PROTOCOL

Assessing the potential of a medieval antibiotic to treat infected diabetic ulcers: a Phase 1 trial in healthy volunteers

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This protocol has regard for current HRA guidance and content

Protocol Amendments:

Amendment No.		Date of Amendment		Date of Approval

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STUDY SUMMARY

Trial Title	Assessment of safety of a medieval antibiotic to treat infected diabetic ulcers: a Phase 1 trial in healthy volunteers
Sponsor ref. number	
Clinical Phase	Phase I
Trial Design	Single centre non-randomised, uncontrolled intervention study
Trial Participants	Healthy volunteers, aged from 18 to 79 years
Planned sample size	100 participants
Treatment Duration	48 hours
Follow-up Duration	48 hours
Planned Trial Period	1 April 2021 to 1 July 2021 (3 months)
Objectives	To aim is to determine the safety of an 'ancientbiotic' (Bald's salve) when applied to the skin of human volunteers. The ancientbiotic is a mixture of natural products used in a historical infection remedy. The specific objective is to determine the frequency of adverse events associated with a patch test of Bald's salve applied to the skin of human volunteers.
Outcomes **Primary** **Secondary**	 Skin irritation assessed at 48 hours. Any other adverse event associated with the patch test application of Bald's salve.

LIST OF ABBREVIATIONS/GLOSSARY

Abbreviation	Explanation
AE	Adverse Event
CI	Chief Investigator
CONSORT	Consolidated *S*tandards *o*f *R*eporting *T*rials
CRF	Case Report Form
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
DFI	Diabetic Foot Ulcer
GCP	Good Clinical Practice
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trial Number
MRSA	Meticillin-resistant *Staphylococcus aureus*
PI	Principal investigator
PPI	Patient & Public Involvement
RCT	Randomised controlled trial
REC	Research Ethics Committee
R&D	Research and Development
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
TSC	Trial Steering Committee
WCTU	Warwick Clinical Trials Unit

1. BACKGROUND

1.1 Burden of diabetic foot ulcers

People with diabetes risk developing ulcers on their lower limbs. Hyperglycaemia, poor circulation and abnormal immune responses mean that ulcers become infected by bacteria. Ten percent of people with Type 1 or Type 2 diabetes develop chronic, non-healing infected ulcers. Diabetic foot infections (DFIs) are a key cause of hospitalisation and severely impact quality of life. Even if a DFI is successfully treated, there is a high chance of recurrence, with up to 66% recurring. Consequences of DFI include amputation and sepsis; up to 12% of people with DFI require an amputation. Approximately half of people with DFIs die within five years of ulcer development. Management of DFIs costs the NHS £600M per year, and the burden of infection will likely increase as the prevalence of Type 2 diabetes rises.[1-3]

Key pathogens in DFIs include common causes of other opportunistic infections, e.g. *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus spp., Enterococcus spp. and Klebsiella pneumoniae*. DFIs typically comprise a diverse community of different bacterial species which live as a multicellular biofilm: a community of bacteria protected by a slime layer that hinders immune clearance and antibiotic penetration.[4-6] Furthermore, widespread antibiotic use has led to bacteria evolving various genetic strategies to become resistant to drugs currently in use. The problem of untreatable DFI will likely worsen due to a scarcity of new antibiotics in development.[3] We desperately need new antibiotics to effectively treat DFIs. Antimicrobial resistance has been highlighted by the World Health Organization (WHO) as a global health emergency.

1.2 The hunt for new antibiotics by studying the past

The research team have used ethnopharmacology (the study of traditional and historical medicine) to find compounds that could be tested and developed into new antibiotics. Medieval European medical texts contain abundant remedies for infections, often using ingredients shown to have antimicrobial qualities in vitro. Some of these have already produced clinical antimicrobials e.g. honey dressings, or *Artemisia spp*, which produce the anti-malarial drug artemisinin.[7,8]

We found that a medieval English remedy for eye infections kills various bacteria found in DFIs and other persistent infections. This 'ancientbiotic' is made by combining garlic, onion, bovine bile and wine, and allowing the mixture to steep for nine days. The recipe was chosen for study as all ingredients are known to have some (limited) antibacterial activity in vitro. The remedy's efficacy depends on preparing and combining ingredients as specified in the original text. While the well-studied compound allicin, from garlic, explains the ability of the remedy to kill bacteria growing in liquid broth culture, allicin alone cannot kill bacteria in biofilms at the concentrations it is found in the remedy.[9,10] This could explain why in vitro antibacterial activity of the individual ingredients does not reliably translate into in vivo potential in clinical trials e.g. garlic.[11] This result is crucial: something happens when the ingredients are combined, generating a cocktail of compounds and/or a novel compound which effectively kills bacteria.

