A pilot, randomised, parallel group, study to assess the safety and debridement efficacy of Aurase Wound Gel (AWG) 24 U/mL compared to Standard of Care in patients with sloughy Venous Leg Ulcers.

Confidentiality Statement

Protocol Number	SCU_VLU_003	
Version Number	5.0	
Amendment Number	#4	
Active Ingredient(s)	Tarumase	
Investigational Product(s)	Aurase Wound Gel (AWG) 24U/mL.	
Trial Phase	Phase IIA	
Trial Acronym:	CLEANVLU2	
Short Title:	Pilot, Parallel Group RCT to assess safety and debridement efficacy of Aurase Wound Gel 24U/mL in VLU.	
Sponsor	SolasCure Ltd Wellington House East Road, Cambridge CB1 1BT	
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SPONSOR AUTHORISATION:

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INVESTIGATOR AGREEMENT

I have read the protocol, appendices, and accessory materials related to Study SC_VLU_003 and agree to the following:

- To conduct this study as described by the protocol and any accessory materials
- To protect the rights, safety, and welfare of the participants under my care
- To provide oversight to all personnel to whom study activities have been delegated
- To control all investigational products provided by the Sponsor and maintain records of the disposition of those products
- To conduct the study in accordance with all applicable local and national regulations, the requirements of the ethics committee of record for my clinical site, and Good Clinical Practices as outlined by ICH E6(R2).
- To obtain approval for the protocol and all written materials provided to participants prior to initiating the study at my site
- To obtain informed consent – and updated consent in the event of new information or amendments – from all participants enrolled at my study site prior to initiating any study-specific procedures or administering investigational products to those participants
- To maintain records of each participant's participation and all data required by the protocol

Name	**Title**	**Institution**
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VERSION CONTROL

Protocol Version	Amendment	Dated	Reason for Issue
1.0	N/A	01 Oct 2024	First Issue
2.0	#1	08 Nov 2024	Request from REC to clarify collection of date of birth (Modified to YYYY only). Request from MHRA to modify trial stopping rules, and to report all Serious Adverse Reactions (SARs)
3.0	#2	15 Jan 2025	Protocol amendment introducing a 12-week follow-up notes review for patients.

4.0	#3	01 Jul 2025	● Amendment to allow extension of treatment through Week 4, permitting up to two additional applications within the existing 4-week treatment period. This change increases the maximum number of treatments from 9 to 11 applications, without extending the overall study duration or the scheduled 4-week follow-up. ● Removal of an assessment of slough recurrence after discontinuation of treatment ● Additional secondary endpoint in respect of proportion of patients achieving a dichotomous (yes / no) of >40% reduction is surface wound area from baseline in 4 weeks. ● Additional secondary endpoint to determine calculated time to closure based on healing rate at week 4. ● Addition of an exploratory endpoint relating to slough thickness ● Reduction in number of patients, based on an amendment to the sample size calculation using mean debridement and partial area healing rather than a dichotomous endpoint of complete debridement. ● Option to continue treating AWG responders through to complete healing or 12 weeks. ● Option to re-enrol non-responders randomised to SOC group into AWG group for 4 weeks of active treatment. ● Change on trial intervention compliance to confirm ± 10% of allocated dose is based on wound size at time of treatment. To the extent the PI subsequently modifies assessment of wound area, then participants will remain compliant. ● If an AWG treated wound is non-responsive to treatment (<10% slough area decrease) after 3 weeks of treatment then extension into 4th week will not occur. ● Confirmation that for practical purposes, the definition of "complete debridement" is any wound containing <10% slough.
5.0	#4	03 Sep 2025	● Removal of the option to continue treating AWG responders through to complete healing or 12 weeks.

			● Clarification of the option to re-enrol non-responders randomised to SOC group into AWG group for 4 weeks of active treatment. ● Aligning the end of trial with LPLV at Week 12 and including a formal interim analysis at Week 4 to evaluate primary and key secondary objectives, with remaining follow-up completed by Week 12.

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1 PROTOCOL SUMMARY

1.1 Protocol Synopsis

1.1.1 Primary and Secondary Objectives and Endpoints

Primary Objective	Primary Endpoint(s)
To assess the systemic and local adverse event profile of the 24U/mL Aurase Wound Gel concentration.	• Frequency and severity of adverse events • Frequency and severity of reference wound related local AEs
Secondary Objectives	**Secondary Endpoints**
To ascertain the debridement efficacy of 24U/mL Aurase Wound Gel compared to standard of care in achieving clinically meaningful wound debridement following 1, 2, 3 and 4 weeks of treatment	• Mean / Median reduction in slough and eschar area of the wound at 1, 2, 3 and 4 weeks compared to baseline slough/eschar area. • Mean/Median reduction in slough and eschar area as a percentage of wound size at 1, 2 ,3 and 4 weeks. • Proportion of patients achieving “complete debridement” after 3, 6, 9 and 11 applications of Aurase Wound Gel 24U/mL compared to standard of care. • Time to achieve “complete debridement”. • Proportion of patients achieving >40% debridement of wound surface area at week 4.
To ascertain the healing potential of 24U/mL Aurase Wound Gel compared to standard of care	• Mean / Median surface area reduction in wounds during weeks 1, 2, 3 and 4 between Aurase Wound Gel and standard care treated wounds • Proportion of patients achieving >40% reduction in wound surface area from baseline at end of week 4. • Mean / Median surface area of granulation tissue (+ healed wound area) as a percentage of baseline wound surface area at 1, 2, 3 and 4 weeks between Aurase Wound Gel and standard care treated wounds. • Linear wound healing rates over 1, 2, 3 and 4 weeks between Aurase Wound Gel and standard care treated wounds • Within-patient linear healing rates over the 2-week run in period vs first 2 weeks of treatment / last 2 weeks of study • Within-patient mean surface area reduction in wounds over the 2-week run in period vs first 2 weeks of treatment / last 2 weeks of study. • Calculated time to complete wound closure from 4-week healing rate.

Primary Objective	Primary Endpoint(s)
To explore the relationship between debridement efficacy and subsequent healing outcomes in wounds treated with 24U/mL Aurase Wound Gel compared to standard of care.	● Proportion of patients achieving complete debridement AND >40% wound surface area reduction at 1, 2, 3 and 4 weeks.
Exploratory Objectives	**Exploratory Endpoints**
Assess the effect of treatment on quality of life	● Changes in the scores of Wound-QOL and Forgotten Wound from baseline to end of study.
Assess the status of the treated wound	● A medical notes review will be conducted at 12 weeks post-treatment.
To assess slough thickness during clinical treatment with Aurase Wound Gel or standard of care	● Clinical assessment of slough thickness over 1, 2, 3 and 4 weeks between Aurase Wound Gel and standard care treated wounds.

1.1.2 Overall Design:

Intervention Model: Randomised, Parallel Design	**Population Type:** Adult patients (including the elderly)
Controls: Autolytic debridement with ActivHeal (hydrogel). All patients to receive good standard of care (advanced moist wound healing dressing + compression)	**Population Diagnosis or Condition:** Venous Leg Ulcer
Blinding: Open	**Treatment arms:** 2
Active Controls: Not Applicable	**Population Age:** ≥ 18 years
Trial Intervention Assignment Method: Randomisation	**Site Distribution:** Single centre trial in UK.

1.1.3 Number of Participants:

- **Group 1**: Standard of Care (ActivHeal + Moist wound healing (secondary dressing) + Compression bandaging): 4 participants
- **Group 2**: Aurase Wound Gel 24U/mL + Moist wound healing (secondary dressing) + Compression bandaging: 16 participants

1.1.4 Study Design

The study consists of 3 Periods:

Screening Period: Screening Period (Visit 1), up to 14 days prior to randomisation during which moist wound healing (secondary dressing) + compression bandaging only will be used at a frequency in accordance with the investigators standard of practice.

Treatment Period: A 4-week treatment period from Day 1 (Baseline, V2) up to Week 4 (Visit 13) in which patients have treatment (Aurase Wound Gel 24U/mL or standard of care autolytic debridement [ActivHeal] re-applied three times weekly (11 applications). Both groups will receive moist wound healing (secondary dressing) + compression bandaging. If during the 4 week treatment period, complete healing is achieved, then Last Observation Carried Forward (LOCF) will be used through to the 4 week efficacy endpoint, and patients will terminate the study prematurely.

Follow-up Period and Medical Record Review: Following completion of the treatment period, participants will complete their involvement in the clinical study and be returned to their standard GP/clinical practice regime and no further visits will be required. At 12 weeks following initiation of treatment, a medical notes review will be performed to understand the current status of the treated wound.

During the study, ongoing assessments of systemic and local safety will be made. If at point in the study ≥15% of patients allocated to Aurase Wound Gel have attributable, “severe” adverse reactions (ADRs) or a single Serious Adverse Reaction (SAR) is reported then a temporary halt will be made to the study whilst a safety review is performed and MHRA will be informed. MHRA & REC approval of the correction plan will be needed before the study could be recommenced. Adverse events will be collected and monitored only until **the end of the follow-up period** and not beyond.

1.1.5 Treatment Arms and Duration:

At baseline, patients will be randomized in a 1:4 ratio to receive standard of care [ActivHeal + moist wound healing (secondary dressing) + compression bandaging] or Aurase Wound Gel 24 U/mL in conjunction with moist wound healing (secondary dressing) + compression bandages for up to 4 weeks.

1.1.6 Key Eligibility Criteria:

Inclusion Criteria:

1) Male or female participants aged 18 years and older at screening who are willing and able to attend and comply with all study visits and study related activities.
2) Provide a signed and dated written informed consent.
3) Presence of ≥ 50% slough or devitalized tissue within the reference ulcer and suitable for debridement therapy.
4) Participants with at least one defined VLU suitable for treatment that is no smaller than 2 cm^2 and no larger than 50 cm^2 and is confirmed as venous in origin by clinical assessments, by Ankle Brachial Pressure Index (ABPI) ≥ 0.8 and/or toe systolic BP pressure > 70mm Hg. Participants with more than one VLU on the target leg can be included, provided other ulcers are at least 1 cm away from the reference ulcer identified for treatment. Selection of the reference ulcer will be at the investigator's discretion, provided it meets all other inclusion/exclusion criteria.
5) Confirmed, clinically diagnosed VLU (ulceration of the lower limb, with no other mechanistic explanation and which has persisted for 6 weeks or more) but which has been present for ≤ 2 years, defined by patient reporting or clinical records.

Exclusion Criteria:

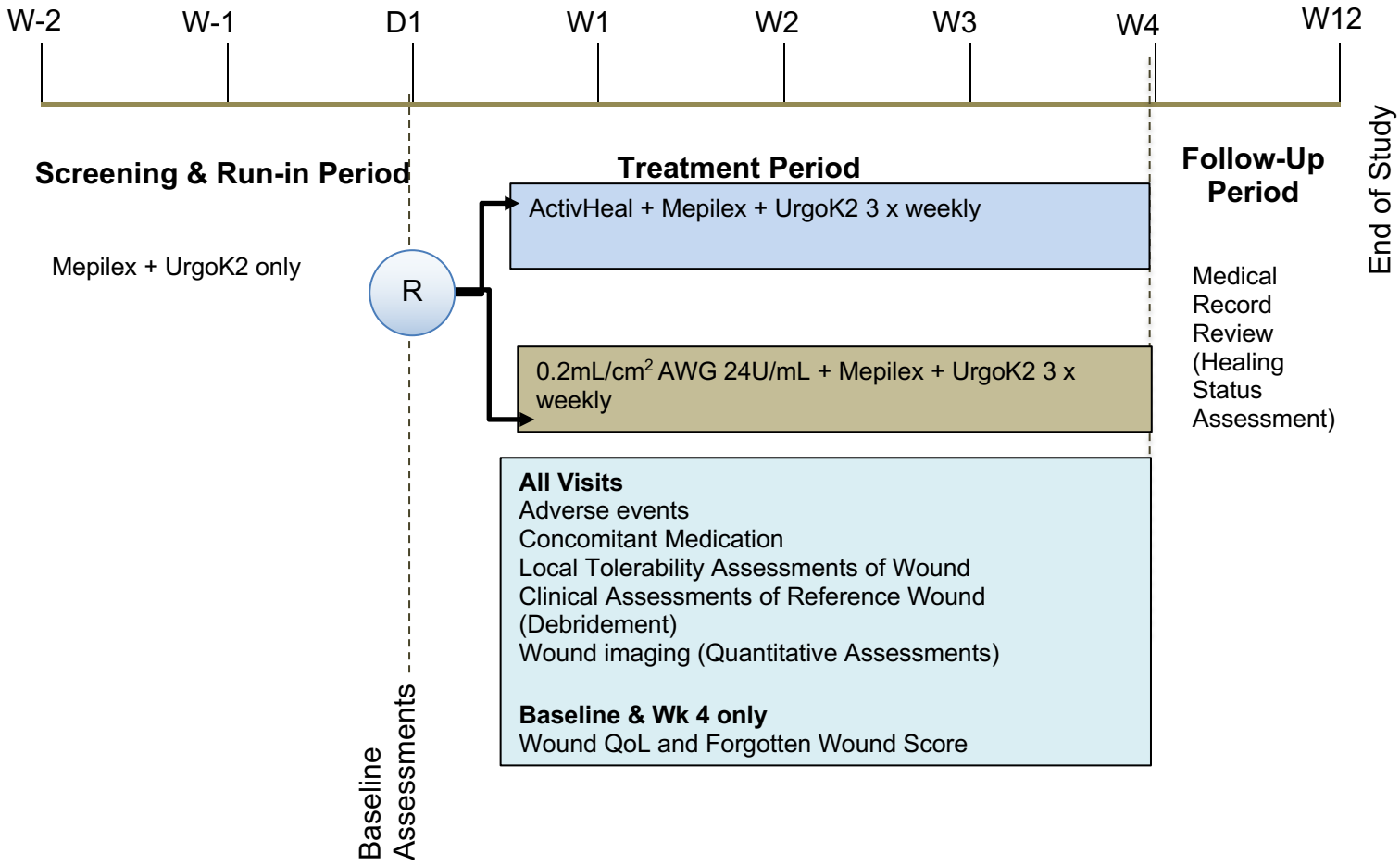
1) Reduction in the wound area of ≥ 40% within the 2-week screening period, when administered standard of care [moist wound healing dressing + compression bandaging] only.
2) Participants who have reported poorly controlled diabetes within 3 months of the screening period
3) Participants with amputation above a trans metatarsal amputation (TMA) in the target leg
4) Reference ulcer has exposed tendons, ligaments, muscle, or bone
5) Reference ulcer [at end of screening] with high levels of exudate, which in the opinion of the investigator, would render the proposed trial management protocol unsuitable.
6) Reference ulcer has active infection at screening determined by the investigator using clinical assessment
7) Active osteomyelitis, cellulitis or gangrene in either leg
8) Participants with current active malignancy [other than basal cell carcinoma] requiring active immune or chemo-therapy treatment.
9) Planned vascular surgery, angioplasty, or thrombolysis procedures within the study period, or 4 weeks before screening
10) Prior skin graft, negative pressure therapy, ultrasound therapy, systemic or cutaneously applied growth factor, other enzymatic debriding agents (e.g. Collagenase, Nexobrid) or live maggot therapy applied to the reference ulcer within 2 weeks before screening.
11) Currently enrolled or has been enrolled in the last 30 days in another investigational device or drug study.
12) Known allergy or hypersensitivity to any component of the investigational product, medication or surgical dressings to be used in the study.

13) Participants lacking capacity to provide informed consent.

14) Any patient which the investigator otherwise considers unsuitable for entry into the study, by reason of acute or chronic mental or physical condition that may interfere with the collection of safety and/or efficacy data.

15) Pregnant or breastfeeding women

1.2 Trial Schema



1.3 Schedule of Activities

Table 1: Schedule of Activities

Study Week	**Screening**	**W1**			**W2**			**W3**			**W4**		**EOT (W4)**	**W12**
	RUN-IN	**TREATMENT**											**FOLLOW-UP**	**MEDICAL RECORD REVIEW**
Visit No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**NA**
Visit Window	**NA**	**0**	**±2**	**±3**	**±2**	**±2**	**±3**	**±2**	**±2**	**±3**	**±2**	**±23**	**±2**	**NA**
Study Days	**-14 to 0**	**1**	**3**	**5**	**8**	**10**	**12**	**15**	**17**	**19**	**22**	**24**	**26**	**NA**
Informed consent	X													
Inclusion /Exclusion	X	X												
Demographics	X													
Medical history	X													
Concomitant	X	X	X	X	X	X	X	X	X	X	X	X	X	

Medications														
Physical examination	X	X											X	
ABPI / Toe systolic pressure	X												X	
Vital Signs (BP, pulse, Temperature)	X	X											X	
Pregnancy test (if applicable)	X													
Standard of Care Compression Bandaging	X	X	X	X	X	X	X	X	X	X	X	X	X	
Randomization		X												
IMP or Hydrogel Dosing		X	X	X	X	X	X	X	X	X	X	X		

Wound Photography	X	X	X	X	X	X	X	X	X	X	X	X	X	
Clinical Assessments	X	X	X	X	X	X	X	X	X	X	X	X	X	
Local Tolerability	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pain NRS	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	
Forgotten Wound Score (QoL)	X	X											X	
Wound-QoL	X	X											X	
Remote Review of Clinical Notes & Medical Records														X

*First dose of treatment administered in Week 1 commences on Day 1 following completion of baseline assessments noted.

2 INTRODUCTION

2.1 Condition Background

Chronic leg ulcers are defined as wounds characterised by either delayed healing (typically more than 6-12 weeks) or by wounds which do not heal at all [1, 2]. Leg ulcers can become chronic for a number of reasons; often they are stalled in the inflammatory stage of the healing process [3] and the presence of elevated levels of matrix metallo-proteases (MMPs) as an underlying cause of wound chronicity has recently been identified [2, 3].

Most leg ulcers arise as a result of chronic venous disease (CVD) which leads to venous insufficiency. Despite ongoing progress in understanding the molecular aspects of CVD the exact mechanism of its development remains unclear. Many different factors may play a role in the pathogenesis of CVD, including changes in hydrostatic pressure, valvular incompetence, increased capillary permeability, endothelial dysfunction, activation of leukocytes, deep venous obstruction, capillary micro-thrombosis, ineffective function of calf muscle pump, biochemical and structural changes in the vessel wall, extracellular matrix alteration, and several other mechanisms [4].

When venous insufficiency occurs, the valves in the veins fail to function correctly and allow blood to pool within the vessel. This eventually leaks out into surrounding tissue which then breaks down to form the ulcer [5, 6]. Local and systemic factors which increase the likelihood of ulceration include poor blood supply, poor venous drainage, age and obesity [5, 7, 8].

