**Association between CD8+ tumor infiltrating lymphocytes and the clinical outcome of patients with operable breast cancer treated with adjuvant dose-dense chemotherapy. A 10 - year follow up report of a Hellenic Cooperative Oncology Group observational study.**

Nikolaos Spathas et al

**Supplementary Materials**

**Supplemental Figure 1**. Distribution of sCD8, iCD8 and total CD8.

**Graphical user interface, table, Excel

Description automatically generated**

**Supplemental Table 1.** Staining protocols and manufacturers for IHC markers. ER1:citric acid-pH6, ER2: EDTA- pH 9, enz2: Proteinase K, RT: Room Temperature

|  |  |  |  |
| --- | --- | --- | --- |
| **IHC Abs** | **Clone/code** | **Manufacturer** | **staining protocol** |
| ER | Clone 6F11, code NCL-L-ER-6F11 | Novocastra, Leica Biosystems Newcastle Upon Tune, UK | Antigen retrieval-20'ER1, Antibody dilution-1:70-20-RT', Bond Refine Detection kit protocol |
| PgR | Clone 1A6, code NCL-L-PGR | Novocastra, Leica Biosystems Newcastle Upon Tune, UK | Antigen retrieval-20'ER1, Antibody dilution-1:70-20'-RT, Bond Refine Detection kit protocol |
| HER2 | polyclonal, Code A 0485 | DAKO, Glostrup, DK | Antigen retrieval-20'ER1, Antibody dilution-1:500-30'-RT, Bond Refine Detection kit protocol |
| Ki 67 | Clone MIB-1, code M 7240 | DAKO, Glostrup, DK | Antigen retrieval-20'ER2, Antibody dilution- 1:70-20'-RT, Bond Refine Detection kit protocol |
| CK5 | Clone XM26, code NCL-L-CK5 | Novocastra, Leica Biosystems Newcastle Upon Tune, UK | Antigen retrieval-20'ER2, Antibody dilution- 1:50-20'-RT, Bond Refine Detection kit protocol |
| CD8 | Clone C8-144B, code M7103 | DAKO, Glostrup, DK | Antigen retrieval-20'ER2, Antibody dilution- 1:80-20'-RT, Bond Refine Detection kit protocol |
| EGFR | Clone 31G7, code 28-005 | Invitrogen Corporation, USA | Antigen retrieval -enz2-8', Antibody dilution-1:50-20'RT, Bond Refine Detection kit protocol |

