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REVIEW

Diagnostic and therapeutic progress of multi-drug resistance with anti-HBV nucleos(t)ide analogues

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Abstract

Nucleos(t)ide analogues (NA) are a breakthrough in the treatment and management of chronic hepatitis B. NA could suppress the replication of hepatitis B virus (HBV) and control the progression of the disease. However, drug resistance caused by their long-term use becomes a practical problem, which influences the long-term outcomes in patients. Liver transplantation is the only choice for patients with HBV-related end-stage liver disease. But, the recurrence of HBV after transplantation often caused by the development of drug resistance leads to unfavorable outcomes for the recipients. Recently, the multi-drug resistance (MDR) has become a common issue raised due to the development and clinical application of a variety of NA. This may complicate the antiviral therapy and bring poorly prognostic outcomes. Although clinical evidence has suggested that combination therapy with different NA could effectively reduce the viral load in patients with MDR, the advent of new antiviral agents with high potency and high genetic barrier to resistance brings hope to antiviral therapy. The future of HBV researches relies on how to prevent the MDR occurrence and develop reasonable and effective treatment strategies. This review focuses on the diagnostic and therapeutic progress in MDR caused by the anti-HBV NA and describes some new research progress in this field.

Key words: Hepatitis B virus; Multi-drug resistance; Nucleos(t)ide analogues; Gene mutation; Liver transplantation

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INTRODUCTION

Globally, it is estimated that about 400 million are chronically infected with hepatitis B virus (HBV). The prevention and control of hepatitis B is an important public health issue. HBV infection may not only result in fulminant, subfulminant, and chronic hepatitis, but may also contribute to the development of liver cirrhosis and hepatocellular carcinoma in the long-term[1]. Decades of clinical experiences have shown that the administration of nucleos(t)ide analogues (NA) could postpone the progression of the disease. NA mainly suppresses the

HBV replication and reduces the damage to hepatocytes by hindering the synthesis of reverse transcriptase (RT), which is the prerequisite for viral replication. The main disadvantage of NA is the recurrence of HBV after therapy interruption. Hence, the long-term treatment becomes a mandate. But, this paves the way for other challenges like HBV genome mutation and clinical drug resistance. The reports of multi-drug resistance (MDR) to NA in the recent years are increasing, and become an important issue for clinicians to modulate the treatment strategies during drug resistance.

MECHANISMS OF ANTIVIRAL RESISTANCE

HBV has a DNA genome. But, it replicates through an RNA intermediated reverse transcription. The lack of proofreading ability of the viral encoded, RNA-dependent DNA polymerase could result in potential mutations at each nucleotide position within the entire genome[2]. This, together with the large number of virions produced (10^{12}-10^{13}/d), indicates that every mutation of the 3.2 kb HBV genome can theoretically be produced daily[3,4]. Under the selective pressure of antiviral agents, such diversity is likely to give rise to drug-resistant mutants[5]. RT is the target of NA and also the chief place of gene mutations. Several factors are associated with the development of antiviral resistance, including viral fitness, potency, and genetic barrier to resistance of the antiviral agents[6]. Studies have shown that there are five pathways leading to antiviral resistance[7]. (1) The L-nucleoside pathway (rtM204V/I), leads to the resistance of lamivudine (LAM), emtricitabine, telbivudine (LdT), and clevudine. This pathway includes entecavir (ETV) resistance in LAM-experienced patients; (2) The acyclic phosphonate pathway (rtN236T), associates with the resistance to adefovir (ADV) and tenofovir disoproxil fumarate (TDF)[8,9]; (3) A shared pathway (rtA181T/V), results in the selection of HBV quasispecies when treated with L-nucleosides or acyclic phosphonates;(4) Naïve entecavir resistance pathway (rtL180M + rtM204V with either rtT184, S202, or M250 codon changes). In this pathway, three mutations are required to appear simultaneously accounting for the very low resistance profile of entecavir[10]; and (5) Multidrug resistance pathway, complex patterns and clusters of specific mutations in HBV polymerase are associated with it.

TDF is recommended since 2008, as first line drug in patients chronically-infected with HBV and led to high rates of virologic success[11,12]. Its effect on HBV DNA suppression appears similar to ETV and LdT and superior to LAM and ADV[13,14].It remains largely active even against ADV-resistant or ETV-resistant mutations[15-17]. However, the rtA194T polymerase mutation has been found in HBV/HIV co-infected patients during TDF treatment and may be associated with TDF resistance[18-20]. Whether the rtA194T mutation truly confers resistance against TDF has remained controversial, as the *in vitro* phenotypic assays showed variable results across laboratories[21,22]. Thus, the potential impact of this mutation on TDF susceptibility deserves further study[20]. The primary antiviral drug resistance mutations in the polymerase gene are listed in Table 1[23].

HBV strains, resistant to at least two anti-HBV agents from different subclasses of NA without a cross-resistance profile, are defined as MDR[24]. The main reasons for MDR are the sequential monotherapy to treat primary resistance and use of agents with similar cross-resistance profiles. The development of MDR is a major challenge for antiviral therapy, and the improper administration of NA may lead to serious outcomes. Thus, more researches on the choice of antiviral agents in treating patients with MDR have been carried out and some significant solutions have been achieved.

THE CURRENT SITUATION AND STRATEGIES OF DIFFERENT TYPES OF MDR

LAM + ADV resistance

LAM, the first oral antiviral agent against HBV, is safe and well tolerated even in patients with decompensated liver cirrhosis[25]. Globally, it has been mostly used with a low genetic barrier to resistance and cumulative incidence of resistance as high as 70% after 5 years of treatment[26,27]. Early studies had suggested that, ADV monotherapy had shown similar antiviral effects to combination therapy with LAM+ADV for LAM-resistant patients in the short-term, and a strategy of switching to ADV monotherapy had widely been adopted[28]. However, recent studies have showed that ADV resistant mutations emerge more frequently during sequential ADV monotherapy in LAM resistance than in treatment-naïve patients[29,30]. The rate of ADV resistance in LAM-resistant patients was shown to be as high as 18% at 1 year, compared with 0% in LAM-naïve patients[31]. Another long-term study reported that the cumulative genotypic resistance and virologic breakthrough at 5 years of sequential ADV monotherapy in LAM-resistant patients were 65.6% and 61.8%, respectively[32]. Fung *et al*[33] reported that the cumulative rate of ADV resistance in LAM-resistant patients at 2 years was 18% for patients who were switched to ADV and 7% for patients who had ADV added to their treatment regimen. In another study of 42 LAM-resistant patients (HBeAg-negative), the ADV resistance rates at 15-18 mo of treatment were 21% (3/14) for patients who were switched to ADV and 0% for patients who had ADV added[34]. It can be assumed that the ADV resistance rate in LAM-resistant patients can be greatly reduced by adding rather than switching to ADV. There are more researches exploring the mechanisms of LAM + ADV dual-resistance, as these two agents were launched early. When the mutations causing resistance to LAM and ADV are not on the same viral genome, a combination therapy of these two agents will likely be effective in suppressing the mutants

