The multi-drug cocktail

Please sit in row K or forward
In honor of Darwin’s birthday...
Random (not entirely biological) fact of the day: trees, languages, folk tales

Language-tree divergence times support the Anatolian theory of Indo-European origin
http://www.nature.com/nature/journal/v426/n6965/full/nature02029.html
Comparative phylogenetic analyses uncover the ancient roots of Indo-European folktales
http://rsos.royalsocietypublishing.org/content/3/1/150645
Midterm Feb 22 in class

- Location: Shan 1430
- Covers material through this week
- 8.5 x 11, handwritten sheet of notes (double sided) permitted
  - You must write your own
  - We will provide the genetic code (if needed)
- Advice for studying
- Review session in class next Monday
Topics for today

• Revisiting the question of why HIV has killed so many people
• Modern treatment approaches: the cocktail
• How we know a treatment works: clinical trials
• Preparing for homework 5: recursion on trees
Question: why has HIV killed so many people?

- Defeating the immune system
  - Attacks the immune system itself
  - Rapid evolution
- Epidemiological factors

The biological basis of the asymptomatic period

• Immune system attacks
  • free virus with antibodies
  • infected cells

• Moderate, but not complete success

Asymptomatic patients are infectious

Thought experiment: in which case will the virus spread to more people?

<table>
<thead>
<tr>
<th>Helper T-cell count (cells per mm³)</th>
<th>HIV RNA copies per mL plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>10⁶</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>10⁵</td>
</tr>
<tr>
<td>AIDS</td>
<td>10⁴</td>
</tr>
<tr>
<td>Death</td>
<td>10³</td>
</tr>
<tr>
<td>Hypothetical variant that kills quickly</td>
<td>Death</td>
</tr>
</tbody>
</table>

Graphs showing the timeline of HIV progression and a hypothetical variant that kills quickly.
Question: why has HIV killed so many people?

• Defeating the immune system
  • Attacks the immune system itself
  • Rapid evolution

• Epidemiological factors: long asymptomatic period allows efficient spread of virus
Topics for today

• Revisiting the question of why HIV has killed so many people
• Modern treatment approaches: the cocktail
• How we know a treatment works: clinical trials
• Preparing for homework 5: recursion on trees
Effect of a single drug regimen

Treatment with single drug (e.g. AZT)
Single drug regimen and HIV abundance

HIV RNA copies per mL plasma vs. weeks and years.

- **Acute**
- **Asymptomatic**
- **AIDS**

**Start of treatment**

- **Untreated**
- **Single drug regimen**
There are many anti-retroviral drugs available

- Targeted points in the viral life cycle
  - Binding
  - Fusion
  - Reverse transcription
  - Integration
  - Proteolytic cleavage

http://dx.doi.org/10.1016/S0140-6736(10)60676-9
You can find many small molecules to bind any given protein:
Effect of multi-drug regimen

Treatment with multi-drug cocktail

(no detectable virus)
Multi-drug regimen and HIV abundance

Why doesn’t resistance evolve?

- Untreated
- Single drug regimen
- Multi-drug regimen

Start of treatment

Detection threshold

HIV RNA copies per mL plasma

Weeks

Years
Federally approved HIV/AIDS medical practice guidelines

Panel's Recommendations

- An antiretroviral regimen for a treatment-naive patient generally consists of two nucleoside reverse transcriptase inhibitors in combination with a third active antiretroviral drug from one of three drug classes: an integrase strand transfer inhibitor, a non-nucleoside reverse transcriptase inhibitor, or a protease inhibitor with a pharmacokinetic enhancer (cobicistat or ritonavir).

- The Panel classifies the following regimens as Recommended regimens for antiretroviral-naive patients:
  - **Integrase Strand Transfer Inhibitor-Based Regimens:**
    - Dolutegravir/abacavir/lamivudine\(^2\) — only for patients who are HLA-B\(^*\)5701 negative (AI)
    - Dolutegravir plus tenofovir disoproxil fumarate (tenofovir/emtricitabine)\(^1\) (AI)
    - Elvitegravir/cobicistat/tenofovir/emtricitabine — only for patients with pre-antiretroviral therapy CrCl > 70 mL/min (AI)
    - Raltegravir plus tenofovir/emtricitabine\(^1\) (AI)

- **Protease Inhibitor-Based Regimen:**
  - Darunavir/ritonavir plus tenofovir/emtricitabine\(^*\) (AI)

- On the basis of individual patient characteristics and needs, an Alternative regimen or, less frequently, an Other regimen, may in some instances be the optimal regimen for a patient. A list of Alternative and Other regimens can be found in Table 6.