Whilst around 70% of leg ulcers result from purely venous insufficiency [9], there can also be an arterial or mixed component to the disease [10] and where this is the case it is important that this is identified so that appropriate treatments can be applied. The two types of disease are differentiated by Doppler analysis of the blood flow in the lower leg [11, 12].

Leg ulcers develop mostly along the medial distal leg and can be very painful. Indeed, one study by Husband *et al* 2001 [13] found that pain was the key symptom of venous ulceration leading patients to seek medical attention. Adding to the complications, these patients are also highly vulnerable to wound necrosis and infections.

Leg ulceration is a common, recurrent condition and is a major cause of prolonged morbidity [14]. Venous Leg Ulcers (VLU) typically affect older people and they are noted to be more common in women than in men. Leg ulcers affect an estimated 1-2% of the global population [14] but this rises to around 5% in the elderly [15]. The estimated prevalence of leg ulcers in the UK population is 1.5 to 3 per 1000 [16]. It is further estimated that 1.5% of adults will experience a leg ulcer at some point in their lives [17].

Compression therapy with dressings and inelastic or elastic bandages remains the gold standard treatment for venous leg ulcers with an ABPI >0.8, and should continue until the ulcers are healed. Gonzalez, 2016 [18] recorded that in a 79-patient study, average healing time for 5 cm ulcers under compression therapy was 14 weeks. Treatment with high compression has also been deployed to reduce recurrence rates [17]. After healing, patients should wear graded compression stockings to prevent ulcer recurrence, however compliance with this is considered to be low. In one study reported by Nelzen *et al* 1997 [19] 44% of patients with healed venous ulcers were noted to have had a recurrence within a period of 54 months. By contrast with venous ulcers, an ABPI value between 0.5-0.8 suggests mixed aetiology disease and typically requires referral to a vascular clinic for further assessment. Compression bandages are largely

avoided under these circumstances, although reduced compression has been used under strict supervision if the ulcer is clinically of venous origin.

In such cases, clinical progress should be checked daily and compression modified accordingly. An ABPI less than 0.5 suggests an arterial ulcer and compression treatment under these circumstances is contra-indicated. In such cases, referral should be made to a vascular clinic for further assessment and possible re-vascularisation.

In common with other chronic wound types, excess wound exudate, necrotic/sloughy wounds, wound infection and wound odour are common in leg ulcers.

2.2 Wound Bed Preparation & TIME

Wound bed preparation and the TIME framework are now well-established concepts within wound care and provide for a standardised approach to managing chronic wounds. This framework involves:

- T – Tissue non-viable or deficient – The wound should be appropriately debrided to restore the viability of the wound base.
- I – Infection or Inflammation – The focus for infection / inflammation should be removed using topical antimicrobials and/or anti-inflammatories to restore bacterial balance and reduce inflammation
- M – Moisture balance – Application of moisture balancing dressings which aid epithelial migration and avoid desiccation of the wound. These dressings also help control excessive fluid and help avoid maceration.
- E – Edge of Wound – non-advancing or undermining. The patient should be continually re-assessed with corrective therapies including further debridement, skin grafts, biological agents and adjunctive therapies.

Within all elements of this framework, wound debridement is a key clinical action.

2.2.1 Wound Debridement

The presence of slough or devitalised tissue in a wound is now well-established to be both a focus for bacteria and a barrier to healing. Debridement occurs naturally within wounds and studies indicate that if this process is accelerated then healing should also be more rapid [20]. Falanga 2001 [21] stresses the importance of both initial and maintenance debridement in the management of chronic wounds.

Repeated debridement of chronic wounds during the healing process has been shown to be clinically beneficial. In one retrospective cohort study reported by Wilcox et al 2013, [22] involving 525 wound care centres (representing 154,644 patients with 312,744 wounds of all causes), the authors report that in regard to time to heal, a significantly higher proportion of wounds that received weekly or more frequent surgical debridement healed in a shorter period of time ($P < 0.001$). Similarly, Cardinal et al 2009, [23] performed a retrospective analysis of two controlled prospective randomised trials of topical wound treatments (one in VLU and one in DFU) and noted that centres where patients were debrided more frequently were also associated with higher rates of wound closure in both clinical studies ($p=0.007$ VLU, $p=0.015$ DFU).

The rationale for repeated debridement is that the pathological and underlying vascular processes causing the original wound frequently continue after initial debridement in the underlying tissues and can create a reservoir for the production of further exudate and necrotic

tissue. These arrest healing and result in further necrosis. Healing does not resume until the 'new' eschar or slough is then removed.

The consequences of not debriding a wound are varied but have been defined by Baharestani 1999 [24]; they include:

- Increased risk of infection;
- Imposition of additional metabolic load;
- Psychological stress;
- Ongoing inflammation;
- Compromised restoration of skin function;
- Abscess formation;
- Odour;
- Inability to fully assess the wound depth;
- Nutritional loss through exudate;
- Sub-optimal clinical and cosmetic outcome;
- Delayed healing

Once it has been established that a wound will benefit from debridement, it is necessary to:

1. Define an aim. This may be to prepare a wound for autologous skin grafting; to receive a skin substitute (e.g., Apligraf, Dermagraft etc); or to proceed with conventional moist wound dressings.

2. Define a timescale – i.e. over what time period should debridement be complete. Typically, a balance has to be made between the risk to surrounding viable tissues from rapid debridement techniques, such as surgical or sharp debridement, and the danger of prolonged exposure to necrotic tissue and its associated bacterial load.

3. Define a method. The choice for the debridement technique is usually determined by the speed of debridement considered appropriate for a particular wound type in combination with practical aspects of debridement e.g. hospital debridement by a surgeon vs community based debridement routinely undertaken by nurses. Other factors which will influence the decision will include wound characteristics, including infection, pain, exudate, involved tissues; the patient's attitude; available skills and resources. Options for wound debridement currently include:

    - **Surgical sharp and conservative sharp debridement**. Typically performed by a skilled practitioner such as a surgeon, this technique is rapid (minutes), selective and highly effective. Debridement tools include a scalpel, curette, scissors, rongeur or forceps, along with more modern devices such as Microwater jets (Versajet®), lasers and/or ultrasonic disruption to remove necrotic tissue from the wound. Although surgical debridement is considered "gold standard", the patient typically requires local or general anaesthesia, debridement must be performed in a suitable surgical environment (e.g. theatre setting) and requires the availability of a skilled surgical practitioner. Thus, whilst more acceptable for hospital-based procedures (e.g. burns wounds) surgical debridement is uncommon in the community setting within the context of VLUs.
    - **Autolytic debridement**. This type of debridement is most commonly used within the UK and comprises standard of care within chronic wound setting, including VLUs.

Autolytic debridement relies on products such as hydrogels and/or advanced wound dressings to modify the healing environment of the wound in favour of "moist wound healing", which facilitates the body's own autolytic processes to break down and remove necrotic tissue and eschar. This type of debridement is generally pain free and can be readily used in the community by non-specialist nurses, however initial wound debridement can be very slow (typically ~4-8 weeks).

- **Mechanical debridement**. This type of debridement utilises irrigation, hydrotherapy, wet-to-dry dressings and/or abrasion techniques (e.g. Debrisoft®) to remove slough or eschar material from the wound. Mechanical techniques are not as rapid or selective as surgical debridement, and remain painful to the patient, but some techniques can be used in the community. Due largely to pain (and the lack of adequate local anaesthesia) mechanical debridement has largely fallen out of favour in many major territories but remains popular in some territories, e.g. United States.
- **Biological debridement.** This debridement technique utilises sterile (live) maggots of *Lucilia sericata* (green bottle fly), which are introduced into the wound either as "free range" maggots or as contained "bags" of maggots (e.g. Biomonde®). Maggots debride through two principal techniques (i) use of their mouth hooks to chew up material and (ii) secretion of proteolytic enzymes that chemically liquify necrotic tissue. By virtue of contained maggots in "bags", Biomonde is limited to essentially proteolytic activity, and thus resembles enzymatic debridement below. Whereas maggot therapy has been shown to be effective and reasonably rapid, the use of the technique is highly limited due to (i) patient acceptability of the use of live maggots and (ii) practical aspects of distributing live maggots.
- **Enzymatic debridement.** This debridement technique involves the application of a prescribed topical agent, which can be readily transported and used either in a hospital or community setting. The enzymes are proteolytic agents that utilise the necrotic tissue or eschar as a substrate, with the aim of chemically liquifying the necrotic material. Two agents are approved in Europe (Nexobrid ® [bromelain] and Iruxol® [collagenase]; importantly neither product is approved for use in the UK:

  Nexobrid® (Bromelain), is a non-specific protease. Although this achieves rapid debridement (hours), the application has been noted to be very painful, non-selective (also acting on viable tissue) and this limits its use to initial wound debridement, typically in hospital environments where the enzyme can be removed after several hours. The approved SmPC limits the use of Nexobrid to a single occasion. Collagenase (Iruxol®) is used in a paraffin (Vaseline) base and although shown to be somewhat effective and selective and less painful than Nexobrid, is constrained in that this enzyme only acts on collagen. As a consequence, wound debridement can still take 4 weeks.

It is therefore considered that a more effective enzymatic debridement agent, that can be used for both hospital and community settings, without specialist surgical training, and which achieves rapid and consistent wound debridement (≤ 3 weeks), and without pain on application remains an unmet medical need in the wound debridement space.

2.3 Product Background

Aurase Wound Gel is intended to be used as part of a new generation of wound bed preparation products; specifically intended for both initial and maintenance debridement of wounds

covered with fibrinous slough or eschar. Wounds showing these characteristics are often found among participants with chronic wounds such as leg ulcers, diabetic foot ulcers, pressure ulcers and also in some participants with sub-acute surgical wounds that have dehisced or become infected.

The investigational product is currently a two component system comprising a proprietary hydrogel for use as a diluent [Component A] and an active solution containing the tarumase enzyme [Component B], which requires reconstitution prior to clinical application.

2.3.1 Component A

Component A is a sterile hydrogel, packaged into a pre-filled 11mL syringe (9mL fill). Component A has been designed to be rheologically comparable to other well-established (and commercially available) autolytic debriding agents. These products are typically marketed as Class IIb medical devices in Europe and Class I (510K exempt) devices in the US and are based on well-established hydrogel technologies that are intended to modify the wound environment.

Notably, the hydrogel polymer (Carpobol 974P) used in Component A effectively acts as an amorphous hydrogel dressing, by virtue of its high-water content. Despite the high levels of water, the gel is actually made up of a three-dimensional network in association with the hydrophilic polymer and thus does not dissolve in the wound entirely; rather the gel donates moisture to the wound bed. This rehydrates wound tissue and holds moisture at the wound surface thereby providing for a moist wound healing environment [3]. This in turn facilitates the body's own processes (including phagocytic action, enzymes and moisture) to break down tough fibrinous eschar and slough.

In line with other autolytic hydrogels, it is anticipated that Component A, will debride sloughy wounds over a 4-6 week treatment period and in this context does not act as a true placebo; instead this represents the vehicle for the active treatment and is essentially consistent with standard of care across many geographical regions for autolytic debridement.

For the purposes of this clinical trial Component A is considered a formulated "diluent" and is therefore an excipient in the final reconstituted Aurase Wound Gel.

2.3.2 Component B

Component B nominally contains 150µg/mL (60U/mL) of tarumase (INN) as the active ingredient; it is a purified (recombinant) fibrinolytic enzyme, developed from maggots of the green bottle fly *Lucilia sericata*, which are themselves often used for the purposes of wound debridement. Tarumase is a trypsin-type serine protease and was selected as an enzymatic debrider for its ability to selectively digest fibrin related materials, a constituent of slough and eschar [3] and which effectively anchors degraded collagen and/or elastin to the wound bed. The enzyme also has some activity directly against collagen and elastin (also major components of wound slough and eschar). Indeed, tarumase is the principal protease component of maggot exudate that is largely responsible for the fibrinolytic effect observed during live (bagged) maggot debridement.

Component B is a sterile solution of tarumase in water, glycerol, poloxamer 188 and citrate buffer made up of citric acid, disodium hydrogen phosphate and sodium chloride, packaged into 2mL blow-fill seal ampoules. The low viscosity nature of the Component B solution facilitates its ready dilution with Component A prior to use and provides for enhanced stability to the tarumase enzyme during long term storage.

2.3.3 Reconstitution / Dilution

Reconstitution to the final concentrations of Aurase Wound Gel (24U/mL) is performed in all cases using 1 syringe [9mL] of Component A [common diluent for all strengths] and three vials of Component B [6mL] for each participant as supplied, using the Osartis Manumix mixing system, according to the procedures set out in the pharmacy manual.

The reconstituted gel is taken up into a sterile (single-use) syringe for administration to the patient. The final diluted gel will be applied (cutaneously) to a fibrinous or sloughy wound at a volume of **0.2mL per cm^2** under specified, standard of care dressings (see section 6.1). 15mL of gel (i.e. volume made from one set of components) is therefore sufficient to treat all sizes of wound in the clinical study. Each ampoule of Component B and syringe of Component A is designed to be used as a single use product; therefore, any remaining Aurase Wound Gel, after application on a single occasion would be discarded as per local regulations.

The final reconstituted gel is designed to remain in contact with the patient's wound for 48-72 hours, facilitating debridement of necrotic and sloughy fibrinous tissue from the wound bed and providing an optimal moist wound environment to encourage rapid healing. Repeated applications of Aurase Wound Gel throughout the debridement and healing process can be administered after removal of any residual gel along with degraded eschar / slough at routine dressing change, by cleansing the wound with sterile saline solution, followed by gently drying the wound with gauze to remove any loose slough.

It is envisaged that the application of the gel to the reference ulcer can be performed by both clinicians or nurses, facilitating use in both the hospital and/or community settings, where appropriate.

2.4 Prior Non-Clinical Experience

Extensive non-clinical toxicology studies have been performed in accordance with ICH M3(R2) and ICH S6(R1) using tarumase drug substance. Further details are provided in the Investigator Brochure In summary:

2.4.1 Pharmacokinetics

A 14-day repeat dose toxicology study was performed with tarumase formulated as Aurase Wound Gel in mini-pigs with full thickness dermal wounds. In this study, 1.2mL of Aurase Wound Gel (equivalent to 0.19mL/cm^2) at concentrations of 105 and 150 µg/g day was applied daily to each of seven full thickness 2.5cm x 2.5cm wound sites in 8m and 8f Gottingen mini pigs. Serial bloods were then taken from the animals on Day 1 of the study (pre-dose [baseline] and at 5mins 15mins, 30 mins and 1h after dosing and again at Day 12 (pre-dose, 5min, 15min, 30min and 1h after dosing). Samples were analysed using a validated LC-MS method; the reported analysis indicates that the tarumase concentration was below the level of quantitation (BLQ), 10ng/mL, in all samples tested.

A 28-day repeat dose toxicology study was also performed with tarumase formulated as Aurase Wound Gel in minipigs on partial thickness wounds. In this study, 28 ± 1 mL of Aurase Wound Gel at concentrations of 3.43 µg/cm^2 and 45.4 µg/cm^2 (54U/mL) tarumase were administered daily to the 150cm^2 wound (equivalent to 514.5 µg/day and 6.81 mg/day) to each of 3m and 3f Gottingen mini-pigs, to bridge and further exceed the maximal application rate to humans, when calculating for equivalent body surface areas. Serial bloods were taken from the animals on Day 1 of the study (pre-dose [baseline], and at 5mins, 15mins, 30 mins and 6 hrs, and again at Day 28 (predose,5 min, 15min, 30 min and 6 hrs after dosing). Samples were analysed using

a validated LC-MS-MS method; the reported analysis indicates that the tarumase concentration at all time points was below the level of quantitation (BLQ), 30ng/mL, in all animals.

The lack of tarumase in the systemic circulation has been attributed to the presence of endogenous protease inhibitors (notably Alpha-1-Antitrypsin [A1AT]) that are present at significantly higher concentrations compared to administered tarumase and rapidly deactivate the tarumase drug substance within 1-2 min.

2.4.2 Toxicology

The toxicology of tarumase has been investigated over a series of studies. These include:

- Non-GLP, single dose maximisation study in mini-pigs, which assessed the toxicity and local tolerability of tarumase formulated as Aurase Wound Gel 150µg/g when administered on a single occasion by topical application to a full-thickness dermal wound with observation period of 2 weeks.
- GLP compliant, repeat dose toxicity study in mini pigs, which assessed the toxicity and local tolerability of tarumase formulated as Aurase Wound Gel 150µg/g when administered daily for 14 days by topical application to seven full thickness 2.5cm x 2.5cm full thickness dermal wound sites (total area 50cm^2) in 8m and 8f Gottingen mini pigs with further observation period of 14 days.
- GLP compliant, repeat dose toxicity study in mini-pigs, which assessed the toxicity and local tolerability of tarumase formulated as Aurase Wound Gel 3.43 µg/cm^2 and 45.4 µg/cm^2 [equivalent to 54U/mL] when administered daily for 28 days by topical application to 150cm^2 partial thickness dermal wound sites in 3m and 3f Gottingen mini-pigs with further observation period of 14 days.
- Non-GLP, dermal administration study (intact skin) in Sprague Dawley rats, to evaluate the toxicity and local tolerability of tarumase formulated as Aurase Wound Gel (150µg/kg/day) when administered by topical application to intact skin on a single occasion followed by a 7-day observation period.
- GLP compliant, 28-day repeat dose, dermal administration (intact skin) in Sprague Dawley rats, to evaluate the toxicity and local tolerability of tarumase formulated as Aurase Wound Gel (150µg/kg/day) when administered by topical application to intact skin.
- Non-GLP single dose toxicity study in Sprague Dawley rats (150µg/kg) to evaluate the systemic toxic potential of tarumase (formulated as a solution) following a single IV administration study, followed by a 7-day observation period.
- GLP compliant, 7-day repeat dose toxicity study in Sprague Dawley rats (up to 150µg/kg) to evaluate the systemic toxic potential of tarumase (formulated as a solution) following IV administration.

The results of these studies are outlined more fully in the Investigator Brochure, however in summary, no clinically relevant toxicological findings (local or systemic) were noted in any of the studies up to and including 45 µg/cm^2 (equivalent to 54U/mL) administered over 150cm^2 (i.e. maximum daily exposure of 6.75mg).

2.5 Prior Clinical Experience

The manner of reporting concentrations for Aurase Wound Gel has changed between the initiation of the FIH clinical trial and this subsequent trial. In the FIH, patients were exposed

to increasing concentrations of tarumase reported as µg/mL (and µg/cm^2) by reference to an LC/MS assay, which has subsequently been found to underreport the content of tarumase compared to a theoretical concentration based on amino acid analysis and subsequently HPLC. In this clinical trial we therefore propose to change the nominal concentrations to reflect the actual HPLC content of tarumase, but also to reflect the biological activity of the product reported in U/mL. A comparison of the concentrations used in the FIH trial and this proposed clinical trial are therefore provided below in Table 2.

