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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2012; published online Dec 10. http://dx.doi.org/10.1016/S0140-6736(12)61425-1.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2012;320:xx-xx

Supplemental Information

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1. Statistical Analysis Supplement

1.1 Patients with cirrhosis at baseline

Multivariable Logistic Regression Model

Univariable logistic regression was used as a first step in characterizing a multivariable model that jointly estimates the most important factors associated with reversal of cirrhosis. The table below includes assessment of the association between various prognostic factors and odds of cirrhosis reversal using a univariable logistic regression model. The initial set of variables selected was based on whether they were considered clinically meaningful and included demographic, laboratory, virologic, and disease-related factors.

Table1 : Univariable Logistic Regression in Assessing Factors Associated with Reversal of Cirrhosis

Variable	Odds Ratio	95% Confidence limit	P-value
Baseline ALT level - IU/liter	1.000	(0.996, 1.004)	0.935
Baseline serum albumin - g/deciliter	1.687	(0.465, 6.120)	0.462
Baseline platelet count - per mm^3	1.007	(0.998, 1.017)	0.138
Genotype D	1.014	(0.405, 2.540)	0.977
Baseline HBV DNA $\log_{10}$ - copies/ml	0.942	(0.677, 1.310)	0.721
Baseline HBsAg $\log_{10}$ - IU/ml	0.960	(0.532, 1.732)	0.893
Years positive for HBV	1.077	(0.996, 1.165)	0.064
Female gender	1.644	(0.427, 6.327)	0.470
Age ≥ 40 – yrs	0.557	(0.185, 1.675)	0.297
Asian ethnicity	2.679	(0.719, 9.987)	0.142
BMI - kg/m^2			
< 25	13.810	(2.985, 63.879)	<0.001
≥25 to < 30	4.048	(1.258, 13.025)	0.019
Baseline Knodell necroinflammatory score	0.955	(0.715, 1.276)	0.756

1. Statistical Analysis Supplement (Continued)

1.1 Patients with cirrhosis at baseline (continued)

Table 1: Univariable Logistic Regression in Assessing Factors Associated with Reversal of Cirrhosis (continued)

Variable	Odds Ratio	95% Confidence limit	P-value
Baseline Ishak fibrosis score	3.619	(0.773, 16.951)	0.103
Previous lamivudine use > 12 weeks	3.345	(0.711, 15.732)	0.126
Prior Interferon-α use	2.133	(0.565, 8.054)	0.264
HBeAg-negative	0.922	(0.348, 2.440)	0.869
Initial treatment arm assignment - TDF	1.699	(0.675, 4.277)	0.261

Multivariable Logistic Regression Model

The table below shows the multivariable model obtained via a forward selection algorithm in which the set of candidate variables included baseline platelet count (/mm^3), BMI category (≤25, ≥25 to <30, ≥30 kg/m^2), Years Positive for HBV, Asian ethnicity, baseline Ishak fibrosis score, and previous lamivudine use for > 12 weeks. Entry into the multivariable model was permitted if the forward selection entry p-value was < 0.10.

Table 2: Multivariable Logistic Regression Identifying Factors Associated with Reversal of Cirrhosis

Variable	Level	Odds Ratio Estimate	95% CI	P-value
BMI (kg/m^2)	BMI < 25 (normal)	18.89	(3.570, 99.953)	<0.001
	BMI ≥ 25 to 30 (overweight)	3.89	(1.071, 14.146)	0.039
	BMI ≥30 (Obese - Reference)	1.00	---	---
Years Positive for HBV	---	1.08	(0.988, 1.177)	0.090
Baseline platelet count (/mm3)	-	1.01		0.068

1. Statistical Analysis Supplement (Continued)

1.2 Patients without cirrhosis at baseline

Multivariable Logistic Regression Model

Univariable logistic regression was used as a first step in characterizing a multivariable model that jointly estimates the most important factors associated with regression of fibrosis, defined as a reduction of at least one point in the Ishak score from baseline. The table below includes assessment of the association between various prognostic factors and odds of cirrhosis reversal using a univariable logistic regression model. The initial set of variables selected was based on whether they were considered clinically meaningful and included demographic, laboratory, virologic, and disease-related factors.

Table 1: Univariable Logistic Regression in Assessing Factors Associated with Regression of Fibrosis

Variable	Odds Ratio	95% Confidence limit	P-value
Baseline ALT level - IU/liter	1.000	(0.997, 1.002)	0.658
Baseline platelet count - per mm^3	1.004	(0.999, 1.009)	0.107
Baseline serum albumin - g/deciliter	0.351	(0.156, 0.787)	0.011
Genotype D	0.978	(0.593, 1.615)	0.932
Baseline HBV DNA $\log_{10}$ - copies/ml	1.294	(1.080, 1.552)	0.005
Baseline HBsAg $\log_{10}$ - IU/ml	1.034	(0.719, 1.487)	0.858
Years positive for HBV	1.007	(0.978, 1.036)	0.653
Female gender	1.696	(0.933, 3.083)	0.083
Age > 40 – yrs	1.230	(0.745, 2.031)	0.418
Asian ethnicity	1.233	(0.700, 2.173)	0.468

Table 1: Univariable Logistic Regression in Assessing Factors Associated with Regression of Fibrosis (Continued)

Variable	Odds Ratio	95% Confidence limit	P-value
BMI – kg/m^2			
<25	0.763	(0.325, 1.792)	0.535
≥25- <30	0.469	(0.191, 1.154)	0.099
Baseline Knodell necroinflammatory score	1.405	(1.237, 1.597)	<0.001
Baseline Ishak fibrosis score	6.058	(3.803, 9.652)	<0.001
Previous lamivudine use > 12 weeks	1.000	(0.489, 2.046)	1.000
Prior Interferon-α use	0.734	(0.392, 1.375)	0.335
HBeAg – negative	0.880	(0.525, 1.476)	0.628
Initial treatment arm assignment – TDF	0.771	(0.445, 1.306)	0.333

