Models of HIV during antiretroviral treatment

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Road Map

- HIV 101
- HIV evolution and phylogenetic models
- What about resistance?
- Complex data in patients with high-level resistance, high viremia and stable CD4 counts
- Application to HIV-1 evolution under fusion inhibitor therapy
- Discussion
Definitions

- **Genotype**: sequence of virus either nucleotide or amino acid
- **Wild-type**: virus without resistance mutations, this is our reference strain
- **Nucleotide**: A, C, T, G
- **Codon**: Set of 3 nucleotides that codes for an amino acid
- **Amino acid**: building blocks of proteins
- **Phenotype**: behavior of the virus
- **Envelope (env)**: gene region involved with viral entry and target of fusion inhibitors
  - gp41: part of env and the target of fusion inhibitors
  - gp120: part of env and a target for the immune system
- **CD4**: Human immune cell and favorite target of HIV
- **Enfuvirtide (ENF)**: fusion inhibitor-disallows HIV entry into cell
- **Coreceptor**: secondary receptor for entry (CCR5 or CXCR4)
- **ART**: anti-retroviral therapy. Anti-HIV drugs
- **HAART**: Highly Active Anti-Retroviral Therapy. A therapeutic regimen consisting of at least 3 drugs in 2 different classes
HIV 101

Classes of drugs

- Reverse transcriptase inhibitors
  - Nucleoside/nucleotide
  - Non-nucleoside inhibitors
- Protease inhibitors
- Fusion inhibitors
- Integrase inhibitors
- Entry inhibitors
Natural History of HIV Infection

- Severity of illness is determined by amount of virus in the body (increasing viral load) and the degree of immune suppression (decreasing CD4 counts).
- Higher the viral load, the sooner immune suppression occurs.

What’s a Viral Load anyway?

- Amount of virus produced every day by viral replication (billions)
- Amount of virus in the body
- Amount of virus destroyed every day by the immune system (billions)
CD4 increases when viremia suppressed but a rapid rebound in virus when HAART stopped.
Motivating example: HIV-1 evolution under fusion inhibitors

- Enfuvirtide (ENF) is a fusion inhibitor that acts on env gp41
- Reserved for patients with high-level resistance
- Patients placed on optimized background + ENF
- Patients quickly developed ENF resistance
- ENF portion only of regimen interrupted (Partial Treatment Interruption (PTI))
- 16 weeks post PTI most patient strains were wildtype

Questions:
- Where does the wildtype come from?
  - Older archived strains?
  - Continued evolution (back-mutation)?
- Does the evolution of gp41 influence the evolution of gp120?
- Does drug resistance attenuate viral virulence?
Intra host HIV-1 evolution
Intra-host HIV evolution

- HIV exists as a quasi-species within a patient
- Intra-host phylogenies can be difficult to resolve
- Two approaches
  - Examine each patient independently and look for common patterns across patients
  - Concatenate the sequences together

1195 env sequences from 9 patients [taken from Rambaut et al 2004]
Trouble with current methods

- Want to know which evolutionary tree is more likely across all K subjects
- Want to know this across gene regions too
- Trouble with current methods:
  - Examine all patients independently
  - Ignores uncertainty
  - Difficult to draw conclusions across subjects
Trouble with current methods

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Trouble with current methods

- **Want to know** which evolutionary tree is more likely across all $K$ subjects
  - Other method: concatenate patient sequences
    - Forces all patients to have the same evolutionary pressures and events

- **Solution**: Hierarchical Phylogenetetic Models (HPMs)
  - A balance between complete independence and concatenated models
Hierarchical Phylogenetic Models (HPMs)

Simultaneously reconstruct evolutionary histories from multiple patients by assuming that within-patient parameters $\theta_k$ are drawn from common across-patient distributions, characterized by population-level estimable parameters $\Phi$:

- For $\theta_k$: pooling/borrowing of strength leads to more efficient within-patient estimates (smaller variances)
- Helpful when one has short sequences with sparse phylogenetic information
Hierarchical Phylogenetic Models (HPMs)

Simultaneously reconstruct evolutionary histories from multiple patients by assuming that within-patient parameters $\theta_k$ are drawn from common across-patient distributions, characterized by population-level estimable parameters $\Phi$:

- Across-patient distributions illuminate common patterns
- Estimate and test tendencies in $\Phi$ while allowing $\theta_k$ in individual patients to vary
Tree Results

**gp41**

**gp120**
Evolutionary Interactions

- Traditional log-linear models explore main effects and interactions in count data that form contingency tables.
- Testing for interactions generalizes the $\chi^2$ test.

