Predictive Factors of Eribulin Activity in Metastatic Breast Cancer Patients

Rita De Sanctis^{a, b} Elisa Agostinetto^a Giovanna Masci^a Emanuela Ferraro^a
Agnese Losurdo^a Alessandro Viganò^b Lidija Antunovic^c Monica Zuradelli^a
Rosalba Maria Concetta Torrisi^a Armando Santoro^{a, d}

^aDepartment of Medical Oncology and Hematology, Humanitas Cancer Center and Research Hospital, IRCCS, Rozzano, Milan, Italy; ^bMolecular and Cellular Networks Lab, Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, "Sapienza" University, Rome, Italy; ^cDepartment of Nuclear Medicine, Humanitas Cancer Center and Research Hospital, IRCCS, Rozzano, Milan, Italy; ^dDepartment of Biomedical Sciences, Humanitas University, Rozzano, Milan, Italy

Keywords

Eribulin · Metastatic breast cancer · Progression-free survival · Discriminant functional analysis

Abstract

Objectives: Predictive factors of response to eribulin are lacking. We aimed to investigate the activity and safety of eribulin in a real-world population of metastatic breast cancer (MBC) patients and to identify possible predictive factors of progression-free survival (PFS) and objective response. **Methods:** We retrospectively analyzed 71 eribulin-treated MBC patients. Best response rate, PFS, and adverse events (AEs) were evaluated. The impact of different clinical-pathological factors on PFS was evaluated using the Cox proportional hazards model. Predictive factors of response were identified by discriminant function analysis (DFA). **Results:** Median PFS was 3.75 months (95% CI, 2.39–4.48); 12 patients (16.90%) achieved partial response (PR), 27 (38.03%) stable disease. The most common AEs were fatigue (25.83%), neutropenia (16.56%), and peripheral neuropathy (13.91%). A worse performance status ($p = 0.025$) and a higher number

of metastatic organ sites ($p = 0.011$) were associated with a worse PFS under eribulin. Overall, in the DFA-predictive model, neutrophil-to-lymphocyte ratio at baseline, estrogen receptor, Ki67, histology, and age were predictive of PR with 100% accuracy. **Conclusions:** Activity and safety profiles of eribulin were consistent with literature data. Performance status and number of metastatic sites were predictive factors of PFS. DFA could be a promising tool to discriminate responses to eribulin among MBC patients.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

Breast cancer is the most common form of cancer among women in Italy and worldwide. Its incidence has been increasing over the past few decades. It is estimated that approximately 12.4% of women will be diagnosed with breast cancer at some point during their lifetime [1, 2]. Despite improvements in treatments, the 5-year survival rate for metastatic breast cancer (MBC) is only 26%. Chemotherapy plays an important role in the advanced

setting of breast cancer treatment; however, there is no standard of care after progression under treatment with anthracyclines and taxanes [3].

Eribulin is a non-taxane microtubule polymerization inhibitor, a structurally simplified synthetic analogue of halichondrin B, a natural product isolated from the marine sponge *Halicondria okadai* [4]. It acts differently from other tubulin-targeted agents, like taxanes and vinca alkaloids. Eribulin inhibits microtubule growth without shortening them, and aggregates soluble tubulin in a non-productive form [5]. This mechanism of action leads to irreversible mitosis arrest and finally to apoptosis. Because of this unique mechanism of action, eribulin may show activity also against taxane-resistant tumor cell lines.

In the phase III, open-label randomized study EMBRACE, eribulin demonstrated a significant increase in overall survival (OS) compared with treatment of physician's choice (TPC) in heavily pretreated patients with MBC [6]. The EMBRACE trial was the first single-agent study of a cytotoxic agent showing significantly increased survival in patients with heavily pretreated advanced breast cancer. In this study, 762 patients who had received between 2 and 5 previous chemotherapy regimens were randomized to receive either eribulin or a TPC. The median OS in women assigned to eribulin was 13.1 months, compared with 10.6 months in the control group [hazard ratio (HR) 0.81, $p = 0.041$]. Objective response rate (ORR) and progression-free survival (PFS) were analyzed as secondary endpoints, with an ORR of 12% and a median PFS of 3.7 months. With regard to safety, eribulin provided manageable toxic effects, in line with those described in earlier studies. This trial led to eribulin approval by the US Food and Drug Administration (FDA) in 2010. In Europe, eribulin is approved as monotherapy for patients with MBC who have received at least one line of chemotherapy for advanced disease, and who have been treated with an anthracycline and a taxane either in an adjuvant or metastatic setting.

However, despite the clear advantage in OS, the benefit in PFS remains limited across the studies. No univocal explanation is currently available to interpret this discrepancy. Although eribulin is now widely employed worldwide, predictive factors of response in clinical trials and in the "real-world" setting are currently lacking. In our retrospective, observational analysis, we aimed to evaluate activity and safety of eribulin in a "real-world" population of patients with MBC, and apply exploratory techniques as the proportional-hazards model and the discriminant function analysis (DFA) to identify possible predictive factors of PFS and response.

