



# A games of T-cells: Baby steps on the paths of curing HIV



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## Abstract:

One person out of 30 million has had a sterilizing cure of HIV. While this cure came through a risky stem cell transplant, it demonstrates that cure of HIV is possible. There is general agreement that latently infected CD4 memory T cells are now the major barrier to a cure, but no agreement on how to measure this “reservoir.” Furthermore the compartment that is easiest to serially sample (blood) likely only contains less than 10% of the reservoir, since most of the reservoir is dispersed in different lymph nodes. Our goal is to correlate blood reservoir measurements with a clinical marker (CD4:CD8 ratio) that is already correlated with immune health and risk of death. We have decreased the time to answer and improved the automation of two types of reservoir measurements TILDA (Tat/rev Induced Limiting Dilution Assay) and Quantitative Viral Outgrowth Assay (QVOA). The TILDA assay we have improved the signal noise by adding a patented sample prep step developed by Salus Discovery. We have qualified ~300 CD4:CD8 ratio responses over decades and are testing the hypothesis that there will be a correlation between reservoir size in the peripheral blood and ratio responsiveness. Normal ratios appear to be different based on age and gender. How variation by the ratio may alter diseases other than HIV is discussed. Rigorous analysis will be needed to determine how the easily available CD4:CD8 ratio may (or may not) be related to the circulating reservoir but circumstantial evidence exists and will be discussed.

## Background:

- CD4 helper/inducer cells and CD8 cytotoxic/suppressor cells are two phenotypes of circulating T lymphocytes.
- The ratio of these two cell types has recently emerged as an indicator of a healthy immune system.
- Generally, ratios between 1.5 and 2.5 are considered normal; however, there are different factors that may impact this ratio, such as sex, age, ethnicity, genetics, and infections. Currently, there is no accepted “normal” CD4/CD8 ratio and definitions of “normal” vary across different hospital systems.
- With the advent of modern ART, the use of absolute CD4 count and HIV viral load no longer serve as accurate indicators of patient risks. The CD4/CD8 ratio may more accurately describe a patient’s risk of immune dysfunction and the co-morbidities associated with this condition.
- Lower ratios are associated with worse outcomes.

## Methods:

A retrospective chart review is currently proposed for 300 Veteran Affairs HIV patients who received care from 1995 to the present day to identify factors associated with normalization or persistently abnormal CD4:CD8 ratio. We are particularly interested in adherence to these medications and pattern of use. An Ethiopian cohort is approved and we are currently converting paper charts to an electronic form. Four patients, who are approved for review, are shown that highlight the potential for improved HIV care by more systemic review of the CD4:CD8 ratio.

