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Evaluation of the androgen receptor in patients with ERα-positive early breast cancer treated with adjuvant tamoxifen ± fluoxymesterone

James N. Ingle[1*], Vera J. Suman[2], Malvika H. Solanki[3], Marie R. Passow[4], Jordan D. Campbell[2], Liewei Wang[5] and Matthew P. Goetz[1]

Abstract

Background Our goal was to evaluate the impact of level of androgen receptor (AR) expression on outcomes in women with estrogen receptor α (ER) positive breast cancer. We sought to corroborate our preclinical findings that AR-agonists were efficacious in patients with ER-positive tumors that also expressed high levels of AR.

Methods Tissue microarrays (TMAs) were prepared from primary tumor blocks from patients entered on a prospective randomized adjuvant trial of tamoxifen (Tam) alone or combined with fluoxymesterone (Flu), an AR-agonist, (NCCTG 89-30-52). TMAs were stained for ER and AR and expression examined in decile increments (0–100%) of positive invasive tumor nuclei. The primary endpoint was relapse-free survival (RFS).

Results 301 (59%) of the 514 patients had sufficient tissue to determine ER and AR expression, where nuclear staining of > 70% was considered "enriched" and nuclear staining of ≤ 70% was considered "poor/moderate". Eleven (4%) of these patients had poor/moderate ER staining and were excluded from these analyses. The proportion of the ER-enriched tumors that also had AR-enriched expression levels was 56.3% in the Tam arm and 51.8% in the Tam + Flu arm**.** Within the AR-enriched patients, the cumulative incidence of RFS events showed an advantage for Tam + Flu over Tam alone that reached significance (Gray's test $p = 0.0472$).

Conclusions Our findings suggest that an AR-agonist may be of value in AR-enriched, ER-enriched breast cancers and should be studied in future trials because of the availability of new, more tolerable AR-agonists.

Keywords Androgen receptor, Estrogen receptor, Tamoxifen, Fluoxymesterone

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Introduction

Estrogen receptor α (ER) is expressed in the majority of breast cancers in women and the vast majority of ER-positive breast cancers also express the androgen receptor (AR), e.g., 74.8% in review by Vera-Badillo [1], 85.9% in the Nurses' Health Study[2], but the AR is not routinely determined in clinical practice. This is despite numerous studies showing expression of AR was associated with improved outcomes [1–5] in early breast cancer. Of note, these studies defined AR positivity in a variety of ways from > 1% (the most common) to as high as 75% of cells staining positive.

Using I-SPY 1, METABRIC, and TCGA, Vidula et al. [6] found that AR expression was correlated with clinicopathologic features, intrinsic subtype and improved outcomes. Recent reviews [7, 8] noted that AR-targeted therapy has shown efficacy in clinical trials. In addition, the AR pathway exhibits cross talk with multiple other signaling pathways including ER, HER2, PI3K/Akt/mTOR, and MAPK.

Several reports have indicated the AR/ER ratio may be a marker of prognosis in ER-positive breast cancer. Cochrane et al. [9] reported a series of 192 patients with early breast cancer who received tamoxifen, that those (13.6%) with an AR/ER ratio ≥ 2 had a significantly worse disease-free survival (DFS) (hazard ratio [HR] = 4.04. $p = 0.002$) and disease-specific survival (DSS) (HR = 2,75, $p = 0.03$). Likewise, Rangel et al. [10] found that 16 (6%) patients out of 284 whose tumors had an AR/ER ratio ≥ 2 had worse disease-free interval and DSS (hazard ratios = 4.96 and 8.69, respectfully with $P \leq 0.004$ for both)..

We recently published laboratory studies that indicated AR levels are differentially associated with breast cancer cell response to AR-agonists vs. AR-antagonists [11]. In relatively AR-high, ER-positive breast cancer cells (CAMA1, HCC1419), dihydrotestosterone, an AR-agonist, showed inhibition of cell proliferation compared with control while enzalutamide (an AR-antagonist) did not have any inhibition compared with control. However, in relatively AR-low, ER-positive breast cancer cells (T47D, MCF7), enzalutamide substantially inhibited cell growth but dihydrotestosterone had no effect on cell growth compared with control.

Hickey et al. [12], utilizing a panel of cell lines and patient-derived models, reported that AR agonism rather than antagonism was the optimal AR-directed therapy in ER-positive breast cancer. From a mechanistic standpoint, they found that AR agonism alters the distribution of ER and its coactivators on chromatin resulting in antagonism of ER-target genes. The use of AR-agonists in combination with standard endocrine therapy improved efficacy in xenograft models in mice.

NCCTG 89-30-52 was a randomized trial that evaluated the addition of the AR-agonist fluoxymesterone (Flu) to tamoxifen (Tam) in women with resected early-stage breast cancer [13]. This trial attempted to corroborate, and was powered by, the findings of superiority for the combination of Tam + Flu over Tam alone seen in a previous randomized trial in metastatic disease [14]. Postmenopausal women with ER-positive early-stage breast cancer were randomized to treatment with Tam (20 mg per day orally for 5 years) alone or combined with Flu (10 mg orally twice per day for 1 year). The primary endpoint was relapse-free survival (RFS). There were 541 eligible patients entered between 1991 and 1995. No significant differences were found between Tam plus Flu and Tam alone in terms of RFS or overall survival [13]. AR status was not determined in this patient cohort at the time this study was conducted because it was known that the vast majority of ER-positive breast cancers also expressed the AR. However, based on the data suggesting that the AR level and AR/ER ratio might be associated with outcomes of patients with ER-positive breast cancer treated with endocrine therapy, we examined whether the time without breast cancer recurrence or death differed with respect to AR level or AR/ER ratio in the postmenopausal women with ER-positive breast cancers enrolled onto NCCTG 89-30-52.

