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Molecular mechanisms of antiviral resistance

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1. The herpesviruses

1.1. Herpes simplex virus (HSV)

Upon initiation of infection by HSV, expression of viral genes occurs in a highly regulated fashion. Three classes of HSV genes have been identified and are classified by both the timing of and the requirements for their expression. These classes are alpha, beta, and gamma. Alpha, or immediate-early, genes are responsible for the regulated expression of the viral genome. They are transcribed in infected host cells in the absence of viral protein synthesis. The beta, or early, gene class requires functional alpha gene products for efficient expression. Beta gene products include proteins which are directly involved in viral DNA synthesis, as well as enzymes involved in nucleotide metabolism. As such, they provide excellent targets for antiviral agents. Examples of such early gene products include viral DNA polymerase and thymidine kinase (TK). While viral DNA polymerase is essential for replication of the HSV genome, viral TK is not essential for viral replication in cell culture. However, in vivo analyses demonstrate that HSV TK plays a pivotal role in

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virulence, pathogenicity, and the ability of HSV to reactivate from a latent state (Field and Wildy, 1978; Tenser and Dunstan, 1979; Coen et al., 1989a; Efstathiou et al., 1989; Jacobson et al., 1993).

The antiviral drug of choice in the treatment of HSV infections is acyclovir (ACV). Additional antiviral agents with activity against HSV include vidarabine (Ara-A), foscarnet (PFA), 1-B-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (BV-ara-U), penciclovir (PCV), and famciclovir (FCV). Acyclovir is converted by the viral TK to its monophosphate derivative, an event that occurs to a much lower extent in uninfected cells (Elion, 1983). Subsequent diphosphorylation and triphosphorylation are catalyzed by cellular enzymes, resulting in ACV triphosphate (ACV-TP) concentrations that are 200 to 1000 times higher in HSV-infected cells than in uninfected cells (Elion, 1982). Acyclovir triphosphate prevents viral DNA synthesis by inhibiting the viral DNA polymerase. In vitro, ACV-TP competes with deoxyguanosine triphosphate as a substrate for viral DNA polymerase. Because ACV-TP lacks the 3′-hydroxyl group required to elongate the DNA chain, the growing chain of DNA is terminated. In the presence of the deoxynucleoside triphosphate complementary to the next template position, the viral DNA polymerase is functionally inactivated (Reardon and Spector, 1989). In addition, ACV-TP is a much better substrate for the viral polymerase than for cellular DNA polymerase alpha, resulting in little incorporation of ACV into cellular DNA.

Viral resistance to ACV can result from mutations in either the viral TK gene or the viral DNA polymerase gene. The phenotypic characterizations of the TK mutants are shown in Table 1. In addition to wild type (TK^+) virus, three categories of TK mutants have been described, based upon the extent of viral TK expression and TK substrate specificity. Thymidine kinase-negative (TK mutants are completely devoid of TK activity. Thymidine kinase-partial (TKp) variants are very low producers of TK, having only 1–15% of normal TK activity. This degree of decrease in TK impairs their ability to phosphorylate ACV effectively. The third category of TK mutants are the TK-altered (TKa) viruses; they are unable to phosphorylate ACV but can still phosphorylate thymidine.

Some overlap exists between TKa and TKp mutants, and the different kinds of TK mutants can be very difficult to distinguish from one another. In particular, different versions of TK enzyme assays and plaque autoradiography assays frequently fail to detect TK activity that is present in HSV-infected cells. As a result, TKp mutants can be misidentified as TK mutants. Despite the potential variability in assay results, it remains useful to consider the TK phenotypes with respect to extent of viral TK expression, as presented in Table 1. Such classification must always be tempered, however, by the realization that the assay systems currently utilized to detect TK activity demonstrate variability. Even mutants that contain nonsense or frameshift mutations resulting in truncated polypeptides can express low levels of TK activity. This observation can have a profound impact

Table 1
Phenotypic characterization of HSV acyclovir-resistant TK mutants

Category	Abbreviation	Definition
TK-negative	TK^-	No TK activity
TK-partial (low TK producers)	TKp	1–15% of normal TK activity
TK-altered	TKa	>15% of normal TK activity, but impaired for ACV-phosphorylation
Wild type	TK^+	40–100% of normal TK activity

upon the biology of HSV; for example, such mutations may permit virus reactivation from a latent state in animal models (Coen et al., 1989b; Hwang et al., 1994). The correlation of these different TK phenotypes with clinical disease remains incomplete.

Mutations within the viral DNA polymerase gene also result in ACV resistance (Larder et al., 1987; Gibbs et al., 1988). These polymerase mutants are less common than TK mutants. All laboratory-derived polymerase mutants analyzed to date, as well as the few clinical isolates identified, result from DNA point mutations. The mechanism(s) by which such mutations confer resistance to ACV is unknown. Possibilities include: 1) alterations in the competitive inhibition between ACV-TP and deoxyguanosine triphosphate; 2) decreased incorporation of ACV-TP into the growing DNA chain; and 3) altered polymerase stability, resulting in decreased likelihood of enzyme inactivation following integration of the ACV-TP molecule into the growing nucleic acid chain. The infrequency with which polymerase mutants are identified in clinical specimens suggests that constraints exist on the number of possible mutations in the DNA polymerase gene that confer ACV resistance yet still produce an enzyme retaining its essential polymerase function. Herpes simplex virus can more readily evade ACV suppression via mutations which reduce or alter TK function. Only two ACV-resistant polymerase mutants have been recovered from patients in the clinical setting (Collins et al., 1989; Sacks et al., 1989).

For both TK^- and TKp variants, the mutations can occur virtually anywhere within the 1.3 kilobase (kb) TK gene. While this may preclude genotypic detection of HSV TK mutants, preliminary data suggest that the mutations are more likely to occur within such "hot spots" as homopolymeric runs of deoxyguanosines or deoxycytosines (Hwang and Chen, 1995). Of those mutants with the TKa phenotype, only a few distinct mutations have been identified to date (Darby et al., 1986; Kost et al., 1993). Based mainly on laboratory-derived isolates, more than 20 different polymerase mutations have been identified in at least six discrete regions of the polymerase gene, spanning more than 1 kb.

Drug-resistant clinical isolates can comprise mixtures of one or more of these

TK and polymerase mutants, as well as drug-susceptible virus. In animal models, such clinical isolates are associated with severe disease that is recalcitrant to ACV therapy (Field, 1982; Field and Lay, 1984; Ellis et al., 1989).

Of the other antiviral agents available for the treatment of HSV, penciclovir (PCV) is the most similar to ACV with respect to mechanism of activation. Like ACV, PCV is converted to its monophosphate form by viral TK. However, PCV is phosphorylated more efficiently than is ACV. Penciclovir monophosphate (PCV-MP) is then converted to the triphosphate form by cellular enzymes. Like ACV-TP, penciclovir triphosphate (PCV-TP) serves as a competitive inhibitor of viral DNA polymerase and is incorporated into the nascent DNA chain. Unlike ACV-TP, PCV-TP is neither an obligate chain terminator nor an inactivator of the DNA polymerase. However, laboratory studies suggest that, once incorporated, PCV-MP does retard the rate of subsequent nucleotide incorporation (Vere Hodge, 1993). The relevance of these observations for clinical efficacy and safety are unknown. PCV-TP is much less potent in inhibiting viral DNA polymerase than is ACV-TP (Earnshaw et al., 1992). However, PCV-TP is present in much higher concentrations and for more prolonged periods in infected cells. Penciclovir-resistant variants with mutations in either TK or DNA polymerase can be selected by passage in vitro. Penciclovir- and ACV-resistant mutants are roughly similar with respect to both phenotypic and genotypic alterations.

