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Increased pathological complete response rate after a long-term neoadjuvant letrozole treatment in postmenopausal oestrogen and/or progesterone receptor-positive breast cancer

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Background: The objective of this study was to determine the optimal scheduling of 2.5 mg daily letrozole in neoadjuvant breast cancer patients to obtain pathological complete response (pathCR) and assess Ki-67 expression as an early predictor of response.

Patients and methods: This single institution study comprised 120 oestrogen receptor (ER)-positive postmenopausal women with primary breast cancer (clinical stage $\geqslant$T2, N0–1), from three sequential cohorts (cohort A of 40, cohort B of 40 and cohort C of 40 patients, respectively) based on different duration of the neoadjuvant letrozole. Biological markers such as ER, progesterone receptor, HER2 and Ki-67 expression were tested at diagnosis and at definitive surgery.

Results: A total of 89 patients (75.4%) achieved an objective response with 44 (37.3%) clinical CRs and 45 (38.1%) partial responses. The clinical CRs were significantly observed in cohort C (23 out of 40 patients, 57.5%) and B (16 out of 38 patients, 42.1%) compared with cohort A (5 out of 40 patients, 12.5%) (P-value for trend <0.001). Letrozole induced a similar significant reduction in Ki-67 index after treatment in all cohorts. The pathCR rate was significantly more frequent in cohort C (7 out of 40 patients, 17.5%) than in cohort A (1 out of 40 patients, 2.5%) and B (2 out of 40 patients, 5.0%) (P-value for trend <0.04).

Conclusion: One-year neoadjuvant letrozole therapy leads to a higher pathCR rate and may be the optimal length of drug exposure.

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Neoadjuvant therapy (NeoT) is commonly used as a preoperative treatment to favour a volume reduction of locally advanced breast cancers, to achieve the highest possible rates of breast-conserving surgery (BCS). Moreover, its use in clinical routine has become increasingly important to test the *in vivo* sensitivity of breast cancer to various therapeutic approaches (Bottini *et al*, 2006; Berruti *et al*, 2008).

The management of the elderly patient with breast cancer is a challenge to the breast care team for a number of reasons. The higher rate of comorbidity in elderly patients increases the risk for complications and mortality following surgery and other adjuvant treatments such as chemotherapy and radiotherapy (Yancik *et al*, 2001). The use of endocrine therapy in the neoadjuvant setting allows disease control and downstaging of tumours to enable less extensive surgery, with less morbidity compared with other available treatments. Aromatase inhibitors have been shown to be superior to tamoxifen and are being tested extensively as NeoT in place of tamoxifen (Eiermann *et al*, 2001; Smith *et al*, 2005). Response rates between 40% and 60% have been reported with aromatase inhibitors in randomised clinical trials (Eiermann *et al*, 2001; Smith *et al*, 2005).

Although endocrine therapy in the NeoT setting is recognised to induce a significant reduction of tumour volume in hormone receptor-positive postmenopausal women after just 3–4-months of treatment (Eiermann *et al*, 2001; Smith *et al*, 2005; Mlineritsch *et al*, 2008), the optimal treatment duration has not been established.

Our group has previously shown that 2.5 mg letrozole administered for 6 months in elderly breast cancer patients receiving primary systemic treatment induced an overall response rate in 41 out of 57 patients (71.9%; 95% CI, 60.8–83.8), with two cases (3.5%) of pathological complete response (pathCR) (Bottini *et al*, 2006). To investigate Ki-67 expression as a surrogate parameter of treatment efficacy (Berruti *et al*, 2011a), we showed that letrozole alone also induced a significant reduction of Ki-67 (evaluated at diagnosis and at definitive surgery) expression after treatment (Bottini *et al*, 2006).

