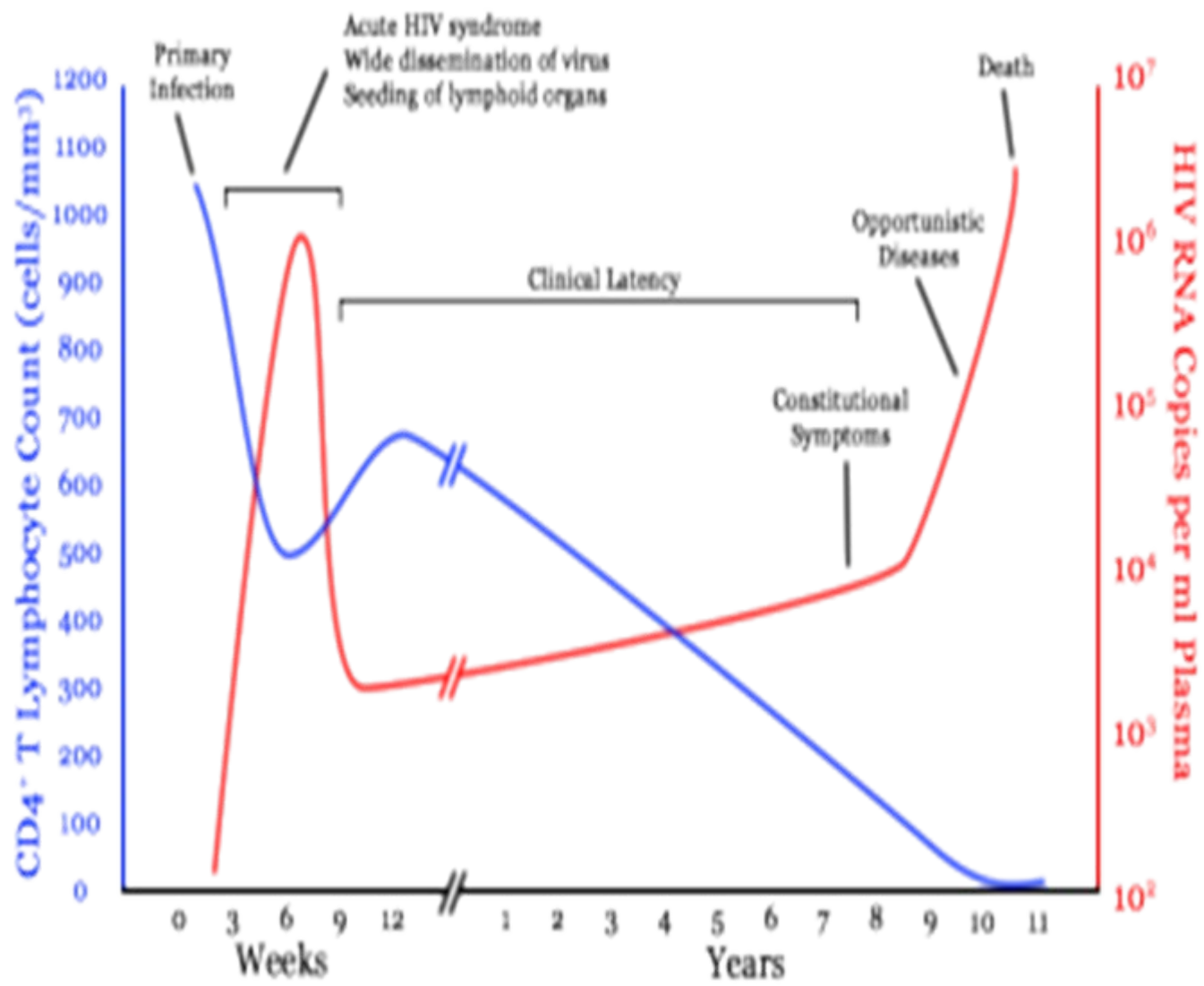


# **HIV Laboratory Monitoring: Including Surrogate Markers for Settings with Limited Access to Regular CD4 and Viral Load Measures**