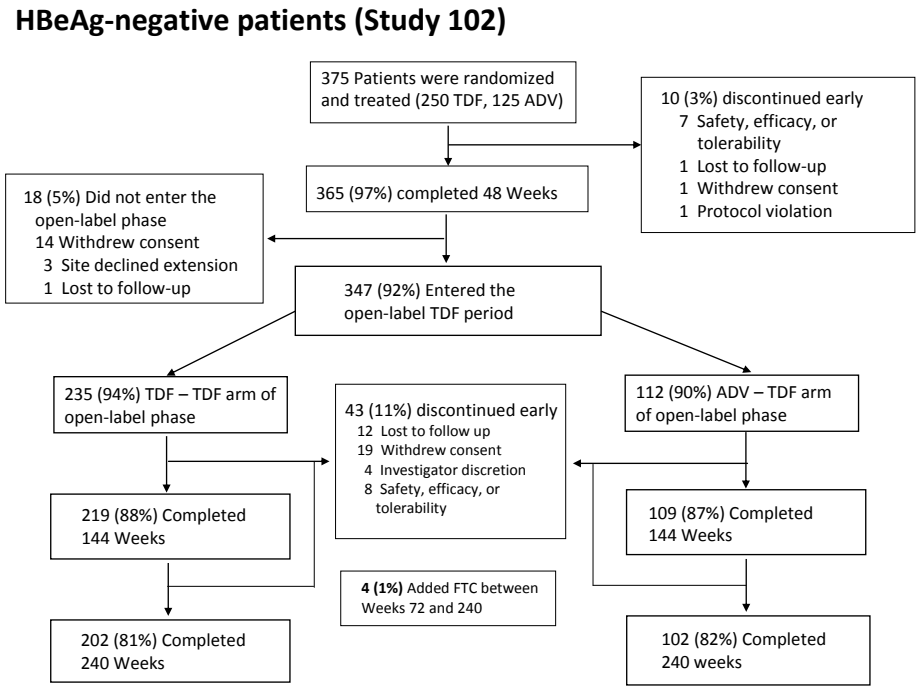
Multivariable Logistic Regression Model

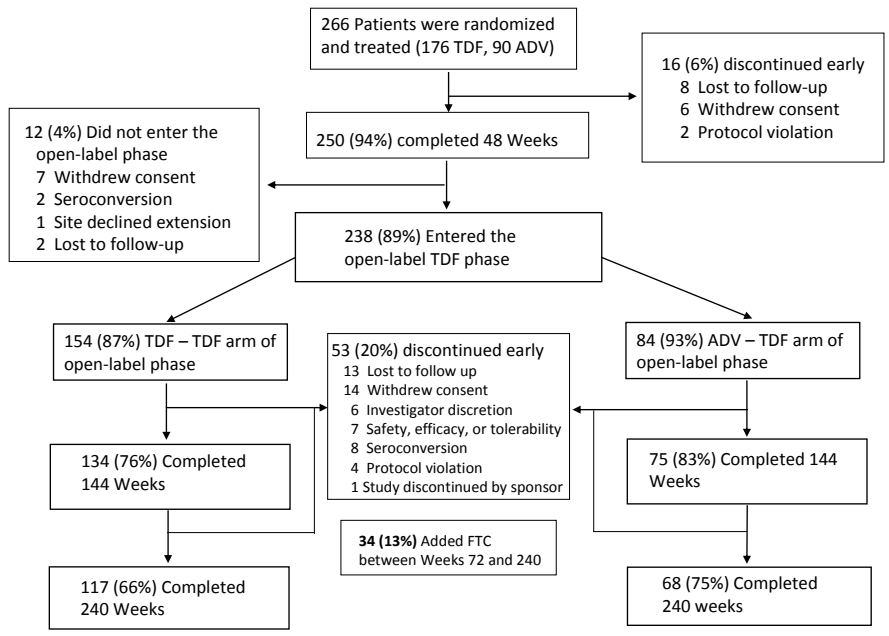
The table below shows the multivariable model obtained via a forward selection algorithm in which the set of candidate variables included baseline platelet count ($/mm^3$), baseline albumin level (g/deciliter), baseline HBV DNA (log_{10} IU/ml), gender, BMI categorical (<25, ≥25 - <30, ≥30 kg/m^2), baseline Knodell necroinflammatory score, and baseline Ishak fibrosis score. Entry into the multivariable model was permitted if the forward selection entry p-value was < 0.10.

Table 2. Multivariable Logistic Regression Identifying Factors Associated with Reversal of Fibrosis

Variable	Odds Ratio Estimate	95% CI	P-value
Baseline Ishak fibrosis score	6.921	(4.162, 11.506)	<0.001
Baseline platelet count ($/mm^3$)	1.009	(1.003, 1.015)	0.004
Baseline HBV DNA (log_{10} IU/ml)	1.240	(0.993, 1.548)	0.058

2. Overall Study Disposition for HBeAg-negative Patients (Study 102) and HBeAg-positive Patients (Study 103)





3. Changes in Ishak Fibrosis Scores at Year 5 for Cirrhotic and Non-cirrhotic Patients

The table below describes the changes in Ishak fibrosis scores for the 348 patients with paired baseline and year 5 liver biopsies (Observed [Missing=Excluded/Data after addition of Emtricitabine Included]). In the group of 96 patients with cirrhosis (Ishak fibrosis score ≥5) at baseline, 71 (74%) of patients had an improvement of 1 unit or more at year 5, while in the group of 252 non-cirrhotic patients at baseline, 105 (41.7%) had an improvement of 1 unit or more at year 5.

