NTM Lecture Series for Providers

April 27-28, 2023
NATIONAL JEWISH HEALTH

Cystic Fibrosis Related NTM Infections

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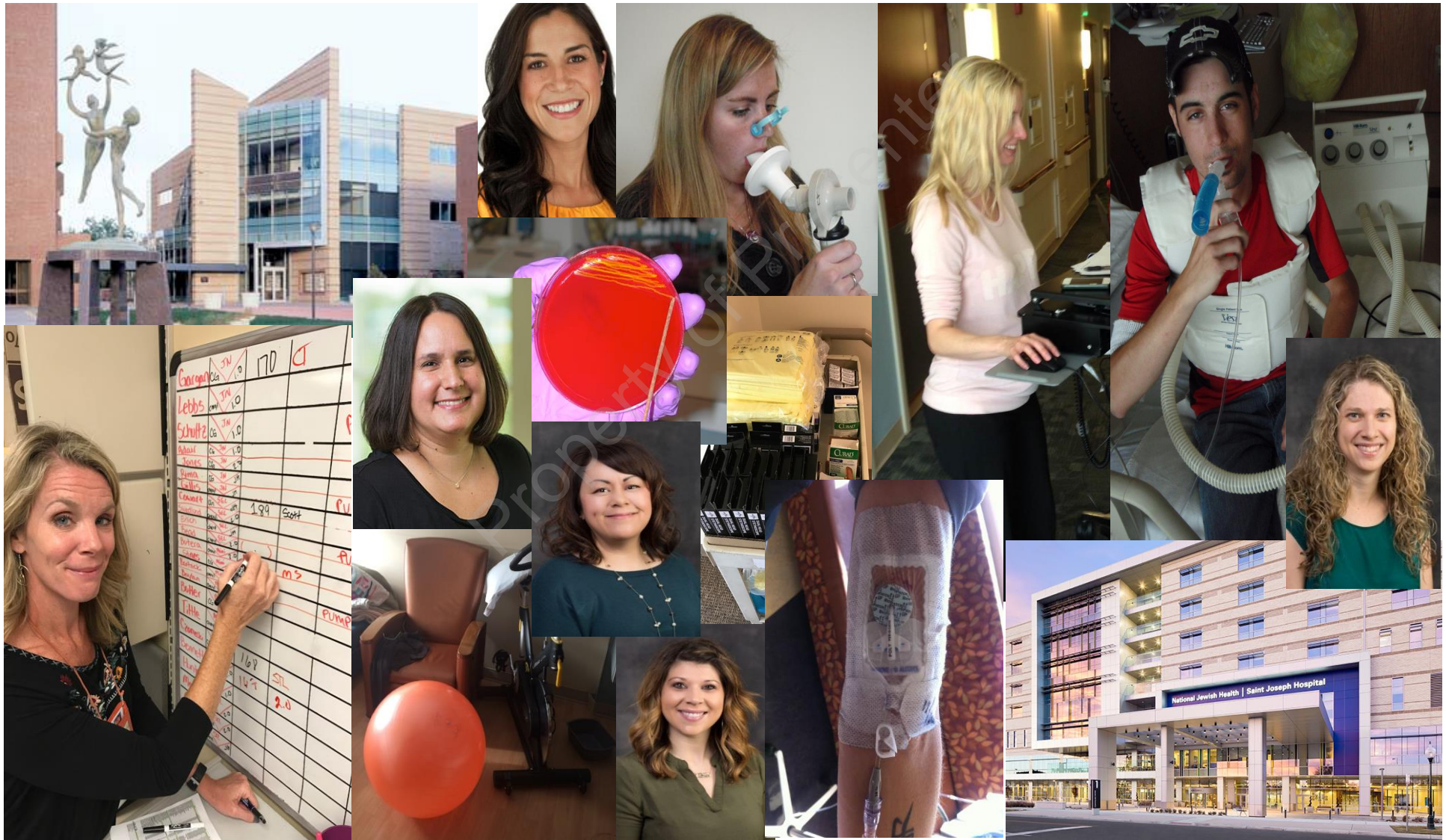
University of Colorado



Disclosures

Conflicts

- **none**

Supported by:

CFF

- Colorado NTM Core Clinical Research Service NRC
- NTM Outcome Measure Advancement Core NRC
- NICK20A0 Urine Lipoarabinomannan as a Marker for Low-risk of NTM Airway Infection
- NICK17K0 NTM-OB-17 Colorado Adult
- NICK21K0 Prospective Evaluation of Markers of NTM and Host Response in Saliva

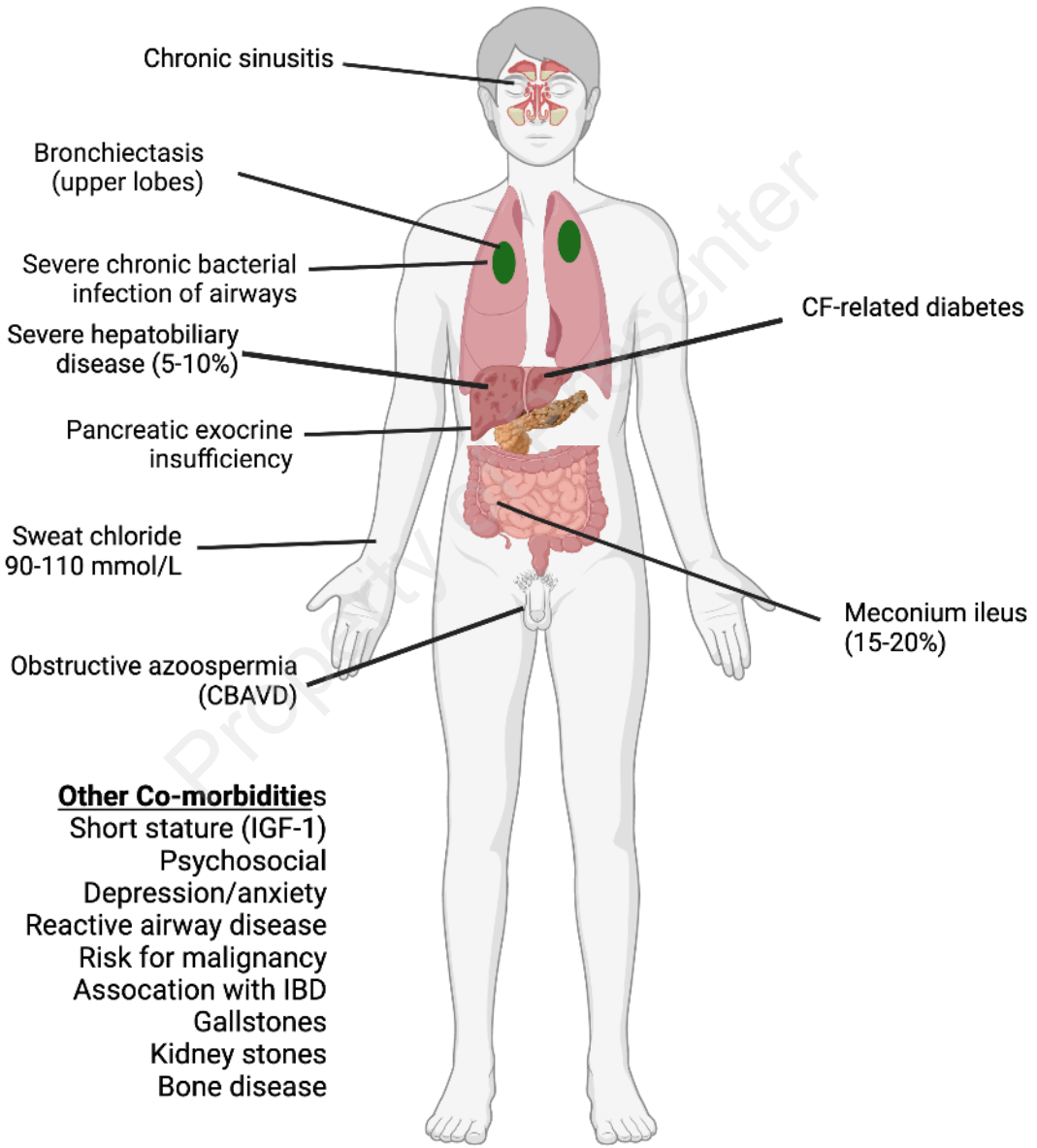
NIH

- R01HL146228 Longitudinal Assessment of Culture-Independent Molecular Airway Markers of Nontuberculous Mycobacteria

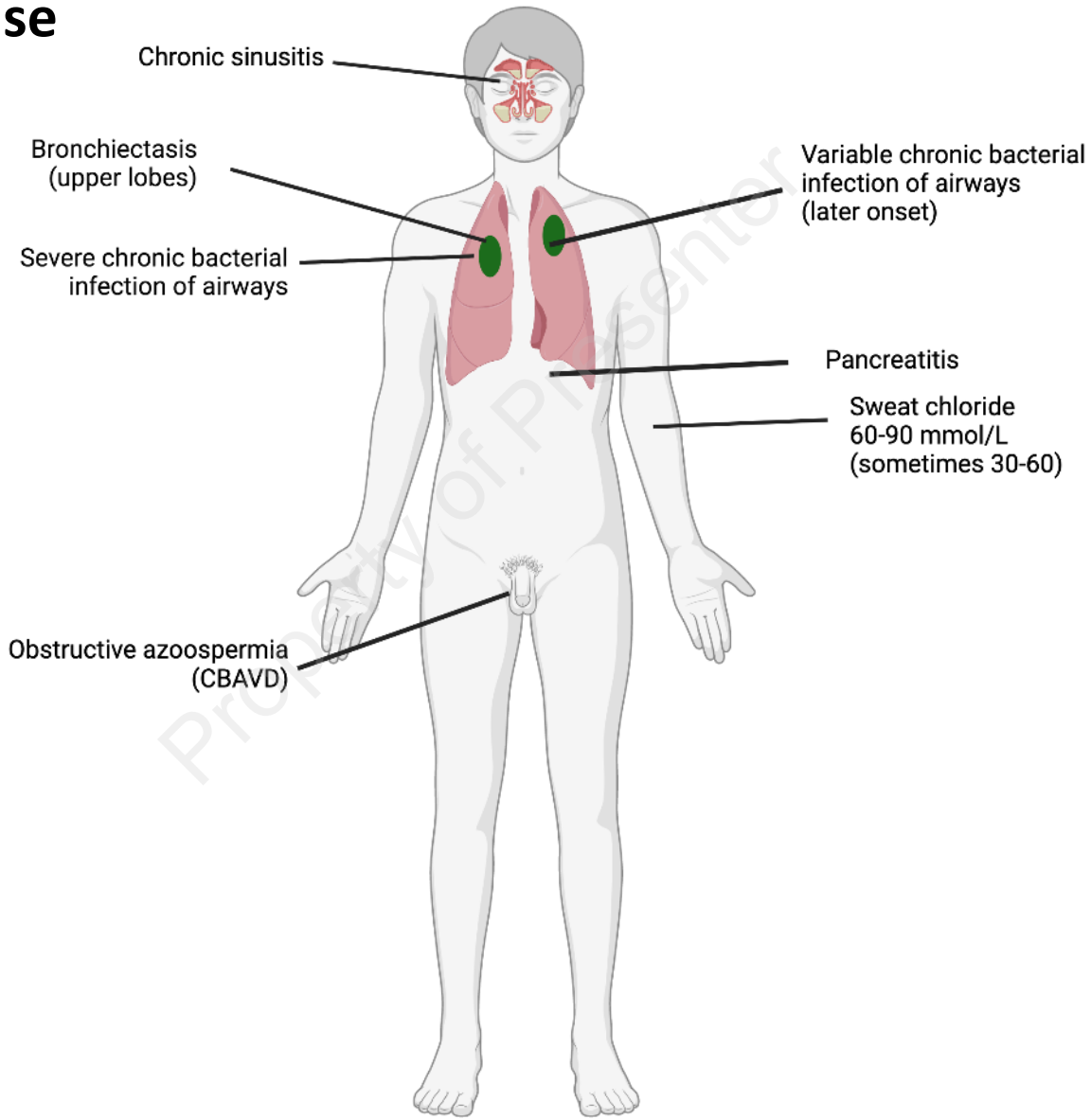
FDA

- R01FD-R-6848 A Phase 1b, Multi-center Study of IV Gallium Nitrate in Patients with Cystic Fibrosis who are colonized with NTM

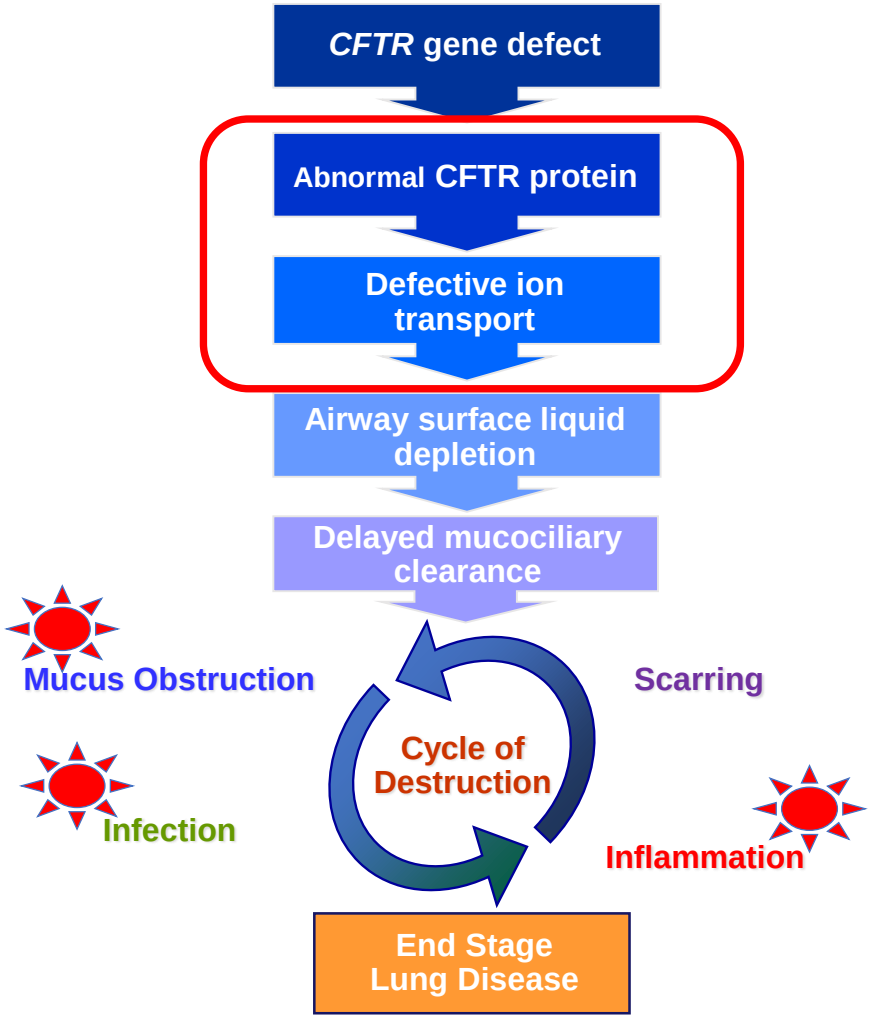
Classic (severe) Disease -childhood diagnosis (≅90%)

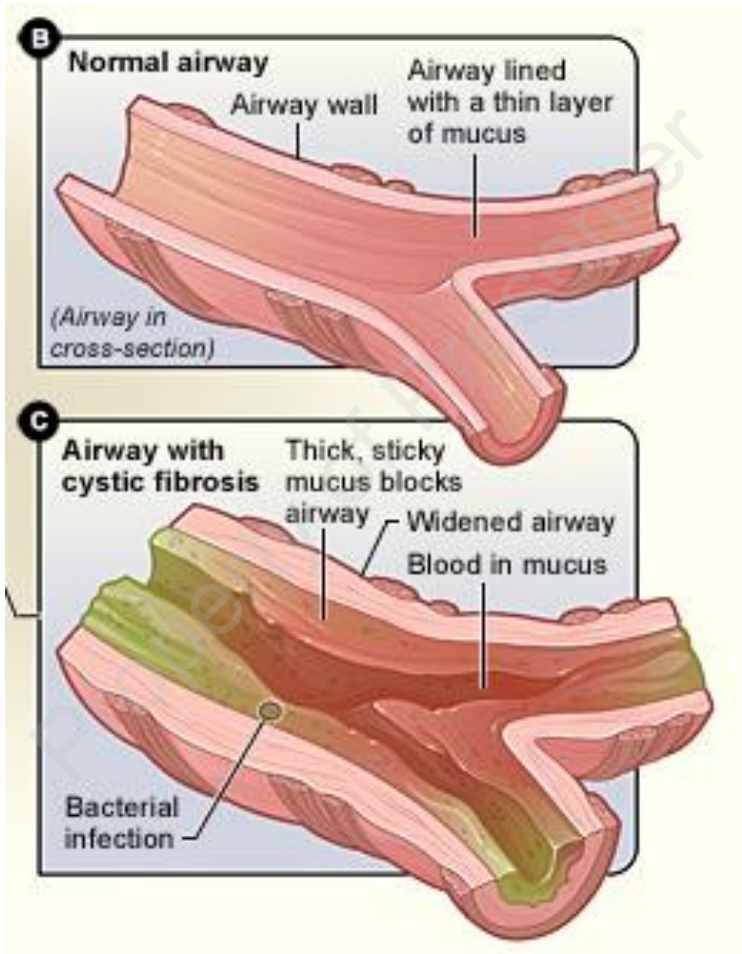


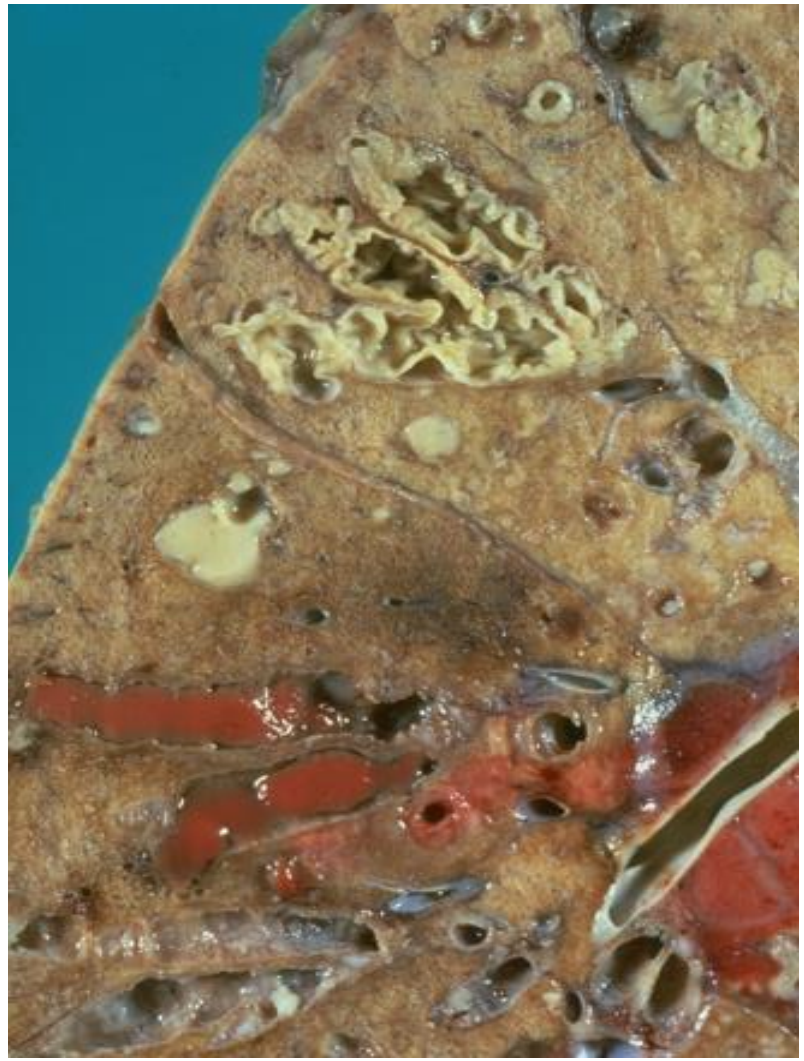
Nonclassic (delayed) Disease -adult diagnosis (≅10%)



Pathogenesis of CF lung disease

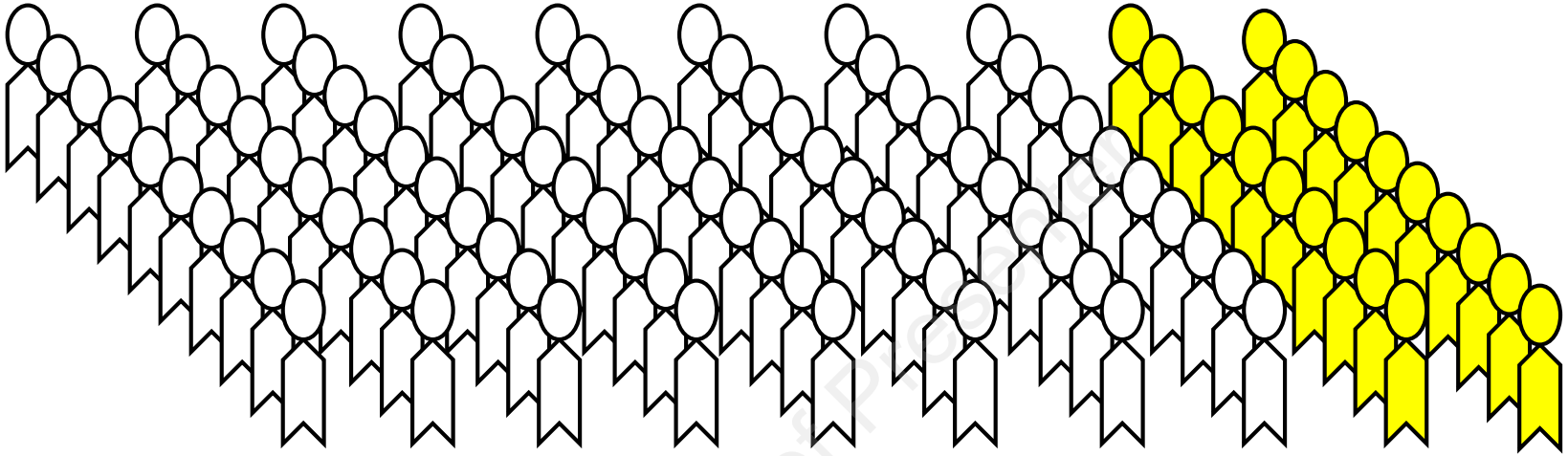






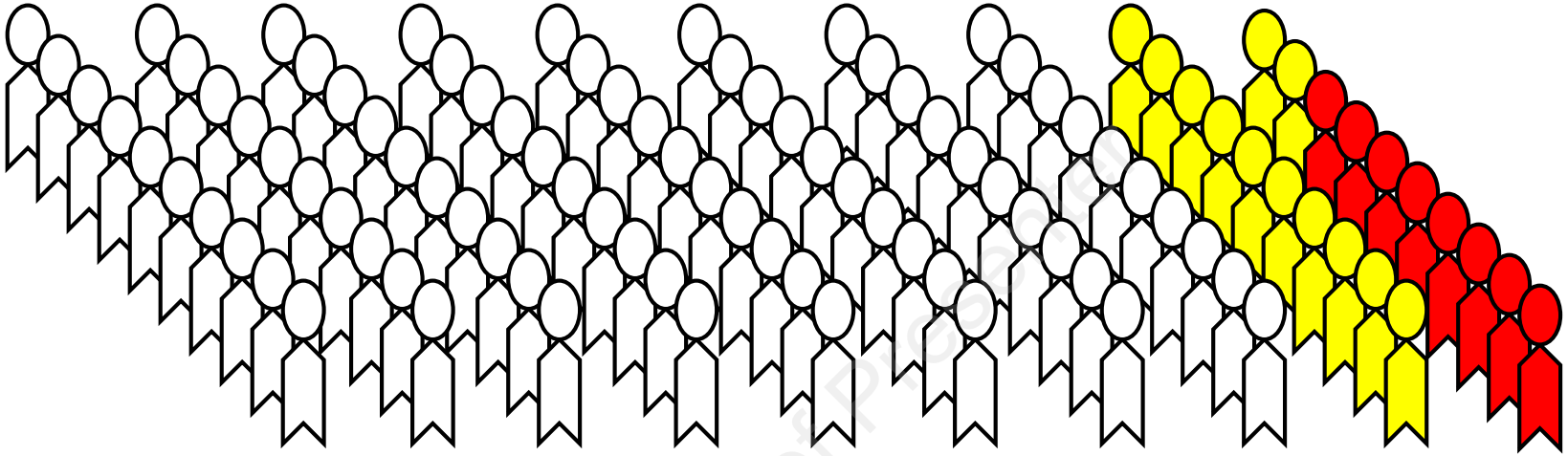
http://www.nhlbi.nih.gov/health/health-topics/topics/cf/signs.html

Epidemiology of NTM in the United States CF Population



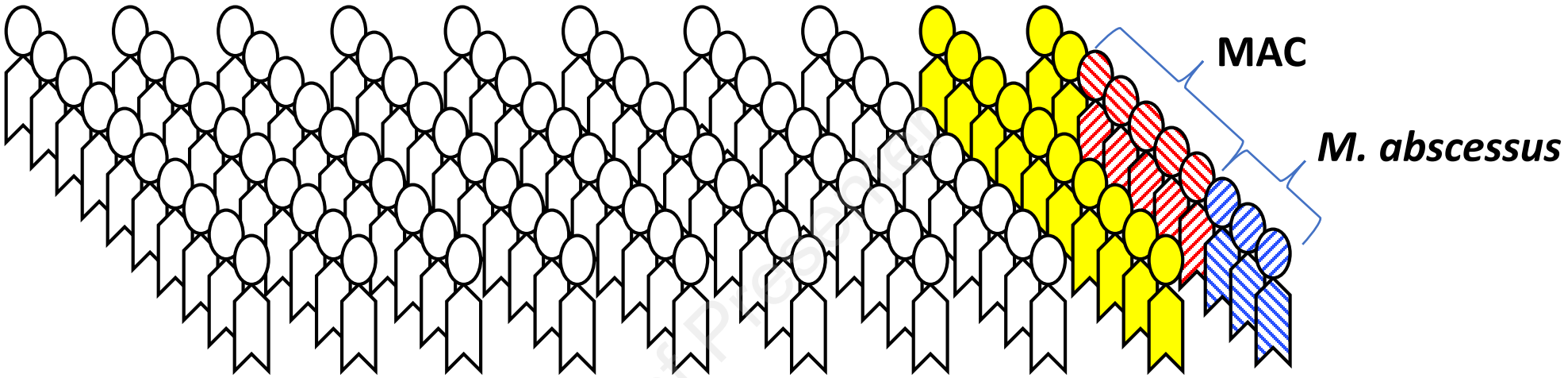
Approximately 20% of CF population will grow NTM over a 5-year period

Epidemiology of NTM in the United States CF Population



25-40% will meet criteria for NTM pulmonary disease

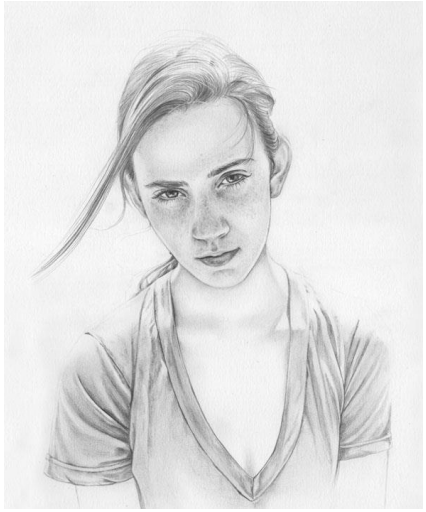
Epidemiology of NTM in the United States CF Population