Table 1 Primary antiviral drug resistance mutations in the polymerase gene[23]

	Domain A	Domain B	Domain C	Domain D	Domain E	Numbers of mutations
Lamivudine and Telbivudine	--	rtV173L rtL180M rtA181T/V	rtM204V/I	--	--	1
Adefovir	--	rtA181T/V		rtN236T	--	1
Tenofovir	--	rtA181T/V		rtN236T	--	?
Entecavir	--	rtL180M rtT184	rtM204I/V rtS202	--	rtM250I/V	3

resistant to each of the drugs. In contrast, when the antiviral resistance mutations are on the same viral genome, the combination treatment may not be adequate[30]. *In vitro* analysis have shown that most of MDR mutations collocate on the same viral genome[35], but the *in vivo* confirmation on the same is lacking. There is no unified clinical treatment strategy for LAM + ADV dual-resistance, but different methods of mono or combination therapy have been carried out.

Due to the limited alternative of NA in the early stage, interferon (IFN) had been tried as a choice for dual-resistance to LAM and ADV. Phenotypic analysis have indicated that IFN-α suppresses equally the mutant strains and wild-type strains *in vitro*[36]. Furthermore, IFN-α also suppresses the replication of LAM-resistant and ADV-resistant mutants *in vivo*[37]. Besides that, IFN-α administration predictably have reduced the resistance to NA when combined with LAM[38,39], as IFN-α exhibits at least two HBV-specific antiviral activities independent of the viral polymerase sequence with one reducing the levels of core protein and replicative intermediates, and the other leading to posttranscriptional degradation of HBV RNA[40]. However, there are certain potential limitations with IFN therapy such as low probability of sustained response, parenteral administration, relatively poor tolerability, and frequent and potential serious adverse effects in patients with advanced liver disease[41]. These deficiencies limited the clinical application of IFN, and more researches focused on the application of oral NA in MDR.

In vitro studies show that the majority of MDR mutations to LAM and ADV collocate on the same viral genome[31]. Therefore, the combination therapy with LAM and ADV may not effectively deal with the patients, who are resistant to these two agents. The advent of ETV enabled a new choice for antiviral therapy. Since TDF is not available in many Asian countries, the 2008 updated guidelines by Asian Pacific Association for the Study of the Liver recommended ETV in LAM and ADV resistant patients[42]. But satisfactory clinical results were not acquired in these patients treated with ETV. Heo *et al*[30] compared the clinical efficacy of LAM + ADV combined therapy and ETV monotherapy in patients with dual-resistance to LAM and ADV. The mean reduction in serum HBV DNA concentration was significantly lower in the LAM + ADV than in the ETV group. But, the difference in mean decline in serum alanine aminotransferase (ALT) levels over 12 mo of treatment and the rate of HBeAg seroconversion at 12 mo did not differ significantly between two groups. Park *et al*[43] reported that ETV monotherapy could not reach an optimal clinical efficacy in ADV-refractory chronic hepatitis B patients with prior LAM resistance. In this long-term study (up to 4 years), the authors have suggested that an early virologic response is essential for a successful ETV monotherapy in this group of patients. Clinically, initial virologic response at 3 mo (IVR-3) is an independent predictor for virologic response, and it may help determine whether to maintain ETV monotherapy or not. Choe *et al*[44] evaluated the antiviral efficacy of ETV in patients, who had failed to achieve viral response during LAM and ADV rescue therapy. The virologic response was achieved in 1 of 18 patients with pre-existing rt204 mutations, whereas it was achieved in all 4 patients without pre-existing rt204 mutations regardless of the presence of rt181 or rt236 mutations. The poor treatment efficacy of ETV in patients with LAM and ADV dual-resistance might have resulted from the pre-existing rt204 mutations, which could further lead to ETV resistance.

As the ideal clinical efficacy was not achieved by ETV monotherapy, researches continued to seek other treatment strategies with ETV combination therapies. A study tried combination of ETV and ADV in LAM-resistant chronic hepatitis B patients with suboptimal response to LAM + ADV[45]. This strategy provided superior virologic response and favorable resistance profiles, when compared with combination therapy of LAM and ADV. But similar to ETV monotherapy, an optimal virologic response still could not be reached. This may likely due to the relatively low antiviral potency of ADV, which suggested to replace ADV with another drug with a similar resistance profile and higher potency against LAM-resistant mutants.

TDF shares the similar molecular structure with ADV. It has higher antiviral potency and lower rate of developing drug resistance. Despite its structural similarity to ADV, TDF partially suppresses ADV-resistant HBV, and it is also highly effective against LAM-resistant virus, suggesting that this drug may be an effective treatment for patients who have previously failed treatment with LAM and ADV[46]. Van Bommel *et al*[47] introduced TDF for LAM-Resistant patients with high HBV DNA level during ADV therapy. The administration of TDF (300 mg daily for all the patients except one) led to an undetectable HBV DNA level in 19 of 20 patients within

a median of 3.5 mo. The only patient who did not become HBV DNA negative during the observation period received a reduced TDF dose (300 mg every second day) because of renal insufficiency. Patterson *et al*[48] reported similar efficacy with TDF in another group of similar patients. But, the virologic response to TDF in this study appeared to be inferior to that observed in treatment-naïve patients. The development of antiviral resistance to TDF in the long-term is uncertain, but the combination therapy with high potency NA without cross-resistance is still a superior strategy for MDR. Recently, Petersen *et al*[49] reported a multicenter study with ETV + TDF as rescue therapy in pretreated chronic hepatitis B patents. HBV DNA was undetectable in 51 out of 57 patients with different types of resistance, and the ALT levels improved in most of them, suggesting a reduction in liver inflammation. Besides, this strategy was efficient, safe, and well tolerated in patients with and without advanced liver disease. The updated 2009 American Association for the Study of the Liver Diseases (AASLD) practice guideline recommended TDF + ETV in patients with sequential LAM and ADV treatment failure[27].

