Among the Devils in the Details Are Protease Sequence, Susceptibility, and Structure in CRF02_AG Viruses

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(See the article by Kinomoto et al. on pages 243–51)

Diverse and distinct subtypes of HIV-1, including complex circulating recombinant forms (CRFs), have been outstripping the prototypic subtype B isolates in much of Africa, Latin America, and Asia. However, in the course of drug development, preclinical in vitro studies, pivotal clinical trials, and analysis of long-term treatment have largely focused on subtype B virus infection. With “scale-up” in global treatment, the pathogenesis and drug susceptibility of non–subtype B HIV-1 infection outside of the United States is a critical question for regional and national treatment guidelines and for clinicians in the United States and Europe who are confronting an increasingly cosmopolitan epidemic.

The work of Kinomoto, Appiah-Opong, and colleagues [1] on the protease inhibitor–susceptibility of Ghanaian viruses of subtype CRF02_AG raises important questions about HIV treatment regimens in West Africa and, more generally, about the susceptibility of non–subtype B viruses. Are the currently approved antiretrovirals, licensed largely on the basis of clinical trials conducted in the United States and Europe, as active against non–subtype B viruses (specifically CRF02_AG, the dominant CRF in West Africa) as they are against subtype B viruses? Do common, subtype-related polymorphisms in gag-pol in subtype CRF02_AG viruses—some of which are associated with drug resistance—significantly impact the effectiveness or the outcome of protease inhibitor–based therapies? Do the modest changes in drug susceptibility, carefully sought through sensitive recombinant phenotypic assays and in structural binding studies, identify an important mechanism for drug failure and selection of resistance in non–subtype B infection?

Despite nearly 10 years of HAART, only a handful of clinical studies compare patients who have subtype B infection with patients who have non–subtype B infection in terms of their response to commonly prescribed regimens. Comparisons of patients infected with different viral subtypes are limited by the diversity of HIV, both within and between subtypes, as well as by differences in site and host factors, including pharmacogenomics, nutritional status, adherence, access, and educational and socioeconomic status. With respect to randomized clinical trials, there is an encouraging trend in the conduct of pivotal clinical trials of HAART. Increasingly, phase III clinical trials are becoming truly global, including sites in Europe, Latin America, Africa, and Asia where non–subtype B viruses predominate. Analysis of these clinical trials may provide insight into the effects of variation in viral subtype and host genomics. However, among the widely divergent and evolving subtypes and CRFs, even large international trials are powered to address only very dramatic differences among subtypes in the efficacy of drugs and drug combinations.

Consistent differences between subtypes are the result of founder effects and immunologic pressure caused by distinct HLA alleles [2]. Differences in the drug susceptibility of non–subtype B viruses rely largely on genotypic analyses of gag-pol sequences and characterizations of the effects of subtype-specific mutations associated with drug resistance in subtype B. Fortunately, the pace of virologic analysis has quickened with increasing international access to drug treatment and resistance testing. Reviewing recent publications through a search of the National Institute of Health’s PubMed database reveals that investigators from Brazil, India, Malaysia, Israel, South Africa, and North America have probed differences in protease inhibitor–associated polymorphisms and gag cleavage site mutations in non-B subtypes. They have described emerging recombinant viruses and have explored relative fitness, distinct codon usage, and...
The current study used just such a strategy, focusing on a subtype B backbone, and the susceptibility to drugs is assessed by measuring virus replication in the presence of drugs. Kinomoto et al. [1] performed sophisticated molecular modeling of structure-function relationships to calculate inhibitor-ligand binding interactions, comparing different enzymes and inhibitors that may require some qualifications. One limitation of the analysis is that the attempt to ascribe the significant difference in inhibition constants to structural differences may need more careful structural analysis of protein-ligand complexes. It is challenging to gain an understanding of the structure/activity relationships at play with even closely related drug structures and/or protein polymorphs. A second limitation is the differences between calculated structures and interactions and those determined empirically by inhibitor-enzyme crystal structures. Minimization routines to calculate ligand binding (including those presented in Kinomoto et al. [1]) are conducted, of necessity, with predetermined constraints placed on certain atoms to limit computing time. However, crystallographers have documented significant changes in protein side-chain packing with mutant structures and different inhibitors. The minimization and docking procedures suggest that a conformational change in the ligand may be the source of the difference in binding between subtypes B and CRF02_AG proteases with certain protease inhibitors. In many structures of HIV protease bound with relatively inflexible inhibitors, it is the side chains of the protein and occasionally the backbone that show movement [11]. Subtle differences in binding may contrast with the huge movements of the “flaps” of protease associated with drug binding as drug and protease are varied. Modeling techniques have been applied to the question of flap movements associated with drug and substrate binding [12]. The modeling presented in Kinomoto et al. [1] shows only subtle differences in binding. Whether the calculated differences in “insertability” of an inhibitor into a mutant structure can account for small differences in the activity of a drug is an intriguing question, but one that is still highly speculative.

Will the results of these studies influence decisions about protease inhibitor therapies in West Africa or in patients infected with subtype CRF02_AG viruses? To a great extent, the guidelines for antiretroviral therapy have already incorporated the recommendation for pharmacologic boosting of protease inhibitors with ritonavir to avoid selecting resistance through subinhibitory drug levels. The ordering of binding coefficients, phenotypic susceptibility, and a predicted genotypic susceptibility to current protease inhibitors for HIV-1 subtype CRF02_AG are important. Combined with drug tolerability, regimen pill burden, cost, and pharmacologic properties, relative drug activity will play a role in optimizing effective regimens to meet the complex challenge of the need for effective HIV treatment in Africa. This is a thoughtful investigation of the mechanism of drug resistance as we enter an era of increasing treatment programs worldwide.

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References


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