|  |  |  |  |
| --- | --- | --- | --- |
|  | Primary resected(n=37) | NeoTx(n=37) | *p* value |
| SexMaleFemale | 21 (58.3%)16 (41.6%) | 20 (54.1%)17 (45.9%) | 0.818 |
| Age (year) | 63.5 (46-82) | 62.6 (47-78) | 0.654 |
| Tumorsize (mm) | 41.5 (3-90) | 37.6 (15-100) | 0.401 |
| T StatusT1-T2T3-T4 | 15 (41.6%)21 (58.3%) | 20 (54.1%)17 (45.9%) | 0.636 |
| N StatusN0N1 | 11 (29.8%)26 (72.2%) | 19 (51.4%)18 (48.6%) | 0.059 |
| M StatusM0M1 | 31 (83.7%)6 (16.3%) | 31 (83.7%)6 (16.3%) | 1.000 |
| GradingG1G2G3 | 2 (5.4%)18 (48.6%)17 (46.0%) | 0 (0%)15 (45.4%)18 (54.6%) | 0.297 |
| Resection StatusR0R1R2 | 18 (54.5%)14 (42.4%)1 (3.1%) | 12 (38.8%)19 (61.2%)0 (0%) | 0.340 |
| Tumor LocalisationHeadCorpusTailHead-corpusCorpus-Tail | 23 (62.2%)10 (27.0%)2 (5.4%)1 (2.7%)1 (2.7%) | 26 (70.2%)5 (13.6%)3 (8.1%)2 (5.4%)1 (2.7%) | 1.000 |
| Surgical operationPancreatic head resectionDistal pancreatectomyTotal pancreatectomy | 21 (56.7%)11 (29.7%)5 (13.5%) | 24 (64.9%)8 (21.6%)5 (13.5%) | 0.634 |
| Clinical manifestationsAbdominal painJaundiceWeight lossNew onset diabetes mellitus | 13 (38.2%)15 (44.1%)9 (26.5%)3 (8.3%) | 17 (65.4%)6 (23%)6 (23%)2 (5.4%) | 0.0380.0930.7680.626 |

|  |  |
| --- | --- |
| NeoCTx regime | N° of patients (n=37) |
| Gemcitabine mono | 6 (16%) |
| Gemcitabine + Erlotinib | 5 (14%) |
| Gemcitabine + Oxaliplatin | 8 (22%) |
| Folfirinox | 13 (34%) |
| Other regimes (e.g. radiotherapy) | 5 (14%) |

**Supplementary Table 1. Clinical characteristics of neoadjuvantly treated vs upfront resected PDAC patients and the types of neoadjuvant regimens.** NeoTx: neoadjuvant treatment

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibody | Catalog Number | Species | Type | Dilution | Company |
| Anti-αSMA  | M0851 | mouse | monoclonal | 1:2000 | DAKO, Hamburg, Germany |
| Anti-S100 | MAB079-1 | mouse | monoclonal | 1:200 | Milipore, Massachusetts, U.S.A |
| Anti-CK19 | sc25724 | rabbit | polyclonal | 1:100 | Santa Cruz, Texas, U.S.A |
| Anti-CD31 | ab-28364 | rabbit | polyclonal | 1:400 | Abcam, Cambridge, UK |
| Anti-CD45 | DB042-1 | rabbit | polyclonal | 1:400 | DB Biotech, Kosice, Slovakia |
| Anti-CD8 | Mob117 | mouse | monoclonal | 1:40 | Diagnostic Biosystems |
| Anti-ITGAE | HPA036313 | rabbit | polyclonal | 1:20 | Sigma, Missouri, U.S.A. |
| Anti-FOXP3 | ab20034 | mouse | monoclonal | 1:100 | Abcam, Cambridge, UK |
| Anti-CD4 | HPA004252 | rabbit | polyclonal | 1:80 | Sigma Prestige, Missouri, U.S.A. |
| Anti-CD33 | SAB4700737 | mouse | monoclonal | 1:100 | Sigma Aldrich, Missouri, U.S.A. |
| Anti-CD11 | Ab52478 | rabbit | polyclonal | 1:100 | Abcam, Cambridge, UK |
| Anti-CD56 | AB5032 | rabbit | polyclonal | 1:600 | Millipore, Massachusetts, U.S.A |
| Anti-CD68 | HPA048982 | rabbit | polyclonal | 1:200 | Sigma, Missouri, U.S.A. |
| Anti-CD68 | Mob167 | mouse | monoclonal | 1:200 | DBiosystems, California, U.S.A. |
| Anti-HLA-DR | sc-56545 | mouse | monoclonal | 1:200 | Santa Cruz, Texas, U.S.A. |
| Anti-MRC1 | HPA004114 | rabbit | polyclonal | 1:200 | Sigma, Missouri, U.S.A. |
| Anti-CD20 | NCL-L-CD20-L26 | mouse | monoclonal | 1:200 | Leica, Wetzlar, Germany |

