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Exploratory analysis of circulating cytokines in patients with metastatic breast cancer treated with eribulin: the TRANSERI-GONO (Gruppo Oncologico del Nord Ovest) study

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ABSTRACT

Background Anticancer drugs can interact with the tumour microenvironment and their effects could be exploited to favour anticancer immune response. Eribulin contributes to tumour vasculature remodelling and transforming growth factor β (TGF-β) modulation in experimental models and in humans. We performed a prospective, translational, exploratory analysis of the levels of circulating cytokines at different time points in patients with metastatic breast cancer treated with eribulin.

Methods TGF-β, tumour necrosis factor α, vascular endothelial growth factor, IL-6, IL-8, IL-10, IL-21 and C-C motif chemokine ligand-2 levels were assessed in peripheral blood samples obtained from seven healthy volunteers and 41 patients at baseline (T_0), after four cycles of eribulin (T_1) and at disease progression (T_{PD}). Baseline values and longitudinal changes in cytokine levels were then related to clinical outcome.

Results In the 41 patients, high IL-6 and IL-8 (above the median) at T_0 significantly correlated with worse survival. At T_1, IL-21 significantly decreased in patients with T_{PD} within the fourth course of treatment, compared with patients without progression. TGF-β and IL-8 above the median and IL-21 below the median at T_1 significantly correlates with worse progression free survival (PFS). Patients exhibiting an increase of TGF-β or a decline of IL-21 between T_0 and T_1 showed a significantly worse PFS. Multivariate Cox regression analysis showed that only plasma TGF-β changes at T_1 correlated with survival. At $T_{PD,}$ TGF-β significantly increased in all patients.

Conclusions We observed a significant correlation between TGF-β decline during eribulin treatment and outcome in patients with metastatic breast cancer. Altogether, our data suggest that eribulin treatment might interfere with the tumour microenvironment.

Key questions

What is already known about this subject?

- Eribulin is a non-taxane inhibitor of microtubule dynamics distinct from other tubulin-targeting drugs such as vinca alkaloid and taxanes.
- Many preclinical in vitro and in vivo data demonstrated off target effects of eribulin including vascular remodelling, increased tumour oxygen saturation and suppression of transforming growth factor β (TGF- β).
- There is only one experience in patients with locally advanced breast cancer treated with eribulin demonstrating a significant reduction of TGF- β after eribulin exposure.

What does this study add?

- The study adds informations about the effect of treatment in a series of cytokines in patients with breast cancer compared with healthy volunteers and their changes over time evaluating the association of each variable with each other variable, best response and progression free survival/overall survival in both univariate and multivariate models.

How might this impact on clinical practice?

- Knowing drugs' off target effects might shed light on the development of new combinations with immunotherapy.

BACKGROUND

Many anticancer agents interact with the tumour microenvironment (TME) and their effects might be exploited to favour anticancer immune response.[1] Therefore, there is an increasing interest in combining conventional chemotherapy with immune therapy.[2 3] However, to design a rational combination, it is necessary to understand which effects can be achieved by each anticancer agent.[4] Eribulin is among the newest drugs used in metastatic breast cancer (mBC). Eribulin is a non-taxane inhibitor

Table 1 Demographic and baseline characteristics

Characteristics	Number (% or range)
Age (median, range)	62 (37–86)
ECOG PS (median, range)	0 (0–2)
De novo disease	14 (34.1%)
ER status:	
Positive	34 (82.9%)
Negative	7 (17.1%)
PgR status:	
Positive	28 (68.3%)
Negative	13 (31.7%)
Triple negative	6 (14.6%)
HER2 status:	
Positive	3 (7.3%)
Negative	38 (92.7%)
Neo/adjuvant chemotherapy*	20 (74.1%)
Adjuvant endocrine therapy*	22 (81.5%)
Number of previous CT lines for advanced disease:	
1	10 (24.4%)
2	20 (48.8%)
3	5 (12.2%)
≥4	6 (14.6%)
Median, range	2 (1–6)
Number of previous ET lines for advanced disease:	
1	6 (14.6%)
2	15 (36.6%)
≥3	11 (26.8%)
Median, range	2 (0–4)
Number of organs involved:	
1	3 (7.3%)
2	14 (34.1%)
≥3	24 (58.5%)
Median, range	3 (1–6)
Most common metastatic sites:	
Bone	31 (75.6%)
Liver	29 (70.7%)
Soft tissues	27 (65.8%)
Lung	17 (41.5%)
Pleura	7 (17.1%)
CNS	5 (12.2%)
Peritoneum	4 (9.7%)

