Studies of mother - infant transmission of HIV-1

- When and how does mother-to-child transmission (MTCT) of HIV occur?
  Studies of breastfeeding transmission from mother-to-infant; 1992-1997

- What interventions are used to try to stop MTCT?
- What impact do these have on infants who become infected?
  Recent study on the impact of treatment to prevent MTCT.

Combination of new lab approaches and epidemiological studies.
  → improve outcomes
  → define mechanisms
Nairobi clinical trial of breast feeding transmission of HIV-1 (Nduati and Kreiss, 1992)

- To determine the frequency and timing of breast milk HIV-1 transmission from a mother to her infant.

- Randomized clinical trial comparing infant HIV-1 infection between breast-fed and formula-fed groups.

- 425 HIV-1 seropositive women in Nairobi, Kenya enrolled at 32 weeks of pregnancy. Mothers and infants were followed from birth through 2 years of life.

The situation in developing countries in 1992-1997:
- treatments to prevent MTCT were not available.
- Breastfeeding was recommended by WHO, even among HIV-1 positive women.
The majority of breastfeeding transmission occurs in the first 6 weeks postpartum.

Breast milk HIV RNA levels correlate with transmission --> They are highest in early milk
Without treatment interventions, 30-40% of infants born to HIV+ women will be infected.

- **in-utero**: ~10%
- **labor & delivery**: ~10%
- **breastfeeding**: ~15-20%

- WHO recommends breastfeeding in cases where women do not have access to safe methods of replacement feeding.
- Most women in developing countries breastfeed their infants even if they are HIV positive.
  - Stigma
  - Lack of clean water for formula
  --> infant death due to other infections.
Current PMTCT strategies:

**Nevirapine (NVP):** Single dose to the woman at the onset of labor and to the infant within 72 hours postpartum.

**Zidovudine (AZT/ZDV):** Daily dose from 36 weeks gestation through labor.

A combination of the two, AZT/sdNVP, is currently recommended in developing countries.
Variable risk of MTCT of HIV (with and without preventive interventions)

- no ARV, breastfeeding
- no ARV, no breastfeeding
- NVP, breastfeeding
- AZT, no breastfeeding
- ARV, no breastfeeding, C-section

0% 25% 50% 75% 100%

- 0% infected
- 25% infected
Randomized trial: NVP vs AZT monotherapy

- Which, if either of these treatments, affect breastmilk viral levels during the critical early breastfeeding period when most transmission occurs?
- Is there a difference in the risk of infant infection in breastfeeding women?
Mean log_{10} HIV-1 RNA in breast milk versus days postpartum

Days Postpartum

Mean log HIV-1 RNA

T_{1/2} = 45-60 hrs

T_{1/2} = 1-2 hrs

= p<0.01
What about transmission?
This was only a phase II trial of 60 mother-infant pairs.
Still saw a difference! (Chung et al, AIDS 2005)

- There was a significant decrease in transmission risk at 6 weeks postpartum for mother-infant pairs who received NVP. P = 0.02.

<table>
<thead>
<tr>
<th>Infant infections:</th>
<th>delivery</th>
<th>week 6</th>
<th>month 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/30</td>
<td></td>
<td>2 (6.8%: 95% CI 0.0-15.9%)</td>
<td></td>
</tr>
<tr>
<td>2/30</td>
<td></td>
<td>8 (30.3%: 95% CI, 12.7-47.9%)</td>
<td></td>
</tr>
</tbody>
</table>
**Single dose NVP**

- **Great option:**
  - Reduces HIV-1 infection during delivery and during early breastfeeding (because it has a long half-life).
  - More practical, since many women in developing countries do not present for care until delivery.

**Any downsides?**
- The long half-life of the drug means that infants are exposed to monotherapy for a long time.
- Leads to antiviral resistance.
Monotherapy - use of one antiviral vs HAART = highly active antiretroviral therapy - a combination of 3 drugs

Time to resistance:
- Fewer rounds of replication, less chance for a mutation that confers resistance to occur
- More drugs means it takes more mutations to develop full resistance: increases the complexity of the puzzle
CD4 receptor

reverse transcriptase

co receptor

make new virus particles

LTR gag pol

vif rev tat

vpu

su tm

envelope

nef
Sequence differences are ~ 100X higher in HIV than in the human genome

Human genome
Person 1

10kb

Person 2

~ 0.1%

HIV genome
Person 1

~ 10-30%

Person 2

LTR gag pol envelope LTR
Single nucleotide polymorphisms confer resistance to NVP

