Supplementary Online Content

Yu NY, Iftimi A, Yau C, et al. Assessment of long-term distant recurrence-free survival associated with tamoxifen therapy in postmenopausal patients with luminal A or luminal B breast cancer. Published online August 8, 2019. *JAMA Oncology*. doi:10.1001/jamaoncol.2019.1856

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

eMETHODS

The Stockholm Tamoxifen (STO-3) trial

The patient subset with formalin-fixed paraffin-embedded (FFPE) tumor material available was well balanced to the original STO-3 trial cohort with regards to tumor characteristics, comparing the original study with the current study subset. For instance, 78% versus 81% of patients had a tumor size less than 20 mm, 78% versus 80% of patients were ER-positive, and 52% versus 50% of patients were assigned to tamoxifen treatment arm.[1]

ER, PR, HER2, and Ki-67 immunohistochemistry

In 2014, formalin-fixed paraffin-embedded (FFPE) tissue sections were sectioned at 4 μm and mounted on plus-coated glass slides. Immunohistochemistry (IHC) was done for ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 at the University of California Davis Medical Center laboratory using DAKO Link48 Autostainer. The antibodies used were: ER (SP1; Spring Bioscience M301), PR (PgR 636; DAKO IR068), HER2 (HercepTest; DAKO SK001), and Ki67 (MIB-1; DAKO M7240). Per-run positive controls were assessed by quantitative image analysis to ensure consistent run-to-run staining intensity. Breast cancer pathologists at the University of California as a part of the ATHENA Breast Health network, scored (on whole-tumor sections with microscopes) the percentage of cancer cells positive for ER, PR, HER2, and Ki-67, where a threshold of 10% or greater was used to define ER and PR receptor positivity (according to the Swedish National guidelines), HER2 positivity was defined as intensity 3+ by immunohistochemistry, and the Ki-67 threshold for positivity was 15% or greater.[2]

Intrinsic subtypes (PAM50)

Tumors were assigned to one of five molecular subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like, Normal-like) using the PAM50 gene expression classification as described in the Parker et al. study.[3] Gene expression was measured using custom designed Agilent arrays containing approximately 32.1K probes, representing around 21.5K unique genes. 652 of the patient samples passed RNA quality check (according to the diagnostic quality model) and were used in the intrinsic subtype analysis. Gene expressions from each chip were log2-scaled and upper quartile normalized. A patient subset was generated using all 113 ER-negative patient samples, with 113 ER-positive patient samples then randomly selected to mirror the ER distribution in the PAM50 classifier training set. Gene expression values of all patient samples were then centered to the median gene values computed from the resulting 226 subsample dataset. Microarray probes from the tumor samples were mapped to the PAM50 classifier by Human Genome Organization Gene Nomenclature committee (HGNC) gene symbols. Expression values from genes represented by multiple probes were computed by averaging expressions from the probes, as per recommended for long oligo platforms.

Statistical Methods

We applied a parametric approach using flexible parametric models as defined by Lambert et al and Royston et al.[4,5] We used a small number of degrees of freedom for the spline in the model in order not to overfit the data. See the R program log file for details on pages 4-5. Further, for the few patients (depending on the specific tumor characteristic – all patients have information on ER and HER2 status, 7 patients are missing PR status, 21 patients are missing information on Ki-67, 5 patients are missing information on tumor grade, and 4 patients are missing information on tumor size) with missing data, we excluded these patients from the flexible parametric analysis.

eRESULTS

In the STO-3 trial, patients who re-consented and were relapse-free after 2 years of tamoxifen treatment were randomized to 3 additional years of tamoxifen or no further therapy. In summary, 92 patients received 5 years of tamoxifen therapy, out of these patients 63 and 29 patients were Luminal A and Luminal B, respectively.

Only lymph node negative patients were included in the STO-3 trial, and the stage distribution is as follows; 125 patients were T1a/bN0 (Luminal A n=111, Luminal B n=14), 245 were T1cN0 (Luminal A n=174, Luminal B n=71), and 88 were T2N0 (Luminal A n=47, Luminal B n=41).

Most patients in the STO-3 trial received mastectomy (n=356 out of 462 patients), out of these patients 248 and 108 patients were Luminal A and Luminal B, respectively. Patients receiving breast-conserving surgery also received radiotherapy (n=106), out of these patients 88 and 18 were Luminal A and Luminal B, respectively.