*Numbers and percentages are based on 27 patients (14 were metastatic de novo).
CNS, central nervous system; CT, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status; ER, oestrogen receptor; ET, endocrine therapy; HER2, Human Epidermal Growth Factor Receptor 2; PgR, progesterone receptor.;

of the mitotic spindle, distinct from other agents with similar target.[5 6] Some non-mitotic effects of the drug on tumour biology have been described recently. Among them, eribulin showed the ability to downregulate transforming growth factor β (TGF-β) in triple negative breast cancer cell lines and in MX-1 tumour xenografts.[7] Moreover, it was shown that eribulin interferes with the tumour vasculature similarly to other inhibitors of the mitotic spindle[8] and it inhibits the epithelial-mesenchymal transition.[9] However, most of these effects have been demonstrated only in vitro or using in vivo models, and their impact on the outcome of patients with mBC is at present unknown. For these reasons, we designed a translational study to assess whether levels of circulating cytokines in patients with mBC change during treatment with eribulin.

We considered eight different cytokines: TGF-β, tumour necrosis factor α (TNF-α), vascular endothelial growth factor (VEGF), IL-6, IL-8, IL-10, IL-21 and C-C motif chemokine ligand 2 (CCL-2).

Among them, TGF-β, VEGF and IL-10 are considered mediators of immune suppression, while TNF-α, IL-6 and CCL-2 are associated with the inflammatory response. IL-8 is also an inflammatory cytokine, but additionally exerts many protumour effects, including induction of neoangiogenesis, neutrophil recruitment and promotion of infiltration, invasion and survival of tumour cells.[10] Similar to IL-8, IL-21 is a 'double-edged sword' cytokine depending on the context.[11] Among favourable biological actions of IL-21 of particular interest are the enhancement of $CD8^+$ and NK (Natural Killer) cell cytotoxicity, M1 polarisation of tumour-associated macrophages (TAM) and the induction of B cell apoptosis.

Finally, we performed an exploratory analysis to describe the correlation between these changes and clinical outcome, aiming at providing a rational basis for the combination of eribulin with immunotherapy.

METHODS

The TRANSERI trial is a multicentre translational study carried out at three Italian institutions. Peripheral blood samples were obtained from patients treated with eribulin (Halaven Eisai) in clinical practice, according to the indication approved by the European Medical Agency.

Study design

Aims of the study. The primary aim was the evaluation of the dynamic changes of plasma TGF-β, TNF-α, VEGF, IL-6, IL-8, IL-10, IL-21 and CCL-2 levels during treatment with eribulin in patients with mBC. The secondary objective was an explorative analysis of the association between the observed cytokines changes, if any, and clinical outcome in terms of clinical benefit (CB), progression free survival (PFS) and overall survival (OS). CB was evaluated by RECIST criteria[12] and defined as the occurrence of complete response, partial response or long-lasting (≥24 weeks) disease stabilisation. PFS was defined as the time elapsed between the first dose of eribulin and progressive disease or death from any cause,

Table 2 Cytokine concentrations (pg/mL) in patients and healthy controls

Cytokine	Median	Range	P value
Patients (n=41)			
T_0 (n=41)			**T_0 vs T1**
TGF-β	204.8	86.5–945.9	Ns
TNF-α	0.4	0.0–737.7	Ns
VEGF	495.2	172.4–1503	Ns
IL-6	4.9	0.8–212.4	Ns
IL-8	16.8	0.6–542.4	Ns
IL-10	2.8	0.4–28.7	Ns
IL-21	32.5	0.0–973.5	Ns
CCL-2	231.4	56.1–995.2	Ns
T_1 (n=41)			**T_1 vs control**
TGF-β	233.9	54.1–1255.4	Ns
TNF-α	2.6	0.0–879.7	Ns
VEGF	523.7	220.6–1248	Ns
IL-6	6.7	1.1–216.1	0.0004*
IL-8	16.5	4.2–240.6	<0.0001*
IL-10	3.5	0.2–49.3	0.0166*
IL-21	22	0.0–742.7	Ns
CCL-2	225.5	74,1–878,1	<0.0001*
T_{PD} (n=35)			**T_{PD} vs T_0**
TGF-β	293.4	99.5–1255.4	0.009***
TNF-α	0.0	0.0–528.5	Ns
VEGF	597.5	220.6–1133.0	Ns
IL-6	7.7	1.6–216.1	Ns
IL-8	16.6	4.2–240.6	Ns
IL-10	2.9	0.7–49.3	Ns
IL-21	0.0	0.0–1000.0	Ns
CCL-2	263.3	74.1–878.1	Ns
Controls (n=7)			**Controls vs T_0**
TGF-β	112.9	83.4–162.4	0.002**
TNF-α	0.0	0–120.5	Ns
VEGF	367.7	311.6–890.8	Ns
IL-6	1.6	0.9–2.28	0.035**
IL-8	3.6	2.24–5	0.0004**
IL-10	2.0	1.52–2.97	Ns
IL-21	0.0	0–143.5	0.03**
CCL-2	32.9	18.49–48.64	<0.0001**