Methods

Study cohort

Candidates for the current study were post-menopausal women with ER-positive breast cancer who met all 89-30-52 eligibility criteria, provided written consent, were randomized, began protocol treatment, and had a paraffin-embedded primary tumor block with sufficient tumor to determine both ER and AR expression levels by our central laboratory. Patients were included in the current study if ER-positive disease was confirmed and there were sufficient tumor cells for an AR determination.

Specimen preparation and evaluation

The tissue microarrays were constructed using the Galileo CK4600 (ISENET) instrument. Up to four 1.0 mm cores were obtained from 378 donor FFPE breast cancer tissue blocks. The cores were placed into recipient blocks using a random arrangement. Control tissues were included in the array including liver for orientation, tonsil, normal breast, normal placenta, normal cervix, and normal prostate.

Immunohistochemical (IHC) staining on 5-micron sections for AR (clone AR27, Leica, 1:50) and ERα (clone SP1, Ventana, predilute) was performed in the CAP/CLIA-certified clinical Immunostains Laboratory at Mayo Clinic Rochester using Ventana Medical Systems

(Roche). Stained slides were scanned at 40 × magnification on the Aperio ScanScope AT2 brightfield instrument (Leica Biosystems). Resolution of the images was 0.25 µm/pixel at 40x. The images were 24-bit contiguous standard pyramid tiled TIFFs compressed via JPEG with a quality setting of 70. The whole slide scanned images were de-arrayed and manually scored using the Xplore software (Philips).

All tissue cores were manually evaluated for the percentage of AR- or ER-immunoreactive/positive invasive tumor nuclei per total evaluable invasive tumor nuclei. This was peformed by two readers (Dr. Solanki, a breast pathologist and Marie Passow, a senior cytotechnologist with expertise in breast biomarkers). This assessment was scored as 0% or in decile increments (1–100%). All scores were recorded electronically in the Xplore software and exported into Microsoft Excel format for use in biostatistical analysis.

Analysis plan

As central laboratory results for ER and AR expression were provided in deciles, the AR/ER ratio was determined by dividing the mid-point of AR-expression decile by the mid-point of the ER-expression decile. For ER and AR, enriched expression was defined as expression levels > 70% and poor/moderate expression was defined as expression levels ≤ 70%. This definition approximates that used for eligibility onto both ACSOG Z1031 (NCT00265759) and ALLIANCE A011106 (NCT01953588), namely, an Allred Score 6–8 or ER IHC expression ≥ 66.7% which has since been referred to as ER-enriched expression. Differences in patient and disease characteristics at study entry were assessed using Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.

The primary outcome of interest, recurrence-free survival (RFS), was defined as the time from registration onto 89-30-52 to documentation of the first of the following events: local recurrence, regional recurrence, distant recurrence, or death from any cause. A number of patients developed a contralateral breast cancer or second primary invasive cancer prior to breast cancer recurrence or death without breast recurrence. As such, a competing risk approach was used to assess whether the likelihood of breast cancer recurrence or death without breast cancer recurrence differed with respect to either AR/ER ratio or AR expression. The cumulative incidence function for the time to breast cancer recurrence or death with breast cancer recurrence was determined considering women diagnosed with a contralateral breast cancer or second primary invasive cancer prior to breast cancer recurrence having a competing event. Women without a second primary cancer diagnosis who died without documentation of disease recurrence or without evidence that they were recurrence-free within six months of death were censored at the time of their last disease evaluation. The six-month window was used as that was the time between disease evaluations for patients enrolled onto 89-30-52. Patients alive without a contralateral breast cancer, a second invasive primary cancer diagnosis or disease recurrence were also censored at the time of their last disease evaluation. Gray's test for the equality of cumulative incidence functions was used to assess whether the likelihood of breast cancer recurrence or death without breast cancer recurrence differed with respect to either AR/ER ratio or AR expression.

Results

Patients in the analyses

Three hundred one (59%) of the 514 eligible patients enrolled onto this trial had sufficient tissue to ascertain both ER and AR expression levels (percent of invasive cancer cells with nuclear IHC staining) (REMARK diagram: Fig. 1).

Eleven patients (4%; 2 on the Tam Arm and 9 on the Tam + Flu Arm) were found to have ER poor/moderate breast cancer by central laboratory testing. As such, the analysis cohort was limited to the 290 patients with ER-enriched breast cancer. The patient and disease characteristics, and clinical outcomes for these 290 patients by assigned treatment arm are provided in Table 1. The patient and disease characteristics of the those patients included in the analysis cohort and those patients not included in the analysis cohort from among the 514 eligible patients enrolled onto 89-30-52 are presented in Supplementary Table S1. The patients not included in the analysis cohort differed from those who were included in the analysis cohort in having a higher nodal burden.

The percentage of tumor cells with AR IHC nuclear staining was 0 in 52 (17.9%) patients; 1–70% in 81 (27.9%) patients, and 71–100% in 157 (54.1%) patients. In this ER-enriched cohort, the resulting AR/ER ratio was 0 in 52 patients (17.9%), 0.1–0.99 in 136 (47.0%) patients and 1.0–1.3 in 102 (35.2%) patients. Table 2 and Fig. 2 provide the distribution of AR IHC nuclear staining and AR/ER ratio by treatment arms. The proportion of patients with AR-enriched tumors was 56.3% in the Tam arm and 51.8% in the Tam + Flu arm. The median (25th, 75th percentile range) of the AR/ER ratio was 0.79 (0.10–1.0) in the Tam arm and 0.79 (0.05–1.0) in the Tam + Flu arm.

