In Search of a Selective Antiviral Chemotherapy

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INTRODUCTION

Viral diseases have for a long time been considered intratable by chemotherapeutic means because of the innate association of viruses with the normal cell machinery. The first, albeit timid, attempts to curtail virus infections date from the early 1950s when the thiosemicarbazones, introduced by Domagk et al. (83) for the treatment of tuberculosis, were found to be active against vaccinia virus as well (93). This work was continued by Bauer (36) and culminated in the demonstration by Bauer et al. (37) in 1963 that the thiosemicarbazone derivative Marboran was effective in the prophylaxis of smallpox infection.

The first antitherpes compound, IDU (idoxuridine), was synthesized by Prusoff (145) in 1959; it was then shown by Kaufman to “cure” herpetic keratitis in rabbits (102) and humans (104) and in the same year (1962), it was approved in the United States for the (topical) therapy of herpetic keratitis. Two years later (1964), TFT ( trifluridine) was introduced for the treatment of herpetic keratitis (103), and both IDU and TFT, now more than 30 years later, are still used for this purpose (as eye drops and, occasionally, as eye ointment).

The first description of the antiviral activity of amantadine (52) and vidarabine (144, 154) also dates from the “avant garde” year 1964. Later, these drugs would be approved for the prophylaxis and treatment of influenza A virus infections (in 1966) and for the therapy of herpes simplex virus (HSV) infections (e.g., herpetic encephalitis) (in 1972).

In the meantime, interferon and interferon inducers [particularly primary and recurrent genital herpes and mucocutaneous herpes simplex virus (HSV) infections (e.g., herpetic encephalitis) (in 1972).

The first synthetic chemical to be credited with broad-spectrum antiviral activity was ribavirin (Virazole). While first described in 1972 as a broad-spectrum antiviral agent active against both DNA and RNA viruses (159), it has proven useful primarily in the treatment of some hemorrhagic fever virus infections (e.g., Lassa fever) and respiratory syncytial virus infections. Although ribavirin (in aerosolized form) has been approved for the latter indication (in 1985), its effectiveness has remained controversial (70).

In retrospect, it can be stated that antiviral chemotherapy came of age with the advent in 1977 of acyclovir [9-(2-hydroxyethoxymethyl)guanine] as the first truly specific antiviral agent (86, 156). The compound was found to effect a selective inhibition of the replication of HSV, essentially because it was phosphorylated by the virus-induced thymidine kinase (TK) (91).

From its chemical structure, it could be hardly anticipated that acyclovir would be recognized as substrate by a TK, whether virus induced or not. At best, the guanine part of acyclovir could be viewed as bearing some resemblance to cytosine, and this would explain why it was recognized by the viral TK, which acts not only as a TK but also as a deoxyctyidine kinase (54).

Empirical search has been the cornerstone of both initial discovery and subsequent improvement of nearly all useful chemotherapeutic agents (155). The first synthetic centrally active drugs, i.e., diuretics, sulfonamides, cardiac glucosides, and antibiotics such as penicillin, were in no way influenced by any fundamental knowledge of the processes with which they interfere (116). This knowledge was acquired later through logic and orderly thinking. It would appear that antiviral chemotherapy has meandered along such a route, with an empirical start and a logical and rational, yet often winding course before arriving (successfully) at its final destination.

(This review is an extended version of the Hoechst Marion Roussel Award Lecture presented at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996.)

SELECTIVE ANTIHERPESVIRUS AGENTS

Shortly after it had been described as a potent and selective inhibitor of the replication of HSV and, to a lesser extent, of varicella-zoster virus (VZV), acyclovir became the drug of choice for the treatment of HSV and VZV infections, particularly primary and recurrent genital herpes and mucocutaneous HSV and VZV infections in immunosuppressed patients. Also, acyclovir proved to be superior to vidarabine in the treatment of herpetic encephalitis and VZV infections and has replaced vidarabine for these indications (63). Because of its limited oral bioavailability (only 20%), acyclovir has, in turn,
been replaced by its prodrug, valaciclovir, in the oral treatment of HSV and VZV infections (47).

The remarkable potency of acyclovir against HSV-1, HSV-2, and to a lesser extent VZV has prompted the development of several structural analogs of acyclovir (Fig. 1) (65). Foremost among these acyclovir congeners are ganciclovir and penciclovir. Ganciclovir has a more pronounced activity against cytomegalovirus (CMV) than acyclovir and became the drug of choice (until the advent of HPMPC [see below]) for the treatment of CMV infections, particularly CMV retinitis, in immunosuppressed patients (121). Penciclovir has a similar activity spectrum and mechanism of action to acyclovir and has been used under its oral prodrug form, famciclovir, for the same indications as valaciclovir, i.e., for the treatment of HSV and VZV infections (88, 182). In addition, penciclovir shows activity against human hepatitis B virus.

Whereas acyclovir emanated from a program centered on examining the substrate specificity of adenosine deaminase (which is known to rapidly convert the antiviral compound vidarabine [ara-A, adenine arabinoside, 9-β-D-arabinofuranosyladenine] to its inactive metabolite ara-Hx [hypoxanthine arabinoside]), brivudin [(E)-5-(2-bromovinyl)-2'-deoxyuridine, BVDU] was originally developed at the Chemistry Department of the University of Birmingham by P. J. Barr, A. S. Jones, and R. T. Walker as a potential radiation-sensitizing agent (assuming that it would be incorporated into DNA). Like acyclovir, BVDU turned out to be a potent and selective anti-HSV agent (74). In its potency and selectivity against HSV-1, BVDU exceeded all other anti-HSV agents, including acyclovir and the older 5-substituted 2'-deoxyuridines such as IDU, TFT, and 5-ethyl-2'-deoxyuridine (EDU) (58) (Fig. 2).

The (E)-bromovinyl group, with the bromine in the trans (or Entgegen) configuration, is crucial for the antiviral selectivity of BVDU, and hence various other compounds that have been synthesized since BVDU and that share with BVDU the

![FIG. 1. Arabinofuranosyl adenine and acyclic nucleoside analogs. Ara-A, vidarabine; 9-β-D-arabinofuranosyladenine; ACV, acyclovir; 9-(2-hydroxyethoxymethyl)guanine; GCV, ganciclovir; 9-(1,3-dihydroxypropoxy)methyl)guanine; PCV, penciclovir; 9-(4-hydroxy-3-hydroxymethyl-but-1-yl)guanine; VACV, valaciclovir; l-valyl ester of acyclovir; FCV, famciclovir, diacetyl ester of β-deoxypenciclovir.]

