1. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Data for this study will be entered into a REDCap database, which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

The general analytical approach for all endpoints will be descriptive in nature. No formal statistical hypothesis testing will be conducted. No p-values will be presented due to the small sample size of this study. Data analyses will be provided for all study subjects combined wherever appropriate.

For continuous variables, summary statistics will include number of subjects, mean, median, standard deviation, minimum, and maximum. For categorical variables, descriptive statistics will include number of subjects and percentages.

In regards to handling of missing data, there will be no imputation of missing values.

1.1 Analysis Populations

Safety Population: all subjects who receive at least 1 dose of study drug and who have at least one post-dosing safety evaluation. All safety endpoints, baseline characteristics, and demographic data will be summarized using the Safety Population.

Efficacy Evaluable Population: all subjects who receive all 3 doses of study drug and have a follow up blood draw for vitamin D levels, or discontinue prior to completing dosing due to a drug-related adverse event. Vitamin D level data will be analyzed using the Efficacy Evaluable Population.

1.2 Study Endpoints

The primary endpoint is to establish the change in vitamin D blood levels following 3 doses of treatment with vitamin D gel.

Secondary study endpoints include assessment of safety, including change in parathyroid hormone level and the number of subjects who have any skin rash and/or other skin reaction following treatment with the vitamin D gel.

1.3 Data Analysis

1.3.1 Subject Disposition

The number and percentage of subjects entering and discontinuing the clinical study will be presented. The reasons for discontinuation will also be summarized.

1.3.2 Demography and Baseline Characteristics

Demographic and baseline characteristics will be descriptively summarized using the Safety Population. Quantitative and/or categorical summaries will be presented for demographics and other baseline characteristics.

1.3.3 Treatment Exposure

Treatment exposure will be summarized. Measures of extent of exposure include the total number of doses per subject, cumulative dose per subject, and compliance (percent of target dose received per cycle). The reason(s) for dose(s) missed will also be summarized.

1.3.4 Safety Analyses

Safety parameters will be listed and summarized using standard descriptive statistics.

Adverse event terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by dose. Incidence of AEs occurring during the study will be summarized by system organ class (SOC) and preferred term. Adverse events will also be summarized by causality and grade. Serious adverse events and AEs leading to discontinuation of study treatment will be listed separately.

Additional analyses will be performed if warranted upon review of the data.

1.3.5 Analysis of Efficacy

The primary outcome will be the change in total Vitamin D following the topical application of 100,000IU Vitamin D3 weekly for three weeks.

1.4 Determination of Sample Size

A change from baseline of 10 μg/L in total 25-OH Vitamin D levels in subjects with Vitamin D deficiency is thought to be a clinically relevant improvement. Assuming that the standard deviation for pre and post measures of total 25-OH Vitamin D is 4 μg/L, then the standard deviation of the difference is 5.6 μg/L so 10 subjects should be adequate to show an effect size of 6.4 μg/L with alpha of .05 and a power of 90%. However the standard deviation of the second measure is unknown since absorption may vary by individual. Assuming the standard deviation of the second measure increases to 12, then 20 subjects would be needed to detect an effect size of 10 μg/L. In order to be most conservative, 20 subjects will be treated in this proof-of-concept study.

ETHICS

1.5 Ethical Considerations

This study will be carried out according to the Declaration of Helsinki, the National Statement on Ethical Conduct in Human Research (2007) and the Notes for Guidance on Good Clinical Practice as adopted by the Australian Therapeutic Goods Administration (TGA; 2000) (CPMP/ICH/135/95) and the ICH GCP Guidelines[i].

1.6 Ethical Review Committee

The Protocol will be submitted for review to the University of Minnesota Investigational Review Board (IRB), and written approval will be obtained from both the IRB and Governance Office (as required), before subjects are recruited and enrolled. The Investigators will receive all the documentation needed for submitting the protocol to the IRB and/or Governance Office. A copy of the respective approval letters will be obtained before starting the study. If approval is suspended or terminated by the IRB or Governance Office, the Sponsor-Investigator will have responsibility for notifying the Food and Drug Administration.

It is the responsibility of the Investigator to report study progress to the IRB or Governance Office as required.

The Sponsor-Investigator, or his/her nominee, will be responsible for reporting any SAEs to the IRB as required.

1.7 Informed Consent

Before recruitment and enrollment into the study, each prospective subject will be given a full explanation of the nature and purposes of the study. Once the essential study information has been provided, and the Investigator is assured that the volunteer understands the implications of participating in the study, the subjects will be asked to give consent to participate in the study by signing the Informed Consent Form (ICF)/Patient information sheet. The consent form will be signed and dated by the appropriate parties. A notation that written informed consent has been obtained will be made on the subject's medical record and CRF. The completed consent form will be retained by the Investigator. A copy of the completed ICF will be provided by the Investigator to the subject.

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[i] ICH Harmonized Tripartite Guideline: Guideline for Good Clinical Practice E6(R1) (10 June 1996). Available from: http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html