

# **Cholangiocarcinoma 2020: the next horizon in mechanisms and management**

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## Supplementary information

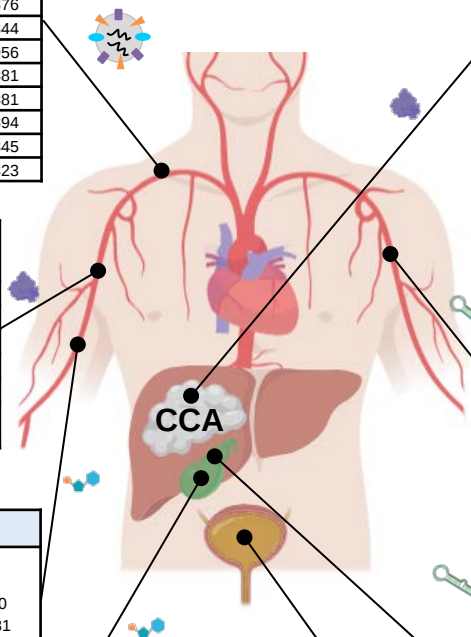
**Supplementary Figure 1 | Diagnostic and prognostic biomarkers for CCA.** Schematic representation of potential biomarkers (i.e. in extracellular vesicles, proteins/cytokines, metabolites and microRNAs) found in serum, bile, urine and tumour tissue with diagnostic and/or prognostic capacity. AUC, area under the curve; CCA, cholangiocarcinoma; EV, extracellular vesicle.

| EV cargo <sup>S1</sup> | Levels | Comparison                            | AUC   |
|------------------------|--------|---------------------------------------|-------|
| AMPN                   | Up     | CCA (n=43) vs healthy controls (n=32) | 0.878 |
| VNN1                   | Up     | CCA (n=43) vs healthy controls (n=32) | 0.876 |
| PIGR                   | Up     | CCA (n=43) vs healthy controls (n=32) | 0.844 |
| FCN2                   | Up     | Early stage CCA (n=13) vs PSC (n=30)  | 0.956 |
| ITIH4                  | Up     | Early stage CCA (n=13) vs PSC (n=30)  | 0.881 |
| FIBG                   | Up     | Early stage CCA (n=13) vs PSC (n=30)  | 0.881 |
| FIBG                   | Up     | iCCA (n=12) vs HCC (n=29)             | 0.894 |
| A1AG1                  | Up     | iCCA (n=12) vs HCC (n=29)             | 0.845 |
| VTDB                   | Up     | iCCA (n=12) vs HCC (n=29)             | 0.823 |

| Protein/<br>Cytokine      | Levels | Comparison                                                      | AUC   |
|---------------------------|--------|-----------------------------------------------------------------|-------|
| MMP7 <sup>S2</sup>        | Up     | CCA (n=44) vs benign biliary tract disease (n=36)               | 0.730 |
| Osteopontin <sup>S3</sup> | Up     | CCA (n=80) vs healthy controls (n=42)                           | 0.964 |
| IL-6 <sup>S4</sup>        | Up     | Bile duct cancer (n=26) vs healthy controls (n=23)              | 0.875 |
| S100A6 <sup>S5</sup>      | Up     | CCA (n=29) vs healthy controls (n=22)                           | 0.909 |
| DKK1 <sup>S6</sup>        | Up     | iCCA (n=37) vs healthy controls (n=50)                          | 0.872 |
| SSP411 <sup>S7</sup>      | Up     | CCA (n=35) vs "choolangitis (n=13) and healthy controls (n=23)" | 0.913 |

| Metabolite <sup>S8</sup> | Levels | Comparison                                        | AUC                      |
|--------------------------|--------|---------------------------------------------------|--------------------------|
| SM(43:2)                 | Up     | iCCA (n=35) vs HCC (n=34)<br><u>Biopsy proven</u> | DIS: 0.900<br>VAL: 0.981 |
| PC(O-16:0/20:3)          | Down   |                                                   |                          |
| PC(O-18:0/18:2)          | Down   |                                                   |                          |
| SM(d18:2/16:0)           | Up     |                                                   |                          |
| Cer(d18:1/16:0)          | Up     |                                                   |                          |
| SM(42:3)                 | Up     | iCCA (n=35) vs PSC (n=35)<br><u>Biopsy proven</u> | DIS: 0.990<br>VAL: 0.995 |
| PC(34:3)                 | Down   |                                                   |                          |
| Histidine                | Down   |                                                   |                          |

