Prevalence and determinants of transmitted antiretroviral drug resistance in HIV-1 infection

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Transmission of drug-resistant HIV-1 variants from antiretroviral treatment-experienced persons has been documented to occur through multiple routes, including sexual intercourse, intravenous drug use and vertically from mother to child. Newly infected persons with transmitted drug resistance (TDR) also act as a source for the onward transmission of resistant variants. Rates of virological suppression and behavioural patterns of treated populations and the relative fitness of drug-resistant variants are important determinants of the prevalence of TDR. Current estimates indicate that the prevalence is highest in regions and populations with long-established use of antiretroviral therapy. Limited data suggest that the incidence of TDR is rising in developing countries where access to therapy is increasing. There are methodological variations between studies, however, including those relative to the selection of the study population and the resistance interpretation system, which can skew prevalence estimates. TDR has important implications for the successful management of antiretroviral therapy. Routine resistance testing of drug-naive persons has been widely adopted in affluent countries and shown to effectively guide the selection of first-line regimens. Genotypic resistance tests offer a practical approach for detecting TDR. However, routine methods can only detect resistant mutants within the dominant quasispecies and fail to detect low-frequency resistant variants, which may become important once selective drug pressure is introduced. More sensitive testing methods are being evaluated but remain research tools at present. In addition, factors such as superinfection and possible differences in resistance patterns between plasma and cellular reservoirs and between anatomical compartments should be considered when evaluating TDR.

Keywords: primary resistance, HAART, newly diagnosed

Introduction

The widespread use of antiretroviral agents for the treatment of HIV-1 infection in developed countries has been accompanied by the emergence of drug resistance, resulting in a large pool of resistant virus variants becoming available to establish new infections. Subsequently, the phenomenon of primary or transmitted drug resistance (TDR) has emerged as a potential threat to the success of antiretroviral therapy. More recently, access to antiretroviral therapy has been increasing in many resource-poor countries where availability of laboratory monitoring is often limited or non-existent. This creates the potential for the emergence and transmission of drug resistance in these settings, with direct implications for the effectiveness of available treatment options. Transmission of drug-resistant viruses has been shown to occur following infection by several different routes, including heterosexual and homosexual intercourse, intravenous drug use and vertically from mother to child.1–3 Furthermore, phylogenetic analyses often detect clusters of recently infected persons with TDR, indicating that those who acquire infection with a drug-resistant virus often act as a source for the onward transmission of resistant variants.

Factors influencing prevalence estimates of TDR

Estimates of the prevalence of TDR vary in different cohorts worldwide, and there are several reasons for this to be the case. Several factors limit reliable comparisons across epidemiology studies, including variations in the study design, geographical location and sample population, definitions and classifications of ‘transmitted’ resistance mutations and the methods used to detect resistance. Antiretroviral treatment strategies influence the rate of TDR by determining the number of patients on treatment, the rates of virological success and the likelihood of
emergence of drug resistance. Given that newly infected persons are an important source of further transmission, the rates of TDR are a further important determinant. The variation may also reflect differences in the selection of the study populations. Prevalence of resistance detectable by standard methods is typically higher among patients presenting with acute seroconversion than in those with established infection, at least in part reflecting the gradual disappearance of TDR from the dominant quasispecies over time. Although testing of acute seroconverters provides a sensitive indicator of current TDR rates, it inevitably skews the composition of the cohort by recruiting patients who present with symptoms of seroconversion or seek more frequent testing. In many Western European countries, targeting of acute seroconverters typically results in study populations heavily biased in favour of white homosexual males, preventing extrapolation to the general HIV-1-infected population. Furthermore, participants who are willing to enrol in a study of drug resistance may be more proactive about their medical care and therefore may have presented voluntarily for HIV testing at an early stage of infection. The use of resistance results produced in routine clinical care may also lead to an overestimation of prevalence rates, by selecting patients more likely to undergo testing because they are perceived to be at the greatest risk of resistance, particularly in locations where financial restrictions limit the generalized provision of resistance testing. In addition, studies generally fail to fully investigate the possibility of undisclosed drug exposure in their populations. The latter may be particularly relevant in patients who have lived in more than one country and often do not have a full medical history available for consultation.

