EUROPEAN JOURNAL OF CANCER 46 (2010) 2027–2035

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Circulating endothelial cells, circulating tumour cells, tissue factor, endothelin-1 and overall survival in prostate cancer patients treated with docetaxel

M.H. Strijbos [a], J.W. Gratama [a], P.I.M. Schmitz [b], C. Rao [c], W. Onstenk [a], G.V. Doyle [c], M.C. Miller [c], R. de Wit [a], L.W.M.M. Terstappen [d], S. Sleijfer [a,*]

[a] Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands
[b] Department of Trials and Statistics, Erasmus Medical Center, Rotterdam, The Netherlands
[c] Veridex LLC, Raritan, NJ, USA
[d] Department of Medical Cell BioPhysics, University of Twente, Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 25 January 2010
Received in revised form 22 March 2010
Accepted 23 March 2010

Keywords:
Prostate cancer
Circulating tumour cells
Circulating endothelial cells
Soluble angiogenic factors
Prognosis

ABSTRACT

Purpose: We investigated whether serum markers of angiogenesis endothelin-1 (ET-1) and tissue factor (TF), and/or markers of vascular damage such as circulating endothelial cells (CECs), or their relative changes during treatment, were prognostic for overall survival (OS) in castration resistant prostate cancer (CRPC) patients. Additionally, we combined these markers with circulating tumour cells (CTCs) to construct a predictive nomogram for treatment outcome.

Patients and methods: One hundred and sixty two CRPC patients treated with a docetaxel containing regimen had blood drawn before and at 2–5 weeks and 6–8 weeks after treatment start. Prospectively determined CTC and CEC levels, and retrospectively measured serum concentrations of ET-1 (pg/mL) and TF (pg/mL) were evaluated to determine their prognostic value for OS.

Results: Baseline CEC, TF and ET-1 were not prognostic for OS. A $\geqslant$3.8-fold increase in CEC 2–5 weeks after treatment initiation was associated with decreased OS (median 10.9 versus 16.8 months; $P = 0.015$), as was any decrease in TF levels compared to baseline levels (median 11.9 versus 21.5 months; $P = 0.0005$). As previously published, baseline and CTC counts $\geqslant$5 at 2–5 weeks were also predictive of decreased OS. Combining CTC with changes in TF and CEC 2–5 weeks after treatment initiation yielded four groups differing in OS (median OS 24.2 versus 16.0 versus 11.4 versus 6.1 months; $P < 0.0001$).

Conclusion: CEC, CTC and TF levels alone and combined can predict early on OS in CRPC patients treated with docetaxel-based therapy. A prospective study to confirm the use of these markers for patient management is needed.

1. Introduction

Prostate cancer is the second leading cause of cancer deaths in males.[1] Today, after failure of local treatment consisting of surgery and/or radiation therapy, androgen deprivation is the therapy of choice, but resistance to hormonal therapies eventually occurs. For these castration resistant prostate cancer (CRPC) patients, standard chemotherapy consists of

* *Corresponding author*: Address: Department of Medical Oncology, Erasmus MC – Daniel den Hoed Cancer Center, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands. Tel.: +31 10 704 17 33; fax: +31 10 704 10 03.
E-mail address: s.sleijfer@erasmusmc.nl (S. Sleijfer).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.03.030

docetaxel plus prednisone resulting in a median overall survival (OS) of 18 months.[2] However given the palliative nature of this treatment and its side-effects, ineffective treatment should be avoided, which underscores the need for markers enabling the clinician to discriminate patients with respect to outcome to therapy.

Historically, prostate specific antigen (PSA) has been used to monitor treatment. More recently, both tumour- and vascular-derived markers have been investigated for this purpose. For example, it was recently shown that the presence of circulating tumour cells (CTCs) prior to and during therapy is associated with OS in patients with metastatic breast, colorectal and also prostate cancer.[3–5] Moreover, in CRPC, CTC counts outperformed serum concentrations of PSA as predictor of OS.[6]

Several studies suggest that markers of angiogenesis could also be of prognostic value in prostate cancer. For example, quantifying angiogenesis by histological assessment of microvessel density appears to be independently associated with survival in prostate cancer.[7,8] But this approach is time consuming, and is therefore less practical in clinical use. Alternative indicators of angiogenesis such as soluble serum proteins endothelin 1 (ET-1) and tissue factor (TF), may provide this information as well, but have not yet been examined in depth in prostate cancer.

ET-1 acts synergistically with vascular endothelial growth factor (VEGF) and is secreted primarily by vascular endothelial cells.[9,10] Following binding to the endothelin A receptor, it induces endothelial cell proliferation, invasion and tubule production. ET-1 levels are associated with microvessel density in several tumour types including breast, colorectal and ovarian cancer.[11–14] TF is a transmembrane glycoprotein derived from platelets, endothelial cells and leukocytes, which is involved in coagulation and tumour neovascularisation.[15] After splicing, it can be measured in the peripheral blood. Both ET-1 and TF receptor antagonists are currently being investigated as anti-angiogenic treatments.[11,12,16,17]

Another promising biomarker with potential prognostic value in cancer patients is the number of circulating endothelial cells (CECs).[18] CECs are mature endothelial cells that have detached from the vessel wall and are considered a marker of vascular damage. The latter is supported by the correlation of CEC numbers with plasma concentrations of thrombomodulin, a putative marker of endothelial injury.[19] CECs are increased in solid cancer patients compared to healthy controls. Furthermore, several studies have suggested that tumour progression is accompanied by an increase in CEC[18,20,21] thereby rendering CEC an attractive marker to be further explored in cancer. However, until now, studies have only been performed in study populations comprising patients with a wide variety of tumour types, and not in a specific tumour type such as prostate cancer.

