Safety and Toxicity of Individual Antiretroviral Agents in Pregnancy

Nucleoside & Nucleotide Analogue Reverse Transcriptase Inhibitors

There are currently seven approved nucleoside analogue reverse transcriptase inhibitors. Data are available from clinical trials in human pregnancy for zidovudine, lamivudine, didanosine**,** and stavudine. Abacavir, emtricitabine, and zalcitabine have not been studied in pregnant women. Tenofovir disoproxil fumarate is the first nucleotide analogue reverse transcriptase inhibitor. The nucleoside analogue drugs require three intracellular phosphorylation steps to form the triphosphate nucleoside, which is the active drug moiety; tenofovir, an acyclic nucleotide analogue drug, contains a monophosphate component attached to the adenine base, and hence only requires two phosphorylation steps to form the active moiety.

Abacavir (Ziagen®, ABC) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Some *in vitro* and *in vivo* mutagenesis and clastogenicity tests are positive. In long-term carcinogenicity studies in mice and rats, malignant tumors of the preputial gland of males and the clitoral gland of females were observed in both species, and malignant hepatic tumors as well as non-malignant hepatic and thyroid tumors were observed in female rats. The tumors were seen at doses in rodents that were 6 to 32 times higher than human exposure at therapeutic doses.
- Reproduction/fertility
  No effect of abacavir on reproduction or fertility in male and female rodents has been seen at doses of up to 500 mg/kg/day (about 8 times that of human therapeutic exposure).
- Teratogenicity/developmental toxicity
  Abacavir is associated with developmental toxicity (decreased fetal body weight and reduced crown-rump length) and increased incidence of fetal anasarca and skeletal malformations in rats treated with abacavir during organogenesis at doses of 1000 mg/kg (about 35 times that of human therapeutic exposure based on area under the curve (AUC)). Toxicity to the developing embryo and fetus (increased resorptions and decreased fetal body weight) occurred with abacavir administration to pregnant rodents at 500 mg/kg/day. The offspring of female rats treated with 500 mg/kg of abacavir beginning at embryo implantation and ending at weaning had an increased incidence of stillbirth and lower body weight throughout life. However, in the rabbit, no evidence of drug-related developmental toxicity was observed and no increase in fetal malformations was observed at doses up to 700 mg/kg (about 8.5 times that of human therapeutic exposure).

  In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to abacavir in humans have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with abacavir. The prevalence of birth defects with first trimester abacavir exposure was 3.2% (95% CI: 1.6–5.6%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.
- Placental and breast milk passage
  Abacavir crosses the placenta and is excreted into the breast milk of lactating rats.
- Human studies in pregnancy
  A phase I study of abacavir in pregnant women indicates that the area under curve drug concentration during pregnancy was similar to that at 6 to 12 weeks postpartum and to non-pregnant individuals *[2]*. Thus, no dose adjustment for abacavir is needed during pregnancy. Serious hypersensitivity reactions have been associated with abacavir therapy in non-pregnant adults and have rarely been fatal; symptoms include fever, skin rash, fatigue, and gastrointestinal symptoms such as nausea, vomiting, diarrhea, or abdominal pain. Abacavir should not be restarted following a hypersensitivity reaction because more severe symptoms will recur within hours and may include life-threatening hypotension and death.

Didanosine (Videx®, ddI) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Long-term animal carcinogenicity screening studies in rodents given didanosine have been negative.

- Reproduction/fertility
  There has been no effect of didanosine on reproduction or fertility in rodents or on preimplantation mouse embryos *[3]*.

- Teratogenicity/developmental toxicity
  No evidence of teratogenicity or toxicity was observed with administration of high doses of didanosine to pregnant rats, mice, or rabbits. Among cases of first trimester didanosine exposure reported to the Antiretroviral Pregnancy Registry, defects have been noted in 6.0% (14/234), compared to a rate of 1.0% (2/193) among those with exposures later in pregnancy. All defects were reviewed in detail by the Registry, and no pattern of defects was discovered. The rate and types of defects will continue to be monitored closely.

- Placental and breast milk passage
  Placental transfer of didanosine was limited in a phase I/II safety and pharmacokinetic study (cord-to-maternal blood ratio, 0.35–0.11) *[4]*. Didanosine is excreted in the milk of lactating rats; it is not known if didanosine is excreted in human breast milk.

- Human studies in pregnancy
  A phase I study (PACTG 249) of didanosine was conducted in 14 HIV-infected pregnant women enrolled at gestational age 26 to 36 weeks and treated through 6 weeks postpartum *[4]*. The drug was well-tolerated during pregnancy by the women and the fetuses. Pharmacokinetic parameters after oral administration were not significantly affected by pregnancy, and dose modification from the usual adult dosage is not needed.

  Cases of lactic acidosis, in some cases fatal, have been described in pregnant women receiving the combination of didanosine and stavudine along with other antiretroviral agents *[5-7]*; the FDA and Bristol Myers Squibb have issued a warning to health care professionals that pregnant women may be at increased risk of fatal lactic acidosis when prescribed the combination of didanosine and stavudine (see "**Pregnancy and mitochondrial toxicity**"). The combination of these two drugs should be prescribed for pregnant women only when the potential benefit clearly outweighs the potential risk; clinicians should prescribe this antiretroviral combination during pregnancy with caution and generally only when other nucleoside analog drug combinations have failed or have caused unacceptable toxicity or side effects.

Emtricitabine (Emtriva®, FTC) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test) or the mouse lymphoma or mouse micronucleus assays. In long-term oral carcinogenicity studies of emtricitabine, no drug-related increases in tumor incidence were found in mice at doses up to 26 times the human systemic exposure at the therapeutic dose of 200 mg/day or in rats at doses up to 31 times the human systemic exposure at the therapeutic dose.

- Reproduction/fertility
  No effect of emtricitabine on reproduction or fertility was observed with doses that produced systemic drug exposures (as measured by area under the curve) approximately 60-fold higher in female mice and 140-fold higher in male mice than observed with human exposure at the recommended therapeutic dose.

- Teratogenicity/developmental toxicity
  The incidence of fetal variations and malformations was not increased with emtricitabine dosing in mice resulting in systemic drug exposure 60-fold higher than observed with human exposure at recommended doses, or in rabbits with dosing resulting in drug exposure 120-fold higher than human exposure.

- Placental and breast milk passage
  It is unknown whether emtricitabine crosses the placenta or is excreted in human milk.

- Human studies in pregnancy
  There have been no studies of emtricitabine in pregnant women or neonates.

Lamivudine (Epivir®, 3TC) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Long-term animal carcinogenicity screening studies in rodents administered lamivudine have been negative.

- Reproduction/fertility
  There appears to be no effect of lamivudine on reproduction or fertility in rodents.

- Teratogenicity/developmental toxicity studies
  There is no evidence of lamivudine-induced teratogenicity. Early embryolethality was seen in rabbits but not in rats at doses similar to human therapeutic exposure.

In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to lamivudine in humans have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with lamivudine. The prevalence of birth defects with first trimester lamivudine exposure was 2.7% (95% CI: 2.0–3.6%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk passage
  Lamivudine readily crosses the placenta in humans, achieving comparable cord blood and maternal concentrations *[8]*. Lamivudine is excreted into human breast milk.

- Human studies in pregnancy
  A small phase I study in South Africa evaluated the safety and pharmacokinetics of lamivudine alone or in combination with zidovudine in 20 HIV-infected pregnant women; therapy was started at 38 weeks gestation, continued through labor, and given for 1 week following birth to the infants *[8]*. The drug was well-tolerated in the women at the recommended adult dose of 150 mg orally twice daily; pharmacokinetics were similar to those observed in nonpregnant adults, and no pharmacokinetic interaction with zidovudine was observed.

  Zidovudine and lamivudine, given in combination orally intrapartum, were well-tolerated. Lamivudine was well-tolerated in the neonates, but clearance was about 50% that of older children, requiring a reduced dosing regimen (4 mg/kg/day in neonates compared to 8 mg/kg/day for infants older than 3 months). There are currently no data on the pharmacokinetics of lamivudine between 2 to 6 weeks of age, and the exact age at which lamivudine clearance begins to approximate that in older children is not known.

Stavudine (Zerit®, d4T) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Some *in vitro* and *in vivo* mutagenesis and clastogenicity tests are positive. In 2-year carcinogenicity studies in mice and rats, d4T was noncarcinogenic in doses producing exposures 39 (mice) and 168 (rats) times human exposure at the recommended therapeutic dose. At higher levels of exposure (250 [mice] and 732 [rats] times human exposure at therapeutic doses), benign and malignant liver tumors occurred in mice and rats and urinary bladder tumors occurred in male rats.

- Reproduction/fertility
  No effect of stavudine on reproduction or fertility in rodents has been seen. A dose-related cytotoxic effect has been observed on preimplantation mouse embryos, with inhibition of blastocyst formation at a concentration of stavudine of 100 μM and of postblastocyst development at 10 μM *[3]*.

- Teratogenicity/developmental toxicity studies
  No evidence of teratogenicity of stavudine has been observed in pregnant rats and rabbits. Developmental toxicity, consisting of a small increase in neonatal mortality and minor skeletal ossification delay, occurred at the highest dose in rats.

  In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to stavudine in humans have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with stavudine. The prevalence of birth defects with first trimester stavudine exposure was 2.7% (95% CI 1.4–4.6%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk passage
  Stavudine crosses the rat placenta *in vivo* and the human placenta *ex vivo*, resulting in a fetal/maternal concentration of approximately 0.50. In primates (pigtailed macaques), fetal/maternal plasma concentrations were approximately 0.80 *[9]*. Stavudine is excreted into the breast milk of lactating rats.

- Human studies in pregnancy
  A phase I/II safety and pharmacokinetic study of combination d4T and 3TC in pregnant HIV-infected women and their infants has been conducted (PACTG 332). Both drugs were well-tolerated, with pharmacokinetics similar to those in non-pregnant adults *[10]*. Data from primate studies also indicated that pregnancy did not affect the pharmacokinetics of d4T *[11]*.

  Cases of lactic acidosis, in some cases fatal, have been described in pregnant women receiving the combination of didanosine and stavudine along with other antiretroviral agents *[5-7]*; the FDA and Bristol Myers Squibb have issued a warning to health care professionals that pregnant women may be at increased risk of fatal lactic acidosis when prescribed the combination of didanosine and stavudine (see "**Pregnancy and mitochondrial toxicity**" on page 6). The combination of these two drugs should be prescribed for pregnant women only when the potential benefit clearly outweighs the potential risk; clinicians should prescribe this antiretroviral combination during pregnancy with caution and generally only when other nucleoside analog drug combinations have failed or have caused unacceptable toxicity or side effects.

