

**Title:**

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

**Running title: TRUSTY: Exploratory biomarker analysis using plasma angiogenesis-related factors and cfDNA**

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## **Supplementary Material**

### **Supplementary Figure legends**

**Supplementary Figure S1. PFS in the biomarker analysis set in the FTD/TPI plus bevacizumab group.**

BEV, bevacizumab; CI, confidence interval; FTD/TPI, trifluridine/tipiracil; PFS, progression-free survival

**Supplementary Figure S2. Kaplan-Meier curves for PFS in the FTD/TPI plus bevacizumab group: A. Angiopoietin-2; B. HGF; C. IFN- $\gamma$ ; D. IL-6; E. IL-8; F. PIGF.**

CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HGF, hepatocyte growth factor, HR, hazard ratio; IFN- $\gamma$ , interferon gamma; IL, interleukin; PFS, progression-free survival; PIGF, placental growth factor

**Supplementary Figure S3. Kaplan-Meier curves for PFS in the FTD/TPI plus bevacizumab group: A. VEGF-A; B. VEGF-D; C. OPN; D. sNeuropilin-1; E. sVEGFR-1; F. sVEGFR-2.**

CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HR, hazard ratio; PFS, progression-free survival; OPN, osteopontin; sNeuropilin-1, soluble neuropilin 1; sVEGFR, soluble vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor

**Supplementary Figure S4. Kaplan-Meier curves for PFS in the FTD/TPI plus bevacizumab group: A. sVEGFR-3; B. TSP-2; C. sICAM-1; D. sVCAM-1; E. TIMP-1; F. cfDNA.**

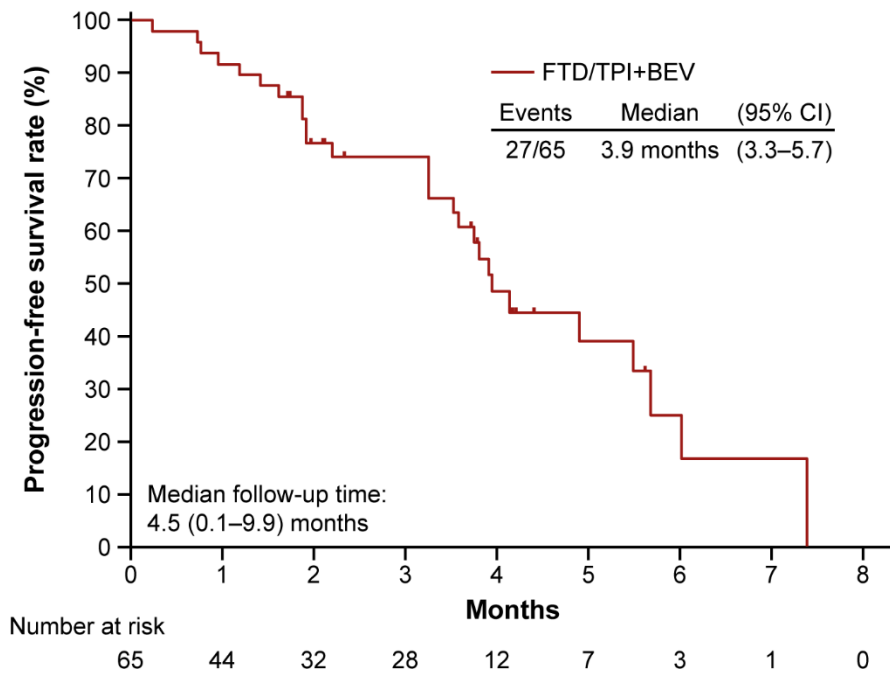
cfDNA, cell-free deoxyribonucleic acid; CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HR, hazard ratio; NE, not estimable; PFS, progression-free survival; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble

vascular cell adhesion molecule-1; sVEGFR, soluble vascular endothelial growth factor receptor; TIMP-1, tissue inhibitor of metalloproteinase-1; TSP-2, thrombospondin-2

**Supplementary Figure S5. Clinical outcomes according to baseline concentrations of angiogenesis-related factors and cfDNA in the control group (fluoropyrimidine plus irinotecan plus bevacizumab): A. DCR; B. PFS.**