Only three groups have explored longer (>6 months) administration and the overall response rate (Krainick-Strobel *et al*, 2008; Dixon *et al*, 2009; Carpenter *et al*, 2010). Dixon *et al* (2009), in their prospective study assessing the response to 3–12 months neoadjuvant treatment with letrozole, showed tumour shrinkage continuing up to 12 months in responders. Krainick-Strobel *et al* (2008)showed that the prolonged treatment could be safely and effectively used for up to 8 months, resulting in further clinical tumour volume reduction (70%) compared with 62.5% obtained in patients treated for 4 months. Although these studies showed that letrozole could be safely and effectively used for at least 8 months in the neoadjuvant setting, to date, there are no data on the pathological response rate using different durations of letrozole to guide optimal therapy for patients in the NeoT context. Therefore, we undertook this study to investigate the potential biological benefits of extended neoadjuvant letrozole therapy comparing 4 months *vs* 8 months *vs* 12 months of letrozole administration in neoadjuvant setting, in sequential patient cohorts, aiming at the identification of the optimal treatment duration to obtain maximum response and identify characteristics associated with pathCR. As pathCR is a prognostic factor for survival (Kaufmann *et al*, 2006; Berruti *et al*, 2011a), the main objective of the study was to evaluate the rate of pathCR after primary systemic treatment with letrozole. Secondary objectives were the rate of clinical and radiological response, tolerability and the variation of Ki-67.

MATERIALS AND METHODS

Patients. Elderly women (age $\geqslant 65$ years) unfit for chemotherapy from April 2004 to February 2011 with clinical T2–4 N0–1 M0 (evaluated with clinical and radiological procedures) and oestrogen receptor-positive (ER+) and/or progesterone receptor-positive (PgR+) breast cancer were eligible, from three sequential patient cohorts (40 each in single cohort) received different duration of the neoadjuvant treatment with letrozole (Femara, Novartis, Milan, Italy) 2.5 mg (one tablet) daily: 4 (cohort A) or 8 (cohort B) or 12 (cohort C) months of treatment. They had an Eastern Cooperative Oncology Group performance status of two or lower, adequate bone marrow reserve (WBC count, $>3.5\times10^9\,l^{-1}$; platelets, $>100\times10^9\,l^{-1}$; haemoglobin, $>10\,g\,dl^{-1}$), hepatic function (AST/ALT bilirubin and alkaline phosphatase levels $<1.25\times$ the upper limit of normal value) and renal function (serum creatinine $<1.25\times$ the upper limit of normal value). The study was approved by the local ethical committee (no. 21391/2012). Written informed consent was obtained before collecting data.

Treatment evaluation and adverse effects. On presentation, an incision biopsy was performed on each patient and a small tissue sample (0.5–0.8 cm^3) removed. Each month, to determine clinical tumour response, the size of the primary tumour was measured with a calipre by the same clinician. The clinician also provided at every visit the monthly letrozole tablets to monitor the patients' adherence to the trial. At baseline and at the end of treatment before definitive surgery, mammography and breast ultrasound were performed. Clinical responses were evaluated according to both radiological (breast ultrasound or mammography) and clinical evaluation, by measuring the largest diameter of the tumour and were graded according to Response Evaluation Criteria In Solid Tumours (RECIST) Version 1.1 (Eisenhauer *et al*, 2009). CR was defined as the disappearance of all known disease. Similarly, partial response (PR) was considered to have occurred if tumour size decreased by at least 30% in the absence of any progression or new lesions. Decreases by $<30\%$ and increases by $<20\%$ were considered to represent stable disease, whereas increases $\geqslant 20\%$ or appearance of new lesions were classed as progressive disease.

PathCR was defined as a total absence of neoplastic cells in the breast and in the axillary lymph nodes after histological examination: the presence of intraductal carcinoma was not required for pathCR. Surgery was planned after full clinical reassessment. Quadrantectomy or radical mastectomy was performed when indicated in association with full axillary node dissection.

Toxicity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE, 2009) Version 4.03 (Rockville, MD, USA). No letrozole dose reduction was planned. Letrozole was planned to be interrupted in case of severe adverse events, defined as any undesirable experience associated with the use of the medical product in a patient.

Immunohistochemistry. Immunohistochemical evaluation was performed on formalin-fixed, paraffin-embedded tumour samples obtained at diagnosis and at definitive surgery. ER, PgR, overexpression of HER2 and Ki-67 staining were carried out at the Pathology Unit of the Azienda Ospedaliera Istituti Ospitalieri of Cremona (Italy). The immunohistochemical methodology is fully described elsewhere (Generali *et al*, 2009). The value of Ki-67 labelling index was used as a cutoff in distinguishing tumours with low ($<14\%$) and high ($\geqslant 14\%$) proliferative fraction (Berruti *et al*, 2011b; Fasching *et al*, 2011; Sheri and Dowsett, 2012).