Tenofovir disoproxil fumarate [DF] (Viread™) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Long-term oral carcinogenicity studies of tenofovir DF in mice and rats were carried out. In female mice, liver adenomas were increased at exposures 16 times that observed in humans at therapeutic doses. In rats, the study was negative for carcinogenic findings at exposures up to 5 times that observed in humans at the therapeutic dose.

- Reproduction/fertility
  Reproductive toxicity has been evaluated in rats and rabbits. Tenofovir had no adverse effects on fertility or general reproductive performance in rats at doses up to 600 mg/kg/day (exposure equivalent to approximately 10 times the human dose based on body surface area comparisons). However, there was an alteration of the estrous cycle in female rats administered 600 mg/kg/day of tenofovir.

- Teratogenicity/developmental toxicity
  No adverse effects on embryo/fetal development were seen when tenofovir was given in doses up to 450 mg/kg/day to pregnant rats and 300 mg/kg/day to pregnant rabbits. When tenofovir was administered to pregnant rats in doses of 450–600 mg/kg/day, which are maternally toxic doses, peri- and post-natal development studies of their offspring showed reduced survival and slight delay in sexual maturation. However, there were no adverse effects on growth, development, behavior, or reproductive parameters when tenofovir was administered to pregnant rodents at doses that were not associated with maternal toxicity (150 mg/kg/day). Chronic exposure of fetal monkeys to tenofovir at a high dose of 30 mg/kg (exposure equivalent to 25 times the AUC achieved with therapeutic dosing in humans) from days 20–150 of gestation did not result in gross structural abnormalities *[12]*. However, significantly lower fetal circulating insulin-like growth factor (IGF)-1 (a primary regulator of linear growth) and higher IGF binding protein (IGFBP)-3 levels were shown and were associated with overall body weights approximately 13% lower than untreated controls. A slight reduction in fetal bone porosity was also observed. Effects on these parameters were observed within 2 months of maternal treatment. Significant changes in maternal monkey bone biomarkers were noted but were primarily limited to the treatment period and were reversible.

  Continued administration of tenofovir at 30 mg/kg/day to the infant monkey postnatally resulted in significant growth restriction and severe bone toxicity in 25% of eight infants and effects on bone biomarkers and defective bone mineralization in all animals. Chronic administration of tenofovir to immature animals of multiple species has resulted in reversible bone abnormalities; these effects were dose-, exposure-, age-, and species-specific. Abnormalities ranged from minimal decrease in bone mineral density and content (with oral dosing in rats and dogs that achieved drug exposures 6 to 10 times that achieved with therapeutic dosing in humans) to severe, pathologic osteomalacia (with subcutaneous dosing given to monkeys). Juvenile monkeys given chronic subcutaneous tenofovir at 30 mg/kg/day (exposure equivalent to 25 times the AUC achieved with therapeutic dosing in humans) developed osteomalacia, bone fractures, and marked hypophosphatemia. However, no clinical or radiologic bone toxicity was seen when juvenile monkeys received subcutaneous dosing of 10 mg/kg/day (exposure equivalent to 8 times the AUC achieved with therapeutic dosing in humans). Evidence of nephrotoxicity was observed in newborn and juvenile monkeys given tenofovir in doses resulting in exposures 12 to 50 times higher than the human dose based on body surface area comparisons.

- Placental and breast milk passage
  Studies in rats have demonstrated that tenofovir is secreted in milk. Intravenous administration of tenofovir to pregnant cynomolgus monkeys resulted in a fetal/maternal concentration of 17%, demonstrating that tenofovir does cross the placenta *[13]*. There are no data on whether tenofovir crosses the placenta or is excreted in breast milk in humans.

- Human studies in pregnancy
  No studies of tenofovir have been conducted in pregnant women or neonates.

Zalcitabine (HIVID®, ddC) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  High doses of zalcitabine (over 1,000 times that of human therapeutic exposure) have been associated with the development of thymic lymphomas in rodents.

- Reproduction/fertility
  No effect of zalcitabine on reproduction or fertility in rodents has been seen. However, there is a dose-related cytotoxic effect on preimplantation mouse embryos, with inhibition at a zalcitabine concentration of 100 μM; no inhibition of postblastocyst development was observed *[3]*.

- Teratogenicity/developmental toxicity
  Teratogenicity (hydrocephalus) occurred in rats given very high doses (over 1,000 times the maximally recommended human exposure) of zalcitabine. Developmental toxicity, consisting of decreased fetal weight and skeletal defects, has been seen in rodents at moderate to high zalcitabine doses. Cytotoxic effects were observed on rat fetal thymocytes at zalcitabine concentrations as low as 10 μM (approximately 100 times human therapeutic exposure).

- Placental and breast milk passage
  In primate and placental perfusion studies, zalcitabine crosses the placenta (fetal-to-maternal drug ratio approximately 0.50 to 0.60) *[14]*. In rodents, zalcitabine concentrates in the fetal kidney and a relatively small proportion (approximately 20%) reaches the fetal brain. It is unknown if ddC is excreted in breast milk.

- Human studies in pregnancy
  No studies of zalcitabine have been conducted in pregnant women or neonates.

Zidovudine (Retrovir®) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Prolonged, continuous, high-dose zidovudine administration to adult rodents is associated with the development of nonmetastasizing vaginal squamous tumors in 13% of female rodents (at estimated drug concentrations 3 and 24 times that of human therapeutic exposure in mice and rats, respectively) *[15]*. In rodents, unmetabolized zidovudine is concentrated in urine with reflux into the vaginal vault. Therefore, vaginal tumors could be a topical effect of chronic zidovudine exposure on the vaginal mucosa. The observation that vaginal squamous cell carcinomas were observed in rodents exposed to 20 mg/mL zidovudine intravaginally is consistent with this hypothesis *[15]*. In humans, only metabolized zidovudine is excreted in the urine. No increase in tumors in other organ sites has been seen in adult rodent studies.

  Two transplacental carcinogenicity studies of zidovudine were conducted in mice, with differing results. In one study, two very high daily doses of zidovudine were administered during the last third of gestation in mice *[16]*. These doses were near the maximum dose beyond which lethal fetal toxicity would be observed and approximately 25 and 50 times greater than the daily dose given to humans (although the cumulative dose was similar to the cumulative dose received by a pregnant woman taking 6 months of zidovudine). In the offspring of zidovudine-exposed pregnant mice at the highest dose level followed for 12 months, a statistically significant increase in lung, liver, and female reproductive organ tumors was observed; the investigators also documented incorporation of zidovudine into the DNA of a variety of newborn mouse tissues, although this did not clearly correlate with the presence of tumors. In the second study, pregnant mice were given one of several regimens of zidovudine, at doses intended to achieve blood levels approximately threefold higher than human therapeutic exposure *[17]*. The daily doses received by the mice during gestation ranged from one-twelfth to one-fiftieth the daily doses received in the previous study. Some of the offspring also received zidovudine for varying periods of time over their lifespan. No increase in the incidence of tumors was observed in the offspring of these mice, except among those that received additional lifetime zidovudine exposure, in which vaginal tumors were again noted.

  Transplacental carcinogenicity studies have not been performed for any of the other available antiretroviral drugs or combinations of drugs. In January 1997, the National Institutes of Health convened an expert panel to review these animal data *[18]*. The panel concluded that the known benefit of zidovudine in reducing vertical transmission of HIV by nearly 70% (7.2 versus 21.9% with placebo) *[19]* far outweighs the theoretical risks of transplacental carcinogenicity. The panel also concluded that infants with *in utero* exposure to zidovudine (or any other antiretroviral) should have long-term follow-up for potential adverse effects. No tumors have been observed in 727 children with *in utero* ZDV exposure followed for over 1,100 person-years *[20]*. While these data are reassuring, follow-up is still limited and needs to be continued into adulthood before it can be concluded that there is no carcinogenic risk.

- Reproduction/fertility
  No effect of zidovudine on reproduction or fertility in rodents has been seen. A dose-related cytotoxic effect on preimplantation mouse embryos can occur, with inhibition of blastocyst and postblastocyst development at a zidovudine concentrations similar to levels achieved with human therapeutic doses *[21]*.

- Teratogenicity/developmental toxicity
  No evidence of teratogenicity or toxicity was observed with administration of doses up to 500 to 600 mg/kg/day of zidovudine to pregnant rats, mice or rabbits. However, marked maternal toxicity and an increase in fetal malformations were noted in rats given a zidovudine dose of 3000 mg/kg/day (near the lethal dose, and 350 times the peak human plasma concentration).

  In humans, in the placebo-controlled perinatal trial PACTG 076, the incidence of minor and major congenital abnormalities was similar between zidovudine and placebo groups and no specific patterns of defects were seen *[19, 22]*. In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to zidovudine have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with zidovudine. The prevalence of birth defects with first trimester zidovudine exposure was 2.9% (95% CI: 2.1–4.0%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk passage
  Zidovudine rapidly crosses the human placenta, achieving cord-to-maternal blood ratios of about 0.80. ZDV is excreted into human breast milk.

- Human studies in pregnancy
  Zidovudine is well-tolerated in pregnancy at recommended adult doses and in the full-term neonate at 2 mg/kg body weight orally every 6 hours *[19, 23]*. Long-term data on the safety of *in utero* drug exposure in humans are not available for any antiretroviral drug; however, short-term data on the safety of zidovudine are reassuring. No difference in disease progression between women in PACTG 076 who received zidovudine and those who received placebo has been seen in follow-up through 4 years postpartum *[24]*. Infants with *in utero* zidovudine exposure followed for nearly 6 years have shown no significant differences from those who received placebo in immunologic, neurologic and growth parameters *[22, 25]*; follow-up of these infants is continuing.

