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<table>
<thead>
<tr>
<th>Speaker Name</th>
<th>Statement</th>
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<tr>
<td>Prof Philippe van de Perre</td>
<td>None</td>
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**COMPETING INTEREST OF FINANCIAL VALUE > £1,000:**

| Date       | November 2013 |
The science of transmission of HIV via breastmilk

Philippe Van de Perre

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BHIVA, London, November 2013
PMTCT research, 1994-2012

1994 U.S. AZT Trial ACTG 076
1998 Thai Bangkok short AZT
1998 Cote d’Ivoire short AP/IP AZT trials (Bfeeding)
1999 PETRA trial AZT + 3TC
1999 HIVNET 012/Uganda single dose NVP (moms & nn)
2000 Thailand PHPT-1 Long vs short AZT
2003 ANRS DITRAME + AZT/3TC/NVP
2004 Thai trial PHPT-2 AZT & NVP
2008 PEPI NVP + short vs long AZT for bfed infant
2008 SWEN NVP PreP for bfed infant
2009 Mma Bana: ART vs Prep CD4<200
2010 BAN: ART vs PreP (CD4>250)
2011 Kesho Bora: ART (CD4>350, Bfeeding)

Source: McIntyre J, Perinatal HIV Clinical Trials
What has been acquired from PMTCT research

• **Prevention of de perinatal HIV transmission:**
  ✓ Early initiation of prophylaxis during pregnancy;
  ✓ Combination ARTs are more effective than monoprophylactic regimens;
  ✓ Some drugs are more efficacious, some may be hazardous (Efavirenz and neurological defects)* ;
  ✓ The target of elimination (MTCT < 5%) seems achievable, if no breastfeeding.

• **Prevention of postnatal (breastfeeding) HIV transmission:**
  ✓ No prophylactic trial covering the whole duration of breastfeeding exposure (= 12 months);
  ✓ Important residual transmission (3.6% at 6 months in the Kesho Bora trial);
  ✓ Concerns about adherence ;
  ✓ The target of elimination seems out of reach.

* Sibiude et al, CROI 2013, Atlanta
WHO guidelines for PMTCT and infant feeding (June 2013)

... but research on breastfeeding transmission should continue!
June 2013 UN guidelines? A critical analysis

- Alarming inflation in the number of WHO-UNICEF PMTCT recommendations (’90s: n=1, 2000s: n=4, 2011-2013: n=2);

- Current WHO PMTCT recommendations are not evidence-based;

- Push for option B+ is based on mathematical models, best guess estimates on feasibility but NOT on measured efficacy or efficiency.

Van de Perre P; BMJ 2013
Option B or B+?

• Suboptimal efficacy on postnatal transmission in the Kesho Bora trial: in mothers with > 350 CD4/μl, 6-month efficacy = 29% (NS)*;

Exception of the « TasP dogma »?

• Suboptimal adherence: in a metanalysis of more than 20,000 pregnant women, adherence of 53% at 12 months post partum**;

• Extremely high rate of resistance in infants who get HIV-infected despite maternal prophylaxis***

* Kesho Bora Study Group, Lancet Infect Dis, 2011
** Nachega et al, AIDS 2012
*** Zeh, PlosMed 2011; Fogel, Clin Infect Dis 2011; Lidström, CROI 2010
Mechanism(s) of breastfeeding transmission of HIV: the moving target

An evolving host

A complex and biologically active source of infection


Graph showing cells/ml over weeks of lactation:
- Macrophages
- Lymphocytes
Portal of entry
Polarised HIV-1 infected cell

Gal Cer

Transcytosis in an enterocyte

Macrophages, lymphocytes and dendritic cells in the \textit{lamina propria}
Transcytosis of HIV-1 across human enterocytes

- Concept of viral synapse
- HIV-1 gp41 recognises a membrane agrin (heparan sulfate proteoglycan) that favour interaction with GalCer and mediate transcytosis through an integrin associated mechanism

