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Management of maternal HIV-1 infection in pregnancy: the experience of the GUIDE clinic

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A thesis submitted for the degree of Doctor of Medicine

Trinity College Dublin

2008

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FIONA LYONS.

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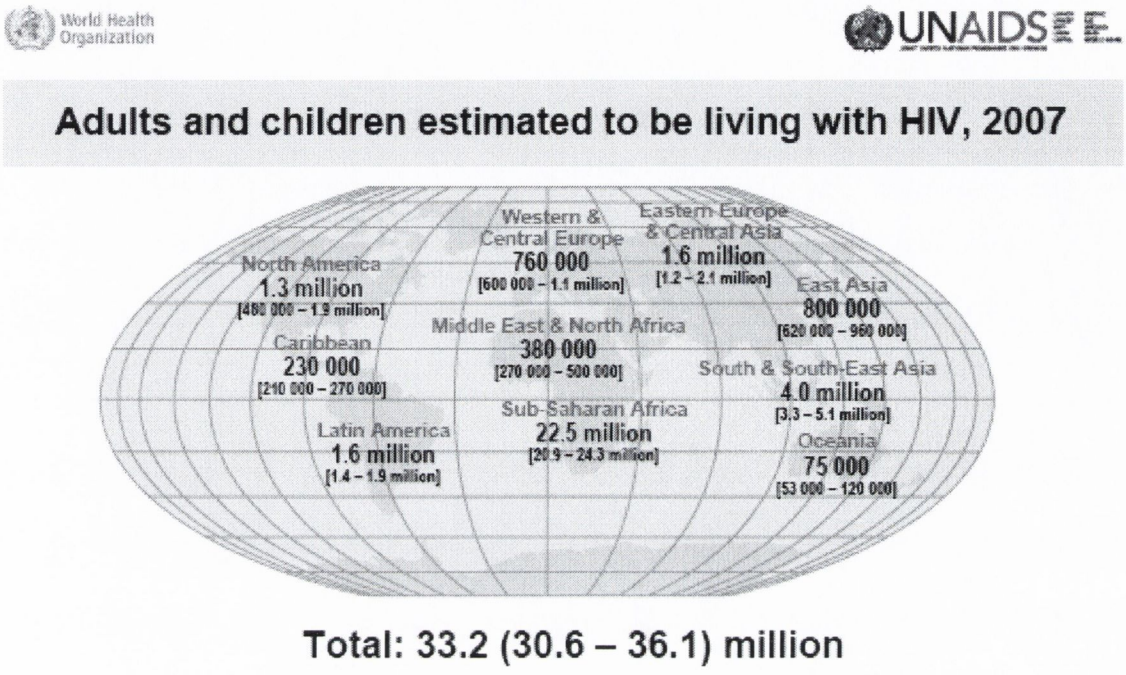
1.1 HIV-1 and AIDS: global estimates and history

As of the end of 2007 there were an estimated 33.2 (30.6 – 36.1) million people worldwide living with HIV-1 or AIDS, see figure 1.1. Almost seven thousand new infections occurred every day in 2007. Children under 15 years of age accounted for 1200 of these new infections and mother-to-child transmission (MTCT) represents the major mode of acquisition in this group[1].

The first human retrovirus (Human T-cell Lymphotropic virus, HTLV-1) was identified in Gallo's laboratory in 1980 from an individual with a T-cell malignancy[2]. Shortly thereafter came the first reports of acquired cellular immunodeficiency in previously healthy homosexual men and injecting drug users[3 4 5]. Similar reports of cellular immunodeficiency followed in infants born to mothers with a history of prostitution and injecting drug use, suggesting that if this acquired cellular immunodeficiency occurred as a result of an infection that sexual, blood borne and vertical transmission were also possible[6 7]. In 1983, Motagnier and colleagues reported the isolation of a human retrovirus in a person at risk for AIDS[8] and one year later a causal link with human T-lymphotropic virus III (then renamed Human Immunodeficiency Virus-1) was made[9].

The strains of HIV-1 are classified into three groups: the "major" group M, the "outlier" group O and the "new" group N. Group O appears to be restricted to west-central Africa and group N, discovered in 1998 in Cameroon, is extremely rare. Worldwide more than 90% of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K. Occasionally, two viruses of different subtypes can form hybrids or "circulating recombinant forms", CRFs. For example, the CRF A/B is a mixture of subtypes

Figure 1.1 Estimated number of people living with HIV/AIDS worldwide 1990 – 2007 (available at www.unaids.org)



A and B. Subtype B is most prevalent in the Americas, whereas clades A, C and D are most prevalent in Sub-Saharan Africa (see figure 1.2).

When untreated, infection with HIV-1 leads to the near complete destruction of the CD4+ T-lymphocyte population. Time to progression of symptomatic infection varies depending on both host and viral factors. Untreated the time between infection and overt immunodeficiency is 7 to 10 years. Profound immunodeficiency is manifested by opportunistic infections and a variety of malignancies. Before the advent of effective treatment studies found that patients with CD4 cell counts above 200 x 10^6/L were at low risk for the majority of HIV related OIs[10]. The risk of an opportunistic infection is significantly increased in individuals with a CD4 count of <50 x 10^6/L[11]. The Central Diseases Centre (CDC) classifies and stages HIV infection on the basis of CD4 cell counts and the presence of specific HIV related conditions (see tables 1.1 and 1.2).

Zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI), was the first licensed treatment for HIV infection in 1987. Early studies demonstrated less opportunistic infections and a survival benefit with zidovudine monotherapy over an 8 to 24 week period[12]. In 1989, zidovudine resistant strains of HIV were found in individuals exposed to zidovudine monotherapy for six months[13]. Over the coming years, more NRTIs became available (see table 1.3) and given the lack of a sustained clinical benefit associated with NRTI monotherapy, combinations of two NRTIs were compared to NRTI monotherapy. These studies demonstrated a clinical advantage of two NRTIs over NRTI monotherapy[14] [15] [16]. In 1996 a new class of antiretrovirals became available, targeting a different part of the HIV life cycle (see figure 1.3) and achieving suppression of the HIV viral load to a much greater extent than previously achievable. This led to the concept of highly active antiretroviral therapy and simultaneous initiation of triple combination antiretroviral therapy was shown to be superior to sequential initiation[17]. In the decade since HAART was first

Figure 1.2 Global distribution of HIV subtypes (reproduced with permission from International AIDS vaccine initiative, www.iavireport.org)

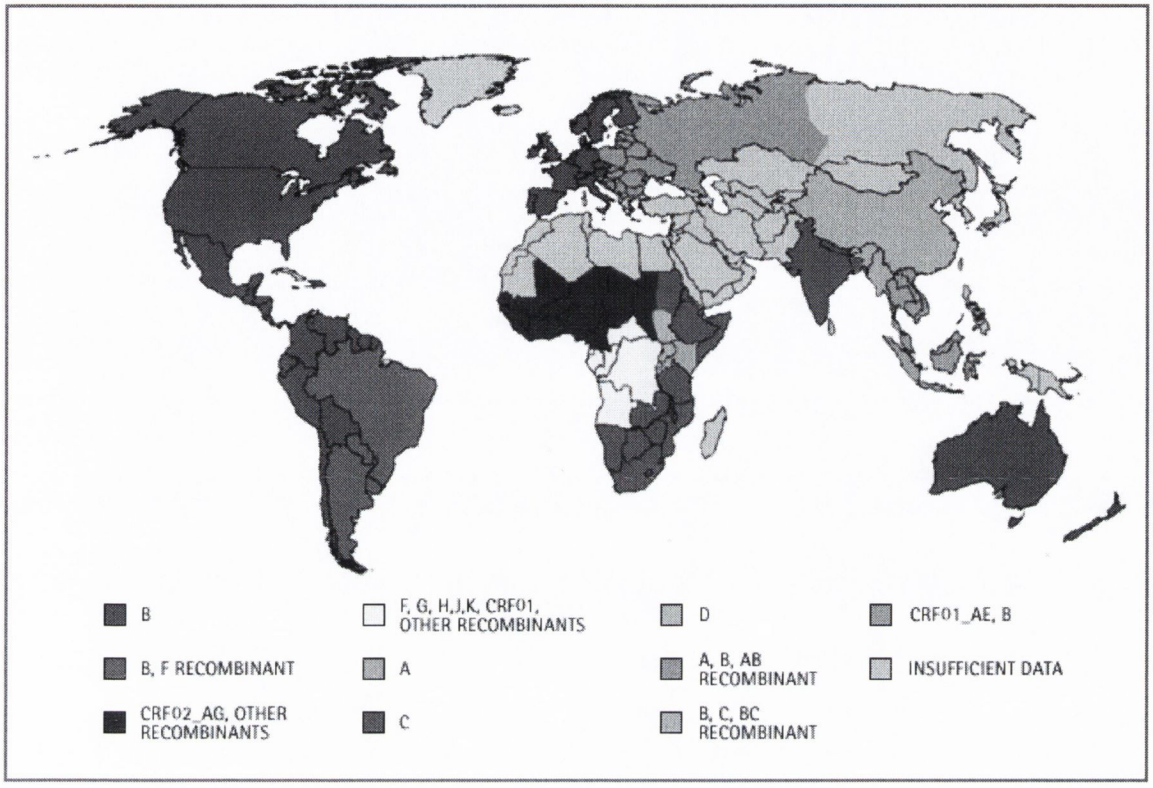


Table 1.1 CDC classification for HIV infected adults and adolescents

	Clinical category		
CD4 category	A (asymptomatic, acute HIV, PGL*)	B** (symptomatic conditions not A or C)	C (AIDS defining illness)
>500 x 10^6/L (1)	A1	B1	C1
200 – 499 x 10^6/L (2)	A2	B2	C2
<200 x 10^6/L (3)	A3	B3	C3

*PGL = persistent generalised lymphadenopathy

**Category B symptomatic conditions are defined as symptomatic conditions occurring in an HIV-infected adolescent or adult that meet at least 1 of the following criteria: a) They are attributed to HIV infection or indicate a defect in cell-mediated immunity b) They are considered to have a clinical course or management that is complicated by HIV infection

Table 1.2 CDC AIDS indicator conditions

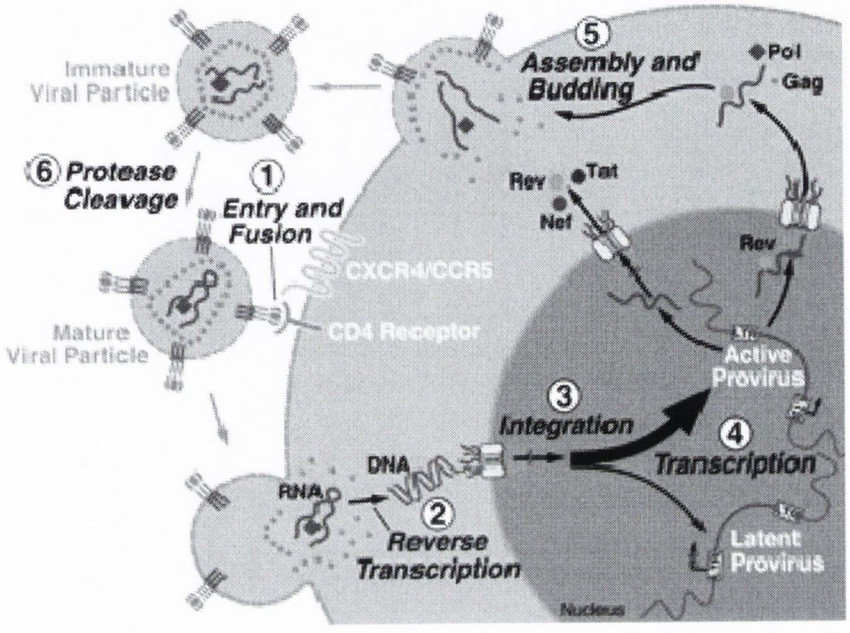
- Bacterial pneumonia, recurrent (≥ 2 episodes in 12 months)
- Candidiasis of the bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical carcinoma, invasive, confirmed by biopsy
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (>1-month duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcers (>1-month duration), or bronchitis, pneumonitis, or oesophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (>1-month duration)
- Kaposi sarcoma
- Lymphoma, Burkitt, immunoblastic, or primary central nervous system
- *Mycobacterium avium* complex (MAC) or *M kansasii* , disseminated or extrapulmonary
- *Mycobacterium tuberculosis* , pulmonary or extrapulmonary
- *Mycobacterium* , other species or unidentified species, disseminated or extrapulmonary
- *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP)
- Progressive multifocal leukoencephalopathy (PML)
- *Salmonella* septicemia, recurrent (nontyphoid)
- Toxoplasmosis of brain
- Wasting syndrome due to HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhea (≥ 2 loose stools per day ≥ 1 month) or chronic weakness and documented fever ≥ 1 month

Table 1.3: antiretroviral drugs, class and year of Food and Drugs Administration (FDA) approval

Drug class	NRTI*	NNRTI**	PI***	Entry/fusion inhibitor	Integrase inhibitor
Year of FDA approval					
1987	Zidovudine				
1991	Didanosine				
1992	Zalcitabine				
1994	Stavudine				
1995	Lamivudine		Saquinavir		
1996		Nevirapine	Ritonavir Indinavir		
1997		Delaviridine	Nelfinavir		
1998	Abacavir	Efavirenz			
1999			Amprenavir		
2000			Lopinavir/r100		
2001	Tenofovir				
2003			Fosamprenavir Atazanavir	Enfuviritide	
2005			Tipranavir/r100		
2006			Darunavir/r100		
2007				Maraviroc	Raltegravir
2008		Etravirine			

* Nucleoside reverse transcriptase inhibitors
** Non-nucleoside reverse transcriptase inhibitors
*** Protease inhibitors

Figure 1.3 HIV life cycle available at ***www.gladstone.ucsf.edu*** *(copyright protected)*



used, major advances have been made in antiretroviral development such that there are now five classes of antiretroviral agents licensed for the treatment of HIV infection: Nucleoside Reverse Transcriptase Inhibitors (NRTIs); Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs); Protease Inhibitors (PIs); Fusion Inhibitors and Integrase Inhibitors (see table 1.3). Highly active antiretroviral therapy (HAART) usually consists of 3 drugs from 2 classes. In individuals initiating antiretroviral therapy for the first time HAART will usually consist of 2 NRTIs plus 1 NNRTI or 1 PI. International guidelines for when to initiate antiretroviral therapy and what to start with are available and are regularly updated[18 19]. The hallmarks of successful antiretroviral therapy are virological suppression to below the limit of detection and immune reconstitution with recovery of CD4 cells.

Similar advances have been made in the prevention of mother to child transmission of HIV-1. In the developed world the risk of vertical transmission of HIV-1 is reduced from 25-35% to ~1% with appropriate interventions. Interventions include antenatal screening; antenatal antiretroviral therapy; selective elective caesarean section; neonatal antiretroviral therapy and where possible avoidance of breastfeeding. HIV per se does not appear to be teratogenic.

1.2 Mother to child transmission of HIV-1

1.2a Mechanisms and timing of mother-to-child transmission of HIV-1

The mechanisms of MTCT of HIV-1 remain incompletely understood. Proposed mechanisms include: transplacental spread at any time in pregnancy[20]; blood borne transmission through micro transfusions with uterine contractions in pregnancy/labour[21];

infection ascending from the lower genital tract to the uterine cavity[22]; mucosal exposure in the peripartum/early postpartum period[23] and through ingestion of cell free or cell associated HIV-1 during breastfeeding[24 25].

Infection can occur at any time before labour/delivery (in utero transmission); during labour and delivery (peripartum transmission) or postnatally through breast milk (postpartum transmission). It is generally accepted that in non-breastfeeding populations up to one third of transmissions occur during pregnancy and the remainder during labour and delivery[26]. In breastfeeding populations it is estimated that the majority of infections occur postpartum[27].

Worldwide the availability, implementation and acceptance of interventions to reduce transmission will have an effect on the timing of MTCT. Over time, with the successful implementation of programmes to reduce MTCT, the proportion of in utero transmissions has increased compared to peripartum transmissions[28]. Viral factors may also have an impact on the timing of transmission with data suggesting that subtype C virus is more likely to lead to in utero transmissions as compared to subtypes A or D[29].

1.2b Risk factors associated with MTCT of HIV-1

Numerous factors have been shown to increase the risk of MTCT of HIV-1. Of the potential maternal factors high plasma HIV viral load around the time of delivery is consistently associated with MTCT[30 31]. The level of maternal viremia and the risk of transmission correlate but a threshold below which transmission cannot occur has not been determined. MTCT has been reported in women with HIV-1 viral loads <1000 copies/ml and <50 copies/ml[32 33]. The presence of maternal genital tract infections may increase the risk of

MTCT of HIV-1[34 35 36]. Obstetric factors associated with intrapartum transmission include longer duration of rupture of the membranes[37 31] and premature delivery[28].

Much of the available data on the factors associated with MTCT of HIV-1 predate the use of HAART in MTCT strategies. The European Collaborative Study, a prospective cohort study across ten European countries, recently reported on risk factors for MTCT[33]. Of the 4525 mother-child pairs, 1983 were enrolled in the HAART era (defined as those enrolled on or after January 1st 1997). In multivariate analysis of those enrolled in the HAART era, maternal HIV viral load around the time of delivery (<1000 copies/ml AOR 1.00; >1000 – 9999 copies /ml AOR 12.1 (95% CI 2.51 – 58.6, p=0.002); >10,000 copies/ml AOR 12.1 (95% CI 2.31 – 63.1, p=0.003) and mode of delivery (ELCS AOR 0.33 (95% CI 0.11 – 0.94, p=0.040) reached significance. Maternal CD4 count, maternal antiretroviral therapy and prematurity were significant factors in the overall cohort but did not reach significance in the HAART era sub cohort. The median duration of membrane rupture was higher amongst infected infants (4.5 hours) than for uninfected infants (1.5 hours, p=0.009). Importantly the small number of infected infants in the mother-child pairs with antenatal HAART use and virological suppression to <50 copies/ml precluded analysis to determine risk factors in this group, thus any potential benefit of an ELCS in women on suppressive HAART remains unknown.

The contribution of breastfeeding has been determined in a randomised controlled trial. In the absence of antiretroviral therapy, the cumulative probability of infection was 36.7% at 24 months (95% CI 29.4 – 44.0) versus 20.5% (95% CI 14.0 – 27.0) in the formula fed arm[38]. The impact of maternal HAART on risk of transmission through breastfeeding remains incompletely understood, thus where safe alternatives are available, breastfeeding should be avoided.

Mother-child genetics have an impact on the risk of transmission. Mother-child human leukocyte antigen (HLA) concordance and maternal HLA homozygosity may increase the risk of vertical transmission of HIV-1 risk by reducing infant immune responses[39]. The increased risk may be due to reduced alloimmunity or less diverse protective immune responses. Furthermore mother-child HLA-A and -B discordance is associated with placental RNase expression and anti-HIV-1 activity[40]. Expression of RNases in the placenta in HLA may contribute to innate host resistance to HIV-1 infection.

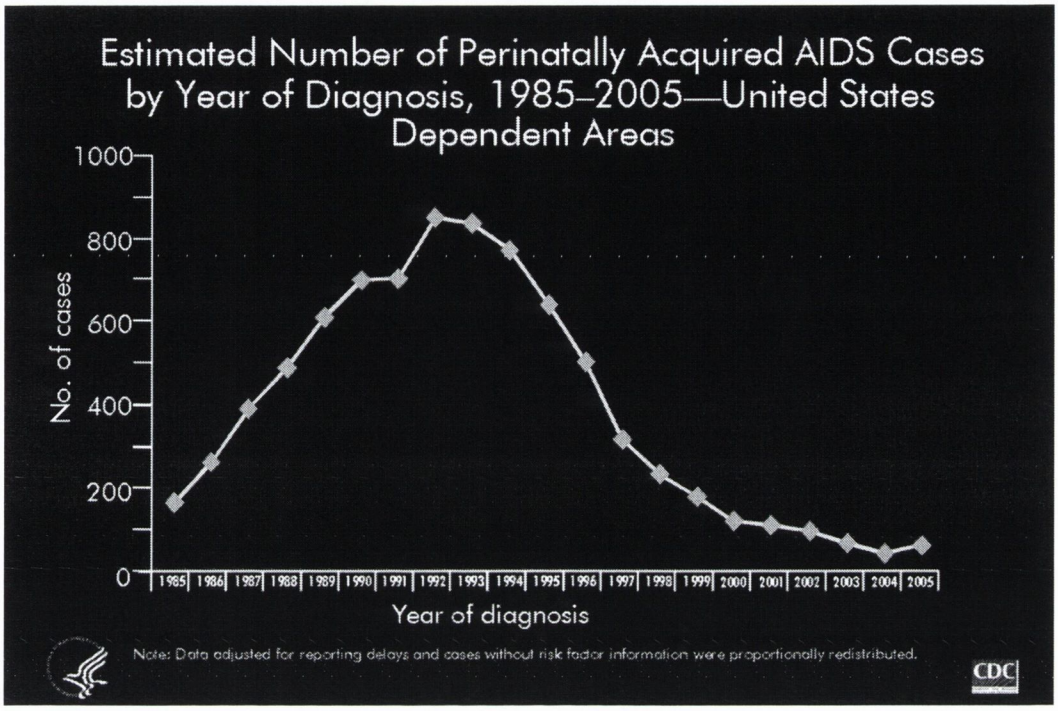
1.2c Interventions to reduce MTCT of HIV-1

Antenatal screening for HIV infection

In the US antenatal screening for HIV infection has been recommended since 1995 by the US Public Health Service (USPHS) [41]. This recommendation came shortly after the publication of the results of the Paediatric AIDS clinical trial group (PACTG) 076 study in 1994[42]. PACTG 076 was the first randomised controlled trial of antiretroviral use in pregnancy. The positive impact of antenatal screening and implementation of interventions to minimise vertical transmission of HIV is eloquently demonstrated by the reduction in perinatally acquired AIDS cases in the United States as seen in Figure 1.4.

In Ireland the National AIDS Strategy Committee (NASC) began anonymous, unlinked HIV antenatal testing on residual blood on women booking for antenatal care in 1992. This was the first programme for anonymous, unlinked HIV antibody testing in Ireland. It sought to track changes in the prevalence of HIV infection in pregnant women and guide on the optimum time to initiate linked screening[43]. The first increase in the number of positive tests was in 1997 with a further dramatic increase in 2000 from 44.0/100,000 in 1999 to

Figure 1.4 Estimated number of perinatally acquired AIDS cases by year of diagnosis, 1985 – 2005 – United States Dependent Areas (available at www.cdc.gov)



124.0/100,000 in 2000 (see table 1.4). In 1999 the NASC recommended that a nationwide "opt-out" antenatal screening policy for HIV infection be introduced. This recommendation was made given the advances that had been made in the prevention of MTCT of HIV coupled with the increase in the numbers of positive tests from anonymous antenatal surveillance. In addition the NASC recommended that anonymous, unlinked screening continue until the voluntary antenatal HIV screening had achieved 90% screening of the target population.

In "opt out" screening all women are screened unless they opt out as compared to "opt in" screening where all women are asked if they would like to have a test. In the US "opt out" antenatal screening for HIV has been shown to be associated with less missed HIV diagnoses in pregnant women versus "opt in" antenatal screening[44].

Antenatal antiretroviral therapy

Antenatal antiretroviral therapy is an important component of the overall strategy to reduce MTCT of HIV-1. The optimum antenatal antiretroviral choice is a balance between that which will achieve maternal immunological recovery (where necessary), successfully prevent MTCT and minimise the risk of toxicity to both mother and child. The suitability of different antiretroviral strategies will vary throughout the world depending on the resources and antiretroviral choices available.

Importantly the long-term effects of in utero exposure (and early neonatal exposure) to antiretrovirals are evolving but currently remain unclear. The Antiretroviral Pregnancy Registry is a voluntary prospective, observational study designed to collect and evaluate data on the outcomes of pregnancy exposures to antiretroviral products. It is sponsored by the pharmaceutical industry and seeks to provide an early signal of any major teratogenic effect associated with a prenatal exposure to the products monitored through the Registry.

Table 1.4 Results of anonymous, unlinked antenatal HIV screening, Ireland (from HPSC available at www.hpsc.ie)

Year	Total Tests Undertaken	Total Negative Tests	Total Tests Confirmed Positive	Rate of Positive Results per 100,000 tests
1993 *	53,480	53,467	13	24.3
1994	51,118	51,112	6	11.7
1995	56,081	56,075	6	10.7
1996	62,008	61,996	12	19.4
1997	64,412	64,385	27	41.9
1998	67,124	67,098	26	38.7
1999	54,089	54,065	24	44.4
2000	56,468	56,398	70	124.0
Total	**464,780**	**464,596**	**184**	**39.6**

* Includes results of 4th Quarter of 1992

In the absence of prospective clinical studies the Antiretroviral Pregnancy Registry is a useful means of improving our knowledge on antiretroviral use in pregnancy. The Antiretroviral Pregnancy Registry can be accessed at **www.apregistry.com**.

It is likely that antiretrovirals exert their effect on reducing MTCT of HIV-1 not only through reducing HIV viral load (and thus the amount of virus the foetus or neonate is exposed to) but also in providing pre-exposure and post-exposure prophylaxis for the foetus/neonate. The relative contribution of these effects in reducing MTCT is unknown and there may be other modes of action as yet not identified.

As mentioned earlier PACTG 076 was the first randomised controlled trial of antiretroviral use in pregnancy. This demonstrated a 66% reduction in the risk of transmission of HIV-1 from mother to baby where women received zidovudine monotherapy orally antenatally, intravenously in labour with a six week oral course to the infant. The efficacy of this strategy has been subsequently confirmed in numerous other studies and epidemiological cohorts[45 46 47 48].

Nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is the other antiretroviral agent that has been studied extensively for use as monotherapy in pregnancy to reduce MTCT. For many reasons it is an ideal candidate for use in MTCT programmes: it is rapidly transferred across the placenta; has a rapid onset of antiviral activity and a long half-life. The HIVNET012 study compared a 2-dose nevirapine strategy (a single dose at the onset of labour and a single dose to the neonate) with oral zidovudine at the onset of labour and three hourly thereafter until delivery in a breastfeeding population. The nevirapine arm was associated with a 41% reduction in the relative risk of transmission through to 18 months of age[49]. In resource limited settings the administration of two oral doses of nevirapine may be more feasible than other more complex protocols.

In the non-pregnant HIV population, before the advent of HAART combinations of two antiretroviral agents were used in the hope of achieving a more durable CD4 and HIV viral load response than that achieved with monotherapy alone. The combination of zidovudine and lamivudine was reported to be efficacious (immunologically and virologically out to 12 months) in antiretroviral naïve patients[50]. In pregnancy, the combination of zidovudine and lamivudine reduces the risk of MTCT of HIV[51 52]. Mandelbrot and colleagues[51] reported a transmission rate (1.6%) that was more favourable than that achieved with the three part zidovudine protocol (as per PACTG 076) that was used as standard of care in France during the same time period. However they also reported the presence of the M184V mutation (conferring resistance to lamivudine) in 52 of 132 (39%) women that were evaluated for antiretroviral resistance 6 weeks after treatment cessation.

In Ireland this report of lamivudine resistance raised concerns that the combination of zidovudine and lamivudine in pregnancy would compromise future maternal antiretroviral options and response. Therefore since January 2002 the Irish Guidelines for the management of HIV-1 in pregnancy recommended that all women be offered HAART in pregnancy regardless of immunological need[53]. Furthermore data published from the Women and Infants' Transmission Study Group (WITS) at this time showed a direct correlation between maternal plasma HIV viral load, the complexity of antiretroviral therapy (both the number of drugs and duration of therapy) and the risk of transmission[54].

Successful HAART will suppress viral replication to below the limit of quantification (<50 copies/ml). In the non-pregnant population the need for HAART is determined by clinical and immunological (CD4 count) parameters. Therefore in HIV infected pregnant women, not requiring therapy for their own health, reducing the risk of MTCT is the primary indication for antiretrovirals. To date the available evidence suggests that for these women

temporary antiretroviral therapy in pregnancy will not have a detrimental effect on maternal health[55]. The physiological changes of pregnancy will lead to a temporary reduction in the absolute CD4 count and may need to be taken into consideration when deciding on the need to continue antiretroviral therapy beyond pregnancy[56 57]. If the baseline pretreatment CD4 is $\leq 200 \times 10^6$/L antiretrovirals for maternal health are indicated and should be initiated as soon as possible after the first trimester and continued post partum. For women presenting with a CD4 count of ≥ 300 10^6/L antiretrovirals are generally not required beyond pregnancy and antiretrovirals initiated in pregnancy are usually discontinued post partum. For women presenting with a CD4 count between 200 and 300 x 10^6/L antiretrovirals may be initiated in pregnancy to reduce MTCT but continuation beyond delivery may not be necessary. For those women not requiring HAART for their own health, the optimum time to commence therapy is that which offers the maximum reduction in MTCT with minimum maternal and foetal drug exposure. The current Irish guidelines recommend that therapy be initiated at around 28 weeks for women not requiring antiretroviral therapy for their own health[53] and favour institution earlier if there is risk of premature delivery. It is interesting to note that, despite a reduction in MTCT rates over time associated with antiretroviral therapy use in pregnancy, the proportion of in utero infections has increased versus intrapartum and early postpartum infections[28]. This suggests that if MTCT rates are to be further reduced antiretroviral therapy may need to be initiated earlier in pregnancy to prevent in utero transmissions. Earlier institution of antiretroviral therapy to reduce in utero MTCT would need to be offset against increased potential toxicity, for mother and foetus/baby with longer antiretroviral exposure.

Elective Caesarean Section

Compared with vaginal delivery, elective Caesarean section (ELCS) has been shown to significantly reduce vertical transmission rates[58 59 60]. ELCS coupled with zidovudine

monotherapy is more effective than zidovudine monotherapy and vaginal delivery (MTCT 0.8% versus 4.3%)[59]. There have been no randomised controlled trials to compare ELCS versus other modes of delivery in women on HAART with an undetectable HIV viral load. Given the low rates of MTCT in women on suppressive HAART, it has been estimated that in order to show a significant effect of ELCS over vaginal delivery, 6345 and 7217 mother child pairs would be needed with vaginal deliveries and ELCS respectively[33]. Thus it appears that in the era of HAART, ELCS remains a significant intervention in preventing MTCT of HIV-1 in women not on suppressive HAART but the role of ELCS in the setting of suppressive HAART remains unknown.

US and UK based guidelines recommend ELCS at 38 weeks gestation[61 62]. However as there is a significant reduction in neonatal morbidity associated with delivery at 39 versus 38 weeks[63] and as premature delivery, in the absence of risk factors, has not been a problem in the Irish cohort[64], timing of ELCS for HIV infected women in Ireland has been at 39 weeks.

Higher morbidity rates following abdominal delivery have been reported in HIV infected women compared to the general population[65 66]. One case control study did not identify an increased rate of postoperative morbidity in HIV infected women, on antiretroviral therapy, undergoing spinal anaesthesia compared to the general population[67]. Many studies describe more postnatal morbidity in HIV infected women following abdominal delivery versus vaginal delivery[68 69]. Within the study from the Women and Infant Transmission Stufy[68] there was a decrease in all postnatal morbidity over time, most likely reflecting improved maternal health and routine use of prophylactic perioperative antibiotics. A recently published study suggests that elective Caesarean section is associated with similar postnatal morbidity to vaginal delivery (OR 1.16, 95% CI, 0.5 – 2.7)[70]. It is noteworthy that many of these studies have been carried out in very different populations at different times and extrapolation to the current Irish situation must be made with caution.

Whilst most of the reported morbidity is minor, postnatal morbidity remains a consideration in mode of delivery decision making in HIV infected women. This is of particular relevance at a time when the additional benefit of elective caesarean section in the setting of suppressive HAART is unknown. Furthermore at a time when more asylum seekers are being repatriated, it is important to recognise that women who have a scar on their uterus may be at greater risk in a future pregnancy if repatriated to a country without access to antenatal care.

Neonatal Antiretroviral Therapy and avoidance of breastfeeding

Neonates commence antiretroviral therapy as soon as possible after birth and continue for the first 4 to 6 weeks of life. The regimen chosen is determined by maternal antiretroviral exposure and HIV viral load close to delivery. In general infants born to mothers who are at low risk for transmission receive zidovudine monotherapy.

Following the first report of transmission of HIV-1 through breast milk the CDC made recommendations around infant feeding in women infected with HIV-1[71]. In the Irish context, where there is access to a clean water supply, women are advised not to breastfeed their infants.

1.3 Potential drawbacks associated with antenatal antiretroviral therapy

1.3a Teratogenicity

Exposure to antiretroviral agents in early intrauterine life has the potential to exert a teratogenic effect. For immunocompromised women presenting for the first time in pregnancy, a decision may be made to withhold antiretroviral therapy until after the first

trimester of pregnancy to ameliorate any potential teratogenic effect. Of all currently available antiretroviral drugs most are FDA (US Food and Drug Administration) class C reflecting the limited data available on antiretroviral use in human pregnancy.

In December 2005 efavirenz was reclassified as an FDA Class D drug (i.e. that there is evidence for harm with exposure in human pregnancies). This was based on four retrospectively reported cases of neural tube defect like abnormalities (three meningomyeloceles and one Dandy-Walker syndrome) in human offspring exposed to efavirenz in utero. Prospective animal experiments had previously demonstrated anencephaly with anophthalmia, microphthalmia and cleft palate in three of twenty cynologous monkeys exposed to the human adult equivalent dose throughout pregnancy[72]. The most recent report from the Antiretroviral Pregnancy Registry has not shown an increased risk of congenital abnormalities as compared to the general population in 364 pregnancies with first trimester exposure to efavirenz[73].

Efavirenz is generally avoided in women contemplating pregnancy and women of child bearing potential should use effective contraception whilst taking efavirenz. Where a woman inadvertently conceives on efavirenz published guidelines give differing advice. The current Irish guidelines recommend that efavirenz is stopped as soon as a pregnancy is diagnosed and substituted with an alternative agent[53]. The British HIV Association (BHIVA) pregnancy guidelines suggest that efavirenz not automatically be discontinued[62]. The rationale for this is that efavirenz has a very long half life and can persist at detectable levels for up to 3 weeks following discontinuation[74]. Thus in a woman with a 28 day menstrual cycle it will be approximately the 15th day of intrauterine life when her period is late. The process of neural tube closure is complete by day 22-24 of intrauterine life. Therefore despite stopping efavirenz when a period is missed there will be ongoing in utero exposure to efavirenz that may extend beyond the period of neural tube closure.

Additionally stopping efavirenz and substituting with an alternative agent may risk loss of virological control with the potential for evolution of antiretroviral resistance and an increased transmission risk. Furthermore initiating another antiretroviral agent exposes the foetus to more drugs, some of which may have an as yet unrecognised teratogenic or detrimental effect.

1.3b Maternal Toxicity

In general pregnancy does not appear to significantly alter tolerability of most antiretroviral agents. There are some combinations that are best avoided and some agents that should be used with caution.

NRTIs can, with varying affinity, inhibit the mitochondrial enzyme DNA polymerase-γ resulting in mitochondrial toxicity. In NRTI exposed individuals mitochondrial toxicity can manifest as myopathy, hepatic steatosis, pancreatitis, lipoatrophy and hyperlactatemia/lactic acidosis. Of the available NRTIs the risk of mitochondrial toxicity is greatest with didanosine and stavudine. Therefore in all HIV infected individuals these agents are avoided where possible. There have been reports of severe maternal morbidity and mortality with didanosine and stavudine exposure in pregnancy[75]. Zidovudine is no longer recommended as first line in HIV individuals initiating antiretroviral therapy[18 19] because of concerns for long term mitochondrial toxicity, particularly lipoatrophy, in those exposed[76 77 78].

Fulminant hepatitis is a rare but well recognised side effect associated with nevirapine. It appears that this risk is gender related and is increased for women with a pretreatment CD4 count of >250 x 10^6/L[79]. In the context of pregnancy nevirapine is generally reserved for women requiring HAART for their own health. In Ireland there have been two maternal deaths in Sub-Saharan African women who initiated a nevirapine containing regimen in pregnancy[80]. It is not known whether women who have had effective immune

reconstitution (to a CD4 count >250 x 10^6/L) with a nevirapine containing regimen are then at a greater risk of going on to develop hepatotoxicity and if so whether pregnancy increases this risk.

