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RECOMBINANT RETROVIRAL SYSTEMS FOR RAPID *IN VIVO* ANALYSIS OF HIV-1 SUSCEPTIBILITY TO REVERSE TRANSCRIPTASE AND PROTEASE INHIBITORS

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Rapid analysis of HIV drug susceptibility is needed to monitor the development of drug resistance in patients receiving approved or experimental antiretroviral therapy. Conventional methods that require virus isolation are time-consuming, labour intensive, and variable. To address these limitations, we have developed two recombinant retroviral systems to rapidly detect and analyse HIV-1 variants resistant to reverse transcriptase (RT) or protease inhibitors. Viral RNA is extracted from plasma and RT or protease/gag coding regions are reverse-transcribed and PCR amplified. A 'library' of infectious molecular clones of HIV-1 is constructed by shotgun cloning of the PCR products into RT- or protease-defective proviral vectors. Infectious recombinant viruses are produced by transfecting MT-2 cells with bulk clonal mixtures or individual subclones. Drug susceptibility is then determined in CD4+ cell lines. DNA sequencing of the cloned RT or protease can identify mutations associated with phenotypic resistance of clonal mixtures or individual subclones. The entire procedure can be completed in 3 weeks. Experiments have shown that (1) the recombinant viruses constructed proportionally represent starting virus mixtures; (2) the emergence of HIV variants resistant to RT or protease inhibitors can be detected in plasma samples; (3) drug-resistant viral quasispecies can be cloned and analysed phenotypically and genotypically at the clonal level; and (4) novel genetic mechanisms of drug resistance can be identified (for example lamivudine/zidovudine co-resistance). These systems can be used to characterize predominant as well as minor drug-resistant species in plasma, and correlate drug susceptibility phenotype with genotype at the clonal level.

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