In this exploratory study in a population in which the association of CTC with outcome was previously assessed,[5,6] we examined whether or not baseline levels of CEC, ET-1 and TF as well as changes in these markers early during therapy are independently associated with OS in CRPC patients treated with docetaxel. Additionally, as each individual marker represents a different process in tumour pathophysiology, we examined whether or not the combined use of these markers – in addition to CTC numbers – would yield a better model for prediction of outcomes in these patients.

2. Patients and methods

2.1. Patients

In a multi-centre prospective study aimed to determine the value of CTC enumeration in CRPC, blood samples were collected from 231 patients with CRPC by venepuncture. Results on CTC levels measured in this group have been previously published.[5] Exploration of CEC numbers with outcome was pre-specified as an exploratory analysis in the study protocol. Although the explorations of TF and ET-1 were not pre-specified in the exploratory end-points of the protocol, the evaluation of altered, over- or under-produced cancer-related molecules was anticipated and stated in the informed consent form which all patients signed. Different chemotherapeutic regimens may largely differ in the extent to which they induce vascular damage, and thereby in their effects on CEC levels. In order to assess a homogenous group of patients with respect to systemic therapy, we selected those who received a docetaxel-containing regimen ($n = 162$) to include in the present study. Of these 162 patients, 12 received docetaxel monotherapy, 139 patients received docetaxel together with an LHRH-analogue and 11 received docetaxel in combination with a monoclonal antibody in the context of clinical trials. Treatment for all patients was continued until progression or unacceptable toxicity. Demographics of the patients included in the present study are shown in Table 1. Main eligibility criteria for all patients participating in the multi-centre prospective study included age $\geqslant$18 years, pathological diagnosis of adenocarcinoma of the prostate, first or later line of chemotherapy, serum testosterone < 1.7 nmol/L (50 ng/mL), Eastern Cooperative Oncology Group (ECOG) performance status 0–2, pretreatment serum PSA $\geqslant$ 5 ng/mL, PSA progression despite androgen deprivation therapy, and ability to sign informed consent. Prior to treatment, all patients had a complete blood count analysis and assessment of serum concentrations of lactate dehydrogenase (LDH), alkaline phosphatase (Alk. Phos), haemoglobin and albumin. Prior to treatment start, and after 2–5 weeks (first follow-up draw) and 6–8 weeks (second follow-up draw) of treatment blood was collected into 2×10 mL CellSave tubes (Veridex, LLC, Raritan, NJ) and 1×6 mL serum tube. One CellSave tube was used to determine the CTC number, the other to determine CEC numbers. The serum tube was used for measuring concentrations of ET-1 and TF. Due to several reasons (e.g. progressive disease and logistics), values of all factors were not available for each time point for each patient. This study was approved by the local Institutional Medical Ethical Review Boards and is in agreement with the Helsinki declaration of 2000. Written informed consent was obtained from all patients prior to participation.

2.2. Enumeration of CTC and CEC

The CellTracks® AutoPrep® and CellTracks Analyzer II® Systems (both Veridex, LLC, Raritan, NJ) were used to count CTC and CEC.[4,22] CTCs were defined as intact cells positive

Table 1 – Patient demographics and laboratory parameters.

Parameter	Baseline	Weeks 2–5	Weeks 6–8
N	162	134	89
Time from baseline (d)			
Means ± SD	0	26.6 ± 6.7	47.7 ± 6.6
Range	0	19–41	42–61
Age (years)			
Means ± SD	69.8 ± 9.6		
Range	45–92		
Race			
White	148		
Black	11		
Hispanic	1		
Asian	2		
ECOG status			
0	74		
1	71		
2	12		
Unknown	5		
Stage at diagnosis			
1	9		
2	24		
3	39		
4	13		
Unknown	77		
Gleason score (means ± SD)	7.1 ± 1.5		
Median + IQR	7 (7–8)		
Range	2–10		
Prior local radiation			
Yes	66		
No	96		
Prior surgery			
Yes	103		
No	59		
Prior chemotherapy			
Mitoxantrone	24		
Estramustine	5		
Gemcitabine	3		
Carboplatin	3		
None	127		
Line of chemotherapy			
1	126		
2	20		
3	12		
4	2		
5	1		
6	1		
Site of metastasis			
Bone involvement	147		
Visceral	65		
Baseline haemoglobin (g/L) [N = 159]	12.4 ± 1.5		
Baseline LDH (IU/mL) [N = 152]	287 ± 212		
Baseline Alk. Phos. (IU/mL) [N = 155]	238.7 ± 280.1		
Baseline albumin (g/dL) [N = 156]	4.0 ± 3.0		
Baseline testosterone (ng/mL) [N = 155]	26 ± 2.0		
PSA (ng/mL)			
N	162	143	122
Median + IQR	128.5 (49–481)	127 (31.8–348)	103 (28.9–285)
Range	1.9–17.800	0.3–17420	0.3–12940

(*continued on next page*)