Another important consideration in the interpretation of prevalence estimates of TDR is the interpretation method used to define resistance. A common approach is to follow the list published by the International AIDS Society USA (IAS-USA), which is updated approximately every 6 months. The list, however, provides an indication of resistance mutations observed under drug pressure and is not especially designed to address TDR. Studies that base their interpretations on the use of different algorithms, such as the Stanford database, should be interpreted accordingly. For example, the reverse transcriptase (RT) mutations E44D, T69N/S and V118I and revertants of T215Y/F (T215rev; e.g. T215C/D/E/L/N/S) are not consistently classified as major mutations in different interpretation systems. One additional confounding factor is the distinction between TDR and naturally occurring polymorphisms, several of which are known to play a role in drug resistance in treatment-experienced persons. Prevalence estimates can be affected significantly by the inclusion or exclusion of mutations such as V118I in RT. These considerations are particularly relevant in persons infected with subtypes other than B, which show numerous polymorphisms relative to the reference subtype B virus. It has therefore been suggested that a separate list for the determination of TDR may be useful. Recently, such a list has been published, incorporating T215 revertants and attempting to distinguish transmitted resistance mutations from polymorphisms. Although the role of many polymorphisms in conferring or facilitating drug resistance remains to be fully elucidated, characterization of sequence variability in non-B subtypes is an important prerequisite to the investigation of TDR in both developing and developed countries with large immigrant populations.

**Epidemiology of TDR**

Data from 2005 and 2006 show rates of TDR ranging from 0% in Sweden and Chile to 24.5% in San Diego, USA (Table 1). Worldwide, the highest prevalence of resistance is observed in regions and populations with well-established use of antiretroviral therapy, including Western Europe, North America and regions of South America. In these settings, the use of mono and dual therapies in the pre-highly active antiretroviral therapy (HAART) era, sequential functional monotherapy and the use of suboptimal regimens in the early HAART era and ongoing difficulties with adherence and tolerability have led to the accumulation of drug resistance in treatment-experienced patients and the subsequent spread of TDR. Initial surveys in some countries reported alarmingly high rates of TDR, but these studies are likely to have suffered from selection bias. Subsequent surveys from the same populations have reported lower prevalence rates, reflecting more comprehensive patient inclusion criteria. In addition, there is evidence that rates of TDR may be genuinely declining in some regions, coinciding with the improved use of therapy and the introduction of ritonavir-boosted-protease inhibitor (PI)-based HAART.

Nonetheless, there remain some striking differences in the reported prevalence of TDR, depending on the geographical region surveyed. For example, the highest prevalence rates of TDR have been reported from the USA, with North Carolina, New York and San Diego being among those recently to report high rates (19.7%, 24.1% and 24.5%, respectively). Comparatively, the rates of TDR appear to be uniformly lower, but still significant in many parts of Western Europe including the UK, Switzerland, Portugal and Germany (7.1%, 7.7%, 8.4% and 9.0% respectively). A large survey of 17 European countries (SPREAD) has recently reported a TDR prevalence of 9.0% among newly diagnosed persons. In these regions, the most prevalent mutations in persons with TDR include the thymidine analogue mutations (found at positions 41, 67, 70, 210, 215 and 219) for the nucleos(t)ide RT inhibitors (NRTIs), the non-nucleoside RT inhibitor (NNRTI) mutations K103N, Y181C and G190A/S and V82A/F and L90M for the PIs. It is not surprising that studies undertaken in Africa and regions of Asia currently show some of the lowest rates of TDR, considering that patients in these regions have been exposed to antiretroviral drugs for a relatively shorter period than those in more developed countries. Preliminary data suggest that resistance may be emerging in countries currently scaling up access to antiretroviral therapy (Table 1). It should be noted however that accurate, large-scale surveys are limited and that problems related to the interpretation of sequence variation in non-B subtypes affect prevalence estimates in these populations.

