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Title:	**Anti-inflammatory and antioxidant effects of oral resveratrol in bronchiectasis**
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Study product name:	Transmax (Resveratrol 500 mg + Polydatin 5 mg + Piperine 5 mg)
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PROTOCOL SYNOPSIS
Anti-inflammatory & antioxidant effects of resveratrol in bronchiectasis: A pilot study

Rationale for research

- Bronchiectasis is a chronic, debilitating disease characterised by recurrent and chronic respiratory infection requiring antibiotics and intense neutrophilic inflammation.[1] Antibiotic resistance is increasing globally, and novel treatments are needed urgently.[2]
- The naturally-occurring antioxidant supplement resveratrol exhibits antibacterial activity against *H. influenzae*[3-5] and suppresses markers of neutrophilic inflammation.[6-8] Antioxidant supplementation has been shown to reduce exacerbations in cystic fibrosis.[9]
- We ultimately plan to undertake a large, randomised, placebo-controlled trial of resveratrol supplementation in bronchiectasis. However, several feasibility issues need to be evaluated before undertaking such a study. These include determining effects of resveratrol treatment on airway inflammation and antioxidant status in patients with bronchiectasis. We will also evaluate tolerability and safety of resveratrol in bronchiectasis.
- Potential benefits of resveratrol treatment for bronchiectasis include fewer exacerbations, improved quality of life and reduced healthcare utilisation.

Aims

Primary aim: To assess whether oral resveratrol has anti-inflammatory effects in adults with bronchiectasis.

Secondary aims: To assess whether oral resveratrol has antimicrobial and antioxidant activity. To determine the optimal dose of resveratrol (1000 mg daily vs 2000 mg daily) in terms of anti-inflammatory, antimicrobial, and antioxidant effects, bioavailability, adherence, tolerability, and safety.

Research design and methods:

Study design: Single centre, single-arm, pre-post, open-label study of oral resveratrol in adults with bronchiectasis, incorporating sub-study of participants randomised to receive either 500 mg twice daily, or 1000 mg twice daily

Study size: 40 participants

Target population: Participants aged ≥18 years with bronchiectasis confirmed on CT chest, with ≥1 exacerbation in the preceding 12 months

Study drug: Participants will take Transmax (resveratrol 500 mg + 5 mg piperine + 5 mg polydatin), either 1 capsule twice a day or 2 capsules twice a day

Duration of treatment: 12 weeks
4 visits: Screening [V1], baseline [V2], 4 weeks [V3], 12 weeks [V4]

Endpoints:

- *Primary endpoint*: Sputum neutrophil elastase
- *Secondary endpoints*:
  Sputum hs-CRP
  Sputum cytokines
  Sputum purulence
  Sputum cathelicidin (LL-37)
  Sputum procalcitonin
  Sputum culture
  Serum & sputum total antioxidant capacity
  Serum & sputum resveratrol (+ metabolite) levels,
  Serum hs-CRP
  Tolerability
  Adherence
  Adverse events
  Exacerbation frequency
  Lung function (spirometry: FEV_1 and FVC)
  Health-related quality of life (SGRQ, BHQ, LCQ)

Schedule of visits and procedures:

Visits	**1**	**2**	**3**	**4**
	Screening	**Treatment**		
	-4 to -2 wks	**0 wks**	**4 wks**	**12 wks**
Informed consent	X			
Demographics	X			
Eligibility criteria	X	X		
Medical history	X			
Physical examination	X	X	X	X
Vital signs, including weight	X	X	X	X
Spirometry	X	X	X	X
Randomisation to 500 mg or 1000 mg twice daily		X		
Blood tests				
FBC	X			X
U+E	X			X
LFT	X			X
hs-CRP		X		X
Cytokines (IL-1β, IL-6, IL-8, GM-CSF, TNF-α)		X		X
Serum resveratrol levels		X		X
Serum total antioxidant capacity		X		X
Sputum analysis				
Neutrophil elastase		X	X	X
hs-CRP		X	X	X
Cytokines (IL-1β, IL-6, IL-8, GM-CSF, TNF-α)		X		X
Sputum purulence		X		X
Sputum cathelicidin (LL37)		X		X
Sputum procalcitonin		X		X
Sputum culture		X		X
Sputum resveratrol levels		X		X
Sputum total antioxidant capacity		X		X
Pregnancy test if applicable (blood)	X			X
Urinary pregnancy test		X		X
Concomitant medication review	X	X	X	X
Record pulmonary exacerbations		X	X	X
Record adverse events			X	X
Health-related Quality of Life				
SGRQ		X	X	X
BHQ		X	X	X
LCQ		X	X	X
MRC & mMRC breathlessness grade	X			
Dietary questionnaires				
Food frequency questionnaire		X		
Issue diary cards	X			
Review and collect diary cards		X	X	X
Issue study medication		X	X	
Collect and account for study drug			X	X
Telephone contact	Weeks 2 and 8			

Background

The increasing importance of bronchiectasis in New Zealand

Bronchiectasis is a chronic, debilitating respiratory condition characterised by productive cough, airway inflammation and recurrent respiratory infections, and a "vicious cycle" of infection, inflammation and lung damage.[10] Bronchiectasis carries a highly significant health burden, with impaired quality of life and associated morbidity and mortality.[11-13] Exacerbations of bronchiectasis commonly require hospital admission and prolonged courses of antibiotic treatment.

