FORMaT

Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

A multi-centre, randomised, multi-arm, adaptive platform trial in people with or without cystic fibrosis Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment (FOR*Ma*T)

MASTER PROTOCOL

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ABBREVIATIONS

ADL	Activities of Daily Living
AE	Adverse Event
AFB	Acid-Fast Bacilli
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
BAR	Bayesian Adaptive Randomisation
BCM	Biased Coin Minimisation
CAG	Consumer Advisory Group
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CI	Chief Investigator
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DISC	Drug and Intervention Selection Committee
DNA	Deoxyribonucleic Acid
eCRF	electronic Case Report Form
eISF	electronic Investigator Site File
eTMF	electronic Trial Master File
FDA	Food and Drug Administration
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GLI	Global Lung Initiative
HRCT	High-Resolution Computed Tomography

HREC	Human Research Ethics Committee
IA	Inhaled Amikacin
ICH-GCP	International Council for Harmonisation Good Clinical Practice Guidelines
ICMJE	International Committee of Medical Journal Editors
iDSMB	Independent Data Safety Monitoring Board
IEC	Independent Ethics Committee
IRB	Independent Review Board
ISO	International Organization for Standardization
IV	Intravenous
IVA	Intravenous Amikacin
LAI	Liposomal Amikacin for Inhalation
LLN	Lower Limit of Normal
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MBS	Medicare Benefits Scheme
MCRI	Murdoch Children's Research Institute
MoOP	Manual of Operating Procedures
MRL	Mycobacterial Reference Laboratory
NTM	Non-Tuberculous Mycobacteria
PBS	Pharmaceutical Benefits Scheme
PI	Principal Investigator
PICF	Participant Information and Consent Form
PK	Pharmacokinetics
QA	Quality Assurance
QoL	Quality of Life
R-Con	Randomisation – Consolidation
RCT	Randomised Control Trial

REDCap	Research Electronic Data Capture
REMAP-CAP	Randomised, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia
R-PI/IC	Randomisation – Prolonged Intensive or Immediate Consolidation
rRNA	ribosomal ribonucleic acid
R-SI	Randomisation – Short Intensive
SAEs	Serious Adverse Events
SAHMRI	South Australian Health and Medical Research Institute
SIV	Site Initiation Visit
SOP	Standard Operating Procedure
SSI	Significant Safety Issues
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TDM	Therapeutic Drug Monitoring
TMC	Trial Management Committee
TPF	Time Point Final
TPST	Time Point Start Treatment
TSC	Trial Steering Committee
ULN	Upper Limit of Normal
USMs	Urgent Safety Matters
UQ	University of Queensland
WGS	Whole Genome Sequencing
6MWD	Six-minute walk distance
6MWT	Six-minute walk test

PRINCIPAL INVESTIGATORS STATEMENT

I confirm that I have read the FOR*Ma*T Master Protocol **Version 4.1, dated 20th February 2024.** As the Principal Investigator, I understand it, and I agree to adhere to the study conduct requirements and agree to conduct this protocol in accordance with International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), the Declaration of Helsinki, the United States (US) Food and Drug Administration (FDA), and local regulations and guidelines. I agree to report all information or data in accordance with the protocol, and in particular I agree to report any serious adverse events. I will accept the monitors', auditors' and regulatory inspectors' oversight of the study. I will promptly submit the protocol to the applicable ethical review board as required.

…….....

Principal Investigator Signature

……..

Principal Investigator Name

……..

Date (dd/mmm/yyyy)

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1 INTRODUCTION

1.1 SYNOPSIS

Background: Mycobacteria in the *Mycobacterium abscessus* group (MABS) are a species of non-tuberculous mycobacteria (NTM) found in water and soil habitats that exhibit high levels of intrinsic multi-drug resistance (1). NTM includes more than 160 species that are recognised opportunistic human pathogens, with chronic pulmonary infections the most common clinical presentation. Individuals with underlying inflammatory lung diseases are more susceptible to MABS pulmonary disease (MABS-PD), but MABS also affects patients with no underlying condition. MABS-PD can result in significant morbidity, increased healthcare utilisation, accelerated lung function decline, impaired quality of life (QoL) (2), more challenging lung transplantation (3), and increased mortality (2). Of particular concern is the increasing prevalence of pulmonary infections occurring worldwide in patients with bronchiectasis and cystic fibrosis (CF), with prevalence between 5 to 20% (2, 4, 5). There is real evidence of this in Queensland, Australia (where the infection is notifiable) with the prevalence increasing from 0.85/100,000 in 2001 to 2.35/100,000 in 2016, where now over 100 cases are reported annually, clearly illustrating the emerging threat of this infection. While the increasing prevalence might be reflective of enhanced surveillance and improved microbiological detection (6-8) (9) the reasons for this changing epidemiology are poorly understood (2, 4, 10, 11). Treatment regimens for MABS are highly variable, not evidence-based and involve complex, expensive, and often poorly tolerated drug combinations for prolonged periods (>12 months). Some individuals will have positive cultures that clear spontaneously, some will initially have positive cultures without obvious MABS-PD but go on to develop disease at a later stage, and some may present with established MABS-PD. MABS-PD can be associated with a rapid decline in health status, and there is evidence that successfully clearing infection is associated with better health outcomes (12, 13). However, toxicity, poor tolerance and the prolonged and complex nature of therapy may increase the reluctance of clinicians to initiate treatment and for patients to accept it. A recent systematic review and meta-analysis revealed that such treatment regimens are often ineffective and may even worsen QoL (14). The costs and treatment burden of NTM infection are high, and highest for MABS-PD, estimated at $AUD12-28,000/month (15) highlighting the need to assess the healthcare costs and cost-effectiveness of therapies to inform health policy around NTM. Pathogen, host, and treatment factors all likely play a role in clinical and microbiological outcomes.

Aims:

1. To build an iterative, standing, platform trial with innovative and adaptive properties to evaluate combinations of therapies for patients with MABS-PD. Initially this will test therapies that are currently used and recommended in published international consensus guidelines and are the basis for the current treatment guidelines for MABS-PD. Once the best combinations have been established the platform described in the Master Protocol will have the capacity to add new treatments and to eliminate therapies because of futility as they either lack efficacy or cause unacceptable toxicity. The data obtained as part of the trial will be used to plan for new waves of the platform trial using novel therapeutic approaches that may be tested against the previously determined optimal approaches, thus leading in an iterative fashion to improving microbiological clearance and health outcomes associated with MABS-PD.

2. To use the opportunities afforded by the clinical trial platform to establish discovery studies to:
    i. Understand the effects of MABS-PD and therapeutic interventions on health-related quality of life and determine the cost effectiveness of proposed therapy combinations;
    ii. To develop strategies for optimising drug dosing using robust pharmacokinetics;
    iii. Understand susceptibility to MABS-PD and develop biomarkers of clinical disease, disease progression and response to therapy;
    iv. Investigate the genomics of human MABS strains and antibiotic resistance genes and impact of therapeutic interventions.
3. To investigate the use of registries to facilitate the long-term monitoring of patient outcomes from MABS-PD and treatment.

Methodology: Entry into the Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment (FOR*Ma*T) trial can occur at two different levels;

1 - participants of any age from their first MABS isolate and not receiving current MABS therapy are eligible to enrol in the Observational Cohort and;

2 - participants of any age meeting the American Thoracic Society (ATS) criteria for the diagnosis of MABS-PD and are untreated for MABS-PD at the time of starting intensive therapy are eligible to enrol in the Intervention Program. Participants initially enrolled into the Observational Cohort who go on to meet the ATS criteria for MABS-PD can transition to the Intervention Program at any time. Intervention Program participants will be randomised to receive MABS-PD therapy combinations and additional outcomes will be assessed.

Participants in both the Observational Cohort and Intervention Programs will contribute the same core data, thus providing the opportunity to examine what happens to both treated and untreated patients with positive cultures longitudinally as well as the transition to MABS-PD.

Primary Outcome

The primary outcome of the Intervention Program is microbiological clearance of MABS with good tolerance of the interventions.

Definition of MABS clearance at final outcome:

Negative MABS cultures from four consecutive sputum samples with one of those sputum specimens collected four weeks after the completion of consolidation therapy, or a MABS negative Bronchoalveolar Lavage (BAL) collected four weeks after completion of consolidation.

Definition of tolerance:

Tolerance is based on the Common Terminology Criteria for Adverse Events (CTCAE version 5.0). Only adverse events that are attributed as either "possibly-", "probably-", or "definitely-" related to study drug will be assessed in the determination of tolerance. "Good" tolerance is defined as no adverse events occurring or only adverse events coded as CTCAE grades 1 and 2. "Poor" tolerance is defined as any adverse events attributed as possibly-, probably-, or definitely-related to study drug coded as CTCAE grades 3, 4, or 5.

The Intervention Program within the FOR*Ma*T platform will adopt an adaptive design with Bayesian Adaptive Randomisation (BAR) when there is randomisation across three or more interventions and may also include interim rules for dropping or adding treatment arms. BAR allows the randomisation probabilities to each of the interventions to be updated during the trial to enable more participants to be randomised to more promising interventions. The probabilities of allocation to each intervention are based on the posterior probability of the intervention being superior to the control (16). The approach used will carry forward the most promising arms that achieve a minimum level of efficacy with acceptable toxicity. If a treatment is carried forward until the end it will be tested against the control/reference group and recommended if the test statistic is above a certain threshold. The Intervention Program will consist of treatment modules specified in Appendix A starting with sequential Phase II trials, including a randomised intensive treatment phase, and a randomised consolidation treatment phase. In the future, interventions reaching a probability threshold of demonstrating success at the end of the Phase II trial will have the potential to seamlessly continue to recruit to a Phase III study to enable the Phase II data to be utilised as part of a Phase III study, thus reducing the resources required to achieve high quality evidence on which to base treatments.

The FOR*Ma*T Master Protocol describes the adaptive platform trial to evaluate microbiological, functional, radiological, and quality of life outcomes of interventions utilised in the treatment of MABS-PD, in combination with the Observational Cohort.

1.2 PROTOCOL STRUCTURE

The FOR*Ma*T trial is designed as a standing iterative, platform trial with innovative and adaptive properties. This is reflected in the multi component structure of the FOR*Ma*T Protocol outlined in Figure 1. The framework for the FOR*Ma*T trial is established within the FOR*Ma*T Master Protocol. The Intervention Program(s), the Observational Cohort, Discovery, Registry Linkage studies, Health Economics and General Statistical Principles are described in specific Appendices.

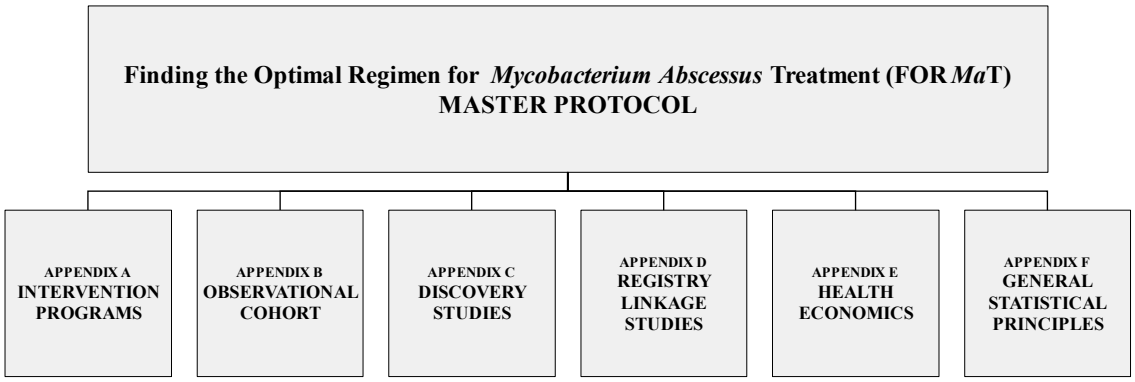

Figure 1 Design of the FOR*Ma*T Master Protocol and Appendices

1.3 FOR*MA*T MASTER PROTOCOL

The FOR*Ma*T Master Protocol outlines the core structure, procedures, and processes of the FOR*Ma*T trial. The information described in the FOR*Ma*T Master Protocol applies to all participants regardless of if they are receiving intervention treatment for MABS-PD or are enrolled in the Observational Cohort, unless otherwise stated.

The Master Protocol has the following structure:

- The background and rationale for determining the optimal treatment regimens for MABS-PD.
- The overarching objectives of the FOR*Ma*T trial (including both the Intervention Program and the Observational Cohort).
- The design of the FOR*Ma*T trial including the two-level approach to assessing eligibility into the trial, the trial endpoints and the general statistical principles to be used in the analysis of the trial.
- The conduct of the FOR*Ma*T trial detailing recruitment methods and consent processes, the core trial timepoints and procedures, safety monitoring processes and data management procedures.
- Details of The FOR*Ma*T trial oversight, ethical and administrative considerations.

1.3.1 FOR*MA*T APPENDICES AND SUPPLEMENTS

The FOR*Ma*T Appendices describe in detail the information specific to the Appendices and the sub-studies and integrated studies nested within them. As such, appendix specific information is not described within the FOR*Ma*T Master Protocol but rather the Master Protocol sets the framework within which the Appendices exist. As the trial progresses, new interventions and methodologies can be added to the FOR*Ma*T trial through the addition of a new appendix. Conversely, as interventions and methodologies are found to be futile the corresponding studies in the appendix can be removed. It is not anticipated that these changes will affect the framework of the Master Protocol. Any changes to appendix specific studies require ethics approval.

The FOR*Ma*T Supplements are additional documents that expand the scope of the relevant appendices. They provide additional detail and information to supplement the specific appendix they are linked to. Changes to supplements do not require ethical approval as they are not a part of the Master Protocol or appendices but are an additional document to elaborate on the specific components contained within an appendix. **FOR*Ma*T Appendices are structured as follows:**

- Each appendix has a theme: for example, Appendix A for Interventions, Appendix B for Observation, Appendix C for Discovery studies, Appendix D for Registry Interactions, Appendix E for Health Economics, and Appendix F for General Statistical Principles.
- Appendix A describes the details for the Intervention Programs of the FOR*Ma*T Trial including intensive and consolidation interventions (Figure 2). For example, Appendix A1 describes the first of such Intervention Programs and if new interventions are added to either the intensive or consolidation phases these would be added in new Appendices A2, A3 etc. Data from previous programs using the same intervention combinations may be incorporated in the analysis of a new program. Thus, data from Appendix A1 could be combined with data from Appendix A2 for example.

- Within some of the Appendices are nested studies that have been designed to investigate specific objective(s).

The components described within each appendix are variable and dependant on the nature of the appendix and the studies nested within it. Overall, FOR*Ma*T Appendices will contain the following components:

- The overall objective specific to that appendix.
- Where relevant, information about the features of the interventions or the study to be investigated.
- Appendix specific eligibility criteria.
- Appendix specific consent requirements.
- Appendix specific procedures and safety measures to be assessed.
- Appendix specific statistical methods and simulations where relevant.

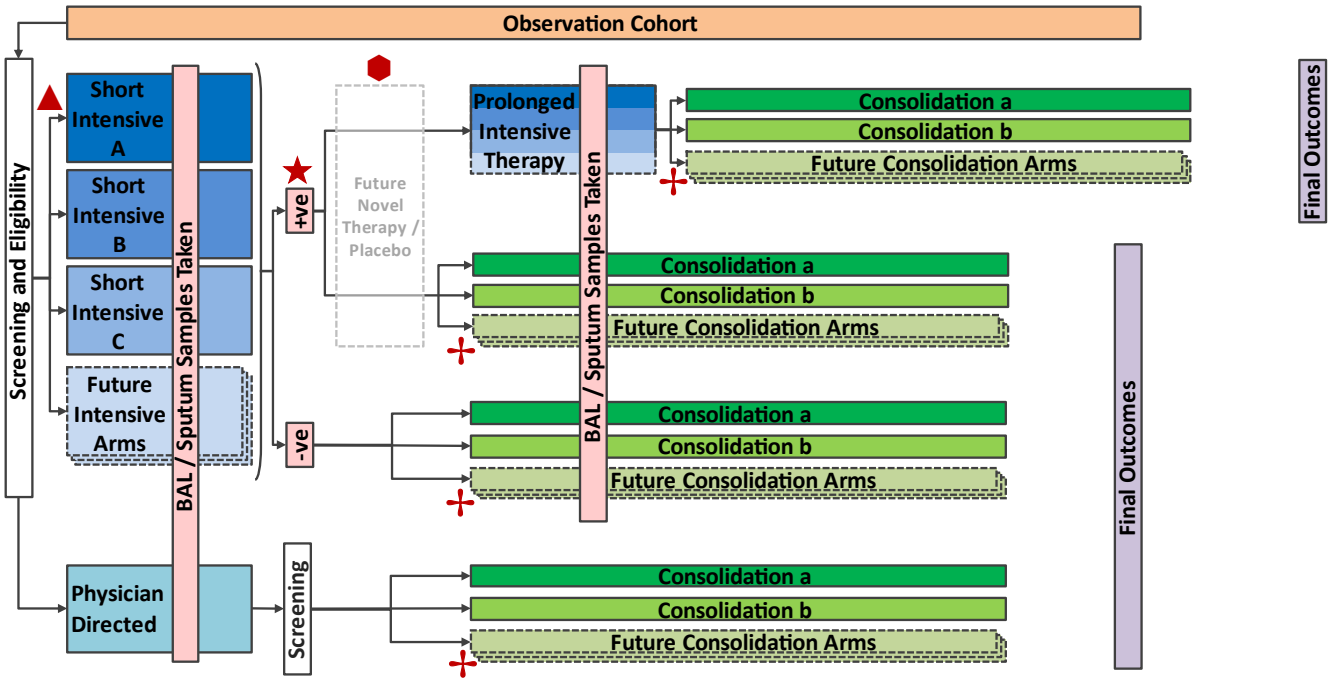

Figure 2 FOR*Ma*T participant flow diagram
Eligibility into the Intervention Program or Observational Cohort is determined at Screening. For the first iteration of the Intervention Program, there will be up to three randomisations between Screening and the Final Outcome Visit. Symbols (▲★⬢✢) indicate possible randomisation points (see the relevant appendix for further information on randomisation).

2 BACKGROUND

2.1 MICROBIOLOGY OF MABS

Of the many pathogenic NTM species, MABS are recognised as causing the most serious pulmonary infections, associated with the greatest problems of antibiotic resistance, toxicity and treatment failure (12, 17). MABS are currently divided into 3 subspecies: *M. abscessus* subspecies *abscessus* (*M. a. abscessus*), subs. *massiliense* (*M. a. massiliense)* and subs. *bolletii* (*M. a. bolletii)*. While there is variation in the prevalence of the subspecies in different populations, *M. a. abscessus* is the most common overall (45-68%), followed *M. a. massiliense* (20-55%) and *M. a. bolletii* (8-25%) (18-20). Progression of MABS-PD due to the different subspecies (21) appears to be similar, although treatment outcomes vary significantly and are partially explained by differential antimicrobial susceptibility to macrolide antibiotics. MABS are intrinsically drug resistant to multiple classes of antibiotics and they can also acquire antibiotic resistance genes to macrolides and aminoglycosides, leading to clearance rates of ≈50% following intensive therapy (19, 22, 23). After apparently successful treatment, relapse or recurrences with new strains occur in 15-33% of patients (19). Nevertheless, macrolides provide the therapeutic backbone of guideline-based MABS treatment (1, 4). Furthermore, they are the only antibiotic class where there is some correlation between *in vitro* susceptibility data and clinical response (14). Macrolide resistance in MABS is either constitutive or inducible (20). The less common, constitutive resistance may be acquired during macrolide therapy and results from mutations in the 23S rRNA gene (*rrl*). Inducible macrolide resistance is related to the MABS ribosomal methyl transferase gene, *erm(41).* Such isolates appear susceptible at day 3 following infection, but resistant by day 14 using prolonged incubation drug susceptibility testing. *M. a. massiliense* has a truncated and dysfunctional *erm(41)* gene, thus making it more susceptible to macrolides, whereas *M. a. abscessus* and *M. a. bolletii* usually, but not invariably, have inducible resistance (20). It is not surprising then that microbiological cure rates of MABS-PD appear partly related to macrolide resistance with clearance rates up to 88% in those with macrolide susceptible isolates, and only 36% in the setting of inducible macrolide resistance (19). Molecular detection of the subspecies, including identifying the *erm(41)* and *rrl* genes is important in understanding treatment response, and potentially targeting novel treatment approaches.

2.2 EPIDEMIOLOGY

NTM can cause both asymptomatic and symptomatic infections in humans (1). Pathogenic strains of MABS have been isolated from potable water, and MABS infections are more prevalent in coastal areas and regions with humid tropical climates (12, 24, 25). While most infections are thought to be acquired from environment aerosols (26), in patients with CF there is evidence supporting the emergence of worldwide dominant clones (of increased virulence) that may be capable of patient-to-patient transmission (18).

2.2.1 HIGH-RISK PATIENT POPULATIONS

CF is an autosomal recessive condition caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Mortality and morbidity of CF patients are predominantly related to chronic suppurative lung disease (27). CF

is a risk factor for MABS-PD (and NTM more broadly) and even carrier status of disease-causing mutation in *CFTR* (24) may also increase risk. Other structural lung diseases, bronchiectasis, chronic obstructive pulmonary disease, previous mycobacterial disease (including Tuberculosis (TB) and NTM), severe gastro-oesophageal reflux and immunosuppression (where dissemination can occur) (11) also increase risk of infection. The age range of CF and non-CF affected patients with MABS-PD overlap, with non-CF patients generally being older than those with CF (>55 vs <30 years) (24).

2.3 CLINICAL PRESENTATION AND DIAGNOSTIC CHALLENGES

Due to their ubiquitous nature, the clinical significance of positive MABS cultures in respiratory specimens can be challenging. It may appear transiently in sputum cultures, persistently colonise the lower airways or progress to MABS-PD. The radiological and clinical features of underlying chronic respiratory disorders overlap considerably with changes attributable to MABS-PD making diagnosis and treatment decisions difficult and care is required to follow the ATS criteria in making the diagnosis of MABS-PD. Inclusion into the intervention program(s) will require meeting all the ATS criteria including both microbiological and clinical criteria.

2.4 TREATMENT REGIMENS GUIDELINES AND CHALLENGES

MABS treatment outcomes differ according to the etiologic organism. Recurrence rates for infection are high, despite successful treatment completion (28) but these approaches have not been evaluated in any trials. Differing treatment outcomes present multiple therapeutic challenges in the treatment of MABS. In recognition of these challenges, the ATS and the United States CF Foundation/European CF Society have published guidelines on NTM pulmonary disease (1, 4). In agreement with the latest Cochrane Review (29), they note there are no drug regimens of proven or predictable efficacy for treating MABS. Therefore, the guidelines are based on expert opinion only and in practice the treatments vary considerably (30). Suggested regimens include an intensive phase of 4-12 weeks (based on microbiological response) of intravenous (IV) antibiotics (usually amikacin, cefoxitin or imipenem + tigecycline) plus an oral macrolide. This is followed by consolidation therapy that includes oral drugs (usually a macrolide, plus others based on antibiograms, tolerability and experience) and an inhaled IV formulation of amikacin for 3 to >12 months. Dosing by individual pharmacokinetic (PK) data and therapeutic drug monitoring (TDM) may lead to optimal drug levels at infection sites and better treatment outcomes, although measuring levels within the lower airways frequently and non-invasively is challenging. Few PK studies involving antibiotics for NTM have been performed and few assays are currently available. Inhaled antibiotics have the potential advantages of achieving higher airway concentrations, while reducing the risk of systemic toxicity.

2.5 HEALTH RELATED QUALITY OF LIFE

A recent systematic review and meta-analysis revealed that MABS treatments are often ineffective and may even worsen QoL (14). The review strongly recommended that "clinical, functional and QoL parameters should be given more emphasis in the evaluation of treatment outcomes" and that "better applications of current antibiotics are

urgently needed" (14, 30). In addition, the costs and the treatment burden of NTM infection are high, and highest for MABS-PD; estimated at $AUD12-28,000/month (15), highlighting the need to assess the healthcare costs and cost-effectiveness of therapies to inform health policy around NTM. Pathogen, host and treatment factors are all likely to play a role in clinical and microbiological outcomes. Consequently, there is an urgent need for evidence to support treatment decision-making for patients with MABS lung infection.

2.6 THE PATIENT VOICE

In October 2015, the Food and Drug Administration (FDA) held a public meeting on NTM infection with patients and carers with the key theme to emerge (31) being the need for better, less toxic treatment with lower therapeutic burden. Participants also prioritised "validating and using tools to measure QoL and developing disease specific activity and severity assessment tools" (32). In January 2017, The James Lind Alliance released their top 10 research priorities for people with CF developed by CF patients, their families, and healthcare providers. The third top priority was "What is the best treatment for NTM, including when to start and what medication?" (33). Patients and healthcare providers are asking for evidence to guide the best approaches to manage this challenging infection.

A consumer representative is a member of the FOR*Ma*T Trial Steering Committee and will be involved in the ongoing oversight of the trial. In addition, the FOR*Ma*T consumer advisory group will enable a range of consumers to participate in the review of the protocol, trial development plans and consumer materials. The consumer group will be able to provide their views and advice which will then be taken on board by the investigators and trial management team.

2.7 GENERATING EVIDENCE USING A RANDOMISED PLATFORM TRIAL DESIGN

Platform trials using Bayesian statistical models, provide the opportunity to efficiently investigate multiple treatments for difficult-to-treat infections (e.g. multi-drug resistant TB (34)) requiring complex drug regimens in a heterogeneous population and can provide an iterative resource facilitating the translation of findings to improve clinical outcomes (35-37). New adaptive trial approaches are now recognised and accepted by regulatory authorities, including the FDA. Such trials are now being used in infectious diseases (38) and TB (34), complex chronic diseases (36) and rare oncology conditions (39). MABS-PD is a serious but relatively rare problem which can benefit from such methodology. FOR*Ma*T provides a common platform that will enable a broad enrolment of patients who both do and do not have cystic fibrosis and across all ages that enables generalisability but also maintains the ability to examine the heterogeneity of treatment responses across specific subgroups, while also enabling a comparison with non-treated patients through an Observational Cohort who are followed up simultaneously. The ability to examine novel therapeutic approaches using a common platform, along with the option to seamlessly move from phase II to phase III if warranted, also reduces the resources and the time required to deliver evidence-based treatments to patients who need it compared with conducting a series of independent trials.

2.8 SUMMARY

There is no evidence currently to guide therapy for MABS-PD, a complex and increasing health problem. The FOR*Ma*T trial seeks to provide answers to key questions by healthcare providers and patients on the timing and nature of treatments for the growing number of people infected with MABS, as well as the potential to model the progression of this condition in both treated and untreated patients. Furthermore, the trial will provide a platform for improving health outcomes for MABS patients and build a solid foundation for future testing of new therapeutics in the treatment of MABS.

3 OBJECTIVES

3.1 PRIMARY OBJECTIVE

To determine the optimal therapy for the treatment of patients with MABS-PD.

3.2 SECONDARY OBJECTIVES

1. To investigate the optimal approaches to antibiotic dosing and therapeutic drug monitoring.
2. To investigate the health-related quality of life and cost effectiveness of proposed therapy combinations.
3. To examine changes in clinical markers such as chest imaging and lung function to predict the onset of MABS-PD and response to therapies.
4. To develop biomarkers to predict the onset of MABS-PD and response to therapies.
5. To understand susceptibility to infection with MABS associated with the development of MABS-PD and host immune responses to infection and with treatment.
6. To characterise the genomics of human MABS strains and antibiotic resistance genes in patients in the observation and intervention studies.

4 TRIAL DESIGN

4.1 ELIGIBILITY CRITERIA

Eligibility criteria for the FOR*Ma*T trial can be applied at two levels:

1. Eligibility into the Intervention Program, or;
2. Eligibility into the Observational Cohort.

Potential participants can only be enrolled in either the Intervention Program or the Observational Cohort at any one time. Provided the eligibility criteria are met, potential participants may either:

1. Enrol directly into the Intervention Program, or;
2. Enrol into the Observational Cohort and transition into the Intervention Program once they satisfy the inclusion criteria for this program which can occur at any time during the trial (see Figure 3).

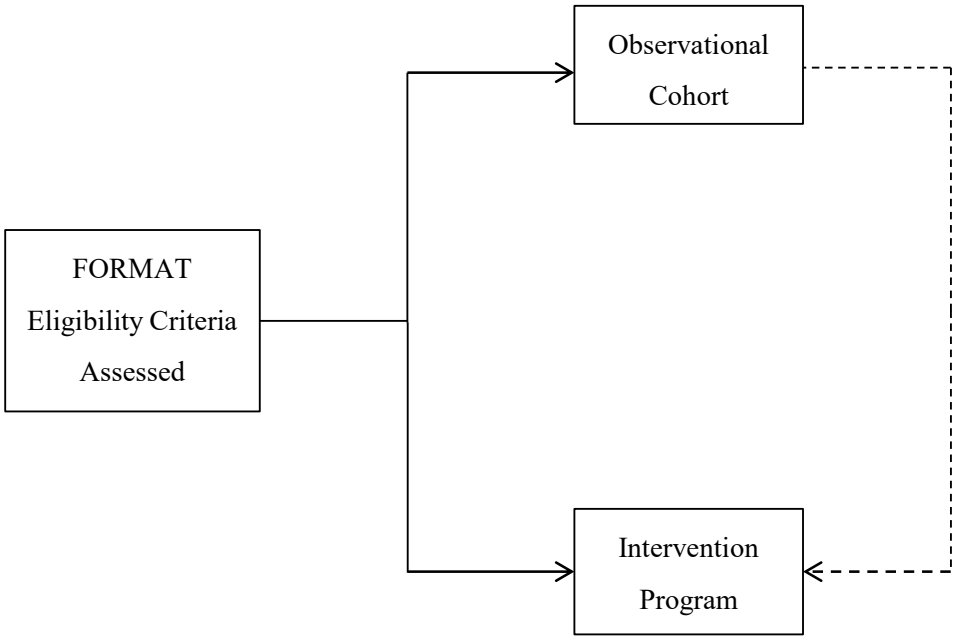

Figure 3 Participant enrolment into the FOR*Ma*T trial.
Eligibility into the FOR*Ma*T trial will be assessed at screening. Observational Cohort participants who go on to meet the Intervention Program eligibility criteria can transition from the Observational Cohort to the Intervention Program.

4.2 INTERVENTION PROGRAM ELIGIBILITY (APPENDIX A)

Potential participants are eligible for the Intervention Program (Appendix A) if the criteria below are met. Eligible participants with mixed NTM infections (slow growers + MABS) or with recurrence of MABS infection following completion of previous treatment will be eligible if they meet the inclusion and exclusion criteria listed below. For eligible participants with mixed NTM infections additional therapy combinations are available as detailed in the relevant appendices.

4.2.1 INTERVENTION PROGRAM INCLUSION CRITERIA

1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD. Defined as:
   a. ***Clinical:*** Pulmonary symptoms and exclusion of other diagnoses.
   b. ***Radiological:*** Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.
   c. ***Microbiological:*** MABS positive culture results from at least two separate expectorated sputum samples.

      or

      Positive culture results from at least one bronchial wash or lavage.

      or

Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli (AFB)) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM.

Screening samples must be collected within the timeframes stated in the relevant appendix.

2. Male or female participants of any age.
3. Participant has not received treatment for MABS-PD in the 12 months preceding assessment of eligibility or as specified in the relevant appendix (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in the FOR*Ma*T Prohibited Drug List Standard Operating Procedure (SOP)).
4. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age.
5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.

4.2.2 INTERVENTION PROGRAM EXCLUSION CRITERIA

- Participants receiving current treatment for MABS (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in the FOR*Ma*T Prohibited Drug List SOP), except for participants taking azithromycin as part of routine treatment for CF or chronic infection-related pulmonary disease, or as specified in the relevant appendix.
- Participants who have a QTc interval of >500 milliseconds (QT interval corrected based on Fridericia method).
- Participants who are pregnant or planning to continue breast feeding.
- Known hypersensitivity or contraindication to any of the therapies for which no alternative option(s) have been provided.

4.3 OBSERVATIONAL COHORT (APPENDIX B)

4.3.1 OBSERVATIONAL COHORT INCLUSION CRITERIA

To be eligible to participate in the Observational Cohort the following criteria must be met:

1. Male and female participants of any age with at least one positive respiratory culture for MABS.
2. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age.
3. Ability to comply with study visits and study procedures as judged by the site investigator.

4.3.2 OBSERVATIONAL COHORT EXCLUSION CRITERIA

Potential participants will be ineligible to participate in the Observational Cohort if any of the following criterion are met:

- Receiving active treatment for MABS within the previous 12 months (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in the FOR*Ma*T Prohibited Drug List SOP, except for participants taking azithromycin as part of routine treatment for CF or chronic infection-related pulmonary disease).

4.4 ADDITIONAL ELIGIBILITY CRITERIA

Mixed NTM infections

Participants who have cultured slow growing NTM of the same species two or more times in the 24 months prior to screening, with one of those cultures within the 6 months prior to screening, will be considered to have mixed NTM infection at the time of screening. The participants must meet all other inclusion criteria and no exclusion criteria to be eligible for participation. Ethambutol may be used in addition to trial therapies to cover mixed NTM infections considered to require treatment by their clinician.

Appendix specific sub-studies and integrated studies

Appendix specific sub-studies and integrated studies may have additional eligibility criteria which are described in each of the relevant appendices.

4.5 TRIAL SETTING AND PARTICIPATING REGIONS

The trial will be conducted in multiple regions and trial sites. There is no limit to the number of countries or participating sites. A list of participating countries and sites are available on the FOR*Ma*T trial website (www.formattrial.com) and the clinical trial registries and will be updated as required.

Race and Ethnicity

Race and ethnicity will be collected as part of the demographic information for each participant. This information may be used to determine whether there are any potentially clinically significant racial and/or ethnic differences in the effects of any trial interventions.

4.6 TRIAL ENDPOINTS

The primary outcome for the FOR*Ma*T Trial (Appendix A Intervention Program) is MABS clearance from respiratory samples with treatment tolerance. MABS clearance is defined as negative MABS cultures from four consecutive sputum samples with one of those sputum specimens collected four weeks after the completion of consolidation therapy or a MABS negative Bronchoalveolar Lavage (BAL) collected four weeks after completion of consolidation therapy.

The Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be applied for the coding of all adverse events (AEs) and serious adverse events (SAEs) that occur during the FOR*Ma*T trial that are categorised as "possibly-", "probably-," or "definitely-" related to study medications and/or interactions between study medications

and concomitant medications. "Good" tolerance will be defined as no adverse events occurring or only adverse events coded CTCAE grades 1 or 2. "Poor" tolerance will be defined as any adverse events coded as CTCAE grades 3, 4, or 5.

The primary outcome of the FOR*Ma*T Trial will be assessed as per the relevant appendices.

5 TRIAL CONDUCT

5.1 SITE INITIATION

The FOR*Ma*T Trial is a multicentre, international clinical trial and the initiation of the FOR*Ma*T Master Protocol (including the Appendices) will be staggered across trial sites. This staggered approach will allow the FOR*Ma*T Trial Management Team to closely examine the effectiveness of the FOR*Ma*T Trial Master Protocol. Any changes required to improve the trial processes can be shared and implemented in other trial sites prior to site initiation. The FOR*Ma*T Trial will open in Australia, and as feasible expand to other countries including centres in Europe, the United Kingdom, the Asia Pacific Region, Canada and North and South America. Site activation and initiation will be performed in accordance with the FOR*Ma*T Site Initiation and Activation SOP.

5.2 TRIAL DURATION

The FOR*Ma*T Master Protocol describes a standing platform trial with an innovative design tailored to the clinical setting that will enable new therapies to be tested as they become available. As such, there is no specific limit to the duration of the FOR*Ma*T trial while there is clinical need for the trial except for logistic issues which include trial funding. A detailed outline of the trial timeline for participants is described in the relevant appendix.

5.3 RECRUITMENT OF POTENTIAL PARTICIPANTS

Potential participants will be identified at study sites by the treating physician. Once identified, potential participants or their parent/guardian will be approached by the FOR*Ma*T Trial site-specific research team or treating physician to discuss the FOR*Ma*T trial and provided with the FOR*Ma*T Participant or Parent/Guardian Information and Consent Forms (PICFs). The FOR*Ma*T Trial site-specific research team members will allow participants and/or their parent/guardian adequate time to read the PICFs and an opportunity to ask any trial related questions and to have these questions answered to the satisfaction of the potential participant or their parent/guardian.

5.4 INFORMED CONSENT

5.4.1 CONSENT TO THE FOR*MA*T MASTER PROTOCOL

In accordance with the International Council for Harmonisation Good Clinical Practice Guidelines (ICH-GCP) and/or the Declaration of Helsinki, consent to the FOR*Ma*T Master Protocol will be obtained by the FOR*Ma*T trial site-specific research team prior to any study related procedures being performed or study data being collected. All

participants, regardless of whether they are enrolled in the Observational or Intervention Program are required to consent to the FOR*Ma*T Master Protocol. If the participant is under 16 years of age or unable to provide consent (due to severe cognitive impairment, an intellectual disability, or a mental illness, including patients with dementia) then informed consent will be obtained from the participant's parent/legal guardian in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 and FDA guidance (40, 41). Where it is expected that the subject population will not understand English, the written participant information and consent forms will be translated into the anticipated specific language that will be understood in accordance with FDA guidance (40). Participants who are enrolled and are under 16 years of age who turn 16 during the study period will require re-consent on the adult consent form. Telephone consent, re-consent, and re-consent via email will be acceptable (using the informed consent checklist outlined in the FOR*Ma*T Trial Site Manual of Operating Procedures (MoOP)) when face-to-face consent is not possible.

The FOR*Ma*T Master Protocol consent form is required to be signed and dated by the individual providing consent or the participant's parent/guardian where appropriate. A copy of the signed consent form will be given to the participant or their parent/guardian for their records with the original signed consent form(s) stored at the site in the participant's medical record and a copy of the signed consent form(s) stored in the participant's study file. Any Independent Review Board (IRB) / Independent Ethics Committee (IEC) / Human Research Ethics Committee (HREC) approved changes to the trial Master Protocol that affect the participant's rights and/or safety will require the participant's reconsent.

5.4.2 CONSENT TO SPECIFIC APPENDIX INTERVENTION PROGRAM(S), OBSERVATION, DISCOVERY SUB-STUDIES AND INTEGRATED STUDIES

If a participant meets the eligibility criteria for the FOR*Ma*T Appendices and/or specific sub-studies and integrated studies, additional appendix specific consent will be required in accordance with the procedures detailed above in section 5.4.1. Appendix specific consent requirements are detailed in the appendices. If additional sub-studies and/or integrated studies are added or removed from an appendix, or any changes are made to the appendix specific sub-studies and/or integrated studies, IRB/IEC/HREC approval for these changes will be sought. Reconsent will be required for additional trial specific procedures and changes that affect the participant's rights and/or safety.

5.5 CORE TRIAL PROCEDURES

Table 1 lists the special considerations that are applicable to Table 2 Core Trial Procedures and Schedule. Scheduling of core trial procedures (including collection time points) are detailed in Table 2 and in the Schedule of Assessment Tables in the relevant appendix.

Table 1 Special Considerations for Core Trial Procedures and Schedule

Symbol	Definition
A	Trial phases relate to various stages of treatment, participant cohorts and/or FOR*Ma*T substudies as outlined in the relevant appendices.
B	Start of trial phase, Interim and End of trial phase visits are required for FOR*Ma*T participants as per the Schedule of Assessments in the relevant appendices.
C	Participant reconsent is required with IRB/ IEC/HREC approved changes to the protocol that affect participants' rights and/or safety and/or if a child turns 18 years old during the trial and must reconsent as an adult participant.
D	MABS-PD status reviewed in accordance with the ATS criteria as per the Schedule of Assessments in the relevant appendices.
E	Respiratory Samples are required to be collected for FOR*Ma*T participants as per the Schedule of Assessments in the relevant appendices.
F	Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.
G	If participants are unable to provide the minimum requested sputum samples as per the Schedule of Assessments in the relevant appendices, then a BAL sample is to be collected.
H	Adult participants require height to be recorded once only during the study (ideally at the Screening Visit).
I	Adult participants do not require weight to be measured at every visit.
J	Chest CT Scan at early withdrawal visit will only be requested if clinically indicated.
K	Safety/Toxicology Monitoring assessments are to be performed as per the Schedule of Assessments in the relevant appendices.
L	Specific Health Related Quality of Life questionnaires are required to be completed by FOR*Ma*T participants as per the Schedule of Assessments in the relevant appendices.
M	Six-minute walk test to be performed in participants ≥ 18 years of age only.

Table 2 Core Trial Procedures and Schedule

Assessments	Screening Visit	Start of Trial Phase [A]	Interim Visit(s)	End of Trial Phase [A]	Final Outcome	Early Withdrawal Visit
Clinic Visit	✓	✓[B]	✓[B]	✓[B]	✓	✓
Informed Consent [C]	✓					
Review Eligibility	✓					
MABS-PD Status	✓		✓[D]		✓[D]	✓[D]
Randomisation		✓				
Medication Review	✓	✓	✓	✓	✓	✓
Respiratory Sample	✓		✓[E/F/G]	✓[E/F/G]	✓[E/F/G]	✓[E/F]
Height [H] and Weight [I]	✓	✓	✓	✓	✓	✓
Spirometry	✓		✓	✓	✓	✓
Chest Computed Tomography	✓				✓	✓[J]
Physical Examination	✓	✓	✓	✓	✓	✓
Safety/Toxicology Monitoring [K]	✓	✓	✓	✓	✓	✓
Health Related Quality of Life Questionnaires [L]	✓		✓	✓	✓	✓
Costs Questionnaire	✓		✓	✓	✓	✓
Six-minute walk test [M]	✓		✓		✓	✓

5.5.1 RESPIRATORY SAMPLES FOR MICROBIOLOGY ASSESSMENT

Respiratory samples are to be collected for participants enrolled in either the Observational Cohort or the Intervention Program. The respiratory samples will be collected as per the relevant appendix.

Collection of respiratory sample(s) for the identification of MABS at screening may not be required if recent sample(s) have been provided by the participant, (within the specified time frames prior to screening stated in the relevant appendix) have been stored and are available as per the local laboratory guidelines (standard of care) to identify and diagnose MABS. If the screening sample has been stored, then the sample will be analysed retrospectively once consent to participate in the FOR*Ma*T trial is given.

All respiratory samples collected for MABS identification are to be sent to local pathology as per local guidelines.

Acceptable methods for collecting a respiratory sample for the microbiological assessment of MABS include:

5.5.1.1 Expectorated Sputum

Participants able to expectorate sputum are required to provide a 1ml expectorated sputum sample in a specimen jar or separate specimen jars if multiple samples are obtained. Specimen jar(s) are to be labelled and sputum samples processed in accordance with site specific pathology requirements for the detection of MABS.

5.5.1.2 Induced Sputum

Sputum induction is indicated for participants incapable of expectorating sputum. Prior to sputum induction a bronchodilator can be administered to minimise bronchospasm. Sputum induction should be conducted as per local guidelines.

5.5.1.3 Bronchoalveolar Lavage (BAL)

A BAL is indicated if an expectorated or induced sputum sample is unobtainable. Where possible, a six-lobe lavage should be collected. If six-lobe lavage is not feasible, a minimum two-lobe lavage from the area most affected on chest CT scan should be collected. All BAL samples are to be processed in accordance with site specific procedures.

5.5.1.4 Lung/Airway Biopsy

Lung/airway biopsy is only acceptable for the initial MABS diagnosis, but not as a routine method for MABS microbiological assessment throughout the trial.

Please note, cough swabs are not an acceptable respiratory sampling technique for the FORMaT Trial.

5.5.2 WHOLE GENOME SEQUENCING

All MABS-positive isolates cultured from the respiratory samples collected in the FOR*Ma*T Trial will be stored and deoxyribonucleic acid (DNA) extracted according to FOR*Ma*T DNA Extraction SOP. The extracted DNA from all samples will undergo whole genome sequencing (WGS) according to the FOR*Ma*T WGS SOP and/or local laboratory standard procedures). The genetic data generated from the WGS of MABS DNA will be stored by the approved collaborator in a mycobacterial genomic library.

5.5.3 CHEST COMPUTED TOMOGRAPHY

Chest CT will be performed at screening and at final outcome. If at screening a recent chest CT scan has already been performed (within 6 months of screening), as part of the standard of care diagnosis of MABS an additional chest CT will not be required. Any additional chest CT scans performed for clinical purposes at other time points while the participant is enrolled in the FOR*Ma*T Trial will also be collected as an outcome measure. Once consent is provided for the FOR*Ma*T trial these images will be accessed from the participant's medical records and included in trial analysis.