Table 2: Comparison of tarumase content and activity used in clinical trials

Description	Volume applied	Concentration (µg/mL)		Concentration (µg/cm^2)		Biological Activity	
		(LC/MS)	(HPLC)	(LC/MS)	(HPLC)	U/mL	U/cm^2
FIH Clinical Trial (SC_VLU_001)							
Cohort 1	0.4mL/cm^2	0	0	0	0	0	0
Cohort 2	0.4mL/cm^2	2.7	3.0	1.08	1.2	1.2	0.5
Cohort 3	0.4mL/cm^2	4.9	5.4	1.94	2.16	2.16	0.9
Cohort 4	0.4mL/cm^2	13.5	15.0	5.4	6.0	6	2.4
Cohort 5	0.4mL/cm^2	24.3	27.3	9.72	10.92	10.9	4.4
This Clinical Trial (SC_VLU_003)							
AWG 24U/mL	0.2mL/cm^2	N/A	60	N/A	12	24	4.8

Note: during the FIH clinical trial the volume of gel administered to the wound at each application was **twice** that anticipated in this clinical study (i.e. 0.4mL/cm^2 vs 0.2mL/cm^2 respectively). Thus although the local concentration of tarumase is intended to be higher in this new clinical study (24U/mL), the daily and total exposure of participants to tarumase will remain largely comparable to that previously studied.

By way of illustration; at the highest concentration administered in the Phase IIA trial (10.92µg/cm^2) and based on the largest wound size enrolled (45.2cm^2), the daily exposure was up to 493.6µg of tarumase per application or 493.6µg x 12 applications = 5.923 mg over the 4-week study duration. By contrast, in this clinical study the exposure at the 24U/mL concentration for a comparable wound size would be 542µg of tarumase per application but only 542 µg x 9 applications = 4.881 mg over the 4 week study duration.

Mean wound size in the initial Phase IIA clinical trial was however much smaller at 16.8 ± 13.7cm^2. Assuming largely comparable wound sizes are enrolled, it is anticipated that participants will receive on average 201.6 µg of tarumase per application and only 201.6 µg x 9 applications = 1.81 mg over the 4 week study duration.

With the above in mind, the results of the initial Phase IIA clinical study are considered highly relevant to the likely safety in this proposed clinical study. In summary, these data indicated:

2.5.1 Safety Results

There was no treatment emergent safety signals arising from escalating the concentration of Aurase Wound Gel from Cohort 1 (vehicle) through to Cohort 5 (10.92µg/cm^2 [10.9U/mL]) dosed at 0.4mL/cm^2. Notably:

- There were no treatment emergent related AEs occurring distant from the site of application, indicative of systemic side effects. All four SAEs reported in the trial (occurring in two patients) were considered unrelated to IMP treatment.
- There were no clinically significant changes noted in systemic blood clotting factors, blood haematology, clinical chemistry, physical checks, and vital signs following treatment with topical Aurase Wound Gel at any concentration.
- Local wound sensations, pain and itch were closely monitored throughout the clinical study and recorded both before application of the IMP, shortly after application (within 15 mins) and between clinical visits (every 2-3 days) using patient reported 11-point Numerical Rating Scales (NRS), where 0 = no pain or itch and 10=worst pain or itch. Although pain and itch were noted to vary significantly between different participants, in all cases the application of tarumase gels at any concentration was not associated with an increase in reported wound pain or itch. Indeed, mean pain and itch scores were noted to reduce over the course of the clinical study, likely correlating with improved clinical outcomes.
- Local wound observations including erythema, oedema, induration, exudate, bleeding and wound infection were also closely monitored. Whilst variations were again observed between different wounds in different participants and at different visits, there were no correlations suggesting an increase in severity of the parameters measured, in response to increased concentrations of tarumase.
- 17/43 (39.5%) of participants receiving treatment with vehicle or Aurase Wound Gel experienced Treatment Emergent AEs (TEAEs) local to the site of application and within these TEAEs 8/22 (36%) were considered by the investigator to be related to the IMP; these included wound maceration, wound erythema, wound inflammation and wound infection. These AEs are commonly observed in patients with VLUs, irrespective of treatment. As with local wound observations, local ADRs were not correlated with increased tarumase concentration, but appeared sporadically throughout the cohorts and assessment timepoints.
- Dose limiting toxicity was not observed in any of the five cohorts. Safety conclusions from this FIH Phase IIA clinical study would therefore support the ability to further escalate the local concentration beyond 10.92µg/cm^2, especially where daily exposure remains largely consistent.

2.5.2 Pharmacokinetic Results

Within the Phase IIA clinical trial, topical exposure to tarumase in VLU wounds, at any concentration, resulted at all time points tested (5, 10, 15, 30 and 60 mins post-application) in values below the limit of quantitation of the assay (i.e. <30ng/mL). Given that the concentrations of Alpha-1-Antitrypsin (A1AT) in human blood have been previously determined to be in the range of 100 – 273 mg/dL [23], and this represents a significant molar excess when compared to the total exposure to tarumase on any single dosing day, it is not surprising that the human PK results obtained within the clinical trial are wholly in line with the reported non-clinical data.

2.5.3 Efficacy Results

Although, the FIH clinical study was first and foremost a (small) dose escalation study, using non-randomised, non-stratified patients and wounds, this trial did provide proof of concept that Aurase Wound Gel was able to debride wounds and support wound healing over a four week period. Notably:

Wound Debridement:

Analyses from the study, whether using both total slough content reduction or percentage of slough reduction (as a proportion of baseline wound size) and irrespective of whether this assessment was performed by an unblinded investigator (based on an on-patient assessment) or a fully blinded independent reader, performing an analysis of standardised 2-D photography, a trend towards both quicker and numerically more complete debridement was observed as the concentration of Aurase Wound Gel was increased throughout the study. This was particularly noted in the results of cohort 5 patients (administered 10.92µg/cm^2 [10.9U/mL]) compared to vehicle and low dose Aurase Wound Gel (administered 1.2µg/cm^2 [1.2U/mL]) when per protocol populations were employed (i.e. no critical deviations from treatment schedule) were evaluated.

Most notably, an ad-hoc analysis of wound debridement of the most sloughy wounds (i.e. wounds that contained more than 50% slough at baseline) and consistent with the patient population planned for this clinical study, a much clearer dose dependent effect with a mean 73% debridement was achieved at 10.9U/mL concentration, and a significant proportion of this effect (approximately 45%) occurred within the first two weeks (i.e. 6 applications).

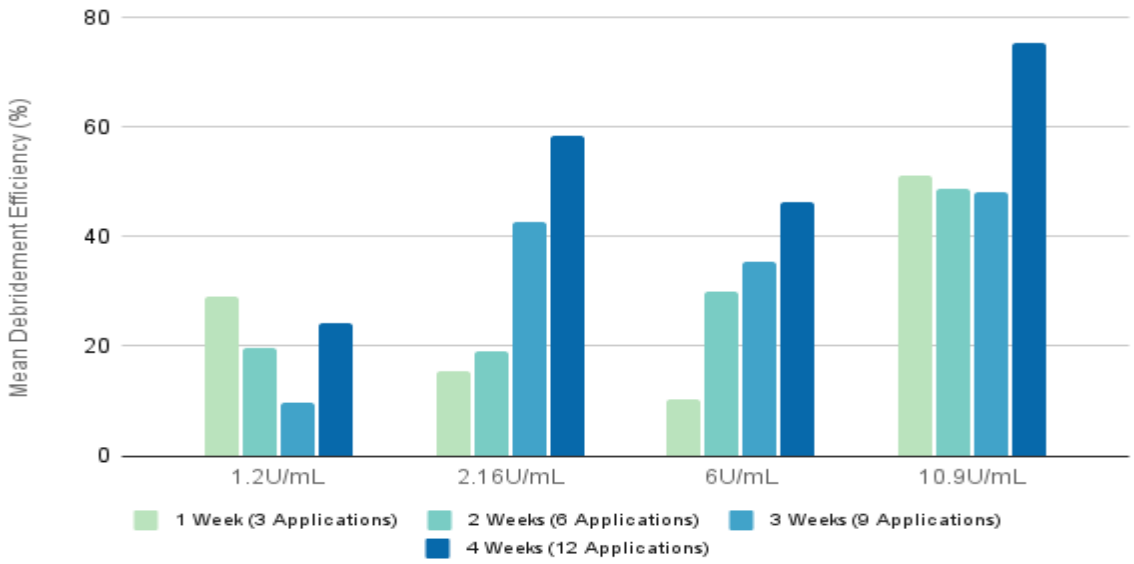


Figure 1: Mean debridement efficiency of Aurase Wound Gel 1.2-10.9/mL over 4 weeks as determined in study SC_VLU_001 in patients with ≥50% slough in the wound bed at baseline.

The target product profile for Aurase Wound Gel envisages that complete debridement can be achieved within a period of 2-4 weeks (6-11 applications), and within this context it is considered that debridement efficacy of the product (and rate of debridement) can be further enhanced by a further increment in the local tarumase concentration. The safety profile reported

in the FIH study, supports the potential to use such higher clinical concentrations, particularly where there is limited systemic absorption and where daily (and total) exposures remain largely comparable with those already tested.

Wound Healing Trajectory:

Consideration of the wound healing trajectory is critical both from a safety perspective, but additionally with the knowledge that regulators have indicated wound healing to be a primary endpoint for later stage (Phase III) clinical trials.

In this context, rate of wound healing in the Phase IIA study was assessed using a variety of techniques, including an assessment of the percentage of granulation tissue relative to baseline, an assessment of the reduction in wound surface area (cm^2) and finally in regard to linear wound healing trajectory using Gilman's equation that accounts for the perimeter (and thus size) of the wound at baseline.

Irrespective of whether the assessment of partial wound healing was performed by the investigator and/or blinded independent reader, general trends were observed indicating a solid wound healing trajectory across all cohorts, indicative that Aurase Wound Gel (and/or vehicle) are not having any deleterious effect on wound healing. Indeed, a trend towards an increased rate of wound healing was observed at the highest concentrations of Aurase Wound Gel tested in the FIH study (i.e. at 6 $\mu g/cm^2$ [6 U/mL] and 10.92 $\mu g/cm^2$ [10.9 U/mL]). As with debridement, this is particularly noted in sub-populations such as the per protocol population where compliance with protocol dosing regimen was assured, or in populations that had very low levels of granulation tissue present at baseline (i.e. ≤. 50%).

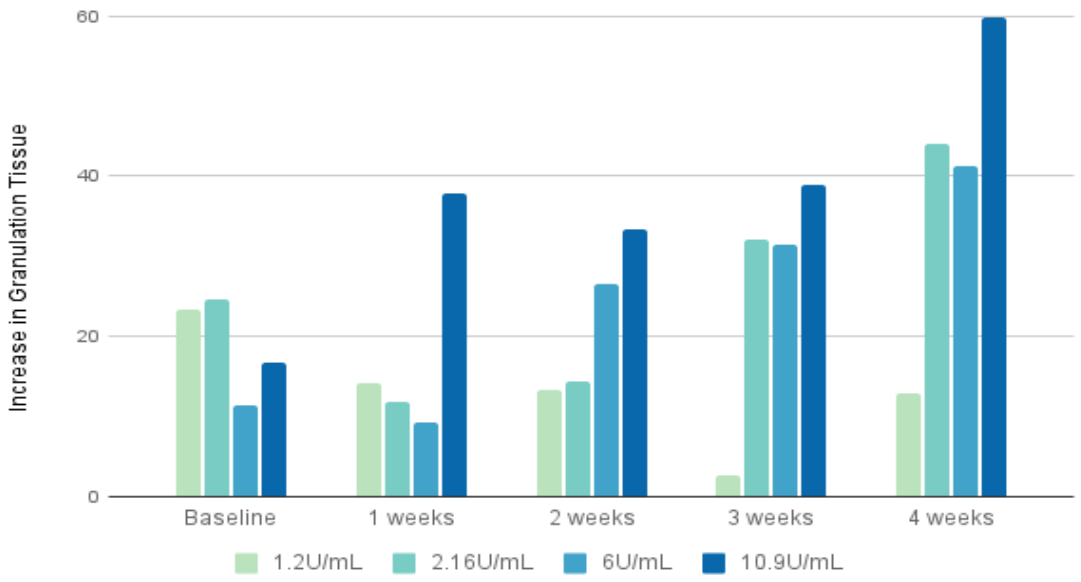


Figure 2: Granulation tissue as a percentage of wound size at baseline and changes at Weeks 1, 2, 3 and 4 in patients with ≤50% granulation tissue at baseline (SC_VLU_001)

Within this context there is an anecdotal link between the rate of wound debridement and the rate of wound healing, bearing in mind that wound slough and eschar have been established as a key barrier to healing. In this sense, it stands that more rapid removal of slough should allow the wound to move from a chronic inflammatory phase to more of an acute wound healing (re-epithelialisation) phase to complete healing in a more rapid period of time.

2.6 Purpose of Trial

This clinical trial seeks to build on the results of the initial Phase IIA FIH clinical trial. Specifically, this clinical trial seeks to assess the local and systemic safety profile of a higher concentration of Aurase Wound Gel (24 U/mL) (primary endpoint) and to explore its efficacy in debridement and wound healing (secondary endpoints) in a relevant VLU patient population. Given the systemic safety profile already observed, combined with the lack of systemic availability (due to the high endogenous A1AT levels) at these daily exposure levels already administered, no further pharmacokinetic analysis is proposed as part of this trial. Systemic safety will however continue to be monitored by reference to the AE profile.

Notably, the first clinical study (by virtue of it being a First-In-Human clinical trial, had significant limitations in its study design, including a lack of randomization (see section 4.3) and/or standard of care control. By contrast this study, seeks to employ a more rigorous randomised, parallel design and using a consistent standard of care, to more definitively assess the safety and treatment effect size of a higher local concentration of Aurase Wound Gel (24U/mL) in adult VLU patients. Participants will therefore be randomised 1:4 to receive either autolytic debridement with a commercially available autolytic debridement hydrogel [ActivHeal®] (+ standard of care) or Aurase Wound Gel 24U/mL (+ standard of care) applied three times weekly (9 applications) over a four-week treatment period. A smaller control group is rationalised on the basis that there is a lot of evidence [including Cochrane reviews] to determine the likely effect size of the control. Moreover, in this study we seek to employ a 2-week run in period to treatment (using standard of care only), so that patients who are highly likely to heal quickly [simply by adopting good standard of compression] are excluded from the patient population. Finally, we seek to explore the utility of running this clinical trial through a community GP clinic setting or home visits, consistent with the treatment pathway for the majority of patients in the UK.

2.7 Summary of Benefits and Risks

As noted in the introduction, the presence of slough and eschar in the wound bed is well-established in the scientific literature to be a barrier to healing, especially in chronic venous leg ulcers. Removal of this material from the wound is therefore a pre-requisite for healing, and/or for treatment with other advanced wound healing products (e.g. matrices, grafts etc). In this context we are seeking to investigate the potential of the investigational treatment to achieve rapid (2-4 week) complete debridement, in a population of patients that have significant amounts of slough and/or devitalised tissue (>50%) in the wound bed, and that otherwise would require either several weeks of autolytic debridement and/or (where possible) referral to surgical clinics for surgical debridement. In this context, it is well established that surgical debridement is a highly painful procedure, requiring anaesthesia. Rapid debridement, that can be readily achieved through local GP clinics, is therefore a significant clinical benefit not only for healthcare systems, but beneficial to the patient, as this can promote more rapid wound healing, in a pain-free manner.

Based on non-clinical data and data from the FIH Phase IIA clinical data, there are minimal risks associated with topical treatment of Aurase Wound Gel. Notably, few adverse events at sites distant from the site of application (i.e. systemic side effects) are expected; this is due to the rapid and complete deactivation of tarumase by A1AT (an endogenous protease inhibitor) which is present in significantly molar excess in the blood stream compared to the levels of tarumase administered, leading to negligible systemic exposure.

Local adverse events do however remain a risk from increased localised concentrations of the enzyme, and these include increased risks of erythema or oedema, increased wound exudate, localised infection and/or further enlargement of the wound. These however are also events that are commonly associated with the underlying disease state (i.e. VLUs associated with patients with peripheral vascular damage) and are therefore to be expected within the patient population; the rates of these events will therefore be carefully assessed throughout the clinical study, by direct comparison with the standard of care (ActivHeal) control group.

3 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS

3.1 Primary / Secondary / Exploratory Objective + Associated Endpoint

Primary Objective	Primary Endpoint(s)
To assess the systemic and local adverse event profile of the 24U/mL Aurase Wound Gel concentration.	● Frequency and severity of adverse events ● Frequency and severity of reference wound related local AEs
Secondary Objectives	**Secondary Endpoints**
To ascertain the debridement efficacy of 24U/mL AWG compared to standard of care in achieving clinically meaningful wound debridement following 1, 2, 3 and 4 weeks of treatment	● Mean / Median reduction in slough and eschar area of the wound at 1, 2, 3 and 4 weeks compared to baseline slough / eschar area ● Mean / Median reduction in slough and eschar area as a percentage of wound size at 1, 2, 3 and 4 weeks. ● Proportion of patients achieving “complete debridement” after 3, 6, 9 and 11 applications of AWG 24 U/mL compared to standard of care. ● Time to achieve “complete debridement”. ● Proportion of patients achieving >40% debridement of wound surface area at week 4.

To ascertain the healing potential of 24U/mL AWG compared to standard of care	● Mean / Median surface area reduction in wounds during weeks 1, 2, 3 and 4 between AWG and standard care treated wounds. ● Proportion of patients achieving >40% reduction in wound surface area from baseline at end of week 4. ● Mean / Median surface area of granulation tissue (+ healed wound area) as a percentage of wound surface area at 1, 2, 3 and 4 weeks between AWG and standard care treated wounds. ● Linear wound healing rates over 1, 2, 3 and 4 weeks between AWG and standard care treated wounds. ● Within-patient linear healing rates over the 2-week run in period vs first 2 weeks of treatment and last 2 weeks of the study ● Within-patient mean surface area reduction in wounds over the 2-week run in period vs 2 weeks of treatment and last 2 weeks of the study. ● Calculated time to complete wound closure from 4-week healing rate.
To explore the relationship between debridement efficacy and subsequent healing outcomes in wounds treated with 24U/mL Aurase Wound Gel compared to standard of care.	● Proportion of patients achieving complete debridement AND >40% wound surface area reduction at 1, 2, 3 and 4 weeks.
Exploratory Objectives	**Exploratory Endpoints**
Assess the effect of treatment on quality of life	● Changes in the scores of Wound-QoL and Forgotten Wound from baseline at 3 weeks of treatment.
Assess the status of the treated wound	● A medical notes review will be conducted at 12 weeks post-treatment.
To assess slough thickness during clinical treatment with Aurase Wound Gel or standard of care	● Clinical assessment of slough thickness over 1, 2, 3 and 4 weeks between Aurase Wound Gel and standard care treated wounds.

