# CLINICAL TRIAL PROTOCOL
# The TEDIV Study
## A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Ca-EDTA added to Inhaled Tobramycin vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation

## Protocol No. TEDIV-001

**Protocol Author(s):**

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## STUDY INFORMATION

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|---|---|
| **Study Name/Number** | The TEDIV Study. Protocol No. TEDIV-001 |
| **Protocol Title** | A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Ca-EDTA added to Inhaled Tobramycin vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation |
| **Principal Investigator** | Dr Barry Clements |
| **Study Monitor** | Dr Clair Lee |
| **Medical Monitor** | Prof Stephen Stick |
| **Laboratories:** | 1. Dr David Reid, Queensland Institute of Medical Research – genomics<br>2. Dr Anthony Keil, Pathwest Laboratory, Princess Margaret Hospital – microbiology<br>3. Dr Ruth Thornton, School of Paediatrics and Child Health, and Telethon Institute of Child Health Research – biofilm imaging and qPCR measurements |
| **Statistical Analysis** | Judy Park - Telethon Institute of Child Health Research |
| **Investigational sites** | Princess Margaret Hospital for Children |

## INVESTIGATOR SIGNATURE PAGE

- I have read, understood and agree to conduct this study as outlined in the protocol and associated study documentation.

- I acknowledge that I am responsible for the conduct of this study at my research site and agree to adhere to the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the International Conference on Harmonisation Guideline for Good Clinical Practice.

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Signature Date

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Name

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| Ca-EDTA | Calcium di-sodium Ethylenediamine tetra-acetate |
| CF | Cystic fibrosis |
| EDTA | Di-sodium Ethylenediamine tetra-acetate |
| FEV1 | Forced expiratory volume in one second |
| MetBL | Mettalo-beta-lactamase |
| mITT | Modified intent to treat |
| N/A | Not applicable |
| *PsA* | *Pseudomonas aeruginosa* |
| qPCR | Quantitative PCR |
| RT-qPCR | Real time quantitative PCR |
| SAE | Serious adverse event |
| SOP | Standard operating procedure |
| DSMC | Data Safety Monitoring Committee |
| ISF | Investigator Site File |
| TRIS | Tris(hydroxymethyl)aminomethane |

# PROTOCOL SYNOPSIS

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|---|---|
| **TITLE** | A Phase IIb, Single-Centre, Randomised, Double-Blind, Comparator-Controlled, Parallel-Group, Pilot Study of Inhaled Tobramycin with added Ca-EDTA vs Tobramycin Alone as Adjunctive therapy to a Course of Standard Treatment for Cystic Fibrosis Children Admitted to Hospital with a *Pseudomonas aeruginosa* Pulmonary Exacerbation |
| **PRINCIPAL INVESTIGATOR** | Dr Barry Clements |
| **CLINICAL PHASE** | Phase IIb |
| **OBJECTIVES** | The objectives of this study are to assess the efficacy, safety and tolerability of adding Ca-EDTA to inhaled tobramycin when used as adjunctive therapy to a course of intravenous (inpatient) followed by nebulised/oral (outpatient) antibiotics in the treatment of a *Pseudomonas aeruginosa* pulmonary exacerbation in children with Cystic Fibrosis |
| **METHODOLOGY** | Randomised, double-blind, comparator-controlled, parallel group, pilot study |
| **TRIAL POPULATION** | Children with Cystic Fibrosis who are infected with *Pseudomonas aeruginosa* and admitted to hospital for treatment of a pulmonary exacerbation |
| **NUMBER OF PATIENTS** | 32 |
| **ELIGIBILITY CRITERIA** | Inclusion Criteria<br>• Male or female children 6 or more years of age with a documented diagnosis of CF (positive sweat chloride test, genotype with two identifiable CF mutations) accompanied by one or more clinical features consistent with the CF phenotype<br>• Current pulmonary exacerbation requiring admission to hospital for intravenous antibiotic therapy<br>• FEV1 $\geq$ 25% of predicted values<br>• Positive sputum or bronchoalveolar lavage culture for *Pseudomonas aeruginosa* in the previous 12 months<br>• Must be able to give informed consent or have legally acceptable representative who can give informed consent in accordance with ICH/GCP<br>• Females of child-bearing potential must agree to use an acceptable method of contraception for the duration of the trial<br>Exclusion Criteria<br>• Known hypersensitivity to the investigational product or its components or known relevant medication allergy<br>• Participation in another study with an investigational drug within 2 months of the planned first dose of investigational product<br>• Known relevant substance abuse<br>• Female patients who are pregnant or lactating<br>• Clinically significant disease or other medical condition other than CF or CF-related conditions that would, in the opinion of the Investigator, compromise the safety of the |

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|---|---|
| | patient or quality of the data |
| **RANDOMISATION** | 1:1 investigational product to comparator |
| **INVESTIGATIONAL PRODUCT** | • Calcium disodium ethylenediamene tetra acetate (Ca-EDTA) will be supplied as a sterile TRIS-buffered solution in a single-use syringe.<br>• 1.5ml Ca-EDTA solution will be added to 2.5ml Tobramycin solution (Tobra-Day®) and administered via a nebuliser<br>• Final concentration: 50mMol<br>• Dose: 75mg Ca-EDTA |
| **REFERENCE THERAPY** | • Placebo will be supplied as a sterile Tris-buffered saline solution in a single-use syringe.<br>• 1.5ml placebo solution will be added to 2.5ml Tobramycin solution (Tobra-Day®) to form the comparator, and administered via a nebuliser. |
| **DURATION OF TREATMENT** | 6 weeks (42 days) – 14 days in conjunction with intravenous antibiotics followed by 28 days as outpatient treatment |
| **STUDY DESIGN** | See Table of Assessments below |
| **EFFICACY CRITERIA** | Relative to comparator:<br>Primary<br>1. Greater reduction in bacterial load<br>Secondary<br>2. Greater reduction in biofilm mass<br>3. Reduced capacity for *PsA* to produce biofilm<br>Exploratory<br>4. Greater reduction in MIC of *PsA* to Tobramycin and other antibiotics<br>5. Greater clinical improvement (questionnaire and FEV1)<br>6. Greater reduction in evidence of inflammation<br>7. Reduction in beta-lactamase production by *PsA*<br>8. Differences in lung microbiome |
| **SAFETY CRITERIA** | • Acute tolerability as measured by respiratory symptoms and signs, as well as FEV1 changes pre-and post-dose.<br>• Systems assessment and vital signs (change pre- and post-dose and change from baseline)<br>• Blood tests – FBC, U&E's, LFT's, Ca and Mg<br>• Treatment emergent adverse events (AE's) up to follow-up visit<br>• Serious Adverse Events (SAE's) up to follow-up visit<br>• Concomitant medications<br>• Monitor *PsA* virulence factors and microbiome changes<br><br>A DSMC will be established to monitor safety |
| **PHARMACOKINETIC CRITERIA** *(optional)* | Not applicable |
| **STATISTICAL METHODOLOGY** | Demographic data will be displayed and summary statistics will be used to describe the study populations.<br><br><u>Efficacy:</u> |

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| | • The first aim would be to establish the standard deviation of within-subject variations. If this standard deviation proves to be similar to previous studies, 32 patients (16 in each group) study will have an 80% power of detecting a difference in the primary efficacy endpoint to the 0.05 level of significance. If not similar, the standard deviation found in our population will guide us as to required numbers for future Phase III studies to demonstrate efficacy.<br><br><u>Safety:</u><br>• Acute changes in respiratory and systemic symptoms and signs, PFTs, and blood parameters will be summarised, with categorical analysis of clinically significant findings.<br>All reported AE's will be coded and grouped by body system. AE's will be tabulated by treatment group and sorted by body system. The incidence of AE's in each treatment group will be tabulated by seriousness, severity and relationship to investigational product.<br><br>• Concomitant medication use will be summarised. |
| **TRIAL SITE** | Princess Margaret Hospital for Children |

