# CORE PROMOTER MUTANT HBV NON-RESPONDING TO ADEFOVIR AFTER VIRAL BREAKTHROUGH ON LAMIVUDINE: RAPID VIROLOGIC RESPONSE TO TENOFOVIR PLUS LAMIVUDINE IN A CIRRHOTIC PATIENT

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**Abstract**

*Background:* In chronic hepatitis B patients undergoing therapy with LAM or ADV, viral breakthrough is possible due to the emergence of drug resistance. LAM resistant HBV strains are susceptible to ADV, while ADV resistant mutants remain sensitive to LAM.

*Case report:* A male patient with HBV-related cirrhosis developed viral breakthrough [HBV DNA > $1.8 \times 10^6$ IU/ml] after 4 1/2 years of treatment with LAM, and therapy was switched to ADV [10mg/d]. After three months, HBV remained highly replicative without any changes of ALT values, and ADV dose was increased [20mg/d]. Because of unchanged VL sequence analysis was performed three months later, which showed the mutation [rtS219A] and the concomitant mutation [sS210R] and 2 mutations in core promoter region [A1762T], [G1764A]. During the sixth month of ADV monotherapy the patient developed liver failure. After administration of TDF plus LAM, HBV DNA became undetectable within 39 days. At day 41, the patient underwent OLT. TDF plus LAM were well tolerated, and the patient maintained undetectable HBV DNA levels, and in addition to HBIG a sustained HBsAg negative status over twenty-eight months post OLT.

*Conclusion:* TDF plus LAM is a safe drug combination in case of viral breakthrough during LAM treatment and subsequent primary non-response to ADV. High VL persisting for ≥6 months of continuous antiviral treatment may indicate drug resistance. Especially in cirrhotic patients with LAM resistance, "add on" of a nucleotide analogue is the right therapeutic strategy even before viral breakthrough gets apparent.

*Key words:* chronic HBV; LAM resistance; ADV non-response; rtS219A, sS210R; TDF

*Abbreviations:* adefovir dipivoxil (ADV); alanine aminotransferase (ALT); lamivudine (LAM); tenofovir disoproxil (TDF); hepatitis B immune globulin (HBIG); hepatocellular carcinoma (HCC); orthotopic liver transplantation (OLT); viral load (VL); polymerase chain reaction (PCR); upper limit of normal (ULN)

## BACKGROUND

A dramatic flare of HBV replication under ongoing antiviral therapy with nucleoside/nucleotide analogues is often associated with the selection of mutant HBV strains. Emergence of virologic resistance with a cumulative rate of 70% after 4 years (Locarnini et al., 2006) has been previously described during antiviral therapy with LAM. Furthermore, it has been shown that LAM resistant mutants remain susceptible to the acyclic nucleotide analogue ADV (Peters et al., 2004). However, mutations causing virologic resistance have also been described for ADV, which can be detected in as many as 29% of patients after 5 years of therapy (Bartholomeusz et al., 2006; Locarnini et al., 2006). However, these variants are mostly susceptible to LAM (Angus et al., 2003; Villeneuve et al., 2003). The need for rescue treatments against LAM resistant mutants non-responding to ADV is warranted in order to prevent liver failure. Non-response to ADV in absence of genotypic HBV resistance against ADV was shown to be treated effectively with tenofovir (Schildgen et al., 2004; van Bommel et al., 2006).

## CASE REPORT

In May 2004, a 51 year old male with chronic hepatitis B developed viral breakthrough with HBV DNA > $1.8 \times 10^6$ IU/ml in real time PCR assay, while being on treatment with LAM [150mg/d] for 4 1/2 years. HBeAg was positive; antibodies against HBeAg, HAV [IgM and IgG], HDV, HCV as well as HIV were not detectable, and ALT values were slightly elevated [threefold the upper limit of normal: 3 ULN]. Medical screening including liver biopsy confirmed cirrhosis CHILD A [Child-Pugh score: 6] and oesophageal varices [grade 2]. Although antiviral therapy was immediately switched to ADV [10 mg/d], HBV DNA and ALT levels remained unchanged until August 2004. Therefore, the dose of ADV was increased [20mg/d].

