The WHO Collaborating Center for Smallpox and other Poxviruses at the Centers for Disease Control and Prevention Atlanta, GA: 2007 report on support for development of second and third generation smallpox vaccines

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Vaccine trials are underway to access the safety and efficacy of novel second generation smallpox vaccines in human subjects. As part of the assessment, 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 assays (PRNTs) were performed on trial sera against a VAR strain originally isolated in Bangladesh. Controls for each assay included neutralization of VAR with positive control sera (multiple vaccinations with Dryvax) and vaccinia immunoglobulin (VIG) as well as naïve human sera samples. PRNT levels were determine by comparison to virus only controls and reported as the endpoint titer achieving 50% or 90% plaque reduction. PRNT assays, which target the mature virus (MV) forms of orthopoxviruses, using VAR, represent one method of efficacy testing for protective antibody responses and may provide a method to compare developing 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 (VAC) and VAR are used as the virus targets for neutralization. Given the fact that historic smallpox vaccines, of a polyclonal nature (i.e. DryVax) were not 100% effective in prevention of smallpox, and the clonal vaccine regimens (i.e., ACAM2000) appear to less effectively neutralize variola, we felt it would be beneficial to do a more comprehensive evaluation (given the very small sample size reported in the Weltzin et. al. study) in order to see if the difference in VAR and VAC neutralization titers is significant.

Method. We have negotiated with NIAID and the VTEU system to evaluate a subset of human sera derived from one of the vaccine trials. The sera are obtained from individuals vaccinated with the control (DryVax), and one of the less-reactogenic vaccines in development (MVA). Paired sera (before and after vaccination) from 30 individuals vaccinated with DryVax, and 30 individuals vaccinated with MVA will be compared at "peak" immunologic response times 30 days post multiple puncture vaccination with DryVax or 30 days post the second of two i.m. vaccinations with MVA per the "standard" doses for each respective vaccine. Sera, with appropriate negative and positive controls, will be evaluated for the ability to neutralize variola, using the same protocol used to establish PRNTs versus vaccinia at the VTEU (Newman et. al. JID 2004).

The assays were performed in a blinded fashion, and the results passed to the NIAID counterparts for unblinding. Statistical support to compare geometric mean titers, or other analytic comparisons, will be performed by NIAID and CDC statisticians.

Results Variola neutralization assays were performed in the BSL-4 facility at the CDC using the same methodologies used by the VTEU. This data has been sent to an independent contractor who is analyzing the anti-vaccinia and anti-variola virus neutralization results. Analysis of the anti-variola virus neutralizing capacity, at peak response, of either the MVA regimens shows that they are at least as effective in elicitation of an anti-variola virus response as the DryVax regimen; some analyses demonstrate superiority.