Development and efficacy of novel nebulized antibiotic formulations for the treatment of *Mycobacterium abscessus* pulmonary infections

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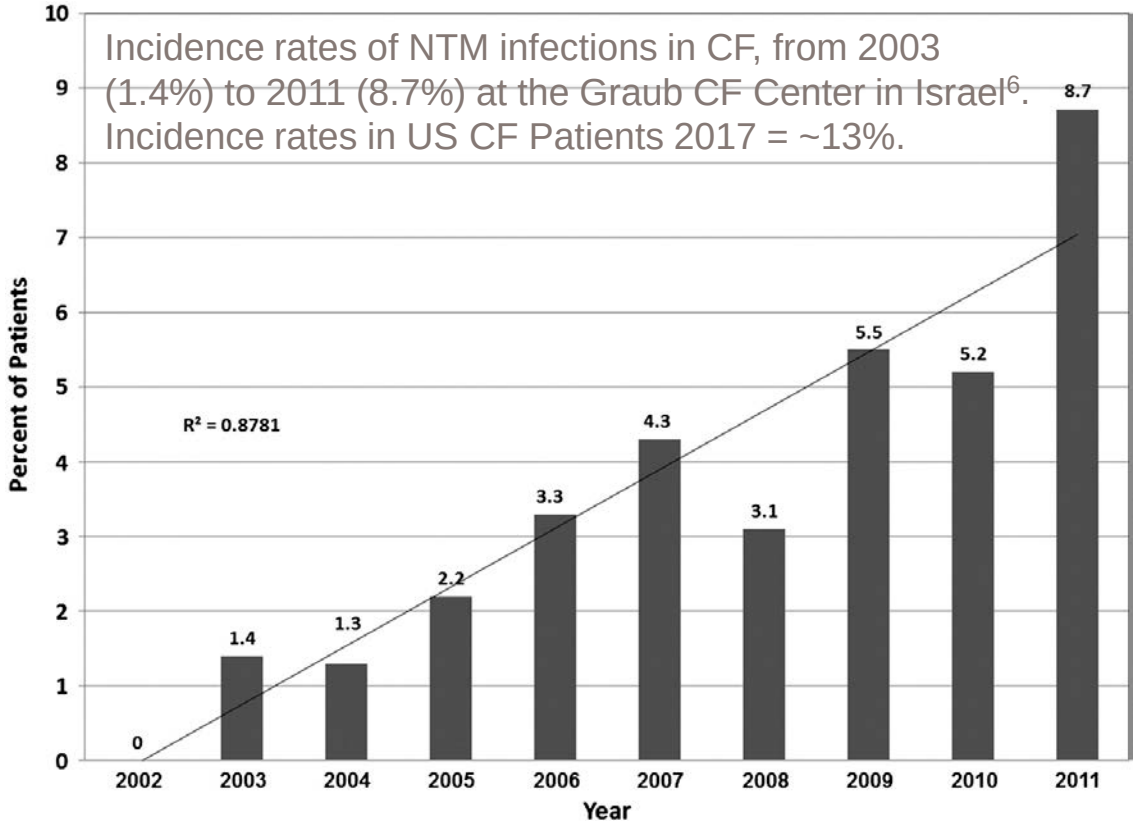
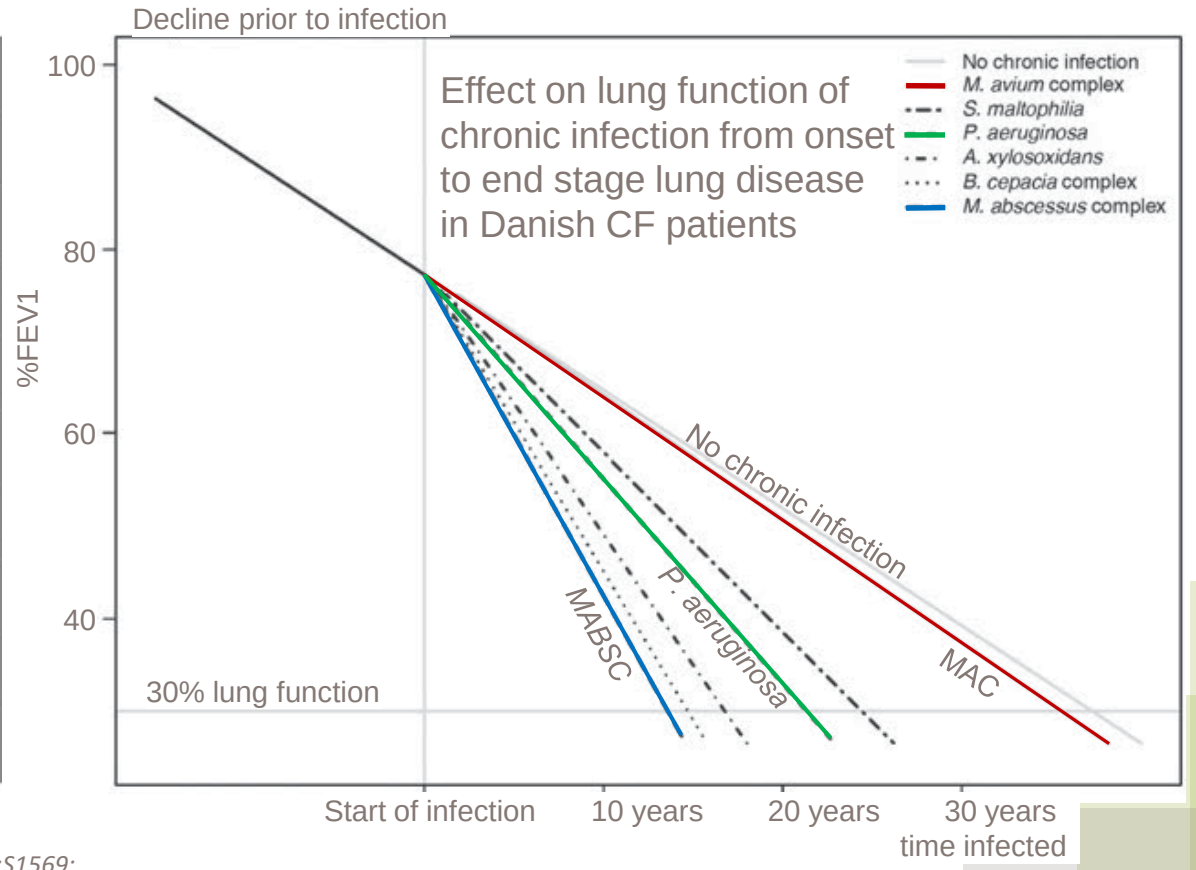
NTM (MABSC and MAC) are destroying lung function, and increasing in prevalence