![FIG. 2. 5-Substituted 2'-deoxyuridines. IDU, idoxuridine; 5-iodo-2'-deoxyuridine; TFT, trifluridine; 5-trifluoromethyl-2'-deoxyuridine; EDU, 5-ethyl-2'-deoxyuridine; BVDU, brivudin; (E)-5-(2-bromovinyl)-2'-deoxyuridine; BVaraU, sorivudine; 1-β-D-arabinofuranosyl-(E)-5-(2-bromovinyl)uracil; C-BVDU, carbocyclic BVDU; BTDU, 5-(5-bromothien-2-yl)-2'-deoxyuridine.]
same 5-(2(E))-bromovinyl substituent demonstrate similar selectivity, potency, and activity spectrum to those of BVDU; these include BVaraU (sorivudine) (119), C-BVDU (carbocyclic BVDU) (97), and S-BVDU (4(thio)-BVDU) (Fig. 2) (185). Also, BTDU (Fig. 2), containing a 5-(5-bromothien-2-yl) substituent with a bromine group in roughly the same position as in BVDU, exhibits similar selectivity and potency to BVDU (188).

The mechanism of action of BVDU (Fig. 3) depends on a specific phosphorylation by the virus-encoded TK, i.e., the TKs induced by HSV-1 and VZV, which convert the compound to its 5'-monophosphate (BVDU-MP) and 5'-diphosphate (BVDU-DP) (5, 79). Upon further phosphorylation by cellular kinase(s), i.e., nucleoside 5'-diphosphate (NDP) kinase, BVDU 5'-triphosphate (BVDU-TP) can act in a dual fashion with the viral DNA polymerase: (i) as a competitive inhibitor with respect to the natural substrate (dTTP) (3) or (ii) as an alternative substrate, which would then allow BVDU-TP to be incorporated (as BVDU-MP) into the DNA chain. This incorporation may, in turn, affect both the stability and the functioning of the DNA. In fact, a close correlation has been found between the incorporation of BVDU into HSV-1 DNA, viral infectivity, and DNA integrity (16, 120).

BVDU displays a remarkable specificity in its antiviral activity spectrum: it is a highly potent inhibitor of HSV-1 but not HSV-2, so that it can be used as a marker for differentiating HSV-2 from HSV-1 strains (57). The reason for the relative inactivity of BVDU against HSV-2 is that the HSV-2-encoded TK is unable to phosphorylate BVDU-MP to BVDU-DP. This results in a substantial reduction in the supply of the active BVDU metabolite, BVDU-TP, in the HSV-2-infected cells (5, 90).

BVDU and its arabinofuranosyl counterpart BVaraU belong to the most potent inhibitors of VZV that have ever been described. At nanomolar concentrations, they inhibit the replication of VZV (4, 158). This is in marked contrast to the established anti-VZV drugs, acyclovir and penciclovir, whose inhibition of VZV replication takes place only at micromolar concentrations.

BVDU is also a potent inhibitor of the replication of Epstein-Barr virus (EBV) in vitro (118), but not CMV or human herpesvirus 6 (HHV-6) and 7 (HHV-7) (148). In addition, BVDU has been found effective against a number of herpesviruses of veterinary importance such as swine herpesvirus type 1 (SHV-1), bovine herpesvirus type 1 (BHV-1), simian varicella virus (SVV), and herpesvirus platyrrhini (HVP) but not equine herpesvirus type 1 (EHV-1) (57). As for HSV-2, the insensitivity of EHV-1 to BVDU could be attributed to an inefficient phosphorylation of BVDU-MP to BVDU-DP in the virus-infected cells (107).

Due to its unique propensity for some virus-encoded TKs, BVDU could also be used as a cytostatic agent, provided that the tumor cells have been transfected by the virus-specific TK. Thus, murine mammary FM3A carcinoma cells transformed with the HSV-1 TK gene are inhibited in their growth by BVDU at 1 ng/ml, a 10,000-fold-lower concentration than that required to inhibit the untransformed cancer cells (12, 13). The cytostatic activity of BVDU against cancer cells that have been transfected by the HSV-1 (or HSV-2) TK gene is due to the inhibitory effect of BVDU-MP (formed intracellularly by the viral TK) on the cellular thymidylate synthase (14, 21). In addition to BVDU, S-BVDU and BTDU (but not BVaraU) can serve as HSV TK-activated cytostatic agents targeted at the thymidylate synthase (24, 41). BVDU and its congeners S-BVDU and BTDU should therefore be considered potential candidate drugs for the treatment of HSV TK gene-transfected tumors.
From a clinical viewpoint, BVDU has been used for many years in the topical treatment (as 0.1% eyedrops) of herpetic keratitis, since it is efficacious against various forms of herpetic keratitis (dendritic and geographic corneal ulcers, and stromal keratitis) that were clinically resistant to other antiviral drugs (i.e. idoxuridine, fluridine, vidarabine, or acyclovir) (122).

Initial (uncontrolled) clinical trials have pointed to the effectiveness of oral BVDU (at a dose of 7.5 mg/kg/day [four 125-mg tablets] in adults or 15 mg/kg/day in children for 5 days) in the treatment of HSV-1 and VZV infections (38, 175, 189). The efficacy of BVDU in the treatment of herpes zoster was then proven in a randomized double-blind trial, in which oral BVDU (at 7.5 mg/kg/day [four 125-mg tablets] for 5 days) was at least as effective as intravenous acyclovir (at 30 mg/kg/day) (122).