Table 1 – *continued*

Parameter	Baseline	Weeks 2–5	Weeks 6–8
CEC (cells/4 mL)			
N	153	134	89
Median + IQR	25 (12–49)	37 (19–94)	50 (27–105)
Range	2–1939	3–1102	0–701
CTC (cells/7.5 mL)			
N	154	142	118
Median + IQR	7 (1–24)	1 (0–9)	0 (0–7)
Range	0–5925	0–525	0–1367
ET-1 (pg/mL)			
N	94	87	80
Median + IQR	11.0 (9.0–14.0)	10.4 (8.5–13.4)	10.9 (8.6–13.1)
Range	2.3–489.7	1.3–200.0	2.5–110.9
TF (pg/mL)			
N	95	88	80
Median + IQR	31.5 (20.0–57.0)	33.1 (20.0–65.3)	20.8 (20.0–37.6)
Range	20.0–243.4	20.0–945.9	20.0–877.6

for the nuclear stain 4′,6-diamidinophenylindole (DAPI), for the epithelial cell adhesion molecule (EpCAM), and for cytokeratins 8, 18 and 19, but with no expression of the panleukocyte marker CD45. CECs were identified as intact cells positive for DAPI, CD146 and CD105 that also lacked expression of CD45. CTC numbers are reported as cells/7.5 mL of whole blood[4–6] and CEC numbers as cells/4.0 mL of whole blood.[22]

2.3. *Assessment of serum levels of TF and ET-1*

Serum concentrations (pg/mL) of ET-1 and TF-1 were determined by ELISA. The ET-1 specific ELISA was purchased from Assay Designs (Ann Arbor, MI, USA) and used according to the manufacturers' instructions. The TF specific ELISA was purchased from AssayPro (St. Charles, MO, USA). To improve the sensitivity of this assay, samples were diluted 2-fold rather than the 4-fold as suggested by the manufacturer. Absorbance was read at 450 nm using a Titertek 212 MS microplate reader (Titertek, Huntsville, AL). Samples were tested in duplicate and related to the standard curves for each assay. The lower detection limits of the assays were 1.3 pg/mL for ET-1 and 20.0 pg/mL for TF.

2.4. *Statistical analysis*

Longitudinal biomarker data were analysed using a random effects linear regression model using the maximum likelihood random effects estimator (the "xtreg, mle" command in STATA). This was done to incorporate both between- and within-subject effects as well as random effects and to assure that the observed alterations in biomarkers were not the result of inter-individual differences. Because the biomarker values were skewed (i.e. non-normally distributed), a logarithmic transformation was applied before the regression analysis. OS was defined as the time (in months) between the first blood draw and the date of death or last contact. Kaplan–Meier survival plots were made using stratified (i.e. categorical) data. For CTC numbers, a cutoff value of $\geqslant 5$ CTC/7.5 mL was used. To determine a cutoff value for survival analysis of CEC numbers and serum concentrations of ET-1 and TF, patient data were first stratified according to percentiles (p0–p25; p25–p50, p50–p75 and p75–p100). Baseline, 2–5 weeks values and changes between baseline and 2–5 weeks were analysed separately. In case of a clear outlier, determined by inspection of the four strata in the Kaplan–Meier plots, the percentiles were used to dichotomise the data in such a way that the overlapping percentiles were grouped together, and the value defining the separating percentile was used as stratifier. If no clear outlier could be observed, the median value was used as stratifier. The logrank test was used to compare survival between strata. Univariate Cox proportional-hazards regression was used to identify baseline parameters associated with survival. Significant baseline parameters (i.e. P-values < 0.05) were subsequently used in multivariate analysis. The predictive accuracy of the multivariate Cox models was assessed by concordance analysis and reported as Harrel's C index. Data are reported as means ± standard deviation (SD) unless stated otherwise. All analyses were performed using STATA v10 software (StataCorp., College Station, TX, USA).

3. Results

3.1. *Alterations in CEC, CTC, ET-1 and TF levels during treatment*

Baseline levels of CEC, CTC, ET-1 and TF are shown in Table 1. A significant increase in CEC numbers was observed at the first follow-up blood draw, taken 2–5 weeks after initiation of docetaxel, when compared to baseline ($P < 0.001$). No further increase was found at the second follow-up draw, 6–8 weeks after treatment initiation (Fig. 1, panel A). The CTC numbers from the subgroup of patients included in this study are depicted in Fig. 1, panel B. CTC decreased significantly after 2–5 weeks of treatment ($P < 0.001$) but remained stable thereafter. In contrast to ET-1 serum concentrations, which remained constant during docetaxel treatment (Fig. 1, panel C), a significant decrease of TF was observed after 6–8 weeks