- **NTM infection in CF approx. 13%, and increasing in prevalence within CF[1,2] and within non CF lung disease[3,4]**



- **MABSC in particular is an aggressive pathogen and leads to rapid loss (>2x) of lung function[5]**



References: *1. Olivier et al. Am J Respir Crit Care Med (2003): 167: 828; 2. Qvist T et al, J Cystic Fibrosis (2014); 14:S1569; 3. Adjemian et al. Am J Respir Crit Care Med (2012); 185: 881.; 4. Prevots DR et al, Am J Respir Crit Care Med (2010); 182: 970; 5. Qvist T et al, J Cystic Fibrosis (2016); 15: 380; 6. Bar-on et al, J Cystic Fibrosis 2015.*

Current NTM therapy options

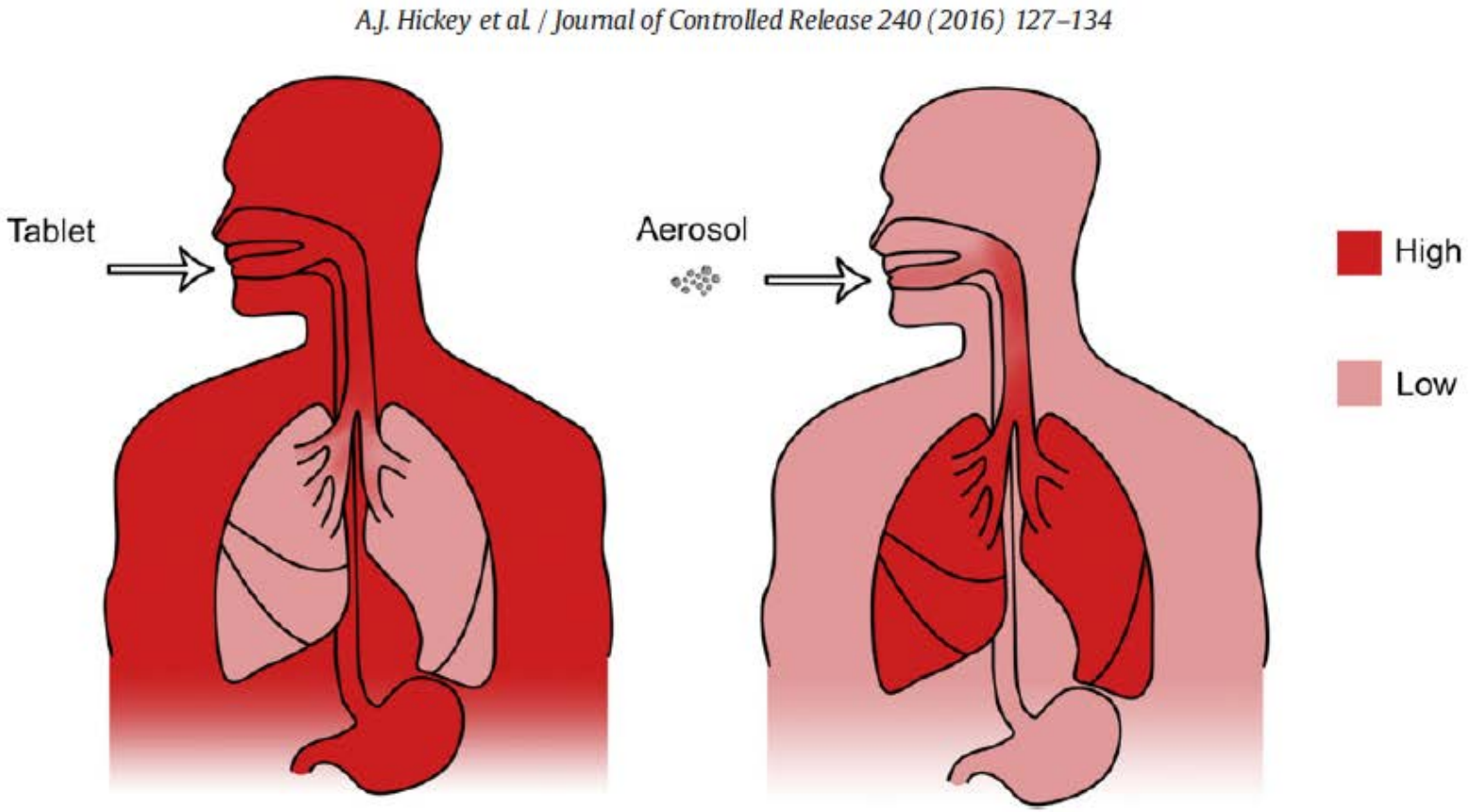
- **NTM are treated with long term oral combination therapy:** current therapeutic options are oral antibiotics with high chronic toxicity[1.] Clofazimine (oral) has skin, GI, neuro, and cardiac side effects.
- **Treatment outcomes are unsatisfactory:** discontinuation is frequent (10-30%) and overall treatment success low (40-60%)[2, 3].
- **Inhaled liposomal Amikacin in development:** Phase 2 results indicate seroconversion of NTM, and focus on MAC[4]

