With variable degrees, drug-resistant HIV-1 is present in approximately 10%–20% of new infections in Western countries and in 60% of patients failing antiretroviral therapy. Non-nucleoside reverse transcriptase inhibitor mutations are the most common transmitted resistance mutations (4.5%-10%); NRTI (4.0%-4.5%) and PI mutations are less common (2.8%-3.4%). Integrase mutations are uncommon but are being monitored in order to evaluate their evolution in the near future. Therefore, monitoring and overcoming HIV-1 drug resistance is crucial for guiding antiretroviral (ARV) treatment.

### 2.1 CLINICAL CONSEQUENCES OF ARV RESISTANCE

The immediate consequence of ARV resistance is a reduction of treatment efficacy with the given ARV drugs. Given the molecular structure similarities within compounds of the same antiretroviral family and their interaction with similar target sites, the emergence of resistance to one drug often leads to variable degrees of cross-resistance to other drugs in the same family. This reduces the therapeutic arsenal available for salvage therapy leading to the prescription of more complex, expensive and often worse tolerated regimens.

### Table 1. Consequences of Antiretroviral Resistance

<table>
<thead>
<tr>
<th>Loss of treatment efficacy</th>
<th>Cross-resistance</th>
<th>Increased mortality (controversial)$^b$</th>
</tr>
</thead>
</table>

Need to prescribe ART that is:
- More complex
- More toxic
- More expensive

<table>
<thead>
<tr>
<th>Shorter duration of antiviral efficacy of subsequent ART$^b$</th>
<th>Increased risk of resistance evolution under subsequent ART</th>
</tr>
</thead>
</table>

With some mutations, hypersusceptibility to certain ARVs$^c$
- In general, reduced viral fitness relative to WT$^c$
- Risk of transmission of resistant HIV

$^a$ART: antiretroviral therapy; ARV: antiretroviral; WT: wild type
$^b$Data generated with cohorts using older drugs. Treatment non-compliance could be a major driver of failure in those patients, besides HIV resistance. This effect could be attenuated with the availability of newer drugs from newer classes
$^c$Clinical significance uncertain
Resistance-associated virological failure creates a ‘vicious circle’ where ARV options are reduced, consecutive treatment lines are associated with progressively reduced duration of antiviral efficacy and each new virological failure is associated with further resistance accumulation (Figure 1). Subjects entering this vicious circle may end up developing viruses with reduced susceptibility to most drug classes.

Certain mutations conferring high-level resistance to one agent may increase viral susceptibility to another compound, resulting in a so-called “hypersusceptible” virus to the other agent. In addition, many resistance-conferring mutations decrease replication capacity in comparison with the wild-type (WT) virus. The clinical correlates of mutation-derived “hypersusceptibility” and replication capacity measurements, however, remain largely unknown.

Two independent studies found that the emergence of antiretroviral resistance among patients starting first-line ART was associated with a nearly 2-fold increased risk of death.\textsuperscript{2-4} Interestingly, emergence of resistance to NNRTIs was associated with a greater risk of subsequent death (3-fold increase) than resistance to any other drug class. These findings have not been confirmed with present ART regimens and might greatly be associated with decreased rates of treatment adherence.

Importantly, development of drug-resistance also increases the probability of transmission of drug-resistant viruses from person to person and constitutes a public health issue.

2.2 CLINICAL MANAGEMENT OF ANTIRETROVIRAL RESISTANCE

2.2.1. Objectives

Antiretroviral drug resistance is both an individual and a public health problem: clinicians must address both levels simultaneously.

- At the individual level, clinicians must seek to maximize the potency and durability of the antiviral activity of ART by providing patients with ARV regimens to which the virus retains maximum susceptibility. Complete viral suppression must be pursued in all individuals, including those with triple-class HIV-1 resistance.
- At the public health level clinicians must seek to reduce the incidence and prevalence of antiretroviral resistance in the society, so that more individuals retain fully susceptible viruses, less transmit resistant viruses, and more can be effectively treated and achieve complete virologic suppression. An HIV-1 genotype must be performed in all treatment-naive subjects before initial ARV therapy commencement, ideally as soon as they enter clinical care in order to maximize detection of transmitted drug-resistant HIV that will decay thereafter in the absence of ART.