**Supplemental Table 2**. Association of iCD8, sCD8 and tCD8 with selected clinicopathological parameters of interest.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **iCD8** | | | | **sCD8** | | | **tCD8** | | |
|  | **Low\*** | | **High\*** | **p-value** | **Low\*** | **High\*** | **p-value** | **Low\*** | **High\*** | **p-value** |
| **Age at diagnosis (years)** | 54.4(29.2,82.7) | | 52.1(29.0,75.4) | 0.085 | 53.2(29.0,82.7) | 53.8(30.2,75.4) | 0.71 | 54.1(29.0,82.7) | 52.7(30.7,76.8) | 0.15 |
| **Ki67** | 27.0(0.00,100.0) | | 60.0(0.00,100.0) | **<0.001** | 28.0(0.00,100.0) | 48.0(0.00,100.0) | **<0.001** | 28.0(0.00,100.0) | 55.0(0.00,100.0) | **<0.001** |
| **TILs** | 5.0(1.00,65.0) | | 20.0(2.0,90.0) | **<0.001** | 5.0(1.00,80.0) | 20.0(2.0,90.0) | **<0.001** | 5.0(1.00,80.0) | 20.0(2.0,90.0) | **<0.001** |
| **Menopausal status** |  | |  | 0.17 |  |  | 0.90 |  |  | 0.54 |
| Premenopausal | 200(42.5) | | 76(48.7) |  | 208(44.2) | 68(43.6) |  | 204(43.3) | 72(46.2) |  |
| Postmenopausal | 271(57.5) | | 80(51.3) |  | 263(55.8) | 88(56.4) |  | 267(56.7) | 84(53.8) |  |
| **Breast surgery** |  | |  | 0.32 |  |  | 0.76 |  |  | 0.22 |
| Modified radical | 257(54.6) | | 78(50.0) |  | 250(53.1) | 85(54.5) |  | 245(52.0) | 90(57.7) |  |
| Partial mastectomy | 214(45.4) | | 78(50.0) |  | 221(46.9) | 71(45.5) |  | 226(48.0) | 66(42.3) |  |
| **Tumor size** |  | |  | 0.82 |  |  | 0.44 |  |  | 0.65 |
| ≤2 cm | 177(37.6) | | 62(39.7) |  | 185(39.3) | 54(34.6) |  | 184(39.1) | 55(35.3) |  |
| 2.1-5 cm | 258(54.8) | | 84(53.8) |  | 250(53.1) | 92(59.0) |  | 254(53.9) | 88(56.4) |  |
| >5 cm | 36(7.6) | | 10(6.4) |  | 36(7.6) | 10(6.4) |  | 33(7.0) | 13(8.3) |  |
| **Nodal status** |  | |  | 0.61 |  |  | 0.97 |  |  | 0.48 |
| 0-3 | 329(70.3) | | 113(72.4) |  | 332(70.8) | 110(71.0) |  | 328(70.1) | 114(73.1) |  |
| ≥4 | 139(29.7) | | 43(27.6) |  | 137(29.2) | 45(29.0) |  | 140(29.9) | 42(26.9) |  |
| **Grade** |  | |  | **<0.001** |  |  | **<0.001** |  |  | **<0.001** |
| I-II | 279(59.2) | | 57(36.5) |  | 277(58.8) | 59(37.8) |  | 273(58.0) | 63(40.4) |  |
| III | 192(40.8) | | 99(63.5) |  | 194(41.2) | 97(62.2) |  | 198(42.0) | 93(59.6) |  |
| **ER/PgR status (central assessment)** |  | |  | **<0.001** |  |  | **<0.001** |  |  | **<0.001** |
| Positive | 371(85.5) | | 107(73.3) |  | 375(85.4) | 103(73.0) |  | 373(85.6) | 105(72.9) |  |
| Negative | 63(14.5) | | 39(26.7) |  | 64(14.6) | 38(27.0) |  | 63(14.4) | 39(27.1) |  |
| **HER2 status (central assessment)** |  | |  | 0.95 |  |  | 0.16 |  |  | 0.16 |
| Positive | 83(17.7) | | 27(17.4) |  | 77(16.4) | 33(21.3) |  | 77(16.4) | 33(21.3) |  |
| Negative | 387(82.3) | | 128(82.6) |  | 393(83.6) | 122(78.7) |  | 393(83.6) | 122(78.7) |  |
|  | |  |  |  |  |  |  |  |  |  |
| \*These values represent median (m), range | |  |  |  |  |  |  |  |  |  |

**Supplemental Table 3**. Sites of relapse in the entire cohort of patients.

|  |  |
| --- | --- |
|  | **Total cohort (N=979)** |
|  | **N (%)** |
| ***Locoregional relapse*** | 19(1.9) |
| Axillary nodes | 6(0.6) |
| Supraclavicular nodes | 4(0.4) |
| Skin | 5(0.5) |
| Residual breast | 5(0.5) |
| ***Distant relapse*** | 151(15.4) |
| Brain | 27(2.8) |
| Bones | 76(7.8) |
| Lung/Pleura | 37(3.8) |
| Liver | 50(5.1) |
| Nodes | 20(2.0) |
| Skin | 1(0.1) |
| Contralateral breast | 6(0.6) |
| Adrenal | 1(0.1) |
| Peritoneal implants | 1(0.1) |
| Cervix | 1(0.1) |
| Abdomen | 1(0.1) |
| Kidney | 1(0.1) |