CCL-2, C-C motif chemokine ligand-2; IL, interleukin; Ns, not statistically significant; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α; VEGF, vascular endothelial growth factor.

whichever occurred first, or at the date of last follow-up for censored patients. OS was defined as the time elapsed between the first dose of eribulin and death from any cause or the date of the time of the last follow-up for censored patients.

Patients and methods. The analysis was conducted on patients with mBC who were candidate to receive eribulin as treatment for advanced disease. Peripheral blood (plasma) samples were obtained from all patients at baseline (T_0), after four cycles of eribulin (d1 cycle 5 before treatment, T_1) and at disease progression (T_{PD}) whenever it occurred. We also collected blood samples in seven healthy volunteers and the results were used as comparator.

Plasma collection. Twelve millilitres of peripheral blood were collected in EDTA-treated Vacutainer (BD, Franklin Lakes, New Jersey, USA). Plasma samples were obtained by centrifugation for 10 min at 340 × g at room temperature (RT) and immediately stored at –80°C.

Cytokine measurement. All cytokines were quantified by an ELISA according to the manufacturer's instructions. Kits were used to measure TNF-α and TGF-β (Enzo Life Sciences, Farmingdale, New York, USA), VEGF-A (Cloud-Clone company, Katy, Texas, USA) and IL-21 (R&D System Minneapolis, Minnesota, USA). After incubations, the reactions were stopped and colorimetric detection was carried out with a spectrophotometer (Multiskan Ascent, Thermo Fisher Scientific, Massachusetts, USA) set at 450 nm with corrections at 570 nm. The measured optical densities were expressed as pg/mL. Concentrations of IL-6, IL-8, IL-10 and CCL-2 were determined in plasma samples using the Ella Simple Plex system (ProteinSimple, San Jose, California, USA). Briefly, a twofold dilution of each plasma sample was spun for 15 min at 1000 × g and added to the Simple Plex cartridge. The cartridge was then inserted into the reactor and run for 90 min at RT. The concentrations were expressed as pg/mL. All samples were analysed centrally at the Translational Research Laboratory ARCO Foundation and assayed in duplicate. The average of each duplicate was used at each point.

Statistical analyses. Due to the exploratory nature of this translational study, no *a priori* sample size calculation and statistical power were performed. For each cytokine, the values measured at any time point were compared with each other and the values of healthy subjects using the Mann-Whitney U test in GraphPad PRISM V.5. In addition, we evaluated the association between the longitudinal changes between T_0 and T_1 of each cytokine and treatment activity in two exploratory analyses (PFS and OS). PFS and OS were compared between patients whose cytokine values at T_1 were higher or lower than T_0. The differences were estimated using the Kaplan-Meyer method and the two groups were compared by the log-rank test using the SPSS V.24.0 software (IBM Corporation, Armonk, New York, USA). HRs of PFS and OS were calculated using the Cox proportional-hazards model in the R software (V.3.5.3 'Great Truth'). A p value lower or equal to 0.05 was considered as significant in all statistical analyses. No correction for multiplicity test was applied.