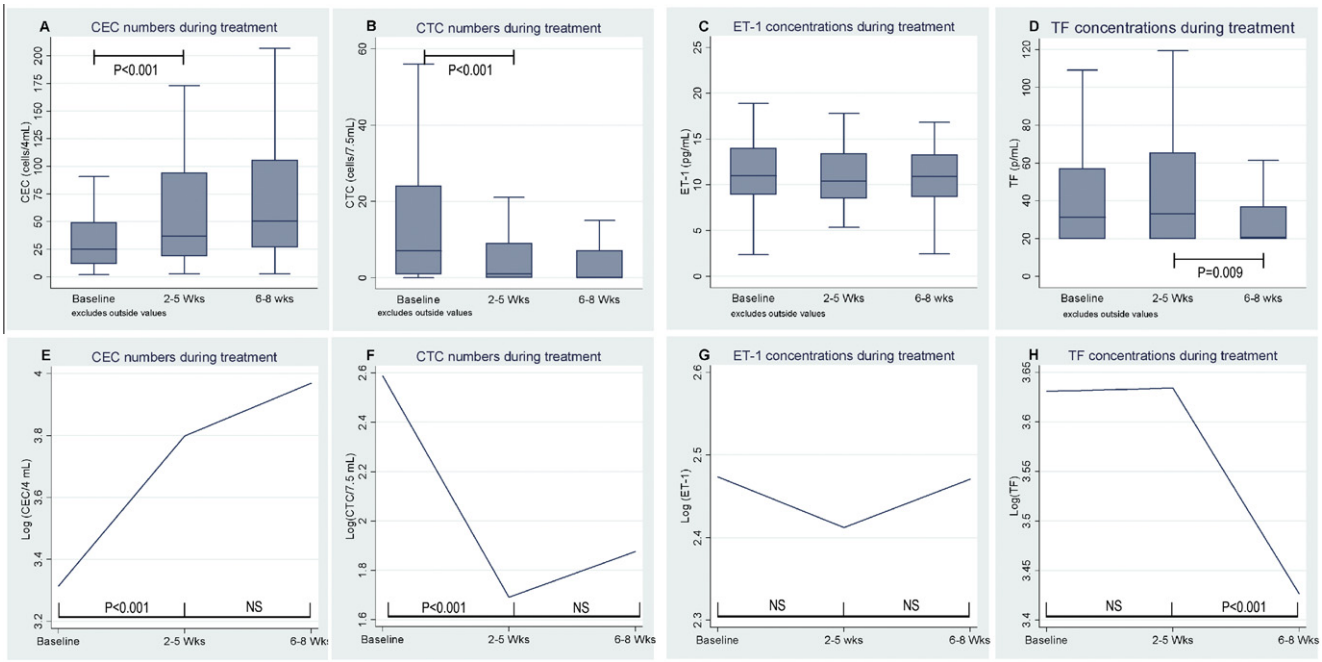

Fig. 1 – Generalised course of tumour and vascular markers during treatment. Panels A–H (E–H log-transformed values): CEC numbers increased significantly within 2–5 weeks of treatment, whereas CTC numbers decreased. Both remained stable thereafter. No treatment effects on ET-1 levels were observed. Serum TF declined after 6–8 weeks of treatment. NS, not significant.

of therapy compared to baseline and first follow-up draw ($P = 0.009$) (Fig. 1, panel D). Repeated linear regression for longitudinal data, performed to correct for inter-individual variation confirmed the observed changes (data not shown).

3.2. *Early changes in CEC, CTC and TF levels are prognostic for poor overall survival*

After dichotomising data around their median baseline values, no prognostic value for OS could be determined for baseline levels of CEC, ET-1 or TF. Similar to results reported for the whole group[5], baseline CTC numbers $\geqslant 5$ cells/7.5 mL in the subgroup analysed in this study were associated with significantly decreased OS compared to patients with baseline CTC numbers <5 cells/7.5 mL (10.9 versus 17.4 months, respectively; $P = 0.0004$, Fig. 2, panel B). With respect to CEC, analyses with various percentiles of the ratio between CEC at 2–5 weeks and CEC at baseline revealed that patients in the p75–p100 percentile (i.e. $\geqslant 3.8$-fold increase in CEC at 2–5 weeks), showed significantly worse OS compared to those with a more limited CEC increase (10.9 versus 16.8 months, respectively, $P = 0.015$, Fig. 2, panel A). No association with OS was revealed for ET-1 levels of changes during treatment, but we found a significantly worse OS in those patients in whom a decrease in TF concentrations was observed after 2–5 weeks of treatment (Fig. 2, panel D; median OS 11.9 months versus 21.5 months; $P < 0.001$). Alterations after 6–8 weeks were not associated with survival.

Additionally, we assessed whether or not the combined use of all markers assessed in this study that were found to be prognostic for decreased OS, i.e. CEC and CTC numbers and TF levels determined at 2–5 weeks after treatment start, could provide additional information on survival. Sixty-nine patients were eligible for this combined analysis, meaning that all clinical and laboratory data were available. First, we assessed any possible relation between each prognostic marker by both uni- and multivariate regression analyses. No significant associations were found, implying the independent prognostic value of each factor (data not shown). Next, we stratified patients based on the number of risk factors for poor survival present after 2–5 weeks of treatment, namely an increase in CEC numbers $\geqslant 3.8$ times the baseline counts, any decrease in TF levels when compared to baseline counts, and CTC counts of $\geqslant 5$ per 7.5 mL of blood. Here, we found a significant decrease in OS as the number of risk factors increased (Logrank test for trend $P < 0.0001$). All data for this analysis are shown in Fig. 3.

3.3. *CEC and TF levels increase the prognostic accuracy of CTC at 2–5 weeks*

To determine which parameters would provide the most accurate survival model, we performed the following procedure. First, we identified all parameters presented in Table 1 that were prognostic at baseline using univariate Cox regression analysis. For CEC, TF and ET-1 levels, the changes after 2–5 weeks of therapy were also univariately evaluated. Parameters found to be significant, which included CTC levels at 2–5 weeks as well as changes in CEC, CTC and TF levels were subsequently used in a multivariate analysis (Table 2). In addition to the baseline LDH level, three risk factors were found to be independently significant in the multivariate analysis; namely CTC counts of $\geqslant 5$ cells/7.5 mL at 2–5 weeks, a $\geqslant 3.8$ increase in CEC from baseline to 2–5 weeks, and any

