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Lamivudine prophylaxis prevents hepatitis B virus reactivation in anti-HBc positive patients under rituximab for non-Hodgkin lymphoma

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ABSTRACT

Backgound: A significant proportion of hepatitis B surface antigen (HBsAg) negative/anti-hepatitis B core antigen (anti-HBc) positive patients with non-Hodgkin lymphoma (NHL) undergoing rituximab-based chemotherapy (R-CT) may suffer hepatitis B virus (HBV) reactivation.
Aims: We wanted to assess efficacy and safety of lamivudine (LMV) prophylaxis to prevent this complication.
Methods: Eighty-five consecutive HBsAg negative/anti-HBc positive NHL patients (71 years, 100% serum HBV DNA undetectable, 74% anti-HBs positive) received LMV coadministered with R-CT and for 18 months after the end of R-CT. Serum ALT, HBsAg, anti-HBs and HBV DNA were assessed every 4 months during and after end of LMV.
Results: During 39 (2–108) months of study period, including 21 months of LMV and 27 additional months after LMV discontinuation, one patient (2%) had HBV reactivation, 31 months after stopping LMV and during administration of new immunosuppressive regimens, without LMV prophylaxis, owing to incomplete oncological response. A 50% decline of anti-HBs titers occurred in 22/63 (35%) patients, including 12 who became anti-HBs seronegative. Five (6%) patients had ALT increase during R-CT but none required R-CT discontinuation. Seventeen (20%) patients died, all for tumour progression.
Conclusion: LMV prophylaxis is safe and effective in preventing HBV reactivation in HBsAg negative/anti-HBc positive NHL patients receiving R-CT.

1. Introduction

Immunosuppression due to anticancer chemotherapy (CT) can induce hepatitis B virus (HBV) reactivation in both HBsAg-positive carriers and those with resolved HBV infection, i.e. HBsAg negative, anti-HBc positive with or without serum anti-HBs [1,2]. Owing to the fact that the clinical manifestations of HBV reactivation range from asymptomatic HBV DNA flares to fulminant or chronic hepatitis with an accelerated clinical course, HBV reactivation poses risks in patient care and survival while causing interruption and/or discontinuation of CT. In the face of recommendations that clearly define management of HBsAg positive patients undergoing immunosuppression who, if left untreated, face a 40%–80% risk of HBV reactivation, there is no standardized care for non-Hodgkin lymphoma (NHL) patients with resolved HBV infection who are treated with rituximab (R)-based CT [3,4]. In these patients, the rate of HBV reactivation in the absence of prophylaxis is up to 24% [5–8]. To reduce this risk, one option is adopting a "pre-emptive therapy", based on monitoring of serum HBV DNA and/or HBsAg

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followed by a rescue treatment with anti-HBV regimens in case of reactivation, or to start "prophylaxis", with the administration of HBV nucleos(t)ides analogs during CT and for a consolidation period after CT. However, patients with lymphoma and resolved HBV infection were not universally protected against HBV reactivation and severe hepatitis B by the "pre-emptive" strategy. Randomized studies, in fact, demonstrated that the risk of HBV reactivation under CT plus R, a monoclonal antibody versus protein CD20, ranges from 10.7% to 18% adopting the "pre-emptive strategy", compared to 0% and 2.4% using prophylaxis with tenofovir disoproxil fumarate or entecavir, respectively [9,10]. Lamivudine (LMV) may represent an attractive alternative strategy for HBV prophylaxis because is widely available and inexpensive in many countries, endowed with an excellent safety profile confirmed by 20 years of use in the setting of patients with chronic hepatitis B. Moreover, the estimated risk of lamivudine resistance is expected to be negligible in patients with minimal HBV replication, as those with resolved infection, who are exposed to short-term immunosuppression.

Aim of this study was to assess the efficacy and safety of lamivudine prophylaxis in NHL patients with resolved HBV infection undergoing rituximab-based chemotherapy.

