New developments in HIV drug resistance

Patricia A. Cane*

Virus Reference Department, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK

Several new antiretroviral drugs have recently been licensed for use in HIV-1-infected patients. These include drugs in two new classes: an integrase inhibitor (raltegravir) and a CCR5 co-receptor antagonist (maraviroc). In addition, two new protease inhibitors, atazanavir and darunavir, which have activity against viruses resistant to other protease inhibitors, have come into clinical use. Finally etravirine, a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) is being increasingly used in patients whose virus is resistant to the earlier NNRTIs. These clinical advances have required the development of novel assays and interpretation systems for detection of resistance to allow the laboratory monitoring of patients receiving these new therapies.

Keywords: new drugs, tropism determination, minority mutant detection, dried blood spots

Introduction

The outlook for patients infected with HIV-1 has greatly improved in recent years due to the introduction of antiretroviral therapy (ART) and there are now >20 drugs licensed for clinical use. Until recently the classes of drugs available included those directed against the viral reverse transcriptase or protease and fusion inhibitors. Reverse transcriptase inhibitors (RTIs) were either nucleoside or nucleotide analogues (NRTIs) or non-nucleoside inhibitors (NNRTIs). Anti-HIV drugs are most effective when used as a triple combination. Such a combination usually consists of two NRTIs together with either a protease inhibitor (PI) or an NNRTI. Modern combination therapy is highly effective in reducing the replication of the virus to undetectable levels and in maintaining that suppression. The rate of therapy failure (as determined by viral load becoming detectable) is ~5% per year. Nevertheless, since therapy is required lifelong, a significant minority of patients are likely to develop resistance to at least one class of drugs and it has been shown that the likelihood of failure is greater with second-line therapy. In addition, patients who started therapy with suboptimal treatments (e.g. patients treated in the 1990s or in Africa more recently) have an increased risk of developing multiclass-resistant virus. Thus, much drug development in recent years has been focused on the discovery of drugs within new classes or that resistance to at least one class of drugs and it has been shown that the likelihood of failure is greater with second-line therapy. In addition, patients who started therapy with suboptimal treatments (e.g. patients treated in the 1990s or in Africa more recently) have an increased risk of developing multiclass-resistant virus. Thus, much drug development in recent years has been focused on the discovery of drugs within new classes or that acting directly on a viral component. In addition, two new protease inhibitors (darunavir and atazanavir) have been approved which show activity against viruses which already show some resistance to other PIs. A further advance has been the approval of etravirine, a novel NNRTI, which shows activity against viruses already resistant to established NNRTIs and which has a high genetic barrier (i.e. requires the acquisition of multiple mutations) for the development of resistance. However, inevitably, it has been found that patients can fail these drugs with the development of resistance. Virological detection of such resistance has required either the development of new assays or the modification of algorithms to interpret sequence data.

Integrase inhibitor resistance

Raltegravir has now been licensed for use in treatment-experienced patients, and a second integrase inhibitor, elvitegravir, is undergoing clinical trials. Resistance to raltegravir has been shown to emerge rapidly if the other components of the patients’ therapy are suboptimal, but data on the mutations conferring resistance are limited. In recent clinical trials, viruses from most patients failing raltegravir therapy have shown ‘signature’ resistance mutations in three integrase sites, namely Y143, Q148 and N155. Each of these mutations can confer resistance alone, but continuing on a failing raltegravir regimen results in the accumulation of more mutations and often the replacement of N155H with Q148H as the predominant population. Further secondary mutations may also emerge which can both increase resistance and restore viral fitness. In vitro studies indicate that resistance to raltegravir will confer resistance to elvitegravir...
and vice versa, though it is not yet known whether the mutations that emerge under therapy with the two drugs will be similar. It is likely that routine detection of resistance to integrase inhibitors will be by sequencing of the appropriate region of the integrase gene. Programs for the analysis of such sequences and their interpretation are available over the internet, e.g. Geno2Pheno (www.geno2pheno.org).

New NNRTI resistance

Etravirine, a new NNRTI, has recently been licensed for use in treatment-experienced patients. It shows activity against viruses resistant to the first-generation NNRTIs, namely nevirapine and efavirenz. However, while nevirapine and efavirenz require only one mutation to confer high-level resistance, resistance to etravirine is mediated by complex combinations of mutations which are only just beginning to be understood. The mutations that have been shown to contribute to etravirine resistance include Y90I, A98G, L100I, K103N/H/P, V106I, E138A, V179C/I/V, Y181C, G190S/A and M230L. It is thought that the presence of three or more of these mutations is required to compromise clinical activity, except for Y181C where only one additional mutation is required. Of note, the presence of K103N, the most common mutation observed conferring resistance to efavirenz, does not compromise etravirine activity. In addition, it has recently been suggested that although etravirine shows a relatively high genetic barrier to the development of resistance, the impact of each mutation requires weighting within a genotypic resistance algorithm.