Having long been considered a rare condition, the worldwide prevalence of bronchiectasis is increasing.[1,14-16] Incidence and prevalence are rising in New Zealand, with substantially higher rates than comparable nations.[17,18] There is a well-documented disproportionate impact of bronchiectasis in Māori and Pacific peoples, and bronchiectasis in these populations is associated with increased severity and mortality.[17-21] Recent New Zealand data highlight this disparity: overall prevalence of bronchiectasis in NZ is 158 per 100,000 people, but more than double in Māori (368 per 100,000) and quadruple in Pacific peoples (686 per 100,000). Rates of hospitalisation due to bronchiectasis increased by 41% from 2000 to 2015; being of Māori, Pacific or Asian ethnicity was the biggest risk factor for hospitalisation, with highest hospitalisation rates seen in Māori aged 15-29 years who had hospitalisation rates 14.5 times higher than non-Māori/Pacific/Asian (non-MPA) people. For all age-groups, Pacific peoples were 8 times more likely to be hospitalised, Māori 4.4 times and Asian peoples 1.6 times more likely, compared to the non-MPA population.[18,22]

In addition to disparity between ethnicities, there is also significant socio-economic disparity in New Zealand: those patients from the most deprived backgrounds are 3.4 times more likely to be admitted to hospital due to bronchiectasis, compared to the least-deprived patients.[22] "Te Hā Ora: The Breath of Life", the New Zealand National Health Strategy released in 2015, highlights the importance of addressing the health inequalities seen in respiratory conditions, specifically bronchiectasis.[23] The estimated cost of hospital admissions for bronchiectasis in NZ is $5.5m per year.[18]

The need for new treatments for bronchiectasis

Recent international guidelines highlight a paucity of high-quality evidence in bronchiectasis, and the need for novel treatments.[2,24] Currently, there are no treatments licensed specifically for the treatment of bronchiectasis.[2] Treatment is mainly limited to courses of antibiotics and techniques to clear sputum from the airway.[1] Chronic respiratory infection is common in bronchiectasis, in particular with the bacteria *Haemophilus influenzae* and *Pseudomonas aeruginosa*, and is strongly linked to worse prognosis.[10,13] Consequently, some such patients require long term antibiotics to reduce the frequency of exacerbations; however, antimicrobial resistance is an increasing global concern and reported in some patients with bronchiectasis receiving long-term antibiotics.[25,26] Developing novel, non-antibiotic treatments for bronchiectasis is therefore crucial.

Oxidative stress and neutrophil-driven inflammation in bronchiectasis

Normal cellular metabolism produces low levels of reactive oxygen species, which play a crucial role in initiating host defence.[27] However, an excess of reactive oxygen species causes cellular damage, inflammation and oxidative stress. Oxidative stress is linked to many chronic diseases, including bronchiectasis.[28-30] A key function of antioxidants is to scavenge reactive oxygen species to reduce oxidative stress and minimise cellular damage.

Neutrophils form the first line of defence against invading bacteria.[31] Bacterial respiratory infection activates cell-signalling pathways, resulting in release of cytokines e.g. TNF-α, interleukin-6 (IL-6), and IL-8. These cytokines recruit neutrophils to the airway, which release reactive oxygen species, including neutrophil elastase.[32-35] This process is dysregulated in bronchiectasis, with excessive reactive oxygen species causing exaggerated inflammation and results in airway damage.[33,34] Levels of inflammatory cytokines are elevated in airways in bronchiectasis and in particular, sputum neutrophil elastase levels correlate with bronchiectasis severity.[11] Furthermore, acute exacerbations of bronchiectasis are driven by intense neutrophilic inflammation that propagates the vicious cycle of lung damage.[10]

Resveratrol as a potential treatment for bronchiectasis

Resveratrol is a naturally-occurring antioxidant with anti-inflammatory and antimicrobial activity. It is found in many foodstuffs; the highest levels are found in red wine, grapes, berries and nuts.[36] The effects of resveratrol are thought to be responsible for the "French Paradox" of reduced cardiovascular disease despite a high-fat diet in traditional French culture.[36,37]

Normal cellular metabolism produces low levels of reactive oxygen species (ROS), which play a crucial role in initiating host defence.[27] Antioxidants scavenge ROS to limit inflammation whereas excessive ROS cause oxidative stress, inflammation and cellular damage, a process which occurs in chronic diseases such as bronchiectasis.[28-30]

Resveratrol acts through multiple cell-signalling pathways and its exact mechanism is not known. Its primary role is thought to be through activation of SIRT1 proteins.[38] Effects of SIRT1 activation include regulation of innate immunity and down-regulation of nuclear factor-κB (NF-κB).[39,40] NF-κB is implicated in neutrophil recruitment, inflammatory cytokine release and lung damage.[32,33,41,42] Resveratrol's other anti-inflammatory mechanisms include inhibition of cyclo-oxygenase (COX) and inducible nitric oxide synthase (iNOS), both of which are linked to a number of inflammatory processes.[43]

Anti-inflammatory properties of resveratrol

The anti-inflammatory and antioxidant effects of resveratrol have been investigated extensively in laboratory studies. Resveratrol has been shown to reduce the release of neutrophil elastase in laboratory studies.[8,44,45] As described above, levels of neutrophil elastase in sputum has been shown to be a marker of severity in bronchiectasis and to predict risk of future severe exacerbations.[11]

Resveratrol has been studied in *ex vivo* studies of white blood cells sampled from patients with COPD. Resveratrol caused a reduction in levels of the highly pro-inflammatory cytokines IL-6 and IL-8,[46-48] and suppresses markers of neutrophilic inflammation in mouse lungs.[7] These same inflammatory cytokines are linked to airway inflammation in bronchiectasis.[49]

Anti-inflammatory effects are also described in murine models of acute lung injury and acute respiratory distress syndrome, with reduced expression of NF-κB and of its downstream pro-inflammatory cytokines.[38,50] These inflammatory cytokines, particularly IL-8, are elevated in airways of patients with bronchiectasis, and attract neutrophils to sites of infection and inflammation.[49]

