

## Supplementary materials

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**Table 1. Spearman correlation between relative eosinophil count and cancer characteristics.**

|                | <b>Spearman correlation coefficient</b> | <b>P value</b> |
|----------------|---|----------------|
| <b>Age</b>     | 0.145                                   | < 0.0001       |
| <b>ER%</b>     | 0.053                                   | 0.116          |
| <b>PgR%</b>    | 0.050                                   | 0.135          |
| <b>Ki67</b>    | -0.050                                  | 0.134          |
| <b>Size mm</b> | -0.036                                  | 0.273          |

ER: Estrogen Receptor; PgR: Progesteron Receptor.

**Table 2. Treatment details in the groups of patients REC-low and REC-high.**

|   | REC < 1,5%<br>(N 393) |       | REC ≥ 1,5%<br>(N 537) |       |
|---|-----------------------|-------|-----------------------|-------|
|   | N of patients         | %     | N of patients         | %     |
| <b>Neoadjuvant treatment</b>                    |                       |       |                       |       |
| Yes   | 146                   | 37.2% | 161                   | 30%   |
| No  | 247                   | 62.8% | 376                   | 70%   |
| <b>Type of neoadjuvant chemotherapy (N 307)</b> |                       |       |                       |       |
| EC --> Ptx/Txt                                  | 89                    | 61%   | 81                    | 50.3% |
| EC --> CBDCA-Ptx                                | 6                     | 4.1%  | 5                     | 3.1%  |
| FEC   | 2                     | 1.4%  | 5                     | 3.1%  |
| FEC --> Ptx/Txt                                 | 37                    | 25.3% | 56                    | 34.8% |
| THC   | 1                     | 0.7%  | -                     | -     |
| Hormonotherapy                                  | 1                     | 0.7%  | 3                     | 1.9%  |
| Other   | 9                     | 6.2%  | 11                    | 6.8%  |
| Unknown   | 1                     | 0.7%  | -                     | -     |
| <b>Adjuvant chemotherapy</b>                    |                       |       |                       |       |
| Yes   | 117                   | 29.8% | 154                   | 28.7% |
| No  | 274                   | 69.7% | 382                   | 71.1% |
| Unknown   | 2                     | 0.5%  | 1                     | 0.2%  |
| <b>Type of adjuvant chemotherapy (N 271)</b>    |                       |       |                       |       |
| EC --> Ptx/Txt                                  | 15                    | 12.8% | 22                    | 14.3% |
| EC --> CBDCA-Ptx                                | 1                     | 0.9%  | -                     | -     |
| FEC   | 46                    | 39.3% | 51                    | 33.1% |
| FEC --> Ptx/Txt                                 | 38                    | 32.5% | 61                    | 39.6% |
| THC/TC  | -                     | -     | 2                     | 1.3%  |
| Capecitabine                                    | 7                     | 6%    | 1                     | 0.6%  |
| Endoxan   | 2                     | 1.7%  | -                     | -     |
| Other   | 6                     | 5.1%  | 14                    | 9.1%  |
| Unknown   | 2                     | 1.7%  | 3                     | 1.9%  |
| <b>Trastuzumab</b>                              |                       |       |                       |       |
| Yes   | 70                    | 1.8%  | 86                    | 16%   |
| No  | 322                   | 81.9% | 451                   | 84%   |
| Unknown   | 1                     | 0.3%  | -                     | -     |
| <b>Radiotherapy</b>                             |                       |       |                       |       |
| Yes   | 309                   | 78.6% | 427                   | 79.5% |
| No  | 82                    | 20.9% | 107                   | 19.9% |
| Unknown   | 2                     | 0.5%  | 3                     | 0.6%  |
| <b>Hormonotherapy</b>                           |                       |       |                       |       |
| Yes   | 265                   | 67.4% | 411                   | 76.5% |
| No  | 125                   | 31.8% | 122                   | 22.7% |
| Unknown   | 3                     | 0.8%  | 4                     | 0.7%  |
| <b>Type of hormonotherapy (N 676)</b>           |                       |       |                       |       |
| SERM  | 106                   | 40%   | 155                   | 37.7% |
| AI  | 127                   | 47.9% | 215                   | 52.3% |
| SERM + AI                                       | 30                    | 11.3% | 35                    | 8.5%  |
| Unknown   | 2                     | 0.8%  | 6                     | 1.5%  |

REC: Relative Eosinophil Count; N: Number; EC: Epirubicin-Cyclophosphamide; Ptx: Paclitaxel; Txt: Docetaxel; CBDCA: Carboplatin; FEC: 5-Fluorouracil- Epirubicin-Cyclophosphamide; THC: Docetaxel-Carboplatin-Herceptin; TC: Docetaxel-Carboplatin; SERM: Selective Estrogen Receptor Modulators; AI: Aromatase Inhibitor.

