**Supplement**

**Table S1:** Summary of baseline characteristics for the current analysis cohort vs. the Remainder of HERA patients

*(\*)Mantel-Haenszel,* (∱*)Fisher’s exact, (∞)Excluding category “Missing”, (£)Excluding category “Unknown”, (¥)Excluding categories “NA”, “ND” & “Missing”, (€)Excluding category “Missing/UK”,( ©)Excluding category “Uncertain”, ( ≠)Excluding category “Not Assessed”, (≏)Excluding category “Unknown”*

| **Baseline characteristics** | **Current analysis cohort (N=862)** | **Remainder of HERA patients (N=4237)** | **Total (N=5099)** | **p-value** |
| --- | --- | --- | --- | --- |
| **Age in yrs** | | | |  |
| Median (IQR) | 50.0 (42.0-57.0) | 49.0 (42.0-56.0) | 49.0 (42.0-56.0) | 0.13\* |
| <35 | 56 (6.50%) | 322 (7.60%) | 378 (7.41%) |  |
| 35-49 | 372 (43.16%) | 1892 (44.65%) | 2264 (44.40%) |  |
| 50-59 | 288 (33.41%) | 1351 (31.89%) | 1639 (32.14%) |  |
| ≥60 | 146 (16.94%) | 672 (15.86%) | 818 (16.04%) |  |
| **Pathological tumor size** | | | |  |
| Median in mm (IQR) | 21.0 (15.0-30.0) | 22.0 (15.0-32.0) | 22.0 (15.0-30.0) |  |
| 0-2 cm | 411 (47.68%) | 1822 (43.00%) | 2233 (43.79%) | <0.001\* |
| >2-5 cm | 395 (45.82%) | 2022 (47.72%) | 2417 (47.40%) | 0.018\*,∞ |
| >5 cm | 44 (5.10%) | 267 (6.30%) | 311 (6.10%) |  |
| Missing | 12 (1.39%) | 126 (2.97%) | 138 (2.71%) |  |
| **PgR Local** | | | |  |
| Negative (-) | 484 (56.15%) | 2614 (61.69%) | 3098 (60.76%) | <0.001∱ |
| Positive (+) | 298 (34.57%) | 1476 (34.84%) | 1774 (34.79%) | 0.29∱,*£* |
| Unknown | 80 (9.28%) | 147 (3.47%) | 227 (4.45%) |  |
| **ER Local** |  |  |  |  |
| Negative (-) | 437 (50.70%) | 2352 (55.51%) | 2789 (54.70%) | 0.0096∱,≏ |
| Positive (+) | 425 (49.30%) | 1884 (44.47%) | 2309 (45.28%) |  |
| Unknown | 0 (0.0%) | 1 (0.02%) | 1 (0.02%) |  |
| **Tumor Grade** | | | |  |
| G1 | 15 (1.74%) | 91 (2.15%) | 106 (2.08%) | 0.30\*,*¥* |
| G2 | 273 (31.67%) | 1379 (32.55%) | 1652 (32.40%) |  |
| G3 | 566 (65.66%) | 2523 (59.55%) | 3089 (60.58%) |  |
| GX | 8 (0.93%) | 217 (5.12%) | 225 (4.41%) |  |
| Not Assessed | 0(0.0%) | 4 (0.09%) | 4 (0.08%) |  |
| Not Done | 0(0.0%) | 22 (0.52%) | 22 (0.43%) |  |
| Missing | - | 1 (0.02%) | 1 (0.02%) |  |
| **Menopausal status** | | | |  |
| Premenopausal | 100 (11.60%) | 617 (14.56%) | 717 (14.06%) | 0.0075∱,€ |
| Postmenopausal | 377 (43.74%) | 1942 (45.83%) | 2319 (45.48%) | 0.14∱,*€,©* |
| Uncertain | 385 (44.66%) | 1676 (39.56%) | 2061 (40.42%) |  |
| Missing/Unknown | - | 2 (0.05%) | 2 (0.04%) |  |
| **Nodal status** | | | |  |
| Not assessed (neo-adjuvant chemotherapy) | 49 (5.68%) | 514 (12.13%) | 563 (11.04%) | <0.001\*,∞ |
| Negative | 288 (33.41%) | 1358 (32.05%) | 1646 (32.28%) | 0.59\*,*∞,≠* |
| 1-3 nodes | 257 (29.81%) | 1207 (28.49%) | 1464 (28.71%) |  |
| ≥4 nodes | 268 (31.09%) | 1157 (27.31%) | 1425 (27.95%) |  |
| Missing | - | 1 (0.02%) | 1 (0.02%) |  |
| **Race** | | | |  |
| Caucasian | 798 (92.58%) | 3456 (81.57%) | 4254 (83.43%) | <0.001∱ |
| Oriental | 56 (6.50%) | 588 (13.88%) | 644 (12.63%) |  |
| Hispanic | 0(0.0%) | 122 (2.88%) | 122 (2.39%) |  |
| Other | 8 (0.93%) | 71 (1.68%) | 79 (1.55%) |  |
| **Region** | | | |  |
| Western and Northern Europe, Canada, South Africa, Australia, New Zealand | 697 (80.86%) | 2948 (69.58%) | 3645 (71.48%) | <0.001∱ |
| Asia Pacific, Japan | 49 (5.68%) | 560 (13.22%) | 609 (11.94%) |  |
| Eastern Europe | 95 (11.02%) | 466 (11.00%) | 561 (11.00%) |  |
| Central and South America | 21 (2.44%) | 263 (6.21%) | 284 (5.57%) |  |
| **Prior neo-adjuvant chemotherapy** | | | |  |
| No anthracyclines | 48 (5.57%) | 254 (5.99%) | 302 (5.92%) | 0.045∱ |
| Anthracyclines, no taxanes | 617 (71.58%) | 2852 (67.31%) | 3469 (68.03%) |  |
| Anthracyclines and taxanes | 197 (22.85%) | 1131 (26.69%) | 1328 (26.04%) |  |
| **ECOG Performance Status** | | | |  |
| 0 | 782 (90.72%) | 3901 (92.07%) | 4683 (91.84%) | 0.35∱ |
| 1 | 80 (9.28%) | 334 (7.88%) | 414 (8.12%) |  |
| Missing | - | 2 (0.05%) | 2 (0.04%) |  |