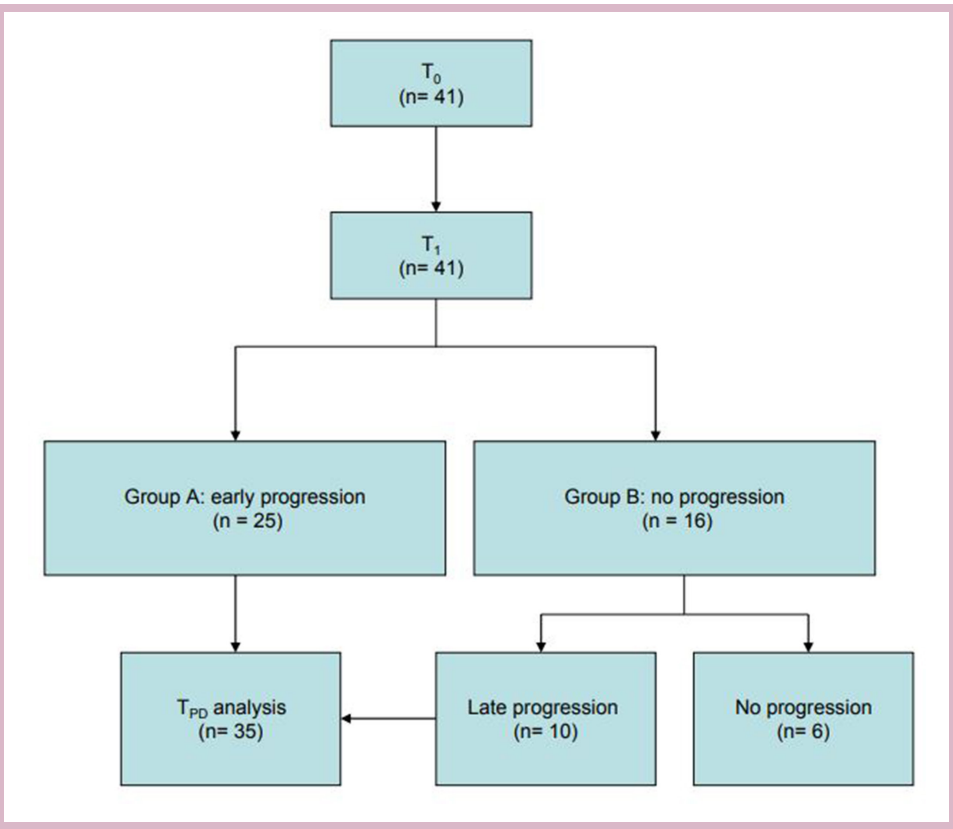

Figure 1 CONSORT diagram.

RESULTS

From April 2016 until August 2018, we collected plasma from 41 patients with mBC treated with eribulin at different time points.

Population and treatment results

Patient characteristics are reported in table 1.

The majority of patients had visceral involvement and three or more metastatic sites. Eribulin was given as third or further line of chemotherapy. Objective response, all partial, was recorded in 10 patients (24.4%). Six patients (14.6%) showed disease stabilisation. Consequently, the CB rate was 39%. At the time of the present analysis (October 2019), two patients were still on therapy. Neither the number of metastatic sites (one to two sites vs three or more) nor previous treatments (one or two vs three or more) significantly correlated with the outcome in this series of patients (data not shown).

Cytokine levels in patients at different time points and in healthy volunteers

Changes in plasma cytokine levels during treatment and their value in seven healthy subjects are reported in table 2.

TNF-α, VEGF and IL-10 were similar between healthy volunteers and patients at T_0, while the remaining cytokines were significantly higher in patients. None of the cytokines significantly differed between T_0 and T_1. Plasma TGF-β levels significantly increased at T_{PD} compared with T_0 (p=0.009).

In order to verify whether the outcome may correlate with modifications of the considered cytokines, we then divided patients at T_1 in two groups: group A, which included patients with progressive disease within the fourth course of eribulin and group B, which included patients without progression and with later progression (>4 cycles) (figure 1). The Cox analysis for PFS and OS between groups A and B underlined a significant risk reduction favouring group B (HR=0.09, 95% CI 0.04 to 0.23; HR=0.46, 95% CI 0.23 to 0.95 for PFS and OS, respectively). Accordingly, the Kaplan-Meyer analysis showed a median PFS of 2.8 and 5.9 months (p=0.000) and a median OS of 9.1 and 17.1 months (p=0.03) in groups A and B, respectively. Only plasma TGF-β and IL-21 values at T_1 were significantly different between groups A and B: plasma TGF-β levels were lower in group B in comparison with A (p<0.001), while IL-21 levels were higher in B compared with A (p<0.05) (figure 2A,B). No significant difference between groups A and B was observed among the remaining cytokines (figure 2). We also compared PFS and OS between patients with values above or below the median of each cytokine at T_1. Patients with TGF-β and IL-8 levels below the median and IL-21 levels above the median showed a statistically significant benefit in PFS (p=0.02, 0.008 and 0.008, respectively) (figure 3A–C). However, considering OS, only plasma IL-8 levels below the median resulted in significantly better survival (p<0.001) (figure 3B), although a similar difference, although not significant, was observed for TGF-β levels below the median (p=0.167) (figure 3A). No significant difference was observed among the remaining cytokines.