A Alfsen, 2005
Breastfeeding transmission of HIV-1: by free virions or by HIV-infected cells?
Cumulative HIV-1 RNA exposure in HIV-1 infected and non-infected infants between 6 weeks and estimated age of HIV acquisition

ANRS 1271 Study / VTS

<table>
<thead>
<tr>
<th>Cumulative HIV-1 RNA exposure until HIV infection</th>
<th>Case</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total; N=36 pairs</td>
<td>19.65 x 10^7</td>
<td>1.30 x 10^7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal antenatal CD4 &gt;350 cells/µl; N=14 pairs</td>
<td>14.86 x 10^7</td>
<td>1.27 x 10^7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Neveu D, Clin Infect Dis 2010
Cell-free and cell-associated HIV-1 are both responsible for breast milk transmission (I Koulińska, 2006)

<table>
<thead>
<tr>
<th>HIV-1 Transmission</th>
<th>Cell-free virus</th>
<th>Cell-associated virus</th>
<th>indetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9 m post p</td>
<td>2</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 9 m post p</td>
<td>11</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>16</td>
<td>11</td>
</tr>
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</table>

p=0.03
Characteristics of T and B lymphocytes from breast milk

Compared to blood, breast milk T and B lymphocytes are

- More frequently memory cells (less naive cells)
- More often activated
- Express markers of homing signing their mucosal origin

(E. Tuaillon et al; J Immunol 2011)
LABORATORY STRATEGY

A

Breast milk cells plus red blood cells of healthy control

Blood

Ficoll Hypaque

Spin

Plasma

Enriched CD4+ T cells

Ficoll-Hypaque

Red blood cells and rosetted cells

Unwanted cells are cross-linked to red blood cells «depletion cocktail »

B

Irradiated cells

Anti-CD28 antibodies

Resting CD4 T cells

Anti-CD3 antibodies

Activated CD4 T cells

HIV-1 antigens

Day

Day 1

Quantification of the HIV-1 DNA by real-time PCR

Enumeration of the HIV-1-Ag SCs by ELISPOT assay

Detection of p24 antigen in supernatants by ELISA
Proportion of latently infected cells able to enter viral cycle

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Breast milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 DNA copies</td>
<td>6.948</td>
<td>4.788</td>
</tr>
<tr>
<td>per 10^6 T CD4+ cells</td>
<td>(2.351-23.043)</td>
<td>(2.590-47.294)</td>
</tr>
<tr>
<td>HIV-1 Ag secreting cells</td>
<td>45 (9-108)*</td>
<td>500 (205-934)*</td>
</tr>
<tr>
<td>per 10^6 T CD4+ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of HIV-1 infected T CD4+ cells entering viral cycle</td>
<td>0.9 - 1.8%</td>
<td>10.4 - 32.4%</td>
</tr>
</tbody>
</table>

Cell activation in breast milk:
- Associated with reactivation of CMV and EBV
- Consistent with cytokine and proteome profiles
**Productively infected CD4⁺ T cells from BM**

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<th>Blood</th>
</tr>
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<tr>
<td>HIV-1 DNA copies/10⁶ CD4⁺ T cells</td>
<td>2886</td>
<td>2240</td>
</tr>
<tr>
<td>HIV-1-Ag-SC/10⁶ CD4⁺ T cells (with undetectable HIV-1 RNA)</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>HIV-1-Ag-SC/10⁶ CD4⁺ T cells (with detectable HIV-1 RNA)</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

**Viral antigens, RNA copies and infectious virus are detected in cell culture supernatants**

*Valea D et al, Retrovirology 2011*

**Cells are either activated within BM and the mammary gland or during migration from mucosal inductor sites**
Productively infected CD4+ T cells are detectable in ART-treated women with undetectable HIV-1 RNA in blood and breast milk.