1.3c Foetal/infant toxicity

As mentioned earlier NRTIs can inhibit mitochondrial DNA polymerase-γ and the association between in utero NRTI exposure and mitochondrial toxicity is well recognised. In a prospective French perinatal cohort eight cases, including two deaths, have been attributed to mitochondrial toxicity following in utero exposure to NRTIs[81]. A subsequent retrospective review of HIV negative or indeterminate children that were exposed to ART and died (n=223) determined that none of the deaths were likely attributable to mitochondrial dysfunction[82]. Notwithstanding the absence of a clear association in this large series there is evidence that mitochondrial DNA is reduced in infants exposed to NRTIs in utero[83 84 85]. The long term effects of asymptomatic mitochondrial DNA depletion are unknown, highlighting the importance of maintaining these children under long term surveillance. Currently the available evidence favours the use of antenatal and neonatal antiretroviral therapy as the benefits in reducing MTCT outweigh potential risks.

Most protease inhibitors do not cross the placenta. Thus the foetus may be protected against potential toxic or teratogenic effects. Atazanavir does cross the placenta with therapeutic levels of atazanavir reported in cord blood of exposed infants[86 87] . One of the anticipated side effects of atazanavir in the general HIV population is unconjugated hyperbilirubinemia caused by competitive inhibition of the uridine diphosphate-glucuronosyl transferase (UGT) 1A1 enzyme by atazanavir. Exposed neonates therefore should be monitored for hyperbilirubinemia. To date dangerous hyperbilirubinemia in exposed neonates has not been significant[88 89].

Anticipated side effects associated with zidovudine exposure include anaemia and neutropenia which usually resolve by 12 weeks of age[64].

1.3d Adverse pregnancy outcome

There are conflicting reports regarding the effects of HAART, particularly protease inhibitor based regimens, on prematurity and low birth weight rates Epidemiological data from Europe indicates an increased incidence of prematurity with a temporal relation to increasing HAART use in pregnancy[90] [91] while data from the US has not demonstrated this risk[92]. A recent report from a single site in the US did find that the risk of prematurity in women receiving combination therapy was greater for those on combination therapy with a protease inhibitor (AOR 1.8, 95% CI 1.1 – 3.0, p=0.03)[93]. Importantly none of the studies have determined the risk of prematurity in situations where the primary indication for HAART is reduction of MTCT and not maternal immune restoration. It may be the case that in immunocompromised women there are unknown confounders that contribute to the risk of prematurity by virtue of advanced maternal disease. Cotter et al[93] indicate that the use of protease inhibitor based regimens in their practice is reserved for women with advanced disease, high plasma HIV viral loads and where there is poor response to other combinations. Additionally the rate of prematurity in women on a combination with protease inhibitors was 70.0% in the time between 1995 and 1997 (when protease inhibitors were first introduced and perhaps more likely to be used in those with advanced disease) versus 33.9% in the period between 1998 and 2002 (when protease inhibitor use may have been more widespread).

If HAART does increase the risk of prematurity the mechanisms for this are unknown. One study has found that in the absence of clinical chorioamnionitis, antenatal HAART that included a protease inhibitor is associated with histological chorioamnionitis and this conferred a tenfold increased risk of prematurity[94]. A recent paper postulates that the

antiretroviral therapy associated modulation of immune responses, which is the opposite to the physiological shift from Th1 to Th2 responses in pregnancy, may in part explain the increased risk of prematurity in women taking HAART in pregnancy[95].

Historically the incidence of pre-eclampsia was lower in HIV infected women[96]. The pathogenesis of pre-eclampsia remains incompletely elucidated but may be mediated through immunological factors or oxidative stress. In the context of HIV, an increased incidence of pre-eclampsia has been reported in the era of HAART use[97 98].

1.3e Antiretroviral Resistance

As the prevalence of transmitted drug resistance[99] at baseline is increasing, pre-treatment testing is now recommended. Genotypic resistance testing is also indicated where a regimen is failing.

In general in the non-pregnant population mono and dual therapy are now avoided because of the risk for development of resistance and lack of clinical durability. The genetic barrier for development of resistance to antiretroviral agents varies but the NNRTIs are particularly susceptible and their long half life may increase this risk. Resistance to NNRTIs has been demonstrated following a single dose of nevirapine at the onset of labour[100 101 102 103]. This may negatively impact on future response to an NNRTI based regimen[104]. Zidovudine has a higher genetic barrier than the NNRTIs but significant resistance associated with prolonged use in patients with advanced HIV disease and following temporary exposure in pregnancy has been reported[13 105]. Both the US and BHIVA guidelines still offer zidovudine monotherapy in selected antenatal patients[61 62]. In the setting of favourable pre-treatment maternal parameters (HIV viral load <10,000 copies/ml and CD4 count >200 x 10^6/L) no significant zidovudine resistance has been

found using both bulk population sequencing and more sensitive cloning for minority quasispecies[106 107].

In women taking temporary HAART in pregnancy the impact of this strategy on evolution of antiretroviral resistance and future antiretroviral response is unknown. It is hypothesized that where women are taking HAART with good virological suppression (<50 copies/ml) that the opportunity for emergence of resistance will be limited, thus preserving future maternal antiretroviral options. The cohort of HIV infected women temporarily exposed to antiretroviral therapy in pregnancy at the GUIDE clinic represents an ideal opportunity to address this question (see chapter 4).

1.3f Therapeutic drug monitoring

In the non-pregnant HIV positive population, therapeutic drug monitoring is a strategy sometimes used to faciliate efficacy of antiretroviral agents and avoid toxicity. The many physiological changes in pregnancy can significantly alter drug handling affecting oral bioavailability; drug disposition (through increased water and fat content and changes in protein levels); metabolism and clearance. These physiological changes may lead to altered pharmacokinetics of antiretroviral drugs in pregnancy but plasma drug levels must be interpreted with caution as the correlation between plasma total concentrations, plasma free concentrations and intracellular concentrations of drugs may be significantly altered from the non-pregnant state.

The available data suggests that for the NRTIs and NNRTIs the pharmacokinetics are either not altered in pregnancy or that small observed alterations in pharmacokinetics do not have an effect on efficacy (where levels may be subtherapeutic) or toxicity (where levels may be too high)[108].

In the non pregnant population there is marked intraindividual variability in the bioavailability of the protease inhibitors. With the exception of indinavir plasma protein binding is >85% and they are lipophilic. Saquinavir and lopinavir are the most widely used

protease inhibitors in pregnancy with increasing reports of atazanavir use. In both the pregnant and non pregnant population these protease inhibitors are administered with low dose (100mg) ritonavir (occasionally atazanavir is administered without ritonavir). Ritonavir is a potent inhibitor of the cytochrome p450 system. Thus co-administration of protease inhibitors with low dose ritonavir boosts their concentrations through inhibition of the cytochrome p450 system (the enzyme system that metabolises protease inhibitors). This leads to more favourable pharmacokinetic profiles, reduces pill burden and in the case of atazanavir facilitates once daily dosing. Studies in different pregnant populations have demonstrated varying results with respect to the pharmacokinetics of the protease inhibitors.

Differing results have been reported for lopinavir/ritonavir soft gel capsules at standard dosing in pregnancy. Low lopinavir levels in the third trimester have been described in a multicentre study from the Unites States[109], while a single centre report from the United Kingdom suggested better steady state levels in the third trimester[110]. Pharmacogenomic differences in the handling of lopinavir/ritonavir in the different cohorts may explain these differing results, highlighting the importance of exercising caution in applying results from one population to another.

At the GUIDE clinic the majority of a cohort of 45 women achieved adequate levels of saquinavir in the third trimester with a median HIV viral load of <50copies/ml at standard saquinavir (1g soft gel capsules BD with ritonavir 100mg BD) dosing[111].

Atazanavir/ritonavir levels, at standard dosing (atazanavir 300mg with ritonavir 100mg once daily) were found in one study to be similar in the third trimester of pregnancy as post partum[87] while another series demonstrated lower levels than that reported for the non pregnant population[88].

As mentioned earlier the objective of antiretroviral therapy is virological control with immunological recovery. It is difficult to interpret reported lower levels of protease inhibitors in the setting of virological control. It is likely that higher levels are required in treatment

experienced individuals who harbour antiretroviral resistance, but slightly reduced levels associated with the physiological changes of pregnancy may be of no consequence in treatment naïve individuals without any antiretroviral resistance.

1.4 Aims of this thesis

In Ireland, the introduction of antenatal screening for HIV infection, coinciding with the increase in asylum seekers from countries of HIV prevalence has led to a dramatic increase in the numbers of HIV infected women being diagnosed for the first time in pregnancy. This thesis will describe the experience of delivering HIV care to pregnant HIV-1 infected women at the Department of Genitourinary Medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin from July 2000 to June 2003 and the research questions and opportunities that arose along the way.

Specific Aims

1) The demographics of the HIV antenatal population have changed since the introduction of antenatal screening for HIV infection. Chapter 3 compares and contrasts the HIV antenatal population and pregnancy outcomes before and since the introduction of antenatal screening.

2) The risk of developing antiretroviral resistance following temporary HAART in pregnancy is unknown and if significant could compromise future maternal antiretroviral options. In chapter 4 the incidence of antiretroviral genotypic resistance in a cohort of women temporarily exposed to HAART in pregnancy using bulk population sequencing will be determined.

3) In chapter 5 maternal toxicity associated with the use of nevirapine as part of antenatal combination antiretroviral therapy in a cohort of HIV infected women, in Dublin, will be evaluated. To identify risk factors for toxicity and make recommendations for use of nevirapine in pregnancy in an Irish context.

4) The results of this work, coupled with international data, will be used to develop nationally relevant guidelines for the management of HIV-1 infection in pregnancy in Ireland.

Chapter 2

The study population and methods

2.1 Introduction

2.2 Methods

2.2 (a) Clinical protocols

2.2 (b) Data collection and management

2.2 (c) Laboratory methods

2.2 (d) Statistical methods

2.2 (c) Ethical approval

2.3 Pregnancies in HIV infected women at the GUIDE clinic 2000 to 2003

2.1 Introduction

The majority of the women studied in this thesis are those that attended the GUIDE clinic for HIV care in pregnancy between the years 2000 and 2003. The author commenced specialist registrar training in Genitourinary Medicine at the GUIDE clinic in 2000. Prior to specialist training in Genitourinary Medicine, the author had completed 4 years post graduate training in Obstetrics and Gynaecology and was thus ideally placed to manage the HIV infected pregnant women at the GUIDE clinic, with consultant supervision.

Data on miscarriages, ectopic pregnancies and pregnancy terminations is incomplete and has been excluded. Thus all pregnancies that reached a viable gestational age (set at 25 weeks) were included.

Inclusion criteria for women from the overall cohort (2000 to 2003), in the different chapters, are described in the relevant chapter. Data from other pregnancies in HIV infected women was included in Chapters 3 and 5. Sourcing and collation of this data is described in these chapters.

2.2 a Clinical protocols

Women found to be HIV positive antenatally were referred to the GUIDE clinic for assessment and management of their HIV. All women at or greater than 28 weeks gestation at the time of their HIV diagnosis were seen urgently in the clinic while women under 28 weeks gestation at the time of their HIV diagnosis were usually seen within 2 weeks of their HIV diagnosis. All HIV infected women (including those diagnosed antenatally and women with a diagnosis of HIV prior to their pregnancy) were seen every four weeks during pregnancy and twice in the first four weeks post initiation of antiretroviral

therapy. Antiretroviral therapy use and recommendations for management of delivery were in accordance with the relevant national guidelines for HIV in pregnancy[53 112].

From July 2000 to 2003 the majority of the women were seen and managed by the author with consultant supervision.

The GUIDE clinic is located in St. James's Hospital, Dublin. There are no on site obstetric services. Thus HIV positive pregnant women attending the GUIDE clinic for HIV care needed to attend a different hospital for obstetric care. The majority of those residing outside Dublin attended the maternity unit closest to their place of residence for obstetric care. This called for regular communication between adult HIV, obstetric and paediatric HIV medical and nursing personnel. FL coordinated this liaison with other specialities.

2.2 (b) Data collection and management

From 1999 all new pregnancies in HIV infected women attending the GUIDE clinic were prospectively registered on a dedicated, password protected, excel spreadsheet. Between 1999 and July 2000 this database was maintained by one of the Specialist Registrars (SC). From July 2000 onwards data was maintained by the author. Relevant demographic, laboratory and clinical data were collated from the medical, pharmacy and laboratory record systems.

The use of peripartum intravenous zidovudine, mode of delivery and paediatric data was obtained by the author from the paediatric database (courtesy of KB, paediatric Infectious Diseases Consultant, Our Lady's Hospital for Sick Children, Crumlin).

Data was regularly checked, by the author, for errors to ensure that inaccuracies were kept to an absolute minimum.

2.2 (c) Statistical methods

The statistical methods employed are outlined in the relevant chapters. Statistical advice and assistance was provided by Dr. Colette Smith, Department of Primary Care and Population Sciences, Royal Free and University College Medical, School, London and Dr. Susan Hopkins, Department of Infection and Immunity, Royal Free Hospital, London.

2.2 (d) Laboratory methods

The laboratory methods employed for genotypic resistance testing in chapter 5 are outlined in that chapter. Ms. Tina Byrne, Dr. Alison Waters and Dr. Suzie Coughlan of the National Virus Reference Laboratory provided assistance, training and supervision to the author for the genotypic resistance testing and sequence analysis.

2.2 (e) Ethical approval

Where necessary, ethical approval was sought from and granted by the Ethics Committee of the Federated Dublin Voluntary Hospitals.

2.3 Pregnancies in HIV infected women at the GUIDE clinic 2000 to 2003

Between 2000 and 2003 there were 220 pregnancies in 177 women attending the GUIDE clinic for HIV care. Detailed analysis of these pregnancies and comparison with those from 1996 to 1999 (the years before the introduction of antenatal screening for HIV) is described and presented in Chapter 3.

Chapter 3

The impact of the introduction of the antenatal HIV antibody screening programme in Ireland on the GUIDE Clinic, Dublin

3.1 Introduction

3.2 Methods

3.2 a Clinical protocols

3.2 b Data collection and management

3.2 c Denominators and definitions

3.2 d Statistical methods

3.3 Results

3.3 a Overall

3.3 b Demographics and characteristics

3.3 c Clinical status

3.3 d Antenatal antiretroviral therapy

3.3 e Pregnancy and infant outcome

3.3 f Maternal response to treatment and follow up

3.4 Discussion

3.1 Introduction

The Department of Genitourinary Medicine and Infectious Diseases (GUIDE Clinic) was first established in 1987 and is the largest adult HIV clinic in the Republic of Ireland. The GUIDE clinic provides HIV care to people from all over the Republic of Ireland.

The publication of the PACTG 076 study in 1994[42] represented the first major breakthrough in reducing MTCT of HIV. In Ireland, following this and before the introduction of routine antenatal HIV testing, women with a perceived risk of HIV infection (women with a history of injecting drug use and women from countries of high HIV prevalence) were offered antenatal HIV testing. Thus, the pregnancies seen in HIV infected women, before routine antenatal screening for HIV, were a selected group of women who were tested in pregnancy, and those known to be HIV infected prior to pregnancy.

Anonymous unlinked HIV antibody testing of all antenatal samples was introduced in 1992 to track changes in HIV prevalence within the antenatal population and determine the optimum time to introduce routine "opt out" antenatal screening for HIV. The first increase in the number of positive tests was in 1997 with a further dramatic increase in 2000 from 44.0/100,000 in 1999 to 124.0/100,000 in 2000 (see table 1.4). In 1999 the National AIDS Strategy Committee recommended that a nationwide "opt-out" antenatal screening policy for HIV infection be introduced. The "opt out" antenatal screening programme for HIV in Ireland thus began in 1999/2000.

National data from linked reporting of all new HIV positive cases to the Health Protection Surveillance Centre, demonstrated that there was a significant increase in the overall

number of HIV diagnoses around this time. As figure 3.1 shows the majority of these infections were heterosexually acquired.

At the GUIDE clinic there were similar increases in the number of heterosexually acquired infections and many of these new infections were diagnosed in non-national individuals (see figure 3.2 and figure 3.3). This most likely reflected the increased number of asylum seekers coming to Ireland from countries of high HIV prevalence. In the late 1990's and early 2000's there was massive growth in migration into Ireland, and a huge growth in the number of people applying for asylum or refugee status, coinciding with an Irish economic boom (see figure 3.4). Between 2001 and 2006, Nigeria was the top country of stated origin for all individuals seeking asylum (between 24.1% and 39.4% of all applications)[113].

For the purposes of monitoring and evaluating the HIV antenatal screening programme, data are voluntarily reported from participating maternity units four times a year to the Health Protection Surveillance Centre. The most recent report is available for the period 2002 to 2006[114] and as table 3.1 demonstrates, the uptake of the offer of screening amongst all pregnant women has been consistently >90%.

The introduction of antenatal screening for HIV, coupled with the increasing number of people arriving in Ireland from countries of high HIV prevalence had an enormous impact on the numbers of people presenting to the GUIDE clinic for HIV care and the numbers of women with HIV in pregnancy. This chapter compares and contrasts the pregnancies in HIV infected women attending the GUIDE clinic before and since the introduction of antenatal screening with respect to the women that were seen, how they were managed and outcomes.

Figure 3.1 New HIV diagnoses in Ireland by risk group 1994 – 2005 (available from newly diagnosed HIV infections in Ireland Q3 and Q4 2005 and 2005 Annual Summary, June 2006, at www.hpsc.ie)

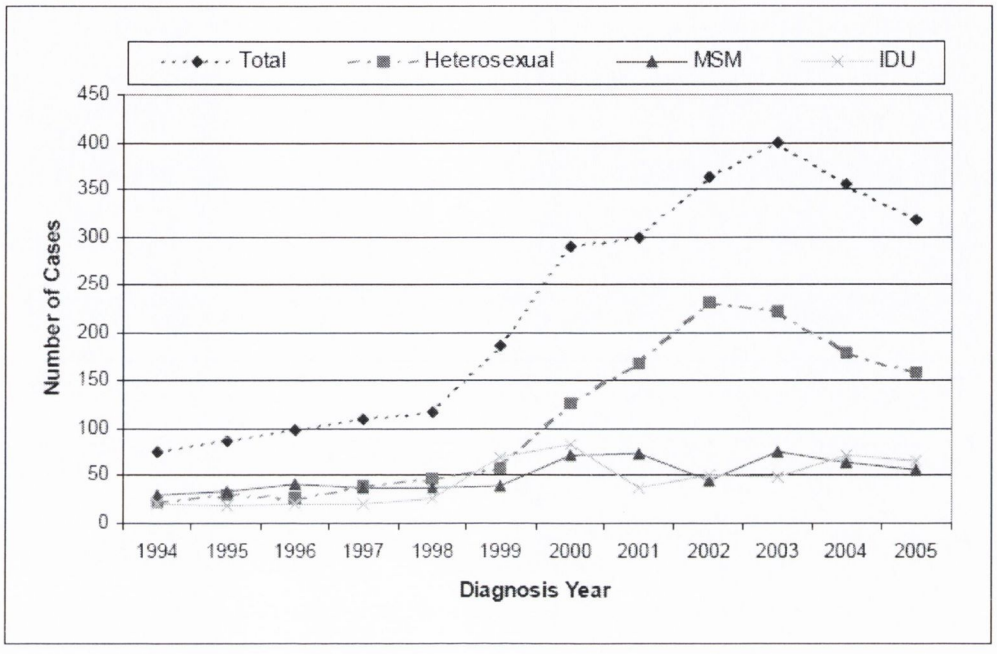


Figure 3.2 New HIV diagnoses by region origin at GUIDE clinic 1999 – 2000 presented at BHIVA, Brighton April 2001
THE HIV EPIDEMIC - IS THIS A TURNING POINT?
(F. Lyons, S. Clarke, S. Hopkins, F. Mulcahy and C. Bergin)

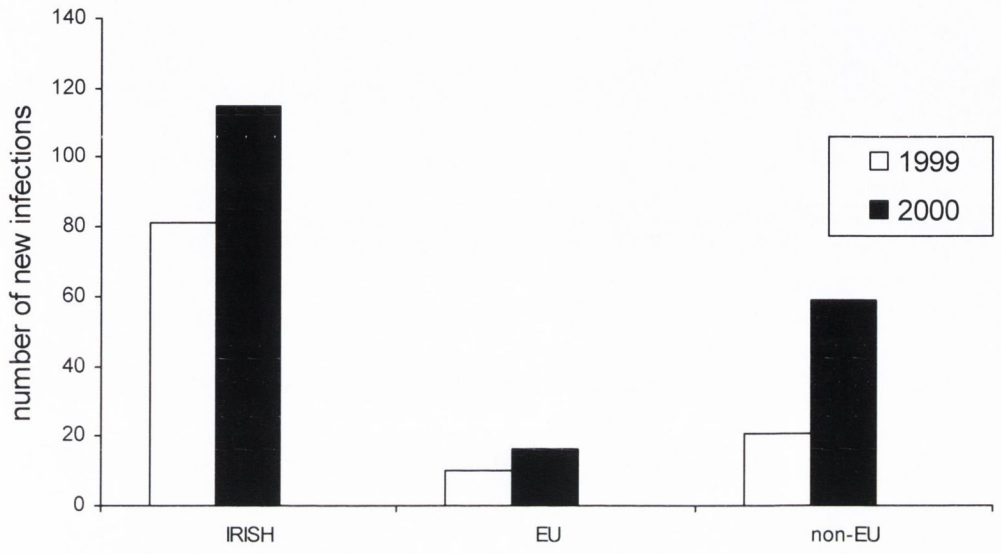


Figure 3.3 New HIV diagnoses by risk group at GUIDE clinic 1999 – 2000 presented at BHIVA, Brighton April 2001 THE HIV EPIDEMIC - IS THIS A TURNING POINT? (F. Lyons, S. Clarke, S. Hopkins, F. Mulcahy and C. Bergin)

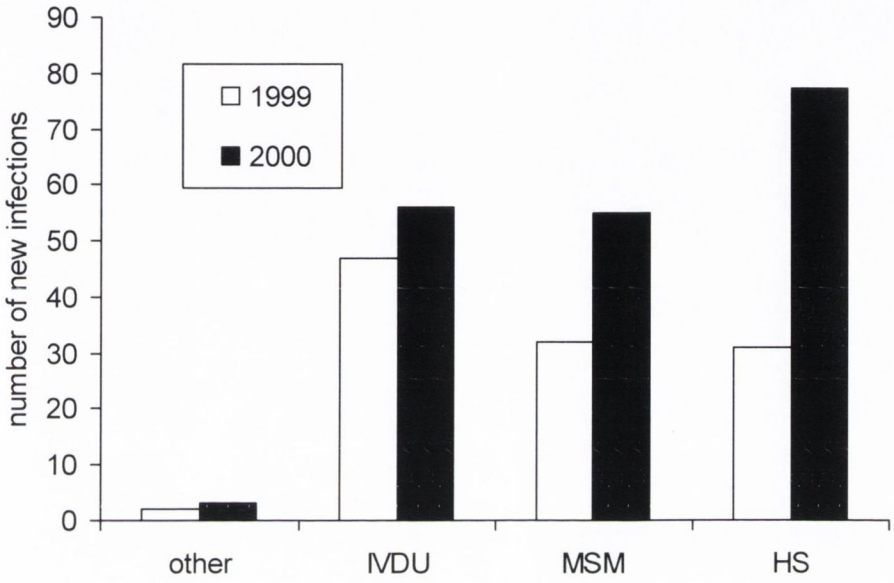


Figure 3.4 Applications for refugee status, Ireland 1992 – 2006
(available from Office of Refugee Applications Commissioner, www.orac.ie.)

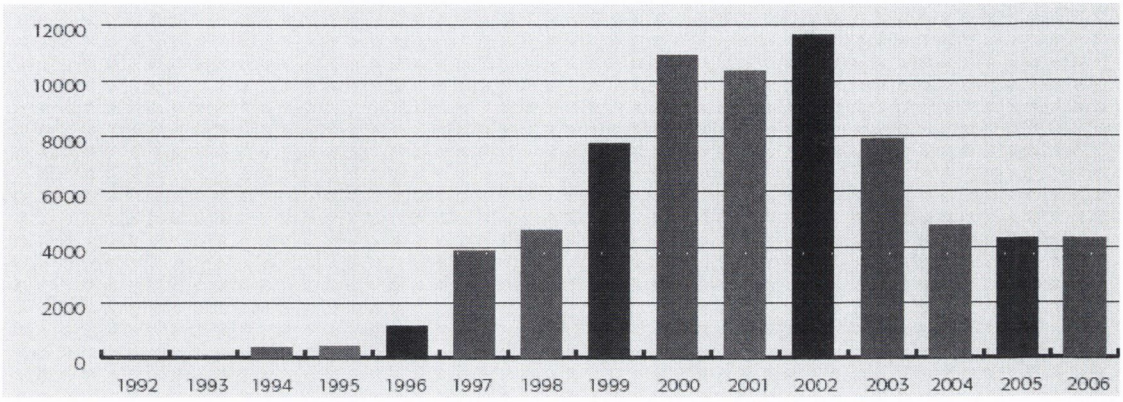


Table 3.1 Antenatal HIV testing 2002 – 2006 (from report on voluntary antenatal HIV testing 2002 – 2006, November 2007, available at www.hpsc.ie)

	Year of testing				
	2002	2003	2004	2005	2006
Number of hospitals providing data	21	21	21	20	19
Number of live births per year[1]	60,503	61,517	61,684	61,042	64,237
Number of women who were offered the HIV antenatal test	54,884	48,274	42,276	44,163	53,802
Number of women who accepted the HIV antenatal test	52,101	46,860	41,588	43,712	50,312
Uptake of HIV antenatal test (%)	94.9%	97.1%	98.4%	99.0%	93.5%
Number of women HIV positive	156	146	103	119	116
Number of women newly diagnosed as HIV positive[2]	113	94	40	43	36

3.2 Methods

For the purposes of comparing the groups the year 2000 is taken as the first year of antenatal screening for HIV. There was an initial roll out of antenatal screening for HIV in some parts of Ireland from April 1999 but 2000 represents the first full year of antenatal screening. The four years before the introduction of antenatal screening (1996 – 1999) were compared to the first four years of antenatal screening (2000 – 2003).There was one known pregnancy in a HIV infected woman in 1995. As outlined in chapter 2 data on miscarriages, ectopic pregnancies and pregnancy terminations is incomplete and has been excluded. Thus all pregnancies that reached a viable gestational age (set at 25 weeks) were included.

3.2 a Clinical protocols

The clinical protocols are described in Chapter 2.

3.2 b Data collection and management

Data collection and management for pregnancies from 1999 to 2003 is described in Chapter 2.

Data on pregnancies between 1996 and 1999 was collated retrospectively from the medical, pharmacy and laboratory record systems and entered on the database by the author (FL).

3.2 c Denominators and definitions

The denominator used varied for different parameters that were examined.

The first pregnancy in the time period (1996 – 2003), n= 201, was used as the denominator for timing of HIV diagnosis; maternal age; mode of HIV acquisition; country of

origin; partner's HIV status, co-morbidities (Hepatitis B and C and syphilis) and maternal follow up post delivery.

Of note hepatitis B, hepatitis C and syphilis status were checked for each pregnancy but the first pregnancy was used as the denominator to determine if there were baseline differences in the types of women seen. Similarly the HIV status of the partner was sought for each pregnancy, reflecting the GUIDE clinic policy of encouraging and facilitating HIV disclosure to sexual partners.

The number of antenatal HIV diagnoses, n=130, was used as the denominator when looking at the gestational age at first HIV visit and the late presenters (>32 weeks).

The number of pregnancies, n=245, was used as the denominator for maternal immunological and virological parameters (baseline and delivery); type of antiretroviral therapy; use of peripartum zidovudine; mode of delivery, gestational age at delivery and prematurity rate.

The number of children, n=247, was used as the denominator for birth weight and infant infection status.

Prematurity rate = percentage of pregnancies delivered before 37 completed weeks of pregnancy
Severe prematurity rate = percentage of pregnancies delivered before 32 completed weeks of pregnancy

MTCT HIV rate = the number of children with confirmed HIV infection

In this non-breastfeeding cohort, infection status of the infant was classified as "infected" if the HIV DNA PCR was confirmed positive on 2 separate occasions, "uninfected" if the HIV DNA PCR was confirmed negative on two separate occasions at least two weeks apart with the second test taken at or greater than 3 months of age and "indeterminate" if the infant died or was lost to follow up before it was possible to confirm their infection status. Vertical HIV transmissions are classified into "in utero"; "peripartum" and "post partum" infections reflecting the timing of infection. In utero = HIV DNA PCR positive within the first 48 hours of life. Peripartum = HIV DNA PCR negative within the first 48 hours of life and positive before 3 months of age. In breastfeeding populations postpartum transmissions are those where the HIV DNA PCR tests are negative within the first ~3 months of life but positive thereafter, within the breastfeeding period.

3.2 d Statistical methods

Comparisons of categorical variables were made using the chi-squared test or Fisher's exact test. This latter test was used when any expected cell frequencies were less than 5. Numerical variables were compared using the Mann-Whitney U test for unpaired data and the signed rank test for paired data. These tests are non-parametric tests, as variables were not normally distributed. All p-values were two-sided and were calculated using the statistical package SAS (SAS Institute Inc., Cary, NC). 95% confidence intervals (CI) for proportions when examining the proportion of children who were HIV-positive were calculated using the exact binomial method (reference: Confidence Interval Analysis Martin J Gardner, Stephen B Gardner and Paul D Winter).

3.3 Results

3.3 a Overall

For the eight year period between 1996 and 2003 (four years before the introduction of antenatal screening for HIV and the first four years of screening) the Department of Genitourinary Medicine and Infectious Diseases at St. James's Hospital, Dublin (GUIDE clinic) provided HIV care to a significant proportion (56%) of all known pregnancies in HIV infected women in Ireland (see figure 3.5). From 1999 to 2000 there was a 250% increase in the total number of pregnancies in HIV infected women in Ireland and a 311% increase in the numbers of pregnancies managed at the GUIDE clinic, reflecting the fact that 2000 was the first complete year in which routine opt-out testing was performed.

At the GUIDE clinic between 1996 and 2003 there were 245 pregnancies in 201 women with 247 births (including 2 sets of twins and 4 intrauterine deaths). 162 women had 1 pregnancy only; 34 had 2 pregnancies each and 5 had 3 pregnancies each during this time.

1 pregnancy = 162 women = 162 pregnancy outcomes

2 pregnancies = 34 women = 68 pregnancy outcomes

3 pregnancies = 5 women = 15 pregnancy outcomes

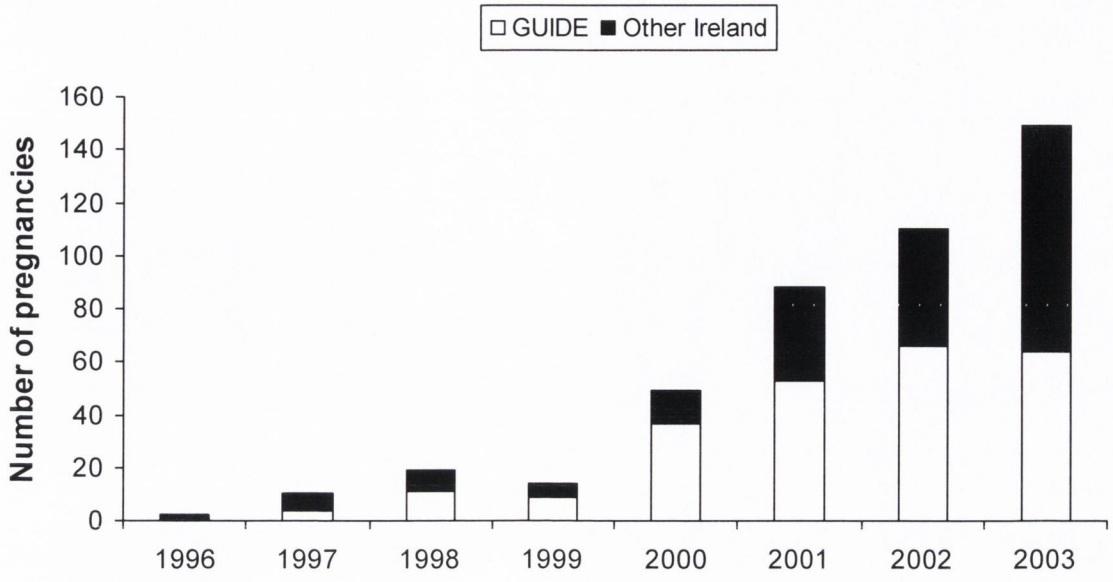
Total = 201 women with 245 pregnancy outcomes

2.3 b Demographics and characteristics

The baseline characteristics of the pregnant women pre and post the introduction of antenatal screening are shown in Table 3.2.

The women seen since the introduction of antenatal screening for HIV were significantly younger than those seen before screening (30 years versus 27 years, $p = 0.009$). The proportion of pregnancies where the HIV diagnosis was made antenatally (50% versus 66%, $p=0.27$) did not change significantly since the introduction of antenatal screening but there was a dramatic increase in the actual numbers of women presenting following an

Figure 3.5 Pregnancies in HIV infected women, Ireland and GUIDE 1996 - 2003



Total pregnancies:								
Ireland	2	10	19	14	49	88	110	149
GUIDE	1	4	11	9	37	53	66	64
% total at GUIDE	50	40	58	64	75	60	60	43

Table 3.2 Baseline demographic characteristics pre and post antenatal screening for pregnancies in HIV infected women at GUIDE, 1996 – 2003

	Pre antenatal screening	Post antenatal screening	Overall	p
Of 201 "first"* pregnancies	24	177	201	
Age (median, years, range)	30 (20 – 38)	27 (16 – 39)	27 (16 – 39)	0.009
Antenatal diagnosis (no. %)	12 (50.0)	118 (66.6)	130 (64.6)	0.27
Risk factor (no. %)				
IVDU	13 (54.1)	23 (12.9)	36 (17.9)	<0.0001
HS	11 (45.8)	153 (86.4)	164 (81.5)	
Other	0 (0)	1 (0.6)	1 (0.5)	
Country of origin (no. %)				
Ireland	17 (70.8)	39 (22.0)	56 (27.8)	<0.0001
*SSA***	7 (29.1)	137 (77.4)	144 (71.6)	
Other	0 (0)	1 (0.6)	1 (0.5)	
Partner's HIV status (no. %)				
Positive	9 (37.5)	18 (10.1)	27 (13.4)	0.001
Negative	5 (20.8)	52 (29.3)	57 (28.3)	
Unknown	10 (41.6)	107 (60.4)	117 (58.2)	
Of 130 antenatal diagnoses	12	118	130	
Gestational age at presentation (median, weeks, range)	32 (16 – 37)	29 (11 – 40)	30 (11 – 40)	0.28
Presenting >32 weeks (no. %)	5 (41.6)	39 (33.0)	44 (33.8)	0.53

* = first pregnancy between 1996 and 2003

** = Sub-Saharan Africa. Women came from 22 countries (Ireland = 55; South Africa = 49; Nigeria = 48; Zimbabwe = 11; other = 37. 36/37 came from other African countries and one woman came from Spain). Other = one woman from Spain

Gestational age at presentation for 1996 and 1997 unavailable

antenatal diagnosis of HIV in the four years after the introduction of antenatal screening for HIV. Of the 130 women diagnosed with HIV antenatally, the median gestational age at presentation was 30 weeks and did not differ significantly before and after the introduction of antenatal screening. One third of those diagnosed antenatally had their first HIV assessment at >32 weeks of pregnancy. The proportion presenting at >32 weeks did not change significantly before and after the introduction of antenatal screening but again there were dramatic shifts in the actual numbers of women seen.

There were significant changes in the HIV risk group and country of origin in the women seen pre and post the introduction of antenatal screening with more heterosexually acquired infection (45.8% versus 86.4%, $p<0.0001$) and more women coming from Sub-Saharan Africa (29.1% versus 77.4%, $p<0.0001$) following the introduction of antenatal screening for HIV. This reflects the increases in migration to Ireland from countries of high HIV prevalence at this time.