*\*Six patients had both locoregional and distant relapse.*

**Supplemental Table 4**. Association of clinicopathological parameters of interest with patients’ outcome.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Event/Total** | **HR (95% CI)** | **p-value** | **Event/Total** | **HR (95% CI)** | **p-value** |
|  | ***DFS*** | | | ***OS*** | | |
| **Age** |  | 1.02 (1.00-1.03) | **0.002** |  | 1.03 (1.01-1.04) | **<.001** |
| **Menopausal status** |  |  |  |  |  |  |
| Postmenopausal | 163/564 | 1.34 (1.04-1.73) | **0.026** | 132/564 | 1.44 (1.08-1.93) | **0.014** |
| Premenopausal | 90/415 | Reference | -- | 69/415 | Reference | -- |
| **Breast surgery** |  |  |  |  |  |  |
| Modified radical | 165/545 | 1.60 (1.24-2.08) | **<.001** | 133/545 | 1.65 (1.23-2.21) | **<.001** |
| Partial mastectomy | 88/434 | Reference | -- | 68/434 | Reference | -- |
| **Tumor size** |  |  |  |  |  |  |
| >5 cm | 28/77 | 2.21 (1.43-3.41) | **<.001** | 24/77 | 2.56 (1.58-4.13) | **<.001** |
| 2.1-5 cm | 149/506 | 1.62 (1.23-2.13) | **<.001** | 122/506 | 1.82 (1.32-2.50) | **<.001** |
| ≤2 cm | 76/395 | Reference | -- | 55/395 | Reference | -- |
| **Nodal status** |  |  |  |  |  |  |
| ≥4 | 118/294 | 2.44 (1.90-3.12) | **<.001** | 101/294 | 2.79 (2.12-3.69) | **<.001** |
| 0-3 | 133/681 | Reference | -- | 98/681 | Reference | -- |
| **Adjuvant HT** |  |  |  |  |  |  |
| Yes | 182/721 | 0.85 (0.65-1.13) | 0.27 | 141/721 | 0.78 (0.58-1.07) | 0.12 |
| No | 69/251 | Reference | -- | 58/251 | Reference | -- |
| **Adjuvant RT** |  |  |  |  |  |  |
| Yes | 191/708 | 1.24 (0.92-1.66) | 0.16 | 156/708 | 1.43 (1.01-2.01) | **0.043** |
| No | 58/259 | Reference | -- | 41/259 | Reference | -- |
| **Grade** |  |  |  |  |  |  |
| I-II | 129/526 | 0.84 (0.66-1.08) | 0.18 | 97/526 | 0.76 (0.58-1.01) | 0.056 |
| III | 124/452 | Reference | -- | 104/452 | Reference | -- |
| **ER/PgR status (central assessment)** |  |  |  |  |  |  |
| Positive | 137/534 | 1.34 (0.84-2.12) | 0.22 | 110/534 | 1.17 (0.72-1.90) | 0.53 |
| Negative | 21/109 | Reference | -- | 19/109 | Reference | -- |
| **HER2 status (central assessment)** |  |  |  |  |  |  |
| Positive | 22/121 | 0.66 (0.43-1.04) | 0.073 | 20/121 | 0.76 (0.47-1.22) | 0.26 |
| Negative | 154/575 | Reference | -- | 125/575 | Reference | -- |

HR, hazard ratio; CI, confidence interval

**Supplemental Table 5**. Association of sCD8, iCD8 and total CD8 with DFS and OS in the entire cohort of patients and among patients with luminal B tumors.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Event/Total** | **HR (95% CI)** | **p-value** | **Event/Total** | **HR (95% CI)** | **p-value** |
|  | ***DFS*** | | | ***OS*** | | |
|  | ***Entire cohort*** | | | | | |
| **sTILs^** |  | 0.94 (0.85-1.04) | 0.23 |  | 0.96 (0.86-1.07) | 0.41 |
| **iCD8** |  |  |  |  |  |  |
| High | 27/156 | 0.58 (0.39-0.88) | **0.011** | 23/156 | 0.63 (0.40-0.98) | **0.043** |
| Low | 133/471 | Reference | -- | 106/471 | Reference | -- |
| **sCD8** |  |  |  |  |  |  |
| High | 31/156 | 0.70 (0.47-1.03) | 0.070 | 26/156 | 0.73 (0.48-1.13) | 0.16 |
| Low | 129/471 | Reference | -- | 103/471 | Reference | -- |
| **tCD8** |  |  |  |  |  |  |
| High | 30/156 | 0.65 (0.44-0.97) | **0.034** | 22/156 | 0.58 (0.37-0.92) | **0.020** |
| Low | 130/471 | Reference | -- | 107/471 | Reference | -- |
|  | ***Luminal B*** | | | | | |
| **sTILs^** |  | 1.00 (0.85-1.17) | 0.95 |  | 1.03 (0.87-1.22) | 0.74 |
| **iCD8** |  |  |  |  |  |  |
| High | 16/68 | 0.74 (0.42-1.30) | 0.30 | 14/68 | 0.78 (0.43-1.42) | 0.41 |
| Low | 54/176 | Reference | -- | 45/176 | Reference | -- |
| **sCD8** |  |  |  |  |  |  |
| High | 14/62 | 0.70 (0.39-1.25) | 0.23 | 12/62 | 0.70 (0.37-1.32) | 0.27 |
| Low | 56/182 | Reference | -- | 47/182 | Reference | -- |
| **tCD8** |  |  |  |  |  |  |
| High | 12/60 | 0.59 (0.32-1.10) | 0.098 | 10/60 | 0.58 (0.29-1.14) | 0.12 |
| Low | 58/184 | Reference | -- | 49/184 | Reference | -- |

HR, hazard ratio; CI, confidence interval

^10% increments