# Supplementary table S1: Full list of proteins in the Olink immuno-oncology assay

|  |  |  |
| --- | --- | --- |
| **Abbreviated protein names** | **Protein names** | **UniProt ID** |
| ADA | Adenosine deaminase | P00813 |
| ADGRG1 | Adhesion G-protein coupled receptor G1 | Q9Y653 |
| ANG-1 | Angiopoietin-1 | Q15389 |
| ANGPT2 | Angiopoietin-2 | O15123 |
| ARG1 | Arginase-1 | P05089 |
| CAIX | Carbonic anhydrase IX (CA9) | Q16790 |
| CASP-8 | Caspase-8 | Q14790 |
| CCL3 | C-C motif chemokine 3 | P10147 |
| CCL4 | C-C motif chemokine 4 | P13236 |
| CCL17 | C-C motif chemokine 17 | Q92583 |
| CCL19 | C-C motif chemokine 19 | Q99731 |
| CCL20 | C-C motif chemokine 20 | P78556 |
| CCL23 | C-C motif chemokine 23 | P55773 |
| CD4 | T cell surface glycoprotein CD4 | P01730 |
| CD5 | T cell surface glycoprotein CD5 | P06127 |
| CD8A | T cell surface glycoprotein CD8 alpha chain | P01732 |
| CD27 | CD27 antigen | P26842 |
| CD28 | T cell-specific surface glycoprotein CD28 | P10747 |
| CD40 | CD40L receptor | P25942 |
| CD40-L | CD40 ligand | P29965 |
| CD70 | CD70 antigen | P32970 |
| CD83 | CD83 antigen | Q01151 |
| CD244 | Natural killer cell receptor 2B4 | Q9BZW8 |
| CRTAM | Cytotoxic and regulatory T cell molecule | O95727 |
| CSF-1 | Macrophage colony-stimulating factor 1 | P09603 |
| CX3CL1 | Fractalkine | P78423 |
| CXCL1 | C-X-C motif chemokine 1 | P09341 |
| CXCL5 | C-X-C motif chemokine 5 | P42830 |
| CXCL9 | C-X-C motif chemokine 9 | Q07325 |
| CXCL10 | C-X-C motif chemokine 10 | P02778 |
| CXCL11 | C-X-C motif chemokine 11 | O14625 |
| CXCL12 | Stromal cell-derived factor 1 | P48061 |
| CXCL13 | C-X-C motif chemokine 13 | O43927 |
| DCN | Decorin | P07585 |
| EGF | Pro-epidermal growth factor | P01133 |
| FASLG | Fas Ligand/Tumor necrosis factor ligand superfamily member 6 | P48023 |
| FGF2 | Fibroblast growth factor 2 | P09038 |
| Gal-1 | Galectin-1 | P09382 |
| Gal-9 | Galectin-9 | O00182 |
| GZMA | Granzyme A | P12544 |
| GZMB | Granzyme B | P10144 |
| GZMH | Granzyme H | P20718 |
| HGF | Hepatocyte growth factor | P14210 |
| HO-1 | Heme oxygenase 1 | P09601 |
| ICOSLG | ICOS ligand | O75144 |
| IFN-beta | Interferon beta | P01574 |
| IFN-gamma | Interferon gamma | P01579 |
| IL-1 alpha | Interleukin-1 alpha | P01583 |
| IL-2 | Interleukin-2 | P60568 |
| IL-4 | Interleukin-4 | P05112 |
| IL-5 | Interleukin-5 | P05113 |
| IL-6 | Interleukin-6 | P05231 |
| IL-7 | Interleukin-7 | P13232 |
| IL-8 | Interleukin 8 | P10145 |
| IL-10 | Interleukin-10 | P22301 |
| IL-12 | Interleukin-12 | P29459, P29460 |
| IL-12RB1 | Interleukin-12 receptor subunit beta-1 | P42701 |
| IL-13 | Interleukin-13 | P35225 |
| IL-18 | Interleukin-18 | Q14116 |
| IL-21 | Interleukin-21 | Q9HBE4 |
| IL-33 | Interleukin-33 | O95760 |
| IL-35 | Interleukin-35 | Q14213, P29459 |
| KLRD1 | Natural killer cells antigen CD94 | Q13241 |
| LAMP3 | Lysosome-associated membrane glycoprotein 3 | Q9UQV4 |
| LAP TGF-beta-1 | Latency-associated peptide transforming growth factor beta-1 | P01137 |
| MCP-1 | Monocyte chemotactic protein 1 | P13500 |
| MCP-2 | Monocyte chemotactic protein 2 | P80075 |
| MCP-3 | Monocyte chemotactic protein 3 | P80098 |
| MCP-4 | Monocyte chemotactic protein 4 | Q99616 |
| MIC-A/B | MHC class I polypeptide-related sequence A/B | Q29983, Q29980 |
| MMP-7 | Matrix metalloproteinase-7 | P09237 |
| MMP-12 | Macrophage metalloproteinase-12 | P39900 |
| NCR1 | Natural cytotoxicity triggering receptor | O76036 |
| NOS3 | Nitric oxide synthase, endothelia | P29474 |
| PDCD1 | Programmed cell death protein 1 | Q15116 |
| PDGF subunit B | Platelet-derived growth factor subunit B | P01127 |
| PD-L1 | Programmed cell death 1 ligand 1 | Q9NZQ7 |
| PD-L2 | Programmed cell death 1 ligand 2 | Q9BQ51 |
| PGF | Placenta growth factor | P49763 |
| PTN | Pleiotrophin | P21246 |
| TIE2 | Angiopoietin-1 receptor | Q02763 |
| TNF | Tumor necrosis factor | P01375 |
| TNFRSF4 | Tumor necrosis factor receptor superfamily member 4 | P43489 |
| TNFRSF9 | Tumor necrosis factor receptor superfamily member 9 | Q07011 |
| TNFRSF12A | Tumor necrosis factor receptor superfamily member 12A | Q9NP84 |
| TNFRSF21 | Tumor necrosis factor receptor superfamily member 21 | O75509 |
| TNFSF14 | Tumor necrosis factor ligand superfamily member 14 | O43557 |
| TRAIL | TNF-related apoptosis-inducing ligand | P50591 |
| TWEAK | Tumor necrosis factor ligand superfamily member 12 | O43508 |
| VEGFA | Vascular endothelial growth factor A | P15692 |
| VEGFC | Vascular endothelial growth factor C | P49767 |
| VEGFR-2 | Vascular endothelial growth factor receptor 2 | P35968 |

# Supplementary table S2: Differential protein expression, pancreatic ductal adenocarcinoma (PDAC) vs. non-PDAC. Proteins listed according to decreasing levels of significance