## TABLE OF STUDY ASSESSMENTS

| | While Admitted to Hospital | | | | While At Home | | |
|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Visit 1** | **Visit 2** | **Visit 3 (Discharge)** | **Visit 4 (TC)** | **Visit 5 (EoT)** | **Follow-up (Safety visit)** |
| **Days ± visit window** | **Day - 3 to 1** | **Day 1** | **Day 8 ± 3** | **Day 15 ± 3** | **Day 29 ± 3** | **Day 43 ± 3** | **Day 71 ± 7** |
| Informed consent | x | | | | | | |
| Eligibility criteria | x | | | | | | |
| Demographics | x | | | | | | |
| Medical history | x | x | | | | | |
| Respiratory symptoms check | x | x | x | x | x | x | x |
| Height and weight | x | | x | x | | x | x |
| Vital signs | x | | x | x | | x | x |
| Physical exam | x | | x | x | | x | x |
| Spirometry | x | x x x[1] | x x | x x | | x | x |
| Sputum collection | x | | | x | | x | x |
| Blood collection | x | | | x | | x | x |
| Concomitant medications | x | x | x | x | x | x | x |
| CF Questionnaire | | | | x | | x | x |
| Dispense medication | | x | | x | | | |
| Adverse events | | x | x | x | x | x | x |
| First dose of study medication | | x | | | | | |
| Patient observation (1/2, 1 and 2 hours post-dose) | | x | | | | | |

**TC = Telephone Call; EoT = End of Treatment**

[1] At visit 1, spirometry will be performed pre-dose and at ½, 1 and 2 hours post-dose
[2] At visits 2 and 3, spirometry will be performed pre-dose and post-dose

# 1. BACKGROUND INFORMATION

## 1.1 Introduction

Cystic fibrosis (CF) individuals are highly susceptible to chronic lung infection by the opportunistic environmental bacteria *Pseudomonas aeruginosa* (*PsA*). This organism is thought to persist by forming biofilms within the relatively hypoxic mucous of the CF lung. Once established, biofilm dwelling bacteria are virtually impossible to eradicate with existing therapies. For some time now it has been recognised that iron is essential not only for growth and proliferation of *PsA*, but also for biofilm creation by the *PsA*.[1-2] In the CF lung (both in epithelial cells and the surrounding mucous) there is increased iron compared to non CF lungs creating an environment conducive to both *PsA* proliferation and biofilm formation.[3] In the light of this, Reid, Moreau-Marquis, and others, have looked at strategies to interfere with *PsA* iron homeostasis on the basis that this would lead to inhibition of biofilm formation thus rendering the *PsA* more vulnerable to eradication with antibiotics. One strategy involves the use of heavy metal chelators which bind available iron making it unavailable to *PsA*.[4-6] EDTA in particular is a well-known and safe heavy metal chelator which by its iron chelating properties has been shown to be very effective in inhibiting biofilm production both in vivo and in vitro.[7-8] As predicted, this in turn has led to markedly reduced minimal inhibitory concentration (MIC) of the *PsA* for a number of antibiotics (including Tobramycin) and a marked increase in killing of the organism in vitro.[9] Importantly, this effect has been shown to be present in low, normal and increased iron environmental circumstances and also in both aerobic and anaerobic conditions.[10] One *in vitro* study showed that the addition of EDTA-Tris to gentamicin improved killing of *PsA* by 1000-fold more than treatment with only gentamicin[30].

In the clinical setting, the veterinary world has for many years recognised the effectiveness of combining EDTA with Tobramycin (as well as certain other antibiotics)[11-13,30]. Chronic *PsA* infection causing persistent discharging ears in dogs, and sinusitis and endometritis in horses, which has failed to be eradicated with prior aggressive antibiotic use, has been successfully cleared in all reported instances by addition of EDTA to antibiotic irrigation solutions.[11-14]As a result of this unequivocal increase in benefit, the combination has now become standard best practice in veterinary guidelines[12]. Human studies have been slow to pick up on this benefit. In 1984 Hillman[15] reported using nebulised (aerosolised) Ca-EDTA in combination with an intravenous antibiotic (Penicillin) in four intubated and ventilated non-CF ICU patients with *PsA* lung infection where prior treatment with a conventional anti-PsA antibiotic (Ticarcillin) had failed to clear the organism. Forty-eight hours after starting the nebulisation of the Ca-EDTA, the *PsA* was eradicated in all four patients with no recurrences subsequently and no reported side effects. The natural progression from all of this is to consider using the combination of EDTA with nebulised antibiotics (particularly Tobramycin) in CF patients chronically colonised with *PsA* to see if this will achieve the same result. The hypothesis being that the iron chelating effect of EDTA will result in reduction in biofilm production thereby rendering the *PsA* more vulnerable to antibiotic exposure and thus hopefully leading to improved bacterial killing of the organism and possibly eradication. This study has been designed to attempt to prove this hypothesis.

An additional mechanism by which heavy metal chelators may improve effectiveness of antibiotics against *PsA* has also been recently reported.[16] Certain *PsA* produce metallo-beta-lactamase (MetBL), an enzyme that neutralises the effect of beta-lactam antibiotics commonly used to treat *PsA* infection. One study in mice showed that nebulised Ca-EDTA, by its zinc chelating effect, resulted in inhibition of the MetBL thus rendering these *PsA* more susceptible to the antibiotics. The same study showed that in addition, EDTA (by this same zinc-chelating effect) also neutralised other tissue-damaging *PsA*-produced metallo-proteases, which contribute to airway inflammation.

## 1.2 Investigational Product

**Di-sodium Ethylenediamine tetra-acetate (EDTA) is a salt which when dissolved in water or normal saline can be administered to humans intravenously or inhaled. Given intravenously EDTA will chelate heavy metal ions such as iron, lead, zinc and mercury and its main use is in treating patients with an excess of these ions, such as in iron overload (which occurs in Thallasaemia) and accidental lead poisoning. Once chelated, the ions are rendered non-toxic and able to be renally excreted. EDTA in its plain form will also chelate calcium ions and therefore if given too rapidly intravenously can result in hypocalcaemia with possible cardiac side effects and even death. For this reason, only the calcium salt of EDTA (Ca-EDTA) is approved for intravenous use by the FDA[17] as it does not remove calcium from the blood, and even in very large doses in small children it has been shown to be safe. Ca-EDTA has also been tried through the nebuliser for removal of heavy metal systemic overload although systemic absorption of the drug from the lung is limited (~10-20%) and therefore massive doses are required to produce a clinically significant effect. Despite these massive doses, no local respiratory or systemic side effects were reported in the studies where this was tried[18-19,26,27]. In our study, we will be using a total daily nebulised dose which will be vastly less than given to those patients (~20 times less) and also – and most importantly - in a concentration proven to be well tolerated in many studies, including a study in CF children. The total daily dose is also vastly less than the intravenous dose used for treating heavy metal overload. In addition, in order to optimise the airway tolerability of the solution, the salt will be dissolved in normal saline and buffered with TRIS-buffer which, when combined with the antibiotic preparation (Tobramycin), will result in a 4ml isotonic solution with a pH of ~7.1.**

## 1.3 Pre-Clinical Studies

**Numerous studies (mentioned earlier) have shown that exposure of *PsA* to EDTA (and other iron chelators) reduces biofilm production, reduces the MIC to various antibiotics (specifically Tobramycin), and results in greater killing of PsA when exposed to these antibiotics.**