TABLE 1. Antiviral activity spectrum of HPMPC

<table>
<thead>
<tr>
<th>Virus</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine polyomavirus</td>
<td>Active</td>
</tr>
<tr>
<td>Human polyomavirus</td>
<td>Active</td>
</tr>
<tr>
<td>Rabbit papillomavirus</td>
<td>Active</td>
</tr>
<tr>
<td>HPV (several types)</td>
<td>Active</td>
</tr>
<tr>
<td>Human adenoviruses (several types)</td>
<td>Active</td>
</tr>
<tr>
<td>HSV-1</td>
<td>Active</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Active</td>
</tr>
<tr>
<td>VZV</td>
<td>Active</td>
</tr>
<tr>
<td>EBV</td>
<td>Active</td>
</tr>
<tr>
<td>HCMV</td>
<td>Active</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Active</td>
</tr>
<tr>
<td>HHV-7</td>
<td>Active</td>
</tr>
<tr>
<td>TK</td>
<td>Active</td>
</tr>
<tr>
<td>VZV</td>
<td>Active</td>
</tr>
<tr>
<td>PK</td>
<td>Active</td>
</tr>
<tr>
<td>HCMV</td>
<td>Active</td>
</tr>
<tr>
<td>SVV</td>
<td>Active</td>
</tr>
<tr>
<td>EHV-1</td>
<td>Active</td>
</tr>
<tr>
<td>BHV-1</td>
<td>Active</td>
</tr>
<tr>
<td>BHV-2</td>
<td>Active</td>
</tr>
<tr>
<td>MCMV</td>
<td>Active</td>
</tr>
<tr>
<td>RCVM</td>
<td>Active</td>
</tr>
<tr>
<td>GPCMV</td>
<td>Active</td>
</tr>
<tr>
<td>HPV (several types)</td>
<td>Active</td>
</tr>
<tr>
<td>Murine adenovirus</td>
<td>Active</td>
</tr>
<tr>
<td>African swine fever virus</td>
<td>Active</td>
</tr>
<tr>
<td>HBV</td>
<td>Inactive</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Active</td>
</tr>
</tbody>
</table>

*TK*, thymidine kinase deficient; *PK*, protein kinase deficient; MCMV, murine CMV; RCVM, rat CMV; GPMCV, guinea pig CMV.  
*Inactive or only slightly active.*
appears to be susceptible (presumably at the transcription level) to the inhibitory effect of S-adenosylhomocysteine hydrolase inhibitors (123). The therapeutic potential of these compounds has yet to materialize.

Starting with DHPA and phosphonoacetic acid, which had long been recognized as an anti-DNA virus agent (and gave rise to phosphonoformic acid [foscarnet] [136], which is now used in the treatment of CMV disease), (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) (Fig. 4) was conceived as a hybrid molecule combining the features of an acyclic nucleoside analog and a phosphonate analog (60). HPMPA was found to be active against a broad spectrum of DNA viruses, including papovaviruses, adenoviruses, herpesviruses, iridoviruses, poxviruses, and hepadnaviruses (75), and its in vivo efficacy was demonstrated in a variety of animal models for HSV and vaccinia virus infection (78).

Starting from HPMPA, several other HPMP analogs were synthesized, and while the hypoxanthine, uracil, and thymine derivatives (termed HPMPHx, HPMPU, and HPMPT, respectively) were virtually inactive, the 2,6-diaminopurine guanine and cytosine derivatives (termed HPMPDAP, HPMPG, and HPMPC, respectively) exhibited marked activity against various DNA viruses (76).

The activity spectrum of HPMPC (Cidofovir) (Fig. 4) encompasses papovaviruses (polyomaviruses and papillomaviruses), adenoviruses, herpesviruses, iridoviruses, and poxviruses (Table 1), and virtually all members of the herpesvirus family, including the TK-deficient HSV and VZV strains and the protein kinase-deficient CMV strains that are resistant to acyclovir and ganciclovir, respectively, have been found to be susceptible to HPMPC (62). The antiviral efficacy of HPMPC has been demonstrated in a large variety of animal models (62). When HPMPC was compared with the “classical” antiviral drugs, it proved clearly more efficacious than acyclovir against HSV infection in mice (71) and more efficacious than ganciclovir against CMV infection in mice (133). Also, in a rabbit model for viral retinitis, HPMPC, following intravitreal injection, afforded a more potent and prolonged antiviral effect than ganciclovir did (89).

HPMPC has been used primarily as an anti-CMV agent: it...
inhibits human CMV (HCMV) replication at a concentration about 1,000-fold lower than the concentration that is toxic to the host cells (160), and this selectivity is also reflected in the 1,000-fold-lower concentration required for HPMPC to inhibit HCMV DNA synthesis than for it to inhibit host cell DNA synthesis (132). To achieve this inhibitory effect on viral DNA synthesis, HPMPC must be converted intracellularly to its active metabolite, the diphosphorylated form of HPMPC, HPMPCpp (Fig. 5). HPMPC enters the cells by endocytosis (46) and is then metabolized, presumably through successive phosphorylation by a pyrimidine nucleoside monophosphate (PNM) kinase and NDP kinase, to HPMPCpp (45, 101). HPMPCpp acts as both a competitive inhibitor and an alternative substrate of dCTP in the viral DNA polymerase reaction, thereby exhibiting a much greater affinity (lower $K_i$) for the DNA polymerases of HSV-1, HSV-2, and HCMV than for the cellular DNA polymerases $\alpha$, $\beta$, and $\gamma$ (43, 98). The incorporation of a single HPMPC molecule into DNA by HCMV DNA polymerase does not lead to chain termination (192). To effectively terminate the DNA chain (Fig. 5), two consecutive HPMPC molecules must be incorporated at the 3' end (191).

HPMPC adds a new dimension to the “art” of antiviral chemotherapy in that it confers a long-lasting antiviral response that lasts for several days (or even weeks) after a single administration, which allows infrequent dosing of the compound (167). This prolonged antiviral response may be attributed to the accumulation inside the cells of the HPMPC metabolites HPMPCp (half-life, 24 h), HPMPCpp (half-life, 65 h), and HPMPCp choline (half-life, 87 h) (1, 98). In particular, the HPMPCp choline adduct would act as a reservoir for the generation of HPMPCpp, thus explaining the prolonged antiviral activity of HPMPC. In keeping with the long intracellular half-life of HPMPCpp and HPMPCp choline, a prolonged elimination phase (up to 36 h) has been observed for HPMPC in monkeys (49), which again supports infrequent doses of the drug for antiviral therapy.

From a clinical viewpoint (Table 2), HPMPC has been pursued primarily for the treatment of CMV, HSV, and human papillomavirus (HPV) infections. The dose-limiting toxicity following systemic (i.e., intravenous) administration of HPMPC has proven to be nephrotoxicity, and this necessitates the concomitant administration of probenecid. The cyclic form of HPMPC, cHPMPC (Fig. 4), is significantly less nephrotoxic.

