

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-003
21-004

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NDA#: 21003, 21004
APPLICANT: Glaxo WELLCOME Inc.
NAME OF DRUG: EPIVIR®-HBV™ (lamivudine) tablets and oral solution
INDICATION: Treatment of Chronic Hepatitis B
DOCUMENTS REVIEWED: Vol. 3.1, 17, 20, 32, 33, 38, 39
MEDICAL INPUT: HFD-530: B. Styrt, M.D.

A. Background

This NDA contains four pivotal, randomized, multicenter, controlled trials to support the proposed indication for the treatment of chronic hepatitis B. All subjects were 16 years or older and had chronic hepatitis B (serum HBsAg positive for at least 6 months), and evidence of HBV DNA replication (serum HBeAg positive and positive for HBV DNA, as measured by a research solution-hybridization assay). Subjects had persistently elevated serum ALT levels and/or chronic inflammation on liver biopsy compatible with a diagnosis of chronic viral hepatitis and they had no signs of hepatic decompensation.

Protocols

US Study: Protocol NUCA3010

Title: "A Study of Lamivudine or Placebo in Patients with Chronic Hepatitis B Infection Who Are Treatment Naive"

This is a double-blind, placebo-controlled study of lamivudine 100 mg once daily versus placebo for 52 weeks, followed by a 16 weeks of no-treatment period, in subjects with chronic hepatitis B in US.

Interferon Non-responder Study: Protocol NUCAB3011

Title: "A Placebo Controlled Study of Lamivudine and Intron A® in non-decompensated Patients with Chronic Hepatitis B Infection who are Interferon α Non-Responders"

This was a partially-blind, placebo-controlled three-arm study primarily in North America and Europe in subjects with chronic hepatitis B who had failed to respond to previous treatment with interferon alfa. The study compared lamivudine 100 mg once daily for 52 weeks, followed by either lamivudine 100 mg once daily, or matching placebo once daily, for 16 weeks (Arm 1) versus placebo once daily for 68 weeks (Arm 2) versus lamivudine 100 mg once daily for 8 weeks, followed by lamivudine 100 mg once daily plus interferon alfa (10 MU subcutaneously three times weekly) for 16 weeks (total treatment regimen of 24 weeks), and then no treatment follow-up for 44 weeks (Arm 3) in US, Canada, Europe and Hong Kong.

Asian Study: Protocol NUCB3009

Title: "A Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of Two Dosage Regimens of Lamivudine in Patients with Chronic Hepatitis B Infection"

This was a double-blind, placebo controlled three arm study that compared lamivudine 25 mg once daily (Arm 1) versus lamivudien 100 mg once daily (Arm 2) versus placebo (Arm 3) for 52 weeks in subjects with chronic hepatitis B who are primarily treatment naïve in Hong Kong, Singapore and Taiwan. In this study subjects were stratified into two groups by the screening HAI score (≥ 3 vs. ≤ 2).

Active-control Study: Protocol NUCB3010

Title: "A Study of Lamivudine and Alpha-Interferon in Patients with Chronic Hepatitis B Infection Who Are Interferon Treatment Naive"

This is a partially-blind, three arm study that compared lamivudine 100 mg once-daily for 52 weeks (Arm 1) versus placebo once daily for 8 weeks followed by placebo once daily plus interferon alfa monotherapy 10 MU subcutaneously three times weekly for 16 weeks (total treatment regimen of 24 weeks) (Arm 2) versus lamivudine 100 mg once daily for 8 weeks followed by interferon alfa 10 MU three times weekly plus lamivudine 100 mg once daily for 16 weeks (total treatment regimen of 24 weeks) (Arm 3) in therapy naïve subjects in Europe, Canada, South Africa, New Zealand and Australia.

Efficacy Endpoints and Analysis Plan

The primary efficacy endpoint for NUCA3010, NUCAB3011 and stratum 1 of NUCB3009 is the histologic response. For stratum 2 of NUCB3009 it is the reduction in HBcAg and liver HBV DNA. For NUCB3010 it is HBeAg seroconversion. However, an abridged analysis plan was submitted upon the request of FDA. In this analysis plan, histology was regarded as the most important endpoint and HBeAg seroconversion was regarded as the most important secondary endpoint.

All efficacy analysis were based on the modified ITT population, which include all subjects randomized with *confirmed* chronic hepatitis B. The definition of 'confirmation' varies among the studies. The primary analysis was conducted at the end of 52 weeks treatment.

For histologic response, responders consisted of subjects with a reduction of Knodell HAI ≥ 2 points at week 52. Missing observations were regarded as non-responders. CMH analysis stratified by center (except NUCA3010, which was not stratified by center) was used for the analysis.

Serology data includes the following marks: HBeAg/HBeAb; HBsAg/HBeAb; HBV DNA and

ALT. HBeAg seroconversion at the end of study was defined as a decrease in HBeAg to undetectable levels, a rise in HBeAb to detectable levels and a decrease in HBV DNA to undetectable levels. Missing observations for HBeAg, HBeAb, HBsAg and HBeAb were imputed using LOCF using only data from scheduled visits. Missing values for ALT and HBV DNA were regarded as failures. Analysis was based on CMH methods.

Subgroup analyses were conducted for the histology response and HBeAg seroconversion using logistic regression. This analysis was performed to determine whether or not the treatment effects could be interpreted as homogeneous across the levels of the baseline covariates. This analysis was also performed on the integrated data of the 4 phase III trials.

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B. Results of the Applicant's Analyses

Baseline Characteristics

The four pivotal studies differ in entry criteria and population studied. The following table describes some of the baseline variables by study.

Baseline Characteristics by Study

Baseline Characteristics		US N=137	IFN Non-responder N=238	Asian N=357	Active-control N=226
Mean Age (years)		41	39	31	34
Gender	Male	83%	81%	73%	74%
	Female	17%	19%	27%	26%
Ethnic Origin	Caucasian	58%	82%	0	63%
	Asian	0%	2%	0%	8%
	Oriental	20%	8%	100%	22%
	Black	17%	3%	0	4%
	Other	5%	5%	0	4%
Mean Weight (kg)		81	76	62	71
Probable Route of Transmission	Horizontal	39%	40%	1%	21%
	Vertical	17%	12%	39%	12%
	Other	13%	14%	1%	12%
	Unknown	33%	34%	60%	55%
Median	Total HAI	10.0	10.0	7.0	4.0
	Necroinflammatory HAI	9.0	7.0	6.0	3.0
	Cirrhosis	10%	19%	5%	7%
	HBV DNA	81.2	96.1	75.2	113.5
	ALT (xULN)	3.1	2.5	1.5	2.4

Horizontal includes sexual contact, transfusion recipient and past IV drug use.

Based on tables on pages 179, 183, 187 and 191 of Clinical Summary section of Vol. 1 of the NDA21003, and Glaxo presentation on the Anti-viral Advisory Committee Meeting held on 10/6/98.

The US Study and the Interferon Non-responder Study are similar in the above baseline characteristics except the composition of ethnic origin where a higher proportion of subjects enrolled were Caucasians in the Interferon Non-responder Study than was in the US Study. Subjects in the Asian Study were generally less ill (lower HAI score, ALT, HBV DNA and fewer cirrhosis), younger and less heavy. Most of the subjects in this study with route of transmission known were due to vertical transmission. Subjects in the Active-control Study had lower baseline HAI scores but higher HBV DNA levels.