IQR: Interquartile range

# 

# Table S2: Summary of Disease-free Survival by subgroup of patients

| **Patient Subgroup** | **No. of patients** | **No. (%) of events** | **8-Year DFS % (95% CI)** | **HR Comb. Trast vs. Obs (95% CI)** |
| --- | --- | --- | --- | --- |
| **Current analysis cohort** | | | | |
| Observation | 301 | 99 (32.9) | 65.8 (60.1, 71.6) |  |
| Combined Trastuzumab | 561 | 150 (26.7) | 72.9 (69.1, 76.7) | 0.74 (0.57, 0.95) |
| Total | 862 | 249 (28.9) | 70.5 (67.3, 73.6) |  |
| **Remainder of HERA pts** | | | | |
| Observation | 1396 | 471 (33.7) | 64.5 (61.9, 67.1) |  |
| Combined Trastuzumab | 2841 | 793 (27.9) | 70.8 (69.0, 72.5) | 0.76 (0.68, 0.85) |
| Total | 4237 | 1264 (29.8) | 68.7 (67.3, 70.2) |  |

**Table S3:** Summary of Overall Survival by subgroup of patients

| **Patient Subgroup** | **No. of patients** | **No. (%) of Deaths** | **8-Year OS % (95% CI)** | **HR Comb. Trast vs. Obs (95% CI)** |
| --- | --- | --- | --- | --- |
| **Current analysis cohort** | | | | |
| Observation | 301 | 53 (17.6) | 81.8 (77.3, 86.3) |  |
| Combined Trastuzumab | 561 | 76 (13.5) | 86.0 (83.1, 89.0) | 0.73 (0.51, 1.03) |
| Total | 862 | 129 (15.0) | 84.5 (82.0, 87.0) |  |
| **Remainder of HERA pts** | | | | |
| Observation | 1396 | 297 (21.3) | 76.4 (74.0, 78.8) | 0.75 (0.65, 0.86) |
| Combined Trastuzumab | 2841 | 476 (16.8) | 81.8 (80.3, 83.4) |  |
| Total | 4237 | 773 (18.2) | 80.1 (78.8, 81.4) |  |

# Table S4: Comparison of median follow-up time (based on reverse censoring for OS)

| **Patient Subgroup** | **Median follow-up time in years (IQR)** | |
| --- | --- | --- |
| **Current analysis cohort (N=862)** | **Remainder HERA patients (N=4237)** |
| Observation | 7.78 (7.13, 8.47) | 7.82 (7.06, 8.35) |
| Combined Trastuzumab | 8.00 (7.08, 8.29) | 8.01 (7.07, 8.19) |
| **Total** | 7.99 (7.08, 8.44) | 8.00 (7.07, 8.24) |

IQR: Interquartile range

# Table S5: Predictive effect of p27 biomarker (Low/High) for DFS: Cox proportional hazards model without adjustment for other variables.