GS-US-174-0102 and GS-US-174-0103 Full Analysis Set Subjects
Observed(Missing=Excluded)/Data After Addition of FTC Included

	Cirrhotic at Baseline			Non-Cirrhotic at Baseline		
	TDF-TDF (N=97)	ADV-TDF (N=55)	Overall (N=152)	TDF-TF (N=325)	ADV-TDF (N=157)	Overall (N=482)
Improvement in Score						
>=1	46/ 59 (78.0%)	25/ 37 (67.6%)	71/ 96 (74.0%)	66/167 (39.5%)	39/ 85 (45.9%)	105/252 (41.7%)
>=2	46/ 59 (78.0%)	24/ 37 (64.9%)	70/ 96 (72.9%)	17/167 (10.2%)	8/ 85 (9.4%)	25/252 (9.9%)
>=3	39/ 59 (66.1%)	17/ 37 (45.9%)	56/ 96 (58.3%)	3/167 (1.8%)	1/ 85 (1.2%)	4/252 (1.6%)
>=4	9/ 59 (15.3%)	6/ 37 (16.2%)	15/ 96 (15.6%)	0/167 (0.0%)	0/ 85 (0.0%)	0/252 (0.0%)
No Change or Worsening in Score						
>=0	13/ 59 (22.0%)	12/ 37 (32.4%)	25/ 96 (26.0%)	101/167 (60.5%)	46/ 85 (54.1%)	147/252 (58.3%)
>=1	1/ 59 (1.7%)	0/ 37 (0.0%)	1/ 96 (1.0%)	8/167 (4.8%)	4/ 85 (4.7%)	12/252 (4.8%)
>=2	0/ 59 (0.0%)	0/ 37 (0.0%)	0/ 96 (0.0%)	2/167 (1.2%)	1/ 85 (1.2%)	3/252 (1.2%)
>=3	0/ 59 (0.0%)	0/ 37 (0.0%)	0/ 96 (0.0%)	1/167 (0.6%)	1/ 85 (1.2%)	2/252 (0.8%)

Cirrhosis defined as Ishak Fibrosis score > 4

4. Characteristics of Patients with and without Liver Biopsy Data at Year 5

Summarized below are the baseline and on treatment characteristics of 641 randomized and treated patients comparing those patients with paired liver biopsies at baseline and year 5 (n=348) to those who had missing biopsy data at year 5 (n=293).

Table. Baseline and On Treatment Characteristics of Patients with and without Biopsy at Year 5*

	Non-Missing at Year 5 (N=348)	Missing at Year 5 (N=293)	P value†
Baseline Characteristic			
Age> 40 years – no. (%)	191 (55)	132 (45)	0.014
Body mass index > 26 – no. (%)‡	135 (39)	106 (36)	0.513
Male sex – no. (%)	276 (79)	197 (67)	<0.001
Asian race – no. (%)	88 (25)	101 (34)	0.012
Alanine aminotransferase – IU/liter	146 ± 119	138 ± 110	0.580
Serum albumin – g/deciliter	4.2± 0.34	4.1± 0.36	0.013
Platelet count – per mm^3	207,000 ± 54,580	213,200 ± 58,820	0.203
Years positive for HBV – no. (%)			
< 4	141 (41)	110 (39)	0.843
4 to 6	35 (10)	30 (10)	
>6	168 (49)	145 (51)	
HBV genotype – no. (%)∫			
A	70 (20)	33 (11)	<0.001
B	34 (10)	40 (14)	
C	44 (13)	68 (23)	
D	182 (52)	133 (45)	
E	4 (1)	8 (3)	
F	5 (1)	4 (1)	
Other¶	9 (3)	7 (2)	
HBV DNA $\log_{10}$ - copies/ml‖	7.5 ± 1.44	7.8 ± 1.55	0.015
HBsAg – IU/ml**	28,587 ± 49,861	35,895 ± 55,728	0.127
Knodell necroinflammatory score	8 ± 2.3	8 ± 2.3	0.858

4. Characteristics of Patients with and without Liver Biopsy Data at Year 5

(Continued)

Table. Baseline and On Treatment Characteristics of Patients with and without Biopsy at Year 5* (Continued)

	Non-Missing at Year 5 (N=348)	Missing at Year 5 (N=293)	P value†
Baseline Characteristic			
Ishak fibrosis score – no. (%)			
0	0	1 (0.3)	0.150
1	10 (3)	10 (3)	
2	126 (36)	122 (43)	
3	79 (23)	74 (26)	
4	37 (11)	23 (8)	
5	19 (5)	10 (3)	
6	77 (22)	46 (16)	
Prior interferon-α use – no. (%)	72 (21)	36 (12)	0.006
Prior lamivudine use for > 12 weeks – no. (%)	54 (15)	21 (7)	0.001
On Treatment Characteristic			
HBV DNA < 400 copies/ml - no./N (%)‡	330/334 (99)	132/136 (97)	0.237
Normal alanine aminotransferase level – no./N (%)	274/337 (81)	110/134 (82)	0.896
Change from baseline in serum albumin – g/deciliter	0.1 ± 0.36	0.2 ± 0.35	0.009
Change from baseline in platelet count – per mm^3	23,500 ± 41,700	21,100 ± 40,300	0.198
HBsAg loss – no./N (%)	6/338 (2)	5/136 (4)	0.308
Change from baseline to Week 12 in HBsAg – IU/ml∫	-8,267 ± 27,189	-14,312 ± 33,454	0.003
HBeAg loss – no. (%)	57/115 (50)	24/50 (48)	0.867