References: *1. Stout et al. (Intl J Infect Dis (2016): 45; 123-134; 2.* Field SK et al, Chest (2004): 126: 566-81; 3. Xu HB et al. (2014) Eur J Clin Microbio Infect Dis 33: 347-58; 4. Olivier et al. AJRCCM (2016) online Oct 17, 2016

MIC Values of QRM Program by Comparison

Drug	*M. avium*	*M. abscessus*	References
Amikacin	4 µg/ml	32 µg/ml	Rose 2014, Ferro 2016, Kaniga 2016
Clofazimine	0.12-0.25 µg/ml	0.5 - 1.3 µg/ml	Shen 2010, van Ingen 2012, Obregon-Henao 2015
QRM-003	1 µg/ml	1 µg/ml	Qrumpharma Data

Inhalation PK/PD

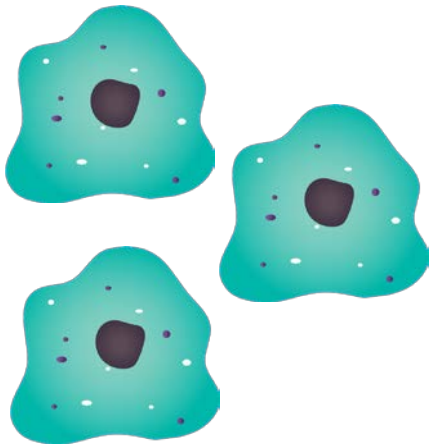


CMC progress, QRM-003 Formulation

- Current Formulation Development Steps
  - Developed a preservative-free, tolerable formulation suitable for delivery by nebulizer
    - Optimized for lung delivery (pH, osmolarity, particle size, airway compatibility)
  - Suspension particle sizes < 2 mm to facilitate use with vibrating mesh and nozzle type nebulizers
  - Goal: 2 years room temperature stable

QRM-003 Macrophage Uptake Assay

- Macrophage uptake relevant for activity vs. NTM – intracellular bacteria



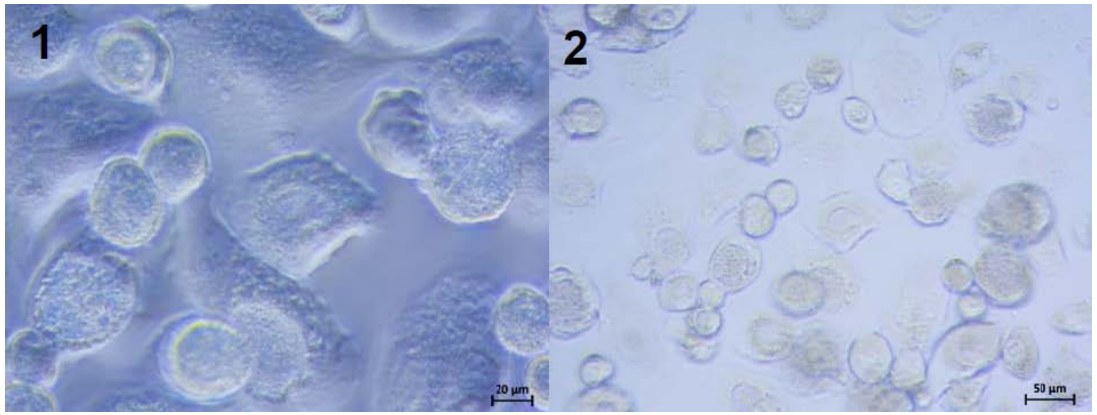
THP-1 derived macrophages incubated with QRM-003 formulation