## Preliminary result:

### Method 3: Ratio can be the measure of the reservoir and possible progression and recovery of HIV disease

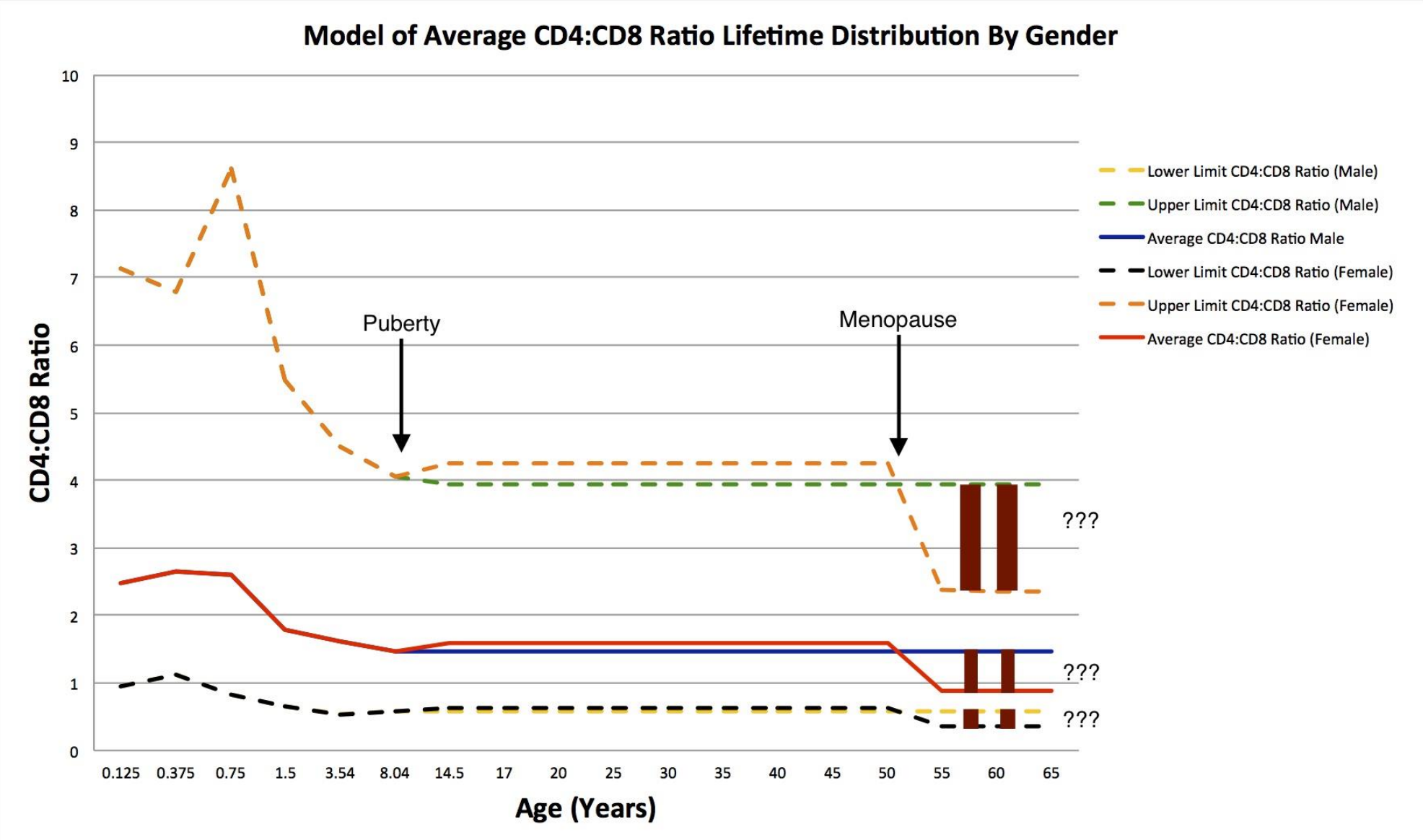


Figure 2: Large variance exists in post-menopausal women and a current range of a normal ratio is unknown (red bars)<sup>1,2</sup>.