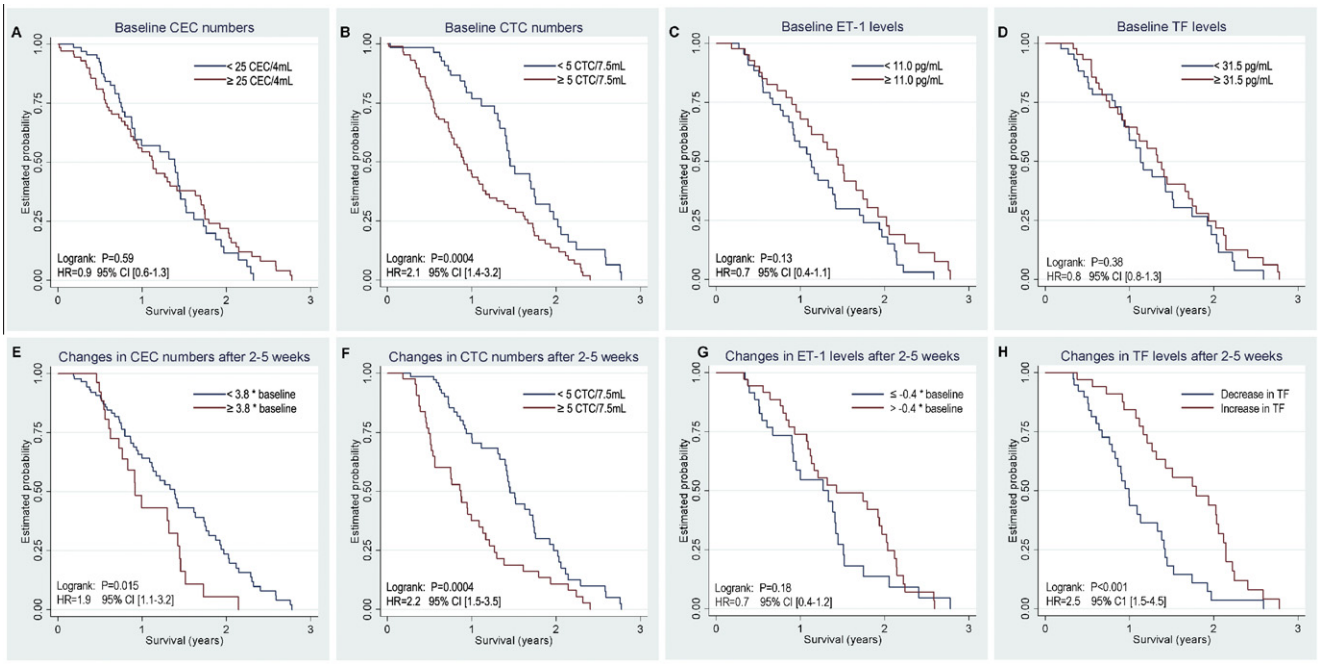

Fig. 2 – Associations of changes in CEC and CTC numbers and ET-1 and TF levels with OS at 2–5 weeks. Panels A–D: Associations of base-line levels with overall survival. Panels E–H: Associations of week 2–5 levels with overall survival. At 2–5 weeks, patients with a ⩾3.8-fold increase in CEC counts, with CTC counts ⩾5 cells/7.5 mL or with a decrease in TF levels were characterized by a markedly worse OS.

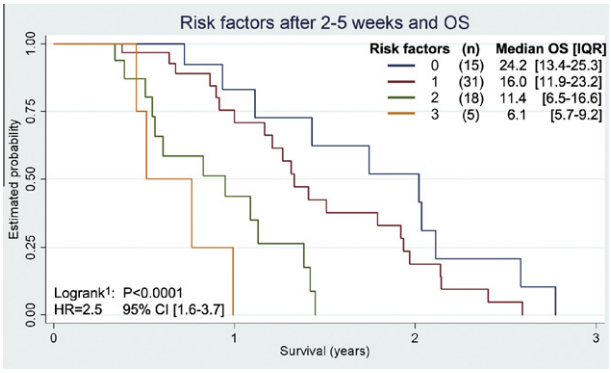

Fig. 3 – Risk factors and overall survival. [1]Test for trend of survivor functions. Association of the number of risk factors present at 2–5 weeks (⩾3.8-fold increase in CEC, a decrease of TF, and a CTC number of ⩾5 cells/7.5 mL) with OS. HR, hazard ratio determined in univariate Cox proportional-hazards regression. CI, confidence interval. *n*, number of patients belonging to the diverse groups.

decrease in TF levels at 2–5 weeks. To assess whether patient stratification yielded a more accurate survival model than the combined use of each individual prognostic parameter, we performed a concordance analysis, which results in a *C* index. Briefly, the *C* index describes how accurate the Cox regression model predicts survival, where a *C* index near 0.5 means that the model does not predict survival and values approaching 1.0 indicate that the model nearly always predicts if a patient has a better prognosis.[23] Here, we found that the stratification based on the number of risk factors present resulted in an increased predictive power fit of the Cox proportional-hazards regression model (Table 3).

3.4. *PSA declines after 2–5 weeks of treatment are not prognostic for poor overall survival*

We also analysed baseline levels of prostate specific antigen (PSA) and decreases in PSA at 2–5 weeks of both 30% and 50% when compared to the baseline values using identical patient stratification as used for the CEC, CTC and soluble marker analyses. Patients with baseline PSA levels >493 ng/mL had a decreased OS (median 9.5 months versus 16.6 months; $P = 0.002$). Neither a 30% nor 50% decrease in PSA levels at 2–5 weeks after start of docetaxel was associated with OS in this cohort (data not shown).

