**Leeds Institute of Rheumatic and Musculoskeletal Medicine**

# FINAL ANALYSIS REPORT

**Study Short Title:** Targeted therapy using intradermal injection of Etanercept for remission induction in Discoid Lupus Erythematosus (TARGET-DLE)

**Study Full Title:** A single arm, phase II open label trial to investigate the efficacy and safety of intra-dermal injection of etanercept for remission induction in discoid lupus erythematosus

**Sponsor Name:** University of Leeds

**Sponsor Number:** RR15/114

**UKCRN ID:** 20582

**REC No:** 15/YH/0257

**MHRA Ref No:** 16767/0279/001-0001

**EudraCT Number:** 2015-001602-33

**ClinicalTrials.gov ID:** NCT02656082

**Chief Investigator:** Prof Paul Emery
**Principal Investigator:** Dr Md Yuzaiful Md Yusof
**CTRU Principal Statistician:** Dr Duncan Wilson
**CTRU Head of Trial Management:** Dr Catherine Fernandez

# Table of Contents

## Introduction

Discoid lupus erythematosus (DLE) is a chronic, autoimmune inflammatory skin condition and a form of chronic cutaneous lupus erythematosus (CCLE). Chronic discoid lesions develop in up to 25% of patients with SLE but may also occur in the absence of any other clinical features of SLE [1]. Patients with DLE usually have only a negative or low titre ANA [2]. In these patients with positive autoantibodies, there is approximately 5 to 10% risk of eventually progressing to SLE, which usually tends to be mild [3]. Hence the pathogenesis of DLE appears to be different from other systemic features of SLE, with a less clear role for circulating autoantibodies.

There is an unmet need for new therapies to control inflammation in DLE. A significant proportion of DLE patients (with or without SLE) are resistant to conventional therapies [4]. There is no clinical guideline or algorithm on how to manage DLE patients who have refractory disease to the first line agents, i.e. anti-malarials. Combination therapy of anti-malarial agents and high dose oral steroid may be effective but will lead to unacceptable complications from excess corticosteroid use including osteoporosis, metabolic consequences and an increased the risk of major cardiovascular events. Importantly, if left untreated, uncontrolled inflammation in DLE will lead to permanent disfiguring and irreversible scarring, thus posing a major cosmetic issue for the patient, which will significantly impair their quality of life [5, 6].

Targeted therapy based on immunopathogenesis of DLE is an attractive approach. Our previous work have shown that DLE may be exacerbated by B-cell depletion therapy with rituximab [7]. Moreover, the common occurrence of DLE in ANA-negative patients without lupus in other organs also suggests that B cell-targeted therapy may not be effective for this manifestation.

TNF is highly expressed in discoid lupus lesions and is implicated in the pathogenesis of DLE [8-10]. A concern with systemic TNF-blocker administration is induction of pathogenic autoantibodies and flare of disease. Approximately 0.5-1.0% of patients treated with systemic TNF-blockers develop high affinity IgG autoantibodies to anti-dsDNA, that were associated with mild lupus-like syndromes [11]. This could be overcome using a low-dose intra-lesional injection, which might be sufficient to neutralise the TNF in lesions. TNF-blockers have been administered using an intra-

lesional injection in other TNF-mediated diseases such as Crohn's [12-14] and ankylosing spondylitis patients with refractory Achilles enthesitis [15], and appear to be safe and similarly effective to systemic administration.

Another important challenge is the problem with outcome measures in DLE. The assessment of disease activity may be difficult owing to the concurrent infection and multiple comorbidities, often present in these patients. Additionally, currently available instruments such as the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) [16], Score of Activity and Damage in DLE (SADDLE) [17] and the mucocutaneous domain of the SLE Disease Activity Index version 2000 (SLEDAI-2K) [18] and British Isles Lupus Assessment group (BILAG)-2004 index [19], rely on subjective assessment. Potential novel objective outcome measures to assess tissue response to therapy including an histology score of skin biopsy, optical coherent tomography (OCT), laser doppler imaging (LDI) and infrared thermography have not been utilised in a clinical trial.

Therefore, the TARGET-DLE trial addressed these problems by (i) administering an existing TNF blocker, etanercept using a novel route of administration (intra-dermal), which would provide local concentration to neutralise TNF in tissue whilst minimised the effect to systemic immunity and (ii) measuring tissue response using the existing outcome measure; the modified limited SADDLE (ML-SADDLE) as well as objective measures such as skin biopsy, OCT, LDI and thermography. The concept and rationale of this study is illustrated in **Figure 1.**

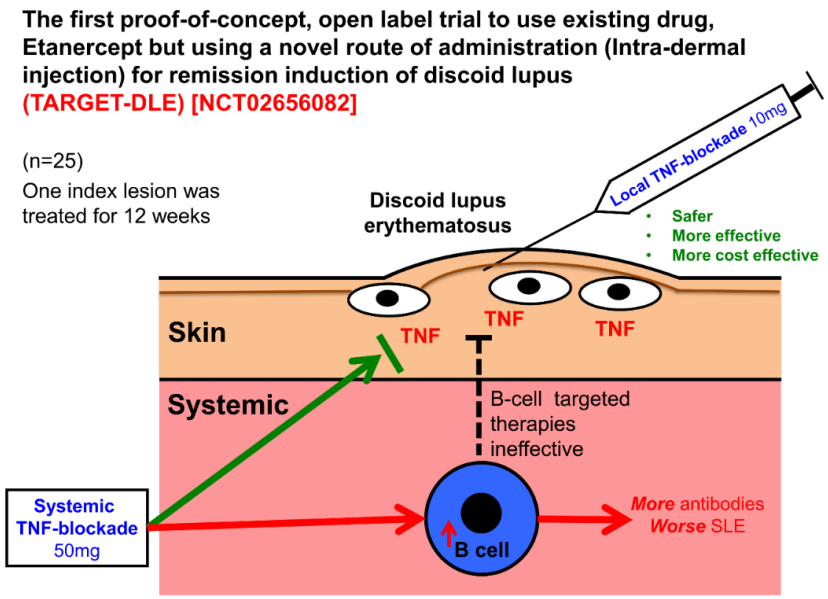


**Figure 1: Concept and rationale of TARGET-DLE**

TNF is implicated in the pathogenesis of DLE, thus should be targeted (green arrow). However, prolonged systemic administration of TNF-blockade therapy may activate B-cells (red arrow pointing upwards) by suppressing the production of Th1 cytokines, thereby driving the immune response towards Th2 cytokine production, IL-10, and IFN-α, a hypothesis called cytokine shift. These cytokines then activate B-cells and lead to increase production of autoantibodies, which may render lupus worse or trigger a lupus-like syndrome. Therefore, we hypothesised that this induction of systemic autoimmunity could be minimised using intra-dermal injection of etanercept in DLE lesion. DLE: discoid lupus erythematosus; IFN-α: interferon-alpha; IL-10: interleukin-10; TNF: tumour necrosis factor; Th1: Helper T-cell type 1; Th2: Helper T-cell type 2

## Hypothesis

Targeting TNF using an intra-dermal injection of etanercept is effective and safe for remission induction in DLE without inducing systemic autoantibody production.

## Objectives

### Primary

To assess the proportion of patients with active DLE that achieved the ML-SADDLE response (defined as reduction ≥20% in total activity from baseline) in the index lesion at Week 12 following treatment with weekly intra-dermal injection of etanercept.

### Secondary

i. To assess other efficacy variables including higher hurdle endpoints such as ML-SADDLE 50 and 70 response rates, physician's VAS of global assessment of disease activity and daily oral corticosteroid requirement
ii. To evaluate patient-reported outcomes including Dermatology Life Quality Index and Patient's VAS
iii. To assess change in lesional OCT, LDI, thermography and histopathology score
iv. To report the safety of therapy in terms of adverse events (AEs), adverse reactions (AR), serious adverse events (SAEs), serious adverse reactions (SAR), suspected unexpected serious adverse reaction (SUSAR)
v. To evaluate the effect of therapy to systemic immunity through development of SLE in patients with DLE only or worsening of SLE disease activities in patients with established SLE
vi. To assess whether intra-dermal delivery of administration is associated with accumulation of drug in systemic circulation

# Methods

## Study design

A prospective single arm, Simon's 2-stage minimax design with Hybrid adaptation, phase II open label trial was conducted in Leeds from 1 February 2016 to 31 December 2017. This study was registered with ClinicalTrials.gov, number NCT02656082.

Simon's 2-stage minimax design was chosen due to the advantage of allowing the minimum total number of patients needed to be treated with a new treatment that might be ineffective [20]. While a hybrid adaptation of the 2-stage design was implemented to allow for recruitment to continue while the results of the first stage of recruitment were generated in the interim analysis [21].

## Ethical approval

All patients provided informed written consent and this study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was gained from the National Research Ethics Committee Yorkshire and Humber, Sheffield [15/YH/0257] and the Medicines and Healthcare products Regulatory Agency [16767/0279/001-0001]. The University of Leeds was contracted with the administrative sponsorship.

## Patients

### Inclusion criteria

i. Adults aged 18-80 years old.
ii. Had at least one active DLE lesion, either diagnosed by skin biopsy or confirmation by Dermatologist/ Rheumatologist.
iii. Patients with DLE only and SLE patients with DLE were included.
iv. Had refractory disease to an anti-malarial for at least 3 months as assessed by Dermatologist or Rheumatologist.
v. Patients receiving anti-malarials must had been receiving them for at least 3 months prior to Screening, with a stable dose regimen for at least 28 days (±1 day) prior to Baseline (the first study drug administration)
vi. Ability to provide an informed consent.

vii. All male and female patients biologically capable of having children agreed to use a reliable method of contraception for the duration of the study and for a period of 3 weeks after their final dose of study drug. Acceptable methods of contraception were surgical sterilisation, oral, implantable or injectable hormonal methods, intrauterine devices or barrier contraceptives.

