Genotypic resistance testing of antiretrovirals in HIV

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The Secretary
Medical Services Advisory Committee
Department of Health and Ageing
Mail Drop 106
GPO Box 9848
Canberra ACT 2601

Enquiries about the content of the report should be directed to the above address.

The Medical Services Advisory Committee (MSAC) is an independent committee which has been established to provide advice to the Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC recommendations do not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

This report was prepared by the Medical Services Advisory Committee with the assistance of Dr Silva Zavarsek, Dr Renea Johnston, Ms Sandra Younie, Associate Professor Anthony Harris, Dr Omar Abdulwadud. Ms Anne Parkhill and Ms Sharon King from the Monash Institute of Health Services Research and the Centre for Health Economics of Monash University. The report was edited by Dr Alana Mitchell, ScienceLink Pty Ltd. The report was endorsed by the Minister for Health and Ageing on 2 March 2005.

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Executive summary

The procedure

Patients with human immunodeficiency virus (HIV) infection are treated with antiretrovirals to reduce viral load and ultimately slow disease progression. Due to the high error rate of reverse transcriptase (the viral enzyme responsible for replicating the viral genome, or genetic material) and the rapid replication rate of HIV, genetic mutations develop in HIV. These mutations may lead to drug resistance. Genotypic resistance testing detects the genetic mutations in HIV that result in drug resistance.

Various assays are available for genotypic resistance testing based on the analysis of mutations associated with HIV drug resistance. Genotypic assays include direct sequencing of the HIV genome and nucleic acid hybridisation using specific wild-type or mutant oligonucleotides. DNA sequencing assays are the most frequently used genotypic assays in Australia.

In general, genotypic assays are performed using RNA obtained directly from the HIV virus, however it is also possible to use viral DNA that has become integrated into the host genome (proviral DNA). When viral RNA is the starting material, it must first be converted to complementary DNA (cDNA). The sequence to be analysed is then amplified by polymerase chain reaction (PCR) to obtain sufficient target DNA. The reverse transcription step is not required when using proviral DNA.

Medical Services Advisory Committee – role and approach

The Medical Services Advisory Committee (MSAC) is a key element of a measure taken by the Australian Government to strengthen the role of evidence in health financing decisions in Australia. MSAC advises the Minister for Health and Ageing on the evidence relating to the safety, effectiveness and cost-effectiveness of new and existing medical technologies and procedures, and under what circumstances public funding should be supported.

A rigorous assessment of the available evidence is thus the basis of decision making when funding is sought under Medicare. A team from Monash University was engaged to conduct a systematic review of literature on genotypic resistance testing of antiretrovirals in HIV. An Advisory Panel with expertise in this area then evaluated the evidence and provided advice to MSAC.

MSAC's assessment of genotypic resistance testing of antiretrovirals in HIV

This assessment was undertaken to provide the broadest possible advice regarding the safety, effectiveness and cost-effectiveness of genotypic resistance testing for antiretrovirals in patients infected with HIV. Evidence was sought for the effectiveness of genotypic resistance testing in patients infected with HIV who are:

(i) adults or children experiencing first or subsequent virological failure, who are planning a change to a new regimen of antiretroviral therapy;

(ii) adults or children naïve to combination antiretroviral therapy having been diagnosed with recent primary HIV infection (less than 12 months ago);

(iii) pregnant women; and

(iv) adults or children whose plasma and other site(s) (eg cerebrospinal fluid, gastrointestinal mucosa or semen) viral load responses are discordant

to predict HIV drug sensitivity and determine the best antiretroviral regimen to achieve virologic success (measured by surrogate biological marker, viral load), slow disease progression (ie AIDS events and death, or measured by surrogate biological markers of viral load and CD4+ T cell count) and improve clinical outcome associated with HIV infection.

Clinical need

The incidence of AIDS and the prevalence of HIV in Australia at the end of 2002 were 1.3 and 67 per 100,000 population, respectively. At the end of 2002, the cumulative AIDS cases and deaths from AIDS in the Australian population were 9,083 and 6,272, respectively. In 2002 alone, 450 new HIV infections were reported. In 2002 an estimated 13,120 people were living with HIV/AIDS. The cumulative number of HIV infections diagnosed was 19,674 at the end of 2002. Approximately 52 per cent of the 13,120 patients living with HIV/AIDS at the end of 2002 were receiving antiretroviral treatment for HIV infection.

In an Australian cohort of 185 patients presenting with acute primary HIV-1 infection between January 1992 and November 2001, at least one mutation associated with resistance was detected in the reverse transcriptase gene in 21.6 per cent of the sequences analysed and at least one mutation associated with resistance was detected in the protease gene in 51.4 per cent of the sequences analysed. Mutations associated with resistance to nucleoside reverse transcription inhibitors (NRTIs) were found in 18.4 per cent and mutations associated with resistance to non-nucleoside reverse transcription inhibitors (NNRTIs) were found in 2.7 per cent of sequences analysed.

The Australian HIV Observational Database (AHOD) reported on the rates of change of combination antiretroviral treatments in Australia between 1997 and 2000. The analyses included 596 patients recruited to the AHOD who had commenced combination antiretroviral treatment after 1 January 1997 and were followed-up for a median of 2.3 years. The reported overall rate of treatment change in this group of patients was 0.45 combinations per year. Multivariate analysis indicated that a low CD4+ cell count at baseline was associated with a higher rate of treatment change. More recent data from the AHOD reporting on 2,218 patients recruited to the AHOD by March 2003 indicated that the total number of patients undergoing follow-up and receiving treatment was 1,443, with 1,345 (93.2%) patients receiving three or more drugs and 848 (63.0%) of these patients being on at least their third regimen. If we assume that the patients in this cohort are representative of the estimated 6,800 patients currently receiving highly active antiretroviral therapy (HAART) in Australia, approximately 4,280 patients are currently on at least their third HAART regimen.

Quality assurance of genotypic resistance testing of antiretrovirals in HIV

External Quality Assessment Schemes performed by the National Serology Reference Laboratory (NRL), Australia, have reported on intra- and inter-laboratory differences in performing, and interpreting the results of, genotypic resistance testing. Whilst eight Australian laboratories were involved in the scheme, there are currently only three authorised prescribers in Australia. The laboratories involved were all able to sequence the entire protease gene of HIV and varying lengths of the reverse transcriptase gene. The assay was highly reproducible with less than a one per cent variation between identical samples in all laboratories. Fifty-five per cent of the laboratories identified 100 per cent of the resistance mutations in the HIV samples. Differences existed in the ability of the laboratories to identify mixtures of wild-type and mutant HIV sequences within the samples. When laboratories used the same criteria to predict the drug resistance patterns from the sequence data, concordance between laboratories was 96.9 per cent. However when laboratories used different interpretation systems, concordance fell to 72.3 per cent.

Reference standard

In the absence of a definitive gold standard to diagnose resistance or susceptibility to therapy, treatment outcome was considered the appropriate reference standard to verify the accuracy of genotypic testing and determination of resistance or susceptibility to therapy.

Comparator

The effectiveness of genotypic resistance testing of antiretrovirals in HIV with expert interpretation of the results was compared with that of:

- standard of care (as defined in the relevant studies); and/or
- genotypic resistance testing without expert interpretation of results; and/or
- drug-susceptibility phenotyping.

Safety

The extensive literature search revealed a lack of safety data for genotypic resistance testing of antiretrovirals in HIV. However, as the test generally only requires a blood sample, the risk to subjects is expected to be minimal.

Effectiveness

Diagnostic accuracy

Evidence of the diagnostic accuracy of genotypic resistance testing of antiretrovirals in HIV was extracted from 10 primary studies of which eight were retrospective and two were prospective in design. Eight of the 10 studies were conducted in Europe, and one each was conducted in Australia and the USA. Each of the studies provided data on

genotypic resistance testing and determination of resistance or susceptibility to various therapies as a predictor of treatment outcome. The results of these studies were presented in a manner that allowed for the calculation of the test's sensitivity, specificity and their derivatives.

Different techniques for genotyping HIV were used in the studies. Seven studies reported treatment outcome as a reference standard to confirm whether baseline resistance to one or more drugs accurately predicted treatment failure. Two studies reported treatment outcome as a reference standard to confirm whether baseline susceptibility to one or more drugs accurately predicted treatment success. The remaining study reported treatment outcome as a reference standard to confirm whether the total number of drug resistance mutations could predict treatment outcome. Treatment outcome was assessed by virologic response in eight studies while two studies assessed treatment outcome with both virologic and immunologic responses. The length of follow-up ranged from six weeks to two years.

A summary of the diagnostic characteristics of genotypic testing was difficult as findings varied across studies. For example:

- All studies examined baseline resistance or sensitivity to a broad range of therapies.

- The predictive value of the presence of resistance to a particular component of HAART therapy may be difficult to ascertain when examining it within the context of a HAART regimen.

- Resistance may develop between the time of genotypic testing and measurement of treatment outcome.

- Measures of treatment outcome and length of follow-up were inconsistent across studies.

The following conclusions were drawn from calculation of the diagnostic characteristics.

- Three of six studies indicated that the presence of baseline resistance mutations to reverse transcriptase inhibitors (RTIs) used in various combination therapies had some use as a predictor of treatment failure to those combination therapies, while the remaining three suggested that the presence of RTI resistance mutations was not a useful predictor of treatment failure.

- Data from one study indicated that the numbers of thymidine analogue, NNRTI and protease inhibitor (PI) mutations present in HIV are of limited use in predicting treatment success.

- Data from one study indicated that the presence of baseline resistance to the protease inhibitors, saquinavir and ritonavir, provided moderate evidence of the likelihood of virologic failure to a HAART regimen of saquinavir and ritonavir plus two RTIs.

- Data from one study indicated that primary and secondary PI mutations are of limited use in predicting treatment failure. However, this study also provided

evidence that the presence of primary PI resistance mutations had some use in predicting treatment failure.

- From one study, data indicated that the presence of RTI or PI baseline resistance was not a useful predictor of treatment failure to HAART.

- Data from two studies indicated that the presence of baseline susceptibility to RTIs was not a useful predictor of treatment success, while data from one of those studies indicated that the presence of baseline susceptibility to PIs was an accurate predictor of treatment success to combination therapy.

Patient outcomes

Randomised controlled trials (RCTs) of the effectiveness of genotypic resistance testing of antiretrovirals in HIV (NHMRC Level II evidence) was found for patient group (i) - adults or children experiencing first or subsequent virological failure, who are planning to change to a new regimen of antiretroviral therapy. There may have been pregnant patients and patients with discordant virologic responses included in the evidence identified, thus there may be benefits in patient groups (iii) and (iv) for genotypic resistance testing, however, it was not possible to extract the data for these patient groups from the available evidence. No evidence was identified for HAART-naïve patients.

Evidence of the clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV was extracted from seven RCTs, one open-label extension of an RCT and one meta-analysis. Four RCTs compared the effectiveness of HIV genotyping with that of standard of care, one RCT compared the effectiveness of genotyping with that of standard of care and drug-susceptibility phenotyping and two RCTs compared the effectiveness of virtual phenotyping with that of drug-susceptibility phenotyping.

Six of the RCTs were conducted in Europe and one in the USA. The length of follow-up in the studies varied from 12 to 48 weeks. Patients included in all trials were HAART experienced, however the degree of previous antiretroviral therapy varied amongst the studies. Three studies specified that patients with foreseeable non-compliance or poor adherence were excluded from the studies. The exclusion of these patients may have biased the results and limited the applicability of these results to clinical practice where non-compliant patients would also undergo the test. The methods used to genotype HIV and the definitions of standard of care and expert interpretation varied across studies.

There were two primary outcomes used to determine the effectiveness of genotype resistance testing of HIV to determine an optimum HAART regimen in patients experiencing virologic failure. The primary outcome of achieving a viral load below the level of detection was used in five trials. The level of detection varied in the studies due to the techniques used to measure viral load. The primary outcome in two trials was the change in viral load from baseline to pre-determined time points following the initiation of therapy.

The major findings of this assessment were:

- All patients enrolled were antiretroviral experienced and failing current therapy, however the degree of previous antiretroviral experience varied across the studies.

- All but one of the RCTs was open-label in design.

- All of the trials based their measure of clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV on virologic outcomes. Each of the studies was powered to detect either a treatment difference between randomised arms in the proportion of patients achieving an undetectable viral load or the mean change in viral load from baseline to a pre-determined time point. Virologic outcomes are an accepted measure of the effectiveness of HAART treatment.

- Deaths and AIDS-related events were not primary outcomes in any of the RCTs and few were reported in the studies. Thus, the studies may not have been powered to detect a difference in the proportion of patients who died or experienced an AIDS-defining event. No statistically significant differences in the number of patients who died or experienced an AIDS-defining event during the course of the studies were found between the treatment arms (genotype versus standard of care, genotype versus drug-susceptibility phenotype and virtual phenotype versus drug-susceptibility phenotype).

- Whilst no differences in the number of deaths and AIDS-related events were found between treatment arms in the studies, results of a meta-analysis to determine the effectiveness of genotype resistance testing compared with standard of care in achieving an undetectable viral load revealed that patients receiving genotype-guided treatment were 1.3 times more likely to achieve plasma HIV RNA below the level of detection than patients treated by standard of care at three months (RR=1.33, 95% CI: 1.14, 1.56; NNT=10, 95% CI: 6, 20) and 1.4 times more likely at six months (RR=1.41, 95% CI: 1.12, 1.77; NNT=9, 95% CI: 6, 25).

- In addition to patients having an increased likelihood of achieving an undetectable viral load when treated by genotype-guided therapy, results of a meta-analysis to estimate the effectiveness of genotype-guided therapy in reducing viral load compared with standard of care revealed that patients receiving genotype-guided therapy had a significantly greater reduction in viral load at three months (–0.23 $\log_{10}$copies/ml, 95% CI: –0.34, –0.12) and this benefit was sustained at six months (–0.23 $\log_{10}$copies/ml, 95% CI: –0.37, –0.08) compared with patients receiving treatment based on standard of care.

- The reported changes in CD4+ cell counts were variable between the RCTs and there is uncertainty pertaining to any treatment differences between genotype-guided therapy and therapy prescribed based on standard of care or drug-susceptibility phenotyping.

- Several differences in the number and/or combinations of antiretroviral drugs prescribed in the genotype and standard of care arms were observed. No differences in the number and/or combinations of drugs prescribed in the HAART regimens were observed between genotyping and drug-susceptibility phenotyping or virtual versus drug-susceptibility phenotyping.

- One study observed no significant differences in the number of active drugs (drugs to which HIV remained susceptible) prescribed between patients receiving genotype-guided therapy and those treated by standard of care.

- No significant differences in the rates of adverse events relating to the toxicities of drugs prescribed in HAART were observed between any of the treatment arms.

- Three of the seven trials reported that patients received multiple genotypic resistance testing if the prescribed treatment was deemed sub-optimal due to patients not achieving a particular level of viral load reduction. The remaining studies did not specify if multiple tests were conducted.

- Each of the studies used different methods to perform genotypic resistance testing and interpret the results of the tests. Results from an Australian quality assessment scheme have indicated that the assay is highly reproducible with less than a one per cent variation between identical samples in all laboratories. However, there is variability in the ability of different laboratories to detect mutations and mixtures of mutations, and the level of concordance in the interpretation of the results of genotypic resistance testing is dependent on the interpretation system used.

- Data from the single arm extension of one RCT appeared to show that patients originally assigned to the genotyping arm showed a maintenance of virologic response and patients originally assigned to standard of care appeared to benefit from having genotyping being made available. Due to the lack of a comparator group, the incremental effectiveness attributable to genotypic resistance testing was difficult to determine.

- The meta-analysis concluded that the results supported the use of a genotypic test in patients experiencing virologic failure during antiretroviral treatment, and that expert interpretation of the test increased the probability of a virologic response.

The following key issues were identified:

- All patients enrolled were antiretroviral experienced and failing current therapy.

- No evidence was found that assessed the effectiveness of genotypic resistance testing in treatment-naïve patients, pregnant women or patients with discordant virologic responses.

- The open-label design of six of the seven trials may have led to bias.

- The follow-up period of the RCTs identified varied from 12 to 48 weeks. There are no long-term data on the clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV.

- All of the trials based their measure of clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV on virologic outcomes. A combination of virologic and immunologic responses to treatment is more effective than virologic responses alone at predicting outcomes of clinical events of patients.

- There was variability in the number of drugs and range of therapies used in each of the studies.

- Whilst no differences were found between the treatment arms for the rates of death and AIDS-defining events, it must be noted that these were not primary outcomes in any of the RCTs. Thus, the studies may not have been powered to detect a difference in the proportion of patients who died or experienced an AIDS-defining event. In addition, the studies were not long enough to allow detection of differences over extended periods of time.

- The differences observed in the number and/or combinations of drugs prescribed between the genotype and standard of care arms in the trials make the incremental benefit of genotype-guided therapy difficult to distinguish from the benefit of the antiretrovirals themselves.

Patient management

The observed differences in the number and/or combinations of antiretrovirals included in the HAART regimens between the genotyping and standard of care arms of the trials appraised in this Assessment Report may indicate changes that may occur in patient management following the introduction of this test into clinical practice. Some of the differences observed included:

- Patients in the genotyping arm were more than four times more likely to be prescribed five or more antiretrovirals in their HAART regimens and were half as likely to be prescribed three or fewer drugs than patients treated by standard of care.

- Patients in the genotyping arm were two times more likely to receive four or more new drugs (antiretrovirals to which they were naïve) and were half as likely to be prescribed three new drugs in their HAART regimen than patients treated by standard of care.

There are two possible explanations for the differences observed between the two groups:

- Genotypic resistance testing results provided information to allow for the identification of a number of drugs to which the patient's virus remained susceptible. As a result, a greater number and selection of drugs were prescribed to patients randomised to genotypic resistance testing.

- The open-label design of the appraised trials may have led to bias:

  - Patient preference in taking an increased number of drugs (and increased likelihood of risking drug-related toxicities) with the knowledge that the selection of suggested antiretrovirals was based on genotypic resistance testing.

  - Prescribers suggesting HAART regimens with an increased number of drugs for patients randomised to genotypic resistance testing.

Given the lack of a double-blind RCT assessing the effectiveness of genotypic resistance testing compared to standard of care, it is difficult to assess the incremental effect of these potential sources of difference on the observed results. Regardless of the reasons for the differences, it is likely that the introduction of genotypic resistance testing into

clinical practice may result in an increased number of antiretrovirals being prescribed in HAART regimens.

Cost-effectiveness

The cost-effectiveness of genotype antiretroviral resistance testing has been calculated using a comparison between standard care plus genotypic antiretroviral testing and standard care for patients failing their first HAART regimen. Standard care was defined as routine specialist clinical care for patients being prescribed antiretroviral treatment. The effectiveness of the test in reducing the probability of virologic failure was estimated at a relative risk of 0.85 and was calculated from the meta-analysis of the three studies considered in the 'Effectiveness' section of the report. The cost of $666.58 for the test was an average of the estimated costs forwarded by the laboratories in response to the Applicant's request.

Based on this cost and effectiveness of the genotype test, the base case cost-effectiveness was estimated at $5,623 per quality adjusted life year (QALY) gained or $38,276 per life year gained. The effectiveness of HAART in reducing the burden of mortality associated with HIV means that the effect of the genotypic test on this outcome is minimal. Nevertheless, the ability of the test to delay a patient's progression to HAART regimens with a reduction in the likelihood of suffering an HIV illness has a considerable impact on the quality of life of a patient with HIV.

It needs to be recognised that the true cost of genotypic antiretroviral resistance testing may be considerably different from that reported in the base case. It will depend particularly on the benefits patients accrue from being in certain health states and also on the actual cost of the test, the true effectiveness of the test in reducing the probability of virologic failure and the rate at which patients fail both primary and secondary HAART therapy. Sensitivity analysis on a combination of these variables suggests a wide range of incremental cost-effectiveness, from a situation where genotype antiretroviral resistance testing is both cheaper and more effective to an extra cost of $132,342 per additional QALY gained.

The economic model confirms that the cost of genotype testing for those failing antiretroviral therapy will be only partly offset by savings in the cost of treatment for those who respond to treatment. There will be gains in quality of life from a reduction in treatment failure and consequent reduction in HIV-related illness.

Expert opinion was ..."Assuming that that the rate of secondary failure is sufficiently low, the effectiveness of the test in practice is within the range estimated in the trials, and the cost of the test is not substantially greater than the estimated average of current laboratory costs, the predicted improvement in survival and quality of life for patients could be regarded to be sufficient to justify the additional cost." However, there is insufficient evidence to support these assumptions.

Recommendation

MSAC found that genotypic resistance testing of antiretrovirals in HIV appeared to be safe and leads to changes in clinical management but there is insufficient evidence on effectiveness and cost-effectiveness to support Medicare funding.

- The Minister for Health and Ageing accepted this recommendation on 2 March 2005.

Introduction

The Medical Services Advisory Committee (MSAC) has reviewed the use of genotypic resistance testing of antiretrovirals in HIV, which is a diagnostic test for patients infected with HIV. MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Scheme in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

MSAC's terms of reference and membership are at Appendix A. MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

This report summarises the assessment of current evidence for the safety, effectiveness and cost-effectiveness of genotypic resistance testing of antiretrovirals in HIV in patients infected with HIV who are:

(i) adults or children experiencing first or subsequent virological failure, who are planning a change to a new regimen of antiretroviral therapy.

No specific evidence was identified to assess the safety, effectiveness and cost-effectiveness of genotypic resistance testing of antiretrovirals in HIV in patients infected with HIV who are:

(ii) adults or children naïve to combination antiretroviral therapy, having been diagnosed with recent primary HIV infection (less than 12 months ago);

(iii) pregnant women; and

(iv) adults or children whose plasma and other site(s) (eg cerebrospinal fluid, gastrointestinal mucosa or semen) viral load responses are discordant.

to predict HIV drug sensitivity and determine the best antiretroviral regimen to achieve virologic success (measured by surrogate biological marker, viral load), slow disease progression (ie AIDS events and death, or measured by surrogate biological markers of viral load and CD4+ T cell count) and improve clinical outcome associated with HIV infection.

Background

Genotypic resistance testing of antiretrovirals in HIV

Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a retrovirus belonging to the lentivirus family (Hoffman & Kamps 2003). The genetic material of retroviruses is single- or double-stranded ribonucleic acid (RNA). The RNA of retroviruses is reverse transcribed into deoxyribonucleic acid (DNA) which is then integrated into the host genome. A HIV virion contains two copies of the viral genome (single-stranded RNA) and three enzymes essential for replication – reverse transcriptase, protease and integrase – surrounded by a lipoprotein membrane (Hoffman & Kamps 2003). There are two strains of HIV, HIV-1 and HIV-2. Both HIV-1 and HIV-2 are further sub-divided into subtypes. Subtype B of HIV-1 is the most prevalent in Australia (Leitner 1996). HIV infects, replicates in and ultimately destroys, cells of the immune system, particularly CD4+ T cells (Hoffman & Kamps 2003).

Natural progression of disease following HIV infection

The natural progression of disease following HIV infection occurs in four stages. During the first stage of primary infection, patients undergo seroconversion which refers to the development of anti-HIV antibodies in the serum. This process is sometimes accompanied by flu-like symptoms and skin rashes, while some patients do not have symptoms. Diagnosis of HIV infection is based on a positive result for the presence of serum antibodies (AFAO 2003). Patients are generally asymptomatic during the second stage of HIV infection, however during symptomatic illness or stage three of infection, HIV-infected patients may experience diarrhoea, minor skin and oral conditions, lack of energy, night sweats and/or persistently swollen glands (AFAO 2003). The fourth stage of progression is to acquired immunodeficiency syndrome (AIDS). Patients with AIDS have a debilitated immune system and are susceptible to opportunistic infections and other illnesses (AFAO 2003).

Surrogate biological markers of disease progression

In a cohort study including 1,604 HIV-1-infected men (1,066 of whom had received antiretroviral treatment) who were followed for 10 years, Mellors et al (1997) examined various potential clinical, serologic, cellular and virologic markers that could be used to predict the progression of HIV infection to AIDS and death. They found that plasma viral load alone was the best predictor of disease progression as 80.0 per cent of patients with a baseline viral load of greater than 30,000 copies/ml and 5.4 per cent of patients with a baseline viral load of less than 500 copies/ml, had progressed to AIDS within six years. In addition, Mellors et al (1997) found that 69.5 per cent of patients with a baseline viral load of greater than 30,000 copies/ml and 0.9 per cent of patients with a baseline viral load of less than 500 copies/ml had died of AIDS within six years. Similarly, in a cohort study of 106 HIV-1-infected infants, those with rapid progression of disease had a higher median viral load than those without rapid disease progression (Shearer et al 1997). Whilst viral load alone strongly predicts disease progression, clinical outcome for

HIV-infected patients is more accurately predicted using a combination of viral load and CD4+ cell count (Mellors et al 1997).

Current treatment for HIV infection

There are no vaccines or cures for HIV infection. Current treatments include the use of antiretroviral drugs that reduce the ability of HIV to replicate and infect new cells, and which increase immune system functions (Gallant, 2000). These drugs are classified as: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and fusion inhibitors (FIs). Features of these drugs are described in Table 1.

Table 1 Description of the class, mode of action, drug name and single tablet formulations of antiretroviral drugs

Class	Mode of action	Drug name	Single tablet combinations
NRTI	Competes with nucleotides for reverse transcriptase binding. Nucleoside reverse transcription inhibitors become incorporated into the elongating viral DNA strand and cause premature termination which disrupts the replication cycle of the virus	1. Zidovudine	5 (Combivir) and 5 & 6 (Trizivir)
		2. Didanosine	
		3. Zalcitabine	
		4. Stavudine	
		5. Lamivudine	1 (Combivir) and 1 & 6 (Trizivir)
		6. Abacavir	1 & 5 (Trizivir)
		7. Tenofovir	
NNRTI	Directly binds and inhibits reverse transcriptase in a non-competitive and direct manner to cause premature termination of the proviral DNA strand and thereby disrupts the replication cycle of the virus	8. Nevirapine	
		9. Delavirdine	
		10. Efavirenz	
PI	Inhibits the cleavage of the polyprotein precursors thereby inhibiting the production of mature virions	11. Saquinavir	
		12. Ritonavir	16 (Kaletra)
		13. Indinavir	
		14. Nelfinavir	
		15. Amprenavir	
		16. Lopinavir	12 (Kaletra)
		17. Atazanavir[a]	
		18. Fosamprenavir[a]	
FI (new class)	Inhibits fusion of the HIV virus with the target cell and entry of the virus into the target cell	19. Enfuvirtide[a]	

[a] Not PBS listed

Abbreviations: NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; FI, fusion inhibitor

Highly active antiretroviral therapy (HAART) consists of a combination of at least three antiretroviral drugs (Gallant 2000). The HAART regimen can be PI-, NRTI- or NNRTI-based. PI-based regimens include combinations of one PI with two NRTIs, NNRTI-based regimens include one NNRTI with two NRTIs, and NRTI-based therapies consist of three NRTIs (Gallant 2000).

HIV drug resistance mutations

The emergence of drug resistant forms of HIV is a growing problem. Drug resistant HIV variants develop due to the high error rate of reverse transcriptase – the viral

enzyme responsible for reproducing the viral genome – coupled with high levels of HIV-1 replication (Sayer et al 2003). Current triple therapy regimens are able to delay the development of drug resistance because they suppress viral replication to undetectable levels. However, problems with adherence to treatment, drug toxicities, differences in drug absorption or metabolism (ie pharmacokinetics) and other host factors can compromise the activity of a HAART regimen. Over time, these factors may allow the accumulation of mutations that confer drug resistance, leading eventually to treatment failure (Sayer et al 2003). Although individual drugs select for specific resistance mutations, the rate at which these mutations emerge is quite variable and often difficult to predict. The specific mutations associated with drug resistant HIV have been determined for a variety of antiretroviral drugs. As a result of which, it is proposed that genotypic resistance testing of HIV, in conjunction with expert interpretation of resistance patterns, may aid in determining future HAART regimens for HAART-failed patients and patients newly infected with drug resistant HIV.

Further complications include the change in drug resistance patterns during the time between genotyping and prescription of a new HAART regimen. Birch et al (2003) examined the evolution of drug resistance mutations in the reverse transcriptase and protease genes of HIV during the time taken to perform drug resistance testing in a sub-population of antiretroviral-experienced patients failing their current regimen who were enrolled in the CREST trial to compare the effectiveness of genotyping with virtual phenotyping. The sub-study examined 30 patients: two patients lost, five patients gained and two patients both lost and gained reverse transcriptase mutations. The protease sequence of one patient changed during failing HAART regimens (Birch et al 2003).

Effects of receiving active antiretrovirals (ie those to which the virus remains susceptible)

Baxter et al (2000) reported the results of a randomised controlled trial (RCT) to assess the effectiveness of genotype resistance testing compared with standard of care. Results of this study showed differences in the numbers and combinations of antiretroviral drugs prescribed in the two treatment arms. *Post-hoc* analyses performed by Baxter et al (2000) indicated that the total number of drugs used, the classes of drugs used and the specific drug regimens did not appear to contribute to the differences observed in patient outcomes. However, the treatment benefits observed in patients receiving genotype-guided therapy may have been due in part to the increased number of drugs to which the patient's virus remained susceptible (active drugs) that these patients received compared with patients treated by standard of care (Baxter et al 2000). Patients in the genotype arm were more likely to receive three active drugs in their HAART regimen than patients treated by standard of care, irrespective of whether the patients were prescribed three, four or five or more drugs in their regimen (Baxter et al 2000).

In addition, there appeared to be an incremental dosage effect for each additional active drug prescribed (Baxter et al 2000, expert opinion). For example, patients receiving one active antiretroviral would achieve better virologic outcomes than patients receiving none, and patients receiving two active antiretrovirals would achieve better virologic outcomes than those receiving one, and so on.

Treatment guidelines

Several Australian guidelines provide information about when to begin therapy and recommended therapies for each stage of disease (see Appendix C, Tables C1-C5). Table

2 summarises the guidelines that provide treatment recommendations for the different patient groups.

Table 2 List of guidelines that provide treatment recommendations for different patient groups in Australia

Patient group	Guidelines
Newly diagnosed patients	Draft 2001 Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, October 2001 (summarised in Table C1) Antiretroviral Therapy for HIV Infection: Principles of use – Standard of Care Guidelines October 1997 HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases (summarised in Table C2) Antiretroviral Therapy for HIV Infection in Women and Children Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, January 1999 (summarised in Table C3) Model of Care for HIV Infection in Adults HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, 1998 (summarised in Table C4)
Patients failing HAART therapy	Draft 2001 Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, October 2001 (summarised in Table C1) Antiretroviral Therapy for HIV Infection: Principles of use – Standard of Care Guidelines October 1997 HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases (summarised in Table C2) Antiretroviral Therapy for HIV Infection in Women and Children Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, January 1999 (summarised in Table C3)
Children	Antiretroviral Therapy for HIV Infection: Principles of use – Standard of Care Guidelines October 1997 HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases (summarised in Table C2) Antiretroviral Therapy for HIV Infection in Women and Children Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, January 1999 (summarised in Table C3)
Pregnant women	Draft 2001 Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, October 2001 (summarised in Table C1) Antiretroviral Therapy for HIV Infection: Principles of use – Standard of Care Guidelines October 1997 HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases (summarised in Table C2) Antiretroviral Therapy for HIV Infection in Women and Children Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee if the Australian National Council on AIDS and Related Diseases, January 1999 (summarised in Table C3)
Post exposure prophylaxis (PEP)	Queensland Management Guidelines for the Detection and Treatment of Sexually Transmissible Disease and Genital Infections. Version II December 2003 (summarised in Table C5)

Surrogate biological markers for monitoring success of highly active antiretroviral therapy (HAART)

Grabar et al (2000) have shown the clinical outcome of patients on HAART is dependent on both immunological (CD4+ cell count) and virological (viral load) responses (Grabar et al 2000). The clinical outcome of patients 24 months after the initiation of HAART was assessed according to immunologic and virologic responses at six months and results of this cohort study of 2,236 HIV-1-infected patients showed that there were no significant differences in the relative risk of clinical progression for patients with both immunological (increased CD4+ cell count) and virological (decreased viral load) responses (described as complete responders) and patients having an immunological

response only (relative risk [RR]=1.55; 95% confidence interval [CI]: 0.96, 2.50). However, patients having a virological response only or having neither an immunological nor virological response (described as non-responders) were more likely to experience clinical progression of disease than complete responders (RR=1.98; 95% CI: 1.26, 3.10) and (RR=3.38; 95% CI: 2.28, 5.02), respectively (Grabar et al 2000). The rate of clinical progression observed in this cohort was 4.8 per cent (95% CI: 3.5%, 6.2%) for complete responders, 7.2 per cent (95% CI: 4.6%, 9.8%) for immunologic responders, 9.5 per cent (95% CI: 6.2%, 12.7%) for virologic responders and 15.9 per cent (95% CI: 11.9%, 19.8%) for non-responders (Grabar et al 2000). Discordance between immunologic and virologic responses is observed frequently in clinical practice and although many patients do not achieve complete early virologic responses, clinical outcome in these patients may be improved if their CD4+ cell count increases (Grabar et al 2000). Grabar et al (2000) therefore proposed that both immunologic and virologic markers be used in assessing clinical treatment failure.

Similarly, results from an RCT in which patients were randomised to antiretroviral treatment with zidovudine and didanosine or zidovudine, didanosine and nevirapine showed that patients with the lowest CD4+ T cell count and the highest viral load at baseline were at the greatest risk of disease progression (Hughes et al 1997). Either viral load or CD4+ T cell count at baseline were predictive of disease progression or death, however the predictive value of these variables was improved when both were considered (Hughes et al 1997). A study by O'Brien & Hartigan (1997) to assess the association between changes in plasma viral load and CD4+ T cell count following antiretroviral treatment and progression to AIDS found that changes in viral load and CD4+ T cell count over time were more strongly associated with progression to AIDS than baseline values in a subset of patients. Management of antiretroviral therapy could be guided by viral load as well as by CD4+ T cell count, as these variables were independently related to clinical outcome (O'Brien & Hartigan 1997).

Guidelines for the use of genotypic resistance testing of antiretrovirals in HIV

Genotypic resistance testing of antiretrovirals in HIV is standard of care in the USA and Europe but not in Australia. Table 3 summarises the recommendations made in the Draft Australian Antiretroviral Guidelines (2001), the EuroGuidelines (2001) and by an International AIDS Society-USA Panel (Hirsch et al 2003).

Table 3 Summary of guidelines for the use of genotypic resistance testing of antiretrovirals in HIV

Guidelines	Recommendations
Draft 2001 Australian Antiretroviral Guidelines	Resistance testing recommended for: Patients with virologic failure on antiretroviral therapy (viral load must be greater than 1,000–2,000 copies/ml) Incomplete supression of viral load after initiation of antiretroviral therapy in antiretroviral naïve patients Resistance testing NOT recommended for: After discontinuation of antiretroviral therapy (drug resistance mutants become minority species) Acute HIV seroconversion (transmission of drug resistant HIV is rare) Chronic HIV infection, prior to initiation of antiretroviral therapy Indications for resistance testing in pregnancy are the same as above
EuroGuidelines 2001	Resistance testing recommended for: Treatment naïve patients where transmission of drug resistance mutations is high or transmission from treated individual is suspected and treatment is to be initiated Chronic infections where transmission of drug resistance mutations is high or transmission from treated individual is suspected Post exposure prophylaxis (PEP): treatment should not be delayed, but if a sample from index case is available, test and modify treatment of recipient Treated patients in all cases where change in therapy is considered. Testing is only useful/valid within the context of complete history and assessment of other reasons for treatment failure Pregnancy: if mother has detectable viral load Paediatrics: infected children born to mothers with detectable viral load while on treatment and in children with virologic treatment failure where testing is only useful/valid within the context of complete history and assessment of other reasons for treatment failure
International AIDS Society-USA Panel 2003	Resistance testing recommended for: Acute or recent HIV infection . Acute infection . HIV infection within previous 12 months (if known) . Sub-optimal HIV-1 RNA response to therapy Before initiation of antiretroviral therapy in established HIV infection . Patients infected within previous two years and possibly longer . First regimen failure . Multiple regimen failures . Pregnancy, if mother has detectable viral load General recommendations: Viral load should be at least 500–1,000 copies/ml No resistance testing technique is recommended over another In patients failing antiretroviral therapy, testing should be performed while they are still on therapy Resistance testing should be performed by laboratories that have appropriate operator training, certification and periodic proficiency assurance Genotypic and phenotypic tests should be interpreted by individuals who are knowledgeable in antiretroviral therapy and drug resistance patterns

The procedure

The Medical Services Advisory Committee (MSAC) has reviewed genotypic resistance testing of HIV with expert interpretation of resistance patterns for the use of antiretrovirals in the treatment of patients with HIV.

Various technologies are available for genotypic resistance testing. Genotypic assays are based on the analysis of mutations associated with HIV drug resistance. They can involve direct sequencing of the HIV genome or nucleic acid hybridisation with specific wild-type or mutant oligonucleotides.

Genotypic assays are most widely performed using HIV RNA viruses, however the technique has also been applied to viruses that have integrated into the host genome (proviral DNA). Where HIV RNA virus is used, the viral RNA is commonly reverse transcribed to complementary DNA (cDNA) after which the sequence to be analysed is amplified by PCR to obtain a sufficient quantity of target DNA. The reverse transcription step is not required when using proviral DNA. Bi et al (2003) analysed the genotype of plasma HIV virus and proviral DNA derived from peripheral mononuclear cells and found that the genetic turnover of the proviral sequences was slower than that of plasma viruses. They concluded that plasma virus should be used preferentially for the early detection of drug resistance during antiretroviral treatment.

Sequencing

DNA sequencing assays are the most commonly used genotypic assays in Australia. The sequencing reaction is carried out by the method of Sanger et al (1997) using four parallel reactions, each containing a primer, the DNA segment to be sequenced, DNA polymerase enzyme, a mixture of the four natural deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP) and one of the four dideoxynucleotide terminators (ddATP, ddCTP, ddGTP and ddTTP). The four dideoxynucleotide terminators lack the 3'-hydroxyl group required for DNA elongation and therefore their incorporation causes termination of the elongation reaction.

The sequencing reaction involves the synthesis of new DNA strands that are initiated at the primer and undergo elongation until one of the four dideoxynucleotide terminators is incorporated into the new DNA strand. The incorporation of the dideoxynucleotide terminators results in DNA fragments of varying lengths whose ends are determined by the sequence of the added terminator. The strands are detected by the inclusion in reaction mixtures of a radio-labelled deoxynucleotide (Sanger et al 1977), a fluorescently-labelled primer, or differentially-labelled fluorescent dideoxynucleotide terminators. The newly-synthesised DNA strands are separated according to strand length by polyacrylamide gel electrophoresis and the sequence is read from the gel. There are several automated commercial systems available in addition to various in-house versions of DNA sequencing.

Nucleic acid hybridisation techniques

Various nucleic acid hybridisation techniques exist for sequencing the HIV reverse transcriptase and protease genes. Nucleic acid hybridisation techniques exploit the complementary base-pairing characteristic of nucleic acids, where adenine (A) is always paired with thymine (T) in DNA or uracil (U) in RNA and cytosine (C) is always paired with guanine (G). Each of the techniques is applied to the search for mutations in a preselected region of sequence.

A limitation of all nucleic acid hybridisation techniques is that all drug resistant mutations of interest must be known and represented with specific probes and/or sequences in order for the mutations to be detected. The techniques would fail to detect a novel mutation.

Southern blotting

The Southern blot hybridisation technique requires electrophoresis of HIV DNA sequences on an agarose gel and their subsequent transfer and binding to a nitrocellulose membrane. Hybridisation of the HIV DNA with oligonucleotides specific for wild-type or corresponding mutant sequences is used to detect the mutations (Richman et al 1991).

GeneChip hybridisation

GeneChip hybridisation uses an array of more than 16,000 unique oligonucleotide probes complementary to the HIV reverse transcriptase (RT) and protease genes, applied to a silicon-glass chip (Wilson et al 2000). The oligonucleotide probes, representing both wild-type and known mutation sequences, are placed on the chip in a precise location or in a grid pattern to which fluorescently-labelled, *in vitro* transcribed HIV RNA products are hybridised. Once hybridisation is complete, the GeneChip is exposed to a laser scanner. The oligonucleotides that best match the HIV sequences yield the highest fluorescence intensity. Specialised software allows determination of the HIV sequence and genotype (Wilson et al 2000).

Line probe assay

The line probe assay (LiPA) uses specific wild-type and mutant oligonucleotide probes of the HIV RT and protease genes immobilised in parallel lines on nitrocellulose membranes (Stuyver et al 1997, Descamps et al 1998). HIV DNA is labelled during PCR. The labelled PCR products are hybridised to the nitrocellulose strips and hybridisation is detected by a colorimetric reaction. Based on the position of the wild-type and mutant oligonucleotide probes on the membranes, the genotype of HIV can be determined.

Point mutation assay

The point mutation assay requires HIV DNA to be labelled during PCR. The labelled PCR products are captured in wells of a microtitre plate and the double-stranded PCR products denatured to generate single-stranded DNA (Clarke et al 2000). The captured single-stranded DNAs are hybridised with specific oligonucleotides that are complementary to the HIV sequence and have a single base missing at the end where there is a potential point mutation (ie a single base change). The PCR products are hybridised to an oligonucleotide in four separate reactions incorporating a radioactively-labelled dNTP (dATP, dCTP, dGTP or dTTP). If the base at the point of interest is complementary to the added radioactively-labelled dNTP, the hybridised oligonucleotide will incorporate the dNTP (Clarke et al 2000). The extent of incorporation of radioactively-labelled dNTPs is measured using a scintillation counter and the incorporated base indicates the complementary base in the HIV sequence.

PCR ligase detection reaction

The PCR ligase detection reaction requires HIV DNA. For each mutation of interest, one oligonucleotide is designed to represent the wild-type sequence and one or two oligonucleotides are designed for the mutation(s). Separate reactions are conducted to detect wild-type and mutant genotypes. The oligonucleotide probes have a latex bead at the start, approximately 20 bases from the base of interest.

The base of interest is complementary to either the wild-type or the mutant base at the end of the oligonucleotide probe (Frenkel et al 1995). Each oligonucleotide also has a labelled 'detector' oligonucleotide designed to hybridise to the HIV sequence adjacent to the oligonucleotide probe. The probe and detector oligonucleotides are hybridised to the PCR product and if the last two bases of the probe and the first two bases of the

detector are complementary to the PCR product, the probe and detector oligonucleotides can be joined (Frenkel et al 1995). The latex beads on the oligonucleotide probes are used to trap the oligonucleotides. Those which have been joined to detector probes are detected colorimetrically to allow for genotype determination.

RNase A mismatch

RNase A is an enzyme that recognises and cleaves single-base mismatches in RNA:RNA and RNA:DNA hybrids (Lopez-Galindez et al 1991). This feature has been exploited to detect point mutations and to analyse the genetic variability of RNA viruses. The RNase A mismatch technique can be applied to either HIV RNA or HIV DNA. Radioactively-labelled RNA probes based on the wild-type sequence are hybridised to HIV RNA or HIV DNA and RNase A added. RNase A cleaves the hybrid if there is a single mismatch between the probe and the target sequence. Hybrids displaying perfect complementarity between probe and target sequences are resistant to RNase A cleavage. After reaction with RNase A, products are separated by electrophoresis on polyacrylamide gels and analysed (Lopez-Galindez et al 1991). A disadvantage of this technique is that the nature of the identified point mutation cannot be determined.

Detection of mixtures of wild-type and mutant sequences

The genotypic resistance testing methods described above have varying abilities to detect mixtures of wild-type and mutant sequences. Sequencing, the most commonly used method in Australia, can detect mixtures of wild-type and mutant sequences when the sequences constitute at least 25 per cent of the total viral population (Schuurman et al 2002). The line probe assay has been reported to detect mixtures where the sequences constitute as little as 0.5 per cent (Clarke et al 2000) and the PCR ligase detection reaction between two and nine per cent (Frenkel et al 1995) of the total viral population. No data were found for the ability of the other genotyping techniques to detect sequence mixtures.

Quality assurance of genotype testing

The National Serology Reference Laboratory (NRL), Australia has conducted several external quality assessment schemes (EQASs) to assess the ability of laboratories to detect antiretroviral drug mutations in the HIV RT and protease genes, to monitor the concordance at the level of sequencing and drug susceptibility and to evaluate intra-laboratory variation over time (Land & Gizzarelli 2003). The seventh EQAS Panel also assisted in the evaluation of the CREST Algorithm version 6.

Eleven laboratories – eight in Australia, one in New Zealand, one in Canada and one in Korea – were involved in Panel 7: GART 2003. Each laboratory was given a panel of four plasma samples collected from antiretroviral-experienced patients. All laboratories were required to genotype HIV by their routine procedures (one laboratory used the TruGene Kit, two laboratories used the ViroSeq kit and the remainder used in-house sequencing methods) and provide the:

- nucleic acid sequence
- antiretroviral susceptibility interpreted by:
  - the manual format of CREST v6, September 2003 (www.nrl.gov.au)

- the online format of CREST v6 (www.nrl.gov.au)
- the Stanford database (SD) (http://hiv-4.stanford.edu/cgi-bin/hivtestweb.pl)
- the laboratories standard protocol if not CREST v6 or the Stanford database.

Results of Panel 7: GART 2003

All of the laboratories were able to sequence the entire protease gene, but varying lengths of the RT gene were sequenced. A total of 160 sites associated with drug resistance were analysed (40 sites per sample) of which 42 contained drug resistance mutations. Six of the 11 laboratories identified 100 per cent of the drug resistance mutations (Land & Gizzarelli 2003).

Laboratories differed in their ability to identify mixtures of wild-type and mutant sequences. Five of the 11 laboratories reported wild-type sequences at some sites that contained drug resistance mutations, particularly codons 74 and 181 of the RT gene. The range of detection of mixtures of wild-type and mutant sequences ranged from 24 to 93 per cent (Land & Gizzarelli 2003).

Between-run reproducibility was also tested on four plasma samples, one of which had previously been tested in EQAS Panel 5. Between-run reproducibility was less than one per cent nucleotide variability and less than two per cent amino acid variability. No alteration in the interpretation of drug susceptibilities resulted from any of these variations (Land & Gizzarelli 2003).

Table 4 summarises the concordance between the laboratories in deducing antiretroviral susceptibilities when using different interpretation systems. Use of the on-line CREST algorithm or Stanford Database to interpret drug susceptibilities from genotype results provided 96.9 per cent concordance between the laboratories. When laboratories used in-house interpretation systems, concordance fell to 72.3 per cent (Land & Gizzarelli 2003).

Table 4 Concordance between antiretroviral susceptibilities deduced by laboratories participating in Panel 7: GART 2003

Antiretroviral drug class	Interpretation system			
	CREST v6[a]	CREST on-line[b] v6	Standford[c]	Various[d]
	Concordance n/N (%)			
Protease inhibitors	28/28 (100.0)	28/28 (100.0)	26/28 (92.9)	20/28 (71.4)
RT inhibitors	30/37 (81.1)	35/37 (94.6)	37/37 (100.0)	27/37 (73.0)
Total	58/65 (89.2)	63/65 (96.9)	63/65 (96.9)	47/65 (72.3)

(Source: Land & Gizzarelli 2003)
[a] CREST Algorithm version 6 manual
[b] CREST Algorithm version 6 on-line
[c] Stanford Database
[d] A variety of in-house interpretation systems used

Intended purpose

Genotypic resistance testing is intended to be used in patients infected with HIV who are:

(i) adults or children experiencing first or subsequent virological failure, who are planning a change to a new regimen of antiretroviral therapy;

(ii) adults or children naïve to combination antiretroviral therapy having been diagnosed with recent primary HIV infection (less than 12 months ago);

(iii) pregnant women; and

(iv) adults or children whose plasma and other site(s) (eg cerebrospinal fluid, gastrointestinal mucosa or semen) viral load responses are discordant

to predict HIV drug sensitivity and determine the best antiretroviral regimen to achieve virologic success (measured by surrogate biological marker, viral load), slow disease progression (ie AIDS events and death, or measured by surrogate biological markers of viral load and CD4+ T cell count) and improve clinical outcome associated with HIV infection.

Clinical need/burden of disease

The morbidity and mortality as a result of HIV/AIDS has been well documented in Australia for both the indigenous and non-indigenous population. The transmission of HIV infection in the Australian population has continued to be mainly through sexual contact between men (National Centre in HIV Epidemiology & Clinical Research 2003).

The incidence of AIDS and HIV prevalence in Australia at the end of 2002 were 1.3 and 67 per 100,000 populations, respectively (National Centre in HIV Epidemiology and Clinical Research 2003). At the end of 2002, the cumulative AIDS cases and deaths from AIDS in the Australian population were 9,083 and 6,272, respectively. In 2002 alone, 450 new HIV infections were reported. In 2002 an estimated 13,120 people were living with HIV/AIDS and the cumulative number of HIV infections diagnosed was 19,674 at the end of 2002 (National Centre in HIV Epidemiology & Clinical Research 2003).

Figure 1 shows the number of diagnosed cases of HIV infection and AIDS in Australia between 1981 and 2002. The annual number of AIDS diagnoses peaked in 1994 at 953, and declined to between 200 and 250 cases from 1999 to 2002. The decrease in the number of AIDS diagnoses has been due to the decline in HIV incidence in the mid 1980s and the use of effective combination antiretroviral therapy for the treatment of HIV infection since 1996 (National Centre in HIV Epidemiology & Clinical Research 2003).

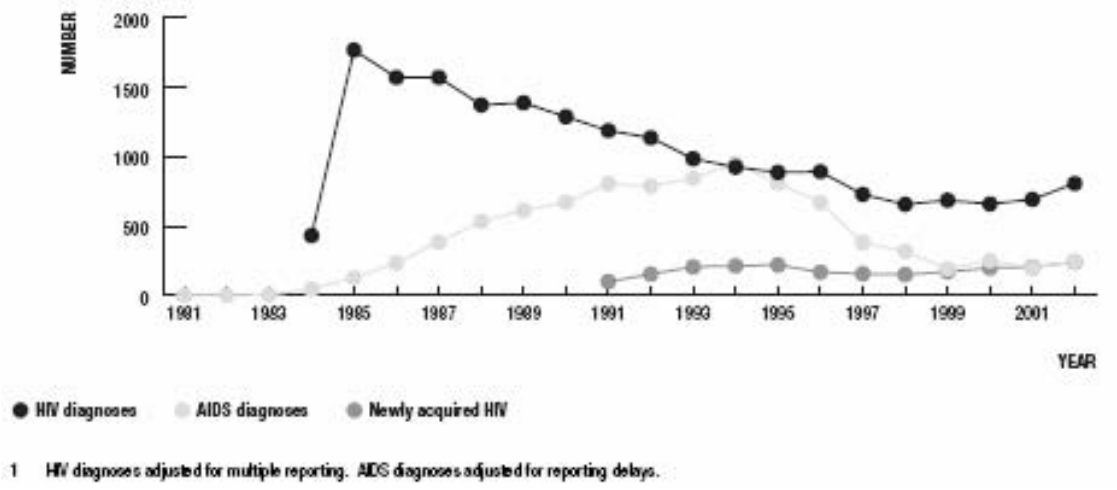

Figure 1 Number of diagnoses of HIV-1 infection and AIDS in Australia

The average annual incidence of diagnoses of HIV infection and AIDS by state/territory in Australia between 1998 and 2002 is shown in Figure 2. Based on the population size, the order of decreasing rates of HIV diagnosis was New South Wales, Victoria, Northern Territory equal to Queensland, the Australian Capital Territory, Western Australia, South Australia and Tasmania (National Centre in HIV Epidemiology & Clinical Research 2003).

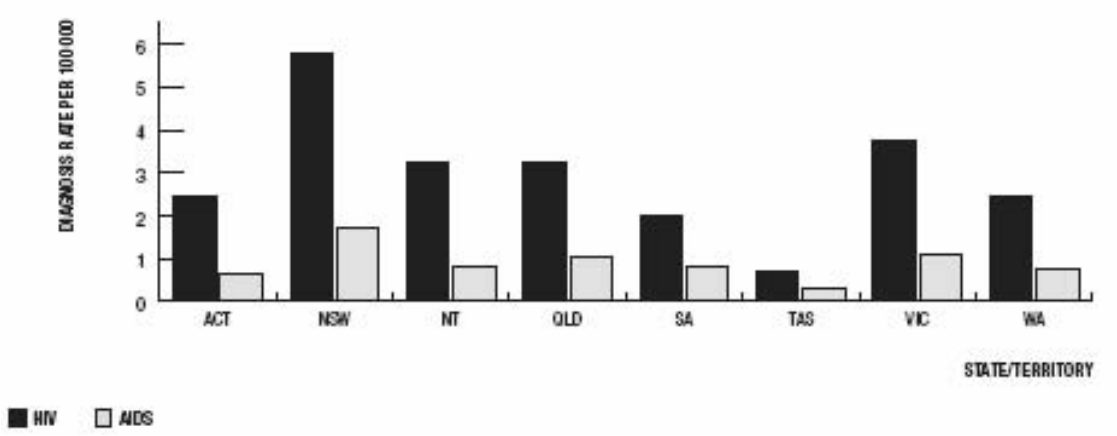

Figure 2 Average annual incidence of diagnoses of HIV and AIDS by state/territory, 1998-2002

The diagnosis of newly-acquired HIV infection rates varied across the states and territories (Figure 3). The rate of diagnosis of newly acquired HIV infection between 1998 and 2002 increased in New South Wales, Queensland and Victoria and remained unchanged in the other states and territories combined (National Centre in HIV Epidemiology & Clinical Research 2003).

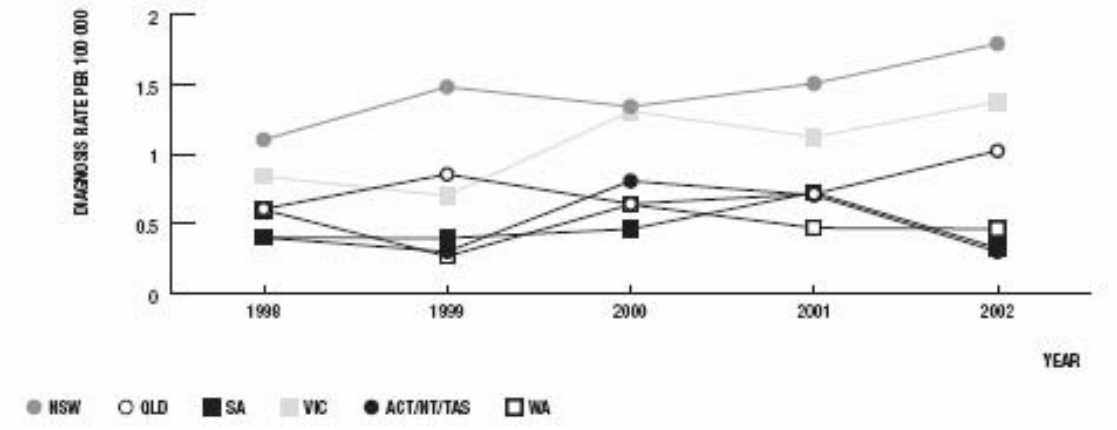

Figure 3 Newly-acquired HIV by state/territory, 1998-2002

The prevalence of drug resistance mutations in patients with acute primary HIV-1 infection has been examined (Ammaranond et al 2003a). In an Australian cohort of 185 patients presenting between January 1992 and November 2001, 21.6 per cent of sequences had at least one mutation associated with resistance in the RT gene and 51.4 per cent of sequences contained at least one mutation associated with resistance in the protease gene. Mutations associated with resistance to NRTIs were found in 18.4 per cent and mutations associated with resistance to NNRTIs were found in 2.7 per cent of sequences. Ammaranond et al (2003a) also found that there was a decrease in the frequency of NRTI mutations from 29.3 to 9.0 per cent between pre- and post-introduction of protease inhibitors (PIs) (designated as January 1996). Three primary protease mutations were also detected in the last 18 months of the study.