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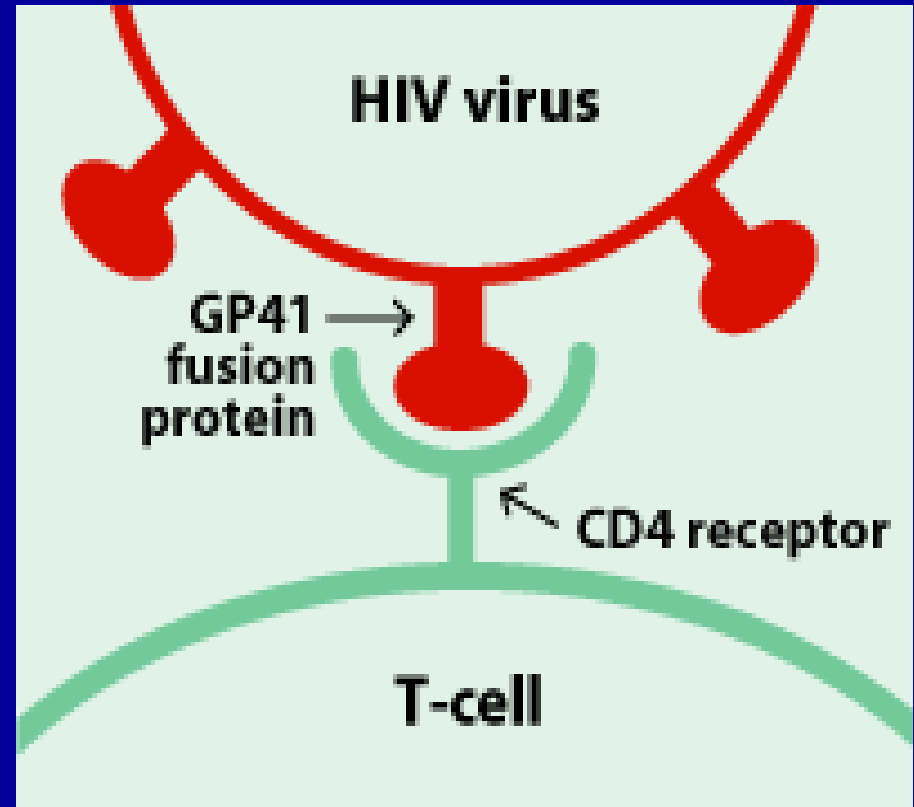
# Lecture Objectives

- Understand the gold standard immunologic and virologic measures for laboratory monitoring of HIV in treated and untreated HIV infection
- Understand the alternatives available for HIV monitoring when CD4 and VL measures are not available
- Recognize the limitations of standard and alternative measurements



# What are CD4 Cells?

- CD4 cells (or T cells or T-helper cells) are the primary targets of the HIV virus.
- Typically ↓ in number over time as HIV infection progresses.
- CD4 and/or the CD4% is the best test for evaluating HIV stage and prognosis, and for monitoring progression to AIDS and the risk of opportunistic illnesses.
- CD4 testing guides differential diagnoses in symptomatic patients, decisions about initiating antiretroviral treatment (ART), and beginning prophylaxis for opportunistic infections.



<http://www.pbs.org/frontlineworld/stories/hongkong/images/sci2.gif>

# CD4 Laboratory Reporting

- The CD4 count is reported as the number of CD4 cells per microliter ( $\mu\text{L}$ ) of blood.
- Most laboratories report the CD4 count as part of a list of several types of lymphocytes, as both an absolute count and a relative percentage.
- Example:

Absolute CD4 count = 240 cells/uL (17%)

# More about CD4

- Significant fluctuations can occur in the absolute CD4 count therefore providers should never draw definitive conclusions based on one value, particularly if different from prior trends or values (>2 or more values)
- Factors may transiently affect CD4 counts (i.e. illness, vaccination, diurnal variation, fluctuating lymphocyte counts, laboratory error, and differences between different laboratories)

# CD4 or CD4% in Children

- In children < 5 years old CD4% is the best measurement to use
- Particularly in the very young <1 year of age, the CD4 count may be markedly higher and fluctuate significantly
  - CD4 count in the very young is less predictive of mortality than CD4%
- In those >5 years of age, CD4 count can be used more reliably

