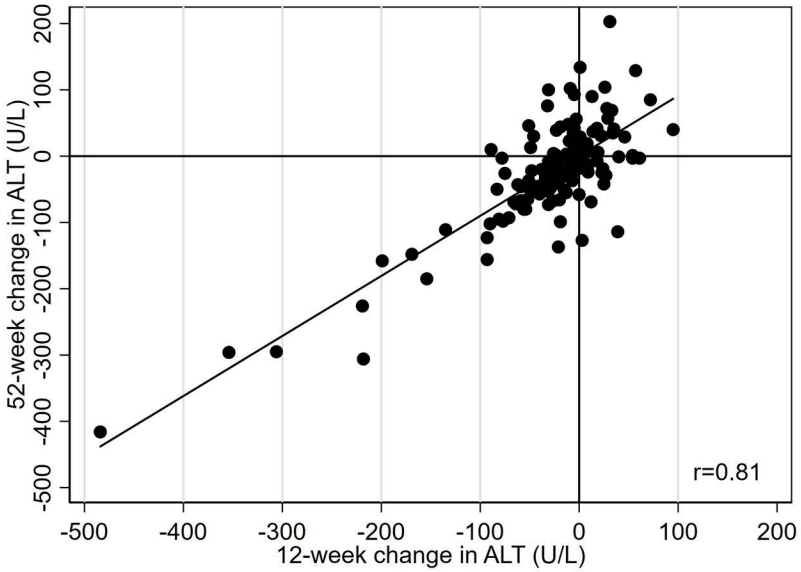
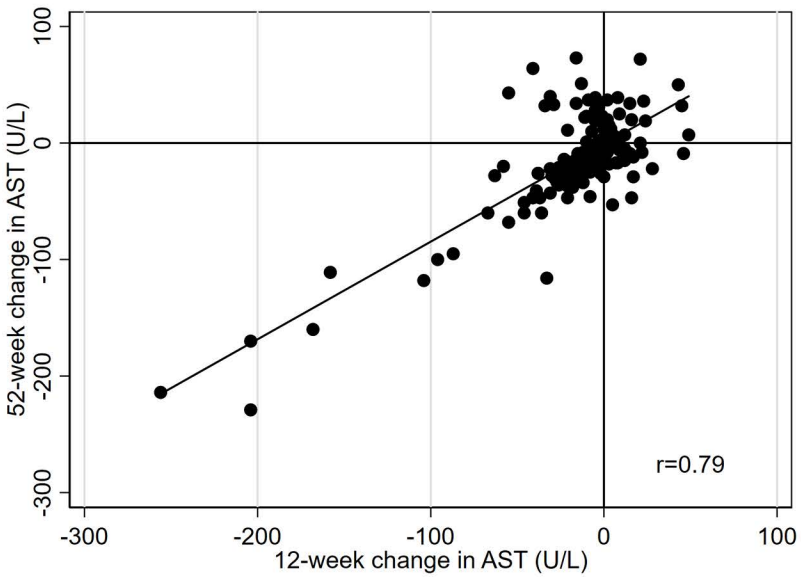
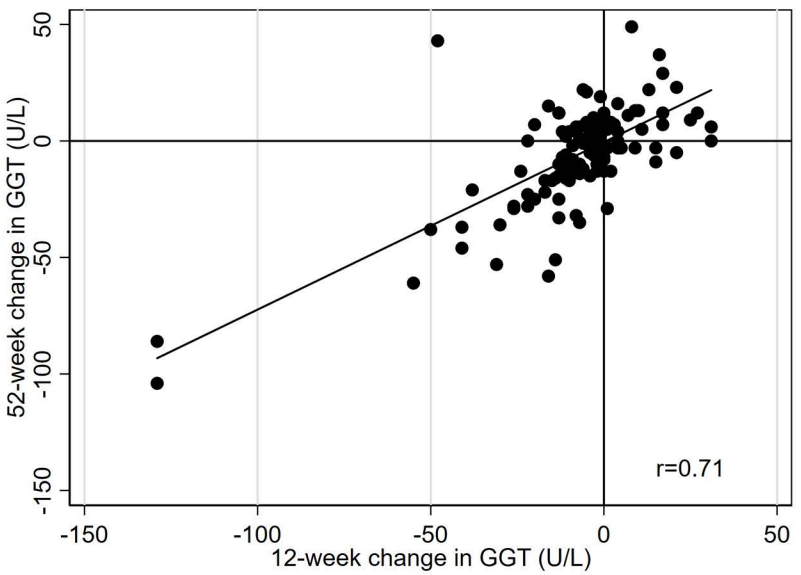
A) ALT (U/L)