Because it does not require activation by the viral TK, vidarabine is less selective in its inhibition of viral DNA synthesis than ACV or PCV. Cellular enzymes convert the drug to its triphosphate form. As a result, activated vidarabine is more likely to interfere with cellular DNA synthesis in uninfected cells than is ACV or PCV. Similarly, foscarnet (PFA) is less selective than either ACV or PCV since it directly inhibits viral DNA polymerase by blocking the pyrophosphate binding site and preventing cleavage of pyrophosphate from deoxynucleotide triphosphates. Mutations in the viral DNA polymerase gene confer resistance to vidarabine and/or PFA. The degree of maximum resistance to vidarabine and PFA

Table 2
Likely cross-resistance profiles for ACV-resistant HSV isolates

Type of mutant	Penciclovir	Foscarnet	Vidarabine
TK-negative	Resistant	Sensitive	Sensitive
TK-partial	Resistant*	Sensitive	Sensitive
TK-altered	Resistant*	Sensitive	Sensitive
Polymerase	Sensitive **	Resistant*	Resistant*
TK + polymerase (double mutants)	Resistant*	Resistant*	Resistant*

* Isolate is usually resistant to the indicated drug, but may be sensitive.
** Isolate is usually sensitive to the indicated drug, but may be resistant.

is 4-fold and 20-fold, respectively. By comparison, the TK and DNA polymerase mutations producing ACV resistance can confer in excess of 100-fold resistance to drug.

Each of the ACV-resistant mutants can demonstrate cross-resistance to other antiviral agents. As shown in Table 2, such cross-resistance profiles can often be predicted based upon the type of phenotypic mutant present. It is important to note that cross-resistance patterns are not uniform for all viral isolates in a given category, and that testing of such isolates is necessary to confirm the susceptibility profile of a particular virus.

The frequency of resistant mutants in both laboratory and clinical isolates from drug-naive patients ranges from below 0.01 to above 0.1%. In conditions that select for high ACV-resistance, TK-deficient mutations predominate. The precise frequency of TK-altered mutations is not known. Polymerase mutants with ≥10-fold resistance to ACV occur at a frequency of about 0.01%. Patient prophylaxis with sufficiently high doses of ACV possibly could limit the viral replicating pool and thereby prevent emergence of resistant isolates; conversely, therapy with insufficient or escalating doses could enhance selection of viruses resistant to drug.

1.2. Cytomegalovirus (CMV)

The two drugs that have been used effectively in the treatment of CMV disease are ganciclovir (GCV) and PFA. Unlike both HSV and VZV, CMV does not encode a thymidine kinase. Rather, the CMV enzyme that catalyzes the initial phosphorylation of GCV in CMV-infected cells is the phosphotransferase encoded by the UL97 gene (Sullivan et al., 1992; Littler et al., 1992). Mutations in either the UL97 gene or the CMV DNA polymerase gene can lead to GCV resistance (Lurain et al., 1992; Sullivan et al., 1993; Lurain et al., 1994a). Additionally, mutations in the DNA polymerase gene can also confer resistance to PFA (D'Aquila et al., 1989; Tatarowicz et al., 1992).

The UL97 phosphotransferase is the protein product of the UL97 open reading frame. This gene encodes a protein comprised of 707 amino acids with a molecular weight of 78,233 daltons. The UL97 phosphotransferase shares homology with the catalytic regions of protein kinases and bacterial aminoglycoside phosphotransferases (Sullivan et al., 1992; Hanks et al., 1988; Chee et al., 1990). It catalyzes the conversion of GCV to its monophosphate form, and cellular enzymes subsequently convert GCV-MP to the active triphosphate form. While the UL97 gene product appears to be an essential enzyme, the exact role that UL97 phosphotransferase plays in CMV replication or pathogenesis is unknown at this time.

Among GCV-resistant clinical and laboratory isolates, little sequence or amino acid diversity exists in the UL97 gene or its protein product (Chou et al., 1995). Such isolates have diminished capacity to phosphorylate GCV to its

monophosphate form, thereby leading to decreased or absent concentrations of the active GCV-TP. Cytomegalovirus isolates with mutations in the UL97 open reading frame make up the predominant phenotype of GCV-resistant isolates (Stanat et al., 1991; Lurain et al., 1994b; Chou et al., 1993; Wolf et al., 1993; Baldanti et al., 1995; Hanson et al., 1994). No null mutants of UL97 have been described to date, suggesting an essential function of this enzyme for CMV growth. Mutations in UL97 which confer GCV resistance are clustered in two highly conserved regions: subdomain VI and subdomain IX. Subdomain VI encompasses an ATP binding site and may play a role in substrate recognition. The methionine at position 460 in subdomain VI appears to be the crucial determinant for GCV monophosphorylation. The exact function of subdomain IX is unknown, but it may be involved in substrate-inhibitor binding. Deletions or point mutations between the alanine at position 590 and the leucine at position 595 have been found to confer GCV resistance (Sullivan et al., 1992; Baldanti et al., 1995; Chou et al., 1995; Wolf et al., 1995). Table 3 summarizes the UL97 mutations which are recognized to confer GCV resistance. Overall, 85% of GCV-resistant mutants have point mutations or, rarely, deletions affecting met_{460}, leu_{595}, or ala_{594}. Because of this high degree of UL97 sequence conservation among clinical strains and the restricted number of mutations, development of a polymerase chain reaction (PCR)-based assay of CMV resistance genotypes is promising (Chou et al., 1995).

As in HSV, the CMV DNA polymerase is an essential enzyme. However, only a minority of GCV-resistant isolates appear to have mutations in the DNA polymerase gene (Sullivan et al., 1993; Lurain et al., 1992). Each of the GCV-resistant polymerase mutants evaluated to date has single amino acid substitutions

Table 3
UL97 mutations conferring ganciclovir resistance

UL97 subdomain	Amino acid change	Codon change	Number of isolates	References
VI	$M_{460}I$	ATG - ATT	3 laboratory isolates	Lurain et al., 1994b
	$M_{460}V$	ATG - GTG	4 clinical isolates	Chou et al., 1993
	$AACR_{593}$ deletion	(GCG GCC TGC CGC) del	1 laboratory isolate	Sullivan et al., 1992
	$A_{594}V$	GCG - GTG	2 clinical isolates	Chou et al., 1993
IX	$L_{595}S$	TTG - TCG	4 clinical isolates	Chou et al., 1993; Wolf et al., 1993
	$L_{595}F$	TTG - TTT	2 clinical isolates	Chou et al., 1993; Wolf et al., 1993
	L_{595} deletion	(TTG) del	1 clinical isolate	Baldanti et al., 1995
VIII	$H_{520}Q$	CAC - CAG	1 clinical isolate	Hanson et al., 1994