1.3 Could an ancientbiotic generate a treatment for infections?

1.3.1 Bald's salve kills a range of clinically-relevant bacteria when grown as biofilms

Bacteria are relatively easy to kill when they are grown as free-swimming cells in liquid cultures in test tubes. Many natural products (compounds made by plants, animals or microbes) can kill pathogenic bacteria when grown in this state. But when bacteria form matrix-embedded, multicellular biofilms – as they do in diabetic ulcers – their tolerance to antibacterial compounds is massively increased. Bacteria that are susceptible to low concentration of antibiotics in a test tube may require a 1,000-fold higher antibiotic concentration for effective biofilm killing, and natural products with promising antibacterial activity in a test tube may fail to kill bacteria in biofilms.

Activity against biofilms is what makes Bald's salve stand out as a promising candidate for clinical development. We have made over 70 batches of the salve, and they are all able to kill mature biofilms of the bacterium *S. aureus* in an *in vitro* model of a diabetic wound biofilm. Mature biofilms comprise approximately 10^8 bacterial cells, but exposure to the salve for 24 hours results in only 100-1,000 bacteria surviving. This represents a 5-6 $\log_{10}$-fold killing effect. In microbiology, a 3 $\log_{10}$-fold killing effect in lab studies is generally taken to represent good bactericidal activity worthy of further investigation.

We have also shown that Bald's salve can kill biofilms of a meticillin-resistant strain of *S. aureus* (MRSA) when biopsies of biofilm-infected tissue from wounded mice are placed into a suspension of the salve, and that the salve kills these tissue-embedded biofilms more quickly than the current gold standard antibiotic for MRSA. Further, we have recently confirmed that Bald's salve can kill biofilms of other important diabetic foot ulcer bacteria: *Acinetobacter baumannii, Stenotrophomonas maltophilia, Staphylococcus epidermi, dis* and *Streptococcus pyogenes.* Activity against *A. baumanii* is especially noteworthy as this is a WHO priority pathogen with extensive intrinsic resistance to numerous antibiotics. While the presence of allicin from garlic in Bald's salve can explain the activity against free-swimming bacterial cells, the salve contains allicin in concentrations much lower than that required to kill biofilms: all ingredients are required to produce the anti-biofilm effect. All of these results have been published.[9-10]

1.3.2 Bald's salve has a promising safety profile in tests with human cells and animal models

We have conducted laboratory research which demonstrates that Bald's salve has a low risk of being toxic to human cells, triggering an excessive inflammatory response, or interfering with wound healing. This work is currently undergoing revision for publication but a preprint is available.[12] The results are summarised below.

First, lab cultures of human skin and immune cells were exposed to the salve. Cultured cells are very sensitive to damage so they are a good 'early warning system' for severe toxicity or immunogenicity. While we saw some cell death, and some release of inflammatory cytokines, this was comparable with death/inflammation induced by exposing the cells to Optrex® antibiotic eyedrops: these contain chloramphenicol, which is known to damage cells grown in the lab although it is perfectly safe to apply to the eye.

We therefore moved to test the salve in an industry-standard *ex vivo* model, and an *in vivo* model, for assessing irritant capacity of substances. The bovine corneal opacity and permeability test is used in the pharmaceutical and cosmetic industries as a standard model of eye irritation. Substances are dropped onto cows' eyes (obtained post-slaughter from a commercial abattoir) and the eyes are then assessed for irritation, visible as a cloudy patch on the cornea which can clear and resolve over time. The salve demonstrated slight irritation to the cornea that resolved within ten minutes. We then used a novel *in vivo* method where test substances are applied to live slugs, and the amount of mucus secreted by the slugs in response to the substance is quantified as a proxy for extent of irritation. A low level of mucus was secreted by slugs exposed to eyesalve, indicating mild mucosal irritation.

Finally, we conducted an *in vivo* wound healing experiment using live mice. A total of 19 mice were administered a full-thickness surgical excision wound and treated by applying 100 µl of the salve (three different batches tested) or 100 µl sterile water to the wound. Each day, the dressing was removed, a further 100 µl of eyesalve or water was added, the wounds were photographed and measured with a wound imaging and documentation system, and the dressing replaced. This continued for 15 days, by which time the wounds had all healed. The area of the wounds reduced daily as shown below, and analysis with linear models showed that there was no difference in healing rates between eyesalve-treated and water-treated groups. All wounds were healed by day 15. There were no visible signs of irritation or inflammation – i.e. there was no reddening of tissue, and the mice did not display signs of irritation. Example images are in press.[12]