4. Discussion

In CRPC patients, docetaxel combined with prednisone is currently the only treatment that yielded an OS benefit in randomised studies.[2] However, the OS gain is relatively modest given a median prolongation of only 2 months compared to mitoxantrone and prednisone, and comes at the expense of potential severe side-effects. With this in mind, many attempts have been made to identify CRPC patients at risk for rapid progression and poor survival.

For this purpose, PSA is the most widely explored marker. Multivariate analysis on baseline PSA levels obtained from the TAX 327 study in CRPC in which patients were randomly assigned to receive docetaxel with prednisone or mitoxantrone with prednisone, demonstrated increased OS for patients with

Table 2 – Multivariate Cox proportional-hazards regression analysis of parameters prognostic for OS after 2–5 weeks of treatment (*N* = 63).

Parameter	OS risk from baseline	
	HR [confidence interval, CI]	*P*-value
2–5 Weeks CTC (⩾5 versus 5 cell/7.5 mL)	2.0 [1.0–4.1]	0.047
2–5 Weeks CEC (⩾3.8 versus <3.8-fold increase CEC/4 mL)	2.3 [1.1–4.6]	0.022
2–5 Weeks TF (decrease versus increase from baseline)	2.4 [1.1–5.0]	0.026
ECOG status (2 versus 1 versus 0)	2.0 [0.9–3.9]	0.06
Baseline haemoglobin (g/L)	1.2 [0.9–1.5]	0.13
Baseline LDH (IU/mL)	3.4 [1.5–7.7]	0.003
Baseline AP (IU/mL)	1.1 [0.7–1.7]	0.78

Table 3 – Combined use markers adds to the predictive strength.

Individual use	HR [CI]	*P*-value	C index
2–5 Weeks CTC (⩾5 versus 5 cell/7.5 mL)	2.0 [1.0–3.8]	0.049	0.74
2–5 Weeks CEC (⩾3.8 versus <3.8-fold increase in CEC/4 mL)	2.5 [.2–4.9]	0.011	
2–5 Weeks TF (decrease versus increase from baseline)	2.9 [1.5–5.6]	0.002	
Baseline LDH (IU/mL)	3.7 [1.7–8.0]	0.001	
Combined use			
Risk factors (3 versus 2 versus 1 versus 0)	2.5 [1.6–3.8]	<0.0001	0.76
Baseline LDH (IU/mL)	3.2 [1.5–6.6]	0.002	

a baseline PSA <114 ng/mL compared to those with higher levels.[24] Furthermore, data from the same study allowed the development of a predictive nomogram for survival. Using baseline parameters such as PSA, LDH, alkaline phosphatase and haemoglobin concentrations, patients likely to have a decreased survival can be identified.[25] In this study, we confirmed the prognostic value of baseline PSA levels but found no prognostic value of baseline CEC, ET-1 or TF levels for survival, three markers that have not previously been assessed in CRPC.

In addition to a marker that provides information on OS prior to initiation of chemotherapy, there is also a great need for markers that discriminate at an early stage during therapy those patients who clearly benefit from chemotherapy from those who do not. Previously, a PSA decrease of 30% after 3 months of treatment was a good surrogate marker for survival in patients treated with docetaxel/estramustine or mitoxantrone/prednisone.[26] Similar results have been observed in the TAX 327 study.[24] In the current study, we found that a ⩾3.8-fold increase in CEC after 1 or 2 cycles of docetaxel was prognostic for decreased OS. CEC numbers are thought to reflect the extent of vascular damage. Several studies have shown an anti-vascular effect for both paclitaxel and docetaxel *in vitro* and in murine models.[27,28] If this holds true for humans as well, then the rise in CEC numbers, which was seen in all patients, is likely the result of vascular damage inflicted by docetaxel. We hypothesise that the additional CEC increase in patients with the worst OS may be attributed to the continued endothelial cell shedding from vessels in tumours progressing during treatment. Furthermore, an increase in TF concentration during the first 2–5 weeks of treatment was associated with a better survival, whereas alterations at 6–8 weeks were not associated with survival. We hypothesise that this observed association is the result of massive shedding from apoptotic tumour cells, which, similar to platelets, endothelial cells and leukocytes, have also been reported to express TF.[29] Expression of TF in the primary tumour tissue was associated with a poorer cause-specific survival in patients with metastatic prostate cancer who received hormonal therapy.[30] Blood TF levels in metastatic prostate cancer however, have to the best of our knowledge not been investigated previously. In localised prostate cancer, elevated plasma TF levels, pre-operatively determined, were associated with a higher risk to relapse.[31] We are not aware of any studies on the role of TF as early marker for response in other tumour types. As previously reported for the whole group from which the subgroup analysed in this study was selected,[5] CTC counts of ⩾5/7.5 mL 2–5 weeks after the initiation of treatment were associated with a poor prognosis.

As all the markers evaluated are considered to represent different processes involved in tumour biology, we explored whether their combined use could aid clinicians in classifying docetaxel-treated CRPC patients into groups differing in OS. Concordance analysis demonstrated that the use of CEC, CTC and TF levels are independent risk factors for OS. Importantly, their combined use after 2–5 weeks yielded four groups with statistically different and clinically relevant differences in OS (median OS: 24.2 versus 16.0 versus 11.4 versus 6.1 months). At the same time point, PSA values were not informative for survival. This suggests that the risk stratification examined in this study outperform PSA levels as early markers for ultimate outcome during docetaxel-based chemotherapy in CRPC.

