# Machine Learning–Based Quantification of Histology Features From Patients* Treated for Chronic Hepatitis B Identifies Features Associated With Viral DNA Suppression and HBeAg Loss

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## Introduction

- Chronic hepatitis B virus (CHB) infection is associated with cirrhosis, liver decompensation, and hepatocellular carcinoma[1]
- HBV DNA suppression and hepatitis B e antigen (HBeAg) loss are important outcomes for patients undergoing treatment for CHB infection; however, the relationships of these outcomes with histology have not been fully elucidated
- Computational pathology leveraging convolutional neural networks could facilitate characterization of histologic features associated with HBV DNA suppression and HBeAg loss

## Objective

- To evaluate associations of histologic features with HBV DNA suppression and HBeAg loss using machine learning (ML)–based computational pathology

## Methods

- Retrospective analysis of adults with CHB infection undergoing treatment with tenofovir disoproxil fumarate in registrational studies GS-US-174-0102 (NCT01277601) and GS-US-174-0103 (NCT00116805)
- Viral parameters (HBV DNA and HBeAg) were measured from blood collected at baseline and every 4 weeks to Week 48 (Year 1), every 6 weeks to Week 96, and then every 12 weeks to Week 240 (Year 5)
  - HBV DNA suppression: ≤69 IU/mL

### Liver Histology

- Liver biopsies at baseline, and Years 1 and 5 were centrally read according to Ishak/Knodell necroinflammatory scoring and Ishak fibrosis staging systems[2]

### ML Assessment of Liver Biopsies (PathAI, Inc., Boston, MA)[3,4]

- Digitized hematoxylin and eosin whole-slide images were split into training (n=1090; 172 patients) and test (n=1060; 170 patients) sets
- Deep convolutional neural networks were trained to quantify liver cell- and tissue-level features using annotations collected from 40 board-certified pathologists
- Quantitative image-level ML features were computed:
  - % Areas of portal inflammation, lobular inflammation, interface hepatitis, steatosis, and hepatocellular ballooning
  - Lymphocyte densities in regions of portal inflammation, lobular inflammation, and interface hepatitis, and within the entire biopsy
- After training, models were deployed on the test set of whole-slide images

### Statistical Analyses

- Associations between ML histologic features with HBV DNA suppression at Year 1 and HBeAg status at Year 5 were determined using regression analysis

## Results

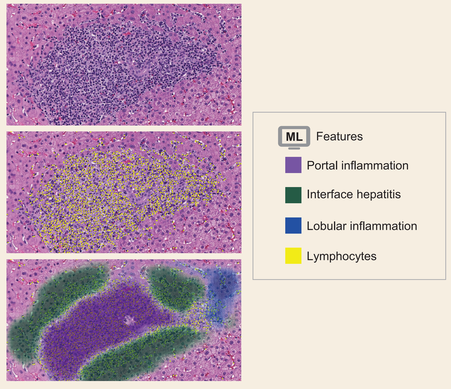
### Baseline Demographics and Clinical Outcomes in Test Set

| | Total N=170 |
|---|---|
| Age ≥50 y, n (%) | 44 (26) |
| Men, n (%) | 142 (84) |
| Mean ALT, U/L (SD) | 140.86 (122) |
| Mean HBV DNA, $\log_{10}$ (SD) | 6.69 (1) |
| HBeAg positive, n (%) | 67 (39) |
| HBV genotype, n (%) | |
| A | 41 (24) |
| B | 15 (9) |
| C | 22 (13) |
| D | 85 (50) |
| E | 3 (2) |
| Median Knodell necroinflammatory score (IQR) | 9 (8, 10) |
| Cirrhosis, n (%) | 43 (25) |
| Ishak 5 | 11 (6) |
| Ishak 6 | 32 (19) |

ALT, alanine aminotransferase; IQR, interquartile range; SD, standard deviation.

- At Year 1, 21% achieved HBeAg loss and 76% achieved HBV DNA suppression (observed data)
- At Year 5, 64% achieved HBeAg loss and 94% achieved HBV DNA suppression (observed data)

### ML Models Detect Features of Inflammation




### Associations Between ML Histologic Features and Pathologist Scores of Inflammation

| Tissue Region | ML Features | CP Ishak HAI Scores | ρ (all p <0.01) |
|---|---|---|---|
| Entire biopsy | Lymphocyte density | Composite score | 0.51 |
| Portal inflammation | Lymphocyte density | Portal inflammation | 0.38 |
| | % Area | | 0.64 |
| Interface hepatitis | Lymphocyte density | Periportal necrosis | 0.27 |
| | % Area | | 0.71 |
| Lobular inflammation | Lymphocyte density | Lobular necrosis | 0.05 |
| | % Area | | 0.60 |

HAI, histologic activity index.

