

M. Louise Markert, M.D., Ph.D., 8/22/01

Review of 0107-488 PI: Rob Roy MacGregor MD, Sponsor: VIRxSYS Corp
“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”

Investigators: Dr. MacGregor and Dr. June are very well qualified to conduct this study.

Brief summary of protocol:

Patients who “fail HAART”, are discontinued from HAART or stop HAART can enroll. The study subjects undergo leukopheresis with CD4 isolation. The vector to be used is a lentivirus which contains an anti-sense sequence targetted to the envelope gene. Its expression should inhibit HIV replication in a transduced cell. CD4 T cells are transduced with this virus and expanded for 8-11 days prior to reintroduction into the patient. The cells are given over 30 minutes intravenously. There are 4 doses.

This protocol was selected for review because it is the first full protocol using a lentiviral vector. This reviewer did not find specific concerns with the vector. There were concerns, however, about protocol rationale, study design, and safety which are detailed below.

Questions:

PRECLINICAL DATA

1. This reviewer appreciates the safety data from the mice as detailed in the study “Safety Study of Vector Transduced Human T cells in SCID Mice” conducted by TherImmune (report # 1173-101).

This reviewer would have included bicarbonate levels in the panel. The PT and PTT likely could not be done because of blood volumes.

Please clarify why all animal weights are decreased on day 2 in Table 2 on page 20. Why are only 5 animals weighed on day 2?

The summary tables eg on page 12 of 52 suffer from small numbers of animals. For instance, in group 1 females there were only 2 animals, one of whom had a high AST. This raises the question as to the health of the animals. Why was the AST so high? This makes the statistical comparisons much less meaningful. The data should have been compared to normative data for the strain of mice used. The variation among the numbers raises questions about the state of health of the mice in the facility used.

Regarding toxicities in the mice, there are occasional elevated ALTs on day 2 (page 27),

occasional ASTs on day 2 (page 29), occasional low WBC (page 31), many low platelets on day 2 (page 33), and 1 very abnormal creatinine on day 30 (page 39). Unfortunately, essentially no creatinines were checked on day 2 in the mice. Parameters of liver function, coagulation, hematologic function, renal and pulmonary function should be incorporated into stopping rules.

What are the white focal splenic lesions in animal 20043 killed on day 2 (group 4), see page 104 (and page 10 from pathology associates)? The interpretation of these lesions may need to be included in the consent document.

What are the pulmonary lesions in animal 20025 sacrificed on day 2 (group 4) and animal 20027 from the same group. (See pathology associates, page 4) What did immunostaining show? These lesions may need to be included in the consent document.

PROTOCOL DESIGN AND METHODS

2 Please provide more information on the CD4 cells.

How many will be removed from the patient?

What is their TCR repertoire (by immunoscope) comparing the initial cells and the final product?

What is their phenotype after 8-11 days of culture? What is the expression of other activation/adhesion markers (besides CD69) such as HLA-DR, CD71, CD122, and CD49b? Is CD95 positive on the cells? Is there evidence for NK-T cells eg CD161+CD3+. What percent of the final product are T cells double positive or double negative for CD4 and CD8? Are they TCR $\alpha\alpha$ or TCR $\alpha\beta$?

What cytokines do these cells make after amplification? How do ELISPOT results compare from the blood as it comes from the patient and as it is ready to be returned to the patient?

Has the investigator looked at telomere length in the cells after expansion?

3. The response to M-II-B.3.f on page 36 states that a secondary outcome measure is “improved lymphocyte function”.

Will anything be measured beside the ELISPOT? What cytokines will be assessed in this assay.

This reviewer would suggest measuring the proliferative response to tetanus toxoid (TT). This is often profoundly suppressed in HIV-seropositive patients with low T cell counts. It would be

interesting to determine if this function improved in these patients.

4. The protocol in section 5.2 on page 12 discusses assignment of subjects to dose levels.

There is no discussion of enlargement of a dose level depending upon adverse events. This is recommended.

5. On page 28 of the response to M-II.B.2.b.(4), the investigator states (in 7.4.3.2) that the LAL results were negative for both cultures.

Please clarify. This reviewer believes that there is always a result that is reported in EU/ml. The allowed dose relates to the number of EU/kg. Please clarify. Table 8 of the protocol doesn't give the lot release criteria.

6. What is TheraSolutions? There is a letter dated July 3, 2001 from Dr. MacGregor to this organization.

7. In the protocol under 8.1, the sponsor outlines SAE reporting.

The sponsor should amend the protocol to include expedited reporting of unanticipated problems in the research subjects. This is required for all GCRC patients under 45CFR46. A copy of the expedited AE reports should be copied to the local GCRC.