To the best of our knowledge, this is the first study that demonstrates a prognostic value for CEC and TF changes during cytotoxic therapy in a well defined study population. Although the number of patients with a decreased OS in our study was relatively small, these results are encouraging.

The combined use of CTC number and relative changes in CEC and TF allows the identification of CRPC patients not responding to docetaxel-based therapy at an early stage during therapy. Whether or not this holds also true for other therapies and other tumour types deserves further study. Although the exploration of CEC numbers with outcome was pre-specified in the study protocol, it is important to realise that this was an exploratory study and that therefore prospective studies should be done to confirm our findings and compare them to conventional biomarkers such as PSA. If confirmed, the model established here may serve as a useful tool for clinical trial design and to tailor patient management.

Conflict of interest statement

Study was financially supported by Veridex LLC, Raritan, NJ, USA

Acknowledgements

Original IMMC-38 Clinical Trial Investigators (and number of patients enrolled): J.S. deBono, D. Dearnaley & C. Parker, Royal Marsden Hosp, Surrey, England (44); H Scher, Memorial Sloan-Kett, New York, NY (16); K.J. Pienta, University of Michigan, Ann Arbor, MI (15); B. Montgomery, University of Washington Medical Center, Seattle, WA (12); M. Carducci and M. Eisenberger, Johns Hopkins Medical Institutions, Baltimore, MD (10); D. Raghavan, R. Dreicer, J. Garcia, and E. Borden, Cleveland Clinic Taussig Cancer Center, Cleveland, OH (9); B. Hellerstedt, Texas Oncology Cancer Center, Austin, TX (8); W.R. Berry, Raleigh Hematology Oncology Clinic, Cary, NC, (7); J. Arseneau, Albany Regional Cancer Center, Albany, NY (6); P. Harper, Guy's Hospital, London, UK (6); A.J. ten Tije, Tergooi Ziekenhuizen, Blaricum, The Netherlands (6); JP Wylie, Christie Hospital, Manchester, UK (6); P. Acevedo, Ocala Oncology Center, Ocala, FL (5); AL Cohn, Rocky Mountain Cancer Centers, Colorado Springs, CO (5); P.J. Flynn, Minnesota Oncology Hematology, St. Paul, MN (5); M. Neubauer, Kansas City Cancer Centers, Kansas City, MO (5); D.A. Smith, Northwest Cancer Specialists-Rose Qtr, Portland, OR (5); G.J. Creemers, Catharina Ziekenhuis, Eindhoven, The Netherlands (4); M. Dhawan, Highland Clinic, Shreveport, LA (4); S.G. Gandhi, CA & Blood Disease Ctr., Lecanto, FL (4); N. Iannotti, Hem/Onc Treasure Coast, Port St. Lucie, FL (4); D.M. Schlossman, Missouri Cancer Associates, Columbia, MO (4); A.K. Chakrabarti, Hope Center, Terre Haute, IN (3); S.A. McKenney, Mamie McFaddin Ward Cancer Center, Beaumont, TX (3); J.G. Moreno, Tri-County Urological Associates - PC, Pottstown, PA (3); R. Reid, Fairfax-Northern Virginia Hem-Onc, PC, Woodbridge, VA (3); G. Robbins, Florida Cancer Institute, New Port Richey, FL (3); T. Smilde, Jeroen Bosch Ziekenhuis, Den Bosch, The Netherlands (3); S.P. Leibowitz, Hematology-Oncology Associates of NJ, Morristown, NJ (2); B. Saidman, Medical Oncology Associates, Kingston, PA (2); J. Vuky, Virginia Mason Medical Center, Seattle, WA (2); R. Anderson, Texas Oncology, PA, Waco, TX (1); T.C. Anderson, Texas Oncology, PA, Bedford, TX (1); V.A. Canfield, Cancer Care Associates, Oklahoma City, OK (1); P.R. Conkling, Virginia Oncology Associates, Hampton, VA (1); N. Gabrail, Gabrail Cancer Center, Canton, OH (1); R. Gersh, Cancer Care Northwest-North, Spokane, WA (1); J.H. Graham, Beatson Oncology Centre, Glasgow, UK (1); L. Klein, Northwest Medical Specialists – PC, Arlington Heights, IL (1); K. Kumar, Pasco Hernando Oncology Assoc., New Port Richey, FL (1); D.M. Loesch, Central Indiana Cancer Centers, Indianapolis, IN (1); T. Lopez, New Mexico Cancer Care Associates, Santa Fe, NM (1); S. McClure, Deke Slayton Cancer Center, Webster, TX (1); K.J. McIntyre, Texas Oncology PA, Dallas, TX (1); P.D. Richards, Onc and Hem Associates of SW VA, Inc., Christiansburg, VA (1); M. Roberts, Hematology Oncology Associates, Phoenix, AZ (1); T. Rogue, Texas Cancer Center, Sherman, TX (1); and P. Vongkovit, Northwestern Carolina Oncology Hematology, Hickory, NC (1).