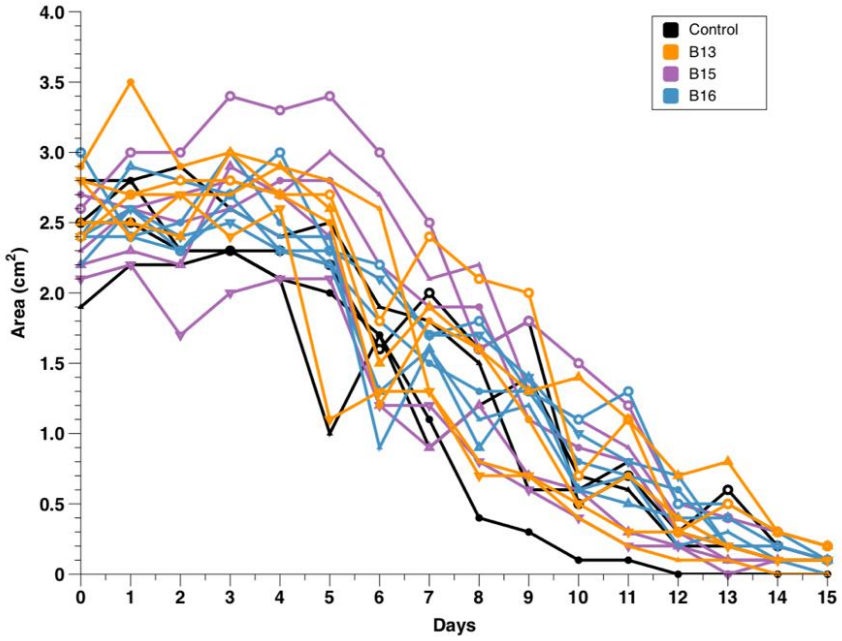

Figure 1. Wound healing over time in mice treated with water (control, black) or Bald's salve (three batches shown in orange, purple and blue – each batch made with fresh ingredients). Each line shows the change in the area of the wound for an individual mouse.

Given the laboratory and animal study evidence for the potential efficacy and safety of the ancientbiotic, the next stage is to test the liquid in a sample of healthy volunteers. We want to assess safety by undertaking a skin patch test, using Bald's salve, in healthy adults to identify adverse events.

1.4 Hypothesis

We hypothesise that a 48-hour exposure to the ancientbiotic will not result in irritation or inflammation in healthy, non-diabetic adults.

1.5 Need for a pilot safety trial

A pilot clinical trial will be conducted with approximately 100 healthy adult volunteers, to assess the safety and tolerability of the ancientbiotic product on human skin. We want to find out whether, after completion of laboratory tests to human cells, there is any suspicion that the ancientbiotic causes inflammation to human cells. We consider this highly unlikely given (1) the data from cytotoxicity, irritation and wound healing assays cited above; and (2) that the product consists entirely of edible household ingredients.

Study Design: non-randomised, uncontrolled, Phase I pilot trial.

Participants: Adults aged 18-79 years, community-dwelling, male or female, without a diagnosis of diabetes, immune-related disease (asthma, eczema, psoriasis), pregnancy, or recent infection will be recruited. People with known dietary allergies will be excluded. People with Covid-19 infection, Covid-19 symptoms, or who are self-isolating due to Covid-19 exposure, will be excluded.

Intervention: Sticking plaster infused with one drop of the liquid Bald's salve ancientbiotic compound, applied to the skin of the upper arm, worn for 48 hours.

Outcomes: Adverse skin effects over 48 hours e.g. redness, heat, swelling or other. Type, number (%) and spread/distribution will be recorded. Digital photographs will be taken of any suspected skin reaction.

1.6 Ethical considerations

The trial will be conducted in full conformance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. It will also comply with all applicable UK legislation and University of Warwick Standard Operating Procedures (SOPs). All data will be stored securely and held in accordance with the General Data Protection Regulation.

Before enrolling people into the study, we will ensure that we have the approval of the Research Ethics Committee (REC). We will not be permitted to enrol people into the trial until written favourable opinion is received from the REC. The sponsor for the trial is the University of Warwick.

The study team will ensure that participants' anonymity is maintained. Participant identifiable information will be stored securely on an electronic database. The consent form will hold participant identifiable information, including name, mobile or home telephone number and email address. We will not hold any other identifiable information and will not access any medical records. Data will be stored securely and separately from other forms that are pseudo-anonymised, thus only labelled with a unique trial ID number. All documents will be stored securely and will only be accessed by study staff and authorised personnel. The study will comply with relevant UK data protection legislation, which requires data to be pseudo-anonymised as soon as it is practical to do so.

Data will be collected at baseline and follow-up assessment after 48 hours, and then entered directly into a secure database developed by WCTU. Paper forms will be on site at WCTU under locked conditions for the duration of the study; these will be considered source documents for the study. Email contact with the research team will be stored on the WCTU M drive server and held for the purposes of data checking and for monitoring purposes only. Direct access to source data and documents will be granted to authorised representatives from the sponsor, host institutions and the regulatory authorities to permit trial related monitoring, audits and inspections.