Patients and Methods

Study Population, Data Collection, and Outcome Measures

All consecutive advanced breast cancer patients who started eribulin at Humanitas Cancer Center between December 2012 and August 2017 were reviewed. Information on clinical history, tumor response, treatment outcome, and safety profile was collected from patients' medical charts.

All patients received eribulin after radiological evidence of progressive disease (PD). Patients included in the present analysis were aged over 18 years, had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 3 , a life expectancy of at least 3 months, absolute neutrophil count at baseline $\geq 1,000/\text{mm}^3$, platelet count $\geq 75,000/\text{mm}^3$, hemoglobin levels $\geq 8 \text{ g/dL}$, normal renal function, and normal liver function defined as normal bilirubin levels, and aspartate aminotransferase and alanine aminotransferase levels less than 2.5 times the upper limit of normal.

Pretreatment evaluation included data on previous treatments, response to treatments, physical examination, blood count, a serum biochemistry panel, radiological tumor assessment by contrast chest and abdomen computed tomography (CT) scan, magnetic resonance imaging (MRI), bone scan or positron emission tomography (PET) according to clinical judgement.

All procedures were conducted in accordance with the Declaration of Helsinki and have been approved by the local ethics committee. Written informed consent to treatment and to the use of clinical data for scientific purposes had been provided by all patients at the time of chemotherapy administration.

Eribulin was administered at a dose of 1.23 mg/m^2 intravenously over 2–5 min on days 1 and 8 of a 21-day cycle after at least 2 prior chemotherapy lines for metastatic disease. The dose was reduced to 0.97 mg/m^2 in case of grade 4 neutropenia lasting more than 7 days, neutropenic fever, grade 4 thrombocytopenia, or grade 3–4 nonhematological toxicities. Granulocyte colony-stimulating factor and erythropoiesis-stimulating agents were permitted only in accordance with American Society of Clinical Oncology guidelines. The treatment continued until disease progression, unacceptable toxicity, or consent withdrawal. Disease evaluations were carried out at baseline (pre-dose, but not more than 3 weeks before initiating treatment with eribulin) and after every three cycles thereafter.

The primary objective of the study was to evaluate the best response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 and Kaplan-Meier estimates of PFS under eribulin (PFS_E). Secondary objectives were to analyze the occurrence of adverse events (AEs) according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.02, and the response evaluation defined by the growth modulation indexes (GMI) induced by eribulin (GMI₁) and carried over by eribulin in the subsequent chemotherapy line (GMI₂). GMI represents the growth of a neoplasm under a determined treatment and measures the action that the treatment has on the natural development of the tumor. Mathematically, it is defined as the PFS during a certain treatment divided by the PFS in the previous line of treatment. GMI of eribulin (GMI₁) was calculated as the ratio between PFS_E and PFS of the previous treatment line (PFS_{PRE}) in each single patient, while GMI post-eribulin (GMI₂) was calculated as the ratio between PFS of the first post-eribulin chemotherapy line (PFS_{POST}) and PFS_E.

Statistical Methods

The study was planned as a retrospective evaluation of treatment with eribulin in a consecutive series of pretreated patients. Actuarial survival curves were generated using the Kaplan-Meier method. PFS was defined as the time from the first day of study treatment until disease progression, as shown by radiological or clinical examination, or death from any cause. Patients without any evidence of PD were censored at the date of their last follow-up.

The variables considered were: age, gender (female vs. male), histology (ductal, lobular, or mixed), hormone receptor expression, human epidermal growth factor receptor 2 (HER2) positivity (protein expression level by immunohistochemistry and/or fluorescent in situ hybridization), proliferation index (Ki67, ≤ 20 vs. $> 20\%$, the most common used staining levels cutoff to dichotomize BC populations [7]), tumor grade (G1–2 vs. G3), disease-free interval between the first diagnosis of breast cancer and the first diagnosis of metastasis (dichotomized around the median value), number of prior chemotherapy lines before eribulin for metastatic disease (2, 3, 4, ≥ 5), taxane-refractory (taxane-sensitive vs. taxane-resistant, defined as primary resistance to a previous taxane treatment), number of metastatic sites (1, 2, 3–4), pattern of metastatic disease (visceral vs. nonvisceral), ECOG PS (0, 1, 2–3), median PFS of the chemotherapy line administered immediately before eribulin, neutrophil-to-lymphocyte ratio (NLR) at the first day of the first cycle of eribulin (≤ 2.5 , 2.5–4, 4–5.5, > 5.5) since NLR has been reported to be associated with prognosis and response to several chemotherapeutic agents in breast cancer (see Discussion section).

Each prognostic factor was submitted to a univariable analysis using a proportional hazards Cox regression, giving data as HR with a 95% confidence interval (CI). Each variable satisfying the criterion $p < 0.25$ was entered into the multivariable model. A multivariable Cox regression model was used to estimate HRs and 95% CIs of the association between the selected predictors and the outcome (PFS).

