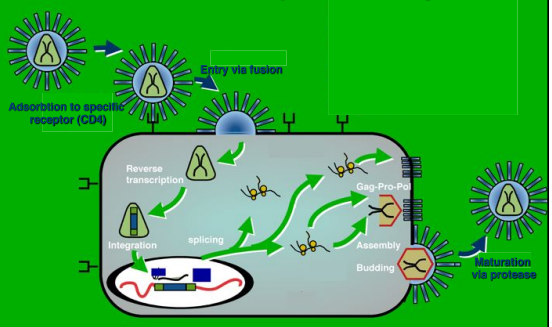
The Retrovirus Replication Cycle



Important Properties of HIV

1. Infection requires CD4 protein on the surface of the cell as receptor.
   - Therefore can only infect CD4+ ("helper") T cells and a few others.
2. Almost all infected cells die within a day or two after infection.
3. Infected CD4 cells make enough virus particles to infect about the same number of new cells (10-100 million).
4. Therefore, the infection in an individual persists by constant, repeated cycles of infection and cell death (about 1 a day).
5. These properties are also found in the benign SIV-monkey infections, but in humans there is a slow loss of total CD4 cells, leading eventually to failure of the immune system.

HIV-Host Interaction

1. After early primary infection, HIV gives lifelong persistent infection leading to AIDS after about 10 years (on average).

2. Persistence is due to constant replication of the virus and killing of 10^7-10^9 infected CD4+ T cells at about 1 cycle/day.

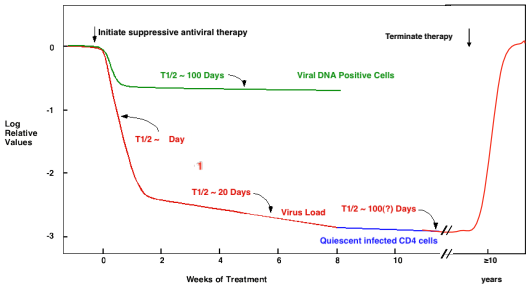
3. Smaller fractions of "latently infected" cells that live much longer after infection are probably unimportant for the natural history of the infection, but very important for foiling treatment.

4. Constant replication day after day, year after year, leads to extensive genetic variation.
   - Antigenic escape.
   - Drug resistance.
   - Variation in coreceptor usage.

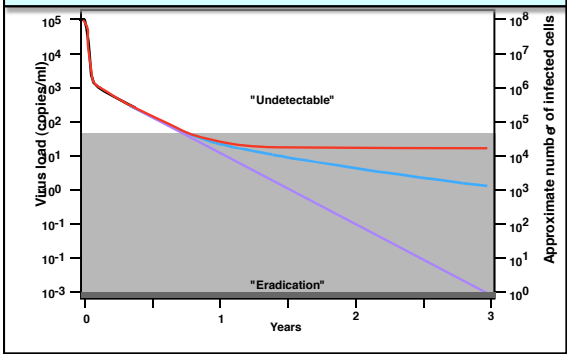
5. The system remains in an extraordinarily robust quasi steady state for thousands of replication cycles before progressing to disease.

6. We still don't know how HIV causes AIDS.

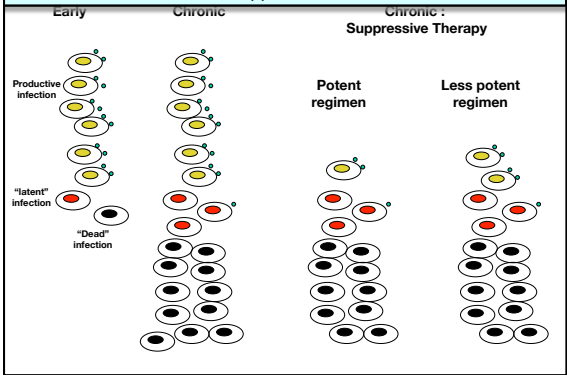
4 Populations of HIV Infected Cells



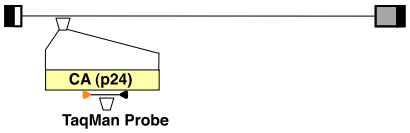
Question: What is happening to the viremia at "undetectable" levels? Ongoing low-level replication or release from latently infected cells?



