ORIGINAL RESEARCH ARTICLE

Exploratory Biomarker Analysis Using Plasma Angiogenesis-Related Factors and Cell-Free DNA in the TRUSTY Study: A Randomized, Phase II/III Study of Trifluridine/Tipiracil Plus Bevacizumab as Second-Line Treatment for Metastatic Colorectal Cancer

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Abstract

Background The TRUSTY study evaluated the efficacy of second-line trifluridine/tipiracil (FTD/TPI) plus bevacizumab in metastatic colorectal cancer (mCRC).

Objective This exploratory biomarker analysis of TRUSTY investigated the relationship between baseline plasma concentrations of angiogenesis-related factors and cell-free DNA (cfDNA), and the efficacy of FTD/TPI plus bevacizumab in patients with mCRC.

Patients and Methods The disease control rate (DCR) and progression-free survival (PFS) were compared between baseline plasma samples of patients with high and low plasma concentrations (based on the median value) of angiogenesis-related factors. Correlations between cfDNA concentrations and PFS were assessed.

Results Baseline characteristics ($n = 65$) were as follows: male/female, 35/30; median age, 64 (range 25–84) years; and *RAS* status wild-type/mutant, 29/36. Patients in the hepatocyte growth factor (HGF)-low and interleukin (IL)-8-low groups had a significantly higher DCR (risk ratio [95% confidence intervals {CIs}]) than patients in the HGF-high (1.83 [1.12–2.98]) and IL-8-high (1.70 [1.02–2.82]) groups. PFS (hazard ratio {HR} [95% CI]) was significantly longer in patients in the HGF-low (0.33 [0.14–0.79]), IL-8-low (0.31 [0.14–0.70]), IL-6-low (0.19 [0.07–0.50]), osteopontin-low (0.39 [0.17–0.88]), thrombospondin-2-low (0.42 [0.18–0.98]), and tissue inhibitor of metalloproteinase-1-low (0.26 [0.10–0.67]) groups versus those having corresponding high plasma concentrations of these angiogenesis-related factors. No correlation was observed between cfDNA concentration and PFS.

Conclusion Low baseline plasma concentrations of HGF and IL-8 may predict better DCR and PFS in patients with mCRC receiving FTD/TPI plus bevacizumab, however further studies are warranted.

Clinical Trial Registration Number jRCTs031180122.

1 Introduction

The 2023 National Comprehensive Cancer Network clinical practice guidelines for colorectal cancer (CRC) recommend chemotherapy (5-fluorouracil [5-FU] and l-leucovorin [l-LV] with oxaliplatin [FOLFOX] or irinotecan [FOLFIRI], or both [FOLFOXIRI]) along with targeted biological agents (e.g., bevacizumab, cetuximab, and panitumumab) as first- or second-line treatment options for metastatic CRC (mCRC) [1]. In addition, the guidelines recommend trifluridine/tipiracil

Key Points

This was an exploratory biomarker analysis that investigated the correlation between the concentrations of angiogenesis-related factors and cell-free DNA and the treatment efficacy of trifluridine/tipiracil plus bevacizumab in patients with metastatic colorectal cancer.

Patients with below-median baseline plasma hepatocyte growth factor and interleukin-8 levels had significantly higher disease control rate and longer progression-free survival than those with above-median levels.

△ Adis

(FTD/TPI) with or without bevacizumab as a treatment option for patients with mCRC who have had progression with standard therapies [1]. The SUNLIGHT trial showed that FTD/TPI plus bevacizumab significantly prolonged survival compared with FTD/TPI alone as third-line or later treatment for patients with mCRC [2]. Furthermore, FTD/TPI plus bevacizumab prolonged survival in patients with mCRC irrespective of the *RAS* status and is the standard third-line treatment in patients with mCRC [2].

Considering the poor outcome of mCRC, there is a need for reliable measures to predict treatment response [3]. Biomarkers can be useful tools for predicting treatment response in patients with mCRC [4]. *RAS* gene mutations are established as a negative predictive factor for the efficacy of anti-epidermal growth factor receptor antibodies such as panitumumab and cetuximab in mCRC [5–8].

Plasma concentrations of angiogenesis factors have been used as biomarkers of response to angiogenesis inhibitors used in first- and second-line chemotherapy [9]. In patients with mCRC who received second-line treatment, high levels of plasma vascular endothelial growth factor (VEGF)-D were identified as a potential predictive biomarker for ramucirumab efficacy, and high serum levels of VEGF-A and placental growth factor (PlGF) were found to underlie the development of resistance to bevacizumab [10, 11]. Increased baseline levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and interleukin (IL)-8 have been associated with a higher risk of death and shorter progression-free survival (PFS) in patients with CRC than in those who had lower levels of these biomarkers [12, 13]. Cell-free DNA (cfDNA) is a minimally invasive and clinically relevant quantitative biomarker of tumor burden that can predict survival in patients with mCRC [14, 15]. However, biomarkers for the efficacy of FTD/TPI plus bevacizumab have not been clearly established.

