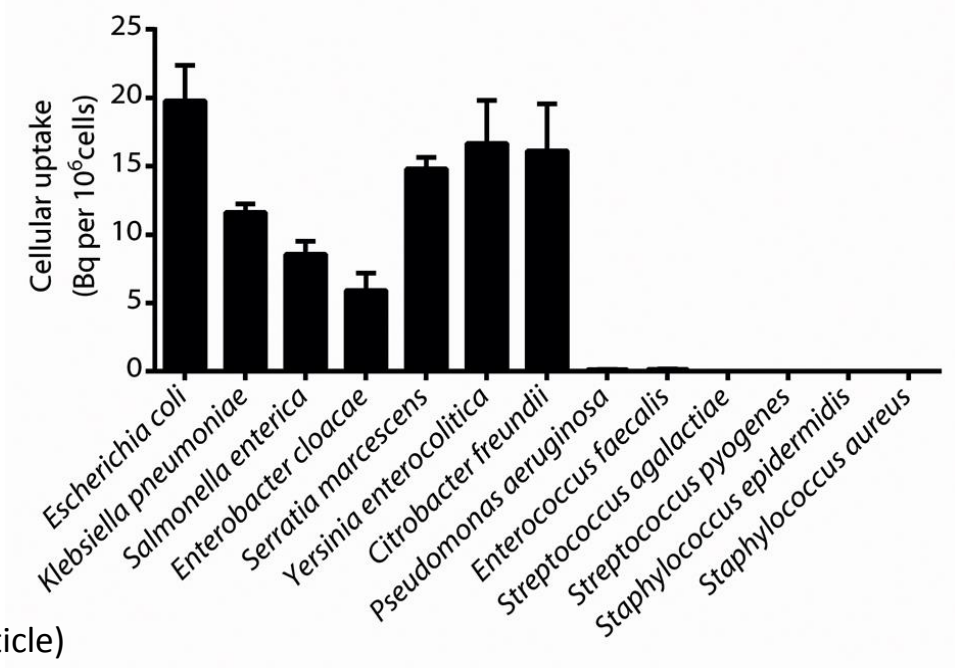
^{18}F-FDS can be synthesized from ^{18}F-FDG (in 30 min) and is specifically accumulated by *Enterobactericacae*