Issues Related to Use of Nucleoside Analogue Drugs and Mitochondrial Toxicity

Nucleoside analogue drugs are known to induce mitochondrial dysfunction, as the drugs have varying affinity for mitochondrial gamma DNA polymerase. This affinity can result in interference with mitochondrial replication, resulting in mitochondrial DNA depletion and dysfunction *[26]*. The relative potency of the nucleosides in inhibiting mitochondrial gamma DNA polymerase *in vitro* is highest for zalcitabine (ddC), followed by didanosine (ddI), stavudine (d4T), lamivudine (3TC), ZDV, and abacavir (ABC). Toxicity related to mitochondrial dysfunction has been reported in infected patients receiving long-term treatment with nucleoside analogues, and generally has resolved with discontinuation of the drug or drugs; a possible genetic susceptibility to these toxicities has been suggested *[27]*. These toxicities may be of particular concern for pregnant women and for infants with *in utero* exposure to nucleoside analogue drugs.

Issues in Pregnancy: Clinical disorders linked to mitochondrial toxicity include neuropathy, myopathy, cardiomyopathy, pancreatitis, hepatic steatosis, and lactic acidosis. Among these disorders, symptomatic lactic acidosis and hepatic steatosis may have a female preponderance *[28]*.

These syndromes have similarities to the rare but life-threatening syndromes of acute fatty liver of pregnancy and hemolysis, elevated liver enzymes and low platelets (the HELLP syndrome) that occur during the third trimester of pregnancy. A number of investigators have correlated these pregnancy-related disorders with a recessively-inherited mitochondrial abnormality in the fetus/infant that results in an inability to oxidize fatty acids *[29-31]*. Since the mother would be a heterozygotic carrier of the abnormal gene, there may be an increased risk of liver toxicity due to an inability to properly oxidize both maternal and accumulating fetal fatty acids *[32]*. Additionally, animal studies show that in late gestation pregnant mice have significant reductions (25%–50%) in mitochondrial fatty acid oxidation, and that exogeneously administered estradiol and progesterone can reproduce these effects *[33, 34]*; whether this can be translated to humans is unknown. However, these data suggest that a disorder of mitochondrial fatty acid oxidation in the mother or her fetus during late pregnancy may play a role in the etiology of acute fatty liver of pregnancy and HELLP syndrome, and possibly contribute to susceptibility to antiretroviral-associated mitochondrial toxicity.

Lactic acidosis with microvacuolar hepatic steatosis is a toxicity related to nucleoside analogue drugs that is thought to be related to mitochondrial toxicity; it has been reported in infected individuals treated with nucleoside analogue drugs for long periods of time (>6 months). Initially, most cases were associated with AZT, but subsequently other nucleoside analogue drugs have been associated with the syndrome, particularly d4T. In a report from the FDA Spontaneous Adverse Event Program of 106 individuals with this syndrome (60 in patients receiving combination and 46 receiving single nucleoside analogue therapy), typical initial symptoms included 1 to 6 weeks of nausea, vomiting, abdominal pain, dyspnea, and weakness *[28]*. Metabolic acidosis with elevated serum lactate and elevated hepatic enzymes was common. Patients in this report were predominantly female gender and high body weight. The incidence of this syndrome may be increasing, possibly due to increased use of combination nucleoside analogue therapy or increased recognition of the syndrome. In a cohort of infected patients receiving nucleoside analogue therapy followed at Johns Hopkins University between 1989 and 1994, the incidence of the hepatic steatosis syndrome was 0.13% per year *[35]*. However, in a report from a cohort of 964 HIV-infected individuals followed in France between 1997 and 1999, the incidence of symptomatic hyperlactatemia was 0.8% per year for all patients and 1.2% for patients receiving a regimen including d4T *[36]*.

The frequency of this syndrome in pregnant HIV-infected women receiving nucleoside analogue treatment is unknown. In 1999, Italian researchers reported a case of severe lactic acidosis in an infected pregnant woman who was receiving d4T/3TC at the time of conception and throughout pregnancy who presented with symptoms and fetal demise at 38 weeks gestation *[37]*. Bristol-Myers Squibb has reported three maternal deaths due to lactic acidosis, two with and one without accompanying pancreatitis, in women who were either pregnant or postpartum and whose antepartum therapy during pregnancy included d4T and ddI in combination with other antiretroviral agents (either a protease inhibitor or nevirapine) *[5, 6]*. All cases were in women who were receiving treatment with these agents at the time of conception and continued for the duration of pregnancy; all presented late in gestation with symptomatic disease that progressed to death in the immediate postpartum period. Two cases were also associated with fetal demise.

It is unclear if pregnancy augments the incidence of the lactic acidosis/hepatic steatosis syndrome reported in non-pregnant individuals receiving nucleoside analogue treatment. However, because pregnancy itself can mimic some of the early symptoms of the lactic acidosis/hepatic steatosis syndrome or be associated with other significant disorders of liver metabolism, these cases emphasize the need for physicians caring for HIV-infected pregnant women receiving nucleoside analogue drugs to be alert for early diagnosis of this syndrome. Pregnant women receiving nucleoside analogue drugs should have hepatic enzymes and electrolytes assessed more frequently during the last trimester of pregnancy, and any new symptoms should be evaluated thoroughly. Additionally, because of the reports of several cases of maternal mortality secondary to lactic acidosis with prolonged use of the combination of d4T and ddI by HIV-infected pregnant women, clinicians should prescribe this antiretroviral combination during pregnancy with caution and generally only when other nucleoside analogue drug combinations have failed or caused unacceptable toxicity or side effects.

Issues with In Utero Exposure: A study conducted in France reported that in a cohort of 1,754 uninfected infants born to HIV-1 infected women who received antiretroviral drugs during pregnancy, eight infants with in utero or neonatal exposure to either ZDV-3TC (four infants) or ZDV alone (four infants) developed indications of mitochondrial dysfunction after the first few months of life *[36]*. Two of these infants (both of whom had been exposed to ZDV-3TC) contracted severe neurologic disease and died, three had mild to moderate symptoms, and three had no symptoms but had transient laboratory abnormalities.

A further evaluation of mitochondrial toxicity was conducted in 4,392 uninfected or HIV-indeterminate children (2,644 with perinatal antiretroviral exposure) followed within the French Pediatric Cohort or identified within a France National Register developed for reporting of possible mitochondrial dysfunction in HIV-exposed children. Evidence of mitochondrial dysfunction was identified in 12 children (including the previous 8 reported cases), all of whom had perinatal antiretroviral exposure, an 18-month incidence of 0.26% *[38]*. Risk was higher among infants exposed to combination antiretroviral drugs (primarily ZDV/3TC) than ZDV alone. All children presented with neurologic symptoms, often with abnormal magnetic resonance imaging and/or a significant episode of hyperlactatemia, and all had an identified deficit in one of the mitochondrial respiratory chain complexes and/or abnormal muscle biopsy histology. An additional 14 children with "possible" mitochondrial dysfunction had unexplained clinical and/or laboratory findings for which mitochondrial dysfunction could be included in the differential diagnosis, although none had respiratory chain enzyme deficits or histologic abnormalities. In a separate publication, the same group reported an increased risk of simple febrile seizures during the first 18 months of life among uninfected infants with antiretroviral exposure *[39]*.

A small study quantified mitochondrial DNA in cord blood and peripheral blood leukocytes at age 1 and 2 years in HIV-exposed infants with (N=10) and without (N=20) perinatal ZDV exposure and infants born to HIV-uninfected women (N=30) *[40]*. Mitochondrial DNA quantity was lower in infants born to HIV-infected women overall compared to those born to uninfected women, and was lowest among those HIV-exposed infants with ZDV exposure compared to those without exposure. In another study, transient hyperlactatemia during the first few weeks of life was reported among 17 HIV-exposed infants with perinatal antiretroviral exposure; lactate levels returned to normal in all children and none developed symptoms of mitochondrial dysfunction during follow-up *[41]*. Thus, the clinical significance of these laboratory findings is unclear, and further studies are needed to validate these findings.

In infants followed through age 18 months in PACTG 076, the occurrence of neurologic events was rare; seizures occurred in one child exposed to ZDV and two exposed to placebo, and one child in each group had reported spasticity. Mortality at 18 months was 1.4% among infants given ZDV compared with 3.5% among those given placebo *[22]*. The Perinatal Safety Review Working Group performed a retrospective review of deaths occurring among children born to HIV-1 infected women and followed during 1986–1999 in five large prospective U.S. perinatal cohorts. No deaths similar to those reported from France or with clinical findings attributable to mitochondrial dysfunction were identified in a database of >16,000 uninfected children born to HIV-1 infected women with and without antiretroviral drug exposure *[42]*. However, most of the infants with antiretroviral exposure had been exposed to ZDV alone and only a relatively small proportion (approximately 6%) had been exposed to ZDV-3TC.

In an African perinatal trial (PETRA) that compared three regimens of ZDV-3TC (during pregnancy starting at 36 weeks' gestation, during labor, and through 1 week postpartum; during labor and postpartum; and during labor only) with placebo for prevention of transmission, data have been reviewed relating to neurologic adverse events among 1,798 children who participated. No increased risk of neurologic events was observed among children treated with ZDV-3TC compared with placebo, regardless of the intensity of treatment *[43]*. The European Collaborative Study reviewed clinical symptoms in 2,414 uninfected children in their cohort, 1,008 of whom had perinatal antiretroviral exposure. The median length of follow-up was 2.2 years (maximum, 16 years). No association of clinical manifestations suggestive of mitochondrial abnormalities was found with perinatal antiretroviral exposure. Of the 4 children with seizures in this cohort, none had perinatal antiretroviral exposure.

Finally, in a study of 382 uninfected infants born to HIV-1 infected women, echocardiograms were prospectively performed every 4 to 6 months during the first 5 years of life; 9% of infants had been exposed to ZDV prenatally *[44]*. No significant differences in ventricular function were observed between infants exposed and not exposed to ZDV.

Thus, there are conflicting data regarding whether mitochondrial dysfunction is associated with perinatal antiretroviral exposure. If this association is demonstrated, the development of severe or fatal mitochondrial disease appears to be extremely rare and should be compared against the clear benefit of antiretroviral prophylaxis in reducing transmission of a fatal infection by 70% or more *[45-47]*. Mitochondrial dysfunction should be considered in uninfected children with perinatal antiretroviral exposure who present with severe clinical findings of unknown etiology, particularly neurologic findings. These results emphasize the importance of the existing Public Health Service recommendation for long-term follow-up for any child with *in utero* exposure to antiretroviral drugs.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Postpartum Hemorrhage, Non-Nucleoside Reverse Transcriptase Inhibitors, and Methergine Use

Women experiencing postpartum hemorrhage due to uterine atony are often managed with oral or parenteral methergine as a first-line agent. However, methergine should not be coadministered with drugs that are potent CYP3A4 enzyme inhibitors, including the non-nucleoside reverse transcriptase inhibitors efavirenz and delavirdine. When uterine atony results in excessive postpartum bleeding in women receiving either delavidine or efavirenz as a component of an antiretroviral regimen, methergine should only be used if alternative treatments (e.g., prostaglandin F 2 alpha, misoprostol, or oxytocin) are not available. If there are no alternative medications available and the need for pharmacologic treatment outweighs the risks, methergine should be used in as low a dosage and for as short a duration as possible.