## 1.4 Clinical Studies

**As also mentioned earlier, in veterinary subjects Ca-EDTA added to antibiotic irrigation solutions has been repeatedly shown to be effective in clearing chronic PsA infection associated with persistent ear discharge, sinusitis, and endometritis, and is now standard best practice[11-14]. In humans, Hillman's**

**study[15] in 1984 used nebulised (aerosolised) Ca-EDTA in combination with an intravenous antibiotic (Penicillin) in four intubated and ventilated ICU patients with PsA lung infection where prior treatment with a conventional anti-PsA antibiotic (Ticarcillin) had failed to clear the organism. Within 48 hours of the introduction of the Ca-EDTA the PsA was eradicated in all four patients with no recurrences subsequently and no reported side effects. This signature study and the veterinary experience together with the highly encouraging pre-clinical studies of Moreau-Marquis and David Reid has instigated the initiation of this trial with the hope that the same result could be achieved in cystic fibrosis patients colonised with *PsA*. In 1984, a study by Brown et al from Sydney using inhaled EDTA (not Ca-EDTA) in 10 CF children, failed to show efficacy in reducing *PsA* counts although in a preliminary study prior to the main study, they successfully eradicated *PsA* in one patient. Possible explanations for this failure are discussed later (see Section 1.6.2 (b)). Importantly however, they used a nebulised concentration of 50mM of EDTA (the same concentration we will be using in our study) twice daily for three months which was well tolerated with no side effects. There are also many studies of inhaled EDTA and Ca-EDTA given in massive doses (much larger than the doses we will be using in our study) relying on systemic absorption from the lung for the purpose of systemic treatment of heavy metal poisoning. None of these reported any local (respiratory) adverse effects and were all well tolerated. Some of these studies are discussed later in the section on Dosing and Safety.**

## 1.5 Potential Risks and Benefit

### (a) Benefit (Summary)

**The in vitro and in vivo, pre-clinical, and clinical evidence presented earlier (with more evidence later in the protocol as well) in both animals and humans supports the fact that Ca-EDTA added to Tobramycin overcomes all of the problems associated with chronic infection of mucosal surfaces with biofilm-forming organisms (such as *PsA*), in that it:**

- **breaks down biofilm**
- **reduces/prevents further biofilm production**
- **reduces the MIC to various antibiotics (in our case, specifically Tobramycin), and**
- **results in greater killing of *PsA* when exposed to certain antibiotics (again in our case, specifically Tobramycin)**

**On the basis of this evidence, we have designed this trial combining Ca-EDTA with the inhaled antibiotic treatment (Tobramycin) with the rationale that, if this drug combination reaches a significant proportion of the resident *PsA* in the CF**

**airway in the right concentration, it will lead to improved *PsA* killing (and possibly eradication) compared to antibiotic alone. This being the case, we would expect that at the completion of the trial, a discernible difference in efficacy outcome measures will be detected relative to comparator. The statistical methods have been chosen accordingly.**

**(b) Risk (Summary)**

**Of all the publications reviewed for this study (both in animals and in humans), we could find none that showed any evidence of local (respiratory) adverse effects of any kind at concentrations under 100mM – except the one Beasley study[22] using patients with exquisitely sensitive airways exhibiting paradoxical bronchoconstriction to bronchodilators containing preservatives – a unique group of patients only found in a small sub-group of asthmatics and never identified in CF. This safety data and further information is discussed in detail in the appropriate sections of this protocol and the Investigator Brochure. On the basis of all of this we have confidently selected a 50mM concentration of Ca-EDTA for our study. No systemic side effects have been reported either at this concentration (or considerably higher concentrations) and we do not foresee any other side effects either. Nevertheless we have set up a very strict safety monitoring component in our study to cater for all possible eventualities, and overall envisage very little potential risk to our patients against the possible benefits.**

## 1.6 Study Rationale

### 1.6.1 Efficacy

**Standard treatment for a *PsA*-colonised CF patient admitted to hospital with a pulmonary exacerbation is two weeks of intravenous anti-pseudomonal antibiotic treatment followed by four weeks of a nebulised antibiotic (commonly Tobramycin) – sometimes in combination with an oral anti-pseudomonal antibiotic (Ciprofloxacin). During the hospital admission a minimum of two intravenous antibiotics are used – generally one beta-lactam (or similar) and an aminoglycoside (commonly Tobramycin). While receiving intravenous antibiotics, many patients may at the same time be prescribed inhaled antibiotics in the form of twice daily nebulised Tobramycin in addition to intravenous Tobramycin. The rationale for this is that it provides a "double-sided" attack on the *PsA* for those two weeks, while not increasing the risk of systemic side effects as the systemic absorption of Tobramycin from the lungs is insignificant. Tobramycin blood levels will be routinely monitored anyway to ensure safety. Past experience has shown that, even when this "double-sided" approach is used, the chronically colonised mucoid form of *PsA* is rarely, if ever, cleared from the**

**sputum of these patients following a course of treatment – or even repeated courses of treatment. In addition, despite significant clinical improvement with a course of treatment, the reduction in the colony count itself is often less than would be anticipated, and usually reverts to pre-treatment levels soon afterwards - together with the whole lung microbiome. Increased antibiotic resistance also invariably develops over time, and as pointed out earlier, a large component of this resistance is attributed to the formation of the protective layer of biofilm as the *PsA* adapts to its environment in the CF airway.**

### 1.6.2 Dosing and Safety

**There is a large amount of data in the literature emphasising the safety of EDTA and particularly Ca-EDTA. We have researched this information carefully with the goal of finding the maximum safe and tolerable concentration (dose) which is likely to achieve concentrations of Ca-EDTA at the airway mucosal surface similar to concentrations which *in vivo* and *in vitro* studies indicate will maximise the chances of achieving the aims for our study. Here is a summary of some of the relevant data we reviewed focusing on systemic and local (respiratory) safety and route of administration.**

#### (a) Systemic Effects

**Systemic effects are best assessed using data from studies involving the intravenous use of EDTA for systemic heavy metal overload/poisoning. Since 2008, the US FDA has only allowed licensing of Ca-EDTA for intravenous use as this compound does not chelate Calcium ions, thereby reducing the risk of depletion of Calcium blood levels and the consequent risk of cardiac side effects. With this risk now removed, significant side effects are negligible despite large doses intravenously – even in children. The recommended dose for lead poisoning in a 20Kg child (40mg/kg/day) would equate to a total dose of 10Gm given over 12 days and this would not be expected to produce any significant side effects. Because the chelated metal ions are renally excreted, caution and possible dose modification is advised in patients with impaired renal function although when renal function is normal, renal toxicity is not expected at doses up to 50mg/kg/day[25]. Systemic absorption from the lung is considered to be around 10% of the dose reaching the airways – possibly up to 20% with more peripheral deposition. At these levels, even massive inhaled doses are extremely unlikely to achieve systemic levels anywhere near those given intravenously for treatment of heavy metal poisoning, and would therefore be considered safe from a systemic point of view. Subsequent similar studies have also shown no evidence of local or systemic side effects[26-27]. No significant gastro-intestinal adverse effects have**

**been reported following oral intake of EDTA and systemic absorption from the gastro-intestinal tract is less than 5%[25].**

**(b) Local (respiratory) effects**

**When considering the tolerability and local effects of inhaled medications, concentration rather than dose is the most appropriate consideration. This is the foundation of all inhaled challenge testing using doubling concentrations until a concentration is reached where provocation results in a measurable effect. The corollary of this principle is that a total dose inhaled over a short time (at a high concentration will result in provocation of local (respiratory) side effects, whereas the same dose, even given repeatedly, over a longer period (at lower concentrations) will not result in any adverse effects. After reviewing all the relevant literature, we have chosen to prescribe a 4ml solution of 50mM concentration of Ca-EDTA based on, amongst others, the studies presented here:**