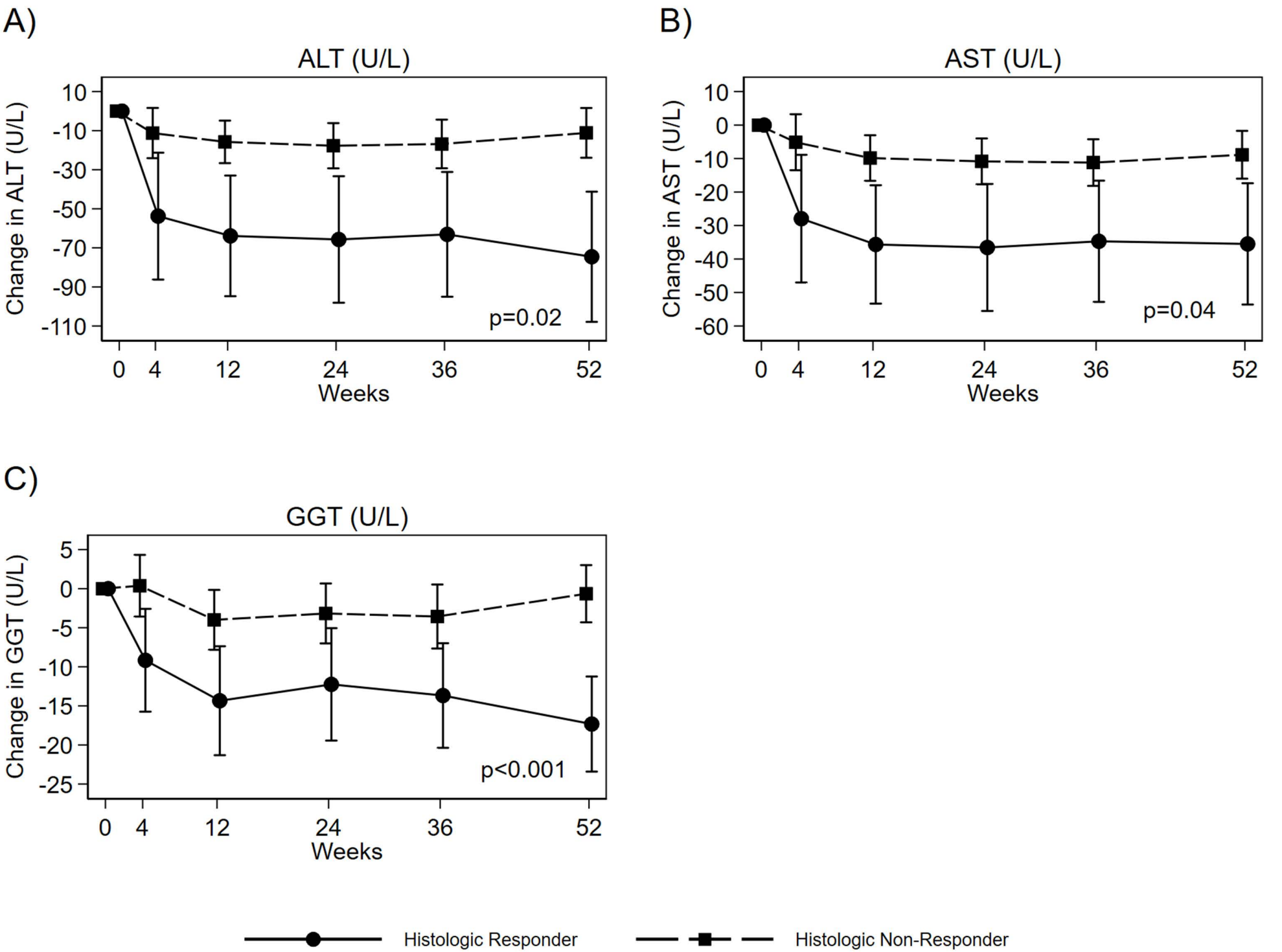


