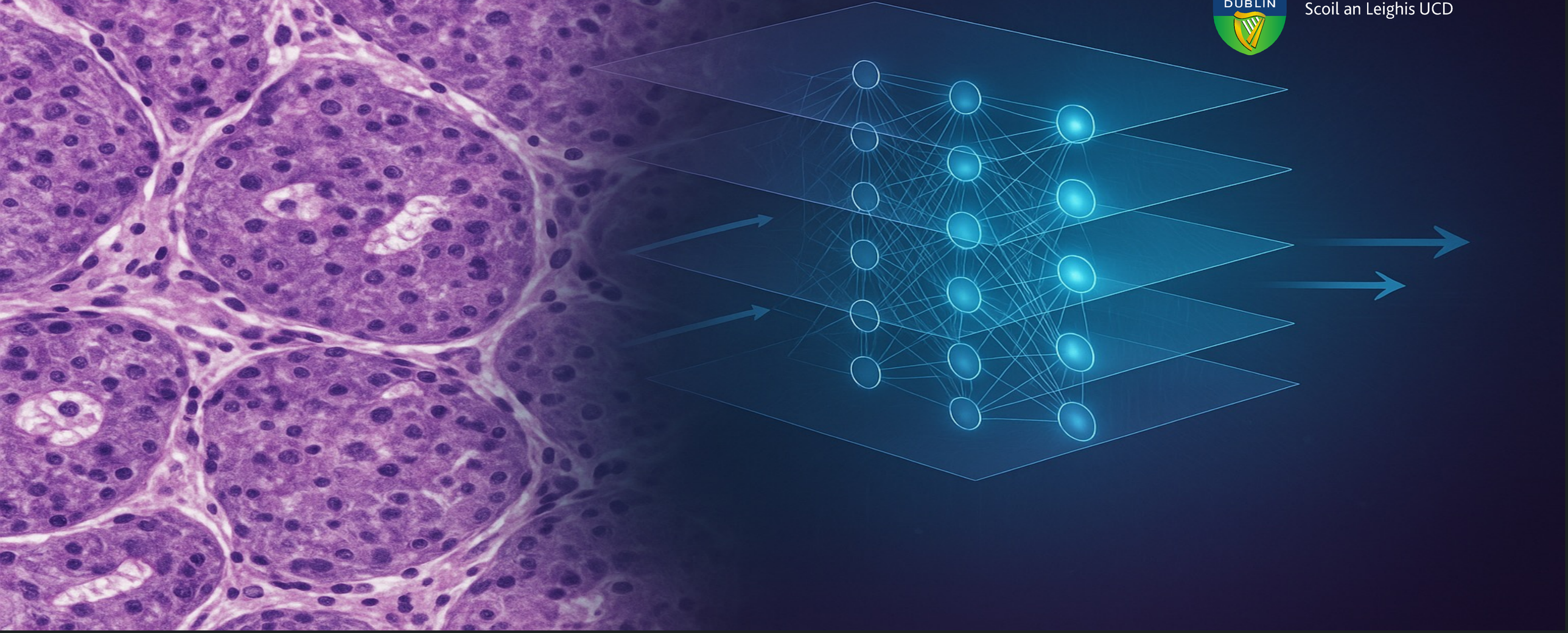

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A Deep Learning Model of Histologic Tumor Differentiation as a Prognostic Tool in Hepatocellular Carcinoma

Anastasiia Deviataieva

Introduction

- Hepatocellular carcinoma (HCC), the most common primary liver cancer and the third most common cause of cancer- related death worldwide.
- "tumor differentiation" refers to the extent to which cancers histologically resemble or recapitulate their normal tissue counterpart.
- Histologic features of tumor differentiation in HCC include cytoarchitecture, immunohistochemistry profile, and reticulin framework.
- Traditional approach: Edmondson-Steiner and World Health Organization (WHO) grading systems.
- AI approach: a supervised deep-learning model to quantify differentiation features digitally, aiming to improve prognostic predictions.

Reference	Grades	Architecture	Cytology	Other features
World Health Organization (21)	Well differentiated	Thin trabecular, frequent acinar structures	Minimal atypia	Fatty change is frequent
	Moderately differentiated	Trabecular (3 or more cells in thickness) and acinar	Abundant eosinophilic cytoplasm, round nuclei with distinct nucleoli	Bile or proteinaceous fluid within acini
	Poorly differentiated	Solid	Moderate to marked pleomorphism	Absence of sinusoid-like blood spaces
	Undifferentiated	Solid	Little cytoplasm, spindle, or round-shaped cells	—
Edmondson and Steiner (13)	Grade I	—	—	Areas of carcinoma where distinction from hyperplastic liver is difficult
	Grade II	Trabecular, frequent acini (lumen varying from tiny canaliculi to large thyroid-like spaces)	Resemblance to normal hepatic cells; larger nuclei; abundant acidophilic cytoplasm	Cell borders sharp and clear cut; acini containing bile or protein precipitate
	Grade III	Distortion of trabecular structure, acini less frequent than grade II	Larger, more hyperchromatic nuclei, granular but less acidophilic cytoplasm	Acini are less frequent; tumor giant cells may be numerous
	Grade IV	Medullary, less trabeculae, rare acini	Highly hyperchromatic nuclei, scanty cytoplasm, with fewer granules	Loss of cell cohesiveness; giant, spindle or short-plump cells can be found

