Evaluation of drug-specific multigene markers to predict aromatase inhibitor efficacy in advanced breast cancer: a cohort study from Danish Breast Cancer Cooperative Group

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Abstract

Background Even with positive oestrogen receptor (ER+) status some advanced breast cancer (ABC) patients fail to benefit from endocrine therapy (ET). A method that previously predicted other drugs in various cancers was evaluated. Here multigene markers based on aromatase inhibitor (AI) effect in vitro were used for prediction of AI benefit in ER+ ABC patients. Simultaneously effects of long-term ET on predictive efficacy was evaluated. Methods The Drug Response Predictors (DRPs) are based on correlations between baseline gene expression and growth inhibition patterns of exemestane, anastrozole and letrozole, respectively, in the National Cancer Institute 60 cell lines. The genes were controlled for expression in 3,500 tumours. In a Danish Breast Cancer Cooperative Group cohort of 695 ABC patients with complete gene expression and time-to-progression (TTP) data, 414 received an AI as monotherapy. Hereof, 57 received anastrozole, 166 received exemestane, and 327 received letrozole. mRNA was isolated from archival formalin-fixed paraffin embedded tumour tissue and run on microarray and 60% of the tumours were from time of primary diagnosis. Medical records of the patients were assessed for TTP for all treatments given for ABC. Results The DRPs were tested in subsets 1) with no adjuvant ET and 2) with adjuvant ET. In 1) the anastrozole DRP predicted benefit of anastrozole (hazard ratio (HR) was 0.21 upper 95%-confidence interval limit (CI) 0.76, p=0.023) but not in 2). Dichotomised by a DRP of 50, the anastrozole DRP did predict benefit (HR=0.16, upper 95%-CI 0.75, p=0.026). Only in 1) the exemestane DRP predicted benefit of exemestane (HR=0.57, upper 95%-CI 1.00, p=0.0497). The letrozole DRP had no predictive value. Additionally, we tested each DRPs ability to predict other AIs. Only the anastrozole DRP predicted benefit of overall AI treatment, in 1) with an HR of 0.76 (upper 95%-CI 0.99, p=0.044) and in 2) with an HR of 0.71 (upper 95%-CI 0.92, p=0.015). The anastrozole DRP did though not predict benefit of letrozole. All tests are one-sided, alpha=5%. Conclusions Among the DRPs for AIs, the anastrozole DRP was strongest with clinically relevant prediction of TTP in AI treated ER+ ABC patients. Trial registration: ClinicalTrials.gov NCT01861496.

Background

Approximately 70% of women with advanced breast cancer (ABC) have oestrogen-receptor positive (ER+) disease and endocrine manipulation is a mainstay in their treatment. The principal source of oestrogens in postmenopausal women is aromatization of adrenal androstenedione and testosterone to oestrogens. Consequently, for postmenopausal women with ER+ breast cancer, treatment will often include an aromatase inhibitor (AI) (1). This removes a main source of growth stimulus for the oestrogen-receptor positive breast cancer. Three AIs are currently used: Anastrozole, letrozole, and exemestane. All are 3rd generation AIs and while anastrozole and letrozole are reversible nonsteroidal inhibitors, exemestane is an irreversible steroidal AI (2). Response rates to AIs as monotherapy vary from 21-32 % for ER+ ABC patients (3–5). Recent successes of combining an AI or fulvestrant with a cyclin-dependent kinase (CDK) 4/6 inhibitor in ER+, human epidermal growth factor receptor 2 negative (HER2-) ABC has moved this combination to first and second line treatment (6,7). Even as the additional benefit of CDK4/6 inhibition

to AIs is very impressive, biomarkers to identify primary endocrine resistance are important in the 20-25 % of patients experiencing early progression on these treatment regimens (6,8).

Predictive biomarkers

In ABC, ER and HER2 analyses have improved the ability to predict which patients to select for treatment with endocrine therapy (ET). Further improvement is needed as no biomarkers predictive of specific treatment outcome have been approved neither in early nor advanced stages of breast cancer, thus, it is currently not possible to select between fulvestrant, tamoxifen and the three AIs in clinical use. Research in the field includes measures of ER, ESR1, the coding gene for ER-α, HER2, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), the coding gene for interleukin 6 signal transducer (IL6ST) and multigene biomarkers. Specific mutations in ESR1 and PIK3CA are clearly linked to resistance to endocrine treatments (9,10). In a meta-analysis of largescale trials ATAC, BIG 1-98 and TEAM HER2- patients appeared to gain significantly greater benefit from treatment with adjuvant AIs compared to adjuvant tamoxifen while there was no difference between treatments in the HER2+ subgroup (11). Prediction of neoadjuvant letrozole response was obtained with 4 genes including IL6ST (12). Lastly, Ueno et al found that low levels of the multigene marker Oncotype Dx® recurrence score (RS) predicted benefit of exemestane in a neoadjuvant trial (13).

In vitro based markers of aromatase inhibition

For postmenopausal patients a large part of the oestrogen production is believed to arise in the tumour itself (14,15); and aromatase expression is confirmed in both stromal cells and carcinoma cells of breast cancer (16). Immunohistochemistry staining for aromatase in primary tumours did, however, not predict clinical outcome of treatment with AIs in the advanced setting (17). Similarly, aromatase expression in ABC patients did not predict efficacy of letrozole (18).

In a study by Sonne-Hansen and Lykkesfeldt oestrogen-dependent MCF-7-breast carcinoma cells survived on serum with testosterone, the substrate for aromatase to make oestrogen. They also showed how AIs exhibit a cytotoxic effect in these cultured breast cancer cells (19). Another recent study showed intra-tumoral amplification of aromatase following clinical resistance to aromatase inhibition (20). Despite a distinct mode of action and well described *in vitro* systems a predictive biomarker for AI efficacy other than ER has not been developed.

New AI predictors

In early breast cancer, the use of complex multigene biomarkers for prognostic purposes has been widely endorsed by the medical oncology societies for clinical practice (21–27). Similar to the complex prognostic markers based on multiple genes we developed multigene profiles specific to AI treatment:

Drug Response Predictors (AI-DRPs). These are based on data from the National Cancer Institute panel of 59 human cancer cell lines (NCI-60). In NCI-60 each tested drug shows a characteristic pattern of sensitive and resistant cell lines (28). Baseline gene expression and protein levels are measured on all cell lines (29).

AIs have been shown to exhibit cytotoxic effects in various cancer cell lines (30,31) and NCI-60 (Fig. 1) (28). While NCI-60 has few breast cancer cell lines Knudsen et al previously showed that it was possible to predict clinical fulvestrant efficacy by use of a fulvestrant specific DRP from NCI-60. Here no additional benefit was obtained by testing in a specific breast cancer cell line panel (32). This may be explained by the presence of oestrogen and derivates in *all* 60 cell lines with surprising abundancy in melanoma and leukaemia cell lines (33).

We thus hypothesize that AI sensitive cancer cells in NCI-60 has a characteristic pattern of gene expression that differs from AI resistant cancer cells and that this pattern, in a modified form, can predict benefit of AIs in clinical practice. By following REMARK guidelines (34) we assess if there is an association between predicted sensitivity to AIs exemestane, anastrozole or letrozole and time-to-progression (TTP) in a Danish Breast Cancer Cooperative Group (DBCG) cohort of ABC patients.