### Table 2. Major clinical indications of HPMPC

<table>
<thead>
<tr>
<th>Topical</th>
<th>Systemic$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucocutaneous HSV lesions (HPMPC gel), particularly when resistant to acyclovir</td>
<td>CMV retinitis (in immunocompromised patients)</td>
</tr>
<tr>
<td>Recurrent genital herpes (HPMPC gel)</td>
<td>Systemic CMV infections (in immunocompromised patients)</td>
</tr>
<tr>
<td>Mucocutaneous HPV lesions (HPMPC gel or intratumoral injections)</td>
<td>HSV and VZV infections (in immunocompromised patients), particularly when resistant to acyclovir</td>
</tr>
<tr>
<td>HSV or adenovirus keratoconjunctivitis (HPMPC eyedrops)</td>
<td>EBV-associated diseases such as hairy leukoplakia and others (to be further explored)</td>
</tr>
</tbody>
</table>

$^a$ Intravenous.

### Table 3. Antiviral activity spectrum of PMEA and PMPA

<table>
<thead>
<tr>
<th>Virus</th>
<th>Activity of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMEA</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>Active</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Active</td>
</tr>
<tr>
<td>VZV</td>
<td>Active</td>
</tr>
<tr>
<td>EBV</td>
<td>Active</td>
</tr>
<tr>
<td>HCMV</td>
<td>Active</td>
</tr>
<tr>
<td>TK HSV</td>
<td>Active</td>
</tr>
<tr>
<td>TK VZV</td>
<td>Active</td>
</tr>
<tr>
<td>Hepadnaviridae</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>Active</td>
</tr>
<tr>
<td>DHBV</td>
<td>Active</td>
</tr>
<tr>
<td>Retroviridae</td>
<td></td>
</tr>
<tr>
<td>HIV-1</td>
<td>Active</td>
</tr>
<tr>
<td>HIV-2</td>
<td>Active</td>
</tr>
<tr>
<td>SIV</td>
<td>Active</td>
</tr>
<tr>
<td>FIV</td>
<td>Active</td>
</tr>
<tr>
<td>Viscna/maedi virus</td>
<td>Active</td>
</tr>
<tr>
<td>Feline leukemia virus</td>
<td>Active</td>
</tr>
<tr>
<td>LP-BMS (murine AIDS) virus</td>
<td>Active</td>
</tr>
<tr>
<td>MSV</td>
<td>Active</td>
</tr>
</tbody>
</table>

$^a$ Inactive or only slightly active.

### Figure 6

Acyclic nucleoside phosphonates. PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PMEDAP, 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine; PMPA, (R)-9-(2-phosphonylmethoxypropyl)adenine; PMPDAP, (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine; bis(POM)-PEMA, bis(pivaloyloxymethyl)ester of PMEA.

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The text continues with detailed explanations and discussions regarding the mechanism of action, clinical applications, and the pharmacological profile of HPMPC.
than HPMPC (39) because it is more efficiently secreted by the kidneys (48), and, since this is reflected by an improved therapeu tic index, cHPMPC has also been selected as a drug candidate for further development.

HPMPC has been recently approved by the U.S. Food and Drug Administration for the systemic (intravenous) treatment of CMV retinitis in patients with AIDS. This approval came after it was found that HPMPC, when administered intravenously at 5 mg/kg once weekly for 2 weeks (induction therapy) and then once every other week (maintenance therapy), extended the median time to progression of retinitis from 22 days (deferred group) to 120 days (treated group) \( P < 0.0001 \) (114). Not only did HPMPC prove efficacious in delaying the progression of previously untreated CMV retinitis, but also it caused a significant delay in the progression of relapsing CMV retinitis (following treatment with ganciclovir). Prolonged arrest of the progression of CMV retinitis has also been obtained following intravitreal injection of HPMPC in patients with AIDS (105, 106). Following a single intravitreal injection of 20 \( \mu \)g of HPMPC, progression of the disease was stopped for a median time of 55 days; if this injection was repeated, it took a median time of 63 days for the disease to progress (105).

HPMPC has been hailed as a new topical treatment for resistant HSV infections (161). In two patients, an AIDS patient with a perineal HSV-2 infection resistant to acyclovir, and a bone marrow transplant recipient with an orofacial HSV-1 infection resistant to both ganciclovir and foscarnet, topical treatment with 1% HPMPC (in Beeler base or Orabase) once daily for three consecutive days resulted in complete regression of the lesions (163). When the HSV lesions recurred, the recurring virus appeared to have regained susceptibility to acyclovir, but it then redeveloped resistance to it, so that the HPMPC treatment had to be reinstituted. At no point during the recurrent herpesvirus episodes did the virus develop resistance to HPMPC (163). These observations point to the usefulness of HPMPC in the topical treatment of acyclovir-resistant HSV infections. The fact that TK \( ^2VZV \), while resistant to acyclovir, remains susceptible to HPMPC (164) also points to the potential usefulness of HPMPC for the treatment of acyclovir-resistant VZV infections.

When intravenously administered HPMPC was being evaluated for its anti-CMV activity based on inhibition of CMV excretion in the urine (112), it was noted that an intercurrent HSV-2 infection that was refractory to acyclovir responded remarkably well to HPMPC treatment (111); a patient with AIDS who had had a 6-month history of acyclovir-resistant

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**FIG. 7. Mechanism of action of PMEA.** Following uptake by the cells and intracellular phosphorylation (by either AMP kinase or 5-phosphoribosyl-1-pyrophosphate [PRPP] synthetase) to the diphosphoryl derivative PMEApp, the latter acts as a DNA chain terminator in the RT reaction.
perineal HSV-2 infection showed, following four weekly doses of intravenous HPMPC at 5 mg/kg/week, an almost complete healing of the lesions. Another patient in this study group had bilateral oral hairy leukoplakia (presumably due to Epstein-Barr virus infection): the lesions resolved within 1 week after a single intravenous administration of HPMPC (5 mg/kg) (112).

