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Antiviral resistance in clinical practice

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1. The Herpesviruses

1.1. Herpes simplex virus (HSV)

Herpes simplex virus is a significant human pathogen that causes a broad spectrum of disease. There are an estimated 40 to 60 million persons with genital herpes in the United States and an incidence of approximately 500,000 new cases per year. Eye infections caused by HSV are the second leading cause of corneal blindness in the United States. Neonatal HSV infections occur in approximately one in 2500 deliveries. Herpes simplex virus infections in the immunocompromised host are common and can be very severe; this is the setting in which antiviral resistance develops most frequently (Ljungman et al., 1990; Gray et al., 1989; Englund et al., 1990).

Acyclovir (ACV) was approved in the United States in 1982. The first report of ACV resistance of HSV in a human being also occurred in 1982 (Crumpacker et al., 1982). This was followed by other isolated cases of ACV-resistant HSV mutants between 1982 and 1989 (Barry et al., 1985; Burns et al., 1982; Sibrack et al., 1982).

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In 1989, a report was published of 12 patients with ACV-resistant HSV isolates, foreshadowing the increasing recognition of resistant virus among immunocompromised individuals (Ehrlich et al., 1989). The early isolated cases of resistance were all found in the setting of severely immunocompromised patients. The mechanisms of ACV-resistance among the clinical isolates were similar to those seen in resistant mutants selected in vitro. Most of the clinical mutants were defined as thymidine kinase-negative (TK^-) isolates (Oliver et al., 1989; Hill et al., 1991). These TK viruses most frequently produced a truncated, nonfunctional TK enzyme (Chatis and Crumpacker, 1991). However, the sites of mutation within the TK gene which produce the truncated enzymes have proven to be very heterogeneous. Characterization of additional clinical specimens utilizing sensitive and informative assays will provide further information regarding the full spectrum of clinical mutants. Thymidine kinase-negative mutants have been found as minority subpopulations (0.001–0.0001%) of wild-type clinical isolates, suggesting that exposure to ACV provides a selective pressure for the emergence of drug-resistant strains as the predominant virus (Parris and Harrington, 1982). In addition, point mutations resulting in amino acid substitutions have also been described in TK^- clinical isolates. Isolates with point mutations which confer an altered TK phenotype (TKa) have been isolated from patients as well; the TKa enzyme has altered substrate specificity, such that it can phosphorylate thymidine but not ACV. Mutations in the second viral enzyme targeted by ACV, DNA polymerase, can also confer ACV resistance. These mutants exhibit altered function in the presence of ACV-triphosphate (KNopf et al., 1981). The DNA polymerase mutants occur clinically much less frequently than TK mutants (Collins et al., 1989; Sacks et al., 1989). It is likely that TK-partial (TKp) isolates will also be found to cause clinical disease as methods of detection of low amounts of TK are improved. It is now clear that low TK producing HSV-2 mutants cause virulent infections in animal models (Ehrlich et al., 1989).

The increasing incidence of ACV-resistant HSV among clinical isolates is a direct consequence of the number of patients infected with the human immunodeficiency virus (HIV) (Erlich et al., 1989). All twelve of the patients reported in 1989 to be infected with ACV-resistant HSV had acquired immunodeficiency syndrome (AIDS). All had been on oral or intravenous ACV therapy, and most had perirectal lesions. These twelve resistant isolates were phenotypically characterized as TK-deficient (the assays were not sensitive enough to distinguish whether they were TK^- or TKp). While the isolates were resistant to both ACV and ganciclovir (GCV), they remained susceptible in vitro to foscarnet (PFA) and vidarabine.

Acyclovir-resistant viruses have been reported to cause meningitis, esophagitis, and pneumonia in immunocompromised patients (Ljungman et al., 1990; Sacks et al., 1989). Patients with documented ACV-resistant virus have demonstrated healing of mucocutaneous lesions following a change of therapy to PFA (Chatis et al., 1989). However, treatment with vidarabine has not been found to be beneficial

even when the isolate is susceptible to both PFA and vidarabine in vitro (Safrin et al., 1991b). In Safrin's study, those patients who failed vidarabine subsequently had healing of lesions upon institution of PFA. This study established that ACV-resistant HSV mutants were clinically significant. Options for treating HSV strains that are resistant to both ACV and PFA are few and are generally unsatisfactory.

