

SUPPLEMENTAL MATERIAL

Supplemental Table 1. Analytical performance characteristics of the IUO assay.

Performance Characteristic	Result
LoB / LoD / LoQ	123 pg/mL; 179 pg/mL; 540 pg/mL
Linear Range	97 pg/mL-10,245 pg/mL
Precision	Repeatability: 3.9%-18.7% CV Within-laboratory precision: 6.2%-23.4%
Multi-site Reproducibility	Between site: 0.0-10.9% CV Reproducibility: 9.4%-22.8% CV
Interference/Cross-reactivity	None of the tested common blood components, drugs or similar molecules resulted in assay interference.
Reference Range	2,323-10,196 pg/mL

CV, coefficient of variation; IUO, investigational use only; LoB, limit of blank; LoD, limit of detection; LoQ, limit of quantitation.

Supplemental Table 2. Association of clinical outcomes with VEGF-D levels in the TR population.

	Overall Survival				Progression-Free Survival			
	High VEGF-D ^b		Low VEGF-D ^c		High VEGF-D ^b		Low VEGF-D ^c	
	RAM+	PL+	RAM+	PL+	RAM+	PL+	RAM+	PL+
	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI
TR Population ^a	<i>N</i> = 171	<i>N</i> = 142	<i>N</i> = 271	<i>N</i> = 294	<i>N</i> = 171	<i>N</i> = 142	<i>N</i> = 271	<i>N</i> = 294
Median, months	13.1	11.1	13.3	12.8	6.0	4.2	5.6	5.6
(5% CI)	(11.6-15.3)	(8.1-11.9)	(12.0-14.6)	(11.8-14.7)	(5.6-7.1)	(3.6-4.5)	(4.7-5.9)	(4.6-5.8)
HR (95% CI) ^d	0.78 (0.60-1.0)		1.00 (0.82-1.2)		0.59 (0.47-0.76)		0.96 (0.80-1.1)	
P value ^e	0.07		0.97		<0.001		0.62	
Interaction P value ^f	0.091			0.001				

^aTranslational research (TR or ME+MC) population (*n* = 878) consists of patients in the RAISE intent-to-treat population from whom a valid assay result has been obtained using the IUO assay.

^bThe high group defined as patients with biomarker values ≥ 5.4 ng/mL (64.4% above cut point).

^cThe low group defined as patients with biomarker values < 5.4 ng/mL (64.4% below cut point).

^dHazard ratio for the time-to-event outcome comparing ramucirumab versus placebo within VEGF-D expression levels.

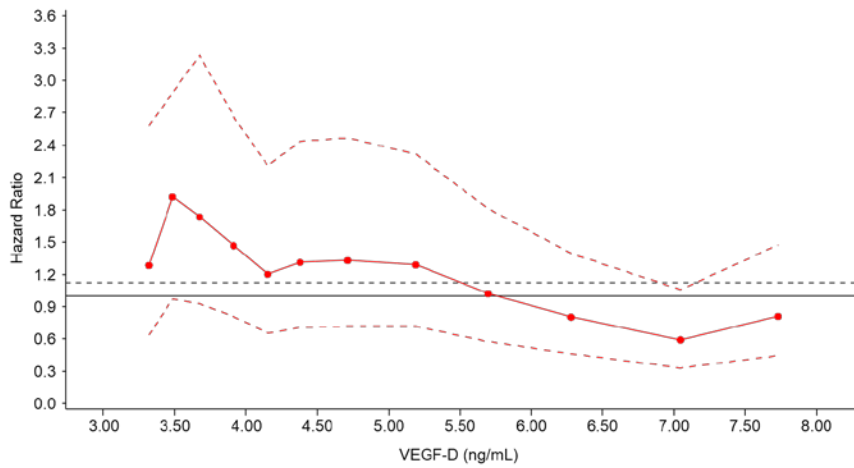
^eP value for treatment effect within VEGF-D expression level obtained using a likelihood ratio test.

^fP value from interaction of treatment by VEGF-D group from separate interaction analyses.