The protocol should be amended to include reporting of AEs to OBA both the expedited reports and the annual report summaries.

8. The protocol does not contain any stopping rules.

Stopping rules and rules with respect to consultation with the FDA with possible increase in cohort size should be incorporated into the protocol based on plasma viral RNA, liver function tests, coagulation parameters, renal function, pulmonary function etc.

9. This phase I protocol should have a DSMB because of its risk. The composition and responsibilities of members need to be detailed.

RATIONALE

10. Under the response to M-II.B.2.b.(2) on page 16, the investigator states that the number of CD4 cells infused is equivalent to 10% of the total bodily CD4 cell mass. The investigator goes on to state "Therefore the upper dose of this phase I protocol represents a dose at which efficacy may be seen."

The logic of this statement is unclear to this reviewer. After expansion of the CD4 T cells, it is likely that there will be a restricted repertoire of CD4 cells to be administered to the research subject. In fact for patients failing HAART, their CD4 T cell repertoire may be restricted prior to expansion. Amplification of a restricted repertoire only leads to more T cells with a restricted repertoire. It is unclear how efficacy can come from this.

11. There is insufficient discussion of the risks of 6 months off HAART.

Could the authors comment on the risk to the immune system and thymus of being off HAART?

The concern of this reviewer is that only a small number of CD4 T cells are harvested. These cells may have a restricted TCR repertoire. After amplification these cells will have the same repertoire. During the 6 months off HAART, the other CD4 T cells in the body could be killed. In addition, the thymus could be irreversibly damaged. If the thymus is damaged, the individual will not be able to generate T cells expressing a more complete repertoire.

My recommendation is that immunoscope be done before and after the 6 month period. It should also be done on the product to be infused. If the product has a limited immunoscope, it would seem that this protocol would be dangerous.

I also recommend a stopping rule based on viral plasma RNA. The scientific abstract states that the protocol “could potentially reduce viral loads”. It would seem to this reviewer that the opposite would occur. Allowing the viral plasma RNA to increase dramatically will only allow the virus to infect more T cells and non-T cells leading to more problems for the research subject afterward.

There likely isn't a good way to assess thymic function. The T cells circulating will likely be mostly related to the treated T cells. These will not have TRECs as they are expanded. It would seem that the new T cells would be destroyed by the virus since no HAART is being used.

11. The response to M-II-B.3.f on page 36 states that a secondary outcome measure is “decrease in the size of the reservoir of replication-competent HIV-1 in resting CD4 T cells or macrophages”.

Please clarify the rationale behind this hypothesis. It would seem that if the virus were allowed to replicate unchecked, that the size of the reservoir in macrophages would increase. There is no mention of the CD4 cells being HIV-specific, thus, there is no reason to suspect that infected macrophages will be cleared.

SAFETY

12. The expansion of T cells under the stimulation of CD3 and CD28 leads to expression of

activation markers. For instance as shown in Figure 30 and Table 20, CD69 expression on CD4 cells increases from less than 5% to over 50%. The cell size increases as shown in figure 28. A concern is that infusion of these cells could result in problems such as pulmonary infiltrates, DIC, etc.

What are the safety data from infusion of similar numbers of activated T cells into humans in other protocols? If this is not available, what happens in large animal models after infusion of large numbers of activated T cells? The relevant dose would be approximately 6×10^8 cells/kg (since the highest dose in the human is 4.3×10^{10} cells assuming a typical 70 kg individual).

This reviewer would recommend inclusion of IL-6 data for all patients.

13. There is insufficient discussion of “failure of” or “resistance to” HAART.

Is failure based on plasma HIV RNA, or CD4 T cell counts? There should be a diagram of the steps taken when the first combination of HAART fails. It would be expected that several different combinations of HAART would be tried prior to entry into this protocol. It would seem that it is risky for research subjects to be allowed to discontinue their medicine and enroll in this study.

In general, the entrance criteria need to be much tighter.

CONSENT

- 14 The consent does not mention the risks of depletion of the TCR repertoire, potential increased risk of infection, damage to the thymus etc.

SUMMARY:

The protocol has a number of safety concerns unrelated to the vector type. The study design should be improved. The underlying rationale is unclear making the assessment of risk versus benefit quite difficult. For individuals to consent to this study, they should be well aware of the potential adverse permanent detrimental effects on the immune system.