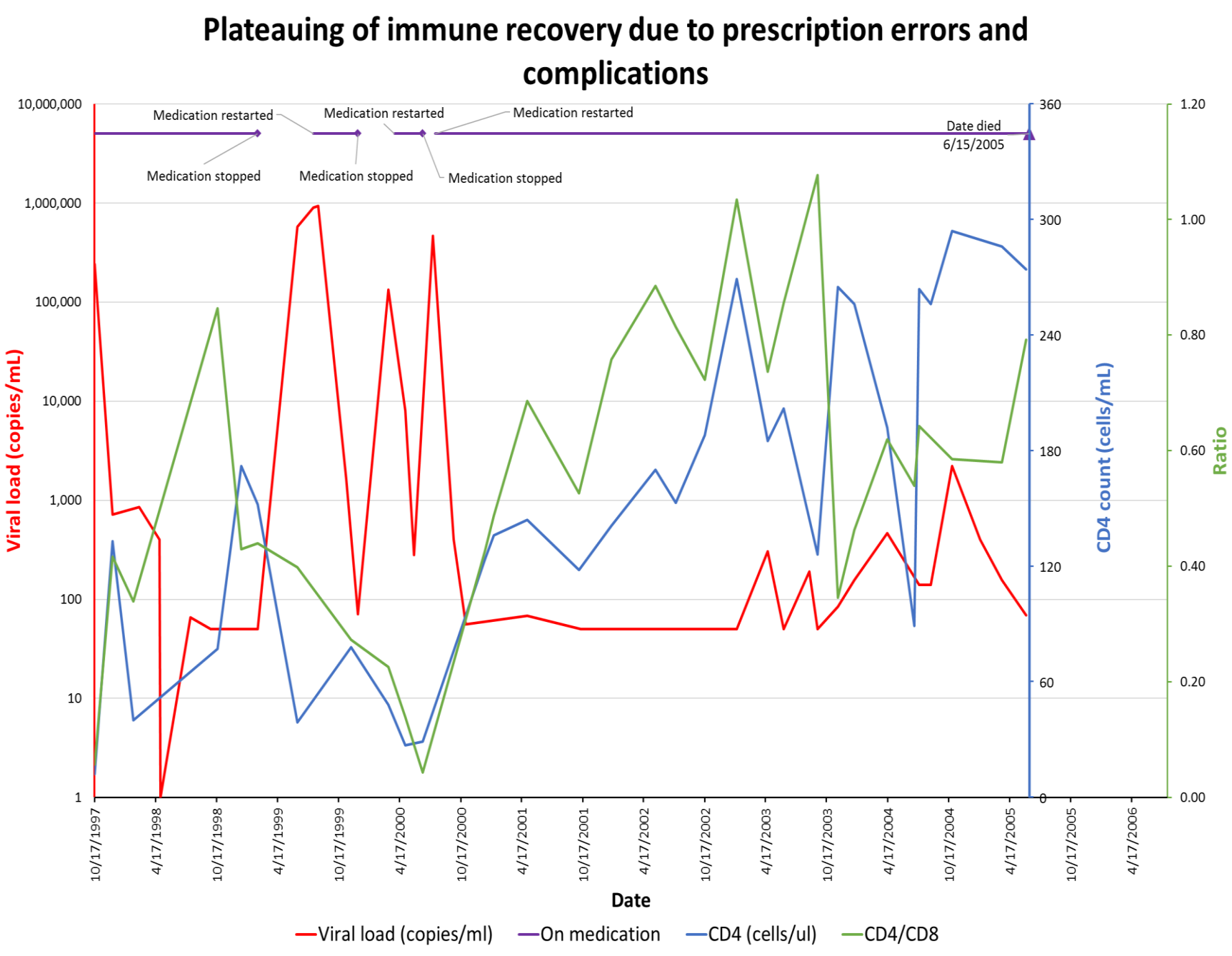


Figure 3: The patient showed good HIV suppression until pancytopenia (potentially due to ganciclovir) reduced both his CD4 and CD8 cells in October 2003. After that, despite the CD4 recovery, the ratio remained low corresponding to the rise in viral load.