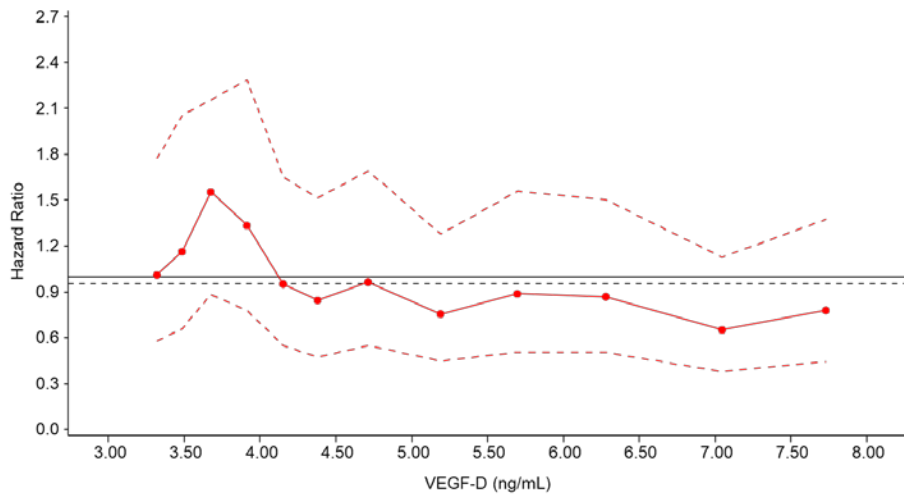
CI, confidence interval; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; HR, hazard ratio; IUO, investigational use only; MC, marker confirmatory; ME, marker exploratory; N , total number of patients in corresponding arm and population; PL, placebo; RAM, ramucirumab; TR, translational research; VEGF-D, vascular endothelial growth factor-D.

Supplemental Figure 1. Subpopulation treatment effect pattern plots of the overall survival (A) and progression-free survival (B) treatment hazard ratios across the range of VEGF-D levels measured with the IUO assay in the ME population. IUO, investigational use only; ME, marker exploratory; VEGF-D, vascular endothelial growth factor D.