After another three months of monotherapy with ADV [20mg/d], ALT values rose to 10 ULN; despite that HBV DNA levels remained unchanged [> $1.8 \times 10^6$ IU/ml in real time PCR assay], nucleotide sequence analysis was performed with the following results:

1. Sequencing of the HBs/pol region [nucleotide 328-619] revealed HBV-genotype A2 / HBsAg subtype *adw2*.
2. The overlapping reverse transcriptase (rt) domain showed the mutation [rtS219A] and a concommittant HBs gene mutation [S210R].

3. ADV resistance mutations [A181V] in domain B or [N236T] in domain D of the polymerase gene were not detected, neither the variants [L217R] and [I233V] (Schildgen et al., 2004; Schildgen et al., 2006).

4. Furthermore, none of the following LAM resistance mutations [rtL180M, rtM204V, rtV173L] (Westland et al., 2005) could be found.

5. Sequence analysis of preS frame showed no further mutations (Gerken et al., 1991).

6. The core promoter sequence showed the two well-known mutations: [A1762T] and [G1764A].

Immediately, therapy was switched to TDF [300mg/d] in combination with LAM [300mg/d], resulting in a drop of HBV DNA by more than 5 $\log_{10}$ [HBV DNA ≤ 356 IU/ml in real time PCR assay] within 39 days. Because of the deteriorating liver function, the patient was listed for OLT, which was performed 41 days after initiating TDF plus LAM therapy.

Beside complete cirrhosis, the histological examination of the explanted liver led to the detection of hepatocellular carcinoma [1cm / pT2, Nx, Mx, G2, V1, L0, R0]. The liver donor was tested negative for antibodies against HAV, HBsAg, HBcAg, HCV and HIV. Twelve days after OLT, the patient developed a biopsy proven episode of acute rejection, which was successfully treated with escalated immune-suppression therapy. At this time point, no sign of HBV reinfection of the transplanted liver could be found. Five weeks after OLT, the patient could be discharged from our hospital. Treatment with HBIG was always adjusted to the current anti-HBs levels.

Eighteen months after OLT, no reinfection of the graft was detected in serological or HBV DNA assays and in liver biopsy during continuous treatment with TDF plus LAM and HBIG. At that time point, the LAM treatment was discontinued, and ten months after discontinuation, HBV DNA and HBsAg still remained undetectable.

## DISCUSSION

Treatment with LAM for ≥ 4 years results in the emergence of resistant mutants in ≥ 70% of all patients (Locarnini et al., 2006). Furthermore, HBV-genotype A2 / HBsAg subtype adw is possibly associated with a significantly higher risk for developing LAM resistance [20-fold increase] as compared to subtype ayw (Zollner et al., 2001). Thus, we believe that in the present case the emergence of LAM resistance mutations should be considered as the most probable reason for developing virological breakthrough after 4 1/2 years of continuous LAM monotherapy, although no sequence analysis was available in that period. The most common LAM resistant mutations have a prevalence of 60% [rtL180M + rtM204V] and 19% [rtL180M + rtM204V + rtV173L], respectively (Westland et al., 2005). In 2004, clinical reports suggested that ADV monotherapy was successful in cases of severe exacerbation of chronic hepatitis B after emerging of LAM resistance (Wiegand et al., 2004), while many investigators confirmed the absence of cross-resistance between LAM and ADV (Delaney et al., 2001); therefore, we switched therapy to ADV.

After six months of uninterrupted therapy with ADV, and despite good compliance, the patient did not respond to ADV. At that time, sequence analysis detected a mutation in polymerase gene [rtS219A] and a concomitant mutation in the open reading frame [ORF] of the HBs gene [sS210R] as well as 2 mutations [A1762T] and [G1764A] in the core promoter region, whereas none of the following resistance mutations against LAM [L180M, M204V] or ADV [N236T, A181V, L217R] could be found (Schildgen et al., 2004; Westland et al., 2005). The recently described mutation [rtI233V] (Schildgen et al., 2006), the impact of which on therapeutic outcomes has been discussed very controversially in the mean time, was also not detected.