We expect all approaches from potential volunteers will be made to the study team directly. We will not apply to access any medical or social care data. We seek signed consent from participants to provide the study team with their personal contact details (telephone number and email) and consent for a photographic image to be taken of the patch test area should any events occur. Participants will be provided with a study email address and telephone number to contact the team straight away for advice.

All participants will be contacted by phone or email after 48 hours and a photo taken of the affected area where any suspected adverse event has occurred. Photographs from a small sample of volunteers with no skin reaction (n=5) will be taken for control purposes only.

Participants who are not fluent in written English will be excluded from the study as we do not have capacity for translation at this stage in the research process. We will exclude adults lacking capacity to consent.

1.7 CONSORT

The trial will be reported in line with the CONSORT (Consolidated Standards *o*f *R*eporting *T*rials) statement (Lancet 2001, **357**: 1191-1194).

1.8 Assessment and management of risk

There will be some risk of experiencing skin redness or irritation from the skin patch test, arising either from application of the Bald's salve or from the sticking plaster. The products within the salve include garlic (active ingredient allicin), onion, wine and bovine bile. We do not anticipate any serious adverse events such as those resulting in death, hospitalisation, life threatening illness or permanent disability. Any participant experiencing any discomfort will be advised to remove the plaster and gently wash the area with soap and water. If symptoms persists, we advise them to visit their GP.

In light of Covid-19, there are also risks associated with contact between participants and the research team. We have taken steps to ensure rules regarding distancing and protection are adhered to. A Perspex sheet will be placed between the attending volunteer and researcher; facial masks will be worn by all personnel and participants. The Perspex sheet will be adapted to allow the participant's upper arm to be accessed for patch test application. Face to face assessment at 48 hours, as originally planned, has been replaced with telephone or video link contact. The study will be administered in keeping with WCTU SOPs for risk assessment and monitoring.

2. TRIAL DESIGN

2.1 Pilot trial

Non-randomised, uncontrolled, Phase I pilot trial.

2.2 Trial summary

Trial overview: Healthy volunteers will be sought by the research team using multiple strategies, including email circular invitations, social media and advertising the study to University of Warwick staff and students during academic term time. We will also seek a broad range of volunteers and will also recruit from the local community via groups (e.g. Women's Institute, activity groups) who have learned of the study via the media or our outreach activities (e.g. FH invited talks planned for January 2021). We will only recruit volunteers who are based in the surrounding locality (Coventry, Leamington, Warwick and Kenilworth). We aim to recruit between 90 to 100 volunteers in total.

After consent and recruitment, a small drop of the Bald's salve (intervention) will be applied onto a sticking plaster and applied to the participant's upper arm.

A fresh batch of the salve will be prepared using garlic and onions sourced from a commercial greengrocer, Pennard Organic white wine sourced from Avalon Vineyard, Somerset, and bovine bile (Merck Millipore catalogue no. B3883 suspended in sterile water). The salve will be passed through a 0.2µm syringe filter prior to use in this study to ensure sterility.

Outcomes will be captured at 48 hours. Due to Covid-19, face to face assessment of all participants will not be undertaken; this will be replaced with telephone or video contact supplemented with email.

Thus, after 48 hours, the participant will be contacted by the research team and asked to report any sign of skin reaction. We will ask participants to send a photographic image of the affected area. Some participants may be contacted by video (MS Teams, Zoom, Google or other video platform at the participant's discretion) for visual assessment of the affected area. We will collect and store photographs only from those reporting signs of redness or irritation and from a small sample of people with no symptoms (n=5), for comparison purposes.

2.3 Aims and objectives

The aim of this trial is to assess the safety profile of an ancientbiotic (Bald's salve), when applied to the skin of healthy human volunteers.

2.3.1 Objectives

The objective is to determine the frequency and character of adverse events associated with a patch test of Bald's salve applied to the skin of 90 to 100 human volunteers.

2.4 Outcome measures

2.4.1 Safety profile of ancientbiotic

Primary Outcome:

Frequency (prevalence) and character of adverse events at 48 hours after application of 100 µl ancientbiotic on a sticking plaster applied to the upper arm.

Follow-up: No further follow-up beyond 48 hours. If there is no skin reaction within 48 hours, we do not anticipate any longer term adverse events. However the study email account will be kept open for up to three months and participants will be advised to contact us if any suspected later reaction occurs.

2.4.2 Safety

The study will be delivered in Warwick Medical School and School of Life Sciences, University of Warwick. See section 4.0 for AE and SAE information.

2.5 Eligibility criteria

Volunteers are eligible to be included in the study if they meet the following criteria:

2.5.1 Inclusion criteria

1. Adults aged 18 years to 79 years.
2. Not known to be pregnant.
3. No history of asthma, eczema, psoriasis, known allergies e.g. to food stuffs, sticking plasters or other allergies, no broken skin on the upper arms.
4. No recent infection in previous 3 months, including Covid-19 infection.
5. Willing to provide signed consent to participate in the study.
6. Fluent in spoken English to allow engagement with study.
7. Willing to be contacted by telephone, email or video-based platform at follow-up.
8. Willing for the research team to hold a photographic image if any skin irritation occurs.