The TRiflUridine/tipiracil in Second-line sTudY (TRUSTY) was a phase II/III randomized controlled trial conducted to determine whether FTD/TPI plus bevacizumab was non-inferior to FOLFIRI plus bevacizumab or S-1 and irinotecan plus bevacizumab (control group) in terms of overall survival (OS) among patients with mCRC; the non-inferiority of FTD/TPI plus bevacizumab was not demonstrated [16]. The purpose of this exploratory biomarker study was to investigate the correlation between the concentrations of angiogenesis-related factors and cfDNA and treatment efficacy of FTD/TPI plus bevacizumab using plasma samples obtained from TRUSTY.

2 Material and Methods

2.1 Study Design and Patients

The design of TRUSTY has been reported previously [17]. Briefly, this was an open-label, multicenter, randomized, comparative, phase II/III study conducted at 65 institutions in Japan. The key inclusion criteria were patients aged ≥ 20 years with histologically confirmed mCRC who did not respond to first-line chemotherapy with fluoropyrimidine (5-FU/l-LV, capecitabine, or S-1) plus oxaliplatin combined with bevacizumab, cetuximab, or panitumumab for patients with *RAS* wild-type tumors; with an Eastern Cooperative Oncology Group performance status of 0 or 1; evaluable lesions, as observed on imaging; and adequate organ function [16]. Written informed consent was obtained from all patients. Eligible patients were subsequently randomized in a 1:1 ratio to either the fluoropyrimidine and irinotecan plus bevacizumab group (control group) or the FTD/TPI plus bevacizumab group (bevacizumab 5 mg/kg by intravenous infusion on days 1 and 15, and oral administration of FTD/TPI 35 mg/m^2 twice daily on days 1–5 and 8–12 in a 28-day cycle). TRUSTY was terminated at the interim analysis for futility because of a low possibility for non-inferiority, even if the study would have been completed [16]. This biomarker study was planned after the registration to TRUSTY began. Only patients who provided separate written informed consent were included in this biomarker analysis.

2.2 Sample Collection and Evaluation of Angiogenesis-Related Factors and Cell-Free DNA (cfDNA)

Blood samples for the control and FTD/TPI plus bevacizumab groups were prospectively collected before the start of study treatment. The collected blood samples were centrifuged at 10,000×*g* for 10 min to separate the plasma samples. The MILLIPLEX® map assay kit (Merck Millipore, Billerica, MA, USA) was used to evaluate 17 factors associated with angiogenesis in the plasma samples of patients with mCRC: angiopoietin-2, hepatocyte growth factor (HGF), interferon gamma (IFN-γ), IL-6, IL-8, PlGF, VEGF-A, VEGF-D, osteopontin (OPN), soluble neuropilin-1 (sNeuropilin-1), soluble VEGF receptor (sVEGFR)-1, sVEGFR-2, sVEGFR-3, thrombospondin-2 (TSP-2), sICAM-1, sVCAM-1, and tissue inhibitor of metalloproteinase-1 (TIMP-1). All assays for proteins were performed according to the manufacturer's recommended protocols. The assay plate was then analyzed using the Luminex® 100/200™ instrument (Luminex Corporation,

Fig. 1 Patient disposition. *BEV* bevacizumab, *FP* fluoropyrimidine, *FTD/TPI* trifluridine/tipiracil, *IRI* irinotecan
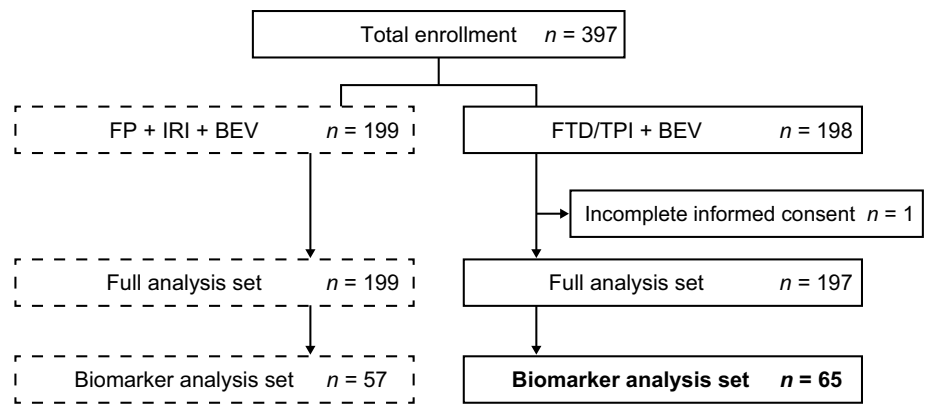
Austin, TX, USA). Total cfDNA was isolated using the QIAamp Circulating Nucleic Acid Kit (50) with the QIAvac (Qiagen, Hilden, Germany). The cfDNA concentration was measured using the TaqMan Copy Number Reference Assay, RNase P with StepOnePlus (Thermo Fisher Scientific, Foster City, CA, USA).