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Protein** | **Test** | **P value** | **PDAC median** | **Non-PDAC median** | **Log2 fold change** |
| CA19-9\* | wilcox test | 5.26×10−55 | 0.308402 | −0.94128 | 1.304516 |
| IL-8 | wilcox test | 1.54×10−45 | 0.091516 | −0.96186 | 1.093563 |
| MMP7 | wilcox test | 9.83×10−37 | 0.447012 | −0.89977 | 1.158354 |
| CCL20 | wilcox test | 1.42×10−32 | 0.121156 | −0.81746 | 0.94427 |
| MCP-3 | wilcox test | 4.15×10−32 | 0.225239 | −0.7784 | 0.938723 |
| IL-10 | wilcox test | 9.3×10−32 | 0.053722 | −0.69331 | 0.808997 |
| IL-6 | wilcox test | 2.7×10−31 | 0.151808 | −0.80065 | 0.933419 |
| HGF | wilcox test | 5.65×10−31 | 0.120868 | −0.73483 | 0.928418 |
| CSF-1 | wilcox test | 6.48×10−30 | 0.296869 | −0.84281 | 1.005507 |
| CASP-8 | wilcox test | 2.91×10−28 | 0.238728 | −1.05129 | 1.003495 |
| MMP12 | wilcox test | 4.64×10−28 | 0.146237 | −0.78247 | 0.871114 |
| Gal-9 | wilcox test | 3.17×10−26 | 0.253217 | −0.64799 | 0.892485 |
| CXCL1 | t test | 3.52×10−24 | 0.189632 | −0.61333 | 0.816986 |
| CCL3 | wilcox test | 8.17×10−24 | 0.120932 | −0.76394 | 0.787062 |
| CCL23 | t test | 4.7×10−23 | 0.159104 | −0.56111 | 0.755342 |
| TNFRSF9 | wilcox test | 5.65×10−21 | 0.164428 | −0.67214 | 0.780979 |
| CXCL9 | wilcox test | 3.01×10−20 | 0.051845 | −0.6923 | 0.761595 |
| FASLG | wilcox test | 1.75×10−19 | −0.22568 | 0.70794 | −0.76841 |
| CD40 | wilcox test | 3.8×10−19 | 0.012528 | −0.6456 | 0.734121 |
| PD-L2 | wilcox test | 5.35×10−19 | 0.202642 | −0.51294 | 0.652796 |
| TRAIL | wilcox test | 9.03×10−18 | −0.21998 | 0.346287 | −0.64747 |
| ADGRG1 | wilcox test | 1.89×10−17 | −0.09893 | −0.67207 | 0.617235 |
| PGF | wilcox test | 2.39×10−17 | 0.095158 | −0.60901 | 0.675727 |
| CD4 | wilcox test | 4.25×10−17 | 0.108487 | −0.6016 | 0.635864 |
| CXCL13 | wilcox test | 1.87×10−16 | 0.103767 | −0.54301 | 0.687041 |
| LAP TGF-beta-1 | wilcox test | 1.04×10−14 | 0.203885 | −0.48381 | 0.649817 |
| ANGPT2 | wilcox test | 3.53×10−14 | 0.143648 | −0.3884 | 0.685824 |
| IL-21 | wilcox test | 7.39×10−14 | 0.131842 | −0.40402 | 0.515412 |
| TNFRSF12A | wilcox test | 8.28×10−14 | 0.062717 | −0.56729 | 0.664295 |
| VEGFA | wilcox test | 1.19×10−13 | 0.089603 | −0.50448 | 0.650807 |
| PD-L1 | wilcox test | 3.91×10−13 | 0.057446 | −0.57906 | 0.483153 |
| CAIX | wilcox test | 5.84×10−13 | −0.0323 | −0.49281 | 0.613624 |
| IL-18 | wilcox test | 1.06×10−12 | 0.114047 | −0.50233 | 0.585666 |
| GZMH | wilcox test | 1.03×10−12 | −0.0297 | −0.54106 | 0.575067 |
| NOS3 | wilcox test | 1.29×10−12 | 0.006415 | −0.48528 | 0.569917 |
| TNFRSF4 | wilcox test | 1.83×10−12 | 0.123013 | −0.50413 | 0.578457 |
| CRTAM | wilcox test | 3.68×10−12 | 0.154801 | −0.41728 | 0.60335 |
| CXCL11 | wilcox test | 4.38×10−12 | 0.02364 | −0.49927 | 0.583937 |
| IL-12RB1 | wilcox test | 7.96×10−11 | 0.05717 | −0.44072 | 0.554226 |
| IL-7 | t test | 1.04×10−10 | 0.17827 | −0.35377 | 0.547567 |
| CCL19 | wilcox test | 1.87×10−10 | −0.00131 | −0.6225 | 0.483045 |
| PTN | wilcox test | 2.11×10−10 | 0.083658 | −0.4312 | 0.513767 |
| MIC-A/B | wilcox test | 7.81×10−10 | 0.352287 | 0.062353 | 0.335938 |
| CD27 | wilcox test | 9.32×10−10 | 0.117753 | −0.48629 | 0.517631 |
| VEGFC | wilcox test | 2.72×10−09 | −0.0842 | 0.441678 | −0.42411 |
| TNFSF14 | t test | 6.73×10−09 | 0.153799 | −0.32217 | 0.51632 |
| TIE2 | t test | 1.66×10−08 | 0.04053 | −0.32166 | 0.476013 |
| KLRD1 | wilcox test | 1.72×10−08 | −0.00923 | −0.43463 | 0.513921 |
| IFN-beta | wilcox test | 1.82×10−08 | −0.10137 | −0.39853 | 0.310112 |
| CX3CL1 | wilcox test | 3.4×10−08 | 0.095325 | −0.33484 | 0.464175 |
| IL-33 | wilcox test | 4.58×10−08 | 0.102935 | −0.38932 | 0.477706 |
| CXCL10 | wilcox test | 4.79×10−08 | −0.02226 | −0.49577 | 0.479347 |
| GZMA | wilcox test | 8.47×10−08 | 0.040437 | −0.36495 | 0.472178 |
| PDCD1 | wilcox test | 1.13×10−07 | 0.028748 | −0.36628 | 0.39726 |
| CCL4 | wilcox test | 1.75×10−07 | 0.052624 | −0.331 | 0.39821 |
| CD83 | wilcox test | 5.49×10−07 | 0.061213 | −0.39254 | 0.423451 |
| CXCL5 | wilcox test | 2.25×10−06 | 0.26047 | −0.22391 | 0.365547 |
| IL-35 | wilcox test | 2.43×10−06 | 0.220433 | −0.22149 | 0.307746 |
| TNFRSF21 | t test | 2.99×10−06 | 0.123641 | −0.2714 | 0.377984 |
| MCP-1 | t test | 3.58×10−06 | 0.086295 | −0.24222 | 0.367079 |
| TWEAK | wilcox test | 4.46×10−06 | −0.1177 | 0.189994 | −0.37713 |
| NCR1 | t test | 5.81×10−06 | 0.144555 | −0.22233 | 0.37184 |
| CD40-L | wilcox test | 2.38×10−05 | 0.147358 | 0.445795 | −0.18184 |
| IFN-gamma | wilcox test | 9.64×10−05 | −0.08808 | −0.29124 | 0.347587 |
| CD70 | wilcox test | 9.83×10−05 | 0.033643 | −0.26994 | 0.354561 |
| CD28 | wilcox test | 0.000534 | −0.07327 | −0.31103 | 0.23914 |
| IL-12 | t test | 0.000729 | 0.079618 | −0.16404 | 0.265183 |
| FGF2 | wilcox test | 0.000754 | −0.14426 | −0.3569 | 0.292939 |
| CXCL12 | wilcox test | 0.001194 | 0.043263 | −0.21577 | 0.271397 |
| DCN | wilcox test | 0.002971 | −0.02301 | −0.21009 | 0.252464 |
| IL-4 | wilcox test | 0.00692 | −0.08742 | −0.24157 | 0.189975 |
| PDGF subunit B | wilcox test | 0.009829 | −0.01674 | 0.125728 | −0.11774 |
| ANG-1 | wilcox test | 0.009997 | 0.144387 | 0.283687 | −0.12392 |
| ADA | wilcox test | 0.012402 | −0.11571 | −0.2109 | 0.286198 |
| VEGFR-2 | wilcox test | 0.019139 | −0.06393 | 0.166679 | −0.20215 |
| TNF | wilcox test | 0.022707 | −0.17845 | −0.28374 | 0.128192 |
| CD8A | wilcox test | 0.026233 | 0.03082 | −0.08207 | 0.125056 |
| CCL17 | wilcox test | 0.046532 | 0.11951 | −0.03425 | 0.146603 |
| EGF | wilcox test | 0.069616 | 0.194952 | 0.355163 | −0.00558 |
| LAMP3 | wilcox test | 0.15295 | −0.03888 | −0.12542 | 0.129346 |
| ARG1 | wilcox test | 0.169932 | −0.04719 | −0.22711 | 0.126799 |
| GZMB | wilcox test | 0.177768 | −0.08974 | −0.18815 | 0.117186 |
| IL-13 | wilcox test | 0.201563 | −0.06659 | −0.0488 | −0.03651 |
| MCP-2 | wilcox test | 0.214124 | 0.105157 | 0.048232 | 0.102853 |
| CD5 | wilcox test | 0.243649 | −0.05615 | −0.14034 | 0.133328 |
| IL-1 alpha | wilcox test | 0.331271 | −0.13943 | −0.12605 | −0.1551 |
| Gal-1 | t test | 0.346793 | −0.01261 | −0.07694 | 0.07657 |
| MCP-4 | t test | 0.468688 | −0.05495 | −0.09023 | 0.061937 |
| IL-5 | wilcox test | 0.571379 | −0.29277 | −0.31719 | 0.087298 |
| ICOSLG | wilcox test | 0.581425 | −0.02269 | −0.0498 | −0.07876 |
| IL-2 | wilcox test | 0.729894 | 0.001159 | 0.054996 | −0.02002 |
| CD244 | wilcox test | 0.795224 | −0.06162 | −0.06536 | 0.05564 |
| HO-1 | wilcox test | 0.945059 | −0.06608 | −0.00039 | −0.01877 |