There were changes in the overall cohort of patients attending the GUIDE clinic with HIV around this time. Of the total (n=357) HIV cohort at the GUIDE clinic in the period 1996 to 1999, 104 (29%) were female. Of these 104 female patients: 67 (64%) acquired infection heterosexually and 36 (35%) acquired infection through injecting drug use. Between 2000 and 2003 325 (43%) of the total cohort (n=756) were female. Of these female patients: 273 (84%) acquired infection heterosexually and 50 (15%) acquired infection through injecting drug use

There were significant differences in the HIV status of partners (positive, negative or unknown) before and after the introduction of antenatal screening. Of the pregnancies in HIV positive asylum seekers attending the GUIDE clinic between January 2000 and March 2001, 60% of the women from Sub Saharan Africa arrived to Ireland unaccompanied and therefore the HIV status of their partner was unknown[115]. Fears of abandonment or violence may have prevented disclosure of HIV status for many of the women and therefore the HIV status of their partner remained unknown.

3.3 c Clinical status

The baseline clinical status of the women is shown in Table 3.3.

Overall the median CD4 count was 382 x 10^6/L, suggesting that the women seen were largely an immune competent group. There was no significant difference in the median baseline CD4 count pre and post the introduction of antenatal screening. The proportion of women seen with a CD4 count <200 x 10^6/L was greater before the introduction of antenatal screening (29% versus 15%) but this did not reach statistical significance, p = 0.14.

Three women had AIDS defining illnesses during the period 1996 – 2003: one woman presented with ocular Kaposi's sarcoma requiring delivery at 36 weeks gestation in 2000; one woman had disseminated mycobacterium avium complex infection diagnosed during pregnancy in 2001 and the third woman presented with cerebral toxoplasmosis early in her second pregnancy in 2003. The woman with cerebral toxoplasmosis had been diagnosed with HIV postnatally in her first pregnancy in 2002 but had defaulted from follow up.

The overall prevalence of infectious Hepatitis B was low at 3.9% and although there was not a significant difference (p=0.6) all the infectious Hepatitis B was seen after the introduction of antenatal screening, reflecting the greater number of women from countries of high HIV and Hepatitis B prevalence since the introduction of antenatal screening. The prevalence of Hepatitis C was higher before the introduction of antenatal screening and the difference before and since almost reached statistical significance (p=0.05). This most likely reflects the greater number of women who acquired HIV infection through injecting drug use before the introduction of antenatal screening.

More women were on ART at conception before the introduction of antenatal screening than since the introduction of antenatal screening (20.8% versus 8.4%, p = 0.07) again probably reflecting the earlier stage of HIV in the women with pregnancies since the introduction of antenatal screening.

Table 3.3 Baseline clinical status pre and post antenatal screening for pregnancies in HIV infected women at GUIDE, 1996 – 2003

	Pre antenatal screening	**Post antenatal screening**	**Overall**	**p**
Of 245 pregnancies	25	220	245	
CD4 (median, 10^6/L, range)	360 (59 – 914)	388 (14 – 1190)	382 (14 – 1190)	0.20
VL (median, copies/ml, range)	10447 (50 – 108000)	4395 (50 – 406722)	4782 (50 – 406722)	0.37
Of 201 "first"* pregnancies	24	177	201	
CD4 <200 x 10^6/L (no. %)	7 (29.1)	27 (15.2)	34 (16.9)	0.14
Hepatitis B surface Ag positive (no. %)	0 (0)	8 (4.5)	8 (3.9)	0.60
Hepatitis C antibody positive (no. %)	8 (33.3)	27 (15.2)	35 (17.4)	0.05
Syphilis positive (no. %)	1 (4.1)	13 (7.3)	14 (6.9)	1.00
ART at conception (no. %)	5 (20.8)	15 (8.4)	20 (9.9)	0.07

*=first pregnancy between 1996 and 2003

3.3 d Antenatal antiretroviral therapy

The overall use of antiretroviral therapy and that pre and post antenatal screening is shown in table 3.4. Table 3.5 and Figure 3.6 show the changes in antiretroviral therapy use by year.

There are no data available on antenatal antiretroviral therapy for the 1 pregnancy in 1996. Of the remaining 244 pregnancies between 1997 and 2003, 20 (8.1%) women had no antenatal ART; 18 (7.4%) women received zidovudine monotherapy; 37 (15.2%) women received dual therapy with zidovudine/lamivudine fixed dose formulation (Combivir®) and 169 (69.3%) women received triple antiretroviral therapy.

Changes in antiretroviral prescribing in pregnancy reflect the changes in guidelines for management of HIV in pregnancy around this time. In 2000 a group of adult and paediatric HIV physicians drew up national guidelines for the management of HIV in pregnancy which became operational in November 2000 and were published in May 2001 (see figure 3.7). In response to international data these guidelines were updated in late 2001 and the new guidelines became operational in January 2002 and were published in May 2006 (see table 3.6). The major difference between the November 2000 guidelines and the January 2002 guidelines was a move away from dual therapy in women not requiring antiretroviral therapy for their own health such that all women, regardless of immunological well being and HIV viral load were offered triple antiretroviral therapy in pregnancy.

No antenatal antiretroviral therapy

There was antenatal antiretroviral therapy exposure in all of the 24 pregnancies with available data before the introduction of antenatal screening. In the first 4 years of antenatal screening there was no antenatal antiretroviral therapy exposure in 20 (9.1%) pregnancies, see table 3.7. Of these 20 pregnancies: 8 were in women diagnosed with HIV postnatally; 6 presented at an advanced gestational age and delivered before they received antiretroviral therapy; 4 women failed to attend for HIV care in one pregnancy

Table 3.4 Antiretroviral therapy pre and post introduction of antenatal screening (n=244)*

	none	mono	dual	triple	***3 NRTI***	***2NRTI + NNRTI***	***2NRTI + PI***
	No. (%)	No. (%)	No. (%)	No. (%)	*No.*	*No.*	*No.*
Pre ANS (n=24)	0 (0)	2 (8.3)	10 (41.6)	12 (50.0)	*0*	*3*	*9*
Post ANS (n=220)	20 (9.1)	16 (7.3)	27 (12.2)	157 (71.4)	*3*	*112*	*42*
Overall (n=244)	20 (8.1)	18 (7.4)	37 (15.2)	169 (69.3)	*3*	*115*	*51*

Type of antiretroviral therapy $p<0.0001$
*no antiretroviral data for the pregnancy in 1996
NRTI = nucleoside reverse transcriptase inhibitor
NNRTI = non- nucleoside reverse transcriptase inhibitor
PI = protease inhibitor
ANS = antenatal screening

*Table 3.5 Antiretroviral therapy by year of delivery (1996 – 2003) n = 244**

No. of drugs	0	1	2	3	*3NRTI*	*2NRTI + NNRTI*	*2NRTI + PI*	Total
1996	na	na	na	na	*na*	*na*	*na*	
1997	0	0	2	2	*0*	*0*	*2*	4
1998	0	0	4	7	*0*	*0*	*7*	11
1999	0	2	4	3	*0*	*3*	*0*	9
2000	3	1	19	14	*1*	*9*	*4*	37
2001	9	9	8	27	*1*	*20*	*6*	53
2002	7	3	0	56	*0*	*53*	*3*	66
2003	1	3	0	60	*1*	*30*	*29*	64
total	20	18	37	169	*3*	*115*	*51*	244

*no antiretroviral data for the pregnancy in 1996
NRTI = nucleoside reverse transcriptase inhibitor
NNRTI = non- nucleoside reverse transcriptase inhibitor
PI = protease inhibitor

Figure 3.6 Antiretroviral therapy by year of delivery, GUIDE clinic 1997 – 2003

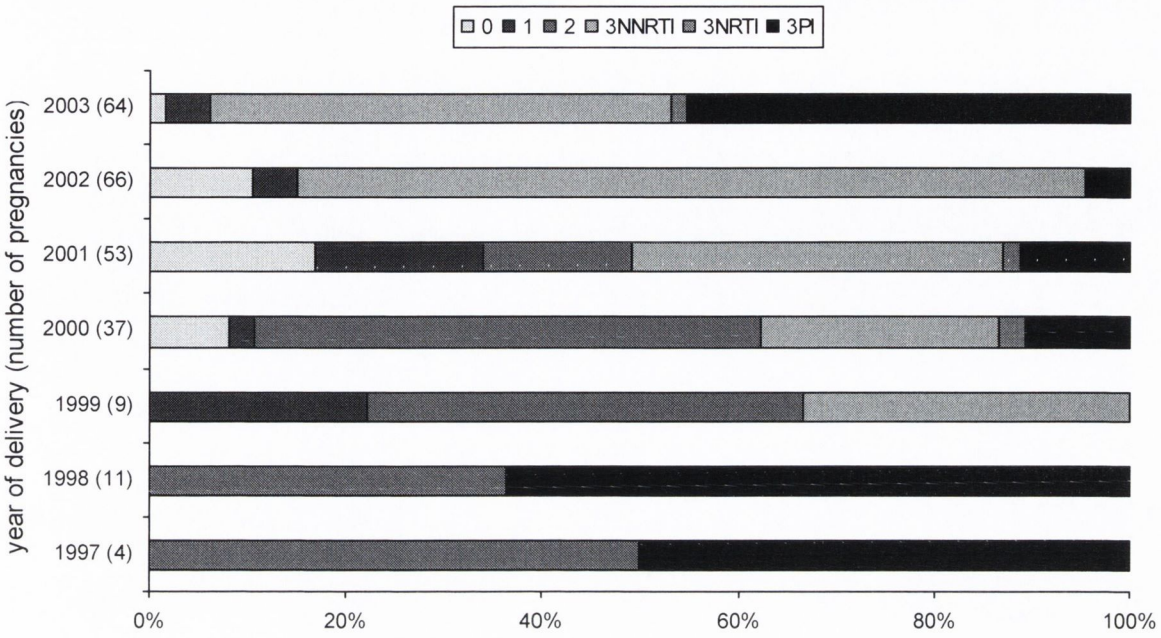


Figure 3.7 Algorithm for antiretroviral therapy use in pregnanc
(S Clarke et al. National guidelines for the active management of HIV in pregnancy. Ir Med J. 2001 May;94(5):137-40)

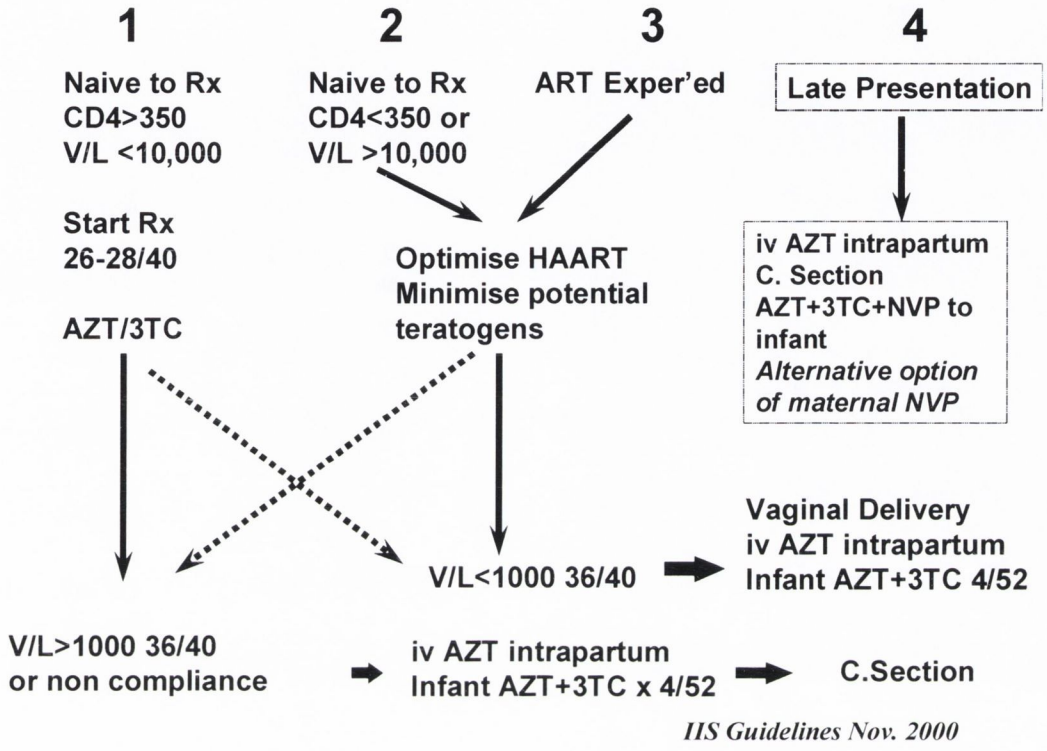


Table 3.6: 2002 guidelines for management of HIV in pregnancy (F Lyons et al,National guidelines for the management of HIV-1 in pregnancy. Ir Med J. 2006 May;99(5):152-4)

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
<28/40	Because of ↓ baseline CD4, mother requires Rx for own health	<200 x 10^6/L	regardless of HIV VL	naive
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT pre-Rx Triple ART to commence ASAP after 1st trimester	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped Start 4 hrs prior to ELCS	continue on triple ART	Await SOL if maternal VL <1000 @ 36/40	ZDV X 4/52 if maternal VL <1000 @36/40 +ROM <12hrs
			ELCS @ 39/40 if maternal VL >1000 @ 36/40	Triple ART x 4/52 if maternal VL >1000 @36/40 + ROM >12hrs

Table 3.6 - continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
<28/40	Mother requires Rx to reduce vertical transmission of HIV to infant but does not require Rx for her own health.	>200 x 10^6/L Some women with CD4 200-300 x 10^6/L will require ART for their own health. This decision rests with the HIV physician	regardless of HIV VL	naive

RECOMMENDATIONS

Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT pre-Rx Triple ART (to commence @ 28/40 for single pregnancy , earlier if multiple pregnancy or h/o prem. labour For selected women ZDV monotherapy may be an option	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped Start 4 hrs prior to ELCS	discontinue post-partum	Await SOL if maternal VL <1000 @ 36/40	ZDV X 4/52 if maternal VL <1000 @36/40 + ROM <12hrs
			ELCS @ 39/40 if maternal VL >1000 @ 36/40	Triple ART 4/52 if maternal VL >1000cpm @36/40 +/or ROM >12hrs

Table 3.6 – continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
>28/40	Because of ↓ CD4 count, mother requires Rx for own health	<200 x 10^6/L	regardless of HIV VL	naive
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT Triple ART to commence ASAP (may be before resistance results available)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped Start 4 hrs prior to ELCS	continue on triple ART	Await SOL if maternal VL <1000 @36/40 and ≥4/52 of Rx	ZDV x 4/52 if mother on Rx for ≥4/52 with VL <1000 + ROM <12hrs
			ELCS @ 39/40 if maternal VL >1000 @ 36/40 or ≤4/52 Rx	Triple ART if mother on Rx. ≤4/52 or VL >1000cpm @36/40 +/or ROM >12hrs

Table 3.6 – continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
>28/40	Mother requires Rx to reduce risk of vertical transmission of HIV to infant but does not require Rx for her own health	>200 x 10^6/L Some women with CD4 200-300 x 10^6/L will require ART for their own health. This decision rests with the HIV physician	regardless of HIV VL	naive

RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT Triple ART to commence ASAP (may be before resistance results available)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped Start 4 hrs prior to ELCS	discontinue post-partum	Await SOL if maternal VL <1000 @36/40 and ≥4/52 of Rx	ZDV x 4/52 if mother on Rx for ≥4/52 with VL <1000 + ROM <12rs
			ELCS @39/40 if maternal VL >1000cpm @36/40 or ≤4/52 Rx	Triple ART if mother on Rx. ≤4 wks Rx or VL >1000cpm @36/40 +/or ROM >12hrs

Table 3.6 – continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
>36/40	Because of ↓ CD4 count, mother requires Rx for HIV	<200 x 10^6/L	regardless	naive
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT Triple ART to commence ASAP (even if resistance results unavailable)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped (Start 4 hrs prior to ELCS)	continue on triple ART	ELCS @ 39/40	Triple ART x 4/52

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
>36/40	Mother requires Rx to reduce risk of VT of HIV to infant but does not require Rx for own health	>200 x 10^6/L	regardless	naive
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
GRT Triple ART to commence ASAP (even if resistance results unavailable)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped (Start 4 hrs prior to ELCS)	discontinue post-partum	ELCS @ 39/40	Triple ART x 4/52

Some women with CD4 200-300 x 10^6/L will require ART for their own health. This decision rests with the HIV physician

Table 3.6 – continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
Attending pre-conception on Rx.	Continue therapy but substitute efavirenz in 1st trimester			
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Virally suppressed @36/40	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped (Start 4 hrs prior to ELCS)	Continue	Await SOL	ZDV x 4/52 if ROM <12hrs
Failing therapy @36/40 Case by case with guidance of GRT	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped (Start 4 hrs prior to ELCS)	Case by case	ELCS @39/40	Case by case

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
Diagnosed in labour	Regardless of status of membranes			
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
	IV ZDV loading 2mg/kg over 1 hr then 1mg/kg/hr until cord is clamped. Consider stat dose NVP	Assessment at adult HIV service post partum	EMCS once loaded with ZDV	Triple ART once maternal HIV confirmed

Table 3.6 - continued

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
Refusing therapy				
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
	Offer IV ZDV loading 2mg/kg over 1 hr then 1mg/kg/hr until cord is clamped. (Start 4 hrs prior to ELCS) Consider Stat dose NVP		Offer ELCS at 39/40	Triple ART

SCENARIO

Gestation at presentation	**Clinical**	**CD4**	**HIV VL**	**Previous ART**
Diagnosed post delivery				
RECOMMENDATIONS				
Antepartum	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
		Assessment at adult HIV service ASAP		Triple ART to commence ASAP (must be commenced within 72 hours of birth)

ART: Antiretroviral therapy
C.S: Caesarean Section
GRT: genotypic resistance testing
SOL: Spontaneous onset of labour
VL: viral load
CPM: Copies per ml
ELCS: Elective caesarean section
NVP: Nevirapine
Rx: therapy
ZDV: Zidovudine or AZT or Retrovir
Triple ART to baby = ZDV + 3TC x 4/52 + stat NVP

Table 3.7 Women who received no antenatal antiretroviral therapy 1996 – 2003, n = 20

	Year	BL CD4 (x 10^6/L)	BL VL (copies/ml)	Antenatal diagnosis	Gestation at presentation (weeks)	Mode of delivery	Infant no of drugs	Infant HIV status	Comment
1	2000	423	22031	Yes	39+	ELCS	3	negative	
2	2000	658		postpartum		ELCS	2	negative	
3	2000			postpartum		SVD	3	negative	
4	2001			postpartum		SVD	0	negative	
5	2001	605	50	Yes	37	EMCS	3	negative	
6	2001	431	591	Yes	38	ELCS	3	negative	
7	2001	612	1788	postpartum		SVD	0	negative	
8	2001	331	2609	Yes	37	ELCS	3	negative	
9	2001	129	4489	Yes	40	ELCS	2	negative	
10	2001	274	38344	Yes	40	ELCS	3	negative	
11	2001			postpartum		EMCS	2	negative	
12	2001	353	50	No	39	ELCS	3	negative	DNA
13	2002	113	10395	Yes	30	SVD	3	negative	DNA
14	2002	14	41012	postpartum		SVD	3	negative	
15	2002			postpartum		EMCS	2	negative	
16	2002	94	49800	postpartum		SVD	3	negative	
17	2002			No		SVD	3	negative	DNA
18	2002	588	911	No		EMCS	3	negative	DNA
19	2002	482	1049	No		ELCS	3	negative	Refused therapy
20	2003	674	14602	No	21	SVD	0	negative	IUD before started ART

BL CD4 = baseline CD4 count, BL VL = baseline viral load, SVD = spontaneous vaginal delivery, EMCS = emergency c section, ELCS = elective c section, DNA = did not attend, IUD = intrauterine death

(one of whom had been diagnosed postnatally in her previous pregnancy); 1 woman refused to take antiretroviral therapy and 1 woman was diagnosed with an intrauterine death at 25 weeks (she had been scheduled to initiate antiretroviral therapy to reduce MTCT at 28 weeks).

Zidovudine monotherapy

In 18 (7.4%) of the pregnancies zidovudine monotherapy was prescribed; 2 (9.1%) before the introduction of antenatal screening and 16 (7.3%) following the introduction of antenatal screening (see table 3.8)

Dual antiretroviral therapy

Dual antiretroviral therapy was used in 37/244 (15.2%) of the pregnancies between 1997 and 2003: 10/24 (41.6%) of those before the introduction of antenatal screening and 27/220 (12.2%) of those following the introduction of antenatal screening. The years 1999 and 2000 were the most popular years for dual antiretroviral therapy use: 4/9 (44.4%) and 19/37 (51.3%) respectively. There was no dual antiretroviral therapy use in 2002 or 2003, in accordance with the January 2002 guidelines.

Triple antiretroviral therapy

Of the 169 women that received triple antiretroviral therapy 3 received a triple NRTI; 115 received an NNRTI based regimen and 51 received a PI based regimen. The triple NRTI regimen was zidovudine/abacavir/lamivudine (fixed dose formulation, Trizivir®) for 2 of the women and abacavir, stavudine and lamivudine for one woman. All NNRTI based regimens were nevirapine based (including one woman who conceived on efavirenz and was interchanged to nevirapine when she presented with a positive pregnancy test). Protease inhibitor based regimens included 1 ritonavir; 2 unboosted indinavir; 3 boosted saquinavir; 2 boosted lopinavir and 53 nelfinavir.

Table 3.8 Women who received zidovudine (ZDV) monotherapy 1997 – 2003, n=18

	Year	BL CD4 (x 10^6/L)	BL VL (copies/ml)	Antenatal diagnosis	Gestation at presentation (weeks)	Stated indication for ZDV	Del VL (copies/ml)	Mode of delivery	Baby HIV status
1	1999	259	1634	Yes	36		BLD	elcs	negative
2	1999	914	BLD	Yes	32	low VL with good CD4	BLD	SVD	negative
3	2000	265	200000	Yes	12	Refused other ART	25693	elcs	negative
4	2001	561	BLD	Yes	30	low VL with good CD4	BLD	SVD	negative
5	2001	398	65	Yes	40	late presenter with low VL	BLD	EMCS	negative
6	2001	772	88	No		Refused other ART	157	EMCS	negative
7	2001	567	113	No			BLD	SVD	negative
8	2001	440	283	No		Refused other ART	100	EMCS	negative
9	2001	365	315	Yes	20	low VL with good CD4	198	SVD	positive (intrauterine transmission)
10	2001	455	BLD	Yes	35	late presenter with undetectable VL	BLD	EMCS	negative
11	2001	367	670	Yes	37	late presenter with low VL	NA	ELCS	negative
12	2001	493	892	Yes	34	late presenter with low VL	96	ELCS	negative
13	2002	840	994	Yes	39	late presenter with low VL	NA	ELCS	negative
14	2002	684	768	No		chaotic IVDU/ETOH with good CD4 and low VL	282	ELCS	negative
15	2002	1190	BLD	Yes	26	Refused to take triple ART	BLD	SVD	negative
16	2003	575	BLD	Yes	38	late presenter with low VL	NA	ELCS	negative
17	2003	922	372	Yes	18	PPROM at 21/40		EMCS	Indeterminate RIP at 3/52
18	2003	432	41159	No		Chaotic IVDU	10358	EMCS	negative

BL CD4 = baseline CD4 count, BLD = below limit of detection, BL VL = baseline viral load, Del VL = delivery viral load, SVD = spontaneous vaginal delivery, EMCS = emergency c section, ELCS = elective c section, IVDU = intravenous drug user, ETOH = alcohol, PPROM = preterm prolonged rupture of the membranes, ZDV = zidovudine

There was a dramatic increase in the proportion of PI based triple antiretroviral regimens between 2002 and 2003 (3% to 48%) and a corresponding drop in the proportion of NNRTI based regimens (95% to 50%), see figure 3.6. This coincides with reports of severe maternal hepatotoxicity (including maternal death from fulminant hepatitis) associated with antenatal nevirapine exposure and a consequent change in prescribing practise.

Peripartum intravenous (IV) zidovudine and mode of delivery

The use of peripartum intravenous zidovudine was high at 85% and did not differ significantly before and after the introduction of antenatal screening, (p=0.5), table 3.9. Similarly the mode of delivery did not change significantly before and after the introduction of antenatal screening (p=0.44), table 3.10.

Table 3.9 Peripartum IV zidovudine pre and post introduction of antenatal screening

IV Zidovudine	Yes	No	Unknown	
	No. (%)	No. (%)	No. (%)	Total
4 years pre ANS	20 (80.0)	4 (16.0)	1 (4.0)	25
4 years post ANS	188 (85.4)	26 (11.8)	6 (2.7)	220
Overall	208 (84.8)	30 (12.2)	7 (2.8)	245

ANS = antenatal screening

Table 3.10 Mode of delivery pre and post introduction of antenatal screening

Mode of delivery	ELCS	EMCS	VD	
	No. (%)	No. (%)	No. (%)	Total
4 years pre ANS	5 (20.8)	2 (8.3)	17 (70.8)	24
4 years post ANS	43 (19.7)	43 (19.7)	132 (60.5)	218
Overall (1996 -2003)	48 (19.8)	45 (18.5)	149 (61.5)	242*

* mode of delivery not known for the pregnancy in 1996, 1 in 2001 and 1 in 2003
P=0.44 (Fishers)
ELCS = Elective Caeserean Section
EMCS = Emergency Caserean Section
VD = vaginal delivery
ANS = antenatal screening

3.3 e Pregnancy and infant outcome

Gestational age at delivery and prematurity rates are shown in table 3.11. The overall median gestational age at delivery was 39 weeks and did not differ before and after the introduction of antenatal screening, p = 1.0. The overall prematurity rate (<37 weeks) was 10.2% and did not differ significantly before and after the introduction of antenatal screening, 4.0% and 10.9% respectively, p=0.49. Similarly there was no significant difference in the rate of severe prematurity (<32 weeks), p=1.00 before and after the introduction of antenatal screening.

The gestational age at delivery and prematurity rates by number of antenatal antiretroviral drugs (0, 1, 2, 3) is shown in table 3.12.

The overall median birth weight at delivery was 3.13kg (0.65 – 5.0kg) and did not differ before and after the introduction of antenatal screening, 3.19kg and 3.13kg respectively, p=0.28.

The infection status of the children is shown in table 3.13. Two hundred and thirty five children were confirmed HIV negative. Four children were infected with HIV and 8 children had an indeterminate HIV status. Six of the 8 indeterminate children died before the 3 month PCR was done and 2 children were lost to follow up. This gives an MTCT rate of 4/247=1.6%; 95% CI 0.4%-4.1%. If the indeterminate children are presumed infected the MTCT rate is 12/247=4.9%; 95% CI 2.5%-8.3%. To date, 9 (3.6%; 95% CI 1.7%, 6.8%) of the children are known to have died, none of them from AIDS (1 x spinal cord tumour; 2 x Sudden Infant Death Syndrome; 4 x intrauterine deaths and 2 other).

HIV infected children

Table 3.11 Gestational age (weeks) and prematurity rates pre and post introduction of antenatal screening

	Median (weeks)	Range	<32 weeks	<37 weeks	Total (pregnancies)
			No. (%)	No. (%)	
4 years pre ANS	39	32 – 42	0 (0.0)	1 (4.0)	25
4 years post ANS	39	25 - 43	6 (2.7)	24 (10.9)	220
Overall	39	25 - 43	6 (2.4)	25 (10.2)	245

* includes 2 sets of twins with gestational age at delivery of 32 and 39 weeks
P=1.00 <32 weeks
P=0.49 <37 weeks
ANS = antenatal screening

Table 3.12 Gestational age at delivery and prematurity rates by number of antenatal antiretroviral drugs

No. of drugs	No. of pregnancies	Median gestational age (weeks, range)	<32 weeks (no. %)	<37 weeks (no. %)
0	20	40 (25 – 42)	1 (5.0)	2 (10)
1	18	40 (30 – 42)	1 (5.5)	1 (5.5)
2	37	39 (31 – 42)	1 (2.7)	5 (13.5)
3	169	39 (25 – 43)	3 (1.8)	17 (10.5)
Overall	244*	39 (25 – 43)	6 (2.4)	25 (10.2)

* no data available on ART for the one pregnancy in 1996

Table 3.13 MTCT pre and post introduction of antenatal screening

	Positive*	negative	Indeterminate	
	No. (%)	No. (%)	No. (%)	Total
4 years pre ANS	0 (0)	26 (96)	1 (3.7)	27
4 years post ANS	4 (1.8)	209 (95)	7 (3.1)	220
Overall	4 (1.6)	235 (95)	8 (3.2)	247

* 3 girls and 1 boy were infected
ANS = antenatal screening

MTCT number 1– PERIPARTUM transmission

A Sub Saharan African woman in her 20's was found to be HIV positive through antenatal screening in the second trimester of her first pregnancy. She had no identified co-morbidities (hepatitis B, hepatitis C and syphilis negative, negative STI screen). Baseline HIV parameters: CD4 = 328 x 10^6/Land HIV viral load = 29696 copies/ml. She commenced antiretroviral therapy at 28 weeks (combivir and nevirapine). The nevirapine was interchanged to nelfinavir 23 days post initiation as part of an elective recall because of concerns for excess maternal toxicity with nevirapine. HIV viral load at the time of interchange (23 days of therapy) = 89 copies/ml. HIV viral load at 36 weeks was 57 copies/ml. Identified issues included complex social circumstances and problems with adherence to antiretroviral therapy. Following spontaneous onset of labour at full term she spontaneously delivered a female infant weighing 2.9kg. She received peripartum IV zidovudine and membranes were ruptured for 3 hours prior to delivery. The infant received zidovudine monotherapy for the first 4 weeks of life and was exclusively formula fed. HIV PCR was negative on day 1 and day 14 of life. The first positive HIV PCR was on day 42 of life (peripartum transmission). The infant commenced combination antiretroviral therapy (zidovudine, abacavir, lamivudine and nevirapine) following the positive HIV PCR result.

MTCT number 2 – IN UTERO transmission

A Sub Saharan African woman in her 20's was found to be HIV positive through antenatal screening in the second trimester. At baseline CD4 = 365 (29%) x 10^6/L and HIV viral load = 315 copies/ml and 473 copies/ml on two separate occasions. She was negative for hepatitis C and syphilis. Hepatitis B core antibody was positive with a negative hepatitis B surface antigen. She initiated zidovudine monotherapy at 32 weeks and at four weeks of therapy HIV viral load was 198 copies/ml. She spontaneously delivered a male infant weighing 2.67kg at 36 weeks following spontaneous onset of labour. She received

peripartum IV zidovudine and the membranes were ruptured for 45 minutes before delivery. Her infant received zidovudine monotherapy until the day 1 PCR (HIV viral load of 50,000 copies/ml) was returned as positive. At that time neonatal ART was changed to abacavir, epivir and ritonavir. The infant was exclusively formula fed.

MTCT number 3 – PERIPARTUM Transmission (prolonged ROM)

A Sub Saharan African woman in her 20's was found to be HIV positive through antenatal screening early in the third trimester of her first pregnancy. At baseline CD4 = 374 (26%) x 10^6/L and HIV viral load = 3490 copies/ml. She was negative for hepatitis C and syphilis. Hepatitis B core antibody was positive with a negative surface antigen. She had a negative STI screen. She initiated combivir and nelfinavir at 29 weeks gestation and at 2 weeks of antiretroviral therapy had an undetectable HIV viral load (<50 copies/ml). At 36 weeks she had an undetectable HIV viral load and one week later (37 weeks gestation) she had spontaneous rupture of the membranes. She was admitted to hospital 24 hours after ruptured membranes and at that time was not in labour. She received IV zidovudine and was delivered of a female infant (2.41kg) by emergency caesarean section. The infant received zidovudine monotherapy for the first 4 weeks of life and was exclusively formula fed. HIV PCR was positive at 4 weeks of age with a viral load of <400 copies/ml. At 6 weeks of life the HIV viral load was 750 000 copies/ml and the infant was commenced on combination antiretroviral therapy with abacavir, lamivudine and nevirapine.

MTCT number 4 – IN UTERO transmission

A Sub Saharan African woman in her 20's was found to be HIV positive through antenatal screening in the second trimester. At baseline her CD4 count = 401 (32%) x 10^6/L and HIV viral load = 34753 copies/ml. She had no identified co-morbidities (hepatitis B, hepatitis C, syphilis and STI screen negative). She refused all treatment despite numerous consultations until 33 weeks when she agreed to commence therapy with combivir and

nevirapine. At approximately 35 weeks she developed pre-eclampsia and was self discontinued her antiretroviral therapy. She was admitted for management of pre-eclampsia and recommenced antiretroviral therapy (combivir and nevirapine) whilst an in-patient. She had an elective caesarean section at 40 weeks with a HIV viral load of 12778 copies/ml on the day of delivery. She was delivered of a female infant weighing 2.21kg. She received peripartum IV zidovudine and her infant received triple antiretroviral therapy (abacavir, epivir and zidovudine). HIV PCR was positive on day 1 of life.

3.3 f Maternal response to treatment and follow up

The median delivery CD4 from1997 to 2003 was 450 x 10^6/L; for the four years before antenatal screening = 438 x 10^6/L and the four years after antenatal screening = 443 x 10^6/L. The median delivery HIV viral load from 1997 to 2003 was 50 copies/ml; for the years pre antenatal screening = 400 copies/ml (range 50 – 11727) and the years since antenatal screening = 50 copies/ml (range 50 – 311111). The proportion of women with delivery HIV viral loads <1000 copies/ml; </=400 copies/ml and <50 copies/ml are shown in Table 3.14. The proportion of women with delivery HIV viral loads <400 copies/ml did not change significantly pre and post the introduction of antenatal screening (p=0.69) but there was an ability to achieve a high proportion of low delivery HIV viral loads (75% <400 copies/ml) despite the dramatic increase in women being cared for following the introduction of antenatal screening.

Table 3.14 Delivery viral load pre and post introduction of antenatal screening

	<1000 cpm*	<400 cpm	<50 cpm	
	No. (%)	No. (%)	No. (%)	Total pregnancies
Pre ANS (1997**-1999)	17 (70.8)	17 (70.8)	7 (29.1)	24
Post ANS (2000 – 2003)	173 (78.6)	164 (74.5)	125 (56.8)	220
Overall	190 (77.8)	181 (74.2)	132 (54.0)	244

<400 copies/ml, p=0.69 (chi-squared)
*no viral load data available for 1996
cpm = copies/ml
ANS = antenatal screening

Maternal follow up

Maternal follow up at 2 years post the first pregnancy is shown in table 3.15. Women who were not seen within 2 years of delivery were deemed lost to follow up (LTFU).

Subsequent pregnancies

Over time there has been an increase in the number of subsequent pregnancies (table 3.16 and figure 3.8).