2.5.2 Exclusion criteria

1. Pregnancy.
2. Diabetes.
3. Any known allergies or recent infection (within 3 months).
4. Any skin condition, including eczema or psoriasis or broken skin.
5. Any diagnosis of asthma.
6. Unable to be contacted by email, phone or video platform within 48 hours of intervention delivery.

2.6 Participant identification / Screening

Participant identification: Volunteers will be identified and screened by the research team:

1. *University of Warwick staff and students:* via advertisement to student groups, before lectures, email circulars to Warwick Medical School and staff from other sections.

2. *Community-based recruitment:* local volunteer groups, Women's Institute (WI), self-referral. The study was advertised during an external event to the WI and people expressed an interest in participating.

A website will be developed, hosted on the University of Warwick server, with information on the study and the participant information sheet (PIS) in downloadable PDF format.

2.7 Staff Training

Up to three members of the study team (Harrison (CI), Bruce, + research assistant under appointment) will be responsible for the administration of the ancientbiotic liquid and application of the sticking plaster. Staff have completed relevant continued professional development (CPD) and good clinical practice (GCP) training. The trial team (Harrison/Bruce) have tested application of the intervention as pre-pilot testing. The clinic will be held over a period of one week initially.

2.8 Consent

Confirmation of eligibility, and participant approach:

1. Volunteer contacts the study team expressing interest in the study.
2. Trial team undertake eligibility screening and volunteer provided with Participant Information Sheet (PIS).
3. Allowed 48 hours to consider participation and ask any questions they may have.
4. Potential participant contacted by email, or phone by a member of the research team.
5. Signed consent obtained via post or at clinic.

Informed consent: volunteers will be invited to a clinic appointment where consent will be taken in person by an appropriately trained member of the clinical or research team.

Adults lacking capacity: will be excluded from the study.

GP notification: We do not intend to inform the participant's GP. It will be up to each participant to inform their own GP that they are taking part in the study. We recommend that anyone experiencing signs of persistent skin irritation, lasting after washing/ beyond 48 hours, should contact their GP.

Timing of consent: Written informed consent will be obtained by a suitably trained member of the research team (FH/JB) at the Warwick Medical School, after allowing sufficient time for the potential

participant to consider their decision and ask questions about the study. Sufficient time for some potential participants may result in a decision to take part in the trial immediately after receiving all the relevant information. Alternatively, if potential participants would like to leave with the information and decide later, they will be free to do so, and can contact the research team at a later time.

New information: We do not anticipate any new information arising that may affect participants' willingness to take part in the study. We will stagger administration initially. If any significant adverse events arise in the first 10 participants, then the study will be halted and the sponsor informed immediately. If minor skin reaction or no reactions occur over 48 hours for the first 10 volunteers, we will continue to roll out administration to the remaining volunteers.

Decline/withdrawal: Participants will have the option to withdraw before treatment starts, if for any reason they change their mind. The participant will remain free to withdraw at any time without giving reasons.

2.9 Randomisation

2.9.1 Randomisation allocation concealment and blinding

This is a non-randomised Phase 1 study with no comparator arm. Eligibility checks will be carried out by the team to ensure that potential participants meet eligibility criteria. Written consent for entry into the trial will be obtained before any salve is applied to the skin. It is not possible to blind participants or practitioners to group allocation due the garlic content in the salve.

2.9.2 Post-randomisation withdrawals and exclusions

Participants may decline to continue involvement in the trial at any time, without prejudice.

Follow up: Core outcomes will be completed over the telephone, if postal copies are not returned. Text messages may also be sent to those participants, who have given their prior consent by initialling the corresponding box on the consent form and providing their mobile telephone number.

Reminders will be sent by text message or email if no contact is made after 48 hours. Paper CRFs containing identifiable data will be stored separately from CRFs that correspond to the participant by participant ID number only.

2.10 Trial treatments / intervention

2.10.1 Trial treatment(s) / intervention

After consent and recruitment, a small drop of the Bald's salve (intervention) will be applied onto a sticking plaster and applied to the participant's upper arm.

2.10.2 Compliance

Follow-up will be after 48 hours. Anyone who we fail to contact after a maximum of ten days will be recorded as a loss to follow-up.

2.11 Blinding and allocation concealment

As this is a non-randomised pilot safety study, we will not randomise participants. There will be no blinding of volunteers or research team. Due to the garlic content in the Bald's salve, it is currently not possible to blind participants to the intervention as the product is identifiable by smell.

2.12 Concomitant illness and medication

2.12.1 Concomitant illness

We will exclude any people with asthma, diabetes, skin conditions or anyone with allergies.