**a.**

| **No. of pts= 862**  **No. of DFS events=249** | **Parameter Estimate** | **Std. Error** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** |  |  |  |
| Combined Trastuzumab vs. Observation | -0.618 | 0.199 | 0.002 |
| **p27 biomarker** |  |  |  |
| High vs. Low | -0.599 | 0.218 | 0.006 |
| Missing vs. Low | -0.334 | 0.305 | 0.27 |
| **Treatment by p27 biomarker interaction** |  |  |  |
| Treatment by p27 int. (p27=High) | 0.589 | 0.281 | 0.036 |
| Treatment by p27 int. (p27=Missing) | 0.334 | 0.408 | 0.41 |

**b.**

| **p27 biomarker level** | **HR Treatment:**  **Comb.Trast vs. Obs.** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| p27 Low | 0.539 | (0.365, 0.796) | 0.0019 |
| p27 High | 0.972 | (0.659, 1.433) | 0.89 |
| p27 Missing | 0.753 | (0.374, 1.514) | 0.43 |

**c.**

| **Treatment arm** | **HR p27 High vs Low** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.549 | (0.358, 0.843) | 0.0061 |
| Combined Trastuzumab | 0.990 | (0.701, 1.400) | 0.96 |

**Table S6:** Predictive effect of p27 biomarker (Low/High) for DFS, adjusted for variables of clinical interest: Multivariate Cox proportional hazards model.

**a.**

| **No. of pts=862**  **No. of DFS events=249** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs Observation | *See Table below* | | <0.001 |
| **p27 biomarker** | | | |
| High vs Low | *See Table below* | | 0.0012 |
| Missing vs Low | 0.17 |
| **Treatment by p27 biomarker interaction** | | | |
| Treatment\*p27 int. (p27=High) | *See Table below* | | 0.0049 |
| Treatment\*p27 int. (p27=Missing) | 0.31 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 1.259 | (0.766, 2.070) | 0.36 |
| Negative vs. ≥4 | 0.328 | (0.232, 0.436) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.532 | (0.389, 0.728) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.670 | (0.328, 1.369) | 0.27 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.806 | (1.263, 2.583) | 0.0012 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.439 | (0.699, 2.960) | 0.32 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.607 | (0.374, 0.987) | 0.044 |
| >2-5 vs. >5 | 0.530 | (0.33, 0.850) | 0.0084 |
| Missing vs. >5 cm | 0.367 | (0.102, 1.317) | 0.12 |

**b.**

| **p27 biomarker level** | **HR Treatment:**  **Comb.Trast vs. Obs.** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| p27 Low | 0.437 | (0.294,0.652) | <0.001 |
| p27 High | 0.972 | (0.658, 1.436) | 0.88 |
| p27 Missing | 0.665 | (0.326, 1.354) | 0.26 |

**c.**

| **Treatment arm** | **HR p27 High vs. Low** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.489 | (0.318, 0.753) | 0.012 |
| Combined Trastuzumab | 1.087 | (0.767, 1.539) | 0.64 |

**Table S7**: Predictive effect of p27 biomarker (Low/High) for DFS, adjusted for variables of clinical interest and the predictive effect of ER Local: Multivariate Cox proportional hazards model

**a.**

| **No. of pts=862**  **No. of DFS events=249** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs. Observation | *See Table below* | | ***<0.001*** |
| **p27 biomarker** | | | |
| High vs. Low | *See Table below* | | 0.081 |
| Missing vs. Low | 0.95 |
| **Treatment by p27 biomarker interaction** | | | |
| Treatment by p27 int. (p27=High) | *See Table below* | | 0.033 |
| Treatment by p27 int. (p27=Missing) | 0.54 |
| **ER Local** |  | |  |
| Positive vs. Negative | - | | 0.52 |
| **ER Local by p27 biomarker interaction** |  | |  |
| ER Local by p27 int. (p27=High) | - | | 0.19 |
| ER Local by p27 int. (p27=Missing) | - | | 0.12 |
| **Treatment by ER Local interaction** |  | |  |
| Treatment by ER Local int. (ER Local=Positive) | - | | 0.033 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 1.303 | (0.789, 2.152) | 0.31 |
| Negative vs. ≥4 | 0.332 | (0.235, 0.470) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.531 | (0.388, 0.725) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.689 | (0.337, 1.409) | 0.31 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.799 | (1.256, 2.578) | 0.0014 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.403 | (0.678, 2.906) | 0.36 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.606 | (0.372, 0.987) | 0.044 |
| >2-5 vs. >5 | 0.531 | (0.330, 0.854) | 0.0090 |
| Missing vs. >5 cm | 0.364 | (0.101, 1.311) | 0.12 |

