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In Vitro Activity of 2,4-Diamino-6-[2-(Phosphonomethoxy)Ethoxy]-Pyrimidine against Multidrug-Resistant Hepatitis B Virus Mutants▽

M. N. Brunelle,[1,2,3] J. Lucifora,[1,2,3] J. Neyts,[4] S. Villet,[1,2,3] A. Holy,[5] C. Trepo,[1,2,3,6] and F. Zoulim[1,2,3,6]*

INSERM, U871, 151 Cours Albert Thomas, 69003 Lyon, France[1]; Université Lyon 1, 69008 Lyon, France[2]; IFR62 Lyon Est, Faculté de Médecine Laennec, 69008 Lyon, France[3]; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium[4]; Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic[5]; and Hospices Civils de Lyon, Hôtel Dieu Hospital, 69002 Lyon, France[6]

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The susceptibilities of drug-resistant hepatitis B virus (HBV) mutants to lamivudine, adefovir, tenofovir, entecavir, and 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (PMEO-DAPym), a novel acyclic pyrimidine analogue, were assessed in vitro. Most drug-resistant mutants, including multidrug-resistant strains, remained sensitive to tenofovir and PMEO-DAPym. Therefore, the latter molecule deserves further evaluation for the treatment of HBV infection.

Treatment of chronic hepatitis B virus (HBV) infection requires the long-term administration of the nucleoside or nucleotide analogs lamivudine [(−)-β-L-2′,3′-dideoxy-3′thiacytidine], adefovir dipivoxil {9-[(2-phosphonylmethoxy)ethyl]adenine}, entecavir {2-amino-1,9-dihydro-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6*H*-purin-6-one, monohydrate}, or telbivudine (β-L-2′-deoxythymidine) (28). However, this leads to the emergence of HBV strains harboring mutations within the reverse transcriptase sequence that confer resistance to these drugs (14, 28, 29). The incidence of resistance increases progressively each year, reaching 70% after 4 years of lamivudine therapy and 29% after 5 years of adefovir dipivoxil therapy (9, 14).

Currently, there are two options for the treatment of patients who carry lamivudine-resistant mutants. Lamivudine can be switched to adefovir dipivoxil or entecavir; however, this results in the risk of development of adefovir resistance (7, 8) or entecavir resistance (4, 19) in the long term. Adefovir dipivoxil can also be added to ongoing lamivudine monotherapy (7, 8) to delay the further development of resistance, as both drugs have a favorable cross-resistance profile when they are used in combination (1, 22, 29). However, the emergence of HBV strains simultaneously harboring lamivudine and adefovir resistance mutations was recently reported within the viral quasispecies of a patient who successively failed lamivudine and lamivudine-adefovir dipivoxil add-on therapy (24). The HBV-resistant mutants that are selected after the successive failure of lamivudine and entecavir therapies are resistant to both drugs (20, 23, 26). Thus, the development of novel HBV inhibitors is needed to overcome HBV drug resistance and to design new combination strategies to delay or prevent the development of drug resistance.

Different nucleoside analogs are currently in development. Recently, 2,4-diamino-6-[(2-phosphonomethoxy)ethoxy]-pyrimidine (PMEO-DAPym), an acyclic pyrimidine nucleoside analog phosphonate, was shown to inhibit human immunodeficiency virus (HIV) and HBV replication in vitro with a potency comparable to those of adefovir and tenofovir {(*R*)-9-[(2-phosphonylmethoxy)propyl]adenine} (2, 11, 27). Tenofovir has been approved as anti-HIV therapy and is in phase III trials for the treatment of HBV infection (25). Moreover, PMEO-DAPym proved to have equipotent activities against wild-type (wt) and lamivudine-resistant rtM204V mutant HBV isolates in inducible transfected hepatoma cell lines (27). In the present study, we investigated in transiently transfected Huh7 cells the cross-resistance profiles of a series of drug-resistant HBV mutants, including multiple-drug-resistant strains, to PMEO-DAPym and to other drugs in parallel assays.