+ QRM-003

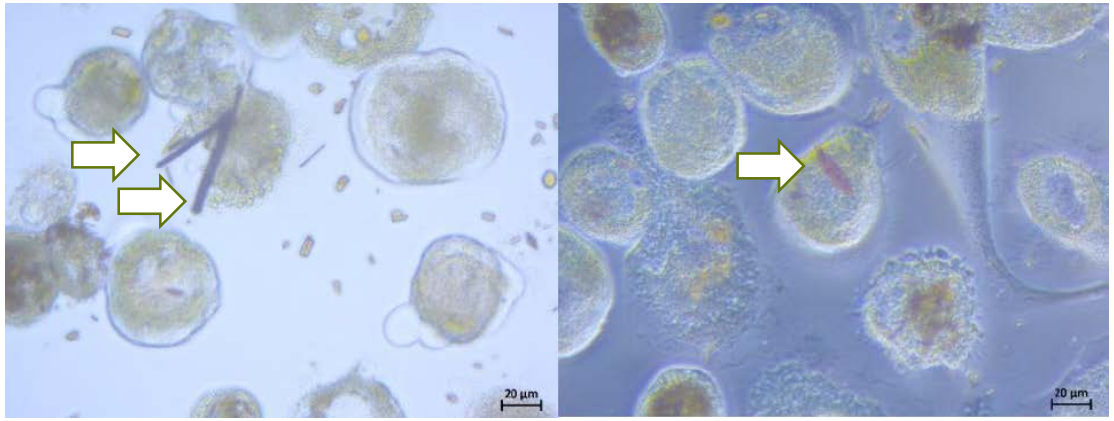
24-hr incubation

Observations via light microscopy

QRM-003 is well phagocytosed



THP-1 cells before incubation with QRM-003. (1: magnification 400x; 2: magnification 200x)



THP-1 cells after 24h of incubation and washing procedure with QRM-003 (both magnification 400x). White arrow = clofazimine crystals

Conclusions

- Clofazimine uptake by macrophages maintained in novel formulation
- Will allow for interaction between clofazimine crystals and intracellular NTM bacteria