- Given the large number of excellent options for initial therapy, selection of a regimen for a particular patient should be guided by factors such as virologic efficacy, toxicity, pill burden, dosing frequency, drug-drug interaction potential, resistance testing results, comorbid conditions, and cost. Table 7 provides guidance on choosing an antiretroviral regimen based on selected clinical case scenarios. Table 8 highlights the advantages and disadvantages of different components in a regimen.

**Rating of Recommendations:** A = Strong; B = Moderate; C = Optional

**Rating of Evidence:** I = Data from randomized controlled trials; II = Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = Expert Opinion

\(^*\) Lamivudine may substitute for emtricitabine or vice versa.
Is multi-drug treatment a cure?

• Half life of virions in blood is on scale of minutes
• Active helper T-cells (the main infected cells producing HIV) have a half life on scale of weeks
• Multi-drug regimen completely stops new infections
Patients must stay on anti-retrovirals for life

- **HIV RNA copies per mL plasma**
  - **Start of treatment**
  - **End of treatment**
  - **Untreated**
  - **Multi-drug regimen**
  - **Detection threshold**

- **Weeks**
- **Years**
Normal scenario for cell infection

Active helper T cell → infection → Infected helper T cell producing new viral particles → Several weeks → Cell dies at end of normal lifetime (or is killed by immune response)
Formation of a latently infected cell: occasionally an actively infected cell converts to a resting memory state.
There is a latent reservoir of HIV in resting memory T cells that spread around the body.
Cells with latent HIV occasionally revert back to an active state. Occurs with a small probability. Cell now producing new viral particles follows its normal course and dies after a few weeks.
- Reservoir of infected long term memory cells forms during the pre-treatment period.

- Reservoir persists even after start of treatment.
  - Reservoir cells return to active state with some small probability; however they cannot restart infection because of presence of drugs.

- Once patient is off drugs reactivating cells quickly restart large scale infection.
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- Revisiting the question of why HIV has killed so many people
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- How we know a treatment works: clinical trials
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How we know a treatment works: clinical trials
Anatomy of a trial: something to compare

Treatment group A
- zidovudine (AZT)
- lamivudine
- indinavir

Nucleoside reverse transcriptase inhibitors
Protease inhibitor

Treatment group B
- zidovudine (AZT)
- lamivudine
- placebo

Nucleoside reverse transcriptase inhibitors
Anatomy of a trial: randomization

577 HIV patients randomized into group A:

- Treatment with two nucleoside reverse transcriptase inhibitors (NRTI)
- Protease inhibitor

579 HIV patients randomized into group B:

- Treatment with two nucleoside reverse transcriptase inhibitors (NRTI) ONLY

Follow until end of trial, or until patient gets AIDS or dies.
Patients on a three drug regimen are less likely to get AIDS or die

<table>
<thead>
<tr>
<th></th>
<th>2 NRTI + protease</th>
<th>2 NRTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>577</td>
<td>579</td>
</tr>
<tr>
<td>AIDS or death</td>
<td>33</td>
<td>63</td>
</tr>
</tbody>
</table>
Patients on a three drug regimen live longer
Patients on a three drug regimen have lower HIV RNA levels
Modern approaches to treatment are based on many clinical trials...
Nucleoside reverse transcriptase inhibitors (NRTIs) were the first AIDS drugs developed. There are numerous types, many of which are well tolerated.

1. The clinical trial we just discussed showed that a three drug cocktail of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor is more effective than a cocktail of two NRTIs. One interpretation of this is that three drugs is (in general) better than two. What is an alternative interpretation?

2. What sort of a clinical trial could you perform to distinguish between these interpretations. In particular, what would be the different treatments you would give the two groups in your trial?

3. The current federal HIV/AIDS treatment guidelines recommend using a multi-drug cocktail with two NRTIs and at least one drug with another mechanism of action (e.g. a non nucleoside RT inhibitor, an integrase inhibitor or a protease inhibitor). Why do you think such cocktails work better?
Nucleoside reverse transcriptase inhibitors (NRTIs) were the first AIDS drugs developed. There are numerous types, many of which are well tolerated.

1. The clinical trial we just discussed showed that a three drug cocktail of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor is more effective than a cocktail of two NRTIs. One interpretation of this is that three drugs is (in general) better than two. What is an alternative interpretation?

   Having a **non-NRTI** (like a protease or integrase inhibitor) in the cocktail provides an especial benefit.