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Counts are unknown
Evolutionary Interactions

- The tree counts ($y_{rc}$) are random (not observed)
- Estimated through sequence data via phylogenetic reconstruction with a twist

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$y_{rc}$
Instead of assuming that the trees $\tau_{ij}$ are independent across patients and gene regions, we place a log-linear motivated prior on them through their summary statistics:

$$y_{rc} = \sum_i 1\{\tau_{ij} = r\}$$

$$y_{rc} \sim \text{Poisson (log linear mean)}$$

Results

- Cell counts were modeled as Poisson
- Bayes Factor for independence over dependence was 3
- Little evidence of evolutionary dependence
- Did not find a relationship with coreceptor usage
- Caveat: small sample size
Results

- Overall, the data across all patients and gene regions supported the Continued Evolution (Tree E) over the Re-emergence (Tree R) hypothesis.
- Bayes Factor=1428, p< 0.0001.
- If we look at gp41 alone, the Bayes Factor was 30.2
- Suggest pooling strengthened conclusions.
Implications

- 5/7 patients strongly support the Evolution tree in each gene region
- Results for gp41 and gp120 both support Evolution hypothesis
- Loss of resistance during ENF interruption often occurs due to ongoing viral evolution (and back-mutation), rather than emergence of archived virus.
- Particularly true in patients whose virus remained diverse or became more diverse during ENF therapy.
- Possible residual antiviral activity of ENF after interruption
- Suggests ongoing immunologic pressure against envelope in advanced disease, with the emergence of virus population that is immunologically more fit than previous generation of viruses.
Evolution of resistance and fitness

- Fitness effect based on circumstantial evidence
  - Resistant strains often exhibit reduced replication capacity in an in vitro assay
  - Virus typically reverts back to a wild-type drug susceptible genotype following treatment cessation
- No direct measurements of the effects of resistance evolution and fitness in vivo
Examined 18 patients with a history of enfuvirtide failure and who were no longer taking the drug

Added ENF for 4 weeks to an existing optimized stable regimen

454 based pyro-sequencing of HIV-1 gp41 performed on plasma on a weekly basis
Frequency resistant over time by patient
CD4 Counts over time
Sequence Data

- Over 38,000 unique sequences of gp41(!)
- Neighbor Joining trees using Tamura-Nei model of evolution
- Detect ultra-minority quasi-species
- Resistance associated mutations characterized according to IAS guidelines
Patient 3109, almost 1000 unique sequences.

- **Black-on T20**
- **Blue- wk 1-6**
- **Light blue wk 8-16**
- **Red: 16-34 weeks**
Results

- At baseline, frequency of resistance was low and quickly expanded when ENF was added.
- CD4 counts were highly positively correlated with ENF resistance.
- 454-based phylogenetic trees support a decrease in diversity following treatment cessation followed by an increase in diversity after 20 weeks.
Conclusions

- Data support the hypothesis that ENF resistance mutations attenuates the virus in vivo.
- Implicate decreased HIV-1 virulence (capacity to cause CD4 depletion) as the principal mechanism driving the sustained immunologic benefit to staying on ENF despite remaining viremic.
Acknowledgements

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HIV Drug resistance

- The drugs work well for most people
- But not everyone...
HIV Drug Resistance

- HIV-1 resistance is the major obstacle to successful treatment
- Estimated that 76% of the HIV infected population in the US has resistance to at least 1 drug (Richman et al AIDS 2004)
Background

- Subset of patients who have high level of cross-resistant HIV
- Rates of immunological and clinical progression is lower in patients with drug-resistant HIV
- Changes in T-cell activation and/or viral fitness may lead to alterations in relationship between viremia and CD4 cell loss
Subjects

- 54 subjects from the Partial Controllers on Antiretroviral Therapy (PCAT) cohort
- Detectable viremia between 200-10,000 copies/ml
- Stable (but not fully suppressive) combination therapy
  - Enrolled before next generation ARVs were available
- Viral load, CD4 T cell count measured every 4 weeks
- Immune activation (CD38/HLA-DR) measured every 16 weeks
Patients have detectable drug-resistant viremia but stable CD4 counts
Log viremia and log CD8 activation over time
Methods

- Multiple Imputation for missing at random data (MCMC method)
- Nonlinear mixed effects models with a random intercept were used to fit the main models
- Spectral decomposition was used to ascertain the presence of an oscillatory signal in a subset of patients
Results-all patients

- Median baseline CD4 counts was 303 cells/mm³
- Median baseline viremia was 3.3 log copies/ml
- 74% failing with a PI regimen
- Patients on boosted regimen has an increase of 20 cells/mm³ while others had an average decrease of 33 cells/mm³
- CD8 activation strongly associated with viremia over time (p<0.01)
- CD4 activation was a strongly associated with CD4 counts over time (p<0.001) controlling for viremia
Subset analysis