Furthermore, a prediction model of response to eribulin was performed by DFA, using response as outcome of interest and clinical characteristics at baseline as potential predictors. Response to treatment was classified as partial response (PR), stable disease (SD), or PD according to the response evaluation criteria in solid tumors (RECIST) 1.1 criteria [8]. DFA is a nonparametric technique that estimates the linear combinations of descriptors maximizing the separability among subjects according to a predefined grouping variable (response to eribulin). Since a large share of variables introduced in the model were categorical (e.g., histology, ECOG PS), a case-wise analysis was conducted without any substitution of missing data (i.e., patients with incomplete data were excluded). DFA was implemented with a forward stepwise design, with an F-value to enter equal to 1 and an F-value to remove equal to 0. Tolerance of variable correlation was set to 0.01. Classification of single cases was performed by using square Mahalanobis distance between groups. Based on the χ^2 distribution, Mahalanobis distance classifies single cases as belonging to distinct groups by measuring the distance of each case (P) from the multidimensional mean (centroid) of a distribution in the multivariate space defined by canonical roots generated by the model's variables. Multidimensional distance is calculated based on a combination of the actual Euclidean distance and covariance of the same distribution. In this manner, the Mahalanobis distance is scaled in the multivariate space by the mutual correlation of the intervening vari-

Table 1. Baseline characteristics of the study population ($n = 71$)

Median age, years (range)	52 (33–72)
Gender	
Female	69 (97.18%)
Male	2 (2.82%)
Histology	
Ductal	57 (80.28%)
Lobular	8 (11.26%)
Mixed	3 (4.23%)
Missing data	3 (4.23%)
Grading	
G1	1 (1.41%)
G2	22 (30.98%)
G3	25 (35.22%)
Missing data	23 (32.39%)
Hormone receptor status	
Positive	53 (74.65%)
Negative	14 (19.72%)
Missing data	4 (5.63%)
HER2 status	
Negative	49 (69.02%)
Positive	8 (11.26%)
Missing data	14 (19.72%)
Triple-negative breast neoplasms	11 (15.49%)
Ki67	
$\leq 20\%$	22 (30.98%)
$> 20\%$	37 (52.11%)
Missing data	12 (16.91%)
Median DFI, months (range)	27.77 (0–300)
No. of prior chemotherapy lines for advanced disease	3 (2–8)
Metastatic sites	
1	42 (59.15%)
2	22 (30.99%)
3	6 (8.45%)
4	1 (1.41%)
Best response	
PR	12 (16.90%)
SD	27 (38.03%)
PD	32 (45.07%)
ECOG PS	
0	16 (22.54%)
1	35 (49.30%)
2	15 (21.13%)
3	5 (7.04%)
NLR before eribulin, median (range)	4 (1.27–44.60)
Median PFS prior to eribulin, months (range)	4.52 (0–28.97)

Mixed histology corresponded to patients with a diagnosis of both ductal and lobular carcinoma. HER2 status was determined based on HER2 protein expression level by immunohistochemistry (IHC). Tumors with HER2 scores of 0 and 1+ were considered as HER2 negative. In tumors with equivocal HER2 IHC test results (2+), gene amplification was evaluated using fluorescence in situ hybridization. Specimens with HER2 3+ scores were considered as HER2 positive. DFI, disease-free interval; PR, partial response; SD, stable disease; PD, progressive disease; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NLR, neutrophil-to-lymphocyte ratio; PFS, progression-free survival.

Fig. 1. Kaplan-Meier curve for PFS in the entire cohort ($n = 71$). Note that the upper and lower (nonbolded) lines are the relative 95% CI.

Fig. 2. Kaplan-Meier curves for PFS in patients treated with eribulin ($n = 71$), according to their Eastern Cooperative Oncology Group Performance Status (ECOG PS) (0, 1, or 2–3). Note that ECOG PS 2 comprises ECOG PS 2 and 3.

ables. P is attributed to the closest group according to that distance [9, 10].

The contribution of variables taken into account by DFA has lately been investigated in a post hoc univariate analysis by non-parametric tests, since the distribution of the selected variables was not Gaussian. The Kruskal-Wallis test and maximum-likelihood χ^2 test were used for continuous and categorical variables. Normality of variable distributions was assessed by the Shapiro-Wilk test. Correlation analysis among variables was performed using Kendall's tau coefficient.

p values resulted from two-sided tests and $p < 0.05$ was considered statistically significant. Analyses were performed with the STATA software package, version 15 and with STATISTICA version 7, StatSoft, Inc.