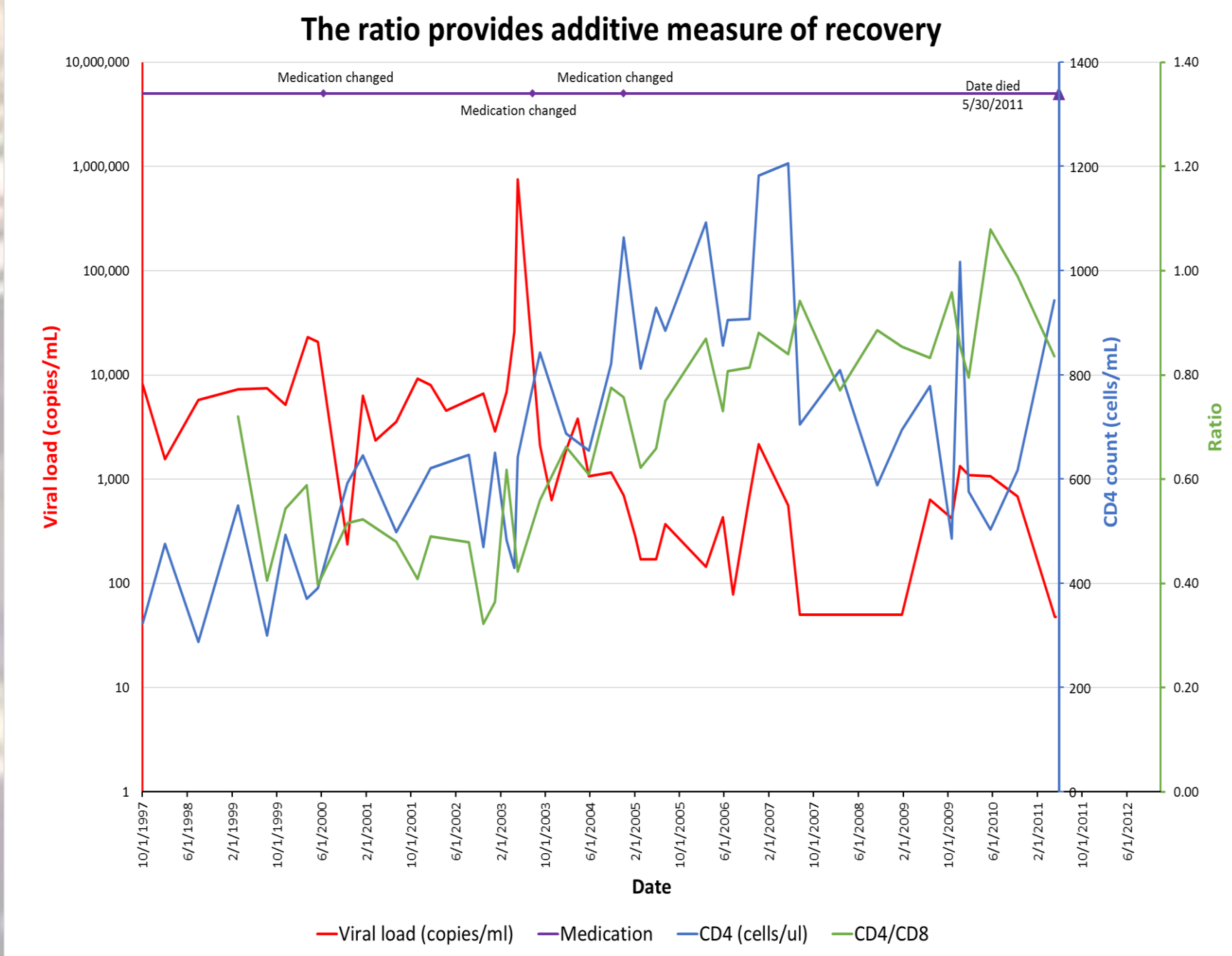
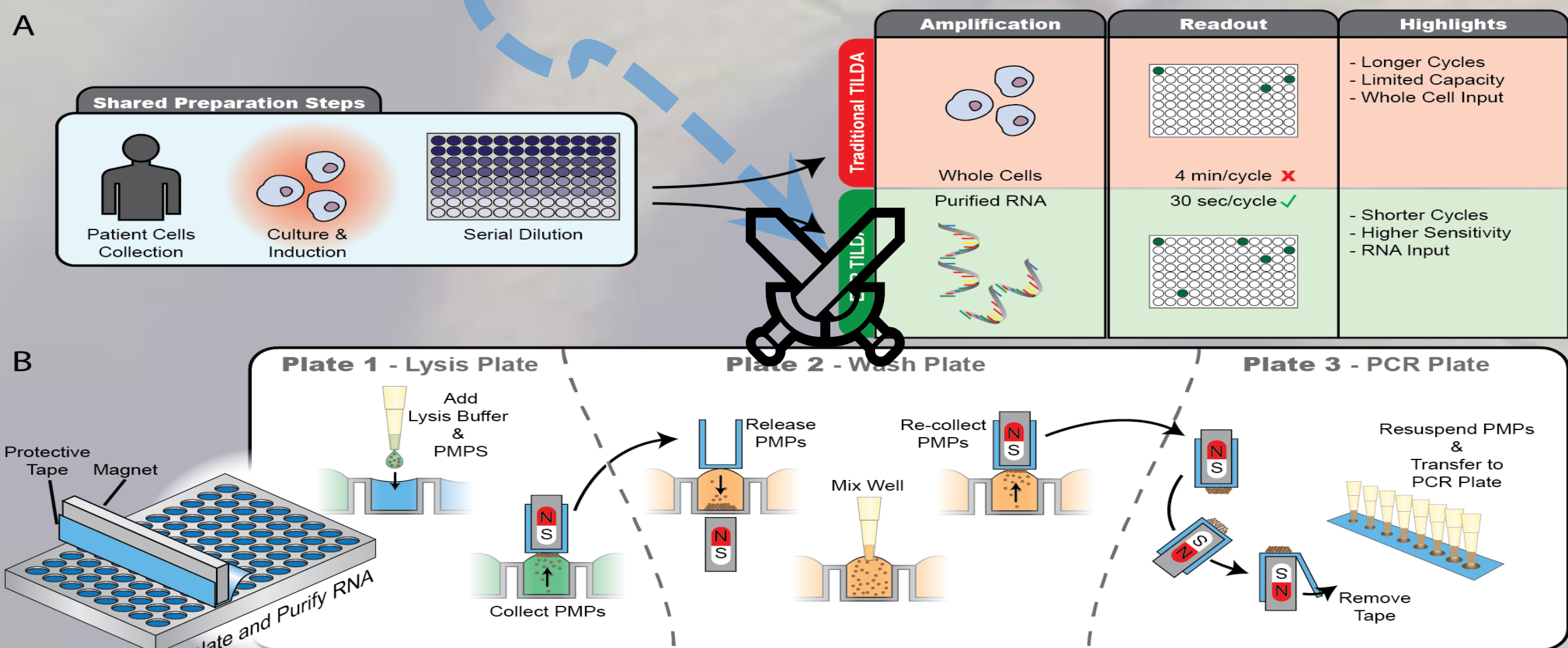