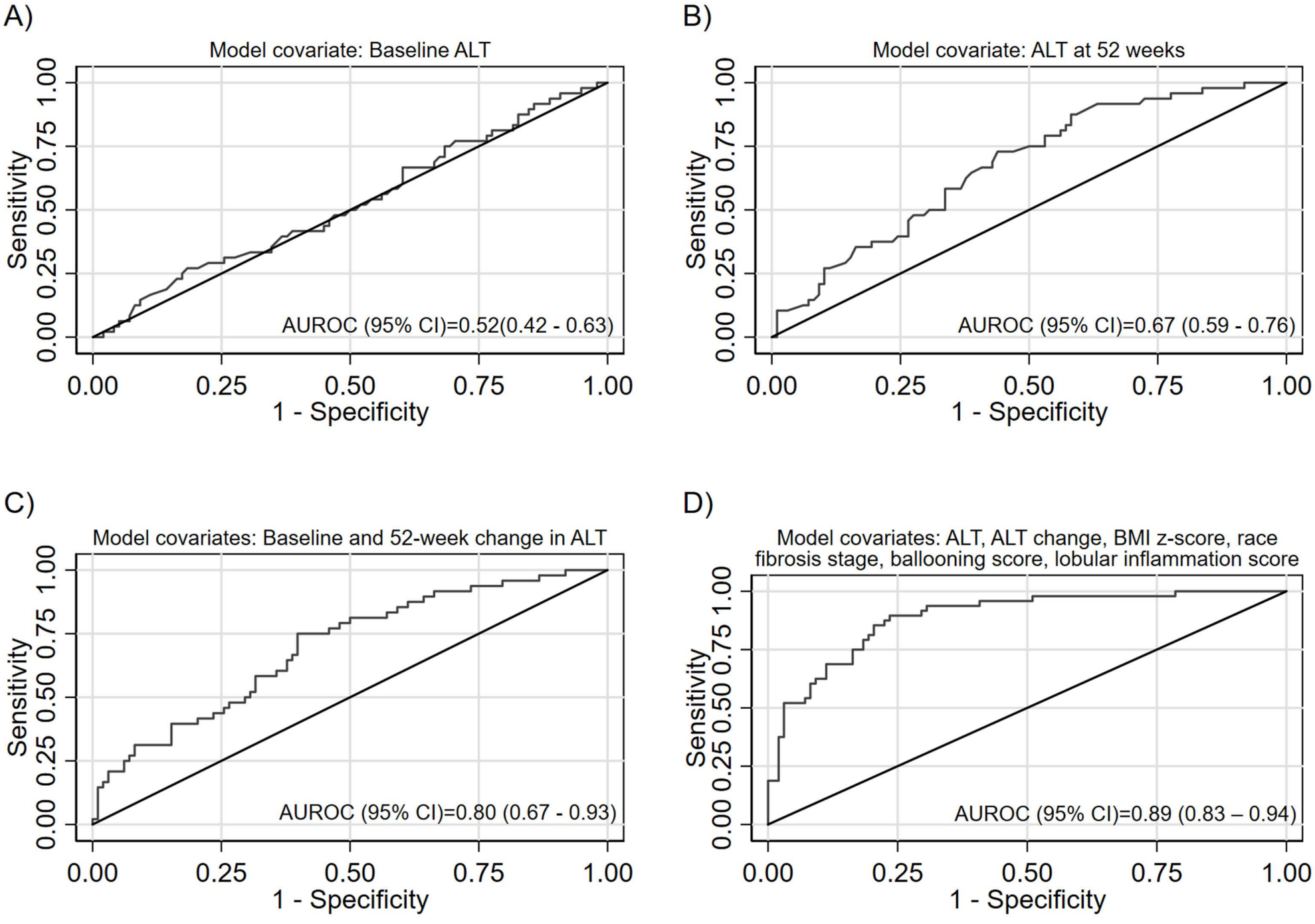
B) AST (U/L)



C) GGT (U/L)