As this is the first clinical trial utilising a more robust randomised, parallel group design, a VLU patient population with >50% slough and/or devitalised tissue, and utilising a run-in period (to exclude patients who are likely to heal solely in response to standard of care) no conclusive estimands for the primary endpoint i.e. achieving complete debridement have been made. Estimands will be included in further Phase II clinical trials on the basis of data from this clinical study.

Notwithstanding the above, this trial has been provisionally powered based on a revised Target Product Profile for both debridement and healing that SolasCure is seeking to achieve; which in turn is based on clinical feedback from Key Opinion Leaders (KOLs) working in the wound care sector. Using an assumption of >60% mean debridement (±25%) and > 60% mean partial area reduction (±25%) within 4 weeks and a mean of 20% debridement and partial area reduction for standard of care, a revised (reduced) sample size of 20 patients is calculated to achieve 80% power with an overall alpha of 5%.

4 TRIAL DESIGN

4.1 Description of Trial Design

This study is initially designed as a prospective, Phase IIA, randomised, parallel group, single centre (UK only) clinical study. The study will be conducted in accordance with the requirements of International Council for Harmonisation Good Clinical Practise (ICH GCP; E6(R2)), ICH E8(R2), the Declaration of Helsinki (revised version of 2013), Good Manufacturing Practice (GMP), and the current national regulations and guidelines, approved by both the local ethics committee (IEC) and regulatory authority (MHRA). The protocol has been developed in accordance with draft ICH M11 guidance.

In overview:

4.1.1 Screening Period

Potential participants assessed as suitable for enrolment into the clinical trial will have a single target VLU selected and will undergo standard of care treatment [moist wound healing dressing + compression bandage] for VLUs for a period of 2 weeks utilising the investigator's normal frequency of visits and dressing changes, but using standardised (pre-specified standard of care dressings). Only participants with wounds that continue to meet the inclusion/exclusion criteria at the end of the 2-week run-in period (i.e. <40% reduction in the surface area of the wound) will be eligible for randomisation to the study.

It is estimated that approximately 20-40% of consented participants will not meet the inclusion/exclusion criteria after the 2-week run-in and as such, will not be randomised. These will be considered as screen failures. Based on these estimates, up to 28 participants are expected to be screened to enrol 20 patients.

Please refer to section 8.1 of the protocol for further information on trial assessments and procedures during the screening period.

4.1.2 Baseline

On day 1, following randomisation but prior to any IMP treatment, all baseline assessments will be made of the wound including determinations of wound size, slough and devitalised (eschar) content of the wound (using digital imaging), and quality of life measures will be completed.

Please refer to section 8.2 of the protocol for further information on trial assessments and procedures to be performed at baseline.

4.1.3 Randomisation

Once eligibility to the study has been confirmed, participants will be randomised into one of two arms in a 1:4 ratio as follows:

- **Group 1: Standard of Care** [comprising ActivHeal + secondary dressing + compression bandaging] (N=4), applied three times weekly at time of routine dressing change, for 4 weeks.
- **Group 2**: **Aurase Wound Gel** [24 U/mL] + secondary dressing + compression bandaging (N=16), applied three times weekly at a volume of 0.2mL/cm^2 at time of routine dressing change for 4 weeks.

4.1.4 Treatment Period: Treatment and Assessments over the first 4 weeks

During the 4-week treatment phase, participants will receive three times weekly dressing changes, wound cleansing and re-application of IMP (or hydrogel) as non-residential visits within a community based, GP practice clinic or during a home visit.

At each of the nine scheduled treatment visits, clinical staff will routinely assess occurrence and severity of AEs (including presence of local AEs at the wound site), the severity of wound pain, and onset of any new concomitant medications. In addition, more detailed clinical assessments of the target VLU wound site will be performed to include:

- A clinical assessment of whether "complete debridement" has been achieved, where "complete debridement" is prospectively defined as an assessment by the investigator that "the wound bed is clean and composed of healthy granulating tissue".
- Determination of wound size (surface area) using digital imaging.
- Slough and eschar content of the wound (as a proportion of wound surface area) using digital imaging.
- Linear wound healing rates using digital imaging.

Please refer to section 8.3.1 of the protocol for further information on trial assessments and procedures to be performed during Weeks 1-4.

Where "complete debridement" as assessed by the investigator occurs during the first 4 weeks, then participants will carry on treatment to the end of week 4 as planned, to assess whether continued treatment has positive effects on the extent of wound healing. Any patients that discontinue treatment early will utilise Last Observation Carried Forward (LOCF) through to the primary endpoint at Week 4.

4.1.5 Follow-up Period and Medical Record Review

At the end of the Treatment Period (i.e. after week 4), all participants will have a final visit at Day 28 (end of Week 4), during which any residual Aurase Wound Gel or ActiveHeal gel will be removed. During this visit, the clinical staff will also assess occurrence and severity of AEs (including presence of local AEs at the wound site), the severity of wound pain, and onset of any new concomitant medications. No AE data will be collected beyond the 4-week study duration, including during the medical notes review at Week 12. In addition, more detailed clinical assessments of the target VLU wound site will be performed to include:

- A clinical assessment of whether "complete debridement" has been achieved [or maintained], where "complete debridement" is prospectively defined as an assessment by the investigator that "the wound bed is clean and composed of healthy granulating tissue".
- Determination of wound size (surface area) using digital imaging.
- Slough and eschar content of the wound (as a proportion of wound surface area) using digital imaging.
- Linear wound healing rates using digital imaging.

End of trial assessments including participant questionnaires will also be provided to participants at this final visit.

At week 12, a follow-up medical records review will be conducted to evaluate the wound healing status of the treated wound. This will involve examining medical records and clinical notes of the participant to assess progress without requiring a physical visit. The aim is to gather essential data on long-term outcomes while minimizing patient burden and ensuring efficient data collection.

4.2 Participant Input into Design

4.2.1 Patient-based Literature

Published studies in patients with VLUs indicate that participants have a poor quality of life compared with age matched controls. In most reported studies, pain is the dominant complaint. Krasner 1998 [25] conducted interviews with 14 people with VLU's to explore the meaning of the experience of living with venous ulcers. Eight themes were identified and one of these key themes was concern over the aspect of starting the pain all over again through [repeated] painful surgical debridement. The cycle of pain and fear left the patients' feeling depressed at times.

Green et al 2018 [26] also carried out initial unstructured interviews with 9 patients suffering with a leg ulcer which revealed significant issues for the patients including the dominance of pain, issues relating to exudate and odour, social isolation and psychological effects. Pain was reported by all nine participants and formed the very core of each interview. Pain dominated the patients' lives and limited their functioning. All spoke of their reluctance to take analgesia, often because they were already taking a cocktail of medications for their comorbidities. Where analgesia was taken, respondents reflected that this was generally ineffective for the type and intensity of pain that their leg ulcers caused. Across the participants, there were similarities in the description of their pain, including its unceasing nature, severity and timing.

Within the context of wound management all of the participants in the Green survey described 'managing' their wound, which showed the prominence that the process of wound care held in their lives. All participants said that "ulcer healing" was the goal of their nursing teams; however, many spoke of accepting that this goal was often elusive and, when achieved, would be difficult to maintain. Some patients had elected to attend a wound care clinic for their dressing changes, while others received care at home.

The reflections made by patients with VLUs from within the literature have therefore been incorporated into the study design; notably:

1. Pain is clearly an overriding symptom in VLU, with ongoing concerns raised about the need for repeated painful surgical or mechanical debridement. In this context, we seek to replace painful surgical debridement procedures with painless application of Aurase Wound Gel and routine assessments of pain will be conducted to confirm that Aurase Wound Gel does not increase the patient's pain burden.

2. The standard of care treatment with VLUs, especially the application of compression bandaging systems, requires specialist nursing. In this context, and based on patient's concerns in regards mobility [i.e. attendance at clinics], or waiting around for nurses [at home application], we do not seek to overburden the patient with dressing changes where these would only be required for IMP treatment. Therefore Aurase Wound Gel in this study will be applied in line with the expectations of routine wound dressing changes (3 times weekly) for the first 3 weeks.

3. All participants enrolled into this clinical trial, and irrespective of randomisation, will receive best standard of UK care involving standardised autolytic debridement moist wound healing dressings combined with compression bandaging, designed to deliver graduated compression (40mmHg at the ankle and 18mm Hg at the knee).

4.2.2 Patient and Public Involvement (PPI) Assessment of the Trial Design

Draft trial designs were initially prepared in consultation with a panel of Key Opinion Leaders (Plastic Surgeons, Dermatological Surgeons and Vascular Surgeons) in chronic wound healing and following consultation with prospective clinical sites in respect of potential enrolment. Subsequently, draft Participant Information Sheet(s), describing the trial design and assessments to be undertaken were prepared and provided to a PPI panel comprising two sequential groups of up to five VLU patients, 1 week in advance of the online meeting. During the PPI meetings the sponsor additionally presented a summary of the clinical protocol to the attending VLU patients in order to obtain feedback in respect of their views on:

- What they thought was good about the clinical trial design
- Whether any aspects of the trial design gave them concerns
- Whether the patients would be happy to attend all visits at a GP community clinic, at home or a mix
- Whether there was anything that the patients considered important to the trial design that the sponsor or KOLs had missed.
- If approached, as a patient, would they have been interested to participate in the study.

Group 1:

Comprised a group of 4 elderly participants (3f and 1m). Overall, the patient panel were overwhelmingly in support of the clinical trial. Preferences were made to indicate treatment in the patient's own home (via community nursing) wherever possibly, although it was accepted that a visit to their local community GP was also acceptable. Less favourable, was the need for extended travel to a specialist clinic. The visit schedule and proposed treatments were also considered acceptable.

Requests were however made, and these largely focussed on the length and type of information provided in the Participant Information Sheet. Notably, these included:

- A request to limit the length of the participant information sheet.
- One participant felt it would be useful to include some information on the prior clinical trial of Aurase Wound Gel.
- One participant felt it would be useful to include some information about the treatment being readily available after the clinical trial ended.

The sponsor agreed to reflect these requests in the participant information sheet, where permitted.

Group 2:

Comprised a group of 2 elderly participants (1m and 1f) video interviews with an additional 2 participants providing written feedback. Focus for discussion was the adequacy of the Participant Information Sheet, the shorter duration of the clinical trial (6 weeks with three weeks of treatment) and travel to a GP surgery clinic. In summary, this panel indicated:

- The participant information sheet / informed consent was clear, understandable, comprehensive and that no additional information was necessary for them to consider volunteering for the clinical trial.

- A shorter duration of clinical study, utilising a 2-week run-in period, 4-week treatment period, although not following out to full healing was suitable to consider the ability of Aurase Wound Gel to "clean their wounds". The patients noted that anything that "kickstarted" the ability of their wounds to heal could be a positive step in achieving an end goal of healing.
- That travel to a local GP surgery for treatment was acceptable. Three weekly visits during the treatment regime was also acceptable, as it would mean that there would be regular good standard of care for their wound, potentially better than routinely available within the NHS.

4.3 Rationale for Trial Design

This clinical study design, seeks to build on clinical results from the previous Phase IIa (First in Human) study of Aurase Wound Gel at concentrations ranging from 1.2 U/mL to 10.9 U/mL. Although this clinical study indicated little concerns in regards safety (either systemic safety or local safety at the application site) from tarumase gels and provided for an indication that increased efficacy could be achieved at higher concentrations of tarumase, the design of the FIH study had significant design limitations, making any definitive conclusions in respect of the efficacy profile difficult. Therefore, prior to larger (double blind) Phase IIB clinical trials, we seek to employ more appropriate trial designs, to further establish proof of concept both for safety and efficacy notably:

- The FIH study utilised a sequential, non-randomised, dose-escalation design, introducing significant bias to the safety and efficacy assessments. In this context, this trial seeks to employ randomisation between the investigational treatment Aurase Wound Gel and control treatment [ActivHeal].
- In the FIH clinical study, the standard of care was not fully harmonised across cohorts, due to the multi-national nature of the clinical trial (driven in part by the availability of different dressings in particular countries). The use of different brands of dressings and their clinical use could potentially affect the rate of wound debridement and/or healing rate. We are therefore now seeking to standardise the standard care treatment arm within this clinical trial to (specified) dressing and compression bandage products.
- The FIH clinical study required a lengthy stay (8h) in a specialised clinic and/or hospital setting, in order to obtain routine blood samples for PK testing, and to follow up patients closely for potential serious safety issues, in line with the principles of FIH clinical trials. Since VLU patients are rarely seen in these specialised clinics, this significantly impacted recruitment within the UK, forcing a multi-national, multi-centre clinical approach to enrol the 45 patients required for the study. This, in turn, introduced additional variability into the clinical study. In the UK, the majority of VLU patients are treated either at home (via community nursing) or in local GP community clinics. To reflect this treatment pathway, we now seek to employ a clinical design which leverages a community-based centre approach through a GP care pathway to treat patients. Patients enrolled to the study, should therefore be more representative of the patient population as a whole.
- The FIH clinical study enrolled all comers that met the inclusion / exclusion criteria in a sequential order; there was no exclusion criteria which looked at prior standard of care treatment or healing of the reference ulcer. Since the initial Phase IIA study, we have been advised that routine standard of care for VLU patients can be highly heterogeneous and in many cases poor. Consequently, in some patients, lack of healing in the chronic wound is more a function of poor standard of care than true chronicity. When these patients are

placed into a clinical trial, with good documented standard of care, these patients go on to heal rapidly. As a result, we propose a 2-week documented run-in period using standardised (and specified) moist wound healing dressings and compression bandaging. To the extent that patients, after this 2-week period, demonstrate a wound surface area reduction of >40%, then this is an indication that the wound has likely been treated inadequately and will go on to heal more rapidly. These patients will be excluded from the proposed clinical trial, in order to minimise potential bias in the clinical results.

- The FIH clinical trial also did not specify a minimum amount of slough and/or devitalised tissue in the wound bed, only that the participant's wound had to be suitable for debridement. During analysis of the FIH clinical data, it was noted that patients were enrolled at baseline with a variety of slough content in the range of 12.2 – 100% of the wound surface area. Those with less slough were found to debride rapidly and completely with standard of care, however Aurase Wound Gel worked better in participants with more slough present. In order to standardise participants, the inclusion criteria has therefore been modified to enrol only participants with ≥ 50% of the wound surface area covered by slough at baseline.
- During the FIH study, clinicians were asked to clean the wound at each dressing change using sterile saline or water only, before re-application of the Aurase Wound Gel; no physical contact with the wound was permitted. In routine clinical practice however, it is usual for clinical staff to use gauze to dry the wound and this additionally enables removal of loose slough from the wound bed. In non-clinical studies, this effect has been found to be synergistic with Aurase Wound Gel, and since this represents standard of care, should be introduced in this next clinical study.

In this context, this follow-up study aims to more definitively assess the safety and efficacy of a higher concentration of Aurase Wound Gel [24U/mL] over a limited treatment period [3 weeks] and a further 1 week follow-up period, using a more robust design and a more targeted patient population. This will help in:

- Establishing a definitive safety profile: This study will prioritize a thorough safety evaluation of the higher concentration, 24U/mL, by closely monitoring for both systemic and local adverse events.
- Further refine the efficacy assessment: We will employ a more rigorous randomised, controlled design to explore the potential for improved debridement and wound healing rate compared to standard of care over the 4 week treatment period. Healing rates at 4 weeks have been reported in the literature to be highly predictive of full wound closure with a positive correlation observed between those that achieve >40% reduction in wound surface area in 4 weeks and the ability to achieve full wound closure within 12 weeks. In this context, an assessment of healing over 4 weeks may provide further evidence to support the wound healing capability of Aurase Wound Gel.

By conducting this focused trial with a higher concentration, we propose bridging the gap between the initial promising Phase IIA results and a more definitive Phase IIb dose selection study.

4.4 Access to Trial Intervention After End of Trial

At the end of the treatment period (4 weeks), participants who have been randomised to Aurase Wound Gel and whose wounds remain sloughy and/or unhealed will revert to the Investigator's standard of care. No continued access to Aurase Wound Gel post study is envisaged, and this is highlighted in the Participant Information Sheet.

Participants randomised to Standard of Care [control] only treatments and who have not responded to standard of care by end of week 4 (i.e. slough remains >50% and wound size remains >60% of initial baseline) may, provided all other inclusion/exclusion criteria continue to be met, the patient continues to consent, and subject to Sponsor approval, be re-enrolled and randomized to 4-week's treatment with AWG.

4.5 Start of Trial and End of Trial

Each participant enrolled and randomised into the study will remain in the study until the end of 12 weeks.

For the purposes of the clinical trial, the start date will be defined as the date on which the first participant provides written informed consent to enrol in the study.

End of trial will be defined as the last participant, last visit at 12 weeks.

The Sponsor reserves the right to terminate the study prior to the end of trial, and in this context, both MHRA and IEC will be advised.

Where temporary suspension of the trial is warranted, both MHRA and IEC will be notified. In such cases, explicit approval of MHRA and IEC will be required before the trial can be re-started.

5 TRIAL POPULATION

5.1 Selection of Trial Population

A maximum of 20 participants will be randomised to treatment after the run-in period. These patients will be sourced from either:

i. Lists of potential patients at the nominated community GP clinical site (South Leicestershire Medical Group [SLMG];

ii. The patient lists of GP community wound hubs utilised as Participant Identification Centres (PIC) (i.e. Leicester Partnership NHS Trust) who have agreed to refer potential participants to the investigational site.

As set out in the inclusion/exclusion criteria (see section 5.3 and 5.4) participants will be adults with pre-existing VLU wounds. We note that there is a higher prevalence of these conditions in the geriatric population, and although not exclusive to this population, it is anticipated that a large majority of the participants enrolled in the trial will be ≥ 65 years old.

5.2 Rationale for Trial Population

A common feature of all chronic wounds, is that many require repeated wound debridements to aid in their healing. There is however a recognition that these non-healing wounds are essentially made up of several distinct clinical populations, notably Venous Leg Ulcers (VLU), Diabetic Foot Ulcers (DFU) and Pressure or Decubitus Ulcers (PU), each with their own pathology, aetiology and standard of care.

Within this context, SolasCure have selected VLU as the first clinical population to be investigated on the grounds that:

1. VLUs often contain fibrinous slough (yellow or green) material rather than black necrotic tissue. This is likely to favour the tarumase enzyme, which is a selective fibrinolytic enzyme.