Methods

Development of the DRP

The method of developing drug response predictor profiles has been described before in development of a DRP for fulvestrant (32). At the National Cancer Institute cell lines are subjected to a drug and growth inhibition values (GI50) are measured and published online (28). These cell lines are referred to as the NCI-60. 49 of these were subjected to anastrozole, 66 to exemestane and 57 to letrozole, searched on NCI-60 database with NSC numbers 719344 (anastrozole), 713563 (exemestane), and 719345 (letrozole) (35). Included are 6 cell lines derived from breast cancer (only 5 in the anastrozole and letrozole treated subsets). On the same cell lines, with no drug present, baseline gene expression has been measured on microarray (29). We used correlations between baseline gene expression and patterns of growth inhibition of anastrozole, exemestane, and letrozole (Fig. 1). Anastrozole, exemestane, and letrozole were tested individually and unique patterns of sensitivity were seen for each drug. When letrozole was applied to the cell lines only 7 out of 57 cell lines had differential GI50 values. The letrozole predictor was thus not expected to perform as well as the other predictors.

Genes that in the cell lines were correlated to a low GI50 of anastrozole were considered sensitive to anastrozole and were therefore biomarkers of sensitivity and retained in the anastrozole DRP. Genes correlated to high GI50 levels were considered resistant to anastrozole and retained as biomarkers of resistance. This was repeated for exemestane and letrozole to produce AI DRPs specific for each AI.

To reduce noise caused by false positive genes, a filter was applied, securing that the selected genes were biologically relevant as the filter removes genes only active in cell lines. The filter consisted of 3,500

tumours of mixed origin (Fig. 1).

For each patient, three DRP scores were calculated as the difference between the mean of all sensitive genes and the mean of all resistant genes for each of the AIs tested. This was compared to a reference population of 819 breast cancer patients. The DRP scores were expressed as a percentile of the reference population. Higher scores predict higher likelihood of sensitivity to anastrozole, exemestane or letrozole, respectively. These final AI drug response predictor scores were tested blinded to outcome. This system has been assessed and validated in various cancer types with several anticancer agents (32,36,37).

Patients

Totally, 1,199 patients with locally advanced or metastatic breast cancer have given informed consent to be enrolled in a screening cohort selecting patients to a phase II trial with liposomal cisplatin (LiPlaCis), the DBCG screening cohort. The phase II trial with LiPlaCis is registered with ClinicalTrials.gov as NCT01861496 (38). Data from medical records for patients enrolled in the DBCG screening cohort was used to test markers for prior treatments as previously described by Buhl and colleagues (39). In brief, patients at least 18 years of age with histologically confirmed locally advanced or metastatic adenocarcinoma of the breast and performance status < 2 with no other malignancy within 5 years prior of the enrolment were eligible. Clinical data was extracted retrospectively from medical records of each patient and included among more information on duration of each treatment for ABC and information on reason for treatment change defined as either progressive disease, toxicity, physician's decision, patient's decision or long-lasting stable disease.

The study was approved by the Regional Committee on Health Research Ethics for the Capital Region Denmark (ID HGH-2016-097). Informed consent for this part was not obtained as the research did not have implications on the health of the research subjects as accepted by the Regional Committee on Health Research Ethics for Capital Region Denmark.

Specimen characteristics

Archival formalin-fixed paraffin-embedded (FFPE) tissue from the patients' breast cancer was used. Of 414 unique patients treated with anastrozole, exemestane, or letrozole tissue blocks were from the original surgery (n = 247), any surgery performed after 4 months from diagnosis including biopsies from metastasis (n = 76) or unknown (n = 91). Sampling was done from tissue block (cores or slices) or from biopsy (core from needle). Tissue was retrieved from the newest possible tumour specimen with control of tumour cell content, necrosis and haemorrhage by certified pathologist Eva Balslev. Only blocks with at least 5 % tumour cells were used.

Assay methods

In the sample from each patient total RNA was extracted using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE samples (Ambion, Austin, Texas) according to manufacturer's protocol. Total RNA was then amplified and applied to microarray Genechip® Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific, Waltham, MA, USA). Samples with *in vitro* transcription yield below 500 ng/µL were discarded.

Study design and statistical analysis plan

Study design was retrospective with no matching control. Prior to unblinding a statistical analysis plan was specified. The plan was to assess any associations of the DRP profiles for anastrozole, exemestane, and letrozole to clinical outcomes on treatment with the same drug. Primary endpoint was time to progression (TTP) defined as time from start of treatment to progression. When primary endpoint was not reached patients were censored on date of latest contact. Other censoring events were death or other outcome such as toxicity, physician's decision or patient's decision to terminate treatment.

Variables considered in the multivariate analysis were DRP, age, adjuvant endocrine treatment, HER2 status, ER status, performance status and number of metastatic sites.

The assessment was done with the cox proportional hazards model with stratification for treatment line and adjusted for previous adjuvant endocrine therapy including an interaction term between this and the DRP. Prespecified in the statistical analysis plan was scoring the DRP as continuous variable with hazard ratio for a difference of 50% points. We also evaluated the DRP grouped by below or above 50%. Only complete cases were used.

As the hypothesis was that high levels of the DRP predict benefit of a drug, all confidence intervals are one sided (upper limit) with a 5% significance level. Model assessments of proportionality and linearity were done using martingale residuals.

Post-hoc analysis included testing each DRP and DRPs for fulvestrant and tamoxifen on all AI treated patients as a random effect for each AI treatment. This was done to assess the specificity and to identify the most robust predictor for future purposes. Other post hoc analysis included predictive models based on the cox proportional hazards model for both the anastrozole DRP in all AI treated and in patients treated with only anastrozole or exemestane.

Statistical calculations were done using SAS (v9.4, SAS Institute, Cary, N.C., USA) and R (R Core Team, Vienna, Austria with packages "rms", "survival" and "survminer") (40). Normalization of mRNA microarray data was performed in R (with package "affy" from Bioconductor) using Robust Multi-array Average (41). A heatmap comparing DRP values for different predictors was constructed with R (R Core Team, Vienna, Austria, v.3.4.3 with packages "ComplexHeatmap" from Bioconductor and "circlize") (42).

Results

Baseline patient characteristics

Patients included in the study population were diagnosed with primary breast cancer between 1979 and 2015 and received an AI in the locally advanced or metastatic setting between June 1997 and November 2016.

In total, 695 ABC patients were evaluable for time-to-progression and had enough tissue for gene expression (Fig. 2). Hereof 414 had received an AI at any given time-point for metastatic disease of which 57 received anastrozole as monotherapy, 166 received exemestane as monotherapy, and 327 received letrozole as monotherapy. This included 19 that received both anastrozole and exemestane, 74 that received letrozole and exemestane, 11 that received letrozole and anastrozole and 16 that received letrozole, exemestane, and anastrozole as monotherapy at separate time points. Anastrozole was administered as the 2nd treatment line (median) for advanced disease and median TTP was 29.3 months, exemestane was administered as 3rd treatment line (median) for advanced disease and median TTP was 8.5 months, whereas letrozole was administered as 1st treatment line (median) and median TTP was 22.2 months (Table 1).

Among all patients treated with anastrozole 32 patients of 56 had received adjuvant ET hereof 50% had received tamoxifen alone (Table 1). For the exemestane treated 102 had received previous adjuvant ET with equal distribution between former treatment with tamoxifen, an AI or both, and in the letrozole treated subset 166 received adjuvant ET hereof approximately 50% received tamoxifen alone (Table 1). The median length of prior adjuvant ET was 3.1 years (range 0.2-7.8 years).

Association between DRPs and baseline characteristics

Baseline characteristics for the AI treated patients are presented in Table 1. Associations between the anastrozole DRP and all variables considered in the multivariate analysis were tested for. Only adjuvant ET status was significantly associated to the anastrozole DRP ($p=0.02$). No other parameters were statistically associated to anastrozole DRP score (p-values were greater than 0.16). Similar results were shown for exemestane ($p>0.14$) with only adjuvant ET status significantly association to the exemestane DRP ($p=0.05$). The letrozole DRP was significantly associated to number of metastatic sites ($p=0.036$) but not to age, ER status or performance status.