\*CA19-9 is a tetrasaccharide and not a protein.

**Abbreviations:** wilcox test – Wilcoxon rank sum test

# Supplementary table S3: Candidate circulating protein signatures for Index I with associated proportion scores. Only signatures with <25 proteins are shown. Signature 14 is Index I

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Abbreviated protein names** | **Signatures** | | | | | | | | | | | | |
| **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** |
| **ADRG1** | X | X | X | X |  |  |  |  |  |  |  |  |  |
| **CASP-8** | X | X | X | X | X | X |  |  |  |  |  |  |  |
| **CCL3** | X | X |  |  |  |  |  |  |  |  |  |  |  |
| **CD4** | X | X | X | X | X | X | X | **X** |  |  |  |  |  |
| **CD40** | X |  |  |  |  |  |  |  |  |  |  |  |  |
| **CD40-L** | X | X | X | X | X |  |  |  |  |  |  |  |  |
| **CRTAM** | X | X | X | X | X | X | X | **X** |  |  |  |  |  |
| **CXCL1** | X | X | X |  |  |  |  |  |  |  |  |  |  |
| **FASLG** | X | X | X | X | X | X | X | **X** | X | X | X | X |  |
| **IL-8** | X | X | X | X | X | X | X | **X** | X |  |  |  |  |
| **IL-10** | X | X | X | X | X | X | X | **X** | X | X |  |  |  |
| **IL-21** | X | X | X | X | X |  |  |  |  |  |  |  |  |
| **LAP TGF-beta-1** | X | X | X | X | X |  |  |  |  |  |  |  |  |
| **MCP-3** | X | X | X | X | X | X | X | **X** | X | X | X |  |  |
| **MMP7** | X | X | X | X | X | X | X | **X** | X | X | X |  |  |
| **MMP12** | X | X | X |  |  |  |  |  |  |  |  |  |  |
| **PDGF subunit B** | X | X |  |  |  |  |  |  |  |  |  |  |  |
| **PD-L2** | X | X | X | X | X | X | X |  |  |  |  |  |  |
| **TRAIL** | X | X | X | X | X | X | X | **X** | X | X | X | X | X |
| **VEGFC** | X | X | X | X | X | X | X | **X** | X | X | X | X |  |
| **CA19-9** | X | X | X | X | X | X | X | **X** | X | X | X | X | X |
| **Proteins (n)** | **21** | **20** | **18** | **16** | **15** | **12** | **11** | **10** | **8** | **7** | **6** | **4** | **2** |
| **Proportion score** | **0.3** | **0.35** | **0.4** | **0.45** | **0.5** | **0.55** | **0.6** | **0.65** | **0.75** | **0.8** | **0.85** | **0.9** | **1.0** |