2. Materials and methods

2.1. Patients

Between October 2009 and June 2014, 248 HBsAg negative/anti-HBc positive patients with onco-hematological diseases in need of anticancer CT were referred to our Liver Centers. Among 92 patients with non-Hodgkin lymphoma, seven patients who started CT without rituximab because anti-CD20 negative were excluded. Therefore, 85 (92%) patients who received rituximab-CT were consecutively enrolled into this retrospective study.

All the included patients started lamivudine prophylaxis at the dose of 100 mg/day whenever the estimated glomerular filtration rate (eGFR) was >50 mL/min, whereas dose was reduced in patients with a eGFR <50 mL/min. LMV was administered before the first dose of R-CT (baseline) to be continued at least for 18 months after the end of R-CT, as per local recommendations [11]: their management had been shared with the hematologists in terms of prophylaxis strategy as well as follow-up. All patients underwent clinical and laboratory examinations in our hospital, including serum HBsAg, anti-HBs, alanine aminotransferase (ALT) and serum HBV DNA by PCR assay every 4 months from baseline, until at least two years after the last dose of lamivudine, the time of last visit (July 2017) or death.

Patients that underwent hematopoietic stem cell transplantation have been included in the analysis until the time of transplantation.

2.2. Serum assays

Serum ALT and aspartate aminotransferase (AST) were measured at 37 °C using an automated method. HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe were assessed using a microparticle enzyme immunoassay (AXSYM, Abbott Laboratories, North Chicago, IL, USA). Anti-HCV was assessed using a second-generation enzyme-linked immunoassay (Ortho Diagnostic System, Raritan, NJ, USA); serum HCV-RNA values were quantified using a commercial assay (Real Time HCV, Abbott Molecular Inc., USA) with a lower limit of detection of 12 IU/mL (LOD). HBV DNA was assessed by the real-time COBAS TaqMan HBV Test (v2.0, Roche Molecular Systems Inc., Branchburg, NJ) with a lower limit of quantification of 10 IU/mL. All patients with virological breakthrough were tested for drug resistance mutations by a line probe assay, INNOLiPA HBV Multi-DR [Innogenetics (Fujirebio Europe), NV Ghent, Belgium] according to the manufacturer's instructions.

Table 1
Baseline demographic, clinical and virological features of the 85 HBsAg negative/anti-HBc positive patients enrolled in the study.

Features	85 patients
Age, yrs[a]	71 (44–88)
Male	54 (64%)
Caucasian	84 (99%)
Anti-HBs positive[b]	63 (74%)
Anti-HBs titre[c], mIU/mL[a]	89 (10–>1000)
Anti-HBe positive	41 (48%)
HBV DNA <10 IU/mL	85 (100%)
ALT[d] <40 IU/L	80 (94%)
HCV RNA positive	4 (5%)

[a] Median (range).
[b] >10 mIU/mL.
[c] In 63 patients with anti-HBs >10 mIU/mL.
[d] Median ALT = 16 (range: 4–39) IU/L in 80 patients with ALT <40 IU/L (upper limit of normal).

2.3. Endpoints

The primary study endpoint was the HBV reactivation rate defined as serum HBV DNA apperance with or without the reappearance of serum HBsAg (HBsAg seroreversion). The secondary endpoints were: the percentage of patients with baseline ALT levels lower than the upper limit of normal (ULN, i.e. 40 IU/L) who showed an increase above the ULN; the percentage of patients with protective anti-HBs titres (>10 mIU/mL) at baseline who showed a >50% reduction during study period; acute hepatitis B, defined as HBsAg seroreversion with a >10-fold increase in serum ALT levels and HBV DNA >5 $\log_{10}$ IU/mL; and chronic HBV infection, defined as the persistence of serum HBsAg for at least six months. Moreover, rates of interruption and/or premature discontinuation of rituximab-based chemotherapy due to HBV reactivation and the overall and liver-related mortality were evaluated.

