Retrospective-prospective blinded evaluation predicting efficacy of epirubicin by a multigene assay in advanced breast cancer within a Danish Breast Cancer Cooperative Group (DBCG) cohort

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Baseline characteristics	N = 135	
Age at diagnosis, years in median (Q1-Q3)	54.9 (46.3-61.9)	
Time to relapse from diagnosis, years in median (Q1-Q3)	4.5 (0.9-8.0)	
ER status		
Positive	119	
Negative	16	
HER 2 status		
Positive	17	
Negative	105	
Data missing	13	
Adjuvant chemotherapy		
CMF	10	
CEF	8	
EC-Tax	11	
None	99	
Data missing	2	
Adjuvant antihormone therapy		
Tamoxifen	19	
Tamoxifen + AI	33	
AI	13	
None	70	
No. of anti-hormone therapies prior to epirubicin (%)		
1	35	26%
2	15	11%
3	10	7.4%
4 or more	10	7.4%
No. of chemotherapies prior to epirubicin (%)		
1	38	28%
2	22	29%
3	7	5.2%
4 or more	9	6.7%
Number of metastatic sites at time of epirubicin treatment (%)		
1	46	34%
2	60	44%
3	17	13%
4 or more	12	9%

***Table 1**: Baseline demographics*

***Abbreviations:** ER: Estrogen Receptor; HER-2: Human Epidermal Growth Receptor 2; CMF: Cyclophosphamide, methotrexate and 5-flourouracil; CEF: Cyclophosphamide, epirubicin and 5-flourouracil; EC-Tax: Epirubicin, cyclophosphamide and docetaxel; AI: Aromatase Inhibitors*

Background

Epirubicin remains a cornerstone in the treatment of primary and advanced breast cancer. The value of the treatment could increase if the sensitive patients were identified.

This study evaluated the predictive effect of a multigene mRNA-based Drug Response Predictor (DRP) in the treatment of advanced breast cancer (ABC). We applied a mathematical method combining *in vitro* sensitivity with gene expression patterns in tumors – see elaboration on *Figure 1*.

Previously the DRP has been broadly validated (1, 2). This includes validation of anthracycline response i.e. epirubicin as monotherapy in 120 breast cancers (3) and doxorubicin as a part of R-CHOP treatment for Diffuse Large B-Cell Lymphoma (4).

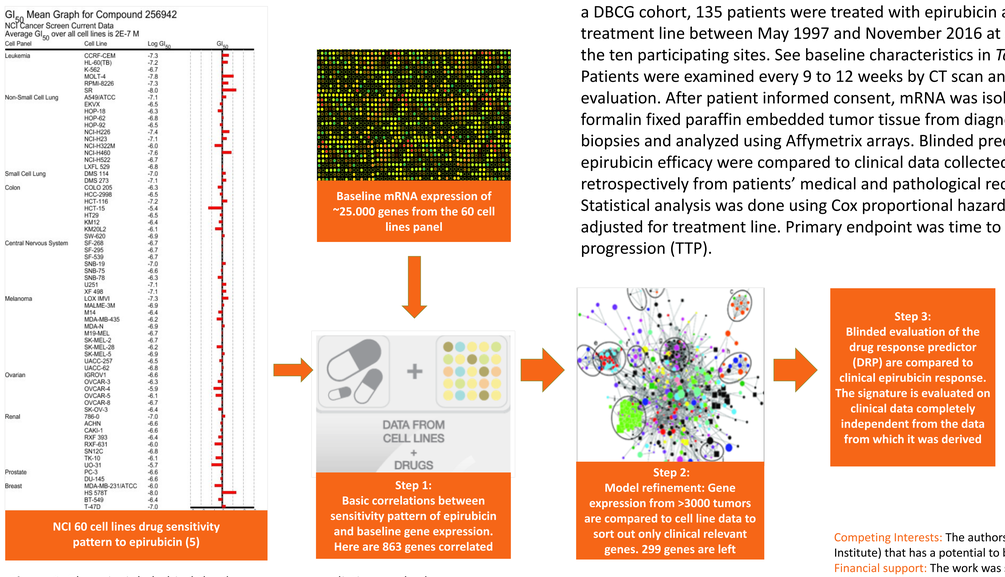

***Figure 1**: The principle behind the drug response prediction method*

Methods

DRP algorithm

The DRP algorithm is based on cell line data from National Cancer Institute, NCI60 (5). Gene expression data from cell lines is correlated to the sensitivity pattern (measured as GI50 values) to epirubicin showing which genes are correlated to sensitivity and which genes are correlated to resistance *in vitro*. To only include the clinical relevant pathways, gene expression from more than 3000 patients tumors of different origin are compared to the raw DRP. Gene expression that are not taking part of any meaningful biological pathway in the 3000 tumors are excluded from the final DRP.