Figure 1. Histological features from Edmondson and Steiner (ES) publication and WHO book. (Martins-Filho et al., 2017)

Materials

Cohort: 99 HCC patients with curative resection (retrospective)

Data: Digitized histopathology slides for each tumor:

- H&E (Hematoxylin & Eosin) for morphology
- Reticulin stain: Reveals the network of supporting fibers; loss of reticulin → poorly differentiated HCC
- IHC for HepPar-1 (marker of hepatocytic differentiation; high expression in well-differentiated cell) and Glypican-3 (oncofetal antigen) tumor differentiation markers

Outcomes Measured: Overall survival, disease-free survival, time to metastasis, local recurrence

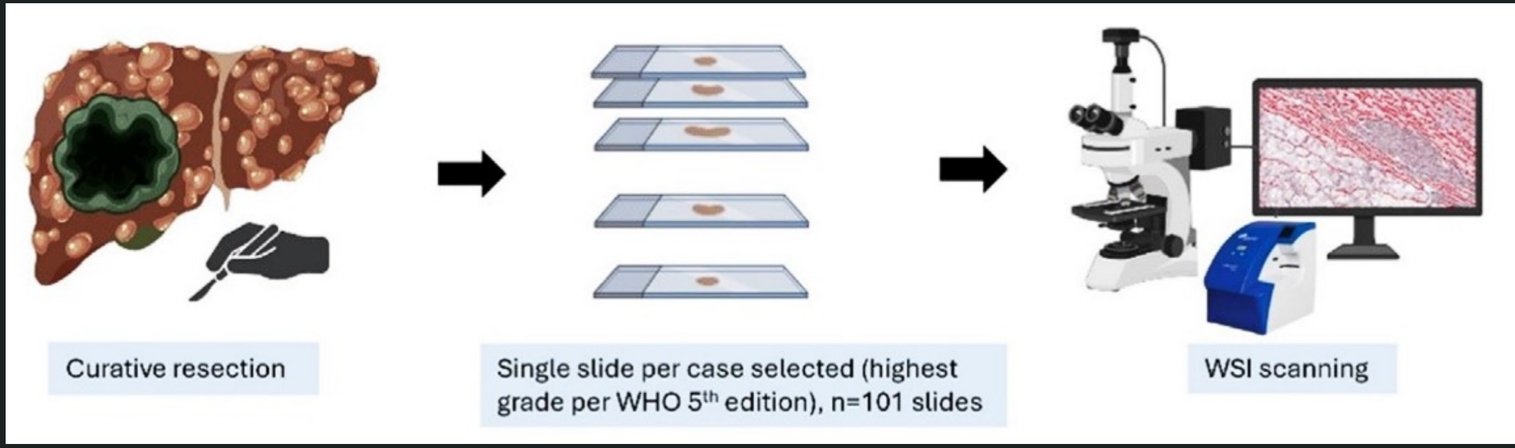


Figure 2. Image extracting workflow. After curative liver resection, one highest-grade slide from each case (n = 101) is selected and digitized through whole-slide imaging for subsequent computational analysis. (Patil et al., 2025). Liver tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 4-mm sections, and stained with H&E, HepPar 1, glypican-3 (GPC) immunostains, and reticulin protocols.

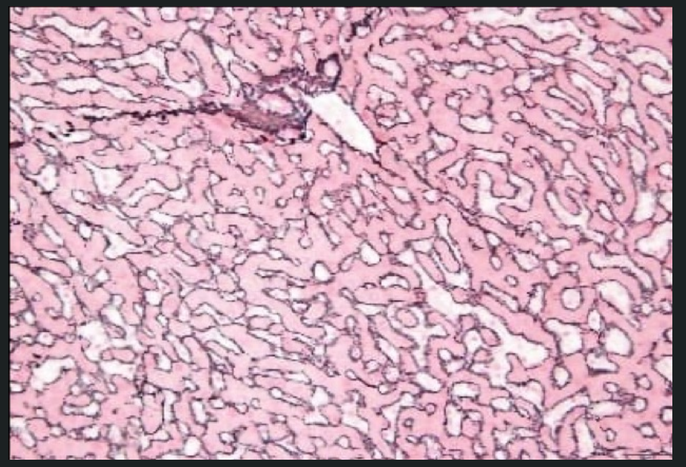


Figure 3. Reticulum II Staining Kit on liver tissue. Reticulum II Staining Kit stains reticulin fibers black against a red pink (Nuclear Fast Red counterstain) background. (ScyTek Laboratories, n.d.)