2. Participants can be selected that are otherwise generally healthy (see proposed inclusion/exclusion criteria) making it more appropriate for Phase II interventional clinical trials. Patients with other chronic ulcer conditions (e.g. Pressure Ulcers are often associated with more serious comorbidities and are often frail and/or very elderly) thereby complicating assessments of local and systemic safety.

3. Some chronic wounds (notably DFUs) may be neuropathic, compromising some of the clinical assessments for safety (e.g. pain on application). VLU's by contrast are typically painful wounds, for which surgical or mechanical debridement may not be appropriate or advisable. As a result lengthy autolytic debridement is the only treatment option.

4. VLU wounds make up one of the largest populations of chronic wounds and production of slough and eschar is common in this clinical population. The protocol therefore addresses a significant clinical need for wound debridement, particularly in the community setting.

5. VLU wounds by contrast with other chronic wounds are largely superficial in terms of wound depth, so that additional complications associated with measuring wound volume (including estimating the degree of undermining etc.) are not an issue that design needs to be addressed. Instead, surface area is commonly used to assess the rate of wound healing.

5.3 Inclusion Criteria

The following inclusion criteria are required to ensure that the participants included in the trial are suitable for entry in terms of their general health and the provision of informed consent:

1) Male or female participants aged 18 years and older at screening who are willing and able to attend and comply with all study visits and study related activities.

2) Provide a signed and dated written informed consent.

3) Presence of ≥ 50% slough or devitalized tissue within the reference ulcer and suitable for debridement therapy.

4) Participants with at least one defined VLU suitable for treatment that is no smaller than 2 cm^2 and no larger than 50 cm^2 and is confirmed as venous in origin by clinical assessments, by Ankle Brachial Pressure Index (ABPI) ≥ 0.8 and/or toe systolic BP pressure > 70mm Hg. Participants with more than one VLU on the target leg can be included, provided other ulcers are at least 1 cm away from the reference ulcer identified for treatment. Selection of the reference ulcer will be at the investigator's discretion, provided it meets all other inclusion/exclusion criteria.

5) Confirmed, clinically diagnosed VLU (ulceration of the lower limb, with no other mechanistic explanation and which has persisted for 6 weeks or more) but which has been present for ≤ 2 years, defined by patient reporting or clinical records.

5.4 Exclusion Criteria

The following criteria are used to exclude participants whose medical history, ongoing conditions or lifestyle could affect the assessment of efficacy or safety during the trial:

1) Reduction in the wound area of ≥ 40% within the 2-week screening period, when administered standard of care [moist wound dressing + compression bandaging] only.
2) Participants who have reported poorly controlled diabetes within 3 months of the screening period
3) Participants with amputation above a trans metatarsal amputation (TMA) in the target leg.
4) Reference ulcer has exposed tendons, ligaments, muscle, or bone.
5) Reference ulcer [at end of screening] with high levels of exudate, which in the opinion of the investigator, would render the proposed trial management protocol unsuitable.
6) Reference ulcer has active infection at screening determined by the investigator using clinical assessment, such as active osteomyelitis, cellulitis or gangrene in either leg
7) Participants with current active malignancy [other than basal cell carcinoma] requiring active immune or chemo-therapy treatment
8) Planned vascular surgery, angioplasty, or thrombolysis procedures within the study period, or 4 weeks before screening.
9) Prior skin graft, negative pressure therapy, ultrasound therapy, systemic or cutaneously applied growth factor, other enzymatic debriding agents (e.g. Collagenase, Nexobrid) or live maggot therapy applied to the reference ulcer within 2 weeks before screening.
10) Currently enrolled or has been enrolled in the last 30 days in another investigational device or drug study [excepting participants previously enrolled into this study and treated with standard of care only and not responded to standard of care – see section 4.4].
11) Known allergy or hypersensitivity to any component of the investigational product, medication or dressings to be used in the study.
12) Participants lacking capacity to provide informed consent.
13) Any patient which the investigator otherwise considers unsuitable for entry into the study, by reason of acute or chronic mental or physical condition that may interfere with the collection of safety and/or efficacy data.
14) Pregnant or breastfeeding women.

5.5 Lifestyle Considerations

Smoking and alcohol abuse are well-established to have a detrimental effect on wound healing. Smoking habits and alcohol use will therefore be recorded as part of the demographic data. Participants who continue to smoke will be encouraged by the clinical site to discontinue smoking and/or to reduce alcohol intake to less than 14 units of alcohol per week. Continued smoking and /or alcohol will not however be an exclusion from the clinical trial.

Exercise is also noted to have a positive effect on wound healing. Participants will be encouraged to be ambulatory, and information on the extent of ambulation will be recorded in the eCRF.

5.6 Screen Failures

Where considered appropriate, participants will be allowed to re-screen for the study once, with the Sponsor's approval.

Once enrolled in the study, participants may not be enrolled for the management of a second ulcer.

6 TRIAL INTERVENTIONS AND CONCOMITANT THERAPY

6.1 Description of Trial Interventions

Experimental IMP	Aurase Wound Gel 24 U/mL
Route of Administration	Cutaneous
Dose	0.18 - 0.22mL per cm^2 of wound surface area (nominal 0.2mL ±10%)
Dose Regimen	Three times weekly. At least 36 hours and no more than 72 hours should occur between applications.
Packaging	Aurase Wound Gel 24 U/mL will be reconstituted by delegated site staff no more than 24 hours prior to administration by reconstituting 3 x 2mL ampoule of Component B with 1 x 9mL syringe of Component A using the Manumix provided. (Total volume 15mL). Reconstituted product should be taken up into one suitably sized syringe, to the volume required to treat the reference ulcer.
Labelling	Each blistered Component A syringe, and blistered Component B ampoule will be labelled with requirements set out in Annex 13, Volume 4 of the Rules Governing Medicinal Products in the European Union. Following reconstitution, but prior to use, the syringe will be labelled with the participant's unique randomisation number and expiry date.
Storage Temperature	At the clinical trial sites, individual Components B ampoules should be stored refrigerated at (2-8°C). Component A may be stored at room temperature (≤25°C). Following reconstitution, and prior to clinical application, reconstituted syringes of Aurase Wound Gel can be stored at room temperature (≤25°C).

Control	ActivHeal® (Advanced Medical Solutions) 
Route of Administration	Cutaneous
Intended Use	ActivHeal Gel is a clear, amorphous hydrogel that gently increases the moisture level within the wound, encouraging moist wound healing through autolytic debridement.
Regimen	During the treatment phase only, the hydrogel will be applied three times weekly.
Packaging	Provided as cartons of 10 tubes containing 15g tubes
Labelling	As commercially released to the UK market. The product is a CE marked Class IIb medical device.
Storage Temperature	As recommended by the Manufacturer

Primary Standard of Care Dressing	Mepilex (Molnlycke) 

Intended Use	Mepilex is intended for a wide range of exuding wounds such as pressure ulcers, leg and foot ulcers, and traumatic wounds such as skin tears and surgical wounds. It can be used on dry and or necrotic wounds in combination with gels.
Regimen	During Run-in; the dressing should be changed at the investigators normal frequency of application. During the treatment phase, the dressing should be changed at each visit through until complete healing or end of study. During the follow-up phase, the dressing should be changed at the investigator's normal frequency of application. Where unscheduled dressing changes are required, details will be recorded in the eCRF.
Packaging	Provided as cartons of 10 dressings.
Labelling	As commercially released to the UK market. The product is a CE marked Class IIb medical device.
Storage Temperature	As recommended by the Manufacturer

Alternative Standard of Care Dressing	Allevyn® Non-Adhesive (Smith & Nephew) 
Intended Use	For the purposes of the study Allevyn Non-Adhesive will be used as an alternative secondary dressing where participants have shown previous sensitivity to the primary Mepilex dressing. Allevyn Non-Adhesive dressings are intended for use under compression therapy in VLU to help manage exudate and risk of leakage.
Regimen	During Run-in; the dressing should be changed at the investigators normal frequency of application.

	During the treatment phase, the dressing should be changed at each visit through until complete healing or end of study. During the follow-up phase, the dressing should be changed at the investigator's normal frequency of application. Where unscheduled dressing changes are required, details will be recorded in the eCRF.
Packaging	Provided as cartons of 10 dressings.
Labelling	As commercially released to the UK market. The product is a CE marked Class IIb medical device.
Storage Temperature	As recommended by the Manufacturer

Standard of Care Dressing when infection is suspected	UrgoClean Ag (Urgo) 
Intended Use	Where infection of the wound is suspected, and either alone or in combination with systemic antibiotics, UrgoClean Ag is intended to reduce bioburden in the infected wound. It can be used under compression.
Regimen	During the treatment phase, and only where infection is clinically observed, Urgoclean Ag may be used as the secondary dressing. The dressing should be changed at each visit. When infection is deemed to be clear, UrgoClean Ag should be stopped and replaced with the primary Mepilex dressing. During the follow-up phase, the dressing should be changed at the investigator's normal frequency of application. Where unscheduled dressing changes are required, details will be recorded in the eCRF.

Packaging	Provided as cartons of 10 dressings.
Labelling	As commercially released to the UK market. The product is a CE marked Class IIb medical device.
Storage Temperature	As recommended by the Manufacturer

Standard of Care	UrgoK2 Compression System (Urgo) 
Intended Use	UrgoK2 is a dual compression system that guarantees continuous, consistent, and comfortable compression. It is indicated for the treatment of venous leg ulcer, venous oedema and lymphedema, which require full compression.
Regimen	During Run-in; the dressing should be changed at the investigators normal frequency of application. During the treatment phase, the compression bandages should be changed at each visit through until complete healing or end of study. During the follow-up phase, the compression bandaging should be changed at the investigator's normal frequency of application.
Packaging	Provided as individual bandages
Labelling	As commercially released to the market

Storage Temperature	As recommended by the Manufacturer

6.1.1 Rationale for Trial Interventions

As noted above in section 2.4.3, proof of concept for the use of Aurase Wound Gel as a debriding agent was tested during the FIH Phase IIA clinical study at 0U/mL (vehicle), 1.2U/mL, 2.16U/mL, 6U/mL and 10.9U/mL. The reported results indicated that over the 4 week period of the study trends towards numerically more and complete debridement and increased granulation tissue / linear wound healing rate was observed.

Notwithstanding these trends, "complete debridement" was only observed in a few patients at the highest concentrations, and this effect was not seen until 4 weeks. Based on an excellent safety profile in these patients it is anticipated that a higher local concentration of Aurase Wound Gel, using a more optimised gel/solution ratio, that faster and more complete debridement can be achieved. In this context, an effect at 24U/mL, representing the highest concentration achievable in the current gel formulation (without compromising viscosity – so that the gel remains in the wound) has been proposed.

ActivHeal, is a CE marked medical device, it is widely used in community GP practice and is a recognized autolytic debridement agent that provides for moisture donation to the wound. With a well-established safety profile, this serves as the control to directly compare the safety and efficacy of the Aurase Wound Gel against a widely employed standard of care.

Throughout the treatment period, and indeed throughout the clinical study, all participants will receive the best standard of care. This includes the primary use of a foam dressing (Mepilex) or an alternative foam (Allevyn Non-Adhesive), where patients may have previously showed sensitivity, that are used for exuding wounds such as VLU in combination with gels. Additionally, all patients will be provided with compression bandaging to aid venous return and to avoid pooling of blood in the lower legs. Such compression systems are typically designed to provide graduated compression; 40-45mmHg at the ankle and 18-20mm Hg at the knee. It is well-established that where compression is not applied properly, there is a risk of increased oedema, exudate and poor wound healing. In this context, the standard of care has been standardised across the clinical study for UrgoK2 Compression, a commonly employed compression system in community clinics.

6.2 Dosing and Administration

At each treatment visit, and irrespective of the randomised treatment, the administration procedures for SOC ActivHeal and Aurase Wound Gel will be standardised as follows:

- The compression bandaging and secondary dressings will be taken down, exposing the reference ulcer.
- The reference ulcer will be cleansed using sterile saline solution or water to wash away any loose slough or eschar.
- The ulcer will be gently dried using a piece of sterile gauze. Sufficient pressure should be applied to the gauze so that any loose slough can be removed, but guided by the patient, pressure should not result in any additional pain.
- Following completion of cleaning [and all subsequent clinical assessments / imaging] Aurase Wound Gel or ActivHeal (as randomised) will be applied to the wound bed.

For ActivHeal the volume of gel applied should be in line with Manufacturer's instructions.

- The actual volume of Aurase Wound Gel to be administered in the clinical trial will be dependent on the surface area of the wound. To calculate the volume of Aurase Wound Gel to be administered, the following calculation will be used:

$$Volume\ (mL) = Surface\ area\ (cm^2) \times 0.2\ (\frac{mL}{cm^2})$$

The surface area will be determined using the Aranz Silhouette Star imaging system. In this context, the absolute quantities of tarumase administered per cm^2 of wound area (and maximal exposure) are as follows:

Table 3: Exposure of tarumase per cm^2 of wound area and maximal exposure based on 0.2mL/cm^2

Group	Label Claim of IMP / Vehicle	Exposure per cm^2 of wound area		Maximal daily exposure**	
		U	µg	U	µg
2	24 U/mL	4.8	12	240	600
** Assumes 50cm^2 maximal wound size in accordance with inclusion/exclusion criteria					

6.3 Treatment of Overdose

Treatment overdose is not anticipated in this clinical study, on the grounds that:

i. Systemic pharmacokinetics at 10.92 µg/cm^2 [10.9 U/mL] in the Phase IIa study in wound sizes up to 50cm^2, administered 3 times weekly for 4 weeks was unable to detect any tarumase in the systemic circulation at any time point. This is due to the rapid deactivation of the enzyme arising from the overwhelming concentration of endogenous protease inhibitors in the circulation, e.g. Alpha-1-Antitrypsin (A1AT) at 100 – 273 mg/dL [23].

ii. No evidence of systemic or localised dose-limiting toxicity was observed in the Phase IIa clinical study at concentrations equivalent to 10.92 µg/cm^2 [10.9U/mL] dosed three times weekly.

iii. The volume of Aurase Wound Gel administered during this clinical trial will be half the volume (0.2mL/cm^2 vs 0.4mL/cm^2) administered in the Phase IIA study. The reduced volume is considered to be further beneficial to the local wound environment and will reduce the risk of localised wound maceration.

Where evidence of localised reactions occur at the wound site, and which are considered attributable to Aurase Wound Gel, and where in the investigator's opinion these become clinically unacceptable to continue application, Aurase Wound Gel may be removed by cleansing the wound with sterile water or saline solution.

If at any point in the study a threshold of >15% attributable "severe" Adverse Drug Reactions (ADRs) or a single attributable Serious Adverse Reaction is reported, then a temporary halt

will be made to the study in order that a safety committee can make an assessment of the safety of treatments and/or to make recommendations on reducing concentrations or frequency of administration. Any temporary halt of the study will be reported to regulatory authorities and REC/IRB, and enrolment will not recommence until further approval is given for any protocol modifications.

6.4 Preparation, Handling, Storage and Accountability

6.4.1 Handling and Storage of Standard of Care Products

All products detailed as part of standard of care, including ActivHeal, Mepilex (or Allevyn Non-Adhesive) and UrgoK2, should be stored and handled in accordance with the Instructions for Use provided by the Manufacturer (see section 6.1).

6.4.2 Preparation of Aurase Wound Gel

IMP components must be stored in a controlled access area, separate from regular medication inventory. The area or container should be locked with access restricted to appropriately delegated clinical trial personnel.

Aurase Wound Gel used in the clinical study can be prepared by a delegated staff member from the clinical site, based on products supplied by the Sponsor. The reconstitution of the IMP should occur in accordance with the pharmacy manual, and not more than 24 hours prior to intended application to the participant's wound.

Aurase Component B (solution) must be diluted into Aurase Component A (gel) in a sterile container (Osartis Manumix) to yield reconstituted Aurase Wound Gel. Ancillary supplies have been selected to ensure complete mixing during the dilution step. All components and the Manumix kit are supplied sterile, and aseptic technique should be used to minimize risk of bioburden contamination during reconstitution.

Reconstituted Aurase Wound Gel should be taken up into an appropriately sized syringe for transfer and administration to the participant. Syringes of reconstituted Aurase Wound Gel (other than at the bedside) will be labelled with the participant's randomisation number and an expiry date (time).

6.4.3 Handling and Storage of Trial Intervention

Aurase Component A

Aurase Component A is provided in blistered [labelled] 11mL syringes [9mL content]. Component A can be stored at room temperature (i.e. below 25°C) until the blister is opened and product is used for the reconstitution of Aurase Wound Gel.

Aurase Component B

Aurase Component B, is provided in blistered [labelled] 2mL ampoules. Component B should be stored refrigerated (2-8°C) until the blister is opened and product is used for the reconstitution of Aurase Wound Gel.

Reconstituted Aurase Wound Gel [and vehicle]

The components of Aurase Wound Gel and vehicle are sterile until the primary containers are opened. Sterile mixing equipment (Osartis Manumix) is supplied to ensure adequate mixing of Aurase Components A and B during the dilution step. Reconstituted Aurase Wound Gel preparations have been shown to be bacteriostatic when stored at room temperature for up to 24 hours.

The preparation should however be performed as close as possible to application, within the maximum 24 hour storage window from the time the first component is unsealed.

6.4.4 Accountability of Trial Intervention

Accountability of IMP will be documented at the clinical site and will be subject to review by the monitor. Accountability will include documentation of the number of Component A syringes, Component B ampoules received, allocated / reconstituted and dispensed for Aurase Wound Gel, and the number of components returned / destroyed.

6.5 Participant Assignment, Randomisation and Blinding

6.5.1 Participant Assignment

A participant screening number will be assigned to all participants as they consent to take part in the trial, in order that screening tests can be undertaken and recorded anonymously within a screening and/or run-in eCRF. The screening reference will take the form of AB/YYYY/ where AB = participant initials and YYYY are the Year of birth. To the extent that multiple participants share initials and year of birth, the screening number will be supplemented with a subsequent integer number.

Once randomised, the screening number will be replaced by a randomisation number for all subsequent assessments. This randomisation number will be allocated sequentially, based on Site and Participant (e.g. 01-001, 01-002 etc meaning site 1, participant 1 and 2). Once a randomisation number has been assigned to a specific participant that number must not be used again, if for example, the participant is later withdrawn from the study or not found to be eligible. If a randomisation number is allocated incorrectly, the trial monitor and Sponsor should be notified as soon as the error is discovered.

6.5.2 Randomisation

Eligible participants to the clinical study will be randomised to a treatment group based on a 1:4 randomisation scheme. A randomisation list will be prepared by or on behalf of the sponsor using a computer programme to randomly allocate patients to treatment Groups 1 and 2 as follows.