Two randomized, double-blind, placebo-controlled trials have recently demonstrated the efficacy of HPMPC topical gel (at 0.3, 1, or 3%) in the treatment of acyclovir-resistant HSV infections in AIDS patients (113) and in the (single-dose) therapy of recurrent genital herpes in immunocompetent patients (153), respectively.

Of major clinical relevance is the activity of HPMPC against HIV-associated lesions, which was first demonstrated in a patient with extensive squamous papillomatous lesions of the hypopharynx and esophagus (180): the lesions showed a complete and permanent regression following a few weekly injections of 1.25 mg of HPMPC into the tumor. A similar complete and permanent regression has been noted following intratumoral injection of HPMPC in a number of patients with recurrent laryngeal papillomatosis (166). Finally, HPMPC topical gel or ointment (at 1%) has been found to induce a complete regression of relapsing anogenital HPV lesions in three patients with AIDS (165), and a double-blind, placebo-controlled trial (in immunocompetent patients) has now been initiated to corroborate the initial clinical findings with HPMPC in the topical treatment of genital warts.

**SELECTIVE INHIBITORS OF RETROVIRUSES AND HEPADNAVIRUSES**

Simultaneously with HPMPA, [9-(2-phosphonylmethoxyethyl)adenine (PMEA) was mentioned as an antiviral agent (75). PMEA (Adefovir) (Fig. 6) can be considered a truncated derivative of carbovir. Whereas the antiviral activity spectrum of PMEA and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) encompasses retroviruses, hepadnaviruses, and herpesviruses, the activity spectrum of PMPA, (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine (PMPDAP), and FPMPA is restricted to retroviruses and hepadnaviruses (Table 3). In particular, PMPA and PMPDAP were found to be highly potent inhibitors of HSV replication in cell culture and MSV-induced tumor formation in mice (22). Subsequently, PMPA and PMPDAP were also found to be highly potent and selective inhibitors of visna virus replication (173), human HBV and duck HBV replication (96), and FIIV infection both in vitro and in vivo in cats (179). From both the mouse and cat experi-
ments, PMPA and PMPDAP emerged as more potent and more selective antiretrovirus agents than PMEA or PMEDAP (22, 179).

PMEA has proved efficacious in the preexposure prophylaxis of SIV infection in macaques. When PMEA was administered subcutaneously at 20 mg/kg/day for 28 days starting 48 h before SIV inoculation, it protected 83% of the macaques from acute SIV infection (177); this compared favorably with the minimal effect seen with zidovudine (AZT) (at a daily dose of 100 mg/kg for 4 weeks), where only 6% of the macaques were protected from infection (176). With PMEA, some toxic side effects in the form of mild skin lesions were observed, and with AZT, there were signs of hematologic toxicity. However, PMPA was investigated under similar experimental conditions (subcutaneous injection of a daily dose of 30 mg/kg for 4 weeks, starting either 48 h before, 4 h after, or 24 h after virus inoculation), it completely prevented the establishment of SIV infection in 100% of the macaques without any sign of toxicity (178). More recent data indicate that PMPA (given at 30 mg/kg/day for 4 weeks) is also effective in the treatment of established SIV infection (2 to 3 log10 unit reduction in the viral load in plasma) (40), and in preventing intravaginal SIV transmission (if applied topically as a 10% PMPA gel) (127).

To exert their antiviral action, PMEA, PMPA, and their congeners must be phosphorylated to their diphosphorylated form (PMEApp, PMPApp, etc.) (Fig. 7). This could occur in either one step, catalyzed by PRPP (5-phosphoribosyl 1-pyrophosphate) synthetase (11), or two consecutive steps, catalyzed by AMP kinase (126). In human lymphoid cells, PMEA would be anabolized to PMEApp mainly through (mitochondrial) AMP kinase (150). In their diphosphorylated form, PMEA, PMPA, and their congeners act as alternative substrates to dATP/chain terminators in the HIV reverse transcriptase (RT) reaction (17, 18). Also, the inhibitory effects of PMEA, PMPA, and their congeners on HBV replication may be attributed to chain termination in the HBV-associated RT reaction. PMEApp and PMPApp apparently possess a greater affinity for HIV (and HBV) RT than for the cellular DNA polymerases α, β, γ, δ, and ε (44, 109), and this may explain their selectivity for retroviruses and hepadnaviruses.

From a clinical viewpoint (Table 4), PMEA, in its oral prodrug from bis(POM)-PMEA, has proved effective in reducing the viral load in HIV-infected individuals (35). Bis(POM)-PMEA has also been the subject of clinical studies in patients with HBV infection and has recently entered a multicentered clinical phase II/III trial for the treatment of HIV infection. Given the potential of PMEA to inhibit herpesviruses (e.g., CMV), HIV-infected individuals treated with bis(POM)-PMEA will be monitored for HIV as well as CMV load reductions.

PMPA has only recently become the subject of clinical trials, but in view of its pronounced therapeutic and prophylactic
while the dideoxynucleosides in their 5'-triphosphate form would inhibit the virus-associated RT reaction by terminating DNA chain elongation (61). These efforts yielded a number of selective HIV inhibitors (65, 66) that have now been approved for the treatment of HIV infections (AIDS), i.e., zidovudine (AZT) (115), didanosine (ddI) (87), zalcitabine (ddC) (187), stavudine (d4T) (117), and lamivudine (3TC) (Fig. 8). Some of these dideoxynucleoside analogues, particularly 3TC, are, like PMEA and PMPA, also active against HBV, and 3TC is being further tested for the treatment of HBV infections.

Other dideoxynucleoside analogs such as FddaraA, 1592U389, and (-)FTC (Fig. 8) are still under development as candidate anti-HIV drugs. The goal of these efforts (66, 67) has been to increase the safety (activity/toxicity) profile of the anti-HIV agents, thereby providing a greater choice of potential candidates for drug combinations. Indeed, it has become increasingly clear that to achieve the greatest benefit in terms of reduction of viral load, increase in CD4 cell counts, delay of resistance development, and clinical outcome, the compounds should be combined in multiple-drug regimens.