2. What sort of a clinical trial could you perform to distinguish between these interpretations. In particular, what would be the different treatments you would give the two groups in your trial?

   One way: compare 3 NRTIs vs. 2 NRTIs
   Another way: 3 NRTIs vs. 2 NRTIs + something else (e.g protease).
   Another way: 2 NRTIs vs. 1 NRTI + something else (e.g protease).

3. The current federal HIV/AIDS treatment guidelines recommend using a multi-drug cocktail with two NRTIs and at least one drug with another mechanism of action (e.g. a non nucleoside RT inhibitor, an integrase inhibitor or a protease inhibitor). Why do you think such cocktails work better?

   If you have three NRTIs, one could imagine that its possible for single mutations to arise which affect the efficacy of more than one drug. This is bad. We’d like it to be impossible for a single mutation to confer resistance to more than one drug.
Topics for today

• Revisiting the question of why HIV has killed so many people
• Modern treatment approaches: the cocktail
• How we know a treatment works: clinical trials
• Preparing for homework 5: recursion on trees
Preparing for homework 5: recursion on trees

exTree = ('anc',
    ('anc',
        ('groody',(),(),3),
        ('anc',
            ('froody',(),(),2),
            ('oody',(),(),1),
        2),
    3),
    ('anc',
        ('snoody',(),(),2),
        ('zoody',(),(),3),
    3),
0)
How many leaves does this tree have?

```python
def leafCount(Tree):
    '''Return the number of leaves in Tree.'''

exTree = ('anc',
          ('anc',
           ('groody',(),(),3),
           ('anc',
            ('froody',(),(),2),
            ('oody',(),(),1),
            2),
            3),
          ('anc',
           ('snoody',(),(),2),
           ('zoody',(),(),3),
          3),
          0)

>>> leafCount(exTree)
5
>>> leafCount(('snoody',(),(),2))
1
```
How many leaves does this tree have?

```python
exTree = ('anc',
    ('anc',
     ('groody',(),(),3),
     ('anc',
      ('froody',(),(),2),
      ('oody',(),(),1),
     2),
    3),
    ('anc',
     ('snoody',(),(),2),
     ('zoody',(),(),3),
    3),
0)

>>> leafCount(exTree)
5
>>> leafCount(('snoody',(),(),2))
1

def leafCount(Tree):
    '''Return the number of leaves in Tree.'''
    if Tree[1] == ():
        return 1
How many leaves does this tree have?

```python
def leafCount(Tree):
    '''Return the number of leaves in Tree.'''
    if Tree[1] == ():
        return 1
    else:
        return leafCount(Tree[1]) + leafCount(Tree[2])

exTree = ('anc',
    ('anc',
        ('groody',(),(),3),
        ('anc',
            ('froody',(),(),2),
            ('oody',(),(),1),
        ),
    ),
    ('anc',
        ('snoody',(),(),2),
        ('zoody',(),(),3),
    ),
0)

>>> leafCount(exTree)
5
>>> leafCount(('snoody',(),(),2))
1
```
Return a list of all the leaves in a tree

exTree = ('anc',
    ('anc',
        ('groody',(),(),3),
        ('anc',
            ('froody',(),(),2),
            ('oody',(),(),1),
        2),
    3),
    ('anc',
        ('snoody',(),(),2),
        ('zoody',(),(),3),
    3),
0)

>>> leafList(exTree)
['groody', 'froody', 'oody', 'snoody', 'zoody']

>>> leafList(('snoody',(),(),2))
['snoody']

def leafList(Tree):
    '''Return a list of all the leaves in a tree.'''
Return a list of all the leaves in a tree

def leafList(Tree):
    '''Return a list of all the leaves in a tree.'''
    if Tree[1] == ():
        return [Tree[0]]
    else:
        return leafList(Tree[1])+leafList(Tree[2])
Return the sum of all branch lengths in a tree

```python
def totalTime(Tree):
    '''Return the sum of all branch lengths in a tree.'''
```

```python
>>> totalTime(exTree)
19
```

```
0 3 3
  3 2 1
    2 3
      1

""groody""
""froody""
""oody""
""snoody""
""zoody"
```
Return the sum of all branch lengths in a tree

```python
def totalTime(Tree):
    '''Return the sum of all branch lengths in a tree.'''
    if Tree[1] == ():
        return Tree[3]
    else:
        return Tree[3] + totalTime(Tree[1]) + totalTime(Tree[2])

>>> totalTime(exTree)
19
```
Hand in your worksheet please!

(and be sure you put your name on it)