Delavirdine (Rescriptor®) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  In vitro screening tests for carcinogenicity have been negative. In rats, delavirdine was non-carcinogenic at all doses studied. In mice, delavirdine was associated with an increase in hepatocellular adenoma and carcinoma in both males and females and urinary bladder tumors in males at systemic exposures 0.5 to 3-fold higher than human exposure at therapeutic doses for female mice and at exposures 0.2 to 4-fold higher in male mice.

- Reproduction/fertility
  Delavirdine does not impair fertility in rodents.

- Teratogenicity/developmental toxicity animal studies
  Delavirdine is teratogenic in rats; doses of 50 to 200 mg/kg/day during organogenesis caused ventricular septal defects. Exposure of rats to doses approximately 5 times human therapeutic exposure resulted in marked maternal toxicity, embryotoxicity, fetal developmental delay, and reduced pup survival.

  Abortions, embryotoxicity, and maternal toxicity were observed in rabbits at doses approximately 6 times human therapeutic exposure.

- Placental and breast milk passage
  Whether delavirdine crosses the placenta is unknown. Delavirdine is excreted in the milk of lactating rats; however, it is unknown if the drug is excreted in human breast milk.

- Human studies in pregnancy
  Delavirdine has not been evaluated in HIV-infected pregnant women. In premarketing clinical studies, the outcomes of seven unplanned pregnancies were reported: three resulted in ectopic pregnancies, three resulted in healthy live births, and one infant was born prematurely with a small muscular ventricular septal defect to a patient who received approximately 6 weeks of treatment with delavirdine and zidovudine early in the course of pregnancy.

Efavirenz (Sustiva®) is classified as FDA pregnancy category D.

- Animal carcinogenicity studies
  In vitro genetic screening tests are negative for mutagenic or clastogenic effects of drug exposure. Long-term animal carcinogenicity studies with efavirenz in mice and rats have been completed. At systemic drug exposures approximately 1.7-fold higher than in humans receiving standard therapeutic doses, no increase in tumor incidence above background was observed in male mice but an increase in hepatocellular adenomas and carcinomas and pulmonary alveolar/bronchiolar adenomas above background were found in female mice. In rats administered systemic drug exposures lower than that in humans receiving therapeutic doses, no increase in tumor incidence above background was observed in male or female rats.

- Reproduction/fertility animal studies
  No effect of efavirenz on reproduction or fertility in rodents has been seen. An increase in fetal resorptions has been observed in rats at doses comparable to or lower than those used to achieve human therapeutic exposure.

- Teratogenicity/developmental toxicity animal studies
  Significant central nervous system malformations were observed in 3 of 20 infants born to pregnant cynomolgus monkeys receiving efavirenz from gestational days 20 to 150 at a dose of 30 mg/kg twice daily (resulting in plasma concentrations comparable to systemic human therapeutic exposure) *[48]*. The malformations included anencephaly and unilateral anophthalmia in one; microphthalmia in another; and cleft palate in the third. Primate teratogenicity studies have not been conducted

for the other non-nucleoside reverse transcription inhibitors, delavirdine or nevirapine.

- Placental and breast milk passage in animal studies
  Efavirenz crosses the placenta in rats, rabbits, and primates, producing cord blood concentrations similar to concentrations in maternal plasma. It is unknown whether efavirenz is excreted in human breast milk.

- Human studies in pregnancy
  No clinical trials with efavirenz in pregnant humans are planned. Efavirenz is classified as FDA Pregnancy Category D and may cause fetal harm when administered to a pregnant woman during the first trimester. In prospectively reported pregnancies with exposure to efavirenz-based regimens in the Antiretroviral Pregnancy Registry through January 2006, birth defects were observed in 6 of 244 live births with first trimester exposure and 1 of 15 births with exposure later in pregnancy; none of the defects in the prospective report were neural tube defects (they included polydactyly, hydronephrosis, bilateral hip dislocation and umbilical hernia, and urinary obstruction secondary to duplicated right collecting system) *[1]*. However, in retrospective case reports, there are 3 cases of neural tube defects in infants born to mothers receiving efavirenz during the first trimester *[49]*, as well as an additional infant with another central nervous system defect (Dandy-Walker malformation). The specific cases include a report of multiple defects, including Dandy-Walker CNS malformation in a fetus from a spontaneous abortion, a fetus with a neural tube defect in a pregnancy with elective termination in second trimester after the defect was diagnosed, and two cases of myelomeningocele in infants born to women who were receiving efavirenz at the time of conception and during the first trimester *[50, 51]*. Although a causal relationship of these events to the use of efavirenz has not been established, similar defects have been observed in preclinical studies of efavirenz (see teratogenicity/developmental toxicity in animal studies).

  Because of the potential for teratogenicity, pregnancy should be avoided in women receiving efavirenz, and treatment with efavirenz should be avoided during the first trimester, which is the primary period of fetal organogenesis. Women of childbearing potential should undergo pregnancy testing prior to initiation of efavirenz and should be counseled about the potential risk to the fetus and need to avoid pregnancy. Different types of contraception have known failure rates in women not receiving antiretroviral drugs; these failure rates may increase with drug interactions between estrogen-progesterone hormonal contraceptives and some antiretroviral drugs, including efavirenz. Alternate antiretroviral regimens that do not include efavirenz should be strongly considered in women who are planning to become pregnant or who are sexually active and not using effective contraception. Barrier contraception should always be used in combination with other methods of contraception (e.g., oral hormonal contraceptives). There are insufficient data on drug interactions with injectable hormones (depo-provera) to make recommendations regarding the need for additional contraception. Theoretically, because hormone levels are much higher with injectable than oral contraceptives, interactions with antiretroviral drugs may be less significant.

Nevirapine (Viramune®) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  In vitro screening tests for carcinogenicity have been negative. Hepatocellular adenomas and carcinomas were increased at all doses in male mice and rats, and at higher doses in female mice and rats. Systemic exposure at all doses studied was lower than systemic exposure in humans receiving therapeutic nevirapine doses.

- Reproduction/fertility
  Evidence of impaired fertility was seen in female rats at nevirapine doses providing systemic exposure comparable to human therapeutic exposure.

- Teratogenicity/developmental toxicity
  Teratogenic effects of nevirapine have not been observed in reproductive studies with rats and rabbits. In rats, however, a significant decrease in fetal weight occurred at doses producing systemic concentrations approximately 50% higher than human therapeutic exposure.

  In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposure to nevirapine in humans have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with nevirapine. The prevalence of birth defects with first trimester nevirapine exposure was 1.9% (95% CI: 0.9–3.5%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk passage
  Nevirapine crosses the placenta and achieves neonatal blood concentrations equivalent to that in the mother

(cord-to-maternal blood ratio approximately 0.90) *[52]*. Nevirapine is excreted into human breast milk; the median concentration in four breast milk samples obtained from three women during the first week after delivery was approximately 76% (range 54 to 104%) of serum levels *[52]*.

- Human studies in pregnancy
  A phase I study (PACTG 250) evaluated the safety and pharmacokinetics of nevirapine, administered to infected pregnant women as a single 200 mg dose at the onset of labor and as a single 2 mg/kg dose to the infant at age 48 to 72 hours *[52]*. No adverse effects were seen in the women or the infants.

Pharmacokinetic parameters in pregnant women receiving intrapartum nevirapine were similar though somewhat more variable than in nonpregnant adults, possibly due to incomplete drug absorption associated with impaired gastrointestinal function during labor. Nevirapine elimination was prolonged in the infants. The regimen maintained serum concentrations associated with antiviral activity in the infants for the first week of life.

The safety, toxicity and pharmacokinetics of nevirapine were also studied in HIV-infected pregnant women beginning chronic therapy late in the third trimester and their infants *[53]*. Initial dose pharmacokinetic profiles in pregnant women were similar to those seen in non-pregnant adults. Serum nevirapine concentrations fell below the 100 ng/mL target concentration by day 7 of life in 4 of 8 infants, suggesting that nevirapine elimination was accelerated in infants whose mother received chronic nevirapine administration compared with newborns whose mothers received only a single intrapartum nevirapine dose.

The HIVNET 012 study in Uganda compared nevirapine (200 mg orally to the mother at the onset of labor and 2 mg/kg to the neonate within 72 hours of birth) with zidovudine (600 mg orally to the mother at the onset of delivery and 300 mg every 3 hours until delivery, and 4 mg/kg orally twice daily for the first 7 days of life to the neonate). In this study, nevirapine lowered the risk of HIV transmission by nearly 50% during the first 14–16 weeks of life compared with zidovudine *[54]*. However, the women in this African trial were not receiving any other antiretroviral therapy.

In the U.S., most infected women who know their HIV status during pregnancy receive combination antiretroviral therapy, usually including ZDV, as well as intravenous ZDV during delivery, with 6 weeks of ZDV given to their infant. A phase III perinatal trial (PACTG 316) conducted in the U.S., Europe, the Bahamas and Brazil evaluated whether the HIVNET 012 single-dose nevirapine regimen in combination with standard antiretroviral therapy (at minimum the PACTG 076 ZDV regimen; 77% of women in the trial received combination therapy) would provide additional benefits in reducing transmission. Transmission was not significantly different between those having the addition of single-dose nevirapine (1.4%) and those who did not (1.6%) *[55]*. Nevirapine resistance can be induced by a single mutation. Nevirapine resistance mutations were detected at 6 weeks postpartum in 19% of antiretroviral naïve women in HIVNET 012 and 15% of a subset of women receiving additional antiretroviral drugs during pregnancy in PACTG 316 who received single-dose nevirapine during labor *[56, 57]*. In HIVNET 012, these mutations were no longer detectable in plasma virus in women at 13-18 months postpartum *[58]*. Evaluation at later time points was not done in PACTG 316.