Chronic infection with *H. influenzae* and *P. aeruginosa* is strongly linked to worse prognosis; resveratrol reduces markers of inflammation in airway epithelial cells that have been exposed to these organisms.[3,5]

Antimicrobial activity of resveratrol

Resveratrol exhibits antibiotic effects against a range of pathogens, including *Haemophilus influenzae and Pseudomonas aeruginosa*, without evidence of inducible resistance in laboratory studies.[4] These organisms are common pathogens in bronchiectasis, that are strongly associated with worse prognosis.[10,13] Resveratrol also up-regulates production of antimicrobial peptides (including cathelicidin), which are produced in response to bacterial infection to enhance bacterial killing.[51,52] However, the effect of increasing cathelicidin (LL-37) levels in bronchiectasis is uncertain as recent data have shown that the antibacterial function of cathelicidin appears to be impaired.[53]

Resveratrol also exerts antiviral effects in rodent models and *in vitro* against important respiratory viruses, including influenza, human metapneumovirus, rhinovirus and respiratory syncytial virus, with decreased virus-induced inflammation and reduced viral replication.[40,54-57] These effects may be important clinically as viral infections are common in bronchiectasis and are associated with up to 49% of exacerbations.[58,59]

Use of resveratrol in clinical trials in humans

Building on extensive *in vitro* research, resveratrol has been studied in clinical trials of *non-respiratory* conditions.[36,60] Meta-analyses suggest that *in vitro* effects can translate to beneficial *clinical* effects on inflammatory markers (e.g. reduced hs-CRP and TNF-α), blood pressure (reduced systolic blood pressure at doses >150 mg per day), diabetes (reduced fasting blood glucose and HbA1c) and stroke (reduced NIH

stroke scale scores, and plasma levels of MMP-2 and MMP-9).[61-66] Resveratrol has also shown efficacy against colorectal, prostate and breast cancer.[67-70] No published trials have evaluated the effects of resveratrol in lung disease.

Safety profile of resveratrol in clinical studies

The dose and preparation of resveratrol in clinical trials varies widely (from 5 mg to 5 g daily) but consistently displays a reassuring safety profile and tolerability profile. Doses up to 5 g daily in healthy volunteers were safe and well-tolerated, with mild adverse effects (gastrointestinal disturbance, headache and myalgia and no severe adverse effects or laboratory abnormalities.[71,72] Minor elevation of bilirubin was seen in an eight week study in liver disease at 3g daily.[73]

Body mass index fell after resveratrol treatment in one study.[74] This may be relevant in bronchiectasis, where a BMI <18.5kg/m^2 is linked to worse outcomes.[10]

Pharmacokinetic profile of resveratrol

Oral bioavailability of resveratrol is low, but wide inter-individual variability exists.[75] It undergoes rapid metabolism to 3 main metabolites (resveratrol-3-*O*-sulphate, resveratrol-4'-*O*-glucuronide and resveratrol-3-*O*-glucuronide).[72,76] Serum metabolite concentrations exceed concentrations of free resveratrol; these metabolites have similar antioxidant effects and may contribute to the activity of resveratrol, with synergistic effects observed.[60,77-79] Assessment of total resveratrol levels (i.e. measuring levels of resveratrol *and* metabolites) is therefore important. Time to maximum resveratrol concentration is 0.8-1.3 hours, and 2.8-3.2 hours for metabolites. Half-life for resveratrol is 5.1 hours and 7.8-8.4 hours for metabolites.[80,81]

Another important consideration is back-conversion of the glucuronated and sulphated metabolites to resveratrol: therefore, the metabolites act as a "reservoir" for resveratrol.[82,83] Resveratrol has been shown to accumulate in tissues (e.g. liver, skeletal muscle), with much higher tissue resveratrol concentrations than in serum; tissue levels of resveratrol may exceed tissue levels of its metabolites.[68,76,82]

Therefore, despite initial low bioavailability, resveratrol and its metabolites may act together to exert localised tissue effects. Sputum levels of resveratrol have not been assessed previously and will be part of this study.

Sputum neutrophil elastase in bronchiectasis

Neutrophils form the first line of defence for the innate immune system through a combination of phagocytosis and the release of reactive oxygen species, enzymes and other proteins.[34] Neutrophil elastase is one such proteolytic enzyme stored in azurophilic granules in the cytoplasm of neutrophils and is released in response to bacterial infection.[11,34] Although this protective mechanism is important in host defence, neutrophil elastase can also have detrimental effects, including airway epithelial damage, stimulation of mucus production and reduced ciliary beat frequency, as well as playing a role in increasing airway inflammation in bronchiectasis.[11]

Sputum neutrophil elastase levels have been studied in a number of respiratory conditions, including bronchiectasis, COPD and cystic fibrosis. Neutrophil elastase levels are elevated in the airways of patients with cystic fibrosis and higher levels are associated with earlier onset of lung disease; in COPD, sputum neutrophil elastase is an important determinant of the extent of lung damage. Agents that reduce levels of neutrophil elastase in the lungs have been proposed as a potential treatment of bronchiectasis and cystic fibrosis.[84]

Scope of a clinical trial of resveratrol supplementation in bronchiectasis

In addition to dietary sources, resveratrol is a readily-available oral supplement, commonly at much lower doses than will be used in this study; average daily dietary intake is reported to be 194 µg per day, which is far lower than we intend to use.[36] It offers a potentially cost-effective, readily-available treatment for bronchiectasis. We hypothesise that its wide-ranging anti-inflammatory, antimicrobial and antioxidant properties will have beneficial effects in the airways of patients with bronchiectasis. The high local prevalence of bronchiectasis within Counties Manukau DHB provides an unparalleled opportunity to undertake this study,[17-19] and will address the following questions:

1) Does resveratrol supplementation have anti-inflammatory, antimicrobial and antioxidant effects in bronchiectasis?