| Metabolite                                                           | Levels     | Comparison                                                                                                | AUC                      |
|----------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------|--------------------------|
| Phosphatidylcholine<br>Bile acids<br>Cholesterol/lipid <sup>S9</sup> | Down       | CCA (n=16) vs<br>begin non-PSC biliary diseases (n=27)                                                    | SEN: 88.9%<br>SPE: 87.1% |
| Glycine-conjugated bile acids<br>Phosphatidylcholines <sup>S10</sup> | Up<br>Down | "Inoperable pCCA (n=3) and dCCA (n=2)" vs<br>non-malignant biliary diseases without<br>cholestasis (n=20) | SEN: 80%<br>SPE: 95%     |



| Gene                        | Expression | Overall survival | Recurrence-free survival |
|-----------------------------|------------|------------------|--------------------------|
| KRAS <sup>S11</sup>         | High       | Low              | Low                      |
| TP53 <sup>S11</sup>         | Low        | Low              | Low                      |
| FASCIN/FSCN1 <sup>S12</sup> | High       | Low              | -                        |
| EGFR <sup>S13</sup>         | High       | Low              | -                        |
| MUC1 <sup>S14</sup>         | High       | Low              | -                        |
| MUC4 <sup>S15</sup>         | High       | Low              | -                        |
| CDKN1B/p27 <sup>S16</sup>   | Low        | Low              | -                        |
| MIR21 <sup>S17, S18</sup>   | High       | Low              | Low                      |

| miRNA                               | Levels | Comparison                             | AUC   |
|-------------------------------------|--------|----------------------------------------|-------|
| miR-21 <sup>S17</sup>               | Up     | iCCA (n=74) vs healthy controls (n=74) | 0.908 |
| miR-192 <sup>S19</sup>              | Up     | CCA (n=11) vs healthy controls (n=9)   | 0.809 |
| miR-26a <sup>S20</sup>              | Up     | CCA (n=66) vs healthy controls (n=66)  | 0.899 |
| miR-150 <sup>S21</sup>              | Up     | iCCA (n=15) vs healthy controls (n=15) | 0.791 |
| miR-194 & miR-483-5p <sup>S22</sup> | Up     | CCA (n=30) vs healthy controls (n=30)  | 0.810 |
| miR-222 & miR-483-5p <sup>S22</sup> | Up     | CCA (n=30) vs PSC (n=30)               | 0.770 |
| miR-126 <sup>S22</sup>              | Down   | CCA (n=30) vs PSC (n=30)               | 0.870 |
| miR-26a <sup>S22</sup>              | Down   | CCA (n=30) vs PSC (n=30)               | 0.780 |
| miR-30b <sup>S22</sup>              | Down   | CCA (n=30) vs PSC (n=30)               | 0.780 |
| miR-122 <sup>S22</sup>              | Down   | CCA (n=30) vs PSC (n=30)               | 0.650 |
| miR-106a <sup>S23</sup>             | Down   | CCA (n=103) vs healthy controls (n=20) | 0.890 |
| miR-1281 <sup>S24</sup>             | Down   | CCA (n=31) vs PSC (n=40)               | 0.830 |