Patients frequently have recurrence of disease with an ACV-sensitive virus following resolution of lesions caused by ACV-resistant virus. This suggests that the original (sensitive) virus can reactivate from the ganglion following clearance of mucocutaneous infection caused by resistant virus. Recurrence of resistant virus has also been documented, however.

Herpes simplex virus isolates demonstrate ACV resistance in approximately 5% of transplant patients and patients with AIDS (Englund et al., 1990; Chatis and Crumpacker, 1992). Among immunocompetent adults, however, only one case of recurrent genital HSV disease caused by an ACV-resistant virus has been reported; this isolate was found to have a TKa phenotype (Kost et al., 1993). In addition, there has been only a single report of a resistant isolate causing disease in a neonate (Nyquist et al., 1994). Foscarnet-resistant clinical isolates have been reported in HIV infected patients (Safrin et al., 1994); mechanisms of resistance are being characterized at this time. With increasing use of antiviral drugs and careful monitoring of resistant HSV, an enhanced understanding of the full clinical potential of antiviral drug resistance in HSV should emerge.

It is reasonable to assume that a resistant isolate is present if lesions have not healed following 5–10 days of adequate ACV therapy. Depending upon the overall clinical status of the patient, empiric initiation of PFA therapy and discontinuation of ACV may be warranted in such a case while awaiting laboratory confirmation of resistance (IC_{50} >2–3 μg/ml) (Balfour et al., 1994). Lesions recurring following a first episode of resistant viral disease are usually caused by ACV-sensitive virus. As such, it is reasonable to initiate ACV therapy upon recurrence of HSV disease in a patient with a single occurrence of an ACV-resistant isolate. If ACV-resistant lesions recur for a second time, use of PFA to treat the second outbreak and all additional outbreaks is warranted, as the likelihood is high that ACV-resistant isolates are present.

1.2. Cytomegalovirus (CMV)

Table 1
Ganciclovir susceptibility ranges for cytomegalovirus isolates

Drug susceptibility	IC_{50} value (μM)
Sensitive	≥5.0
Partial resistance	6.0–12.0
Resistant	>12.0

Cytomegalovirus causes a broad range of clinical disease. It is the major cause of congenital viral infection in developed countries, resulting in serious sequelae in an estimated 7,600 infants each year in the United States. Among immunocompromised persons, CMV is also an important pathogen, causing severe disease in persons with AIDS and in recipients of organ transplants. Immunocompromised patients frequently receive prolonged therapy with either GCV or PFA as CMV prophylaxis or treatment. With prolonged therapy, the likelihood of developing clinical resistance to antiviral therapy increases, with associated disease progression and recovery of resistant strains of CMV. Ganciclovir susceptibility ranges for cytomegalovirus isolates are shown in Table 1.

Clinical specimens of CMV are composed of mixed collections of isolates, all of which have differing susceptibilities to GCV. As the proportion of isolates that are relatively less susceptible to GCV increases in the viral population, the IC_{50} for the entire population increases. Such an increase in resistance (that is, an increase in IC_{50}) can be expected to occur under the selective pressure of GCV exposure. As a clinical correlate, increasing IC_{50}s to GCV correspond with increasing levels of CMV antigenemia and CMV DNA as detected by the polymerase chain reaction (PCR) or branched DNA (bDNA) assays. As a result, these tools can be used as surrogate markers for resistance when cultures remain negative.

The prevalence of antiviral resistance has been evaluated in AIDS patients receiving GCV for CMV retinitis (Drew et al., 1991). Of 72 AIDS patients receiving GCV, no GCV-resistant CMV isolates were detected during the first three months of therapy. After three months of therapy, five of 13 (38%) tested isolates demonstrated GCV resistance, as defined by an IC_{50} in excess of four times the mean for pretherapy isolates; an additional three isolates demonstrated intermediate susceptibility. Following five or more months of GCV therapy, only 20% of patients' urine cultures were positive; therefore, it was assumed that most of the patients remained infected by presumed drug-sensitive strains. Given the low rate of viral recovery, the overall prevalence of resistant strains in patients with AIDS taking GCV for more than three months was estimated to be 7.6% (38% of 20%).