Statistical analysis. Data were evaluated for non-normality distribution using graphical and descriptive techniques. A test for overall comparison was employed for each outcome variable across all three cohorts (A, B and C), and if a significant difference was detected, we proceeded to pairwise comparisons (i.e., A *vs* B, B *vs* C, A *vs* C) using the χ^2 test or Fisher's exact test when indicated for dichotomous variables, and Mann–Whitney *U*-test for continuous variables. Changes in Ki-67 labelling index within cohorts were

tested with the Wilcoxon signed-rank test. All tests were two sided; $P<0.05$ was considered as statistically significant. Data were analysed using the Statistica software (StatSoft Inc., Tulsa, OK, USA).

RESULTS

The median age at the start of treatment for all patients was 78.4 years (range 65.0–95.4 years). Tumoral characteristics at baseline were: 100% (120 out of 120 cases) positive ER whose intensity was >90%; 82.5% (99 out of 120 cases) positive PgR; and 6.7% (8 out of 120 cases) positive HER2. Median Ki-67 was 14.5% (range 2–90%): 48.3% (58 out of 120 cases) with low, and 51.7% (62 out of 120 cases) with high proliferative fraction. The most frequent histological type was infiltrating ductal carcinoma (85 out of 120 cases, 70.8%) followed by infiltrating lobular carcinoma (18 out of 120 cases, 15.0%), mixed ductal and lobular carcinoma (8 out of 120 cases, 6.7%) and others (9 out of 120, 7.5%).

Patients' baseline characteristics according to cohorts are outlined in Table 1. The three cohorts of patients were comparable in all variables.

Treatment toxicity. Adverse events occur in 29 out of 120 patients (24.2%). The most frequent relevant adverse events (grade 2) recorded were hot flashes in 13 patients (44.8%), osteoarticular pain in 6 patients (20.7%), dizziness in 3 patients (10.3%), weight gain in 2 patients (6.9%) and headache in 2 patients (6.9%). No patients interrupted or delayed the treatment. In two patients, grade 3 adverse events were monitored: one with dizziness and one with osteoarticular pain. In these patients, treatment was discontinued after definite surgery.

Treatment response. The clinical response recorded for each cohort is described in Table 2. The majority of PR and CR were observed in cohort C (38 out of 40, objective response rate (ORR) = 95.0% (95% confidence interval, CI: 83.1–99.4%)) as opposed to cohort B (33 out of 38, ORR = 86.8% (95% CI: 71.9–95.6%)) or A (18 out of 40, ORR = 45.0% (95% CI: 29.3–61.5%)). The clinical ORR was higher in cohort C compared with A (*P*-value for trend <0.00001), and B compared with A (*P*-value for trend <0.001). The clinical CR rate compared with non-CR rate was confined to cohorts C and B rather than cohort A (*P*-value for trend <0.001). Six out of 120 patients, mainly confined in cohort A (five patients), who received the shorter administration of letrozole, progressed under treatment, suggesting the possible primary/*de novo* activation of pathways involved in aromatase inhibitors' resistance (Margariti *et al*, 2011; Cavazzoni *et al*, 2012).

ORR was 49.6% (58 out of 117 cases (95% CI: 40.2–59.0%)) at month 4, 85.3% (64 out of 75 cases (95% CI: 75.3–92.4%)) at month 8 and 95.0% (38 out of 40 cases (95% CI: 83.1–99.4%)) at month 12 of treatment compared with baseline (Table 3).

The pathCR was confined to the cohort C (7 out of 40 cases, 17.5%), with respect to cohort B (2 out of 40 cases, 5.0%) and cohort A (1 out of 40 cases, 2.5%) (*P*-value for trend <0.04).

As previously shown by our group (Fiorentino *et al*, 2001), the grade of response of breast cancer to primary chemotherapy, showed by mammography and echography (data not shown), was less marked than the grade of response seen at clinical examination. The frequency of early treatment discontinuation, resulting from disease progression and patient refusal, did not occur among the treatment cohorts.