- **Group 1**: Standard of Care [comprising ActivHeal, Mepilex (or Allevyn Non-Adhesive) and UrgoK2 dual layer compression system], applied three times weekly for three weeks.
- **Group 2**: Aurase Wound Gel 24U/mL administered 0.2mL/cm^3 administered in combination with standard of care [comprising Mepilex (or Allevyn Non-Adhesive) and UrgoK2 dual layer compression system], three times weekly for 3 weeks.

6.5.3 Blinding

Due to the clear differences in the presentation and appearance of Aurase Wound Gel and ActivHeal it is not possible to blind this study. This study will therefore be open designed.

6.6 Trial Intervention Compliance

Participants will be deemed to be compliant with the study protocol for treatment intervention compliance if:

- They receive 9-11 applications of IMP within the four week treatment period that are not less than 36 hours and not more than 72 hours apart.

- That at each dosing visit, they receive Aurase Wound Gel at a dose of 0.2mL/cm^2 of reference ulcer surface area ± 10% (i.e. 0.18-0.22 mL/cm^2). To the extent that the wound area is modified on review of the images by the Principal Investigator [after the patient has been treated], dosing will still be considered to be compliant if the dose applied was 0.2mL/cm^2 ± 10% of the determined area at time of dosing.

6.7 Concomitant Therapy

All concomitant medications taken by the participant between the screening visit until the final visit at week 4 will be recorded in the subject's electronic Case Record Form (eCRF).

6.7.1 Prohibited Concomitant Therapy

Prohibited concomitant therapy during the clinical trial include:

- Negative Pressure Wound Therapy (NPWT)
- Ultrasound therapy
- Systemic or cutaneously applied growth factors
- Other locally applied enzymatic debriding agents (e.g. collagenase, Bromelain)
- Live maggot therapy
- Local antiseptics, including hydrogen peroxide, hypochlorous acid and iodine containing products such as Betadine or Cadexomer iodine.

Use of any prohibited concomitant medication will result in the participant being withdrawn from the study.

6.7.2 Permitted Concomitant Therapy

Participants who were on stable doses of medications for 2 weeks prior to enrolment into the clinical trial and not excluded by the protocol (see criteria 5.4) may continue on these medications throughout the clinical study and are permitted.

Participants who develop clinical signs of a local infection should preferentially be administered with systemic antibiotics as per the clinical investigators standard practice. Where deemed clinically required, locally applied (Aurase Wound Gel compatible) a silver containing dressing (UrgoClean Ag) may also be utilised to replace standard Mepilex dressings.

6.7.3 Rescue Therapy

In the event that participants are withdrawn from the study, either at their own request or on the recommendation of the investigator, treatment will be stopped and participants will revert to standard of care provided by their own healthcare practitioner.

6.7.4 Other Therapy

Where the investigator considers it a clinical necessity, by virtue of the fragility of the participant's surrounding intact skin (i.e. peri-wound area), and in an effort to prospectively minimise potential maceration of the surrounding intact skin either from excess exudate and/or other liquids from the wound, the investigator may at their discretion utilise a skin protectant on the peri-wound skin. Where this is used, this will be recorded in the eCRF.

7 DISCONTINUATION OF TRIAL INTERVENTION AND PARTICIPANT WITHDRAWAL FROM TRIAL

7.1 Discontinuation of Trial Intervention

Discontinuation of the trial intervention will be defined as stopping (or having to remove) the Aurase Wound Gel prior to the end of the clinical study as defined in Section 4.4.

7.1.1 Criteria for Permanent Discontinuation of Trial Intervention

The participant will be permanently discontinued from the clinical trial if:

- The participant withdraws informed consent to the study;
- Any prohibited concomitant therapy is used as set out in section 6.7.1;
- The participant experiences a Suspected Unexpected Serious Adverse Reaction (SUSAR) that is considered as attributable to the IMP.
- The wound is deemed non-responsive to AWG treatment, defined as <10% slough area reduction compared to baseline after 3 weeks of treatment, at which point discontinuation may be advised by the investigator.

7.1.2 Temporary Discontinuation or Interruption of Trial Intervention

Venous Leg Ulcers are highly susceptible to localised wound infections that can temporarily increase the exudate production from the wound and/or slough and eschar at the wound site. In addition, wound infections can negatively impact the wound, resulting in an increase in the wound size. Where in the investigator's opinion there is significant localised wound infection (based on clinical symptoms of exudate, erythema, oedema and purulence) in combination with increases in wound size, treatment intervention with Aurase Wound Gel or hydrogel may be temporarily stopped in the best interests of the patient. These will be recorded as AE/SAE as appropriate. The investigator can decide to re-start treatment once the wound is stabilised.

Where temporary discontinuation of the IMP is required, this should be noted in the eCRF.

7.2 Participant Withdrawal from the Trial

Participants may withdraw their consent to participate in the trial at any time, for any reason without prejudice to their future medical care. The Investigator may also withdraw subjects from the trial, if they are not compliant with the trial protocol or on clinical judgement and feedback from the participant on tolerability of pain level or other side effects.

Participants who are withdrawn from IMP treatment will be asked to complete (observational) weekly assessments, following treatment withdrawal, for the purposes of safety follow-up.

The reason for withdrawal will be recorded in the participant's medical record and in the electronic Case Report Form (eCRF). If a participant is withdrawn for more than one reason, each reason will be documented in the source documents and the most clinically relevant reason should be entered into the eCRF. Any comments (spontaneous or elicited) or complaints made by the participant and the reason for termination, date of stopping investigational product and the total amount of investigational product taken must be recorded in the CRF and source documents.

Participants who are randomised and subsequently withdrawn for safety will not be replaced. Participants who withdraw from the clinical study for other reasons may be replaced with the permission of the Sponsor.

The Sponsor reserves the right to terminate the trial at any time if deemed appropriate. In such an event, the necessary regulatory authorities, investigators and institutes will be informed promptly.

7.2.1 Lost to Follow-Up

At least three documented attempts will be made to contact any participant lost to follow-up, one of which must include sending a certified letter to the subject's last known address, requesting that they return to the study site for final safety evaluations.

7.3 Trial Stopping Rules

During the study ongoing assessments of safety will be made. If during the study a threshold level of 15% "severe" attributable Adverse Drug Reactions (ADRs) is met or a single attributable Serious Adverse Reaction (SAR) is reported then a temporary halt will be made to the study whilst a safety committee is asked to assess the continued safety of treatments and/or to make recommendations on reducing concentration or frequency of administration.

Prior approval of MHRA and IEC will be required before the trial can be recommenced.

8 TRIAL ASSESSMENTS AND PROCEDURES

A summary of the trial assessments and procedures to be undertaken throughout the clinical study is provided by reference to Table 1. In overview, these include:

8.1 Screening and Baseline Only Procedures:

8.1.1 Inclusion / Exclusion Criteria

The investigator will assess the potential participant against the inclusion / exclusion criteria set out in sections 5.3 and 5.4 of this protocol, using open questions to the patient and by applying the results of other assessments set out below, to determine whether the participant is eligible for inclusion in the study.

8.1.2 Demographic data

The following demographic data will be recorded into the eCRF:

- Year of birth
- Age
- Sex
- Race
- Height
- Weight (a BMI will be calculated based on height and weight)

8.1.3 Medical History & Concomitant Medication

The participant's medical history and concomitant medication will be recorded by the investigator (or a delegated member of the clinical staff), utilising open questions to the participant to determine relevant medical history both for ongoing chronic conditions, and acute medical history over the prior 3 months. Particular attention will be taken to a history of the reference wound and factors that may impact healing including:

- Wound duration
- Prior treatments to the wound
- Alcohol intake
- Smoking history
- Extent of ambulation

The participant's medical history will also be checked by reference to a request to the participant's GP for relevant medical history.

8.1.4 Physical Examination

A physical examination will be conducted by the Investigator (or an appropriately delegated member of the clinical staff) to assure the health of the participants enrolled into the trial. This will include an assessment of the following systems according to normal clinical practice:

- Cardiovascular health
- Respiratory health
- Gastrointestinal health
- Musculoskeletal health
- Neurological health
- Dermatological health
- Lymphatic health

8.1.5 Vital Signs

Blood pressure and pulse will be measured using a digital blood pressure monitor. The resting position will be recorded in the eCRF. Temperature will also be collected following the standard procedure of the investigator (or appropriately delegated member of the clinical staff) (e.g. using skin temperature, oral temperature, etc).

8.1.6 ABPI / Toe Systolic Pressure

The ankle-brachial pressure index (ABPI) is a non-invasive method of assessing peripheral arterial perfusion in the lower limbs. ABPI is a ratio composed of the blood pressure of the upper arm (brachial artery) and the blood pressure of the lower limb (dorsalis pedis and the posterior tibial artery). The measures are performed with a sphygmomanometer cuff over the arm proximal to the brachial artery and on the ankle over the posterior tibial artery (PTA), which is located posterior to the medial malleolus and over the dorsalis pedis artery (DP) of the foot, which is located lateral to the extensor hallucis longus tendon. The higher of the latter two pressures are used for calculating the ABPI as follows:

ABPI = (highest pressure of either PTA or DP) / (highest brachial pressure).

As an alternative to ABPI, a toe systolic pressure can be determined using an automated system, based on photoplethysmography (PPG).

Where the participant has already had an ABPI or toe systolic pressure related to the reference wound, and this has been documented within the last 3 months, it is acceptable to transcribe the ABPI or toe pressure into the eCRF from the patient's records.

8.1.7 Pregnancy Testing

In WOCBP, a urine pregnancy test strip for the determination of hCG in urine specimens will be used to obtain a visual, qualitative result of pregnancy at screening and Day 1 (pre-treatment) to assure that the patient is not pregnant prior to dosing. If pregnancy is suspected by either test, the patient will not be enrolled for treatment.

8.2 Clinical Procedures

8.2.1 Wound Cleansing

Cleansing of the wound should be performed at each dressing change. Once the dressing has been taken down, the wound should be cleaned with sterile saline or sterile water and then gently dried using a piece of gauze to remove any loose slough. Sufficient pressure should be used to remove any loose slough but, guided by the patient, insufficient pressure to result in increased pain to the patient.

Wound cleansing should be performed before any efficacy assessment of the wound is performed.

8.2.2 IMP Dosing

IMP dosing of Aurase Wound Gel 24U/mL (or ActivHeal as part of standard of care) will be performed at the end of each visit, after all efficacy and safety assessments are performed and prior to redressing the wound. As set out previously, dosing occurs at each of the treatment visits; three times weekly. IMP will be reconstituted not more than 24-hours prior to clinical use, according to the procedures set out in the Pharmacy Manual. The reconstituted material will be presented in a syringe, utilising a volume determined by a recommended dose of 0.2mL/cm^2 (see section 6.2).

8.2.3 Standard of Care

Following IMP or ActivHeal application, all wounds included in the study, should be re-dressed using Mepilex dressing (or an alternative dressing in accordance with section 6.7.2) followed by UrgoK2 dual layer compression system as a standardised compression bandage. Dressings should be performed in accordance with the Manufacturer's instructions.

8.3 Efficacy Assessments and Procedures

8.3.1 Clinical Assessment of Complete Debridement

The investigator (or an appropriately delegated member of clinical staff) will be asked at each visit to make a dichotomous (Yes / No) clinical assessment as to whether the wound has achieved "complete debridement" using the following definition:

"*Complete debridement will be considered to have been achieved when the wound bed is clean, and composed of healthy granulating tissue. For the practical purposes of this protocol, this corresponds to the removal of >90% of visible slough."*

8.3.2 Clinical Assessment of Complete Closure

The investigator (or an appropriately delegated member of clinical staff) will also be asked at each visit to make a dichotomous (Yes / No) clinical assessment of whether the wound has achieved complete wound closure, where the following definition is used:

"*Complete wound closure is defined as skin re-epithelialization without drainage or dressing requirements*".

At week 12, a remote review of clinical notes and medical records will be conducted to assess wound closure status.

8.3.3 Clinical Assessment of Thickness of the Slough

The investigator (or an appropriately delegated member of clinical staff) will be asked at each visit to assess the overall thickness of slough present in the wound bed and grade it using the following qualitative categories:

- None (0) – an absence of slough in which the wound is comprised solely of granulating tissue or re-epithelialised tissue.
- Very thin (1) – granulation tissue is clearly visible beneath the any remaining slough.
- Thin (2) – early granulation tissue is beginning can be seen to be emerging to emerge beneath the slough.
- Medium – granulation tissue is not visible beneath the slough.
- Thick – a dense layer of slough likely exceeding 1mm in depth covers the wound surface.

This assessment will be documented at each time point to support evaluation of debridement progression over the course of treatment.

8.3.4 Wound photographs may also be analysed using image analysis software to estimate slough thickness. The height of the visible slough collar relative to surrounding tissue may be used as a surrogate measure of thickness.

8.3.5 Wound Imaging

Wound imaging will be performed by a trained and appropriately delegated member of the clinical team using the Aranz Silhouette Star Camera provided by the Sponsor. This is a CE Marked medical device that has been clinically validated for tracking the healing (surface area / volume) and condition of chronic wounds. Specifically, the imaging system will be used in this clinical trial to determine:

- The surface area of the wound (cm^2) [for calculating volume to dose]
- Mean and median surface area reduction of the wounds [i.e. Partial Area Reductions] from screening to baseline and from baseline to weeks 1, 2, 3 and 4.
- Mean and median changes in perimeter length of the wound (mm) [Linear Wound Healing] from screening to baseline and from baseline to weeks 1, 2, 3 and 4
- The percentage of slough and eschar in the wound relative to surface area (%) at each visit
- The amount of slough and eschar in the wound (cm^2) at each visit
- The percentage of granulation tissue in the wound relative to surface area (%) at each visit
- The amount of granulation tissue in the wound (cm^2) at each visit
- The height of the visible slough collar (mm) at each visit.

The imaging system consists of a hand-held 3D camera as shown in Figure 3.

Figure 3: Illustrative image of the Aranz Silhouette Star Camera System

The imaging system allows for the easy capturing of standardised wound assessment, without the need for directly contacting the wound. The device uses laser line pointers to guide users to ensure correct focus and to take consistent images over time; the automatic flash built into the camera also provides for consistent lighting conditions.

Silhouette uses the three lasers to additionally measure the profile of the wound-bed and the distance of the camera from the wound. Once the distance of the camera from the wound is known, then the scale of the image can be automatically determined without contact with the wound, and the various wound measurements are calculated using algorithms within the camera system.

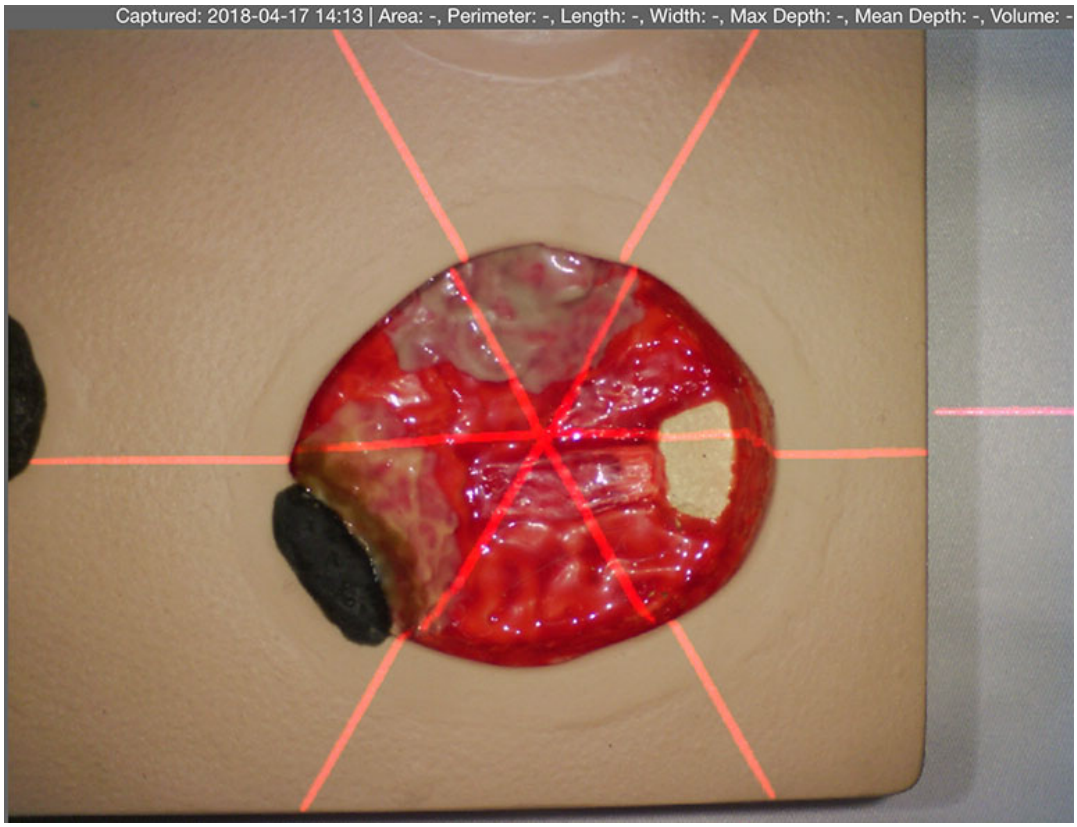


Figure 4: Representative image capture using laser pointers.

The captured image is uploaded to a tablet or laptop and can be either uploaded directly to Silhouette Central immediately (where Wi-Fi is available) or at a later timepoint (if Wi-Fi is not immediately available). Once uploaded to Silhouette Central, the image can be accessed and viewed by an Investigator for assessment purposes, and by the sponsor for monitoring / auditing purposes. All images uploaded to the server are audit trailed in accordance with the requirements of CFR 21 Part 11.

The system nevertheless permits further review and amendment of the automated boundaries within Silhouette Central for clinical staff (log-in / password restriction), allowing for corrections to be made by appropriately delegated clinical staff. Similarly, corrections for content of sloughy tissue (yellow) and/or granulation tissue (red) can be made to accurately track wound slough / granulation tissue and wound size at each visit.

