A TECHNICAL REVIEW

LabCorp
Laboratory Corporation of America

GenoSure PRIme®

Detection of HIV-1 protease, reverse transcriptase, and integrase inhibitor resistance

Introduction

Acquired immune deficiency syndrome (AIDS) was first reported in the US in June 1981, and the causative agent, human immunodeficiency virus type 1 (HIV-1) was identified in 1983. It is estimated that more than one million people in the US are currently infected with HIV-1, and approximately 50,000 new HIV-1 infections occur annually.[1]

The US Food and Drug Administration (FDA) approved the first antiretroviral (ARV), azidothymidine (AZT, zidovudine [ZDV]) for the treatment of HIV/AIDS in March 1987. Since then, more than thirty ARV agents comprising six drug classes have received FDA approval for the treatment of HIV/AIDS.[2] Despite the number of available ARV agents, successful treatment (ie, complete and sustained suppression of viral replication) may be compromised by the emergence of virus strains that exhibit resistance to one or more of these therapeutics.

Furthermore, achievement of complete and sustained viral suppression typically necessitates the simultaneous administration of three to four potent ARV agents to prevent the emergence of multidrug-resistant strains generated by the high rate and error-prone nature of HIV-1 replication. The selection of optimal combinations of ARVs is routinely guided by DNA sequencing-based drug resistance assays designed to detect mutations in HIV-1 that are associated with drug resistance.

Drug resistant HIV-1 can be transmitted at the time of infection or acquired in response to suboptimal ARV treatment. Prevalence rates of transmitted drug-resistant (TDR) virus in ARV treatment-naïve patients have increased, as measured and reported by the Centers for Disease Control (CDC) and numerous other laboratories. An analysis puplished in 2000 identified overall ARV drug resistance in 5% of ARV treatment-naïve individuals residing in six US cities.[3] CDC surveillance data from 2007 reported one or more transmitted drug resistance mutations in 16% of newly documented HIV-1 infections.[4] In San Francisco, analysis of a large cohort of newly diagnosed patients identified transmitted drug resistance at a prevalence of 15% by 2009.[5]

Fluctuating trends in HIV-1 drug resistance have been reported and are likely indicative of changes in clinical treatment practices as new ARVs — as well as new ARV drug classes — become available for patient management. At the time of the 1998 CDC report, TDR virus most often involved HIV-1 strains characterized by resistance to nucleoside reverse transcriptase inhibitors (NRTI).[3] By 2003 to 2006, TDR involving strains exhibiting resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs) had increased to 6.9% from 2% in an earlier analysis.[3,6] Irrespective of whether ARV drug resistance was transmitted or acquired during treatment, a recent analysis of resistance trends conducted on a large data base comprised of drug resistance test results performed between 2007 and 2010 illustrates the impact of the availability of new ARV therapies on the emergence of drug-resistant HIV-1.[7]

As an example, the percentage of virus strains manifesting reduced susceptibility to any member of the protease inhibitor (PI) drug class declined from 49% in 2007 to 26% in 2010. This was accompanied by a decrease in the frequency of PI mutations associated with resistance to first-generation PIs and a concomitant increase in PI mutations associated with resistance to more recently developed PIs that are more potent and tolerable.[7]

In a recently published commentary that described the identification of TDR strains exhibiting resistance to specific ARV drug classes, TDR emerged at four and three years, respectively, following the broad adoption of NNRTI and PI use in clinical practice.[8] The first IN strand transfer inhibitor (INSTI), raltegravir (Isentress®, Merck Laboratories), received FDA approval in 2007, and a similar pattern of TDR appears to be emerging with this new ARV drug class. Three cases of TDR to INSTIs have been documented roughly four years following the widespread adoption of raltegravir use in clinical practice.[9–11]

Laboratory Method

The GenoSure PRIme (PR-RT-IN) assay evaluates the HIV-1 polymerase region (*pol* gene), including the complete PR and IN coding regions and amino acids 1 to 400 of RT. (See figure 1.) RT-PCR amplification and population sequencing are used to generate nucleotide sequences in the *pol* region, from which the amino acid sequences of the PR, RT, and IN proteins are derived. These amino acid sequences are compared to wild-type (susceptible) reference sequences to identify amino acid substitutions/mutations associated with PI, NRTI, NNRTI, and/or INSTI resistance. Subsequent analyses of these mutation profiles are performed using proprietary algorithms to derive net assessments (ie, predictions) of susceptibility to commercially available NRTIs, NNRTIs, PIs, and INSTIs. Net assessments include sensitive (S), resistance possible (RP), and resistant (R) determinations.