**Impact of TDR on responses to first-line therapy**

It was previously assumed that transmitted mutations detected at diagnosis would become irrelevant over time, as fitter wild-type strains became the more dominant quasispecies. However, transmitted drug-resistant mutants can persist for years as dominant quasispecies and for even longer as a minority quasispecies in plasma RNA and as archived resistance within proviral DNA in peripheral blood mononuclear cells (PBMCs), indicating that TDR can have a long-term potential to impact on responses to
Table 1. Prevalence of antiretroviral drug resistance among drug-naive HIV-1-infected persons worldwide

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Years</th>
<th>Country</th>
<th>Prevalence (%)</th>
<th>IS</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Untreated patients              | 85    | 2000–05     | Swedish West Coast             | 0              | European guidelines/Stanford | Aridezden et al.  
|                                 |       |             |                                |                |                                               |
| Untreated patients              | 157   | 2000–05     | Chile                          | 0              | Stanford          | Rios et al.                                   |
| Untreated patients              | 18    | 2005        | Nigeria                        | 0              | NG               | Agwale et al.                                 |
| Untreated patients              | 100   | 2003–04     | Malaysia                       | 1.0            | IAS-USA          | Tse et al.                                    |
| Untreated patients              | 48    | 2004–05     | São Tome e Principe            | 2.0            | Stanford          | Vergne et al.                                 |
| Consecutive seroconverters      | 108   |             | Brazil                         | 3.1            | IAS-USA          | Rodrigues et al.                              |
| Untreated patients              | 359   | 2002–03     | Peru                           | 3.3            | NG               | Lama et al.                                   |
| All new diagnoses               | 182   | 2004–05     | Spain                          | 3.8            | IAS-USA          | Martinez-Picado et al.                        |
| New diagnoses                   | 77    | 2000–04     | Slovenia                       | 3.9            | IAS-USA          | Babic et al.                                  |
| New diagnoses                   | 690   | 2000–04     | Denmark                        | 3.9            | IAS-USA          | Jørgensen et al.                              |
| New diagnoses                   | 575   | 2003–04     | Japan                          | 4.0            | IAS-USA          | Gatanaga et al.                               |
| Untreated patients              | 70    | 2002        | Democratic Republic of Congo   | 4.3            | Stanford          | Vidal et al.                                  |
| New diagnoses                   | 49    | 2004        | Czech Republic                 | 6.0            | IAS-USA          | Bruckova et al.                               |
| Untreated patients              | 140   | 2005–06     | UK (London)                    | 6.4            | IAS-USA          | Fox et al.                                    |
| New diagnoses enrolled in a     | 149   |             | Paris (102)                    | 9.4            | IAS-USA          | Wensing et al.                                |
| study                           |       |             |                                |                |                                               |
| Untreated patients              | 55    | 2004        | San Francisco, USA             | 8              | IAS-USA          | Truong et al.                                 |
| Seroconverters (<12 months)     | 49    |             |                                | 12             | IAS-USA          |                                               |
| New diagnoses                   | 1084  | 2000–01     | Canada                         | 8.1            | IAS-USA          | Jayaraman et al.                              |
| New diagnoses                   | 199   | 2001–03     | Burkino Faso (97)              | 8.3            | Stanford          | Bonim et al.                                  |
|                                |       |             | Cameroon (102)                 | 7.8            | IAS-USA          |                                               |
| A representative proportion of  | 178   | 2003        | Portugal                       | 8.4            | IAS-USA          | Palma et al.                                  |
| all new diagnoses               |       |             |                                |                |                                               |
| New diagnoses                   | 310   | 2004–05     | Galicia and Basque Country, Spain | 8.7      | Stanford          | Pérez-Alvarez et al.                          |
| Chronically infected patients   | 831   | 2001–05     | Germany                        | 9.0            | IAS-USA          | Oette et al.                                  |
| New diagnoses                   | 1050  | 2002–03     | 17 European countries (SPREAD studyb) | 9.0      | IAS-USA          | Wensing et al.                                |
| A representative proportion of  | 101   | 2002–03     | Greece                         | 9.0            | IAS-USA          | Paraskevis et al.                             |
| all new diagnoses               |       |             |                                |                |                                               |
| Untreated patients              | 568   | 2004        | Canada                         | 9.7            | Stanford/IAS-USA | Brooks et al.                                 |
| Untreated patients enrolled in a | 1795  | 2000–04     | USA (33 states)                | 10             | IAS-USA          | Ross et al.                                   |
| trial                           |       |             |                                |                |                                               |
| Untreated patients              | 698   | 1999–2003   | Europe-wide                    | 10             | IAS-USA          | Van de Vijver et al.                          |
| New diagnoses and chronically    |       |             |                                |                |                                               |
| infected patients               | 2208  | 1996–2002   | 19 European countries (SPREAD studyb) | 10.4    | IAS-USA          | Wensing et al.                                |
| Untreated patients              | 223   | 2003–05     | Belgium                        | 10.8           | IAS-USA          | Vercauteren et al.                            |
| Seroconverters (<12 months)     | 198   | 1997–2004   | Spain                          | 12.1           | IAS-USA          | de Mendoza et al.                             |
| New diagnoses                   | 323   | 2003–04     | France                         | 12.3           | French algorithm | Chaix et al.                                  |

Continued
antiretroviral therapy. Reduced responses to therapy permit ongoing viral replication under selective drug pressure, thus promoting the further evolution of resistance.