Li *et al*. *Mol Imaging Biol* 2008

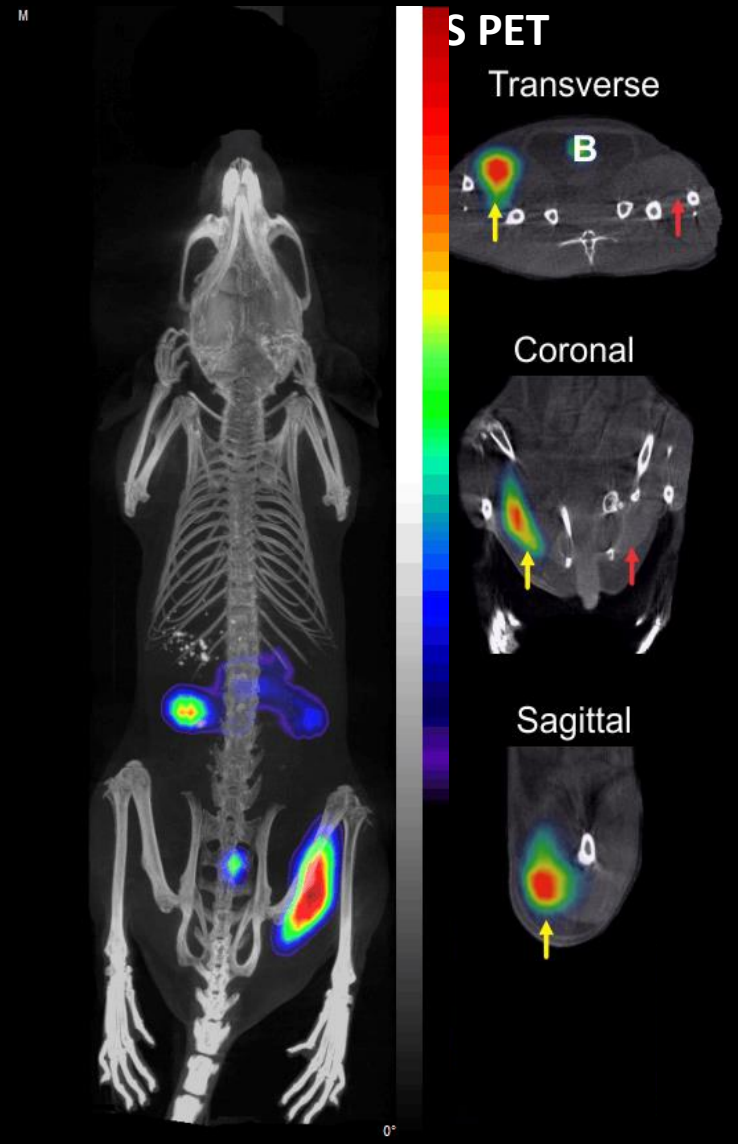






Weinstein and Ordonez *et al*. *Sci Transl Med* 2014 (Cover article)

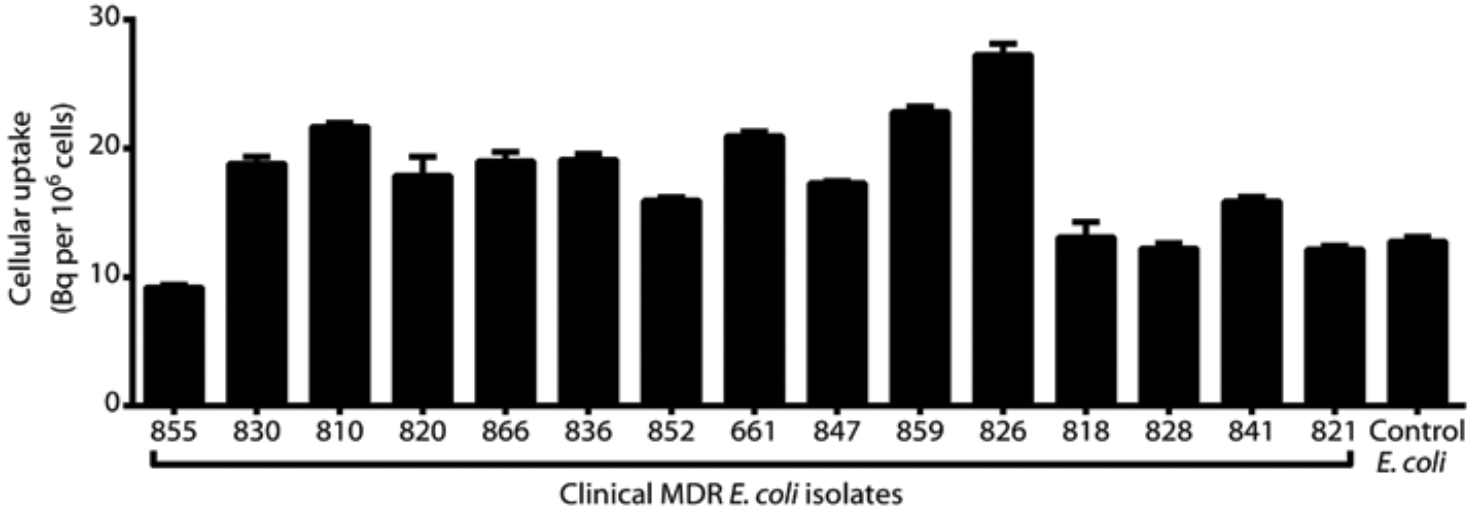
^{18}F-FDS PET can differentiate infection sites from sterile inflammation



Weinstein and Ordonez *et al*. *Sci Transl Med* 2014 (Cover article)