Schematic of positions of mutations in reverse transcriptase that are known to confer resistance to NVP (and other drugs of a similar class- called NNRTIs)
High prevalence of drug resistance in mothers and infants treated with intrapartum sdNVP

~ 20% of women had virus with NVP resistant mutations at 6-8 weeks postpartum
~ 45% of infected infants at 8 weeks of age had virus with NVP resistant mutations

- Eshleman SH, et al. 
- JAIDS 2004; 35; 126-30.
Single dose NVP

- Major downside for an infant who does get infected despite NVP prophylaxis:
  - They may harbor a virus that is resistant to NVP.
  - The recommended combination (HAART) regimens in developing countries include NVP plus two other drugs.
    - A NVP-exposed infected infant may start out being treated with basically only two drugs if the NVP is not effective --> easier path to resistance to the other drugs.
In a multivariate analysis, that included all women who had received intrapartum NVP, detectable NNRTI and NRTI resistance mutations were not associated with virologic failure (p=0.28 and p=0.31, respectively), after adjustment for viral load at treatment initiation.

*Jourdain et al NEJM 351(3): 229-240.*
New study Objective:

OPH6-12: Grace-John Stewart, Dalton Wamalwa

- To compare response to therapy in NVP-exposed infants randomized to NVP-containing vs. NVP sparing HAART.
Current study: Should maternal PMTCT / ARV therapy influence the HAART regimen chosen for infants who become infected despite PMTCT treatment?

Failed NVP PMTCT
Infant is infected
High risk of resistant virus

Enroll 6-12 mo. old infants who do not have detectable NVP resistance by genotyping (population sequencing) N=200.

NVP sparing:
AZT + 3TC + PI
(needs refrigeration, less palatable, costly)

NVP containing:
AZT + 3TC + NVP

- Infant response – clinical, immunologic, virologic
- Is there a threshold where resistance to one drug in the combination does not impact treatment outcome?
ARV Regimens 1st line infants

**First line NVP unexposed:** (WHO & Kenya)
- ZDV or D4T + 3TC + NVP

**First line NVP exposed:** (Kenyan Ministry of Health)
- ZDV + 3TC + PI (protease inhibitor: Kaletra)

Some countries are still unsure how best to treat NVP exposed infants and guidelines have been changing
# Nevirapine vs. PI's

<table>
<thead>
<tr>
<th></th>
<th>NVP</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability at room temperature</td>
<td>Good</td>
<td>Poor refrigerate</td>
</tr>
<tr>
<td>Palatability</td>
<td>Good</td>
<td>Unpalatable</td>
</tr>
<tr>
<td>Adherence</td>
<td>Potentially better</td>
<td>Challenging</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Future options</td>
<td>PI's</td>
<td>Less</td>
</tr>
</tbody>
</table>
Current study: lab aspects

Failed NVP PMTCT
Infant is infected
Need method of detecting infant infection

Enroll 6-12 mo.old infants who do not have detectable NVP resistance by genotyping (population sequencing)
Need method of detecting resistance

NVP sparing:
AZT + 3TC + PI
(needs refrigeration, less palatable, costly)

NVP containing:
AZT + 3TC + NVP

• Infant response – clinical, immunologic, virologic
• Is there a threshold where resistance to one drug in the combination does not impact treatment outcome?

Need quantitative assay to detect resistance mutations at low levels
Issues with off the shelf assays

- They were designed to detect the strains of HIV in the US and Europe, which differ from strains of HIV in Africa by up to 30% in sequence.
- They are designed for ‘developed world payment systems’:
  - Drug resistance assay ~ $500
  - Diagnosing an infant as HIV+ ~ $50
  - The good thing is drugs are free through PEPFAR

Develop ‘home-brew’ methods here that are affordable and work with strains in Africa.

Transfer to Kenya
Diagnosing infants

Issues with early diagnosis of HIV-1 in infants

- Infants <18 months harbor maternal antibodies - can’t use ELISA
- Moms and infants do not want to provide a vial of blood
  need a good collection method
- Needs to be inexpensive and amenable to high throughput

PCR detection of HIV in dried blood spots collected on filter paper (FP)-DBS

Routine DBS PCR still involves intricate DNA extraction.