2.3 Outcomes

Disease control rate (DCR) and PFS were compared between patients with high and low concentrations of each biomarker in the FTD/TPI plus bevacizumab and control groups. In addition, correlations between the cfDNA concentration and PFS were evaluated. DCR was defined as the proportion of complete or partial responses or stable disease for more than 6 weeks from the initiation of study treatment. PFS was defined as the period from the date of enrollment to the earliest date of disease progression or death due to any cause, whichever occurred first.

2.4 Statistical Analysis

Using the median value of each angiogenesis-related factor and cfDNA as the cut-off, patients were divided into the high and low plasma concentration groups. The median value was calculated for all patients, including patients in both the control and FTD/TPI plus bevacizumab groups. For IFN-γ, IL-6, and VEGF-A, patients were divided into high (detectable) and low (undetectable) groups because > 50% of patients had baseline plasma concentrations below the lower limit of detection. For DCR, risk ratio (RR) and the corresponding 95% confidence intervals (CIs) were calculated. PFS was estimated using the Kaplan–Meier method. Hazard ratios (HRs) and CIs were calculated using the Cox proportional hazards model with only category (< median, ≥ median) as a covariate. Correlations between the cfDNA concentration and

PFS were assessed using Spearman's rank correlation coefficient. All statistical analyses were performed using SAS® software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

3 Results

3.1 Patient Disposition

Patients were enrolled in TRUSTY between 2 October 2017 and 16 July 2020 [16]; those who were enrolled after 5 September 2019 were included in this biomarker study. Of the 397 patients enrolled in TRUSTY, 198 were randomized to the FTD/TPI plus bevacizumab group and 199 to the control group. Plasma samples were available for 65 patients in the FTD/TPI plus bevacizumab group and 57 patients in the control group (biomarker analysis population) (Fig. 1).

3.2 Demographics and Baseline Clinical Characteristics

The biomarker analysis population had similar baseline characteristics to those of the intention-to-treat population [16]. In the FTD/TPI plus bevacizumab group, 35 (53.8%) patients were male and the median (range) age was 64 (25–84) years. *RAS* mutation was present in 36 (55.4%) patients and 43 (66.2%) patients had two or more metastatic lesions. Bevacizumab was used as a first-line treatment in 48 (73.8%) patients. The demographics and baseline clinical characteristics of patients in the control group were generally similar to those of patients in the FTD/TPI plus bevacizumab group (Table 1). The median values for each angiogenesis-related factor and cfDNA at baseline are presented in electronic supplementary material (ESM) Table S1.

Table 1 Baseline characteristics of patients in the FTD/TPI plus BEV group and the control group in the biomarker analysis population and the ITT population

Characteristics	Biomarker analysis population		ITT population	
	FTD/TPI plus BEV [n = 65]	Control group [n = 57]	FTD/TPI plus BEV [n = 197]	Control group [n = 199]
Sex				
Male	35 (53.8)	32 (56.1)	94 (47.7)	99 (49.7)
Female	30 (46.2)	25 (43.9)	103 (52.3)	100 (50.3)
Age, years	64.0 (25–84)	68.0 (32–81)	67.0 (25–84)	68.0 (32–82)
< 65	33 (50.8)	23 (40.4)	80 (40.6)	75 (37.7)
≥ 65	32 (49.2)	34 (59.6)	117 (59.4)	124 (62.3)
ECOG performance status				
0	38 (58.5)	32 (56.1)	120 (60.9)	124 (62.3)
1	27 (41.5)	25 (43.9)	77 (39.1)	75 (37.7)
RAS status				
Wild-type	29 (44.6)	24 (42.1)	79 (40.1)	79 (39.7)
Mutant	36 (55.4)	33 (57.9)	118 (59.9)	120 (60.3)
Primary tumor location[a]				
Left side	52 (80.0)	44 (77.2)	150 (76.1)	149 (74.9)
Right side	13 (20.0)	13 (22.8)	47 (23.9)	50 (25.1)
Number of metastatic lesions				
0 or 1	22 (33.8)	27 (47.4)	70 (35.5)	82 (41.2)
≥ 2	43 (66.2)	30 (52.6)	127 (64.5)	117 (58.8)
PFS in first-line treatment				
≥ 9 months	44 (67.7)	42 (73.7)	130 (66.0)	131 (65.8)
< 9 months	21 (32.3)	15 (26.3)	67 (34.0)	68 (34.2)
Biologics used in first-line treatment				
Anti-EGFR antibody	17 (26.2)	12 (21.1)	37 (18.8)	35 (17.6)
BEV	48 (73.8)	45 (78.9)	160 (81.2)	164 (82.4)