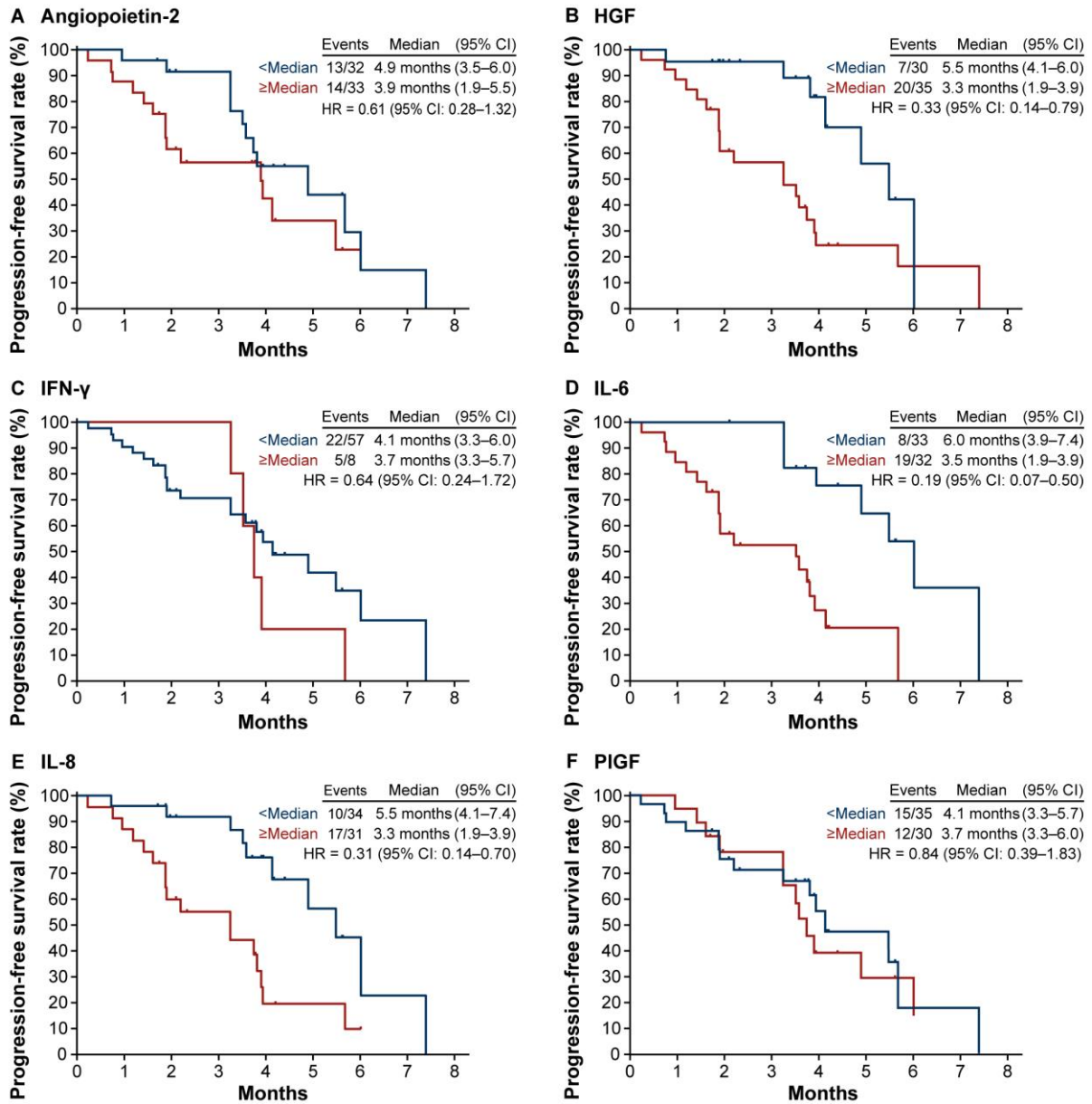
cfDNA, cell-free DNA; CI, confidence interval; DCR, disease control rate; HGF, hepatocyte growth factor; HR, hazard ratio; IFN- $\gamma$ , interferon gamma; IL, interleukin; mPFS, median progression-free survival; NE, not estimable; PFS, progression-free survival; OPN, osteopontin; PIGF, placental growth factor; RR, risk ratio; sICAM-1, soluble intercellular adhesion molecule-1; sNeuropilin-1, soluble neuropilin 1; sVCAM-1, soluble vascular 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