Luminal A tumors as compared to Luminal B tumors were significantly smaller in size (85.8% vs. 67.5 %, $p < 0.001$), of lower tumor grade (28.4% vs. 4.1%, $p < 0.001$), and a higher proportion of the tumors were PR-positive (75.2% vs. 61.6%, $p < 0.001$), HER2-negative (99.1% vs 96.0%, $p = 0.038$), and Ki-67-negative (86.2% vs. 59.5%, $p < 0.001$).

eAppendix. R program log file

```
fpm <- stpm2(Surv(Met_25yr, MetBC25yr) ~ tamoxifen + PAM50 + factor(Prstatus_WT) + factor(gradenm_TMA) + factor(size20nm) + AGE1 + factor(YR1_5) + factor(Ki67status_WT), data, df = 2, tvc = list(tamoxifen = 1, PAM50 = 1), stata.stpm2.compatible=TRUE)

summary(fpm)
# Maximum likelihood estimation
#
# Call:
#
#   mle2(minuslogl = negll, start = coef, eval.only = TRUE, vecpar = TRUE,
#       gr = function (beta)
#       {
#         localargs <- args
#         localargs$init <- beta
#         localargs$return_type <- "gradient"
#         return(.Call("model_output", localargs, PACKAGE = "rstpm2"))
#       }, control = list(parscale = c(1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1), maxit = 300),
#       stata.stpm2.compatible = TRUE,
#       lower = -Inf, upper = Inf)

# Coefficients                                  Estimate      Std. Error    z value      Pr(z)
#  (Intercept)                                  -7.8030559    1.4844695     -5.2565      1.469e-07 ***
#   tamoxifen                                   -2.1481634    0.6738435     -3.1879      0.0014330 **
#   PAM50LumB                                   2.7788008     0.6317294     4.3987       1.089e-05 ***
#   factor(Prstatus_WT)Negative                 0.0240383     0.2246660     0.1070       0.9147925
#   factor(Prstatus_WT)Unknown                  1.2065816     0.6073980     1.9865       0.0469805 *
#   factor(gradenm_TMA)1                        -0.1156723    0.3020945     -0.3829      0.7017932
#   factor(gradenm_TMA)3                        0.3220720     0.2832506     1.1371       0.2555145
#   factor(gradenm_TMA)99                       0.7574823     0.7462152     1.0151       0.3100587
#   factor(size20nm)1                           0.5585301     0.2328439     2.3987       0.0164519 *
#   AGE1                                        0.0040418     0.0200771     0.2013       0.8404549
#   factor(YR1_5)2                              0.1356378     0.2223183     0.6101       0.5417915
#   factor(YR1_5)1                              0.2468390     0.2989114     0.8258       0.4089215
#   factor(Ki67status_WT)Positive               0.0301318     0.2569623     0.1173       0.9066528
#   factor(Ki67status_WT)Unknown                -0.4311872    0.6045443     -0.7132      0.4756952
#   nsx(log(Met_25yr), df = 2)1                 9.4860791     1.2389566     7.6565       1.911e-14 ***
#   nsx(log(Met_25yr), df = 2)2                 3.6490210     0.4422382     8.2513       < 2.2e-16 ***
#   tamoxifen:nsx(log(Met_25yr), df = 1)        1.7471926     0.8617266     2.0275       0.0426063 *
#   PAM50LumB:nsx(log(Met_25yr), df = 1)        -2.9281925    0.7902118     -3.7056      0.0002109 ***
#   ---------------------------------------------------------------------------------------------------------------------------------------------
#   Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
#
#   -2 log L: 982.6006

pred <- predict(fpm, type="hr", newdata=data.frame(Met_25yr=c(5,10,15,16,20,25), tamoxifen=0,PAM50="LumA", Prstatus_WT="Positive", gradenm_TMA=2,size20nm=0,AGE1=mean(bc_data$AGE1), YR1_5=3, Ki67status_WT="Negative"), var= "tamoxifen", se.fit = TRUE)  #var gives the hazard ratio by tamoxifen

t <- c(5,10,15,20,25)

cbind(t,pred)
# t             Estimate        lower           upper
# 1    5        0.3316150       0.2033062       0.5409010
```

```
# 2   10        0.4682142       0.3056646       0.7172060
# 3   15        0.5721210       0.3477929       0.9411416
# 4   20        0.6499228       0.3667970       1.1515896
# 5   25        0.7081655       0.3772551       1.3293346
```