2.2.2 Management Principles

Resistance-associated virological failure must be prevented by:\textsuperscript{5}

a. identifying pre-existing or primary resistance in antiretroviral naïve subjects;

b. tailoring first-line ART to ensure that all components of the drug regimen retain full antiretroviral activity;

c. using only preferred regimens with the highest level of evidence proven in randomized clinical trials;

d. maximizing the antiviral potency of the regimen alongside its tolerability and convenience in order to ensure adequate long-term adherence;
e. detecting virological failure early and identifying emerging resistance mutations in order to select the the most active subsequent combinations;
f. managing virological failure aggressively, i.e., switching early to a new ARV regimen tailored according to drug resistance testing, preferably including drugs with high barrier to attain resistance;
g. Expert advise must be obtained in the management of complex treatment failures.6

These individual-based measures should exert public health benefits by:
a. decreasing HIV transmission overall, because more subjects would remain aviremic, and
b. decreasing transmission of ARV-resistant HIV, given that fewer resistant viruses should circulate among human populations with adequate access to ART.

How ARV drug resistance will evolve in countries scaling up ARV remains uncertain.

2.2.3 Identifying Antiretroviral Resistance

The goal of resistance testing is to provide information to assist in the selection of the antiretroviral regimen(s) that will more likely achieve and maintain viral suppression.5,7 The current international consensus on the clinical indications of HIV resistance testing is well summarized by the International AIDS Society USA recommendations (Table 2);
Table 2. Summary of clinical situations in which resistance testing is recommended (Modified from IAS-USA, 2016).^5

<table>
<thead>
<tr>
<th>Clinical setting</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before initiation of therapy</strong></td>
<td></td>
</tr>
<tr>
<td>Primary (acute and early) infection</td>
<td>Resistance testing is recommended, including for patients for whom therapy is delayed, because plasma wild-type isolates may replace drug-resistant virus with time in the absence of treatment.</td>
</tr>
<tr>
<td>First evaluation of chronic HIV-1 infection</td>
<td>Resistance testing is recommended as soon as the patient enters in care. Initial therapy may be altered based on resistance test results.</td>
</tr>
<tr>
<td>Treatment initiation for chronic HIV-1 infection</td>
<td>Resistance testing is recommended because of a rising prevalence of baseline HIV-1 drug resistance in untreated patients with chronic infection, unless preexisting data or stored samples for testing are available.</td>
</tr>
<tr>
<td><strong>In antiretroviral-treated patients</strong></td>
<td></td>
</tr>
<tr>
<td>Treatment failure</td>
<td>Resistance testing is recommended and must always include tropism testing. The decision to change therapy should integrate treatment history, new and prior resistance results (if available), and evaluation of adherence and possible drug interactions. Resistance testing must include the HIV integrase in all subjects treated with integrase inhibitors.</td>
</tr>
<tr>
<td><strong>In specific settings</strong></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Resistance testing is recommended before initiation of therapy to effectively treat the mother and prevent mother-to-child transmission.</td>
</tr>
</tbody>
</table>
Other considerations and general recommendations

<table>
<thead>
<tr>
<th>Clinical setting</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postexposure prophylaxis should consider treatment history and resistance data from the source, when available;</td>
<td></td>
</tr>
<tr>
<td>A sudden increase in HIV-1 plasma RNA may reflect treatment withdrawal or super-infection, possibly with drug-resistant virus;</td>
<td></td>
</tr>
<tr>
<td>Plasma samples to be tested for drug resistance should contain at least 200 HIV-1 RNA copies/mL to ensure successful PCR amplification required for all sequencing approaches;</td>
<td></td>
</tr>
<tr>
<td>It is preferable that the blood sample for resistance testing be obtained while the patient is receiving the failing regimen, if possible;</td>
<td></td>
</tr>
<tr>
<td>Resistance testing should be performed by laboratories that have appropriate operator training, certification, and periodic proficiency assurance;</td>
<td></td>
</tr>
<tr>
<td>Genotypic and phenotypic test results should be interpreted by individuals knowledgeable in antiretroviral therapy and drug resistance patterns;</td>
<td></td>
</tr>
<tr>
<td>Inhibitory quotient testing is not recommended for clinical decision-making.</td>
<td></td>
</tr>
</tbody>
</table>

*If resistance test results are available from before the pregnancy, clinical judgment should guide whether retesting for resistance is necessary.*

- HIV drug resistance testing should be performed when HIV-infected persons enter clinical care, whether or not they will be treated immediately (Table 2). This strategy attempts to maximize the chances of detecting transmitted drug resistance. In those individuals in whom treatment is largely deferred, resistance testing should be preferably repeated before therapy initiation. Genotypic testing is preferable to phenotypic testing because of lower cost, faster turnaround time, and a greater sensitivity for detecting mixtures of WT and resistant viruses. In addition, genotypic resistance testing is recommended for all untreated pregnant women prior to initiation of therapy and for those entering pregnancy with detectable HIV RNA levels while on therapy.

