# Clinical response to primary letrozole therapy in elderly patients with early breast cancer: Possible role for p53 as a biomarker

#Veerabhadram Garimella[1,2], #Tasadooq Hussain[1,2,4], #Vijay Agarwal[1,3,4], Selvi Radhakrishna[1,2], John N Fox[2], Peter J Kneeshaw[2], Ervine D Long[5], Tapan K Mahapatra[2], Penelope L McManus[2], Michael J Lind[1,3,4], Philip J Drew[1,2,4], *Lynn Cawkwell[1,4]

[1]Postgraduate Medical Institute of the University of Hull, Hull, UK.

[2]Breast Care Unit, Hull and East Yorkshire NHS Trust, Hull, UK.

[3]Queens Centre for Oncology and Haematology, Hull and East Yorkshire NHS Trust, Hull, UK.

[4]Hull York Medical School, Hull, UK.

[5]Histopathology Department, Hull and East Yorkshire NHS Trust, Hull, UK.

# Contributed equally

*__Address for Correspondence:__

Dr Lynn Cawkwell PhD, Daisy Building, Castle Hill Hospital, Hull, HU16 5JQ, UK.

L.Cawkwell@hull.ac.uk Tel (+44) 1482 461850 Fax (+44) 1482 461874

__Article type:__ Original

__Short Title:__ Garimella Primary letrozole in elderly breast cancer patients

**Abstract**

Primary tamoxifen therapy has been widely used to treat elderly women with ER-positive breast cancer in the past. Aromatase inhibitors may be more beneficial than tamoxifen when used as primary endocrine therapy in elderly patients. We aimed to retrospectively evaluate a series of elderly women with ER-positive breast cancer treated with primary letrozole therapy as sole therapy with a minimum of 5 years follow-up. To identify possible predictive biomarkers a pilot immunohistochemical analysis was performed to assess the expression of PR, HER2, EGFR, BCL2 and p53.

A total of 45 women, aged more than 70 years with a diagnosis of ER-positive breast cancer that was treated with primary letrozole therapy were identified. A case note review was undertaken to obtain clinical information. Formalin fixed paraffin embedded tumour tissue from diagnostic core biopsies was available for all patients. Immunohistochemical analysis was performed to establish the protein expression status of p53, PR, HER2, EGFR and BCL2.

The mean age of the 45 patients was 87 years (range 70-101). Clinical benefit was seen in 60% of the patients. Median progression free survival was 53 months (95% CI – 34 to 72) and the median time to progression was 43 months (95% CI – 22 to 64). BCL2 was expressed in 45/45 (100%); PR in 38/45 (84%); EGFR in 13/45 (28%); HER2 in 9/45 (20%) and p53 in 5/45 (11%) of tissue samples. Positive expression of p53 was associated with poor progression free survival (p=0.03) in this pilot study.

This study demonstrates that letrozole as sole treatment appears to be a suitable treatment option for elderly patients with ER-positive breast cancer who are not fit for, or decline, surgery. The analysis of p53 in a larger study is warranted in order to assess its role as a biomarker in this patient group.

***Keywords:*** aromatase inhibitor, breast cancer, elderly, letrozole, ER, p53

**Introduction:**

Breast cancer is one of the most common cancers worldwide, with more than one third of them occurring in women over 70 years of age in the UK [1]. In this cohort (>70 years), breast cancer predominantly present as palpable cancers (mostly T2-4) and are frequently hormone receptor positive [2-7]. In the absence of clinical recommendations, treatment of women in this age group has often been based upon studies conducted in younger patients, since few women over the age of 65 are recruited into major trials [1, 7, 8].