We first determined the effects of the compounds on Huh7 cell viability by determining the concentration of drug that reduced the uptake of neutral red dye by 50% (the 50% cell cytotoxic concentration [CC_{50}]), as described before (10). Transient transfection of Huh7 cells was then performed, as described previously (6), with plasmids containing 1.1 genome unit of a wt or a mutant HBV strain under the control of the chicken beta-actin promoter. One group of constructs contained the genome of HBV laboratory strains (genotype D, serotype ayw), including wt and resistant HBV mutants, obtained by site-directed mutagenesis (lamivudine-resistant mutant rtL180M/M204V, adefovir-resistant mutant rtN236T, and lamivudine- and adefovir-resistant mutant rtL180M/M204V/N236T) (3, 6, 17). The second group of constructs contained HBV genomes cloned from the viral quasispecies of two patients chronically infected with HBV who failed sequential therapy with currently approved HBV inhibitors (23, 24). The following clinical isolates (cloned HBV genomes) were studied: lamivudine-resistant mutants rtL180M/M204V, rtL180M/A181V, and rtV173L/L180M/M204V; lamivudine- and adefovir-resistant mutants rtV173L/L180M/A181V, rtV173L/L180M/A181V/M204V, rtV173L/L180M/A181V/M204V/N236T, and rtV173L/L180M/A181V/N236T; and entecavir-resistant mutant rtL180M/S202G/M204V. Antiviral assays with transfected cells, purification of intracellular HBV DNA, and analysis of HBV

* Corresponding author. Mailing address: INSERM, U871, 151 Cours Albert Thomas, 69424 Lyon cedex 03, France. Phone: 33 4 72 68 19 70. Fax: 33 4 72 68 19 71. E-mail: zoulim@lyon.inserm.fr.
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TABLE 1. Activities of PMEO-DAPym and selected compounds against wild-type HBV replication and cell viability in Huh7 cells

Compound	EC_{50} (μM)[a]	CC_{50} (μM)[b]
Lamivudine	1 ± 1.1	>1,000
Entecavir	0.3 ± 0.42	125 ± 35
Adefovir	13 ± 29	365 ± 120
Tenofovir	16 ± 7.9	>1,000
PMEO-DAPym	4.9 ± 0.6	>1,000

[a] For each drug, the EC_{50} value is the mean of the EC_{50}s for wild-type HBV presented in Tables 2 and 3.
[b] CC_{50}s are mean values ± SDs for three independent experiments performed in quadruplicate.

DNA by Southern blotting were performed as described previously (3, 6).

As shown in Table 1, in Huh7 cells, PMEO-DAPym had little or no effect on cell viability (CC_{50}, >1,000 μM), as was also the case for lamivudine and tenofovir. The CC_{50} values for entecavir and adefovir were 125 ± 35 μM and 365 ± 120 μM, respectively. Furthermore, PMEO-DAPym had no effect on HBsAg production by wt HBV transfected cells (data not shown). When the anti-HBV activity was assessed, entecavir proved to be the most potent compound, with the lowest 50% effective concentration (EC_{50}), followed by lamivudine, PMEO-DAPym, adefovir, and tenofovir. The EC_{50} of PMEO-DAPym was three- to fourfold lower than those of adefovir and tenofovir under our in vitro conditions (Table 1). The EC_{50} of PMEO-DAPym was higher under our in vitro conditions with Huh7 cells than the EC_{50}s obtained with a stable cell line derived from HepG2 cells (27). These types of EC_{50} variations between Huh7 and HepG2 cells have already been observed previously with other nucleoside analogs (17); however, the ranking of antiviral potency was not affected. This may indicate that the intracellular metabolism, including entry, transport, phosphorylation, and pumping out of this nucleoside analog, may depend on the cell lines used for the experiment.

PMEO-DAPym inhibited the replication of both laboratory and clinical lamivudine-resistant HBV variants rtL180M/M204V and rtV173L/L180M/M204V as efficiently as it did with wt HBV (Tables 2 and 3). The rtL180M/A181V mutant displayed a 4.8-fold decreased susceptibility to PMEO-DAPym. However, among the drugs studied, tenofovir was the only one which inhibited this mutant as well as wt HBV (Table 3). Lamivudine-resistant HBV strains show decreased susceptibility to entecavir compared with the susceptibilities of the wt HBV strains (Tables 2 and 3). Interestingly, laboratory HBV strain rtL180M/M204V, which was engineered by site-directed mutagenesis (Table 2), was more susceptible to entecavir than its counterpart derived from one patient (Table 3). Discrepancies between the susceptibilities to entecavir of laboratory- or patient-derived HBV rtL180M/M204V strains have already been observed (20) and may be explained by differences in the genetic backgrounds of the strains outside of the polymerase region that has been cloned.