In a review of the literature, Ammaranond et al (2003b) reported that most studies estimated that at least 10 per cent of patients with primary HIV-1 infection carry virus with resistance to at least one of the antiretroviral drugs while they are naïve to treatment, which suggests that these patients had been infected with resistant virus. Mutations in the RT gene are transmitted more commonly than mutations in the protease gene, however this may vary according to the risk factors associated with transmission (Ammaranond et al 2003b).

The Australian HIV Observational Database (AHOD) reported on the rates of change of combination antiretroviral treatments in Australia between 1997 and 2000 (AHOD 2002). The analyses included 596 patients recruited to the AHOD who had commenced combination antiretroviral treatment after 1 January 1997 and were followed-up for a median of 2.3 years. Patients remained on their first regimen for a median of 656 days and 322 of 596 patients (54.0%) progressed to a second regimen which was maintained for a median of 623 days. The 149 of 322 patients(46.3%) who progressed to a third regimen maintained it for a median of 392 days (AHOD 2002).

The reported overall rate of treatment change in this group of patients was 0.45 combinations per year. Multivariate analysis indicated that a low CD4+ cell count at baseline was associated with a higher rate of treatment change. Conversely, patients receiving NNRTIs in their combination regimens had slower rates of treatment change than patients receiving combination therapies that included a PI (AHOD 2002). Data from 2,218 patients recruited to the AHOD by March 2003 indicated that the total

number of patients undergoing follow-up and receiving treatment was 1,443 (AHOD 2003). Of these, 1,345 patients (93.2%) receiving three or more drugs and 848 (63.0%) were on at least their third regimen. Approximately 52 per cent of the 13,120 patients living with HIV/AIDS at the end of 2002 were receiving combination antiretroviral therapy (National Centre in HIV Epidemiology & Clinical Research 2003). If we assume that the patients in the AHOD (2003) are representative of the estimated 6,800 patients currently receiving HAART in Australia, approximately 4,280 patients will be on at least their third regimen.

Existing procedures

Antiretroviral regimens for patients with HIV are currently prescribed according to treatment guidelines for patients naïve to treatment. Patients requiring a change of regimen due to treatment failure, drug toxicity or non-adherence are currently prescribed according to treatment guidelines, patient's treatment history and prescriber's best judgement. Although genotypic resistance testing has no specific MBS item number, it is performed and subsidised by HIV funding from the Australian and State Governments. Usage data from five authorised prescribers in Australia are presented in Table 5.

Table 5 Genotypic usage data for genotypic resistance testing of antiretrovirals in HIV by Authorised Prescribers from December 2000 to December 2003

Authorised Prescriber	Number of resistance tests performed						
	2000	2001		2002		2003	
	Dec	Jan-Jun	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun	Jul-Dec
1	6	34	45	35	41	66	27[b]
2	17	98	117	0	0	151[c]	
3	0	88	131	179	171	201	237
4[a]	0	64	60	47	74	83	0
5[a]	0	77[d]	82	39[e]	0	0	0

[a] Authorised prescribers whose authorisation has expired
[b] July to October
[c] April to September
[d] February to June
[e] January to March
Information provided by the Clinical Section, Office of Devices, Blood and Tissues, Therapeutic Goods Administration June 2004

Reference standard

In the absence of a definitive gold standard to diagnose resistance or susceptibility to therapy, treatment outcome was considered the appropriate reference standard to verify the accuracy of genotypic testing and determination of resistance or susceptibility to therapy.

Comparator

The effectiveness of genotypic resistance testing of antiretrovirals in HIV with expert interpretation of the results was compared with that of:

- Standard of care (as defined in the relevant studies) and/or;

- Genotypic resistance testing without expert interpretation of results and/or;
- Drug-susceptibility phenotyping.

Marketing status of the technology

In-house and commercial kits used to genotype HIV are available and in use in Australia, however, none are listed with the Therapeutic Goods Administration.

Current reimbursement arrangement

There are no current specific reimbursement arrangements for genotype resistance testing in Australia. The states receive funding from the Australian Government for HIV programs, however the allocation of the funding is determined by the individual states. Therefore there is no equity of access for this testing procedure throughout Australia.

Approach to assessment

Review of literature

The medical literature was searched to identify relevant studies and reviews for the period between 1966 and 2004. Searches were conducted via the following electronic databases (Table 6).

Table 6 Electronic databases used in this review

Database	Period covered
Cochrane Library	April 2004
Medline	1966–April 2004
Medline in-process & other non-indexed citations	March 31 2004
EMBASE	1980–2004 Week 13
Australasian Medical Index	2001–April 2004
Biological Abstracts	1980–2004 Week 10
CINAHL	1982–2004 Week 10

In order to identify all the relevant information published in journal articles, we performed the search as a number of separate strategies all of which are detailed in Appendix D.

The core terms of the searches were all the terms that can be used to describe genotypic resistance testing (Appendix D).

For searches on safety, we combined all of the terms for safety, complications and adverse events with the core terms using the Boolean operator "AND".

For searches on effectiveness, we included the diagnostic filter (Appendix D) with the core terms for determination of the diagnostic accuracy of the test. We also included the RCT and systematic review filter (Appendix D) and the core terms for patient management and health outcomes. These were combined with the core terms using the Boolean operator "AND".

For cost-effectiveness, we included the terms for economics, costs, pricing and quality adjusted life years (QALYs) with the core terms.

There may be a large overlap between the records for these individual search strategies as journal articles may cover more than one aspect of genotypic resistance testing of HIV with expert interpretation of resistance patterns.

Other search strategies

Relevant Health Technology Assessment websites (Appendix E) were searched to identify completed reviews or economic evaluations of genotypic resistance testing of HIV with expert interpretation of resistance patterns. Relevant clinical trial register websites (Appendix E) were also searched to identify clinical trials currently under way.

Selection Criteria

The following criteria were developed *a priori* to determine eligibility of relevant studies that assessed diagnostic accuracy of genotypic resistance testing (Table 7) and for studies that assessed patient outcomes following testing (Table 8). The criteria listed were based on those agreed upon by MSAC and the members of the Advisory Panel.

Table 7 Inclusion and exclusion criteria for diagnostic accuracy of genotypic resistance testing

Part 1: Test Accuracy: **What is the diagnostic accuracy of genotypic resistance testing of HIV with expert interpretation of resistance patterns in determining HIV drug resistance and treatment outcome ofto an antiretroviral regimen?**		
Characteristics	**Inclusion**	**Exclusion**
Patients	Patients infected with HIV and who are: Adults or children experiencing first or subsequent virological failure, who are planning a change to a new regimen of antiretroviral therapy. Adults or children naïve to combination antiretroviral therapy having been diagnosed with recent primary HIV infection (less than 12 months ago). Pregnant women. Adults or children whose plasma and other site(s) (eg cerebrospinal fluid, gastrointestinal mucosa or semen) viral load responses are discordant.	None defined.
Test	Genotypic resistance testing of HIV with expert interpretation of resistance patterns. Included tests: sequencing (both in-house and commercial kits) and other techniques (such as Southern blotting, GeneChip hybridisation, Line Probe Assay, point mutation assay, PCR-ligase detection reaction and RNase A mismatch). Definitions of expert interpretation will be as defined in the studies.	Not genotypic resistance testing.
Reference standard	Clinical follow-up to measure treatment outcome or disease progression in patients undergoing genotypic resistance testing (Knottnerus & Muris 2002).	Phenotypic resistance testing.
Outcomes	Information should be available to allow the construction of the diagnostic two by two table with its four cells: true positive, true negative, false positive and false negative to assess the accuracy of genotypic resistance testing of HIV (sensitivity, specificity and derivatives) in predicting treatment outcome to an antiretroviral regimen.	None defined.
Study design	Cross-sectional studies that report the diagnostic characteristics in an independent blind comparison of genotypic resistance testing of HIV with expert interpretation of the resistance patterns to an antiretroviral regimen and an appropriate reference standard (clinical follow-up) in a consecutively-selected group of patients. If no such studies existed, studies that report diagnostic characteristics in an independent blind or objective comparison in non-consecutively selected patients or studies that report diagnostic characteristics in which the reference standard was not applied to all patients would have been included. If none of the above exist, studies that report diagnostic accuracy without a reference standard in a consecutively selected case series may have been considered for inclusion.	Narrative reviews, editorials and other opinion pieces, articles identified as preliminary reports when results are published in later versions, articles in abstract form only, case reports.
Publication	English-language articles.	None-defined.

Table 8 Inclusion and exclusion criteria for patient management and health outcomes following genotypic resistance testing and an antiretroviral regimen

Part 2: Patient management and health outcomes following genotypic resistance testing with expert interpretation of the resistance patterns to a prescribed antiretroviral regimen: **What are the effects of genotypic resistance testing with expert interpretation of the resistance patterns on patient management and health outcomes?**		
Characteristics	**Inclusion**	**Exclusion**
Patients	Patients infected with HIV and who are: Adults or children experiencing first or subsequent virological failure, who are planning a change to a new regimen of antiretroviral therapy. Adults or children naïve to combination antiretroviral therapy having been diagnosed with recent primary HIV infection (less than 12 months ago). Pregnant women. Adults or children whose plasma and other site(s) (eg cerebrospinal fluid, gastrointestinal mucosa or semen) viral load responses are discordant.	None defined.
Intervention	Genotypic resistance testing of HIV with expert interpretation of resistance patterns.	Not genotypic resistance testing.
Comparator	Standard care: treatment history, clinical picture and standard immunological and virological parameters. (No genotypic resistance testing and no expert interpretation of the resistance patterns) and/or; Genotypic resistance testing without expert interpretation of resistance patterns and/or; Drug-susceptibility phenotype assays	None defined.
Outcomes	Patient health outcomes following genotypic resistance testing of HIV with expert interpretation of the resistance patterns to an antiretroviral regimen, eg morbidity, mortality, quality of life, virologic response (and surrogate marker of), disease progression (and surrogate markers of), and change in the drugs prescribed. Surrogate biological markers that will be used include: viral load as a measure of virologic response; and viral load and CD4+ cell count as a measure of disease progression. Adverse events relating to genotypic resistance testing and relating to the toxicity of the new antiretroviral regimen.	None defined.
Study design	Health technology assessments, systematic reviews, meta-analyses and randomised controlled trials were sought initially. If these were unavailable, other controlled trials, comparative studies and cohort studies may have been assessed. In the event that these too were unavailable, case series of consecutively selected patients may have been considered for inclusion.	Narrative reviews, editorials and other opinion pieces, articles identified as preliminary reports when results are published in later versions, articles in abstract form only, case reports.
Publication	English-language articles, or well-designed RCTs published in any language.	None defined.

Assessment of validity

Articles meeting inclusion criteria for assessment of effectiveness underwent critical appraisal to evaluate the potential for bias of their study designs. Critical appraisal was performed using the following methods.

Effectiveness

Two factors are considered in the determination of the effectiveness of a diagnostic test:

- accuracy of the test, ie diagnostic characteristics; and
- patient management and outcomes following the test, ie the usefulness of the test in improving outcomes for patients.

Part 1 Diagnostic accuracy of genotypic resistance testing

The most rigorous study design for assessing the validity of diagnostic tests is considered to be a cross-sectional study comparing blindly and independently the test with the most appropriate reference standard in consecutively selected patients from a relevant clinical population (Jaeschke et al 1994a, Sackett et al 2000). The Cochrane Methods Working Group on Systematic Review of Screening and Diagnostic Tests (1996) expand on this definition and recommend six criteria for assessing the validity of evidence. Based on these criteria, the validity of the methodology of included articles was assessed against the following checklist presented in Table 9. Studies meeting all the criteria are considered the most rigorous and least susceptible to bias, compared to studies that do not meet all these criteria.

Table 9 Criteria and definitions for assessing validity of diagnostic studies

Validity criterion	Definition
Test is compared with an appropriate reference standard	Patients in the study should have undergone both the diagnostic test in question and a reference test that would provide confirmatory proof that they do or do not have the target disorder.
Appropriate spectrum of consecutive patients	Study included patients that the test would normally be used on in clinical practice, ie patients covering the spectrum of mild to severe cases of the target disorder, early and late cases, and patients with other, commonly confused, diagnoses. An inappropriate spectrum compares patients already known to have the disorder with a group of normal non-diseased patients or with patients diagnosed with another condition.
Masked assessment of study and reference tests results	The study test and the reference test should be interpreted separately by persons unaware of the results of the other (avoidance of review bias).
All study subjects tested with both study and reference tests	The reference test should be applied regardless of a positive or negative result from the study test (avoidance of work-up / verification bias).
Study test measured independently of clinical information	The person interpreting the test should be masked to clinical history and results of any other tests performed previously.
Reference test measured prior to any interventions	No treatment interventions were initiated prior to the application of the reference (or study) test.

In the absence of a definitive gold standard to diagnose resistance or susceptibility to therapy, treatment outcome assessed during follow-up of patients was considered the appropriate reference standard to verify the accuracy of genotypic testing and determination of resistance or susceptibility to therapy.

Thus, the delayed-type cross-sectional study was considered the most appropriate study design to assess the accuracy of genotypic resistance testing for identifying resistance or sensitivity to therapy and its accuracy in predicting the outcome of treatment to these therapies as failure or success. Prospective studies are preferable to retrospective studies because they minimise selection bias, however it was more likely that the retrospective design was used for practical and ethical reasons to assess the predictive value of baseline resistance to therapy and treatment outcome.

Reporting accuracy outcomes

The accuracy of a diagnostic test is primarily determined by its ability to identify the target disorder compared to the most appropriate reference standard. Accuracy is measured by diagnostic characteristics such as sensitivity and specificity. The diagnostic characteristics of genotypic resistance testing were reviewed, subject to the availability of sufficient data to compute diagnostic two-by-two tables. Minimum requirements for computing sensitivity are sufficient data to compute the proportion of subjects with the disorder whose tests were correctly identified as positive. For specificity, data are required to compute the proportion of patients without the disorder whose tests were correctly identified as negative.

Diagnostic test results are summarised in two-by-two tables (Table 10). Individuals who test positive for the disease in both the study test under investigation and the reference test are represented in cell "a" and are called true positives (TP). Individuals without the disease who test negative in both tests (the "d" cell) are called true negatives (TN).

A diagnostic test may produce discordance between the test result and the true disease status of the subject. When this occurs, a false result is reported. Cells "b" and "c" in Table 10 illustrate these situations. In the former case the test is positive in individuals without the disease and in the latter case the test is negative in individuals with the disease. These two sets of false results are called false positives (FP) and false negatives (FN), respectively.

Table 10 The generic relationship between results of the diagnostic test and disease status

Study test result	True disease status (Reference standard)		Total
	Diseased	Not diseased	
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

Abbreviations: a=number of diseased individuals detected by the test; b=number of individuals without disease detected by the test; c=number of diseased individuals not detected by the test; d=number of individuals withoutdisease not detected by the test; a+b=total number of individuals testing positive; c+d=total number of individuals testing negative; a+c=total number of diseased individuals; b+d=total number of individuals without disease; a+b+c+d=total number of individuals studied.

Genotypic resistance testing determines the pattern of detectable resistance mutations in HIV to antiretroviral drugs. Hence, we constructed a two-by-two table (Table 11) using the following methods to extract the appropriate data in each of the four cells:

- proportion of patients resistant to one, two, three, etc drugs prescribed and with treatment failure/disease progression (true positives);
- proportion of patients resistant to one, two, three, etc drugs prescribed and with treatment success/no disease progression (false positives);

- proportion of patients not resistant to one, two, three, etc drugs prescribed and with treatment failure/disease progression (false negatives); and

- proportion of patients not resistant to one, two, three, etc drugs prescribed and with treatment success/no disease progression (true negatives).

Table 11 The relationship between baseline resistance to therapy determined from genotypic testing and treatment outcome

Resistance to one, two, three, etc, of the prescribed drugs in HAART	Treatment outcome	
	Failure	Success
Yes	True positives	False positives
No	False negatives	True negatives

Whilst genotypic resistance testing determines the pattern of detectable resistance mutations in HIV to antiretroviral drugs, the test can also be used to infer susceptibility of HIV to antiretroviral drugs. Hence, for studies examining this relationship we constructed a two-by-two table (Table 12) using the following methods:

- proportion of patients susceptible to one, two, three, etc drugs prescribed and have treatment success/no disease progression (true positives);

- proportion of patients sensitive to one, two, three, etc drugs prescribed and have treatment failure/disease progression (false positives);

- proportion of patients not susceptible to one, two, three, etc drugs prescribed and have treatment success/no disease progression (false negatives); and

- proportion of patients not susceptible to one, two, three, etc drugs prescribed and have treatment failure/disease progression (true negatives).

Table 12 The relationship between baseline susceptibility to therapy determined from genotypic testing and treatment outcome

Sensitivity to one, two, three, etc, of the prescribed drugs in HAART?	Treatment outcome	
	Success	Failure
Yes	True positives	False positives
No	False negatives	True negatives

Sensitivity is the proportion of diseased individuals who test positive, or in this case the proportion of individuals with baseline resistance or susceptibility who have treatment failure or success. It is a measure of the probability of correctly diagnosing someone with baseline resistance, or the probability that any given case will be identified by the test. Referring to Tables 10, 11 and 12,

$$Sen = \frac{a}{a+c} = \frac{TP}{TP+FN}$$

Specificity is the proportion of individuals without disease who test negative, or in this case, the proportion of individuals without baseline resistance (without susceptibility) to

therapy who do not fail therapy (who fail therapy). It is the probability of correctly identifying a person without resistance with genotypic resistance testing.

$$Spe = \frac{d}{b+d} = \frac{TN}{TN+FP}$$

The complement of specificity is called the false positive rate (FPR).

$$FPR = 1 - Spe$$

Likelihood ratios (LRs), which indicate by how much a given diagnostic test result will raise or lower the pre-test probability of the target disorder, were also computed if appropriate data could be extracted from individual articles. Likelihood ratios express the odds that a given level of a test result would be expected in a patient with the condition compared to one without the condition, or in this case the odds that a person with baseline resistance would have treatment failure or a person with baseline susceptibility would have treatment success. The LR for a positive test result expresses the odds that a given finding (eg baseline resistance) would occur in a patient with, as opposed to without, the target condition (eg treatment failure) and is related to sensitivity and the false positive rate by:

$$LR+ = \frac{Sen}{FPR}$$

The LR for a negative test result expresses the odds that a given finding (eg, baseline resistance) would not occur in a patient without, as opposed to with, the target condition (treatment failure) and is calculated by:

$$LR- = \frac{1 - Sen}{Spe}$$

Jaeschke et al (1994b) have provided a general guide to interpreting LRs. Large positive LRs of 10 or more, and small negative LRs of <0.1 indicate large, and often conclusive changes in disease likelihood, ie large changes from pre- to post-test probability of having the condition. Positive LRs of 5–10 and negative LRs of 0.2–0.1 indicate moderate changes in pre- to post-test probability. Positive LRs of 2–5 and negative LRs of 0.5–0.2 indicate small but sometimes clinically important changes in probability. If the LR for a positive test result is below two and the LR for a negative test result is above 0.5, then there is little or no likelihood that the presence of disease will be diagnosed as a result of the test.

Part 2 Patient outcomes following genotypic resistance testing

Detection of the pathology of the diagnostic procedure under consideration is not the only indicator of the usefulness of the test. Unless application of the procedure improves patient management options, and, ultimately, patient health outcomes, its usefulness is considered limited (Sackett et al 2000). The ideal method for assessing patient outcomes following use of the diagnostic test is an RCT that compares outcomes of patients who have had the test with outcomes from those patients who have not had the test, and follows up patients for an appropriate length of time to measure patient-relevant morbidity, quality of life and mortality. Thus, RCTs were sought which compared

treatment outcomes in patients allocated to baseline genotypic resistance testing with patients allocated to care without baseline resistance testing.

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC 2000).

These dimensions (Table 13) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. The last two require expert clinical input as part of their determination.

Table 13 Evidence dimensions

Type of evidence	Definition
Strength of the evidence	
Level	The study design used, as an indicator of the degree to which bias has been eliminated by design.[a]
Quality	The methods used by investigators to minimise bias within a study design.
Statistical precision	The *p*-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Size of effect	The distance of the study estimate from the "null" value and the inclusion of only clinically important effects in the confidence interval.
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.

[a]See Table 14

The three sub-domains (level, quality and statistical precision) are collectively a measure of the strength of the evidence. The designations of the levels of evidence are shown in Table 14.

Table 14 Designations of levels of evidence

Level of evidence[a]	Study design
I	Evidence obtained from a systematic review of all relevant randomised controlled trials
II	Evidence obtained from at least one properly-designed randomised controlled trial
III-1	Evidence obtained from well-designed pseudorandomised controlled trials (alternate allocation or some other method)
III-2	Evidence obtained from comparative studies (including systematic reviews of such studies) with concurrent controls and allocation not randomised, cohort studies, case-control studies, or interrupted time series with a control group
III-3	Evidence obtained from comparative studies with historical control, two or more single arm studies, or interrupted time series without a parallel control group
IV	Evidence obtained from case series, either post-test or pre-test/post-test

[a]Modified from NHMRC, 1999

Included articles underwent critical appraisal to evaluate aspects of the study design for susceptibility to bias. A list of criteria used to evaluate the validity of the primary research evidence included in this report is outlined in Table 15. These criteria are based on a list assembled by the NHS Centre for Reviews and Dissemination (2001) to evaluate the validity of evidence from various study designs.

Table 15 Validity criteria according to study design

Study design	Validity criteria[a]
Randomised controlled trial	Randomised method; allocation concealment; blinding of patients, investigators and outcome assessors; proportion lost to follow-up; intention to treat analysis
Cohort	Prospective/ retrospective; comparable groups at inception; identification and adjustment for confounding factors; blind outcome assessment; sufficient duration of follow-up; proportion lost to follow-up
Case-control	Explicit definition of cases; adequate details of selection of controls; comparable groups with respect to confounding factors; interventions and other exposures assessed in same way for cases and controls; appropriate statistical analysis
Case series	Indication was comparable across patients; disease severity was comparable across patients; explicit entry criteria; outcome assessed in all patients; follow-up time uniform; outcomes assessed objectively; outcomes assessed in a blinded manner; outcome measures quantified

[a] Modified from NHS Centre for Reviews and Dissemination (2001)

Data extraction

Data were extracted using standardised instruments created for the assessment. Two reviewers examined each article and any discrepancies in evaluation were discussed and resolved through consensus.

Data analysis

Data provided in the original publications were analysed with Intercooled Stata 7.0 for Windows 95/95/NT (Stata Corporation). Intention-to-treat analyses were performed with all randomised patients included and missing results treated as treatment failures.

Conduct of meta-analyses

Meta-analysis of data provided in the original publications was performed using Review Manager 4.2.2 (The Cochrane Collaboration, Wintertree Software Inc). A fixed effects model was used as no significant statistical heterogeneity between the studies (p-value ≥0.1) was observed. The fixed effects approach is an average measure of the treatment effect observed in the studies for which a statistically significant result indicates that there is an effect in at least one of the studies included in the analysis (Clarke & Oxman, 2003).

Expert advice

An Advisory Panel with expertise in HIV medicine, diagnostics, pharmaceuticals and consumer issues was established to evaluate the evidence and provide advice to MSAC from a clinical perspective. In selecting members for Advisory Panels, MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations and consumer bodies for nominees. Membership of the Advisory Panel for the current assessment report is provided in Appendix B.

Results of assessment

Search results - effectiveness

A flow chart indicating the numbers of articles identified and excluded from, or included in, the search strategies is provided in Figure 4. To assess the effectiveness of genotypic resistance testing, two search strategies were employed. The first was designed to identify relevant literature reporting the diagnostic accuracy of the test and the second to identify relevant literature reporting patient health outcomes following use of the test.

Of the 1,893 articles identified in the accuracy search, 1,810 were excluded upon inspection of the abstracts and 82 were inspected in full text. Of these, 72 were subsequently excluded and 10 were included for critical appraisal. Of the 1,886 articles identified in the patient outcomes search, 1,725 were excluded upon inspection of the abstracts and 160 were inspected in full text. Of these, 151 were subsequently excluded and nine were included for critical appraisal.

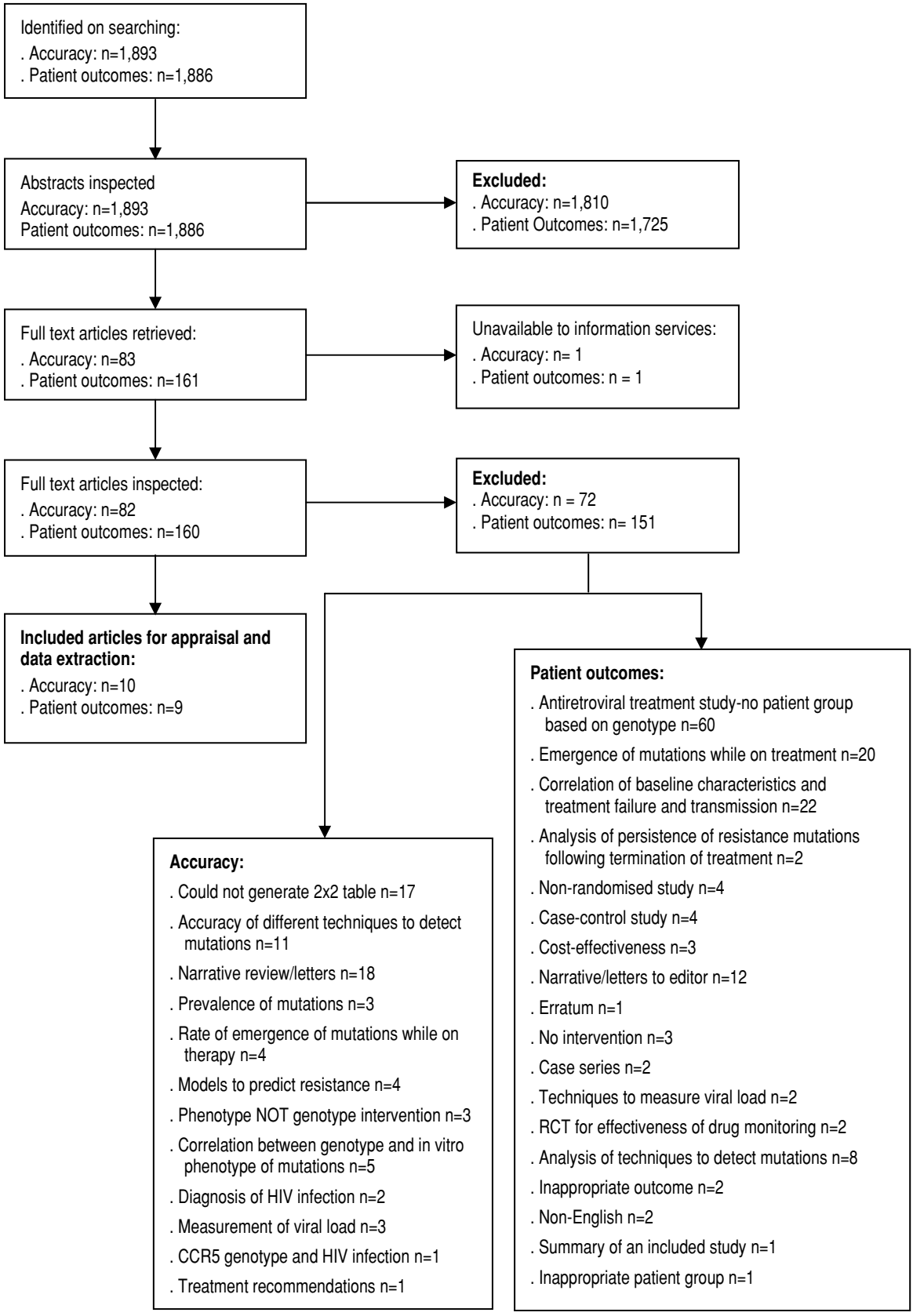

Figure 4 Process for selection of articles assessing the effectiveness of genotypic resistance testing of antiretrovirals in HIV

Is it safe?

An extensive literature search revealed a lack of safety data for genotypic resistance testing of antiretrovirals in HIV. However, as the test generally requires only a blood sample, the risks are expected to be minimal.

Whilst no safety data were found pertaining to the test itself, data regarding the adverse events related to drug toxicity resulting from HAART regimens prescribed in the treatment arms were available from RCTs. No significant differences between the treatment arms for the rates of occurrence of drug-related adverse events were reported in any of the trials (see the section 'Effectiveness – patient outcomes' for details).

Is it effective?

Ten primary studies reporting on the accuracy of genotypic resistance testing were identified as meeting inclusion criteria for critical appraisal and are discussed in the following section, 'Part 1 – Diagnostic accuracy of genotypic testing'. Seven RCTs, one single-arm extension of a RCT and one meta-analysis comparing outcomes in patients undergoing treatment with genotypic resistance testing and patients undergoing treatment without genotypic testing met inclusion criteria and are discussed in the section, 'Part 2 – Patient health outcomes following genotypic resistance testing'.

Part 1 Diagnostic accuracy of genotypic testing

Description of included studies and subjects

The accuracy of genotypic resistance testing of antiretrovirals in predicting treatment outcome was assessed from 10 primary studies that met inclusion criteria. These studies reported on resistance or sensitivity to various therapies as a predictor of treatment outcome and provided sufficient data to allow computation of the test properties sensitivity, specificity and their derivatives. Descriptive characteristics of the studies and patient selection criteria are outlined in Tables 16 and 17, respectively. Two studies (Perez et al 2001, Pellegrin et al 2003) were prospective (Table 16) and eight were retrospective in design (Kaufmann et al 2001, Venturi et al 1999, Van Vaerenbergh et al 2000, Cinque et al 2001, Setti et al 2001, Van Laethem et al 2002, Van Vaerenbergh et al 2002, Vray et al 2003).

Eight of the studies were conducted in Europe (Table 16) – four in Italy (Cinque et al 2001, Setti et al 2001, Van Laethem et al 2002, Venturi et al 1999), two in France (Pellegrin et al 2003, Vray et al 2003), one in Belgium (Van Vaerenbergh et al 2002) and one multicentre study in Belgium, Spain and Luxembourg (Van Vaerenbergh et al 2000). In addition, one study was conducted in Australia (Kaufmann et al 2001) and one in the USA (Perez et al 2001). The smallest study included 15 patients (Cinque et al 2001) while the largest included 518 patients (Vray et al 2003).

Table 16 Descriptive characteristics of included studies

Study	Study design	Location	Enrolment period	Study population		
				Sample size	Age in years mean (SD), range	Number of males (%)
Cinque et al (2001)	Retrospective cohort	Italy	1997–1999	15[a]	40 (9), 30-61	12 (80)
Kaufmann et al (2001)	Retrospective sample from an observational cohort	Sydney, Australia	1998	42[b]	42 (7)	42 (100)
Pellegrin et al (2003)	Prospective cohort	Bordeaux, France	Sep 2000–Sep 2001	75[c]	Median 42 [37, 50][d]	63 (84)
Perez et al (2001)	Prospective cohort	USA	Jan 1996–Oct 1999	26	Median 8, 1-17	13 (50)
Setti et al (2001)	Retrospective cohort	Genoa, Italy	Not reported	62	Not reported	Not reported
Van Laethem et al (2002)	Retrospective cohort	Rome, Italy	Apr 1999–Jun 2000	240[e]	Median 38, 18-69	170 (71)
Van Vaerenbergh et al (2000)	Retrospective cohort	Multicentre[f]	Not reported	88[g]	Not reported	68 (77)
Van Vaerenbergh et al (2002)	Retrospective cohort	Belgium	Sep 1998–Jul 1999	41	Responders: 41 (8.7) Non-responders: 43 (4.7)	Responders: 27 (87) Non-responders: 7 (70)
Venturi et al (1999)	Retrospective cohort	Siena, Italy	Not reported	39	Not reported	Not reported
Vray et al (2003)	Retrospective cohort (originally from RCT)	France	Apr–Oct 1999	518	41	420 (81)

[a]Diagnosis data available for n=14
[b]n=56 in original cohort, data published elsewhere
[c]Subgroup receiving abacavir therapy, diagnosis data available for n=55
[d]25th, 75th percentiles
[e]Subgroup, diagnosis data available for n=185
[f]Belgium, Spain, Luxembourg
[g] n=107 enrolled, n=88 included
Abbreviations: RCT, randomised controlled trial

Table 17 outlines the patient inclusion and exclusion criteria in each study. Nine of the studies included patients with HIV-1 infection. Only Van Vaerenbergh et al (2002) did not specify HIV-1 infection. Kaufmann et al (2001), Pellegrin et al (2003), Van Laethem et al (2002), Van Vaerenbergh et al (2002) and Vray et al (2003) included treatment-experienced adults. Those specifically failing current treatment were included in Kaufmann et al (2001), Van Laethem et al (2002) and Vray et al (2003). Setti et al (2001), Van Vaerenbergh et al (2000) and Venturi et al (1999) also included treatment-experienced subjects, but their age was not stated. Cinque et al (2001) studied adults with neurological disease, some with treatment experience, and measured plasma and cerebrospinal fluid viral load responses to therapy. Perez et al (2001) included children and adolescents previously treated with reverse transcriptase inhibitors (RTIs) who were commencing a new combination therapy consisting of a PI and one or two RTIs.

Table 17 Patient selection criteria of included studies

Study	Selection criteria	
	Inclusion	Exclusion
Cinque et al (2001)	Adult HIV-1 patients with neurological disease, admitted to hospital, measured plasma and CSF responses to therapy	Patients with opportunistic brain infections that may affect CSF HIV RNA levels, eg, cryptococcal or tuberculosis meningitis; patients whose adherence to treatment was judged as uncertain by the treating physician
Kaufmann et al (2001)	Adults with HIV-1, part of an observational cohort of 56 subjects with a high virological failure rate, treated with saquinavir, ritonavir, 2 RTIs, with stored baseline plasma samples	Patients in which the HIV genotype could not be determined: no stored samples (n=8), insufficient sample specimens (n=3), genotype assay failed (n=3). Excluded subjects had similar baseline characteristics to included subjects
Pellegrin et al (2003)	Adults with HIV-1, HAART-experienced, successfully treated with triple-therapy (2 RTIs plus 1 PI) for at least 6 months, with HIV-1 RNA levels <50 copies/ml for at least 6 months. Patients either continued current therapy or switched to abacavir-based triple RTI regimen (same 2 RTIs)	Not reported
Perez et al (2001)	HIV-1 infected children and adolescents aged 1–18 years, naïve to PI therapy (treated previously with RTIs but were naïve to ≥1 RTI in new treatment protocol), immune compromised (CDC immune stage 2 or 3), with plasma virus levels >4.0 log10 copies/ml, enrolled in a treatment protocol consisting of combination therapy with 1 PI and 1–2 RTIs	Not reported
Setti et al (2001)	HIV-1 patients referred to a centre of diagnosis and treatment, eligible for HAART (2 RTIs, 1 PI), previously treated with an RTI for at least 2 years, or eligible for HAART due to newly discovered but presumably old infection. Absolute compliance to treatment (presumably assessed via patient report)	Patients who did not receive alternative treatment based on resistance testing due to presence of resistance to all RTIs (n=4)
Van Laethem et al (2002)	Adults with HIV-1, failing HAART (viral load ≥1000 RNA copies/ml), for which genotyping was performed, undergoing salvage therapy for at least 3 months	Treatment discontinuations (n=70). Excluded subjects had similar baseline characteristics to included subjects
Van Vaerenbergh et al (2000)	HIV-1 infected patients from 3 European countries, treated solely with RTIs, starting or changing a single RTI	Patients receiving stavudine as a newly added NRTI (as no resistance-related mutations for stavudine could be analysed with the genotype test used), absence of amplification (n=14), absence of hybridisation of the codon of interest (n=5)
Van Vaerenbergh et al (2002)	HIV-infected patients on HAART treatment including a PI for at least one month, aged 18 years or over and able to read Dutch, French or English	Patients not fulfilling inclusion criteria and those suffering from opportunistic infection or malignancy
Venturi et al (1999)	HIV-1 infected patients pre-treated with NRTI monotherapy with zidovudine for at least 3 months, adding a second NRTI - didanosine, zalcitabine or lamivudine to therapy	Not reported
Vray et al (2003)	HIV-1 infected patients failing (>1,000 copies/ml) a PI-containing regimen. Previous exposure to at least one PI for at least 3 months. Unchanged antiretroviral regimen for the 2 months preceding. Age over 18 years. Karnofsky score >70%	Not reported in this paper, but reported in Meynard et al (2002): Active opportunistic infection, previous resistance testing, estimated poor adherence, blood haemoglobin <8 g/dl, blood neutrophils <750 x 10^6/l, serum creatinine >150μmol/l, serum amylase >3 times the upper limit of normal, liver aminotransferase >5 times the upper limit of normal

Abbreviations: CDC, Centre for Disease Control; CSF, cerebrospinal fluid; HAART, highly active antiretroviral therapy; PI, protease inhibitor; RTI, reverse transcriptase inhibitor

The techniques used for genotypic resistance testing are outlined in Table 18. Seven studies (Cinque et al 2001, Kaufmann et al 2001, Perez et al 2001, Van Laethem et al 2002, Van Vaerenbergh et al 2002, Venturi et al 1999, Vray et al 2003) reported the use of genotypic assays using HIV RNA viruses and direct DNA sequencing of the HIV genome. Pellegrin et al (2003) applied the assay to proviral DNA rather than HIV RNA viruses. Setti et al (2001), Van Vaerenbergh et al (2000) and Van Vaerenbergh et al (2002) used the line probe assay in addition to sequencing to identify a limited number of mutations that confer resistance to specific RTIs. The line probe assay is limited in the number of mutations it detects such that there may be mutations at other, unexamined, sites.

Cinque et al (2001), Kaufmann et al (2001), Pellegrin et al (2003), Setti et al (2001), Van Vaerenbergh et al (2002), Van Vaerenbergh et al (2000) and Venturi et al (1999), used treatment outcome as a reference standard to confirm whether baseline resistance to one or more drugs accurately predicted failure to respond to therapy (Table 18). Conversely, Perez et al (2001) and Van Laethem et al (2002) determined the susceptibility of patients to particular therapies, based on the absence of mutations to those therapies, and followed up patients to determine treatment outcome as a reference standard to confirm if these susceptibilities resulted in treatment success (Table 18). Vray et al (2003) examined the number of thymidine analogue, NNRTI and PI mutations and their usefulness in predicting treatment outcome.

The length of follow-up differed across studies, ranging from six weeks (Cinque et al 2001) to two years (Van Vaerenbergh et al 2002). Definitions of treatment failure or treatment success also varied across studies. The majority of studies (Cinque et al 2001, Kaufmann et al 2001, Pellegrin et al 2003, Van Laethem et al 2002, Van Vaerenbergh et al 2000, Van Vaerenbergh et al 2002, Venturi et al 1999, Vray et al 2003) used virologic response as a measure of treatment outcome. Perez et al (2001) and Setti et al (2001) used virologic and immunologic responses.

Table 18 Description of genotypic testing and treatment outcome

Study	Genotype test	Treatment outcome
Cinque et al (2001)	Sequencing DNA: nucleotides 1–684 of the RT and nucleotides 1–297 of the protease genes. RTI and protease resistance mutations were obtained from published reports	Follow-up (retrospective) of patients after median 6 weeks (range 2–20 weeks) of HAART to measure if presence of baseline resistance mutations to RTIs and PIs predicted failure of plasma and CSF virologic responses to these therapies. Plasma virologic response: decrease in HIV-1 RNA to below detection limit of 2.60 $\log_{10}$ copies/ml or decrease of more than 1.0 $\log_{10}$ copies/ml; CSF virologic response: decrease in HIV-1 RNA to below detection limit or ΔCSF/Δplasma viral load cut-off value of 0.6 from baseline
Kaufmann et al (2001)	DNA sequencing: HIV-1 RNA extracted and reversed transcribed to cDNA followed by amplification of cDNA sequences with PCR	Follow-up (retrospective) of one year to measure if presence of baseline resistance mutations to RTIs or PIs predicted virologic failure to these therapies. Virologic failure: non-responders who always had a detectable viral load and intermittent responders whose plasma viral load was only intermittently reduced to undetectable levels; virologic success: individuals with a sustained undetectable viral load (<400 copies/ml) over the 1-year period
Pellegrin et al (2003)	DNA sequencing of RT and protease (published elsewhere) genotypes. Used published guidelines to evaluate RT resistance to abacavir plus 2 RTIs: resistant if RT gene had Q151M mutation, insertion at codon 69 alone or ≥ 6 of the following mutations (partial resistance with 4–5 mutations), M41L, K65R, D67N, K70R, L74V, Y115F, M184V/I, L210W, T215Y/F, K219Q/E	Follow-up (prospective) of 6 months to measure if baseline resistance to RTIs (RT gene mutations) predicted virologic failure to a regimen of abacavir plus 2 RTIs. Virologic success: plasma HIV-1 RNA remained at <50 copies/ml for 6-month follow-up; virologic failure: plasma HIV-1 RNA increased to >50 copies/ml during 6 mo follow-up; blip: intermittent plasma HIV-1 RNA 50-1000 copies/ml then return to undetectable levels (failure includes blips)
Perez et al (2001)	RT and protease genotypes assessed from baseline plasma samples; cDNA amplification by RT-PCR was followed by combination PCR/sequencing reactions and the products analysed by automated DNA sequencing. Detectable amino acid polymorphisms at positions in RT or protease that are associated with reduced susceptibility to drugs were scored as resistant	Follow-up of 48 weeks (prospective) to measure if protease or RT susceptibility at baseline predicts successful therapy outcome to 1 PI + 1–2 RTIs in PI-naïve patients. Therapy outcome: virologic success (VS): decrease in plasma viral RNA by >1.5$\log_{10}$/ml during first 4 weeks of therapy followed by sustained suppression at <400 copies/ml ≥16 weeks; immunologic success (IS): increase in CD4+ T cell count by ≥1 CDC stage by 24 weeks of therapy. Three 3 possible outcomes: VS/IS, VF/IS, VF/IF
Setti et al (2001)	Viral RNA extracted from plasma, RT region amplified with a kit and mutations identified by LiPA (INNO-LiPA HIV-1 RT). Used published guidelines to detect 7 sites where mutations are associated with an increase in resistance and 13 associated with known drug resistance, plus a computerized algorithm to determine a pattern of possible drug resistance	Follow up (retrospective design) of 3 months to measure if presence of RTI resistance mutations at baseline predict failure to respond to HAART. Complete response (CR): fall in viral load to undetectable levels and an increase in CD4+ count by ≥ 25% at 3 months; partial response (PR): viral load detectable but <10,000 or CD4+ count increased but to <25%, or both; no response: no viral load decrease to undetectable levels and no CD4+ increase by 25%
Van Laethem et al (2002)	Manufacturer's kit for DNA sequencing (unclear). Genotype resistance at baseline used in an algorithm to give a susceptibility score to salvage therapy (stavudine)	Follow-up of 3 months (retrospective) to measure if presence of susceptibility to salvage therapy predicts treatment success of salvage therapy. Treatment success = virologic success: <500 RNA copies/ml

Table 18 (cont'd) Description of genotypic testing and treatment outcome

Study	Genotype test	Treatment outcome
Van Vaerenbergh et al (2000)	HIV-1 RT LiPA (INNO-LiPA HIV-1 RT), which selects the following resistance mutations: M41L, T69D, K70R, L74V, M184V, T215F/Y in the RT gene. The following resistance mutations were scored: didanosine, positions 74, 184; zalcitabine, positions 69, 74, 184; lamivudine, position 184; ZDV, positions 41, 70, 215	Follow-up (retrospective design) of 1–3 months to measure if presence of RTI resistance to a newly added RTI at baseline predicts viral load responses (failure to respond) to adding or starting that RTI in therapy regimen. Responders: decrease in viral load of ≥ $0.5\log_{10}$ 1–3 months after therapy[a]
Van Vaerenbergh et al (2002)	Line probe assay (LiPA HIV-1 RT), selecting the following resistance mutations: M41L, T69D, K70R, L74V, M184V, T215F/Y in the RT gene. A research version of LiPA was used for scoring mutations in the protease gene, selecting for the following resistance mutations: D30N, M46I, G48V, I50V, I54A/V, V82A/F/T/I, I84V and L90M. ARMS-151 was performed as described elsewhere Sequencing of the protease and RT genes from either the HIV Genotyping System (HGS 2.2; Perkin Elmer Biosystems) or from an in-house sequencing method	Follow-up (retrospective design) of 2 years to measure if presence of RT, PI and primary PI mutations accurately predict non-response to combination therapy. Response to therapy defined as a drop in viral load below 50 copies/ml over almost the entire period of HAART monitoring. Non-response defined as patients never achieving an undetectable viral load or having a rising viral load above 50 copies/ml after the initial response
Venturi et al (1999)	HIV-1 DNA polymerase gene mutations associated with RTI resistance: region corresponding to RT amino acids 30–230 was amplified by nested PCR. ZDV resistance was scored if any combination of the following RT amino acid changes were present: M41L, D67N, K70R, L210W, T215Y/F, K219Q/E. Genotypes that confer low levels of resistance to ZDV (eg mutation K70R alone) were scored as resistant or sensitive on the basis of failure of patient to respond to a second RTI. Lamivudine resistant genotypes: mutation at M184V/I. Resistance to didanosine: the K65R mutation; resistance to zalcitabine: the T69D mutation. Resistance to both didanosine and zalcitabine: mutation at K65R or M184V/I. Multidrug resistance to all RTIs: Q151M with or without any of A62V, V75I, F77L, F116Y	Follow-up (retrospective design) of 24 weeks to measure if presence of RT mutations (substantial resistance to ZDV[b]) accurately predicts non-response to combination therapy (ZDV plus one other NRTI). Response to therapy: $>0.5\log_{10}$ decrease in HIV RNA load at week 24; non response: $< 0.5\log_{10}$ decrease in HIV RNA load at week 24
Vray et al (2003)	RT-PCR of plasma derived HIV RNA using the TruGene assay. Mutations defined according to ANRS algorithm defined in 1998 and used in 1999. When mixed sequences were detected, the corresponding codon was considered mutated	Follow up (retrospective design) of 12 weeks to measure the variables associated with virologic success (<200 copies/ml) and/or failure

[a]CD4+ was also measured but did not differ between patients with resistance mutations and those without, and was not used in the definition of responder or non-responder
[b]Substantial resistance to ZDV defined as presence of any combination of ZDV resistance mutations or of the T215Y/F amino acid change only (ie does not include low ZDV resistance, defined as presence of the K70R mutation only)
Abbreviations: CSF, cerebrospinal fluid; HAART, highly active antiretroviral therapy; LiPA, line probe assay; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor; ZDV, zidovudine

Validity of the included studies

Critical appraisal of the included studies against criteria for assessing the validity of diagnostic studies is summarised in Table 19. Lijmer et al (1999) conducted an observational study of primary studies assessing accuracy of diagnostic tests to determine methodological features associated with bias in estimates of diagnostic accuracy. The

study reported that diagnostic accuracy was overestimated in studies with the following methodological features: case-referent designs (comparison of test results in subjects known to have the target disorder and subjects without the disorder) compared to designs in which subjects were selected across the spectrum likely in clinical practice; application of differential reference standards to verify positive and negative index test results; assessment of reference test results unblinded to the results of the index test and inadequate description of the index test or the population.

Non-consecutive selection of patients compared to consecutive selection and retrospective data collection compared to prospective collection were found not to be associated with a bias in the diagnostic accuracy (Lijmer et al 1999).

The included studies used the delayed-type cross-sectional study design to measure the association between genotypic testing to identify resistance or sensitivity to therapy and treatment outcome in response to these therapies. Diagnostic data from the included studies could be extracted in the form of two-by-two tables. The cross-sectional study design is considered the most valid for determining the accuracy of a diagnostic test (Knottnerus & van Weel 2002).

The reference standard employed in all studies was treatment outcome. In the absence of a definitive gold standard to diagnose resistance or susceptibility to therapy, treatment outcome was considered the appropriate reference standard to verify the accuracy of genotypic testing and determination of resistance or susceptibility to therapy. All patients in all studies underwent verification with the reference standard, thus the potential for partial verification bias was minimised. In addition, as all subjects in all studies had their genotypic test results verified with the one reference standard, regardless of the presence of baseline resistance mutations, it is unlikely that results would have been subject to differential verification bias.

The majority of studies were retrospective which theoretically could render them susceptible to selection bias, as it cannot be determined if patients were selected on the basis of the presence of resistance mutations or response to therapy. However, retrospective compared to prospective designs may not be associated with a significant bias in diagnostic accuracy results (Lijmer et al 1999). Although there is potential for selection bias on the basis that studies did not report consecutive selection of all eligible subjects into the studies, Lijmer et al (1999) report that the impact of non-consecutive selection of subjects on estimates of diagnostic accuracy may be minimal. In any case, to assess if baseline resistance is predictive of treatment failure, it is more likely that studies would be designed retrospectively for practical and ethical reasons.

Studies selected patients from appropriate spectra of clinical populations. All studies included treatment-experienced subjects and Kaufmann et al (2001), Van Laethem et al (2002) and Vray et al (2003) included patients who were failing current treatment. Cinque et al (2001), Setti et al (2001) Van Laethem et al (2002) and Vray et al (2003) excluded non-compliant patients and Van Vaerenbergh et al (2002) excluded patients with opportunistic infections and malignancies. The impact of these exclusions on estimates of diagnostic accuracy is uncertain. Exclusion of these patients may limit the patient spectrum that would use the test in practice and thus may limit the generalisability of the results to settings outside those of the studies.

Most studies did not report if assessors of the reference standard results (ie response to therapy) were masked to the genotypic test results and one study reported retrospective

results from an open-label RCT, which may overestimate diagnostic accuracy (Lijmer et al 1999). However, this overestimation should be minimal, particularly as the response to therapy was measured objectively. In all studies, the genotypic test results were interpreted independently of clinical history or previous testing. The potential for test review bias is minimal since the test is objective. All but three studies (Cinque et al 2001, Kaufmann et al 2001, Setti et al 2001) reported that responses to therapy following genotypic testing were measured without additional changes in therapy. If therapies were altered before measurement of treatment response, it would be difficult to interpret the genotypic test results.

Table 19 Validity of included studies

Study	Design	Verification of test result with appropriate reference	Subjects tested with both study test & reference	Appropriate spectrum of consecutive patients	Masked assessment of study and reference results	Study test measured independent of clinical information	Reference measured before change in intervention
Cinque et al (2001)	Retro	Yes: virologic outcomes	Yes	Not explicit if consecutive	Not reported	Yes	Not reported
Kaufmann et al (2001)	Retro	Yes: virologic outcomes	Yes	Not explicit if consecutive[a]	Not reported	Yes	Not reported
Pellegrin et al (2003)	Prosp	Yes: virologic outcomes	Yes	Not explicit if consecutive[a]	Not reported	Yes	Yes
Perez et al (2001)	Prosp	Yes: virologic and immunologic outcomes	Yes	Implies consecutive	Not reported	Yes	Yes
Setti et al (2001)	Retro	Yes: virologic and immunologic outcomes	Yes	Not explicit if consecutive	Not reported	Yes	Not reported
Van Laethem et al (2002)	Retro	Yes: virologic outcomes	Yes	Not explicit if consecutive[a]	Not reported	Yes	Yes
Van Vaerenbergh et al (2000)	Retro	Yes: virologic outcomes[b]	Yes	Not explicit if consecutive	Not reported	Yes	Yes
Van Vaerenbergh et al (2002)	Retro	Yes: virologic outcomes[b]	Yes	Not explicit if consecutive	Not reported	Yes	Yes
Venturi et al (1999)	Retro	Yes: virologic outcomes	Yes	Not explicit if consecutive	Not reported	Yes	Yes
Vray et al (2003)	Retro	Yes: virologic outcomes	Yes	Not explicit if consecutive	Not reported	Yes	Yes

[a]Diagnostic data available for subgroup; characteristics of the subgroup not reported
[b]Although CD4+ and virologic outcomes were measured, CD4+ count was not used in the definition of responder or non-responder
Abbreviations: Prosp, prospective; Retro, retrospective

Findings and interpretations

Diagnostic characteristics of genotypic resistance testing varied across studies (Table 20), thus it is difficult to summarise the findings in a meaningful way. One possible reason for the variation in results is that although all studies examined baseline resistance or susceptibility to particular therapies derived from resistance mutations in the genotype to predict treatment outcome, there was little consistency in which therapies were evaluated across studies. Furthermore, results may have been confounded by the design of studies that measured resistance to particular therapies but measured outcome to those therapies

in combination with other therapies (or were unclear if the therapies tested for resistance were included in the treatment regimen). A further confounder that would be difficult to control by study design is the development of resistance between the time of genotypic testing and measurement of treatment outcome (Birch et al 2003). In addition, measures of treatment outcome and length of follow-up were inconsistent across studies (see Table 18).

Six of 10 studies (Kaufmann et al 2001, Pellegrin et al 2003, Setti et al 2001, van Vaerenbergh et al 2000, Van Vaerenbergh et al 2002,Venturi et al 1999) examined mutations in the RT gene to establish baseline resistance to RTIs and its relationship to virologic failure of treatment.

Kaufmann et al (2001) examined genotypic testing to establish baseline resistance to RTIs as a predictor of virologic failure to a HAART regimen consisting of the two PIs saquinavir and ritonavir in combination with two RTIs. Data reported in this study allowed calculation of a sensitivity of 79 per cent and a specificity of 28 per cent. Based on these estimates, the positive LR of detecting a resistance to an RTI in a patient with virologic failure was 1.1 and the negative LR of detecting the absence of resistance in a patient without failure was 0.75. These LRs indicate little change in the post-test probability of the treatment outcome compared to the pre-test probability. This result is difficult to interpret however, as although Kaufmann et al (2001) report which specific mutations each patient had at baseline (ie, mutations conferring resistance to zidovudine, lamivudine and stavudine), the authors do not report if these were the RTIs patients actually received.

Similarly, Van Vaerenbergh et al (2002) assessed the predictive value of baseline resistance mutations to RTIs to predict virologic failure. Data from Van Vaerenbergh et al (2002) resulted in a sensitivity and specificity of 100 per cent and 15 per cent, respectively, and a positive predictive value of 1.2, indicating that the presence of RTI mutations are of limited use in predicting treatment failure. This result is difficult to interpret as the authors do not report if patients received the RTIs to which they were resistant.

Setti et al (2001) examined genotypic testing that showed resistance to one or two RTIs included in a HAART regimen as a predictor of virologic failure to that HAART regimen. Sensitivity and specificity calculated from data in this study were 70 per cent and 95 per cent, respectively. The positive LR was 13.6, indicating the presence of resistance to one or two RTIs to be included in HAART was a useful predictor of virologic failure and the negative LR was 0.2, indicating that the absence of one or two resistances may have some use in the prediction of virologic success. Specificity was 100 per cent in the subgroup of patients with baseline resistance to two RTIs included in their therapy regimen, ie the absence of resistance to two RTIs included in a HAART treatment regimen accurately predicted treatment success.

Pellegrin et al (2003), Van Vaerenbergh et al (2000) and Venturi et al (1999) also examined baseline resistance to RTIs and the relationship to virologic failure of combination therapy (including the RTIs). Pellegrin et al (2003) measured baseline resistance to abacavir and the prediction of treatment failure of a treatment regimen consisting of abacavir plus two other RTIs. Sensitivity and specificity were seven per cent and 100 per cent, respectively. The high specificity indicates that the absence of resistance to abacavir accurately predicted virologic success to abacavir-based triple RTI

therapy, but a negative LR of 0.9 limits the use of testing for abacavir resistance in these patients for predicting the outcome of patients without resistance to abacavir.

Van Vaerenbergh et al (2000) measured the predictive value of the presence of baseline resistance to a newly added RTI (didanosine, zalcitabine, lamivudine or zidovudine) to therapy and treatment outcome. Sensitivity and specificity in this setting were 20 per cent and 97 per cent, respectively. A positive LR of 6.4 indicated that the presence of baseline resistance to an RTI to be added to therapy has moderate usefulness in predicting treatment failure, but a negative LR of 0.8 indicates that the absence of a mutation does not give a good indication of treatment success.