Subject Accountability

The following table presents the disposition of subjects for four studies by Week 52.

Subject Status and Reason Discontinued by Treatment Group and Study

Treatment	US		IFN Non-responder			Asian			Active-control		
	LAM100	PLA	LAM100	LAM+IFN	PLA	LAM100	LAM25	PLA	LAM+IFN	LAM	IFN
Total Randomized	71	72	119	63	56	143	142	73	77	82	71
ITT	66	71	119	63	56	143	142	72	75	82	69
Withdrawals (%)	14	18	8	16	18	3	6	4	19	21	16
Adverse Event	2	1	1	2	7	1	1	1	3	5	0
Lack of Efficacy	0	1	2	0	2	0	0	0	0	0	0
Failed to Return	8	8	2	8	5	1	4	1	8	10	10
Other	5	7	3	6	4	1	1	1	8	6	6

Based on tables on pages 179, 183, 187 and 191 of Clinical Summary section of Vol. 1 of the NDA21003 and submitted electronic dataset

The Asian Study had relatively low withdrawal rates in each treatment arm. The rates for the treatment arms in the other three studies ranged from 8% to 21%. Little differentiation in reasons for withdrawals between treatment arms was seen.

Efficacy Endpoints

The table below displays the results for the primary efficacy endpoints: histologic improvement and HBeAg seroconversion at Week 52. The missing seroconversion rates were filled in with the last available seroconversion status. The missing histologic evaluations were considered failures.

Week 52 Histologic Improvement (≥ 2 points) by Treatment and Study

Missing values treated as failures

n/N (%)

Study	PLA	LAM100	LAM+IFN	IFN	p-value*
US	16/71 (23)	34/66 (52)	n/a	n/a	<0.001
IFN Nonresponder	14/56 (25)	62/119 (52)	20/63 (32)	n/a	0.002
Asian	18/72 (25)	74/143 (52)	n/a	n/a	<0.001
Active-control	n/a	31/82 (38)	21/75 (28)	25/69 (36)	n/a

* LAM100 vs. PLA

Based on tables on page 195 of Clinical Summary section of Vol. 1 of the NDA21003.

Week 52 HBeAg Seroconversion by Treatment and Study
Missing Data Imputed Using LOCF
n/N (%)

Study	PLA	LAM100	LAM+IFN	IFN	p-value*
US	4/69 (6)	11/63 (17)	n/a	n/a	0.036
IFN Nonresponder	7/53 (13)	19/108 (18)	7/57 (12)	n/a	0.022
Asian	3/70 (4)	22/140 (16)	n/a	n/a	n/a
Active-control	n/a	14/80 (18)	20/68 (29)	12/64 (19)	n/a

* LAM100 vs. PLA

Based on tables on page 198 of Clinical Summary section of Vol. 1 of the NDA21003.

The histologic comparisons between lamivudine 100 mg and placebo were statistically significant in each of the three placebo-controlled studies (US, IFN Non-responder and Asian). Lamivudine 100 mg treatment significantly enhanced HBeAg seroconversion rates compared with placebo in the Asian Study and the US study in primarily treatment naïve subjects.

Subgroup Analysis

The subgroup analysis was conducted for the histologic improvement and HBeAg seroconversion using logistic regression with all four studies combined. Factors investigated are: baseline ALT (≤ 2 vs. >2 xULN), baseline HBV DNA (0-50, >50-75, >75-100, >100 pg/mL), ethnic origin (Oriental, Caucasian and Other), age (0-16, 17-40, 41-64, ≥ 65), gender, baseline Knodell HAI (0-4, 5-9, 10-14, 15-22), weight (0-50, >50-75, >75-100, >100 kg), Body Mass index (BMI) (<20, 20-24, 25-29, >29 kg/m²), Cirrhosis (Yes, No) and Bridging Fibrosis (Yes, No). For histologic response, none of these appeared to be related to the treatment differences. For HBeAg seroconversion, treatment differences were found to be related to baseline HBV DNA (p=0.023) and Gender (p=0.018). Further analysis will be done in the reviewer's analysis.

C. Statistical Reviewer's Comments

Among the four principal Phase III studies, the Asian Study and the Active-control Study were not conducted under . The protocol-specified primary endpoints for these two studies differ from those of the US Study and the IFN Non-responder Study. Per FDA requests, the applicant conducted analyses for the two non-IND studies similarly to the two IND studies. In this review, Histologic improvement will be considered the primary endpoint and HBeAg seroconversion will be regarded the primary secondary endpoint.

Some subjects who did not exactly meet the entry criteria were admitted into the trials and were included in the intent-to-treat population. To assess the impact the sponsor conducted analyses on subjects who met entry criteria and did not have other protocol violations (Per Protocol). The consistency of the results will be discussed in the reviewer's analysis.

There were a few subjects in the ITT population who actually received treatment other than the randomized one. The possible influence of these subjects on the primary endpoints will be assessed.

The handling of missing HBeAg, HBeAb and HBeAg seroconversion using last-observation-carried-forward (LOCF) was considered not acceptable. The reason being that these responses were not durable among subjects with follow-up data. Of the subjects who ever had a HBeAg negative, 37% had a positive value later; of the subjects who ever became HBeAg positive, 39% had a negative value later. For this reason, missing will be treated as failures in the reviewer's analysis. Other ways of handling missing values will be explored to assess the sensitivity of the primary outcome results.

Since the applicant is seeking an indication for lamivudine 100 mg daily, the reviewer's analyses will focus primarily on lamivudine 100 mg vs. placebo comparison.

The sponsor identified baseline HBV DNA and Gender as potential modifiers of serologic response. In the analyses below, response rates for subgroups defined by these three factors plus age and race will be listed and compared by study.

In addition, exploratory analysis will be presented to examine the relationship between histologic improvement and seroconversion status; to compare serologic responses on treatment and off treatment; to compare serologic responses for continued treatment and stopped treatment; to examine responses overtime for serologic and virologic endpoints; to study baseline predictors of Week 52 response; to predict Week 52 responses using HBV DNA patterns during treatment; and to study the association of HBV DNA response patterns and the emergence of YMDD mutants.

D. Statistical Reviewer's Analyses

D.1 Primary efficacy results

Histologic Improvement

Histologic improvement is defined to be a decrease of the total Knodell score by 2 points or more at Week 52 from baseline. Missing histologic improvement could be due to either missing baseline or Week 52 Knodell score. The histologic improvement rates presented in the table below are identical to the applicant's results with the addition for the two strata of the Asian study.

Histologic Improvement Rates

Study	LAM100			PLA			IFN+LAM			IFN			LAM25		
	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis
US	66	51.5	22.7	71	22.5	25.4									
IFN Non-rspd	119	52.1	25.2	56	25.0	23.2	63	31.7	30.2						
Asian	143	51.7	15.4	72	25.0	16.7							142	47.9	15.5
Stratum 1	100	60.0	16.0	49	28.6	20.4							100	58.0	16.0
Stratum 2	43	32.6	14.0	23	17.4	8.7							42	23.8	14.3
Active-control	82	37.8	23.2				75	28.0	24.0	69	36.2	21.7			

Yes means subject had a two or more points improvement in total Knodell score

Mis. means either the Week 52 or the baseline total Knodell score is missing

Stratum 1: subjects with modified HAI score ≥ 3 at baseline. Modified HAI is the total of periportal and intralobular scores.