As reported previously, the rtN236T mutation identified in patients who failed adefovir dipivoxil therapy decreased the sensitivity to adefovir by 3.2- to 7.3-fold (1, 3, 22) and that to tenofovir by 4.5-fold (3) (Table 2). The rtL180M/S202G/M204V mutant, identified in a patient who failed successive lamivudine and entecavir therapies (23), displayed a 210-fold resistance to entecavir and a >100-fold resistance to lamivudine (Table 3). Interestingly, both adefovir- and entecavir-resistant HBV strains were sensitive to PMEO-DAPym (Tables 2 and 3).

All four mutants resistant to lamivudine-adefovir, characterized in a patient who failed sequential therapy, displayed 2.1- to 5.1-fold decreased susceptibilities to PMEO-DAPym, depending on the combination of mutations that they harbored (Table 3). The EC_{50}s of PMEO-DAPym for mutants rtV173L/L180M/A181V, rtV173L/L180M/A181V/M204V, and rtV173L/L180M/A181V/M204V/N236T were lower than that of tenofovir and similar to that of tenofovir for mutant rtV173L/L180M/A181V/N236T. However, the resistance factor observed for all four lamivudine-adefovir-resistant mutants was slightly higher for PMEO-DAPym compared to that for tenofovir. PMEO-DAPym and tenofovir had greater inhibitory activities than lamivudine and entecavir against these multiple-drug-resistant mutants; the factors for resistance to adefovir for these mutants were slightly higher, but the in vivo pharmacological characteristics of adefovir preclude its use at higher dosages (15). The inhibitory activities of the evaluated compounds against the rtV173L/L180M/A181V/N236T mutant (lamivudine and adefovir escape mutant) were in the following order of potency: tenofovir > PMEO-DAPym > entecavir > adefovir > lamivudine.

Our results provide direct information regarding the cross-

TABLE 2. Effects of selected anti-HBV drugs on replication of wt HBV and HBV laboratory strains of genotype D carrying lamivudine, adefovir, or lamivudine-adefovir resistance mutations[a]

HBV strain[b]	LAM[c]		ADV[c]		TDF[c]		ETV[c]		PMEO-DAPym	
	EC_{50} (μM)	FR[d]	EC_{50} (μM)	FR	EC_{50} (μM)	FR	EC_{50} (μM)	FR	EC_{50}[e] (μM)	FR
wt	2.48 ± 0.67	1	15.8 ± 1.9	1	10.3 ± 1.3	1	0.8 ± 0.1	1	4.0 ± 0.51	1
ADV^r	2.65 ± 0.52	1.06	50.3 ± 11	3.2	46 ± 6	4.5	0.7	0.88	4.5 ± 0.35	1.1
LAM^r	>100	>40	15.5 ± 1.8	0.98	35.2 ± 5.1	3.4	5 ± 0.25	6.25	4.7 ± 1.12	1.2
LAM^r + ADV^r	>100	>40	100 ± 20	6.3	45.5 ± 6.1	4.4	5 ± 0.7	6.25	5.7 ± 0.77	1.4

[a] LAM, lamivudine; ADV, adefovir; TDF, tenofovir; ETV, entecavir.
[b] Lamivudine-resistant (LAM^r) mutant rtL180M/M204V, adefovir-resistant (ADV^r) mutant rtN236T, and lamivudine-adefovir-resistant mutant (LAM^r + ADV^r) rtL180M/M204V/N236T.
[c] Data were reported previously (3).
[d] FR, fold resistance, which is equal to (mutant EC_{50})/(wt EC_{50}).
[e] The values represent the means of at least three independent experiments, each of which was performed in triplicate. For each experiment, the drug-resistant HBV strains and their corresponding wt strain were treated simultaneously with the same range of drug concentrations (from 0 to 100 μM for PMEO-DAPym, lamivudine, adefovir, and tenofovir; from 0 to 10 μM for entecavir), and all the samples were extracted and analyzed by Southern blotting in parallel.