**Table 3. TTF and BCSS according to REC, RLC and ELP at baseline.**

|             |                                | REC                 |        | RLC                 |         | ELP                 |        |
|-------------|--------------------------------|---------------------|--------|---------------------|---------|---------------------|--------|
|             |                                | < 1.5%              | ≥ 1.5% | < 17.5%             | ≥ 17.5% | < 36.5              | ≥ 36.5 |
| <b>TTF</b>  | <b>5y-TTF</b>                  | 74%                 | 84%    | 51%                 | 75%     | 74%                 | 84%    |
|             | <b>10y-TTF</b>                 | 67%                 | 76%    | 51%                 | 71%     | 67%                 | 86%    |
|             | <b>P value (Log-Rank test)</b> | 0.001               |        | < 0.0001            |         | 0.003               |        |
|             | <b>HR (95% CI)</b>             | 0.610 (0.458-0.812) |        | 0.421 (0.262-0.677) |         | 0.646 (0.484-0.861) |        |
| <b>BCSS</b> | <b>5y-BCSS</b>                 | 86%                 | 90%    | 74%                 | 89%     | 86%                 | 90%    |
|             | <b>10y-BCSS</b>                | 79%                 | 87%    | 62%                 | 85%     | 79%                 | 87%    |
|             | <b>P value (Log-Rank test)</b> | 0.021               |        | < 0.0001            |         | 0.003               |        |
|             | <b>HR (95% CI)</b>             | 0.632 (0.433-0.923) |        | 0.350 (0.200-0.614) |         | 0.572 (0.392-0.834) |        |

TTF: Time to Treatment Failure; BCSS: Breast Cancer Specific Survival; REC: Relative Eosinophil Count; RLC: Relative Lymphocyte Count; ELP: Eosinophil-Lymphocyte Product; y: years; HR: Hazard Ratio; CI: Confidence Interval.

**Table 4. Association of relapse with REC, RLC and ELP at baseline.**

|                       | <b>Relapse<br/>N of patients (%)</b> | <b>No relapse<br/>N of patients (%)</b> | <b>p value (Chi-Square<br/>test)</b> |
|-----------------------|--------------------------------------|---|--------------------------------------|
| <b>REC &lt; 1,5%</b>  | 97 (24.7%)                           | 296 (75.3%)                             | 0.005                                |
| <b>REC ≥ 1,5%</b>     | 92 (17.1%)                           | 445 (82.9%)                             |                                      |
| <b>RLC &lt; 17.5%</b> | 19 (35.8%)                           | 34 (64.2%)                              | 0.004                                |
| <b>RLC ≥ 17.5%</b>    | 170 (19.4%)                          | 707 (80.6%)                             |                                      |
| <b>ELP &lt; 36,5</b>  | 85 (24.8%)                           | 258 (75.2%)                             | 0.01                                 |
| <b>ELP ≥ 36.5</b>     | 104 (17.7%)                          | 483 (82.3%)                             |                                      |

N: Number; REC: Relative Eosinophil Count; RLC: Relative Lymphocyte Count; ELP: Eosinophil-Lymphocyte Product.