- In HIV-infected individuals receiving antiretroviral therapy, resistance testing should be performed in the presence of virological failure. Virological failure is defined as a confirmed viral load ≥50 HIV-1 RNA copies/mL. However, successful amplification is technically limited in samples with viral load 50-200 HIV-1 RNA copies/mL. For practical purposes, some guidelines define virological failure at ≥200 HIV-1 RNA copies/mL.

- Testing at low viral loads. A substantial proportion of subjects fail therapy with sustained low viral loads (<1000 copies/mL). Virological failure must be detected and managed early. Even though most commercial HIV-1 genotypic
resistance assays claim a limit of detection of 1000 copies/mL, current genotyping can successfully be performed below a viral load of 1000 copies/mL. Therefore, this must not be a limitation to order these tests.

- Importantly, given that drug resistance mutations wane after treatment interruption, drug resistance testing in the setting of virologic failure should be performed preferably while the patient is taking antiretroviral drugs, or within 4 weeks after discontinuing therapy.

### 2.2.4 Interpreting ARV Resistance Testing Results

Drug resistance testing interpretation is difficult because genotypic data used to infer phenotypic susceptibility in vivo is fragmentary.

- HIV genotypes obtained through viral population sequencing are consensus sequences that do not capture infrequent viral variants (i.e. those at levels below 15-20% of the viral population).
- Genotypic analyses frequently focus on discrete regions of the HIV genome focusing on the RT, PRO and integrase. However, missing substitutions in outside regions may also modulate resistance or viral fitness (e.g. connection and RNase H domains of RT or PR cleavage sites in Gag or Gap-Pol or the nef gene).
- Mutational interactions may modify the effect of each mutation by itself; i.e., some mutations conferring resistance to particular drugs may increase susceptibility to others, may simply be additive or may boost the individual effect of them. The interaction of complex patterns of mutations requires computational models for their interpretation. The most frequently used one in clinical practice is the free and continuously updated HIV Drug Resistance Database of the Stanford University (available at http://hivdb.stanford.edu/).
- Often, interpretation rules are based on expert opinion, which has the potential for information biases, or on complex mathematical algorithms and machine-learning methods, where computer programs extract rules from comparisons between genotypes paired with phenotypes or with clinical efficacy data.

Fortunately, a fair amount of clinically relevant information can be extracted from this complex picture. Moreover, incorporating such information into the clinical management of HIV-infected patients clearly improves their chances to do well on therapy. Table 3 outlines the basic mechanisms of resistance. The principal aspects of resistance interpretation relevant for clinical management may be found in other chapters of this issue.
### Table 3. Principal Mechanisms of Antiretroviral Drug Resistance

<table>
<thead>
<tr>
<th>Drug Family</th>
<th>Mechanism of Action</th>
<th>Mechanism of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTIs and NtRTIs</td>
<td>NRTIs and NtRTIs are chain terminators. They are incorporated into the nascent chain of viral DNA. Because they lack a 3’ hydroxyl group, no additional nucleotides can be appended. Newer NRTIs act as translocation inhibitors.</td>
<td>Impaired nucleotide incorporation: M184V, K65R and the Q151M complex selectively impair RT’s ability to incorporate an analogue into DNA. Nucleotide excision: TAMs allow ATP to bind RT near the 3’ end of viral DNA terminated by the incorporation of a nucleoside analogue. ATP then excises the analogue from viral DNA, allowing reverse transcription to proceed normally</td>
</tr>
<tr>
<td>NNRTIs</td>
<td>Small molecules with strong affinity for a hydrophobic pocket located near the catalytic domain of RT. Inhibitor binding affects the flexibility of the enzyme, thereby blocking its ability to synthesize DNA.</td>
<td>Most NNRTI resistance mutations affect residues that are directly involved in inhibitor binding. Few have been found to act indirectly, by changing the position or the orientation of the aminoacids involved with direct contact with the inhibitor. Etravirine (ETR), rilpivirine (RPV) and doravirine (DOR) are diarylpirimidines or pyridones with some conformational isomerism that can bind RT in multiple conformations, allowing for a more robust interaction between ETR and the enzyme, even in the presence of some mutations (K103N)</td>
</tr>
<tr>
<td>PIs</td>
<td>PIs mimic the structure of the natural viral substrates of the HIV PR, competing with them for binding in the enzyme’s active site.</td>
<td>Mutations in direct contact with the inhibitor or of distant aminoacids that modify the overall shape of the PR cavity disrupt fitting of the PI within the cavity and induce resistance. Mutations in the uncleaved Gag and the cytoplasmic tail (CT) of the Env protein could also be selected by protease inhibitors, well outside of the protease itself, and promote protease inhibitor resistance.</td>
</tr>
</tbody>
</table>
InSTI

**Mechanism of Action**

Raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG) and bictegravir (BIC) are DNA strand transfer inhibitors that block the joining of the processed viral DNA ends into the host chromosome.