| miRNA                   | Levels | Comparison                                                 | AUC   |
|-------------------------|--------|------------------------------------------------------------|-------|
| miR-640 <sup>S24</sup>  | Up     | PSC-CCA (n=12) vs PSC (n=52)                               | 0.810 |
| miR-412 <sup>S24</sup>  | Up     | PSC-CCA (n=12) vs PSC (n=52)                               | 0.810 |
| miR-1537 <sup>S24</sup> | Up     | PSC-CCA (n=12) vs PSC (n=52)                               | 0.780 |
| miR-3189 <sup>S24</sup> | Up     | PSC-CCA (n=12) vs PSC (n=52)                               | 0.800 |
| miR-9 <sup>S25</sup>    | Up     | Biliary tract cancer (n=9) vs<br>choledocholithiasis (n=9) | 0.975 |
| miR-145 <sup>S25</sup>  | Up     | Biliary tract cancer (n=9) vs<br>choledocholithiasis (n=9) | 0.975 |

| miRNA                           | Levels | Comparison                            | AUC   |
|---------------------------------|--------|---------------------------------------|-------|
| miR-21 & miR-192 <sup>S26</sup> | Up     | CCA (n=22) vs healthy controls (n=21) | 0.849 |

**Supplementary Table 1 |Selected ongoing and completed clinical trials of therapeutic agents for cholangiocarcinoma**

| Clinical trial name     | Study arms                                       | <i>n</i>                   | Primary endpoint                                                                                       | Secondary endpoint                                                      |
|-------------------------|--------------------------------------------------|----------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| BCAT                    | Observation alone vs gemcitabine                 | 225 (all extrahepatic CCA) | Overall survival; no differences                                                                       | No benefit in RFS; no differences based on lymph node and margin status |
| PRODIGE-12              | Observation alone vs gemcitabine and oxaliplatin | 186 (156 CCA)              | RFS and HRQOL: no differences in RFS or time to deterioration of HRQOL                                 | Overall survival was not different between arms                         |
| BILCAP                  | Observation alone vs capecitabine                | 447 (368 CCA)              | Overall survival; benefit showed in the pre-specified sensitivity analysis (HR, 0.71; <i>P</i> = 0.01) | RFS HR 0.75, <i>P</i> = 0.033                                           |
| ACTICCA-1 (NCT02170090) | Observation alone vs cisplatin and gemcitabine   | Target: 781; ongoing       | Disease-free survival at 24 months                                                                     | —                                                                       |

CCA, cholangiocarcinoma; HRQOL, health-related quality of life; HR, hazard ratio; RFS, relapse-free survival.