**Supplementary Table 2. Specifications and dilutions of the primary antibodies used in our experimental pipeline**

|  |  |  |
| --- | --- | --- |
|  | Histological feature | Formula |
| Desmoplastic reaction | Collagen ratio | Collagen area (µm2)/total area (µm2) |
| αSMA ratio | αSMA area (µm2)/ total area (µm2) |
| Activated stroma Index (ASI) | αSMA area (µm2)/collagen area (µm2) |
| Angiogenesis | Endothelial area (EA) | CD31 area (µm2)/ total area (µm2) |
| Microvessel lumen area (MLA) | Microvessel lumen (µm2) area/ total area (µm2) |
| Neurogenesis | Neural density | N° of nerves/ total area (µm2) |
| Neural area (%) | Area of nervesx100 (µm2)/ total area (µm2) |
| Neural hypertrophy (µm2) | Area of nerves (µm2)/ total N° of nerves  |
| Neural Invasion | Perineural invasion (PNI) index | N° of nerves with PNI/ total N° of nerves |
| Endoneural invasion (ENI) index | N° of nerves with ENI/ total N° of nerves |
| Neural invasion (NI) index | 2x N° of nerves with ENI + N° of nerves with PNI/ total N° of nerves |

**Supplementary Table 3. Histopathological variables used in the analysis of the PCa stroma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Median survival (mo) | p value univariate | p value multivariate | Hazard ratio (95% CI) |
| Nodal status (N0/N1) | 24.0/14.0 | 0.0793 | 0.001 | 0.618 (0.361-1.058) |
| Surgical margins (R0/R1-2) | 24.0/14.5 | 0.0232 | 0.023 | 0.537 (0.314-0.918) |
| Differentiation (G1-2/G3) | 28.0/14.0 | 0.0316 | 0.032 | 0.552 (0.322-0.949) |
| ASI | 24.5/13.0 | 0.0157 | 0.027 | 0.488 (0.273-0.873) |
| NI index | 24.0/13.0 | 0.0392 | 0.732 | 0.488 (0.247-0.965) |
| CD4+ T cell density | 16.0/23.0 | 0.0475 | 0.052 | 1.989 (1.008-3.927) |
| CD56+ NK cells | 24.0/13.5 | 0.0514 | 0.038 | 0.591 (0.340-1.025) |

**Supplementary Table 4. Univariate and multivariate survival analysis for prognostically relevant clinical and histological features.**  Lymph node status, surgical margins and grade of differentiation were divided into negative and positive groups, while the infiltration with immune cells were divided into low and high groups using the median as cut-off value for ASI, DCs and NK cells, the 25 percentile for CD4+ T cells and the 75 percentile for NI as they appeared to predict more accurately the tumoral behaviour. We used the log-rank test for the univariate analysis and the calculation of the hazard ratio and a Cox regression for the multivariate analysis. CI: confidence interval.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  NeoTx | Primary resected | Figure |
| Inflammation |  |  |  1 |
| Leukocytes |  |  | 1 |
| CD8+ Cytotoxic T cells |  |  | 1 |
| CD103+ CD8+ Dendritic cells  |  |  | 1 |
| CD68+ macrophages |  |  | 1 |
| HLA-DR+CD68+ M1 macrophages |  |  | 1 |
| CD206+CD68+ M2 macrophages |  |  | 2 |
| CD4+ T helper cell density |  |  | 2 |
| Foxp3+CD4+ Treg |  |  | 2 |
| CD56+ NK cells |  |  | 2 |
| CD20+ B lymphocytes |  |  | 2 |
| CD33+CD11+ MDSC |  |  | 2 |
| Proportion CD8+ to MDSC |  |  | 3 |
| Tumor index (%vital tumor cells) |  |  |  3 |
| Ki67+ tumor cells |  |  |  5 |
| Neural invasion index |  |  |  5 |
| Angiogenesis |  |  |  4 |
| Stromal activity |  |  |  4 |
| aSMA+ area |  |  |  4 |
| Collagen+ area |  |  |  4 |
| Activated stroma index  |  |  |  4 |

**Supplementary Table 5. Summary of the histopathology results in the comparison of pancreatic cancer (PCa) tissues after neoadjuvant therapy (neoTx) vs. after primary resection.** Indicates lower amount, indicates higher amount,

 stands for no significant difference in amount.

|  |  |
| --- | --- |
| NeoCTx regime | N° of patients (n=37) |
| Gemcitabine mono | 6 (16%) |
| Gemcitabine + Erlotinib | 5 (14%) |
| Gemcitabine + Oxaliplatin | 8 (22%) |
| Folfirinox | 13 (34%) |
| Other regimes (e.g. radiotherapy) | 5 (14%) |

**Supplementary Table 6. Neoadjuvant therapy regimes**