Results

Patients' Characteristics

From December 2012 to August 2017, 71 patients with MBC treated at Humanitas Cancer Center were included in the analysis. Demographics and main clinical and baseline pathological characteristics of the patients are reported in Table 1. Median age was 52 years (range, 33–72). De novo metastatic patients were 12 (16.91%). Twenty-five (35.22%) patients had a G3 tumor, and 37 (52.11%) had a high proliferation index (Ki67 >20%); 53 (74.65%) had a hormone-positive disease, 8 (11.26%) had HER2-positive disease, while 11 (15.49%) patients had a triple-negative breast cancer.

Before receiving eribulin, patients had received a median of 3 previous chemotherapy lines for metastatic disease (range 2–8). The median value of the NLR in our sample at baseline (NLR1) was 4 (range 1.2–44.6). Median follow-up was 47.47 months [interquartile range (IQR) 30.38–73.51 months] and 7.4 months (IQR 2.92–12.56 months) from the first diagnosis of metastatic disease and from the first dose of eribulin, respectively. Median PFS from the first dose of eribulin was 3.57 months (95% CI, 2.39–4.48 months; Fig. 1). OS data were not mature at the time of the analysis with 4 events reported. The ORR was 16.90% with a 54.93% clinical benefit rate.

Safety Profile

All 71 patients were included in the safety analysis, and a total of 345 cycles were evaluable for toxicity. Treatment with eribulin was generally well tolerated. The most common eribulin-related toxicities were fatigue, neutropenia, and peripheral neuropathy with a global incidence of 25.83, 16.56, and 13.91%, respectively. Grade 3–4 AEs comprised neutropenia (8.61%) and fatigue (5.30%), while febrile neutropenia was reported with an incidence of 2.64%. Dose reduction occurred in 22 patients for grade 3–4 toxicities (Table 2).

Cox Proportional Hazards Regression

To investigate the impact of different clinical-pathological factors on PFS, we first conducted a univariable

Table 2. Adverse events according to NCI-CTC ($n = 71$)

	G1	G2	G3–4	Total
Any	80 (52.98%)	38 (25.17%)	33 (21.85%)	151 (100.00%)
Hematological				
Anemia	7 (4.63%)	–	1 (0.66%)	8 (5.29%)
Neutropenia	3 (1.99%)	9 (5.96%)	13 (8.61%)	25 (16.56%)
Thrombocytopenia	3 (1.99%)	–	–	3 (1.99%)
Febrile neutropenia	–	–	4 (2.64%)	4 (2.64%)
Nonhematological				
Fatigue	19 (12.58%)	12 (7.95%)	8 (5.30%)	39 (25.83%)
Peripheral neuropathy	9 (5.96%)	9 (5.96%)	3 (1.99%)	21 (13.91%)
CINV	9 (5.96%)	5 (3.32%)	–	14 (9.27%)
Constipation	9 (5.96%)	1 (0.66%)	1 (0.66%)	11 (7.28%)
Diarrhea	3 (1.99%)	–	–	3 (1.99%)
Mucositis	1 (0.66%)	2 (1.32%)	3 (1.99%)	6 (3.97%)
Conjunctivitis	2 (1.32%)	–	–	2 (1.32%)
Arthralgia	12 (7.95%)	–	–	12 (7.95%)
Headache	3 (1.99%)	–	–	3 (1.99%)

One patient may have experienced more than one adverse event. The percentage is expressed on the total of the grand total of the events. CINV, chemotherapy-induced nausea and vomiting.

Cox proportional hazards model. At univariable analysis, an ECOG PS of 2 or 3 and a number of organ sites involved equal or superior to 3 were associated with a worse PFS_E ($p = 0.025$ and $p = 0.011$, respectively). In particular, median PFS resulted significantly different in patients with an ECOG PS of 0, 1, or 2–3 (6.03 vs. 4.3 vs. 1.47 months; $p = 0.05$; Fig. 2). Conversely, no significant differences were observed in PFS according to the hormone receptor status ($p = 0.082$), the expression of HER2 ($p = 0.363$), previous treatments, or NLR levels (Table 3A). Multivariate analysis confirmed that PFS was affected by having a worse ECOG PS and a greater number of metastatic involved sites ($p = 0.023$ and $p = 0.012$, respectively; Table 3B).

Discriminant Function Analysis

In the DFA, histology, age, NLR1, Ki67, estrogen receptor levels (ER), progesterone receptor levels (PgR), number of previous lines of chemotherapy for metastatic disease, taxane resistance, number of involved metastatic sites and ECOG PS were entered into the model as potential predictors. PgR, ECOG PS, taxane resistance, number of prior chemotherapeutic lines and number of involved organ sites were excluded by DFA from the model as not significant in discriminating between different outcomes.