REFERENCES

1. ACS. American Cancer Society: Cancer Statistics 2007 Presentation. Atlanta; 2008.
2. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004;**351**(15):1502–12.
3. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**(19):3213–21.
4. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004;**351**(8):781–91.
5. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2008;**14**(19):6302–9.
6. Scher HI, Jia X, de Bono JS, et al. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol* 2009;**10**(3):233–9.
7. Offersen BV, Borre M, Overgaard J. Quantification of angiogenesis as a prognostic marker in human carcinomas: a critical evaluation of histopathological methods for estimation of vascular density. *Eur J Cancer* 2003;**39**(7):881–90.
8. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* 1993;**143**(2):401–9.
9. Bagnato A, Spinella F, Rosano L. The endothelin axis in cancer: the promise and the challenges of molecularly targeted therapy. *Can J Physiol Pharmacol* 2008;**86**(8):473–84.
10. Spinella F, Rosano L, Di Castro V, Natali PG, Bagnato A. Endothelin-1 induces vascular endothelial growth factor by increasing hypoxia-inducible factor-1alpha in ovarian carcinoma cells. *J Biol Chem* 2002;**277**(31):27850–5.
11. Asham E, Shankar A, Loizidou M, et al. Increased endothelin-1 in colorectal cancer and reduction of tumour growth by ET(A) receptor antagonism. *Br J Cancer* 2001;**85**(11):1759–63.
12. Salani D, Rosano L, Di Castro V, et al. ABT-627, a potent endothelin receptor A antagonist, inhibits ovarian carcinoma growth in vitro. *Clin Sci (Lond)* 2002;**103**(Suppl. 48):318S–21S.
13. Shankar A, Loizidou M, Aliev G, et al. Raised endothelin 1 levels in patients with colorectal liver metastases. *Br J Surg* 1998;**85**(4):502–6.
14. Wulfing P, Kersting C, Tio J, et al. Endothelin-1-, endothelin-A-, and endothelin-B-receptor expression is correlated with vascular endothelial growth factor expression and

angiogenesis in breast cancer. *Clin Cancer Res* 2004;**10**(7):2393–400.
15. Yu J, May L, Milsom C, et al. Contribution of host-derived tissue factor to tumor neovascularization. *Arterioscler Thromb Vasc Biol* 2008;**28**(11):1975–81.
16. Lalich M, McNeel DG, Wilding G, Liu G. Endothelin receptor antagonists in cancer therapy. *Cancer Invest* 2007;**25**(8):785–94.
17. Wang HY, Yang YM, Zhuang Y, et al. The effect of celecoxib on tissue factor expression in pancreatic cancer cells. *Chin Med J (Engl)* 2007;**120**(20):1753–6.
18. Strijbos MH, Gratama JW, Kraan J, et al. Circulating endothelial cells in oncology: pitfalls and promises. *Br J Cancer* 2008;**98**(11):1731–5.
19. Strijbos MH, Rao C, Schmitz PI, et al. Correlation between circulating endothelial cell counts and plasma thrombomodulin levels as markers for endothelial damage. *Thromb Haemost* 2008;**100**(4):642–7.
20. Beerepoot LV, Mehra N, Vermaat JS, et al. Increased levels of viable circulating endothelial cells are an indicator of progressive disease in cancer patients. *Ann Oncol* 2004;**15**(1):139–45.
21. Beerepoot LV, Radema SA, Witteveen EO, et al. Phase I clinical evaluation of weekly administration of the novel vascular-targeting agent, ZD6126, in patients with solid tumors. *J Clin Oncol* 2006;**24**(10):1491–8.
22. Rowand JL, Martin G, Doyle GV, et al. Endothelial cells in peripheral blood of healthy subjects and patients with metastatic carcinomas. *Cytometry A* 2007;**71**(2):105–13.
23. Xiang A, Lapuerta P, Ryutov A, Buckley J, Azen S. Comparison of the performance of neural network methods and Cox Regression for censored survival data. *Comput Stat Data Anal* 2000;**34**(2):243–57.
24. Berthold DR, Pond GR, de Wit R, Eisenberger M, Tannock IF. Survival and PSA response of patients in the TAX 327 study who crossed over to receive docetaxel after mitoxantrone or vice versa. *Ann Oncol* 2008;**19**(10):1749–53.
25. Armstrong AJ, Garrett-Mayer ES, Yang YC, et al. A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. *Clin Cancer Res* 2007;**13**(21):6396–403.
26. Petrylak DP, Ankerst DP, Jiang CS, et al. Evaluation of prostate-specific antigen declines for surrogacy in patients treated on SWOG 99-16. *J Natl Cancer Inst* 2006;**98**(8):516–21.
27. Grant DS, Williams TL, Zahaczewsky M, Dicker AP. Comparison of antiangiogenic activities using paclitaxel (taxol) and docetaxel (taxotere). *Int J Cancer* 2003;**104**(1):121–9.
28. Hotchkiss KA, Ashton AW, Mahmood R, et al. Inhibition of endothelial cell function in vitro and angiogenesis in vivo by docetaxel (Taxotere): association with impaired repositioning of the microtubule organizing center. *Mol Cancer Ther* 2002;**1**(13):1191–200.
29. Gouault-Helimann M, Josso F. Initiation in vivo of blood coagulation. The role of white blood cells and tissue factor (author's transl). *Nouv Presse Med* 1979;**8**(40):3249–53.
30. Akashi T, Furuya Y, Ohta S, Fuse H. Tissue factor expression and prognosis in patients with metastatic prostate cancer. *Urology* 2003;**62**(6):1078–82.
31. Langer F, Chun FK, Amirkhosravi A, et al. Plasma tissue factor antigen in localized prostate cancer: distribution, clinical significance and correlation with haemostatic activation markers. *Thromb Haemost* 2007;**97**(3):464–70.