Documented Prevalence of HIV Type 1 Antiretroviral Transmitted Drug Resistance in Ireland from 2004 to 2008

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Abstract

HIV-1-infected individuals with transmitted HIV drug resistance (TDR) begin antiretroviral therapy (ART) with a lower genetic barrier to resistance and a higher risk of both virological failure and of developing further resistance. TDR surveillance informs HIV-1 public health strategies and first line ART. TDR has not been studied nationally in an Irish population. This study includes all new HIV diagnoses from January 2004 to September 2008 from the National Virus Reference Laboratory, University College Dublin. HIV-1 protease and reverse transcriptase sequences were generated, and resistance mutations identified using the Siemens TRUGENE HIV-1 Genotyping System. Subtypes were determined using web-based genotyping tools. The study comprised 1579 patients. There were 305 new diagnoses in 2004 (173 male; 132 female), 298 in 2005 (175M; 123F), 321 in 2006 (197M; 124F), 297 in 2007 (184M; 113F), and 358 (235M; 123F) in 2008. HIV-1 RNA was sequenced from 158/305 patients in 2004, 199/298 in 2005, 225/321 in 2006, 203/297 in 2007, and 275/358 in 2008. The overall TDR rate was 6.3%, peaking in 2006 at 10.4% and declining to 5.3% in 2008. The majority of TDR was seen in Irish born individuals with HIV-1 subtype B infection. The TDR rate in Ireland is comparatively low. Thus, a health technology assessment is required to ascertain the most cost effective use of genotypic antiretroviral resistance testing (GART) in the future: the current approach of performing baseline GART on all new diagnoses, or perhaps a more targeted approach that focuses on patients commencing nonnucleoside reverse transcriptase inhibitor (NNRTI)-based ART.

Introduction

Human immunodeficiency virus type 1 (HIV-1) was introduced into humans during the first half of the twentieth century.1 While there are now more than 25 antiviral agents available for the treatment of HIV infection, and the prognosis for those infected—in the developed world at least—continues to improve, effective control of the HIV pandemic remains elusive. Although there are a number of reasons for this, one of the most significant is drug resistance, which may be primary (transmitted) or secondary (acquired).

Transmitted (or primary) HIV drug resistance occurs when a virus already possessing resistance-associated mutations infects a drug-naive individual. Although both acquired and transmitted HIV-1 drug resistance (TDR) are public health concerns, it is the latter that has the potential to more significantly impact the effectiveness of first-line antiretroviral therapy (ART) at the population level. Persons with TDR begin ART with a lower genetic barrier to resistance, a higher risk of virological failure, and a higher risk of developing further resistance, even to those drugs in their treatment regimen that were originally fully active.2 Consequently, HIV drug resistance surveillance has been advocated and programs funded by the WHO and the European Union established to support public health bodies in designing education and prevention programs, to minimize the development and transmission of drug-resistant viruses, and to support the rational use of ART by treatment programs, clinicians, and policy makers.

Studies in Europe and North America have reported TDR prevalence of between 5% and 15% in newly diagnosed individuals, and from 10% to 25% in acutely infected individuals.2 For this reason, routine genotypic antiretroviral resistance testing (GART) is recommended for all newly
diagnosed HIV-1 infected individuals prior to commencing ART. Indeed, this recommendation was adopted in Ireland by clinical consensus as best practice in 2004. To date no comprehensive study of primary resistance in the Irish population has been performed, and therefore, the prevalence of TDR at a national level is unknown. Thus, the aim of this study was to determine the prevalence of HIV-1 genotypic antiretroviral drug resistance in newly diagnosed patients (i.e., TDR) in Ireland from 2004 to 2008.

Materials and Methods

Study duration and design

The study focused on patients diagnosed with HIV infection in Ireland from January 2004 to December 2008 inclusive. Study data were collated in the National Virus Reference Laboratory (NVRL), University College Dublin (UCD). The NVRL is the primary center in Ireland for the serological diagnosis and/or confirmation of HIV-1 infection: the NVRL also performs 85–90% of HIV-1 RNA (viral load) testing in Ireland, and all GART for HIV-1-infected individuals.