The site and scanner specific protocols for chest CTs as well as the evaluation of trial CTs are described in the relevant appendix. The site and scanner specific protocols are recommended to be used to perform the core trial chest CT scans where feasible at sites that are certified and trained to use the site and scanner specific protocols.

5.5.4 SPIROMETRY

Spirometry will be measured in all participants from three years of age in accordance with the American Thoracic Society and European Respiratory Society standards (42) and according to the appendix specific schedule of assessments where possible. The multi-ethnic global lung initiative (GLI) reference values for all spirometry indices will be applied to participants 3-95 years of age (43).

5.5.5 PHYSICAL EXAMINATION AND VITAL SIGNS

A physical examination of all body systems and vital signs will be completed in accordance with appendix specific schedule of assessments (where possible) and with assessment of any adverse event(s). A physical examination is to be completed by a site investigator or delegate. The physical examination includes a review of the following systems: head/neck/thyroid, eyes/ears/nose/throat, respiratory, cardiovascular, lymph nodes, abdomen, skin, musculoskeletal and neurological systems. Breast, anorectal, and genital examinations will be performed only when medically indicated. After screening, any new clinically significant abnormal findings in physical examinations will be reported as adverse events (AEs), see section 5.8.

Vital signs include blood pressure (systolic and diastolic), temperature, pulse rate and respiration rate will be assessed following a 5-minute rest in the seated or supine position.

5.5.6 SIX MINUTE WALK TEST

There are several modalities available for the objective evaluation of functional exercise capacity. The 6-minute walk test (6MWT) is a clinical exercise test that is tolerated by those with chronic respiratory disease and is reflective of activities of daily living. This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes (6-minute walk distance (6MWD)). The strongest indication for the 6MWT is to measure response to medical interventions in patients with moderate to severe heart or lung disease. Most 6MWTs will be performed before and after an intervention to determine whether the patient has experienced a clinically significant improvement. The change in 6MWD is expressed as an absolute value.

The 6MWT is to be conducted in adult participants only and performed according to the protocol in the ATS Statement: Guidelines for the Six-Minute Walk Test and/or local standard clinical practice guidelines (44). The ATS guideline includes absolute contraindications, relative contraindications and precautions to completing the test.

5.5.7 MEDICATION REVIEW

Current medication use including both prescription, over-the counter medication, herbal remedies and preparations will be recorded from time of consent until final outcome visit as per the schedule of assessments in the relevant appendix. Trade drug names, start and stop dates, dose, route, and indications for use should be recorded in the relevant Case Report Form (CRF).

5.6 QUALITY ASSURANCE (QA)

The FOR*Ma*T trial will be conducted in accordance with the current approved Master Protocol and Appendices. To improve Master Protocol adherence as well as ensure complete data entry, the following QA procedures will be implemented:

1. A FOR*Ma*T trial start-up meeting for research coordinators and site investigators prior to the submission of ethical approval and any other required approvals.
2. A Site Initiation Visit (SIV) once the FOR*Ma*T site has obtained ethics and any other required approvals, but prior to participant recruitment.
3. A CRF Completion Guide detailing all the data to be collected in the CRFs/electronic CRFs (eCRFs).
4. Regular and timely validation of entered data, queries and corrections by the FOR*Ma*T Trial Management Team.
5. Trial monitoring (onsite, remote, central, and/or local) as described in Section 7.7 Monitoring and according to the FOR*Ma*T Monitoring SOP and Monitoring Plan.

5.6.1 MICROBIOLOGY AND DNA EXTRACTION QA

Microbiology is of key importance and samples are processed in each of the participating countries in the local mycobacterial reference laboratories (MRLs). The FOR*Ma*T trial management team will work collaboratively with each MRL to ensure standard approaches for sample processing are followed where feasible.

5.7 NOTES ON SPECIFIC TRIAL VISITS

The timepoint definitions outlined below are overarching concepts that are applicable to each of the intervention studies described within the relevant appendices.

5.7.1 SCREENING

Screening is a window of time between the Date of Consent and the date of first randomisation in which a participant's eligibility into the trial is assessed and screening assessment data is collected according to the schedule of assessments in the relevant Appendices.

5.7.2 TIME POINT START TREATMENT (TPST)

Participants in the Intervention program(s) will commence on the treatment arm allocated by randomisation. Time Point Start Treatment (TPST) is defined as the date the participant undergoes the randomisation allocating a treatment arm. Each separate randomisation will have its own TPST as defined in the relevant appendix.

5.7.3 TIME POINT FINAL (TPF)

Participants will be reviewed at the end of each trial phase (Time Point Final (TPF)) and assessments will be performed as per the appendix specific schedule of assessments. Each treatment phase that follows a randomisation will have its own separate TPF as defined in the relevant appendix.

5.7.4 EARLY WITHDRAWAL VISIT

Participants who are withdrawn or who withdraw from the trial will be asked to attend an Early Withdrawal Visit. Where practical, all efforts should be made for the participants to complete the early withdrawal trial procedures detailed in the appendix specific schedule of assessments. Where possible, if there are any ongoing AEs these should be followed and monitored in accordance with the FOR*Ma*T Master Protocol irrespective of withdrawal from study.

5.7.5 UNSCHEDULED VISIT

Study visits that are conducted in addition to those listed in the appendix specific assessment schedule are known as Unscheduled Visits. If the Investigator deems that a participant should attend the study site to follow up an AE, repeat laboratory testing or any other study-related reason, then this visit should be documented in the FOR*Ma*T Research Electronic Data Capture (REDCap) database via the Unscheduled Visit CRF.

5.8 SAFETY MONITORING

Occurrence of AEs due to the underlying MABS infection and treatments used are well recognised and expected during the FOR*Ma*T trial. As such, AE monitoring procedures outlined in the relevant appendices will be implemented for participants enrolled in the Intervention Program or Observational Cohort. Toxicology monitoring procedures will be implemented for Intervention Program participants.

5.8.1 DEFINITION OF ADVERSE EVENTS

An AE is any untoward medical occurrence in a clinical investigation of a participant enrolled in FOR*Ma*T who may or may not be treated with an investigational product.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the participant's medical record or source documents. AEs will be recorded in the AE or SAE eCRF. AEs will be described by duration (start and stop dates), severity, outcome, treatment, and relationship to study medication (where relevant), or if unrelated, the cause.

AEs will be collected from the time of informed consent until the final study visit.

All AEs will be reviewed by the FOR*Ma*T trial Pharmacovigilance Team and coded in accordance with the CTCAE version 5. For any AE that is unable to be categorised in accordance with the CTCAE, the independent data safety monitoring board (iDSMB) will be required to review and categorise the AE.

5.8.2 ATTRIBUTION OF ADVERSE EVENTS IN THE FOR*MA*T TRIAL

The relationship, or attribution, of an AE to the trial therapies will be determined by the Investigator. The relationship of the AE to the investigational product should be coded according to the following definitions:

Unrelated: The adverse event is clearly not related to the investigational product.

Unlikely: The adverse event is doubtfully related to the investigational product.

Possibly: The adverse event may be related to the investigational product.

Probably: The adverse event is likely related to the investigational product.

Definitely: The adverse event is clearly related to the investigational product.

5.8.3 DEFINITION OF SERIOUS ADVERSE EVENTS

SAEs will be defined as any untoward medical occurrence that:

1. Results in death.
2. Is considered life threatening (i.e., in the view of the Investigator the adverse experience places the participant at immediate risk of death from the reaction, as it occurred; it **does not** include a reaction that, had it occurred in a more severe form, might have caused death).

3. Requires hospital admission or prolongation of an existing hospitalisation.
4. Results in persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions).
5. Is a congenital anomaly/birth defect.
6. Is an important medical event (i.e., when based upon appropriate medical judgment, the adverse experience may jeopardise the participant and may require medical or surgical intervention to prevent one of the above listed outcomes).

5.8.4 REPORTING OF SAFETY EVENTS

Study sites will document all SAEs that occur after informed consent is obtained until the final study visit in an SAE Report (whether or not related to study treatment). All SAEs will be reported to the FOR*Ma*T Trial Management Team within 24 hours of becoming aware of the event. Exclusions to the expedited SAE reporting include:

1. Infective exacerbation of an underlying lung condition requiring hospitalisation or home intravenous antibiotic treatment does not require expedited reporting to the FOR*Ma*T Trial Management Team;
2. Any planned and/or elective hospital admissions does not require expedited reporting;
3. Any planned and/or elective medical procedures does not require expedited reporting.

The site will complete and submit an initial SAE report form via the FOR*Ma*T trial database. Any follow up information about the SAE is to be reported on an SAE follow up report form and submitted in the FOR*Ma*T trial database as soon as relevant information is available.

All SAEs will be reviewed by the FOR*Ma*T trial Pharmacovigilance Team and coded in accordance with the CTCAE version 5. For any SAE that is unable to be categorised in accordance with the CTCAE, the iDSMB will be required to review and categorise the SAE.

Suspected unexpected serious adverse reactions (SUSARs) will be reported on the SAE form via the FOR*Ma*T Trial database. Urgent safety measures (USMs) and significant safety issues (SSIs) will be reported via email to the FOR*Ma*T Trial Management Team and submitted in the FOR*Ma*T trial database (if the USM or SSI is also an SAE). USMs, SSIs, and SUSARs are to be reported by the FOR*Ma*T Trial Management Team and/or the local site investigator to the relevant regulatory bodies within their required timeframes.

The FOR*Ma*T Trial Management Team, as the Sponsor delegate, will notify the Sponsor of all SUSARs, USMs and SSIs as required and submit an Annual Safety Report to the Sponsor. Site investigators are required to report all AEs that are related to study intervention(s) and/or interaction with study intervention(s) within 2 weeks of becoming aware of the event. All other AEs and laboratory abnormalities that are not related to study intervention(s) or interactions with study intervention(s) are to be reported to the FOR*Ma*T Trial Management by the end of specified TPF outlined in the relevant appendix.

Refer to FOR*Ma*T Safety Monitoring and Reporting SOP for detailed procedure(s) for safety reporting.

5.8.5 CODING OF ADVERSE EVENTS FOR ANALYSIS

CTCAE, version 5.0 will be applied for the database coding of all AEs including SAEs but only those categorised as at least "possibly", "probably", or "definitely" related to study medications and/or interactions between study medications and concomitant medications will be used to assess tolerance. Coding for analysis will be completed by the FOR*Ma*T Pharmacovigilance Team. Refer to section 4.6 for the definition of tolerance. Coding and grading is performed in accordance with the FOR*Ma*T Coding and Grading Work Instruction.

If an AE is unable to be coded and/or graded by the pharmacovigilance team or if coding/grading cannot be agreed upon, the iDSMB will be consulted to assess and code and/or grade the AE.

5.8.6 TOXICOLOGY THRESHOLDS

Toxicology monitoring will use the thresholds that match the grading from CTCAE, Version 5.0 as described in Table 3 and toxicity monitoring procedures for trial interventions will be as specified in the relevant intervention appendix. New interventions will require a separate toxicity monitoring plan including any requirements for central laboratory testing of samples.

5.9 PARTICIPANT WITHDRAWAL AND DISCONTINUATION OF TREATMENT

5.9.1 WITHDRAWAL OF CONSENT

All participants are free to withdraw from the study at any time, with or without a specified reason and without prejudice. Where possible, the participant or the participant's parent/legal guardian is required to sign the withdrawal of consent form, formally documenting the withdrawal process including the reason for withdrawal if they choose to provide this information. In the event of a participant withdrawing from the trial, an early withdrawal visit should be completed within the specified window of time from withdrawal if the participant is agreeable. If for any reason the participant is unable to complete the early withdrawal visit this should be noted on the Study Completion eCRF.

5.9.2 DISCONTINUATION OF TREATMENT

Participants enrolled in the Intervention Program can discontinue treatment at any time during the trial if:

- The participant is no longer able to comply with the FOR*Ma*T Master Protocol and relevant appendix, including completing required study assessments for safety requirements.
- The site investigator believes that treatment is no longer in the participant's best interests (due to safety or tolerance concerns).
- The participant no longer wants to continue treatment.

Participants discontinuing treatment prematurely but who do not withdraw consent or assent will be encouraged to continue with the trial according to the specific appendix intervention study and schedule of assessments in accordance with the relevant appendices with the final outcome respiratory sampling assessments to be prioritised.

Table 3: Safety critical monitoring thresholds

Investigations					
	Grade				
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Alanine aminotransferase increased	>ULN - 3.0 x ULN if baseline was normal, ***or;*** 1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 - 5.0 x ULN if baseline was normal, ***or;*** >3.0 - 5.0 x baseline if baseline was abnormal.	>5.0 - 20.0 x ULN if baseline was normal, ***or;*** >5.0 - 20.0 x baseline if baseline was abnormal.	>20.0 x ULN if baseline was normal, ***or;*** >20.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an increase in the level of alanine aminotransferase (ALT or SGPT) in the blood specimen.					
Anaemia	Haemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN – 6.2 mmol/L; <LLN – 100 g/L.	Hgb <10.0 – 8.0 g/ dL; <6.2 – 4.9 mmol/L; <100 - 80 g/L.	Hgb <8.0 g/ dL; <4.9 mmol/L; < 80 g/L; transfusion indicated.	Life-threatening consequences; urgent intervention indicated.	Death
Definition: A disorder characterized by a reduction in the amount of haemoglobin in 100 ml of blood. Signs and symptoms of anaemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.					
Aspartate aminotransferase increased	>ULN - 3.0 x ULN if baseline was normal, ***or;*** 1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 - 5.0 x ULN if baseline was normal, ***or;*** >3.0 – 5.0 x baseline if baseline was abnormal.	>5.0 - 20.0 x ULN if baseline was normal, ***or;*** >5.0 – 20.0 x baseline if baseline was abnormal.	>20.0 x ULN if baseline was normal, ***or;*** >20.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an increase in the level of aspartate aminotransferase (AST or SGOT) in the blood specimen.					
Blood bilirubin increased	>ULN - 1.5 x ULN if baseline was normal, ***or;*** >1.0 – 1.5 x baseline if baseline was abnormal.	>1.5 - 3.0 x ULN if baseline was normal, ***or;*** >1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 – 10.0 x ULN if baseline was normal, ***or;*** >3.0 – 10.0 x baseline if baseline was abnormal.	>10.0 x ULN if baseline was normal, ***or;*** >10.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an abnormally high level of bilirubin in the blood. Excess bilirubin is associated with jaundice.					
Electrocardiogram QT corrected interval prolonged	Average QTc 450 - 480 ms	Average QTc 481 - 500 ms	Average QTc >= 501 ms; >60 ms change from baseline.	Torsade de pointes; polymorphic ventricular tachycardia; signs/symptoms of serious arrhythmia.	-
Definition: A disorder characterised by Electrocardiogram T wave amplitude changes.					

Investigations					
Grade					
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Hearing impaired	**Adults** enrolled on a Monitoring Program (on a 1, 2, 4, 3, 6, and 8 kHz audiogram): Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear; **Adults** not enrolled on a Monitoring Program: Subjective change in hearing in the absence of documented hearing loss; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift >20 dB hearing loss (HL) (i.e., 25 dB HL or greater); sensorineural hearing loss (SNHL) above 4 kHz (i.e., 6 or 8 kHz) in at least one ear.	**Adults** enrolled on a Monitoring Program (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear; **Adults** not enrolled on a Monitoring Program: Hearing loss with hearing aid or intervention not indicated; limiting instrumental ADL; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift >20 dB at 4 kHz in at least one ear.	**Adults** enrolled on a Monitoring Program (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear; therapeutic intervention indicated; **Adults** not enrolled on a Monitoring Program: Hearing loss with hearing aid or intervention indicated; limiting self care ADL; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Hearing loss sufficient to indicate therapeutic intervention, including hearing aids; threshold shift >20 dB at 2 to < 4 kHz in at least one ear.	**Adults**: Decrease in hearing to profound bilateral loss (absolute threshold >80 dB HL at 2 kHz and above); nonservicable hearing. **Pediatric**: Audiologic indication for cochlear implant; > 40 dB HL (i.e., 45 dB HL or more); SNHL at 2 kHz and above.	-
Definition: A disorder characterized by partial or complete loss of the ability to detect or understand sounds resulting from damage to ear structures					
Leukocytosis	-	-	>100,000/mm^3	Clinical manifestations of leucostasis; urgent intervention indicated.	-
Definition: A disorder characterised by laboratory results that indicate an increased number of white blood cells in the blood.					
Lymphocyte count decreased	<LLN - 800/mm^3; <LLN - 0.8 x 10^9/L	<800 – 500/mm^3; <0.8 - 0.5 x 10^9/L	<500 – 200/mm^3; <0.5 - 0.2 x 10^9/L	< 200/mm^3; < 0.2 x 10^9/L	-

Investigations					
	Grade				
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Definition: A finding based on laboratory test results that indicate a decrease in the number of lymphocytes in a blood specimen.					
Neutrophil count decreased	<LLN - 1500/mm^3; <LLN - 1.5 x 10^9/L	<1500 - 1000/mm^3; <1.5 - 1.0 x 10^9/L	<1000 - 500/mm^3; <1.0 - 0.5 x 10^9/L	<500/mm^3; < 0.5 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the number of neutrophils in a blood specimen.					
Platelet count decreased	<LLN - 75,000/ mm^3; <LLN - 75.0 x 10^9/L	<75,000 - 50,000/ mm^3; <75.0 - 50.0 x 10^9/L	<50,000 - 25,000/ mm^3; <50.0 - 25.0 x 10^9/L	<25,000/ mm^3; <25.0 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the levels of pancreatic enzymes in a biological specimen.					
Tinnitus	Mild symptoms; intervention not indicated.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by noise in the ears, such as ringing, buzzing, roaring or clicking.					
Vertigo	Mild symptoms.	Moderate symptoms; limited instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by a sensation as if the external world were revolving around the patient (objective vertigo) or as if he himself were revolving in space (subjective vertigo).					
Vestibular Disorder	-	Symptomatic; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by dizziness, imbalance, nausea and vision problems.					
White blood cell decreased	<LLN - 3000/ mm^3; <LLN – 3.0 x 10^9/L	<3000 - 2000/ mm^3; <3.0 – 2.0 x 10^9/L	<2000 - 1000/ mm^3; <2.0 – 1.0 x 10^9/L	<1000/ mm^3; <1.0 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the number of white blood cells in a blood specimen.					

Adapted from CTCAE, version 5.0

5.10 DATA MANAGEMENT

5.10.1 DATA COLLECTION, ENTRY AND STORAGE

Trial data will be collected from various sources including, but not limited to, medical records, participant questionnaires, output from lab results, study assessments, correspondence and CT scans. These documents and any documents where data is first recorded for a participant will form the source documents. FOR*Ma*T trial site staff will be trained in the collection of the required data during the site initiation visit and data collection instructions will be stored in the site's electronic Investigator Site File (eISF).

The site investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of all trial data reported. All source documents are to be viewable in a neat, legible manner to ensure accurate interpretation of data. The site investigators will maintain adequate case histories of trial participants, including source documentation.

Sites will enter the data required for the trial directly into an eCRF located in a study specific Research Electronic Data Capture (REDCap) FOR*Ma*T database. Data for the eCRF will be obtained directly from the medical record or source documents.

Due to the adaptive design of the trial all **data is to be entered within 14 days from the specified TPF as defined in the relevant appendix unless otherwise stated (e.g. expedited AE/SAE reporting) Data** cleaning will be performed at regular intervals for the trial to ensure that the data are cleaned and ready for interim analyses.

The REDCap FOR*Ma*T database will be hosted on Murdoch Children's Research Institute (MCRI) infrastructure and is subject to the same security and backup regimen as other systems at the MCRI (e.g. the network file servers). Data is backed up nightly to a local backup server, with a monthly backup taken to tape and stored offsite. All data transmissions between users and the REDCap server are encrypted. Regular data quality checks, such as automatic range checks, will be performed to identify data that appear inconsistent, incomplete, or inaccurate.

Access to REDCap is managed by the system administrator. The permissions granted to each user within each REDCap project is controlled by and is the responsibility of the FOR*Ma*T trial management team. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. REDCap maintains an audit trail of data created/updated/deleted that is accessible to project users that are granted permission to view it.

The SiteDocs Portal is an online tool hosted by TrialDocs Clinical Research Document Management and The University of Queensland (UQ) that is compliant with FDA requirements and General Data Protection Regulation (GDPR) which will securely host and track all key trial documents for the FOR*Ma*T Trial, including the electronic Trial Master File (eTMF). SiteDocs includes features such as version control, document protection, temporary remote monitoring access, alerts, archiving and a full audit trail of data and users.

Trial sites will have individual secure access to their site's eISF via SiteDocs, where protocols, templates, trial documents and correspondence will be hosted. Sites will be able to collaborate with the Sponsor and/or their delegate to upload site-specific clinical trial documents or deidentified participant documents to their eISF, receive notifications of changes and easily access the most up-to-date document versions.

Trial sites will have access to their own separate site-specific Portal for SiteDocs where they can upload and manage identifiable source documents. Sites will be responsible for granting access to their site-specific Portal, including temporary access to external monitors.

5.10.2 DATA STORAGE AND RETENTION

Each site is required to maintain source documents for a minimum of 15 years post completion of the trial. If site specific requirements or relevant legislation dictate data retention for periods longer than 15 years, trial sites will be required to adhere to these requirements.

6 STATISTICAL ANALYSIS PRINCIPLES

This section of the Master Protocol provides an outline and summary of the general statistical methods and principles used for FOR*Ma*T. The detailed statistical methods and sample size simulations relevant to each of the Intervention Programs and discovery studies are outlined in the relevant appendix.

6.1 BAYESIAN ANALYSIS AND BAYESIAN ADAPTIVE RANDOMISATION (BAR)

Interim monitoring for the Intervention Program in the FOR*Ma*T trial will use a Bayesian analysis approach (45). This approach will calculate the (posterior) probability of an intervention being found to be superior to the reference arm during the trial. As new data is generated the probability will be updated. This updating will occur at the time of the meetings of the iDSMB, who will have access to blinded and unblinded data and will ask the Trial Steering Committee (TSC) to ratify the updated blinded probabilities. Initially there are no plans to stop any arms of the study arms due to superiority, but such rules may be established as the trial continues and will be detailed in the relevant appendices. The iDSMB may however make a recommendation to the TSC about stopping current interventions if they show poor promise or futility. Stopping rules will be defined to guide the use of the posterior probabilities.

Adaptive randomisation allows intervention arm allocation ratios to be adapted based on interim analyses undertaken during the trial to favour the intervention arm with the highest posterior probability of success (46). This approach can lead to increased efficiency of the trial and reduce patient exposures to less promising or more toxic therapies compared with non-adaptive randomisation. In the FOR*Ma*T trial, BAR will be used for updating allocation probabilities in randomisations when there are more than two interventions being compared at any one of the randomisation stages. BAR will be implemented after every 60 participants have been randomised so that there is sufficient information available to determine the adaptation. Success of an intervention (and hence the adaptations) will be determined by the primary outcome; microbiological clearance with tolerability (refer to sections 1.1 Synopsis and 4.6 Trial Endpoints for definitions of clearance and tolerance).

6.2 RANDOMISATION

6.2.1 BLINDING OF TREATMENT ALLOCATION

The FOR*Ma*T trial may include placebo controlled double blind randomised interventions in the future, but the initial Intervention Program detailed in Appendix A1 is randomised and open label.

Each stage of the FOR*Ma*T intervention program(s) requires randomisation and is detailed in the relevant appendix. The allocated treatment must start on the same day as the randomisation where feasible, or as soon as possible after the randomisation has been performed. Any delays to starting an allocated treatment after randomisation has occurred must be reported to the FOR*Ma*T Trial Management Team.

6.3 MINIMISATION

To ensure balance between arms in important patient characteristics, randomisation at the different stages of the trial will use minimisation with a random element. Minimisation is a dynamic randomisation approach used in clinical trials to balance allocation to treatment arms with respect to a number of important stratification factors. In minimisation, the first participant is allocated to their treatment arm at random. Subsequent participants are assigned to a treatment arm by first selecting the preferred arm that would best improve the balance of participants across the arms based on the stratification variables of interest in terms of the numerical difference in the sample size in each of the treatment arms across all of the stratification factors (47, 48). The preferred arm is then selected with a probability of 0.7, with rest of the probability split between the alternative arms.

Following updating the randomisation allocations through BAR, minimisation will be conducted using Biased Coin Minimisation (BCM) (49). Under this method, the same methodology as standard minimisation will be used to determine the preferred intervention, but the randomisation probabilities will be altered to reflect the minimisation probabilities and the updated allocation ratios from the BAR as detailed in Han et al (49).

Randomisation will be conducted electronically through the trial database following completion of all the specific required data entry by the study team at each site. Participants will be randomised according to the stratification criteria described below using to the weights specified for each factor:

1. Macrolide resistance*: Yes or no (weight = 50% in randomisations 1 and 2 (Randomisation-Short Intensive (R-SI) and Randomisation-Prolonged Intensive or Immediate Consolidation (R-PI/IC), respectively), 25% in randomisation 3 (Randomisation-Consolidation (R-Con))).

   Any of these measurement methods are acceptable for defining macrolide resistance (in order of preference):

   a. Inducible at 14 days or constitutive at 3 days, and/or;
   b. *Erm*(41) status: Functional or dysfunctional, and/or;
   c. MABS subspecies: *M. a. abscessus* & *M. a. bolletii* combined or *M. a. massiliense*.
2. Age: <12 years, 12-30 years and >30 years of age (weight = 20%).
3. Sex: Male or Female (weight = 7.5%).
4. Location: Asia Pacific as one stratum (includes Australia, New Zealand, Singapore and other Asian Pacific countries), United Kingdom and Republic of Ireland as one stratum, Europe as another stratum (includes

Denmark, France, Netherlands), and Canada and the Americas as one stratum (weight = 7.5%). Parts of the world not listed above can be added into the regions based on closest proximity to the regions longitudinally.

5. Cystic Fibrosis Status: Yes or no (weight = 7.5%).
6. Mixed NTM infections at enrolment: Yes or no (weight =7.5%).
7. MABS positive culture (at initial randomisation to intensive therapy (R-SI) and for R-PI/IC all participants will have a positive culture, so this factor will not be required. However, it will be required for R-Con): Yes or no (weight = 25%).

6.4 ADDING AND STOPPING INTERVENTIONS

New interventions, which may include new therapeutics or new timelines either during the intensive or consolidation phase, may be added as a treatment arm either for the intensive or for the consolidation phase as determined by the trial Drug and Intervention Selection committee (DISC) with approval of the TSC. No new therapies will be added until at least 60 subjects have completed the trial phase associated with the proposed new intervention.

Interventions may be stopped early due to a lack of benefit at interim analyses. Interim analyses will be conducted after every 60 participants have completed the trial phase. The statistical team, acting in confidence, will present the results from such analyses only to the iDSMB who will provide guidance around stopping particular interventions. Pre-defined triggers for stopping an intervention will be specified for both intensive and consolidation interventions separately, and for the combination of intensive and consolidation. If a single intervention, or combination of interventions, has less than a 0.01 posterior probability of being an optimal intervention in that phase then that intervention will be regarded as inferior and should be recommended to be discontinued.

For new interventions in the future, if an intervention has a high posterior probability (to be pre-specified prior to the new intervention being added) of being an optimal therapy this invention will be considered as superior, in which case the iDSMB might recommend that the randomisation stage be stopped for superiority. Stopping rules for superiority will be detailed in the relevant section of the appendices. Following the iDSMB meeting, the recommendations from the iDSMB will be notified to the Trial Management Committee (TMC) and TSC. The TSC will consider the recommendations and make a decision regarding the potential to stop the randomisation stage for superiority. If any interventions are stopped, the TMC will have the responsibility of developing a public disclosure as is practical through presentation of results and publications.

6.5 SEAMLESS PHASE II TO PHASE III

The FOR*Ma*T platform trial provides a resource to enable promising therapies to be more rapidly evaluated and has the potential to facilitate seamless transition from Phase II to a Phase III study. Initially the platform will support a phase II study, but new interventions being evaluated in the future may be considered for the potential to move seamlessly from this Phase II study to a Phase III (50). The principles guiding this move will include planning for the potential of Phase III in the design of the Phase II study and with agreement between the Platform trial team and sponsors of the new therapy around management and access to data. The move to a Phase III trial will be guided by a pre-defined threshold for the probability of a successful intervention during phase III, which would need to be

established in planning the Phase II trial and agreed to by regulatory authorities for pivotal studies. If a seamless transition to Phase III is considered feasible, the TMC along with the trial statistics team will provide the iDSMB and TSC with a Phase III proposal, and if this is approved by the iDSMB and TSC then further recruitment to the target number determined would be undertaken. This transition would require approval from relevant IRB / IEC / HREC and any other relevant regulatory bodies before implementation.

6.6 SIMULATIONS AND STATISTICAL POWER

The design (including stopping rules**)** and sample size of each intervention program will be informed by simulations using Monte Carlo methods to give a range of power according to different scenarios and taking into account the probabilities of variable responses to intensive and/or consolidation interventions. Simulations will be updated with the addition of any new interventions. The details of these simulations can be found in the relevant appendix.

6.7 GENERAL ANALYSIS PRINCIPLES

The analyses will be conducted according to the statistical analysis methods described in the relevant appendices, statistical principles appendix and the statistical analysis plan.

7 STUDY OVERSIGHT

7.1 OVERVIEW

The FOR*Ma*T Trial will have several committees established to provide oversight of the FOR*Ma*T Trial.

7.2 TRIAL STEERING COMMITTEE

The TSC is the executive decision-making group and provides overall supervision of the FOR*Ma*T trial. The Terms of Reference (available on request) outlines the roles and responsibilities of the TSC members. Membership to the TSC will consist of independent and non-independent members from a variety of backgrounds including clinical and statistical as well as a member from the general community. The Chair of the TSC will be independent of the FOR*Ma*T Trial.

7.3 INDEPENDENT DATA SAFETY MONITORING BOARD

The iDSMB will review interim analyses, monitor for effectiveness and safety, along with trial conduct. They will meet prior to the commencement of the trial at least annually and at the time of interim analysis after every 60 participants have completed each stage of the trial. The iDSMB will provide advice to the TSC and the Trial Management Committee (TMC). The iDSMB Charter (available on request) outlines the roles and responsibilities of the iDSMB members. Membership to the iDSMB will consist of clinicians and statisticians who are independent of the FOR*Ma*T Trial.

The iDSMB will be responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review and voting procedures prior to initiating any data review. The iDSMB will be responsible for

maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it.

The iDSMB will review each version of the FOR*Ma*T Master Protocol (including any new or updated appendices). During the trial, the iDSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, iDSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The iDSMB will also assess the performance of overall study operations and any other relevant issues, as necessary.

Items to be reviewed by the iDSMB may include:

- Interim/cumulative data for evidence of study-related AEs.
- Interim/cumulative data for evidence of efficacy and futility according to pre-established statistical guidelines.
- Data quality, completeness and timeliness.
- Performance of individual centres/countries.
- Adequacy of compliance with goals for recruitment and retention, including those related to the participation of women and minorities.
- Adherence to the protocol.
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol deviations, unmasking, etc.).
- Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.
- AEs that are unable to be categorised by the FOR*Ma*T Pharmacovigilance Team in accordance with the CTCAE.

The iDSMB will conclude each review with their recommendations to the TSC and the FOR*Ma*T Chief Investigator (CI) as to whether the study should continue without change, be modified, or terminated. Recommendations regarding modification of the design and conduct of the study could include:

- Modifications of the Master Protocol (including appendices) based upon the review of the safety data.
- Suspension or early termination of the study or of one or more study arms because of serious concerns about subjects' safety, inadequate performance or rate of enrolment.
- Suspension or early termination of the study or of one or more study arms because study objectives have been obtained according to pre-established statistical guidelines.
- Corrective actions regarding a study centre whose performance appears unsatisfactory or suspicious.

Confidentiality must always be maintained during all phases of iDSMB review and deliberations.

Meeting sessions can be either open or closed at the request of the iDSMB Chair or may involve periods of each. The lead investigators of FOR*Ma*T can attend the open session along with other investigators as requested.

The closed session will only include the iDSMB members who have voting rights and they will formulate recommendations regarding the study to the TSC. The FOR*Ma*T CI may be invited to the closed session to present an update of the trial but will not be present for any other agenda items.

Reports will be provided to iDSMB that include an open report and a closed report. Reports for the iDSMB will be prepared by the Clinical Epidemiology and Biostatistics Unit at MCRI.

Open reports will include information on the study conduct such as accrual data, demographics and baseline characteristics, site performance and protocol compliance and quality control issues along with general toxicity and safety data presented across the study as a whole with no reference to treatment arm.

Closed reports will include the same data as the open reports as well as data on efficacy outcomes at the time of each interim analysis (every 60 patients in randomisation levels with more than two interventions) and will be presented by (masked) treatment arm.

Reports from the iDSMB will provide details of any items that require urgent action as well as any recommendations made by the iDSMB and will be provided to the FOR*Ma*T CI and the TSC and notified to other participating organisations as well as to the IRB/IEC/HRECs involved.

7.4 TRIAL MANAGEMENT COMMITTEE

The Trial Management Committee (TMC) is responsible for reviewing the day-to-day management of the trial and assist the FOR*Ma*T project team with any issues that arise during the study, as well as provide advice to the TSC and iDSMB where relevant. The TMC will include the FOR*Ma*T Trial CI, the Senior Trial Project Managers, appropriate representatives across all the key areas (Statistics, Database, Pharmacovigilance, Drug and Intervention Selection Committee (DISC), and Microbiology), and a representative from each participating country or region. To facilitate and ensure adequate communication across the large number of team members and a wide geographic spread, information from the TMC may be shared on the FOR*Ma*T trial website and SiteDocs. The TMC will report to the TSC. Day-to-day management will be overseen by the FOR*Ma*T CI, the senior trial project managers and the core trial clinical lead physicians. A Terms of Reference (available on request) outlines the roles and responsibilities of the TMC.

7.5 FOR*MA*T PHARMACOVIGILANCE TEAM

All AEs and SAEs will be reviewed by the FOR*Ma*T Pharmacovigilance Team and coded in accordance with the CTCAE, version 5.0. Members of the FOR*Ma*T Pharmacovigilance Team include:

- Senior Clinical Pharmacist (Lead).
- Non-independent senior paediatric physician.
- Non-independent senior adult physician.
- FOR*Ma*T Trial database representative (if required).
- Statistician (If required).

For further detail refer to the Pharmacovigilance Team Terms of Reference (available on request).

7.6 DRUG AND INTERVENTION SELECTION COMMITTEE (DISC)

The DISC includes the DISC chair, FOR*Ma*T CI, FOR*Ma*T Trial Management Team, a representative from each participating country and experts external to the trial. The role of the DISC includes assessment of new intervention or therapies to be considered for inclusion in a new Intervention Program. The DISC will provide a report supported by the Statistical Analysis Team and reviewed by the trial management team, for consideration by the TSC and if approved will lead to a new therapy program being included. For further detail refer to the DISC Terms of Reference (available on request).

7.7 CONSUMER ADVISORY GROUP (CAG)

The purpose of the FOR*Ma*T consumer advisory group (CAG) is to provide a consumer and community perspective to various aspects of the FOR*Ma*T trial. This includes advice on participant facing documents such as consent forms, future research plans including proposals for new treatments to be added to the study, grant applications requesting funding support for the study, and lay summaries of the study and any study results. Members of the CAG may also be invited to join various trial committee meetings to provide their perspective.

7.8 MONITORING

The Sponsor and its delegates will have overall responsibility for monitoring. An SIV will occur prior to sites commencing the recruitment of trial participants. The purpose of the SIV is to train site investigators and local study coordinators with regards to the Master Protocol, the Appendices, data entry, management of trial documentation, and safety monitoring and reporting. The site will be activated once the SIV has been presented and all the essential documentation from the local site has been collected according to FOR*Ma*T Site Initiation and Activation SOP. Monitoring visits will ensure that the study is being conducted according to good clinical practice (GCP), the Master Protocol and relevant appendices, that study participants' safety, rights and well-being are being protected and that data entry is accurate and verifiable from source documentation. These will be conducted via central, remote, local or on-site monitoring. Visits may be conducted either face-to-face or through on-site electronic facilities, at intervals specified in the FOR*Ma*T Trial Monitoring SOP and Trial Monitoring Plan. The FOR*Ma*T trial will accommodate any requests to be audited by ethical, regulatory and/or other relevant authorities.

8 ETHICAL AND ADMINISTRATIVE CONSIDERATIONS

The trial will be conducted according to the Declaration of Helsinki, the International Conference on Harmonization - Good Clinical Practice E6 (ICH-GCP) and with the laws and regulations of the country in which the research is conducted, whichever represents the greater protection of the individual.

8.1 RESEARCH ETHICS APPROVAL AND SITE-SPECIFIC GOVERNANCE

Prior to trial initiation the FOR*Ma*T Master Protocol, appendices, information sheets and consent forms, trial questionnaires and any other patient facing material will be reviewed and approved by the relevant IRB/IEC/HREC

for each participating country and/or site. Approved delegates will keep the IRB/IEC/HREC informed as to the progress of the trial and comply with annual reporting requirements.

The IRB/IEC/HREC must approve any revisions to FOR*Ma*T trial documents and be informed of any serious and/or unexpected AEs occurring during the trial as required and of any new information that may adversely affect the safety of the participants or the conduct of the trial. Reporting requirements are to be adhered to in accordance with local IRB/IEC/HREC and additional site-specific requirements.

8.1.1 FOR*MA*T MASTER PROTOCOL AND TRIAL DOCUMENT AMENDMENTS

Any amendments to the approved IRB/IEC/HREC FOR*Ma*T trial documents may not be initiated without prior written IRB/IEC/HREC approval except when necessary to eliminate immediate hazards to the participants. Amendments will be submitted in writing to relevant IRB/IEC/HRECs and written approval will be obtained before the updated version is implemented.

8.1.2 PROTOCOL DEVIATIONS

A protocol deviation occurs when there is any deviation from the study procedures or treatment plans as specified in the IRB/IEB/HREC approved protocol. Examples of protocol deviations may include non-compliance with GCP, study visits outside of set windows and missing assessments outlined in the protocol. Participants enrolled in the intervention cohort where protocol deviations have occurred involving modifications to the study drug regimen are able to continue to be enrolled and assessed for the remainder of the study.

Protocol deviations may be minor or major. Minor protocol deviations do not carry significant ethical or administrative consequences. Major protocol deviations are those that affect participant's rights, safety or wellbeing and/or accuracy and reliability of the study data. An example of a minor protocol deviation is visit non-compliance (i.e. study visit and assessments are conducted outside of the required timeframe, or a procedure is missed) and there are no participant safety concerns.

Examples of major protocol deviations include:

a. Randomisation of an ineligible participant.
b. Visit non-compliance (e.g., study visit and assessments are conducted outside of the required timeframe, or visit or assessment is missed) and there are participant safety concerns.
c. Dispensing or dosing error of IMP.
d. Not reporting SAEs.

When a major protocol deviation occurs, it will be discussed with the site investigator(s) and site PI and a Protocol Deviation Form detailing the deviation will be generated. This form, outlining the major protocol deviation, will be signed by the site investigator. A copy of the form will be filed in the investigator site file and details of the deviation will be entered onto the study database.

8.1.2.1 Reporting Requirements

Minor protocol deviations do not need to be reported to the lead IRB/IEB/HREC at the time they occur. All minor deviations must be recorded in the protocol deviation log and reported to the Sponsor (if applicable).

Major protocol deviations may need to be reported to the lead IRB/IEC/HREC and to the Sponsor (if applicable) as per local requirements and a protocol deviation form detailing the event completed. The protocol deviation CRF is to be completed, signed by the site investigator, and entered in the database.

All protocol deviations (major or minor) must be recorded in a protocol deviation log and reported as per local ethical and regulatory requirements.

8.2 CONFIDENTIALITY

The Investigator must ensure that a participant's anonymity will be respected throughout the study and that their identities are protected from unauthorised parties. A participant's privacy and confidentiality will be maintained by the assignment of a unique identification number. On CRFs and other documents submitted to review committees and the Sponsor participants will not be identified by their names, rather their unique identification number. These numbers will be used to collect, store and report participant information, including in the trial database. The local site should keep a Subject Identification Log showing codes, names and dates of birth of the participants. Confidentiality and protection of data will be maintained according to local regulatory requirements. All information disclosed or obtained during the trial is confidential. The site PI and any person under his/her authority must maintain this confidentiality and must not disclose the information to any third party without the prior written approval of the CI.

8.3 SITE REIMBURSEMENT

Sites will be reimbursed according to each participating site's contract. Please see the relevant appendix for further information.

8.4 DATA SHARING

All de-identified raw data measured during the trial will be made available on request to relevant regulatory authorities, recognised academic institutions and clinical teams. Deidentified, aggregated data sets will be hosted on an approved public research data platform. Data requests must be made in writing with a proposal for data usage to the CI. Upon the approval for data sharing by the CI, the requester will be required to sign a data agreement.

8.5 PUBLICATION POLICY

The results of this trial (positive, negative and/or inconclusive) will be published and/or presented at scientific meetings. The preparation and submission for publication of manuscripts containing the trial results shall only be done if prior consent is obtained by the CI. Any manuscript requests can be submitted to the FOR*Ma*T Trial

Management Team. Authorship will be granted according to the recommendations from the International Committee of Medical Journal Editors (ICMJE)(51).

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FORMaT

Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

APPENDIX A1- INTERVENTION PROGRAM

Combined Intensive and Consolidation Therapies

Appendix A1 – Intervention Program	Version 1.1
Appendix Date	26 November 2024
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
EudraCT Number	2020-000050-10
EU-CT Number	2023-506575-99-00-EU CT
IRAS Project Number	1007146
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AE	Adverse Event
AFB	Acid-Fast Bacilli
ATS	American Thoracic Society
AUC	Area Under the Curve
BAL	Bronchoalveolar Lavage
BAR	Bayesian Adaptive Randomisation
BTS	British Thoracic Society
CEACS	Cost-effectiveness acceptability curves
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CHU9D	Child Health Utility 9D
Cmax	Maximum serum concentration
Cmin	Minimum serum concentration
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DVA	Dynamic Visual Acuity
ECG	Electrocardiogram
eCRF	electronic Case Report Form
FEV1	Forced Expiratory Volume in one second
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HIT	Head Impulse Test
HRCT	High-Resolution Computed Tomography
HRQoL	Health Related Quality of Life
IA	Inhaled Amikacin
ICD	International Statistical Classification of Diseases

ICERS	Incremental cost-effectiveness ratios
IV	Intravenous
IVA	Intravenous Amikacin
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MAC	*Mycobacterium avium* Complex
MARS-5	5-item Medication Adherence Rating Scale
MIC	Minimum Inhibitory Concentration
MoOP	Manual of Operating Procedures
MPR	Medication Possession Ratio
NMB	Net Monetary Benefit
NTM	Non-Tuberculous Mycobacteria
PedsQL™	Pediatric Quality of Life Inventory
QALY	Quality-Life Adjusted Years
QoL	Quality of Life
RCT	Randomised Control Trial
REDCap	Research Electronic Data Capture
R-Con	Randomisation – Consolidation
R-PI/IC	Randomisation – Prolonged Intensive/Immediate Consolidation
R-SI	Randomisation – Short Intensive
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SF-36	Short form-36
SGRQ	St George Respiratory Questionnaire
SOP	Standard Operating Procedure
TDM	Therapeutic Drug Monitoring
TPF	Time Point Final
TPF-PI	Time Point Final – Prolonged Intensive

TPF-SI	Time Point Final – Short Intensive
TPF-WK12	Time Point Final – at Week 12 Visit
TPST	Time Point Start Treatment
TPST-Con	Time Point Start Treatment – Consolidation
TPST-SI	Time Point Start Treatment – Short Intensive
VAS	Visual Analogue Scale
vHIT	Video Head Impulse Test
VOR	Vestibulo-ocular Reflex
WTP	Willingness To Pay
6MWD	Six Minute Walk Distance
6MWT	Six Minute Walk Test

APPENDIX A: INTERVENTION PROGRAM

Appendix A contains the Intervention Program modules, with new modules created as interventions are added to either intensive or consolidation phases of the trial. They are numbered sequentially, A1, A2, A3 etc, with Appendix A1 describing the first iteration of the Intervention Program. The relevant detailed statistical methods and simulations for each Intervention Program module will be described in Appendix F: General Statistical Principles. Separate consent procedures may be required for each appendix. Of note, in some cases data collected as part of one appendix may be incorporated in the analysis of another appendix, if collected to address the same objective. If this occurs, it will be detailed in the relevant appendices. Should an intervention arm be dropped, or a new intervention arm added either to intensive therapy or consolidation therapy this will require a new appendix and a new statistical analysis plan.

