**Supplementary material**

**Supplementary Table 1.** STM measurements stratified by PD, SD and PR subgroups.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | **PD (N=35)** | **PR (N=128)** | **SD (N=100)** | **ALL (N=263)** |
| **CA 125 (U/mL)** |   |   |   |   |
| Mean | 411.88 | 78.45 | 65.09 | 117.74 |
| SD | 739.25 | 399.06 | 89.28 | 405.58 |
| Median | 109.2 | 26.34 | 33.79 | 32.29 |
| Min-Max | 5.23-3395.00 | 7.27-4528.00 | 6.29-529.60 | 5.23-4528.00 |
| **CA 15-3 (U/mL)** |   |   |   |   |
| Mean | 120.56 | 37.69 | 54.74 | 55.2 |
| SD | 117.19 | 38.89 | 67.49 | 70.41 |
| Median | 58.47 | 30.26 | 31.8 | 32.74 |
| Min-Max | 10.14-300.00 | 9.35-300.00 | 8.42-300.00 | 8.42-300.00 |
| **CEA (ng/mL)** |   |   |   |   |
| Mean | 58.12 | 37.73 | 35.91 | 39.75 |
| SD | 148.59 | 119.13 | 62.69 | 106.22 |
| Median | 9.44 | 4.79 | 9.06 | 6.36 |
| Min-Max | 1.11-675.30 | 0.31-1000.00 | 1.05-317.20 | 0.31-1000.00 |
| **CYFRA 21-1 (ng/mL)** |   |   |   |   |
| Mean | 14.09 | 3.19 | 4.89 | 5.28 |
| SD | 19.42 | 5.53 | 5.09 | 9.28 |
| Median | 7.37 | 2.13 | 3.04 | 2.72 |
| Min-Max | 1.11-71.26 | 0.50-60.49 | 0.38-34.53 | 0.38-71.26 |
| **NSE (ng/mL)** |   |   |   |   |
| Mean | 23.36 | 17.33 | 18.4 | 18.54 |
| SD | 14.28 | 7.55 | 8.29 | 9.16 |
| Median | 17.67 | 15.47 | 17.02 | 16.35 |
| Min-Max | 11.36-70.65 | 7.04-57.16 | 8.50-62.19 | 7.04-70.65 |
| **SCC (ng/mL)** |   |   |   |   |
| Mean | 5.68 | 1.91 | 2.34 | 2.58 |
| SD | 13.56 | 1.47 | 3.49 | 5.57 |
| Median | 1.52 | 1.56 | 1.6 | 1.55 |
| Min-Max | 0.10-70.00 | 0.20-9.55 | 0.34-33.12 | 0.10-70.00 |
| **proGRP (pg/mL)** |   |   |   |   |
| Mean | 76.31 | 49.55 | 82.45 | 65.62 |
| SD | 196.95 | 28.63 | 338.38 | 221.24 |
| Median | 39 | 41.5 | 45 | 42 |
| Min-Max | 12.00-1195.00 | 16.00-255.00 | 18.00-3427.00 | 12.00-3427.00 |

HE4 was excluded as we saw a strong influence of eGFR on biomarker values.

**Supplementary Table 2.** Results of univariate and combination analyses of the prognostic values of CYFRA 21-1, CA 125, CEA, CA 15-3, NSE, ProGRP and SCC in patients with stable disease (ADC + SCC with interaction term [n=100]) at the first CT scan after the second cycle for progression-free survival (A) and overall survival (B).