Patient selection

Subjects eligible for inclusion comprised newly diagnosed, antiretroviral-naive individuals with HIV infection in Ireland over the study period. The date of diagnosis for each patient was taken as the first date upon which a blood sample from that patient (plasma or serum) tested positive for either HIV-specific antibody or HIV-1 RNA in the NVRL.

Branched DNA testing

HIV-1 (RNA) viral load measurement was performed using the Siemens VERSANT HIV-1 RNA 3.0 Assay (bDNA) as per the manufacturer’s instructions.

Genotypic antiretroviral resistance testing

HIV-1 genotypic resistance testing was performed using the Siemens TRUGENE HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System as per the manufacturer’s instructions.

Identifying mutations indicative of transmitted drug resistance

TDR-associated mutations were defined as per Shafer and colleagues for the specific purpose of enabling physicians and public health officials to perform HIV-1 drug resistance surveillance. This list has since proven useful in a number of published surveys of TDR performed in sub-Saharan Africa and South and Southeast Asia. However, as a result of the introduction of several new drugs, and the identification of several new drug resistance mutations since the original publication, the 2007 list has recently been updated. Both the original list and the 2009 update were used to ascertain the percentage prevalence of HIV-1 transmitted drug resistance in newly diagnosed individuals in Ireland in this study.

GART-based HIV subtyping

HIV subtyping was performed by submitting GART sequences to three well-established and highly regarded online web-based genotyping tools: three tools were chosen to ensure consensus. These were the Stanford University HIV Drug Resistance database (http://hivdb.stanford.edu), the National Center for Biotechnology Information (NCBI) Viral Genotyping Tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi), and the Katholieke Universiteit Leuven REGA HIV-1 Automated Subtyping Tool (http://www.bioafrica.net/subtypetool/html/subtypinghiv.html). Consensus was defined as any two tools yielding the same subtype result for a particular HIV sequence.

Statistical analysis

Differences in mean HIV-1 viral load data were analyzed using the Student’s t-test, while significance in changes in proportions over time was determined using the chi-squared test. Both applications were performed on raw data (as against means/percentages) in Microsoft EXCEL.

Results

New diagnoses 2004 to 2008

Over the course of the 5-year study period, there were 1579 new diagnoses identified, 615 (39%) female and 964 (61%) male. The yearly totals were 305 in 2004 [132 (43.3%) female; 173 (56.7%) male], 298 in 2005 [123 (41.3%) F; 175 (58.7%) M], 321 in 2006 [124 (38.6%) F; 197 (61.4%) M], 297 in 2007 [113 (38%) F; 184 (62%) M], and 358 in 2008 [123 (34.4%) F; 235 (65.6%) M]. The mean age at diagnosis for the entire cohort (N=1579) was 33.9 years.

HIV sequence data

Of 1579 new diagnoses, GART was performed in 1060 (67.1%) individuals, comprising 392 (37%) females and 668 (63%) males. Of 1060 sequenced samples over the 5 years studied, HIV subtype B accounted for 548 (51.7%). Subtype C was the second most prevalent subtype in the study cohort, accounting for 273 (25.8%) individuals, followed by 59 CRF02_AG (5.6%), 36 subtype G (3.4%), 35 CRF01_AE (3.3%), 33 subtype A [3.1% (32 A1 and 1 A2)], 15 CRF06_CPX (1.4%), 11 subtype D (1%), 7 subtype F [0.6% (all F1)], 4 CRF14_BG (0.4%), and 1 each of CRF18_CPX and subtype H; 37 sequences were unassigned.

Antiretroviral drug resistance

Of the 1060 HIV sequences obtained, country of birth and mode of HIV acquisition were known for a cohort of 864 individuals: eight sequences were incomplete, meaning that some codons at which drug-associated mutations may occur were not sequenced; therefore baseline resistance data for 856 patients was available. Reverse transcriptase (RT) and protease (PR) resistance mutations and polymorphisms identified by the TRUGENE system are presented in Tables 1 and 2. One hundred and forty-two reverse transcriptase mutations were identified in the cohort of 107 newly diagnosed individuals: 749 had no RT mutations detected. The most common mutations were V118I (40), K103N (28), V179D (19), and M41L (7). Of note, not all of these mutations confer resistance, but as they are different to the consensus reference HIV B sequence, they are identified as such by the resistance software. Those mutations that are genuinely associated with...
resistance are marked with an asterisk (Table 1). In contrast to findings from the RT region, a far greater number of PR mutations were identified, with 2659 mutations in 794 new diagnoses (62 individuals had no mutations detected). The most prevalent mutations identified were M36I (458), L63P (424), H69K (281), and L89M (246). However, as with the RT, not all of these PR mutations confer resistance. In fact, the majority of the mutations listed in Table 2 are polymorphisms (natural variations in the HIV genome) rather than resistance-associated mutations.