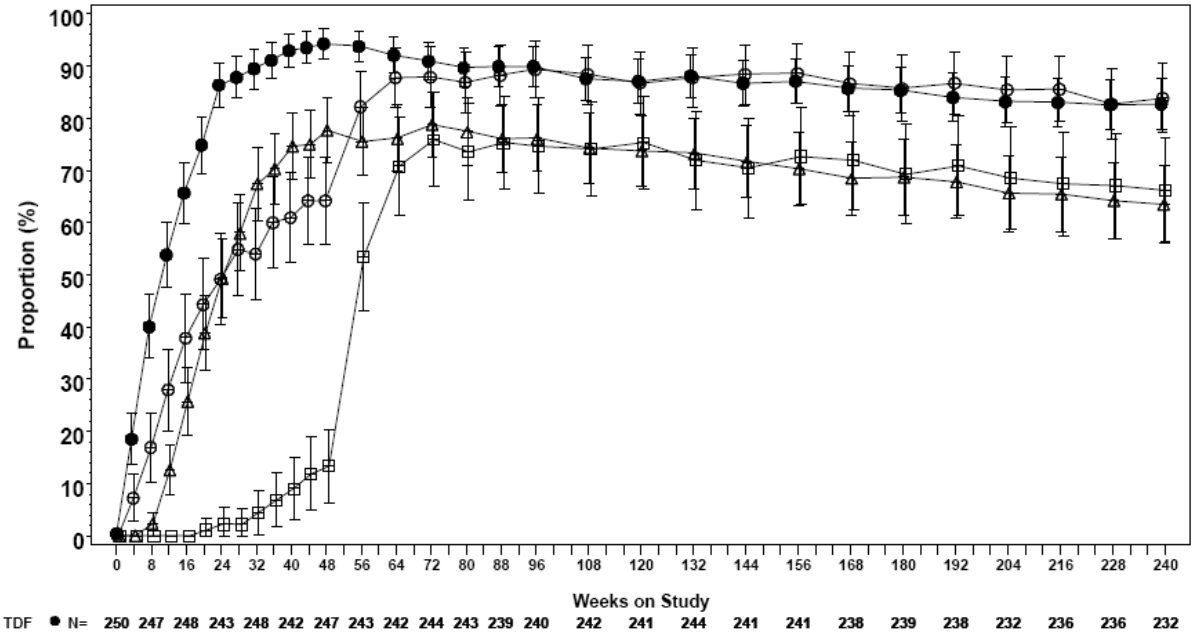
*Plus-minus values are means ± SD unless otherwise indicated. Percentages may not total 100 because of rounding.
†P values for the comparison of categorical data from the Fisher's exact test, and for continuous data from a two-sided Wilcoxon rank-sum test. No adjustments for covariates were performed.
‡Body mass index is the weight in kilograms divided by the square of the height in meters.
∫The HBV genotype was determined with the use of an enzyme-linked immunosorbent assay (ELISA).
¶Includes genotypes G, H and unable to genotype.
‖HBV DNA levels were measured with the use of the COBAS TaqMan assay (Roche Molecular Systems, Inc., Branchburg, NJ) which has a lower limit of quantitation of 29 IU/ml (169 copies/ml).
**HBsAg levels were measured with the use of the Architect HBsAg quantitative assay (Abbott) which has a lower limit of quantitation of 1 IU/ml

5. Additional Efficacy Results in HBeAg-Positive (Study 103) and HBeAg-Negative (Study 102) Patients

In both studies, patients were initially randomized (2:1) to receive TDF or adefovir dipivoxil (ADV) in a double-blind fashion.[25] Patients who completed 48 weeks of double-blind treatment and underwent a liver biopsy were eligible to continue on open-label TDF for 7 years. Patients with confirmed HBV DNA ≥ 400 copies/ml on or after week 72 were permitted to have emtricitabine (FTC) added to TDF. Four patients (1%) added FTC in Study 102 and 34 (13%) added FTC in Study 103. Additional efficacy results through 5 years are provided below.

5.1. Proportion (95% confidence interval) of Patients with HBV DNA Levels in Plasma Below 400 copies/ml (69 IU/ml) Through Week 240 (Year 5) – Full Analysis Set

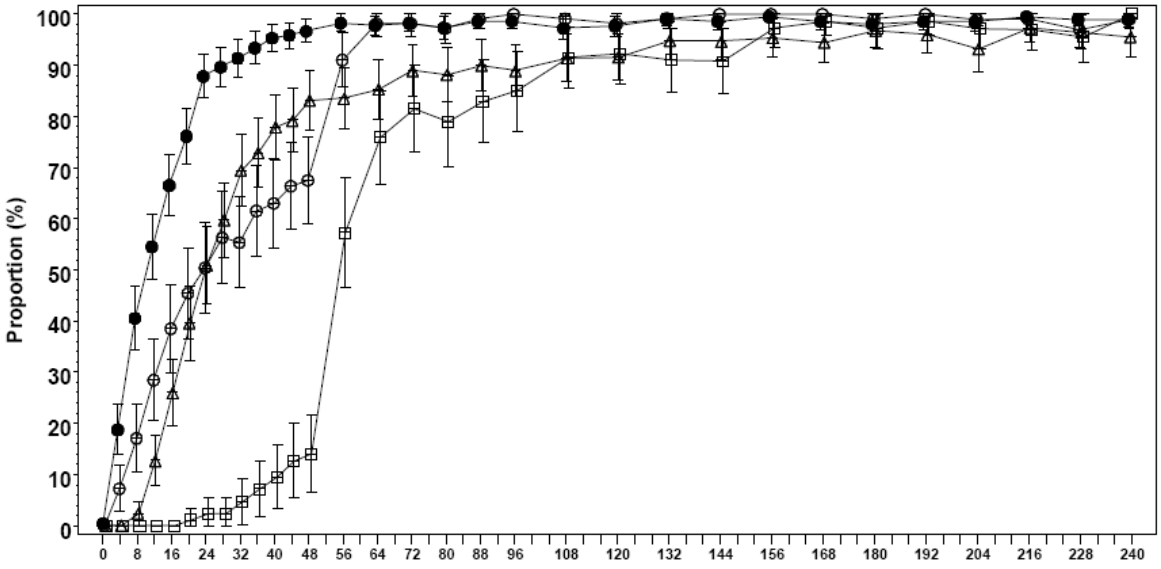
Intent to Treat, Missing = Failure/Adition of Emtricitabine = Failure