Clinical events in patients excreting GCV-resistant CMV isolates suggest that these strains are virulent. In studies of patients receiving GCV for treatment of CMV retinitis, recovery of resistant virus was associated with progression of retinal disease (Erice et al., 1989; Jacobson et al., 1991). However, clinical deterioration has also been noted in patients who excreted sensitive strains. Therefore, the clinical significance of detection of CMV strains with high level or intermediate resistance is unclear. Cytomegalovirus cultures in patients with CMV retinitis are frequently negative. Among patients diagnosed with their first episode of CMV retinitis, only 70% have positive cultures. Of those positive cultures, almost all isolates are susceptible to GCV. With first progression of retinitis, cultures are rarely positive. In at least some cases, these progressions may be attributable to poor compliance or inadequate dosage of drug. Moreover, the eye provides a protected

anatomical site for viral replication and drug delivery, allowing for focal heterogeneity among the CMV isolates present in a host. Resistant isolates may be present at one site (i.e. the eye) but not another (i.e. the blood or urine).

Preliminary data suggest that GCV-resistant subpopulations exist within GCV-sensitive isolates. If this is true, GCV resistance at the clinical level could be a selective and progressive phenomenon, as ACV resistance is for HSV. However, further analysis of CMV isolates from GCV-naive patients is needed for confirmation. Following cessation of drug, the GCV-resistant mutants may or may not revert to GCV-susceptibility. Use of ACV does not appear to induce GCV resistance in AIDS patients, even with prolonged exposure.

Management of immunocompromised patients with CMV disease can be difficult due to the infrequency with which CMV cultures are positive even when GCV-resistant virus is present. Pending development of more rapid susceptibility tests, the quantitative amounts of antigenemia, PCR-amplified DNA, or bDNA can be determined serially to evaluate efficacy of treatment. If levels of these surrogate markers do not decrease, the possibility of emergence of antiviral resistance should be entertained. One should assume that an isolate is resistant if cultures of blood or urine are positive in a patient who is failing GCV therapy, as about 40% of excreters of CMV who have been on GCV therapy for three or more months shed resistant virus. Among patients with progression of CMV disease while on maintenance GCV therapy, an increase in the GCV dose to induction levels is reasonable. If disease progression should continue, an empiric change from GCV to PFA may be warranted, even if cultures remain negative (Jacobson et al., 1991). The role of intravitreal therapy is being evaluated currently in clinical trials; in these studies, GCV or PFA may be repeatedly injected into the eye, potentially providing a means of overcoming resistant viral isolates locally. Preliminary studies of combination antiviral regimens have found them to be less toxic than anticipated; however, their efficacy is still unknown. The impact of the recently approved oral formulation of GCV upon the emergence of resistant CMV is a potentially serious concern and merits systematic investigation.

1.3. Varicella-zoster virus (VZV)

To date, all patients with ACV-resistant VZV infections have had impairment of their immune systems due to HIV infection and have been on prolonged oral ACV therapy (Jacobson et al., 1990; Hoppenjans et al., 1990; Pahwa et al., 1988; Linnemann et al., 1990). All individuals have had CD4 counts of less than 100, and most have had CD4 counts below 25. In an evaluation of ACV susceptibilities, 49 VZV isolates were recovered from 33 patients with AIDS who had apparent clinical ACV failure. Of these 49 isolates, 25 were ACV-sensitive and 24 were ACV-resistant. Phenotypic analysis of the 24 resistant strains found that 22 of them were TK mutants, with 17 being TK and 5 being TKa. In addition, two DNA polymerase

variants were identified. One of these was a VZV strain resistant to both PFA and ACV that had been isolated from a patient following PFA therapy. The second DNA polymerase mutant identified in the study was among a mixed isolate that also contained TK virus; this ACV-resistant virus was recovered after eight months of oral or intravenous ACV therapy. Ten patients shed only ACV-sensitive virus.

Drug-resistant VZV in immunocompromised patients presents as chronic varicella or zoster, though it lacks the classic features of either infection. Multiple dermatomes are often involved, but the infection does not disseminate to visceral organs. The course is indolent, with slow progression of widely scattered atypical lesions which can be hyperkeratotic or ulcerative. Isolates of virus from treatment failures may prove susceptible to ACV (IC_{50} <2 μg/ml) or exhibit in vitro resistance (IC_{50} >10 μg/ml). Regardless of the IC_{50}, most of these clinical failures seem to respond to intravenous PFA. Foscarnet resistance has been reported as well (Safrin et al., 1991a). Some VZV isolates with in vitro resistance to PFA have responded in vivo to PFA therapy. In addition, individual cases have responded to continuous infusion ACV. Following apparent resolution of the lesions with PFA treatment, most recur quickly; recurrent viruses are often sensitive to ACV. Isolation of virus from lesions is difficult because the lesions are only rarely vesicular and contain low levels of virus; excisional biopsy is often required to culture the resistant VZV isolate.