Severe, life-threatening, and in some cases, fatal hepatotoxicity, including fulminant and cholestatic hepatitis, hepatic necrosis, and hepatic failure, and severe, life-threatening hypersensitivity skin reactions, including Stevens-Johnson syndrome, have been reported in HIV-infected patients receiving nevirapine in combination with other drugs for treatment of HIV disease and in a small number of individuals receiving nevirapine as part of a combination regimen for post-exposure prophylaxis of nosocomial or sexual HIV exposure *[59]*. These toxicities have not been reported in women or infants receiving two-dose nevirapine (the HIVNET 012 regimen) for prevention of perinatal transmission. The greatest risk of severe rash or hepatic events occurs during the first 6 to 18 weeks of therapy, although the risk of toxicity continues past this period and monitoring should continue at frequent intervals.

The development of severe nevirapine-associated skin rash has been reported to be 5.5 to 7.3 times more common in women than men, and has been reported in pregnant women *[60-62]*. Other studies have found that hepatic adverse events with systemic symptoms (often rash) were 3.2 fold more common in women than men *[63]*. The degree of risk for hepatic toxicity varies with $CD4^+$ cell count. In a summary analysis of data from 17 clinical trials of nevirapine therapy, women with $CD4^+$ counts greater than 250 cells/mm^3 were 9.8 times more likely than women with lower $CD4^+$ counts to experience symptomatic, often rash-associated, nevirapine-related hepatotoxicity *[63]*. Higher $CD4^+$ cell counts have also been associated with increased risk of severe nevirapine-associated skin rash *[61]*. In controlled clinical trials, clinical hepatic events, regardless of severity, occurred in 4.0% (range 2.5-11.0%) of patients who received nevirapine; however, the risk of nevirapine-associated liver failure or hepatic mortality has been lower, ranging between 0.04-0.40% *[63, 64]*. Severe or life threatening rash occurs in approximately 2% of patients receiving nevirapine *[64]*.

Although deaths due to hepatic failure have been reported in HIV-infected pregnant women receiving nevirapine as part of a combination antiretroviral regimen, it is unknown if pregnancy increases the risk of hepatotoxicity in women receiving nevirapine or other antiretroviral drugs *[65, 66]*. Women initiating nevirapine with $CD4^+$ counts > 250 cells/mm^3, including pregnant women receiving antiretroviral drugs solely for prevention of transmission, have an increased risk of developing symptomatic, often rash-associated, nevirapine-related hepatotoxicity, which can be severe, life-threatening, and in some cases fatal *[34]*. Nevirapine should therefore be used as a component of a combination regimen in this setting only if the benefit clearly outweighs the risk. Women with $CD4^+$ counts below 250/mm^3 can receive nevirapine-based regimens, and women who enter pregnancy on nevirapine regimens and are tolerating the regimens well may continue therapy, regardless of $CD4^+$ count. Hepatic toxicity has not been seen in women receiving single dose nevirapine during labor for prevention of perinatal transmission of HIV-1.

Because pregnancy itself can mimic some of the early symptoms of hepatotoxicity, health care providers caring for women receiving nevirapine during pregnancy should be aware of this potential complication and conduct frequent and careful monitoring of clinical symptoms and hepatic transaminases (i.e., alanine aminotransferase, ALT and aspartate aminotransferase, AST), particularly during the first 18 weeks of therapy. Some clinicians measure serum transaminases at baseline, every 2 weeks for the first month, monthly through 4 months, and every 1 to 3 months thereafter [**Adult Antiretroviral Guidelines**]; in patients with pre-existing liver disease, monitoring should be performed more frequently when initiating therapy, and then monthly *[67]*. Transaminase levels should be checked in all women who develop a rash while receiving nevirapine. Patients who develop suggestive clinical symptoms accompanied by elevation in serum transaminase levels (ALT and/or AST), or have asymptomatic but severe transaminase elevations, should stop nevirapine and not receive nevirapine therapy in the future.

PROTEASE INHIBITORS

Issues Related to the Use of Protease Inhibitors

Hyperglycemia and Diabetes Mellitus

Hyperglycemia, new onset diabetes mellitus, exacerbation of existing diabetes mellitus, and diabetic ketoacidosis have been reported with administration of protease inhibitor antiretroviral drugs in HIV-infected patients *[68-71]*. In addition, pregnancy is itself a risk factor for hyperglycemia; it is unknown if the use of protease inhibitors will exacerbate the risk for pregnancy-associated hyperglycemia. Clinicians caring for HIV-infected pregnant women who are receiving protease inhibitor therapy should be aware of the risk of this complication, and closely monitor glucose levels. Symptoms of hyperglycemia should be discussed with pregnant women who are receiving protease inhibitors.

Combination Therapy and Pregnancy Outcome: There are limited data concerning combination antiretroviral therapy in pregnancy. A retrospective Swiss report evaluated the pregnancy outcome in 37 HIV-infected pregnant women treated with combination therapy; all received two reverse transcriptase inhibitors and 16 received one or two protease inhibitors *[72]*. Almost 80% of women developed one or more typical adverse effects of the drugs such as anemia, nausea/vomiting, aminotransferase elevation, or hyperglycemia. A possible association of combination antiretroviral therapy with preterm births was noted, as 10 of 30 babies were born prematurely. The preterm birth rate did not differ between women receiving combination therapy with or without protease inhibitors. The contribution of maternal HIV disease stage and other covariates that might be associated with a risk for prematurity were not assessed. Furthermore, some studies have shown elevated preterm birth rates in HIV-infected women who have not received any antiretroviral therapy *[73-75]*.

The European Collaborative Study and the Swiss Mother + Child HIV-1 Cohort Study investigated the effects of combination retroviral therapy in a population of 3,920 mother - child pairs. Adjusting for $CD4^+$ T-lymphocyte count ($CD4^+$ count) and intravenous drug use, they found a 2.6-fold (95% confidence interval [CI] = 1.4 - 4.8) increased odds of preterm delivery for infants exposed to combination therapy with or without protease inhibitors compared with no treatment; women receiving combination therapy that had been initiated before their pregnancy were twice as likely to deliver prematurely as those starting therapy during the third trimester *[76]*. However, combination therapy was received by only 323 (8%) women studied. Exposure to monotherapy was not associated with prematurity.

In contrast, in a French open-label study of 445 HIV-1-infected women receiving ZDV who had lamivudine (3TC) added to their therapy at 32 weeks' gestation, the rate of preterm delivery was 6%, similar to the 9% rate in a historical control group of women receiving only ZDV *[77]*. Additionally, in a large meta-analysis of seven clinical studies that included 2,123 HIV-infected pregnant women who delivered infants during 1990-1998 and had received antenatal antiretroviral therapy and 1,143 women who did not receive antenatal antiretroviral therapy, use of multiple antiretroviral drugs as compared with no treatment or treatment with one drug was not associated with increased rates of preterm labor, low birth weight, low Apgar scores, or stillbirth *[78]*.

Until more information is known, it is recommended that HIV-infected pregnant women who are receiving combination therapy for treatment of their HIV infection should continue their provider-recommended regimen. They should receive careful, regular monitoring for pregnancy complications and for potential toxicities.

Postpartum Hemorrhage, Protease Inhibitors, and Methergine Use

Women experiencing postpartum hemorrhage due to uterine atony are often managed with oral or parenteral methergine as a first-line agent. However, methergine should not be coadministered with drugs that are potent CYP3A4 enzyme inhibitors, including protease inhibitors. The concomitant use of ergotamines and protease inhibitors has been associated with exaggerated vasoconstrictive responses. When uterine atony results in excessive postpartum bleeding in women receiving protease inhibitors as a component of an antiretroviral regimen, methergine should only be used if alternative treatments (e.g., prostaglandin F 2 alpha, misoprostol, or oxytocin) are not available. If there are no alternative medications available and the need for pharmacologic treatment outweighs the risks, methergine should be used in as low a dosage and for as short a duration as possible.

Individual Agents: Protease Inhibitors

Amprenavir (Agenerase®) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  In vitro screening tests for carcinogenicity have been negative. An increase in benign hepatocellular adenomas and hepatocellular carcinomas was observed in male mice and rats at the highest doses evaluated, which produced systemic exposures in mice 2-fold and in rats 4-fold higher than systemic exposure in humans receiving therapeutic doses of amprenavir. Female mice and rats were not affected.

- Reproduction/fertility
  No effect has been seen on reproductive performance, fertility, or embryo survival in rats at exposures about twice those of human therapeutic exposure.

- Teratogenicity/developmental toxicity
  In pregnant rabbits, administration of amprenavir resulting in systemic exposures about one-twentieth of that observed with human therapeutic exposure was associated with abortions and an increased incidence of minor skeletal variations resulting from deficient ossification of the femur, humerus trochlea and humerus. In rat fetuses, thymic elongation and incomplete ossification of bones were also attributed to amprenavir at systemic exposures about one-half that associated with the recommended human dose. Reduced body weights of approximately 10–20% were observed in offspring of rodents administered amprenavir from day 7 of gestation to day 22 of lactation (exposures approximately twice that observed with the human therapeutic dose). However, the subsequent development of the offspring, including fertility and reproductive performance, was not affected by maternal administration of amprenavir.

- Placental and breast milk passage
  Whether amprenavir crosses the placenta is unknown. Amprenavir is excreted in the milk of lactating rats; it is not known if it is excreted in human milk.

- Human studies in pregnancy
  There have been no studies of amprenavir in pregnant women or neonates. Amprenavir oral solution contains high levels of excipient propylene glycol in the oral solution vehicle; this is not true for the capsular formulation. Propylene glycol is metabolized by the alcohol and aldehyde dehydrogenase enzyme pathway. Some patients, including infants and children below the age of four years, pregnant women, patients with hepatic or renal failure, and

patients treated with disulfiram or metronidazole, are not able to adequately metabolize and eliminate propylene glycol, thereby leading to its accumulation and potential adverse events. Thus, while the capsule formulation of amprenavir may be used in pregnancy, amprenavir oral solution is contraindicated in pregnant women and infants and in children under the age of four years.

Atazanavir (Reyataz$^{®}$, ATV) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Two-year carcinogenicity studies in mice and rats were conducted with atazanavir. In female mice, the incidence of benign hepatocellular adenomas was increased at systemic exposures 7.2–fold higher than those in humans at the recommended therapeutic dose. There were no increases in the incidence of tumors in male mice at any dose. In rats, no significant positive trends in the incidence of neoplasms occurred at systemic exposures up to 5.7–fold higher than those in humans at the recommended therapeutic dose.

- Reproduction/fertility
  No effect of atazanavir on reproduction or fertility in male and female rodents was seen at systemic drug exposures (as measured by area under the curve) up to two times those achieved in humans at the recommended therapeutic dose.