Overall, the model run on 55 patients with no missing data. Model efficacy in predicting response to eribulin

was significant [Wilk's lambda = 0.633; $F(10, 84) = 2.154$, $p = 0.03$]. Partial Wilk's lambda (PWL) values revealed the respective contributions of included covariates to the prediction model and, in increasing order, they were age (PWL: 0.87), histology (PWL: 0.87), Ki67 (PWL: 0.91), ER (PWL: 0.93), and NLR1 (PWL: 0.94). Canonical analysis generated two roots (Root 1: eigenvalue = 0.38, canonical R = 0.52, Wilk's lambda = 0.63, $\chi^2 = 20.08$, $df = 10$, $p = 0.028$; Root 2: eigenvalue = 0.14, canonical R = 0.35, Wilk's lambda = 0.87, $\chi^2 = 5.79$, $df = 4$, $p = 0.21$). Mahalanobis distance correctly classified 33 cases out of 55 included in the model. However, the DFA model was able to discriminate the 100% of PR cases from SD and PD with the contribution of Root 1 (Fig. 3).

Post hoc Univariate Analysis

Among all the variables included in the model, only NLR1 and ECOG PS were different among outcome groups. The outcome groups (PR, SD, PD) differed for NLR1 value ($H(2, n = 65) = 13, 78$; $p = 0.001$). The NLR1 value was significantly different between PR and PD. The NLR1 value was significantly lower for PR patients in comparison with patients experiencing SD and PD (PR vs. SD, $p = 0.02$; PR vs. PD, $p = 0.006$). No difference was found between SD and PD patients ($p = 0.64$). See Table 4 for NLR values. Moreover NLR1 was also negatively correlated with PFS (correlation coefficient -0.3 , $p = 0.001$).

Table 3. Univariable and multivariable Cox analysis of progression-free survival under eribulin treatment

	HR	95% CI	<i>p</i> value		HR	95% CI	<i>p</i> value
A Univariable analysis				B Multivariable analysis			
Age	0.98	0.95–1.01	0.190	Pattern of disease			
Gender				Nonvisceral	1.00		
Female	1.00	–	–	Visceral	0.76	0.39–1.47	0.417
Male	0.63	0.15–2.63	0.534	ECOG PS			
Histology				0	1.00	–	–
Ductal	1.00	–	–	1	1.77	0.88–3.56	0.109
Lobular	0.84	0.36–1.98	0.694	2–3	2.36	1.15–4.98	0.025
Mixed	1.56	0.47–5.12	0.466	PFS prior to eribulin			
Hormone receptor expression				≥6 months	1.00		
Negative	1.00	–	–	<6 months	0.64	0.37–1.11	0.116
Positive	1.95	0.92–4.16	0.082	NLR before eribulin			
HER2 status				≤2.5	1.00		
Negative	1.00	–	–	2.5 <NLR ≥4	0.73	0.34–1.58	0.428
Positive	1.47	0.64–3.38	0.363	4 <NLR ≥5.5	0.48	0.19–1.22	0.125
Ki67				NLR >5.5	0.61	0.28–1.37	0.235
≤20%	1.00	–	–	B Multivariable analysis			
>20%	1.16	0.63–2.11	0.635	Number of metastatic sites			
Grading				1	1.00	–	–
G1-G2	1.00	–	–	2	1.23	0.68–2.23	0.498
G3	0.72	0.38–1.34	0.301	3–4	3.27	1.30–8.22	0.012
DFI				ECOG PS			
>2 years	1.00	–	–	0	1.00	–	–
≤2 years	0.80	0.48–1.33	0.396	1	1.63	0.81–3.30	0.174
Number of previous CT lines				2–3	2.38	1.12–5.04	0.023
2	1.00	–	–	Note that histological characteristics (histology, ER, PgR, HER2, Ki67, grading) refer to specimens obtained at the time of the first diagnosis of breast cancer. Mixed histology corresponded to patients with a diagnosis of both ductal and lobular carcinoma. HR, hazard ratio; CI, confidence interval; DFI, disease-free interval; CT, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group Performance Status; PFS, progression-free survival; NLR, neutrophil-to-lymphocyte ratio.			
3	1.07	0.55–2.09	0.833				
4	1.30	0.59–2.85	0.507				
≥5	0.58	0.27–1.24	0.160				
Taxane refractory							
No	1.00	–	–				
Yes	1.30	0.72–2.37	0.383				
Number of metastatic sites							
1	1.00	–	–				
2	1.23	0.68–2.23	0.489				
3–4	2.98	1.31–8.04	0.011				

Table 4. Neutrophil-to-lymphocyte ratio (NLR) values recorded before eribulin treatment (NLR1), at the best response (NLR2), and at progression of disease (NLR3)

Outcome groups	NLR1	NLR2	NLR3
PR	2.64 (1.27–4.26)	1.27 (0.46–4.13)	2.06 (1.19–6.57)
SD	4.17 (1.39–11.67)	3.31 (0.96–13.75)	3.44 (0.80–18.78)
PD	4.74 (2.14–44.60)	2.81 (2.81–2.81)	3.12 (0.25–32.0)

Values are expressed as median (range). PR, partial response; SD, stable disease; PD, progressive disease.