Epirubicin sensitivity predictor

Among 716 consecutive patients with advanced breast cancer from a DBCG cohort, 135 patients were treated with epirubicin at any treatment line between May 1997 and November 2016 at one of the ten participating sites. See baseline characteristics in *Table 1*. Patients were examined every 9 to 12 weeks by CT scan and clinical evaluation. After patient informed consent, mRNA was isolated from formalin fixed paraffin embedded tumor tissue from diagnostic biopsies and analyzed using Affymetrix arrays. Blinded predictions of epirubicin efficacy were compared to clinical data collected retrospectively from patients' medical and pathological records. Statistical analysis was done using Cox proportional hazards model adjusted for treatment line. Primary endpoint was time to progression (TTP).

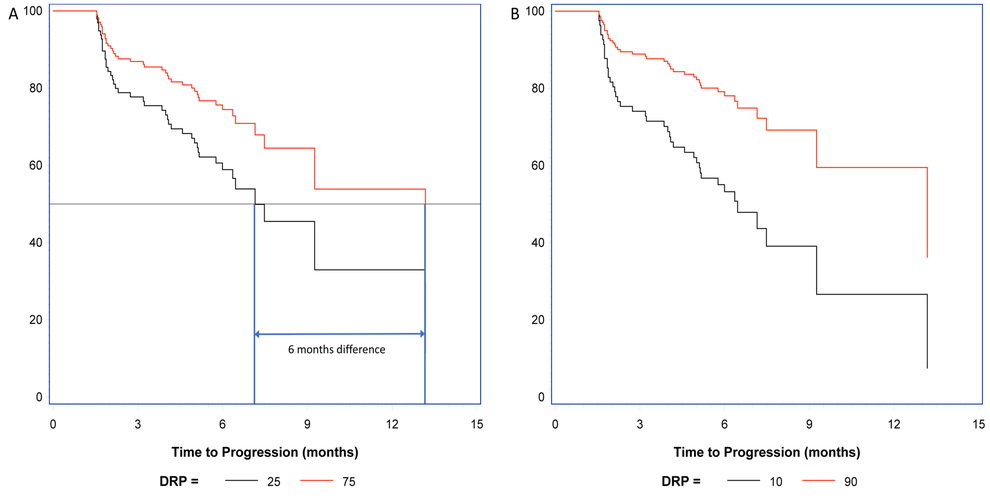

***Figure 2:** Cox regression with DRP-values of A) 25% and 75% and B) 10% and 90%.*
In A) the black horizontal line represents the median. Blue lines points out the 6 months difference at median time to progression.

Results

Median time to progression was 9.3 months (95% CI: 7.2-13.2). Of the 135 patients, four received epirubicin more than once.

Scoring the DRP as a continuous covariate demonstrated that the DRP was significantly associated to TTP (p = 0.02). By comparing two patients with DRP scores differing by 50 percentage points the hazard ratio was 0.54 (90% CI: 0.35-0.89). The estimated median time to progression for a patient with a DRP value of 75% was 13 months whereas this was reduced to 7 months for a patient with a DRP value of 25%, i.e. a 6 months difference as demonstrated with blue lines in *Figure 2A*. *Figure 2B* shows the same analysis with a 80 percentage points difference and a hazard ratio of 0.35 (90% CI: 0.15-0.82) suggesting an even stronger separation in the risk between extreme DRP values.

Conclusion

The current study demonstrates a potential clinical value by being able to select patients that benefit from epirubicin against patients predicted not to benefit sparing the last patients unnecessary toxicity.

Competing Interests: The authors have read and adhere to ASCO's policy and have the following conflicts: authors ASKB IJC AR AH SK PBJ declare past or present primary employment or advisory role or ownership in a company (Medical Prognosis Institute) that has a potential to benefit from these results. Medical Prognosis Institute holds a patent on the subject matter. This does not alter the authors' adherence to all policies on sharing data and materials.
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