The paramount importance of a healthy immune system in the containment and clearance of resistant VZV is apparent from the fact that the resistant virus has only caused disease in immunocompromised individuals. The immune status of most patients is adequate to clear resistant virus. Cancer patients and bone marrow transplant (BMT) recipients have not been afflicted with resistant VZV infections to date, perhaps because their immunity is impaired severely for only a short period of time. Post-BMT zoster usually does not occur until several months after the BMT, by which time engraftment has taken place and more immune competence presumably exists; thus, viral replication and spread is limited by the more fully reconstituted immune system.

Since immunocompetence is usually sufficient to resolve VZV infections even in the absence of treatment, zoster is a common and early marker for immune attrition in the setting of HIV infection. Because VZV does not replicate to high titers, infections are often characterized by limited viral burden. With less virus present from which resistant clones can be selected, a reduced opportunity exists for resistant clones to emerge under the selected pressure of therapy. This also reduces the likelihood for resistance to emerge during "salvage" therapy with PFA. It may be reasonable, therefore, to treat VZV infections in immunocompromised patients promptly and with higher or more sustained doses of antiviral drugs to reduce the potential for resistance to emerge. As resistance appears to emerge quite suddenly in immunocompromised patients, it is possible that a very small proportion of TK-

positive VZV can confer overall susceptibility. By the time clinical resistance occurs, between 90% and 100% of the purified plaques in an isolate are already resistant to ACV.

One should consider the possibility of emergence of ACV-resistant VZV if cutaneous lesions continue to appear and progress after 5–10 days of IV ACV, regardless of the isolate's in vitro susceptibility. Since the infection is so atypical in the immunocompromised host, the diagnosis of VZV resistance should be confirmed by culture and in vitro susceptibility testing; excisional biopsy may be necessary to recover virus. While awaiting culture and susceptibility results, therapy can be changed empirically from ACV to PFA (Balfour et al., 1994). Treatment should be continued until healing of the lesions is complete, a process which may take 3–6 weeks or longer. Some experts suggest obtaining serial cultures while monitoring clinical response.

Following resolution of the initial episode of ACV-resistant VZV infection, the optimal approach to reinstitution of suppressive therapy is unknown. The fact that most of the first-round recurrences following PFA therapy are again ACV-sensitive suggests that reinstitution of suppressive ACV therapy is a reasonable approach; however, no consensus exists on this issue at this time.

Acyclovir resistance among VZV isolates is relatively uncommon given the number of immunocompromised patients receiving ACV. As such, application of broad strategies to prevent emergence of resistance may not be warranted at this time. Should the prevalence of ACV resistance increase, however, several possible strategies exist. One approach is to avoid ACV-suppression for any indication in patients with CD4 counts less than 100. Another strategy is to not treat the first occurrence of VZV because the course is so indolent, often not even being painful. Alternatively, one could utilize PFA as initial therapy of VZV infection in the severely immunocompromised host. Ideally, one would choose combination therapies including drugs with mechanisms of action different than that of ACV.

2. Influenza

As noted previously, resistance to amantadine and rimantadine in influenza A viruses is conferred by point mutations and corresponding amino acid substitutions in the membrane-spanning portion of the M2 protein. In animal models, resistant isolates emerge rapidly during treatment, are genetically stable, can be transmitted, and are pathogenic in contacts. Such resistant strains are capable of competing with wild-type viruses for transmission even in the absence of selective drug pressure. Recovery of drug-resistant viruses from patients receiving amantadine or rimantadine has been documented, and resistant virus has even been recovered in non-treated contacts, though this has been detected less frequently. In the detection of resistant variants, use of a breakpoint of 1.0 μg/ml in the ELISA assay is probably the most relevant; this is the concentration of either amantadine or rimantadine that

is achievable in the blood or respiratory secretions (Douglas, 1990).