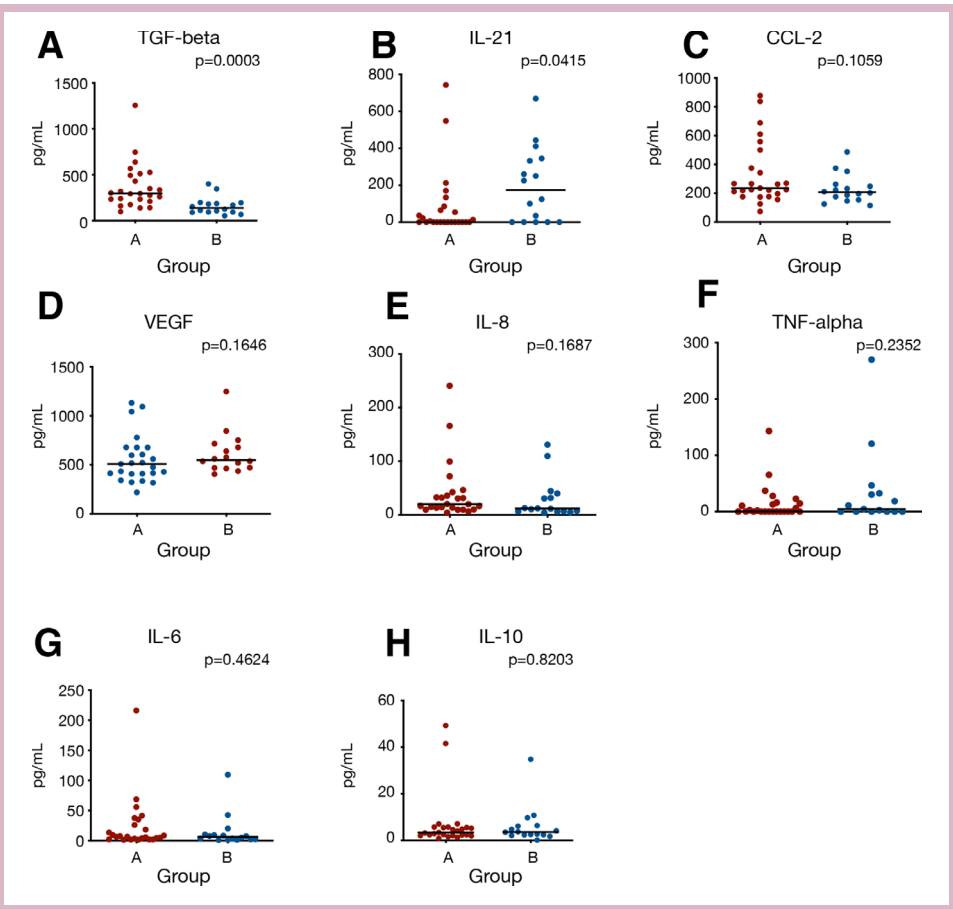

Figure 2 Changes of the plasma levels of the eight cytokines studied in two groups of patients. Group A: patients with disease progression within the fourth course of eribulin. Group B: patients with disease progression after 4 courses of eribulin. CCL-2,C-C motif chemokine ligand-2; IL, interleukin; TGF,transforming growth factor; TNF, tumour necrosis factor; VEGF, vascularendothelial growth factor.

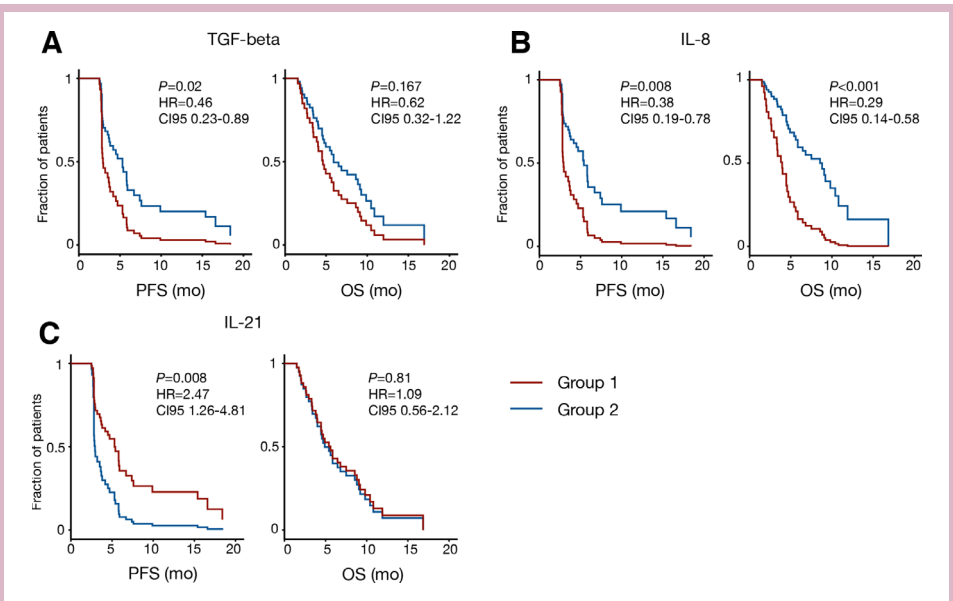

Figure 3 Cox regression model for TGF-β, IL-8 and IL-21 at T_1 divided in two groups. Group 1: patients with cytokine levels above the median. Group 2: patients with cytokine levels below or equal to the median. IL,interleukin; OS, overall survival; PFS, progression free survival; TGF-β,transforming growth factor β.