**Exclusion criteria**

i. Any prior treatment with TNF-blockade therapies.
ii. Intramuscular or intra-dermal corticosteroid within 28 days of the Screening visit.
iii. Corticosteroid of greater than 10mg prednisolone daily equivalent, or change in oral steroid dose within 28 days prior to Baseline Visit.
iv. A change in the dose of other immunosuppressant including methotrexate, azathioprine and mycophenolate mofetil within 28 days (±1 day) prior to Baseline Visit.
v. Concomitant therapies with any alkylating agents (e.g. cyclophosphamide, chlorambucil), other immunosuppressant including sulfasalazine and leflunomide, other biological agent particularly anakinra and abatacept and other experimental drug. If patients were on any of these, they had to be off therapies for at least 28 days prior to Baseline Visit to allow for washout.
vi. Evidence of an immunosuppressive state, including an active HIV infection, agammaglobulinaemias, T-cell deficiencies or Human T cell Lymphotrophic Virus Type 1 (HTLV-1).
vii. Chronic active infection such as hepatitis B or hepatitis C and tuberculosis. Patients with latent tuberculosis could be included if treated with chemoprophylaxis for at least 2 months before starting the study, and to continue chemoprophylaxis for a total of 6 months.
viii. A history of cancer within the last 5 years except for squamous or basal cell skin carcinoma, which had been completely excised and treated cervical carcinoma *in situ.*
ix. Demyelinating diseases.
x. Moderate to severe heart failure based on New York Heart Association (NYHA) functional class III and IV.
xi. Pregnancy.

xii. Breastfeeding.

xiii. Planned surgery within the study period which was expected to require omission of study medication of 28 days or more.

xiv. Receipt of live attenuated vaccine within 28 days prior to Baseline Visit.

## Treatment

The investigational medicine product (IMP) used in this study was etanercept (Enbrel). Etanercept is a recombinant human TNF-receptor fusion protein. It interferes with the inflammatory cascade by binding to TNF, thereby blocking its interaction with cell-surface receptors. The usual route of administration in its licensed indications i.e. rheumatoid arthritis, juvenile inflammatory arthritis, psoriatic arthritis, psoriasis and axial spondyloarthropathies is via subcutaneous injection, given every weekly. However in this study, a novel route of administration using an intra-dermal delivery of the drug to DLE lesion for remission induction was investigated.

The non-IMP used was any anti-malarial agent including hydroxycholoroquine 200mg daily, chloroquine 150mg daily or combination therapy with hydroxychloroquine 200mg and mepacrine 100mg (alternate days). Patients would have had to receive any of the therapy above for at least 3 months. This non-IMP was continued during the trial as well as after the study had been completed at 12 weeks, for maintenance of disease control.

One index lesion was identified (i.e. the lesion with the highest ML-SADDLE score at baseline) and treated with weekly intra-dermal injection of etanercept for up to 12 weeks. The same lesion was injected at each time point.

If remission (as defined by modified limited SADDLE activity score = 0) was achieved earlier than expected, the study treatment would be ceased. The injection was administered by the investigators or qualified research nurses at the Day Case Unit (Ward 5), Chapel Allerton Hospital, Leeds.

## Selection of dose and dose modification

The usual dose of an intradermal administration of a therapy such corticosteroid is 0.2ml per injection, with repeated injections used to cover a larger lesion. Etanercept

was available in a 10mg vial, which was made up to 1ml so that each 0.2ml dose would contain 2mg.

We estimated that this dose should adequately neutralise typical concentrations of TNF in DLE lesion. The estimated TNF concentration in an inflamed tissue would be up to 500-5000 ng/mL [22]. The dose of TNF-blocker required to neutralise this would be 100 times the concentration of TNF. We estimated that a dose of 500µg of etanercept would be adequate to treat 1ml of inflamed tissue. When adjusting for residual volume retained in the syringe and backflow of volume out of the skin, the following dosing guide as specified in **Table 1** was used, with multiple injections spread across a larger lesion.

**Table 1: Intra-dermal injection of etanercept dosing guide**

| **Lesion radius (cm)** | **Volume of inflamed skin for 0.5cm thickness ($cm^{3)}$** | **Estimated Etanercept concentration required (mg)** | **Volume of Etanercept to be injected (ml) [10mg/ml]** | **Number of 0.2ml doses required** |
|---|---|---|---|---|
| 1 | 1.57 | 0.80 | 0.1 | 0.5 |
| 1.5 | 3.53 | 1.80 | 0.2 | 1 |
| 2 | 6.28 | 3.14 | 0.3 | 1.5 |
| 2.5 | 9.81 | 4.90 | 0.5 | 2.5 |
| 3 | 14.14 | 7.10 | 0.7 | 3.5 |
| 3.5 | 19.24 | 9.62 | 1.0 | 5.0 |

For safety and tolerability purposes, the first dose acted as a test dose using etanercept 1mg dose irrespective of the size of the lesion. As etanercept was used for an unlicensed condition in this study, we had capped a ceiling therapy of 10mg per injection at one treatment visit for a discoid lesion ≥3.5 cm radius. This is in line with clinical practice where up to 10mg of triamcinolone (corticosteroid) is injected intra-lesionally to discoid lupus at one session [23].

## Prior and concomitant medications

Concomitant medications were kept to a minimum during the study. However, should these were considered necessary for the patients' welfare and were unlikely to interfere with the investigational products, they could be given at the discretion of the investigator and recorded.

## Prohibited prior medications

- Any prior treatment with TNF-blockade therapies.
- Intramuscular or intra-dermal corticosteroid within 28 days of the Screening visit.

## Permitted concomitant medications

If the patients were prescribed oral prednisolone for maintenance, the dose must had been ≤10mg (or equivalent) and were stable for at least 28 days prior to Baseline visit.

Those who were prescribed anti-malarials must had been receiving them for at least 3 months prior to Screening, with a stable dose regimen for at least 28 days (±1 day) prior to Baseline visit.

Permitted other concomitant csDMARDs include methotrexate, azathioprine and mycophenolate mofetil. The patients must had been on a stable dose of this DMARDs for at least 28 days (±1 day) prior to Baseline visit.

## Prohibited concomitant medications

- Any topical corticosteroid preparation
- Any alkylating agents (e.g. cyclophosphamide, chlorambucil).
- Certain csDMARDs including sulfasalazine and leflunomide.
- Any other bDMARDs particularly anakinra and abatacept.
- Any experimental drug.
- Vaccination with live attenuated vaccines.

## Assessment

### Study schematic

The study schematic of TARGET-DLE trial is summarised in **Figure 2.**

### Summary schedule of study assessments

The schedule of study assessment of the 15-week study is summarised in **Table 2.**