It is unknown if resveratrol's anti-inflammatory, antimicrobial and antioxidant effects translate to patients with bronchiectasis. Resveratrol suppresses neutrophil-driven inflammation and decreases neutrophil elastase release and levels of pro-inflammatory cytokines found in excessive levels in airways of patients with bronchiectasis.[3,6-8,44,46-48] In laboratory studies, resveratrol has antimicrobial effects against pathogens that are important in bronchiectasis.[4,85] Resveratrol intake increases markers of antioxidant status, including total antioxidant capacity (TAC).[86,87] High serum TAC has been associated with less severe disease in COPD and better lung function in asthma.[88,89] Serum TAC is low in bronchiectasis,[30] but sputum TAC has not been investigated previously. *We hypothesise that resveratrol will decrease neutrophil elastase activity and other markers of inflammation and infection, and increase total antioxidant capacity*. Such effects would have the potential to reduce pulmonary exacerbations, which are inflammatory events associated with worse prognosis in bronchiectasis.

2) What is the optimal dose of resveratrol in bronchiectasis?

A wide range of doses and preparations of resveratrol have been used in clinical trials, from 5 mg to 5 g total daily dose.[36] Studies that have found beneficial effects (outside of cancer trials) have used total daily doses of 150 mg to 2000 mg;[36,63] much higher serum levels of resveratrol and its metabolites obtained using total daily doses of 2000 mg.[74] Randomised controlled trials evaluating the effects of resveratrol on inflammatory biomarkers have used doses of up to 800 mg a day.[61] Since *in vitro* studies of resveratrol report dose-dependent effects, we hypothesise that a dose of 2000 mg a day (taken as two 500mg capsules, twice a day) will be superior to 1000 mg a day (one 500 mg capsule, twice a day).

3) *Is high-dose resveratrol safe and well-tolerated in bronchiectasis?*

Resveratrol has a good safety and tolerability profile in clinical trials, up to doses of 5 grams per day.[36,74,90] Mild to moderate adverse effects may occur at doses of more than 500 mg a day, and are reversible.[90] The pharmacokinetic profile has not been studied in patients with bronchiectasis, and may differ from the profile of other conditions: in addition to characteristic localised airway inflammation, bronchiectasis is also a systemic inflammatory condition.[91] As this study will use doses of up to 1000 mg twice a day, it will be important to assess the safety and tolerability of resveratrol in this population.

Study product:

We will use a formulation manufactured by Biotivia LLC, New York, USA ("Transmax"), that is a commercially available, high-purity resveratrol product, in use in two randomised trials.[92,93] It is available as a 500 mg capsules, taken twice a day. It contains 98% resveratrol, with added piperine and polydatin: these additives enhance the bioavailability of resveratrol. Polydatin is a precursor of resveratrol, while piperine (an extract of black pepper) has been shown to increase serum resveratrol concentrations.[83,94] Participants will be randomised to receive either 500 mg twice a day or 1000 mg twice a day.

In vitro studies of resveratrol report dose-dependent effects, with greater anti-inflammatory and antimicrobial effects at higher resveratrol concentrations;[4] the dose of many commercially available products is 100 mg daily. In some smaller clinical studies, a single 500 mg dose of resveratrol can achieve serum concentrations similar to the concentrations that have demonstrated potentially beneficial effects *in vitro*; doses greater than 2g daily have been associated with more gastrointestinal disturbance.[71-73]

Research Design and Methods

Hypothesis
We hypothesise that resveratrol taken twice daily will demonstrate anti-inflammatory and antioxidant effects in the airways of patients with bronchiectasis, and that these effects will be dose-dependent. The primary hypothesis is that resveratrol will induce a decrease in sputum neutrophil elastase levels, and in one or more pro-inflammatory sputum biomarkers (cytokines): IL1β, IL-6, IL-8, GM-CSF and TNFα.

Aims
Primary aim: To assess whether resveratrol treatment has anti-inflammatory effects in adults with bronchiectasis.

Secondary aims: To assess whether resveratrol has antimicrobial and antioxidant activity in adults with bronchiectasis; to determine the optimal dose of resveratrol in terms of anti-inflammatory, antimicrobial, and antioxidant effects, bioavailability, adherence, tolerability and safety in adults with bronchiectasis.

Study design
Single centre, single-arm, pre-post, open-label study in patients with stable bronchiectasis over 12 weeks. The study will incorporate a sub-study, whereby participants with be randomised to receive either 500 mg twice daily, or 1000 mg twice daily

Study centre and recruitment
The study site is Middlemore Hospital, South Auckland. The hospital serves a population of approximately 510,000 people with relatively large Māori (16%) and Pacific (23%) populations. Patients will be identified using a departmental research database of patients with bronchiectasis, and using clinical coding data (ICD-10) for patients attending outpatient clinics and discharged from hospital with a diagnosis of bronchiectasis.

We plan to recruit 40 patients for this study.