New protease inhibitor resistance

Two new PIs, darunavir and tipranavir, have recently been licensed for use with ritonavir boosting. These drugs are active against viruses that have acquired resistance to earlier PIs and also have a high genetic barrier to the development of resistance, as has already been observed with another PI, lopinavir. The standard of care for HIV-1-infected patients to date has been combination therapy comprising three drugs. The observation of the high genetic barrier to resistance of the new PIs led to the idea that it might be possible to use these agents as monotherapy. In addition, it is rare for patients failing lopinavir therapy to show development of resistance mutations in the protease gene. Initial trials using lopinavir/ritonavir as the only therapy from the beginning of treatment gave very variable results and this approach is now seldom used.

ACTG 5201 was a pilot study which examined whether a single PI (atazanavir) could be used as maintenance therapy in patients who have undetectable viral load as induced by use of combination therapy. Patients with suppressed viral load on treatment with two NRTIs and a first PI for >48 weeks initially had their PI switched to atazanavir/ritonavir and then, after a further 6 weeks, the NRTIs were discontinued. Of the 36 patients, three experienced virological failure by week 24 but, in two of the three virological failures, atazanavir drug levels were not detected. Thus in this relatively short-term trial, monotherapy with a PI was effective as maintenance therapy in >90% of the patients. Further larger scale trials are now underway to examine this treatment strategy. If such monotherapy can be shown to be effective, then it will be a useful way of reducing drug treatment, so reducing toxicity and long-term costs.

Co-receptor antagonists

Maraviroc has recently been licensed for use in treatment-experienced patients. It is a CCR5 co-receptor antagonist and is the first example of an anti-HIV drug blocking a cellular rather than a viral function. HIV-1 uses CCR5 and/or CXCR4 as co-receptors to gain entry to cells expressing CD4. Viruses are designated R5 if they use CCR5, X4 if they use CXCR4, and R5/X4 if they are dual tropic or a mixture of R5 and X4 variants. Different cell tropisms were defined long before the discovery of a co-receptor for HIV-1 and those designations largely reflect the co-receptor use. R5 viruses are equivalent to those previously called macrophage tropic or non-syncytium inducing while X4 viruses are equivalent to the T-cell-tropic, syncytium-inducing grouping. It has previously been shown that R5 viruses are the most prevalent variant early in infection while X4 viruses emerge later in the course of disease in about half of untreated patients and are associated with rapid CD4+ cell decline and disease progression. It is not known whether the switch from R5 to X4 tropism is the cause or consequence of disease progression, which has led to some concerns that use of R5 inhibitors could select for X4 variants with consequent acceleration of disease.

The relative prevalence of R5 and X4 viruses in treated and naive patients is summarized in Table 1 which shows data from eight cohorts, three of treatment-naive patients and five of treatment-experienced patients. Dual/mixed or X4 virus appears to be less prevalent among those who have yet to receive treatment. Nevertheless, even in the naive setting, 12%-19% of individuals had detectable D/M or X4 virus. In those with more treatment experience, ~20%-50% of subjects had detectable D/M or X4 virus. It could therefore be concluded that there will be more patients eligible for use of an R5 inhibitor (i.e. with no detectable D/M or X4 virus) among less treatment-experienced subjects who are often in the earlier stages of disease.

Since maraviroc targets only R5 viruses, it has been shown to be vital to determine the tropism of the virus infecting individual patients before starting therapy. Determination of tropism can be by phenotypic or genotypic assays. Phenotypic assays

Table 1. Prevalence of co-receptor tropisms in treated and untreated patients

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample (n)</th>
<th>R5 only</th>
<th>Dual/mixed</th>
<th>X4 only</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>325</td>
<td>88%</td>
<td>12%</td>
<td>0%</td>
<td>10</td>
</tr>
<tr>
<td>Naive</td>
<td>979</td>
<td>82%</td>
<td>18%</td>
<td>0.1%</td>
<td>11</td>
</tr>
<tr>
<td>Naive</td>
<td>402</td>
<td>81%</td>
<td>19%</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>Experienced</td>
<td>117</td>
<td>67%</td>
<td>28%</td>
<td>5.0%</td>
<td>10</td>
</tr>
<tr>
<td>Experienced</td>
<td>125</td>
<td>78%</td>
<td>22%</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>Experienced</td>
<td>724</td>
<td>50%</td>
<td>48%</td>
<td>2.0%</td>
<td>13</td>
</tr>
<tr>
<td>Experienced</td>
<td>391</td>
<td>49%</td>
<td>47%</td>
<td>4.0%</td>
<td>14</td>
</tr>
<tr>
<td>Experienced</td>
<td>1076</td>
<td>56%</td>
<td>44%</td>
<td>44%</td>
<td>15</td>
</tr>
</tbody>
</table>