**Human studies:**

1. **The Brown study[20] from Sydney in 1984 is the only study using nebulised EDTA in CF patients and provides us with the best basis for choosing the most appropriate concentration of Ca-EDTA for our study. They used a concentration of 50mM EDTA nebulised twice daily (the same as our study) for six months (three months on EDTA and three months on placebo in a double-blinded crossover fashion) in 10 patients. Their patients showed no adverse effects compared to placebo in any parameters measured including cough frequency, sputum volume, and spirometry values (FEV1, FVC and FEF25-75). In preliminary studies they did however show that 100mM concentrations produced unacceptable coughing in some patients and therefore chose the 50mM concentration for their formal study. Incidentally, their formal study showed no efficacy although in the preliminary study, one patient was cleared of *PsA* and remained clear for a year. In their own analysis and our analysis, the following comments may explain this failure of efficacy:**
    - **They used Tetracycline as their concomitant antibiotic – one not recognized as an anti-pseudomonal drug and one with well-recognised variable penetration into mucous and no correlation with serum levels. (Tetracycline was apparently chosen purely because it had shown good synergy with EDTA *in vitro*)[23].**
    - **The Tetracycline was given orally whereas inhaled antibiotics provide a much higher MIC at the mucosal surface – even compared to those which show good penetrance from serum into mucous.**

- **Tobramycin has been shown to have amongst the highest level of synergy with EDTA (killing of *PsA* increased 1,000-fold to gentamicin alone in one *in vitro* study[30]).**
- **Current nebulisers have much improved efficiency – particularly in the amount and distribution of particle deposition - compared to nebulisers used at the time of their study. In addition, particle size is smaller (2-3 microns compared to 5-7 microns) which results in better penetration into mucous and a longer half-life.**
- **Numbers in the study were small**
- **Increased frequency of dosing may be necessary**

2. **Hillman's study[15] used a total daily dose of 600mg/day as a continuous low concentration aerosolized solution for three days with no evidence of adverse respiratory effects – again supporting the fact that a lower concentration given over a longer time is not likely to cause problems.**

3. **Petrovic[18] showed that when workers from a lead factory inhaled massive doses of aerosolized Ca-EDTA (up to 2.4Gms total daily dose) in order to reduce elevated blood lead levels, no systemic or local (respiratory) side effects were reported.**

4. **Similarly, Horiuchi[26] gave 4Gm EDTA inhaled over 20 minutes for 4 days with no respiratory side effects.**

5. **Ashbel[27] nebulised 5ml of 10% solutipn Ca-EDTA twice daily in 60 lead workers with no respiratory side effects. All evidence of Ca-EDTA in the body had cleared within 24-30 hours.**

6. **A study by Kumar[28] compared inhaled dry powder nanoparticles of Ca-EDTA to micronized (3-5micrometers in diameter) particles and showed the micronized particles (similar in size to our nebulised particles) had good distribution through the lung with ~50% cleared by 1 hour and virtually all cleared by 4 hours. This supports the fact that 50mM concentration given 4 times a day (for the 2 week inpatient period) should not result in dose accumulation at the deposition site as the Ca-EDTA is likely to be cleared by the time of next dose.**

7. **The Asmus[21] study using highly sensitive asthmatics only went to a total dose of 2.4mg EDTA using a concentration of 600micrograms/ml for four doses because he was only trying to prove safety beyond the concentrations of EDTA found in bronchodilator solutions at the time (~600micrograms/ml). He showed no reduction in FEV1 up to that level but in fact may well have been able to go to much higher dose levels before eliciting a response – or**

**possibly never unless he increased the concentration. Therefore this study cannot be used to negate or dictate the concentration chosen for our study. Additionally, even the placebo in this study showed more bronchoconstrictor activity than EDTA – remembering that the patients used were highly sensitive asthmatics.**

8. **The Beasley[22] study involved six patients who had shown paradoxical bronchoconstriction to an inhaled bronchodilator containing small amounts of the preservative benzalkonium chloride (a know bronchoconstrictor) and EDTA. Paradoxical bronchoconstriction is extremely rare and occurs only in a small subgroup of highly sensitive asthmatics – almost always as a result of the benzalkonium chloride and not the EDTA. It is unique to asthma and has never been described in CF. We consider that, outside of this rare group of patients, the chances of finding this degree of airway hypersensitivity in any patient, including CF patients) are negligible. However, Beasley does also show that this bronchoconstriction can be successfully attenuated by prior administration of (preservative-free) albuterol (salbutamol), and on this basis, we have modified our protocol to include this option if necessary (see Section 7.2 Assessment of Safety). The Beasley study also used plain EDTA (not Ca-EDTA), unbuffered and with unknown tonicity – all of which could have added to the bronchoconstrictor activity, and all of which are corrected in our study drug. Another comment in the paper estimates that EDTA (in this form) is ~50 times weaker than histamine as a bronchoconstrictor, although again this is based on this uniquely sensitive group of patients.**

9. **However, Beasley also performed another study[29] looking at 18 "normal" asthmatics (not with paradoxical bronchoconstriction) using inhaled formoterol and ipatropium bromide (Duovent) with and without 05.mg/ml of EDTA. No significant difference in airway response was seen among the solutions.**

**Animal studies**

10. **The Beasley paper quotes a study on Basenji dogs* where Calcium chelation is believed to contribute to bronchoconstriction. This is almost certainly also a factor in humans which in our study will be avoided by using Ca-EDTA which does not chelate Calcium ions.**

11. **In a study in mice, an inhaled dose 100 times our proposed dose per kilogram was used with no evidence of side effects – again systemic or local.[16]**

12. **Dogs receiving an ultrasonic mist on Ca-EDTA at 100mM for 6 hours daily for two weeks*, and sheep receiving similar concentrations* showed no ill**

**effects until the dose was raised to 250mM when cough was observed at intervals.**

13. **In these same sheep, mucociliary transport rates in the trachea were not affected at concentrations of 100mM or less, although at 250mM transport rates were halved and returned to normal after 24 hours*.**

14. **Lung sections from animals exposed repeatedly to 100mM aerosolised Ca-EDTA, including examination by electron microscopy, showed no evidence of pulmonary toxicity*.**

**Data and Safety Monitoring**

An independent Data Safety Monitoring Committee (DSMC) will monitor safety and address any specific safety issues during the conduct of the trial**.** Formal review guidelines have been set out in the DSMC charter filed in the ISF.

## 2. STUDY OBJECTIVES

The objectives of this study are to demonstrate that the addition of Ca-EDTA to inhaled Tobramycin will – relative to the comparator – result in:

### 2.1 Efficacy

Primary
1. Greater reduction in bacterial load

Secondary
1. Reduced capacity of the *PsA* to produce biofilm
2. Reduced biofilm mass

Exploratory
1. Greater reduction in MIC of *PsA* to Tobramycin and other antibiotics
2. Greater reduction in evidence of inflammation
3. Reduction in beta-lactamase production by *PsA*
4. Greater clinical improvement (questionnaire and spirometry – FEV1)
5. Change in microbiome

### 2.2 Safety

1. No adverse effect on respiratory symptoms or lung function.
2. No evidence of systemic reaction or side effects.

## 3. STUDY DESIGN

## 3.1 Overview of Design

**This is a single-centre, randomised, double-blind, parallel-group, comparator study of inhaled Tobramycin with added Ca-EDTA vs inhaled Tobramycin alone for children with cystic fibrosis (CF) admitted to hospital for intravenous and nebulised antibiotic treatment of a *PsA* exacerbation.**

