**Background:** Defining the effect of antiretroviral regimens on breast milk HIV-1 levels is useful to inform the rational design of strategies to decrease perinatal HIV-1 transmission. Methods Pregnant HIV-1 seropositive women (CD4 >250 and <500 cells/mm3) electing to breastfeed in Nairobi, Kenya were randomized to HAART (zidovudine, lamivudine, and nevirapine) during pregnancy and 6 months postpartum or to short-course zidovudine plus single-dose nevirapine (ZDV/NVP). Breast milk samples were collected 2-3 times per week in the first month postpartum. **Findings:** Between November 2003 and April 2006, 444 breast milk samples were collected from 58 randomized women during the first month after delivery. Between 3 and 14 days postpartum, women in the HAART and ZDV/NVP arms had a similar prevalence of undetectable breast milk HIV-1 RNA. From 15 to 28 days postpartum, women in the HAART arm had significantly lower levels of breast milk HIV-1 RNA than women randomized to ZDV/NVP (1.7 log10 copies/ml (limit of detection) vs. > 2.10 log10 copies/ml, P < 0.001). In contrast to breast milk HIV-1 RNA, suppression of plasma HIV-1 RNA during the neonatal period was consistently several log10 greater in the HAART arm compared to the ZDV/NVP arm. **Conclusions:** HAART resulted in lower breast milk HIV-1 RNA than ZDV/NVP, however, ZDV/NVP yielded comparable breast milk HIV-1 RNA levels in the first 2 weeks postpartum. Breast milk HIV-1 RNA remained suppressed in the ZDV/NVP arm despite increased plasma HIV-1 levels, which may reflect local drug-effects or compartmentalization.