

Letter

To the Editor –

Greg Podsakoff's commentary (1) on the review of our clinical protocol (protocol #0107-488 entitled: A Phase I, Open-Label Clinical Trial of the Safety and Tolerability of Single Escalating Doses of Autologous CD4 T-Cells Transduced with VRX496 in HIV-Positive Subjects) at the National Institutes of Health Recombinant DNA Advisory Committee meeting on September 6th, 2001 requires clarification on several points.

First, Podsakoff describes our VSV-G pseudotyped vector as being a "first-generation" construct. The first-generation HIV vector system initially described by Verma and colleagues consists of a three plasmid system with HIV accessory genes present in one of the two helper plasmid constructs (2). In contrast, our single helper plasmid construct does not contain any accessory genes such as Vif, Vpr, Vpu or Nef. We engineered our helper plasmid construct to encode for both HIV structural and VSV-G envelope proteins with the open reading frames transcriptionally partitioned by strong poly A signals and synthetic pause sites (3) to thwart transcriptional read-through. The two-plasmid packaging system is useful for scale-up of vector manufacturing because significantly more HIV vector can be produced from 293 cells using the two-plasmid system rather than the three-plasmid system. Additional refining features of our packaging system include degeneration of certain regions in the helper that are common to the vector so as to decrease the likelihood of recombination, and insertion of a stop codon in the vector to prevent gag-pol translation if recombination between the vector and helper (or wt-HIV) should occur.

The commentary notes correctly that our fully-gutted vectors could transduce primary CD4 T lymphocytes to greater than 93% and inhibited wt-HIV replication by 3-logarithmic units of p24 at an MOI of 0.001. However, it was incorrectly reported that the inhibitory effects may be abrogated by higher MOIs. At the meeting, we stated that we have seen similar inhibition of wt-HIV replication at higher MOIs, up to an MOI of 0.1.

The commentary also incorrectly reports that our therapeutic strategy relies upon mobilization of the vector. Our therapeutic approach uses an HIV vector to inhibit wt-HIV (4), and can optimally use vector mobilization to increase anti-HIV efficacy. In fact, we have chosen VRX496, a vector with poor mobilization characteristics, because of the perceived safety risks of using a mobilizable vector for the first lentiviral vector clinical trial. Even though we have vectors that mobilize more efficiently and display greater anti-HIV efficacy, we purposely chose VRX496, which mobilizes poorly for greater safety in the first clinical trial of a lentiviral vector.

Podsakoff noted correctly that one of the reviewers raised concerns about a 28-day follow-up period prior to dose escalation, but failed to report our response. CD4 T cells consist of two cell types: short-lived and long-lived cells. Short-lived cells are generally activated cells and have a short life-span of less than two weeks. Long-lived cells are generally quiescent and can survive for years. Our patient monitoring scheme addresses both types of cells – short-term monitoring at 24, 48 & 72 hours, 7, 14 & 28 days, and long-term monitoring at 3 & 6 months post-infusion and then yearly for the life of the patient. During the meeting we explained that a 28-day follow-up period prior to dose escalation is appropriate for this study because most of the infused CD4 T cells will be activated cells, capable of supporting wt-HIV replication or presumably the replication of a putative HIV recombinant that may produce an adverse event. In contrast, long-lived cells would be incapable of supporting productive HIV replication while in their quiescent state and would support HIV replication only upon their sporadic activation by specific antigen over the long-term. Since the number of activated cells containing VRX496 is highest immediately after infusion, the greatest risk for an adverse event is short-term rather than long-term. Nevertheless, our patient follow-up scheme will include long-term follow-up at 3 & 6 months and then yearly for life to look for any long-term adverse events that may arise.

As it is widely recognized, the major concern for the use of a VSV-G pseudotyped HIV vector is the formation of a replication-competent recombinant virus, particularly with VSV-G envelope sequences. To address this safety issue, we have proposed to the RAC and the FDA the use of sensitive molecular assays for detection of functional VSV-G DNA molecules in the final T cell product. These assays would be used in addition to and in conjunction with biological assays analogous to those used for replication-competent retrovirus detection in the murine retroviral vector system. We concur that these assays are critical for the use of HIV vectors in the clinic.

Finally, it was noted that one of the reviewers stated that the clinical endpoints of increasing CD4 levels and decreasing HIV-1 viral loads seem unrealistic. It is important to note that the proposed trial is a phase I safety trial and not an efficacy trial, so safety is the singularly important goal of the trial. We recognize that a successful T cell therapeutic approach would require sufficient numbers of VRX496 modified T cells to survive long-term in vivo so as to decrease viral loads and promote the anti-HIV immune response. Several reports indicate that ex vivo expanded T cells can indeed survive long-term in vivo (5, 6). Several clinical options are available to further address efficacy issues. A future efficacy trial could look at infusion of multiple doses of VRX496-modified T cells or use vectors that mobilize more efficiently. Other approaches could include conditioning regimens for patient-subjects that would endow the modified cells with an advantage over endogenous unmodified cells. Moreover, our target cell population need not be restricted to CD4 T cells -- VRX496 could also be delivered into hematopoietic stem cells by bone marrow transplantation, where the modified stem cells could then differentiate into mature T cells. However, no matter the approach, we believe that our preclinical data presented at the meeting demonstrates that HIV-derived vectors offer a unique opportunity for the treatment and management of HIV infection. As with any new class of vector, safety issues are paramount. We are working to address these issues to the satisfaction of the RAC and FDA. We are optimistic that once safety is established, this new class of vectors will make a significant therapeutic impact in the treatment of AIDS and other diseases.

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