# Supplementary table S4: Results for all 19 candidate signatures for Index I, 95% CIs are provided in parentheses

a) Discovery cohort

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Signa ture** | **Proportion score** | **Number of proteins** | **AUC** | **BPse** | **BPsp** | **PPV** | **NPV** | **BPt** | **FSEse** | **FSEsp** | **FSEt** | **FSPse** | **FSPsp** | **FSPt** |
| **1** | 0 | 78 | 0.92 (0.88–0.95) | 0.87 (0.77–0.96) | 0.80 (0.70–0.94) | 0.92 (0.89–0.97) | 0.71 (0.59–0.89) | 0.67 | 0.95 (0.92–0.97) | 0.70 (0.61–0.79) | 0.46 | 0.58 (0.52–0.64) | 0.95 (0.91–0.98) | 0.89 |
| **2** | 0.05 | 62 | 0.92 (0.89–0.95) | 0.85 (0.79–0.96) | 0.85 (0.74–0.94) | 0.93 (0.90–0.97) | 0.69 (0.62–0.87) | 0.70 | 0.95 (0.92–0.97) | 0.70 (0.61–0.79) | 0.44 | 0.62 (0.55–0.68) | 0.95 (0.91–0.98) | 0.87 |
| **3** | 0.1 | 53 | 0.92 (0.89–0.95) | 0.84 (0.80–0.96) | 0.86 (0.73–0.93) | 0.94 (0.90–0.97) | 0.68 (0.62–0.88) | 0.71 | 0.96 (0.93–0.98) | 0.69 (0.59–0.78) | 0.38 | 0.64 (0.57–0.70) | 0.95 (0.91–0.98) | 0.88 |
| **4** | 0.15 | 36 | 0.92 (0.89–0.95) | 0.88 (0.79–0.97)0.88 (0.79–0.97) | 0.80 (0.70–0.91) | 0.92 (0.89–0.96) | 0.72 (0.62–0.90) | 0.60 | 0.95 (0.92–0.97) | 0.70 (0.60–0.79) | 0.41 | 0.61 (0.55–0.67) | 0.96 (0.92–1.0) | 0.87 |
| **5** | 0.2 | 33 | 0.92 (0.89–0.95) | 0.93 (0.78–0.97) | 0.76 (0.70–0.92) | 0.91 (0.89–0.96) | 0.81 (0.60–0.90) | 0.50 | 0.95 (0.92–0.97) | 0.69 (0.60–0.79) | 0.37 | 0.60 (0.54–0.66) | 0.97 (0.94–1.0) | 0.89 |
| **6** | 0.25 | 27 | 0.92 (0.89–0.95) | 0.89 (0.80–0.97) | 0.80 (0.70–0.91) | 0.92 (0.89–0.96) | 0.74 (0.63–0.91) | 0.63 | 0.96 (0.93–0.98) | 0.69 (0.60–0.78) | 0.33 | 0.61 (0.55–0.68) | 0.96 (0.92–1.0) | 0.89 |
| **7** | 0.3 | 21 | 0.92 (0.89–0.95) | 0.90 (0.80–0.97) | 0.79 (0.70–0.91) | 0.92 (0.89–0.96) | 0.75 (0.62–0.92) | 0.59 | 0.97 (0.94–0.99) | 0.68 (0.58–0.78) | 0.31 | 0.62 (0.56–0.68) | 0.95 (0.91–0.98) | 0.89 |
| **8** | 0.35 | 20 | 0.92 (0.89–0.95) | 0.90 (0.79–0.97) | 0.79 (0.70–0.92) | 0.92 (0.89–0.967) | 0.75 (0.61–0.91) | 0.59 | 0.97 (0.94–0.99) | 0.68 (0.58–0.78) | 0.31 | 0.62 (0.56–0.68) | 0.95 (0.91–0.98) | 0.89 |
| **9** | 0.4 | 18 | 0.92 (0.89–0.95) | 0.91 (0.83–0.97) | 0.79 (0.70–0.89) | 0.92 (0.89–0.95) | 0.77 (0.65–0.93) | 0.54 | 0.97 (0.95–0.99) | 0.67 (0.58–0.77) | 0.29 | 0.60 (0.53–0.66) | 0.96 (0.93–1.0) | 0.89 |
| **10** | 0.45 | 16 | 0.92 (0.89–0.96) | 0.92 (0.81–0.99) | 0.78 (0.67–0.91) | 0.91 (0.88–0.96) | 0.80 (0.64–0.98) | 0.51 | 0.97 (0.94–0.99) | 0.70 (0.60–0.79) | 0.33 | 0.58 (0.52–0.64) | 0.96 (0.92–1.0) | 0.90 |
| **11** | 0.5 | 15 | 0.93 (0.89–0.96) | 0.91 (0.80–0.99) | 0.79 (0.70–0.92) | 0.92 (0.89–0.96) | 0.77 (0.63–0.96) | 0.59 | 0.95 (0.93–0.97) | 0.71 (0.62–0.80) | 0.37 | 0.58 (0.52–0.64) | 0.96 (0.92–1.0) | 0.90 |
| **12** | 0.55 | 12 | 0.93 (0.89–0.96) | 0.93 (0.79–0.99) | 0.77 (0.69–0.92) | 0.91 (0.89–0.96) | 0.81 (0.62–0.97) | 0.495 | 0.98 (0.96–0.99) | 0.70 (0.60–0.79) | 0.34 | 0.55 (0.50–0.62) | 0.96 (0.92–1.0) | 0.89 |
| **13** | 0.6 | 11 | 0.93 (0.90–0.96) | 0.93 (0.79–0.99) | 0.77 (0.69–0.92) | 0.91 (0.89–0.96) | 0.82 (0.62–0.97) | 0.47 | 0.97 (0.96–0.99) | 0.70 (0.61–0.79) | 0.34 | 0.56 (0.50–0.62) | 0.96 (0.92–1.0) | 0.89 |
| **14** | 0.65 | 10 | 0.93 (0.89–0.96) | 0.86 (0.82–0.98) | 0.85 (0.71–0.92) | 0.93 (0.89–0.96) | 0.70 (0.65–0.94) | 0.64 | 0.97 (0.94–0.98) | 0.73 (0.62–0.82) | 0.37 | 0.55 (0.49–0.61) | 0.95 (0.91–0.98) | 0.90 |
| **16** | 0.75 | 8 | 0.92 (0.89–0.95) | 0.91 (0.81–0.99) | 0.78 (0.68–0.91) | 0.91 (0.89–0.96) | 0.77 (0.64–0.98) | 0.51 | 0.98 (0.97–1.0) | 0.70 (0.61–0.79) | 0.36 | 0.56 (0.50–0.62) | 0.95 (0.91–0.98) | 0.89 |
| **17** | 0.8 | 7 | 0.92 (0.89–0.96) | 0.92 (0.81–0.98) | 0.79 (0.70–0.91) | 0.92 (0.89–0.96) | 0.79 (0.64–0.94) | 0.48 | 0.96 (0.93–0.98) | 0.75 (0.66–0.84) | 0.41 | 0.55 (0.48–0.61) | 0.96 (0.92–1.0) | 0.90 |
| **18** | 0.85 | 6 | 0.93 (0.90–0.96) | 0.92 (0.89–0.97) | 0.83 (0.74–0.89) | 0.93 (0.90–0.96) | 0.81 (0.74–0.93) | 0.53 | 0.96 (0.93–0.98) | 0.77 (0.68–0.85) | 0.45 | 0.60 (0.54–0.67) | 0.95 (0.91–0.98) | 0.86 |
| **19** | 0.9 | 4 | 0.91 (0.88–0.94) | 0.85 (0.71–0.94) | 0.83 (0.73–0.95) | 0.93 (0.89–0.97) | 0.68 (0.55–0.83) | 0.56 | 0.95 (0.92–0.97) | 0.53 (0.43–0.64) | 0.37 | 0.58 (0.51–0.64) | 0.97 (0.94–1.0) | 0.84 |
| **21** | 1 | 2 | 0.90 (0.86–0.93) | 0.89 (0.69–0.92) | 0.75 (0.70–0.94) | 0.90 (0.88–0.97) | 0.72 (0.52–0.80) | 0.51 | 0.95 (0.92–0.97) | 0.48 (0.38–0.59) | 0.39 | 0.62 (0.56–0.68) | 0.95 (0.91–0.98) | 0.80 |