Each program in Appendix A will stipulate which of the Discovery studies and Registry linkages is applicable. The Intervention Program modules in Appendix A will include the following:

- A description of the primary and secondary objectives.
- A description of the intervention trial design including any specific inclusion or exclusion criteria specific to the Intervention Program modules.
- A description of the interventions and dosing.
- The methods for assigning treatment arms to the Intervention Program participants.
- Consent requirements.
- Specific Intervention Program trial procedures, monitoring and safety requirements and schedule of assessments.
- A description of relevant nested studies.
- A description of cost effectiveness methodology and analysis as appropriate will be detailed in Appendix E: Health Economics.
- A description of statistical analyses and simulations will be detailed in Appendix F: General Statistical Principles.
- The Discovery studies applicable to the module (if relevant).

APPENDIX A1 – COMBINED INTENSIVE AND CONSOLIDATION MODULE

Appendix A1 describes the initial *Mycobacterium abscessus* Pulmonary Disease (MABS-PD) intervention platform for the FOR*Ma*T trial. Within Appendix A1 there are intensive and consolidation therapy nested studies which are governed by the trial design and conduct described below.

<table>
<tr><th colspan="4">FORMaT Appendix A1 Summary</th></tr>
<tr><td rowspan="4">Treatment combinations:</td><td colspan="3">Intensive therapy arms:</td></tr>
<tr><td>Arm A</td><td>Arm B</td><td>Arm C</td></tr>
<tr><td>1. IV amikacin, and;
2. IV tigecycline, and;
3. IV imipenem or IV cefoxitin, and;
4. Oral azithromycin or oral clarithromycin, and;
5. Oral clofazimine.</td><td>1. Inhaled amikacin, and;
2. IV tigecycline, and;
3. IV imipenem or IV cefoxitin, and;
4. Oral azithromycin or oral clarithromycin, and;
5. Oral clofazimine.</td><td>1. IV amikacin, and;
2. IV tigecycline, and;
3. IV imipenem or IV cefoxitin, and;
4. Oral azithromycin or oral clarithromycin.</td></tr>
<tr><td colspan="3">Consolidation therapy arms:</td></tr>
<tr><td></td><td colspan="2">Arm a</td><td>Arm b</td></tr>
<tr><td></td><td colspan="2">1. Oral clofazimine, and;
2. Oral azithromycin or clarithromycin, and;
3. In combination with one to three of the following oral antibiotics:
• Linezolid,
• Trimethoprim / sulfamethoxazole (co-trimoxazole),
• Bedaquiline,
• Rifabutin.
• Doxycycline
• Moxifloxacin</td><td>1. Inhaled amikacin
2. Oral clofazimine, and
3. Oral azithromycin or clarithromycin, and;
4. In combination with one to three of the following oral antibiotics:
• Linezolid,
• Trimethoprim / sulfamethoxazole (co-trimoxazole),
• Bedaquiline,
• Rifabutin.
• Doxycycline
• Moxifloxacin</td></tr>
<tr><td></td><td colspan="3">A mixed Non-Tuberculous Mycobacteria (NTM) infection (slow grower + Mycobacterium abscessus (MABS)) can include the use of ethambutol in either/both the intensive or consolidation phase/s of treatment.</td></tr>
<tr><td>Appendix A1-specific eligibility:</td><td colspan="3">Inclusion and exclusion criteria as per Master Protocol section 4.1 and below.</td></tr>
<tr><td>Appendix A1-specific inclusions:</td><td colspan="3">1. Positive MABS-PD diagnosis meeting all three American Thoracic Society (ATS) clinical, radiological and microbiological diagnostic criteria for MABS-PD. Defined as;
Clinical: Pulmonary symptoms and exclusion of other diagnoses.
Radiological: Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.
Microbiological: MABS positive culture results from at least two separate expectorated sputum samples.
or;
Positive culture results from at least one bronchial wash or lavage.
or;</td></tr>
</table>

FOR*Ma*T Appendix A1 Summary	
	Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli (AFB)) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM. 2. Male or female participants of any age. 3. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age. For those participating in both the intensive and consolidation modules a combined consent form may be used. 4. Participant has not received MABS-PD treatment in the 12 months preceding assessment of eligibility (this includes drugs prescribed for treatment of other mycobacteria and/or other indications that may have activity against MABS as specified in FOR*Ma*T Prohibited Drug List SOP). 5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.
Appendix A1-specific exclusions:	1. Participants receiving current treatment for MABS within the previous 12 months (this includes drugs prescribed for treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP), except for participants taking azithromycin as part of routine treatment for Cystic Fibrosis (CF) or chronic infection-related pulmonary disease). 2. Positive pregnancy test at screening or any time during the FOR*Ma*T trial for females of childbearing potential. 3. Breast-feeding. 4. An unwillingness to comply with the acceptable methods of contraception defined in the relevant section of Appendix A1. 5. QTc>500 milliseconds (QT interval to be corrected based on Fridericia method). 6. Known hypersensitivity or contraindication to any of the therapies for which no alternative option(s) have been provided.
Target recruitment:	300 participants
Outcome measures:	***Primary outcome:*** MABS clearance from respiratory sample(s) with tolerance at the Final Outcome. ***Secondary outcomes:*** 1. Probability of MABS clearance at Final Outcome irrespective of toxicity according to participant's treatment pathway. 2. Safety of treatment combinations, including changes in microbiological resistance. 3. Change in FEV1 z-score at Final Outcome compared with Screening in participants who do and do not clear MABS at Final Outcome. 4. Phenotype of the structural abnormalities of chest CTs and changes in chest CT scores between Screening and Final Outcome between participants who clear or do not clear MABS at Final Outcome. 5. Predictive value of structural abnormalities on Screening CT scans for sputum conversion and for progression of structural changes in relation to treatment. 6. Change in 6-minute walk distance (6MWD) for adult participants from Screening to Final Outcome according to treatment pathway and in participants who do and do not clear MABS at Final Outcome. 7. Change in HRQoL for participants from Screening to Final Outcome according to treatment pathway and in participants who do and do not clear MABS at Final Outcome. 8. Cost effectiveness of the treatment combinations across intensive and consolidation phases of the trial.

FOR*Ma*T Appendix A1 Summary		
	9. Causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives. ***Exploratory outcomes:*** 1. Participant's MABS clearance status 12 months after Final Outcome.	
Timepoints:	***Screening:***	Up to Minus 42 days from Date of Randomisation-Short Intensive (R-SI).
	Time Point Start Treatment-Short Intensive (TPST-SI):	R-SI.
	Time Point Final (TPF):	End of treatment plus four weeks off-treatment (Final Outcome Visit date).
Nested studies	***Intensive Therapy Modules:***	
	Nested study A1.1:	Type of Short intensive Therapy
	Nested study A1.2:	Duration of intensive therapy for patients completing short intensive treatment with ongoing positive MABS cultures collected at 4 weeks and randomised to either a further 6 weeks intensive therapy or immediate consolidation.
	Consolidation Therapy Module:	
	Nested study A1.3:	The use of oral therapy only or oral therapy and inhaled amikacin for consolidation therapy.

Intensive Therapy Module Nested Studies		
Nested study A1.1: Type of Short Intensive Therapy		
Timepoints:	***Screening:***	Up to Minus 42 days from Date of R-SI.
	TPST-SI:	R-SI
	Time Point Final-Short Intensive (TPF-SI)*:	Date of Randomisation Prolonged Intensive or Immediate Consolidation (R-PI/IC) minus 1 day for those allocated prolonged intensive or Randomisation-Consolidation (R-Con) minus 1 day for those allocated to immediate consolidation.
	*The most accurate method of determining TPF for this trial phase is calculating the time immediately preceding randomisation to the next treatment phase i.e. date of next randomisation minus 1 day.	
Nested study A1.2: Duration of intensive therapy for patients with ongoing positive MABS cultures		
Timepoints:	***Screening:***	Up to Minus 42 days from R-SI.
	TPST-SI:	Date of R-SI
	TPF-PI* or TPF-WK12:	Date of R-Con minus 1 day (for those allocated to prolonged intensive) or Week 12 visit date (for those allocated to immediate consolidation).
	*The most accurate method of determining TPF for this trial phase is calculating the time immediately preceding randomisation to the next treatment phase i.e. date of next randomisation minus 1 day.	

Consolidation Therapy Module Nested Study		
Nested study A1.3: Use of oral therapy +/- inhaled amikacin for consolidation therapy		
Timepoints:	***Time Point Start Treatment-Consolidation (TPST-Con):***	Date of R-Con
	TPF:	End of treatment plus 4 weeks off treatment (Final Outcome Visit date).

1 INTRODUCTION

The probability of microbiological clearance with acceptable toxicity for treatment combinations tested in FOR*Ma*T, inclusive of both Appendix A1 intensive and Appendix A1 consolidation for patients with *Mycobacterium abscessus* pulmonary disease (MABS-PD) will be determined.

The probability of microbiological clearance with acceptable toxicity of treatment combinations will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies (*M. a. abscessus/ M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense)* and those with constitutive macrolide resistance and those with mixed NTM infections).

The best therapy combinations may then form the control arms for new intervention studies which can be added as new arms within Appendix A.

Sites may undertake both intensive and consolidation modules or if they are unable to conduct the intensive therapy module, then they can partner with a FOR*Ma*T site that is able to manage the intensive therapy module, and only undertake the consolidation module themselves. Sites that are unable to undertake the intensive therapy module themselves and are unable to partner with a site that is able to manage the intensive therapy module, can choose to only undertake the consolidation module (see Appendix A2 for detailed information).

2 OBJECTIVES

2.1 PRIMARY OBJECTIVES

The primary objective for Appendix A1 is to determine the optimal treatment for MABS-PD. Optimal treatment is defined by MABS clearance from respiratory samples with tolerance at 56 weeks (denoted Final Outcome) for participants who received short intensive therapy or at 62 weeks (denoted Final Outcome) for participants who received prolonged intensive therapy.

Definition of tolerance:

Tolerance is based on the Common Terminology Criteria for Adverse Events (CTCAE version 5.0). Only adverse events that are attributed as either “possibly”, “probably”, or “definitely” related to study drug will be assessed in the determination of tolerance. “Good” tolerance is defined as no adverse events occurring or only adverse events coded as CTCAE grades 1 and 2. “Poor” tolerance is defined as any adverse events attributed as possibly, probably, or definitely related to study drug coded as CTCAE grades 3, 4, or 5.

MABS clearance at final outcome will be defined as:

Negative MABS cultures from four consecutive sputum samples with one of those sputum specimens collected four weeks after the completion of consolidation therapy (either week 56 or 62, depending on treatment arm randomisation). Or, a MABS negative Bronchoalveolar Lavage (BAL) collected four weeks after completion of consolidation (either week 56 or 62, depending on treatment arm randomisation).

2.2 SECONDARY OBJECTIVES

1. To examine the probability of microbiological clearance at Final Outcome (irrespective of toxicity) for participants according to treatment path.
2. To describe the safety of the treatment combinations in patients with MABS.
3. To examine the change in Forced Expiratory Volume in one second (FEV1) z-score at Final Outcome compared with Screening in patients who do and who do not clear MABS at Final Outcome.
4. To phenotype the structural abnormalities of chest Computed Tomography (CT)s of MABS patients and examine changes in chest CT scores (bronchiectasis, trapped air, % disease) between Screening and Final Outcome between those who clear and those who do not clear MABS at Final Outcome.
5. To examine the predictive value of structural abnormalities on Screening CTs for sputum conversion and for progression of structural changes in relation to therapy.
6. To examine change in 6MWD for adult participants from Screening to Final Outcome according to treatment pathway and in participants who do and do not clear MABS at Final Outcome.
7. To examine the change in Health-Related Quality of Life (HRQoL) for participants with CF (using the Cystic Fibrosis Questionnaire-Revised (CFQ-R)) at Final Outcome compared with Screening according to treatment path and in those that do and those that do not clear MABS at Final Outcome.
8. To examine general HRQoL at Final Outcome compared with Screening according to treatment path and in those who do and who do not clear MABS at Final Outcome.
9. To examine the cost effectiveness of the proposed treatment combinations across both intensive and consolidation phases of the trial.
10. To examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives.

2.3 EXPLORATORY OBJECTIVES

- To examine MABS clearance status at twelve (12) months after Final Outcome.

3 DESIGN

Appendix A1 describes the intensive and consolidation modules (Figure A1.1) and will test therapies that are currently used and are the basis for the current treatment guidelines.

The intensive and consolidation modules function as nested studies within the trial.

Sites that are unable to participate in intensive therapy (nested A1 studies A1.1 and A1.2) but are able to undertake consolidation (nested study A1.3) may still be included as a trial site and partner with other trial sites that are able to undertake intensive therapy to facilitate trial participation (see FOR*Ma*T Shared Trial Sites SOP for further information on management of shared trial sites).

Intensive Therapy Module

Appendix A1.1: Short Intensive Therapy:

A1.1.1: Use of Inhaled Amikacin (IA) During Intensive Therapy to Replace Intravenous Amikacin (IVA) in the Treatment of MABS-PD.

A1.1.2: The Use of Additional Clofazimine to Standard Intravenous Therapies during Intensive Therapy in the Treatment of MABS-PD.

Appendix A1.2: Duration of Intensive Therapy for Patients with Ongoing Positive MABS cultures after completing 4 weeks vs 10 weeks of Intensive Therapy.

Consolidation Therapy Module

Appendix A1.3: The use of oral therapy only or oral therapy and inhaled amikacin for Consolidation Therapy. Note, participants recruited as part of Appendix A2 will also be included in the analysis of the objectives of this nested study.

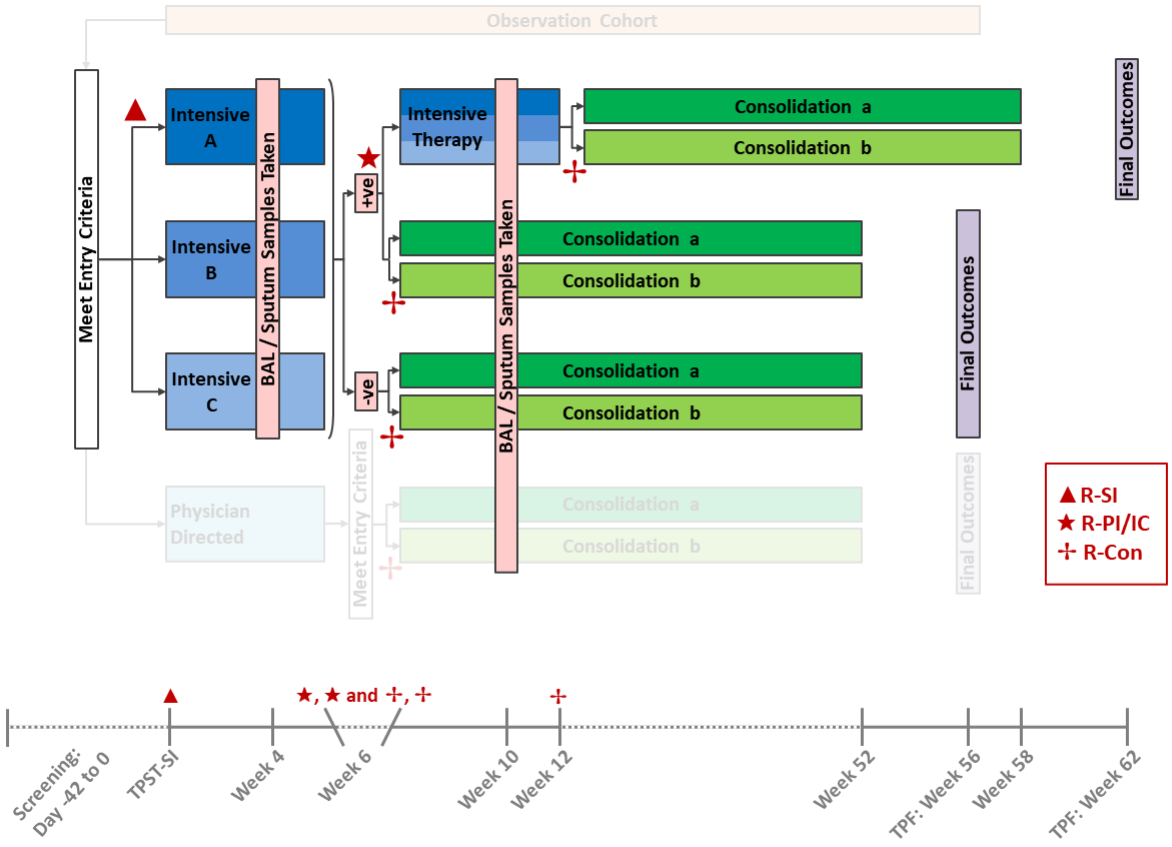

Figure 1 Flow Diagram for Appendix A1, Intervention Program Eligibility into the Intervention Program is determined at screening. At Randomisation-Short Intensive (R-SI) (▲); participants are randomised between the different intensive therapy arms (Intensive A, Intensive B and Intensive C) for a period of 6 weeks. At the end of intensive therapy, it will be determined if participants are still MABS positive, or MABS negative (cleared). Randomisation-Prolonged Intensive or Immediate Consolidation (R-PI/IC) (★) will ONLY be for participants who are still MABS positive based on respiratory samples taken at 4 weeks and will allocate participants to either 1) continue intensive therapy or 2) immediately commence consolidation therapy. Randomisation-Consolidation (R-Con) (✢) allocates participants to the consolidation therapy arms either at week 6 (for those allocated to immediate consolidation) or at week 12 (for those allocated to prolonged intensive therapy). Refer to Appendix A2 for information regarding Consolidation Only intervention program module where participants receive physician directed intensive therapy prior to enrolment into Consolidation Only program.

4 ELIGIBILITY CRITERIA

Participants are eligible for Appendix A1 if the following criteria are met:

4.1 INCLUSION CRITERIA

1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD. Defined as:

   Clinical: Pulmonary symptoms and exclusion of other diagnoses.

   Radiological: Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.

   Microbiological: MABS positive culture results from at least two separate expectorated sputum samples.

or

Positive culture results from at least one bronchial wash or lavage.

or

Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli, (AFB))) and positive culture for NTM ***or*** biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM.

2. Male or female participants of any age.
3. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age. For those participating in both the intensive and consolidation modules at the same site a combined consent may be used. For those sites only participating in the consolidation module, a specific consolidation consent may be required.
4. Participant has not received MABS-PD treatment in the 12 months preceding assessment of eligibility (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP).
5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.

4.2 EXCLUSION CRITERIA

- Participants receiving treatment for MABS within the previous 12 months (this includes drugs prescribed for treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP), except for participants taking azithromycin as part of routine treatment for CF or chronic infection-related pulmonary disease.
- Positive pregnancy test at any time during the FOR*Ma*T trial for females of childbearing potential.
- Breast-feeding.
- An unwillingness to comply with the acceptable methods of contraception, as described in Section 5 of this appendix.
- Participants with a QTc interval >500 milliseconds (QT interval corrected based on Fridericia method).
- Known hypersensitivity or contraindications to any of the therapies for which no alternative option(s) have been provided. This includes:
    - Amikacin,
    - Tigecycline (Tigecycline is only indicated in ages ≥8 years, as per the prescribing information),
    - Macrolide antibiotics, and
    - Clofazimine.

4.3 ADDITIONAL CRITERIA

4.3.1 MIXED NTM INFECTIONS

Participants who have cultured slow growing NTM of the same species two or more times in the 24 months prior to screening, with one of those cultures within the 6 months prior to screening will be considered to have mixed NTM infection at the time of screening. If the participant meets all other inclusion criteria and no exclusion criteria they will be eligible for participation. Ethambutol may be used in addition to trial therapies to cover mixed infections considered to require treatment by their clinician.

4.3.2 CO-ENROLMENT WITH OTHER TRIALS

Co-enrolment is not permitted when there is a potential interaction between trial interventions, any compromise to the validity of either trial or impact on participants' rights and/or safety. However, co-enrolment may be permitted in the instance where all trials that the participant is enrolled or to be enrolled in have mutually agreed to the co-enrolment arrangement.

5 ACCEPTABLE METHODS OF CONTRACEPTION

The effects of some drugs used during the Intervention Program on the unborn child and on the newborn baby are not known. Because of this, it is important that participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project.

It is therefore important that highly effective birth control is used while in this study. Site investigators should discuss effective methods of contraception with participants. Both male and female participants are to use effective contraception, used correctly with every act of sexual intercourse, from at least 14 days before the first dose of MABS-PD therapy, during the course of the trial and for a period of 90 days after the last dose of MABS-PD therapy. Acceptable methods of contraception for participants enrolled in Appendix A1 include:

1) Male vasectomy 6 months or more previously, with a documented negative post-vasectomy semen analysis for sperm.
2) Female bilateral tubal ligation performed at least 6 months previously.
3) Female continuous use of an intrauterine device (non-hormone releasing or hormone releasing) for at least 90 days before the first dose of MABS-PD therapy.
4) Female combined (estrogen and progestogen-containing) or progestogen-only oral, injected, implanted or vaginal hormonal contraception associated with inhibition of ovulation.

The barrier contraception methods listed below are not acceptable and are only to be used in special circumstances where the investigator determines that barrier contraception is appropriate.

1) Male and female condom with spermicide (either as a single product if commercially available and/or as allowed according to local regulations; otherwise condom and spermicide as separate products).
2) Male condom with female diaphragm, cervical cap, or vaginal sponge, each with spermicide.

For female participants using birth control pills

The use of birth control pills for contraception must be discussed and approved by the local site investigator at the start of each phase of the trial and when any changes in therapy occur, as some therapies used during the trial may interact with the effectiveness of birth control pills. Birth control pills should be in successful use from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

Female participants who change their method of contraception to birth control pills during the trial must continue to use a second form of approved contraception for at least 60 days after starting the use of birth control pills.

For female participants using hormonal injected, implanted or vaginal contraception

Injected, implanted or vaginal hormonal contraception should be used successfully from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

6 TRIAL CONDUCT

6.1 INFORMED CONSENT

In addition to providing consent to the FOR*Ma*T Master Protocol, participants enrolling in the Intervention Program are required to sign and date the relevant Appendix A1 consent. Consent to Appendix A1 includes consent to all Appendix A1 nested studies. Consent will be obtained from participants or their parent/guardian in accordance with the policies described in section 5.4 of the FOR*Ma*T Master Protocol.

6.2 PREGNANCY INFORMATION CONSENT

If a FOR*Ma*T trial participant becomes pregnant or is the biological father of a child conceived while enrolled in this Intervention Program the site investigators are requested to provide the participant with the FOR*Ma*T pregnancy information and consent form. The FOR*Ma*T pregnancy and consent form requests to follow the participant and the child for 12 months after conception. Please refer to FOR*Ma*T Safety Monitoring and Reporting SOP for further detail for safety monitoring and reporting of pregnancies and pregnancy outcomes in female trial participants or female partners who are pregnant to male participants in the Intervention Program.

6.3 METHODS OF ASSIGNING PARTICIPANTS TO TREATMENT ARMS

Participants will be randomised into different treatment arms for both the intensive and consolidation phase of treatment as described in the Master Protocol section 6.2 using the randomisation technique minimisation, initially in a 1:1:1 ratio for R-SI and 1:1 ratio for R-PI and R-Con. As described in the Master Protocol, all randomisations will be conducted via Research Electronic Data Capture (REDCap) at each FOR*Ma*T trial site. At each stage, study staff will enter the subject demographic data and the stratification factors (see section 6.3 in the Master Protocol) into REDCap, which will then inform them of the participant's treatment allocation for that stage.

Following interim analysis, after 60 participants have completed 6 weeks of intensive therapy, if appropriate, Bayesian Adaptive Randomisation (BAR) will be implemented for R-SI to implement the allocation probabilities which will be used until the data support either early stopping for futility, or a maximum sample size is attained.

Refer to Appendix F: General Statistical Principles for detailed information regarding randomisation and statistical principles for Appendix A1.

7 INTERVENTION PROGRAM PROCEDURES AND SAFETY MONITORING

In addition to the core trial procedures described in the Master Protocol, participants enrolled in the Intervention Program are required to undertake procedures and regular toxicology monitoring. The type of toxicology monitoring procedure required will be determined by the treatment arm the participant is allocated to, and in accordance with the schedule of assessments outlined in tables 12 to 14. Toxicology thresholds will be defined in accordance with CTCAE criteria as outlined in the Master Protocol, section 5.9.2. The outcome of all the assessments below will be documented in the corresponding Case Report Form (CRF) and entered into REDCap.

7.1 SERUM/URINE PREGNANCY TEST

All female participants of childbearing potential are required to undergo regular pregnancy testing while enrolled in the Intervention Program. Childbearing potential is defined as a premenopausal female capable of becoming pregnant, and includes females on oral, injectable, or mechanical contraception; females who are single and females whose male partners have been vasectomised or are using mechanical contraception (1). A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A serum pregnancy test will be performed at screening and at the final study visit (either week 56 or 62 depending on treatment arm allocation) and if applicable, at the early withdrawal visit. A urine β-hCG test or serum pregnancy is acceptable for regular pregnancy monitoring from day 1 in accordance with the schedule of assessments outlined in tables 12 to 14. Both urine and serum pregnancy tests are to be performed in accordance with site specific procedures.

7.2 CHEST CT SCAN

The chest CT scan for screening is to be performed ideally within the three months prior to screening for Intervention Program participants (maximum of six months earlier).

7.3 RESPIRATORY SAMPLING

For screening a minimum of 2 sputum samples (with at least 1 collected within 6 months prior to screening and other samples collected within 12 months prior to screening) or 1 BAL collected within 6 months prior to screening is required for Intervention Program participants. Tables 12 to 14 outline the minimum respiratory sample collection timepoints during the trial; however, monthly sputum sample collections are recommended.

7.4 AUDIOGRAM

To monitor aminoglycoside induced ototoxicity, regular audiometry assessments will be undertaken in accordance with the schedule of assessments outlined in tables 12 to 14. The battery of assessments to be completed should comply with the site-specific requirements. Assessments can be conducted in either a hospital or community setting.

7.5 VESTIBULAR MONITORING

Vestibular toxicity monitoring will document any vestibular symptoms (motion-induced oscillopsia, postural instability and gait unsteadiness) associated with prolonged aminoglycoside use. Assessments will take place in accordance with the schedule of assessments outlined in tables 12 to 14. Testing can be completed either by the treating physician or a physiotherapist. Additional vestibular monitoring may be required if deemed necessary by the Site investigator.

7.5.1 DYNAMIC VISUAL ACUITY TESTING (DVA)

Ask the patient to read a visual acuity chart (e.g., Snellen) while sitting still at recommended distance. This result is their static visual acuity. Repeat task while oscillating the patient's head horizontally or vertically at 1 to 2 Hz. An abnormal DVA is defined as loss of at least three lines of visual acuity compared with static condition (horizontal and/or vertical).

7.5.2 HEAD IMPULSE TESTING (HIT)

Stand in front of the seated patient, facing them, and ask the patient to focus on a target directly in front of them. Briskly rotate the patient's head horizontally approximately 10 to 20° amplitude, watching the patient's eyes closely. In normal subjects, the patient's eyes remain still as they remain on target. However, in a patient with impaired vestibulo-ocular reflex (VOR), the patient's eyes drift off the target and require a corrective 'catch-up' saccade to re-fixate on the target and stabilise vision. This catch-up saccade is a small amplitude horizontal eye movement in the opposite direction of the head turn and should occur with every head impulse (repeatable).

If available, Video Head Impulse Test (vHIT) is recommended as this has a higher sensitivity than the traditional bedside head impulse test at detecting impaired VOR. M=The main benefits include detecting covert (hidden) catch up saccades and peer review.

7.5.3 ROMBERG ON FOAM TEST

On a foam surface, ask the participant to stand still with two feet together. The participant should be able to stand steady with their eyes open. If the participant is not able to perform this task, ask them to separate their feet to minimal distance that allows them to do so. Repeat the task this time with their eyes closed. Record if the participant falls (positive Romberg test) or does not (negative Romberg test).

Document vestibular test results and any further actions (if results indicate significant vestibular impairment) in the CRF.

7.6 PHYSICAL EXAMINATION

The physical examination will be performed in accordance with the procedures outlined in the FOR*Ma*T Master Protocol, section 5.5.5. in accordance with the schedule of assessments outlined in tables 12 to 14.

7.7 ELECTROCARDIOGRAM (ECG)

A standard 12 lead ECG will be performed in accordance with the relevant tables and site-specific procedures after the participant has been supine for at least 5 minutes. A site investigator will interpret, sign and date the ECG. The QTc interval and the clinical interpretation will be recorded in the electronic case report form (eCRF) and ECGs will be required to be scanned and stored in the CRF. The QT interval at Screening is to be corrected using the Fridericia method. For any subsequent QTc intervals which are 'abnormal' the Fridericia method is to be used to confirm the QTc value.

7.8 SIX-MINUTE WALK TEST

A 6-minute walk test (6MWT) will be performed in adult participants only, at the time points outlined in tables 12 to 14 if the testing is available at the trial site. The 6MWT will be performed according to the protocol in the ATS Statement: Guidelines for the Six Minute Walk Test (2) and/or local standard procedures. Results of the 6MWT will be recorded in the relevant eCRF in the FOR*Ma*T database by the end of the Final Outcome visit.

7.9 BLOOD SAMPLING

Blood samples will be collected in accordance with tables 12 to 14. Liver function, full blood count and chemistry and renal function tests (refer to FOR*Ma*T Monitoring Blood Parameters SOP for specific blood parameters to be measured) are to be performed in accordance with the local pathology requirements. All relevant de-identified blood pathology reports are to be uploaded in the appropriate eCRFs in the FOR*Ma*T database by the end of the Week 6, Week 12 and Final Outcome Visits. Any blood abnormalities that meet Adverse Event (AE) or Serious Adverse Event (SAE) criteria are to be reported as an AE/SAE within the specified timeframes.

7.10 STUDY MEDICATION REVIEW

Participant adherence to MABS-PD treatment in the intervention cohort will be measured via two indirect methods. Self-reported adherence will be assessed using a questionnaire, the 5-item Medication Adherence Rating Scale (MARS-5)(3, 4) at the timepoints outlined in tables 12 to 14 and results recorded in the Medication Adherence Questionnaire CRF. Data from pharmacy prescription refill records and prescription claims databases will be obtained (where possible) and used to calculate the refill adherence measure, Medication Possession Ratio (MPR) (5-8).

7.11 AMIKACIN ADMINISTRATION AND MONITORING

7.11.1 NEBULISER TYPE FOR IA

Amikacin for injection preparation (not liposomal amikacin) is to be used for inhalation in participants randomised to inhaled amikacin. A low preservative preparation, if available, is advised to reduce the risks of bronchospasm. The brand of amikacin preparation, if known, is to be recorded in the study drug CRF. Five hundred milligrams of

amikacin should be administered according to local standard practices. Prior to administration the patient should receive a bronchodilator, for example salbutamol, to reduce the risk of coughing and bronchospasm. This can be given nebulised or by metered dose inhaler. Amikacin for injection may only be mixed with sodium chloride 0.9%. Sodium Chloride 0.9% is a commercial product and will be locally sourced and supplied by the study site. It must not be nebulised as a mixture with other nebulised drugs (e.g. salbutamol, dornase alfa or other nebulised antibiotics).

High efficiency nebulisers are required to nebulise amikacin (IV formulation). A suitable nebuliser with a filter attachment (e.g., SideStream Plus with filter attachment, or Pari LC Plus with filter attachment) are to be used to prevent environmental deposition of nebulised antibiotics and to reduce the risk of developing antibiotic resistant organisms. Air flow of 6-10 L/min is required to achieve effective nebulisation of amikacin.

7.11.2 AMIKACIN THERAPEUTIC DRUG MONITORING

TDM of intravenous amikacin will be required to reduce and monitor toxicity. There is currently variation across trial sites surrounding amikacin TDM methods. To reflect this, acceptable amikacin TDM strategies that can be used in this trial include;

1) Trough amikacin levels (independent of minimum inhibitory concentration (MIC) using nomogram for next dose);
2) Maximum serum concentration (C_{max})/MIC target;
3) Area under the curve (AUC) target;
4) C_{max}/MIC and minimum serum concentration (C_{min});
5) AUC/MIC and C_{min}.

The chosen amikacin TDM strategy used must be recorded in the CRF and adhered to consistently for that participant.

For subjects randomised to Intensive Therapy Arm B, an amikacin level should be collected at week one, 90 minutes (range 60 to 120 minutes) post completion of the inhaled dose. The time of starting and completing the inhaled therapy and time of blood collection should be documented. No adjustment of the inhaled amikacin dose should be undertaken.

7.12 HEALTH RELATED QUALITY OF LIFE AND UTILITY MEASURES

HRQoL will be assessed in all participants (where possible) according to the schedule of assessments in the relevant appendix. The questionnaires are required to be completed prior to any clinical assessment and are dependent on the participant's age and whether they have CF. The age appropriate HRQoL questionnaire issued to the participant at the start of the study will continue to be used throughout the study even if the participant progresses to a different age range. HRQoL questionnaires will be made available to study sites. All questionnaires will be made available in English or if available the local language version will be sourced. Questionnaires can be completed by the participant and/or their parent/carer via an online link to the questionnaire(s) on the trial database or if unable to access the electronic form these may be completed using a paper-based questionnaire. Responses from paper-based questionnaires are to be entered into the trial database by trial site staff.

7.12.1 CYSTIC FIBROSIS QUESTIONNAIRE-REVISED (CFQ-R)

The CFQ-R has been developed specifically for use in people with CF. This questionnaire measures the impact of CF on overall health, daily life, perceived well-being and symptoms. Age-appropriate questionnaires have been developed; CFQ-R teen/adult for adolescents and adults 14 years of age and older, the CFQ-R child for those 6-13 years of age, and the CFQ-R parent for the parents of those aged 6-13.

In children ≤16 years of age, the CFQ-R should be administered after the PedsQL™.

7.12.2 EQ-5D-5L

The EQ-5D-5L questionnaire is a standardised measure of health status in adults 18 years of age and older. This questionnaire can be applied as a generic measure of health for clinical and economic appraisal, including the calculation of quality-life adjusted years (QALYs). The EQ-5D-5L measures five dimensions:

1. Mobility;
2. Self-care;
3. Usual activities;
4. Pain/discomfort;
5. Anxiety/depression.

Each dimension has five possible answers: no problems, slight problems, moderate problems, severe problems and extreme problems. The respondent is asked to indicate his/her health state by selecting the most appropriate statement from a list.

7.12.3 EQ-5D-Y

The EQ-5D-Y is the child friendly version of the EQ-5D-5L questionnaire. The EQ-5D-Y has been developed for use in children 8 to 17 years of age. The dimensions and the visual analogue scale (VAS) measured are the same as the EQ-5D-5L questionnaire, but with child friendly wording. For children 4 to 7 years of age the proxy 1 version of the EQ-5D-Y can be utilised allowing the respondent (parent/carer) to evaluate participants quality of life (QoL) from respondents' own view.

7.12.4 ST. GEORGE RESPIRATORY QUESTIONNAIRE (SGRQ)

The SGRQ is to be completed by all non-CF participants 18 years of age and older. The SGRQ is a supervised self-administered 50-item questionnaire measuring health status across three domains: symptoms, activity and impacts (psycho-social) in people with airway obstruction.

7.12.5 SHORT FORM- 36 HEALTH SURVEY

The SF-36 health survey is a self-reported questionnaire applicable in all participants 16 years and older. Covering eight health concepts the SF-36 health survey is a generic outcome measure designed to examine a person's perceived health status.

7.12.6 PEDIATRIC QUALITY OF LIFE INVENTORY (PEDSQL™)

The PedsQL™ child health questionnaire is a non-preference-based measure to assess HRQoL for children and adolescents from 2 years up to 16 years of age. Children less than 16 years of age at screening will continue to use the PedsQL™ up until the end of the trial, rather than change to using the SF-36 if they turn 16 years of age during the study.

Developmentally appropriate child self-report questionnaires are available (ages 5-7, 8-12, 13-18) together with parent/carer proxy-reports (ages 2-4, 5-7, 8-12 and 13-18). If feasible, the PedsQL™ should be completed *before* the respondents complete any other health data forms and *before* they see their physician or healthcare provider.

Parents/carers, children (aged 8-12) and young people (aged >12 years) may self-administer the PedsQL™ after the FOR*Ma*T site researcher has provided instructions. If it is determined by the FOR*Ma*T site researcher that the child, young person or parent/carer is unable to self-administer the PedsQL™ the questionnaire should be administered, word for word by the FOR*Ma*T site researcher. If the child has difficulty understanding the age appropriate PedsQL™ the preceding age questionnaire may be used. The parent and the child must complete the questionnaires independently of each other and in accordance with the PedsQL™ administration guidelines (available via hyperlink or refer to relevant section in FOR*Ma*T Trial Site Manual of Operating Procedures (MoOP)).

7.12.7 CHILD HEALTH UTILITY 9D (CHU9D)

The CHU9D is a generic preference-based measure of paediatric HRQoL for use in children 7 to 17 years of age. The use of a descriptive system and a set of preference weights allows for the calculation of quality adjusted life years (QALYs) for use in economic evaluation.

7.13 MABS CLEARANCE FOLLOW-UP QUESTIONNAIRE

Site investigators will be requested to complete a follow up questionnaire for eligible intervention participants to assess microbiological clearance of MABS at 12 months after final outcome.

8 SITE REIMBURSEMENT

For participants enrolled in Appendix A1, trial sites will be reimbursed on a per participant basis at each of the following time points;

1. Screening;
2. End of intensive;
3. End of consolidation;
4. Final study visit.

Sites will be reimbursed according to their contract. Payments will be paid for each time point once the data is entered into the trial database and all queries finalised. Invoices should be prepared at a minimum of every 6 months.

9 INTENSIVE THERAPY NESTED STUDIES

9.1 NESTED STUDY A1.1: SHORT INTENSIVE THERAPY

9.1.1 PRIMARY OBJECTIVE

To compare the efficacy of intensive therapies on microbiological clearance of MABS with good tolerance at end of short intensive. Respiratory samples will be collected at 4 weeks to allow microbiological outcomes to be available at end of short intensive and tolerance will be assessed at the end of short intensive. Treatment will continue up until the end of short intensive (6 weeks). Specifically, the effects of inhaled amikacin (IA) versus intravenous amikacin (IVA) will be examined (A1.1.1) and the efficacy of additional oral clofazimine to standard intravenous therapy will be examined (A1.1.2).

Investigation of the efficacy of intensive therapy on microbiological clearance with acceptable toxicity of treatment combinations will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/ M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).

MABS clearance at the end of the short intensive therapy will be defined as negative MABS cultures from all 3 sputum samples or from one BAL sample collected at week 4.

Additional studies nested within Appendix A1.1 are:

A1.1.1: Use of IA During Intensive Therapy to Replace IVA in the Treatment of MABS-PD;

A1.1.2: The use of Additional Clofazimine to Standard IV Therapies During Intensive Therapy in the Treatment of MABS-PD.

9.1.1.1. Study A1.1.1: Use of Inhaled Amikacin (IA) During Intensive Therapy to Replace Intravenous Amikacin (IVA) in the Treatment of MABS-PD

Introduction

Amikacin is an aminoglycoside recommended in guideline-based therapy for the treatment of MABS-PD. It can be administered either intravenously or nebulised for inhalation (using the IV formulation). Inhaled aminoglycosides have the potential advantages of achieving higher airway concentrations, while reducing the risk of systemic toxicity. However, amikacin for inhalation is not currently available commercially for the indication of treating MABS and clinicians have used an "off-label" IV form of amikacin (delivered via a nebuliser). A Phase II Randomised Control Trial (RCT) (9) of amikacin liposome inhalation suspension (Arikayce) sponsored by Insmed Inc., investigated the safety and efficacy of Arikayce to treat NTM infection (36% MABS) in addition to highly variable multi-drug therapy in 89 patients with (19%) and without (81%) CF in patients who had failed to clear infection using their current treatment. The trial did not meet the primary endpoint of decreased mycobacterial load, but the treatment group had a higher proportion of subjects with >1 negative sputum (32% vs 9%, p=0.006) and improved 6- minute walk distance, suggesting a potential benefit from adding Arikayce to consolidation therapy. Arikayce has now been approved for the treatment of patients infected with *Mycobacterium avium* complex (MAC) who do not respond to conventional treatment. This trial highlighted the difficulties and inefficiencies of undertaking conventional clinical trials using the "one population, one drug, one disease" in a disease where there are relatively small patient numbers, a heterogeneous population and complex, inconsistent drug combinations. These factors greatly limit the clinical information obtained and contribute to making conventional trials in this patient population difficult to interpret. This contrasts with innovative platform trials, which have advantages for efficiently evaluating multiple treatment combinations (e.g. multi-drug resistant TB (10)) requiring complex drug regimens in a heterogeneous population.

Eligibility Criteria

No additional eligibility criteria are required for participation in A1.1.1 from those described in Appendix A1, Section 4.

Objectives

Primary Objective

To compare the microbiological clearance of MABS from respiratory samples collected at 4 weeks with good tolerability assessed at the end of short intensive therapy between the use of IA (Arm B) and the use of IVA (Arm A) given during intensive phase.

Secondary Objectives

1. Microbiological clearance at 4 weeks (irrespective of toxicity) with use of IA (Arm B) compared with use of IVA (Arm A) in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies M. a. abscessus/ M. a. bolletii [inducible macrolide resistance] and M. a. massiliense and those with constitutive macrolide resistance).
2. Safety of using IA to replace IVA in the short intensive therapy phase.

3. Change in FEV1 z-score at end of short intensive therapy versus at Screening with use of IA (Arm B) compared with use of IVA (Arm A).
4. Change in HRQoL (CFQ-R) for participants at end of short intensive therapy versus at Screening with use of IA (Arm B) compared with use of IVA (Arm A).
5. To examine general HRQoL between Screening and end of short intensive therapy in participants with use of IA (Arm B) compared with use of IVA (Arm A).
6. To examine the cost effectiveness of IA compared with IVA during short intensive therapy.
7. To examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives with use of IA (Arm B) compared with use of IVA (Arm A).

Statistical Analysis

Details of the simulations (sample size calculation) for Appendix A1 are outlined in Section 6 of the Master Protocol and the relevant section of Appendix F: General Statistical Principles and the Statistical Analysis Plan (SAP).

9.1.1.2. Study A1.1.2: The Use of Additional Clofazimine to Standard Intravenous Therapies During Intensive Therapy in the Treatment of MABS-PD

Introduction

Clofazimine is approved for use in leprosy. It has recognised antibacterial and anti-inflammatory effects in the management of leprosy and has been used to treat erythema nodosum related to leprosy. There is no current clinical trial evidence to support the use of clofazimine in the treatment of MABS-PD. Clofazimine use has however increased clinically, potentially driven by the difficulty in achieving microbiological clearance and reports of *in vitro* synergy between combinations of clofazimine and amikacin (11, 12).

Eligibility Criteria

No additional eligibility criteria are required for participation in nested study A1.1.2 from those described in Appendix A1, Eligibility Criteria.

Objectives

Primary Objective

To compare the microbiological clearance of MABS from respiratory samples collected at 4 weeks with good tolerability assessed at the end of short intensive therapy between standard IV therapy without clofazimine (Arm C) and with clofazimine (Arm A) given during intensive phase.

Secondary Objectives

1. Microbiological clearance from respiratory samples collected at 4 weeks with acceptable toxicity of intensive therapy at the end of short intensive therapy without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment will be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies M. a. abscessus/ M. a. bolletii [inducible macrolide resistance] and M. a. massiliense) and those with constitutive macrolide resistance).
2. Microbiological clearance (irrespective of toxicity) at 4 weeks without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment.
3. Microbiological clearance (irrespective of toxicity) at 4 weeks without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment will be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies M. a. abscessus/ M. a. bolletii [inducible macrolide resistance] and M. a. massiliense) and those with constitutive macrolide resistance).
4. Safety of treatment without (Arm C) and with the addition of clofazimine (Arm A) in the short intensive therapy phase.
5. Change in FEV1 z-score between Screening and end of short intensive therapy in participants treated without (Arm C) and with the addition of clofazimine (Arm A).
6. Changes in HRQoL (CFQ-R) for participants with CF between Screening and end of short intensive therapy in participants treated without (Arm C) and with the addition of clofazimine (Arm A).
7. To examine general HRQoL between Screening and end of short intensive therapy in participants treated without (Arm C) and with the addition of clofazimine (Arm A).