Table 3.15 Maternal follow-up at 2 years post delivery pre and post introduction of antenatal screening (for first pregnancy only, n = 201)

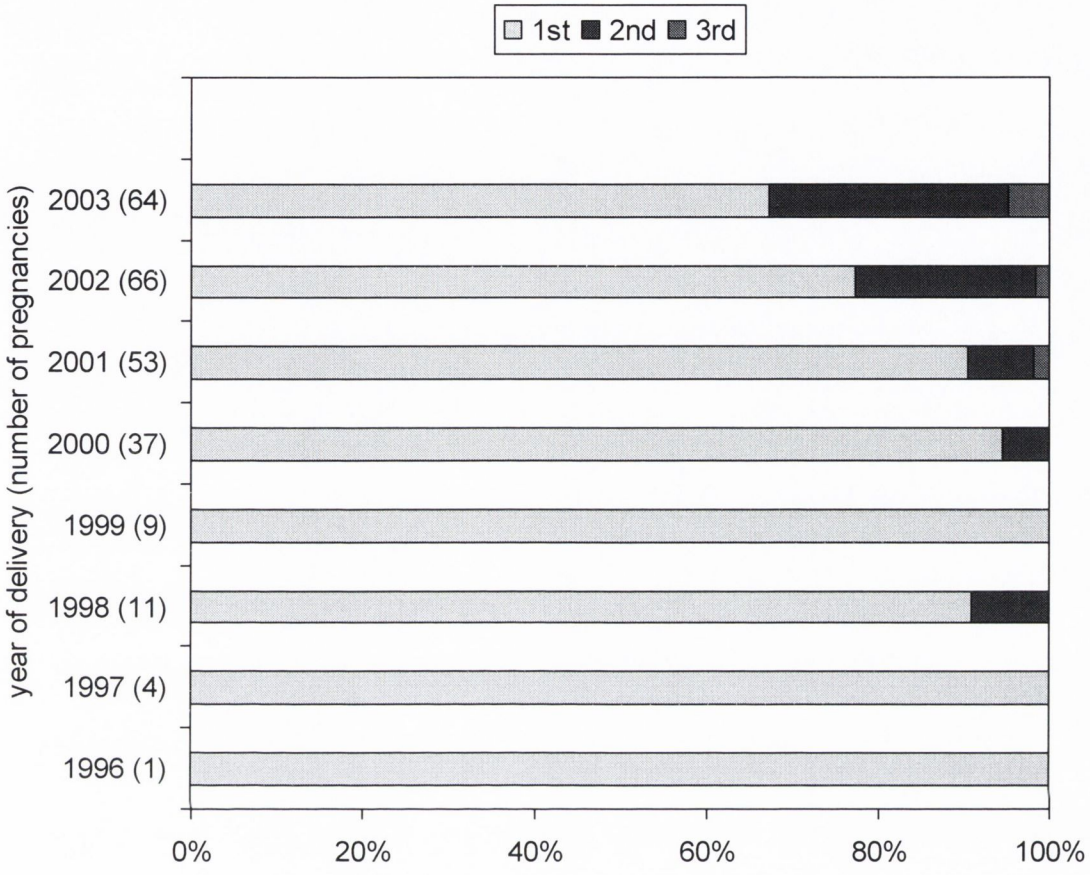
	Active	LTFU	RIP	Transfer	
	No. (%)	No. (%)	No. (%)	No. (%)	Total
4 years pre ANS	21 (87.5)	3 (12.5)	0 (0)	0 (0)	24
4 years post ANS	141 (79.6)	29 (16.3)	3 (1.7)	4 (2.2)	177
Overall	162 (80.5)	32 (15.9)	3 (1.4)	4 (1.9)	201

Active = still attending for care
LTFU = lost to follow up
RIP = dead
Transfer = transferred care to other unit
ANS = antenatal screening

Table 3.16 Subsequent pregnancies in HIV infected women at the GUIDE clinic, 1996 – 2003

Year	1st	2nd	3rd	total
1996	1	0	0	1
1997	4	0	0	4
1998	10	1	0	11
1999	9	0	0	9
2000	35	2	0	37
2001	48	4	1	53
2002	51	14	1	66
2003	43	18	3	64
Overall	201	39	5	245

Figure 3.8 Subsequent pregnancies at GUIDE clinic 1996 to 2003



3.4 Discussion

Antenatal screening for HIV forms an integral part of any programme for preventing MTCT of HIV. In Ireland the introduction of antenatal screening has been very successful with uptake rates consistently >90% for the years 2002 to 2006. The introduction of antenatal screening coincided with a massive increase in migration to Ireland of individuals from countries of high HIV prevalence and an economic boom referred to as the "Celtic Tiger". Antenatal screening for HIV provides an opportunity to identify women with HIV before their infants are born and thus make interventions to reduce perinatal transmission. Notwithstanding the obvious benefits of this, for many women it represents a very traumatic time in their lives. Many of the women that attended the GUIDE clinic were recent arrivals to Ireland, pregnant and previously unaware of their HIV status. Overall 33.8% of the women diagnosed with HIV through antenatal screening were seen for their first HIV assessment at >32 weeks gestation. This left little opportunity for women to reflect on their HIV status before needing to start antiretroviral therapy and little time for staff to provide education and information on HIV, its natural history, antiretroviral therapy and interventions required to reduce perinatal transmission. Oftentimes cultural implications of a HIV diagnosis for many of the non-national women presented further challenges for health care staff. This has since formed the basis for a qualitative study of African women's experience of the Irish health service following a positive HIV diagnosis during pregnancy in Ireland. The results of this study indicated that women were devastated by their diagnosis and identified a need to provide culturally appropriate education and counselling in this circumstance[116]. Furthermore a recent audit of children infected with HIV in England between 2002 and 2005 identified the need to address the complex social needs of HIV infected women in pregnancy as part of the overall strategy in preventing perinatal transmission of HIV[117].

Notwithstanding the challenges outlined above, the majority of the women before and after the introduction of HIV were clinically well. Of the 201 women, only 3 (14.9%) had AIDS

defining illnesses and all three women were diagnosed with HIV in pregnancy (one in the early post partum period). In addition to reducing perinatal transmission of HIV, it could be hypothesised that antenatal screening for HIV has the potential to identify women at an earlier stage of their HIV disease. Whilst the proportion of women with a baseline CD4 count of $<200 \times 10^6/L$ was less after the introduction of antenatal screening (15% versus 29%), this did not reach statistical significance, p=0.14.

Effective antiretroviral therapy has revolutionised HIV disease and forms an integral part of preventing perinatal transmission of HIV. In the four years after the introduction of antenatal screening there was no antiretroviral exposure in 20 (9.1%) of the pregnancies at the GUIDE clinic, see table 3.7. Fortunately all of these children are HIV negative. In eight of the pregnancies the diagnosis of HIV was made postnatally and all but one of the infants received combination post exposure prophylaxis. Rapid and same day testing for HIV in the setting of the unbooked, HIV positive mother provides an opportunity to reduce transmission by 6 – 13% with peri and post partum interventions[27].

Four of the women failed to attend the GUIDE clinic in pregnancy despite repeated efforts on the part of multiple health care staff to facilitate their engagement in care. One of the challenges at this time was the policy of dispersal of newly arrived asylum seekers to resettle outside of the Dublin area. This was implemented in April 2000 in response to the shortage of accommodation in the Dublin area. Coincident with the policy of dispersal was "direct provision" such that as part of the resettling outside the Dublin area, newly arrived asylum seekers were given full board accommodation and a small amount of money on a weekly basis. At this time there were no adult HIV services outside of Dublin and Cork such that asylum seekers with HIV often needed to travel to attend for HIV clinic appointments. The need for travel and lack of money may have contributed to individuals' inability to attend for HIV care.

One woman refused antiretroviral therapy despite several, multidisciplinary consultations. She believed that God would intervene and ensure that her child would not be infected with HIV and furthermore that prayer would eventually clear her HIV infection. Eventually she agreed to an elective caesarean section, neonatal antiretroviral therapy and formula feeding her infant. This case became the subject of a high court case and the infant was made a ward of court to ensure administration of antiretroviral therapy.

Zidovudine was the first antiretroviral drug used in pregnancy and shown in a randomised controlled trial to be effective in reducing MTCT of HIV[42]. Recent data on 5027 mother-child pairs from the UK and Ireland showed no difference in transmission rates between those on HAART with an elective caesarean section (0.7%), those on HAART with a spontaneous vaginal delivery (0.7%) and those on zidovudine coupled with an elective caesarean section (0.0%, p=0.15)[118]. Furthermore there is accumulating evidence that in women with low pre treatment HIV viral loads, the risk of developing resistance following antenatal exposure to zidovudine monotherapy is remote[106 107].

At the GUIDE clinic, 16 of the 18 (88%) women that received zidovudine monotherapy had pretreatment CD4 counts >350 x 10^6/L and 88% had low pretreatment HIV viral loads. Three of the women refused more complex antiretroviral therapy. This coupled with the recent data on efficacy and safety with respect to evolution of antiretroviral resistance, outlined earlier, suggests that zidovudine monotherapy may need to be considered as an option in future Irish guidelines for the management of HIV in pregnancy.

Of note vertical transmission of HIV occurred in one of the 18 pregnancies where zidovudine monotherapy was used. This woman had a good pretreatment CD4 count and low pretreatment HIV viral load. This was an in utero transmission and more likely

occurred as a result of the timing of initiation of antiretroviral therapy rather than choice of antiretroviral therapy (see discussion on infected children).

The majority (115/169, 68%) of the women that received triple antiretroviral therapy were on a nevirapine based regimen. Advantages of including nevirapine as part of antenatal antiretroviral therapy include low pill burden, good gastrointestinal tolerability, rapid viral decay[119] and the fact that it crosses the placenta. Rapid viral load decay and crossing the placenta are particularly useful in women presenting late in pregnancy. Unfortunately in Dublin in 2002 there were two maternal deaths from fulminant hepatitis attributed to nevirapine as part of combination antiretroviral therapy in pregnancy. This prompted a review of all nevirapine use in pregnancy in Dublin and forms the basis of chapter 5.

The overall vaginal delivery rate was 61.5% which is much higher than an overall vaginal delivery rate of 22.2% in a recent report of over 5000 mother-child pairs[118]. Advantages of a vaginal delivery in women with HIV include the absence of a scar on the uterus. This may be of particular importance for women who have come to Ireland seeking asylum but have failed the asylum seeking process and face deportation to a country that may not be able to offer the same degree of obstetric care as in the developed world. Furthermore as outlined earlier there have been reports of increased morbidity in HIV infected women undergoing delivery by caesarean section versus vaginal delivery though this appears to be decreasing over time.

In recent years there have been conflicting reports on the impact of antenatal antiretroviral exposure on pregnancy length. In general, reports from this side of the Atlantic have suggested that prematurity rates are higher with more complex antiretroviral exposure[90 91] whilst reports from the United States[92] have not demonstrated this effect. In the GUIDE cohort the overall median gestational age at delivery and prematurity rates have not

changed in the four years before and the four years after the introduction of antenatal screening for HIV (see table 3.11). Similarly the gestational age at delivery and prematurity rates did not differ with increasingly complex antiretroviral therapy (see table 3.12). It is important to acknowledge that this cohort is small and did not control for many of the factors known to be associated with premature delivery (for example previous premature delivery, current injecting drug use, smoking and genitourinary tract infection).

Between 1996 and 2003, of 247 births (243 live births) four children have been confirmed HIV positive, giving an overall confirmed transmission rate of 1.6%. In the developed world vertical transmission of HIV, where the mother's HIV status is known before delivery, is fortunately an uncommon event. When transmissions occur careful examination of the case is required to ensure that any identified systems failures are addressed and lessons learned are used to prevent future infections. Of the four confirmed infections, two occurred in utero and two peripartum. In the case of the in utero infections, antiretroviral therapy was not initiated until 32 and 33 weeks (one because of physician decision and one because of refusal on the part of the woman to take antiretroviral therapy). The recent audit of perinatal transmission of HIV[117] identified that in a number of cases therapy was not started until 32 weeks as the 2001 BHIVA guidelines recommended that therapy be started "by 32 weeks". Of note the 2008 British guidelines recommend that therapy be initiated between 20 and 28 weeks and that "commencing before foetal viability may be prudent".

Complex social circumstances, difficulties engaging in care and adhering to antiretroviral therapy were contributory factors in three of the four cases of MTCT. At present, at the GUIDE clinic, there are two Liaison Nurses (one for non-national patients and one for injecting drug users) who work closely with HIV positive pregnant women and Liaison Midwives in infection and substance abuse to facilitate women's adherence with

recommendations and improve their understanding of the rationale for the recommendations in a culturally appropriate way.

In one of the peripartum transmissions the membranes were ruptured for 24 hours before delivery. The risk of transmission increases the longer the membranes are ruptured[37]. Since this case, triple neonatal antiretroviral therapy has been recommended in circumstances where the membranes are ruptured for more than 12 hours.

Maternal follow up at 2 years post delivery has not changed significantly since the introduction of antenatal screening. Potential factors that could influence maternal attendance following delivery include inability to travel long distances to the GUIDE clinic or unwillingness to engage in care following a traumatic antenatal diagnosis of HIV. Furthermore the Citizenship Referendum, 2004, changed the right to citizenship in Ireland such that an individual was no longer entitled to citizenship of Ireland on the basis of being born on the island of Ireland. The Irish Born Child scheme (IBC 2005) gave parents of children born in Ireland prior to 2005 the option to apply for leave to remain for an initial period of 2 years (with renewal in 2007). There has been an overall decrease in the numbers of people applying for refugee status since 2002 (see figure 3.4) and a reduction in the number of first pregnancies in non-national HIV infected women at GUIDE.

It is interesting to note the number of subsequent pregnancies in the last number of years (see table 3.16). It is a testament to the success of antiretroviral therapy in ensuring disease free survival and reducing vertical transmission of HIV that HIV infected women are able to embark on more than one pregnancy. The challenge for physicians caring for women having more than one pregnancy, especially when starting and stopping antiretroviral therapy more than once, lies in ensuring that repeated courses of temporary antiretroviral therapy do not lead to antiretroviral resistance with attendant implications for

future maternal antiretroviral options.

Chapter 4

Emergence of antiretroviral resistance in HIV-1 infected women exposed to temporary combination antiretroviral therapy in pregnancy.

4.1 Introduction

4.2 Methods

4.2 a Patients

4.2 b Clinical protocol

4.2 c Laboratory methods

4.2 d Allocation of subtype and interpretation of mutations

4.2 e Data collection and statistical methods

4.3 Results

4.4 Discussion

4.1 Introduction

Published guidelines for the management of HIV-1 in pregnancy offer a strategy of temporary HAART to reduce MTCT of HIV-1[53 61 62]. The impact of this strategy on the emergence of genotypic resistance and future ART response is unknown. Emergence of genotypic resistance may also pose a risk for MTCT of resistant virus. This study was designed to ascertain the incidence of antiretroviral resistance following a temporary course of HAART in pregnancy. It was hypothesised that there would be a low risk of antiretroviral resistance developing in the presence of good virological control with three antiretroviral drugs.

4.2 Methods

4.2 a Patients

From the cohort of pregnant HIV-1 infected women attending the GUIDE clinic, this study included all those asymptomatic HIV infection with a pre-treatment CD4 count of >300 x 10^6/L who initiated HAART in pregnancy and discontinued post partum, in accordance with the Irish guidelines for the management of HIV-1 in pregnancy[53].

4.2 b Clinical protocol

All women initiating HAART in pregnancy were counseled about therapy adherence with reinforcement at each visit (FL). Women were asked to contact the clinic if they experienced medication difficulties and not to stop therapy until review. Antiretroviral drugs were dispensed from the dedicated HIV pharmacy within the GUIDE clinic. Attendance records were maintained and non-attendees were contacted and another appointment scheduled (FL). Women were seen two weekly for the first month of therapy and four weekly thereafter.

Nevirapine is a nucleoside reverse transcriptase inhibitor. It has a longer half life than the nucleoside reverse transcriptase inhibitors (NRTIs). Furthermore nevirapine has a low genetic barrier to resistance and monotherapy is associated with a high incidence of resistance[100 101 102 103]. In recognition of these factors pregnant women taking temporary regimens that included nevirapine were asked to stop nevirapine immediately post-partum and continue the NRTI backbone for five days. To facilitate this process, women were given separate "post partum packs" (containing five days of the NRTI backbone) at their last visit to the GUIDE clinic before delivery (circa 36 weeks gestation). Women were asked to disregard the rest of their antiretroviral therapy following delivery and just take the medication in the "post partum pack". Furthermore the importance of staggered therapy

cessation was highlighted in communication with Obstetric colleagues for women taking nevirapine containing regimens.

Post-partum women were asked to return to the clinic as close as possible to six weeks following treatment cessation for genotypic resistance testing. Six week assessment post treatment cessation was chosen to allow for some virological rebound in the absence of antiretroviral pressure and to optimise identification of mutations before reversion to wild type virus. Where resistance mutations were identified on post partum samples, pre-treatment samples were examined to ascertain if the mutations were present at baseline.

4.2 c Laboratory methods

Immunological parameters

All CD4 counts were processed in the Immunology Laboratory, St. James's Hospital, Dublin as part of standard care.

Virological parameters

All virological samples were processed at the National Virus Reference Laboratory, University College Dublin, Dublin as part of standard care. Plasma HIV-1 RNA was quantified using VERSANT HIV-1 bDNA 3.0 (Bayer, Berkeley, California, USA, limit of detection 50 copies/ml) or Amplicor HIV-1 Monitor v1.5 (Roche Molecular Systems, Branchburg, New Jersey, USA, limit of detection 400 copies/ml).

Genotypic antiretroviral resistance testing

Samples for post partum resistance testing were taken at the GUIDE clinic with routine bloods at the time of the first clinic visit following delivery. The plasma was separated in the microbiology laboratory at St. James's and transported with routine clinical specimens to the National Virus Reference Laboratory, University College Dublin, Dublin. Antiretroviral resistance testing was performed using the Bayer/VGI TruGene HIV-1 Genotyping Kit. This kit is licensed for use on plasma samples with a HIV viral load of at least 1000 copies/ml. For the purposes of this study a HIV viral load of 500 copies/ml or greater was chosen.

Assay Principle

All assay procedures were performed according to the manufacturer's instructions. This assay provides automated DNA sequencing of the entire protease (PR) gene and the majority of the reverse transcriptase (RT) gene (codons 1 – 246) from a 1.3-kb fragment of copy DNA (cDNA) of the HIV-1 *pol* gene. It examines the sequence from the dominant viral population and mutations must be present in at least 20% of the population sequenced in order to be identified. There are five major steps in the assay.

1. **Extraction** of nucleic acid from plasma samples using the QIAGEN viral RNA extraction kit (QIAGEN, Hilden, Germany).
2. **Reverse transcription** of target RNA to generate complimentary DNA (cDNA) using RT-PCR amplification of target cDNA using HIV-1 specific primers
3. Coupled **amplification and sequencing** (CLIP^{TM} sequencing) of the PCR amplicons using HIV-1 specific primers
4. **Separation** of the CLIP^{TM} sequencing reactions by electrophoresis on a polyacrylamide gel, and detection by laser-induced fluorescence
5. **Analysis** of the forward and reverse CLIP^{TM} sequences using the OpenGene DNA System Software.

1. RNA Extraction (QIAamp Viral RNA Mini Kit, QIAGEN, Hilden, Germany)

- Centrifuge 140µl of plasma at maximum speed at 4°C for 1 hour
- Remove supernatant
- Resuspend pellet in 140µl elution buffer (AVE) and vortex
- Add 14µl proteinase K and 1µl RNasin, vortex and incubate at 55°C for 30 minutes
- Add 560µl lysis buffer (AVL) to tubes , vortex and incubate at room temperature for 10 minutes
- Add 560µl 100% ethanol and vortex
- Add 630µl to the Qiagen mini spin column and centrifuge at 8000 rpm for 1 minute and discard flow-through
- Add the remaining solution in each tube to the Qiagen mini spin column and centrifuge at 8000 rpm for 1 minute and discard flow-through
- Change collection tube
- Add 500µL buffer AW1 to the Qiagen mini spin column and centrifuge at 8000 rpm for 1 minute and discard flow-through
- Add 500µL buffer AW2 to the Qiagen mini spin column and centrifuge at 8000 rpm for 1 minute and discard flow-through
- Change collection tube
- Centrifuge the Qiagen mini spin column at 14, 000 rpm for 1 minute, discard flow through
- Change collection tube
- Elute RNA from Qiagen mini spin column in 60µL elution buffer (AVE) by centrifugation at 8,000 rpm for 1 minute
- The extracted RNA is placed on ice and used immediately or stored at -80°C

2. Reverse Transcription and PCR amplification

The reverse transcription and PCR amplification are done in the same reaction tube. The reverse transcription reaction is performed with genetically modified Murine Leukemia Virus reverse transcriptase (to convert the extracted RNA to complimentary DNA). The amplification reaction is performed with a thermostable DNA polymerase.

- Thaw TruGene reagents (excluding enzymes) at room temperature
- Vortex each component (except enzymes) for 5 seconds, microcentrifuge for 5 seconds and place on ice. Remove enzymes from freezer immediately prior to use
- Label two 0.5ml tubes for master mix 1 and 2 and eight 0.2ml individual thin wall PCR tubes for each specimen and control. Place the PCR tubes in a rack on ice
- Prepare RT PCR master mix 1 by adding the following in the order below

Master Mix 1		
	1X	8X
RT-PCR primers	6.75μl	54μl
dNTP solution	1.7μl	13.5μl
DTT solution	1.1μl	9.0μl
RNase inhibitor	0.56μl	4.5μl

- Vortex the mix and aliquot 9μl into the bottom of each of the 0.2ml tubes on ice. Close the lids
- Prepare Master Mix 2 by adding the following reagents in the order below

Master Mix 2		
	1X	8X
RT-PCR buffer	11.25μl	90μl
RNase-Inhibitor	0.56μl	4.5μl

RT Enzyme	1.1μl	9.0μl
DNA Polymerase	2.8μl	22.5μl

- Vortex briefly and keep on ice
- Take the ice bucket containing the 8 tubes (containing master mix 1) and the tube containing the master mix 2 to a separate work area
- Keeping the tubes on ice add 17μl of each of the extracted RNA samples the positive control and the negative control to the bottom of the respective PCR tubes containing master mix 1
- Mix by pipetting up and down making sure to change tips between each addition. Only open one tube at a time
- Check that the tube caps are closed before proceeding
- The PCR tubes, containing sample and master mix 1 are moved to a separate area the RT-PCR cycle program

RT-PCR cycle program			
1 cycle of	20 cycles of	17 cycles of	1 cycle of
90°C for 2'	94°C for 0.5'	94°C for 0.5'	68°C for 7'
50°C for 60'	57°C for 0.5'	60°C for 0.5'	4°C hold
94°C for 2'	68°C for 2'	68°C for 2.5'	

All ramp times at 1°C/second
' = 1 minute

- Preheat the DNA Engine to 90°C and press PAUSE
- Place the tubes in the DNA Engine and press PROCEED. Cycling will begin with 2 mins at 90°C and one hour to 50°C

- After 5 mins at 50°C, pause the thermocycler. Add 14μl of RT-PCR Master Mix 2 to the bottom of each tube and mix by pipetting up and down. Change the pipette tube after the addition of Master Mix 2 to each tube. Keep the tubes in the block while adding the mix. Close the lid. Continue the cycling programme

3. CLIP™ sequencing reaction

12 PCR tubes are required for each specimen and control. A 96 well plate containing 0.2ml thin walled tubes specific to the thermocycler is set up as follows.

	Protease				RT beginning				RT middle			
Sample 1	A	C	T	G	A	C	T	G	A	C	T	G
Sample 2	A	C	T	G	A	C	T	G	A	C	T	G
Sample 3	A	C	T	G	A	C	T	G	A	C	T	G
Sample 4	A	C	T	G	A	C	T	G	A	C	T	G
Sample 5	A	C	T	G	A	C	T	G	A	C	T	G
Sample 6	A	C	T	G	A	C	T	G	A	C	T	G
Pos control	A	C	T	G	A	C	T	G	A	C	T	G
Neg control	A	C	T	G	A	C	T	G	A	C	T	G

- Transfer 7μl of the appropriate CLIP™ terminator mix (A,C,T or G) provided in the TruGene Kit to the bottom of the respective PCR tube
- Label eight 0.5ml tubes one for each specimen and control
- Prepare the CLIP™ master mix in a 1.5ml microcentrifuge tube by adding the reagents in the order listed below. Keep the CLIP ENZ on ice and add last.
- Vortex the CLIP™ mastermix before use

CLIP™ Master Mix		
	1X	8X
Molecular grade H2O	59.4μl	475μl
CLIP™ Buffer	15μl	120μl
CLIP™ ENZ	2.8μl	22.5μl

- Aliquot 71μl of this Master mix into the eight labelled tubes. Keep on ice

CLIP™ sequencing of the amplified DNA

- Remove the tubes containing the RT-PCR product from the thermocycler. Vortex and place on ice
- Using filtered pipette tips, add 4μl of the RT-PCR product into the appropriately labeled tube containing the CLIP™ master mix: the RT-PCR product from sample 1 goes into tube 1, RT-PCR product from sample 2 goes into tube 2 and so on
- Vortex the tubes for 5 seconds and store on ice
- From this diluted RT-PCR/Clip mixture aliquot 5μl into each of the 12 termination mixes for each sample and control prepared in step 5.18. Add the 5μl to the side of the tube at an angle, without touching the terminator mix in the bottom of the well to avoid contamination. Close the tube caps and mix the contents by tapping gently on the bench

CLIP™ sequencing cycle program		
1 cycle of	30 cycles of	1 cycle of
94°C for 5'	94°C for 20 secs	70°C for 5'
	56°C for 20 secs	4°C hold
	70°C for 1.5'	

All ramp times at 1°C/second

- Pre-heat the thermocycler to 94°C
- Firmly tap the plate on the bench to collect the reagents to the bottom of the wells
- Place the plate onto the DNA Engine plate holder and continue with the CLIP programme

- When the cycling reaction is complete remove the plate from the thermocycler and move to the sequencing area
- Add 14μl of vortexed Stop Solution to each well
- Mix the reactions thoroughly by tapping the plate on the bench to collect the reagent at the bottom of the well
- Denature the samples by placing the plate in the thermocycler at 85°C for 3mins
- Keep the samples on ice until ready to load onto the MicroCel 500 cassette. The CLIP™ sequencing samples can be stored at 2-8°C overnight

4. Separation of the CLIP sequences by electrophoresis

- Take the SureFill 6% sequencing Gel Cartridge from the fridge and equilibrate to room temperature
- Turn on the computer and start the GeneObjects software. Refer to the TRUGENE HIV-1 Module Guide for instructions on setting up and initiating a sequencing run
- Once the computer is on the Long Read Tower sequencer may be turned on.
- Use the following Sequencer Control settings

Gel Temperature	60°C
Gel Voltage	2000 V
Laser Power	50%
Run Clock (sampling interval)	0.5sec
Run Clock (Run duration)	50mins

- To assemble a MicroCel 500 cassette, insert comb at the top of the plate and secure with clips provided. Ensure that two wells are positioned in every lane

- Open the drawer of the toaster and insert the splash guard into the bottom of the fill fixture unit of the toaster
- Attach the SureFill injector to the fill fixture unit and remove any air bubbles by placing a lint tissue to the exit point on the fill fixture unit and squeezing the injector trigger gently until liquid exits in a stream
- Place the assembled MicroCel cassette in the Fill Fixture unit. Tighten the thumbscrew by turning counter-clockwise. Fill MicroCel cassette with acrylamide by gently and evenly squeezing the trigger until the acrylamide fills the cassette and floods the wells
- Close the drawer on the gel toaster unit and press the start button on the gel toaster polymerisation unit cycle. When polymerisation is complete press the reset button and open the drawer
- Remove the clips and comb from the MicroCel cassette taking care not to damage the wells. Wipe off the excess acrylamide from the plate
- Visualize the gel and ensure that it does not contain any bubbles. Make sure the lower area of the plate is clean by wiping with lint tissue
- Place the prepared MicroCel 500 cassette in the Long-Read Tower sequencer with the wells facing outwards and add 1X TBE buffer to fill both upper and lower buffer chambers
- Close the door of the Long-Read Tower sequencer and pre-run the cast gel
- Immediately prior to loading the samples flush out the wells with the 1X TBE buffer to remove residual urea and air bubbles
- Using a pipette load 1.5μl of the first four tubes of a sample (for example ACTG protease) into the first four wells. Close the door for 10-15 seconds
- Change pipette tips

- Load the next 4 tubes from a sample and again close the door for 10-15 seconds. Continue until the gel is completely loaded. Load one complete gel per patient sample
- Place the condensation cover on the upper reservoir and close the sequencer door. Start the run by pressing the RUN button on the front of the Long Read Tower

4.2 d Allocation of subtype and interpretation of mutations

HIV-1 subtype was determined using the web based National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) subtyping algorithm (available at www.ncbi.nlm.nih.gov). Mutations were classified according to the IAS/USA drug resistance mutations group (available at www.iasusa.org) and Guidelines v6.0 (Bayer/VGI). At the time of writing up this for submission (2007) sequences were also submitted to the Stanford University HIV resistance database (available at http://hivdb.stanford.edu/) to determine if there were significant differences in the allocation of subtype and reporting of mutations between 2004 and 2007.

4.2 e Data collection and statistical methods

Demographic information, previous ART exposure, current ART exposure, immunological parameters, virological parameters, HIV-1 subtypes and genotypic resistance results were maintained in a secure excel file. Statistical analysis was performed using SPSS, version 11.

4.3 Results

Fifty women were eligible for inclusion. The median age was 27 years (range 16-32 years). Forty six (92%) were from Sub Saharan Africa. The median pre-treatment CD4 count was 480 x 10^6/L (range 300-1083 x 10^6/L) and HIV viral load was 2689 copies/ml (range 50-34753 copies/ml). Four women (8%) had been exposed to antiretroviral therapy in a previous pregnancy (1 x zidovudine monotherapy; 3 x zidovudine/lamivudine dual therapy).

Of the cohort (n = 50), 39 (78%) reverse transcriptase (RT) sequences and 36 (72%) protease sequences were generated. All the protease sequences generated were in women in whom RT sequences were obtained. In two samples the *pol* gene was not amplifiable and nine samples did not have genotypic resistance testing performed because the post partum HIV viral load was <500 copies/ml. The baseline characteristics of the total cohort, those with sequences and those excluded (because of low viral load or failed amplification) are shown in table 4.1.

Of the 39 women with obtainable post-partum sequences, 28 women initiated zidovudine, lamivudine and nevirapine; ten initiated zidovudine, lamivudine and nelfinavir. One woman initiated didanosine, zidovudine and nevirapine. She had a history of zidovudine and lamivudine dual therapy in a previous pregnancy with the M184V mutation identified post-partum. No changes were made to the NRTI component of any of the initiated regimens. Eight women switched from nevirapine to nelfinavir: 1 rash; 2 rash and abnormal liver enzymes; 5 electively switched from nevirapine to nelfinavir because of concerns for maternal toxicity with nevirapine exposure in pregnancy[80]. These interchanges had no adverse virological or immunological consequences.

The median duration of antiretroviral exposure before delivery was seventy days (range 3 – 114). The median viral load reduction was 1.76 $\log_{10}$ copies/ml (range 0 – 2.74). The viral load was <1000 copies/ml in 31 of 32 (97%) available results at thirty six weeks, one

Table 4.1 Baseline characteristics of women exposed to temporary HAART in pregnancy

	Total cohort (n=50)	Sequence (n=39)	No sequence* (n=11)	p
BL CD4 (x 10^6/L, median, range)	480 (300 – 1082)	428 (300 – 1082)	741 (322 – 1003)	ns
BL VL (copies/ml, median range)	2688 (49 – 34753)	3708	1425 (49 – 6275)	ns
BL VL (log, median, range)	3.42 (1.70 – 4.54)	3.56 (1.70 – 4.54)	3.15 (1.7 – 3.8)	ns
Black African (no., %)	46 (92)	35/39 (90)	11/11 (100)	ns

* 9/11 (82%) had viral loads <500 copies/ml and were therefore not sequenced. It was not possible to generate sequences in 2/11 (viral load 961 and 476 copies/ml)

BL CD4 = baseline CD4

BL VL = baseline HIV viral load

viral load was 12,788 copies/ml. Seven women (18%) had no viral load from 36 weeks gestation: three very late presentation, three delivered before final appointment and one non-attender.

The median time from ART cessation to genotypic resistance testing was forty two days (IQR 57-33).

HIV Subtype

HIV subtype according to the BLAST subtyping algorithm, 2004, is shown in table 4.2. When the sequences were submitted to the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu/) in 200, alterations in subtype allocation were made for 4 of the sequences (see table 4.3).

Mutations

- Reverse Transcriptase gene: Seven primary mutations were detected in the reverse transcriptase segment in 5 of 39 (13%) post-partum sequences – see Table 4.4. All five were ART naïve before this pregnancy. In four women pre-treatment samples did not demonstrate any relevant mutations. In one woman pre-treatment sequencing was unavailable (pre-treatment HIV viral load <500 copies/ml).
- Protease gene: Of the thirty five protease sequences generated six (17%) demonstrated M36I (subtype C (1), G (1), CRFO6 (1), CRFO2_AG (2) and CRFO2_AG/G (1), two demonstrated L10I (5.7%) (subtype G (1), CRFO2_AG (1)) and one demonstrated K20R (2.8%) (subtype C). All women were protease inhibitor naive.

Table 4.2 Subtype distribution in women exposed to temporary HAART in pregnancy cohort, n=39

Subtype	Number	%
C	16	41.0%
CRFO2_AG	9	23.0%
G	5	12.8%
B	3	7.6%
CRFO6	2	5.1%
CRFO2/G	2	5.1%
CRFO5	1	2.5%
D	1	2.5%
Total	39	100%

Table 4.3 Comparison of subtype output from BLAST 2004 and Stanford 2007. 39 sequences submitted, 4 subtypes altered

BLAST 2004	Stanford 2007
CRFO5_DF	Protease: D, RT: B
CRFO2_AG/G	G
CRF06	CRFO2_AG/G
CRF06	CRFO2_AG/G

Table 4.4 Characteristics of the 5 women with primary reverse transcriptase mutations post partum

BL CD4	BL VL	ART	Days ART	Del VL	Mutations	Subtype
428	33,372	ZDV/3TC/NVP	84	73	V106A	CRFO2_AG
300	3708	ZDV/3TC/NVP	56	<400	Y181, G190A, T215S	CRFO2_AG/G
557	83	ZDV/3TC/NVP	26	na	M184V	CRFO6
306	1335	ZDV/3TC/NVP*	95	<50	K101E	CRFO6
542	1056	ZDV/3TC/NVP	13	na	Y181C	G

(BL = baseline, Del = delivery, na = not available)

* Nevirapine was interchanged to nelfinavir after 59 days of therapy because of a change in protocol prompted by concerns for excess maternal morbidity toxicity nevirapine use in pregnancy. A viral load of <50 cpm had been documented before the interchange.

- When the sequences were submitted to the Stanford University HIV Drug Resistance Database in 2006 (http://hivdb.stanford.edu/) no clinically relevant additional mutations were identified. A number of atypical codons and atypical mutations of uncertain significance were identified in the reverse transcriptase gene in eight of the sequences (M230V, K103IK, L234FLPS, M230I, L234LP, K238FL, K238KQ, K65EK, L210FL, T69N). In the protease gene L33F was found in one sequence and M46V was found in another.

Those with no mutations post-partum did not differ from those that did with respect to pre-treatment CD4 count, pre-treatment viral load, duration of ART, pre-delivery viral load or time from treatment cessation to genotypic resistance testing using the Mann Whitney test for two independent samples.

4.4 Discussion

Emergence of significant mutations in women taking temporary ART in pregnancy may jeopardise future maternal HIV care. Furthermore in the event that MTCT occurs the infant may be infected with resistant virus. This study identified five women (13% of the cohort) with primary RT mutations following temporary triple ART. None of these mutations were detectable pre-treatment. We had postulated that these women would be at low-risk for emergence of genotypic resistance given their good immunological parameters and a low median pre-treatment viral load. Statistically those that developed resistance did not differ from those that did not when comparing pre-treatment immunological and virological parameters, duration of therapy and virological response to therapy.

Those with post-partum RT mutations were on the same regimen (zidovudine 300mg twice daily, lamivudine 150mg twice daily and nevirapine 200mg once daily for the first fourteen days of therapy and 200mg twice daily thereafter). In four of the 5 women (90%) the primary RT mutations conferred NNRTI resistance; one woman had 2 NNRTI associated mutations.

Nevirapine has a low genetic barrier for emergence of resistance and therefore nevirapine monotherapy is not advised[120]. Nevirapine is metabolised primarily by the CYP3A4 family of the cytochrome p450 system. Additionally it induces this group of enzymes, effectively inducing its own metabolism. This auto induction decreases the terminal phase plasma half-life from approximately 45 hours after a single dose to approximately 25-30 hours when steady state is achieved after multiple dosing[120]. There are conflicting reports on the steady state pharmacokinetics of nevirapine in pregnancy with some authors reporting unaltered plasma concentrations and a more recent study reporting reduced steady state plasma nevirapine levels in late pregnancy[121 122]. The NRTIs have shorter half-lives. These pharmacokinetic differences may predispose to nevirapine monotherapy when ART is stopped simultaneously. To avoid this, in our study, women continued NRTIs for five days

following nevirapine discontinuation postpartum. Mackie et al adopted the same principle in a study of nine individuals (including two pregnant women) stopping a nevirapine based regimen[123]. This study confirmed the long half life of nevirapine but did not demonstrate any de novo mutations when plasma samples were examined at first viral rebound post treatment interruption While our study did not measure plasma nevirapine levels after nevirapine cessation a possible explanation for the difference in the results could be that the women that developed mutations in our study had detectable nevirapine levels for longer than those in Mackie's study. Gender related or pharmacogenomic differences in nevirapine elimination may predispose to longer periods of nevirapine monotherapy. Gender differences in nevirapine pharmacokinetics have been described but racial differences have not[124]. The viral subtype may play a role in the risk of developing antiretroviral resistance, the majority of the individuals in Mackie's study had subtype B HIV infection while this study group who developed mutations had nonB HIV infection. Women with protease segment mutations were protease inhibitor naïve. The M36I mutation was identified in 17% of the sequences generated. This has previously been described as a polymorphism with high prevalence rates in non-B HIV clades[125] and may predispose to failure of protease inhibitor regimens through emergence of the L90M mutation[126]. The L10I and K20R accessory mutations may have similar implications for protease inhibitor response.