Primary tamoxifen therapy has been widely used to treat elderly women with ER-positive breast cancer in the past. However, a recent Cochrane systematic review of 6 randomized control trials (n=157; events 869) has shown that primary surgery (with or without adjuvant endocrine therapy) resulted in better progression free survival (PFS) compared to tamoxifen, but there was no difference in overall survival [9]. A randomized control trial in the UK comparing surgery plus tamoxifen versus tamoxifen alone in 455 women over 70 years of age with a median follow up of more than 12 years showed the surgery plus tamoxifen treatment arm to have longer time to treatment failure and improved survival (overall mortality and mortality from breast cancer) compared to tamoxifen alone [10]. The Italian phase III randomized GRETA trial compared surgery plus adjuvant tamoxifen (n=239) to primary tamoxifen alone (n=235) in women over 70 years of age with breast cancer and demonstrated no benefit in the overall survival or breast cancer survival, but improved rates of local progression in the surgery plus tamoxifen treatment arm compared to tamoxifen alone (p=0.0001) [11]. Further, the clinical results from three major trials (CRC,

GRETA and Nottingham 2 study) that looked into the role of surgery plus adjuvant endocrine treatment to primary endocrine treatment alone indicate the overall survival (OS) for the primary endocrine treatment is comparable to the surgery plus adjuvant treatment arm (Table 1).

Aromatase inhibitors (AI's) such as letrozole, anastrozole or exemestane may be more beneficial than tamoxifen when used as primary endocrine therapy in elderly patients. Letrozole has been shown to be beneficial in older patients in two large adjuvant trials, MA.17 and BIG 1-98 [12-14] and the use of AI's for primary therapy in postmenopausal women with ER-positive breast cancer has also shown promising results in various trials (Table 2). The P024 trial compared 4 months of pre-operative tamoxifen versus letrozole in post-menopausal women and demonstrated that the overall response to letrozole was significantly better than tamoxifen (55% versus 36%; p=0.001) and resulted in increased breast-conserving surgery (45% versus 35%; p=0.022) [15-16]. Other studies such as The IMPACT trial and the PROACT trial compared pre-operative treatment with anastrozole versus tamoxifen and showed that there was no statistically significant difference between anastrozole and tamoxifen in overall response and rates of breast-conserving surgery [17-18]. In a study of elderly patients with ER-positive breast cancer treated with either primary tamoxifen or anastrozole no significant difference in clinical benefit between the two groups was identified [19]. Two small phase II trials found that the use of exemestane as primary therapy was effective in post-menopausal women with ER-positive breast cancer and was a feasible therapy option for elderly patients [20-21]. Recent recommendations for the management of elderly breast cancer patients include the use of primary tamoxifen or AI therapy in unfit patients with short life expectancy or for those who refuse surgery [7].

To further increase the efficacy of AI's as primary or neoadjuvant treatment in breast cancer, the identification of predictive biomarkers of response and improved survival is paramount. In the P024 trial, letrozole showed similar efficacy when administered to patients with HER2-positive, HER2-negative, EGFR-positive and EGFR-negative tumours. However, ER-positive patients who demonstrated double positivity for EGFR and HER2 had higher response rates to letrozole therapy compared to patients who were double negative (88% versus 54%; p=0.018) [22]. In a similar study using anastrazole, HER2 status was not associated with response to treatment [23]. In another study of 305 patients, which included patients from the P024 study as well as from the Edinburgh Breast Unit, response to letrozole was seen irrespective of the HER2 status which was assessed by FISH [24]. In a study involving elderly patients treated with primary anastrazole treatment, HER2 positivity was associated with shorter PFS (p=0.003) whilst EGFR expression was not associated with survival [19]. Further data on the use of primary AI therapy in the treatment of elderly patients with ER-positive breast cancer is required.

**Aims of the Study:**

We aimed to retrospectively evaluate a series of elderly women with ER-positive breast cancer treated at our Breast Unit with primary letrozole therapy as sole therapy and with a minimum of 5 years follow-up. To further identify possible predictive biomarkers of response, a pilot immunohistochemical analysis was performed to assess the tumour expression of PR, HER2, EGFR, BCL2 and p53.