Contemporary pandemic strains of influenza virus have been sensitive to amantadine and rimantadine (Hayden, 1994). Surveys of epidemic isolates have only infrequently found evidence of resistant isolates recovered from untreated patients (Belshe et al., 1989). During the 1990-1992 influenza seasons, for example, fewer than 1% of the clinical isolates recovered from untreated patients were resistant (Hayden, 1994). The potential for de novo resistance among influenza A viruses is best seen in the analysis of H1N1 isolates recovered from patients between 1933 and 1945: drug resistance associated with a position 31 mutation in M2 were reported, even though these isolates were obtained decades before the first use of amantadine (Hayden, 1994).

Emergence of resistance occurs rapidly during amantadine or rimantadine therapy (Belshe et al., 1989; Hayden et al., 1989). Up to 30% of drug-treated children and adults shed resistant virus within 3–5 days of initiation of therapy (Hayden et al., 1991). The clinical significance of shedding resistant virus for treated patients is unclear, as there is no rebound or prolongation in illness (Hayden et al., 1991). In addition, risk factors for the shedding of resistant variants have not been identified.

Both amantadine and rimantadine appear to be effective for postexposure prophylaxis in families when index cases are not treated (Hayden et al., 1989). Trials in which the index case is also treated have found only minimal efficacy of postexposure prophylaxis, possibly due to spread within the household of resistant viruses. However, the effect of treatment of index cases on postexposure prophylactic efficacy has not been directly assessed in clinical trials (Hayden, 1994).

In nursing-home settings, amantadine treatment without isolation of treated index cases has been associated with multiple prophylaxis failures (Mast et al., 1991). Resistant viruses have been recovered from these prophylaxis failures. These resistant isolates appear to be as pathogenic as wild-type influenza virus in terms of illness severity (Hayden et al., 1989; Mast et al., 1991). Resistant viruses are usually shed for 5–7 days from initiation of therapy, although recovery of resistant viruses for weeks to months has been documented in several immunocompromised patients (F. Hayden, personal communication). Such patients can serve as a reservoir for spread of resistant viruses even in the absence of selective drug pressure.

Epidemiologic evaluation suggests that selection and apparent transmission of resistant isolates occur when close contact exists between treated ill persons and those receiving prophylaxis. Both the immune selection of antigenic variants and the disappearance of previously circulating strains reduce the likelihood of emergence of drug-resistant epidemic strains. If such resistant epidemic strains did arise, it is unknown if they would be biologically stable and capable of competing with wild-type viruses for transmission in the absence of selective drug pressure. In addition, the degree of selective drug pressure during an epidemic necessary to cause substantial transmission of resistant viruses at the community level is

unknown.

The rapid emergence of drug-resistant isolates is not a contraindication to the therapeutic use of amantadine or rimantadine. Vaccination, however, remains the primary means of prevention of influenza A disease. Additional precautions include avoiding contact between treated and susceptible high-risk patients. Since children transmit virus to household contacts at a higher rate than do adults (Hayden et al., 1989), one could consider not treating the index case when it is a child; rather, prophylaxis of household contacts should be the focus. In general, use of both treatment and postexposure prophylaxis in the same household should be avoided. When contacts are at high-risk for development of influenza disease (elderly, immunocompromised individuals, chronic pulmonary diseases, etc.), emphasis should be on their protection by placing them on prophylaxis and not treating the index case. When the index case is an adult and the contacts are not in a high-risk category, it is acceptable to treat the ill index case without providing prophylaxis to the contacts. This recommendation is based upon the relatively low risk of secondary transmission events (20–30% in the case of H3N2 subtype viruses) (Hayden, 1994). The combined use of vaccination and antiviral prophylaxis can provide protection to patients at risk of acquiring influenza infection. Ribavirin provides an alternative investigational agent which has been used in some severely ill patients who have deteriorated while on amantadine or rimantadine. In addition, the topical neuraminidase inhibitor GG167 may prove efficacious in the treatment or prevention of influenza disease in humans.