In one study of individuals with acquired drug-resistant virus, poor virological responses were observed following the introduction of therapy with potent regimens.\textsuperscript{61} Even the presence of revertants, which represent intermediates between drug-resistant variants and wild-type virus, appears to have a negative impact on virological responses. The significance of T215rev has been well established.\textsuperscript{In vitro} virus variants with T215C/D show no significant phenotypic resistance but can develop resistance to zidovudine more rapidly than wild-type virus when cultured in the presence of the drug.\textsuperscript{62} The virus variants require a single base change to convert to T215Y when compared with the two base changes required for a wild-type virus, thus accelerating the emergence of drug resistance. Likewise, variants with the RT mutations D67N and K219E, which alone do not confer phenotypic resistance, have been shown to develop faster progression to zidovudine resistance than wild-type virus when cultured \textit{in vitro}.\textsuperscript{63}

In one study, virological failure of first-line therapy with thymidine analogues was observed in 47% of patients with T215rev when compared with 30% of patients lacking the mutations.\textsuperscript{64} The greatest impact of TDR is likely to be seen with the NNRTIs efavirenz and nevirapine, as a single mutation can abrogate their activity. Conversely, there may be a less detrimental effect for ritonavir-boosted PIs, where the high plasma concentrations are sufficient to overcome the low-to-intermediate PI resistance usually seen in persons with TDR. Nonetheless, the few PI mutations detected may mask more extensive PI resistance. Furthermore, the genetic barrier of the PI-based regimen will be lowered by the presence of resistance mutations, and this can facilitate the emergence of further resistance and treatment failure in some patients with suboptimal adherence (Figure 1). However, most persons with TDR have preserved treatment options. By using resistance testing to guide the choice of a first-line regimen, it has been shown that even those with TDR can achieve good immunological and virological responses.\textsuperscript{57,65}

### Source of drug-resistant virus variants

Treatment practices are a major determinant of the rate of drug resistance in drug-experienced patients and therefore a key predictor of the rate of TDR in newly infected persons. A Spanish surveillance study conducted over 7 years showed that the prevalence of TDR in persons with acute HIV-1 seroconversion was correlated with the proportion of chronically infected individuals with a detectable HIV-1 RNA load in plasma.\textsuperscript{5} Indeed, in one Madrid study, it was shown that of 31 seroconverters from 1996 to 1999, the prevalence of TDR was 25.8% when compared with 3.8% in 26 seroconverters between 2000 and 2001, with the authors suggesting that new infections in the latter group were more likely to be derived from HIV-1-infected persons unaware of their status and hence antiretroviral naive.\textsuperscript{66} A recent study suggested that the stable and low rates of TDR observed in