**Methods:**

Following approval by Hull and East Riding Research Ethics Committee, retrospective data was assessed from elderly patients (age >70 years) who had received primary letrozole therapy for ER-positive breast carcinoma between 2002

and 2004. Diagnostic core biopsies and clinical information was required for all patients. A case note review was undertaken to obtain information regarding tumour characteristics, response to therapy and disease progression. All patients had undergone triple assessment including clinical examination, mammogram, ultrasound scan and histological assessment of core biopsy samples to establish the diagnosis of breast cancer. Each case had been discussed at a multidisciplinary team meeting before patients were offered the treatment options. Primary letrozole therapy was employed for ER/PR positive elderly patients with significant co-morbidity or those who had declined surgery. All patients received clinical followed up at three monthly intervals and at the first sign of loss of local tumour control or metastases other treatment options had been discussed with the patient.

***Endocrine therapy:***

All elderly post-menopausal patients with early breast cancer not fit for surgery were treated with letrozole 2.5 mg daily as first line agent.

***Assessment of response:***

Clinical end points such as PFS, time to progression (TTP) and response rate were calculated. PFS was defined as the time from biopsy until tumour progression or death due to any cause. As a significant number of patients in this cohort had died due to causes unrelated to breast cancer TTP was also calculated and defined as the time from biopsy until tumor progression. Progressive disease was defined as an increase in the primary breast tumor size by clinical caliper examination of more than 20% or development of metastatic disease. Clinical benefit was defined when tumour response or stable disease was achieved following treatment.

***Immunohistochemistry:***

Formalin fixed, paraffin embedded core biopsies taken at the time of breast cancer diagnosis were retrieved for 45 tumours. Immunohistochemical results for ER and

PR, performed at the time of diagnosis and scored according to the method of Allred [25], were obtained from histology reports. Immunohistochemical staining for BCL2, EGFR, HER2 and p53 was carried out using an avidin-biotin complex protocol described previously [26-27]. In brief, antigenic sites were retrieved by boiling in a 1:100 Antigen Unmasking Solution (Vector Laboratories Ltd) in a pressure cooker for 3 minutes at 15 psi. Non-specific binding was blocked with 1x casein (Vector Laboratories Ltd) and endogenous avidin and biotin were blocked by 15 minute incubations with biotin and avidin, respectively (Avidin Biotin Blocking Kit, Vector Laboratories Ltd). Sections were incubated at room temperature for 2 hours with primary antibody. A final dilution of 1:50 was used for BCL2 (#MS-123, Neomarkers) and EGFR (#MS-316, Neomarkers). A final dilution of 1:100 was used for p53 (#554293, BD Biosciences). HER2 (#NCL-L-CBE-356, Novocastra Vision Biosystems) was used at 1:50 without heat retrieval. A negative control was also included in each batch of slides, in which the primary antibody was omitted. The slides were scored by 2 independent investigators. Sections were scored positive for p53 expression if strong clonal immunoreactivity was observed in greater than 10% of tumour nuclei and positive for the anti-apoptotic protein BCL2 if strong expression was seen in greater than 10% of tumour cytoplasm as previously described [27]. Similarly, sections were scored positive for EGFR and HER2 if strong cytoplasmic/membrane expression was observed in greater than 10% of malignant cells.

***Statistical analysis:***

Statistical analysis was performed using SPSS software version 19.0 (SPSS, Chicago, USA). PFS and TTP were estimated using Kaplan Meier curves. Univariate survival analysis was performed for biomarker expression using Kaplan Meier curves with log rank analysis. Fisher's exact test was performed to assess biomarkers

predictive of response to letrozole. A two tailed p-value of <0.05 was considered to be statistically significant.

**Results:**

The patient characteristics in the study are described in the Figure 1. A total of 45 patients' core biopsies were analysed using IHC. This sample represents 3% (45/1450) of all the breast cancers treated at our institution during the three year study period. The median age of study patients was 87 years (range 70-101). Triple assessment revealed a clinical tumour stage T2 or greater in 66% (30/45) of cases. Histological characteristics revealed that the majority (57%; 26/45) of tumours were of non-specific subtype. Invasive ductal carcinoma represented 37% (17/45) of cases and invasive lobular carcinoma represented 4% (2/45) of cases.