MAC is the predominant NTM in the United States CF population

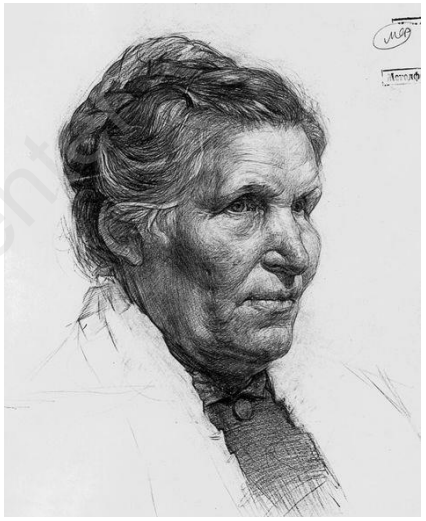
NTM pulmonary infection and disease: Cystic Fibrosis *vs.* Non-CF

Cystic Fibrosis



- CF Care Center
- Lifelong sputum culture surveillance = Earlier detection without clinical features
- MAC or *M. abscessus*
- Always co-infection with other bacteria
- Uniform pathogenesis
- Greater variability in drug PK
- Evidence of diminished response to treatment
- Uncertainty regarding the effect of highly effective modulator therapy

Non-CF Bronchiectasis

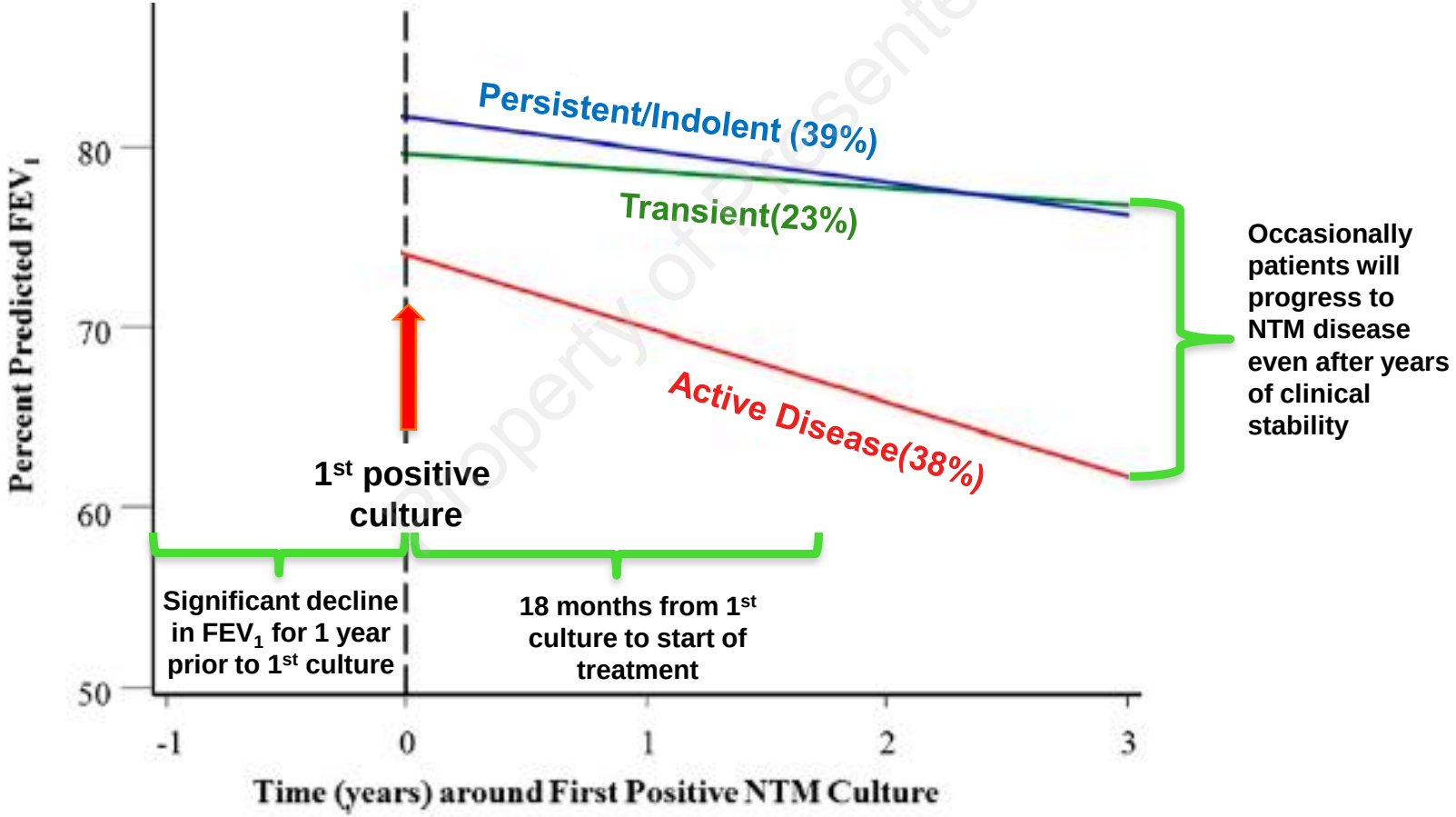


- Community-based care
- Infrequent airway cultures= Clinical symptoms prompt cultures
- Greater spectrum of NTM infections
- NTM may be primary infection
- Mix of underlying diseases and co-morbidities

Clinical Significance of a First Positive NTM Culture in CF

Change in FEV_1 pre- and post first positive NTM culture

Average time of follow-up 4.4 yrs. (n=96)



Martiniano AATS, 2014

Diagnosis of NTM Pulmonary Disease: CF-specific considerations

Other CF pathogens and co-morbidities should be considered as potential contributors to a patient's symptoms and radiological features when determining the clinical significance of NTM positive cultures

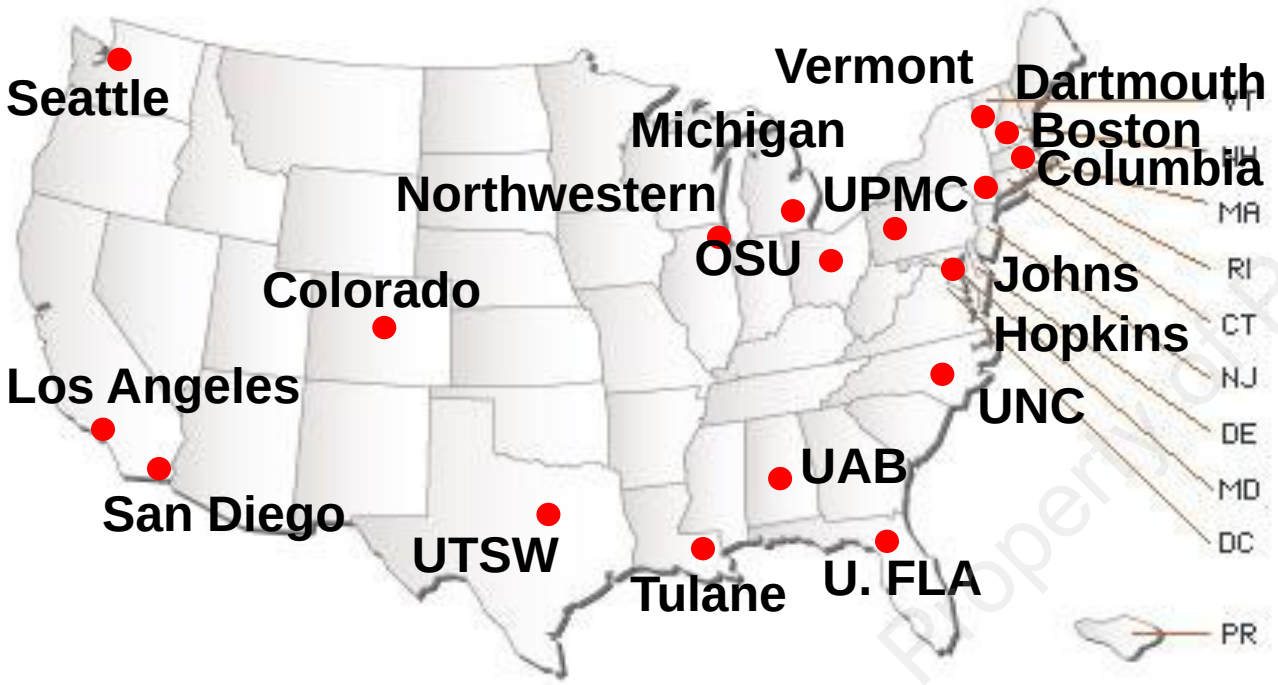
Suboptimal CF care
Pulmonary Exacerbations
- -Usual CF pathogens
- -New bacterial infection

Co-morbidities:
- -Allergic bronchopulmonary aspergillosis (ABPA), asthma
- -CF-related diabetes
- -Sinus disease
- -Gastroesophageal reflux
- -Chronic aspiration
- -Nutritional deficiencies

CFF/ECFS Guidelines: NTM treatment **should be considered** for individuals with CF who have ATS/IDSA defined NTM pulmonary disease.

ATS/IDSA Statement: "Diagnosis, Treatment, and Prevention of NTM Disease" AJRCCM 2007;
US CFF/ECFS "Consensus recommendations for the management of NTM in individuals with CF, Thorax, 2016

Cystic Fibrosis NTM Consortium 2023



2013

- National Jewish Health, Children's Hospital Colorado

2018

- University of Washington
- University of Michigan
- University of Texas Southwestern
- University of Alabama Birmingham (Adult Program)
- Johns Hopkins University
- University of North Carolina
- Columbia University

2022

- University of Florida (Gainesville)
- Rady Children's Hospital, University of California San Diego
- Children's Hospital of Pittsburgh of UPMC & University of Pittsburgh Medical Center
- University of Vermont Children's Hospital (Adult)
- Nationwide Children's Hospital (Columbus, OH)
- Dartmouth Hitchcock Medical Center
- Northwestern University (Adult)
- Boston Children's
- Los Angeles Children's Hospital, USC Adult Program
- Tulane University

Prospective Evaluation of NTM Disease in Cystic Fibrosis (PREDICT Study)

- Prospective, single-center, observational trial at the Pediatric and Adult Colorado CF Care Center
  - Single diagnostic algorithm based on CF Foundation and European CF Society Guidelines (*Thorax*, 2016)

- **Inclusion:**
  - Diagnosis of CF
  - Positive NTM culture (last 2 years) with *M. avium* complex (MAC) or *M. abscessus* complex (MABSC)
- **Exclusion:**
  - Recent or current treatment of NTM

PATIENCE: Objectives

Prospective, open-label, treatment trial

Primary Study Goals:

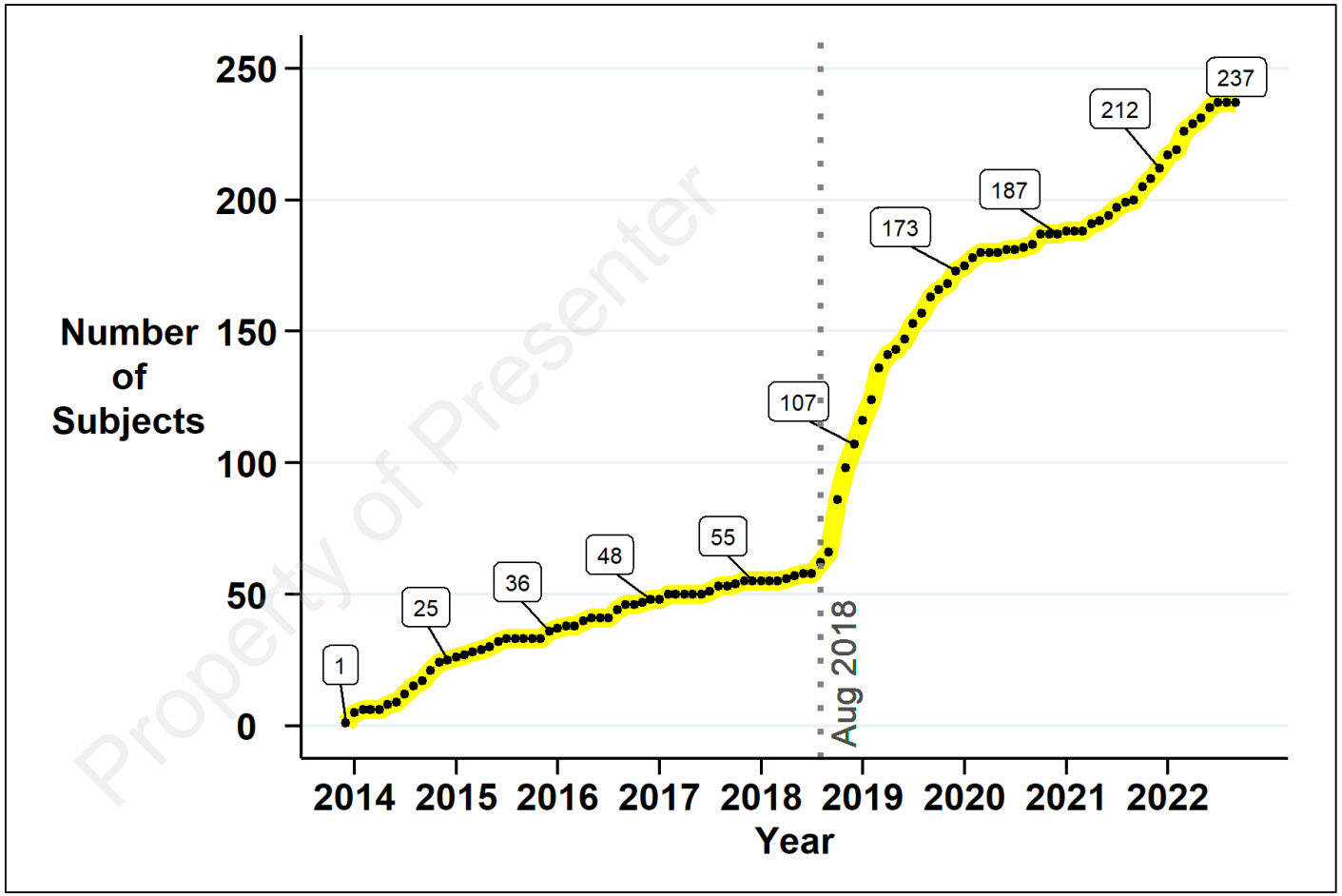
- To implement a standardized approach to the initial treatment of NTM pulmonary disease (NTM-PD) in CF patients

Secondary Study Goals:

- Define an expected rate of clinical response and tolerance of treatment of NTM-PD
- Establish foundation for future NTM treatment trials

PREDICT: Ever Enrolled

Current number of PREDICT participants "ongoing study procedures": n=151

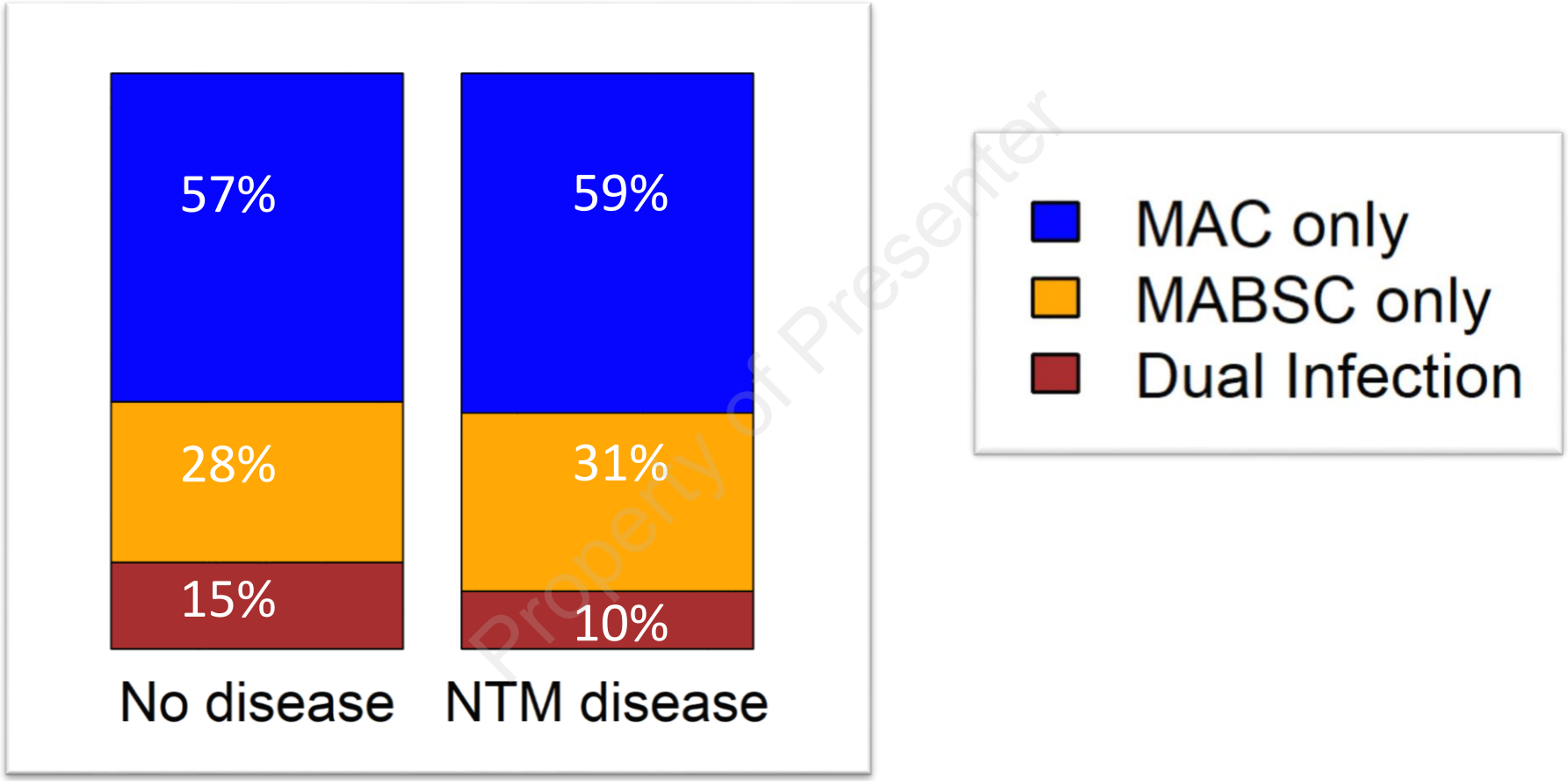


PREDICT: Clinical Comparisons by NTM Disease Status

	Total (n=237)	No NTM disease (n=181; 76%)	NTM disease (n=56; 24%)	P-value
Male sex (%)	40%	43%	30%	0.58
Enrollment FEV% predicted, mean (SD)	79 (24)	80 (24)	75 (23)	0.18
Enrollment age, mean (SD)	30 (14)	31 (14)	25 (13)	0.01
Enrollment age distribution 6 to 12 years >12 to 18 years >18 to 30 years > 30 years	 8% 11% 43% 38%	 7% 9% 43% 42%	 13% 18% 43% 27%	0.05
First Lifetime NTM culture age, mean (SD)	27 (13)	27 (13)	22 (13)	0.02

*** No differences in CF genotype, race, ethnicity**

NTM Species Distribution

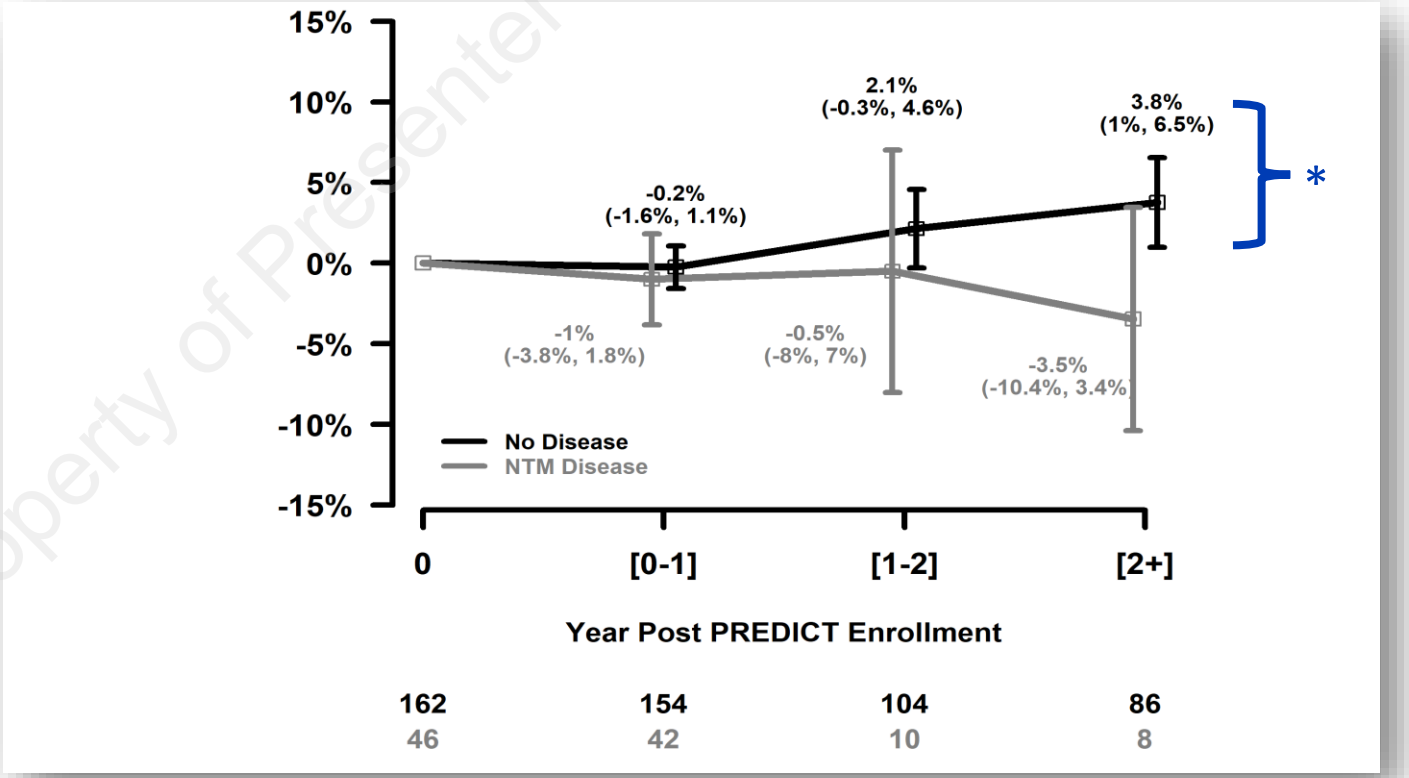


PREDICT: Follow Up & Change in FEV1% Predicted

Mean Follow-up Time:

No NTM Disease:
30 months
4 clinic visits per year

NTM Disease Diagnosis:
12 months
6 clinic visits per year



* For those with NTM-PD diagnosis 2 years post enrollment, FEV1pp was 7 % lower (95% CI 0% to 14%, p=0.05) compared to no NTM-PD

Prospective Algorithm for Treatment of NTM in Cystic Fibrosis (PATIENCE)

Prospective, open-label, treatment trial at the Colorado CF Care Center

- Single treatment algorithm based on CF Foundation and European CF Society Guidelines (*Thorax*, 2016)

Inclusion: confirmed diagnosis of CF

- Age 7 years or greater
- Diagnosis of NTM disease (via PREDICT or Provider)
- Intention to treat the NTM disease, based on the judgment of the CF clinic physician that the patient may benefit from treatment

Exclusion:

- Pregnant
- History of transplantation
- Currently undergoing treatment for NTM infection
- Prior treatment failure for current NTM species, as defined by positive sputum cultures within 12 months of discontinuation of antibiotic treatment

I. Stenzel
http://www.thebreathingroom.org/
Photo by Derek Powazek

NCT02419989

PATIENCE: Objectives

Prospective, open-label, treatment trial

Primary Study Goals:

- To implement a standardized approach to the initial treatment of NTM pulmonary disease (NTM-PD) in CF patients

Secondary Study Goals:

- Define an expected rate of clinical response and tolerance of treatment of NTM-PD
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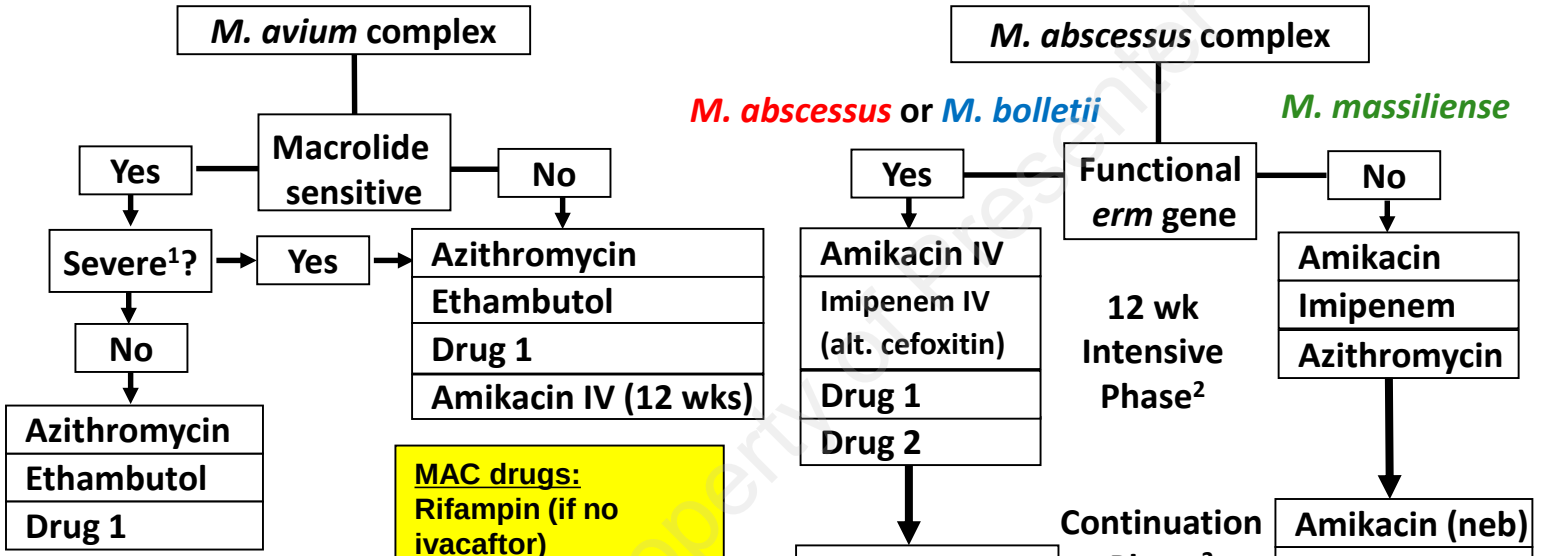
PATIENCE Treatment Protocols



[1] **Severe Disease:**
- Smear positive +/or
- Cavitary infection radiographically +/or
- Systemically ill