**Mechanism of Resistance**

Binding requires divalent metal and resistance is metal dependent with active site mutants displaying resistance only when the enzymes are evaluated in the context of Mg2+. There is extensive cross-resistance between RAL and EVG, while DTG remains active in many RAL and EVG resistant clones, particularly if the codon Q148 is preserved. Bictegravir has an improved resistance profile compared to DTG *in vitro*, particularly for isolates with high-level INSTI resistance containing combinations of mutations such as E92Q+N155H or G140C/S+Q148R/H/K.

**Fusion Inhibitors** (Enfuvirtide)

**Mechanism of Action**

Enfuvirtide (ENF) is a 36-mer synthetic oligopeptide that binds to the trimeric HR-1 complex, preventing the association of HR-1 with HR-2 and inhibiting fusion.

**Mechanism of Resistance**

Changes in a conserved amino acid triad (GIV) at positions 36–38 and in amino acids 39-45 in the HR1 region of gp41 prevent ENF binding.

**CCR5 Antagonists**

**Mechanism of Action**

CCR5 antagonist binding alters the conformational state of the human CCR5 receptor, inhibiting the binding of gp120 to CCR5 by an allosteric mechanism.

**Mechanism of Resistance**

Resistant viruses acquire the ability to recognize receptor conformations stabilized by CCR5 antagonists.

**Attachment inhibitors**

**Mechanism of Action**

GSK3684934 binds to the HIV-1 gp120, blocking viral attachment to host CD4 cells, before co-receptor binding and fusion.

**Mechanism of Resistance**

Resistance mutations involve the HIV-1 gp120 gene. Its activity is not affected by co-receptor tropism changes.

RT: reverse transcriptase; PR: protease; IN: integrase; HR1: first heptad repeat; HR2: second heptad repeat; NRTI: nucleoside reverse transcriptase inhibitor; NtRTI: Nucleotide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor; InSTI: Integrase Strand Transfer Inhibitor.
2.2.5 Management of Primary Antiretroviral Resistance

The clinical management goal for ARV resistance in treatment-naïve subjects is to ensure durable virological suppression with first-line ART by identifying pre-existing ARV resistance and avoiding prescription of regimens to which the virus is not fully susceptible (Figure 1). This is particularly relevant for drugs or combinations with low barriers to resistance (only shown for nevirapine and efavirenz).

2.2.5.1 Origin of Primary Resistance

- Resistant viruses can either be spontaneously generated or transmitted from person-to-person through contact with blood or blood products, sexual intercourse or from mother-to-child.\textsuperscript{21, 22}
- Resistant viruses are usually transmitted less efficiently than wild type,\textsuperscript{23} although multidrug-resistant variants are sometimes transmitted.\textsuperscript{21, 24-26}
- Current knowledge suggests that only a few variants present in the “donor” viral population -known as "founder" viruses- are able to establish primary HIV infection, even if transmission occurs through direct blood-to-blood contact.
- Because resistant variants are often transmitted alone, the viral population in the recipient subject is mostly conformed by resistant viruses, which remain predominant until wild-type revertants are generated through spontaneous mutation in the absence of antiretroviral pressure.\textsuperscript{27}
- Conversely, resistant mutants generated through replication errors often co-exist and compete with the WT in the quasispecies. As a result, mutants often become extinct or, sometimes, persist in the viral quasispecies at very low frequency, as predicted from the Poisson distribution.
- Whereas transmitted resistant variants can contain several resistance mutations in various genes, mutants generated spontaneously in the absence of ART pressure rarely accumulate more than 2 resistance-associated substitutions in the same genome.

2.2.5.2 Prevalence of Primary Resistance

The prevalence of primary resistance (Table 4) varies as more effective and suppressive ART is introduced in a given population.

- Resistance does not evolve nor is transmitted in populations with no ART available.
- Increased rates of secondary resistance in the treated population due to suboptimal therapy and/or inadequate therapeutic monitoring tend to be followed by increases in the prevalence of primary resistance in the population level, thus becoming a public health issue.
- As ART becomes more potent, includes drugs with higher barrier against resistance development and the ART coverage of subjects in need of treatment increases, more individuals remain aviremic and fewer harbor resistant viruses at the time of treatment failure. At this point, the prevalence of primary resistance may remain stable or start to decline.
Table 4. Factors Influencing the Prevalence of Primary Resistance in a Given Population