b) Replication cohort

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Signa ture** | **Proportion score** | **Number of proteins** | **AUC** | **BPse** | **BPsp** | **PPV** | **NPV** | **BPt** | **FSEse** | **FSEsp** | **FSEt** | **FSPse** | **FSPsp** | **FSPt** |
| **1** | 0 | 78 | 0.94 (0.90–0.97) | 0.96 (0.74–1.0) | 0.78 (0.68–0.98) | 0.91 (0.87–0.98) | 0.90 (0.62–1.0) | 0.59 | 0.96 (0.93–0.99) | 0.78 (0.66–0.88) | 0.59 | 0.67 (0.59–0.76) | 0.96 (0.90–1.0) | 0.89 |
| **2** | 0.05 | 62 | 0.93 (0.89–0.97) | 0.95 (0.75–1.0) | 0.78 (0.66–0.96) | 0.91 (0.87–0.97) | 0.88 (0.62–1.0) | 0.57 | 0.95 (0.91–0.99) | 0.78 (0.66–0.88) | 0.57 | 0.57 (0.49–0.66) | 0.98 (0.94–1.0) | 0.91 |
| **3** | 0.1 | 53 | 0.94 (0.90–0.98) | 0.83 (0.78–1.0) | 0.90 (0.70–0.96) | 0.95 (0.88–0.98) | 0.69 (0.64–1.0) | 0.80 | 0.95 (0.91–0.99) | 0.76 (0.64–0.88) | 0.50 | 0.66 (0.57–0.74) | 0.96 (0.90–1.0) | 0.91 |
| **4** | 0.15 | 36 | 0.92 (0.88–0.97) | 0.88 (0.80–0.99) | 0.84 (0.70–0.94) | 0.92 (0.88–0.97) | 0.76 (0.66–0.97) | 0.64 | 0.96 (0.93–0.99) | 0.76 (0.64–0.86) | 0.52 | 0.46 (0.37–0.55) | 0.96 (0.90–1.0) | 0.94 |
| **5** | 0.2 | 33 | 0.92 (0.88–0.97) | 0.96 (0.76–0.99) | 0.76 (0.70–0.96) | 0.90 (0.88–0.98) | 0.90 (0.62–0.97) | 0.53 | 0.96 (0.93–0.99) | 0.76 (0.64–0.88) | 0.53 | 0.44 (0.35–0.54) | 0.96 (0.90–1.0) | 0.95 |
| **6** | 0.25 | 27 | 0.93 (0.88–0.97) | 0.90 (0.85–0.99) | 0.86 (0.74–0.94) | 0.93 (0.89–0.97) | 0.80 (0.72–0.-97) | 0.63 | 0.95 (0.92–0.99) | 0.80 (0.68–0.90) | 0.56 | 0.50 (0.40–0.59) | 0.96 (0.90–1.0) | 0.94 |
| **7** | 0.3 | 21 | 0.91 (0.86–0.97) | 0.94 (0.87–1.0) | 0.80 (0.70–0.92) | 0.91 (0.88–0.96) | 0.85 (0.73–1.0) | 0.59 | 0.98 (0.95–1.0) | 0.72 (0.60–0.84) | 0.46 | 0.41 (0.33–0.50) | 0.96 (0.90–1.0) | 0.96 |
| **8** | 0.35 | 20 | 0.91 (0.86–0.97) | 0.89 (0.83–1.0) | 0.82 (0.68–0.92) | 0.92 (0.87–0.96) | 0.77 (0.68–1.0) | 0.65 | 0.96 (0.93–0.99) | 0.72 (0.58–0.84) | 0.47 | 0.39 (0.31–0.48) | 0.96 (0.90–1.0) | 0.96 |
| **9** | 0.4 | 18 | 0.91 (0.86–0.97) | 0.93 (0.84–1.0) | 0.80 (0.68–0.92) | 0.91 (0.87–0.96) | 0.83 (0.70–1.0) | 0.61 | 0.96 (0.92–0.99) | 0.72 (0.60–0.84) | 0.45 | 0.37 (0.28–0.46) | 0.98 (0.94–1.0) | 0.96 |
| **10** | 0.45 | 16 | 0.92 (0.87–0.97) | 0.89 (0.77–1.0) | 0.82 (0.68–0.94) | 0.92 (0.87–0.97) | 0.77 (0.63–1.0) | 0.64 | 0.98 (0.95–1.0) | 0.72 (0.60–0.84) | 0.45 | 0.59 (0.50–0.67) | 0.96 (0.90–1.0) | 0.93 |
| **11** | 0.5 | 15 | 0.92 (0.87–0.97) | 0.88 (0.77–1.0) | 0.82 (0.68–0.94) | 0.92 (0.87–0.97) | 0.76 (0.63–1.0) | 0.67 | 0.96 (0.93–0.99) | 0.74 (0.62–0.86) | 0.51 | 0.63 (0.55–0.72) | 0.96 (0.90–1.0) | 0.92 |
| **12** | 0.55 | 12 | 0.92 (0.88–0.97) | 0.93 (0.76–1.0) | 0.78 (0.68–0.96) | 0.90 (0.87–0.97) | 0.83 (0.62–1.0) | 0.60 | 0.96 (0.93–0.99) | 0.74 (0.62–0.86) | 0.48 | 0.65 (0.56–0.73) | 0.96 (0.90–1.0) | 0.90 |
| **13** | 0.6 | 11 | 0.92 (0.88–0.97) | 0.90 (0.75–1.0) | 0.82 (0.68–0.96) | 0.92 (0.87–0.97) | 0.79 (0.62–1.0) | 0.65 | 0.95 (0.92–0.99) | 0.74 (0.62–0.86) | 0.51 | 0.65 (0.55–0.74) | 0.96 (0.90–1.0) | 0.90 |
| **14** | 0.65 | 10 | 0.92 (0.87–0.97) | 0.95 (0.77–1.0) | 0.76 (0.68–0.94) | 0.90 (0.87–0.97) | 0.88 (0.64–1.0) | 0.50 | 0.95 (0.91–0.99) | 0.76 (0.64–0.88) | 0.52 | 0.64 (0.55–0.72) | 0.96 (0.90–1.0) | 0.90 |
| **16** | 0.75 | 8 | 0.92 (0.88–0.97) | 0.90 (0.78–1.0) | 0.82 (0.68–0.94) | 0.92 (0.87–0.97) | 0.79 (0.64–1.0) | 0.63 | 0.98 (0.95–1.0) | 0.68 (0.54–0.80) | 0.46 | 0.64 (0.55–0.72) | 0.96 (0.90–1.0) | 0.89 |
| **17** | 0.8 | 7 | 0.92 (0.88–0.97) | 0.87 (0.79–0.98) | 0.86 (0.72–0.96) | 0.93 (0.88–0.98) | 0.74 (0.65–0.95) | 0.74 | 0.96 (0.92–0.99) | 0.66 (0.52–0.80) | 0.50 | 0.52 (0.43–0.61) | 0.98 (0.94–1.0) | 0.93 |
| **18** | 0.85 | 6 | 0.92 (0.87–0.97) | 0.87 (0.80–0.97) | 0.86 (0.74–0.96) | 0.93 (0.88–0.98) | 0.74 (0.65–0.91) | 0.72 | 0.95 (0.91–0.99) | 0.66 (0.52–0.78) | 0.52 | 0.53 (0.44–0.61) | 0.96 (0.90–1.0) | 0.93 |
| **19** | 0.9 | 4 | 0.94 (0.90–0.98) | 0.90 (0.79–0.95) | 0.90 (0.84–1.0) | 0.95 (0.93–1.0) | 0.80 (0.67–0.90) | 0.67 | 0.95 (0.91–0.99) | 0.66 (0.52–0.78) | 0.51 | 0.80 (0.72–0.87) | 0.96 (0.90–1.0) | 0.71 |
| **21** | 1 | 2 | 0.92 (0.88–0.96) | 0.79 (0.72–0.90) | 0.96 (0.86–1.0) | 0.97 (0.93–1.0) | 0.67 (0.60–0.81) | 0.70 | 0.95 (0.91–0.99) | 0.58 (0.45–0.72) | 0.50s | 0.79 (0.72–0.86) | 0.96 (0.90–1.0) | 0.70 |

**Abbreviations:**

AUC: The “area under the ROC curve” score.

BPse: Sensitivity for the best point (Youdens index).

BPsp: Specificity for the best point (Youdens index).