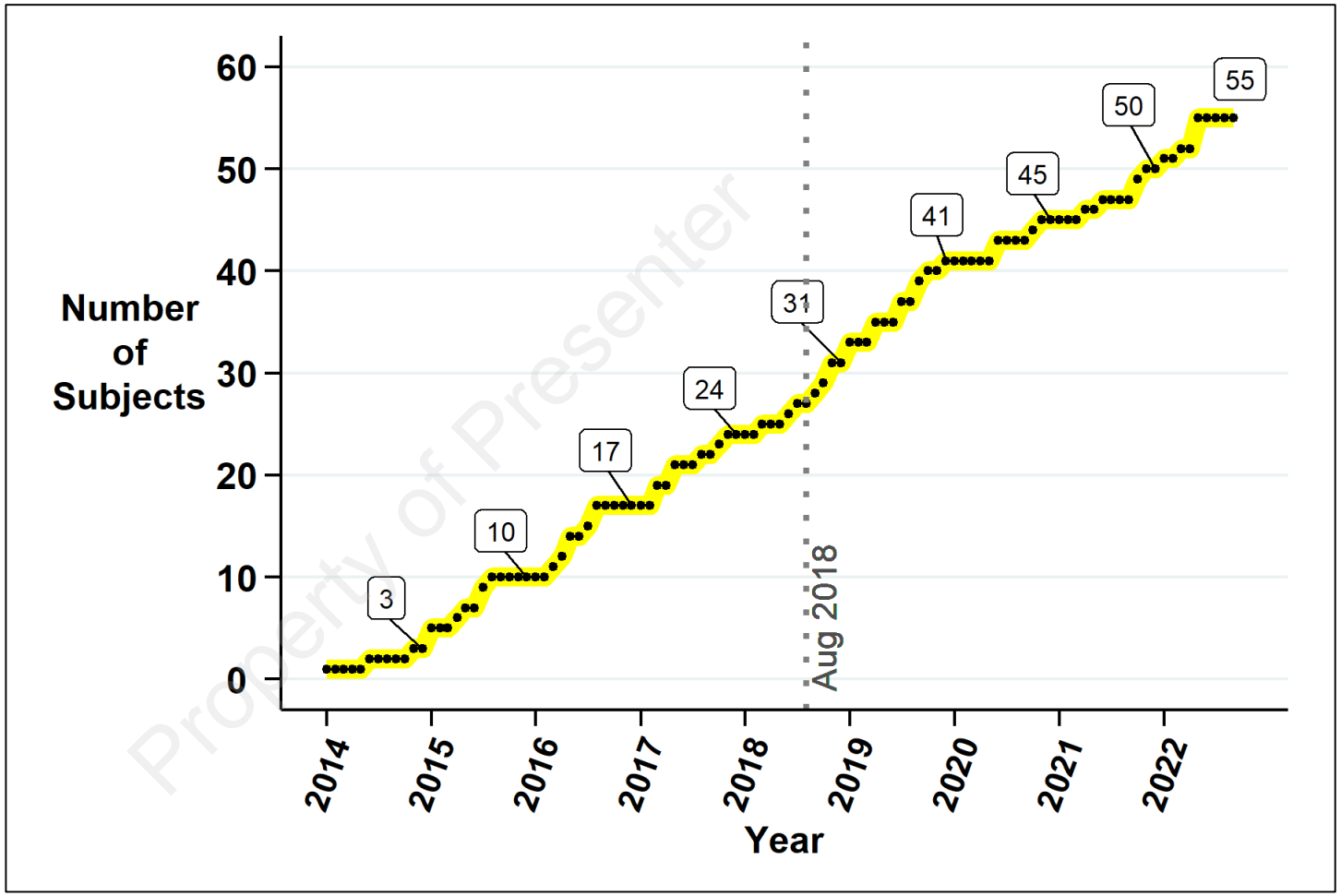
MAC drugs:
Rifampin (if no ivacaftor)
Clofazimine
ALIS>Amikacin (inh)
Tedazolid>Linezolid
Bedaquiline

MAB alternates:
Clofazimine
Omadacylin > Tigecylcine
Tedazolid > Linezolid
Bedaquiline
Moxifloxicin

All NTM isolates are evaluated by WGS by the Colorado NRC Molecular Core

PATIENCE: Ever Enrolled

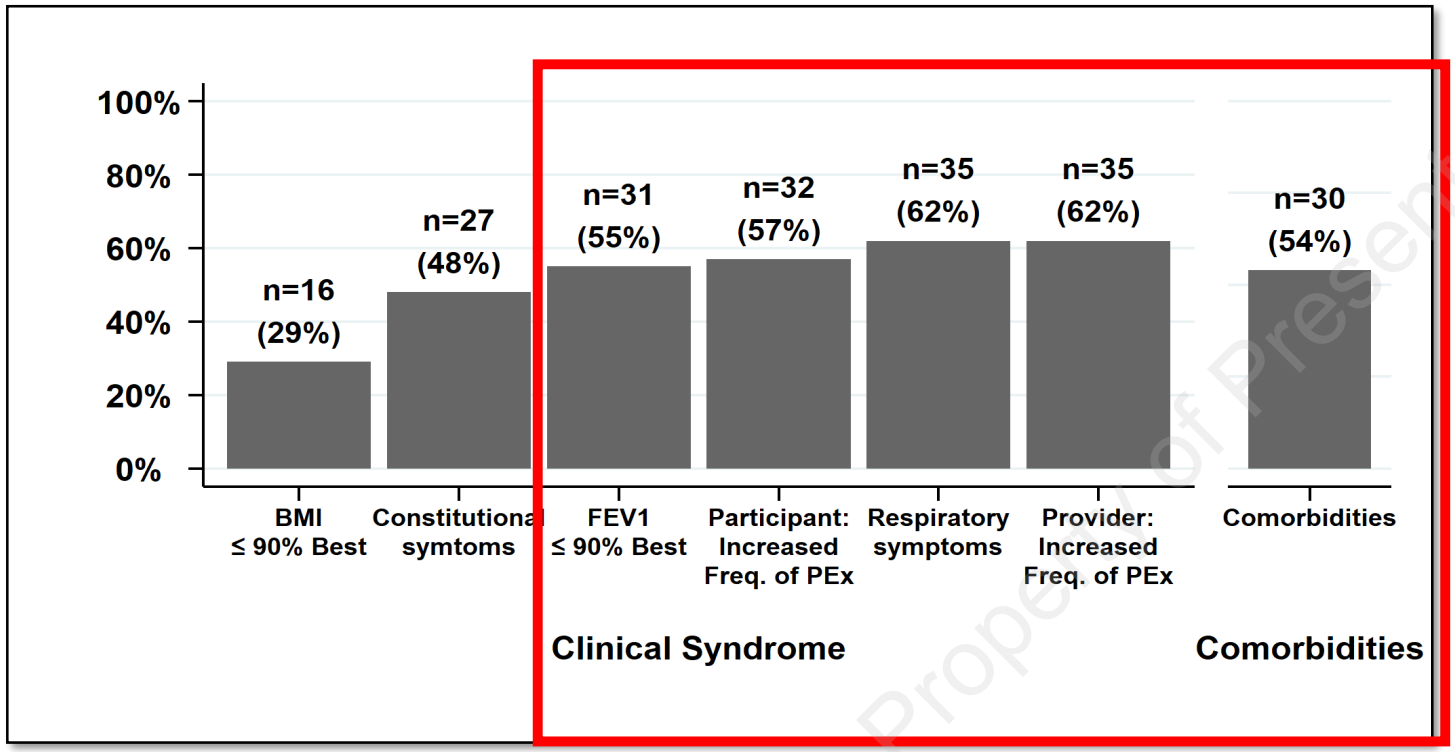
Current number of PATIENCE participants "ongoing study procedures": n=15

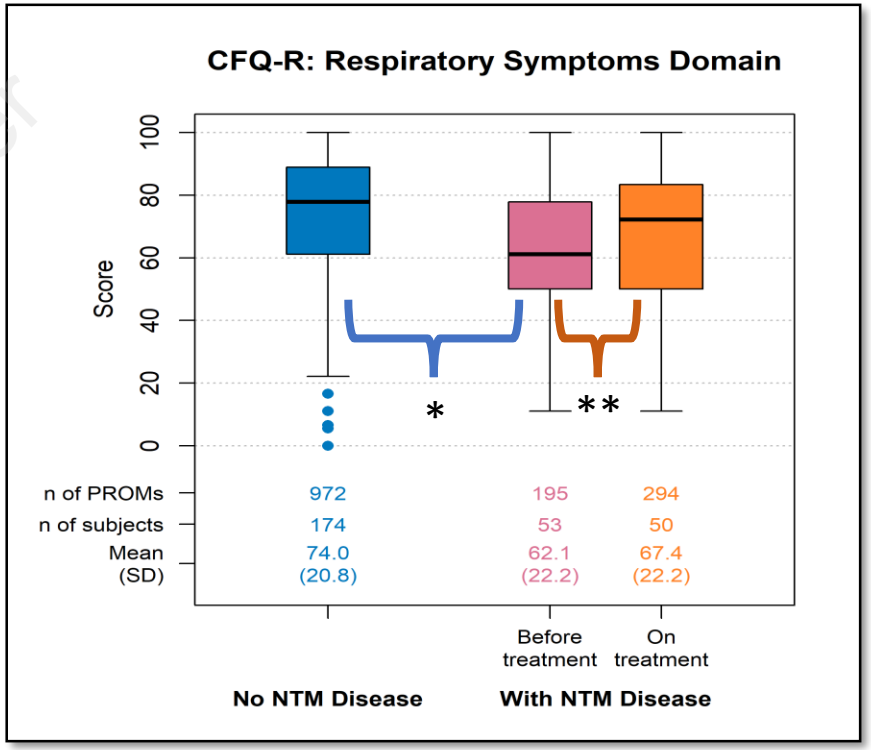


PATIENCE Demographics: MAC & MABSC

	Total (n=55)	MAC (n=33; 60%)	MABSC (n=22; 40%)
Male sex (%)	31%	24%	41%
Enrollment FEV% predicted, mean (SD)	72 (24)	76 (24)	64 (21)
Enrollment age, mean (SD)	25 (12)	26 (14)	25 (10)
Enrollment age distribution			
6 to 12 years	9%	12%	5%
>12 to 18 years	18%	18%	18%
>18 to 30 years	49%	42%	59%
> 30 years	24%	27%	18%
CF Genotype			
F508del homozygous	46%	46%	46%
F508del heterozygous	38%	39%	36%
other	17%	12%	18%

Clinical Syndrome & CFQ-R at Time of NTM Disease Diagnosis

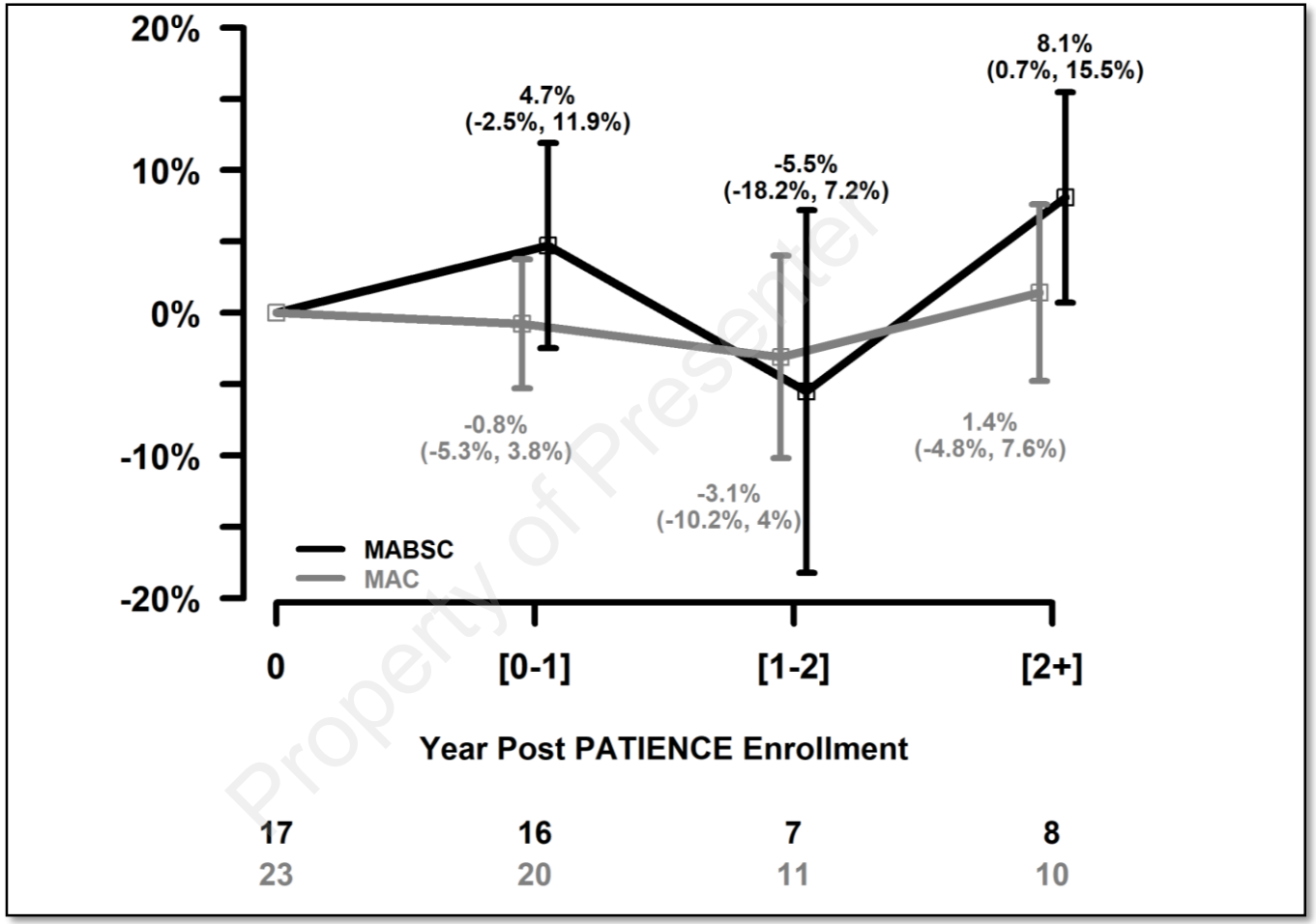




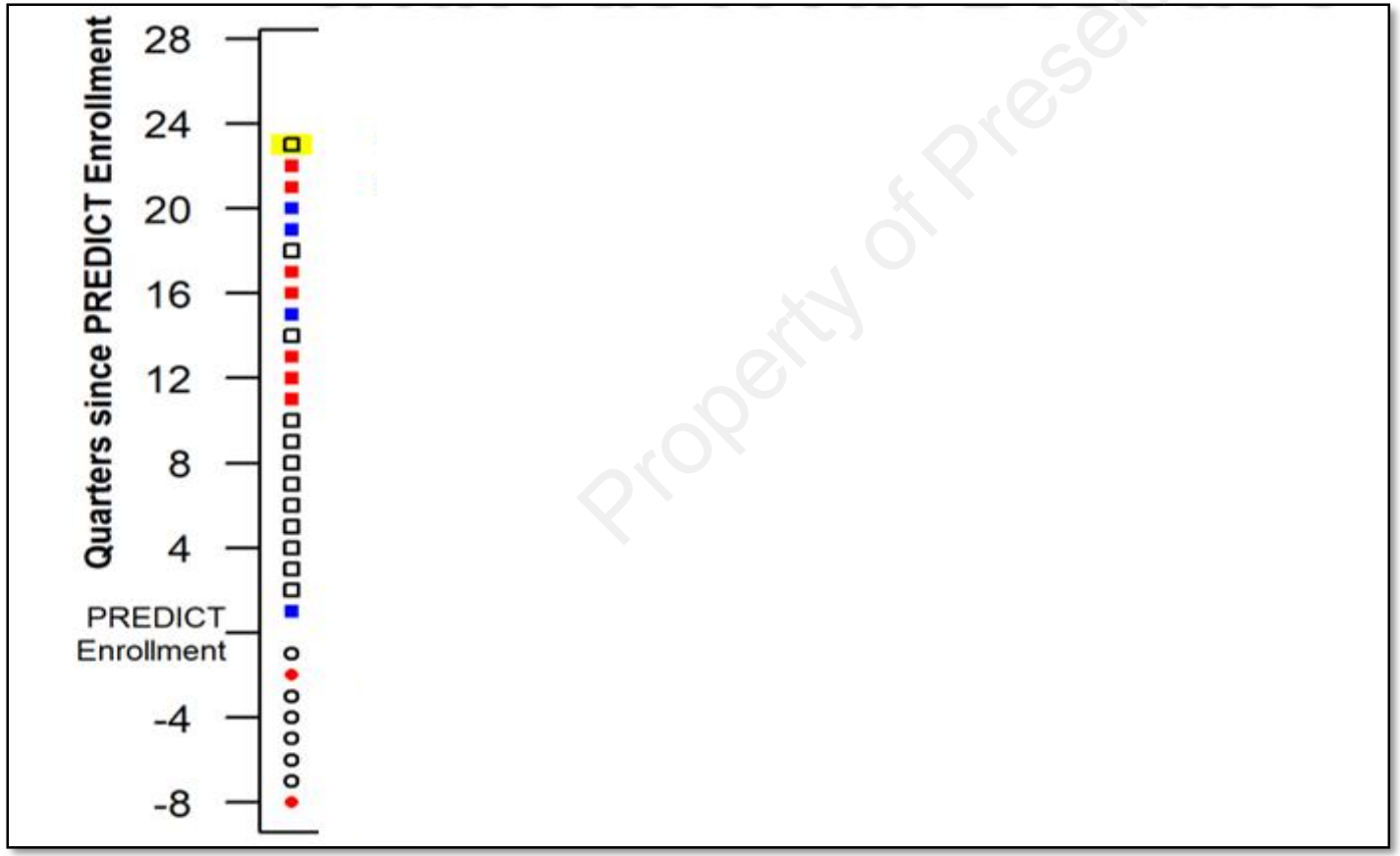
*Respiratory symptom domain is 14 points lower after NTM disease onset prior to treatment, 95% CI 8 to 20, p<0.001.

**Respiratory symptom domain 10 points higher post treatment in those with NTM disease, 95% CI 6 to 13, p<0.001.

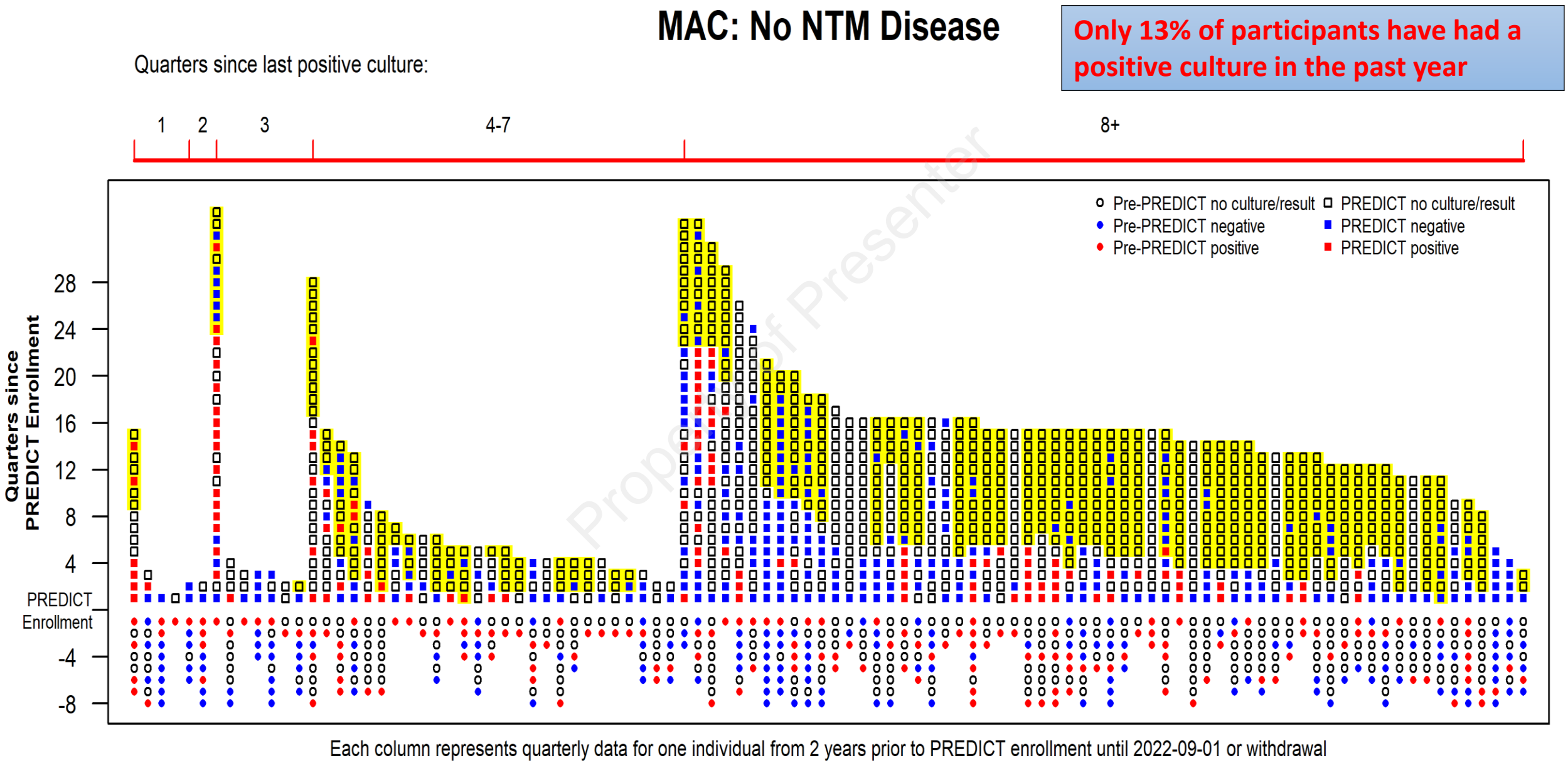
PATIENCE: Change in FEV1 % Predicted



Culture Data among PREDICT Subjects without NTM Disease



Culture results: PREDICT subjects without NTM Disease



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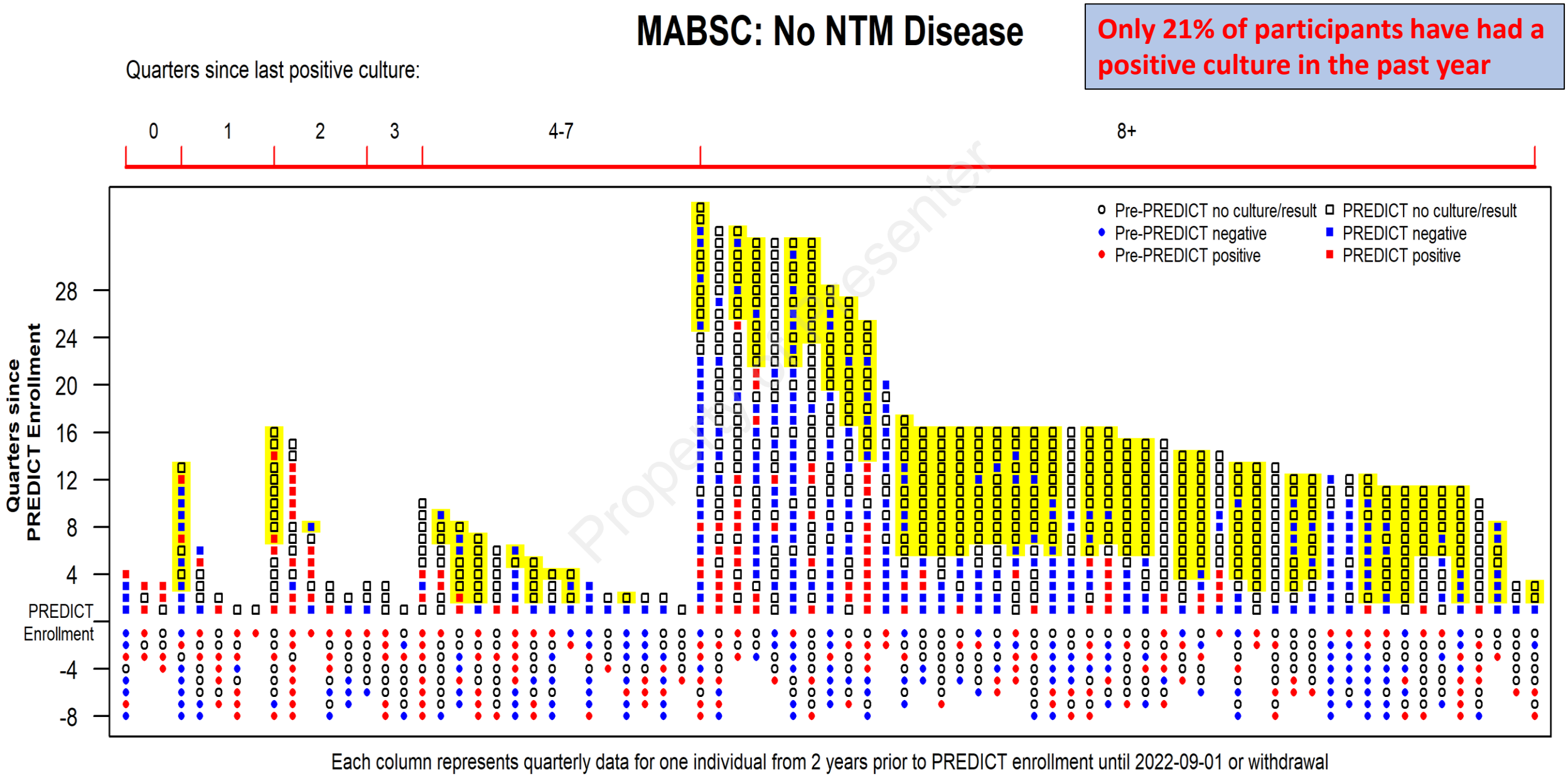
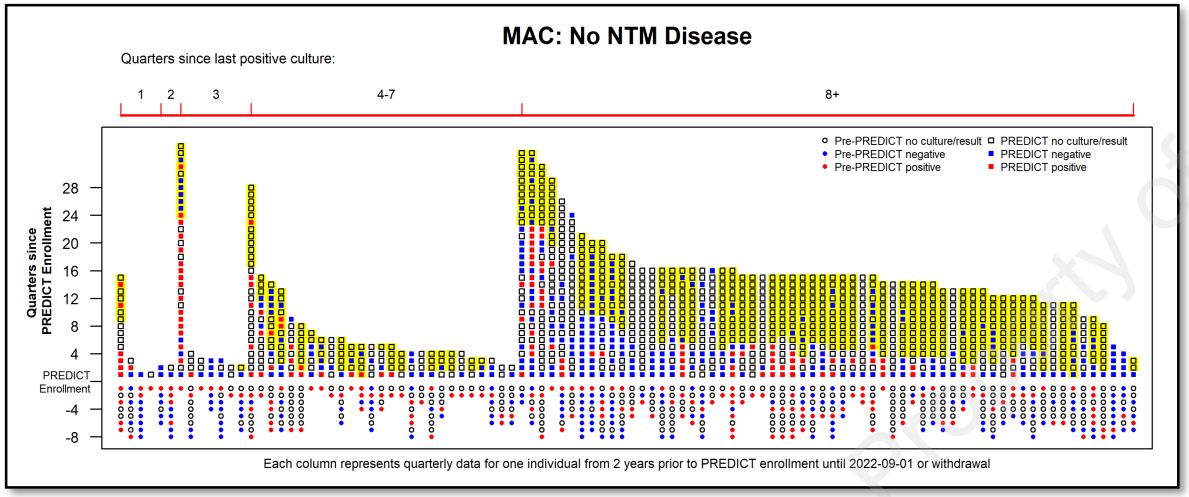
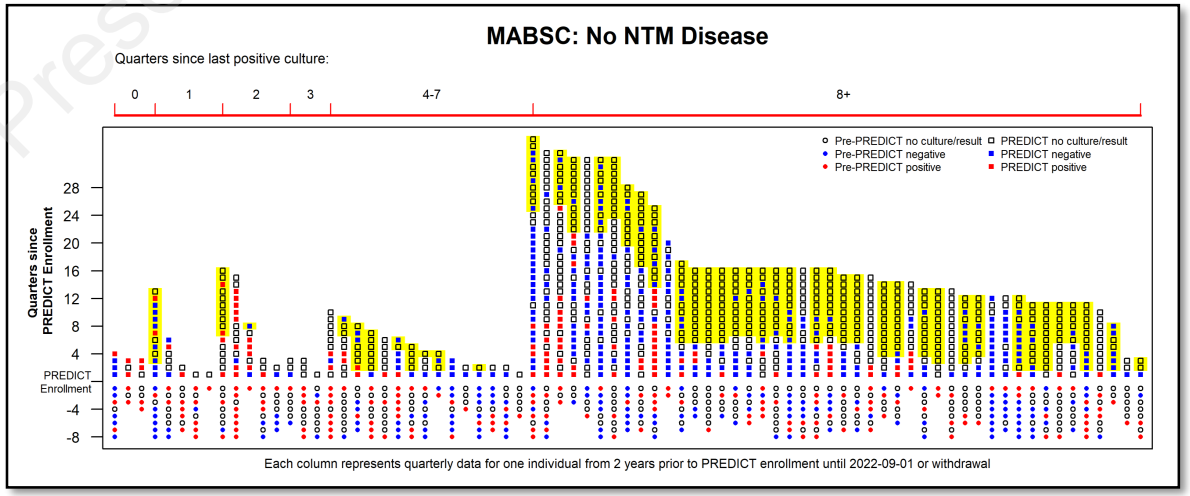