Table 4
DNA polymerase mutations conferring ganciclovir resistance

Selective agent	Amino acid substitution	Strain	Polypeptide conserved region	Drug susceptibility phenotype	Reference
GCV	$G_{987}A$	GDG^r P53 - Lab (759^r D100 parent)	V	GCV^r ACV^s PFA^s HPMPC^r HPMPA^r	Sullivan et al., 1993
GCV	$L_{501}I$	D6/3/1 - Lab D1/3/4	between IV and A	GCV^r ACV^s PFA^s HPMPC^r HPMPA^r	Lurain et al., 1992
GCV	$F_{412}V$	D10/3/2 - Lab	IV	GCV^r ACV^s PFA^s HPMPC^r HPMPA^r	Lurain et al., 1992
None	$D_{301}N$	D16 - clinical	upstream of IV	GCV^r ACV$^{s\pm}$ PFA^s HPMPC^r HPMPA^r	Lurain et al., 1994b
None	$I_{503}T$	D16 - clinical	between IV and A	GCV^r ACV^s PFA^s HPMPC^r HPMPA^r	Lurain et al., 1994b
PFA	ND	PFA^r B300 - Lab	ND	GCV^s ACV^r PFA^r HPMPC^s HPMPA^s	Sullivan and Coen, 1991

in the DNA polymerase protein product (Table 4). Reports in the literature regarding CMV DNA polymerase mutants include only four laboratory isolates and one clinical isolate. Each of the four laboratory isolates contained mutations in both the UL97 and DNA polymerase genes (Sullivan et al., 1993; Lurain et al., 1992; Lurain et al., 1994b). The single clinical isolate was present as less than 1% of the virus population recovered from the bronchial brushing of a heart transplant recipient prior to GCV therapy; it is not clear if this patient had previously been treated with ACV (Tatarowicz et al., 1992). This virus mixture consisted of two variants, each with a single mutation in the polymerase gene (Lurain et al., 1994a). Given the very small number of CMV polymerase mutants so far identified, insufficient information exists regarding the need or feasibility for development of rapid detection assays. In addition to GCV, mutations in the CMV DNA polymerase can confer resistance to PFA. As the use of PFA increases, more information will become available regarding both the frequency of polymerase mutations in PFA-treated patients and the specific locations of these mutations within the polymerase gene.

1.3. Varicella-zoster virus (VZV)

Though the use of famciclovir (FCV) for the treatment of VZV disease has

increased to roughly 15% of the U.S. market, ACV remains the antiviral agent of choice for the treatment of VZV infections at this time. The VZV TK phosphorylates ACV to its monophosphate form. Cellular protein kinases then further phosphorylate the drug to its active triphosphate form, which competes with dGTP as a substrate for the viral DNA polymerase. Acyclovir-triphosphate inactivates the DNA polymerase, resulting in premature termination of the growing viral DNA chain. Of the other antiviral agents available for the treatment of VZV infections, PCV, BV-ara-U, and the PCV prodrug, FCV, also require initial phosphorylation by viral TK; vidarabine and PFA have no such requirement.

The VZV TK enzyme is also referred to as a deoxypyrimidine kinase because it phosphorylates deoxycytidine more efficiently than thymidylate or thymidine. The VZV TK shares 31% amino acid identity with its HSV TK counterpart. A greater degree of amino acid homology exists at the two active sites of the VZV TK enzyme. The VZV TK ATP-binding site, comprised of amino acids 12 to 29, shares a 56% amino acid identity with its HSV counterpart. Similarly, the nucleoside-binding site, consisting of amino acids 129 to 145, shares 65% amino acid homology with its HSV counterpart. The VZV TK is less sensitive to ACV than is the HSV TK. However, it is more sensitive to the 5′-pyrimidine analogs, including BV-ara-U.

As is the case with HSV, ACV resistance in VZV is predominantly conferred by mutations in the TK gene. As is shown in Table 5, three phenotypes of the VZV TK have been described. Wild-type virus (TK^+) is capable of expressing fully active TK that can phosphorylate thymidine, deoxycytidine, and ACV. Viral mutants that express inactive TK (TK^-) are unable to phosphorylate these three substrates. Such isolates usually produce truncated TK proteins due to a stop codon in the gene. Viral mutants that express altered TK (TKa) can phosphorylate thymidine but not deoxycytidine or ACV (Talarico et al., 1993; Boivin et al., 1994). Such altered substrate specificity is usually due to a point mutation in the TK gene, though

Table 5
Phenotypic characterization of VZV acyclovir-resistant TK mutants

Category	Abbreviation	Definition
TK-negative	TK^-	Inactive TK that is unable to phosphorylate either thymidine or deoxycytidine
TK-altered	TKa	Enzyme with altered substrate specificity: it can phosphorylate thymidine with up to full efficiency, but cannot phosphorylate deoxycytidine with the same efficiency as the wild-type enzyme
Wild type	TK^+	Fully active TK which phosphorylates both thymidine and deoxycytidine

truncated mutations at the carboxy terminal end have also been reported with this phenotype.

Both laboratory and clinical isolates resistant to ACV due to mutations in the viral TK have been described (Sawyer et al., 1988; Lacey et al., 1991; Roberts et al., 1991; Boivin et al., 1994). Of the laboratory isolates, eight point mutations have been reported (Sawyer et al., 1988; Mori et al., 1988; Lacey et al., 1991; Suzutani et al., 1992). Three are located in the ATP-binding site, one is located in the nucleoside-binding site, and four are located at other sites. Frameshift mutations in the TK gene have been generated in vitro; these mutations result in truncated TK gene products. Because of lack of an animal model for VZV, it is unknown whether these viruses have reduced virulence and altered transmissibility in vivo.

All cases of clinical ACV-resistant VZV TK mutants have occurred in immunosuppressed patients with AIDS receiving prolonged oral ACV therapy (Boivin et al., 1994; Talarico et al., 1993; Sawyer et al., 1988; Pahwa et al., 1989; Safrin et al., 1991; Jacobson et al., 1990; Linnemann et al., 1990). The ACV-resistant clinical isolates evaluated to date have a mean ED_{50} (50% effective dose) that is 4–100-fold higher than that of wild-type virus. The mutant enzymes phosphorylate deoxycytidine very poorly, if at all. In addition, the TK^- isolates are cross-resistant to other antivirals that require activation by TK, such as PCV and BV-ara-U. The TKa isolates, however, may or may not be resistant to other TK-dependent antivirals. Clinical isolates can contain mixtures of wild-type and mutant viruses (Pahwa et al., 1989; Safrin et al., 1991). In addition, mutants with different genotypes and with different antiviral susceptibilities can be obtained from a single culture specimen (Pahwa et al., 1989).

Different point mutations at a single codon can confer varying antiviral resistance profiles. For example, changing Arg_{143} to Lys_{143} confers resistance to ACV but not BV-ara-U or PCV (Talarico et al., 1993). Alternatively, changing Arg_{143} to Gly_{143} results in resistance to ACV and PCV, as well as decreased susceptibility to BV-ara-U. As opposed to the laboratory isolates discussed above, ten point mutations within the VZV TK gene have been described among ACV-resistant clinical isolates. Two are located in the ATP-binding site and can confer either a TK or a TKa phenotype. Six point mutations are located in the nucleoside-binding site; in one of these six, a point mutation at amino acid 130 produced a mutant enzyme that phosphorylated thymidine, deoxycytidine, and ACV much less efficiently (<1%) than did wild-type virus (Roberts et al., 1991). Two additional point mutations are located at other sites. One VZV isolate contained two point mutations (amino acids 138 and 242).

