**STATISTICAL ANALYSIS PLAN**

Randomised controlled trial to determine the efficacy of thermotherapy in comparison with intralesional meglumine antimoniate to treat cutaneous leishmaniasis in an operational setting in Syria.

Short Title: CL in Syria.

Version 1.0

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Written by:

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Bob Taylor, coI Date

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## Background & Rationale

Cutaneous leishmaniasis (CL) is a vector-borne (sand fly) disease that is caused by a protozoan of the genus Leishmania. Syria has Old World Cutaneous Leishmaniasis (OWCL) caused by *Leishmania tropica* and *L. major*. The *L. tropica* to *L. major* ratio varies by country but *L. tropica* predominates in Afghanistan, northern Syria and Turkey. The sand fly transmits CL between humans and animals (e.g. dogs and rodents) as a zoonotic infection, or between humans, an anthroponotic infection (McGwire BS and Satoskar AR 2014). The latter mode of transmission explains the epidemic nature of L. tropica, especially in crowded environments.

OWCL thrives wherever there is poverty, war, destruction of buildings and population displacement resulting in increased exposure to sand flies. This has been and continues to be seen in Afghanistan 1 2 and in the Syrian 3 and Yemeni conflicts 4.

OWCL lesions may heal spontaneously but *L. tropica* takes longer, ≥ 1 year compared to 2-8 months for *L. major*. With effective treatment healing times are reduced but these depend on disease severity e.g. size and depth of skin lesions/ulcers, their number and location. OWCL can cause scarring leading to social stigmatization, exclusion and psychological problems 5.

The treatment of OWCL centres on the antimonials, like sodium stibogluconate or meglumine 6 7, and thermotherapy has shown promise in some trials 8 9.

## Study synopsis

|  |  |
| --- | --- |
| **Study Design** | An open randomised controlled non-inferiority treatment trial  |
| **Study Participants** | Patients with cutaneous leishmaniasis presenting at health facilities and leishmaniasis mobile treatment clinics in Idleb governorate, North-West Syria |
| **Planned Sample Size** | 580 participants, 290/arm  |
| **Aim** | The study aims to gather evidence on the use of intralesional antimoniates and thermotherapy in Old World cutaneous leishmaniasis in war-torn Syria to inform health policies. |
|  | **Objectives** | **Outcome Measures** |
| **Primary** | Compare the efficacy of thermotherapy treatment to an IL course of meglumine antimoniate (MA) for the treatment of cutaneous leishmaniasis in Syria. | Complete re-epithelization for ulcerated lesions, and obvious flattening for non-ulcerated lesions measured at 3 months post treatment completion.  |
| **Secondary** | Determine tolerability | Adverse events |
|  | Determine leishmania species, genotypes & molecular markers of antimonial resistance | PCR of kinetoplast DNA (species), sequence internal transcriber spacer 2 and cytochrome b genes (genotype), gene expression of e.g. aquaglyceroporin 1, multidrug resistance protein A |

## Analysis considerations

The main strategy will be the intention to treat (ITT) principle in which all patients will be included in the analysis according to the arm of randomisation. These will be followed by per protocol (PP) analyses. Patients wrongly randomised or lost to follow-up will be excluded from the PP analysis. For efficacy, only subjects with a parasitological (skin scrape and/or PCR) diagnosis will be included. For the safety evaluation, patients will be included if had their thermotherapy and, for the MA arm, received at least one injection of IL MA.

A CONSORT chart will be presented to summarize the patient flow from recruitment to study end.

All analyses will be two sided and p value of <0.05 is considered statistically significant. Analyses will be done in Stata 18 or higher; or R software.

### Baseline summaries

Normally distributed baseline characteristics will be summarised using means and standard deviations while skewed continuous data will be summarised using medians and ranges. Proportional data will be summarised using counts and percentages.

### Cure rate of miltefosine treated CL at six months

As per the EMRO recommendations and those of a recent paper (Olliaro, Grogl et al. 2018) on standardising endpoints in CL trials, the clinical criteria for clinical cure by three months are:

* re-epithelization for ulcerated lesions, and
* flattening for non-ulcerated lesions.

The proportion of participants who achieve the cure definition above will be obtained from the Kaplan Meier method and summarised by group. A Kaplan Meier plot will be plotted to visually show the time to cure for the two groups. In these analyses, participants with partial information e.g., those lost to follow-up etc will be censored at the last timepoint when they were observed. Hazard ratios will be obtained from the Cox proportional Hazards Model. The proportional analyses will also be done for a 3-month end point. A binomial logistic regression model will also be used to assess factors affecting cure.

Other WHO efficacy definitions will include:

* treatment failure is defined as increasing skin lesion ≤ 14 days starting treatment
* relapse rate - reappearance of original skin lesion ≤ 6m after ‘cure’

The safety analysis will centre on adverse events, using standard definitions.

## Analysis of categorical variables

These will be analysed by:

* Chi squared or
* Fisher’s exact test, as appropriate.

## Analysis of continuous data

These analyses will depend on the distribution. For two group comparisons, the unpaired t test will be used for normally distributed data and the Mann Whitney U test for skewed data.

Longitudinal data (e.g. lesion surface area over time, ECG intervals over time) will be analysed by mixed effects linear modelling. Factors associated with longitudinal data will be examined using multivariable analysis.

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