Stratum 2: subjects with modified HAI score ≤ 2 at baseline.

CMH analyses were conducted to compare lamivudine 100mg vs. placebo, the results are summarized below.

Histologic Improvement Rates with Missing as Failures

	US	IFN Non-responder	Asian	Asian: Stratum 1	Asian: Stratum 2
LAM100	51.5	52.1	51.7	60.0	32.6
PLA	22.5	25.0	25.0	28.6	17.4
p-value	0.001	0.002*	0.001**	0.001**	0.218**

* Stratified by investigator

** Stratified by the randomization stratum and investigator

Among subjects with missing histologic improvement, 28% had missing baseline Knodell score. These subjects had similar screening HBV DNA, baseline HBV DNA, Week 52 HBV DNA, Week 52 HBV DNA imputed with LOCF, baseline ALT (xULN), Week 52 ALT (xULN) and Week 52 total Knodell score when compared to the subjects with baseline Knodell score. It does not appear that subjects without baseline Knodell scores were different from those with baseline Knodell score in baseline disease severity or week 52 outcome. Under the assumption that the

treatment effect sizes for histologic improvement were comparable for subjects with and without baseline Knodell score, we can exclude them from histology analysis without affecting the interpretation of the results. The table below lists the results for this analysis.

Histologic Improvement Rates

Subjects with Missing Baseline Knodell Score Excluded

Study	LAM100			PLA			IFN+LAM			IFN			LAM25		
	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis
US	62	54.8	17.7	62	25.4	15.9									
IFN Non-rspd	110	56.4	19.1	54	25.9	20.4	59	33.9	25.4						
Asian	131	56.5	7.6	68	26.5	11.8							134	50.7	10.4
Stratum 1	89	67.4	5.6	45	31.1	13.3							93	62.4	9.7
Stratum 2	42	33.3	11.9	23	17.4	8.7							41	24.4	12.2
Active-control	81	38.3	22.2				72	29.2	20.2	67	37.3	19.4			

Yes means subject had a two or more points improvement in total Knodell score

Mis. means the Week 52 total Knodell score is missing

Stratum 1: subjects with modified HAI score ≥ 3 at baseline.

Stratum 2: subjects with modified HAI score ≤ 2 at baseline.

The statistical comparisons based on this population are summarized below.

Histologic Improvement Rates with Missing as Failures

Subjects with Missing Baseline Knodell Score Excluded

	US	IFN Non-responder	Asian	Asian: Stratum 1	Asian: Stratum 2
LAM100	54.8	56.4	56.5	67.4	33.3
PLA	25.4	25.9	26.5	31.1	17.4
p-value	0.001	0.001*	0.001**	0.001**	0.204**

* Stratified by investigator

** Stratified by the randomization stratum and investigator

Based on either analysis, the US Study, the IFN Non-responder Study and the Asian Study demonstrated a significant treatment difference at 0.05 level. The stratum 2 of the Asian Study showed a treatment difference favoring lamivudine 100mg but it is not statistically significant.

Note that in the first table the missing rates ranged from 8.7% to 25.4% while the treatment effects for the three placebo controlled studies and Stratum 1 of Asian Study are greater than 26%. Sensitivity analysis to investigate different ways of classifying missing values showed that the statistical significance was robust. For example, when half of the missing observations in the placebo arms are regarded as success while all other missing values are treated as failures, the statistical analyses still achieved the 0.05 level. Excluding subjects with missing baseline histologic value (the third and fourth table) will make the estimated treatment effects larger and reduce the missing rates, making the statistical comparison less influenced by how missing values are treated.

A few subjects started treatment other than the randomized one during the first 52 weeks of treatment. In the IFN Non-responder Study, one subject was randomized to the lamivudine monotherapy group but actually received lamivudine and interferon combination. In the Active-control Study, three subjects randomized to the combination therapy and one subject randomized to the interferon monotherapy received the lamivudine monotherapy; two subjects randomized to lamivudine monotherapy received the combination therapy. The influence of these subjects on the efficacy evaluation is small.

The results based on the Per Protocol analysis, which uses only subjects who satisfy the entry criteria and without protocol violations during the trial, were nearly identical to the analysis based on the ITT population.

Overall we see evidence that lamivudine 100mg dose induces a statistically higher proportion of histologic responses than placebo after 52 weeks of treatment with the exception of the stratum 2 of the Asian Study. Since few subjects in the other three studies were similar to stratum 2 of the Asian study, there is not sufficient information to conclude that there is a treatment benefit for these subjects. However, as will be seen in an exploratory analysis later, the histologic response in stratum 2 is consistent with the finding that lower baseline Knodell score is associated with less histologic improvement.

HBeAg Seroconversion

HBeAg seroconversion at Week 52 was defined to be HBeAg negative, HBeAb positive and HBV DNA below assay limit at Week 52. The HBeAg seroconversion rates presented in the table below differs from the applicant's results in that missing values were not imputed. Instead, the missing values are presented as a separate category. However, it does agree with the FDA requested analysis with missing as failures presented by the applicant for each study. The results for the two strata of the Asian study are also presented below.

HBeAg Seroconversion Rates

Study	LAM100			PLA			IFN+LAM			IFN			LAM25		
	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis	N	Yes	Mis
US	63	17.5	15.9	69	5.8	15.9									
IFN Non-rspd	108	14.8	16.7	53	13.2	18.9	57	5.3	17.5						
Asian	140	15.7	4.3	70	4.3	4.3							135	12.6	5.9
Stratum 1	97	21.6	4.1	47	6.4	6.4							93	18.3	4.3
Stratum 2	43	2.3	4.7	23	0.0	0.0							42	0.0	9.5
Active-control	80	12.5	25.0				68	26.5	19.1	64	17.2	12.5			

Yes means subject was HBeAg negative, HBeAb positive and HBV DNA above the assay limit at Week 52

Mis means the HBeAg seroconversion status at Week 52 could not be determined

Stratum 1: subjects with modified HAI (Sum of periportal and nebular Knodell components) score ≥ 3 at baseline.

Stratum 2: subjects with modified HAI score ≤ 2 at baseline.

Note the sample sizes for this table differ from the tables for histology because subjects with negative HBeAg or HBV DNA were excluded for this analysis.

The statistical comparisons for week 52 results between lamivudine 100mg and placebo are summarized below.

HBeAg Seroconversion rates at Week 52 with Missing as Failures

	US	IFN Non-responder	Asian	Asian: Stratum 1	Asian: Stratum 2
LAM100	17.5	14.8	15.7	21.6	2.3
PLA	5.8	13.2	4.3	6.4	0.0
p-value	0.036	0.527*	0.017**	0.023**	0.450**

* Stratified by investigator

** Stratified by the randomization stratum and investigator

The US Study showed a treatment difference that just passes statistical significance level 0.05. Since 16% of observations were missing for Week 52 seroconversion for both treatment arms, a slightly different treatment of missing values for the two treatment arms will lead to non-significance for this comparison. Therefore this result is very sensitive to how missing values are handled.

For the IFN Non-responder Study, the two arms showed comparable seroconversion rates and the difference was not statistically significant.

For the Asian Study, there is an overall statistically significant difference between the two arms. But this is mostly due to the difference seen in the Stratum 1. There was less missing values in this study and the result is more robust. For the Stratum 2 very few subjects were HBeAg seroconverters at Week 52 and the difference is not statistically significant.

