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ORIGINAL ARTICLE

Vasoactive peptides associate with treatment outcome of bevacizumab-containing therapy in metastatic colorectal cancer

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ABSTRACT

Background: Hypertension is a common early adverse event of anti-angiogenic treatment of cancer and may associate with treatment response. However, blood pressure measurement as a surrogate response biomarker has methodological limitations, and predictive biomarkers of angiogenesis inhibitors are lacking. In disease associated with hypertension, vasoactive peptides have been linked to cardiovascular pressure load. Here, we have explored potential associations between circulating levels of vasoactive peptides and tumor response during bevacizumab-containing treatment of colorectal cancer.

Material and methods: Metastatic colorectal cancer (mCRC) patients with available best objective response (ORR) and time to tumor progression (TTP) data were included from a randomized clinical trial investigating maintenance therapy after first line chemotherapy plus bevacizumab. Midregional-pro-adrenomedullin (MR-proADM), midregional-pro-atrial-natriuretic-peptide (MR-proANP), and C-terminal-prepro-vasopressin (Copeptin) vasoactive peptide concentrations were measured in plasma at baseline and after 6 weeks of chemotherapy and bevacizumab treatment ($n = 97$). We determined associations among clinical outcome (ORR and TTP), peptide levels, and hypertension (NCI-CTCAE 4.0 criteria), using Spearman's test, multiple linear regression, and Mann–Whitney's test.

Results: Increasing levels of vasoactive peptides from baseline and after six weeks of treatment were associated with improved treatment outcome (MR-proADM: ORR, $p = .0003$; TTP, $p = .05$; MR-proANP: ORR, $p = .05$; TTP, $p = .03$; Copeptin: ORR, $p = .10$; TTP, $p = .02$). Patients with increasing levels of all three peptides (n = 28) versus increasing levels of one or two peptides ($n = 59$) showed a median TTP of 284 and 225 d, respectively ($p = .02$).

Conclusions: Our results suggest that increasing systemic levels of vasoactive peptides associate with improved tumor response and TTP in mCRC patients treated with a bevacizumab-containing regimen. These findings support the proposed link between the tumor vasculature and the cardiovascular system of the host. This should motivate further studies that investigate the potential role of vasoactive peptides as a novel class of dynamic biomarkers in the treatment of cancer.

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Introduction

Angiogenesis, i.e., the formation of new blood vessels, is a hallmark and requirement of tumor development and metastasis. The concept of anti-angiogenic treatment of cancer has been extensively investigated for almost half a century. Bevacizumab, an antibody targeting the vascular endothelial growth factor (VEGF), is commonly used in combination with chemotherapy in several advanced cancer types including metastatic colorectal cancer (mCRC) [1]. However, a significant limitation of anti-angiogenic drugs is the current lack of biomarkers to predict treatment response [2]. Therefore, the identification of biomarkers that could separate responding patients from patients with no clinical benefit of anti-angiogenic drugs remains a challenge of high clinical relevance.

Angiogenesis and blood pressure are interconnected, and hypertension is a common adverse event of bevacizumab and other anti-angiogenic agents. It has been proposed that VEGF inhibition by bevacizumab increases peripheral vascular resistance through down-regulation of vasodilators, e.g., nitrous oxide, and through a functional decrease of arterioles and capillaries, together resulting in increased cardiovascular pressure load and hypertension [3]. Importantly, some studies have shown an association between the anti-tumoral effect of bevacizumab treatment and increased blood pressure [4–9]. However, a large comprehensive analysis found that in six out of seven studies, hypertension did not associate with improved clinical benefit from bevacizumab treatment [10]. These discrepancies probably reflect the intrinsic limitations of blood pressure measurement as a surrogate biomarker

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due to, e.g., diurnal variation, white coat effect, and the methodological variability of the test.

Several vasoactive peptides that reflect cardiovascular pressure load and blood pressure have been identified [11–13]. Whether this class of peptides respond to angiogenesis inhibitors and associate with their anti-tumoral activity has never been studied. We have investigated stable fragments of three different vasoactive peptide hormones: Midregional-pro-adrenomedullin (MR-proADM), midregional-pro-atrial-natriuretic-peptide (MR-proANP), and C-terminal-prepro-vasopressin (Copeptin) that were selected on basis of their link to angiogenesis, cardiovascular stress, microalbuminuria, hypertension, and diseases characterized by blood pressure instability, such as syncope and sepsis [11–16]. The study was designed to explore the association between vasoactive peptide levels and efficacy of bevacizumab-containing first line treatment of mCRC patients included in a clinical trial.