Venturi et al (1999) examined baseline resistance to the RTI zidovudine as a predictor of treatment outcome to zidovudine plus one other RTI. A sensitivity of 76 per cent and a specificity of 83 per cent were calculated from their data. However, a positive LR of 4.6 and a negative LR of 0.3 indicated that testing for baseline resistance in this setting is likely to be of limited use in predicting treatment outcome.

Kaufmann et al (2001) also examined genotypic testing to establish baseline resistance to the PIs saquinavir and ritonavir as a predictor of virologic outcome to a HAART regimen consisting of those two protease inhibitors in combination with two RTIs. From these data, we calculated a sensitivity and specificity of 46 per cent and 94 per cent respectively and positive and negative LRs of 8.3 and 0.6, respectively. These LRs indicate that the presence of PI resistance provides moderate evidence of virologic failure in these patients (but the absence of resistance is not an accurate predictor of virologic success).

Similarly, Van Vaerenbergh et al (2002) assessed the predictive value of all baseline resistance mutations in addition to only primary resistance mutations to PIs for virologic failure. When observing all protease resistance mutations, data from Van Vaerenbergh et al (2002) resulted in a sensitivity and specificity of 100 per cent and 23 per cent, respectively, and a positive predictive value of 1.3, indicating that the presence of primary and secondary resistance mutations to PIs is of limited use in predicting treatment failure. Conversely, when only primary PI mutations were considered, the sensitivity and specificity were calculated as 60 per cent and 92 per cent, respectively, and positive and negative LRs were 7.8 and 0.4, respectively. The positive LR indicates that the presence of primary PI resistance mutations is of some use in predicting treatment failure and that the absence of primary PI resistance mutations is of limited use in predicting treatment success. However, as stated previously, this result is difficult to interpret, as the authors do not report if patients received the PIs to which they were resistant.

Cinque et al (2001) measured baseline resistance to RTIs or PIs and virologic outcome in cerebrospinal fluid to HAART. Positive and negative LRs calculated from these data were 1.3 and 0.9, respectively, indicating little use for resistance testing in these patients in predicting treatment outcome.

Two studies (Perez et al 2001, Van Laethem et al 2002) measured baseline susceptibility derived from genotypic resistance testing, rather than resistance to therapy as a predictor of treatment outcome. Perez et al (2001) examined genotypic testing to determine sensitivity to PIs or RTIs in PI-naïve children as predictors of treatment outcome of combination therapy of one PI plus one or two RTIs. There were three possible treatment outcomes, viral success and immune success, viral failure and immune success, or viral failure and immune failure.

Furthermore, diagnostic characteristics varied with the definition of treatment success (Table 20). Perez et al (2001) reported that in children, the immunologic response was more closely related to longer term clinical outcomes (AIDS-defining disease or death) than the virologic response. Thus, we calculated diagnostic characteristics using immunologic success, with or without virologic success, as the definition of treatment success. This gives a sensitivity of genotypic testing to determine baseline sensitivity to RTI therapy as a predictor of treatment success of 47 per cent and a specificity of 75 per cent. Calculation of positive and negative LRs (1.9 and 0.7, respectively) indicated that detection of susceptibility to RTIs was of limited use in predicting treatment outcome to the combination therapy. Conversely, susceptibility to PIs at baseline as a predictor of immunologic success gave a sensitivity of 62 per cent and a specificity of 100 per cent. The specificity indicates that the absence of susceptibility to PIs was an accurate predictor of immunologic failure to combination therapy in these patients.

Van Laethem et al (2002) measured susceptibility to salvage therapy (with the RTI stavudine) derived from genotypic testing as a predictor of virologic outcome to the therapy. Diagnostic data calculated from this study were sensitivity, 70 per cent and specificity, 46 per cent. Positive and negative LRs were 1.3 and 0.7, respectively, indicating detection of susceptibility to salvage therapy was of limited use in predicting treatment outcome in these patients.

Vray et al (2003) examined the baseline characteristics of patients enrolled in an open-label RCT comparing the effectiveness of standard of care, genotyping and phenotyping (Meynard et al 2002) and their use in predicting virologic success. Whilst the RCT had three treatment arms, Vray et al (2003) observed the baseline characteristics of the study population retrospectively as a cohort. The sensitivity and specificity of predicting virologic success when comparing patients with HIV with fewer than three, and three or more thymidine analogue mutations, were 49 per cent and 72 per cent, respectively, with a positive LR of 1.8 and a negative LR of 0.7, indicating that the number of thymidine analogue mutations present in HIV is of limited use in predicting treatment outcome.

Similarly, the absence or presence of NNRTI mutations in predicting virologic success resulted in 84 per cent sensitivity and 38 percent specificity, with positive and negative LRs of 1.4 and 0.4, respectively. The total number of PI mutations (<5 or ≥ 5) in predicting virologic success had a sensitivity of 58 per cent and a specificity of 71 per cent with positive and negative LRs of 2.0 and 0.6, respectively, indicating that these are also of limited use in predicting treatment outcome.

Data from Vray et al (2003) should be interpreted with caution as more than 66 percent of the patients in this cohort received antiretroviral treatment based on genotype or drug-susceptibility phenotype results. This could lead to an underestimation of the predictive value of the total number of thymidine analogue, NNRTI and PI mutations, as patients with increased numbers of mutations treated according to genotype or drug-susceptibility phenotype results would presumably have received treatment that did not include antiretroviral drugs to which they were resistant. Additionally, the total number and types of mutations provides limited information regarding the number of drugs patients were resistant to, or the proportion of mutations that were primary rather than secondary or contributory.

Table 20 Diagnostic characteristics of genotypic resistance testing

Study	Test and reference	n	Diagnostic characteristics			
			Sensitivity (%)	Specificity (%)	LR+	LR-
Cinque et al (2001)	Baseline RT or protease resistance as predictor of virologic failure (in CSF) to HAART	14	33	75	1.3	0.9
Kaufmann et al (2001)	Baseline resistance to PIs as a predictor of virologic failure to HAART[a]	42	46	94	8.3	0.6
	Baseline resistance to (any) RTIs as a predictor of virologic failure to HAART	42	79	28	1.1	0.75
Pellegrin et al (2003)	Baseline resistance to the RTI abacavir as predictor of virologic failure to abacavir + 2 other NRTIs	49	7	100	-	0.9
Perez et al (2001)	Baseline susceptibility to RTIs as predictor of treatment success (VSIS)[b] to 1 PI+ 1–2 RTIs	23	33	50	0.7	1.3
	Baseline susceptibility RTI as predictor of VSIS or VFIS[c]	23	47	75	1.9	0.7
	Baseline PI susceptibility as predictor of VSIS	26	89	71	3.0	0.2
	Baseline PI susceptibility as predictor of VSIS or VFIS	26	62	100	-	0.4
Setti et al (2001)	Resistance to 1 or 2 RTIs in HAART as predictor of virologic failure[d] to HAART	62	70	95	13.6	0.2
	Resistance to 1 RTI in HAART as predictor of virologic failure to HAART	56	59	95	11.5	0.4
	Resistance to 2 RTIs in HAART as predictor of virologic failure to HAART	19	46	100	-	0.5
Van Laethem et al (2002)	Susceptibility score to RTI salvage therapy with stavudine as predictor of virologic success to stavudine	185	70	46	1.3	0.7
Van Vaerenbergh (2000)	Resistance to newly-added RTI (didanosine, zalcitabine, lamivudine or zidovudine) to combination RTI therapy as predictor of virologic failure to therapy[e]	88	20	97	6.4	0.8
Van Vaerenbergh et al (2002)	Resistance to RTIs as a predictor of virologic failure	27	100	15	1.2	-
	Resistance to PIs as a predictor of virologic failure	26	100	23	1.3	-
	Presence of primary PI mutations as a predictor of virologic failure	26	60	92	7.8	0.4
Venturi et al (1999)	RTI (ZDV) resistance as predictor of virologic failure to ZDV plus one other RTI	39	76	83	4.6	0.3

Table 20 (cont'd) Diagnostic characteristics

Study	Test and reference	n	Diagnostic characteristics			
			Sensitivity (%)	Specificity (%)	LR+	LR-
Vray et al (2003)	Number of thymidine analogue mutations (<3 compared with ≥3) as predictor of virologic success	518	49	72	1.8	0.7
	Number of NNRTI mutations (0 compared with ≥1) as predictor of virologic success	518	84	38	1.4	0.4
	Number of PI mutations (<5 compared with ≥5) as predictor of virologic success	518	58	71	2.0	0.6

[a]Patients treated with saquinavir, ritonavir and 2 RTIs
[b]Defining treatment success as VSIS (virologic success and immunologic success) and treatment failure as VFIF (virologic failure and immunologic failure) or VFIS (virologic failure and immunologic success)
[c]Defining treatment success as VSIS or VFIS and treatment failure as VFIF; clinical outcomes at 24-48 weeks indicate that VFIF patients experienced new AIDS-defining diseases or death, VSIS and VFIS patients experienced no new AIDS-defining diseases or death
[d]Response failure: no response or partial response (see Table 18 for definitions); HAART: 2 RTIs + 1 PI
[e]Although CD4+ was measured it did not differ between patients with resistance mutations and those without, and was not used in the definition of responder or non-responder
Abbreviations: CSF, cerebrospinal fluid; HAART, highly active antiretroviral therapy; LR+, likelihood ratio of a positive test; LR–, likelihood ratio of a negative test; PI, protease inhibitor; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor; ZDV, zidovudine

Additional outcomes reported in studies

Some studies reported the use of regression analysis (Kaufmann et al 2001, Pellegrin et al 2003, Perez et al 2001, Van Laethem et al 2002, Van Vaerenbergh et al 2002, Venturi et al 1999, Vray et al 2003) or other statistical techniques (Cinque et al 2001, Setti et al 2001, Van Vaerenbergh et al 2000) to measure the association between resistance or susceptibility to therapies at baseline (and other factors) and treatment outcome. These models are more suited to the testing of hypotheses than determining the clinical usefulness of the test, which may explain some of the discrepancies between the conclusions of the authors and the results reported in Table 20.

For example, Van Laethem et al (2002) conclude that the susceptibility to stavudine therapy at baseline is strongly associated with virologic outcome, but the LRs indicate that baseline susceptibility was in fact of little use as a predictor of virologic outcome. The conclusion of Venturi et al (1999) that genotypic resistance to zidovudine at baseline is a significant predictor of failure of combination therapy that includes zidovudine is not supported by calculation of the diagnostic data (Table 20). Similarly, Vray et al (2003) conclude that increased numbers of thymidine analogue and PI mutations are associated with a decreased likelihood of virologic success, however this is not supported by the diagnostic data (Table 20).

However, there are some similarities between authors' conclusions of the predictive value of the presence of baseline mutations to therapies and outcome and the conclusions reported in Table 20. However, the statistical models tend to overstate the relationship compared to the diagnostic characteristics, in particular the LRs. For example, Kaufmann et al (2001) and Perez et al (2001) conclude that the presence of baseline mutations to PIs is significantly associated with virologic failure, which concurs with the results reported in Table 20. Similarly, the conclusions of Pellegrin et al (2003), Setti et al (2001) and Van Vaerenbergh et al (2000) that baseline resistance mutations to RTIs are associated with treatment failure are supported to some extent by the LRs reported in Table 20, indicating some usefulness of the test.

Summary of diagnostic accuracy of genotypic resistance testing

- Diagnostic characteristics of genotypic resistance testing to establish the presence of baseline resistance or susceptibility to therapy as a predictor of treatment outcome were extracted from 10 primary studies. Studies were conducted in Europe, the USA and Australia, in adults or children infected with HIV.

- Eight studies were retrospective in design, potentially making them susceptible to selection bias, although the impact on estimates of diagnostic accuracy is unclear and may be minimal. The authors failed to report if assessors of the reference standard results (ie response to therapy) were masked to the genotypic resistance testing results. The lack of masking would introduce a potential source of bias that could overestimate diagnostic accuracy. However, all studies met other important validity criteria, including ensuring that all genotypic test results of patients were verified by the appropriate reference standard. In this instance, in the absence of a definitive gold standard to diagnose resistance to therapy, follow-up to determine treatment outcome following baseline resistance testing is the most appropriate reference standard.

- It was difficult to summarise diagnostic characteristics of genotypic testing due to the variation in findings across the included studies. Possible reasons for the variation include: lack of consistency in the way therapies were evaluated across studies; possible confounding of results by study designs that measured resistance to particular therapies but measured outcome to those therapies in combination with other therapies; the possibility that resistance may develop in the interval between genotypic testing and measurement of treatment outcome and inconsistency in the measures of treatment outcome and length of follow-up across the included studies.

- The following tentative conclusions were drawn from calculation of the diagnostic characteristics.

  - Three of six studies indicated that the presence of baseline resistance mutations to RTIs used in various combination therapies has some use as a predictor of treatment failure to those combination therapies. The remaining three studies suggested that the presence of RTI resistance mutations was not a useful predictor of treatment failure.

  - Data from one study indicated that the numbers of thymidine analogue, NNRTI and PI mutations present in HIV are of limited use in predicting treatment success. These results may however underestimate the predictive value of the number of mutations on treatment success as patients who had received treatment based on genotype and drug-susceptibility phenotype results would have presumably been prescribed antiretrovirals to which they were not resistant.

  - Data from one study provided moderate evidence that the presence of baseline resistance to the PIs saquinavir and ritonavir predicts virologic failure to a HAART regimen of saquinavir and ritonavir plus two RTIs.

  - Data from one study indicated that the presence of any primary and secondary PI mutations are of limited use in predicting treatment failure.

However, this study also provided evidence that the presence of primary PI resistance mutations had some use in predicting treatment failure.

- From one study, data indicated that the presence of RTI or PI baseline resistance was not a useful predictor of treatment failure to HAART.

- Data from two studies indicated that the presence of baseline susceptibility to RTIs was not a useful predictor of treatment success, while data from one of these indicated that the presence of baseline susceptibility to PIs was an accurate predictor of treatment success to combination therapy.

Part 2 Patient health outcomes following testing

This report assessed the effectiveness of HIV genotyping with expert interpretation of the resistance results compared with standard of care and drug-susceptibility phenotype analysis in prescribing a HAART regimen following the critical appraisal of seven RCTs, one single-arm extension of a RCT and one meta-analysis.

Randomised controlled trials

Four RCTs (Baxter et al 2000, Cingolani et al 2002, Durant et al 1999, Tural et al 2002) compared the effectiveness of HIV genotyping with that of standard of care, two RCTs compared the effectiveness of virtual phenotyping with that of drug-susceptibility phenotyping (Mazzotta et al 2003, Perez-Elias et al 2003) and Meynard et al 2002 compared the effectiveness of genotyping with that of standard of care and drug susceptibility phenotyping. The descriptive characteristics of these studies are listed in Table 21. Six of the RCTs were conducted in Europe and one was conducted in the USA. The length of follow-up in the studies varied from 12 to 48 weeks. The majority of patients enrolled in each study were males (ranging from 68.7% to 87.6% of the study population, excluding Tural et al 2002 which did not provide this information) and were of similar ages.

Table 21 Descriptive characteristics of randomised controlled trials

Study	NHMRC level and study design	Location	Enrolment period	Maximum length of follow-up	Study population		
					Sample size	Number of males (%)	Age (years)
Baxter et al (2000) GART	Level II RCT	US	Jul 1997–Dec 1998	12 weeks	153	134 (87.6)	Mean: 40.9
Cingolani et al (2002) ARGENTA	Level II RCT	Italy	Apr 1999–Feb 2000	6 months	174	129 (74.1)	Median: 37
Durant et al (1999) Viradapt	Level II RCT	France	Mar 1997–Mar 1998	6 months	108	81 (75.0)	Mean (SD) All: 39.6 (7.8) Control: 40.1 (7.5) Genotype: 39.4 (8.2)
Mazzotta et al (2003) Gen-Phe-Rex	Level II RCT	Italy	May–Jul 2000	48 weeks	201	138 (68.7)	Mean (SD) Drug-susceptibility phenotype: 38.8 (6.04) Virtual phenotype: 39.82 (7.49)
Meynard et al (2002) NARVAL	Level II RCT	France	Apr–Oct 1999	36 weeks	541	438 (81.0)	Mean (SD) All: 41 (8) Control: 41 (8) Genotype: 42 (8) Drug-susceptibility phenotype: 39 (8)
Perez-Elias et al (2003)	Level II RCT	Spain	Feb 2000–Feb 2001	48 weeks	300	214 (71.3)	Median (IQR) Drug-susceptibility phenotype: 37 (34–41) Virtual phenotype: 38 (31-45)
Tural et al (2002) Havana	Level II RCT	Spain	Mar 1999–Feb 2000	24 weeks	326	Not reported	Mean (SD) No genotype: 36.6 (7.2) Genotype: 37.6 (7.8) No expert advice: 37.5 (8.2) Expert advice: 36.6 (6.6)

Patient selection criteria for randomised controlled trials

Patients were enrolled in each of the trials if they met the eligibility criteria determined for each of the studies. Eligibility criteria for each of the trials are listed in Table 22.

Five studies (Baxter et al 2000, Durant et al 1999, Mazzotta et al 2003, Meynard et al 2002, Tural et al 2002) specified that patients be infected with HIV-1 and two studies (Cingolani et al 2002, Perez-Elias et al 2003) did not. Three studies included patients that were at least 18 years of age (Durant et al 1999, Meynard et al 2002, Perez-Elias et al 2003), Baxter et al (2000) included patients who were at least 13 years of age and the remaining three (Cingolani et al 2002, Mazzotta et al 2003, Tural et al 2002) did not specify the age of the patients. Patients included in all trials were HAART-experienced,

however the degree of previous antiretroviral therapy varied among the studies. Baxter et al (2000) required patients to be experiencing virologic failure on a HAART regimen that included one of the PIs indinavir, nelfinavir, saquinavir or ritonavir, and to have had at least 12 months of cumulative antiretroviral therapy. Cingolani et al (2002) and Meynard et al (2002) included patients experiencing virologic failure while on a HAART regimen for at least two months and also required that patients had had prior exposure to at least one PI for at least three months. Patients experiencing virologic failure after at least three months of treatment with a PI and at least six months of treatment with nucleoside analogues were included in Durant et al (1999), whilst patients experiencing virologic failure on a stable HAART regimen for three or six months were included in Perez-Elias et al (2003) and Tural et al (2002), respectively. Mazzotta et al (2003) required that included patients had at least two years of HAART exposure and that patients had been exposed to at least six drugs during their treatment history.

Durant et al (1999), Perez-Elias et al (2003) and Tural et al (2002) specified that patients with foreseeable non-compliance or poor adherence be excluded from the studies. The exclusion of these patients may have biased the results and limited their applicability to clinical practice where non-compliant patients would also undergo the test.

Table 22 Patient selection criteria for randomised controlled trials

Study	Inclusion	Exclusion
Baxter et al (2000) GART	Patients with HIV-1 infection At least 13 years old Experiencing virologic failure on a combination antiretroviral regimen containing a single PI (indinavir, nelfinavir, saquinavir or ritonavir) For virologic failure, four conditions had to be met: . patient taking a current triple regimen for at least 16 weeks . a locally determined screening HIV-1 RNA level >20,000 copies/ml by Roche Amplicor HIV-1 assay or >10,000 copies/ml by the Chiron branched chain (bDNA) assay within 6 weeks before a required baseline visit . documentation that the screening HIV-1 RNA level was three-fold greater than the nadir HIV-1 RNA level while on triple drug regimen or that the nadir was <500 copies/ml . a centrally determined HIV-1 RNA level >5,000 copies/ml by the Chiron 2.0 bDNA assay At least 12 months of cumulative antiretroviral therapy Screening CD4+ cell count between 50 and 500 x 10^6 cells/l	Used an antiretroviral drug other than those in the qualifying regimen in the 16 weeks before the baseline visit Access to previous genotypic or phenotypic test results
Cingolani et al (2002) ARGENTA	Patients on a highly active antiretroviral regimen (concomitant use of three or more antiretroviral agents) for at least 2 months Either a plasma viral load greater than 2,000 copies/ml in at least two consecutive determinations or less than 1 $\log_{10}$ reduction in HIV RNA more than two months after initiation of the last prescribed regimen	None defined

Table 22 (cont'd) Patient selection criteria for randomised controlled trials

Study	Inclusion	Exclusion
Durant et al (1999) Viradapt	Plasma HIV-1 RNA of more than 10,000 copies/ml despite at least 6 months treatment with nucleoside analogues and at least 3 months treatment with a PI At least 18 years of age Karnofsky score > 50%	Haemoglobin concentration <6 mmol/L Absolute neutrophil count <0.8 x 10^9/L Creatinine concentration of >200 μmol/L Liver aminotransferase values of >five times the normal upper limit Patients with foreseeable non-compliance
Mazzotta et al (2003) Gen-Phe-Rex	Patients with at least 2 years of previous exposure to antiretrovirals and more than six experienced drugs in the treatment history Plasma HIV-1 RNA load >1000 copies/ml On a stable antiretroviral HAART for >6 months	None specified
Meynard et al (2002) NARVAL	Plasma HIV-1 RNA level >1,000 copies/ml Previous exposure to at least one PI for at least 3 months Unchanged antiretroviral regimen for the 2 months preceding Age over 18 years Karnofsky score >70%	Active opportunistic infection Previous resistance testing Estimated poor adherence Blood haemoglobin <8 g/dl Blood neutrophils <750 x 10^6/L Serum creatinine >50μmol/L Serum amylase >3 times the upper limit of normal Liver aminotransferase >5 times the upper limit of normal
Perez-Elias et al (2003)	Adult patients (>18 years old) failing their prescribed antiretroviral regime Must have received their current regimen for at least 3 months	Naïve to antiretroviral therapy Suspected of being poorly adherent to their treatment Off antiretroviral therapy for >21 days
Tural et al (2002) Havana	Plasma HIV-1 RNA ≥1,000 copies/ml On stable antiretroviral therapy combination for more than 6 months	Substantial related adverse events history Poor adherence Active drug abuse as reported by treating physician

Table 23 describes the interventions, definitions of expert interpretation and comparator(s) used in the studies. Four studies used the TruGene Assay (Cingolani et al 2002, Durant et al 1999, Meynard et al 2002, Tural et al 2002), two studies used virtual phenotyping performed by Virco (Mazzotta et al 2003, Perez-Elias et al 2003) and Baxter et al (2000) and Durant et al (1999) (in addition to the TruGene Assay) used in-house genotyping methods to genotype HIV.

Four studies (Baxter et al 2000, Cingolani et al 2002, Durant et al 1999, Tural et al 2002) compared the effectiveness of genotype testing with that of standard of care in recommending a new HAART regimen. The definitions of standard of care varied across studies. Baxter et al (2000) and Cingolani et al (2002) proposed HAART regimens based on treatment history and Durant et al (1999) and Tural et al (2002) proposed HAART regimens based on current optimum care according to published guidelines and clinicians' best judgement, respectively. Meynard et al (2002) compared the effectiveness of genotype testing with both standard of care and drug-susceptibility phenotyping, where the standard of care arm had HAART regimens proposed based on treatment history and the phenotyping arm based on the drug-susceptibility phenotype results. Mazzotta et al (2003) and Perez-Elias et al (2003) compared virtual and drug-susceptibility phenotyping.

The interpretation of genotype test results and the experts used to prescribe or recommend HAART regimens following genotype testing varied across the studies. Of the five studies using genotype testing, two interpreted resistance results using written algorithms (Baxter et al 2000, Meynard et al 2002), one used a consensus statement on antiretroviral resistance testing (Durant et al 1999), one used reports from the manufacturer of TruGene (Cingolani et al 2002) and one used Retrogram Interpretation Software (Tural et al 2002).

Recommendations of HAART regimens to be prescribed were performed using a panel of experts with three virologists (Baxter et al 2000), one physician and two virologists (Cingolani et al 2002) or four clinicians and two virologists (Tural et al 2002). Whilst Baxter et al (2000) had a panel of three virologists to recommend a HAART regimen, clinicians made the final decision and were not required to use any of the recommended regimens. Clinicians recommended HAART regimens in Meynard et al (2002). Durant et al (1999) did not report on how the HAART regimens were recommended. In the two studies where virtual phenotyping methods were used to genotype HIV (Mazzotta et al 2003, Perez-Elias et al 2003), interpretation of the mutation patterns was performed by Virco using Vircogen I or II software that predicts drug-susceptibility phenotypes from a database of previously determined viral genotype and phenotype test results. A panel of three clinicians or a single clinician provided recommendations for potential HAART in Mazzotta et al (2003) and Perez-Elias et al (2003), respectively.

Table 23 Descriptive characteristics of the interventions and comparator(s)

Study	Intervention	Comparator
Baxter et al (2000) GART	Genotyping . RT-PCR of plasma derived HIV-1 RNA: PCR product encompassed whole protease gene and first 250 amino acids of the RT gene . Standard dideoxytermination sequencing (ABI) . Mixture of wild-type and mutant nucleotides at a particular position was called where peak height of minor peak was ≥30% of total signal in both sequencing directions Expert interpretation . Three virologists suggested up to 4 treatment regimens (clinicians not required to follow these treatment suggestions) based on: characterisation of virus as sensitive (absence of resistance mutation linked to drug), possibly resistant (presence of 'minor' or secondary mutations) and resistant (presence of primary mutation) based on a written algorithm (Appendix in paper); patient treatment history and treatment contradictions	Standard of care . Prescription was proposed by the site clinician before patient randomisation based on current consensus treatment guidelines, which included expert recommendation for changing therapy based on treatment history

Table 23 (cont'd) Descriptive characteristics of the interventions and comparator(s)

Study	Intervention	Comparator
Cingolani et al (2002) ARGENTA	Genotyping . RT-PCR of plasma derived HIV RNA using the TruGene assay: 1.3kb PCR product encompassing the entire protease gene and majority of RT gene and CLIP sequencing . Sequence analysed with GeneObjects and compared to the wild-type HIV-1HBX2 sequence using GeneLibrarian (Visible Genetics). Sequences manually proofread to verify accuracy. Sequence compared to a database of known mutations associated with drug resistance Expert interpretation . Panel (one physician and two experts in the interpretation of genotypic resistance results) evaluated results and prescribed a regimen based on: treatment history; clinical picture; standard immunologic and virologic parameters and classification of drug as either active (no resistance to specific antiretroviral drug) or resistant (detection of ≥1 primary mutation) according to updated reports from the manufacturer . A genotypic sensitivity score was calculated for each regimen. A sensitivity score of 1 was given to each drug that was not associated with any resistance mutations identified as primary according to the Visible Genetics interpretation system, hiv.gnl (September 1999)	Standard of care . Panel (one physician and two experts in the interpretation of genotypic resistance results) prescribed a regimen based on: treatment history; clinical picture and standard immunologic and virologic parameters
Durant et al (1999) Viradapt	Genotyping Two methods used: . RT-PCR of HIV-1 RNA: 800bp RT gene PCR product and a 350bp protease gene PCR product and ABI sequencing. Amplification of desired sequence with nested PCR . RT-PCR of plasma derived HIV-1 RNA using the TruGene assay: 1.3kb PCR product encompassing the entire protease gene and majority of RT gene and CLIP sequencing . Sequence analysed with GeneObjects and assembled using GeneLibrarian (Visible Genetics) and compared to a database of known mutations associated with drug resistance Expert interpretation . Mutations classified as primary, secondary and polymorphism, associated or not associated with decreased drug sensitivity, according to the consensus statement on antiretroviral resistance testing	Standard of care . Current optimum care according to published guidelines

Table 23 (cont'd) Descriptive characteristics of the interventions and comparator(s)

Study	Intervention	Comparator
Mazzotta et al (2003) Gen-Phe-Rex	Virtual phenotyping . Performed by Virco Expert interpretation . Expert panel of 3 clinicians provided independent advice on treatment options based on: resistance testing; treatment history; clinical picture and standard virologic and immunologic parameters . Technical cut-offs: <4-, 4–10- and >10-fold resistance were used for decision making. After completion of enrolment, biologic cut-offs were created that took into account the natural variability (2 standard deviations) in drug susceptibility. A sensitivity score for each regimen (the number of drugs to which the virus was sensitive) was calculated for each patient	Drug-susceptibility phenotyping . Antivirogram performed by Virco Expert interpretation . Expert panel of 3 clinicians provided independent advice on treatment options based on: resistance testing; treatment history; clinical picture and standard virologic and immunologic parameters . Technical cut-offs: <4-, 4–10- and >10-fold resistance were used for decision making. After completion of enrolment, biologic cut-offs were created that took into account the natural variability (2 standard deviations) in drug susceptibility. A sensitivity score for each regimen (the number of drugs to which the virus was sensitive) was calculated for each patient
Meynard et al (2002) NARVAL	Genotyping . RT-PCR of plasma derived HIV RNA using the TruGene assay Expert interpretation . Investigators received a standardised form listing mutations known to be associated with drug resistance and classified each drug as either having no evidence of resistance, possible resistance and resistance, according to ANRS algorithm defined in 1998 and used in 1999 (Appendix II of the paper) . When mixed sequences detected, the corresponding codon was considered mutated	Standard of care . Antiretroviral treatment chosen by clinician according to treatment history Drug-susceptibility phenotyping . Performed using a recombinant virus assay based on PCR amplification of protease and RT HIV sequences from plasma and evaluation of drug susceptibility of recombinant HIV in a single cycle of infection . Drug-susceptibility phenotype determined for 12 drugs and resistance was calculated as the fold increase in 50% or 90% inhibitory concentration relative to reference virus NL_{4-3} . Clinicians received resistance rankings within each class of drugs and an indication of which drugs were 'best choice', 'second choice' or 'not recommended' using an algorithm based on a cut-off of four-fold for PI and five-fold for NRTI and NNRTI
Perez-Elias et al (2003)	Virtual phenotyping . RT-PCR of plasma derived HIV-1 RNA: PCR product encompassed whole protease gene and first 250 amino acids of the RT gene . Standard dideoxytermination sequencing (ABI) Expert interpretation . Sequencing results analysed by Virco using Vircogen I or II software that predicts the drug susceptibility phenotype from a database of previously determined viral genotypes and phenotype test results . Characterised HIV as sensitive (<4-fold increase), intermediate (4–10-fold increase) or resistant (>10-fold increase)	Drug-susceptibility phenotype . Recombinant virus assay (Antivirogram) performed by Virco . Results expressed as the fold increase of IC_{50} relative to the wild-type virus strain HIVIIIB/LAI . Characterised HIV as sensitive (<4-fold increase), intermediate (4–10-fold increase) or resistant (>10-fold increase)

Table 23 (cont'd) Descriptive characteristics of the interventions and comparator(s)

Study	Intervention	Comparator
Tural et al (2002) Havana	Genotyping . RT-PCR of plasma derived HIV RNA using the TruGene assay. PCR product encompassed whole protease gene and codons 37–248 of the RT gene and CLIP sequencing . Analysed sequence with GeneObjects and compared sequence with a known, reference HIV-1 sequence (LAV-1) for identification of mutations . Classified mutations based on Hirsch 2000 Genotype with no expert interpretation . Retrogram Interpretation Software that compares sequence to that of the NL_{4-3} reference strain and contains approximately 200 rules relating base substitutions in HIV to reported effects on drug response . Drugs are classified as (A) 'can be used', (B) 'consider if no class A is available', (C) 'consider if no class A or B drugs are available', (D) 'consider if neither drugs in A, B, or C are available', or (U) 'unranked, insufficient data available'. Drugs are ranked depending on pattern of mutation(s) Genotype with expert interpretation . Made up of 4 clinicians and 2 virologists with >10 years' experience in HIV virology/ clinical care. Treatment decisions based on: previous drug history, T cell counts, viral load changes, adverse reactions to antiretroviral drugs and drug adherence in addition to the results from the Retrogram Interpretation Software	Standard of care with no expert interpretation . Patients managed according to clinician's best judgement Standard of care with expert interpretation . Made up of 4 clinicians and 2 virologists with >10 years' experience in HIV virology/ clinical care. Treatment decisions based on: previous drug history, T cell counts, viral load changes, adverse reactions to antiretroviral drugs and drug adherence

Validity assessment of randomised controlled trials

The validity assessment of the seven trials included in the critical appraisal are summarised in Table 24.

Table 24 Validity of randomised controlled trials

Study	Validity					Outcome measures
	Method of randomisation	Concealment of allocation	Inclusion of randomised participants	Masking	Losses to follow-up (%)	
Baxter et al (2000) GART	Randomly assigned 1:1; stratified according to CD4+ cell count: 50–199 x 10^6 cells/L and 200–500 x 10^6 cell/L and PI in failing regimen: indinavir, nelfinavir, ritonavir or saquinavir. Eight strata in total. Randomisation schedules for each stratum was prepared using permuted blocks	Clinician informed of patients assignment at randomisation visit Concealment of allocation for patients and outcome assessors was not reported	No – 1 patient in GART group had viral load measurements missing at both 4 and 8 weeks and was excluded from primary endpoint analysis	Clinicians – NA Not reported for patients and outcome assessors	Week 4: 2.0 Week 8: 3.3 Week 12: 3.3	Primary: Change in plasma HIV-1 RNA (log_{10}) from baseline (the geometric mean of measurements from the baseline and randomisation visits) to the average (geometric mean) of the measurements at 4 and 8 weeks post-randomisation Secondary: Change in plasma HIV-1 RNA through 12 weeks, and a change in CD4+ cell count through the average of 4 and 8 weeks and through 12 weeks
Cingolani et al (2002) ARGENTA	Consecutively randomly assigned 1:1 to either genotyping or standard of care	None – open trial	Yes	NA	Not reported	Primary: Proportion of patients with less than 500 HIV RNA copies/ml of plasma at 3 and 6 months Secondary: Changes from baseline: HIV-1 RNA levels and CD4+ cell counts
Durant et al (1999) Viradapt	Permutation table in blocks of six, with a two/three ratio in the control and treatment groups, respectively	Adequate	Yes	None – open trial	SOC Month 3: 4.7 Month 6: 7.0 Genotype Month 3: 4.6 Month 6: 9.2	Primary: Variation of HIV-1 RNA from baseline to month 3 and month 6 (log transformed) Secondary: CD4+ cell count and the proportion of patients with plasma HIV-1 RNA lower than the limit of detection (200 copies/ml)
Mazzotta et al (2003) Gen-Phe-Rex	Consecutively assigned 1:1 to either drug-susceptibility or virtual phenotyping by a coordinating centre	None – open trial	No – 14 patients in each arm dropped out before starting therapy	NA	19.9 – 14 from each arm before initiation of treatment and 12 treatment interruptions 40/201	Primary: Proportion of patients with HIV plasma viral load <400 copies/ml at 48 weeks Secondary: Absolute plasma viral load change, proportion of patients with a plasma viral load reduction >0.5 log_{10} copies/ml and absolute CD4+ cell change

Table 24 (cont'd) Validity of randomised controlled trials

Study	Validity					Outcome measures
	Method of randomisation	Concealment of allocation	Inclusion of randomised participants	Masking	Losses to follow-up (%)	
Meynard et al (2002) NARVAL	Not reported	None Investigators and patients were told of arm assignment on day 0 of the trial	No	NA	SOC Week 12: 4.6 Week 24: 9.4 Week 36: 23.9 Phenotype Week 12: 1.6 Week 24: 3.7 Week 36: 24.7 Genotype Week 12: 3.1 Week 24: 5.2 Week 36: 20.8	Primary: Percentage of patients with plasma HIV-1 RNA <200 copies/ml at week 12 Secondary: Percentage of patients with plasma HIV-1 RNA <20 copies/ml at week 12; changes in plasma HIV-1 RNA and CD4+ cell counts between day 0 and week 12; percentage of patients with plasma HIV-1 RNA <200 copies/ml at 12 and 24 weeks; percentage of patients with plasma HIV-1 RNA <200 copies/ml at 12, 24 and 36 weeks
Perez-Elias et al (2003)	Centrally randomised 1:1 to drug-susceptibility or virtual phenotype. Stratification based on previous antiretroviral treatment with one or two versus three drug classes	Adequate	No	Patients, clinicians and statisticians	Drug-susceptibility phenotype 6.7 Virtual phenotype 9.3	Primary: Per cent of patients with HIV RNA suppression (ie with <400 copies/ml after 24 weeks) Secondary: Median HIV RNA concentration and change from baseline in HIV RNA concentration
Tural et al (2002) Havana	Factorial study with two randomisations, stratified by whether the patient had one, two or three or more treatment failures	None – open trial	Yes	NA	Week 12: 14.7 Week 24: 23.3	Primary: Proportion of patients with plasma HIV-1 RNA load <400 copies/ml at 24 weeks Secondary: Change in plasma viral load at 12 and 24 weeks from baseline

Abbreviations: NA, not applicable; SOC, standard of care

Randomisation, allocation concealment and masking

All but one of the trials (Meynard et al 2002) reported the methods used to randomise patients, however only one trial (Mazzotta et al 2003) reported the method by which the randomisation sequence was generated (which was by a co-ordinating centre). Four studies randomised patients 1:1 to the genotype/virtual phenotype and comparator arms (Baxter et al 2000, Cingolani et al 2002, Mazzotta et al 2003, Perez-Elias et al 2003).

Durant et al (1999) randomised patients in a two/three ratio to the standard of care and genotype arms and Tural et al (2002) performed a factorial study with two randomisations, the first was for genotyping or no genotyping and the second for expert advice or no expert advice. Baxter et al (2000), Perez-Elias et al (2003) and Tural et al (2002) stratified patients according to CD4+ cell count and PI in failing regimens, previous antiretroviral treatment with one or two versus three drug classes, and whether the patient had one, two or three or more treatment failures, respectively.

Six trials were open-label (Baxter et al 2000, Cingolani et al 2002, Durant et al 1999, Mazzotta et al 2003, Meynard et al 2002 Tural et al 2002). Concealment of allocation and continued masking of patients, clinicians and outcome assessors are important in limiting conscious and unconscious bias in the selection of patients for each treatment arm and subsequent interpretation of results from clinical trials. It has been reported that inadequate or unclear concealment of allocation may exaggerate or overestimate treatment effects by approximately 30 per cent compared with trials that adequately conceal allocation, while trials that are not blinded exaggerate treatment effects by 17 per cent compared with blinded or masked trials (Shulz et al 1995). Given that the outcomes of interest in each of the trials are objective (changes in viral load and CD4+ cell counts), the effects of a lack of concealment of allocation and masking may be minimised. Whilst the study by Durant et al (1999) was an open trial, treatment allocation to the genotype and standard of care arms was concealed using opaque envelopes to minimise selection bias. Perez-Elias et al (2003) adequately concealed allocation and masking of patients, clinicians and statisticians by the generation of a unique, blinded report.

Follow-up and intention-to-treat

Follow-up of patients in the RCTs are summarised in Table 25. Follow-up was achieved for a high proportion of patients in three trials (Baxter et al 2000, Durant et al 1999, Perez-Elias et al 2003). At conclusion of the trials, Meynard et al (2002) and Tural et al (2002) had followed-up fewer than 80 per cent of the randomised patients at 36 and 24 weeks, respectively. Mazzotta et al (2003) reported that 14 patients in each arm dropped out of the study before the initiation of therapy, however they did not report any further losses to follow-up during the study. Similarly, Cingolani et al (2002) did not report the number of patients lost to follow-up.

Three studies used intent-to-treat analysis (including all patients randomised to the different treatment groups and analysing them within their randomisation group, regardless of losses to follow-up) with the last observation carried forward (Cingolani et al 2002, Durant et al 1999, Tural et al 2002).

The other four studies also reported using intention-to-treat analysis. Their definitions of this type of analysis were not strictly correct. All four studies analysed patients within their randomisation groups, however drop-outs or losses to follow-up as the trials progressed were not included.

Baxter et al (2000) analysed patients within their randomisation groups, however the denominator changed, indicating that losses to follow-up were not included as the trial progressed.

Mazzotta et al (2003) randomised 100 patients to the virtual phenotyping and 101 patients to the drug-susceptibility phenotyping arms, however 14 patients from each treatment arm dropped out of the study before initiation of therapy, leading to the

authors basing their intention-to-treat analysis with last observation carried forward on 86 and 87 patients in the virtual and drug-susceptibility phenotyping arms, respectively. The baseline characteristics reported in this study reflect those of the 86 and 87 patients that initiated therapy. The baseline characteristics of the 28 patients who dropped out before starting therapy were not reported. On-treatment analysis was also completed that included only patients who had measurements at each follow-up time point and remained on the treatment regimen recommended at trial initiation.

Meynard et al (2002) performed their primary analysis on observed values and completed a secondary analysis using intention-to-treat analysis in which patients with missing data were considered to be treatment failures.

Perez-Elias et al (2003) also analysed patients within their randomisation groups. This study randomised 151 and 149 patients to the virtual and drug-susceptibility phenotyping arms, respectively. Of the 151 patients randomised to virtual phenotyping, eight patients had an invalid genotype result and subsequently one patient in this group had treatment guided by drug-susceptibility phenotyping, leaving 142 actually guided by virtual phenotyping. Despite this, all patients randomised to the virtual phenotyping group were analysed within this group. A total of 14 patients were not evaluated in the virtual phenotyping arm and the total number of patients analysed was 137. Of the 149 patients randomised to the drug-susceptibility phenotyping arm, 26 patients had an invalid drug-susceptibility phenotype result, leading to 127 and 19 patients having treatment guided by drug-susceptibility and virtual phenotyping, respectively. Despite this, all patients randomised to the drug-susceptibility phenotyping group were analysed within this group. Ten patients were not evaluated and the total number of patients analysed was 139. Therefore, patients who were lost to follow-up before starting treatment and patients who died before receiving test results were excluded from the analysis. A secondary as-treated analysis was also performed in which only patients who were tested by the method assigned to them at randomisation were included.

Table 25 Patient follow-up in the randomised controlled trials

Study	Follow-up period	Genotype n/N (%)	Comparator		All n/N (%)
			SOC n/N (%)	Phenotype n/N (%)	
Baxter et al (2000)	4 weeks				150/153 (98.0)
	8 weeks				148/153 (96.7)
	12 weeks				148/153 (96.7)
Cingolani et al (2002)					
Durant et al (1999)	3 months	62/65 (95.4)	41/43 (95.4)		103/108 (95.4)
	6 months	59/ 65 (90.8)	40/43 (93.0)		99/108 (91.7)
Mazzotta et al (2003)	Before starting	86/100 (86.0)		87/101 (86.1)	173/201 (86.1)
Meynard et al (2002)	12 weeks	186/192 (96.9)	152/159 (95.6)	187/190 (98.4)	525/541 (97.0)
	24 weeks	182/192 (94.8)	144/159 (90.6)	183/190 (96.3)	509/541 (94.1)
	36 weeks	152/192 (79.2)	121/159 (76.1)	143/190 (75.3)	416/541 (76.9)
Perez-Elias et al (2003)	To 48 weeks	139/149 (93.3)		137/151 (90.7)	276/300 (92.0)
Tural et al (2002)	12 weeks				278/326 (85.3)
	24 weeks				250/326 (76.7)

Shading: light grey, not reported; dark grey, not applicable

Outcomes

There were two main primary outcomes used to determine the effectiveness of genotype resistance testing of HIV to determine an optimum HAART regimen in patients experiencing virologic failure. The primary outcome of achieving a viral load below the level of detection was used in five trials (Cingolani et al 2002, Mazzotta et al 2003, Meynard et al 2002, Perez-Elias et al 2003, Tural et al 2002). The level of detection varied in the studies due to the techniques used to measure viral load. A change in viral load from baseline to pre-determined time points following the initiation of therapy was the primary outcome in two trials (Baxter et al 2000, Durant et al 1999). Baxter et al (2000) had a primary endpoint of a change in viral load from baseline defined as the geometric mean of the baseline and randomisation visits and the average (geometric mean) of viral loads at four and eight weeks post-randomisation. This endpoint was determined to reflect the duration of the prescribed regimen – patients were requested to remain on the regimen for eight weeks (unless experiencing toxicity). The regimen could be changed at this point if the four week measurements were considered suboptimal.

Secondary outcome effectiveness measures included a change in viral load from baseline to pre-determined time points following the initiation of therapy in five studies (Cingolani et al 2002, Mazzotta et al 2003, Meynard et al 2002, Perez-Elias et al 2003, Tural et al 2002). The secondary outcome measure in both Baxter et al (2000) and Durant et al (1999) was the proportion of patients achieving a viral load below the level of detection. Five studies (Baxter et al 2000, Cingolani et al 2002, Durant et al 1999, Mazzotta et al 2003, Meynard et al 2002) also analysed the change in CD4+ cell counts from baseline to pre-determined time points following the initiation of therapy as a secondary effectiveness outcome.

Sample size and power

Four studies (Mazzotta et al 2003, Meynard et al 2002, Perez-Elias et al 2003, Tural et al 2002) provided calculations of the sample size required to detect differences between genotype and comparator groups for the primary outcome of the trials.

Mazzotta et al (2003) reported that a χ^2 test with a one-sided significance level of 0.05 would have 87 per cent power to detect the difference between a Group 1 proportion of 0.400 and a Group 2 proportion of 0.200 when the sample size in each group was 80.

Meynard et al (2002) reported that the study had 80 per cent power with a type-I error of 0.05 and two-sided tests to detect a difference of 15 per cent between the genotype and standard of care arm and between the drug-susceptibility phenotype and genotype arms.

An estimated sample size of 300 patients was necessary to show equivalent effectiveness between virtual and drug-susceptibility phenotyping (Perez-Elias et al 2003). A two-sided χ^2 test was performed with an α error of 0.05 and a β error of 0.10 for detecting a difference of 20 per cent between the two tests with an estimated loss to follow-up of 10 per cent.

Tural et al (2002) calculated that 326 patients were necessary to obtain 80 per cent power to detect a difference of 50 per cent (using a 0.05 level test) between treatment arms for the primary outcome for the two-factorial comparisons of genotype versus no genotype and expert advice versus no expert advice.

Findings and interpretations

The ultimate goal of treating patients infected with HIV with HAART has been to reduce mortality and morbidity. The number of patients in each treatment arm of each study included in the critical appraisal who died or experienced an AIDS-defining event are summarised in Table 26. No statistically significant differences were found between the treatment arms (genotype versus standard of care; genotype versus drug-susceptibility phenotype; virtual versus drug-susceptibility phenotype) in the number of patients who died or experienced an AIDS-defining event during the course of the studies. Whilst no differences were found, it must be noted that this outcome was not a primary outcome in any of the RCTs. Thus, the studies may not have been powered to detect a difference in the proportion of patients who died or experienced an AIDS-defining event, nor were the studies long enough to detect differences over extended periods of time.

Table 26 Proportion of patients that died or experienced an AIDS defining event

Study	Event	Genotype n/N (%)	Comparator(s)		Relative risk (95% CI)	NNH (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Baxter et al (2000) GART	Death	1/78 (1.3)	1/75 (1.3)		0.96 (0.06, 15.10)	NA
	AIDS					
Cingolani et al (2002) ARGENTA	Death					
	AIDS					
Durant et al (1999) Viradapt	Death	2/65 (6.2)	2/43 (9.3)		0.66 (0.10, 4.50)	NA
	AIDS	2/65 (3.1)	4/43 (9.3)		0.33 (0.06, 1.73)	NA
Mazzotta et al (2003) Gen-Phe-Rex	Death	2/100 (2.0)		3/101 (3.0)	0.67 (0.11, 3.94)	NA
	AIDS	0/100 (0.0)		4/101 (4.0)	0.11 (0.01, 3.94)	NA
Meynard et al (2002) NARVAL	Death	1/192 (0.5)	1/159 (0.6)	2/190 (1.1)	Genotype vs SOC 0.83 (0.05, 13.13)	NA
					Genotype vs drug-susceptibility phenotype 0.49 (0.05, 5.41)	NA
	AIDS	7/192 (3.6)	4/159 (2.5)	8/190 (4.2)	Genotype vs SOC 1.45 (0.43, 4.86)	NA
					Genotype vs drug-susceptibility phenotype 0.87 (0.32, 2.34)	NA
Perez-Elias et al (2003)	Death	1/151 (0.6)		3/149 (2.0)	0.33 (0.03, 3.13)	NA
	AIDS	12/151 (7.9)		11/149 (7.4)	1.08 (0.49, 2.36)	NA
Tural et al (2002) Havana	Death					
	AIDS					

Abbreviations: 95% CI, 95% confidence interval; NA, not applicable since not statistically significant; NNH, number needed to harm; SOC, standard of care ; NNH, number needed to harm; 95% confidence interval; n/a, not applicable since not statistically significant
Shading: light grey, not reported; dark grey, not applicable

Plasma HIV RNA below the level of detection

Achieving plasma HIV RNA levels below the level of detection was a primary outcome in five studies (Cingolani et al 2002, Mazzotta et al 2003, Meynard et al 2002, Perez-Elias

et al 2003, Tural et al 2002). The proportion of patients achieving an undetectable viral load in these studies are summarised in Table 27.

Of the studies where achieving an undetectable viral load was the primary outcome, Cingolani et al (2002) showed that patients whose treatment was guided by genotype resistance testing were twice as likely to achieve an undetectable viral load at three months compared to those being treated by standard of care (RR= 2.09, 95% CI: 1.14, 4.21; NNT=7, 95% CI: 4, 33), however, this treatment benefit was not maintained at six months (RR=1.26, 95% CI: 0.68, 2.33). Similarly, Tural et al (2002) showed that at 24 weeks, patients whose treatment was guided by genotype resistance testing were 33 per cent more likely to achieve undetectable viral loads than patients treated by standard of care (RR=1.33, 95% CI: 1.03, 1.72; NNT=8, 95% CI: 4, 100), however, no differences between the treatment arms were evident at 12 weeks (secondary outcome).

Results from Meynard et al (2002) showed no significant differences between genotyping and standard of care for a viral load of less than 200 copies/ml at 12 weeks (primary outcome) or at 12, 24 and 36 weeks (secondary outcome), however a significant difference was observed between patients in the genotype and standard of care arms for patients with an undetectable viral load at 12 and 24 weeks (RR=1.50, 95% CI: 1.02, 2.20; NNT=10, 95% CI: 5, 100; secondary outcome). No significant differences between the genotype and drug-susceptibility phenotype arms were reported for any time point (Meynard et al 2002).

The two studies comparing the effectiveness of virtual and drug-susceptibility phenotyping (Mazzotta et al 2003, Perez-Elias et al 2003) revealed that there were no significant differences in the likelihood of achieving plasma HIV RNA below the level of detection between the two treatment arms at 24 weeks (Perez-Elias et al 2003) or 48 weeks (Mazzotta et al 2003, Perez-Elias et al 2003).

In the remaining two studies (Baxter et al 2000, Durant et al 1999) which specified this outcome as a secondary endpoint, Baxter et al (2000) showed a significant difference between genotyping and standard of care at four weeks (RR=1.98, 95% CI: 1.22, 3.22; NNT=5, 95% CI: 3, 13), eight weeks (RR=2.19, 95% CI: 1.39, 3.45; NNT=3, 95% CI: 2, 7) and the average of four and eight weeks (RR=1.92, 95% CI: 1.10, 3.36; NNT=6, 95% CI: 3, 33) but not at 12 weeks (RR=1.56, 95% CI: 0.91, 2.67). Durant et al (1999) showed a significant difference between the genotyping and standard of care arms at six months (RR=2.32, 95% CI: 1.02, 5.26; NNT=6, 95% CI: 3, 33) but not at three months (RR=2.09, 95% CI: 0.91, 4.82).

The results of this analysis show a great deal of variation across the studies in terms of the proportion of patients achieving plasma HIV RNA below the level of detection. Baxter et al (2000) and Cingolani et al (2002) showed that genotyping was effective in achieving this outcome at earlier time points but that this effect was not maintained and Durant et al (1999) and Tural et al (2002) showed that whilst the proportion of patients achieving this outcome at three months was not statistically significantly different between treatment arms, a significant difference was observed at six months.

Table 27 Proportion of patients achieving undetectable plasma viral RNA levels – intention to treat analysis

Study	Plasma HIV RNA below level of detection[a]	Genotype n/N (%)	Comparator		Relative risk (95% CI)	NNT (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Baxter et al (2000) GART	<500 copies/ml at 4 weeks[b]	35/78 (44.9)	17/75 (22.7)		1.98 (1.22, 3.22)	5 (3, 13)
	<500 copies/ml at 8 weeks[b]	41/78 (52.6)	18/75 (24.0)		2.19 (1.39, 3.45)	3 (2, 7)
	<500 copies/ml average of 4 and 8 weeks[b]	28/78 (35.9)	14/75 (18.7)		1.92 (1.10, 3.36)	6 (3, 33)
	<500 copies/ml at 12 weeks[b]	26/78 (33.3)	16/75 (21.3)		1.56 (0.91, 2.67)	NA
Cingolani et al (2002) ARGENTA	<500 copies/ml at 3 months[c]	23/85 (27.1)	11/89 (12.4)		2.19 (1.14, 4.21)	7 (4, 33)
	<500 copies/ml at 6 months[c]	18/85 (21.2)	15/89 (16.9)		1.26 (0.68, 2.33)	NA
Durant et al (1999) Viradapt	<200 copies/ml at 3 months[b]	19/65 (29.2)	6/43 (14.0)		2.09 (0.91, 4.82)	NA
	<200 copies/ml at 6 months[b]	21/65(32.3)	6/43 (14.0)		2.32 (1.02, 5.26)	6 (3, 33)
Mazzotta et al (2003) Gen-Phe-Rex	<400 copies/ml at 4 weeks[b]	10/100 (10.0)		11/101 (11.0)	0.92 (0.41, 2.07)	NA
	<400 copies/ml at 16 weeks[b]	10/100 (10.0)		8/101 (8.0)	1.26 (0.52, 3.07)	NA
	<400 copies/ml at 32 weeks[b]	9/100 (9.0)		7/101 (7.0)	1.30 (0.50, 3.35)	NA
	<400 copies/ml at 48 weeks[c]	8/100 (8.0)		5/101 (5.0)	1.62 (0.55, 4.77)	NA
Meynard et al (2002) NARVAL	<200 copies/ml at 12 weeks[c]	82/192 (42.7)	55/159 (34.6)	65/190 (34.2)	Genotype vs SOC 1.23 (0.94, 1.62)	NA
					Genotype vs phenotype 1.25 (0.97, 16.61)	NA
	<200 copies/ml at 12 and 24 weeks[b]	56/192 (29.2)	31/159 (19.5)	42/190 (22.1)	Genotype vs SOC 1.50 (1.02, 2.20)	10 (5, 100)
					Genotype vs phenotype 1.32 (0.93, 1.87)	NA
	<200 copies/ml at 12, 24 and 36 weeks[b]	38/192 (19.8)	23/159 (14.5)	29/190 (15.3)	Genotype vs SOC 1.37 (0.85, 2.20)	NA
					Genotype vs phenotype 1.30 (0.84, 2.01)	NA
	<400 copies/ml at week 24[c,d]	78/161 (48.4)	60/165 (36.4)		1.33 (1.03, 1.72)	8 (4, 100)

Table 27 (cont'd) Proportion of patients achieving undetectable plasma viral RNA levels – intention to treat analysis

Study	Plasma HIV RNA below level of detection[a]	Genotype n/N (%)	Comparator		Relative risk (95% CI)	NNT (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Perez-Elias et al (2003)	<400 copies/ml at 24 weeks[c]	77/151 (51.0)		65/149 (43.6)	1.17 (0.92, 1.49)	NA
	<400 copies/ml at 48 weeks	65/151 (43.0)		50/149 (33.6)	1.28 (0.96, 1.72)	NA
Tural et al (2002) Havana	<400 copies/ml at week 12[b,d]	88/161 (54.6)	77/165 (46.7)		1.17 (0.94, 1.45)	NA

[a] Levels of detection varied between studies
[b] Secondary endpoint of study
[c] Primary endpoint of study
[d] Numbers of patients include those with and without expert advice
Abbreviations: 95% CI, 95% confidence interval; NA, not applicable since not statistically significant; NNT, number needed to treat; SOC, standard of care
Shading: not applicable

Due to the variation observed between studies and the fact that some of the studies may not have been powered to detect a difference in the proportion of patients with plasma HIV RNA below the level of detection at three and six months, a meta-analysis was performed to determine the effectiveness of genotype resistance testing compared with standard of care in achieving an undetectable viral load at three months (Figure 5). Five studies were included in the analysis which revealed that patients receiving genotype-guided treatment were 1.3 times more likely to achieve plasma HIV RNA below the level of detection than patients treated by standard of care (RR=1.33, 95% CI: 1.14, 1.56; NNT=10, 95% CI: 6, 20).