### Table 1. Continued

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Years</th>
<th>Country</th>
<th>Prevalence (%)</th>
<th>IS</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated patients\textsuperscript{a}</td>
<td>214</td>
<td>2002–06</td>
<td>Southern California, USA,</td>
<td>12.6</td>
<td>Stanford</td>
<td>Drumright \textit{et al.}\textsuperscript{45}</td>
</tr>
<tr>
<td>All new diagnoses consenting to study</td>
<td>186\textsuperscript{a}</td>
<td>1992–2002</td>
<td>Sweden</td>
<td>12.9</td>
<td>IAS-USA</td>
<td>Ohlis \textit{et al.}\textsuperscript{46}</td>
</tr>
<tr>
<td>Documented seroconverters and new diagnoses</td>
<td>743</td>
<td>1996–2005</td>
<td>Germany</td>
<td>14.0</td>
<td>IAS-USA</td>
<td>Kücherer \textit{et al.}\textsuperscript{47}</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>2357</td>
<td>1996–2003</td>
<td>UK</td>
<td>14.2</td>
<td>Stanford</td>
<td>Cane \textit{et al.}\textsuperscript{48}</td>
</tr>
<tr>
<td>New diagnoses</td>
<td>787</td>
<td>2003–04</td>
<td>USA</td>
<td>14.5</td>
<td>IAS-USA</td>
<td>Bennett \textit{et al.}\textsuperscript{49}</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>195</td>
<td>1999–2003</td>
<td>USA (six major cities)</td>
<td>15.9</td>
<td>NG</td>
<td>Eshleman \textit{et al.}\textsuperscript{50}</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>96</td>
<td>2002–03</td>
<td>Mexico</td>
<td>16.0</td>
<td>Stanford</td>
<td>Escoto-Delgadillo \textit{et al.}\textsuperscript{51}</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>79</td>
<td>NG</td>
<td>Cameroon</td>
<td>16.5</td>
<td>Stanford</td>
<td>Ndembí \textit{et al.}\textsuperscript{52}</td>
</tr>
<tr>
<td>Recently infected 12–24 year olds</td>
<td>55</td>
<td>2004</td>
<td>USA</td>
<td>18.2</td>
<td>IAS-USA</td>
<td>Viani \textit{et al.}\textsuperscript{53}</td>
</tr>
<tr>
<td>Acute or recent acute</td>
<td>127</td>
<td>1998–2004</td>
<td>USA (North Carolina)</td>
<td>19.7</td>
<td>IAS-USA</td>
<td>Hicks \textit{et al.}\textsuperscript{54}</td>
</tr>
<tr>
<td>Untreated patients\textsuperscript{c}</td>
<td>27</td>
<td>1994–97</td>
<td>Brazil (Rio de Janeiro)</td>
<td>22.2</td>
<td>Stanford/</td>
<td>Maia Teixeira \textit{et al.}\textsuperscript{55}</td>
</tr>
<tr>
<td>Seroconverters (&lt;12 months)</td>
<td>112\textsuperscript{d}</td>
<td>1999–2001</td>
<td>USA (New York)</td>
<td>24.1</td>
<td>IAS-USA</td>
<td>Shet \textit{et al.}\textsuperscript{56}</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>106</td>
<td>2003–04</td>
<td>San Diego, USA</td>
<td>25.2</td>
<td>IAS-USA</td>
<td>Smith \textit{et al.}\textsuperscript{5}</td>
</tr>
</tbody>
</table>

IS, interpretation system; NG, not given.
\textsuperscript{a}Only homosexual males included.
\textsuperscript{b}Strategy to control SPREAD of HIV drug resistance.
\textsuperscript{c}Only intravenous drug users included.
\textsuperscript{d}Ninety-eight per cent homosexual males.
drug-naive Danish patients are a reflection of the declining rates of treatment failure, and hence emergence of resistant virus, observed among persons on HAART in recent years. Data from Swiss seroconverters showed that TDR had declined from 14.6% in 1997 to 5% in 1999. The authors concluded that this was likely to be due to both the increasing prevalence of non-B subtypes and the increasing numbers of patients with undetectable viral loads in their HIV-1-infected population. More recent data from the Swiss HIV cohort showed phylogenetic clustering of sequences from treatment-experienced persons—the potential transmitters—and newly diagnosed drug-naive patients. A New York-based study of persons with acute seroconversion also found phylogenetic clustering of cases of TDR, suggesting that those with multiresistant virus act as a common source to multiple persons. Fluctuations in TDR have also been linked to rates of drug resistance in treated patients by a small Italian study which notes that although the prevalence of TDR declined from 22.5% in 1996–97 to 9.5% in 1998–99, it then increased 23.9% in 2000–01. This pattern was partially attributed to the number of treated patients with drug resistance, which had initially declined and then increased over the same time period. This hypothesis has been supported by several mathematical models, showing that it can be predicted that rates of TDR may rise or fall according to recommended treatment regimens and the replication fitness of drug-resistant viruses.