8. To examine the cost effectiveness of treatment without (Arm C) and with the addition of clofazimine (Arm A) during short intensive therapy in addition to standard IV treatment.
9. To examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment.

Statistical Analysis

Details of the sample size calculation for this study are presented in Section 6.0 of the Master Protocol and the relevant section of Appendix F: General Statistical Principles and in the SAP.

9.2 NESTED STUDY A1.2: DURATION OF INTENSIVE THERAPY FOR PATIENTS WITH ONGOING POSITIVE MABS CULTURES AFTER 4 WEEKS OF INTENSIVE THERAPY.

9.2.1 INTRODUCTION

Current published guidelines for the treatment of MABS-PD (13-15) are based on expert opinion, and in practice treatments vary considerably (16). Suggested regimens include an intensive phase of 4-12 weeks of IV antibiotics with duration based on clinical and microbiological response. Whether extending the length of intensive therapy improves microbiological clearance of MABS is not known but the strategy is used clinically in patients with ongoing positive cultures. Toxicity and costs are thought to be related to length of treatment and need to be balanced against the potential for better microbiological outcomes.

9.2.2 ELIGIBILITY CRITERIA

Participants are required to have completed 6 weeks of intensive therapy and will have at least one out of 3 sputum cultures or one BAL culture positive for MABS collected at week 4 (± 3 days).

No other additional eligibility criteria are required for participation in nested study A1.2 from those described in Section 4 of Appendix A1.

9.2.3 OBJECTIVES

9.2.3.1. Primary Objective

To compare the microbiological clearance from samples collected at 10 weeks with good tolerability between those who are allocated to prolonged intensive therapy and those allocated to immediate consolidation following short intensive therapy.

MABS clearance, assessed at the end of prolonged intensive therapy (for those allocated to prolonged intensive) or at 12 weeks (for those allocated to immediate consolidation) will be defined as negative MABS cultures from all 3 sputum samples or from one BAL sample collected at 10 weeks.

9.2.3.2. Secondary Objectives

1. To compare microbiological clearance from samples collected at 10 weeks (irrespective of toxicity) in prolonged intensive therapy compared with short intensive and immediate consolidation therapy in patients who had MABS positive cultures from samples collected at 4 weeks.
2. Safety of prolonged intensive therapy compared with short intensive + immediate consolidation therapy.
3. Change in FEV1 z-score between Screening and end of prolonged intensive therapy or 12 weeks in participants that received prolonged intensive compared with short intensive and immediate consolidation who had MABS positive cultures from samples collected at 4 weeks.
4. Change in FEV1 z-score between Screening and end of prolonged intensive therapy or 12 weeks between those participants still culture positive for MABS at 10 weeks compared with those who have cleared MABS at 10 weeks.

5. Change in 6MWD between Screening and end of prolonged intensive therapy or 12 weeks in adult participants who receive prolonged intensive compared with short intensive and immediate consolidation therapy who had MABS positive cultures from samples collected at 4 weeks.

6. Change in HRQoL (CFQ-R) for participants with CF between Screening and end of prolonged intensive therapy or 12 weeks in participants who received prolonged intensive compared with short intensive and immediate consolidation therapy who had MABS positive cultures from samples collected at 4 weeks.

7. Changes in general HRQoL in prolonged intensive therapy compared with short intensive and immediate consolidation therapy in patients who had MABS positive cultures at 4 weeks.
8. Change in CT scan parameters (bronchiectasis, mucus plugging, airway wall thickening, --atelectasis, % disease and air trapping), between Screening and end of prolonged intensive therapy (for those allocated to prolonged intensive) or 12 weeks (for those allocated to immediate consolidation) taking into account microbiological clearance based on samples collected at 4 and at 10 weeks.
9. To examine the cost effectiveness of prolonged intensive compared with short intensive and immediate consolidation for those who remain MABS positive based on samples collected at 4 weeks.
10. To examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives in prolonged intensive compared with short intensive and immediate consolidation in patients who had MABS positive cultures based on samples collected at 4 weeks.

9.2.4 STATISTICAL ANALYSIS

Details of the sample size calculation for this study are presented in Section 6.0 of the Master Protocol and the relevant section of Appendix F: General Statistical Principles and in the SAP.

9.3 INTENSIVE THERAPY DOSING REGIMEN

At R-SI, participants will be randomised to one of three treatment arms during the intensive phase and will receive drug therapy in accordance with the dosing tables below. Drug therapy, administration and duration is dependent on the treatment arm (Intensive A, Intensive B, Intensive C) the participant is randomised to. Drug dosing is based on the participants age (<18 or ≥18 years of age). The start and end points of each drug therapy, the dose of each drug therapy used, any changes in dosing and all concomitant medications used will be required to be entered into the CRF.

There are currently three proposed treatment arms in the intensive therapy phase. Randomisation – Short Intensive dictates the drug therapy that participants will be randomised to. Treatment Arm A is the reference arm (i.e., control).

Intensive Arm A	**Intensive Arm B**	**Intensive Arm C**
1. IV amikacin, and; 2. IV tigecycline*, and; 3. IV imipenem/cilastatin or IV cefoxitin, and; 4. Oral azithromycin or oral clarithromycin, and; 5. Oral clofazimine.	1. Inhaled amikacin, and; 2. IV tigecycline*, and; 3. IV imipenem/cilastatin or IV cefoxitin, and; 4. Oral azithromycin or oral clarithromycin, and; 5. Oral clofazimine.	1. IV amikacin, and; 2. IV tigecycline*, and; 3. IV imipenem/cilastatin or IV cefoxitin, and; 4. Oral azithromycin or oral clarithromycin.

*Tigecycline is only indicated in ages ≥8 years as per prescribing information.

For participants with confirmed mixed NTM infections (slow growers + MABS), ethambutol can be added to the treatment arms (in accordance with the dosing tables below) if required by the treating physician.

The intensive therapy dosing regimen tables outlined below are separated by age (adult, paediatric) and by intensive treatment arm (Arm A, Arm B, Arm C). The recommended doses and frequencies are a guideline and participant dosing must also take into account clinical judgement and relevant prescribing information. These medications have the potential for drug-drug interactions. The treating physician must consider drug and non-drug interactions, special warnings and precautions for use prior to prescribing these medications according to the relevant prescribing information.

Table 1 Intensive therapy dosing regimen for Intensive Arm A in adults

Intensive Arm A: Adult Dosing [A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
IV amikacin	15mg/kg **OR** 20-25mg/kg	Once daily Thrice weekly
Dosing will be made in accordance with British Thoracic Society (BTS) guidelines (13) and is dependent on the physiology, site and therapeutic drug monitoring (TDM) outcomes of each participant. In overweight participants use the ideal body weight calculator or in cases of extremes of actual body weight where body weight is greater than 20% above ideal use the adjusted body weight calculator available in the MoOP. To determine ideal body weight for amputees, refer to for the table in the MoOP describing the percentage of total weight contributed by individual body parts.		
IV tigecycline	25mg increasing by 5mg every two doses until either 50mg reached or until patient is unable to tolerate.	Twice daily or same total daily dose over a 24-hour infusion
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	**≥50kg** 1g (dose based on imipenem component)	Twice – four times daily infused over 1-4 hours as tolerated
	<50kg 15 – 25mg/kg (dose based on imipenem component). Maximum 1g.	Twice – four times daily infused over 1-4 hours as tolerated
IV cefoxitin *Cefoxitin only for use if imipenem/cilastatin not tolerated.*	2 – 4g	Thrice daily infused over 1-4 hours as tolerated, or same total daily dose over a 24-hour infusion.
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*	250 – 500mg **<40kg or poorly tolerated** 250mg	Once daily Once daily
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
Oral clofazimine	100 – 300mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (rounded to account for tablet strength) **OR** 25mg/kg (rounded to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 2 Intensive therapy dosing regimen for Intensive Arm B in adults

Intensive Arm B: Adult Dosing [A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) **(IV formulation)**	500mg	Twice daily
IV tigecycline	25mg increasing by 5mg every two doses until either 50mg reached or until patient is unable to tolerate.	Twice daily or same total daily dose over a 24-hour infusion
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	**≥50kg** 1g (dose based on imipenem component)	Twice – four times daily infused over 1-4 hours as tolerated
	<50kg 15 – 25mg/kg (dose based on imipenem component). Maximum 1g.	Twice – four times daily infused over 1-4 hours as tolerated
IV cefoxitin *Cefoxitin only for use if imipenem/cilastatin not tolerated.*	2 – 4g	Thrice daily infused over 1-4 hours as tolerated, or same total daily dose over a 24-hour infusion.
Oral azithromycin	250 – 500mg	Once daily
If azithromycin not tolerated, use oral clarithromycin.	**<40kg or poorly tolerated** 250mg	Once daily
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
Oral clofazimine	100 – 300mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 3 Intensive therapy dosing regimen for Intensive Arm C in adults

Intensive Arm C: Adult Dosing [A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
IV amikacin	15 mg/kg **OR** 20-25mg/kg	Once daily Thrice weekly
Dosing will be made in accordance with British Thoracic Society (BTS) guidelines (13) and is dependent on the physiology, site and therapeutic drug monitoring (TDM) outcomes of each participant. In overweight participants use the ideal body weight calculator or in cases of extremes of actual body weight where body weight is greater than 20% above ideal use the adjusted body weight calculator available in the MoOP. To determine ideal body weight for amputees, refer to for the table in the MoOP describing the percentage of total weight contributed by individual body parts.		
IV tigecycline	25mg increasing by 5mg every two doses until either 50mg reached or until patient is unable to tolerate.	Twice daily or same total daily dose over a 24-hour infusion
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	**≥50kg** 1g (dose based on imipenem component) **<50kg** 15 – 25mg/kg (dose based on imipenem component). Maximum 1g.	Twice – four times daily infused over 1-4 hours as tolerated Twice – four times daily infused over 1-4 hours as tolerated
IV cefoxitin *Cefoxitin only for use if imipenem/cilastatin not tolerated.*	2 – 4g	Thrice daily infused over 1-4 hours as tolerated, or same total daily dose over a 24-hour infusion.
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*	250 – 500mg **<40kg or poorly tolerated** 250mg	Once daily Once daily
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 4 Intensive therapy dosing regimen for Intensive Arm A in paediatrics

Intensive Arm A: Paediatric Dosing [A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
IV amikacin		15-30mg/kg, max 1500mg initial dose	Once daily
Dosing will be made in accordance with BTS guidelines (13) and is dependent on the physiology, site and TDM outcomes of each participant. In obese participants, use the ideal body weight calculator or in cases of extremes of actual body weight where body weight is greater than 20% above ideal body weight use the adjusted body weight calculator available in the MoOP. To determine ideal body weight or adjusted body weight for amputees, refer to for the table in the MoOP describing the percentage of total weight contributed by individual body parts.			
IV tigecycline (ages ≥8 years)	Day 1: (50% of optimal dose)	0.6mg/kg, max 25mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6mg/kg, max 25mg	In the morning
		1.2mg/kg, max 50mg	At night
	Day 3: (100% of optimal dose)	1.2mg/kg, max 50mg	Twice daily (12 hourly)
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	Day 1-2	15 - 25mg/kg, max 1g (dose based on imipenem component)	Twice daily (12 hourly)
	Day 3	15 - 25mg/kg, max 1g (dose based on imipenem component)	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV cefoxitin *Only for use if imipenem/cilastatin not tolerated.*		40mg/kg, max 2g	Four times daily (6 hourly)
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
Oral clofazimine Dosing may be rounded to account for capsule strength.		**<40kg** 3-5mg/kg, max 100mg	Once daily
		≥40kg 100mg	
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 5 Intensive therapy dosing regimen for Intensive Arm B in paediatrics

Intensive Arm B: Paediatric Dosing [A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) (IV formulation)		500mg	Twice daily
IV tigecycline (ages ≥8 years)	Day 1: (50% of optimal dose)	0.6mg/kg, max 25mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6mg/kg, max 25mg	In the morning
		1.2mg/kg, max 50mg	At night
	Day 3: (100% of optimal dose)	1.2mg/kg, max 50mg	Twice daily (12 hourly)
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	Day 1-2	15 - 25mg/kg, max 1g. (dose based on imipenem component)	Twice daily (12 hourly)
	Day 3	15 - 25mg/kg, max 1g (dose based on imipenem component)	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV cefoxitin *Only for use if imipenem/cilastatin not tolerated.*		40mg/kg, max 2g	Four times daily (6 hourly)
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
Oral clofazimine Dosing may be rounded to account for capsules strength.		**<40kg** 3-5mg/kg, max 100mg	Once daily
		≥40kg 100mg	
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 6 Intensive therapy dosing regimen for Intensive Arm C in paediatrics

Intensive Arm C: Paediatric Dosing [A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
IV amikacin		15-30mg/kg, max 1500mg initial dose	Once daily
Dosing will be made in accordance with BTS guidelines (13) and is dependent on the physiology, site and TDM outcomes of each participant. In obese participants, use the ideal body weight calculator or in cases of extremes of actual body weight where body weight is greater than 20% above ideal body weight use the adjusted body weight calculator available in the MoOP. To determine ideal body weight or adjusted body weight for amputees, refer to for the table in the MoOP describing the percentage of total weight contributed by individual body parts.			
IV tigecycline (ages ≥8 years)	Day 1: (50% of optimal dose)	0.6mg/kg, max 25mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6mg/kg, max 25mg	In the morning
		1.2mg/kg, max 50mg	At night
	Day 3: (100% of optimal dose)	1.2mg/kg, max 50mg	Twice daily (12 hourly)
IV imipenem/cilastatin *IV imipenem/cilastatin is preferred but if not tolerated, use IV cefoxitin.*	Day 1-2	15 - 25mg/kg, max 1g. (dose based on imipenem component)	Twice daily (12 hourly)
	Day 3	15 - 25mg/kg, max 1g (dose based on imipenem component)	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV cefoxitin *Only for use if imipenem/cilastatin not tolerated.*		40mg/kg, max 2g	Four times daily (6 hourly)
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

9.3.1 DRUG SUPPLY, STORAGE AND DISTRIBUTION DURING INTENSIVE THERAPY

Supply, distribution and storage of drugs will be the responsibility of the site coming from site/institution pharmacy stocks as per standard of care and in accordance with site specific guidelines and requirements. Any medication the participant is prescribed which are not investigational products, including adjunctive treatments, for example, medication to treat nausea, will be commercially available products and are to be sourced and supplied locally by study sites according to local standard practices.

9.3.2 OPTIONS FOR REDUCING NAUSEA DURING INTENSIVE THERAPY

Titration of dosing for imipenem and tigecycline is described in tables 1 to 6. Imipenem should also be given over 2-3 hours (where possible) to reduce nausea. Pharmacological treatment options to manage nausea are at the discretion of the treating physician and are to be prescribed according to relevant local clinical guidelines.

10 NESTED STUDY A1.3: CONSOLIDATION THERAPY

10.1 INTRODUCTION

Currently, the guidelines for consolidation therapy (13) suggest that patients with isolates that are sensitive to macrolides or that have inducible resistance should be managed with a combination of between one and three oral antibiotics based on drug susceptibility of the isolate and tolerance in combination with IA. Those with isolates that have constitutive resistance should use a combination of between two and four oral drugs based on susceptibility and tolerance in combination with IA. In addition, guidelines suggest that patients with isolates that have amikacin resistance could substitute IA for an alternative oral antibiotic. It is suggested that treatment should continue for 12 months after culture conversion.

There is currently no evidence for any of these guidelines. IA is costly and requires time and effort for maintenance of hygienic practice and drug delivery. Furthermore, adherence to inhaled therapies over the longer term is variable (17-19). This study will examine the effects of IA in addition to the oral only thus providing some evidence regarding the use of additional IA during consolidation. The timing of consolidation therapy is only 46 weeks for this trial. Microbiological clearance will be determined from culture of respiratory samples (sputum (expectorated or induced)) collected ***at least one week apart*** or BAL as follows:

Participants who continue to produce sputum samples during the consolidation phase are requested to provide additional sputum samples monthly. Three consecutive sputum samples will be collected at least one week apart over the last 6 weeks of consolidation therapy. A final sputum sample collected four weeks after cessation of consolidation therapy will together determine the final outcome of microbiological clearance. All four samples will need to be clear to determine clearance.

For participants who continue to have positive sputum cultures at the end of consolidation, a gap in therapy to complete final outcomes may not be required if ongoing treatment is clinically required as microbiological clearance will not have occurred.

Participants must cease treatment for 4 weeks at the end of consolidation prior to the final outcome measures to enable assessment of microbiological clearance without antibiotic suppression.
For participants who are unproductive of sputum and/or induced sputum during the consolidation phase, or who become unproductive of sputum and/or induced sputum during the course of consolidation therapy, a BAL is to be performed at final outcome (4 weeks after completing consolidation therapy).

10.2 OBJECTIVES

10.2.1 PRIMARY OBJECTIVE

To compare the microbiological clearance with good tolerability of MABS between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral treatment and additional IA at Final Outcome.

10.2.2 SECONDARY OBJECTIVES

1. To compare microbiological clearance (irrespective of toxicity) at Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral treatment and additional IA taking into account microbiological status at the start of consolidation.
2. Difference in safety between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
3. Change in FEV1 z-score between start of consolidation therapy and Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA taking into account microbiological clearance at Final Outcome.
4. Change in 6MWD between Week 12 of the trial and Final Outcome between adult participants allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA taking into account microbiological clearance at Final Outcome.
5. Change in CT scan parameters (bronchiectasis, mucus plugging, airway wall thickening, atelectasis, % disease and air trapping), between 12 weeks and Final Outcome taking into account microbiological clearance at start of consolidation therapy between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
6. Changes in HRQoL (CFQ-R) for participants with CF between start of consolidation therapy and Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
7. To examine general HRQoL between start of consolidation therapy and Final Outcome in adults (CF and non-CF) between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
8. To compare the cost effectiveness of consolidation between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
9. To examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.

10.3 ELIGIBILITY CRITERIA

Participants who have been randomised to intensive therapy in Appendix A1 and have either completed intensive therapy or who are unable to complete Intensive therapy due to a lack of tolerance.

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10.4 STATISTICAL ANALYSIS

Details of the sample size calculation for this study are presented in Section 6.0 of the Master Protocol and the relevant section of Appendix F: General Statistical Principles and in the SAP.

10.5 CONSOLIDATION THERAPY DOSING REGIMEN

Following R-Con, participants will receive consolidation treatment in accordance with the dosing tables below. Drug therapy, administration and duration is dependent on the treatment arm (Consolidation Arm a and Consolidation Arm b), age, and/or weight of the participant. The start and end points of each drug therapy, the dose of each drug therapy used, any changes in dosing, and all concomitant medications used will be required to be entered into the CRF.

There are currently two proposed treatment arms that participants will be randomised to during consolidation therapy.

Consolidation Arm a	**Consolidation Arm b**
1. Oral clofazimine, and; 2. Oral azithromycin or oral clarithromycin, and; In combination with one to three of the following oral antibiotics: • Oral linezolid, • Oral trimethoprim / sulfamethoxazole, • Oral bedaquiline, • Oral rifabutin, • Oral doxycycline, • Oral moxifloxacin.	1. Inhaled amikacin 2. Oral clofazimine, and; 3. Oral azithromycin or oral clarithromycin, and; In combination with one to three of the following oral antibiotics: • Oral linezolid, • Oral trimethoprim / sulfamethoxazole, • Oral bedaquiline, • Oral rifabutin, • Oral doxycycline, • Oral moxifloxacin.

For participants with confirmed mixed NTM infections (slow growers + MABS), ethambutol can be added to the treatment arms (in accordance with the dosing tables below) if required by the treating physician.

The consolidation therapy dosing regimen tables outlined below are separated by age (adult, paediatric) and by consolidation treatment arm (Arm a and Arm b). The recommended doses and frequencies are a guideline and participant dosing must also take into account clinical judgement and relevant prescribing information.

These medications have the potential for drug-drug interactions. The treating physician must consider drug and non-drug interactions, special warnings and precautions for use prior to prescribing these medications according to the relevant prescribing information.

Table 7 Consolidation therapy dosing regimen for Consolidation Arm a in adults

Consolidation Arm a: Adult Dosing[A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
Oral clofazimine	100 – 300mg	Once daily
Oral azithromycin	250 – 500mg **OR** 500mg	Once daily Thrice weekly
If azithromycin not tolerated, use oral clarithromycin.	**<40kg or poorly tolerated** 250mg	 Thrice weekly
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.		
Oral linezolid	600mg	Once daily
Oral trimethoprim with sulfamethoxazole	160mg/800mg	Twice daily
Oral bedaquiline *(Weighing at least 30kg)*	**First 2 weeks** 400mg	Once daily
	For remaining 22 weeks 200mg Max duration 6 months	Thrice weekly At least 48 hours between doses
Oral rifabutin	5mg/kg, max 450mg	Once daily
Oral doxycycline	100mg	Once daily
Oral moxifloxacin	400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 8 Consolidation therapy dosing regimen for Consolidation Arm b in adults

Consolidation Arm b: Adult Dosing [A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) **(IV formulation)**	500mg	Twice daily
Oral clofazimine	100 – 300mg	Once daily
Oral azithromycin	250 – 500mg **OR** 500mg	Once daily Thrice weekly
If azithromycin not tolerated, use oral clarithromycin.	**<40kg or poorly tolerated** 250mg	 Thrice weekly
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.		
Oral linezolid	600mg	Once daily
Oral trimethoprim with sulfamethoxazole	160mg/800mg	Twice daily
Oral bedaquiline *(Weighing at least 30kg)*	**First 2 weeks** 400mg	Once daily
	For remaining 22 weeks 200mg Max duration 6 months	Thrice weekly At least 48 hours between doses
Oral rifabutin	5mg/kg, max 450mg	Once daily
Oral doxycycline	100mg	Once daily
Oral moxifloxacin	400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 9 Consolidation therapy dosing regimen for Consolidation Arm a in paediatrics

Consolidation Arm a: Paediatric Dosing [A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
Oral clofazimine Dosing may be rounded to account for capsules.		**<40kg** 3-5mg/kg, max 100mg	Once daily
		≥40kg 100mg	
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral linezolid	1 month – 9 years	10mg/kg, max 450mg	Twice daily
	10 – 12 years	10mg/kg, max 600mg	Daily
	>12 years	600mg	Daily
Oral trimethoprim with sulfamethoxazole		5mg/kg trimethoprim max 160mg trimethoprim	Twice daily
Oral bedaquiline (age ≥5 years)	**Weeks 1 and 2**		
	≥15kg - <20kg	160mg	Once daily
	≥20kg - <30kg	200mg	
	≥30kg	400mg	
	Weeks 3-24 - Max Duration 6 Months		
	≥15kg - <20kg	80mg	Thrice weekly. At least 48 hours between doses
	≥20kg - <30kg	100mg	
	≥30kg	200mg	
Oral rifabutin		5mg/kg, max 300mg	Once daily
Oral doxycycline (ages ≥8 years)		2mg/kg, max 100mg	Once daily
Oral moxifloxacin Dosing may be rounded to account for capsules.		10-15mg/kg, max 400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 10 Consolidation therapy dosing regimen for Consolidation Arm b in paediatrics

Consolidation Arm b: Paediatric Dosing [A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) (IV formulation)		500mg	Twice daily
Oral clofazimine Dosing may be rounded to account for capsules.		**<40kg** 3-5mg/kg, max 100mg	Once daily
		≥40kg 100mg	
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral linezolid	1 month – 9 years	10mg/kg, max 450mg	Twice daily
	10 – 12 years	10mg/kg, max 600mg	Daily
	>12 years	600mg	Daily
Oral trimethoprim with sulfamethoxazole		5mg/kg trimethoprim max 160mg trimethoprim	Twice daily
Oral bedaquiline (age ≥5 years)	**Weeks 1 and 2**		
	≥15kg - <20kg	160mg	Once daily
	≥20kg - <30kg	200mg	
	≥30kg	400mg	
	Weeks 3-24 - Max Duration 6 Months		
	≥15kg - <20kg	80mg	Thrice weekly. At least 48 hours between doses
	≥20kg - <30kg	100mg	
	≥30kg	200mg	
Oral rifabutin		5mg/kg, max 300mg	Once daily
Oral doxycycline (ages ≥8 years)		2mg/kg, max 100mg	Once daily
Oral moxifloxacin Dosing may be rounded to account for capsules.		10-15mg/kg, max 400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

10.5.1 DRUG SUPPLY, STORAGE AND DISTRIBUTION DURING CONSOLIDATION THERAPY

Supply, distribution and storage of drugs will be the responsibility of the site coming from site/institution pharmacy stocks as per standard of care and made in accordance with site-specific guidelines and requirements. Medications the participant is taking which are not investigational products, including adjunctive treatments, for example, medication to treat nausea, will be commercially available products and are to be sourced and supplied locally by study sites according to local standard practices.

10.5.2 DRUG COMPLIANCE DURING CONSOLIDATION THERAPY

The procedures for distribution of consolidation therapy are site dependent. Drug compliance during consolidation therapy will be captured from pharmacy records of drugs dispensed to the participant while enrolled in FOR*Ma*T Appendix A1.

11 SCHEDULE OF ASSESSMENTS

Table 11 lists the special considerations that are applicable to all Schedule of Assessment tables. Table 11 must be used in conjunction with Tables 12 to 14. Tables 12 to 14 show the schedules of assessment tables for participants enrolled in Appendix A1. Table 12 shows the schedule of assessments for all participants from Screening to end of Week 6, Table 13 shows the schedule of assessments from Week 7 to Week 12 for participants randomised to Prolonged Intensive and Table 14 shows the schedule of assessments for all participants during the Consolidation therapy phase and at the Final Outcome. Acceptable study visit windows are also outlined in tables 12 to 14.

Table 11 Special considerations for Schedule of Assessments

Symbol	Definition
A	Participant reconsent is required with IRB/ IED/HREC approved changes to the protocol that affect the participants rights and/or safety and/or if a child turns 18 years old during the trial and must reconsent as an adult participant.
B	Visits may be conducted while an inpatient or at home if participant is receiving home-based care.
C	To be eligible to enrol in the Intervention Program, the participant is required to have either two (2) MABS-positive sputum samples or one (1) MABS-positive BAL sample. Refer to section 7.3 for further information.
D	To determine MABS clearance following four (4) weeks of intensive therapy, three sputum samples or one BAL sample are required to be collected in Week 4 (±3 days) to ensure results are available by week 6 of intensive therapy to inform randomisation.
E	Adult height is to be recorded once (preferably at the screening visit). Paediatric height must be measured at least every six weeks.
F	Only required if the screening assessments were reviewed more than two (2) weeks earlier than Day 1.
G	Refer to section 7.2 for eligibility criteria for the screening visit chest CT.
H	Only required in female participants of childbearing potential. A serum pregnancy test is required at screening and final study visit. A urine or serum pregnancy test is acceptable at all other times.
I	Screening audiology and screening vestibular assessment can be performed if a participant has commenced intensive therapy but must be performed within three (3) days of first receiving treatment.
J	QT interval at screening is to be corrected using the Fridericia method. Any subsequent abnormal QTc intervals are to be confirmed using the Fridericia method.
K	Six-minute walk test to be performed in participants ≥18 years of age only.
L	Plasma levels post inhaled amikacin are to be collected 90 minutes post dose (range 60 to 120 minutes). Please record inhaled amikacin start and stop times.
M	IV Amikacin Therapeutic Drug Monitoring to be completed within 48 hours of dosing according to site protocol.
N	The EQ-5D-5L is to be administered to participants ≥18 years of age whereas the EQ-5D-Y is to be administered to participants 8 to 17 years of age. The EQ-5D-Y Proxy is to be administered to parents/carers of participants 4 to 7 years of age.
O	The SF-36 is to be administered to participants ≥16 years of age at time of Screening. For participants aged <16 years at Screening, the PedsQL™ is to be used for entire duration of trial.
P	The PedsQL™ is only to be assessed in participants <16 years of age at time of Screening. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R.
Q	The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen (≥14 years of age), CFQ-R child (6 to 13 years of age) and CFQ-R parent (parent/carer of participant 6 to 13 years of age).
R	The Child Health Utility is only to be assessed in participants 7 to 17 years of age.

S	The SGRQ is only to be assessed in non-CF participants 18 years of age and older.
T	Costs questionnaire only required for participants that are randomised to immediate consolidation.
U	MARS-5 is only to be completed in participants in the intervention cohort on outpatient based MABS-PD treatment.
V	Week 12 chest CT scan is optional and requires participant to consent to FOR*Ma*T Sub-Study C3: Imaging. The site must have approval to conduct this additional scan, be certified to perform the scan using the scanner specific protocol and the participant must provide additional consent.
W	To determine MABS clearance following ten (10) weeks of intensive therapy, three sputum samples or one BAL sample are required to be collected in Week 10 (±3 days).
X	Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.
Y	Participants who have produced sputum samples during Weeks 18, 28, and 38 are requested to provide three additional sputum samples collected at least one week apart nearer to the end of Weeks 52. For participants who were unproductive (intermittent or continual) during Weeks 18, 28, 38 and 52, a BAL sample is to be collected during Week 56.
Z	Clinic visit and some assessments at Week 10 can be completed at any time between Week 10 and Week 12.
1	Chest CT scan at early withdrawal visit will only be requested if clinically indicated.

Table 12 Schedule of Assessments for Intervention Program Participants: Intensive Therapy (Screening and first six weeks of intensive therapy)

ASSESSMENT		SCREENING VISIT	INTENSIVE THERAPY Week 1 - Week 6						
		Day 0 *-42 Days*	**Day 1** *+24 hours*	**Week 1** *+3 Days*	**Week 2** *±3 Days*	**Week 3** *±3 Days*	**Week 4** *±3 Days*	**Week 5** *±3 Days*	**Week 6** *±3 Days*
Informed consent for Appendix A1[A]		✓							
Clinic visit [B]		✓	✓	✓	✓	✓	✓	✓	✓
Review eligibility		✓							
Randomisation			✓						✓
Adverse event monitoring			✓	✓	✓	✓	✓	✓	✓
Respiratory sample	Sputum (expectorated or induced) ***or;***	✓[C]					✓ x3[D]		
	BAL	✓[C]					✓[D]		
Height [E] and Weight		✓							✓
Medication Review		✓	✓[F]	✓	✓	✓	✓	✓	✓
Spirometry		✓							✓
Chest CT		✓[G]							
Pregnancy Test and Breastfeeding Status [H]		✓	✓		✓		✓		✓
Audiogram		✓[I]					✓		
Vestibular Monitoring		✓[I]					✓		
ECG [J]		✓			✓				✓
6-minute walk test [K]		✓							
Blood collection:									
1. Chemistry and Renal Function		✓		✓	✓	✓	✓	✓	✓
2. Liver Function Tests		✓		✓	✓	✓	✓	✓	✓
3. Full Blood Count		✓		✓	✓	✓	✓	✓	✓
Physical examination		✓	✓[F]						✓
Amikacin monitoring	Therapeutic Drug Monitoring, ***or;***		✓[M] Arm A & C		✓ Arm A & C				✓ Arm A & C
	Post dose levels [L]			✓ Arm B only					
Health-related quality of life questionnaires:									
1. EQ-5D-5L or -Y [N]		✓							✓
2. SF36 [O]		✓							✓
3. PedsQL™ [P]		✓							✓
4. CFQ-R [Q]		✓							✓

ASSESSMENT	SCREENING VISIT	INTENSIVE THERAPY Week 1 - Week 6						
	Day 0 *-42 Days*	**Day 1** *+24 hours*	**Week 1** *+3 Days*	**Week 2** *±3 Days*	**Week 3** *±3 Days*	**Week 4** *±3 Days*	**Week 5** *±3 Days*	**Week 6** *±3 Days*
5. Child Health Utility 9D [R]	✓							✓
6. SGRQ [S]	✓							✓
Costs Questionnaire	✓							✓
Medication Adherence Questionnaire [U]								✓

Table 13 Schedule of Assessments for Intervention Program Participants: Prolonged Intensive Therapy(Total of 12 weeks of intensive therapy)

ASSESSMENT		PROLONGED INTENSIVE THERAPY Week 7- Week 12					
		Week 7 ***±3 Days***	**Week 8** ***±3 Days***	**Week 9** ***±3 Days***	**Week 10** ***±3 Days***	**Week 11** ***±3 Days***	**Week 12** ***±3 Days***
Clinic visit [B]		✓	✓	✓	✓	✓	✓
Randomisation							✓
Adverse event monitoring		✓	✓	✓	✓	✓	✓
Respiratory sample	Sputum (expectorated or induced) ***or;***				✓ x3[W]		
	BAL				✓[W]		
Height [E] and Weight							✓
Medication Review		✓	✓	✓	✓	✓	✓
Spirometry							✓
Chest CT							Optional [V]
Pregnancy Test and Breastfeeding Status [H]				✓			✓
Audiogram			✓				✓
Vestibular Monitoring			✓				✓
ECG[J]							✓
6-minute walk test [K]							✓
Blood collection:							
7. Chemistry and Renal Function		✓	✓	✓	✓	✓	✓
8. Liver Function Tests		✓	✓	✓	✓	✓	✓
9. Full Blood Count		✓	✓	✓	✓	✓	✓
Physical examination							✓
Amikacin Therapeutic Drug Monitoring							✓ Arm A & C
Health-related quality of life questionnaires:							
10. EQ-5D-5L or -Y [N]							✓
11. SF36 [O]							✓
12. PedsQL™ [P]							✓

ASSESSMENT	PROLONGED INTENSIVE THERAPY Week 7- Week 12					
	Week 7 ***±3 Days***	**Week 8** ***±3 Days***	**Week 9** ***±3 Days***	**Week 10** ***±3 Days***	**Week 11** ***±3 Days***	**Week 12** ***±3 Days***
13. CFQ-R [Q]						✓
14. Child Health Utility 9D [R]						✓
15. SGRQ [S]						✓
Costs Questionnaire						✓
Medication Adherence Questionnaire [U]						✓

Table 14 Schedule of Assessments for Consolidation Therapy and Final Outcome

ASSESSMENT		CONSOLIDATION THERAPY										FINAL STUDY VISIT	Early Withdrawal Visit
		Immediate consolidation *After completing six weeks (short) intensive therapy*						Delayed consolidation *After completing 12 weeks (prolonged) intensive therapy*					
		Week 10 *±3 days*	**Week 12** *±3 days*	**Week 18** *±30 days*	**Week 28** *±30 days*	**Week 38** *±30 days*	**End of consolidation (Week 52)** *+5 days*	**Week 18** *±30 days*	**Week 28** *±30 days*	**Week 38** *±30 days*	**End of consolidation (Week 58)** *+5 days*	**+4 weeks post End of consolidation** *+14 days*	**+30 days**
Clinic visit [B]		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Adverse event monitoring		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Respiratory sample	Sputum (expectorated or induced) ***or;***	✓ x3[W]		✓[X]	✓[X]	✓[X]	✓[X]	✓[X]	✓[X]	✓[X]	✓[X]	✓[Y]	✓
	Broncho-alveolar lavage (BAL)	✓[W]											
Height [E] and Weight		✓[Z]	✓		✓		✓		✓		✓	✓	✓
Medication Review		✓[Z]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Spirometry			✓									✓	✓
Chest CT			Optional [V]									✓	✓[1]
Pregnancy Test and Breastfeeding Status [H]			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Audiogram			✓		✓				✓			✓	✓
Vestibular Monitoring			✓		✓				✓			✓	✓
ECG [J]			✓		✓				✓			✓	✓
6-minute walk test [K]			✓									✓	✓
Blood collections:													
1.	Chemistry and Renal Function	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2.	Liver Function Tests	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3.	Full Blood Count	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical examination			✓									✓	✓
Health-related quality of life questionnaires:													
4.	EQ-5D-5L or -Y [N]		✓									✓	✓
5.	SF-36 [O]		✓									✓	✓
6.	PedsQL™ [P]		✓									✓	✓
7.	CFQ-R [Q]		✓									✓	✓
8.	Child Health Utility 9D [R]		✓									✓	✓
9.	SGRQ [S]		✓									✓	✓
Costs Questionnaire			✓		✓				✓			✓	✓
Medication Adherence Questionnaire [U]			✓		✓		✓		✓		✓		

12 RANDOMISATION

There will be three stages of randomisation in the Intervention Program Appendix A, dictating the treatment the participant will receive:

***Randomisation to Short Intensive (R-SI)*:** The first randomisation will be at the start of the intensive phase, with all participants randomised between the different intensive therapy arms according to the study specific Appendix.

Randomisation to Prolonged Intensive or Immediate Consolidation (R-PI/IC): The second randomisation will ONLY be for participants who are still MABS positive at the end of short intensive therapy (based on respiratory sampling collected at 4 weeks) and are able to continue with intensive therapy. Randomisation will occur at the end of short intensive therapy and will allocate participants to either;

> Continue intensive therapy which will be followed by consolidation (participants remain on the same intensive therapy drug regimen if randomised to prolonged intensive therapy),
>
> or immediately commence consolidation therapy.

Randomisation to Consolidation (R-Con): This randomisation will allocate participants to the consolidation therapy arms. Each randomisation will function as a 'quasi-separate' trial (as described in the relevant sections of Appendix A), as well as being considered in combination (intensive + consolidation). Randomisation at each level will be conducted using the method of minimisation (described in the relevant sections of the Master Protocol and Appendix F: General Statistical Principles). Each randomisation level will be planned to enable flexibility via pre-planned adaptations as described above.

13 APPLICABLE DISCOVERY STUDIES AND REGISTRY LINKAGE FOR APPENDIX A1

Intervention Program participants may be eligible to enrol in the following FOR*Ma*T Sub-Studies and Integrated Studies:

1) Appendix C Discovery
   i. C1: Pharmacokinetics
      i) C1.1 Steady state pharmacokinetics of Amikacin
      ii) C1.2 Microsampling and non-blood matrix validation for amikacin TDM
      iii) C1.3 Pharmacokinetics of MABS-PD therapies
      iv) C1.4 Pharmacokinetics of CFTR modulator therapy in persons with CF on MABS therapy
   ii. C2: Immune factors and biomarkers
      i) C2.1 Macrophage function
      ii) C2.2 Mitochondrial stress
      iii) C2.3 T-cell function
      iv) C2.4 Gene expression
      v) C2.5 Serology
   iii. C3: Imaging
2) Appendix D Registry Linkage
   i. D1: Australian cystic fibrosis data registry
3) Appendix E Health Economics
   i. E1: Cost effectiveness and Resource utilisation

Please see the relevant sections of the applicable appendix for further information, including additional eligibility criteria.

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APPENDIX A2 - INTERVENTION PROGRAM

Consolidation Only Therapy

Appendix A2 – Intervention Program (Consolidation Only Therapy)	Version 1.1
Appendix Date	26 November 2024
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
EudraCT Number	2020-000050-10
EU-CT Number	2023-506575-99-00-EU CT
IRAS Project Number	1007149
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AE	Adverse Event
AFB	Acid-Fast Bacilli
BAL	Bronchoalveolar Lavage
BAR	Bayesian Adaptive Randomisation
CEAC	Cost-Effectiveness Acceptability Curves
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CHU9D	Child Health Utility 9D
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DVA	Dynamic Visual Acuity
ECG	Electrocardiogram
eCRF	electronic Case Report Form
FEV1	Forced Expiratory Volume in one second
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HIT	Head Impulse Test
HRCT	High-Resolution Computed Tomography
HRQoL	Health Related Quality of Life
IA	Inhaled Amikacin
ICD-10	International Statistical Classification of Diseases
ICER	Incremental Cost-Effectiveness Ratio
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MARS-5	5-item Medication Adherence Rating Scale

MoOP	Manual of Operating Procedures
MPM	Medication Possession Ratio
NMP	Net Monetary Benefit
NTM	Non-Tuberculous Mycobacteria
PedsQL™	Pediatric Quality of Life Inventory
QALY	Quality-Life Adjusted Years
QoL	Quality of Life
REDCap	Research Electronic Data Capture
R-Con	Randomisation – Consolidation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SF-36	Short form-36
SGRQ	St George Respiratory Questionnaire
SOP	Standard Operating Procedure
TDM	Therapeutic Drug Monitoring
TPF	Time Point Final
TPS	Time Point Screening
TPST-Con	Time Point Start Treatment - Consolidation
VAS	Visual Analogue Scale
vHIT	Video Head Impulse Test
VOR	Vestibulo-ocular Reflex
WT	Willingness to Pay

APPENDIX A: INTERVENTION PROGRAM

Appendix A contains the Intervention Program modules, with new modules created as interventions are added to either intensive or consolidation phases of the trial. They are numbered sequentially, A1, A2, A3 etc, with Appendix A1 describing the first iteration of the Intervention Program. The relevant detailed statistical methods and simulations for each Intervention Program module will be described in Appendix F: General Statistical Principles. Separate consent procedures may be required for each appendix. Of note, in some cases data collected as part of one appendix may be incorporated in the analysis of another appendix, if collected to address the same objective. If this occurs, it will be detailed in the relevant appendices. Should an intervention arm be dropped or a new intervention arm added either to intensive therapy or consolidation therapy this will require a new appendix and a new statistical analysis plan.

Each program in Appendix A will stipulate which of the Discovery studies and Registry linkages is applicable. The Intervention program modules in Appendix A will include the following:

- A description of the primary and secondary objectives.
- A description of the intervention trial design including any specific inclusion or exclusion criteria specific to the Intervention program modules.
- A description of the interventions and dosing.
- The methods for assigning treatment arms to the intervention program participants.
- Consent requirements.
- Specific intervention program trial procedures, monitoring and safety requirements and schedule of assessments.
- A description of relevant nested studies.
- A description of cost effectiveness methodology and analysis as appropriate will be detailed in Appendix E: Health Economics.
- A description of statistical analyses and simulations will be detailed in Appendix F: Statistical Principles.
- The Discovery studies applicable to the module (if relevant).

APPENDIX A2 CONSOLIDATION ONLY THERAPY

Appendix A2 describes the MABS-PD consolidation only intervention platform for the FOR*Ma*T Trial. This Appendix applies to sites that are unable to undertake the FOR*Ma*T intensive therapy module themselves and are also unable to partner with a site that is able to manage the FOR*Ma*T intensive therapy phase. Such sites can choose to undertake the FOR*Ma*T Consolidation Only therapy module after any physician led intensive therapy.

Note, the data collected as part of this Appendix will be combined with data from Appendix A1.3 to address the objectives stated in Appendix A1.3.

FOR*Ma*T Appendix A2 Summary	
Treatment combinations	Consolidation therapy arms only listed in Appendix A2. ***Reference:*** Consolidation arm a ***Experimental:*** Consolidation arm b
	A mixed NTM infection (slow grower + MABS) can include the use of ethambutol in the consolidation phase of treatment.
Appendix A2-specific eligibility:	Inclusion and exclusion criteria as per Master Protocol section 4.1 and below.
Appendix A2-specific inclusions:	1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD at the time of starting physician-directed intensive therapy defined as; a. Clinical: Pulmonary symptoms and exclusion of other diagnoses. b. Radiological: Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules. c. Microbiological: MABS positive culture results from at least two separate expectorated sputum samples. ***or;*** Positive culture results from at least one bronchial wash or bronchoalveolar lavage (BAL). ***or;*** Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli (AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM. 2. Male or female participants of any age. 3. Informed consent for consolidation therapy signed by participant or parent/legal guardian if participant is under 18 years of age. 4. Participant has not received MABS-PD treatment in the 12 months prior to receiving the physician-directed intensive therapy (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Standard Operating Procedure (SOP) 14). 5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.