- Teratogenicity/developmental toxicity
  Atazanavir did not produce teratogenic effects in rabbits with maternal dosing producing systemic drug exposure equal to (rabbits) or twice that (rats) achieved in humans at the recommended therapeutic dose. In developmental toxicity studies in rats, maternal dosing that resulted in maternal toxicity and produced systemic drug exposure twice that achieved in humans at the recommended therapeutic dose resulted in weight loss or suppression of weight gain in the offspring. However, offspring were unaffected at lower maternal doses that produced systemic drug exposure equivalent to that observed in humans at the recommended therapeutic dose.

  Elevation in indirect (unconjugated) bilirubin attributable to atazanavir-related inhibition of hepatic uridine diphosphate glucuronosyltransferase enzyme occurs frequently during treatment with atazanavir. It is unknown whether treatment during pregnancy will exacerbate physiologic hyperbilirubinemia in the neonate.

- Placental and breast milk passage
  It is unknown whether atazanavir crosses the placenta. Atazanavir is excreted in the milk of lactating rats; it is not known if it is excreted in human milk.

- Human studies in pregnancy
  There have been no studies of atazanavir in pregnant women or neonates.

Darunavir (Prezista™) is classified as FDA category B.

Animal carcinogenicity studies
Long-term carcinogenicity studies in rodents have not been completed. Darunavir tested negative in the *in vitro* Ames reverse mutation assay and *in vitro* chromosomal aberration assay in human lymphocytes. Darunavir did not induce chromosomal damage in the micronucleus test in mice.

Reproduction/fertility
No effects on fertility and early embryonic development were seen with darunavir in rats.

Teratogenicity/developmental toxicity
No embryotoxicity or teratogenicity was seen in mice, rats, or rabbits. Because of limited bioavailability of darunavir in animals and dosing limitation, the plasma exposures were approximately 50% (mice and rats) and 5% (rabbits) of those obtained in humans. In the rat pre- and postnatal development study, a reduction in pup weight gain was observed with darunavir alone or with ritonavir during lactation due to exposure of pups to drug substances via the milk. Sexual development, fertility or mating performance of offspring were not affected by maternal treatment. No data are available in humans.

Placental and breast milk passage
No animal studies of placental passage of darunavir have been reported. As noted above, passage of darunavir into breast milk has been noted in rats. It is unknown if placental or breast milk passage of darunavir occurs in humans.

Human studies in pregnancy
No studies of darunavir have been conducted in pregnant women or neonates.

Fosamprenavir (Lexiva™) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Carcinogenicity studies of fosamprenavir in rats and mice are in progress. Results of studies with amprenavir showed an increase in the incidence of benign hepatocellular adenomas and the combined incidence of benign hepatocellular adenomas and carcinoma in males in both species at the highest doses tested, approximately two to four times the human exposure. Female mice and rats were not affected. No other benign or malignant neoplasms were increased. Fosamprenavir and amprenavir were not mutagenic or genotoxic in a battery of in vitro and in vivo assays.

- Reproduction/fertility
  No impairment of fertility or mating was seen in rats at doses providing three to four times the human exposure to fosamprenavir alone or exposure similar to that with fosamprenavir and ritonavir dosing in humans. No affect was seen on the development or maturation of sperm in rats at these doses.

- Teratogenicity/developmental toxicity
  Fosamprenavir was studied in rabbits at 0.8 and in rats at two times the exposure in humans to fosamprenavir alone and at 0.3 (rabbits) and 0.7 (rats) times the exposure in humans to the combination of fosamprenavir and ritonavir. At these doses, the incidence of abortion was increased in rabbits, but no embryo-fetal effects were seen. In contrast, administration of amprenavir at a lower dose in rabbits was associated with abortions and an increased incidence of minor skeletal variations from deficient ossification of the femur, humerus, and trochlea. Fosamprenavir was associated with a reduction in pup survival and body weights in rats. F1 female rats had an increased time to successful mating, an increased length of gestation, a reduced number of uterine implantation sites per litter, and reduced gestational body weights compared to controls.

- Placental and breast milk passage
  It is unknown whether fosamprenavir crosses the placenta. Fosamprenavir is excreted in the milk of lactating rats; it is not known if it is excreted in human milk.

- Human studies in pregnancy
  There have been no studies of fosamprenavir in pregnant women or neonates.

Indinavir (Crixivan®) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  In vitro screening tests for carcinogenicity have been negative. No increased incidence of any tumor types occurred in long-term studies in mice. At the highest dose studied in rats (1.3-fold higher than systemic exposure at human therapeutic doses), thyroid adenomas were seen in male rats.

- Reproduction/fertility
  No effect of indinavir has been seen on reproductive performance, fertility, or embryo survival in rats.

- Teratogenicity/developmental toxicity
  There has been no evidence of teratogenicity of indinavir in rats, rabbits or dogs. In rats, developmental toxicity manifested by an increase in supernumerary and cervical ribs was observed at doses comparable to those administered to humans. No treatment-related external, visceral or skeletal changes were seen in rabbits (fetal exposure limited, approximately 2% of maternal levels) or dogs (fetal exposure approximately 50% of maternal levels). Indinavir was administered to Rhesus monkeys during the third trimester of pregnancy (at doses up to 160 mg/kg twice daily) and to neonatal Rhesus monkeys (at doses up to 160 mg/kg twice daily). When administered to neonates, indinavir caused an exacerbation of the transient physiologic hyperbilirubinemia seen in this species after birth; serum bilirubin values were approximately fourfold above controls at 160 mg/kg twice daily. A similar exacerbation did not occur in neonates after *in utero* exposure to indinavir during the third trimester of pregnancy. In Rhesus monkeys, fetal plasma drug levels were approximately 1–2% of maternal plasma drug levels approximately 1 hour after maternal dosing at 40, 80, or 160 mg/kg twice daily.

- Placental and breast milk passage
  Significant placental passage of indinavir occurs in rats and dogs, but only limited placental transfer occurs in rabbits. In a phase I study in pregnant women and their infants (PACTG 358, see below), transplacental passage of indinavir was minimal *[79]*. Additionally, in a study of cord blood samples from 21 women treated with indinavir during pregnancy, the cord blood concentration of indinavir was below the assay limit of detection in samples from all women *[80]*. Indinavir is excreted in the milk of lactating rats at concentrations slightly above maternal levels (milk-to-plasma ratio 1.26 to 1.45); it is not known if indinavir is excreted in human milk.

- Human studies in pregnancy
  The optimal dosing regimen for use of indinavir in pregnant patients has not been established. A phase I/II safety and pharmacokinetic study (PACTG 358) of indinavir (800 mg tid) in combination with zidovudine and lamivudine in pregnant HIV-infected women and their infants was conducted (the infants did not receive indinavir in this study). Data are available from 16 HIV-infected pregnant patients at 14 to 28 weeks of gestation at enrollment *[79]*. The mean indinavir plasma area under the curve ($AUC_{0\text{-}8hr}$) at weeks 30-32 of gestation (n=11) was 9231 nM•hr, which is 74% (95% CI: 50%, 86%) lower than that observed 6 weeks postpartum. Six of these 11 (55%) patients had mean indinavir plasma concentrations 8 hours post-dose (Cmin) below assay threshold of reliable quantification. The pharmacokinetics of indinavir in these 11 patients at 6 weeks postpartum were generally similar to those observed in non-pregnant patients in another study. In another pharmacokinetic study of two pregnant HIV-infected women receiving combination therapy including indinavir (800 mg tid), a marked difference was noted between the AUC indinavir exposure between the third trimester and postpartum evaluations *[81]*. The AUC during the third trimester was reduced by 63% in one and 86% in the other woman when compared to 9-12 week postpartum evaluations in the same women. Similar reductions in maximum plasma indinavir concentrations were observed. Therefore, given the substantially lower antepartum exposures observed in these studies and the generally limited data in this patient population, use of indinavir as a sole protease inhibitor is not recommended in HIV-infected pregnant patients; data are not yet available regarding the pharmacokinetics of indinavir with low dose ritonavir boosting in pregnant women.

Lopinavir + Ritonavir (Kaletra™) is classified as FDA pregnancy category C.

- Animal carcinogenicity studies
  Lopinavir/ritonavir combination was evaluated for carcinogenic potential by oral gavage administration to mice and rats for up to 104 weeks. Results showed an increase in the incidence of benign hepatocellular adenomas and an increase in the combined incidence of hepatocellular adenomas plus carcinoma in both males and females in mice and males in rats at doses that produced approximately 1.6-2.2 times (mice) and 0.5 times (rats) the human exposure at the recommended therapeutic dose (based on AUC 0-24hr measurement). Administration of lopinavir/ritonavir did not cause a statistically significant increase in the incidence of any other benign or malignant neoplasm in mice or rats.

- Reproduction/fertility
  Lopinavir in combination with ritonavir at a 2:1 ratio produced no effects on fertility in male and female rats with exposures approximately 0.7-fold for lopinavir and 1.8-fold for ritonavir of the exposures in humans at the recommended therapeutic dose.

- Teratogenicity/developmental toxicity
  There has been no evidence of teratogenicity with administration of lopinavir + ritonavir to pregnant rats or rabbits. In rats treated with maternally toxic dosage (100 mg lopinavir/50 mg ritonavir/kg/day), embryonic and fetal developmental toxicities (early resorption, decreased fetal viability, decreased fetal body weight, increased incidence of skeletal variations and skeletal ossification delays) were observed; drug exposure in the pregnant rats was 0.7-fold for lopinavir and 1.8-fold for ritonavir of the exposures in humans at the recommended therapeutic dose. In a peri- and postnatal study in rats, a decrease in survival of pups between birth and postnatal day 21 occurred with exposures of 40 mg lopinavir/20 mg ritonavir/kg/day or greater. In rabbits, no embryonic or fetal developmental toxicities were observed with maternally toxic dosage, where drug exposure was 0.6-fold for lopinavir and 1.0-fold for ritonavir of the exposures in humans at recommended therapeutic dose.

- Placental and breast milk passage
  Data on placental passage of lopinavir in animals are not available. For ritonavir, data in humans indicates only minimal transplacental passage (see Ritonavir). Lopinavir and ritonavir are secreted in the breast milk of lactating rats; it is not known if either drug is excreted in human milk.