**Table 5. Multivariate analysis for relapse.**

| Variable  | Chi Square test               | Mann-Whitney U test/Kruskal Wallis test | Multivariate analysis | OR (95% CI)         |
|---|-------------------------------|---|-----------------------|---------------------|
| Age   | -                             | 0.664                                   |                       |                     |
| ER (pos/neg)<br>ER%   | <0.0001*<br>-                 | -<br><0.0001 <sup>¶*</sup>              | 0.843                 |                     |
| PgR (pos/neg)<br>PgR%   | <0.0001*<br>-                 | -<br><0.0001 <sup>¶*</sup>              | 0.800                 |                     |
| HER2  | 0.176                         | -                                       |                       |                     |
| Ki67 (</≥ 20%)<br>Ki67%   | 0.004*<br>-                   | -<br><0.0001 <sup>¶*</sup>              | 0.053                 |                     |
| T stage<br>Size mm  | <0.0001*<br>-                 | -<br><0.0001 <sup>¶*</sup>              | 0.434                 |                     |
| N stage   | <0.0001 <sup>¶*</sup>         | -                                       | 0.004*                | 2.392 (1.324-4.321) |
| Tumor grade   | 0.023 <sup>¶*</sup>           | -                                       | 0.857                 |                     |
| Histology   | 0.056 <sup>¶</sup>            | -                                       | 0.915                 |                     |
| Lymphovascular invasion   | <0.0001 <sup>¶*</sup>         | -                                       | 0.449                 |                     |
| Chemotherapy  | 0.002 <sup>¶*</sup>           | -                                       | 0.689                 |                     |
| Anti-HER2 treatment   | 0.092 <sup>¶</sup>            | -                                       | 0.232                 |                     |
| Radiotherapy  | 0.464                         | -                                       |                       |                     |
| Hormonotherapy  | <0.0001 <sup>¶*</sup>         | -                                       | 0.529                 |                     |
| BRCA mutation   | 0.082 <sup>¶</sup>            | -                                       | 0.100                 |                     |
| PLT   | -                             | 0.234                                   |                       |                     |
| Relative neutrophils<br>Absolute neutrophils                                  | -<br>-                        | 0.053<br>0.029* <sup>¶</sup>            | 0.979                 |                     |
| Relative lymphocytes <sup>§</sup><br>Absolute lymphocytes<br>RLC <sup>§</sup> | -<br>-<br>0.004 <sup>¶*</sup> | 0.093<br>0.616<br>-                     | 0.376                 |                     |
| Relative monocytes<br>Absolute monocytes                                      | -<br>-                        | 0.236<br>0.684                          |                       |                     |
| Relative eosinophils <sup>§</sup><br>Absolute eosinophils<br>REC <sup>§</sup> | -<br>-<br>0.005 <sup>¶*</sup> | 0.007*<br>0.037*<br>-                   | 0.704                 |                     |
| Relative basophils<br>Absolute basophils                                      | -<br>-                        | 0.158<br>0.834                          |                       |                     |

<sup>¶</sup> Variables included in the multivariate analysis

\*Statistically significant results

<sup>§</sup>Relative lymphocyte and relative eosinophils are considered as continuous variables, while RLC and REC are considered as dichotomic variables with 17.5% as cut-off for RLC and 1.5% for REC

OR: Odds Ratio; CI: Confidence Interval; ER: Estrogen Receptor; PgR: Progesteron Receptor; N: Number; PLT; Platelet; RLC: Relative Lymphocyte Count; REC: Relative Eosinophil Count.

**Table 6. TTF and BCSS according to REC, RLC and ELP after surgery.**

|             |                      | REC                 |        | RLC                 |         | ELP                 |        |
|-------------|----------------------|---------------------|--------|---------------------|---------|---------------------|--------|
|             |                      | < 1.5%              | ≥ 1.5% | < 17.5%             | ≥ 17.5% | < 36.5              | ≥ 36.5 |
| <b>TTF</b>  | <b>5y-TTF</b>        | 67%                 | 79%    | 51%                 | 75%     | 66%                 | 74%    |
|             | <b>10y-TTF</b>       | 63%                 | 75%    | 51%                 | 75%     | 66%                 | 70%    |
|             | <b>Log-Rank test</b> | 0.187               |        | 0.209               |         | 0.179               |        |
|             | <b>HR (95% CI)</b>   | 0.771 (0.526-1.129) |        | 0.755 (0.487-1.172) |         | 0.776 (0.535-1.125) |        |
| <b>BCSS</b> | <b>5y-BCSS</b>       | 78%                 | 86%    | 69%                 | 86%     | 79%                 | 86%    |
|             | <b>10y-BCSS</b>      | 72%                 | 82%    | 51%                 | 83%     | 69%                 | 82%    |
|             | <b>Log-Rank test</b> | 0.025               |        | 0.666               |         | 0.018               |        |
|             | <b>HR (95% CI)</b>   | 0.583 (0.361-0.940) |        | 0.874 (0.472-1.616) |         | 0.571 (0.357-0.913) |        |

TTF: Time to Treatment Failure; BCSS: Breast Cancer Specific Survival; REC: Relative Eosinophil Count; RLC: Relative Lymphocyte Count; ELP: Eosinophil-Lymphocyte Product; y: years; HR: Hazard Ratio; CI: Confidence Interval.