Predictive value of the markers

The patients assessed were those treated with an AI in any treatment line at any time for ABC. We grouped the patients into those that had received prior adjuvant ET or not, stratified for treatment line and with random effect for treatment (anastrozole, exemestane or letrozole) when assessing the DRP in all AI treated patients. The following variables were adjusted for in the multivariate model: adjuvant ET, treatment line, ER status, number of metastatic sites (1, 2, 3, >3), age (<50, >50-60, >60-70, >70) and

performance status (0-1 vs 2-). Each of the AI DRPs were tested on the treatment they were developed on, anastrozole DRP was then as a primary objective tested in the anastrozole treated patients, the exemestane DRP in the exemestane treated patients and so forth. Secondarily we evaluated each of the DRPs in the entire subset of AI treated patients from the DBCG cohort (Table 3).

The anastrozole DRP

In the anastrozole treated patients that had not received adjuvant ET the anastrozole DRP predicted TTP with a HR of 0.21 (upper 95%-CI 0.76, p=0.023) (Table 2). The anastrozole DRP was scored continuously, with HR presented for a 50-point difference of the DRP score. The performance of the anastrozole DRP remained significant in a multivariate model. When dichotomising the anastrozole DRP by 50 the HR was 0.16 (upper 95%-CI 0.75, p=0.026) (Fig. 3) in the patients that had not received adjuvant ET. In the subset of anastrozole treated patients that had received adjuvant ET previously the anastrozole predictor did not predict TTP (HR 1.05, p=0.54) (Table 2 and Additional File 1: Fig. S1).

Univariate analysis of the anastrozole DRP, the exemestane DRP, and the letrozole DRP in anastrozole treated patients, in exemestane treated patients, in letrozole treated patients, respectively. The HRs are for continuously scored values of the DRP for a 50-point difference in DRP score. One-sided, upper CI-level of 95% with corresponding p-values are shown. Primary analyses are marked in bold. Significant p-values of 5 % one-sided level are marked with *. Abbreviations: CI = confidence interval, DRP = Drug Response Predictor, ET = endocrine therapy, HR = Hazard ratio.

The exemestane DRP

In the exemestane treated patients naïve to adjuvant ET, the exemestane DRP predicted TTP with an HR=0.56 (upper 95%-CI 1, p=0.0497) (Table 2). This was robust through all treatment lines with exemestane given for advanced disease and in a multivariate model with an HR of 0.47 (95%-CI upper-limit 0.87, p=0.023). Otherwise only performance status was statistically significant. When patients in this subset were dichotomised by exemestane DRP > 50 versus exemestane DRP < 50 HR was 0.43 (upper 95%-CI .79, p=0.022) in a cox proportional hazards model with stratification for treatment line (Fig. 4). No predictive effect of the exemestane DRP was found in patients who had received adjuvant ET (Table 2 and Additional File 1: Fig. S2). Some early results for the exemestane DRP was presented at American Society of Clinical Oncology Annual Meeting 2017 (43).

The letrozole DRP

No effect of the letrozole DRP was present in the letrozole treated patients (Table 2).

The DRPs in all AI treated patients

To assess any ability to predict treatment outcomes of treatment with other AIs we did additional post-hoc analysis evaluating each predictor in each subset of AI treated patients.

The anastrozole DRP predicted benefit of treatment with either anastrozole, exemestane or letrozole with an HR of 0.76 (upper 95%-CI 0.99, p=0.044) in the subset that had not received adjuvant ET (Table 3, Overall performance). The overall performance of the anastrozole DRP remained significant in a multivariate model with an HR of 0.73 (upper 95%-CI 0.95, p=0.025). Otherwise number of metastatic sites (p=0.025) was statistically significant in the multivariate model. We dichotomised the anastrozole DRP at 50% points showing that HR was 0.82 (upper 95%-CI 1.08, p=0.11) (presented in individual subsets in Fig. 3, and in Additional File 1: Fig. S3a and S3c).

The anastrozole DRP also predicted TTP in the subset of AI treated patients that had received previous adjuvant ET. Scored continuously HR for a 50-point difference was 0.71 (upper 95%-CI 0.92, p=0.015) (Table 3 and Additional File 1: Fig. 1 and S3b and S3d). However, the anastrozole DRP was not predictive of treatment with letrozole in either subset (Table 3).

The exemestane DRP did not predict benefit of treatment with neither anastrozole nor letrozole (p-values ranged from 0.26-0.98) (Table 3). The letrozole DRP appeared to predict some benefit of exemestane in the subset that had received adjuvant ET previously (HR=0.51, p=0.004) but was otherwise not predictive of TTP (Table 3).

As the anastrozole DRP had an improved performance in both the anastrozole treated and the exemestane treated subsets and a better overall performance than the exemestane and letrozole DRPs, the anastrozole DRP was chosen for further evaluation.

Predictive models

Based on the multivariate cox model, a predictive model was specified for all AI treated in the subset with no previous adjuvant ET. The model predicted outcomes for a given DRP level of the anastrozole DRP, no other variables were included in this predictive model. In all AI treated estimation of median TTP for a DRP of 10 was 16 months (95%-CI 13-20 months), for a DRP of 50 the estimation was 19 months (95%-CI 16-22 months), and for a DRP of 90 estimation was 23 months (95%-CI 18-30 months). As there was no effect of the anastrozole DRP in the letrozole treated patients we specified a model post-hoc without the letrozole treated patients as well. In this model the estimated TTP for an anastrozole DRP score of 10 was 6.2 months (95%-CI 3.5-17.8 months), for a DRP of 50 the estimation was 17.8 months (95%-CI 8.5-25.3 months) and the estimated TTP for a DRP of 90 was 27.0 months (95%-CI 18.0 months – not possible to estimate).

Comparison of predictors

As an explorative analysis we compared the prediction of the AI DRPs to DRPs based on fulvestrant and tamoxifen on the AI treated patients (32). Neither the fulvestrant DRP nor the tamoxifen DRP predicted any overall benefit of AI treatment (p-values ranged 0.35-0.93). Subgroup analysis showed that the fulvestrant DRP was able to predict long TTP in the letrozole treated ABC patients that had received adjuvant ET (for continuous scoring with a difference of 50 HR was 0.42, p=0.0014).

The Pearson correlation between the anastrozole DRP scores and the exemestane DRP scores was modest, r=0.25. Thus, the predictors appeared to identify different patients as benefitting treatment which was also obvious in the differential effect of the two DRPs. Fig. 5 shows a heatmap of exemestane and anastrozole treated patients comparing individual scores for a DRP for fulvestrant, the anastrozole DRP, and the exemestane DRP. The heatmap is presented with continuous scoring and complete Euclidean clustering of columns (patients). The higher the score, the higher the likelihood of benefit of the treatment. Red areas represent patients with predicted benefit. The majority of the patients predicted to benefit from either AIs or fulvestrant are found in the left side of the figure.

Discussion

Postmenopausal patients with ER+ breast cancer who have not received an AI in the adjuvant setting are generally recommended an AI following distant metastases. Our study suggests that those who are likely to benefit from exemestane or anastrozole may be identified by DRPs based on exemestane or anastrozole, respectively. The basis for the DRP scores is gene expression using FFPE tumour tissue from either the primary tumour (60%) or unknown or a later recurrence (40%) (no further details were available). Either may have been excised from the patients many years prior to initiation of the treatment we are assessing. Prediction across all AI treatments was attempted and the anastrozole DRP appeared to predict benefit of exemestane in addition to anastrozole in both the subset treated with adjuvant ET and in the subset that did not receive adjuvant ET. The predictive effect of the anastrozole in exemestane treated patients points to a similar mechanism of action in the AIs. However, no prediction of benefit of letrozole was possible with either of the AI DRPs. We did not find any interactions between letrozole treatment and baseline characteristics nor any issues with proportionality that could explain this.