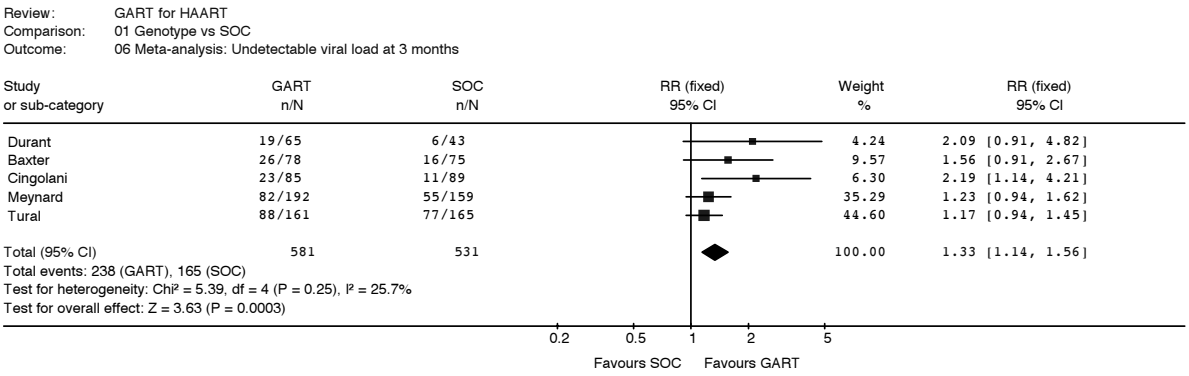

Figure 5 Meta-analysis of the proportion of patients with plasma HIV RNA below the level of detection at three months

Figure 6 shows the results of a meta-analysis to determine the effectiveness of genotype resistance testing compared with standard of care in achieving an undetectable viral load at six months. Three studies were included in the analysis and the results revealed that patients receiving genotype-guided treatment were 1.4 times more likely to achieve plasma HIV RNA below the level of detection than patients treated by standard of care (RR=1.41, 95% CI: 1.12, 1.77; NNT=9, 95% CI: 6, 25).

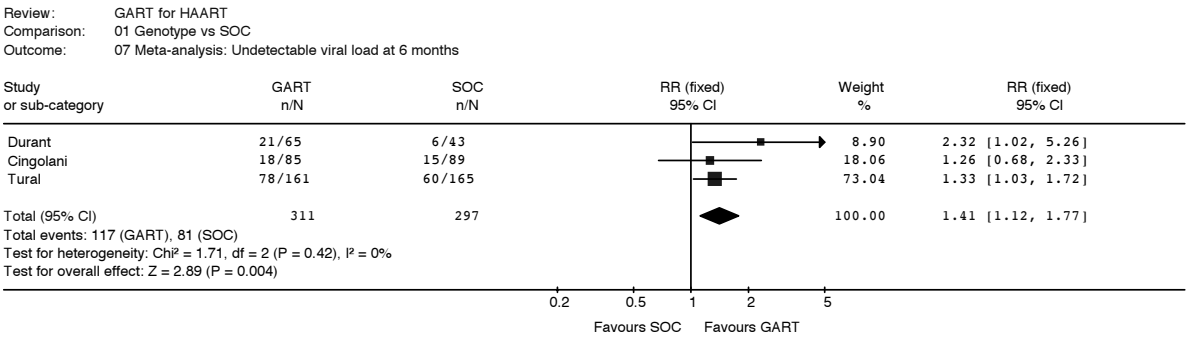

Figure 6 Meta-analysis of the proportion of patients with plasma HIV RNA below the level of detection at six months

Although the studies appeared to have a great deal of variation for the outcome of plasma HIV RNA below the level of detection (Table 27), the forest plots (Figures 5 and 6) show that each of the trials had similar confidence intervals and that while some studies did not reach statistical significance, they each had a trend favouring genotype over standard of care. An additional measure of heterogeneity between studies is the I^2 statistic. The meta-analysis for data at three months (Figure 5) resulted in an I^2 value of 25.7 per cent and at six months (Figure 6) I^2 was zero per cent. An I^2 value of less than 50 per cent suggests that there is no significant heterogeneity between the studies (Alderson et al 2003).

Two studies completed sub-group analysis (Cingolani et al 2002, Tural et al 2002). Cingolani et al (2002) analysed the proportion of patients in the genotype and standard of care arms who were failing their first or second HAART regimen and those failing their third (Table 28). Patients failing their first or second HAART regimen were twice as likely to achieve a viral load below the level of detection if the HAART regimen was prescribed based on genotyping results than if based on standard of care at three months (RR=2.01, 95% CI: 1.04, 3.89; NNT=6 95% CI: 3, 100), however this benefit was not sustained at six months (RR=1.24, 95% CI: 0.67, 2.30). No significant differences between the treatment arms were observed at three or six months for patients failing their third HAART regimen (RR= 6.84, 95% CI: 0.39, 119.46) and (RR=3.80, 95% CI: 0.19, 74.6), respectively.

Table 28 Proportion of patients achieving undetectable plasma viral RNA levels – subgroup analysis by treatment history

Study	Patient group	Plasma HIV RNA <500 copies/ml	Genotype n/N (%)	SOC n/N (%)	Relative risk (95% CI)	NNT (95% CI)
Cingolani et al (2002) ARGENTA	Patients failing first or second regimen	3 months	19/61 (31.1)	11/71 (15.5)	2.01 (1.04, 3.89)	6 (3, 100)
		6 months	16/61 (26.2)	15/71 (21.1)	1.24 (0.67, 2.30)	NA
	Patients failing three or more regimens	3 months	4/24 (16.7)	0/18 (0.0)	6.84 (0.39, 119.46)	NA
		6 months	2/24 (8.3)	0/18 (0.0)	3.80 (0.19, 74.6)	NA

[Source: Cingolani et al 2002)
Abbreviations: SOC, standard of care; 95% CI, 95% confidence interval; NNT, number needed to treat; NA, not applicable since not statistically significant

The effectiveness of expert advice on patients achieving undetectable plasma HIV RNA levels was analysed by Tural et al (2002). As summarised in Table 29, the proportion of patients with an undetectable viral load receiving expert advice (in addition to or without genotype testing) was not significantly different from patients not receiving expert advice

(in addition to or without genotype testing) at 12 weeks (RR=1.10, 95% CI: 0.89, 1.37) and 24 weeks (RR=1.25, 95% CI: 0.96, 1.61).

Table 29 Proportion of patients achieving undetectable plasma viral RNA levels – subgroup analysis by expert advice

Study	Plasma HIV RNA <400 copies/ml	Expert advice n/N (%)	No expert advice n/N (%)	Relative risk (95% CI)	NNT (95% CI)
Tural et al (2002) Havana	12 weeks	87/164 (53.0)	78/162 (48.1)	1.10 (0.89, 1.37)	NA
	24 weeks	77/164 (47.0)	61/162 (37.7)	1.25 (0.96, 1.61)	NA

[Source: Tural et al 2002)
Abbreviations: 95% CI, 95% confidence interval; NA, not applicable since not statistically significant; NNT, number needed to treat; SOC, standard of care

In addition to analysing the effect of expert advice on achieving plasma HIV RNA below the level of detection, Tural et al (2002) also analysed the proportion of patients who had failed one, two or three HAART regimens, irrespective of the treatment group they had been assigned to in the trial. Table 30 summarises this data and shows that there were no statistically significant differences between patients failing one or two previous HAART regimens in achieving an undetectable viral load at 12 weeks (RR=0.98, 95% CI: 0.77, 1.25) or 24 weeks (RR=1.10, 95% CI: 0.83, 1.47).

However, significant differences were observed between patients who had failed one or two compared to three previous HAART regimens. Patients having failed one HAART regimen were more likely to achieve an undetectable viral load at 12 weeks (RR=1.61, 95% CI: 1.25, 2.08) and 24 weeks (RR=1.88, 95% CI: 1.40, 2.53) than patients who had failed three regimens. Similarly, patients who had failed two HAART regimens were more likely to achieve an undetectable viral load at 12 weeks (RR=1.65, 95% CI: 1.30, 2.10) and 24 weeks (RR=1.71, 95% CI: 1.27, 2.30) than patients who had failed three regimens.

Table 30 Proportion of patients achieving undetectable plasma viral RNA levels – subgroup analysis by treatment history

Study	Plasma HIV RNA <400 copies/ml	Patients failing n/N (%)			Relative risk (95% CI)	
		First regimen	Second regimen	Third regimen		
Tural et al (2002) Havana	12 weeks	41/64 (64.1)	51/78 (65.4)	73/184 (39.7)	1st vs 2nd failure:	0.98 (0.77, 1.25)
					1st vs 3rd failure:	1.61 (1.25, 2.08)
					2nd vs 3rd failure:	1.65 (1.30, 2.10)
	24 weeks	38/64 (59.4)	42/78 (53.8)	58/184 (31.5)	1st vs 2nd failure:	1.10 (0.83, 1.47)
					1st vs 3rd failure:	1.88 (1.40, 2.53)
					2nd vs 3rd failure:	1.71 (1.27, 2.30)

[Source: Tural et al 2002)

Change in plasma HIV RNA levels

In addition to achieving plasma HIV RNA below the level of detection, the mean change in viral load was also reported and was the primary outcome in two studies (Baxter et al 2000, Durant et al 1999). Table 31 summarises the mean changes in viral load in patients whose treatment was guided by genotyping, standard of care and drug-susceptibility phenotyping. The two studies in which this outcome was the primary endpoint of the

trial showed significant differences between the genotype and standard of care arms in the trials.

Baxter et al (2000) showed a significant difference between the genotype and standard of care groups for the mean change in viral load from baseline to the average of the values at four and eight weeks. Patients in the genotype group reduced their viral load by an average of –0.58 $\log_{10}$copies/ml (95% CI: –0.83, –0.33) more than patients treated by standard of care. Similar treatment differences were observed between the two groups at four weeks (–0.51, 95% CI: –0.76, –0.26), eight weeks (–0.60, 95% CI: 0.88, –0.32) and 12 weeks (–0.47, 95% CI: –0.75, –0.19).

Similarly, Durant et al (1999) showed that patients receiving treatment that was guided by genotype results had a significantly greater decrease in viral load at three months (–0.58 $\log_{10}$copies/ml, 95% CI: –1.01, –0.15) and six months (–0.48 $\log_{10}$copies/ml, 95% CI: –0.95, –0.01) than patients treated by standard of care.

Of the studies where the mean change in viral load was a secondary endpoint measure, only Tural et al (2002) showed that patients receiving genotype-guided treatment had a significantly greater reduction in viral load than patients treated by standard of care at 24 weeks (–0.21 $\log_{10}$copies/ml, 95% CI: –0.38, –0.04). A lack of statistical difference in the other studies may have resulted in a lack of power to detect any differences. Meynard et al (2002) showed no significant differences between patients receiving genotype-guided therapy or drug-susceptibility phenotype-guided therapy (–0.02, 95% CI: –0.23, 0.19).

Table 31 Change in plasma viral RNA levels

Study	Change in viral load	Genotype log_{10}copies/ml	Comparator		Treatment difference (95% CI)
			SOC log_{10}copies/ml	Phenotype log_{10}copies/ml	
Baxter et al (2000) GART[a]	Mean (SD) change from baseline to 4 weeks[c]	–1.26 (0.79) n=77	–0.75 (0.77) n=73		–0.51 (–0.76, –0.26)
	Mean (SD) change from baseline to 8 weeks[c]	–1.12 (0.95) n=75	–0.52 (0.77) n=73		–0.60 (–0.88, –0.32)
	Mean (SD) change from baseline to the average of 4 and 8 weeks[b]	–1.19 (0.79) n=77	–0.61 (0.78) n=75		–0.58 (–0.83, –0.33)
	Mean (SD) change from baseline to week 12[c]	–0.94 (0.96) n=76	–0.47 (0.76) n=72		–0.47 (–0.75, –0.19)
Cingolani et al (2002) ARGENTA	Mean (SD) change from baseline to month 3[c]	–0.62 (1.16) n=85	–0.38 (0.96) n=89		–0.24 (–0.56, 0.08)
	Mean (SD) change from baseline to month 6[c]	–0.57 (1.09) n=85	–0.39 (1.04) n=89		–0.18 (–0.50, 0.14)
Durant et al (1999) Viradapt[a]	Mean (SD) change from baseline to month 3[b]	–1.04 (1.13) n=65	–0.46 (1.11) n=43		–0.58 (–1.01, –0.15)
	Mean (SD) change from baseline to month 6[b]	–1.15 (1.20) n=65	–0.67 (1.25) n=43		–0.48 (–0.95, –0.01)
Mazzotta et al (2003) Gen-Phe-Rex					
Meynard et al (2002) NARVAL	Mean (SD) change from baseline to week 12	–0.95 (1.03) n=186	–0.76 (1.01) n=152	0.93 (1.07) n=187	Genotype vs SOC –0.19 (–0.41, 0.03)
					Genotype vs drug-susceptibility phenotype –0.02 (–0.23, 0.19)
Perez-Elias et al (2003)	Median decrease from baseline to week 24[c]	1.3 n=137		1.0 n=139	NA
Tural et al (2002) Havana	Mean (SD) change from baseline to week 12[c,d]	–0.92 (0.8) n=161	–0.80 (0.7) n=165		–0.12 (–0.28, 0.04)
	Mean (SD) change from baseline to week 24 [c,d]	–0.84 (0.8) n=161	–0.63 (0.8) n=165		–0.21 (–0.38, –0.04)

[a] Presented as mean (standard error) which was converted to mean (standard deviation)
[b] Primary endpoint of study
[c] Secondary endpoint of study
[d] Numbers of patients include those with and without expert advice
Abbreviations: NA, could not calculate as the standard deviation was not reported
Shading: light grey, not reported; dark grey, not applicable

Meta-analyses were carried out to estimate the effectiveness of genotype-guided therapy in reducing viral load compared with standard of care at three and six months. The meta-analyses were performed using a weighted mean difference as each of the studies measured viral load on the same scale of log_{10}copies/ml (Alderson et al 2003). Figures 7 and 8 depict the meta-analyses performed with results of the mean changes in viral load between the two groups at three and six months, respectively. Overall, patients receiving genotype-guided therapy had a significantly greater reduction in viral load at three months (–0.23 log_{10}copies/ml, 95% CI: –0.34, –0.12) and this benefit was sustained at six months (–0.23 log_{10}copies/ml, 95% CI: –0.37, –0.08) compared with patients receiving

treatment based on standard of care. The results of each of the studies were homogenous as the I^2 values for the three- and six-month meta-analyses were 45 and zero per cent, respectively.

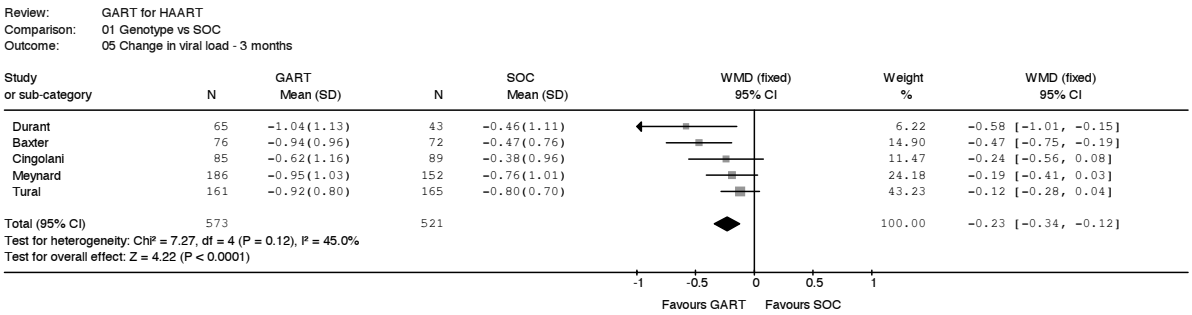

Figure 7 Meta-analysis of the mean change in HIV RNA at three months

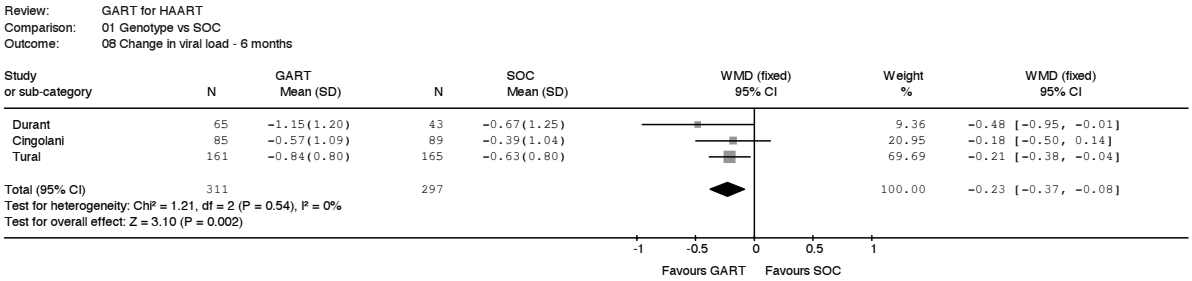

Figure 8 Meta-analysis of the mean change in HIV RNA at six months

Changes in CD4+ cell counts

Table 32 summarises the changes in CD4+ cell count experienced by patients in the genotype, standard of care and drug-susceptibility phenotype arms of the trials. Many of the studies did not report standard deviations around the mean change from baseline in viral load for the treatment groups which precluded analysis of the differences and the performance of meta-analyses. Analysis of the changes in CD4+ cell count between patients receiving treatment based on genotyping or standard of care from data reported by Durant et al (1999) showed that patients receiving genotype-guided therapy had a significant increase in CD4+ cell counts compared with patients treated by standard of care at three months (treatment difference [genotype - standard of care]=18 cells/μl, 95% CI: 10.44, 25.56). However, at six months, patients receiving treatment by standard of care had a significantly greater increase in CD4+ cell number compared with patients receiving genotype-guided therapy (treatment difference = –12 cells/μl, 95% CI: –19.50, –4.50). Meynard et al (2002) did not report any significant differences between an increase in CD4+ cell count for the genotype and standard of care groups at 12 weeks (treatment difference=–13 cells/μl, 95% CI: –34.64, 8.64), however patients in the drug-susceptibility phenotype-guided arm experienced a significantly greater increase in CD4+ cell counts than the genotype arm (treatment difference= –26 cells/μl, 95% CI: –46.98, –5.02).

Table 32 Changes in CD4+ cells – secondary outcome measure

Study	CD4+ cell number	Genotype cells/μl	Comparator		Treatment difference (95% CI)
			SOC cells/μl	Phenotype cells/μl	
Baxter et al (2000) GART[a]	Average change from baseline to the average of 4 and 8 weeks	+23 n=77	+22 n=75		NA
	Average change from baseline to week 12	+25 n=76	+18 n=72		NA
Cingolani et al (2002) ARGENTA	Mean (95% CI) change from baseline to month 3	+9 (–18, +27) n=85	+19 (–2, +39) n=89		NA
	Mean (95% CI) change from baseline to month 6	+15 (–10, +39) n=85	+22 (–4, +49) n=89		NA
Durant et al (1999) Viradapt	Mean (SD) change from baseline to month 3	+36 (19) n=65	+18 (20) n=43		18 (10.44, 25.56)
	Mean (SD) change from baseline to month 6	+21 (18) n=65	+33 (21) n=43		–12 (–19.50, –4.50)
Mazzotta et al (2003) Gen-Phe-Rex	Mean change from baseline to week 4	+42.7 n=81		+48.4 n=82	NA
	Mean change from baseline to week 16	+52.6 n=77		+43.0 n=75	NA
	Mean change from baseline to week 32	+77.7 n=76		+22.7 n=77	NA
	Mean change from baseline to week 48	+94.4 n=64		+41.6 n=60	NA
Meynard et al (2002) NARVAL	Mean (SD) change from baseline to week 12	14 (113) n=186	27 (83) n=152	40 (92) n=187	Genotype vs SOC –13 (–34.64, 8.64)
					Genotype vs Drug-susceptibility phenotype –26 (–46.98, –5.02)
Perez-Elias et al (2003)	Median at week 24	391 n=137		407 n=139	NA
Tural et al (2002) Havana					

[a] Uncertainty about number of patients
Abbreviations: NA, not applicable – could not calculate due to standard deviations, standard errors, or variance not being reported
Shading: light grey, not reported; dark grey, not applicable

Number and/or combinations of drugs prescribed

There were substantial differences in several trials that reported the number and/or combinations of drugs prescribed in each of the treatment arms (Table 33).

Results from the analysis of data from Baxter et al (2000) showed that there were significantly fewer patients in the genotype arm compared with the standard of care arm who had been prescribed three or fewer drugs (RR=0.46, 95% CI: 0.30, 0.72), three new drugs (RR=0.53, 95% CI: 0.32, 0.87), a regimen including NRTIs and PIs (RR=0.50, 95% CI: 0.30, 0.81) and, more specifically, a regimen including NRTIs and a single PI (RR=0.38, 95% CI: 0.18, 0.82).

Conversely, significantly more patients in the genotyping compared with the standard of care arm were prescribed five or more antiretrovirals (RR=4.42, 95% CI: 1.77, 11.0), four

or more drugs to which patients were naïve (RR=2.19, 95% CI: 1.16, 4.12), a regimen including NRTIs, NNRTIs and two PIs (RR=3.85, 95% CI: 1.79, 8.27), a regimen containing an NNRTI (RR=1.41, 95% CI: 1.11, 1.79) and hydroxyurea (RR=3.53, 95% CI: 1.81, 6.86). There were no significant differences between genotyping and standard of care arms in the prescription of four drugs (RR= 1.16, 95% CI0.79, 1.69), two or few newer drugs (RR=1.05, 95% CI: 0.74, 1.49), all three drug classes (RR=1.31, 95% CI: 0.98, 1.75), a regimen including NRTIs, NNRTIs and a single PI (RR=0.69, 95% CI: 0.44, 1.11), a regimen containing NRTIs and two PIs (RR=0.67, 95% CI: 0.30, 1.46), a regimen containing NRTIs and NNRTIs (RR=3.37, 95% CI: 0.72, 15.69), other regimens (RR=1.25, 95% CI: 0.35, 4.48) or NRTIs (RR=0.99, 95% CI: 0.91, 1.07) or PIs (RR=0.92, 95% CI: 0.85, 1.00).

Cingolani et al (2002) reported that the number of active drugs (defined as drugs to which the patients do not have resistance-associated mutations) prescribed to patients in the genotype and standard of care arms did not differ significantly. Patients in the genotype group had a mean of 2.3 active drugs at both 3 and 6 months, and patients in the standard of care group had a mean of 2.1 and 2.2 active drugs at 3 and 6 months, respectively (Cingolani et al 2002). The mean genotypic sensitivity scores for the genotype and standard of care groups was 1.8 and 1.7, respectively. Genotypic sensitivity scores were calculated by designating a sensitivity score of one to each drug in the regimen that was not associated with primary mutations.

Durant et al (1999) reported that significantly fewer patients receiving genotype-guided therapy were prescribed two NRTIs and one PI (RR=0.59, 95% CI: 0.36, 0.99) compared with the patients treated by standard of care. No significant differences were observed between the two treatment groups in the prescription of two NRTIs and two PIs (RR=1.12, 95% CI: 0.63, 1.97), one NRTI and two PIs (RR= 1.98, 95% CI: 0.42, 9.38), one drug from each class (RR=1.59, 95% CI: 0.60, 4.19), two NRTIs and one NNRTI (RR=1.98, 95% CI: 0.21, 18.46) or other regimens (RR=1.32, 95% CI: 0.25, 6.91), where other regimens included two PIs and three or four NRTIs, two PIs and one NNRTI, or two or three NRTIs and one NNRTI.

Meynard et al (2002) reported that significantly fewer patients in the genotype compared with the standard of care arm were prescribed at least three new drugs (RR=0.36, 95% CI: 0.26, 0.48) or prescribed drugs belonging to the three different classes (RR=0.53, 95% CI: 0.41, 0.70) No significant differences between the genotyping and drug-susceptibility phenotyping arms were observed for prescription of at least three new drugs (RR=0.99, 95% CI: 0.66, 1.48) or prescription of drugs from the three classes (RR=1.11, 95% CI: 0.80, 1.55).

Tural et al (2002) reported that there were no significant differences in the number of drugs included in the prescribed therapies for patients in the genotype group and no-genotype group. They were four (standard deviation, 0.9) for the genotype group and four (standard deviation, 0.8) for the no-genotype group.

No significant differences were reported by Mazzotta et al (2003) for the combinations of drugs prescribed to patients in the virtual and drug-susceptibility phenotyping arms (Table 33). Similarly, Perez-Elias et al (2003) found no significant differences between the use of ritonavir to boost the prescribed PI (RR=1.09, 95% CI: 0.96, 1.24) or the prescription of PI-sparing regimens (RR=0.82, 95% PI 0.60, 1.11) between the drug-susceptibility and virtual phenotyping groups.

Table 33 Number and/or combinations of drugs prescribed in each treatment group – intention to treat analysis

Study	Drug combinations	Genotype n/N (%)	Comparator		RR (95% CI)	RD (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Baxter et al (2000) GART	Three or fewer	20/78 (25.6)	41/75 (54.7)		0.46 (0.30, 0.72)	-0.29 (-0.44, -0.14)
	Four	35/78 (44.9)	29/75 (38.7)		1.16 (0.79, 1.69)	0.06 (-0.09, 0.22)
	Five or more	23/78 (29.5)	5/75 (6.7)		4.42 (1.77, 11.0)	0.23 (0.11, 0.34)
	Two or fewer new[a]	36/78 (46.2)	33/75 (44.0)		1.05 (0.74, 1.49)	0.02 (-0.14, 0.18)
	Three new[a]	17/78 (21.8)	31/75 (41.3)		0.53 (0.32, 0.87)	-0.19 (-0.34, -0.05)
	Four or more new[a]	25/78 (32.1)	11/75 (14.7)		2.19 (1.16, 4.12)	0.17 (0.04, 0.30)
	NRTI, NNRTI, PI	49/78 (62.8)	36/75 (48.0)		1.31 (0.98, 1.75)	0.15 (-0.007, 0.30)
	NRTI, NNRTI, 1 PI	21/78 (26.9)	29/75 (38.7)		0.69 (0.44, 1.11)	-0.12 (-0.27, 0.03)
	NRTI, NNRTI, 2 PI	28/78 (35.9)	7/75 (9.3)		3.85 (1.79, 8.27)	0.27 (0.14, 0.39)
	NRTI, PI	17/78 (21.8)	33/75 (44.0)		0.50 (0.30, 0.81)	-0.22 (-0.37, -0.08)
	NRTI, 1 PI	8/78 (10.3)	20/75 (26.7)		0.38 (0.18, 0.82)	-0.16 (-0.28, -0.43)
	NRTI, 2 PI	9/78 (11.5)	13/75 (17.3)		0.67 (0.30, 1.46)	-0.06 (-0.17, 0.05)
	NRTI, NNRTI	7/78 (9.0)	2/75 (2.7)		3.37 (0.72, 15.69)	0.06 (-0.01, 0.14)
	Other regimens	5/78 (6.4)	4/75 (5.3)		1.25 (0.35, 4.48)	0.01 (-0.06, 0.09)
	NRTI	73/78 (93.6)	71/75 (94.7)		0.99 (0.91, 1.07)	-0.01 (-0.09, 0.06)
	PI	70/78 (89.7)	73/75 (97.3)		0.92 (0.85, 1.00)	-0.08 (-0.15, 0.001)
	NNRTI	60/78 (76.9)	41/75 (54.7)		1.41 (1.11, 1.79)	0.22 (0.07, 0.37)
	Hydroxyurea	33/78 (42.3)	9/75 (12.0)		3.53 (1.81, 6.86)	0.30 (0.17, 0.44)
Cingolani et al (2002) ARGENTA						
Durant et al (1999) Viradapt	2 NRTI, 1 PI	18/65 (27.7)	20/43 (46.5)		0.59 (0.36, 0.99)	-0.19 (-0.37, -0.003)
	2 NRTI, 2 PI	22/65 (33.8)	13/43 (30.2)		1.12 (0.63, 1.97)	0.04 (-0.14, 0.22)
	1 NRTI, 2 PI	6/65 (9.2)	2/43 (4.7)		1.98 (0.42, 9.38)	0.05 (-0.05, 0.14)
	1 NRTI, 1 NNRTI, 1 PI	12/65 (18.5)	5/43 (11.6)		1.59 (0.60, 4.19)	0.07 (-0.66, 0.20)
	2 NRTI, 1 NNRTI	3/65 (4.6)	1/43 (2.3)		1.98 (0.21, 18.46)	0.02 (-0.05, 0.09)
	Other combinations[b]	4/65 (6.2)	2/43 (4.7)		1.32 (0.25, 6.91)	0.02 (-0.07, 0.10)
Mazzotta et al (2003) Gen-Phe-Rex	NNRTI	10/86		10/87	1.01 (0.44, 2.31)	-0.00 (-0.09, 0.10)
	PI	49/86		56/87	0.89 (0.70, 1.13)	-0.07 (-0.22, 0.07)
	RTV-boosted	43/49		43/56	1.14 (0.96, 1.37)	0.11 (-0.04, 0.25)
	Triple class	8/86		6/87	1.35 (0.49, 3.72)	0.02 (-0.06, 0.11)
	Other regimens	19/86		15/87	1.28 (0.70, 2.35)	0.05 (-0.07, 0.17)

Table 33 (cont'd) Number and/or combinations of drugs prescribed in each treatment group – intention to treat analysis

Study	Drug combinations	Genotype n/N (%)	Comparator		RR (95% CI)	RD (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Meynard et al (2002) NARVAL	At least three new drugs	38/192 (19.8)	87/159 (54.7)	38/190 (20.0)	Genotype vs SOC 0.36 (0.26, 0.48)	-0.35 (-0.44, -0.25)
					Genotype vs Drug-susceptibility phenotype 0.99 (0.66, 1.48)	-0.002 (-0.08, 0.08)
	Drugs belonging to three different classes	54/192 (28.1)	84/159 (52.8)	48/190 (25.3)	Genotype vs SOC 0.53 (0.41, 0.70)	-0.24 (-0.35, -0.15)
					Genotype vs Drug-susceptibility phenotype 1.11 (0.80, 1.55)	0.03 (-0.06, 0.12)
Perez-Elias et al (2003)[c]	PI boosted with ritonavir	119/151 (78.8)		108/149 (72.5)	1.09 (0.96, 1.24)	0.06 (-0.03, 0.16)
	PI-sparing regimens	48/151 (31.8)		58/149 (38.9)	0.82 (0.60, 1.11)	-0.07 (-0.18, 0.04)
Tural et al (2002) Havana						

[a]Drugs to which the patient was naïve, including hydroxyurea
[b]Other combinations includes 2 PI and 3 or 4 NRTI; 2 PI and 1 NNRTI; 2 or 3 NRTI and 1 NNRTI
[c]Numbers of patients receiving treatments include more than those included in trial
Abbreviations: NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor
Shading: light grey, not reported; dark grey, not applicable

Drug toxicities associated with HAART regimens prescribed according to treatment arm

No significant differences in the number of drug toxicity adverse events occurred between any of the treatment groups: genotype versus standard of care [RR=1.32, 95% CI: 0.56, 3.10 (Baxter et al 2000) and RR=0.99, 95% CI: 0.30, 3.31 (Durant et al 1999)]; or virtual versus drug-susceptibility phenotype: [RR=0.25, 95% CI: 0.03, 2.22 (Mazzotta et al 2003)] (Table 34). Meynard et al (2002) did not specify the nature of the adverse events reported, however no differences were observed in the occurrence of adverse events between the genotype and standard of care arms (RR=0.74, 95% CI: 0.51, 1.07) or the genotype and drug-susceptibility phenotype arms (RR=0.86, 95% CI: 0.59, 1.25).

Mazzotta et al (2003) reported that 25 patients were no longer on prescribed HAART at the end of the study due to adverse events. Tural et al (2002) reported that 22/326 (6.7%) patients experienced drug-related adverse events. The numbers of patients experiencing these events in each of the treatment groups was not reported. Perez-Elias et al (2003) reported no drug-related adverse events.

Table 34 Adverse events occurring in each treatment group through the course of the studies

Study	Event	Genotype n/N (%)	Comparator		Relative risk (95% CI)	NNH (95% CI)
			SOC n/N (%)	Phenotype n/N (%)		
Baxter et al (2000) GART	Discontinuation due to drug toxicity	11/78 (14.1)	8/75 (10.7)		1.32 (0.56, 3.10)	NA
Cingolani et al (2002) ARGENTA						
Durant et al (1999) Viradapt	Treatment modification due to drug related side effects	6/65 (9.2)	4/43 (9.3)		0.99 (0.30, 3.31)	NA
Mazzotta et al (2003) Gen-Phe-Rex	Treatment interruption due to drug toxicity	1/100 (1.0)		4/101 (4.0)	0.25 (0.03, 2.22)	NA
Meynard et al (2002) NARVAL	Severe adverse events[a]	40/192 (20.8)	45/159 (28.3)	46/190 (24.2)	Genotype vs SOC 0.74 (0.51, 1.07) Genotype vs drug-susceptibility phenotype 0.86 (0.59, 1.25)	NA
Perez-Elias et al (2003)						
Tural et al (2002) Havana						

[a]The nature of the adverse events was not reported
Abbreviations: NA not applicable since not statistically significant; NND, number needed to harm; SOC, standard of care
Shading: light grey, not reported; dark grey, not applicable

Additional outcomes reported in studies:

Baxter et al (2000) performed subgroup analysis to examine the effects of several baseline characteristics of the patients on the effectiveness of genotype-guided therapy compared with standard of care in terms of a change in viral load from baseline. The effectiveness of genotype-guided therapy remained consistent across the baseline subgroups defined by the PI in existing regimen prior to change, CD4+ cell count, baseline HIV viral load, number of prior PIs used before, the presence or absence of major drug resistance-associated mutations and the inclusion of NNRTIs in the new regimen. The mean decrease in viral load ranged from –0.3 to –0.8 $\log_{10}$copies/ml for each of the subgroups examined. The authors also performed subgroup analysis on post-randomisation characteristics and found that the viral load response was associated with the number of active drugs prescribed with each additional active drug associated with a –0.37 $\log_{10}$ (95% CI: –0.51, –0.22) change and each inactive drug associated with a –0.17 $\log_{10}$ change (95% CI: –0.34, 0.01). Examination of the effects of the implementation of treatment recommendations by the experts showed that in centres where greater than 80 per cent of the patients received therapies recommended by the expert panel there was a significantly greater decrease in viral load than in centres where fewer than 60 per cent were prescribed one of the suggested regimens.

Cingolani et al (2002) examined possible predictors of virologic success using bivariate logistic regression and found that transmission through injecting drug users, a greater number of experienced HAART regimens, a greater baseline viral load, patient-reported

non-adherence, the presence of protease mutation L90M and the total number of PI mutations were all associated with a decreased odds ratio of achieving a viral load of 500 copies/ml at three months. Conversely, a previous history of a viral load of less than 500 copies/ml and the absence of drug resistance-associated mutations to all drugs in the regimen were associated with an increased odds ratio of achieving a viral load of 500 copies/ml at three months.

Mazzotta et al (2003) reported that using univariate analysis with intention-to-treat with last observation carried forward, the variables associated with a plasma HIV RNA level of less than 400 copies/ml were baseline CD4+ cell count, baseline viral load, adherence and the number of drugs in the regimen to which the patients remained susceptible. Using on-treatment analysis, adherence was the only factor independently associated with virologic outcome.

Meynard et al (2002) assessed plasma drug concentrations in a subset of the trial population at week 12. A significant difference between the genotyping and standard of care arms was observed with respect to the percentage of patients in each arm having effective plasma drug concentrations of all PIs and NNRTIs evaluated – more of the patients receiving genotype-guided therapy had effective drug concentrations than those treated by standard of care.

Perez-Elias et al (2003) performed a separate analysis of each stratum. Patients with a history of use of only one or two classes of antiretrovirals showed a trend towards a better virologic response at 24 weeks in the drug-susceptibility and virtual phenotyping arms. In patients treated previously with all three classes, a greater benefit was seen in patients treated by virtual compared with drug-susceptibility phenotyping. An as-treated analysis was also performed as some patients in the drug-susceptibility and virtual phenotyping arms had treatment guided by virtual or drug-susceptibility phenotyping, respectively. Although the data were not shown, the authors reported that there was no difference between intention-to-treat and as-treated analysis.

Tural et al (2002) performed a multivariate analysis and observed that the factors associated with a higher probability of achieving a plasma viral load of less than 400 copies/ml at 24 weeks were HIV-1 genotyping and expert advice in patients failing a second antiretroviral regimen. Patients who had failed three or more regimens were more likely to have virologic failure, regardless of the treatment arm (genotyping or no genotyping) to which they were assigned. However, in patients who had failed three or more regimens, a significant difference in the mean decrease of plasma viral load in the combined 12- and 24-week analyses was observed between the genotyping and no genotyping arms (genotyping –0.84±0.9 and no genotyping –0.7±0.7), but not between the expert and no expert advice arms.

Summary of patient outcomes of genotypic resistance testing from randomised controlled trials

- The effectiveness of genotypic resistance testing in regard to patient outcomes was extracted from seven RCTs.
- The trials included antiretroviral-experienced adults and adolescents and were conducted in Europe and the USA. The majority of the RCTs were open-label in design.

- Methods of genotypic resistance testing and interpretation of the resistance patterns varied across studies.

- Measures of treatment outcome and length of follow-up were inconsistent across studies.

- There were no significant differences in the rates of death or AIDS-defining events between any of the treatment arms during the course of the studies. The lack of any differences may be due to the studies not being powered to observe a difference and the limited time (12–48 weeks) of follow-up.

- Results from the individual trials gave varying results on the effectiveness of genotype-resistance testing compared with standard of care for the proportion of patients achieving an undetectable viral load. The meta-analysis performed in this report showed that overall, genotype-guided therapy was more effective than therapy guided by standard of care for the proportion of patients achieving an undetectable viral load at three months (RR=1.33, 95% CI: 1.14, 1.56; NNT=10, 95% CI: 6, 20) and at six months (RR=1.41, 95% CI: 1.12, 1.77; NNT=9, 95% CI: 6, 25).

- Patients with extensive previous antiretroviral experience were less likely to achieve an undetectable viral load, regardless of genotypic resistance testing.

- Results from the individual trials gave varying results on the effectiveness of genotype-resistance testing compared with standard of care for the change in viral load measured in $\log_{10}$ copies/ml. The meta-analysis performed in this report showed that overall, genotype-guided therapy was more effective than therapy guided by standard of care for reducing plasma HIV RNA levels, measured in $\log_{10}$ copies/ml with a mean difference of –0.23 $\log_{10}$copies/ml (95% CI: –0.34, –0.12) at three months and –0.23 $\log_{10}$copies/ml (95% CI: –0.37, –0.08) at six months.

- No significant differences were observed for the outcomes of achieving an undetectable viral load or mean change in plasma HIV RNA levels between the genotyping and drug-susceptibility phenotyping arms, or between virtual and drug-susceptibility phenotyping at any time point examined.

- Changes in CD4+ cell counts from baseline were significantly different between genotyping and standard of care arms in two studies. One study showed that patients in the genotyping arm had a significantly greater increase in CD4+ cell count at three months, however at six months, patients treated by standard of care had a significantly greater increase in CD4+ cell count. Another study found no significant differences between the genotyping and standard of care arms at three months, however, a significant difference between the genotyping and drug-susceptibility phenotyping arms was evident at three months, with patients receiving drug-susceptibility phenotype-guided therapy achieving a significantly greater increase in CD4+ cell count.

- Some of the trials allowed genotypic resistance to be performed multiple times throughout the follow-up period in the event of sub-optimal virologic responses.

- Each of the studies used different methods to perform genotypic resistance testing and interpret the results of the tests. Results from an Australian quality assessment scheme have indicated that the assay is highly reproducible with less than a one per cent variation between identical samples in all laboratories. However, there is variability in the ability of different laboratories to detect mutations and mixtures of mutations, and the level of concordance in the interpretation of genotypic resistance testing results is dependent on the interpretation system used.

- Significant differences were found in the number and/or combinations of antiretroviral drugs prescribed in the genotyping and standard of care arms of several trials, however no differences were observed between the genotyping and phenotyping or the virtual versus drug-susceptibility phenotyping arms.

- One study observed no significant differences in the number of active drugs prescribed. This is surprising as genotypic resistance testing should have provided information regarding the drugs to which the patient's virus was susceptible.

- No significant differences in drug toxicity-related adverse events were observed in any of the treatment arms over the course of the studies.

The following key issues were identified:

- All patients enrolled were antiretroviral experienced and failing current therapy.

- No evidence was found for the effectiveness or otherwise of genotypic resistance testing in treatment-naïve patients, pregnant women or patients with discordant virologic responses.

- Six of the seven trials were open-label in design which may lead to bias.

- The follow-up period of the identified RCTs varied from 12 to 48 weeks. There are no long-term data on the clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV.

- All of the trials based their measure of clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV on virologic outcomes. A combination of virologic and immunologic responses to treatment is more effective at predicting clinical outcome of patients than virologic responses alone.

- Whilst no differences were found between the treatment arms for the rates of death and AIDS-defining events, it must be noted that these were not defined as primary outcomes in any of the RCTs. Thus, the studies may not have been powered to detect a difference in the proportion of patients who died or experienced an AIDS-defining event, nor were the studies long enough to detect differences over extended periods of time.

- HIV medicine is an evolving field and new antiretrovirals and treatments for HIV are being developed. The studies were performed at different times and in different countries where there may have been differences in the numbers and types of antiretrovirals available for use.

- The differences observed in the number and/or combinations of drugs prescribed between the genotype and standard of care arms in the trials make the incremental benefit of genotype-guided therapy difficult to distinguish from the benefit of the antiretrovirals themselves.

Open label extension of a randomised controlled trial

Clevenbergh et al (2000) reported on the 48 week follow-up of patients enrolled in the Viradapt trial (Durant et al 1999). Following the 24 weeks of follow-up reported in Durant et al (1999), genotype-guided treatment was offered to all participants with a further six months of follow-up. Of the patients randomised to the genotype arm in the trial, 60/65 (92.3%) completed the nine months and 56/65 patients (86.2%) completed 12 months of follow-up. Of the 43 patients randomised to standard of care, 39 (90.7%) and 36 (83.7%) completed the nine and 12 month follow-up, respectively.

Table 35 summarises the number of patients in each of the original treatment arms who received a genotype-guided treatment change during the extended follow-up. Numbers of patients from the genotype arm represent those patients receiving a third or fourth treatment change and those from the standard of care arm represent those receiving one or two genotype-guided treatment changes.

Table 35 Proportion of patients receiving a genotype-guided treatment change during follow-up

Genotype guided treatment change	Patients originally assigned to:	
	Genotype[a] n/N (%)	Standard of care[b] n/N (%)
Entire follow-up	48/65 (73.8)	30/43 (69.8)
Month 6	31/65 (47.7)	18/43(41.9)
Month 9	32/65 (49.2)	23/43 (53.5)

[a] Numbers of patients from the genotype arm represent those patients receiving a third or fourth treatment change
[b] Numbers from the standard of care arm represent those receiving one or two genotype-guided treatment changes

Reduction in viral load

The primary endpoint in the Viradapt trial (Durant et al 1999) was a mean change in viral load from baseline to months three and six. Table 36 shows that patients from the genotype arm of the trial were able to maintain the reduction in viral load observed at month six through to month 12. With the availability of genotype-guided therapy, patients originally assigned to the standard of care arm were able to achieve a further reduction in viral load [mean (SD): –0.67 $\log_{10}$copies/ml (1.25) at month six to –0.98 $\log_{10}$copies/ml (1.44) at month 12]. Whilst this appeared to be an improvement, the incremental benefit of genotype-guided therapy is difficult to measure due to the lack of a comparator group.

Table 36 Mean decrease in plasma HIV RNA levels: comparing patients originally assigned to genotype testing or standard of care

Follow-up	Mean change of viral load from baseline $\log_{10}$ copies/ml (SD) for patients originally assigned to:	
	Genotype	Standard of care
Month 6	–1.15 (1.20)	–0.67 (1.25)
Month 12	–1.15 (1.37)	–0.98 (1.44)

Undetectable viral load

The proportion of patients achieving plasma HIV RNA below the level of detection was a secondary outcome in Durant et al (1999). Clevenbergh et al (2000) reported the proportion of patients originally assigned to the genotype and standard of care arms who maintained, and/or subsequently achieved, undetectable viral loads at months nine and 12 after genotype-guided therapy was made available to all study participants. It appears from the data presented that per protocol rather than intention-to-treat analysis was performed (Table 37). With the availability of genotype-guided therapy, more patients originally assigned to the standard of care arm were able to achieve undetectable viral loads – 6/43 (14.0%) at month six and 11/43 (25.6%) at month 12. Whilst this appears to be an improvement, the incremental benefit of genotype-guided therapy is difficult to measure due to the lack of a comparator group. The proportion of patients originally assigned to genotype-guided therapy who achieved an undetectable viral load was 21/65 (32.3%) at month six and 17/65 (26.2%) at month 12.

Table 37 Proportion of patients achieving plasma HIV RNA below the level of detection during follow-up

Follow-up	Patients achieving plasma HIV <200 copies/ml, originally assigned to:			
	Genotype		Standard of care	
	ITT analylsis	PP analysis	ITT	PP analysis
Month 6	21/65 (32.3)		6/43 (14.0)	
Month 9	19/65 (29.2)	19/60 (31.7)	5/43 (11.6)	5/39 (12.8)
Month 12	17/65 (26.2)	17/56 (30.4)	11/43 (25.6)	11/36 (30.6)

Abbreviations: ITT, intention-to-treat; PP, per protocol

Critical appraisal of published meta-analysis

Focused question

The systematic review of Torre & Tambini (2002) focussed on a clear research question and provided a statement of the patient group, interventions (phenotypic and genotypic tests) and outcome (virologic response). Explicitly, the review focused on evaluating the influence of resistance tests (both phenotypic and genotypic), on virologic response to antiretroviral therapy in patients failing one or more courses of potent antiretroviral therapy.

Inclusion and exclusion criteria

Torre & Tambini (2002) reported their inclusion and exclusion criteria but did not provide explicit *a priori* details of the studies that were to be included and excluded. Likewise, they did not describe how the studies were selected or the number of reviewers who performed the selection of studies.

Explicit comprehensive search strategy

The search strategy described by Torre & Tambini (2002) was limited and may have missed studies. The search was confined to one electronic database (Medline) and the authors did not describe or fully report the sources or specific Internet sites searched. In addition, the authors did not examine all of the conference proceedings and appeared to restrict the search to English language articles. However, the authors attempted to identify unpublished articles from conference proceedings which may have minimised publication bias.

Assessed validity of included trials

Torre & Tambini (2002) did not report a method for assessing or describe how the included studies were assessed for validity. In addition, they did not state the number of reviewers performing the validity assessment. Hence, it is probable that the validity of the included studies was not assessed in this review

Results of meta-analysis

The meta-analysis by Torre & Tambini (2002) evaluated six RCTs with a total sample size of 1,471 patients. Four of them assessed the virologic response in a total of 708 patients receiving treatment based on genotypic test results against standard of care. One RCT with 541 patients evaluated both phenotypic and genotypic tests to standard of care while another examined only a phenotypic test in 221 patients. Of the total 551 patients treated on the basis of genotypic results, expert advice was provided for 143 patients. The authors summarised the main findings on genotypic testing as follows:

- Based on six RCTs that assessed virologic response at three months, the proportion of patients with undetectable viral load after three months was 42.6 per cent (234 of 549) of patients treated on the basis of genotype test results, and 33.2 per cent (163 of 506) of those treated on the basis of a clinician's decision (OR 1.7; 95% CI: 1.3, 2.2; $p<0.0001$; p for heterogeneity=0.60).

- In four RCTs that assessed virologic response at six months, the proportion of patients with undetectable viral load after six months was 38.8 per cent (168 of 432) for those treated on the basis of genotype results and 28.7 per cent (115 of 400) for those treated on the basis of clinician decision (OR 1.6; 95% CI: 1.2, 2.2; $p=0.0005$; p for heterogeneity=0.65).

- When no expert advice was provided and the clinicians interpreted genotypic test results, undetectable viral load was achieved in 36.4 per cent (145 of 398) of genotype-tested patients and in 31.5 per cent (133 of 422) of patients treated with standard of care (OR 1.4; 95% CI: 1.0-1.9; $p=0.0053$; p for heterogeneity=0.27).

- When clinicians were assisted by expert advice, undetectable viral load was found in 72 of 142 (50.7%) genotype-tested, expert-advised patients versus 77 of 215 (35.8%) patients who were treated with standard of care, irrespective of expert advice (OR 2.4; 95% CI: 1.5-3.7; $p=0.0001$; p for heterogeneity=0.30).

Discussion of meta-analysis

The authors of the review concluded that the results supported the use of genotypic testing in patients experiencing virologic failure during antiretroviral treatment and that expert interpretation of the test increased the probability of a virologic response. Nonetheless, the authors believe a number of key issues remain to be clarified:

- All RCTs included patients with virologic failure during triple antiretroviral therapy but not patients with primary HIV infection or pregnant patients.

- Despite the use of genotype resistance tests, virologic response to antiretroviral therapy at six months in the current review was about 44 per cent less frequent than response to the first triple combination therapy reported by a different meta-analysis.

- Better virologic response was observed when expert advice was provided, indicating that genotypic data should be carefully evaluated before clinical use, even if computerized interpretation is provided.

- Virologic outcome was assessed at three and six months only, but the real potential for resistance testing is over the longer term when a new treatment strategy is under consideration.

- In some of the RCTs, efficacy of resistance testing may be influenced by the greater number of new drugs used in the genotypic group compared to the standard of care group.

- There are issues related to concordance and cross-validation of the various drug resistance assays currently used in clinical research and the clinical setting. Different phenotypic and genotypic assays may give highly concordant results, although operator experience may correlate with assay performance.

Other limitations of the review reported by the authors were:

- Four of the six included RCTs used commercial kits to genotype HIV and two used in-house tests.

- Although all patients had experienced triple antiretroviral therapy, the number of drugs previously used and the duration of previous therapy differed widely among RCTs, ranging from patients treated with a single triple-antiretroviral regimen to heavily pre-treated patients (median: seven drugs per patient).

- Virologic response was the only outcome assessed in five of the six RCTs.

- The follow up period in the RCTs was short, ranging from 3 to 6 months.

Selection of patients for therapy

The authors assert that because response to a new antiretroviral treatment after virologic failure remains far less frequent than response to the first antiretroviral treatment, correct evaluation by resistant testing and expert advice of the time to begin treatment and of patient characteristics such as compliance and adherence is strongly recommended in clinical practice.

There were differences in the point estimates for the relative risk of achieving an undetectable viral load reported in Torre & Tambini (2002) and those of the meta-analyses performed in this Assessment Report. The differences stem from the included studies in each of the meta-analyses. Torre & Tambini (2002) included data from two abstracts. The meta-analyses described in this report included the subsequent published data from one of these abstracts (Tural et al 2002) and excluded data from the other abstract.

What are the economic considerations?

Literature Review

In addition to the process used to identify any literature analysing the cost-effectiveness outlined in the 'Approach to assessment' section of this report a search of economic databases including EconLit, NHSEED, HTA and DARE was also done. There are eight economic evaluations analysis of genotype resistance testing for HIV patients and two reviews. These are:

Author	Title
Sax et al 2002	Should resistance testing be done in antiretroviral naïve patients? A cost-effectiveness analysis.
Chaix et al 2000	Economic evaluation of drug resistance genotyping for the adaptation of treatment in HIV-infected patients in the VIRADAPT study.
Anis et al 1999a	Optimising Drug Treatment: A cost-effectiveness analysis of HIV/AIDS Drug Resistance Testing.
Anis et al 1999b	The cost-effectiveness of immediate ritonavir-saquinavir therapy versus resistance testing and a drug holiday in HIV patients failing protease including regimens.
Weinstein et al 1999	Resistance testing to guide the choice of second-line antiretroviral therapy in HIV: Clinical impact and cost-effectiveness.
Weinstein et al 2001	Use of genotypic resistance testing to guide HIV therapy: Clinical impact and cost-effectiveness.
Chaix-Couturier et al 2000	HIV-1 Drug Resistance Genotyping: A Review of Clinical and Economic Issues.
Corzillius et al 2004	Cost effectiveness analysis of routine use of genotypic antiretroviral resistance testing after failure of antiretroviral treatment for HIV.
Lauria et al 2003	Cost-effectiveness analysis of using antiretroviral drug resistance testing.

Only studies with a formal economic evaluation were included in the review therefore the articles by Lauria et al (2003) and Chaix-Couturier et al (2000) which are themselves reviews are not included and on this basis neither are Sax et al (2002), Anis et al (1999a, 1999b), Weinstein et al (1999), which are abstracts presented at conferences, and contain little detail of the methodology used. The two articles on cost effectiveness relevant to this review are Corzillius et al (2004) and Weinstein et al (2001).

Weinstein et al (2001) used a state transition model in which patients could randomly make transition between health states at monthly intervals (first-order Monte Carlo). Outcomes measures were life expectancy, quality-adjusted life expectancy and cost-effectiveness in dollars per quality-adjusted life-year (QALY) gained. Results were expressed as an incremental cost effectiveness ratio per quality-adjusted life expectancy.

A societal perspective was adopted and costs and clinical benefits were discounted at three per cent per year.

Weinstein et al (2001) defined health states in his model by using patient's current and maximum HIV RNA viral load, CD4+ cell count, time receiving HAART, history of effective and ineffective antiretroviral therapy and previous opportunistic infections. HIV viral loads and CD4+ cell counts were divided into six strata and disease progression was modelled as monthly transitions between these health states. Patients could enter or exit temporary health states corresponding to acute episodes of defined opportunistic infections from which they would die or survive in which case they would transit to a new chronic state. The probability of each opportunistic infection was estimated as a function of the current CD4+ cell count. The CD4+ cell count, which was used as a surrogate marker of disease progression, was also used to predict the rates of opportunistic infections and HIV-related death. Virologic failure was defined as an increase in HIV RNA levels for two consecutive months while receiving HAART. The two arms of the model were that of clinical judgement guided by genotype testing and clinical judgement alone. One million lives were simulated and followed to death. Before treatment HIV RNA levels were assumed to be at a steady state value.

Clinical data was based on the Multicenter AIDS cohort study, this included the distribution of HIV RNA levels among patients and monthly decreases in CD4+ cell count in the absence of HAART and as a function of HIV RNA level set point (steady state value). Cost data related to HIV-related care was obtained from the AIDS Costs and Services Ultization Survey. To estimate costs for each geographic area, a ratio of charges to costs was estimated for each area and applied to the charge costs in the survey data. Drug prices were obtained directly from the 1998 Red Book. The costs of tests were obtained directly from hospital cost-accounting systems. Patient time and non-medical costs were excluded. Costs were in 1998 prices. Health-related utilities for the chronic and acute health states were obtained by transforming quality of life data.

Both primary resistance testing (resistance testing to guide the choice of initial therapy) and secondary resistance testing (to guide the choice of subsequent therapy after initial HAART failure) analyses were performed.

Weinstein et al (2001) found that secondary resistance testing increased life expectancy by three months, at a cost of $17,900 USD per QALY gained. The cost-effectiveness of primary resistance testing was $22,300 USD per QALY gained with a 20 per cent prevalence of primary resistance but increased to $ 69,000 USD per QALY gained with four per cent prevalence.