***Follow-up, response and survival to primary letrozole therapy:***

All patients were followed up for a minimum of 5 years, with a median follow up period of 92 months (range 4–106 months). All patients during the FU were evaluated by clinical examination. Clinical benefit was seen in 60% (27/45) of the patients and 40% (18/45) of patients had progressed on letrozole therapy. At the end of last follow up, 68% (31/45) of the patients had died. The median PFS was 53 months (95% CI – 34 to 72) and the median TTP was 43 months (95% CI – 22 to 64).

***Molecular markers predicting response or improved survival to primary letrozole therapy:***

The expression of BCL2 was seen in 100% (45/45) of the tumour samples. The expression of PR was seen in 84% (38/45) of cases; EGFR in 28% (13/45); HER2 in 20% (9/45) and p53 in 11% (5/45). In this pilot study p53 staining was used as a surrogate to identify p53 gene mutation. In a univariate analysis, the positive

expression of p53 was significantly found associated with a poor PFS (median survival 20 months *versus* 54 months in p53 negative; p=0.03; Figure 2). Clinical benefit was seen in only 1 out of 5 patients harbouring a p53-positive tumour. The expression of PR, EGFR and HER2 was not associated with survival. Correlation of protein expression with treatment response using Fishers exact test was not statistically significant.

**Discussion:**

In this study, we have described a series of 45 elderly patients with ER-positive breast cancer who were treated with primary letrozole therapy and followed up for a minimum period of 5 years. Our study demonstrated clinical benefit in 60% of the patients, with 40% having progressed on letrozole at last follow up or death. There are few published reports describing the clinical experience of using primary letrozole therapy in elderly breast cancer patients. A previous case series from the Edinburgh Breast Unit reported their findings from 182 post-menopausal patients, of whom 63 had continued on primary letrozole therapy beyond 3 months [28]. The median follow up of this study was 3 years and disease control was achieved in over 70% of the 63 patients using letrozole. Another recently published retrospective study evaluated the overall survival and response rate of 104 elderly patients with ER/PR-positive breast cancer who were treated with primary letrozole therapy [16]. Some of the earlier described studies using tamoxifen as the primary hormone therapy showed disease progression and poor survival after two years even in patients who initially responded to treatment. In contrast, findings from our study showed only one patient progressed within the first year and four more within two years of commencing treatment (11%; 5/45).

Previously, breast cancer studies in elderly evaluated the role of p53, HER2 and EGFR as potential biomarkers of therapy responses and prognosis with trends towards less frequent overexpression of biomarkers favouring an improved survival and more desirable tumour biology (5, 22, 24, 29, 30). Our pilot study showed similar trend of low frequency of overexpression of these protein markers. Previously, HER2 positivity has been demonstrated to associate with shorter PFS in elderly patients who received primary anastrazole therapy [19]. However, in our pilot study, we found p53 expression as a surrogate for mutation status which was found to be associated with poor PFS. From the above findings, we hypothesise that if validated further, p53 may be useful in screening patients for primary letrozole therapy. Mutations in p53 may result in a stable p53 protein that can be identified by immunohistochemistry, however not all mutations are associated with stabilised protein [31]. Further studies using DNA sequencing-based methods, may therefore also be required to assess p53 mutation status in order to validate the effect of p53 mutation on PFS in patients treated with letrozole as primary therapy.

The study however has certain limitations. For a study of an exploratory nature, the included patient sample in the study is small and has a potential to impact on the study power. However, despite the above limitation, a clear statistical difference was detected in the study for p53 responder group versus the non- responders. The three other biomarkers (BcL-2, EGFR and HER2) explored in the study however, failed to reach a statistical difference in the responder group. This may well be due to a type 2 statistical error from a limited sample of patients analysed in the study. Further, authors acknowledge that the non-availability of biopsy material in 35% (24/69) of potentially eligible patients preclude the ability of the study to recommend the use p53 as a screening tool. Lastly, only a small proportion of patients (0.3%; 5/1450; 11% of 3%) from the study group were shown to benefit from using p53 as a therapy

biomarker, larger studies evaluating the role of p53 in primary endocrine therapy patient selection are therefore now required.