3. Picornaviruses

In clinical trials of the capsid-binding agents, three compounds have demonstrated activity in clinical studies. Phase II clinical trials of the antiviral compound WIN 54954 demonstrated it to be effective against a coxsackie virus A21 infection in volunteers. However, this compound was not efficacious when evaluated in three human rhinovirus challenge studies. These conflicting results are presumably due to differences in the amounts of compound reaching the site of infection in the study volunteers, since the in vitro susceptibilities were similar in these studies. Studies of another capsid-binding compound, R77975, have found that it must be given 6 times per day via the intranasal route in order to be efficacious. Efficacy was not seen when the drug was administered less frequently (i.e. 3 times per day). These results may be due to the rapid rate of metabolism of the compound; additionally, mucociliary clearance mechanisms may also play a role in the short half-life. Double-blind, placebo-controlled trials of the related antiviral compound, R61837, have shown it to be effective in suppressing colds in human volunteers challenged with rhinovirus type 9 (Al-Nakib et al., 1989). However, this agent is limited by a restricted spectrum of activity.

Evidence for the emergence of antiviral resistance during these clinical trials has

been quite variable. In the WIN 54954 phase II study noted above, none of the 23 volunteers evaluated developed resistant virus with treatment, including three volunteers who developed symptomatic infection while on the drug. In a study of the antiviral compound R61837, on the other hand, resistant virus was isolated from eight of the nine patients infected with rhinovirus type 9 and treated with R61837 (Dearden et al., 1989). Four of the nine subjects shed resistant virus prior to treatment; four of the nine developed resistant virus during therapy; and one of the nine had resistant virus become sensitive during the course of treatment. Only one of these isolates was highly resistant. Difficulty in separating drug from virus following isolation from patients may have artifactually biased these results. A third clinical study utilizing rhinovirus type 2 mutants that were both drug-dependent and drug resistant found that drug-dependent virus did not cause infection if the drug was not present.In the presence of drug, however, drug resistant virus caused infection and produced disease, although its infectivity was significantly reduced compared to wild-type virus (Yasin et al., 1990). The clinical importance of drug resistance to capsid-binding anti-rhinoviral agents, as well as soluble intercellular adhesion molecule-1 (ICAM-1) (Arruda et al., 1994), remains to be determined.

4. Human immunodeficiency virus-1 (HIV-1)

Antiviral resistance in HIV disease is more complex than in other human viral infections. The chronic and multifactorial nature of HIV disease complicates the establishment of the relative interactions between in vitro drug resistance, clinical drug failure, and disease progression. Clarification of these interactions is made more difficult by the fact that, with the extensive genetic variation of HIV, most mutations do not confer phenotypic resistance. In addition, the degree of phenotypic resistance for many drugs varies depending upon the combinations of mutations present within the reverse transcriptase (RT) gene. Furthermore, mixtures of virus with different drug resistance genotypes and phenotypes often exist simultaneously within a patient. Such focal heterogeneity may be related to differences in viral turnover rates in the different anatomic sites. The rate of emergence of antiviral resistance in HIV infection is a function of both duration of viral infection and disease state; the higher viral replicative rates occurring in patients with more advanced disease are associated with more rapid emergence of resistance (Richman et al., 1993). Zidovudine (AZT) resistance is particularly complicated, especially in comparison to non-nucleoside antiretrovirals (St. Clair et al., 1991; D'Aquila et al., 1995; Japour et al., 1995; Richman, 1993).

Zidovudine resistance was initially described in clinical isolates in 1989 (Larder et al., 1989). In these initial reports, approximately 30% of patients developed AZT-resistant mutants, with more than 100-fold increases in IC_{50} being detected after more than six months of therapy. These isolates remained sensitive to 2′,3′-dideoxycytosine (ddC), 2′,3′-dideoxydidehydrothymidine (d4T), and PFA. Among