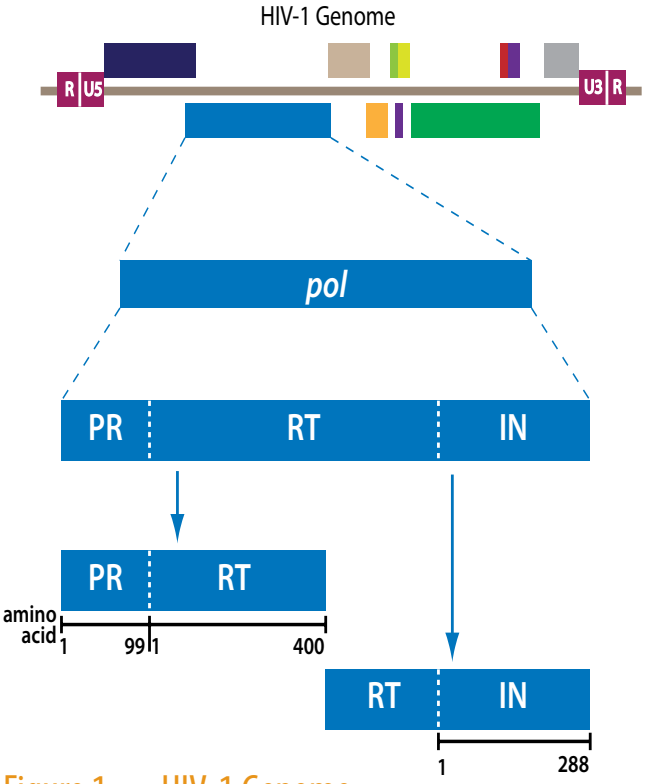

Figure 1 — HIV-1 Genome

(*pol*=HIV-1 polymerase region, PR=protease coding region, RT=reverse trranscriptase coding region, IN=integrase coding region)

An expansive data base consisting of more than 100,000 paired genotypic susceptibility determinations and phenotypic susceptibility measurements is the foundation of the GenoSure PRIme proprietary algorithms.[12] Sophisticated statistical analyses—comprehensive review of the scientific literature and conference presentations, expert opinion, and directed experimental validations of novel patterns—contribute to regular updates of the algorithm.

GenoSure PRIme has the ability to detect minor drug-resistant variants present at levels as low as 10% of the population. For amino acid positions with a mixture of both wild-type and drug-resistant variants, these positions are considered resistant in the determination of the net assessment (ie, S, RP, R). A minimum HIV-1 RNA viral load of 500 copies/mL is required for analysis.

GenoSure PRIme Clinical Application

Current guidelines issued by the Department of Health and Human Services (DHHS) and the International Antiviral Society (IAS-USA) recommend genotypic resistance testing when a person with HIV-1 enters into care, whether or not treatment is initiated.[18,19] Current genotypic drug resistance assays routinely identify mutations in the PR and RT regions. Evaluation of the IN region is considered when there is concern that a particular TDR strain may exhibit resistance to the INSTI class of ARV drugs (eg, in cases of poor responses to an initial PI-NRTI or NNRTI-NRTI regimen). As previously noted, the first cases of transmitted INSTI (raltegravir) resistant virus have been confirmed and documented and appear to be emerging consistent with cases of TDR involving the PI, NRTI, and NNRTI classes.[8–11]

The concern for transmitted IN resistance and the need to screen for INSTI-resistant variants will continue to grow as the use of INSTI continues to increase in clinical settings. It is anticipated, therefore, that the incorporation of INSTI resistance testing into baseline genotypic resistance testing on initial diagnosis of HIV-1 infection will become increasingly warranted. Raltegravir, the first in class INSTI is approved by the FDA for use in ARV treatment-experienced and ARV treatment-naïve settings. Raltegravir is also a component of one of the preferred first-line regimens recommended by the DHHS Guidelines and IAS-USA.[18,19]

The integrase inhibitor elvitegravir (Gilead Sciences) was approved in August 2012 and dolutegravir (GlaxoSmithKline) was approved in August 2013. As these promising integrase inhibitors become commercially available, the use of this class is likely to expand due to more favorable dosing schedules and increased potency.[13-17,20]

Genotypic ARV drug resistance testing is also recommended following suboptimal virologic suppression or virologic failure during the course of early-stage or late-stage ARV treatment regimens. These regimens may or may not include INSTIs. If a patient is failing an INSTI-containing regimen, the DHHS guidelines recommend consideration of resistance testing to determine IN inhibitor resistance mutations.[18] In such cases, providers may consider supplementing routine genotypic PR/RT resistance testing with a separate genotypic resistance test for INSTI.[18]

The availability of GenoSure PRIme enables the evaluation of PR, RT, and IN inhibitor resistance from one sample in a single assay, providing a comprehensive assessment of HIV-1 ARV drug resistance on one report. The information provided by the GenoSure PRIme assay can guide the selection of effective HIV-1 treatments that establish or reëstablish complete suppression of HIV-1 replication, which in turn will slow, arrest, or reverse HIV-1 disease progression.

Relevant Assays*

Test Name	Test Nº
Human Immunodeficiency Virus 1 (HIV-1) GenoSure PRIme®	**551700**
Human Immunodeficiency Virus 1 (HIV-1), Quantitative, Real-time PCR (Graphical) With Reflex to HIV-1 GenoSure PRIme®	**550630**
Human Immunodeficiency Virus 1 (HIV-1), Quantitative, Real-time PCR (Nongraphical) With Reflex to HIV-1 GenoSure PRIme®	**550655**

*For the most current information regarding test options, including specimen requirements, please consult the online *Directory of Services and Interpretive Guide* at https://www.labcorp.com/wps/portal/testmenu.

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