DRAFT QUESTIONS FOR OCTOBER 26:
Questions Specific to VIRxSYS' Proposed Clinical Trial

1. Is the VRX496 vector proposed for use in the clinical trial by VIRxSYS designed and manufactured in a manner to sufficiently address safety concerns relevant to generation of RCL? Please consider that the vector will be used in HIV-positive subjects.

   How does the use of a transient transfection system vs. a stable packaging cell line for vector production affect the rate of recombination in a manner that would sufficiently compensate for the use of one plasmid to encode all helper functions?

2. Please discuss whether any additional safety testing of VRX496 should be performed prior to initiating the proposed clinical trial. In particular, please discuss the following:
   a) Should an in vitro assay for detection of functional LTR-*gag-pol*-LTR recombination intermediates be used as a lot release assay?
   b) Is the RCL infectivity assay of sufficient sensitivity? Is the positive control for the assay adequate for determining the sensitivity?
   c) Are there additional in vivo studies that need to be performed?
   d) When VRX496-transduced cells are challenged with wild-type HIV, a "breakthrough" virus is observed to replicate to high titers after a lag of 2-3 weeks. Is it necessary to characterize the molecular nature of the "breakthrough" virus prior to starting a clinical trial?

3. Please discuss whether vector mobilization is considered an advantage or a safety concern for the proposed clinical trial? Please consider the following:
   a) Are the data available from the assays to assess vector mobilization by wild-type HIV sufficient? Are there additional preclinical studies to assess vector mobilization that should be performed? If so, please discuss the optimal study design.
   b) Should assays for assessment of vector mobilization in the study subjects be developed? If so, please discuss the optimal assay design.

4. Please discuss whether there are any additional assays that should be used for safety assessment of the subjects in this clinical trial. In particular, should VIRxSYS monitor HIV variants present in the subject prior to and after treatment?