Patients with AR-enriched tumors were less likely to have prior exposure to exogenous estrogens (10.5% vs. 22.6%; $p = 0.010$) or positive lymph nodes (29.3% vs. 40.6%; $p = 0.048$) than women with AR-poor/moderate tumors. However, patients with AR-enriched tumors and patients with AR-poor/moderate tumors were not found

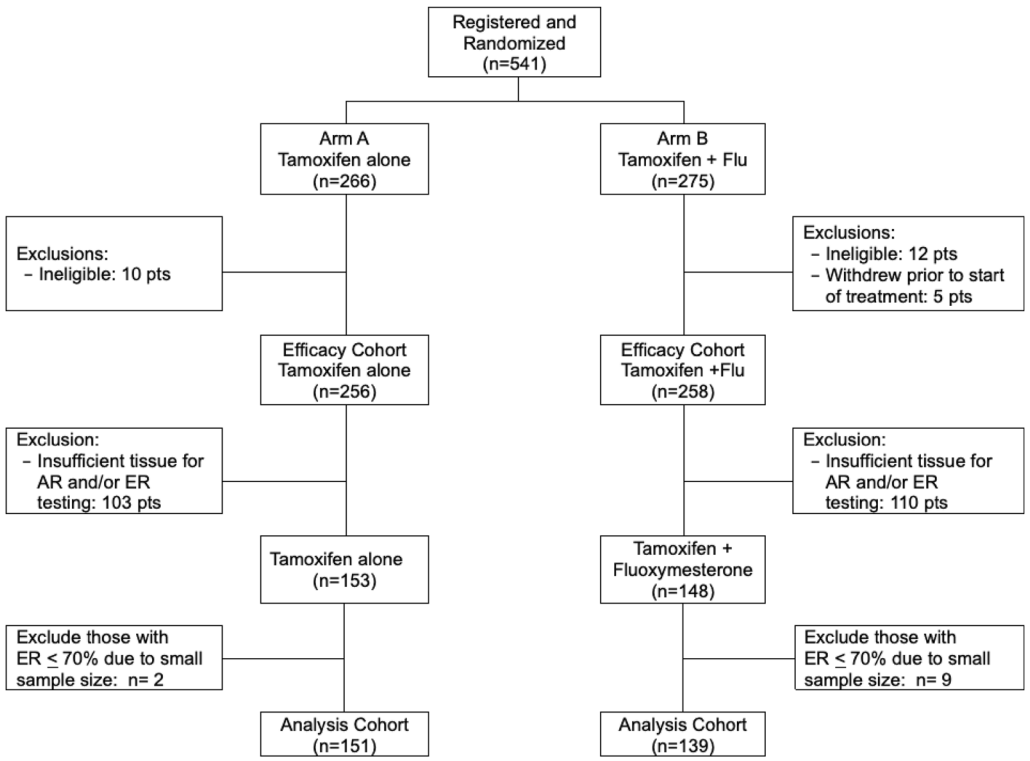

Fig. 1 REMARK diagram

to differ significantly in the proportion of patients with tumors > 3 cm (21.0% vs. 22.6%; $p = 0.776$) or age at study entry (median: 68; range: 48–84 vs. median: 68; range: 42–89; $p = 0.768$).

The median length of follow-up among the 103 patients still alive was 20.4 years (range: 3.0–23.6 years). The first disease events reported included breast cancer recurrence in 59 women (Tam: 35; Tam + Flu: 22): breast cancer recurrence concurrent with a second primary diagnosis in 2 women (Tam: 1; Tam + Flu: 1); and contralateral breast cancer or second primary diagnosis in 42 women (Tam: 22; Tam + Flu: 20).

Patient outcomes

Among the 187 women who have died (Tam: 100; Tam + Flu: 87), the causes of death were breast cancer in 46 women (Tam: 25; Tam + Flu: 21); another cancer in 15 women (Tam: 5; Tam + Flu: 10); some other non-cancer cause in 117 women (Tam: 66; Tam + Flu: 51) and unknown in 9 women (Tam: 4; Tam + Flu: 5).

Association between AR status and likelihood of breast cancer recurrence or death within each treatment arm

The association between AR expression and the cumulative incidence of a breast cancer recurrence or death (CI-RecDeath) was first examined by categorizing AR expression as enriched or poor/moderate. The CI-RecDeath was not found to differ with respect to AR cateogory for patients enrolled onto the Tam arm (Gray's test $p = 0.718$) or patients enrolled onto the Tam + Flu arm (Gray's test $p = 0.257$). As these patients had ER-enriched tumors, we next examined whether CI-RecDeath differed between those with AR-enriched/ER-enriched breast cancers and those with AR-poor/moderate/ER-enriched breast cancers. The CI-RecDeath was not found to differ with respect to whether a patient's tumor was AR-enriched or not in the Tam arm (Fig. 3A, Gray's test $p = 0.445$) or in the Tam + Flu arm (Fig. 3B, Gray's test $p = 0.126$).

The CI-RecDeath was also not found to differ with respect to whether the AR/ER ratio ≥ 1.0 or not in the Tam arm (Fig. 4A, Gray's test $p = 0.768$) or in the Tam + Flu arm (Fig. 4B, Gray's test $p = 0.656$).