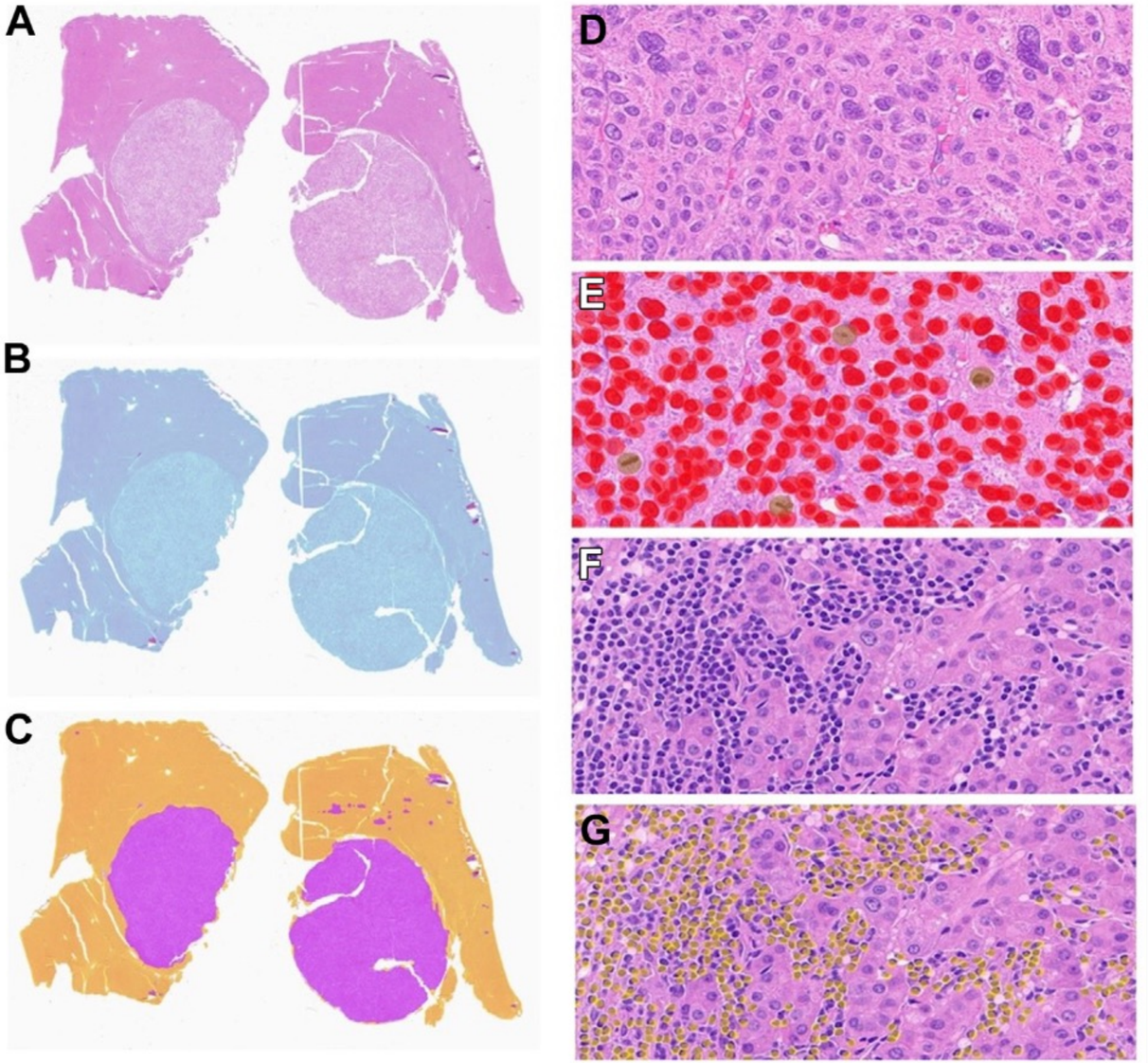


Figure 4. AI model for automated recognition of histologic features of HCC. (A) Hematoxylin and eosin stain, (B) general tissue detector algorithm with automated artifact exclusion (light blue), and (C) automated tumor (purple) and nonneoplastic liver tissue (yellow) detection. (D) HCC hematoxylin and eosin stain (E) with object recognition of hepatocyte nuclei (red) and mitotic figures (brown) and (F) intratumoral lymphocytes (G) with automated tumor-infiltrating lymphocyte detection. (H) HCC reticulin stain and (I) automated quantitative analysis of reticulin fibers. HCC immunostains with automated quantitative analysis of (J) glypican-3 and (K) HepPar 1. HCC, hepatocellular carcinoma. (Patil et al., 2025)