LAM + ETV resistance

ETV is an effective antiviral agent with high potency and high genetic barrier to resistance. In NA-naïve patients, the 5-year cumulative probability of genotypic ETV resistance and genotypic ETV resistance associated with virologic breakthrough was only 1.2% and 0.8%, respectively[50]. However, the emergence of resistance to ETV occurs more frequently in LAM-refractory population. Based on the previous reports, the resistance to ETV in LMV-refractory patients was detected in 8% of patients after 12 mo, 43% after 48 mo, and 51% after 60 mo of treatment[51,52]. In a clinical study, the cumulative rates of ETV genotypic resistance in patients with LAM resistance are 6%, 15%, 36%, 46%, and 51% from years 1 to 5, respectively[53]. The resistance to ETV in the sequential monotherapy in LAM-refractory patients shares the same pathway (mutation of rtM204V/I) with LAM resistance. Yatsuji *et al*[54] reported a patient with dual-resistance to LAM and ETV, but was effectively treated with ADV. Interestingly, the typical mutation strains of ETV (rtL180M + M204V + S202G) were observed in this patient. An *in vitro* study indicated that the rtL180M + M204V + S202G mutant had no resistance against ADV, and this case report confirmed this view clinically. Another study included 12 patients with dual-resistance to LAM and ETV, and half of them reached complete virological response with the combination therapy of ADV and ETV after 18 mo. In addition, no enrolled patients developed virologic breakthrough and had mutations resistant to ADV at the end of follow-up. However, not all the patients realized virologic response, which may due to the low potency of ADV and high pretreatment levels of HBV DNA[55]. This combination therapy strategy may be helpful as ETV is effective to rtA181T related mutant and ADV is effective to rtM204V related mutant. The optimal strategy for such dual-resistance patients has not been determined due to less availability of literature data.

LAM + ADV + ETV resistance

Recently, MDR to three NA has been described in case reports. Liu *et al*[56] reported LAM + ADV could be helpful in a patient, who was resistant to LAM, ADV, and ETV. It could be speculated that the HBV DNA replication had been suppressed due to LAM and ADV. The immune response against the MDR strains might also have contributed to the clearance of these strains. The exact effect of this strategy remains to be observed due to the short follow-up period. Sayan *et al*[24] reported a case of another patient, who was effectively treated with ETV and TDF. Three primer drug resistance mutations in different domains of HBV viral polymerase, such as rtA181V/T, rtL180M + rtM204V mutations and the rtN236T, were characterized in the same genome, which might explain the MDR profile. In another study of MDR, amino acid changes consisting of L80V, L91I, M204I, S219A, N238D, and Y245H were found on the same dominant viral genome strain. These newly discovered mutation types may also relate to MDR[57]. This type of resistance, usually caused by sequential monotherapy, may be effectively treated by combination therapy. However, the mechanisms and preventive methods for MDR to three NA need to be studied.

Treatment strategy of MDR in liver transplant recipients

Liver transplant is an effective method for patients with HBV-related end-stage liver disease. In a liver transplant setting, three different clinical phases have to be considered: (1) treatment of HBV infection during waiting list; (2) prophylaxis of hepatitis B recurrence after liver transplant; and (3) treatment of recurrent hepatitis B when prophylactic measures have failed[58]. The main factors associated with HBV recurrence were HBeAg status at transplant listing and serum HBV-DNA level at transplant[59]. The goals of antiviral therapy in the pretransplant patients include the reduction of viral load to low or nondetectable serum HBV DNA levels[60]. The development of drug resistance for patients in the waiting list for liver transplant is a common problem. Osborn *et al*[61] found that the antiviral therapy failure in patients with HBV in the waiting list did not impair clinical outcomes when recognized early and also when the salvage therapy was promptly initiated and neither the survival rate with transplant nor without transplant was negatively impacted by antiviral therapy failure.

However, the recurrence of HBV after transplant is still a troublesome problem and may influence the long-term outcomes. The combination of both LAM and hepatitis B immunoglobulin (HBIg) has emerged as the most effective prophylactic strategy in HBV transplant recipients with a 3-year recurrence rate of 0-10%[58,62,63]. But, the drug resistance to this combination therapy has also emerged. In addition to the typical resistance to LAM, it was detected in 45% of the immunosuppressed

patients within the first year following liver transplant[64]. The escape mutation from anti-HBs occurs in the common α determinant region of the surface gene, which is a highly conservative region of the HBsAg protein[65]. IFN is not currently used for the treatment of hepatitis B after transplantation, given the risk of graft rejection[66,67]. The availability of ADV has changed the clinical course of LAM-resistant patients, and it is effective and safe among liver transplant recipients[68-70]. Researches showed that ADV could be an effective rescue therapy for patients with LAM-resistant hepatitis B post-liver transplant[71,72]. In a retrospective review, viral recurrence was noted in 5 out of 23 liver transplant recipients, who are on combination prophylaxis of LAM and HBIg with 1 patient receiving TDF and 4 receiving ADV. Only 1 death related to HBV recurrence was reported in this population, who had been switched to ADV[73].

Apart from ADV, ETV appears to be a more attractive candidate than ADV for use in the transplant setting[74]. A study of 30 patients receiving a combination of ETV and low-dose HBIg confirmed the efficacy and safety of ETV in preventing recurrence of HBV after liver transplant[75]. Another small study of 8 patients showed that ETV was safe and effective as prophylaxis after liver transplant with no interactions observed with the immunosuppressive medications[76]. Fung *et al*[77] reported that although only 26% of patients had complete viral suppression at the time of transplant and 91% of patients underwent loss of HBsAg after 2 years of follow-up. Also, 98.8% of patients achieved undetectable HBV DNA levels with ETV as the sole antiviral agent after liver transplant. It can be inferred that ETV may play an effective role in recipients resistant to both LAM and HBIg.