TABLE 3. Effects of selected anti-HBV drugs on replication of HBV mutants derived from the viral quasispecies of chronically infected patients[a]

HBV strain[b]	LAM		ADV		TDF		ETV		PMEO-DAPym	
	EC_{50} (μM)	FR[c]	EC_{50} (μM)	FR	EC_{50} (μM)[d]	FR	EC_{50} (μM)[d]	FR	EC_{50} (μM)[d]	FR
wt 1	0.64 ± 0.17[d]	1	13.6 ± 4.08[d]	1	13.6 ± 4	1	0.09 ± 0.03	1	4.4 ± 0.5	1
wt 2	0.1 ± 0.2[e]	1	10 ± 3[e]	1	25 ± 7.1	1	0.06 ± 0.01	1	5.6 ± 1.0	1
LAM^r										
rtL180M/M204V	>100[e]	>1,000	15 ± 6[e]	1.5	27 ± 10	1.1	10.5 ± 2.2	175	5.3 ± 0.88	0.9
rtL180M/A181V	80 ± 9[e]	800	27 ± 16[e]	2.7	36 ± 13	1.4	1.5 ± 0.6	28	27 ± 10.3	4.8
rtV173L/L180M/M204V	>100[d]	>156	9.8 ± 2.5[d]	0.7	16 ± 5.8	1.2	3.7 ± 1.4	43	4.1 ± 0.4	0.9
LAM^r + ADV^r										
rtV173L/L180M/A181V	100 ± 5[e]	1,000	48 ± 19[e]	4.8	42 ± 8.1	1.6	2.75 ± 1.2	50	24 ± 4.5	4.3
rtV173L/L180M/A181V/M204V	>100[e]	>1,000	40 ± 20[e]	4.0	45 ± 21.3	1.8	≥50 ± 15.9	≥800	18 ± 7.0	3.2
rtV173L/L180M/A181V/M204V/N236T	>100[e]	>1,000	77 ± 20[e]	7.7	46 ± 18.3	1.8	25.4 ± 5.5	461	12 ± 3.0	2.1
rtV173L/L180M/A181V/N236T	>100[e]	>1,000	>100[e]	>10	28 ± 5.6	1.1	0.5 ± 0.14	9.0	29 ± 5.3	5.1
ETV^r										
rtL180M/S202G/M204V	>100[d]	>156	15 ± 4.5[d]	1.1	27 ± 9.8	2	18 ± 8.7	210	4.5 ± 0.9	1.0

[a] LAM, lamivudine; ADV, adefovir; TDF, tenofovir; ETV, entecavir.

[b] LAM^r, lamivudine resistant; LAM^r + ADV^r, lamivudine-adefovir resistant; ETV^r, entecavir resistant.

[c] FR, fold resistance, which is equal to (mutant EC_{50})/(wt EC_{50}). For mutants rtV173L/L180M/M204V and rtL180M/S202G/M204V, the corresponding wt strain is wt1 (genotype H) and the fold resistance is equal to (mutant EC_{50})/(wt1 EC_{50}). For the other mutants, the corresponding wt strain is wt2 (genotype E) and the fold resistance is equal to (mutant EC_{50})/(wt2 EC_{50}).

[d] Values represent the means of at least three independent experiments, each of which was performed in triplicate. For each experiment, the drug-resistant HBV strains and their corresponding wt strains were treated simultaneously with the same range of drug concentrations (from 0 to 100 μM for PMEO-DAPym, lamivudine, adefovir, and tenofovir; from 0 to 10 μM for entecavir), and all the samples were extracted and analyzed by Southern blotting, in parallel.

