# Measurement of Protease Activity Using Novel Plasma Biosensors Can Accurately Detect HCC from Healthy Controls

Tram Tran, Ben Holmes, Christopher Gulka, Anatoly Myaskovsky, Andrea Shepard, Mackenzie Rowe, Sophie Cazanave, Faycal Touti, Jina Lee, Alejandro Balbin, Wendy Winckler

*Glympse Bio, Cambridge, United States*

## BACKGROUND

### Disease Overview[1]

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

### Clinical Paradigm[1]

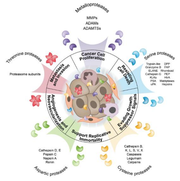
- Surveillance using abdominal ultrasound (US) with or without alpha-fetoprotein (AFP) is recommended in at-risk patients.
- Current modalities have poor sensitivity for detecting HCC at an early stage.
- Early detection of HCC results in a 5-year survival of 60-80% after curative therapies vs. < 10%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

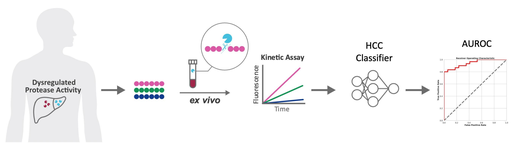
Tumor Proteolytic Landscape[2]



- 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.



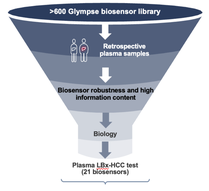

## AIM

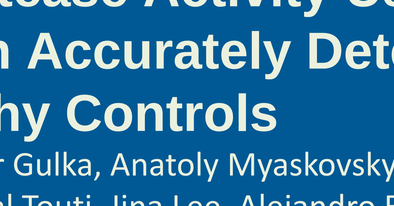
- Develop a panel of Glympse biosensors specific for discrimination of HCC plasma samples from healthy controls.
- Assess protease activity changes in plasma of diagnosed HCC patients compared to healthy controls.
- Develop a highly accurate machine learning classifier to identify patients diagnosed with HCC from healthy controls, using protease activity measured from plasma samples.

## METHOD

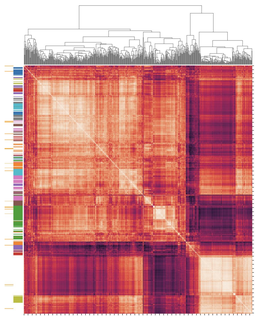
- HCC biosensor panel was selected from the full Glympse library using patient-derived plasma samples.
- We created a panel with reliable and biologically diverse detection capabilities by selecting biosensors that balance signal repeatability as measured in HCC vs healthy samples and maximize signal 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 analysis with HCC patients and healthy controls.
- 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




UMAP projection of correlation matrix into 2D. Clustered probes only. Selected probes represented with black dots.



Clustered correlation matrix for 614 probes

## CONCLUSIONS

- In this study of two independently tested, diverse cohorts of patients with known HCC vs. healthy controls, Glympse's plasma liquid biopsy platform using protease biosensors was highly effective at detecting HCC, with AUCs above 0.94.
- In the future, this technology could be used in surveillance strategies for earlier, easier, and more accurate diagnosis of HCC.

## REFERENCES

1 **Marrero JA et al.** Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68(2):723-750.
2 **Vizovisek M et al.** The Tumor Proteolytic Landscape: A Challenging Frontier in Cancer Diagnosis and Therapy. *Int. J. Mol. Sci.* 2021, 22, 2514. https:// doi.org/10.3390/ijms22052514
3 **Scheau C et al.** The Role of Matrix Metalloproteinases in the Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma. Analytical cellular pathology (Amsterdam), 2019, 9423907. https://doi.org/10.1155/2019/9423907
4. **Boccardi V et al.** Serum CD26 levels in patients with gastric cancer: a novel potential diagnostic marker. *BMC Cancer* 15, 703 (2015). https://doi.org/10.1186/s12885-015-1757-0
5 **De Chiara L et al**. Postoperative Serum Levels of sCD26 for Surveillance in Colorectal Cancer Patients. PLoS ONE 2014, 9(9): e107470. https://doi.org/10.1371/journal.pone.0107470
6 **Zhang GP et al.** Cathepsin C Interacts with TNF-α/p38 MAPK Signaling Pathway to Promote Proliferation and Metastasis in Hepatocellular Carcinoma. *Cancer Res Treat*. 2020;52(1):10-23. doi:10.4143/crt.2019.145

