Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

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HIV Drug-Resistance and Resistance Assays

HIV replication is a continuous process in most untreated patients, leading to the daily production of billions of virions. The goal of combination antiretroviral therapy (cART) is to suppress HIV replication as rapidly and fully as possible, as indicated by a reduction in plasma HIV RNA to below the limit of detection using the most sensitive assays available. Unfortunately, mutations in HIV RNA arise during viral replication because HIV reverse transcriptase (RT) is a highly error-prone enzyme. Consequently, ongoing replication in the presence of antiretroviral (ARV) drugs, as occurs in suboptimal adherence, readily and progressively selects for strains of HIV with mutations that confer drug resistance. Viruses harboring resistance-associated mutations can be transmitted in both perinatal and non-perinatal infection, underscoring the importance of resistance testing at the time of HIV diagnosis before cART initiation.1,2

Drug-resistance detection methods vary depending on the class of ARV agents. Both genotypic assays and phenotypic assays currently are used to detect the presence of virus that is resistant to inhibitors of the HIV RT, integrase (IN), or protease (PR) enzymes. Clinical experience with testing for viral resistance to other agents is more limited, but genotypic assays that assess mutations in gp41 (envelope) genes also are commercially available. Experience is also limited with the use of commercially available genotypic and phenotypic assays in the evaluation of drug resistance in patients infected with non-B subtypes of HIV.3,4 Table 19 summarizes the indications for using available resistance testing.
**Genotypic Assays**

Genotypic assays for resistance to RT and PR inhibitors and IN strand transfer inhibitors are based on polymerase chain reaction amplification and analysis of the RT, PR, and IN coding sequences present in HIV RNA extracted from plasma. Genotypic assays can detect resistance mutations in plasma samples containing approximately 1,000 copies/mL or more of HIV RNA and results generally are available within 1 to 2 weeks of sample collection. Not all available genotypic tests include IN resistance; it may need to be specifically requested. Interpretation of test results requires knowledge of the mutations selected by different ARV drugs and of the potential for cross resistance to other drugs conferred by certain mutations. For some drugs, the genetic barrier to the development of resistance is low and a single nucleotide mutation is enough to confer high-level resistance sufficient to remove any clinical utility of the drug. This is exemplified by resistance to nevirapine and efavirenz resulting from mutations in the HIV RT (e.g., K103N). Other mutations lead to drug resistance but simultaneously impair HIV replication. Clinically useful activity of the ARV agent may therefore remain, as demonstrated by evidence of continued clinical benefit from lamivudine in individuals with evidence of the high-level lamivudine resistance engendered by the M184V RT mutation. By contrast, HIV evolution to high-level resistance to some drugs is associated with the emergence of mutations that confer resistance as well as compensatory mutations that allow the virus to replicate more efficiently in the presence of the ARV agent. In addition, polymorphisms that occur naturally or in the presence of drug and are not significant alone may confer clinically significant drug resistance when present with other polymorphisms or major resistance mutations.

The International AIDS Society-USA (IAS-USA) and the Stanford University HIV Drug Resistance Database maintain lists of resistance mutations that confer resistance to currently available ARV drugs (see [http://www.iasusa.org/resistance_mutations](http://www.iasusa.org/resistance_mutations) or [http://hivdb.stanford.edu](http://hivdb.stanford.edu)). A variety of online tools analyze the simultaneous effect of all mutations detected in a patient in order to assist the provider in interpreting genotypic test results. Although the response to cART in children and adolescents is not always predicted by the results of genotypic resistance assays, clinical trials in adults have demonstrated the benefit of resistance testing combined with consultation with specialists in HIV drug resistance in improving virologic outcomes. Given the potential complexity of interpretation of genotypic resistance, it is recommended that clinicians consult with a pediatric HIV specialist for assistance in the interpretation of genotypic results and design of an optimal new regimen.

**Phenotypic Assays**

Phenotypic resistance assays provide a more direct assessment of the impact on ARV susceptibility of viral replication of mutations that are present in an individual’s HIV variants. As they are most often performed, phenotypic assays involve PCR amplification of the predominant RT, IN, PR, or gp41 envelope gene sequences from patient plasma and insertion of those amplified patient sequences into the backbone of a cloned strain of HIV that expresses a reporter gene. Replication of this recombinant virus in the presence of a range of drug concentrations is monitored by quantifying expression of the reporter gene and is compared with replication of a reference drug susceptible HIV variant. The drug concentration that inhibits viral replication by 50% (i.e., the mean inhibitory concentration [IC₅₀]) is calculated, and the ratio of the IC₅₀ of test and reference viruses is reported as the fold increase in IC₅₀ (i.e., fold resistance change). Automated, recombinant phenotypic assays that can produce results in 2 to 3 weeks are commercially available; however, they are more costly than genotypic assays.