**SELECTIVE INHIBITORS OF HIV-1**

While the dideoxynucleosides in their 5'-triphosphate form and the acyclic nucleoside phosphonates, PMEA and PMPA, in their diprophosphorylated form interact as competitive inhibitors or alternative substrates for the substrate-binding site of the HIV RT, nonnucleoside RT inhibitors (NNRTIs) interact with a non-substrate-binding site that is located in the close vicinity of the substrate-binding site. NNRTIs can be readily differentiated from the nucleoside type of RT inhibitors in that they inhibit HIV-1 but not HIV-2 replication. The first compounds that were found to behave in this way were the 1-[2-(hydroxyethyl)methyl]-6-(phenylthio)thymine (HEPT) derivatives (6, 128) and the tetrahydroimidazo[4,5,1-k][1,4]-benzodiazepin-2(1H)-one and -thione (TIBO) derivatives (53, 140). These compounds were then shown to exert this unique selectivity for HIV-1 through a specific interaction with the HIV-1 RT that, unlike the interaction of the nucleoside type of RT inhibitors, appeared to be noncompetitive with regard to the normal substrates (7, 8, 53).

Since the descriptions of HEPT and TIBO, literally dozens of different classes of NNRTIs have been described, and all these compounds were found to inhibit HIV-1 but not HIV-2 replication in vitro at concentrations well below the cytotoxicity threshold: dipyridodiazepinones (e.g., nevirapine) (125), pyridinones (e.g., L-697,661) (92), bis(heteroaryl)piperazines (e.g., BHAP U-88204) (151), 2,5'-bis-(O-(tet-butylmethylsilyl)-3'-spiro-5''-(4''-amino-1''-2''-oxathiole-2''-dioxide)pyrimidine (TSAO) derivatives (e.g., TSAO-m-T) (20), α-anilinophenacylamides (α-APA) (141), quinoxalines (e.g., S-2720) (108), and PETT derivatives (2). Meanwhile, the original HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and

<table>
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<th>NNRTI</th>
<th>Wild type (HIV-1 H100)</th>
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<th>HIV-1 K103N</th>
<th>HIV-1 V106A</th>
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| * Data from references 27, 30, 32, 34a, and 72.  
| * Yes, EC₅₀ ≤ 1 μM; No, EC₅₀ > 1 μM.  

**TABLE 5. Antiviral activity spectrum of NNRTIs**

While the dideoxynucleosides in their 5'-triphosphate form and the acyclic nucleoside phosphonates, PMEA and PMPA, in their diprophosphorylated form interact as competitive inhibitors or alternative substrates for the substrate-binding site of the HIV RT, nonnucleoside RT inhibitors (NNRTIs) interact with a non-substrate-binding site that is located in the close vicinity of the substrate-binding site. NNRTIs can be readily differentiated from the nucleoside type of RT inhibitors in that they inhibit HIV-1 but not HIV-2 replication. The first compounds that were found to behave in this way were the 1-[2-(hydroxyethyl)methyl]-6-(phenylthio)thymine (HEPT) derivatives (6, 128) and the tetrahydroimidazo[4,5,1-k][1,4]-benzodiazepin-2(1H)-one and -thione (TIBO) derivatives (53, 140). These compounds were then shown to exert this unique selectivity for HIV-1 through a specific interaction with the HIV-1 RT that, unlike the interaction of the nucleoside type of RT inhibitors, appeared to be noncompetitive with regard to the normal substrates (7, 8, 53).

Since the descriptions of HEPT and TIBO, literally dozens of different classes of NNRTIs have been described, and all these compounds were found to inhibit HIV-1 but not HIV-2 replication in vitro at concentrations well below the cytotoxicity threshold: dipyridodiazepinones (e.g., nevirapine) (125), pyridinones (e.g., L-697,661) (92), bis(heteroaryl)piperazines (e.g., BHAP U-88204) (151), 2,5'-bis-(O-(tet-butylmethylsilyl)-3'-spiro-5''-(4''-amino-1''-2''-oxathiole-2''-dioxide)pyrimidine (TSAO) derivatives (e.g., TSAO-m-T) (20), α-anilinophenacylamides (α-APA) (141), quinoxalines (e.g., S-2720) (108), and PETT derivatives (2). Meanwhile, the original HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and 8-HEPT and TIBO parent compounds had given rise to new and more active congeners, i.e., I-EBU (MKC-442) (9) and
FIG. 10. Positioning of NNRTI in its “pocket” site in the HIV-1 RT heterodimer (p66-p51).

(a) RT heterodimer colored by crystallographic B-factor: the regions colored blue are the most rigid (B < 30 Å²), those in white are intermediate, and the red regions are the most flexible (B > 30 Å²). Regions marked A and A' (α12) are residues 421 to 428 in the p66 and p51 subunits, respectively, while B and B' mark residues 218 to 241 in each subunit (β9 to β11 in p66); these two regions differ markedly between the two subunits. Reprinted from reference 146 with permission of the publisher.

(b) Overlap of the three NNRTIs TIBO R86183, nevirapine, and α-APA R95845 in their complexes with RT. The secondary-structure elements which form the pocket site are depicted as ribbons. The inhibitors are shown in ball-and-stick models: TIBO, green; α-APA, yellow; and nevirapine, light purple. Also shown are the side chains of the surrounding amino acid residues. Reprinted from reference 81 with permission of the publisher.

(c) Schematic representation of the NNRTIs showing the butterfly shape (with the two wings) and the interactions between different positions of the butterfly and the surrounding amino acid residues. All amino acid residues making contact with the NNRTIs are from the p66 subunit, except for E138, which is from the p51 subunit. Reprinted from reference 81 with permission of the publisher.
may lead to cross-resistance among the different NNRTI classes, this is not inevitably so. As shown in Table 5, most of the NNRTIs retain sufficient activity (here, arbitrarily set at a 50% effective concentration of \( \leq 1 \mu M \)) against RT-mutant HIV-1 strains that have become resistant to other NNRTIs (27, 30, 72). In particular, the quinoxalines (S-2720 and HBY 097) and thiocarboxanilides (UC-82 and UC-781) at concentrations below 1 \( \mu M \) (or even below 0.1 \( \mu M \) [32], i.e., concentrations that are readily attainable therapeutically, have proved active against various HIV mutant strains (L100I, K103N, V106A, E138K, and Y181C).

