The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, GA: 2009 report on support for development of second and third generation smallpox vaccines

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Vaccine trials are underway to assess the safety and activity of novel third generation smallpox vaccines in human subjects. As part of the assessment, serum samples were submitted to the Centers for Disease Control and Prevention for testing the ability to neutralize variola virus (VAR) *in vitro*. Plaque reduction and neutralization tests (PRNTs) were performed on trial sera against VAR strain Solamain, originally isolated in Bangladesh. Controls for each assay included neutralization of VAR with vaccinia immunoglobulin (VIG) as well as naïve human sera samples. PRNT levels were determined by comparison to virus only controls and reported as the endpoint titer achieving 60% or 90% plaque reduction. PRNT assays, which target the mature virus (MV) forms of orthopoxviruses, represent one method of activity testing for protective antibody responses and may provide a method to compare vaccine candidates. Additional methods (comet reduction or extracellular enveloped virus (EEV) titration) will focus on the ability to neutralize EEV, implicated in the dissemination of orthopoxviruses within the host.

As early as 1958 (Downie and McCarthy, J Hyg.1958), observations have suggested that there is not a 1:1 correlation in PRNT assays when vaccinia virus (VAC) and VAR are used as the virus targets for neutralization. Given the fact that the third generation clonal vaccine regimens (*i.e.*, MVA) are still being evaluated for antigenicity, we felt it would

be beneficial to evaluate VAR neutralization capacity (compared to VAC neutralization) derived from an ACAM3000 MVA vaccination regimen.

Methods. We have negotiated with NIAID to evaluate a subset of human sera derived from one of the vaccine trials (DMID 05-0010). Initial evaluations using VAR will focus on the higher dose ACAM3000 MVA subcutaneous and intradermal (ID) arms of the trial. This is based on preliminary results from Dr. Baden and colleagues, which demonstrated particularly good neutralization parameters of VAC WR using the ID route. The sera were obtained from individuals vaccinated with a two dose regimen of ACAM3000 MVA; several subjects were subsequently challenged with DryVax 6 – 12 months after the first dose of ACAM3000 MVA. Initially, 5-7 samples per volunteer will be studied ranging from day 0 to 1 year post-vaccination [day 0 (MVA Vac #1), 14, 42 (11 days post MVA Vac #2), 180 (Dryvax administered), 194, and 365] and assayed for VAR MV neutralization capacity.

Results Variola neutralization assays were performed in the biosafety level 4 maximum containment facility at the CDC on 5-7 sera samples each from 23 subjects. Dilution series were based on results gathered from the previous analysis of DMID02-017. Analysis is ongoing to define the sera titers to achieve 60 % and 90% neutralization capacity of VAR. Preliminary analysis suggests that further assays may be required to obtain more detailed data surrounding the 60% and 90% neutralization capacity.