**Figure 2 Study schematic of TARGET-DLE trial**

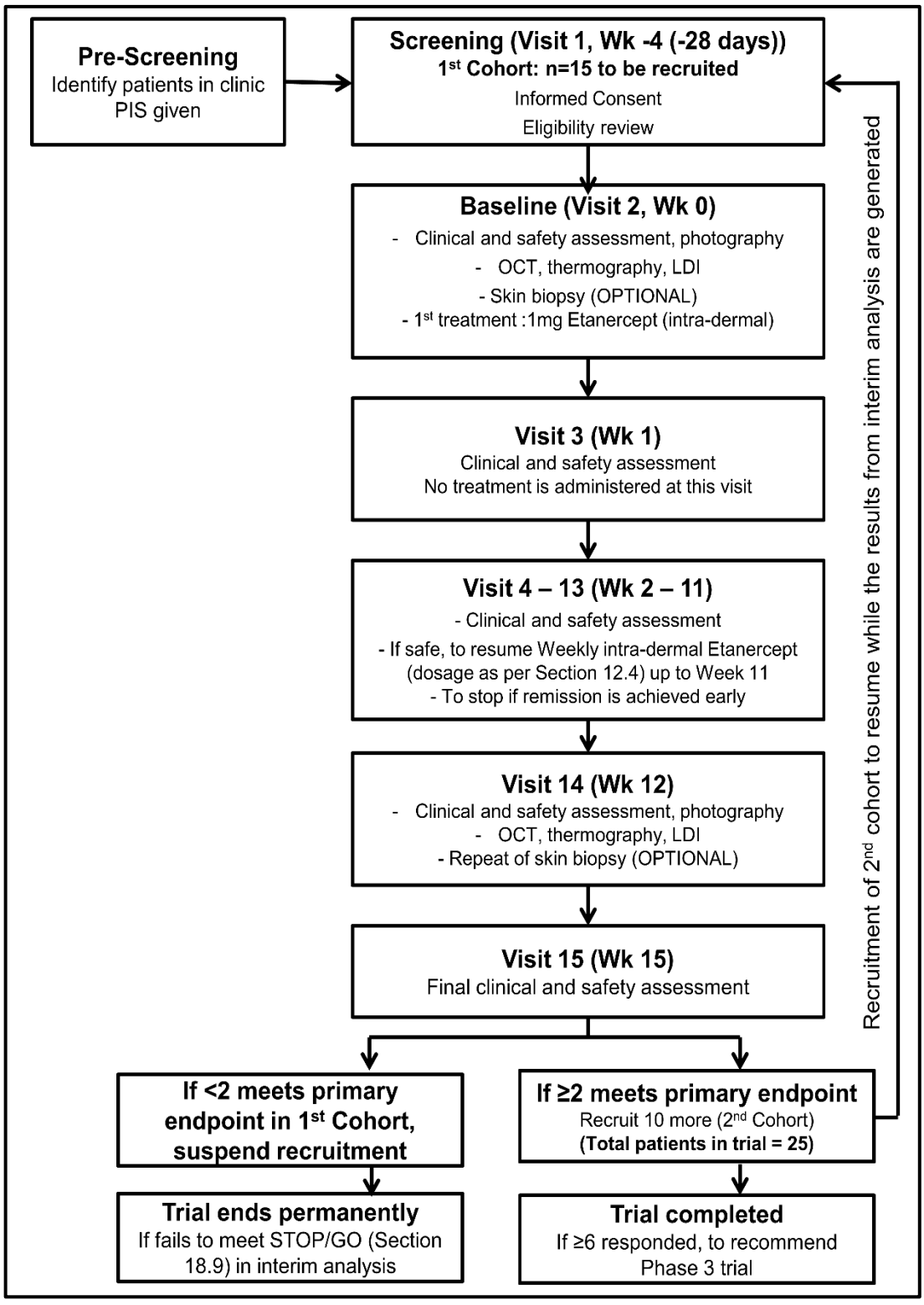

**Table 1 Summary schedule of study assessments for TARGET-DLE trial**

| Assessment (Procedure/Activity) | Screening Visit 1 | Baseline Visit 2 | Clin. Visit 3 | Clin. Visit 4 | Clin. Visit 5 | Clin. Visit 6 | Clin. Visit 7 | Clin. Visit 8 | Clin. Visit 9 | Clin. Visit 10 | Clin. Visit 11 | Clin. Visit 12 | Clin. Visit 13 | Clin. Visit 14 | Clin. Visit 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week (Wk) | Wk -4 (-28 days) | Wk 0 | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 | Wk 7 | Wk 8 | Wk 9 | Wk 10 | Wk 11 | Wk 12 | Wk 15 |
| Treatment - Etanercept intra-dermal injection[a] | | x | | x | x | x | x | x | x | x | x | x | x | | |
| Informed Consent | x | | | | | | | | | | | | | | |
| Inclusion / Exclusion Criteria | x | x | | | | | | | | | | | | | |
| Demographic Data | x | | | | | | | | | | | | | | |
| Medical/Surgical History | x | x | | | | | | | | | | | | x | x |
| Concomitant Medication | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Adverse Event check | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Physical examination & Vital signs | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Urinalysis | x | x | | | | | | | x | | | | | | x |
| Pregnancy test (urine) | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Chest X-ray (if not already done within 6 months) | x | | | | | | | | | | | | | | |
| HIV screen | x | | | | | | | | | | | | | | |
| Hepatitis screen | x | | | | | | | | | | | | | | |
| Quantiferon test | x | | | | | | | | | | | | | | |
| Immunology (ANA,anti-dsDNA, ENAs and ACA) | x | | | | | | | | x | | | | | | x |
| Complement (C3 and C4) | x | | | | | | | | x | | | | | | x |
| Immunoglobulins | x | | | | | | | | x | | | | | | x |
| Haematology | x | | | | | | | | x | | | | | | x |
| Biochemistry | x | | | | | | | | x | | | | | | x |
| Etanercept level[b] | | x | | | | x | | | | | | | | | |
| Blood for exploratory biomarkers | | x | | | | | | | | | | | | | |
| Medical photography of the skin lesions[c] | | x | | | | | | | | | | | | x | x |
| Skin Biopsy[d] | | x | | | | | | | | | | | | x | |
| Optical Coherence Tomography (OCT) | | x | | | | | | | | | | | | x | |
| Infrared thermography | | x | | | | | | | | | | | | x | |
| Laser Doppler Imaging (LDI) | | x | | | | | | | | | | | | x | |
| Modified Limited SADDLE | | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| BILAG 2004 (for SLE patients with DLE) | | x | | | | | | | x | | | | | | x |
| SLEDAI (for SLE patients with DLE) | | x | | | | | | | x | | | | | | x |
| Physician's Global Assessment (VAS) | | x | | | | | | | | | | | | x | x |
| Patient's Global Assessment (VAS) | | x | | | | | | | | | | | | x | x |
| Dermatology Life Quality Index (DLQI) | | x | | | | | | | | | | | | x | |

## Primary efficacy variable

SADDLE score [17] was chosen as the primary efficacy variable instead of other instruments such CLASI [16] because it accounted for the three important morphologies of DLE; erythema, scaling and induration [24]. The items for activity include erythema, scaling and induration while for damage are scarring or atrophy and dyspigmentation. Each item is graded between 0 to 3 in 13 parts of the body with a total score ranges between 0 and 195. SADDLE index has been shown to be valid, correlates well with other global assessment scores and has been used in clinical studies [25, 26].

In this study, a ML-SADDLE score was used; limited to only one index lesion and the efficacy was judged based on total score in activity only.

## Secondary efficacy variables

### Physician's VAS for global assessment of disease activity

The investigator rated the overall disease activity status of the patient on the day of the visit, with respect to the DLE signs and symptoms and the functional capacity of the patient, using a 100mm VAS where 0 was "very good, asymptomatic, and no limitation of normal activities" and 100 was "very poor, very severe symptoms which were intolerable, and inability to carry out all normal activities."

### Requirement for daily oral corticosteroid

The patients reported daily oral prednisolone intake at each visit. Tapering of oral corticosteroids after Week 3 (Visit 5) to a target dose of ≤5 mg/day prednisolone equivalent was encouraged during the study. Steroid dose adjustments should be avoided during Weeks 9 to 12 (Visit 11 to 14).

A temporary increase in oral corticosteroids up to a maximum of 25% above Baseline levels was allowed, if needed, at the discretion of the investigator should the patients develop skin flare with therapy. Flare was defined as an increase in disease activity in skin compared to previous assessment in a patient previously improving or stable, requiring a change in treatment. Those who had increment in oral corticosteroids >25% of Baseline levels were considered non-responders.

### BILAG-2004 (For SLE patients with DLE)

The classic British Isles Lupus Assessment Group (BILAG) was originally developed to match the physician's intention to change therapy [27]. The revised BILAG Index (version 2004) measures disease activity (scored from grade A to E) in 9 body or organ systems affected by SLE based on clinical assessments and laboratory results [19]. The BILAG-2004 index covers 97 items as opposed to 86 items in the classic BILAG and records disease activity occurring over the past 4 weeks. Each domain or system is then given an overall grade of: 0 = not present, 1 = improving, 2 = same, 3 = worse, or 4 = new. The grading is detailed **Table 3.** This assessment was only undertaken in SLE patients with DLE at Baseline, Week 7 and Week 15.

**Table 3: Grade and definition of BILAG-2004 index**

| Category | Definition |
|---|---|
| A | Severely active disease (sufficient to require disease-modifying treatment ie: >20mg/day prednisolone, immunosuppressant and cytotoxics) |
| B | Moderately active disease (requires only symptomatic therapy, for example, prednisolone ≤20mg/day prednisolone, or anti-malarials |
| C | Mild stable disease (no indication for changes in treatment) |
| D | Inactive now but previously active |
| E | Never affected |

### SLEDAI-2K (For SLE patients with DLE)

The SLEDAI-2K (version 2000) is a modified validated instrument that measures disease activity within the last 10 days. It is a global index and includes 24 clinical and laboratory variables that are weighted by the type of manifestation but not by severity [18]. The total score falls between 0 and 105, with higher scores representing increased disease activity. The SLEDAI-2K has been shown to be a valid and reliable disease activity measure in multiple patient groups [28, 29].

Similar to BILAG-2004 index, this assessment was only undertaken in SLE patients with DLE at Baseline, Week 7 and Week 15.

**Dermatology Life Quality Index (DLQI)**

The DLQI was the first dermatology-specific health-related quality of life (HRQoL) questionnaire developed in 1994 [30]. This instrument consisted of 10 questions concerning patients' perception regarding the impact of skin diseases on different aspects of their health related quality of life over the last one week. The items of the DLQI encompassed aspects such as symptoms and feelings, daily activities, leisure, work or school, personal relationships and the side effects of treatment.

Each question was scored on a 4-point Likert scale: Not at all/Not relevant=0, A little=1, A lot=2 and Very much=3. Scores of individual items (0-3) were added to yield a total score (0-30).

**Patient's VAS for global assessment of disease activity**

The patients rated the global assessment of their DLE disease activity on the day of the visit in response to the question "Considering all the ways your DLE affects you, please mark a vertical line on the scale below for how are you feeling today?" using a 100mm VAS where 0 was "very good, no symptoms" and 100 was "very poor, very severe symptoms."

**Immunological assessments**

Blood samples for measurement of immunological parameters were collected at the time points specified in the schedule of study assessments as per in **Table 2.**

Lupus-associated immunological parameters:

- ANA
- anti-dsDNA
- anti-ENAs (anti-SM, anti-RNP, anti-Ro, anti-La and anti-chromatin antibodies)
- anti-cardiolipin antibody (ACA)
- Complement levels (C3 and C4)

**Optical Coherent Tomography**

"Virtual skin biopsy" using OCT, is a novel quantitative imaging biomarker that has the potential for use in monitoring disease activity in the skin. This non-invasive technique produces two-dimensional (2D) images of optical scattering from internal tissues that enable visualisation of micromorphological structures at the epidermis and the upper dermis [31].

In this study, the OCT scans were performed by a rheumatologist using the VivoSight machine (Michelson Diagnostics) which comprised four parallel Swept-Source OCT systems, using a laser with central wavelength of 1310 and 150 nm laser sweep. For each index lesion that was scanned, the handheld OCT probe was used to capture 100 OCT 6 mm B-scans with an inter-frame spacing of 4 μm. The resulting image (4×0.4×2 mm) was reviewed in real-time before being stored for later analysis.

Previous studies have shown that the OCT parameters correlated with the histopathology of cutaneous lupus in skin biopsy: (i) thickening and disruption of the entrance signal correlated with hyperkeratosis (ii) thinning of layer below the entrance signal correlated with atrophy of epidermis (iii) patchy hyporeflective zones in the epidermis correlated with lymphocytic infiltrates in the upper dermis and (iv) wide signal free cavities in the upper dermis correlated with dilated vessels in the upper dermis [32]. At the end of this study, the OCT images were scored by an independent rheumatologist, who was blinded to the patients' clinical status. These four OCT parameters were each graded using a scale of 0-3; 0=none, 1=slight, 2=moderate and 3=strong; with a possible maximum total OCT score of 12.

**Laser Doppler Imaging**

Laser Doppler imaging (LDI) is a non-invasive imaging modality that is used to monitor blood perfusion in dermal tissue. Alteration in peripheral blood flow has been shown to correlate with the degree of inflammation in skin psoriasis and other cutaneous manifestation of rheumatic diseases [33, 34].

An area with the highest ML-SADDLE score and non-lesional area were evaluated using a high resolution LDI system (moorLDI2-IR, Moor Instruments UK) by a rheumatologist; who was trained in the operation of the LDI and was blinded to the patients' clinical information.

All scans were performed in a designated assessment room after the patients were acclimatised in a room temperature (22ºCelcius) for 15 minutes. Images were acquired at a distance between 40-70 cm from the selected areas using a bandwidth between 250Hz-15KHz and the scan speed of less than 5ms/pixel. The region of interests (ROIs) were selected and analysed using Moor LDI2-IR version 5.0 software. The absolute difference in the mean perfusion between active and non-active CLE lesions was calculated and expressed in perfusion unit (PU).

**Infrared thermography**

Thermography is a non-invasive technique that detects infrared radiation to provide an image of the temperature distribution across skin surface. This skin temperature image is influenced by the state of the skin vasculature or heat generated in deeper tissues (inflamed). This tool has been used to assess disease activity in cutaneous manifestation of connective tissue disease [35].

The protocol for thermography and LDI was nearly identical, so these two tests were performed one after the other. The difference was that the former detected the temperature of the skin whereas the latter studied blood flow (perfusion) to the skin. Two areas were evaluated using the FLIR C2 compact thermal imaging system; active DLE lesion and non-lesion areas by a rheumatologist, who was blinded to the patients' clinical information. The absolute difference in temperatures between these lesions was calculated in real-time and expressed in ºCelcius.

**Histology score of skin biopsy**

The patients were invited to undergo skin punch biopsies at Baseline and post-treatment (Visit 14, Week 12). A separate section in the Consent Form was provided for the consenting patients.