Valea D et al, Retrovirology 2011
Antiretroviral drugs in breast milk
Antiretroviral drugs in breast milk of HIV-1 infected women:

- **NRTI**
  - RTV (11%)
  - LPV (11-21%)
  - NFV (21%)
  - NVP (67-82%)

- **NNRTI**
  - NVP (67-82%)

- **PI**
  - NFV (21%)

- **3TC** (300-420%)
  - ZDV (117-140%)
  - d4T (173%)
  - 3TC (300-420%)

* = detectable levels in baby’s blood but at very low concentration

Rezk NL, Ther Drug Monit 2008
Schneider S, JAIDS 2008
Miroschnick M, AACT 2009
Shapiro RL, JID 2006
**Infant PreP (Option A)?**

- **Until now, unknown efficacy** if infant PreP is extended during the whole duration of exposure (12 months breastfeeding recommended by WHO);

- **Adherence and tolerance** uncompletely explored;

- **Results of the ANRS 12274-PROMISE-PEP trial**
BAN trial (Malawi)

- HIV-infected pregnant women, CD4>250/μl, breastfeeding for max 28 weeks, N=2,369
- Comparison
  - mothers: AZT/3TC/[NVP or NFV or LPV/r]
  - infant: PreP NVP (max 28 weeks)
  - control: perinatal prophylaxis only

- **At 28 w:**
  - **Postnatal transmission (2 to 28 w)**
  - **Inf HIV+ or death**

<table>
<thead>
<tr>
<th></th>
<th>ART in moms</th>
<th>PreP in infants</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ART in moms</strong></td>
<td>2.9% (1.9-4.4) (n=21)</td>
<td>1.7% (1.0-2.9) (n=12)</td>
<td>5.7% (4.1-8.0) (n=32)</td>
</tr>
<tr>
<td><strong>PreP in infants</strong></td>
<td>4.1% (2.9-5.8)</td>
<td>2.6% (1.7-4.1)</td>
<td>7.0% (5.1-9.4)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>2.9% (1.9-4.4) (n=21)</td>
<td>1.7% (1.0-2.9) (n=12)</td>
<td>5.7% (4.1-8.0) (n=32)</td>
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<td>2.6% (1.7-4.1)</td>
<td>7.0% (5.1-9.4)</td>
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Chasela CS, NEJM 2010
ANRS 12174 trial – preliminary data

- Randomised trial of infant PreP extended up to 12 months, 3TC versus LPV/r; Burkina Faso/Uganda/Afrique du Sud/Zambie
- N=1273; Follow up will be completed in April 2013;
- July 2012: unblinded analyses on transmission, tolerance and mortality on the 788 infants aged 12 months or more;
- D7-M12 HIV-1 Transmission rate: 1.1% (95% CI: 0.6-2.2), including 6/9 infections after 6 months (D7-M6 transmission: 0.3%)
- Overall MTCT rate: 1.8%, well within the target of elimination!
- 12 months mortality: 3.2 per 100 inf-yr (95% CI: 1.8-4.5)
- 12 months HIV-free survival : 96% (95% CI: 94-97)
- SAE: 188, none attributable to PreP

Conclusions:
- Transmission rate is the lowest ever observed;
- Compared efficacy and tolerance of the 2 PreP regimens will be known in December 2013

Tylleskär T et al, CROI 2013, Atlanta
Conclusions (1)

1. Do not throw Infant PreP (option A) with the baby’s bath

2. Evidence based versus best guess or model-based international recommendations?

3. Future research?
   - How to operationalise the access to prevention and therapy within national programs?
   - How to optimise existing PMTCT regimens?
   - Infant PreP: a place for long acting ARV drugs?
What about tomorrow?

STR-based ART in all HIV infected pregnant women eligible

+  

Infant PreP with a long acting drug covering the whole duration of breastfeeding

Examples: Rilpivirine LA*, GSK744**

* Van ‘t Klooster G, AAC 2010
** Andrews C et al, CROI 2013, Atlanta
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