The analyses based on Per Protocol population yielded very similar response rates. However, the p-value for the US study is 0.054 in this population and is not statistically significant.

Overall, there was no evidence of treatment effects for the IFN Non-responder Study and subjects with mild disease in the Asian Study. There was marginal evidence for the US Study. And there was better evidence for the severe subjects in the Asian Study.

D.2 Subgroup Analysis

In this subsection we will analyze the histologic improvement and seroconversion status in subgroups defined by gender, age, race and baseline HBV DNA.

The following tables summarize the histologic results by gender and treatment with missing regarded as failures.

Subgroup Analysis by Gender and Treatment

Study	Gender	Histologic Improvement				HBeAg Seroconversion			
		LAM100		Placebo		LAM100		Placebo	
US	Male	49.1%	28/57	21.1%	12/57	14.6%	8/55	3.6%	2/55
	Female	66.7%	6/9	28.6%	4/14	37.5%	3/8	14.3%	2/14
IFN-Nrspd	Male	51.5%	51/99	24.5%	12/49	16.9%	15/89	8.7%	4/46
	Female	55.0%	11/20	28.6%	2/7	5.3%	1/19	42.9%	3/7
Asian	Male	52.8%	56/106	28.9%	15/52	17.3%	18/104	3.9%	2/51
	Female	48.7%	18/37	15%	3/20	11.1%	4/36	5.3%	1/19
Active-ctrl	Male	41.4%	24/58			15.5%	9/58		
	Female	29.2%	7/24			4.6%	1/22		

We see typically that the sample sizes for females are small in all four studies. Response rates are sometimes higher for females and sometimes for males. Testing of histologic response rates in males and females showed no significant differences (p -value > 0.25 for each of the four studies). There is no evidence that gender is a significant modifier of the histologic treatment effects as evidenced by testing of gender and treatment interaction using Breslow-Day test (p -values ≥ 0.4 for the three placebo-controlled studies).

For seroconversion, the response rate is higher in females than in males for the US Study (p -value=0.03) but the difference is not statistically significant in other studies. However, in view of the number of comparisons performed, even the difference in the US Study is not conclusive after adjusting for multiple comparisons. The Breslow-Day test of gender by treatment interaction yielded p -values of 0.86 for the US Study, 0.006 for the IFN Non-responder Study and 0.55 for the Asian Study. When all three studies are combined, the resulting p -value is 0.012. Therefore the applicant's results for the gender by treatment interaction is primarily due to the interaction in the IFN Non-responder Study.

Overall, the treatment effects may be different for males and females in IFN Non-responders. But the US Study and Asian study do not support this conclusion.

Similar analyses for age and ethnic origin showed little evidence that these two factors are modifiers of the histologic improvement or seroconversion rates or their treatment effects.

The table below summarizes the response rates for subgroups defined by baseline HBV DNA (≤ 100 vs. >100 , subjects with missing baseline HBV DNA excluded).

Subgroup Analysis by HBV DNA and Treatment

Study	HBV DNA	Histologic Improvement				HBeAg Seroconversion			
		LAM100		Placebo		LAM100		Placebo	
US	≤100	61.3%	19/31	27.5%	11/40	21.4%	6/28	7.9%	3/39
	>100	40.6%	13/32	16.0%	4/25	12.5%	4/32	0.0%	0/25
IFN-Nrspd	≤100	60.0%	33/55	31.0%	9/29	25.0%	11/44	23.1%	6/26
	>100	44.3%	27/61	19.2%	5/26	6.6%	4/61	3.8%	1/26
Asian	≤100	52.8%	47/89	22.2%	8/36	18.6%	16/86	5.9%	2/34
	>100	50.0%	27/54	27.8%	10/36	11.1%	6/54	2.8%	1/36
Active-ctrl	≤100	45.2%	14/31			27.6%	8/29		
	>100	33.3%	17/51			3.9%	2/51		

We see from the table that subjects with baseline HBV DNA ≤100pg/mL typically had better histologic improvement and seroconversion rates at Week 52 than subjects with baseline HBV DNA >100pg/mL. This difference is statistically significant for both histology (p=0.02 based on CMH test adjusting for study and treatment) and seroconversion (p=0.001) for lamivudine 100mg groups. However, similar patterns were seen for placebo treated subjects, too.

To see if the treatment effects are homogeneous across different levels of baseline HBV DNA, we compare the treatment effects in the two HBV DNA strata. In the US Study, the treatments effects are (61.3 – 27.5) = 33.8% in the lower stratum (≤100) vs. (40.6 – 16.0) = 24.6% in the upper stratum (>100) for histologic improvement. The other two placebo-controlled studies showed very similar patterns, suggesting that effects on histologic improvement may be larger for the lower stratum. However, testing of HBV DNA strata by treatment interaction using the Breslow-Day test yielded p-values > 0.3, suggesting that this relative difference in treatment effects on histology could be due to chance. Similar conclusion can be reached for HBeAg seroconversion.

For histology, the above conclusion of no significant interaction is consistent with the applicant's results. However, for HBeAg seroconversion, it differs from the applicant's result. Recall that the interaction of baseline HBV DNA by treatment was statistically significant with p-value 0.023 in the applicant's analysis. Since this result could not be reproduced with the applicant-specified model (logistic regression with treatment and baseline HBV DNA and their interaction in the model. Baseline HBV DNA was classified into 4 categories but was treated as continuous in the model. The resulting p-value for interaction is 0.407), the applicant's results will be ignored.

D.3 Exploratory Analysis

The histologic and seroconversion endpoints are surrogate markers for long term outcome. The clinical relevance of these surrogates have not been fully established. Further, the trial was only

one year long and it is not clear if this constitutes an adequate treatment period. In this section the reviewer will attempt to address these issues.

1. Relationship between Histologic Improvement and Seroconversion at Week 52

Since histology and seroconversion were regarded as the most important endpoints for this submission, it is of interest to see how these two measures correlate. Especially since biopsy is invasive it is of interest to know how well seroconversion can predict histologic outcome. This relationship is summarized in the table below for lamivudine 100mg and placebo treated subjects with all four studies combined. Subjects with missing baseline Knodell score or with negative eAg or negative HBV DNA have been excluded.

Seroconversion Status vs. Histologic Improvement at Week 52

Number of Subjects

	With seroconversion status?	Improved in total Knodell score by 2 points or more?			
		Yes	No	Missing	Total
LAM100	Yes	39	12	6	57
	No	141	106	18	265
	Missing	8	4	34	46
	Total	188	122	58	368
PLA	Yes	6	7	1	14
	No	39	95	13	147
	Missing	0	3	14	17
	Total	45	105	28	178

For lamivudine 100mg treated subjects, $39/(39+12) = 76\%$ of the subjects with seroconversion status also had 2 or more points of histologic improvement. However, for subjects without the seroconversion status, $141/(141+106) = 57\%$ of them also had the histologic improvement. Even though the difference between the two proportions is statistically significant, indicating an association of these two measures, the fairly small magnitude of the difference here suggests that histologic outcome could not be well predicted using seroconversion status.

Few seroconverters were available to make a meaningful comparison for the placebo group.

