Through both global profiling and targeted quantitation, we identified 11 N-glycans that were statistically significant between HCC cases and patients with liver cirrhosis in two cohorts (Egypt and US) as well as two complementary LC-ESI-MS quantitation approaches (global profiling and targeted quantitation).

### Study Population
- The samples in this study were obtained from participants recruited in Egypt and the US.
- The Egyptian participants consist of 89 adult patients (40 HCC cases and 49 patients with liver cirrhosis) recruited at the Tanta University Hospital, Tanta, Egypt (TU cohort; Table 1).
- The US participants are comprised of 94 adult patients (48 HCC cases and 46 patients with liver cirrhosis), recruited at MedStar Georgetown University Hospital, Washington, DC, USA (GU cohort; Table 2).

### Study Design
- Each cohort analyzed in four batches (GU1/GU2, TU1/TU2, TU3/TU4) in the TU cohort; GU1/GU2/GU3/GU4 in the GU cohort.
- Balanced assignment of cases (HCC) and controls (CIR) into each batch in terms of age, race, gender, smoking, alcohol, and BMI.
- Samples within the same batch prepared together and LC-ESI-MS analysis performed following a randomized order to avoid systematic biases.

### LC-MS Profiling of N-Glycans from Human Serum for HCC Biomarker Discovery

#### Candidate N-Glycan Biomarkers

<table>
<thead>
<tr>
<th>Microarray</th>
<th>Color</th>
<th>Quantiation</th>
<th>RT (min)</th>
<th>Annotation</th>
<th>q-value</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[H]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[H]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[H]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### LC-MS Data Acquisition
- **Global profiling**: Thermo Scientific LTQ Orbitrap Velos mass spectrometer coupled to the Ultimate Dionex 3000 HPLC system (Nano LC 350 nL/min).
- **Five MS/MS scans per MS scan on positive mode**.
- **Targeted quantitation**: Thermo Scientific TSQ Vantage mass spectrometer (Q1 and Q3 operated at a unit resolution) coupled to the Ultimate Dionex 3000 HPLC system.
- Cycling time of 213 transition channels was 2.7s on average.

#### LC-MS Data Analysis
- **LC-ESI-MS data by global profiling**: In-house-developed algorithms and open-source software tools were used to preprocess LC-ESI-MS data. Steps include desialylation of mass spectra (DecoTools), peak detection, peak alignment (SIMA) and normalization.
- Peak lists from four batches in each cohort were matched, resulting in 1628 and 1500 common peaks in the TU and GU cohort, respectively.
- ANOVA model-based statistical analysis revealed 18 and 11 significant N-glycans in the TU and GU cohort, respectively (Table 3).
- ANOVA model-based statistical analysis identified 11 and five significant N-glycans in the cohort-specific five MS/MS scans quantitation.

#### Summary
- These glycans can be grouped into four clusters by their structures (Figure 4).
- Through both global profiling and targeted quantitation, we identified 11 N-glycans with statistically significant differences between HCC cases and cirrhotic controls.
- Most of the identified biomarkers were present in the cohort-specific five MS/MS scans quantitation.
- The results of this study demonstrate the power of combining global profiling and targeted quantitation methods for a comprehensive glycomic analysis, to investigate changes in glycan levels between HCC cases and patients with liver cirrhosis in two cohorts.

### Acknowledgment
This work was supported by NIH Grants R01CA143420 and R01GM086746.