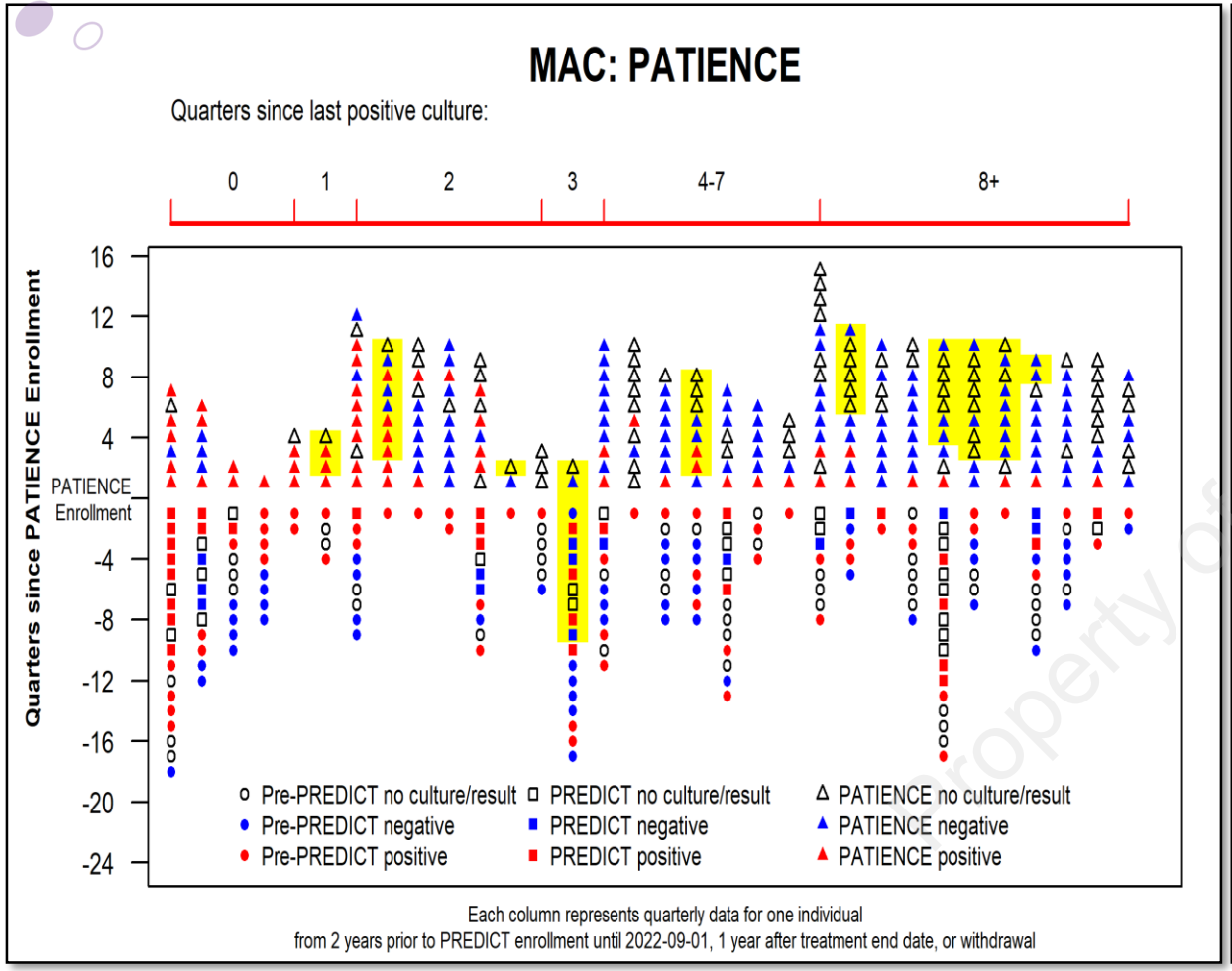
Culture Data among PREDICT Subjects without NTM Disease

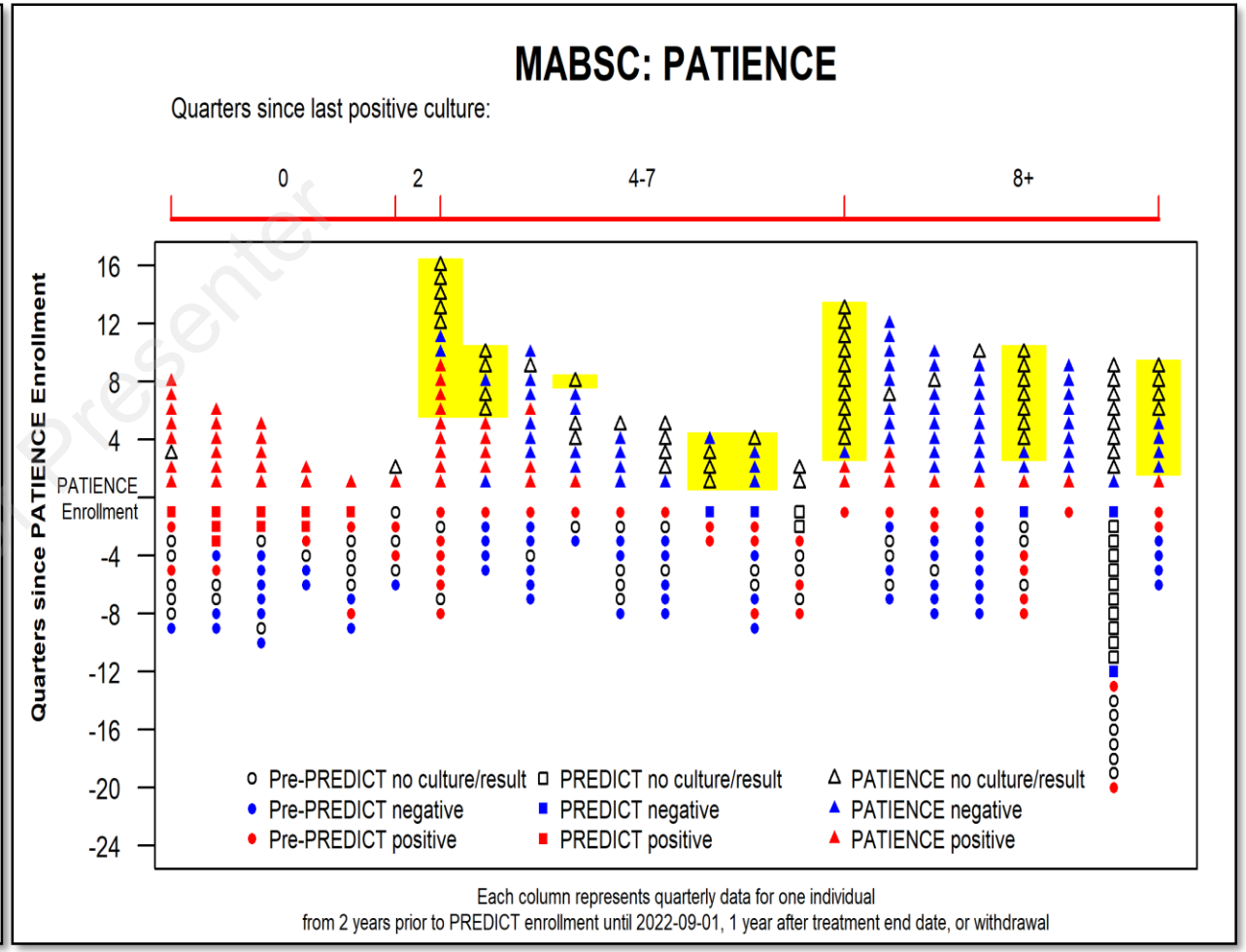




- 23% of participants have had NTM culture obtained in the last 6 months
- 44% of participants have had NTM culture obtained in the last year
- Most missing cultures likely due to elexacaftor/tezacaftor/ivacaftor (Trikafta)

Culture Data: Treatment Response





MAC (n=33)
34% culture negative > 2 years
69% most recent culture negative
13% remain culture positive
6% inconclusive due to missing cultures

***M. abscessus* (n=22)**
35% culture negative > 2 years
70% most recent culture negative
22% remain culture positive
4% inconclusive due to missing cultures

So what's wrong with NTM airway cultures?

(context of CF lung disease)

Low sensitivity due to low NTM burden

- Reduced yield due to sputum decontamination
- NTM disease (PATIENCE Trial) 65% positive
- Indolent infection (PREDICT Trial) 38% positive
- Following E/T/I (PREDICT Trial) 18% positive

Lack of sputum samples

- Expectorated sputum rare in children and adults with mild disease
- Dramatic reduction due to E/T/I
- BAL not recommended for asymptomatic screening

The reasons we need biomarkers are also why validation is so challenging!

Colorado NTM Core Clinical Research Service

- Analysis of **unprocessed airway samples**
- **Culture** and **molecular identification** of isolates
- Expanded and custom **drug susceptibility testing**
- SOP for **remote sputum sample collection**
- NTM **biorepository**
- Custom **PK/PD** testing
- Custom **whole genome sequencing (WGS)** of isolates
- WGS **comparisons** to extensive CF and non-CF isolates
- **Database coordination** with WGS and phenotypes
- Clinical trial **design and outcome** analysis

NTM Outcome Measure Advancement Core

- Custom **PK/PD assays** from sputum, tissue, other biosamples
- Gene or isolate testing for **pre- and post-trial changes** in DST
- **Consulting** on preclinical studies (industry & academics)
- **Phylogenetic analysis** of NTM isolates to distinguish between reactivation, reinfection or polyclonal infections
- Panels of CF and non-CF **NTM isolates** for drug candidate screening.
- Quantitative/ culture independent assessment of **NTM bacterial burden**
- Provide ***in vitro*** **testing** of agents against NTM
  - biofilm phenotype, intracellular systems, mouse models

Clinical Trials Assisted by the NTM NRCs

PREDICT Trial (NCT02073409) and **PATIENCE** Trial (NCT02419989) (Nick, Martiniano)
PAINLESS Trial: (NCT04324088)(Nick)
PIVOT Trial: Prospective evaluat**I**on of sali**V**a c**O**mpared to sputum.
ABATE Trial: A Phase 1b, Multi-center Study of IV Gallium Nitrate in Patients with Cystic Fibrosis who are Colonized with Nontuberculous Mycobacteria (CFF and FDA) (Goss, Nick, Singh)
HALT NTM Study: (NCT04024423)(Gross)
ENCORE Trial: (NCT03597347) (Nick)
FORMaT Trial: (ACTRN12618001831279p) (Wainwright)
Improving Treatment of NTM Infection in CF (NCT02372383) (Martiniano)
Protocol For **Bacteriophage Treatment** of *Mycobacteria abscessus* through An Investigational New Drug Application (IND)

27 additional planned or pre-clinical trials also receiving NTM NRC assistance

National Jewish Health
Breathing Science is Life.

J Nick, MD | C Daley, MD | M Strong, PhD | R Davidson, PhD

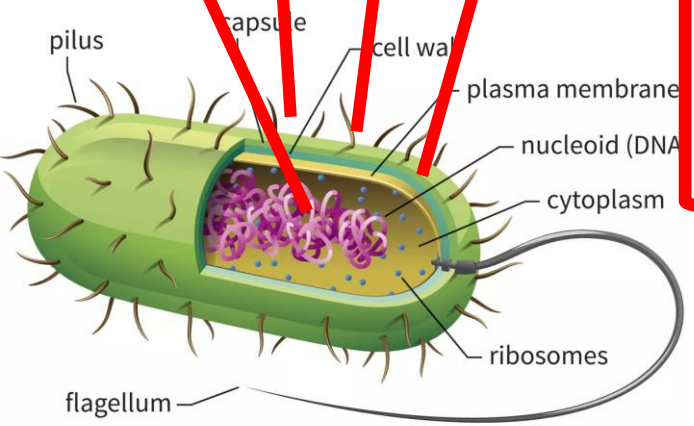
Targets for NTM Detection

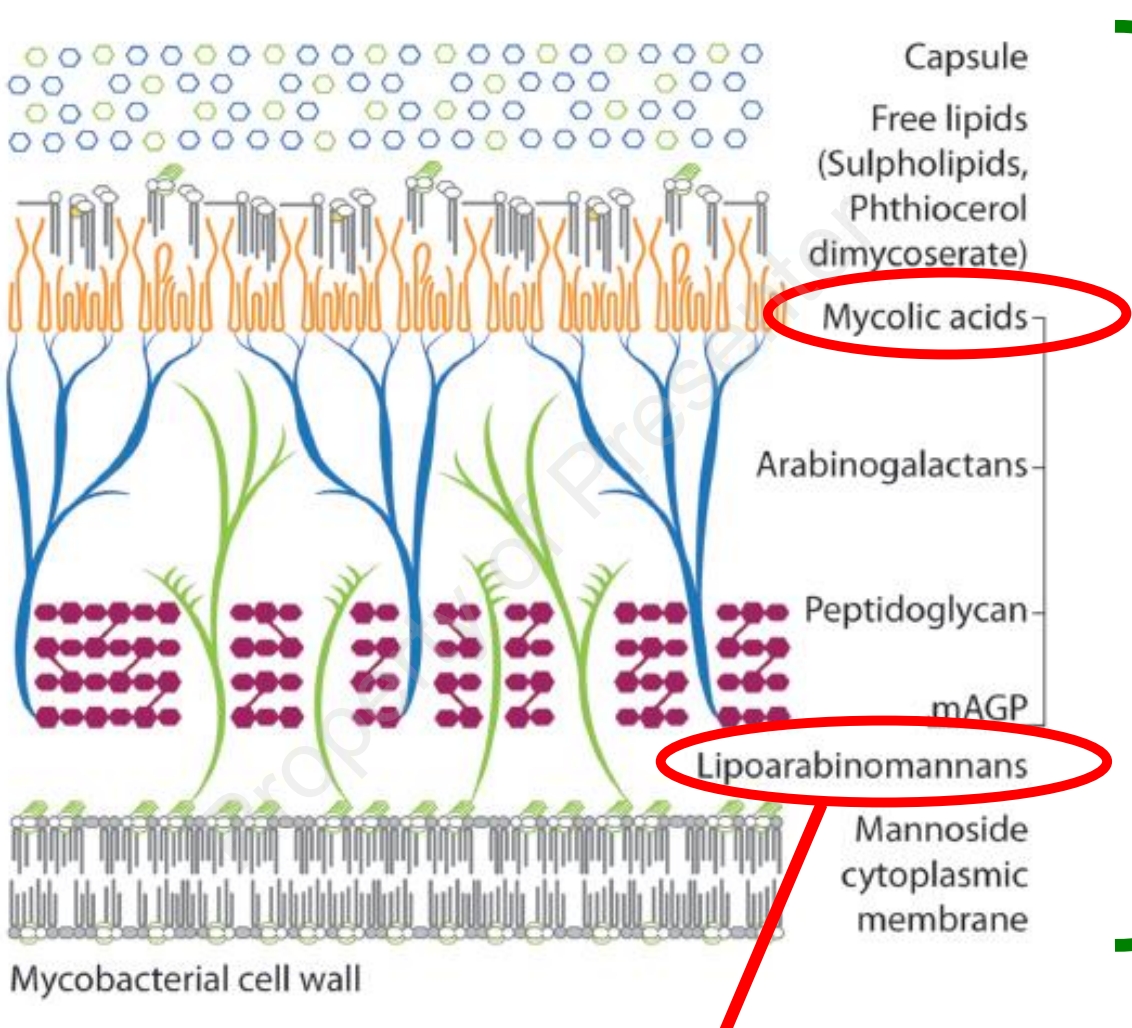
Volatile metabolites

Culture

Cell Wall

DNA





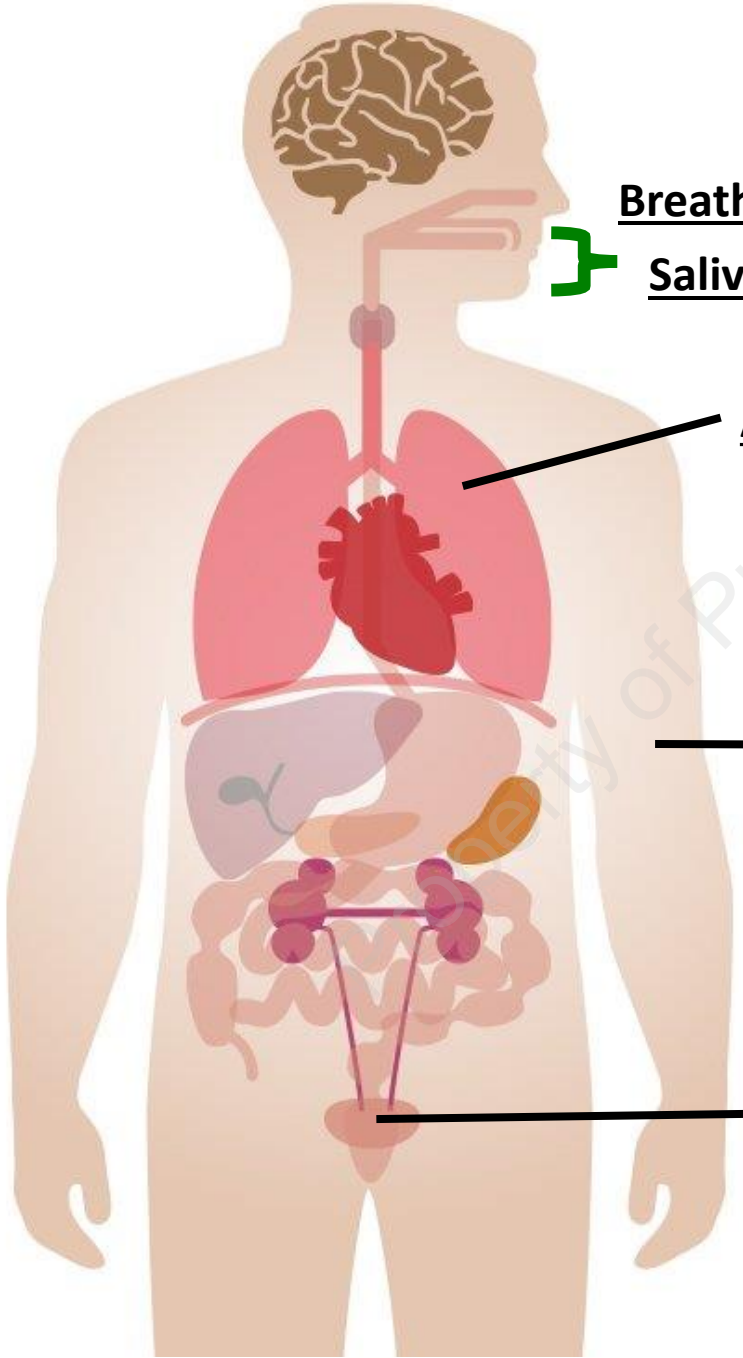
Direct Measure of Antigens

Host response

Leukocyte RNA expression

Immunoglobulins

Sites and Specimens for NTM Detection



Breath: volatile metabolites

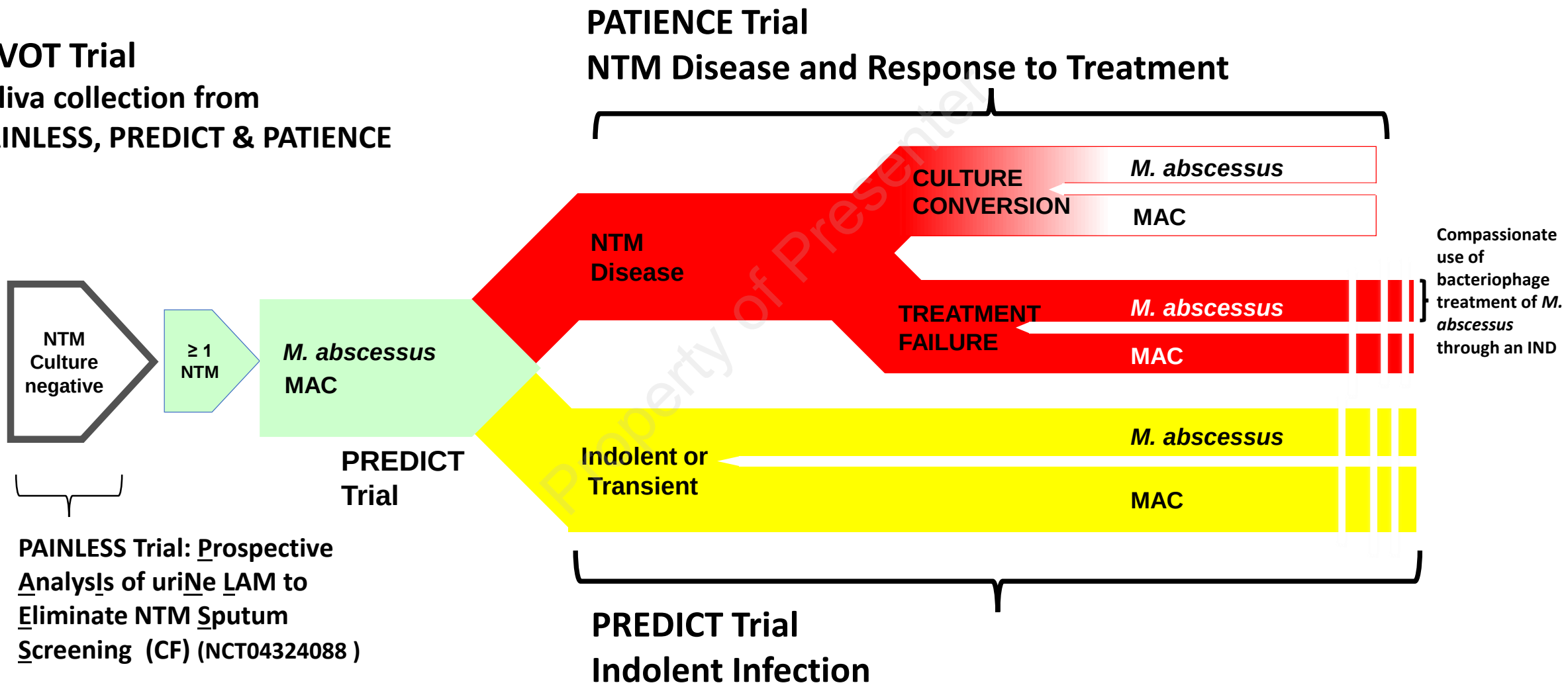
Saliva: Immunoglobulin, DNA

Airway (sputum, BAL): Culture, DNA

Circulation:
DNA
Leukocyte RNA expression
Immunoglobulins
LAM

Urine: LAM

Utilizing prospectively identified NTM cohorts to replace single sputum cultures in marker studies



Biomarker studies through Colorado NTM Outcome Measure Advancement Core NRC

NTM Markers Currently Being Tested in Trials

NTM genome: Colorado Adult P&P Award

- ***WGS*** (Michael Strong, PhD, Rebecca Davidson, PhD NJH).

Radiographic predictors: CFF CRSP Award

- ***HRCT*** (Stacey Martiniano, MD, CHCO, David Lynch, MD, NJH).

Sputum: NIH-funded ancillary study (R01 HL146228)

- ***Microbiome*** (Rebecca Davidson, PhD, NJH).
- ***Volatile sputum metabolites*** (Jane Hill, PhD, University of British Columbia).

Urine: CFF IRI Clinical Trial Award (PAINLESS Trial)

- ***Urine lipoarabinomannan*** (Delphi Chatterjee, PhD, CSU).

Saliva: CFF Clinical Trial Award (pending)(PIVOT Trial)

- ***Targeted amplicon*** (Rebecca Davidson, PhD, NJH).
- ***Antibodies*** (Kara Calhoun, MD, UCD).

NTM Markers Under Evaluation to be Added to Trials

Breath: CFF Clinical Pilot Award

- ***Volatile breath metabolites*** (Jane Hill, PhD, University of British Columbia).

Whole Blood: CFF Clinical Pilot Award

- ***Circulating leukocyte RNA signatures*** (Mimi Saavedra, MD, NJH).

Plasma: CFF Clinical Pilot Award

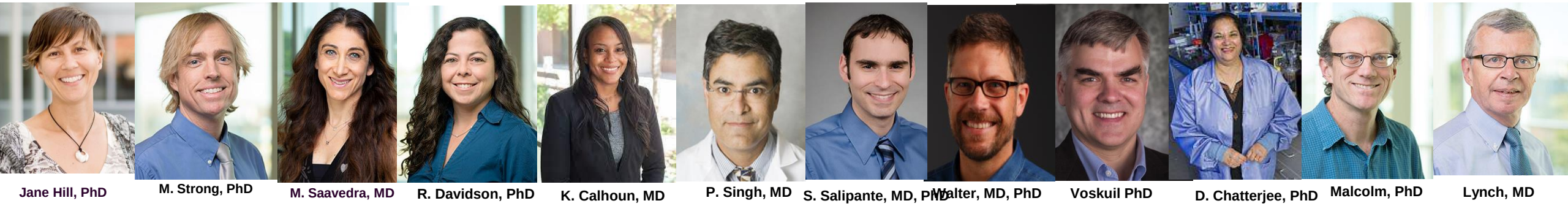
- ***Circulating DNA signatures*** (Pradeep Singh, MD, Steve Salipante, MD, PhD, University of Washington).