Impact of addition of flu to tam in the AR-enriched, ER-enriched cohort

We examined the impact of the addition of Flu to Tam in patients with both AR- and ER-enriched tumors. We observed no significant differences in the age at study entry ($p = 0.514$), the proportion of women with prior to exposure to exogenous estrogens (Tam: 11.8% vs. Tam + Flu: 9.7%; $p = 0.799$), proportion of women whose

Table 1 Patient characteristics and outcomes in 290 ER-enriched (> 70% Nuclear staining) breast cancers

	Tamoxifen (n = 151)	**Tamoxifen + Fluoxymesterone n = 139)**
Disease characteristics at Study Entry		
Age		
Median	68	68
25–75th percentile	63–74	62–74
Range	42–84	51–89
Race (self-reported)		
Black/African American	3 (2.0%)	2 (1.4%)
Native American	1 (0.7%)	0
White	143 (94.7%)	136 (97.8%)
Not provided	4 (2.7%)	1 (0.7%)
Hispanic	0	1 (0.7%)
Exogenous estrogens	23 (15.2%)	24 (17.1%)
ECOG PS		
0	129 (85.4%)	123 (88.5%)
1–2	22 (14.6%)	16 (11.5%)
ER		
71–80%	6 (2.0%)	4 (2.9%)
81–90%	12 (8.0%)	5 (3.6%)
91–100%	133 (88.1%)	130 (93.5%)
AR		
0	20 (13.2%)	32 (23.0%)
1–30%	27 (17.9%)	18 (12.9%)
31–70%	19 (12.6%)	17 (12.2%)
71–80%	10 (6.6%)	11 (7.9%)
81–90%	14 (9.3%)	21 (15.1%)
91–100%	61 (40.4%)	40 (28.8%)
AR/ER ratio		
0	20 (13.2%)	32 (23.0%)
– 0.24	26 (17.2%)	13 (9.4%)
0.25 – 0.49	12 (7.9%)	11 (7.9%)
0.50 – 0.74	7 (4.6%)	11 (7.9%)
0.75 – 0.99	25 (16.6%)	31 (22.3%)
1.0	59 (39.1%)	41 (29.5%)
1.1–1.24	2 (1.3%)	0
PR		
0%	11 (7.3%)	16 (11.5%)
1–10%	24 (15.9%)	24 (17.3%)
> 10%	113 (74.8%)	99 (71.2%)
Not obtained	3 (2.0%)	0
Her2		
0	48 (31.8%)	57 (41.0%)
1 +	67 (44.4%)	53 (38.1%)
2 +	23 (15.2%)	17 (12.2%)
3 +	9 (6.0%)	11 (7.9%)
Not obtained	4 (2.6%)	1 (0.7%)
Extent of surgery		
Mastectomy	125 (82.8%)	113 (81.3%)
Breast conserving	26 (17.2%)	26 (18.7%)
Primary		

Table 1 (continued)

	Tamoxifen (n = 151)	Tamoxifen + Fluoxymesterone n = 139)
< 3 cm	113 (74.8%)	114 (82.0%)
≥ 3 cm	38 (25.2%)	25 (18.0%)
Number of positive lymph nodes		
0	97 (64.2%)	93 (66.9%)
1–3	39 (25.8%)	36 (25.9%)
4–9	10 (6.6%)	4 (2.9%)
≥ 10	5 (3.3%)	6 (4.3%)
Outcomes		
Follow-up status		
Alive	51	52
Death due to breast cancer	25	21
Death due to another cancer	5	10
Other	66	51
Unknown	4	5

Table 2 AR and ER results among the 290 ER-enriched breast cancer patients

AR stain	ER stain	Tamoxifen (n = 151)	Tamoxifen + Fluoxymesterone (n = 139)
< 1%	71–80	3 (2.0%)	2 (1.4%)
< 1%	81–90	3 (2.0%)	1 (0.7%)
< 1%	91–100	14 (9.2%)	29 (20.9%)
1–10	71–80	0	2 (1.4%)
1–10	81–90	1 (0.7%)	1 (0.7%)
1–10	91–100	14 (9.3%)	6 (4.3%)
11–20	71–80	1 (0.7%)	0
11–20	81–90	1 (0.7%)	0
11–20	91–100	9 (6.0%)	4 (2.9%)
21–30	91–100	1 (0.7%)	5 (3.6%)
31–40	81–90	1 (0.7%)	0
31–40	91–100	5 (3.3%)	5 (3.6%)
41–50	81–90	2 (1.3%)	1 (0.7%)
41–50	91–100	5 (3.3%)	1 (0.7%)
51–60	71–80	2 (1.3%)	0
51–60	91–100	1 (0.7%)	5 (3.6%)
61–70	81–90	1 (0.7%)	0
61–70	91–100	2 (1.3%)	5 (3.6%)
71–80	80–91	0	1 (0.7%)
71–80	91–100	10 (6.6%)	10 (7.2%)
81–90	81–90	0	1 (0.7%)
81–90	91–100	14 (9.3%)	20 (14.4%)
91–100	81–90	2 (1.3%)	0
91–100	91–100	59 (39.1%)	40 (28.8%)

tumor size > 3 cm (Tam: 21.2% vs. Tam + Flu: 20.8%; $p = 0.999$), proportion of women with positive lymph nodes (Tam: 28.4% vs. Tam + Flu: 30.6%; $p = 0.861$) between the treatment arms. The CI-RecDeath for these patients was greater in women on the Tam arm than women on the Tam + Flu arm. (Fig. 5A, Gray's test $p = 0.0472$).