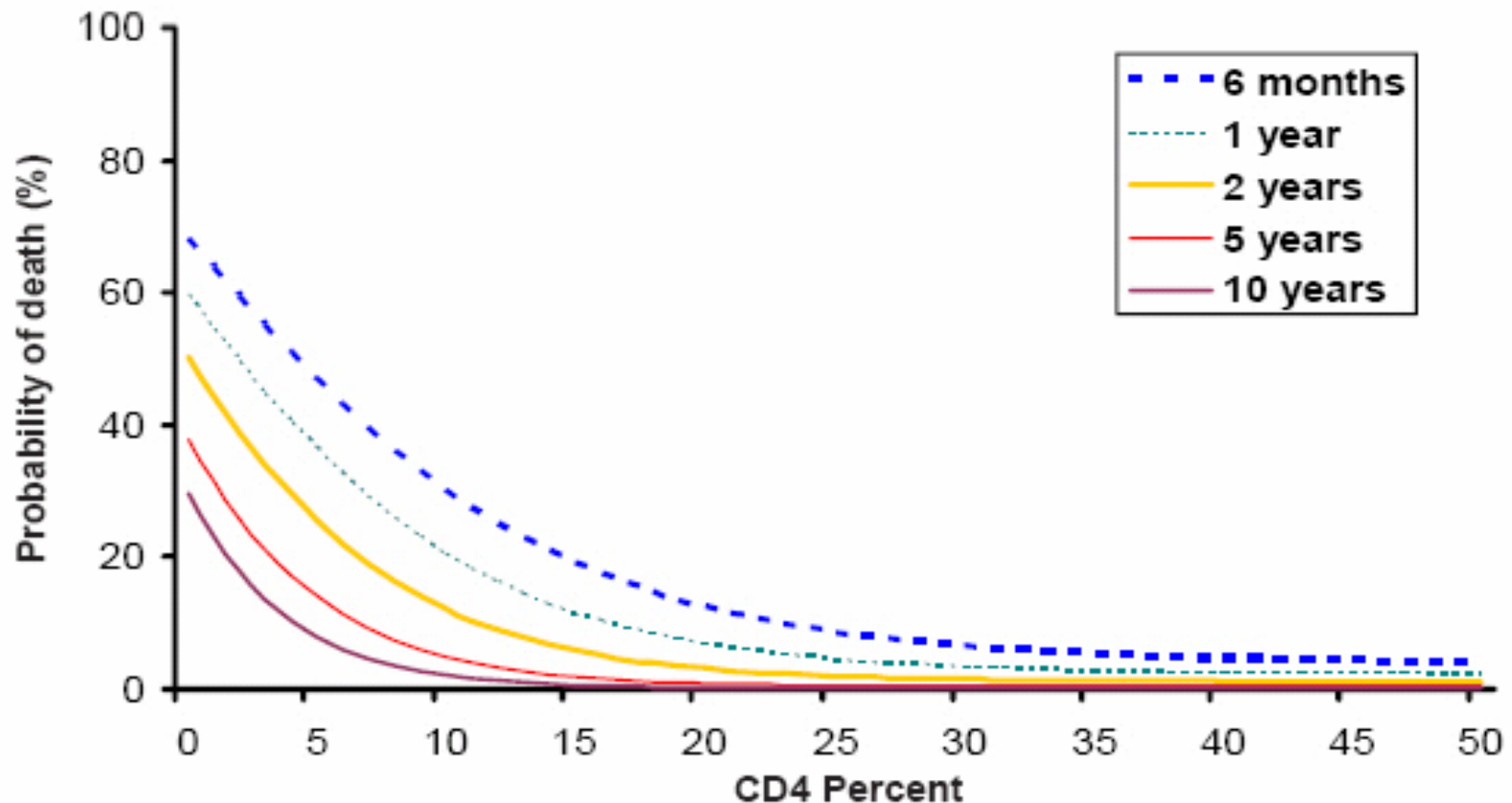
## 7.2.1 Using CD4 count

**Table 3: WHO classification of HIV-associated immunodeficiency using CD4 count**

Classification of HIV-associated immunodeficiency	Age-related CD4 values			
	$\leq 11$ months (CD4%)	12–35 months (CD4%)	36–59 months (CD4%)	$\geq 5$ years (cells/mm <sup>3</sup> or CD4%)
Not significant	> 35	> 30	> 25	> 500
Mild	30–35	25–30	20–25	350–499
Advanced	25–29	20–24	15–19	200–349
Severe	< 25	< 20	< 15	< 200 or < 15%