Material and methods

Patient population

Patients were treated within the randomized clinical trial Nordic ACT2 (ClinicalTrials.gov: NCT01229813) [17]. This study was performed in accordance with the Declaration of Helsinki and all patients signed separate written informed consent to be part of the biomarker study. Main inclusion criteria were untreated mCRC, $\geq$18 years of age, performance status ECOG 0-1, and adequate hematological, hepatic, and renal function. Uncontrolled hypertension, significant active cardiovascular disease, and active use of anticoagulants for therapeutic purpose were not allowed. For the present study, patients were selected for biomarker analyses based on two well-defined, pre-determined criteria: (1) reason for end of treatment (EOT) in ACT2 specified as progressive disease (PD) according to Response Evaluation In Solid Tumours (RECIST) 1.0, and (2) available plasma samples at baseline before initiation of treatment and at approximately 6 weeks from treatment start. At the time of the plasma sample inventory, eight patients who fulfilled the above-mentioned second criteria were still on treatment in the ACT2 study and had not yet reached EOT. These patients' samples were included for vasoactive peptides measurements in order to maximize the final biomarker cohort. In time for the statistical analysis, all these patients had reached EOT; however, six of them for other reasons than tumor progression, and thus did not fulfill the first pre-determined inclusion criteria mentioned above (see Figure 1). Accordingly, the sample size was determined by a clear-cut definition of the endpoint time to tumor progression (TTP), and by our aim to minimize any exclusion of subjects due to lack of necessary data.

Anti-tumoral treatment regimens

First-line induction treatment was given for a maximum of 18 weeks with a fluoropyrimidine in combination with oxaliplatin or irinotecan (XELOX/XELIRI or FOLFOX/FOLFIRI according to investigator's choice) plus standard dosing of bevacizumab (equal to 2.5 mg/kg body weight per week) [17]. Patients without PD by the second tumor evaluation after 18 weeks of induction treatment were then eligible for randomization to maintenance treatment. Thus, tumor response evaluation was performed twice during the induction treatment for all patients that started maintenance phase. Patients with Kirsten rat sarcoma oncogene (KRAS) wild-type (wt) tumors were randomized between bevacizumab alone (7.5 mg/kg) once every 3 weeks (arm wt-B) or in combination with oral erlotinib 150 mg once daily continuously (arm wt-BE). Patients with KRAS mutated (mut in codons 12 or 13 of exon 2) tumors were randomized to bevacizumab alone (arm mut-B), or metronomic capecitabine 500 mg twice daily (arm mut-C).

Tumor evaluation and clinical data

A computed tomography (CT) scan of the thorax and abdomen was performed within 28 d before enrollment in the ACT2 trial as baseline assessment. According to the ACT2 protocol two CT evaluations were planned in the induction treatment phase: One after 8–12 weeks of induction treatment, and for patients that did not have PD and continued treatment in the study a second CT scan was performed after a total of 18 weeks of induction treatment. At this time point, patients without PD were eligible for randomization to maintenance treatment. CT scans were performed every nine weeks during the maintenance treatment phase (see Supplementary Figure 1 for a schematic presentation of interventions and assessments).

Blood pressure (BP) was measured with the patient in resting position for at least 5 min at the start of each treatment course. Verification of BP by repeated measurement should be undertaken if systolic BP $\geq$140 and/or diastolic BP $\geq$90 was recorded. Patient-specific BP data were used to grade hypertension retrospectively according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0 [18].

Vasoactive peptide analyses

Blood was collected at baseline before initiation of induction treatment with chemotherapy and bevacizumab (sample A), and approximately after 6 weeks of induction treatment (sample B), i.e., prior to cycle three or four depending on the chosen induction treatment regimen schedule (Supplementary Figure 1). Blood (4–7 ml) was collected in an EDTA tube, and centrifuged after resting for 30 min, aliquoted into 1.5 ml cryovials and stored at -70° C until assayed. Absolute levels (pmol/l) of stable fragments of the vasoactive peptides MRpro-ANP, MR pro-ADM, and Copeptin were determined in EDTA plasma using a standardized, commercial fully automated immunoassay (KRYPTOR, Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany) involving the Time-Resolved Amplified Cryptate Emission (TRACE) technology, which has been evolved from the originally described immunoassays [19–21]. The assays were performed and