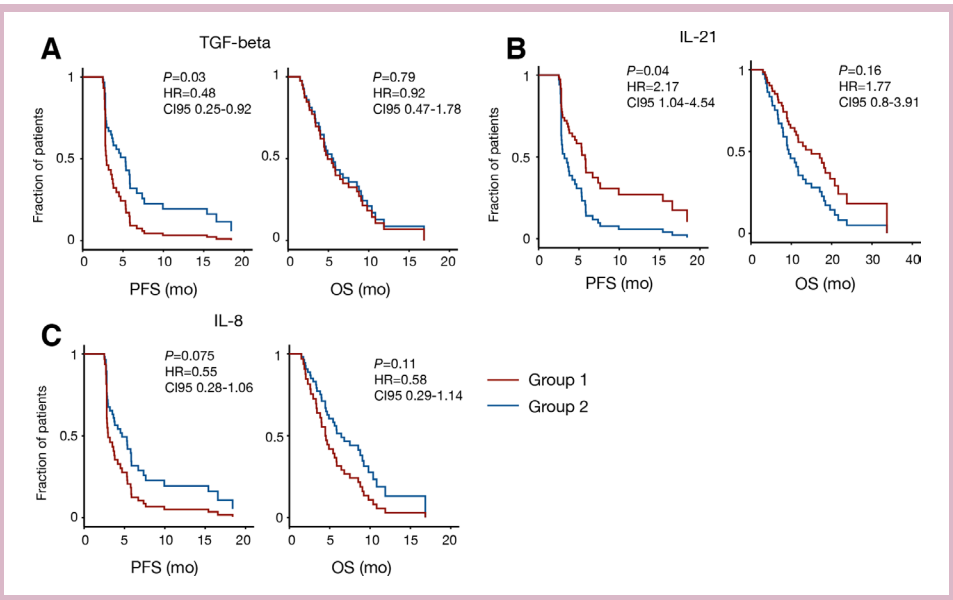

Figure 4 Cox regression model for TGF-β, IL-21 and IL-8 comparing T_0 with T_1. Group 1: patients with $T_1 > T_0$. Group 2: patients with $T_1 \leq T_0$. IL,interleukin; OS, overall survival. PFS, progression free survival;TGF, transforming growth factor.

Longitudinal analyses of cytokine values at different time points

The longitudinal analysis of changes in plasma cytokine levels between T_0 and T_1 allowed us to identify two groups of patients: those in which the value of certain cytokines at T_1 was higher than the value of the same cytokine at T_0 (group 1) and patients in which the value at T_1 was lower than T_0 (group 2), regardless of the value at T_0 in each patient. Noteworthy, a significant difference in PFS between group 1 and 2 was evidenced when considering plasma TGF-β and IL-21 levels (p=0.03 and 0.04, respectively) (figure 4A,B). In addition, we also observed a similar difference for IL-8, although it was not statically significant (p=0.075) (figure 4C). OS was similar between groups 1 and 2 for all the three cytokines (figure 4A–C). We did not find any statistically significant difference among the remaining cytokines. Interestingly, considering the previous defined groups A and B, we observed a significant reduction of median TGF-β plasma level between T_0 and T_1 in group B (from 213.7 to 139.4, p=0.03). On the contrary, median TGF-β plasma level significantly increased between T_0 and T_1 in group A (from 192.6 to 297.3, p=0.04). No other significant difference between T_0 and T_1 was recorded in groups A and B for the remaining cytokines.

Correlation between the cytokine values at T_0 or T_1 and outcome

Finally, we investigated whether the baseline value of the cytokines could have a prognostic role. Considering the values above or below the median recorded at T_0, IL-21 above the median significantly correlated only with better PFS in a Cox univariate analysis (HR=0.3; 95% CI 0.1 to 0.6). Plasma values below the median of IL-6 and IL-8 significantly correlated with better OS (p=0.004; HR=0.34; 95% CI 0.2 to 0.7 and p=0.03; HR 0.35; 95% CI 0.2 to 0.7, respectively) (table 3).