A

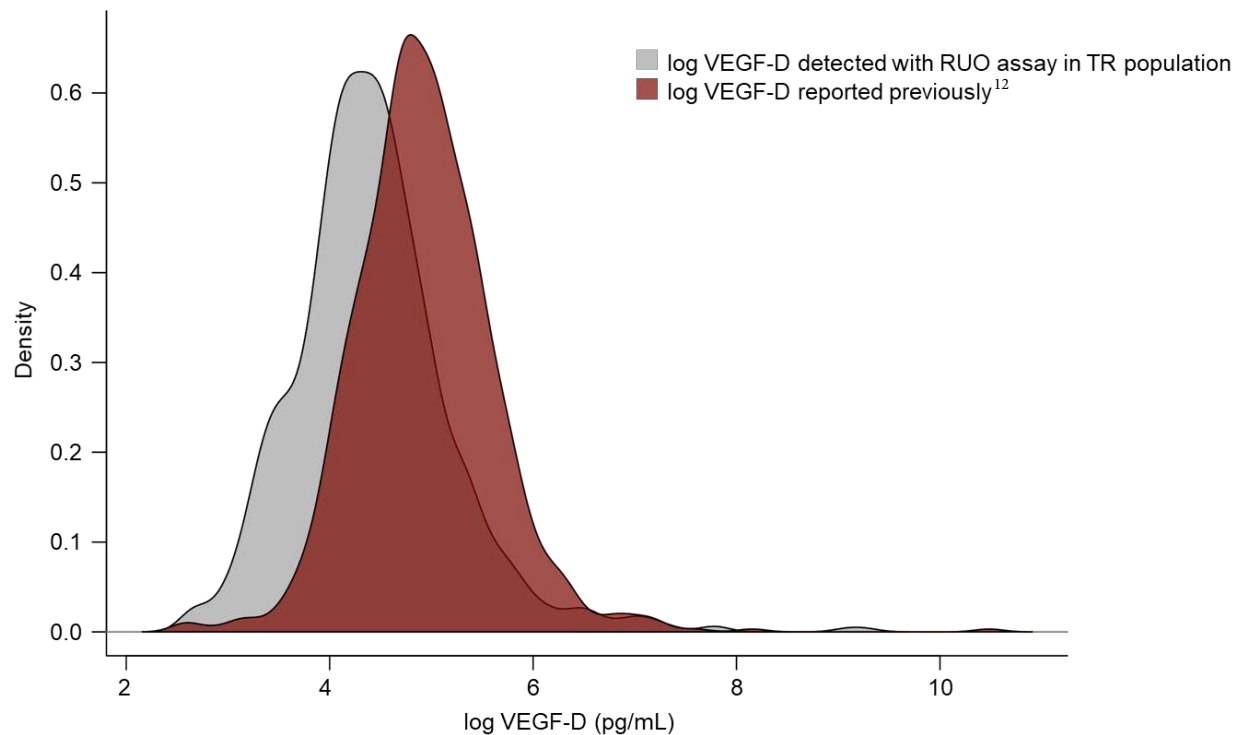


B



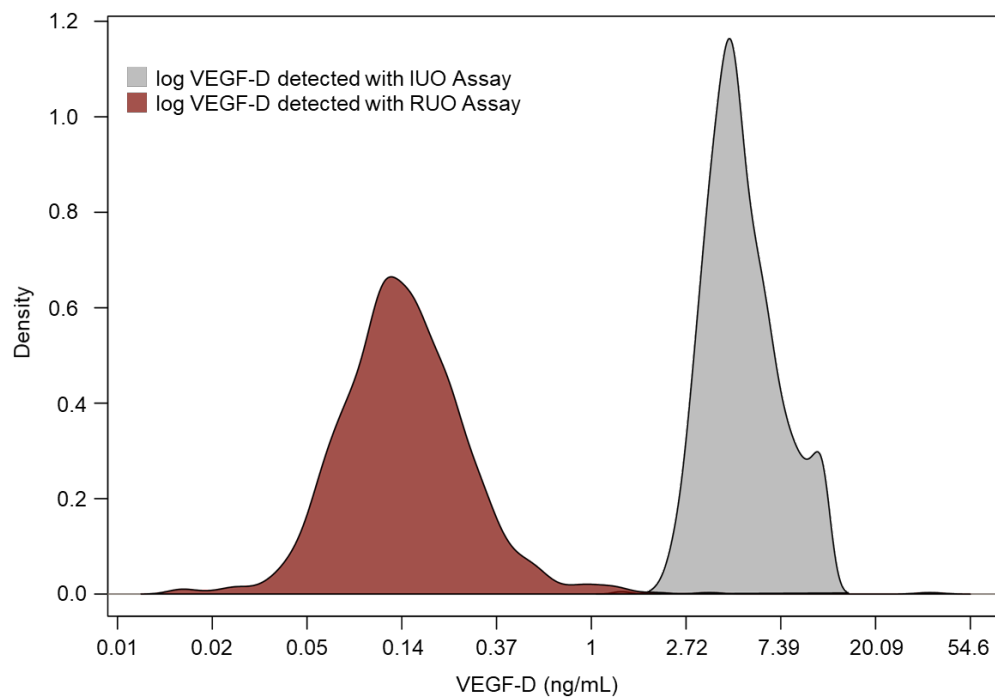
Supplemental Figure 2. Distribution of VEGF-D in plasma from the TR population measured with the RUO assay compared with previously reported VEGF-D levels. The distribution of VEGF-D detected with the RUO assay in the RAISE TR population compared with the previously reported distribution of VEGF-D in the RAISE TR population detected with the RUO assay (pg/mL).¹²

Note: The TR population in the current study has 6 fewer patients than the TR population from the previously reported results.¹² RUO, research use only; TR, translational research; VEGF-D, vascular endothelial growth factor D.