2. Serologic responses: On Treatment vs. Off Treatment

Should subjects who had seroconversion status be continuously treated or should they be taken off the drug? This question really can not be adequately answered with these four trials because of the limited number of subjects who were followed with no treatment after lamivudine 100mg, and more importantly, because of the unavailability of long term clinical endpoints. Lacking such data, we will investigate if subjects who had seroconversion status are more likely to maintain their serologic responses on treatment than off treatment. Because of the limited parallel data

regarding on and off treatment, we will compare the probability of re-emergence of HBeAg and HBV DNA, and loss of HBeAb and HBeAg seroconversion during the last 16 weeks of lamivudine 100mg treatment (Weeks 36 – 52) vs. the first 16 weeks of follow-up without any active treatment (Weeks 52 – 68). The table below summarizes the results.

Kaplan-Meier Probability for Loss of Response after 16 Weeks

Subjects treated with lamivudine 100mg for 52 weeks followed with placebo or no treatment

Response Variable	Responders at	Number of subjects			Probability for Loss of Response (Standard Error)
		Total	Failed	Censored	
Seroconversion Status	Week 36	38	13	25	0.370 (0.082)
	Week 52	52	12	40	0.464 (0.104)
HbeAg Negative	Week 36	78	9	69	0.123 (0.038)
	Week 52	77	14	63	0.299 (0.068)
HbeAb Positive	Week 36	45	6	39	0.150 (0.057)
	Week 52	83	15	68	0.412 (0.086)
HBV DNA Negative	Week 36	260	90	170	0.355 (0.030)
	Week 52	207	65	142	0.628 (0.049)

For example, the rows for “Seroconversion Status” show that at Week 36, 38 subjects had HBeAg seroconversion. From Week 36 to Week 52, 13 of these subjects lost this status at least at one visit while the remaining 25 subjects either completed the treatment or withdrew without observing loss of this status. The Kaplan-Meier estimation of probability of the loss of status, which assumes withdraw or lost to follow-up is not related to loss of seroconversion status, is 37.0% with standard error 8.2%. Similarly, there were 52 subjects with seroconversion status at Week 52 and an estimated 46.4% (with standard error 10.4%) lost seroconversion status at least during one visit from Week 52 to 68. Due to the lack of an adequate control, no tests of significance are provided.

We see from the table that the probabilities for loss of responses were lower while the subjects were on treatment (Weeks 36 – 52) than off treatment (weeks 52 – 68). In particular, the re-emergence of HBV DNA was significantly lower while the subjects were on treatment. However, in the next table we will see that this difference was primarily due to the difference in subjects without seroconversion status. The rates for subjects with seroconversion status were similar.

To see if subjects with seroconversion status are more likely to have sustained HBV DNA response than only with HBV DNA negative, we further divide the subjects with negative HBV DNA into two groups according to seroconversion status. The re-appearance of HBV DNA is summarized below.

Kaplan-Meier Probability for Loss of HBV DNA Response after 16 Weeks

Subjects treated with lamivudine 100mg for 52 weeks followed with placebo or no treatment

Responders at	Seroconversion Status	Number of subjects			Probability for Loss of Response (Standard Error)	p-value
		Total	Failed	Censored		
Week 36	Yes*	38	10	28	0.274 (0.074)	0.25
	No**	222	80	142	0.368 (0.033)	
Week 52	Yes*	52	9	43	0.337 (0.095)	<0.001
	No**	155	56	99	0.742 (0.052)	

* Re-emergence of HBV DNA among subjects with HBeAg seroconversion status.

** Re-emergence of HBV DNA among subjects with negative HBV DNA but without seroconversion status.

From the table we see that while on the lamivudine 100mg treatment during Weeks 36 – 52, subjects with or without seroconversion status had similar probabilities of maintaining negative HBV DNA. The 95% confidence interval for the difference of the probabilities is (-25.3%, 6.5%), which is too wide to be conclusive.

From Week 52 – 68 while subjects were not on any active treatment, subjects who had seroconversion status at Week 52 were significantly more likely to maintain HBV DNA response than those with only negative HBV DNA at Week 52.

Note that for subjects with seroconversion status at Week 36, 27.4% were estimated to have re-appearance of HBV DNA during the next 16 weeks while on treatment. This rate is not much different from the rate of 33.7% for subjects with seroconversion status at Week 52 during off treatment.

3. Continued Treatment vs. Stopped Treatment

Subjects who were randomized to receive lamivudine 100mg daily during the first 52 weeks in the IFN Non-responder Study and Asian Study were re-randomized either to continue to receive lamivudine 100mg dose or were switched to placebo. For the IFN Non-responder Study this lasted for additional 16 weeks. For the Asian Study the information is available for one year (NUCB3018). Additionally, subjects in the US Study were followed for 16 weeks and subjects in the Active-control Study were followed for 12 weeks off treatment.

Biopsies were not available past Week 52 except for a few subjects in the Asian Study at Week 104, therefore analysis beyond Week 52 will not be performed for histologic endpoints. Since quite a few subjects who were randomized to continue lamivudine treatment actually received placebo treatment, analysis based on both the randomized treatment assignment and actual assignment will be presented.

The first table compares the change of seroconversion status from Week 52 to Week 68 for the subjects who were previously treated with lamivudine 100mg but were then randomized to receive either lamivudine 100mg or placebo.

**Seroconversion Status: Continued Treatment vs. Stopped Treatment
IFN Non-responder Study**

Based on Randomized Treatment Assignment

		LAM/LAM			
# of Subjects		Week 68			
		Success	Failure	Unknown	Total
Week 52	Success	6	3	1	10
	Failure	4	31	1	36
	Unknown	2	4	7	13
	Total	12	38	9	59

		LAM/PLA			
# of Subjects		Week 68			
		Success	Failure	Unknown	Total
Week 52	Success	4	3	0	7
	Failure	3	31	4	38
	Unknown	0	2	4	6
	Total	7	36	8	51

Among 59 subjects who were randomized to continue lamivudine therapy (LAM/LAM) at Week 52, 10 had seroconversion status, 36 did not have such a status and the status of the remaining 13 subjects could not be determined. The numbers for subjects who were switched to placebo (LAM/PLA) were similar. In LAM/LAM group, of the 10 subjects who had seroconversion status, 6 maintained the status, 3 lost the status and 1 subject did not have Week 68 measurements. On the other hand, of the 36 subjects who did not have the seroconversion status at Week 52, 4 gained it at Week 68. For the LAM/PLA group, 3 lost and 3 gained the status.

The two tables differed little in numbers, which suggest that there is not sufficient evidence to support a hypothesis that continued treatment is beneficial. From Week 52 to Week 68, the seroconversion rate (Unknowns regarded as failures) increased 3% from $10/59 = 17\%$ to $12/59 = 20\%$ for the LAM/LAM group, for the LAM/PLA group, there is no change. Therefore there is a difference of 3% between the two groups. A formal statistical testing of this difference (testing of interaction) yielded a p-value of 0.63 and a 95% confidence interval of (-11%, 17%), indicating that the trial size may be too small to draw any conclusion.

When this data is analyzed according to the actual treatments received, a similar testing of interaction showed a p-value of 0.24 and a 95% confidence interval of (-6%, 22%). Considering that the seroconversion rate at Week 52 was only 7% for the LAM/LAM arm, again there is not sufficient data to draw any conclusions of continued vs. stopped therapy.