**Supplementary Figure S1. PFS in the biomarker analysis set in the FTD/TPI plus bevacizumab group.**



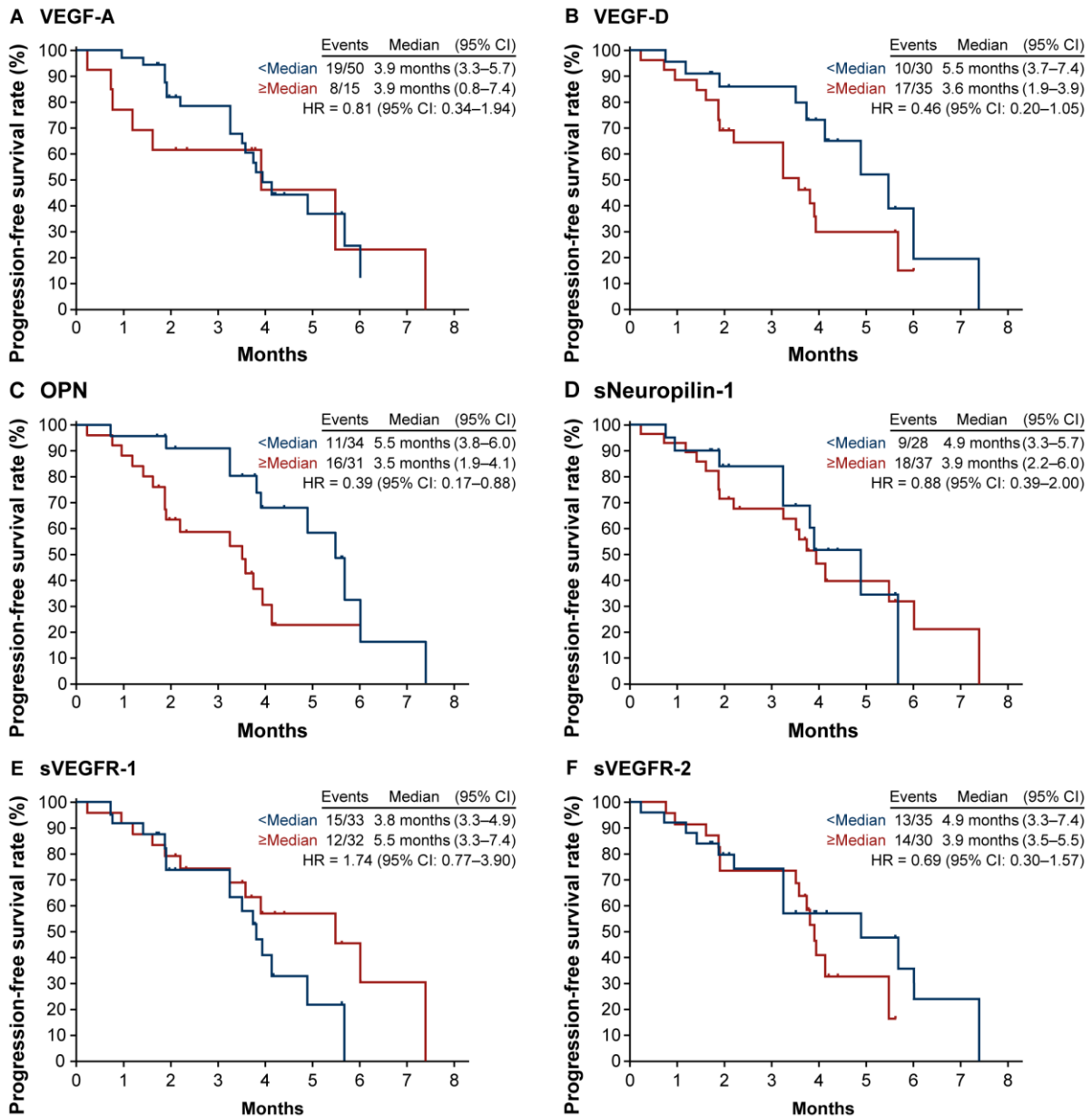
BEV, bevacizumab; CI, confidence interval; FTD/TPI, trifluridine/tipiracil; PFS, progression-free survival