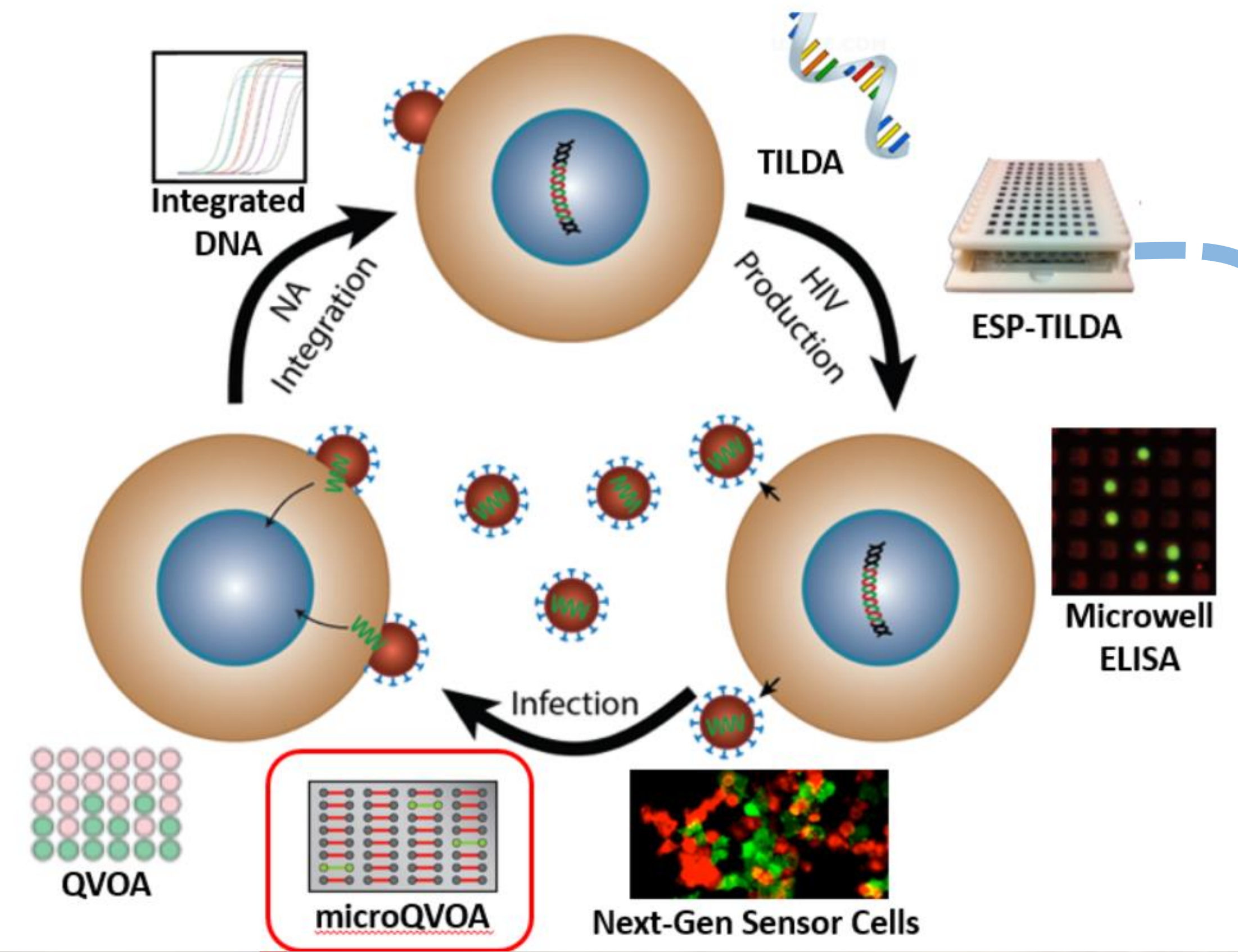