**Transmitted drug resistance**

These data were cross-referenced with an established list of previously published resistance-associated mutations. The mutations with asterisks in Tables 1 and 2 have been previously documented as significant evidence of prior ART exposure or TDR. A total of 54 cases with probable TDR were identified in the cohort of 856, giving an overall prevalence of 6.3% for the 5 years studied. The annual TDR rates were 3/91 (3.3%) in 2004, 10/172 (5.8%) in 2005, 19/183 (10.4%) in 2006, 10/182 (5.5%) in 2007, and 12/228 (5.3%) in 2008, suggesting that TDR prevalence peaked in 2006 (Fig. 1). However, while there was a significant increase in the prevalence of TDR between 2004 and 2006 ($p = 0.04$), over the entire study period, there was no significant change in the prevalence of TDR ($p = 0.12$).

Nineteen individuals with probable TDR were female and 35 male, with mean ages of 32 and 33 years, respectively. The modes of acquisition of HIV infection were heterosexual transmission (HT: $N = 20$), injection drug use (IDU: $N = 19$), sex between men (MSM: $N = 12$), mother to child (MTCT: $N = 2$), and receipt of an infected blood transfusion (BT: $N = 1$) (Fig. 1). Thirty-three patients were Irish-born, 10 European, 10 Sub-Saharan Africans, and 1 Southeast Asian. Accordingly, the predominant subtype in which resistance was detected was subtype B, accounting for 37 (67.3%) of the infections: there were seven (12.7%) subtype C, two each (3.6%) of subtypes A, F, and CRF14_BG, and one each of CRF01_AE, CRF02_AG and CRF_06cpx; one subtype was unassigned.

Eighty-one (69 RT and 12 PR) surveillance drug resistance mutations (SDRM) were identified in this cohort. Forty-four patients had a single mutation, five patients had two mutations, two patients had three mutations, two patients had four mutations, and one patient had more than four (13) mutations. As a result, forty-nine individuals would have had resistance to a single class of antiretroviral therapy, four would have had resistance to two classes of ART, and one individual had triple-class resistance. The most common mutations in the RT region were K103N (28), M184V (7), and M41L, while in the PR region M46I (6) and F53L (2) accounted for 75% of the total number of PR mutations detected (Table 2).

**Discussion**

Transmitted drug resistance (TDR) occurs when a virus already possessing resistance-associated mutations infects a drug-naive individual. In the newly infected individual, due

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**Table 1. HIV Reverse Transcriptase Mutations (Trugene) in Newly Diagnosed Infections in Ireland 2004–2008 (n = 856)**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Frequency</th>
</tr>
</thead>
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<tr>
<td>M41L*</td>
<td>7</td>
<td>K101E*</td>
<td>1</td>
<td>Y181C*</td>
<td>1</td>
<td>T215F*</td>
<td>1</td>
</tr>
<tr>
<td>A62V</td>
<td>1</td>
<td>K101Q</td>
<td>1</td>
<td>Y181I*</td>
<td>1</td>
<td>T215Y*</td>
<td>2</td>
</tr>
<tr>
<td>D67N*</td>
<td>2</td>
<td>K103N*</td>
<td>28</td>
<td>M184V*</td>
<td>7</td>
<td>K219Q*</td>
<td>2</td>
</tr>
<tr>
<td>T69D*</td>
<td>3</td>
<td>V106M*</td>
<td>1</td>
<td>Y188C*</td>
<td>1</td>
<td>P225H*</td>
<td>2</td>
</tr>
<tr>
<td>V75M*</td>
<td>1</td>
<td>V108I</td>
<td>3</td>
<td>G190A*</td>
<td>3</td>
<td>F227L</td>
<td>1</td>
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<tr>
<td>F77I*</td>
<td>2</td>
<td>V118I</td>
<td>40</td>
<td>H208Y</td>
<td>1</td>
<td>M230L*</td>
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<td>W88G</td>
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<td>V179D</td>
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<td>Total</td>
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</tr>
<tr>
<td>A98G</td>
<td>5</td>
<td>V179E</td>
<td>1</td>
<td>T215C*</td>
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</tr>
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</table>

Asterisks indicate those mutations that are associated with prior antiretroviral therapy (ART) exposure and may indicate transmitted drug resistance (TDR).

