

## Supplementary Online Content

Esserman LJ, Yau C, Thompson CK, et al. Use of molecular tools to identify patients with indolent breast cancers with ultralow risk over 2 decades. *JAMA Oncol*. Published online June 29, 2017. doi:10.1001/jamaoncol.2017.1261

### **eMethods**

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This supplementary material has been provided by the authors to give readers additional information about their work.

## **eMethods**

Additional detail on calculating PAM50 result: Specifically, log<sub>2</sub>-scaled upper quartile normalized expression data received from Agendia was used; and to adjust for population assumption inherent to the PAM50 classifier, we first generated a subsample of our cohort balanced for ER status comprising all 113 ER- and a randomly selected 113 ER+ patients. We then computed the median of each gene across this subsample; and centered the expression levels within each sample to this median. Centered expression data was mapped by gene symbol to the genes within the PAM50 classifier. Genes represented by multiple probes were collapsed by averaging (as per recommended for long oligo platforms).

**eTable.** Patient and tumor characteristics at primary tumor diagnosis

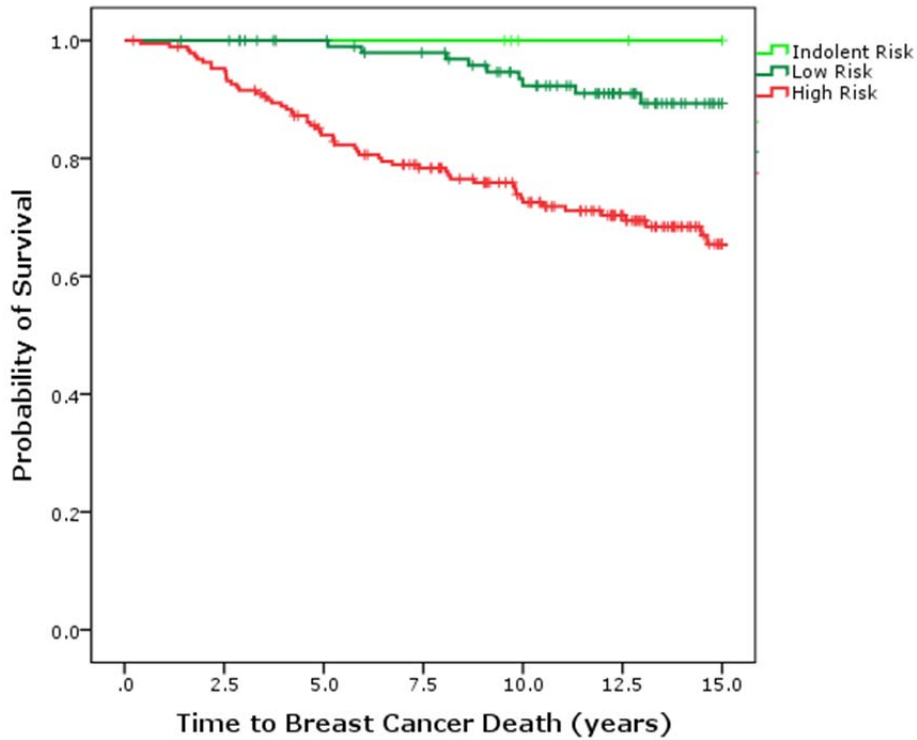
|   |  | Randomized trial arm |         |                   |         |                           |         |      |
|---|--|----------------------|---------|-------------------|---------|---------------------------|---------|------|
|   |  | Untreated            |         | Tamoxifen treated |         | Association test          | Total   |      |
|   |  | Number               | Percent | Number            | Percent | P-value                   | Number  |      |
|   |  |                      |         |                   |         | $\chi^2$ , Fisher's exact | Percent |      |
| <b>Patients</b>                             |  | 313                  | 48.0    | 339               | 52.0    |                           | 652     | 100  |
| <b>Patient characteristics</b>              |  |                      |         |                   |         |                           |         |      |
| <u>Calendar period of primary diagnosis</u> |  |                      |         |                   |         | 0.97, 1.00                |         |      |
| 1976-1984                                   |  | 162                  | 51.8    | 175               | 51.6    |                           | 337     | 51.7 |
| 1985-1991                                   |  | 151                  | 48.2    | 164               | 48.4    |                           | 315     | 48.3 |
| <u>Age at primary diagnosis, years</u>      |  |                      |         |                   |         | 0.61, 0.63                |         |      |
| 45-54                                       |  | 38                   | 12.1    | 33                | 9.7     |                           | 71      | 10.9 |
| 55-64                                       |  | 156                  | 49.8    | 172               | 50.8    |                           | 328     | 50.3 |
| 65-74                                       |  | 119                  | 38.1    | 134               | 39.5    |                           | 253     | 38.8 |
| <b>Primary tumor characteristics</b>        |  |                      |         |                   |         |                           |         |      |
| <u>Type of surgery</u>                      |  |                      |         |                   |         |                           |         |      |
| Breast conserving surgery and RT            |  | 66                   | 21.1    | 70                | 20.7    | 0.89, 0.92                | 136     | 20.9 |
| Mastectomy                                  |  | 247                  | 78.9    | 269               | 79.3    |                           | 516     | 79.1 |
| <u>Estrogen receptor status</u>             |  |                      |         |                   |         | 0.59, 0.87                |         |      |
| Positive                                    |  | 257                  | 82.1    | 281               | 83.1    |                           | 538     | 82.6 |
| Negative                                    |  | 56                   | 17.9    | 57                | 16.9    |                           | 113     | 17.4 |
| Unknown                                     |  | 0                    | -       | 1                 | -       |                           | 1       | -    |
| <u>Progesterone receptor status</u>         |  |                      |         |                   |         | 0.88, 0.88                |         |      |
| Positive                                    |  | 174                  | 56.5    | 195               | 58.4    |                           | 369     | 57.5 |
| Negative                                    |  | 134                  | 43.5    | 139               | 41.6    |                           | 273     | 42.5 |
| Unknown                                     |  | 5                    | -       | 5                 | -       |                           | 10      | -    |
| <u>HER2 status</u>                          |  |                      |         |                   |         | 0.42, 0.43                |         |      |
| Positive                                    |  | 30                   | 9.6     | 23                | 6.8     |                           | 53      | 8.2  |
| Negative                                    |  | 282                  | 90.4    | 315               | 93.2    |                           | 597     | 91.8 |
| Unknown                                     |  | 1                    | -       | 1                 | -       |                           | 2       | -    |
| <u>Ki67 status</u>                          |  |                      |         |                   |         | 0.28, 0.28                |         |      |
| Positive                                    |  | 93                   | 30.9    | 85                | 26.6    |                           | 178     | 28.7 |

