Preexposure prophylaxis (PrEP) with emtricitabine plus Background. tenofovir disoproxil fumarate (FTC/TDF) or TDF alone reduces the risk of human immunodeficiency virus (HIV) acquisition. Understanding the risk of antiretroviral resistance selected by PrEP during breakthrough infections is important because of the risk of treatment failure during subsequent antiretroviral use. Within the largest randomized trial of FTC/TDF versus TDF as Methods. PrEP, plasma samples were tested for HIV with resistance mutations associated with FTC (K65R and M184IV) and TDF (K65R and K70E), using 454 sequencing. Of 121 HIV seroconverters, 25 received FTC/TDF, 38 received Results. TDF, and 58 received placebo. Plasma drug levels in 26 individuals indicated PrEP use during or after HIV acquisition, of which 5 had virus with resistance mutations associated with their PrEP regimen. Among those with PrEP drug detected during infection, resistance was more frequent in the FTC/TDF arm (4 of 7 [57%]), compared with the TDF arm (1 of 19 [5.3%]; P = .01), owing to the FTC-associated mutation M184IV. Of these cases, 3 had unrecognized acute infection at PrEP randomization, and 2 were HIV negative at enrollment. These results suggest that resistance selected by PrEP is Conclusions. rare but can occur both with PrEP initiation during acute seronegative HIV infection and in PrEP breakthrough infections and that FTC is associated with a greater frequency of resistance mutations than TDF.