- Human studies in pregnancy
  The capsule formulation of lopinavir/ritonavir is no longer available; it has been replaced by a new tablet formulation of lopinavir 200 mg/ritonavir 50 mg that is heat stable and does not have a food requirement. However, pharmacokinetic data on lopinavir in pregnancy are currently available only for the capsule formulation, although studies of the tablet formulation in pregnant women are underway.

  The pharmacokinetics of lopinavir/ritionavir capsules were evaluated in the second and third trimester of pregnancy in protocol P1026s. At standard adult capsule doses (3 lopinavir 133 mg/ritonavir 33 mg capsules twice daily), lopinavir levels during the third trimester were significantly lower compared to postpartum levels and those in non-pregnant adults *[82]*. Only 18% of 17 women evaluated during the third trimester had lopinavir area under the curve (AUC) concentrations above the 10th percentile for non-pregnant adults, and none exceeded the 50th percentile; in contrast, 79% of these women evaluated postpartum had AUC values above the 10th percentile. As with ritonavir, placental passage of lopinavir was limited.

Increasing the dose of lopinavir/ritonavir in the third trimester to 4 capsules twice daily provided adequate lopinavir exposure during the third trimester, but resulted in higher levels by 2 weeks postpartum *[83]*. However, a separate study in London of 16 pregnant HIV-infected primarily antiretroviral-naïve women receiving standard dosing of lopinavir/ritonavir capsules throughout pregnancy found that the median trough level of lopinavir in the third trimester was 3,660 ng/mL and that 94% had trough levels >1,000 ng/mL (the minimum trough required to inhibit wild type HIV); 14/16 (88%) of women had virologic suppression *[84]*. Data for AUC were not provided, so these data are not comparable with P1026s data. These investigators suggested therapeutic drug monitoring during the third trimester to determine if an increased dose would be required for the capsule formulation.

The tablet is the currently available formulation of lopinavir/ritonavir. Plasma concentrations of lopinavir and ritonavir after administration of two 200/50 mg lopinavir/ritonavir tablets are similar to those achieved with three 133.3/33.3 mg lopinavir/ritonavir capsules given with food, but with less pharmacokinetic variability.

P1026s is currently evaluating standard dosing of the new lopinavir/ritonavir tablet formulation (2 tablets twice daily) until 30 weeks gestation, followed by an increase to 3 tablets twice daily until postpartum hospital discharge, when return to standard dosing occurs. However, no data are yet available on the tablet formulation to confirm if an increased dose will be required in late pregnancy or on the safety of increased dosing, and therefore no specific recommendations can be made until further data are available.

Once daily dosing of lopinavir/ritonavir capsules or tablets is not recommended in pregnancy, as there are no data to address whether drug levels are adequate with such administration.

Nelfinavir (Viracept®) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Nelfinavir is negative for mutagenicity and clastogenicity in *in vitro* and *in vivo* tests. However, thyroid follicular cell adenomas and carcinomas were increased over baseline in male rats receiving 300 mg/kg/day or higher (equal to a systemic exposure similar to that in humans at therapeutic doses) and female rats receiving 1000 mg/kg/day (equal to a systemic exposure 3-fold higher than that in humans at therapeutic doses) of nelfinavir.

- Reproduction/fertility
  No effect of nelfinavir has been seen on reproductive performance, fertility, or embryo survival in rats at exposures comparable to human therapeutic exposure.

- Teratogenicity/developmental toxicity
  No evidence of teratogenicity has been observed in pregnant rats and rabbits. Developmental toxicity, consisting of small increase in neonatal mortality and minor skeletal ossification delay, occurred at the highest dose in rats. In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to nelfinavir have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with nelfinavir. The prevalence of birth defects with first trimester nelfinavir exposure was 3.7% (95% CI: 2.3–5.6%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk transfer
  In a phase I study in pregnant women and their infants (PACTG 353, see below), transplacental passage of nelfinavir was minimal *[85]*. Additionally, in a study of cord blood samples from 38 women who were treated with nelfinavir during pregnancy, the cord blood nelfinavir concentration was below the assay limit of detection in 24 (63%), and the cord blood concentration was low (median, 0.35 ug/mL) in the remaining 14 women *[80]*. Nelfinavir is excreted in the milk of lactating rats; it is not known if it is excreted in human milk.

- Human studies in pregnancy
  A phase I/II safety and pharmacokinetic study (PACTG 353) of nelfinavir in combination with ZDV and lamivudine in pregnant HIV-infected women and their infants was conducted *[85]*. Nelfinavir

administered at a dose of 750 mg tid produced drug exposures in the first nine pregnant HIV-infected women enrolled in the study that were variable and generally lower than those reported in non-pregnant adults for both tid and bid dosing. Therefore, the study was modified to evaluate an increased dose of nelfinavir given twice daily, 1250 mg bid, which resulted in adequate levels of nelfinavir in pregnancy.

Ritonavir (Norvir®) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  In vitro mutagenicity and clastogenicity screening tests are negative for ritonavir. Carcinogenicity studies in mice and rats have been completed. In male mice, at levels of 50, 100 or 200 mg/kg/day, a dose-dependent increase in liver adenomas and combined adenomas and carcinomas was observed; based on AUC, exposure in male mice at the highest dose was approximately fourfold that in male humans at the recommended therapeutic dose. No carcinogenic effects were observed in female mice with exposures ninefold that of female humans at the recommended therapeutic dose. No carcinogenic effects were observed in rats at exposures up to 0.7-fold that of humans at the recommended therapeutic dose.

- Reproduction/fertility
  No effect of ritonavir has been seen on reproductive performance or fertility in rats at drug exposures 40% (male) and 60% (female) of that achieved with human therapeutic dosing; higher doses were not feasible due to hepatic toxicity in the rodents.

- Teratogenicity/developmental toxicity
  No ritonavir-related teratogenicity has been observed in rats or rabbits. Developmental toxicity was observed in rats, including early resorptions, decreased body weight, ossification delays, and developmental variations such as wavy ribs and enlarged fontanelles; however, these effects occurred only at maternally toxic dosages (exposure equivalent to 30% of human therapeutic exposure). In addition, a slight increase in cryptorchidism was also noted in rats at exposures equivalent to 22% of the human therapeutic dose. In rabbits, developmental toxicity (resorptions, decreased litter size, and decreased fetal weight) was observed only at maternally toxic doses (1.8 times human therapeutic exposure).

  In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester exposures to ritonavir have been monitored to be able to detect at least a two-fold increase in risk of overall birth defects and those in the more common classes, cardiovascular and genitourinary systems. No such increase in birth defects has been observed with ritonavir. The prevalence of birth defects with first trimester ritonavir exposure was 3.1% (95% CI: 1.4–5.8%) compared with total prevalence of birth defects in the U.S. population based on CDC surveillance of 3.1% *[1]*.

- Placental and breast milk transfer
  Transplacental passage of ritonavir has been observed in rats with fetal tissue to maternal serum ratios >1.0 at 24 hours post-dose in mid- and late-gestation fetuses. In a human placental perfusion model, the clearance index of ritonavir was very low, with little accumulation in the fetal compartment and no accumulation in placental tissue *[86]*. In a phase I study in pregnant women and their infants (PACTG 354, see below), transplacental passage of ritonavir was minimal *[87]*. Additionally, in a study of cord blood samples from 6 women treated with ritonavir during pregnancy, the cord blood concentration was below the assay limit of detection in 83%, and was only 0.38 *u*g/mL in the remaining woman *[80]*. Ritonavir is excreted in the milk of lactating rats; it is unknown if it is excreted in human milk.

- Human studies in pregnancy
  A phase I/II safety and pharmacokinetic study (PACTG 354) of ritonavir in combination with zidovudine and lamivudine in pregnant HIV-infected women and their infants is being conducted, but complete data are not yet available. Preliminary data indicate minimal, if any, placental passage of ritonavir.

Saquinavir (Invirase® [Hard Gel Capsule] is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  In vitro screening tests have been negative. Carcinogenicity studies found no indication of carcinogenic activity in rats and mice administered saquinavir for approximately 2 years, at plasma exposures approximately 60% of those obtained in humans at the recommended therapeutic dose (rats) and at exposures equivalent to those in humans at the recommended therapeutic dose (mice).

- Reproduction/fertility
  No effect of saquinavir has been seen on reproductive performance, fertility, or embryo survival in rats. Administration of low doses of saquinavir to newborn rats was associated with gastrointestinal toxicity,

including inflammation at the rectoanal junction and red anal fluid; mortality was seen at very high doses (1200 mg/kg/day).

- Teratogenicity/developmental toxicity
  No evidence for embryotoxicity or teratogenicity of saquinavir has been found in animal studies.

- Placental and breast milk transfer
  Placental transfer of saquinavir in the rat and rabbit was minimal. In a phase I study in pregnant women and their infants (PACTG 386, see below), transplacental passage of saquinavir was minimal *[88]*. Additionally, in a study of cord blood samples from 8 women treated with saquinavir during pregnancy, the cord blood concentration of saquinavir was below the assay limit of detection in samples from all women *[80]*. Saquinavir is excreted in the milk of lactating rats; it is not known if it is excreted in human milk.

- Human studies in pregnancy
  A phase I/II safety and pharmacokinetic study (PACTG 386) of saquinavir-soft gel capsule (SGC) in combination with ZDV and lamivudine in pregnant HIV-infected women and their infants was conducted. The standard adult dose of saquinavir (1200 mg TID) as a single protease inhibitor was not sufficient to produce adequate drug levels in the first four pregnant HIV-infected women enrolled in the study compared to those obtained with standard dosing in non-pregnant adults. Thus, the study was modified to evaluate the combination of saquinavir-SGC (800 mg) plus ritonavir (100 mg), both administered BID. This regimen was well-tolerated and achieved adequate saquinavir levels in the women *[88, 89]*. However, saquinavir-SGC is no longer manufactured, as the saquinavir-hard gel capsule (HGC) is better tolerated and requires fewer daily capsules.