NA, not available.
include the Trofile recombinant virus assay provided by Monogram Biosciences (San Francisco, USA) which is currently the most widely used test as it has had clinical validation. An enhanced version of the Trofile is now available which has improved sensitivity for detection of X4-using viruses within a mixture. However, this assay is relatively slow and expensive compared with genotypic methods and requires stringent conditions for collection and processing of samples, and a significant minority of samples fail to provide a result. Consequently, consideration is now being given to the use of genotypic tests for prediction of tropism.

The strongest genotypic determinants for tropism lie in the V3 loop of the envelope protein and this is the area being focused on for the development of genotypic assays based on nucleotide sequencing. At present there is variable correlation between results obtained by genotyping and phenotyping, especially for non-B subtypes, and there is also increased sensitivity shown by phenotyping for detection of minority populations of X4 virus. It has recently been reported that virological outcomes are predicted more accurately by use of a Trofile assay with enhanced sensitivity.

There are two mechanisms by which resistance to CCR5 antagonists emerges. First, viruses that use X4 or are dual tropic emerge during therapy, and these are thought to grow out from a pre-existing reservoir. This occurs in about two-thirds of failures and has not been associated with a rapid fall in CD4 count. The second mechanism is via alterations in the amino acid sequence of the V3 loop which then allow the virus to bind to the CCR5 with the inhibitor bound in place. A possible mechanism which has not been observed is the evolution of the V3 loop such that it becomes CXCR4 tropic, a parallel situation to that observed during the normal course of disease.

Sensitive genotyping methods

Standard genotyping methods use population sequencing of the overall virus population. The sensitivity of this approach for detection of minority species of mutants is limited to ~20%. In contrast allele-specific PCR methods can detect mutants present as only 0.1%–1% of the population, although this approach can only be used to detect specific mutations not provide overall nucleotide sequence or linkage of mutations. Johnson et al. used this approach to examine the prevalence of transmitted resistance with respect to a panel of mutations in treatment-naive patients and found that there was increased detection of resistance. The tests showed 1–3 minority drug resistance mutations in 34/205 (17%) of newly diagnosed patients who had wild-type virus by conventional sequencing. Also, 30/303 (10%) of samples which showed mutations by population sequencing were found to have at least one additional mutation when tested using the more sensitive assays. There is some preliminary evidence that this may have some clinical significance since 7/95 (7%) of patients with wild-type baseline virus by standard methods who experienced virological failure had minority drug resistance mutations at baseline while such mutations were detected in only 2/221 (0.9%) of treatment successes. In a second study, four patients who experienced early virological failure were found to have harboured drug-resistant viruses at low frequencies before treatment. Thus it seems likely that minority populations of drug-resistant viruses present at baseline can rapidly emerge and become the dominant virus population and subsequently lead to early failure of first-line therapy, especially in patients in receipt of ART with a low genetic resistance barrier. However, the level at which minority mutations become clinically significant remains to be determined.

Another approach for detection of minority mutants is the use of ‘ultra-deep’ sequencing, though this remains firmly in the research realm at present. This technique involves sequencing of up to 100000 individual amplicon molecules so allowing detection of minute levels of mutants. However, this in turn can produce false-positive results due to the inherent rate of error in HIV-1 replication together with the error rate in the enzymes used in the assay.

Resistance testing in developing countries

The roll-out of ART in the developing world has transformed the outlook for the millions of HIV-infected patients requiring treatment. However, in many instances treatment is being applied without concomitant laboratory monitoring of either viral load or resistance. In part this is due to the logistical problems of storing or transporting blood or plasma samples from remote areas without good communications and/or a reliable electricity supply. One solution to this aspect is to use dried blood spots which are considerably more stable than plasma and can be shipped at ambient temperature. The WHO has established a network of laboratories to give guidance on use of dried blood spots and monitor the transmission of drug-resistant HIV.

Conclusions

Treatment of HIV-1 infection has been radically altered over the past year by the introduction of new drugs. This has necessitated the development of new methods for the detection of resistance. In addition, technological advances have made it possible to detect resistant viruses even when they are present as low-level minority species. The challenge now is to determine the clinical utility of the new methods and to implement the new assays as appropriate into routine clinical use.

Transparency declaration

The author has served as an advisor to Pfizer Inc.

References