Table 5. Contingency table reporting Eastern Cooperative Oncology Group Performance Status (ECOG PS) and RECIST response at the post hoc univariate analysis

ECOG PS	PR	SD	PD	Total
0	2	10	4	16
1	9	11	15	35
2	0	6	9	15
3	1	0	4	5
Total	12	27	32	71

PR, partial response; SD, stable disease; PD, progressive disease.

As for ECOG PS, a worse PS was associated with a worse response ($p = 0.01$; Table 5).

Histology, age, Ki67, and ER were not significantly different among outcome groups. Histology groups were uniformly distributed among outcome groups according to the M-L χ^2 test ($p = 0.42$). For age, Kruskal-Wallis was equal to $H(2, n = 71) = 1.278$; $p = 0.53$. Ki67 had $H(2, n = 59) = 1.709$; $p = 0.42$. ER had $H(2, n = 67) = 0.211$; $p = 0.90$.

NLR during Eribulin Treatment

Since NLR had a predominant role in the DFA model in predicting response to treatment, we also analyzed the course of NLR throughout the therapy with eribulin. Kruskal-Wallis reported a global $H(2, n = 38) = 8.02$ ($p = 0.02$), and, after post hoc comparison, the NLR2 value differed significantly between PR and SD ($p = 0.01$). For complete values, see Table 4.

Growth Modulation Index

GMI1 median was 0.59 (IQR: 0.28–1.69), while GMI2 was 0.49 (IQR: 0.31–1.35). No significant difference was found between GMI1 and GMI2 ($p = 0.49$).

Discussion

Nowadays, overall response rate remains the main evaluable treatment endpoint in an everyday clinical setting for MBC. Nevertheless, treatment prioritization according to clinical characteristics and consequent different treatment strategies may also influence post-progression survival and OS. One can argue that chemotherapy can have a different impact on the outcome of a metastatic patient according to the sequences of administration.

Fig. 3. Scatterplot of 55 cases according to Root 1 and Root 2, the unique two roots produced by the model. The model found a single significant root (Root 1), based on canonical analysis, that was able to predict the PR outcome versus SD and PD. PR, partial response; SD, stable disease; PD, progressive disease.

Eribulin is a non-taxane microtubule dynamics inhibitor, but recent literature data have shown its complex nonmitotic effects on tumor biology [11–13]. Eribulin's mechanism of action includes tumor vasculature remodeling [14], reversal of epithelial-mesenchymal transition, and decreased capacity for migration and invasion [15]. To date, in vivo measurements of these effects are still challenging.

According to the working hypothesis that, after eribulin, residual tumors are phenotypically different and probably less aggressive, we studied the post-progression survival and compared it with PFS_E (GMI2) and the ratio between PFS_E and PFS prior to eribulin (GMI1) [16]. However, no significant difference was found between GMI1 and GMI2. On the other hand, our population was heavily pretreated, and almost all patients had received paclitaxel and capecitabine (data not shown), which may interact with eribulin's mechanism of action, albeit we have only preclinical data on this topic [14].

As a consequence of eribulin-induced epithelial-mesenchymal transition, eribulin could also influence cancer immunoediting [17, 18]. As a potential epiphenomenon of chemotherapy-induced immunomodulation, we also studied NLR which has been reported to be associated with prognosis and response to chemothera-

peutic agents in breast cancer in several studies [19–22]. In the present study, NLR was dynamically analyzed during eribulin treatment: at baseline, at best response and at progression. Baseline NLR had a predominant role in maximizing the separation between response groups in the DFA model. Of note, since no steroid premedication for eribulin was routinely provided before blood test at day 1 of each cycle, the relatively high median NLR in our population should have been very little influenced by concomitant medications. Moreover, NLR at best response significantly differed between patients reporting PR or SD. Therefore, beyond a predictive role of NLR at baseline, NLR is also influenced by the treatment and its immunoediting effect. To the best of our knowledge, this is the first report of a potential predictive role of NLR in patients with MBC receiving eribulin.

Several studies have evaluated the activity of eribulin according to molecular profiles of breast cancer. According to the subgroup analysis of the EMBRACE trial, eribulin demonstrated a significant increase in OS in all molecular subtypes, either HER2-positive or -negative tumors [6]. In another study, Rossi et al. [3] analyzed possible predictive criteria in order to select patients that might benefit the most from eribulin. A longer PFS was found in patients with hormone-positive tumors ($p = 0.0051$) and in HER2-negative cases ($p = 0.037$). Gamucci et al. [23], in their evaluation of eribulin activity in a real-world patient population, showed a significant improvement in terms of partial response and clinical benefit in patients with HER2-negative tumors ($p = 0.01$ and $p = 0.004$, respectively). On the other hand, the ERIBEX trial, a retrospective, international, multicenter study involving 258 patients receiving eribulin for a locally advanced or metastatic breast cancer, HER2 positivity seemed to be a predictive factor, with a significant increase in clinical benefit rate in HER2 positive tumors compared to those HER2 negative (HR 0.38, $p = 0.02$). This could be probably due to the systematic use of trastuzumab in association with eribulin, in the population of ERIBEX study [24]. In our Cox proportional hazards model, there was no significant difference in PFS according to hormone receptor expression and HER2 positivity, even if HR values for hormone-positive disease were near or superior to 1, thus suggesting potentially worse PFS curves in these patients.