Data are expressed as *n* (%) or median (range)

BEV bevacizumab, *ECOG* Eastern Cooperative Oncology Group, *EGFR* epidermal growth factor receptor, *FTD/TPI* trifluridine/tipiracil, *ITT* intention-to-treat, *PFS* progression-free survival, *RAS* rat sarcoma virus

[a]Tumors located in the cecum, ascending colon, and transverse colon were considered right-sided; tumors located within the splenic flexure and beyond were considered left-sided

3.3 Disease Control Rate (DCR) and Progression-Free Survival (PFS) in the Biomarker Analysis Set

The median duration of follow-up was 4.5 months (range 0.1–9.9) as of 16 July 2020 (data cut-off). For the biomarker analysis population, the DCR was 55.4% (95% CI 42.5–67.7) and the median PFS was 3.9 months (95% CI 3.3–5.7) in the FTD/TPI plus bevacizumab group (ESM Table S2 and ESM Fig. S1).

3.4 DCR and PFS According to the Plasma Concentrations of Angiogenesis-Related Factors and cfDNA at Baseline in the Biomarker Analysis Set

In the FTD/TPI plus bevacizumab group, patients in the HGF-low (RR 1.83, 95% CI 1.12–2.98) and IL-8-low (RR 1.70, 95% CI 1.02–2.82) groups had significantly better DCR than those in the HGF-high and IL-8-high groups. No significant difference was observed between the high and low groups for the remaining angiogenesis-related factors and cfDNA (Fig. 2). Similar to the DCR results, PFS was significantly longer in the HGF-low (5.5 vs. 3.3 months; HR 0.33, 95% CI 0.14–0.79) and IL-8-low groups (5.5 vs. 3.3 months; HR 0.31, 95% CI 0.14–0.70) than in the HGF-high and IL-8-high groups. Patients with low
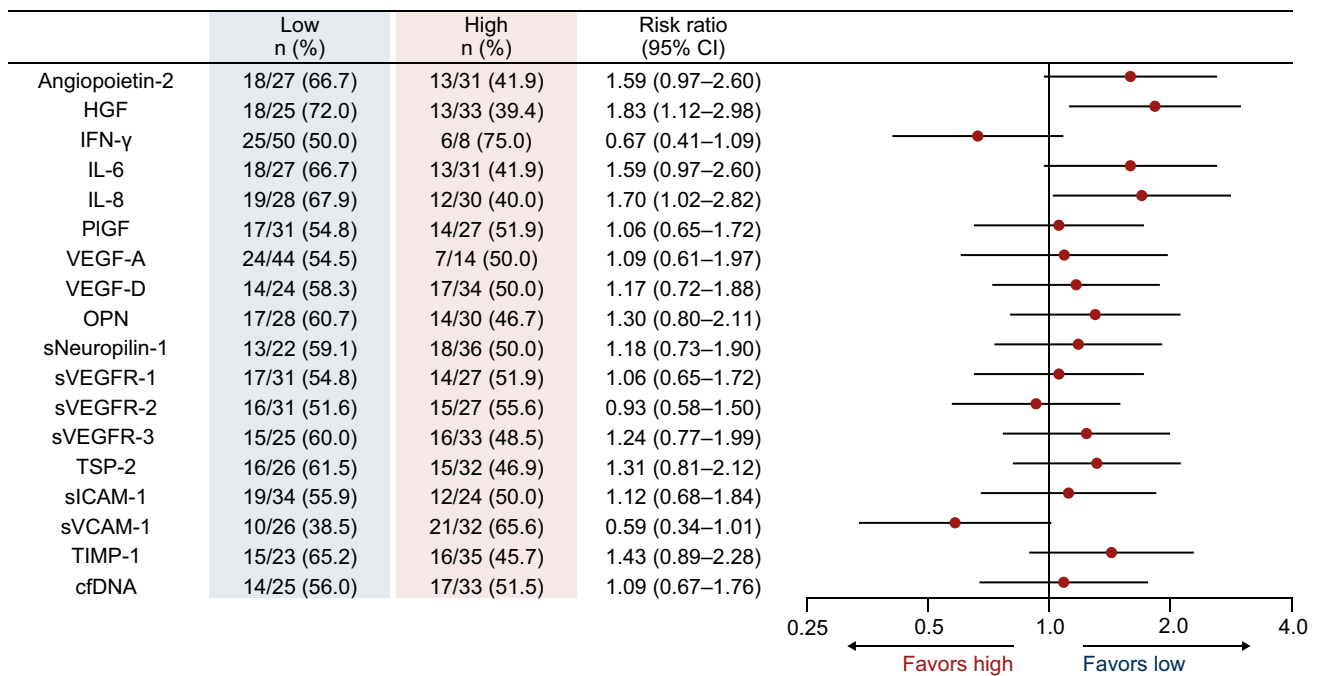
	Low n (%)	High n (%)	Risk ratio (95% CI)	
Angiopoietin-2	18/27 (66.7)	13/31 (41.9)	1.59 (0.97–2.60)	
HGF	18/25 (72.0)	13/33 (39.4)	1.83 (1.12–2.98)	
IFN-γ	25/50 (50.0)	6/8 (75.0)	0.67 (0.41–1.09)	
IL-6	18/27 (66.7)	13/31 (41.9)	1.59 (0.97–2.60)	
IL-8	19/28 (67.9)	12/30 (40.0)	1.70 (1.02–2.82)	
PIGF	17/31 (54.8)	14/27 (51.9)	1.06 (0.65–1.72)	
VEGF-A	24/44 (54.5)	7/14 (50.0)	1.09 (0.61–1.97)	
VEGF-D	14/24 (58.3)	17/34 (50.0)	1.17 (0.72–1.88)	
OPN	17/28 (60.7)	14/30 (46.7)	1.30 (0.80–2.11)	
sNeuropilin-1	13/22 (59.1)	18/36 (50.0)	1.18 (0.73–1.90)	
sVEGFR-1	17/31 (54.8)	14/27 (51.9)	1.06 (0.65–1.72)	
sVEGFR-2	16/31 (51.6)	15/27 (55.6)	0.93 (0.58–1.50)	
sVEGFR-3	15/25 (60.0)	16/33 (48.5)	1.24 (0.77–1.99)	
TSP-2	16/26 (61.5)	15/32 (46.9)	1.31 (0.81–2.12)	
sICAM-1	19/34 (55.9)	12/24 (50.0)	1.12 (0.68–1.84)	
sVCAM-1	10/26 (38.5)	21/32 (65.6)	0.59 (0.34–1.01)	
TIMP-1	15/23 (65.2)	16/35 (45.7)	1.43 (0.89–2.28)	
cfDNA	14/25 (56.0)	17/33 (51.5)	1.09 (0.67–1.76)	