Hypothesis: More potent therapeutic regimens will give a lower level of viremia at maximal suppression



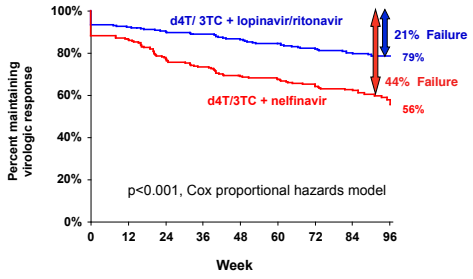
Single-Copy Assay (SCA)



- Real-time RT-PCR assay to measure viral RNA levels down to a single copy in 3 ml
- 50 to 75 times more sensitive than commercial assays
- Can monitor previously "undetectable" viremia
- This assay is being used to define the origin of persistent viremia

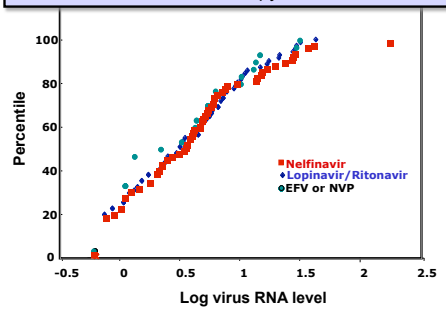
Palmer et al. JCM 2003

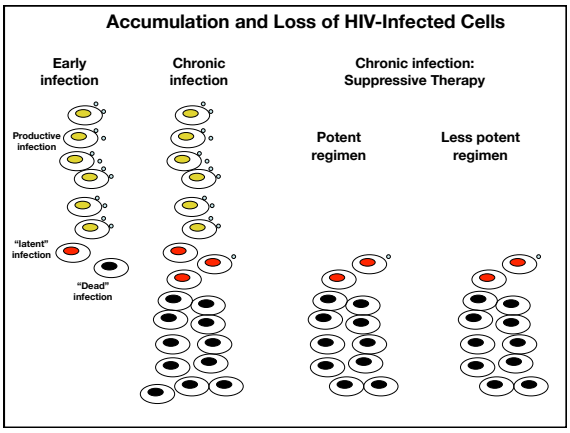
Question: Is the difference in potency in the two arms of the trial reflected in the level of viremia?



King MS, et al. JID 2004;190:280-4

Conclusion: Distribution of persistent viremia among patients is independent of PI or NNRTI Regimen. Implies that virus comes from cells infected before therapy.

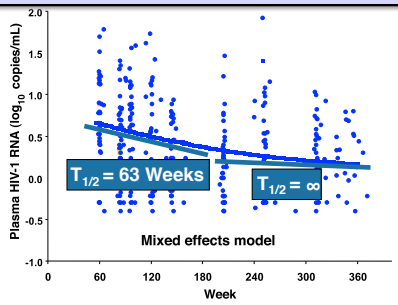




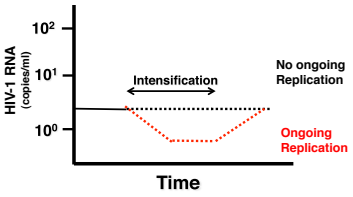
How Long Can Detectable Viremia Persist on Therapy?

- The Abbott 720 trial:
  - 41 patients on suppressive LPV/r-based therapy
  - No viremia > 50 copies RNA/ ml for more than 7 years
  - ca 10 samples each analyzed by SCA

Conclusion: Viremia persists for more than 7 years. Longitudinal analysis reveals an additional third and fourth phase of viral decay

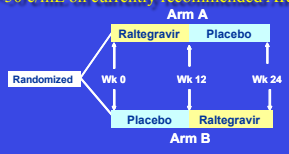


Probing the Mechanism of Chronic Viremia Using Antiretroviral Intensification



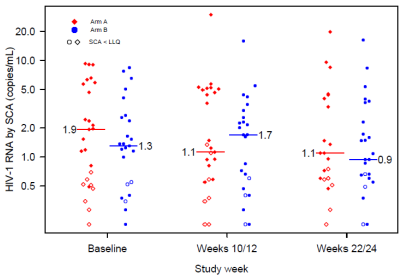
Intensification with Raltegravir (ACTG 5244)