	6. For randomisation the participant must provide respiratory samples (three sputums (expectorated or induced) or one BAL, two (2) weeks before completing the physician-directed intensive therapy and have culture results available for randomisation.
Appendix A2-specific exclusions:	Participants receiving current treatment for MABS within the previous 12 months prior to commencing physician-directed intensive therapy (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP, except for participants taking azithromycin as part of routine treatment for cystic fibrosis (CF) or chronic infection-related pulmonary disease. 1. Positive pregnancy test at any time during the FOR*Ma*T trial for females of childbearing potential. 2. Breast-feeding. 3. An unwillingness to comply with the acceptable methods of contraception defined in the Appendix A2 Section 5. 4. QTc> 500 milliseconds (QT interval to be corrected based on Fredericia method) 5. Known hypersensitivity or contraindications to any of the therapies for which no alternative option(s) have been provided. This includes: a. Amikacin, b. Macrolide antibiotics, and c. Clofazimine.
Target recruitment:	300 participants across the combination of Appendix A1 and Appendix A2
Outcome measures:	***Primary outcome:*** MABS clearance from respiratory sample(s) with tolerance at Final Outcome. ***Secondary outcomes:*** 1. Probability of MABS clearance at Final Outcome irrespective of toxicity according to participant's treatment pathway. 2. Safety of oral versus oral plus inhaled treatment, including changes in microbiological resistance. 3. Change in FEV1 z-score between Screening and Final Outcome between participants allocated to oral consolidation treatment versus oral treatment and additional IA taking into account MABS clearance at Final Outcome. 4. Changes in chest CT parameters between 6 weeks and Final Outcome taking into account MABS clearance at Final Outcome between participants allocated to oral consolidation treatment versus oral treatment and additional IA. 5. Change in 6-minute walk distance (6MWD) from Screening to Final Outcome between participants allocated to oral consolidation treatment versus oral treatment and additional IA. 6. Change in HRQoL for participants from Screening to Final Outcome between participants allocated to oral consolidation treatment versus oral treatment and additional IA. 7. Cost effectiveness of consolidation treatment between participants allocated to oral consolidation treatment versus oral treatment and additional IA. 8. Examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives. Exploratory Outcomes: 1. Participant's MABS clearance status 12 months after Final Outcome.
Timepoints:	***Screening:*** From the Date of consent to the Date of Randomisation-Consolidation (R-Con). ***Time Point Start Treatment-Consolidation (TPST-Con):*** Randomisation-Consolidation (R-Con).

	Time Point Final (TPF):	End of 46 weeks of treatment plus 4 weeks off treatment (Final Outcome Visit date).

1 INTRODUCTION

Currently, the guidelines for consolidation therapy (1) suggest that patients with isolates that are sensitive to macrolides or that have inducible resistance should be managed with a combination of between one and three oral antibiotics based on drug susceptibility of the isolate and tolerance in combination with inhaled amikacin (IA). Those with isolates that have constitutive resistance should use a combination of between two and four oral drugs based on susceptibility and tolerance in combination with IA. In addition, guidelines suggest that patients with isolates that have amikacin resistance could substitute IA for an alternative oral antibiotic. It is suggested that treatment should continue for 12 months after culture conversion.

There is currently no evidence for any of these guidelines. IA is costly and requires time and effort for maintenance of hygienic practice and drug delivery. Furthermore, adherence to inhaled therapies over the longer term is variable (2-4). This study will examine the effects of adding IA to oral treatment thus providing some evidence regarding the use of IA during consolidation therapy. The timing of consolidation therapy is only 46 weeks for this trial and patients are free to continue treatments after completion of the trial. Microbiological clearance will be determined from culture of respiratory samples (sputum (expectorated or induced) or BAL) collected ***at least one week apart*** as follows:

1. Participants who continue to produce sputum samples during the consolidation only therapy phase are requested to provide additional sputum samples (preferably monthly). Three consecutive sputum samples will be collected at least one week apart over the last 6 weeks of consolidation and a final sputum sample collected four weeks after cessation of consolidation therapy. These samples will together determine the final outcome microbiological clearance. All four samples will need to be clear to confirm clearance.
2. For participants who continue to have positive sputum cultures at the end of 46 weeks of consolidation, a gap in therapy to final outcome visit may not be required if ongoing treatment is clinically indicated as microbiological clearance will not have occurred.
3. Participants who have negative cultures on treatment at end of consolidation will need to cease treatment for 4 weeks to enable assessment of microbiological clearance without antibiotic suppression at the final outcome visit.
4. For participants who are unproductive of sputum during the consolidation phase, or who become unproductive of sputum during the course of consolidation therapy, a BAL is to be performed at final outcome (4 weeks after completing consolidation).

Sites that are unable to undertake the intensive therapy module themselves and are also unable to partner with a site that is able to manage the intensive therapy phase, can choose to only undertake the Consolidation Only therapy module.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE

The objective of Appendix A2 is to enable more rapid comparison of the microbiological clearance of MABS with good tolerability between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral treatment and additional IA at Final Outcome (46 weeks of consolidation therapy plus 4 weeks off treatment) by recruiting eligible participants from sites that are unable to undertake Appendix A1 intensive therapy. The data collected as part of this appendix (A2) will be combined with the data from Appendix A1.3 to address the same primary (and secondary) objectives as stated in Appendix A1.3 and in A2.

Definition of tolerance:

Tolerance is based on the Common Terminology Criteria for Adverse Events (CTCAE version 5.0). Only adverse events that are attributed as either "possibly", "probably", or "definitely" related to study drug will be assessed in the determination of tolerance. "Good" tolerance is defined as no adverse events occurring or only adverse events coded as CTCAE grades 1 and 2. "Poor" tolerance is defined as any adverse events attributed as possibly, probably, or definitely related to study drug coded as CTCAE grades 3, 4, or 5.

MABS clearance at Final Outcome will be defined as:

Negative MABS cultures from four consecutive sputum samples with one of those sputum specimens collected four weeks after the completion of consolidation therapy (at Final Outcome) or, a MABS negative BAL collected four weeks after completion of consolidation (at Final Outcome).

2.2 SECONDARY OBJECTIVES

As stated above, the data collected in this Appendix will contribute to the assessment of the secondary objectives stated in Appendix A1.3, namely:

1. To compare microbiological clearance (irrespective of toxicity) at completion of consolidation therapy between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA at Final Outcome.
2. Differences in safety between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.
3. Change in forced expiratory volume in one second (FEV1) z-score between Screening and Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA taking into account microbiological clearance at end of physician led intensive therapy.
4. Change in computed tomography (CT) scan parameters (bronchiectasis, mucus plugging, airway wall thickening, atelectasis, % disease and air trapping), between 6 weeks and at Final Outcome taking into account microbiological clearance at end of physician led intensive therapy between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.
5. Change in 6-minute walk distance (6MWD) between Screening and Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.

6. Changes in health-related quality of life (HRQoL) (Cystic Fibrosis Questionnaire-Revised (CFQ-R)) for participants with CF between Screening and Final Outcome in those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.
7. To examine general HRQoL between Screening and Final Outcome between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.
8. To compare the cost effectiveness of consolidation between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA.
9. Examine causes for early withdrawal from MABS-PD treatment due to reasons other than poor tolerance as defined in the primary objectives between those allocated to consolidation therapy with oral treatment and those allocated to oral treatment with addition of IA at Final Outcome.

2.3 EXPLORATORY OBJECTIVES

The data collected in this Appendix will contribute to the assessment of the exploratory objective stated in Appendix A1.3, namely:

- To examine MABS clearance status at 12 months after Final Outcome.

3 DESIGN

Appendix A2 describes the consolidation only module and will test therapies that are currently used and are the basis for the current treatment guidelines (Figure A2.1).

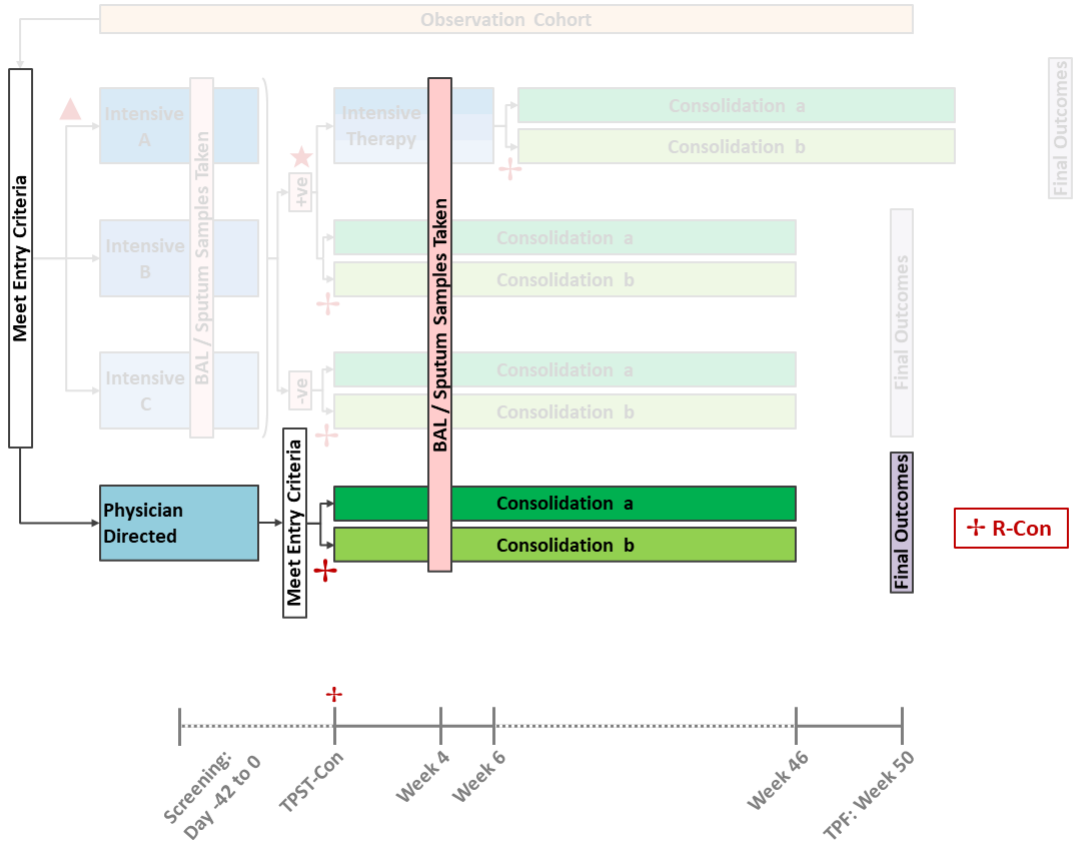

Figure 1 Flow Diagram for Appendix A2: Consolidation Only Therapy program Eligibility into the consolidation only therapy program is determined at screening. Randomisation-Consolidation (R-Con)(✛) allocates participants to the consolidation therapy arms depending on MABS status at 2 weeks prior to entry into Consolidation Only therapy module.

4 ELIGIBILITY CRITERIA

Participants are eligible for Appendix A2 if the following criteria are met in addition to those outlined in section 4.2 in the FOR*Ma*T Master Protocol:

4.1 INCLUSION CRITERIA

1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD prior to starting physician directed intensive therapy for MABS eradication. Defined as:

   - ***Clinical:*** Pulmonary symptoms and exclusion of other diagnoses.
   - ***Radiological:*** Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.
   - ***Microbiological:*** MABS positive culture results from at least two separate expectorated sputum samples.

   or

   Positive culture results from at least one bronchial wash or lavage.

   or

   Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli, AFB) and positive culture for NTM ***or*** biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM.

2. Male or female participants of any age.
3. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age. For those participating in the consolidation only therapy Appendix A2, a specific consolidation only therapy consent is required.
4. Participant has not received MABS-PD treatment in the 12 months preceding any physician-directed intensive therapy (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP.
5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.
6. Participant must provide respiratory samples (three sputums (expectorated or induced) or one BAL) two (2) weeks before completing the physician-directed intensive therapy and have culture results available for the randomisation. If participant has not received any physician-directed intensive therapy the screening respiratory samples may be used for the randomisation.

4.2 EXCLUSION CRITERIA

- Participants receiving current treatment for MABS within the previous 12 months of commencing physician-directed therapy (this includes drugs prescribed for treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP, except for participants taking azithromycin as part of routine treatment for CF or chronic infection-related pulmonary disease.
- Positive pregnancy test at any time during the FOR*Ma*T trial for females of childbearing potential.
- Breast-feeding.
- An unwillingness to comply with the acceptable methods of contraception, as described in Section 5.
- Participants with a QTc interval >500 milliseconds (QT interval corrected based on Fridericia method).
- Known hypersensitivity or contraindications to any of the therapies for which no alternative option(s) have been provided. This includes:
    - Amikacin,
    - Macrolide antibiotics, and
    - Clofazimine.

4.3 ADDITIONAL CRITERIA

4.3.1 MIXED NTM INFECTIONS

Participants who have cultured slow growing NTM of the same species two or more times in the 24 months prior to screening, with one of those cultures within the 6 months prior to screening, will be considered to have mixed NTM infection. If the participants meet all other inclusion criteria and no exclusion criteria they will be eligible for participation. Ethambutol may be used in addition to trial therapies to cover mixed NTM infections considered to require treatment by their clinician.

4.3.2 CO-ENROLMENT WITH OTHER TRIALS

Co-enrolment is not permitted when there is a potential interaction between trial interventions, any compromise to the validity of either trial or impact on participants' rights and/or safety. However, co-enrolment may be permitted in the instance where all trials that the participant is enrolled or to be enrolled in have mutually agreed to the co-enrolment arrangement.

5 ACCEPTABLE METHODS OF CONTRACEPTION

The effects of some drugs used during the Intervention Program on the unborn child and on the newborn baby are not known. Because of this, it is important that participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project.

It is therefore important that birth control is used while in this study. Site investigators should discuss effective methods of contraception with participants. Both male and female participants are to use effective contraception, used correctly with every act of sexual intercourse, from at least 14 days before the first dose of MABS-PD therapy,

during the course of the trial and for a period of 90 days after the last dose of MABS-PD therapy. Acceptable methods of contraception for participants enrolled in Appendix A2 include:

1) Male vasectomy 6 months or more previously, with a documented negative post-vasectomy semen analysis for sperm.
2) Female bilateral tubal ligation performed at least 6 months previously.
3) Female continuous use of an intrauterine device (non-hormone releasing or hormone releasing) for at least 90 days before the first dose of MABS-PD therapy.
4) Female combined (estrogen and progestogen-containing) or progestogen-only oral, injected, implanted or vaginal hormonal contraception associated with inhibition of ovulation.

The barrier contraception methods listed below are not acceptable and are only to be used in special circumstances where the investigator determines that barrier contraception is appropriate.

1) Male and female condom with spermicide (either as a single product if commercially available and/or as allowed according to local regulations; otherwise condom and spermicide as separate products).
2) Male condom with female diaphragm, cervical cap, or vaginal sponge, each with spermicide.

For female participants using birth control pills

The use of birth control pills for contraception must be discussed and approved by the local site investigator at the start of each phase of the trial and when any changes in therapy occur as, some therapies used during the trial may interact with the effectiveness of birth control pills. Birth control pills should be in successful use from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

Female participants who change their method of contraception to birth control pills during the trial must continue to use a second form of approved contraception for at least 60 days after starting the use of birth control pills.

For female participants using hormonal injected, implanted or vaginal contraception

Injected, implanted or vaginal hormonal contraception should be used successfully from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

6 TRIAL CONDUCT

6.1 INFORMED CONSENT

In addition to providing consent to the FOR*Ma*T Master Protocol, participants enrolling in the intervention program are required to sign and date the relevant Appendix A2 consent. Consent will be obtained from participants or their parent/guardian in accordance with the policies described in section 5.4 of the FOR*Ma*T Master Protocol.

6.2 PREGNANCY INFORMATION CONSENT

If a FOR*Ma*T trial participant becomes pregnant or is the biological father of a child conceived while enrolled in this intervention program the site investigators are requested to provide the participant with the FOR*Ma*T pregnancy information and consent form. The FOR*Ma*T pregnancy and consent form requests to follow the participant and the child for 12 months after conception. Please refer to FOR*Ma*T Safety and Monitoring Plan SOP for further detail for safety monitoring and reporting of pregnancies and pregnancy outcomes in female trial participants or female partners who are pregnant to male participants in the intervention program.

6.3 METHODS OF ASSIGNING PARTICIPANTS TO TREATMENT ARMS

Participants will be randomised into different treatment arms for the consolidation only phase of treatment as described in Appendix A2, Section 131 and Master Protocol section 6 using the randomisation technique minimisation in a 1:1 ratio. As described in the Master Protocol, all randomisations will be conducted via Research Electronic Data Capture (REDCap) at each FOR*Ma*T trial site. Study staff will enter the subject demographic data and the stratification factors (see section 6.3 in the Master Protocol) into REDCap, which will then inform them of the participant's treatment allocation.

7 CONSOLIDATION ONLY PROGRAM PROCEDURES AND SAFETY MONITORING

In addition to the core trial procedures described in the Master Protocol, participants enrolled in the Intervention program are required to undertake procedures and regular toxicology monitoring. The type of toxicology monitoring procedure required will be determined by the treatment arm the participant is allocated to, and in accordance with the schedule of assessments outlined in Table 6. Toxicology thresholds will be defined in accordance with CTCAE criteria as outlined in the Master Protocol, section 5.8.6. The outcome of all the assessments below are to be documented in the corresponding case report form (CRF) and entered into REDCap.

7.1 SERUM/URINE PREGNANCY TEST

All female participants of childbearing potential are required to undergo regular pregnancy testing while enrolled in the intervention program. Childbearing potential is defined as a premenopausal female capable of becoming pregnant, and includes females on oral, injectable, or mechanical contraception; females who are single and females whose male partners have been vasectomised or were using mechanical contraception (5). A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A serum pregnancy test will be performed at screening and at the final study visit and if applicable, at the early termination visit. A urine β-hCG test or serum pregnancy is acceptable for regular pregnancy monitoring from day 1 in accordance with the schedule of assessments outlined in Table 6. Both urine and serum pregnancy tests are to be performed in accordance with site specific procedures.

7.2 CHEST CT SCAN

The chest CT scan for screening is be ordered ideally within three months (up to maximum of six months) prior to commencement of physician-directed intensive therapy or screening if participant has not received any intensive therapy. Table 5 outlines the time points for when a chest CT scan is to be performed.

7.3 RESPIRATORY SAMPLING

For screening a minimum of 2 sputum samples (with at least 1 collected within 6 months prior to screening and other samples collected within 12 months prior to screening) or 1 BAL collected within 6 months prior to commencing physician-directed intensive therapy will be required to assess eligibility according to the Appendix A2 eligibility criteria. For randomisation, respiratory samples (three sputums (expectorated or induced) or one BAL) are to be collected ideally two (2) weeks prior to completing physician-directed intensive therapy with culture results available for randomisation. For participants who have not undergone any intensive therapy, the screening respiratory samples may be used for randomisation. Table 6 outlines the minimum respiratory sample collection timepoints; however, monthly sputum sample collections are recommended.

7.4 AUDIOGRAM

To monitor aminoglycoside and/or azithromycin induced ototoxicity, regular audiometry assessments will be undertaken in accordance with the schedule of assessments outlined in Table 6. The battery of assessments to be completed should comply with the site-specific requirements. Assessments can be conducted in either a hospital or community setting.

7.5 VESTIBULAR MONITORING

Vestibular toxicity monitoring will document any vestibular symptoms (motion-induced oscillopsia, postural instability and gait unsteadiness) associated with prolonged aminoglycoside use. Assessments will take place in accordance with the schedule of assessments outlined in Table 6. Testing can be completed either by the treating clinician.

Additional specific vestibular monitoring may be required if deemed necessary by the site investigator, see below.

7.5.1 DYNAMIC VISUAL ACUITY TESTING (DVA)

Ask the patient to read a visual acuity chart (e.g. Snellen) while sitting still at recommend distance. This result is the static visual acuity. Repeat task while oscillating the patient's head horizontally or vertically at 1 to 2 Hz. An abnormal DVA is defined as loss of at least three lines of visual acuity compared with static condition (horizontal and/or vertical).

7.5.2 HEAD IMPULSE TESTING (HIT)

Stand in front of the seated patient, facing them, and ask the patient to focus on a target directly in front of them. Briskly rotate the patient's head horizontally approximately 10 to 20° amplitude, watching the patient's eyes closely. In normal subjects, the patient's eyes remain still as they remain on target. However, in a patient with impaired vestibulo-ocular reflex (VOR), the patient's eyes drift off the target and require a corrective 'catch-up' saccade to re-

fixate on the target and stabilise vision. This catch-up saccade is a small amplitude horizontal eye movement in the opposite direction of the head turn and should occur with every head impulse (repeatable).

If available, Video Head Impulse Test (vHIT) is recommended as this has a higher sensitivity than the traditional bedside head impulse test at detecting impaired VOR. M=The main benefits include detecting covert (hidden) catch up saccades and peer review.

7.5.3 ROMBERG ON FOAM TEST

On a foam surface, ask the participant to stand still with two feet together. The participant should be able to stand steady with their eyes open. If the participant is not able to perform this task, ask them to separate their feet to minimal distance that allows them to do so. Repeat the task this time with their eyes closed. Record if the participant falls (positive Romberg test) or does not (negative Romberg test).

Document vestibular test results and any further actions (if results indicate significant vestibular impairment) in the CRF.

7.6 PHYSICAL EXAMINATION: INTERVENTION

The physical examination will be performed in accordance with the procedures outlined in the FOR*Ma*T Master Protocol, section 5.5.5 in accordance with the schedule of assessments outlined in Table 6.

7.7 ELECTROCARDIOGRAM (ECG)

A standard 12 lead ECG will be performed in accordance with the relevant tables and site-specific procedures after the participant has been supine for at least 5 minutes. The site investigator will interpret, sign and date the ECG. The QTc interval and the clinical interpretation will be recorded in the eCRF and ECGs will be required to be scanned and stored in the eCRF. The QT interval at Screening is to be corrected using the Fridericia method. For any subsequent QTc intervals which are 'abnormal' the Fridericia method is to be used to confirm the QTc value.

7.8 SIX MINUTE WALK TEST

A 6-minute walk test (6MWT) will be performed in adult participants only at the time points outlined in Table 6 if the testing is available at the trial site. The 6MWT will be performed according to the protocol in the ATS Statement: Guidelines for the Six Minute Walk Test (6) and/or local standard procedures. Results of walk test will be recorded in the relevant eCRF in the FOR*Ma*T database by the end of the Final Outcome visit.

7.9 BLOOD SAMPLING

Blood samples will be collected in accordance with Table 6. Liver function, full blood count and chemistry and renal function tests (refer to FOR*Ma*T Monitoring Blood Parameters SOP for specific blood parameters to be measured) are to be performed in accordance with the local pathology requirements. All relevant de-identified blood pathology reports are to be uploaded in the appropriate eCRFs in the FOR*Ma*T database by the end of the Final Study Visit. Any blood abnormalities that meet adverse event (AE) or serious adverse event (SAE) criteria are to be reported as an AE/SAE within the specified timeframes.

7.10 STUDY MEDICATION REVIEW

Participant adherence to MABS-PD treatment will be measured via two indirect methods. Self-reported adherence will be assessed using a validated questionnaire, the 5-item Medication Adherence Rating Scale (MARS-5)(7, 8) at the timepoints outlined in Table 6 and results recorded in the Medication Adherence Questionnaire CRF. Data from pharmacy prescription refill records and prescription claims databases will be obtained (where possible) and used to calculate the refill adherence measure, Medication Possession Ratio (MPR) (9-12).

7.11 HEALTH RELATED QUALITY OF LIFE AND UTILITY MEASURES

HRQoL will be assessed in all participants (where possible) according to the schedule of assessments in the relevant appendix. The questionnaires are required to be completed prior to any clinical assessment and are dependent on the participant's age and whether they have CF. The age appropriate HRQoL questionnaire issued to the participant at the start of the study will continue to be used throughout the study even if the participant progresses to a different age range. HRQoL questionnaires will be made available to study sites. All questionnaires will be made available in English or if available the local language version will be sourced. Questionnaires can be completed by the participant and/or their parent/carer via an online link to the questionnaire(s) on the trial database or if unable to access the electronic form these may be completed using a paper-based questionnaire. Responses from paper-based questionnaires are to be entered into the trial database by trial site staff.

7.11.1 CYSTIC FIBROSIS QUESTIONNAIRE-REVISED

The CFQ-R has been developed specifically for use in people with CF. This questionnaire measures the impact of CF on overall health, daily life, perceived well-being and symptoms. Age-appropriate questionnaires have been developed; CFQ-R teen/adult for adolescents and adults 14 years of age and older, the CFQ-R child for those 6-13 years of age, and the CFQ-R parent for the parents of those aged 6-13.

In children ≤16 years of age, the CFQ-R should be administered after the PedsQL™.

7.11.2 EQ-5D-5L

The EQ-5D-5L questionnaire is a standardised measure of health status in adults 18 years of age and older. This questionnaire can be applied as a generic measure of health for clinical and economic appraisal, including the calculation of quality-life adjusted years (QALYs). The EQ-5D-5L measures five dimensions:

1. Mobility;
2. Self-care;
3. Usual activities;
4. Pain/discomfort;
5. Anxiety/depression.

Each dimension has five possible answers; no problems, slight problems, moderate problems, severe problems and extreme problems. The respondent is asked to indicate his/her health state by selecting the most appropriate statement.

7.11.3 EQ-5D-Y

The EQ-5D-Y is the child friendly version of the EQ-5D-5L questionnaire. The EQ-5D-Y has been developed for use in children 8 to 17 years of age. The dimensions and the visual analogue scale (VAS) measured are the same as the EQ-5D-5L questionnaire, but with child friendly wording. For children 4 to 7 years of age the proxy 1 version of the EQ-5D-Y can be utilised allowing the respondent (parent/carer) to evaluate participants QOL from respondents' own view.

7.11.4 ST. GEORGE RESPIRATORY QUESTIONNAIRE (SGRQ)

The SGRQ is to be completed by all non-CF participants 18 years of age and older. The SGRQ is a supervised self-administered 50-item questionnaire measuring health status across three domains; symptoms, activity and impacts (psycho-social) in people with airway obstruction.

7.11.5 SHORT FORM- 36 HEALTH SURVEY

The SF-36 health survey is a self-reported questionnaire applicable in all participants 16 years and older. Covering eight health concepts the SF-36 health survey is a generic outcome measure designed to examine a person's perceived health status.

7.11.6 PEDIATRIC QUALITY OF LIFE INVENTORY

The PedsQL™ child health questionnaire is a non-preference-based measure to assess HRQoL for children and adolescents from 2 years up to 16 years of age. Children less than 16 years of age at screening will continue to use the PedsQL™ up until the end of the trial, rather than change to using the SF-36 if they turn 16 years of age during the study.

Developmentally appropriate child self-report questionnaires are available (ages 5-7, 8-12, 13-18) together with parent/carer proxy-reports (ages 2-4, 5-7, 8-12 and 13-18). If feasible, the PedsQL™ should be completed *before* the respondents complete any other health data forms and *before* they see their physician or healthcare provider.

Parents/carers, children (aged 8-12) and young people (aged >12 years) may self-administer the PedsQL™ after the FOR*Ma*T site researcher has provided instructions. If it is determined by the FOR*Ma*T site researcher that the child, young person or parent/carer is unable to self-administer the PedsQL™ the questionnaire should be administered, word for word by the FOR*Ma*T site researcher. If the child has difficulty understanding the age appropriate PedsQL™ the preceding age questionnaire may be used. The parent and the child must complete the questionnaires independently of each other and in accordance with the PedsQL™ administration guidelines (available via hyperlink or refer to relevant section in FOR*Ma*T Trial Site Manual of Operating Procedures (MoOP)).

7.11.7 CHILD HEALTH UTILITY 9D (CHU9D)

The CHU9D is a generic preference-based measure of paediatric HRQoL for use in children 7 to 17 years of age. The use of a descriptive system and a set of preference weights allows for the calculation of QALYs for use in economic evaluation.

7.12 MABS CLEARANCE FOLLOW-UP QUESTIONNAIRE

Site investigators will be requested to complete a follow up questionnaire for eligible intervention participants to assess microbiological clearance of MABS at 12 months after final outcome.

8 SITE REIMBURSEMENT

For participants enrolled in Appendix A2, trial sites will be reimbursed on a per participant basis at each of the following time points;

1. Screening;
2. End of consolidation;
3. Final study visit.

Sites will be reimbursed according to their contract. Payments will be paid for each time point once the data is entered into the trial database and all queries finalised. Invoices should be prepared at a minimum of every 6 months.

9 CONSOLIDATION ONLY THERAPY DOSING REGIMENS

Following R-Con, participants will receive consolidation treatment in accordance with the dosing tables below. Drug therapy, administration and duration is dependent on the treatment arm (Consolidation Arm a and Consolidation Arm b), age, and/or weight of the participant. The start and end points of each drug therapy, the dose of each drug therapy used, any changes in dosing, as well as all concomitant medications used, will be required to be entered into the relevant CRF.

There are currently two proposed treatment arms that participants will be randomised to during consolidation therapy.

Consolidation Arm a	**Consolidation Arm b**
1. Oral clofazimine, and; 2. Oral azithromycin or oral clarithromycin, and; In combination with one to three of the following oral antibiotics: • Oral linezolid, • Oral trimethoprim / sulfamethoxazole (co-trimoxazole), • Oral bedaquiline, • Oral rifabutin, • Oral doxycycline, • Oral moxifloxacin.	1. Inhaled amikacin 2. Oral clofazimine, and; 3. Oral azithromycin or oral clarithromycin, and; In combination with one to three of the following oral antibiotics: • Oral linezolid, • Oral trimethoprim / sulfamethoxazole (co-trimoxazole), • Oral bedaquiline, • Oral rifabutin, • Oral doxycycline, • Oral moxifloxacin.

For participants with confirmed mixed NTM infections (slow growers + MABS), ethambutol can be added to the treatment arms (in accordance with the dosing tables below) if required by the treating physician.

The consolidation therapy dosing regimen tables outlined below are separated by age (adult, paediatric) and by consolidation treatment arm (Arm a and Arm b). The recommended doses and frequencies are a guideline and participant dosing must also take into account clinical judgement and relevant prescribing information.

These medications have the potential for drug-drug interactions. The treating physician must consider drug and non-drug interactions, special warnings and precautions for use prior to prescribing these medications according to the relevant prescribing information.

Table 1 Consolidation Only therapy dosing regimen for Consolidation Arm a in adults

Consolidation Arm a: Adult Dosing[A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
Oral clofazimine	100 – 300mg	Once daily
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*	250 – 500mg **OR** 500mg **<40kg or poorly tolerated** 250mg	Once daily Thrice weekly Thrice weekly
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.		
Oral linezolid	600mg	Once daily
Oral trimethoprim with sulfamethoxazole	160mg/800mg	Twice daily
Oral bedaquiline *(Weighing at least 30kg)*	**First 2 weeks** 400mg **For remaining 22 weeks** 200mg Max duration 6 months	Once daily Thrice weekly At least 48 hours between doses
Oral rifabutin	5mg/kg, max 450mg	Once daily
Oral doxycycline	100mg	Once daily
Oral moxifloxacin	400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 2 Consolidation Only therapy dosing regimen for Consolidation Arm b in adults

Consolidation Arm b: Adult Dosing[A]		
Drug	**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) **(IV formulation)**	500mg	Twice daily
Oral clofazimine	100 – 300mg	Once daily
Oral azithromycin	250 – 500mg **OR** 500mg	Once daily Thrice weekly
If azithromycin not tolerated, use oral clarithromycin.	**<40kg or poorly tolerated** 250mg	 Thrice weekly
Oral clarithromycin *Clarithromycin only for use if azithromycin not tolerated.*	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.		
Oral linezolid	600mg	Once daily
Oral trimethoprim with sulfamethoxazole	160mg/800mg	Twice daily
Oral bedaquiline *(Weighing at least 30kg)*	**First 2 weeks** 400mg	Once daily
	For remaining 22 weeks 200mg Max duration 6 months	Thrice weekly At least 48 hours between doses
Oral rifabutin	5mg/kg, max 450mg	Once daily
Oral doxycycline	100mg	Once daily
Oral moxifloxacin	400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.		
Oral ethambutol Ethambutol should be dosed on ideal body weight.	15mg/kg (round to account for tablet strength) **OR** 25mg/kg (round to account for tablet strength)	Once daily Thrice weekly

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 3 Consolidation Only therapy dosing regimen for Consolidation Arm a in paediatrics

Consolidation Arm a: Paediatric Dosing[A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
Oral clofazimine Dosing may be rounded to account for capsules.		**<40kg** 3-5mg/kg, max 100mg	Once daily
		≥40kg 100mg	
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral linezolid	1 month – 9 years	10mg/kg, max 450mg	Twice daily
	10 – 12 years	10mg/kg, max 600mg	Daily
	>12 years	600mg	Daily
Oral trimethoprim with sulfamethoxazole		5mg/kg trimethoprim max 160mg trimethoprim	Twice daily
Oral bedaquiline (age ≥5 years)	**Weeks 1 and 2**		
	≥15kg - <20kg	160mg	Once daily
	≥20kg - <30kg	200mg	
	≥30kg	400mg	
	Weeks 3-24 - Max Duration 6 Months		
	≥15kg - <20kg	80mg	Thrice weekly. At least 48 hours between doses
	≥20kg - <30kg	100mg	
	≥30kg	200mg	
Oral rifabutin		5mg/kg, max 300mg	Once daily
Oral doxycycline (ages ≥8 years)		2mg/kg, max 100mg	Once daily
Oral moxifloxacin Dosing may be rounded to account for capsules.		10-15mg/kg, max 400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

Table 4 Consolidation Only therapy dosing regimen for Consolidation Arm b in paediatrics

Consolidation Arm b: Paediatric Dosing[A]			
Drug		**Recommended Dose (per dose)**	**Frequency**
Inhaled amikacin (IA) (IV formulation)		500mg	Twice daily
Oral clofazimine Dosing may be rounded to account for capsules.		**<40kg** 3-5mg/kg, max 100mg **≥40kg** 100mg	Once daily
Oral azithromycin *If azithromycin not tolerated, use oral clarithromycin.*		10mg/kg, max 500mg	Once daily
Oral clarithromycin *Only for use if azithromycin not tolerated.*	**Children 1 month – 11 years of age**		
	<8 kg	7.5mg/kg	Twice daily
	8-11 kg	62.5mg	
	12-19 kg	125mg	
	20-29 kg	187.5mg	
	30-40 kg	250mg	
	Children 12-18 years of age		
	Dosing independent of weight	500mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral linezolid	1 month – 9 years	10mg/kg, max 450mg	Twice daily
	10 – 12 years	10mg/kg, max 600mg	Daily
	>12 years	600mg	Daily
Oral trimethoprim with sulfamethoxazole		5mg/kg trimethoprim max 160mg trimethoprim	Twice daily
Oral bedaquiline (age ≥5 years)	**Weeks 1 and 2**		
	≥15kg - <20kg	160mg	Once daily
	≥20kg - <30kg	200mg	
	≥30kg	400mg	
	Weeks 3-24 - Max Duration 6 Months		
	≥15kg - <20kg	80mg	Thrice weekly. At least 48 hours between doses
	≥20kg - <30kg	100mg	
	≥30kg	200mg	
Oral rifabutin		5mg/kg, max 300mg	Once daily
Oral doxycycline (ages ≥8 years)		2mg/kg, max 100mg	Once daily
Oral moxifloxacin Dosing may be rounded to account for capsules.		10-15mg/kg, max 400mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral ethambutol Ethambutol should be dosed on ideal body weight.		15mg/kg, max 1200mg (round to account for tablet strength)	Once daily

[A] The recommended doses and frequencies are a guideline and participant dosing must also consider clinical judgement and relevant prescribing information.

10 DRUG SUPPLY, STORAGE AND DISTRIBUTION DURING CONSOLIDATION THERAPY

Supply, distribution and storage of drugs will be the responsibility of the site, coming from site/institution pharmacy stocks as per standard of care in accordance with site-specific guidelines and requirements. Any medication the participant is prescribed which are not investigational products, including adjunctive treatments, for example, medication to treat nausea, will be commercially available products and are to be sourced and supplied locally by study sites according to local standard practices.

10.1 DRUG COMPLIANCE DURING CONSOLIDATION THERAPY

The procedures for distribution of consolidation therapy are site dependent. Drug compliance during consolidation therapy will be captured from pharmacy records of drugs dispensed to the participant while enrolled in FOR*Ma*T Appendix A2.

11 AMIKACIN GUIDELINES

11.1 NEBULISER TYPE FOR IA

Amikacin for injection preparation (not liposomal amikacin) is to be used for inhalation in participants randomised to inhaled amikacin. A low preservative preparation, if available, is advised to reduce the risk of bronchospasm. The brand of amikacin preparation, if known, is to be recorded in the study drug CRF. Five hundred milligrams (500mg) of amikacin should be administered according to local standard practices. Prior to administration the patient should receive a bronchodilator, for example, salbutamol, to reduce the risk of coughing and bronchospasm. This can be given nebulised or by metered dose inhaler. Amikacin for injection may only be mixed with sodium chloride 0.9%. Sodium Chloride 0.9% is a commercial product and will be locally sourced and supplied by the study site. It must not be nebulised as a mixture with other nebulised drugs (e.g., salbutamol, dornase alfa or other nebulised antibiotics).

High efficiency nebulisers are required to nebulise amikacin (IV formulation). A suitable nebuliser with a filter attachment (e.g. SideStream Plus with filter attachment, or Pari LC Plus with filter attachment) are to be used to prevent environmental deposition of nebulised antibiotics and to reduce the risk of developing antibiotic resistant organisms. Air flow of 6-10 L/min is required to achieve effective nebulisation of amikacin.

11.2 THERAPEUTIC DRUG MONITORING FOR IA

For subjects randomised to Consolidation Only Arm b, an amikacin level should be collected at week four (4), 90 minutes (range 60 to 120 minutes) post completion of the inhaled dose. The time of starting and completing the inhaled therapy and time of blood collection should be documented. No adjustment of the inhaled amikacin dose should be undertaken.

12 SCHEDULE OF ASSESSMENTS

Table 5 lists all the special considerations for the Schedule of Assessments for participants enrolled in Appendix A2: Consolidation Only. Table 5, "Special considerations for Schedule of Assessments" must be used in conjunction with Table 6.

Table 5 Special considerations for Schedule of Assessments

Symbol	Definition
A	Participant reconsent is required with IRB/ IED/HREC approved changes to the protocol that affect the participants rights and/or safety and/or if a child turns 18 years old during the trial and must reconsent as an adult participant.
B	Consent may be performed at any time between MABS-PD Screening and Day 0.
C	Visits may be conducted while an inpatient or at home if participant is receiving home-based care.
D	Clinic visit and some assessments at Week 4 can be completed at any time between Week 4 and Week 6.
E	Refer to Section 4.0 'Eligibility Criteria" for detailed information on all eligibility criteria.
F	Refer to section 7.3 for eligibility criteria for the respiratory sampling for screening and pre-randomisation.
G	Respiratory samples (three sputums (expectorated or induced) or one BAL) to be collected at least 14 days prior to completing physician directed intensive therapy and respiratory sample culture results must be available for randomisation.
H	To determine MABS clearance, three sputum samples or one BAL sample are required to be collected in Week 4 (±3 days).
I	Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.
J	Participants who have produced sputum samples during Weeks 12, 22, and 32 are requested to provide three additional sputum samples collected at least one week apart nearer to the end of Weeks 46. For participants who were unproductive (intermittent or continual) during Weeks 12, 22, 32 and 46, a BAL sample is to be collected during Week 50.
K	Adult height is to be recorded once (preferably at the screening visit). Paediatric height must be measured at least every six weeks.
L	Refer to section 7.2 for eligibility criteria for the screening visit chest CT
M	Week 6 chest CT scan is optional and requires participant to consent to FOR*Ma*T Sub-Study C3: Imaging. The site must have approval to conduct this additional scan, be certified to perform the scan using the scanner specific protocol and the participant must provide additional consent.
N	Chest CT scan at early withdrawal visit will only be requested if clinically indicated.
O	Only required in female participants of childbearing potential. A serum pregnancy test is required at screening and final study visit. A urine or serum pregnancy test is acceptable at all other times.
P	Screening audiology and screening vestibular assessment can be performed if a participant has commenced consolidation therapy but must be performed within three (3) days of first receiving treatment.
Q	QT interval at Screening is to be corrected using the Fridericia method. Any subsequent abnormal QTc intervals are to be confirmed using the Fridericia method.
R	Six-minute walk test to be performed in participants ≥18 years of age only.
S	Plasma levels post inhaled amikacin are to be collected 90 minutes post dose (range 60 to 120 minutes). Please record inhaled amikacin start and stop times.
T	The EQ-5D-5L is to be administered to participants ≥18 years of age whereas the EQ-5D-5Y is to be administered to participants 8 to 17 years of age. The EQ-5D-Y Proxy is to be administered to parents/carers of participants 4 to 7 years of age.

U	The SF-36 is to be administered to participants ≥16 years of age at time of Screening. For participants aged <16 years at Screening, the PedsQL™ is to be used for entire duration of trial.
V	The PedsQL™ is only to be assessed in participants <16 years of age at time of Screening. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R.
W	The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen (≥14 years of age), CFQ-R child (6 to 13 years of age) and CFQ-R parent (parent/carer of participant 6 to 13 years of age).
X	The Child Health Utility is only to be assessed in participants 7 to 17 years of age.
Y	The SGRQ is only to be assessed in non-CF participants 18 years of age and older.
Z	MARS-5 is only to be completed in participants in the intervention cohort on outpatient based MABS-PD treatment.

Table 6 Schedule of Assessments for Intervention Program Participants: Consolidation Only Therapy

ASSESSMENT		MABS-PD SCREENING (-42 days from start of physician directed therapy)	PRE-RANDOMISATION SCREENING (-14 days from completion of physician directed therapy)	PRE-RANDOMISATION SCREENING Day 0 ±3 days	CONSOLIDATION THERAPY ONLY Day 1 +24 hours	Week 4 ±3 days	Week 6 ±3 days	Week 12 ±30 days	Week 22 ±30 days	Week 32 ±30 days	End of consolidation (Week 46) +5 days	FINAL STUDY VISIT +4 weeks after end of consolidation (Week 50) +14 days	EARLY WITHDRAWAL VISIT +30 days
Informed consent for Appendix A2 [A]		✓[B]											
Clinic visit [C]				✓	✓	✓[D]	✓	✓	✓	✓	✓	✓	✓
Review eligibility		✓[E]		✓[E]									
Randomisation					✓								
Adverse event monitoring				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Respiratory sample	Sputum (expectorated or induced) ***or;***	✓[F]	✓ x3 [G]			✓ x3[H]		✓[I]	✓[I]	✓[I]	✓[I]	✓[J]	✓
	Broncho-alveolar lavage (BAL)	✓[F]	✓[G]			✓[H]							
Height [K] and Weight				✓		✓[D]	✓		✓			✓	✓
Medication Review		✓		✓	✓	✓[D]	✓	✓	✓	✓	✓	✓	✓
Spirometry				✓			✓					✓	✓
Chest CT		✓[L]					Optional [M]					✓	✓[N]
Pregnancy Test and Breastfeeding Status [O]				✓			✓	✓	✓	✓	✓	✓	✓
Audiogram				✓[P]			✓		✓			✓	✓
Vestibular Monitoring				✓[P]			✓		✓			✓	✓
ECG [Q]				✓			✓		✓			✓	✓
6-minute walk test [R]				✓								✓	✓
Blood collections:													
1. Chemistry and Renal Function				✓		✓	✓	✓	✓	✓	✓	✓	✓
2. Liver Function Tests				✓		✓	✓	✓	✓	✓	✓	✓	✓
3. Full Blood Count				✓		✓	✓	✓	✓	✓	✓	✓	✓
Amikacin Monitoring	Post dose levels [S]					✓ Arm B only							
Physical examination				✓			✓					✓	✓

ASSESSMENT	MABS-PD SCREENING (-42 days from start of physician directed therapy)	PRE-RANDOMISATION SCREENING		CONSOLIDATION THERAPY ONLY							FINAL STUDY VISIT +4 weeks after end of consolidation (Week 50) *+14 days*	EARLY WITHDRAWAL VISIT +30 days
		(-14 days from completion of physician directed therapy)	Day 0 *±3 days*	Day 1 *+24 hours*	Week 4 *±3 days*	Week 6 *±3 days*	Week 12 *±30 days*	Week 22 *±30 days*	Week 32 *±30 days*	End of consolidation (Week 46) *+5 days*		
Health-related quality of life questionnaires:												✓
4. EQ-5D-5L or -Y [T]			✓			✓					✓	✓
5. SF-36 [U]			✓			✓					✓	✓
6. PedsQL™ [V]			✓			✓					✓	✓
7. CFQ-R [W]			✓			✓					✓	✓
8. Child Health Utility 9D [X]			✓			✓					✓	✓
9. SGRQ [Y]			✓			✓					✓	✓
Costs Questionnaire			✓			✓		✓			✓	✓
Medication Adherence Questionnaire [Z]						✓		✓		✓		

13 STATISTICAL ANALYSIS AND SIMULATIONS FOR APPENDIX A2

13.1 RANDOMISATION

There will be one randomisation (R-Con) in the intervention program Appendix A2, dictating the treatment the participant will receive. Randomisation-Short Intensive and Randomisation-Prolonged Intensive will not apply to Appendix A2 participants. The R-Con randomisation will allocate participants to the consolidation therapy arms. Randomisation will be conducted suing the method of minimisation described in Appendix F: General Statistical Principles. The consolidation phase power simulations and statistical analysis are also detailed in Appendix F: General Statistical Principles and the Statistical Analysis Plan (SAP).