**b.**

| **p27 biomarker level** | **HR Treatment:**  **Comb.Trast vs. Obs.** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| p27 Low | 0.363 | (0.235, 0.560) | <0.001 |
| p27 High | 0.689 | (0.415, 1.144) | 0.15 |
| p27 Missing | 0.468 | (0.223, 0.984) | 0.045 |

**c.**

| **Treatment arm** | **HR p27 High vs. Low** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.647 | (0.396, 1.056) | 0.081 |
| Combined Trastuzumab | 1.223 | (0.763, 1.976) | 0.40 |

**Table S8:** Predictive effect of p27 biomarker (Low/High) for DFS, adjusted for variables of clinical interest and the predictive effect of ESR1: Multivariate Cox proportional hazards model

**a.**

| **No. of pts=862**  **No. of DFS events=249** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs. Observation | *See Table below* | | <0.001 |
| **p27 biomarker** | | | |
| High vs. Low | *See Table below* | | 0.0055 |
| Missing vs. Low | 0.75 |
| **Treatment by p27 biomarker interaction** | | | |
| Treatment by p27 int. (p27=High) | *See Table below* | | 0.019 |
| Treatment by p27 int. (p27=Missing) | 0.50 |
| **ESR1 gene expression** |  | |  |
| 2nd vs. 1st tertile | - | | 0.065 |
| 3rd vs. 1st tertile | - | | 0.14 |
| Missing vs. 1st tertile | - | | 0.16 |
| **Treatment by ESR1 gene expression interaction** |  | |  |
| Treatment by ESR1 int. (ESR1=2nd tertile) | - | | 0.24 |
| Treatment by ESR1 int. (ESR1=3rd tertile) | - | | 0.047 |
| Treatment by ESR1 int. (ESR1=Missing) | - | | 0.046 |
| **p27 biomarker by ESR1 gene expression interaction** |  | |  |
| p27 cat by ESR1 int. (p27=High, ESR1=2nd tertile) | - | | 0.015 |
| p27 cat by ESR1 int. (p27=High, ESR1=3rd tertile) | - | | 0.91 |
| p27 cat by ESR1 int. (p27=High, ESR1=Missing) | - | | 0.29 |
| p27 cat by ESR1 int. (p27=Missing, ESR1=1st tertile) | - | | 0.57 |
| p27 cat by ESR1 int. (p27=Missing, ESR1=2nd tertile) | - | | 0.73 |
| p27 cat by ESR1 int. (p27=Missing, ESR1=3rd tertile) | - | | 0.44 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 1.182 | (0.713, 1.961) | 0.52 |
| Negative vs. ≥4 | 0.323 | (0.228, 0.457) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.512 | (0.374, 0.702) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.670 | (0.325, 1.382) | 0.28 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.878 | (1.301, 2.712) | 0.0080 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.694 | (0.807, 3.554) | 0.16 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.605 | (0.369, 0.991) | 0.046 |
| >2-5 vs. >5 | 0.522 | (0.322, 0.845) | 0.0082 |
| Missing vs. >5 cm | 0.344 | (0.095, 1.250) | 0.10 |

**b.**

| **p27 biomarker level** | **HR Treatment:**  **Comb.Trast vs. Obs.** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| p27 Low | 0.269 | (0.149, 0.487) | <0.001 |
| p27 High | 0.537 | (0.269, 1.072) | 0.078 |
| p27 Missing | 0.358 | (0.145, 0.883) | 0.025 |

**c.**

| **Treatment arm** | **HR p27 High vs. Low** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.357 | (0.173, 0.739) | 0.0055 |
| Combined Trastuzumab | 0.712 | (0.361, 1.402) | 0.33 |

**Table S9:** Predictive effect of cyclin D1 biomarker (continuous) for DFS: Multivariate Cox proportional hazards model without adjustment for other variables.

**a.**

| **No. of pts=757**  **No. of DFS events=214** | **Parameter Estimate** | **Std. Error** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** |  |  |  |
| Combined Trastuzumab vs. Observation | -0.659 | 0.175 | <0.001 |
| **Cyclin D1 biomarker** |  |  |  |
| Cyclin D1 | -0.034 | 0.013 | 0.0091 |
| **Treatment by cyclin D1 biomarker interaction** |  |  |  |
| Treatment by Cyclin D1 int. | 0.039 | 0.014 | 0.0056 |

**b.**

| **Cyclin D1 biomarker** | **HR Treatment:**  **Comb. Trast vs. Obs** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Cyclin D1=10.56\* | 0.779 | (0.580, 1.045) | 0.10 |

\*mean value of Cyclin D1

**c.**

| **Treatment arm** | **HR Cyclin D1** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.967 | (0.943, 0.992) | 0.0091 |
| Combined Trastuzumab | 1.005 | (0.994, 1.016) | 0.35 |

# Table S10: Predictive effect of cyclin D1 (continuous) biomarker for DFS, adjusted for variables of clinical interest: Multivariate Cox proportional hazards model.