  Several small studies have evaluated the pharmacokinetics of saquinavir-HGC combined with low dose ritonavir in pregnant women. In a pharmacokinetic study of 4 pregnant women receiving saquinavir-HGC 1,000 mg/ritonavir 100 mg based regimen twice daily, trough concentrations ranged from 656 to 2,169 ng/mL and peak concentrations from 845 to 4,002 ng/mL; the minimum trough concentration for wild-type virus is 100 ng/mL *[90]*. In a separate pharmacokinetic study of 15 pregnant women receiving a saquinavir-HGC 1,000 mg/ritonavir 100 mg based regimen twice daily, the projected median trough level was 1,041 ng/mL (range 96–2,238 ng/mL); one woman had a trough level below <100 ng/mL but achieved adequate levels at an increased dose of 1,200 mg saquinavir-HGC/100 mg ritonavir *[91]*. Finally, in a study of 2 women who received saquinavir-HGC 1,200 mg/ritonavir 100 mg given once daily, trough levels were 285 and 684 ng/mL and the area under the curves (AUC_{0-24}) were 28,010 and 16,790 ng•hour/mL, above the target AUC of 10,000 ng•hour/mL *[92]*. Thus, the limited available data suggest that 1,000 mg saquinavir-HGC/100 mg ritonavir given twice daily should achieve adequate trough levels in HIV-infected pregnant women; data are too limited to recommend once-daily dosing. Saquinavir-HGC should always be given with low dose ritonavir boosting.

Tipranavir (Aptivus$^{®}$) is classified as FDA category C.

- Animal carcinogenicity studies
  Long term carcinogenicity bioassays are currently in progress. Tipranavir showed no evidence of mutagenicity or clastogenicity in a battery of five tests including the Ames bacterial reverse mutation assay, unscheduled DNA synthesis in rat hepatocytes, induction of gene mutation in Chinese hamster ovary cells, a chromosome aberration assay in human peripheral lymphocytes, and a micronucleus assay in mice.

- Reproduction/fertility
  Tipranavir had no effect on fertility or early embryonic development in rats at dose levels similar to human exposures.

- Teratogenicity/developmental toxicity
  No teratogenicity was detected in studies of pregnant rats and rabbits at exposure levels approximately 1.1-fold and 0.1-fold human exposure. In rats exposed to 400 mg/kg/day (~0.8-fold human exposure) and above, fetal toxicity (decreased ossification and body weights) was observed. Fetal toxicity was not seen in rats and rabbits at levels of 0.2-fold and 0.1-fold exposures in humans. In rats, no adverse effects were seen on development at levels of 40 mg/kg/day (~0.2-fold human exposure), but growth inhibition in pups and maternal toxicity were seen at 400 mg/kg/day (~0.8-fold human exposure).

- Placental and breast milk transfer
  No animal studies of placental or breast milk passage of tipranavir have been reported. It is unknown if placental or breast milk passage of tipranavir occurs in humans.

- Human studies in pregnancy
  No studies of tipranavir have been conducted in pregnant women or neonates.

FUSION INHIBITORS

Enfuvirtide, which requires subcutaneous administration, is the first of the fusion inhibitor class of antiretroviral drugs; these drugs inhibit binding or fusion of HIV to host target cells. Binding of the viral envelope glycoprotein gp120 to the $CD4^+$ receptor induces conformational changes that enable gp120 to interact with a chemokine receptor on the host cell; binding of gp120 to the coreceptor causes subsequent conformational changes in the viral transmembrane glycoprotein gp41, exposing the "fusion peptide" of gp41, which inserts into the cell membrane. A helical region of gp41, called HR1, then interacts with a similar helical region, HR2, on gp41, resulting in a "zipping" together of the two helices and mediating the fusion of cellular and viral membranes. Enfuvirtide is a synthetic 36 amino acid peptide derived from a naturally occurring motif within the HR2 domain of viral gp41. As a molecular mimic of the HR2 region, the drug binds to the HR1 region, preventing the HR1-HR2 interaction and correct folding of gp41 into its secondary structure, thereby inhibiting virus-cell fusion. Enfuvirtide was approved for use in combination with other antiretroviral drugs to treat advanced HIV infection in adults and children aged 6 years or older.

Enfuvirtide (Fuzeon™, T-20) is classified as FDA pregnancy category B.

- Animal carcinogenicity studies
  Long-term animal carcinogenicity studies of enfuvirtide have not been conducted. Enfuvirtide was neither mutagenic or clastogenic in a series of in vitro and animal *in vivo* screening tests.

- Reproduction/fertility animal studies
  Reproductive toxicity has been evaluated in rats and rabbits. Enfuvirtide produced no adverse effects on fertility of male or female rats at doses up 30 mg/kg/day administered subcutaneously (1.6 times the maximum recommended adult human daily dose on a m^2 basis).

- Teratogenicity/developmental toxicity animal studies
  Studies in rats and rabbits revealed no evidence of harm to the fetus from enfuvirtide administered in doses up to 27 times and 3.7 times, respectively, the adult human daily dose on a m^2 basis.

- Placental and breast milk passage
  Studies of radio-labeled enfuvirtide administered to lactating rats indicated radioactivity was present in the milk; however, it is not known if this reflected radio-labeled enfuvirtide or from radio-labeled metabolites (e.g., amino acid and peptide fragments) of enfuvirtide. It is not known if enfuvirtide is crossed the human placenta or is excreted in human milk.

- Human studies in pregnancy
  No studies of enfuvirtide have been conducted in pregnant women or neonates.

MISCELLANEOUS AGENTS

Hydroxyurea is classified as FDA pregnancy category D.

Hydroxyurea is a cytotoxic and antimitotic agent that inhibits DNA synthesis and has been used for treatment of myeloproliferative disorders and sickle cell anemia. It has recently been studied for treatment of HIV disease in combination with nucleoside analogue antiretroviral agents. By inhibiting ribonucleotide reductase, it depletes the pool of deoxynucleoside triphosphates, particularly dATP, thereby potentiating the incorporation of the nucleoside analogue drugs into viral DNA and increasing their antiretroviral effect. However, the drug has significant toxicities and its role in HIV therapy is not well defined.

- Animal carcinogenicity studies and human data
  Hydroxyurea is genotoxic in a wide range of *in vitro* and *in vivo* animal test systems, causes cellular transformation to a tumorigenic phenotype, and is a transspecies carcinogen, which implies a potential carcinogenic risk to humans. Conventional long-term animal carcinogenicity studies have not been performed. However, intraperitoneal administration of 125 to 250 mg/kg of hydroxyurea (approximately 0.6 to 1.2 times the maximum recommended human oral dose on a mg/m^2 basis) three times weekly for 6 months to female rats increased the incidence of mammary tumors in rats surviving to 18 months compared to controls.

  In humans receiving long-term hydroxyurea for myeloproliferative disorders such as polycythemia vera, secondary leukemias have been reported. It is unknown whether this leukemogenic effect is secondary to hydroxyurea or is associated with the patients' underlying disease. Skin cancer has also been reported in patients receiving long-term therapy.

- Reproduction/fertility
  Hydroxyurea administered to male rats at doses of 60 mg/kg/day (about 0.3 times the maximum recommended human daily dose on a mg/m^2 basis)

produced testicular atrophy, decreased spermatogenesis, and significantly reduced their ability to impregnate females.

- <u>Teratogenicity/developmental toxicity</u>
  Potent teratogenic effects have been observed in all animal species tested, with defects reported in multiple organ systems *[93-99]*. Administration of hydroxyurea to pregnant rats at doses as low as 180 mg/kg/day (about 0.8 times the maximum recommended human daily dose on a mg/m^2 basis) and pregnant rabbits at 30 mg/kg/day (about 0.3 times the maximum recommended human daily dose on a mg/m^2 basis) was associated with embryotoxicity and fetal malformations. In pregnant rats administered doses ranging from 185 to 1000 mg/kg body weight, fetal defects that have been observed include central nervous system, cardiovascular, ocular, craniofacial, and skeletal anomalies, limb deformities, and diaphragmatic hernia, with the pattern of defects dependent on gestational day of exposure *[93, 96, 97]*. Exposure early in gestation was associated with embryo death in a large percentage of cases. In pregnant rats, single doses of 375 mg/kg body weight or more (about 1.7 times the maximum recommended human daily dose on a mg/m^2 basis), were associated with growth retardation and impaired learning ability in their offspring. In hamsters, neural tube defects and cardiovascular abnormalities were produced after a 50 mg dose of hydroxyurea was given intravenously *[94]*. In pregnant rhesus monkeys administered a cumulative dose greater than 500 mg/kg body weight, multiple skeletal, genitourinary, cardiac, and ocular anomalies were found in their offspring *[96]*. Teratogenicity was also demonstrated in pregnant cats given a single oral dose of 50 or 100 mg/kg body weight *[95]*.

- <u>Placental and breast milk passage</u>
  Hydroxyurea has been shown to cross the placenta in animals. Hydroxyurea is excreted in human milk *[100]*.

- <u>Human studies in pregnancy</u>
  Published reports of hydroxyurea during human pregnancy include 16 women, all treated for primary hematologic illnesses (e.g., chronic myeloid leukemia, sickle cell anemia, primary thrombocytopenia) *[101]*. Doses ranged from 0.5 to 3 g/day and 13 women had first trimester exposure. No fetal anomalies were seen and normal pregnancy outcomes were reported, except for one stillbirth with eclampsia at 26 weeks gestation and four elective pregnancy terminations.

Because of concerns raised by the significant anomalies seen in multiple animal species exposed to hydroxyurea and limited human information, as well as the uncertain role of hydroxyurea in HIV therapy, hydroxyurea use as an antiretroviral regimen component should be avoided during pregnancy. Clinicians should counsel women of childbearing potential about potential risks of teratogenicity if they are treated with hydroxyurea and become pregnant, and encouraged to use effective contraception and avoid becoming pregnant while being treated with hydroxyurea.

ANTIRETROVIRAL PREGNANCY REGISTRY

The Antiretroviral Pregnancy Registry is an epidemiologic project to collect observational, nonexperimental data on antiretroviral exposure during pregnancy for the purpose of assessing the potential teratogenicity of these drugs. Registry data will be used to supplement animal toxicology studies and assist clinicians in weighing the potential risks and benefits of treatment for individual patients. The registry is a collaborative project of the pharmaceutical manufacturers with an advisory committee of obstetric and pediatric practitioners.

It is strongly recommended that health care providers who are treating HIV-1-infected pregnant women and their newborns report cases of prenatal exposure to antiretroviral drugs (either alone or in combination) to the Antiretroviral Pregnancy Registry. The registry does not use patient names, and birth outcome follow-up is obtained by registry staff from the reporting physician.

Referrals should be directed to
Antiretroviral Pregnancy Registry
Research Park
1011 Ashes Drive
Wilmington, NC 28405
Telephone: 1–800–258–4263
Fax: 1–800–800–1052
Internet access www.APRegistry.com.

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