Impact of addition of flu to tam in the AR-poor/moderate, ER-enriched cohort

Among women with AR-poor/moderate, ER-enriched tumors, no significant differences in age at study entry ($p = 0.516$), the proportion of women with prior to exposure to exogenous estrogens (Tam: 19.7% vs. Tam + Flu: 25.4%; $p = 0.535$), or the proportion of women with positive lymph nodes (Tam: 45.5% vs. Tam + Flu: 35.8%; $p = 0.292$) were found between the treatment arms. However, the proportion of women whose tumor was > 3 cm was significantly greater in the Tam arm than the Tam + Flu arm (Tam: 30.3% vs. Tam + Flu: 14.9; $p = 0.039$). The CI-RecDeath in the AR-poor/moderate cohort was not found to differ with treatment arm (Fig. 5B, Gray's test $p = 0.7084$).

Discussion

This study included women with early-stage ER-enriched breast cancer who enrolled on a prospective randomized trial of adjuvant therapy with Tam alone or combined with Flu [13]. The majority (86%) were entered on the clinical trial based on a dextran-coated charcoal assay (two thirds with ≥ 50 fmols/mg cytosol protein) with the remainder entered based on an immunostain positive for ER. For the current analyses ER expression levels were ascertained from a TMA created from the primary tumor blocks collected 29 to 33 years ago. A striking finding was the high ER expression levels in this cohort. Because

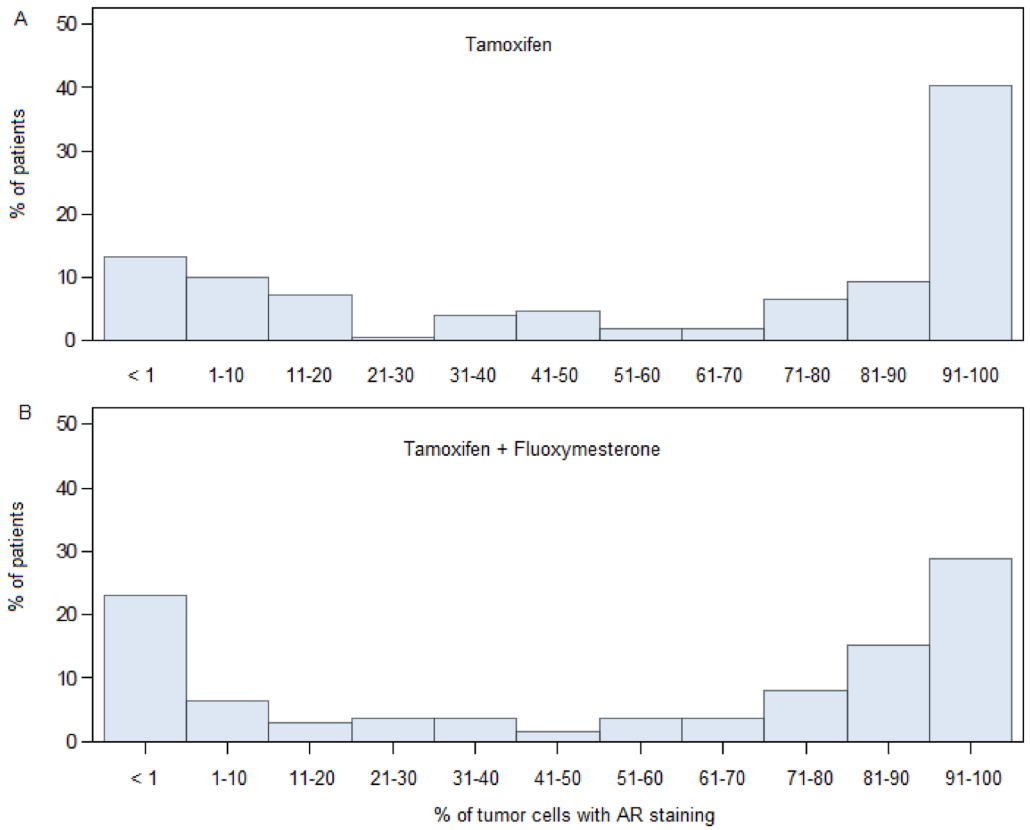

Fig. 2 Percentage of tumor cells with androgen receptor immunohistochemical nuclear staining for the tamoxifen cohort (**A**) and the tamoxifen + fluoxymesterone cohort (**B**)

only 11 (4%) patients had ER nuclear staining in ≤ 70% of tumor cells, we restricted our analysis of CI-RecDeath to the 290 patients with ER nuclear staining in > 70% of the tumor cells (ER-enriched breast cancers).

In a retrospective cohort of 284 women with ER + / HER2 − primary invasive breast cancers from a single institution receiving physician-directed therapy unspecified endocrine therapy alone (69.7%) or with chemotherapy (29.4%), Rangel [10] found 17 (6%) women had cancers with an AR/ER ratio ≥ 2 and these women had worse disease-free interval and disease-specific survival. In contrast, our study cohort is composed of women with ER-enriched BC randomized between 2 endocrine treatments, Only 2 of these patients had an AR/ER ratio > 1.0 where both patients had AR staining of 91–100% and ER staining of 81–90%. Dischotomizing the AR/ER ratio at 1.0, we did not find the CI-RecDeath differed with respect to whether the AR/ER ratio ≥ 1.0 or not in the Tam arm (Fig. 4A, Gray's test $p = 0.768$) or in the Tam + Flu arm (Fig. 4B, Gray's test $p = 0.656$).