Inclusion criteria
- Aged ≥ 18 years
- Able to provide written informed consent
- Able to provide spontaneous sputum sample at visit 2 (week 0)
- High-resolution CT (HRCT) chest scan confirming diagnosis of bronchiectasis within 5 years
- Clinically stable during baseline period, for 4 weeks prior to commencement of treatment (defined as the absence of clinical worsening beyond normal daily variation, with no need for increasing habitual medications or taking antibiotics or prednisone, with stable spirometry)
- History of one or more pulmonary exacerbations requiring antibiotics in the past 12 months.
- Patients with asthma and COPD will be included if the primary diagnosis is bronchiectasis

***Exclusion criteria*:**
- Patient using current oral, intravenous or inhaled antibiotics within 4 weeks prior to commencing study drug
- Patients taking continuous macrolide therapy (≥3 months) within 3 months of screening
- Patients taking continuous oral corticosteroids (>6 weeks)
- Bronchiectasis exacerbation or respiratory infection requiring oral or intravenous antibiotic treatment within 4 weeks prior to commencing study treatment
- Use of prescribed or dietary antioxidant supplement within 1 month of screening
- Body mass index <18.5 kg/m^2
- Patients with a history of non-compliance with medications
- Patients with significant medical conditions other than bronchiectasis:
    - A significant disease is one that would, in the opinion of the investigator, put the participant at risk through participation in the study, or a disease which may influence the results of the study, or the participant's ability to participate in the study.
- Patients with cystic fibrosis
- Patients with primary ciliary dyskinesia
- Patients with hypogammaglobulinaemia

- Patients with allergic bronchopulmonary aspergillosis (defined as the presence of asthma, total serum IgE > 1000 IU/ml, with detectable specific IgE to Aspergillus or positive Aspergillus skin test)
- Patients taking immunosuppressive agents (e.g. azathioprine, methotrexate, cyclophosphamide)
- Patients with other primary or acquired immunodeficiency
- Patients with evidence of active or suspected cancer and patients having undergone cancer treatment including resection, radiation therapy or chemotherapy within the last 2 years (patients with basal cell carcinoma and squamous cell carcinoma are allowed)
- Pregnant or lactating women
- Participation in a separate clinical or device trial within 4 weeks of screening
- Allergy to any of the components of Transmax

Reasons for discontinuation of study medication

- Participant request to discontinue treatment
- Adverse event that contraindicated further dosing, in the opinion of the investigator
- Risk to subject, as judged by the investigator
- Pregnancy
- Discontinuation of the study at the request of the Data Monitoring Committee or regulatory agency, institutional review board or independent ethics committee

Participants who prematurely discontinue treatment should continue in the study until the final visit, if possible. It is highly recommended that all study procedures, including any scheduled visits, are continued until the final visit, if possible.

Withdrawal of participants from the study

- Participant request to withdraw from study
- Lost to follow-up
- Investigators deem that participants are at significant risk of harm from study medication or procedures
- The Data Monitoring Committee requests patient withdrawal due to major safety concerns

Criteria for termination of the study

- Investigators deem that participants are at significant risk of harm from study medication or procedures
- Investigators reserve the right to terminate the study if adequate resources or funding are no longer available to continue the study

Study medication

Investigational products

Substance:	Resveratrol 500 mg + piperine 5 mg + polydatin 5 mg (“Transmax”)
Form:	Capsule
Unit strength:	500 mg
Route of administration:	Oral

Dosage regimen

Oral resveratrol will be commenced at a dose of either 500 mg twice daily or 1000 mg twice daily.

Treatment will continue at this dose for the duration of the study (12 weeks). Patients will be advised to take the study product with food to maximize absorption.[79,95]

Test drug dispensing

Participants will be issued with resveratrol at visits 2 (week 0) and 3 (week 4). 40 participants will each receive a blister pack supply of 500 mg capsules for 4 weeks at visit 2, and for a further 8 weeks at visit 3, according to the dosage regime to which they are allocated.

Participants will be advised to contact the research team if adverse effects develop. If adverse effects are experienced, patients can opt to *reduce by one dose step* after consultation at either a clinic visit or following telephone communication. Dose steps will be 2000 mg a day, 1000 mg a day and 500 mg a day.

Adherence will be monitored and measured by keeping a record of the number of tablets issued and returned. Patient's adherence with the proper use of the study drug will be assessed at visits 3 and 4, and patients will be reminded to bring all study medication pack(s) (used or unused) and completed diaries to these visits.

Packaging and labelling
Biotivia LLC, New York, USA will provide the capsules containing 500 mg resveratrol. The following will be printed on each compliance pack: trial number, medication number, packet number, participant initials, directions for use, expiry date, caution statement, storage instructions, space for "date dispensed", and name and address of host institution. All used trial medication packs are to be returned to the patient treatment box for drug accountability.

Concomitant Medications
Any medication that patients take other than the study drug, including herbal medicine or supplement, is considered as concomitant medication and must be recorded in the worksheets.

At screening (visit 1), patients will be asked about all medications they have taken during the last 12 months with special attention to the use of antibiotics, corticosteroids, antioxidant and dietary supplements, and bronchodilators. These should be reviewed at every subsequent study visit.

Safety Parameters

Adverse events
An adverse event (AE) is defined as any untoward medical occurrence, including an exacerbation of an existing condition in a trial participant receiving a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment.

A serious adverse event is defined as any AE which results in death, is immediately life threatening, results in persistent or significant disability, requires or prolongs participant hospitalisation, is a congenital anomaly or birth defect, or is an important medical event that may jeopardise the patient or require intervention to prevent one of the above outcomes.

It is the responsibility of the investigator to record and report all AEs (both serious and non-serious) occurring during the course of the clinical trial (from signing the informed consent form through to the end of the trial). It will be derived by spontaneous, unsolicited reports of patient, by observation and by routine open questionings such as "Has there been any change in your health since I last saw you".

All adverse events will be fully documented on the worksheet with the onset, intensity, seriousness, outcome and action taken, including change in dosage of study drug.

All serious adverse events and unexpected serious adverse reactions (SUSARs) will be reported to the study Data Monitoring Committee. The Health and Disability Ethics Committee (HDEC) do not require notification of SAEs and SUSARs but *urgent safety measures* (to protect participants from a serious, immediate hazard to their health and safety) will be notified to the HDEC within 7 days.