0.25 0.5 1.0 2.0 4.0
← Favors high   Favors low →

Fig. 2 DCR according to baseline plasma concentrations of angiogenesis-related factors and cfDNA in the FTD/TPI plus bevacizumab group. *cfDNA* cell-free DNA, *CI* confidence interval, *DCR* disease control rate, *FTD/TPI* trifluridine/tipiracil, *HGF* hepatocyte growth factor, *IFN-γ* interferon gamma, *IL* interleukin, *OPN* osteopontin, *PIGF* placental growth factor, *sICAM-1* soluble intercellular adhesion molecule-1, *sNeuropilin-1* soluble neuropilin-1, *sVCAM-1* soluble vascular cell adhesion molecule-1, *sVEGFR* soluble vascular endothelial growth factor receptor, *TIMP-1* tissue inhibitor of metalloproteinase-1, *TSP-2* thrombospondin-2, *VEGF* vascular endothelial growth factor

plasma concentrations of IL-6 (6.0 vs. 3.5 months; HR 0.19, 95% CI 0.07–0.50), OPN (5.5 vs. 3.5 months; HR 0.39, 95% CI 0.17–0.88), TSP-2 (4.9 vs. 3.6 months; HR 0.42, 95% CI 0.18–0.98), and TIMP-1 (7.4 vs. 3.6 months; HR 0.26, 95% CI 0.10–0.67) also had longer PFS than those with high plasma concentrations of these biomarkers (ESM Figs. S2–S4; Fig. 3). No correlation was observed between the cfDNA concentration and PFS (Spearman, *R* = 0.074) (Fig. 4).

In the control group, angiopoietin-2 showed a contrary trend to that in the FTD/TPI plus bevacizumab group. Patients in the VEGF-D-low group had a significantly higher DCR than those in the VEGF-D-high group. As for PFS, angiopoietin-2 and sVEGFR-2 showed a contrary trend to that in the FTD/TPI plus bevacizumab group. Patients who had low plasma concentrations of IL-8 and sICAM-1 had a significantly longer PFS than those who had high plasma concentrations of these biomarkers (ESM Fig. S5).

4 Discussion

This is the first exploratory biomarker analysis to investigate the correlation between baseline concentrations of plasma angiogenesis-related factors and cfDNA with the efficacy of FTD/TPI plus bevacizumab in patients with mCRC. Patients with baseline plasma concentrations of HGF and IL-8 below the median had a higher DCR than those with plasma concentrations above the median. In addition, patients with baseline HGF, IL-6, IL-8, OPN, TSP-2, and TIMP-1 levels below the median had a longer PFS than those with baseline levels above the median. Thus, HGF and IL-8 can be used as predictive biomarkers to preselect patients with mCRC who might respond to FTD/TPI plus bevacizumab.