## CD4 Percent

Figure 2: Estimated Probability of Death Within 12 Months by Age and CD4 Percentage in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [modified from *Lancet* 2003;362:1605-11]



# CD4 in Untreated/Treated Patients

- As untreated HIV infection progresses, the CD4 count decreases by  $\sim 4\%$  per year.
- With successful ART, the CD4 count may increase by  $>50$  cells/ $\mu\text{L}$  within weeks after viral suppression, and then increases by 50-100 cells/ $\mu\text{L}$  per year thereafter until a threshold is reached.
  - Note: In some patients, CD4 counts may not increase this quickly or steadily, even with durable viral load suppression.

# Recommendations for CD4 Monitoring

- CD4 count is one of many factors (including clinical status, viral load status, and medication adherence) that should be assessed before starting or changing ART.
- Ideally the CD4 count should be repeated approximately every 2-4 months in patients on stable ART and every 6 months in untreated patients (DHHS and WHO)
- In some situations, the CD4 count may need to be checked more frequently (i.e. infants)

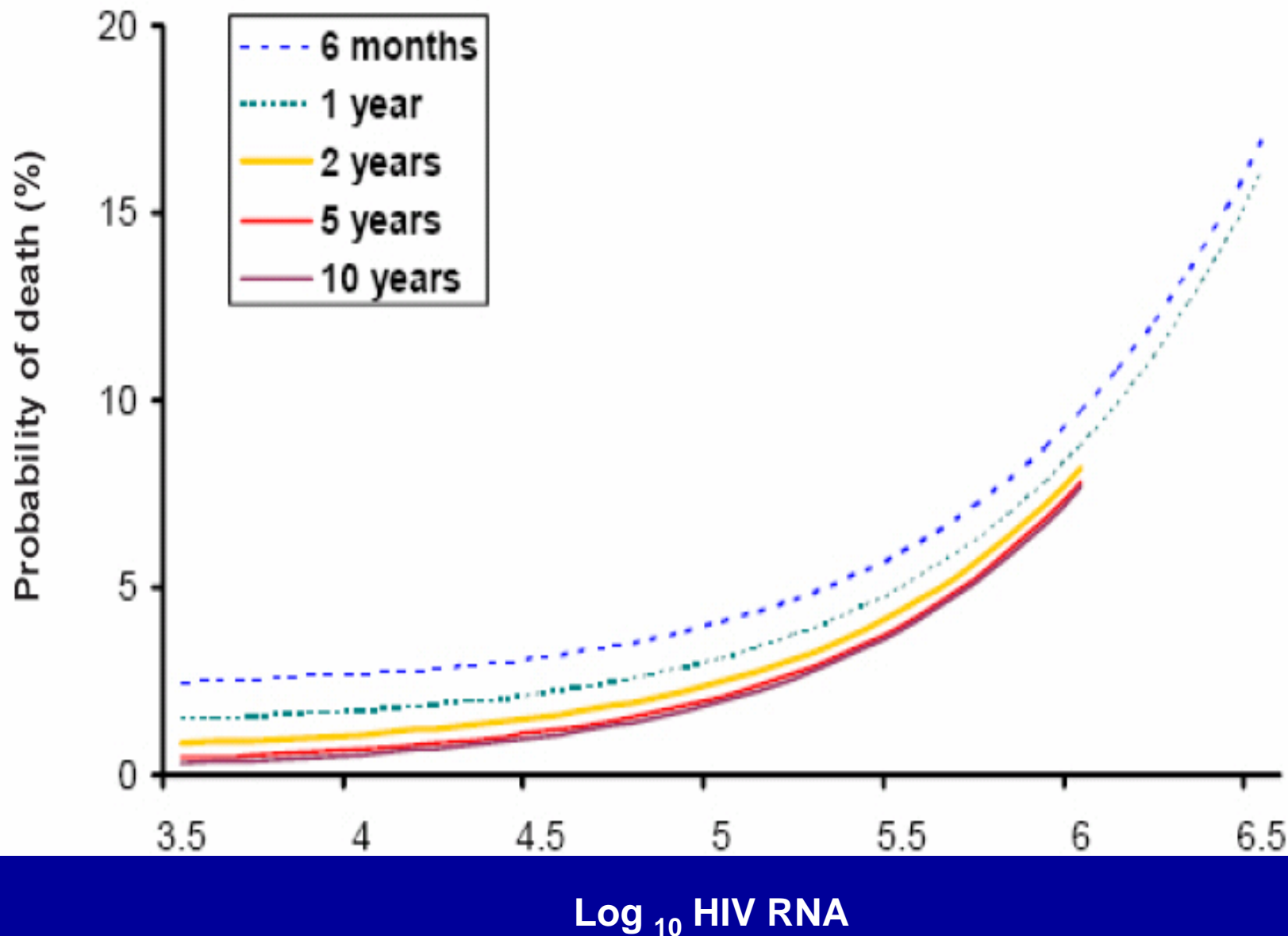
# Disadvantages of CD4 Testing

- Expensive
- Limited availability
- Laboratory variability

# HIV Viral Load

- The HIV viral load is the best indicator of how active HIV is in the patient's body.
- Higher amounts of viral activity has been correlated with more rapid disease progression as there is more HIV available to attack the CD4 cells
- When HIV antibody tests may be negative or misleading (i.e. acute HIV infection or neonatal infection), the HIV viral load may be used to help diagnose HIV infection.
- ARVs are used in combination to reduce the amount of virus in the body of someone with HIV.
- The goal of therapy is to lower the activity of HIV in the body and lower the viral load.
- With less HIV present, the body is able to produce more CD4 cells and improve the immune system.

Figure 4: Estimated Probability of Death Within 12 Months by Age and HIV RNA Copy Number in HIV-Infected Children Receiving No Therapy or Zidovudine Monotherapy [modified from *Lancet* 2003;362:1605-11]



# More about Viral Loads

- The goal of therapy is to have an undetectable viral load does meaning that the virus cannot be detected in the blood, although it exists in other parts of the body.