Figure 4: The CD4/CD8 ratio can be a potential additive measure of the recovery. Even though the absolute CD4 cell counts decreased, the ratio remained stable corresponding to the stability of the disease.

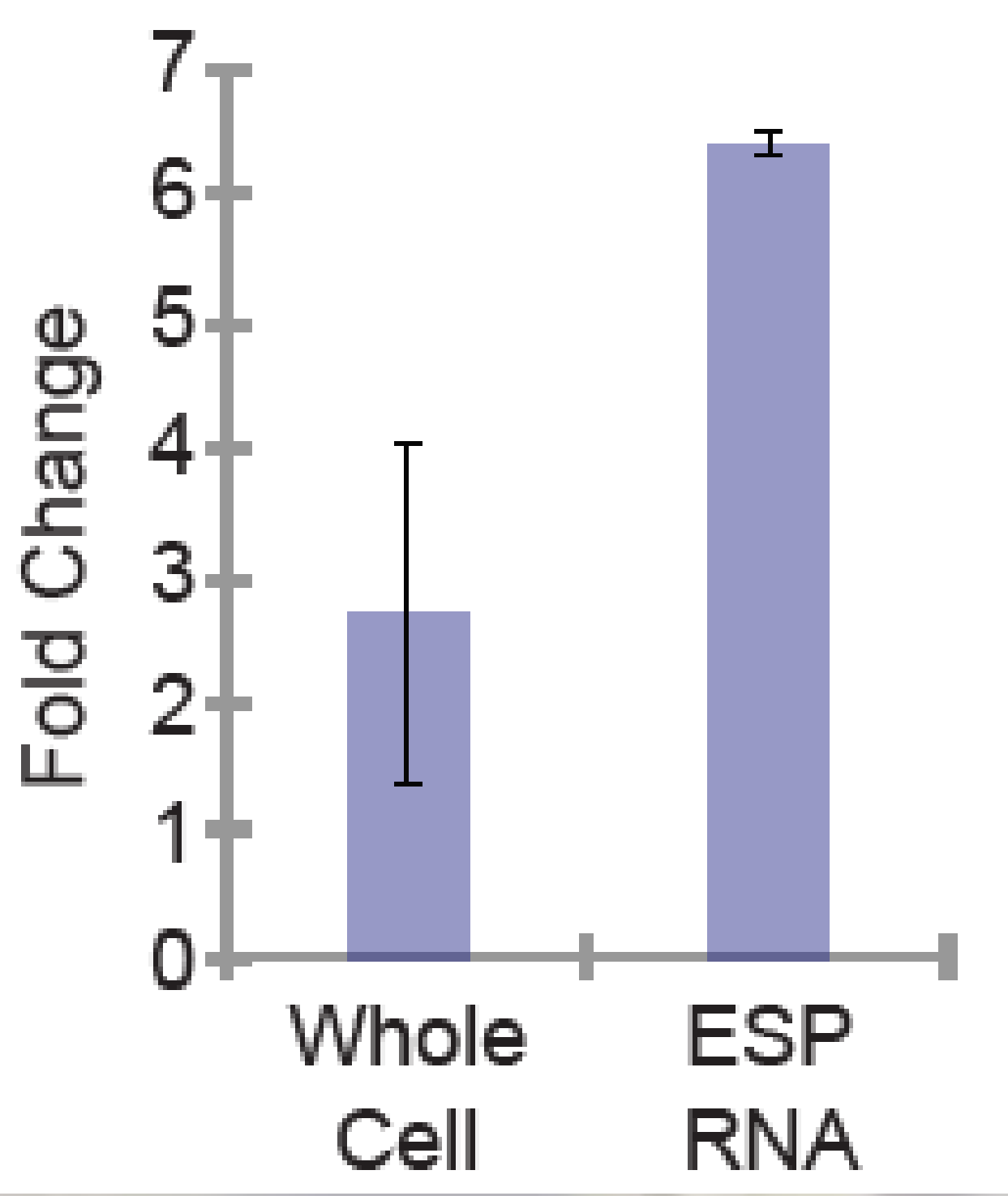
Figure 1: Measurement of the reservoir can be performed at various points of the HIV life cycle using techniques such as QVOA and TILDA.