The angiogenesis-related factors are considered predictive factors for the efficacy of bevacizumab. In this study, although both groups included bevacizumab, the angiogenesis-related factors showing significance differed. Angiogenesis-related factors correlated with bevacizumab might be influenced by the concurrent use of cytotoxic drugs. In the MAX trial, in which bevacizumab was administered in combination with capecitabine and mitomycin to patients with mCRC, patients with a higher VEGF-D expression had a shorter PFS with bevacizumab than those with a lower VEGF-D expression [18]. Furthermore, the predictive value of VEGF-D expression for bevacizumab may depend on the chemotherapy backbone used [18].

The inherent complexity of angiogenesis has been a substantial hurdle in the attempts to develop response-predictive biomarkers to anti-angiogenesis drugs, which may
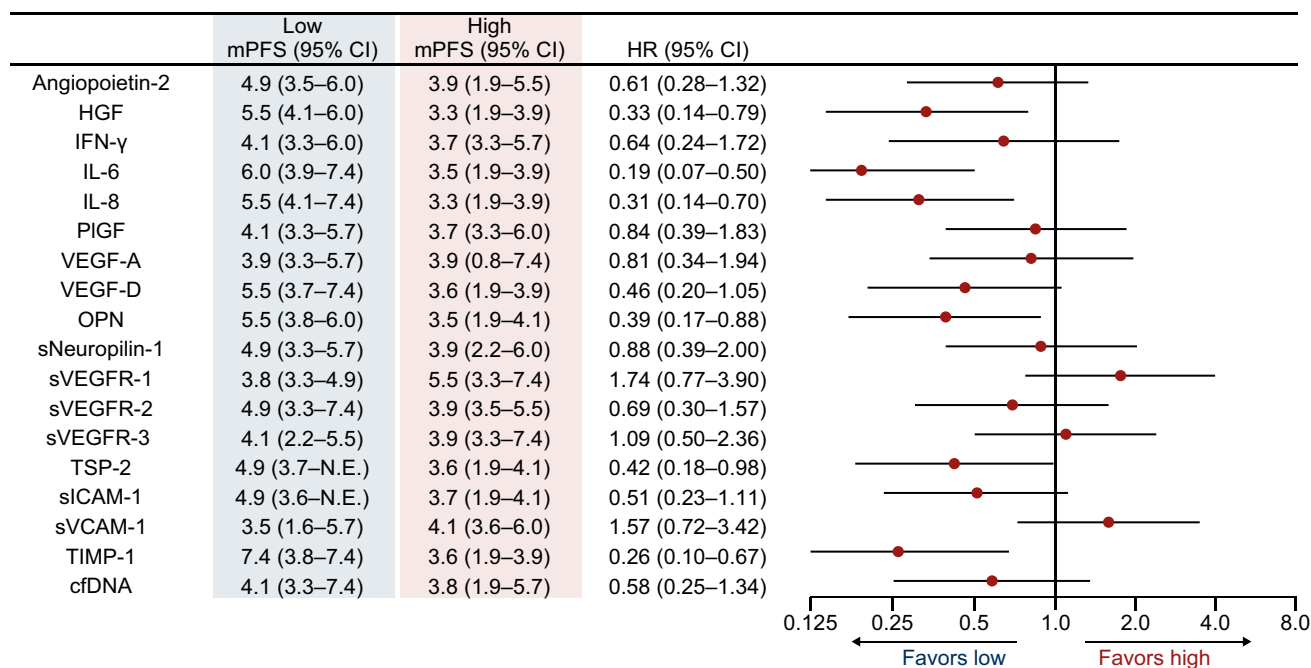
	Low mPFS (95% CI)	High mPFS (95% CI)	HR (95% CI)	
Angiopoietin-2	4.9 (3.5–6.0)	3.9 (1.9–5.5)	0.61 (0.28–1.32)	
HGF	5.5 (4.1–6.0)	3.3 (1.9–3.9)	0.33 (0.14–0.79)	
IFN-γ	4.1 (3.3–6.0)	3.7 (3.3–5.7)	0.64 (0.24–1.72)	
IL-6	6.0 (3.9–7.4)	3.5 (1.9–3.9)	0.19 (0.07–0.50)	
IL-8	5.5 (4.1–7.4)	3.3 (1.9–3.9)	0.31 (0.14–0.70)	
PlGF	4.1 (3.3–5.7)	3.7 (3.3–6.0)	0.84 (0.39–1.83)	
VEGF-A	3.9 (3.3–5.7)	3.9 (0.8–7.4)	0.81 (0.34–1.94)	
VEGF-D	5.5 (3.7–7.4)	3.6 (1.9–3.9)	0.46 (0.20–1.05)	
OPN	5.5 (3.8–6.0)	3.5 (1.9–4.1)	0.39 (0.17–0.88)	
sNeuropilin-1	4.9 (3.3–5.7)	3.9 (2.2–6.0)	0.88 (0.39–2.00)	
sVEGFR-1	3.8 (3.3–4.9)	5.5 (3.3–7.4)	1.74 (0.77–3.90)	
sVEGFR-2	4.9 (3.3–7.4)	3.9 (3.5–5.5)	0.69 (0.30–1.57)	
sVEGFR-3	4.1 (2.2–5.5)	3.9 (3.3–7.4)	1.09 (0.50–2.36)	
TSP-2	4.9 (3.7–N.E.)	3.6 (1.9–4.1)	0.42 (0.18–0.98)	
sICAM-1	4.9 (3.6–N.E.)	3.7 (1.9–4.1)	0.51 (0.23–1.11)	
sVCAM-1	3.5 (1.6–5.7)	4.1 (3.6–6.0)	1.57 (0.72–3.42)	
TIMP-1	7.4 (3.8–7.4)	3.6 (1.9–3.9)	0.26 (0.10–0.67)	
cfDNA	4.1 (3.3–7.4)	3.8 (1.9–5.7)	0.58 (0.25–1.34)	