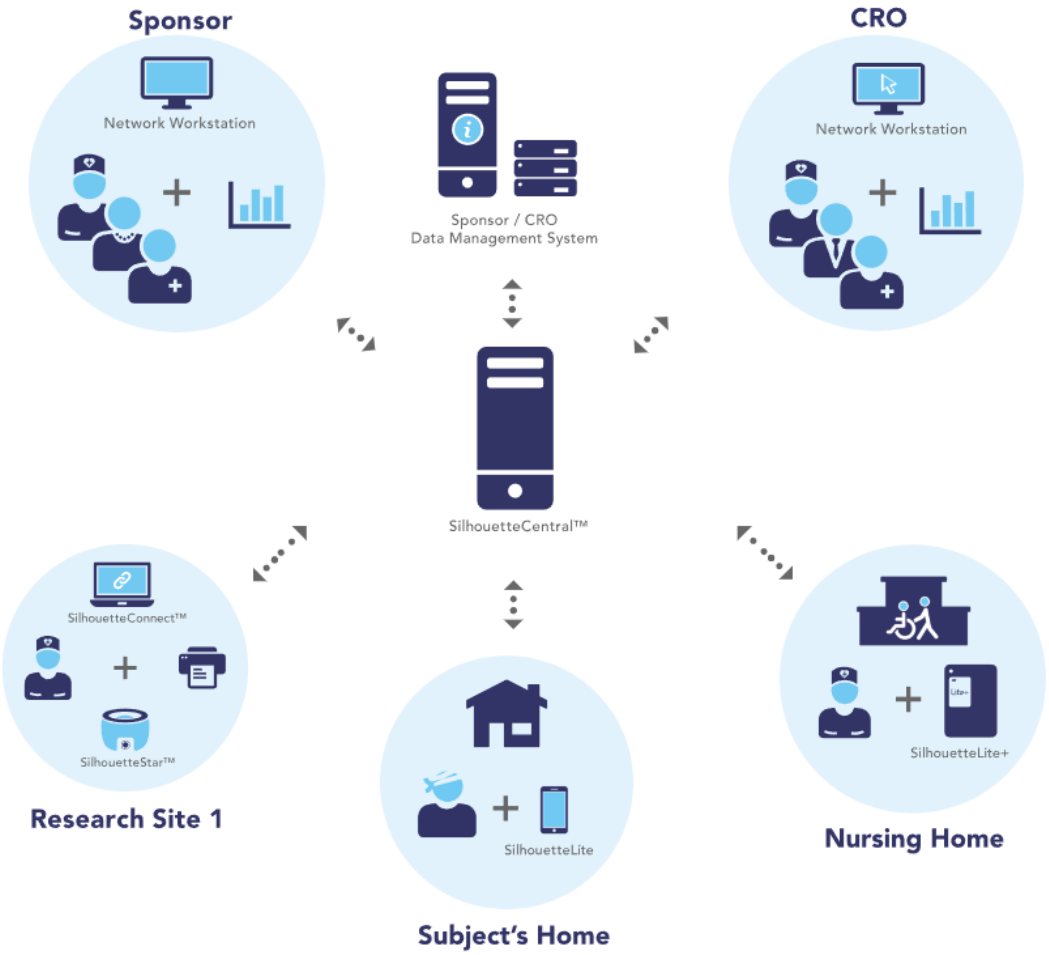


Figure 5: Silhouette Central as used in Decentralised Clinical Trials

An example of one such determination (from clinical trial SC_VLU_001) is shown below:

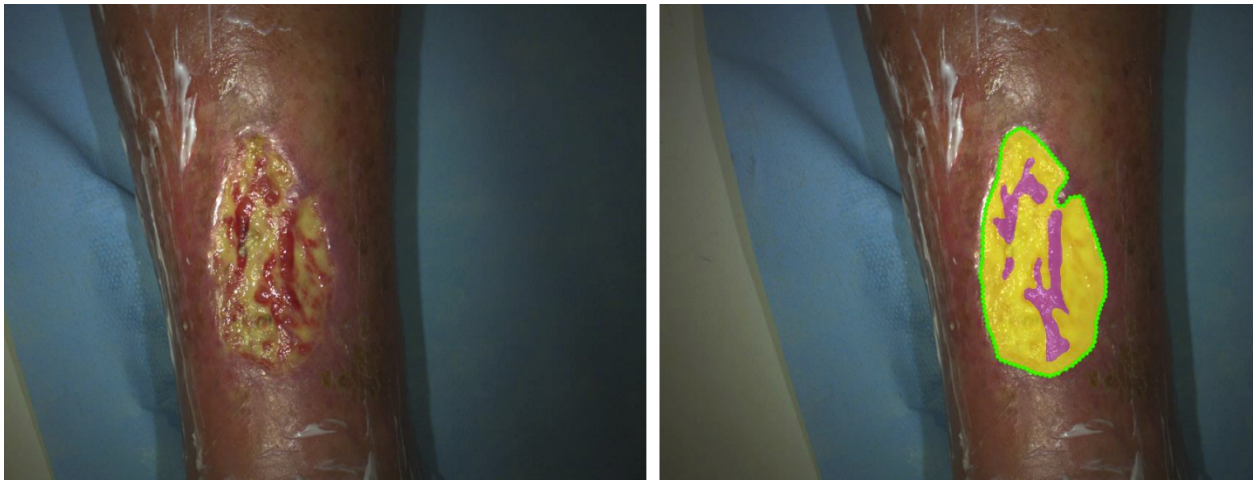


Figure 6: Illustrative review of a non-viable tissue (a) original photo (b) marked areas by automated detection and investigator

The accuracy of the Aranz Silhouette Camera has been validated in numerous independent research studies, clinical studies and in numerous healthcare settings globally. These data indicate that Silhouette Star derived measurements are within:

- 2% for surface area measurements
- 1% for wound perimeter lengths
- 5% for average wound depths
- 5% for volume determinations.

The data from the Silhouette Central database will be exported into a password protected .csv file and will then be imported into the EDC system.

8.3.6 Independent Reader Assessment

In addition to a clinical assessment of the wound by the investigator, an independent reader (Key Opinion Leader- KOL) in chronic wound care will be asked to assess each of the wound images taken with the Silhouette Star imaging system. The independent reader will not reassess wound size but will be asked to assess, based solely on the image to determine:

- Has complete debridement been achieved (Yes / No) in accordance with the definition provided in 8.3.1.
- In the opinion of the KOL, is the wound ready for a skin or tissue graft (Yes / No).

Data will be collected in a password protected .csv file and will then be imported into the EDC system.

8.3.7 Linear Wound Healing Rate

Using data acquired from the Aranz Silhouette Star imaging system, the within patient mean daily wound healing rate over the 2-week run-in period will be compared with the mean daily wound healing rate in the first 2 week-treatment period and with the last 2 weeks of the study, to directly assess treatment related changes in the daily wound healing rate.

The healing rate will also be determined based on Gilman's equation which assesses the linear advancement of wound margins toward the centre of the wound, to calculate healing over time, taking into account the wound's baseline size and shape [27].

The equation is the change in area divided by the average change in perimeter:

$$D = \frac{(Ab - Aa)}{\frac{[pa + pb]}{2}}$$

Where

- D (in millimetres) equals the average distance of the linear advancement of the margins in a direction toward the wound centre
- A equals area (in mm^2);
- a equals the beginning of the observation period (screening)
- b equals the end of the observation period (Day 1 baseline or Week 2)
- p equals the average of the wound perimeter (in mm) over the observation period.

A daily rate of linear healing can then be determined using the calculation:

$$Daily\ healing\ rate = \frac{D}{n}$$

Where:

- D (in millimetres) equals the average distance of the linear advancement of the margins in a direction toward the wound centre as calculated above
- n = number of days over which the observation was performed (14)

In addition to the within-patient linear healing rate, the mean linear healing rate will also be calculated to assess any differences in the healing rates between Aurase Wound Gel and standard of care observed over the four week period of the study.

8.3.8 Wound-QoL

A quality of life measure will be performed using the Wound-QOL (17) instrument as shown below

Wound-QoL questionnaire on quality of life with chronic wounds

With the following questions, we aim to find out how your chronic wound(s) affect(s) your quality of life.

Please tick one box per line!

	In the last seven days ...	not at all	a little	moderately	quite a lot	very much
1	...my wound hurt	O	O	O	O	O
2	...my wound had a bad smell	O	O	O	O	O
3	...there was a disturbing discharge from the wound	O	O	O	O	O
4	...the wound has affected my sleep	O	O	O	O	O
5	...the treatment of the wound has been a burden to me	O	O	O	O	O
6	...the wound has made me unhappy	O	O	O	O	O
7	...I have felt frustrated because the wound is taking so long to heal	O	O	O	O	O
8	...I have worried about my wound	O	O	O	O	O
9	...I have been afraid of the wound getting worse or of new wounds appearing	O	O	O	O	O
10	...I have been afraid of knocking the wound	O	O	O	O	O
11	...I have had trouble moving about because of the wound	O	O	O	O	O
12	...climbing stairs has been difficult because of the wound	O	O	O	O	O
13	...I have had trouble with day-to-day activities because of the wound	O	O	O	O	O
14	...the wound has limited my leisure activities	O	O	O	O	O
15	...the wound has forced me to limit my activities with others	O	O	O	O	O
16	...I have felt dependent on help from others because of the wound	O	O	O	O	O
17	...the wound has been a financial burden to me	O	O	O	O	O

"Wound-QoL" questionnaire on Health-related Quality of Life in Chronic Wounds | Version English (UK), Augustin et al. 2017, Blome et al. 2014

Figure 7 : Wound QoL Questionnaire

The Wound QOL comprises 17 questions, across three subscales; "Body" (Questions #1 – 5), "Psyche" (Question #6-10), "Everyday life" (Questions #11-16) and "Financial burden" (Question 17) each scored on a 0-4 scale where 0 = "not at all" to 4 = "very much" and a total Wound-QoL score summing the individual scores.

Assessment of the Wound QoL will be scored at baseline (before IMP treatment) and at the end of Weeks 4 to assesses the participant's quality of life over the previous 7 days.

The difference in total scores and differences for each of the sub-scales will be calculated (baseline – observation). Lower scores indicate an improved QoL outcome for the patient.

The minimal important difference (MID) in Wound-QoL-17 overall score has previously been determined in a German sample of 227 patients with chronic wounds [28]. MID estimates ranged from 0.47 to 0.52 with a mean MID being determined to be 0.50. This means that a decrease of the Wound-QoL-17 total score of 0.50 or more (i.e., HRQoL improvement) has been used to indicate patient-relevant change.

8.3.9 Forgotten Wound Score

An additional QoL measure "The Forgotten Wound Score" will also be performed as shown in the table below:

Table 4: Forgotten Wound Score

Please tick one box per line

Are you aware of your wound?	Never	Almost Never	Seldom	Sometimes	Mostly	Does not apply
…in bed at night?						
…when sitting on a chair for more than 1 hour?						
…when you are walking for more than 15 minutes?						
…when taking a bath / shower?						
…when travelling in a car or on public transport?						
…when dressing or undressing?						
…when getting up in the morning?						
…when standing for long periods of time?						
…when doing housework, working or preparing meals?						

Table 4: Forgotten Wound Score

Please tick one box per line

Are you aware of your wound?	Never	Almost Never	Seldom	Sometimes	Mostly	Does not apply
…when doing your favourite activity?	☐	☐	☐	☐	☐	☐
…if/when it smells bad?	☐	☐	☐	☐	☐	☐
…if/when it hurts?	☐	☐	☐	☐	☐	☐
Scores						

Mean Score: _____

Total Score: _____

Unlike the Wound-QoL, this PRO focusses on asking the patients about their experience of having to think about their wound under a set of proscribed situations. The total score is calculated:

- By summing the responses where never=0, almost never = 1, Seldom = 2, sometimes = 3 and Mostly = 4. The sum is then divided by the number of completed items to derive a mean score for the patient.
- Questions that are answered "do not apply to me" are treated as a missing value. Where there are more than 4 lines checked in this category, the questionnaire cannot be used.
- The mean score obtained is subsequently multiplied by 25 to obtain a total score in the range of 0-100
- Finally, the total score is subtracted from 100 to change the direction of the final score in such a way that high scores indicate a high degree of "forgetting" the wound.

8.4 Safety Assessments and Procedures

8.4.1 Adverse Events (AEs)

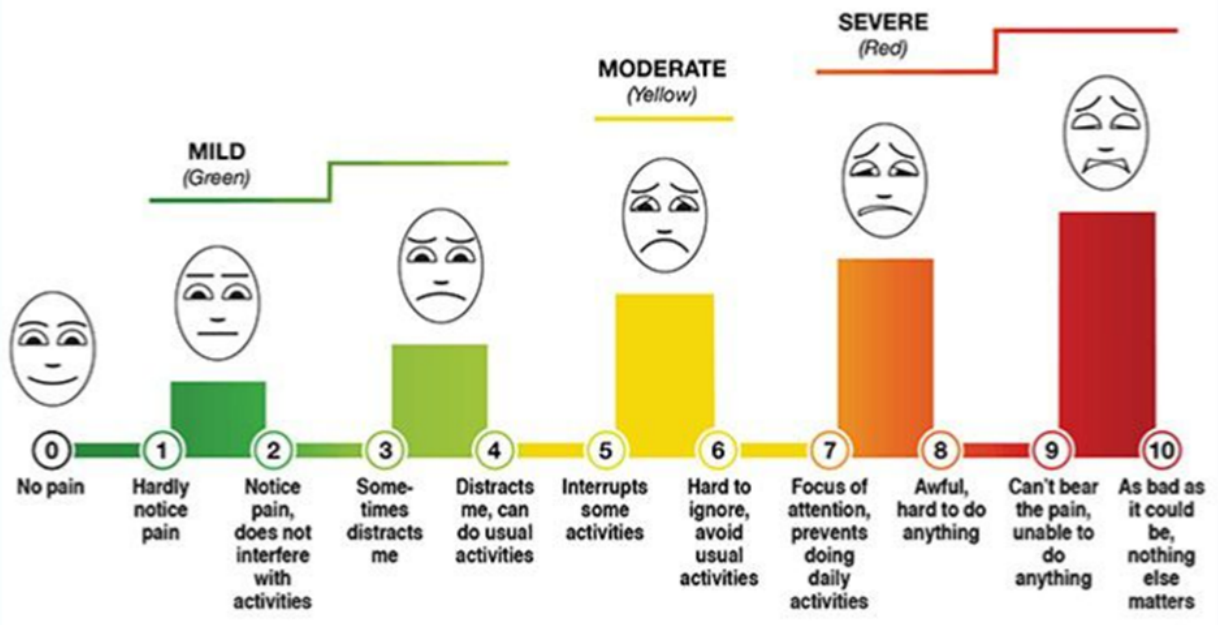
The Investigator will assess any AEs experienced by the participant at each assessment using a general open question such as "How are you feeling?" In addition, AEs will be assessed by the investigator upon examination of the reference ulcer and peri-wound site.

AEs will be recorded and assessed as described in Section 8.5 and Appendix (Section 12).

8.4.2 Assessments of Pain

An assessment of pain at the wound site will be completed twice by the participant at each dosing visit (i) shortly prior to the wound dressing change and (ii) within 15 mins of application of the gel.

The assessment of pain will utilise an 11-point NRS as set out overleaf:



8.4.3 Local Tolerability Assessments

When dressings are taken down at each scheduled visit and the wound cleansed, the investigator (or clinically designated member of the clinical team) will be asked to perform a clinical assessment of the wound for each of the following localised wound characteristics, using 5-point Likert scales, scored as zero (none) through to 4 (severe):

Erythema as:

0 - None (normal skin)

1 - Mild (faintly detectable erythema, very light pink)

2 - Moderate (pink and distinguishable)

3 - Marked (dull red, clearly distinguishable)

4 - Severe (deep dark red and spreading from the wound margins)

Oedema as:

0- None

1- Mild (<2mm swelling)

2 - Moderate (2-5mm swelling, localised to wound margins)

3 - Marked (>5mm swelling, spreading from wound margins)

4 - Severe (>5mm swelling, deep oedema, spreading extensively from wound margins)

Exudate as:

0 - None

1 - Mild (wound tissues moist)

2- Moderate (wound tissues wet)

3 - Marked (wound tissues saturated)

4 - Severe (wound tissues bathed in fluid and drainage freely expressed - requires ≥1 daily dressing change)

Induration as: -

0 - None

1 - Mild (Induration <2cm around the wound)

2 - Moderate (Induration 2-4cm extending <50% around the wound)

3 - Marked (Induration 2-4cm extending ≥50% around the wound)

4 - Severe (Induration >4cm in any area)

In addition, the parameters of bleeding and infection will be assessed as either absent (score = 0) or present (score =1).

8.5 Adverse Events and Serious Adverse Events

8.5.1 Definitions of AE and SAE

An Adverse Event (AE) will be defined as any untoward medical occurrence in a clinical trial participant who has been administered an interventional treatment and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, disease or exacerbation of a pre-existing condition temporally associated with the use of a medicinal (investigational) product. Please refer to section 12.1 for further details.

A Serious AE (SAE) is defined as any untoward medical occurrence that at any dose results:

- in death;
- is life threatening;
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability / incapacity;
- or results in a congenital anomaly / birth defect.

Please refer to section 12.2 for further details.

8.5.2 Time Period and Frequency for Collecting AE and SAE Information

AEs and SAEs will be collected at each visit from consenting of the participant through to the last visit at Week 4.

8.5.3 Identifying AEs and SAEs

The responsibility for identifying AEs and SAEs will lie with the investigator (or delegated clinical site staff). At each of the respective assessment timepoints the investigator will assess any AEs experienced by the participant at each assessment using a general open question such as “How are you feeling?”, combined with the investigators own assessment of the wound (including events occurring at the wound site).

8.5.4 Recording of AEs and SAEs

All AEs which occur between informed consent and the end of week 4 will be recorded in the eCRF such that study related interventions as well as IMP related events can be captured. As patients end direct involvement in the study at week 4, and as the week 12 medical notes review is intended solely to determine the “healed status” of the treated wound, no AEs will be collected between week 5 and 12.

8.5.5 Follow up of AEs and SAEs

Follow-up of the AE or SAE, after the date of therapy discontinuation, is required if the AE or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilise at a level acceptable to the Investigator or their designated representative

8.5.6 Reporting of SAEs

All serious adverse events (as defined below) regardless of treatment group or suspected relationship to trial drug must be reported within 24 hours, by emailing a completed Serious Adverse Event / Follow-up form to the Medical Monitor, at:

Safety@SolasCure.com

The responsible Medical Monitor, in combination with Sponsor's Responsible Physician, will assess all serious adverse events and where appropriate, report any Serious Adverse Reaction (SAR) i.e. a SAE considered attributable to treatment, to the regulatory authorities and ethics committee according to standard operating procedures.

8.5.7 Regulatory Reporting Requirements for SAEs

All SAEs will be recorded in a SAE log and trended on a periodic basis to ascertain any trends and/or changes required to the Reference Safety Information for the Aurase Wound Gel.

8.5.8 Serious Adverse Reaction (SAR) Reporting

SAEs which have causality assessed as possible, probable or almost definitely related to the Aurase Wound Gel (i.e. SARs), will be reported to MHRA, Ethics Committee (s) and Clinical Investigators. Initial reports of SARs will be made within 7 calendar days for deaths or life-threatening conditions and within 15 days for all other SARs in accordance with regulatory requirements.