|  |
| --- |
| **A) Progression-free survival** |
| **Biomarker**  | **C-index** | **HR (high vs low risk SD)**  |
| **Optimized cut-off** | **Median cut-off** |
| **Univariate analysis** |  |  |  |
| CYFRA 21-1 | 0.67 | 3.73 | 2.48 |
| CA 125 | 0.63 | 3.16 | 2.44 |
| CEA | 0.59 | 2.23 | 1.53 |
| CA 15-3 | 0.56 | 1.81 | 1.22 |
| NSE | 0.54 | 1.73 | 1.16 |
| ProGRP | 0.53 | 1.63 | 1.53 |
| SCC | 0.55 | 1.45 | 1.09 |
| **Combination analysis** |  |  |  |
| CA 125 + CA 15-3 | 0.63 | 3.21 | 1.95 |
| CA 125 + CA 15-3 + CEA | 0.66 | 2.95 | 2.07 |
| CA 125 + CA 15-3 + CYFRA 21-1 | 0.68 | 3.48 | 2.85 |
| CA 125 + CA 15-3 + NSE | 0.63 | 3.02 | 1.89 |
| CA 125 + CA 15-3 + ProGRP | 0.63 | 3.21 | 2.04 |
| CA 125 + CA 15-3 + SCC | 0.64 | 2.97 | 2.19 |
| CA 125 + CEA | 0.66 | 2.77 | 2.37 |
| CA 125 + CEA + NSE | 0.66 | 2.68 | 2.51 |
| CA 125 + CEA + ProGRP | 0.66 | 2.59 | 2.28 |
| CA 125 + CEA + SCC | 0.67 | 3.04 | 2.42 |
| CA 125 + CYFRA 21-1 | 0.68 | 3.78 | 2.92 |
| CA 125 + CYFRA 21-1 + CEA | 0.70 | 4.17 | 2.41 |
| CA 125 + CYFRA 21-1 + NSE | 0.69 | 3.58 | 2.81 |
| CA 125 + CYFRA 21-1 + ProGRP | 0.68 | 3.78 | 2.92 |
| CA 125 + CYFRA 21-1 + SCC | 0.68 | 4.27 | 2.65 |
| CA 125 + NSE | 0.63 | 2.93 | 2.41 |
| CA 125 + NSE + ProGRP | 0.63 | 2.94 | 2.30 |
| CA 125 + NSE + SCC | 0.64 | 2.82 | 2.40 |
| CA 125 + ProGRP | 0.63 | 2.94 | 2.22 |
| CA 125 + SCC | 0.64 | 2.52 | 2.30 |
| CA 125 + SCC + ProGRP | 0.64 | 3.02 | 2.24 |
|  |  |  |  |
| CA 15-3 + CEA | 0.61 | 1.89 | 1.54 |
| CA 15-3 + CEA + CYFRA 21-1 | 0.70 | 3.99 | 2.78 |
| CA 15-3 + CEA + NSE | 0.61 | 1.94 | 1.56 |
| CA 15-3 + CEA + ProGRP | 0.61 | 1.98 | 1.55 |
| CA 15-3 + CEA + SCC | 0.62 | 2.50 | 1.86 |
| CA 15-3 + CYFRA 21-1 | 0.67 | 3.24 | 2.69 |
| CA 15-3 + CYFRA 21-1 + NSE | 0.68 | 3.37 | 2.25 |
| CA 15-3 + CYFRA 21-1 + ProGRP | 0.67 | 3.24 | 2.75 |
| CA 15-3 + CYFRA 21-1 + SCC | 0.67 | 3.04 | 2.23 |
| CA 15-3 + NSE | 0.57 | 2.06 | 1.25 |
| CA 15-3 + NSE + ProGRP | 0.57 | 2.31 | 1.27 |
| CA 15-3 + NSE + SCC | 0.58 | 2.14 | 1.33 |