0.125 0.25 0.5 1.0 2.0 4.0 8.0

← Favors low   Favors high →

Fig. 3 PFS according to baseline plasma concentrations of angiogenesis-related factors and cfDNA in the FTD/TPI plus bevacizumab group. *cfDNA* cell-free DNA, *CI* confidence interval, *FTD/TPI* trifluridine/tipiracil, *HGF* hepatocyte growth factor, *HR* hazard ratio, *IFN-γ* interferon gamma, *IL* interleukin, *mPFS* median progression-free survival, *N.E.* not estimable, *OPN* osteopontin, *PFS* progression-free survival, *PlGF* placental growth factor, *sICAM-1* soluble intercellular adhesion molecule-1, *sNeuropilin-1* soluble neuropilin-1, *sVCAM-1* soluble vascular cell adhesion molecule-1, *sVEGFR* soluble vascular endothelial growth factor receptor, *TIMP-1* tissue inhibitor of metalloproteinase-1, *TSP-2* thrombospondin-2, *VEGF* vascular endothelial growth factor

be attributed to the tumor microenvironment comprising tumor-infiltrating cells, vasculature, extracellular matrix, and other matrix-associated molecules [19, 20]. Fibroblasts are the predominant constituents of the tumor stroma, and HGF is a major component of fibroblast secretome [21]. By binding with c-Met (tyrosine-protein kinase Met or HGF receptor), HGF activates a variety of downstream signaling cascades, such as the ERK-MAPK and PI3K-Akt pathways, consequently triggering cancer cell proliferation, invasion, metastasis, and angiogenesis [22]. Overexpression of c-Met at the protein or messenger RNA (mRNA) level is associated with poor prognosis in various cancers, including CRC [23–27]. High levels of circulating HGF have been reported to be a poor prognostic factor in lung cancer, gastric cancer, and melanoma [28–30].

IL-8 is differentially produced by tumor or stromal components, depending on the CRC genetic background [31]. IL-8 binds to the C-X-C chemokine receptor type 2 (CXCR2), thereby promoting tumor angiogenesis, growth, and metastasis [32, 33]. High levels of IL-8 have been reported to be a poor prognostic factor in various types of cancers [34–36]. Furthermore, high IL-8 levels are significantly correlated with a shorter OS and PFS in CRC [31]. Thus far, no previous studies have reported the association between these factors and FTD/TPI plus bevacizumab; this

is the first study to investigate this association, however confirmation of the results in further studies is required.

The GI-SCREEN CRC UKIT study evaluated the strength of interactions of pretreatment levels of 17 plasma angiogenesis factors (dichotomized according to the median value) on PFS in patients with mCRC treated with chemotherapy in combination with angiogenesis inhibitors [37]. Patients treated with chemotherapy plus bevacizumab and low PlGF had significantly longer PFS, and patients with high VEGF-A or low VEGF-D tended to have a better PFS [37, 38]. However, no difference in PFS was observed between the high and low groups based on the PlGF, VEGF-A, and VEGF-D levels in this biomarker analysis. In accordance with the findings of the current study, the AFFIRM (Study of Aflibercept and Modified FOLFOX6 as First-Line Treatment in Patients with Metastatic Colorectal Cancer) trial found that high circulating plasma concentrations of IL-8 at baseline were correlated with a reduced PFS [39]. In a retrospective exploratory analysis of the biomarker subgroups in the CORRECT (patients with metastatic COloRectal cancer treated with REgorafenib or plaCebo after failure of standard Therapy) trial, a significant association between plasma IL-8 concentrations and OS was noted in a multivariable analysis after controlling for various clinical factors [40].