**Table 7. RLC in the group of 741 patients without relapse.**

|                             |                         | RLC baseline   | RLC post-surgery | RLC 1y follow-up | RLC 2y follow-up | RLC 5y follow-up | RLC 10y follow-up | Friedman test |
|-----------------------------|-------------------------|----------------|------------------|------------------|------------------|------------------|-------------------|---------------|
| <b>N pts</b>                |                         | 741            | 532              | 600              | 554              | 408              | 172               |               |
| <b>Median</b>               |                         | 29%            | 24.8%            | 27.2%            | 29.8%            | 30.5%            | 31%               | < 0.0001      |
| <b>SD</b>                   |                         | 7.80           | 7.91             | 7.69             | 8.18             | 8.76             | 8.83              |               |
|                             |                         | <b>p value</b> |                  |                  |                  |                  |                   |               |
| <b>Bonferroni-Dunn test</b> | <b>RLC baseline</b>     | -              | < 0.0001         | 0.023            | 0.083            | 0.001            | 0.001             | NA            |
|                             | <b>RLC post-surgery</b> | < 0.0001       | -                | < 0.0001         | < 0.0001         | < 0.0001         | < 0.0001          | NA            |
|                             | <b>RLC 1y follow-up</b> | 0.023          | < 0.0001         | -                | < 0.0001         | < 0.0001         | < 0.0001          | NA            |
|                             | <b>RLC 2y follow-up</b> | 0.083          | < 0.0001         | < 0.0001         | -                | 1                | 0.437             | NA            |
|                             | <b>RLC 5y follow-up</b> | 0.001          | < 0.0001         | < 0.0001         | 1                | -                | 1                 | NA            |
|                             | <b>RLC10y follow-up</b> | 0.001          | < 0.0001         | < 0.0001         | 0.437            | 1                | -                 | NA            |

RLC: Relative Lymphocyte Count; y: years; N: Number; SD: Standard Deviation; NA: Not Applicable.

**Table 8. RLC in the group of 189 patients with relapse.**

|                              |                          | RLC baseline                        | RLC post-surgery | RLC 1y follow-up | RLC 2y follow-up | RLC 5y follow-up | RLC 10y follow-up | RLC relapse | Friedman test |
|------------------------------|--------------------------|-------------------------------------|------------------|------------------|------------------|------------------|-------------------|-------------|---------------|
| <b>N pts</b>                 |                          | 189                                 | 142              | 123              | 97               | 45               | 4                 | 158         |               |
| <b>Median</b>                |                          | 27.4%                               | 24.5%            | 26.6%            | 28.7%            | 32.9%            | 26.6%             | 23.4%       | < 0.0001      |
| <b>SD</b>                    |                          | 8.74                                | 8.76             | 8.30             | 8.87             | 9.84             | 6.11              | 10.45       |               |
|                              |                          | <b>p value Bonferroni-Dunn test</b> |                  |                  |                  |                  |                   |             |               |
| <b>Bonferro ni-Dunn test</b> | <b>RLC baseline</b>      | -                                   | 0.055            | 1                | 1                | 0.264            | 1                 | 0.001       | NA            |
|                              | <b>RLC post-surgery</b>  | 0.055                               | -                | 1                | 0.001            | < 0.0001         | 1                 | 1           | NA            |
|                              | <b>RLC 1y follow-up</b>  | 1                                   | 1                | -                | 0.551            | 0.063            | 1                 | 0.103       | NA            |
|                              | <b>RLC 2y follow-up</b>  | 1                                   | 0.001            | 0.551            | -                | 1                | 1                 | < 0.0001    | NA            |
|                              | <b>RLC 5y follow-up</b>  | 0.264                               | < 0.0001         | 0.063            | 1                | -                | 1                 | < 0.0001    | NA            |
|                              | <b>RLC 10y follow-up</b> | 1                                   | 1                | 1                | 1                | 1                | -                 | 1           | NA            |
|                              | <b>RLC relapse</b>       | 0.001                               | 1                | 0.103            | < 0.0001         | < 0.0001         | 1                 | -           | NA            |

RLC: Relative Lymphocyte Count; y: years; N: Number; SD: Standard Deviation; NA: Not Applicable.