- ML % area features correlated more strongly with pathologist scores than ML lymphocyte density features, suggesting % area features better recapitulate pathologist scores
- ML lymphocyte density features may provide additional information not readily captured by pathologist scores

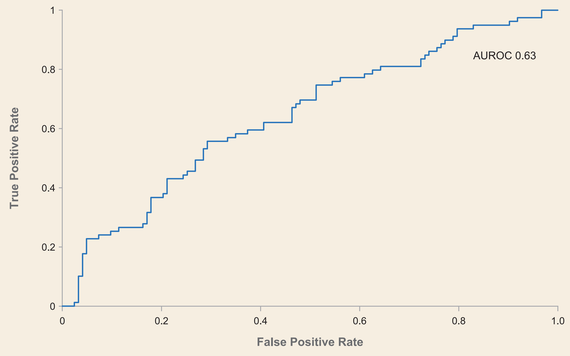
### Associations of Baseline ML Histologic Features With HBeAg Status at Year 5

| Baseline ML Histologic Features | | HBeAg Loss at Year 5* | | |
|---|---|---|---|---|
| Histologic | Type | Yes | No | p-Value |
| Portal inflammation | % Area | 3.18 (3.14) | 3.40 (2.76) | 0.43 |
| | Lymphocyte density | 5309 (2585) | 4889 (2582) | 0.11 |
| Lobular inflammation | % Area | 2.26 (1.89) | 2.61 (2.00) | 0.50 |
| | Lymphocyte density | 2337 (696) | 2115 (929) | **0.03** |
| Interface hepatitis | % Area | 2.00 (3.06) | 2.36 (1.77) | 0.40 |
| | Lymphocyte density | 3536 (1294) | 3344 (1884) | 0.32 |
| Entire biopsy | Lymphocyte density | 691 (457) | 557 (334) | **0.01** |
| Steatosis | % Area | 0.21 (0.71) | 0.08 (0.22) | 0.37 |
| Hepatocellular ballooning | % Area | 0.03 (0.11) | 0.03 (0.04) | 0.42 |

*Median (IQR); *bold:* p <0.05.

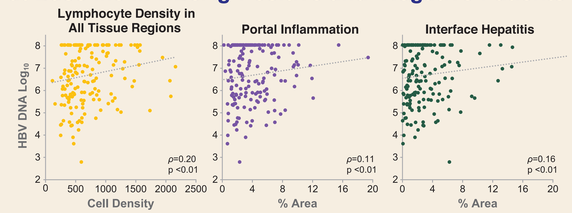
- Greater ML lymphocyte density in entire biopsy and regions of lobular inflammation was associated with HBeAg loss at Year 5

### A Model Incorporating ML Histologic Features at Baseline Was Moderately Predictive of HBeAg Loss at Year 5*



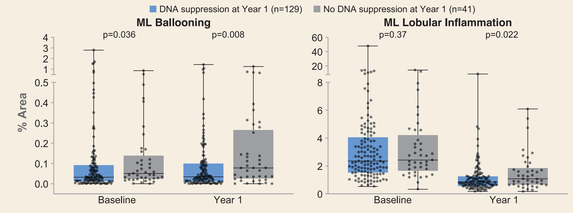

*Model derived using multivariate model incorporating baseline ML features of lymphocyte density, portal inflammation, lobular inflammation, and interface hepatitis. AUROC, area under receiver operating characteristic curve.

### Correlation of ML Histologic Features With HBV DNA at Baseline in HBeAg-Positive and -Negative Patients




- ML-calculated features of lymphocyte density, and % areas of portal inflammation and interface hepatitis were significantly correlated with HBV DNA levels
- Other ML histologic features were not significantly correlated with HBV DNA level at baseline

### Assocations of ML Histologic Features With HBV DNA Suppression in HBeAg-Positive and -Negative Patients




- ML-calculated histologic features of lymphocyte density, portal inflammation, and interface hepatitis at baseline and Year 1 were not associated with HBV DNA suppression
- At baseline, patients who did not achieve HBV DNA suppression had greater % areas of ballooning and similar % areas of lobular inflammation (p=0.036 and p=0.37, respectively) compared with those who achieved HBV DNA suppression
- At Year 1, patients who did not achieve HBV DNA suppression had greater % areas of ballooning and lobular inflammation (p=0.008 and 0.022, respectively)
- When controlling for ALT normalization, only % area of ballooning at Year 1 was significantly greater in patients who did not suppress HBV DNA (data not shown)

## Conclusions

- ML-model quantification of histopathologic features from biopsies of patients in clinical trials GS-US-174-102/103 revealed cell and tissue level changes that were undetectable using traditional pathology
- Greater ML lymphocyte density at baseline was associated with HBeAg loss
- Lack of HBV DNA suppression was associated with significantly greater % areas of hepatocellular ballooning and lobular inflammation at Year 1
- An ML approach for evaluating liver histology in patients with CHB can provide mechanistic insight into HBV pathogenesis, HBeAg loss, and HBV DNA suppression

*Study subjects in clinical trials.

**References:** **1.** Mittal S, et al. J Clin Gastroenterol 2013;47:S2-6; **2.** Marcellin P, et al. Lancet 2013;381:468-75; **3.** Pokkalla H, et al. AASLD 2019, abstr 0187; **4.** Younossi ZM, et al. AASLD 2019, abstr 1718.

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