pred <- predict(fpm, type="hr", newdata=data.frame(Met_25yr=c(5,8,10,15,20,25), tamoxifen=0,PAM50="LumB", Prstatus_WT="Positive", gradenm_TMA=2,size20nm=0,AGE1=mean(bc_data$AGE1), YR1_5=3, Ki67status_WT="Negative"), var= "tamoxifen", se.fit = TRUE) #var gives the hazard ratio by tamoxifen

t <- c(5,10,15,20,25)

cbind(t,pred)

```
# t             Estimate        lower           upper
# 1  5          0.3758089       0.2392528       0.5903058
# 2 10          0.6588990       0.3670301       1.1828670
# 3 15          1.0348026       0.3799409       2.8183763
# 4 20          1.3909355       0.3513922       5.5058184
# 5 25          1.5751722       0.3470134       7.1500637
```

eREFERENCES

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4. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. *Stata J.* 2009;9(2):265-290.
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eTable 1. Patient and tumor characteristics by Luminal A and Luminal B subtypes as identified by PAM50 gene expression analysis

		STO-3 trial		
	Luminal A	**Luminal B**		
	No. (%)	**No. (%)**	**P***	**Total No. of patients**
STO-3 trial arm			0.53	
Treated	183 (54.5)	64 (50.8)		247
Untreated	153 (45.5)	62 (49.2)		215
Patient characteristics				
Calendar period of primary diagnosis			0.53	
1976-1984	166 (49.4)	67 (53.2)		233
1985-1990	170 (50.6)	59 (46.8)		229
Age at primary diagnosis, years			0.55	
45-54	29 (8.6)	10 (7.9)		39
55-64	166 (49.4)	56 (44.5)		222
65-73	141 (42.0)	60 (47.6)		201
Primary tumor characteristics				
Progesterone receptor status			< 0.001	
Positive	248 (75.2)	77 (61.6)		325
Negative	82 (24.8)	48 (38.4)		130
Unknown	6 (-)	1 (-)		7
HER2 status[£]			0.038	
Positive	3 (0.9)	5 (4.0)		8
Negative	333 (99.1)	121 (96.0)		454
Unknown	0 (-)	0 (-)		0
Ki-67 status[€]			< 0.001	
Positive	44 (13.8)	49 (40.5)		93
Negative	276 (86.2)	72 (59.5)		348
Unknown	16 (-)	5 (-)		21
Tumor grade			< 0.001	
1	95 (28.4)	5 (4.1)		100
2	222 (66.5)	78 (63.4)		300
3	17 (5.1)	40 (32.5)		57
Unknown	2 (-)	3 (-)		5
Tumor size			< 0.001	
pT < 20 mm	285 (85.8)	85 (67.5)		370
pT ≥ 20 mm	47 (14.2)	41 (32.5)		88
Unknown	4 (-)	0 (-)		4
*Fishers exact test				
[£]HER2 positive defined as 3+ by immunohistochemistry				
[€]Ki-67 cutoff for positivity at 15%				

eTable 2. Time-dependent relative hazard ratio by trial arm (tamoxifen treated versus untreated) for luminal A and luminal B subtype using flexible parametric survival models

STO-3 trial			**Risk of distant breast cancer recurrence**			
Patients included	**Trial arm**	**No.**	**Distant-recurrence 25-year No.**	**Years since diagnosis**	**Crude estimates adjusted for age and period of diagnosis HR (95% CI)***	**Adjusted estimates for patient and tumor characteristics HR (95% CI)*£**
Luminal A£	Treated	183	18	**5**	**0.33 (0.20-0.54)**	**0.33 (0.20-0.54)**
				10	**0.48 (0.31-0.73)**	**0.47 (0.31-0.72)**
				15	**0.59 (0.36-0.97)**	**0.57 (0.35-0.94)**
				20	0.68 (0.38-1.20)	0.65 (0.37-1.15)
				25	0.74 (0.40-1.39)	0.71 (0.38-1.33)
	Untreated	153	39		1.0 ref.	1.0 ref.
Luminal B£	Treated	64	18	**5**	**0.38 (0.24-0.59)**	**0.38 (0.24-0.59)**
				10	0.69 (0.39-1.23)	0.66 (0.37-1.18)
				15	1.13 (0.41-3.11)	1.04 (0.38-2.82)
				20	1.58 (0.38-6.54)	1.39 (0.35-5.51)
				25	1.81 (0.38-8.70)	1.58 (0.35-7.15)
	Untreated	62	25		1.0 ref.	1.0 ref.

*HR = hazard ratio, CI = confidence interval

£Flexible parametric survival models adjusting for treatment arm, age and period of diagnosis, progesterone receptor status, Ki-67 status, tumor grade, and tumor size