**Supplementary Figure S2. Kaplan-Meier curves for PFS in the FTD/TPI plus bevacizumab group: A. Angiopoietin-2; B. HGF; C. IFN- $\gamma$ ; D. IL-6; E. IL-8; F. PIGF.**



CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HGF, hepatocyte growth factor, HR, hazard ratio; IFN- $\gamma$ , interferon gamma; IL, interleukin; PFS, progression-free survival; PIGF, placental growth factor

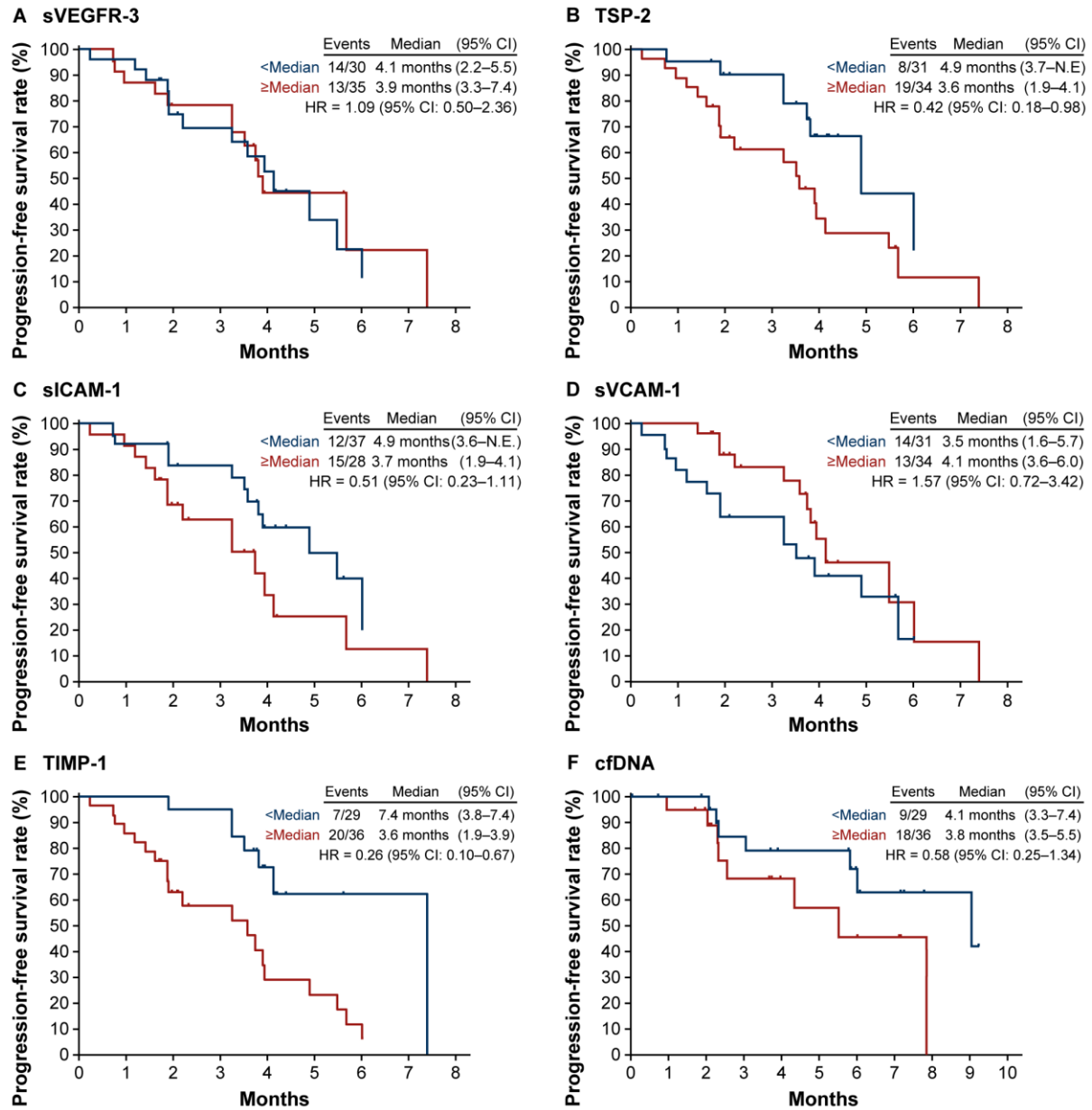
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CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HR, hazard ratio; PFS, progression-free survival; OPN, osteopontin; sNeuropilin-1, soluble neuropilin 1; sVEGFR, soluble vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor



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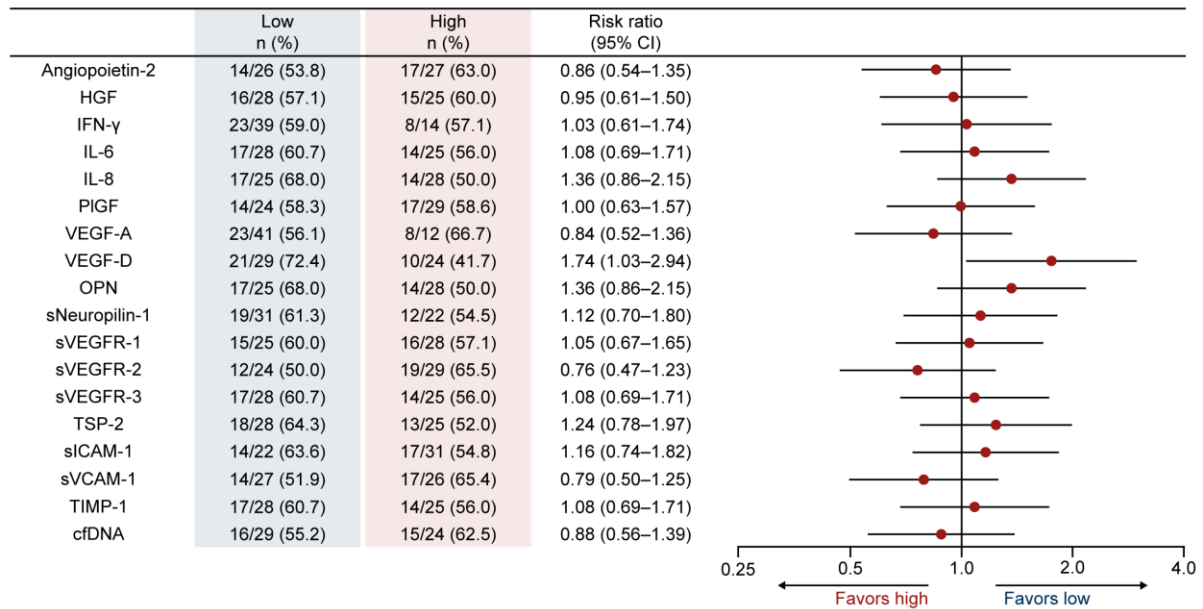


cfDNA, cell-free DNA; CI, confidence interval; FTD/TPI, trifluridine/tipiracil; HR, hazard ratio; NE, not estimable; PFS, progression-free survival; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion

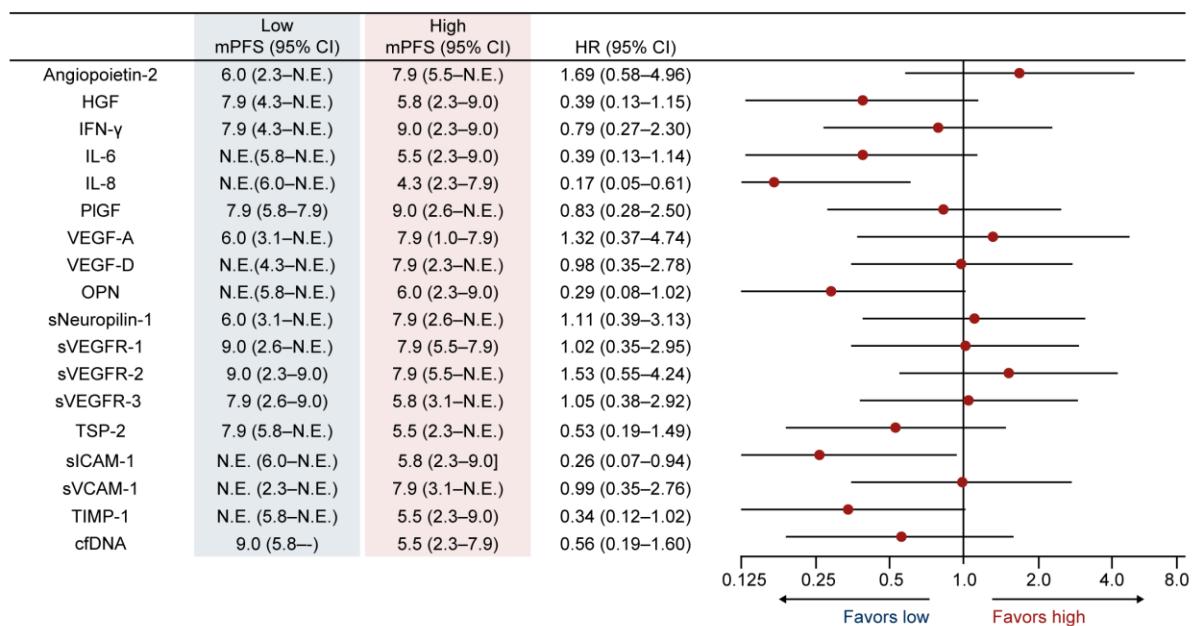
molecule-1; sVEGFR, soluble vascular endothelial growth factor receptor; TIMP-1, tissue inhibitor of metalloproteinase-1; TSP-2, thrombospondin-2

