Statistical Analysis Plan for the Primary and Secondary Endpoint
Preventing Diabetic Foot Ulcers through Cleaner Feet
VA DFU Clinical Trial – HP-79894
March 3, 2023

1. Aims

a. Primary. To determine if daily use of chlorhexidine on the feet for a year reduces new foot complications including chronic foot ulcer, foot infection or full or partial foot amputation.

b. Secondary. To determine if daily use of chlorhexidine on the feet for a year increases antibiotic resistance among bacterial pathogens on feet.

2. Endpoints

a. Primary. Time from randomization until either 1) a new chronic (present 28 days from initial diagnosis regardless of use of the intervention) foot ulcer or wound or 2) a moderate or severe foot infection (as defined by IDSA Diabetic Foot Infection Severity classification) not from an existing ulcer or 3) a full or partial foot amputation for a new ulcer. Participants are followed for the outcome from the date of randomization until either 53 weeks after randomization, the date of their V6 study visit, the date of their death or the date of their first outcome, whichever comes first.

b. Secondary. Minimum inhibitory concentration (MIC) to chlorhexidine and other key antibiotics of *E. cloacae, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* and *E. faecium* (ESKAPE) and other diabetic foot infection pathogens.

3. Study Design

This is a single center, randomized, double blind (participant, outcome assessor), parallel group clinical trial comparing daily foot care with cloths containing 2% chlorhexidine compared to daily foot care with cloths not containing chlorhexidine. The target population is adults (age ≥ 18) with a clinical diagnosis of diabetes who are at risk (see protocol) for a new diabetic foot ulcer.

4. Randomization

Study participants will be assigned to two experimental groups (chlorhexidine and placebo) using blocked randomization with a 1:1 allocation ratio and variable block size. Randomization will not be stratified by site or other covariate.

5. Baseline Demographic and Clinical Characteristics

The study population description will include demographic characteristics, foot hygiene activities and relevant lab values at the time of enrollment and health indicators, comorbidities, recent medication use, current foot characteristics and past and current foot complications at the time of randomization. All descriptions will be stratified by treatment group. Participant characteristics will be described by mean and standard deviation, by median and interquartile range, or by frequency and percentage as appropriate.

6. Analysis Populations

a. Intention to Treat Population. All participants randomized into the study. Participants will be grouped based on the intervention that was assigned at randomization regardless of their participation in the intervention. Unless participants withdrew permission to access their medical records, outcome status based on medical record review

will be available on partially withdrawn participants. Data on fully withdrawn participants will be in the intention-to-treat analysis up to the time of withdrawal and then censored.

b. Per-Protocol Population. All randomized participants who were at least 80% adherent. Adherence is defined as the number of wipes the participant reported using divided by the number of days the participant was at risk of the outcome. Participants will be grouped based on the intervention that was assigned at randomization.

7. Handling of Missing Data

Due to low frequency of missing data, no imputation is planned.

8. Accounting for Multiple Comparisons

The test of the primary hypothesis is a single log-rank test with an alpha level of 0.05 (two-sided). Significance tests for the secondary aim will not be adjusted for multiple comparisons. The secondary outcomes (MICs to specific antimicrobials in specific bacterial pathogens) are safety outcomes and unadjusted p-values are more liberal in detecting a safety concern.