Twelve clinical specimens with frameshift mutations in the TK gene have also been described. Such mutations result in truncated TK gene products due to single, double, or quadruple nucleotide insertions or deletions scattered throughout the TK gene. Four of the 12 mutants contained a stop codon at amino acid 231, making this

the most common site of mutation in the ACV-resistant VZV isolates identified to date (Talarico et al., 1993; Boivin et al., 1994).

In comparing clinical VZV TK mutants and HSV TK mutants, ACV-resistant VZV mutants appear to be more likely to express full length TK than are HSV mutants. In addition, there is greater heterogeneity of mutations within the TK gene among ACV-resistant VZV than in ACV-resistant HSV isolates. Since there are no "hot-spot" sites of mutations within the VZV TK, identification of resistant isolates entails growth and phenotypic characterization in vitro, followed by functional assessment of the viral TK for the natural substrates. Confirmation of TK mutations can only be achieved by sequencing of the entire VZV TK gene.

As with HSV, mutations in VZV DNA polymerase can confer ACV resistance. The VZV DNA polymerase protein shares a 57% amino acid identity with its HSV homologue. Both VZV and HSV DNA polymerase enzymes have seven highly conserved regions (I–VII) (Larder et al., 1987). Mutations in or near six of these seven regions in HSV have been shown to affect ACV susceptibility. The DNA polymerase gene is more highly conserved among VZV strains than among HSV strains. Comparison of three VZV strains showed only one nucleotide change and no amino acid changes in the resulting gene product, while comparison of four HSV strains showed 43 nucleotide differences and 16 amino acid changes. Varicella-zoster virus strains selected in the laboratory for drug resistance to ACV, phosphonoacetic acid (PAA), or vidarabine have been shown to contain mutations in the DNA polymerase gene. In each case, these drug-resistant polymerases contained point mutations located in regions I, II, and III. The laboratory strain selected for resistance to ACV has a point mutation in region III and is hypersensitive to PFA and vidarabine. A laboratory strain with resistance to vidarabine has a mutation in region II; this isolate is sensitive to ACV and hypersensitive to PFA. A laboratory strain with resistance to PAA has a mutation in region I and is resistant to both ACV and vidarabine. The lack of a good animal model for VZV has precluded characterization of the in vivo virulence or transmissibility of these laboratory mutants. Among VZV clinical specimens that are resistant to ACV, only two isolates with mutations in the DNA polymerase gene have been reported. Such mutants are believed to occur only very rarely in the clinical setting. Table 6 summarizes the estimated distribution frequencies of mutations which confer antiviral resistance among the herpesviruses.

2. Influenza

The antiviral agents of choice in the treatment of influenza virus infections are amantadine and rimantadine. The target of the inhibitory action for both of these drugs is the influenza A virus M2 protein. The M2 protein is an integral membrane protein consisting of 23 extracellular residues, a 19 residue transmembrane domain, and a 54 residue cytoplasmic tail. The protein functions as an ion channel

Table 6
Estimated distribution frequencies of mutations from clinical isolates which confer antiviral resistance among the herpesviruses

Herpesvirus	Type of mutation			
	TK^-	TKa	DNA polymerase	UL97
HSV[a]	~90%[b]	~10%	<1%[c]	NA[d]
VZV[a]	74%	22%	4%	NA[d]
CMV[e]	NA[d]	NA[d]	4%	96%

[a] Resistance emerging with the selective pressure of ACV therapy.
[b] As assays are developed that are able to detect low levels of TK, some of these isolates may be found to be TKp.
[c] Only two DNA polymerase mutants have been reported in the literature.
[d] NA: not applicable.
[e] Resistance emerging with the selective pressure of GCV therapy.

and is activated by pH (Pinto et al., 1992; Lamb et al., 1994; Tosteson et al., 1994; Wang et al., 1994; Chizhmakov et al., 1995). The pH sensor may be the histidine residue at position 37 of the transmembrane domain. The M2 channel permits ions to enter the virion during the process of uncoating. This results in destabilization of protein-protein bonds, allowing the viral DNA to be transported into the nucleus (reviewed in Lamb et al., 1994). In addition, the M2 channel acts to modulate the pH of intracellular compartments, particularly the Golgi apparatus (reviewed in Hay, 1992; Lamb et al., 1994). In some species, this stabilizes the influenza A virus hemagglutinin (HA) during intracellular transport (Takeuchi and Lamb, 1994; Takeuchi et al., 1994; Grambas et al., 1992). These activities of the M2 ion channel are blocked by both rimantadine and amantadine (Pinto et al., 1992; Wang et al., 1993).

Mutations in the M2 protein which confer resistance to amantadine and rimantadine occur in the transmembrane domain. This region of the M2 protein has a highly conserved amino acid sequence among all human, swine, equine, and avian strains of influenza A virus (reviewed in Hay, 1992). Among amantadine-resistant mutants, substitution of residues in the putative α-helix of the M2 transmembrane domain alters properties of the M2 ion channel (Holsinger et al., 1994). Amantadine inhibits channel activity by decreasing both the amplitude and the frequency of M2 ion channel opening, effectively blocking the pH channel. The M2 proteins from amantadine-resistant isolates can contain modifications of an N-linked carbohydrate chain at an asparagine residue within the transmembrane domain (Grambas et al., 1992; Holsinger et al., 1994).

A deletion of residues 28 to 31 in the transmembrane domain of the M2 protein

results in loss of activation by pH (Pinto et al., 1992; Wang et al., 1994). Such laboratory mutants are resistant to amantadine and possess an M2 channel that is activated by voltage. This altered ion channel appears to exist in pentameric form, rather than the typical tetramer (Holsinger et al., 1994). In addition, in vitro substitution of the alanine at residue 30 with threonine also confers amantadine resistance and significantly attenuates the low pH-activation response (Holsinger et al., 1994).

Resistance of influenza A clinical isolates to amantadine and rimantadine is conferred by single amino acid substitutions at one of five positions of the transmembrane domain of M2 (Belshe et al., 1988). These sites of mutation include amino acids 26, 27, 30, 31, and 34. The same spectrum of mutations is also noted among in vitro isolates passaged in the presence of either antiviral agent (Hay, 1992). Such resistant isolates emerge readily in cell culture in the presence of either amantadine or rimantadine. Amantadine and rimantadine appear to demonstrate identical pharmacodynamics with respect to M2 channel activity (Wang et al., 1993). As inferred from the Hill coefficient, one amantadine molecule blocks one M2 channel complex (Wang et al., 1993).

3. Picornaviruses

The picornavirus family is comprised of both the enterovirus genus and the rhinovirus genus. Seventy distinct serotypes of enteroviruses have been identified, while 100 different types of rhinoviruses are known to exist. One class of antiviral agents which has demonstrated activity against picornaviruses is the capsid-binding compounds. Members of this class of drugs have widely different structures, yet all bind in the same site on the virion capsid. The inhibitors of this class tend to be very insoluble and are rapidly and extensively metabolized by the host. As such, it has been difficult to find compounds with properties suitable for clinical development.