Clinical trial participants alert card
Participants will also be issued with a clinical trial participant's alert card containing information of the study and contact phone numbers of the study staff, to carry throughout the duration of the study.

Study Procedures

There will be 4 visits (screening [visit 1]; treatment commencement [visit 2]; 4 weeks [visit 3]; and 12 weeks [visit 4]).

There will be a 2-4 week baseline period between screening (visit 1) and commencing treatment (visit 2). At visit 2, patients will be randomised to receive either 500 mg twice daily, or 1000 mg twice daily. Participants will be randomized in a ratio of 1:1, with 20 participants in each group of the sub-study. Randomisation will be performed with the use of a computer- generated sequence with random block sizes of 2 and 4.

Participants have to be clinically stable for at least 4 weeks with no event-based exacerbations before commencing treatment to ensure clinical stability.

Clinical assessment **(visits 1, 2, 3, 4)**
This will include documentation of subject characteristics, medications, and measurement of vital signs (pulse, blood pressure, and temperature at every visit. Physical examination (general, cardiovascular, respiratory, abdominal examinations) will be done by an investigator at all study visits.

Simple blood tests **(visits 1 and 4)**
Blood samples will be taken to measure full blood count (FBC), urea & electrolytes (U&E), liver function tests (LFT) and highly-sensitive C-reactive peptide (hs-CRP) at visits 1 and 4. For female participants of child-bearing potential, a serum β-hCG level (pregnancy test) will be measured at screening (visit 1). A urinary pregnancy test will be done at visit 2 prior to commencement of study medication and again at visit 4.

Sputum analysis
Participants will be asked to provide a baseline spontaneous sputum sample at visit 2. Participants who are productive of only a small volume of sputum or have difficulty expectorating will be asked to provide a baseline sputum sample within one week of visit 2. Samples are also required at visits 3 and 4.

Sputum will be processed using standardised guidelines in keeping with the European Respiratory Society Task Force document regarding sputum induction and processing.[96,97] Spontaneous sputum specimens (sputum plus saliva) will be obtained at visit 2. If participants are unable to provide a sputum sample at visit 3 or 4, induced sputum samples will be collected (see below); samples will be processed within 2 to 4 hours of collection.

Sputum supernatant for measurement of neutrophil elastase, cytokines, hs-CRP and total antioxidant capacity will be separated and frozen at -70°C for measurement at the completion of the study.

Induction of sputum
This will be performed to obtain sputum samples only in patients who are unable to expectorate sputum at visits 3 and 4. After baseline FEV1 and FVC measurements, salbutamol will be given by inhalation of 400 µg of salbutamol given by volumatic spacer device. Post-bronchodilator FEV1 and FVC will be performed after 15 minutes. Induction of sputum will not be performed if the post-bronchodilator FEV1 is less than 50% of predicted. Participants will then inhale hypertonic (4.5%) saline, nebulized for periods of progressively increasing length (0.5, 1, 2, 4, 8 min) until 1-2 ml of sputum has been provided. FEV1 will be remeasured 1 min after each inhalation period.[98] An ultrasonic nebulizer will be used to nebulize the saline solutions.

Sputum collection method and processing
The entire sputum sample will be emptied into a sterile Petri dish and sputum plugs will be selected from the saliva, using sterilised fine forceps. Plugs will be transferred to a Petri dish lid. Using larger blunt-end forceps, the sputum plugs will be gathered into one mass and then condensed by moving around the lid with small circular motions. The saliva-free sputum will be transferred using the blunt-end forceps into a polypropylene centrifuge tube with screw top. Sputum weight (g) x 2 volumes of Dulbecco's phosphate buffered saline (D-PBS) will be added and the sputum will then be dispersed by repeated gentle aspiration into a plastic pipette, vortexed for 15 seconds and set on a bench rocker for 30 minutes (on ice). Samples will then be centrifuged at 790 g for 10 minutes at 4°C. Four volumes of the supernatant will then be removed to a clean 15 mL polypropylene tube, centrifuged at 1500 x g for 10 minutes at 4°C and the supernatant sub-aliquoted and stored at -70°C for subsequent biomarker analyses and measurement of total antioxidant capacity and resveratrol levels.

Markers of airway inflammation*:*

- *Sputum neutrophil elastase and sputum hs-CRP* (visits 2, 3, 4)

A portion of sputum will be prepared for measurement of neutrophil elastase. Sputum neutrophil elastase levels will be determined by ProteaseTag Active NE Immunoassay (ProAxis Ltd, Belfast, UK).[11,99] Sputum hs-CRP will be by DEIA-XY2160 ELISA kit (Creative Diagnostics, Shirley, USA)

- *Sputum cytokines* (visits 2 and 4)

Similarly, a portion of sputum will be tested for the cytokines GM-CSF, IL-1β, IL-6, IL-8, and TNF-α, determined using *Luminex® Cytokine Human 5-Plex Panel* (Life Technologies, New York, USA).

- *Sputum purulence*

Sputum purulence will be determined according to the sputum purulence chart, which is a validated measure of neutrophilic inflammation in the airways of patients with bronchiectasis.[100,101]

Markers of airway infection

- *Sputum culture, sputum cathelicidin (LL-37) and sputum procalcitonin* (visits 2 and 4)

A portion of the sputum will be sent for bacterial culture at visits 2 and 4. Another portion of sputum will be tested for cathelicidin levels using LL-37 ELISA Test Kit (Hycult Biotechnology, Uden, Netherlands).