2.13 End of trial

The trial will end when data collection and statistical analysis has been completed. We will notify the relevant REC within 90 days of the end of the study.

3 METHODS AND ASSESSMENTS

3.1 Schedule of data collection

Table 1. Trial visits/assessments

Visit	WMS Clinic / Phone	WMS Clinic	WMS Clinic
Time-point	Pre-consent	Day 0	Day 2
Eligibility checks (in person or on phone)	**X**		
Written/verbal information about study	**X**		
Written informed consent	**X**	**X**	
Digital photograph image, if indicated			**X**
Skin check at baseline		**X**	
Outcome assessment			**X**
Any other adverse event		**X**	**X**

3.2 Long term follow-up

Not applicable. We do not anticipate any longer term adverse events beyond 48 hours. However, we will keep the resource email account open for a period of three months beyond the end of recruitment and advise participants they can contact the study team by email if any suspected adverse event occurs in the longer term.

3.2 ADVERSE EVENT MANAGEMENT

3.3 Definitions

3.3.1 Adverse Events (AE)

An Adverse Event (AE) is defined as any untoward medical occurrence involving a participant, which does not necessarily have a causal relationship with the intervention or trial.

Expected AEs, related to the ancientbiotic intervention, may include:

- Skin redness
- Skin itchiness
- Skin discomfort

We will capture symptoms arising within and at 48 hours.

Unexpected AEs related to the ancientbiotic will be recorded via self-report by the participant up to 48 hours or at 48 hours when contacted by the research team.

3.3.2 Serious Adverse Events (SAEs)

We do not anticipate any serious adverse events (SAE) as the liquid is made up of common food stuffs. We consider an SAE to be an untoward medical occurrence that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is an important medical condition

3.4 Reporting related and unexpected SAEs

All SAEs will be entered onto the appropriate reporting form and returned to WCTU. All SAE reports will be recorded within 24 hours of the investigator being made aware. The CI will compile all the necessary information. WCTU is responsible for reporting any related and unexpected SAEs to the sponsor and REC within required timelines. All SAEs will be recorded for inclusion in the annual reports to the REC. The CI and CoIs will review causality.

The causality of SAEs (i.e. relationship to trial intervention) will be assessed by the investigator(s) using the SAE form (table 2).

Table 2. SAE Causal relationship

Relationship to trial medication	Description

Unrelated	There is no evidence of any causal relationship
Unlikely to be related	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial intervention). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possible relationship	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial intervention). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probable relationship	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely related	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

To establish causality, the following information should be collected for each SAE:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to intervention), in the opinion of the CI
- whether the event would be considered expected or unexpected.

SAEs that are deemed to be unexpected and possibly, probably or definitely related to the trial interventions or outcomes assessments, will be notified to the Research Ethics Committee (REC) within 15 days. All such events will be reported to the wider research group. All SAEs that occur between the date the intervention is delivered and 48 hours will reported. We do not anticipate new adverse events to occur beyond 48 hours if none are apparent within 48 hours. Nevertheless, the resource account email address will remain active for three months beyond the end of the last 48 hour follow-up.

Any change of condition or other follow-up information should be communicated to the Sponsor as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed until resolution or a final outcome has been reached. A member of the study team will be instructed to closely monitor each participant who experiences a SAE, until the outcome of the SAE has been determined.

Annual reporting: All SAEs will be recorded for inclusion in annual reports to the Research Ethics Committee.

3.5 Responsibilities

Co-Investigators:

1. Checking for AEs when participants attend for follow-up.
2. Using clinical judgement in assigning seriousness, causality and expectedness.
3. Ensuring that all SAEs are recorded and reported to the Sponsor within 24 hours of becoming aware of the event, and providing further follow-up information as soon as available.
4. Ensuring that AEs are recorded and reported to the Sponsor in line with the requirements of the protocol.

Chief Investigator / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using clinical judgement in assigning seriousness, causality and expectedness of SAEs where it has not been possible to obtain local medical assessment.
3. Using clinical judgement in assigning expectedness.
4. Immediate review of all related and unexpected SAEs
5. Review of specific SAEs in accordance with the trial risk assessment, protocol as detailed in the Trial Monitoring Plan.
6. Production and submission of annual reports to the relevant REC.

Sponsor:

1. Central data collection and verification of AEs, and SAEs, according to the trial protocol.
2. Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
3. Reporting safety information to the independent oversight committees identified for the trial (DMC and TSC) according to the Trial Monitoring Plan.
4. Expedited reporting of related and unexpected SAEs to the REC within required timelines.
5. Notifying Investigators of related and unexpected SAEs that occur within the trial.

External Independent Monitoring:

As this is a Phase 1 study, no external committees have been formed (Trial Steering or Data Monitoring Committee).

3.6 Notification of deaths

We do not anticipate any death arising within the study period of 48 hours. However, any death will be reported to the sponsor irrespective of whether the death is related to the intervention, or an unrelated event.