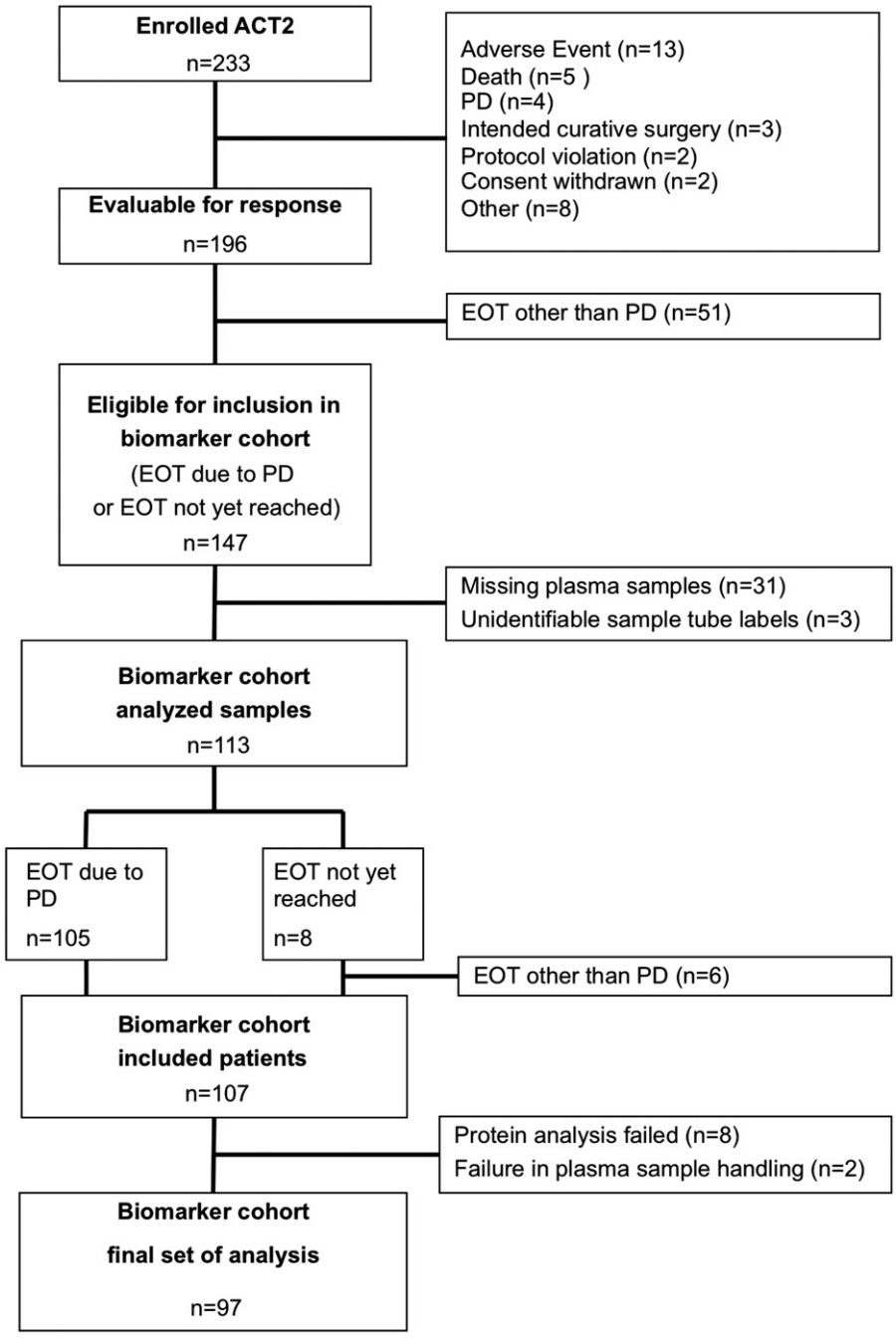

Figure 1. CONSORT diagram of the ACT2 biomarker cohort. PD: progressive disease; EOT: end of treatment in ACT2 study. See Supplementary Figure 1 for further details.

reported by assisting personnel blinded to the patient clinical data.

Statistical methods

TTP was defined as the time in days from start of first treatment cycle in the induction phase until the date of PD recorded in the ACT2 study, either in the induction treatment phase or for the randomized patients during maintenance treatment. The change in blood pressure (with baseline measurement as reference) was expressed as the grade of hypertension (0–1, 2, or 3) according to CTCAE 4.0 before the third or the fourth cycle of induction treatment, approximately 6 weeks from treatment start. Due to the low threshold for grade 1 hypertension, also denoted "pre-hypertension" in the CTCAE 4.0 document, patients with grade 0 hypertension (blood pressure below 120/80 mm Hg, $n = 3$) were pooled with patients classified with hypertension grade 1 into one group. Hypertension grade 4 or 5 was not observed. Changes in peptide concentrations from baseline (sample A) to approximately 6 weeks from treatment start (sample B) were analyzed as log concentration ratios (B/A), base 2. Spearmańs rank correlation test was used to investigate the associations between peptide concentrations at baseline and TTP, hypertension and TTP, and hypertension

grade versus peptide concentration ratios. In addition to Spearman's test, simple linear regression was used to describe peptide concentration ratios versus clinical outcome in terms of TTP and objective tumor response (ORR). ORR was defined as the best objective tumor response, PR (partial response), SD (stable disease), or PD (progressive disease) (according to RECIST 1.0), observed during induction treatment with bevacizumab and chemotherapy. The relationship between TTP and the three peptide concentration ratios, dichotomized at 1.00, i.e., increasing (B/A >1.00) versus non-increasing (B/A ≤ 1.00) concentrations, was studied with *t*-test and analyzed simultaneously using a multiple linear regression model. The patients were then classified into three groups depending on number of peptides with increasing concentrations: 0, 1 to 2, or 3. The TTP-distributions for these three groups were compared overall using the 2-df Kruskal–Wallis test and pairwise using the Wilcoxon–Mann–Whitney rank sum test. All statistical analyses were done with STATA, version 14 (StataCorp, College Station, TX, USA). A two-sided *p* value of $<.05$ was considered statistically significant.