Breast surgery. All patients treated with letrozole in each cohort underwent surgery no later than 30 days after taking the last dose of study drug. The use of letrozole in neoadjuvant setting enabled a more conservative surgical approach: 101 (84.2%) out of 120 patients underwent BCS (lumpectomy). In cohort A, 32 (80.0%)

Table 1. Patients' characteristics (absolute and percentage value) according to single cohort

	Cohort A	Cohort B	Cohort C
Age (years)			
Median	79.4	75.0	78.51
Range	67.5–93.8	67.2–95.4	65.0–88.4
No. of patients	40	40	40
PgR status			
PgR +	35 (87.5)	32 (80.0)	32 (80.0)
PgR −	5 (12.5)	8 (20.0)	8 (20.0)
HER-2 status			
HER2 +	2 (5.0)	5 (12.5)	1 (2.5)
HER2 −	38 (95.0)	35 (87.5)	39 (97.5)
Ki-67 expression			
Median	13	13.5	15
Range	5–35	2–90	2–32
<14%	21 (52.5)	20 (50.0)	17 (42.5)
⩾14%	19 (47.5)	20 (50.0)	23 (57.5)
Grade			
I	2 (5.0)	0	0
II	24 (60.0)	27 (67.5)	23 (57.5)
III	13 (32.5)	13 (32.5)	17 (42.5)
NA	1 (2.5)	0	0
Histotype			
DIC	24 (60.0)	32 (80.0)	29 (72.5)
LIC	8 (20.0)	5 (12.5)	5 (12.5)
Mixed DIC-LIC	3 (7.5)	2 (5.0)	3 (7.5)
Other	5 (12.5)	1 (2.5)	3 (7.5)
Clinical stage			
T2	35 (87.5)	30 (75.0)	28 (70.0)
T3	0	4 (10.0)	3 (7.5)
T4	4 (10.0)	6 (15.0)	8 (20.0)
NA	1 (2.5)	0	1 (2.5)
N0	31 (77.5)	32 (80.0)	33 (82.5)
N1	5 (15.0)	8 (20.0)	4 (10.0)
NA	3 (7.5)	0	3 (7.5)

Abbreviations: DIC = ductal infiltrating carcinoma; LIC = lobular infiltrating carcinoma; NA = not available; PgR = progesterone receptor. The three cohorts of patients were comparable in terms of age at the start of neoadjuvant treatment, basal clinical dimensions, tumoral grade, histotype, PgR and HER2 status, and Ki-67 value.

Table 2. Distribution of clinical disease response (absolute and percentage value) at individual end according to single cohort

	Cohort A	Cohort B	Cohort C
No. of patients	40	40	40
NA	0	2	0
Progressive disease	4 (10.0)	1 (2.6)	0
Stable disease	18 (45.0)	4 (10.5)	2 (5.0%)
Partial response	13 (32.5)	17 (44.8)	15 (37.5%)
Complete response	5 (12.5)	16 (42.1)	23 (57.5)
Overall response rate	45.0%	86.8%	95.0%
95% CI	29.3–61.5	71.9–95.6	83.1–99.4

Abbreviations: CI = confidence interval; NA = not available.

Table 3. Distribution of clinical disease response (absolute and percentage value) according to months of treatment

	Month 4	Month 8	Month 12
No. of patients	117	75	40
NA	3	5	0
Progressive disease	5 (4.3)	1 (1.3)	0
Stable disease	54 (46.2)	10 (13.4)	2 (5.0%)
Partial response	37 (31.6)	33 (44.0)	15 (37.5%)
Complete response	21 (17.9)	31 (41.3)	23 (57.5)
Overall response rate	49.6%	85.3%	95.0%
95% CI	40.2–59.0	75.3–92.4	83.1–99.4

Abbreviations: CI = confidence interval; NA = not available.

out of 40, in cohort B, 34 (85.0%) out of 40 and in cohort C, 35 (87.5%) out of 40 patients received lumpectomy, respectively. However, no statistical differences were observed between cohorts in relation to the type of surgery performed.

Changes in Ki-67 expression. Baseline and post-treatment Ki-67 expression in 120 cases within each cohort is depicted in Figure 1. At baseline, no difference in Ki-67 index between treatment arms was observed. Letrozole-based treatment resulted in an overall significant reduction in Ki-67 index after treatment ($P<0.000001$). At post-letrozole treatment, residual histology Ki-67 index was significantly lower compared with baseline in all cohorts ($P<0.00001$, $P<0.00001$ and $P<0.0001$, respectively), but no significant differences were detected between cohorts (Table 4).