Two x 4mm biopsies were obtained from the DLE lesion of the consenting patients, of which ½ x 4mm was fixed in 10% formalin before staining with haematoxylin and eosin whilst another ½ x 4mm was kept in Michel's transport medium for immunofluorescence staining. The samples were rated in real-time by a histopathologist, with over 10 years' experience in reporting DLE cases and who was blinded to the patients' clinic status. Since there was no standardised histological scoring system for DLE, the histopathologist scored the biopsy based on their classic histological features including (i) interface dermatitis; (ii) inflammatory cell infiltrate in a perivascular, periappendageal or subepidermal location; (iii) vacuolar alteration of the basal layer; (iv) thickening of the basement membrane; (v) follicular plugging; (vi) the presence of immunofluorescence and (vii) dermal mucin deposition [24]. The first two parameters were rated using a graded scale of 0-2; 0=absent, 1=mild and 2=strong while the remaining five parameters were rated using a binary scale; 0=absent, 1=present, with a possible maximum total score of 9. Finally, since these parameters were not weighted for clinical significance, an overall histology grade was then assigned for each biopsy sample using a graded scale of 0-2; 0=non active,

1=mild and 2=active. This histology grade was used as a gold standard for measuring DLE activity and for comparison with other instruments.

The remaining 4.0 mm biopsy sample was cryopreserved using the optimum cutting temperature compound and stored at the University laboratory, Chapel Allerton Hospital for later analysis and future research.

**Photograph of DLE**

The photograph of the index lesion was taken at baseline and post-therapy using a macro digital camera, Canon EOS 600D.

**Pharmacokinetics**

The pharmacokinetic (PK) profile of etanercept was assessed to determine whether intra-dermal route drug delivery led to accumulation of etanercept in systemic circulation. Blood samples for determination of serum etanercept were collected at two time points: (i) before the first dose at Baseline and (ii) trough levels (prior to treatment at Week 4) as specified in the schedule of study procedures (Table 2). These bloods were stored as serum at the University laboratory, Chapel Allerton Hospital. At the end of the study, these serum were tested for etanercept concentration using the Promonitor® Etanecept ELISA according to the manufacturer's instructions. A positive test (as determined by the manufacturer) was etanercept concentration >175 µg/mL.

**Safety**

Safety variables including AEs, ARs, SAEs, SARs and SUSARs were recorded at each visit throughout the study period. All AEs were graded according to the National Cancer Institute's Common Terminology Criteria (version 4.0) [36].

## Withdrawal criteria

The patients were permitted to withdraw from the study at any time, without prejudice to their continued care.

They could be withdrawn from the study for the following reasons:

- At their own request – they might (i) withdrew from having treatment only but were happy to be followed up; or (ii) withdrew consent for further trial treatment and follow-up, but were willing to have any available follow-up information collected from healthcare records; or (iii) withdrew from further trial treatment, and follow-up

information to be collected.

- At the request of their legally authorised representative.
- If, in the opinion of the investigator or the Data Monitoring and Ethics Committee (DMEC), continuation in the study was detrimental to the patients' well-being.

They must be discontinued from study medication based on the following circumstances:

- Pregnancy or constant failure to use a medically acceptable form of birth control in the 4 months of the study period (every attempt must be made to follow up patients who became pregnant to determine the outcome of the pregnancy).
- Grade 3 or 4 systemic toxicity [36] or SAEs thought to be related to study treatment and not alleviated by symptomatic treatment after cessation the patient's medication of up to 4 weeks.
- Serious infection requiring parenteral (intravenous, intramuscular) antimicrobial agent or hypotension suggestive of impending sepsis syndrome.
- Acute or re-activation of tuberculosis or hepatitis infection.
- Confirmed blood dyscrasia or a demyelinating disorder (such as multiple sclerosis or optic neuritis).
- Progression to SLE in patients with DLE only.
- Worsening in BILAG-2004 in organ systems other than mucocutaneous compared to baseline in SLE patients with DLE.
- The patients' compliance. If they were to miss the treatment by 4 or more consecutive injections, then they would be withdrawn from further therapy.

## Primary endpoint

Since the patients had DLE lesions that were refractory to the standard therapy with anti-malarial agents, there was no other proven effective second line agent for this condition. Therefore, a relatively low hurdle was set. Hence, treatment with intra-dermal injection of etanercept was deemed successful if there was a decrease of 20% from baseline at Week 12 in the ML-SADDLE score. This was also in line with response criteria for other autoimmune rheumatic diseases (ARDs) such as rheumatoid arthritis (RA) and psoriatic arthritis where a reduction of 20% from baseline

in the American College of Rheumatology (ACR) disease activity index was used as primary endpoints in clinical trials [37, 38].

Therefore, the primary endpoint for this study was at least 6 patients achieving the ML-SADDLE 20 response (defined as reduction ≥20% in total activity from baseline) at Week 12 for a phase III trial to be recommended.

## Secondary endpoints

i. Proportion of patients with ML-SADDLE-50 and 70 responses.
ii. Change in physician's VAS and daily oral prednisolone requirement at Week 12.
iii. Change in patient-reported outcomes; DLQI and patient's VAS at Week 12.
iv. Change in the total score of OCT score at Week 12.
v. Change in the difference in temperature between active DLE and non-active areas using thermography at Week 12.
vi. Change in the difference in perfusion between active DLE and non-active areas using LDI at Week 12.
vii. Change in the overall histology grade of skin biopsy at Week 12.
viii. Incidence of AEs, ARs, SAEs, SARs and SUSARs.
ix. New development or worsening of positive auto-antibodies titres: ANA, anti-dsDNA, anti-ENAs and ACA at Week 7 and 15.
x. Change in complement (C3 and C4) levels to below the normal limit (if normal at baseline) at Week 7 and 15.
xi. For SLE patients with DLE, change in disease activity as assessed using the BILAG-2004 score and SLEDAI-2K indices at Week 7 and 15.
xii. Proportion of patients with detectable trough etanercept level in serum post-therapy.

## Statistical analyses

### Sample size calculation and STOP/GO criteria

Based on the current evidence [4] and from clinical experience, after treatment failure to an anti-malarial agent, there is no second line agent which is effective. Most patients would have exhausted various conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) such as acitretin, thalidomide, retinoids, dapsone, methotrexate and mycophenolate mofetil; all with limited benefit.

Therefore, we considered that if intra-dermal injection of etanercept could reduce the ML-SADDLE score by 20% or more from baseline score in 30% or more patients who were refractory to other treatments, then this would be worthwhile to assess further in a phase III trial. However, if the response rate was lower than 10%, then intra-dermal injection of etanercept in DLE should be rejected from further consideration.

Using a maximum significance level of 5% and power of 80% in a minimax design required 15 patients to be recruited in the first cohort. If 2 or more patients were considered responders from the interim analysis, then a second cohort of 10 patients would be recruited. While the outcomes data for each of the first 15 patients were collected, recruitment of second cohort of patients would continue. This accrual would stop if a total of 25 patients had been recruited prior to a complete evaluation of the results from the first cohort. Should this occur, then study would be treated as per a single-stage design with no interim analyses required. Otherwise, a formal interim analysis would take place.

During the interim analysis, should there be less than two responders, then the accrual of second cohort would be suspended. The interim analysis would take into account data from the second cohort of patients that had been collected. In this circumstance, the stopping rule for permanently terminating accrual could be calculated using the formula:

$c^* \approx r1\,(1 - (n^*/n2)) + r2\,(n^*/n2);$

where c* was the maximum number of responders required for trial termination, r1 was the maximum number of responders for terminating the trial in the first stage based on the original minimax design, n* was the number of patients (with complete data) that had been accrued in the second cohort, r2 was the total maximum number of responders in the first and second stages that would result in a phase III trial not going

ahead based on the original minimax design, and n2 was the number of patients in the second cohort.

For example, if there were less than 2 responders when the first 15 patients were analysed while 5 further patients had completed follow-up in the second cohort, then the criteria for permanently stopping recruitment in the interim analysis, C* would be = 1(1-(5/10))+5(5/10) = 3. Hence, if 3 or fewer of the 20 patients responded to treatment in the first cohort, then further recruitment would be permanently terminated.

Once the second cohort had been recruited and the study was completed, if 6 or more of the combined 25 patients were considered responders, a phase III RCT could be recommended.

**Missing data**

Attempts were made to retrieve all missing data via a thorough data cleaning process.

Missing data were expected for the efficacy and safety analyses due to frequency of visits in this trial i.e. 15 visits within 3 months follow-up, concurrent infection or AEs leading to omission of treatment at a particular visit, participants' non-compliance and if the participants withdraw early from further study treatment only.

In general, missing data were described and summarised through looking at proportions of missing-ness for each endpoint, missing data patterns and reasons for missing data. After exploring the missing-ness, appropriate methods could be considered such as complete case analysis or imputation methods (e.g. regression/stochastic/multiple) or including as a 'missing' category in the relevant endpoint.

If a participant has completed a predefined minimum amount of treatment i.e. did not meet criteria for early discontinuation of study by not missing the treatment by 4 or more consecutive injections, but did not attend the End of Treatment Assessment (Week 12, Visit 14) due to reasons that did not satisfy criteria for early discontinuation of study, (s)he should be included in the denominator. The conservative option was to assume that the participant did not respond and this would be the primary analysis assumption. However, secondary analysis could take into account the missing-ness by imputation, which was equivalent to assuming that (s)he had the same chance of response as those for whom measurements were available and who had similar measurements up to the time of withdrawal. The previous ML-SADDLE scores

recorded can be used and the sensitivity of results to assumptions about the missing outcomes to be evaluated, particularly if the decision was close.

**Analyses**

All data including patients who withdrew from the therapy were included in the final analysis (full analysis set). Descriptive summary statistics including number of patients, mean, standard deviation, median, 25% and 75% quartiles were reported for all continuous variables. Frequency distributions were provided for categorical data. The number of cases that met the primary and secondary endpoints were summarised using proportion and 95% CIs. Continuous variables were compared either using Student's T-tests or Mann-Whitney U tests whilst Fisher's exact test was used for categorical variables. Correlation between two continuous variables was assessed using Pearson's correlation.

Line listings of all AEs, SARs, SAEs and SUSARs were provided. Statistical analyses were performed using IBM SPSS Statistics v21.0 (IBM Corp, Armonk, New York, USA) and Stata v.13.1 (StataCorp College Station, Texas, USA) for Windows.