This study may underestimate the number of mutations present following temporary ART in pregnancy. The range of time to performance of post-partum resistance testing is wide with a median of forty two days and one woman not returning until 198 days postpartum. The longer the time to performance of post-partum resistance testing the greater the likelihood that the virus will have reverted to wild type. Furthermore the profile of mutations detected may change in the days and weeks after ART cessation with selection and fading of mutations at different time points[127]. Therefore genotypic resistance testing at more than one time point may be more fruitful.

There are a number of limitations to this study. The small sample size may contribute to the inability to detect differences between those that developed mutations and those that did not. Adherence to therapy was not formally assessed. Previous authors have identified poor adherence rates with ART in pregnancy, citing that teenagers and minority groups had poorer adherence rates versus white women and those on ART before pregnancy[128]. In our cohort forty six women (92%) came from Sub Saharan Africa and all those with post partum mutations came from Sub Saharan Africa. Furthermore all those with mutations were diagnosed with HIV infection through antenatal screening in the index pregnancy. The absence of genotypic resistance data on all women pre-treatment is a further drawback as significant mutations may be present pre treatment. However, the majority of the women came from Sub Saharan Africa where acquired resistant virus is less prevalent than in countries with more widespread antiretroviral use. Resistance may evolve spontaneously and there is conflicting data on the prevalence of naturally occurring mutations within the non-B subtypes of HIV-1. One study found no mutations in a cohort of pregnant women with clade C infection[129] while other workers found more frequent genetic variation at the NNRTI resistance associated positions in naïve C subtypes versus naïve B subtypes[130]. The impact of these sequence changes on subsequent treatment response is unknown but may alter frequency and pattern of drug resistance mutations selected under NNRTI pressure.

Genotypic resistance testing has limitations; it is poorly sensitive and detection of mutations is dependant on 20 - 25% of the prevailing viral population having the mutation present. More sensitive resistance testing methods have been developed but their clinical utility as yet remains undetermined[131].

In conclusion this study identified a significant number of primary mutations in the RT gene in a cohort of women taking nevirapine containing regimens in pregnancy. Women on nelfinavir containing regimens did not have mutations detected but this may be due to small sample size. This study suggests that the strategy of using triple ART in pregnancy

may not protect drugs with known low genetic barriers such as nevirapine. The clinical implications of this remain to be seen. Larger studies of the temporary use of triple ART in pregnancy with drugs with higher genetic barriers than nevirapine are warranted.

Chapter 5

Maternal hepatotoxicity with nevirapine as part of combination antiretroviral therapy in pregnancy.

5.1 Introduction

5.2 Maternal Deaths associated with nevirapine use in pregnancy: case summaries

5.3 Methods

5.3 a Patients included and data collection

5.3 b Statistical methods

5.3 Results

5.4 Discussion

5.1 Introduction

Use of antenatal antiretroviral therapy is integral to any strategy to reduce mother to child transmission of HIV-1. The optimum regimen is that which exerts maximum virological effect with minimum toxicity to the mother and foetus/infant. A combination of zidovudine, lamivudine (together as Combivir®) and nevirapine was the most popular choice for HIV-1 infected women in pregnancy in Ireland up until the spring of 2003. Reasons for this included: low pill burden; good tolerability and documented transplacental passage (thus offering the foetus pre-exposure prophylaxis).

Unfortunately in the spring of 2003 two women died from fulminant hepatitis. Both were taking Combivir® and nevirapine, in pregnancy, to reduce the risk of MTCT. Severe hepatotoxicity is a recognised but uncommon side effect associated with nevirapine exposure.

The two maternal deaths prompted an emergency meeting of adult and paediatric HIV physicians, in Dublin, to discuss the use of nevirapine as part of combination therapy in pregnancy. Both deaths occurred in individuals with good pre-treatment CD4 counts (506 x 10^6/L and 473 x 10^6/L). This coupled with prior reports of severe hepatotoxicity in HIV negative health care workers following occupational needle stick injuries[132], raised concerns that being immune competent increased the risk of severe hepatotoxicity. Therefore it was agreed that all HIV-1 infected pregnant women with pre-treatment CD4 counts of >300 x 10^6/L taking nevirapine be recalled with a view to discontinuing nevirapine. Until the potential risk was quantified nevirapine use in pregnancy was restricted to those with CD4 counts of <300 x 106/L.

Furthermore it was agreed that a retrospective study be undertaken of all women prescribed nevirapine, as part of combination antiretroviral therapy in pregnancy, at three adult HIV clinics in Dublin between October 2000 and February 2003. The purpose of this study was to determine the incidence and identify risk factors for hepatotoxicity in those

with normal baseline liver biochemistry. The author was nominated as the person to identify these women, collate and analyse the data and write the paper with recommendations for use of nevirapine in pregnancy in Ireland.

5.2 *Maternal Deaths associated with nevirapine use in pregnancy: case summaries*

Case 1

A 30-year-old black South African woman was found to be HIV-1 antibody positive through antenatal screening at 28 weeks gestation. At presentation she had a CD4 count of 506 x 10^6/L and HIV viral load of 1895 copies/ml (Chiron, bDNA). Allergy to co-trimoxazole was documented. She was hepatitis A IgG negative, hepatitis B immune and hepatitis C antibody and PCR negative. She received 10 days of Procaine Penicillin for latent treponemal infection (positive syphilis serology (TPPA 1:80), which was also detected on antenatal screening. At 32 weeks of pregnancy she initiated combination antiretroviral therapy with Combivir ® (zidovudine and lamivudine) one tablet twice daily and nevirapine 200mg once daily. Nevirapine was initiated at 200mg once daily for the first 2 weeks and dose escalated to 200mg twice daily thereafter. She reported no symptoms when she was reviewed 2 weeks and 4 weeks after initiating antiretroviral therapy. Clinical examination and liver biochemistry were normal at baseline, 2 weeks and 4 weeks of antiretroviral therapy. Thirty-two days after initiating antiretroviral therapy she presented to the maternity services with a generalised skin rash and oral mucosal ulceration. Liver biochemistry and serum lactate were normal. Foetal assessment necessitated delivery by emergency caesarean section under spinal anaesthesia and a healthy female infant was delivered. Nevirapine was discontinued and Combivir® was continued for five days for pharmacokinetic reasons. Ten days post-partum the mother was noted to have jaundice, fever and abnormal liver biochemistry. Eighteen days post-partum she was transferred to the national liver unit where her admission parameters were: INR 3.9, bilirubin 213 umol/L (1-21 umol/L) and AST 2850 U/L (8-40 U/L). She further deteriorated developing fulminant hepatic failure with grade 3 hepatic encephalopathy. She was listed for urgent liver transplantation which was performed 22 days post-partum. Following graft reperfusion she developed progressive hypotension and despite maximum support expired on the

operating table. Histological examination of her liver confirmed fulminant hepatitis consistent with drug toxicity. Diffuse subendocardial haemorrhage was noted but histological examination of her heart and associated structures was normal.

Case 2

A 31-year-old black South African woman was found to be HIV-1 antibody positive through antenatal screening at 30 weeks gestation. At presentation she had a CD4 count of 473 x 10^6/L and HIV viral load of 724 copies/ml. She was hepatitis B surface antigen and hepatitis C antibody negative. She was commenced on Combivir®, one tablet twice daily and nevirapine 200mg once daily. Liver biochemistry was normal at baseline and at 2 weeks at which time nevirapine was dose escalated. Four weeks after commencing antiretroviral therapy she complained of fatigue, nasal congestion and fever. Clinical examination and liver biochemistry were normal and symptoms resolved within 24 hours. One week later she presented to the maternity services with fever, nausea, vomiting and a generalised skin rash. She was treated with intravenous antibiotics and a caesarean section, under general anaesthetic, was performed 5 days later for foetal distress. Liver enzymes were not checked before delivery but were noted to be abnormal after delivery and antiretroviral therapy was discontinued. However it was subsequently identified that she may have continued to take her own supply of antiretroviral therapy for another 5 days. She was transferred to the national liver unit and on admission: ALT 1005 U/L (1-50 U/L); AST 1276 U/L (8-40 U/L) and serum bilirubin 100 umol/L (1-21 umol/L). Serum lactate was 8.4mmol/L with normal arterial pH. A transjugular liver biopsy showed pan-acinar necrosis and electron microscopy showed pools of free ribosomes in the cytoplasm of hepatocytes. There was no evidence of mitochondrial damage. Haemofiltration was commenced. She developed grade 3 hepatic encephalopathy and met standard criteria for urgent liver transplantation. She was not listed for transplantation due to the concern that she had multi-organ drug toxicity but was treated with Molecular Adsorption Recycling

System (MARS) haemofiltration[133]. Despite this she continued to deteriorate with development of hypotension, seizures and renal failure. She died 7 days after admission to the liver unit. Post mortem examination demonstrated acute hepatic necrosis.

5.3 Methods

5.3 a Patients included and data collection

The author identified women who initiated nevirapine based regimens in pregnancy from the overall cohort of HIV infected pregnant women at the GUIDE clinic as outlined in chapter 2. In the other two centres women who initiated a nevirapine containing regimen in pregnancy were identified on reviewing dedicated HIV pharmacy records of all nevirapine prescriptions and cross referencing these with known pregnancies between October 2000 and February 2003. Case notes, laboratory results and pharmacy records were reviewed to extract data.

All toxicities experienced were graded according to the Division of AIDS (DAIDS) toxicity guidelines for adults (available at http://rcc.tech-res-intl.com/tox_tables.htm).

Women with abnormal baseline liver enzymes (n = 7) were excluded from the analysis as this study sought to determine the risks for hepatotoxicity in women with normal liver biochemistry at baseline. Women with incomplete liver biochemistry data (less than pre-treatment and at least two on treatment values) were also excluded from this analysis (n=31).

5.3 b Statistical methods

Statistical analysis was carried out using STATA V8.0.

Women who were included in this analysis were compared to those that were excluded.

Of those included, individuals with serious hepatotoxicity (grade 3 or 4) were compared to those with no hepatotoxicity or grade 1 or 2 hepatotoxicity. Medians and ranges were computed for each continuous variable. All continuous variables were analysed using the

Mann Whitney U test. Categorical variables were analysed using Chi-squared tests. Statistical significance was set at p=0.05. Multivariate analyses were planned if more than one continuous or categorical variable had a significance level of 0.10.

5.3 Results

123 women who initiated nevirapine as part of combination antiretroviral therapy in pregnancy between October 2000 and February 2003 were identified. The baseline demographic and clinical characteristics are shown in Table 5.1.

The most frequently prescribed NRTI backbone was zidovudine plus lamivudine (fixed dose combination formulation, Combivir® one table twice daily) coupled with nevirapine (n= 119). Antiretroviral regimens and changes made are shown in Table 5.2.

There was one intrauterine death at 37 weeks gestation in a woman co-infected with HIV and Hepatitis B. At 32 weeks gestation, four weeks after initiating Combivir® and nevirapine, she presented with symptoms and signs consistent with cholestasis of pregnancy. There was no evidence of a rash or mucosal ulceration. Liver enzymes were abnormal and nevirapine was stopped and interchanged to nelfinavir. Unfortunately despite numerous efforts to contact her by the author she defaulted from care until she presented to the obstetric services at 37 weeks with reduced foetal movements. An intrauterine death was diagnosed at that time.

There was one documented vertical transmission of HIV in the 123 women that initiated a nevirapine containing regimen in pregnancy.

Seven women (5.6%) had a grade 1 liver enzyme abnormality prior to nevirapine initiation and were therefore excluded from the analysis of risk factors for hepatotoxicity. None of these women subsequently developed more severe liver enzyme abnormalities.

In 31 women incomplete data precluded their inclusion in the analysis of risk factors for hepatotoxicity. Those excluded from this analysis did not differ from those included with respect to baseline characteristics (see Table 5.3).

Table 5.1 Demographics and baseline clinical characteristics of the cohort exposed to nevirapine containing regimens in pregnancy(n=123)

Age (median, range, years)	27 (16-39)
Race/ethnicity	
White Caucasian	16
Black African	107
Duration of nevirapine exposure (median, range, days)	75 (3 – 207)
Pre-treatment CD4 (median, range, $x10^6$/L)	324 (14-1082)
Pre-treatment HIV viral load (median, range, copies/ml)	6600 (50-406 x 10^3)
HCV antibody positive (%)	8 (6.5%)
HBV surface antigen positive (%)	2 (1.6%)

Table 5.2 Antiretroviral regimens in the cohort exposed to nevirapine containing regimens in pregnancy(n=123)

Initial regimen	No.	Changed regimen	Indication	No.	comment
ZDV, 3TC, EFV	1	ZDV, 3TC, NVP	Teratogeniticy risk		
ZDV, 3TC, NLF	1	DDI, D4T, NVP	Viral escape		No toxicity
D4T, 3TC, NVP	1	ZDV, 3TC, NVP	To include ZDV		No toxicity
DDI, ZDV, NVP	1	No change	Previous M184V		No toxicity
ZDV, 3TC, NVP	119				
		DDI, D4T, NLF	Viral escape	2	No toxicity
			Hepatotoxicity	7	
			Rash	6	
			Elective withdrawal*	4	
			Other	2	

* Women with pre-treatment CD4 >300 x 10^6/L were recalled to review nevirapine in light of concerns for severe hepatotoxicity with nevirapine in pregnancy

Table 5.3 Demographics and clinical characteristics of those included in analysis of risk factors for hepatotoxicity versus those excluded

	Included (n=85)	Excluded (n=38*)	p
Age (median, range, years)	27 (16-39)	28 (18-39)	NS
Black African (%)	75 (88)	32 (84)	NS
HCV a/body positive (%)	6 (7)	2 (5.7)	NS
HBV sAg positive (%)	2 (2.3)	0 (0)	NS
Baseline CD4 (median, range)	322 (14–1082)	327 (90–1056)	NS
Baseline HIV VL (median, range)	7049 (50–406722)	4235 (50 – 58990)	NS

* Of 38: 7 abnormal baseline liver biochemistry; 31 incomplete data available

Of the 85 women included in the analysis for hepatotoxicity eight (9.4%) developed grade 3 or 4 hepatotoxicity, including two women who died of fulminant hepatitis. Fourteen (16.4%) developed grade 2 hepatotoxicity, seven (8.2%) grade 1 hepatotoxicity and 56 (65.8%) did not experience any hepatotoxicity.

Thirteen (10.5%) women had a skin rash documented. Of these six developed a skin rash but had normal liver biochemistry. Five women with grade 3 or 4 hepatotoxicity; one woman with grade 2 hepatotoxicity and one woman with grade 1 hepatotoxicity also had a skin rash.

Thirteen women (10.5%) discontinued nevirapine during pregnancy because of toxicity; three because of grade 3 or 4 hepatotoxicity with a rash; one because of grade 1 hepatotoxicity with a rash; one because of grade 4 hepatotoxicity with no rash; two because of grade 2 hepatotoxicity with no rash and six because of rash with normal liver biochemistry. An additional four women had nevirapine electively withdrawn because of concerns about excessive hepatotoxicity associated with nevirapine use in pregnancy following the two maternal deaths from fulminant hepatitis.

Risk factors for hepatotoxicity

A univariate analysis was performed to identify risk factors associated with hepatotoxicity among variables collected. Relative risks were calculated. Results of the univariate analysis are shown in table 5.4. Multivariate analysis, to adjust for potential confounders, was planned if the univariate analysis identified statistically significant factors for hepatotoxicity.

Table 5.4 Univariate analysis for risk factors for hepatotoxicity women exposed to nevirapine containing regimens in pregnancy

	RR	95% CI	P value
HIV diagnosis pre-conception	0.8	0.1, 4.0	0.1
Black African	1.0	0.1, 8.3	0.96
Age <27 years (median age)	0.8	0.2, 3.5	0.8
Baseline CD4<250	0.7	0.6, 0.8	0.05

The pre-treatment CD4 count was significantly different between those that had normal or developed grade 1 or 2 hepatotoxicity (n= 77, median = 310 x 10^6/L, 14-1082) versus those that developed grade 3 or 4 hepatotoxicity (n=8, median = 493 x 10^6/L, 303 - 790), p=0.010. There were no significant differences between these groups with respect to age at initiation of antiretroviral therapy, pre-treatment HIV viral load, gestational age at initiation of antiretroviral therapy or duration of exposure to antiretroviral therapy.

5.4 Discussion

This study was prompted by two maternal deaths from fulminant hepatitis associated with nevirapine use in pregnancy. To date there have been four reported cases of fulminant hepatitis culminating in maternal death associated with nevirapine as part of combination antiretroviral therapy in pregnancy, including the two from this cohort[80 134 135]. All four cases occurred in women of black race, within four weeks of nevirapine initiation and in three of the four cases the pre-treatment CD4 count was >250 x 10^6/L. All women had normal liver biochemistry prior to initiation of antiretroviral therapy and none of the women were co-infected with hepatitis B or C.

Similar cases of nevirapine associated hepatitis, two of which were life-threatening, one requiring liver transplantation, have previously been reported in HIV negative health care workers (n=30) receiving nevirapine as part of post-exposure prophylaxis[136].

Both women in this cohort were delivered by emergency Caesarean section for foetal distress; the first case was done under spinal anaesthesia and the second case under general anaesthesia. Hepatitis has been reported following the use of general anaesthetic agents, particularly halothane[137 138]. Halothane may have contributed to but is unlikely to be solely responsible for the hepatitis in this case as the clinical scenario prior to the general anaesthetic and temporal relation to initiation of nevirapine are consistent with other reported cases.

Hepatotoxicity associated with antiretrovirals may be multifactorial in aetiology. In particular mitochondrial toxicity manifesting as hepatic steatosis is associated with the nucleoside reverse transcriptase inhibitors through inhibition of mitochondrial DNA polymerase γ. Indeed there have been concerns for the use of the nucleoside reverse transcriptase inhibitors, didanosine and stavudine, in pregnancy with severe morbidity and mortality from lactic acidosis and pancreatitis reported[75]. In the second case of fulminant hepatitis mitochondrial toxicity related to nucleoside analogues was considered because of

documented hyperlactemia (lactate = 8.4mmol/L) at the time of admission to the liver unit. However electron microscopy of liver tissue from the transjugular liver biopsy did not demonstrate any evidence of mitochondrial damage.

Hepatotoxicity is a recognised potential side effect of nevirapine therapy. In this cohort abnormal liver biochemistry and hepatotoxicity were most likely related to nevirapine and, with the exception of the two maternal deaths, when nevirapine was stopped or interchanged to an alternative agent liver biochemistry returned to normal. Previously identified potential risk factors for hepatotoxicity with nevirapine include co-infection with either hepatitis B or C[139] ; female sex and black African race[140] and higher pre-treatment CD4 counts[79]. In women, a pre-treatment CD4 count of >250 x 10^6/L, may increase the risk for hepatotoxicity. In our cohort, women who experienced grade 3 and grade 4 hepatotoxicity had higher pre-treatment CD4 counts compared to those with no hepatotoxicity or those with less severe hepatotoxicity (grade 1 or 2). As has been reported in other cohorts[134] all of the women with grade 3 or 4 hepatotoxicity had pre-treatment CD4 counts of greater than 250 x 10^6/L (median 494 x 10^6/L, range 303 – 790). Severe hypersensitivity to nevirapine can manifest as fulminant hepatitis or as a systemic syndrome with predominant cutaneous manifestations referred to as hypersensitivity syndrome or drug rash with eosinophilia and systemic symptoms. It may be that female gender, higher baseline CD4 count, black race and pregnancy predispose to such severe hypersensitivity. Previous reports suggest that severe rash is more likely in females[141]. In our cohort thirteen women (10.5%) developed a generalised skin rash whilst on nevirapine in pregnancy. Six of these did not have associated liver biochemistry abnormalities but one of the six required admission for observation with the rash. The retrospective nature of the study made it difficult to grade the severity of the rash as it was not always clearly documented in the medical notes.

The physiological changes of pregnancy may alter the pharmacokinetic handling of nevirapine and contribute to more toxicity. There are conflicting reports on the steady state pharmacokinetics of nevirapine in pregnancy with some authors reporting unaltered plasma concentrations and a more recent study reporting reduced steady state plasma nevirapine levels in late pregnancy[121 122]. Furthermore there are conflicting reports as to whether or not toxicity with nevirapine is related to plasma concentration[142 143].

Since we first reported the two maternal deaths there have been numerous reports from many groups seeking to determine the risk of serious toxicity associated with nevirapine exposure in pregnancy[144 145]. There have been inconsistencies in these reports. There are many potential factors that explain these inconsistencies. Firstly serious toxicity from nevirapine is an uncommon event and therefore small sample size may preclude elucidation of the true risk. Additionally the reports come from many disparate populations. Different populations may have different inherent risks for serious nevirapine toxicity. Associations between nevirapine hypersensitivity and HLA type have been reported but the clinical utility of HLA testing in this context is undetermined[146 147]. Many of the studies in the literature, including our study, are retrospective in nature and as such are subject to shortcomings. Firstly there is a potential to bias towards selection of complicated cases. However, in our study the number of pregnancies where nevirapine was used is likely to be accurate, since in one centre the data on pregnancies was already being collected and in the other two centres detailed review of all nevirapine prescriptions through the dedicated HIV pharmacy, cross referencing with known pregnancies identified all women. Secondly the data has been collected from review of case notes, laboratory and pharmacy records. In our study, as there was not a predefined study protocol for data collection much information is unavailable. This is reflected in the exclusion of 31 of the women from the analysis because of incomplete data on liver biochemistry. Of note the 31 women were similar to the rest of the cohort with respect to demographics and baseline clinical

parameters. Furthermore it is likely that they did not experience significant toxicity as this would have been captured in the medical records.

The absence of a control group of non-pregnant women in the available studies makes it difficult to determine the potential contribution of pregnancy itself to toxicity with nevirapine.

Serious toxicity from nevirapine, both in pregnancy and the general HIV population, is fortunately an uncommon event. Risk factors that increase the likelihood of developing serious toxicity appear to vary in different populations studied. In the context of nevirapine use in pregnancy in Ireland this study has demonstrated that serious hepatotoxicity is more likely in those with higher pre-treatment CD4 counts. This experience is reflected in the Irish guidelines for management of HIV in pregnancy where nevirapine, as part of combination antiretroviral therapy in pregnancy, is no longer recommended when the primary indication for therapy is reduction of MTCT of HIV and not maternal immune status.

Importantly the information from this data cannot be extrapolated to the use of single dose nevirapine (which is often the only intervention available in resource limited settings) where there have been no similar reports of hepatotoxicity.

Chapter 6

National Guidelines for the management of HIV-1 infection in pregnancy in Ireland, 2008

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- requiring antiretroviral therapy
- not requiring antiretroviral therapy

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premature labour

On effective HAART

- <34/40
- >34/40

Failing HAART or not on any antiretroviral therapy

- <34/40
- >34/40

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- <34/40
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Conclusion

6.1 Introduction

Multidisciplinary management of HIV-1 in pregnancy has reduced the mother-to-child-transmission (MTCT) rate from 25-30% to <2% in the developed world, including Ireland. In Ireland, in the 9 year period between January 1999 and December 2007, there were 874 live births in HIV infected mothers, including 9 twin pregnancies. The vertical transmission rate was 1% [95% CI 0.98 – 1.019][148], which compares very favourably with international data.

The most recent report on voluntary antenatal HIV testing indicates that between 2002 and 2006 uptake rates for antenatal HIV screening have consistently been >90%. In 2006, of 53,802 women offered antenatal HIV testing, 51,144 proceeded with testing giving an uptake rate of 93.5%. 116 were identified as HIV positive and of these, 36 (31%) were previously unaware of their HIV status, giving a HIV prevalence of 0.23% in pregnant women[114]. The prevalence is higher in the HSE Eastern region (0.33%) compared to the rest of the country (0.14%). The successful nationwide implementation of antenatal screening for HIV coupled with the policy of geographic dispersal of asylum seekers adopted in 2000 has resulted in HIV infected women delivering in obstetric units throughout the country. There is a need for clear and accessible, nationally relevant guidelines for the management of HIV in pregnancy so that the current success in preventing vertical transmission can be maintained.

These guidelines replace previously published guidelines for the management of HIV in pregnancy in Ireland[53], and offer a broad management outline for HIV positive pregnant women. Ultimately, each woman must be assessed by a multidisciplinary team and an individualised plan determined.

6.2 Methods

There are few randomised controlled trials to guide the management of HIV in pregnancy. Nonetheless both antiretroviral therapy prescribing and obstetric management of HIV infected women have changed significantly since the publication of the pivotal PACTG 076 study in 1994[42]. Many of these changes have been informed by observational data coupled with international expert opinion.

These guidelines are based on currently available peer-reviewed international data (both published and conference data) and other published guidelines for the management of HIV in pregnancy. The experience gained and lessons learned from the management of HIV infection in pregnancy in Ireland are used to provide nationally relevant management options. Various scenarios have been drawn up to reflect the diversity of clinical presentations.

These draft guidelines have been written by the author (FL) in consultation with adult and paediatric HIV physicians and obstetricians (Professor Fiona Mulcahy, Professor Karina Butler and Professor Fionnuala McAuliffe). Following a period of consultation where the draft guidelines are made available for review and comment to all obstetricians and HIV physicians the final agreed guidelines will be submitted for publication.

6.3 Principles of management

6.3 a Antenatal screening

In Ireland the opt-out antenatal HIV screening programme began in April 1999 with more extensive national coverage in 2000. This programme recommends that all women booking for antenatal care be offered a HIV test in recognition of the significant impact that interventions in pregnancy have in reducing mother to child transmission of HIV. The most

recent report on uptake of antenatal screening indicates that uptake rates have been >90% between 2002 and 2006.

- When a woman tests positive for HIV through antenatal screening there should be a clear mechanism for informing women of their test result in a timely manner and for onward referral to adult HIV services.
- Women who decline testing should be made aware of the benefits of knowing their HIV status and encouraged to proceed with testing.
- Women who decline testing for themselves should be offered, and encouraged to avail of, HIV testing of their infant following delivery and when the infant is three months of age.
- Women with ongoing risk factors for acquisition of HIV (active injecting drug use, known HIV infected partner or partner with identified risks for HIV infection and unknown HIV status) and an initial negative test should be made aware of the symptoms of HIV seroconversion and offered repeat testing throughout pregnancy.
- Women who book for antenatal care at a gestational age greater than 24 weeks should have their HIV test performed urgently with a clear mechanism for management of positive results. In the event of a positive test refer the woman to adult HIV services as soon as possible, without waiting for the results of a confirmatory HIV test.
- Women presenting unbooked in labour should have a HIV test performed urgently with availability of results within 24 hours (in larger obstetric units availability of a result within hours can be anticipated but this may be unrealistic in peripheral units). FDA and CE (Conformité Européene) point of care HIV tests are now commercially available and offer good sensitivity and specificity. Where it is not possible to have rapid laboratory testing for HIV, use of rapid point of care HIV testing should be considered. Reactive point of care tests must be followed up with confirmatory serum tests but offer sensitivities and specificities that allow rapid

clinical decision making in the unbooked woman presenting in labour. Of note there have been recent reports of unacceptably high false positive rates with one of the approved oral fluid rapid tests (OraSure Advance® Rapid HIV-1/2 antibody test, OraSure Technologies, Bethlehem Pennsylvania)[149]. These false positive rates have not been seen with the approved whole blood finger prick tests.

6.3 b Maternal HIV care

The delivery of HIV care to women in pregnancy requires a multidisciplinary approach to optimise the chances of ensuring good maternal health and a HIV negative infant.

6.3 b (i) Assessment of maternal health

All pregnant HIV infected women should be assessed and managed at a specialist adult HIV service. In the non-pregnant population the need for antiretroviral therapy is guided by the CD4 count and HIV related symptoms. International guidelines for initiation of antiretroviral therapy are available and regularly updated[18 19].

The current US and British guidelines for the management of HIV in pregnancy recommend that the need for maternal antiretroviral therapy should be in line with the guidelines for the non-pregnant population[61 62]. Currently these guidelines identify a need for antiretroviral therapy at a CD4 count of $<350 \times 10^6$/L with a need for antiretroviral therapy and prophylaxis against opportunistic infections with a CD4 count of $<200 \times 10^6$/L. The physiological changes of pregnancy may predispose to a temporary decline in maternal CD4 count in pregnancy through haemodilution. This phenomenon has been described and reported in cohorts of HIV infected pregnant women in Ireland and Sub Saharan Africa[56 57]. Therefore for some pregnant women with a pre-treatment CD4 count of $<350 \times 10^6$/L there may not be a need for antiretroviral therapy beyond pregnancy. For

women with a pre-treatment CD4 count of <200 x 10^6/L prophylaxis against opportunistic infections should be as per the non-pregnant population.

HIV-1 RNA levels (viral load) are measured at baseline and in response to antiretroviral therapy. There are a number of different assays available which generally correlate but differences in RNA copy number of 0.5 to 1.0 log have been described[150]. Therefore it is important that the same assay be used when determining response to antiretroviral therapy. Where this is not possible the results should be interpreted with caution.

There have been reports that some assays underestimate the HIV viral load in nonB virus subtypes. Where there is discrepancy between the anticipated HIV viral load, CD4 count and clinical status (i.e. undetectable viral load in an untreated patient or low viral load in an individual with low CD4 count or symptomatic disease) in an individual infected with nonB virus, it is advisable to use an alternative assay so that significant viremia is not missed.

Genotypic antiretroviral resistance testing is performed at baseline in all newly diagnosed HIV infected people in Ireland. Between 2002 and 2003, of over 1200 new HIV diagnoses across Europe, the prevalence of acquired HIV resistance was 9.1%[151] supporting this practise. Similarly, in pregnancy, all women should have baseline genotypic resistance testing performed, where possible before initiation of antiretroviral therapy. However where a woman presents at an advanced gestational age initiation of antiretroviral therapy should not be delayed pending results of baseline genotypic resistance testing.

For women failing antiretroviral therapy in pregnancy, genotypic resistance testing is indicated as per the non-pregnant population. Results may need to be sought urgently in order to optimise the chances of achieving virological control before delivery.

Temporary antiretroviral therapy in pregnancy may predispose to development of antiretroviral resistance. In a cohort of women attending the GUIDE clinic 13% developed resistance following temporary exposure to an NNRTI based regimen in pregnancy (see chapter 4)[152]. For women discontinuing antiretroviral therapy after delivery, post partum

resistance testing should be performed around six weeks after delivery to guide future antiretroviral regimens.

6.3 b (ii) Maternal antiretroviral therapy

The single most important determinant of whether or not a woman transmits HIV to her infant is maternal ARV therapy. In the non-pregnant HIV population the hallmarks of effective antiretroviral therapy are complete virological suppression and immunological recovery. There are currently five classes of antiretroviral agents licensed for the treatment of HIV infection: Nucleoside Reverse Transcriptase Inhibitors (NRTIs); Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs); Protease Inhibitors (PIs); Fusion Inhibitors and Integrase Inhibitors (see table 1.3). Highly active antiretroviral therapy (HAART) usually consists of 3 drugs from 2 classes. In individuals initiating antiretroviral therapy for the first time HAART will usually consist of 2 NRTIs plus 1 NNRTI or 1 PI. In pregnancy HIV infected women may require antiretroviral therapy for their own health or solely to reduce the risk of vertical transmission of HIV.

Monotherapy

The seminal breakthrough in the development of strategies for the prevention of MTCT was the PACTG076 study, published in 1994[42]. This randomised controlled trial demonstrated that compared with placebo, oral antenatal zidovudine, intravenous peripartum zidovudine and oral postpartum zidovudine reduced the risk of MTCT by 66% Following the advent of HAART circa 1997 there was a move towards use of more combination antiretroviral therapy in pregnancy with some data suggesting that the more complex the regimen the lower the risk of transmission. However recently published data on over 5000 mother-child pairs in the UK and Ireland did not demonstrate a difference between zidovudine monotherapy coupled with an elective caesarean section and HAART in preventing MTCT[118] (see table 5.1). The other concern surrounding the use of

Table 6.1 MTCT UK and Ireland 2000 to 2006, AIDS. 2008 May 11;22(8):973-81

	No (%)
Overall (95% CI 0.9 – 1.5)	16/5151 (1.2)
Any ART for at least 14 days	40/4864 (0.8)
HAART + elective caesarean section	17/2286 (0.7)*
HAART + planned vaginal delivery	4/559 (0.7)*
Zidovudine + elective caesarean section	0/464 (0)*

* p=0.150

zidovudine monotherapy was that monotherapy would increase the risk of evolution of antiretroviral resistance. In selected women with low pre treatment HIV viral loads, the risk of antiretroviral resistance is low when assessed with genotypic population sequencing and more sensitive cloning[106 107]. At the GUIDE clinic between 1997 and 2003, 20 women received antenatal zidovudine monotherapy. Maternal concerns for more complex antiretroviral exposure, difficulty with pill burden and women with very low level pre-treatment viraemia and normal CD4 counts were amongst the reasons cited for opting for zidovudine monotherapy. The recent data from the Institute of child health support the use of zidovudine monotherapy, coupled with elective caesarean section, in selected women with normal pre-treatment CD4 counts and low pre-treatment HIV viral loads.

Nevirapine a non-nucleoside reverse transcriptase inhibitor is the other antiretroviral agent that has shown efficacy in reducing the risk of MTCT when used as a single agent. It rapidly crosses the placenta and has a long half life and has been shown to reduce the risk of MTCT in breastfeeding populations when given as a single dose to the mother in labour and to the neonate within the first 72 hours of life[49 153]. Unfortunately nevirapine has a low genetic barrier to resistance and maternal resistance to nevirapine has been demonstrated in up to 40% of exposed women after a single dose[154]. Therefore single dose nevirapine to the mother and neonate is now reserved as a strategy in resource limited settings where there are limited alternative options for reducing the risk of MTCT. Because nevirapine rapidly crosses the placenta and nevirapine containing regimens have demonstrated rapid virological decay, single dose nevirapine may be considered as an option where women present late in pregnancy and can initiate antiretroviral therapy at the same time and continue until they have virological suppression. Single dose nevirapine may also be considered in women with high HIV viral loads and previously documented NNRTI resistance who present late in pregnancy in an effort to reduce the amount of HIV exposure.

Dual therapy

The use of dual antiretroviral therapy with zidovudine and lamivudine is not recommended because of the potential to induce lamivudine resistance with this strategy[51].

Triple therapy

Since January 2002 the Irish Guidelines for the management of HIV in pregnancy have recommended that all HIV infected pregnant women be offered triple antiretroviral therapy regardless of immunological well being[53]. For women not requiring antiretroviral therapy treatment is discontinued following delivery.

When to start therapy in pregnancy?

Timing of initiation of antiretroviral therapy will depend on maternal well being and obstetric factors. For women requiring antiretroviral therapy for their own health, it may be prudent to wait until after the first trimester to initiate antiretroviral therapy in order to minimise potential teratogenicity. Where the sole indication for antiretroviral therapy is prevention of MTCT, the optimum time for initiation of antiretroviral therapy is that which offers the best chance of avoiding transmission balanced against minimisation of exposure (both in utero and maternal) and toxicity whilst ensuring preservation of future maternal antiretroviral options. Previous Irish guidelines have recommended that antiretroviral therapy be started at approximately 28 weeks in women not requiring antiretroviral therapy for their own health. In the recent report on over 5000 pregnancies in Ireland and the UK, there were 3 transmissions in women with an undetectable HIV viral load at delivery[118]. Interestingly all of these transmissions were in utero and perhaps earlier initiation of antiretroviral therapy would have prevented transmission. This data also demonstrated that the risk of transmission reduced by 0.9% for every additional week of antenatal antiretroviral therapy. Furthermore as the rates of MTCT have declined the proportion of in utero transmissions

has increased[28]. A recent report on risk factors for transmission in Thailand found that initiation of antiretroviral therapy after 31.4 weeks gestation was an independent risk factor for transmission of HIV[155]. The current British guidelines recommend that therapy be initiated between 20 and 28 weeks, suggesting that initiation of therapy before fetal viability may be prudent[62], whilst the current US guidelines suggest initiating antiretroviral therapy any time after 10 to 12 weeks of pregnancy in those requiring therapy solely to reduce the risk of MTCT[61].