| CA 15-3 + ProGRP | 0.55 | 2.14 | 1.17 |
| CA 15-3 + SCC | 0.57 | 2.05 | 1.17 |
| CA 15-3 + SCC + ProGRP | 0.56 | 1.90 | 1.09 |
|  |  |  |  |
| CEA + CYFRA 21-1 | 0.69 | 4.05 | 2.79 |
| CEA + CYFRA 21-1 + NSE | 0.70 | 3.61 | 3.04 |
| CEA + CYFRA 21-1 + ProGRP | 0.69 | 4.33 | 2.68 |
| CEA + CYFRA 21-1 + SCC | 0.70 | 4.32 | 2.91 |
| CEA + NSE | 0.59 | 2.16 | 1.56 |
| CEA + NSE + ProGRP | 0.60 | 2.31 | 1.51 |
| CEA + NSE + SCC | 0.62 | 2.32 | 1.92 |
| CEA + ProGRP | 0.59 | 2.57 | 1.45 |
| CEA + SCC | 0.62 | 2.32 | 2.00 |
| CEA + SCC + ProGRP | 0.62 | 2.68 | 1.89 |
|  |  |  |  |
| CYFRA 21-1 + NSE | 0.68 | 3.28 | 2.58 |
| CYFRA 21-1 + NSE + ProGRP | 0.68 | 3.28 | 2.58 |
| CYFRA 21-1 + NSE + SCC | 0.68 | 3.21 | 2.58 |
| CYFRA 21-1 + ProGRP | 0.67 | 3.73 | 2.54 |
| CYFRA 21-1 + SCC | 0.67 | 3.97 | 2.48 |
| CYFRA 21-1 + SCC + ProGRP | 0.68 | 3.97 | 2.16 |
|  |  |  |  |
| NSE + ProGRP | 0.55 | 1.55 | 1.31 |
| NSE + SCC | 0.56 | 1.47 | 1.01 |
| NSE + SCC + ProGRP | 0.56 | 1.58 | 1.25 |
|  |  |  |  |
| SCC + ProGRP | 0.54 | 1.47 | 1.13 |
| **B) Overall survival** |
| **Univariate analysis** |  |  |  |
| CYFRA 21-1 | 0.69 | 3.91 | 2.31 |
| CA 125 | 0.65 | 2.49 | 2.50 |
| CEA | 0.55 | 1.64 | 1.38 |
| CA 15-3 | 0.60 | 1.94 | 1.50 |
| NSE | 0.53 | 1.69 | 1.25 |
| ProGRP | 0.52 | 1.64 | 1.10 |
| SCC | 0.54 | 1.45 | 1.31 |
| **Combination analysis** |  |  |  |
| CA 125 + CA 15-3 | 0.66 | 2.68 | 2.16 |
| CA 125 + CA 15-3 + CEA | 0.65 | 3.01 | 2.31 |
| CA 125 + CA 15-3 + CYFRA 21-1 | 0.71 | 3.87 | 3.10 |
| CA 125 + CA 15-3 + NSE | 0.65 | 2.68 | 2.08 |
| CA 125 + CA 15-3 + ProGRP | 0.66 | 2.56 | 2.50 |
| CA 125 + CA 15-3 + SCC | 0.66 | 2.91 | 2.95 |
| CA 125 + CEA | 0.64 | 2.80 | 2.30 |
| CA 125 + CEA + CYFRA 21-1 | 0.70 | 3.99 | 2.08 |
| CA 125 + CEA + NSE | 0.64 | 2.59 | 2.52 |
| CA 125 + CEA + ProGRP | 0.65 | 2.89 | 2.64 |
| CA 125 + CEA + SCC | 0.65 | 2.82 | 2.82 |
| CA 125 + CYFRA 21-1 | 0.70 | 3.51 | 2.49 |
| CA 125 + CYFRA 21-1 + NSE | 0.71 | 3.90 | 2.49 |
