Antibody-based protection against HIV infection by vectored immunophylaxis

1. What is the context of the paper?
   - The lentivirus HIV infects and lyses helper T cells, macrophages, and dendritic cells. The severe reduction of immune system allows opportunistic infections and cancers to thrive.
   - Previous research has identified numerous antibodies which undergo high levels of affinity maturation, allowing for an incremental increase in affinity for the ever developing antigens of HIV over time.
   - A practical alternative immunization with HIV antigens is the usage of vector based gene transfer. The latter method could be used to engineer life long secretion of broadly neutralizing antibodies at large concentrations from muscle tissue. The researchers call this method VIP (vectored immunophylaxis) and it was administered using the Adeno-associated virus (AAV) vector.
     - Unlike previous work by Johnson et al involving the usage scAAV to express artificially fused antibody fragments, this paper focused on expressing full length human antibodies (4E10, b12, 2G12, 2F5, and VRC01) in the gastrocnemius muscle mice.

2. Summarize big picture aspect.
   - Due to the results of their trials in successfully utilizing VIP to engineer and produce the b12 antibody in the muscular tissue of live immunosuppressed mice against high doses of the NL4-3 strain of HIV, the researchers suggest that this approach might be used in humans as well.

3. What is the central hypothesis to be tested?
   - The researcher hypothesized that using an AAV to introduce a secreted human antibody gene into mice muscle cells might help the mouse control an HIV infection if the antibody that was introduced was a broadly neutralizing antibody.
   - The antibody gene or a luciferase gene was placed in the vector along with the powerful CMV enhancer. In order to account for codon usage bias the gene was codon optimized for the mouse. Luciferase was used to report that the vector can be administered by the AAV and that it remains in the transformed cells for an extended period of time.
   - In all these transformed immunosuppressed humanized (contain human PBMCs) mice can be given dosages of the HIV strain NL4-3 and the effectiveness of the antibody can be determined by measuring the percentage of helper T cells.

4. FOR each figure—what are the conclusions based upon?
   - **Figure 1 and Supplementary Figure 1:**
     - The conclusion is that mice can be genetically engineered by AAV to produce large quantities of the codon optimized gene product for an extended period of time.
       - BLI of immunodeficient Rag2/yc mouse depicts concentrated luciferase reactions around the gastrocnemius muscle. This demonstrates that the AAV is capable of delivering a vector to those cells. Supplementary figure 1a confirms that the vector remains active for at least 56 straight weeks. In addition the production of 4E10 also persisted for about 64 weeks straight.
       - Quantification of human IgG by ELISA shows that immunodeficient mice and immunocompetent mice produced at least 100 ug/ml of b12 by week4. The transformed cells readily produced b12 for 52 weeks straight.
     - The other conclusion is that VIP protects mice from HIV in vivo over time.
       - Figure 1c indicates that an average concentration of 100 ug/ml of b12 is produced by the humanized immunosuppressed mice when the codon optimized antibody gene is
delivered to muscle tissue by AAV. The antibody circulates the blood stream. The control luciferase vector shows undetectable levels of the IgG within similar mice.

- Figure 1d demonstrates that depletion of CD4 cells by the HIV virus is inhibited by the expression of b12. The percentages of CD4 remain about the same while most of mice that received the luciferase vector were unable to stop the depletion. As indicated by supplementary the percentage of CD4 cells in humanized mice reaches near zero by week 7 when they are infected with HIV.

- **Figure 2:**
  - The **conclusion** is that the broadly neutralizing antibody b12 completely protects CD4 cells from HIV infection. The 2G12, 4E10, and 2F5 antibodies partially protect helper T cells.
  - Figure 2a demonstrates that all four antibodies can be expressed and circulate the bloodstream of NSG mice. In supplementary figure 3b we can see that in vitro expression of 4E10 and 2F5 is much higher than in vivo expression (fig 2a).
  - Figure 2b illustrates that the percentage of CD4 cells rapidly decrease in response to 5 ng of p24 NL4-3 HIV and is close to zero when the luciferase vector is present. The percentage is higher in mice expressing 4E10, 2G12, and 2F5. However it is at its highest (above 75%) when the NSG mice are expressing b12.
  - Immunohistochemical staining in figure 2c shows that the p24 antigen of HIV is absent when the b12 antibody is being expressed. Further quantification of the p24 antigen expressing cells reveals that their levels are nigh undetectable when b12 is being expressed. In comparison to the control luciferase mice the expression of 2G12 and 4E10 significantly decrease the amount of p24 expressing cells.

- **Figure 3:**
  - The **conclusion** was that transgene toxicity does not contribute to CD4 cell death and that mice expressing b12 were capable of protecting CD4 cells at HIV doses 100 fold higher than those needed to most of the kill the control mice.
  - As indicated by supplementary fig. 6 the humanized immunodeficient mice being used were all expressing about of 100 ug/ml of b12. The mock infection in figure three demonstrates that both the luciferase vectors and b12 vectors have no effect on the percentage of CD4 cells. However the addition of the HIV virus shows a steady decrease for the mice expressing luciferase, while those expressing b12 were protected even at extremely high doses of 125 ng.

- **Figure 4:**
  - **Conclusion-** VRC01 confers full protection of CD4 at high doses like b12.
  - Figure 4 shows that VRC01 antibody can be introduced into the mouse using AAV and that expression can last for weeks. However only an AAV dose of 2.5 x 10^10 and higher gave full protection of CD4 in an 10 ng HIV infection.

5. What are the controls used in each figure?

- **Figure 1:**
  - Luciferase was used as a control in:
    - fig. 1c to indicate that although it is expressed readily, it does not circulate the bloodstream like b12 antibody.
    - fig. 1d to demonstrate that CD4 depletion is due to fact that a broad acting antibody such as b12 was not present on that luciferase vector.

- **Figure 2:**
  - Luciferase was used as a control in
• fig. 2b to demonstrate that CD4 depletion is due to fact that broad acting antibodies were not present on that luciferase vector.
• fig. 2c to show that p24 expression by HIV is because the vector did not contain the b12 antibody.
• fig 2d to illustrate that reduction in p24 expressing cells is due to the expression of broad acting antibodies from the vector.

**Figure 3:**
• Luciferase was used as a control to demonstrate that expression of b12 can mitigate the depletion CD4 by HIV. Luciferase did not offer such protection when high concentrations of HIV were introduced.

**Figure 4:**
• Luciferase was used as a control to show that it has no effect on CD4 depletion, while the vector containing the VRC01 antibody does.

6. What is **WRONG** with the data/interpretation?
• One of the luciferase expressing mice in figure 1d is capable of protecting CD4 cells against a HIV infection (10 ng) at a similar level as that of a b12 expressing mouse. Similarly in figure 3, two of the luciferase expressing mice being exposed to 5 ng of HIV were also protected. Their CD4 cells increased. It is odd considering that these mice were all immunosuppressed. Perhaps there these mice are no longer deficient or maybe they accidentally inserted the vector containing the antibody instead of luciferase into the mice.
• Figure 2 indicates that although the antibody 2F5 is capable of partly protecting CD4 cells, there is no significant reduction in p24 expressing cells in comparison to the control luciferase. Quantification reveals that the amount of cells remain about the same. Perhaps this antibody does not partially protect the against the strain of the HIV virus. Does it have a high affinity for p24?

7. What experiment would you do to check?
• Using an ELISA they could measure the concentration of human IgG in the circulatory system of the mice in question. Or they could use BLI to test for luciferase activity.
• An ELISA could be done to test the binding ability of p24 with 2F5? The affinity constant should also be measured.