A)
ALT (U/L)
Change in ALT (U/L)
10
-10
-30
-50
-70
-90
-110
p=0.02
0
4
12
24
36
52
Weeks
B)
AST (U/L)
Change in AST (U/L)
10
0
-10
-20
-30
-40
-50
-60
p=0.04
0
4
12
24
36
52
Weeks
C)
GGT (U/L)
Change in GGT (U/L)
5
0
-5
-10
-15
-20
-25
p<0.001
0
4
12
24
36
52
Weeks
Histologic Responder
Histologic Non-Responder


A)
Model covariate: Baseline ALT
Sensitivity
0.00 0.25 0.50 0.75 1.00
AUROC (95% CI)=0.52(0.42 - 0.63)
0.00 0.25 0.50 0.75 1.00
1 - Specificity
B)
Model covariate: ALT at 52 weeks
Sensitivity
0.00 0.25 0.50 0.75 1.00
AUROC (95% CI)=0.67 (0.59 - 0.76)
0.00 0.25 0.50 0.75 1.00
1 - Specificity
C)
Model covariates: Baseline and 52-week change in ALT
Sensitivity
0.00 0.25 0.50 0.75 1.00
AUROC (95% CI)=0.80 (0.67 - 0.93)
0.00 0.25 0.50 0.75 1.00
1 - Specificity
D)
Model covariates: ALT, ALT change, BMI z-score, race
fibrosis stage, ballooning score, lobular inflammation score
Sensitivity
0.00 0.25 0.50 0.75 1.00
AUROC (95% CI)=0.89 (0.83 – 0.94)
0.00 0.25 0.50 0.75 1.00
1 - Specificity

Supplemental Table 1. Baseline characteristics of CyNCh patients with baseline and end of treatment biopsies

	CBDR (N=71)	Placebo (N=75)	Total (N=146)	P
Demographics				
Age (years)	13.6 (2.8)	13.6 (2.5)	13.6 (2.6)	0.98
Male	52 (73%)	51 (68%)	103 (71%)	0.59
Race				0.92
White	43 (61%)	43 (57%)	86 (59%)	
Non-white	10 (14%)	12 (16%)	22 (15%)	
Refusal/not stated	18 (25%)	20 (27%)	38 (26%)	
Hispanic ethnicity	56 (79%)	52 (69%)	108 (74%)	0.26
Weight stratum				0.97
≤65 kg	21 (30%)	21 (28%)	42 (29%)	
>65-80 kg	9 (13%)	9 (12%)	18 (12%)	
>80 kg	41 (58%)	45 (60%)	86 (59%)	
Liver chemistries – median (IQR)				
Alanine aminotransferase (U/L)	92 (67-161)	83 (57-122)	86 (62-140)	0.18
Aspartate aminotransferase (U/L)	54 (40-91)	49 (36-71)	51 (38-79)	0.43
γ-glutamyl transpeptidase (U/L)	37 (26-65)	34 (27-51)	35 (27-60)	0.39
Metabolic factors				
Weight (kg)	84 (26)	84 (25)	84 (26)	0.98
Body-mass index (kg/m^2)	32 (7)	32 (6)	32 (6)	0.79
Body-mass index z-score	2.2 (0.5)	2.2 (0.4)	2.2 (0.4)	0.54
Liver histology findings				
NAFLD activity score*	4.7 (1.3)	4.6 (1.4)	4.7 (1.4)	0.87
Steatosis score	2.3 (0.8)	2.5 (0.7)	2.4 (0.7)	0.13
Lobular inflammation score	1.8 (0.7)	1.6 (0.7)	1.7 (0.7)	0.06
Hepatocellular ballooning score	0.6 (0.7)	0.6 (0.8)	0.6 (0.7)	0.96
Portal inflammation score†	1.1 (0.5)	1.1 (0.5)	1.1 (0.5)	0.95
Fibrosis stage				0.83
0, none	21 (30%)	24 (32%)	45 (31%)	
1a, mild, zone 3 perisinusoidal	5 (7%)	6 (8%)	11 (8%)	
1b, moderate, zone 3 perisinusoidal	4 (6%)	5 (7%)	9 (6%)	
1c, portal/periportal only	18 (25%)	19 (25%)	37 (25%)	
2, zone 3 and periportal, any combination	7 (10%)	11 (15%)	18 (12%)	
3, bridging	15 (21%)	10 (13%)	25 (17%)	
4, cirrhosis	1 (1%)	0 (0%)	1 (1%)	
Steatohepatitis				0.43
No	22 (31%)	18 (24%)	40 (27%)	
Borderline Zone 3 pattern	13 (18%)	9 (12%)	22 (15%)	
Borderline Zone 1 pattern	19 (27%)	27 (36%)	46 (32%)	
Definite	17 (24%)	21 (28%)	38 (26%)	
Resolution of Borderline Zone 1 pattern	9 (47%)	10 (37%)	19 (41%)	0.55

Data are n (%) or mean (SD), unless otherwise noted.