174-0102 / TDF	● N=	250	247	248	243	248	242	247	243	242	244	243	239	240	242	241	244	241	241	238	239	238	232	236	236	232
174-0102 / ADV	○ N=	125	124	124	124	124	123	123	124	123	124	122	119	122	121	121	123	121	123	120	119	121	117	118	116	118
174-0103 / TDF	△ N=	176	173	175	170	172	174	171	168	168	165	164	163	168	167	168	165	166	165	165	163	165	163	162	162	162
174-0103 / ADV	□ N=	90	89	90	88	90	88	90	88	89	87	87	89	87	89	89	89	88	88	89	88	86	86	86	85	86

5.1. Proportion (95% confidence interval) of Patients with HBV DNA Levels in Plasma Below 400 copies/ml (69 IU/ml) Through Week 240 (Year 5) – Full Analysis Set

Observed, Missing = Excluded/Addition of Emtricitabine = Included

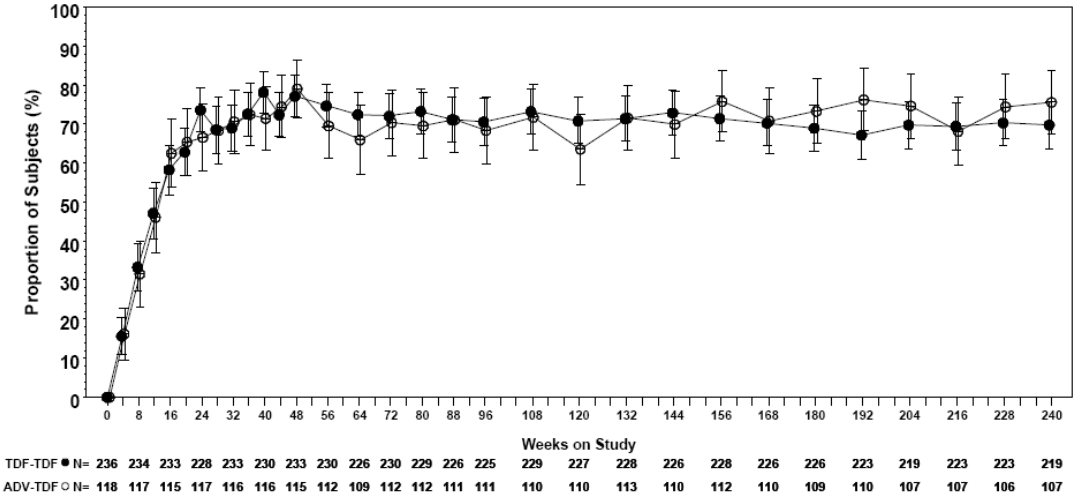


174-0102 / TDF	● N=	250	244	245	239	243	236	241	232	228	226	224	219	220	219	217	219	215	214	209	210	205	198	199	199	195
174-0102 / ADV	○ N=	125	123	122	121	121	119	117	112	110	111	109	106	109	108	107	109	107	109	104	103	105	101	102	99	100
174-0103 / TDF	△ N=	176	171	173	165	167	167	160	152	149	145	144	140	145	140	142	136	133	129	126	123	124	118	113	114	111
174-0103 / ADV	□ N=	90	88	88	85	86	84	85	82	83	81	81	82	80	82	78	78	76	74	72	72	70	69	67	68	64

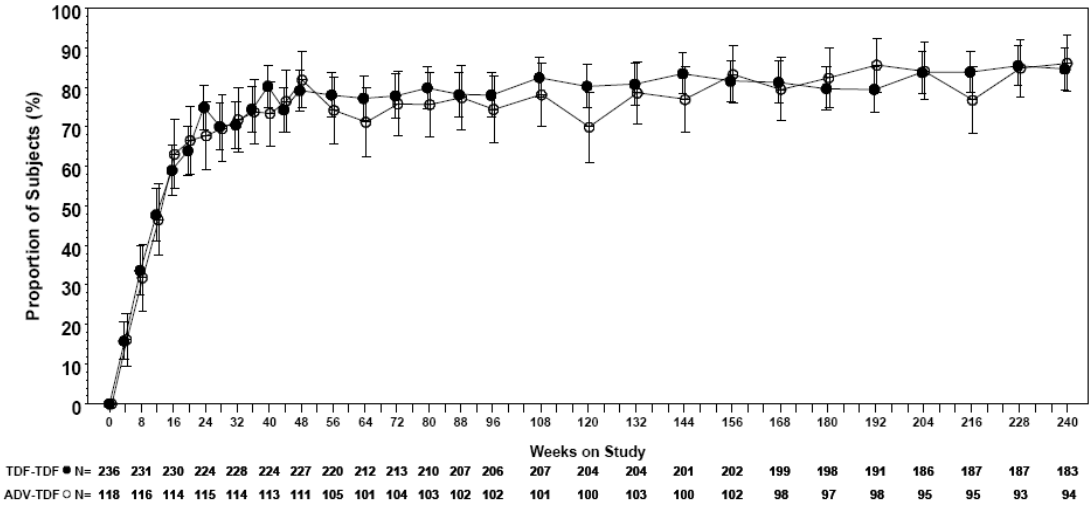
5.2. ALT Normalization (Biochemically Evaluable Analysis Set)

In the biochemically evaluable analysis set, only patients with baseline ALT values greater than the upper limit of normal (ULN) are included. Normal ALT is defined at having an ALT value at or below ULN.