The mutation [S219A], which occurs at a polymorphic site within the reverse transcriptase, has been previously reported in association with LAM resistance (Cane et al., 1999). This mutation can be selected more favorably amongst HBV patients with genotype A during LAM treatment (Locarnini et al., 2006). However, according to the same source the [rtS219A] is a naturally occurring polymorphic substitution that can often be found at baseline in HBV isolates of patients who respond to LAM therapy, and therefore, it is unlikely to be associated itself with a LAM resistant phenotype (Locarnini et al., 2006). The presence of [rtS219A] six months after initiation of ADV led to the question whether this polymorphic substitution might have been emerged under ADV selection pressure, and therefore, it might also predict resistance to ADV. In this regard, two randomized studies reported that the [rtS219A] belonged to a group of substitutions at polymorphic site, which occurred at very low frequencies (<1.6%) during treatment with ADV in therapy-naïve patients and were not associated with HBV DNA increases (Westland et al., 2003). Furthermore, phenotypic assays in vitro showed that in HBV clones, which contained the mutation [rtS219A], the susceptibility to ADV did not change post therapy relative to baseline indicating that neither this polymorphic substitution nor other concomitant mutations elsewhere in HBV genome conferred resistance to ADV (Westland et al., 2003)

In the present case, the persistent high VL over six months despite continuous and even escalated ADV therapy on the one hand, and the absence of ADV resistance mutations against ADV on the other hand suggested primary non-response to ADV. Interestingly, it has been shown that primary non-response to ADV occurs in up to 15% of patients infected with LAM resistant hepatitis B virus, and most often with genotype A. The reasons for this still remain unclear (Peters et al., 2004). I our patient, we presume that the viral breakthrough was caused by LAM resistance mutations, which in turn became undetectable due to subsequent treatment with ADV over six months. Because blood samples from the year 2004 were no longer available during the period of treatment with ADV we could not retrospectively perform subspecies analysis within the virus population to rule out the presence of a significant minor population bearing ADV or LAM resistance mutations.

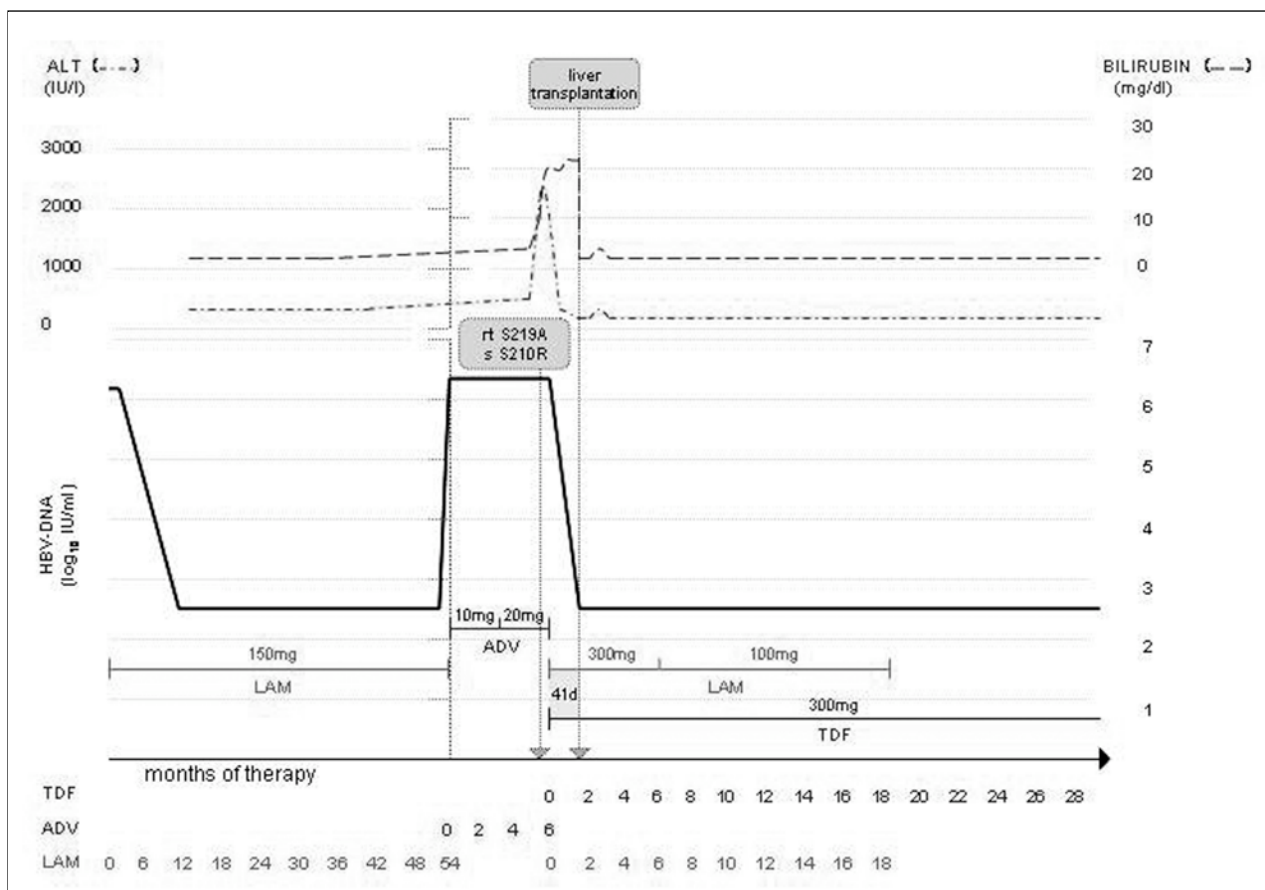Persistent high viraemia over six months was associated with the development of acute on chronic liver