PPV: The positive predictive value (PPV) for the best point. Describes how many of the predicted positive that are true positive.

NPV: The negative predictive value (NPV) for the best point describes how many of the predicted negative that are true negative.

BPt: Threshold for the best point.

FSEse: Sensitivity for the cut–off with focus on sensitivity.

FSEsp: Specificity for the cut–off with focus on sensitivity.

FSEt: The threshold for the focus on sensitivity.

FSPse: Sensitivity for the cut–off with focus on specificity.

FSPsp: Specificity for the cut–off with focus on specificity.

FSPt: The threshold for the focus on specificity.

# Supplementary table S5: Proteins in the two indices with regression coefficients contributing to the model and rank according to weight

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Index I** | **Regression coefficients**  **(weight, numerical)** | **Rank** | **Index II** | **Regression coefficients**  **(weight, numerical)** | **Rank** | **Below limit of detection (<LOD) or not significantly different in univariate analyses** |
| CA19–9 | 1.125929 | 1 | CA19–9 | 1.5358887 | 1 |  |
| MCP–3 | 0.824344 | 2 | MMP7 | 0.4121379 | 2 |  |
| CD4 | 0.509974 | 3 | TRAIL\* | 0.389802x | 3 |  |
| TRAIL\* | 0.50469 | 4 | FASLG\* | 0.3830535 | 4 |  |
| IL–8 | 0.344613 | 5 | CASP–8 | 0.2953009 | 5 |  |
| VEGFC\* | 0.31168 | 6 | CCL20 | 0.2599806 | 6 |  |
| FASLG\* | 0.31540 | 7 | VEGFC\* | 0.2483147 | 7 |  |
| CRTAM | 0.273446 | 8 | CD4 | 0.1784059 | 8 |  |
| MMP7 | 0.269671 | 9 | IL–10 | 0.1751496 | 9 |  |
| IL–10 | 0.138478 | 10 | LAMP3 | 0.1592336 | 10 | Non–significant in univariate analyses |
|  |  |  | CRTAM | 0.1442537 | 11 |  |
|  |  |  | CXCL1 | 0.1387618 | 12 |  |
|  |  |  | MCP–3 | 0.1250681 | 13 |  |
|  |  |  | IL–33 | 0.0670252 | 14 | <LOD |
|  |  |  | CCL23 | 0.0507365 | 15 |  |
|  |  |  | IL–8 | 0.050109 | 16 |  |
|  |  |  | CD40L | 0.0448391 | 17 |  |
|  |  |  | IL–1 alpha | 0.0391283 | 18 | Non–significant in univariate analyses  and <LOD |
|  |  |  | MIC–A/B | 0.0380305 | 19 |  |
|  |  |  | CCL3 | 0.0344368 | 20 |  |
|  |  |  | CSF–1 | 0.0326415 | 21 |  |
|  |  |  | PD–L2 | 0.0263208 | 22 |  |
|  |  |  | IL–5 | 0.0219836 | 23 | <LOD |
|  |  |  | IL–12 | 0.0035492 | 24 |  |