<table>
<thead>
<tr>
<th>Epidemiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of acquired (secondary) HIV resistance in the population</td>
</tr>
<tr>
<td>Fraction of subjects on ART who remain viremic</td>
</tr>
<tr>
<td>ART coverage of the population in need of treatment</td>
</tr>
<tr>
<td>Characteristics (particularly, barrier to attain resistance and excellence of regimens commonly used) of the drug regimens given to the population</td>
</tr>
<tr>
<td>Existence of HIV transmission “hotspots”, i.e. clusters of individuals frequently engaging in high-risk transmission practices</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutational bias toward G to A replacements during viral replication mainly related with HIV-1 genotypye prevalence</td>
</tr>
<tr>
<td>Fitness cost of resistance mutations, which influences their persistence and detectability in plasma</td>
</tr>
<tr>
<td>Persistence of transmitted mutants through ‘compensatory fixation’</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technical or methodological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity of the assay used to detect resistance</td>
</tr>
<tr>
<td>Definition of ARV resistance</td>
</tr>
<tr>
<td>Sample selection and representativity</td>
</tr>
</tbody>
</table>

The prevalence of primary resistance in well-resourced countries ranges from 8% to 19% for any drug, 5% to 12 % for NRTIs, 2% to 8% for NNRTIs and 3 to 7 % for PIs.\textsuperscript{24, 28-33} After a period of steady increases, the overall prevalence of primary ARV resistance seems to have stabilized around 10% in most industrialized countries. By drug class, only resistance to NNRTIs has clearly increased in the last decade, probably due to the widespread use of first generation NNRTIs and their low barrier to resistance, coupled with the minimal impact of NNRTI resistance mutations on virus fitness. Fortunately, the prevalence of primary dual or triple drug resistance has remained at low levels (<2%). Primary resistance to new ARV drugs like the integrase strand-transfer inhibitors (InSTIs) is virtually zero due to their recent introduction in clinical management, but needs to be monitored prospectively.\textsuperscript{34} Some cases with transmitted integrase resistance have been reported.\textsuperscript{33} Primary INSTI resistance is beginning to arise in ART-naïve subjects in Spain, thus reinforcing the need for Europewide surveillance of transmitted HIV integrase resistance.
Table 5. Prevalence of Antiretroviral Resistance Mutations in US and Europe ART-Naïve HIV-Infected Patients

<table>
<thead>
<tr>
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<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Major resistance mutations</td>
<td>9%</td>
<td>5%</td>
<td>20%</td>
<td>13%</td>
<td>20%</td>
<td>13%</td>
<td>7.5%</td>
</tr>
<tr>
<td>NRTI</td>
<td>3%</td>
<td>4%</td>
<td>4%</td>
<td>5%</td>
<td>2%</td>
<td>2-5%</td>
<td>3.5%</td>
</tr>
<tr>
<td>NNRTI</td>
<td>6%</td>
<td>2%</td>
<td>15%</td>
<td>8%</td>
<td>6%</td>
<td>4-6%</td>
<td>3.3%</td>
</tr>
<tr>
<td>PI</td>
<td>2%</td>
<td>2%</td>
<td>3%</td>
<td>3%</td>
<td>0%</td>
<td>0-2%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Dual class</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Triple class</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>0%</td>
<td>0%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

In a large cohort of 3542 ART-naïve HIV-infected patients from 36 US states and District of Columbia enrolling into clinical trials between 2001 and 2007, the prevalence of primary resistance evolved increased from 2001 to 2007, mainly at the expense of the prevalence of primary NNRTI resistance. It has thereafter remained stable in Europe until 2010.33


2.2.5.3 Antiretroviral Resistance is Underestimated by Viral Population Genotypic Assays

Current viral population sequencing assays only detect viral variants present in more than 15-20% of the viral quasispecies. Several primary resistance surveillance studies using ultrasensitive resistance assays have demonstrated increases in detection of HIV-1 variants with primary resistance of at least 2 to 3-fold relative to standard population-based sequencing. However, ultrasensitive resistance testing to help design more effective ARV therapy should currently only reduce the rate of virological failure to initial NNRTI-based triple ART (efavirenz or nevirapine). While their clinical value is still being further elucidated, their technical improvements and greatest easy of performance are forcing their massive implementation in routine clinical practice.
2.2.5.4 Clinical Implications of Primary Resistance

While transmitted drug resistance increases the rates of virological failure to initial ART based on the first generation NNRTIs NVP and EFV, their impact on second generation NNRTI-, boosted PI-, or IN- based ART has not been proven. A recent study in 10,056 patients from 25 European cohorts demonstrated that antiretroviral naïve subjects with mutations conferring resistance to at least one drug of their first-line regimen were more than 3-fold more likely to develop virological failure than those with no preexisting resistance to their initial ARV regimen. Such increased risk was observed both for NNRTIs and ritonavir-boosted PIs. However, a study of the Swiss HIV Cohort did not detect that patients harboring viruses with minor PI mutations had a worse treatment outcome in subtype B infection. Further on this, the prevalence of transmitted resistance against currently preferred initial ART regimens in the modern era is very low.