9. Analysis of the Primary Outcome

The primary analysis will be of the intent-to-treat population and will follow the principle of intent-to-treat. The primary hypothesis is specifically that the chlorhexidine group will have longer times to new foot complication event (chronic foot ulcer, foot infection, or foot amputation) than the placebo group and equivalently that the hazard rate for foot complications will be reduced in the chlorhexidine group. We will non-parametrically describe the distribution of event times, from randomization until diagnosis of foot complication, in each group with a Kaplan-Meier curve as well as calculate the 75th percentile and median event time, if possible. The log-rank test will test the null hypothesis of no difference in the distribution of times to foot complication between the two arms. In primary analyses, death will generally be treated as a non-informative censoring mechanism due to the low death rate over the observation period. Effect size will be measured with the hazard ratio and 95% confidence interval from a simple, unadjusted Cox regression model. The proportional hazards assumption will be assessed by analysis of residuals. A participant's medical chart will continue to be monitored for trial outcomes even if the patient stops participating in treatment (chlorhexidine or placebo) unless the patient withdraws consent for the study to continue to review their medical chart. For chronic foot ulcers, the event time will be time to first diagnosis of the ulcer that is still present 28 days later (i.e., that is ultimately chronic). Participants who develop a foot ulcer within the last 28 days of their time in the trial (observation period) will be followed to determine chronicity.

10. Secondary Analyses

a. Analysis of the Secondary Outcome

The null hypothesis that the chlorhexidine group will not be colonized with *E. cloacae, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* and *E. faecium* (ESKAPE) and other diabetic foot infection pathogens with a higher minimum inhibitory concentration (MIC) to chlorhexidine than the placebo group will be tested with the nonparametric Wilcoxon Rank Sum test performed on normalized MICs from all detected ESKAPE organisms from each participant. MIC levels will be normalized by subtracting the MIC50 from the literature for each organism from the observed MIC on a $\log_2()$ scale. Effect sizes will be expressed in terms of means on a $\log_2()$ scale as the mean as an effect size metric will be more sensitive to group differences than the median. In addition, 95% confidence intervals will be calculated around effect size estimates to quantify the precision of estimates. All study participants colonized with pathogens at the V7 study visit will be included in the analysis. The V7 study visit occurred approximately 4 weeks after stopping the intervention. For participants who did not have the

primary outcome, the V7 study visit occurred approximately 13 months after randomization. For participants who had the primary outcome, the V7 study visit may have occurred sooner than 13 months after randomization.

b. Subgroup and Adjusted Analyses

The primary subgroup of interest is participants who had a resolved foot complication prior to randomization in the current study. The pre-specified subgroup will be defined specifically as participants with a prior foot complication defined as at least one of the following at any point in the past: foot ulcer or wound, partial foot amputation, or major foot infection. For the primary outcome (time until foot complication) a Cox regression model will be used to test whether there is a differential effect between randomized treatment groups between those who had and did not have a prior foot complication. Independent variables in this model will include treatment group indicator, subgroup indicator, and interaction between the group indicators. A significant interaction term would indicate a differential treatment effect depending on subgroup. Power will likely be subpar to detect an interaction, but hazard ratios and 95% confidence intervals will be presented overall and by subgroup as well. A similar strategy will be used for the secondary outcomes using linear regression, but again difference of means with 95% confidence intervals will be presented overall and by subgroup. Also, if there is a significant treatment arm imbalance on a variable predictive of the primary outcome, such as having a prior foot complication, the primary outcome will also be analyzed with Cox regression adjusting for this variable. An additional analysis will be to test the null hypothesis of no difference in healing between randomized treatment groups among participants with an existing foot ulcer at randomization. The outcome for this analysis is the healing of the existing foot ulcer prior to end of study follow up. A Fisher's exact test will be used to test this hypothesis.

13. Data Processing and Statistical Analysis Software

All final statistical analyses will be completed in Stata version 15 (College Station, TX), SAS (9.4) and R (4.2.1).

14. Sample Size Justification

Sample size is based on the primary objective and outcome. We will test the null hypothesis for the primary objective that there is no difference in time to new foot complication between the control and intervention groups using a log-rank test (two-tailed, 5% significance level). We assume that 10% of participants will die prior to one year of follow-up. We used the Freedman method for sample size calculation reported in Machin et al. (1997) using NCSS PASS software (2011). Prior data suggest that the annual proportion of individuals with new foot complications among controls will be about 0.4. If the true proportion for experimental participants is 0.2 one year after randomization, power of the log-rank test will be 0.87 to detect the difference if 100 patients are randomized to each study arm (i.e. total N=200).