**Supplementary Figure S5. Clinical outcomes according to baseline concentrations of angiogenesis-related factors and cfDNA in the control group (fluoropyrimidine plus irinotecan plus bevacizumab): A. DCR; B. PFS.**

**A**



**B**



cfDNA, cell-free DNA; CI, confidence interval; DCR, disease control rate; HGF, hepatocyte growth factor; HR, hazard ratio; IFN-γ, interferon gamma; IL, interleukin; mPFS, median progression-free survival; NE, not estimable; PFS, progression-free

survival; OPN, osteopontin; PlGF, placental growth factor; sICAM-1, soluble intercellular adhesion molecule-1; sNeuropilin-1, soluble neuropilin 1; sVCAM-1, soluble vascular 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

**Supplementary Table S1. Plasma concentrations of angiogenesis-related factors and cfDNA at baseline in the biomarker analysis set**

	<b>Median (ng/mL)</b>	<b>Range</b>
Angiopoietin-2	1,025.0	37–4,100
HGF	170.0	60–898
IFN- $\gamma$	2.0	2.0–887
IL-6	2.0	2.0–120
IL-8	7.95	2.0–187
PlGF	17.55	3.0–75.7
VEGF-A	30.0	30–1170
VEGF-D	433.0	60–2,760
OPN	17,600.0	855–95,700
sNeuropilin-1	335,500.0	140,000–1,000,000
sVEGFR-1	147.5	50–950
sVEGFR-2	9,285.0	3,330–21,300
sVEGFR-3	4,440.0	1,500–25,500
TSP-2	7,375.0	500–127,000
sICAM-1	369,500.0	104,000–2,130,000
sVCAM-1	908,000.0	487,000–2,210,000
TIMP-1	261,000.0	135,000–2,090,000
cfDNA <sup>a</sup>	0.8631	0.090–20.535

<sup>a</sup>Concentration is mg/L

cfDNA, cell-free DNA; FTD/TPI, trifluridine/tipiracil; HGF, hepatocyte growth factor; IFN- $\gamma$ , interferon gamma; IL, interleukin; OPN, osteopontin; PlGF, placental growth

factor; sICAM-1, soluble intercellular adhesion molecule-1; sNeuropilin-1, soluble neuropilin 1; sVCAM-1, soluble vascular adhesion molecule-1; sVEGFR, soluble vascular endothelial growth factor receptor; TIMP-1, tissue inhibitor of metalloproteinase-1; TSP-1, thrombospondin-2; VEGF, vascular endothelial growth factor

**Supplementary Table S2. Best overall response in the biomarker analysis set**

<b>FTD/TPI plus BEV</b> <b>(<i>n</i> = 65)</b>	<b><i>n</i> (%)</b>
CR	0 (0.0)
PR	2 (3.1)
SD	34 (52.3)
PD	10 (15.4)
NE	19 (29.2)
Response rate (CR + PR)	2 (3.1)
95% CI	0.4–10.7
DCR	36 (55.4)
95% CI	42.5–67.7

BEV, bevacizumab; CI, confidence interval; CR, complete response; DCR, disease control rate; FTD/TPI, trifluridine/tipiracil; NE, not estimable; PD, progressive disease; PR, partial response; SD, stable disease