- Randomized cross-over trial of RAL intensification in patients with HIV RNA <50 c/mL on currently recommended ART



- Primary objective: To compare HIV RNA level by SCA averaged between wks 10/12 in subjects who add RAL to subjects who do not add RAL to their background regimen

No Reduction in Low-Level Residual Viremia after Raltegravir Intensification



♦ Arm A: RAL first (immediate intensification)
● Arm B: Placebo first (deferred intensification)

Conclusions

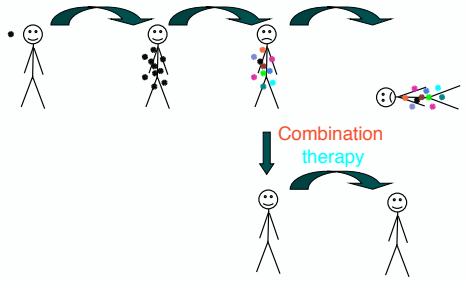
1. No effect of intensification on persistent viremia could be detected in either the aggregated patient group or in any individual patient in either study.
2. This result is independent of study site, treatment regimen (4 different) or intensifying agent (4 different).
3. The result is inconsistent with persistent replication as the source of persistent low level viremia.
4. Thus, all indications are that persistent, low level, viremia comes from cells infected prior to the start of therapy.
5. The nature of these cells remains to be determined, but the prime suspects are latently-infected CD4+ T cells.

HIV Drug Resistance Program

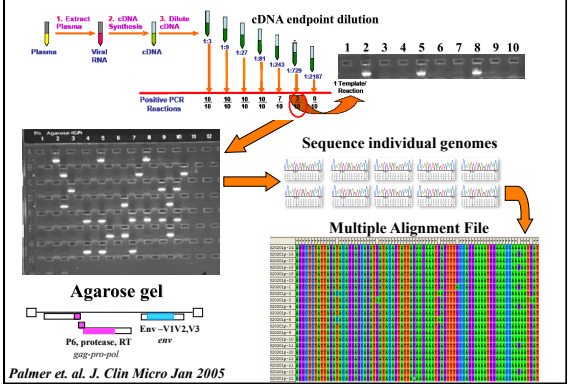
National Cancer Institute at Frederick

Genetic Diversity of HIV-1 Populations in Infected Individuals

Within-Host Virus Evolution



Single-Genome Sequencing



Palmer et. al. J. Clin Micro Jan 2005

Antiretroviral Therapy and Chronic Infection

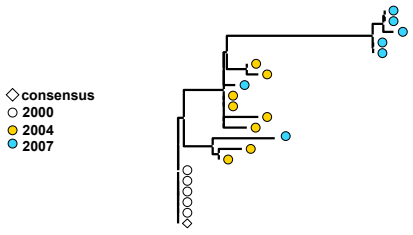
- Chronic infection is characterized by relatively stable levels of viremia comprising highly diverse virus populations for long periods of time.

  - How does the virus population evolve under these conditions?

- Therapy leads to profound reduction in HIV-1 RNA levels relative to on-therapy steady state viremia

  - How does the genetic structure of the virus population change with time?

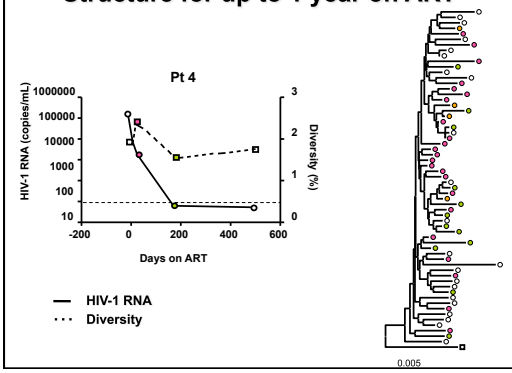
HIV-1 Populations Evolve within Patients Over Years of Infection



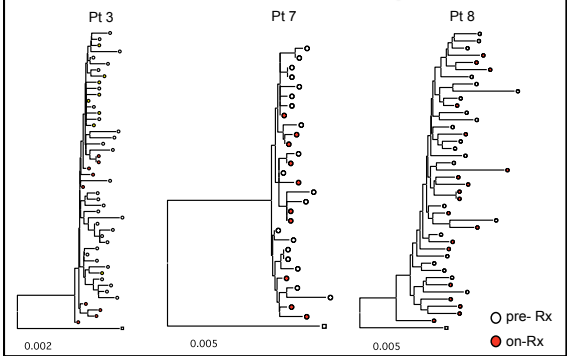
What is the Impact of Antiretroviral Therapy (ART) on HIV-1 Genetic Diversity in Plasma?