**Eligible admitted patients will all receive our standard institutional regime of dual intravenous and twice daily nebulised antibiotic regime for 2 weeks while in hospital followed by four weeks of outpatient twice daily nebulised antibiotic (Tobramycin). At the discretion of the treating physician, some patients may also have oral antibiotics during the outpatient four week phase. The choice of intravenous antibiotics and (if necessary) any additional anti-bacterial and/or anti-fungal antibiotics (both intravenous and/or oral, and in and/or out of hospital) will be made on clinical and microbiological grounds by the treating physician and will not influence or be influenced by the trial. Thus the only medication change as a result of participating in the trial will be the randomised double-blinded addition of either Ca-EDTA or placebo (normal saline) to the nebulised antibiotic (Tobramycin) throughout the 6-week treatment period. Screening will take place on admission to hospital with the permission of the treating physician, and if the participant meets the inclusion and exclusion criteria, he/she will start Day 1 of the trial that same day or the next day with randomisation of study drug and baseline investigations. The study will involve 32 children aged equal to or >6 years and be divided into three phases – the hospital admission phase (during which both intravenous and nebulised antibiotics will be given; the outpatient phase (nebulised antibiotic phase); and the follow-up phase (safety and ongoing efficacy). During the 2-week intravenous phase of treatment (while admitted) participants will be given study treatment (Ca-EDTA or placebo) four times daily – twice daily combined with their inhaled antibiotic, and twice daily on its own in between the inhaled antibiotic doses. The rationale behind the two extra daily doses of Ca-EDTA (or placebo) during the intravenous phase is to increase the period of Ca-EDTA airway activity while antibiotic is being presented to the airway mucosa in a continuous fashion via the bloodstream over the 24 hour period of each day, in addition to the twice daily nebulised Tobramycin. Following discharge from hospital - during the 4 week outpatient phase - the participants will continue to receive study drug (inhaled Tobramycin with added Ca-EDTA or placebo) twice daily. Baseline assessments and measurements as well as safety and tolerability will be evaluated on Day 1 followed by safety and efficacy assessments weekly on Day 8 and Day 15) until discharge. Subsequent assessments in the outpatient phase will be by telephone call visits on Day 29, and a clinic visit on Day 43. Clinic visits will monitor safety and efficacy parameters while telephone call**

**visits will be used to follow general progress focusing on tolerability, side effects and adherence. For the four weeks after the end of the treatment period, participants will enter the follow-up phase with a follow-up clinic visit on day 71 for final safety and efficacy assessment.**

## 3.2 Endpoints

### 3.2.1 Efficacy

Primary

- Greater reduction in bacterial load relative to comparator

Secondary

- Reduced capacity of *PsA* to produce biofilm relative to comparator
- Reduced biofilm mass relative to comparator

Exploratory

- Greater reduction in MIC of *PsA* to Tobramycin and other antibiotics relative to comparator
- Greater reduction in evidence of inflammation relative to comparator
- Reduction in beta-lactamase production by *PsA*
- Greater clinical improvement relative to comparator (respiratory symptoms check and spirometry – FEV1)
- Differences in microbiome relative to comparator
-

### 3.2.2 Safety

- No adverse effect on respiratory symptoms or lung function.
- No evidence of systemic reaction or side effects.

## 3.3 Randomisation

**The pharmacist prior to commencement of the study will prepare a permuted-block randomisation schedule. The statistician will hold the full randomisation code so that all investigators, study staff and participants remain blinded. Access to a patient's allocated treatment will be available in a sealed envelope kept in the pharmacy with the investigational product should there be a need for un-blinding.**

## 3.4 Investigational Product

The Investigational Product is the Calcium form of disodium ethylene diaminetetraacetate (Ca-EDTA). Investigational product will be manufactured and stored in the Princess Margaret Hospital Pharmacy Department.

### 3.4.1 Active Study Drug

Calcium disodium ethylene diaminetetraacetate (Ca-EDTA) TRIS-buffered to pH~7.1 in 1.5ml normal saline added to 2.5 ml Tobramycin solution to make a 50Mmol solution and nebulised four times daily for two weeks during the admission phase followed by two times daily for the four week outpatient phase.

### 3.4.2 Comparator

TRIS-buffered normal saline 1.5ml added to 2.5 ml Tobramycin solution and nebulised four times daily during the two week admission phase followed by twice daily for the four week outpatient phase of the study.

## 3.5 Duration of Therapy

Study participants will receive Ca-EDTA or placebo in combination with Tobramycin nebulised twice daily from Day 1 through to Day 43. For the first two weeks while admitted to hospital, participants will receive an extra two doses of study drug (Ca-EDTA or placebo) mixed with normal saline (instead of the Tobramycin) in between the standard two doses mixed with Tobramycin (see above for explanation and rationale). Following discharge from hospital, subsequent doses will be self-administered twice daily in the participant's home for four weeks, unless the participant elects to discontinue treatment. After completion of the 42 day treatment period participants will be monitored for a further 28 days for safety and ongoing efficacy. Participants will continue in the study until all follow-up assessments are completed.

## 3.6 Investigational Product Discontinuation

**Investigational product will be discontinued for a participant who meets any of the criteria below:**

- **Investigator believes that continuing treatment with investigational product is no longer in the participant's best interests (eg. due to significant AE related to investigational product).**
- **Participant withdraws assent.**
- **Participant's legal guardian withdraws consent.**
- **Pregnancy.**
- **Participant is unable to comply with the protocol, including completing required treatments, procedures, tests and/or examinations.**

**If a participant discontinues therapy with the investigational product they should be encouraged to continue with the study visit schedule if possible as they will be included in the Intention to Treat (ITT) analysis.**

## 3.7 Study and Site Discontinuation

### 3.7.1 Study Discontinuation

**Participants may be withdrawn from the study at any time at the Investigator's discretion, upon request from the Medical Monitor or at the participant's request. A participant or their legal guardian may withdraw consent at any time**

**without giving reasons. Withdrawal of consent to continue study participation shall not lead to any form of discrimination against the participant, in particular regarding medical care. If a participant withdraws their assent to participate in the study this will be respected by the Investigator, even if the participant's legal guardian maintains their consent for participation.**

**If a participant does not wish to continue with the study visits, study staff should attempt to schedule an Early Termination visit unless the participant discontinues at visit 1.**

**The reason for discontinuation should be recorded in the participant's source documents. If withdrawal is due to an AE the participant should be monitored for safety until the AE resolves or stabilises.**

### 3.7.2 Site Discontinuation

The Investigator or the Therapeutic Goods Administration may stop the study for any reason. In the event that the study is terminated prematurely, written notification will be sent to the Human Research Ethics Committee (HREC) as soon as possible. The Investigator will promptly inform all participants and make arrangements for appropriate medical follow-up. All study materials will be returned to the study site by participants.

### 3.7.3 Replacement of Participants

**Participants who prematurely discontinue from the study for any reason will not be replaced.**

## 3.8 Investigational Product Accountability and Disposition of Clinical Study Supplies

**During the 2-week inpatient phase, an accountability log will be attached to each individual trial patient's medication chart with full explanation and documentation to nursing staff on administration and recording of trial drug. Should the patient be discharged on "Home IV", he/she will be given the remainder of the 2-week inpatient trial drug with full explanation of how it should be administered. At the 2-week review visit, the patient will be required to return all used and unused trial drug items, when pharmacy will perform a standard accountability log, before dispensing the 4-week outpatient batch of trial drug. At the 6-week review visit (4 weeks after cessation of IV antibiotic treatment)) the patient will again be requested to return all used and unused items for standard pharmacy accountability logging. A spare 3-day reserve supply will be issued to each patient to be kept separate from the main batch for use if the main batch is mislaid or damaged. Pharmacy will arrange appropriate**

**disposal of all collected used and unused trial drug items as per standard procedure.**

## 3.9 Study Blind and Un-blinding

**This is a double-blind study, therefore neither the participants, study staff nor medical monitor will know which treatment has been assigned. The randomisation schedule will be generated by the statistician and kept securely by the pharmacist. The pharmacist will keep sealed envelopes with the treatment assignment for each participant to expedite un-blinding of individual participants if required.**