HBeAg-negative patients (Study 102)
Missing = Failure/Addition of Emtricitabine = Failure

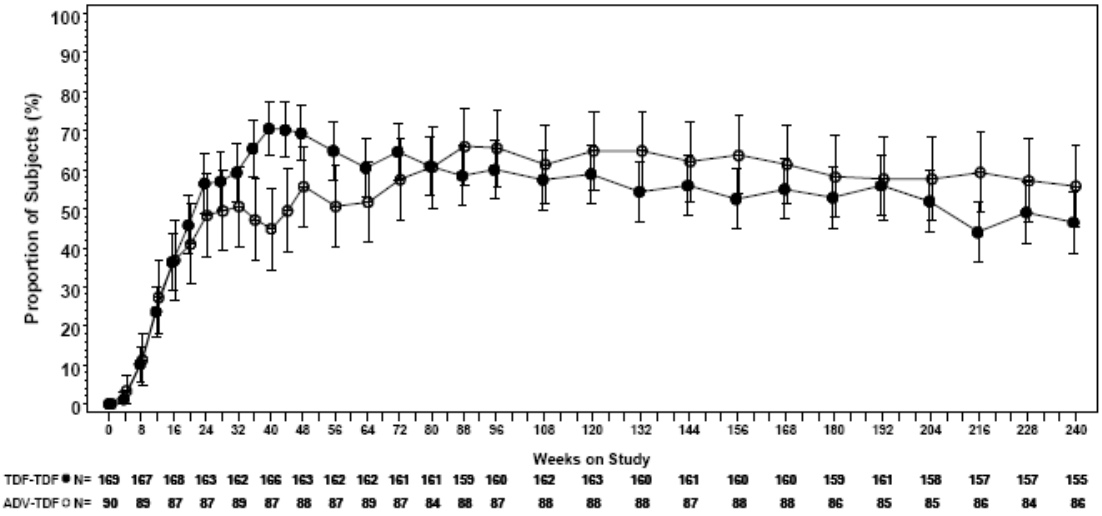


Observed, Missing = Excluded/Addition of Emtricitabine = Included

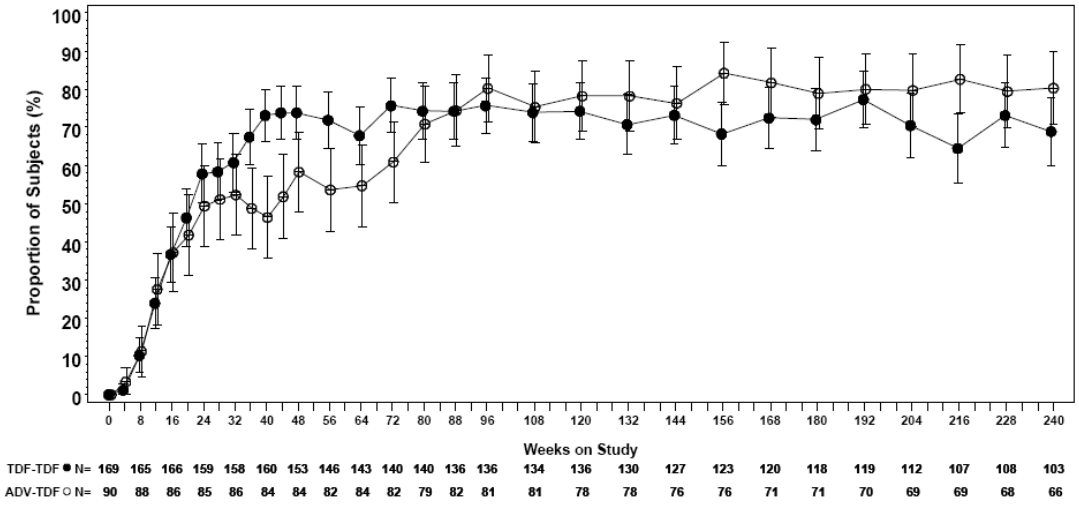


5.2 ALT Normalization (Biochemically Evaluable Analysis Set) - continued

HBeAg-positive Patients (Study 103)
Missing = Failure/Addition of Emtricitabine = Failure