patients with highly AZT-resistant isolates, there were no sudden increases in p24 antigen or abrupt clinical deteriorations. The clinical consequences of AZT resistance have been best addressed in the ACTG 116B/117 trial (D'Aquila et al., 1995). In this study, patients who had received at least 16 weeks of AZT treatment were randomized either to continued AZT treatment or to switch to 2',3'-dideoxyinosine (ddI). Although patients randomized to ddI experienced a lower rate of AIDS or death than those remaining on AZT, the results showed no evidence that duration of prior AZT therapy affected the benefit of switching from AZT to ddI. Furthermore, high-level AZT resistance was a poor prognostic sign, with patients who had high-level resistance at baseline having an almost three-fold increase in risk of HIV disease progression regardless of whether they remained on AZT or were switched to ddI. This suggests that the complexity of the relationship between the failing host and the population of AZT-resistant viruses is incompletely understood. In addition, those patients who were randomized to receive ddI experienced a 60% lower likelihood of disease progression, independent of whether their isolate was resistant to AZT. Because AZT-sensitive and -resistant viruses are equally susceptible to ddI, there is no obvious explanation for this observation. These results suggest that patients with advanced HIV-1 disease may benefit from switching from AZT to ddI monotherapy, regardless of whether high-level HIV-1 AZT resistance is present. In addition, laboratory assessment of AZT resistance is not necessary to decide when to switch monotherapy from AZT to ddI. Data for resistance to ddI, ddC, d4T, and combination therapies are limited at this time (St. Clair et al., 1991; Fitzgibbon et al., 1992; Gu et al., 1992, 1994; Gao et al., 1993; Slade et al., 1993; Schinazi et al., 1993; Tisdale et al., 1993; Lacey and Larder, 1994; Zhang et al., 1994).

While the ACTG 116B/117 study illustrates some of the confusing aspects of AZT's antiretroviral activities, additional peculiarities exist. For instance, CD4, p24, and RNA responses tend to return to baseline after several months of AZT therapy even in the absence of in vitro antiretroviral resistance. Also, wild-type and resistant RTs are enzymatically indistinguishable to inhibition by AZT triphosphate. In addition, many AZT resistance mutations are not located near the catalytic site of the RT enzyme. Among isolates with mutations at amino acid 215 (a site which is known to confer resistance to AZT but not other antiretroviral agents), the nucleoside analogues ddI and ddC and the non-nucleoside RT inhibitor nevirapine all reduce plasma levels to a lesser degree than with wild-type HIV isolates. Clearly, aspects of the interactions between the host and the AZT-resistant virus are incompletely understood. The ACTG 116B/117 study of resistance established for the first time that high level resistance to AZT predicts clinical disease progression and death, when controlling for other varibales (D'Aquila et al., 1995). This study establishes that resistance to AZT is highly clinically significant.

Resistance to non-nucleoside RT inhibitors (NNRTIs) is much more straightforward. Resistance to NNRTIs results in the loss of drug activity. Clinical trials have

confirmed that the rapid selection of NNRTI-resistant viruses seen in vitro also occurs in vivo (Saag et al., 1993; Richman et al., 1994). An initial reduction in the levels of the p24 antigen and an elevation of CD4 cell counts within 1 or 2 weeks is followed by a loss of these responses associated with the emergence of the drug-resistance phenotype. Genotypic analysis of these isolates reveals one or more mutations at residues 100, 103, 106, 108, 181, 188 and 190. Combination trials revealed that the emergence of NNRTI-resistant virus was not impeded by the concurrent administration of AZT. While the uniform and rapid emergence of resistance to NNRTIs might call into question their clinical utility, preliminary results suggest that these resistant isolates do not cause disease progression when there is no change in antiretroviral therapy (Havlir et al., 1995). Specifically, some patients given high doses of nevirapine (400 mg daily) and delaviridine have sustained elevations of CD4 cell counts and reductions in p24 antigenemia and RNA. The mechanism by which this sustained antiretroviral activity occurs despite drug resistance has not been elucidated. It is possible that plasma levels of drug could exceed the susceptibility of the resistant mutants. Alternatively, mutations conferring drug resistance could also attenuate the replicative capacity of the resistant virus, resulting in a less virulent infection. In addition, with the convergent combination of nevirapine and delaviridine (both of which are directed at a common target), mutations induced by one compound could constrain the evolutionary options for acquisition of resistance to the second compound or could increase susceptibility to the second drug.

Resistance to the protease inhibitors (PIs) is being recognized as these compounds move into clinical trials (Emini et al., 1994; Jacobsen et al., 1994). At this time, however, data on resistance to PIs are very limited. Characterization of resistance mechanisms from clinical HIV isolates undoubtedly will grow as these agents are used in additional clinical studies.

Transmission of drug-resistant viruses is increasingly being recognized (Erice et al., 1993). Transmission of AZT-resistant HIV isolates has been documented via sexual, percutaneous, and maternal/fetal routes. In addition, the prevalence of AZT-resistance at HIV seroconversion has been increasing in Europe and North America. Data for transmission of ddI, ddC, or multidrug resistance are not available at this time.

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Herpes simplex virus

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