[e] The data were reported previously (24).

resistance profiles of the lamivudine-, lamivudine-adefovir-, and entecavir-resistant HBV strains isolated from patients who failed sequential therapy. It is noteworthy that entecavir may not represent the best anti-HBV agent for the treatment of patients who have failed a lamivudine therapy, as lamivudine may lead to the emergence of HBV variants harboring the rtL180M/M204V or rtL180M/A181V mutation, which impairs the antiviral effect of entecavir (Tables 2 and 3). Moreover, long-term entecavir treatment of patients infected with lamivudine-resistant HBV strains leads to the selection of secondary mutations that, in the presence of a genetic background of lamivudine-resistant mutations, confer increased resistance to entecavir (20, 23). Nevertheless, entecavir may be valuable for the treatment of patients who have failed adefovir dipivoxil therapy since mutants harboring the rtN236T mutation, in the absence of the lamivudine resistance mutation rtM204V, retained susceptibility to entecavir (Tables 2 and 3) (3). Tenofovir displayed activity against wt HBV similar to that of adefovir (Table 1) and efficiently inhibited the replication of a series of lamivudine-, adefovir-, lamivudine-adefovir-, and entecavir-resistant HBV strains (Tables 2 and 3). Clinically, tenofovir has been used successfully for the treatment of patients who successively failed lamivudine and lamivudine-adefovir dipivoxil therapy (16, 23, 24). Several clinical reports have suggested the potent anti-HBV activity of tenofovir in patients who failed adefovir therapy and, moreover, the better anti-HBV activity of tenofovir over that of adefovir in patients who failed lamivudine therapy (13, 21), which may be due to the better pharmacokinetic properties of tenofovir. Whether tenofovir may select for drug-resistant mutants in patients remains a matter of controversy (5, 18).

The development of novel strategies for HBV therapy that may be based on the combination of various nucleoside analogs with different cross-resistance profiles will require the discovery of novel HBV inhibitors. We recently demonstrated the in vitro potency of 2′,3′-dideoxy-3′-fluoroguanosine for the inhibition of wt, lamivudine-, adefovir-, and lamivudine-adefovir-resistant laboratory HBV strains (12). In the present study, we confirmed the findings of previous studies that showed that PMEO-DAPym is a potent inhibitor of wt HBV in vitro (27). Interestingly, we provide new information showing that PMEO-DAPym inhibits the replication of lamivudine-, entecavir-, adefovir-, and lamivudine-adefovir-resistant mutants almost as efficiently as it does that of wt HBV (Tables 2 and 3). The in vitro cross-resistance profile of PMEO-DAPym for the laboratory and clinical strains studied here proved to be more favorable than the cross-resistance profiles of lamivudine, adefovir, and entecavir and was more or less comparable to that of tenofovir.

Interestingly, PMEO-DAPym efficiently inhibited all HBV variants harboring the rtL180M/M204V mutation, which is the most frequently observed lamivudine resistance-conferring mutation found in patients (14, 30) (Tables 2 and 3). Until now, only purine analogs, such as adefovir or tenofovir, have shown activity against the replication of the lamivudine-resistant rtL180M/M204V mutant, which is resistant to lamivudine and all known pyrimidine L-nucleosides (29). Thus, although PMEO-DAPym does not carry a purine base, it exhibits the same cross-resistance profile as purine-based nucleoside phosphonate analogs. This supports our earlier assumption (based on molecular modeling) that the 2,4-diamino-substituted pyrimidine ring of PMEO-DAPym can be viewed as a open-ring analog of the purine system in the 2,6-diaminopurine acyclic nucleoside phosphonate derivatives (27). Although most adefovir- and lamivudine-adefovir-resistant HBV strains retained some degree of susceptibility to adefovir in vitro (Table 2 and 3), the clinical efficacy of adefovir is limited by its nephrotoxicity when the daily dose of adefovir dipivoxil is increased from 10 to 30 mg (15). Under our experimental conditions, tenofovir and PMEO-DAPym exhibited the most favorable in vitro cross-resistance profiles as inhibitors of the replication of multiple-drug-resistant HBV genomes derived from clinical strains

from patients who failed sequential therapy with currently approved HBV inhibitors. Therefore, it will be interesting to determine the pharmacodynamics of PMEO-DAPym in vivo.

In conclusion, the broad inhibitory activity of PMEO-DAPym against HBV drug-resistant mutants and its favorable cytotoxicity profile, observed in tissue culture experiments, warrants the further preclinical evaluation of this compound in animal models of hepadnavirus infections.