2.4. Statistical analysis

The qualitative variables are expressed as counts and percentages, and the discrete variables as median values and ranges. The differences in their distribution were evaluated using respectively Fisher's exact test and Wilcoxon's rank sum test. All of the p values are two tailed, and a value of 0.05 was considered statistically significant. The data were statistically analysed using STATA software (release 10.3, Stata Corporation, College Station, TX).

3. Results

The analysis reflects the three time periods in which the study can be divided: admininstation of lamivudine during and after the end of rituximab-based chemotherapy and follow-up after lamivudine discontinuation (Fig. 1).

3.1. Clinical and virological features at baseline

Table 1 shows baseline demographic, clinical and virological characteristics of the 85 patients included in the study. The majority were aged males with diffuse large B-cell lymphoma (DLBCL) who received R-CHOP (rituximab, cyclophosphamide, doxorubicine, vincristine and prednisone) or R-CVP (rituximab, cyclophopshamide, vincristine, prednisone) or R-bendamustine (Table 2). All patients were negative for HBsAg and positive for anti-HBc, 74% were anti-HBs positive, 48% anti-HBe positive, while 100% were serum HBV DNA negative by PCR assay. Twelve

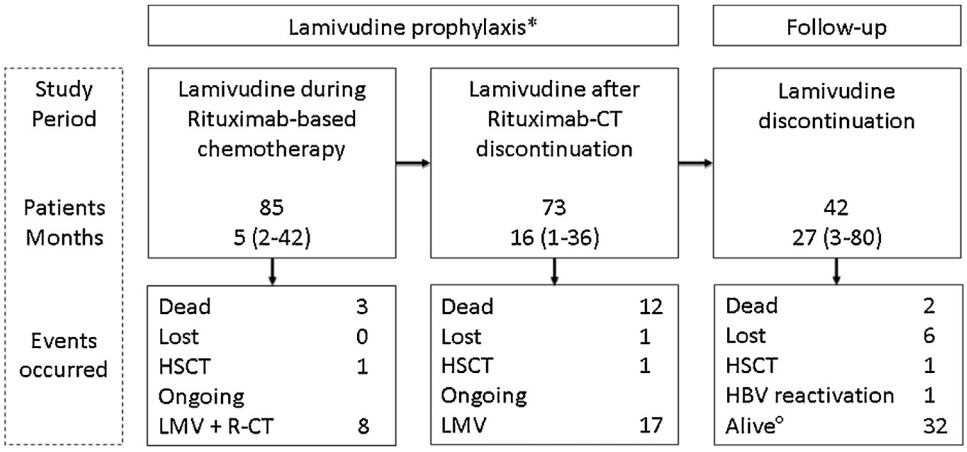

Fig. 1. Patient disposition during lamivudine prophylaxis and follow-up period. HSCT: hematopoietic stem cell transplantation; LMV: lamivudine; R-CT: rituximab-based chemotherapy. *Lamivudine administered during rituximab-based chemotherapy and for 18 months after R-CT discontinuation, with surveillance blood tests every 4 months, as for internal protocol. °Complete remission of non-Hodgkin's lymphoma in 24, partial remission in 5 and progression in 3 patients.

Table 2
Types of non-Hodgkin's lymphoma and rituximab-based chemotherapy regimens in the 85 HBsAg negative/anti-HBc positive patients enrolled in the study.

Variables	85 patients
Type of NHL:	
DLBCL	47 (55%)
Follicular/marginal	32 (38%)
Mantle cell	6 (7%)
Ann Arbor stage (%):	
I	8 (9%)
II	11 (13%)
III	21 (25%)
IV	45 (53%)
B symptoms	15 (18%)
LDH > ULN (%)[a]	45 (53%)
Type of R-CT regimen[e]:	
R-CHOP	40 (47%)
R-CVP	15 (18%)
R-bendamustine	15 (18%)
R-COMP	4 (5%)
R-DHAP	4 (5%)
R-BAC	3 (4%)
Others[d]	4 (4%)
Cycles of R-CT[b]	6 (1–15)
Duration of R-CT, months[b]	4 (1–31)
Duration of R maintenance therapy[c], months[b]	19 (17–35)