- Does HIV-1 diversity decline as the virus load is reduced on ART?
- What is the impact of long-term ART on the diversity and structure of HIV-1 populations?
- What is the genetic relatedness of rebound virus populations compared to pre-therapy virus?

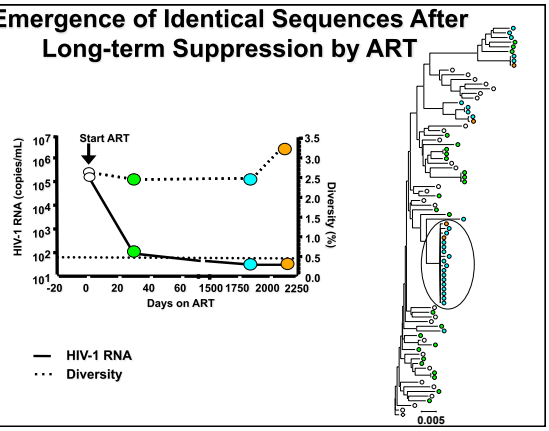
No Obvious Change in Population Structure for up to 1 year on ART



No Obvious Change in Population after One Year on ART in 9/10 Subjects



Emergence of Identical Sequences After Long-term Suppression by ART

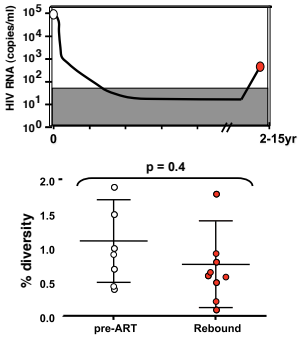


Change in Population Structure After Long-term ART in 7/8 Subjects

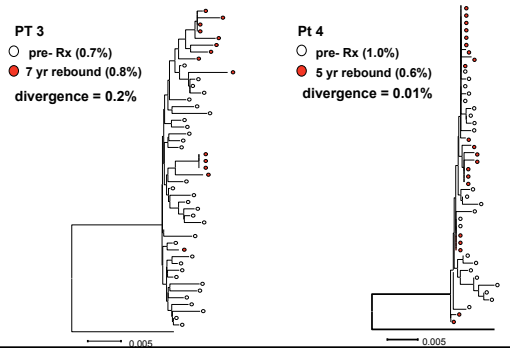
What is the Impact of Antiretroviral Therapy (ART) on HIV-1 Genetic Diversity in Plasma?

- Does HIV-1 diversity decline as the virus load is reduced on ART?
  - NO
- What is the impact of long-term ART on the diversity and structure of HIV-1 populations?
  - Emergence of identical sequences
- What is the genetic relatedness of rebound virus populations compared to pre-therapy virus?

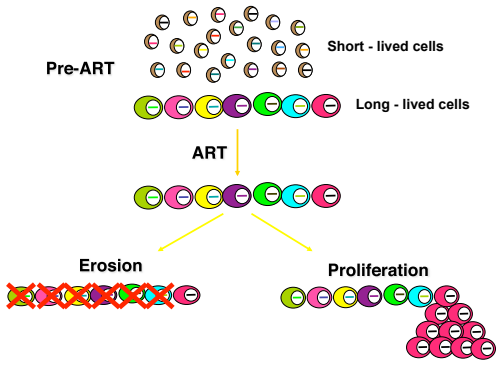
No Significant Change in HIV Diversity or Divergence of Rebound Viremia



Little Divergence in Rebound Viremia from Pre-therapy After Long-term ART



Suggested Model



Conclusions:
Can we Cure HIV Infection?

