# A dual filtration method for single cell transcriptomic analysis of circulating tumor cells and clusters in hepatocellular carcinoma

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## **Introduction and Aims**

- Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death
- Circulating tumor (CT) cells and clusters have prognostic and functional significance in cancer including HCC
- "Liquid biopsy" has attracted interest in obtaining molecular data about HCC, as biopsy is not standard of care
- This is a pilot study evaluating feasibility of single cell transcriptomics on circulating tumor cells and clusters

## Methods

- CT cells/clusters isolated using a dual filtration system designed to capture CT cells and clusters while allowing WBCs and RBCs to pass (Fig. 1).
- Single CT cells/clusters were picked with a micropipette and placed in lysis buffer
- We then conducted single cell RNAseq
- RT/PCR with a modified SmartSeq2 protocol
- Library prep and sequencing at the University of Michigan Advanced Genomics Core with an Illumina MiSeq V3 platform (51 bp, 150 cycles)
- Alignment to transcriptome using STAR
- Downstream analysis using Seurat



Fig. 1. Schematic of dual-filtration system

### Results Total of 8 patients enrolled Median age 64, 7/8 male, 7/8 Caucasian, 7/8 cirrhosis High tumor burden: median tumor size 11.6 cm, BCLC stage C for 7/8 CT cells/clusters found in 6/8 patients. 76 cells/clusters in total Cells fell into two "groups" on UMAP projection (Fig. 2). On single cell transcriptomic analysis, these groups had distinct patterns of gene expression (Fig. 3). Group 1 was enriched for epithelial (EPCAM, ASGR2, KRT8), EMT (CD44), stemness (PROM1 [CD133], POU5F1, *NOTCH1, STAT3*), and proliferation (*CCND1*) genes (p < 0.05 for all). Fig. 3. Expression of candidate genes in Group 1 vs. 2





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Presence of Group 1 CT cells/clusters was associated with decreased overall survival

## **Findings and Next Steps**

- Molecular characterization allowed for poorer prognosis
- stages of HCC and a larger sample size



#### Fig. 4. Overall survival based on CTC status





A dual-filtration system identified CT cells and clusters in most patients with advanced stage HCC identification of a group of cells associated with

Further studies will expand these analyses to earlier

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