The assessed patients are treated with anastrozole, exemestane or letrozole in any treatment line at any time for advanced disease and the effects of the DRPs were not reduced with later treatment lines or with other endocrine treatments for advanced disease.

We planned and conducted the analysis stratified by prior adjuvant ET status because we had hypothesised that long term exposure to endocrine therapies would alter gene expression in a way that we could no longer use samples from before the exposure. The tissue blocks used in the study were obtained from archival samples of the current breast cancer from each patient. The samples represent a pre-treatment baseline gene expression level and patients received 3 years (median) of adjuvant ET. The inability of the exemestane DRP to predict outcome when patients had received adjuvant antihormonal therapy could be caused by the anticipated substantial change in genes in the tumour after several years

of endocrine treatment (44). To assess whether the exemestane DRP is useful in patients with previous adjuvant endocrine treatment using a new biopsy after adjuvant treatment will be necessary.

Letrozole had a marginal effect on the cell lines in NCI-60 as only 7 of the 57 cell lines had differential GI50 values. Therefore, it was not very likely to succeed as a predictor of benefit in patients. We conclude that new experiments at NCI-60 or on other cell lines are needed to improve this predictor. Additional examinations and validations in the letrozole treated patients are necessary as our current results are conflicting: the fulvestrant DRP appeared to predict in letrozole treated patients that had received adjuvant ET.

Limitations of the current study include the retrospective and non-randomised study design and thus lack of an untreated control group. A comparator group could enlighten whether the markers are predictive of AI treatment or prognostic in this subset of patients. Another limitation is the small sample sizes in subgroups but also in the anastrozole treated subset altogether. Strengths include that this is a blinded, independent validation of locked-down versions of the DRPs.

As approximately 20% of ER+ patients are de-novo resistant to AI treatment accuracy in patient selection can be improved (8). In our cohort of AI treated ABC patients all but 9 patients are ER+. As the DRP from anastrozole can discriminate patients with differential benefit of anastrozole and exemestane in this cohort, it is evident that ER status is not enough to select patients for treatment. However, no biomarkers predicting choice of specific endocrine treatment are approved for clinical use.

Currently the treatment algorithm for advanced hormone-receptor positive disease patients is rapidly changing with CDK4/6 inhibitors and fulvestrant or AI being current first line (6). Fulvestrant appears superior to AIs (45,46), but we hypothesize that some patients may gain more effect by AIs than fulvestrant, as the marker predicts differential outcome between the two drugs in an all ER+ population (Fig. 5). A biomarker to predict response to either fulvestrant or AIs would be of great clinical use. A similar DRP for fulvestrant has been shown to predict benefit of fulvestrant in the DBCG cohort and retrospectively in a neoadjuvant trial with fulvestrant (32,47). As the DRP system has previously been found to be predictive in settings with multiple drugs (48,49), this system could then potentially point to which antihormonal treatment would be preferable: fulvestrant or AIs in the first line setting of combinations with CDK4/6 inhibitors.

Conclusion

The anastrozole DRP may predict benefit of exemestane and anastrozole in ABC patients. The prediction was best in patients that had not received adjuvant ET. No prediction of letrozole benefit was found.

In this cohort predictions of benefit were made from archival FFPE tumour tissue most of which were from time of primary disease. The overall predictive effect of the anastrozole DRP could aid physicians in choice of ET in ABC patients.

Abbreviations

ABC = advanced breast cancer, AI = aromatase inhibitor, AnaDRP = anastrozole based Drug Response Predictor, CDK4/& = cyclin dependent kinase 4/6, CEF = cyclophosphamide, epirubicine, 5-fluorouracil, CI = confidence interval, CMF = cyclophosphamide, methotrexate and 5-fluorouracil, DBCG = Danish Breast Cancer Cooperative Group, DRP = Drug Response Predictor, EC-Tax = epirubicine, cyclophosphamide, paclitaxel/docetaxel, ER = oestrogen receptor, ESR1 = coding gene for ER- α, ET = endocrine therapy, ExeDRP = exemestane based Drug Response Predictor, FFPE = formalin-fixed paraffin-embedded, GI50 = growth inhibition 50, HER2 = human epidermal growth factor receptor 2, HR = hazard ratio, IL6ST = coding gene for interleukin 6 signal transducer, NCI-60 = National Cancer Institute 60 cell lines, OV = Oncology Venture, PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, RS = recurrence score, TTP = time-to-progression.

Declarations

Ethics, consent and permissions

The experiments comply with the current laws of Denmark in which they were performed. Informed consent was obtained for sampling of the tissue in the Phase II trial on LiPlaCis, registered with ClinicalTrials.gov, number NCT01861496. Informed consent was not obtained for this additional retrospective comparison of gene expression and treatment outcome, as the research did not have implications on the health of the research subjects and this was accepted by the Regional Committee on Health Research Ethics for Capital Region Denmark and is in accordance with the 1964 Helsinki declaration and its later amendments.

Consent for publication

Not applicable.

Availability of data and material

The datasets analysed during this study are available through DBCG on reasonable request (dbcg@dbcg.dk).

Competing interests

The authors declare the following potential competing interest: authors IKB, ASKB, UHB, SK, and PBJ declare past or present primary employment or leadership position in a company (Oncology Venture) that has a potential to benefit from these results. Authors IKB (immediate family), ASKB, IJC, UHB, SK, and PBJ declare ownership in Oncology Venture. Author IJC declares advisory role to Oncology Venture.

Author BE declares funding to institution by Oncology Venture and other outside the submitted work. Authors ASK and EHJ declare consultancy roles outside the submitted work. Authors TDC, EB, IK, HD, AAL, STL, SL, JB, VG, and DN declare that they have no competing interests.

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Author's contributions

Study design: ASK, BE, HD, AAL, STL, SL, EHJ, JB, VG, PBJ, DN. Principal investigators: EB, ASK, BE, IK, HD, AAL, STL, SL, EHJ, JB, VG, DN. Collection of data: ASKB, TDC, EB. Curation of data: IKB, IJC. Data analysis: IKB, IJC, SK. Data interpretation: IKB, TDC, ASKB, IJC, ASK, BE, IK, UHB, SK, PBJ, DN. Initial draft of manuscript: IKB, IJC. Revising and final approval of manuscript: all authors.

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References

1. Kümler I, Knoop AS, Jessing CAR, Ejlertsen B, Nielsen DL. Review of hormone-based treatments in postmenopausal patients with advanced breast cancer focusing on aromatase inhibitors and fulvestrant. ESMO open. 2016;1(4):e000062.

2. Brueggemeier RW. Overview of the pharmacology of the aromatase inactivator exemestane. Breast Cancer Res Treat. 2002;74(2):177–85.

3. Mouridsen H, Gershanovich M. The role of aromatase inhibitors in the treatment of metastatic breast cancer. Semin Oncol. 2003 Aug 1;30:33–45.

4. Mouridsen H, Gershanovich M, Sun Y, Pérez-Carrión R, Boni C, Monnier A, et al. Phase III Study of Letrozole Versus Tamoxifen as First-Line Therapy of Advanced Breast Cancer in Postmenopausal Women: Analysis of Survival and Update of Efficacy From the International Letrozole Breast Cancer Group. J Clin Oncol. 2003 Jun 1;21(11):2101–9.

5. Paridaens RJ, Dirix LY, Beex L V, Nooij M, Cameron DA, Cufer T, et al. Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: the European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. J Clin Oncol. 2008 Oct 20;26(30):4883–90.