The following table shows the change of seroconversion status from Week 52 to Week 104 for subjects who received lamivudine 100mg daily during the first 52 weeks of the trial. Note that subjects who experienced HBV DNA rebound were given open-label 100mg daily treatment. 90% of the LAM/PLA subjects switched to open-label therapy.

**Seroconversion Status: Continued Treatment vs. Stopped Treatment
Asian Study**

Based on Randomized Treatment Assignment

		LAM/LAM			
# of Subjects	Week 104				
	Success	Failure	nknow	Total	
Week 52	Success	13	3	2	18
	Failure	7	62	6	75
	Total	20	65	8	93

		LAM/PLA			
# of Subjects	Week 104				
	Success	Failure	nknow	Total	
Week 52	Success	1	1	1	3
	Failure	1	35	2	38
	Total	2	36	3	41

The p-value for the interaction test is 0.41 with 95% confidence interval (-6%, 16%). Again, no conclusion can be drawn based on this comparison.

Overall, the trials conducted by the applicant were insufficient to either conclude or reject a benefit of continued treatment.

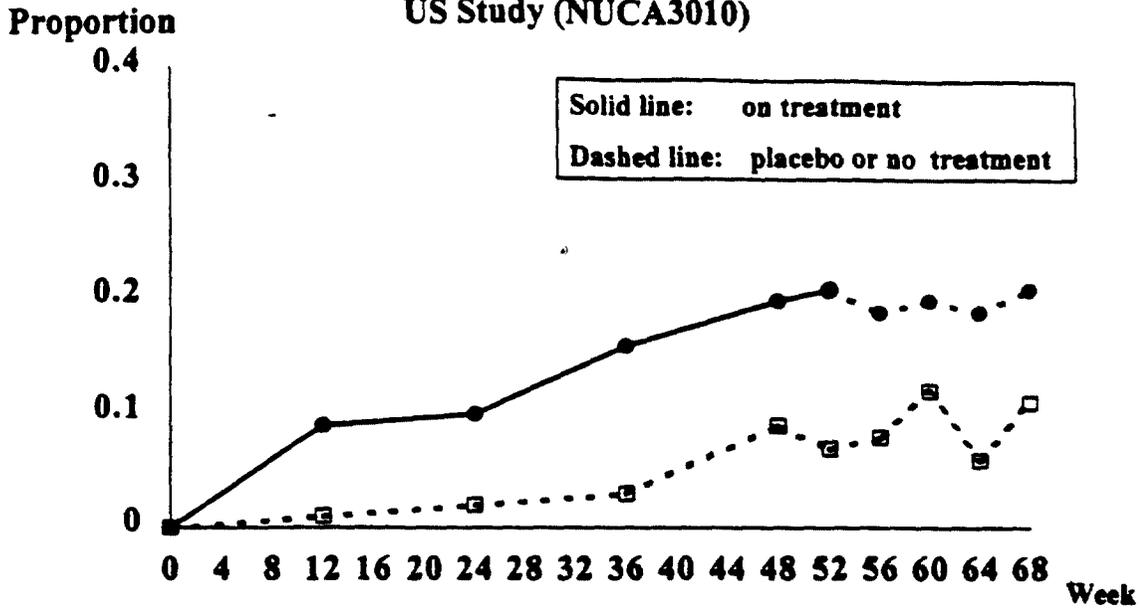
4. Responses over Time

The primary analyses were conducted at Week 52. To understand how soon the treatment response occurred and if the responses are durable, response rates over the course of the trial were plotted. Since biopsy results were available almost exclusively at baseline and Week 52, this analysis could not be performed for this endpoint. Instead, the analyses will focus on seroconversion and its three components (eAg, eAb and HBV DNA).

First we will see how seroconversion rates change over the course of the trial for the US Study in the graph below. In this graph and future graphs a solid line represents active treatment and a dashed line represents either placebo or no treatment. From the plot we see that over the course of the trial, the proportion of subjects with seroconversion status increased in both the lamivudine and placebo groups. Recall that the statistical comparison at week 52 was not robust, In fact the difference varies before and after Week 52. This reflects changes brought about by a small number of subjects.

Patterns in the other two placebo-controlled studies are similar and will not be presented.

**Seroconversion Status
US Study (NUCA3010)**

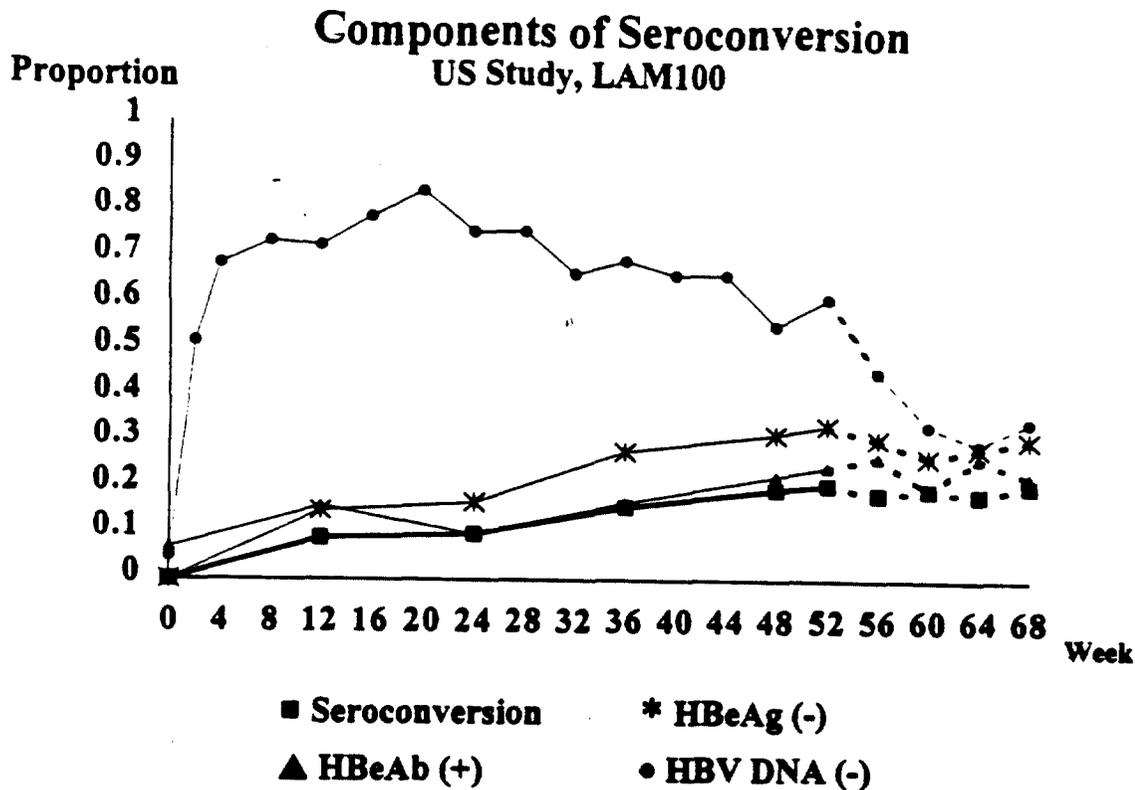


• LAM/PLA (N=63)

■ PLA (N=69)

To understand how the seroconversion rates were driven by its components (including HBeAg, HBeAb and HBV DNA) over time, the components are presented in the next graph for the Lamivudine 100mg arm of the US Study.