*Fig. 1:* Virological and biochemical course before and after liver transplantation. LAM: lamivudine; ADV: adefovir dipivoxil; TDF: tenofovir disoproxil. [PCR limit of detection: 356 IU/ml]

failure in the patient, who had histologically proven complete cirrhosis, probably co-favored by the presence of core promoter mutations as well. Indeed, it has been described that in patients with severe hepatic decompensation, high replicative HBV strains might be selected from HBV quasispecies during LAM-treatment, preferentially in HBV genotype A. Among these strains, those with the core promoter mutations [A1762T and G1764A] are most likely to be associated with severe clinical exacerbation (Chen et al., 2006; Zhang et al., 2005).

In HBV/HIV patients, it has been reported that the overlapping coincidence of LAM resistance and HBV genotype A may predict resistance to ADV (Peters et al., 2004) and therefore, therapy change to TDF should be considered. A non-randomized study demonstrated that treatment with TDF was successful in patients with high replicative [HBeAg positive] chronic hepatitis B non-responding to ADV monotherapy after the emergence of LAM resistance, and without any detectable ADV resistance mutations (Schildgen et al., 2004). Furthermore, it has been shown that patients with incomplete response to ADV and no evidence of HBV genotypic resistance against ADV were treated effectively with TDF (van Bommel et al., 2006). Considering these reports and the twice illustrated shortcoming of serial monotherapy as well as the absence of drug resistance mutations after six months of continuous non-response to ADV treatment, we decided to salvage the patient with the combi-

nation TDF plus LAM. Since the molecular configuration of TDF is not too different than ADV, we assume that TDF plus LAM suppressed VL very effectively, mainly because of the stronger antiviral potency of TDF relative to ADV. In particular, in vitro experiments demonstrated that TDF has a greater inhibitory potency compared to ADV in equal intracellular concentrations [pmols/106cells], although both drugs have a quite similar intracellular half life time (Delaney et al., 2006). Moreover, clinical trials (Van Bommel et al., 2004; Peters et al., 2006) showed a greater decline of viral load using TDF (245mg/d) over 48 weeks compared to ADV (10mg/d). According to the actual guidelines for HBV treatment, combination therapy is recommended in case of non-response to single drug therapy for ≥6 months (Stroffolini et al., 2007; Cronberg et al., 2007). Moreover, results from an Italian group (Lampertico et al., 2005) evaluating the impact of combination therapy on chronic HBV infection in LAM resistant patients confirmed that the addition of a second drug in case of emerged drug resistance is the right way to go even before viral breakthrough gets apparent. As our case demonstrated, a prompt switch to TDF plus LAM achieved a rapid and safe viral suppression (Fig. 1), and therefore should be considered as a rescue therapy option. As a consequence, this case proves that cirrhotic patients with lamivudine resistance should preferably be treated with combination therapy in order to achieve a fast suppression of HBV DNA.

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