QRM Aerosol Safety and Tolerability

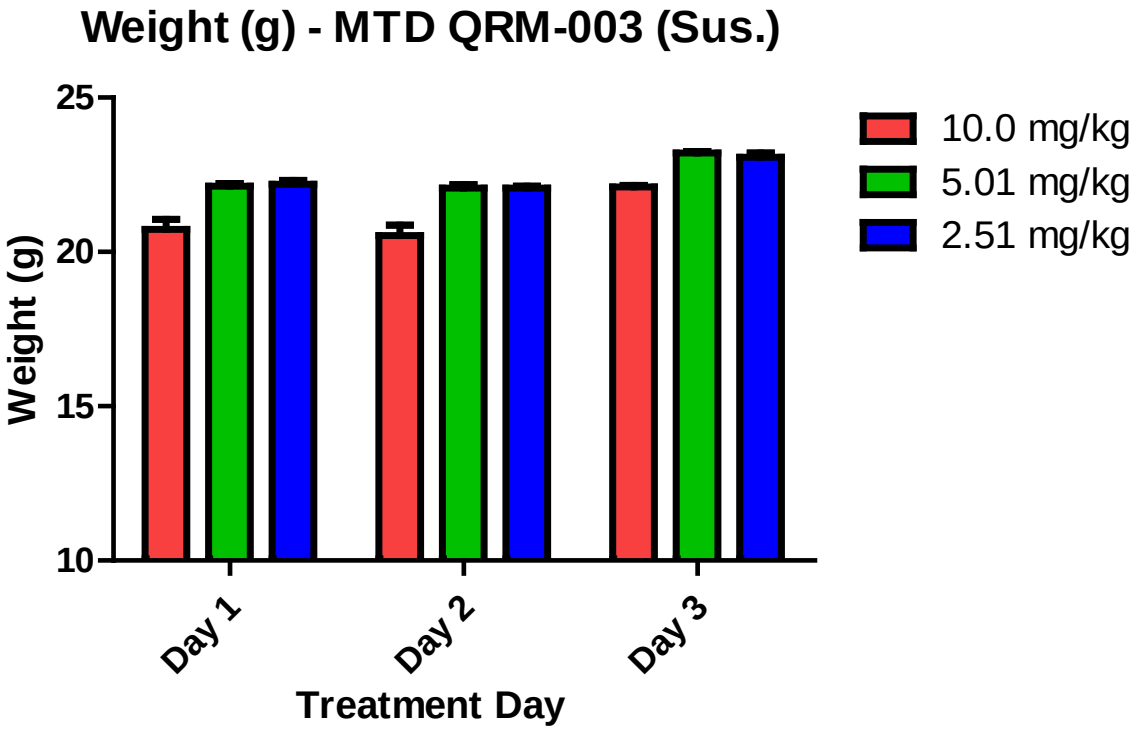
3 Treatments;
1x/2 days

Balb/C Mouse

Day 1 QRM-003 (Sus.)	Day 3 QRM-003 (Sus.)	Day 5 QRM-003 (Sus.)
1. 10.0 mg/kg	1. 10.0 mg/kg	1. 10.0 mg/kg
2. 5.01 mg/kg	2. 5.01 mg/kg	2. 5.01 mg/kg
3. 2.51 mg/kg	3. 2.51 mg/kg	3. 2.51 mg/kg

*all drug concentrations are calculated on the assumption of a 25g mouse

MTD, Safety Results – QRM-003



Treatment Groups	Day 1	Day 2	Day 3
Group 1	BAR	BAR	BAR
10.0 mg/kg	BAR	BAR	BAR
	BAR	BAR	BAR
Group 2	BAR	BAR	BAR
5.01 mg/kg	BAR	BAR	BAR
	BAR	BAR	BAR
Group 3	BAR	BAR	BAR
2.51 mg/kg	BAR	BAR	BAR
	BAR	BAR	BAR

Observations
BAR = bright, responsive, alert (Normal mouse)

Figure 4. Weight (g) of female Balb/C mice following instillation of QRM-003 (Sus.). (n = 3)

*all drug concentrations are calculated on the assumption of a 25g mouse

QRM Program Efficacy vs. *M. abscessus* – SCID mouse model

SCID Mouse

Intratracheal Infection with ***M. abscessus* 103**

Day 1
3 Mice Culled
(MABSC Uptake)

8 Treatments;
1x/2 days

Euthanasia

Day 2 – Tx Initiation
Treatment groups:

a) Control (Saline)
b) Amikacin (150 mg/kg SQ)
c) Clofazimine (20 mg/kg, Oral)
d) QRM-003 (Sus.) (10.0 mg/kg, IT)

Bacterial Recovery (Tissue Homogenate):