* NAFLD activity score was assessed on a scale of 0-8, with higher scores showing more severe disease (the components of this measure are steatosis [assessed on a scale of 0-3], lobular inflammation [assessed on a scale of 0-3], and hepatocellular ballooning [assessed on a scale of 0-2]).

† Portal inflammation was assessed on a scale of 0-2, with higher scores showing more severe inflammation.

Supplemental Table 2. ALT, AST, and GGT at baseline, 52-weeks, and change from baseline, by histologic response

	Histologic Responder (N=43)	Histologic Non-responder (N=103)	P*
ALT (U/L)			
Baseline			
Geometric mean (95% CI)	105 (82, 135)	90 (81, 101)	0.53
Min, Max	18, 522	24, 499	
52-weeks			
Geometric mean (95% CI)	44 (32, 59)	77 (67, 87)	<0.001
Min, Max	7, 374	16, 341	
Change from baseline			
Mean (95% CI)	-75 (-108, -41)	-11 (-24, 2))	<0.001
Min, max	-416, 104	-296, 203	
AST (U/L)			
Baseline			
Geometric mean (95% CI)	62 (51, 76)	54 (48, 59)	0.67
Min, Max	19, 330	13, 280	
52-weeks			
Geometric mean (95% CI)	33 (26, 41)	44 (39, 49)	<0.001
Min, Max	11, 221	15, 234	
Change from baseline			
Mean (95% CI)	-35 (-54, -17)	-9 (-16, -2)	<0.001
Min, max	-229, 64	-170, 73	
GGT (U/L)			
Baseline			
Geometric mean (95% CI)	41 (34, 48)	39 (35, 43)	0.30
Min, Max	13, 147	17, 224	
52-weeks			
Geometric mean (95% CI)	26 (22, 31)	37 (33, 42)	<0.001
Min, Max	9, 76	11, 202	
Change from baseline			
Mean (95% CI)	-17 (-23, -11)	-1 (-4, 3)	<0.001
Min, max	-104, 6	-86, 49	

* P-values determined from ANCOVA models, regressing laboratory measure (ALT, AST, GGT) on histologic response, using robust regression to down weight outliers. 52-week values and change from baseline values were adjusted for the laboratory value at baseline.

Supplemental Table 3. Changes in liver chemistries over time by histologic improvement and treatment group

	Cysteamine			Placebo			
Change from baseline – Adjusted means (95% CI)	**Improved (N=25)**	**Not improved (N=46)**	**P**	**Improved (N=18)**	**Not improved (N=57)**	**P**	**Inter-action P**
ALT (U/L)							
4 weeks	-67 (-91, -44)	-34 (-52, -17)	0.03	-15 (-46, 15)	+2 (-16, 19)	0.34	0.19
12 weeks	-71 (-95, -47)	-44 (-63, -26)	0.09	-35 (-53, -16)	-1 (-11, 10)	0.003	0.78
24 weeks	-70 (-94, -46)	-46 (-64, -27)	0.12	-37 (-60, -14)	-3 (-15, 10)	0.01	0.69
36 weeks	-77 (-99, -55)	-43 (-59, -26)	0.02	-28 (-52, -5)	-2 (-15, 12)	0.05	0.50
52 weeks	-81 (-103, -59)	-36 (-53, -20)	0.002	-35 (-65, -5)	-1 (-17, 16)	0.06	0.70
AST (U/L)							
4 weeks	-37 (-52, -21)	-18 (-30, -7)	0.06	-7 (-25, 12)	+3 (-8, 14)	0.38	0.26
12 weeks	-40 (-52, -27)	-28 (-37, -19)	0.13	-19 (-29, -9)	0 (-6, 6)	0.002	0.57
24 weeks	-40 (-54, -25)	-27 (-38, -15)	0.16	-21 (-34, -7)	-2 (-9, 5)	0.02	0.74
36 weeks	-44 (-54, -34)	-26 (-34, -19)	0.006	-13 (-26, 1)	-2 (-10, 5)	0.17	0.33
52 weeks	-42 (-55, -29)	-24 (-33, -14)	0.03	-13 (-29, 3)	-1 (-10, 8)	0.19	0.50
GGT (U/L)							
4 weeks	-11 (-21, -1)	+1 (-6, 9)	0.06	-6 (-13, 0)	-1 (-4, 3)	0.11	0.40
12 weeks	-17 (-23, -10)	-9 (-14, -4)	0.08	-10 (-16, -4)	-1 (-4, 3)	0.01	0.71
24 weeks	-16 (-24, -8)	-5 (-11, 1)	0.03	-5 (-11, 2)	-2 (-5, 2)	0.44	0.25
36 weeks	-17 (-25, -10)	-8 (-13, -3)	0.03	-8 (-14, -2)	0 (-4, 3)	0.04	0.80
52 weeks	-20 (-26, -14)	-5 (-9, -1)	<0.001	-12 (-19, -4)	+2 (-2, 6)	0.002	0.81