The mechanisms by which these agents mediate viral inhibition have been evaluated. The drug binds in the VP1 hydrophobic pocket that lies beneath the canyon which runs around each of the 5-fold axes of symmetry of the virus (Smith et al., 1986). Such binding results in inhibition of uncoating of all picornaviruses studied to date (Shepard et al., 1993). In addition, the presence of drug in the viral pocket blocks attachment of those picornaviruses that bind to ICAM (Pevear et al., 1989).

The four in vitro systems in which resistance mechanisms have been evaluated are rhinovirus 14, rhinovirus 1A, poliovirus, and coxsackie virus B3. Evaluation of the rhinovirus 14 model revealed two types of resistance (Shepard et al., 1993; Heinz et al., 1989; Badger et al., 1989). High level resistance results in a 200-fold increase in IC_{50}, with all such isolates having mutations which map to two sites in the drug binding pocket. Mutations to larger side chains in the pocket result in

exclusion of the drug from the pocket. Isolates with low level resistance, on the other hand, exhibit only a 3–10-fold increase in IC_{50}. The mechanism by which these isolates confer resistance is less well understood. None of these mutants has mutations in binding pocket, and all appear to increase the receptor binding affinity. This increase in receptor binding affinity is thought to result in the compound no longer being able to block attachment of viruses.

In the rhinovirus 1A model, all of the resistant mutants have drug-requiring phenotypes. Namely, they have markedly decreased stability in the absence of drug, and require drug binding to restore wild-type stability and infectivity. All of the mutations are outside of the pocket, mapping to the pseudo 3-fold axis. While of interest from the standpoint of understanding the biology of rhinoviruses and their mechanisms of antiviral resistance, drug-requiring mutants should not pose clinical problems since they cannot effectively be transmitted to individuals who are not receiving antiviral therapy.

Unlike the other two model systems, the virulence of the coxsackie virus B3 mutants can be assessed in experimental murine models. Mutations to larger side chains in the coxsackie virus B3 pocket appear to exclude the drug from access to the binding site. Of the ten exclusion mutants isolated to date, mutations have been identified only at amino acids 1092 and 1207. As with rhinovirus 1A, these mutants are less stable than wild-type virus. In the mouse model, these mutants are approximately 10-fold less virulent than wild-type virus. These results suggest that mutations to the drug resistant phenotype result in a virus with compromised stability which, in turn, results in reduced virulence.

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

The three major classes of inhibitors of HIV-1 replication are: 1) nucleoside analog inhibitors of the viral reverse transcriptase (RT) enzyme; 2) non-nucleoside inhibitors of the viral RT enzyme; and 3) inhibitors of the viral protease. The main factor that influences in vivo resistance of HIV-1 to these antiviral compounds is the extraordinarily and persistently high rate of viral replication which is unique to HIV. Other factors include the high viral mutation rate which is typical of all RNA viruses, the high viral load in infected persons (up to 10^6–10^7 genomic copies/ml plasma), and the rapid turnover of the viral population (half-life of approximately 2 days).

The nucleoside analog RT inhibitors act by mediating premature termination of nascent viral DNA synthesis. All antiviral drugs currently approved for treatment of HIV-1 infection belong to this class of inhibitors. Resistant viral variants have been isolated both in vitro and in vivo for all of the analogs studied to date, though the resistance to 2′,3′-dideoxycytosine (ddC) and 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T, stavudine) appears to be of borderline magnitude and significance. Loss of susceptibility appears to be due to RT amino acid

substitutions that map near the enzyme's active site or to the finger domain of the p66 subunit. Such mutations appear to affect the enzyme's interaction/association with the primer and substrate/template, resulting in a decreased ability of the enzyme-template-primer complex to recognize inhibitor. However, the wild-type RT and zidovudine (AZT)-resistant RT are enzymatically indistinguishable, suggesting that other differences between the two exist. Different combinations of substitutions result in varying degrees of viral resistance (Larder and Kemp, 1989). Many individual substitutions or combinations of substitutions produce variants that are resistant to multiple inhibitors. Amino acid substitutions in the reverse transcriptase which can confer nucleoside analog resistance are summarized in Appendix A.

The non-nucleoside RT inhibitors (NNRTIs) are a structurally diverse group of compounds that bind to the same site on the enzyme. Despite the variability in structure of these agents, they are functionally essentially identical. All are highly specific for the HIV type 1 RT, but not the HIV type 2 RT or simian immunodeficiency virus (SIV) RT. They bind to a small pocket located proximal to the RT's active site. Binding of drug appears to result in premature dissociation of the enzyme-template-primer complex. Resistant viral variants have been isolated both in vitro and in vivo for all of the NNRTIs studied to date (Nunberg et al., 1991; Richman et al., 1991). Amino acid substitutions which confer resistance to these compounds are located within the inhibitor binding pocket and probably alter the enzyme's ability to interact with the drug. As with the nucleoside RT inhibitors, different amino acid substitutions confer varying degrees of antiviral resistance. Many resistant variants exhibit cross-resistance to other members of the NNRTI class; these variants express either multiple or cross-active substitutions. Amino acid substitutions that confer loss of susceptibility to non-nucleoside compounds are summarized in Appendix A.

The HIV-1 protease mediates cleavage of the viral polypeptides during the process of virion maturation. Inhibition of protease activity by protease inhibitors (PIs) results in production of noninfectious, immature viral particles. All PIs described to date bind to the enzyme's active site. Viral variants that are resistant to PIs have been isolated in vitro (Ho et al., 1994; Kaplan et al., 1994; Otto et al., 1993; El-Farrash et al., 1994). Analysis of such variants suggests that certain amino acid substitutions appear to be associated with loss of susceptibility. However, the phenotypic influences of individual substitutions await the results of confirmatory genetic studies. Amino acid substitutions can occur at residues that clearly interact with the PI. Alternatively, other substitutions are located at residues that are distal to the interaction between the compound and the enzyme but that still influence the enzyme's association with the drug. Viral isolates obtained from patients following prolonged treatment with the protease inhibitor L735,524 express notable cross-resistance to multiple, structurally diverse PIs. The genotypic basis of this apparent cross-resistance is complex and is not fully understood at this time (Condra et al.,

in press). The amino acid substitutions in the protease enzyme that have been isolated from resistant viral variants are summarized in Appendix A.

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2. Appendix A

Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance *

* Adapted from: Mellors, J.W., Larder, B.A. and Schinazi, R.F. (1995) Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance. Int. Antivir. News, 3(1): 8-13. Reproduced with permission from *International Antiviral News*.

