Novel and accurate measurement of differential protease activity in diagnosed HCC patients compared to non-HCC cirrhotic patients

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BACKGROUND

Disease Overview[1]

- Hepatocellular carcinoma (HCC) is the most common form of liver cancer (>80%) and one of the leading cause of death in patients with compensated cirrhosis.
- Cirrhosis from any etiology is the strongest risk factor for HCC, with disease burden primarily driven by viral hepatitis (B/C), excessive alcohol consumption and NASH

Clinical Paradigm[1]

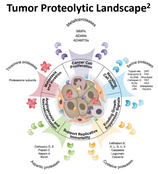
- Surveillance using abdominal ultrasound (US) with or without alpha-fetoprotein (AFP) is recommended in at-risk patients.
- Current modalities have poor sensitivity for detecting HCC at an early stage.
- Early detection of HCC results in a 5-year survival of 60-80% after curative therapies vs. < 10% for HCC detected at late-stage

Suboptimal adherence to routine surveillance and late diagnosis limit treatment options, thus new diagnostic options are needed for at-risk patients

Proteases Linked to Cancer Pathway

- Proteases play mechanistic roles in all the hallmarks of cancer; there are families of proteases that may be part of biological pathways involved in HCC including tumor invasion of extracellular matrix, matrix remodeling, inflammation and fibrinolysis.

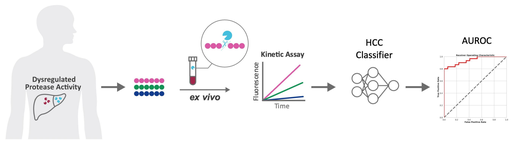
Tumor Proteolytic Landscape[2]



- Metalloproteases target a broad range of extracellular matrix proteins, contributing to cancer development, progression, invasive growth and spread of cancer cells and their elevated activity has so far been detected in almost all types of cancer, including HCC[3]
- Serine proteases, such as dipeptidyl peptidases and kallikreins, are the second largest family of proteolytic enzymes and have shown prognostic value in several types of cancers[4,5]
- Cathepsin C has been reported to maintain malignant biological properties in various cancers and may play an important role in the growth and metastasis of HCC[6]

Ex vivo Platform Development for HCC

Glympse's novel liquid biopsy (LBx) technology uses fluorogenic biosensors and machine learning to sensitively measure protease activity in plasma samples.



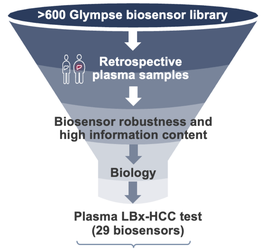
AIM

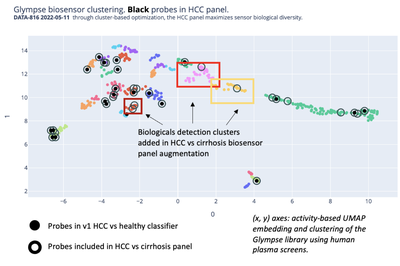
- We previously showed a panel of biosensors that interrogate protease activity in biological pathways implicated in HCC pathogenesis was highly effective at differentiating patients with HCC from healthy controls, with AUCs > 0.94 (Tran et al. EASL 2022, Poster SAT564)
- The aim of this study was to further assess accuracy of protease biosensor biomarker panel to distinguish patients with HCC from those with cirrhosis without HCC

METHOD

- HCC biosensor panel was selected from the full Glympse library using patient-derived plasma samples.
- We created a panel with reliable and biologically diverse detection capabilities by selecting biosensors that balance signal repeatability as measured in HCC vs healthy samples and maximize signal independence of selected probes.
- Biological relevance and coverage of the protease space was determined by cross-referencing protease targets and known biomarkers in HCC and cancer.
- 21 biosensors were nominated for the final panel in the previously conducted analysis with HCC patients and healthy controls .
- CCL4 mouse fibrosis model was used to add 8 new biosensors into our library which could differentiate between fibrosis and HCC, specifically.
- The final panel of 29 biosensors was then applied to the analysis with HCC vs. cirrhosis patients.
- Protease biosensor cleavage was assayed from human plasma by fluorimetry, and the relative signal was used for classification by regularized logistic regression using 100 cross-validation (80% train, 20% validation splits).
- Independent classifier models were developed for each cohort and cross-tested without retraining in the second cohort to assess robustness.

Plasma LBx-HCC Biosensor Selection Process





CONCLUSIONS

- The LBx-HCC classifier leverages a novel platform to non-invasively measure protease activity essential to biological disease processes for HCC development.
- In this pilot biomarker study, the LBx-HCC classifier assay accurately differentiated HCC from cirrhosis.
- If validated in larger studies, the LBx-HCC classifier assay may improve early HCC detection.

REFERENCES

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CONTACT INFORMATION

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RESULTS

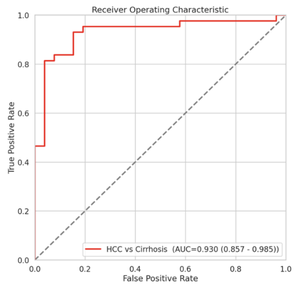
Patent Clinical Characteristics

Plasma samples were obtained from 43 HCC cases and 26 cirrhosis cases and tested using the LBx-HCC assay. Demographics of the 69 patients are summarized below:

	HCC (n=43)	Cirrhosis (n=26)
Gender	28 Male 15 Female	8 Male 18 Female
Mean Age, years	58 ± 15	57 ± 9
Ethnicity, n	26 Asian 17 White	26 White
Etiology, n	21 HBV 3 HCV 19 unknown	26 NASH
Tumor stage (TNM), n	18 Stage 1 13 Stage 2 6 Stage 3 5 Stage 4 1 unknown	N/A

Plasma LBx-HCC Performance HCC vs. Cirrhosis

The panel of protease biosensors had high accuracy for differentiating patients with HCC from those with cirrhosis (AUC 0.930 [CI 0.857-0.985], sensitivity 0.837, specificity 0.846).



Plasma LBx-HCC-Predicted

Histology / Model Prediction	Cirrhosis	HCC
Clinical		
HCC	8	35
Cirrhosis	24	2

Among all 29 biosensors included in the panel, 8 biosensors were most important in differentiating activities between HCC and cirrhosis patients.

HCC Biosensor	Log Ratio Activity	
H8721	-1.737291	
H3636	-0.719729	Suggests cirrhosis activity
H6639	-0.653693	
H6934	-0.481537	
H6938	0.166839	
H7087	0.559350	Suggests HCC activity
H9409	1.000388	
H6521	HCC activity only*	

* Negligible activity in healthy plasma

Biosensor Performance by Clinical Parameters

Plasma LBx-HCC classifier results were evaluated with respect to gender, age range, TNM stage and etiology showing no difference in performance.