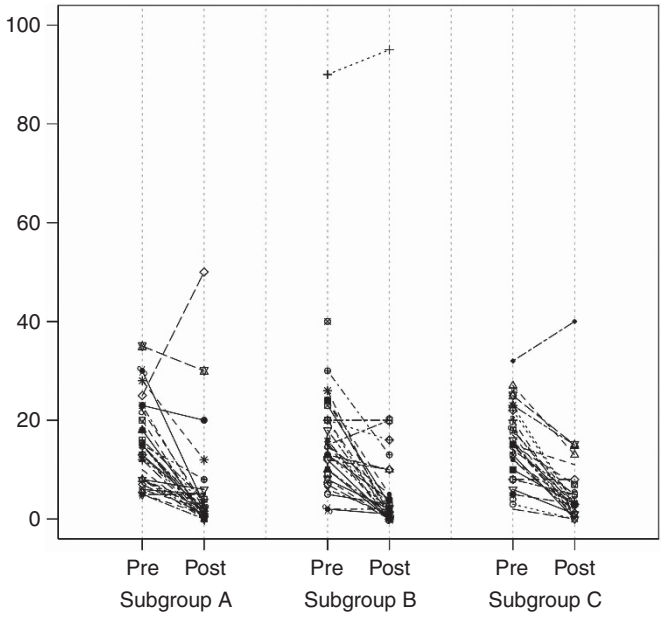

Figure 1. Changes in Ki-67 expression for individual patients at baseline and post-treatment histology according to cohort. The large majority of patients receiving letrozole, independently of the cohort they belong, showed a suppression of Ki-67 expression after treatment.

Table 4. Ki-67 expression at baseline and after treatment

Description		*P*-value	
Overall			
Pre-treatment Post-treatment	Median 14.5% (range 2–90%) Median 2% (range 0–95%)	$P<0.000001$	
Cohort A			
Pre-treatment Post-treatment	Median 13% (5–35%) Median 2% (0–50%)	$P<0.00001$	
Cohort B			
Pre-treatment Post-treatment	Median 13.5% (2–90%) Median 2% (0–95%)	$P<0.00001$	$P=$ not significant
Cohort C			
Pre-treatment Post-treatment	Median 15% (2–32%) Median 3% (0–40%)	$P<0.0001$	

Letrozole induced an overall significant reduction of Ki-67 expression after treatment. The significant reduction was detected in each single cohort. No significant differences in Ki-67 suppression after treatment were observed among each cohort.

DISCUSSION

Historically, neoadjuvant endocrine therapy has been limited to patients who were unsuitable for chemotherapy and surgery. Earlier phase II studies with tamoxifen that focused primarily on elderly and/or frail patients often unselected for hormone receptor status of the tumour showed a response rate ranging from 49% to 68% (Abrial *et al*, 2006). Indeed, the third-generation AIs are now acknowledged as the gold standard in the endocrine treatment of oestrogen-responsive postmenopausal breast cancer, in both the neoadjuvant and adjuvant settings (Smith and Dowsett, 2003). However, the superiority in efficacy of AIs over tamoxifen as neoadjuvant treatment for postmenopausal patients with ER+/PgR+ breast cancer is well known (Eiermann *et al*, 2001; Smith *et al*, 2005); among the aromatase inhibitors, the three commercially available agents are clinically and biologically equivalent and therefore likely to have similar neoadjuvant and adjuvant activities (Ellis *et al*, 2011). Our group has previously shown a very high response rate associated with a significant reduction of Ki-67 index after treatment in neoadjuvant setting based on AI treatment in elderly patients with locally advanced breast cancer (Bottini *et al*, 2006).

However, the question regarding the optimal duration of neoadjuvant treatment that is able to modulate significantly the biology of the tumour beyond tumour shrinkage and conservative surgery based on AIs is still unknown. Recently, Krainick-Strobel *et al* (2008) published the prolonged treatment for up to 8 months can result in further tumour volume reduction in some patients with a favourable overall safety and tolerability profile; however, a clear information about the optimum duration of the treatment along with its biological effect is still to be determined. On the basis of the clinical data, it was recently agreed by an International Consensus Panel that neoadjuvant hormonal therapy should be continued for not <4 months (Berruti *et al*, 2011b; Kaufmann *et al*, 2012; Zambetti *et al*, 2012). However, to provide hard data for enabling evidence-based best practice our group have addressed this issue, from both a clinical and biological point of view, collecting data from patients who have received letrozole

2.5 mg day^{-1} of different duration in the neoadjuvant setting for hormone-responsive locally advanced breast cancer.