Serum: CFF Clinical Pilot Award

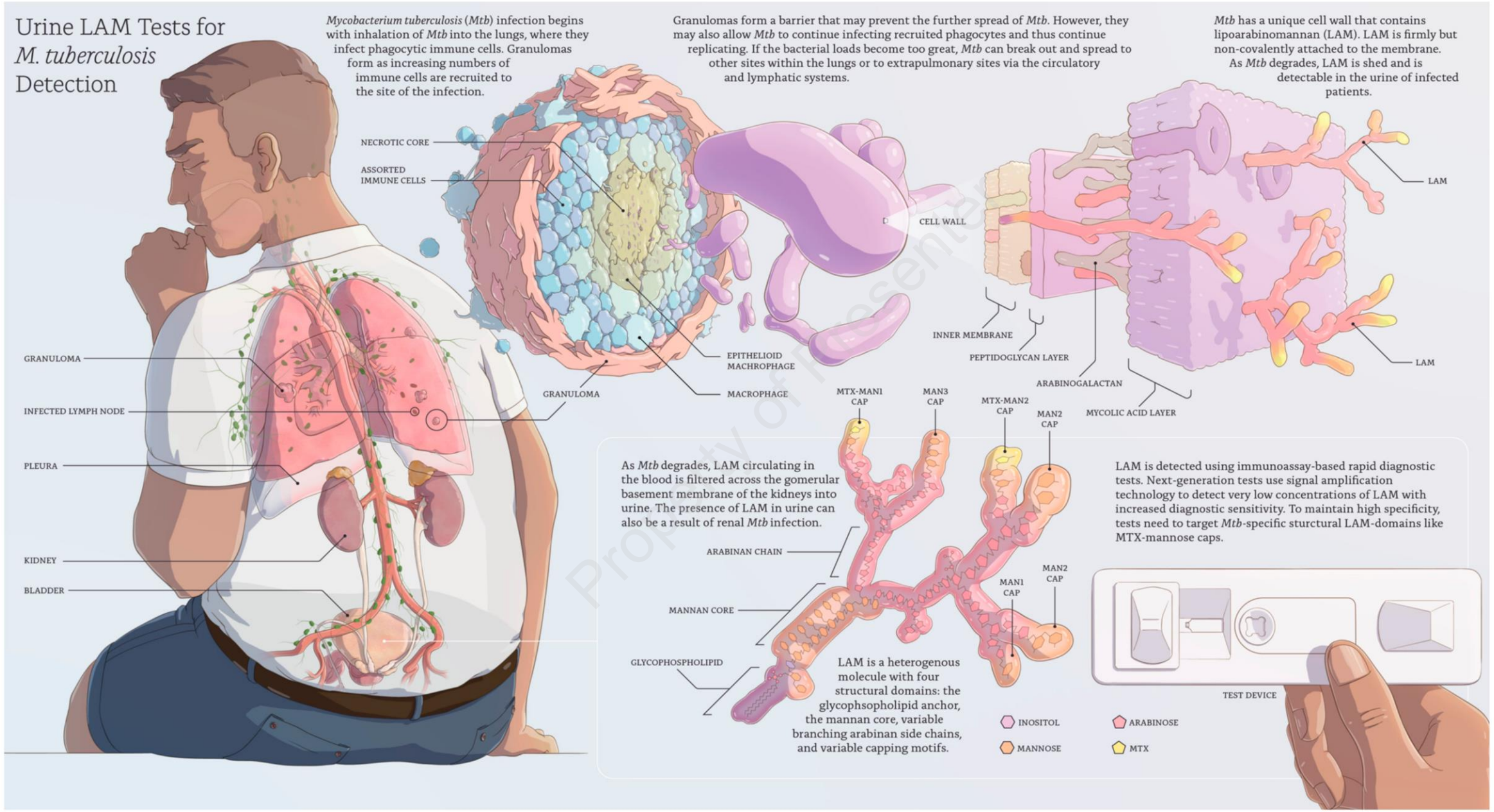
- ***Antibodies and inflammatory markers*** (Ken Malcolm, PhD, NJH).
- ***Mycolic Acid Antibodies*** (Diagnostig, UK).
- ***Cholesterol metabolites*** (Jen Philips, MD, PhD, Washington Univ).

Sputum

- **Bacterial RNA (R/S ratio)**(Nick Walter, MD, PhD, Martin Voskuil, PhD, University of Colorado



Urine Lipoarabinomannan (LAM)



J. Clin. Med.

Urine LAM

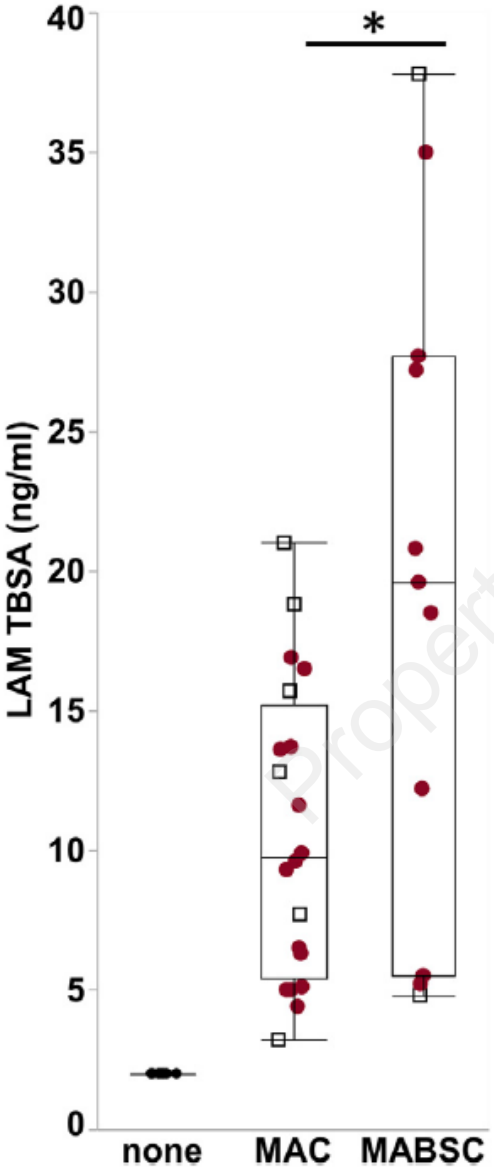
Advantages

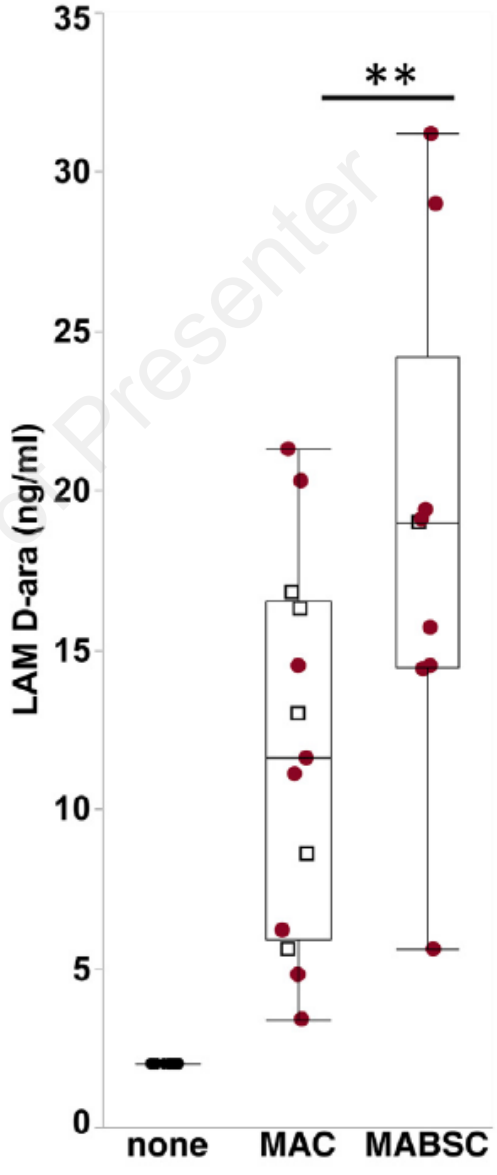
- Sample is readily available from all subjects
- Test is well validated in TB among individuals coinfected with HIV
- May reflect global infectious burden (all lobes)

Challenges

- Very low burden of infection in CF versus TB
- LAM from NTM differs from TB
- Generally below LOD for approved immunoassays
- Nonspecific for species or subspecies of NTM

Urine lipoarabinomannan (D-ara or TBSA) analyzed by GC/MS correlates with past NTM culture history



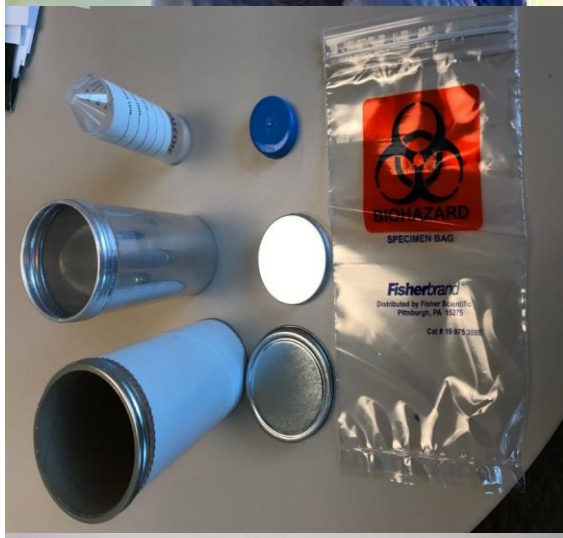


D. Chatterjee (CSU)

J Cyst Fibros. 2020 Sep;19(5):

PAINLESS Trial: Prospective Analysis of uriNe LAM to Eliminate NTM Sputum Screening (CF) (NCT04324088)

- Never culture positive of NTM by chart review **and** minimum of 3 cultures in last 3 years
- Annual urine LAM compared to sputum cultures during clinical care
- Electronic consent
- Sputum collection by mail (or in person)



E. Armantrout, RN

V. Lovell, RN

PAINLESS Trial: Prospective Analysis of uriNe LAM to Eliminate NTM Sputum Screening (NCT04324088)

Concordance of a positive urine LAM result and with previous airway cultures for NTM

	Urine LAM Pos (+)	Urine LAM Neg (-)
Pos. NTM History	n=4 True Pos.	n=0 False Neg.
Neg. NTM History	n=10 False Pos.	n=96 True Neg.

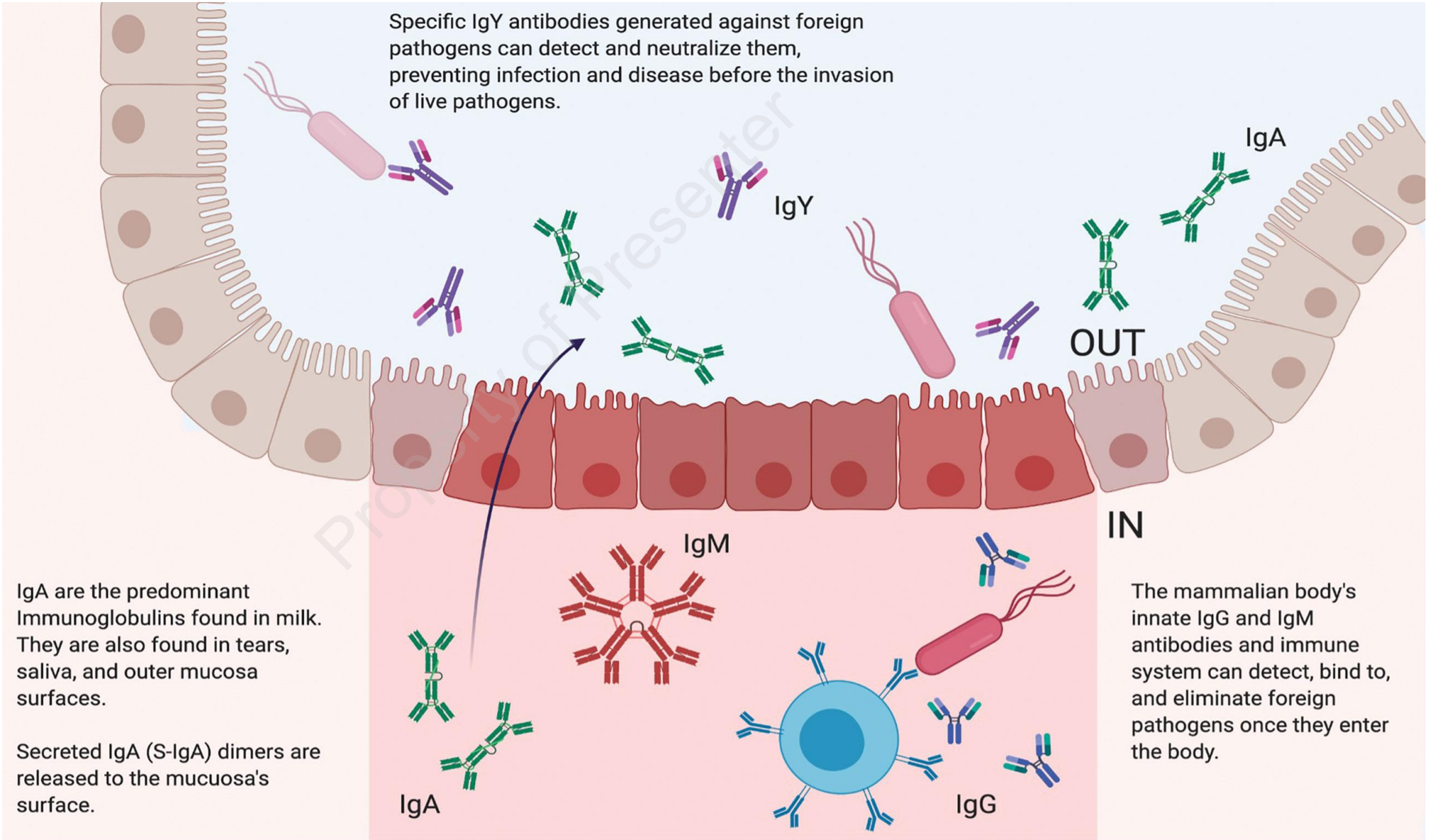
Negative Predictive Power = 100%
Sensitivity = 100%
Specificity = 90.6%
Positive Predictive Power = 28.6%

Next Steps

CFF-NICK20A0 (Nick, PI) Urine Lipoarabinomannan as a Marker for Low-risk of NTM Airway Infection **–cap at n=100**
Expansion of urine LAM screening to multiple sites

Longitudinal Assessment of Urine Lipoarabinomannan as a Culture-Independent Systemic Marker of Pulmonary Nontuberculous Mycobacteria *Urine LAM analysis from PREDICT and PATIENCE*

Immunoglobulin titers (serum and/or saliva)



Immunoglobulin titers (serum and/or saliva)

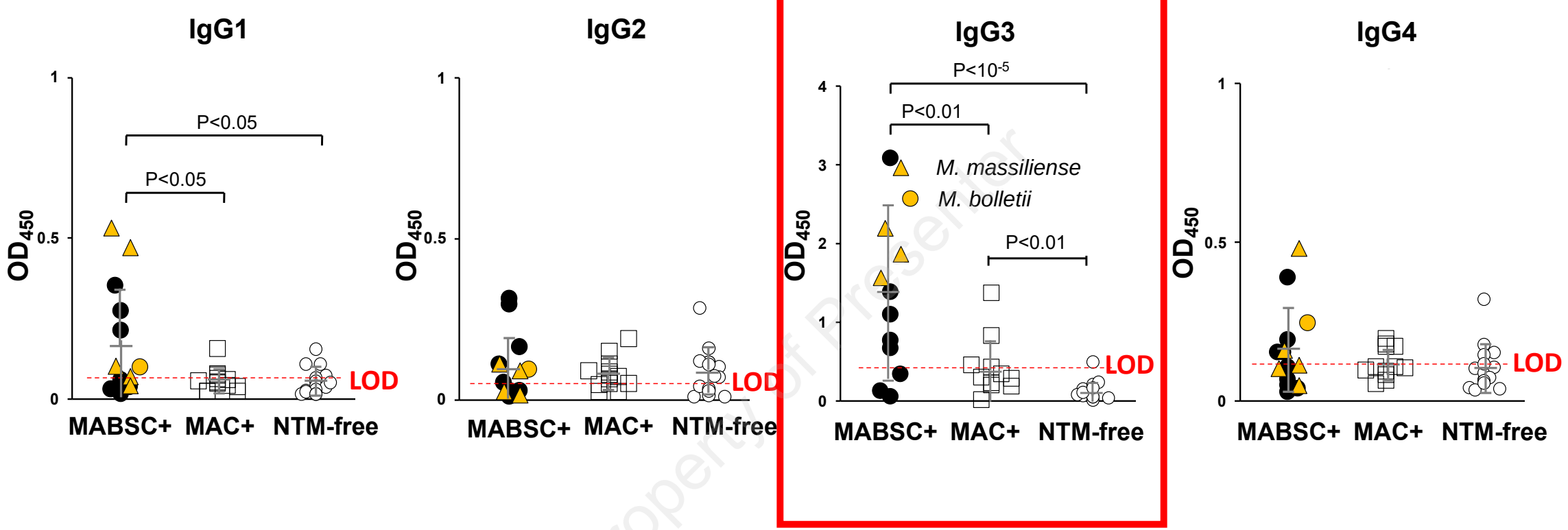
Advantages

- Sample(s) are readily available from all subjects
- Potentially highly sensitive

Challenges

- Depends on functioning immune system
- Nonspecific for species

Serum IgG titers

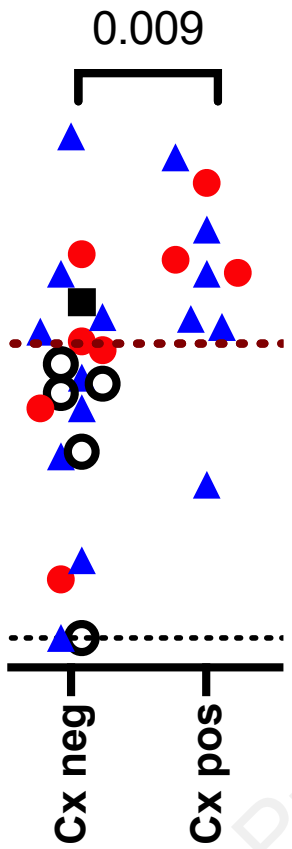


ELISA using NTM whole cell lysates
Culture status over a 5-year period
Duel infections assigned to MABSC

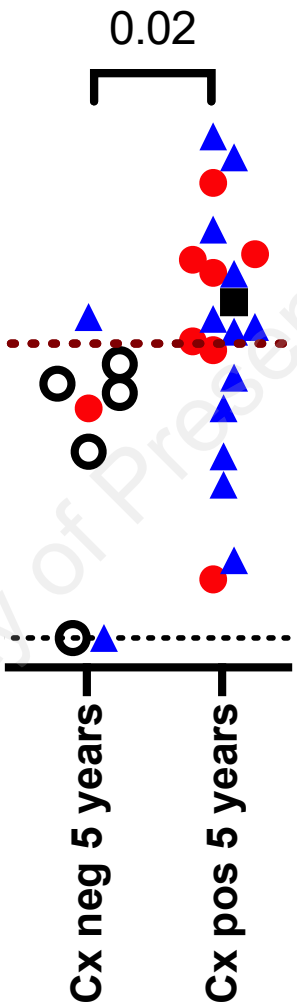
K. Malcolm (NJH)

Malcolm, KC (Microbiol Spectr. 2022**)**

Saliva: IgG + IgA against NTM cell lysates



Sens 0.58
Spec 0.93



Sens 0.92
Spec 0.47

K. Calhoun, MD

Molecular Detection of NTM from raw sputum (and saliva)

1. **Quantitative real-time PCR (qPCR)**
   - *M. abscessus* or MAC assays
   - Bacterial DNA quantification compared to standard curve

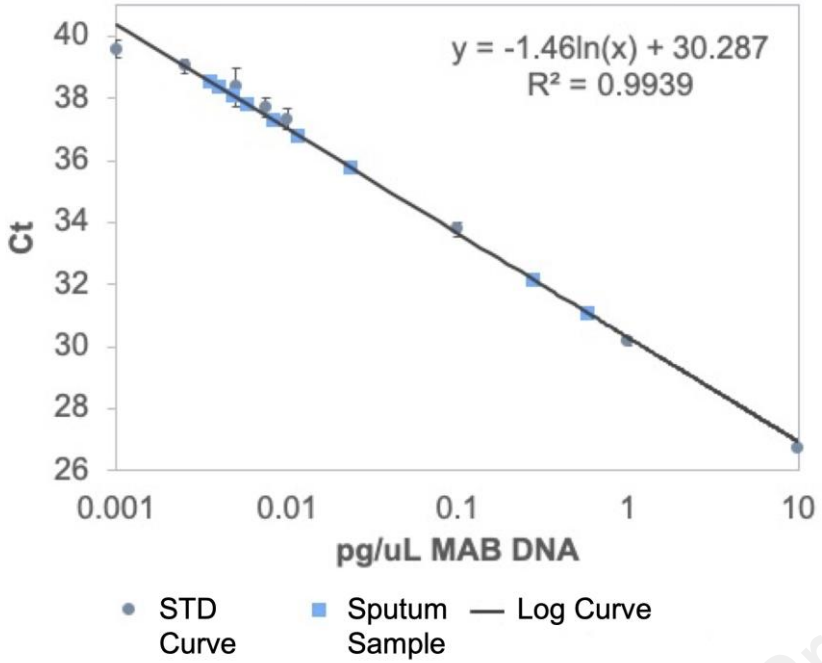
2. **Targeted Amplicon Sequencing Panel**
   - Simultaneous detection of multiple NTM species
   - More sensitive than qPCR

Advantages:
- Minimal processing of samples (no decontamination)
- Low DNA input for assays
- Samples are direct from the site of infection
- Easy to obtain (saliva)
- Highly sensitive for species identification

Challenges:
- Very low burden of infection (saliva)
- Low availability of samples (sputum)
- Unable to distinguish between living/dead bacteria
- Water contaminants in saliva

M. abscessus qPCR tested in 120 sputum samples

- from 86 subjects in the PREDICT and PATIENCE trials



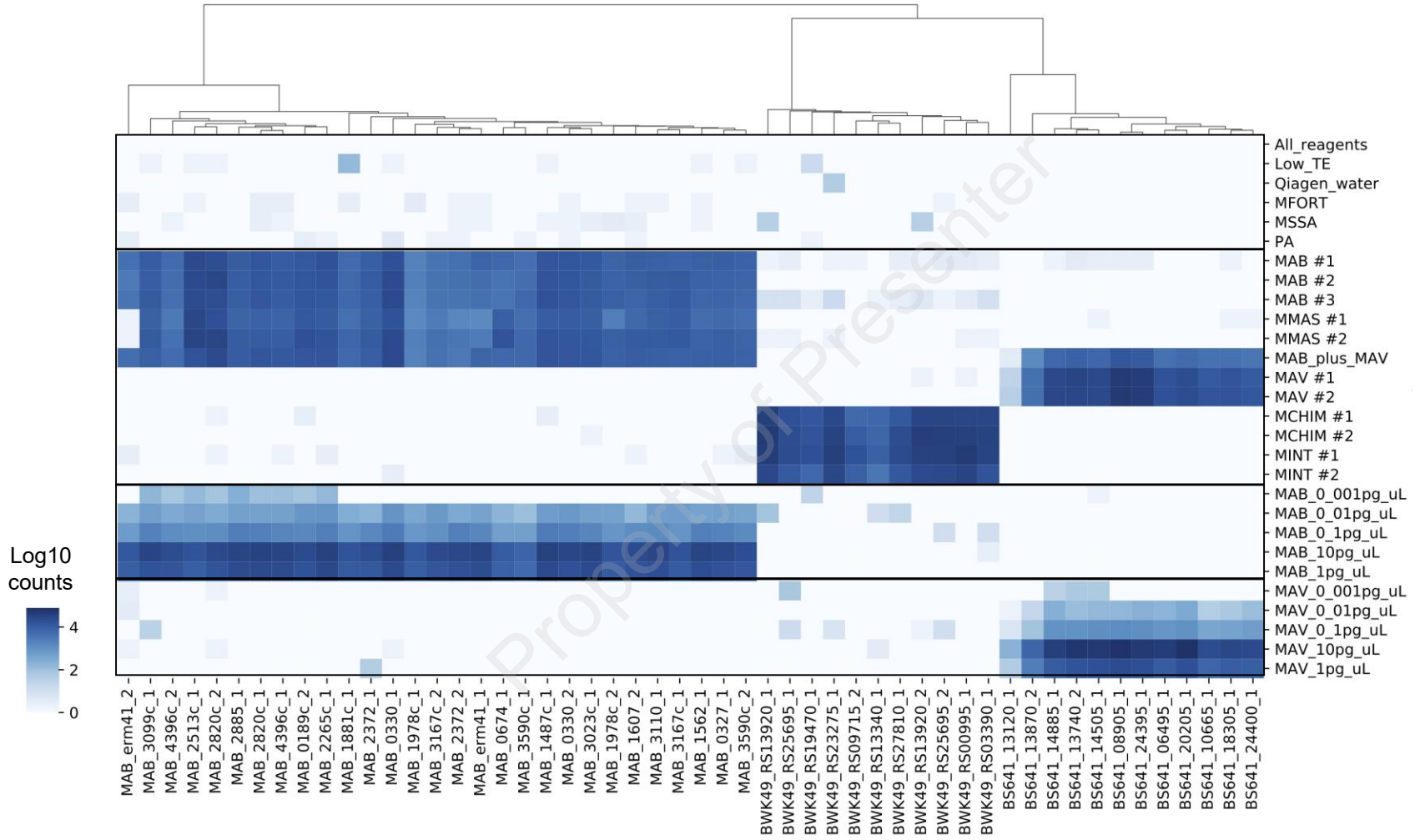
	Sens	Spec	PPV	NPV
Matched culture (n=90)	0.79	0.91	0.61	0.96

High cycle threshold but
Excellent fit within standard curve

R. Davidson (NJH)

Targeted Amplicon Sequencing for NTM

59 amplicons in sequencing panel



Negative controls

On target NTM species

MAB dilution series

MAV dilution series

Columns = amplicons
Rows = QI samples

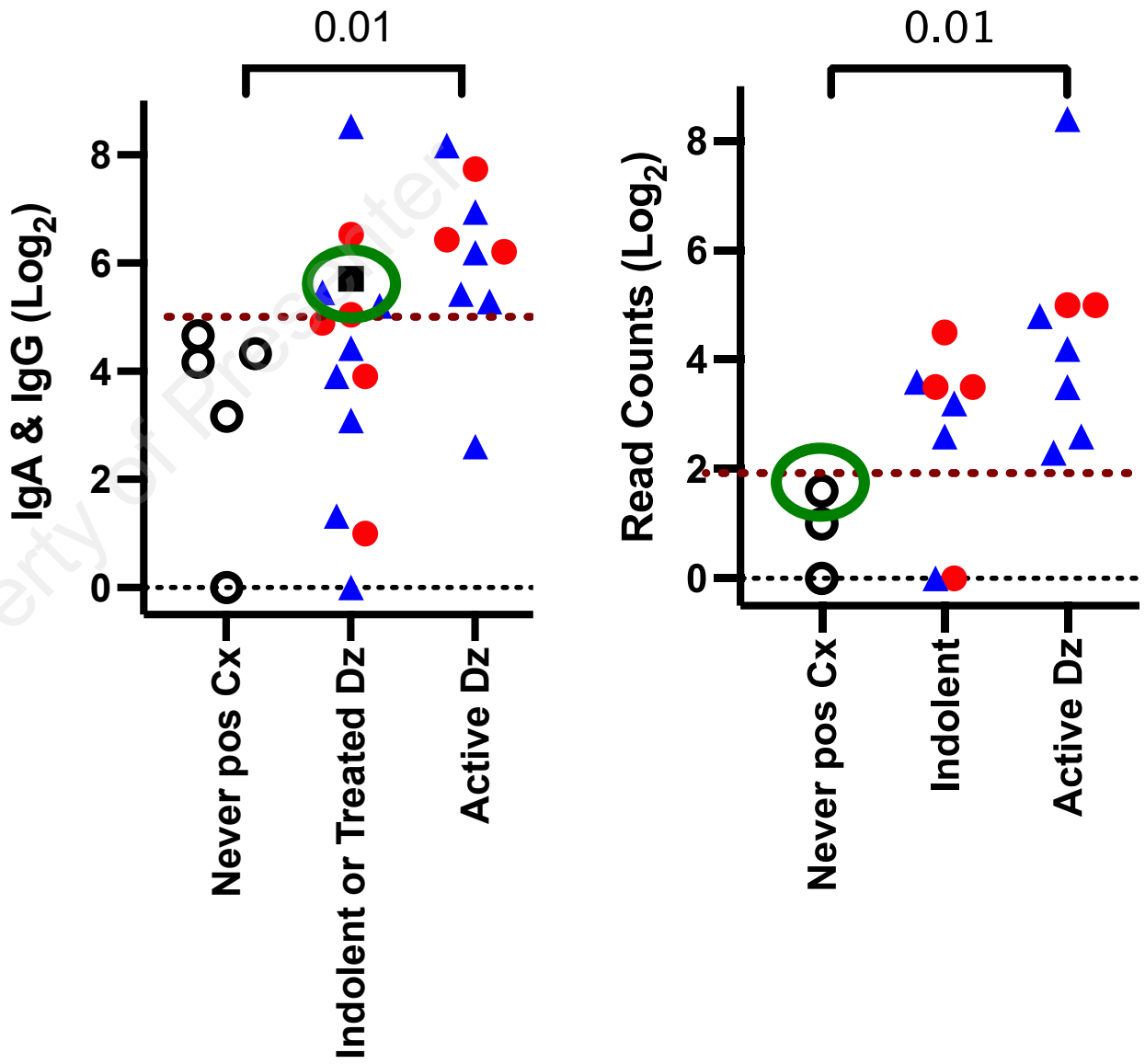
R. Davidson (NJH)

Combined Immunoglobulin and Molecular markers for NTM in saliva

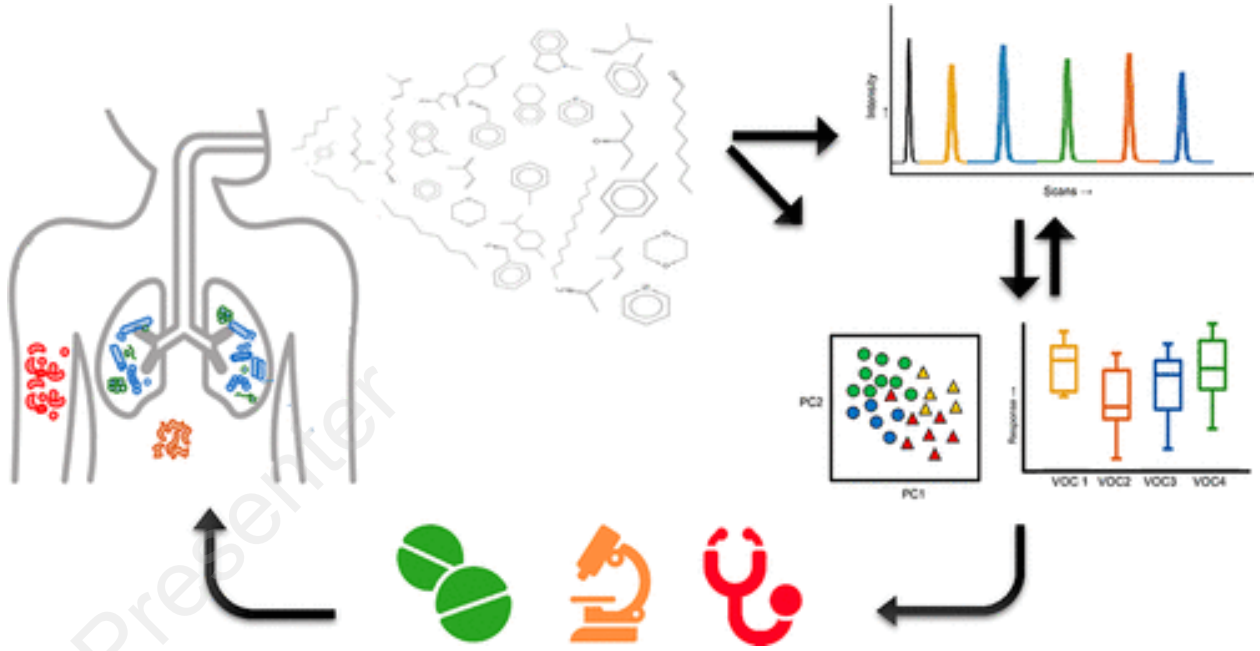
Sum of Ig and Mol positive
Active Dz Sensitivity 0.80

Either Ig or Mol positive
Specificity 1.0

Subject with *M. lentiflavum* correctly identified as having a positive NTM culture by Immunoglobulin assay, but neither MAC or *M. abscessus* by molecular assay.