Initiation of antiretroviral therapy around fetal viability probably offers the optimum balance between limiting in utero and maternal exposure, limiting the potential for prematurity with more prolonged exposure whilst offering enough protection against in utero and peripartum transmission. In certain circumstances confounding factors such as a history of previous prematurity, twin or higher order pregnancy, maternal infections (e.g. malaria or infectious syphilis) may warrant even earlier initiation of antiretroviral therapy.

What therapy to start in pregnancy?

All HIV infected women should be offered antiretroviral therapy in pregnancy, regardless of gestational age at first presentation or pre-treatment immunological and virological parameters. Furthermore all women should be appraised of the potential risks and unknowns associated with antiretroviral therapy in general and in pregnancy.

For women requiring antiretroviral therapy for their own well being the primary objective is to achieve virological control and immunological recovery. The choice of regimen will be based on maternal CD4 count, HIV viral load, co-morbidities and previous antiretroviral exposure and resistance. Whilst there is limited data around the use of many antiretroviral drugs in pregnancy, particularly newer agents, the absence of data should not preclude use of agents for which there is a clear maternal indication.

In circumstances where the primary indication for therapy is reducing MTCT, the objective is to minimise fetal exposure to virus, whilst maintaining future maternal antiretroviral options.

In general zidovudine should be included in antenatal antiretroviral regimens where possible. Reasons for this include that zidovudine is triphosphorylated to its active form in the placenta and is readily transmitted to the foetus. In the foetus it may act as pre-exposure prophylaxis around the time of labour and delivery, when the risk for exposure to HIV is greatest. Additionally international experience with zidovudine use in pregnancy is greater than for other agents.

At the GUIDE clinic between 1997 and 2003, 149 of 242 (61.5%) pregnancies were delivered vaginally and the option of having a vaginal delivery has been included in both of the previous Irish Guidelines for the management of HIV in pregnancy. Where women would like to have a vaginal delivery triple antiretroviral therapy is recommended.

Where women do not have a requirement for antiretroviral therapy and the pre treatment viral load is <5000 copies, zidovudine monotherapy may be an option. In this situation an elective caesarean section is indicated (for timing of elective caesarean section see 5.3 c (iii)).

The commonest antiretroviral regimen in pregnancy is combivir (fixed dose formulation zidovudine 300mg plus lamivudine 150mg) 1 tablet BD coupled with a boosted protease inhibitor (lopinavir/ritonavir, saquinavir/ritonavir or atazanavir/ritonavir).

In Ireland, the use of nevirapine in combination antiretroviral therapy in pregnancy has been associated with an increased risk of serious hepatotoxicity (including 2 maternal deaths from fulminant hepatitis) with higher pre treatment CD4 counts[156]. The inclusion of nevirapine in antenatal antiretroviral regimens is reserved for women with pre-treatment CD4 counts of <250 x 10^6/L. Nevirapine containing regimens may be particularly useful for women initiating antiretroviral therapy late in pregnancy as it is associated with more rapid viral decay than the protease inhibitors[119]. Furthermore nevirapine has good transplacental

transfer, thus offering pre-exposure prophylaxis to the fetus/neonate which may be particularly useful in preventing peripartum transmissions in women initiating antiretroviral therapy late in pregnancy.

How to stop antiretroviral therapy post partum?

In women taking antiretroviral therapy solely to reduce the risk of MTCT, therapy can be stopped post partum. In general with protease inhibitor based regimens all agents can be stopped at the same time. In women taking nevirapine-based regimens, the long half-life of nevirapine must be taken into consideration and a staggered cessation of the regimen is recommended. The rationale for this approach is that discontinuation of nevirapine immediately post partum and continuing the nucleoside back bone for 7 days will avoid effective nevirapine monotherapy and reduce the risk of developing NNRTI resistance. In the Irish setting, despite this strategy, 5 of 39 (13%) women exposed to temporary ART in pregnancy developed significant resistance post partum[152]. In one report from a non-pregnant population staggered cessation of NNRTI containing regimens has not been associated with the development of significant resistance[123].

In situations where women initiate antiretroviral therapy late in pregnancy, it may be prudent to continue antiretroviral therapy until virological suppression has been achieved to reduce the risk of emergence of resistance.

Adverse effects of antiretroviral therapy in pregnancy

Potential adverse effects associated with antiretroviral therapy to be considered include teratogenicity; toxicity (both fetal/neonatal and maternal) and the effect of antiretroviral therapy in pregnancy on prematurity rates.

The antiretroviral pregnancy registry is an international voluntary, prospective reporting system that tracks congenital abnormalities in children exposed to antiretroviral therapy in

utero. Reports are issued twice a year and are accessible at www.apregistry.com. The most recent report is covers the period January 1st 1989 to January 31st 2007 (see table 6.2)[73].

Previous Irish guidelines have suggested that efavirenz be substituted with an alternative agent if a woman conceives on efavirenz. This recommendation is based on animal studies which have shown in that cynomolgous monkeys in utero exposure to efavirenz is associated with neural tube defects. Additionally, in response to four retrospective case reports of neural tube type defects associated with human in utero exposure to efavirenz, the FDA reclassified efavirenz as a class D drug (i.e. positive evidence of harm) in December 2004. The antiretroviral pregnancy registry has not demonstrated any increased risk of teratogenicity in prospective reporting of in utero first trimester exposure to efavirenz (see table 6.2). Importantly there are now sufficient numbers reported to the registry to give sufficient power to detect a two-fold increased risk of teratogenicity compared to the general population. Given the absence of prospective human data suggesting significant teratogenicity with in utero exposure to efavirenz it may be reasonable to continue efavirenz in some women who conceive whilst on an efavirenz containing regimen. Furthermore efavirenz has a long half life and can persist at detectable levels for up to 3 weeks following discontinuation[74]. Thus in a woman with a 28 day cycle it will be approximately the 15th day of intrauterine life when her period is late. The process of neural tube closure is complete by day 22-24 of intrauterine life and therefore despite stopping efavirenz when a period is missed there will be ongoing in utero exposure to efavirenz that may extend beyond the period of neural tube closure. Additionally stopping efavirenz and substituting with an alternative agent may risk loss of virological control with the potential for evolution of antiretroviral resistance and an increased transmission risk. Furthermore initiating another antiretroviral agent exposes the

Table 6.2 First trimester exposures reported to APR January 1st 1989 to January 31st 2008 (www.apregistry.com)

	First Trimester Exposure	
Regimen	*Defects/Live Births*	*Prevalence (95% confidence interval)*
Zidovudine	87/2808	3.1% (2.5%, 3.8%)
Lamivudine	85/2784	3.1% (2.4%, 3.8%)
Nelfinavir	33/972	3.4% (2.3%, 4.7%)
Nevirapine	18/737	2.4% (1.5%, 3.8%)
Stavudine	19/651	2.9% (1.8%, 4.5%)
Ritonavir	16/628	2.5% (1.5%, 4.1%)
Abacavir	17/512	3.3% (1.9%, 5.3%)
Tenofovir	11/491	2.2% (1.1%, 4.0%)
Efavirenz	10/364	2.7% (1.3%, 5.0%)
Didanosine	16/353	4.5% (2.6%, 7.3%)
Lopinavir	6/328	1.8% (0.7%, 3.9%)
Indinavir	6/272	2.2% (0.8%, 4.7%)

foetus to more drugs, some of which may have an as yet unrecognised teratogenic or detrimental effect.

Fetal/Neonatal toxicity

Nucleoside reverse transcriptase inhibitors (NRTIs) can inhibit mitochondrial DNA polymerase-gamma and the association between NRTI exposure and mitochondrial toxicity is well recognised. In a prospective French perinatal cohort eight cases, including two deaths, have been attributed to mitochondrial toxicity following in utero exposure to NRTIs[81]. A subsequent retrospective review of HIV negative or indeterminate children (n=223) that were exposed to ART and died determined that none of the deaths were likely attributable to mitochondrial dysfunction[82]. Notwithstanding the absence of a clear association in this large series there is evidence that mitochondrial DNA is reduced in infants exposed to NRTIs in utero[83 84 85]. The long term effects of asymptomatic mitochondrial DNA depletion are unknown, highlighting the importance of maintaining these children in long term surveillance.

Tenofovir crosses the placenta and has been associated with alterations in bone biomarkers in rhesus monkeys exposed to tenofovir in utero[157].

Atazanavir has been shown to cross the placenta with therapeutic atazanavir levels in cord blood of exposed infants[86 87]. Atazanavir inhibits the uridine diphosphate-glucuronosyl transferase 1A1 enzyme resulting in an unconjugated hyperbilirubinemia in exposed people. Exposed neonates therefore should be monitored for hyperbilirubinemia but the available evidence to date suggests that dangerous hyperbilirubinemia in exposed neonates is not significant[88 89].

Maternal toxicity

In the developed world current guidelines for the use of antiretroviral therapy in the general adult HIV population do not recommend zidovudine as first line. This change from previous

guidelines reflects increasing data suggesting that zidovudine can cause significant mitochondrial toxicity and lipoatrophy[76 77 78]. Current guidelines recommend tenofovir or abacavir coupled with lamivudine or emtricitabine as first line nucleoside or nucleotide backbones in antiretroviral naïve individuals. Notwithstanding this, zidovudine remains one of the recommended first line agents for the management of HIV in pregnancy. For those taking therapy temporarily in pregnancy toxicity associated with zidovudine may be transient and reversible. For women continuing antiretroviral therapy following delivery a switch from zidovudine to either tenofovir or abacavir after delivery should be considered. More data on the use of tenofovir and abacavir in pregnancy may lead to a change in this stance in future pregnancy guidelines.

As in the non-pregnant population, didanosine and stavudine should be avoided where possible. It is not clear whether or not pregnancy increases the risk of serious toxicity associated with these agents but maternal mortalities have been reported[75].

Fulminant hepatitis is a rare but well recognised side effect associated with nevirapine. It appears that the risk for severe nevirapine induced hepatotoxicity is gender related and increased in women with a pre-treatment CD4 count of >250 x 10^6/L[79]. In the context of pregnancy nevirapine is generally reserved for women requiring HAART for their own health. In Ireland there have been two maternal deaths in Sub-Saharan African women who initiated a nevirapine containing regimen in pregnancy and in a review of all nevirapine containing regimens in pregnancy over a two and a half year period in Dublin more severe hepatotoxicity was seen in women with higher pre-treatment CD4 counts (see Chapter 4)[156].

Following initiation of antiretroviral therapy in pregnancy it is recommended that women are seen twice within the first month of therapy and monthly thereafter to monitor for potential toxicity and assessment of virological (and immunological) response.

Adverse effects pregnancy length

Epidemiological data from the European Collaborative Study indicates an increased incidence of prematurity with a temporal relation to increasing HAART use in pregnancy. Furthermore this risk, in women delivering vaginally or by emergency Caesarean section, is greatest when HAART has been started pre-pregnancy (AOR 4.0, 95% CI 2.26 – 7.08)[91]. A recent report from a single site in the United States found that the risk of prematurity in women receiving combination therapy was greater for those on combination therapy with a protease inhibitor (AOR 1.8, 95% CI 1.1 – 3.0, p=0.03)[93]. In Ireland, at the GUIDE clinic between 1997 and 2003, the median gestational age at delivery in 244 pregnancies was 39 weeks with an overall prematurity rate of 10.2%. The prematurity rates did not differ significantly with more complex antiretroviral therapy (see table 3.12).

Therapeutic drug monitoring in pregnancy

The many physiological changes of pregnancy can significantly change drug handling and therefore pharmacokinetic data in pregnancy must be interpreted with caution as the correlation between plasma total concentrations, plasma free concentrations and intracellular concentrations of drugs may be significantly altered.

Various studies in different pregnant populations have demonstrated varying results with respect to the pharmacokinetics of the protease inhibitors.

Lopinavir/ritonavir is one of the more commonly used protease inhibitors in pregnancy. Differing results have been reported for lopinavir/ritonavir soft gel capsules at standard dosing in pregnancy. Low lopinavir levels in the third trimester have been described in a multicentre study from the Unites States[109], while a single centre report from the United

Kingdom found adequate steady state levels in the third trimester[110]. Pharmacogenomic differences in the handling of lopinavir/ritonavir in the different cohorts may explain these differing results, highlighting the importance of exercising caution in applying results from one population to another.

Saquinavir/ritonavir is also widely used as part of temporary HAART in pregnancy. At the GUIDE clinic the majority of a cohort of 45 women achieved adequate levels of saquinavir in the third trimester with a median HIV viral load of <50copies/ml at standard saquinavir (1g soft gel capsules BD with ritonavir 100mg BD) dosing[111].

Atazanavir/ritonavir levels, at standard dosing (atazanavir 300mg with ritonavir 100mg once daily) were found in one study to be similar in the third trimester of pregnancy as post partum[87] while another series demonstrated lower levels than that reported for the non pregnant population[88].

At present there is a multicentre study of the pharmacokinetics of protease inhibitors in pregnancy ongoing in Irish and United Kingdom sites, when available these results will provide nationally relevant data.

6.3 b (iii) Management of co-morbidities

All pregnant HIV infected women should have serological screening for hepatitis B, hepatitis C and syphilis. All pregnant HIV infected women should have at least one STI screen performed in pregnancy. Syphilis serology should be repeated in the third trimester and a repeat STI screen considered if the history identifies a new risk for acquisition of an STI.

Hepatitis B

Hepatitis B transmission from mother to baby is related to the level of Hepatitis B viraemia and can be prevented by vaccination and administration of hepatitis B immunoglobulin to the neonate. It is not clear whether or not HIV co-infection increases the risk of hepatitis B transmission but HIV co-infection may increase the level of hepatitis B DNA[158159].

When HIV/HBV co-infected surface Ag positive women are commencing combination antiretroviral therapy in pregnancy it is prudent to include two agents with hepatitis B activity (tenofovir with lamivudine or emtricitabine) to reduce the risk of developing resistance to hepatitis B agents. Additionally the use of antiretroviral agents with activity against hepatitis B may reduce the risk of hepatitis B transmission[160]. Children born to women who are hepatitis B surface antigen positive should be administered hepatitis B immunoglobulin and accelerated hepatitis B vaccination.

Hepatitis B surface antigen is a very sensitive marker of viral activity in individuals who are hepatitis B core antibody positive (indicating previous hepatitis B exposure). Therefore it is not necessary to use antiretroviral agents with hepatitis B activity in all women who are hepatitis B core antibody positive but hepatitis b surface antigen negative. Furthermore it is not necessary to administer hepatitis B immunoglobulin and accelerated vaccination to their infants born to women who are hepatitis core antibody positive but surface antigen negative.

Hepatitis B is a notifiable disease and should be reported to public health. Household contacts of people who are Hepatitis B surface antigen positive should be screened and offered hepatitis B vaccination. As part of the national vaccination programme all HIV exposed infants should be vaccinated against Hepatitis B (see 6.3 d (v) Infant vaccination).

Hepatitis C

In hepatitis C monoinfected women vertical transmission rates of up to 6% have been reported[161] [162]. In the monoinfected group up to ~50% of children will go on to spontaneously clear infection. Maternal HIV/HCV co-infection increases the risk of hepatitis C transmission and this risk increases with higher hepatitis C viral loads[163] [164].

Little is known about the mechanism(s) and timing of hepatitis C transmission from mother to child but transmission risk may increase the longer the membranes are ruptured suggesting that intrapartum transmission is significant[165]. Data from the European Paediatric Hepatitis C network suggests that in hepatitis C monoinfected women, ELCS does not reduce the risk of transmission[166] [167]. The earlier of these two reports suggests that in HIV/HCV co-infected women ELCS may reduce the risk of Hepatitis C transmission but this is not seen in the later report. Furthermore the later report suggests that the use of HAART in HIV/HCV co-infected women reduces the risk of hepatitis C transmission (OR 0.26, 95% CI 0.07 – 1.01). It is noteworthy that there are no randomised controlled trials on interventions such as the use of HAART or ELCS to reduce the risk of hepatitis C transmission. In Ireland, HIV/HCV co-infected women with ongoing hepatitis C viral replication have generally been offered an ELCS to reduce the risk of hepatitis C transmission.

Syphilis

Untreated early infectious syphilis in pregnancy can lead to still birth in up to 33% of cases. Treatment of syphilis in HIV infected pregnant women should be in accordance with treatment guidelines for syphilis in pregnancy and syphilis in HIV infected persons. Maternal syphilis infection may increase the risk of HIV MTCT and this risk appears to be greater with primary, secondary or early latent syphilis infection[168] [169]. Syphilis serology should be repeated in the third trimester in all HIV infected women.

Genital herpes infection

Maternal genital herpes rarely results in disseminated neonatal herpes simplex virus infection. The risk of neonatal infection is greatest with an episode primary of genital herpes in the third trimester of pregnancy. The presence of genital ulcers or a clinical diagnosis of genital herpes has been associated with an increased risk of transmission of HIV from mother to baby[170] [171]. In HIV positive non-pregnant populations herpes suppressive therapy has been associated with a reduction in both plasma and genital tract HIV viral loads[172] [173]. It is not known whether or not the use of herpes suppressive therapy in HIV infected women in pregnancy reduces the risk of vertical transmission of HIV.

In general HIV infected women with genital herpes in pregnancy should be managed as per the HIV negative population. The use of herpes suppressive therapy from 36 weeks to delivery may provide some benefit in reducing the risk of HIV transmission. Acyclovir is not licensed for use in pregnancy but reported in utero exposure has not been associated with an increased risk of teratogenicity[174].

Other sexually transmitted infections

All pregnant HIV infected women should have an STI screen performed at baseline in pregnancy. STI screens should be repeated thereafter if risk of a new infection is identified. Infections should be treated and managed as per the HIV negative population with contact tracing to prevent onward transmission and re-infection.

6.3 b (iv) Management of opportunistic infections

In Ireland, fortunately, the majority of HIV infected women in pregnancy have been relatively immune competent (median CD4 at presentation for women attending the GUIDE clinic for the years 2000 to 2003 inclusive was 388 x 10^6/L) and the incidence of

opportunistic infections in pregnancy to date is low. By and large opportunistic infections in pregnancy should be managed as per the non-pregnant population. Where possible medications with less teratogenicity should be used but efficacy should not be compromised.

6.3 b (v) Psychosocial support

Whilst antenatal screening for HIV is integral to a strategy to reduce MTCT, a new diagnosis of HIV in pregnancy is traumatic for those diagnosed and their families. Qualitative research in African women diagnosed with HIV through antenatal screening in Ireland has demonstrated that they experience significant trauma requiring support, education and counseling at this time. Furthermore this work identified a need to develop culturally appropriate support mechanisms for this group of women[116].

For asylum seekers in "direct provision" attendance for hospital appointments can be limited by inability to pay for transport, particularly for women dispersed to centres in rural areas.

Pregnant HIV infected women with a history of past or current substance abuse often find themselves in challenging social circumstances with multiple stressors. This can have a negative impact on their attendance for hospital appointments and adherence with antiretroviral therapy.

A new diagnosis of HIV brings with it the need to identify, inform and offer HIV testing to other parties, potentially at risk for HIV infection and for HIV infected women in pregnancy this will include sexual partners and other children, born before the diagnosis of HIV is made. The Irish Medical Council gives clear guidance on the need for disclosure where a party may be at risk of an infection. Every effort must be made to facilitate self-disclosure in a timely manner but where this is not possible the Irish Medical Council endorses breaching an individual's confidentiality in order to achieve disclosure[175]. It is important to

recognise that a newly diagnosed pregnant woman may face abandonment or domestic violence following disclosure of HIV and that inappropriate handling of disclosure could result in the woman losing trust in the multidisciplinary team and defaulting from care.
The identification, management and alleviation of the many psychosocial challenges facing HIV infected pregnant women requires a multidisciplinary team approach with clear channels of communication between members of the multidisciplinary team.

6.3 c Obstetric care

6.3 c (i) Antenatal care

In obstetric centres where significant numbers of HIV infected women are seen they are generally managed at a dedicated clinic.

In general there is no need for increased antenatal surveillance for HIV infected women in pregnancy as HIV itself does not appear to have a detrimental effect on pregnancy outcome. There may be an increased risk of pre-eclampsia associated with immune reconstitution in pregnancy[97 98] and protease inhibitors are known to increase the risk of diabetes through insulin resistance[176]. As with all pregnant women periconceptual folic acid is recommended as a measure to reduce the risk of neural tube defects. For women in receipt of co-trimoxazole for PCP prophylaxis it may be prudent to give high dose folic acid (5mg versus 400microgram), since co-trimoxazole is a dihydrofolate reductase inhibitor.

Reports that predate the availability of HAART suggest that invasive prenatal testing increases the risk of vertical transmission of HIV[177]. More recently data from the HAART

era has reported lower transmission rates associated with invasive pre natal testing, suggesting that antiretroviral therapy may reduce the risk of HIV transmission associated with invasive pre natal testing[178] [179]. HIV infection and antiretroviral therapy may alter serum human chorionic gonadotrophin and alpha fetoprotein levels and thus make interpretation of serum markers difficult[180].

In many circumstances the management of obstetric problems in HIV positive women is as per the HIV negative population, in particular the use of antenatal steroids to promote fetal lung maturity should be administered where obstetrically indicated. Potential toxicity associated with administration of antiretroviral therapy may be difficult to distinguish from obstetric disorders such as cholestasis of pregnancy or the HELLP (**H**aemolysis, **e**levated **l**iver enzymes and **l**ow **p**latelets) syndrome.

Prematurity remains an independent risk factor for transmission of HIV[181] and the management of women presenting in premature labour or threatened premature labour needs to be individualised, balancing the potential morbidity and mortality associated with early delivery against the risk of HIV infection. Where there is a clear obstetric indication, tocolysis should be used and the management of the HIV risk can then be tailored to the situation (see scenarios). Genital tract infection increases the risk of prematurity and women presenting in premature labour should be screened for genital tract infection.

In the general obstetric population, because of an increased risk of intrauterine death associated with prolonged pregnancy, arrangements are made for delivery once a pregnancy has gone beyond 41+ weeks. Delivery can be achieved by induction of labour with the administration of vaginal prostaglandins, artificial rupture of the membranes and intravenous syntocinon or by elective caesarean section. In the context of HIV, there is an association between transmission of HIV and longer duration of ruptured membranes. This data predates the widespread use of HAART in pregnancy and importantly the impact of

duration of ruptured membranes on transmission in women on HAART with an undetectable viral load is unknown. Of note one of the peripartum transmissions in Ireland occurred in the context of ruptured membranes for 24 hours where the mother was taking HAART with an undetectable HIV viral load (see chapter 3, 3.3 e pregnancy and infant outcome). The risk for ascending infection and development of chorioamnionitis increases the longer the membranes are ruptured. The presence of inflammation in the genital tract may lead to an increase in the HIV viral load in the genital tract, despite undetectable levels in the plasma and HAART. Thus there are multiple reasons why it is prudent to avoid early rupture of the membranes in the context of HIV infection.

For women on HAART with an undetectable viral load who wish to have a vaginal delivery, in whom the cervix is favourable for induction (perhaps particularly women who have delivered vaginally before), a pragmatic approach may be to commence intravenous zidovudine, induce labour with a view to delivery as quickly as possible with minimum duration of ruptured membranes. Where the membranes have been ruptured for longer than anticipated, consideration should be given to triple neonatal antiretroviral therapy (see 5.3 d (ii) infant antiretroviral therapy).

6.3 c (ii) Management in labour

In Ireland HIV infected pregnant women are offered the option of a vaginal delivery where there has been an adequate duration of and response to antiretroviral therapy in pregnancy. Of 242 deliveries in women attending the GUIDE clinic for HIV care between 1996 and 2003, 61.5% were vaginal deliveries.

Previous Irish guidelines have recommended peripartum intravenous zidovudine regardless of antenatal antiretroviral therapy and peripartum HIV viral load. Furthermore

this is an achievable objective with 85% of all the women that attended the GUIDE clinic in pregnancy between 1996 and 2003 receiving intravenous zidovudine before delivery. There is no data on how much zidovudine was received intravenously and the correlation with other potential confounders such as the duration of ruptured membranes. The current British guidelines suggest that in women on HAART with an undetectable HIV viral load, there may not be an additional benefit in administering peripartum intravenous zidovudine. The US guidelines continue to recommend peripartum zidovudine for all pregnancies, regardless of antiretroviral therapy. Recent data from the French cohort did not identify a difference in transmission rate in women with a delivery HIV-1RNA <400 copies/ml by whether or not they received peripartum zidovudine (0% versus 0.6%, p=1)[181]. Conversely in women where the delivery HIV RNA was >/=10,000 copies/ml there was a significant benefit in administering intravenous zidovudine (22.7% versus 5.3%, p=0.009). On logistic regression analysis the absence of intrapartum prophylaxis was associated with an increased risk of transmission (OR 4.72, 95% CI 1.42 – 15.71, p=0.011). In the absence of definitive data on the need for intravenous zidovudine in women who have been on HAART for at least 4 weeks with a HIV viral load of <50 copies/ml, it is reasonable to continue with this recommendation, particularly in situations where the membranes have spontaneously ruptured or have been artificially ruptured to induce or augment labour. For women being delivered by elective caesarean section, intravenous zidovudine should be started approximately 4 hours before the anticipated delivery time.

As outlined earlier the risk of transmission increases the longer the membranes are ruptured. Therefore it is best to avoid artificial rupture of the membranes where possible in HIV infected women in labour and triple antiretroviral therapy should be given to the neonate if the membranes have been ruptured for more than 12 hours. It is generally accepted that interventions that could be associated with a risk of transmission in labour, namely fetal scalp electrodes and fetal blood sampling are best avoided. Where an

instrumental delivery is deemed necessary, consideration should be given to triple neonatal antiretroviral therapy if there is any evidence of trauma with breach of the neonatal skin at delivery.

6.3 c (iii) Elective Caesarean section

When is an elective caesarean section of benefit?

Before the use of combination antiretroviral therapy in preventing MTCT, elective caesarean section was shown to be of benefit in preventing transmission in women not on antiretroviral therapy and in women on zidovudine monotherapy[58] [59] [60]. Now that transmission rates are low it has been estimated that a randomised trial would require over 6000 mother-child pairs in each arm to determine if there was an additional transmission benefit associated with elective caesarean section in the setting of suppressive HAART[33] . Recent data from the UK and Ireland of over 5000 mother child pairs failed to demonstrate a difference in transmission between women on HAART who were delivered vaginally or by elective caesarean section (see table 6.1)[118]. Similarly the French cohort did not demonstrate an additional benefit associated with elective caesarean section in women with a delivery viral load of <400 copies/ml (0.4% for elective caesarean section versus 0.5% for other modes of delivery, p = 0.35)[181].

Elective caesarean section is indicated for women on zidovudine monotherapy. For women on HAART, the optimum duration of therapy before delivery and the delivery viral load threshold below which elective caesarean section is unlikely to be of benefit is not known. In the recent report from the UK and Ireland, the overall transmission rate in women who received at least 14 days of any type of antiretroviral therapy was 0.8% and the risk of transmission in women on HAART declined with each additional week of antiretroviral therapy (AOR = 0.90 per week of HAART, p=0.007)[118]. Interestingly in the

French cohort, the proportion of in utero transmissions was higher in women transmitting with low viral loads at delivery (42% with delivery viral load of <400 copies/ml; 43% for delivery viral load of 400 – 9999 copies/ml and 21% for delivery viral load of >10,000copies/ml), suggesting that in this circumstance an elective caesarean section may not be of additional benefit as transmission has already occurred. The previous guidelines have recommended that women be offered an elective caesarean section if they have not received at least 4 weeks of antiretroviral therapy and if the 36 week viral load is >1000 copies/ml. Cautiously extrapolating from the UK and Ireland and French cohort data it may be reasonable to reduce the minimum requirement for antiretroviral therapy from 4 weeks to 2 weeks but reduce the viral load threshold for elective caesarean section from <1000 copies/ml to <400 copies/ml. This position would place the Irish guidelines between that taken in the current British (elective caesarean section for all if viral load >50 copies/ml at 36 weeks) and US (elective caesarean section if viral load >1000copies/ml at 36 weeks) guidelines for management of HIV in pregnancy.

Is an elective caesarean section safe in HIV infected women?

Higher morbidity rates following delivery have been reported in HIV infected women compared to the general population[65 66]. One case control study did not identify an increased rate of postoperative morbidity in HIV infected women undergoing spinal anaesthesia compared to the general population[67]. Many studies describe more postnatal morbidity in HIV infected women following abdominal delivery versus vaginal delivery[68 69]. Within the study from the Women and Infant Transmission Stufy[68] there was a decrease in all postnatal morbidity over time, most likely reflecting improved maternal health and routine use of prophylactic perioperative antibiotics. A recently published study suggests that elective Caesarean section is associated with similar postnatal morbidity to vaginal delivery (OR 1.16, 95% CI, 0.5 – 2.7)[70]. It is noteworthy that many of these studies have been carried out in very different populations at different times and extrapolation to the

current Irish situation must be made with caution. Whilst most of the reported morbidity is minor, postnatal morbidity must remain a consideration in mode of delivery decision-making in HIV infected women. This is of particular relevance at a time when the additional benefit of elective caesarean section in the setting of suppressive HAART is unknown. Furthermore in an environment where more and more asylum seekers are being repatriated, it is important to recognise that women who have a scar on their uterus may be at greater risk in a future pregnancy, without access to antenatal care.

Timing of elective caesarean section

In the general obstetric population, elective caesarean section is scheduled for 39 weeks in recognition of the reduced risk of transient tachypnea of the newborn associated with an additional week in utero[63]. Current British and US guidelines recommend that where an elective caesarean section is being performed to reduce the risk of transmission of HIV that it be performed at 38 rather than 39 weeks in order to avoid spontaneous rupture of the membranes or labour in a woman scheduled for a caesarean section. There is an association between antiretroviral therapy and earlier onset of labour with a concern that longer exposure to more complex antiretroviral therapy increases the risk of premature delivery. The experience at the GUIDE clinic has been that there was not an increased risk of prematurity with more complex antiretroviral therapy (none, one, two or three drugs) and the median gestational age at delivery in 242 pregnancies between 1996 and 2003 was 39 weeks. This supports the previous recommendation for scheduling of elective caesarean sections for 39 weeks rather than 38 weeks in the Irish setting. In situations where an elective caesarean section is anticipated and where there is a concern that a woman may go into spontaneous labour or have spontaneous rupture of the membranes between 38 and 39 weeks it would be prudent to do schedule delivery for 38 weeks.

6.3 d Infant care

6.3 d (i) general care

In general the management of HIV exposed infants should not differ to that of non-exposed. HIV exposure per se is not an indication for admission to a special care baby unit. Infants should be bathed before leaving the labour ward to remove any maternal blood. Infants should commence antiretroviral therapy as soon as possible following delivery (see below).

6.3 d (ii) Infant Feeding

Although breast milk is the ideal nutritional source for infants, breastfeeding is associated with a 10 – 15 % increased risk of HIV transmission. The risk appears greatest in the first 6 – 8 weeks post partum but continues throughout all of the time of breastfeeding. Maternal seroconversion during breastfeeding is associated with a particularly high transmission risk. Studies have shown that mixed feeding is associated with the significantly higher transmission rate compared with exclusive breast or formula feeding[182] [183]. However in some settings, the benefit of preventing breast feeding associated HIV transmission is effectively negated by the excess mortality in children undergoing replacement feeding[182]. In resource constrained countries this additional risk of breastfeeding has to be balanced against the very significant morbidity that can be associated with replacement feeding[184]. Feeding recommendations for infants born to HIV infected will therefore vary depending on the local setting. Studies are ongoing to determine the impact of either maternal or infant antiretroviral therapy in preventing breastfeeding associated transmission[185] [186]. Pending the results of these studies, the recommendation must stand that in Ireland, as in other developed countries, where there are safe alternative options, infants of HIV positive mothers should not be breast fed.

6.3 d (iii) Infant antiretroviral therapy

After delivery, infants commence ART as soon as possible, at least within 4 hours of birth and continue for 4 weeks. The regimen chosen is determined by maternal antiretroviral exposure and HIV viral load close to delivery. In general infants born to mothers who are at low risk for transmission receive zidovudine syrup as monotherapy. This is administered twice daily for four weeks. Triple ART, usually with zidovudine, lamivudine and nevirapine, is recommended for infants at higher risk of transmission i.e.

- where the mother has received <4 weeks of combination ART
- where there have been concerns for maternal adherence to antiretroviral therapy
- where the maternal HIV viral load is >1000 copies/ml prior to delivery
- where there has been ruptured membranes for more than 12 hours
- or where other factors associated with increased risk of transmission are identified

Infants who are unable to tolerate zidovudine syrup orally should receive intravenous zidovudine. For infants prescribed triple therapy, lamivudine is given orally at the same time as zidovudine for four weeks. Nevirapine is the third agent in common use for neonatal triple antiretroviral therapy. The timing of neonatal nevirapine administration depends of whether or not the mother has received nevirapine. If the mother has not been prescribed nevirapine a first dose is given to the infant as soon as possible following delivery and a second dose given 24 – 48 hours later. If the mother has received nevirapine the first neonatal dose is given at 48 to 72 hours with a second dose the following day. Two doses only of nevirapine are used. The efficacy of the programme has been demonstrated with a vertical transmission rate of just 1% in 874 deliveries in Ireland over the years 1999 to 2007 inclusive[148]. In selected situations, e.g. where there is evidence of maternal antiretroviral resistance, alternative neonatal antiretroviral regimens may be used.

Although the data is limited, there is evidence to support initiation of antiretroviral therapy as post exposure prophylaxis for HIV exposed infants even where mothers have received no antiretroviral therapy[187] [188] . Thus infants of women diagnosed at delivery or in the immediate (<72hrs) postpartum period should receive triple ARV therapy as post exposure prophylaxis. The value of ARV in preventing HIV transmission if > 72 hours post delivery had elapsed is not proven. In this situation the emphasis should be on avoiding breast feeding and early HIV testing for infection in the infant.

6.3 d (iv) Monitoring for toxicity

Anticipated side effects associated with zidovudine include anaemia and neutropenia which usually resolve by 12 weeks of age. A full blood count should be obtained on Day 1, at 2, 6 and 12 weeks of age. Liver transaminases should also be checked post delivery. Although asymptomatic hyperlactatemia has been reported in infants exposed to antiretroviral therapy in utero, routine monitoring of lactate levels is not indicated.

6.3 d (v) Infant vaccination

BCG vaccine should be deferred until HIV PCR test results are available. Infants with negative HIV PCR tests at birth, 2 and 6 weeks of age can proceed to BCG vaccination.
HIV exposed infants should receive all of the routine primary immunisations including the hexavalent (6 in 1) vaccine (DaPT/HIB/HBV/IPV), meningococcal C, Prevnar and MMR vaccines.
As outlined earlier infants born to hepatitis B surface antigen positive mothers should receive their first dose of hepatitis B vaccine together with hepatitis B immunoglobulin as soon as possible following delivery. Further doses of HBV vaccine will be given as components of the hexavalent vaccine at 2, 4, and 6 months of age. Testing for Hepatitis B

surface antigen (to exclude infection) and hepatitis B surface antibody (to ensure adequacy of protective response) should be carried out between 8 and 12 months of age.

6.3 d (vi) PCP prophylaxis

Co-trimoxazole, 240mg, given once daily every Monday, Wednesday and Friday should be initiated at 6 weeks of age for:-

- All HIV infected infants
- All HIV exposed infants considered at higher risk of infection (i.e. all infants who received triple ARV prophylaxis).

PCP prophylaxis is not required for HIV exposed infants born to mothers who were receiving HAART and who were virally suppressed at delivery.

6.3 d (vii) Infant testing for HIV infection

Cord blood sampling should be avoided

Blood for HIV PCR testing should be obtained on day 1, week 2, 6, 12 and month 6 months of life. HIV antibody testing is carried out at 18 months of age.