The article by Corzillius et al (2004) reports on a German HTA assessment of genotype antiretroviral resistance testing. A decision-analytic Markov model was used to estimate lifetime clinical and economic outcomes in a cohort of HIV patients starting with initiation of HAART. Outcome measures were lifetime costs, life expectancy and cost-effectiveness expressed in euros (€) per life year (LY). Results were expressed in incremental cost effectiveness (€/LYs).

The model structure was designed so that patients transited from one health state to another in six monthly cycles based on their response to antiretroviral therapy. Health States were defined as the differing HAART regimens. Those patients with primary failure (failure to respond to the HAART therapy) were switched to another HAART regimen while those patients who responded stayed on their HAART regimen; when

they failed (secondary failure) they then switched to another HAART. Success was defined as an undetectable viral load (<500 RNA copies/ml). Baseline CD4+ cell counts were defined as 350/μl followed by a mean rise of 150/μl under successful HAART. Only four HAART regimens were modelled as it was argued that the probability of achieving a viral load <500 copies/ml after this is practically nil. The relative risk reduction of primary treatment failure using genotype resistance testing was assumed to be constant across the HAART regimens. The cohort was described so as to resemble those patients enrolled in the Swiss Cohort Study from which most of the probabilities on rates of treatment failure, estimates of GART effectiveness and data on disease progression were derived. Additional data from published trials was also used to estimate some of the transition probabilities. For example probabilities for the progression to AIDS as predicted by viral load were derived from data that refer to wild-type virus in accordance with some of the literature reporting that multiresistant HIV strains often lead to slower decline in CD4+ cell counts due to reduced viral fitness. It was assumed that patients died two years after a diagnosis of AIDS, reflecting data from prior to the HAART era. Corzillius et al (2004) also assumes that once the patient gets AIDS, survival would be the same whether or not GART had been used earlier.

The results were that genotypic antiretroviral resistance testing (GART) after treatment failures increased life expectancy by nine months and undiscounted life-time costs per case by 16,406 euros. The discounted incremental cost effectiveness ratio was 22,510 euros per life-year gained. Best and worst-case scenarios yielded 16,512 euros/LY and 42,900 euros/LY, respectively. GART prior to the initiation of HAART would be equally cost effective if it could reduce the probability of first HAART failure by at least 36 per cent.

These studies are of limited value in assessing the cost effectiveness of genotype testing in Australia. The study by Weinstein et al (2001) is limited by its reliance on a disease progression model based on surrogate markers (CD4+ count and viral load) that precedes the introduction of triple therapy. The study by Corzillius et al (2004) is heavily reliant on evidence for a Swiss observational study that may not be relevant to Australia as much greater numbers acquired HIV through intravenous drug use in the Swedish study. Therefore a greater proportion are female compared to Australian HIV positive patients and a greater proportion have co-morbid conditions such as Hepatitis C and B.

Review of the model from the Applicant

The approach taken in the submission from the Applicant is to estimate the direct cost of a genotype test. The number of people eligible for the genotype test is estimated by taking all people on HAART who fail who have the test and multiplying them by the test costs in a two year period. The assumptions are:

- 6,000 people are on HAART in Australia.
- Patients included in the model are those on their first through to sixth combination.
- Data from the Australian HIV Observational Database (AHOD) was used to determine the rate at which patients moved combinations.
- 27.45 per cent of all patients on HAART will experience failure/rebound in a six month period.

- Only 50 per cent of those patients experiencing failure/rebound will choose to have the test.

- Not all patients who change therapies do so for reasons of resistance.

- The cost of the test is $450.00.

- No naïve patients commence on treatment in the two year span of the model. To counteract any undercounting of possible test numbers, the application assumes that no patient dies or stops treatment during this two year period.

- HIV viral load below detection is defined as ≤400 copies/ml.

- Rebound is defined as patients who sometime on their current therapy fell to below 400 copies/ml and subsequently had a viral load greater than 400 copies/ml.

- Failing is defined as last HIV RNA load measures on current therapy >400 copies/ml and never recorded a viral load ≤400 copies/ml.

Using these assumptions the Applicant estimates that 1647 will fail to control their HIV viral load below detection and if 50 per cent choose to have the test, this corresponds to 824 potential tests in a six month period for a cost of $370,661. It is then assumed that these people would change their combination while others will change due to toxicities; with these assumptions another 929 assays might be needed in the following six months. The total cost for the year was estimated at $788,972. Using a similar method the Application estimates the cost for the second year at $901,980.

Summary of assessment of submitted model

The conclusion of the economic evaluation undertaken in the Application is that if genotype resistance testing allows better use of expensive but vital medicines (estimated to cost around $80 million dollars per year) then the cost to the government of genotype listing on the MBS will be worthy of consideration. The claim is poorly supported by evidence in the Application.

- The model provided in the Application does not attempt to calculate the cost-effectiveness of introducing genotype testing into the therapeutic regimen of HIV positive patients on HAART. A comparison with current standard treatment is not provided, and neither incremental costs nor benefits estimated.

- The model does not attempt to qualify or value any benefits that may accrue to patients as a result of being on a HAART regimen to which they are not resistant.

- The model uses an estimate of the cost of the test ($450). According to the laboratory estimates provided in the summary table of laboratory cost estimates on page 89 of the Application the cost of the test should fall within the range $528-$890.70.

- The model is not based on the requested listing for genotype resistance testing. That is, the test is requested for patients prior to starting their first regimen, who are failing their first regimen or pregnant women. The model makes the assumption that of

patients failing their first regimen only 50 per cent will choose to have the test. No evidence is provided on take up of the test, it maybe closer to 100 per cent

- More specifically it was difficult to clearly identify some of the assumptions used in the model as they are not clearly spelt out, no spreadsheets were provided and the data is generated from the AHOD database which cannot be independently verified. In particular it was not possible to determine the rate at which patients move from one combination therapy to another, only a snapshot of where patients were at six months, 12 months or 18 months is provided and the rate at which patients change therapies for others reasons (such as toxicity, non-adherence), while used in the formula to estimate the potential number of tests that may be required, was not documented.

- While the Application provides evidence that patients have a greater propensity to fail HAART the more HAART regimens they have been on, this information is not used to adjust model outcomes.

The Application believes that its model may overestimate those patients with virological failure because some patients may not have been on a combination for long enough at the time of entry into AHOD to have achieved viral suppression. This could not be verified.

Clinical setting

According to the 2003 Annual Surveillance Report on HIV/AIDS (National Centre in HIV Epidemiology and Clinical Research 2003) there were 13,120 people living with HIV/AIDS in 2002 of which an estimated 52 per cent were being treated with antiretroviral therapy (6,822). The estimated AIDS incidence and HIV prevalence were 1.3 and 67 per 100,000 population respectively. Survival following AIDS in Australia increased from 17.4 months for cases diagnosed in 1993 to 38.4 months for cases diagnosed in 1999. The introduction of triple combination therapy and subsequent control of viral replication has had a marked effect on the rate of AIDS survival in Australia. Figure 9 uses data from the AHOD database to illustrate the effect that improved treatment for opportunistic infection and the introduction of combination therapy have had on this improved survival.

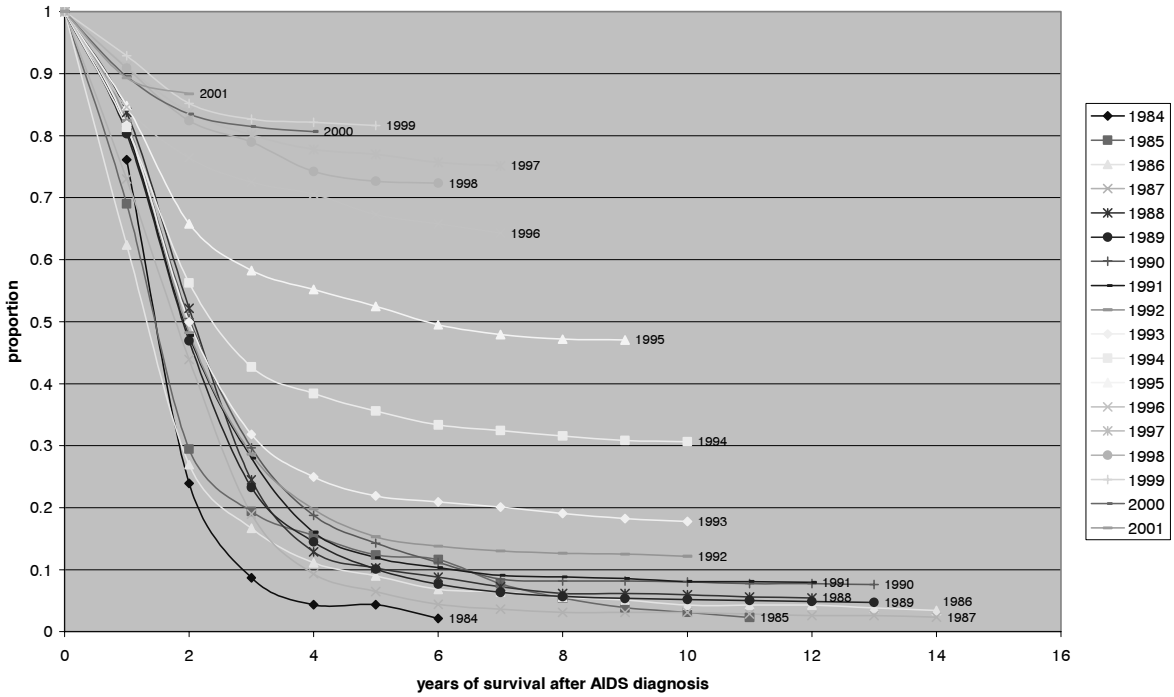

Figure 9 **Effect of improved treatment for opportunistic infection and introduction of combination antiretroviral therapy on improved survival**

Cost-Effectiveness Model

This section presents the structure of a decision analytic model to compare genotype resistance testing in HIV patients who are failing antiretroviral with standard care (that is those patients who do not have the test).

The aims are:

- To calculate the additional cost of using a HIV genotype test for patients failing initial HAART therapy and every time a patients fails HAART (as requested by the Advisory Panel) compared to standard care. Costs are calculated from a health system perspective and include not only the cost of the test but also the cost of HIV/AIDS related treatments.

- To calculate the additional survival and quality of life associated with genotype test assisted anti-retroviral treatment compared to treatment that is not assisted.

- To estimate the incremental cost per life year saved and per quality adjusted life year associated with genotype assisted anti-retroviral therapy in a hypothetical cohort of Australian HIV patients who have commenced on HAART.

- To place a likely range around the estimate of the incremental cost effectiveness of genotype testing for patients on HIV therapy in Australia.

- To predict the net financial cost of genotype testing over the next five years.

Cost-effectiveness analysis in a decision analytic framework allows consideration of the potential cost-effectiveness of genotypic testing over the course of HIV infection. In the absence of high quality randomised controlled trial of long term evidence of effectiveness or high quality prospective cost data decision analytic modelling provides information on the likely costs and health outcomes in clinical practice. The randomised trials assessed in the results section present surrogate outcomes only, that is changes in plasma HIV-1 RNA (log_{10}) from baseline to the endpoint or the proportion of patients with undetectable HIV plasma viral load (reported as <200, <400 or <500 copies/ml) at a given endpoint. There are no trials of genotype testing with final endpoints such as survival. Cost-effectiveness analysis allows identification of a range of potential costs and outcomes associated with genotypic testing and the uncertainties associated with each. Modelling provides a means by which surrogate outcomes can be extrapolated beyond the time period of the clinical trials (which relative to the natural history of HIV are very short) to their effect on final outcomes. The results of the analysis will remain subject to considerable uncertainty given the quality of the underlying clinical and economic data but provide the basis for an assessment of the potential for the technology to provide health gain at an acceptable cost.

Initially it was proposed that three strategies: genotype testing in antiretroviral naïve patients prior to commencing their first regimen who have seroconverted in the previous 12 months; HIV patients failing their first HAART regimen; and pregnant women, would be considered. Evidence of the effictiveness of genotype resistance testing was found only for patients who are failing their first HAART regimen. Consequently only the cost effectiveness of genotype test assisted therapy for this group has been modelled. A threshold analysis of genotype resistance testing in the antiretroviral naïve cohort will be done to estimate at what level of endemic HIV resistance the use of genotype testing may be cost-effective when initiating first HAART.

HIV disease progression and the model structure

Figure 10 is a diagrammatic presentation of the model structure. The purpose of using the genotype test is to reduce the number of patients who fail to respond to their HAART therapy due to resistance, and the model has been designed specifically to simulate this effect.

The model has fifteen health states through which a cohort of patients move over time at three monthly intervals. Each health state is associated with a resource cost and a level of health status. The cost and the health outcome of each of the two strategies (genotype assisted therapy and standard antiretroviral therapy) are estimated as the sum of costs and health status in each three month cycle over a 50 year period from age 36. The health states reflect the progression of disease and treatment over a lifetime of HIV illness from the time of initial anti-retroviral therapy. There are 13 health states based on stages of antiretroviral therapy and two ways of exiting the model — death from HIV related disease and death from other causes. Treatment naive patients commence antiretroviral therapy in health state HAART1 with a cost of antiretroviral therapy in the first three months. Those who respond move to the health state 'HAART1 continue', while those who fail—primary failure—move to a new HAART regime—'HAART2'. This movement based on responding or not responding to therapy is repeated throughout the model until patients have moved through six HAART regimens. Each period patients have an age related likelihood of dying from HIV related or other causes and exit the model. Upon failing the sixth regimen they remain in a health state called 'salvage' and

continue to receive antiretroviral therapy. The six treatment regimens reflect the experience of patients in the AHOD (Australian HIV Observational Database) database.

Patients who experience treatment failure are divided into those experiencing 'primary failure' that is they do not respond to their new HAART regimen and those experiencing 'secondary failure' that is patients who initially responded to their HAART regimen but after a period of months or years are no longer responding. This reflects the findings from observational database studies that patients who respond to treatment experience lower failure rates than the primary failure rates (Corzillius et al 2004, Ledergerber et al 1999). Patients are also separated into different health states, according to response to therapy or not, to assign different probabilities of clinical disease progression associated with their differing responses to HAART therapy as has been reported in the literature (Ledegerber et al 1999, Hogg et al 2001). Primary failure of HAART therapy increases the greater the number of combinations a patient has experienced while secondary failure is assumed to remain constant (Corzillius et al 2004, AHOD 2002).

The purpose of anti-retroviral therapy is to suppress HIV viral load and maintain CD4+ cell count in order to avoid HIV related morbidity and mortality. Failure of therapy at any point increases the risk of HIV related morbidity and mortality. The model assumes that in each three month cycle the probability of HIV related morbidity is higher if the patient has failed to respond to anti-retroviral therapy. An HIV related morbidity event in any three month period is assumed to lead to an additional cost of treatment, a reduction in quality of life, and an increase in the risk of dying.

The effect of genotype testing is modelled by reducing the risk of primary failure of the subsequent regimen.

Specifically the model makes the following assumptions:

1) All patients entering the model are treatment naïve.

2) All patients commencing treatment have CD4+ cell counts of >200 and <350 per μl as recommended in the Australian literature (Hoy & Lewin 2004). It is acknowledged that this will not be a true reflection of patients commencing their first antiretroviral therapy, some patients will have lower CD4+ cell counts.

3) The average age of the patients is 36 years of age. This age was estimated from de-identified AHOD data of the age of patients when diagnosed with HIV. The time horizon of the model is 50 years. This time horizon was used because of clinical advice that many patients with HIV who are responding and adhering to their antiretroviral treatment can expect to have a normal life expectancy.

4) No explicit assumptions about the gender of the cohort is made though it is assumed that the cohort will be primarily male reflecting that in Australia HIV is primarily transmitted through male on male sex. When sourcing disease progression probabilities outside Australia attempts were made to source from countries with similar cultural and health standards. Nevertheless, differences exist with greater numbers of intravenous drug users infected in some European countries, this differing mode of acquiring HIV will necessarily also change the gender mix of the HIV positive population and may impact on any probabilities used from this population.

5) The model is three monthly cycles to mimic the frequency of plasma viral load testing.

6) Six HAART regimens are used.

7) Patients are defined as responders if they have an undetectable HIV RNA viral load of <400 copies/ml (or 500 copies/ml) for the first three HAART regimens and if they have a HIV-1 RNA viral load of <1000 copies/ml after HAART3 and a CD4+ count of >250 μl. Non-responders are those patients who do not achieve either an undetectable viral load when commencing a new HAART regimen, experience a decrease by less than a factor of 10 in HIV-1 RNA level by week eight or an increase by a factor of more than 10 above nadir measurement (and >2000 copies/ml within 24 weeks). Patients who experience viral rebound are defined as patients who have previously responded to an HAART regimen and are now experiencing a HIV-1 RNA level above 400 copies/ml in a subject with two previous measurements of less than 400 copies/ml for two consecutive months.

8) Only patients, who are defined as non responders within the three month cycle, receive a genotype test before switching to a new HAART regimen. Patients, who initially respond and then subsequently fail, are assumed not to receive a genotype test because the probability of failure is not modified by the test.

9) The absolute risk of failing HAART1 in the first three months is based on the probability of first virological failure of the most effective HAART regimen (zidovudine, lamivudine and efavirenz) reported in Figure 3 of Robbins et al (2003). The assumption that the primary failure rate for HAART2 through to HAART6 increases by 50 per cent each therapy change is based on expert opinion and is the assumption used by Corzillius et al (2004) based on his observations of the Swiss Cohort Study. A more conservative figure of 25 per cent would be consistent with the increase in failure rates in the AHOD (AHOD 2002) that showed data that report treatment rates of change per follow up year of 39 per cent, 45 per cent and 60 per cent for first second and third combinations respectively since 1997. However these rates most likely include both higher initial and lower subsequent failure rates and as a snapshot are not representative of a cohort of patients moving through a treatment regimen.

10) The constant risk of failing HAART1 each subsequent three months after initially responding (viral rebound or secondary failure) was estimated by assuming an exponential survival function fitted to the data in Figure 3d in Robbins et al (2003).

11) The rate of secondary failure to therapy is assumed to be constant for the HAART2 to HAART6 regimen. This is consistent with what was reported by Corzillius et al (2004) from the Swiss Observational Database, and from comments made in the Application based on the AHOD that "there was no difference in the rate of viral rebound as the number of combinations tried increased" (pg 66). The constant 10.7 per cent rate of secondary failure was estimated simply by observing the different percentage of patients still on their second combination between 12 and 18 months, as reported in the Application, and estimating the rate of change for three months (pg 69). The survival curves presented in the Application and in the article analysing the rates of combination change from the AHOD database (AHOD 2002) could not be used to estimate the rate of secondary failure because different groups of patients are represented by the first and second and third failure curves. This figure of a 10.7

per cent probability of secondary failure is conservative with respect to the cost effectiveness of genotype testing as it is likely to overestimate the risk of failing a regimen after responding, Corzillius et al (2004) estimate a probability of failing after initially responding of 15 per cent each six month period.

12) The three month probability of a patient experiencing toxic effects from HAART1 is based on the first three month probability of the survival curve reporting toxic or severe events in Figure 4 of Robbins et al (2003); this probability was used as the probability of a toxic event in the initial three months for each subsequent HAART regimen. The ongoing probability of experiencing toxicity each three month cycle, after the initial three month period, on any HAART regimen, was estimated using an exponential approximation of the survival curve reporting a toxic or severe event after the first three months (Robbins et al 2003). The rate at which patients will change their HAART regimen in response to a toxic event was estimated at 50 per cent for HAART1 (based on expert advice) but this percentage was reduced for each subsequent HAART regimen, as the number of antiretrovirals to which patients could switch diminished. After HAART3 the rate of change in regimen following a toxic event is assumed to be constant at 10 per cent.

13) The effect of the genotype resistance test was calculated as the relative risk reduction of having an undetectable viral load (<500 copies/ml). This relative risk was calculated using a meta-analysis of three randomised controlled studies (Durant et al 1999, Cingolani et al 2002, Tural et al 2002) which had an undetectable viral load as either their primary or secondary endpoint. The sensitivity and specificity of the test are not included directly in the model because the genotype antiretroviral resistance test is used as a treatment modifier rather than as a diagnostic test. The RR of 0.85 is considered conservative. Corzillius et al (2004) used a RR of 0.79 from Durant et al (1999) and Weinstein et al (2001) 0.79- 0.85. It is assumed that this relative risk reduction is constant even though the absolute risk of failing initial HAART treatment increases with each subsequent HAART regimen. This assumption is justified because the VIRADAPT study estimated the mean effect of genotype resistance testing across a patient population that included patients on their first, second or third HAART regimens (Durant et al (1999). From a logical perspective one would expect the functionality of genotype resistance testing in assisting clinician's choice of therapy to increase the greater the number of HAART regimens experienced by the patient and the fewer antiretroviral choices still available. The effect of the genotype test is assumed to only occur within the first three months of exposure to a new HAART regimen.

14) Patients, both responders and non-responders can exhibit clinical progression by moving through a temporary health state called HIV morbidity. This health state includes patients with co-morbidities, such as viral hepatitis and neurological conditions, for which the presence of HIV and in particular high plasma viral loads can reduce health status. Transition probabilities into this state are based on the probability of disease progression, as defined by experiencing an AIDS illness or dying, and not the probability of experiencing any illness associated with HIV. Accordingly the probabilities used most likely result in an underestimate of the overall level of morbidity experienced by this group. The rate of morbidity experienced by these patients is adjusted up by a factor of 1.51 for those patients who are 50 years of age or older (Egger et al 2002). As patients experience ongoing treatment failures both viral load and transition probabilities to clinical progression increases. To reflect this clinical picture, transition probabilities to HIV morbidity

from HAART4 onwards were modified using the study by Lawrence et al (2003) that included patients with multiresistant virus. In that study, the control group received optimal therapy while the other group (treatment interrupted) received no therapy for four months and then optimal therapy. It was assumed that patients experiencing no initial response to a new HAART regime from HAART4 onwards had similar rates of clinical disease progression as those patients in the study by Lawrence et al (2003) who received treatment interruption for four months. Patients in the model who had initially responded to their HAART regimen but who subsequently experienced viral rebound, from HAART4 onwards in the model, were assumed to have similar rates of disease progression as the control group in the Lawrence study (Lawrence et al 2003). In making this assumption there may be an underestimate of the numbers of patients in this group who will experience disease progression as the control group in the Lawrence study included responders and non responders to optimal HAART therapy.

15) The likelihood of dying from causes other than HIV was assumed to be the age specific all cause mortality rate (Australian Bureau of Statistics 2002), unadjusted for HIV deaths. The HIV mortality rate used is an excess death rate (EDR) calculated from the Swiss Cohort Study (Jaggy et al 2003), normalised for age and sex. It was thought more realistic to use an EDR reflecting as it does lifestyle factors that may contribute to higher rates of death for this cohort in addition to AIDS deaths. These EDR, which have been calculated according to whether patients were classified as responders, experiencing viral rebound or had never responded, have been adjusted up by a factor of 3.09 for those patients who are 50 years or older (Egger et al 2002). In adjusting up the risk of dying of AIDS after 50, those deaths counted in the EDR that may not increase with age, have also been adjusted up. This transition probability will overestimate the number of patients with HIV morbidity who die after age 50. The EDR rate calculated from the Swiss Cohort Study may also overestimate the death rate experienced by HIV patients in Australia as the Swiss Study appears to include a greater proportion of patients who acquired HIV through intravenous drug use, and who also suffer from viral hepatitis. These factors in combination may point to a cohort of Swiss patients who engage in riskier behaviours than the Australian HIV cohort in the model.

16) While it is acknowledged that some non-responses are due to non-adherence (for reasons other than toxicity) expert clinical advice suggested that this group was not significant enough to separate them out from non-responders in general, and as a group they would not be expected to impact on the ICER.

17) Life years are reported are quality adjusted life years. Utility weights used were from a meta-analysis of pooled utilities reported in Tengs 2002 (Tengs & Lin 2002). Utilities weights were not reported for each of the temporary health states used in our model. The study reported utility rates only for asymptomatic HIV 0.94, symptomatic HIV 0.82 and AIDS 0.70 therefore an adjustment of these utility weights is done to apply utility weights to those temporary health states that lay between asymptomatic HIV responder and HIV morbidity non-responders (which was assumed to equate to the AIDS utility weight). A utility weight of 0.90 is applied to asymptomatic HIV for non-responders; it is assumed that failing to respond to a HAART regimen will incur some disutility. Similarly for patients who are responding but experiencing toxic/severe effects such that they may need to change therapy, it was assumed that the disutility experienced may by significant and they were assigned a utility weight of 0.82, for patients who have HIV morbidity but are responding to HAART their

utility weight was adjusted up 0.04 (from 0.70 for non responders) as it was judged that their response to therapy and the possibility of possible future immune reconstitution resulting from their response would give them extra utility. All weights remain within the range reported by Tengs & Lin (2002).

18) Costing data is from a number of sources. To derive resource use for the temporary health states, that is the asymptomatic states, the recommendations of the HIV Model of Care Working Group subcommittee of the Clinical Trials and Treatment Advisory Committee (CTACC) of the Australian National Council on AIDS and Related Disease (September 1998) were used as a guide. In following these recommendations, costing for tests or monitoring undertaken that did not differ between the different patient categories, and the time at which these tests would be instigated would in part be based on the individual being assessed and not necessarily their HIV viral load or CD4+ count, were not included only resource use that was clearly recommend based on the different categorisation of the patient according to HIV viral load and CD4+ count was included. The use of prophylactic treatment for opportunistic infections was based on the recommendations of this Working Group and the more recent HIV Management in Australia (Hoy & Lewin 2004). All drugs costs, both HAART and for opportunistic infections was sourced from the Schedule of Pharmaceutical Benefits (1 May 2004). Recommended dosages were obtained from MIMS or from HIV Management in Australia. The drug regimen included are examples for costing purposes only, and are not intended to reflect what would be the actual HAART regimen.

19) To obtain a cost for the temporary health state, HIV morbidity, Australian Diagnostic Related Groupings AR-DRGs were used (Department of Health, Australian Refined Diagnosis Related Groups (2000-1). All patients who were discharged from hospital (alive or dead), who were admitted under one of the HIV categories, are recorded and average costs per episode (and average length of stay) calculated for 2000-2001. These separations were weighted by number of separations and an average weighted cost per hospital admission calculated, (these costs were updated to 2002-03 costs using the CPI-health group (Australian Bureau of Statistics 2004). Included in these DRGs are patients with HIV who have associated co-morbidities, which may be aggravated by their HIV and for which they may need to be admitted, for example patients with both HIV and viral hepatitis. Although it may be assumed that an assumption that all patients with HIV morbidity require hospital resources may overestimate the cost of patients in this health state, the weighting includes over 50 per cent of separations who were recorded as being in the HIV same day category, therefore less than 50 per cent of patients in this category required ongoing in-patient treatment, to the extent this represents patients with HIV morbidity these costs may not be an overestimate. Data on the proportion of patients who have a HIV morbidity that require hospitalisation is not available to validate this assumption. Only active treatment for opportunistic infections was assumed to occur in hospital, therefore any ongoing monitoring of patients post discharge and requirement for prophylactic treatment was considered additional costs. In 1995 a study was undertaken by Hurley et al (1995) to describe the patterns of health-service usage and the resulting costs in 1992-93 for Australian men. Because of the significant changes to HIV care that has occurred in the decade since this report was written it was decided not to use these figures in the model, but where possible to use the figures to validate results obtained from the model. The cost of the genotype

test was calculated as an average of the laboratory prices submitted by the Applicant, the lowest and most expensive price quoted were used in the sensitivity analysis.

The model was structured using clinical rather than virological endpoints, such that patients probabilities of moving into temporary health states were not based on a matrix of HIV viral load and CD4+ count but by using published studies that estimated the rate of observed disease progression based on patient's response to HAART therapy. An alternative approach is taken by Weinstein et al (2001). Their model allows patients to move between different strata of HIV RNA levels and CD4+ count, from their 'set point' in response to or failing HAART therapy. There are inherent complexities in trying to model the rate of a patients disease progression based on a matrix of HIV-1 viral load and CD4+ count, not the least appears to be that the direction of CD4+ counts are not always predictable. For example, Durant et al (1999) report that at three months patients receiving genotype-guided therapy had a significant increase in CD4+ cell counts compared to patients treated with standard care, while at six months the opposite was found; patients receiving standard care had a significantly greater increase in CD4+ cell count. Additionally, as reported by Hogg et al (2001), in a cohort of patients starting triple-drug antiretroviral therapy (similar to the cohort modelled), uniformly low rates of disease progression to AIDS and death were observed but that progression to death was clustered among patients starting therapy with CD4+ cell counts less than 200/μl and that rate of disease progression and death, in this cohort of individuals receiving antiretroviral therapy was independent of age, sex, prior AIDS diagnosis, protease inhibitor use, and plasma HIV RNA levels. They conclude that the fact that CD4+ cell count remains the single independent predictor of survival in this population-based cohort of treated individuals would suggest that there is a threshold beyond which immune reconstitution may be compromised. Based on this recent literature it was decided that using HIV and CD4+ strata to model disease progression was not the most appropriate way to model HIV disease progression. It is implied in the model that patients develop AIDS illnesses because their CD4+ cell counts are lower than the illness threshold, and patients who respond to therapy will have an improvement in their CD4+ cell counts.

The approach to modelling the cost effectiveness of genotype assisted HAART is shown in Figure 10.

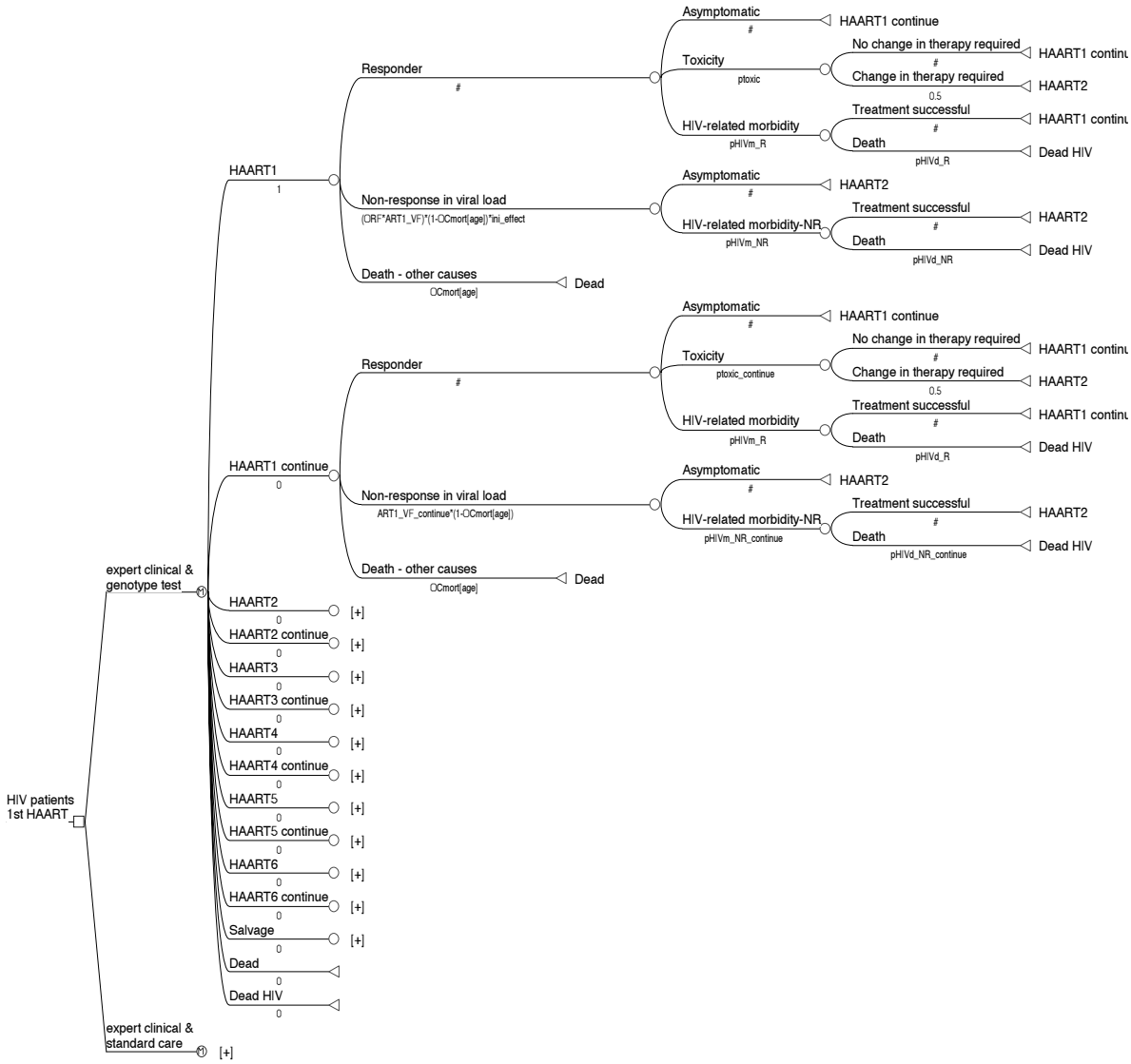

Figure 10 Diagrammatic representation of the model structure

Table 38 Parameter values for the Markov model in each three-month cycle

Variable	Name	Minimum	Base case	Maximum	Source
	Transition probabilities				
Probability of failing first 3 months of first HAART	ART1_VF	0.0	0.0562	0.10	Robbins et al (2003)
3 month probability of HAART failure after at least 3 months initial success	ART1_VFcontinue	0.001	0.0170	0.04	Robbins et al (2003)
Probability of failing first 3 months of second HAART	ART2_VF	0.05	0.0843	0.15	50% increase from HAART1-
Probability of failing each 3 months HAART after at least 3 months initial success	ART2_VFcontinue	0.05	0.107	0.15	AHOD database
Probability of failing the first 3 months of HAART 3	ART3_VF	0.06	0.1265	0.18	50% increase over HAART2
Probability of failing each 3 months HAART after at least 3 months initial success	ART3_VFcontinue	0.05	0.107	0.15	
Probability of failing the first 3 months of HAART4	ART4_VF	0.1	0.1897	0.30	50% increase over HAART3
Probability of failing each 3 months HAART after at least 3 months initial success	ART4_VFcontinue	0.05	0.107	0.15	
Probability of failing the first three months of HAART5	ART5_VF	0.14	0.2846	0.42	50% increase over HAART4
Probability of failing each 3 months HAART after at least 3 months initial success	ART5_VFcontinue	0.05	0.107	0.15	
Probability of failing the first 3 months of HAART6	ART6_VF	0.22	0.4269	0.64	50% increase over HAART5
Probability of failing each 3 months HAART after at least 3 months initial success	ART6_VFcontinue	0.05	0.107	0.15	
Age specific all cause mortality	OCmort				ABS.Deaths. Catalogue (Australian Bureau of Statistics 2002) 3302.0 2002
Probability of a toxic reaction to therapy in first 3 months of any HAART regimen	pToxic		0.16		Robbins et al (2003)
Probability of toxic reaction each 3 months HAART after at least 3 months exposure	pToxic_continue		0.0457		Robbins et al (2003)
Probability of disease progression each 3 months for patients who achieved and maintained undetectable viral load on current therapy (or considered responders in later HAART)[a]	pHIVm-R[a]	0.004589	0.006578 ≥ 50 years (0.009917)[b]	0.008563	Ledergerber et al (1999)

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name	Min	Base case	Max	Source
	Transition probabilities				
Probability of disease progression in the first 3 months for patients who failed to achieve undetectable VL on early HAART (used for HAART 1 & 2 & 3)[a]	pHIVm-NR	0.015184	0.019899 ≥ 50 years (0.029895)[b]	0.024593	Ledergerber et al (1999)
Probability of disease progression in first 3 months for patients who failed to respond to respond to HAART therapies (used for HAART 4 & 5 & 6)	pHIVm_NR_LD (late disease)		0.034395 ≥ 50 years (0.051478)[b]		Lawrence et al (2003)
Probability of disease progression each 3 months for patients who achieved undetectable VL and then suffered viral rebound, early HAART therapy (used for HAART 1 & 2 &3)[a]	pHIVm_NR_continue‡ (used for HAART 1&2&3)	0.005485	0.00896 (3-monthly) ≥ 50 years (0.013498)[b]	0.012422	Ledergerber et al (1999)
Probability of disease progression each 3 months for patients who responded to HAART therapy and then suffered viral rebound, who have received a number of HAART combinations (used for HAART 4 & 5 & 6)[a]	pHIVm_NR_continue_LD		0.012422 ≥ 50 years (0.018698)[b]		Lawrence et al (2003)
Mortality rate each 3 months for those who respond to HAART	pHIVd_R		0.002223 ≥ 50 years (0.006852)[b]		Jaggy et al (2003)
Mortality rate each 3 months for those who do not have an initial response to any HAART	pHIVd_NR		0.028287 ≥ 50yrs (0.08485)[b]		Jaggy et al (2003)
Mortality rate each 3 months for those who initially responded to HAART therapy and then suffered viral rebound	pHIVd_NR_continue		0.003868 (3-monthly) ≥ 50 years (0.011902)[b]		Jaggy et al (2003)

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name Transition probabilities	Min	Base case	Max	Source	Comments
Costs						
Cost of genotype test	cTest	$528.00	$666.58	$890.70	Average cost of laboratory cost estimates submitted	
Cost of other tests each 3 months for those who respond to HAART and are asymptomatic	cAsymp_R		$247.1		(Department of Health and Ageing 2004, HIV Model of Care Wprking Party 1998). MBS 69381	assume <1000 copies/ml) and CD4+ count >250 viral load test every 3 months)
Cost of other tests for primary failure who do not respond to early HAART and are asymptomatic (used for HAART 1 & 2 & 3)	cAsymp_NR_initial		$843.75		Department of Health and Ageing 2004, HIV Model of Care Wprking Party 1998. MBS	Assume patients have CD 4 count <250 cells/μl and HIV viral load >400 copies/ml) Viral load test monthly + lymphocyte surface marker (CD4+;CD%) every 3 months
Cost of other tests for primary failure who do not respond to later HAART and are asymptomatic (used for HAART 4&5&6)	cAsymp_NR_inital_LD		$933.75		Department of Health and Ageing 2004, HIV Model of Care Wprking Party 1998. MBS	HAART3 onwards. Assume HIV viral load >10000 copies/ml and CD4+<200 cells/μl Additional costs for prophylactic therapy for PCP, toxoplasmosis
Cost of other tests each 3 months for those who respond to HAART for at least 3 months and then suffer viral rebound	cAsymp_NR		$843.75		Department of Health and Ageing 2004, HIV Model of Care Wprking Party 1998 MBS	For patients with viral rebound assume viral load >400 copies/ml and CD4+ >250 cells/μl
Cost of other tests each 3 months for those who receive salvage therapy	cAsymp_S		$933.75		Department of Health and Ageing 2004, HIV Model of Care Wprking Party 1998. MBS	More active monitoring viral load levels are higher in patients with multi-resistant virus. CD4+>200 cells/μl PCP & toxo prophylactic therapy

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name Transition probabilities	Min	Base case	Max	Source	Comments
Cost of treatment for toxic reaction	cToxic		$212.80		MBS	Specialist visit
Cost of treatment for HIV related illness for those who have responded to HAART	cHIVmorb_R	**********	$6,784.00	**********	AR-DRG S60Z-S64B ABS CPI-Health group catalogue 64010 PBS **********	Weighted average of hospital separations using total average costs. Updated to 2002-03 costs + follow-up costs- as for Asymp_R + prophylactic treatment for PCP & toxo ********** Sensitivity analysis
		$6,813	$10,938	$15,474	Hurley et al (1995)	Using update of Hurley figures (minus drugs for opportunistic infection) mean monthly costs average in-patient days 3.33
Cost of treatment for HIV related illness for those who have not responded to HAART 1, 2 or 3	cHIVmorb_NR	********	$7290.85	**********	AR-DRG S60Z-S64B ABS CPI-Health group catalogue 64010 PBS **********	Weighted average of hospital separations for using total average costs updated to 2002-03 costs. Plus follow-up costs as for Asymp_NR+prophylaxtic treatment PCP, toxo ********** Sensitivity analysis
		$6,813	$10,938	$15,474	Hurley et al (1995)	Using update of Hurley figures as above

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name	Min	Base case	Max	Source	Comments
	Transition probabilities					
Cost of treatment for HIV related illness for those who have not responded to HAART 4, 5 or 6	cHIVmorb_NR_LD		$8,524.65		AR-DRG S60Z-S64B MBS PBS	Weighted average of hospital separation as for cHIVmorb_NR + prophylactic treatment for MAC (CD4+ count <100 cells/µl, ophthalmological screening + MAC prophylaxis – used HAART3 onwards
Cost of an HIV related death	cDeath	********	$21,193.6 **********	*********	AR-DRG S62Z **********	Weighted average cost HIV-related Malignancy (ALOS12.53) & HIV-related infection +Ccc (ALOS 23.08) from AR-DRG **********
	3-monthly	$5,139	$40,356	$54,837	Hurley et al (1995)	Sensitivity analysis Update of Hurley figs using health component of CPI underestimate (minus drugs for opportunistic infection) mean monthly costs average in-patient days 15.78 days
	6-monthly	$30,834	$121,068	$164,511		
	monthly	$1,713	($13,452)	$18,279		
Cost of first triple anti-retroviral therapy (HAART1) for 3 months	cART1		$3,093.72		PBS	Zidovudine (150 mg) + Lamivudine(300 mg, fixed dose bd) Efavirenz (600 mg nightly)
Cost of second and third triple anti-retroviral therapy (HAART2 and HAART3) for 3 months	cART2&ART3		$3,831.75		PBS	Didanosine (400 mg daily (250 mg for <60kg) Stavudine (40 mg bd) or (30 mg for <60 kg) Nelfinavir (1,250 mg bd)
Cost of second and third quadruple anti-retroviral therapy (HAART4, 5 and 6) for 3 months	cART4&ART5 &ART6		$4,549.29		PBS	Didanosine (40 mg bd), Lamivudine(150 mg bd), Efavirnez (600 mg daily), Indinavir (800 mg td)

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name	Min	Base case	Max	Source	Comments
	Transition probabilities					
Cost of salvage therapy for 3 months	csalvage		$5,520.00		PBS	Stavudine (40 mg bd), Didanosine (400 mg EC cap daily), Lamivudine(150 mg BD), Efavirnez (600 mg daily), Amprenavir (1200mg daily), Ritonavir 200 mg daily (Deeks et al 2003)
Quality of Life Adjustments (Utility of perfect health=1 and death = 0)						
Utility of asymptomatic patient who has responded to HAART	uAsymp_R	0.846	0.94	1.0	Tengs & Lin (2002)	Asymptomatic HIV Sensitivity analysis +/- 10%
Utility of asymptomatic patient who did not respond to HAART	uAsymp_NR		0.90			Sensitivity analysis, set equal to asymptomatic responder and varied as with this variable
Utility of patient who has a toxic reaction	uToxic		0.82		Tengs & Lin (2002)	Utility symptomatic HIV
Utility of patient who responds to HAART but has an HIV-related illness	uHIVmorb_R		0.74			Sensitivity analysis; set equal to HIV morbidity not responding and varied as with this variable
Utility of patient who does not respond to HAART but has an HIV-related illness	uHIVmorb_NR	0.63	0.70	0.77	Tengs & Lin (2002)	Utility of AIDS
Utility of a patient on salvage therapy	uSalvage	0.738	0.82	0.902		Sensitivity analysis +/- 10%
Other Parameters						
Efficacy of genotype test on the rate of failure of HAART in the first 3 months	Effect_initial (RR)	0.76	0.85	0.95	Durant et al (1999), Cingolani et al (2002), Tural et al (2002)	Meta-analysis of 3 RCT, Durant, Cingolani, Tural-RR of undetectable viral load
Starting age of cohort	Initial age		36		AHOD database	
Discount rate	Discount rate	0	0.05	0.08		5% pa, (sensitivity 0–8%)

Table 38 (cont'd) Parameter values for the Markov model in each three-month cycle

Variable	Name	Min	Base case	Max	Source	Comments
	Transition probabilities					
Percentage of those who switch from a HAART 1 regimen following a toxic reaction	Change therapy due toxicity HAART1		0.5		Expert advice	
Percentage of those who switch from a HAART 2 regimen following a toxic reaction	Change therapy due to toxicity, HAART2		0.25		Expert advice	
Percentage of those who switch from a HAART 3, 4, 5 or 6 regimen following a toxic reaction	Change therapy due to toxicity HAART3 onwards		0.1		Expert advice	
Increased risk of HIV-related illness with age	Hazard ratio of AIDS by age	1.12 (>40 yrs)	1.51 (≥50 yrs)		Egger et al (2002)	
Increased risk of HIV-related mortality with age	Hazard ratio of death by age	1.41 (>40 years)	3.09 (≥50 years)		Egger et al (2002)	
Overall rate of failure of population with population subgroup with resistance (15%-25%)	ORF		0.987–0.963			Parameter only used to investigate the additional cost of testing all patients commencing HAART

[a]Undetectable viral load—<400 copies/ml
[b]Eggers et al
[c]Patients who started HAART between Sept 1 1995 and Nov 30 1998. The overall mortality rate for all patients in the cohort was 4.2/100 patient-years
[d]Viral rebound —Two consecutive measurements of >400 copies/ ml after an undetectable reading
[e]Subgroup from Swiss Cohort Study for which HCV serology available, in this HIV population—42% were HCV positive.
EDR for this HIV population: 23.9/1000 patient-years (had at least 6 months' treatment with HAART). EDR also includes death due to myocardial infarction as a result of HAART (Jaggy et al (2003) citing Friis-Moller)

Results

Table 39 Base case cost-effectiveness of genotype assisted HIV therapy compared to standard therapy over 50 years at five per cent discount rate

Strategy	Cost	Incremental Cost	Effect	Incremental Effect	Cost/ Effectiveness	ICER
Standard of care	$287.3K		10.162 QALYs		28,268 $/QALYs	
			14.3966 LYs		19,953 $/LYs	
Genotype test	$287.9K	$0.6K	10.274 QALYs	0.112 QALYs	28,021 $/QALYs	5,623 $/QALYs
			14.4131 LYs	0.0165 LYs	19,974. $/LYs	38,276 $/LYs

The results in Tables 39 and 40 are for patients who enter the model on their first HAART. The cost effectiveness analysis in Table 39 shows that neither the genotype test nor standard care dominated the other strategy. The genotype anti-retroviral resistance testing demonstrated greater effectiveness in both life years gained and quality adjusted life-years but at a greater cost. The greatest gain in effectiveness was in quality adjusted life years, reflecting the efficacy of the genotype test, through reducing primary failure, in maintaining greater numbers of patients in an asymptomatic state and hence delaying their progression onto subsequent HAART regimens with increased probability of virological failure and subsequent HIV morbidity or mortality. There is a small saving

in life years acquired through a delay in switching to later HAART regimens, and providing the opportunity for greater numbers of patients in the genotype arm to die from age-related mortality instead of HIV.

Table 40 Base case cost-effectiveness of genotype assisted HIV therapy compared to standard therapy over 50 years at five per cent discount rate –genotype test include in salvage therapy

Strategy	Cost	Incremental Cost	Effect	Incremental Effect	Cost/ Effectiveness	ICER
Standard of care	$287.3K		10.162 QALYs		28,268 $/QALYs	
			14.3966 LYs		19,953 $/LYs	
Genotype test	$288.5K	$1.2K	10.274 QALYs	0.112 QALYs	28,078 $/QALYs	10,804 $/QALYs
			14.4131 LYs	0.0165 LYs	20,015 $/LYs	73,540 $/LYs

Table 40 shows the results of including a genotype test each time a patient on salvage therapy develops a HIV illness. As is shown from the table, while there is an increase in costs, there is no increase in effectiveness.

Sensitivity Analysis

There is considerable uncertainty around many of the key parameters in the model. To the extent that the base case results are likely to be sensitive to the assumptions about these values, the estimated cost effectiveness of genotype testing may not be a reliable. We have tested the robustness of the model by first varying each of the key parameters in turn and examining the influence on the value of genotype testing.

Table 41 Sensitivity of ICER to key variables

	Base case ICER = $5,623/QALY	
Values	Incr C/E (ICER)	
	Low value parameter	High value parameter
Responder failing HAART2-6 (5-15%)	441 $/QALY	9,761 $/QALY
Primary failure of HAART6 (22-64%)	8,659 $/QALYs	3,429 $/QALYs
Absolute risk of primary failure of HAART increase over previous HAART (25%-75%)	17,362$/QALYs	Genotype Test dominant[a]
Initial effect of the test (RR=76-95%)	Genotype test dominant	40,527 $/QALYs
Cost of the test ($528-890.7)	2,019 $/QALYs	11,450 $/QALYs
Discount rate (0-8%)	5,446 $/QALYs	6,604 $/QALYs
Primary failure of HAART1 (3%--10%)	7,245 $/QALYs	3,306 $/QALYs
Cost of HIV mortality non responder * ($6813-15,474)	5,837 $/QALYs	5,880 $/QALYs
Cost of HIV mortality responder *($6813-15,474)	5,643 $/QALYs	6,093 $/QALYs
Cost of dying ($10,599-$31,790)	5,762 $/QALYs	5,484 $/QALYs
Utility of receiving salvage therapy (0.738-0.902)	8761$/QALY	4,144$/QALY

Note: Using the Hurley figures, the base case amount for both HIVm_R and HIVm_NR is changed to $10,938 for both, therefore there is no difference between these two parameters for the base case.

[a]Dominant refers to the situation where the genotype test is found to be both more effective and cheaper than standard care.

Univariate sensitivity analysis of the ICER shown in Table 41 confirm that the results are sensitive to individual assumption on parameters of the rate of failure and the effectiveness of the test in reducing that rate of failure at a given cost per test. Variation in any of these variables alone appears to confirm the robustness of the model. In using an update of the Hurley's cost data (Hurley et al 1995), both responders and non-responders who develop a HIV illness are costed the same at the higher cost of $10,038 because these costs were not separated in the original Hurley study. The model does not appear that sensitive to changes in the values included for the costs of HIV morbidity. Increasing the cost of dying for patients has a positive effect on the cost effectiveness of the genotype testing, the greater the cost of a patient dying the more attractive genotype testing becomes in the model. However it may be that a likely combination of variation in these parameters in practice will lead to a less favourable cost effectiveness ratio.

The model is sensitive to a 10 per cent +/- change in utility weight of those on salvage therapy, in particular the 10 per cent decrease in the utility weight results in a 56 per cent increase in the cost per QALY over the base case. This 10 per cent decrease in the benefit of receiving ongoing salvage therapy without a HIV illness would bring the utility weight to be on par with the disutility of having an HIV illness, a very unlikely scenario.

Table 42 Multivariate sensitivity analysis

	Base case ICER = $5,623/QALY	
	Incremental C/E (ICER)	
Value	**Lowest value parameter**	**Highest value parameter**
Test (0.76-0.95) Cost of Test (528-890)	3,141$/QALY	58,105$/QALY
Probability of disease progression for responders (0.00458-0.008563) Probability of disease progression those who do not achieve undetectable viral load (0.01518-0.024593)	5,499$/QALY	5,661$/QALY
Three month probability of HAART 1 failure after at least 3 months initial success (0.001-0.04) Three month probability of HAART 2 failure after at least 3 months initial success (0.05-0.15)	1,400$/QALY	8,080$/QALY
Utility of asymptomatic patients both equal irrespective of whether responding to HAART (0.846-1.00)	6,571$/QALY	631$/QALY
Utility of HIV related illness both equal irrespective of whether responding to HAART (0.63-0.77)	4,003$/QALY	9,487$/QALY
Multivariate sensitivity analysis varying the three month probability of HAART failing after at least 3 months initial success (probability of secondary failure) (0.05-0.15)	442$/QALY	9,762$/QALY
Three way sensitivity analysis of cost of the test ($890), effectiveness of the test (0.95) and secondary failure (0.15)		78,374$/QALY 585,623$/LY
A four way sensitivity analysis of cost of test ($890),effectiveness of the test (0.95), secondary failure (0.15) and utility of HIV morbidity (0.77)		132,342$$/QALY 585,263$$/LYs

Table 42 presents the results of the sensitivity analysis varying cost and efficacy of the test as well as the probability of disease progression. The upper and lower confidence intervals of the meta-analysis of the three RCTs of the genotype test were used as the range within which to explore the effect of the efficacy on the ICER, while the highest and lowest price of the test presented by the laboratories is used to vary the cost of the test. The model is sensitive to these two parameters varied together, if the test has little or no effect and is priced at the upper quote from the laboratories then the genotype test does not represent value for money. Varying the probability of clinical disease progression for responders and for those who do not achieve undetectable viral load in the first three months do not appear to have much affect on the model. These figures are very small and would be driven by the percentage of the cohort who are assumed to respond to HAART.

Varying both the probability of continuing to respond to HAART1 and 2 after initially responding to treatment, does impact on the ICER considerably. There was an increase in incremental costs with the highest probability of failing but there was also an

associated gain in QALYs of 0.013. An assumption is made in the model, based on the literature, that the rate of viral rebound is constant after patients have initially responded. To reflect this assumption a multivariate sensitivity analysis was done so that all probabilities of secondary failure were varied together. Changing these parameters increases the ICER by over 95 per cent a significant change. It is these parameters that appear to have the greatest impact on the model, for which the data is least certain.

The utility values associated with each of the health states are varied, to test the sensitivity of the model results to the benefits associated with each health state. Firstly, the utilities for each of the asymptomatic states are set equal and then varied +/- 10 per cent. Secondly, the utility associated with having a HIV morbidity is set equal for both of these health states and varied +/- 10 per cent. Confidence intervals around the utility weights could not be used for sensitivity testing as only significance was reported in the meta-analysis by Tengs & Lin (2002). The base value was only varied by 10 per cent in the sensitivity analysis because greater percentages would have taken either their minimum or maximum value beyond the basecase value for the next lower/higher health state, an illogical outcome. The effect of setting the utilities of the asymptomatic states equal to each other, and the utilities of the HIV morbidity states equal to each other, is to increase the overall cost per QALY by $26 (these results are not shown). In respect of the asymptomatic health states, reducing the utility does increase the cost per QALY, but not markedly, but, as expected, making utility equal to one significantly reduces the cost per QALY, both more QALYs are acquired with a greater incremental effectiveness over standard care. Reducing the utility weight for HIV morbidity results in a lower cost per QALY compared to the base case. Increasing the utility weight for the HIV morbidity health state results in substantially higher cost per QALY. The model is sensitive to the utility weights but not necessarily in the direction anticipated.

The sensitivity of the base case results are further demonstrated if more than two variables for which we are uncertain are varied at once. Firstly, the cost of the test and its effectiveness along with the rate of secondary failure of HAART are set equal to the values in Table 38 that are most unfavourable to the cost effectiveness of genotype testing. We assume that the cost of the test is $890.70, that the effectiveness of the test in reducing the probability of virological failure is only five per cent, and that the secondary failure rate is 15 per cent every three months. Under this scenario the ICER is $78,374 per QALY or $585,623 per extra life year. To complete the multiway sensitivity analysis HIV morbidity utility is set equal to 0.77. This combination of assumptions results in an increase in the incremental cost per QALY to $132,342, but no increase in cost per life years saved. This scenario results in an increase in quality of life for both genotype testing and the standard care strategies, but the incremental effectiveness is reduced resulting in a greater ICER.

The number of antiretrovirals in each HAART regimen was assumed to be the same between the two arms of the model; this was not varied in a sensitivity analysis. Although Cingolani et al (2002) found that patients in the genotype arm showed a significant mean increase in the number of ARV drugs used compared to control this finding was not confirmed by the studies done by Durant et al (1999) nor Tural et al (2002).

Financial implications for the health system over the next five years

The cost of funding genotype testing for HIV patients who are failing anti-retroviral therapy has been calculated using the model over the next five years. The number of

people on therapy in Australia has been estimated at 6,000, with 27 per cent on HAART1, 20 per cent on HAART2, 17 per cent on HAART3, 11 per cent on HAART4, 10 per cent on HAART5, 15 per cent on HAART6. (AHOD reported in Application). The additional financial cost of genotype testing for all those who fail therapy for the first five years of the model for an initial cohort of 6,000 distributed in these regimens, including the cost of the test and the net costs associated with HIV related disease is $2,574,000.