3.7 Reporting urgent safety measures

If any urgent safety measures are taken, the CI/Sponsor shall immediately and, in any event, no later than 3 days from the date the measures are taken, give written notice to the relevant REC of the measures taken and the circumstances giving rise to those measures.

4 DATA MANAGEMENT

Personal data collected during the trial will be handled and stored in accordance with the General Data Protection Regulation. All members of the study team have completed compulsory data security training as per University of Warwick guidelines.

Personal identifying information will be sent to and stored at WCTU for follow up purposes. Handling of personal data will be clearly documented in the participant information sheet and consent obtained.

Disclosure of confidential information will only be considered if there is an issue which may jeopardise the safety of the participant or another person, according to WCTU SOPs (WCTU SOP 15 part 1) and the UK regulatory framework. There is no reason to expect this situation to occur in this trial more than any other.

4.1 Data collection and management

The CRFs have been developed by the research team to collect all required trial data. Suitably trained members of the research team will complete the CRFs and enter the data onto a secure password-protected MS Excel sheet in accordance with the WCTU SOPs. Paper CRFs will be returned to WCTU for data checking and quality assurance. Various methods will be used to chase missing data/unreturned questionnaires including post, phone, text and email, the procedures for managing this will be outlined in the data management plan and appropriate consent will be sought to contact participants. Data will still be collected for participants who discontinue or deviate from the intervention protocol, unless they withdraw their consent.

4.2 Database

An MS Excel database will be used to store trial data. All database variables, validation checks, screens will be agreed between the research team and the trial statistician. The database will be held on the secure M drive on the University of Warwick server.

4.3 Data storage

All essential documentation and trial records will be stored at WCTU in conformance with the applicable regulatory requirements and Warwick SOPs. Access to stored information (paper and electronic) will be restricted to authorised personnel. All data will be stored in a designated storage facility within the WCTU. Electronic data will be stored on password protected university computers in a restricted access building for the recommended 10 years as per Warwick SOPs.

4.4 Data access and quality assurance

All data will be pseudo-anonymised after the collection of the baseline demographic data for each participant. Confidentiality will be strictly maintained and names or email addresses will not be disclosed to anyone other than the staff involved in running the trial. All electronic participant-identifiable information will be held on a secure, password-protected database accessible only to authorised personnel. Paper forms with participant-identifiable information will be held in secure, locked filing cabinets within a restricted area of WCTU. Participants will be identified by a participant

number only on a consecutive basis. Direct access to source data/documents will be available for trial-related monitoring or audit by WCTU for internal audit or regulatory authorities.

Direct access to source data/documents will be required for trial-related monitoring. For quality assurance, the data and results will be statistically checked. A full data management plan will be produced by the trial manager and statistician will outline the data monitoring checks required.

4.5 Data Shared with Third Parties

Requests for data sharing will be managed in accordance with University of Warwick/WCTU policy on data sharing. The datasets generated during and/or analysed during the current study are/will be available upon request after publication of the main study results. The trial results and trial data will be in line with the NIHR standard terms and will follow WCTU SOP 22: Publication & Dissemination.

4.6 Archiving

Trial documentation and data will be archived for at least ten years after completion of the trial.

5 STATISTICAL ANALYSIS

5.1 Power and sample size

No sample size was calculated for this phase 1 study. The aim is to assess safety of the salve. The primary outcome will be adverse skin effects over 48 hours e.g. redness, heat, swelling or other. Type, number (%) and spread/distribution will be recorded.

5.2 Statistical analysis of efficacy and harms

5.2.1 Statistics and data analysis

All data will be analysed and reported in accordance with the CONSORT statement.

5.2.2 Planned recruitment rate

We plan to recruit over a period of four weeks. This will be extended if necessary. The first phase will recruit five to ten participants and then, after completion of follow-up, the remainder will be recruited.

5.2.3 Statistical analysis plan

Type, number (%) and spread/distribution of any adverse events will be recorded.

5.2.4 Summary of baseline data and flow of patients

Baseline data of sex and age will be summarised. We will report numbers screened and characteristics of those eligible and those ineligible, also those eligible but withholding consent. A CONSORT chart illustrating participant flow throughout the study will also be produced. Standard statistical summaries will be presented for the primary outcome measure.

5.2.5 Primary outcome analysis

Data will be summarised and reported in accordance with CONSORT guidelines for RCTs [50]. Type, number (%) and spread/distribution of any adverse events will be reported by age and sex characteristics. Analysis will be undertaken by a senior statistician (NP).

5.2.6 Procedure(s) to account for missing or spurious data

Whilst every effort will be made to ensure compliance and data collection, it is inevitable that some data will be missing. Careful monitoring of missingness will be conducted. We do not intend to use multiple imputation methods for this pilot trial. Some data may not be available due to voluntary withdrawal of participants, lack of completion of individual data items or general loss to follow-up. Where possible the reasons for data 'missingness' will be ascertained and reported. The nature and pattern of the missingness will be carefully considered, including whether data can be treated as missing completely at random.