Volatile Molecules (breath or sputum)



Advantages

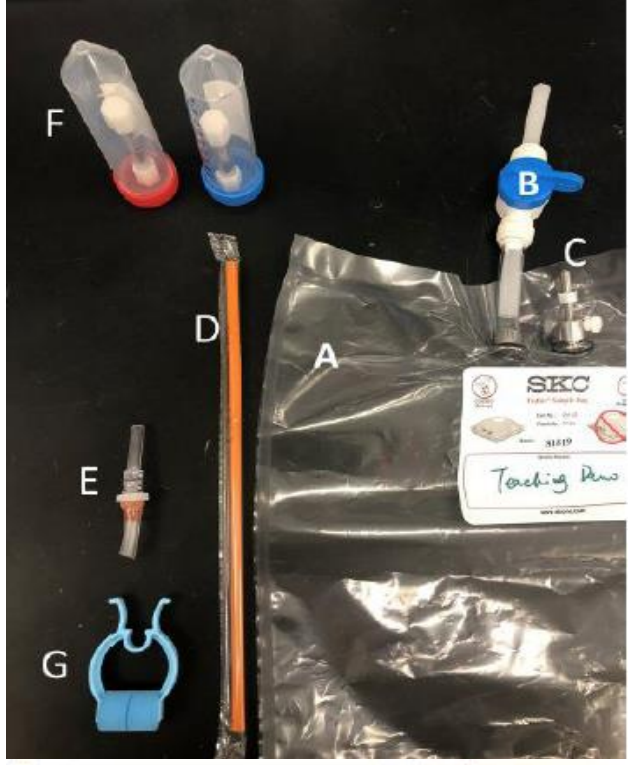
- Non-invasive sample from airway
- Can be applied to sputum samples
- Potentially highly sensitive
- Successful reports in TB and a variety of airway infections

Challenges

- Method and interpretation not standardized or widely available

Volatile Molecules (breath or sputum)

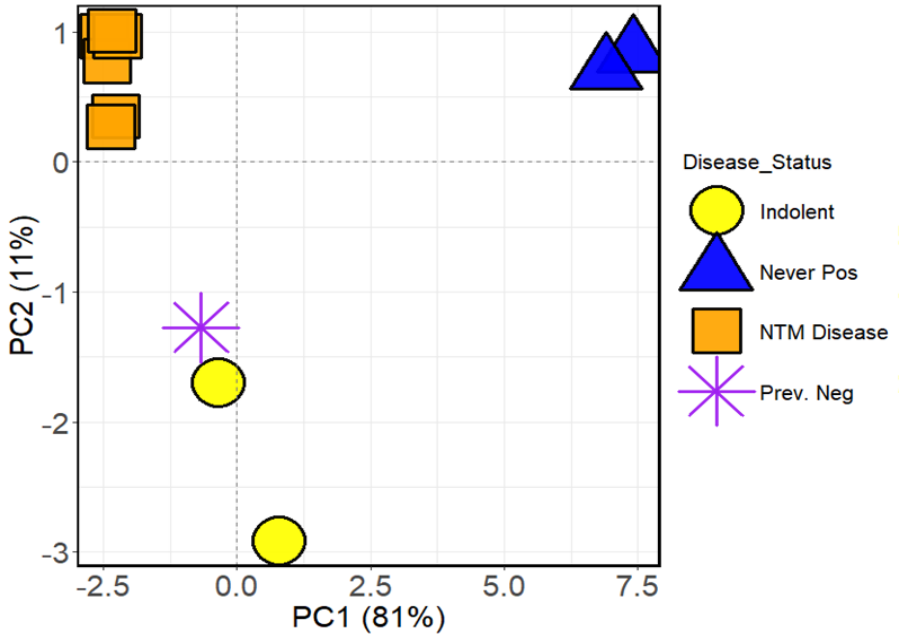
- Subjects breath into 1.5L Tedlar bags
- Breath is drawn through a filter onto 3-bed thermal desorption tubes (TDT) via a vacuum pump
- TDT contents are desorbed at 330 °C into a cryogenically cooled (-120 °C) inlet liner
- Analyzed by 2-D gas chromatography time of flight mass spectrometer (GC×GC-TOFMS)
- Method can be modified to sample “headspace” of sputum in a tube



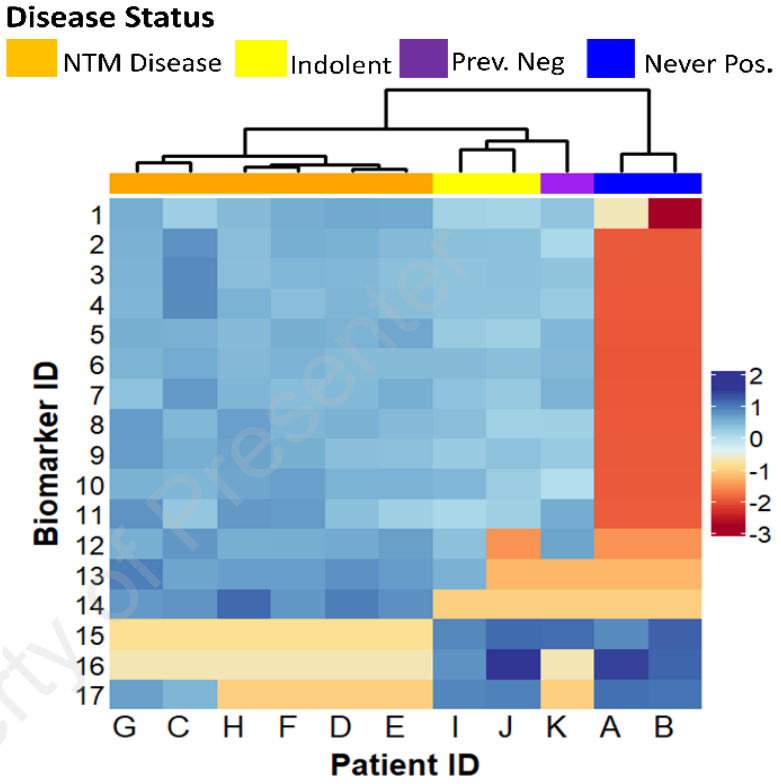
S. Caceres, MS

K. Poch, BS

Volatile molecules in exhaled breath distinguished between disease states



Principal component score plot constructed using the normalized, transformed and scaled expression profile of 17 features selected by the Boruta method



Dendrogram and heatmap using the 17-compound breathprint

Analysis of stored sputum from PREDICT and PATIENCE Trials

R01HL146228

Jane Hill, (UBC)

J Breath Res. 2022 May 13;16(3)

Markers of Treatment Response?

26 y/o male with Cystic Fibrosis

- *M. abscessus* in 2016
- Enrolled in the PREDICT Trial
- Met diagnostic criteria in early 2017
- Enrolled in the PATIENCE Trial
- Refractory to antibiotic treatment
- Two effect phages identified
- Initiated on phage therapy Sept 2020
- Culture conversion January 2021
- Lung Transplant October 2021
- Completed phage (and antibiotics) March 2022

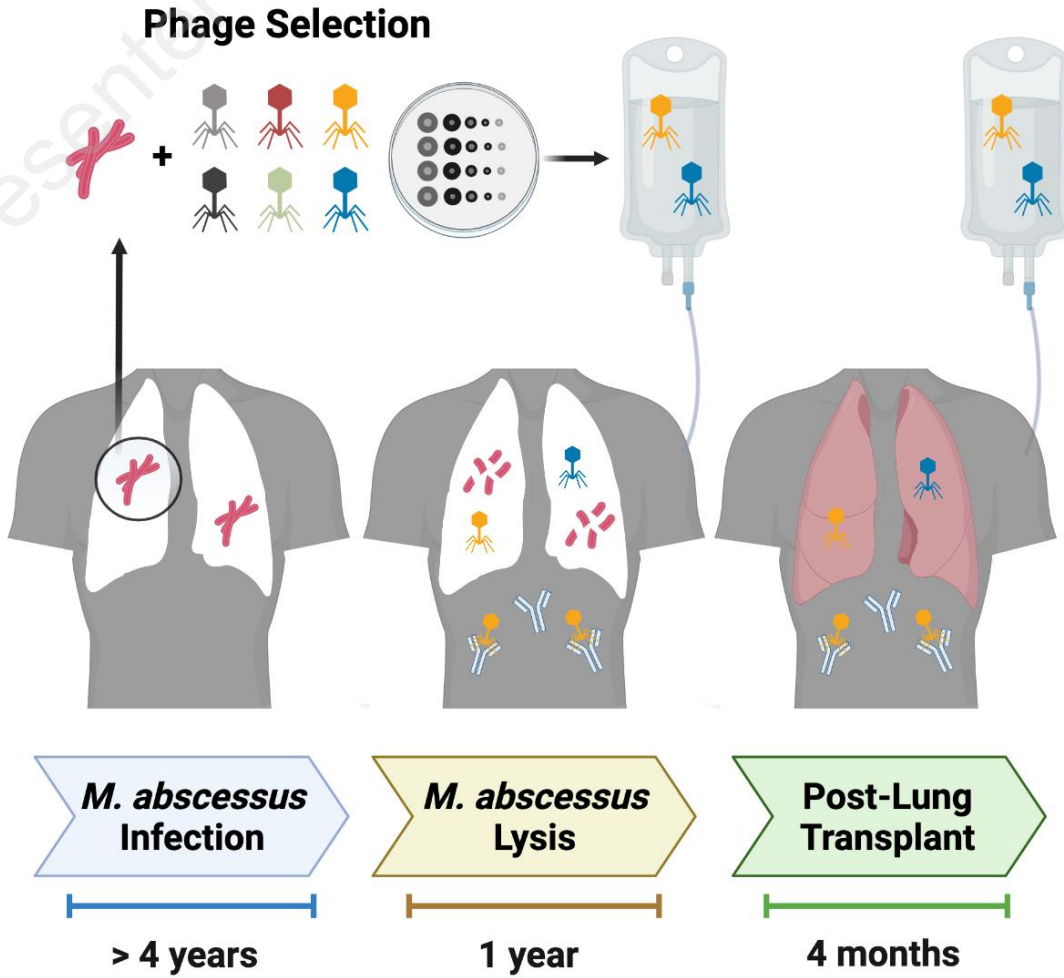
G. Hatfull (Pitt)

R. Dedrick (Pitt)

Cell

Article

Host and pathogen response to bacteriophage engineered against *Mycobacterium abscessus* lung infection

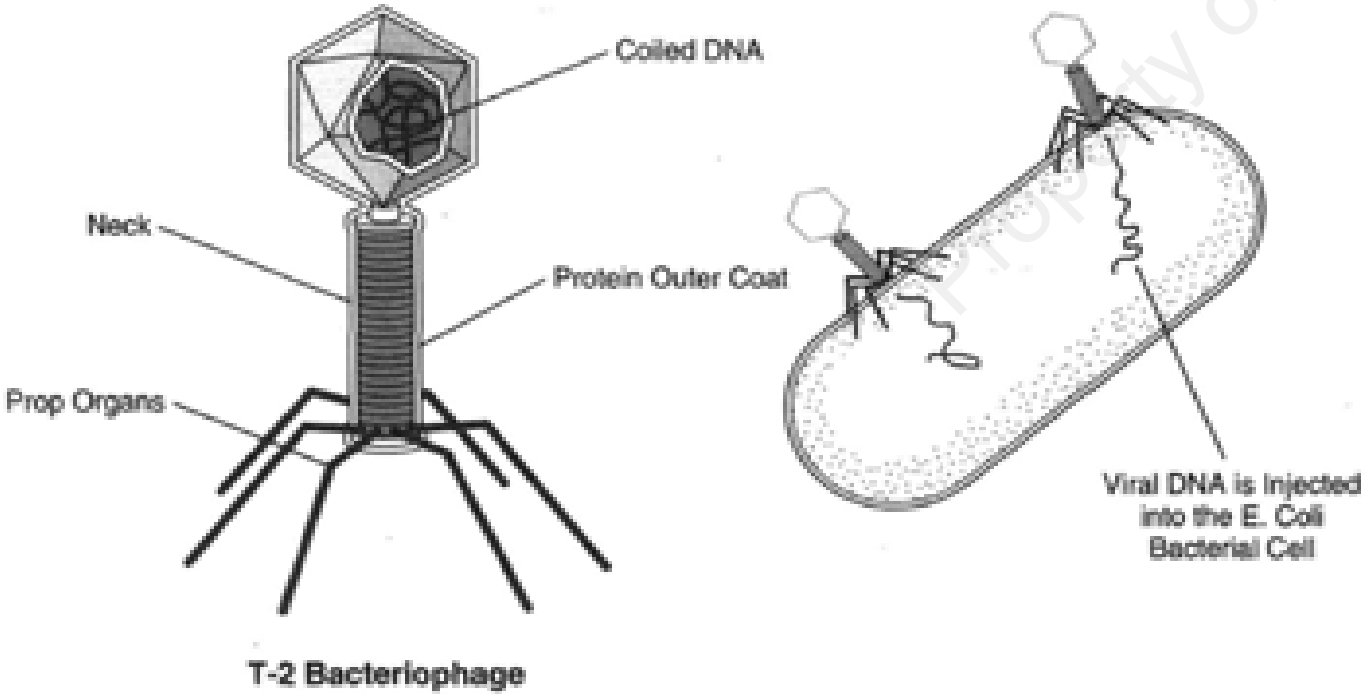


Cell, 2022, 185(11)

Bacteriophages

- Natural predators of bacteria
- Most abundant organism on earth (10^{31})
- 10^{23} phage infections per second

- Highly specific host range-species/genus
- Incapable of infecting human cells



T-2 Bacteriophage

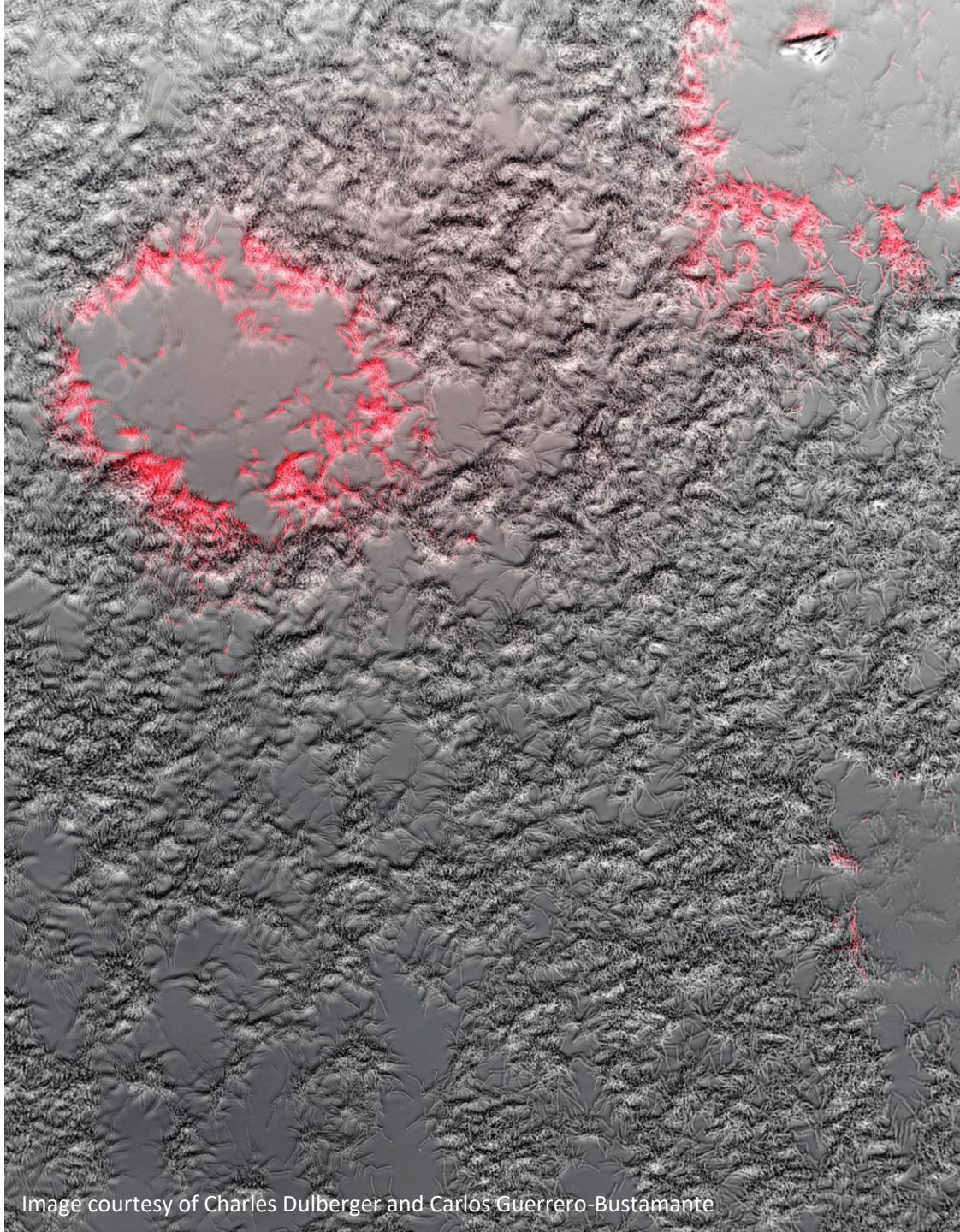
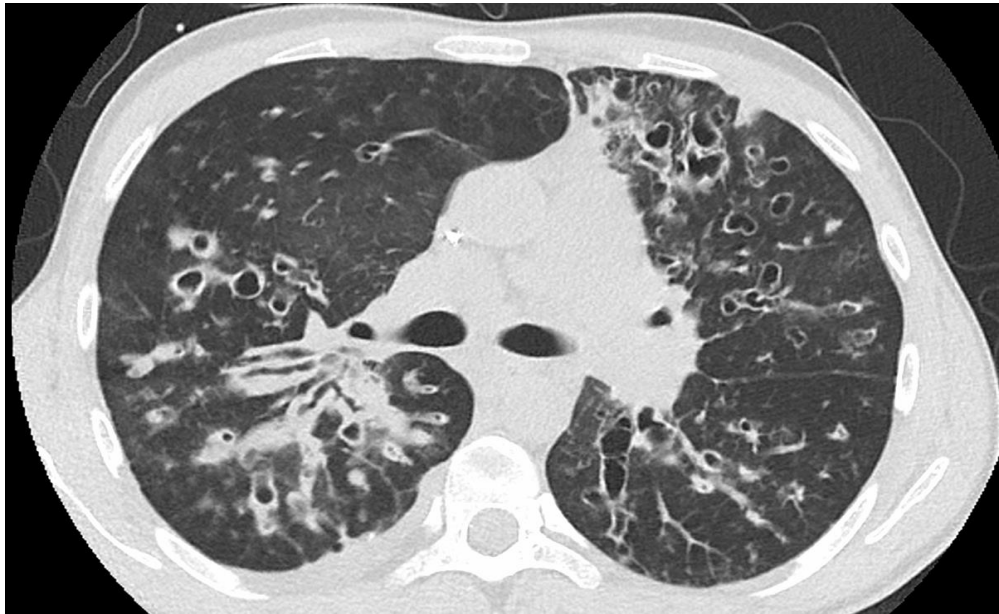


Image courtesy of Charles Dulberger and Carlos Guerrero-Bustamante

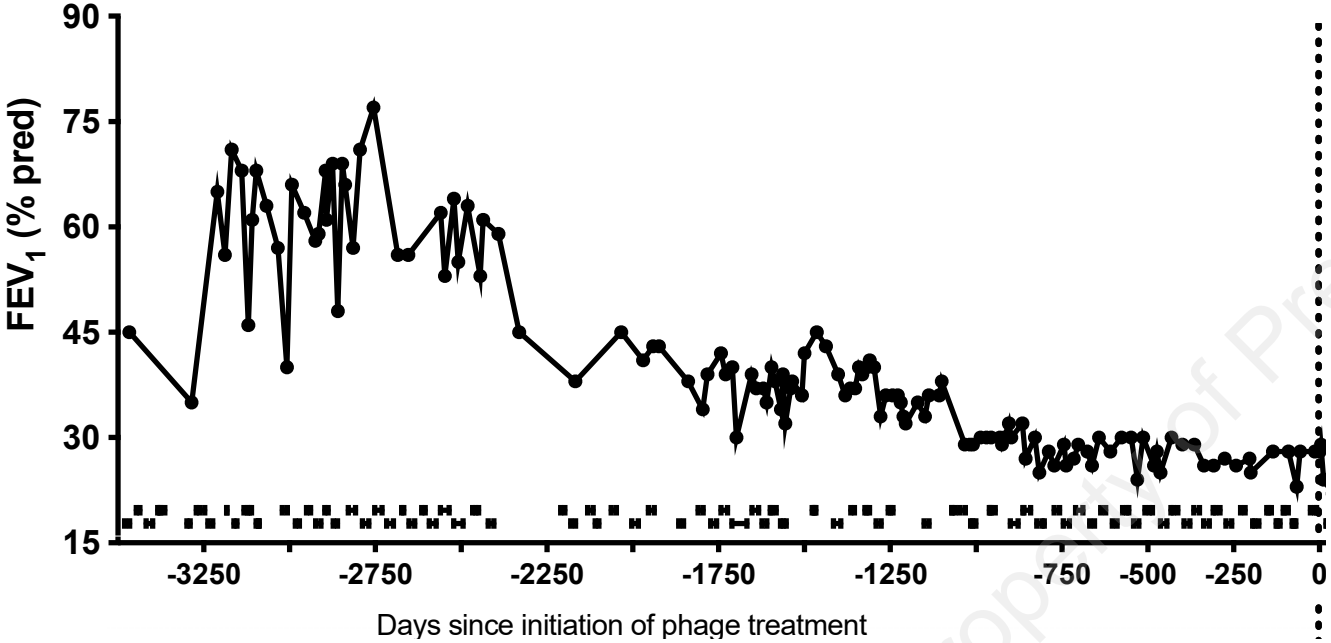
Case

JJ is 25-year-old man with cystic fibrosis (CF) and treatment refractory *Mycobacterium abscessus* lung disease

- Genotype H199Y/2184insA- no approved CFTR modulator
- Chronic infection with multi-drug resistant *P. aeruginosa* and methicillin-resistant *S. aureus.*
- Pancreatic insufficiency
- CF-related diabetes
- CF-related sinus disease
- Malnutrition with nighttime feeding via PEG
- Not currently listed for lung transplant