|                    |     |      |     |      |            |     |      |
|--------------------|-----|------|-----|------|------------|-----|------|
| Negative           | 208 | 69.1 | 235 | 73.4 |            | 443 | 71.3 |
| Unknown            | 12  | -    | 19  | -    |            | 31  | -    |
| <u>Tumor grade</u> |     |      |     |      | 0.47, 0.48 |     |      |
| 1                  | 60  | 19.4 | 61  | 18.3 |            | 121 | 18.8 |
| 2                  | 173 | 55.8 | 202 | 60.7 |            | 375 | 58.3 |
| 3                  | 77  | 24.8 | 70  | 21.0 |            | 147 | 22.9 |
| Unknown            | 3   | -    | 6   | -    |            | 9   | -    |
| <u>Tumor size</u>  |     |      |     |      | 0.42, 0.44 |     |      |
| pT < 2 cm          | 244 | 79.2 | 255 | 75.9 |            | 499 | 77.5 |
| pT ≥ 2 cm          | 64  | 20.8 | 81  | 24.1 |            | 145 | 22.5 |
| Unknown            | 5   | -    | 3   | -    |            | 8   | -    |
|                    |     |      |     |      |            |     |      |

Table 1S: Demographics

The clinical features of the patients in the randomized STO-3 trial are shown. The type of surgery changed over time, where breast conservation increased and mastectomy decreased. Chi-square and Fisher's exact test were used to evaluate the differences in clinical characteristics between the arms.