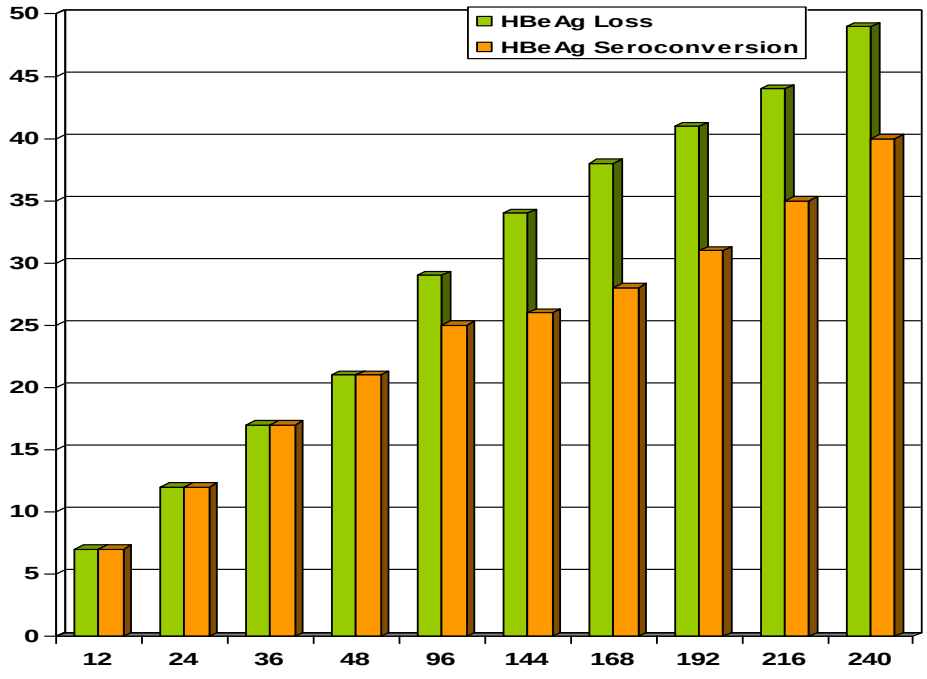


Observed, Missing = Excluded/Addition of Emtricitabine = Included



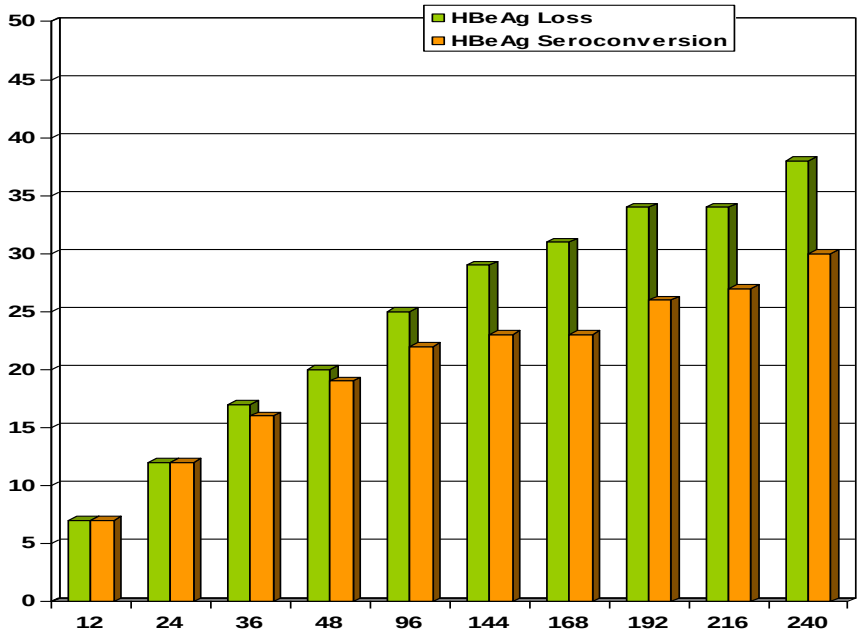
5.3 HBeAg Loss and Seroconversion

In Study 103, rates of HBeAg loss and seroconversion to anti-HBe are depicted in the figure below (Observed [On treatment] analysis, missing = excluded,/Addition of Emtricitabine Included). The Y axis is percentage of patients and the X axis is study visit (in weeks). At week 240 (year 5), 81/165 (49%) and 66/164 (40%) of patients experienced HBeAg loss and seroconversion, respectively.



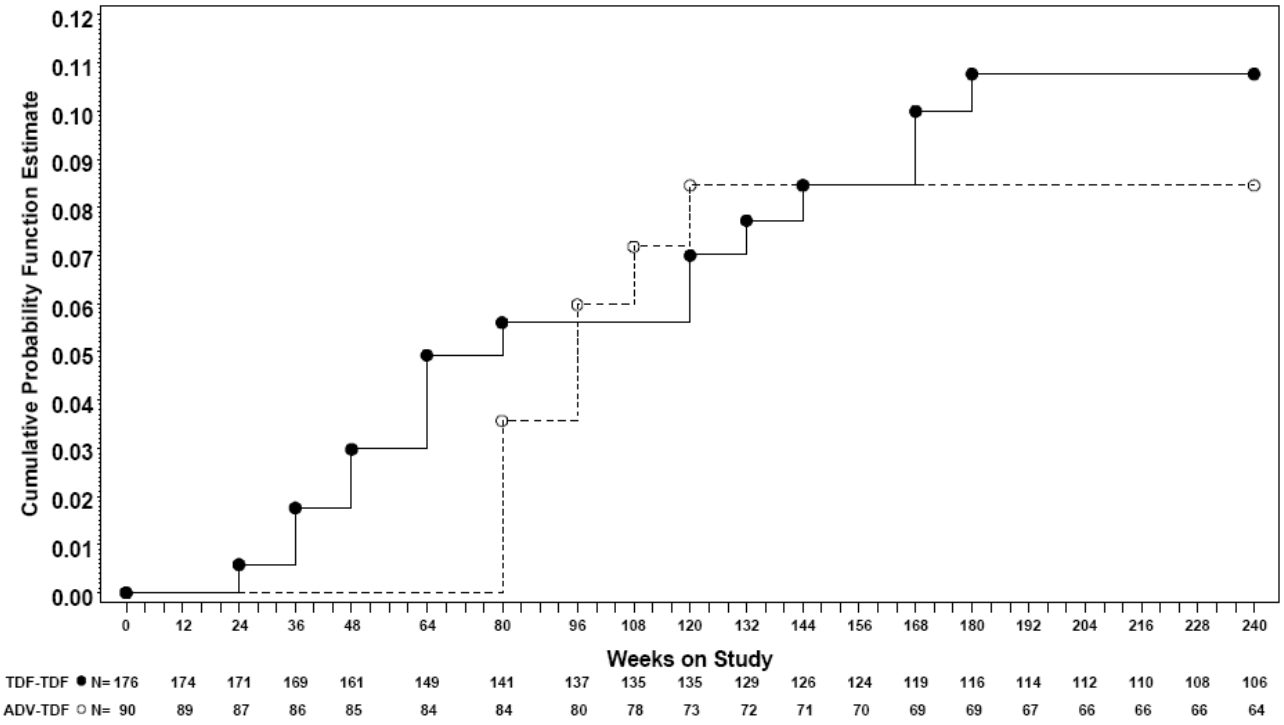
5.3 HBeAg Loss and Seroconversion (Continued)

In Study 103, rates of HBeAg loss and seroconversion to anti-HBe are depicted in the figure below (Intent to treat [Missing = Failure/Addition of Emtricitabine =Failure] LTE-TDF analysis). The Y axis is percentage of patients and the X axis is study visit (in weeks). At week 240 (year 5), 92/244 (38%) and 73/243 (30%) of patients experienced HBeAg loss and seroconversion, respectively.