Abbreviations for Appendix A

Amino acids: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenyalanine; G, glycine, H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Appendix A: Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
					Nucleoside RT inhibitors		
AZT	M41L	ATG to TTG or CTG	ND	Yes	M41L; 4-fold resistance: M41L/T215Y; 60–70-fold: K67N/K70R/T215Y/K219Q; 120-fold: M41L/K67N/K70R/T215Y; 180-fold.	Yes	Larder and Kemp, 1989 Larder et al., 1991
	D67N	GAC to AAC	Yes	Yes		Yes	Kellam et al., 1992
	K70R	AAA to AGA	Yes	Yes	Effect of T215Y is reversed by a ddI resistance mutation (L74V), NNRTI mutations (L100I;Y181C) or (−)-FTC/3TC mutations (M184I/V)	Yes	
	T215Y	ACC to TAC	Yes	Yes		Yes	
	T215F	ACC to TTC	ND	Yes		Yes	
	K219Q	AAA to CAA	ND	Yes		Yes	
	K219E	AAA to GAA	Yes	No		Yes	
ddI	L74V	TTA to GTA	No	Yes	5–10-fold resistance; cross-resistance to ddC; can reverse effect of T215Y, AZT resistance mutation observed with D4T selection; cross-resistance to ddI, ddC, d4C, (−)-FTC	Yes	St. Clair et al., 1991
	V75T	GTA to ACA	Yes	Yes		Yes	Lacey and Larder, 1994
ddI	M184V	ATG to GTG	Yes	Yes	5–10-fold resistance; cross-resistance to ddC	Yes	Gu et al., 1992
ddC	K65R	AAA to AGA	Yes	Yes	4–10-fold resistance; observed in patients receiving ddI or ddC	Yes	Gu et al., 1994a Zhang et al., 1994
	T69D	ACT to GAT	No	Yes	5-fold resistance	Yes	Fitzgibbon et al., 1992
	L74V	TTA to GTA			Observed with ddI therapy	Yes	St. Clair et al., 1991
	V75T	GTA to ACA			Observed with d4T selection *in vitro*	Yes	Lacey and Larder, 1994
	M184V	ATG to GTG			Observed with ddI, 3TC therapy; cross-resistance to ddC	Yes	Gu et al., 1992
	Y215C	TTC to TGC	No	Yes	4-fold resistance; arises on background of T215Y AZT resistance mutation	Yes	Slade et al., 1993
D4T	I50T	ATT to ACT	Yes	Unknown	30-fold resistance	Yes	Gu et al., 1994b

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
	V75T	GTA to ACA	Yes	Yes	7-fold resistance; cross-resistance to ddI, ddC, d4C and (−)-FTC	Yes	Lacey and Larder, 1994
3TC or (−)-FTC	M184V	ATG to GTG or GTA	Yes	Yes	>100-fold resistance; M184V and M184I can suppress effects of AZT resistance mutations	Yes	Schinazi et al., 1993 Tisdale et al., 1993 Gao et al., 1993
	M184I	ATG to ATA	Yes	Yes		Yes	Schinazi et al., 1993 Tisdale et al., 1993 Gao et al., 1993
1592U89	K65R	AAA to AGA	Yes	No	3-fold resistance	Yes	Tisdale et al., 1994a
	L74V	TTA to GTA	Yes	No	4-fold resistance	Yes	
	Y115F	TAT to TTT	Yes	No	2-fold resistance	Yes	
	M184V	ATG to GTG	Yes	No	3-fold resistance	Yes	
					K65R/M184V; 8-fold resistance: L74V/M184V; 9-fold resistance: L74V/Y115F/M184V; 11-fold resistance		
					HIV-1-specific RT inhibitors		
Nevirapine	A98G	GCA to GGA	No	Yes		Yes	Richman et al., 1994
	L100I	TTA to ATA	No	Yes		Yes	Richman, 1993
	K103N	AAA to AAC	No	Yes		Yes	Richman, 1993
	V106A	GTA to GCA	Yes	Yes	~100-fold resistance; no effect on AZT resistance	Yes	Richman et al., 1994 Richman, 1993 Larder, 1992 Balzarini et al., 1993a
	V108I	GTA to ATA	No	Yes		Yes	Richman, 1993
	Y181C	TAT to TGT	Yes	Yes	>100-fold resistance; cross-resistance to other NNRTI; can suppress effects of AZT resistance mutations	Yes	Richman et al., 1994 Nunberg et al., 1991 Richman et al., 1991 Mellors et al., 1992

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
Nevirapine	Y181I	TGT to ATT	No	Yes	High-level resistance observed in one nevirapine-treated patient		Shaw et al., 1994
	Y188C	TAT to TGT	No	Yes		Yes	Richman, 1993
	G190A	GGA to GCA	No	Yes		Yes	Richman et al., 1994
TIBO R82150	L100I	TTA to ATA	Yes	Unknown	>100-fold resistance; can reverse effects of AZT resistance mutations	Yes	Mellors et al., 1993 Balzarini et al., 1993b Byrnes et al., 1993a
TIBO R82913	L100I	TTA to ATA	Yes	Unknown		Yes	Larder, 1992
	K103N	AAA to AAC	Yes	Unknown	>100-fold resistance	Yes	Balzarini et al., 1993a
	V106A	GTA to GCA	Yes	Unknown	~100-fold resistance	Yes	Larder, 1992
	E138K	GAG to AAG	Yes	Unknown	Found in combination with L100I		Balzarini et al., 1993b
	Y181C	TAT to TGT	Yes	Unknown	>100-fold resistance	Yes	Larder, 1992
	Y188H	TAT to CAT	Yes	Unknown		Yes	Balzarini et al., 1993b
	Y188L	TAT to TTA	No	Yes		Yes	Vandamme et al., 1994
L-697,593	K103N	AAA to AAC	Yes	Unknown	20-fold resistance	Yes	Nunberg et al., 1991
	Y181C	TAT to TGT	Yes	Unknown	>100-fold resistance	Yes	Nunberg et al., 1991
L-697,661	A98G	GCA to GGA	No	Yes	8-fold resistance	Yes	Byrnes et al., 1993b
	L100I	TTA to ATA	Yes	No	2-fold resistance	Yes	Byrnes et al., 1993b
	K101E	AAA to GAA	No	Yes	8-fold resistance	Yes	Byrnes et al., 1993b
	K103N	AAA to AAC	Yes	Yes	8-fold resistance	Yes	Byrnes et al., 1993b
	K103Q	AAA to CAA	No	Yes	8-fold resistance	Yes	Saag et al., 1993
L-697,661	V108I	GTA to GCA	Yes	Yes	4-fold resistance	Yes	Byrnes et al., 1993b
	V179D	GTT to GAT	No	Yes	4-fold resistance	Yes	Byrnes et al., 1993b
	V179E	GTT to GAG	No	Yes	8-fold resistance	Yes	Byrnes et al., 1993b
	Y181C	TAT to TGT	Yes	Yes	>30-fold resistance	Yes	Byrnes et al., 1993b
BHAP U-90152 delaviridine	P236L	CCT to CTT	Yes	Unknown	P236L sensitizes RT ~10-fold to nevirapine, TIBO R82913, and L-697,661	Yes	Dueweke et al., 1993