ETV could be a choice in liver transplant recipients, and TDF may also be effective when used alone or in combination therapy. Villet *et al*[78] reported a recipient who was resistant to LAM, ADV, and HBIg. The combination therapy of LAM and TDF effectively suppressed the HBV DNA replication. Karlas *et al*[79] reported that the combination therapy with ETV and TDF may prevent post-liver transplant hepatitis B recurrence even without HBIg maintenance therapy. He illustrated that the combination oral antiviral therapy might substitute for HBIg as indefinite prophylactic regimen due to profound antiviral efficacy and low risk of viral resistance. The optimal combination method for MDR in liver transplant recipients is still uncertain, and the therapeutic experiences in non-transplant patients could be learned.

NEW PERSPECTIVES ON THE RESEARCH OF MDR

There are still some limitations on the current research on drug resistance. For instance, the genetic analysis of resistance to NA inhibitors is usually focused on the RT domain, and only infrequently takes into consideration of the whole genome of intra-host HBV variants[80-82]. Thai *et al*[83] reported that the rtM204I/V substitution is insufficient for establishing resistance against LAM. The analysis of 639 HBV whole-genome sequences obtained from 11 patients showed that rtM204I/V was independently acquired by more than one intra-host HBV variant, indicating the convergent nature of LAM resistance. Currently, the most commonly used methods for detecting HBV drug-resistance mutations are direct sequencing and reverse hybridization[84]. However, these methods do not enable haplotype analysis, and hence, they cannot be used to determine the collocation of mutations on the same viral genome. This limits the accurate identification of viral mutants that are resistant to drugs with high genetic barrier[85]. Recently, several next-generation sequencing technologies, such as ultra-deep pyrosequencing, are available to generate more data[85-88]. These methods may offer significant advantages in explaining and predicting the responses of HBV patients to antiviral therapy and enable quantification of HBV quasispecies variants.

Recently, some studies analyzed the mutation pattern in relation to the HBV genotypes and found that the rtL180M mutation is significantly connected to the rtM204V mutation in genotypes A, B, and C. Also, the HBV genotypes differ in their mutation pattern of LAM resistance[89]. Another study indicated the association of genotype C with higher rates of hepatitis B recurrence after transplant due to LAM resistance[90]. It can be inferred that HBV genotypes may play an important role in the progression of HBV-related liver disease and response to antiviral therapy. The assessment of HBV genotype prior to the treatment may help to individualize the antiviral therapy and reduce the incidence of treatment failures and complications[91,92]. However, the relationship between HBV genotypes and antiviral resistance is unclear. Extensive researches may provide new perspectives for the prevention and optimal rescue therapy to patients with drug resistance.

PREVENTION OF MDR

Treatment failure in anti-HBV therapy could be regarded as an iatrogenic factor, and a judicious use of NA in chronic hepatitis B patients is the most effective prophylaxis against the development of MDR. Thus, proper strategy should be applied by clinicians at the beginning of therapy. An antiviral agent with the highest potency and a high genetic barrier to resistance should be selected[93,94]. The pros and cons of initiating the treatment with combination therapy in minimizing the development of antiviral resistance are currently being investigated[95,96]. Avoiding the sequential use of NA monotherapy is an effective preventive method for MDR[24]. A roadmap suggested that HBV DNA concentration at week 12 after initial treatment should be checked to identify patients with primary treatment failure, which is defined by < 1 log copies/mL reduction of HBV DNA concentration. For patients with primary treatment failure with good drug compliance, addition of another NA is indicated. The second assessment of HBV DNA should then be

Table 2 Patterns and pathways of antiviral drug resistance in chronic hepatitis B in the context of cross-resistance[99]

Pathway	Amino acid substitution in the rt domain	LAM	LdT	ETV	ADV	TDF
	WT	S	S	S	S	S
L-nucleoside (LAM/LdT)	M204I/V	R	R	I	S	S
Acyclic phosphonate (ADV)	N236T	S	S	S	R	I
Shared (LAM, LdT, ADV)	A181T/V	R	R	S	R	I
Double(ADV, TDF)	A181T/V + N236T	R	R	S	R	R
D-Cyclopentane (ETV)	L180M + M204V/I ± I169 ± T184 ± S202 ± M250	R	R	R	S	S

I: Intermediate sensitivity; R: Resistant; S: Sensitive based on cell culture and clinical; LAM: Lamivudine; LdT: Telbivudine; ETV: Entecavir; TDF: Tenofovir; ADV: Adefovir.

Table 3 Management of antiviral-resistant hepatitis B virus in updated guidelines

	AASLD 2009[27]	EASL 2012[101]
LAM-resistance	Add ADV or TDF In patients with no prior exposure to other NA: Add LAM or ETV	Switch to TDF or add ADV In patients with no prior exposure to other NA: Switch to ETV or TDF
ADV-resistance	In patients with prior LAM resistance: Switch to TDF plus LAM or ETV	In patients with prior LAM resistance: Switch to TDF and another NA
ETV-resistance	Switch to TDF	Switch to or add TDF
TDF-resistance	--	In patients with no prior exposure to LAM: Switch to ETV In patients with prior LAM resistance: Add ETV

LAM: Lamivudine; LdT: Telbivudine; ETV: Entecavir; TDF: Tenofovir; ADV: Adefovir; NA: Nucleos(t)ide analogues.

done at week 24[97,98]. Treatment strategies in patients with partial virologic response are based on the potency and genetic barrier of the antiviral agent. Patients receiving NA with a high genetic barrier can remain the treatment beyond 48 wk. Patients receiving a less potent NA should continue treatment and be re-assessed at week 48, and those, who receive NA with a low genetic barrier, should add a more potent drug due to the high risk of resistance when the treatment is not adapted[26]. To avoid MDR, the combination therapy of NA with cross-resistance should be avoided. The patterns and pathways of antiviral drug resistance in chronic hepatitis B in the context of cross-resistance are listed in Table 2[99]. Patient's adherence to antiviral therapy is another important factor in avoiding resistance. Adherence may be monitored using patient reports, dispensed medication counts, or HBV DNA detection[93]. If low response or virologic breakthrough is observed with the primary treatment, gene sequencing should be done to find out the type and location of mutations, in order to guide the optimal rescue therapy. Recently, An *et al*[100] reported that the family history, negative conversion time of HBV DNA, and different NA were independent risk factors of gene resistant mutation, which provided a theoretical basis for predicting drug resistance and salvage treatment.