Focusing on survival, a Cox multivariate analysis to correct for confounding interactions among the cytokines revealed that only plasma TGF-β levels above the median at T_1 correlated with shorter OS (p=0.001) (table 4).

DISCUSSION

In this exploratory study, we have quantified the plasma levels of eight different cytokines (TGF-β, TNF-α, VEGF, IL-6, IL-8, IL-10, IL-21 and CCL-2) in 41 patients with mBC treated with eribulin.

Considering the whole population, eribulin treatment did not affect the concentration at T_1 compared with T_0, of any of the cytokines examined in this study, but plasma TGF-β levels significantly increased at T_{PD}. Although in the initial phase of cancer development, TGF-β represents an important antitumour cytokine, it also exerts several protumour activities during cancer progression. Among them, TGF-β reduces dendritic cell maturation and antigen presentation, $CD8^+$ cell proliferation and effector function; favours the conversion of Th1 into T_{reg} cells; reduces cytotoxicity of NK cells and induces TAM M2 polarisation. Therefore, the TGF-β increase observed at T_{PD} may represent a general worsening of the TME, and, if so, plasma TGF-β levels might represent a biomarker of the TME status. In line with this hypothesis, we observed that plasma TGF-β levels below the median at T_1 were associated with a significant benefit in PFS.

Moreover, to further support the hypothesis, a significant decrease of median TGF-β plasma level was observed in group B at T_1 compared with T_0 while group A showed opposite behaviour.

With the limited statistical power associated with the small number of patients included in the study, our longitudinal analysis of cytokine levels changes, between T_0 and T_1, revealed that only TGF-β and IL-21 were significantly associated with PFS. In addition, we were not able

Table 3 OS univariate Cox model

Variable	Group	n	HR	95 CI	P value
# Sites	>2	24	1.00	0.42 to 1.65	0.61
	≤2	17	0.84		
Sites	Visceral	35	1.00	0.34 to 2.26	0.78
	Bone/soft tissue	6	0.87		
# Lines	>2	31	1.00	0.66 to 2.94	0.39
	2	10	1.39		
TGF-β (T_0)	>Median	20	1.00	0.38 to 1.45	0.38
	≤Median	21	0.74		
TGF-β (T_1)	>Median	20	1.00	0.32 to 1.22	0.17
	≤Median	21	0.62		
TNF-α (T_0)	>Median	20	1.00	0.58 to 2.18	0.75
	≤Median	21	1.12		
TNF-α (T_1)	>Median	20	1.00	0.43 to 1.61	0.58
	≤Median	21	0.83		
VEGF (T_0)	>Median	20	1.00	0.67 to 2.70	0.40
	≤Median	21	1.35		
VEGF (T_1)	>Median	20	1.00	0.64 to 2.42	0.53
	≤Median	21	1.24		
IL-6 (T_0)	>Median	20	1.00	0.16 to 0.70	0.004
	≤Median	21	0.34		
IL-6 (T_1)	>Median	20	1.00	0.20 to 0.78	0.007
	≤Median	21	0.39		
IL-8 (T_0)	>Median	20	1.00	0.17 to 0.70	0.03
	≤Median	21	0.35		
IL-8 (T_1)	>Median	20	1.00	0.14 to 0.58	<0.001
	≤Median	21	0.29		
IL-10 (T_0)	>Median	20	1.00	0.28 to 1.07	0.08
	≤Median	21	0.55		
IL-10 (T_1)	>Median	20	1.00	0.30 to 1.16	0.13
	≤Median	21	0.59		
IL-21 (T_0)	>Median	20	1.00	0.59 to 2.29	0.66
	≤Median	21	1.17		
IL-21 (T_1)	>Median	20	1.00	0.55 to 2.12	0.81
	≤Median	21	1.09		
CCL-2 (T_0)	>Median	20	1.00	0.36 to 1.36	0.29
	≤Median	21	0.70		
CCL-2 (T_1)	>Median	20	1.00	0.31 to 1.55	0.37
	≤Median	21	0.70		

CCL-2, C-C motif chemokine ligand-2; IL, interleukin; OS, overall survival; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α; VEGF, vascular endothelial growth factor.