**eFigure 1.** Breast Cancer Specific Survival (BCSS) of Transbig- Test Data set

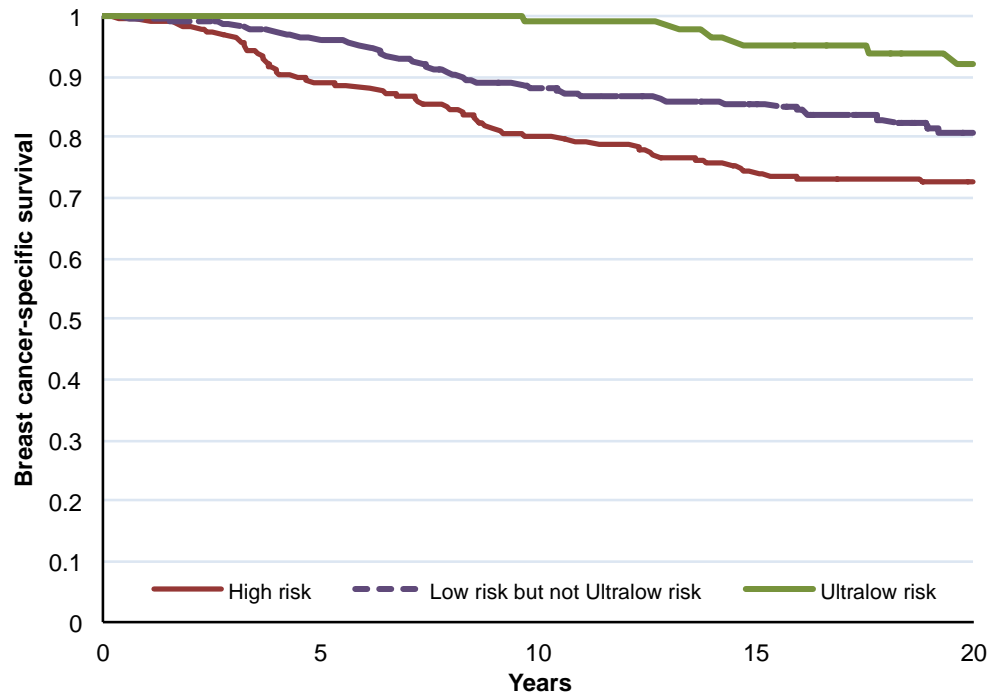


|                  | Number at risk at different BCSS time points |     |     |     |     |      |    |
|------------------|--|-----|-----|-----|-----|------|----|
|                  | 0  | 2.5 | 5   | 7.5 | 10  | 12.5 | 15 |
| <b>Indolent</b>  | 5  | 5   | 5   | 5   | 2   | 2    | 1  |
| <b>Low Risk</b>  | 106  | 105 | 98  | 92  | 79  | 58   | 35 |
| <b>High Risk</b> | 191  | 180 | 152 | 133 | 108 | 80   | 39 |

The Kaplan Meier plot with 15 year outcomes of the Transbig data set are shown stratified by Ultralow risk/Indolent threshold vs. low but not indolent, and high risk (according to the original MammaPrint designation). The threshold for the ultralow risk was set where there were no deaths from breast cancer at 20 years [1].

eFIGURE 2. Kaplan Meier plots of breast cancer-specific survival (not censored at contralateral breast cancer)

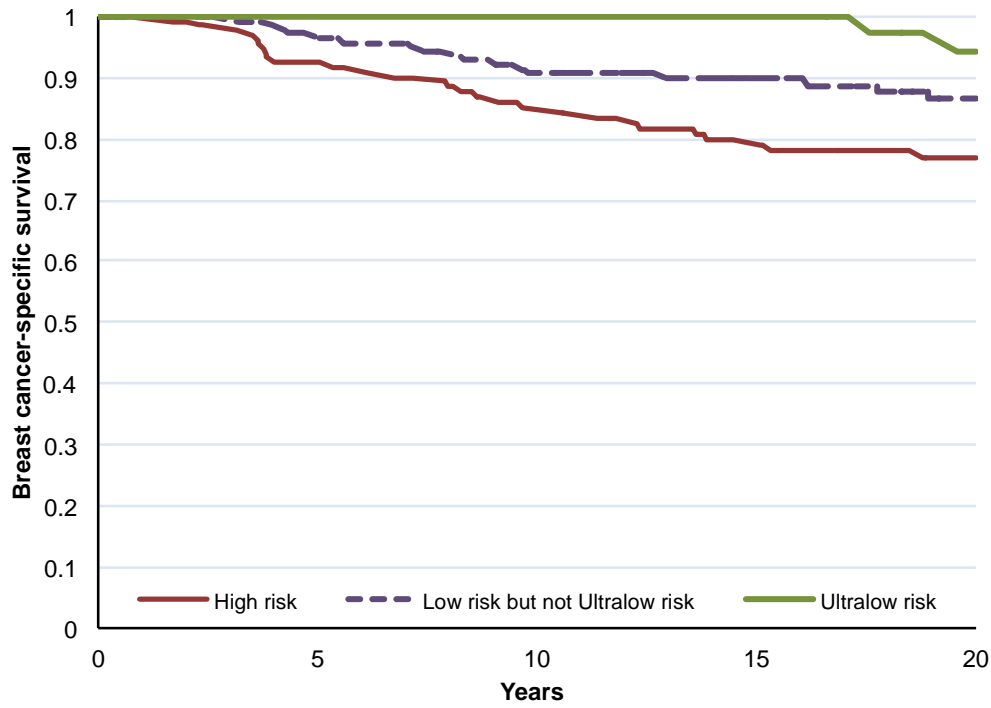
(A)