Results

Patient characteristics of the biomarker cohort

Of the 196 patients evaluable for response in the ACT2 study, 147 were potentially eligible for vasoactive peptide analyses according to pre-determined selection criteria, and from this group, 113 full sets of plasma samples were identified. The main reason for exclusion was missing plasma sample after 6 weeks of treatment. To minimize the risk of pre-analytical bias, the study group decided to exclude two patients at one specific Swedish study site before statistical analysis due to improper validation of sample handling. At the time of the statistical analysis, six of the selected patients were excluded due to reason for EOT specified as other than PD, i.e., they did not fulfill the criteria for biomarker measurements. The peptide analyses from this biomarker cohort ($n = 107$) yielded reliable data for all three peptides, MR-proADM, MR-proANP, and Copeptin, at both time-points (A and B) for 97 patients, who were included in the statistical analyses (Figure 1).

Patient characteristics of the final biomarker cohort are presented in Table 1. Approximately, 81% ($n = 79$) of the patients were randomized after induction therapy, the majority of which continued on bevacizumab as part of their maintenance treatment ($n = 58$), whereas the remaining patients received metronomic capecitabine as anti-angiogenic maintenance treatment until progression ($n = 21$). Ten patients had either SD ($n = 8$) or PR ($n = 2$) as best response at first CT evaluation but later progressed at the second evaluation in the induction phase, and thus were not randomized to maintenance treatment.

The median TTP of the biomarker cohort was 238 days (range 57–643 d) from start of induction treatment. For the eight patients of the biomarker cohort that had PD at first CT evaluation, the TTP range was 57–65 d with a median of 8 weeks and 4 d. Importantly, the time interval from start of induction treatment to the first CT evaluation (8–12 weeks) as pre-specified in the protocol to allow for expected treatment cycle delays, thus had only marginal effects on TTP results. The baseline plasma sample A was taken within 7 d before start of induction treatment according to the ACT2 study protocol. The median time from start of first cycle of induction treatment to date of sample B (at start of treatment cycle three or four) was 42 d (total range 35–75 d, inter-quartile range 42–49 d). The main reason for delay of induction treatment cycles was toxicity of chemotherapy, accounting for the longer interval between induction treatment start and sample B in some patients.

Table 1. Baseline patient characteristics of the ACT2 biomarker cohort ($n = 97$).

Characteristic	No. of patients	Percentage (%)
Age, years, median (range)	64 (37–79)	
Gender, F/M	37/60	38/62
ECOG, PFS 0/1	66/31	68/32
HT diagnosis at baseline, yes/no	41/56	42/58
Induction regimen		
Bev + FOLFOX/XELOX	17/35	54
Bev + FOLFIRI/XELIRI	36/9	46
Best response to induction treatment		
PR	50	52
SD	39	40
PD	8	8
KRAS status		
wild type	46	47
mutant	49	51
unknown	2	2
Randomized, yes/no	79/18	81/19
Maintenance regimen arms	(n = 79)	
wt-BE	20	25
wt-B/mut-B	18/20	48
mut-C	21	27

ECOG PFS: Eastern Cooperative Oncology Group performance score; HT: hypertension; induction chemotherapy regimen (maximum 18 weeks): Bev: bevacizumab; FOLFOX/XELOX: oxaliplatin +5FU/capecitabine, FOLFIRI/XELIRI: irinotecan +5FU/capecitabine; PR: partial response; SD: stable disease; PD: progressive disease; KRAS: Kirsten rat sarcoma oncogene; definitions of maintenance regimen arms: wt/mut = KRAS wild type/mutant; B: bevacizumab; E: erlotinib; C: metronomic capecitabine.

Peptide concentrations and clinical outcome

Initially, we addressed potential associations between baseline concentrations (sample A) of each peptide and TTP. Negative and non-significant correlations were found for all the three peptides (MRpro-ADM: $r_s = -.08$; $p = .42$, MRpro-ANP: $r_s = -.05$; $p = .60$, Copeptin: $r_s = -.06$; $p = .56$). Thus, none of the peptide marker concentrations measured before treatment start did predict outcome in terms of TTP. Changes in vasoactive peptides, expressed as the ratio (B/A) between the 6-week sample (sample B) and baseline sample (A), were then correlated with clinical outcome in terms of ORR (PR, SD, or PD, respectively) (Figure 2). The results revealed negative correlation coefficients between each peptide ratio and the ordered objective response variable, coded 1 = PR, 2 = SD, and 3 = PD, i.e., an increasing peptide concentration was associated with a better ORR. The rank correlation was statistically significant for MRpro-ADM ($r_s = -.36$; $p = .0003$, Figure 2(A)) and MRpro-ANP ($r_s = -.20$; $p = .05$, Figure 2(B)). A slightly weaker and non-significant association was observed for Copeptin ($r_s = -.17$; $p = .10$, Figure 2(C)). In accordance with these results, we found a positive association between an increasing peptide concentration and