5.4 HBsAg Loss in Study 103

HBsAg loss was estimated by the Kaplan-Meier method. Data after the addition of emtricitabine are included. No patients in the ADV arm experienced loss of HBsAg during the double-blind (48 week) phase of Study 103. One patient (not shown) had confirmed loss of HBsAg at week 240 in Study 102.



5.5 Summary of Efficacy Results at Year 5 for HBeAg-positive (Study 103) and HBeAg-negative (Study 102) Patients by Treatment Arm and Overall

Summary of Efficacy Results at Year 5

	HBeAg+ Patients			HBeAg- Patients		
	TDF-TDF	**ADV-TDF**	**Overall**	**TDF-TDF**	**ADV-TDF**	**Overall**
ALT						
Normalized ALT[a,b]	69% (71/103)	80% (53/66)	73% (124/169)	85% (155/183)	86% (81/94)	85% (236/277)
Mean ± SD (U/L)	42 ± 43	32 ± 15	38 ± 35	31 ± 16	31 ± 16	31 ± 16
HBV DNA						
< 400 copies/mL[c] (intent-to-treat)	64% (103/162)	66% (57/86)	65% (160/248)	83% (192/232)	84% (99/118)	83% (291/350)
< 400 copies/mL[b] (on treatment)	96% (106/111)	100% (64/64)	97% (170/175)	99% (193/195)	99% (99/100)	99% (292/295)
< 169 copies/mL[c] (intent-to-treat)	63% (102/162)	66% (57/86)	64% (159/248)	82% (191/233)	84% (99/118)	83% (290/351)
Serology						
HBeAg loss[b]	50% (52/104)	48% (29/61)	49% (81/165)	na[d]	na	na
HBeAg seroconversion[b]	40% (41/103)	41% (25/61)	40% (66/164)	na	na	na
HBsAg Loss[e,f]	11% (6.7, 17.0)	8% (4.1, 16.9)	10% (6.8, 14.7)	-	-	-[g]
HBsAg Seroconversion[e]	8% (4.4, 13.6)	8% (4.1, 17.0)	8% (5.1, 12.5)	-	-	-
Histology						
Histologic improvement[b,h,i]	88% (67/76)	90% (43/48)	89% (110/124)	87% (131/150)	85% (63/74)	87% (194/224)

[a]Biochemically evaluable dataset (ALT>ULN at baseline);
[b]Observed analysis (missing=excluded; addition of FTC included);
[c]LTE-TDF (missing=failure; add FTC=failure);
[d]na = not applicable; [e]KM = Kaplan-Meier analysis (%, 95% CI);
[f]N=23 patients; 1 patient had transient confirmed HBsAg loss but became HBsAg+ again 24 weeks after seroconversion;
[g]One HBeAg-negative patient had confirmed HBsAg loss at week 240 (year 5);
[h]Defined as reduction in Knodell necroinflammation score of ≥2 points with no worsening of fibrosis
[i]Overall, results at year 5 for patients who did not add FTC (N=331) showed 88% with histologic improvement

6. Patients who Developed Hepatocellular Carcinoma While on Studies 102 and 103 Through 5 Years

Table. Summary of 12 Patients who Developed Hepatocellular Carcinoma (HCC) in Studies 102 and 103 through 5 Years

Patient No.	Demographic and Disease Characteristics[a]	Ishak Fibrosis Score (BL /Wk 48 /Wk 240)[b]	HCC Onset (Study Week)	Therapy for HCC	HCC Outcome[c]
Patients with cirrhosis[d]					
1	63 yo white male, HBeAg-negative, genotype D	6 / 6 / NA	50	surgery	unresolved
2	64 yo Asian male, HBeAg-negative, genotype C	6 / 6 / NA	114	radiofrequency ablation	unresolved
3	46 yo white male, HBeAg-negative, genotype D	6 / 6 / 4	114	surgery	unresolved
4	29 yo white male, HBeAg-positive, genotype F	6 / 3 / NA	125	chemotherapy	unresolved
5	52 yo white male, HBeAg-positive, genotype D	6 / 6 / NA	175	chemotherapy	unresolved
6	61 yo black male, HBeAg-positive, genotype E	6 / 6 / NA	194	supportive	patient expired
7	52 yo Asian male, HBeAg-positive, genotype C	3 / 3 / NA[e]	206	surgery	resolved
Patients without cirrhosis[f]					
8	66 yo Maori male, HBeAg-negative, genotype C	3 / 3 / NA	17	surgery	resolved
9	49 yo Asian male, HBeAg-negative, unable to genotype	2 / 2 / NA	28	surgery	resolved
10	65 yo white male, HBeAg-negative, genotype D	4 / 4 / NA	46	surgery	patient expired
11	50 yo Asian male, HBeAg-positive, genotype C	4 / 4 / NA	112	radiofrequency ablations x 3	resolved
12	62 yo white male, HBeAg-negative, genotype D	2 / 3 / NA	172	supportive care only	patient expired

[a]Age is relative to the time of diagnosis of HCC, HBV genotype was determined at study baseline.
[b]BL refers to the baseline (pre-study) biopsy, Week 48 (Yr 1) and Week 240 (Yr 5) biopsies; NA = data not available
[c]Patient status as of Week 240 (Yr 5)
[d]Ishak fibrosis score ≥ 5
[e]Patient was diagnosed with cirrhosis based on tissue obtained during surgical resection of HCC (Week 210)
[f]Ishak fibrosis score ≤ 4