Analytic techniques have also been developed to use the genotype to predict the likelihood of a drug-resistant phenotype. This bioinformatic approach, currently applicable for RT, IN, and PR inhibitor resistance only, matches the pattern of mutations obtained from the patient sample with a large database of samples for which both genotype and phenotype are known. Therefore, the sample is assigned a predicted phenotype susceptibility (or virtual phenotype) based on the data from specimens matching the patient’s genotype.
**Tropism (Viral Coreceptor Usage) Assays**

HIV enters cells by a complex, multistep process that involves sequential interactions between the HIV envelope protein molecules and the CD4 T lymphocyte (CD4) receptor, and then with either the CCR5 or CXCR4 coreceptor molecules, culminating in the fusion of the viral and cellular membranes. Viruses initially are CCR5 tropic in the majority of untreated individuals, including infants and children perinatally infected with HIV. However, a shift in coreceptor tropism often occurs over time, from CCR5 usage to either CXCR4 or dual or mixed [D/M] tropism. Viral coreceptor (tropism) assays are used to detect virus with tropism that will (CCR5 tropism) or will not (CXCR4 tropism or D/M tropism) be blocked by CCR5 antagonists. Detection of viral variants with CXCR4 or D/M tropism indicates resistance to CCR5 antagonists. ARV-treated patients with extensive drug resistance are more likely to harbor detectable CXCR4- or D/M-tropic virus than untreated patients with comparable CD4 counts. Studies of heavily treated perinatally infected children and adolescents have shown CXCR4 tropism rates of 19% to 80%; however, most of the studies have used genotypic testing to determine tropism, which may be flawed, as discussed below.

Resistance to CCR5 antagonists is detected using specialized phenotypic assays (Phenoscript [VIRalliance] and Trofile™ [Monogram Biosciences, Inc.]). These assays involve the generation of recombinant viruses bearing patient-derived envelope proteins (gp120 and gp41). The relative capacity of these pseudoviruses to infect cells bearing the cell surface proteins CCR5 or CXCR4 is based on the expression of a reporter gene. Detection of X4 or D/M tropism is a contraindication to the use of the CCR5 antagonists as part of a therapeutic regimen. Coreceptor assays must be performed before a CCR5 inhibitor is used and should be considered in patients exhibiting virologic failure on a CCR5 inhibitor such as maraviroc.

The Trofile™ assay takes about 2 weeks to perform and requires a plasma viral load ≥1000 copies/mL and at least 3 mL of plasma. The initial version of the Trofile™ assay used during the clinical trials that led to the licensure of maraviroc was able to detect X4-tropic virus with 100% sensitivity when present at a frequency of 10% of the plasma virus population, but only 83% sensitivity when the variant was present at a frequency of 5%. In initial clinical trials of CCR5 antagonist drugs, this sensitivity threshold was not always sufficient to exclude the presence of clinically meaningful levels of X4- or D/M-tropic virus in patients initiating a CCR5 inhibitor-based regimen. The current enhanced sensitivity version of the Trofile™ assay (Trofile-EST™) is able to detect X4- or D/M-tropic virus representing as little as 0.3% of the plasma virus. One of the tropism assays can also be performed following amplification of HIV sequences from peripheral blood DNA (Trofile-DNA™ [Monogram Biosciences, Inc.]) and may be most useful when a change to a regimen containing a CCR5 antagonist is being considered for individuals with plasma viral load below 1,000 copies/mL and can be used even when the viral load is undetectable (e.g., if single-drug substitution for toxicity).

**Limitations of Current Resistance and Tropism Assays**

Limitations of the genotypic, phenotypic, and phenotype-prediction assay approaches include lack of uniform quality assurance testing and high cost. In addition, drug-resistant variants are likely to exist at low levels in every HIV-infected patient. Drug-resistant viruses that constitute <10% to 20% of the circulating virus population or are present in the reservoir of latently infected cells may not be detected by any of the currently available commercial resistance assays. A comprehensive review of the past use of ARV agents and the virologic responses to those agents, and all prior resistance mutations (i.e., cumulative genotype), even if not present on the current genotype, is important in making decisions regarding the choice of new agents for patients with virologic failure.