# Results

## Study summary

All 25 patients were recruited within the 18-month period set for this study. Of this, 17 patients completed the primary efficacy assessment [Did not attend Week 12 visit=1, early withdrawals=7 (personal choice=2, AE=2, worsening of DLE=1, non-compliance to protocol=1 and pregnant=1)]. The study summary is illustrated in **Figure 3.**

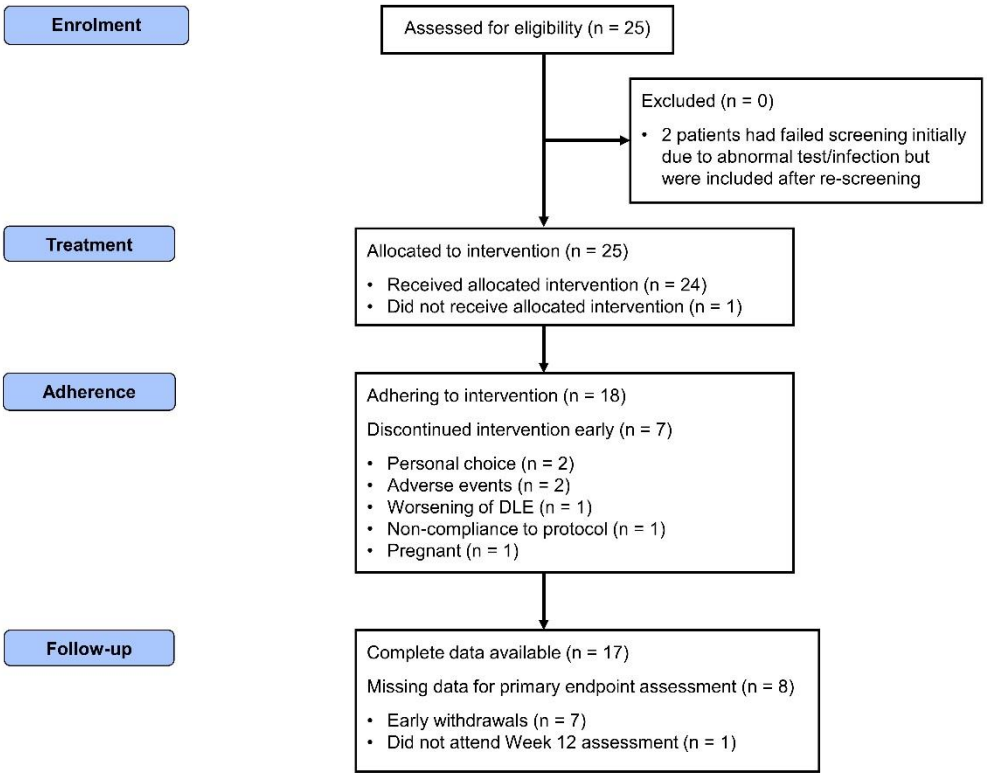


**Figure 3: CONSORT flow diagram of TARGET-DLE trial**

All 25 patients were recruited into the study over a period of 18 months. Of this, 24/25 received the intervention. 7 patients discontinued treatment early. At the end of the study, complete data were available for 17 patients. However, for primary endpoint assessment, the results were reported based on Full Analysis Set.

## Patient characteristics

Baseline characteristics are summarised in **Table 4.** Notably, this cohort comprised resistant DLE patients with median (range) number of previous systemic therapies (csDMARDs and biological DMARDs (bDMARDs)) of 5 (1-16).

**Table 4: Baseline characteristics of 25 patients recruited in TARGET-DLE trial**

| Characteristic | Values |
|---|---|
| Mean Age (SD), Years | 47.3 (12.2) |
| Female, n (%) | 18 (72) |

| Characteristic | Values |
|---|---|
| Race, n (%) | |
| Caucasian | 18 (72) |
| South Asian | 6 (24) |
| Afro-Caribbean | 1 (4) |
| Median DLE duration (IQR), Years | 9.8 (3.3 – 16.0) |
| Previous positive skin biopsy for DLE, n (%) | 19 (76) |
| Concurrent SLE, n (%) | 6 (24) |
| ANA positive, n (%) | 9 (36) |
| anti-dsDNA | 2 (8) |
| anti-Ro | 3 (12) |
| anti-La | 2 (8) |
| anti-Sm | 1 (4) |
| anti-Chromatin | 1 (4) |
| anti-RNP | 0 |
| anti-Ribosomal P | 0 |
| anti-Cardiolipin/anti-B2-Glycoprotein | 0 |
| Low C3 or C4 complement levels, n (%) | 1 (4) |
| Concomitant csDMARDs excluding anti-malarials, n (%) | 6 (24) |
| Methotrexate | 2 (8) |
| Thalidomide | 2 (8) |
| Azathioprine | 1 (4) |
| Mycophenolate mofetil | 1 (4) |
| Concomitant anti-malarial agents, n (%) | 14 (56) |
| Concomitant prednisolone, n (%) | 7 (28) |
| Median no. previous cs and bDMARDs (Range) | 5 (1 – 16) |
| Family history of ARDs, n (%) | 6 (24) |
| Ever smoked, n (%) | 20 (80) |
| Current | 15 (60) |
| Previous | 5 (20) |

ARD: autoimmune rheumatic disease; bDMARDs: biological disease modifying anti-rheumatic drugs; cs: conventional synthetic; DLE: discoid lupus erythematosus; dsDNA: double-stranded deoxyribonucleic acid; RNP: ribonucleic peptide

## Treatment characteristics

In terms of feasibility of therapy administration, 8/25 (32%) adhered to all 11 injections planned. The median percentage treatment compliance was 82%. Details of treatment characteristics are described in **Table 5.**

**Table 5: Treatment characteristics and compliance to therapy**

| Treatment characteristic | Values |
|---|---|
| Median diameter DLE index lesion at baseline (IQR), cm | 3.3 (2.0 – 4.9) |
| Proportion of patients who completed all 11 intra-dermal injections including the test dose, n (%) | 8 (32) |
| Median percentage treatment compliance i.e. number of injections received/expected number of injections received, (IQR) | 82 (36 – 100) |
| Reason for treatment interruption, n | |
| Concurrent infection | 8 |
| Other concurrent adverse events | 3 |
| Early withdrawals | 7 |
| Logistics issues | 2 |
| Other Personal reasons | 13 |
| Dose modification that violated the trial protocol, n | 0 |

## Primary endpoint

Since all 25 patients were recruited prior to complete evaluation of the results from the first cohort i.e. the first 15 patients, no formal interim analysis was undertaken. Therefore, this study was treated as per a single-stage design. Nevertheless, the decision of moving to a phase III trial remained the same, which was to be guided by whether or not 6 or more responses were observed over the full trial.

At 12 weeks, there were 13 responders, 4 non-responders and 8 had missing data. Of those with missing data, 7 were due to early withdrawals and 1 did not attend the primary endpoint assessment visit as described in **Figure 3.**

Therefore, using the conservative approach by assuming that those with missing data were non-responders, in the Full Analysis Set, the primary endpoint was met with

13/25 (52%, 95% CI 31-73) meeting the ML-SADDLE 20 response rate at week 12. Photographs examples of two responders are illustrated in **Figures 4-5.**

At Week 15, in the Full Analysis Set, the ML-SADDLE 20 response rate was sustained at 52% (95% CI 31-73).

Complete remission (as defined by ML-SADDLE score=0) was achieved in 1/25 (4%) at Week 12 and 2/25 (8%) at Week 15.

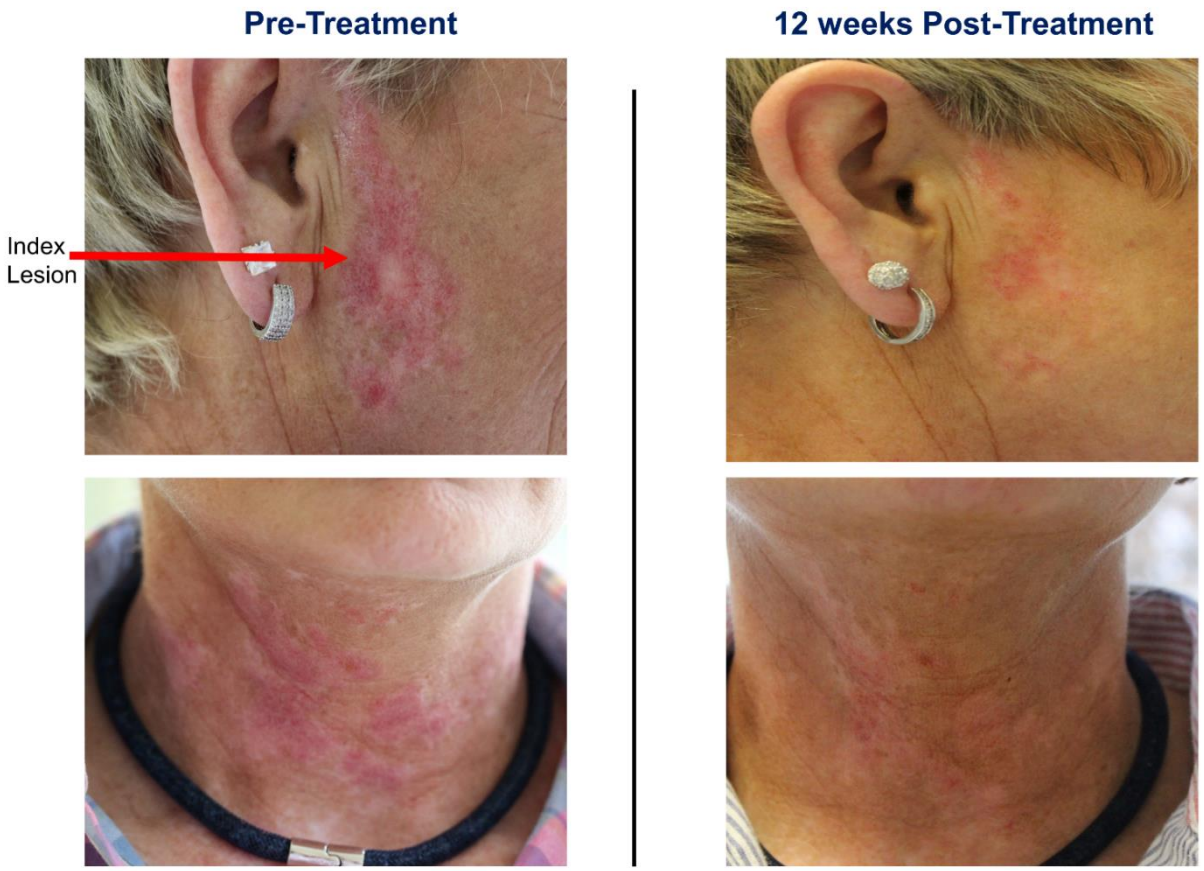


**Figure 4: Photographs of Patent 05 who responded to intra-dermal injection of etanercept**

Photos of a patient who responded to the therapy. Red arrow denotes the index lesion, the site where the injection was given.