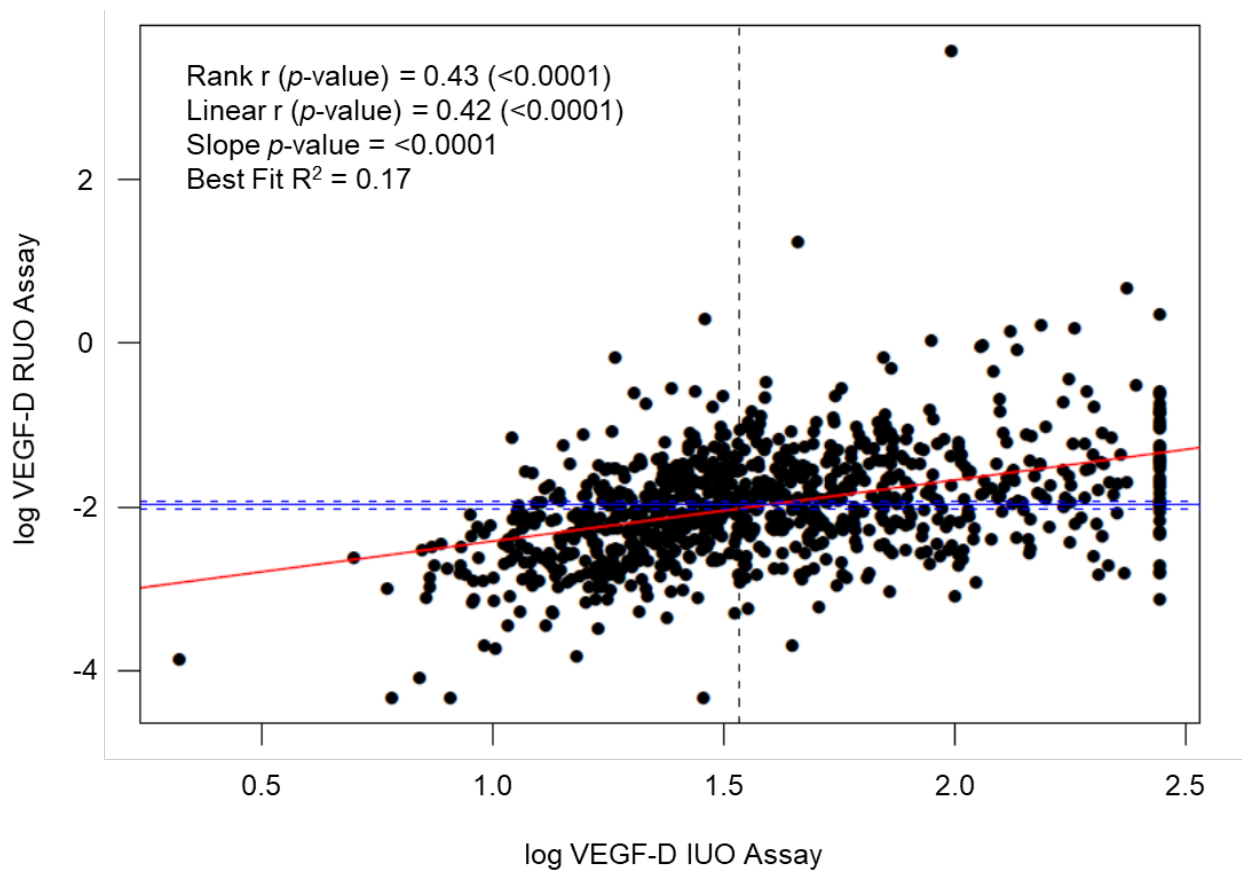
Supplemental Figure 3. Distribution of VEGF-D measured in plasma from the TR population with the IUO assay compared with previously reported data from the RUO assay. The distribution of VEGF-D detected with the IUO assay in the RAISE TR population compared with the previously reported distribution of VEGF-D in the RAISE TR population detected with the RUO assay (ng/mL).¹²

Note: The TR population in the current study has 6 fewer patients than the TR population from the previously reported results.¹² IUO, investigational use only; RUO, research use only; TR, translational research; VEGF-D, vascular endothelial growth factor D.



Supplemental Figure 4. Scatterplot of VEGF-D levels from plasma in the TR population detected with the IUO assay versus outcomes from the RUO assay reported previously. A scatterplot is shown of the log VEGF-D levels detected in plasma from the RAISE TR population with the IUO assay (x-axis) versus the previously reported log VEGF-D levels in the TR population detected with the RUO assay.¹²

Note: The TR population samples analyzed in this study has 6 fewer patients than the TR population samples previously reported.¹² IUO, investigational use only; RUO, research use only; TR, translational research; VEGF-D, vascular endothelial growth factor D.



Supplemental Figure 5. Distribution of VEGF-D from the ME and MC populations detected with the RUO or IUO assays. The distribution of VEGF-D detected utilizing the IUO assay in the ME (grey) or MC (red) populations are shown as well as the distribution of VEGF-D (ng/mL) detected utilizing the RUO assay in the ME (blue) or MC (purple) populations as previously reported.¹²

Note: The MC population in the current study has 6 fewer patients than the MC population from the previously reported results.¹² IUO, investigational use only; MC, marker confirmatory; ME, marker exploratory; RUO, research use only; VEGF-D, vascular endothelial growth factor D.