The primary limitations of phenotypic assays are that their predictive power depends upon the sensitivity of the genotypic methods used and the number of matches to the patient’s genotype. These tests also are more costly than genotypic testing; therefore, their use should be reserved for clinical settings in which the information they provide will add benefit (see Table 19).

Genotypic assays to assess tropism have been proposed as an alternative approach to determining the tropism...
of plasma HIV. However, they are not currently recommended because the limited experience with this approach indicates that the sensitivity may be lower than phenotypic tropism assays, particularly in the setting of CCR5 antagonist interruption where reversion to wild-type may occur.\textsuperscript{22,23}

Although drug resistance may be detected in the circulating plasma of infants, children, and adults who are not receiving therapy at the time of the assay, loss of detectable resistance and reversion to predominantly wild-type virus often occur in the first 4 to 6 weeks after ARV drugs are stopped.\textsuperscript{24-26} As a result, resistance testing is of greatest value when performed prior to or within 4 weeks after drugs are discontinued, or as soon after diagnosis as possible.\textsuperscript{27} The absence of detectable resistance to a drug at the time of testing does not ensure that future use of the drug will be successful,\textsuperscript{1,28} especially if the agent shares cross resistance with drugs previously used. It may be prudent to repeat resistance testing if an incomplete virological response to a new treatment regimen is observed in an individual with prior treatment failure(s) (see Management of Children Receiving Antiretroviral Therapy).

**Use of Resistance Assays in Determining Initial Treatment**

Transmission of drug-resistant strains to newly infected individuals (via perinatal and non-perinatal transmission of HIV) has been well documented and is associated with suboptimal virologic response to initial cART if this resistance is not taken into account when designing the initial regimen.\textsuperscript{29-33} Drug-resistant variants of HIV may persist for months after birth in infected infants\textsuperscript{34} and impair the response to cART.\textsuperscript{35} Consequently, ARV drug-resistance testing is recommended for all treatment-naive children before therapy is initiated. Standard genotypic testing is preferred in this setting because it may reveal the presence of both RT and PR resistance mutations and polymorphisms that facilitate the replication of drug-resistant virus. Genotypic testing for integrase resistance mutations prior to initial treatment is only recommended in special circumstances (e.g., acquisition of HIV from an individual treated with an integrase inhibitor with concern for transmission of integrase resistance).

**Use of Resistance Assays in the Event of Virologic Failure**

Several studies in adults\textsuperscript{5,8-14} have indicated that early virologic responses to salvage regimens were improved when results of resistance testing were available to guide changes in therapy, compared with responses observed when changes in therapy were guided only by clinical judgment. Although not yet confirmed in children,\textsuperscript{36} resistance testing appears to be a useful tool in selecting active drugs when changing ARV regimens in cases of virologic failure. Resistance testing also can help guide treatment decisions for patients with suboptimal viral load reduction because virologic failure in the setting of cART may be associated with resistance to only one component of the regimen.\textsuperscript{3} Poor adherence is the most common reason for virologic failure, regardless of whether resistance develops. It should always be suspected, confirmed, and addressed, especially when no evidence of resistance to a failing regimen is identified (see Management of Children Receiving Antiretroviral Therapy).

**Table 19. Recommendations for Use of Available Resistance Testing**

<table>
<thead>
<tr>
<th>Resistance Test</th>
<th>Initial Treatment</th>
<th>Virologic Failure</th>
</tr>
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<tbody>
<tr>
<td>Standard genotype (RT, PR)</td>
<td>Resistance testing indicated</td>
<td>Resistance testing indicated</td>
</tr>
<tr>
<td>Integrate phenotype/genotype</td>
<td>Only if concern for acquisition of virus with resistance</td>
<td>If failure on integrate inhibitor</td>
</tr>
<tr>
<td>Trufire\textsuperscript{TM}</td>
<td>Only if considering CCR5 antagonist as part of initial treatment</td>
<td>Only if considering CCR5 antagonist for subsequent regimen</td>
</tr>
<tr>
<td>Phenotype (RT, PR)</td>
<td>Not recommended prior to initial treatment unless genotypic evidence that multi-drug resistance was acquired</td>
<td>In the setting of extensive drug resistance, may assist in determining most active cART regimen. Must be used in conjunction with cumulative genotypic resistance results and cART history and response</td>
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**Key to Acronyms:** cART = combination antiretroviral therapy; PR = protease; RT = reverse transcriptase
References


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