Fitness of drug-resistant virus variants

Virus variants carrying drug-resistance mutations in genes encoding the RT, protease and, more recently, the gp41 envelope protein generally display a reduction in fitness relative to wild-type virus. Virus fitness can be defined as the overall capacity of a virus to infect, replicate and produce mature infectious progeny in a defined host environment. Virus variants with resistance-associated mutations should therefore have reduced transmission efficiency relative to wild-type virus. One study estimated that the transmission of resistant variants occurs ~80% less frequently than expected when related to the amount of antiretroviral resistance in the population of treated persons. Along the same lines, one study reported that the prevalence of resistance mutations was only 10.5% among newly diagnosed patients when compared with 72.4% among drug-experienced patients from the same geographical region. Variations in fitness of drug-resistant mutants have been well described. For example, the lamivudine and emtricitabine RT mutations M184V/I confer a significant reduction in viral fitness, whereas the NNRTI mutations K103N and Y181C have little impact. Published evidence supports the concept that differential transmission of resistant mutants occurs. The M184V and T215rev occur more frequently in TDR, consistent with their improved fitness relative to T215Y/F.
Recently in New York, the case of a recently infected person found to harbour a virus with multiple resistance mutations in RT and protease received widespread media coverage following the patient’s rapid decline in CD4 count after infection.63 Using the Replicative Capacity Assay (Monogram Biosciences, formerly Virologic, USA), which assesses the contribution of RT and protease to virus fitness using a recombinant virus, the patient’s virus showed a replicative capacity of 136% when compared with the wild-type virus. It should be noted, however, that even drug-resistant virus variants showing a low replicative capacity by the recombinant virus assay can establish infection.66 Taken together, these data indicate that drug-resistant variants can achieve sufficient fitness for efficient transmission and pathogenicity, probably through the acquisition of compensatory changes in RT and protease, as well as other viral regions such as Env, Gag, and Gag cleavage sites.

Transmitted resistant variants may persist for many months or even years in the absence of drug pressure.59,81,87–90 In particular, several mutations considered less detrimental to viral fitness, such as M41L, T69D/N, K103N, G190S, L210W, T215rev and K219Q in RT and I84V and L90M in protease, show little reversion to wild-type over time.81,91 The proposed explanation for this finding is that infection with a highly homogenous drug-resistant virus quasispecies does not allow the rapid outgrowth of wild-type virus. Consistent with this model, one study found a highly homogenous HIV-1 population when comparing viral RNA in plasma with proviral DNA in PBMC.90 In this scenario, the emergence of wild-type virus would require reversion of the drug-resistant mutants. This process of reversion can be slow, as the transmitted variants may be genetically locked and unable to revert without at least transient loss of fitness.

However, reversion does occur over time. For example, in the absence of selective drug pressure, the RT mutations T215Y/F are replaced by the fitter T215rev variants, which act therefore as markers of transmitted NRTI resistance.92 Other mutations that show reversion include K70R, M184V and less rapidly D67N, K219N and Y181C.91 Clearly, the different rates of reversion influence the overall detection of TDR and the mutational patterns observed.

**Effect of TDR on CD4 count**

Consistent with the reduced fitness and pathogenicity of drug-resistant variants, patients with TDR have been shown to have a higher initial CD4 count than persons with wild-type virus,93 although this difference tends to disappear over time, possibly coinciding with the emergence of fitter variants. However, evidence is contradictory. One other study reported that patients with TDR had a faster decline in CD4 count than persons with wild-type virus during the first year following infection, but not in subsequent years.94 In another cohort, patients with TDR showing persistence of resistant mutants over time showed a lower CD4 count than those with reverting quasispecies.91 We recently reported that newly diagnosed patients with TDR had a significantly higher CD4 count than persons with wild-type virus.27 Although this may have suggested a reduced pathogenicity of drug-resistant virus, an alternative explanation is that the association simply reflected the effect of time since infection on the detection of TDR by routine genotyping methods.

**Detection of low-frequency-transmitted drug-resistant virus variants**

Routine genotypic resistance testing methods (also known as population sequencing) are only able to detect resistant mutants within the dominant quasispecies and are unlikely to identify low-frequency mutants representing <20% to 30% of the total viral population. More sensitive approaches are being evaluated in research settings. One approach is based on the detection of archived quasispecies in proviral DNA of PBMC.69 In addition, allele-specific real-time PCR and single genome sequencing can detect drug-resistant mutants in plasma with a sensitivity of ≤0.1% to 0.2% of the total viral population.96–98 These techniques have been used successfully to detect resistance mutations among patients lacking evidence of resistance by standard genotyping. One recent study analysed the prevalence of three common resistance mutations—K103N and M184V in RT and L90M in the protease gene by allele-specific PCR. The mutations were detected in 10 of 49 patients using allele-specific PCR, compared with only 5 patients by population sequencing.97 One other study showed that in a population already known to have TDR by conventional methods, additional resistance mutations could be found using allele-specific PCR and clonal sequencing.98 Recently, another study also found that allele-specific PCR detected at least 20% more multidrug-resistant viruses than population sequencing.96