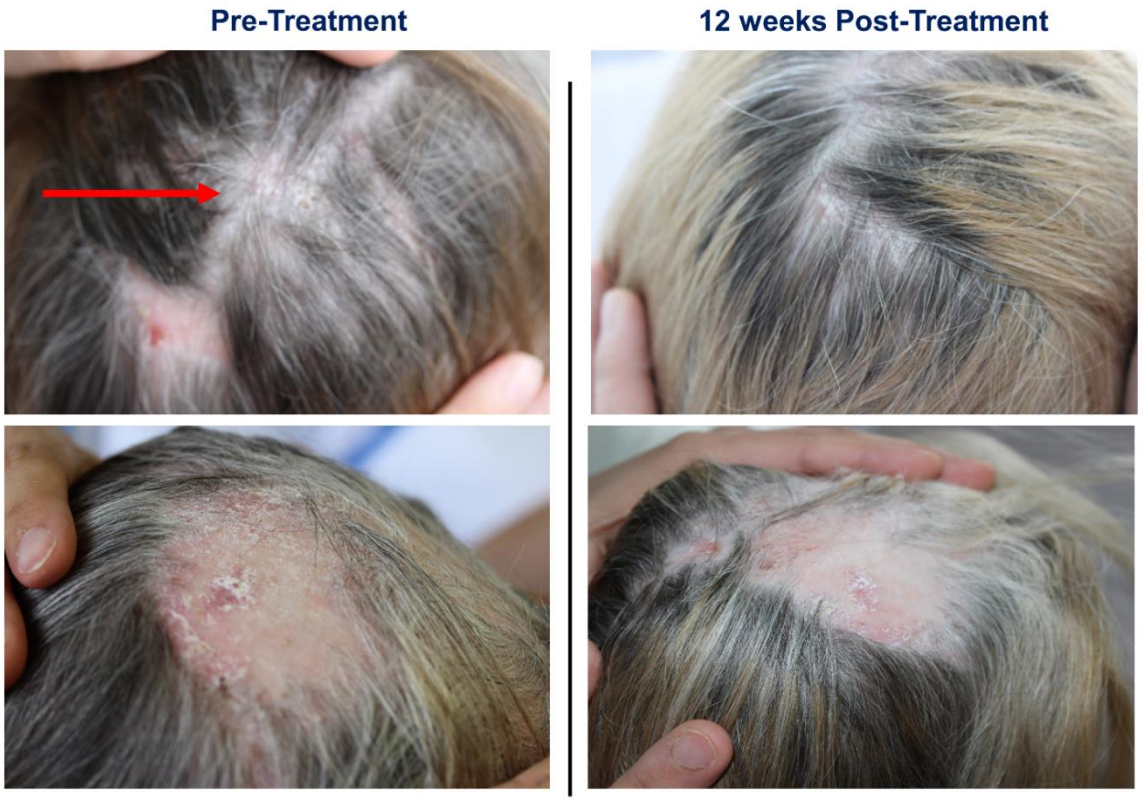


**Figure 5: Photographs of Patient 07 who responded to intra-dermal injection of etanercept**

This patient met the ML-SADDLE 70 response at Week 12. This was an exceptional case where her scarring alopecia did improve with therapy. The red arrow denotes the index lesion where the injection was given. ML-SADDLE: Modified Limited Score of Activity and Damage in Discoid Lupus Erythematosus

## Secondary endpoints

### Clinical endpoints

*Higher hurdle ML-SADDLE response rates*

At Week 12, 12 patients achieved a reduction in the modified limited SADDLE score by ≥50% (ML-SADDLE 50), 5 non-responders and 8 had missing data. Therefore, in the Full Analysis Set, the ML-SADDLE 50 response rates at Week 12 and Week 15 week were both 12/25 (48%, 95% CI 27-69).

At Week 12, 5 patients achieved a reduction in the modified limited SADDLE score by ≥70% (ML-SADDLE 70), 12 non-responders and 8 had missing data. Therefore, in the Full Analysis Set, the ML-SADDLE 70 response rates at week 12 and week 15 week were 5/25 (20%, 3-37) and 6/25 (24%, 6-42) respectively.

*Change in daily oral prednisolone requirement at Weeks 12 and 15*

In the Full Analysis Set, of 7/25 patients who were on daily oral prednisolone at baseline, none of them had their dose either reduced or increased at Week 12.

At Week 15, 2/7 had their dose doubled by the medical team since they were hospitalised due to infections. Of 18 patients who were not on daily oral prednisolone at baseline, none of them required treatment with steroid throughout the trial.

*Change in physician's VAS at Weeks 12*

In the Complete Case Analysis (n=17), the mean (SD) of physician's VAS at Baseline and Week 12 were 53.1 (16) and 23.2 (20) respectively.

There was a significant improvement in the physician's VAS at Week 12 from Baseline; mean difference 29.9 (95% CI 19.4 to 40.4), p<0.001.

### Patient-reported endpoints

*Change in DLQI at Weeks 12*

In the Complete Case Analysis (n=17), the mean (SD) of DLQI at Baseline and Week 12 were 11.4 (6.87) and 6.5 (6.21) respectively.

There was a significant improvement in the DLQI at Week 12 from Baseline; mean difference 4.9 (95% CI 2.6 to 7.1), p<0.001.

*Change in patient's VAS at Week 12*

In the Complete Case Analysis (n=17), the mean (SD) of patient's VAS at Baseline and Week 12 were 56.9 (28) and 29.7 (28) respectively.

There was a significant improvement in the patient's VAS at Week 12 from Baseline; mean difference 27.2 (95% CI 12.2 to 40.1), p=0.001.

**Objective outcome measures**

*Change in infrared thermography parameter at Week 12*

In the Complete Case Analysis (n=17), the mean (SD) of the absolute difference in temperature between active DLE and non-active areas using thermography at Baseline and Week 12 were 1.92 (1.17) and 1.08 (1.05) respectively.

There was a significant improvement in the absolute difference in temperature between active DLE and non-active areas using thermography at Week 12 from Baseline; mean difference 0.84 (0.30 to 1.39), p=0.005.

*Change in LDI parameter at Week 12*

In the Complete Case Analysis (n=17), the mean (SD) of the absolute difference in perfusion unit between active DLE and non-active areas using the LDI at Baseline and Week 12 were 495.1 (224) and 376.2 (223) respectively.

There was a significant improvement in the absolute difference in perfusion unit between active DLE and non-active areas using the LDI at Week 12 from Baseline; mean difference 118.9 (23.7 to 214.0), p=0.018. An LDI image of a responder is depicted in **Figure 6.**

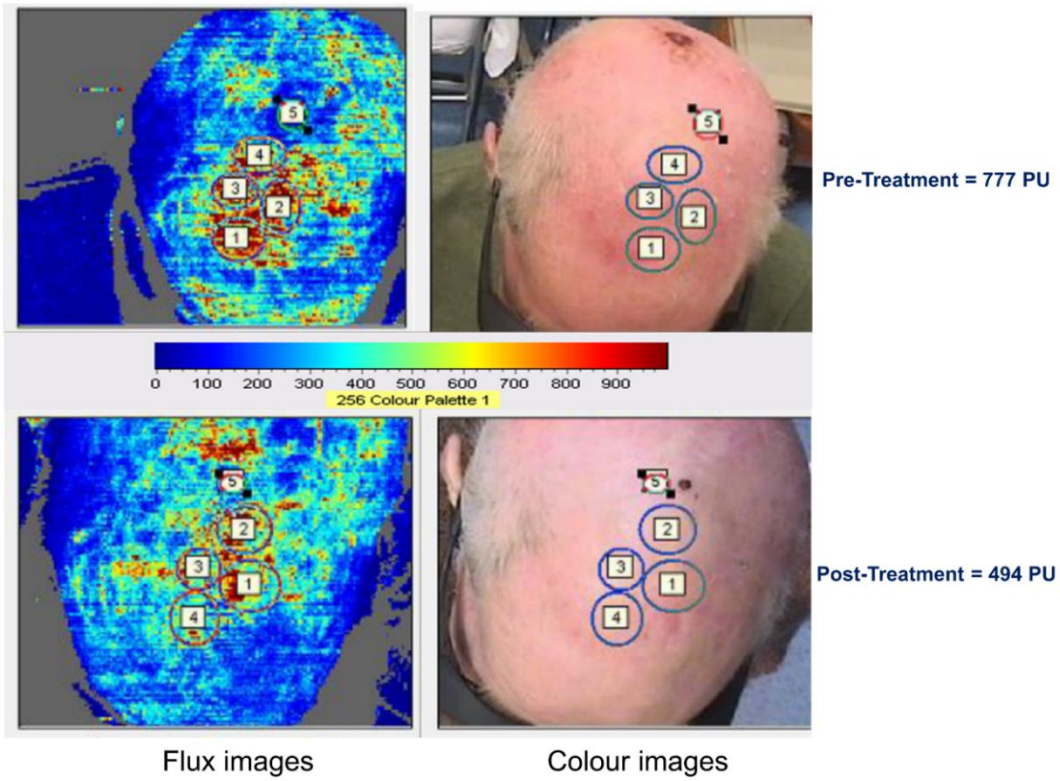


**Figure 6 Images from laser Doppler imaging of Patient 25 who had a partial reduction in ML-SADDLE score post-treatment**

This patient had a partial reduction in ML-SADDLE score from 5 at baseline to 2 at week 12. This partial improvement as rated by clinical assessment was supported by LDI. The blue circles drawn outside the numbers 1-5 represent regions of interest (ROI) in the analyses. The ROI with the highest score was chosen. ML-SADDLE: Modified Limited Score of Activity and Damage in Discoid Lupus Erythematosus; PU: perfusion unit

*Change in OCT score at Week 12*

In the Complete Case Analysis (n=17), the mean (SD) of the total OCT score in DLE lesions at Baseline and Week 12 were 4.4 (1.77) and 3.7 (1.94) respectively.

There was no significant improvement in the total OCT score in DLE lesions at Week 12 from Baseline; mean difference 0.7 (-0.3 to 1.7), p=0.144.

*Change in the overall grade of histology score of skin biopsy at Week 12*

Thirteen patients underwent skin biopsy procedures at baseline. Of these, 6/13 had paired pre- and post-biopsy samples. Of those with paired biopsy samples, 2/6 had histology score improved, 2/6 remained the same and 2/6 had worsening score at week 12.

The estimated correlation between total histology score and ML-SADDLE score at baseline was r=0.50; p=0.085.