2.2.5.5 Recommendations for the Clinical Management of Primary Resistance

Resistance testing (genotype) must be done in the initial visit in all HIV-infected subjects as soon as acute HIV infection is diagnosed, even if treatment is not initiated. In case it cannot be performed, a plasma sample should be stored for future testing. If genotypic testing is not available, a ritonavir-boosted PI or DTG should be included in the first-line regimen, and a first-generation non-nucleoside or rilpivirine should be avoided.

To date, first-line ART with efavirenz and lamivudine (two drugs with low barrier) plus another NRTI is an ART option more resilient to virological failure than a lopinavir-based one (plus 2 NRTIs), even though resistance develops more frequently in those who develop efavirenz failure. This shows that:

- Drugs with higher barrier do not necessarily lead to higher treatment success rates overall.
- Antiretroviral resistance is only one relevant factor influencing ART outcome: tolerance, convenience, toxicity, and adherence are equally important to maximize the success of first-line ART.
- Another important factor is to plan for second and future ART lines in the event of virological failure. On this regard, virological failure to first-line NNRTI-based ART is more frequently associated with detection of resistance to NRTIs and to the “third regimen” than failure to boosted-PI regimens.

Thereby, given the high number of alternatives for first-line ART, clinicians should use ARV resistance information to:

a) Design ART combinations that incorporate drugs to which the virus is fully susceptible.
b) Plan for the best second and further ART lines in the event of treatment failure.
c) In the infrequent event of transmission of multidrug-resistant HIV:
   i. Investigate the resistance profile of the source, if available.
   ii. Strongly suspect the existence of other mutations to commonly used regimens that have not been captured by the assay.
iii. Maximize the predicted antiviral activity of the regimen. If partial antiviral activity is expected, prioritize the inclusion of drugs with high barrier to resistance development.

d) In every case, ART design should integrate resistance information alongside considerations regarding tolerance, convenience, and toxicity of regimens, as well as the patient’s ability to adhere to ART in the long term.

2.2.6 Management of Acquired or Secondary ARV Resistance

The goal of ARV resistance management in treatment-experienced subjects is to minimize as much as possible the accumulation of resistance mutations during virological failure by detecting failure as soon as possible and withdrawing the selective pressure of the failing ART (partially those with a low barrier to resistance development), and to regain virological suppression <50 copies/mL as soon as possible with a new ARV regimen. The ultimate objectives of these actions are:

a. to preserve treatment options at the time of virological failure to the greatest extent,
b. to regain and maintain viral suppression below 50 copies/mL for the longest time possible, and
c. to prevent the development of MDR HIV. (Figure 3)

A public health goal derived from these individual-based objectives is to reduce the prevalence and prevent transmission of drug-resistant HIV in the population.

2.2.6.1 Recommendations for Clinical Management of Acquired Resistance

The management of acquired resistance should be based on the following points:

a. When designing ART for the first time, clinicians should only use preferred regimens with high rates of success documented in randomized clinical trials.
b. All efforts should be undertaken to diagnose virological failure early to avoid accumulation of mutations and cross resistance.
c. Genotypic resistance testing should be ordered as soon as virological failure is detected. All expert laboratories should be able to produce genotypic testing data from plasmas with HIV-1 RNA levels >1000 copies/mL. If HIV-1 RNA levels are below 1000 copies/mL genotypic results can frequently be obtained when HIV-1 RNA is extracted from 3mL of plasma after centrifugation, particularly with HIV-1 RNA levels >400 copies/mL.
d. Salvage therapy should be initiated as soon as virological failure is confirmed. Treatment design should be based on the analysis of complete ART history and the prediction of the most likely resistance patterns to occur, the results from all the compiled genotypic tests data, and an external expert advice when necessary.6
e. To maximize the success of the new fully suppressive regimen, clinicians should preferably prescribe drugs from new families without cross resistance to previous drug exposure (integrase inhibitors, or the CCR5 antagonist Maraviroc), drugs with high barrier to resistance (e.g. boosted protease
inhibitors like darunavir/ritonavir, second-generation NNRTIs like etravirine, and dolutegravir), and seek for the highest antiviral activity of the regimen. In settings with full availability of new ARVs, it should often be possible to design a suppressive regimen containing three drugs to which the virus remains fully susceptible even in subjects with multidrug resistance.

f. Clinicians should plan for potential ARV schemes to be used in the event of virological failure to new salvage regimen and, if possible, preserve active compounds for subsequent treatment lines.