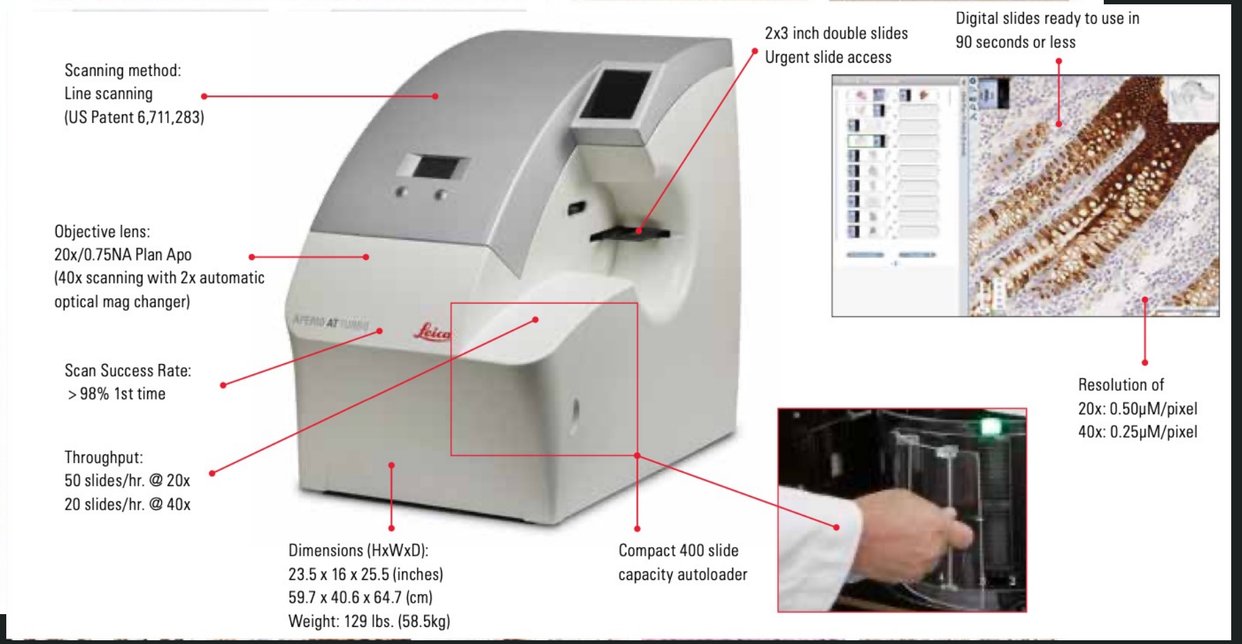


Figure 5. Aperio ScanScope AT Turbo, a bright- field scanning instrument by Leica Biosystem (ScyTek Laboratories, n.d.).

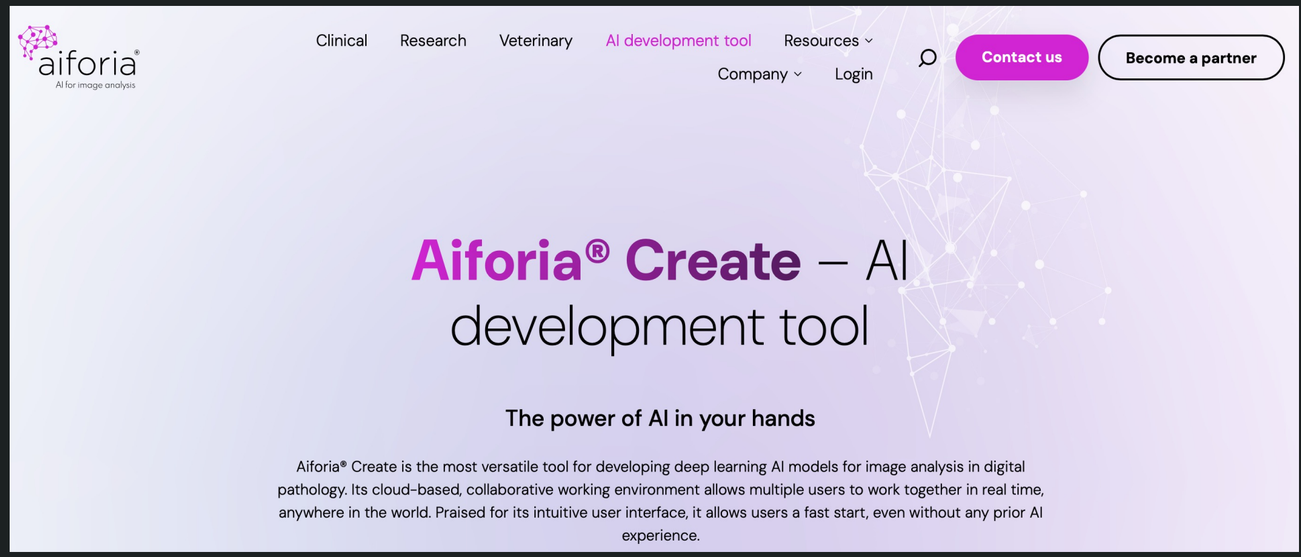
AI Model Workflow

Platform: Used a cloud-based convolutional neural network platform (Aiforia Technologies) for supervised learning.

Tumor segmentation: model distinguished tumor tissue from benign (tissue detector, artifact removal).

Feature detection:

- Nuclear morphology: density (cellularity), nuclear area, shape circularity, chromatin texture, cell pleomorphism
- Mitoses: automated detection of mitotic figures (cell divisions)
- IHC: percentage of tumor cells positive for HepPar-1 and for Glypican-3
- Reticulin framework: proportion of area with reticulin fibers (comparison to normal liver)



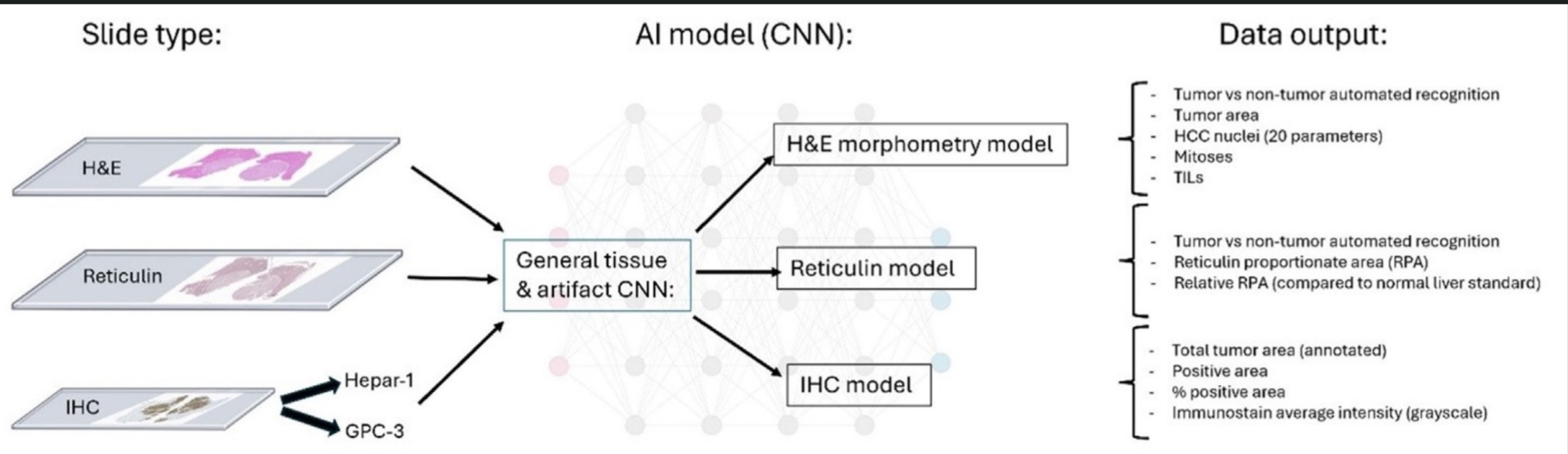


Figure 6. AI model development (Patil et al., 2025)

CNNs Architecture

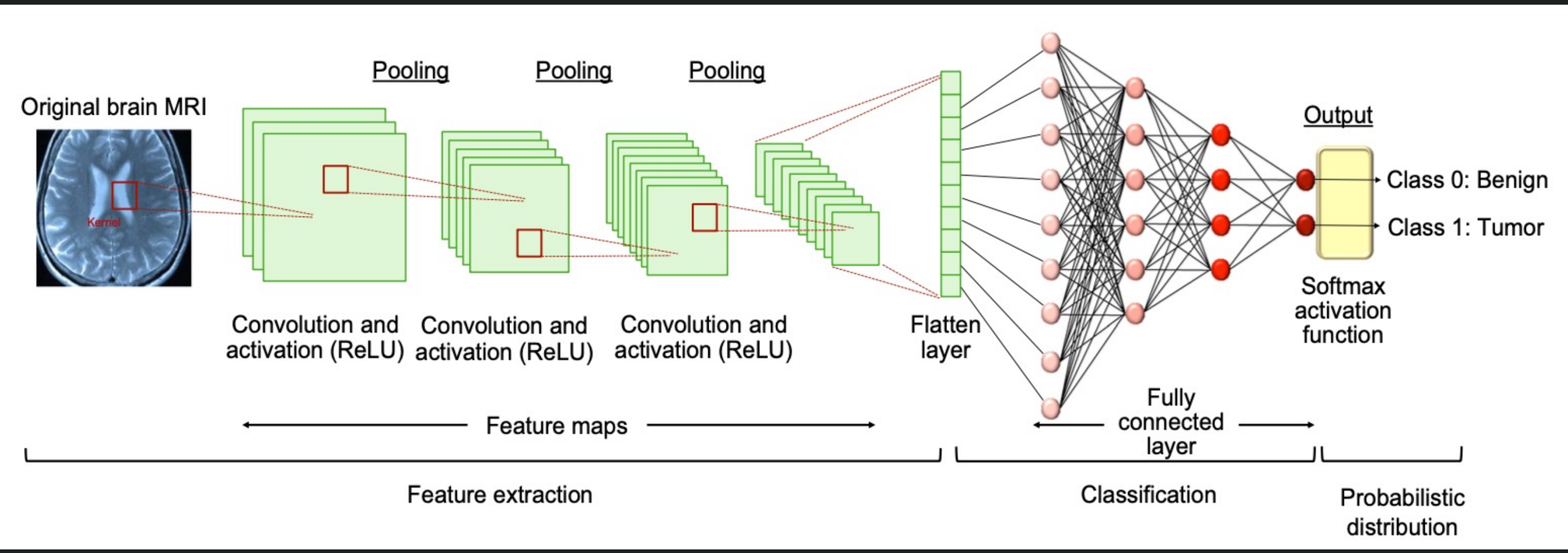


Figure 7. An example of medical image analysis using CNN architecture (brain MRI). (Takahashi et al., 2024)

CNN Pipeline — Generic Form:

- Input – raw image or multi-channel data array
- Convolution + Activation (e.g., ReLU) – sliding kernels generate local feature maps
- Pooling / Sub-sampling – shrink spatial size while retaining salient responses
- Stacked Convolutional Blocks – wider receptive fields, progressively abstract features
- Flatten or Global Pooling – convert 3-D feature tensor to 1-D feature vector
- Fully Connected layer(s) – integrate all learned features
- Output head (Softmax, Sigmoid, etc.) – produces final class probabilities or regression values

Statistical Analysis

Modeling: Built Cox proportional-hazards models for outcomes (overall survival, disease-free survival, metastasis, recurrence).

Comparisons: Tested models using routine data vs. models including AI-derived features.

Metrics: Evaluated using concordance index (C-index) – higher is better predictive accuracy.

Validation: Performed bootstrap resampling to estimate confidence intervals

Feature selection: Applied LASSO regression to select the most informative features and avoid overfitting

Statistical model	Variables included
Base Path	• WHO grade • AJCC pT stage
AI Path	• Up to 5 selected AI-quantified features
Full Path	• WHO grade • AJCC pT stage • Up to 5 selected AI-quantified features
Base ClinPath	• WHO grade • AJCC pT stage • Age • Sex
Full ClinPath	• WHO grade • AJCC pT stage • Age (y) • Sex • Up to 5 selected AI-quantified features

AI, artificial intelligence; AJCC, American Joint Committee on Cancer; Path, pathology; WHO, World Health Organization.

Figure 8. Clinical and pathologic variables included in each statistical model. (Patil et al., 2025)

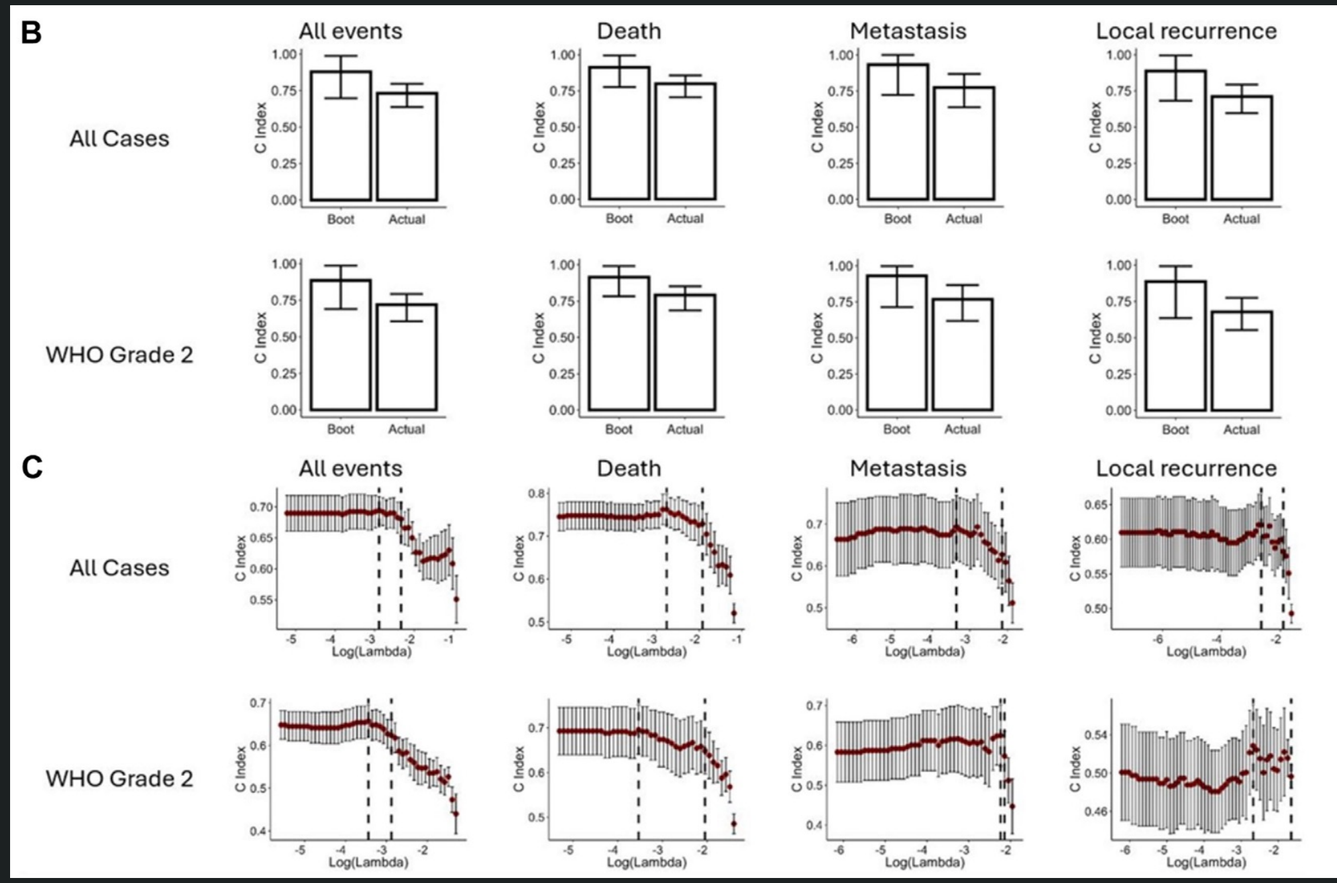


Figure 9. B) Comparative analysis of the C-index across 4 clinical outcomes. C-index is shown for models evaluated on bootstrapped samples ("boot") versus the actual data set ("actual"). (C) LASSO model showing optimization of lambda penalty parameter. Each panel displays the C-index for varying degrees of regularization imposed by log(lambda), highlighting the model's predictive accuracy as lambda values change. (Patil et al., 2025)

Results

Statistical models that included these AI-based variables outperformed models with combined clinical pathologic features:

- overall survival (C-indexes of 0.81 vs 0.68),
- disease-free survival (C-indexes of 0.73 vs 0.68),
- metastasis (C-indexes of 0.78 vs 0.65),
- local recurrence (C-indexes of 0.72 vs 0.68)

for all cases, with similar results in the subgroup analysis of WHO grade 2 HCCs.

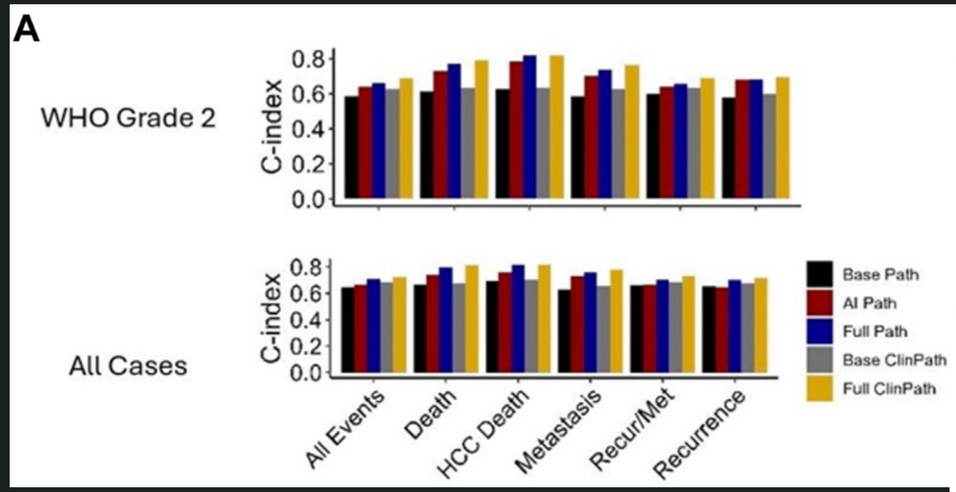


Figure 10. C-index for statistical models, including different combinations of clinical (routine), pathologic, and AI modele generated data. (Patil et al., 2025)