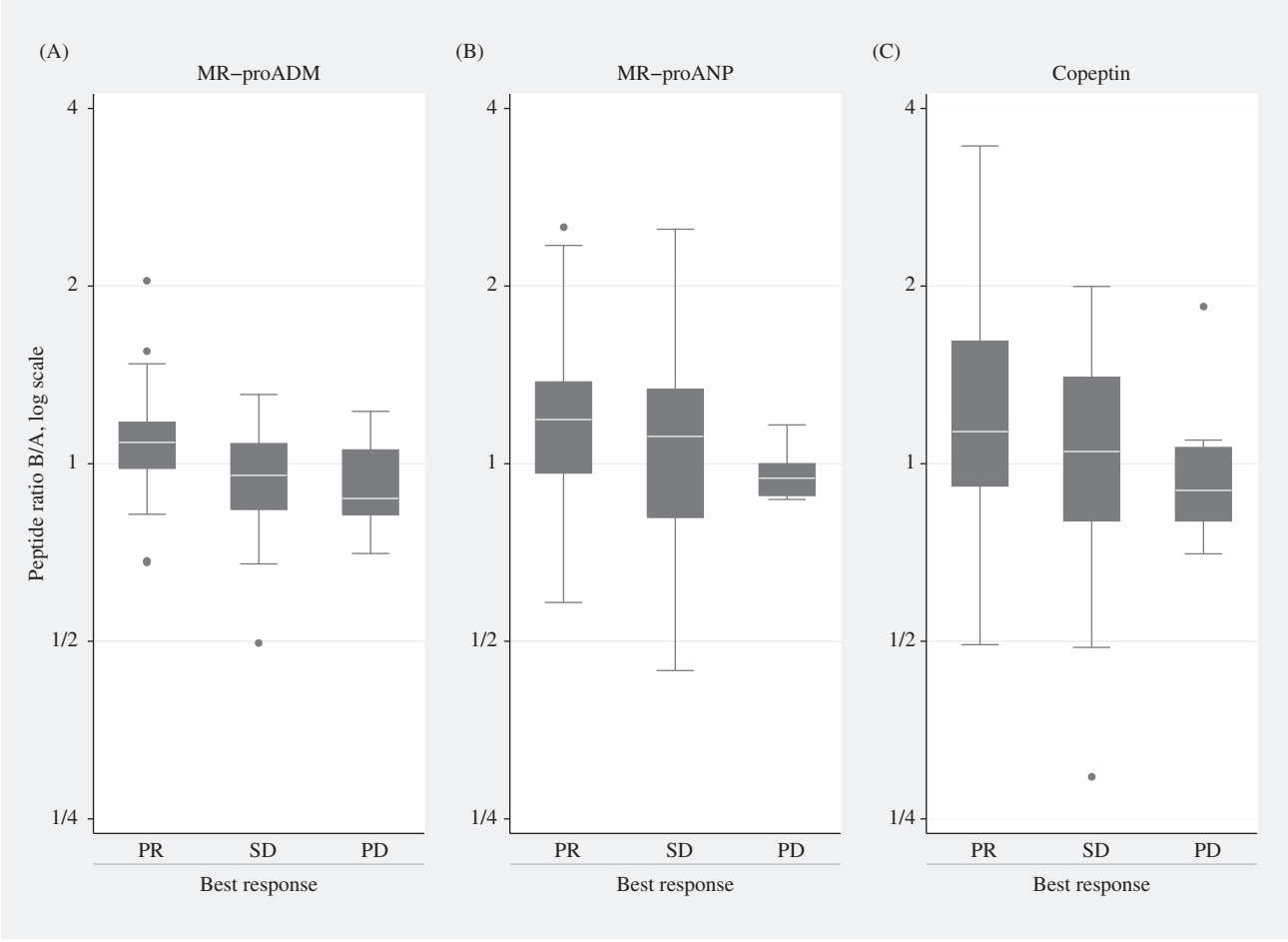

Figure 2. Peptide ratio (B/A) versus clinical outcome (best response). (A) MR-pro-adrenomedullin: $r_s = -.36$; $p = .0003$. (B) MR-pro-atrial-natriuretic peptide: $r_s = -.20$; $p = .05$. (C) Copeptin: $r_s = -.17$; $p = .10$. MR: mid-regional. r_s = Spearman's rank correlation coefficient. Peptide ratio = vasoactive peptide plasma concentrations ratio of sample B to A (at approximately 6 weeks/baseline) associate with best objective tumor response recorded in the induction phase. PR: partial response; SD: stable disease; PD: progressive disease.

prolonged TTP for all three peptides (MRpro-ADM: $r_s = .20$; $p = .05$; MRpro-ANP: $r_s = .22$; $p = .03$; and Copeptin: $r_s = .23$; $p = .02$) (Figure 3). To better illustrate a possible clinical impact of these associations, we also used *t*-tests to study relationships between dichotomized peptide ratios (above versus below 1.00) and TTP. Patients with increasing values of MRpro-ADM (B/A ratio >1.00, $n = 53$) had on average 45 d longer TTP than patients with decreasing levels of this peptide (B/A ratio <1.00, $n = 44$), (mean TTP 269 versus 224 d, 95% CI: 0.6–90, $p = .05$). Similar data for increasing MRpro-ANP (B/A ratio >1.00, $n = 65$) were 41 d longer mean TTP (262 versus 221 d, 95% CI: −6.6 to 88, $p = .09$), and for Copeptin (B/A ratio >1.00, $n = 57$) 39 d longer mean TTP (265 versus 225 d, 95% CI: −6.1 to 85, $p = .09$) than patients with decreasing peptide levels for the respective peptide.