- A subset of patients (N=11) had more extensive analysis
- Subjects had on average 25 time points (range 20-28) over up to 120 weeks (range 95-120) follow up
- No significant difference between the subset and main dataset in clinical parameters.
- Spectral analysis can detect cyclical patterns in time-series data using a Fourier transform.
  - Time series decomposed as a mixture of sine functions
  - Examined viremia, CD4 / CD8 activation and viremia
Results - Spectral analysis

- Spectral analysis showed a significant oscillatory signal in CD8 and CD4 activation as well as viremia.
- Complex forms of mixtures of 0-7 sine waves
Nonlinear mixed models-subset

- CD8 activation was a significant predictor of viremia over time (p=0.0007)
- Patients whose maintenance regimen did not include a PI had a stronger oscillatory signal (p=0.04)
Conclusions

- Immune activation is a strong predictor of immunologic outcomes in stable patients with highly resistant HIV.
- The oscillatory relationship between virus and T cells is most readily explained by a shift in predator-prey dynamics.
- These data support prior conceptual models suggesting that reductions in viral fitness will lead to paradoxical CD4 outcomes by preserving target cells.
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Can long-term suppression restore CD4 counts to a normal level?

- Normal level defined to be a CD4+ T cell count > 500 cells/mm3
- Antiretroviral therapy has ability to fully restore CD4 counts, is this true for all patients who can maintain suppression?
- Not been tested with long-term follow up
Inclusion Criteria

- Achieve suppression < 1000 copies/ml within first 48 weeks of therapy initiation
- Maintain suppression < 1000 copies/ml for at least 4 years
- Patients allowed to modify antiretroviral regimen as long as suppression < 1000 copies/ml
CD4 T-cell counts over time in patients with 10 years of continual suppression and had a pre-therapy CD4 count < 200 cells/mm³
Statistical Methods-longitudinal data

- Spline-smoothing regression fit due to potential non-linearity over time
- Found to have 1 knot at 4 years
- Piece-wise linear with knot at 4 years
- Mixed-effects model
- Other cofactors: Baseline CD4 count, nadir CD4 count, CD4 count at year 4, hepatitis C co-infection. Use of boosted protease regimen, year of HAART initiation, timing of initial response to HAART, pre-HAART exposure to antiretrovirals, proportion of visits with “blips”.
- CD4 square root transformed.
- Fit using R
Time to immune reconstitution analysis

- Fit using the method of Kaplan Meier
- Endpoint: immunologic restoration, defined as 2 consecutive CD4 counts > 500 cells/mm3
- Strata compared using log rank test
  - Year 4 CD4 count
  - Baseline CD4 count
  - Nadir CD4 count
Results

- 151 (41%) patients had CD4 < 500 at year 4
- 61/151 (40%) eventually had increases to > 500 cells/mm3
- 0/48 patients who started with CD4 < 200 cells at baseline achieve immunologic restoration after 10 years of suppression
Average CD4 slopes after year 4

- Overall change in CD4 slope after year 4 was 17 cells/mm³ per year (95% CI: 11-21 yr)
- If stratify by year 4 CD4 count:
  - < 350: 21 cells (12-31)
  - 350-499: 17 cells (6-28)
  - > 500: 11 cells (3-17)
- 19% of CD4 < 350 has slope not different from zero
- 27% of CD4 350-500 had slope not different from zero
Time to immunologic restoration by year 4
CD4 count

![Graph showing the probability of immunologic restoration over months on HAART for different CD4 count categories: N=216 for CD4 count <350, N=76 for 350-500, and N=74 for >500.](image)
Time to immunologic restoration by baseline CD4

- 95% of patients with a pre-HAART baseline > 300 cells were able to achieve immunologic restoration
- 25% of patients with a baseline CD4 between 100-200 cells did NOT achieve restoration
- 44% of patients with baseline CD4 > 100 did NOT achieve restoration
Time to immunologic restoration by baseline CD4 count

- N=60
- N=50
- N=67
- N=72
- N=101
Percentage of patients with immunologic restoration by CD4 nadir

![Graph showing percentage of patients with CD4+ T cell count >500 cells/mm³ over years of HAART, divided by initial CD4 cell count categories: >350 cells/mm³ (closed black circles), 200-350 cells/mm³ (gray open circles), and <200 cells/mm³ (white open circles).](image)
Discussion

- There appears to be a threshold for which the immune system cannot fully recover
- Suggests initiation of therapy before CD4 decline below 200
- Small subset of people who are not able to achieve immunologic restoration
Caveats

- **Selection bias**
  - Only patients with durable long-term suppression included

- Level of suppression rather high. Now can get is < 50 copies/ml

- Intermittent viremia not significant in models

- Unmeasured confounders

- Immune activation, other co-infections
  - Age was a significant predictor of CD4 decline over time