**Conclusion:**

In conclusion, our pilot study demonstrated that the positive expression of p53 was associated with poor PFS in patients treated with primary letrozole therapy. This biomarker may be used as a useful screening tool to select patients who would benefit from primary letrozole therapy.

**Acknowledgments**

None.

**Conflict of Interest Statement**

The authors declare that they have no competing interests.

**Role of the Funding Source**

There was no external funding body hence they had no role in study design; collection, analysis or interpretation of data; writing of the manuscript; or in the decision to submit the manuscript for publication.

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# Table 1: Surgery plus adjuvant endocrine therapy *vs* primary endocrine therapy

| Trial | Median Follow up | Surgery (n/N) | Primary Endocrine Therapy (n/N) | HR (95% CI) |
|---|---|---|---|---|
| **Surgery plus endocrine therapy *vs* primary endocrine therapy** | | | | |
| *Mortality (OS)* | | | | |
| CRC | 13 years | 159/225 | 187/230 | 0.78 (0.63-0.98) |
| GRETA | 7 years | 130/239 | 144/235 | 0.98 (0.77 -1.25) |
| Nottingham 2 | 5 years | 8/53 | 14/94 | 0.80 (0.73-2.32) |
| *Progression Free survival (PFS)* | | | | |
| CRC | 13 years | NR | NR | NR |
| GRETA | 7 years | 14/239 | 188/235 | 0.65 (0.53-0.81) |
| Nottingham 2 | 5 years | NR | NR | NR |
| *Local Progression (LR) or LR as first event* | | | | |
| | 13 years | 36/225 | 115/230 | 0.25 (0.19-0.32) |
| CRC | 7 years | 27/239 | 95/235 | 0.38 (0.25-0.57) |
| GRETA | 5 years | 2/53 | 30/94 | Not estimable |
| Nottingham 2 | | | | |

**Table 1:** The above table summarises the findings of surgery plus adjuvant endocrine treatment versus primary endocrine treatment alone from the three important randomised controlled trials. In all the three trials, the overall survival (OS) for the primary endocrine treatment arm was found similar to surgery and adjuvant treatment arm (Hind D et al. Br J Cancer. 2007 Apr 10; 96(7):1025-9)

# Table 2: Aromatase Inhibitor Trials

| Trial | Patient Number (n) | Comparator | | ORR | BCS |
|---|---|---|---|---|---|
| P024 | n= 337 | Letrozole | Tamoxifen | 55% vs 36% | 45% vs 35% |
| (Eiermann 2001) | | | | *P= <0.001* | *P = 0.022* |
| IMPACT | n= 330 | Anastrazole | Tamoxifen | 37% vs 36% | 44% vs 31% |
| (Smith 2005) | | | | *P= 0.87* | *P= 0.23* |
| PROACT (Cataliotti 2006) | n=451 | Anastrazole | Tamoxifen | 40% vs 35% | 38% vs 30% |
| | | | | *P= 0.29* | *P= 0.11* |
| (Semiglazov 2007) | n=239 | Anastrazole / Exemestane | Chemotherapy | 65% vs 64% | 33% vs 24% |
| | | | | *P= >0.5* | *P= 0.58* |

**Table 2:** The above table shows findings from the four major aromatase inhibitor trials namely P024, IMPACT, PROACT and Semiglazov study. Of the four trials, three compared anastrozole with standard tamoxifen and/or chemotherapy treatment and one trial compared letrozole

with tamoxifen for attainment of objective response (ORR) and breast conserving surgery (BCS) rates. Findings from the studies clearly demonstrate letrozole to be the superior therapy to attain ORR and BCS rates compared to other AIs and chemotherapy.

