**Infiltration by interleukin-22 producing T cells promotes neutrophil recruitment and predicts favorable clinical outcome in human colorectal cancer**

 **Table 1S: Testing TMA. Characteristics of CRC patient cohort (n=522)\***

|  |  |  |
| --- | --- | --- |
| **Characteristics** | **N or mean** | **(% or range)** |
|  |  |  |  |
| **Age** | **years (median, mean)** | 71, 69.7 | (40-96) |
|  |  |  |  |
| **Diameter** | **mm (median, mean)** | 50, 51.7 | (5-170) |
|  |  |  |  |
|  |  |  |  |
| **Gender** | **Female (%)** | 264 | (50.6) |
|  | **Male (%)** | 258 | (49.4) |
|  |  |  |  |
|  |  |  |  |
| **Tumor location** | **Left-sided (%)** | 337 | (64.6) |
|  | **Right-sided (%)** | 184 | (35.2) |
|  |  |  |  |
|  |  |  |  |
| **pT stage** | **T1 (%)** | 17 | (3.3) |
|  | **T2 (%)** | 67 | (12.8) |
|  | **T3 (%)** | 355 | (68.0) |
|  | **T4 (%)** | 69 | (13.2) |
|  |  |  |  |
|  |  |  |  |
| **pN stage** | **N0 (%)** | 255 | (48.9) |
|  | **N1 (%)** | 146 | (28.0) |
|  | **N2 (%)** | 100 | (19.2) |
|  |  |  |  |
|  |  |  |  |
| **Tumor grade** | **G1 (%)** | 11 | (2.1) |
|  | **G2 (%)** | 462 | (88.5) |
|  | **G3 (%)** | 34 | (6.5) |
|  |  |  |  |
|  |  |  |  |
| **UICC stage** | **Stage IA (%) T1N0** | 11 | (2.1) |
|  | **Stage IB (%) T2N0** | 47 | (9.0) |
|  | **Stage IIA (%) T3N0** | 172 | (33.0) |
|  | **Stage IIB-C (%) T4N0** | 19 | (3.6) |
|  | **Stage III (%) >N0** | 246 | (49.4) |
|  |  |  |  |
|  |  |  |  |
| **Tumor border** | **Infiltrative (%)** | 360 | (69.0) |
|  | **Pushing (%)** | 146 | (28.0) |
|  |  |  |  |
|  |  |  |  |
| **Vascular invasion** | **No (%)** | 364 | (69.7) |
|  | **Yes (%)** | 144 | (27.6) |
|  |  |  |  |
|  |  |  |  |
| **Microsatellite Stability** | **Proficient (%)** | 439 | (84.1) |
|  | **Deficient (%)** | 83 | (15.9) |
|  |  |  |  |
| **Rectal cancers** | **Rectal cancers (%)** | 179 | (34.3) |
|  | **Rectosigmoid cancers (%)** | 31 | (5.9) |
|  |  |  |  |
| **Overall survival time** | **months** | 64.5 | 0-152 |
|  |  |  |  |
| **5-year survival rate** | **5-years survival % (95%CI)**  | 0.49 | 0.44 – 0.53 |
|  |  |  |  |

\*percentage may not add to 100% due to missing values of same variables; age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ2 test. Survival analysis was performed using the Kaplan-Meier method.

**Table 2S: Validation TMA. Characteristics of CRC patient cohort (n=89)\***

|  |  |  |
| --- | --- | --- |
| **Characteristics** | **N or mean** | **(% or range)** |
|  |  |  |  |
| **Age** | **years (median, mean)** | 76, 73.2 | (40-98) |
|  |  |  |  |
| **Diameter** | **mm (median, mean)** | 40, 43.3 | (8-90) |
|  |  |  |  |
|  |  |  |  |
| **Gender** | **Female (%)** | 36 | (40.4) |
|  | **Male (%)** | 53 | (59.6) |
|  |  |  |  |
|  |  |  |  |
| **pT stage** | **T1 (%)** | 6 | (6.7) |
|  | **T2 (%)** | 20 | (22.5) |
|  | **T3 (%)** | 41 | (46.1) |
|  | **T4 (%)** | 22 | (24.7) |
|  |  |  |  |
|  |  |  |  |
| **pN stage** | **N0 (%)** | 43 | (48.4) |
|  | **N1 (%)** | 29 | (32.6) |
|  | **N2 (%)** | 16 | (18.0) |
|  |  |  |  |
|  |  |  |  |
| **Tumor grade** | **G2 (%)** | 60 | (67.4) |
|  | **G3 (%)** | 24 | (32.6) |
|  |  |  |  |
|  |  |  |  |
| **Tumor border configuration** | **Infiltrative (%)** | 360 | (69.0) |
|  | **Pushing (%)** | 146 | (28.0) |
|  |  |  |  |
| **Overall survival time** | **months** | 30-35.8 | 1-93 |