[a] 222 IU/L, LDH upper normal limit.
[b] Median (range).
[c] In 21 patients, 8 (1–11) cycles of rituximab (R)-consolidation.
[d] R-cyclophosphamide (n = 2), R-CD (n = 2).
[e] R-CHOP: R-cyclophosphamide + doxorubicine + vincristine + prednisone; R-CVP: R-cyclophosphamide + vincristine + prednisone; R-COMP: R-cyclophosphamide + non-pegylated liposomal doxorubicine + vincristine + prednisone; R-DHAP: R-dexamethasone + cytarabine + cisplatin; R-BAC: R-bendamustine + cytarabine; R-CD: R-cyclophosphamide + dexamethasone.

(14%) patients had anti-HCV seropositivity but only 4 (5%) had detectable serum HCV RNA levels (700,689; 2,284,341; 2,847,184 and 4,559,558 IU/mL). Five patients (6%) had ALT >ULN (median 66 IU/L, range: 43–92 IU/L).

3.2. Virological and clinical events during lamivudine prophylaxis and rituximab-CT

Overall, 85 patients received 6 (range: 1–15) cycles of rituximab-CT lasting for a median of 4 (1–31) months; 21 (25%) patients underwent rituximab maintenance monotherapy for 8 (1–11) cycles lasting for a median of 19 (17–35) months. During R-CT and LMV prophylaxis none of the patients experienced HBV DNA increase nor HBsAg seroreversion although 4 out of 63 (6%) patients with baseline protective anti-HBs titres had a >50% decline of anti-HBs levels, including one patient who became anti-HBs negative. Five patients with baseline normal ALT levels experienced an increase of ALT >ULN (to the maximum values of 4, 3, 3.2, 2.3 and 2× the ULN) which however was not related to HBV reactivation. Among the 5 patients with baseline ALT levels >ULN, including 4 patients with chronic HCV infection, ALT normalized in 2 patients.

None of the patients required interruption or discontinuation of chemotherapy due to HBV reactivation or liver related events, nor experienced any lamivudine-related side effects (Table 3).

Table 3
Outcome of the 85 HBsAg negative/anti-HBc positive patients during 27 (range: 3–80) months of follow-up.

Events	N = 85
HBsAg seroreversion	1 (1%)
Detectable serum HBV DNA	1 (1%)
Decline of anti-HBs titers >50%[a]	21 (33%)
Loss of anti-HBs levels[a]	12 (19%)
ALT ≥40 IU/L	5 (6%)[b]
Side effects related to LMV	0
Overall death	17 (20%)
Liver-related death	0

[a] Among the 63 patients with positive anti-HBs titers at baseline.
[b] None related to HBV reactivation and all occurring during rituximab-CT (3 patients) or rituximab maintenance monotherapy (2 patients).

3.3. Virological and clinical events during lamivudine prophylaxis and after rituximab-CT discontinuation

During 16 (1–36) months after R-CT discontinuation, none of the 73 patients on lamivudine prophylaxis experienced HBV reactivation. Twelve (19%) anti-HBs positive patients had a >50% decline of anti-HBs levels, including 8 patients who later become anti-HBs negative (Table 3). None of the patients had ALT flares nor lamivudine-related side effects.

Overall, lamivudine prophylaxis was maintained for a median of 21 (2–75) months, while 17 patients are still on lamivudine at the last visit (Fig. 1).