**Table 2. HIV Protease Mutations (Trugene) in Newly Diagnosed Infections in Ireland 2004–2008 (n = 856)**

<table>
<thead>
<tr>
<th>Mutation</th>
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<th>Mutation</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Frequency</th>
<th>Mutation</th>
<th>Frequency</th>
</tr>
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<tr>
<td>L10F</td>
<td>2</td>
<td>K20V</td>
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<td>K43T</td>
<td>1</td>
<td>H69K</td>
<td>281</td>
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<tr>
<td>L10I</td>
<td>68</td>
<td>D30N*</td>
<td>1</td>
<td>M46I*</td>
<td>6</td>
<td>A71T</td>
<td>74</td>
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<tr>
<td>L10M</td>
<td>1</td>
<td>V32I*</td>
<td>1</td>
<td>M46V*</td>
<td>1</td>
<td>A71V</td>
<td>2</td>
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<tr>
<td>L10V</td>
<td>64</td>
<td>L33I</td>
<td>2</td>
<td>I47V*</td>
<td>1</td>
<td>G73I</td>
<td>1</td>
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<tr>
<td>I13V</td>
<td>154</td>
<td>L33I</td>
<td>5</td>
<td>F53L*</td>
<td>2</td>
<td>T74S</td>
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<td>I15V</td>
<td>146</td>
<td>L33V</td>
<td>20</td>
<td>Q95E*</td>
<td>5</td>
<td>V77I</td>
<td>87</td>
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<tr>
<td>G16E</td>
<td>46</td>
<td>E34Q</td>
<td>1</td>
<td>D61E</td>
<td>43</td>
<td>I94V*</td>
<td>1</td>
</tr>
<tr>
<td>L19I</td>
<td>3</td>
<td>E35G</td>
<td>2</td>
<td>I62V</td>
<td>81</td>
<td>L89I</td>
<td>5</td>
</tr>
<tr>
<td>K20I</td>
<td>95</td>
<td>M36I</td>
<td>458</td>
<td>L63P</td>
<td>424</td>
<td>L89M</td>
<td>246</td>
</tr>
<tr>
<td>K20M</td>
<td>1</td>
<td>M36L</td>
<td>10</td>
<td>L63T</td>
<td>51</td>
<td>I93L</td>
<td>169</td>
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<tr>
<td>K20R</td>
<td>78</td>
<td>M36V</td>
<td>2</td>
<td>L63V</td>
<td>1</td>
<td>Total</td>
<td>2659</td>
</tr>
</tbody>
</table>

Asterisks indicate those mutations that are associated with prior ART exposure and may indicate transmitted drug resistance (TDR).
The average prevalence of TDR in the Irish cohort studied here was 6.3%, ranging from 3.3% in 2004 to 10.4% in 2006. Despite a significant increase in TDR rates between 2004 and 2006 ($p=0.04$), over the entire study period, there was no significant change in the prevalence of TDR ($p=0.12$). The gender breakdown of the overall cohort was essentially maintained in the TDR, with 19 (35.2%) females and 35 (64.8%) males. Furthermore, the TDR prevalence was equivalent between females (6.2%) and males (6.4%). Not surprisingly, resistance was most often detected in Irish-born individuals infected with HIV-1 subtype B. However, seven other subtypes were represented, demonstrating that TDR occurs in non-B infection. While previous studies have reported lower levels of TDR in persons infected with non-B subtypes of HIV-1, in Europe and the UK at least, it is probable that this difference is due primarily to the importation of non-B HIV-1 infection from countries where antiretroviral therapy is not widely available. This is almost certainly the case in Ireland as well.