## Safety

### Incidence of AEs, ARs, SAEs, SARs and SUSARs

In the Full Analysis Set, there were 51 AEs recorded in 20 patients as described in **Table 6.** Of these, 28/51 were treatment-emergent recorded in 13 patients. The most common reason for an AE was lower respiratory tract infection (LRTI); n=5. Injection-site reaction or infection cases were recorded in 4 patients.

Grade 3 or higher systemic toxicity AEs were recorded in 2 patients (LRTI = 1, Presumed infection, source unidentified = 1, Heart failure = 1, Worsening of chilblains = 1). One patient became pregnant after receiving one dose of intra-dermal injection of etanercept and she had to be withdrawn from the study. The outcomes for both mother and baby were uneventful. Withdrawals due to AEs were recorded in 2 patients.

**Table 6: Adverse events recorded in TARGET-DLE trial (n=25)**

| Characteristic | Values |
|---|---|
| All Adverse Events (AEs), n | 51 |
| Treatment-emergent AEs, n | 28 |
| Infection | |
| Lower respiratory tract infection | 5 |

| Characteristic | Values |
|---|---|
| Presumed infection | 1 |
| Urinary tract infection | 1 |
| Injection related-skin infection | 1 |
| Pharyngitis | 1 |
| Otitis externa | 1 |
| Skin | |
| Pruritus | 1 |
| Injection related swelling/oedema | 3 |
| Worsening of subacute cutaneous lupus | 1 |
| Nervous system | |
| Headache | 4 |
| Dizziness | 1 |
| Respiratory | |
| Upper respiratory tract infection | 1 |
| Cough | 1 |
| Pleuritic chest pain | 1 |
| Sore throat | 1 |
| Cardiovascular | |
| Heart failure | 1 |
| Gastrointestinal | |
| Vomiting | 1 |
| Faecal incontinence | 1 |
| General | |
| Fatigue | 1 |
| Grade 3 or higher AE, n | 4 |
| AE of special interest: Pregnancy, n | 1 |
| All Serious Adverse Events (SAEs), n | 4 |
| Presumed Infection – source/organism unidentified, n | 1 |
| Lower respiratory tract infection, n | 1 |
| Heart failure, n | 1 |
| Worsening of chilblains lupus, n | 1 |
| AE leading to discontinuation of study, n | 2 |
| AR, SAR and SUSAR, n | 0 |
| Deaths, n | 0 |

**Immunological parameters**

No patient had new development of ANA or clinically significant worsening of autoantibodies titres (anti-dsDNA, anti-ENAs and ACA) from Baseline to Week 15.

One patient (4%) had Anti-B2 glycoprotein antibody positivity detected at Week 7; 21.00 U/mL from 14.70 U/mL at Baseline (normal <19.99 U/mL). There was no history of venous or arterial thrombosis observed. Her ANA remained negative. At Week 15, the Anti-B2 glycoprotein antibody reverted back to normal.

The one patient with low baseline complement levels had his levels normalised at Early Withdrawal visit (week 7). Two patients (8%) had changes in complement levels to < lower limit of normal (LLN) at Week 7 but only one (4%) had persistently low levels at Week 15.

**SLE disease activity**

Of 6 patients with concurrent SLE, only 4 completed the study. Those who withdrew early did not have deterioration in either BILAG-2004 or SLEDAI-2K scores at the Withdrawal Visits.

Of 4 patients who completed the study, only 1 patient had increased in SLEDAI-2K score from 8 to 10 points due to worsening of complement levels at week 7 and week 15. However, her BILAG-2004 activities improved at week 15. Details are as below:

- Her Baseline BILAG Activities were: (i) Grade B Mucocutaneous (Mild skin eruption – worse; mild and severe alopecia – worse), and (ii) Grade B Musculoskeletal (Mild and moderate arthritis – same). Her SLEDAI-2K score was 8 points (rash, alopecia and arthritis).
- At week 7, she had (i) Grade B Mucocutaneous (mild rash – same; mild and severe alopecia – same), and (ii) Grade B Musculoskeletal (mild and moderate arthritis – same). Her SLEDAI-2K score had increased at Week 7 to 10 points (rash, alopecia, arthritis and new low complement)
- At week 15, her BILAG activities improved; (i) Grade C Mucocutaneous (Mild skin eruption – improving, mild and severe alopecia – improving) and (ii) Grade B Musculoskeletal (Mild and Moderate arthritis – same). Her SLEDAI-2K score remained at 10 points (arthritis, rash, alopecia, low complement).

### Serum etanercept level

At baseline, as expected, etanercept levels were undetected in all 24 patients with available data. Of 23 patients with pre- and post-trough serum etanercept levels available, 6/23 (26%) had detectable serum etanercept level post-therapy.

## Discussion

This report presented the results from a phase II open label trial, which was the first to evaluate the efficacy and safety of an existing drug, etanercept, licensed for other indications but using a novel route of administration using intra-dermal injection for remission induction in DLE. The primary endpoint as assessed using ML-SADDLE 20 response rate was achieved and the therapy was well tolerated.

In this study, just over half of the patients responded to intra-dermal injection of etanercept. This response rate was particularly notable because of the inclusion of cohort, which comprised patients who were refractory to various systemic therapies as well as median disease duration of about a decade. A Cochrane review in 2017 only identified a small number of formal studies that had been undertaken in this field including topical therapies (n=4) and one study compared hydroxychloroquine and acitretin [39]. The response rates reported by the authors based on variable outcome measures with these therapies ranged from 10% to 68% [40-44]. However, none of these trials were of high quality when they were assessed using the Grading of Recommendation, Assessment, Development and Evaluation (GRADE) [45]. In contrast, TARGET-DLE was a well-designed trial and this treatment exhibited efficacy across multiple endpoints including patient-reported outcomes and objective outcome measures.

This study offers insights into the pathogenesis of DLE and help direct future therapies. Deposition of immune complexes containing IgM, IgG and complement C3 at the dermo-epidermal junction is pathognomonic in DLE. However, this direct immunofluorescence test can also be detected in non-lesional biopsies [46]. This observation suggests that although autoantibodies are involved in the formation of skin lesions, additional mediators are needed for DLE lesions to develop. This may also explain the failure of treatment with B-cell depleting agent in this particular subtype of CLE [7]. TNF is a major pro-inflammatory cytokine that is overexpressed in the kidney

and skin lesions from patients with SLE [47]. Research in animals studies showed that intradermal injection of lupus serum into the skin of TNF-deficient mice failed to induce an inflammatory response [48], suggesting the importance of this cytokine in the development of skin lesions. Thus translating findings from bench to bedside, this study shows that TNF-blockade is effective in inducing remission of active DLE. Analysis of skin biopsy samples for TNF and expression of other cytokines are in progress and may help stratify those who will respond to this therapy.

There were no major safety signals from administration of TNF-blockade therapy using intra-dermal injection in this study. Although the number of AEs reported were high, only 28 were treatment-emergent. These could be attributed to frequency of research and treatment visits as well as over 3/4 of the patients were either current or previous smokers. None of the patients had progression or worsening of lupus from immunological and disease activity perspectives. In addition, the frequency of injection-site reaction was very low. Compliance to treatment was also satisfactory with patients receiving the treatment on average 82% of the time. However, just over a quarter of the patients withdrew early in this study with 4/25 (16%) of them discontinued due to reasons other than adverse events or pregnancy. Thus, a more refined drug delivery of TNF-blockade either using topical or microneedles [49] may help resolve these issues. With regards to cost, treatment with intra-dermal injection is cost-saving. A 12-week course of treatment up to 10mg of etanercept weekly costs 5 times cheaper than systemic etanercept administration as well as without inducing systemic autoantibody production.

This study has some limitations. First, this was an open label trial. Hence, our results could be influenced by reporting bias from both the participants and investigators. However, evidences of efficacy were also supported by objective measures including the LDI and thermography. Second, 19/25 (76%) were on concomitant csDMARDs or anti-malarials, thus efficacy could not be contributed to etanercept alone. Lastly, this study was designed for remission induction using a short course regimen. Although the ML-SADDLE response rate was maintained at week 15, longitudinal follow-up is needed.

## Conclusion

A low dose intradermal injection of etanercept up to 10mg substantially reduced clinical activity, met its primary and most secondary endpoints including patient-reported outcomes and objective measures. This therapy was tolerable in DLE patients who were refractory to anti-malarials and other systemic therapies. The results support further development of therapy in multi-centre trials. Analyses of histological biomarkers are in progress and may help stratifying patients for response.

## Key messages

i. Administration of etanercept (potentially harmful in SLE) using a novel route, intra-dermal injection is effective for remission induction in refractory DLE.

ii. No major safety signals were observed including induction of systemic autoantibody production.

iii. The results from this trial will be used to power a phase III trial.