- HIV diversity does not decline following initiation of ART
  - indicating that both short- and long-lived cells are infected with similarly diverse virus populations
- A restricted group of HIV-1 variants (identical sequences) emerges after years of suppressive ART
  - suggests that there is either slow loss of chronically-infected cells or expansion of one or more chronically-infected cells
- Rebound viremia after long-term therapy has little divergence from pre-therapy virus populations
  - implicating long-lived cells infected before therapy as the source for viral rebound
- Long-lived cells must be further characterized and targeted to eradicate HIV-1 infection
  - Strategies to reverse latency by altering histone modification are being avidly pursued

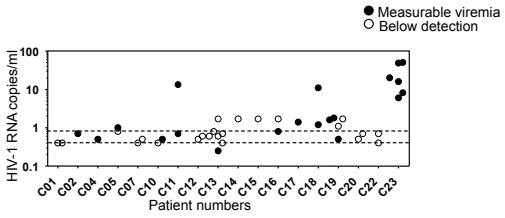
Elite Controllers

- 0.5% of HIV-1 infected population spontaneous HIV-1 RNA<50 copies/ml
- Known as HIV-1 controllers/elite controllers/elite suppressors
- Low level viremia <50 copies/ml by standard assay
- Co-cultivation with PBMC yields replication competent virus
- Plasma viruses contain no gross genetic defects and replicate well in culture
- Superinfecting virus replicates to high levels
  - Ongoing replication *in vivo*?

Study Subjects

- 21 HIV-1 controllers – Danish HIV-1 database
- 25 non controllers from an NIH cohort
- Min. 3 HIV-1 RNA<50 cp/ml within 1 year + no therapy
- Median duration of infection 11 years (IQR 7-18)
- Total of 257 plasma samples – median of 14 plasma samples available per patient
- HLA available in 16 individuals – 6/16 (38%)B*5701/27 positive

Viremia by SCA

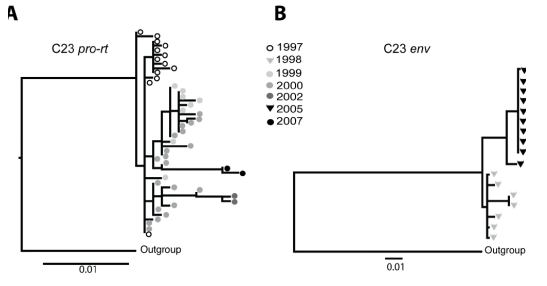


Minimum median: 0.3 copies/ml
Maximum median: 0.8 copies/ml

Ongoing Replication?

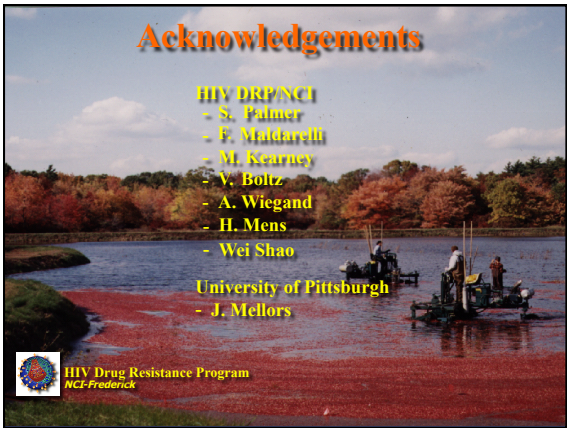
- SGS from 1-5 ml of plasma
- Amplification success in ~1/3 of samples
- A total of 337 single genome sequences of p6-rt and env
- SGS data on >2 time points in a total of 15 patients

Time Dependent Clustering Rooted ML trees



Conclusions

- Evidence of evolution:
  - Increasing root-to-tip distances of rooted maximum likelihood trees
  - 3-4 fold lower rate of replication compared to non-controllers
- Implies eplication and adaptation to specific cellular immune responses but not humoral immune responses in HIV-1 controllers
- Suggests that, unlike patients on ART, the virus undergoes full cycles of replication in HIV-1 controllers
- Most likely reflects anunusual host-virus relationship in which there is a CTL response against one or a few virus epitopes from which the virus cannot easily escape except at great cost to its replicative fitness
- Despite their superficial similarities to patients on therapy, elite controllers are not good models for patients with drug-suppressed viremia

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