There is still no consensus statement on the management of MDR in current HBV treatment guidelines. However, the guidelines for primary treatment failure could be used in order to prevent MDR. The management of antiviral-resistant HBV in guidelines is listed in Table 3[27,101]. In these updated guidelines, the combination therapy was not recommended in all circumstances. The antiviral agents with high potency and high genetic barrier to resistance, such as ETV and TDF, could also be used alone.

CONCLUSION

In summary, MDR to NA is a thorny issue in the anti-HBV therapy. The clinical efficacy in patients has been improved to some extent by the combination therapy. ETV and TDF can be regarded as the optimal choice for patients with MDR. Further investigations on the mechanisms and optimal treatment modalities are still lacking. The progress and emergence of new genetic testing technology will probably improve the anti-HBV therapy. The newly discovered forms of gene mutations to resistance may provide useful clues to solve the problem of MDR. The efficacy of IFN in patients with MDR needs further exploration. The development of new anti-HBV agents, which act not only on RT, but also on other targets of HBV, may be a new approach to prevent MDR in NA.

REFERENCES

1 **Baumert TF**, Thimme R, von Weizsäcker F. Pathogenesis of hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 82-90

2 **Murray JM**, Purcell RH, Wieland SF. The half-life of hepatitis B virions. *Hepatology* 2006; **44**: 1117-1121

3 **Zoulim F**, Buti M, Lok AS. Antiviral-resistant hepatitis B virus: can we prevent this monster from growing? *J Viral Hepat* 2007; **14** Suppl 1: 29-36

4 **Alazawi W**, Foster GR. Advances in the diagnosis and treatment of hepatitis B. *Curr Opin Infect Dis* 2008; **21**: 508-515

5 **Leemans WF**, Janssen HL, Niesters HG, de Man RA. Switching patients with lamivudine resistant chronic hepatitis B virus from tenofovir to adefovir results in less potent HBV-

DNA suppression. *J Viral Hepat* 2008; **15**: 108-114
6 **Richman DD**. The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. *Hepatology* 2000; **32**: 866-867
7 **Locarnini S**. Primary resistance, multidrug resistance, and cross-resistance pathways in HBV as a consequence of treatment failure. *Hepatol Int* 2008; **2**: 147-151
8 **Angus P**, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, Brosgart C, Colledge D, Edwards R, Ayres A, Bartholomeusz A, Locarnini S. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* 2003; **125**: 292-297
9 **van Bömmel F**, Zollner B, Moller B, Huppe D, Feucht HH, Wiedenmann B, Berg T. Is tenofovir effective in treatment of adefovir resistant hepatitis B virus (HBV) infections? *Hepatology* 2006; **44** Suppl 1: 567A
10 **Suzuki F**, Akuta N, Suzuki Y, Yatsuji H, Sezaki H, Arase Y, Kawamura Y, Hosaka T, Kobayashi M, Ikeda K, Kobayashi M, Watahiki S, Kumada H. Selection of a virus strain resistant to entecavir in a nucleoside-naive patient with hepatitis B of genotype H. *J Clin Virol* 2007; **39**: 149-152
11 **Locarnini S**, Bowden S. Drug resistance in antiviral therapy. *Clin Liver Dis* 2010; **14**: 439-459
12 **Dupouey J**, Gerolami R, Solas C, Colson P. Hepatitis B virus variant with the a194t substitution within reverse transcriptase before and under adefovir and tenofovir therapy. *Clin Res Hepatol Gastroenterol* 2012; **36**: e26-e28
13 **Tacke F**, Trautwein C. Hepatitis B goes globe: Telbivudine as a new treatment option. *Hepatology* 2008; **47**: 1786-1787
14 **Hoofnagle JH**, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; **45**: 1056-1075
15 **Tan J**, Degertekin B, Wong SN, Husain M, Oberhelman K, Lok AS. Tenofovir monotherapy is effective in hepatitis B patients with antiviral treatment failure to adefovir in the absence of adefovir-resistant mutations. *J Hepatol* 2008; **48**: 391-398
16 **Leemans WF**, Niesters HG, van der Eijk AA, Janssen HL, Schalm SW, de Man RA. Selection of an entecavir-resistant mutant despite prolonged hepatitis B virus DNA suppression, in a chronic hepatitis B patient with preexistent lamivudine resistance: successful rescue therapy with tenofovir. *Eur J Gastroenterol Hepatol* 2008; **20**: 773-777
17 **Choe WH**, Kwon SY, Kim BK, Ko SY, Yeon JE, Byun KS, Kim GH, Lee CH. Tenofovir plus lamivudine as rescue therapy for adefovir-resistant chronic hepatitis B in hepatitis B e antigen-positive patients with liver cirrhosis. *Liver Int* 2008; **28**: 814-820
18 **Amini-Bavil-Olyaee S**, Herbers U, Sheldon J, Luedde T, Trautwein C, Tacke F. The rtA194T polymerase mutation impacts viral replication and susceptibility to tenofovir in hepatitis B e antigen-positive and hepatitis B e antigen-negative hepatitis B virus strains. *Hepatology* 2009; **49**: 1158-1165
19 **Reijnders JG**, LA Janssen H. New approaches in the management of chronic hepatitis B: role of tenofovir. *Infect Drug Resist* 2009; **2**: 13-26
20 **Pastor R**, Habersetzer F, Fafi-Kremer S, Doffoel M, Baumert TF, Gut JP, Stoll-Keller F, Schvoerer E. Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naive patients. *World J Gastroenterol* 2009; **15**: 753-755
21 **Sheldon J**, Camino N, Rodés B, Bartholomeusz A, Kuiper M, Tacke F, Núñez M, Mauss S, Lutz T, Klausen G, Locarnini S, Soriano V. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. *Antivir Ther* 2005; **10**: 727-734
22 **Delaney WE**, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, Miller MD. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. *Antimicrob Agents Chemother* 2006; **50**: 2471-2477
23 **Gish R**, Jia JD, Locarnini S, Zoulim F. Selection of chronic hepatitis B therapy with high barrier to resistance. *Lancet Infect Dis* 2012; **12**: 341-353
24 **Sayan M**, Hulagu S, Karatayli SC. Multidrug-resistant hepatitis B virus strain in a chronic Turkish patient. *Hepat Mon* 2010; **10**: 141-146
25 **Villeneuve JP**, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, Leduc R, Peltekian K, Wong F, Margulies M, Heathcote EJ. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *Hepatology* 2000; **31**: 207-210
26 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242
27 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662
28 **Peters MG**, Hann Hw Hw, Martin P, Heathcote EJ, Buggisch P, Rubin R, Bourliere M, Kowdley K, Trepo C, Gray Df Df, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2004; **126**: 91-101
29 **Fung SK**, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, Hussain M, Lok AS. Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; **44**: 283-290
30 **Heo NY**, Lim YS, Lee HC, Chung YH, Lee YS, Suh DJ. Lamivudine plus adefovir or entecavir for patients with chronic hepatitis B resistant to lamivudine and adefovir. *J Hepatol* 2010; **53**: 449-454
31 **Lee YS**, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, Chung YH, Lee YS, Yoo W, Kim SO. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006; **43**: 1385-1391
32 **Lee JM**, Park JY, Kim do Y, Nguyen T, Hong SP, Kim SO, Chon CY, Han KH, Ahn SH. Long-term adefovir dipivoxil monotherapy for up to 5 years in lamivudine-resistant chronic hepatitis B. *Antivir Ther* 2010; **15**: 235-241
33 **Fung J**, Lai CL, Yuen JC, Wong DK, Tanaka Y, Mizokami M, Yuen MF. Adefovir dipivoxil monotherapy and combination therapy with lamivudine for the treatment of chronic hepatitis B in an Asian population. *Antivir Ther* 2007; **12**: 41-46
34 **Rapti I**, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* 2007; **45**: 307-313
35 **Yim HJ**, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. *Hepatology* 2006; **44**: 703-712
36 **Brunelle MN**, Jacquard AC, Pichoud C, Durantel D, Carrouée-Durantel S, Villeneuve JP, Trépo C, Zoulim F. Susceptibility to antivirals of a human HBV strain with mutations conferring resistance to both lamivudine and adefovir. *Hepatology* 2005; **41**: 1391-1398
37 **Nafa S**, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, Merle P, Abidi H, Trépo C, Zoulim F. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. *Hepatology* 2000; **32**: 1078-1088
38 **Marcellin P**, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004; **351**: 1206-1217
39 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-posi-