Sputum levels of procalcitonin will be measured using the Vidas BRAHMS PCT assay (bioMérieux, Lyon, France). Procalcitonin is a biomarker that is sensitive and specific for bacterial infection;[102] our previous studies have found elevated levels of procalcitonin in sputum of patients with bronchiectasis, and with 10-fold higher levels compared to serum during exacerbations.[103]

Markers of systemic inflammation

- *Serum hs-CRP* (visits 2 and 4)

Serum hs-CRP will be assessed at visits 2 and 4 (by DEIA-XY2160 ELISA kit (Creative Diagnostics, Shirley, USA). We will also measure cytokines in serum (GM-CSF, IL-1β, IL-6, IL-8, and TNF-α, determined using *Luminex® Cytokine Human 5-Plex Panel* (Life Technologies, New York, USA).

- *Serum cytokines* (visits 2 and 4)

Serum will be tested for the cytokines GM-CSF, IL-1β, IL-6, IL-8, and TNF-α, determined using *Luminex® Cytokine Human 5-Plex Panel* (Life Technologies, New York, USA).

Measurement of antioxidant status and resveratrol levels

- *Sputum and serum total antioxidant capacity (TAC)* (visits 2 and 4)

The TAC Assay (Cell BioLabs, San Diego, USA) is a widely-used measure of overall antioxidant status, and has previously been used in studies involving resveratrol.[86] Serum and sputum samples will be tested.

- *Sputum and serum resveratrol levels* (visits 2 and 4)

Sputum resveratrol levels (and levels of its metabolites) will be determined using Q-Exactive UPLC-mass spectrometry (ThermoFisher, Masachussetts, USA) to quantify levels of resveratrol and its metabolites. The tests will be developed and validated, and performed at the Liggins Institute, University of Auckland.

On the day of each visit, participants will be instructed to take the morning resveratrol dose 30 minutes prior to the visit; time of dosing will be recorded. Levels will be taken at 90 minutes post-dose.

***Spirometry* (all visits)**

Spirometry will be performed at all visits, using EasyOne spirometer (ndd Medical Technologies, Zurich), in accordance with American Thoracic Society (ATS) and European Respiratory Society (ERS) standards and using predicted values from Global Lung Initiative (GLI) 2012.[104,105] Each participant will use the same spirometer during the entire study period.

Before spirometry testing, respiratory medications will be withheld according to the following schedule: long-acting anticholinergic agents, 24 hours; short-acting beta-agonists, 4 hours; short-acting theophyllines and long-acting beta-agonists, 12 hours; and once daily theophyllines 48 hours.

At all visits, pre-bronchodilator spirometry will be performed initially, followed immediately by 400 µg of salbutamol given by volumatic spacer device. Reversibility will be tested by measuring spirometry after 15 minutes.

***Health-related quality of life (HR-QoL) questionnaires* (Visits 2 and 4)**

Three different HR-QOL questionnaires will be used, to assess differing but common symptoms experienced by patients with bronchiectasis.

- The *St George's Respiratory Questionnaire (SGRQ)* is validated for use in bronchiectasis and includes 56 items (76 weighted responses) across 3 domains - symptoms, activity and impact.[106,107] Component scores from each domain and a total score will be examined. The total score is between 1 and 100; high scores indicate poor health. Thresholds for a clinically significant change in health status using SGRQ

are: i) a difference of 4 units, which indicates effective treatment ii) a difference of 8 units, which indicates very effective treatment.[108]

- The *Bronchiectasis Health Questionnaire (BHQ)* is the most recent HR-QoL questionnaire developed for use in bronchiectasis. It consists of 10 items, with higher scores corresponding to better HRQoL.[109] It has very good internal consistency and strong convergent validity with the SGRQ. Strengths of the BHQ include easy interpretation, using a single total health score, and availability in 11 different languages (English, French, Spanish, Dutch, German, Italian, Japanese, Mandarin, Belgian, US-Spanish and US English).

- The *Leicester Cough Questionnaire (LCQ)* is a further HR-QoL questionnaire, developed for patients with chronic cough, which is a key symptom for patients with bronchiectasis. The LCQ consists of 19 items, is self-administered and assesses three domains over the preceding two weeks: physical, psychological and social. Total severity score ranges from 3 to 21; a lower score reflects worse quality of life relating to cough. It is validated for use in bronchiectasis and scores correlate with SGRQ. The minimal clinically important difference for LCQ is 1.3 points.[110,111]

- *Medical research council (MRC) breathlessness score and modified MRC (MMRC)* are brief, single answer questionnaires that quantify a patient's degree of breathlessness. They have been widely administered for a range of respiratory conditions since the MRC was developed in 1952, which evolved into MMRC.[112] MMRC score is a component of important markers of severity scoring systems in bronchiectasis.[113]

Food frequency questionnaire **(Visit 2)**
The amount of resveratrol in the study product far exceeds dietary intake, but it is important to consider the contribution of dietary resveratrol and other dietary antioxidants to overall levels and antioxidant status.