2.2.7 Management of MDR HIV Infection

- Until the recent advent of new drugs and classes, between 5-10% of subjects in the clinic had MDR HIV and no ART options left to regain virological suppression. Since all six drug classes are available, and with attachment inhibitors in late-stage development (accessible through clinical trials or compassionate use), it is now very rare to encounter patients in whom a potent, suppressive regimen can not be mustered.
- Only in areas where newer drug classes and new generation PIs and NNRTIs are still not available, physicians encounter situations where there are no or few treatment options.
- In rare cases of toxicities, pharmacokinetic interactions, severe compliance issues, and only in subjects with high CD4+ T-cell counts, it might be reasonable to continue a simple non-suppressive antiretroviral regimen until newer and presumably active agents become available in order to preserve immune responses and delay clinical progression. The major risk of this approach is ongoing viral evolution and the loss of future drug options as well as a progressive CD4 cell decline.
- Alternatively, simplified approaches with only NRTIs aiming to preserve viruses with reduced fitness can be attempted. However, these bridging regimens must only be used while awaiting the availability of new active drugs, or while solving the adherence problems of the subject that impeded the initiation of a fully-suppressive salvage regimen. These strategies rarely are preferred in developed countries.
- By no means should ART be interrupted in subjects without better treatment options because this is clearly associated with higher morbidity and mortality than remaining on a failing regimen.48, 49

2.3 CLINICAL RELEVANCE OF MINORITY HIV-1 RESISTANT VARIANTS

2.3.1 Minority Variants in Therapy-Naïve Patients

Most studies show that detection of pre-existing minority NNRTI-resistant variants in ART-naïve subjects increase the risk of virological failure to first-line NNRTI-based
regimens (efavirenz or nevirapine) more than 3-fold. This has been also demonstrated in all strata of treatment adherence with first-generation NNRTIs.

At least one low-frequency resistant variant against NNRTIs was found in 17% of newly diagnosed ART-naïve persons with no resistance detected with standard population sequencing using RT-PCR based assays. The same study showed that 7% of the persons who experienced virological failure had minority drug resistance mutations at baseline compared to 0.9% of treatment success cases.

Similarly, Metzner et al. suggested that minority variants, which remain undetected by population sequence, can become the major viral population and, thus, lead to early therapy failure in treatment-naïve patients with low barrier to resistance treatments based on RT inhibitors. Of interest, a dose–effect relationship between virological failure and mutational load of minority variants has been found. Similar results were found by Paredes et al. and Balduin for Y181C and K103N mutants respectively, detected using ASPCR. UDS technique has also found that a significantly larger proportion of HIV-infected patients harboured drug-resistant viral variants when compared to standard population sequencing and that this resulted in a higher risk of virological failure in patients initiating an ART regimen which combined nucleoside and non-nucleoside RT inhibitors.

Conversely, the existence of minority drug-resistant variants did not significantly increase the risk of viral failure when treatment regimens could include protease inhibitors and when minority variants included TAMs or any of the following mutations: K103N, M184V, or L90M. The absence of minority variants against integrase inhibitors has precluded the analysis of its influence against dolutegravir-based ART.

A recent pooled analysis of 10 major studies addressing the clinical value of minority variants, including 985 ART-naïve individuals initiating solely NNRTI-based regimens, showed that only low-frequency HIV-1 mutations involving NNRTI resistance were significantly associated with a dose-dependent increased risk of virologic failure with first-line ART. Interestingly, a significantly increased risk of virological failure was found both for higher (>95%) and lower (<95%) treatment adherence with a combined HR of 2.5 and 3 respectively.

On the other hand, there is no indication that minority variants are statistically associated with ART failure of Protease Inhibitors containing regimens, probably due to higher barriers against resistance development. In the Simen’s study the presence of minority PI-resistance was not significantly associated with higher rates of virological failure in subjects receiving mainly unboosted PIs (nelfinavir or indinavir). However, all subjects with minority PI resistant variants (5 out of 109 individuals) experienced virological failure to their unboosted PI containing ART. Although all 5 experienced VF, the sample size was not adequate to assess any association between the presence of minority PI-resistance and subsequent VF, and those 5 individuals could also harbour resistance to other drug classes.

In a subanalysis of the CASTLE study, primary drug resistance mutations were common (30.5%); B and non-B subtypes had similar rates of drug resistance and, overall, minority drug resistant variants did not affect virologic response for subjects on a boosted
PI (atazanavir or lopinavir) by week 48; however, a small subset of subjects with extensive NRTI backbone resistance patterns experienced virologic failure.