to demonstrate any significant association of OS with longitudinal changes of the cytokines. Considering that the majority of patients received further therapies, which may affect survival after eribulin, our results suggest that TGF-β and IL-21 might be influenced by treatment. In agreement with our findings, it was demonstrated that in patients with locally advanced breast cancer, a single dose of eribulin is able to significantly reduce TGF-β levels in the plasma.[13] Interestingly, we found that an increase of plasma IL-21 levels between T_0 and T_1 had a positive association on PFS, while the opposite effect was observed for TGF-β. The latter effect is intuitive, given the immunosuppressive role of TGF-β in tumours. In contrast, IL-21 drives both protumour and antitumour effects depending on the context. Therefore, our findings suggest that the ability of the 'context' to switch the role of IL-21 from positive to negative and vice versa might be driven by TGF-β. This observation supports the hypothesis that, in

Table 4 OS multivariate Cox model

Variable	Size	SE	HR	95 CI	P-value
TGF-β (T_1)	>Median	0.48	1.00	0.08 to 0.53	0.001
	≤Median		0.21		
IL-6 (T_0)	>Median	0.52	1.00	0.14 to 1.07	0.07
	≤Median		0.39		
IL-6 (T_1)	>Median	0.51	1.00	0.15 to 1.12	0.08
	≤Median		0.41		
IL-8 (T_0)	>Median	0.51	1.00	0.32 to 2.32	0.76
	≤Median		0.51		
IL-8 (T_1)	>Median	0.47	1.00	0.17 to 1.09	0.08
	≤Median		0.47		
IL-10 (T_0)	>Median	0.64	1.00	0.16 to 1.93	0.35
	≤Median		0.55		
IL-10 (T_1)	>Median	0.54	1.00	0.50 to 4.13	0.50
	≤Median		1.44		
CCL-2 (T_1)	>Median	0.39	1.00	0.33 to 1.52	0.38
	≤Median		0.39		

CCL-2, C-C motif chemokine ligand-2; OS, overall survival; TGF, transforming growth factor.

patients showing a benefit from treatment with eribulin, the TME may be polarised towards a less immunosuppressive status and TGF-β could represent a major driver of TME.

Our analysis of cytokine levels at T_0 showed that IL-6 and IL-8 levels below the median were associated with better OS, while only IL-21 above the median correlated with better PFS. In contrast, TGF-β and IL-8 levels below the median and plasma IL-21 level above the median at T_1 were associated with longer PFS. Plasma IL-6 and IL-8 levels below the median at T_1 were associated with longer OS.

Overall, these data suggest that both IL-6 and IL-8 might have a prognostic role.

This hypothesis is supported by previous studies. Dethlefsen *et al*[14] observed that upregulation of IL-6 correlates with low survival in patients with breast cancer. Sanmamed *et al*[15] suggested that IL-8 level is directly related to tumour burden in patients with non-small cell lung cancer, melanoma, renal cell carcinoma and hepatocellular carcinoma and is associated with poor survival. More recently, a negative prognostic role of IL-8 was also suggested for patients with breast cancer.[16]

Finally, we performed a Cox multivariate analysis focusing on survival, considering all the eight cytokine profiled in this study. The limitation of this analysis is the large number of variables considered with respect to the limited number of events. However, we decided to select all the variables under the threshold of p=0.2 from the univariate analysis, as previously suggested.[17 18] After correcting for confounding variables, only TGF-β levels correlated with OS. This result supports the hypothesis of a central role of TGF-β in our series of patients.

Our data suggest that the benefit induced by eribulin is associated, in responding patients, with TGF-β reduction. This observation might support the hypothesis of combining eribulin and immunotherapy in patients showing a reduction of TGF-β after four courses of therapy.

A recent study combining eribulin with the immune checkpoint inhibitor pembrolizumab showed no benefit for the combination.[19] However, the authors of this study did not select patients for the treatment, thus the negative result could be explained by a dilution effect induced by eribulin non-responder patients.

We are aware that our study includes a small number of patients, but it is due to its exploratory nature. In addition, a limitation is that we cannot distinguish whether the effects observed are due to eribulin itself or due to the response/non-response to treatment regardless of the drug used. However, published data showed that eribulin induces modulation of TGF-β in humans[20] and in experimental models,[21] supporting the hypothesis that the observed effects might be eribulin related.

CONCLUSIONS

The combination of conventional chemotherapy with immune therapy represents a promising field of investigation. Among the multiple chemotherapies in clinical practice, eribulin is an interesting drug due to some supposed mechanisms of action interfering with immune response. Our findings suggest that eribulin could affect the TME and might modulate TGF-β in patients achieving a CB. However, due to the exploratory nature of our study, we are aware of the limits of our results that should be regarded primarily as hypothesis generating. An ongoing study is investigating the effects of other drugs on the same cytokines in mBC.

The purpose is to clarify whether the observed effects may be attributed to eribulin or if they represent a more generic effect, related to treatment response.

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