3.4. Virological and clinical events after lamivudine discontinuation

Among 42 patients who were followed for 27 (3–80) months after lamivudine discontinuation, all maintained undetectable

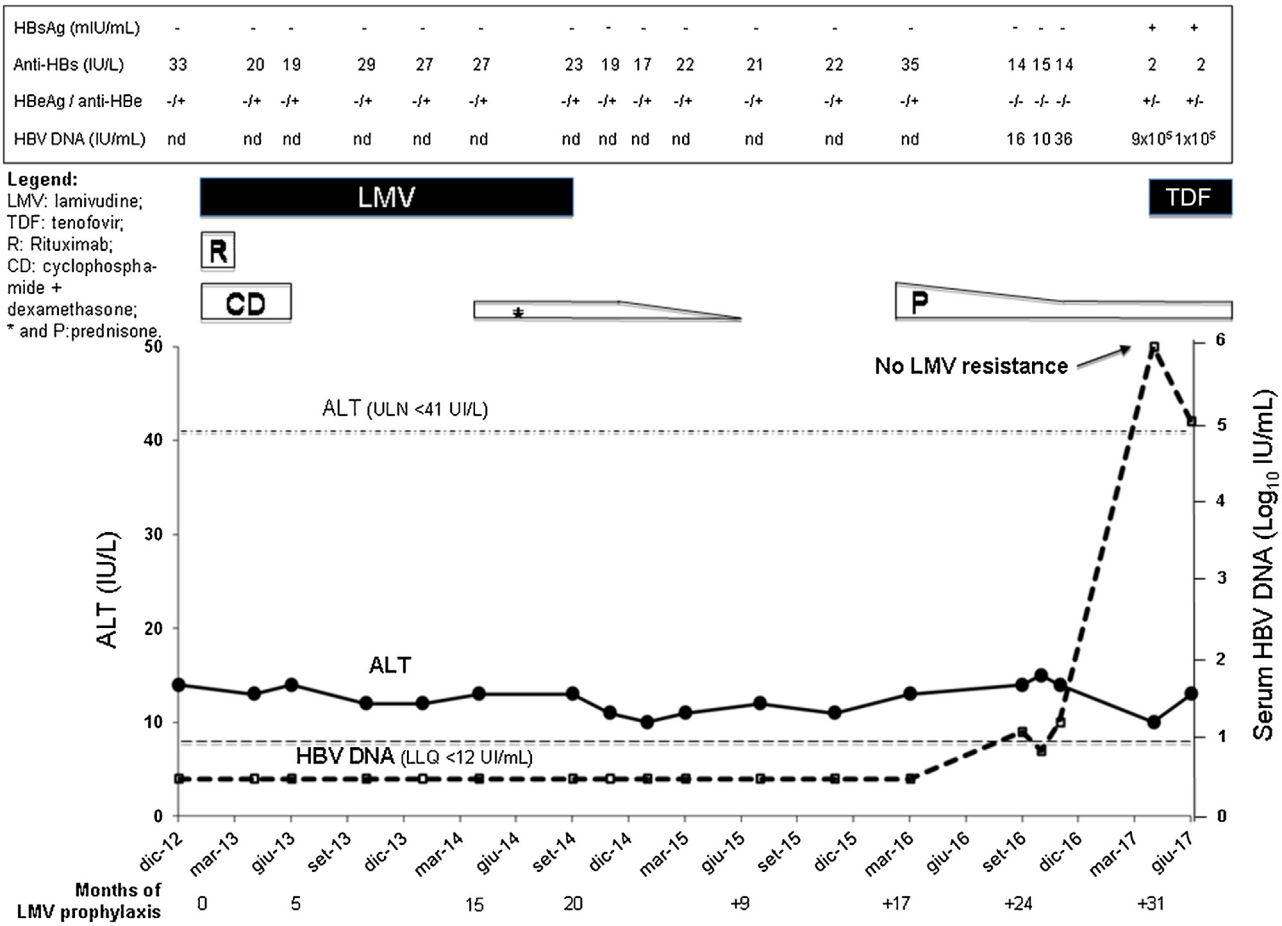

Fig. 2. Time course of virological and biochemical variables during and after lamivudine prophylaxis in a 84-year old female who seroreverted to HBsAg 31 months after lamivudine withdrawal. Prompt administration of tenofovir disoproxil fumarate rapidly suppressed viral replication in 4 months preventing ALT flare (data not shown).