We examined the association between AR status and likelihood of a breast cancer recurrence or death within each treatment arm and found the CI-RecDeath did not differ with respect to whether a patient's tumor was AR-enriched or not in the Tam arm (Fig. 3A, Gray's test $p = 0.445$) or in the Tam + Flu arm (Fig. 3B, Gray's test $p = 0.126$). Regarding the Tam alone arm, Amicis et al. [15] reported that AR overexpression induced resistance to Tam but we did not find a worse outcome in ER-enriched/AR-enriched relative to the ER-enriched/AR-poor/moderate Tam-treated patients.

We then proceeded to examine whether the AR-agonist Flu provided benefit in those patients with ER-enriched/AR-enriched tumors and what impact, if any, in those patients with ER-enriched/AR-poor/moderate tumors. Among the patients with ER-enriched/AR-enriched, tumors, the CI-RecDeath was greater in those on the Tam arm than those on the Tam + Flu arm (Fig. 5A, Gray's test $p = 0.0472$). However, among ER-enriched/AR-poor/moderate tumors, we found no evidence that CI-RecDeath was greater in those on the Tam arm than those on the Tam + Flu arm (Fig. 5B, Gray's test $p = 0.708$).

Interest in the use of agents that are AR agonistic has been heightened by the advent of selective AR modulators (SARMs) that are AR-agonists but do not have the

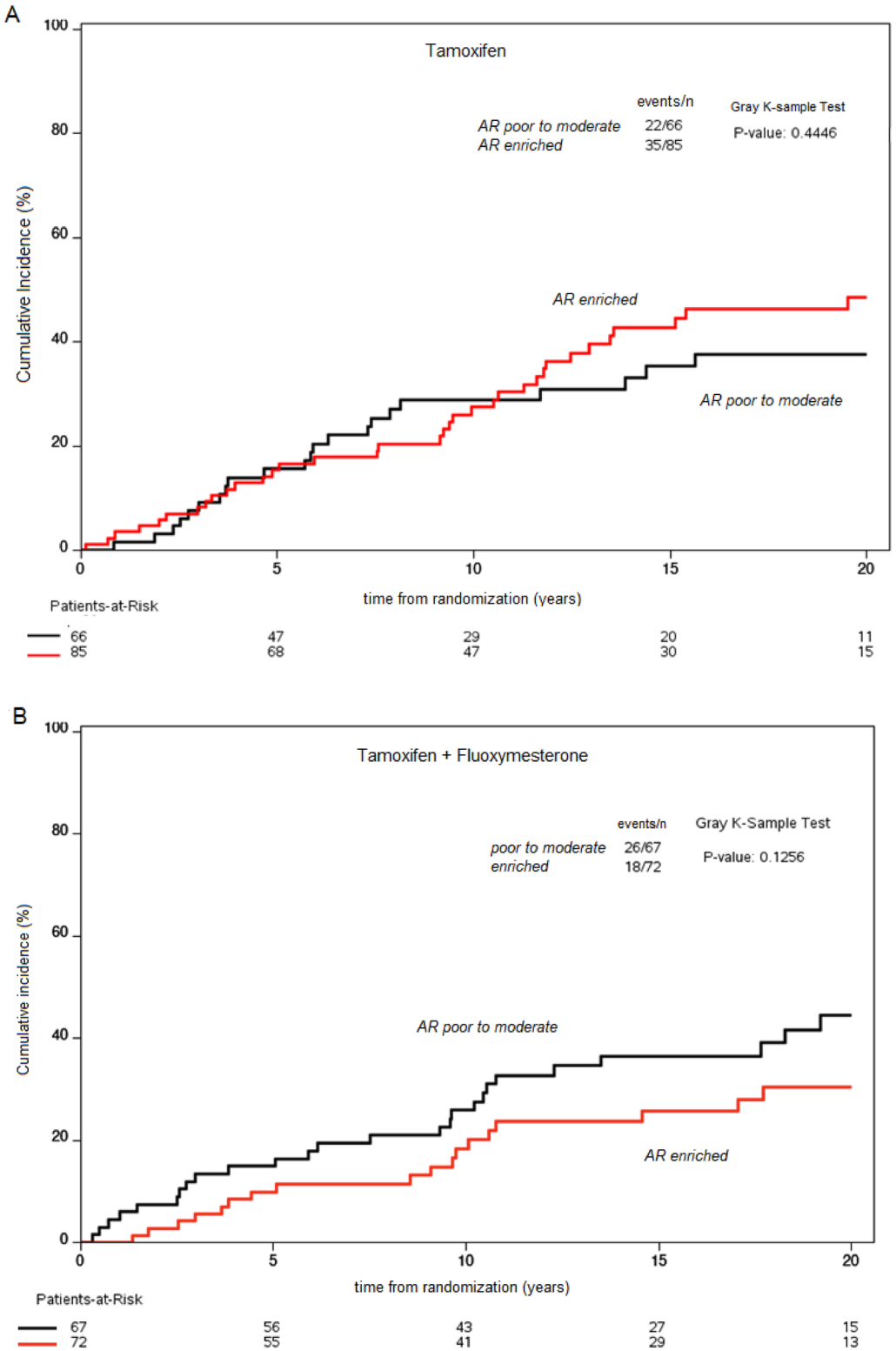

Fig. 3 Cumulative incidence of breast cancer recurrence or death by androgen receptor expression (AR poor to moderate [black line]; AR enriched [red line]) for the tamoxifen cohort (**A**) and the tamoxifen + fluoxymesterone cohort (**B**)