*P-values derived from ANCOVA, regressing the change in liver chemistry on histologic improvement and the baseline value of the liver chemistry.

Supplemental Table 4. Association between 12-week change in ALT and 52-week change in ALT

	β	95% CI	P
Outcome: Δ ALT (52-week-BL, U/L)			
12-week change in ALT	0.9	0.8, 1.0	<0.001
Treatment group (Cyst vs. Plbo)	-8.1	-25.3, 9.2	0.36
Intercept	3.8		
Outcome: Δ AST (52-week-BL, U/L)			
12-week change in AST	0.8	0.7, 0.9	<0.001
Treatment group (Cyst vs. Plbo)	-6.0	-16.0, 4.0	0.24
Intercept	1.5		
Outcome: Δ GGT (52-week-BL, U/L)			
12-week change in GGT	0.7	0.6, 0.8	<0.001
Treatment group (Cyst vs. Plbo)	-4.0	-9.0, 1.1	0.12
Intercept	1.3		

* Treatment group*12-week change in liver chemistry interaction p-value: ALT P=0.69; AST P=0.19; GGT P=0.07.

Supplemental Table 5. Logistic regression analysis of histologic improvement on liver chemistries (ALT, AST, and GGT)

Outcome: Histologic Improvement	Odds ratio	95% CI	P
MODEL 0: Univariable logistic regression models			
Baseline (per 10 U/L increase)			
ALT (U/L)	1.04	1.00 – 1.07	0.04
AST (U/L)	1.06	0.99 – 1.13	0.07
GGT (U/L)	1.02	0.91 – 1.14	0.73
Change (per 10 U/L decrease from baseline to 52 weeks)			
Δ ALT (U/L)	1.10	1.04 – 1.17	<0.001
Δ AST (U/L)	1.14	1.04 – 1.24	0.004
Δ GGT (U/L)	1.60	1.26 – 2.03	<0.001
MODEL 1: Multivariable logistic regression of baseline ALT, AST, GGT, and 52-week changes			
Baseline (per 10 U/L increase)			
ALT (U/L)	0.99	0.82 – 1.21	
AST (U/L)	1.03	0.71 – 1.49	
GGT (U/L)	0.75	0.56 – 1.00	
Change (per 10 U/L decrease from baseline to 52 weeks)			
Δ ALT (U/L)	1.12	0.90 – 1.40	
Δ AST (U/L)	0.86	0.62 – 1.20	
Δ GGT (U/L)	1.88	1.16 – 3.04	
Intercept	0.66	0.29 – 1.54	
P for baseline ALT, Δ ALT (2 d.f.):			0.44
P for baseline AST, Δ AST (2 d.f.):			0.56
P for baseline GGT, Δ GGT (2 d.f.):			0.04
P for baseline and Δ for ALT, AST, GGT (6 d.f.):			<0.0001
MODEL 2: Best AIC model from candidate set: baseline ALT, AST, GGT, and 52-week changes			
Baseline GGT (per 10 U/L increase)	0.74	0.60 – 0.91	0.005
Δ GGT (per 10 U/L decrease from baseline to 52 weeks)	2.22	1.54 – 3.21	<0.0001
P for baseline and Δ GGT			<0.0001
Model 3: Best AIC model from all possible subsets of 24 candidate variables, added to Model 2 variables (GGT and ΔGGT)			
Baseline GGT (per 10 U/L increase)	0.55	0.40 – 0.77	<0.001
Δ GGT (per 10 U/L decrease from baseline to 52 weeks)	2.25	1.45 – 3.50	<0.001
Baseline lobular inflammation	6.31	2.70 – 14.74	<0.001
Baseline alkaline phosphatase (per 10 U/L increase)	1.08	1.02 – 1.15	0.01
Baseline LDL (per 10 mg/dL increase)	1.19	1.01 – 1.42	0.04
Hypertension at baseline	4.37	0.97 – 19.66	0.05
Age (years)	1.22	0.96 – 1.56	0.11