A multiple linear regression model was then fitted for prediction of TTP using peptide ratios (B/A) for MR-proADM, MR-proANP, and Copeptin dichotomized at 1.00, i.e., increasing versus non-increasing concentrations. According to this model, the expected TTP is 192 d if all the three peptide ratios are decreasing. The expected TTP increase is 35 d [95% CI: −11 to +80] if MR-proADM increases, adjusted for the status of the other two peptides in the model. Similarly, the adjusted expected TTP increase is 30 d [95% CI: −18 to +78] if MR-proANP increases, and 30 d [95% CI: −15 to 76] if Copeptin increases, summing to 35 + 30 + 30 = 95 d longer TTP for patients with increasing levels of all the three peptides compared to patients with decreasing levels. To further illustrate the impact of simultaneous change in peptide concentrations on TTP, patients were divided into three groups: non-increasing concentrations for all three peptides ($n = 10$), increasing concentrations for only one or two of the peptides ($n = 59$), and increasing concentrations for all the three peptides ($n = 28$). The median TTP for these three groups were 222, 225, and 284 d ($p = .04$, Kruskal –Wallis test) with a stronger evidence of different TTP distributions for the two latter groups ($p = .02$, Mann –Whitney test) (Table 2).

These data suggest that increasing levels of three separate vasoactive peptides between baseline and approximately 6 weeks of treatment with a bevacizumab-containing chemotherapy regimen were associated with improved outcome in terms of TTP and ORR.

Hypertension and clinical outcome

To address whether the above data were independent on associations between patient outcome and hypertension,