Following the cohort of HAART patients for five years, the model estimates that, over the five years, on average each patient will require one genotype test.

Validation of model

The model has been calibrated on AHOD data for the percentage of patients on later therapy and is similar to that reported in AHOD (2002) at 44 months (15 cycles).

The change in the distribution of patients who were on therapy at the beginning of the AHOD data period is shown in Table 43 along with the model predictions of the percentage who remain in each therapy if they begin in a particular regimen at the start of the model simulation. The model predicts that patients will remain on a particular combination for longer than the AHOD data suggests. This is to be expected. In estimating the probability of failure of HAART1 (both primary and secondary failure), the results of a randomised controlled trial by Robbins et al (2003), in which four of the latest antiretroviral regimens were tested, was used and from these results only the most effective regimen included in the model. On the other hand the AHOD database will include patients on multiple different first antiretroviral combinations used since 1997 (including some no longer in use), and as such the rates of failure must be higher than what has been used in the model. We did not use the rates of failure in the AHOD database because we firstly wished to use a regimen that was representative of current use and secondly it was not possible to separate out primary and secondary failure. The AHOD data is a snapshot of patients on therapy rather than a cohort as modelled. Since some will have been on therapy for some time it is expected that the six month retention on therapy would be lower than in the model. By two years the results are similar at least for second and subsequent regimens.

Table 43 Comparison of model prediction of change in combination with AHOD data

% of patients still on combination (AHOD sept 2000)				Patients still on a particular combination model data				
	at 6 mths	at 12mths	at 18 mths		at 6 mths	at 12mths	at 18 mths	at 24mths
first	0.7	0.54	0.45	first	0.83	0.76	0.7	0.65
second	0.69	0.51	0.4	second	0.77	0.6	0.47	0.36
third or more	0.58	0.37	0.37	third or more	0.76	0.6	0.47	0.36

The rate of change of combination therapy after the first regimen is reported in AHOD (2002) as 0.45 per follow up year. If we distribute the initial cohort in the same proportions as the AHOD the model, the average number of tests is 1.02 in the first 15 cycles, corresponding to a rate of change of combination in the first 45 months of 0.27 per person per year. At 30 cycles the cumulative number of tests is 1.62 per person but only 48 per cent are on HAART1 – HAART6. The rate of genotype testing at 120 months (and therefore the change of combination) per person year on HAART1 through HAART6 is 0.32. The model predictions on the rate of change in combinations shown in

Table 43 are consistent with the AHOD data, but do not favour genotype testing. Rather they are biased towards standard therapy in the cost effectiveness analysis compared to the AHOD data.

Primary resistance testing

There is no evidence for effectiveness of providing a genotype antiretroviral resistance test to all patients commencing their first HAART. In order to provide some information on the possible cost effectiveness of antiretroviral resistance testing in treatment naïve patients, an indicative model has been constructed for a cohort of patients commencing first HAART. A subgroup of patients in the HIV population, comprising 9% of that population (Ammaranond et al 2003a) is assumed to have some degree of primary resistance. If resistance testing reduces the probability of failure in this subgroup by 15 per cent, a 1.35 per cent overall reduction in failure rate of first HAART might be expected.

Table 44 Overall reductions in failure rate in population with subgroups of resistance

	Overall reduction in failure in the population	
Effectiveness of genotype test in reducing probability of failing HAART	**Resistance in population (9%)**	**Resistance in population (15%)**
15%	1.35%	2.25%
20%	1.8%	3%
25%	2.25%	3.75%

The decision analytic model was then re-run, using the same assumptions that generated the base case, but now including the effect of a primary test on the failure rate of the first HAART. The effect of including primary testing on the number of patients who fail their first HAART is predicted from a number of possible assumptions about resistance in the population and the effectiveness of genotype testing in reducing the probability of failing HAART (Table 44). Each patient incurs a cost of $666.58 for the genotype test. The results are shown in Table 45.

Table 45 Incremental cost per QALY, varying the effectiveness of the test and level of resistance in the population, compared to standard of care

Resistance in population	Effective-ness of test	Strategy	Cost	Incr Cost	Effect QALYs	Incr Eff QALYs	C/E $/QALYs	Incr C/E $/QALYs
9%	15%	SOC	$287.3K		10.162		28,268	
		GT	$288.5K	$1.3K	10.275	0.113	28,081	11,244
	25%	SOC	$287.3K		10.162		28,268	
		GT	$288.5K	$1.3K	10.276	0.114	28,077	11,035
15%	15%	SOC	$287.3K		10.162		28,268.34	
		GT	$288.5K	$1.3K	10.276	0.114	28,077.31	11,035.43
	25 %	SOC	$287.3K		10.162		28,268.34	
		GT	$288.5K	$1.2K	10.277	0.115	28,071.63	10,692.78

Abbreviations: SOC, standard of care; GT, genotype test

Table 46 presents the cost effectiveness of testing each patient before commencing HAART and subsequently after each primary failure for different combinations of the genotype test effectiveness and endemic HIV resistance levels. The incremental cost per

extra quality adjusted life year gained compared to no testing ranges between $10,693 and $11,244. The results in Tables 39 and 45 compare genotype testing regimes (naïve patients and those who have failed HAART) against no testing. If however we compare these two regimes the incremental cost per extra quality adjusted life year gained is $600,000 per QALY (Table 46). In other words the incremental cost per extra quality adjusted life year gained of testing all patients commencing HAART and then subsequently when they fail, compared to testing only those who fail HAART is $600,000. The large increase in the ICER is due to the low prevalence of endemic resistance to antiretrovirals and the consequent small effect of testing on first failure to respond to HAART.

Table 46 Incremental cost-effectiveness of testing all patients commencing HAART compared to patients failing their first HAART assuming a RR of achieving an undetectable viral load of 0.85 and nine per cent resistance in the population.

Strategy	Cost	Incremental Cost	Effect	Incremental Effect	Cost/Effectiveness	Incremental C/E
Genotype test all patients	$288.5K		10.275 QALYs		$28,081 /QALYs	
Genotype test patients failing first HAART	$287.9K		10.274 QALYs		$28,021 $/QALYs	
		$0.6K		0.001		$600,000/QALY

Discussion

The economic analysis has used a variety of data sources and a decision analytic model to predict the cost and outcomes from the use of genotype testing for HIV in a cohort of Australian patients when they fail each HAART regimen. The base case estimate suggests that genotype testing has an incremental cost per extra QALY of $5,623 /QALY and an incremental cost per extra life year of $38,276. An indicative analysis suggests that if HAART naïve patients are included in genotype testing then the incremental cost per extra QALY compared to no testing ranges between $10,693 and $11,244. However there is no evidence of the efficacy of genotype testing in patients prior to receiving HAART. The incremental cost per extra quality adjusted life year gained of testing all patients commencing HAART and then subsequently when they fail, compared to testing only those who fail HAART is $600,000 per QALY. On this basis it might be argued that there is a case for restricting testing to those who have failed HAART.

The model is capable of providing an indication of the likely costs and outcomes, it needs to be recognised however that there are a number of limitations on the analysis. The most important is the lack of high quality evidence on many of the key parameters in the model. This is particularly the case since the parameters of the model are taken from disparate sources with unknown measurement errors in populations that may not be similar to that simulated in the estimate of costs effectiveness. The single variable sensitivity analysis suggests that the results are robust within the parameter range tested. The model structure and base case assumptions on regimen switching without genotype testing appear to have some validity insofar as they are consistent with Australian data on the number of patients on each regimen through time. However when a number of the parameters were varied simultaneously, to levels unfavourable to genotype testing, the cost effectiveness ratio increased six-fold.

Conclusions

Safety

The extensive literature search revealed a lack of safety data for genotypic resistance testing of antiretrovirals in HIV. However, as the test generally requires only a blood sample, the risks to subjects would be expected to be minimal.

Effectiveness

Diagnostic accuracy

Evidence of the diagnostic accuracy of genotypic resistance testing of antiretrovirals in HIV was extracted from 10 primary studies, eight of which were retrospective and two of which were prospective in design. Each of the studies provided data on genotypic resistance testing and determination of resistance or susceptibility to various therapies as a predictor of treatment outcome in a manner which allowed for the calculation of the test's sensitivity and specificity and their derivatives.

Seven studies reported treatment outcome as a reference standard to confirm whether baseline resistance to one or more drugs accurately predicted treatment failure. Two studies reported treatment outcome as a reference standard to confirm whether baseline susceptibility to one or more drugs accurately predicted treatment success and the remaining study reported treatment outcome as a reference standard to confirm whether the total number of drug resistance mutations could predict treatment outcome. Treatment outcome was assessed by virologic response in eight studies, while two studies assessed treatment outcome with both virologic and immunologic responses. The length of follow-up between the studies ranged from six weeks to two years.

It was difficult to summarise the diagnostic characteristics of genotypic testing as findings varied across the studies. Possible reasons for the variation include: although all studies examined baseline resistance or sensitivity to particular therapies derived from resistance mutations in the genotype to predict treatment outcome, there was little consistency in which therapies were evaluated across studies; the potential confounding of results by the design of studies that measured resistance to particular therapies but measured outcome to those therapies in combination with other therapies; the possibility that resistance developed between the time of genotypic testing and measurement of treatment outcome and the inconsistency of measures of treatment outcome and length of follow-up.

The following tentative conclusions were drawn from calculation of the diagnostic characteristics.

- Of the studies that assessed the value of RTI resistance mutations in predicting virologic failure, 50 per cent indicated that the presence of baseline resistance mutations to RTIs used in various combination therapies has some use as a predictor of treatment failure to those combination therapies. The remaining 50

per cent of studies suggested that the presence of RTI resistance mutations was not a useful predictor of treatment failure.

- Data from one study indicated that the numbers of thymidine analogue, NNRTI and PI mutations present in HIV are of limited use in predicting treatment success.

- Data from one study indicated that the presence of baseline resistance to the PIs saquinavir and ritonavir provided moderate evidence of the likelihood of virologic failure to a HAART regimen of saquinavir and ritonavir with two RTIs.

- Data from one study indicated that the presence of any primary and secondary PI mutations are of limited use in predicting treatment failure. However, this study also provided evidence that the presence of primary PI resistance mutations was of some use in predicting treatment failure.

- Data from one study indicated that the presence of RTI or PI baseline resistance was not a useful predictor of treatment failure to HAART.

- Data from two studies indicated that the presence of baseline susceptibility to RTIs was not a useful predictor of treatment success, while data from one of those studies indicated that the presence of baseline susceptibility to PIs was an accurate predictor of treatment success to combination therapy.

Patient outcomes

Evidence of the clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV was extracted from seven RCTs, one open-label extension of an RCT and one meta-analysis. Four RCTs compared the effectiveness of HIV genotyping with that of standard of care, one RCT compared the effectiveness of genotyping with that of standard of care and drug-susceptibility phenotyping and two RCTs compared the effectiveness of virtual phenotyping with that of drug-susceptibility phenotyping. Patients included in all trials were HAART-experienced, however the degree of previous antiretroviral therapy varied amongst the studies. The definitions of standard of care varied across studies, as did the interpretation of genotype test results and the experts used to prescribe or recommend HAART regimens following genotype testing.

There were two main primary outcomes used to determine the effectiveness of genotypic resistance testing of HIV to determine an optimum HAART regimen in patients experiencing virologic failure. The primary outcome of achieving a viral load below the level of detection was used in five trials. The level of detection varied in the studies due to the techniques used to measure viral load. A change in viral load from baseline to pre-determined time points following the initiation of therapy was the primary outcome in two trials.

The clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV were extracted from seven RCTs, one single arm extension of an RCT and one meta-analysis. The major findings of this assessment are as follows:

- Few deaths and AIDS-related events were reported in any of the studies. No statistically significant differences were found between the treatment arms

(genotype versus standard of care; genotype versus drug-susceptibility phenotype; virtual versus drug-susceptibility phenotype) in the number of patients that died or experienced an AIDS-defining event during the course of the studies.

- Whilst no differences in the number of deaths and AIDS-related events were found between treatment arms in the studies, results of a meta-analysis to determine the effectiveness of genotype resistance testing compared with standard of care in achieving an undetectable viral load revealed that patients receiving genotype-guided treatment were 1.3 times more likely to achieve plasma HIV RNA below the level of detection than patients treated by standard of care at three months (RR=1.33, 95% CI: 1.14, 1.56; NNT=10, 95% CI: 6, 20) and 1.4 times more likely at six months (RR=1.41, 95% CI: 1.12, 1.77; NNT=9, 95% CI: 6, 25).

- In addition to patients having an increased likelihood of achieving an undetectable viral load when treated by genotype-guided therapy, results of a meta-analysis to estimate the effectiveness of genotype-guided therapy in reducing viral load compared with standard of care revealed that patients receiving genotype-guided therapy had a significantly greater reduction in viral load at three months (–0.23 $\log_{10}$copies/ml, 95% CI: –0.34, –0.12) and this benefit was sustained at six months (–0.23 $\log_{10}$ copies/ml, 95% CI: –0.37, –0.08) compared with patients receiving treatment based on standard of care.

- The reported changes in CD4+ cell counts was variable between the RCTs and there is uncertainty pertaining to any treatment differences between genotype-guided therapy and therapy prescribed based on standard of care or drug-susceptibility phenotyping.

- There were several differences observed in the number and/or combinations of antiretroviral drugs prescribed in the genotype and standard of care arms.

- There were no differences observed in the number and/or combinations of drugs prescribed in the HAART regimens between genotyping and phenotyping or drug-susceptibility versus virtual phenotyping.

- There were no significant differences observed in the rates of adverse events involving toxicity of any drugs prescribed in HAART between any of the treatment arms.

- Three of the seven trials reported that patients received multiple genotypic resistance testing if the prescribed treatment was deemed sub-optimal due to patients not achieving a particular level of viral load reduction. The remaining studies did not specify if multiple tests were conducted.

- Each of the studies used different methods to perform genotypic resistance testing and interpret the results of the tests. Results from an Australian quality assessment scheme have indicated that the assay is highly reproducible, with less than a one per cent variation between identical samples in all laboratories. However, there is variability between laboratories in the detection of mutations and mixtures of mutations, and the level of concordance in the interpretation of genotypic resistance testing results (which depends on the interpretation system used).

- Data from the single-arm extension of an RCT appeared to show a maintenance of virologic response in patients originally assigned to the genotyping arm and patients originally assigned to standard of care appeared to benefit from having genotyping being made available. It is not possible to determine the incremental effectiveness attributable to genotypic resistance due to the lack of a comparator group.

- The meta-analysis concluded that the results supported the use of a genotypic test in patients experiencing virologic failure during antiretroviral treatment, and that expert interpretation of the test increased the probability of a virologic response.

The following key issues were identified:

- All patients enrolled were antiretroviral experienced and failing current therapy.

- No evidence of the effectiveness of genotypic resistance testing in treatment-naïve patients, pregnant women or patients with discordant virologic responses was found.

- Six of the seven trials were open-label in design which may lead to bias.

- The follow-up period of the RCTs identified varied from 12 to 48 weeks. There are no long-term data to assess the clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV.

- All of the trials based their measure of clinical effectiveness of genotypic resistance testing of antiretrovirals in HIV on virologic outcomes. It has been well documented that a combination of virologic and immunologic responses to treatment are more effective at predicting outcomes of clinical events of patients.

- There was variability in the number of drugs and range of therapies used in each of the studies.

- Whilst no differences were found between the treatment arms for the rates of death and AIDS-defining events, these were not primary outcomes in any of the RCTs. Thus, the studies may not have been powered to detect a difference in the proportion of patients who died or experienced an AIDS-defining event. In addition, the studies were not designed to detect differences over extended periods.

- The differences observed in the number and/or combinations of drugs prescribed between the genotype and standard of care arms in the trials make the incremental benefit of genotype-guided therapy difficult to distinguish from the benefit of the antiretrovirals themselves.

Cost-effectiveness

There is plausible evidence of the efficacy of genotype testing in patients who fail HAART treatment. There is no evidence of the efficacy of genotype testing in patients before initiation of HAART or in pregnant women. A decision analytic model of

genotype testing in a cohort of patients on first HAART when they fail each regimen was used to calculate the incremental cost and incremental benefits of testing in terms of quality adjusted survival and reduced cost of treatment for HIV-related disease.

The results suggest that under plausible assumptions, genotype testing at a cost of $666.58 for patients who fail antiretroviral therapy would have an incremental cost per QALY of $5,623. The effect of genotype testing is to reduce the rate of failure of HAART and slow disease progression. In the context of the Australian population, in which HAART has already led to a fall in disease progression and HIV-related morbidity and mortality, the effect of genotype testing on survival and quality of life is muted. Nevertheless, maintaining even a small number of patients on a HAART regimen for longer was found to slow disease progression, reduce morbidity (and the associated large unit costs of health care) and have some impact on quality of life.

Overall, the economic model confirms that the cost of treatment for a small number of patients and the improvement in the quality of life of those who respond to treatment are likely to counter-balance the cost of the test. Expert opinion was ..."Assuming that that the rate of secondary failure is sufficiently low, the effectiveness of the test in practice is within the range estimated in the trials, and the cost of the test is not substantially greater than the estimated average of current laboratory costs, the predicted improvement in survival and quality of life for patients could be regarded to be sufficient to justify the additional cost." However, there is insufficient evidence to support these assumptions.

Recommendation

MSAC found that genotypic resistance testing of antiretrovirals in HIV appeared to be safe and leads to changes in clinical management but there is insufficient evidence on effectiveness and cost-effectiveness to support Medicare funding.

- The Minister for Health and Ageing accepted this recommendation on 2 March 2005.

Appendix A MSAC terms of reference and membership

MSAC's terms of reference are to:

- advise the Minister for Health and Ageing on the strength of evidence pertaining to new and emerging medical technologies and procedures in relation to their safety, effectiveness and cost-effectiveness and under what circumstances public funding should be supported;
- advise the Minister for Health and Ageing on which new medical technologies and procedures should be funded on an interim basis to allow data to be assembled to determine their safety, effectiveness and cost-effectiveness;
- advise the Minister for Health and Ageing on references related either to new and/or existing medical technologies and procedures; and
- undertake health technology assessment work referred by the Australian Health Ministers' Advisory Council (AHMAC) and report its findings to AHMAC.

The membership of MSAC comprises a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member	Expertise or Affiliation
Dr Stephen Blamey (Chair)	general surgery
Associate Professor John Atherton	cardiology
Professor Sydney Bell	pathology
Dr Michael Cleary	emergency medicine
Dr Paul Craft	clinical epidemiology and oncology
Dr Gerry FitzGerald	Australian Health Ministers' Advisory Council representative
Dr Kwun Fong	thoracic medicine
Dr Debra Graves	medical administrator
Professor Jane Hall	health economics
Professor John Horvath	Chief Medical Officer, Department of Health and Ageing
Ms Rosemary Huxtable	Medicare Benefits Branch, Department of Health and Ageing
Dr Terri Jackson	health economics
Professor Brendon Kearney	health administration and planning
Dr Ray Kirk	health research
Dr Michael Kitchener	nuclear medicine
Professor Alan Lopez	medical statistics and population health
Associate Professor Donald Perry-Keene	endocrinology
Dr Ewa Piejko	general practice
Mrs Sheila Rimmer	consumer representative
Professor Jeffrey Robinson	obstetrics and gynaecology
Professor John Simes	clinical epidemiology and clinical trials
Professor Michael Solomon	colorectal surgery and clinical epidemiology
Professor Ken Thomson	radiology
Dr Doug Travis	urology

Appendix B Advisory Panel

Advisory Panel for MSAC application 1067
Genotype resistance testing for use of antiretrovirals in HIV

Professor Sydney Bell (Chair) MD, BS, FRCPA, FAFPHM (RACP) Area Director of Microbiology South East Sydney Area Health Service (SEALS) Randwick, NSW	MSAC member
Dr Ewa Piejko MBBS, FRACGP, DRANZCCG General practitioner The Circle Surgery North Altona, VIC	MSAC member
Associate Professor Andrew Carr MBBS, MD, FRACP, FRCPA Senior staff specialist St Vincent's Hospital, Sydney Associate Professor of Medicine University of NSW, Sydney	Nominated by the Royal Australasian College of Physicians
Mr John Daye Treatments spokesperson, National Association of People Living with AIDS Board Director, Alfred Hospital Board Director, Victorian AIDS Council President, People Living with HIV/AIDS	Nominated by the Health Consumers' Health Forum of Australia
Dr Roger Garsia MBBS (Hons), PhD, FRACP, FRCPA Senior staff specialist, Clinical Immunology Director, Central Sydney Area Health AIDS Service Royal Prince Alfred Hospital Camperdown, NSW	Nominated by Royal College of Pathologists of Australasia
Dr Geoff Higgins MBBS, PhD, FRCPA Deputy Head of Virology Infectious Diseases Laboratories Institute of Medical and Veterinary Science Adelaide, SA	Co-opted Advisory Panel member

A/Professor Jennifer Hoy MBBS, FRACP Head, Clinical Research Unit Infectious Diseases The Alfred Hospital Prahran, VIC	Nominated by the Australasian Society for HIV Medicine
Dr Ruth Lopert BSc, BMed, MMedSci Medical Advisor, Pharmaceutical Benefits Branch Department of Health and Aging Canberra, ACT	observer
A/Professor Anne Mijch MBBS (Honours), FRACP, OAM, Diploma Epidemiology & Biostats Head Victorian Aids Service Infectious Diseases Unit The Alfred Hospital Prahran, Victoria	Nominated by the Australasian Society for Infectious Diseases
Professor Lloyd Sansom Dip (Pharma), BSc, PhD Chair, Pharmaceutical Benefits Advisory Committee Woden, ACT	Co-opted Advisory Panel member

Appendix C Treatment guidelines

Table C1 DRAFT 2001 Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, October 2001

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
Patients with established HIV infection: Group A: Symptomatic HIV disease with any CD4+ count and any HIV RNA level Group B: Asymptomatic with CD4+ cells <200/μl and any HIV RNA level Group C: Asymptomatic with CD4+ cells 200–350/μl and any HIV RNA level Group D: Asymptomatic with CD4+ cells >350/μl and viral load >50,000 copies/ml (RT-PCR)[a] Group E: Asymptomatic HIV with CD4+ cells >350/μl and viral load <50,000 copies/ml (RT-PCR)[a, b]	Group A: Treat with potent antiretroviral therapy Group B: Treat with potent antiretroviral therapy Group C: Offer treatment Group D: Treatment recommended Group E: Defer treatment Treatment consisting of one of the following nucleoside analogue combinations: Zidovudine + didanosine Zidovudine + lamivudine Stavudine + didanosine Stavudine + lamivudine Didanosine + lamivudine PLUS One of the following NNRTI or PIs: Efavirenz Indinavir (or indinavir with ritonavir boost) Nelfinavir Lopinavir (with ritonavir boost) Saquinavir with ritonavir Abacavir (if the viral load is <100,000 copies/ml) Nevirapine	If cause of treatment failure is drug intolerance and undetectable viral load is achieved, the single drug causing the adverse effects can be exchanged for another antiretroviral agent If the cause of treatment failure is significant increases in plasma viral load or the inability to achieve an undetectable viral load, change as many drugs in the regimen as possible. Do not change a single drug or add a single drug to a failing regimen – sequential monotherapy sets the stage for rapid development of resistance to the new agent. Avoid exchanging NNRTI in a failing regimen because of high-level cross-resistance (may be possible to exchange efavirenz for nevirapine if Y181C mutation is cause of resistance). Changing ritonavir for indinavir should also be avoided as these drugs share high-level cross-resistance For first regimen failure, change all the drugs in the regimen. If the first regimen was a PI-containing regimen, assess the contribution of poor adherence or poor pharmacokinetics to virologic failure. Significant PI resistance is unlikely in this setting. Intensification of the regimen by adding ritonavir for pharmacokinetic enhancement is recommended if no significant resistance is found on assay. If the first regimen was an NNRTI-containing regimen, then a complete change of all drugs is warranted. It is known that the response to second and subsequent regimens is inferior to that of an initial regimen Individuals who have experienced virologic failure on two treatment regimens require consideration of salvage therapy regimens. An assessment of adherence, strategies to improve adherence and suboptimal pharmacokinetic interactions may provide improved treatment efficacy if there is no development of resistance. The ability to achieve undetectable viral loads with salvage therapy is less likely than with first regimens, however there is a 60–70% chance if a new regimen can be constructed which contains a class to which the individual has not been exposed It is recommended that patients who have exhausted their treatment options and have virologic failure continue the failing therapy as the treatment retains some of its antiviral activity, and data suggest that some drug resistant viruses are 'less fit'	

Table C1 (cont'd) DRAFT 2001 Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, October 2001

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
Patients with primary HIV infection (HIV seroconversion illness)	Insufficient clinical trial data to make firm recommendations of particular antiretroviral regimens. Similar considerations to those made for treatment of established HIV infection in the asymptomatic individual should be employed in selecting an appropriate regimen. The duration of treatment for primary infection is unknown	NA	The long-term clinical outcome benefit of potent combination antiretroviral therapy for the treatment of acute HIV infection is unknown at this stage At this time, all patients commenced on antiretroviral therapy during primary infection should remain on treatment until further research is completed The goal of potent antiretroviral therapy during primary infection is the complete and durable suppression of plasma HIV RNA to below limits of detection of current tests
HIV infected pregnant women and paediatric HIV infection	Not included in the Guidelines	Not included in the Guidelines	

[a]If using bDNA assays, the equivalent viral load level is 30,000 copies/ml

[b]In Australia, eligibility for Section 100 reimbursement for antiretroviral therapy remains at the indication for therapy of < 500 CD4+ cells/μl and/or a plasma viral load greater than 10,000 copies/ml

Abbreviations: NA, not applicable

Table C2 Antiretroviral Therapy for HIV Infection: Principles of use - Standard of Care Guidelines October 1997. HIV/AIDS Clinical Trials & Treatments Advisory Committee, of the Australian National Council on AIDS & Related Diseases

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
General	Offer combination therapy early in the course of HIV disease In general, 2 NRTIs together with either a PI or an NNRTI are required for adequate treatment of HIV infection The selection of antiretroviral agents needs to be individualised for each patient to account for the effects of compliance, toxicity, dosing requirements, prior therapies, concomitant therapies and disease stage	Viral load counts should be used in conjunction with CD4+ cell counts to determine treatment failure. Other such as patients life style, side effects of drugs, compliance and commitment to treatment should also be considered In case of resistance to treatment: change at least two drugs rather than sequentially add one new drug (poor compliance, particularly with PIs and NNRTIs, has been shown to be a major contributor to the development of resistance) Benefit may be obtained from any three- or four-drug regimen, which contains at least two drugs to which the individual has not been previously exposed, where these drugs possess synergistic or additive *in vitro* activity and there are no overlapping toxicities or adverse pharmacokinetic interactions The regimen would ideally include two NRTIs and a PI. The role of NNRTIs in salvage therapy is unknown Alteration in antiretroviral therapy should be done in consultation with a practitioner experienced in HIV medicine	The following antiretroviral combinations cannot be recommended for reasons of safety or because of insufficient supporting data: didanosine & zalcitabine (increased risk of peripheral neuropathy/pancreatitis) stavudine & zalcitabine (increased risk of peripheral neuropathy/pancreatitis) zalcitabine & lamivudine (*in vitro* evidence of competition for phosphorylation) zidovudine & stavudine (*in vitro* evidence of competition for phosphorylation) Combining therapy with the PIs ritonavir and indinavir is not currently recommended. Combinations of NNRTIs – nevirapine, loviride and delavirdine – cannot be recommended, primarily because of *in vitro* cross-resistance patterns There are no data to provide firm guidelines for the optimum HIV RNA level at which antiretroviral therapy should commence or change, and rates of disease progression will vary between individuals. Therefore, treatment decisions should be individualised according to the level of risk indicated by HIV RNA levels and CD4+ counts Based on available data, antiretroviral treatment is recommended for patients with <500 CD4+ cells or a viral load >10,000 copies/ml. Viral load counts of 500 to 10,000 copies/ml indicates low viral load, although treatment options could be considered. Less than 500 copies/ml indicates very low viral load, with adequate control Although the goal of antiretroviral therapy is to achieve undetectable HIV RNA in plasma, this will not be possible in all patients (especially those with extensive prior antiretroviral therapy). Therefore it is important that the clinician and patient do not have unrealistic expectations

Table C2 (cont'd) Antiretroviral Therapy for HIV Infection: Principles of use - Standard of Care Guidelines October 1997. HIV/AIDS Clinical Trials & Treatments Advisory Committee, of the Australian National Council on AIDS & Related Diseases

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
Patients with primary infection	A minimum combination of two nucleoside analogues plus a PI is recommended. All antiretroviral drugs should be started together At this time, all patients commenced on antiretroviral therapy during primary infection should remain on treatment until further research is completed The duration of treatment for primary infection is unknown	NA	Viral load should be monitored frequently, with the goal of achieving levels below detection The possibility that primary infection may have been with a drug-resistant virus (eg zidovudine) needs to be considered when selecting the antiretroviral combination Patients should be monitored closely for drug tolerance and treatment compliance The management of primary HIV infection should be undertaken in consultation with a practitioner experienced in HIV medicine
Patients who are HIV antibody positive, but in whom viral load is low and no laboratory markers of immune activation or immune deficiency are present	Treatment not required at present for these patients. However, they should be monitored	NA	
Treatment-naïve HIV-positive patients with one or more laboratory markers of immune activation or immune deficiency (see Section 1.5) or uncontrolled viral replication (high or increasing viral load)	Commence therapy with three or more agents (usually two nucleoside analogues and a potent PI) Initiation of therapy should be undertaken in consultation with a practitioner experienced in HIV medicine	NA	

Table C2 (cont'd) Antiretroviral Therapy for HIV Infection: Principles of use - Standard of Care Guidelines October 1997. HIV/AIDS Clinical Trials & Treatments Advisory Committee, of the Australian National Council on AIDS & Related Diseases

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
HIV-infected pregnant women	For reduction of HIV infection risk to the infant, all pregnant HIV-infected women should be encouraged to be on therapy which should include the 076 regimen: zidovudine 200 mg three times daily during pregnancy zidovudine 2.0 mg/kg IV during first hour of labour and 1.0 mg/kg per hour thereafter until delivery zidovudine syrup to infant 2 mg/kg every six hours from eight hours after birth for six weeks Most women should be receiving combination antiretroviral therapy. In women planning pregnancy or currently pregnant, combinations including zidovudine, nevirapine, didanosine, lamivudine and saquinavir currently appear to be the safest options	Not stated in the guidelines	Maternal care should be optimised, aiming to achieve the lowest possible plasma HIV RNA viral load, the highest CD4+ count and the best foetal growth possible. All women should be fully informed of the benefits of the 076 zidovudine regimen and of the limitations of the data in relation to those with prior experience of antiretroviral therapy or those contemplating treatment in the first trimester of pregnancy Women should be informed of the benefits of triple combination antiretroviral therapy in terms of survival benefits, prevention of opportunistic infection and reduction in HIV viral load for themselves The lack of clinical efficacy and toxicity data needs to be carefully explained to the women involved
Paediatric infection	Whilst the principles of management are the same for children and adults, paediatric HIV infection is a specialised area and the child should be referred to a paediatric physician with experience in HIV medicine	Not stated in the guidelines	

Abbreviations: IV, intravenous; NA, not applicable

Table C3 Antiretroviral Therapy for HIV Infection in Women and Children: Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, January 1999

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
HIV-infected woman contemplating pregnancy or the HIV-infected pregnant woman and her infant	To reduce perinatal HIV transmission: Time: Antenatal (initiated from 14 to 34 weeks of gestation). Continue throughout pregnancy Target: Mother (not applicable for infant) Treatment: ZDV 100 mg orally, 5 times a day Time: Onset of labour Target: Mother (not applicable for infant) Treatment: Loading dose: ZDV 2 mg/kg IV over 1 hour. Maintenance dose: Continuous infusion of ZDV 1 mg/kg/hr until delivery Time: For elective caesarean section Target: Mother (not applicable for infant) Treatment: Commence intravenous infusion of ZDV 4 hours prior to delivery Time: After delivery review commence ZDV syrup[a], Target: Mother and infant Treatment: Review mother. For infant, commence ZDV syrup[a], 2 mg/kg/dose orally every 6 hours 8–12 hours after birth for 6 weeks NB If infant is not allowed oral intake, give IV ZDV 1.5 mg/kg, 6-hourly	Not stated in the guidelines	

Table C3 (cont'd) Antiretroviral Therapy for HIV Infection in Women and Children: Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, January 1999

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
HIV-infected children	Suggested initial drug regimens: . ZDV + 3TC + nelfinavir, or d4T + 3TC + nelfinavir is the regimen of choice . ZDV + 3TC + nevirapine + ritonavir (or nelfinavir), or d4T + 3TC + nevirapine + ritonavir (or nelfinavir) is suggested newborns with high viral load (with forethought given to adherence issues Points to note: . ZDV and d4T cross into the central nervous system. They are therefore advantageous in a drug combination regimen but should not be used together . ddI and ddC should not be used together . Resistance to ddI precludes the subsequent use of ddC (and vice versa)	When treatment failure occurs (eg progression of clinical symptoms and/or increasing viral load or evidence of worsening immune function), adherence to therapy should be carefully considered and potentially treatable reasons for failure need to be excluded (eg concurrent opportunistic infections). When using viral load as a guide to management, a change in therapy should be made only if the HIV RNA level has risen by more than 0.5 $\log_{10}$ and is more than 4 $\log_{10}$ units (as only a limited number of options for new drug regimens exists at the present time) When changing therapy, the new combination should include a change of at least two drugs, each of which should be from a different category, and a change in PI A drug-free period between the cessation of a failed regimen and the commencement of another combination of drugs has been suggested. Some of the panelists disagreed with this idea on the basis that once resistant mutants emerge, they will persist even if the drugs are stopped. If, however, the child or family requests a drug-free period, then 4 weeks is considered to be appropriate. It is considered less harmful to suspend treatment of all drugs simultaneously than to miss doses of individual drugs	Monitoring: . Monthly viral loads and CD4+ lymphocyte counts when initiating or changing therapy; then at three-monthly intervals . Wait up to six months before changing therapy regimens if there is a downward trend in viral load . If CD4+ lymphocyte counts improve and the patient is clinically well but viral load is not responding, the current drug regimen does not always need to be changed . Growth and neurological assessments are integral parts of clinical monitoring Special situations: Encephalopathy: A child developing encephalopathy while on a drug combination that includes oral ZDV is considered to have disease progression. d4T should then be used as part of the new combination regimen rather than IV ZDV HIV prophylaxis in sexual abuse in a high-risk sexual abuse situation (eg anal penetration) by a perpetrator of unknown HIV status: post-exposure prophylaxis should be similar to that of individuals who have incurred needle stick injuries ie ZDV + 3TC (with or without a PI) for four weeks (28). The prophylactic regimen should be commenced as soon as possible and preferably no more than 72 hours after the incident

[a]Some patients felt that ZDV syrup 4 mg/kg/dose, twice a day, would also be acceptable due to prolonged intracellular half-life over serum half-life of ZDV
Abbreviations: IV, intravenous

Table C4 Model of Care for HIV Infection in Adults HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases, 1998

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
Group A: Nadir CD4+ count >500/ml and/or viral load <10,000 copies/ml Group B: Nadir CD4+ count 500–200/ml and/or viral load >10,000 copies/ml Group C: Nadir CD4+ count 200–100/ml and viral load >10,000 copies/ml Group D: Nadir CD4+ count <100/ml and viral load >10,000 copies/ml These guidelines do not address the issue of the diagnosis or management of primary or acute HIV infection, nor do they address post-exposure prophylaxis	Group A: Consider antiretrovirals; consult Antiretroviral Guidelines Group B: Consider antiretroviral therapy for all patients, particularly if the CD4+ count is declining; consult Antiretroviral Guidelines Group C: Antiretroviral therapy is recommended for all patients; consult Antiretroviral Guidelines Group D: Antiretroviral therapy is recommended for all patients; consult Antiretroviral Guidelines	Not available in the guidelines	

Table C5 Queensland Management Guidelines for the Detection and Treatment of Sexually Transmissible Diseases and Genital Infections. Version II December 2003, page 96 Queensland Government, Queensland Health

Patient group	Recommended treatment		Remarks related to treatment
	Newly-diagnosed patients	Patients with treatment failure	
Post exposure prophylaxis (PEP) of HIV	Persons exposed to significant risk of HIV infection through occupational exposure or non-occupational exposure may benefit from administration of combination antiretroviral therapy for one month, provided drugs are commenced within 72 hours of exposure Current antiretroviral treatment strategies recommend the use of combined treatment with 3 or 4 drugs	Not stated in the guidelines	Direct evidence of the effectiveness of PEP is lacking, but a substantial body of indirect epidemiological evidence now exists for the effectiveness of occupational PEP People seeking PEP may present to emergency departments or sexual health clinics where starter packs of appropriate antiretroviral drugs are available. They should then be followed up within a few days for counseling and further prescription of antiretroviral drugs for one month, if appropriate, by accredited HIV prescribers Counseling for PEP involves exploring with the patient the known or suspected risks of HIV infection against the risks and known side effects of one month's antiretroviral therapy

Appendix D Search strategies

Core terms

Table D1 Core terms for MEDLINE and MEDLINE In-Process & other non-indexed citations

1	exp HIV/
2	HIV.mp.
3	HIV-1.mp.
4	HIV-2.mp.
5	human immun$ virus$.mp.
6	(human adj immun$ adj virus$).mp.
7	HIV infections/vi
8	Proviruses/
9	provir$.mp.
10	8 or 9
11	or/1-7
12	10 and 11
13	11 or 12
14	Genotype/
15	genotyp$.mp.
16	Polymerase Chain Reaction/ or Reverse Transcriptase Polymerase Chain Reaction/
17	Nucleic Acid Hybridization/ or Oligonucleotide Array Sequence Analysis/
18	exp Sequence Analysis/
19	(resist$ adj2 test$).mp.
20	DNA sequenc$.mp.
21	RNA sequenc$.mp.
22	Southern blot$.mp. or Blotting, Southern/
23	(PCR adj2 ligase adj2 detect$ adj2 reaction$).mp.
24	(RNase$ adj2 mismatch$).mp.
25	(point$ adj2 mutat$ adj2 assay$).mp.
26	(line$ adj2 prob$ adj2 assay$).mp.
27	(gene$ adj2 chip$ adj2 hybridi$).mp.
28	nucleic acid hybridi$.mp.
29	(drug adj2 resist$ adj2 test$).mp.
30	TruGene.mp.
31	ViroSeq.mp.
32	virtualphenotyp$.mp.
33	(virtual adj phenotyp$).mp.
34	GART.mp.
35	GRT.mp.
36	or/14-35
37	13 and 36

Table D1 (cont'd) Core terms for MEDLINE and MEDLINE In-Process & other non-indexed citations

34	GART.mp.
35	GRT.mp.
36	or/14-35
37	13 and 36

$=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
adj=terms must be close to one another in the record
.vi=virology
.mp = textword, keyword in the text of the title, abstract or subject heading fields
TEST TERM/=[MeSH] Medical Subject Headings, Medline's subject descriptors
and/or=Boolean operators "AND" and "OR"

Table D2 Diagnostic filter for MEDLINE and MEDLINE In-Process & other non-indexed citations

1	exp "sensitivity and specificity"/
2	(predictive and value$).tw
3	or/1-2

$=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
TEST TERM/=[MeSH] Medical Subject Headings, Medline's subject descriptors
.tw = textword, search term used as free text keyword anywhere in the Medline record
and/or=Boolean operators "AND" and "OR"

Table D3 Randomised controlled trial and systematic review filter for MEDLINE and MEDLINE In-Process & other non-indexed citations

1	randomised controlled trial.pt.
2	meta-analysis.pt.
3	controlled clinical trial.pt.
4	clinical trial.pt.
5	random$.tw.
6	(meta-anal$ or metaanaly$ or meta analy$).tw.
7	((doubl$ or singl$) and blind$).tw.
8	exp clinical trials/
9	crossover.tw.
10	or/1-9

$=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
TEST TERM/=[MeSH] Medical Subject Headings, Medline's subject descriptors
.tw = textword, search term used as free text keyword anywhere in the Medline record
.pt=publication type
and/or=Boolean operators "AND" and "OR"

Table D4 Safety filter for MEDLINE and MEDLINE In-Process & other non-indexed citations, Biological Abstracts and CINAHL

1	Safety/
2	ae.fs
3	or/1-2

TEST TERM/=[MeSH] Medical Subject Headings, Medline's subject descriptors
ae=adverse events
.fs=floating subheading
and/or=Boolean operators "AND" and "OR"

Table D5 Core terms for Biological Abstracts and CINAHL

1	HIV.mp.
2	HIV-1.mp.
3	HIV-2.mp.
4	(human adj immun$ adj virus$).mp.
5	or/1-4
6	Genotype.mp. or genotype.sh.
7	polymerase chain reaction.sh. or polymerase chain reaction.mp.
8	reverse transcriptase polymerase chain reaction.mp.
9	(nucleic adj acid adj2 hybridi$).mp.
10	sequence analysis.sh. or sequence analysis.mp.
11	(resist$ adj2 test$).mp.
12	DNA sequenc$.mp.
13	RNA sequenc$.mp.
14	(southern adj2 blot$).mp.
15	polymerase chain reaction.mp. or Polymerase Chain Reaction/
16	PCR.mp.
17	RN$ mismatch$.mp.
18	mutation.mp. or MUTATION/
19	(resistan$ adj2 test$).mp.
20	nucleic acid hybridi$.mp.
21	or/6-20
22	5 and 21
23	provirus$.mp.
24	(point$ adj2 mutat$ adj2 assay$).mp.
25	(line$ adj2 prob$ adj2 assay$).mp.
26	(gene$ adj2 chip$ adj2 hybridi$).mp.
27	(drug$ adj2 resist$ adj2 test$).mp.
28	TruGene.mp.
29	ViroSeq.mp.
30	virtualphenotyp$.mp.
31	(virtual adj phenotyp$).mp.
32	GART.mp.
33	GRT.mp.
34	5 and 23
35	or/24-33
36	21 or 35
37	5 and 36

$=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
adj=terms must be close to one another in the record
.mp = textword, keyword in the text of the title, abstract or subject heading fields
TEST TERM/=[MeSH] Medical Subject Headings
and/or=Boolean operators "AND" and "OR"

Table D6 Diagnostic filter for Biological Abstracts

1	sensitivity.tw.
2	specificity.tw.
3	1 and 2
4	(predictive and value$).tw.
5	3 or 4

( )= nested terms to be searched together
adj=terms must be close to one another in the record
.tw = textword, search term used as free text keyword anywhere in the Medline record
and/or=Boolean operators "AND" and "OR"

Table D7 Randomised controlled trial filter for Biological Abstracts

1	random$.tw.
2	(meta-anal$ or metaanaly$ or meta analy$).tw.
3	crossover.tw.
4	(clinical adj trial$).mp.
5	or/1-4

$=truncation symbol to represent a series of letters at the end of a word segment
.mp = textword, keyword in the text of the title, abstract or subject heading fields
.tw = textword, search term used as free text keyword anywhere in the Medline record
and/or=Boolean operators "AND" and "OR"

Table D8 Diagnostic filter for CINAHL

1	"sensitivity and specificity"/
2	sensitivity.tw.
3	specificity.tw.
4	exp DIAGNOSIS/
5	exp Validity/
6	exp observer bias/
7	Nursing Assessment/
8	or/1-7

.tw = textword, search term used as free text keyword anywhere in the Medline record
TEST TERM/=[MeSH] Medical Subject Headings
exp=explode subject heading
and/or=Boolean operators "AND" and "OR"

Table D9 Randomised controlled trial filter for CINAHL

1	clinical trial.pt.
2	random$.tw.
3	(meta-anal$ or metaanaly$ or meta analy$).tw.
4	((doubl$ or singl$) and blind$).tw.
5	exp clinical trials/
6	crossover.tw.
7	clin$ trial.tw.
8	(control$ and (trial$ or stud$)).tw.
9	((singl$ or doubl$ or tripl$ or trebl$) and (blind$ or mask$)).tw.
10	placebo.tw.
11	research design/
12	comparative study/
13	exp Literature Review/
14	exp Literature Searching/
15	(systematic adj2 review$).mp. [mp=title, cinahl subject headings, abstract, instrumentation]
16	or/1-15

$=truncation symbol to represent a series of letters at the end of a word segment.
( )= nested terms to be searched together.
adj=terms must be close to one another in the record.
.mp = textword, keyword in the text of the title, abstract or subject heading fields
.tw = textword, search term used as free text keyword anywhere in the Medline record.
.pt=publication type
TEST TERM/=[MeSH] Medical Subject Headings
and/or=Boolean operators "“AND"” and "“OR"”

Table D10 Core terms for EMBASE

1	HIV.mp. or Human Immunodeficiency Virus
2	(human$ adj immuno$ adj virus$).mp
3	HIV infection$.mp. or exp Human Immunodeficiency Virus Infection/
4	or/1-3
5	genotyp$.mp. or exp Genotype/
6	(sequence adj analy$).mp.
7	DNA sequenc$.mp.
8	RNA sequenc$.mp.
9	(drug$ adj2 resist$ adj2 test$).mp.
10	TruGene.mp.
11	ViroSeq.mp.
12	virtualphenotyp$.mp.
13	(virtual adj phenotyp$).mp.
14	GRT.mp.
15	or/5-14
16	4 and 15

$=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
adj=terms must be close to one another in the record
.mp = textword, keyword in the text of the title, abstract or subject heading fields
TEST TERM/=[MeSH] Medical Subject Headings
and/or=Boolean operators "AND" and "OR"

Table D11 Diagnostic filter for EMBASE

1	(sensitivity adj2 specificity).mp.
2	(predictive adj2 value$).mp.
3	or/1-2

$=truncation symbol to represent a series of letters at the end of a word segment.
( )= nested terms to be searched together
adj=terms must be close to one another in the record
.mp = textword, keyword in the text of the title, abstract or subject heading fields
and/or=Boolean operators "AND" and "OR"

Table D12 Randomised controlled trial and systematic review filter for EMBASE

1	random$.mp.

$=truncation symbol to represent a series of letters at the end of a word segment
.mp = textword, keyword in the text of the title, abstract or subject heading fields

Table D13 Core terms for Australasian Medical Index

1	HIV
2	HIV 1
3	HIV 2
4	HIV infection*
5	Human immunodeficiency virus*
6	Provirus*
7	#1 or #2 or #3 or #4 or #5 or #6
8	gart
9	GART
10	grt
11	Genotyp*
12	polymerase chain reaction*
13	PCR
14	nucleic acid hybridi*
15	sequence analysis
16	resistance test*
17	DNA sequenc*
18	RNA sequenc*
19	southern blot*
20	PCR ligase* detect*
21	RNA mismatch*
22	point* mutat* assay*
23	line* prob* assay*
24	gene chip hybridi*
25	nucleic acid hybridi*
26	drug resist*
27	genotyp*
28	mutat*
29	TruGene
30	ViroSeq
31	virtualphenotyp*
32	virtual phenotyp*
33	#8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32
34	#7 and #33

*=truncation symbol to represent a series of letters at the end of a word segment
and/or=Boolean operators "AND" and "OR"

Table D14 Diagnostic filter for Australasian Medical Index

1	“sensitivity and specificity”
2	sensitivity
3	specificity
4	diagnosi*
5	#1 or #2 or #3 or #4

*=truncation symbol to represent a series of letters at the end of a word segment
and/or=Boolean operators "AND" and "OR"

Table D15 Randomised controlled trial and systematic review filter for Australasian Medical Index

1	randomised controlled trial
2	meta-analysis
3	controlled clinical trial
4	clinical trial*
5	random*
6	metaanaly*
7	meta-analy*
8	meta analy*
9	double blind*
10	single blind*
11	crossover
12	clin* trial*
13	control* trial*
14	single mask*
15	double mask*
16	tripl* mask*
17	trebl* mask*
18	placebo
19	research design*
20	comparative stud*
21	control* stud*
22	tripl* blind*
23	trebl* blind*
24	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23

*=truncation symbol to represent a series of letters at the end of a word segment
and/or=Boolean operators "AND" and "OR"

Table D16 Safety filter for Australasian Medical Index

1	case-control stud*
2	cohort stud*
3	risk*
4	odds ratio*
5	causality
6	side effect*
7	adverse event*
8	adverse effect*
9	etiolog
10	poison*
11	toxic*
12	prevention and control
13	epidemiolog*
14	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13

*=truncation symbol to represent a series of letters at the end of a word segment
and/or=Boolean operators "AND" and "OR"

Table D17 Terms for Cochrane

1	HIV explode all trees (MeSH)
2	HIV
3	HIV 1
4	HIV 2
5	human next immune* next virus*
6	provirus*
7	#1 or #2 or #3 or #4 or #5 or #6
8	GENOTYPE single term (MeSH)
9	genotyp*
10	POLYMERASE CHAIN REACTION explode all trees (MeSH)
11	NUCLEIC ACID HYBRIDIZATION single term (MeSH)
12	OLIGONUCLEOTIDE ARRAY SEQUENCE ANALYSIS single term (MeSH)
13	SEQUENCE ANALYSIS explode all trees (MeSH)
14	(resist* next test*)
15	(dna next sequenc*)
16	(rna next sequenc*)
17	(southern next blot*)
18	(pcr next ligase next detect* next reaction*)
19	(rnase* next mismatch*)
20	(point* next mutat* next assay*)
21	(line* next prob* next assay*)
22	(gene* next chip* next hybridi*)
23	(nucleic next acid next hybridi*)
24	trugene
25	viroseq
26	virtualphenotyp*
27	gart
28	grt
29	#8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28
30	#7 and #29

*=truncation symbol to represent a series of letters at the end of a word segment
( )= nested terms to be searched together
next=terms must be close to one another in the record
(MeSH) Medical Subject Headings
and/or=Boolean operators "AND" and "OR"

Cost terms

Table D18 Cost-effectiveness terms for Medline

1.	COST BENEFIT ANALYSIS/
2.	cost$.mp.
3.	price$.mp.
4.	pricing.mp
5.	COST AND COST ANALYSIS/
6.	ECONOMICS/
7.	economic$.mp.
8.	ECONOMICS, PHARAMCEUTICAL/
9.	pharmacoeconomic$.mp.
10.	(expenditure$ not energy).mp.
11.	(value adj money).mp
12.	budget$.mp.
13.	BUDGETS/
14.	preference$.mp
15.	QUALITY ADJUSTED LIFE YEARS/
16.	qaly.mp.
17.	practice guideline.pt
18.	og.xs
19.	sn.xs
20.	or/ 1-19

Appendix E Internet sites searched

Relevant HTA websites

Agence d'Evaluation des Technologies et des Modes d'Intervention en Santé (Aetmis)
http://www.aetmis.gouv.qc.ca/en/

Agency for Healthcare Research and Quality – technology assessments (AHRQ)
http://www.ahcpr.gov/clinic/techix.htm

Alberta Heritage Foundation for Medical Research (AHFMR)
http://www.ahfmr.ab.ca/hta/

BCBS Technology Evaluation Center
http://www.bcbs.com/tec/index.html

Canadian Coordinating Office for Health Technology Assessment (CCOHTA)
http://www.ccohta.ca/

Center for Health Services and Policy Research (CHSPR)
http://www.chspr.ubc.ca/

Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)
http://www.sst.dk/Planlaegning_og_behandling/Medicinsk_teknologivurdering.aspx?lang=en

EUROSCAN: The European Information Network on New and Changing Health Technologies
http://www.publichealth.bham.ac.uk/euroscan/

Finnish Office for Health Care Technology Assessment
http://www.stakes.fi/finohta/

Health Council of the Netherlands
http://www.gr.nl/

HSTAT : Health Services/Technology Assessment Text
http://hstat.nlm.nih.gov/hq/Hquest/screen/HquestHome/s/35548

Health Technology Assessment (HTA) Database
http://nhscrd.york.ac.uk/htahp.htm

Health Technology Assessment Unit at McGill University Health Center
http://www.mcgill.ca/tau/

Institute for Clinical Systems Improvement (ICSI)
http://www.icsi.org/index.asp

Institute of Technology Assessment of the Austrian Academy of Science
http://www.oeaw.ac.at/ita/welcome.htm

International Network of Agencies for Health Technology Assessment
http://www.inahta.org/

Medical Technology Assessment Group (M-TAG)
http://www.m-tag.net/flash_index.htm

The National Coordinating Centre for Health Technology Assessment (NCCHTA)
http://www.hta.nhsweb.nhs.uk/

National Horizon Scanning Centre
http://www.publichealth.bham.ac.uk/horizon/

National Institute for Clinical Excellence (NICE)
http://www.nice.org.uk/Cat.asp?pn=professional&cn=toplevel&ln=en

The Norwegian Center for Health Technology Assessment
http://www.oslo.sintef.no/smm/News/FramesetNews.htm

NZHTA Clearing House
http://nzhta.chmeds.ac.nz/

SBU Evaluates Health Care Technology
http://www.sbu.se/www/index.asp

Swiss Network for Health Technology Assessment (SNHTA)
http://www.snhta.ch/home/portal.php

West Midlands Health Technology Assessment Collaboration (WMHTAC)
http://www.publichealth.bham.ac.uk/wmhtac/

Relevant economic evaluation databases

NHS Economic evaluation database
http://www.york.ac.uk/inst/crd/crddatabases.htm

Health Economics Evaluation Database, Office of Health Economics

Clinical trial register websites

AIDS Clinical Trials Information Service
http://www.aidsinfo.nih.gov/

CentreWatch clinical trials listing service
http://www.centerwatch.com/

ClinicalTrials.com
http://www.clinicaltrials.com/

ClinicalTrials.gov
http://www.clinicaltrials.gov/

Current Controlled Trials
http://www.controlled-trials.com/

NHMRC Clinical Trials Centre
http://www.ctc.usyd.edu.au/trials/registry/registry.htm

Society for Clinical Trials
http://www.sctweb.org/

TrialsCentral
http://www.trialscentral.org/

UK The National Research Register
http://www.update-software.com/national/

Appendix F Studies included in the review

Part1: Diagnostic accuracy

Cinque, P., Presi, S., Bestetti, A., Pierotti, C., Racca, S., Boeri, E., Morelli, P., Carrera, P., Ferrari, M. & Lazzarin, A. 2001. 'Effect of genotypic resistance on the virological response to highly active antiretroviral therapy in cerebrospinal fluid', *AIDS Research & Human Retroviruses*, 17 (5), 377–383.

Kaufmann, G.R., Suzuki, K., Cunningham, P., Mukaide, M., Kondo, M., Imai, M., Zaunders, J. & Cooper, D.A. 2001. 'Impact of HIV type 1 protease, reverse transcriptase, cleavage site, and p6 mutations on the virological response to quadruple therapy with saquinavir, ritonavir, and two nucleoside analogs', *AIDS Research & Human Retroviruses*, 17 (6), 487–497.

Pellegrin, I., Caumont, A., Garrigue, I., Merel, P., Schrive, M.H., Fleury, H., Dupon, M., Pellegrin, J.L. & Ragnaud, J.M. 2003. 'Predictive value of provirus load and DNA human immunodeficiency virus genotype for successful abacavir-based simplified therapy', *Journal of Infectious Diseases*, 187 (1), 38–46.

Perez, E.E., Rose, S.L., Peyser, B., Lamers, S.L., Burkhardt, B., Dunn, B.M., Hutson, A.D., Sleasman, J.W. & Goodenow, M.M. 2001. 'Human immunodeficiency virus type 1 protease genotype predicts immune and viral responses to combination therapy with protease inhibitors (PIs) in PI-naive patients', *Journal of Infectious Diseases*, 183 (4), 579–588.

Setti, M., Bruzzone, B., Ansaldi, F., Borrelli, P., Indiveri, F. & Icardi, G. 2001. 'Identification of key mutations in HIV reverse transcriptase gene can influence the clinical outcome of HAART', *Journal of Medical Virology*, 64 (3), 199–206.