NNRTIs owe their specific inhibitory effect on HIV-1 RT to a specific interaction with the enzyme at a site that is both functionally and spatially associated with, but distinct from, the substrate binding site in the palm domain of the p66 subunit of the HIV-1 RT p66-p51 heterodimer (172). The NNRTI-binding site could best be described as a flexible hydrophobic pocket that would open only in the presence of the inhibitor (82, 172). As shown for TIBO, HEPT, nevirapine, and \( \alpha \)-APA, the NNRTIs can easily dock into this pocket (Fig. 10a), largely based on hydrophobic interactions (146). Within the pocket, the NNRTIs would roughly overlie each other and assume a remarkably similar conformational shape (147), reminiscent of a butterfly (81) (Fig. 10b and c). The side chains of the amino acid residues surrounding the pocket could adapt to each bound inhibitor so as to make tight van der Waals contacts (110) and thus account for the highly specific interaction between the NNRTI and its HIV-1 RT target site.

Of the NNRTIs, only one (nevirapine) has so far been formally approved for the treatment of HIV-1 infections, and the approval of a second one (delavirdine) is pending. Clinical studies with other NNRTIs are either far advanced (loviride), under way (HBY 097), just started (MKC-442), or about to start (thiocarboxanilides). The development of the NNRTIs for clinical use has been hampered by poor oral bioavailability (TIBO R82913 [80]), rapid development of viral resistance (nevirapine [149], pyridinone L-697,661 [152]), or toxic side effects such as rash and pruritus (nevirapine [94]). However, loviride (R89439) does not seem to suffer from these drawbacks (170), and this justifies the further study of this compound in the treatment of HIV infections. Loviride should be used in drug combinations rather than monotherapy so as to counteract the rapid emergence of drug-resistant virus (169).

NNRTIs are highly potent inhibitors of HIV-1 replication that could be used in the prevention and therapy of HIV-1 infections, including those that have become resistant to other...
drugs such as AZT, ddI, ddC, d4T, or 3TC. The NNRTIs should be used as part of a drug combination regimen whose administration should be started as soon as possible after the infection at doses that are sufficiently high to completely suppress virus replication (see the section on knocking out the virus, below).

In parallel with the NNRTIs, inhibitors of the HIV protease have been investigated as therapeutic modalities to block the HIV replicative cycle. The HIV protease is encoded by the viral genome and is responsible for the cleavage of the gag-pol precursor and pol precursor proteins to the mature viral proteins (i.e., the capsid [gag] proteins [p17, p24, p7, and p6], protease [p11], RT [p51], RNase H [p15], and integrase [p34]). This proteolytic cleavage is needed for the maturation and hence the infectivity of the virus particles, and, thus, HIV protease inhibitors may be expected to suppress virus production and infectivity.

The search for specific inhibitors of the HIV protease has yielded a wealth of products that are now available for clinical use (saquinavir, ritonavir, and indinavir) or are in clinical development (nelfinavir, VX-478, KNI-272, DMP-323, and U-140690, among others) (Fig. 11). Most of these compounds (except for DMP-323 and U-140690, which are cyclic urea and dihydropyrene derivatives) can be considered “peptidomimetics,” mimicking the peptide bond that is normally cleaved by the HIV protease.

In clinical trials, saquinavir (135), ritonavir, indinavir, and other protease inhibitors (such as nelfinavir) (129, 157) have been found to achieve a marked and sustained reduction in viral load, particularly when combined with the RT inhibitor AZT or ddC (or 3TC), and it is anticipated that drug combination regimens including one or more protease inhibitors, when complied with properly, may be able to suppress virus replication and prevent drug resistance from emerging for an extended or indefinite period.

**KNOCKING OUT THE VIRUS**

All antiviral agents that are targeted at a specific viral protein (or function) may be expected to lead sooner or later to the development of drug resistance. For the NNRTIs, emergence of drug-resistant virus strains occurs quite rapidly, and (the fear for) this resistance has markedly slowed the clinical and commercial development of these anti-HIV-1 agents. Yet the problem of HIV-1 resistance to NNRTIs can be prevented or circumvented by the following strategies (64, 68, 69): (i) switching from one compound to another if there is no cross-resistance between the two compounds (Table 5); (ii) combining different compounds, particularly those that show an antagonistic resistance pattern (i.e., mutually counteracting resistance mutations); (iii) using from the start sufficiently high (“knocking-out”) concentrations of the individual compounds so as to prevent the breakthrough of any virus, whether resistant or not; and (iv) using from the start sufficiently high concentrations of different compounds combined, which could then achieve a knocking out of the virus at lower concentrations than if the compounds were used individually.

What seems to be the best strategy to overcome HIV-1 resistance to NNRTIs, and may also be applicable to other anti-HIV agents (e.g., HIV protease inhibitors) and antiviral compounds in general, is to use these compounds from the beginning at sufficiently high concentrations so as to knock out, i.e., completely prevent breakthrough of, the virus. Thus, TIBO R82913, nevirapine, pyridinone L-697,661, and BHAP U-88204 were found to prevent virus breakthrough (as attested by the absence of several viral parameters such as viral cytopathicity, p24 antigen, viral envelope glycoproteins, and proviral DNA) for at least 40 days if added at 2.5 or 10 μg/ml to HIV-1-infected cell cultures (23). Also, BHAP U-88204 at 1 μM was found to completely block HIV-1 replication in cell culture for at least 15 days (181), and BHAP U-90152 (delavirdine) has been shown to prevent viral spread for at least 85
days when added to the HIV-1-infected cells from the start at a concentration of 3 μM (84).

Whereas nevirapine and BHAP U-88204 had to be added to the HIV-1-infected cell cultures at 10 and 2.5 μg/ml, respectively, to completely prevent virus breakthrough, the thiocarboxanilide UC-10 did so at concentrations of only 0.5 to 1 μg/ml (30). For the quinoxaline S-2720, the minimum concentration required to completely prevent virus breakthrough was 0.1 μg/ml, and for the quinoxaline HBY 097 it was even lower (0.01 μg/ml). That the quinoxalines at these concentrations really knocked out the virus was ascertained by the absence of proviral DNA (25). Also, the newer thiocarboxanilide derivatives, UC-10, UC-781, and UC-82, were found to completely prevent virus breakthrough when used at concentrations as low as 0.05 to 0.1 μg/ml (32). Thus, the quinoxaline S-2720 and HBY-097 and the thiocarboxanilides UC-781, UC-82, and UC-10 (Fig. 12) were able to knock out the virus in vitro at a concentration (0.1 μg/ml) that should be readily attainable in vivo.