Method 1: Schematic overview of whole cell TILDA vs. ESP RNA TILDA process (B). The ESP assay demonstrated higher sensitivity as compared to the whole cell assay

## References:

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2. Shao, Ming-Jun, Yi-Jun Zhu, Ting-Er Qiu, Min Hu, and Yun-Qin He. "Changes in the Level of Immunoglobulins and CD4/CD8 Ratio in Young and Aged Mice with Estradiol Deficiency." *Immunological Investigations* 46.3 (2017): 305-13. Web.
3. Procopio, Francesco Andrea, Raomi Fromentin, Deanna A. Kulpa, et al. "A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-Infected Individuals." *EBioMedicine* 2.8 (2015): 874-83. Web.



There are currently three methods to quantify the viral reservoir:  
1. Method 1: TILDA  
2. Method 2: Q-VOA  
3. Method 3: CD4/CD8 Ratio

## Possible Spoilers:

- HIV AB quantification may be an easy way to link ratio with reservoir.
- Treatment discontinuation occurs “naturally”. Unfortunately, after prison discharge, will it be protective of viral relapse?
- Veteran’s Affairs data contains NK immunological cell counts. Can machine learning uncover a new ratio?
- Should the goal of a cure be a supranormal ratio or balance?

## Acknowledgement:

