Characteristics of the tumor microenvironment in IDH1-mutated cholangiocarcinoma patients from ClarIDHy trial

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INTRODUCTION

- Somatic isocitrate dehydrogenase 1 mutations (IDH1m) convert α-ketoglutarate to the oncogenic metabolite R-2-hydroxyglutarate (2-HG).IDH1m are detected in approximately 13% of intrahepatic cholangiocarcinomas (CCAs).[1]
- Ivosidenib (IVO), an oral inhibitor of the IDH1m protein inhibits 2-HG, and data suggests restores immune response in CCA.[2]
- In the global, phase 3 ClarIDHy study evaluating IVO vs placebo (PBO) in patients with nonresectable or metastatic IDH1m CCA (ClarIDHy, ClinicalTrials.gov NCT02989857) [3] :
  - IVO demonstrated a favorable safety profile
  - IVO significantly improved progression-free survival vs PBO (HR = 0.37, p < 0.0001)

OBJECTIVE

Using machine learning models to quantify histologic features[4] of the CCA pre-treatment tissue sections, we aimed to identify correlates of tumor microenvironment associated with:

- IDH1 mutation status
- Early disease progression (patients experienced progression or death within 1.54 months)
- Plasma 2-HG levels (median, 630 ng/ml)

METHODS

A set of H&E images, including from ClarIDHy,[3] a phase 3 PBO controlled clinical trial of ivosidenib in IDH1m CCA, were split into training/validation (n=200) and test sets for model development. Whole slide images were annotated by GI pathologists to identify and quantify more than 150 different human interpretable features (HIFs), including cell (cancer cell, lymphocyte, macrophage, plasma cell, fibroblast) and tissue (cancer epithelium, stroma, necrosis) features. Utilizing IDH1m and wild type (WT) screening samples, multivariate logistic regression models were trained to predict IDH1m. P-values were calculated by univariate logistic regression and corrected for multiple comparisons via adjustment for FDR. FDR corrected p values were displayed for different clusters. Uncorrected P values were displayed for representative HIFs.

ACKNOWLEDGEMENTS

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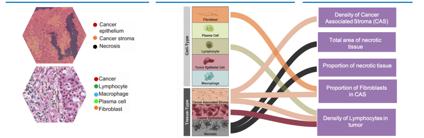
TECHNOLOGY AND VALIDATION

Figure 1: Predicting and quantifying Human-interpretable Image Features (HIFs)


Cell type and spatial information
Model predictions
Quantification of >150 HIFs
Cancer epithelium
Cancer stroma
Necrosis
Cancer
Lymphocyte
Macrophage
Plasma cell
Fibroblast
Cell-Type
Tissue-Type
Density of Cancer Associated Stroma (CAS)
Total area of necrotic tissue
Proportion of necrotic tissue
Proportion of Fibroblasts in CAS
Density of Lymphocytes in tumor

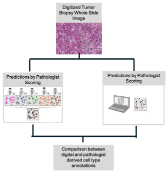
Step1: Train deep learning models to obtain tissue and cell type spatial information

Step 2: Extract HIFs and Model prediction

Step 3: Train classical machine learning models to find relationships with clinical data:HIFs were identified and clustered to reduce dimensionality on pre-treatment CCA patient sample H&Es

Adapted from Diao *et al.* 2021. Human-interpretable image features derived from densely mapped cancer pathology slides predict diverse molecular phenotypes. *Nature Communications*

Figure 2: Model derived predictions of cell types correlated with pathologist consensus labels



Images adapted from Baxi et al., *Association of artificial intelligence-powered and manual quantification of programmed death-ligand 1 (PD-L1) expression with outcomes in patients treated with nivolumab ± ipilimumab*

Predictions in pre-screening CCA tissue sections*

Cell Type	Model predicted count versus pathologist consensus (spearman correlation, ρ, 95% CI)	Each individual pathologist consensus versus the remaining consensus (spearman correlation, ρ, 95% CI)
Cancer cells	0.85 [0.81, 0.88]	0.85 [0.82, 0.88]
Plasma cells	0.49 [0.38, 0.58]	0.52 [0.46, 0.58]
Macrophages	0.35 [0.23, 0.45]	0.36 [0.31, 0.41]
Lymphocytes	0.76 [0.7 , 0.81]	0.74 [0.71, 0.77]
Fibroblasts	0.56 [0.46, 0.64]	0.59 [0.54, 0.63]

*240 frames from 77 whole slide images were used for the validation analysis (300X300 pixel size)

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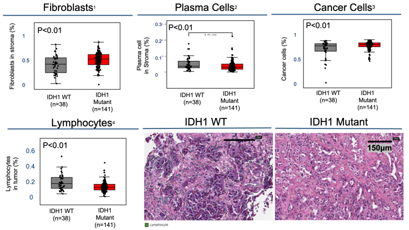
RESULTS

- The multivariate model is able to predict IDH1 mutation status with high accuracy (147/179; 82.1%). 4/10 HIF clusters identified were associated with IDH1 mutations at the baseline after FDR correction.

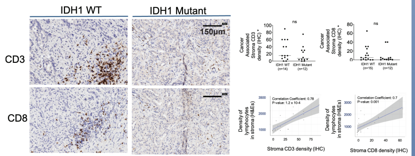
Figure 3: IDH1 mutated CCA have distinctive TME characteristics



HIF clusters with FDR corrected p-value ≤0.05: [1]Fibroblasts in close proximity to immune cells in stroma; [2] Plasma cell features [3] Cancer cell features in tumor [4] Lymphocyte features relative to all cells

- Density of T lymphocytes in stroma detected by CD3 and CD8 immunohistochemistry correlates with the HIF findings on H&E images.

Figure 4: Lower T Lymphocyte infiltration patterns in cancer associated stroma in IDH1 mutated CCA

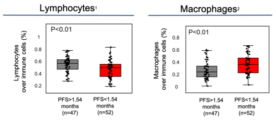


* Entire whole slide images are reviewed. The tumor ROI includes cancer regions and cancer-associated stroma regions per patient slide. Cancer-associated stroma regions: includes both intratumoral and peritumoral stroma. Intratumoral stroma is defined as stromal elements (fibroblasts, immune cells) that are intervening through cancer cells (present as single cells or clusters/nests).Peritumoral stroma is defined as stromal elements (fibroblasts, immune cells) that are contiguous with cancer cells at the edge of the tumor mass.

RESULTS

- 2/9 HIF clusters identified were associated with early disease progression. After correcting for the effect of the drug (AG-120 vs. placebo), higher proportions of lymphocytes throughout the tumor was still significantly associated with improved PFS.

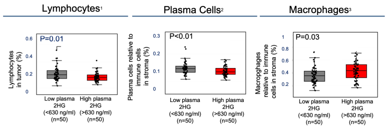
Figure 5: Different immune cell compositions in patients with early disease progression



HIF clusters with FDR corrected p-value <0.05: [1]Lymphocyte features relative to all immune cells; [2] Lymphocyte features relative to all immune cells

- 3/9 HIF clusters identified may be be associated with elevated 2HG levels.

Figure 6: Lower percentage of lymphocytes and plasma cells; higher percentage of macrophages in patients with elevated 2HG



HIF clusters p-value ≤0.03: [1]Lymphocyte features relative to all cells, FDR corrected P value: 0.07 ; [2] Plasma cell features in stroma, FDR corrected P value 0.07; [3]Macrophage features relative to all immune cells.

SUMMARY AND CONCLUSIONS

- Machine learning models in CCA were derived and cell type predictions correlated with pathologist consensus labels
- Tumor microenvironment of IDH1m CCA tumors have colder/immune-excluded TME characteristics.
  - Lower lymphocytes (FDR p<0.01) and plasma cells (FDR p<0.05); higher cancer cells (FDR p<0.01) and plasma cells (FDR p<0.05); higher cancer cells (FDR p<0.01) and fibroblasts in stroma (FDR p<0.01) are associated with IDHm.
  - Trend of lower percentages of CD8/CD3 positivity observed in IDH1 mutant cancer-associated stroma regions.
- Higher percentage of lymphocytes relative to all immune cells is associated with PFS longer than 1.54 months (FDR p<0.05), while higher percentage of macrophages relative to all immune cells, is associated with early disease progression (<1.54 months) (FDR p<0.05).
- Elevated 2-HG levels may be correlated with lower proportions of lymphocytes (FDR p=0.07), plasma cells (FDR p=0.07), and higher proportions of macrophages relative to all immune cells (FDR p=0.07).
- Findings from this analysis support exploration of combination with immune checkpoint inhibitors.