# Figure 1: THE STUDY CONSORT

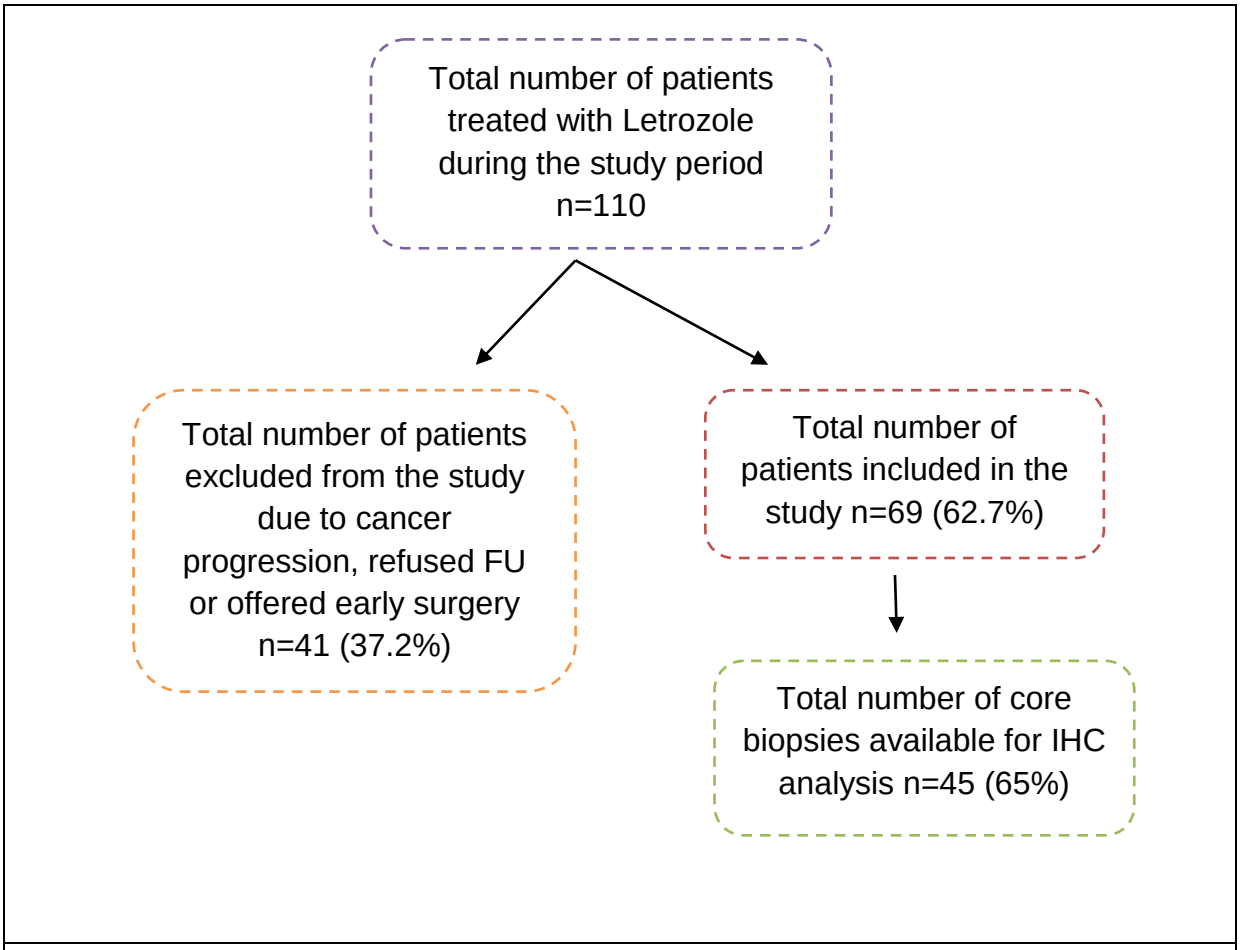


**Figure 1:**

Consort chart for the pilot study showing a total of 110 patients who were treated with letrozole as primary therapy as identified from the prospectively maintained chemotherapy database. After case-note review, 41 patients were excluded from the study as they had metastatic disease at presentation, refused follow-up after their first visit or they chose early surgery without completing the treatment course. Of the 69 remaining patients, 45 patients had core biopsy samples available for assessment.

**Figure 2:** Kaplan Meier plot showing univariate analysis of p53 expression (p=0.03, log rank). The median survival was 20 months in p53-positive cases (n=5; dotted line) *versus* 54 months for p53-negative cases (n=40; solid line).