HBsAg and HBV DNA levels but one (2%). An 83-year-old female with diffuse large B-cell lymphoma with positive anti-HBe and anti-HBs (33 mIU/mL), undetectable serum HBV DNA (<12 IU/mL) and negative for HCV, started LMV prophylaxis concomitantly with R-CT (rituximab + cyclophosphamide + dexamethasone) in January 2013. She received only two administrations of rituximab due to adverse effects, and five months of cyclophosphamide + dexamethasone therapy. LMV prophylaxis was discontinued in September 2014, i.e. 15 months after cyclophosphamide + dexamethasone withdrawal and 19 months after R withdrawal. The virological profile remained unchanged during these 20 months of LMV prophylaxis. Owing to incomplete oncological response, low dose steroids (prednisone 10 mg/day) were given between April 2014 and June 2015. No changes in anti-HBs levels were observed in this period. A second prednisone course (25 mg/day) was started on February 2016, 17 months after LMV withdrawal. In September 2016, 24 months after LMV withdrawal, HBV DNA tested positive (16 IU/mL) for the first time and anti-HBs levels decreased to 14 mIU/mL while anti-HBe became negative and HBsAg remained negative. As the patient failed to comply to the recommended strict monitoring of blood test, the next available visit was in March 2017, 31 months after LMV withdrawal. At this time point, both HBsAg and HBeAg turned positive, HBV DNA increased to 935,000 IU/mL while ALT levels remained within the normal range (13 IU/L). Molecular analysis of the circulating HBV strains did not show any evidence of lamivudine resistance; HBV genotype was D. Administration of tenofovir disoproxil fumarate rapidly and efficiently suppressed viral replication within 4 months (Fig. 2).

Five (6%) additional patients with baseline protective anti-HBs titres had a >50% decline of anti-HBs levels including 3 patients who became anti-HBs negative (Table 3). No patient experienced an ALT flare.

3.5. Survival and outcome

Overall, during 39 (2–108) months of study period, 17 patients (20%) died after a median of 12 (2–47) months from the start of R-CT. All died due to progression of the onco-hematological disease. Three patients underwent allogenic hematopoietic stem cell transplantation (HSCT) and 7 were lost to follow-up after 27 (13–47) months. Among 32 patients who are still alive after 33 (3–80) months of follow-up, haematotological disease was in complete remission in 24, in partial remission in 5 and in progression in the remaining.

4. Discussion

Our study demonstrates that universal prophylaxis with lamivudine is an effective and safe strategy to prevent HBV reactivation in HBsAg negative/anti-HBc positive patients with non-Hodgkin lymphoma receiving rituximab-based chemotherapy, since none of the patients experienced HBV reactivation or treatment-related side effects during lamivudine. Only one patient seroreverted to HBsAg 31 months after LMV withdrawal, but this was not related to LMV failure. To the best of our knowledge, this is the first large, long-term longitudinal cohort study assessing the effeciveness and safety of lamivudine prophylaxis in immuno-

suppressed Caucasian patients with onco-hematolgical disease receiving rituximab-containing regimens.

The importance of antiviral therapy to prevent HBV reactivation in HBsAg positive patients receiving rituximab-CT for onco-hematological diseases is well established [11–14]. However, HBV may also reactivate in up to 27% of NHL patients with resolved HBV infection, following rituximab-CT [2–4,5–8,15,16], with a cumulative HBV reactivation rates at months 6, 12, and 18 after chemotherapy of 8%, 11.2%, and 25.9% without prophylaxis [10]. Viral reactivation in such patients may be associated with a mortality rate of up to 50% even if antiviral treatment is started at the time of reactivation, and/or with the interruption or premature discontinuation of CT which by itself may negatively impact on patient survival [4,8,15,16]. This is not a trivial point owing to the fact that 1.6 billion people worldwide have a resolved HBV infection and the proportion of patients with concomitant onco-hematological disease and resolved HBV infection ranges between 20% in Western countries and up to 80% in highly endemic areas for HBV [17,18].