It is important to note that transmitted resistant mutants that present at low frequency within the quasispecies can rapidly become dominant once selective drug pressure is introduced. Emerging evidence suggests that, for the NNRTIs, the detection of low-frequency-resistant viruses in treatment-experienced persons is associated with reduced virological responses to antiretroviral therapy.99 Among treatment-naïve persons, one study targeting the RT mutations K103N, Y181C and M184V by allele-specific PCR found a strong correlation between the presence of the mutants as low-frequency quasispecies and virological failure of first-line NNRTI-based HAART.100

Although population sequencing remains currently the most widely available and well-validated method for HIV-1 resistance testing, taken together these findings indicate that more sensitive methods of resistance testing are likely to become important in the evaluation of treatment-naïve persons, particularly where NNRTI-based therapy is being considered. A further potential application includes the detection of ‘less fit’ mutations such as M184V, which are rarely detected in drug-naïve patients by population sequencing. The impact of low-frequency-transmitted PI mutations on responses to first-line and subsequent therapy with ritonavir-boosted PIs and the clinical utility of detecting low-frequency protease resistance mutations remains to be determined.

**Guidelines on resistance testing in treatment-naïve persons**

In the light of the possibility that TDR may become undetectable by routine testing over time, resistance testing guidelines, including British, US and European guidelines, recommend testing for TDR using the earliest available sample after diagnosis.101–103 This is consistent with evidence that the use of genotypic resistance testing can be cost-effective in this setting.104 In addition, in patients without evidence of TDR, it is generally
recommended that a suboptimal virological response early after the initiation of first-line therapy may be an indication of undiscovered TDR and should prompt a further resistance test.\textsuperscript{101} Genotypic resistance testing is regarded as cost-effective in the detection of TDR as it is widely available and inexpensive. In addition, it is more sensitive than phenotypic testing in the detection of TDR, as it allows for the detection of ‘sentinel’ mutations that have little impact on phenotypic resistance, as seen in the case of T215rev.

The phenomenon of superinfection poses further potential problems in the detection and interpretation of TDR. Although the true magnitude of the risk remains to be clearly demonstrated, cases have been reported of drug resistance acquired through superinfection, where the newly acquired drug-resistant virus has become dominant over the initial drug-susceptible virus.\textsuperscript{105–107} Superinfection with wild-type virus following infection with a resistant virus has also been documented—a prospect that should be considered when interpreting genotypic resistance test results as the wild-type virus may become dominant masking the mutations of the drug-resistant virus.\textsuperscript{106,108}

According to the current guidelines, once testing of the baseline sample has been performed, repeat testing prior to starting therapy is not considered cost-effective and not recommended. Nonetheless, future recommendations may change should evidence indicate that repeat testing of multiple samples prior to initiation of therapy provides beneficial information. The current British HIV Association (BHIVA) guidelines indicate that repeat testing may be considered if clinical and behavioural indicators suggest that superinfection is likely (e.g. sudden changes in the CD4 count or plasma viral load in individuals with high-risk behaviour).\textsuperscript{101}

Conclusions

The transmission of drug-resistant HIV-1 has important implications for the successful management of antiretroviral therapy among infected individuals, restricting drug options and increasing the risk of suboptimal treatment outcomes. In addition, if the pool of drug-resistant viral strains available to infect more individuals grows, a vicious circle leading to even higher rates of TDR can develop. In the past, TDR has been mainly restricted to more affluent countries; however, with the use of antiretroviral drugs in developing countries becoming more widespread, the incidence of transmitted resistance worldwide is set to increase further. Currently, resistance testing is limited or non-existent in resource-poor settings. However, it is important that surveillance for the emergence of drug resistance in both treated and newly diagnosed persons is carried out in these populations. The current surveillance plan run by the World Health Organization will be critical to monitoring the incidence of TDR and ensuring that national treatment guidelines are adapted appropriately.\textsuperscript{109}

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