Transmitted drug resistance in many published studies from the developed world was identified mainly among recently infected MSM, although not limited to this group. Reasons for this pattern probably relate to the majority of HT infections in these populations historically originating in countries where ART was not available; in addition, IDU were traditionally perceived to be a poorly compliant patient group, and therefore may have been underrepresented in ART treatment cohorts. Conversely, the Irish data presented here would suggest that the majority of TDR is occurring in IDU with a prevalence of 9.7% (19/199), followed by MSM at 5.6% (12/214) and HT at 4.7% (20/424). These findings are consistent with the historical approach to the treatment of HIV-1-infected IDU in Ireland, where there prevailed a well-structured clinical practice of treating IDU—in partnership with drug (addiction) treatment centers—with once-daily ART, which in the late 1990s in Ireland comprised predominantly NNRTI-based regimens, despite the concern about pharmacological interactions with methadone. Indeed, in support of the data presented, three of the current authors have previously reported higher rates of virological failure in IDU, with significant levels of NNRTI and 3TC resistance. Furthermore, the rate of TDR in MSM seems to be declining while in IDU and HT, overall 2008 rates are comparable to those seen in 2005, albeit with some fluctuation in the intervening period (Fig. 1). These contrasting findings reinforce the importance of each country performing its own studies of this type despite the fact that Ireland contributes data to the SPREAD study, the findings from that large cohort do not in fact mirror those seen at the national level.

Baseline GART has been the standard of care for newly diagnosed HIV-1-infected individuals since the turn of the century. Treatment guidelines recommend genotypic testing in antiretroviral-naive patients to detect the presence of TDR and to adapt their first-line treatment accordingly. While the benefit of GART was initially demonstrated in patients failing their first ART regimen, subsequent studies have confirmed this benefit in ART-naive individuals. Indeed, even in the setting of patients receiving a three-drug ART regimen in which the virus has lost susceptibility to only one drug, TDR has been associated with virological failure.

Of greater concern in this context is the fact that there also appears to be a higher risk of virological failure in patients...
starting a regimen of two NRTIs plus one NNRTI if the patient harbors a virus with TDR, even if the prescribed regimen is predicted to be fully active. These findings may be partly explained by the presence of additional low-level or minority NNRTI-resistant strains, which have also been demonstrated to increase the risk of virological failure. To date, minority protease inhibitor-resistant strains have not been shown to have the same impact. In the cohort presented here, K103N was detected (using standard GART) in 28 of 54 individuals, accounting for 52% of probable TDR cases. While it has been proposed that assays capable of detecting NNRTI minority resistant variants be developed for routine clinical use, at present such assays are not available. Consequently, where an NNRTI-based regimen is being considered as first-line ART, it is arguably vital that at the very least baseline (standard) GART be performed.

In 2001, Weinstein and colleagues published a cost-effectiveness study on the use of standard GART to guide the choice of ART, concluding that GART was cost effective in the setting of both failing patients and ART-naive patients. Weinstein calculated the cost per QALY (quality adjusted life year) of GART in drug-naive patients at US $22,300, assuming a TDR prevalence of 20%. As the assumed TDR prevalence rate was decreased to 4%, the cost per QALY increased to US $69,000. The present study is the first in Ireland to attempt to ascertain the usefulness and the cost effectiveness (based on the TDR prevalence rate) of baseline GART. While it would be inappropriate to determine the cost effectiveness of this policy on the basis of a decade-old study from a different health system and jurisdiction, the low TDR prevalence observed suggests (especially in the current economic climate) that a health technology assessment should be performed as a priority to ascertain the wisdom of the current approach. One feasible alternative approach to that currently in place would be the performance of baseline GART only for those patients commencing NNRT-based ART. While it is accepted that ART-related decisions may not be made at diagnosis, plasma could be stored at this time, and subsequently tested, if indicated, before commencing ART. That being said, as more data become available regarding the clinical relevance of minority variants, it may be appropriate to wait and perform such an assessment when next generation GART assays become available.

This study is the largest undertaken to date in the Irish HIV-1-infected population. However, it must be acknowledged that new diagnoses have been reported here rather than new infections. Thus, the data probably predominantly represent chronically infected rather than newly infected individuals. This inevitably leads to an underestimation of the true TDR rate: in addition, as this study has not followed up the cohort of patients without TDR mutations at baseline through their primary ART regimen, it is not possible to determine how many cases of TDR have been missed through reversion.

**Author Disclosure Statement**

No competing financial interests exist.

**References**


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