Fig. 4 PFS by baseline cfDNA concentration in the FTD/TPI plus bevacizumab group. *cfDNA* cell-free DNA, *FTD/TPI* trifluridine/tipiracil, *PFS* progression-free survival, *qPCR* quantitative polymerase chain reaction
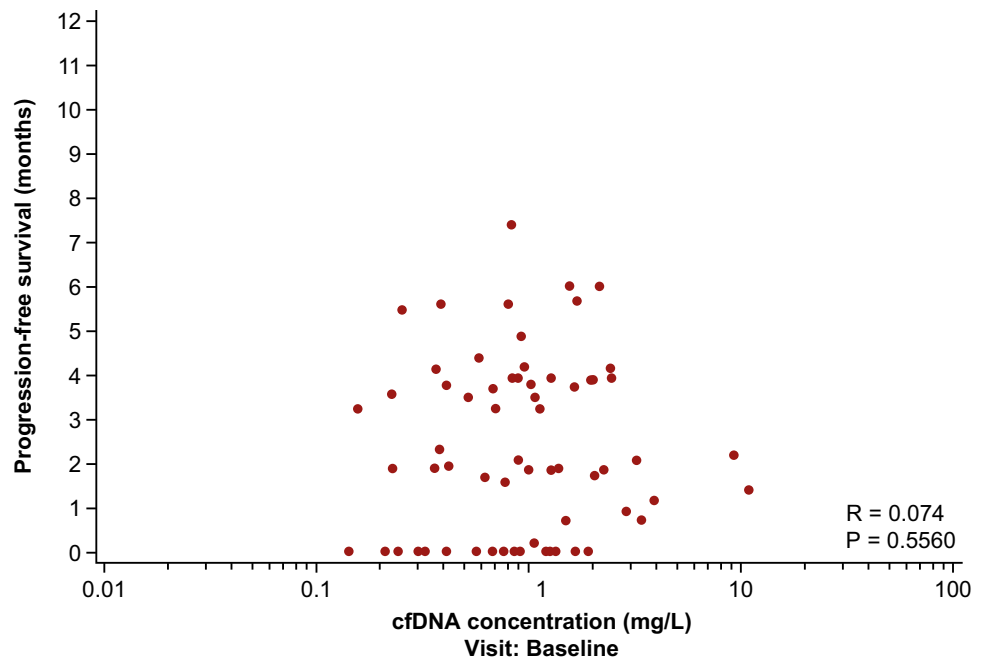
In addition to HGF and IL-8, low levels of IL-6, OPN, TSP-2, and TIMP-1 were found to correlate with a better PFS in the present study. During CRC progression, IL-6 is produced mainly by tumor-associated macrophages, mesenchymal stem cells, or colon cancer-associated fibroblasts [41]. Patients with mCRC who had high baseline serum IL-6 concentrations had a shorter median OS than those who had low baseline serum IL-6 concentrations (16.6 vs. 26.0 months, $p < 0.001$) [42]. OPN is a multifunctional phosphorylated protein widely involved in solid tumor progression, and its expression is negatively correlated with the prognosis of patients with colorectal and head and neck cancers [43]. Thrombospondin is an adhesive protein that stimulates tumor cell invasion into the surrounding tissue [44]. A direct correlation between the plasma thrombospondin levels and degree of venous invasion has been reported in patients with CRC [44]. TIMP-1 is a glycoprotein regulating metalloproteinases and may play a key role in tumor progression [45]. Serum levels of TIMP-1 were elevated in patients with advanced CRC, and patients with high TIMP-1 levels had worse prognosis [46].

The baseline plasma concentration of cfDNA is related to the tumor burden and is a strong prognostic factor in patients with mCRC, with higher levels showing a poor prognosis [47]. However, the current biomarker study found no correlation between cfDNA concentration and PFS. Treatment with FTD/TPI plus bevacizumab may be clinically effective regardless of the tumor volume. Our study suggests that FTD/TPI plus bevacizumab may be

the standard of care for patients with mCRC who have a worse prognosis.

This study has some limitations. First, the follow-up was conducted for a short period owing to the early termination of TRUSTY. Therefore, the relationship between OS and the measured biomarkers could not be evaluated. Second, because only a few blood samples were available, it was not considered appropriate to compare the FTD/TPI plus bevacizumab and control groups. Therefore, this biomarker analysis examined only prognostic factors, not predictive factors, for FTD/TPI plus bevacizumab. Third, this biomarker analysis was exploratory in nature, and a validation study is needed. Lastly, although the median was used as the cut-off value for this biomarker analysis, there may be other appropriate cut-off values.

5 Conclusions

Low baseline plasma concentrations of HGF and IL-8 may better serve as predictors for a higher DCR than high plasma concentrations. Moreover, low plasma concentrations of HGF, IL-6, IL-8, OPN, TSP-2, and TIMP-1 may better serve as predictors of a longer PFS than high plasma concentrations in patients with mCRC treated with second-line FTD/TPI plus bevacizumab, although further studies are warranted.

Declarations

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