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
BHAP U-87201 ateviridine	K101E	AAA to GAA	No	Yes	K103N and Y181C observed with U-87201 monotherapy; K101E, Y188H, E233Y and K238T observed with U87201/AZT combination therapy	No	Demeter et al., 1993
	K103N	AAA to AAC	No	Yes		No	
	Y181C	TAT to TGT	No	Yes		No	Demeter et al., 1993
	Y188H	TAT to CAT	No	Yes		No	Demeter et al., 1993
	E233V	GAA to GTA	No	Yes		No	Demeter et al., 1993
	P236L	CCT to CTT	Yes	No		Yes	Dueweke et al., 1993
	K238T	AAA to ACA	No	Yes		No	Demeter et al., 1993
BHAP U-88204	L100I	TTA to ATA	Yes	Unknown			Balzarini et al., 1993a Vasudevachari et al., 1992
	V106A	GTA to GCA	Yes	Unknown		Yes	Vasudevachari et al., 1992
	Y181C	TAT to TGT	Yes	Unknown		Yes	Vasudevachari et al., 1992
	Y181I	TGT to ATT	Yes	Yes	Appeared after treatment of Y181C-mutated virus with BHAP. High-level resistance to BHAP, nevirapine and TIBO. Observed in one nevirapine-treated patient		Balzarini et al., 1994
HEPT	Y188C	TAT to TGT	Yes	Unknown			Balzarini et al., 1993c
E-EBU	Y181C	TAT to TGT	Yes	Unknown			Balzarini et al., 1993c
E-EBU-dM	Y106A	GTA to GCA	Yes	Unknown			Balzarini et al., 1993c
E-EPU and E-EPSeU	Y181C	TAT to TGT	Yes	Unknown	Y188C is the predominant mutation for E-EPSeU; Y188C confers greater resistance to E-EPSeU/E-EPU than Y181C	Yes	Nguyen et al., 1994
	Y188C	TAT to TGT	Yes	Unknown		Yes	Nguyen et al., 1994
α-APA R18893	Y181C	TAT to TGT	Yes	Unknown		Yes	de Béthune et al., 1993
S-2720	G190E	GGA to GAA	Yes	Unknown	Mutation decreases RT activity and viral replication competency	Yes	Kleim et al., 1993

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
TSAO	E138K	GAG to AAG	Yes	Unknown	>100-fold	Yes	Balzarini et al., 1993d Balzarini et al.,1993e
BM+51.0836	Y181C	TAT to TGT	Yes	Unknown		Yes	Maas et al., 1993
					Protease inhibitors		
A-77003	R8Q	CGA to CAA	Yes	Unknown	10-fold viral resistance	Yes	Ho et al., 1994 Kaplan et al., 1994
	R8K	CGA to AAA	Yes	Unknown	10-fold viral resistance	Yes	Ho et al., 1994
	V32I	GTA to ATA	Yes	Unknown	7-fold enzyme resistance	Yes	Kaplan et al., 1994
	M46I	ATG to ATA	Yes	Unknown	No effect on susceptibility but improves replication competency of R8Q mutant	Yes	Ho et al., 1994
	M46L	ATG to TTC	Yes	Unknown	2–3-fold enzyme resistance	Yes	Kaplan et al., 1994
	M46F	ATG to TTC	Yes	Unknown	4-fold enzyme resistance	Yes	Kaplan et al., 1994
	G48V	GGG to GTG	Yes	Unknown	R8K/M46I/G48V: 20-fold viral resistance		Tisdale et al., 1994b
	V82I	GTC to ATC	Yes	Unknown	No resistance alone but V32I and V82I are synergistic mutations yielding 20-fold enzyme resistance	Yes	Kaplan et al., 1994
	V82A	GTC to GCC	Yes	Unknown	Rare; seen with M46F		Swanstrom et al., 1994
	I82T	ATC to ACC	Yes	Unknown	G48V/I82T combined produce 100-fold resistance (82T was derived from *in vitro* passage of 82I)		Swanstrom et al., 1994
A-75925	V32I	GTA to ATA	Yes	Unknown	40-fold viral resistance		Maschera et al, 1994
ABT-538	V82F	GTC to TTC	Yes	Unknown	V82F/I84V: 8–10-fold viral resistance	Yes	Kempf et al., 1994
	I84V	ATA to GTA	Yes	Unknown	M46I/L63P/A71V/V82F/I84V: 27-fold resistance		
BILA 1906 BS	V32I	GTA to ATA	Yes	Unknown	V32I/A71V; 3-fold viral resistance: V32I/		Lamarre et al., 1994
	M46L	ATG to TTG	Yes	Unknown	A71V/M46I/I84V; 5-fold: V32I/A71V/		
	A71V	GCT to GTT	Yes	Unknown	M46I/I84V; 1000-fold (mutation also		
	I84V	ATA to GTA	Yes		detected in p6/p7 cleavage site)		

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
BMS 186,318	A71T	GCT to ACT	Yes	Unknown	A71T/V82A; 15-fold viral resistance	Yes	Rose et al., 1994
	V82A	GTC to GCC	Yes	Unknown	4-fold cross resistance to A77003	Yes	
L-735,524	L10R	CTC to CGC	No	Yes	M46I/L63P/V82T; 4-fold viral resistance:	Yes	Emini et al., 1994
	M46I	ATG to ATA	No	Yes	L10R/M46I/L63P/V82T; 4-fold viral resis-		
	L63P	CTC to CCC	No	Yes	tance: L10R/M46I/L63P/V82T/I84V;		
	V82T	GTC to ACC	No	Yes	8-fold viral resistance; cross-resistance to		
	I84V	ATA to CTA	No	Yes	XM-323 (15-fold), A-80987 (4-fold), Ro-31-8959 (8-fold), VX-478 (8-fold), SC-52151 (8-fold)		
	V32I	GTA to ATA	Yes	Unknown	V32I/M46L/V82A; 3-fold viral resistance:		Tisdale et al., 1994b
	M46I	ATG to ATA	Yes	Unknown	V32I/M46L/A71V/V82A: 14-fold viral resis-		
	A71V	GCT to GTT	Yes	Unknown	tance		
	V82A	GTC to GCC	Yes	Unknown			
P9941	V82A	GTC to GCC	Yes	Unknown	6–8-fold resistance	Yes	Otto et al., 1993
Ro 31-8959	G48V	GGG to GTG	Yes	Yes		Yes	Jacobsen et al., 1994
	I84V	ATA to GTA	Yes	Unknown			Tisdale et al., 1994b
	L90M	TTG to ATG	Yes	Yes	G48V/L90M combined yield >100-fold enzyme resistance, but double mutant rare in vivo; L90M most common in vivo; G48V/I84V/L90M: 30-fold viral resistance		Jacobsen et al., 1994 Tisdale et al., 1994b
RPI-312	I84V	ATA to GTA	Yes	Unknown	5-fold viral resistance	Yes	El-Farrash et al., 1994
SC-52151	L24V	TTA to GTA	Yes	Unknown	G48V alone, G48V/V82A, G48V/L63P/ V82A or I54T: 10–20-fold viral resistance		Potts et al., 1994 Pillay et al., 1994
	G48V	GGG to GTG	Yes	Unknown			
	A71V	GCT to GTT	Yes	Unknown	A71V/V75I/P81T: 20–30-fold viral resis- tance		