- Lung
- Spleen
- Liver

*all drug concentrations are calculated on the assumption of a 25g mouse

Aerosol QRM-003 suspension resulted in a 3 log reduction in lung CFU

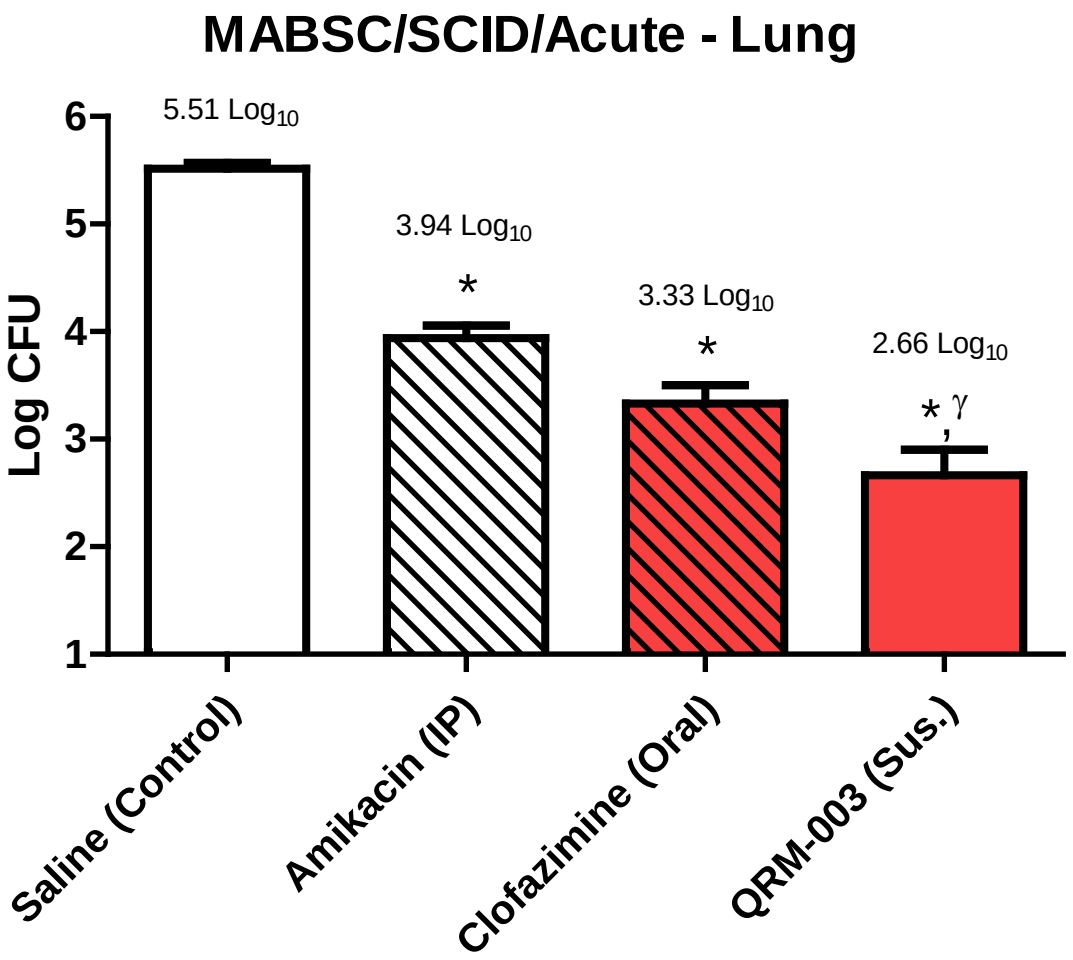


Figure 6. Bacterial counts in the lungs of SCID mice after pulmonary infection with 1x10^6 CFUs of *M. abscessus.* (n = 6)

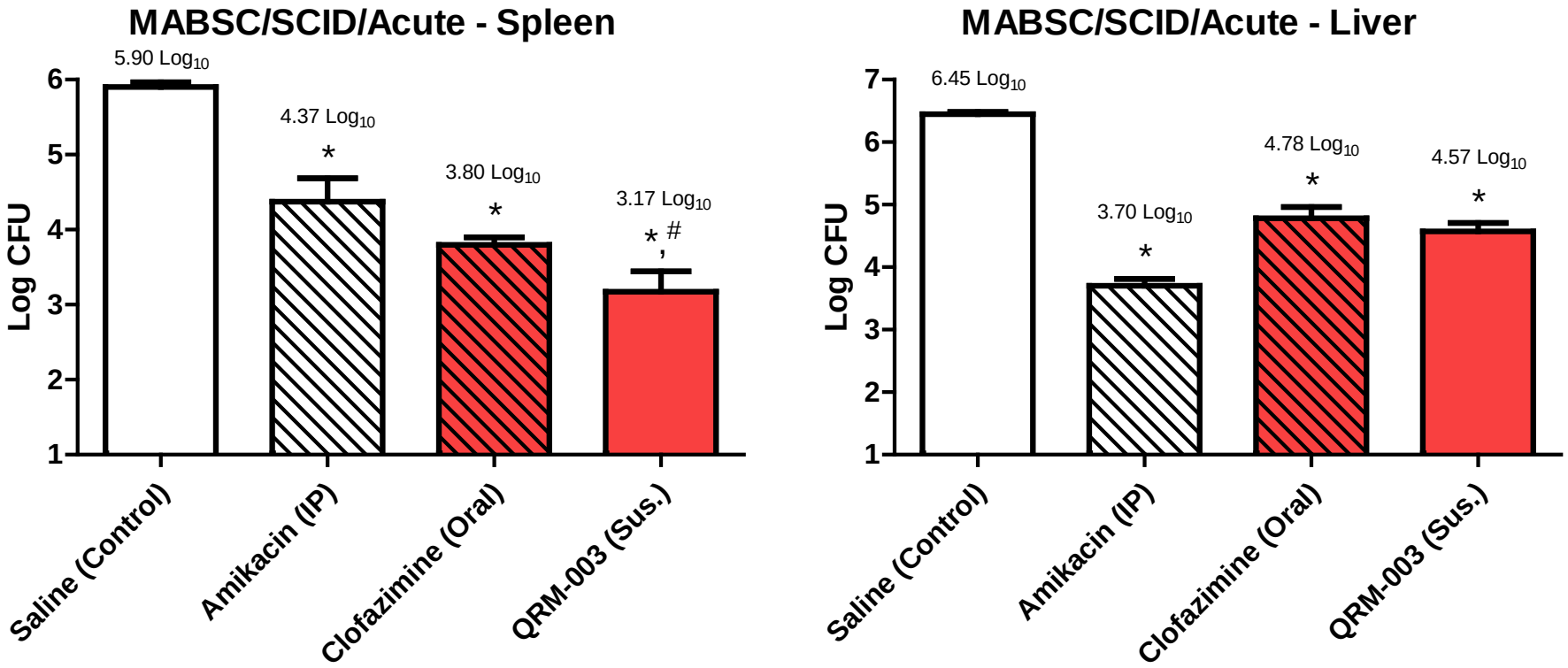
- Received 8 total treatments, administered every other day
- Treatment Groups:
  - Saline (IT) (White bar);
  - Amikacin 150 mg/kg (SQ) (White striped);
  - Clofazimine 20 mg/kg (gavage) (Red striped);
  - QRM-003 (Sus.) 10 mg/kg (IT) (Red);

- Results represent the average bacterial recovery, expressed as average Log_{10} CFU (± SEM) cells. Statistical analysis performed by one-way ANOVA.