Van Laethem, K., De Luca, A., Antinori, A., Cingolani, A., Perna, C.F. & Vandamme, A.M. 2002. 'A genotypic drug resistance interpretation algorithm that significantly predicts therapy response in HIV-1-infected patients', *Antiviral Therapy*, 7 (2), 123–129.

Van Vaerenbergh, K., De Geest, S., Derdelinckx, I., Bobbaers, H., Carbonez, A., Deschamps, A., De Graeve, V., De Saar, V., Ceunen, H., De Smet, K., Maes, B., Peetermans, W., Schrooten, Y., Desmyter, J., De Clercq, E., Van Ranst, M., Van Wijngaerden, E. & Vandamme, A.M. 2002. 'A combination of poor adherence and a low baseline susceptibility score is highly predictive for HAART failure', *Antiviral Chemistry & Chemotherapy*, 13 (4), 231–240.

Van Vaerenbergh, K., Van Laethem, K., Van Wijngaerden, E., Schmit, J.C., Schneider, F., Ruiz, L., Clotet, B., Verhofstede, C., Van Wanzeele, F., Muyldermans, G., Simons, P., Stuyver, L., Hermans, P., Evans, C., De Clercq, E., Desmyter, J. & Vandamme, A.M. 2000. 'Baseline HIV type 1 genotypic resistance to a newly added nucleoside analog is predictive of virologic failure of the new therapy', *AIDS Research & Human Retroviruses*, 16 (6), 529–537.

Venturi, G., Romano, L., Catucci, M., Riccio, M.L., De Milito, A., Gonnelli, A., Rubino, M., Valensin, P.E. & Zazzi, M. 1999. 'Genotypic resistance to zidovudine as a predictor of failure of subsequent therapy with human immunodeficiency virus type-1 nucleoside reverse-transcriptase inhibitors', *European Journal of Clinical Microbiology & Infectious Diseases*, 18 (4), 274–282.

Vray, M., Meynard, J.L., Dalban, C., Morand-Joubert, L., Clavel, F., Brun-Vezinet, F., Peytavin, G., Costagliola, D., Girard, P.M. & Narval Trial G. 2003. 'Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1-infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS 088)', *Antiviral Therapy*, 8 (5), 427–434.

Part 2: Patient outcomes

Baxter, J.D., Mayers, D.L., Wentworth, D.N., Neaton, J.D., Hoover, M.L., Winters, M.A., Mannheimer, S.B., Thompson, M.A., Abrams, D.I., Brizz, B.J., Ioannidis, J.P. & Merigan, T.C. 2000. 'A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS', *AIDS*, 14 (9), F83–93.

Cingolani, A., Antinori, A., Rizzo, M.G., Murri, R., Ammassari, A., Baldini, F., Di Giambenedetto, S., Cauda, R. & De Luca, A. 2002. 'Usefulness of monitoring HIV drug resistance and adherence in individuals failing highly active antiretroviral therapy: a randomized study (ARGENTA)', *AIDS*, 16 (3), 369–379.

Clevenbergh, P., Durant, J., Halfon, P., del Giudice, P., Mondain, V., Montagne, N., Schapiro, J.M., Boucher, C.A. & Dellamonica, P. 2000. 'Persisting long-term benefit of genotype-guided treatment for HIV-infected patients failing HAART. The Viradapt Study: week 48 follow-up', *Antiviral Therapy*, 5 (1), 65–70.

Durant, J., Clevenbergh, P., Halfon, P., Delgiudice, P., Porsin, S., Simonet, P., Montagne, N., Boucher, C.A., Schapiro, J.M. & Dellamonica, P. 1999. 'Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial.[see comment][erratum appears in Lancet 1999 Sep 25;354(9184):1128]', *The Lancet*, 353 (9171), 2195–2199.

Mazzotta, F., Lo Caputo, S., Torti, C., Tinelli, C., Pierotti, P., Castelli, F., Lazzarin, A., Angarano, G., Maserati, R., Gianotti, N., Ladisa, N., Quiros-Roldan, E., Rinehart, A.R., Carosi, G. & Genotipo-Fenotipo di Resistenza Group of the Italian Management Standardizzato di Terapia Antiretrovirale C. 2003. 'Real versus virtual phenotype to guide treatment in heavily pretreated patients: 48-week follow-up of the Genotipo-Fenotipo di Resistenza (GenPheRex) trial', *Journal of Acquired Immune Deficiency Syndromes*, 32 (3), 268–280.

Meynard, J.L., Vray, M., Morand-Joubert, L., Race, E., Descamps, D., Peytavin, G., Matheron, S., Lamotte, C., Guiramand, S., Costagliola, D., Brun-Vezinet, F., Clavel, F., Girard, P.M. & Narval Trial G. 2002. 'Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial', *AIDS*, 16 (5), 727–736.

Perez-Elias, M.J., Garcia-Arota, I., Munoz, V., Santos, I., Sanz, J., Abraira, V., Arribas, J.R., Gonzalez, J., Moreno, A., Dronda, F., Antela, A., Pumares, M., Marti-Belda, P., Casado, J.L., Geijos, P., Moreno, S. & Realvirfen study g. 2003. 'Phenotype or virtual phenotype for choosing antiretroviral therapy after failure: a prospective, randomized study', *Antiviral Therapy*, 8 (6), 577–584.

Torre, D. & Tambini, R. 2002. 'Antiretroviral drug resistance testing in patients with HIV-1 infection: a meta-analysis study', *HIV Clinical Trials*, 3 (1), 1–8.

Tural, C., Ruiz, L., Holtzer, C., Schapiro, J., Viciana, P., Gonzalez, J., Domingo, P., Boucher, C., Rey-Joly, C., Clotet, B. & Havana Study G. 2002. 'Clinical utility of HIV-1 genotyping and expert advice: the Havana trial', *AIDS*, 16 (2), 209–218.

Appendix G Studies excluded from critical appraisal

Part1: Diagnostic accuracy

Could not generate two-by-two table

Barreiro, P., Camino, N., de Mendoza, C., Valer, L., Nunez, M., Martin-Carbonero, L., Gonzalez-Lahoz, J. & Soriano, V. 2002. 'Comparison of the efficacy, safety and predictive value of HIV genotyping using distinct ritonavir-boosted protease inhibitors', *International Journal of Antimicrobial Agents*, 20 (6), 438–443.

Clevenbergh, P., Bozonnat, M.C., Kirstetter, M., Durant, J., Cua, E., del Giudice, P., Montagne, N., Simonet, P. & Group I 2003. 'Variable virological outcomes according to the center providing antiretroviral therapy within the PharmAdapt clinical trial', *HIV Clinical Trials*, 4 (2), 84–91.

Cohen, N.J., Oram, R., Elsen, C. & Englund, J.A. 2002. 'Response to changes in antiretroviral therapy after genotyping in human immunodeficiency virus-infected children', *Pediatric Infectious Disease Journal*, 21 (7), 647–653.

Gingeras, T.R., Prodanovich, P., Latimer, T., Guatelli, J.C., Richman, D.D. & Barringer, K.J. 1991. 'Use of self-sustained sequence replication amplification reaction to analyze and detect mutations in zidovudine-resistant human immunodeficiency virus', *Journal of Infectious Diseases*, 164 (6), 1066–1074.

Harrigan, P.R., Hertogs, K., Verbiest, W., Pauwels, R., Larder, B., Kemp, S., Bloor, S., Yip, B., Hogg, R., Alexander, C. & Montaner, J.S. 1999. 'Baseline HIV drug resistance profile predicts response to ritonavir-saquinavir protease inhibitor therapy in a community setting', *AIDS*, 13 (14), 1863–1871.

Imrie, A., Carr, A., Duncombe, C., Finlayson, R., Vizzard, J., Law, M., Kaldor, J., Penny, R., Cooper, D.A. & Group SPHIS. 1996. 'Primary infection with zidovudine-resistant human immunodeficiency virus type 1 does not adversely affect outcome at 1 year', *Journal of Infectious Diseases*, 174 (1), 195–198.

Lorenzi, P., Opravil, M., Hirschel, B., Chave, J.P., Furrer, H.J., Sax, H., Perneger, T.V., Perrin, L., Kaiser, L. & Yerly, S. 1999. 'Impact of drug resistance mutations on virologic response to salvage therapy. Swiss HIV Cohort Study', *AIDS*, 13 (2), F17–21.

Marcelin, A.G., Lamotte, C., Delaugerre, C., Ktorza, N., Ait Mohand, H., Cacace, R., Bonmarchand, M., Wirden, M., Simon, A., Bossi, P., Bricaire, F., Costagliola, D., Katlama, C., Peytavin, G., Calvez, V., & Genophar Study G. 2003. 'Genotypic inhibitory quotient as predictor of virological response to ritonavir-amprenavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients', *Antimicrobial Agents & Chemotherapy*, 47 (2), 594–600.

Masuhr, A., Mueller, M., Simon, V., Zwingers, T., Kurowski, M., Jessen, H., Lauenroth-Mai, E., Moll, A., Schranz, D., Moecklinghoff, C. & Arasteh, K. 2002. 'Predictors of treatment failure during highly active antiretroviral therapy (racing trial)', *European Journal of Medical Research*, 7 (8), 341–346.

Mathews, W.C., Cole, J., Ballard, C., Colwell, B., Haubrich, R., Barber, E. & Lew, T. 2002. 'Early adoption of HIV-1 resistance testing in the San Diego County Ryan White CARE Act Program: predictors and outcome', *AIDS Patient Care & STDs*, 16 (7), 337–348.

Perez-Olmeda, M., Rodriguez-Rosado, R., Gomez-Cano, M., Briones, C. & Soriano, V. 1999. 'Usefulness of genotypic analysis of resistance to nucleoside analogues in the clinical setting', *European Journal of Clinical Microbiology & Infectious Diseases*, 18 (6), 448–449.

Piketty, C., Race, E., Castiel, P., Belec, L., Peytavin, G., Si-Mohamed, A., Gonzalez-Canali, G., Weiss, L., Clavel, F. & Kazatchkine, M.D. 1999. 'Efficacy of a five-drug combination including ritonavir, saquinavir and efavirenz in patients who failed on a conventional triple-drug regimen: phenotypic resistance to protease inhibitors predicts outcome of therapy.[see comment]', *AIDS*, 13 (11), F71–77.

Sabin, C.A., Wilkins, E., Murphy, M., Fisher, M., de Ruiter, A., Easterbrook, P.J., Leen, C., Vlahakis, E., Cane, P.A., Li, X., Pillay, D. & Programme UKEAA. 2003. 'Drug resistance genotypes predict response to amprenavir-containing regimens in highly drug-experienced HIV-1-infected patients', *Antiviral Therapy*, 8 (4), 301–308.

Servais, J., Hainaut, M., Schmitz, V., Maes, P., Fransen, K., Vaira, D., Brichard, B., Arendt, V., Schneider, F., Hemmer, R. & Schmit, J.C. 2002. 'Resistance testing in children changing human immunodeficiency virus type 1 protease inhibitor', *Pediatric Infectious Disease Journal*, 21 (3), 214–220.

Servais, J., Lambert, C., Plesseria, J.M., Fontaine, E., Robert, I., Arendt, V., Staub, T., Hemmer, R., Schneider, F. & Schmit, J.C. 2002. 'Longitudinal use of a line probe assay for human immunodeficiency virus type 1 protease predicts phenotypic resistance and clinical progression in patients failing highly active antiretroviral therapy', *Antimicrobial Agents & Chemotherapy*, 46 (6), 1928–1933.

Van Vaerenbergh, K. 2001. 'Study of the impact of HIV genotypic drug resistance testing on therapy efficacy', *Verhandelingen - Koninklijke Academie voor Geneeskunde van Belgie*, 63 (5), 447–473.

Zolopa, A.R., Shafer, R.W., Warford, A., Montoya, J.G., Hsu, P., Katzenstein, D., Merigan, T.C. & Efron, B. 1999. 'HIV-1 genotypic resistance patterns predict response to saquinavir-ritonavir therapy in patients in whom previous protease inhibitor therapy had failed', *Annals of Internal Medicine*, 131 (11), 813–821.

Accuracy of different techniques to detect mutations

Beck, I.A., Mahalanabis, M., Pepper, G., Wright, A., Hamilton, S., Langston, E. & Frenkel, L.M. 2002. 'Rapid and sensitive oligonucleotide ligation assay for detection of mutations in human immunodeficiency virus type 1 associated with high-level resistance to protease inhibitors', *Journal of Clinical Microbiology*, 40 (4), 1413–1419.

Eastman, P.S., Boyer, E., Mole, L., Kolberg, J., Urdea, M. & Holodniy, M. 1995. 'Nonisotopic hybridization assay for determination of relative amounts of genotypic human immunodeficiency virus type 1 zidovudine resistance', *Journal of Clinical Microbiology*, 33 (10), 2777–2780.

Edelstein, R.E., Nickerson, D.A., Tobe, V.O., Manns-Arcuino, L.A. & Frenkel, L.M. 1998. 'Oligonucleotide ligation assay for detecting mutations in the human immunodeficiency virus type 1 pol gene that are associated with resistance to zidovudine, didanosine, and lamivudine', *Journal of Clinical Microbiology*, 36 (2), 569–572.

Frater, A.J., Chaput, C.C., Beddows, S., Weber, J.N. & McClure, M.O. 2001. 'Simple detection of point mutations associated with HIV-1 drug resistance', *Journal of Virological Methods*, 93 (1-2), 145–156.

Frenkel, L.M., Wagner, L.E. 2nd, Atwood, S.M., Cummins, T.J. & Dewhurst, S. 1995. 'Specific, sensitive, and rapid assay for human immunodeficiency virus type 1 pol mutations associated with resistance to zidovudine and didanosine', *Journal of Clinical Microbiology*, 33 (2), 342–347.

Gunthard HF, Wong JK, Ignacio CC, Havlir DV & Richman DD, 1998. 'Comparative performance of high-density oligonucleotide sequencing and dideoxynucleotide sequencing of HIV type 1 pol from clinical samples'. *AIDS Research & Human Retroviruses,* 14 (10), 869-76.

Lindstrom, A. & Albert, J. 2003. 'A simple and sensitive 'in-house' method for determining genotypic drug resistance in HIV-1', *Journal of Virological Methods*, 107 (1), 45–51.

Shafer, R.W., Levee, D.J., Winters, M.A., Richmond, K.L., Huang, D. & Merigan, T.C. 1997. 'Comparison of QIAamp HCV kit spin columns, silica beads, and phenol-chloroform for recovering human immunodeficiency virus type 1 RNA from plasma', *Journal of Clinical Microbiology*, 35 (2), 520–522.

Shafer, R.W., Warford, A., Winters, M.A. & Gonzales, M.J. 2000. 'Reproducibility of human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase sequencing of plasma samples from heavily treated HIV-1-infected individuals', *Journal of Virological Methods*, 86 (2), 143–153.

Shafer, R.W., Winters, M.A., Mayers, D.L., Japour, A.J., Kuritzkes, D.R., Weislow, O.S., White, F., Erice, A., Sannerud, K.J., Iversen, A., Pena, F., Dimitrov, D., Frenkel, L.M. & Reichelderfer, P.S. 1996. 'Interlaboratory comparison of sequence-specific PCR and ligase detection reaction to detect a human immunodeficiency virus type 1 drug resistance mutation', *Journal of Clinical Microbiology*, 34 (7), 1849–1853.

Van Laethem, K., Van Vaerenbergh, K., Schmit, J.C., Sprecher, S., Hermans, P., De Vroey, V., Schuurman, R., Harrer, T., Witvrouw, M., Van Wijngaerden, E., Stuyver, L., Van Ranst, M., Desmyter, J., De Clercq, E. & Vandamme, A.M. 1999. 'Phenotypic assays and sequencing are less sensitive than point mutation assays for detection of resistance in mixed HIV-1 genotypic populations'. *Journal of Acquired Immune Deficiency Syndromes*, 22 (2), 107–118.

Narrative review / letters

Aceti, A. & Carosi, G. 2003. 'Theme 1. Genotypic resistance tests in the management of the HIV-infected patient at virological failure', *Scandinavian Journal of Infectious Diseases Supplementum. Issue*, 106, 61–104.

Anonymous, 1997. 'Diagnostic tests for HIV', *Medical Letter on Drugs & Therapeutics*, 39 (1008), 81–83.

Bakker, A. 2000. 'Predictive value of genotypic and phenotypic resistance in 2 nelfinavir-experienced patients', *Journal of Infectious Diseases*, 181 (5), 1862–1864.

Bean, P., Robbins, D.J., Hamdan, H. & Alcorn, T.M. 2000. 'HIV genotyping by DNA sequencing', *American Clinical Laboratory*, 19 (4), 18–20.

Beaubien, G. 1997. 'Test of integrity', *Positively Aware: the Monthly Journal of the Test Positive Aware Network*, 8 (5), 33–35, 39.

Blaise, P., Clevenbergh, P., Vaira, D., Moutschen, M. & Dellamonica, P. 2002. 'HIV resistance to antiretroviral drugs: mechanisms, genotypic and phenotypic resistance testing in clinical practice', *Acta Clinica Belgica*, 57 (4), 191–201.

Clumeck, N. & De Wit, S. 2000. 'Update on highly active antiretroviral therapy: progress and strategies', *Biomedicine & Pharmacotherapy*, 54 (1), 7–12.

Cowley, S. 2001. 'The biology of HIV infection', *Leprosy Review*, 72 (2), 212–220.

Deeks, S.G. & Abrams, D.I. 1997. 'Genotypic-resistance assays and antiretroviral therapy', *Lancet*, 349 (9064), 1489–1490.

Dunn, D., Cormack, S., Babiker, A. & Darbyshire, J. 2000. 'Tracker trials. Introduction of resistance testing might be an inappropriate use of resources', *British Medical Journal*, 320 (7251), 1727.

Grady, C. & Vogel, S. 1993. 'Laboratory methods for diagnosing and monitoring HIV infection', *Journal of the Association of Nurses in AIDS Care*, 4 (2), 11–21; quiz 22–23.

Johnson, V.A., Brun-Vezinet, F., Clotet, B., Conway, B., D'Aquila, R.T., Demeter, L.M., Kuritzkes, D.R., Pillay, D., Schapiro, J.M., Telenti, A., Richman, D.D. & International AS-USADRMG. 2003. 'Drug resistance mutations in HIV-1', *Topics in HIV Medicine*, 11 (6), 215–221.

Klimkait, T. 2002. 'A sensitive replicative system to assess HIV-1 drug resistance', *American Clinical Laboratory*, 21 (1), 20–24.

Sherer, R. & Rush Medical College CIUSA, 2001. 'Limitations and uncertainties of resistance testing: a review of "negative" trials', *Iapac Monthly*, 7 (8), 237–241.

Sninsky, J.J. & Kwok, S. 1993. 'The application of quantitative polymerase chain reaction to therapeutic monitoring', *AIDS*, 7 (Suppl 2), S29–34.

Tobin, N.H. & Frenkel, L.M. 2002. 'Human immunodeficiency virus drug susceptibility and resistance testing', *Pediatric Infectious Disease Journal*, 21 (7), 681–683.

Van Houtte, M. 2001. 'Update on resistance testing', *Journal of HIV Therapy*, 6 (3), 61–64.

Project Inform. 1999, *Ultrasenstive PCR approved* [Internet]. Available from: www.thebody.com/pinf/april99/ois.html [Accessed 2 May 2004]

Prevalence of mutations

Birk, M. & Sonnerborg, A. 1998. 'Variations in HIV-1 pol gene associated with reduced sensitivity to antiretroviral drugs in treatment-naive patients', *AIDS*, 12 (18), 2369–2375.

Brindeiro, R.M., Diaz, R.S., Sabino, E.C., Morgado, M.G., Pires, I.L., Brigido, L., Dantas, M.C., Barreira, D., Teixeira, P.R., Tanuri, A. & The BNfDRS, 2003. 'Brazilian Network for HIV Drug Resistance Surveillance (HIV-BResNet): A survey of chronically infected individuals', *AIDS*, 17, 1063–1069.

Machado, D.M., Delwart, E.L., Diaz, R.S., de Oliveira, C.F., Alves, K., Rawal, B.D., Sullivan, M., Gwinn, M., Clark, K.A. & Busch, M.P. 2002. 'Use of the sensitive/less-sensitive (detuned) EIA strategy for targeting genetic analysis of HIV-1 to recently infected blood donors', *AIDS*, 16 (1), 113–119.

Rate of emergence of mutations while on therapy

Brun-Vezinet, F., Boucher, C., Loveday, C., Descamps, D., Fauveau, V., Izopet, J., Jeffries, D., Kaye, S., Krzyanowski, C., Nunn, A., Schuurman, R., Seigneurin, J.M., Tamalet, C., Tedder, R., Weber, J. & Weverling, G.J. 1997. 'HIV-1 viral load, phenotype, and resistance in a subset of drug-naive participants from the Delta trial. The National Virology Groups. Delta Virology Working Group and Coordinating Committee.[see comment]', *Lancet*, 350 (9083), 983–990.

Johann-Liang, R., Lee, S.E., Fernandez, A., Cervia, J. & Noel, G.J. 2000. 'Genotypic characterization of human immunodeficiency virus type 1 isolated from vertically infected children with antiretroviral therapy experience', *Pediatric Infectious Disease Journal*, 19 (4), 363–364.

Leal, M., Rey, C., Torres, Y., Relimpio, F., Pino, R., Lissen, E., Sanchez-Quijano, A. & Luque, F. 1996. 'Rate of development of mutation at codon 215 of HIV-1 reverse transcriptase and its predictive factors at the time of initiation of zidovudine therapy', *European Journal of Clinical Investigation*, 26 (6), 476–480.

Relimpio, F., Rey, C., Pineda, J.A., Leal, M., Caruz, A., Sanchez-Quijano, A. & Lissen, E. 1997. 'Viraemia and p24 antigenaemia are independent risk factors for the emergency of a zidovudine-resistant genotype in nucleoside analogue-treated HIV-1 infection', *Antiviral Therapy*, 2 (2), 99–104.

Models to predict resistance

Beerenwinkel, N., Lengauer, T., M DA, Kaiser, R., Walter, H., Korn, K., Hoffmann, D. & Selbig, J. 2003. 'Methods for optimizing antiviral combination therapies', *Bioinformatics*, 19 (1), i16–i25.

Foulkes, A.S. & De, G.V. 2002. 'Characterizing the relationship between HIV-1 genotype and phenotype: prediction-based classification', *Biometrics*, 58 (1), 145–156.

Vergu, E., Mallet, A. & Golmard, J.L. 2002. 'The role of resistance characteristics of viral strains in the prediction of the response to antiretroviral therapy in HIV infection', *Journal of Acquired Immune Deficiency Syndrome*, 30 (3), 263–270.

Wang, D. & Larder, B. 2003. 'Enhanced prediction of lopinavir resistance from genotype by use of artificial neural networks', *Journal of Infectious Diseases*, 188 (5), 653–660.

Phenotype not genotype intervention

Greene, R.A., Japour, A.J., Brewster, F., Joseph, R.A., Chung, P.H., Kasila, P.A. & Chatis, P.A. 1996. 'Determination of HIV-1 susceptibility to reverse transcriptase (RT) inhibitors by a quantitative cell-free RT assay', *Clinical & Diagnostic Virology*, 7 (2), 111–119.

Perez-Elias, M.J., Lanier, R., Munoz, V., Garcia-Arata, I., Casado, J.L., Marti-Belda, P., Moreno, A., Dronda, F., Antela, A., Marco, S. & Moreno, S. 2000. 'Phenotypic testing predicts virological response in successive protease inhibitor-based regimens', *AIDS*, 14 (9), F95–101.

Servais, J., Plesseria, J.M., Lambert, C., Fontaine, E., Robert, I., Arendt, V., Staub, T., Schneider, F., Hemmer, R. & Schmit, J.C. 2002. 'Longitudinal use of phenotypic resistance testing to HIV-1 protease inhibitors in patients developing HAART failure', *Journal of Medical Virology*, 67 (3), 312–319.

Correlation between genotype and in vitro phenotype of mutations

Richman, D.D., Guatelli, J.C., Grimes, J., Tsiatis, A. & Gingeras, T. 1991. 'Detection of mutations associated with zidovudine resistance in human immunodeficiency virus by use of the polymerase chain reaction', *Journal of Infectious Diseases*, 164 (6), 1075–1081.

Rubsamen-Waigmann, H., Schroder, B., Biesert, L., Bauermeister, C.D., von Briesen, H., Suhartono, H., Zimmermann, F., Brede, H.D., Regeniter, A., Gerte, S. & et al. 1991. 'Markers for HIV-disease progression in untreated patients and patients receiving AZT: evaluation of viral activity, AZT resistance, serum cholesterol, beta 2-microglobulin, CD4+ cell counts, and HIV antigen', *Infection*, 19 (Suppl 2), S77–82.

Schmidt, B., Walter, H., Moschik, B., Paatz, C., van Vaerenbergh, K., Vandamme, A.M., Schmitt, M., Harrer, T., Uberla, K. & Korn, K. 2000. 'Simple algorithm derived from a geno-/phenotypic database to predict HIV-1 protease inhibitor resistance', *AIDS*, 14 (12), 1731–1738.

Schmidt, B., Korn, K., Moschik, B., Paatz, C., Uberla, K. & Walter, H. 2000. 'Low level of cross-resistance to amprenavir (141W94) in samples from patients pretreated with other protease inhibitors', *Antimicrobial Agents & Chemotherapy*, 44 (11), 3213–3216.

Torti, C., Quiros-Roldan, E., Keulen, W., Scudeller, L., Lo, C.S., Boucher, C., Castelli, F., Mazzotta, F., Pierotti, P., Been-Tiktak, A.M., Buccoliero, G., De G.M., Carosi, G. & Tinelli, C. 2003. 'Comparison between rules-based human immunodeficiency virus type 1 genotype interpretations and real or virtual phenotype: Concordance analysis and correlation with clinical outcome in heavily treated patients', *Journal of Infectious Diseases*, 188, 194–201.

Diagnosis of HIV infection

Epstein, L.D., Munoz, A. & He, D. 1996. 'Bayesian imputation of predictive values when covariate information is available and gold standard diagnosis is unavailable', *Statistics in Medicine*, 15 (5), 463–476.

Sarr, A.D., Hamel, D.J., Thior, I., Kokkotou, E., Sankale, J.L., Marlink, R.G., Coll-Seck, E.M., Essex, M.E., Siby, T., I ND, Mboup, S. & Kanki, P.J. 1998. 'HIV-1 and HIV-2 dual infection: lack of HIV-2 provirus correlates with low CD4+ lymphocyte counts', *AIDS*, 12 (2), 131–137.

Measuring viral load

de Mendoza, C., Lu, W., Machuca, A., Sainz, M., Castilla, J. & Soriano, V. 2001. 'Monitoring the response to antiretroviral therapy in HIV-1 group O infected patients using two new RT-PCR assays', *Journal of Medical Virology*, 64 (3), 217–222.

Palmer, S., Wiegand, A.P., Maldarelli, F., Bazmi, H., Mican, J.M., Polis, M., Dewar, R.L., Planta, A., Liu, S., Metcalf, J.A., Mellors, J.W. & Coffin, J.M. 2003. 'New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma', *Journal of Clinical Microbiology*, 41 (10), 4531–4536.

Schmitt, Y. 2001. 'Performance characteristics of quantification assays for human immunodeficiency virus type 1 RNA', *Journal of Clinical Virology*, 20 (1-2), 31–33.

CCR5 genotype and HIV infection

Taylor, J.M., Wang,Y., Ahdieh, L., Chmiel, J.S., Detels, R., Giorgi, J.V., Kaslow, R., Kingsley, L. & Margolick, J. 2000. 'Causal pathways for CCR5 genotype and HIV progression', *Journal of Acquired Immune Deficiency Syndromes*, 23 (2), 160–171.

Treatment recommendations

Sandstrom, E., Uhnoo, I., Ahlqvist-Rastad, J., Bratt, G., Berglund, T., Gisslen, M., Lindback, S., Morfeldt, L., Stahle, L., Sonnerborg, A. & Swedish Consensus G. 2003. 'Antiretroviral treatment of human immunodeficiency virus infection: Swedish recommendations', *Scandinavian Journal of Infectious Diseases*, 35 (3), 155–167.

Unavailable information

Craig, C., Race, E., Sheldon, J., Whittaker, L., Gilbert, S., Moffatt, A., Rose, J., Dissanayeke, S., Chirn, G.W., Duncan, I.B. & Cammack, N. 1998. 'HIV protease genotype and viral sensitivity to HIV protease inhibitors following saquinavir therapy', *AIDS*, 12 (13), 1611–1618.

Part 2: Patient outcomes

Inappropriate patient group

Wegner, S.A., Wallace, M.R., Aronson, N.E., Tasker, S.A., Blazes, D.L., Tamminga, C., Fraser, S., Dolan, M.J., Stephan, K.T., Michael, N.L., Jagodzinski, L.L., Vahey, M.T., Gilcrest, J.L., Tracy, L., Milazzo, M.J., Murphy, D.J., McKenna, P., Hertogs, K., Rinehart, A., Larder, B., Birx, D.L., Education RVCf & Research on Therapeutics Study T. 2004. 'Long-term efficacy of routine access to antiretroviral-resistance testing in HIV type 1-infected patients: results of the clinical efficacy of resistance testing trial', *Clinical Infectious Diseases*, 38 (5), 723–730.

Inappropriate outcomes

Baxter, J.D., Merigan, T.C., Wentworth, D.N., Neaton, J.D., Hoover, M.L., Hoetelmans, R.M., Piscitelli, S.C., Verbiest, W.H., Mayers, D.L. & AIDS CSTftTBCPfCRo. 2002. 'Both baseline HIV-1 drug resistance and antiretroviral drug levels are associated with short-term virologic responses to salvage therapy', *AIDS*, 16 (8), 1131–1138.

Durant, J., Clevenbergh, P., Garraffo, R., Halfon, P., Icard, S., Del Giudice, P., Montagne, N., Schapiro, J.M. & Dellamonica, P. 2000. 'Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study', *AIDS*, 14 (10), 1333–1339.

Antiretroviral treatment study – no patient or group treated based on genotype

Ait-Khaled, M., Rakik, A., Griffin, P., Cutrell, A., Fischl, M.A., Clumeck, N., Greenberg, S.B., Rubio, R., Peters, B.S., Pulido, F., Gould, J., Pearce, G., Spreen, W., Tisdale, M., Lafon, S. & Team CNAIS. 2002. 'Mutations in HIV-1 reverse transcriptase during therapy with abacavir, lamivudine and zidovudine in HIV-1-infected adults with no prior antiretroviral therapy', *Antiviral Therapy*, 7 (1), 43–51.

Ait-Khaled, M., Rakik, A., Griffin, P., Stone, C., Richards, N., Thomas, D., Falloon, J., Tisdale, M. & Team CNAIS. 2003. 'HIV-1 reverse transcriptase and protease resistance mutations selected during 16-72 weeks of therapy in isolates from antiretroviral therapy-experienced patients receiving abacavir/efavirenz/amprenavir in the CNA2007 study', *Antiviral Therapy*, 8 (2), 111–120.

Andreoni, M., Parisi, S.G., Sarmati, L., Nicastri, E., Ercoli, L., Mancino, G., Sotgiu, G., Mannazzu, M., Trevenzoli, M., Tridente, G., Concia, E. & Aceti, A. 2000. 'Cellular proviral HIV-DNA decline and viral isolation in naive subjects with <5000 copies/ml of HIV-RNA and >500 x 10(6)/l CD4+ cells treated with highly active antiretroviral therapy', *AIDS*, 14 (1), 23–29.

Atkinson, B., Isaacson, J., Knowles, M., Mazabel, E. & Patick, A.K. 2000. 'Correlation between human immunodeficiency virus genotypic resistance and virologic response in patients receiving nelfinavir monotherapy or nelfinavir with lamivudine and zidovudine', *Journal of Infectious Diseases*, 182 (2), 420–427.

Bardeguez, A.D., Shapiro, D.E., Mofenson, L.M., Coombs, R., Frenkel, L.M., Fowler, M.G., Huang, S., Sperling, R.S., Cunningham, B., Gandia, J., Maupin, R., Zorrilla, C.D., Jones, T., O'Sullivan, M.J. & Pediatrics ACTGPT. 2003. 'Effect of cessation of zidovudine prophylaxis to reduce vertical transmission on maternal HIV disease progression and survival', *Journal of Acquired Immune Deficiency Syndromes*, 32 (2), 170–181.

Barreiro, P., Camino, N., de Mendoza, C., Valer, L., Nunez, M., Martin-Carbonero, L., Gonzalez-Lahoz, J. & Soriano, V. 2002. 'Comparison of the efficacy, safety and predictive value of HIV genotyping using distinct ritonavir-boosted protease inhibitors', *International Journal of Antimicrobial Agents*, 20 (6), 438–443.

Bogner, J.R., Eberle, J., Troendle, U. & Goebel, F.D. 2003. 'Amprenavir in pre-treated patients: virological and immunological response in a cohort of 45 patients', *European Journal of Medical Research*, 8 (2), 49–55.

Bongiovanni, M., Bini, T., Adorni, F., Meraviglia, P., Capetti, A., Tordato, F., Cicconi, P., Chiesa, E., Cordier, L., Cargnel, A., Landonio, S., Rusconi, S. & d'Arminio Monforte, A. 2003. 'Virological success of lopinavir/ritonavir salvage regimen is affected by an increasing number of lopinavir/ritonavir-related mutations', *Antiviral Therapy*, 8 (3), 209–214.

Brites, C., Gilbert, M.J., Pedral-Sampaio, D., Bahia, F., Pedroso, C., Alcantara, A.P., Sasaki, M.D., Matos, J., Renjifo, B., Essex, M., Whitmore, J.B., Agosti, J.M. & Badaro, R. 2000. 'A randomized, placebo-controlled trial of granulocyte-macrophage colony-stimulating factor and nucleoside analogue therapy in AIDS', *Journal of Infectious Diseases*, 182 (5), 1531–1535.

Brun-Vezinet, F., Boucher, C., Loveday, C., Descamps, D., Fauveau, V., Izopet, J., Jeffries, D., Kaye, S., Krzyanowski, C., Nunn, A., Schuurman, R., Seigneurin, J.M., Tamalet, C., Tedder, R., Weber, J. & Weverling, G.J. 1997. 'HIV-1 viral load, phenotype, and resistance in a subset of drug-naive participants from the Delta trial', *Lancet*, 350 (9083), 983–990.

Casado, J.L., Dronda, F., Hertogs, K., Sabido, R., Antela, A., Marti-Belda, P., Dehertogh, P., Moreno, S. & Study N. 2001. 'Efficacy, tolerance, and pharmacokinetics of the combination of stavudine, nevirapine, nelfinavir, and saquinavir as salvage regimen after ritonavir or indinavir failure', *AIDS Research & Human Retroviruses*, 17 (2), 93–98.

Casado, J.L., Moreno, A., Marti-Belda, P., Sabido, R., Pinheiro, S., Bermudez, E., Antela, A., Dronda, F., Perez-Elias, M.J. & Moreno, S. 2003. 'Overcoming resistance: virologic response to a salvage regimen with the combination of ritonavir plus indinavir', *HIV Clinical Trials*, 4 (1), 21–28.

Chavanet, P., Piroth, L., Grappin, M., Buisson, M., Gourdon, F., Cabie, A., Duong, M., Brunel-Dalmas, F., Peytavin, G. & Portier, H. 2001. 'Randomized salvage therapy with saquinavir-ritonavir versus saquinavir-nelfinavir for highly protease inhibitor-experienced HIV-infected patients', *HIV Clinical Trials*, 2 (5), 408–412.

Churchill, D.R., Pym, A.S., Galpin, S., Foxall, R., Stainsby, C., Clarke, J.R., Kaye, S., Bloor, S., Larder, B.A., Wills, B., Sun, E., Babiker, A.G., Back, D.J. & Weber, J.N. 1999. 'The rabbit study: ritonavir and saquinavir in combination in saquinavir-experienced and previously untreated patients', *AIDS Research & Human Retroviruses*, 15 (13), 1181–1189.

de Mendoza, C., Ramos, J.T., Ciria, L., Fortuny, C., Garcia, F.J., de Jose, M.I., Asensi, F. & Soriano, V. 2002. 'Efficacy and safety of stavudine plus didanosine in asymptomatic HIV-infected children with plasma HIV RNA below 50,000 copies per milliliter', *HIV Clinical Trials*, 3 (1), 9-16.

de Mendoza C, Soriano V, Perez-Olmeda M, Rodriguez-Rosado R & Gonzalez-Lahoz J, 2000. 'Efficacy and safety of didanosine and lamivudine both once daily plus indinavir in human immunodeficiency virus-infected patients'. *Journal of Human Virology,* 3 (6), 335-40.

de Requena, D.G., Nunez, M., Gallego, O., Jimenez-Nacher, I., Gonzalez-Lahoz, J. & Soriano, V. 2002. 'Does an increase in nevirapine plasma levels cause complete virologic suppression in patients experiencing early virologic failure?', *HIV Clinical Trials*, 3 (6), 463–467.

Descamps, D., Flandre, P., Joly, V., Meiffredy, V., Peytavin, G., Izopet, J., Tamalet, C., Zeng, A.F., Harel, M., Lastere, S., Aboulker, J.P., Yeni, P., Brun-Vezinet, F. & Group NS. 2002. 'Effect of zidovudine resistance mutations on virologic response to treatment with zidovudine or stavudine, each in combination with lamivudine and indinavir', *Journal of Acquired Immune Deficiency Syndromes*, 31 (5), 464–471.

Eastman, P.S., Shapiro, D.E., Coombs, R.W., Frenkel, L.M., McSherry, G.D., Britto, P., Herman, S.A. & Sperling, R.S., 1998. 'Maternal viral genotypic zidovudine resistance and infrequent failure of zidovudine therapy to prevent perinatal transmission of human immunodeficiency virus type 1 in pediatric AIDS Clinical Trials Group Protocol 076', *Journal of Infectious Diseases*, 177 (3), 557–564.

Eshleman, S.H., Krogstad, P., Jackson, J.B., Wang, Y.G., Lee, S., Wei, L.J., Cunningham, S., Wantman, M., Wiznia, A., Johnson, G., Nachman, S. & Palumbo, P. 2001. 'Analysis of human immunodeficiency virus type 1 drug resistance in children receiving nucleoside analogue reverse-transcriptase inhibitors plus nevirapine, nelfinavir, or ritonavir (Pediatric AIDS Clinical Trials Group 377)', *Journal of Infectious Diseases*, 183 (12), 1732–1738.

Falloon, J., Ait-Khaled, M., Thomas, D.A., Brosgart, C.L., Eron, Jr. J.J., Feinberg, J., Flanigan, T.P., Hammer, S.M., Kraus, P.W., Murphy, R., Torres, R., Masur, H. & Team CNAS. 2002. 'HIV-1 genotype and phenotype correlate with virological response to abacavir, amprenavir and efavirenz in treatment-experienced patients', *AIDS*, 16 (3), 387–396.

Falloon, J., Piscitelli, S., Vogel, S., Sadler, B., Mitsuya, H., Kavlick, M.F., Yoshimura, K., Rogers, M., LaFon, S., Manion, D.J., Lane, H.C. & Masur, H. 2000. 'Combination therapy with amprenavir, abacavir, and efavirenz in human immunodeficiency virus (HIV)-infected patients failing a protease-inhibitor regimen: pharmacokinetic drug interactions and antiviral activity', *Clinical Infectious Diseases*, 30 (2), 313–318.

Ferrer, E., Podzamczer, D., Arnedo, M., Fumero, E., McKenna, P., Rinehart, A., Perez, J.L., Barbera, M.J., Pumarola, T., Gatell, J.M., Gudiol, F. & Combine Study T. 2003. 'Genotype and phenotype at baseline and at failure in human immunodeficiency virus-infected antiretroviral-naive patients in a randomized trial comparing zidovudine and lamivudine plus nelfinavir or nevirapine', *Journal of Infectious Diseases*, 187 (4), 687–690.

Foudraine, N.A., de Jong, J.J., Jan Weverling, G., van Benthem, B.H., Maas, J., Keet, I.P., Jurriaans, S., Roos, M.T., Vandermeulen, K., de Wolf, F. & Lange, J.M. 1998. 'An open randomized controlled trial of zidovudine plus lamivudine versus stavudine plus lamivudine', *AIDS*, 12 (12), 1513–1519.

Frissen, P.H., van der Ende, M.E., ten Napel, C.H., Weigel, H.M., Schreij, G.S., Kauffmann, R.H., Koopmans, P.P., Hoepelman, A.I., de Boer, J.B., Weverling, G.J. & et al. 1994. 'Zidovudine and interferon-alpha combination therapy versus zidovudine monotherapy in subjects with symptomatic human immunodeficiency virus type 1 infection', *Journal of Infectious Diseases*, 169 (6), 1351–1355.

Garcia, F., Alonso, M.M., Romeu, J., Knobel, H., Arrizabalaga, J., Ferrer, E., Dalmau, D., Ruiz, I., Vidal, F., Frances, A., Segura, F., Gomez-Sirvent, J.L., Cruceta, A., Clotet, B., Pumarola, T., Gallart, T., O'Brien, W.A., Miro, J.M. & Gatell, J.M. 2000. 'Comparison of immunologic restoration and virologic response in plasma, tonsillar tissue, and cerebrospinal fluid in HIV-1-infected patients treated with double versus triple antiretroviral therapy in very early stages: The Spanish EARTH-2 Study', *Journal of Acquired Immune Deficiency Syndromes*, 25 (1), 26–35.

Garcia, F., Knobel, H., Sambeat, M.A., Arrizabalaga, J., Aranda, M., Romeu, J., Dalmau, D., Segura, F., Gomez-Sirvent, J.L., Ferrer, E., Cruceta, A., Gallart, T., Pumarola, T., Miro, J.M., Gatell, J.M. & Spanish SSG, 2000. 'Comparison of twice-daily stavudine plus once- or twice-daily didanosine and nevirapine in early stages of HIV infection: the scan study', *AIDS*, 14 (16), 2485–2494.

Garcia, F., Romeu, J., Grau, I., Sambeat, M.A., Dalmau, D., Knobel, H., Gomez-Sirvent, J.L., Arrizabalaga, J., Cruceta, A., Clotet, B.G., Podzamczer, D., Pumarola, T., Gallart, T., O'Brien, W.A., Miro, J.M. & Gatell, J.M. 1999. 'A randomized study comparing triple versus double antiretroviral therapy or no treatment in HIV-1-infected patients in very early stage disease: the Spanish Earth-1 study', *AIDS*, 13 (17), 2377–2388.

Grodesky, M., Acosta, E.P., Fujita, N., Mason, S. & Gerber, J.G. 2001. 'Combination therapy with indinavir, ritonavir, and delavirdine and nucleoside reverse transcriptase inhibitors in patients with HIV/AIDS who have failed multiple antiretroviral combinations', *HIV Clinical Trials*, 2 (3), 193–199.

Gulick, R.M., Mellors, J.W., Havlir, D., Eron, J.J., Meibohm, A., Condra, J.H., Valentine, F.T., McMahon, D., Gonzalez, C., Jonas, L., Emini, E.A., Chodakewitz, J.A., Isaacs, R. & Richman, D.D. 2000. '3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine', *Annals of Internal Medicine*, 133 (1), 35–39.

Havlir, D.V., Hellmann, N.S., Petropoulos, C.J., Whitcomb, J.M., Collier, A.C., Hirsch, M.S., Tebas, P., Sommadossi, J.P. & Richman, D.D. 2000. 'Drug susceptibility in HIV infection after viral rebound in patients receiving indinavir-containing regimens', *Journal of the American Medical Association*, 283 (2), 229–234.

Hoogewerf, M., Regez, R.M., Schouten, W.E., Weigel, H.M., Frissen, P.H. & Brinkman, K. 2003. 'Change to abacavir-lamivudine-tenofovir combination treatment in patients with HIV-1 who had complete virological suppression', *The Lancet*, 362 (9400), 1979–1980.

Kahn, J., Lagakos, S., Wulfsohn, M., Cherng, D., Miller, M., Cherrington, J., Hardy, D., Beall, G., Cooper, R., Murphy, R., Basgoz, N., Ng, E., Deeks, S., Winslow, D., Toole, J.J. & Coakley, D. 1999. 'Efficacy and safety of adefovir dipivoxil with antiretroviral therapy: a randomized controlled trial', *Journal of the American Medical Association*, 282 (24), 2305–2312.

Katlama, C., Clotet, B., Plettenberg, A., Jost, J., Arasteh, K., Bernasconi, E., Jeantils, V., Cutrell, A., Stone, C., Ait-Khaled, M. & Purdon, S. 2000. 'The role of abacavir (ABC, 1592) in antiretroviral therapy-experienced patients: results from a randomized, double-blind, trial', *AIDS*, 14 (7), 781–789.

Katlama, C., Ingrand, D., Loveday, C., Clumeck, N., Mallolas, J., Staszewski, S., Johnson, M., Hill, A.M., Pearce, G. & McDade, H. 1996. 'Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy', *Journal of the American Medical Association*, 276 (2), 118–125.

Katlama, C., Pellegrin, J.L., Lacoste, D., Aquilina, C., Raffi, F., Pialoux, G., Vittecoq, D., Raguin, G., Lantz, O., Mouroux, M., Calvez, V., Trylesinski, A., Montestruc, F., Dohin, E., Goehrs, J.M. & Delfraissy, J.F. 2001. 'MIKADO: a multicentre, open-label pilot study to evaluate the antiretroviral activity and safety of saquinavir with stavudine and zalcitabine', *HIV Medicine*, 2 (1), 20–26.

Kleim, J.P., Winters, M., Dunkler, A., Suarez, J.R., Riess, G., Winkler, I., Balzarini, J., Oette, D. & Merigan, T.C. 1999. 'Antiviral activity of the human immunodeficiency virus type 1-specific nonnucleoside reverse transcriptase inhibitor HBY 097 alone and in combination with zidovudine in a phase II study', *Journal of Infectious Diseases*, 179 (3), 709–713.

Kuritzkes, D.R., Quinn, J.B., Benoit, S.L., Shugarts, D.L., Griffin, A., Bakhtiari, M., Poticha, D., Eron, J.J., Fallon, M.A. & Rubin, M. 1996. 'Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients', *AIDS*, 10 (9), 975–981.

Kuritzkes, D.R., Sevin, A., Young, B., Bakhtiari, M., Wu, H., St Clair, M., Connick, E., Landay, A., Spritzler, J., Kessler, H. & Lederman, M.M. 2000. 'Effect of zidovudine resistance mutations on virologic response to treatment with zidovudine-lamivudine-ritonavir: genotypic analysis of human immunodeficiency virus type 1 isolates from AIDS clinical trials group protocol 315', *Journal of Infectious Diseases*, 181 (2), 491–497.

Lafeuillade, A., Poggi, C., Chadapaud, S., Hittinger, G., Chouraqui, M., Pisapia, M. & Delbeke, E. 2001. 'Pilot study of a combination of highly active antiretroviral therapy and cytokines to induce HIV-1 remission', *Journal of Acquired Immune Deficiency Syndromes*, 26 (1), 44–55.

Lalezari, J.P., Eron, J.J., Carlson, M., Cohen, C., DeJesus, E., Arduino, R.C., Gallant, J.E., Volberding, P., Murphy, R.L., Valentine, F., Nelson, E.L., Sista, P.R., Dusek, A. & Kilby, J.M. 2003. 'A phase II clinical study of the long-term safety and antiviral activity of enfuvirtide-based antiretroviral therapy', *AIDS*, 17 (5), 691–698.

Lazzarin, A., Clotet, B., Cooper, D., Reynes, J., Arasteh, K., Nelson, M., Katlama, C., Stellbrink, H.J., Delfraissy, J.F., Lange, J., Huson, L., DeMasi, R., Wat, C., Delehanty, J., Drobnes, C., Salgo, M. & Group TS. 2003. 'Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia', *New England Journal of Medicine*, 348 (22), 2186–2195.

Manfredi, R., Calza, L. & Chiodo, F. 2003. 'Prospective comparison of first-line nelfinavir therapy versus nelfinavir introduction in rescue antiretroviral regimens', *AIDS Patient Care & STDs*, 17 (3), 105–114.

Manfredi, R., Rizzo, E., Calza, L. & Chiodo, F. 2001. 'The use of efavirenz as a part of late rescue antiretroviral treatment', *HIV Clinical Trials*, 2 (5), 413–420.

McCoig, C., Castrejon, M.M., Castano, E., De Suman, O., Baez, C., Redondo, W., McClernon, D., Danehower, S., Lanier, E.R., Richardson, C., Keller, A., Hetherington, S., Saez-Llorens, X. & Ramilo, O. 2002. 'Effect of combination antiretroviral therapy on cerebrospinal fluid HIV RNA, HIV resistance, and clinical manifestations of encephalopathy', *Journal of Pediatrics*, 141 (1), 36–44.

Middleton, T., Smith, D., Larder, B., Law, M. & Birch, C. 2001. 'Baseline antiretroviral drug susceptibility influences treatment response in patients receiving saquinavir-enhancing therapy', *HIV Clinical Trials*, 2 (6), 445–452.

Para, M.F., Glidden, D.V., Coombs, R.W., Collier, A.C., Condra, J.H., Craig, C., Bassett, R., Leavitt, R., Snyder, S., McAuliffe, V. & Boucher, C. 2000. 'Baseline human immunodeficiency virus type 1 phenotype, genotype, and RNA response after switching from long-term hard-capsule saquinavir to indinavir or soft-gel-capsule saquinavir in AIDS clinical trials group protocol 333', *Journal of Infectious Diseases*, 182 (3), 733–743.

(PENTA) PENfToA, 2004. 'Highly active antiretroviral therapy started in infants under 3 months of age: 72-Week follow-up for CD4+ cell count, viral load and drug resistance outcome', *AIDS*, 18 (2), 237–245.

Quattro, Steering & Committee. 1999. 'A randomized trial comparing regimens of four reverse transcriptase inhibitors given together or cyclically in HIV-1 infection--the Quattro Trial', *AIDS*, 13 (16), 2209–2217.

Quattro, Steering & Committee. 2002. 'Observations of HIV-1 genotypic drug resistance in a trial of four reverse transcriptase inhibitors', *Antiviral Therapy*, 7 (1), 11–20.

Rozenbaum, W., Katlama, C., Massip, P., Bentata, M., Zucman, D., Delfraissy, J.F., Trepo, C., David, F., Lanier, E.R., Vavro, C. & Mamet, J.P. 2001. 'Treatment intensification with abacavir in HIV-infected patients with at least 12 weeks previous lamivudine/zidovudine treatment', *Antiviral Therapy*, 6 (2), 135–142.

Rubsamen-Waigmann, H., Huguenel, E., Shah, A., Paessens, A., Ruoff, H.J., von Briesen, H., Immelmann, A., Dietrich, U. & Wainberg, M.A. 1999. 'Resistance mutations selected in vivo under therapy with anti-HIV drug HBY 097 differ from resistance pattern selected in vitro', *Antiviral Research*, 42 (1), 15–24.

Schapiro, J.M., Winters, M.A., Lawrence, J. & Merigan, T.C. 1999. 'Clinical cross-resistance between the HIV-1 protease inhibitors saquinavir and indinavir and correlations with genotypic mutations', *AIDS*, 13 (3), 359–365.

Sevin, A.D., DeGruttola, V., Nijhuis, M., Schapiro, J.M., Foulkes, A.S., Para, M.F. & Boucher, C.A. 2000. 'Methods for investigation of the relationship between drug-susceptibility phenotype and human immunodeficiency virus type 1 genotype with applications to AIDS clinical trials group 333', *Journal of Infectious Diseases*, 182 (1), 59–67.

Shafer, R.W., Kozal, M.J., Winters, M.A., Iversen, A.K., Katzenstein, D.A., Ragni, M.V., Meyer, W.A., 3rd., Gupta, P., Rasheed, S., Coombs, R. & et al. 1994. 'Combination therapy with zidovudine and didanosine selects for drug-resistant human immunodeficiency virus type 1 strains with unique patterns of pol gene mutations', *Journal of Infectious Diseases*, 169 (4), 722–729.

Sirivichayakul, S., Ruxrungtham, K., Ungsedhapand, C., Techasathit, W., Ubolyam, S., Chuenyam, T., Emery, S., Cooper, D., Lange, J. & Phanuphak, P. 2003. 'Nucleoside analogue mutations and Q151M in HIV-1 subtype A/E infection treated with nucleoside reverse transcriptase inhibitors', *AIDS*, 17 (13), 1889–1896.

Staszewski, S., Massari, F.E., Kober, A., Gohler, R., Durr, S., Anderson, K.W., Schneider, C.L., Waterbury, J.A., Bakshi, K.K., Taylor, V.I. & et al. 1995. 'Combination therapy with zidovudine prevents selection of human immunodeficiency virus type 1 variants expressing high-level resistance to L-697,661, a nonnucleoside reverse transcriptase inhibitor', *Journal of Infectious Diseases*, 171 (5), 1159–1165.

Staszewski, S., Miller, V., Rehmet, S., Stark, T., De Cree, J., De Brabander, M., Peeters, M., Andries, K., Moeremans, M., De Raeymaeker, M., Pearce, G., Van den Broeck, R., Verbiest, W. & Stoffels, P. 1996. 'Virological and immunological analysis of a triple combination pilot study with loviride, lamivudine and zidovudine in HIV-1-infected patients', *AIDS*, 10 (5), F1–7.

Sullivan, A.K., Nelson, M.R., Shaw, A., Moyle, G.J., Mandalia, S., Gazzard, B.G. & Asboe, D. 2000. 'Efficacy of a nelfinavir- and nevirapine-containing salvage regimen', *HIV Clinical Trials*, 1 (1), 7–12.

Valer, L., De Mendoza, C., De Requena, D.G., Labarga, P., Garcia-Henarejos, A., Barreiro, P., Guerrero, F., Vergara, A., Soriano, V. & Fortogene Spanish Study G. 2002. 'Impact of HIV genotyping and drug levels on the response to salvage therapy with saquinavir/ritonavir', *AIDS*, 16 (14), 1964–1966.

Emergence of mutations while on treatment

Birch, C., Middleton, T., Hales, G., Cooper, D., Law, M., Crowe, S., Hoy, J. & Emery, S. 2003. 'Limited evolution of HIV antiretroviral drug resistance-associated mutations during the performance of drug resistance testing', *Journal of Acquired Immune Deficiency Syndromes*, 32 (1), 57–61.

Calderon, E.J., Torres, Y., Medrano, F.J., Luque, F., Larder, B., Rey, C., Sanchez-Quijano, A., Lissen, E. & Leal, M. 1995. 'Emergence and clinical relevance of mutations associated with zidovudine resistance in asymptomatic HIV-1 infected patients', *European Journal of Clinical Microbiology & Infectious Diseases*, 14 (6), 512–519.

Cho, Y.K., Sung, H., Lee, H.J., Joo, C.H. & Cho, G.J. 2001. 'Long-term intake of Korean red ginseng in HIV-1-infected patients: development of resistance mutation to zidovudine is delayed', *International Immunopharmacology*, 1 (7), 1295–1305.

Conway, B., Wainberg, M.A., Hall, D., Harris, M., Reiss, P., Cooper, D., Vella, S., Curry, R., Robinson, P., Lange, J.M. & Montaner, J.S. 2001. 'Development of drug resistance in patients receiving combinations of zidovudine, didanosine and nevirapine', *AIDS*, 15 (10), 1269–1274.

Craig, C., Race, E., Sheldon, J., Whittaker, L., Gilbert, S., Moffatt, A., Rose, J., Dissanayeke, S., Chirn, G.W., Duncan, I.B. & Cammack, N. 1998. 'HIV protease genotype and viral sensitivity to HIV protease inhibitors following saquinavir therapy', *AIDS*, 12 (13), 1611–1618.