14 APPLICABLE DISCOVERY STUDIES AND REGISTRY LINKAGE FOR APPENDIX A2

Appendix A2 participants may be eligible to enrol in the following FOR*Ma*T Sub-Studies and Integrated Studies:

1. Appendix C Discovery
    i. C1: Pharmacokinetics
        i. C1.2 Microsampling and non-blood matrix validation for amikacin therapeutic drug monitoring (TDM)
        ii. C1.3 Pharmacokinetics of MABS-PD therapies
        iii. C1.4 Pharmacokinetics of CFTR modulator therapy in persons with cystic fibrosis on MABS therapy
    ii. C2: Immune factors and biomarkers
        i. C2.1 Macrophage function
        ii. C2.2 Mitochondrial stress
        iii. C2.3 T-cell function
        iv. C2.4 Gene expression
        v. C2.5 Serology
    iii. C3: Imaging
2. Appendix D Registry Linkage
    i. D1: Australian cystic fibrosis data registry
3. Appendix E Health Economics
    i. E1: Cost effectiveness and Resource utilisation

Please see the relevant sections of the applicable appendix for further information, including additional eligibility criteria.

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FORMaT

Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

APPENDIX B - OBSERVATIONAL COHORT

Appendix B – Observational Cohort	Version 1.2
Appendix Date	20 February 2024
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
EudraCT Number	2020-000050-10
EU-CT Number	2023-506575-99-00-EU CT
IRAS Project Number	1007146
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AE	Adverse Event
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CHU9D	Child Health Utility 9D
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
FEV1	Forced Expiratory Volume in one second
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HRQoL	Health Related Quality of Life
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
PedsQL™	Pediatric Quality of Life Inventory
QALY	Quality-Life Adjusted Years
QoL	Quality of Life
SAEs	Serious Adverse Events
SF-36	Short form-36
SOP	Standard Operating Procedure
SGRQ	St George Respiratory Questionnaire
VAS	Visual Analogue Scale

1 INTRODUCTION

Appendix B describes the schedule of assessments for participants enrolled in the Observational Cohort arm of Finding the Optimal Regimen for Mycobacterium *abscessus* Treatment (FOR*Ma*T) study.

2 OBJECTIVES

1. Describe the following outcomes for participants in the Observational Cohort:
    - Microbiological clearance of *Mycobacterium abscessus* (MABS),
    - Clinical markers including health related quality of life (HRQoL) and adverse effects on health.
    - Host immune factors and biomarkers.
2. Describe any changes associated with the development of MABS pulmonary disease (MABS-PD) in the following:
    - Changes in airway microbiology,
    - Change in clinical markers including HRQoL, FEV_1 z-score and CT scores,
    - Change in host immune factors and biomarkers.
3. Characterise the genomics of human MABS strains and antibiotic resistance genes in participants enrolled in the Observational Cohort.

The Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be applied for the coding and grading of all adverse events/serious adverse events (AE/SAEs) that occur during the FOR*Ma*T trial.

3 ELIGIBILITY CRITERIA

3.1 INCLUSION CRITERIA

To be eligible to participate in the observational cohort the following criteria must be met:

1. Male and female participants of any age with at least one positive respiratory culture for MABS in the 12 months prior to enrolment.
2. Informed consent signed by participant or their parent/legal guardian if participant is under 18 years of age.
3. Ability to comply with study visits and study procedures as judged by the site investigator.

3.2 EXCLUSION CRITERIA

Potential participants will be ineligible to participate in the observational cohort if any of the following criterion are met:

- Receiving active treatment for MABS within the previous 12 months (this includes drugs prescribed for the treatment of other mycobacteria and/or other indications that may have activity against MABS, as specified in FOR*Ma*T Prohibited Drug List SOP, except for participants taking azithromycin as part of routine treatment for cystic fibrosis (CF) or chronic infection-related pulmonary disease.

Participants already enrolled in the observational cohort who go on to meet MABS-PD criteria and require treatment for MABS-PD may transition to the Intervention Program if they meet eligibility criteria. However, if they are unable to be enrolled into the Intervention Program but do commence any physician led MABS-PD treatment, they are no longer eligible to continue participating in the observational cohort.

4 TRIAL CONDUCT

4.1 INFORMED CONSENT

In addition to providing consent to the FOR*Ma*T Master Protocol, participants enrolling in the Observational Cohort are required to sign and date an additional Observational Cohort consent form. If the participant is <18 years of age, then the parent/guardian will provide consent on behalf of the participant.

4.2 OBSERVATIONAL COHORT PROCEDURES

In addition to the core trial procedures listed in the FOR*Ma*T Master Protocol (Master Protocol Section 5.5 Core Trial Procedures), the Observational Cohort participants are required to undergo further testing detailed below and in Table 2.

4.2.1 RESPIRATORY SAMPLING

Collection of respiratory sample(s) for the identification of MABS at screening may not be required if recent sample(s) have been provided by the participant within the 12 months prior to screening and has been stored and is available as per the local laboratory guidelines (standard of care) to identify and diagnose MABS infection. If the screening sample has been stored, then the sample will be analysed retrospectively once consent to participate in the FOR*Ma*T trial is given.

The minimum respiratory sampling collection timepoints have been outlined in Table 2; however, monthly sputum sample collections are recommended for participants who continue to be productive. For participants who become non-productive between screening and Week 12, one Bronchoalveolar Lavage (BAL) between Week 10 to Week 12 can be collected in the absence of sputum samples.

4.2.2 MABS-PD STATUS

Participants enrolled in the Observational Cohort are required to have their MABS-PD status reviewed in accordance with the American Thoracic Society (ATS) criteria for MABS-PD diagnosis (Master Protocol Section 4.2.1). The MABS-PD status of Observational Cohort participants is required to be reviewed as per Table 2 and, if applicable the early termination visit by the treating physician. This information is to be documented in the MABS-PD status question contained in the relevant Visit Case Report Forms (CRFs). If an observation participant develops MABS-PD they become eligible for the intervention program. If felt appropriate by the site investigator and informed consent is obtained, the participant can move from the Observational Cohort into the intervention program.

4.2.3 SIX MINUTE WALK TEST

A 6-minute walk test (6MWT) will be performed in adult participants only, at the time points outlined in Table 2, if the testing is available at the trial site. The 6MWT will be performed according to the protocol in the ATS Statement:

Guidelines for the Six Minute Walk Test (1) and/or local standard procedures. Results of the 6MWT will be recorded in the relevant eCRF in the FOR*Ma*T database by the end of the Final Outcome visit.

4.2.4 HEALTH RELATED QUALITY OF LIFE (HRQOL) AND UTILITY MEASURES

HRQoL will be assessed in all participants (where possible) according to the schedule of assessments in the relevant appendix. The questionnaires are required to be completed prior to any clinical assessment and are dependent on the participant's age and whether they have CF. The age-appropriate HRQoL questionnaire issued to the participant at the start of the study will continue to be used throughout the study even if the participant progresses to a different age range. HRQoL questionnaires will be made available to study sites. All questionnaires will be made available in English or if available the local language version will be sourced. Questionnaires can be completed by the participant and/or their parent/carer via an online link to the questionnaire(s) on the trial database or completed using a paper-based questionnaire. Responses from paper-based questionnaires are to be entered into the trial database by trial site staff.

4.2.4.1 Cystic fibrosis Questionnaire-revised (CFQ-R)

The CFQ-R has been developed specifically for use in people with CF. This questionnaire measures the impact of CF on overall health, daily life, perceived well-being and symptoms. Age-appropriate questionnaires have been developed; CFQ-R teen/adult for adolescents and adults 14 years of age and older, the CFQ-R child and the CFQ-R parent are to be completed by the child (6-13 years of age) and the parent/carer respectively of children with CF.

In children ≤16 years of age, the CFQ-R should be administered after the PedsQL™.

4.2.4.2 EQ-5D-5L

The EQ-5D-5L questionnaire is a standardised measure of health status in adults 18 years of age and older. This questionnaire can be applied as a generic measure of health for clinical and economic appraisal, including the calculation of quality-life adjusted years (QALYs). The EQ-5D-5L measures five dimensions:

- Mobility;
- Self-care;
- Usual activities;
- Pain/discomfort;
- Anxiety/depression.

Each dimension has five possible answers: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by selecting the most appropriate statement.

4.2.4.3 EQ-5D-Y

The EQ-5D-Y is the child friendly version of the EQ-5D-5L questionnaire. The EQ-5D-Y has been developed for use in children 8 to 17 years of age. The dimensions and the visual analogue scale (VAS) measured are the same as the EQ-5D-5L questionnaire, but with child friendly wording. For children 4-7 years of age the proxy 1 version of

the EQ-5D-Y can be utilised allowing the respondent (parent/carer) to evaluate participants quality of life (QoL) from respondents' own view.

4.2.4.4 St George Respiratory Questionnaire (SGRQ)

The SGRQ is to be completed by all non-CF participants 18 years of age and older. The SGRQ is a supervised self-administered 50-item questionnaire measuring health status across three domains: symptoms, activity and impacts (psycho-social) in people with airway obstruction.

4.2.4.5 Short Form 36 (SF-36)

The SF-36 health survey is a self-reported questionnaire applicable in all participants 16 years and older. Covering eight health concepts the SF-36 health survey is a generic outcome measure designed to examine a person's perceived health status.

4.2.4.6 PedsQL™

The PedsQL™ child health questionnaire is a non-preference-based measure to assess HRQoL for children and adolescents from 2 years up to 16 years of age. Children less than 16 years of age at screening will continue to use the PedsQL™ up until the end of the trial, rather than change to using the SF-36 if they turn 16 years of age during the study.

Developmentally appropriate child self-report questionnaires are available (ages 5-7, 8-12, 13-18) together with parent/carer proxy-reports (ages 2-4, 5-7, 8-12 and 13-18). If feasible, the PedsQL™ should be completed *before* the respondents complete any other health data forms and *before* they see their physician or healthcare provider.

Parents/carers, children (aged 8-12) and young people (aged >12 years) may self-administer the PedsQL™ after the FOR*Ma*T site researcher has provided instructions. If it is determined by the FOR*Ma*T site researcher that the child, young person or parent/carer is unable to self-administer the PedsQL™ the questionnaire should be administered, word for word by the FOR*Ma*T site researcher. If the child has difficulty understanding the age appropriate PedsQL™ the preceding age questionnaire may be used. The parent and the child must complete the questionnaires independently of each other and in accordance with the PedsQL™ administration guidelines (available via hyperlink or in relevant section of the FOR*Ma*T Trial Site Manual of Operating Procedures (MoOP)).

4.2.4.7 Child Health Utility 9D (CHU9D)

The CHU9D is a generic preference-based measure of paediatric HRQoL for use in children 7 to 17 years of age. The use of a descriptive system and a set of preference weights allows for the calculation of quality adjusted life years (QALYs) for use in economic evaluation

5 SCHEDULE OF ASSESSMENTS

Table 1 lists the special considerations that are applicable to Table 2 Schedule of Assessment for Observational Cohort. Table 1 must be used in conjunction with Table 2 Schedule of Assessments for Observational Cohort.

Table 1 Special Considerations for Schedule of Assessments

Symbol	Definition
A	Participant reconsent is required with IRB/ IED/HREC approved changes to the protocol that affect the participants rights and/or safety and/or if a child turns 18 years old during the trial and must reconsent as an adult participant.
B	Visits may be conducted while an inpatient or at home if participant is receiving home-based care.
C	To be eligible to enrol in the Observational Cohort, the participant is required to have at least one (1) MABS-positive respiratory sample.
D	Observational Cohort participants are requested to provide three sputum samples at Weeks 6 and 12. If the participant is unable to provide sputum samples, one BAL is to be collected at Week 12 for microbiology assessment.
E	Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.
F	Participants who have produced sputum samples during Weeks 18, 28, and 38 are requested to provide three additional sputum samples collected at least one week apart nearer to the end of Week 56. For participants who were unproductive (intermittent or continual) during Weeks 18, 28, and 38 a BAL sample is to be collected during Week 56.
G	Adult height is to be recorded once (preferably at the screening visit). Paediatric height must be measured at least every six weeks.
H	Week 12 chest CT scan is optional and requires participant to consent to FOR*Ma*T Appendix C3: Imaging. The site must have approval to conduct this additional scan, be certified to perform the scan using the scanner specific protocol, and the participant must provide additional consent. Chest CT scan may be done as part of standard of care for clinical purposes, results of these chest CT scans will be collected for participants consenting to Master Protocol.
I	Chest CT scan at early withdrawal visit will only be requested if clinically indicated.
J	Six-minute walk test to be performed in participants ≥18 years of age only.
K	The EQ-5D-5L is to be administered to participants ≥18 years of age whereas the EQ-5D-5Y is to be administered to participants 8 to 17 years of age. The EQ-5D-Y Proxy is to be administered to parents/carers of participants 4 to 7 years of age.
L	The SF-36 is to be administered to participants ≥16 years of age at time of Screening. For participants aged <16 years at Screening, the PedsQL™ is to be used for entire duration of trial.
M	The PedsQL™ is only to be assessed in participants <16 years of age at time of Screening. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R.
N	The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen (≥14 years of age), CFQ-R child (6 to 13 years of age) and CFQ-R parent (parent/carer of participant 6 to 13 years of age).
O	The Child Health Utility is only to be assessed in participants 7 to 17 years of age.
P	The SGRQ is only to be assessed in non-CF participants 18 years of age and older.

Table 2 describes the schedule of assessments for the Observational Cohort as well as the acceptable study visit windows.

Table 2 Schedule of Assessments for Observational Cohort

ASSESSMENT		**SCREENING VISIT**	**INTERIM VISITS**					**FINAL OUTCOME VISIT**	**EARLY WITHDRAWAL VISIT**
		Day 0 ***-42 days***	**Week 6** ***±14 days***	**Week 12** ***±14 days***	**Week 18** ***±30 days***	**Week 28** ***±30 days***	**Week 38** ***±30 days***	**Week 56** ***±14 days***	***+30 days***
Informed Consent for Appendix B [A]		✓							
Clinic Visit [B]		✓	✓	✓	✓	✓	✓	✓	✓
Review Eligibility		✓							
Adverse Event Monitoring			✓	✓	✓	✓	✓	✓	✓
Respiratory Sample	Sputum (expectorated or induced) ***or;***	✓[C]	✓x3[D]	✓x3[D]	✓[E]	✓[E]	✓[E]	✓[F]	✓
	Broncho-alveolar lavage (BAL)	✓[C]		✓[D]				✓[F]	
Height [G] and Weight		✓	✓	✓		✓		✓	✓
MABS-PD Status		✓	✓	✓				✓	✓
Medication Review		✓	✓	✓	✓	✓	✓	✓	✓
Spirometry		✓	✓	✓				✓	✓
Chest CT		✓		Optional[H]				✓	✓[I]
Physical Examination		✓	✓	✓				✓	✓
6-Minute Walk Test [J]		✓		✓				✓	✓
Health-related Quality of Life Questionnaires:									
1. EQ-5D-5L or -Y [K]		✓	✓	✓				✓	✓
2. SF-36 [L]		✓	✓	✓				✓	✓
3. PedsQL™ [M]		✓	✓	✓				✓	✓
4. CFQ-R [N]		✓	✓	✓				✓	✓
5. Child Health Utility 9D [O]		✓	✓	✓				✓	✓
6. SGRQ [P]		✓	✓	✓				✓	✓
Costs Questionnaire		✓	✓	✓		✓		✓	✓

6 SITE REIMBURSEMENT

For participants enrolled in Appendix B, trial sites will be reimbursed on a per participant basis at each of the following time points;

1. Screening;
2. End of week 6;
3. Final study visit.

Sites will be reimbursed according to their contract. Payments will be paid for each time point once the data is entered into the trial database and all queries finalised. Invoices should be prepared at a minimum of every 6 months.

7 STATISTICIAL ANALYSIS

The objectives will be addressed by a descriptive analysis of changes in clinical status, immune status, biomarkers, MABS genomics and antimicrobial resistance genes between Day 0 and final outcome. The following data will be used:

- Adverse events occurring between Day 0 and final outcome,
- Microbiological clearance of MABS between Day 0 and final outcome,
- Airway microbiology between Day 0 and final outcome
- Change in HRQoL between Day 0 and final outcome,
- Change in forced expiratory volume in one second (FEV1) z-score, between Day 0 and final outcome,
- Change in chest computed tomography (CT) scores, between Day 0 and final outcome,
- Immune factors and biomarkers (refer to the relevant substudy appendix for further detail on statistical analyses),
- Genomics of human MABS strains and antibiotic resistance genes.

8 APPLICABLE DISCOVERY STUDIES AND REGISTRY LINKAGE

Observational Cohort participants may be eligible to enrol in the following FOR*Ma*T Sub-Studies and Integrated Studies:

1. Appendix C Discovery
    i. C2: Immune factors and biomarkers
        i. C2.1 Macrophage function
        ii. C2.2 Mitochondrial stress
        iii. C2.3 T-cell function
        iv. C2.4 Gene expression
        v. C2.5 Serology
    ii. C3: Imaging

2. Appendix D Registry Linkage
    i. D1: Australian cystic fibrosis data registry
3. Appendix E: Health Economics
    i. E1: Cost effectiveness and Resource utilisation

Please see the relevant sections of the applicable appendix for further information, including additional eligibility criteria.

9 REFERENCES

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Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

APPENDIX C1 – DISCOVERY

Pharmacokinetics

Appendix C1 – Discovery	Version 1.0
Appendix Date	29 March 2023
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
IRAS Project ID	1007146
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AUC	Area Under the Curve
BAL	Bronchoalveolar Lavage
CART	Correlation and Regression Tree
C_{max}	Maximum serum concentration
CRF	Case Report Form
DBS	Dried Blood Spot
FDA	Food and Drug Administration
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
FBC	Full blood count
IV	Intravenous
LLOQ	Lower Limit of Quantification
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MDR-TB	Multi Drug Resistant-Tuberculosis
MIC	Minimum Inhibitory Concentration
MRL	Mycobacterial Reference Laboratory
NTM	Non-Tuberculous Mycobacteria
PD	Pharmacodynamics
PK	Pharmacokinetics
pwCF	Persons with cystic fibrosis
REDCap	Research Electronic Data Capture
TB	Tuberculosis
TDM	Therapeutic Drug Monitoring
UQCCR	University of Queensland Centre for Clinical Research

APPENDIX C: DISCOVERY

Appendix C: Discovery, consists of research studies/programs that may be added over time and that are aimed at gaining understanding through collaboration with the FOR*Ma*T trial but are not specific components of the trial. Participation in each discovery study may require additional consent. Consent requirements are detailed within each of the discovery sub-studies. Discovery studies may be applicable to all participants or only to specific sites or groups of participants. Each study will be described in the following format:

- The named Investigator leads for the sub-study;
- The primary and secondary objectives of the sub-study;
- An introduction to the background of the sub-study;
- Additional eligibility and consent requirements;
- Sub-study specific trial procedures and schedule of assessments;
- Analysis plan.

Appendix C1: Pharmacokinetics.

Appendix C2: Immune Factors and Biomarkers.

Appendix C3: Imaging.

APPENDIX C1: PHARMACOKINETICS

Investigator Leads

Dr Andrew Burke and Professor Jason Roberts

1 INTRODUCTION

Appendix C1, Pharmacokinetics, addresses a key research question posed by the FOR*Ma*T trial relating to the optimal use of drug therapies in *Mycobacterium abscessus* (MABS infection). Answering this question has been hampered by the lack of pharmacokinetic (PK) studies in people with mycobacterial lung disease. Nested within Appendix C1 are four PK sub-studies:

SUB-STUDY C1.1: Steady State Pharmacokinetics of Amikacin;

SUB-STUDY C1.2: Micro Sampling and Non-Blood Matrix Validation for Amikacin Therapeutic Drug Monitoring;

SUB-STUDY C1.3: Pharmacokinetics of *Mycobacterium abscessus* Pulmonary Disease (MABS-PD) Therapies;

SUB-STUDY C1.4: Pharmacokinetics of CFTR Modulators in Persons with Cystic Fibrosis (pwCF) on MABS Therapy.

2 SUB-STUDY C1.1: STEADY STATE PHARMACOKINETICS OF AMIKACIN

2.1 PRIMARY OBJECTIVE

Determine the best practice therapeutic drug monitoring recommendations for use of intravenous amikacin to maximise antimicrobial effect and minimise toxicity in the treatment of MABS-PD.

2.2 INTRODUCTION

The optimal use of amikacin in the treatment of MABS-PD is hampered by the lack of PK studies in all people with mycobacterial lung disease (1). There are no current validated maximum serum concentration (C_{max}) or area under the curve (AUC) targets for amikacin use in the management of non-tuberculous mycobacteria (NTM) disease, with or without concomitant Minimum Inhibitory Concentration (MIC) data. The development of novel anti-mycobacterial drug assays at the University of Queensland Centre for Clinical Research (UQCCR) and Pathology Queensland will allow accurate measurement of drug concentrations in plasma and other body fluids including bronchoalveolar lavage (BAL), sputum, and oral fluids. These data can then be analysed to inform further understanding of the PK of amikacin in MABS-PD patients. It will also allow for the rational design of future studies on TDM for amikacin which has a narrow therapeutic window. The increased use of TDM has been suggested in the treatment of multidrug resistant tuberculosis (MDR-TB) as well as for complicated NTM infections. A better understanding of the PK of amikacin will therefore allow optimisation of dosing to minimise toxicity while maximising the likelihood of cure.

2.3 ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the Master Protocol, participants are required to meet the following inclusion criteria:

- Informed consent by participant or their parent/guardian.
- Receiving intravenous amikacin as part of FOR*Ma*T Intervention Program,
- Availability of patient demographics including weight and height,
- Accurate recording of time of drug administration and infusion time.

2.4 INFORMED CONSENT

Informed consent will be obtained to use information already collected as standard of care amikacin therapeutic drug monitoring and in accordance with the policies described in the FOR*Ma*T Master Protocol for the purposes of this sub-study C1.1.

2.5 AMIKACIN TDM PROCEDURES

There is currently variation in practice surrounding amikacin dosing and TDM and this is likely to be reflected in the recruiting centres for the FOR*Ma*T trial. Some patients will be receiving intermittent (second or third daily dosing) and others once daily dosing. The current TDM strategies that can be used in the FOR*Ma*T trial include:

1. Trough amikacin levels (independent of MIC) using nomogram for next dose (equivalent to $C_{min/}$ trough),
2. C_{max}/MIC target,
3. AUC target,
4. C_{max}/MIC and minimum serum concentration ($C_{min/}$ trough)
5. AUC/MIC target.
6. C_{max} (peak)

As TDM for amikacin is already standard of care and is incorporated into the FOR*Ma*T Appendix A1, all sampling events will be recorded in the FOR*Ma*T trial Research Electronic Data Capture (REDCap) database. Investigators will be free to use the dosing and TDM method that is their usual practice, however it will be documented what approach is being used to allow comparison between different strategies.

Participants will have blood drawn through a suitable venous access device and the sample will be processed at local laboratory as per usual practice for that trial site. Results to be recorded include timing and dose of amikacin, and frequency of dosing. Same day serum creatinine concentrations as well as admission weight and height will also be recorded.

All amikacin levels accompanied by a serum creatinine concentration within 24 hours of sampling will be included in analysis. Relevant demographic data e.g., weight and height, time that study drugs have been administered and method of TDM utilised by treating clinicians will be recorded in the case report form (CRF).

2.6 PHARMACOKINETIC ANALYSIS

To describe amikacin concentrations, one and two-compartment models will be evaluated using the Nonparametric Adaptive Grid (NPAG) algorithm within the Pmetrics software package for R (Los Angeles, CA). Elimination from the central compartment, and intercompartmental distribution into the peripheral compartment (two compartment model) will be modelled as first-order processes. Discrimination between different models will use comparison of the -2 log likelihood (-2LL). A p-value of <0.05 will be considered statistically significant.

Age, sex, body weight, renal function descriptors and other clinical descriptors will be tested as covariates. Covariate selection will be performed using a stepwise linear regression from R on all covariates and Bayesian posterior parameters. Potential covariates will be separately entered into the model and statistically tested by use of the -2LL values. If inclusion of the covariate results in a statistically significant improvement in the -2LL values ($p<0.05$) and/or improved the goodness-of-fit plots, then it will be retained in the final model.

2.7 MODEL DIAGNOSTICS

Goodness-of-fit will be assessed by linear regression, with an observed-predicted plot, coefficients of determination, and log-likelihood values. Predictive performance evaluation will be based on mean prediction error (bias) and the mean bias-adjusted squared prediction error (imprecision) of the population and individual prediction models. The internal validity of the population pharmacokinetic model will be assessed by the bootstrap resampling method (n=1000) and normalised prediction distribution errors (NPDEs). Using visual predictive check (VPC) method, parameters obtained from the bootstrap method will be plotted with the observed concentrations.

Once the pharmacokinetic model has been developed, amikacin exposure in enrolled patients will be simulated as area under the concentration-time curve (AUC) from 0-24 hours and peak concentration (C_{max}) during a dosing interval. The association of clinical cure versus amikacin exposure will be tested using a Mann-Whitney U-test. A classification and regression tree (CART) analysis will be used to propose breakpoints (e.g., C_{max}, C_{max}/MIC, AUC, AUC/MIC) which quantify the exposures associated with maximal effectiveness and can be used to guide future TDM processes.

3 SUB-STUDY C1.2: MICRO SAMPLING AND NON-BLOOD MATRIX VALIDATION FOR AMIKACIN THERAPEUTIC DRUG MONITORING

3.1 PRIMARY OBJECTIVE

Compare less intrusive micro sampling strategies to the gold standard methods for amikacin TDM.

3.2 INTRODUCTION

Patients are on treatment for MABS-PD for months and repeated blood sampling for TDM and PK analysis currently requires venepuncture and on-site centrifuge prior to transport offsite for testing. If same day testing cannot be done, then the sample requires freezing. These factors cause discomfort to the patient and impose logistical barriers to PK studies in clinical trials as well as limiting the availability of TDM and its ability to influence prescribing decisions through real time feedback to clinicians.

Less intrusive sampling strategies have been proposed, however, it is unknown whether these strategies can accurately measure amikacin levels in a diverse range of body fluids. Dried blood spot (DBS) and Volumetric Absorbent Micro sampling (VAMS) performed through finger prick testing may be a practical surrogate for traditional methods of obtaining plasma from venepuncture. Other sampling technologies that will be trialled include: hemaPEN, safeClinitube, and Drummond plasma gel separator tubes.

As NTM infection is situated in the lower respiratory tract, the concentration of drugs in the respiratory epithelial fluid is the PK endpoint of most relevance. Sampling this compartment has traditionally required bronchoscopy with BAL. As well as being an invasive procedure requiring sedation, lung inflammation and mucous impaction as found in CF may contribute to sampling and dilutional error. Suitable alternatives to be investigated include sputum and oral fluid to specimens obtained from BAL. Simpler and more reliable sampling methods testing oral fluid drug concentrations on salivette cotton swabs are already an established and well tolerated method for obtaining cortisol levels in clinical practice but further investigation into the utility of these samples in MABS-PD is required.

3.3 ELIGIBILITY CRITERIA

Participants at nominated Australian FOR*Ma*T trial sites will be eligible to participate in sub-study C1.2. In addition, participant eligibility will be based on the following inclusion criteria:

- Receiving intravenous amikacin as part of the FOR*Ma*T Intervention Program,
- Availability of suitable intravenous access to facilitate sample collection,
- Patients may be receiving other antibiotics concurrently,
- Informed consent by participant or their parent/guardian.

3.4 INFORMED CONSENT

Additional blood samples are required as part of the study procedures for sub-study C1.2, therefore additional consent will be obtained in accordance with the policies described in the FOR*Ma*T Master Protocol.

3.5 MICRO SAMPLING PROCEDURES

The day of testing will be determined by the site investigator and will be after **the second dose** of IV amikacin.

1. Participants receiving IV amikacin will have samples taken using two micro sampling techniques (detailed below) in addition to whole blood collection through phlebotomy.
2. In addition to the amikacin trough and peak levels collected for standard of care, samples will be collected at three time points. Specific time points used will be dependent on amikacin frequency of administration and current amikacin assay validation. Time points are determined by the lead investigators of the PK sub-studies.
    a. For adults, this will be three (3) time points at 0 hours (pre-dose level), and 1 hour and 1.5 hours after the start of amikacin infusion.
    b. Paediatric patients may have collections at these time points, or alternatively samples may be collected at two (2) opportunistic time points that align with their standard of care blood collection for amikacin TDM.
3. At each time point the following blood samples will be collected from the participant: 1 venous whole blood sample (4ml EDTA tube) and a sample from two micro sampling devices.
4. Micro sampling devices to be tested include:
    - 1 finger prick sample using 1 Guthrie card for dried blood spot (DBS) ~ 50 µl of blood, OR;
    - 1 finger prick sample using 2 Mitra® VAMS (a fraction of a bead of blood (10µL) x2), OR;
    - 1 finger prick sample using haemaPEN (a fraction of a bead of blood (2.5µL) x4), OR;
    - safeClinitube (50 µL equivalent to a drop of blood), OR;
    - Drummond plasma gel separator tubes (50 µL equivalent to a drop of blood).
5. Whole blood will be drawn through a suitable venous access device kept at ambient temperature and sent via courier with other microsamples to UQCCR for processing.
6. Same day full blood count (FBC) and Chem-20 tests will be performed by a local laboratory as part of the standard of care for the trial site to monitor haematocrit, albumin and creatinine levels.
7. A brief patient or care giver questionnaire will be undertaken to determine level of discomfort with micro sampling techniques compared to venepuncture as well as degree of technique acceptability to research nurse.
8. Amikacin assay results and other relevant pathology results will be recorded as will time, dose, and frequency of dosing of IV amikacin in the PK sub-study REDCap database.

3.5.1 CHOICE OF MICRO SAMPLING DEVICE

The choice of which micro sampling devices to be used will be determined by the lead Investigator for the PK sub-studies. To maintain consistency in technique and to ensure recruitment of sufficient numbers in each testing arm, centres will be allocated micro sampling devices to test.

Once participants have consented to sub-study C1.2, the micro sampling devices allocated to that participant, along with the TDM strategy employed, will be recorded by the trial site in the participant's record in the Pharmacokinetic Study REDCap database.

Once 25 separate paired amikacin samples have been collected further testing on each device will cease pending results of analysis. 15-20 subjects will be required per device depending on the TDM strategy used. Once a study site has completed target enrolment in the allocated microsampling device, they will be allocated another device to trial. Devices will be allocated in the order of: VAMS, DBS, haemaPEN, safeClinitube and Drummond plasma gel separator tubes.

3.6 OPPORTUNISTIC BAL SAMPLING

For those participants having bronchoscopy as part of their normal clinical care, BAL samples will be collected for the measurement of drug concentrations. Before sedation or anaesthesia, a patient will be asked to provide a saliva sample and to perform a salivette test. Blood will also be taken as close as possible to the time of BAL and usually while under sedation/anaesthesia with the time bloods were taken documented. If not performed previously, two (2) samples using microsampling techniques will be collected as outlined above at the time of blood collection. The use of the salivette swab is to determine whether saliva can be used as a surrogate for BAL concentrations. Paired whole blood and micro samples will be transported to UQCCR, with the saliva, BAL and paired blood samples being transported to Pathology Queensland, Herston, Brisbane, and drug assays will be performed for study drugs including amikacin. Bronchoscopy will only be performed based on clinical need and not for the primary purpose of PK studies.

3.7 STATISTICAL ANALYSIS PLAN

The aim of the microsampling study will be to assess the utility and validate different microsampling devices. A formal PK analysis will not be performed on these specimens as this will have already been conducted on plasma samples. Validation of microsampling techniques will be performed with reference to the Food and Drug Administration (FDA) document on bioanalysis. Intra and inter-assay precision and accuracy will be determined for each technique as will the lower limit of quantification (LLOQ), linearity, matrix effects, recovery, selectivity, and stability of dried matrix.

4 SUB-STUDY C1.3: PHARMACOKINETICS OF MABS-PD THERAPIES

4.1 PRIMARY OBJECTIVE

Assess steady state pharmacokinetics in participants enrolled in the Intervention Program for the treatment of MABS-PD.

4.2 INTRODUCTION

Population pharmacokinetic modelling of samples across one dosing interval can give more reliable PK information than traditional PK analysis. This allows for a more efficient and streamlined study on a smaller number of patients. Given the increasing treatment burden for MABS-PD, a more comprehensive understanding of the PK of these drugs is imperative in the development of safer and more effective therapies for patients with MABS-PD. There are no data currently available relating to pharmacokinetics of drugs commonly used in the management of MABS-PD.

4.3 ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the FOR*Ma*T Master Protocol, participants are required to meet the inclusion criteria outlined below to enrol in sub-study C1.3:

1. Enrolled in the Intervention Program at an Australian site,
2. Availability of suitable intravenous access to facilitate sample collection,
3. Patients may be receiving other antibiotics concurrently,
4. Availability of same day (preferably pre-dose) FBC and Chem-20 test results,
5. Informed consent by participant or their parent/guardian.

4.4 INFORMED CONSENT

Additional blood samples are required as part of the study procedures for sub-study C1.3, therefore additional consent will be obtained in accordance with the policies described in the FOR*Ma*T Master Protocol.

4.5 SUB-STUDY SPECIFIC PROCEDURES

1. The day of study will be determined by the site investigators and will be after **5 half-lives** (at least 48hrs after the patient is established on the planned treatment dose) of the drug with the longest half-life (excluding azithromycin which has a prolonged half-life) to ensure testing is done at **steady state**. There should have been no dose adjustments in the 48hrs prior to sampling.
2. Participants will have up to 5 ml (adults) or 3ml (paediatric participants) whole blood drawn through a cannula at the following times on a single day of intensive therapy treatment:
    i. immediately pre-dose (trough level).
    ii. post- dosing: at approximately 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, and 8 hours after the administration of the first infusion or oral administration of study medications on the day of sampling.
    iii. Children who cannot have a separate IV line for frequent sampling may have opportunistic testing of blood concentrations based on samples that are already being collected as part of routine care (i.e.,

"opportunistic" bloods). The timing of these samples may be chosen to align with expected trough and peak times of infused antibiotics as determined by the lead investigator at site in collaboration with the PK sub-study lead. In most cases this will include a blood draw at the end of infusion.

3. The time of each blood draw will be recorded in the PK sub-study REDCap database and on the study-specific clinical trial pathology form. If the participant is on multiple IV antibiotics, the commencement of the infusion of each IV antibiotic will occur at different times. The timings for commencement and completion of each IV drug will be recorded and entered into the PK sub-study REDCap database. Administration of the IV drug with the longest half-life first (e.g., tigecycline before imipenem) is recommended so that timing aligns.
4. On the same day, a salivette swab will be inserted into the mouth at each time point where blood is being collected. The salivette swab will be removed after being chewed for 2 minutes and will be transported to Pathology Queensland, Herston Brisbane with all other samples on either the same or the next day.
5. All samples must be processed within 1 hour of collection and stored frozen at -80°C until transfer to Pathology Queensland, Herston Brisbane.
6. **Opportunistic BAL sampling:** For participants having bronchoscopy as part of their normal clinical care, BAL samples will be collected for the measurement of drug concentrations. Before sedation/anaesthesia the patient will be asked to perform a salivette test. Blood will be taken as close as possible to the time of BAL usually under sedation/anaesthesia with the time clearly documented. These samples will be transported to Pathology Queensland, Herston Brisbane and drug assays will be performed for study drugs including amikacin. The use of the salivette swab is to determine whether saliva can be used as a surrogate for BAL concentrations. Bronchoscopy will only be performed based on clinical need and not for the primary purpose of PK studies.
7. **Opportunistic clofazimine sampling:** For participants that have participated in the intensive day of sampling as per study procedures above, regular clofazimine drug concentration testing will be conducted to compare drug levels between steady state during initial intensive therapy and the drug levels at the end of consolidation therapy. The 5 ml sample required to perform the analysis will be taken at the same time blood is collected during study visits for standard of care tests. Samples will be collected by the local phlebotomy service at the hospital where study visits are conducted, and samples will be processed by the local laboratory. These bloods will be collected at weeks 6, 12, 18, 28, 38, and the end of consolidation (week 52 or 58, depending on randomisation allocation of immediate consolidation or delayed consolidation).

4.6 STATISTICAL ANALYSIS

Enrolment targets for sub-study C1.3 are: 50 participants ≥18 years of age and 30 participants <18 years of age. Participants will be on different antibiotic therapies; therefore, this allocation of participants will allow for a minimum of 20 participants per antibiotic.

PK indices will include volumes of distribution, C_{max}, AUC and clearance. A pharmacokinetic model will also be developed using a population pharmacokinetic approach. This technique will enable the effect of patient and

treatment characteristics on altered pharmacokinetics (e.g., patient age, weight, renal function, hepatic function, interacting drugs) to be quantified. This model can then be used to assess the adequacy of current dosing regimens and can also be used for dosing simulations to define optimised dosing regimens for these patients.

PK analysis will be conducted with Pmetrics software package for R (version 1.4.1., Los Angeles, CA). Non-linear mixed effect modelling approach will be used. A stepwise analysis will be performed to first determine the structural base model by fitting the concentration-time data to one, two and three compartment models. Testing of additive, proportional or a combination of additive and proportional models will be conducted to select the best fit statistical error model. Following population PK analysis, Monte Carlo simulations will be performed using MICs for target NTM pathogens derived from clinical specimens tested at Pathology Queensland's Mycobacterial Reference laboratory (MRL). These PK/PD parameters can be used to estimate appropriate doses in clinical practice.

5 SUB-STUDY C1.4: PHARMACOKINETICS OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) MODULATORS IN PERSONS WITH CYSTIC FIBROSIS (CF) ON MYCOBACTERIUM ABSCESSUS THERAPY

5.1 PRIMARY OBJECTIVE

Assess steady state pharmacokinetics of CFTR modulators in participants enrolled in the Intervention Program for the treatment of MABS-PD and compare the plasma concentration area under the curve (AUC) of CFTR modulators before MABS-PD therapy and while on MABS-PD therapy.

5.2 INTRODUCTION

There is marked interpatient PK variability in CFTR modulators in people with CF which may result in variations in therapeutic response and toxicity. In addition, there are significant CFTR modulator drug-drug interactions (DDIs) mediated through P450 enzyme pathways that complicate choice of MABS regimen (2-4). Given the unpredictability of DDIs in an individual, patient therapeutic drug monitoring has been proposed as a means of optimising treatment. In order to determine the feasibility and utility of this, the steady state PK of CFTR modulators needs to be determined across multiple time points. This may then allow the development of limited (i.e., less frequent) sampling strategies, in order to assist with TDM in real-world ambulatory settings. Validated CFTR modulator drug assays are being developed by Pathology Queensland, Australia to assist with this study and provide a national reference laboratory for CFTR modulator TDM.

5.3 ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the FOR*Ma*T Master Protocol, participants are required to meet the inclusion criteria outlined below to enrol in sub-study C1.4:

1. Enrolled in the Intervention Program at an Australian site,
2. Have cystic fibrosis and be on CFTR modulator therapy for at least 4 weeks prior to enrolling into the Intervention Program,
3. Availability of suitable intravenous access to facilitate sample collection,
4. Patients may be receiving other antibiotics concurrently,
5. Informed consent by participant or their parent/guardian.

5.4 INFORMED CONSENT

Additional blood samples are required as part of the study procedures for sub-study C1.4, therefore additional consent will be obtained in accordance with the policies described in the FOR*Ma*T Master Protocol.

5.5 SUB-STUDY SPECIFIC PROCEDURES

1. Patients will have CFTR modulator PK plasma samples collected on two separate days.

2. The initial pre-MABS treatment sampling day will likely be the day before MABS treatment is commenced in order to define baseline steady state CFTR modulator PK prior to treatment.
3. The second sampling day of this study will be determined by the site investigator and will be after **5 half-lives** (at least 48hrs after the patient is established on the planned treatment dose) of the antibiotic with the longest half-life (excluding azithromycin which has a prolonged half-life) to ensure testing is done at **steady state**. There must be no dose adjustments in the 48hrs prior to sampling. The CFTR modulator sub study (C1.4) second day of sampling can occur on the same day as the antibiotic PK study (C1.3) as this will allow CFTR modulator samples to be taken at the same time.
4. Adult participants will have up to 5 ml whole blood drawn through a cannula at the following times on 2 separate days: (1) the day prior to MABS treatment starting and (2) on a single day of intensive MABS therapy treatment:
    i. immediately pre CFTR modulator dose (trough level),
    ii. post dosing: at approximately 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours after the oral administration of CFTR modulator medications on the day of sampling.
5. Paediatric participants will have 3mL whole blood drawn through a cannula at the following times on 2 separate days: (1) the day prior to MABS treatment starting and (2) on a single day of intensive MABS therapy treatment:
    i. Immediately pre CFTR modulator dose (trough level),
    ii. Post dosing: at approximately 3 hours and 6 hours after the oral administration of CFTR modulator medications on the day of sampling.
6. Additional blood samples for this study may be taken at the same time as other samples taken for routine care or other sub-studies (C1.2 and 1.3).
7. The time of each blood draw will be recorded in the PK sub-study REDCap database and on the study-specific clinical trial pathology form.
8. All samples must be processed within 1 hour of collection and stored frozen at -80°C until transfer to Pathology Queensland, Herston Brisbane.

5.6 STATISTICAL ANALYSIS

Enrolment targets for sub-study C1.4 are: 20 participants ≥18 years of age and 10 participants <18 years of age.

PK indices will include volumes of distribution, C_{max}, AUC and clearance. A pharmacokinetic model will also be developed using a population pharmacokinetic approach. This technique will enable the effect of patient and treatment characteristics on altered pharmacokinetics (e.g., patient age, weight, renal function, hepatic function, interacting drugs) to be quantified. This model can then be used to assess the adequacy of current dosing regimens and can also be used for dosing simulations to define optimised dosing regimens for these patients.

PK analysis will be conducted with Pmetrics software package for R (version 1.4.1., Los Angeles, CA). Non-linear mixed effect modelling approach will be used. A stepwise analysis will be performed to first determine the structural base model by fitting the concentration-time data to one, two and three compartment models. Testing of additive,

proportional or a combination of additive and proportional models will be conducted to select the best fit statistical error model. Following population PK analysis, Monte Carlo simulations will be performed. The likelihood of reaching the effective concentration to achieve 90% of the maximal clinical effect on lung function (EC90) for CFTR modulators both on and off MABS therapy will be determined. These PK/PD parameters can be used to estimate appropriate doses in clinical practice.

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APPENDIX C2 – DISCOVERY

Immune Factors and Biomarkers

Appendix C2 – Discovery	Version 1.1
Appendix Date	20 February 2024
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
EudraCT Number	2020-000050-10
EU-CT Number	2023-506575-99-00-EU CT
IRAS Project Number	1007146
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

BAL	Bronchoalveolar Lavage
CCA	Canonical Correspondence Analysis
CCHR	Centre for Children's Health Research
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CLR	C-type Lectin Receptor
CM	Central memory
CT	Computed Tomography
EM	Effector Memory
EMRA	Effector Memory RA+
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
GPLs	Glycopeptidolipids
ID	Identification
IQR	Interquartile Range
M1	Type 1 Macrophage
M2	Type 2 Macrophage
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MABS-R	Rough MABS
MABS-S	Smooth MABS
MDMs	Monocyte-Derived Macrophages
NTM	Non-Tuberculous Mycobacteria
PBMCs	Peripheral Blood Mononuclear Cells
PCA	Principal component analysis
PPD	Purified Protein Derivative
R	Rough

RDA	Redundancy Analysis
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
S	Smooth
SOP	Standard Operating Procedure
TLRs	Toll Like Receptors

APPENDIX C: DISCOVERY

Appendix C: Discovery, consists of research studies/programs that may be added over time and that are aimed at gaining understanding through collaboration with the FOR*Ma*T trial but are not specific components of the trial. Participation in each discovery study may require additional consent. Consent requirements are detailed within each of the discovery sub-studies. Discovery studies may be applicable to all participants or only to specific sites or groups of participants. Each study will be described in the following format:

- The named Investigator leads for the sub-study;
- The primary and secondary objectives of the sub-study;
- An introduction to the background of the sub-study;
- Additional eligibility and consent requirements;
- Sub-study specific trial procedures and schedule of assessments;
- Analysis plan.