tive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695
40 **Rang A**, Günther S, Will H. Effect of interferon alpha on hepatitis B virus replication and gene expression in transiently transfected human hepatoma cells. *J Hepatol* 1999; **31**: 791-799
41 **Papatheodoridis GV**, Manolakopoulos S, Dusheiko G, Archimandritis AJ. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. *Lancet Infect Dis* 2008; **8**: 167-178
42 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283
43 **Park JW**, Kim HS, Seo DD, Jang JS, Shin WG, Kim KH, Jang MK, Lee JH, Kim HY, Kim DJ, Lee MS, Park CK. Long-term efficacy of entecavir in adefovir-refractory chronic hepatitis B patients with prior lamivudine resistance. *J Viral Hepat* 2011; **18**: e475-e481
44 **Choe WH**, Hong SP, Kim BK, Ko SY, Jung YK, Kim JH, Yeon JE, Byun KS, Kim KH, Ji SI, Kim SO, Lee CH, Kwon SY. Evolution of hepatitis B virus mutation during entecavir rescue therapy in patients with antiviral resistance to lamivudine and adefovir. *Antivir Ther* 2009; **14**: 985-993
45 **Lim YS**, Lee JY, Lee D, Shim JH, Lee HC, Lee YS, Suh DJ. Randomized trial of entecavir plus adefovir in patients with lamivudine-resistant chronic hepatitis B who show suboptimal response to lamivudine plus adefovir. *Antimicrob Agents Chemother* 2012; **56**: 2941-2947
46 **van Bömmel F**, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, Erhardt A, Hüppe D, Stein K, Trojan J, Sarrazin C, Böcher WO, Spengler U, Wasmuth HE, Reinders JG, Möller B, Rhode P, Feucht HH, Wiedenmann B, Berg T. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. *Hepatology* 2010; **51**: 73-80
47 **van Bömmel F**, Zöllner B, Sarrazin C, Spengler U, Hüppe D, Möller B, Feucht HH, Wiedenmann B, Berg T. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level during adefovir therapy. *Hepatology* 2006; **44**: 318-325
48 **Patterson SJ**, George J, Strasser SI, Lee AU, Sievert W, Nicoll AJ, Desmond PV, Roberts SK, Locarnini S, Bowden S, Angus PW. Tenofovir disoproxil fumarate rescue therapy following failure of both lamivudine and adefovir dipivoxil in chronic hepatitis B. *Gut* 2011; **60**: 247-254
49 **Petersen J**, Ratziu V, Buti M, Janssen HL, Brown A, Lampertico P, Schollmeyer J, Zoulim F, Wedemeyer H, Sterneck M, Berg T, Sarrazin C, Lutgehetmann M, Buggisch P. Entecavir plus tenofovir combination as rescue therapy in pre-treated chronic hepatitis B patients: an international multicenter cohort study. *J Hepatol* 2012; **56**: 520-526
50 **Kim SS**, Cheong JY, Cho SW. Current Nucleos(t)ide Analogue Therapy for Chronic Hepatitis B. *Gut Liver* 2011; **5**: 278-287
51 **Sherman M**, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, Sette H, Tsai N, Tenney DJ, Vaughan J, Kreter B, Hindes R. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology* 2008; **48**: 99-108
52 **Ustundag Y**, Topalak O. Think twice if you consider entecavir treatment in cases with lamivudine refractoriness. *Hepatology* 2010; **52**: 397-398
53 **Tenney DJ**, Pokornowski KA, Rose RE, Baldick CJ, Eggers BJ, Fang J, Yang JY, Xu D, Brett-Smith H, Kreter B, Colonno RJ, Neo BL. Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance. *J Gastroenterol Hepatol* 2008; **23** Suppl 5: A29
54 **Yatsuji H**, Hiraga N, Mori N, Hatakeyama T, Tsuge M, Imamura M, Takahashi S, Fujimoto Y, Ochi H, Abe H, Maekawa T, Suzuki F, Kumada H, Chayama K. Successful treatment of an entecavir-resistant hepatitis B virus variant. *J Med Virol* 2007; **79**: 1811-1817
55 **Jeon JW**, Shin HP, Lee JI, Joo KR, Cha JM, Park JJ, Lim JU, Lim K, Kim S. Efficacy of entecavir and adefovir combination therapy for patients with lamivudine- and entecavir-resistant chronic hepatitis B. *Dig Dis Sci* 2012; **57**: 1358-1365