**In the case of a medical emergency where knowledge of the treatment assignment is essential to the welfare of the participant, the Principal Investigator (or appropriately- qualified designee) may request un-blinding of that participant by the pharmacist. A Treatment Un-blinding form will be completed by the Investigator to document the reason(s) for which it was necessary to break the blind.**

**The DSMC will have access to un-blinded data if required. Further information is provided in the DSMC Charter filed in the ISF.**

# 4. SUBJECT SELECTION

**Once a decision is made by the treating physician to admit a cystic fibrosis patient to hospital and commence a standard course of intravenous and nebulised antibiotic treatment for a pulmonary exacerbation, the patient will be approached (with the treating physician's approval) to participate in the trial provided they meet the criteria outlined below.**

## 4.1 Inclusion Criteria

- Male or female 6 years of age or older with a documented diagnosis of CF (positive sweat chloride test, genotype with two identifiable CF mutations) accompanied by one or more clinical features consistent with the CF phenotype.
- Current pulmonary exacerbation requiring antibiotic therapy.
- If older than 6 years, must be able to perform acceptable spirometric manoeuvres.
- FEV1 > 25% of predicted values (if older than 6 years of age).
- Positive sputum or bronchoalveolar lavage culture for *Ps aeruginosa* in the past 12 months.
- Must be able to give informed consent or have legally acceptable representative who can give informed consent in accordance with ICH/GCP.
- Females of child-bearing potential must agree to use an acceptable method of contraception for the duration of the trial.

## 4.2 Exclusion Criteria

- Known hypersensitivity to the investigational product or its components or known relevant medication allergy.
- Participation in another study with an investigational drug within 2 months of the planned first dose of investigational product.
- Known relevant substance abuse.
- Female patients who are pregnant or lactating
- Clinically significant disease or other medical condition other than CF or CF related conditions that would, in the opinion or the Investigator, compromise the safety of the patient or quality of the data.

Please note: The presence of additional bacterial or fungal organisms on sputum culture and/or the prescription of additional antibiotics (oral, intravenous, anti-pseudomonal, or non-anti-pseudomonal) at any stage through the trial will NOT affect inclusion into the trial)

## 4.3 Screening Failures and Rescreening

**Patients who fail screening may be approached again at the next admission to hospital for treatment of an exacerbation provided the factor(s) responsible for the first screen failure are thought to be not present any more.**

# 5. TREATMENT OF SUBJECTS

## 5.1 Study Drug Administration

**Patients will be randomly assigned to study drug or placebo. Study drug will consist of 2 ampoules – one containing Tobramycin 500 mg in 5 ml from which 2.5 ml will be withdrawn for each dose and second will be an ampoule containing 1.5 ml of a solution of Ca-EDTA and TRIS buffered saline from which 1.5 ml will be added to the 2.5 ml of Tobramycin immediately prior to use. The combined 4 ml will be nebulised until completed (~15 minutes). The comparator will also have 2 ampoules – Tobramycin (the same as in the treatment arm) and 1.5 ml of TRIS-buffered saline without Ca-EDTA.**

## 5.2 Study Drug Compliance

**Compliance will be monitored at each visit with the use of charts, simple questioning, diary cards and medication counts.**

## 5.3 Concomitant Therapies

In addition to the inhaled study drug, patients will continue with their standard CF care and medications (including any additional antibiotics at the discretion of the treating physician, as well as other forms of inhaled therapy such as Pulmozyme and hypertonic saline if necessary). All concomitant medications will be recorded throughout the study.

## 6. STUDY EVALUATIONS

**The initial screening visit will assess patient eligibility for the study and ensure that the patient is fully aware of the aims, risks and possible benefits of the study before obtaining signed consent. At each subsequent visit, evaluations will be focused on collecting data and assessing the patient for (a) safety and (b) efficacy.**

**Screening visit:**

**(a) Safety:**
- **Ensure patient is willing, fit and able to participate in and likely to complete the study**
- **Meets eligibility criteria**
- **Has signed consent**
- **Has no conditions likely to be complicated by participating in the study**
- **Treating physician agrees to patient participating in trial**
- **History, physical assessment, vital signs, spirometry**
- **Baseline safety blood tests performed**

**(b) Efficacy:**
- **nil**

**Visit 1: (within 48 hours of Screening visit)**

**(a) Safety:**
- **Patient observed and respiratory signs and symptoms checked pre-, and half, 1 and 2 hours post-, first dose**
- **Spirometry pre-, and half, 1 and 2 hours post-, first dose**
- **First dose of medication given**
- **Blood test – Tobramycin serum levels (at appropriate time post-IV and neb dose)**
- **Systems and vital signs check pre-, and half, 1 and 2 hours post-, first dose**
- **Symptom diary provided to patient/parent**
- **Concomitant medications noted**

**(b) Efficacy:**
- **Baseline sputum, clinical assessment and Spirometry performed prior to first dose**
- **Study medication written up and dispensed for duration of two week hospital stay – to be administered by nursing staff and monitored by pharmacy as per protocol provided.**

**Visit 2: (1 week after starting treatment)**

**(a) Safety:**

- **Respiratory signs and symptoms checked pre- and half hour post-dose**
- **Spirometry pre- and half hour post-dose**
- **Tolerability, adverse events and diary card check**
- **History, system examination and vital signs checked**
- **Concomitant medications noted**

**(b) Efficacy**

- **nil**

**Visit 3: (at discharge from hospital and end of IV antibiotic treatment)**

**(a) Safety:**

- **Respiratory signs and symptoms checked pre- and half hour post-dose**
- **Spirometry pre- and half hour post-dose**
- **Tolerability, adverse events and diary card check**
- **History, system examination and vital signs checked**
- **Safety blood tests performed**
- **Concomitant medications noted**
- **CF Questionnaire applied**
- **Medication for 4-week home treatment period dispensed with appropriate instructions regarding storage and use as per protocol provided and explained to parent/patient.**
- **Emergency plan and phone numbers provided in case of emergency**

**(b) Efficacy:**

- **Respiratory symptoms and signs noted**
- **Spirometry recorded**
- **Sputum sample collected**
- **CF Questionnaire applied**

**Visit 4: (telephone call)**

**(a) Safety:**

- **Respiratory symptoms check**
- **Tolerability and adverse events check**
- **Concomitant medications noted**

**(b) Efficacy:**

- **Nil**

**Visit 5: (end of treatment visit – 4 weeks after discharge/6 weeks after starting treatment)**

**(a) Safety:**

- **Respiratory signs and symptoms checked**
- **Spirometry**
- **Tolerability, adverse events and diary card check**

- **History, system examination and vital signs checked**
- **Safety blood tests performed**
- **Concomitant medications noted**
- **CF Questionnaire applied**

**(b) Efficacy:**

- **Respiratory symptoms and signs noted**
- **Spirometry recorded**
- **Sputum sample collected**
- **CF Questionnaire applied**

**Visit 6 (follow-up safety and efficacy visit – 4 weeks after stopping trial medication)**

**(a) Safety:**

- **Respiratory signs and symptoms checked**
- **Spirometry**
- **Tolerability, adverse events and diary card check**
- **History, system examination and vital signs checked**
- **Safety blood tests performed**
- **Concomitant medications noted**
- **CF Questionnaire applied**

**(b) Efficacy:**

- **Respiratory symptoms and signs noted**
- **Spirometry recorded**
- **Sputum sample collected**
- **CF Questionnaire applied**