**Table 3S: Clinico-pathological features of the TCGA collective (n=597 CRC).**

|  |  |  |
| --- | --- | --- |
| **Characteristics** | **N or mean** | **(% or range)\*** |
| **Age** | **years (median)** | 68 |  |
| **Gender** | **Female (%)** | 275 | (46) |
| **Male (%)** | 322 | (54) |
| **Tumor location** | **Colon & (mucosal type) (%)** | 373 & (60) | (62.5) & (10) |
| **Rectum & (mucosal type) (%)** | 140 & (13) | (23.5) & (2) |
| **pT stageTumor stage** | **T1 (%)** | 103 | (17) |
| **T2 (%)** | 215 | (36) |
| **T3 (%)** | 174 | (29) |
| **T4 (%)** | 85 | (14) |
| **Not assessed (%)** | 20 | (3) |
| **Perineural invasion** | **Absent (%)** | 170 | (28.5) |
| **Present (%)** | 59 | (10) |
| **Lymphatic invasion** | **Absent (%)** | 326 | (55) |
| **Present (%)** | 210 | (35) |
| **Venous invasion** | **Absent (%)** | 393 | (66) |
| **Present (%)** | 125 | (21) |
| **Microsatellite instability** | **Absent (%)** | 105 | (18) |
| **Present (%)** | 11 | (2) |

\* percentages may not add to 100% since calculated with respect to the overall collective of 597 CRC cases and/or because of rounding.

**Table 4S: Primers list used in the study**

|  |  |  |
| --- | --- | --- |
|  | **code/seq** | **company** |
| **CCL5** | Hs00982282\_m1 | Applied Biosystems |
| **CCL20** | Hs00171125\_m1 | " |
| **CCL22** | Hs00171080\_m1 | " |
| **CXCL1** | Hs00236937\_m1 | " |
| **CXCL2** | Hs00236966\_m1 | " |
| **CXCL3** | Hs00171061\_m1 | " |
| **CXCL8** | Hs00174103\_m1 | " |
| **CXCL9** | Hs00171065\_m1 | " |
| **CXCL10** | Hs99999049\_m1 | " |
| **CXCL11** | Hs00171138\_m1 | " |
| **CX3CL1** | Hs00171086\_m1 | " |
| **IL17A** | Hs99999082\_m1 | “ |
| **IL22** | Hs01574154\_m1 | “ |
| **IL22Ra** | Hs00222035\_m1 | " |
| **IL10Rb** | Hs00175123\_m1 | “ |
| **GAPDH** | Hs02786624\_g1 | “ |
| **CD66b** (Forward) | 5’ TCA AAG CAT TTG CAA TCA | Microsynth |
| **CD66b (**Reverse) | 5’ GTG GGC AAC TTC ACA AAG | “ |
| **GAPDH** (Forward) | 5’ GCT CCT CCT GTT CGA CAG TCA | “ |
| **GAPDH (**Reverse) | 5’ ACC TTC CCC ATG GTG TCT GA | “ |

**Table 5S: Uni- and Multivariate\* Hazard Cox regression survival analysis in the validation TMA** (\*performed with 81 cases – 5 excluded because treated with neoadjuvant chemotherapy, further 3 not included because of missing values)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **HR (95% CI)** | **P-Values** | **HR (95% CI)** | **P-Values** |
|  |  |  |  |  |
| **IL22 low vs high** | **0.32** (0.12-0.89) | **0.03** | **0.22** (0.06-0.79) | **0.021** |
|  |  |  |  |  |
| **Adjuvant chemotherapy** | **3.08** (1.17-8.17) | **0.23** | **1.39** (0.42-4.60) | **0.586** |
|  |  |  |  |  |
| **Gender (men vs women)** | **0.82** (0.32-2.13) | **0.68** | **0.59** (0.19-1.86) | **0.368** |
|  |  |  |  |  |
| **Age** | **1.02** (0.97-1.07) | **0.44** | **1.02** (0.97-1.07) | **0.499** |
|  |  |  |  |  |
| **Tumor Grade** | **3.49** (1.28-9.53) | **0.02** | **2.62** (0.91-7.61) | **0.077** |
|  |  |  |  |  |
| **UICC (1-2 vs 3-4)** | **8.63** (1.97-37.82) | **0.004** | **7.44** (1.38-7.61) | **0.019** |
|  |  |  |  |  |