56 **Liu Y**, Wang C, Zhong Y, Chen L, Li X, Ji D, Wang H, Xin S, Zoulim F, Xu D. Evolution and suppression of HBV strains with multidrug resistance to lamivudine, adefovir dipivoxil and entecavir in a patient with chronic hepatitis B. *Antivir Ther* 2010; **15**: 1185-1190
57 **Karatayli E**, Karatayli SC, Cinar K, Gokahmetoglu S, Güven K, Idilman R, Yurdaydin C, Bozdayi AM. Molecular characterization of a novel entecavir mutation pattern isolated from a multi-drug refractory patient with chronic hepatitis B infection. *J Clin Virol* 2012; **53**: 130-134
58 **D'Avola D**, Herrero JI. Prophylaxis and treatment of hepatitis B infection in the setting of liver transplantation. *Rev Esp Enferm Dig* 2011; **103**: 142-149
59 **Subramanian AK**. Antimicrobial prophylaxis regimens following transplantation. *Curr Opin Infect Dis* 2011; **24**: 344-349
60 **Buchanan C**, Tran TT. Current status of liver transplantation for hepatitis B virus. *Clin Liver Dis* 2011; **15**: 753-764
61 **Osborn MK**, Han SH, Regev A, Bzowej NH, Ishitani MB, Tran TT, Lok AS. Outcomes of patients with hepatitis B who developed antiviral resistance while on the liver transplant waiting list. *Clin Gastroenterol Hepatol* 2007; **5**: 1454-1461
62 **Katz LH**, Fraser A, Gafter-Gvili A, Leibovici L, Tur-Kaspa R. Lamivudine prevents reactivation of hepatitis B and reduces mortality in immunosuppressed patients: systematic review and meta-analysis. *J Viral Hepat* 2008; **15**: 89-102
63 **Loomba R**, Rowley AK, Wesley R, Smith KG, Liang TJ, Pucino F, Csako G. Hepatitis B immunoglobulin and Lamivudine improve hepatitis B-related outcomes after liver transplantation: meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 696-700
64 **Jiang L**, Jiang LS, Cheng NS, Yan LN. Current prophylactic strategies against hepatitis B virus recurrence after liver transplantation. *World J Gastroenterol* 2009; **15**: 2489-2499
65 **Ueda Y**, Marusawa H, Egawa H, Okamoto S, Ogura Y, Oike F, Nishijima N, Takada Y, Uemoto S, Chiba T. De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation. *Antivir Ther* 2011; **16**: 479-487
66 **Baid-Agrawal S**, Pascual M, Moradpour D, Frei U, Tolkoff-Rubin N. Hepatitis C virus infection in haemodialysis and kidney transplant patients. *Rev Med Virol* 2008; **18**: 97-115
67 **Walter T**, Dumortier J, Guillaud O, Hervieu V, Paliard P, Scoazec JY, Boillot O. Rejection under alpha interferon therapy in liver transplant recipients. *Am J Transplant* 2007; **7**: 177-184
68 **Schiff E**, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann H, Samuel D, Zeuzem S, Villeneuve JP, Arterburn S, Borroto-Esoda K, Brosgart C, Chuck S. Adefovir dipivoxil for wait-listed and post-liver transplantation patients with lamivudine-resistant hepatitis B: final long-term results. *Liver Transpl* 2007; **13**: 349-360
69 **Akyildiz M**, Karasu Z, Zeytunlu M, Aydin U, Ozacar T, Kilic M. Adefovir dipivoxil therapy in liver transplant recipients for recurrence of hepatitis B virus infection despite lamivudine plus hepatitis B immunoglobulin prophylaxis. *J Gastroenterol Hepatol* 2007; **22**: 2130-2134
70 **Limquiaco JL**, Wong J, Wong VW, Wong GL, Tse CH, Chan HY, Kwan KY, Lai PB, Chan HL. Lamivudine monoprophylaxis and adefovir salvage for liver transplantation in chronic hepatitis B: a seven-year follow-up study. *J Med Virol* 2009; **81**: 224-229
71 **Wai CT**, Prabhakaran K, Wee A, Lee YM, Dan YY, Sutedja DS, Mak K, Isaac J, Lee KH, Lee HL, Da Costa M, Lim SG. Adefovir dipivoxil as the rescue therapy for lamivudine-re-