## 7. ASSESSMENT OF ENDPOINTS

### 7.1 Assessment of Efficacy

**Essentially, we will be comparing the efficacy of the trial medication relative to the comparator with regards to biofilm formation and *PsA* growth in sputum. For this purpose, sputum will be collected using the Induced Sputum Method (see protocol – Addendum A) at Screening, Visit3, Visit 5 and Follow-up. In patients where sputum is not able to be obtained by this method, a naso-pharyngeal swab will be performed although it is recognised that this may limit the number and interpretation of some of the tests performed on this specimen. Some patients may for clinical reasons require bronchoalveolar lavage in which case these samples will be used. The collection, handling and storage of the sputum specimens is outlined in the Standard Operating Procedure (SOP) document included as Addendum B at the end of this protocol. Essentially each**

**specimen will be homogenised with an equal volume of sputolysin, separated into measured aliquots for each of the specific tests or collection of tests, and stored in appropriate preservative media at -80 degrees centigrade for batch testing once all specimens have been collected. For most of the tests (particular the molecular testing) only very small quantities (50-100 microlitres) are needed.**

- **Bacterial load will be assessed by:**
  - **Quantitative PCR (qPCR) using established RT-qPCR assays based on the housekeeping genes *oprL* and *clpX*. These will be performed at the Menzies Institute in Darwin through the Infectious Diseases laboratory at the Telethon Institute of Child Health research, and at Dr David Reid's laboratory in Queensland.**
  - **CFU counts (bacterial colony forming unit counts) performed in our microbiology laboratory at Princess Margaret Hospital.**

  **Routine cultures for clinical assessment (if and as required by the treating physician) will be performed separately and will not be affected by this study.**

- **Biofilm production will be assessed by:**
  - **Imaging techniques using confocal light microscopy using a recently validated procedure at TICHR**
  - **David Reid's laboratory:**
    - **pyoverdin and pyochelin assays will be used as a measure of iron utilisation in biofilm production.**
    - ***PsA* expression of iron-related virulence genes and RT-qPCR assays based on the housekeeping genes *oprL* and *clpX*.**
    - **In addition, as added insurance to exclude generation of a more aggressive *PsA* as a result of iron starvation, they will be quantifying the transcripts for the following virulence genes: *algD* (alginate synthesis), *hcnB* (cyanogenesis), *lasI* and *rhII* (quorum sensing regulators of biofilm production) and *lasB* (proteolytic enzyme)**

  - **Morphological characteristics of biofilm on culture**

- **Changes in MIC to a range of antibiotics (including particularly Tobramycin) will be done in our laboratory at PMH using standardised commercially available kits.**

- **Clinical comparisons using:**
  - **a standardised CF questionnaire**
  - **assessment of respiratory signs and symptoms at each visit**
  - **changes in FEV1 on spirometry measurements**

**Additional exploratory endpoints (subject to results of above endpoints and availability of future funding) may include:**

- **Assessment of inflammatory markers performed in our lab at TICHR**
- **Measurement of expression of genetic markers of beta-lactamase production by *PsA* – again using genetic markers validated at Dr Reid's laboratory in Brisbane.**

## 7.2 Assessment of Safety

- Systemic
  - blood tests: FBC, LFT's, U&E's, Ca, Mg
  - history and general physical examination (including vital signs) at visits
- Local respiratory:
  - Symptoms – CF questionnaire
  - Symptoms (cough, wheeze and chest tightness) and physical examination (respiratory) at visits pre- and half-hour post-dose
  - Spirometry: change in FEV1 between pre- and half, 1, and 2 hours post-dosing with the first dose, and then pre- and half hour post-dosing at subsequent visits.
- Microbiome:
  - Monitoring of possible changes in *PsA* virulence factors, iron utilization and microbiome as a result of treatment (See paragraph above "Biofilm production assessed …." In Dr Reid's laboratory in Brisbane

## Notes on Pulmonary Function Testing as part of the safety assessment

- The study will include patients 6 years and older although only patients 7 years and older will be required to perform spirometry.
- While in hospital spirometry will be performed at the patient's bedside using a portable spirometer by a qualified technician using Knudsen reference values and according to ATS/ERS guidelines. During clinic visits spirometry will be performed using the same spirometer in the Respiratory Laboratory at Princess Margaret Hospital.
- A drop in post-dose FEV1 of >15% after the first dose or any subsequent dose will result in the patient been given appropriate treatment with Ventolin and if the event is deemed to be possibly or definitely related to the drug, he/she will be withdrawn from the study.
- Similarly, if any patient who is unable to perform spirometry exhibits any adverse respiratory or systemic signs or symptoms after the first or any subsequent dose, the event will be treated appropriately, and, if the event is deemed to be possibly or definitely related to the drug, he/she will be withdrawn from the study.

## Notes on Blood testing:

- **Patients will be offered EMLA cream prior to blood being taken**
- **Approximately 2.5ml blood will be needed on each occasion.**
- **Blood will be taken by a qualified phlebotomist from the Princess Margaret Hospital haematology/biochemistry laboratory.**

## 7.3 Assessment of Pharmacokinetic Endpoints

**Not applicable.**

# 8 RATIONALE FOR STUDY

### 8.1 Rationale for study design

(Already discussed previously)

### 8.2 Rationale for Efficacy Assessments

Bacterial Load: Quantitative PCR (qPCR) is now well recognised as a sensitive and accurate quantitative measurement of bacterial load in standardised sputum specimens[31,32]. Both Dr Thornton (TICHR/SPACH) and Dr David Reid (QIMR Brisbane) have validated this measurement in their laboratories. However, it is still not clearly established as to whether qPCR is superior to CFU and therefore we will be using both of these methods on all of our specimens. This will fortuitously allow us the opportunity of comparing qPCR results between two laboratories and qPCR against CFU which in itself will act as a valuable quality assurance exercise. Correlation between the tests will be analysed using the appropriate statistical method, such as Blandt-Altman plot. At this stage, it is planned to use the CFU results in the statistical analysis for the efficacy component of the study as it is probably still recognized as the "gold standard" for measurement of bacterial load[34].

Biofilm measurements: We are most fortunate that Dr Ruth Thornton's laboratory is a leader in the use of confocal light microscopy imaging for the assessment and quantification of biofilm in sputum. In addition, Dr David Reid's laboratory in Brisbane has developed and validated RNA probes for the assessment of the expression of genetic markers of biofilm production and virulence factors in *PsA* organisms. If our study is successful, these probes will show that the expression of these genetic markers for biofilm production will be significantly down regulated relative to the comparator.

# 9. ADVERSE EVENT DEFINITIONS AND REPORTING REQUIREMENTS

## 9.1 Adverse Events

### 9.1.1 Definition of Adverse Events

**Per Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95):**
**"An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product.**

**AE's include:**

- **Exacerbation or worsening of symptoms of a pre-existing medical condition or disease – in particular, cystic fibrosis.**

- **Increase in frequency or intensity or a pre-existing episodic medical condition or disease.**
- **Disease or medical condition that is newly diagnosed during the study, even though symptoms may have been present prior to participation in the study.**
- **Overdose of investigational product or concomitant medication – whether this produces signs or symptoms, or not.**
- **Events that the Investigator considers to be related to protocol-required procedures.**
- **Abnormal assessments (including laboratory test abnormalities) that represent a clinically significant finding that was not present at baseline, worsened during the study, or led to discontinuation of investigational product.**

**Cystic Fibrosis symptoms that worsen during the study will be recorded as AE's.**

**AE's <u>do not</u> include:**

- **Medical or surgical procedures. The condition that leads to the procedure is the AE.**
- **Pre-existing medical condition or disease that does not worsen or increase in frequency.**
- **Situations in which an AE has not occurred (e.g. hospitalisation for social or convenience reasons).**
- **Changes resulting from normal growth and development (e.g. teething) that do not change significantly in severity or frequency from expected levels.**