Developed a method that requires little processing
The method we developed for use in our lab in Seattle requires two rounds of PCR (gag assay)- increases chances of contamination
Develop a new assay that is just one round (pol assay)
METHOD OF HIV-1 DNA FP PCR

- One rounds of Pol PCR
- Two rounds of Gag PCR
- Gel electrophoresis
Optimizing pol FP PCR

- Since DBS are limited, assay must be sensitive down to 1 copy of HIV since only a small volume is sampled.
- Blood can cause issues with inhibition and therefore the maximum amount that can be added to the PCR must be defined.
- It must work across the diverse HIV strains in Africa.
FP DBS
- FP’s spiked with a known copy number of HIV-1 template

Expects product : 166 bp
Summary of results using filter paper that has HIV negative blood spiked with HIV-1 + cells

<table>
<thead>
<tr>
<th>Cell count</th>
<th>Real-time copies/rx after lysis</th>
<th>No. positive qualitative PCR</th>
<th>Avg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>45/80</td>
<td>56.2</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>59/80</td>
<td>73.7</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>32/40</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>8.0</td>
<td>79/80</td>
<td>98.7</td>
</tr>
</tbody>
</table>
Validation of the ‘in-house’ FP PCRs versus other gold standard assays

Sensitivity & Specificity of the single round pol FP PCRs

<table>
<thead>
<tr>
<th>Defined by:</th>
<th>PCR type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma RNA</td>
<td>Pol</td>
<td>89%</td>
<td>97%</td>
</tr>
<tr>
<td>Gag PCR</td>
<td>Pol</td>
<td>89%</td>
<td>97%</td>
</tr>
</tbody>
</table>
Assays for single nucleotide polymorphisms confer resistance to NVP

Pol/Rt

<table>
<thead>
<tr>
<th>Bases</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td>Lysine (K)</td>
</tr>
<tr>
<td>AAG</td>
<td></td>
</tr>
<tr>
<td>AAC</td>
<td>Asparagine (N)</td>
</tr>
<tr>
<td>AAT</td>
<td></td>
</tr>
</tbody>
</table>

NVP resistant mutations

~ 400 bp
**HIV-1 drug resistance sequencing assay**

1. extract HIV-1 RNA
2. cDNA synthesis
3. Two rounds of PCR
4. sequencing
Once the drug is removed, wild type virus may outcompete the resistant virus.

Most drug resistant HIV strains do not replicate as well as their wild type counterparts.
Standardizing the ‘in-house’ genotyping resistance assay: sequencing of mixed plasmids Q23  wild-type : k103n
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to view this picture.
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.
Sequencing of mixed wild-type and mutant plasmids

WT: K103N

100:0

80:20

50:50

20:80
Is there a more standard way to define whether the peak is mixed?
Patterns of antiretroviral resistance after short-course monotherapy?

Does resistance persist at low levels, below the level we can detect with sequencing assays?
Challenges for this approach

• Our goal is to detect rare variants, not just dominant ones
  realistically detecting a resistant variant at 0.1-1% would be great, as we can’t usually sample more than ~ 1000 copies

• We need to be able to deal with background variation
  HIV is genetically diverse

• The assay must be quantitative
Generate a mutant template that has the resistance mutation

Primer for mutant

Wild type template

Wild type

mutant

Mutant template

Primer for mutant

Template amplifies in PCR
There are at least 3 different subtypes (clades) of HIV-1 in Kenya.

Results of analyses of viruses from >300 women in Nairobi. Neilson, 1999

- **Subtype A**: 5-12% intrasubtype diversity
- **Subtype D**: 20-30% intersubtype diversity
NVP resistant mutations:

K103N mutation (NVP)
Allele-specific PCR using primers that match the resistant virus

<table>
<thead>
<tr>
<th>Template copy number</th>
<th>WT</th>
<th>HIV-negative controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>$10^5$</td>
<td>HS DNA</td>
</tr>
<tr>
<td>$10^4$</td>
<td>$10^4$</td>
<td>Human genomic</td>
</tr>
<tr>
<td>$10^3$</td>
<td></td>
<td>Human cell line</td>
</tr>
<tr>
<td>$10^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Template:
- Resistant
- WT
- HIV-negative controls
Allele-specific real time assay to quantify mutant virus sequences

Real time PCR with subtype A-specific primers can amplify down to 10 copies of subtype A mutant template and does not detect up to $10^5$ copies of wild-type template.
Is there a threshold below which the presence of a resistance mutation does not impact future treatment?

Infant rapidly fails combination therapy that includes NVP

Infant does well on combination therapy that includes NVP

Level of detection of sequence assays ~ 20%

NVP\textsuperscript{R} virus is present at 5%

NVP\textsuperscript{R} virus is present at 0.2\%