| CA 125 + CYFRA 21-1 + ProGRP | 0.69 | 3.55 | 2.55 |
| CA 125 + CYFRA 21-1 + SCC | 0.71 | 3.44 | 3.10 |
| CA 125 + NSE | 0.64 | 2.56 | 2.16 |
| CA 125 + NSE + ProGRP | 0.65 | 2.75 | 2.22 |
| CA 125 + NSE + SCC | 0.66 | 2.95 | 2.59 |
| CA 125 + ProGRP | 0.66 | 2.93 | 2.63 |
| CA 125 + SCC | 0.66 | 2.78 | 2.73 |
| CA 125 + SCC + ProGRP | 0.66 | 2.81 | 2.39 |
|  |  |  |  |
| CA 15-3 + CEA | 0.62 | 2.66 | 1.92 |
| CA 15-3 + CEA + CYFRA 21-1 | 0.71 | 3.35 | 2.82 |
| CA 15-3 + CEA + NSE | 0.62 | 2.10 | 1.91 |
| CA 15-3 + CEA + ProGRP | 0.62 | 2.20 | 1.81 |
| CA 15-3 + CEA + SCC | 0.62 | 2.29 | 1.67 |
| CA 15-3 + CYFRA 21-1 | 0.70 | 4.17 | 2.56 |
| CA 15-3 + CYFRA 21-1 + NSE | 0.71 | 3.39 | 2.48 |
| CA 15-3 + CYFRA 21-1 + ProGRP | 0.70 | 3.75 | 2.15 |
| CA 15-3 + CYFRA 21-1 + SCC | 0.70 | 4.17 | 2.32 |
| CA 15-3 + NSE | 0.61 | 2.35 | 1.37 |
| CA 15-3 + NSE + ProGRP | 0.62 | 2.19 | 1.60 |
| CA 15-3 + NSE + SCC | 0.60 | 2.46 | 1.56 |
| CA 15-3 + ProGRP | 0.62 | 2.85 | 1.90 |
| CA 15-3 + SCC | 0.60 | 1.97 | 1.59 |
| CA 15-3 + SCC + ProGRP | 0.61 | 1.78 | 1.66 |
|  |  |  |  |
| CEA + CYFRA 21-1 | 0.70 | 4.32 | 2.61 |
| CEA + CYFRA 21-1 + NSE | 0.71 | 3.77 | 2.53 |
| CEA + CYFRA 21-1 + ProGRP | 0.69 | 3.53 | 2.51 |
| CEA + CYFRA 21-1 + SCC | 0.70 | 3.69 | 2.80 |
| CEA + NSE | 0.60 | 1.81 | 1.36 |
| CEA + NSE + ProGRP | 0.56 | 1.69 | 1.52 |
| CEA + NSE + SCC | 0.57 | 1.72 | 1.55 |
| CEA + ProGRP | 0.56 | 1.63 | 1.57 |
| CEA + SCC | 0.57 | 1.82 | 1.42 |
| CEA + SCC + ProGRP | 0.57 | 1.76 | 1.55 |
|  |  |  |  |
| CYFRA 21-1 + NSE | 0.70 | 3.33 | 2.38 |
| CYFRA 21-1 + NSE + ProGRP | 0.70 | 3.62 | 2.88 |
| CYFRA 21-1 + NSE + SCC | 0.71 | 3.89 | 2.31 |
| CYFRA 21-1 + ProGRP | 0.69 | 3.84 | 2.00 |
| CYFRA 21-1 + SCC | 0.69 | 3.91 | 2.31 |
| CYFRA 21-1 + SCC + ProGRP | 0.68 | 3.84 | 2.13 |
|  |  |  |  |
| NSE + ProGRP | 0.52 | 1.72 | 1.12 |
| NSE + SCC | 0.55 | 1.62 | 1.42 |
| NSE + SCC + ProGRP | 0.54 | 1.81 | 1.28 |
|  |  |  |  |
| SCC + ProGRP | 0.55 | 1.61 | 1.57 |