#### 9.1.2 Reporting of Adverse Event Data

**Participants will be interviewed in a non-directed manner to elicit potential AE's from the first dose of investigational product until the day 56 (follow-up) visit. A diary card will be provided to participants to record AE's experienced between clinic visits. The occurrence of an AE will be deduced from:**

- **history and physical examination at the time of clinic visits as per Clinic Visit Monitoring Sheet (attached to this protocol as an Addendum)**
- **Diary card**
- **CF Questionnaire**
- **Phone call visit assessments**
- **Any changes in the participant's physical examination, lung function, and/or signs and symptoms at any stage**

**AE's will be followed until resolution while the participant remains on the study. AE's that are considered to be related to investigational product will be followed until resolved or clinically stable.**

**All AE's will be recorded in the participant's Case Report Form (CRF).**

#### 9.1.3 Intensity of Adverse Events

**The Investigator will use the Common Terminology Criteria for Adverse Events (CTCAE) version 4.02 to grade the severity of AE's. The general clinical descriptions for each grade are as follows:**
**Grade 1 – Mild AE, transient and easily tolerated.**
**Grade 2 – Moderate AE, causes discomfort and interrupts participant's ADL**
**Grade 3 – Severe AE, causes considerable interferences with ADL, could be life-threatening or incapacitating.**
**Grade 4 – Life-threatening or disabling AE.**
**Grade 5 – Death related to AE**

#### 9.1.4 Relationship to Investigational Product

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

**Unrelated:** The adverse event is due to an underlying or concurrent illness or effect of another drug and is clearly not related to the investigational product (eg. has no temporal relationship to study drug or has a much more likely alternative aetiology).

**Unlikely:** The adverse event has little or no temporal relationship to the study drug and a more likely aetiology exists.

**Possibly:** The adverse event has a strong temporal relationship to study drug and an alternative aetiology is equally or less likely compared to the potential relationship to study drug.

**Probably:** The adverse event has a strong temporal relationship to study drug or recurs on re-challenge. Aetiology is unlikely or significantly less likely.

The Investigator should attempt to provide an alternative aetiology for all AE's considered to be unrelated, unlikely or possibly related to study drug. This will be recorded in the source documentation.

For the purposes of reporting to the relevant regulatory agencies, unrelated and unlikely AE's will be classified as Not Related, while Possibly and Probably related will be reported as Related.

### 9.2 Serious Adverse Events

#### 9.2.1 Definition of Serious Adverse Events

A SAE or reaction is any untoward medical occurrence that results in one or more of the following:

- results in death
- is life-threatening
- requires inpatient hospitalization or results in prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a medically important event or reaction

Death is an outcome of an AE and not an AE in itself.

A "life threatening" event is any adverse therapy experience that, in the view of the Investigator, places the participant at immediate risk of death from the reaction as it occurred.

An "inpatient hospitalization" occurs when a participant is formally admitted to hospital for any length of time. It does not refer to Emergency Room or Accident & Emergency visits where the patient is observed and not admitted.

Medical and scientific judgment should be exercised in deciding whether other situations than those listed above should be considered serious. This may include important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the participant or might require intervention to prevent one of the other outcomes listed above.

Regardless of the relationship of the event to the investigational product, the event must be reported as a SAE if it meets any of the definitions listed in this section.

### 9.2.2 Reporting of Serious Adverse Events

**SAE's will be reported from the time of informed consent until the Follow-up visit. All SAE's will be reported to the Human Research Ethics Committee (HREC) and Medical Monitor within 1 business day of site staff becoming aware of the event. The SAE Report Form will be completed and submitted with all information known at the time. A minimum of the SAE name, onset date and date of last dose of investigational product should be provided, and if possible, the form should be reviewed and signed by an Investigator prior to submission.**

### 9.2.3 Follow up of Serious Adverse Events

If site staff becomes aware of new information regarding an SAE, a follow-up report should be completed and forwarded to the HREC within 5 days. New information may include the acquisition of relevant hospital records, reports and/or laboratory results.

SAE's that are ongoing at the end of the study should be followed up until a final assessment is possible, and until all queries have been resolved.

### 9.2.4 Reporting to Regulatory Authorities

It is important that all known information at the time of the SAE is reported to the Medical Monitor within the protocol-required time frame, as expedited reporting to the appropriate regulatory authorities may be required based on the following criteria:

**No Reporting**

This requirement applies if the SAE is deemed not serious by the Medical Monitor.

**Standard Reporting**

This requirement applies if the SAE is classified as any of the following:
Serious, expected, and related to the investigational product
Serious, expected and *not* related to the investigational product
Serious, *unexpected* and *not* related to the investigational product

**Expedited Reporting**

This requirement applies if the SAE is classified as serious, unexpected and related to the investigational product. During these circumstances, the appropriate health authorities must be informed within 15 days, however fatal or life-threatening events must be reported within 7 days.

# 10. STATISTICAL ANALYSIS

## 10.1 Statistical Methods

### 10.1.1 Demographic data

Demographic data will be displayed and summary statistics will be used to describe the study populations.

### 10.1.2 Efficacy

All efficacy analysis will be performed using the modified Intent to Treat (mITT) population, which is defined as all randomised participants who received at least one dose of investigational product. Selected efficacy analyses will be repeated in the per-protocol population, which is defined as participants having completed ≥ 75% of doses with not more than 3 consecutive missed doses.

As this is a pilot/proof of concept study, the results will in the first instance be used afterwards to assess the standard deviation of the within-subject variations. If this is similar to previous studies, we expect a sample size of 32 patients will have an 80% power at one standard deviation to detect a difference of 2.6 $\log^{10}$ CFU per gram in the primary efficacy endpoint between treatment groups after 6 weeks at the 0.05 level of significance. If however, the standard deviation is higher than in previous studies, the methodology in any further follow-up studies will have to be reviewed in the light of this. On the other hand, if the standard deviation is lower than in previous studies, this will lend more power to the statistical significance.

### 10.1.3 Safety

All safety analyses will be performed using the safety population which is defined as per the efficacy population.

Acute changes in respiratory and systemic symptoms and signs, PFT's, and blood parameters will be summarised, with categorical analysis of clinically significant findings.

Safety and tolerability will be analysed in all participants who have received investigational product.

All reported AE's will be coded using the CTCAE and grouped by body system. The incidence of AE's in each treatment arm will be tabulated by seriousness, severity and relationship to investigational product. If an AE is reported more than once during the study for a given participant the greatest severity and worst-case relationship will be reported in the tables. AE's will also be listed for individual participants. AE's which lead to withdrawal from the study will be listed and summarised per treatment arm. A separate tabulation and listing of SAE's will be provided.

Concomitant medication use will be summarised descriptively using frequency and percentage of participants by treatment arm.

### 10.1.4 Data Handling Conventions

In general, values for missing data will not be imputed unless specified. Details of missing data conventions will be provided in the statistical analysis plan.

## 11. QUALITY CONTROL AND QUALITY ASSURANCE

The study will be conducted under standard GCP principles. The hospital Ethics committee will be kept informed of progress and adverse events. Pharmacy will adhere to standard recommended prescribing and accountability rules.

Inter-laboratory comparison of qPCR measurements (Dr Thornton's and Dr Reid's laboratories) and the comparison of these measurements with the matched 10 CFU samples will serve as Quality controls for the measurement of bacterial load in the specimens provided for these tests.

A Data Safety Monitoring Committee (DSMC) will be established for this trial to monitor safety and address any specific safety issues during the conduct of the trial. The DSMC will be independent from the sponsor and will consist of two Respiratory Physicians experienced in research (at least one of whom will be paediatric) and a biostatistician. The DSMC will make recommendations to the sponsor/PMH Ethics Committee about safety data, and whether to continue, modify or terminate the study. Details on the composition of the DSMC, operating procedures, and interactions will be provided in a charter filed in the Investigator site file (ISF).

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