In a large study of 40,685 individuals, the mean dietary resveratrol intake in Spain was 194 µg/day. In this population, the highest source of resveratrol was red wine, followed by other wines, fresh grapes, grape juice, peanuts and pistachios.[114] The highest levels are found in red wines (up to 14.3 mg/L), considerably lower than the doses used in this study.[36] Interestingly, annual red wine consumption in Spain and NZ is similar (21.7L and 20.3L per capita per year respectively).[115]

Similarly, a wide range of other antioxidants are available from dietary sources. The antioxidant content of different foodstuffs has been studied in great detail and dietary intake of antioxidants may impact on many diseases, including bronchiectasis.[29]

We will assess variation in antioxidant intake in study participants using a food frequency questionnaire (FFQ), developed for this study. Our FFQ will focus on foodstuffs containing high levels of antioxidants, and foodstuffs that account for most variation in antioxidant intake.[116-118] The FFQ will also specifically ask participants about their intake of the ten foodstuffs/food groups containing the highest quantities of resveratrol.[114,119-123]

Participants will be divided into tertiles according to their reported intake, classified as "highest", "moderate" or "lowest" consumers, based on their responses. Two scores will be calculated: an "Overall Antioxidant Score" and a "Resveratrol Score". Scoring for all questions will range from 0 to 8 (0 for "never or less than once per month"; 8 for "6+ times per day"). All questions will be included for the "Overall Antioxidant Score". The 10 foodstuffs containing resveratrol are annotated with the letter "R" on the questionnaire. Scoring will be performed in identical fashion for these ten items to provide the "Resveratrol Score".

Diary cards **(screening to visit 4)**
Symptoms will be recorded using daily diary cards and will be reviewed at each clinic visit. Symptom severity (cough, wellbeing, sputum volume, sputum colour, dyspnoea) will be recorded. Use of community-based services, and any associated costs paid by patients themselves, will be recorded in the daily diary card. This will include services provided by GPs, practice nurse, physiotherapists and any other primary health providers that patients access directly. Sputum colour will be assessed using a validated and reliable sputum colour chart which is available for use in bronchiectasis.[100,101] Details including descriptive features will be located in the diary card.

Phone contact **(weeks 2 and 8)**
Patients will be contacted by telephone in between visits to remind them of follow up appointments and completion of diary cards, to discuss adherence to study medication, and to record any exacerbations or adverse events.

Patients will also be instructed to contact study staff if they have been admitted to hospital. Pulmonary exacerbation will be defined in accordance with an international consensus agreement: "a deterioration in three of more of the following key symptoms for at least 48 hours, in at least three of: cough, sputum volume and/or consistency, sputum purulence, breathlessness and/or exercise tolerance, fatigue or malaise, haemoptysis – AND a clinician determines that a change in bronchiectasis treatment is required".[124]

These key symptoms will make up the patient diary card discussed above, helping to standardize pulmonary exacerbation diagnosis. Information on the use of any hospital-based services will be recorded by study personnel. Full details of any treatments received in the hospital setting will be recorded from their electronic medical record.

Tolerability will be recorded dichotomously and defined as successful completion of the study. Adherence will be described as percentage of capsules taken calculated from prescribed and returned medication. We will record participation consent probability, recruitment and drop-out events.

End points

Primary end point: Change in sputum neutrophil elastase

Secondary end points:

Sputum hs-CRP

Sputum cytokines – GM-CSF, IL-1β, IL-6, IL-8, TNF-α

Sputum purulence

Sputum cathelicidin (LL-37)

Sputum procalcitonin

Sputum culture

Sputum and serum total antioxidant capacity

Sputum and serum resveratrol levels (and metabolites)

Pulmonary exacerbation frequency

Lung function (spirometry: FEV1, FVC)

Health-related quality of life

- St George's Respiratory Questionnaire
- Bronchiectasis Health Questionnaire
- Leicester Cough Questionnaire

Adverse events

Adherence, tolerability and safety of oral resveratrol supplementation

Requirement for induction of sputum at Visit 4

Statistical Methods

Analysis plan
The analysis will take place in an intention-to-treat analysis set. Our primary aim is addressed by the hypothesis that the primary outcome (*sputum neutrophil elastase levels*) at visit 4 will decline from high values at visit 2 (baseline), or remain stable from low values at visit 2. Accordingly, the primary analysis will consist in a regression of the change at visit 4 from baseline on the value at baseline, using a suitable parametric model, a flexible smooth model for the baseline value and appropriate adjustments (including adjustments for dosage in interaction with baseline).

The alternative hypothesis then translates as a negative relationship between baseline and difference at visit 4. The model is provisionally identified as lognormal, inferred from the literature, but a data review will be effected to confirm.

Safety data will be reported in detail by arm and stratified by severity and causality, using Common Terminology Criteria for Adverse Events (CTCAE) standard. *Tolerability and adherence* will be regressed on the dosage arm using logistic and linear regression respectively. As this is a signal-finding study, tests will be conducted at a significance level of 10% against two-sided alternatives, with no adjustment for multiplicity.

Sample size
We have based our sample size on the baseline values from two highly important papers assessing neutrophil elastase levels in bronchiectasis.[11,101] One study reported median sputum neutrophil elastase levels of 15 µg/ml (IQR 3-23 µg/ml); in the other, levels varied according to stable or exacerbation state, with median of 0.39 µg/ml (IQR 0-23.5 µg/ml).[11,101] Converting these values to approximate mean and standard deviation using standard techniques yielded estimated lognormal parameters on which we based our simulations. With these parameters and assuming a drop-out rate of 10%, we ran extensive power simulations to establish a detectable decrease at visit 4 from baseline of 40% with 80% power (under any dosage). The detectable difference corresponds to a decrease from 5 to 3 µg/ml between baseline and visit 4. The same sample size enables us to detect a doubling of the efficacy of resveratrol under the 2000 mg vs 1000 mg per day dosage with 80% power, provided the larger dose decreases sputum neutrophil elastase by 67% or more.

Data Monitoring Committee
A data monitoring committee will comprise 3 external members.

- *Christin Coomarasamy* (Biostatistician, Ko Awatea, Counties Manukau DHB; Independent) – Chair of committee
- *Assoc. Professor Lata Jayaram* (Respiratory Physician, Western Health, Melbourne; Co-investigator)
- *Dr Leon Chang* (Respiratory Physician, Department of Respiratory Medicine, Middlemore Hospital; Independent)

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