The knocking-out strategy has also been shown to work with HIV-1 RT mutant strains: in cells infected with the TSAO-resistant E138K mutant, virus replication could be completely suppressed by any NNRTI other than TSAO (i.e., TIBO R82913, nevirapine, and BHAP U-88204) if added at sufficiently high concentrations (2.5, 10, and 10 μg/ml, respectively) (26). Also, HIV protease inhibitors are able to knock out the virus: long-term treatment of HIV-infected MT-4 cells with saquinavir at 0.1 μM resulted in a complete “sterilization” of the cells, so that after 3 months of treatment the drug could be withdrawn safely and HIV was cleared demonstrably from the cell culture (134).

When different anti-HIV agents are combined, e.g., NNRTIs with one another or with AZT or 3TC, they are able to prevent virus breakthrough for a longer time and at lower drug concentrations than when they are used as single agents (Fig. 13). This has been clearly demonstrated for combinations of different NNRTIs (i.e., TSAO-m3T, MKC-442, delavirdine, or thiocarboxanilide) with 3TC (34) and also for the combination of MKC-442 with AZT (138). For example, when TSAO-m3T and 3TC were used individually at 0.4 and 0.1 μg/ml, respectively, they delayed virus breakthrough for 13 days. If, however, the drugs at these concentrations were used in combination, they prevented virus breakthrough for more than 52 days (Fig. 13a), and upon further examination, the cells appeared to be cleared of the virus (34).

Also, combinations of two different NNRTIs, e.g., TSAO-m3T with the thiocarboxanilide UC-42, result in a much longer suppression of virus breakthrough (>77 days) than do the two compounds used individually (16 to 20 days) (30). If a third drug (e.g., delavirdine) is added to this dual drug combination, virus breakthrough is suppressed for an even longer time and at lower drug concentrations than with the single drugs or two-drug combinations (30). In fact, several three-drug combinations, e.g., containing 3TC, TSAO-m3T, or UC-42 and delavirdine or MKC-442, have been found to prevent virus breakthrough for more than 45 days under conditions in which the individual compounds suppressed virus replication for only 9 to 16 days (34).
OUTLOOK FOR ANTI-HIV DRUGS

Could the knocking-out principle be expected to work in vivo in the HIV-infected individual? Recent studies have indicated that viremia in HIV-1 infection is sustained by rapid, high-level viral replication, accompanied by a rapid cell turnover, with a composite life span (half-life) of virus in plasma and virus-infected cells of only 2 days (100, 186). Further kinetic analyses (143) indicated that the extent of viremia depends on the rate of virus production, independent of the disease stage. The higher level of viremia and, by inference, the higher rate of virus production are associated with a higher risk of disease progression and thus with a poorer prognosis (124). Kinetic analyses (143) also indicated that the extent of viremia and, by inference, the higher rate of virus production are associated with a higher risk of disease progression and thus with a poorer prognosis (124).

Here, a cautionary note should be added. When extrapolating from in vitro data on knocking-out concentrations to the in vivo setting, one should be aware of the various pharmacokinetic factors that may affect the drug concentrations that are eventually achieved at the site(s) of virus replication. These factors include the bioavailability of the drug from its portal of entry (i.e., the gastrointestinal tract if the drug is given orally), distribution of the drug through the body fluids and tissues, its metabolism (i.e., by the liver) and excretion (i.e., by the kidneys), its binding to plasma proteins, and its interaction (i.e., by the kidneys) with the metabolism of other drugs. All these factors may significantly influence the drug concentrations that are eventually achieved at the site(s) where they are expected to suppress (knockout) virus replication.

Several studies (42, 124, 137, 139) have addressed whether viral load is correlated with disease progression, and these studies have invariably demonstrated that long-term nonprogressive HIV infection is associated with a low viral load (42, 139). Both a decrease in the HIV-1 RNA level in plasma and an increase in the CD4⁺ T-cell count, taken together, may be valid predictors of the clinical outcome following anti-HIV drug treatment (137), but a decrease in the HIV-1 RNA level is a better predictor of outcome than is an increase in the CD4⁺ T-cell count. The extent of viremia, monitored as HIV-1 RNA, can be considered the best marker of HIV-1 disease progression (124) (Fig. 14) (99), which implies, from a therapeutic viewpoint, that there should be a close correlation between the reduction in HIV-1 RNA levels in plasma in response to antiretroviral therapy and the clinical outcome.

The data that have so far been obtained from clinical studies with both NNRTIs and HIV protease inhibitors support the guidelines deduced from the in vitro studies. Thus, at higher drug dosage levels, both NNRTIs (e.g., nevirapine) and HIV protease inhibitors (e.g., saquinavir and ritonavir) offer a more durable antiviral response and a longer delay in the emergence of drug-resistant virus (68). In three studies where a three-drug combination regimen (AZT plus ddI plus nevirapine, AZT plus ddI plus delavirdine, or AZT plus 3TC plus lopinavir) was compared with the two-drug combination (AZT plus ddI or AZT plus lopinavir), the three-drug combination regimen invariably proved superior in reducing the viral titers in plasma (50, 51, 171). It would now seem imperative to examine how long the antiviral response could be extended and how long the appearance of drug-resistant mutants could be delayed, by using combinations of different drugs at sufficiently high (knocking-out) doses.

When combinations of different drugs are used at knocking-out concentrations, they should not enable the virus to develop resistance. Consequently, no drug-resistant virus may emerge, and the patient may be cleared of actively replicating virus. The problem is, however, how much latent virus may remain in the organism and where would it hide? As long as it is not expressed, the latent proviral DNA will not be affected by the knocking-out approach. However, by the same token, as long as it stays latent, the proviral DNA will not affect the host either. Only when expressed, thus generating a productive infection, will the proviral DNA be harmful and endanger the host immune system, but at that point, the virus will become susceptible to the antiviral drugs.

What, therefore, could be expected from anti-HIV drugs in general, and NNRTIs in particular, is that when they are administered from the beginning at sufficiently high doses in multiple-drug combination regimens, they may achieve a pronounced and sustained reduction in viral load. The result may even be a complete suppression of HIV production for an indefinite period, so that the risk for progression to AIDS (Fig. 14) would be substantially reduced or even annihilated.

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