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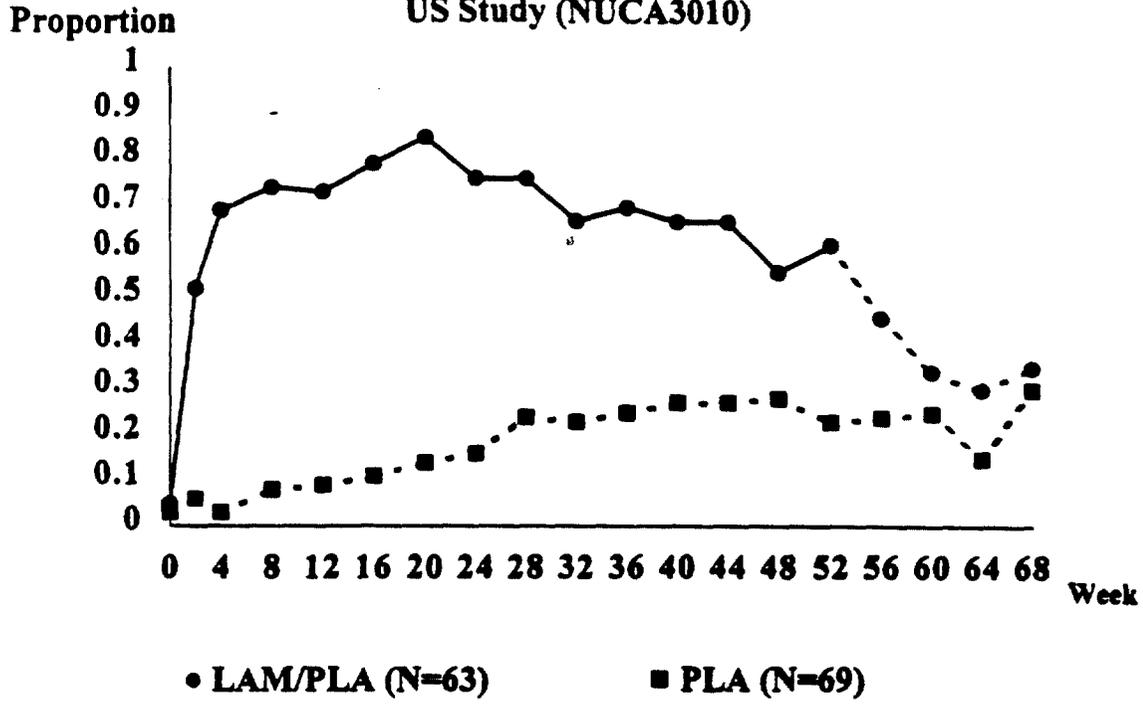


From the plot we see that among the three components, HBeAg and HBeAb are very similar to the composite, but the HBV DNA component is different. While the seroconversion rates and rates of HBeAg negative and HBeAb positive increase over the course of the trial, the proportion of subjects with HBV DNA below assay limit increased initially and then is seen to decrease even during the active treatment. For this reason we will single out HBV DNA for further analysis.

The plot below compares lamivudine and placebo for the US Study. The lamivudine line was shown on the previous plot. We see that there is an initial abrupt rise in the proportion of subjects with HBV DNA below assay limit, after that, there is a continual decrease and this decrease began well before the end of the active treatment. In placebo group, the proportion of HBV DNA below assay limit rises gradually over time. This graph raises the possibility that there may be a loss of relative efficacy well before the discontinuation of treatment.

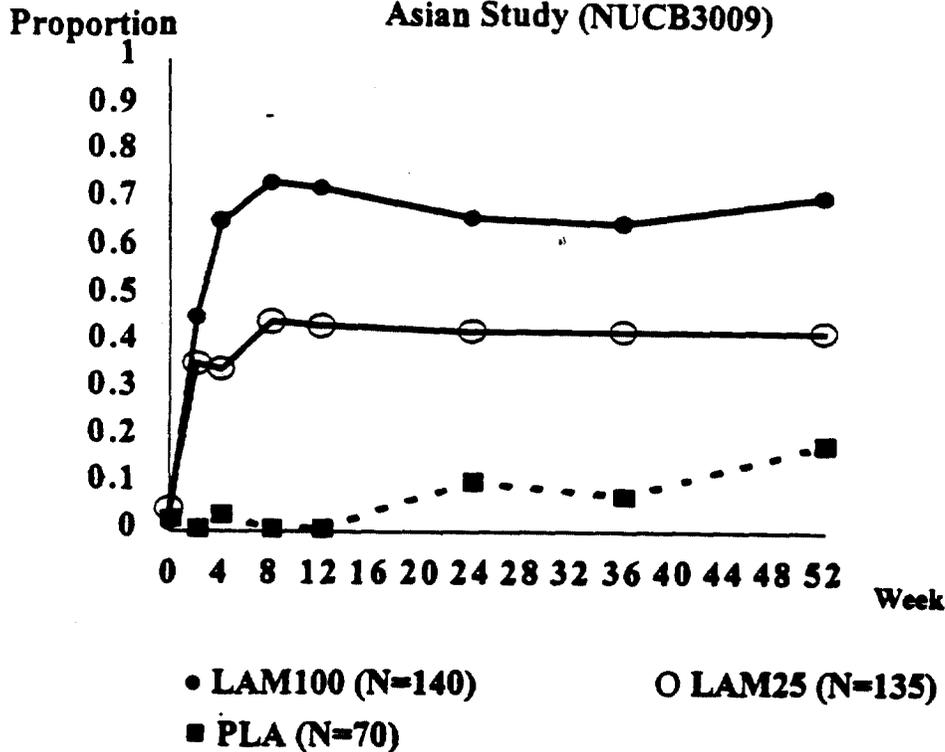
The IFN Non-responder Study is similar. The Asian Study is different. Contrary to the pattern we have seen in the US Study, the proportion of HBV DNA below assay limit does not decline after its initial rise. Rather the proportions of HBV DNA below assay limit for both the 100mg dose and the 25mg dose peak around week 8 with no apparent subsequent decline. The placebo group is similar in pattern to the other two studies

HBV DNA Below Assay Limit US Study (NUCA3010)



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HBV DNA Below Assay Limit Asian Study (NUCB3009)



5. Predictors of Week 52 responses at Baseline

Baseline disease status and subject characteristics including baseline total Knodell score, baseline Fibrosis, baseline ALT, baseline AST, baseline HBV DNA, Age, Gender, Weight and BMI (Body Mass Index) were studied for possible association with histologic improvement, seroconversion status and its three components (HBeAg, HBeAb and HBV DNA) at Week 52. This was examined by calculating the Spearman correlation and its associated p-value for testing if this correlation is equal to 0 using all subjects in the four pivotal trials. Considering the number of comparisons here (45 total), only p-values below $0.05/45 \approx 0.001$ will be considered statistically significant.

Higher baseline Knodell score, higher baseline Fibrosis score, higher ALT, higher AST and lower HBV DNA are associated with more frequent histologic improvement, meeting seroconversion criteria, HBeAg negative, HBeAb positive and HBV DNA below assay limit. Age, gender, BMI and weight had no significant influence on these outcomes.