An infant is considered HIV "infected" if the HIV DNA PCR is confirmed positive on 2 separate occasions An infant is considered HIV "uninfected" if HIV is not detected by PCR testing of two blood samples, separated by at least a 2 week interval, the second of which is at of after 3 months of age. The uninfected status is confirmed by testing for HIV antibody at 18 months of age, by which time seroreversion or loss of maternal antibody will generally have occurred. With the improved sensitivity of the new generations of HIV antibody test, on occasion, it is possible to detect some faint residual antibody at 18 months of age but this will has disappeared if testing is repeated at two years. An infant's HIV status is considered indeterminate if the infant dies or is lost to follow up before their HIV status can be confirmed.

6.4 Scenarios

6.4 (a) Women on antiretroviral therapy at conception

In general women on suppressive HAART should not make any changes to their antiretroviral therapy during pregnancy.

It may be reasonable to continue efavirenz in pregnancy, particularly if the physician is not aware of the pregnancy until after the neural tube has closed and if a change in antiretroviral therapy could lead to loss of virological control or exposure to other antiretrovirals with less data on teratogenicity. Efavirenz should be avoided in women of child bearing potential who are not using effective contraception. Recently presented audits from both London and Dublin demonstrated extremely poor documentation of contraception in women prescribed efavirenz, highlighting the need to include discussion regarding family planning in women of child bearing potential being prescribed antiretroviral therapy, particularly efavirenz[189 190]

Women on a non-suppressive regimen should have genotypic resistance testing performed to guide further antiretroviral regimens with a view to achieving an undetectable viral load as soon as possible.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

6.4 (b) Women presenting <24/40

Baseline CD4 count <200 x 106/L, regardless of HIV viral load

Prophylaxis against Pneumocystis jiroveci (formerly pneumocystis carinii, PCP) infection should be initiated as soon as possible. Where co-trimoxazole is used consider co-administration of folic acid 5mg once daily to reduce theoretical risk of a neural tube defect secondary to inhibition of dihydrofolate reductase with co-trimoxazole. Antiretroviral therapy is indicated for maternal health and should be initiated as soon as possible after the first trimester. Baseline genotypic resistance testing should be performed to guide antiretroviral choices. Given the wealth of experience with zidovudine in pregnancy it should be included in antenatal antiretroviral regimens wherever possible. A nevirapine based regimen may be an option in women with a baseline CD4 count of <250 x 10^6/L.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

Baseline CD4 count >200 x 10^6/L but <350 x 10^6/L

For some women in this situation the CD4 count may be spuriously low because of the physiological changes associated in pregnancy and for others there will be a clear need for antiretroviral therapy for maternal well being. Where the CD4 count is deemed spuriously low because of pregnancy, initiation of antiretroviral therapy can be delayed to between 20 and 24 weeks. Where there is a need for antiretroviral therapy for maternal well being, antiretroviral therapy should be initiated as soon as possible after the first trimester.

Baseline genotypic resistance testing should guide antiretroviral choices.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

Baseline CD4 count >350 x 10^6/L baseline HIV viral load <5000 copies/ml

In general women with a baseline CD4 count of >350 x 10^6/L will not require antiretroviral therapy for their own health and can initiate therapy between 20 and 24 weeks. Baseline genotypic resistance testing should guide antiretroviral choices. If the baseline HIV viral load is <5000 copies/ml women may opt for 1) zidovudine monotherapy plus an elective caesarean section or 2) HAART with a view to awaiting spontaneous onset of labour.

All women opting for zidovudine monotherapy should be delivered by elective caesarean section. In women opting for HAART final recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

Baseline CD4 count >350 x 10^6/L baseline HIV viral load >5000 copies/ml

In general women with a baseline CD4 count of >350 x 10^6/L will not require antiretroviral therapy for their own health but because the baseline HIV viral load is >5000 copies/ml, HAART is recommended and can be initiated between 20 and 24 weeks.

Baseline genotypic resistance testing should guide antiretroviral choices.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

6.4 (c) Women presenting >24/40

Women presenting for antenatal care after 24/40 should have their HIV test processed as quickly as possible. Where the initial test is positive it may be prudent to refer women for adult HIV assessment without waiting for the results of a second confirmatory HIV test.

Baseline CD4 count <200 x 10^6/L, regardless of HIV viral load

Prophylaxis against Pneumocystis jiroveci (formerly pneumocystis carinii, PCP) infection and HAART should be initiated as soon as possible. It may not be possible to wait for results of baseline genotypic resistance testing before initiating therapy and choices should be guided by previous antiretroviral exposure and likelihood of transmitted resistance at baseline. A nevirapine based regimen may be a prudent choice, particularly with presentation in the third trimester given its rapid onset of action and effective transfer across the placenta.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by duration of antiretroviral therapy, HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

Baseline CD4 count >200 x 10^6/L but <350 x 10^6/L, regardless of HIV viral load

For some women in this situation the CD4 count may be spuriously low because of the physiological changes associated in pregnancy and for others there will be a clear need for antiretroviral therapy for maternal well being. Regardless of maternal need HAART should be initiated as soon as possible in all women presenting for the first time after foetal viability. It may not be possible to wait for results of baseline genotypic resistance testing before initiating therapy and choices should be guided by previous antiretroviral exposure and likelihood of transmitted resistance at baseline.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by duration of antiretroviral therapy, HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

The decision to continue or discontinue HAART beyond pregnancy will be based on individual maternal need.

Baseline CD4 count >350 x 10^6/L, regardless of baseline HIV viral load

In general women with a baseline CD4 count of >350 x 10^6/L will not require antiretroviral therapy for their own health but because they have presented after foetal viability, HAART is recommended and should be initiated as soon as possible. It may not be possible to wait for results of baseline genotypic resistance testing before initiating therapy and choices should be guided by previous antiretroviral exposure and likelihood of transmitted resistance at baseline.

Recommendations regarding the mode of delivery and neonatal antiretrovirals will be determined by duration of antiretroviral therapy, HIV viral load at 36 weeks and duration of ruptured membranes prior to delivery (see Table 6.3).

6.4 (d) Women presenting in premature labour or threatened premature labour

In the HAART era, prematurity remains an independent risk for vertical transmission of HIV. Management of the HIV positive woman presenting in premature labour or threatened premature labour needs to be individualised, balancing the morbidity (and mortality) associated with prematurity with the risk for transmission of HIV and requires multidisciplinary consultation. Genital tract infection, which may have led to premature labour, should be identified and treated.

On effective HAART <34/40

Maternal steroids should be administered to promote foetal lung maturity and tocolysis may be indicated to delay delivery and facilitate further maturation in utero. Maternal antiretroviral therapy should be continued and an urgent HIV viral load test sent. A single dose of nevirapine given to the mother should be considered to pre-load the neonate with nevirapine. If tocolysis has successfully delayed delivery, consideration should be given to caesarean section once steroids have taken effect, depending on the estimated foetal weight and gestational age at that time.

Triple antiretroviral therapy should be administered to the neonate where the membranes have been ruptured for more than 12 hours before delivery or the urgent HIV viral load is >1000 copies/ml but may be limited by unsuitability of some premature neonates for enteral therapy.

On effective HAART >34/40

Maternal antiretroviral therapy should be continued and an urgent HIV viral load test sent. Intravenous zidovudine should be commenced and if delivery is not imminent, particularly where the membranes have ruptured, consideration should be given to delivery by emergency caesarean section. A single dose of nevirapine given to the mother should be considered to pre-load the neonate with nevirapine.

Where possible triple antiretroviral therapy should be administered if the membranes have been ruptured for more than 12 hours before delivery or the urgent HIV viral load is >1000 copies/ml.

Failing HAART or not on antiretroviral therapy <34/40

Maternal steroids should be administered to promote foetal lung maturity and tocolysis may be indicated to delay delivery and facilitate further maturation in utero. Intravenous

zidovudine should be administered as soon as possible. Maternal HAART should be commenced or optimised (in the setting of a failing regimen) as soon as possible with baseline bloods sent for urgent HIV viral load and CD4 count. Where the CD4 count is known to be <250 x 10^6/L and there is no history of NNRTI resistance a nevirapine based regimen should be considered given rapid onset of action and effective placental transfer. Where the CD4 count if known to be >250 x 10^6/L or there is known NNRTI resistance a protease inhibitor based regimen should be initiated as soon as possible and there may be value in administering a single dose of nevirapine to the mother even in the setting of previously documented NNRTI resistance. If tocolysis has successfully delayed delivery, consideration should be given to caesarean section once steroids have taken effect, depending on the estimated foetal weight and gestational age at that time.
Triple antiretroviral therapy should be administered to the neonate but may be limited by unsuitability of some premature neonates for enteral therapy.

Failing HAART or not on antiretroviral therapy >34/40

An urgent HIV viral load (and genotypic resistance testing) should be sent and for newly diagnosed women an urgent CD4 count should be sent. Intravenous zidovudine should be administered as soon as possible. Where time permits maternal HAART should be commenced or optimised (in the setting of a failing regimen) as soon as possible. Where the CD4 count is known to be <250 x 10^6/L and there is no history of NNRTI resistance a nevirapine based regimen should be considered given rapid onset of action and effective placental transfer. Where the CD4 count if known to be >250 x 10^6/L or there is known NNRTI resistance a protease inhibitor based regimen should be initiated and there may be value in administering a single dose of nevirapine to the mother even in the setting of previously documented NNRTI resistance. If delivery is not imminent, consideration should be given to delivery by emergency caesarean section, particularly if the membranes have ruptured.

Triple antiretroviral therapy should be administered to the neonate but may be limited by unsuitability of some premature neonates for enteral therapy.

6.4 (e) Women presenting with pre-labour ruptured membranes

The risk of transmission increases with the duration of membrane rupture. This has been clearly demonstrated for women in active labour but definitive studies on women with premature rupture of the membranes (i.e. rupture of the membranes without onset of labour) are not available. It is advisable, however, to minimise duration of membrane rupture i.e. expedite delivery unless the risk to the infant from premature delivery outweighs the risk of HIV acquisition. Genital tract infection should be identified and treated.

<34/40 on effective HAART

Maternal steroids should be administered to promote foetal lung maturity. Maternal antiretroviral therapy should be continued and an urgent HIV viral load test sent. Consideration should be given to delivery by caesarean section once steroids have taken effect, depending on the estimated foetal weight and gestational age at that time. Intravenous zidovudine should be administered around delivery and a single dose of nevirapine given to the mother should be considered to pre-load the neonate with nevirapine.

Triple antiretroviral therapy should be administered where the membranes have been ruptured for more than 12 hours before delivery or the urgent HIV viral load is >1000 copies/ml but may be limited by unsuitability of some premature neonates for enteral therapy.

<34/40 failing HAART or not on antiretroviral therapy

Maternal steroids should be administered to promote foetal lung maturity. Intravenous zidovudine should be administered as soon as possible. Maternal HAART should be initiated or optimised (in the setting of a failing regimen) as soon as possible. Where the CD4 count is known to be <250 x 10^6/L and there is no history of NNRTI resistance a nevirapine based regimen should be considered given rapid onset of action and effective placental transfer. Where the CD4 count if known to be >250 x 10^6/L or there is known NNRTI resistance a protease inhibitor based regimen should be initiated and there may be value in administering a single dose of nevirapine to the mother even in the setting of previously documented NNRTI resistance. Consideration should be given to delivery by caesarean section once steroids have taken effect, depending on the estimated foetal weight and gestational age at that time.

Triple antiretroviral therapy should be administered to the neonate but may be limited by unsuitability of some premature neonates for enteral therapy.

>34/40 on effective HAART

Maternal antiretroviral therapy should be continued and an urgent HIV viral load test sent. Intravenous zidovudine should be commenced and delivery should be expedited by caesarean section or induction of labour.

Triple antiretroviral therapy should be administered where the membranes have been ruptured for more than 12 hours before delivery or the urgent HIV viral load is >1000 copies/ml but may be limited by unsuitability of some premature neonates for enteral therapy.

>34/40 failing HAART or not on antiretroviral therapy

An urgent HIV viral load (and genotypic resistance testing) should be sent and for newly diagnosed women an urgent CD4 count should be sent. Intravenous zidovudine should be administered as soon as possible. Where time permits maternal HAART should be

commenced or optimised (in the setting of a failing regimen) as soon as possible. Where the CD4 count is known to be <250 x 10^6/L and there is no history of NNRTI resistance a nevirapine based regimen should be considered given rapid onset of action and effective placental transfer. Where the CD4 count if known to be >250 x 10^6/L or there is known NNRTI resistance a protease inhibitor based regimen should be initiated and there may be value in administering a single dose of nevirapine to the mother even in the setting of previously documented NNRTI resistance. If delivery is not imminent, consideration should be given to delivery by caesarean section, particularly if the membranes have ruptured. Triple antiretroviral therapy should be administered to the neonate but may be limited by unsuitability of some premature neonates for enteral therapy.

6.4 (f) Women presenting in labour at term

Labour may represent the first opportunity to implement measures to reduce transmission of HIV in two circumstances: either a woman presents unbooked in labour and has an urgent HIV test performed or where a positive result was identified earlier in the pregnancy but the woman did not attend for the result. Where a woman has defaulted from antenatal care and is thus unaware of her HIV status, the delivery suite and neonatal staff should be aware of the situation and a proposed management plan should be in place before the estimated due date for the woman.

Intravenous zidovudine should be commenced as soon as possible and consideration given to a single dose of nevirapine to the mother. If delivery is not considered likely to occur within a reasonable time frame, particularly where the membranes have ruptured, consideration should be given to an emergency caesarean section. The neonate should be commenced on triple antiretroviral therapy as soon as possible after birth. The mother's HIV status should be confirmed as soon as possible with early referral to an adult HIV service.

6.4 (g) Women diagnosed post partum

Women who are delivered before admission to hospital or shortly after admission, should have their HIV test sent urgently to ensure that there is an opportunity to initiate neonatal triple antiretroviral therapy as soon as possible and before 72 hours of life. The mother's HIV status should be confirmed as soon as possible with early referral to an adult HIV service.

6.4 (h) Women refusing antiretroviral therapy

Where women refuse to engage in care it is important that they understand and appreciate the benefits associated with interventions to reduce MTCT. It is important to give culturally competent verbal and written information with access to an interpretive service when required. Where women have refused interventions the Irish Courts have made these infants a ward of court to ensure administration of antiretroviral therapy and avoidance of breastfeeding. It is prudent to have a management plan in place well in advance of the anticipated date of delivery.

Conclusion

The overall management of HIV in pregnancy requires a multidisciplinary approach between adult and paediatric HIV specialists and obstetric services. The changing demographics of the population in Ireland provide medical, social and cultural challenges for the management of HIV. Equally, the management of subsequent pregnancies in mothers who have previously received short term antiretrovirals to reduce MTCT adds further complexities to their management.

While these guidelines can be used as a model for the management of HIV in pregnancy, ultimately each patient is managed individually, and a unique antenatal, intrapartum, and postpartum path is determined for each patient

Table 6.3 – scenarios in the management of HIV in pregnancy

SCENARIO

Gestation at presentation: Attending pre-conception on antiretroviral therapy			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Virally suppressed throughout pregnancy Continue PCP prophylaxis if CD4 <200		<50	In general continue HAART Substituting efavirenz for alternative may not be of benefit depending on gestational age when presents	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	Await SOL	ZDV x 4/52 if ROM <12hrs
Failing therapy anytime Continue PCP prophylaxis if CD4 <200		>50	Optimise HAART with guidance of GRT and previous ART history	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Case by case	ELCS @39/40 if VL >1000 cpm at 36/40 Await SOL if VL <1000 cpm at 36/40	Case by case

Table 6.3 – continued

SCENARIO

Gestation at presentation: <24/40			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Because of ↓ baseline CD4, mother requires HAART and PCP prophylaxis for own health	<200	regardless of HIV VL	Await GRT before commencing HAART Commence PCP prophylaxis ASAP HAART to commence ASAP after 1st trimester	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped In all scenarios If for ELCS start IV ZDV 4 hours prior to delivery	Continue	Await SOL if maternal VL <1000 @ 36/40	ZDV X 4/52 if maternal VL <1000 @36/40 +ROM <12hrs
						ELCS @ 39/40 if maternal VL >1000 @ 36/40	Triple ART x 4/52 if maternal VL >1000 @36/40 + ROM >12hrs

Table 6.3 – continued

SCENARIO

Gestation at presentation: <24/40			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Mother requires treatment for her own health	>200 but <350 x 10^6/L	regardless of HIV VL	Await GRT before commencing HAART ASAP after 1st trimester	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	ELCS @ 39/40 if maternal VL >1000 @ 36/40	Triple ART x 4/52 if maternal VL >1000 @36/40 + ROM >12hrs
CD4 attributed to physiological changes of pregnancy		<5000	Await GRT before commencing Rx at 20-24/40 May opt for zidovudine monotherapy plus ELCS or HAART plus SVD		discontinue post-partum and see for GRT at ~6 weeks	Await SOL if maternal VL <1000 @ 36/40	ZDV X 4/52 if maternal VL <1000 @36/40 +ROM <12hrs or ZDV mono and ELCS
		>5000	Await GRT before commencing HAART at 20-24/40			ELCS @39/40 if ZDV monoRx	

Table 6.3 – continued

SCENARIO

Gestation at presentation: <24/40			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Mother requires Rx to reduce vertical transmission of HIV to infant but does not require Rx for her own health.	>350	<5000	Await GRT before commencing Rx at 20-24/40 May opt for zidovudine monotherapy and ELCS or HAART plus SVD	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	discontinue post-partum and see for GRT at ~6 weeks	ELCS @39/40 if ZDV monoRx	ZDV x 4/52
		>5000	Await GRT before commencing HAART at 20-24/40			Await SOL if maternal VL <1000 @ 36/40	ZDV X 4/52 if maternal VL <1000 @36/40 + ROM <12hrs
						ELCS @ 39/40 if maternal VL >1000 @ 36/40	Triple ART x 4/52 if maternal VL >1000 @36/40 +/or ROM >12hrs

Table 6.3 – continued

SCENARIO

Gestation at presentation: >24/40			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
Because of ↓ baseline CD4, mother requires Rx and PCP prophylaxis for own health	<200	regardless of HIV VL	GRT Commence PCP prophylaxis asap HAART to commence ASAP (depending on gestation at presentation may commence before resistance results available, consider nevirapine especially if very late presentation)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	continue on triple ART	Await SOL if maternal VL <1000 @36/40 and ≥4/52 of Rx	ZDV x 4/52 if mother on Rx for ≥4/52 with VL <1000 + ROM <12hrs
						ELCS @ 39/40 if maternal VL >1000 @ 36/40 or ≤4/52 Rx	Triple ART x 4/52 if mother on Rx. ≤4/52 or VL >1000cpm @36/40 +/or ROM >12hrs
Mother requires treatment for her own health	>200 but < 350	regardless of HIV VL	GRT HAART to commence ASAP (depending on gestation at presentation may commence before resistance results available)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	Await SOL if maternal VL <1000 @36/40 and ≥4/52 of Rx	ZDV x 4/52 if mother on Rx. for ≥4/52 with VL <1000 + ROM <12hrs

Table 6.3 – continued

SCENARIO

Gestation at presentation: >24/40			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
CD4 attributed to physiological changes of pregnancy	>200 but < 350		GRT HAART to commence ASAP (depending on gestation at presentation may commence before resistance results available)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	discontinue post-partum and see for GRT at ~6 weeks	ELCS @ 39/40 if maternal VL >1000 @ 36/40 or ≤4/52 Rx	Triple ART if mother on Rx ≤4/52 or VL >1000cpm @36/40 +/or ROM >12hrs
Mother requires Rx to reduce vertical transmission of HIV to infant but does not require Rx for her own	>350	regardless of HIV VL	GRT HAART to commence ASAP (depending on gestation at presentation may commence before resistance results available)	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	discontinue post-partum and see for GRT at ~6 weeks	Await SOL if maternal VL <1000 @36/40 and ≥4/52 of Rx	ZDV x 4/52 if mother on Rx for ≥4/52 with VL <1000 + ROM <12hrs
						ELCS @ 39/40 if maternal VL >1000 @ 36/40 or ≤4/52 Rx	Triple ARTx 4/52 if mother on Rx. ≤4/52 or VL >1000cpm @36/40 +/or ROM >12hrs

Table 6.3 – continued

SCENARIO

Gestation at presentation: Premature labour on effective HAART			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
<34/40		<50	Steroids Consider tocolysis Continue HAART	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	Consider CS after steroids	Triple ART x 4/52 where possible if ROM >12 hours
>34/40			Continue HAART	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	Consider CS if delivery not imminent	Triple ART x 4/52 where possible if ROM >12 hours

Table 6.3 – continued

SCENARIO

Gestation at presentation: Premature labour failing HAART or not on HAART			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
<34/40		>50	GRT Steroids Consider tocolysis Consider sdNVP if CD4 >250 plus PI based HAART Consider NVP based regimen if CD4 <250	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue if indicated for maternal health Discontinue if not indicated for maternal health. Staggered stop if NVP based. GRT at ~6 weeks	Consider CS after steroids	Triple ART x 4/52where possible
>34/40			GRT Consider sdNVP if CD4 >250 plus PI based HAART Consider NVP based regimen if CD4 <250			Consider CS if delivery not imminent	

Table 6.3 – continued

SCENARIO

Gestation at presentation: Prelabour ruptured membranes at term			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
On effective HAART		<50	Continue HAART	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue	Expedite delivery, aim for shortest duration of ROM	ZDV x 4/52 if ROM <12 hours Triple ART x 4/52 if ROM >12 hours
Failing HAART or not on HAART		>50	GRT Consider sdNVP if CD4 >250 plus PI based HAART Consider NVP based regimen if CD4 <250		Continue if indicated for maternal health Discontinue if not indicated for maternal health. Staggered stop if NVP based. GRT at ~6 weeks		Triple ART x 4/52

Table 6.3 – continued

SCENARIO

Gestation at presentation: Prelabour ruptured membranes pre term			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
On effective HAART <34/40			Steroids Continue HAART Urgent VL Consider sdNVP to mother to pre load infant	IV ZDV 2mg/kg loading over 1 hour then 1mg/kg until cord is clamped	Continue if on effective HAART Optimise HAART if failing therapy	<34/40 consider CS once steroids effective >34/40 expedite delivery aiming for shortest duration of ROM	Triple ART where possible if ROM >12 hours or mother on failing regimen
On effective HAART >34/40			Continue HAART				
Failing HAART or not on HAART <34/40			GRT Steroids Consider sdNVP if CD4 >250 plus PI based HAART Consider NVP based regimen if CD4 <250				
Failing HAART or not on HAART >34/40			Consider sdNVP if CD4 >250 plus PI based HAART Consider NVP based regimen if CD4 <250				

Table 6.3 – continued

SCENARIO

Gestation at presentation: Diagnosed in labour			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
				As above Consider sdNVP	Urgent confirmation of HIV status Assessment at adult HIV service post partum	Expedite delivery	Triple ART where possible and urgent confirmation of maternal HIV

SCENARIO

Gestation at presentation: Diagnosed post partum			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
					Assessment at adult HIV service post partum		Triple ART where possible

Table 6.3 – continued

SCENARIO

Gestation at presentation: Refusing interventions to reduce MTCT			**RECOMMENDATIONS**				
Clinical	**CD4**	**HIV VL**	**Antepartum**	**Intrapartum or prior to CS**	**Postpartum mother**	**Mode of delivery**	**Postpartum infant**
				Offer IV zidovudine and consider sdNVP	Offer adult HIV service assessment	Offer ELCS	Triple ART, infant to be made ward of court if required

ART: Antiretroviral therapy
ELCS: Elective caesarean section
SOL: Spontaneous onset of labour
VL: viral load

HIV VL measured in copies per ml
GRT: genotypic resistance testing
Rx: therapy
ZDV: Zidovudine or AZT or Retrovir

C.S: Caesarean Section
NVP: Nevirapine
Triple ART to baby = ZDV + 3TC x 4/52 + stat NVP
CD4 units = x 10^6/L

References

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[1] 2007 Report on the Global AIDS Epidemic, available at www.unaids.org

[2] Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A. 1980 Dec;77(12):7415-9

[3] Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med. 1981 Dec 10;305(24):1425-31

[4] Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Murray HW, Cunningham-Rundles S. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med. 1981 Dec 10;305(24):1431-8

[5] Siegal FP, Lopez C, Hammer GS, Brown AE, Kornfeld SJ, Gold J, Hassett J, Hirschman SZ, Cunningham-Randles C, Adelsberg BR et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. NEJM 1981, 305:1439 -1444

[6] Unexplained Immunodeficiency and Opportunistic Infections in Infants - New York, New Jersey, California Vol 31, No MM49; 665, December 17, 1982

[7] Cowan MJ, Hellmann D, Chudwin D, Wara DW, Chang RS, Ammann AJ. Maternal transmission of acquired immune deficiency syndrome. Pediatrics. 1984 Mar;73(3):382-6

[8] Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983, May 20;220(4599):868-71

[9] Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, et al. Frequent detection and isolation of cytopathic retroviruses (HTLVIII) from patients with AIDS and at risk for AIDS. Science 1984: 2; 500-502

[10] Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban clinical cohort. Ann Intern Med. 1996 Apr 1;124(7):633-42

[11] Miller V, Mocroft A, Reiss P, Katlama C, Papadopoulos AI, Katzenstein T, van Lunzen J, Antunes F, Phillips AN, Lundgren JD. Relations among CD4 lymphocyte count nadir, antiretroviral therapy, and HIV-1 disease progression: results from the EuroSIDA study. Ann Intern Med. 1999 Apr 6;130(7):570-7

[12] Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Schooley RT, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med. 1987 Jul 23;317(4):185-91

[13] Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science. 1989 Mar 31;243(4899):1731-4

[14] Delta Coordinating Committee. Delta: a randomised double-blind controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. Delta Coordinating Committee. Lancet. 1996 Aug 3;348(9023):283-91

[15] Hammer SM, Katzenstein DA, Hughes MD, Gundacker H, Schooley RT, Haubrich RH, Henry WK, Lederman MM, Phair JP, Niu M, Hirsch MS, Merigan TC. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS Clinical Trials Group Study 175 Study Team. N Engl J Med. 1996 Oct 10;335(15):1081-90

[16] Eron JJ, Benoit SL, Jemsek J, MacArthur RD, Santana J, Quinn JB, Kuritzkes DR, Fallon MA, Rubin M Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. N Engl J Med. 1995 Dec 21;333(25):1662-9

[17] Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, Jonas L, Meibohm A, Holder D, Schleif WA, Condra JH, Emini EA, Isaacs R, Chodakewitz JA, Richman DD. Simultaneous vs sequential initiation of therapy with indinavir, zidovudine, and lamivudine for HIV-1 infection: 100-week follow-up. JAMA. 1998 Jul 1;280(1):35-41

[18] Public Health Service Task Force Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents – January 29 2008. Available at www.aidsinfo.nih.gov

[19] British HIV association guidelines for the treatment of HIV-1 infected adults with antiretroviral therapy, 2008. Available at www.bhiva.org

[20] Soilleux EJ, Morris LS, Lee B, Pohlmann S, Trowsdale J, Doms RW, Coleman N. Placental expression of DC-SIGN may mediate intrauterine vertical transmission of HIV. J Pathol. 2001 Dec;195(5):586-92

[21] Kaneda T, Shikari K, Hirano K. Detection of maternofetal transfusion by placental alkaline phosphatase. J Paediatr 1997; 130: 730-735

[22] Mwanyumba F, Gaillard P, Inion I, Verhofstede C, Claeys P, Chohan V, Vansteelandt S, Mandaliya K, Praet M, Temmerman M. Placental inflammation and perinatal transmission of HIV-1. J Acquir Immune Defic Syndr. 2002 Mar 1;29(3):262-9

[23] Gaillard P, Verhofstede C, Mwanyumba F, Claeys P, Chohan V, Mandaliya K, Bwayo J, Plum J, Temmerman M. Exposure to HIV-1 during delivery and mother-to-child transmission. AIDS 2000 Oct 20;14(15):2341-8

[24] Lewis P, Nduati R, Kreiss JK, John GC, Richardson BA, Mbori-Ngacha D, Ndinya-Achola J, Overbaugh J. Cell-free human immunodeficiency virus type 1 in breast milk. J Infect Dis. 1998 Jan;177(1):34-9

[25] Koulinska IN, Villamor E, Chaplin B, Msamanga G, Fawzi W, Renjifo B, Essex M. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. J Acquir Immune Defic Syndr. 2006 Jan 1; 41(1):93-9

[26] Kourtis A, Butlerys S, Nesheim S et al. Understanding the timing of HIV transmission from mother to infant. JAMA. 2001; 285(6): 709 – 712

[27] Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, Bulterys M. Mother-to-child transmission of HIV-1: timing and implications for prevention. Lancet Infect Dis. 2006 Nov;6(11):726-32

[28] Magder LS, Mofenson L, Paul ME, Zorrilla CD, Blattner WA, Tuomala RE, LaRussa P, Landesman S, Rich KC. Risk factors for in utero and intrapartum transmission of HIV. J Acquir Immune Defic Syndr. 2005 Jan 1;38(1):87-95

[29] Renjifo B, Gilbert P, Chaplin B, Msamanga G, Mwakagile D, Fawzi W, Essex M; Tanzanian Vitamin and HIV Study Group Preferential in-utero transmission of HIV-1 subtype C as compared to HIV-1 subtype A or D. AIDS 2004 Aug 20;18(12):1629-36

[30] Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, Burchett SK, Kornegay J, Jackson B, Moye J, Hanson C, Zorrilla C, Lew JF. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group. N Engl J Med. 1999 Aug 5;341(6):394-402

[31] No authors listed. Maternal viral load and vertical transmission of HIV-1: an important factor but not the only one. The European Collaborative Study. AIDS. 1999 Jul 30;13(11):1377-85

[32] Ioannidis JP, Abrams EJ, Ammann A, Bulterys M, Goedert JJ, Gray L, Korber BT, Mayaux MJ, Mofenson LM, Newell ML, Shapiro DE, Teglas JP, Wilfert CM. Perinatal

transmission of human immunodeficiency virus type 1 by pregnant women with RNA virus loads <1000 copies/ml. J Infect Dis. 2001 Feb 15;183(4):539-45

[33] European Collaborative Study. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. Clin Infect Dis. 2005 Feb 1;40(3):458-65

[34] Landesman SH, Kalish LA, Burns DN, Minkoff H, Fox HE, Zorrilla C, Garcia P, Fowler MG, Mofenson L, Tuomala R. Obstetrical factors and the transmission of human immunodeficiency virus type 1 from mother to child. The Women and Infants Transmission Study. N Engl J Med. 1996 Jun 20; 334(25):1617-23.

[35] Newell ML, Dunn DT, Peckham CS, Semprini AE, Pardi G. Vertical transmission of HIV-1: maternal immune status and obstetric factors. The European Collaborative Study. AIDS. 1996 Dec;10(14):1675-81

[36] Van Dyke RB, Korber BT, Popek E, Macken C, Widmayer SM, Bardeguez A, Hanson IC, Wiznia A, Luzuriaga K, Viscarello RR, Wolinsky S. The Ariel Project: A prospective cohort study of maternal-child transmission of human immunodeficiency virus type 1 in the era of maternal antiretroviral therapy. J Infect Dis. 1999 Feb;179(2):319-28

[37] International Perinatal HIV Group. Duration of ruptured membranes and vertical transmission of HIV-1: a meta-analysis from 15 prospective cohort studies. AIDS. 2001 Feb 16;15(3):357-68

[38] Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwatha A, Ndinya-Achola J, Bwayo J, Onyango FE, Hughes J, Kreiss J. Effect of breastfeeding and formula

feeding on transmission of HIV-1: a randomized clinical trial. JAMA. 2000 Mar 1;283(9):1167-74

[39] Mackelprang RD, John-Stewart G, Carrington M, Richardson B, Rowland-Jones S, Gao X, Mbori-Ngacha D, Mabuka J, Lohman-Payne B, Farquhar C. Maternal HLA homozygosity and mother-child HLA concordance increase the risk of vertical transmission of HIV-1. J Infect Dis. 2008 Apr 15; 197(8):1156-61

[40] Bedoya VI, Jaimes FA, Delgado JC, Rugeles C, Usuga X, Zapata W, Castaño ME, Boasso A, Shearer G, Rugeles MT. Fetal-maternal HLA-A and -B discordance is associated with placental RNase expression and anti-HIV-1 activity. Curr HIV Res. 2008 Jun; 6(4):380-7

[41] U.S. Public Health Service Recommendations for Human Immunodeficiency Virus Counseling and Voluntary Testing for Pregnant Women. MMWR Morb Mortal Wkly Rep July 7 1995/44 (RR-7); 1 – 15

[42] Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, VanDyke R, Bey M, Shearer W, Jacobson RL, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med. 1994 Nov 3;331(18):1173-80

[43] Report of the Surveillance sub-committee of the National AIDS strategy committee on anonymous unlinked antenatal HIV screening in Ireland. Results for the period 4th Quarter 1992 to 4th Quarter 2000. Available at www.hpsc.ie

[44] Centers for Disease Control and Prevention. HIV testing among pregnant women--United States and Canada, 1998-2001. MMWR Morb Mortal Wkly Rep. 2002 Nov 15;51(45):1013-1016

[45] Lallemant M, Jourdain G, Le Coeur S, Kim S, Koetsawang S, Comeau AM, Phoolcharoen W, Essex M, McIntosh K, Vithayasai V. A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type Perinatal HIV Prevention Trial (Thailand) Investigators. N Engl J Med. 2000 Oct 5;343(14):982-91

[46] Wade NA, Birkhead GS, Warren BL, Charbonneau TT, French PT, Wang L, Baum JB, Tesoriero JM, Savicki R. Abbreviated regimens of zidovudine prophylaxis and perinatal transmission of the human immunodeficiency virus. N Engl J Med. 1998 Nov 12;339(20):1409-14

[47] Shaffer N, Chuachoowong R, Mock PA, Bhadrakom C, Siriwasin W, Young NL, Chotpitayasunondh T, Chearskul S, Roongpisuthipong A, Chinayon P, Karon J, Mastro TD, Simonds RJ. Short-course zidovudine for perinatal HIV-1 transmission in Bangkok, Thailand: a randomised controlled trial. Bangkok Collaborative Perinatal HIV Transmission Study Group.Lancet. 1999 Mar 6;353(9155):773-80

[48] Mayaux MJ, Teglas JP, Mandelbrot L, Berrebi A, Gallais H, Matheron S, Ciraru-Vigneron N, Parnet-Mathieu F, Bongain A, Rouzioux C, Delfraissy JF, Blanche S. Acceptability and impact of zidovudine for prevention of mother-to-child human immunodeficiency virus-1 transmission in France. J Pediatr. 1997 Dec;131(6):857-62

[49] Jackson JB, Musoke P, Fleming T, Guay LA, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Owor M, Ducar C, Deseyve M, Mwatha A, Emel L, Duefield C, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Gigliotti M, Bray D, Mmiro F. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. Lancet. 2003 Sep 13;362(9387):859-68

[50] Katlama C, Ingrand D, Loveday C, Clumeck N, Mallolas J, Staszewski S, Johnson M, Hill AM, Pearce G, McDade H. Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group. JAMA. 1996 Jul 10;276(2):118-25

[51] Mandelbrot L, Landreau-Mascaro A, Rekacewicz C, Berrebi A, Benifla JL, Burgard M, Lachassine E, Barret B, Chaix ML, Bongain A, Ciraru-Vigneron N, Crenn-Hebert C, Delfraissy JF, Rouzioux C, Mayaux MJ, Blanche S; Agence Nationale de Recherches sur le SIDA (ANRS) 075 Study Group. Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. JAMA. 2001 Apr 25;285(16):2083-93

[52] Clarke SM, Mulcahy F, Healy CM, Condon S, Butler KM. The efficacy and tolerability of combination antiretroviral therapy in pregnancy: infant and maternal outcome. Int J STD AIDS. 2000 Apr;11(4):220-3

[53] Lyons F, Butler K, Coulter Smith S, Mulcahy F. National guidelines for the management of HIV-1 in pregnancy. Ir Med J. 2006 May;100(1):152-4

[54] Cooper ER, Charurat M, Mofenson L, Hanson IC, Pitt J, Diaz C, Hayani K, Handelsman E, Smeriglio V, Hoff R, Blattner W; Women and Infants' Transmission Study Group. Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr. 2002 Apr 15;29(5):484-94

[55] Martin F, Navaratne L, Khan W, Sarner L, Mercey D, Anderson J, Noble H, Fakoya A, Hawkins DA, De Ruiter A, Taylor GP. Pregnant women with HIV infection can expect healthy survival: three-year follow-up. J Acquir Immune Defic Syndr. 2006 Oct 1;43(2):186-92

[56] D Ekouev, A Inwoley, B Tonwe-Gold, C Danel, R Becquet, T N'dri-Yoman, F Rouet, V Leroy, Xavier Anglaret, and F Dabis. Criteria for HAART Should Be Revisited in HIV-infected Pregnant Women in Resource-limited Settings. Poster 704a, 13th Conference of Retroviruses and Opportunistic Infections, Denver, February 2006

[57] Mulcahy F, Wallace E, Woods S, Hanlon M, Clarke S, Bergin B, and Jackson J. CD4 Counts in Pregnancy Do not Accurately Reflect the Need for Long-term HAART. Poster 704b, 13th Conference of Retroviruses and Opportunistic Infections, Denver, February 2006

[58] Stringer JS, Rouse DJ, Goldenberg RL. Prophylactic cesarean delivery for the prevention of perinatal human immunodeficiency virus transmission: the case for restraint. JAMA. 1999 May 26;281(20):1946-9

[59] No authors listed. Elective caesarean-section versus vaginal delivery in prevention of vertical HIV-1 transmission: a randomised clinical trial. The European Mode of Delivery Collaboration. Lancet. 1999 Mar 27;353(9158):1035-9

[60] No authors listed. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1--a meta-analysis of 15 prospective cohort studies. The International Perinatal HIV Group. N Engl J Med. 1999 Apr 1;340(13):977-87

[61] Public Health Service Task Force Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States - November 2, 2007. Available at www.aidsinfo.nih.gov

[62] British HIV association and Children's HIV association guidelines for the management of HIV infection in pregnant women, 2008. Available at www.bhiva.org

[63] No Authors listed. ACOG educational bulletin. Assessment of fetal lung maturity. Number 230, November 1996. Committee on Educational Bulletins of the American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet. 1997 Feb;56(2):191-8

[64] O'Meara M, Goode M, Hayes E, Griffin R and Butler K. Simplification of the neonatal component of regimens to prevent perinatal HIV transmission (PHT). Poster 70, 10th Conference on Retroviruses and Opportunistic Infections, Boston 2003, P70

[65] Fiore S, Newell ML, Thorne C; European HIV in Obstetrics Group. Higher rates of post-partum complications in HIV-infected than in uninfected women irrespective of mode of delivery. AIDS. 2004 Apr 9;18(6):933-8

[66] Lapaire O, Irion O, Koch-Holch A, Holzgreve W, Rudin C, Hoesli I; The Swiss Mother and Child HIV Cohort Study. Increased peri- and post-elective cesarean section morbidity in women infected with human immunodeficiency virus-1: a case-controlled multicenter study. Arch Gynecol Obstet. 2006 Jun;274(3):165-169

[67] Avidan MS, Groves P, Blott M, Welch J, Leung T, Pozniak A, Davies E, Ball C, Zuckerman M. Low complication rate associated with cesarean section under spinal anesthesia for HIV-1-infected women on antiretroviral therapy. Anesthesiology. 2002 Aug;97(2):320-4

[68] Read JS, Tuomala R, Kpamegan E, Zorrilla C, Landesman S, Brown G, Vajaranant M, Hammill H, Thompson B; Women and Infants Transmission Study Group. Mode of delivery and postpartum morbidity among HIV-infected women: the women and infants transmission study. J Acquir Immune Defic Syndr. 2001 Mar 1;26(3):236-45

[69] Marcollet A, Goffinet F, Firtion G, Pannier E, Le Bret T, Brival ML, Mandelbrot L Differences in postpartum morbidity in women who are infected with the human immunodeficiency virus after elective cesarean delivery, emergency cesarean delivery, or vaginal delivery. Am J Obstet Gynecol. 2002 Apr;186(4):784-9

[70] Duarte G, Read JS, Gonin R, Freimanis L, Ivalo S, Melo VH, Marcolin A, Mayoral C, Ceriotto M, de Souza R, Cardoso E, Harris DR; NISDI Perinatal Study Group.

