Official Title: A Randomized Pilot Study to Evaluate the Effects of a Short Course of Metformin versus No Therapy in the Period Prior to Hysterectomy for Grade 1-2 Adenocarcinoma of the Endometrium in Obese Non-Diabetic Women

Protocol - Statistical Analysis Plan, v10 dated 5/24/2016

Data Handling and Recordkeeping

The PI will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. The data will be kept in an electronic database in the office of clinical trials at the Winthrop P. Rockefeller Cancer Institute. Only the PI and Co-I, CRAs, regulatory personnel, biostatistician and lab personnel performing the analyses will have access to the data. In the event that a subject fails screening, only the eligibility criteria case report form will be completed.

Monitoring

Medical Monitor: The Medical Monitor, Principal Investigator and study staff will meet quarterly, once the PI has monitored the data, to review safety data (adverse events, serious adverse events and other scientific observations).

Study Monitor: The study will be monitored to ensure that the rights and well-being of human subjects are protected, that the data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with currently approved protocol/amendments on a quarterly basis.

Statistical Considerations

General approach: For each tissue endpoint, the tumor-NTU difference will be calculated in each patient as the paired difference in values between tumor and adjacent NTU tissue. For each blood endpoint, the endpoint response to treatment will be calculated in each patient as the paired difference between post-study and pre-study values. All data will be summarized by treatment arm as the mean, standard deviation (SD), median, and range, and will be graphed as box plots. Tumor-NTU differences and endpoint responses will be compared against zero via signed-rank test within each treatment arm. Treatment arms will be compared to each other for differences via Wilcoxon rank-sum tests, with additional details provided below. All statistical comparisons will be two-sided, and will employ a 10% alpha to reduce Type II error in this pilot proof-of-concept study.

Analysis plan for tolerability: Meformin-related adverse events will be summarized by type and treatment arm, both as proportions at each grade and as average grades (i.e., mean scores). For each type, the Wilcoxon rank-sum test will be used to compare treatment arms for differences in average grade.

Analysis plan for the IHC-based primary endpoints: The IHC-based primary endpoints will be measured in tissue, and will include markers of proliferation (Ki67, phospho-histone H3, ERα, PR, and hTERT), anti-proliferation and apoptosis (PTEN, KLF9, cleaved caspase-3 and TUNEL staining), and insulin-mediated signalling (total and phosphorylated versions of Akt, Erk1/2, AMPK, and receptors for Insulin, IGF-I, and IGF-

II). Endpoints measured in tumor tissue, adjacent NTU tissue, and tumor-NTU differences will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoints measured in tumor, and likewise endpoints measured in adjacent NTU tissue, for differences between the metformin and no-metformin treatment arms.

Analysis plan for secondary endpoints measured in blood or plasma: The secondary endpoints measured in blood will be the levels of HbA_1c, cholesterol, HDL-C, LDL-C, and triglycerides. The secondary endpoints measured in plasma will be the plasma hormone levels (Insulin, IGF-I, IGF-II, leptin, and adiponectin) and the plasma-measured expression levels of mRNAs and miRs. Endpoints measured at pre-study and post-study, and endpoint responses to treatment will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoint responses to treatment for differences between the metformin and no-metformin treatment arms.

Analysis plan for secondary endpoints measured in tissue: The secondary endpoints measured in tissue will be the expression levels of mRNAs and miRs. Endpoints measured in tumor tissue, adjacent NTU tissue, and tumor-NTU differences will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoints measured in tumor, and likewise endpoints measured in adjacent NTU tissue, for differences between the metformin and no-metformin treatment arms.

Sample Size and Power: There will be 20 subjects on each treatment arm, yielding a total of 40 subjects. The two-sided Wilcoxon rank-sum test will be used at 10% alpha to compare all endpoints and endpoint responses for differences between the metformin arm and the no-metformin arm. This study will be considered sufficiently powered to meet research objectives if the Wilcoxon rank-sum test has >90% power at 10% alpha to detect a treatment-arm difference of 1.0 SDs or more. Statistical power of the Wilcoxon rank-sum test was calculated using the 2-sample t-test module of PASS 2011 by selecting options corresponding to the worst-case scenario of underlying normal distributions with unknown SDs. Under this worst case scenario, 20 subjects per treatment arm (for a total of 40 subjects) give the two-sided Wilcoxon rank-sum test 91.6% power at 10% alpha to detect a difference of 1.0 SDs between the metformin and no-metformin arms, thereby establishing that this study is sufficiently powered to meet research objectives.

Dissemination of Data

The results will be analyzed after the analysis of all the blood and tissue samples. Then the two groups will be compared and the statistical analysis will be performed. The results will be submitted for presentation at national meetings, manuscripts will be written for publication and the data will be used to apply for additional funding from federal agencies including the National Institutes of Health/National Cancer Institute (NIH/NCI).