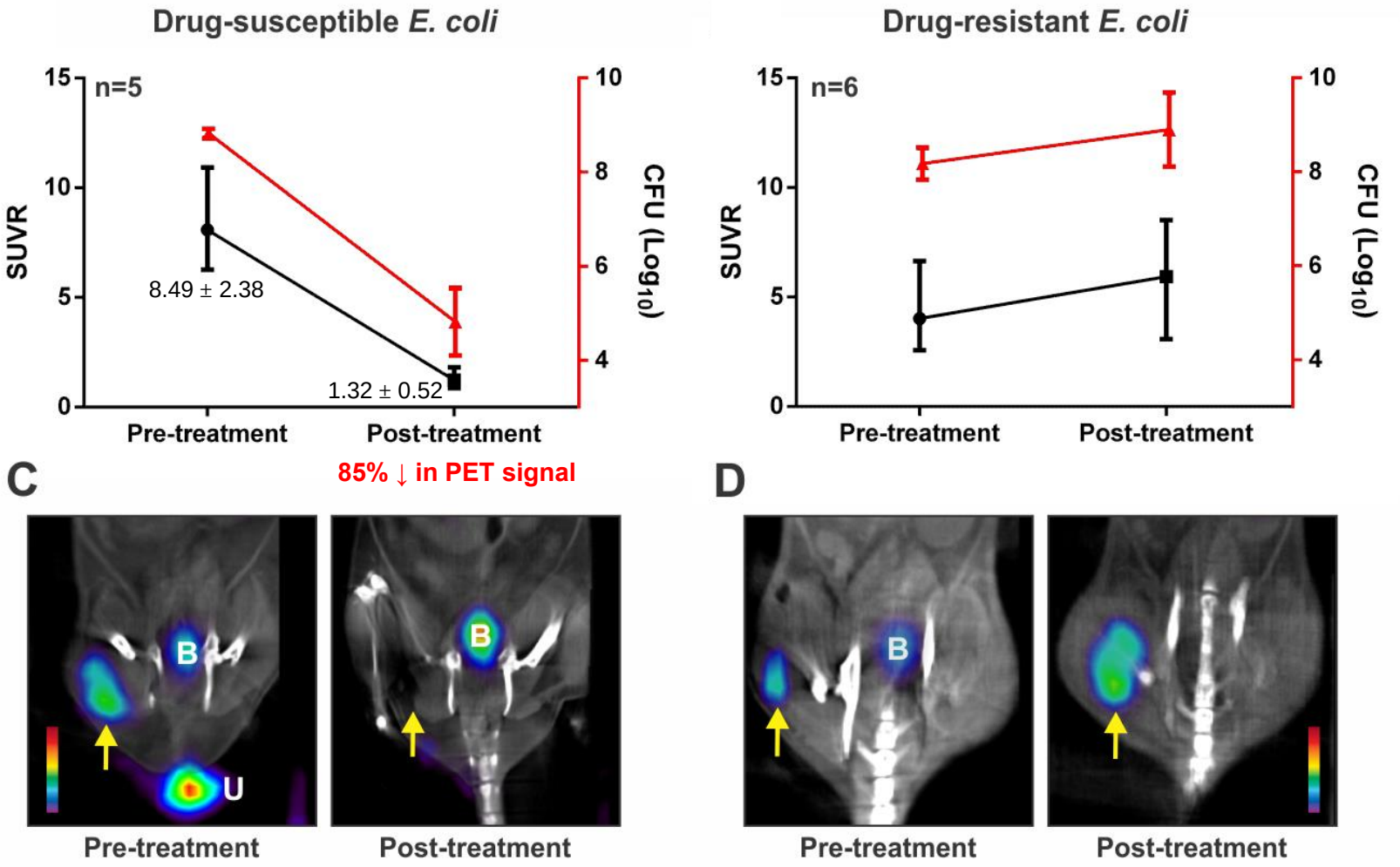
^{18}F-FDS Uptake by 15 Random *Clinical* Multidrug-Resistant *Enterobacteriaceae* strains

- Notorious source of life-threatening nosocomial infections due to multi-drug resistant organisms including:
  - Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*
  - Carbapenem-resistant *Enterobacteriaceae* (CRE)
  - *Enterobacteriaceae* resistant to colistin ("last line of defense")
- All 15 random ESBL-producing clinical strains (clinical MDR *E. coli*), demonstrated substantial ^{18}F-FDS uptake



Weinstein and Ordonez *et al*. *Sci Transl Med* 2014 (Cover article)

Monitoring antimicrobial efficacy in multidrug-resistant infections *in situ*



An increase in bacterial load corresponds with disease progression to sepsis and death in seriously ill patients

Weinstein and Ordonez *et al*. *Sci Transl Med* 2014 (Cover article)

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XDR-TB report

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