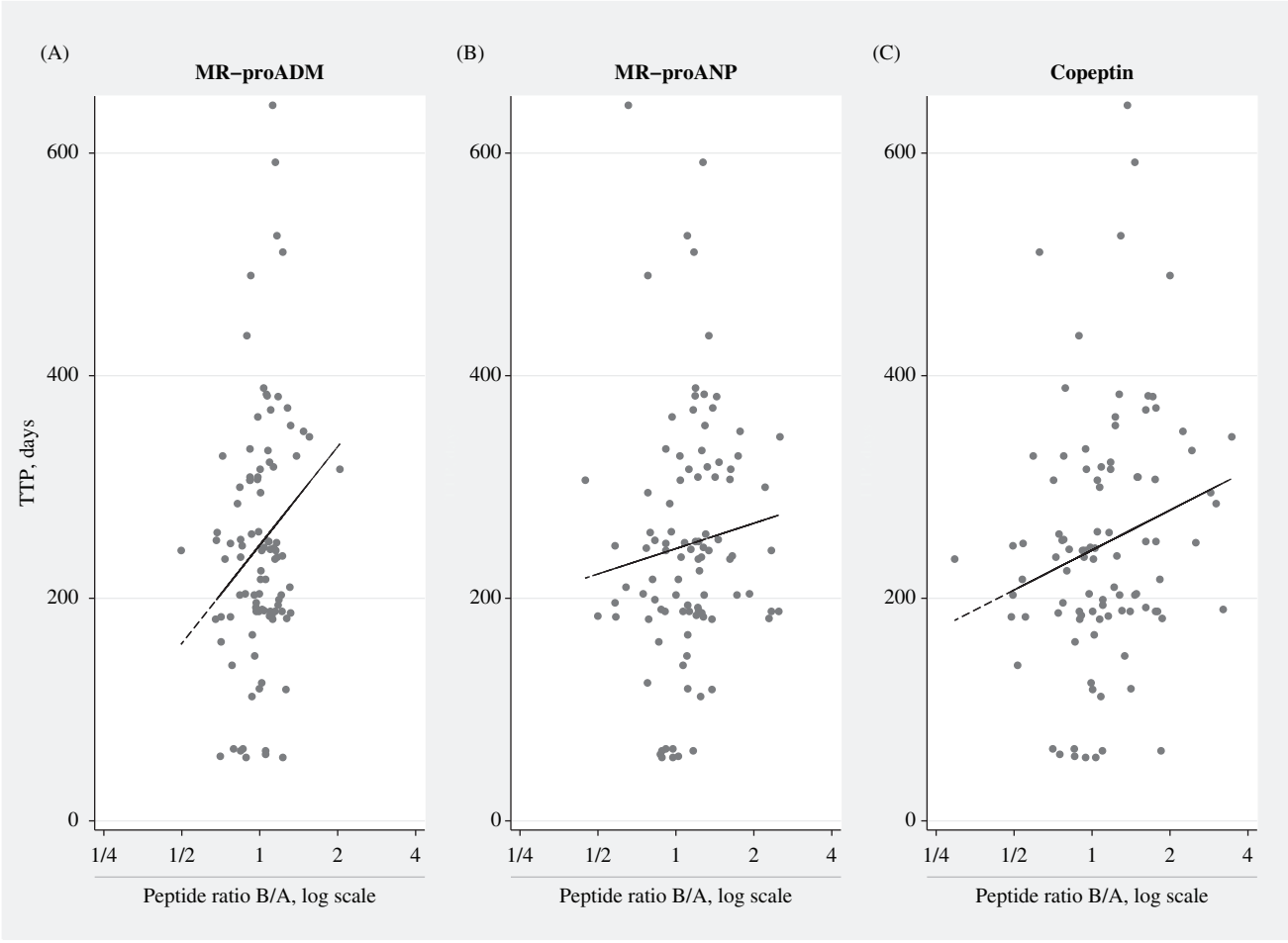

Figure 3. Clinical outcome (TTP) versus peptide ratio (B/A). (A) MR-pro-adrenomedullin: $r_s = .20$; $p = .05$. (B) MR-pro-atrial-natriuretic peptide: $r_s = .22$; $p = .03$. (C) Copeptin: $r_s = 0.23$; $p = .02$. MR: mid-regional. r_s = Spearman's rank correlation coefficient. Peptide ratio = vasoactive peptide plasma concentrations ratio of sample B to A (at approximately 6 weeks/baseline) associate with TTP: time to tumor progression.

Table 2. Dichotomized peptide ratio score ($n = 97$).

	n	Median TTP (days)	IQR (days)	*p*
All three peptide ratios ≤1 (levels equal or decreasing)	10	222	183-249	.70[a]
One or two peptide ratios >1 (1–2 out of the three peptide levels increasing)	59	225	60-436	
All three peptide ratios >1 (levels increasing)	28	284	188-382	.02[a]
				.04[b]

[a]Mann–Whitney-rank-sum test comparing median TTP pairwise using patients with one or two of the three peptide B/A ratios >1.00 as a reference.
[b]Overall 2df-test of equal TTP in the three groups (Kruskal–Wallis test).
TTP: time to tumor progression; IQR: inter quartile range; peptides: MR: mid-regional; pro-ADM: pro-adrenomedullin; pro-ANP: pro-atrial-natriuretic peptide; copeptin: C-terminal-prepro-vasopressin; peptide ratio: vasoactive peptide concentration of sample B (at approximately 6 weeks) versus A (at baseline).

we next correlated available blood pressure data with TTP. The median TTP for patients with and without diagnosis of hypertension at baseline (yes/no) was 239 and 238 d, respectively, with a rank correlation close to zero between TTP and the binary hypertension variable ($r_s = -.05$; $p = .64$, Spearman's test) (Supplementary Figure 2(A)). A non-significant trend towards shorter TTP with an increasing grade of hypertension at 6 weeks was observed (median TTP: grade 0–1: 251 d, grade 2: 243 d, and grade 3: 194 d; $r_s = -.18$; $p = .07$) (Supplementary Figure 2(B)). Accordingly, no association could be found between an early rise in blood pressure and increased TTP in this cohort.

Peptide concentrations and hypertension

Finally, we investigated a possible link between increasing levels of vasoactive peptides and hypertension. However, we found no evidence of an association between peptide ratios and hypertension grade at approximately 6 weeks in our cohort (MRpro-ADM: $r_s = .005$; $p = .96$, MRpro-ANP: $r_s = -.03$; $p = .74$, Copeptin: $r_s = -.04$; $p = .66$) (Supplementary Figure 3).

Discussion

In this prospective-retrospective study, we found that early changes of circulating levels of three separate vasoactive

peptides, MR-proADM, MR-proANP, and Copeptin, are associated with an improved treatment outcome in mCRC patients receiving first-line treatment with a bevacizumab-containing regimen. Substantial efforts have been directed at the identification of biomarkers predicting the efficacy of anti-angiogenic therapy, including tumor phenotype characteristics, circulating angiogenesis-related proteins, circulating tumor cells, and endothelial progenitor cells. Pharmacogenetic studies of polymorphisms in genes of angiogenesis pathways, and new imaging guided criteria to predict treatment effects have also been explored [22]. Although these studies have resulted in an increased understanding of tumor angiogenesis, it currently remains uncertain whether any of these approaches will provide a useful baseline biomarker to predict the effect of anti-angiogenic treatment and change clinical praxis. In the present study, we have explored an alternative approach based on the hypothesis that dynamic effects of angiogenesis inhibition on the cardiovascular system of the host are correlated with effects on the tumor vasculature.