Mode of delivery and postpartum morbidity in Latin American and Caribbean countries among women who are infected with human immunodeficiency virus-1: the NICHD International Site Development Initiative (NISDI) Perinatal Study. Am J Obstet Gynecol. 2006 Jul;195(1):215-29

[71] Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome. MMWR Morb Mortal Wkly Rep. 1985;34:721-731

[72] Re: Important Change in SUSTIVA (efavirenz) Package Insert — Change from Pregnancy Category C to D, Bristol-Myers Squibb Company, Dear Health Care Provider Letter March 2005

[73] Antiretroviral Pregnancy Registry International Interim Report for 1 January 1989 – 31 January 2008. Available at www.apregistry.com

[74] Taylor S, Allen S, Fidler S et al. Stop Study. After discontinuation of Efavirenz, plasma concentrations may persist for 2 weeks or longer. Abstract 131, 11th Conference on Retroviruses and Opportunistic Infections, San Francisco, 2004

[75] Sarner L, Fakoya A. Acute onset lactic acidosis and pancreatitis in the third trimester of pregnancy in HIV-1 positive women taking antiretroviral medication. Sex Transm Infect. 2002 Feb;78(1):58-9

[76] Martin A, Smith DE, Carr A, Ringland C, Amin J, Emery S, Hoy J, Workman C, Doong N, Freund J, Cooper DA; Mitochondrial Toxicity Study Group. Reversibility of lipoatrophy in

HIV-infected patients 2 years after switching from a thymidine analogue to abacavir: the MITOX Extension Study. AIDS. 2004 Apr 30;18(7):1029-36

[77] Mallal SA, John M, Moore CB, James IR, McKinnon EJ. Contribution of NRTIs to subcutaneous fat wasting in patients with HIV infection. AIDS. 2000 Jul 7;14(10):1309-16

[78] Saint-Marc T, Partisani M, Poizot-Martin I, Bruno F, Rouviere O, Lang JM, Gastaut JA, Touraine JL. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. AIDS. 1999 Sep 10;13(13):1659-67

[79] Stern JO, Robinson PA, Love J, Lanes S, Imperiale MS, Mayers DL. A comprehensive hepatic safety analysis of nevirapine in different populations of HIV infected patients. J Acquir Immune Defic Syndr. 2003 Sep;34 Suppl 1:S21-33

[80] . Hopkins, A. Mc Geary, G. Sheehan, C. Bergin, and F. Mulcahy. Nevirapine tolerability in HIV infected women in pregnancy – A word of caution. Late breaker 27, 2nd IAS conference on HIV pathogenesis and treatment. Paris, France July 2003

[81] Blanche S, Tardieu M, Rustin P, Slama A, Barret B, Firtion G, Ciraru-Vigneron N, Lacroix C, Rouzioux C, Mandelbrot L, Desguerre I, Rötig A, Mayaux MJ, Delfraissy JF. Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues. Lancet. 1999 Sep 25;354(9184):1084-9

[82] Bulterys M, Nesheim S, Abrams EJ, Palumbo P, Farley J, Lampe M, Fowler MG; Perinatal Safety Review Working Group. Lack of evidence of mitochondrial dysfunction in the offspring of HIV-infected women. Retrospective review of perinatal exposure to

antiretroviral drugs in the Perinatal AIDS Collaborative Transmission Study. Ann N Y Acad Sci. 2000 Nov;918:212-21

[83] Poirier MC, Divi RL, Al-Harthi L, Olivero OA, Nguyen V, Walker B, Landay AL, Walker VE, Charurat M, Blattner WA; Women and Infants Transmission Study (WITS) Group. Long-term mitochondrial toxicity in HIV-uninfected infants born to HIV-infected mothers. J Acquir Immune Defic Syndr 2003 Jun 1;33(2):175-83

[84] Barret B Tardieu M, Rustin P, Lacroix C, Chabrol B, Desguerre I, Dollfus C, Mayaux MJ, Blanche S; For the French Perinatal Cohort Study Group. Persistent mitochondrial dysfunction in HIV-1-exposed but uninfected infants: clinical screening in a large prospective cohort. AIDS 2003 Aug 15;17(12):1769-85

[85] Divi RL, Walker VE, Wade NA, Nagashima K, Seilkop SK, Adams ME, Nesel CJ, O'Neill JP, Abrams EJ, Poirier MC. Mitochondrial damage and DNA depletion in cord blood and umbilical cord from infants exposed in utero to Combivir AIDS. 2004 Apr 30;18(7):1013-21

[86] Lechelt M, Lyons F, Clarke A, Magaya V, Issa R, de Ruiter A. Human placental transfer of atazanavir: a case report. AIDS. 2006 Jan 9;20(2):307

[87] Ripamonti D, Cattaneo D, Maggiolo F, Airoldi M, Frigerio L, Bertuletti P, Ruggeri M, Suter F. Atazanavir plus low-dose ritonavir in pregnancy: pharmacokinetics and placental transfer. AIDS. 2007 Nov 30; 21(18):2409-15

[88] Elay, T, Vandeloise E, Child M et al for the atazanavir 182 pregnancy study group. Steady state pharmacokinetics and safety of atazanavir after treatment with ATV300mg

once daily/ritonavir 100mg once daily + ZDV/3TC during the third trimester in HIV+ women. Poster 624, 15th Conference of Retroviruses and Opportunistic Infections, Boston 2008

[89] Ferreira C, F Loch-Tudal C, Meier F et al. Atazanavir in pregnancy: influence on neonatal hyperbilirubinemia. Poster 625, 15th Conference of Retroviruses and Opportunistic Infections, Boston 2008

[90] Thorne C, Patel D, Newell ML.Increased risk of adverse pregnancy outcomes in HIV-infected women treated with HAART in Europe. AIDS. 2004 Nov 19;18(17):2337-9

[91] Townsend CL, Cortina-Borja M, Peckham CS, Tookey PA. Antiretroviral therapy and premature delivery in diagnosed HIV-infected women in the United Kingdom and Ireland. AIDS. 2007 May 11;21(8):1019-26

[92] Tuomala RE, Watts DH, Li D, Vajaranant M, Pitt J, Hammill H, Landesman S,Zorrilla C,Thompson B; Women and Infants Transmission Study. Improved obstetric outcomes and few maternal toxicities are associated with antiretroviral therapy, including HAART during pregnancy. J Acquir Immune Defic Syndr. 2005 Apr 1;38(4):449-73

[93] Cotter AM, Garcia AG, Duthely ML, Luke B, O'Sullivan MJ. Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth? J Infect Dis. 2006 May 1;193(9):1195-201

[94] Cotter A, Duthely M, O'Sullivan MJ, Romanguera R. Antiretroviral therapy and preterm delivery: another clue in the puzzle?. Am J Obstet Gynecol 2004; 191: S59

[95] Fiore S, Newell ML, Trabattoni , Thorne C, Gray L, Savasi V, Tibaldi C, Ferrazzi E and Clerici M. Antiretroviral therapy-associated modulation of Th1 and Th2 immune responses in HIV-infected pregnant women. J Reprod Immunol. 2006 Jun;70(1-2):143-50

[96] Stratton P, Tuomala RE, Abboud R, Rodriguez E, Rich K, Pitt J, Diaz C, Hammill H, Minkoff H. Obstetric and newborn outcomes in a cohort of HIV-infected pregnant women: a report of the women and infants transmission study. J Acquir Immune Defic Syndr Hum Retrovirol. 1999 Feb 1;20(2):179-86

[97] Wimalasundera RC, Larbalestier N, Smith JH, de Ruiter A, McG Thom SA, Hughes AD, Poulter N, Regan L, Taylor GP Pre-clampsia, antiretroviral therapy, and immune reconstitution. Lancet. 2002 Oct 12;360(9340):1152-4

[98] Suy A, Martı´nez E, Coll O, Lonca M, Palacio M, de Lazzari E, Larrousse M, Milinkovic A, Herna´ndez S, Blanco J, Mallolas J, Leo´n A, Vanrell J and Gatell M. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS. 2006 Jan 2;20(1):59-66

[99] Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, Koup RA, Mellors JW, Connick E, Conway B, Kilby M, Wang L, Whitcomb JM, Hellmann NS, Richman DD. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med. 2002 Aug 8;347(6):385-94

[100] Eshleman SH, Mracna M, Guay LA, Deseyve M, Cunningham S, Mirochnick M, Musoke P, Fleming T, Glenn Fowler M, Mofenson LM, Mmiro F, Jackson JB. Selection and fading

of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS 2001 Oct 19;15(15):1951-7

[101] Jackson JB, Becker-Pergola G, Guay LA, Musoke P, Mracna M, Fowler MG, Mofenson LM, Mirochnick M, Mmiro F, Eshleman SH. Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. AIDS 2000 Jul 28;14(11):F111-5

[102] Cunningham CK, Chaix ML, Rekacewicz C, Britto P, Rouzioux C, Gelber RD, Dorenbaum A, Delfraissy JF, Bazin B, Mofenson L, Sullivan JL. Development of resistance mutations in women receiving standard antiretroviral therapy who received intrapartum nevirapine to prevent perinatal human immunodeficiency virus type 1 transmission: a substudy of pediatric AIDS clinical trials group protocol 316. J Infect Dis 2002 Jul 15;186(2):181-8

[103] Palmer S,Boltz V,Martinson N,Maldarelli F,Gray G, McIntyre J, Mellors J, Morris L Coffin J. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. Proc Natl Acad Sci USA. 2006 May 2;103(18):7094-9

[104] Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, Leechanachai P, Ariyadej S, Leenasirimakul P, Hammer S, Lallemant M; Perinatal HIV Prevention Trial Group. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med 2004 Jul 15;351(3):229-40

[105] Welles S, Pitt J, Colgrove R, Mcintosh K et al. HIV-1 genotypic zidovudine drug resistance and the risk of maternal-infant transmission in the Women and Infants Transmission Study. AIDS 100;14:263-271

[106] Larbalestier N, Mullen J, O'Shea S, Cottam F, Sabin CA, Chrystie IL, Welch J, Zuckerman M, Hay P, Rice P, Taylor GP, de Ruiter A. Drug resistance is uncommon in pregnant women with low viral loads taking zidovudine monotherapy to prevent perinatal HIV transmission. AIDS 2003 Dec 5;17(18):2665-7

[107] Read P, Costello S, Mullen J, O'Shea S, Lyons F, et al. Does zidovudine monotherapy in pregnancy predispose to emergence of resistance? HIV Medicine 2006; 7 (suppl 1): 2 (O5)

[108] M Mirochnick and E Capparelli. Pharmacokinetics of antiretrovirals in pregnant women. Clin Pharmacokinet. 2004;43(15):1071-87

[109] Stek AM, Mirochnick M, Capparelli E, Best BM, Hu C, Burchett SK, Elgie C, Holland DT, Smith E, Tuomala R, Cotter A, Read JS. Reduced lopinavir exposure during pregnancy. AIDS. 2006 Oct 3;20(15):1931-9

[110] Lyons F; Lechelt M; De Ruiter A. Steady-state lopinavir levels in third trimester of pregnancy. AIDS 11 May 2007: 21(8); 1053–1054

[111] Hanlon M, Ward D, O'Dea S, Lyons F, Mulcahy F, Clarke S. Pharmacokinetics of saquinavir in pregnancy. HIV Med 2008 May; 9 (suppl 1) P62

[112] Clarke S, Butler K, Bergin C, Lyons F, Sheehan G, Horgan M, Mulcahy F; Irish Infection Society. National guidelines for the active management of HIV in pregnancy. Ir Med J 2001 May;94(5):137-40

[113] Annual Reports 2001 to 2006, Office of the Refugee Commissioner, www.orac.ie

[114] Voluntary Antenatal HIV Testing in Ireland: Results of the Screening Programme 2002 – 2006, A Report by the Health Protection Surveillance Centre, November 2007, available at www.hpsc.ie

[115] Lyons F, Clarke S, Mulcahy F, Bergin C. Pregnancies in HIV positive asylum seekers in Dublin. Poster presentation, 7th Conference of the International Society of Travel Medicine Innsbruck, Austria, May 2001

[116]O'Dea S. African Women's Experiences of the Irish Health Service following a Positive HIV Diagnosis during Pregnancy. Unpublished Masters Thesis, 2006. Global Health Library, Trinity College, Dublin

[117] AIAU, NSHPC, CHIVA. Perinatal transmission of HIV in England 2002-2005, Executive Summary and Recommendations, October 2007. Available at www.nshpc.ucl.ac.uk.

[118] Townsend CL, Cortina-Borja M, Peckham CS, de Ruiter A, Lyall H, Tookey PA. Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006. AIDS 2008 May 11;22(8):973-81

[119] C Bell, M Douglas, Y Gilleece, N Desmond, and G Taylor Early Viral Load Reduction in Pregnant Women Differs according to Antiretroviral Regimen. Poster 656, 15th Conference on Retroviruses and Opportunistic Infections, Boston 2008

[120] Viramune Tablets. Summary of Product Characteristics, United Kingdom and Republic of Ireland

[121] Aweeks F, Lizak P, Frenkel L, Au S, Watts H, McNamara J, Stevens L, Hitti J and PACTG 1022 study team. Steady state nevirapine pharmacokinetics during second and third trimester pregnancy and postpartum: PACTG 1022. Abstract 932, 11th Conference on retroviruses and opportunistic infections, San Francisco 2004

[122] von Hentig N, Carlebach A, Gute P, Knecht G, Klauke S,Rohrbacher M, Stocker H, Kurowski M, Harder S, Staszewski S, Haberl A. A comparison of the steady-state pharmacokinetics of nevirapine in men, nonpregnant women and women in late pregnancy. Br J Clin Pharmacol. 2006 Nov;62(5):552-9

[123] Mackie NE, Fidler S, Tamm N, Clarke JR, Back D, Weber JN, Taylor GP Clinical implications of stopping nevirapine-based antiretroviral therapy: relative pharmacokinetics and avoidance of drug resistance. HIV Med 2004 May;5(3):180-4

[124] de Maat M, Nellen J, Huitema A, Wit F,Mulder J, Prins J and Beijnen J. Race Is Not Associated with Nevirapine Pharmacokinetics. Ther Drug Monit. 2004 Aug;26(4):456-8

[125] Turner D, Brenner B, Spira B, Schapiro J, Songok J, Wainberg MA. Novel drug resistance profiles in Non-B subtype HIV-1 infections. Abstract 144, 10th Conference on retroviruses and opportunistic infections, Boston 2003

[126] Perno CF, Cozzi-Lepri A, Forbici F, Bertoli A, Violin M, Mura MS et al. Minor mutations in HIV protease at baseline and appearance of primary 90M in patients for whom their first protease-inhibitor antiretroviral regimens failed. J Infect Dis. 2004 Jun 1;189(11):1983-7

[127] Eshleman SH, Guay LA, Mwatha A, Cunningham SP, Brown ER, Musoke P, Mmiro F, Jackson JB. Comparison of nevirapine (NVP) resistance in Ugandan women 7 days vs. 6-8 weeks after single-dose nvp prophylaxis: HIVNET 012. AIDS Res Hum Retroviruses. 2004 Jun;20(6):595-9

[128] Laine C, Newschaffer CJ, Zhang D, Cosler L, Hauck WW and Turner B. Adherence to antiretroviral therapy by pregnant women infected with Human Immunodeficiency Virus: A pharmacy claims-based analysis. Obstet Gynecol. 2000 Feb;95(2):167-73

[129] Pillay C, Bredell H, McIntyre J, Gray G and Morris L. HIV-1 Subtype C reverse transcriptase sequences from drug naïve pregnant women in South Africa. AIDS Res Hum Retroviruses. 2002 May 20;18(8):605-10

[130] Grossman Z, Istomin V, Levi I, Risenberg K, Chowers M, Shahar E et al. Greater genetic variation at NNRTI-associated resistance positions in patients infected by subtype C. Abstract 624, 10th Conference on retroviruses and opportunistic infections, Boston 2003

[131] Kearney M, Palmer S, Maldarelli F, Bixby C, Bazmi H, Rock D et al. Single-genome sequencing is more sensitive than standard genotype analysis for detection of HIV-1 drug resistance mutations. Abstract 695, 11th Conference on retroviruses and opportunistic infections, San Francisco 2004

[132] No authors listed. Serious adverse events attributed to nevirapine regimens for post exposure prophylaxis after HIV exposures - Worldwide, 1997-2000 MMWR Morb Mortal Wkly Rep. 2001 Jan 5; 49 (51-52):1153-6

[133] Heemann U, Treichel U, Loock J, Philipp T, Gerken G, Malago M, Klammt S, Loehr M, Liebe S, Mitzner S, Schmidt R, Stange J. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. Hepatology. 2002 Oct;36(4 Pt 1):949-58

[134] For the PACTG 1022 Study Team. Maternal Toxicity with Continuous Nevirapine in Pregnancy: Results from PACTG 1022. J Acquir Immune Defic Syndr 2004 Jul 1; 36(3):772-776

[135] Langlet P, Guillaume MP, Devriendt J et al. Fatal Liver failure associated with nevirapine in a pregnant HIV patient: the first reported case. Gastroenterology 2000; 118: A1461

[136] Patel SM, Johnson S, Belknap SM, Chan J, Sha BE, Bennett C. Serious adverse cutaneous and hepatic toxicities associated with nevirapine use by non-HIV-infected individuals. J Acquir Immune Defic Syndr 2004 Feb 1; 35(2):120-5

[137] Kumar GP, Bhat VJ, Sowdi V. Fulminant hepatic failure following halothane anaesthesia. J Clin Forensic Med. 2005 Oct;12(5):271-3

[138] Otedo AE. Halothane induced hepatitis: case report. East Afr Med J. 2004 Oct;81(10):538-9

[139] Sulkowski MS, Thomas DL, Chaisson RE, Moore DE. Hepatotoxicity after introduction of antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA. 2000 Jan 5;283(1):74-80

[140] Sanne I on behalf of the FTC-302 Study Investigators and the FTC-302 Independent Clinical Steering Committee. Severe liver toxicity in patients receiving two nucleoside analogues and a non-nucleoside reverse transcriptase inhibitor. AIDS 2000; 14(suppl 4): S12

[141] Wong KH, Chan KC, Lee SS. Sex differences in nevirapine rash. Clin Infect Dis. 2001 Dec 15;33(12):2096-8

[142] Dailly E, Billaud E, Reliquet V, Breurec S, Perre P, Leautez S, Jolliet P, Bourin M, Raffi F. No relationship between high nevirapine plasma concentration and hepatotoxicity in HIV-1-infected patients naive of antiretroviral treatment or switched from protease inhibitors. Eur J Clin Pharmacol. 2004 Jul; 60(5):343-8

[143] Gonzalez de Requena D, Nunez M, Jimenez-Nacher I, Soriano V. Liver toxicity caused by nevirapine. AIDS. 2002 Jan 25;16(2):290-1

[144] João EC, Calvet GA, Menezes JA, D'Ippolito MM, Cruz ML, Salgado LA, Matos HJ. Nevirapine toxicity in a cohort of HIV-1-infected pregnant women. Am J Obstet Gynecol. 2006 Jan;194(1):199-202

[145] Marazzi MC, Germano P, Liotta G, Guidotti G, Loureiro S, da Cruz Gomes A, Valls Blazquez MC, Narciso P, Perno CF, Mancinelli S and Palombi L. Safety of nevirapine-containing antiretroviral triple therapy regimens to prevent vertical transmission in an African cohort of HIV-1-infected pregnant women. HIV Med. 2006 Jul;7(5):338-44

[146] Littera R, Carcassi C, Masala A, Piano P, Serra P, Ortu F, Corso N, Casula B, La Nasa G, Contu L, Manconi PE. HLA-dependent hypersensitivity to nevirapine in Sardinian HIV patients. AIDS 2006 Aug 1;20(12):1621-6

[147] Martin AM, Nolan D, James I, Cameron P, Keller J, Moore C, Phillips E, Christiansen FT, Mallal S. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1*0101 and abrogated by low CD4 T-cell counts. AIDS. 2005 Jan 3;19(1):97-9

[148] Ferguson W, Goode M, Walsh A, Gavin P, Butler K. Four weeks neonatal antiretroviral therapy is sufficient to optimally prevent mother to child transmission of HIV. Abstract ?, IDSA/ICAAC annual meeting, Washington DC 2008

[149] Cumiskey J, Mavinkurve M, Paneth-Pollak R, Borrelli J, Kowalski A, Blank S, Branson B. False-positive oral fluid rapid HIV tests, New York City, 2005 – 2008. MMWR Morb Mortal Wkly Rep June 20, 2008/57 (24); 660 – 665

[150] Braun P, Ehret R, Wiesmann F, Zabbai F, Knickmann M, Kühn R, Thamm S, Warnat G, Knechten H. Comparison of four commercial quantitative HIV-1 assays for viral load monitoring in clinical daily routine. Clin Chem Lab Med. 2007;45(1):93-9

[151] SPREAD Programme. Transmission of drug-resistant HIV-1 in Europe remains limited to single classes. AIDS 2008 Mar 12;22(5):625-35

[152] Lyons FE, Coughlan S, Byrne CM, Hopkins SM, Hall WW, Mulcahy FM Emergence of antiretroviral resistance in HIV-positive women receiving combination antiretroviral therapy in pregnancy. AIDS 2005 Jan 3;19(1):63-7. Erratum in: AIDS. 2007 Jul 31;21(12):1671

[153] Moodley D, Moodley J, Coovadia H, Gray G, McIntyre J, Hofmyer J, Nikodem C, Hall D, Gigliotti M, Robinson P, Boshoff L, Sullivan JL; South African Intrapartum Nevirapine Trial (SAINT) Investigators. A multicenter randomized controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early postpartum mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis. 2003 Mar 1;187(5):725-35

[154]Arrivé E, Newell ML, Ekouevi DK, Chaix ML, Thiebaut R, Masquelier B, Leroy V, Perre PV, Rouzioux C, Dabis F; Ghent Group on HIV in Women and Children Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Int J Epidemiol. 2007 Oct; 36(5):1009-21.

[155] Jourdain G, Mary JY, Coeur SL, Ngo-Giang-Huong N, Yuthavisuthi P, Limtrakul A, Traisathit P, McIntosh K, Lallemant M; Perinatal HIV Prevention Trial Group, Thailand.

Risk factors for in utero or intrapartum mother-to-child transmission of human immunodeficiency virus type 1 in Thailand. J Infect Dis. 2007 Dec 1;196(11):1629-36

[156] Lyons F, Hopkins S, Kelleher B, McGeary A, Sheehan G, Geoghegan J, Bergin C, Mulcahy FM, McCormick PA. Maternal hepatotoxicity with nevirapine as part of combination antiretroviral therapy in pregnancy. HIV Med 2006 May;7(4):255-60

[157] Tarantal AF, Castillo A, Ekert JE, Bischofberger N, Martin RB Fetal and maternal outcome after administration of tenofovir to gravid rhesus monkeys (Macaca mulatta). J Acquir Immune Defic Syndr. 2002 Mar 1;29(3):207-20.

[158] Gilson RJ, Hawkins AE, Beecham MR, Ross E, Waite J, Briggs M, McNally T, Kelly GE, Tedder RS, Weller IV. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. AIDS. 1997 Apr;11(5):597-606

[159] Colin JF, Cazals-Hatem D, Loriot MA, Martinot-Peignoux M, Pham BN, Auperin A, Degott C, Benhamou JP, Erlinger S, Valla D, Marcellin P. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology. 1999 Apr;29(4):1306-10.

[160] van Zonneveld M, van Nunen AB, Niesters HG, de Man RA, Schalm SW, Janssen HL. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. J Viral Hepat. 2003 Jul;10(4):294-7

[161] Gibb DM, Goodall RL, Dunn DT, Healy M, Neave P, Cafferkey M, Butler K.

Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. Lancet 2000 Sep 9;356(9233):904-7

[162] Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. Hepatology 2000 Mar;31(3):751-5

[163] Polis CB, Shah SN, Johnson KE, Gupta A Impact of maternal HIV coinfection on the vertical transmission of hepatitis C virus: a meta-analysis. Clin Infect Dis 2007 Apr 15; 44(8):1123-31

[164] Mariné-Barjoan E, Berrébi A, Giordanengo V, Favre SF, Haas H, Moreigne M, Izopet J, Tricoire J, Tran A, Pradier C, Bongain A HCV/HIV co-infection, HCV viral load and mode of delivery: risk factors for mother-to-child transmission of hepatitis C virus? AIDS 2007 Aug 20; 21(13):1811-5

[165] Dobosz S. The risk of vertical HCV transmission in children born to HIV infected mothers. Przegl Epidemiol. 2007;61(2):349-56

[166] European Paediatric Hepatitis C Virus Network. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. European Paediatric Hepatitis C Virus Network. BJOG 2001 Apr;108(4):371-7

[167] European Paediatric Hepatitis C Virus Network. A significant sex--but not elective cesarean section--effect on mother-to-child transmission of hepatitis C virus infection. J Infect Dis. 2005 Dec 1;192(11):1872-9

[168] Mwapasa V, Rogerson SJ, Kwiek JJ, Wilson PE, Milner D, Molyneux ME, Kamwendo DD, Tadesse E, Chaluluka E, Meshnick SR. Maternal syphilis infection is associated with increased risk of mother-to-child transmission of HIV in Malawi. AIDS 2006 Sep 11;20(14):1869-77

[169] Lee MJ, Hallmark RJ, Frenkel LM, Del Priore G Maternal syphilis and vertical perinatal transmission of human immunodeficiency virus type-1 infection. Int J Gynaecol Obstet. 1998 Dec; 63(3):247-52

[170] Drake AL, John-Stewart GC, Wald A, Mbori-Ngacha DA, Bosire R, Wamalwa DC, Lohman-Payne BL, Ashley-Morrow R, Corey L, Farquhar C. Herpes simplex virus type 2 and risk of intrapartum human immunodeficiency virus transmission. Obstet Gynecol. 2007 Feb;109(2 Pt 1):403-9. Erratum in: Obstet Gynecol. 2007 Apr;109(4):1002-3

[171] Chen KT, Segú M, Lumey LH, Kuhn L, Carter RJ, Bulterys M, Abrams EJ; New York City Perinatal AIDS Collaborative Transmission Study (PACTS) Group.Genital herpes simplex virus infection and perinatal transmission of human immunodeficiency virus. Obstet Gynecol. 2005 Dec;106(6):1341-8

[172] Ouedraogo A, Nagot N, Vergne L, Konate I, Weiss HA, Defer MC, Foulongne V, Sanon A, Andonaba JB, Segondy M, Mayaud P, Van de Perre P. Impact of suppressive herpes

therapy on genital HIV-1 RNA among women taking antiretroviral therapy: a randomized controlled trial. AIDS 2006 Nov 28; 20(18):2305-13

[173] Nagot N, Ouédraogo A, Foulongne V, Konaté I, Weiss HA, Vergne L, Defer MC, Djagbaré D, Sanon A, Andonaba JB, Becquart P, Segondy M, Vallo R, Sawadogo A, Van de Perre P, Mayaud P; ANRS 1285 Study Group. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus N Engl J Med. 2007 Feb 22; 356(8):790-9

[174] Acyclovir and Valacyclovir in pregnancy registry final report. April 1999. available at http://pregnancyregistry.gsk.com/acyclovir.html

[175] A guide to ethical conduct and behaviour, 6th edition 2004, available at www.medicalcouncil.ie

[176] Justman JE, Benning L, Danoff A, Minkoff H, Levine A, Greenblatt RM, Weber K, Piessens E, Robison E, Anastos K. Protease inhibitor use and the incidence of diabetes mellitus in a large cohort of HIV-infected women. J Acquir Immune Defic Syndr. 2003 Mar 1; 32(3):298-302

[177] Mandelbrot L, Mayaux MJ, Bongain A, Berrebi A, Moudoub-Jeanpetit Y, Bénifla JL, Ciraru-Vigneron N, Le Chenadec J, Blanche S, Delfraissy JF. Obstetric factors and mother-to-child transmission of human immunodeficiency virus type 1: the French perinatal cohorts. SEROGEST French Pediatric HIV Infection Study Group. Am J Obstet Gynecol. 1996 Sep;175(3 Pt 1):661-7

[178] Somigliana E, Bucceri AM, Tibaldi C, Alberico S, Ravizza M, Savasi V, Marini S, Matrone R, Pardi G; Italian Collaborative Study on HIV Infection in Pregnancy. Early invasive diagnostic techniques in pregnant women who are infected with the HIV: a multicenter case series. Am J Obstet Gynecol 2005 Aug;193(2):437-42

[179] Coll O, Suy A, Hernandez S, Pisa S, Lonca M, Thorne C, Borrell A. Prenatal diagnosis in human immunodeficiency virus-infected women: a new screening program for chromosomal anomalies. Am J Obstet Gynecol. 2006 Jan;194(1):192-8

[180] Franklin J, Douglas M, Clarke A, Nartey G, Tookey P, Cortina-Borja M, Smith J, and Taylor G. Interpretation of Serum a-Fetoprotein, Human Chorionic Gonadotrophin, and the Risk of Down's Syndrome in Pregnant Women Infected with HIV. Abstract 655, 15th Conference of retroviruses and opportunistic infections, Boston 2008

[181] Warszawski J, Tubiana R, Le Chenadec J, Blanche S, Teglas JP, Dollfus C, Faye A, Burgard M, Rouzioux C, Mandelbrot L; ANRS French Perinatal Cohort. Mother-to-child HIV transmission despite antiretroviral therapy in the ANRS French Perinatal Cohort. AIDS. 2008 Jan 11;22(2):289-99

[182]Coovadia HM, Rollins NC, Bland RM, Little K, Coutsoudis A, Bennish ML, Newell ML. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet 2007 Mar 31; 369 (9567):1107-16.

[183] Thea D et al. HIV Exclusive Breastfeeding Associated with Significantly Lower Postnatal Transmission: Zambia. Late breaker, 14th Conference of retroviruses and opportunistic infections, Los Angeles, 2007

[184] Thior I, Lockman S, Smeaton LM, Shapiro RL, Wester C, Heymann SJ, Gilbert PB, Stevens L, Peter T, Kim S, van Widenfelt E, Moffat C, Ndase P, Arimi P, Kebaabetswe P, Mazonde P, Makhema J, McIntosh K, Novitsky V, Lee TH, Marlink R, Lagakos S, Essex M; Mashi Study Team. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA 2006 Aug 16; 296(7):794-805.

[185] Six Week Extended-Dose Nevirapine (SWEN) Study Team, Bedri A, Gudetta B, Isehak A, Kumbi S, Lulseged S, Mengistu Y, Bhore AV, Bhosale R, Varadhrajan V, Gupte N, Sastry J, Suryavanshi N, Tripathy S, Mmiro F, Mubiru M, Onyango C, Taylor A, Musoke P, Nakabiito C, Abashawl A, Adamu R, Antelman G, Bollinger RC, Bright P, Chaudhary MA, Coberly J, Guay L, Fowler MG, Gupta A, Hassen E, Jackson JB, Moulton LH, Nayak U, Omer SB, Propper L, Ram M, Rexroad V, Ruff AJ, Shankar A, Zwerski S. Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in Ethiopia, India, and Uganda: an analysis of three randomised controlled trials. Lancet 2008 Jul 26;372(9635):300-13

[186] Schneider S, Peltier A, Gras A, Arendt V, Karasi-Omes C, Mujawamariwa A, Ndimubanzi PC, Ndayisaba G, Wennig R. Efavirenz in human breast milk, mothers', and newborns' plasma. J Acquir Immune Defic Syndr 2008 Aug 1;48(4):450-4

[187] Wade NA, Zielinski MA, Butsashvili M, McNutt LA, Warren BL, Glaros R, Cheku B, Pulver W, Pass K, Fox K, Novello AC, Birkhead GS. Decline in perinatal HIV transmission in New York State (1997-2000). J Acquir Immune Defic Syndr 2004 Aug 15; 36(5):1075-82.

[188] Taha TE, Kumwenda NI, Gibbons A, Broadhead RL, Fiscus S, Lema V, Liomba G, Nkhoma C, Miotti PG, Hoover DR. Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: NVAZ randomised clinical trial. Lancet 2003 Oct 11;362 (9391):1171-7.

[189] Hanlon M, Clarke S, Mulcahy F, Lyons F. Documentation of contraception in HIV-1 infected women of child bearing potential prescribed efavirenz. HIV Med 2008 May; 9 (suppl 1) P91

[190] Abu Bakar MS, Surah S, Lyons F, Hurley F, de Ruiter A. An audit of efavirenz prescribing in women of child bearing potential attending an HIV unit. HIV Med 2008 May; 9 (suppl 1) P22