## Supplementary References

- S1 Arbelaiz, A. *et al.* Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. *Hepatology* **66**, 1125-1143, doi:10.1002/hep.29291 (2017).
- S2 Leelawat, K., Sakchinabut, S., Narong, S. & Wannaprasert, J. Detection of serum MMP-7 and MMP-9 in cholangiocarcinoma patients: evaluation of diagnostic accuracy. *BMC Gastroenterol* **9**, 30, doi:10.1186/1471-230X-9-30 (2009).
- S3 Loosen, S. H. *et al.* Elevated levels of circulating osteopontin are associated with a poor survival after resection of cholangiocarcinoma. *J Hepatol* **67**, 749-757, doi:10.1016/j.jhep.2017.06.020 (2017).
- S4 Cheon, Y. K. *et al.* Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* **102**, 2164-2170, doi:10.1111/j.1572-0241.2007.01403.x (2007).
- S5 Onsurathum, S. *et al.* Proteomics detection of S100A6 in tumor tissue interstitial fluid and evaluation of its potential as a biomarker of cholangiocarcinoma. *Tumour Biol* **40**, 1010428318767195, doi:10.1177/1010428318767195 (2018).
- S6 Shi, R. Y. *et al.* High expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery. *Cancer* **119**, 993-1003, doi:10.1002/cncr.27788 (2013).
- S7 Shen, J. *et al.* Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma. *PLoS One* **7**, e47476, doi:10.1371/journal.pone.0047476 (2012).
- S8 Banales, J. M. *et al.* Serum Metabolites as Diagnostic Biomarkers for Cholangiocarcinoma, Hepatocellular Carcinoma, and Primary Sclerosing Cholangitis. *Hepatology*, doi:10.1002/hep.30319 (2018).
- S9 Albiin, N. *et al.* Detection of cholangiocarcinoma with magnetic resonance spectroscopy of bile in patients with and without primary sclerosing cholangitis. *Acta Radiol* **49**, 855-862, doi:10.1080/02841850802220092 (2008).
- S10 Sharif, A. W. *et al.* Metabolic profiling of bile in cholangiocarcinoma using in vitro magnetic resonance spectroscopy. *HPB (Oxford)* **12**, 396-402, doi:10.1111/j.1477-2574.2010.00185.x (2010).
- S11 Nepal, C. *et al.* Genomic perturbations reveal distinct regulatory networks in intrahepatic cholangiocarcinoma. *Hepatology* **68**, 949-963, doi:10.1002/hep.29764 (2018).
- S12 Mao, X. *et al.* Differential expression of fascin, E-cadherin and vimentin: Proteins associated with survival of cholangiocarcinoma patients. *Am J Med Sci* **346**, 261-268, doi:10.1097/MAJ.0b013e3182707108 (2013).
- S13 Yoshikawa, D. *et al.* Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br J Cancer* **98**, 418-425, doi:10.1038/sj.bjc.6604129 (2008).
- S14 Boonla, C. *et al.* MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. *World J Gastroenterol* **11**, 4939-4946, doi:10.3748/wjg.v11.i32.4939 (2005).
- S15 Shibahara, H. *et al.* MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology* **39**, 220-229, doi:10.1002/hep.20031 (2004).
- S16 Fiorentino, M. *et al.* Low p27 expression is an independent predictor of survival for patients with either hilar or peripheral intrahepatic cholangiocarcinoma. *Clin Cancer Res* **7**, 3994-3999 (2001).
- S17 Wang, L. J. *et al.* MiR-21 promotes intrahepatic cholangiocarcinoma proliferation and growth in vitro and in vivo by targeting PTPN14 and PTEN. *Oncotarget* **6**, 5932-5946, doi:10.18632/oncotarget.3465 (2015).
- S18 Chusorn, P. *et al.* Overexpression of microRNA-21 regulating PDCD4 during tumorigenesis of liver fluke-associated cholangiocarcinoma contributes to tumor growth and metastasis. *Tumour Biol* **34**, 1579-1588, doi:10.1007/s13277-013-0688-0 (2013).
- S19 Silakit, R. *et al.* Circulating miR-192 in liver fluke-associated cholangiocarcinoma patients: a prospective prognostic indicator. *J Hepatobiliary Pancreat Sci* **21**, 864-872, doi:10.1002/jhbp.145 (2014).
- S20 Wang, L. J. *et al.* Serum miR-26a as a diagnostic and prognostic biomarker in cholangiocarcinoma. *Oncotarget* **6**, 18631-18640, doi:10.18632/oncotarget.4072 (2015).
- S21 Wang, S. *et al.* Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma. *Oncol Rep* **33**, 819-825, doi:10.3892/or.2014.3641 (2015).
- S22 Bernuzzi, F. *et al.* Serum microRNAs as novel biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. *Clin Exp Immunol* **185**, 61-71, doi:10.1111/cei.12776 (2016).
- S23 Cheng, Q. *et al.* Circulating miR-106a is a Novel Prognostic and Lymph Node Metastasis Indicator for Cholangiocarcinoma. *Sci Rep* **5**, 16103, doi:10.1038/srep16103 (2015).

- S24 Voigtlander, T. *et al.* MicroRNAs in Serum and Bile of Patients with Primary Sclerosing Cholangitis and/or Cholangiocarcinoma. *PLoS One* **10**, e0139305, doi:10.1371/journal.pone.0139305 (2015).
- S25 Shigehara, K. *et al.* Real-time PCR-based analysis of the human bile microRNAome identifies miR-9 as a potential diagnostic biomarker for biliary tract cancer. *PLoS One* **6**, e23584, doi:10.1371/journal.pone.0023584 (2011).
- S26 Silakit, R. *et al.* Urinary microRNA-192 and microRNA-21 as potential indicators for liver fluke-associated cholangiocarcinoma risk group. *Parasitol Int* **66**, 479-485, doi:10.1016/j.parint.2015.10.001 (2017).