Clinical features



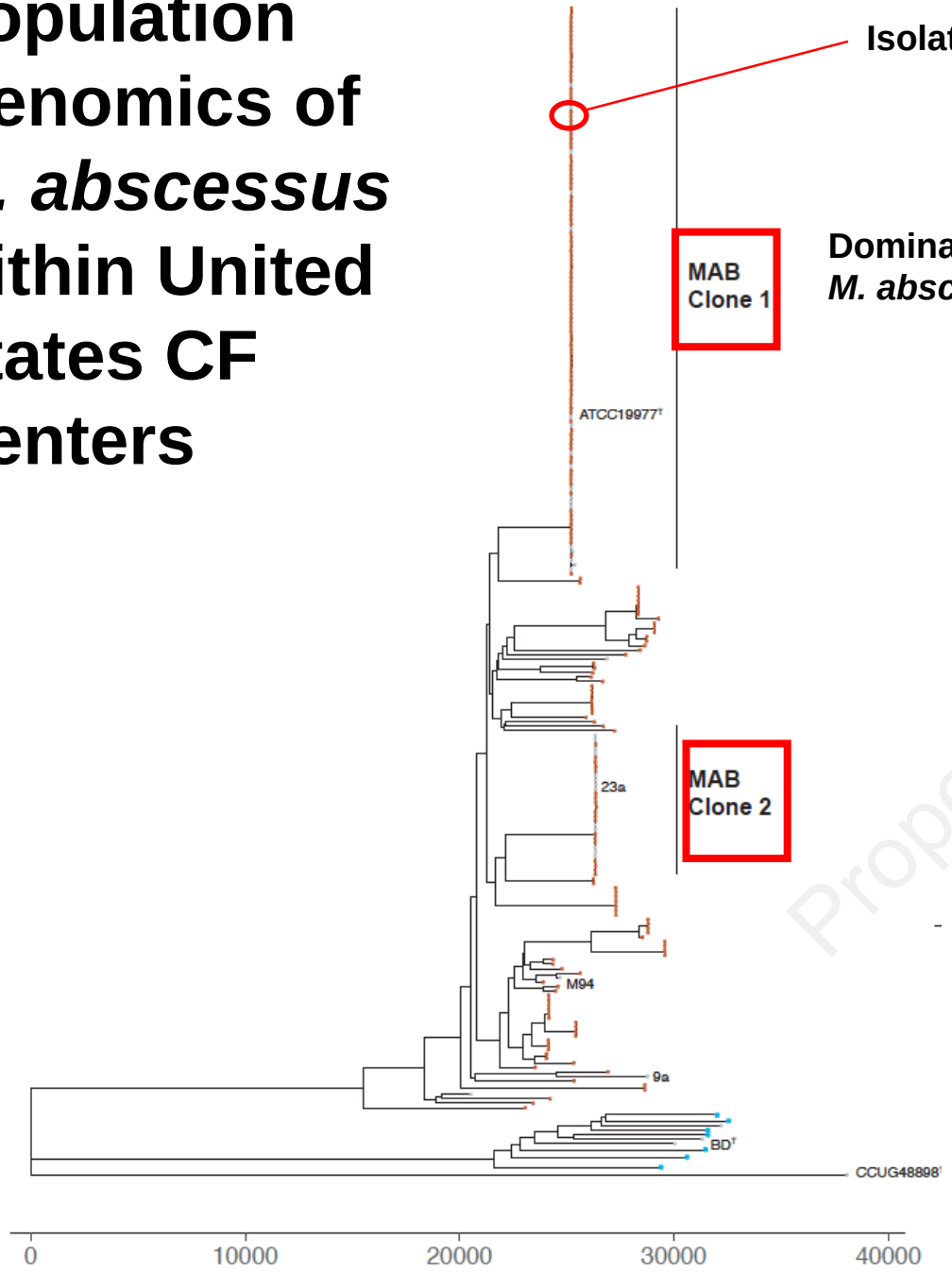
Lung Function

Forced Expiratory Volume in 1 second (FEV_1) (%predicted)

Hospitalizations

***Cell*, 2022, 185(11)**

Population Genomics of *M. abscessus* within United States CF Centers



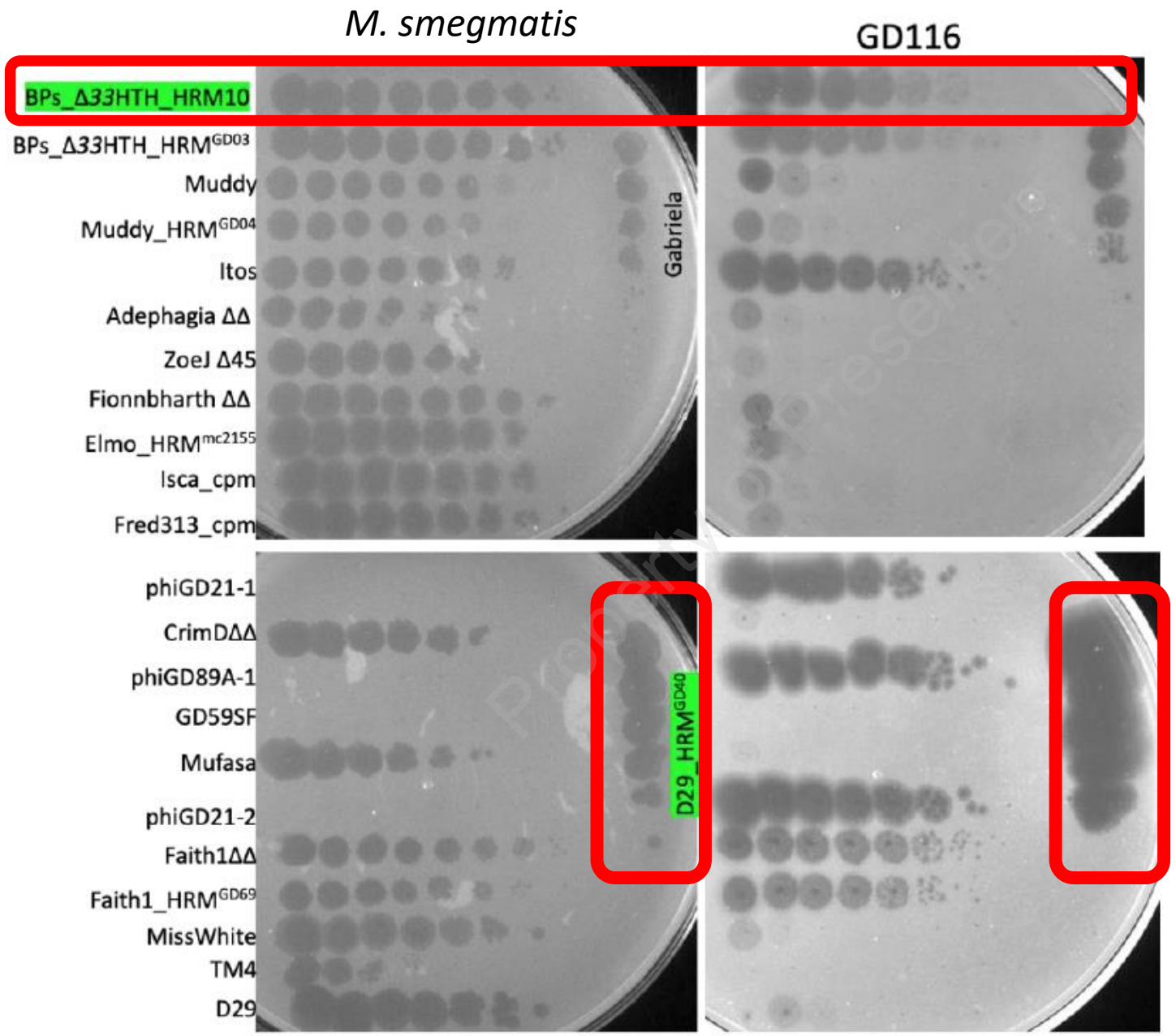
Dominant circulating clone of *M. abscessus ssp. abscessus*

Ann Am Thorac Soc. 2021 Dec;18(12):1960-1969

R. Davidson, PhD

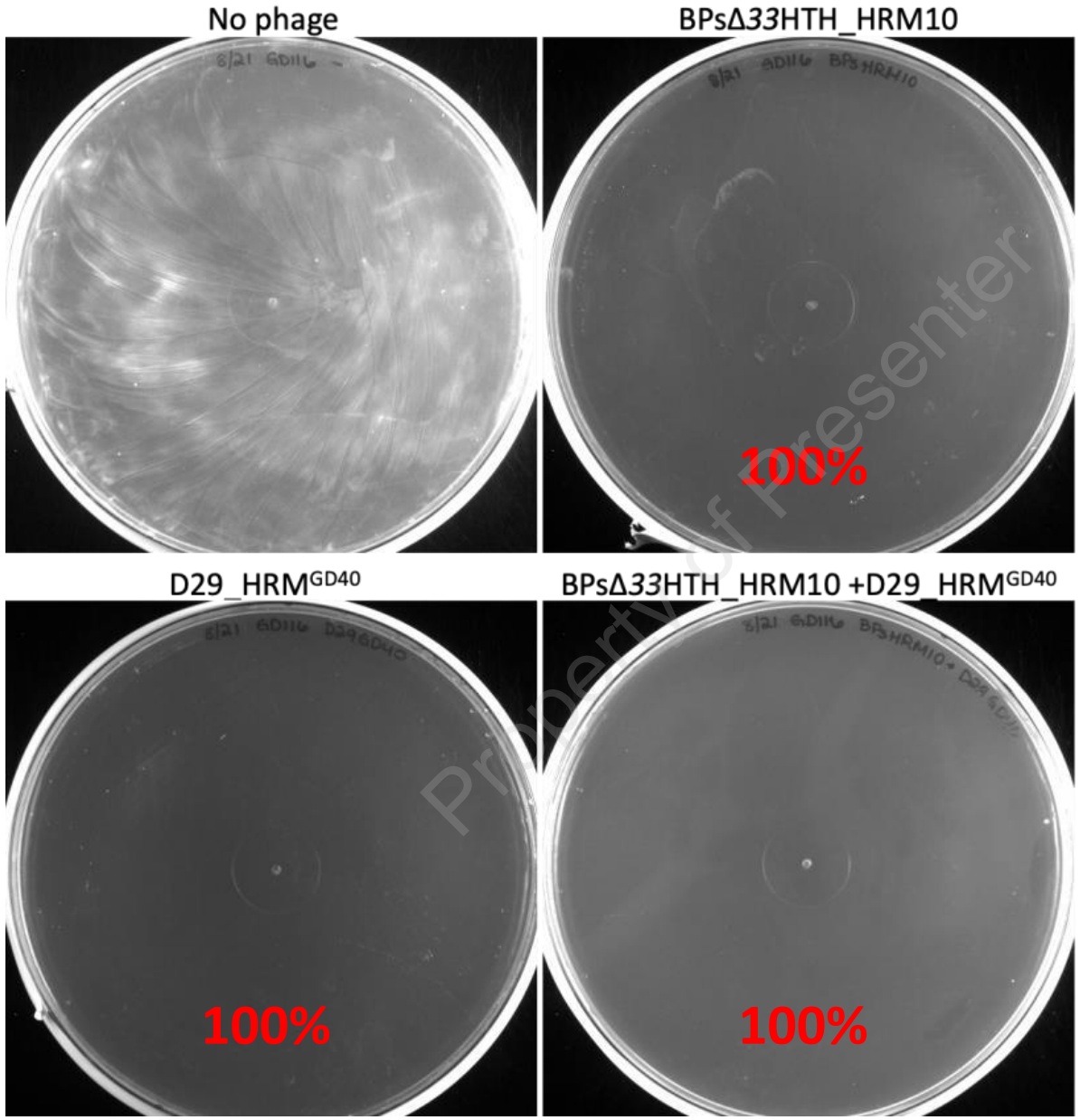
N. Hasan, PhD

Phage-selection



G. Hatfull (Pitt) R. Dedrick (Pit

Efficiencies of phage killing of *M. abscessus* GD116 *in vitro*



G. Hatfull (Pitt) R. Dedrick (Pit

Regulatory Requirements for Bacteriophage Treatment of *M. abscessus*

- Material Transfer Agreement (MTA) between Univ. Pittsburgh and NJH
- Write a treatment protocol
- Investigational New Drug (IND) approval from the FDA
- IRB approval for phage adminstration
- Informed consent of the patient
- Protocol with St. Joseph Pharmacy to dilute the stock in PBS and package the phage in syringes
- IRB approval for banking of isolates (PREDICT and PATIENCE Trials)
- IRB approval for specimen collection
- Protocol for explanted lung analysis
- IRB approval for analysis of explanted lungs
- Biohazard protocol for phage administration at St. Joes and UCH

Keira A Cohen, M.D.

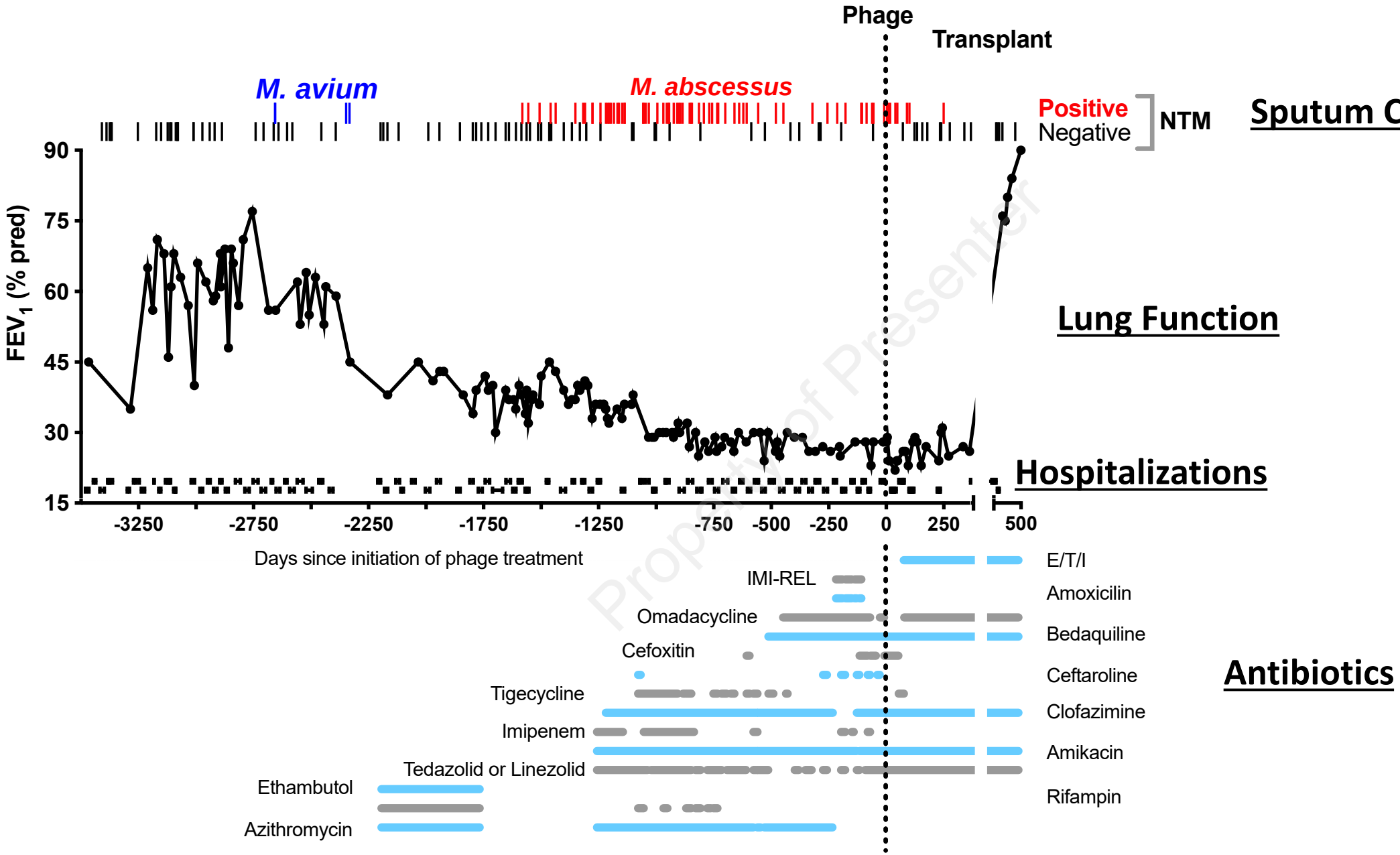
M. Jones, RN

V. Lovell, RN

Protocol For Bacteriophage Treatment of *M. abscessus*

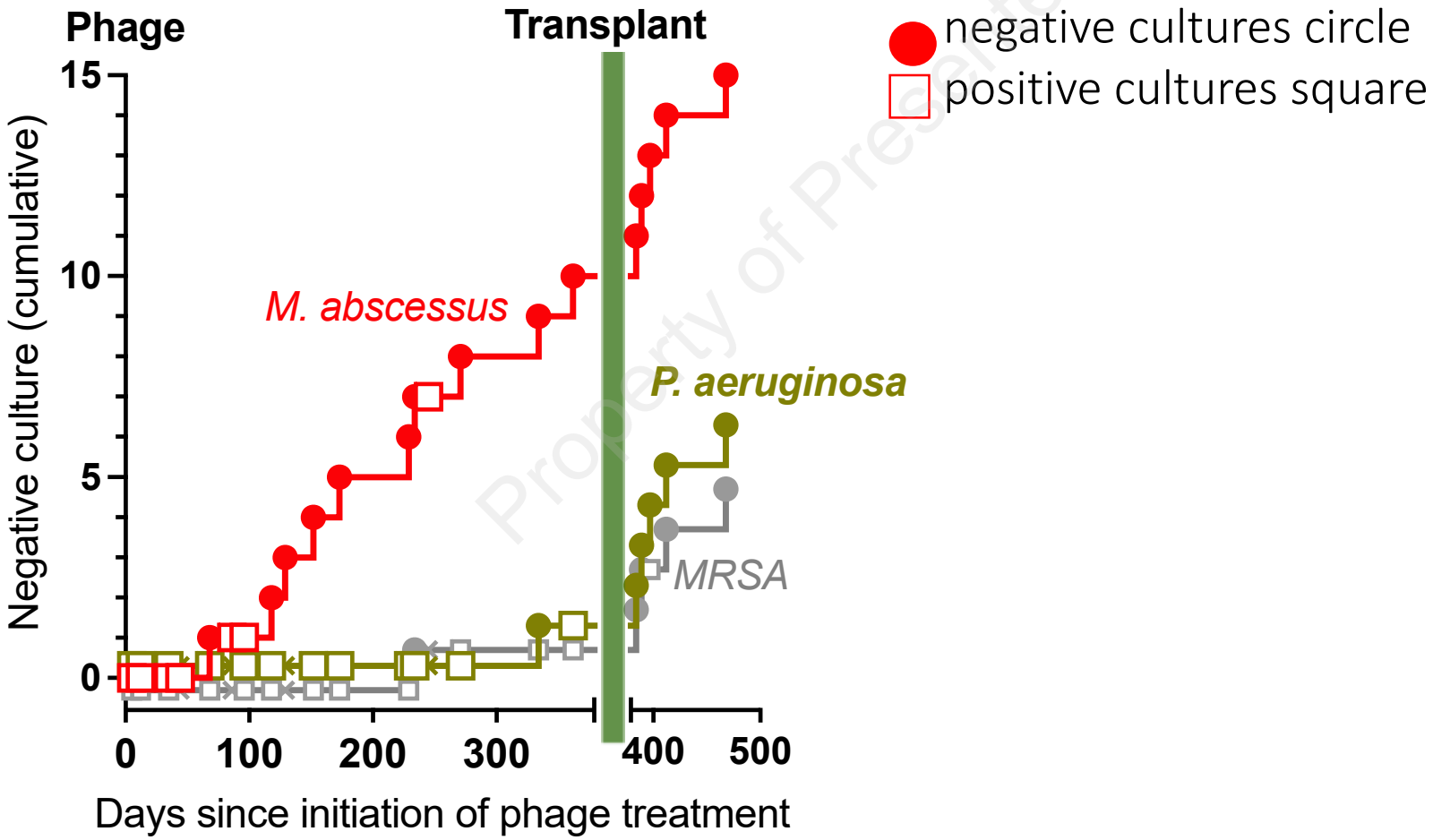
- Initial doses while monitored (St. Joseph)
- 5 ml (10^8 to 10^9 PFU) solution of bacteriophage cocktail (BPsΔ*33*HTH_HRM10 and D29_HRM_{GD40}) IV via slow push twice daily via mediport.
- Continuation of intensive antibiotic treatment
- Phage administered by patient when home
- Weekly telehealth or clinic, and visiting nurse and labs.
- Initial approval requested for 24 weeks- extended indefinitely
- Monitoring of cultures and radiographs per standard level of care.

Clinical features

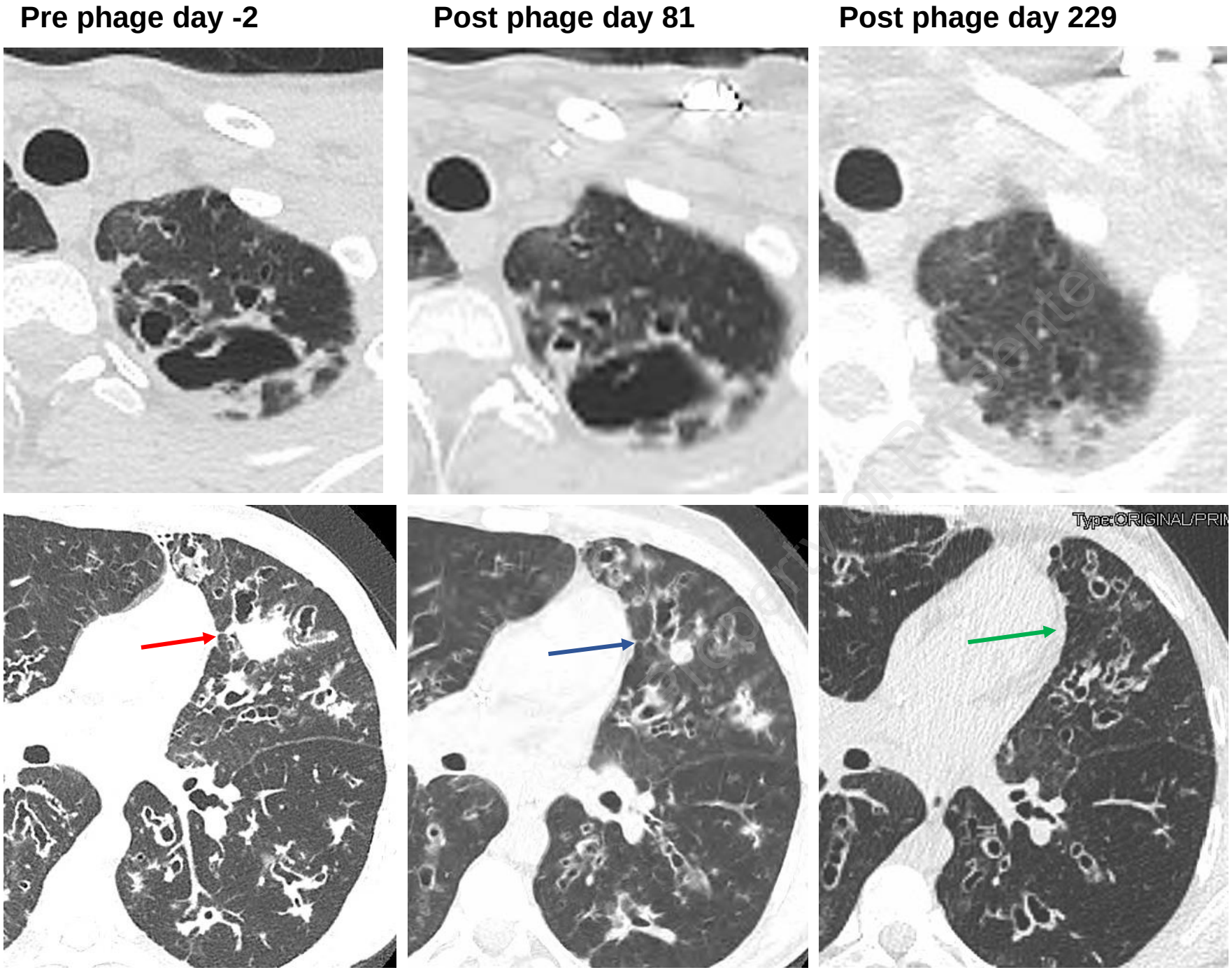


Cell, 2022, 185(11)

Cumulative negative airway cultures following initiation of phage therapy

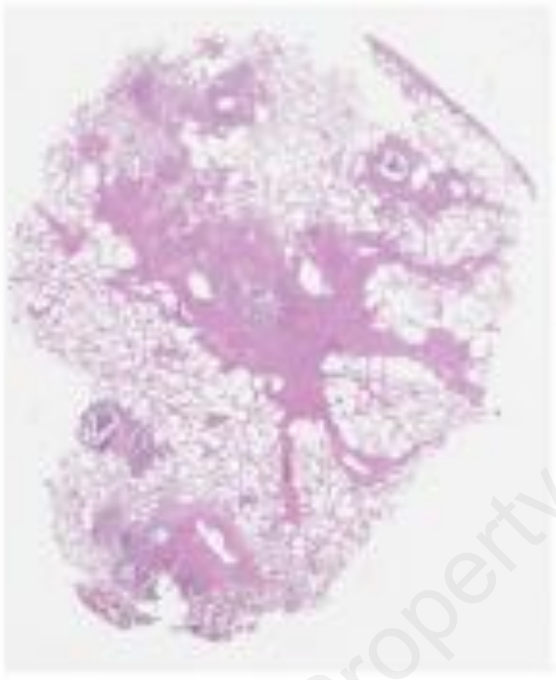


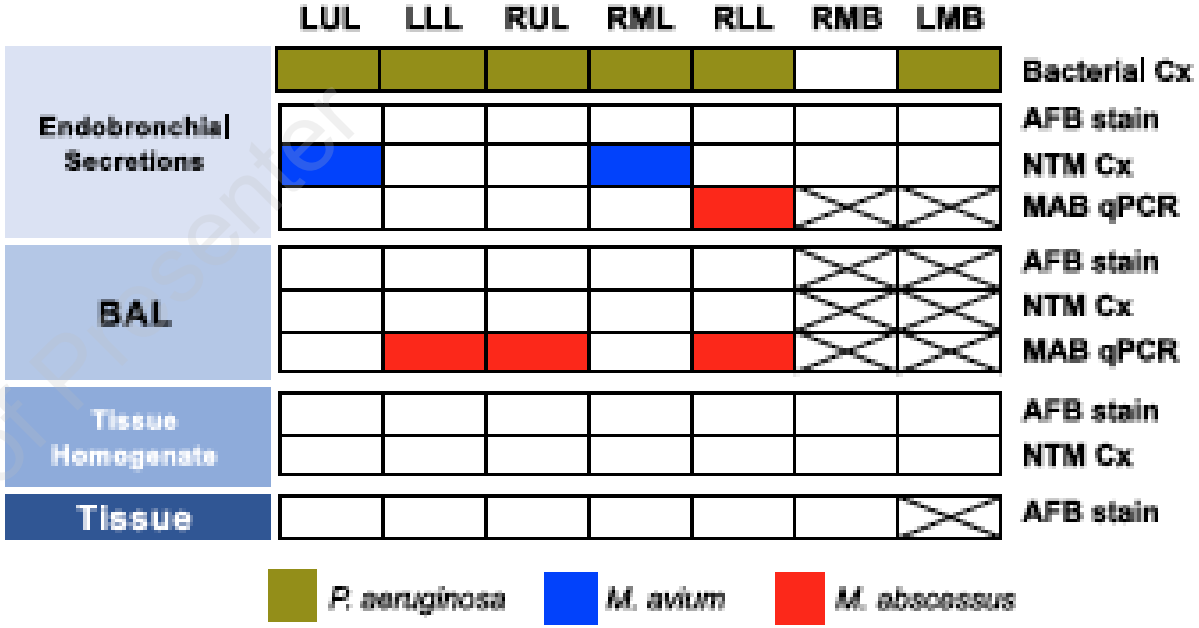
Radiologic Changes During Phage Therapy



https://doi.org/10.17632/jtdvbm2tzx.1

Systematic analysis of explanted lung tissue for *M. abscessus*



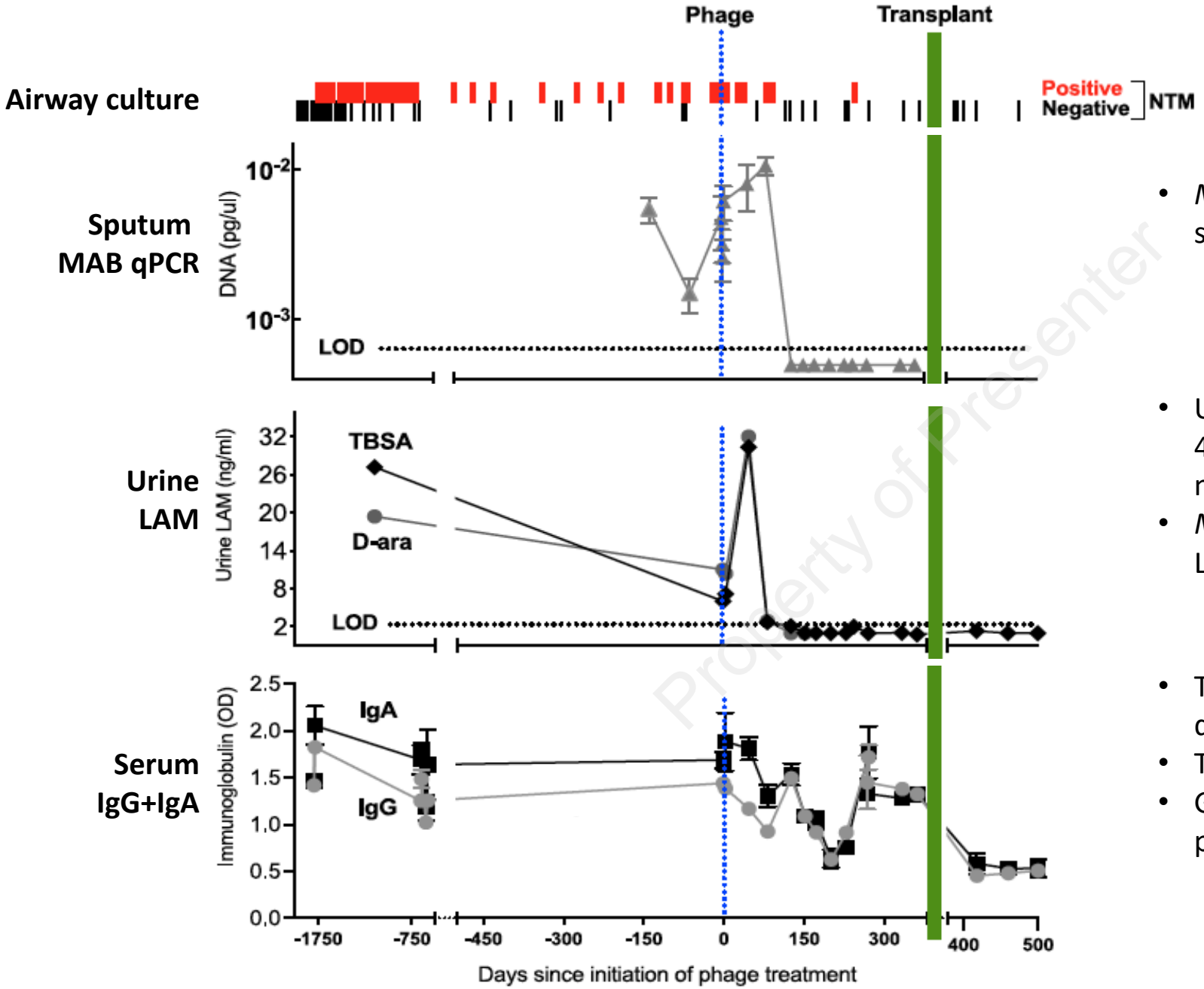


M. avium **grown from endobronchial secretions.**

M. avium **last recovered from airway cultures 6.4 years pre-transplant**
(1 year of successful treatment)

139 cultures negative for *M. avium*

Markers of Treatment Response



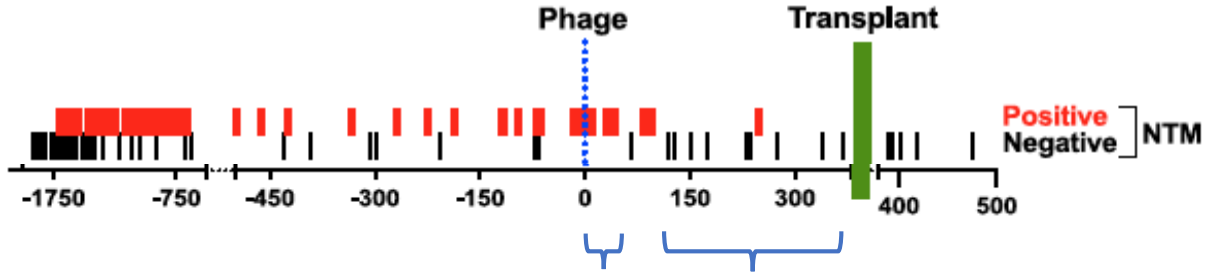
- *M. abscessus*-specific qPCR closely reflected sputum culture results