  - Lack of detection does not mean a cure!
  - When therapy is stopped the HIV activity increases again
- As viral load declines, the patient's CD4 count usually increases and he or she may be protected from infections and other illnesses related to HIV.

# Viral Load Testing

- Where available, the viral load reported as copies/mL or  $\log_{10}$  HIV RNA is a standard tool used to monitor viral activity and ARV response
- Many different types of VL assays (i.e. HIV RNA polymerase chain reaction (Amplicor HIV-1 Monitor; Roche Laboratories), branched chain DNA (Versant HIV-1 RNA assay; Bayer), and nucleic acid sequence-based amplification (NucliSens HIV-1 QT test; bioMerieux).
- Lowest level of detection varies for each test (most commonly 400 copies/ml).
- Ultrasensitive assays (which are preferred in most circumstances when patients are on therapy) measure viral loads to 50-80 copies/mL depending on the specific test

# Interpreting Viral Load Results

- When a viral load is below the level of detection ("undetectable") it means that at the limit of detection of that specific assay, the virus was not detected.
  - Virus may be detected with more sensitive assays
  - It does NOT mean that there is no virus in the body
- Suppressing HIV RNA to an undetectable level (<50-75 copies/mL as measured by the ultrasensitive assay) is an important goal of ART.
- The different assays also have different values for the highest levels of detection, usually between 500,000 copies/mL and 750,000 copies/mL. Higher viral loads are reported, as >500,000 copies/mL for example.

# Interpreting Viral Load Results

- Viral loads can vary considerably in (laboratory variation, assay fluctuations, in acute illness or recent vaccinations)
- Variations less than approximately 0.5 log<sub>10</sub> copies/mL (3-fold) usually are not clinically significant
- As with CD4, viral load results that are inconsistent with previous trends should be repeated, and treatment decisions usually should be based on 2 or more similar values.
- When patients have had recent illnesses or vaccinations, viral load measurement should be deferred for 4 weeks, if possible.
- Viral load should be checked at least twice at baseline, before starting an ART regimen.