iv. This trial also confirms the role of TNF in the pathogenesis of DLE

## References:


1. Pistiner M, Wallace DJ, Nessim S, Metzger AL, Klinenberg JR. Lupus erythematosus in the 1980s: a survey of 570 patients. Seminars in arthritis and rheumatism. 1991 Aug; 21(1):55-64.
2. Provost TT. The relationship between discoid and systemic lupus erythematosus. Arch Dermatol. 1994 Oct; 130(10):1308-1310.
3. Healy E, Kieran E, Rogers S. Cutaneous lupus erythematosus — A study of clinical and laboratory prognostic factors in 65 patients. Irish Journal of Medical Science. 1995 1995/04/01; 164(2):113.
4. Jessop S, Whitelaw DA, Grainge MJ, Jayasekera P. Drugs for discoid lupus erythematosus. The Cochrane database of systematic reviews. 2017 May 5; 5:Cd002954.
5. Martins PR, Skare T, Ferrari TA, Silva AP, Alessio BF. Comparative analysis of the quality of life of patients with discoid lupus erythematosus and systemic lupus erythematosus with skin injuries. Anais brasileiros de dermatologia. 2012 Mar-Apr; 87(2):326-328.
6. Klein R, Moghadam-Kia S, Taylor L, Coley C, Okawa J, LoMonico J, et al. Quality of life in cutaneous lupus erythematosus. Journal of the American Academy of Dermatology. 2011; 64(5):849-858.
7. Vital EM, Wittmann M, Edward S, Md Yusof MY, MacIver H, Pease CT, et al. Brief report: responses to rituximab suggest B cell-independent inflammation in cutaneous systemic lupus erythematosus. Arthritis Rheumatol. 2015 Jun; 67(6):1586-1591.
8. Toro JR, Finlay D, Dou X, Zheng S, LeBoit PE, Connolly M. Detection of type 1 cytokines in discoid lupus erythematosus. Archives of Dermatology. 2000; 136(12):1497-1501.
9. Wenzel J, Zahn S, Tuting T. Pathogenesis of cutaneous lupus erythematosus: common and different features in distinct subsets. Lupus. 2010 Aug; 19(9):1020-1028.
10. Nabatian AS, Bashir MM, Wysocka M, Sharma M, Werth VP. Tumor necrosis factor alpha release in peripheral blood mononuclear cells of cutaneous lupus and dermatomyositis patients. Arthritis Res Ther. 2012 Jan 4; 14(1):R1.
11. Petri M, Singh S, Tesfasyone H, Dedrick R, Fry K, Lal P, et al. Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. Lupus. 2009 Oct; 18(11):980-989.
12. Hendel J, Karstensen JG, Vilmann P. Serial intralesional injections of infliximab in small bowel Crohn's strictures are feasible and might lower inflammation. United European gastroenterology journal. 2014; 2(5):406-412.
13. Adegbola SO, Sahnan K, Tozer PJ, Phillips RK, Faiz OD, Warusavitarne J, et al. Review of local injection of anti-TNF for perianal fistulising Crohn's disease. International journal of colorectal disease. 2017; 32(11):1539-1544.
14. Tonelli F, Giudici F, Asteria CR. Effectiveness and safety of local adalimumab injection in patients with fistulizing perianal Crohn's disease: a pilot study. Diseases of the colon and rectum. 2012 Aug; 55(8):870-875.
15. Huang Z, Cao J, Li T, Zheng B, Wang M, Zheng R. Efficacy and safety of ultrasound-guided local injections of etanercept into entheses of ankylosing spondylitis patients with refractory Achilles enthesitis. Clin Exp Rheumatol. 2011 Jul-Aug; 29(4):642-649.
16. Albrecht J, Taylor L, Berlin JA, Dulay S, Ang G, Fakharzadeh S, et al. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an

outcome instrument for cutaneous lupus erythematosus. J Invest Dermatol. 2005 Nov; 125(5):889-894.
17. Wahie S, McColl E, Reynolds NJ, Meggitt SJ. Measuring disease activity and damage in discoid lupus erythematosus. Br J Dermatol. 2010 May; 162(5):1030-1037.
18. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002 Feb; 29(2):288-291.
19. Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, et al. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. Rheumatology (Oxford). 2005 Jul; 44(7):902-906.
20. Simon R. Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials. 1989; 10(1):1-10.
21. Herndon JE, 2nd. A design alternative for two-stage, phase II, multicenter cancer clinical trials. Control Clin Trials. 1998 Oct; 19(5):440-450.
22. Aringer M, Smolen JS. Cytokine expression in lupus kidneys. Lupus. 2005 2005/01/01; 14(1):13-18.
23. Rothfield N, Sontheimer RD, Bernstein M. Lupus erythematosus: systemic and cutaneous manifestations. Clin Dermatol. 2006 Sep-Oct; 24(5):348-362.
24. David-Bajar KM, Bennion SD, DeSpain JD, Golitz LE, Lee LA. Clinical, Histologic, and Immunofluorescent Distinctions Between Subacute Cutaneous Lupus Erythematosus and Discoid Lupus Erythematosus. Journal of Investigative Dermatology. 1992 1992/09/01/; 99(3):251-257.
25. Wahie S, Meggitt SJ. Long-term response to hydroxychloroquine in patients with discoid lupus erythematosus. Br J Dermatol. 2013 Sep; 169(3):653-659.
26. Gordon H, Chandran A, Vandal AC, Yung A, Jarrett P. The relationship between disease severity and quality of life in discoid lupus erythematosus. British Journal of Dermatology. 2016 2017/10/01; 177(4):1134-1135.
27. Isenberg DA, Gordon C. From BILAG to BLIPS—disease activity assessment in lupus past, present and future. Lupus. 2000 2000/11/01; 9(9):651-654.
28. Yee C-S, Farewell VT, Isenberg DA, Griffiths B, Teh L-S, Bruce IN, et al. The use of Systemic Lupus Erythematosus Disease Activity Index-2000 to define active disease and minimal clinically meaningful change based on data from a large cohort of systemic lupus erythematosus patients. Rheumatology. 2011; 50(5):982-988.
29. Uribe AG, Vila LM, McGwin G, Jr., Sanchez ML, Reveille JD, Alarcon GS. The Systemic Lupus Activity Measure-revised, the Mexican Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and a modified SLEDAI-2K are adequate instruments to measure disease activity in systemic lupus erythematosus. J Rheumatol. 2004 Oct; 31(10):1934-1940.
30. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clinical and experimental dermatology. 1994 May; 19(3):210-216.
31. Abignano G, Aydin SZ, Castillo-Gallego C, Liakouli V, Woods D, Meekings A, et al. Virtual skin biopsy by optical coherence tomography: the first quantitative imaging biomarker for scleroderma. Annals of the Rheumatic Diseases. 2013; 72(11):1845-1851.
32. Gambichler T, Hyun J, Moussa G, Tomi NS, Boms S, Altmeyer P, et al. Optical coherence tomography of cutaneous lupus erythematosus correlates with histopathology. Lupus. 2007 2007/01/01; 16(1):35-38.
33. Hendriks AG, Steenbergen W, Hondebrink E, van Hespen JC, van de Kerkhof PC, Seyger MM. Whole field laser Doppler imaging of the microcirculation in psoriasis and clinically unaffected skin. J Dermatolog Treat. 2014 Feb; 25(1):18-21.

34. Murray AK, Herrick AL, King TA. Laser Doppler imaging: a developing technique for application in the rheumatic diseases. Rheumatology (Oxford). 2004 Oct; 43(10):1210-1218.
35. Martini G, Murray KJ, Howell KJ, Harper J, Atherton D, Woo P, et al. Juvenile-onset localized scleroderma activity detection by infrared thermography. Rheumatology (Oxford). 2002 Oct; 41(10):1178-1182.
36. NCI. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. [online] Available at: https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf (accessed June 1 2018).
37. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B, et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. Arthritis Rheum. 1993 Jun; 36(6):729-740.
38. Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. Lancet. 2000 Jul 29; 356(9227):385-390.
39. Yusof MM, Britton J, Hensor E, Goodfield M, Wittmann M, Emery P, et al. SAT0647 Laser doppler imaging: an objective outcome measure for assessment of cutaneous lupus erythematosus. Annals of the Rheumatic Diseases. 2017; 76(Suppl 2):1019-1020.
40. Roenigk HH, Jr., Martin JS, Eichorn P, Gilliam JN. Discoid lupus erythematosus. Diagnostic features and evaluation of topical corticosteroid therapy. Cutis. 1980 Mar; 25(3):281-285.
41. Ruzicka T, Sommerburg C, Goerz G, Kind P, Mensing H. Treatment of cutaneous lupus erythematosus with acitretin and hydroxychloroquine. Br J Dermatol. 1992 Nov; 127(5):513-518.
42. Barikbin B, Givrad S, Yousefi M, Eskandari F. Pimecrolimus 1% cream versus betamethasone 17-valerate 0.1% cream in the treatment of facial discoid lupus erythematosus: a double-blind, randomized pilot study. Clinical and experimental dermatology. 2009 Oct; 34(7):776-780.
43. Jemec GB, Ullman S, Goodfield M, Bygum A, Olesen AB, Berth-Jones J, et al. A randomized controlled trial of R-salbutamol for topical treatment of discoid lupus erythematosus. Br J Dermatol. 2009 Dec; 161(6):1365-1370.
44. Kuhn A, Gensch K, Haust M, Schneider SW, Bonsmann G, Gaebelein-Wissing N, et al. Efficacy of tacrolimus 0.1% ointment in cutaneous lupus erythematosus: a multicenter, randomized, double-blind, vehicle-controlled trial. J Am Acad Dermatol. 2011 Jul; 65(1):54-64, 64.e51-52.
45. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008; 336(7650):924-926.
46. Deng GM, Tsokos GC. Pathogenesis and targeted treatment of skin injury in SLE. Nat Rev Rheumatol. 2015 Nov; 11(11):663-669.
47. Zampieri S, Alaibac M, Iaccarino L, Rondinone R, Ghirardello A, Sarzi-Puttini P, et al. Tumour necrosis factor alpha is expressed in refractory skin lesions from patients with subacute cutaneous lupus erythematosus. Ann Rheum Dis. 2006 Apr; 65(4):545-548.
48. Deng GM, Liu L, Kyttaris VC, Tsokos GC. Lupus serum IgG induces skin inflammation through the TNFR1 signaling pathway. J Immunol. 2010 Jun 15; 184(12):7154-7161.

49. Rzhevskiy AS, Singh TRR, Donnelly RF, Anissimov YG. Microneedles as the technique of drug delivery enhancement in diverse organs and tissues. Journal of Controlled Release. 2018 2018/01/28/; 270:184-202.

## Appendix

**Modified Limited Score of Activity and Damage in DLE (SADDLE)**

| **Affected Area** (Only score area to be included in study → with the highest baseline activity score) | **Activity (0-3)** | | | **Damage (0-3)** | |
|---|---|---|---|---|---|
| | **Erythema** | **Induration** | **Scaling** | **Scarring/ Atrophy** | **Pigment Change** |
| Forehead | | | | | |
| Right Cheek | | | | | |
| Left Cheek | | | | | |
| Muzzle | | | | | |
| Nose | | | | | |
| Ears | | | | | |
| Scalp | | | | | |
| Neck | | | | | |
| Arms (Exc. hands) | | | | | |
| Hands | | | | | |
| Torso | | | | | |
| Legs (Exc. feet) | | | | | |
| Feet | | | | | |
| Total Score | | | | | |

**Notes:**

**Grading:**

**0** = absent; the sign cannot be detected even after careful inspection
**1** = present but subtle; the sign is certainly present but careful inspection is required
**2** = present and immediately apparent
**3** = the sign is very prominent and severe

**Definition of individual lesion characteristics:**

**Erythema** = A red colouration of the skin due to the presence of an increased amount of blood within capillaries. It will therefore blanch on pressure
**Induration** = Begins as coarsening of the skin lesion and its surface markings and progresses to thickening of the lesion, best assessed by palpation
**Scaling** = The presence of abnormal epidermal hyperkeratosis of varying thickness
**Scarring** = A mark left on the skin indicating damage following the presence of inflammatory lesion, usually manifesting as a localized permanent change in skin contour ie: atrophy due to dermal damage. In the scalp, it is also represented by hair loss
**Dyspigmentation** = Either an increase or a decrease in normal skin pigment following an inflammatory lesion