Yet, the management of HBV reactivation in patients with resolved HBV infection is not standardized, leaving doubts concerning the choice between different strategies, i.e pre-emptive anti-HBV therapy versus anti-HBV prophylaxis, and between different nucleos(t)ide analaogs, i.e. the cheapest and less potent lamivudine versus the newer, more expensive, entecavir or tenofovir disoproxil fumarate.

In contrast with national guidelines that recommended anti-HBV prophylaxis in patients with NHL undergoing R-CT chemotherapy since 2007 [11], international societies have changed their recommendations gradually over the years. The 2012 European Association for the Study of Liver (EASL) clinical practice guidelines (CPG) suggested universal prophylaxis of such patients [19], an approach that was further strengthened in the 2015 EASL HBV special conference and finally recommended in the recently published 2017 EASL HBV CPG [20,21]. Likewise, American Gastroenterology Association and American Association for the Study of Liver Disease recognized HBsAg negative/anti-HBc positive patients receiving R-CT and/or hematopoietic stem cell transplantation to be at high risk of HBV reactivation [22–24] and therefore liable to receive specific prophylaxys. While recognising this as a relevant clinical problem, in the Asian Pacific Association for the Study of the Liver guidelines the optimal preventive strategy for HBV reactivation in these patients remains undetermined, as further studies are needed to compare the efficacy and cost-effectiveness of the two different preventive strategies [25]. Three Asian uncontrolled studies in 447 patients with lymphoma and resolved HBV infection undergoing R-CHOP demonstrated that pre-emptive therapy with entecavir prevented severe HBV-related hepatitis in almost all patients (99%) leaving however a residual risk of HBV DNA flare (10%) and HBsAg seroreversion (2.6%), at the price of aggressive (monthly) and expensive monitoring of serum HBV DNA [5–7]. In two randomized controlled studies, rates of HBV reactivation and HBsAg seroconversion were lower in Taiwanese patients with lymphoma and resolved HBV infection undergoing R-CT randomized to entecavir prophylaxis compared to entecavir pre-emptive therapy (2.4% vs 18%, $p = 0.027$) [10].

One retrospective study that evaluated prophylaxis with lamivudine in elderly patients (median age 80 years) with NHL and resolved HBV infection treated with R-CT demonstrated a 10% risk of HBV reactivation [26]. However, the interpretation of this study is hampered by the lack of virological resistance test in virological breakthrough cases which does not allow to exclude a poor adherence to therapy. Moreover, the duration of lamivudine prophylaxis is not provided, and the relationship between HBV DNA flares and lamivudine administration is unclear. Aggressive lymphoproliferative bulky disease, coupled with the advanced age, may have had a role in facilitating viral replication by further reducing the immune control of these patients. One recent study that evaluated the efficacy and safety of lamivudine prophylaxis for 18 months post chemotherapy in 68 HBsAg-negative/anti-HBc-positive patients with onco-hematological disease (68% NHL), reported HBV reactivation 1–7 months after the lamivudine discontinuation in 2 patients with chronic lymphocytic leukaemia and in one with Waldenstrom's disease, but no case in patients with NHL [27]. However, in this study the patients were monitored with HBsAg and not with serum HBV DNA, thus making less sensitive the diagnosis of HBV reactivation.