6 TRIAL ORGANISATION AND OVERSIGHT

6.1 Sponsor and governance arrangements

The University of Warwick will sponsor the trial. The day-to-day running of the trial will be managed by WCTU according to WCTU SOPs.

6.2 Ethical approval

All ethical approvals will be sought using the Integrated Research Application System. The trial will be conducted in accordance with relevant regulations and guidelines. Before enrolling people into the trial, we will ensure that have full ethical approval. If there are any substantial protocol amendments (e.g. changes to eligibility criteria, outcomes, analyses) will be communicated by the study team to relevant parties i.e. investigators, RECs, participants, NHS Trusts and trial registries once approved. Annual reports will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The REC and sponsor will be notified of the end of the trial (whether the study ends at the planned time or prematurely). The CI will submit a final report to the required authorities with the results, including any publications, within one year ending the trial.

6.3 Trial Registration

The trial will be registered with the International Standard Randomised Controlled Trial Number (ISRCTN) Register: ISRCTN (to be allocated).

6.4 Notification of serious breaches to GCP and/or trial protocol

A "serious breach" is a breach which is likely to effect, to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial;

(b) the scientific value of the trial

If a serious breach occurs, the sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase

6.5 Indemnity

The University of Warwick provides indemnity for any harm caused to participants by the design of the research protocol.

6.6 Trial timetable and milestones

Table 1. Trial timetable and milestones

Month	Activity	Milestone	Team
-3-0	Ethics submission & approval	Approval	CI/CoCI/RA
1-2	1. Start project 2. Finalise protocol & CRFs 3. Pilot with 5-10 participants	1. Final versions approved	
3	1. Continue recruitment 2. 48 hour follow-up 3. Continue follow-up on all participants	1.Recruit to target Assessment of follow-up rates	CI/CoCI/RA
4-5	End recruitment		
	Statistical analysis		Statistician
	End of study	Final reports	CI/CoCI/RA

6.7 Administration

The trial will be managed at WCTU, University of Warwick.

6.8 Trial Management Group (TMG)

We have not formed a Trial Management Group. The chief and co-investigators involved in the day-to-day running of the trial, will meet regularly throughout the project. Significant issues arising from management meetings will be referred to the wider study team (co-applicants), as appropriate.

6.9 Trial Steering Committee (TSC) / Data Management Committee (DMC)

Not applicable for a Phase 1 study.

6.10 Essential Documentation

A Trial Master File will be set up according to Warwick SOP 11 and held securely at the coordinating centre.

6.11 Financial Support

The trial has been funded by Diabetes UK: 17/129/02.

7 MONITORING, AUDIT AND INSPECTION

The study will be monitored by the Quality Assurance team at WCTU as representatives of the trial coordinating centre and academic lead, to ensure that the study is being conducted as per protocol, adhering to Research Governance and GCP. The approach to, and extent of, monitoring will be specified in a trial monitoring plan determined by the risk assessment undertaken prior to the start of the study. A Trial Monitoring Plan will be developed and agreed based on the trial risk assessment. Processes to be considered in the monitoring plan will include participant enrolment, consent, eligibility; adherence to trial interventions and policies to protect participants, including reporting of harm and completeness, accuracy, and timeliness of data collection. The plan will be available from the WCTU and will also be lodged with the sponsor. Whilst the monitors work in the same institution as the CI and trial team (WCTU), they will act independently in this role.

The sponsor will ensure investigator(s) and/or institutions will permit trial-related monitoring, audits and REC review, providing direct access to source data/documents as required. Monitoring will be performed by exploring the trial dataset or performing central monitoring procedures, as defined in the trial monitoring plan.

8 PATIENT AND PUBLIC INVOLVEMENT (PPI)

None at this stage. Lay co-applicants were involved with the original grant application.

9 DISSEMINATION AND PUBLICATION

Results of the study will be prepared by the research team and submitted to Diabetes UK as a final report. Findings will be submitted to peer-reviewed journals and disseminated to the medical and exercise rehabilitation communities. Papers will be published in open-access journals, in accordance with recommended guidance for transparent reporting, the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org), the NIHR standard terms, and Warwick SOP 22: Publication & Dissemination. Abstracts will be submitted to national and international conferences.

A lay summary will be produced for volunteers involved via the WMS website. Results will be publicised via the trial website and social media e.g. Twitter. Commercial outputs are not expected from this publically funded trial, but intervention materials will be copyrighted as per institutional practice.

HRA guidance on information for participants at the end of a trial will be followed:
https://www.hra.nhs.uk/about-us/consultations/closed-consultations/guidance-participant-information-end-study-consultation/

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