Appendix C1: Pharmacokinetics.

Appendix C2: Immune Factors and Biomarkers.

Appendix C3: Imaging

APPENDIX C2: IMMUNE FACTORS AND BIOMARKERS

1 INTRODUCTION

Appendix C2 sub-studies will take advantage of careful clinical and microbiological phenotyping of participants enrolled in the FOR*Ma*T Master Protocol to examine immunological susceptibility to infection with MABS-PD providing an opportunity to develop new approaches to assessing risk of infection as well as approaches to management in the future. A summary of sample requirements and volumes are outlined in Table 6 and 7.

SUB-STUDY C2.1: Macrophage Function.

SUB-STUDY C2.2: Mitochondrial Stress.

SUB-STUDY C2.3: T-Cell Function.

SUB-STUDY C2.4: Gene Expression Signatures.

SUB-STUDY C2.5: Serology for MABS.

2 SUB-STUDY C2.1: MACROPHAGE FUNCTION

2.1 INVESTIGATOR LEADS

Professor Peter Sly and Dr Abdullah Tarique.

2.2 PRIMARY OBJECTIVE

Determine susceptibility to *Mycobacterium abscessus* (MABS) infection in participants enrolled in the Observational Cohort compared to those with *Mycobacterium abscessus* pulmonary disease (MABS-PD) enrolled in the Intervention Program by examining impaired function of MABS infected macrophages.

2.3 SECONDARY OBJECTIVES

1. Determine efficiency of macrophage phagocytosis and killing of MABS.
2. Determine whether decreased efficiency of macrophage phagocytosis and killing of MABS contributes to the likelihood of chronic infection with MABS-PD.
3. Determine whether inflammatory signalling pathways activated following MABS-PD infection are dysregulated in macrophages.
4. Examine the association between aberrant phagocytosis and enhanced mitochondrial reactive oxygen species (ROS) production in macrophages in participants following MABS infection.

2.4 INTRODUCTION

MABS exists in two morphotypes, rough (R) and smooth (S), depending on the presence of surface glycopeptidolipids (GPLs) (1). Rough MABS (MABS-R), which lack surface GLP expression, and smooth MABS (MABS-S) are both phagocytized by human monocytes and macrophages. MABS-R have greater ability for intracellular multiplication and adhere more tightly to the phagosomal membrane (2). Macrophages play critical roles in the initiation and resolution of pulmonary inflammation. Following microbial infection, macrophages recognise the invading bacteria via specific surface receptors including Toll-like receptors (TLRs) to engulf and phagocytose them into phagosomes, a compartment that restricts the mobility of the invading bacteria. Phagosomes then fuse with acidic lysosomal compartments to initiate bacterial killing. TLR signalling initiates inflammatory responses to the pathogen. MABS-S are recognised via both Dectin-1, a C-type lectin molecule and TLR2 (3, 4). A physical interaction between Dectin-1 and TLR2 is required to initiate phagocytosis. FAM19A4, a recently described chemokine released by macrophages following bacterial infection, is involved in phagocytosis regulation in macrophages (5).

Macrophages in people with Cystic Fibrosis (CF) have a reduced ability to phagocytose and kill bacteria. Murine studies showed that functional Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is indispensable for phagolysomal killing of bacteria (6). Lysosomes from CFTR knockout mice were less acidic than from wild-type mice, which led to reduced bacterial killing. Inhibiting CFTR channel activity with specific inhibitors during phagocytosis significantly reduced lysosomal acidification, similar to reduced bacterial killing by human macrophages (7). Furthermore, both murine and human studies showed CFTR protein as a regulator of inflammatory responses to bacterial infection (8, 9). Loss of CFTR protein or function led to uncontrolled inflammation in CF.

Macrophages from healthy individuals often display "don't eat me" signals by surface expression of CD31 and CD47 molecules (10, 11). Expression of TLR2, Dectin-1, regulators of phagocytosis and "don't eat me" molecules in macrophages from patients with CF have not been adequately studied.

2.5 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites will be eligible to participate in sub-study C2.1. In addition, participants' eligibility will be based on the following inclusion criteria:

1. Aged six years and older.
2. Ability to provide a minimum 5ml whole blood sample (this blood sample will be used for sub-studies C2.1, C2.2 and C2.3).
3. Participant is enrolled at a FOR*Ma*T Trial site in Queensland that can comply with sampling and shipping requirements.
4. Informed consent by participant or their parent/guardian.

2.6 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study C2.1, therefore additional consent will be obtained in accordance with the policies described in the FOR*Ma*T Master Protocol.

2.7 SUB-STUDY SPECIFIC PROCEDURES

5-10ml of whole blood is collected in participants 6-11 years of age. Participants 12 years and older will be asked to provide up to a 20ml whole blood sample. If participants 12 years and older are unable to provide a 20ml whole blood sample, a 10ml whole blood sample is acceptable. Whole blood samples will be collected into an EDTA collection tube and transported to the Centre for Children's Health Research (CCHR), South Brisbane **within two hours of collection**. Trial identification (ID) number, date and time of collection are to be recorded on the sample tube.

Samples will be collected in accordance with Table 1: Sub-study C2.1 Schedule of Assessments.

Table 1: Sub-study C2.1 Schedule of Assessments

ASSESSMENT	STUDY VISITS			
	Trial Start	**Week 6**	**Week 12**	**Final Outcome^**
Observation Cohort Participant Timeframes	Screening (Day 0; -42 days to +3 days)	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	Screening (Day 0; -42 Days to +3 days)	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	Day 1; -14 Days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50.

2.8 STATISTICAL ANALYSIS

Based on previous experience, the data generated in these experiments are likely to be non-normally distributed (using Shapiro-Wilk test), in which case grouped data will be described by group median (27^{th} and 75^{th} percentile). Comparisons between groups will be undertaken using Mann-Whitney U-Test (Wilcoxon rank sum test), Kruskal-Wallis test on ranks (non-parametric alternative to one-way analysis of variance), or Friedman test (non-parametric alternative to analysis of variance with repeated measures), as appropriate. Regression analyses will be used to control for likely confounder or modifier variables, including age, sex, previous treatment, current CFTR corrector/potentiators therapy. The techniques used for regression analyses will depend on the final sample size. If large enough, parametric methods will be used as the key assumption is that the residuals of the regression are normally distributed, and the Central Limit Theorem states that as the sample size increases all distributions of residuals tend towards normality. If the sample size is not sufficient a multivariable model using quantile regression, in which the median difference and 95% CI between groups, will be determined.

3 SUB-STUDY C2.2: MITOCHONDRIAL STRESS

3.1 INVESTIGATOR LEADS

Professor Peter Sly and Dr Abdullah Tarique

3.2 PRIMARY OBJECTIVE

Determine susceptibility to MABS infection in participants enrolled in the observational program compared to those with MABS-PD enrolled in the Intervention Program by examining mitochondrial function.

3.3 SECONDARY OBJECTIVES

Examine the association between aberrant phagocytosis and reduced mitochondrial function in macrophages in participants following MABS infection.

3.4 INTRODUCTION

Macrophages recognise the invading bacteria via specific surface receptors to engulf and phagocytose them into phagosomes, a compartment that restrict the mobility of the invading bacteria. Phagosomes then fuse with acidic lysosomal compartments to initiate bacterial killing. Concurrent with this, specific bacterial products activate pattern recognition receptors such as the TLRs and C-type Lectin Receptors (CLRs) to induce both antimicrobial and inflammatory responses to the pathogen. MABS are recognised by the CLR, Dectin-1, as well as TLR2 (3, 4). A physical interaction between Dectin-1 and TLR2 is required to initiate phagocytosis. CFTR-dependent dysfunction in Monocyte-Derived Macrophages (MDMs) in CF that result in a decreased ability of type 1 macrophages (M1s) to kill phagocytosed bacteria and to polarise into the type 2 (M2) phenotype has been described (7). These defects are related to a post-transcriptional defect of surface expression of the IL-13Rα, required for polarisation into M2s. This defect is CFTR-dependent, as it is also seen when CFTR function is inhibited in MDMs from healthy controls. In macrophages, CFTR functions as a charge shunt lowering the pH of lysosomes sufficiently to enable bacterial killing. However, CFTR function is also required for generation of ROS, an important component of intracellular bacterial killing (12). Thus, macrophages with deficient CFTR function are likely to have limited ability to kill and clear MABS.

Macrophages use a variety of methods for bacterial killing, including phagocytosis-coupled ROS generation and lysosome-mediated degradation. As noted above, CFTR function is important for these rapid antimicrobial responses. In addition, TLR signalling induces a suite of late-stage antimicrobial responses that facilitate clearance of persistent intracellular bacteria (13). These include redistribution of intracellular zinc for metal ion poisoning of bacteria (14) as well as mitochondrial reprogramming to generate antimicrobial products such as itaconic acid (15) and mitoROS (16). Mitochondria are dynamic organelles that, depending on the cellular environment, can exist as a complex network (driven by mitochondrial fusion) or as fragmented organelles (driven by mitochondrial fission, hereafter fission). TLR signalling induces fission in macrophages (17), and recently fission was identified as a novel antimicrobial response in macrophages (unpublished). It was also found that professional intra-macrophage pathogens subvert fission, and strategies were devised to overcome this subversion to enable macrophage-mediated bacterial clearance (unpublished). Specifically, deacetylation of mitochondrial fusion-promoting mitofusin-1 by

histone deacetylase 6 (HDAC6) constrains fission (18). Thus, inhibiting HDAC6 with a specific inhibitor (tubastatin A) greatly enhances fission and mitoROS generation in bacterially-infected macrophages. However, the roles of antimicrobial mechanisms such as mitoROS and fission in MDM responses against MABS, and whether these responses are affected in CF MDMs, are unknown.

3.5 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites will be eligible to participate in sub-study C2.2. Furthermore, samples required for sub-study C2.2 are derived from the whole blood sample obtained in sub-study C2.1. Therefore, only participants enrolled in sub-study C2.1 are eligible for sub-study C2.2. In addition, participants' eligibility will be based on the following inclusion criteria:

1. Enrolled in sub-study C2.1.
2. Aged six years and older.
3. Ability to provide a minimum 5ml whole blood sample (this blood sample will be used for sub-studies C2.1, C2.2 and C2.3).
4. Participant is enrolled at a FOR*Ma*T Trial site in Queensland that can comply with sampling and shipping requirements.
5. Informed consent by participant or their parent/guardian.

3.6 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional consent will be obtained for sub-study C2.2 in accordance with the policies describe in the FOR*Ma*T Master Protocol.

3.7 SUB-STUDY SPECIFIC PROCEDURES

Mitochondrial samples are obtained from the 5-20 ml (age dependent) whole blood samples collected in sub-study C2.1. Refer to the above section for a description of the procedures. No additional samples are required. Trial ID number, date and time of collection are to be recorded on the sample tube.

Samples will be collected in accordance with Table 2: Sub-study C2.2 Schedule of Assessments.

Table 2: Sub-study C2.2 Schedule of Assessments

ASSESSMENT	STUDY VISITS			
	Trial Start	**Week 6**	**Week 12**	**Final Outcome^**
Observation Cohort Participant Timeframes	Screening (Day 0; -42 days to +3 days)	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	Screening (Day 0; -42 Days to +3 days)	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	Day 1; -14 Days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50. N.B. Mitochondrial samples will be obtained from the whole blood sample collected in sub-study C2.1. No additional blood samples are required.

3.8 STATISTICAL ANALYSIS

Based on previous experience, the data generated in these experiments are likely to be non-normally distributed (Shapiro-Wilk test), in which case grouped data will be described by group median (25^{th} and 75^{th} percentile). Comparisons between groups will be undertaken using Mann-Whitney U-Test, Kruskal-Wallis test on ranks (non-parametric alternative to one-way analysis of variance), or Friedman test, as appropriate. Regression analyses will be used to control for likely confounder or modifier variables, including age, sex, previous treatment and current CFTR corrector/potentiators therapy. The techniques used for regression analyses will depend on the final sample size. If large enough, parametric methods will be used as the key assumption is that the residuals of the regression are normally distributed, and the Central Limit Theorem tells us that as the sample size increases all distributions of residuals tend towards normality. If the sample size is not sufficient a multivariable model using quantile regression, in which the median difference and 95% CI between groups, will be determined.

4 SUB-STUDY C2.3: T-CELL FUNCTION

4.1 INVESTIGATOR LEADS

Associate Professor David Reid and Associate Professor Severine Navarro

4.2 PRIMARY OBJECTIVE

Determine susceptibility to MABS infection in in CF and non-CF patients by examining T-cell function participants enrolled in the Observational Cohort and Intervention Program.

4.3 INTRODUCTION

T cells play a critical role in MABS sensing and MABS clearance. It has been hypothesised that CF patients susceptible to MABS infection may have one or more failures in immune control. Using high dimensional immunoprofiling, this hypothesis was validated and found CF patients with current or past MABS infection showed significant distortions in T cell subtypes and in T cell function (19). *Ex vivo* differences in Treg percentages and in T cell activation (CD25) and exhaustion (CTLA-4 & PD-1) markers were observed, as were differences in polyfunctionality of CD4+ T cells (TNF-α, IFN-γ & IL-2); revealing 17 new immune biomarkers for MABS susceptibility. Using these new immune biomarkers, a regression model was generated that produced an AUC of 1. Notably, TNF-α secretion appeared to be defective in CD4+ T cells of CF patients susceptible to MABS-PD infection. This is important given TNF-α directly activates macrophages to restrict mycobacterial growth and induces apoptosis of infected macrophages leading to bacterial killing (20). TNF-α is also essential for granuloma formation and disease restriction (21) and mice deficient in TNF-α are highly susceptible to disseminated forms of mycobacteria (22). These published data around immune failures in CF patients susceptible to MABS could point the way to immune modulating therapies to complement antibiotics and possibly predict therapeutic outcome, but this remains speculative.

The FOR*Ma*T trial provides a unique opportunity to examine T cell phenotype in a much larger population of individuals with and without CF than has been previously possible. The projected number of new MABS-PD diagnoses will allow a comprehensive study of T cell function and allow the conduct of the critical functional experiments that are required to pave the way for new immunomodulating therapeutics and clinical biomarkers. To allow better targeting of studies of immune dysfunction in CF and non-CF individuals, further analyses will be undertaken with new immunoprofiling methods available through collaboration with A/Prof. Navarro, initially using historical stored peripheral blood mononuclear cells from CF patients with chronic MABS infection, as well as historical samples from patients who spontaneously cleared MABS infection and patients who underwent successful eradication of MABS infection and compare immunoprofiles in these disease states to that of normal, healthy controls.

4.4 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites and in sub-studies C2.1 and C2.2 will be eligible to participate in sub-study C2.3. This is because the samples required for sub-study C2.3 are derived from the samples obtained in sub-study C2.1 and C2.2. These inclusion criteria are reflected below:

1. Enrolled in sub-study C2.1 and C2.2.
2. Aged six years and older.
3. Ability to provide a minimum 5ml whole blood sample (this blood sample will be used for sub-studies C2.1, C2.2 and C2.3).
4. Participant is enrolled at a FOR*Ma*T Trial site in Queensland that can comply with sampling and shipping requirements.
5. Informed consent by participant or their parent/guardian.

4.5 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional consent will be obtained for sub-study C2.3 in accordance with the policies outlined in the FOR*Ma*T Master Protocol.

4.6 SUB-STUDY SPECIFIC PROCEDURES

2 x 10^6 peripheral blood mononuclear cells (PBMCs)/ml and 5ml of plasma will be separated from the whole blood sample provided in sub-study C2.1. No additional samples are required. Trial ID number, date and time of collection are to be recorded on the sample tube. All samples will be processed at the CCHR, South Brisbane. Samples will be batched and sent to QIMR Berghofer Institute of Medical Research as requested.

High-dimensional immunoprofiling will be performed by multiparametric flow cytometry followed by RNA sequencing of T cell populations from the same participants examined in sub-study C2.1 and C2.2. Custom flow cytometry panels will be designed based on the strongest T cell biomarkers identified by RNAseq (A/Prof Coin – refer to sub-study C2.4 below). To further determine susceptibility to MABS infection, we will perform T-cell receptor (TCR) sequencing analysis using 10x Genomics single cell V(D)J kits. The diversity of TCR sequences will inform on the true biological diversity of T cells as well as the molecular genetic determinants of antigen specificity. The different T cell clonotypes will be characterised and confirmed where possible, using real-time PCR and flow cytometry. Two or more aliquots of PBMCs will be available from each participant. One aliquot will be used for T cell analysis and one aliquot used for macrophage analysis. Multiparametric flow cytometry will be conducted on major T cell subsets, including central memory (CM), effector memory (EM) and effector memory RA+ (EMRA). Both CD8+ and CD4+ subsets will be profiled along antigen-specific populations using recombinant purified protein derivative (PPD) as a stimulus. Multiplex assay systems (Human Immune Monitoring 65-Plex ProcartaPlex Panel, ThermoFisher) will be utilised to analyse up to 65 protein targets (cytokines, chemokines, growth factors) on tiny volumes of sample (25 - 50µL) from patients in duplicate.

Samples will be collected in accordance with Table 3: Sub-study C2.3 Schedule of Assessments.

Table 3: Sub-study C2.3 Schedule of Assessments

ASSESSMENT	STUDY VISITS			
	Trial Start	**Week 6**	**Week 12**	**Final Outcome^**
Observation Cohort Participant Timeframes	Screening (Day 0; -42 days to +3 days)	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	Screening (Day 0; -42 Days to +3 days)	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	Day 1; -14 Days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50. N.B.- Tcell samples will be obtained from the whole blood sample collected in sub-study C2.1. No additional blood samples are required.

4.7 STATISTICAL ANALYSIS

Flow cytometry data will be analysed using Cytobank which will include heatmap, SPADE, viSNE, dot and histogram overlays and CITRUS outputs. ScRNAseq analysis of 7000 cells has >95% power to detect up to 10 clusters with a minimum of 50 cells per cluster, assuming the smallest subset exists at 1% of the total. Identified functional pathways involving the different T cell subsets will be further interrogated further using the SINCERA pipeline. Cell clusters will be identified using hierarchical clustering with average linkage and Pearson's correlation-based distance. Genes will be considered differentially expressed if they have an average log fold-change of at least 0.25 and a Bonferroni-adjusted *p* value of 0.05 or lower. Cell type assignment will be based on cell type-specific marker gene expression and validated using functional enrichment of cluster-specific differentially expressed genes. Cluster-specific differentially expressed genes will be identified using the two-group Welch's *t*-test-based method in SINCERA.

5 SUB-STUDY C2.4: GENE EXPRESSION SIGNATURES

5.1 INVESTIGATOR LEAD

Associate Professor Lachlan Coin

5.2 PRIMARY OBJECTIVE

Identify biomarker signatures for MABS clearance at the end of intensive therapy and at the final outcome, distinguishing MABS colonisation from MABS-PD between the Observational Cohort and Intervention Program participants.

5.3 INTRODUCTION

In addition to serology biomarkers, development of gene expression signatures may provide an important adjunct for diagnosing and monitoring MABS infection, disease progression and response to treatment. High quality evidence obtained from the FOR*Ma*T trial will guide decisions for starting treatment and measuring disease severity in patients with MABS-PD, influencing global practice.

5.4 ADDITIONAL ELIGIBILITY CRITERIA

All Observational Cohort and Intervention Program participants are eligible to participate in sub-study C2.4 in accordance with the FOR*Ma*T Master Protocol eligibility and if they are able tomeet the following inclusion criteria:

- Ability to provide a 2.5ml whole blood sample.
- Participant is enrolled at a FOR*Ma*T Trial site that can comply with sampling and shipping requirements.
- Informed consent by participant or their parent/guardian.

5.5 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study C2.4, therefore additional consent will be obtained in accordance with the policies outlined in the FOR*Ma*T Master Protocol.

5.6 SUB-STUDY SPECIFIC PROCEDURES

Whole blood samples of up to 2.5ml will be collected in 10ml PAXgene tubes optimised for ribonucleic acid (RNA). Whole blood samples for gene expression signature biomarkers will be collected in accordance with Table 4, Sub-Study C2.4 Schedule of Assessments. Trial ID number, date and time of collection are to be recorded on the sample tube. Blood RNA tubes are to be transported to a central study laboratory at the Centre for Children's Health Research, Queensland, for temporary storage at -20°C prior to batch shipping the samples to the Peter Doherty Institute, University of Melbourne. Alternatively, the samples can be sent directly to the Peter Doherty Institute, University of Melbourne, Australia for processing. The FOR*Ma*T trial management team will assist trial sites in coordinating transportation of samples according to FOR*Ma*T Biological Sample Management SOP.

Samples will be collected in accordance with Table 4: Sub-study C2.4 Schedule of Assessments.

Table 4: Sub-study C2.4 Schedule of Assessments

ASSESSMENT	STUDY VISITS			
	Trial Start	**Week 6**	**Week 12**	**Final Outcome^**
Observation Cohort Participant Timeframes	Screening (Day 0; -42 days to +3 days)	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	Screening (Day 0; -42 Days to +3 days)	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	Day 1; -14 Days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50.

5.7 STATISTICAL ANALYSIS

5.7.1 Identifying a biomarker signature for MABS-PD microbiological cure (clearance)

RNA-seq will be conducted on bloods taken at baseline and at their final outcome assessment, generating ~30m reads per sample. We will use Salmon (23) to calculate transcript abundance from these data, and Limma-Voom pipeline (24) to identify statistically significant differences in expressed transcripts between acute and convalescent time-points. Using the ssizeRNA package in R we can estimate that this approach provides 99.5% power at a false discovery rate of 5% to identify genes with a 1.4 fold-change between conditions, and 50% power at a fold-change of 1.2. We will use the elastic net (25) to identify a biomarker signature for predicting recovery, as well as the FS-PLS algorithm we have described previously for identifying minimal biomarker signatures of recovery (26). Identified biomarkers will be measured on the intermediate time-points, using the NanoString platform in order to investigate the variability of the biomarker signature over time. The identified biomarkers will be validated using the NanoString platform on blood collected from participants with MABS infection.

5.7.2 Identifying a biomarker signature of MABS-PD vs simple colonisation:

RNAseq will be performed on blood collected from 100 patients recruited into the Observational Cohort. Approximately half of these are expected to later develop MABS-PD. As above, we will use Salmon (23) to quantify expression, and Limma-Voom (24) to identify significant differences in expressed transcripts between these two groups, as well as the elastic net (25) and FS-PLS to identify biomarkers of infection vs colonisation. These signatures will be validated using nanoString on a sample of a further 100 samples. As above, this approach has a 99.5% power to identify genes with a 1.4 fold-change.

6 SUB-STUDY C2.5: SEROLOGY FOR MABS

6.1 INVESTIGATOR LEADS

Professor Niels Høiby and Dr Tavs Qvist

6.2 PRIMARY OBJECTIVE

Identify serological markers that predict 1) the natural history of MABS colonisation and 2) progression to MABS-PD as well as 3) treatment failure in different patient subpopulations.

6.3 INTRODUCTION

Targeted therapeutic approaches to maximise clinical benefit are required in the treatment of MABS-PD. The development of serological assessments to help predict and identify patients who may benefit from early treatment and identify successful responses to treatment is required (27). In doing so, unnecessary treatment associated toxicity and costs are reduced, improving outcomes for MABS-PD patients.

6.4 ADDITIONAL ELIGIBILITY CRITERIA

All Observational Cohort and Intervention Program participants are eligible to participate in sub-study C2.5 in accordance with the FOR*Ma*T Master Protocol eligibility. In addition, participants are required to meet the following inclusion criteria:

- Ability to provide a minimum 1ml serum sample.
- Enrolled at a FOR*Ma*T Trial site that can comply with sampling and shipping requirements.
- Informed consent by participant or their parent/guardian.

6.5 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study C2.5, therefore additional consent will be obtained in accordance with the policies outlined in the FOR*Ma*T Master Protocol.

6.6 SUB-STUDY SPECIFIC PROCEDURES

Up to 4mL of blood is obtained by venepuncture and left to clot at ambient temperature. Centrifuging is carried out to pellet the blood cells and serum is then collected by pipetting to a plastic tube (e.g., NAGLE no. 479-3222 from VWR) with an airtight screw cap. Serum samples will be collected in accordance with Table 5, Sub-Study C2.5 Schedule of Assessments. Trial ID number, date and time of collection are to be recorded on the sample tube. Once the sample is processed the tube containing the sample can be stored on site at -20°C. If -20°C storage is not available onsite samples can be transported to a central laboratory located at the Centre for Children's Health Research, Queensland, for temporary storage. All serum samples will be batched and shipped to the Department of Clinical Microbiology, Copenhagen regularly (approximately every three months) by the FOR*Ma*T trial management team, although this may be more or less frequent depending on recruitment and site storage ability.

Table 5: Sub-study C2.5 Schedule of Assessments

ASSESSMENT	STUDY VISITS			
	Trial Start	**Week 6**	**Week 12**	**Final Outcome^**
Observation Cohort Participant Timeframes	Screening (Day 0; -42 days to +3 days)	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	Screening (Day 0; -42 Days to +3 days)	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	Day 1; -14 Days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50.

6.7 STATISTICAL ANALYSIS

Analysis will be descriptive and examine changes across both successful and failed treatment courses in the different subgroups and differences between those who develop MABS-PD and those who are colonized. Validation studies will be developed in the future.

Baseline data will be reported as median and interquartile range (IQR) for non-normally distributed continuous variables, and as percentages for categorical variables. Group comparisons will be made using Kruskal-Wallis test on ranks (non-parametric alternative to one-way analysis of variance) and Dunn's multiple comparison test. A p-value of ≤0.05 will be considered statistically significant. Longitudinal anti-MABS IgG kinetics during the Intervention Program will be compared to the Observational Cohort and effect of treatment will be assessed. A diagnostic algorithm will be constructed based on the principle of risk stratification. The premise of the algorithm will be the predictive value of one routine serum sample for anti-MABS per patient per year. Multilevel predictive values, likelihood ratios and a receiver operating curve for sensitivity and specificity will be constructed. All measures will be reported with confidence intervals. Participants will be divided into phenotype groups on the basis of NTM culture results and clinical data captured from patient records:

1. Participants with MABS-PD disease who are not receiving treatment (at enrolment in the Intervention Program or remaining in the Observation Cohort without treatment).
2. Those that clear infection with treatment during FOR*Ma*T.
3. Those that do not clear infection with treatment during FOR*Ma*T.
4. Participants with positive MABS cultures but no pulmonary disease in the Observation Cohort.
5. Control samples will also be available from patients with no known history of NTM infection and current negative NTM culture results. These control samples are available through samples currently being collected as part of other clinical studies such as: CF control samples - Early Life Origins of CF lung disease CFFT grant (SLY18KO) that includes samples from adults and children (estimated 200 blood samples will be available matched with NTM cultures performed and full clinical history). Non-CF control samples will also be sourced from other approved studies.

6. Recognised previous NTM infections will be documented at enrolment in the FOR*Ma*T Intervention Program and Observation Cohort and any new NTM infections detected during the study will also be documented. The presence of past or present infection with another NTM on MABS serology will be examined.

Coefficient of variance will be reported. The study will be reported in accordance with the guidelines for reporting studies on diagnostic accuracy (STARD).

7 SUMMARY OF C2 SAMPLE COLLECTION

Table 6: Sample collection time frames for C2 sub-studies

Assessment	Study Visit			
	Screening Visit (Day 0)	**Week 6**	**Week 12**	**Final Outcome^**
Review Eligibility for Sub-Studies	✓			
Observation Cohort Participant Timeframes	-42 days to +3 days	+14 Days	±14 Days	±14 Days
Combined Intensive and Consolidation Therapies Participant Timeframes	-42 days to +3 days	±3 Days	±3 Days	+14 Days
Consolidation Only Therapy Participant Timeframes	-42 days to +3 days			+14 Days

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62. For Intervention Program participants receiving Consolidation Only therapy, this is collected at Week 50.

Table 7: Sample volume requirements for C2 sub-studies

Assessment	Sample and Volume Needed
Macrophage, Mitochondrial and T-cells Samples	5-10ml (6-11y) whole blood 10-20ml (12+y) whole blood
Serology	1ml serum (from 2-4ml whole blood)
Gene Expression Signatures	2.5ml whole blood

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FORMaT

Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

APPENDIX C3 – DISCOVERY

Imaging

Appendix C3 – Discovery	Version 1.2
Appendix Date	20 February 2024
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
EudraCT Number	2020-000050-10
EU-CT Number	2023-506575-99-00-EU CT
IRAS Project Number	1007146
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

ATS	American Thoracic Society
CF	Cystic Fibrosis
CT	Computed Tomography
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HRCT	High-Resolution Computed Tomography
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MAC	*Mycobacterium avium* Complex
NTM	Non-tuberculous mycobacteria

APPENDIX C: DISCOVERY

Appendix C: Discovery consists of research studies/programs that may be added over time and that are aimed at gaining understanding through collaboration with the FOR*Ma*T trial but are not specific components of the trial. Participation in each discovery study may require additional consent. Consent requirements are detailed within each of the discovery sub-studies. Discovery studies may be applicable to all participants or only to specific sites or groups of participants. Each study will be described in the following format:

- The named Investigator leads for the sub-study;
- The primary and secondary objectives of the sub-study;
- An introduction to the background of the sub-study;
- Additional eligibility and consent requirements;
- Sub-study specific trial procedures and schedule of assessments;
- Analysis plan.

Appendix C1: Pharmacokinetics.

Appendix C2: Immune Factors and Biomarkers.

Appendix C3: Imaging.

APPENDIX C3: IMAGING

Investigator Leads

Professor Harm Tiddens, Dr Daan Caudri, Dr Taryn Reddy

1 OBJECTIVES

1.1 PRIMARY OBJECTIVES

To examine the changes in structural lung disease as detected on chest computed tomography (CT) scan (PRAGMA-CF %disease, %bronchiectasis, %mucus plugging and %trapped air) across the FOR*Ma*T Intervention Program associated with and without clearance of *Mycobacterium abscessus* (MABS) at 6 and 12 weeks and at final outcome.

1.2 SECONDARY OBJECTIVES

1. Compare the progression of structural changes on chest CT scan (PRAGMA %disease, %bronchiectasis, %mucus plugging and %trapped air) in patients in the Intervention Program and patients in the Observational Cohort with and without *Mycobacterium abscessus* pulmonary disease (MABS-PD).
2. Compare progression of structural changes on chest CT scan (PRAGMA % disease, %bronchiectasis, %mucus plugging and %trapped air) associated with the development of MABS-PD.
3. Compare progression of structural changes on chest CT scan as assessed by the fully automatic LungQ (Thirona, Nijmegen the Netherlands) Bronchus-Artery (BA) analysis (Airway lumen diameter (A_{lumen}), Airway Outer diameter (A_{out}), Airway wall thickness (A_{wt}), Artery diameter (A), A_{lumen}/A, A_{out}/A, A_{wt}/A) associated with the development of MABS-PD.
4. Develop an automated scoring system for chest CT imaging to support diagnosis and monitoring of MABS-PD.
5. Identify CT related biomarkers to predict treatment response.
6. Determine variability in protocolling of CT studies in people with unknown or suspected non-tuberculous mycobacterium (NTM) pulmonary disease (including MABS-PD) across different institutions and develop optimal protocols for CT imaging of MABS-PD.

2 INTRODUCTION

CT scans are regarded as important components of establishing the diagnosis of MABS-PD according to the ATS criteria and are recommended in international guidelines as part of the assessments to be undertaken when starting and ending treatment in patients with MABS-PD (1). There is also some evidence that patients who clear *Mycobacterium avium* Complex (MAC) infection have improved radiological outcomes compared with those who do not clear infection (2) and that early radiological improvement is a predictor of converting to negative sputum cultures. In this study a relative crude image analysis was used, furthermore image acquisition was not standardised. Hence, it is likely that chest CT outcomes using standardised chest CT protocols and more sensitive image analysis systems such as PRAGMA-CF and the BA-analysis can improve the predictive power for sputum conversion and radiological resolution of MABS-PD related structural changes. This trial provides an opportunity to include standardised chest CT scan according to American Thoracic Society (ATS) criteria for MABS-PD for phenotyping of MABS-PD patients.

Using CT related phenotyping the FOR*Ma*T trial provides an opportunity to document the spectrum and changes in structural lung disease as detected on chest CT scan in patients with MABS who do or do not clear infection as well as progression in those with positive MABS cultures that do and do not develop MABS-PD. From a safety and clinical perspective, MABS infection may be cleared from the sputum but this response might be dissociated from progression of structural lung disease as it is questionable whether sputum is reflective of consolidated lung areas.

There are no specific scoring systems for MABS-PD and the FOR*Ma*T trial provides a unique opportunity for the development of a radiological scoring system specific for patients with MABS who do or do not have cystic fibrosis (CF) and this will be one of the Discovery Substudies PRAGMA-CF is currently being automated for monitoring CF lung disease. For FOR*Ma*T all CTs need to be manually annotated. Next these annotated CTs can be used to adjust and train the algorithm future automated image analysis of MABS patients.

Currently there is wide variability in the protocolling of CT studies for the investigation and follow-up of NTM pulmonary disease, including MABS-PD. Therefore, a CT questionnaire has been designed to ascertain the extent of variability in protocolling of these CT studies across different institutions, in order to formulate a more standardised approach in future.

3 ELIGIBILITY CRITERIA

No additional eligibility criteria to those outlined in the FOR*Ma*T Master Protocol apply to sub-study C3.

4 INFORMED CONSENT

All CT scans are performed as part of the FOR*Ma*T Master Protocol. Additional consent is required for the Appendix C3 study specific chest CT scan performed at Week 6 (for Appendix A2 participants) and at Week 12 (for Appendix A1 participants and Appendix B participants).

5 CT TRIAL PROCEDURES

An inspiratory and expiratory helical chest CT scan with High-Resolution Computed Tomography (HRCT) reconstruction will be performed for all FOR*Ma*T trial participants in accordance with the trial procedures outlined in the relevant Schedule of Assessment tables.

Each FOR*Ma*T site participating in the additional Appendix C3 study specific CT scan will have a CT scanning protocol developed by the Erasmus team, taking into account site specific scanning requirements, optimising the balance between radiation and image quality. Site staff will undergo web-based training. Appendix C3 study specific chest CT scans must be performed with the FOR*Ma*T specific CT scanning protocol using optimisation of lung volumes to provide standardisation longitudinally.

Standard of care chest CT scans are strongly recommended to be performed with the FOR*Ma*T specific CT scanning protocol with optimisation of lung volumes where feasible.

Images must be de-identified by the certified imaging provider and sent to the FOR*Ma*T trial management team who will send coded CT images electronically to Erasmus Medical Centre. Alternatively, deidentified coded CT images

will be sent directly from the certified imaging provider to Erasmus Medical Centre for scoring using the PRAGMA-CF scoring system and the LungQ BA-analysis.

To improve understanding of lung disease from infection and develop better analysis of lung images, data from de-identified images may also be sent for further analysis and stored for research purposes by other approved collaborative research groups.

A CT protocol questionnaire will be sent to the relevant radiology department at approved trial sites and contracted imaging providers to determine the current scanning protocols used for unknown pathology and for suspected/proven NTM.

6 CT SCORING

All scans will be manually scored initially using the PRAGMA-CF scoring system which has been extensively validated for quantitative analysis of CF lung disease in children and adolescents (3, 4). Compared with other scoring systems, PRAGMA-CF has increased sensitivity for detecting and monitoring changes in mild as well as in advanced disease (including inflammatory changes, such as mucus plugging, "tree-in-bud", consolidation and bronchiectasis seen in MABS-PD) and has a known expected change with time for CF, which is important for maintaining discriminatory ability and sensitivity to potential change from additional disease (MABS-PD). For example, PRAGMA-CF has been used as outcome measure in a large Phase III clinical trial of Ataluren in 207 children and adults with CF. Longitudinal analysis showed that %Disease at start of study was 9.8% and that it progressed significantly over the study duration (5). No difference was detected between the ataluren and placebo group. This lack of effectiveness was later confirmed in a second Ataluren phase III trial and used in a Phase III clinical trial of Ataluren.

Scoring of coded CTs will be undertaken blinded to treatment allocation and clinical data by the Erasmus Medical Centre LungAnalysis team.

The development of automated scoring will require an initial discovery approach using all the known clinical markers and manual and automated scoring analysis. The protocol developed for scoring will then require validation in two independent data sets with blinding as to disease progression and background disease e.g. cystic fibrosis, COPD, non-CF bronchiectasis, previous tuberculosis etc. One of the data sets will be from prospective FOR*Ma*T participants including observation and intervention participants the other will be from a separate non-FORM*a*T data set.

7 NUMBERS OF AVAILABLE SCANS

There will be 300 paired scans (N=600) from enrolment to completion of the FOR*Ma*T trial for the initial intervention program. We estimate from discussions with patients and from other studies that approximately 200 participants will consent to a further scan at 12 weeks. The risk related to the extra radiation of the third CT scan is low and is estimated to be in the order of 3-6 months background radiation (6). New MABS notifications in Queensland Australia now average >100/year (≈1/3rd CF). An audit of 51 adults with CF and MABS infection in Queensland found 42% did not receive treatment and so would potentially enter the observation arm, although reasons for this were variable. The Royal Brompton Hospital London (pers. com. Prof Elborn and Dr. Jones) suggested 4/49 (8%)

patients in their cohort culturing MABS had single isolates and therefore would not meet ATS criteria but would be eligible for the observation arm. For the Observational Cohort we estimate that 1/3 of subjects will not meet ATS criteria (estimate 50 paired scans) and 1/3 would start in the observation arm but develop MABS-PD and move into the trial subsequently (estimate 50 paired scans) and potentially 1/3 have MABS-PD and not receive treatment through choice (estimate 50 paired scans). In an observation study with 180 subjects 82% of subjects had sputum conversion of MAC on treatment and this was associated with a significant improvement ($P<0.0001$) in structural lung disease (2). This suggests that the numbers of scans available in this trial will support the planned analyses.

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APPENDIX E1 – HEALTH ECONOMICS

Cost Effectiveness and Resource Utilisation

Appendix E1 – HEALTH ECONOMICS	Version 1.0
Appendix Date	29 March 2023
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
IRAS Project ID	1007146
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AE	Adverse event
CAHE	Centre for Applied Health Economics
CEAC	Cost effectiveness acceptability curve
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HRQoL	Health Related Quality of Life
ICD-10	International Statistical Classification of Diseases
ICER	Incremental cost-effectiveness ratios
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
NMB	Net monetary benefit
NTM	Non-tuberculous mycobacterium
PI	Principal Investigator
QALY	Quality adjusted life years
UK	United Kingdom
WTP	Willingness to pay

APPENDIX E: HEALTH ECONOMICS

Appendix E: Health Economics consists of components aimed at gaining understanding of the healthcare costs of Mycobacterium *abscessus* pulmonary disease (MABS-PD) through collaboration with the Finding the Optimal Regimen for *Mycobacterium abscessus* treatment (FOR*Ma*T) trial. Additional Health Economics components may be added during the trial. Participation in each Health Economics component may require additional consent and are detailed within each of the Health Economics appendices or supplements. The supplements are additional documents that expand on the scope of the appendices and provide additional detail and information to supplement the specific appendix they are linked to. Health Economics components may be applicable to specific sites or groups of participants. Each Health Economics appendix will be described in the following format:

- The named Investigator leads;
- The primary and secondary objectives;
- An introduction to the background;
- Additional eligibility and consent requirements;
- Appendix specific trial procedures;
- Analysis plan.

Appendix E1: Cost Effectiveness and Resource Utilisation.
Supplement 1: Australian Health Economics and Data Linkage

APPENDIX E1: COST-EFFECTIVENESS AND RESOURCE UTILISATION

Investigator Leads

Associate Professor Joshua Byrnes and Ms Lauren Ward, Centre for Applied Health Economics (CAHE), Griffith University.

1 INTRODUCTION

Of the different non-tuberculous mycobacterium (NTM) infections, MABS-PD is reported to have the highest associated treatment costs. To help inform health policy about the treatment of MABS-PD the healthcare costs and cost-effectiveness of therapies are required. Participants who do not meet eligibility criteria for Appendix A1 or A2 or choose not to undergo treatment may still enrol in the Observational Cohort, further informing the overall burden of MABS-PD.

2 METHODOLOGY

To capture cost utilisation data in the treatment of MABS-PD the complete resource utilisation data set is required in a minimum of 20% of participants enrolled in each Intervention Program(s) and the Observational Cohort. This includes country specific linked administrative healthcare utilisation data where available, treatment arm allocation, study medications, adverse event (AE) data, and responses to health-related quality of life (HRQoL) and costs questionnaires. These data will be entered in the FOR*Ma*T REDCap database by the FOR*Ma*T trial site and Health Economics research team members. All other participants will require the minimum resource utilisation data set to be entered in the FOR*Ma*T REDCap database. Minimum data includes country specific linked administrative healthcare utilisation data where available, HRQoL questionnaire responses and costs questionnaire responses.

A health resource utilisation and cost questionnaire will be completed by all consenting FOR*Ma*T participants. The survey will be administered at the time points outlined in the Schedule of Assessments tables in the relevant appendices. The questionnaire is based on the 'Annotated cost questionnaire' originally designed for the United Kingdom (UK) setting but will be appropriately adapted for each country by the health economics lead investigator and lead Principal Investigator (PI) for each respective country. Within the health resource utilisation and cost questionnaire, participants will be provided an opportunity to provide feedback. This feedback will be used as an initial quality improvement measure and to confirm face and content validity. Participants who indicate their willingness to be contacted to improve the questionnaire will be contacted by the health economics team and an unstructured interview conducted to receive feedback and suggestions for greater clarity and/or additional content to the questionnaire. Interviews will be conducted via a convenient and remote mode that is available to the participant (e.g., phone or videoconference).

3 COST-EFFECTIVENESS AND RESOURCE UTILISATION FOR APPENDIX A1

3.1 COST-EFFECTIVENESS ANALYSIS

The primary cost-effectiveness analysis will be conducted using a cost-utility analysis for each of the participating countries' health system perspective based on trial participant data. In addition to analyses for a) health system costs only, a broader perspective will also be applied to also include b) costs for both direct and indirect health care costs including patient/carer productivity losses.

Comparative analysis will be completed on an intention-to-treat strategy where patients are analysed within the treatment group to which they were randomly allocated, regardless of, for example, whether or not they received the treatment medication they were allocated to. Results will be presented as incremental cost-effectiveness ratios (ICERs) with their corresponding 95% confidence intervals and cost-effectiveness acceptability curves (CEACs). The ICER represents the additional cost for an incremental improvement in outcome when comparing between groups, Equation 1:

$$Eq(1) \qquad ICER = \left(Cost_{exp} - Cost_{comp}\right) \Big/ \left(Effect_{exp} - Effect_{comp}\right)$$

Where:
Cost = sum costs associated with treatment and consequences of treatment
Effect = summary health outcome measure of interest
exp = experimental group of interest
comp = experimental comparator group

All current treatment paths that include the intensive phase within the platform trial will be evaluated simultaneously prior to adjustment in the platform trial design. Primary analysis (i) will be with respect to which therapy combination (including both the initial and consolidation phases) is most cost-effective. Additional analyses will also consider which therapy combination is the most cost-effective with respect to initial treatment (ii); which therapy combination is the most cost-effective for those who are clear of Mycobacterium *abscessus* (MABS) at 4 weeks (iii); and which therapy combination is the most cost-effective for those who have remained positive for MABS at 4 weeks (iv). Cost effectiveness analyses within each of the four analysis sets will be completed simultaneously with ICER results presented relative to the treatment group with the lowest cost. An intervention arm will be considered cost-effective where the ICER is less than or equal to each countries respective cost effectiveness threshold (λ), the value of an incremental improvement in effect. However, there is considerable uncertainty as to any given countries λ. As such, cost effectiveness acceptability curves (CEACs) will be presented as a way to examine the probability of the intervention being cost effective, given various values of a countries λ.