Under the conditions of our study, the majority of patients responded to letrozole treatment by 12 months. Also, the ORR was significantly higher with this prolonged duration of administration compared with 4 months, indicating additional benefit from prolonged letrozole treatment. These data are validated by Krainick-Strobel *et al* (2008), who observed increased overall clinical palpation response rates in intention-to-treat analysis of 72.4% (CR, 6.9%; PR, 65.5%), suggesting incremental benefit from letrozole treatment beyond 4 months' duration (Krainick-Strobel *et al*, 2008). As far as a comparison is possible, our data also agree with the findings obtained by Renshaw *et al* (2004),who reported clinical responses from 3 to 12 months neoadjuvant treatment with letrozole 2.5 mg day^{-1} in 142 postmenopausal women with large operable or locally advanced ER-rich breast cancer (Renshaw *et al*, 2004).

This tendency of an increased ORR with the increased duration of the treatment administration was also reflected in the BCS rate from 80.0% in cohort A to 87.5% in cohort C, respectively. This underlines the beneficial effect of neoadjuvant letrozole treatment for longer than 4 months; considering also that the observed adverse events related to letrozole were not unexpected and mostly mild-to-moderate in severity, even the treatment was longer.

The response to neoadjuvant treatment is frequently utilised as a surrogate of outcome with chemotherapy, as it has been shown to be associated with a longer disease-free survival and overall survival compared with no response (US Department of Health and Human Services, 2012). In particular, it has been assumed that pathological complete remission is a valid surrogate of long-term survival and cure from breast cancer (Fisher *et al*, 1998; Guarneri *et al*, 2006; Akashi-Tanaka *et al*, 2007; Berruti *et al*, 2011a). However, pathCR can be achieved only in a minority of patients with ER-positive disease irrespective of the treatment adopted (chemotherapy or hormone therapy). Recent consensus papers on neoadjuvant therapy in breast cancer indicate that pathCR rates ranges from 2% to 10% in those patients whose tumours that express ER (Berruti *et al*, 2011b; Kaufmann *et al*, 2012; Zambetti *et al*, 2012). However, our study showed for the first time that to achieve an optimal pathCR rate in ER-positive disease, the duration of the endocrine treatment administration is critical, increasing up to 17.5% with 12 months treatment.

In this study, we have treated elderly patients with high level of tumour ER expression and it is possible that higher tumour ER levels correlate with a higher probability of response, as reported in two randomised neoadjuvant trials in postmenopausal patients with ER-positive disease (Ellis *et al*, 2001; Smith *et al*, 2005). It is also recognised in advanced disease that it may take many months to obtain the maximum response, but that has not as yet translated into practice for primary cancers. The pathCR obtained with 12 months letrozole therapy in our study is indeed superior than that expected with chemotherapy and is preferable in this setting. All these data suggest the oncologists should administer hormone therapy of longer duration to obtain not only the best tumour response for breast-conserving surgery but also to achieve a pathCR to offer the best outcome as possible to their breast cancer patients.

From the biological point of view, as expected, letrozole reduced significantly Ki-67 expression after treatment in all arms. A positive significant correlation between the ER level and the degree of Ki-67 suppression after 2 and 12 weeks of endocrine treatment has been reported (Dowsett *et al*, 2005). We hypothesise that the level of expression of ER, and maybe also PgR (only few patients were negative), might also be correlated with the probability of response to neoadjuvant hormone therapy with prolonged therapy. In retrospective studies, high baseline Ki-67 was found to be an independent factor predictive for pathCR at multivariate analyses (Petit *et al*, 2004; Colleoni *et al*, 2010). Even though we did not find any correlations with basal expression of Ki-67 (cutoff used $>14\%$) and pathCR in any arm, it might be possible to consider that the high baseline expression of Ki-67 combined with higher ER expression could be helpful for oncologists in selecting patients who would benefit from longer administration of letrozole in neoadjuvant setting. It is worth noting that changes in Ki-67 in our series did not differ comparing patients who received 12-month letrozole therapy with those with shorter drug exposure. This observation suggests that reduction in Ki-67 is not predictive of pathCR and underlines that treatment-induced changes in proliferative activity and apoptosis follow independent mechanisms.

In conclusion, prolonged neoadjuvant letrozole treatment is well tolerated with a favourable toxicity profile and results in further tumour volume reduction, and thus may provide incremental benefit to patients for conservative surgery and the induction of a high rate of pathCR.

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