**a.**

| **No. of pts=757**  **No. of DFS events=214** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs. Observation | See Tables below | | <0.001 |
| **Cyclin D1 biomarker** | | | |
| Cyclin D1 | See Tables below | | 0.0082 |
| **Treatment by cyclin D1 biomarker interaction** | | | |
| Treatment by Cyclin D1 int. | See Tables below | | 0.0046 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 0.952 | (0.528, 1.716) | 0.87 |
| Negative vs. ≥4 | 0.306 | (0.210, 0.446) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.488 | (0.351, 0.680) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.670 | (0.312, 1.437) | 0.30 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.858 | (1.257, 2.745) | 0.0019 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.449 | (0.588, 3.567) | 0.42 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.644 | (0.383, 1.086) | 0.099 |
| >2-5 vs. >5 | 0.530 | (0.318, 0.882) | 0.015 |
| Missing vs. >5 cm | 0.425 | (0.115, 1.578) | 0.20 |

**b.**

| **Cyclin D1 biomarker** | **HR Treatment:**  **Comb. Trast vs. Obs** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Cyclin D1=10.56\* | 0.736 | (0.545, 0.993) | 0.045 |

**c.**

| **Treatment arm** | **HR Cyclin D1** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.964 | (0.939, 0.991) | 0.0082 |
| Combined Trastuzumab | 1.005 | (0.995, 1.016) | 0.31 |

\*mean value

**Table S11:** Predictive effect of cyclin D1 biomarker (continuous) for DFS, adjusted for variables of clinical interest and the predictive effect of ER Local: Multivariate Cox proportional hazards model

**a.**

| **No. of pts=757**  **No. of DFS events=214** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs. Observation | *See Tables below* | | <0.001 |
| **Cyclin D1 biomarker** | | | |
| Cyclin D1 | *See Tables below* | | 0.10 |
| **Treatment by cyclin D1 biomarker interaction** | | | |
| Treatment by cyclin D1 int. | *See Tables below* | | 0.028 |
| **ER Local** |  | |  |
| Positive vs. Negative | - | | 0.028 |
| **Treatment by ER Local interaction** |  | |  |
| Treatment by ER Local int. (ER Local=Positive) | - | | 0.0025 |
| **Cyclin D1 biomarker by ER Local interaction** |  | |  |
| Cyclin D1 by ER local int. (ER Local=positive) | - | | 0.45 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 0.962 | (0.533, 1.737) | 0.90 |
| Negative vs. ≥4 | 0.307 | (0.211, 0.447) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.486 | (0.349, 0.676) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.655 | (0.304, 1.409) | 0.28 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.894 | (1.276, 2.811) | 0.0015 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.471 | (0.596, 3,630) | 0.40 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.630 | (0.372, 1.065) | 0.084 |
| >2-5 vs. >5 | 0.527 | (0.315, 0.880) | 0.014 |
| Missing vs. >5 cm | 0.454 | (0.122, 1.689) | 0.12 |

**b.**

| **Cyclin D1 biomarker** | **HR Treatment:**  **Comb. Trast vs. Obs** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Cyclin D1=10.56\* | 0.472 | (0.321, 0.714) | <0.001 |

**c.**

| **Treatment arm** | **HR Cyclin D1** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.978 | (0.952, 1.005) | 0.10 |
| Combined Trastuzumab | 1.009 | (0.992, 1.027) | 0.31 |

\*mean value

**Table S12:** Predictive effect of cyclin D1 biomarker (continuous) for DFS, adjusted for variables of clinical interest and the predictive effect of ESR1: Multivariate Cox proportional hazards model