## CONTACT INFORMATION

Tram T. Tran, MD ttran@glympsebio.com

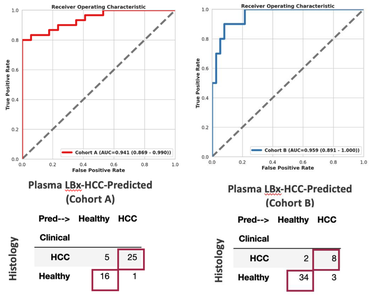
## RESULTS

### Patent Clinical Characteristics

Plasma samples were obtained from two independent cohorts with tissue confirmed diagnosis of HCC and healthy controls and tested using the LBx-HCC assay. Demographics of the 94 patients from both cohorts are summarized below:

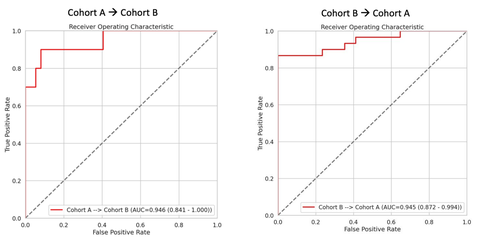
| Cohort A | Healthy (n=17) | HCC (n=30) | Cohort B | Healthy (n=37) | HCC (n=10) |
|---|---|---|---|---|---|
| Gender, n | 10 Male / 7 Female | 23 Male / 7 Female | Gender, n | 24 Male / 13 Female | 5 Male / 5 Female |
| Age, years | 35.8 ± 12.6 | 55.8 ± 9.9 | Age, years | 59.2 ± 9.4 | 59.0 ± 15.4 |
| BMI, kg/m$^2$ | 28.2 ± 4.5 | 22.3 ± 2.3 | BMI, kg/m$^2$ | 25.8 ± 2.9 | 23.6 ± 4.7 |
| Ethnicity, n | 10 White / 4 Black / 3 Asian | 29 Asian / 1 other | Ethnicity, n | 37 White | 9 White / 1 other |
| Etiology, n | N/A | 24 HBV or HCV | Etiology, n | N/A | 0 HBV or HCV |
| Tumor Stage (TNM), n | N/A | 16 Stage 1, 6 Stage 2, 6 Stage 3, 1 Stage 4, 1 unknown | Tumor Stage (TNM), n | N/A | 1 Stage I, 4 Stage 2, 1 Stage 3, 4 Stage 4 |

### Plasma LBx-HCC Performance HCC vs. Healthy




Protease biosensors were highly effective at differentiating between patients with HCC and healthy controls in both cohorts (Cohort A: AUC 0.94 [CI 0.87 – 0.99] and Cohort B: AUC 0.96 [CI 0.89 – 1.00]).

### Plasma LBx-HCC Performance Reproducibility




The AUC remains above 0.94 even when naively applying a pre-trained classifier in one cohort to the other despite the differences in the disease etiology of each cohort.

### Biologically diverse signals were observed in HCC

| HCC Biosensor | P adjusted |
|---|---|
| H4618 | 9.330449e-03 |
| H9409 | 5.780665e-03 |
| H7087 | 5.704095e-05 |
| H5803 | 4.357435e-03 |
| H5798 | 4.326146e-03 |
| H3457 | 4.326146e-03 |
| H8721 | 3.912910e-04 |
| H3636 | 2.770575e-03 |
| H7201 | 2.085539e-10 |
| H5025 | 2.085539e-10 |
| H9844 | 2.079535e-08 |
| H2595 | 1.064813e-03 |
| H6639 | 1.026560e-02 |

Among all 21 biosensors included in the panel, 13 biosensors were most important in differentiating activities between HCC and healthy patients.