\* Negative weights in the models

# Supplementary table S6: Proteins included in Index I and the top 10 ranking proteins in Index II, and relations to cancer and inflammation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Known relation to**  **Abbrevi–**  **ated protein names** | **Biological process** | **Cancer** | **Inflammation** | **Associated with** |
| CASP–8 | Apoptosis/cell killing | Low expression of CASP–8 has been shown to promote tumor formation, progression, and treatment resistance in several types of cancers. Plays a key role in the regulation of apoptosis (12). Evasion from apoptosis is considered a hallmark of cancer (13). | Required for lymphocyte activation (14).  Part of the caspase pathway leading to apoptosis. CASP–8 is activated when FASLG binds to the Fas receptor on the cell surface as part of the death–inducing signaling complex (DISC) (15,16). | FASLG,  TRAIL |
| CCL20  = Macrophage Inflammatory Protein–3  (MIP3A) | Chemotaxis  Suppress immune reactions on tumors | CCL20 has been found to work in a paracrine fashion leading to an increased recruitment of peripheral blood mononuclear cells (PBMCs), which in turn mediated apoptosis resistance in **PDAC** cells (17). | The CCL–20 receptor, CCR6, is selectively expressed on immature dendritic cells and memory T cells, and these two bind exclusively to one another (18). Involved in several inflammatory conditions, including inflammatory bowel disease, psoriasis, and arthritis (19,20). |  |
| CD4  = T cell surface glycoprotein CD4 | Suppress immune reactions on tumors | Infiltration of CD4+ lymphocytes in **PDAC** tumor tissue has been found to be associated with improved OS at 12 months (21). However, CD4+ T cells have also been shown to be skewed towards **suppressive** lineages in **PDAC** tumors, and regulatory CD4+ Foxp3+ T cells (Tregs) are found expanded nearly 2–fold in the peripheral blood of treatment–naïve stage I–III human PDAC as well as accumulate in pancreatic cancer tissue compared with both inflammatory and non–inflammatory pancreatic tissues. (22). These immunosuppressive Tregs secrete immunosuppressive cytokines such as IL–10 and TGF–β, and the density of these Tregs in pancreatic cancer tissue is correlated with lymph node metastases and poor survival in PDAC (22). | Regulates the adaptive immune response. The activity of cytotoxic CD8+ cells can be regulated by CD4+ T cells. | IL–10 |
| CRTAM  = Class I MHC Restricted T Cell Associated Molecule, CD355 | Promote immune reactions on tumors | Activated NK cells and CD8+ T cells recognize the tumor suppressor Necl–2 through the cell surface receptor CRTAM. NK cells express CRTAM upon encountering tumor cells susceptible to lysis. De novo expression of CRTAM promotes NK cell killing of tumor cells expressing Necl–2 (23). | Transmembrane protein expressed on the cell surface of *activated* NK, CD8+ and CD4+ T cells. Expression of CRTAM identifies activated subpopulations of cells with pro–inflammatory properties. Correlated with cell adhesion, cytotoxicity, and cytokine production (24). |  |
| FASLG  = CD95L | Apoptosis/cell killing | Immune cells can use FASLG–binding to FAS as a mechanism to kill cancer cells; however, most cancer cells are immune to FASLG–mediated apoptosis (25). The cancer cells either have decreased surface expression of FAS, or at the level of the DISC, inhibits the interaction of, e.g., CASP–8 with the DISC (25). Serum FASLG has been shown to be correlated with progression and poor prognosis in many cancer types (25), including **PDAC** (26,27) where it has been shown to be elevated in the serum of patients (26). | Apoptosis–induction is the most well–established activity of FASLG/FAS.  FASLG is predominantly expressed in the activated T lymphocyte and NK–cells. The receptor, FAS, is expressed throughout the body (25). | TRAIL,  CASP–8 |
| IL–8  = CXCL8 | Chemotaxis  Suppress immune reactions on tumors | Involved in tumorigenesis in various cancers, including **pancreatic cancer** (28). Stimulates **PDAC** cell proliferation (29). Associated with short OS in patients with **PDAC** (30). | Pro–inflammatory chemokine stimulated by IL–1, IL–6, hypoxia, and more (28). Known to be overexpressed a number of inflammatory–mediated diseases, including autoimmune pancreatitis (28,31). Recruits neutrophils at the site of infection and induces a neutrophil response to eliminate inflammatory stimulus and increase bacterial clearance, hence protecting the host from further infection and tissue damage (28). | IL–6,  IL–10 |
| IL–10 | Suppress immune reactions on tumors | IL–10 has paradoxical effects on tumors. On one hand, it promotes the proliferation and cytotoxicity of CD8+ T cells in a tumor environment, on the other hand, it inhibits antigen presentation on APCs, which may impede its antitumor activity (32).  Secreted by Tregs in the TME contributing to a local immunosuppressive environment ideal for tumor growth in **PDAC** (33). IL–10 participates in the stimulation of macrophages toward M2 anti–inflammatory state, which again promotes angiogenesis, matrix remodeling, and tumor metastasis in **PDAC** (33).  Associated with short OS in patients with **PDAC** (30). | Anti–inflammatory cytokine targeting both innate and adaptive immune responses. Exerts immunosuppressive functions to reduce tissue damage caused by excess and uncontrolled inflammatory responses (32). High or dysregulated productions of IL–10 may contribute to chronic infections (32). | IL–6,  IL–8 |
| LAMP3  = Dendritic cell lysosomal associated membrane glycoprotein (DC–LAMP) | Suppress immune reactions on tumors | Has been found to be overexpressed in esophageal squamous cell carcinoma (34) as well as in uterine cervical cancer, colon cancer, ovarian cancer, breast cancer, and liver cancer (35). Overexpression has found to be associated with drug and stress resistance (e.g. radiotherapy), cancer progression, migration, and metastasis (36). | Serves as a specific marker for the maturation of dendritic cells, and levels rise steeply upon activation of these (37). During maturation of dendritic cells LAMP3 co–localizes with MHC class II molecules within the MHC class II compartments, suggesting a role for LAMP3 in the antigen presentation process (36).  LAMP expression is induced in hypoxic conditions (36). |  |
| MCP–3  = Chemokine (C–C motif) ligand 7 (CCL7) | Chemotaxis | Produced by macrophages and some tumor cells (38). MCP–3 has been shown to have anti–tumor potential in different tumor models, including animal models of **PDAC**, where MCP–3 induces infiltration and activation of NK and monocytic cells (39). | MCP–3 is known to attract a variety of innate immune cells (including dendritic cells, NK cells, T cells and eosinophils) because of its ability to interact with at least three chemokine receptors (CCR1, –2 and –3), which are expressed by a large subset of leucocytes (38,39). |  |
| MMP–7  = pump–1 protease (PUMP–1) | Apoptosis/cell killing  Suppress immune reactions on tumors | Expressed by tumor cells in many adenocarcinomas (40). MMP–7 has been shown to be elevated in **PDAC** patients compared to healthy subjects and those with chronic pancreatitis (41), and also associated with early tumor progression, suggesting a value as potential early diagnostic marker (42,43). Significant predictor of survival of patients with PDAC (43). | Expressed predominantly in epithelial cells of glandular tissue (43). MMP–7 is found in both acute and chronic pancreatitis (44). In inflammation MMP–7 activates tumor necrosis factor (TNF) and promotes neutrophil influx by creation of chemokine gradients (44). | Stat3,  TNF |
| TRAIL | Apoptosis/cell killing | Have been found to have a capacity to selectively kill tumor cells, without killing any normal cell types (45). However, the effect seems to be reversed in KRAS–driven cancer cells. It has been found to stimulate tumor invasion and metastasis in the presence of oncogenic KRAS–mutations in colorectal cancer cell lines (46). Known to activate non–apoptotic signaling pathways in cancer cell lines, such as NF–κB, MAP–K, and ERK (47,48). Its receptor, TRAIL–R2, has been shown to be highly expressed on **PDAC** human tissue and stimulation promoted cancer progression, invasion, and metastasis (47). | TRAIL is a cytokine and is expressed mainly by cells of the immune system (49).  Binding of TRAIL to TRAIL–R1 (=DR4) and TRAIL–R2 (=DR5) triggers formation of the death–inducing signaling complex (DISC), activating CASP–8 and apoptosis (49). | CASP–8,  FASLG |
| VEGFC | Vascular remodeling | The expression of VEGF–C is detected in a variety of human tumors and the increased level of VEGF–C is significantly correlated with lymph node metastasis, distant metastasis, and poor prognosis (50). | Binds to VEGFR3 and induces downstream signaling which mediates cell survival and lymphangiogenesis (50). Lymphangiogenesis often occurs in chronic inflammatory tissues and is largely driven by VEGF–C/VEGFR3 interaction (50). |  |

**Abbreviations:** APCs: Antigen Presenting Cells; CP: Chronic Pancreatitis; CRP: C–reactive Protein; DISC: Death–inducing signaling complex; ECM: Extracellular matrix; NK cells: Natural killer cells; PD–1: Programmed cell death protein 1; ; PDAC: pancreatic ductal adenocarcinoma; TME: tumor microenvironment; Tregs: T regulatory immune cells; TWEAK: TNF–related weak inducer of apoptosis; VEGFR: Vascular endothelial growth factor receptors.