**a.**

| **No. of pts=757**  **No. of DFS events=214** | **Hazard Ratio** | **95% CI** | **p-value** |
| --- | --- | --- | --- |
| **Treatment** | | | |
| Combined Trastuzumab vs Observation | See Tables below | | <0.001 |
| **Cyclin D1 biomarker** | | | |
| Cyclin D1 | See Tables below | | 0.16 |
| **Treatment by cyclin D1 biomarker interaction** | | | |
| Treatment by cyclin D1 int. | See Tables below | | 0.038 |
| **ESR1 gene expression** |  | |  |
| 2nd vs. 1st tertile | - | | 0.26 |
| 3rd vs. 1st tertile | - | | 0.053 |
| Missing vs. 1st tertile | - | | 0.21 |
| **Treatment by ESR1 gene expression interaction** |  | |  |
| Treatment by ESR1 int. (ESR1=2nd tertile) | - | | 0.12 |
| Treatment by ESR1 int. (ESR1=3rd tertile) | - | | 0.0083 |
| Treatment by ESR1 int. (ESR1=Missing) | - | | 0.019 |
| **Cyclin D1 biomarker by ESR1 gene expression interaction** |  | |  |
| Cyclin D1 by ESR1 int. (ESR1=2nd tertile) | - | | 0.57 |
| Cyclin D1 by ESR1 int. (ESR1=3rd tertile) | - | | 0.58 |
| Cyclin D1 by ESR1 int. (ESR1=Missing) | - | | 0.50 |
| **Nodal status** | | | |
| Not assessed vs. ≥4 | 0.873 | (0.481, 1.583) | 0.65 |
| Negative vs. ≥4 | 0.292 | (0.200, 0.427) | <0.001 |
| 1-3 Positive vs. ≥4 | 0.470 | (0.337, 0.665) | <0.001 |
| **Region** | | | |
| Asia Pacific, Japan vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 0.658 | (0.302, 1.435) | 0.29 |
| Eastern Europe vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.983 | (1.331, 2.593) | <0.001 |
| Central & South America vs. W/N Europe, Canada, South Africa, Australia, New Zealand | 1.598 | (0.640, 3.989) | 0.31 |
| **Pathological tumor size** | | | |
| 0-2 cm vs. >5cm | 0.662 | (0.393, 1.116) | 0.12 |
| >2-5 vs. >5 | 0.520 | (0.311, 0.869) | 0.013 |
| Missing vs. >5 cm | 0.415 | (0.112, 1.544) | 0.20 |

**b.**

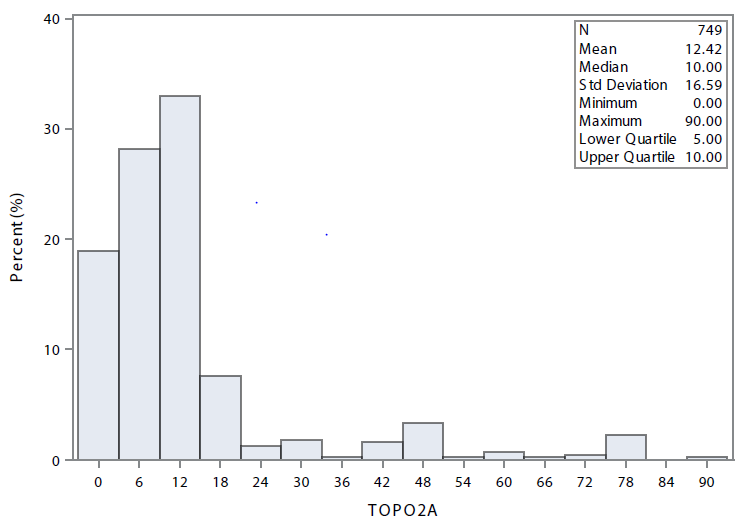
| **Cyclin D1 biomarker** | **HR Treatment:**  **Comb. Trast vs Obs** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Cyclin D1=10.56\* | 0.348 | (0.189, 1.011) | <0.001 |

**c.**

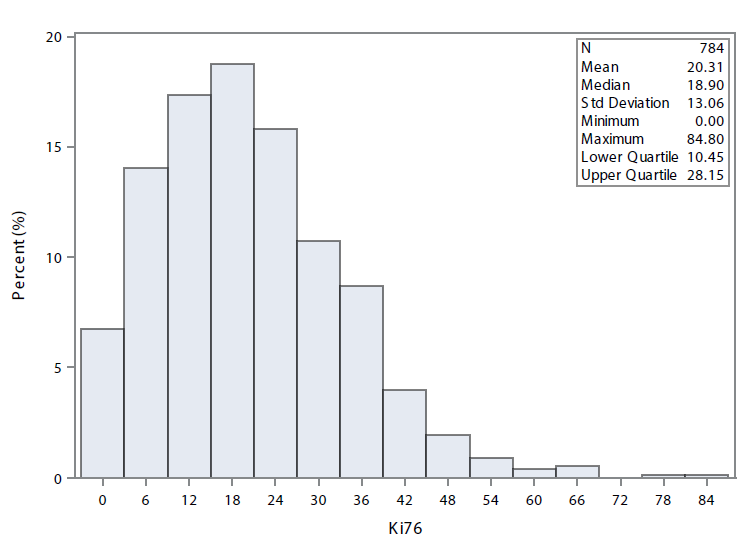
| **Treatment arm** | **HR Cyclin D1** | **95% C.I.** | **p-value** |
| --- | --- | --- | --- |
| Observation | 0.974 | (0.938, 1.011) | 0.17 |
| Combined Trastuzumab | 1.004 | (0.973, 1.036) | 0.81 |