6. Cardoso F, Costa A, Senkus E, Aapro M, André F, Barrios CH, et al. 3rd ESO-ESMO international consensus guidelines for Advanced Breast Cancer (ABC 3). Breast. 2017 Feb 1;31:244–59.

7. Im S-A, Lu Y-S, Bardia A, Harbeck N, Colleoni M, Franke F, et al. Overall Survival with Ribociclib plus Endocrine Therapy in Breast Cancer. N Engl J Med. 2019 Jun 4;NEJMoa1903765.

8. Finn RS, Martin M, Rugo HS, Jones S, Im S-A, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016 Nov 17;375(20):1925–36.

9. Reinert T, Gonçalves R, Bines J. Implications of ESR1 Mutations in Hormone Receptor-Positive Breast Cancer. Curr Treat Options Oncol. 2018 May 17;19(5):24.

10. Ellis MJ, Perou CM. The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov. 2013 Jan;3(1):27–34.

11. Bartlett JMS, Ahmed I, Regan MM, Sestak I, Mallon EA, Dell'Orto P, et al. HER2 status predicts for upfront AI benefit: A TRANS-AIOG meta-analysis of 12,129 patients from ATAC, BIG 1-98 and TEAM with centrally determined HER2. Eur J Cancer. 2017 Jul;79:129–38.

12. Turnbull AK, Arthur LM, Renshaw L, Larionov AA, Kay C, Dunbier AK, et al. Accurate Prediction and Validation of Response to Endocrine Therapy in Breast Cancer. J Clin Oncol. 2015 Jul 10;33(20):2270–8.

13. Ueno T, Masuda N, Yamanaka T, Saji S, Kuroi K, Sato N, et al. Evaluating the 21-gene assay Recurrence Score® as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer. Int J Clin Oncol. 2014 Aug 8;19(4):607–13.

14. Simpson E. Sources of estrogen and their importance. J Steroid Biochem Mol Biol. 2003 Sep 1;86(3–5):225–30.

15. Sasano H, Suzuki T, Miki Y, Moriya T. Intracrinology of estrogens and androgens in breast carcinoma. J Steroid Biochem Mol Biol. 2008 Feb 1;108(3–5):181–5.

16. Brodie A, Lu Q, Nakamura J. Aromatase in the normal breast and breast cancer. J Steroid Biochem Mol Biol. 1997 Apr 1;61(3–6):281–6.

17. de Jong PC, Blankenstein MA, Nortier JW., Slee PHT., van de Ven J, van Gorp JMH., et al. The relationship between aromatase in primary breast tumors and response to treatment with aromatase inhibitors in advanced disease. J Steroid Biochem Mol Biol. 2003 Nov 1;87(2–3):149–55.

18. Lykkesfeldt AE, Henriksen KL, Rasmussen BB, Sasano H, Evans DB, Møller S, et al. In situ aromatase expression in primary tumor is associated with estrogen receptor expression but is not predictive of response to endocrine therapy in advanced breast cancer. BMC Cancer. 2009 Dec 16;9(1):185.

19. Sonne-Hansen K, Lykkesfeldt AE. Endogenous aromatization of testosterone results in growth stimulation of the human MCF-7 breast cancer cell line. J Steroid Biochem Mol Biol. 2005 Jan 1;93(1):25–34.

20. Magnani L, Frige G, Gadaleta RM, Corleone G, Fabris S, Kempe MH, et al. Acquired CYP19A1 amplification is an early specific mechanism of aromatase inhibitor resistance in ERα metastatic breast cancer. Nat Genet. 2017 Mar;49(3):444–50.

21. Duffy MJ, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E, et al. Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). Eur J Cancer. 2017 Apr;75:284–98.

22. Harris LN, Ismaila N, Mcshane LM, Andre F, Collyar DE, Gonzalez-Angulo AM, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2016;34:1134–50.

23. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer. N Engl J Med. 2004;351(27):2817–26.

24. van de Vijver MJ, He YD, van ’t Veer LJ, Dai H, Hart AAM, Voskuil DW, et al. A Gene-Expression Signature as a Predictor of Survival in Breast Cancer. N Engl J Med. 2002 Dec 19;347(25):1999–2009.

25. Buus R, Sestak I, Kronenwett R, Denkert C, Dubsky P, Krappmann K, et al. Comparison of EndoPredict and EPclin With Oncotype DX Recurrence Score for Prediction of Risk of Distant Recurrence After Endocrine Therapy. J Natl Cancer Inst. 2016 Nov 10;108(11):djw149.

26. Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. J Clin Oncol. 2013 Aug 1;31(22):2783–90.

27. Sestak I, Buus R, Cuzick J, Dubsky P, Kronenwett R, Denkert C, et al. Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor–Positive Breast Cancer. JAMA Oncol. 2018 Feb 15;

28. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer. 2006 Oct;6(10):813–23.

29. Shankavaram UT, Reinhold WC, Nishizuka S, Major S, Morita D, Chary KK, et al. Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study. Mol Cancer Ther. 2007 Mar 1;6(3):820–32.

30. Stoppoloni D, Salvatori L, Biroccio A, D'Angelo C, Muti P, Verdina A, et al. Aromatase Inhibitor Exemestane has Antiproliferative Effects on Human Mesothelioma Cells. J Thorac Oncol. 2011 Mar 1;6(3):583–91.

31. Hassan F, El-Hiti GA, Abd-Allateef M, Yousif E. Cytotoxicity anticancer activities of anastrozole against breast, liver hepatocellular, and prostate cancer cells. Saudi Med J. 2017 Apr;38(4):359–65.

32. Knudsen S, Jensen T, Hansen A, Mazin W, Lindemann J, Kuter I, et al. Development and Validation of a Gene Expression Score That Predicts Response to Fulvestrant in Breast Cancer Patients. Xu W, editor. PLoS One. 2014 Feb 5;9(2):e87415.

33. Xu X, Veenstra TD. Concentration of endogenous estrogens and estrogen metabolites in the NCI-60 human tumor cell lines. 2012.

34. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumor MARKer prognostic studies (REMARK). Breast Cancer Res Treat. 2006 Oct 24;100(2):229–35.

35. Databases & Tools | Developmental Therapeutics Programme at National Cancer Institute [Internet]. [cited 2019 Jun 18]. Available from: https://dtp.cancer.gov/dtpstandard/dwindex/index.jsp

36. Vangsted AJ, Helm-Petersen S, Cowland JB, Jensen PB, Gimsing P, Barlogie B, et al. Drug response prediction in high-risk multiple myeloma. Gene. 2018 Feb 20;644:80–6.

37. Wang W, Baggerly KA, Knudsen S, Askaa J, Mazin W, Coombes KR. Independent validation of a model using cell line chemosensitivity to predict response to therapy. J Natl Cancer Inst. 2013;105(17):1284–91.

38. Jakobsen EH, Nielsen D, Danoe H, Linnet S, Hansen J, Lassen UN, et al. Liposomal cisplatin response prediction in heavily pretreated breast cancer patients: A multigene biomarker in a prospective phase 2 study. In: ASCO Annual Meeting. Alexandria, VA 22314: American Society of Clinical Oncology; 2018.

39. Buhl ASK, Christensen TD, Christensen IJ, Nelausen KM, Balslev E, Knoop AS, et al. Predicting efficacy of epirubicin by a multigene assay in advanced breast cancer within a Danish Breast Cancer Cooperative Group (DBCG) cohort: a retrospective-prospective blinded study. Breast Cancer Res Treat. 2018 Aug 11;1–10.

40. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.

41. Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy - Analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004;20(3):307–15.

42. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 2016 Sep 15;32(18):2847–9.

