IRB#202790 Phase II HSIL PepCan Trial Statistical Analysis Plan

8/4/23

Overview

PepCan, the HPV therapeutic vaccine developed by our group, consists of 4 cGMP-grade synthetic peptides covering the HPV 16 E6 protein, along with *Candida* as a novel vaccine adjuvant. This combination is designed to boost anti-HPV E6 responses, which have been associated with clearance of HPV and regression of cervical lesions.[1-3] *Candida* is being used as a vaccine adjuvant because injections of recall antigen into common warts induces wart regression[4-10] and anti-HPV T-cell responses.[9] *In vitro* studies showed that the vaccine's HPV 16 E6 peptides induce maturation of Langerhans cells, the main antigen-presenting cells in skin, and that *Candida* stimulates proliferation of T-cells.[11]

This is a Phase II randomized double-blind clinical trial for treatment of cervical intraepithelial neoplasia 2/3 (CIN2/3) with two treatment arms: PepCan and *Candida*. Because the Phase I results showed Th1 promotion and better clearance of non-HPV 16 infection compared to HPV 16 infection, a *Candida*-only arm was also included. The design of this clinical trial closely resembles the latest guidelines for treating young women with HSILs.[12] Potential study participants with recent Pap smear results that indicate HSILs or that cannot rule out HSILs are being recruited locally and widely through community-based efforts. The patient's eligibility for vaccination is assessed by explaining the inclusion and exclusion criteria, obtaining medical history (including a list of current medications), and performing a physical examination. The inclusion criteria are (1) women ages 18–50, (2) ability to provide informed consent, (3) willingness to comply with requirements of the protocol, and (4) recent Pap smear report of HSIL or "cannot rule out HSIL" or colposcopy-guided biopsy report of HSIL. The exclusion criteria are (1) history of disease or treatment that has caused immunosuppression, (2) pregnancy or attempting to be pregnant, (3) breast feeding, (4) allergy to *Candida* antigen, (5) history of severe asthma requiring emergency room visit or hospitalization <5 years, (6) history of invasive squamous cell carcinoma of the cervix, and (7) history of having received PepCan. Colposcopy with biopsy is then performed by a study gynecologist, and a ThinPrep sample for HPV-DNA testing is collected. A blood sample is drawn for a complete blood cell count (CBC; white blood cells [needs to be ≥3K], hemoglobin, hematocrit, platelets [≥50K]) and complete metabolic panel (CMP; ALT, AST, albumin, alkaline phosphatase, total bilirubin, total protein, sodium, potassium, chloride, CO_2, BUN, creatinine, calcium, glucose). Subjects with biopsy-confirmed CIN2/3 then proceed to vaccination. The vaccine assignment is randomized to PepCan and *Candida* (1:1) and is double-blinded. Injections are given intradermally in limbs every 3 weeks, for a total of 4 injections. A urine pregnancy test is performed before each vaccination. CBC, CMP, and immunomonitoring are repeated during visits 1 and 3 and visits at 6 month and 12 month. PepCan is prepared by a research pharmacist by reconstituting lyophilized peptides with sterile water and drawing them in a syringe, along with 300 μL of *Candida*. Because PepCan and *Candida* are both colorless, no additional procedures are required for blinding. A nurse administers the vaccine intradermally in a limb. Any immediate AEs are recorded. Subjects are asked to record their symptoms in a diary. At the 6-month visit, colposcopy is performed and biopsies are taken if suspicious for progressing disease. ThinPrep sample for Papanicolaou smear and HPV testing is collected. If the disease is persistent or progressing, subjects would be provided an option to proceed to loop electrical excision procedure (LEEP). At 12-month visit, histological response is assessed by colposcopy-guided 4-quadrant biopsies. Results from each treatment arm will be compared to those from a historical placebo group.[13]

The unblinding process can start when biopsy results are available for the last subject are available. However, research laboratory analyses will be ongoing for several months, so the laboratory personnel (including the PI) will be blinded until the last laboratory testing is complete (expected in May 2023).

I. Baseline Characteristics

a. Age, race, ethnicity, baseline histology, number of cervical quadrants involved, body mass index, serum albumin, serum total protein, receipt of HPV prophylactic vaccine, education level, parity, number of sexual partners, smoking, oral contraceptive use will be tabulated for the PepCan group, *Candida* group, and both combined.
b. Welch's t-test will be used to compare age, number of cervical quadrants, and body mass index. Fisher's exact test will be used to compare remaining comparisons.

II. Efficacy

a. Histological response rates will be assessed for PepCan group, *Candida* group, and the historical placebo group based in the intention-to-treat (ITT) and per-protocol populations. The ITT analysis will include subjects who qualified for the study, and were randomized. Per-protocol analysis will include subjects who completed the 12-month assessment (15-month from treatment initiation).
b. If there are multiple biopsies, the diagnosis of the highest degree will be used. For example, if a subject has quadrant biopsy results of benign, CIN1, CIN2, and CIN3, then CIN3 would be resulted. Any subjects with the diagnosis of CIN2 and CIN3 would be considered to be a "histological non-responder", subjects with the diagnosis of CIN1 would be considered to be a "histological partial responder", and subjects with the diagnosis of "no CIN" would be considered to be a "histological complete responder". A stringent criterion would consider "histological complete responders" as responders and "histological non-responders" and "histological partial responders" as non-responders. A lenient criterion would consider "histological complete responders" and "histological partial responders" as responders and "histological non-responders" as non-responders.
c. Each treatment group (PepCan and *Candida*) will be compared to the historical placebo group using the exact binomial test (two-sided). The PepCan and *Candida* groups will be compared using Pearson's χ^2-tests (two-sided). The Clopper-Pearson method will be used to determine the 95% confidence intervals.
d. For the historical placebo group, only responses based on the stringent criterion are available. Its ITT response rate was 22.8% (34 of 149 subjects), and the per-protocol response rate was 29.1% (34 of 117 subjects). For per-protocol analysis, information based on entry diagnosis was available. The response rate was 27.3% (18 of 66 subjects) for CIN2, and 31.4% (16 of 51 subjects) for >CIN2.

III. Safety

a. AEs are being assessed based on Common Terminology Criteria for Adverse Events (CTCAE) v 4.03 from the time of consent to the last study visit. They will be grouped separately into PepCan and *Candida* groups after unblinding.
b. A table with commonly (recorded with $\geq$5% of injections) observed treatment-related AEs will be prepared. Comparisons of such AEs between the PepCan and *Candida* groups will be made using Fisher's exact test (two-sided).
c. Another table with all AEs recorded will be created dividing them between the PepCan and *Candida* groups.

References

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