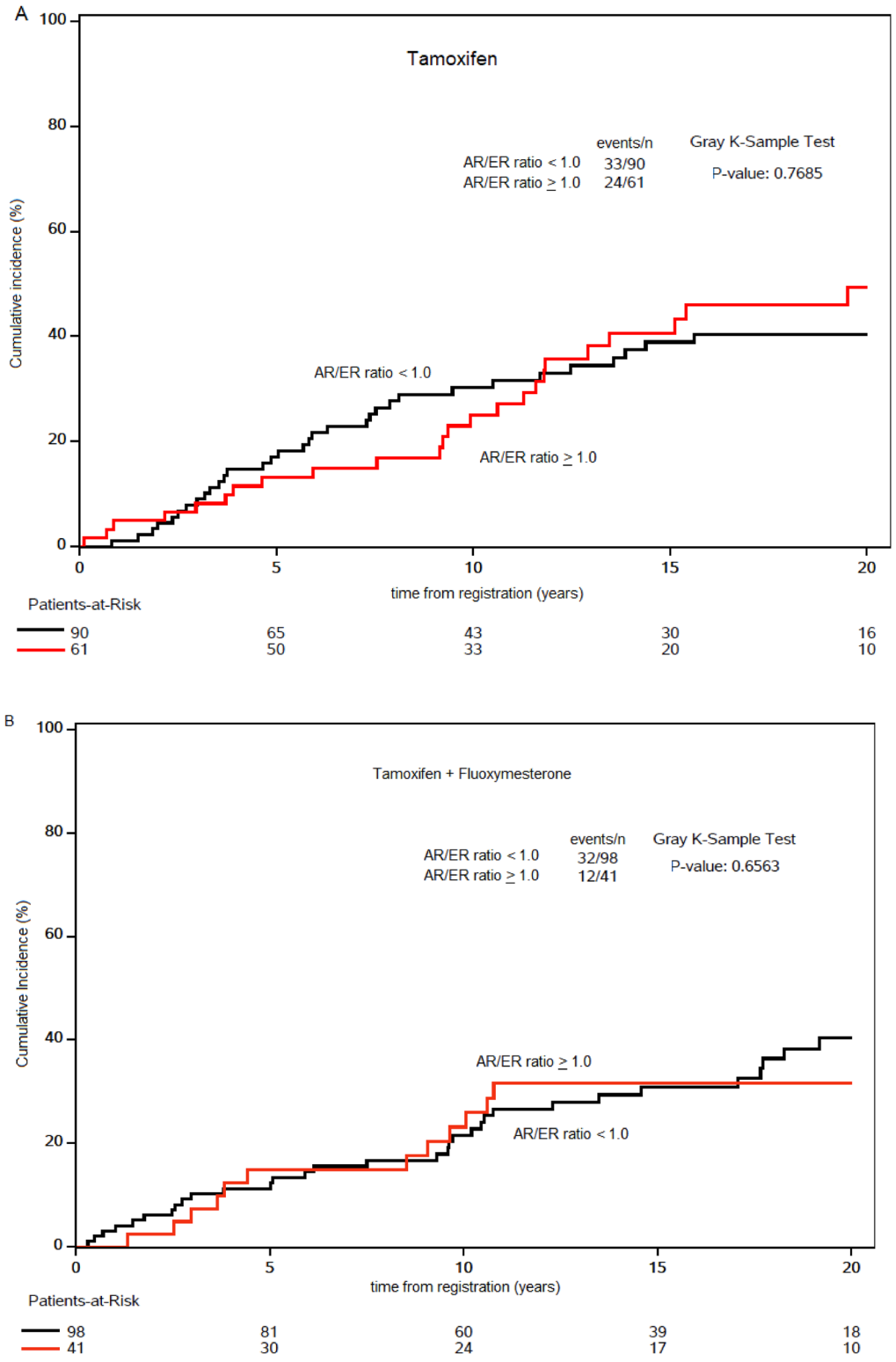

Fig. 4 Cumulative incidence of breast cancer recurrence or death by androgen receptor/estrogen receptor (AR/ER) ratio (less than 1 [black line] vs. 1 or more [red line]) for the tamoxifen cohort (**A**) and the tamoxifen + fluoxymesterone cohort (**B**)