*p<0.05 vs. Saline control
$^{\gamma}$p<0.05 QRM-003 vs. Amikacin

*all drug concentrations are calculated on the assumption of a 25g mouse

Aerosol QRM-003 suspension resulted in bacterial reduction in Spleen, Liver



- Saline (IT) (White bar);
- Amikacin 150 mg/kg (SQ) (White striped);
- Clofazimine 20 mg/kg (gavage) (Red striped);
- QRM-003 (Sus.) 10 mg/kg (IT) (Red);

Figure 7. Bacterial recovery from a) spleen, and b) liver of SCID mice with following pulmonary infection with 1x10^6 CFUs of *M. abscessus* 103.

*p<0.05 vs. Saline control

QRM Program Efficacy vs. *M. avium* – Beige Mouse Model

Beige Mouse

Aerosol Infection with ***M. avium* 2285**

Day 1
3 Mice Culled
(MAC Uptake)

10 Treatment Days;
1x/2 days

Day 7 – Tx Initiation
Treatment groups:

a) Control (Saline)
b) Clofazimine (20 mg/kg, Oral)
c) QRM-003 (Sus.) (10.0 mg/kg, IT)

Euthanasia

Bacterial Recovery (Tissue Homogenate):

- Lung
- Spleen
- Liver

*all drug concentrations are calculated on the assumption of a 25g mouse

Aerosol delivery of QRM-003 resulted in 1.7 log reduction in lung CFU

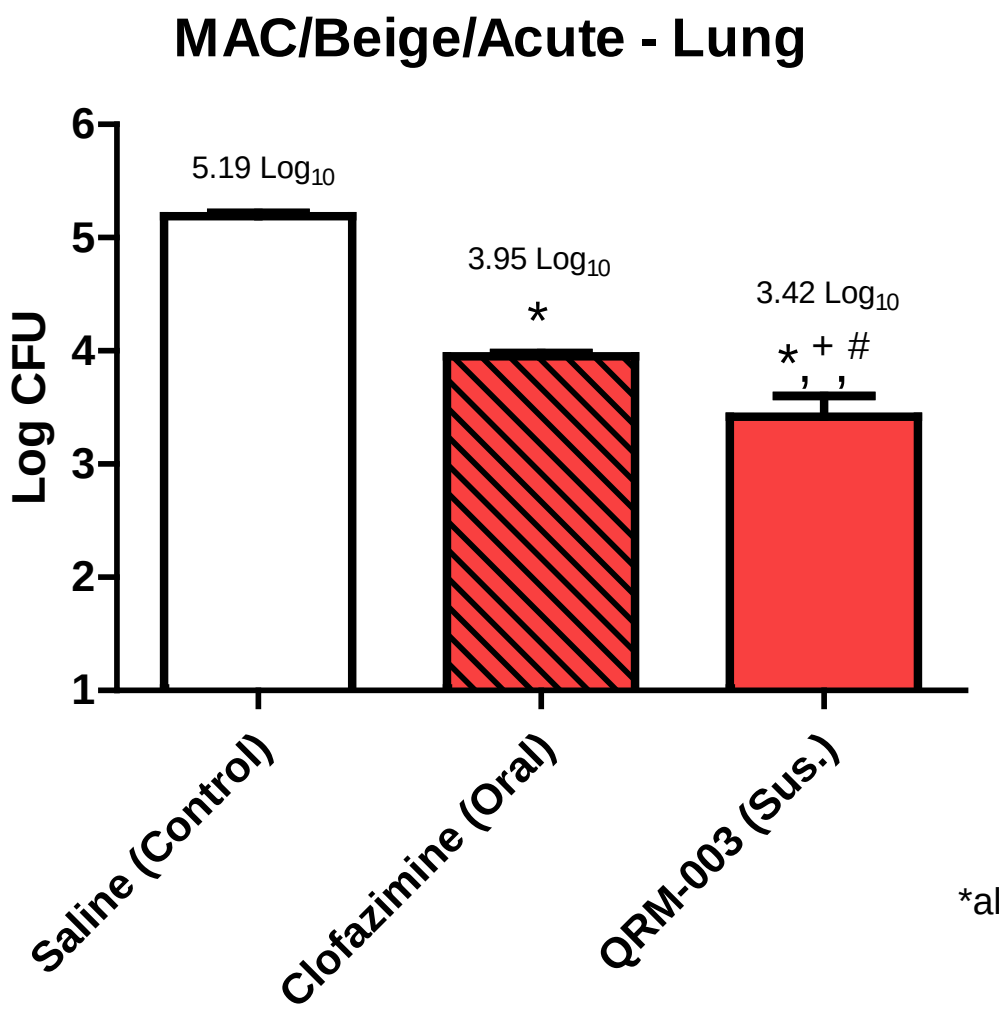


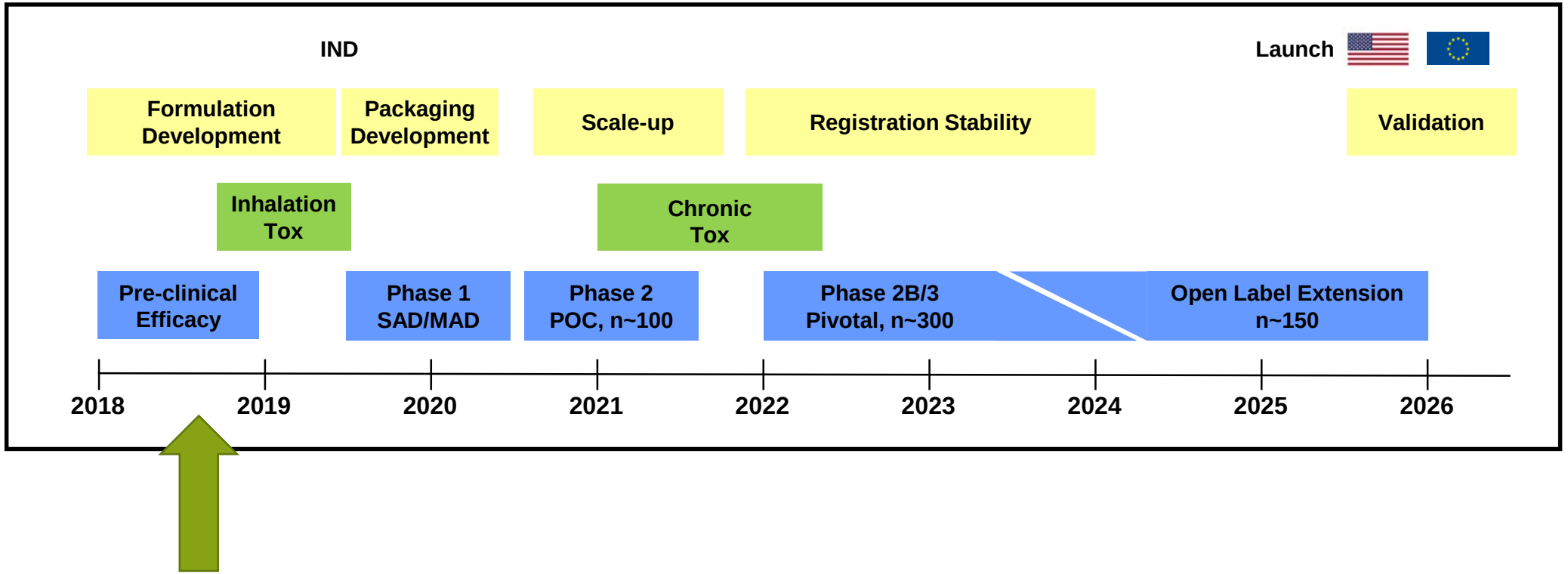
Figure 8. Bacterial counts in the lungs of Beige mice after high-dose aerosol infection with 1x10^8 CFUs of *M. avium* 2285. (n = 6)