\*mean value

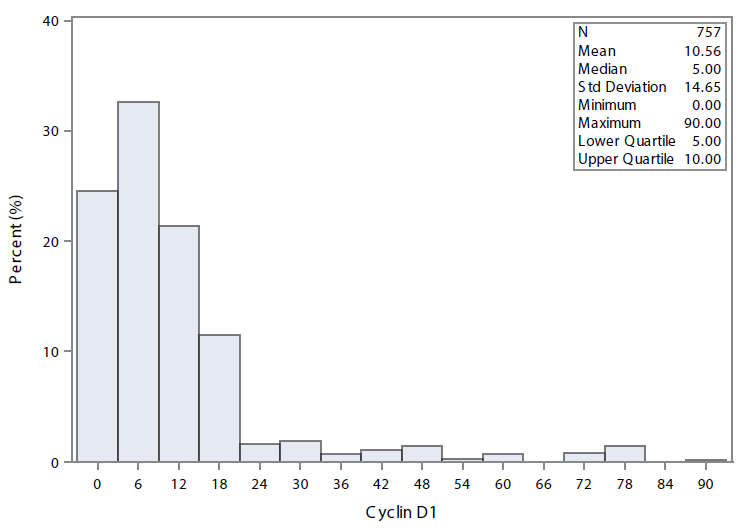
# Figure S1. A: Histogram of TOPO2A biomarker