43. Christensen TD, Buhl ASK, Christensen IJ, Nelausen KM, Balslev E, Knoop AS, et al. Prediction of exemestane benefit in patients with advanced breast cancer based on diagnostic biopsy mRNA analysis. In: J Clin Oncol. 2017. p. e12532.

44. Mukhopadhyay K De, Liu Z, Bandyopadhyay A, Kirma NB, Tekmal RR, Wang S, et al. Aromatase Expression Increases the Survival and Malignancy of Estrogen Receptor Positive Breast Cancer Cells. Tan M, editor. PLoS One. 2015 Apr 2;10(4):e0121136.

45. Ellis MJ, Llombart-Cussac A, Feltl D, Dewar JA, Jasiówka M, Hewson N, et al. Fulvestrant 500 mg Versus Anastrozole 1 mg for the First-Line Treatment of Advanced Breast Cancer: Overall Survival Analysis From the Phase II FIRST Study. J Clin Oncol. 2015 Nov 10;33(32):3781–7.

46. Robertson JFR, Bondarenko IM, Trishkina E, Dvorkin M, Panasci L, Manikhas A, et al. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. Lancet (London, England). 2016 Dec 17;388(10063):2997–3005.

47. Christensen TD, Buhl ASK, Christensen IJ, Nelausen KM, Balslev E, Knoop AS, et al. Fulvestrant response prediction from transcriptome data obtained from primary breast cancer biopsies. In: Journal of Clinical Oncology. 2016. p. abstract e12056.

48. Knudsen S, Hother C, Grønbæk K, Jensen T, Hansen A, Mazin W, et al. Development and Blind Clinical Validation of a MicroRNA Based Predictor of Response to Treatment with R-CHO(E)P in DLBCL. PLoS One. 2015;10(2):e0115538.

49. Buhl IK, Santoni-Rugiu E, Ravn J, Hansen A, Christensen IJ, Jensen T, et al. Molecular prediction of adjuvant cisplatin efficacy in Non-Small Cell Lung Cancer (NSCLC)—validation in two independent cohorts. Ahmad A, editor. PLoS One. 2018 Mar 22;13(3):e0194609.

Tables

Due to technical limitations, tables 1-3 are only available as a download in the supplemental files section

Figures

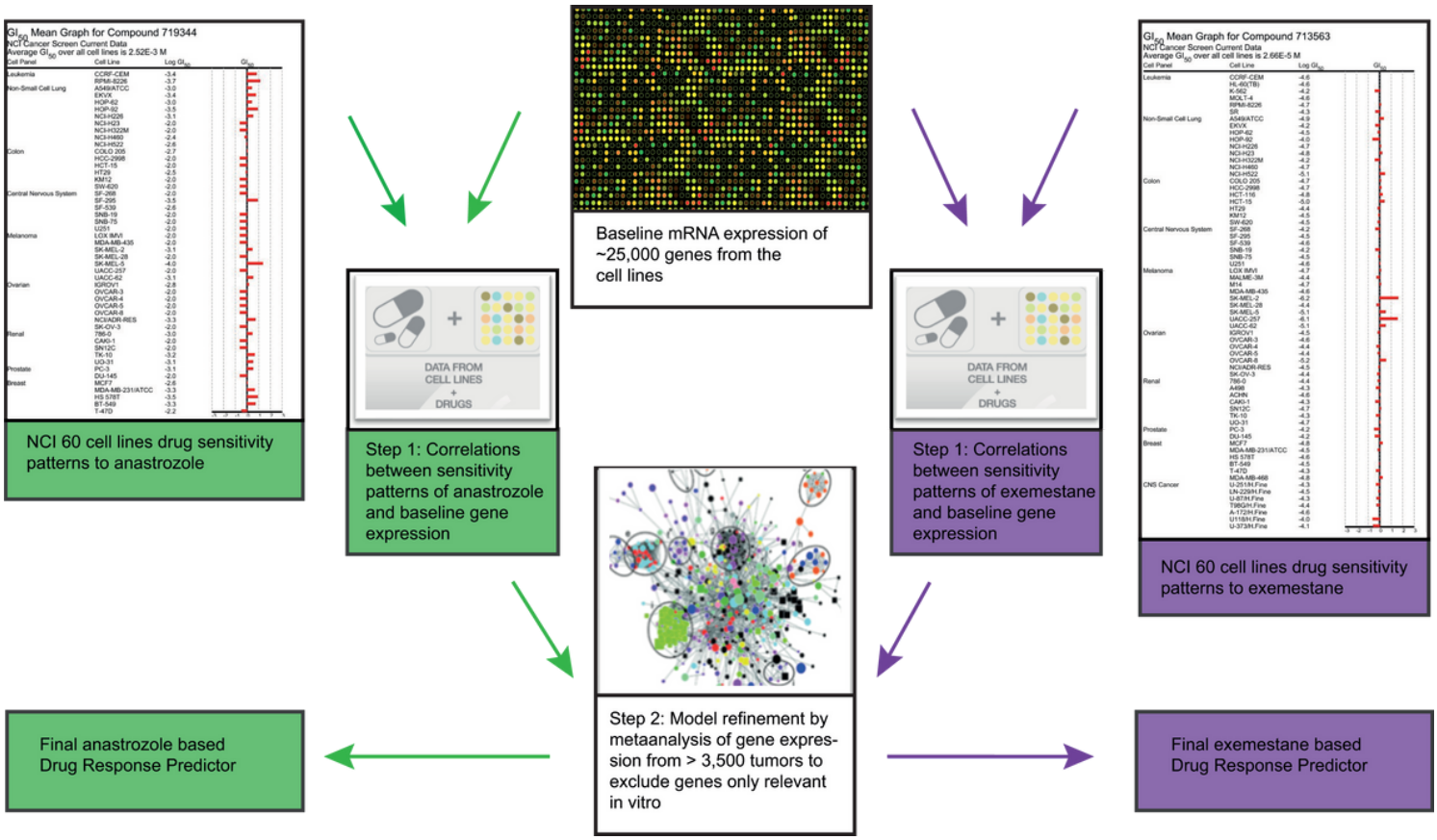

Figure 1

Drug Response Predictor development The figure is a modified form of a figure by Buhl ASK and colleagues (39).