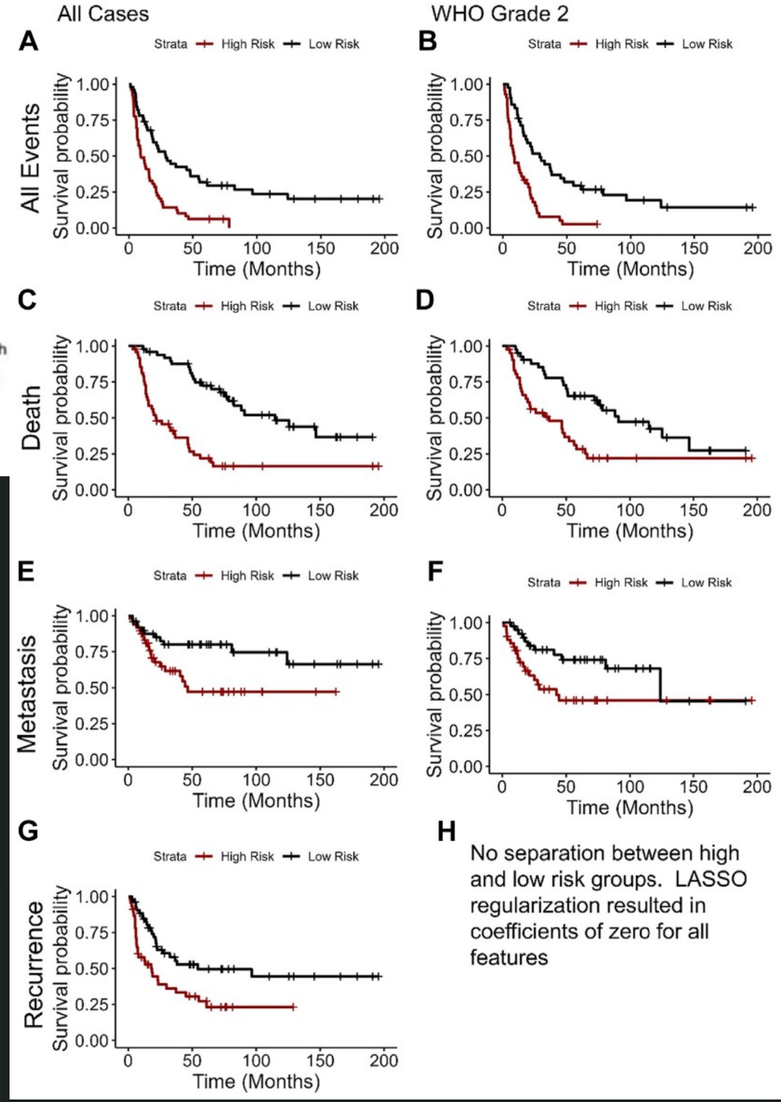


Figure 11. Kaplan-Meier survival curves for high-risk and low-risk groups across clinical outcomes. Survival probabilities over time for patients stratified into high-risk and low-risk groups based on model predictions for both all cases and WHO grade 2 cases specifically..(Patil et al., 2025)

Variable	Description
1. pT stage	"T" component of the *AJCC Cancer Staging Manual*, eighth edition
2. World Health Organization grade	Global tumor grading according to the WHO Classification of Tumours, fifth edition
3. Nuclear area percent (AI model)	Ratio between total tumor nuclear area/total tumor area, by AI-based automated recognition
4. Hepar-low/GPC-3–positive phenotype (AI model)	HepPar 1 expression in <50% of tumor and any degree of glypican-3 expression by AI-based automated recognition
5. r-RPA (AI model)	RPA (ratio of reticulin area/total tumor area) by AI-based automated recognition

Features 3 to 5 are based on automated recognition and quantitative analysis by our HCC AI model.
AI, artificial intelligence; HCC, hepatocellular carcinoma; RPA, reticulin proportionate area.

Figure 12. Pathologic variables identified as most significant for prognostic assessment of various HCC-related clinical outcomes across our statistical models. (Patil et al., 2025)

Conclusion

Proof-of-concept	Demonstrated that digital pathology with deep learning can objectively quantify HCC differentiation features to aid prognosis.
Independent prognostic value:	Several AI-derived metrics (nuclear area, reticulin loss, HepPar-1/GPC3 status) independently predicted HCC outcomes.
Implications	This approach could complement traditional grading, providing standardized, reproducible biomarkers.
Future work	Larger cohorts and integration into clinical workflows will be needed for validation.

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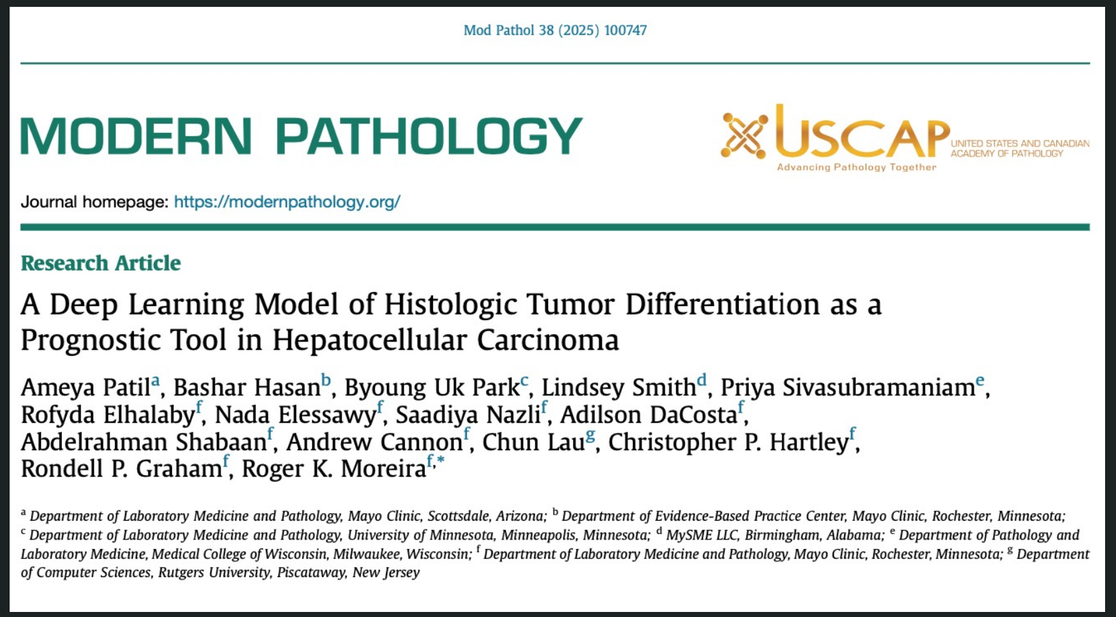

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Research Article

A Deep Learning Model of Histologic Tumor Differentiation as a Prognostic Tool in Hepatocellular Carcinoma

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