# Figure S1.B Histogram of Ki67 biomarker

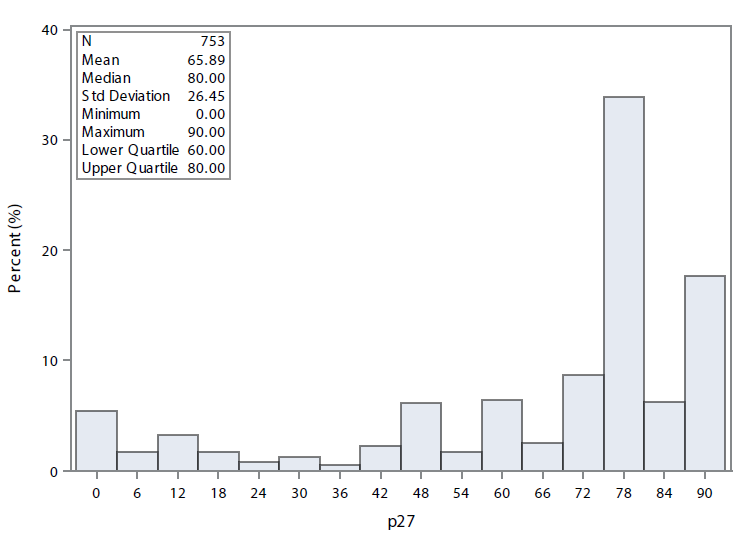


# Figure S1.C: Histogram of Cyclin D1 biomarker

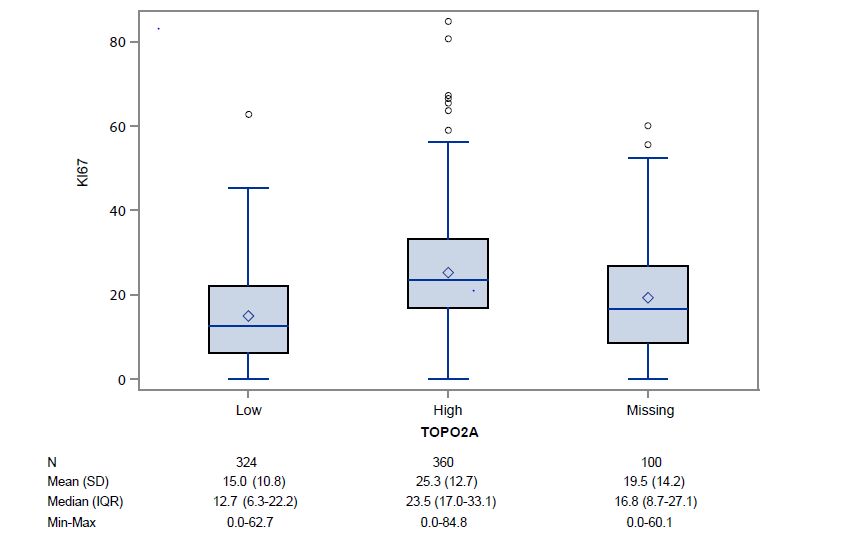


# 

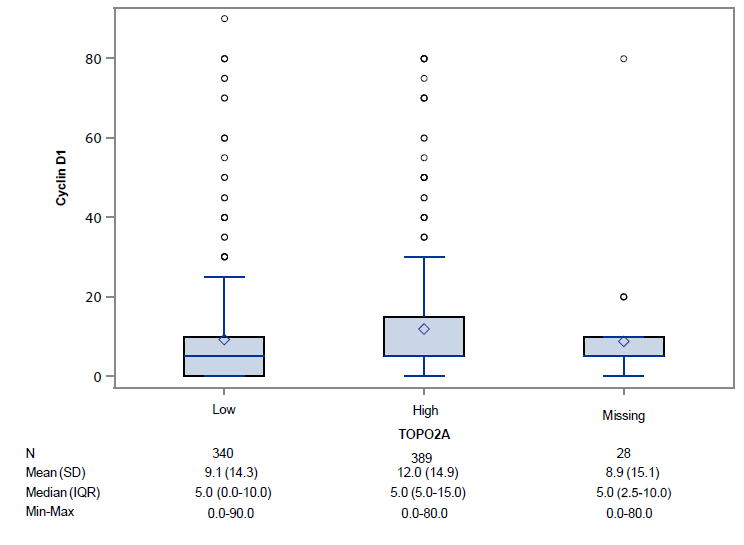
# Figure S1.D: Histogram of p27 biomarker



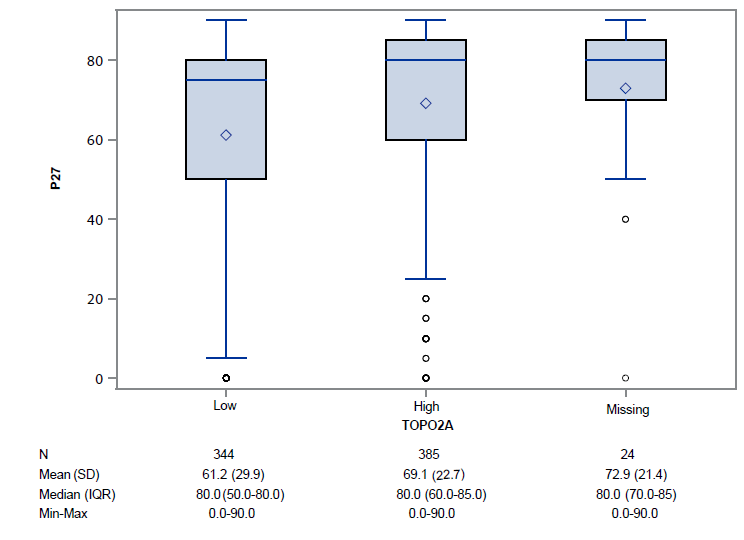
**Figure S2.A:** Ki67 distribution by TOPO2A level



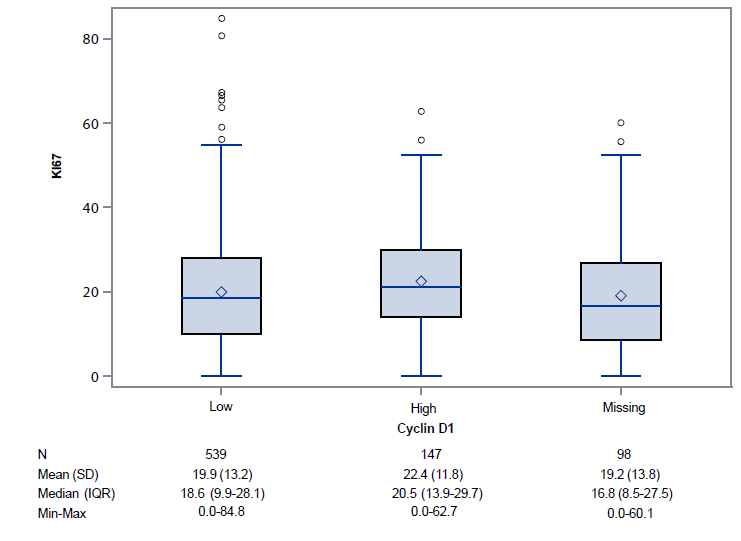
**Figure S2.B:** Cyclin D1 distribution by TOPO2A level



**Figure S2.C:** p27 distribution by TOPO2A level



**Figure S3.A**: Ki67 distribution by Cyclin D1 level



**Figure S3.B:** p27distribution by Cyclin D1 level