 HE4 was excluded as we saw a strong influence of eGFR on biomarker values.

**Supplementary Table 3.** Results of univariate and combination analyses of the prognostic values of CYFRA 21-1, CA 125 and CEA in patients with stable disease at the first CT scan after the second cycle for progression-free survival (A) and overall survival (B).

1. **Progression-free survival**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **ADC (n=76)** | **SCC (n=24)** | **ADC + SCC with interaction term (n=100)** |
|  |  | **C-index** | **HR (high vs low risk SD)a** | **C-index** | **HR (high vs low risk SD)a** | **C-index** | **HR (high vs low risk SD)a** |
| **Median biomarker cutoff** |
| **Univariate analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 |  | 0.69 | 2.30 | 0.61 | 2.58 | 0.67 | 2.48 |
| CA 125 |  | 0.63 | 1.93 | 0.68 | 3.47 | 0.63 | 2.44 |
| CEA |  | 0.60 | 1.61 | 0.51 | 0.76 | 0.59 | 1.53 |
| **Combination analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 + CA 125 |  | 0.69 | 2.75 | 0.69 | 3.65 | 0.68 | 2.92 |
| CYFRA 21-1 + CA 125 + CEA |  | 0.71 | 2.87 | 0.70 | 3.65 | 0.70 | 2.41 |
| **Optimized biomarker cut-off** |
| **Univariate analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 |  | 0.69 | 4.04 | 0.61 | 2.93 | 0.67 | 3.73 |
| CA 125 |  | 0.63 | 2.76 | 0.68 | 4.31 | 0.63 | 3.16 |
| CEA |  | 0.60 | 2.359 | 0.51 | 0.60 | 0.59 | 2.23 |
| **Combination analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 + CA 125 |  | 0.69 | 4.04 | 0.69 | 3.95 | 0.68 | 3.78 |
| CYFRA 21-1 + CA 125 + CEA |  | 0.71 | 4.59 | 0.70 | 4.63 | 0.70 | 4.17 |
| 1. **Overall survival**
 |  |  |
|  |  | **ADC (n=76)** | **SCC (n=24)** | **ADC + SCC with interaction term (n=100)** |
|  |  | **C-index** | **HR (high vs low risk SD)a** | **C-index** | **HR (high vs low risk SD)a** | **C-index** | **HR (high vs low risk SD)a** |
| **Median biomarker cutoff** |
| **Univariate analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 |  | 0.71 | 2.53 | 0.61 | 1.36 | 0.69 | 2.31 |
| CA 125 |  | 0.66 | 2.51 | 0.62 | 3.48 | 0.65 | 2.450 |
| CEA |  | 0.56 | 1.12 | 0.51 | 1.06 | 0.55 | 1.38 |
| **Combination analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 + CA 125 |  | 0.73 | 2.99 | 0.62 | 1.55 | 0.70 | 2.489 |
| CYFRA 21-1 + CA 125 + CEA |  | 0.72 | 2.64 | 0.67 | 1.51 | 0.70 | 2.08 |
| **Optimized biomarker cut-off** |
| **Univariate analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 |  | 0.71 | 3.94 | 0.61 | 1.52 | 0.69 | 3.91 |
| CA 125 |  | 0.66 | 3.02 | 0.62 | 3.48 | 0.65 | 2.49 |
| CEA |  | 0.56 | 1.877 | 0.51 | 1.49 | 0.55 | 1.64 |
| **Combination analysis** |  |  |  |  |  |  |  |
| CYFRA 21-1 + CA 125 |  | 0.73 | 3.94 | 0.62 | 3.309 | 0.70 | 3.507 |
| CYFRA 21-1 + CA 125 + CEA |  | 0.72 | 4.38 | 0.67 | 3.34 | 0.70 | 3.99 |

aPatients with SD stratified according to biomarker value above or below median or optimized cut-offs. The C-index was calculated based on a model that included the continuous form of the biomarkers.

**Supplementary Figure 1.** Workflow for assessing the prognostic power of individual and combinations of biomarkers.


ADC, adenocarcinoma; CA 125, cancer antigen 125; CA 15-3, cancer antigen 15-3; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragment 21-1; HE4, Human Epididymis Protein 4; NSE, neuron-specific enolase; ProGRP, progastrin-releasing peptide; SCC, squamous cell carcinoma.

**Supplementary Figure 2.** Best biomarker performance in the analysis of PFS in patients with SD after the first CT scan after Cycle 2. (**A**) Patients with ADC stratified by CYFRA 21-1 above or below median; (**B**) Patients with SCC stratified by CA 125 above or below median; (**C**) Patients with SD regardless of histology stratified by CYFRA 21-1 and CA 125 above or below median; (**D**) Patients with SD regardless of histology stratified by CYFRA 21-1, CA 125 and CEA above or below median.



The C-index was calculated based on a model that included the continuous form of the biomarkers. ADC, adenocarcinoma; CI, confidence interval; HR, hazard ratio; PFS, progression-free survival; SCC, squamous cell carcinoma; SD, stable disease.