Hypertension is a common clinical manifestation of anti-angiogenic treatment, and some studies have linked this side effect to tumor response [4–9]. Accordingly, systemic factors associated with blood pressure regulation should represent interesting candidates for response biomarkers of angiogenesis inhibition. We thus investigated early changes in circulating vasoactive peptides to monitor effects on cardiovascular pressure load and explore associations with treatment effects caused by angiogenesis inhibition. In a subgroup of patients who had increased levels for all three peptides, a prolongation of 2 months in TTP was demonstrated as compared with the group with an increase in only one or two of the peptide levels (Table 2). Although these results support the hypothesis of a link between treatment-induced effects on the systemic and tumor vasculature, the combined peptide analysis was based on small subgroups and the clinical impact of these findings should be interpreted with caution.

The biological actions of ADM and ANP are similar in their ability of inducing vasodilatation, diuresis and natriuresis, whereas vasopressin, as measured by Copeptin, is a vasoconstrictor. Interestingly, in addition to their effects on vascular tone and salt and water balance, vasoactive peptides may have direct effects on angiogenesis. ADM is a potent, pro-angiogenic factor, and genetic or pharmacological targeting of ADM resulted in reduced angiogenic and tumorigenic potential in CRC xenograft studies [23]. Our finding that an increased level of MR-proADM was associated with better clinical outcome thus appears paradoxical. However, ADM can act as a potent promoter of endothelial barrier stabilization, a process known as vascular normalization that supports improved tumor bioavailability of cytostatic agents [24,25]. A strong vasoactive peptide response to the vascular rarefaction and increased cardiovascular pressure load induced by VEGF-inhibition may thus open a window of opportunity for better synergy with chemotherapy. This notion is supported by our finding of an association between increased peptide levels and ORR during the induction phase.

In line with the comprehensive analysis by Hurwitz et al. [10], we found no significant correlation between the grade of hypertension during early induction treatment phase and treatment response in terms of TTP. Also, we found no significant association between changes in peptide concentrations and hypertension grade. Hypertension was graded retrospectively by using the CTCAE version 4.0, which besides variations in blood pressure also takes changes in antihypertensive medication into account, i.e., the risk of underestimating the grade of hypertension should be low. Notably, a single blood pressure measurement defined as hypertension grade 1 according to CTCAE 4.0 would not necessarily be considered as significant hypertension in clinical oncology. Moreover, grade 1 reflects the high normal/pre-hypertensive state, as defined by the European and American guidelines for blood pressure monitoring and antihypertensive treatment [18]. Consequently, we chose to analyze grade 0 and grade 1 hypertension as one group. The use of different classification systems, e.g., NCI-CTCAE 3.0 versus CTCAE 4.0, diverging cut off values and frequencies of blood pressure monitoring may partly explain contradictions in the literature. Further, variations in the patient's position, stress reaction in the treatment situation, compliance to antihypertensive drugs, and method of measurement introduce significant methodological bias, altogether pointing at blood pressure measurement as an unreliable surrogate biomarker of anti-angiogenic treatment response in clinical praxis.

The present study was based on a randomized controlled trial, which ascertains high-quality clinical data, and excluded patients with significant cardiovascular disease that could have obscured peptide measurement data. The reason for using OR and TTP rather than overall survival as clinical endpoints was to avoid possible bias by second and third line treatment effects. Due to the design of the original clinical trial, however, direct links between vasoactive peptides and anti-angiogenesis could not be investigated, since all patients received bevacizumab. Also, different induction chemotherapy schedules were allowed, and in the maintenance phase patients were randomized to receive bevacizumab alone or in combination with erlotinib, or to single low-dose capecitabine. However, increased cardiovascular pressure load and hypertension are uncommon side-effects of chemotherapy. Therefore, it is conceivable that the early changes in vasoactive peptides were mainly caused by bevacizumab, although effects on the cardiovascular system and cardiotoxicity by e.g., fluoropyrimidines, during induction treatment cannot be excluded. Another potential criticism of the design could be that the group treated with metronomic capecitabine as maintenance ($n = 21$) only received bevacizumab in the induction phase. On the contrary, quantified peptide ratios and ORR in induction are independent of maintenance therapy, and results from the original ACT2 trial showed no significant difference in PFS, OS or median duration of maintenance treatment when comparing bevacizumab alone with metronomic capecitabine as maintenance treatment [17]. The inclusion criteria of the present study were based on an intent to explore potential associations between vasoactive peptides and treatment outcome in patients with a well-defined progressive disease during the course of first-line treatment, which limit the generalizability of the results. Clearly, the role of vasoactive peptides as possible

biomarkers of the response to anti-angiogenic agents needs to be investigated in further studies using broader inclusion criteria as well as in patients with other tumor types including treatment with other anti-angiogenic agents.

In summary, we conclude that circulating, vasoactive peptides may reflect the patient's vascular response to anti-angiogenic therapy and could represent a novel class of early response biomarkers with potential to improve clinical benefit of angiogenesis inhibition.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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