8.5.9 Adverse Events of Special Interest

In line with the Investigator Brochure, and consistent with commitments made, special attention will be given to any sub-acute reactions, observed in participants, which may be indicative of a delayed immunogenic reaction including delayed onset of fever, rash, arthralgia, myalgia, observable haematuria, proteinuria, serositis, central nervous system complications and haemolytic anaemia. In such cases, SolasCure would commit to expedited reporting of these suspected ADRs.

8.5.10 Disease-related Events or Outcomes NOT Qualifying as AEs or SAEs

Localised symptoms at the wound site (e.g. wound erythema, wound oedema, wound exudate, wound induration, wound pain, bleeding, wound maceration) are all common features of VLUs and can be influenced not only by trial interventions but by disease related events and their management under standard of care. Small changes in reported levels of wound symptoms are expected and will not be considered as adverse events, unless and to the extent that the investigator considers that the severity of increase is greater than would be expected given the baseline condition of the wound.

8.6 Pregnancy and Post-partum Information

8.6.1 Participants Who Become Pregnant During the Trial

Based on non-clinical and clinical evidence, there is little or no systemic availability of tarumase following cutaneous application. No effects on reproductive effects are therefore anticipated.

Pregnancy testing will nevertheless be performed on WOCBP to confirm that participants are not pregnant on the first day of dosing. If patients subsequently report become pregnant during the clinical trial, dosing for that patient will be stopped and the participant withdrawn from the clinical trial. Participants that become pregnant will however be followed up to ascertain a normal birth.

8.6.2 Participants Whose Partners Become Pregnant

Based on non-clinical and clinical evidence, there is little or no systemic availability of tarumase following cutaneous application. No effects on reproductive effects are therefore anticipated. Consequently, no anticipated follow-up of participants, whose partners become pregnant is planned in this study.

8.7 Medical Device Product Complaints for Drug/Device Combination Products

Medical devices including standard of care dressings such as ActivHeal, Mepilex [Allevyn Non-Adhesive] and UrgoK2 dual layer compression system will be used as part of standard of care in this clinical trial. Any complaints or adverse events reported as part of this clinical study, and specifically relating to the medical devices will be forwarded to the device Manufacturer (AMS Health, Molnlycke Healthcare, Smith & Nephew or Urgo) respectively.

8.8 Pharmacokinetics

Data from prior non-clinical testing and the Phase IIA clinical safety study indicate that topically applied tarumase at up to 10.92 $\mu g/cm^2$ could not be detected within the systemic circulation as a result of absorption. As the total daily exposure in this study is largely consistent ($12\mu g/cm^2$) with the Phase IIa clinical trial, no additional pharmacokinetic testing is proposed as part of this clinical study.

8.9 Genetics

No genetics information will be collected in this clinical study. This section is therefore not applicable.

8.10 Biomarkers

No biomarker information will be collected in this clinical study. This section is therefore not applicable.

8.11 Medical Resource Utilisation and Health Economics

No medical resource utilisation and/or health economic data will be collected in this clinical study. This section is therefore not applicable.

9 STATISTICAL CONSIDERATIONS

Data management and statistical analysis for this clinical trial will be undertaken by an independent clinical statistician according to defined standard operating procedures.

The statistical analyses of the clinical study will be performed according to a Statistical and Analysis Plan (SAP), which will be finalised prior to unblinding of the database and will be included as an appendix to the Clinical Study Report. Any ad-hoc analysis and/or data mining undertaken following unblinding of the trial will be fully reported in the Clinical Study Report.

9.1 Analysis Sets

Four patient population sets are planned for analysis:

a) **Enrolled Population**: Consisting of all subjects consented to the clinical trial at screening, and involved in the run-in period, including screen failures and subjects that did not go onto receive interventional treatment.

b) **Safety population** - Consisting of all subjects administered at least one dose of investigational product and have at least one post-dose safety assessment.

c) **Intent to Treat (ITT) population** - Consisting of subjects who are randomised, receive at least one dose of investigational product, and have at least one assessment of efficacy measures.

d) **Per Protocol population** – A subset of the ITT population consisting of subjects who do not have any major protocol violations or missing primary efficacy data.

9.2 Analysis Supporting Primary Objective

9.2.1 Statistical Model, Hypothesis and Method of Analysis

The primary analysis of safety will be based solely on descriptive statistics (frequency of events, and severity of events) in the respective patient populations.

The key analyses for efficacy (secondary objective) will be undertaken on the mean reduction of slough area [as a proportion of baseline slough area] achieved over the 4 week period (i.e. the debridement efficiency of treatment) and the mean reduction in wound surface area (cm^2) compared to the baseline surface area (i.e. the partial area reduction of the wound). An additional secondary endpoint will assess the proportion of patients that achieve “complete debridement”.

The null hypothesis is that there is no difference between the two arms (Group 1 and Group 2).

The alternative hypothesis is that there is a significant difference between at the two arms.

The detailed statistical model for analysis will be prepared by an independent statistician and will be set out in the SAP.

9.2.2 Handling of Missing Data

Any data missing from the primary analysis will be managed as follows:

- Any participants achieving “complete debridement” within the 4 week period, will have their data imputed as Last Observation Carried Forward; i.e. “complete debridement” will be assumed for the remaining observations in the 4 week period through to the primary endpoint.

- Where patients discontinue treatment prior to the end of the 4 week period, remaining visits will be imputed based on Last Observation Carried Forward.

9.2.3 Sensitivity Analysis

The primary efficacy analysis will be performed using the ITT population; a sensitivity analysis will be performed using the PP population.

9.3 Interim Analysis

A formal interim analysis will be conducted after the last patient completes Week 4 to evaluate safety, debridement outcomes, and partial wound healing, addressing the primary and key secondary objectives of the study. Follow-up in the study will however continue through until end of trial; Last Participant Last Visit at Week 12 (patient review), at which point exploratory outcomes, including complete wound closure, will be assessed.

9.4 Sample Size Determination

Using an assumption of >60% mean debridement (±25%) and >60% mean partial area reduction (±25%) within 4 weeks for Aurase Wound Gel and a mean of 20% debridement and partial area reduction for standard of care (ActivHeal), a revised (reduced) sample size of 20 patients is calculated to achieve 80% power with an overall alpha of 5%.

9.5 Protocol Deviations

Protocol deviations will be recorded by the Investigator in the eCRF and/or by the Clinical Monitor within a deviations log maintained during the live phase of the clinical trial. Protocol deviation categories will be prospectively defined by the Sponsor into a deviation category list as either “Not clinically important” = minor protocol deviations or “Clinically important” = major protocol deviation. Reported deviations will be categorised accordingly, unless there is sufficient clinical justification for reclassifying them.

10 GENERAL CONSIDERATIONS: REGULATORY, ETHICAL AND TRIAL OVERSIGHT

10.1 Regulatory and Ethical Considerations

The Sponsor will ensure that local regulatory requirements including approval of MHRA and IEC are met before the start of the study. The Sponsor will be responsible for the preparation, submission and confirmation of receipt of MHRA and IEC approvals before IMP shipment to the trial centres.

This protocol will comply with the Declaration of Helsinki (2008).

This protocol also accords with the principles of the 18th World Medical Assembly (Helsinki 1964) and amendments of the 29th (Tokyo 1975), the 35th (Venice 1983), the 41st (Hong Kong 1989) and the 48th (Somerset West [South Africa] 1996), the 53rd (Washington 2002), 55th (Tokyo 2004) and 59th (Seoul 2008) World Medical Assemblies.

10.2 Committees

If during the study a threshold level of 15% “severe” attributable Adverse Drug Reactions (ADRs) is met or a single attributable Serious Adverse Reaction (SAR) is reported then a temporary halt will be made to the study whilst a safety committee is asked to assess the

continued safety of treatments and/or to make recommendations on reducing concentration or frequency of administration.

The safety committee (SC) will include the Sponsor's Responsible Medical Officer, Medical Monitor, Trial Manager, and Principal Investigator as a minimum.

The SC will be responsible for performing and assessing the outcome of safety assessments (adverse event, local tolerability reactions) for enrolled patients and for making recommendations on either reducing the concentration of tarumase, reducing the dosing frequency and/or identifying an alternative recommendation, including permanently ceasing the clinical trial. The outcome of the safety committee will be presented to MHRA and IEC and approval must be received before the trial can be recommenced.

10.3 Informed Consent Process

It is the responsibility of the investigator (or delegated clinical staff) to obtain written Informed Consent from subjects in accordance with all applicable local, national and international regulations and guidance. All consent documentation must be in accordance with applicable regulations and ICH GCP. Each participant is requested to sign the Informed Consent Form after the participant has received and read written information and received an explanation of what the trial involves, including but not limited to: the objectives, potential benefits and risk, inconveniences and the patient's rights and responsibilities. A copy of the informed consent documentation (Participant Information and the Consent Form, as applicable) must be given to the participant. Signed consent forms must remain in each participant's medical notes and must be available for verification by study monitors at any time.

10.4 Data Protection

Data collected during this trial may be used to support the development, registration or marketing of Aurase Wound Gel within this specific indication. The Sponsor will control all data collected during the trial, and will abide by the UK Legal Requirements on Data Privacy concerning the processing and use of participant's personal data. For the purpose of UK data privacy legislation, SolasCure Ltd will be the data controller.

After subjects have consented to take part in the trial their medical records and the data collected during the trial will be reviewed by the Sponsor or their representatives. These records and data may, in addition, be reviewed by the following: independent auditors who validate the data on behalf of Sponsor; third parties with whom Sponsor may develop, register or market Aurase Wound Gel; national regulatory authorities and the IEC which gave their approval for this trial to proceed.

Although subjects will be known only by a unique patient randomisation number, their initials and date of birth will also be collected and used to assist Sponsor to verify the accuracy of the data. The results of this trial containing the unique number, initials, date of birth and relevant medical information may be recorded and transferred to and used in other countries throughout the world, which may not afford the same level of protection that applies within the UK. The purpose of any such transfer would be to support regulatory submissions made by SolasCure in order to market Aurase Wound Gel in other countries.

10.5 Early Site Closure or Trial Termination

The Sponsor retains the right to terminate the clinical trial at any point and/or to initiate early site closure. When the study is terminated prematurely, the regulators and IEC/IRBs will be notified accordingly.

11 GENERAL CONSIDERATIONS: RISK MANAGEMENT AND QUALITY ASSURANCE

11.1 Quality Tolerance Limits

No quality tolerance limits have been pre-specified in the study except in regard to treatment intervention compliance (please refer to section 6.6).

The Sponsor will ensure that all persons involved in the preparation, execution and/or reporting of this clinical study will be appropriately GCP-trained. Copies of GCP training will be maintained.

11.2 Data Quality Assurance

SolasCure will undertake their roles for this trial in compliance with all applicable regulations, ICH GCP Guideline E6(R2), ICH E8(R2), with UK SI 2004/1031 (The Medicines for Human Use (Clinical Trials) Regulations 2004, as amended and in accordance with sponsor's (or delegates) Standard Operating Procedures.

In this context, the Sponsor will appoint monitors to conduct the monitoring of this clinical trial, which will be conducted according to agreed monitoring conventions. The monitor will review the source documents for accuracy of data recording and for compliance with the protocol, ICH GCP guidelines and applicable regulatory guidelines.

Records and data may additionally be reviewed by Quality Assurance auditors or by regulatory authorities.

11.3 Source Data

Source Data will be information in the participant's medical notes (SystmOne medical record), hard copy of the CRF [completed contemporaneously with treatment] or information directly captured in the eCRF and/or digital imaging software (Silhouette Connect). Source data will include all information in source documents (original records, certified copies of original records and electronic records) and include all clinical findings, observations, or other activities during the study necessary for the reconstruction and evaluation of the study.

Source data verification (SDV) is the comparison of source data other than the eCRF to the data recorded in the eCRF. SDV will be performed to ensure that information transcribed into the eCRF was complete and consistent with source documents and to meet the protocol requirements. SDV will be performed by the monitor during each on-site monitoring visits and will included a review of:

- Informed Consent
- Visit Date
- Demographics
- Medical History
- History of Diagnosis
- Eligibility
- Physical Examination
- Vital Signs
- Urine Pregnancy Test
- Assessments of Pain
- Investigational Medicinal Product Preparation & Dosing
- Wound Assessments including wound imaging and clinical assessments.

- Completion Withdrawal
- Concomitant Medications
- Adverse Events
- Concomitant Procedures
- Protocol Deviations

12 APPENDIX: ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS – DEFINITIONS, SEVERITY AND CAUSALITY

12.1 Further Details and Clarifications of the AE Definition

An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject who has been administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, disease or exacerbation of a pre-existing condition temporally associated with the use of a medicinal (investigational) product [29].

Events involving adverse drug reactions (ADRs), illnesses that onset during the trial, or exacerbations of pre-existing illnesses should be recorded. Exacerbation of pre-existing illness is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the trial. It may include worsening or increase in severity of signs or symptoms of the illness increase in frequency of signs and symptoms of an intermittent illness, or the appearance of a new manifestation/complication. Exacerbation of a pre-existing illness should be considered when a patient requires new or additional concomitant drug or non-drug therapy for the treatment of that illness during the trial. Lack of or insufficient clinical response, benefit, efficacy, therapeutic effect or pharmacological action, should not be recorded as an AE. The Investigator must make the distinction between exacerbation of pre-existing illness and lack of therapeutic efficacy.

In addition, abnormal objective test findings that result in a change in trial drug dosage or in discontinuation of the drug, or require intervention or diagnostic evaluation to assess the risk to the volunteer should be recorded as AEs. Clinically significant changes in physical examination findings should also be recorded as AEs.

For all AEs, the Investigator must pursue and obtain adequate information both to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious adverse event (SAE) requiring immediate notification to SolasCure.

For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE (i.e. investigational product or other illness). The Investigator is required to assess causality and indicate that assessment on the eCRF. Follow-up of the AE, after the date of therapy discontinuation, is required if the AE or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilise at a level acceptable to the Investigator or their designated representative.

The Investigator must supply all subjects with a card detailing the trial protocol number and the Investigator's name. This card should provide details of 24-hour contact numbers that can be called in the event of a medical emergency.

12.2 Further Details and Clarifications of the SAE Definition

A SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening;
- Requires hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability / incapacity;
- Results in a congenital anomaly / birth defect.

Medically significant events that may not result in death, be life-threatening, or require hospitalisation may be considered serious adverse drug experiences when, based upon appropriate medical judgement, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalisation or the development of drug dependency or drug abuse.

Serious Adverse Events which have causality assessed as possible, probable or almost definitely related to the IMP will be reported as Serios Adverse Reactions (SARs) to MHRA, IEC and Clinical Investigators. Initial reports of SARs will be made within 7 calendar days for deaths or life-threatening conditions and within 15 days for all other SARs.

12.3 Severity

For the purposes of the clinical study, AE severity will be classified by the investigator according to the following definitions:

- **MILD**:Discomfort noticed but does not interfere with the patient's daily routines.
- **MODERATE**: Discomfort sufficient to interfere with some aspect of the patient's daily routines.
- **SEVERE**: Discomfort so severe it prevents patient continuing daily routines. Note that the term 'serious' is usually associated with events that pose a threat to the patient's life or functioning. The term 'severe' is used to describe the intensity of a specific event but the event may itself be of minor clinical significance e.g. a headache.

12.4 Causality

Determining the relationship of an AE to the investigational drug or study procedure will be the responsibility of the investigator and is based on clinical judgement. The following guidelines should be used, which are based on the Karch-Lasagna classification [30]:

- **ALMOST DEFINITE:** Distinct temporal relationship with drug treatment. Known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot be explained by patient's clinical state or other factors.
- **PROBABLE:** Reasonable temporal relationship with drug treatment. Likely to be known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot easily be explained by patient's clinical state or other factors.
- **POSSIBLE**: Reasonable temporal relationship with drug treatment. Event could be explained by patient's clinical state or other factors.

- **UNLIKELY**: Poor temporal relationship with drug treatment and/or Event easily explained by subject's clinical state or other factors.
- **UNRELATED**: Event occurred before dosing or Event or intercurrent illness due wholly to factors other than drug treatment.

13 APPENDIX: DEFINITIONS AND SUPPORTING OPERATIONAL DETAILS

13.1 Contraception and Pregnancy Testing

13.1.1 Definitions Relating to Childbearing Potential

Pregnancy testing and contraception are not required for women not of child-bearing potential, including postmenopausal women or those with documented hysterectomy or bilateral oophorectomy. Postmenopausal women must be amenorrhoeic for at least 12 months in order not to be considered of child-bearing potential. Postmenopausal status will be confirmed by measurement of FSH.

13.1.2 Contraception

For the purposes of the study, all WOCBP, must utilise highly effective contraceptive measures for the duration of the clinical study. These includes stable use of combined (oestrogen and progestogen containing) hormonal contraception (oral, intravaginal, transdermal) or progestogen-only hormonal contraception (oral, injectable, implantable) associated with inhibition of ovulation initiated 2 or more menstrual cycles prior to screening; intrauterine device (IUD); intrauterine hormone-releasing system (IUS); bilateral tubal ligation; vasectomised partner; and sexual abstinence.

Note: Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient.

13.1.3 Pregnancy Testing

For WOCBP, a urine pregnancy test strip for the determination of hCG in urine specimens will be used to obtain a visual, qualitative result of pregnancy in all WOCBP at screening and Day 1 (immediately pre-treatment) to assure that the patient is not pregnant prior to dosing. If pregnancy is suspected, the patient will not be randomised to treatment.

13.2 Prior Protocol Amendments

Not applicable. This is the original version of the study.

14 APPENDIX: GLOSSARY OF TERMS

ADR	Adverse Drug Reaction
AE	Adverse Event
BMI	Body Mass Index
cm	Centimetres
eCRF	Electronic Case Record Form
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HCG	Human Chorionic Gonadotropin
ICH GCP	International Conference on Harmonisation : Harmonised Tripartite Guideline for Good Clinical Practice
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
ITT	Intention-to-Treat Population
LOCF	Last Observation Carried Forward
LPFV	Last Patient First Visit
LPLV	Last Patient Last Visit
Max	Maximum
MedDRA	Medical Dictionary for Drug Regulatory Affairs
MCV	Mean Cell Volume
Min	Minimum
mm	Millimetre
Mo	Month (in this trial refers to a period of 28 days)
µl	Microlitre
PP	Per Protocol Population
SAE	Serious Adverse Event
SDV	Source Data Verification
SUSAR	Suspected Unexpected Serious Adverse Reaction

TMF	Trial Master File
VLU	Venous Leg Ulcers
WOCBP	Women of Child Bearing Potential
UK	United Kingdom of Great Britain and Northern Ireland

15 APPENDIX: REFERENCES

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