Full model P:			<0.0001
Comparison of Models	**AUROC**	**95% CI**	**P**
Model 2: Baseline GGT, Δ GGT	0.79	0.71 – 0.87	
Model 3: Baseline GGT, Δ GGT, lobular inflammation, alkaline phosphatase (U/L), LDL (mg/dL), hypertension, age (years)	0.89	0.82 – 0.95	
P-value: Model 3 vs. Model 2			0.01

April 30, 2020

]Supplemental Table 6. Logistic regression analysis of resolution of Zone 1, periportal pattern on liver chemistries (ALT, AST, and GGT)

Outcome: Resolution of borderline Zone 1 steatohepatitis	**Odds ratio**	**95% CI**	**P**
MODEL 0: Univariable logistic regression models			
Baseline (per 10 U/L increase)			
ALT (U/L)	0.91	0.80 – 1.04	0.16
AST (U/L)	0.84	0.66 – 1.07	0.16
GGT (U/L)	0.72	0.54 – 0.96	0.02
Change (per 10 U/L decrease from baseline to 52 weeks)			
Δ ALT (U/L)	1.13	1.01 – 1.27	0.04
Δ AST (U/L)	1.20	0.97 – 1.48	0.09
Δ GGT (U/L)	1.11	0.85 – 1.45	0.43
MODEL 1: Multivariable logistic regression of baseline ALT, AST, GGT, and 52-week changes			
Baseline (per 10 U/L increase)			
ALT (U/L)	0.67	0.38 – 1.17	
AST (U/L)	1.51	0.55 – 4.16	
GGT (U/L)	0.79	0.48 – 1.30	
Change (per 10 U/L decrease from baseline to 52 weeks)			
Δ ALT (U/L)	1.73	0.97 – 3.11	
Δ AST (U/L)	0.56	0.21 – 1.54	
Δ GGT (U/L)	1.01	0.53 – 1.93	
Intercept	4.97	0.60 – 41.36	
P for Δ ALT, Δ AST, Δ GGT (3 d.f.):			0.07
P for baseline ALT, Δ ALT (2 d.f.):			0.18
P for baseline AST, Δ AST (2 d.f.):			0.52
P for baseline GGT, Δ GGT (2 d.f.):			0.60
Full model P:			0.007
MODEL 2: Best AIC model from candidate set: baseline ALT, AST, GGT, and 52-week changes			
Baseline ALT (per 10 U/L increase)	0.71	0.55 – 0.92	0.009
Δ ALT (per 10 U/L decrease from baseline to 52 weeks)	1.38	1.10 – 1.74	0.006
Full model P:			0.0004
Model 3: Best AIC model from all possible subsets of 24 candidate variables, added to Model 2 variables (ALT and ΔALT)			
Baseline ALT (per 10 U/L increase)	0.60	0.40 – 0.90	0.01
Δ ALT (per 10 U/ L decrease from baseline to 52 weeks)	2.03	1.27 – 3.25	0.003
LDL (mg/dL)	0.94	0.90 – 0.99	0.008
White vs. non-white	22.20	1.60 – 307.39	0.02
Full model P:			<0.0001

Comparison of Models	AUROC	95% CI	P
Model 2: Baseline ALT, Δ ALT	0.80	0.67 – 0.93	
Model 3: Baseline ALT, Δ ALT, LDL (mg/dL), white race	0.91	0.83 – 0.99	
P-value: Model 3 vs. Model 2			0.03