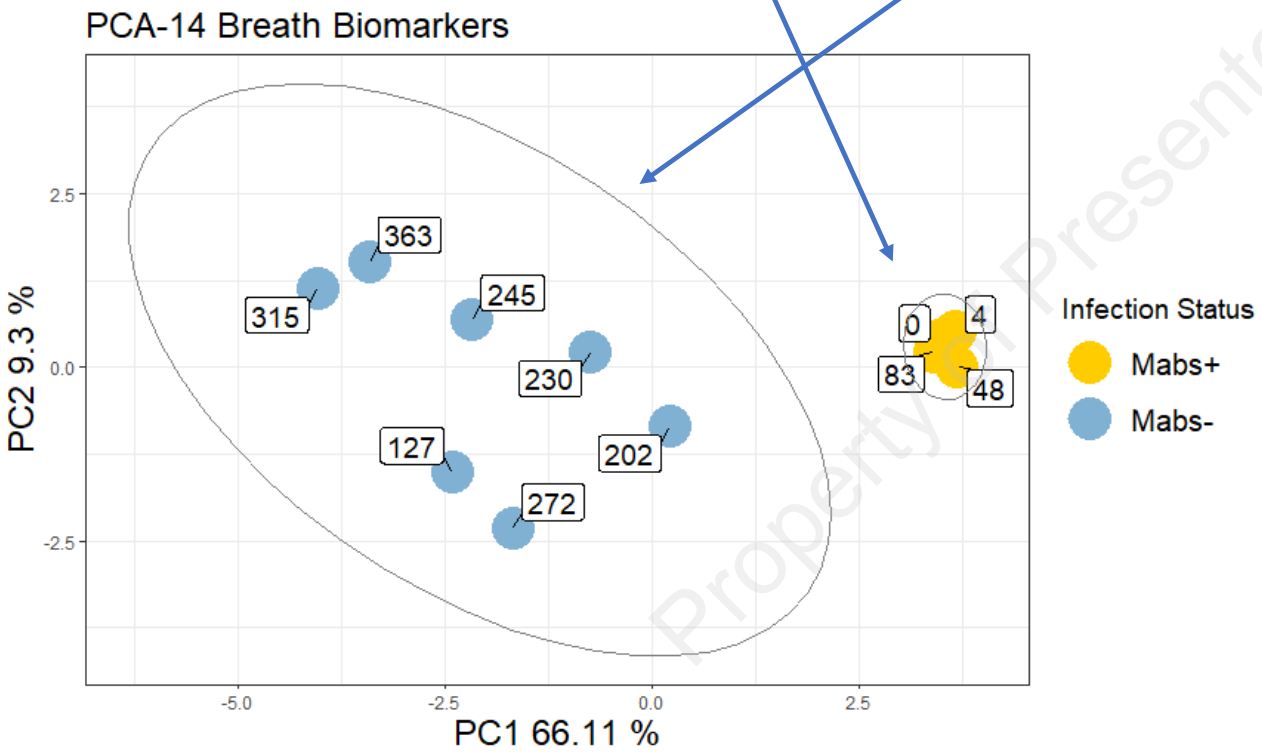
- Urine LAM signaled dramatic NTM lysis at day 44, and fell below LOD when cultures turned negative.
- *M. avium* infection apparently below urine LAM LOD

- Titers fell rapidly with initial treatment, possibly due to binding of LAM?
- Titers rose at time of late positive culture
- Greatest decrease seen post-transplant, possibly due to elimination of M. avium

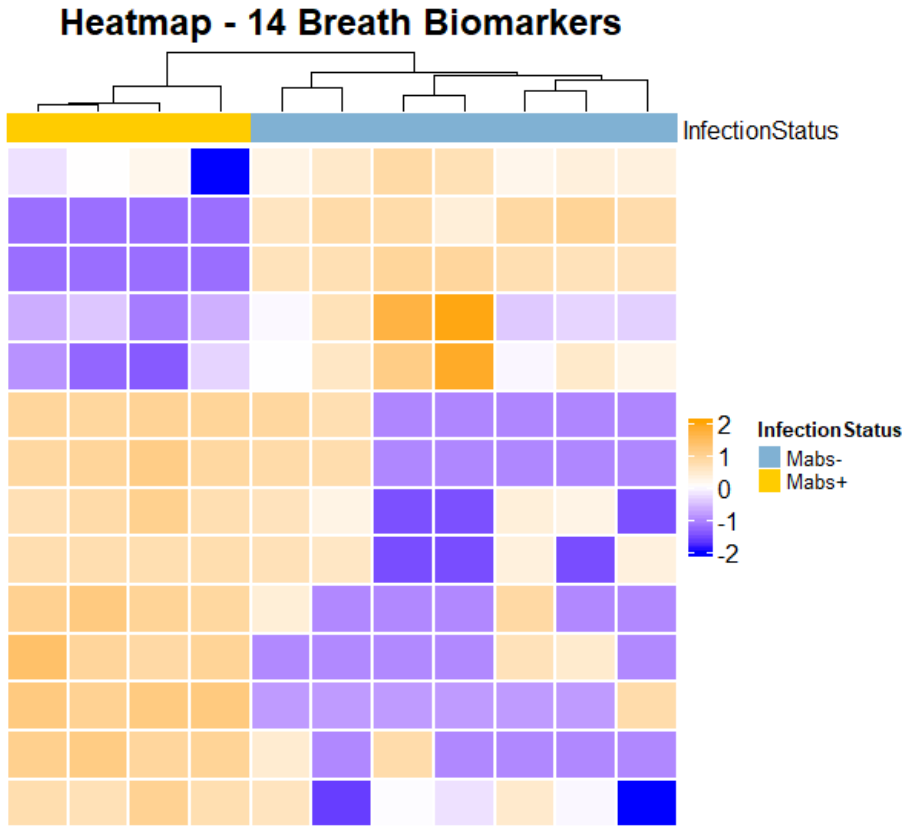
Markers of Treatment Response?

Airway culture



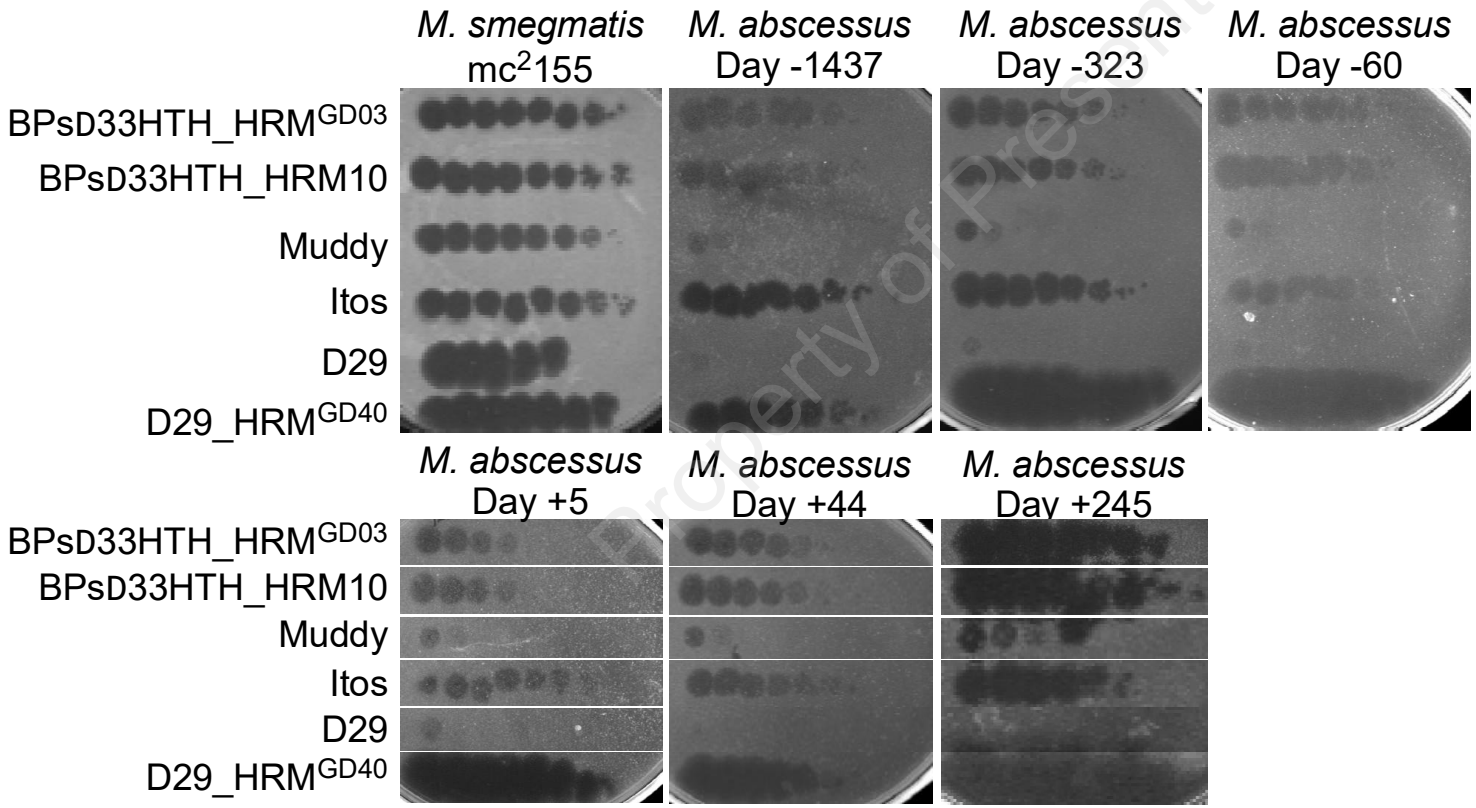


- *Volatile metabolites from breath* closely reflected sputum culture results



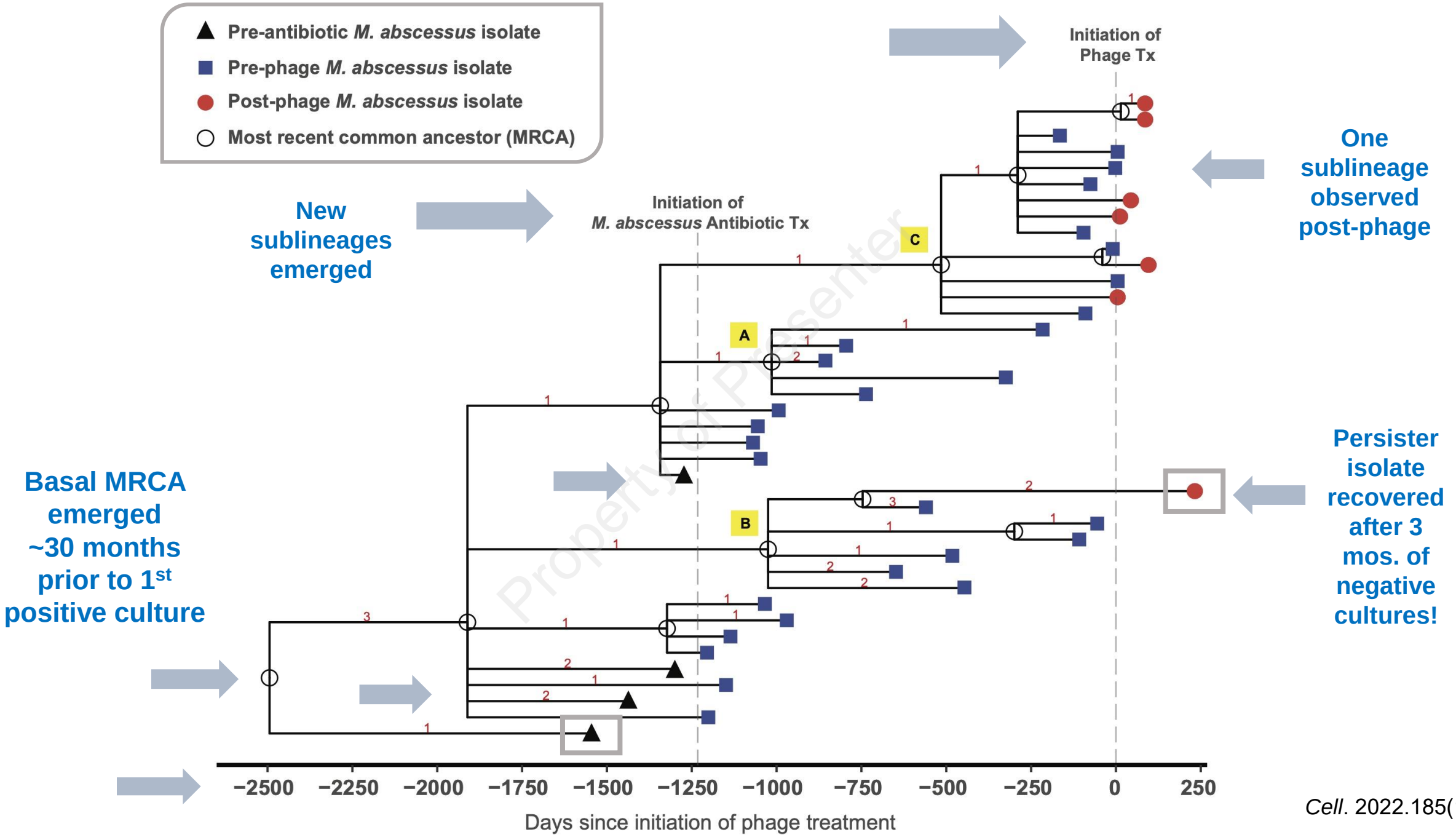
Jane Hill, (UBC)

Phage Therapy Did Not Select for Phage-resistant Isolates



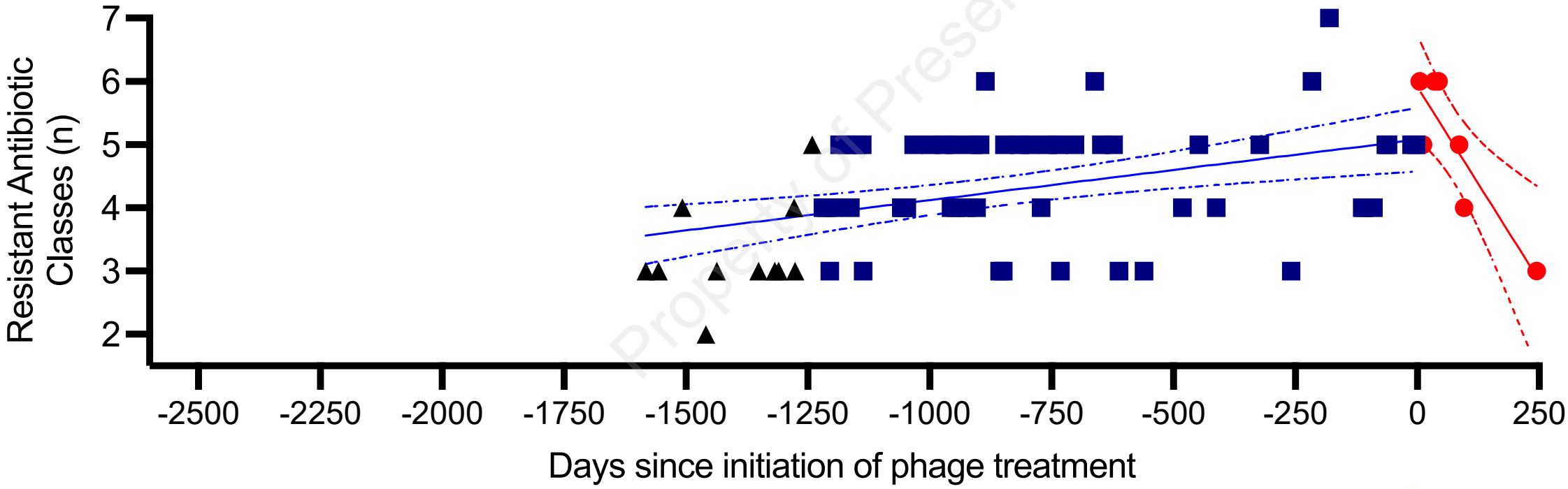
G. Hatfull, PhD

R. Dedrick, PhD



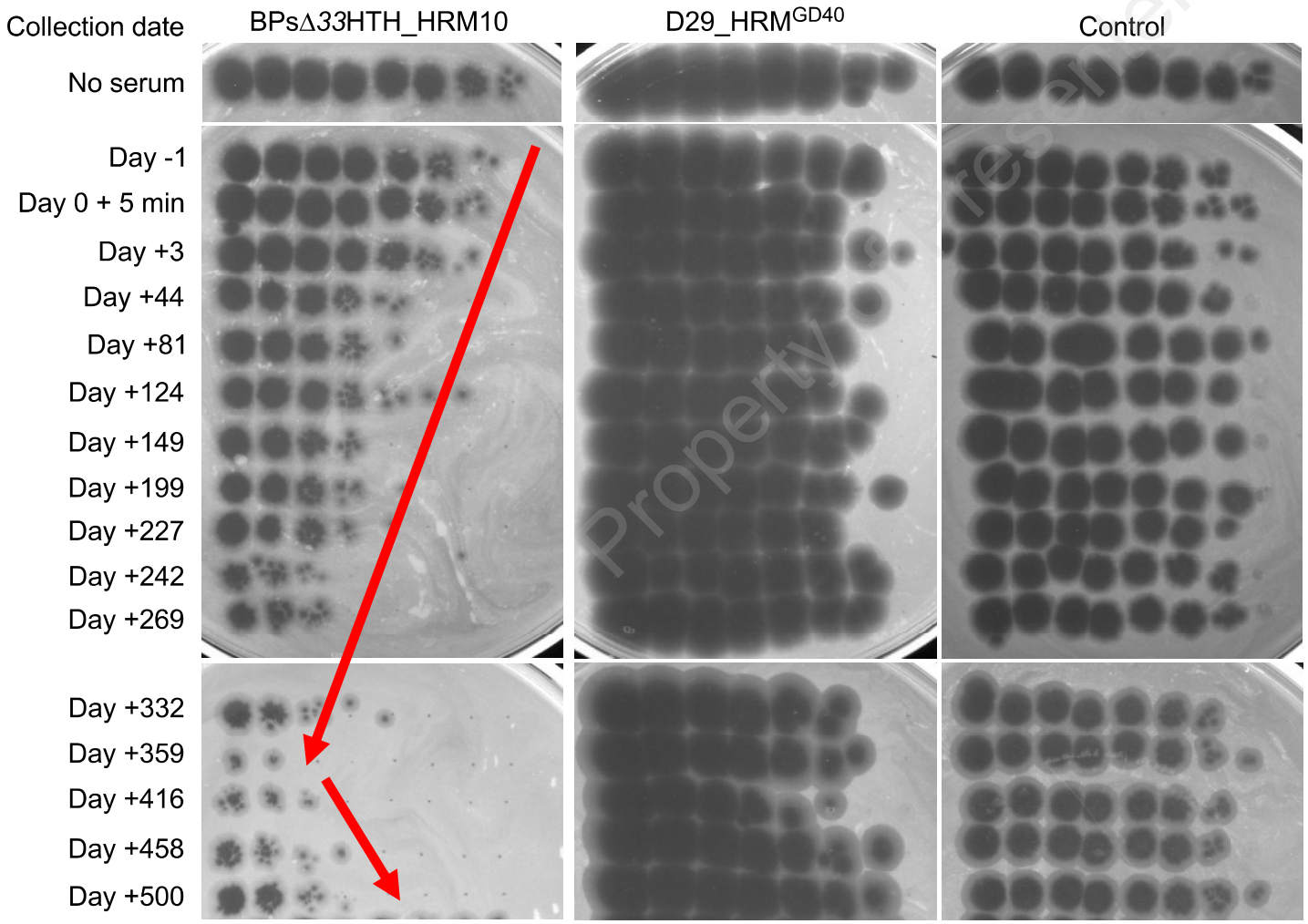
Cell. 2022.185(11)

Antibiotic Sensitivity of *M. abscessus* Isolates Following Phage Therapy



National Jewish Health®
Breathing Science is Life.®

Antibody Binding to Phages Pre- and Post-Phage Treatment Demonstrates Late Development of Anti-phage Neutralizing Antibodies.



G. Hatfull, PhD

R. Dedrick, PhD

Conclusions

- People with CF are at high risk for NTM infection
- Unique aspects of CF care has led to a high proportion of individuals with apparently indolent infection, making identification of who will benefit from treatment more challenging.
- Low sensitivity of sputum cultures combined with CFTR-modulator therapy have significantly reduced our ability to screen for NTM or monitor any aspect of disease or treatment.
- Culture-independent markers are urgently needed for clinical care and clinical trials in this population.
- Phage combined with antibiotic treatment resulted in apparent eradication of *M. abscessus,* allowing for a successful lung transplant, without evidence of post-transplant infection.

PREDICT & PATIENCE Study Team

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Phuong Vu
Sonya Helshe

TDNCC Data Management
Claire Chapdu
Jing Xie
Missy Cianciola
Rafael Kuttner

Budget and Contracts
Lisya VanHousen

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Delphi Chatterjee

Study RCs
Emily Armantrout
Katie Poch
Silvia Caceres
Valerie K. Lovell

CSU Analytical Team
Ibrahim Aboellail
Prithwiraj De

Study Statistician
Brian Vestal

CYSTIC FIBROSIS FOUNDATION

CYSTIC FIBROSIS FOUNDATION THERAPEUTICS DEVELOPMENT NETWORK

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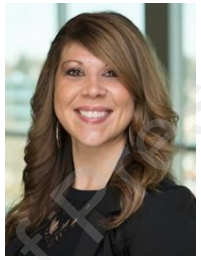
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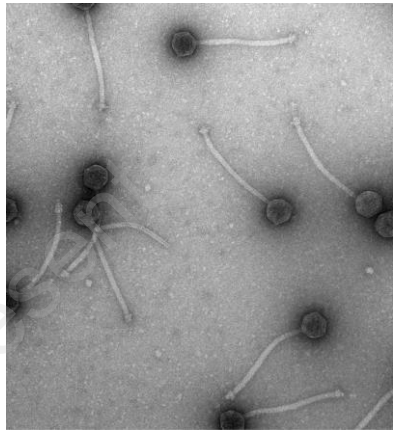
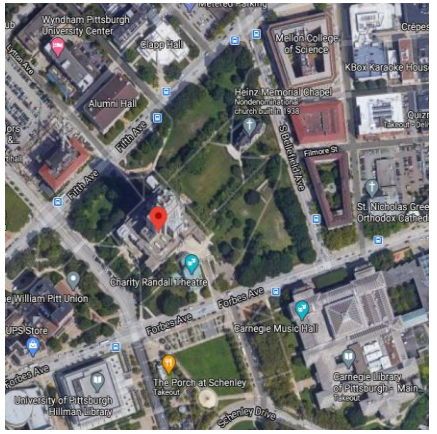
Phage engineering

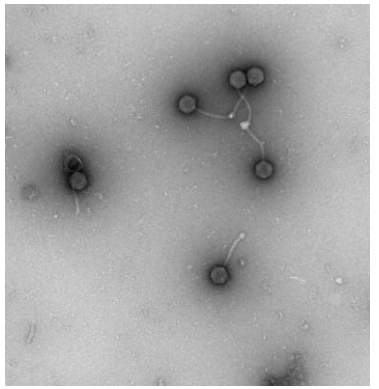
- BPs was isolated using *Mycobacterium smegmatis* as a host.
- BPs is temperate
- BPs△*33*HTH was constructed in which the helix-turn-helix DNA binding domain of the repressor gene (gene *33*) is deleted, rendering the phage lytic for *Mycobacterium smegmatis*
- Mutant BPs△*33*HTH_ HRM10 was isolated and is able to efficiently infect specific *M. abscessus* strains
- Not considered a GMO by the European Union





Pittsburgh, Pennsylvania

- D29 is a lytic phage, was isolated using *Mycobacterium smegmatis* as a host
- Native truncated, non-functional, repressor gene which does not allow for temperate lifestyle



Los Angeles, CA

https://phagesdb.org/