As the ICER is a ratio and multiple treatment strategies are being explored simultaneously, several difficulties may arise in its interpretation. A positive ratio can represent both higher costs and better outcomes in the experimental

group, and lower costs and less favourable outcomes (compared to the control group). Moreover, a negative ratio can indicate higher costs coupled with worse outcomes (comparator dominates the intervention) as well as lower costs and better outcomes (intervention dominates the comparator). As such, the net monetary benefit (NMB) of each intervention will also be estimated. Whereby, net benefit is estimated by multiplying the outcome by λ, and subtracting costs, as per Equation 2:

$$Eq(2) \qquad NMB = Effect * \lambda - \text{Cost}$$

Where:
Cost = sum costs associated with treatment and consequences of treatment
Effect = summary health outcome measure of interest
λ = the value of an incremental improvement in effect

For each intervention the NMB will be estimated and ranked according to the largest NMB. Again, given the uncertainty as to any given countries λ, the probability of an intervention being the most cost effective will be explored over a range of estimates of λ.

3.2 EFFECT MEASURE

The summary health outcome measure to be included in the analysis is Quality Adjusted Life Years (QALYs). QALYs are estimated based on the sum of the duration of time living in respective health states. Where health states are described by responses to the EQ-5D and CHU9D surveys for adults and children respectively. The utility values for each health state will be informed by country specific utility valuation sets and applied respectively for each country specific analysis. Generally, QALYs are estimated based on Equation 3:

$$Eq(3) \qquad QALY = \sum_{t=a}^{a+L} \frac{Q_t}{(1+r)^{t-a}}$$

Where:
QALY = quality adjusted life years
a = current age
L = lifetime or model duration
t = time period within that lifetime/model duration
Q = vector of health-related quality of life weights for each time period
r = discount rate

In this analysis, individual QALYs will be estimated using an area under the curve approach (1). For each individual, QALYs are estimated based on the change in health-related quality of life assuming change in health state occurs linearly between measurements. The unadjusted difference in QALYs between the randomisation groups is calculated as per Equation (2) in which the mean difference between health related quality of life utility scores are multiplied with the time in years between observations and divided with 4:

$$Eq(4) \qquad AUC_i = \frac{1}{2}\sum_{j=1}^{n_i}(t_{j+1} - t_j)(Q_j + Q_{j+1})$$

Where:
Q_{ij} = health-related quality of life weights based on quality of life survey responses
t_{ij} = represents the observation time period,
j = 1, …*ni* observations
i = 1, …, *m* participants

3.3 COSTS

Costs will include both medication and diagnostic costs associated with treatment and monitoring as well as costs associated with community, hospital and emergency department presentations during treatment. This will enable the inclusion of costs associated with treatment related adverse events.

Descriptive data for treatment related and all other health care utilisation will be presented with respect to counts at an aggregated descriptor level. For example, International Statistical Classification of Diseases (ICD-10) codes for admitted patient separations, anatomical therapeutic chemical classification for prescription medications and major diagnostic classification for emergency department presentations. More formally, costs for both experimental and comparator groups will be estimated as Equation 5:

$$Eq\ (5) \qquad Total\ Cost = \sum_{t=a}^{a+L}\frac{Cost_t}{(1+r)^{t-a}}$$

Where:
Cost = cost incurred in period
a = current age
L = lifetime or model duration
t = time period within that lifetime/model duration
r = discount rate

3.4 COST UTILITY ANALYSIS

Analysis of the cost-utility of the interventions will be conducted with the use of a Seemingly Unrelated Regression (2). The system of Seemingly Unrelated Regression equations is being used as it has been argued that such methods are generally robust to skewed data and to allow for any correlation between costs and effects. Multivariate regression analysis of the NMB will be undertaken with the intervention group as the primary explanatory variable of interest. Linear compared to general model specification will be determined based on comparison of summary goodness of fit measures including Akaike's Information Criteria and Bayesian information criterion as well as Pregibon and Modified Park Test. Separate regression models will be fitted for a) the entire population b) block-randomised sub-groups. Within these analytical frameworks it is possible to control for baseline scores, and confounding variables (3, 4). Bootstrapping will be used to obtain multiple estimates for the group difference, allowing a probabilistic interpretation of results: the percentage of estimates of the group difference greater than one

– indicating better results for the experimental group – is plotted for each value of willingness to pay (WTP), resulting in the CEAC (5).

All models will take into account clustering by site. Missing data will be addressed using multiple imputation (6). A blinded data review will inform the imputation strategy and the selection of multivariable models. To assess the effect of missing data, economic analyses will be performed on the imputed data, and on complete cases. A complete-case analysis will only include participants if complete cost and outcome data at all time points are available for that participant.

4 COST EFFECTIVENESS AND RESOURCE UTILISATION FOR APPENDIX A2

The primary analysis for this arm will be with respect to which consolidation therapy is most cost-effective. Specifically, cost effectiveness analyses for each of the consolidation treatment groups will be completed simultaneously with ICER results presented relative to the treatment group with the lowest cost. The NMB of each intervention will also be estimated with intervention strategies ranked by highest NMB.

4.1 COST-EFFECTIVENESS ANALYSIS

The primary cost-effectiveness analysis will be conducted using a cost-utility analysis for each of the participating countries' health system perspective based on trial participant data. In addition to analyses for a) health system costs only, a broader perspective will also be applied to also include b) costs for both direct and indirect health care costs including patient/carer productivity losses.

Comparative analysis will be completed on an intention-to-treat strategy where patients are analysed within the treatment group to which they were randomly allocated, regardless of, for example, whether or not they received the treatment medication they were allocated to. Results will be presented as incremental cost-effectiveness ratios (ICERs) with their corresponding 95% confidence intervals and cost-effectiveness acceptability curves (CEACs). The ICER represents the additional cost for an incremental improvement in outcome when comparing between groups, Equation 1:

$$Eq(1) \qquad ICER = \left(Cost_{exp} - Cost_{comp}\right) \Big/ \left(Effect_{exp} - Effect_{comp}\right)$$

Where:
Cost = sum costs associated with treatment and consequences of treatment
Effect = summary health outcome measure of interest
exp = experimental group of interest
comp = experimental comparator group

4.2 EFFECT MEASURE

The summary health outcome measure to be included in the analysis is QALYs. QALYs are estimated based on the sum of the duration of time living in respective health states. Where health states are described by responses to the EQ-5D and CHU9D surveys for adults and children respectively. The utility values for each health state will be informed by country specific utility valuation sets and applied respectively for each country specific analysis. Generally, QALYs are estimated based on Equation 2:

$$Eq(2) \qquad QALY = \sum_{t=a}^{a+L} \frac{Q_t}{(1+r)^{t-a}}$$

Where:
QALY = quality adjusted life years
a = current age

L = lifetime or model duration
t = time period within that lifetime/model duration
Q = vector of health-related quality of life weights for each time period
r = discount rate

In this analysis individual QALYs will be estimated using an area under the curve approach (1). For each individual, QALYs are estimated based on the change in health-related quality of life assuming change in health state occurs linearly between measurements. The unadjusted difference in QALYs between the randomisation groups is calculated as per Equation 2 in which the mean difference between health-related quality of life utility scores are multiplied with the time in years between observations and divided with 3:

$$Eq(3) \qquad AUC_i = \frac{1}{2}\sum_{j=1}^{n_i}(t_{j+1} - t_j)(Q_j + Q_{j+1})$$

Where:
Q_{ij} = health-related quality of life weights based on quality-of-life survey responses
t_{ij} = represents the observation time period,
j = 1, …*ni* observations
i = 1, …, *m* participants

4.3 COSTS

Costs for the consolidation group will include both medication and diagnostic costs associated with treatment and monitoring as well as costs associated with community, hospital and emergency department presentations during treatment phase. This will enable the inclusion of costs associated with treatment related adverse events.

Descriptive data for treatment related and all other health care utilisation will be presented with respect to counts at an aggregated descriptor level. For example, ICD-10 codes for admitted patient separations, anatomical therapeutic chemical classification for prescription medications, major diagnostic classification for emergency department presentations. More formally, costs for consolidation therapies will be estimated as, equation 4:

$$Eq(4) \qquad Total\ Cost = \sum_{t=a}^{a+L}\frac{Cost_t}{(1+r)^{t-a}}$$

Where:
Cost = cost incurred in period
a = current age
L = lifetime or model duration
t = time period within that lifetime/model duration
r = discount rate

4.4 COST UTILITY ANALYSIS

Analysis of the cost-utility of the consolidation therapies will be conducted with the use of a Seemingly Unrelated Regression (2). The system of Seemingly Unrelated Regression equations is being used as it has been argued that such methods are generally robust to skewed data and to allow for any correlation between costs and effects. Here, separate regression models will be fitted for a) the entire population b) block-randomised sub-groups. Within these analytical frameworks it is possible to control for baseline scores, and confounding variables (3, 4). Bootstrapping will be used to obtain multiple estimates for the group difference, allowing a probabilistic interpretation of results: The percentage of estimates of the group difference greater than one – indicating better results for the experimental group – is plotted for each value of WTP, resulting in the CEAC (5).

All models will take into account clustering by site. Analyses will be shown for a) health system costs only and b) costs including direct and indirect health care costs including for example, patient/carer productivity losses. Missing data will be addressed using multiple imputation (6). A blinded data review will inform the imputation strategy and the selection of multivariable models. To assess the effect of missing data, economic analyses will be performed on the imputed data, and on complete cases. A complete-case analysis will only include participants if complete cost and outcome data at all time points are available for that participant.

5 COST-EFFECTIVENESS AND RESOURCE UTILISATION FOR APPENDIX B

5.1 COSTS

Costs will include both medication and diagnostic costs associated with monitoring as well as costs associated with community, hospital and emergency department presentations during this time. This will enable the inclusion of costs associated with adverse events.

Descriptive data for MABS infection related and all other health care utilisation will be presented with respect to counts at an aggregated descriptor level. For example, ICD-10 codes for admitted patient separations, anatomical therapeutic chemical classification for prescription medications, and major diagnostic classification for emergency department presentations. More formally, costs will be estimated as Equation 5:

$$Eq\ (5) \qquad Total\ Cost = \sum_{t=a}^{a+L} \frac{Cost_t}{(1+r)^{t-a}}$$

Where:
Cost = cost incurred in period
a = current age
L = lifetime or model duration
t = time period within that lifetime/model duration
r = discount rate

5.2 COST ANALYSIS

A cost analysis will be undertaken with a descriptive analysis of the total cost per person, the total cost per month of follow up between Day 0 and Final outcome. In addition, the expected cost per adverse event by adverse event type will be estimated.

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FORMaT

Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment

APPENDIX F

General Statistical Principles

Appendix F – General Statistical Principles	Version 1.0
Appendix Date	29 March 2023
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
IRAS Project ID	1007146
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
Sponsor	The University of Queensland
Contact	FORMaTtrial@uq.edu.au

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ABBREVIATIONS

AE	Adverse Event
AI	Adaptive intervention
BAL	Bronchoalveolar Lavage
BAR	Bayesian Adaptive Randomisation
CI	Confidence Interval
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DTR	Dynamic treatment regimen
FEV1	Forced Expiratory Volume in one second
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
HRQoL	Health Related Quality of Life
IA	Inhaled Amikacin
ICE	Intercurrent event
iDSMB	Independent Data and Safety Monitoring Board
ITT	Intention To Treat
IV	Intravenous
IVA	Intravenous Amikacin
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
OR	Odds ratio
R-Con	Randomisation – Consolidation
R-PI	Randomisation – Prolonged Intensive
R-SI	Randomisation – Short Intensive
SAE	Serious Adverse Event

SAP	Statistical analysis plan
SMART	Sequential Multiple Assignment Randomised Trial

1 INTRODUCTION

Appendix F: General Statistical Principles, provides details of the general statistical principles of the Intervention Program(s) (Appendix A). The statistical principles pertaining to the Observational Cohort, discovery sub-studies and integrated studies are detailed in the relevant appendices. The Statistical Analysis Plan(s) (SAP) for Appendix A will provide a detailed plan of the statistical analysis of the Appendix A intervention program(s).

2 RANDOMISATION

The initial design of the FOR*Ma*T trial detailed in Appendix A1 has 3 stages of randomisation (see Figure 1 in Appendix A1). Details of the randomisation process at the 3 stages can be found in sections 6.3 and 12.1 of Appendix A1.

2.1 BLINDING OF TREATMENT ALLOCATION

The initial Intervention Program detailed in Appendix A1 is randomised and open label, however the FOR*Ma*T trial may include placebo controlled double blind randomised interventions in the future. If this occurs, details of the blinding will be provided in the relevant appendix.

2.2 MINIMISATION

To ensure balance between arms in important patient characteristics, randomisation at each stage of the trial will use minimisation with a random element. Minimisation is a dynamic randomisation approach used in clinical trials to balance allocation to treatment arms with respect to a number of important stratification factors. In minimisation, the first participant is allocated to their treatment arm at random. Subsequent participants are assigned to a treatment arm by first selecting the preferred arm that would best improve the balance of participants across the arms based on the stratification variables of interest in terms of the numerical difference in the sample size in each of the treatment arms across all of the stratification factors (1, 2). The preferred arm is then selected with a probability of 0.7, with the rest of the probability split between the alternative arms.

Where the randomisation consists of 3 or more interventions, the allocation probabilities will be updated using Bayesian Adaptive Randomisation (BAR, see section 2.3). Following BAR, minimisation will be conducted using Biased Coin Minimisation (BCM) (3). Under this method, the same methodology as standard minimisation will be used to determine the preferred intervention, but the randomisation probabilities will be altered to reflect the minimisation probabilities and the updated allocation ratios from the BAR as detailed in Han et al (3).

Randomisation will be conducted electronically through the trial database following completion of all the specific required data entry by the study team at each site. Participants will be randomised according to the stratification criteria described below using the weights specified for each factor:

1. Macrolide resistance*: Yes or no (weight = 50% in randomisations 1 and 2 (Randomisation-Short Intensive (R-SI) and Randomisation-Prolonged Intensive (R-PI), respectively), 25% in randomisation 3 (Randomisation-Consolidation (R-Con))).

   *Any of these measurement methods are acceptable for defining macrolide resistance (in order of preference): Inducible at 14 days or constitutive at 3 days, and/or; *Erm*(41) status: Functional or dysfunctional, and/or; MABS subspecies: *M. a. abscessus* & *M. a. bolletii* combined or *M. a. massiliense*.
2. Age: <12 years, 12-30 years and >30 years of age (weight = 20%).
3. Sex: Male or Female (weight = 7.5%).
4. Location: Asia Pacific as one stratum (includes Australia, New Zealand, Singapore and other Asian Pacific countries), United Kingdom and Republic of Ireland as one stratum, Europe as another stratum (includes Denmark, France, Netherlands) and Canada and the Americas as one stratum (weight = 7.5%). Parts of the world not listed above can be added into the regions based on closest proximity to the regions longitudinally.
5. Cystic Fibrosis Status: Yes or no (weight = 7.5%).
6. Mixed NTM infections at enrolment: Yes or no (weight =7.5%).
7. MABS positive culture (at first randomisation to intensive therapy (R-SI) and for R-PI all participants will have a positive culture, so this factor will not be required. However, it will be required for R-Con: Yes or no (weight = 25%).

2.3 BAYESIAN ADAPTIVE RANDOMISATION

When a randomisation stage involves randomising participants between 3 or more interventions, BAR will be used to update the allocation ratios between the intervention arms. When the Intervention Program (Appendix A1) initially begins, there will be 3 interventions in the intensive phase (R-SI), hence BAR will be used in this phase, but not the other randomisations. As treatments are added into the later randomisation stages it may also be used at other randomisation stages (see Randomisation sections in Appendix A1 and A2). In the intensive phase, after achieving the minimum number of 100 subjects to be recruited into this randomisation stage, BAR will be implemented at two interim updates (after 100 and 200 participants have been randomised), unless the data overwhelmingly supports early stopping for futility, or the maximum sample size is attained (see section 3).

For the BAR, the following will be used to update the allocation ratios in the first randomisation. Let p_A, p_B and p_C represent the true probability of six-week clearance with tolerability on arms A, B and C respectively. At each of the interim analyses the posterior probabilities of:

1) Inhaled amikacin (IA) being superior to intravenous amikacin (IVA) with clofazimine, $P(p_B > p_A)$ and

2) IVA without clofazimine being superior to IVA with clofazimine, $P(p_C > p_A)$

will be estimated. To compute the posterior probabilities in 1) and 2), a prior distribution on the success probabilities (p_A, p_B and p_C) will be specified. A beta prior distribution, $beta(0.2,0.8)$, will be used and the posterior probabilities $P(p_B > p_A)$ and $P(p_C > p_A)$ will be computed using formula (2) in Wason and Trippa (4). The parameters of the prior are chosen so that the prior distribution is centred around an initial guess p_A^*.

These will then be used to set the allocation ratio for randomising the next stage of participants respectively to arms A, B and C namely π_A, π_B, π_C. The formula used to set the allocation is:

$$\pi_A = 1/3$$
$$\pi_B = (2/3) \times \frac{P(p_B > p_A)^{\gamma(n/N)}}{P(p_B > p_A)^{\gamma(n/N)} + P(p_C > p_A)^{\gamma(n/N)}}$$
$$\pi_C = (2/3) \times \frac{P(p_C > p_A)^{\gamma(n/N)}}{P(p_B > p_A)^{\gamma(n/N)} + P(p_C > p_A)^{\gamma(n/N)}},$$

where $\gamma(n/N) = 12 * (\left(\frac{n}{N}\right))^{2.5}$, n/N represents the proportion of planned sample size recruited so far (1/3 at first interim and 2/3 at second interim). This procedure follows the one recommended by Wason and Trippa (4).

3 STATISTICAL SIMULATIONS FOR POWER CALCULATIONS

The design of this trial, including the stopping rules and the planned sample size, will be guided by simulating data from realistic scenarios for the outcomes and the treatment effects obtained from discussions with content experts. The initial simulations will incorporate outcomes from the 3 stages of the trial simultaneously and will be used to explore the frequentist operating characteristics of the trial at the end of each stage. In this section, we provide details of the initial simulations used to develop the trial design and sample size for Appendix A.

We have powered the trial assuming 300 Intervention Program trial participants are recruited in total. The sample size of 300 participants is based on the feasibility of recruitment and providing reasonable power for plausible effect sizes based on the results of the simulations. Two interim analyses to update the allocation probabilities using BAR in randomisation stage 1 (R-SI) will be conducted after 100 and 200 patients have their outcome data available.

We have considered six different scenarios for the probability of clearance with tolerability outcome at the three different randomisation stages. Tables 1 to 3 show these probabilities for each scenario.

Table 1: Intensive phase six-week outcome probabilities

Arm	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6

Intensive A: IVA with clofazimine (reference arm)	0.5	0.45	0.45	0.5	0.7	0.50
Intensive B: IA with clofazimine	0.5	0.65	0.45	0.7	0.5	0.65
Intensive C: IVA without clofazimine	0.5	0.45	0.65	0.7	0.5	0.65

Table 2: Continued intensive vs not 12-week outcome probabilities

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
1. MABS positive, Prolonged intensive, Consolidation a (Oral only)	0.5	0.5	0.7	0.5	0.5	0.30
2. MABS positive, Prolonged intensive, Consolidation b (Oral+IA)	0.5	0.5	0.7	0.5	0.5	0.30
3. MABS positive, Immediate Consolidation a	0.5	0.7	0.5	0.6	0.4	0.55
4. MABS positive, Immediate Consolidation b	0.5	0.7	0.5	0.6	0.4	0.55

Table 3: Consolidation phase long-term outcome probabilities

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
1. MABS positive, Prolonged intensive, Consolidation a	0.5	0.5	0.30	0.7	0.60	0.3
2. MABS positive, Prolonged intensive, Consolidation b	0.5	0.7	0.45	0.5	0.80	0.5
3. MABS positive, Immediate Consolidation a	0.5	0.5	0.30	0.7	0.55	0.5
4. MABS positive, Immediate Consolidation b	0.5	0.7	0.45	0.5	0.75	0.7

5. MABS negative, Immediate Consolidation a	0.5	0.5	0.30	0.7	0.75	0.7
6. MABS negative, Immediate Consolidation b	0.5	0.7	0.45	0.5	0.95	0.9

3.1 SCENARIO DEFINITIONS

- Scenario 1 represents the null scenario where all treatment strategies (i.e. all interventions at each stage) have the same effect.
- Scenario 2 represents a scenario where one intensive phase treatment provides an advantage in probability of clearance with tolerability, prolonged intensive is worse than immediate consolidation at 12 weeks, and oral+IA provides an advantage over oral for consolidation for each type of patient.
- Scenario 3 represents a similar scenario to Scenario 2 except prolonged intensive is better than immediate consolidation at 12 weeks and consolidation probabilities are lower.
- In Scenario 4, both arm B and C provide advantages over arm A at 6 weeks, with oral being superior to oral+IA at the long-term outcome, immediate consolidation is better than prolonged intensive at 12 weeks with lower probabilities than in Scenario 2.
- In Scenario 5, arm A is superior to B and C. Consolidation probabilities are highly variable by participant pathway (albeit with oral+IA having the same absolute improvement in each case), prolonged intensive is better than immediate consolidation at 12 weeks with a lower probability of clearance with tolerability.
- Scenario 6 is similar to Scenario 5, with each pathway having generally lower probability of clearance with tolerability at the long-term outcome, both arm B and C provide advantages over arm A at 6 weeks with lower probability of clearance with tolerability and prolonged intensive is worse than immediate consolidation at 12 weeks with lower probabilities than in Scenarios 2 and 4.

For each scenario, 100,000 simulation replicates were conducted. In each replicate, 300 patients have their pathways through the trial simulated with 6-week outcome, 12-week outcome and long-term outcome generated with a binomial distribution with probability according to the relevant scenario in the above tables.

3.2 INTENSIVE PHASE POWER SIMULATIONS

The intensive phase data is analysed (in simulations) with a test of proportions assuming a normal approximation. Note the analysis models used in the simulations are unadjusted for covariates as it is difficult to incorporate covariates into a trial simulation. For the final statistical analysis, adjustments will be made for the variables used in the randomisation procedure (see section 2). It is anticipated that these adjustments will increase the statistical power.

Table 4 shows the power of the trial for testing the two primary research questions in the intensive phase under the different scenarios.

Table 4: Intensive phase outcome power for different hypotheses, 100,000 simulation replicates

Comparison	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
B vs A	0.060	0.870	0.048	0.81	0.87	0.56
C vs A	0.059	0.052	0.870	0.81	0.86	0.56

Generally, for cases where there is ≥20% difference between arms, the trial with 300 participants is well powered. The BAR procedure is advantageous when one experimental arm provides an advantage but not the other (as more patients are randomised to the effective arm).

3.3 PROLONGED INTENSIVE VS SHORT INTENSIVE (IMMEDIATE CONSOLIDATION) AT 12 WEEKS POWER SIMULATIONS

Table 5 shows the power for the design to conclude an improvement at 12 weeks from randomising non-cleared patients between prolonged intensive and immediate consolidation in terms of final clearance outcome, for different scenarios. When the difference between groups (prolonged intensive vs not) is greater than 25% (scenario 6) the trial with 300 participants has 80% power (row 1). The adjustment for different recruitment stages (row 2, Model 1) does not reduce the power. These simulations use an analysis model that is not adjusted for randomisation variables. In the final analysis adjusting for minimisation variables will be done which is expected to increase power (see section 7).

Within the simulation study different ways of analysing the data were investigated to allow adjustments for the study stage and treatment groups. Row 1 shows results using a test of proportions. The models used for analysis in the other rows are given below. Rows 1 and 2 show the probability of detecting a difference between prolonged intensive and short intensive (immediate consolidation) at week 12, unadjusted and adjusted for stage (using Model 1) respectively. Rows 3 and 4 show the probability of detecting a difference between prolonged intensive treatment and moving directly to consolidation arms a and b respectively (adjusted for stage using Model 2). These simulations indicate that Model 1 results in similar power to the fully unadjusted analysis model, and a model testing for interaction between path and stage does not have enough power (results not shown here).

Table 5: 12 weeks outcome power for different hypotheses, 100,000 simulation replicates

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
Prolonged vs short intensive, 12 weeks (unadjusted)	0.052	0.67	0.67	0.18	0.21	0.80
Prolonged vs short intensive, 12 weeks (adjusted for stage using Model 1)	0.054	0.67	0.67	0.19	0.21	0.80

Immediate Consolidation a vs Prolonged intensive (adjusted for stage using Model 2)	0.049	0.48	0.50	0.13	0.15	0.62
Immediate Consolidation b vs Prolonged intensive (adjusted for stage using Model 2)	0.051	0.48	0.50	0.13	0.15	0.62

Where:

Model 1: $logit(P = 1|treat, stage) = \alpha_0 + \alpha_1\, treat + \alpha_2 stage_2 + \alpha_3 stage_3$

Treat=0 if a patient with a positive sputum (after six weeks) had an extended intensive treatment.

Treat=1 if a patient with a positive sputum started the oral consolidation directly (either oral consolidation alone or plus IA). Stage corresponds to the three recruitment periods. $\boldsymbol{Stage_2 = 1}$ if a patient was recruited during the second period, $\boldsymbol{Stage_3 = 1}$ if a patient was recruited during the third period, and $\boldsymbol{Stage_2 = Stage_3 = 0}$ if a patient was recruited during the first period.

Model2:$logit(P = 1|path, stage) = \alpha_0 + \alpha_1\, path_2 + \alpha_2 path_3 + \alpha_3 stage_2 + \alpha_4 stage_3$, where path corresponds to one of the three pathways (immediate consolidation a, immediate consolidation b, prolonged intensive) followed by each patient after six weeks. $\mathbf{Path_2} = 1$ if a patient with a positive sputum after six weeks started consolidation a , $\mathbf{path_3} = 1$ if a patient with a positive sputum after six weeks started consolidation b, and $\mathbf{Path_2} = \mathbf{Path_3} = 0$ if a patient with a positive sputum after six weeks had an extended intensive treatment. Stage is defined as before.

3.4 CONSOLIDATION PHASE POWER SIMULATIONS

Table 6 shows the power of the design to detect differences between Consolidation a (oral consolidation) vs Consolidation b (oral + IA consolidation). It appears that the planned study design has good power to detect differences for the scenarios considered. The consolidation phase data is analysed using a test of proportions assuming a normal approximation. Again, note that the simulations are unadjusted for randomisation covariates. In the final analysis we plan on adjusting for these and this adjustment is expected to result in increased power (see section 7).

Table 6: Consolidation Power for different hypotheses, 100,000 simulation replicates

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
Consolidation b vs a 1: 3+5 vs 4+6	0.051	0.86	0.63	0.83	0.95	0.90
Consolidation b vs a 2: 1+3+5 vs 2+4+6	0.051	0.95	0.77	0.95	0.98	0.95

4 ESTIMANDS

The analytical approaches for the primary and secondary outcomes will be detailed using the estimand framework. An estimand is a precise description of the treatment effect reflecting the clinical question posed by a given clinical trial objective. It has 5 attributes: population, treatment, variable of interest (e.g. outcome), summary measure, and possible intercurrent events (ICEs; an event that can occur post-randomisation and preclude or affect the interpretation of the variable of interest e.g. discontinuation of treatment).

When defining an estimand, it must be made clear how possible ICEs will be handled in the analysis. Different approaches can be taken towards handling ICEs and are described below:

i) Hypothetical: a strategy which envisages a scenario in which the ICE would not occur, e.g. if participants had not switched treatment or if death had not occurred.

ii) Treatment policy: a strategy which seeks to understand the treatment effect on the variable regardless of the intercurrent event, i.e. an outcome is of interest whether or not the ICE occurred prior to the outcome, e.g. the final outcome is of interest irrespective of whether the participant takes additional medication.

iii) Composite: a strategy which considers the occurrence of the ICE as informative about the participants outcome. Under this strategy the ICE is included in the endpoint definition, e.g. classifying the use of rescue medication as failure, in addition to disease progression, in a time-to-event analysis.

iv) Principal Stratification: a strategy wherein treatment effects are assessed in the stratum of participants who would have a specific status with respect to the ICE e.g. examining the effect of treatment in participants who would not require rescue medication.

v) While-on-treatment: a strategy where the interest is in the response to treatment prior to the occurrence of the ICE. For repeated measures, values up to the ICE are of interest but not values after the ICE. Generally this strategy is only useful if the duration of treatment is not relevant, either because it is not clinically relevant or because the rate of an event or outcome is constant over time e.g. the rate of adverse events, where one assumes a constant hazard.

Within the SAP, the estimand for each objective will be described using the 5 attributes above, and will specify how possible ICEs will be handled in the analysis. Possible ICEs that will be considered in this study will be;

i) Treatment discontinuation due to an adverse event (AE) with a Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher or a serious adverse event (SAE).

ii) Treatment discontinuation due to other reasons, including AEs below CTCAE grade 3.

iii) Treatment non-compliance (intermittent or partial treatment adherence not resulting in discontinuation).

iv) Treatment discontinuation for any reason except death.

v) Death.

5 POPULATIONS OF ANALYSIS

The interim analyses and the primary analysis for all sub-studies will be conducted on the Intention-To-Treat (ITT) population. This population will consist of all randomised participants, with participants analysed according to the intervention group to which they were randomised irrespective of the intervention received.

It is possible that participants in the intervention trial may cease one or more of the intervention treatments for their allocated arm because of toxicity and may need alternate therapy introduced. In addition, it is recognised that clinicians will have concerns if patients remain MABS culture positive through the duration of the trial and may wish to change or add treatments. All changes in the intervention treatment allocated will be captured through the study database and any AEs will be reported. Irrespective of whether treatments are ceased or altered, participants will remain in their assigned arm for analysis. Participants who cease or alter their treatment during a study stage may continue into the next stage of the trial once they complete the previous stage. For example, a participant who was unable to tolerate intravenous tigecycline in the first stage might cease tigecycline and an alternative treatment may be included in the intensive therapy stage. Once the intensive stage has been completed, they may still be randomised at stage 2 or 3. Change of treatment required for toxicity/AE or failure to clear MABS would be reflected in the primary outcome of clearance of MABS with good tolerance as they would fail to achieve this outcome for that stage of the trial. Details of how the ICEs (e.g. treatment discontinuation due to toxicity) will be addressed in the analysis will be detailed in the SAP using the estimand framework described in section 4.

6 INTERIM ANALYSES, DATA MONITORING AND STOPPING GUIDELINES

When a randomisation stage has 3 or more intervention arms, BAR will be used to update allocation ratios as detailed in section 2 using data from an interim analysis (4). Initially this will only occur in the first randomisation stage. Interim analyses of the stage 1 data will be performed after every 100 participants have completed 6 weeks of intensive therapy. BAR will be guided by the (posterior) probability of an intervention being found to be superior to the reference arm, which will be used to update the allocation probability at two interim analyses (see details in section 2.3).

The results of these analyses will be presented to an independent data and safety monitoring board (iDSMB) who will have access to unblinded data. Initially there are no plans to stop any arms of the study due to superiority, but such rules may be established as the trial continues. These will be detailed in the relevant appendix and will also be based on the (posterior) probability of an intervention being found to be superior to the reference arm. The iDSMB may however make a recommendation about stopping current interventions if they show poor promise or futility. If stopping rules are introduced, they will be defined to guide the use of these posterior probabilities.

7 FINAL ANALYSIS

In the FOR*Ma*T trial, there will be two analyses of interest; the comparison of interventions pertaining to each stage of randomisation and the comparison of dynamic treatment regimens that consider all treatment combinations in the trial. The final analyses will take place after the completion of each study stage. For instance, the analyses pertaining to study A1.1; the comparison of short intensive therapy arms, will take place once the required number of participants (initially planned for a sample size of 300 participants in the intensive program, which may potentially change with updates following interim analysis) complete the first 6 weeks of intensive treatment in one of the three therapy arms.

7.1 INTERVENTION COMPARISONS WITHIN A RANDOMISATION STAGE

The analyses of interventions within a randomisation stage will be conducted using standard frequentist methodology. When formal statistical testing is carried out the results will be presented as group differences along with 95% confidence intervals (CI) and p-values. The p-values will be interpreted as the strength of evidence against the null hypothesis of no group differences between treatment arms.

For binary outcomes, the effect measure of interest will be the odds ratio (OR) of each intervention compared with the control, estimated via a logistic regression model fitted to the outcome with a fixed effect of treatment assignment. Models will be adjusted for variables used in the randomisation process (i.e. the minimisation factors). The model will be as follows:

$$\boldsymbol{logit(P) = \beta_0 + \beta_t\, treatment + \beta_1 Z_1 + \cdots + \beta_k Z_k} \qquad (1)$$

Where P is the probability of success, logit(P)=log(P/(1-P)), treatment is a categorical variable representing the intervention arm participants are assigned to, $Z_1,\ldots,Z_k$ are the variables that will be adjusted for. Results will be presented as the Adjusted ORs for each treatment compared to the control (exp $(\boldsymbol{\beta_t})$) along with the 95% CIs and p-values. An attempt will be made to adjust for all minimisation factors, however there may have to be variables dropped if there are rare categories (i.e. less than 5 events per variable). As a sensitivity analysis, an unadjusted analysis will also be performed.

For continuous outcomes multiple regression models will be used where the effect measure of interest will be a difference in means of each intervention compared with the control. Denoting the continuous outcome Y, and other notation as above, the following model will be fitted:

$$\boldsymbol{Y = \beta_0 + \beta_t\, treatment + \beta_1 Z_1 + \cdots + \beta_k Z_k + \varepsilon} \qquad (2)$$

Adjusted regression coefficients $(\boldsymbol{\beta_t})$ for the difference in mean outcomes between treatment arms, 95% CIs and p-values will be presented.

7.1.1 HANDLING MISSING DATA

For the final analysis of any of the intervention comparisons, missing data in the primary and secondary outcomes (including any data set missing due to ICEs) will be handled using multiple imputation if the proportion of missing outcome data is greater than 5% on average across the primary and secondary outcomes. As missing data will be in outcomes measured at repeated intervals following enrolment, multiple imputation will enable exploitation of all information including that in partially observed cases. Multiple imputation will be conducted using chained equations, also known as fully conditional specification. Ideally this would be conducted using a single imputation model for all outcomes, although it may be necessary to impute outcomes using separate models for each outcome type including all the time points. Ordinal variables will be imputed using ordinal regression and continuous variables (forced expiratory volume in one second (FEV1) Z-score, CT scores) will be imputed using linear regression, or predictive mean matching if non-normal. Baseline variables will be included as auxiliary variables in the imputation model. Imputation will be carried out separately by cohort, to ensure that any treatment effects are maintained, using 50 imputed datasets. As a sensitivity analysis, a delta-adjusted analysis will also be conducted where the incomplete outcomes will be allowed to be different to the observed outcomes by an amount, delta. This analysis will be conducted using multiple imputation via the not at random fully conditional specification (NARFCS) procedure (5). Potential values for delta will be elicited from content experts. Details of this analysis and the elicitation process will be provided in the SAP.

7.1.2 MANAGING CARRY-OVER EFFECTS

It is not expected that carryover will affect the statistical properties of subsequent stages of treatment as randomising at each stage will be independent of the other stages, hence there is no plan to adjust for carryover effects in the primary analysis. However, if it appears that treatments given at previous treatment stages may affect results of subsequent treatment stages, and this will taken into account in a sensitivity analysis by including a treatment indicator for treatment allocation at the earlier stage(s) as a covariate in equations 1 and 2 above. Of note, the simulations conducted to govern the design of this study take into account the effects of different treatment pathways through the trial and thus allows for the possibility of carryover effects in terms of the power of the trial.

7.1.3 MANAGING POPULATION TIME TRENDS

BAR can be subject to time trends if the patient characteristics change during the trial. Recent work (6) has, however, shown that the change in treatment outcomes must be extremely high to affect the statistical properties more than a negligible amount. As a secondary analysis time will be adjusted for in order to mitigate the effect of any potential trial trends by including time of intensive randomisation (defined as months from randomisation of first patient) as an additional adjustment variable (Z_t) in the models described in section 7.1 above (equations 1 and 2).

7.1.4 ADJUSTMENT FOR MULTIPLICITY

No formal adjustments for multiplicity are planned in evaluating the primary or secondary outcomes. The results will be interpreted based on the strength of evidence of the treatment effects.

7.2 COMPARISON OF DYNAMIC TREATMENT REGIMENS

The FOR*Ma*T trial will use a Sequential Multiple Assignment Randomised Trial (SMART) analysis approach to identify a sequence of treatment options that leads to the highest MABS clearance to form the basis of treatment guidelines for MABS-PD.

A SMART is a multi-stage trial design that can be used to construct effective dynamic treatment regimens (DTR) also known as adaptive interventions (AI) or adaptive treatment strategies. AI provide a sequence of decision rules that specify whether, how, and/or when to alter the type, dosage, or delivery of the intervention at a critical decision point based on individual characteristics and behaviour (7).

For the FOR*Ma*T trial, the critical decision point corresponds to the intermediate response at six weeks, when the patients will be tested for the clearance of the bacterium. At this time point, a patient can have a successful clearance (-) or non-successful clearance (+). An AI consists of intervention options (Intensive A, Intensive B, Intensive C, additional intensive therapy, consolidation phase), critical decision points at which an individual is assessed and treatment decisions can be made (clearance status at six weeks) and tailoring a variable (successful/non-successful clearance).

The aim of the SMART analysis is to identify which AI gives the highest MABS clearance during the whole trial (analysed at the final outcome time point). Figure 1 below depicts different routes that a given patient can follow.

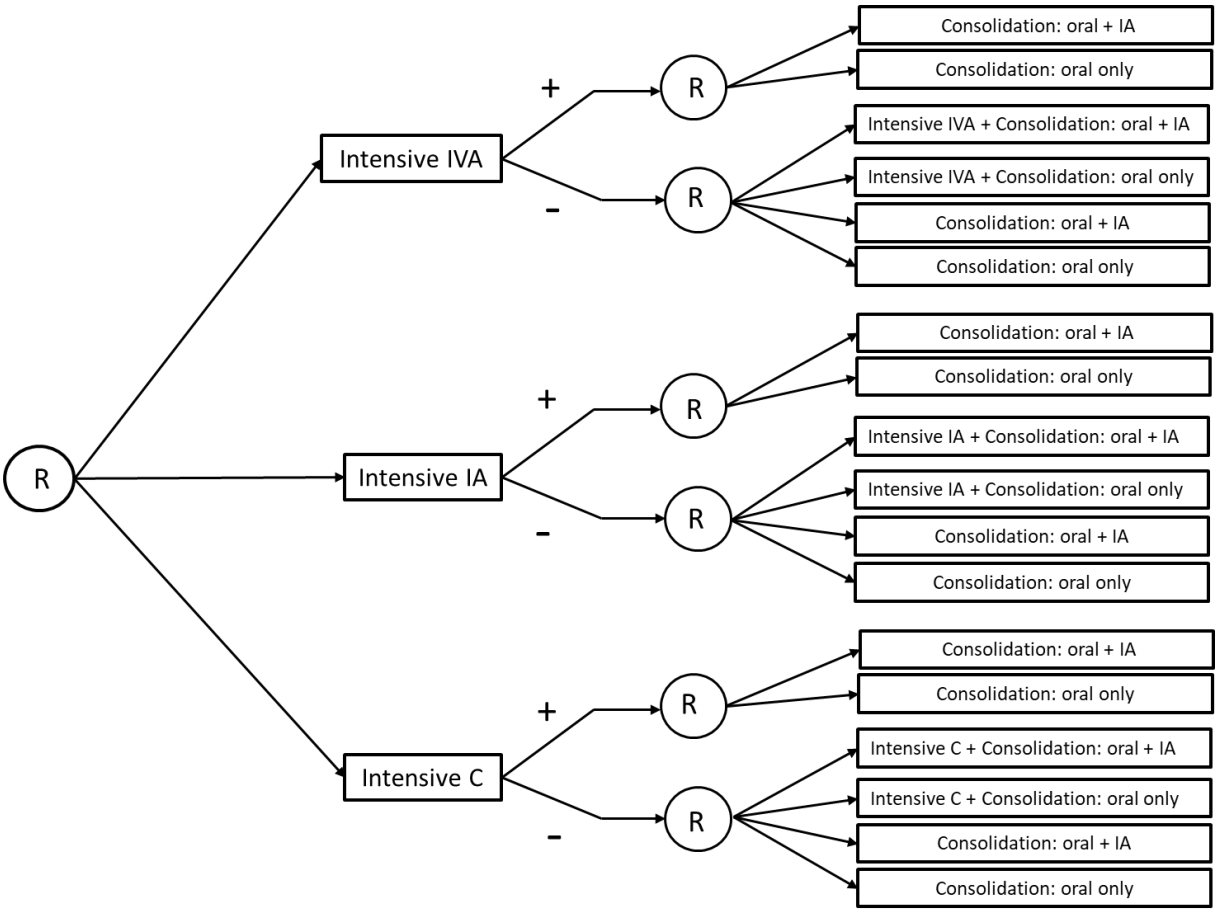

Figure 1: SMART design for the FOR*Ma*T study.
The R inside a circle denotes randomisation; A denotes Intensive A (Arm A); B denotes Intensive B (Arm B); C denotes Intensive C (Arm C); - denotes non-successful clearance; + denotes successful clearance.

Each AI is represented as a triplet of the form (X_1, X_2, X_3) where X_1 is the first-stage intervention, X_2 the second-stage intervention for responders (successful clearance) and X_3 the second-stage intervention for non-responders (non-successful clearance). For instance, for the first AI below it can be stated: a patient whose initial treatment is Intensive IVA (first stage treatment), if they achieve MABS clearance (response) at six weeks, then the second-stage treatment is "Consolidation: oral + IA", or else if they don't achieve MABS clearance (non-response) then the second-stage treatment is "Intensive A + Consolidation a".

There are 24 embedded AIs in the initial design of FOR*Ma*T (listed below). Given this high number of AIs, any subgroup analysis or secondary outcomes will not be considered in the SMART analysis.

#AI 1: (Intensive IVA, Consolidation: oral + IA, Intensive IVA + Consolidation: oral + IA")

#AI 2: (Intensive IVA, "Consolidation: oral + IA", "Intensive IVA + Consolidation: oral")

#AI 3: (Intensive IVA, "Consolidation: oral + IA", "Consolidation: oral + IA")

#AI 4: (Intensive IVA, "Consolidation: oral + IA", "Consolidation: oral")

#AI 5: (Intensive IVA, "Consolidation: oral", "Intensive IVA + Consolidation: oral + IA")

#AI 6: (Intensive IVA, “Consolidation: oral”, “Intensive IVA + Consolidation: oral”)

#AI 7: (Intensive IVA, “Consolidation: oral”, “Consolidation: oral + IA”)

#AI 8: (Intensive IVA, “Consolidation: oral”, “Consolidation: oral”)

#AI 9: (Intensive IA, “Consolidation: oral + IA”, “Intensive IA + Consolidation: oral + IA”)

#AI 10: (Intensive IA, “Consolidation: oral + IA”, “Intensive IA + Consolidation: oral”)

#AI 11: (Intensive IA, “Consolidation: oral + IA”, “Consolidation: oral + IA”)

#AI 12: (Intensive IA, “Consolidation: oral + IA”, “Consolidation: oral”)

#AI 13: (Intensive IA, “Consolidation: oral”, “Intensive IA + Consolidation: oral + IA”)

#AI 14: (Intensive IA, “Consolidation: oral”, “Intensive IA + Consolidation: oral”)

#AI 15: (Intensive IA, “Consolidation: oral”, “Consolidation: oral + IA”)

#AI 16: (Intensive IA, “Consolidation: oral”, “Consolidation: oral”)

#AI 17: (Intensive C, “Consolidation: oral + IA”, “Intensive C + Consolidation: oral + IA”)

#AI 18: (Intensive C, “Consolidation: oral + IA”, “Intensive C + Consolidation: oral”)

#AI 19: (Intensive C, “Consolidation: oral + IA”, “Consolidation: oral + IA”)

#AI 20: (Intensive C, “Consolidation: oral + IA”, “Consolidation: oral”)

#AI 21: (Intensive C, “Consolidation: oral”, “Intensive C + Consolidation: oral + IA”)

#AI 22: (Intensive C, “Consolidation: oral”, “Intensive C + Consolidation: oral”)

#AI 23: (Intensive C, “Consolidation: oral”, “Consolidation: oral + IA”)

#AI 24: (Intensive C, “Consolidation: oral”, “Consolidation: oral”)

The aim of this analysis is to identify which AI gives the highest MABS clearance during the whole trial (analysed at the final outcome time-point). Given the high number of pairwise comparisons, the MABS clearance rate for each of the 24 AIs will be estimated. The AI with the highest clearance rate will be chosen (8). This analysis will include patients, with complete data at all timepoints. The primary outcome will be analysed in the ITT population. More details of the statistical methodology will be provided in the SAP.

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