# Disadvantages of VL Testing

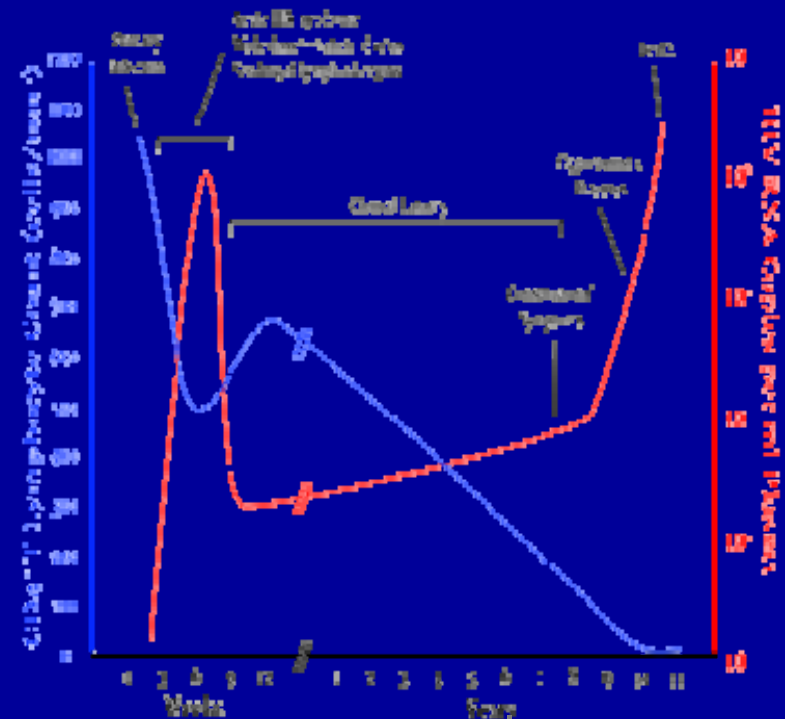
- Expensive
- Not widely available
- Labor intensive
- Laboratory variability

# Recommendations for VL Monitoring

- Where available, viral load measurement should be performed at regular intervals
- Usual interval is every 3-4 months
- Closer intervals if: new therapy or changes in therapy, significant changes in viral load or CD4 count, declining clinical status

# Putting CD4 and VL Together

- Absolute CD4 cell count is more predictive of clinical disease progression than is the baseline viral load
- Studies have shown that patients with high plasma viral loads have an increased risk of progression to symptomatic disease and AIDS compared with patients who have low or undetectable levels (rationale for DHHS guidelines).
- In acute seroconversion and in advanced disease viral loads may be markedly elevated >500,000 copies/mL, while in chronic infection usually viral loads are lower
- Concerns re: CD4 monitoring alone



**How can you monitor HIV  
disease progression when CD4  
and VL are unavailable?**

# Other Proposed Laboratory Parameters

- Total lymphocyte count (TLC)
- Hemoglobin
- Serum albumin
- p24 antigen
- When analyzed, low TLC and albumin independently predicted mortality
- WHO recommends TLC as alternative to CD4 for disease progression, but NOT for assessment of response to treatment