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
	V75I	GTA to ATA	Yes	Unknown	L24V/G48V/A71V/V75V/P81T: 1000-fold,		
	P81T	CCT to ACT	Yes	Unknown	some cross-resistance to SC55389A and		
	V82A	GTC to GCC	Yes	Unknown	Ro 31-8959, but not to L-735,524		
	N88D	AAT to GAT	Yes	Unknown	N88D alone, or I11V/M46I/F53L/A71V/ N88D: 10–20-fold resistance		
SC-55389A	L10F	CTC to CGC	Yes	Unknown	N88S alone: 20-fold viral resistance, no cross-resistance to SC-52151		Potts et al., 1994 Pillay et al., 1994
	N88S	AAT to AGT	Yes	Unknown	N88S/L10F: 10-fold viral resistance, no cross-resistance to SC-52151		
VB 11,328	L10F	CTC to GGC	Yes	Unknown	L10F/I84V: 8-fold viral resistance		Partaledis et al., 1994
VB 11,328	M46I	ATG to ATA	Yes	Unknown	I50V/M46I/I47V: 20-fold viral resistance	Yes	Tisdale et al., 1994b Partaledis et al.,1994
	I47V	ATA to CTA	Yes	Unknown			
	I50V	ATT to GTT	Yes	Unknown	3-fold viral resistance	Yes	Tisdale et al., 1994b
	I84V	ATA to GTA	Yes	Unknown			
XM323	L10F	CTC to CGC			L10F/V82A: 2-fold viral resistance; L10F/	Yes	King et al., 1995
	K45I	AAA to ATA			K45I/I84V: 50-fold		Tisdale et al., 1994b
	G48V	GGG to GTG	Yes	Unknown		Yes	King et al., 1995
	V82A	GTC to GCC	Yes	Unknown	V82A/M46L; 7-fold resistance: V82A/ M46L/L97V; 11-fold resistance	Yes	King et al., 1995
	V82I	GTC to ATC	Yes	Unknown	<2-fold viral resistance	Yes	King et al., 1995
	V82F	GTC to TTC	Yes	Unknown	see below	Yes	King et al., 1995
	I84V	ATA to GTA	Yes	Unknown	12-fold resistance alone; V82F/I84V: 92-fold resistance; cross-resistant to P9941, but not A77003 or Ro 31-8959		Tisdale et al., 1994b King et al., 1995
	L97V	TTA to GTA	Yes	Unknown	no resistance alone; V82A/L97V: 3-fold resistance	Yes	King et al., 1995

Compound	Amino acid change	Codon change	In vitro	In vivo	Comments	Confirmed by site-directed mutagenesis	References
					Pyrophosphate analogue RT inhibitor		
Foscarnet	W88S	TGG to TCG	No	Yes	4-fold resistance	Yes	Mellors et al., 1994
Foscarnet	E89G	GAA to GGA	Yes	No	isolated by screening RT clones for ddGTP resistance; 14-fold viral resistance	Yes	Prasad et al., 1991
	E89K	GAA to GGA	Yes	No	E89K and L92I cause increased susceptibility		Tachedjian et al., 1994
	L92I	TTA to ATA	Yes	No	to AZT and HIV-1 specific RTI		Tachedjian et al., 1994
	S156A	TCA to GCA	Yes	No			Tachedjian et al., 1994
	Q161L	CAA to CTA	Yes	Yes	10-fold resistance	Yes	Mellors et al., 1994
	H208Y	CAT to TAT	Yes	Yes	2-fold resistance	Yes	Mellors et al., 1994
					Q161L + H208Y cause increased susceptibility to AZT (100-fold), nevirapine (20-fold), and TIBO R82150 (30-fold)		

Abbreviations for Appendix A (contd.):

1592U89, (1*S*,4*R*)-4-[2-amino-6-cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol succinate; 3TC, (–)-b-l-2′,3′-dideoxy-3′-thiacytidine; a-APA 18893, α-nitroanilinophenylacetamide; A-77003, C2 symmetry-based protease inhibitor (Abbott); A-75925, C2 symmetry-based protease inhibitor (Abbott); ABT-538, C2 symmetry-based protease inhibitor (Abbott); AzddU, 3′-azido-2′,3′-dideoxyuridine; AZT-p-ddI, 3′-azido-3′-deoxythymidilyl-(5′,5′)-2,3,-dideoxy-5′-inosinic acid; AZT, 3′-azido-2′,3′-dideoxythymidine, BHAP, bis-heteroaryl-piperazine; BILA1906, protease inhibitor (Boehringer-Ingelheim); BM+51.0836, thiazolo-isoindolinone derivative; BMS 186,318, aminodiol derivative HIV-1 protease inhibitor (Bristol-Myers-Squibb); d4C, 2′,3′-didehydro-2′,3′-deoxycytidine; d4T, 2′,3′-didehydro-3′-deoxythymidine; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; EBU-dM, 5-ethyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)uracil; E-EBU, 5-ethyl-1-ethoxymethyl-6-benzyluracil; E-EPSeU, 1-(ethoxymethyl)-(6-phenylselenyl)-5-ethyluracil; E-EPU, 1-(ethoxy-methyl)-(6-phenyl-thio)-5-ethyluracil; (–)-FTC, (–)-b-l-2′,3′-dideoxy-5-fluoro-3′-thiacytidine; HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine; HIV-1, human immunodeficiency virus type 1; L'697,593; 5-ethyl-6-methyl-3-(2-phthalimidoethyl)pyridin-2-(1*H*)-one; L'735,524, hydroxyaminopentane amide HIV-1 protease inhibitor (Merck); L'697,661, 3-{[(-4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2-(1*H*)-one; l-FDDC, (–)-b-l-5-fluoro-2′,3′-dideoxycytidine; l-FDDC, (–)-b-l-5-fluoro-dioxolane cytosine; ND, not determined; Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyridol[3,2-*b*:2′,3′-*e*]diazepin-6-one; NNRTI, non-nucleoside reverse transcriptase inhibitor; P9941, protease inhibitor (Dupont Merck), [2-pyridylacetyl-IlePheAla-y(CHOH)]-2; PFA, phosphonoformate (foscarnet); PMEA, 9-(2-phosphonylmethoxyethyl)-adenine; Ro 31-8959, hydroxyethylamine derivative HIV-1 protease inhibitor (Roche); RPI-312, peptidyl protease inhibitor, 1-[(3*s*)-3-(*n*-α-benzyloxycarbonyl)-1-asparaginyl)-amino-2-hydroxy-4-phenylbutyryl]-*n*-*tert*-butyl-1-proline amide; RT, reverse transcriptase; S-2720, 6-chloro-3,3-dimethyl-4-(isopropenyloxy-carbonyl)-3,4,-dihydroquinoxalin-2(1*H*)-thione; SC-52151, hydroxyethylurea isostere protease inhibitor (Searle); SC-55389A, hydroxyethylurea isostere protease inhibitor (Searle); TIBO R82150, (+)-(5*S*)-4,5,6,7,-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-thione; TIBO 82913, (+)-(5S)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-*jk*]-[1,4]benzodiazepin-2(1*H*)-thione; TSAO-m3T, [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-*spiro*-5′-(4′-amino-1′,2′-oxa-thiole-2′,2′-dioxide)]-b-d-pentofuranosyl-*N*3-methylthymine; U90152, 1-[3-[(1-methylethyl)amino]-2-pyridinyl]-4-[[5-[(methylsulphonyl)amino]-1*H*-indol-2-yl]-carbonyl]piperazine; VB 11,328, hydroxyethylsulphonamide protease inhibitor (Vertex); VX-478,

hydroxyethylsulphonamide protease inhibitor (Vertex); XM 323, cyclic urea protease inhibitor (Dupont Merck).

References for Appendix A

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