# Supplementary table S7: Recent studies exploring proteins for diagnosis of pancreatic ductal adenocarcinoma

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Year** | **Country** | **Examined proteins, n** | **Chosen proteins, n** | **Test sets** | | **PDAC, n** | **CP, n** | **IPMN, n** | **Healthy, n** | **AUC** | **Sensitivity** | **Specificity** | **Notes** |
| Brand RE et al., *Clin Cancer Res* (51) | 2011 | USA | 83 | 3 (CA19–9, ICAM–1, OPG) | Training set | 160 | | 74\* | | 107 | 0.93 PDAC vs. healthy | 0.88 | 0.90 | \*Benign pancreatic conditions |
| 3 (CA19–9, ICAM–1, OPG) | Validation set | 173 | | 70\* | | 120 | 0.91 PDAC vs. healthy | 0.78 | 0.94 |
| Park HD et al., *Proteomics* (52) | 2012 | Korea | 8 | 3 (CA19–9, Cathepsin D, MMP–7) | Training set | 109 | | 30 | – | 40 | 0.904 PDAC vs. all controls | 0.88 | 0.80 |  |
| Validation set | 139 | | 72 | – | 74 | 0.91 PDAC vs. all controls | 0.89 | 0.77 |  |
| Makawita S. et al., *BMC Cancer* (53) | 2013 | Canada | 5 | 3 (CA19–9, SYCN, REG1B) | Sample set A  (test set) | 100 | | – | – | 92 | 0.87 PDAC (stage I+II) vs. disease free controls | 0.39 | 0.95 |  |
| Sample set B  (training set) | 82 | | 70\* | 41^ | 47¨ | 0.92 PDAC (stage I+II) vs. disease free controls | 0.74 | 0.95 | \*Other cancers (primarily GI cancers)  ^Non–malignant (including 10 patients with IPMN and 21 patients with CP)  ¨Disease–free |
| Zhang P. et al., *Int J Cancer* (54) | 2014 | China | 61^ | 4 (CA19–9, ALB, CRP, IL–8) | Training | 163 | | 109\* | | 200 | 0.984 PDAC vs. healthy | 0.99 | 0.90 | ^61 clinical parameter, including 25 tumor biomarkers and inflammation markers  \*Benign patients |
| Cross–validation | 129 | | 95\* | | 183 | 0.969 PDAC vs. healthy | 0.94 | 0.90 | \*Benign patients  Furthermore, for evaluation of cancer selectivity: 77 breast cancer, 72 cervical cancer, 101 colon cancer, 138 gastric cancer, 108 prostate cancer, 132 lung cancer |
| Shaw V. et al., *Mol Cancer* (55) | 2014 | UK | 27 | 3 (CA19–9, IL–8, IL–1b) | Training set | 84 | | 32 | 13\* | 29 | 0.984 PDAC vs. healthy | 0.941 (median of the combined panels) | 1.00 (median of the combined panels) | \*Benign biliary obstruction |
| Test set | 43 | | 17 | 7\* | 16 | 0.997 PDAC vs. healthy |
| Mirus JE et al., *Clin Cancer Res* (56) | 2015 | USA | >4000 | 3 (ERBB2, TNC, ESR1) | Pre–diagnostic samples\* | 87 | | – | – | 87 | 0.68 PDAC vs. healthy | – | – | \*From women who later succumbed to PDAC |
| 4 (+ CA19–9) | Diagnostic samples | 24 | | 24 | 0.97 PDAC vs. healthy | – | – |  |
| Capello M et al., *J Natl Cancer Inst* (57) | 2017 | USA | 18 | 7 | Triage set | 75 | | 19 | – | 27 | – | – | – | Differentially expressed protein in Triage set –> combined validation set and test set |
| 7 | 3 (CA19–9, LRG1, TIMP1) | Combined validation set | 73 | | 60 | 14\* | 60 | 0.949 PDAC vs. healthy, | 0.849 | 0.95 | \*Benign pancreatic cyst cases |
| 3 (CA19–9, LRG1, TIMP1) | 3 (CA19–9, LRG1, TIMP1) | Test set | 39 | | – | – | 83 | 0.887 PDAC vs. healthy | 0.667 | 0.95 |  |
| Park J et al., *Oncotarget* (58) | 2017 | Korea | 1000 | 54 | Discovery, Verification | 50 | | – | 34 | 50 |  |  |  |  |
| 54 | 3 (LRG1, TTR, CA19–9) | Validation (1 training and 5 test sets) | 401 | | *149^* | 109\* | 349 | 0.93 PDAC vs. healthy and benign pancreatic diseases | 0.82 |  | ^Other cancers (thyroid, breast and colorectal)  \*Benign pancreatic diseases |
| Park J et al., *Oncotarget* (59) | 2017 | Korea | 90 | 9 | Discovery | 116 | | 31\* | – | 35 | – | – | – | \*Pancreatitis |
| 9 | 3 (CA19–9, APOA4, TIMP1) | Validation | 292 | | 71\* | – | 94 | 0.934 PDAC vs. pancreatitis | 0.86 | 0.90 |
| Balasenthil S et al., *J Natl Cancer Inst* (60) | 2017 | USA | 3 | 3 (TFPI, TNC–FN III–C, CA19–9) | CLIA analysis | 20 | | – | – | 20 | 0.92 PDAC vs. healthy | – | – |  |
| Early stage prevalidation cohort 1 | 85 | | – | – | 30\* | 0.97 stage II PDAC vs. healthy\* | – | – | \*GI screening controls |
| Early stage blinded validation cohort 2 | 23 | | 24 | – | 17\* | 0.86 stage IIb PDAC vs. CP | – | – |
| Early stage blinded validation cohort 3 | 98 | | 62 | 31\* | 61 | 0.79 stage Ia, Ib, IIa PDAC vs. healthy | 0.73 | 0.82 | \*Acute biliary obstruction controls |
| Mellby LD et al., *J Clin Oncol* (61) | 2018 | Sweden | 349 | 29 | Discovery cohort (DK) | 443 | | – | 8 | 888 | 0.96 PDAC stage I+II vs. healthy | 0.95 | 0.96 |  |
| Validation cohort (USA) | 143 | | 57 | 20 | 219 | 0.96 PDAC stage I+II vs. healthy | 0.93 | 0.95 |  |
| Dong et al., *Cancer Sci* (62) | 2018 | China | 4 | 3 (POSTN, CA242, CA19–9) | Training set | 125 | | 21 | 11\* | 37 | 0.98 PDAC early stage vs. healthy | 0.967 | 0.946 | \*Other pancreatic diseases, including IPMN |
| Validation set | 144 | | 28 | 15\* | 37 | 0.98 PDAC early stage vs. healthy | 0.921 | 0.973 |
| Aronsson L et al., *Scand J Gastroenterol* (63) | 2018 | Sweden | 1000 | 10^ | Discovery | 8 | | – | – | 8 | – | – | – | ^Top 10 glycosylated proteins |
| 10 | 4 | Verification | 49 | | – | 13 | 47 | 0.974 PDAV vs. healthy | – | 0.90 |  |
| Jahan et al., *EBioMedicine* (64) | 2019 | USA | 4 | 4 (TFF1, TFF2, TFF3, CA19–9) | Training | 153 | | 47 | 104\* | – | 0.94 PDAC vs. benign control  0.94 PDAC vs. CP | 0.95  /  0.90 | 0.92  /  0.92 | \*Benign control |
| Validation | 23 | | 27 | 8\* |  | 0.85 PDAC vs. benign control  0.85 PDAC vs. CP | 0.63  /  0.84 | 1.00  /  0.41 |
| Wu X et al., *Proteomics Clin Appl* (65) | 2019 | China | 869 | 55^ | Discovery group | 25 | | – | – | 15 | – | – | – | ^Differentially expressed proteins |
| 6^ | 2 (TNFRSF6B, PROZ) | Training group | 112 | | 20\* | | 20 | 0.966 PDAC vs. benign/healthy | 0.875 | 1.0 | ^Top 5 upregulated proteins + CA19–9  \*Benign controls: benign pancreatic tumors |
| 3 | 3 (TNFRSF6B, PROZ, CA19–9) | Validation group | 46 | | 20\* | | 20 | 0.932 PDAC stage I vs. benign/healthy | 0.761p | 1.0 |
| Stage 1 patient group | 82 | | 40\* | | 40 | 0.919 PDAC stage I vs. benign/healthy | 0.756 | 0.95 |

Abbreviations:

ALB – Albumin

APOA4 – Apolipoprotein A–IV

CA125 – Cancer antigen 125

CRP – C–reactive protein

ERBB2 – v–erb–b2 erythroblastic leukemia viral oncogene homolog 2

ESR1 – Estrogen receptor 1

ICAM–1 – Inter–cellular adhesion molecule 1

LAMC2 – Laminin gamma C

LRG1 – Leucine–rich alpha–2–glycoprotein 1

MIC–1 – Macrophage inhibitory cytokine 1

MUC5AC – Mucin 5AC

OPN / OPG – Osteopontin

POST – Periostin

PROZ – Vitamin K–dependent protein Z

REG1B – Regenerating islet–derived 1 beta

SYCN – Syncollin

TFF1 – Trefoil factor 1

TFF2 – Trefoil factor 2

TFF3 – Trefoil factor 3

TFPI – Plasma tissue factor pathway inhibitor

TIMP1 – Tissue inhibitor of metalloproteinase 1

TNC – Tenascin C

TNFRSF6B – Tumor necrosis factor receptor superfamily member 6b

TTR – Transthyretin