## 7.2.2 Using total lymphocyte count (TLC)

**Table 4: Diagnosing severe immunodeficiency using TLC (optional if CD4 is not available)**

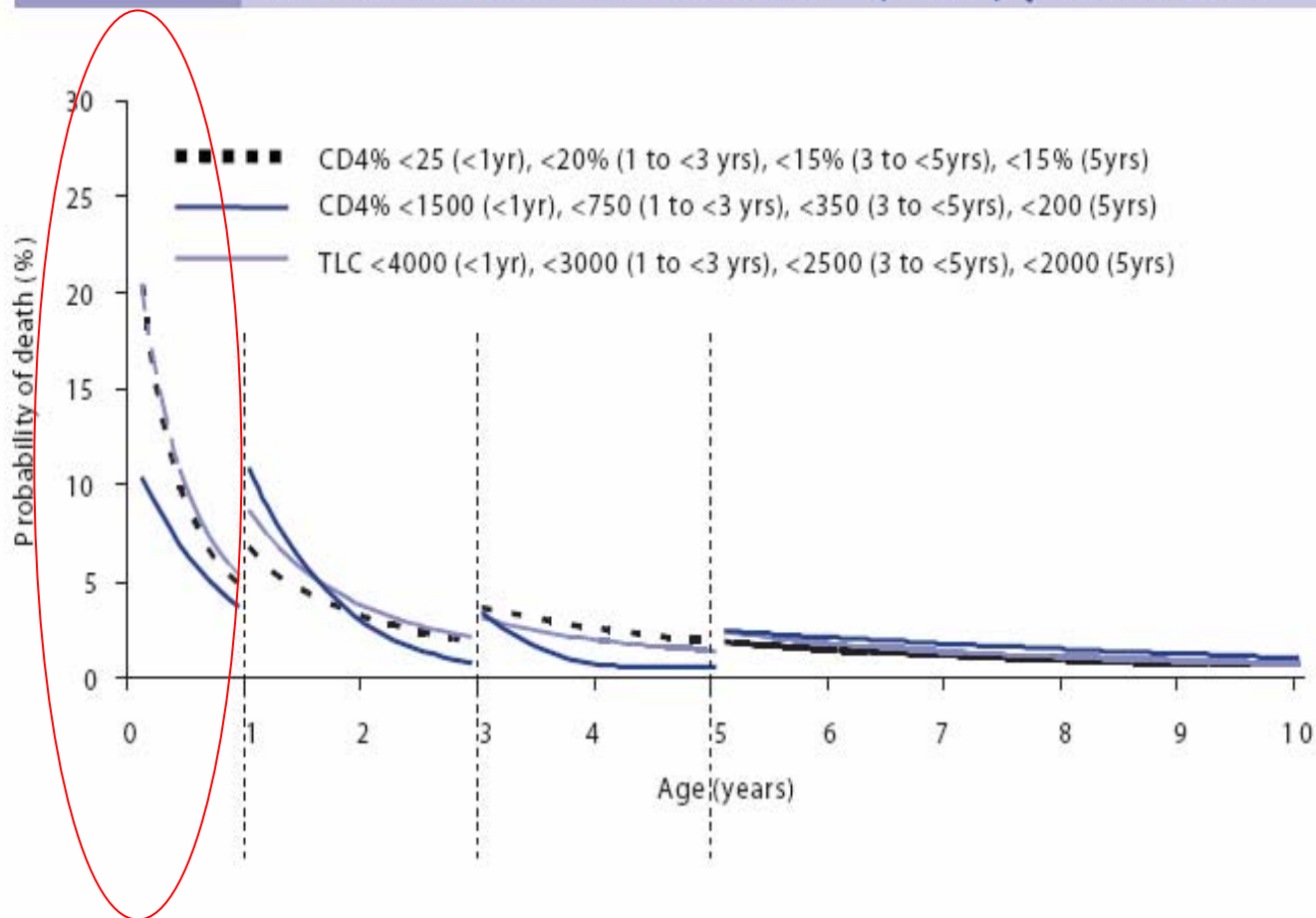
Classification of HIV-associated immunodeficiency	Age-related TLC values (cells/mm <sup>3</sup> )			
	< 11 months	12–35 months	36–59 months	≥ 5 years
TLC	< 4000	< 3000	< 2500	< 2000
CD4 count	< 1500	< 750	< 350	< 200

- The TLC is an option that is used only if CD4 measurement is not available in children with WHO clinical stage 2 disease. It cannot be used in asymptomatic children. The TLC is also not useful for monitoring ART.
- Calculation of TLC = % lymphocytes x total white blood cell (WBC) count. Annex C (*see p. 85*) shows the 12-month mortality risk at selected thresholds for CD4%, absolute CD4 cell count and TLC.

ANNEX

C

# 12-MONTH MORTALITY RISK AT SELECTED THRESHOLDS FOR CD4%, ABSOLUTE CD4 COUNT AND TOTAL LYMPHOCYTE COUNT (TLC), BY AGE



# Monitoring Children Without Laboratory Parameters

Clinical parameters are useful:

- Improvement in growth in children with prior growth failure
- Improvement or resolution of neurological symptoms and development in children with encephalopathy or delay
- Attainment of developmental milestones in those previously with developmental delay
- Decrease in frequency of infections (i.e. bacterial infections, oral thrush and/or other opportunistic infections)

# References

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