In contrast, our study included all patients with non-Hodgkin lymphoma and resolved HBV infection who were consecutively given lamivudine prophylaxis before starting rituximab-CT, and planned to last for 18 months afterthe end of CT as recommended by Italian guidelines published in 2007 [11]. Our study has a regular monitoring of HBV DNA, HBsAg, anti-HBs and ALT both during prophylaxis and after lamivudine discontinuation. Although one-third of patients with protective anti-HBs titres at baseline showed a significant reduction in titres during R-CT treatment and 12 patients became anti-HBs negative, no HBsAg seroreversion occurred during lamivudine prophylaxis. Only one case seroreverted to HBsAg after lamivudine withdrawal but this was not related to lamivudine failure as this event occurred more than 2 years after lamivudine withdrawal in the absence of any of molecular evidence of drug-resistant strain. Most likely, this late event was due to the combination of incomplete oncological response and the administration of immunosuppressive regimens in a patient who did not restart anti-HBV prophylaxis. To the best of our knowledge, this is the first large and long-term cohort study that addresses this clinical point which is relevant not only because of the burden of serum anti-HBc positive patients worldwide, but also because of the increased use of R-CT in patients with onco-hematological disorders. Lamivudine prophylaxis represents a low cost, user friendly drug with a well consolidated safety profile. As a matter of fact, our study reported no treatment related adverse events.

The major limitation of the study relates to the fact thatis a retrospective one, with all the possible bias of patients' selection in a tertiary hematological division, the referral to the hepalogist, and the absence of a control group left without lamivudine prophylaxis. Anyway, features of our treated cohort are comparable with those of 39 Asian untreated patients (72% males, mean age of 69 years, 82% with diffuse large B-cell lymphoma) that faced a 25.9% cumulative risk of HBV reactivation after 18 months [10]. Moreover, the strenghts of this study are the homogeneity of the enrolled patients in terms of hematological disease, HBV profile, and prophylaxis strategy, and the sample size the largest to date. Further, virological markers were tested in a single laboratory using sensitive replication markers. Moreover, patients' management had been shared with the hematologists in terms of prophylaxis strategy and follow-up. This strategy has allowed us to have no missing data on HBV DNA as well as on HBsAg and anti-HBs levels (591 virological samples were performed over the entire study period). An additional strength of the study was the safety and efficacy of lamivudine prophylaxis also in those patients who received R-based immunosuppression as long-term maintenance therapy.

What is less clear is whether safety and efficacy lamivudine prophylaxis in this clinical setting are comparable to strategies based on entecavir or tenofovir disoproxil fumarate. Important variables to be considered are the duration and the depth of the immunosuppression as new, potent molecular target drugs will be administered for a long period of time in some cases. Indeed, two cases of lamivudine resistance emerging after 5 years of lamivudine prophylaxis in highly immunocompromised onco-hematological patients have been recently reported [28]. In such patients, treated with different immunosuppressant after ritux-

imab and therefore with a high probability of further reactivation, the possibility to administer lamivudine beyond the recommended terms is clinically sound, as well as an extended virological monitoring. Prophylaxis with entecavir, tenofovir disoproxil fumarate or the recently approved tenofovir alafenamide in these patients could also be considered although this strategy would be more expensive, and it is not currently refunded by many National Health Systems including our. Only long-term prospective studies in large cohort of such patients aimed to define the risk and predictors of lamivudine failure, will shed new lights on this relevant issue.

In conclusion, lamivudine is an efficacious and safe prophylaxis for Caucasian patients with non-Hodgkin Lymphoma and resolved HBV infection undergoing rituximab-based chemotherapy. This notwithstanding, monitoring of virological markers during lamivudine prophylaxis to intercept and promptly rescue the few failures, is mandatory. For selected patients requiring long-term CT, those with detectable HBV DNA level or whenever regular HBV DNA monitoring cannot be implemented, prophylaxis with entecavir, tenofovir disoproxil fumarate or tenofovir alafenamide could be considered as alternative strategy.

Conflict of interest

Mauro Viganò is on the speakers' bureau of Roche and Bristol–Myers Squibb. He is also in the speakers' bureau of and received grants from Gilead. Pietro Lampertico advises, is on the speakers' bureau of Bristol–Myers Squibb, Roche, Gilead, GlaxoSmithKline, MSD and Abbvie. Mariagrazia Rumi is on speaker bureau of AbbVie and received research grants from MSD. The other authors declare that they have no competing interests.

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