The main result from our time-to-event analysis was the association of tumor burden and clinical conditions with PFS. Even if patients with an ECOG PS of 2 or 3 and with 3 or more metastatic organ sites involved were rela-

tively few in our population, these results strengthen the role of these clinical parameters as major clinical determinants in the real-world decision-making.

Beyond studying PFS, we analyzed RECIST response by DFA run using as covariates the parameters obtained by both the Cox regression analysis and literature data. The main finding was that DFA managed to distinguish PR patients from the others (SD, PD). In the model, NLR at baseline, ER, Ki67, histology, and age were more relevant factors in defining the discriminant function with NLR as the principal one. In the post hoc subanalysis of the role of NLR1, we found not only that NLR1 is a predictive factor of response to eribulin but also that patients experiencing PR significantly differed in terms of NLR1 with respect to patients reporting SD and PD. Furthermore, the NLR values of PR patients during eribulin treatment remained different from SD patients and, probably (even if the low sample size hampers a statistical conclusion), from patients reporting PD. This suggests that future studies with an adequate patient number could identify a cut-off of the NLR predictor of response and/or that the value of NLR could be studied during treatment to monitor the response as a tumor marker.

The safety profile of eribulin in our cohort was consistent with AEs previously reported [6, 25–27].

Some limitations of the present analysis are worth to be mentioned: the limited number of patients, the retrospective nature of the study and the enrollment of patients with missing data. There could also be an ascertainment bias, with the GMI influenced by frequency of tumor evaluation during different chemotherapy regimens. Furthermore, at a first glance, it is odd to obtain different results regarding predictors by DFA and Cox in the same population. However, the two analyses focused on different outcome measures (response and PFS, respectively). Moreover, the fact that DFA excluded from the model the two predictors that resulted from the Cox model can be explained in at least two ways. First, the DFA is a punctual and not a time-to-event analysis, and it is very sensitive to missing data. However, methodological studies have demonstrated that using mean substitution of missing data instead of case-wise approach (as we did) does not lead to better predictions [28]. Alternatively, predictors of response are not necessarily associated with the course of the disease, and we can observe a ceiling effect of response, that is, a clinical benefit may be achieved in a high-risk patient (according to our Cox model) but, despite the good response, the time to progression remains poor.

Conclusions

In this “real-world” experience, the activity and safety profiles of eribulin were both consistent with literature data. Considering promising OS curves but not statistically significant improvement in PFS data of eribulin, in the present study we focused on potential predictive factors of response and PFS. Apart from well-known clinical predictors of clinical outcome, as ECOG PS and tumor burden, our results also showed that immunological imbalance and hormonal status are the main factors in the predictive model by DFA, suggesting that they may be relevant for the progression of MBC under eribulin treatment, either solely or in combination. In this context, DFA seems to be an interesting statistical tool for identifying predictive factors in MBC patients.

References

- 1 AIOM (Italian Association of Medical Oncology) breast cancer guidelines. <http://www.aiom.it/>.
- 2 Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tata-lovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds): SEER Cancer Statistics Review, 1975–2014, National Cancer Institute. Bethesda. https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
- 3 Rossi S, Cassano A, Strippoli A, Schinzari G, D’Argento E, Basso M, Barone C: Prognostic and predictive factors of eribulin efficacy in heavily pretreated patients affected by metastatic breast cancer: correlation with tumor biology and previous therapies. *Drugs Context* 2017;6:212506.
- 4 Vahdat LT, Pruitt B, Fabian CJ, Rivera RR, Smith DA, Tan-Chiu E, Wright J, Tan AR, Dacosta NA, Chuang E, Smith J, O’Shaughnessy J, Shuster DE, Meneses NL, Chandrawansa K, Fang F, Cole PE, Ashworth S, Blum JL: Phase II study of eribulin mesylate, a halichondrin B analog, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. *J Clin Oncol* 2009;27:2954–2961.
- 5 Gourmelon C, Frenel JS, Campone M: Eribulin mesylate for the treatment of late-stage breast cancer. *Expert Opin Pharmacother* 2011;12:2883–2890.
- 6 Cortes J, O’Shaughnessy J, Loesch D, Blum JL, Vahdat LT, Petrakova K, Chollet P, Manikas A, Diéras V, Delozier T, Vladimirov V, Cardoso F, Koh H, Bougnoux P, Dutcus CE, Seegobin S, Mir D, Meneses N, Wanders J, Twelves C; EMBRACE (Eisai Metastatic Breast Cancer Study Assessing Physician’s