sistant hepatitis B post liver transplant. *Transplant Proc* 2004; **36**: 2313-2314
72 **Herreros de Tejada Echanojáuregui A**, Moreno Planas JM, Rubio González E, Portero Azorin F, López Monclús J, Revilla Negro J, Lucena de la Poza JL, Sánchez Turrión V, Barrios Peinado C, Cuervas-Mons Martínez V. Adefovir dipivoxil therapy in liver transplant recipients with lamivudine-resistant hepatitis B virus. *Transplant Proc* 2005; **37**: 1507-1508
73 **Neff GW**, O'brien CB, Nery J, Shire N, Montalbano M, Ruiz P, Nery C, Safdar K, De Medina M, Tzakis AG, Schiff ER, Madariaga J. Outcomes in liver transplant recipients with hepatitis B virus: resistance and recurrence patterns from a large transplant center over the last decade. *Liver Transpl* 2004; **10**: 1372-1378
74 **Angus PW**, Patterson SJ. Liver transplantation for hepatitis B: what is the best hepatitis B immune globulin/antiviral regimen? *Liver Transpl* 2008; **14** Suppl 2: S15-S22
75 **Xi ZF**, Xia Q, Zhang JJ, Chen XS, Han LZ, Wang X, Shen CH, Luo Y, Xin TY, Wang SY, Qiu de K. The role of entecavir in preventing hepatitis B recurrence after liver transplantation. *J Dig Dis* 2009; **10**: 321-327
76 **Jiménez-Pérez M**, Sáez-Gómez AB, Mongil Poce L, Lozano-Rey JM, de la Cruz-Lombardo J, Rodrigo-López JM. Efficacy and safety of entecavir and/or tenofovir for prophylaxis and treatment of hepatitis B recurrence post-liver transplant. *Transplant Proc* 2010; **42**: 3167-3168
77 **Fung J**, Cheung C, Chan SC, Yuen MF, Chok KS, Sharr W, Dai WC, Chan AC, Cheung TT, Tsang S, Lam B, Lai CL, Lo CM. Entecavir monotherapy is effective in suppressing hepatitis B virus after liver transplantation. *Gastroenterology* 2011; **141**: 1212-1219
78 **Villet S**, Pichoud C, Villeneuve JP, Trépo C, Zoulim F. Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient. *Gastroenterology* 2006; **131**: 1253-1261
79 **Karlas T**, Hartmann J, Weimann A, Maier M, Bartels M, Jonas S, Mössner J, Berg T, Tillmann HL, Wiegand J. Prevention of lamivudine-resistant hepatitis B recurrence after liver transplantation with entecavir plus tenofovir combination therapy and perioperative hepatitis B immunoglobulin only. *Transpl Infect Dis* 2011; **13**: 299-302
80 **Khudyakov Y**. Coevolution and HBV drug resistance. *Antivir Ther* 2010; **15**: 505-515
81 **Cassino L**, Laufer N, Salomon H, Campos R, Quarleri J. Hepatitis B precore/core promoter mutations in isolates from HBV-monoinfected and HBV-HIV coinfected patients: a 3-yr prospective study. *J Clin Virol* 2009; **46**: 354-359
82 **Wang F**, Wang H, Shen H, Meng C, Weng X, Zhang W. Evolution of hepatitis B virus polymerase mutations in a patient with HBeAg-positive chronic hepatitis B virus treated with sequential monotherapy and add-on nucleoside/nucleotide analogues. *Clin Ther* 2009; **31**: 360-366
83 **Thai H**, Campo DS, Lara J, Dimitrova Z, Ramachandran S, Xia G, Ganova-Raeva L, Teo CG, Lok A, Khudyakov Y. Convergence and coevolution of hepatitis B virus drug resistance. *Nat Commun* 2012; **3**: 789
84 **Zoulim F**, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009; **137**: 1593-1608.e1-2
85 **Ko SY**, Oh HB, Park CW, Lee HC, Lee JE. Analysis of hepatitis B virus drug-resistant mutant haplotypes by ultra-deep pyrosequencing. *Clin Microbiol Infect* 2012; **18**: E404-E411
86 **Shendure J**, Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 2008; **26**: 1135-1145
87 **Metzker ML**. Sequencing technologies - the next generation. *Nat Rev Genet* 2010; **11**: 31-46
88 **Rodriguez-Frías F**, Tabernero D, Quer J, Esteban JI, Ortega I, Domingo E, Cubero M, Camós S, Ferrer-Costa C, Sánchez A, Jardí R, Schaper M, Homs M, Garcia-Cehic D, Guardia J, Esteban R, Buti M. Ultra-deep pyrosequencing detects conserved genomic sites and quantifies linkage of drug-resistant amino acid changes in the hepatitis B virus genome. *PLoS One* 2012; **7**: e37874
89 **Damerow H**, Yuen L, Wiegand J, Walker C, Bock CT, Locarnini S, Tillmann HL. Mutation pattern of lamivudine resistance in relation to hepatitis B genotypes: hepatitis B genotypes differ in their lamivudine resistance associated mutation pattern. *J Med Virol* 2010; **82**: 1850-1858
90 **Lo CM**, Cheung CK, Lau GK, Yuen MF, Liu CL, Chan SC, Fan ST, Wong J. Significance of hepatitis B virus genotype in liver transplantation for chronic hepatitis B. *Am J Transplant* 2005; **5**: 1893-1900
91 **Yıldız O**, Aygen B, Demirtürk N, Demirdal T, Inan D, Yıldırmak T, Kantürk A, Tütüncü E. Lamivudine resistance mutations in patients infected with hepatitis B virus genotype D. *World J Gastroenterol* 2011; **17**: 4987-4992
92 **Wong VW**, Sung JJ. Diagnosis and personalized management of hepatitis B including significance of genotypes. *Curr Opin Infect Dis* 2012; **25**: 570-577
93 **Zoulim F**. Hepatitis B virus resistance to antiviral drugs: where are we going? *Liver Int* 2011; **31** Suppl 1: 111-116
94 **Tujios SR**, Lee WM. New advances in chronic hepatitis B. *Curr Opin Gastroenterol* 2012; **28**: 193-197
95 **Ghany MG**, Doo EC. Antiviral resistance and hepatitis B therapy. *Hepatology* 2009; **49**: S174-S184
96 **Ayoub WS**, Keeffe EB. Review article: current antiviral therapy of chronic hepatitis B. *Aliment Pharmacol Ther* 2011; **34**: 1145-1158
97 **Yuen MF**, Fung J, Wong DK, Lai CL. Prevention and management of drug resistance for antihepatitis B treatment. *Lancet Infect Dis* 2009; **9**: 256-264
98 **Keeffe EB**, Zeuzem S, Koff RS, Dieterich DT, Esteban-Mur R, Gane EJ, Jacobson IM, Lim SG, Naoumov N, Marcellin P, Piratvisuth T, Zoulim F. Report of an international workshop: Roadmap for management of patients receiving oral therapy for chronic hepatitis B. *Clin Gastroenterol Hepatol* 2007; **5**: 890-897
99 **Zoulim F**, Locarnini S. Management of treatment failure in chronic hepatitis B. *J Hepatol* 2012; **56** Suppl 1: S112-S122
100 **An P**, Bian L, Yin B, Lv D, Cao YX, Wu X. Risk factors of gene-resistant mutations in different nucleosides. *Hepatogastroenterology* 2012; **59**: 228-230
101 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185

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