- Received 10 total treatments, administered every other day
- Treatment Groups:
  - Saline (IT) (White bar);
  - Clofazimine 20 mg/kg (gavage) (Red striped);
  - QRM-003 (Sus.) 10.0 mg/kg (IT) (Red);
- Results represent the average bacterial recovery, expressed as average Log_{10} CFU (± SEM) cells. Statistical analysis performed by one-way ANOVA.

*p<0.05 vs. Saline control
$^+$p<0.05 QRM-003 vs. clofazimine (gavage)

*all drug concentrations are calculated on the assumption of a 25g mouse

Timeline for Inhaled Clofazimine Development



Status:

- Formulation, CMC and animal studies (pre-clinical efficacy)

Next steps:

- CMC upscaling and inhalation tox to prepare IND

Conclusions

- QRM-003 (aerosolized) demonstrates optimal anti-NTM activity
  - Safe at highest possible dose *in vivo (multiple of clinical dose)*
  - Greatest reduction of bacteria in the lung in all experimental models (MAC and MABSC; various formulations)
  - Significantly reduces NTM bacteria from lung/spleen/liver
  - QRM-003 Clofazimine is taken up by macrophages in vitro

QRM-003 (Clofazimine) has been chosen as lead therapeutic candidate, and will be taken into toxicology and IND