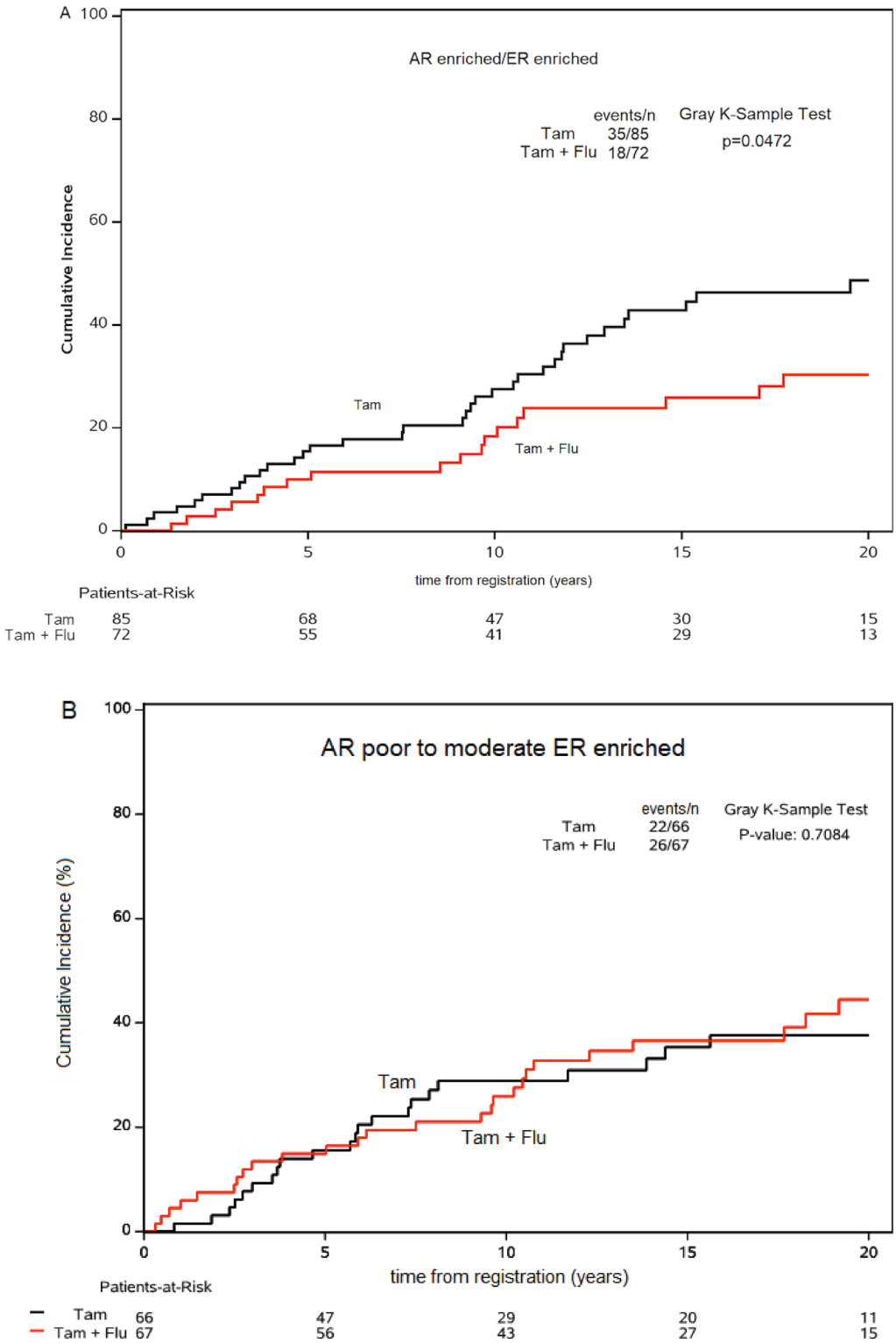

Fig. 5 Cumulative incidence of breast cancer recurrence or death of tamoxifen (Tam) alone vs tamoxifen plus fluoxymesterone (Tam + Flu) in patients with AR-enriched, ER-enriched tumors (**A**) and AR-poor to moderate, ER-enriched tumors (**B**)

virilizing adverse events typical of androgenic therapies such as Flu [16, 17]. Enobosarm is an oral SARM that has recently been reported to have anti-tumor activity and an acceptable safety profile in previously treated AR-positive, ER-positive, HER2-negative breast cancer [18]. Of note is that the antitumor activity of enobosarm was greater in women with moderate to high AR expression (≥40%) than women with no to low AR expression (≤40%) [19].

A strength of our study is that the patients included were from a prospective randomized trial with long-term (median 20.4 years) follow-up. Limitations include the relatively small sample size overall and the small number of patients with no AR expression. The level of ER staining was exceedingly high with 96% having > 70% nuclear staining and we were unable to address the impact of AR expression in women with lower levels of ER expression. An additional limitation was the relatively short duration of administration of the AR-agonist (Flu). We planned for patients randomized to the Tam + Flu arm to receive only one year of Flu because of concern regarding tolerability, particularly virilization. A limitation of 89–30-52 is that we did not collect data on compliance with Flu but given the toxicities observed it is likely that some patients discontinued Flu before the one-year mark. Despite the limitations we were still able to show an advantage of adding Flu to Tam in ER-enriched/AR-enriched breast cancers.

Conclusions

Despite the small sample size we found that the cumulative incidence of breast cancer recurrence or death in patients with ER-enriched/AR-enriched primary cancers was lower among those receiving the AR-agonist Flu along with Tam than than those treated with Tam alone. This suggests that the AR level may be important when one examines the use of AR-agonist therapy. Given that the planned duration of Flu was for only one year, longer administration of AR-agonist therapy would be expected to provide a greater level of benefit. The availability of AR-agonists without androgenic adverse events, i.e., SARMs, raises the opportunity to examine the impact of the AR expression level on efficacy of these agents in ER-positive breast cancer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13058-025-01992-0.

Additional file 1.

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Author contributions
JNI, LW, MPG contributed to the study conception and design. Material preparation, data collection and analysis were performed by MHS, MRP, VJS, and JDC. The first draft of the manuscript was written by JNI and VJS and all authors commented on the manuscript, All authors read and approved the final manuscript.

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Availability of data and materials
De-identified data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed consistent with the principles of the Declaration of Helsinki. Approval was granted by the Mayo Clinic Institutional Review Board.

Consent for publication
Not applicable.

Competing interests
LW reports that she is a co-founder and stockholder in OneOme, LLC; MPG reports personal fees for CME activities from Research to Practice, Clinical Education Alliance, Medscape, and MJH Life Sciences; personal fees serving as a panelist for a panel discussion from Total Health Conferencing and personal fees for serving as a moderator for Curio Science; personal fees from Ideology; consulting fees to Mayo Clinic from ARCTherapeutics, AstraZeneca, Biotheranostics, Blueprint Medicines, Lilly, Rna Diagnostics, Sanofi Genzyme, Seattle Genetics and Engage Health Media; and grant funding to Mayo Clinic from Lilly, Pfizer, Sermonix, Loxo, AstraZeneca and ATOSSA. The other authors declare no financial interests.

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