- Choice Versus E7389) investigators: Eribulin monotherapy versus treatment of physician’s choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. *Lancet* 2011;377:914–923.
- 7 Dowsett M, Nielsen TO, A’Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotirou C, Smith IE, Viale G, Zujewski JA, Hayes DF; International Ki-67 in Breast Cancer Working Group: Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *J Natl Cancer Inst* 2011;103:1656–1664.
 - 8 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–247.
 - 9 Lachenbruch PA. Discriminant diagnostics. *Biometrics* 1997;53:1284–1292.
 - 10 Hill T, Lewicki P: Statistics: Methods and Applications StatSoft, ed 1. Tulsa, StatSoft Inc, 2005.
 - 11 Jordan MA, Kamath K, Manna T, Okouneva T, Miller HP, Davis C, Littlefield BA, Wilson L: The primary antimitotic mechanism of action of the synthetic halichondrin E7389 is suppression of microtubule growth. *Mol Cancer Ther* 2005;4:1086–1095.
 - 12 Kuznetsov G, Towle MJ, Cheng H, Kawamura T, TenDyke K, Liu D, Kishi Y, Yu MJ, Littlefield BA: Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. *Cancer Res* 2004;64:5760–5766.

Acknowledgments

Medical writing was performed by Luca Giacomelli, PhD, Ambra Corti, and Aashni Shah on behalf of Content Ed Net; this assistance was supported by Eisai. The supporting company was not offered the opportunity to review the manuscript, and had no role in the decision to submit.

Disclosure Statement

The authors declare no conflict of interest.

- 18 Bracci L, Schiavoni G, Sistigu A, Belardelli F: Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ* 2014; 21:15–25.
- 19 Liu X, Qu J-K, Zhang J, Yan Y, Zhao XX, Wang JZ, Qu HY, Liu L, Wang JS, Duan XY: Prognostic role of pretreatment neutrophil to lymphocyte ratio in breast cancer patients: a meta-analysis. *Medicine (Baltimore)* 2017; 96:e8101.
- 20 Marín Hernández C, Piñero Madrona A, Gil Vázquez PJ, Galindo Fernández PJ, Ruiz Merino G, Alonso Romero JL, Parilla Paricio P: Usefulness of lymphocyte-to-monocyte, neutrophil-to-monocyte and neutrophil-to-lymphocyte ratios as prognostic markers in breast cancer patients treated with neoadjuvant chemotherapy. *Clin Transl Oncol* DOI: 10.1007/s12094-017-1732-0.
- 21 Ethier J-L, Desautels D, Templeton A, Shah PS, Amir E: Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. *Breast Cancer Res* 2017;19:2.
- 22 Chen Y, Chen K, Xiao X, Nie Y, Qu S, Gong C, Su F, Song E: Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: a retrospective study. *BMC Cancer* 2016;16:320.
- 23 Gamucci T, Michelotti A, Pizzuti L, Mentuccia L, Landucci E, Sperduti I, Di Lauro L, Fabi A, Tonini G, Sini V, Salesi N, Ferrarini I, Vaccaro A, Pavese I, Veltri E, Moscetti L, Marchetti P, Vici P: Eribulin mesylate in pretreated breast cancer patients: a multicenter retrospective observational study. *J Cancer* 2014;5: 320–327.
- 24 Dell'Ova M, De Maio E, Guiu S, Roca L, Dalenc F, Durigova A, Pinguet F, Bekhtari K, Jacot W, Pouderoux S: Tumour biology, metastatic sites and taxanes sensitivity as determinants of eribulin mesylate efficacy in breast cancer: results from the ERIBEX retrospective, international, multicenter study. *BMC Cancer* 2015;15:659.
- 25 Garrone O, Montemurro F, Saggia C, La Verde N, Vandone AM, Airolidi M, De Conciliis E, Donadio M, Lucio F, Polimeni MA, Oletti MV, Giacobino A, Merlano MC: Eribulin in pretreated metastatic breast cancer patients: results of the TROTTER trial-a multicenter retrospective study of eribulin in real life. *Springerplus* 2016;5:59.
- 26 Poletti P, Ghilardi V, Livraghi L, Milesi L, Rota Caremoli E, Tondini C: Eribulin mesylate in heavily pretreated metastatic breast cancer patients: current practice in an Italian community hospital. *Future Oncol* 2014;10: 233–239.
- 27 Ramaswami R, O'Cathail SM, Brindley JH, Silcocks P, Mahmoud S, Palmieri C: Activity of eribulin mesylate in heavily pretreated breast cancer granted access via the Cancer Drugs Fund. *Future Oncol* 2014;10:363–376.
- 28 De Sanctis R, Viganò A, Giuliani A, De Paoli A, Navarria P, Quagliuolo V, Santoro A, Colosimo A: Unsupervised versus supervised identification of prognostic factors in patients with localized retroperitoneal sarcoma (RPS): a data clustering and the Mahalanobis Distance approach. *Biomed Res Int*, in press.