Delaugerre, C., Valantin, M.A., Mouroux, M., Bonmarchand, M., Carcelain, G., Duvivier, C., Tubiana, R., Simon, A., Bricaire, F., Agut, H., Autran, B., Katlama, C. & Calvez, V. 2001. 'Re-occurrence of HIV-1 drug mutations after treatment re-initiation following interruption in patients with multiple treatment failure', *AIDS*, 15 (16), 2189–2191.

Eron, J.J., Vernazza, P.L., Johnston, D.M., Seillier-Moiseiwitsch, F., Alcorn, T.M., Fiscus, S.A. & Cohen, M.S. 1998. 'Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission', *AIDS*, 12 (15), F181–189.

Fitzgibbon, J.E., Gaur, S., Walsman, S.M., Janahi, M., Whitley-Williams, P. & John, J.F., Jr. 2001. 'Emergence of drug resistance mutations in a group of HIV-infected children taking nelfinavir-containing regimens', *AIDS Research & Human Retroviruses*, 17 (14), 1321–1328.

Gibb, D.M., Walker, A.S., Kaye, S., De Rossi, A., Ait-Khaled, M., Pillay, D., Munoz-Fernandez, M.A., Loveday, C., Compagnucci, A., Dunn, D.T. & Babiker, A.G. 2002. 'Evolution of antiretroviral phenotypic and genotypic drug resistance in antiretroviral-naive HIV-1-infected children treated with abacavir/lamivudine, zidovudine/lamivudine or abacavir/zidovudine, with or without nelfinavir (the PENTA 5 trial)', *Antiviral Therapy*, 7 (4), 293–303.

Holguin, A., Dietrich, U., Immelmann, A. & Soriano, V. 1998. 'Genotypic and phenotypic resistance to stavudine after long-term monotherapy', *Antiviral Therapy*, 3 (3), 183–186.

Izopet, J., Sailler, L., Sandres, K., Pasquier, C., Bonnet, E., Aquilina, C., Puel, J., Massip, P. & Marchou, B. 1999. 'Intermittent selection pressure with zidovudine plus zalcitabine treatment reduces the emergence in vivo of zidovudine resistance HIV mutations', *Journal of Medical Virology*, 57 (2), 163–168.

Jacobsen H, Hanggi M, Ott M, Duncan IB, Owen S, Andreoni M, Vella S & Mous J, 1996. 'In vivo resistance to a human immunodeficiency virus type 1 proteinase inhibitor: mutations, kinetics, and frequencies'. *Journal of Infectious Diseases,* 173 (6), 1379-87.

Karmochkine, M., Si Mohamed, A., Piketty, C., Ginsburg, C., Raguin, G., Schneider-Fauveau, V., Gutmann, L., Kazatchkine, M.D. & Belec, L. 2000. 'The cumulative occurrence of resistance mutations in the HIV-1 protease gene is associated with failure of salvage therapy with ritonavir and saquinavir in protease inhibitor-experienced patients', *Antiviral Research*, 47 (3), 179–188.

Larder, B.A., Kohli, A., Bloor, S., Kemp, S.D., Harrigan, P.R., Schooley, R.T., Lange, J.M., Pennington, K.N. & St Clair, M.H. 1996. 'Human immunodeficiency virus type 1 drug susceptibility during zidovudine (AZT) monotherapy compared with AZT plus 2',3'-dideoxyinosine or AZT plus 2',3'-dideoxycytidine combination therapy', *Journal of Virology*, 70 (9), 5922–5929.

Mulato, A.S., Lamy, P.D., Miller, M.D., Li, W.X., Anton, K.E., Hellmann, N.S. & Cherrington, J.M. 1998. 'Genotypic and phenotypic characterization of human immunodeficiency virus type 1 variants isolated from AIDS patients after prolonged adefovir dipivoxil therapy', *Antimicrobial Agents & Chemotherapy*, 42 (7), 1620–1628.

Rusconi, S., De Pasquale, M.P., Milazzo, L., Moscatelli, G., Bulgheroni, E., Citterio, P., d'Arminio-Monforte, A., Moroni, M. & Galli, M. 1997. 'Loss of antiviral effect owing to zidovudine and lamivudine double resistance in HIV-1-infected patients in an ongoing open-label trial', *Antiviral Therapy*, 2 (1), 39–46.

Schooley, R.T., Clumeck, N., Haubrich, R., Thompson, M., Danner, S.A., van Der Ende, M.E., Sereni, D., Antunes, F., Blake, D., Myers, R.E., Tisdale, M., Millard, J., Mustafa, N. & Nacci, P. 2001. 'A dose-ranging study to evaluate the antiretroviral activity and safety of amprenavir alone and in combination with abacavir in HIV-infected adults with limited antiretroviral experience', *Antiviral Therapy*, 6 (2), 89–96.

Squires, K., Pozniak, A.L., Pierone, G., Jr., Steinhart, C.R., Berger, D., Bellos, N.C., Becker, S.L., Wulfsohn, M., Miller, M.D., Toole, J.J., Coakley, D.F., Cheng, A. & Study T. 2003. 'Tenofovir disoproxil fumarate in nucleoside-resistant HIV-1 infection: a randomized trial', *Annals of Internal Medicine*, 139 (5 Pt 1), 313–20.

Vergne, L., Kane, C.T., Laurent, C., Diakhate, N., Gueye, N.F., Gueye, P.M., Sow, P.S., Faye, M.A., Liegeois, F., Ndir, A., Laniece, I., Peeters, M., Ndoye, I., Mboup, S. & Delaporte, E. 2003. 'Low rate of genotypic HIV-1 drug-resistant strains in the Senegalese government initiative of access to antiretroviral therapy', *AIDS*, 17 (Suppl 3), S31–38.

Vidal, C., Arnedo, M., Garcia, F., Mestre, G., Plana, M., Cruceta, A., Capon, A., Gallart, T., Miro, J.M., Pumarola, T. & Gatell, J.M. 2002. 'Genotypic and phenotypic resistance patterns in early-stage HIV-1-infected patients failing initial therapy with stavudine, didanosine and nevirapine', *Antiviral Therapy*, 7 (4), 283–287.

Correlation of baseline characteristics and treatment failure and transmission

Bacheler, L., Jeffrey, S., Hanna, G., D'Aquila, R., Wallace, L., Logue, K., Cordova, B., Hertogs, K., Larder, B., Buckery, R., Baker, D., Gallagher, K., Scarnati, H., Tritch, R. & Rizzo, C. 2001. 'Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy', *Journal of Virology*, 75 (11), 4999–5008.

Brown, A.J., Gunthard, H.F., Wong, J.K., D'Aquila, R.T., Johnson, V.A., Kuritzkes, D.R. & Richman, D.D. 1999. 'Sequence clusters in human immunodeficiency virus type 1 reverse transcriptase are associated with subsequent virological response to antiretroviral therapy', *Journal of Infectious Diseases*, 180 (4), 1043–1049.

Casado, J.L., Moreno, S., Hertogs, K., Dronda, F., Antela, A., Dehertogh, P., Perez-Elias, M.J., Moreno, A. & study N. 2002. 'Plasma drug levels, genotypic resistance, and virological response to a nelfinavir plus saquinavir-containing regimen', *AIDS*, 16 (1), 47–52.

Clotet, B., Ruiz, L., Martinez-Picado, J., Negredo, E., Hill, A. & Popescu, M. 2002. 'Prevalence of HIV protease mutations on failure of nelfinavir-containing HAART: a retrospective analysis of four clinical studies and two observational cohorts', *HIV Clinical Trials*, 3 (4), 316–323.

DeGruttola, V., Dix, L., D'Aquila, R., Holder, D., Phillips, A., Ait-Khaled, M., Baxter, J., Clevenbergh, P., Hammer, S., Harrigan, R., Katzenstein, D., Lanier, R., Miller, M., Para, M., Yerly, S., Zolopa, A., Murray, J., Patick, A., Miller, V., Castillo, S., Pedneault, L. & Mellors, J. 2000. 'The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan', *Antiviral Therapy*, 5 (1), 41–48.

Gehringer, H., Bogner, J.R., Goebel, F.D., Nitschko, H. & von der Helm, K. 2000. 'Sequence analysis of the HIV-1 protease coding region of 18 HIV-1-infected patients prior to HAART and possible implications on HAART', *Journal of Clinical Virology*, 17 (2), 137–141.

Harrigan, P.R., Hertogs, K., Verbiest, W., Larder, B., Yip, B., Brumme, Z.L., Alexander, C., Tilley, J., O'Shaughnessy, M.V. & Montaner, J.S. 2003. 'Modest decreases in NNRTI susceptibility do not influence virological outcome in patients receiving initial NNRTI-containing triple therapy', *Antiviral Therapy*, 8 (5), 395–402.

Izopet, J., Bicart-See, A., Pasquier, C., Sandres, K., Bonnet, E., Marchou, B., Puel, J. & Massip, P. 1999. 'Mutations conferring resistance to zidovudine diminish the antiviral effect of stavudine plus didanosine', *Journal of Medical Virology*, 59 (4), 507–511.

Johann-Liang, R., Lee, S.E., Fernandez, A., Cervia, J. & Noel, G.J. 2000. 'Genotypic characterization of human immunodeficiency virus type 1 isolated from vertically infected children with antiretroviral therapy experience', *Pediatric Infectious Disease Journal*, 19 (4), 363–364.

Kempf, D.J., Isaacson, J.D., King, M.S., Brun, S.C., Sylte, J., Richards, B., Bernstein, B., Rode, R. & Sun, E. 2002. 'Analysis of the virological response with respect to baseline viral phenotype and genotype in protease inhibitor-experienced HIV-1-infected patients receiving lopinavir/ritonavir therapy', *Antiviral Therapy*, 7 (3), 165–174.

Lafeuillade, A., Khiri, H., Chadapaud, S., Hittinger, G. & Halfon, P. 2001. 'Persistence of HIV-1 resistance in lymph node mononuclear cell RNA despite effective HAART', *AIDS*, 15 (15), 1965–1969.

Lafeuillade, A., Poggi, C., Chadapaud, S., Hittinger, G., Chouraqui, M., Delbeke, E. & Apollo, S. 2001. 'HIV-1 induction-maintenance at the lymph node level: the "Apollo-97" Study', *Journal of Acquired Immune Deficiency Syndromes*, 28 (2), 154–157.

Lawrence, J., Mayers, D.L., Hullsiek, K.H., Collins, G., Abrams, D.I., Reisler, R.B., Crane, L.R., Schmetter, B.S., Dionne, T.J., Saldanha, J.M., Jones, M.C., Baxter, J.D. & AIDS STotTBCPfCRo. 2003. 'Structured treatment interruption in patients with multidrug-resistant human immunodeficiency virus.[see comment]', *New England Journal of Medicine*, 349 (9), 837–846.

Leriche-Guerin, K., Trabaud, M.A., Cotte, L., Bissuel, F., Deruelle, E., Rougier, P. & Trepo, C. 1997. 'Correlation between antiretroviral resistance mutations, biological parameters, and clinical evolution in zidovudine-treated patients infected with human immunodeficiency virus type 1', *European Journal of Clinical Microbiology & Infectious Diseases*, 16 (9), 660–668.

Linde, R., Funk, M.B., Schuster, T., Wintergerst, U., Notheis, G., Klingebiel, T. & Kreuz, W. 2001. 'Low incidence of genotypic and phenotypic resistance in paediatric HIV-infected patients on long-term first-line antiretroviral triple therapy', *AIDS*, 15 (8), 1077–1079.

Miller, V., Phillips, A., Rottmann, C., Staszewski, S., Pauwels, R., Hertogs, K., Bethune, M.-P.D., Kemp, S.D., Bloor, S., Harrigan, P.R. & Larder, B.A. 1998. 'Dual resistance to zidovudine and lamivudine in patients treated with zidovudine-lamivudine combination therapy: Association with therapy failure', *Journal of Infectious Diseases*, 177 (6), 1521–1532.

Shulman, N.S., Machekano, R.A., Shafer, R.W., Winters, M.A., Zolopa, A.R., Liou, S.H., Hughes, M., Katzenstein, D.A. & Team ACTGS. 2001. 'Genotypic correlates of a virologic response to stavudine after zidovudine monotherapy', *Journal of Acquired Immune Deficiency Syndromes*, 27 (4), 377–380.

Torti, C., Quiros-Roldan, E., Scudeller, L., Lo Caputo, S., Tomasoni, L., Castelli, F., Poggio, A., Delle Foglie, P., Chirianni, A., Sighinolfi, L., Mazzotta, F., Carosi, G. & GenPheRex Group of the Ma STRC. 2003. 'Characterization of viro-immunological responses in a closely followed cohort of heavily pretreated patients: evidence from the GenPheRex Study', *HIV Medicine*, 4 (3), 263–270.

van der Ende, M.E., Prins, J.M., Brinkman, K., Keuter, M., Veenstra, J., Danner, S.A., Niesters, H.G., Osterhaus, A.D. & Schutten, M. 2003. 'Clinical, immunological and virological response to different antiretroviral regimens in a cohort of HIV-2-infected patients', *AIDS*, 17 (Suppl 3), S55–61.

Verhofstede, C., Reniers, S., Van Wanzeele, F. & Plum, J. 1994. 'Evaluation of proviral copy number and plasma RNA level as early indicators of progression in HIV-1 infection: correlation with virological and immunological markers of disease', *AIDS*, 8 (10), 1421–1427.

Vray, M., Meynard, J.L., Dalban, C., Morand-Joubert, L., Clavel, F., Brun-Vezinet, F., Peytavin, G., Costagliola, D., Girard, P.M. & Narval Trial G. 2003. 'Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1-infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS 088)', *Antiviral Therapy*, 8 (5), 427–434.

Wainberg, M.A., Tremblay, M., Rooke, R., Fanning, M., Tsoukas, C., Montaner, J.S., O'Shaughnessy, M. & Ruedy, J. 1992. 'Characterization of zidovudine resistant variants of HIV-1 isolated from patients on prolonged therapy', *Drugs Under Experimental & Clinical Research*, 18 (7), 283–290.

Analysis of persistence of resistance mutations following treatment termination

Birk, M., Svedhem, V. & Sonnerborg, A. 2001. 'Kinetics of HIV-1 RNA and resistance-associated mutations after cessation of antiretroviral combination therapy', *AIDS*, 15 (11), 1359–1368.

Smith, M.S., Koerber, K.L. & Pagano, J.S. 1994. 'Long-term persistence of zidovudine resistance mutations in plasma isolates of human immunodeficiency virus type 1 of dideoxyinosine-treated patients removed from zidovudine therapy', *Journal of Infectious Diseases*, 169 (1), 184–188.

Non-randomised study

Badri, S.M., Adeyemi, O.M., Max, B.E., Zagorski, B.M. & Barker, D.E. 2003. 'How does expert advice impact genotypic resistance testing in clinical practice?',*Clinical Infectious Diseases*, 37 (5), 708–713.

De Luca, A., Cingolani, A., Di Giambenedetto, S., Trotta, M.P., Baldini, F., Rizzo, M.G., Bertoli, A., Liuzzi, G., Narciso, P., Murri, R., Ammassari, A., Perno, C.F. & Antinori, A. 2003. 'Variable prediction of antiretroviral treatment outcome by different systems for interpreting genotypic human immunodeficiency virus type 1 drug resistance', *Journal of Infectious Diseases*, 187 (12), 1934–1943.

Haupts, S., Ledergerber, B., Boni, J., Schupbach, J., Kronenberg, A., Opravil, M., Flepp, M., Speck, R.F., Grube, C., Rentsch, K., Weber, R., Gunthard, H.F. & Swiss HIVCS. 2003. 'Impact of genotypic resistance testing on selection of salvage regimen in clinical practice', *Antiviral Therapy*, 8 (5), 443–454.

Zollner, B., Feucht, H.H., Weitner, L., Adam, A., Schroter, M., Schafer, P. & Laufs, R. 2001. 'Application of HIV-1 genotypic-resistance testing prevents the evolution of further resistance mutations in heavily pretreated patients', *Journal of Clinical Virology*, 21 (1), 37–45.

Case-control study

Descamps, D., Flandre, P., Calvez, V., Peytavin, G., Meiffredy, V., Collin, G., Delaugerre, C., Robert-Delmas, S., Bazin, B., Aboulker, J.P., Pialoux, G., Raffi, F. & Brun-Vezinet, F. 2000. 'Mechanisms of virologic failure in previously untreated HIV-infected patients from a trial of induction-maintenance therapy. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team).[see comment][erratum appears in JAMA 2000 Sep 27;284(12):1518]', *Journal of the American Medical Association*, 283 (2), 205–211.

Englund, J.A., Raskino, C., Vavro, C., Palumbo, P., Ross, L.L., McKinney, R., Nikolic-Djokic, D., Colgrove, R.C., Baker, C.J. & Pediatric ACTGPT. 2004. 'Mutations linked to drug resistance, human immunodeficiency virus type 1 biologic phenotype and their association with disease progression in children receiving nucleoside reverse transcriptase inhibitors', *Pediatric Infectious Disease Journal*, 23 (1), 15–22.

Langmann, P., Weissbrich, B., Desch, S., Vath, T., Schirmer, D., Zilly, M. & Klinker, H. 2002. 'Efavirenz plasma levels for the prediction of treatment failure in heavily pretreated HIV-1 infected patients', *European Journal of Medical Research*, 7 (7), 309–314.

Walsh, J.C., Pozniak, A.L., Nelson, M.R., Mandalia, S. & Gazzard, B.G. 2002. 'Virologic rebound on HAART in the context of low treatment adherence is associated with a low prevalence of antiretroviral drug resistance', *Journal of Acquired Immune Deficiency Syndromes*, 30 (3), 278–287.

Cost-effectiveness

Chaix, C., Grenier-Sennelier, C., Clevenbergh, P., Durant, J., Schapiro, J.M., Dellamonica, P. & Durand-Zaleski, I. 2000. 'Economic evaluation of drug resistance genotyping for the adaptation of treatment in HIV-infected patients in the VIRADAPT study', *Journal of Acquired Immune Deficiency Syndromes*, 24 (3), 227–231.

Chaix-Couturier, C., Holtzer, C., Phillips, K.A., Durand-Zaleski, I. & Stansell, J. 2000. 'HIV-1 drug resistance genotyping. A review of clinical and economic issues', *Pharmacoeconomics*, 18 (5), 425–433.

Weinstein, M.C., Goldie, S.J., Losina, E., Cohen, C.J., Baxter, J.D., Zhang, H., Kimmel, A.D. & Freedberg, K.A. 2001. 'Use of genotypic resistance testing to guide hiv therapy: clinical impact and cost-effectiveness.[see comment]', *Annals of Internal Medicine*, 134 (6), 440–450.

Narrative/letters to editor

Anonymous, 1999. 'HIV resistance testing proves its value', *Project Inform Perspective*, (28), 14.

Anonymous, 2003. 'Resistance data on 908'. *AIDS Patient Care & STDs*, 17 (9), 483–486.

Cadman, J. 1998. 'Reevaluating initial therapy', *Gmhc Treatment Issues: the Gay Men's Health Crisis Newsletter of Experimental AIDS Therapies*, 12 (2), 1–5.

Iafrate, A.J. 2004. 'Clinical Testing for HIV-1 Drug Resistance', *Clinical Microbiology Newsletter*, 26 (5), 33–37.

Loveday, C., Dunn, D., McCormack, S. & Babiker, A. 1999. 'The use of HIV resistance assays--random or randomised?', *Sexually Transmitted Infections*, 75 (3), 140–141.

Manfredi, R. & Calza, L. 2002. 'HIV genotype mutations evoked by nelfinavir-based regimens: frequency, background, and consequences on subsequent treatment options', *Journal of Acquired Immune Deficiency Syndromes*, 30 (2), 258–260.

Montroni, M., D'Arminio Monforte, A., Butini, L., Constantini, A. & Regnery, C.M. 2003. 'Theme 5. Genotypic resistance tests for the management of structured therapeutic interruptions after multiple drug failure', *Scandinavian Journal of Infectious Diseases Supplementum. Issue*, 106, 79–81.

Narciso, P., Lazzarin, A. & Tozzi, V. 2003. 'Theme 2. Genotypic resistance tests for the clinical management of patients with primary HIV infection'. *Scandinavian Journal of Infectious Diseases Supplementum. Issue*, 106, 66–70.

Price, N., Smit, E. & Pillay, D. 2002. 'Resistance tests: what do clinical trials tell us?', *Journal of HIV Therapy*, 7 (3), 80–86.

Simmons, P. 1999. 'Resistance testing debate: does it make the grade?', *Research Initiative, Treatment Action: Rita*, 5 (4), 9–12.

Stebbing, J. & Gazzard, B.G. 2002. 'Clinical utility of resistance testing', *Journal of HIV Therapy*, 7 (4), 75–79.

Visco-Comandini, U. & Balotta, C. 2003. 'Theme 4. Genotypic resistance tests for the management of the HIV-infected patient with non-B viral isolates', *Scandinavian Journal of Infectious Diseases Supplementum. Issue*, 106, 75–78.

Erratum

Durant, J. & et al., 1999. 'Erratum: Drug-resistance genotyping in HIV-1 therapy: The VIRADAPT randomised controlled trial', *The Lancet*, 354 (9184), 1128.

No intervention

Anastos, K., Gange, S.J., Lau, B., Weiser, B., Detels, R., Giorgi, J.V., Margolick, J.B., Cohen, M., Phair, J., Melnick, S., Rinaldo, C.R., Kovacs, A., Levine, A., Landesman, S., Young, M., Munoz, A. & Greenblatt, R.M. 2000. 'Association of race and gender with HIV-1 RNA levels and immunologic progression', *Journal of Acquired Immune Deficiency Syndromes*, 24 (3), 218–226.

Andreoni, M., Sarmati, L., Ercoli, L., Nicastri, E., Giannini, G., Galluzzo, C., Pirillo, M.F. & Vella, S. 1997. 'Correlation between changes in plasma HIV RNA levels and in plasma infectivity in response to antiretroviral therapy', *AIDS Research & Human Retroviruses*, 13 (7), 555–561.

Bauer, G.R., Welles, S.L., Colgrove, R.R., Pitt, J., Women & Infants Transmission Study T. 2003. 'Zidovudine resistance phenotype and risk of perinatal HIV-1 transmission in zidovudine monotherapy-treated mothers with moderately advanced disease.[see comment]'. *Journal of Acquired Immune Deficiency Syndromes*, 34 (3), 312–319.

Case series

Eshleman, S.H., Mracna, M., Guay, L.A., Deseyve, M., Cunningham, S., Mirochnick, M., Musoke, P., Fleming, T., Glenn Fowler, M., Mofenson, L.M., Mmiro, F. & Jackson, J.B. 2001. 'Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012)', *AIDS*, 15 (15), 1951–1957.

Quiros-Roldan, E., Moretti, F., Airoldi, M., Fausti, C., Chiodera, A., Castelli, F. & Carosi, G. 2001. 'Long-term benefit of genotypic-guided therapy and prevalence of multinucleoside resistance in an Italian group of antiretroviral multiexperienced patients', *Journal of Clinical Laboratory Analysis*, 15 (3), 127–130.

Techniques to measure viral load

Bruisten, S.M., Koppelman, M.H., Roos, M.T., Loeliger, A.E., Reiss, P., Boucher, C.A. & Huisman, H.G. 1993. 'Use of competitive polymerase chain reaction to determine HIV-1 levels in response to antiviral treatments', *AIDS*, 7 (Suppl 2), S15–20.

Mellors, J.W., Kingsley, L.A., Rinaldo, C.R., Jr., Todd, J.A., Hoo, B.S., Kokka, R.P. & Gupta, P. 1995. 'Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion', *Annals of Internal Medicine*, 122 (8), 573–579.

RCT for effectiveness of drug monitoring

Clevenbergh, P., Garraffo, R., Durant, J. & Dellamonica, P. 2002. 'PharmAdapt: a randomized prospective study to evaluate the benefit of therapeutic monitoring of protease inhibitors: 12 week results', *AIDS*, 16 (17), 2311–2315.

Clevenbergh, P., Bozonnat, M.C., Kirstetter, M., Durant, J., Cua, E., del Giudice, P., Montagne, N., Simonet, P. & Group I. 2003. 'Variable virological outcomes according to the center providing antiretroviral therapy within the PharmAdapt clinical trial', *HIV Clinical Trials*, 4 (2), 84–91.

Analysis of techniques to detect mutations

Beddows, S., Galpin, S., Kazmi, S.H., Ashraf, A., Johargy, A., Frater, A.J., White, N., Braganza, R., Clarke, J., McClure, M. & Weber, J.N. 2003. 'Performance of two commercially available sequence-based HIV-1 genotyping systems for the detection of drug resistance against HIV type 1 group M subtypes', *Journal of Medical Virology*, 70 (3), 337–342.

Clarke, J.R., Kaye, S., Babiker, A.G., Hooker, M.H., Tedder, R. & Weber, J.N. 2000. 'Comparison of a point mutation assay with a line probe assay for the detection of the major mutations in the HIV-1 reverse transcriptase gene associated with reduced susceptibility to nucleoside analogues', *Journal of Virological Methods*, 88 (2), 117–124.

Cunningham, S., Ank, B., Lewis, D., Lu, W., Wantman, M., Dileanis, J.A., Jackson, J.B., Palumbo, P., Krogstad, P. & Eshleman, S.H. 2001. 'Performance of the applied biosystems ViroSeq human immunodeficiency virus type 1 (HIV-1) genotyping system for sequence-based analysis of HIV-1 in pediatric plasma samples', *Journal of Clinical Microbiology*, 39 (4), 1254–1257.

Descamps, D., Calvez, V., Collin, G., Cecille, A., Apetrei, C., Damond, F., Katlama, C., Matheron, S., Huraux, J.M. & Brun-Vezinet, F. 1998. 'Line probe assay for detection of human immunodeficiency virus type 1 mutations conferring resistance to nucleoside inhibitors of reverse transcriptase: comparison with sequence analysis', *Journal of Clinical Microbiology*, 36 (7), 2143–2145.

Dunne, A.L., Mitchell, F.M., Coberly, S.K., Hellmann, N.S., Hoy, J., Mijch, A., Petropoulos, C.J., Mills, J. & Crowe, S.M. 2001. 'Comparison of genotyping and phenotyping methods for determining susceptibility of HIV-1 to antiretroviral drugs', *AIDS*, 15 (12), 1471–1475.

Hanna, G.J., Johnson, V.A., Kuritzkes, D.R., Richman, D.D., Martinez-Picado, J., Sutton, L., Hazelwood, J.D. & D'Aquila, R.T. 2000. 'Comparison of sequencing by hybridization and cycle sequencing for genotyping of human immunodeficiency virus type 1 reverse transcriptase', *Journal of Clinical Microbiology*, 38 (7), 2715–2721.

Mracna, M., Becker-Pergola, G., Dileanis, J., Guay, L.A., Cunningham, S., Jackson, J.B. & Eshleman, S.H. 2001. 'Performance of Applied Biosystems ViroSeq HIV-1 Genotyping System for sequence-based analysis of non-subtype B human immunodeficiency virus type 1 from Uganda', *Journal of Clinical Microbiology*, 39 (12), 4323–4327.

Scudeller, L., Torti, C., Quiros-Roldan, E., Patroni, A., Lo Caputo, S., Moretti, F., Mazzotta, F., Donati, E., Vivarelli, A., Carosi, G. & GenPheRex Group of the MC. 2003. 'HIV susceptibility to amprenavir: phenotype-based versus rules-based interpretations', *Journal of Antimicrobial Chemotherapy*, 52 (5), 776–781.

Summary of an included study

Alberta Heritage Foundation for Medical Research, 1999. 'Genotypic HIV resistance testing', *Edmonton: Alberta Heritage Foundation for Medical Research (AHFMR).*

Non-English

Bossi, P., Tubiana, R., Calvez, V., Peytavin, G., Katlama, C. & Bricaire, F. 2001. '[Management of therapeutic failure in HIV-infected patients]', *Presse Medicale*, 30 (16), 806–812.

Corzillius, M., Muhlberger, N., Sroczynski, G., Peeters, J., Siebert, U., Jager, H. & Wasem, J. 2003. 'Evaluation of the genotypic and phenotypic definition of resistance in the treatment of patients infected by HIV - Medical efficacy and economic efficiency (project)', *German Agency of Health Technology Assessment at German Institute for Medical Documentation and Information (DAHTA@DIMDI) (DAHTA),*

Unavailable to information services

Roberts, N.A. 1995. 'Drug-resistance patterns of saquinavir and other HIV proteinase inhibitors', *Aids, Supplement*, 9 (2), S27–32.

Abbreviations

AHOD	Australian HIV Observational Database
AIDS	acquired immune deficiency syndrome
AR-DRGs	Australian Diagnostic Related Groupings
ART	antiretroviral therapy
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
DNA	deoxyribonucleic acid
EDR	excess death rate
EQAS	external quality assessment scheme
FI	fusion inhibitor
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
ICER	incremental cost-effectiveness ratio
LR-	likelihood ratio of a negative test
LR+	likelihood ratio of a positive test
LY	life year
ml	millilitre
MSAC	Medical Services Advisory Committee
NHMRC	National Health and Medical Research Council
NNH	number needed to harm
NNRTI	non-nucleoside reverse transcriptase inhibitor
NNT	number needed to treat
NRTI	nucleoside reverse transcriptase inhibitor
PCR	polymerase chain reaction
PI	protease inhibitor
PR	protease
QALY	quality adjusted life years
RCT	randomised controlled trial
RD	risk difference
RNA	ribonucleic acid
RR	relative risk
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
SOC	standard of care
VFIF	viral failure and immune failure
VFIS	viral failure and immune success
VSIS	viral success and immune success

References

AFAO, 2003. Australian Federation of AIDS Organisations. [Internet] Available from: http://www.afao.org.au [Accessed 13 November 2003]

AHOD, 2002. 'Rates of combination antiretroviral treatment change in Australia, 1997-2000', *HIV Medicine*, 3, 28–36.

Alderson, P., Green, S., Higgins, J.P.T. editors. Cochrane Reviewers' Handbook 4.2.2 [Internet] (updated December 2003). Available from: http://www.cochrane.org/resources/handbook/hbook.htm [Accessed 11 May 2004].

Ammaranond, P., Cunningham, P., Oelrichs, R., Suzuki, K., Harris, C., Leas, L., Grulich, A., Cooper, D.A. & Kelleher, A.D. 2003a. 'No increase in protease resistance and a decrease in reverse transcriptase resistance mutations in primary HIV-1 infection: 1992-2001', *AIDS*, 17 (2), 264–267.

Ammaranond, P., Cunningham, P., Oelrichs, R., Suzuki, K., Harris, C., Leas, L., Grulich, A., Cooper, D.A. & Kelleher, A.D. 2003b. 'Rates of transmission of antiretroviral drug resistant strains of HIV-1', *Journal of Clinical Virology*, 26 (2), 153–161.

Anis, A.H., Wang, X., Harrigan, R., Hogg, R.S. Yip B., O'Shaughnessy M.V., Schechter M.T. & Montaner J.S.G. 1999a. 'Optimising Drug Treatment: A cost-effectiveness analysis of HIV/AIDS Drug Resistance Testing. [abstract] 21st Annual Meeting of the Society for Medical Decision Making, Reno, Nevada.'

Anis., A.H., Wang, X.., Harrigan, R.. 1999b 'The cost-effectiveness of immediate ritonavir-saquinavir therapy versus resistance testing and a drug holiday in HIV patients failing protease including regimens [abstract]. Med Decision Making. Reno (NV): Society for Medical Decision Making, Oct 3-6'.

Antiretroviral Therapy for HIV Infection: Principles of use - Standard of Care Guidelines October 1997 HIV/AIDS Clinical Trials and Treatments Advisory Committee of the Australian National Council on AIDS and Related Diseases. [Internet] Available from: http://ashm.org.au [Accessed 11 March 2004]

Antiretroviral Therapy for HIV Infection in Women and Children Standard of Care Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee if the Australian National Council on AIDS and Related Diseases, January 1999. [Internet] Available from: http://www.health.gov.au/pubhlth/ancard/pdf/hivwomen.pdf [Accessed 11 March 2004]

Australian Antiretroviral Guidelines HIV/AIDS Clinical Trials and Treatments Advisory Committee if the Australian National Council on AIDS and Related Diseases, October 2001. [Internet] Available from: http://www.ashm.org.au/uploadFile/GUIDELINES2.pdf [Accessed 11 March 2004]

Australian Bureau of Statistics (2002). In Catalogue 3302.0AGPS, Canberra.

Australian Bureau of Statistics (2004). Consumer Price Index: Australia. Australian Bureau of Statistics, Canberra.

Bi X, Gatanaga H, Ida S, Tsuchiya K, Matsuoka-Aizawa S, Kimura S & Oka S, 2003. 'Emergence of protease inhibitor resistance-associated mutations in plasma HIV-1 precedes that in proviruses of peripheral blood mononuclear cells by more than a year'. *Journal of Acquired Immune Deficiency Syndromes: JAIDS,* 34 (1), 1-6.

Birch C, Middleton T, Hales G, Cooper D, Law M, Crowe S, Hoy J & Emery S, 2003. 'Limited evolution of HIV antiretroviral drug resistance-associated mutations during the performance of drug resistance testing'. *Journal of Acquired Immune Deficiency Syndromes: JAIDS,* 32 (1), 57-61.

Baxter, J.D., Mayers, D.L., Wentworth, D.N., Neaton, J.D., Hoover, M.L., Winters, M.A., Mannheimer, S.B., Thompson, M.A., Abrams, D.I., Brizz, B.J., Ioannidis, J.P. & Merigan, T.C. 2000. 'A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS', *AIDS*, 14 (9), F83–93.

Chaix, C., Grenier-Sennelier, C. Clevenberg, P. Durant J., Schapiro J. M., Dellamonica P., Durand-Zaleski I. 2000. 'Economic evaluation of drug resistance genotyping for the adaptation of treatment in HIV-infected patients in the VIRADAPT study', *Journal of Acquired Immune Deficiency Syndromes,* 24 (3) 227–231.

Chaix-Couturier, C., Holtzer, C., Phillips, K.A., Durand-Zaleski, I. & Stansell, J. 2000. 'HIV-1 Drug Resistance Genotyping: A Review of Clinical and Economic Issues', *Pharmacoeconomics*, 18 (5) 425–433.

Cingolani, A., Antinori, A., Rizzo, M.G., Murri, R., Ammassari, A., Baldini, F., Di Giambenedetto, S., Cauda, R. & De Luca, A. 2002. 'Usefulness of monitoring HIV drug resistance and adherence in individuals failing highly active antiretroviral therapy: a randomized study (ARGENTA)', *AIDS*, 16 (3), 369–379.

Cinque, P., Presi, S., Bestetti, A., Pierotti, C., Racca, S., Boeri, E., Morelli, P., Carrera, P., Ferrari, M. & Lazzarin, A. 2001. 'Effect of genotypic resistance on the virological response to highly active antiretroviral therapy in cerebrospinal fluid', *AIDS Research & Human Retroviruses*, 17 (5), 377–383.

Clarke, J.R., Kaye, S., Babiker, A.G., Hooker, M.H., Tedder, R. & Weber, J.N. 2000. 'Comparison of a point mutation assay with a line probe assay for the detection of the major mutations in the HIV-1 reverse transcriptase gene associated with reduced susceptibility to nucleoside analogues', *Journal of Virological Methods*, 88 (2), 117–124.

Clarke, M. & Oxman, A. 2003. Cochrane Reviewers' Handbook 4.2.0, In: *The Cochrane Library*, Update Software, Oxford.

Clevenbergh, P., Durant, J., Halfon, P., del Giudice, P., Mondain, V., Montagne, N., Schapiro, J.M., Boucher, C.A. & Dellamonica, P. 2000. 'Persisting long-term benefit of genotype-guided treatment for HIV-infected patients failing HAART. The Viradapt Study: week 48 follow-up', *Antiviral Therapy*, 5 (1), 65–70.

Cochrane Methods Working Group. 1996. *Systematic Review of Screening and Diagnostic Tests: Recommended Methods.* [Internet]. Available from: http://www.cochrane.org/docs/sadtdoc1.htm [Accessed 4 January, 2002]

Corzillius, M., Muhlberger, N., Sroczynski, G., Jaeger, H., Wasem, J. & Siebert, U. 2004. 'Cost effectiveness analysis of routine use of genotypic antiretroviral resistance testing after failure of antiretroviral treatment for HIV', *Antiviral Therapy*, 9, 27–36.

Deeks, S.G., Grant, R.M., Wrin, T., Paxinos, E., Liegler, T., Hoh, R., Martin, J. N. & Petropoulos, C. J. 2003. 'Persistence of drug-resistant HIV-1 after a structured treatment interruption and its impact on treatment response', *AIDS*, 7, 361–370.

Department of Health. 2000-01, *Australian Refined Diagnosis Related Groups* [Internet]. Department of Health, Available from: [Accessed 25 June 2004].

Department of Health and Aging. 2004. *Medicare Benefits Schedule* [Internet]. Available from: http://www.health.gov.au/medicare/index.htm [Accessed 26 June 2004].

Descamps, D., Calvez, V., Collin, G., Cecille, A., Apetrei, C., Damond, F., Katlama, C., Matheron, S., Huraux, J.M. & Brun-Vezinet, F. 1998. 'Line probe assay for detection of human immunodeficiency virus type 1 mutations conferring resistance to nucleoside inhibitors of reverse transcriptase: comparison with sequence analysis', *Journal of Clinical Microbiology*, 36 (7), 2143–2145.

Durant, J., Clevenbergh, P., Halfon, P., Delgiudice, P., Porsin, S., Simonet, P., Montagne, N., Boucher, C.A., Schapiro, J.M. & Dellamonica, P. 1999. 'Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial.[see comment][erratum appears in *The Lancet* 1999 Sep 25;354(9184):1128]', *The Lancet*, 353 (9171), 2195–2199.

Egger, M., May, M., Chene, G. Phillips, A. N.,Ledergerber, B., Dabis, F.,Costagliola, D., D'Arminio Monforte, de Wolf, F., Reiss, P., Lundgren, J. D., Justice, A. C., Staszewski, S., Leport, C., Hogg, R. S., Sabin, C. A., Gill, M. J., Salzberger, B. & Sterne, J. A. 2002. 'Prognosis of HIV-1 infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies', *The Lancet*, 360 (9327), 119–129.

Frenkel, L.M., Wagner, L.E., 2nd, Atwood, S.M., Cummins, T.J. & Dewhurst, S. 1995. 'Specific, sensitive, and rapid assay for human immunodeficiency virus type 1 pol mutations associated with resistance to zidovudine and didanosine', *Journal of Clinical Microbiology*, 33 (2), 342–347.

Gallant, J.E. 2000. 'Strategies for long-term success in the treatment of HIV infection', *Journal of the American Medical Association*, 283 (10), 1329–1334.

Grabar, S., Le Moing, V., Goujard, C., Leport, C., Kazatchkine, M.D., Costagliola, D. & Weiss, L. 2000. 'Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy.[see comment]', *Annals of Internal Medicine*, 133 (6), 401–410.

Hirsch, M.S., Brun-Vezinet, F., Clotet, B., Conway, B., Kuritzkes, D.R., D'Aquila, R.T., Demeter, L.M., Hammer, S.M., Johnson, V.A., Loveday, C., Mellors, J.W., Jacobsen, D.M. & Richman, D.D. 2003. 'Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel', *Clinical infectious diseases,* 37 (1), 113–128.

HIV Model of Care Working Party. 1998. *Model of Care for HIV infections in adults*. Clinical Trials and Treatment Advisory Committee (CTTAC), Canberra.

Hoffman, C. & Kamps, B.S. 2003. *HIV medicine* [Internet]. Flying Publisher, Available from: www.HIVMedicine.com/pdf/hivmedicine2003.pdf
[Accessed 19th December 2003].

Hogg, R., Yip, B., Chan, K. Wood, E., Craib, K. J., O'Shaughnessy, M. V. & Montaner, J. S. 2001. 'Rates of Disease Progression by Baseline CD4 Cell Count and Viral Load After Initiating Triple-Drug Therapy', *Journal of the American Medical Association*, 286 (20), 2568–2577.

Hoy, J. & Lewin, S. (Eds.) 2004. *HIV Management in Australasia: a guide for clinical care*. Australasian Society for HIV Medicine Inc, Adelaide.

Hughes, M.D., Johnson, V.A., Hirsch, M.S., Bremer, J.W., Elbeik, T., Erice, A., Kuritzkes, D.R., Scott, W.A., Spector, S.A., Basgoz, N., Fischl, M.A. & D'Aquila, R.T. 1997. 'Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. ACTG 241 Protocol Virology Substudy Team'. *Annals of Internal Medicine*, 126 (12), 929–938.

Hurley, S., Kaldor, J., Carlin, J., Gardiner, S., Gardiner, S. Chondros, P., Hoy, J., Spelman, D., Spicer, W. J., Wraight, H. & Evans, D. 1995. 'The usage and costs of health services for HIV infection in Australia', *AIDS*, 9, 777–785.

Jaeschke, R., Guyatt, G. & Sackett, D. 1994a. 'Users' guide to the medical literature III: how to use an article about a diagnostic test. A. Are the results of the study valid?', *Journal of the American Medical Association*, 271, 389–391.

Jaeschke, R., Guyatt, G.H. & Sackett, D.L. 1994b. 'Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group', *Journal of the American Medical Association*, 271 (9), 703–707.

Jaggy, C., von Overbeck, J., Ledergerber, B. Schwarz C, Egger, M., Rickenbach, M., Furrer, H.J., Telenti, A, Battegay, M., Flepp, M., Vernazza. P., Bernasconi, E. & Hirschel, B. 2003. 'Mortality in the Swiss HIV Cohort Study (SHCS) and the Swiss general population', *The Lancet*, 362 (9387) 877–878.

Kaufmann, G.R., Suzuki, K., Cunningham, P., Mukaide, M., Kondo, M., Imai, M., Zaunders, J. & Cooper, D.A. 2001. 'Impact of HIV type 1 protease, reverse transcriptase, cleavage site, and p6 mutations on the virological response to quadruple therapy with saquinavir, ritonavir, and two nucleoside analogs', *AIDS Research & Human Retroviruses*, 17 (6), 487–497.

Knottnerus, J.A. & Muris, J.W. 2002, 'Assessment of the accuracy of diagnostic tests: the cross-sectional study', In: Knottnerus JA. *The evidence base of clinical diagnosis*, BMJ Books, London, 39–60.

Knottnerus, J.A & van Weel, C. 2002, 'General introduction: evaluation of diagnostic procedures', In: Knottnerus JA. *The evidence base of clinical diagnosis*, BMJ Books, London, 1-18.

Land, S. & Gizzarelli, L. 2003. *HIV-1 genotypic anti-retroviral testing external quality assessment scheme: report on testing GART 2003 Panel* [Internet]. Available from: www.nrl.gov.au/dir185/NRLAttach.nsf/SImages/GART2003report.pdf/$file/GART2003report.pdf [Accessed 7 May 2004].

Lauria, F.N. & Angeletti, C. 2003. 'Cost-effectiveness analysis of using antiretroviral drug resistance testing', *Scandinavian Journal of Infectious Diseases Supplement*, Dec; 35 Suppl 106 54–57.

Lawrence, et al, Mayers, D., Hullsiek, K., Collins, G., Abrams, D. Reisler, R. B., Crane, L. R., Schmetter, B. S., Dionne, T. J., Saldanha, J. M., Jones, M. C. & Baxter, J. D. 2003. 'Structured Treatment Interruption in Patients with Multidrug-Resistant Human Immunodeficiency virus'. *New England Journal of Medicine*, 349, 837–846.

Ledergerber, B., Egger, M., Opravil, M. L., Telenti, A., Hirschel, B., Battegay, M., Vernazza, P., Sudre, P., Flepp, M., Furrer, H., Francioli, P. & Weber R. 1999. 'Clinical Progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study', *The Lancet*, 353, 863–868.

Leitner, T. 1996. Genetic subtypes of HIV. [Internet] Available from: http://www.hiv.lanl.gov/content/hiv-db/COMPENDIUM/1996/PART-III/3.pdf [Accessed 11 November 2003].

Lijmer, J.G, Mol, B.W., Heisterkamp, S., Bonsel, G.J., Prins, M.H., van der Meulen, J.H. & Bossuyt, P.M. 1999. 'Empirical evidence of design-related bias in studies of diagnostic tests', *Journal of the American Medical Association*, 282 (11), 1061–1066.

Lopez-Galindez, C., Rojas, J.M., Najera, R., Richman, D.D. & Perucho, M. 1991. 'Characterization of genetic variation and 3'-azido-3'-deoxythymidine- resistance mutations of human immunodeficiency virus by the RNase A mismatch cleavage method', *Proceedings of the National Academy of Sciences of the United States of America*, 88 (10), 4280–4284.

Mazzotta, F., Lo Caputo, S., Torti, C., Tinelli, C., Pierotti, P., Castelli, F., Lazzarin, A., Angarano, G., Maserati, R., Gianotti, N., Ladisa, N., Quiros-Roldan, E., Rinehart, A.R., Carosi, G. & Genotipo-Fenotipo di Resistenza Group of the Italian Management Standardizzato di Terapia Antiretrovirale C. 2003. 'Real versus virtual phenotype to guide treatment in heavily pretreated patients: 48-week follow-up of the Genotipo-Fenotipo di Resistenza (GenPheRex) trial', *Journal of Acquired Immune Deficiency Syndromes*, 32 (3), 268–280.

Mellors, J.W., Munoz, A., Giorgi, J.V., Margolick, J.B., Tassoni, C.J., Gupta, P., Kingsley, L.A., Todd, J.A., Saah, A.J., Detels, R., Phair, J.P. & Rinaldo, C.R., Jr. 1997. 'Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection'. *Annals of Internal Medicine*, 126 (12), 946–954.

Meynard, J.L., Vray, M., Morand-Joubert, L., Race, E., Descamps, D., Peytavin, G., Matheron, S., Lamotte, C., Guiramand, S., Costagliola, D., Brun-Vezinet, F., Clavel, F., Girard, P.M. & Narval Trial G. 2002. 'Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial', *AIDS*, 16 (5), 727–736.

Model of Care for HIV Infection in Adults HIV/AIDS Clinical Trials and Treatments Advisory Committee if the Australian National Council on AIDS and Related Diseases, 1998. [Internet] Available from: http://www.health.gov.au/pubhlth/ancard/pdf/model.pdf [Accessed 11 March 2004]

National Centre in HIV Epidemiology and Clinical Research (NCHECR). 2003. *Australian HIV Observational Database: biannual report* [Internet]. Sydney. Available from: www.med.unsw.edu.au/ncher [Accessed 12 November 2003]

National Centre in HIV Epidemiology and Clinical Research (NCHECR). 2003. *HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia. Annual Surveillance Report.* [Internet] Available from: http://www.med.unsw.edu.au/nchecr [Accessed 12 November 2003].

NHMRC. 1999. *A guide to the development, implementation and evaluation of clinical practice guidelines,* National Health and Medical Research Council, Canberra.

NHMRC. 2000. *How to use the evidence: assessment and application of scientific evidence,* National Health and Medical Research Council, Canberra.

NHS Centre for Reviews and Dissemination 2001. *Undertaking systematic review of research on effectiveness. CRD's guidance for those carrying out or commissioning reviews* [Internet]. NHS Centre for Reviews and Dissemination Available from: http://www.york.ac.uk/inst/crd/report4.htm [Accessed 23 June 2003].

O'Brien, W. & Hartigan, P. 1997. 'Changes in plasma HIV RNA levels and CD4 lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure', *Annals of Internal Medicine*, 126 (12), 939–945.

Pellegrin, I., Caumont, A., Garrigue, I., Merel, P., Schrive, M.H., Fleury, H., Dupon, M., Pellegrin, J.L. & Ragnaud, J.M. 2003. 'Predictive value of provirus load and DNA human immunodeficiency virus genotype for successful abacavir-based simplified therapy', *Journal of Infectious Diseases*, 187 (1), 38–46.

Perez, E.E., Rose, S.L., Peyser, B., Lamers, S.L., Burkhardt, B., Dunn, B.M., Hutson, A.D., Sleasman, J.W. & Goodenow, M.M. 2001. 'Human immunodeficiency virus type 1 protease genotype predicts immune and viral responses to combination therapy with protease inhibitors (PIs) in PI-naive patients', *Journal of Infectious Diseases*, 183 (4), 579–588.

Perez-Elias, M.J., Garcia-Arota, I., Munoz, V., Santos, I., Sanz, J., Abraira, V., Arribas, J.R., Gonzalez, J., Moreno, A., Dronda, F., Antela, A., Pumares, M., Marti-Belda, P., Casado, J.L., Geijos, P., Moreno, S. & Realvirfen study g. 2003. 'Phenotype or virtual phenotype for choosing antiretroviral therapy after failure: a prospective, randomized study', *Antiviral Therapy*, 8 (6), 577–584.

Queensland Management Guidelines for the Detection and Treatment of SexuallyTransmissible Disease and Genital Infections. Version II, December 2003. [Internet] Available from: http://www.health.qld.gov.au/sexhealth/documents/22040_HIV_AIDS.pdf [Accessed 11 March 2004]

Richman, D.D., Guatelli, J.C., Grimes, J., Tsiatis, A. & Gingeras, T. 1991. 'Detection of mutations associated with zidovudine resistance in human immunodeficiency virus by use of the polymerase chain reaction', *Journal of Infectious Diseases*, 164 (6), 1075–1081.

Robbins, G., Gruttola, V., Shafer, R.., Smeaton, L., Snyder, S., Robbins, G. K., De Gruttola, V., Shafer, R. W., Smeaton, L. M., Snyder, S. W., Pettinelli, C., Dube, M. P., Fischl, M. A., Pollard, R. B., Delapenha, R., Gedeon, L., van der Horst, C., Murphy, R. L., Becker, M., I. D'Aquila, R. T., Vella, S. Merigan, T. C. & Hirsch, M. S. 2003. 'Comparison of sequential three-drug regimens as initial therapy for HIV-1 infection', *New England Journal of Medicine*, 349 (24), 2293–2203.

Sackett, D., Strauss, S., Richardson, W., Rosenberg, W. & Haynes, R. 2000, 'Diagnosis and screening.' In: *Evidence-Based Medicine: How to Practice and Teach EBM*, Churchill Livingstone, Edinburgh, 67–93.

Sanger, F., Nicklen, S. & Coulson, A.R. 1977. 'DNA sequencing with chain-terminating inhibitors', *Proceedings of the National Academy of Sciences of the United States of America*, 74 (12), 5463–5467.

Sayer, D.C., Land, S., Gizzarelli, L., French, M., Hales, G., Emery, S., Christiansen, F.T. & Dax, E.M. 2003. 'Quality assessment program for genotypic antiretroviral testing improves detection of drug resistance mutations', *Journal of Clinical Microbiology*, 41 (1), 227–236.

Sax, P.E. R.I., Losina, E., Weinstein, M.C., Cohen, C.J. & Freedberg, K.A. 2002. 'Should resistance testing be done in antiretroviral naïve patients? A cost-effectiveness analysis', In: World AIDS, Barcelona.

Schuurman, R., Brambilla, D., de Groot, T., Huang, D., Land, S., Bremer, J., Benders, I. & Boucher, C.A. 2002. 'Underestimation of HIV type 1 drug resistance mutations: results from the ENVA-2 genotyping proficiency program', *AIDS Research & Human Retroviruses*, 18 (4), 243–248.

Setti, M., Bruzzone, B., Ansaldi, F., Borrelli, P., Indiveri, F. & Icardi, G. 2001. 'Identification of key mutations in HIV reverse transcriptase gene can influence the clinical outcome of HAART', *Journal of Medical Virology*, 64 (3), 199–206.

Schulz KF, Chalmers I, Hayes RJ and Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. Jama.1995. 273. 5. 408-12.

Shearer, W.T., Quinn, T.C., LaRussa, P., Lew, J.F., Mofenson, L., Almy, S., Rich, K., Handelsman, E., Diaz, C., Pagano, M., Smeriglio, V. & Kalish, L.A. 1997. 'Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group', *New England Journal of Medicine*, 336 (19), 1337–1342.

Stuyver, L., Wyseur, A., Rombout, A., Louwagie, J., Scarcez, T., Verhofstede, C., Rimland, D., Schinazi, R.F. & Rossau, R. 1997. 'Line probe assay for rapid detection of drug-selected mutations in the human immunodeficiency virus type 1 reverse transcriptase gene', *Antimicrobial Agents & Chemotherapy*, 41 (2), 284–291.

Tengs, T. & Lin, T. 2002. 'A Meta-Analysis of Utility Estimates of HIV/AIDS', *Medical Decision Making*, 22, 475–481.

The EuroGUidelines Group for HIV resistance. 2001. 'Clinical and laboratory guidelines for the use of HIV-1 drug resistance testing as part of treatment management: recommendations for the European setting', *AIDS*, 15 (3), 309–320.

Torre, D. & Tambini, R. 2002. 'Antiretroviral drug resistance testing in patients with HIV-1 infection: a meta-analysis study', *HIV Clinical Trials*, 3 (1), 1–8.

Tural, C., Ruiz, L., Holtzer, C., Schapiro, J., Viciana, P., Gonzalez, J., Domingo, P., Boucher, C., Rey-Joly, C., Clotet, B. & Havana Study G. 2002. 'Clinical utility of HIV-1 genotyping and expert advice: the Havana trial', *AIDS*, 16 (2), 209–218.

Van Laethem, K., De Luca, A., Antinori, A., Cingolani, A., Perna, C.F. & Vandamme, A.M. 2002. 'A genotypic drug resistance interpretation algorithm that significantly predicts therapy response in HIV-1-infected patients', *Antiviral Therapy*, 7 (2), 123–129.

Van Vaerenbergh, K., De Geest, S., Derdelinckx, I., Bobbaers, H., Carbonez, A., Deschamps, A., De Graeve, V., De Saar, V., Ceunen, H., De Smet, K., Maes, B., Peetermans, W., Schrooten, Y., Desmyter, J., De Clercq, E., Van Ranst, M., Van Wijngaerden, E. & Vandamme, A.M. 2002. 'A combination of poor adherence and a low baseline susceptibility score is highly predictive for HAART failure', *Antiviral Chemistry & Chemotherapy*, 13 (4), 231–240.

Van Vaerenbergh, K., Van Laethem, K., Van Wijngaerden, E., Schmit, J.C., Schneider, F., Ruiz, L., Clotet, B., Verhofstede, C., Van Wanzeele, F., Muyldermans, G., Simons, P., Stuyver, L., Hermans, P., Evans, C., De Clercq, E., Desmyter, J. & Vandamme, A.M. 2000. 'Baseline HIV type 1 genotypic resistance to a newly added nucleoside analog is predictive of virologic failure of the new therapy', *AIDS Research & Human Retroviruses*, 16 (6), 529–537.

Venturi, G., Romano, L., Catucci, M., Riccio, M.L., De Milito, A., Gonnelli, A., Rubino, M., Valensin, P.E. & Zazzi, M. 1999. 'Genotypic resistance to zidovudine as a predictor of failure of subsequent therapy with human immunodeficiency virus type-1 nucleoside reverse-transcriptase inhibitors', *European Journal of Clinical Microbiology & Infectious Diseases*, 18 (4), 274–282.

Vray, M., Meynard, J.L., Dalban, C., Morand-Joubert, L., Clavel, F., Brun-Vezinet, F., Peytavin, G., Costagliola, D., Girard, P.M. & Narval Trial G. 2003. 'Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1-infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS 088)', *Antiviral Therapy*, 8 (5), 427–434.

Weinstein, M.C., Goldie, S.F., Cohen, C., Weinstein M.C., Goldie, S. F., Cohen, C., Losina, E., Zhang, H., Kimmel, A. D. & Freedberg, K.A. 1999. ' Resistance testing to guide the choice of second-line antiretroviral therapy in HIV: Clinical impact and cost-effectiveness', 21st Annual Meeting of the Society for Medical Decision Making 1999, Reno, Nevada.'

Weinstein, M.C., Goldie, S.J., Losina, E. Cohen, C., Baxter, J.D., Zhang, H. Kimmel, A. D. & Freedberg, K.A. 2001. 'Use of genotypic resistance testing to guide HIV therapy: Clinical impact and cost-effectiveness', *Annals of Internal Medicine*, 134, 440–450.