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REFERENCES

1. **Angus, P., R. Vaughan, S. Xiong, H. Yang, W. Delaney, C. Gibbs, C. Brosgart, D. Colledge, R. Edwards, A. Ayres, A. Bartholomeusz, and S. Locarnini.** 2003. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology **125:**292–297.
2. **Balzarini, J., C. Pannecouque, E. De Clercq, S. Aquaro, C. F. Perno, H. Egberink, and A. Holy.** 2002. Antiretrovirus activity of a novel class of acyclic pyrimidine nucleoside phosphonates. Antimicrob. Agents Chemother. **46:** 2185–2193.
3. **Brunelle, M. N., A. C. Jacquard, C. Pichoud, D. Durantel, S. Carrouee-Durantel, J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2005. Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir. Hepatology **41:**1391–1398.
4. **Chang, T. T., R. G. Gish, S. J. Hadziyannis, J. Cianciara, M. Rizzetto, E. R. Schiff, G. Pastore, B. R. Bacon, T. Poynard, S. Joshi, K. S. Klesczewski, A. Thiry, R. E. Rose, R. J. Colonno, and R. G. Hindes.** 2005. A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. Gastroenterology **129:**1198–1209.
5. **Delaney, W. E. T., A. S. Ray, H. Yang, X. Qi, S. Xiong, Y. Zhu, and M. D. Miller.** 2006. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob. Agents Chemother. **50:**2471–2477.
6. **Durantel, D., S. Carrouee-Durantel, B. Werle-Lapostolle, M. N. Brunelle, C. Pichoud, C. Trepo, and F. Zoulim.** 2004. A new strategy for studying in vitro the drug susceptibility of clinical isolates of human hepatitis B virus. Hepatology **40:**855–864.
7. **Fung, S. K., P. Andreone, S. H. Han, K. Rajender Reddy, A. Regev, E. B. Keeffe, M. Hussain, C. Cursaro, P. Richtmyer, J. A. Marrero, and A. S. Lok.** 2005. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J. Hepatol. **43:**937–943.
8. **Fung, S. K., H. B. Chae, R. J. Fontana, H. Conjeevaram, J. Marrero, K. Oberhelman, M. Hussain, and A. S. Lok.** 2006. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J. Hepatol. **44:** 283–290.
9. **Hadziyannis, S. J., N. C. Tassopoulos, E. J. Heathcote, T. T. Chang, G. Kitis, M. Rizzetto, P. Marcellin, S. G. Lim, Z. Goodman, J. Ma, S. Arterburn, S. Xiong, G. Currie, and C. L. Brosgart.** 2005. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N. Engl. J. Med. **352:**2673–2681.
10. **Hantz, O., C. Borel, C. Trabaud, F. Zoulim, J. Dessolin, M. Camplo, P. Vlieghe, M. Bouygues, C. Trépo, and J. L. Kraus.** 1997. Selective inhibition of the duck hepatitis B virus by a new class of tetraazamacrocycles. Antimicrob. Agents Chemother. **41:**2579–2581.
11. **Holy, A., I. Votruba, M. Masojidkova, G. Andrei, R. Snoeck, L. Naesens, E. De Clercq, and J. Balzarini.** 2002. 6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with antiviral activity. J. Med. Chem. **45:**1918–1929.
12. **Jacquard, A. C., M. N. Brunelle, C. Pichoud, D. Durantel, S. Carrouee-Durantel, C. Trepo, and F. Zoulim.** 2006. In vitro characterization of the anti-hepatitis B virus activity and cross-resistance profile of 2′,3′-dideoxy-3′-fluoroguanosine. Antimicrob. Agents Chemother. **50:**955–961.
13. **Lacombe, K., J. Gozlan, P. Y. Boelle, L. Serfaty, F. Zoulim, A. J. Valleron, and P. M. Girard.** 2005. Long-term hepatitis B virus dynamics in HIV-hepatitis B virus-co-infected patients treated with tenofovir disoproxil fumarate. AIDS **19:**907–915.
14. **Lai, C. L., J. Dienstag, E. Schiff, N. W. Leung, M. Atkins, C. Hunt, N. Brown, M. Woessner, R. Boehme, and L. Condreay.** 2003. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin. Infect. Dis. **36:**687–696.