6. Predicting Week 52 response using response pattern during treatment

The pattern of response for the HBV DNA up to Week 52 is divided into three categories:

- Sustained:** HBV DNA became negative (with confirmation within 16 weeks) and never had two consecutive positive values (except for week 52 where a single positive value is sufficient) later
- Rebounded:** HBV DNA became negative (with confirmation) followed by at least two consecutive positive values not separated by more than 16 weeks (except for week 52 where a single positive value is sufficient).
- No response:** HBV DNA never became negative (with confirmation)

To see if the observed HBV DNA patterns during the 52 weeks of therapy predicts the outcome at or after Week 52 for different treatments, the 4 studies were first combined and response measures were compared between the three DNA patterns for lamivudine 100 subjects. The response measures analyzed include histologic improvement, HBeAg seroconversion, HBeAg, HBeAb, ALT at Week 52 and HBV DNA during follow-up.

Since dropouts affect the "No response" group more than any other groups, the first analysis will exclude subjects who did not complete the 52 weeks of the studies. The response rates for lamivudine 100mg are listed in the table below.

HBV DNA Pattern and Post-treatment Responses
Lamivudine 100mg for 52 weeks
 Completed 52 weeks of therapy

HBV DNA pattern	N	Drop outs [†]	Histology	HBeAg seroconversion	HBeAg negative	HbeAb negative	ALT \leq ULN	HBV DNA negative during FU* (N)
No response	40	16	47.6%	2.5%	7.5%	15.0%	50.0%	0.0% (14)
Rebounded	125	2	41.6%	3.2%	12.0%	10.4%	42.4%	9.5% (74)
Sustained	217	10	59.9%	26.7%	35.0%	35.5%	77.0%	43.0% (100)

* Asian study excluded. Subjects who continued on lamivudine 100mg were also excluded.

† Subjects who did not complete 52 weeks of therapy. Excluded from denominator in the calculation of percent of responders.

The "No response" group and the "Rebounded" group appears to be similar with regard to the first five response variables in the table ($p > 0.38$). However, there is marginal evidence that subjects who "Rebounded" during the treatment is more likely to have negative HBV DNA during follow-up than subjects who never achieved confirmed negative HBV DNA ($p = 0.005$). Note that there are 6 response variables here and 3 possible comparisons for each response variable, therefore 18 comparisons were conducted here. With adjustment of multiple comparison, the statistical significance level will be set at for each comparison.

The "Sustained" group had a higher response rate than the other two groups for each response variable in the table. The differences are statistically significant when compared to the "Rebounded" group ($p < 0.001$) for each response variable. When compared to the "No response" group, the statistical significance is achieved ($p < 0.002$) with the exception for the histologic improvement ($p = 0.15$).

The conclusions based on the ITT population are the same.

To see if the same relationship holds for placebo treated subjects, the same analysis was conducted below for placebo groups in the four studies.

**HBV DNA Pattern and Post-treatment Responses
Lamivudine 100mg for 52 weeks**

Placebo

HBV DNA pattern	N	Drop outs [†]	Histology	HBeAg seroconversion	HBeAg negative	HBeAb negative	ALT \leq ULN	HBV DNA negative during FU* (N)
No response	134	18	22.4%	2.2%	2.2%	6.7%	25.4%	1.4% (72)
Rebounded	15	0	46.7%	6.7%	20.0%	20.0%	20.0%	16.7% (12)
Sustained	31	1	35.5%	35.5%	45.2%	41.9%	58.1%	48.1% (27)

* Asian study excluded.

[†] Subjects who did not complete 52 weeks of therapy. Excluded from denominator in the calculation of percent of responders.

The "No response" group again did not show significant difference from the "Rebounded" group ($p > 0.07$) for each response variable. The differences between the "sustained" group and the "No response" group were in favor of the "Sustained" group. The comparisons are statistically significant ($p < 0.0007$) with the exception for histologic improvement ($p = 0.16$). The sample sizes for the "Rebounded" and the "Sustained" group are too small to permit any conclusive comparisons ($p = 0.47$ for histology, $0.005 < p < 0.11$ for other response variables in favor of the "Sustained" group).

Overall, we see that for the lamivudine 100mg arms, subjects who became HBV DNA negative during therapy and subsequently did not rebound had better response rates in histology, HBeAg seroconversion, HBeAg negative, HBeAb positive, normalized ALT at Week 52 and negative HBV DNA during follow-up than other subjects. The results for the placebo arms did not contradict this conclusion.

7. HBV DNA Response Patterns and Emergence of YMDD Mutants

As was seen earlier, there was a decline in the proportion of HBV DNA below assay limit even during active lamivudine treatment in the US Study and the IFN Non-responder Study. This rebound of HBV DNA could be due to the emergence of resistant mutants. In this section we will explore this possible association.

The table below shows the association of the HBV DNA pattern and the resistance status at Week 52 for the lamivudine 100mg and placebo group. Subjects whose resistant statuses were unknown because of dropouts were also listed.

Genotype at Week 52

	DNA Pattern	WT	Mix	Res	0	Missing		Total
						Completers	Dropouts	
LAM100	No Response	25	3	4	1	7	16	56
	Rebounded	45	16	34	11	19	2	127
	Sustained	116	13	3	55	30	10	227
PLA	No Response	117	0	0	1	16	18	152
	Rebounded	12	0	0	1	2	0	15
	Sustained	18	0	0	12	1	1	32

Relatively few subjects who had sustained HBV DNA below assay limit developed resistance mutants (including mixed mutants and fully resistant mutants). The rate is $(13+3)/227 = 7.0\%$. For subjects who achieved HBV DNA below assay limit and then rebounded before Week 52, this rate is $(16+34)/127 = 39.4\%$. For subjects who never went below assay limit before Week 52, $(3+4)/56 = 12.5\%$ developed resistant mutants. The rates for the rebounders are significantly higher than the other two groups ($p < 0.001$).

As expected, none of the placebo treated subjects were known to have developed resistant mutants.

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E: Statistical Reviewer's Overall Assessment

Based on the placebo-controlled studies NUCA3010, NUCAB3011 and NUCB3009, the following statistical conclusions can be drawn:

A significantly higher percentage of subjects in the lamivudine 100mg group achieved histologic improvement than those in the Placebo group. This conclusion is relatively robust with respect to how missing values are handled.

A higher percentage of subjects in the lamivudine 100mg group met HBeAg seroconversion criteria (HBeAg negative, HBeAb positive and HBV DNA negative) at Week 52 than those in the Placebo group in NUCA3010 and NUCB3009. The conclusion for the first study is sensitive to assumptions made on missing data because of the smaller treatment effect with respect to biopsy, and the greater amount of missing biopsy data. The conclusion for the latter study is more robust. The rates in NUCAB3011 are similar for the two groups.

The trials did not demonstrate that treatment past 52 weeks is necessary. Additionally, it is uncertain if the development of resistance will erode both the histologic and HBeAg seroconversion effects over time.

IS
Greg Soon, Ph.D.
Mathematical Statistician
12/16/98

Concur: Dr. Flyer *DF 12/16/98*

cc:

Archival NDA #21-003

HFD-530

HFD-104/Ms. Townsend (via team links)

HFD-530/Dr. Jolson (via team links)

HFD-530/Dr. Dempsey(via team links)

HFD-530/Dr. Styrt

HFD-530/Dr. Kukich

HFD-530/Mr. ~~Zeccola~~ *CRESOEM21*

HFD-725/Dr. Flyer

HFD-725/Dr. Soon

HFD-725/Dr. Huque

HFD-725/Ms. Shores

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This review contains 26 pages