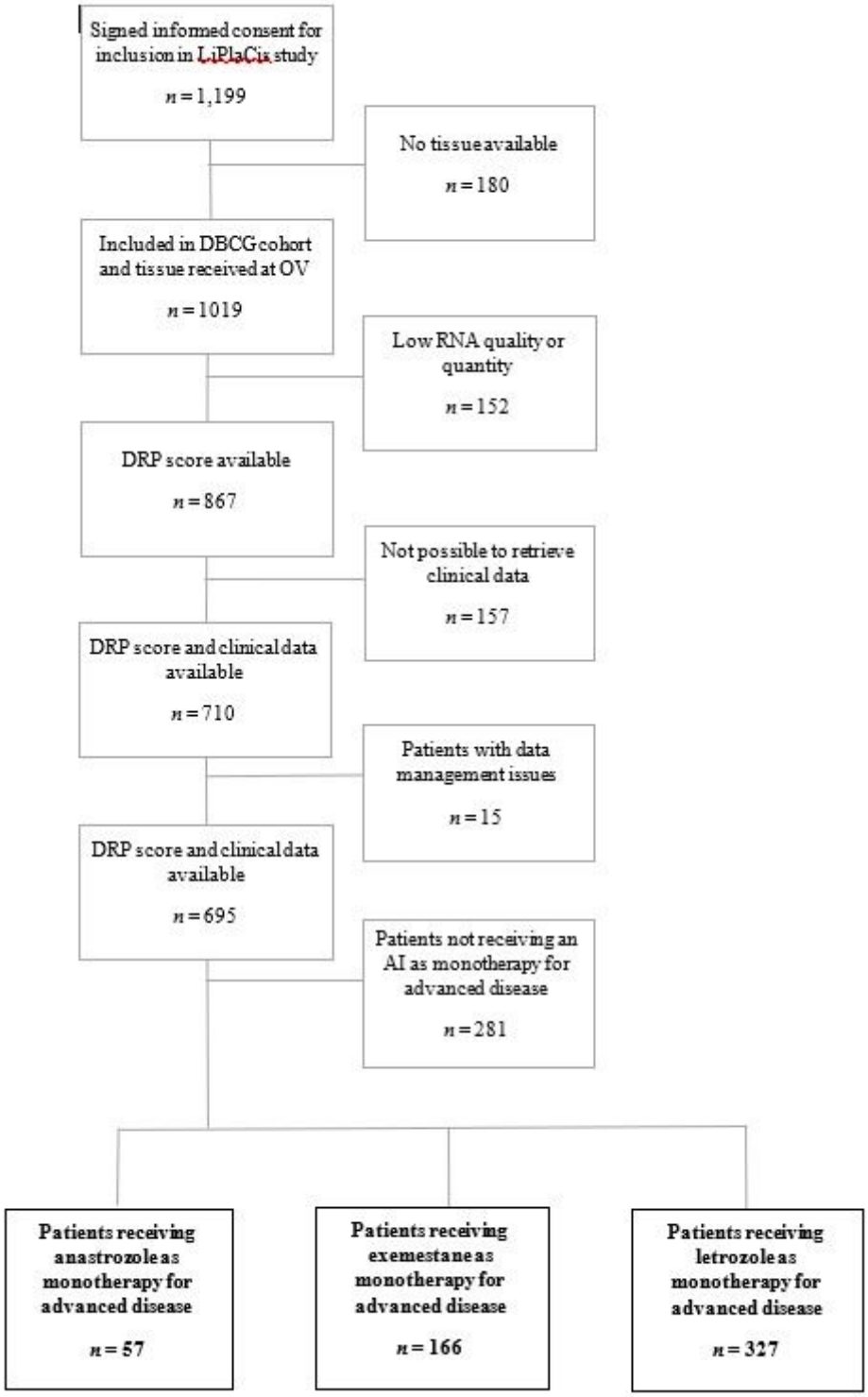

Figure 2

Inclusion and exclusion of patients for final analysis A flowchart describing the inclusion and exclusion of patients for final analysis. As some patients received more than one AI treatment as monotherapy at separate timepoints, the sum of all three subsets is higher than the individual patients receiving AI monotherapy (n = 414). The included patients are in bold. Abbreviations: ABC = advanced breast cancer, DBCG = Danish Breast Cancer Cooperative Group, DRP = Drug Response Predictor, OV = Oncology Venture.

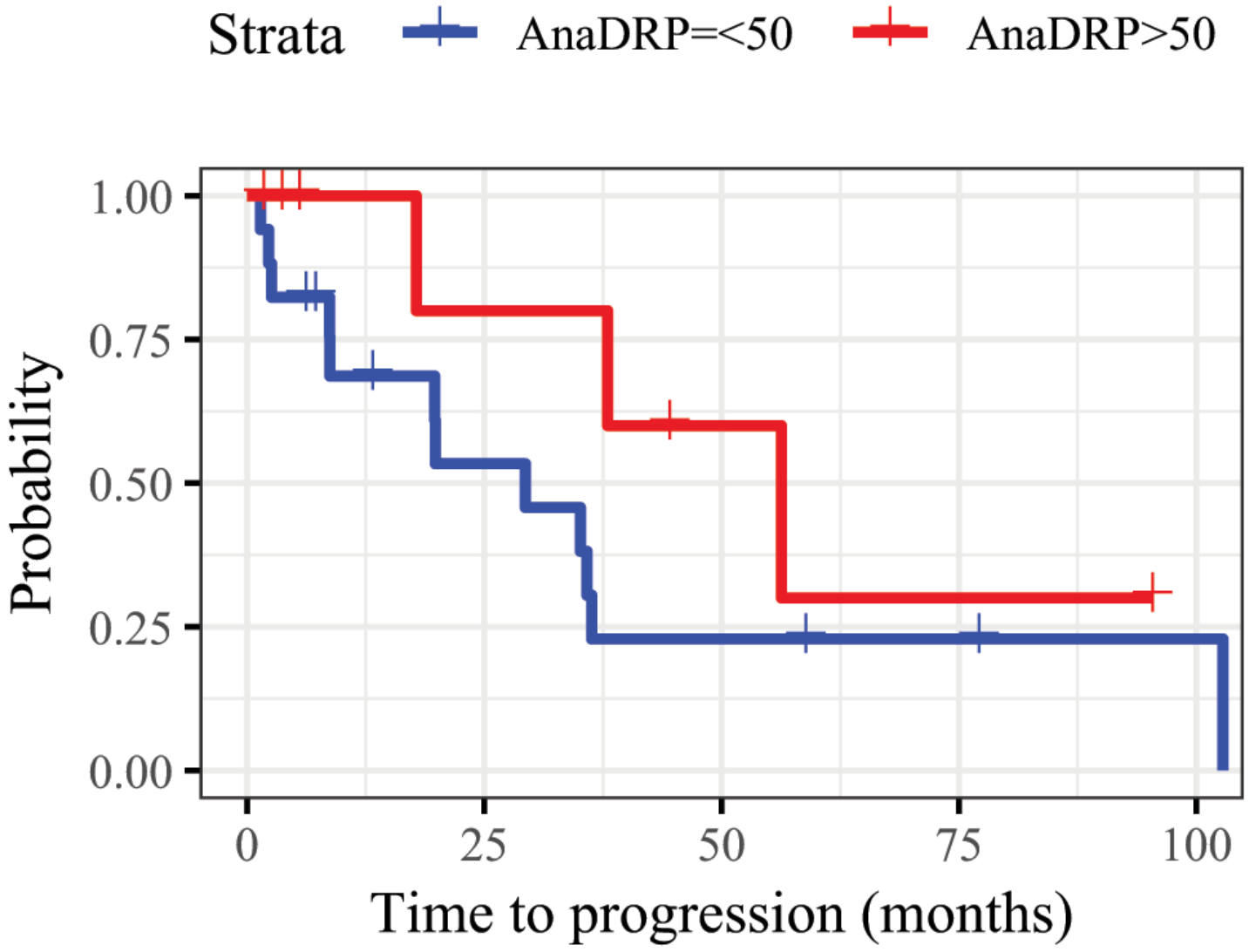

Figure 3

Kaplan-Meier plots of the anastrozole DRP in anastrozole treated patients Kaplan-Meier plots of the anastrozole DRP score dichotomised at 50% in anastrozole treated patients that had not received adjuvant ET, endpoint TTP. Abbreviations: AI = aromatase inhibitor, AnaDRP = anastrozole based Drug Response Predictor, ET = endocrine therapy, TTP = time to progression.