15. **Marcellin, P., T. T. Chang, S. G. Lim, M. J. Tong, W. Sievert, M. L. Shiffman, L. Jeffers, Z. Goodman, M. S. Wulfsohn, S. Xiong, J. Fry, and C. L. Brosgart.** 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N. Engl. J. Med. **348:**808–816.
16. **Ratziu, V., V. Thibault, Y. Benhamou, and T. Poynard.** 2006. Successful rescue therapy with tenofovir in a patient with hepatic decompensation and adefovir resistant HBV mutant. Comp. Hepatol. **5:**1.
17. **Seigneres, B., C. Pichoud, P. Martin, P. Furman, C. Trepo, and F. Zoulim.** 2002. Inhibitory activity of dioxolane purine analogs on wild-type and lamivudine-resistant mutants of hepadnaviruses. Hepatology **36:**710–722.
18. **Sheldon, J., N. Camino, B. Rodes, A. Bartholomeusz, M. Kuiper, F. Tacke, M. Nunez, S. Mauss, T. Lutz, G. Klausen, S. Locarnini, and V. Soriano.** 2005. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. Antivir. Ther. **10:**727–734.
19. **Sherman, M., C. Yurdaydin, J. Sollano, M. Silva, Y. F. Liaw, J. Cianciara, A. Boron-Kaczmarska, P. Martin, Z. Goodman, R. Colonno, A. Cross, G. Denisky, B. Kreter, and R. Hindes.** 2006. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology **130:**2039–2049.
20. **Tenney, D. J., S. M. Levine, R. E. Rose, A. W. Walsh, S. P. Weinheimer, L. Discotto, M. Plym, K. Pokornowski, C. F. Yu, P. Angus, A. Ayres, A. Bartholomeusz, W. Sievert, G. Thompson, N. Warner, S. Locarnini, and R. J. Colonno.** 2004. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. Antimicrob. Agents Chemother. **48:**3498–3507.
21. **van Bommel, F., B. Zollner, C. Sarrazin, U. Spengler, D. Huppe, B. Moller, H. H. Feucht, B. Wiedenmann, and T. Berg.** 2006. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level during adefovir therapy. Hepatology **44:**318–325.
22. **Villeneuve, J. P., D. Durantel, S. Durantel, C. Westland, S. Xiong, C. L. Brosgart, C. S. Gibbs, P. Parvaz, B. Werle, C. Trepo, and F. Zoulim.** 2003. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J. Hepatol. **39:**1085–1089.
23. **Villet, S., A. Ollivet, C. Pichoud, J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2007. Stepwise process for the development of entecavir resistance in a chronic hepatitis B virus infected patient. J. Hepatol. **46:**531–538.
24. **Villet, S., C. Pichoud, J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2006. Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient. Gastroenterology **131:**1253–1261.
25. **Wong, S. N., and A. S. Lok.** 2006. Tenofovir disoproxil fumarate: role in hepatitis B treatment. Hepatology **44:**309–313.
26. **Yim, H. J., M. Hussain, Y. Liu, S. N. Wong, S. K. Fung, and A. S. Lok.** 2006. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology **44:**703–712.
27. **Ying, C., A. Holy, D. Hockova, Z. Havlas, E. De Clercq, and J. Neyts.** 2005. Novel acyclic nucleoside phosphonate analogues with potent anti-hepatitis B virus activities. Antimicrob. Agents Chemother. **49:**1177–1180.
28. **Zoulim, F.** 2006. Antiviral therapy of chronic hepatitis B. Antivir. Res. **71:**206–215.
29. **Zoulim, F.** 2004. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antivir. Res. **64:**1–15.
30. **Zoulim, F., T. Poynard, F. Degos, A. Slama, A. El Hasnaoui, P. Blin, F. Mercier, P. Deny, P. Landais, P. Parvaz, and C. Trepo.** 2006. A prospective study of the evolution of lamivudine resistance mutations in patients with chronic hepatitis B treated with lamivudine. J. Viral Hepat. **13:**278–288.