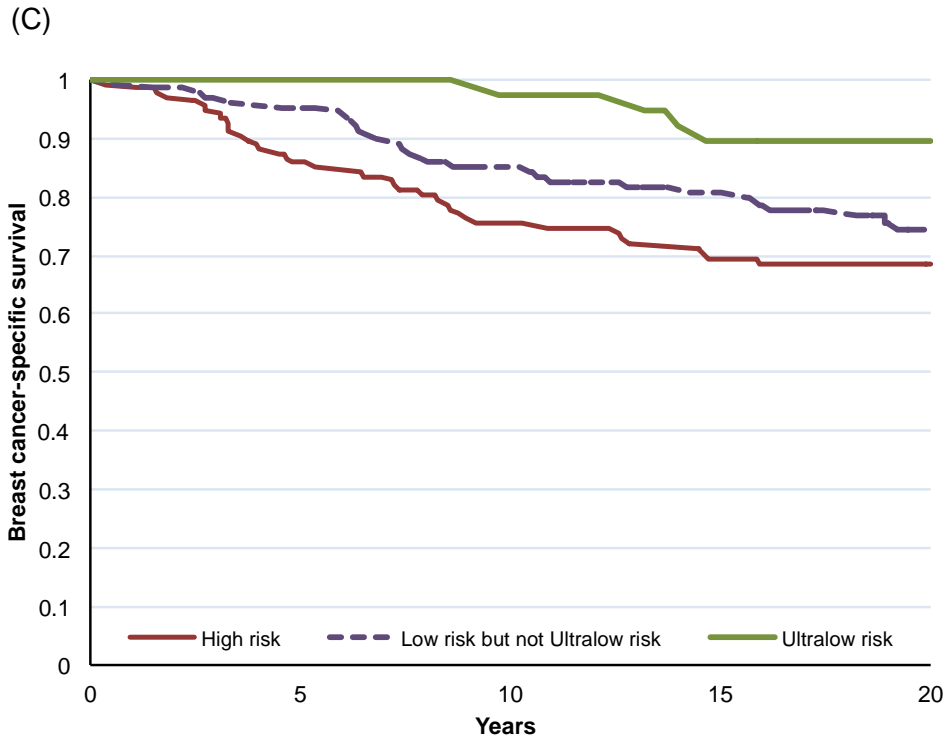
| Numbers at risk<br>(COD BC years) | 0   | 5   | 10  | 15  | 20  |
|-----------------------------------|-----|-----|-----|-----|-----|
| High risk                         | 275 | 231 | 191 | 160 | 129 |
| Low risk but not<br>Ultralow risk | 279 | 259 | 222 | 182 | 131 |
| Ultralow risk                     | 98  | 95  | 88  | 74  | 51  |

P-log rank=  
<0.0001

(B)



| Numbers at risk                | 0   | 5   | 10  | 15 | 20                    |
|--------------------------------|-----|-----|-----|----|-----------------------|
| (COD BC years)                 |     |     |     |    |                       |
|                                |     |     |     |    | P-log rank=<br>0.0046 |
| High risk                      | 137 | 119 | 101 | 84 | 66                    |
| Low risk but not Ultralow risk | 148 | 137 | 119 | 96 | 71                    |
| Ultralow risk                  | 54  | 52  | 49  | 41 | 29                    |



| Numbers at risk                | 0   | 5   | 10  | 15 | 20                   |
|--------------------------------|-----|-----|-----|----|----------------------|
| (COD BC years)                 |     |     |     |    |                      |
|                                |     |     |     |    | P-log rank=<br>0.014 |
| High risk                      | 138 | 112 | 90  | 76 | 63                   |
| Low risk but not Ultralow risk | 131 | 122 | 103 | 86 | 60                   |
| Ultralow risk                  | 44  | 43  | 39  | 33 | 22                   |

STO-3 trial stratified based on the 70-gene score into three categories (Ultralow risk, Low risk but not ultralow risk, and High risk). Tamoxifen treated patients have the same excellent survival. Women with second cancers appear to be at slightly higher risk after 10-15 years, perhaps reflecting the preventive impact of tamoxifen.

- A. All patients
- B. Tamoxifen treated
- C. Without tamoxifen treatment



## eReference

1. 6. Delahaye L, Dreezen C, Witteveen AT, et al. A breast cancer gene signature for indolent disease [published online April 27, 2017] *Breast Cancer Res Treat*. doi:10.1007/s10549-017-4262-0 Medline:28451965