The role of low-frequency HIV-1 drug-resistance mutations against integrase inhibitors still remains to be established. In a longitudinal analysis, our group showed that HIV-1 is able to escape from a suboptimal regimen including raltegravir through selection of pre-existing raltegravir drug-resistant mutants. More recently, minority integrase resistant variants (more than 1% of the population) have been found in 4.8-9.6% of treatment-naive subjects in Spain, depending on the platform used for their interpretation. This underscores the importance of using fully active antiretroviral regimens to treat all HIV-1-infected subjects.

2.3.2 Minority Variants in ART-Experienced Patients

Published studies on the impact of minority variants in treatment-experienced patients underline the importance of the sensitivity of the technique used for resistance assessment when tailoring patient ART. However, no full picture of the impact of such variants on the success of ART is provided.

Le et al. showed that minority variants in ART-failing subjects increased the genotypic resistance for 77% of the cases, and 50% of them involved resistance to a new antiretroviral drug. Nevertheless, the correlation of the presence of minority variants with historical ART use was better than with the failing antiretroviral drugs, pointing out that detection of such variants can provide important historical resistance information for the better planning of new ART.

In addition, Kapoor et al. found that minority variants resistant to PI which were present at therapy change became major variants at later time points, therefore suggesting that the detection of such minority variants at viral failure would improve new regimen’s design. Similarly, Roquebert et al. found that PI resistant variants, which remained undetectable to population sequencing at viral failure to nelfinavir-containing ART, emerged during salvage therapy treatment containing a boosted PI.

Regarding the new-generation NNRTIs, and particularly etravirine, it has been shown that multiple additional NNRTI-resistant minority variants were found in subjects with only K103N demonstrated in population sequencing, thus increasing the degree of estimated etravirine resistance. This finding is clinically relevant, as it suggests that etravirine may not be fully active in patients with acquired K103N as the only acquired mutation identified by standard population sequencing. At the contrary, UDPS did not detect additional major NNRTI-resistant mutations in treatment-naive patients with only K103N demonstrated in population sequencing.

To explore the potential of deep HIV-1 sequencing for adding clinically relevant information relative to viral population sequencing in heavily pre-treated HIV-1-infected subjects, our group recently compared deep sequencing to population sequencing in 7 HIV-1-infected individuals with previous triple-class virological failure who also developed virologic failure to deep salvage therapy including, at least, darunavir, tipranavir, etravirine or raltegravir. Deep sequencing detected all mutations found
by population sequencing and identified additional resistance mutations in all but one individual, predominantly after virological failure to deep salvage therapy. Additional genotypic information led to consistent decreases in predicted susceptibility to etravirine, efavirenz, nucleoside reverse transcriptase inhibitors and indinavir in 2, 1, 2 and 1 subject, respectively. However, deep sequencing data did not consistently modify the susceptibility predictions achieved with population sequencing for darunavir, tipranavir or raltegravir.

More recently, in a retrospective, multicentre, cohort study with 132 ART-experienced subjects, ultrasensitive HIV-1 genotyping has also shown statistically significant improvements in genotyping sensitivity score-based predictions of virological outcomes of salvage ART relative to the last available population genotype. This study suggests that more sensitive genotypic HIV drug resistance assays, such as deep HIV sequencing, may help clinicians design antiretroviral treatment combinations better suited for patients infected with multidrug-resistant viruses, particularly when compiled historical genotype results are not available.

2.4 SUMMARY: CLINICAL OPTIONS TO PREVENT AND MANAGE ANTIRETROVIRAL DRUG RESISTANCE.

The above considerations can be summarized in the following recommendations to prevent and manage ARV drug resistance in the clinic:

a. Systematically screen for the presence of primary antiretroviral resistance in all subjects entering clinical care, preferably as soon after HIV infection as possible. Obtaining a genotype from a subject as soon as he/she enters into clinical care may allow an increased detection of transmitted resistant viruses, which becomes harder to detect with time.

b. Adjust the design of first-line regimens to the genotypic resistance information obtained if needed. Never initiate an NNRTI-based regimen in a treatment-naive subject without having the results of a genotypic resistance test.

c. Once primary resistance is ruled out, good adherence, forgiving pharmacology (including both pharmacokinetic and pharmacodynamic favourable characteristics), potency and high barrier against resistance development are the principal factors associated with reduced emergence of antiretroviral resistance. Ritonavir-boosted PI and dolutegravir-containing regimens are associated with low rates of resistance at treatment failure and lower rates of NRTI resistance than NNRTI-based regimens. On the other hand, first-line efavirenz-based regimens are more resilient to virological failure than lopinavir-ritonavir, possibly due to lower compliance on PI regimens because of side effects.

d. It is crucial to detect virological failure early and change failing therapy as soon as failure is confirmed, with the aim to re-suppress viral replication to < 50 copies/mL.

e. Use of 3 new agents is paramount to achieve durable viral suppression and prevent the future emergence of multidrug-resistant viruses.
References