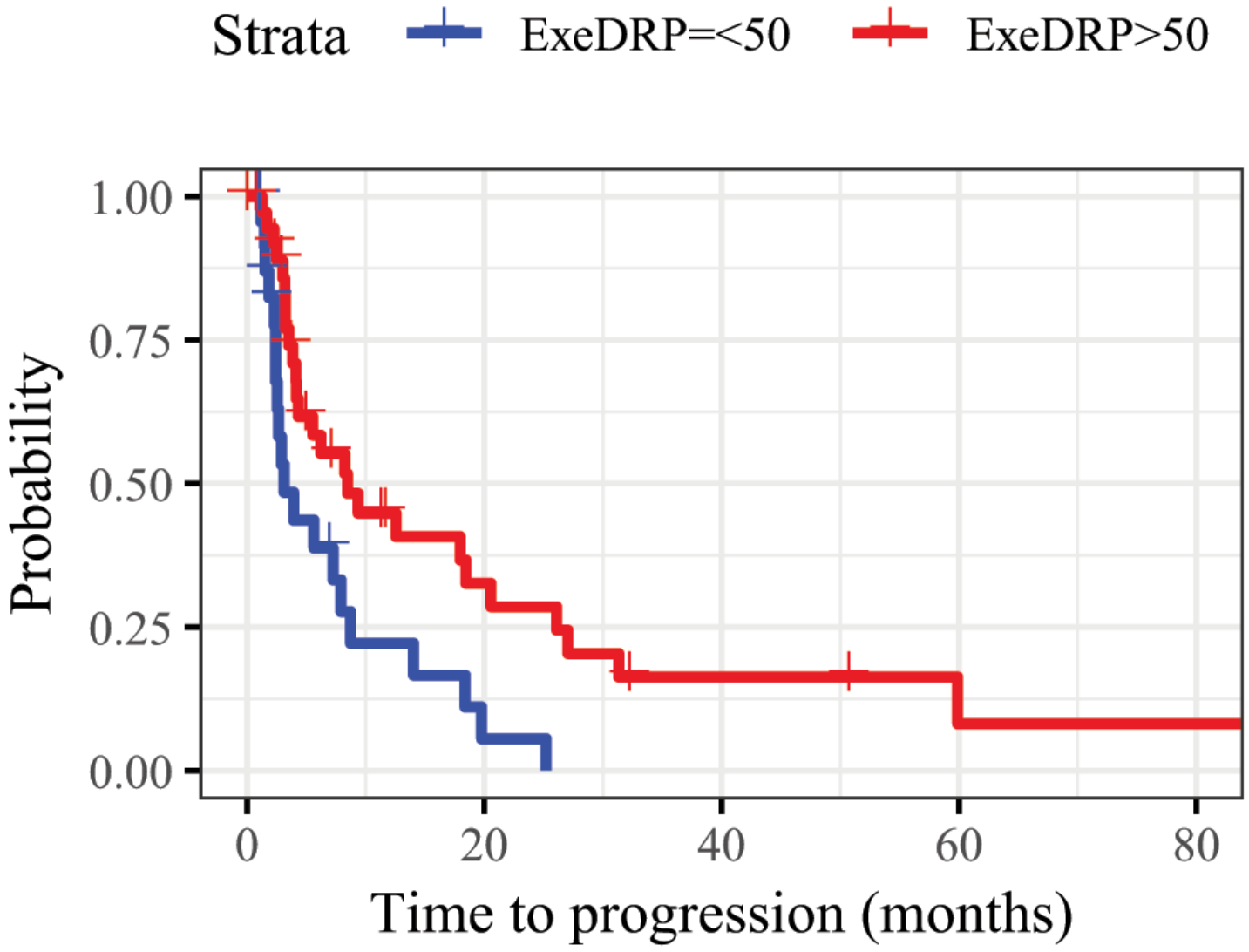

Figure 4

Kaplan-Meier plot of the exemestane DRP in exemestane treated patients Kaplan-Meier plots of the exemestane DRP score dichotomised at 50% in exemestane treated that had not received adjuvant ET, endpoint TTP. Abbreviations: ET = endocrine therapy, ExeDRP = exemestane based Drug Response Predictor, TTP = time to progression.

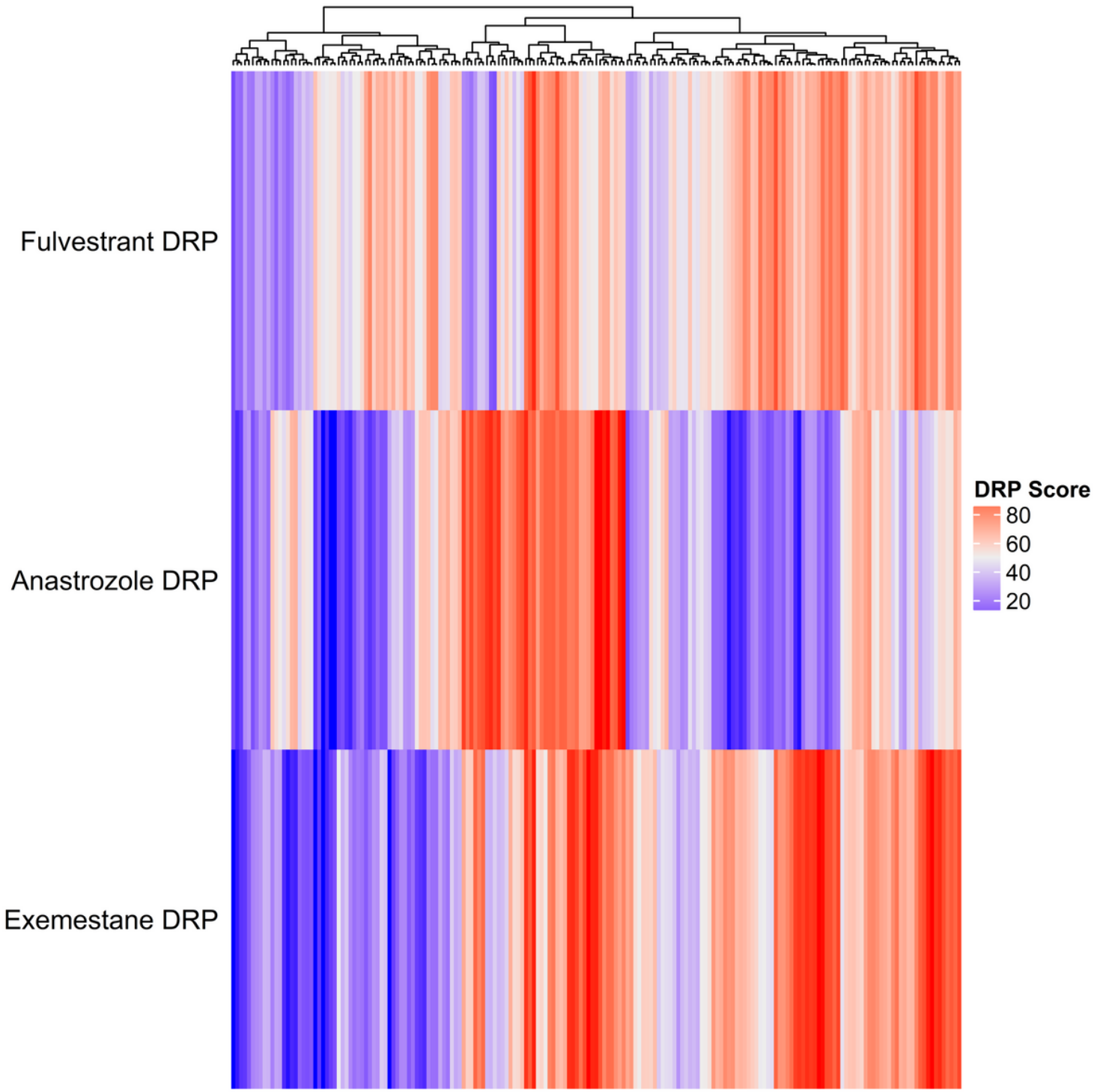

Figure 5

Heatmap comparing scores of separate DRPs A heatmap used for comparison of individual scores on three separate markers: the fulvestrant DRP (top), the anastrozole DRP (middle) and the exemestane DRP (bottom) for each patient treated with exemestane or anastrozole for ABC. Each column presents the values of each marker for one patient. Abbreviations: ABC = advanced breast cancer, DRP = Drug Response Predictor.

Supplementary Files

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