

## Updated analysis of tislelizumab plus fruquintinib and FMT in MSS mCRC

**Table S1.** Baseline characteristics of mCRC patients in real-world third-line or above immunotherapy-based treatment (n = 94)

Characteristics	Patients, No. (%)
Age, median (IQR), years	59 (51-66)
> 60	38 (40.4)
≤ 60	56 (59.6)
Sex	
Male	63 (67.0)
Female	31 (33.0)
ECOG PS	
0-1	88 (93.6)
2	6 (6.4)
Primary tumor site	
Left-sided	76 (80.9)
Right-sided	18 (19.1)
Type of metastasis	
With liver metastasis	62 (66.0)
With lung metastasis	57 (60.6)
Previous treatment	
Surgery	75 (79.8)
Radiotherapy	42(44.7)
Chemotherapy	94 (100.0)
Anti-VEGF(R) therapy	83 (88.3)
Anti-EGFR therapy	19 (20.2)
Lines of prior treatment	
2	53 (56.4)
≥ 3	41 (43.6)
Current treatment patterns	
ICI alone	6 (6.4)
ICI+CT±RT	7 (7.4)
ICI+anti-VEGF(R)/EGFR/HER2 mAb±CT±RT	24 (25.5)
ICI+TKI±CT±RT <sup>a</sup>	55 (58.5)
ICI+anti-VEGF(R)/EGFR/HER2 mAb+TKI±CT±RT	2 (2.1)
RAS/BRAF gene status	
WT	28 (29.8)
MT	41 (43.6)
Unknown	25 (26.6)
MSI status	
MSS/pMMR	94 (100.0)

Abbreviations: CT, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor; IQR, interquartile range; mAb, monoclonal antibody; MSI, microsatellite instability; MSS, microsatellite stable; MT, mutated type; pMMR, mismatch repair proficient; RT, radiotherapy; TKI, tyrosine kinase inhibitor; VEGF(R), vascular endothelial growth factor (receptor); WT, wild type. <sup>a</sup>TKI: VEGFRi (Regorafenib, Fruquintinib, Sulfatinib, Apatinib, Anlotinib), BRAFi (Dabrafenib, Vemurafenib), MEKi (Trametinib), HER2i (Pyrotinib, Lapatinib, Tucatinib), ALKi (Lorlatinib).

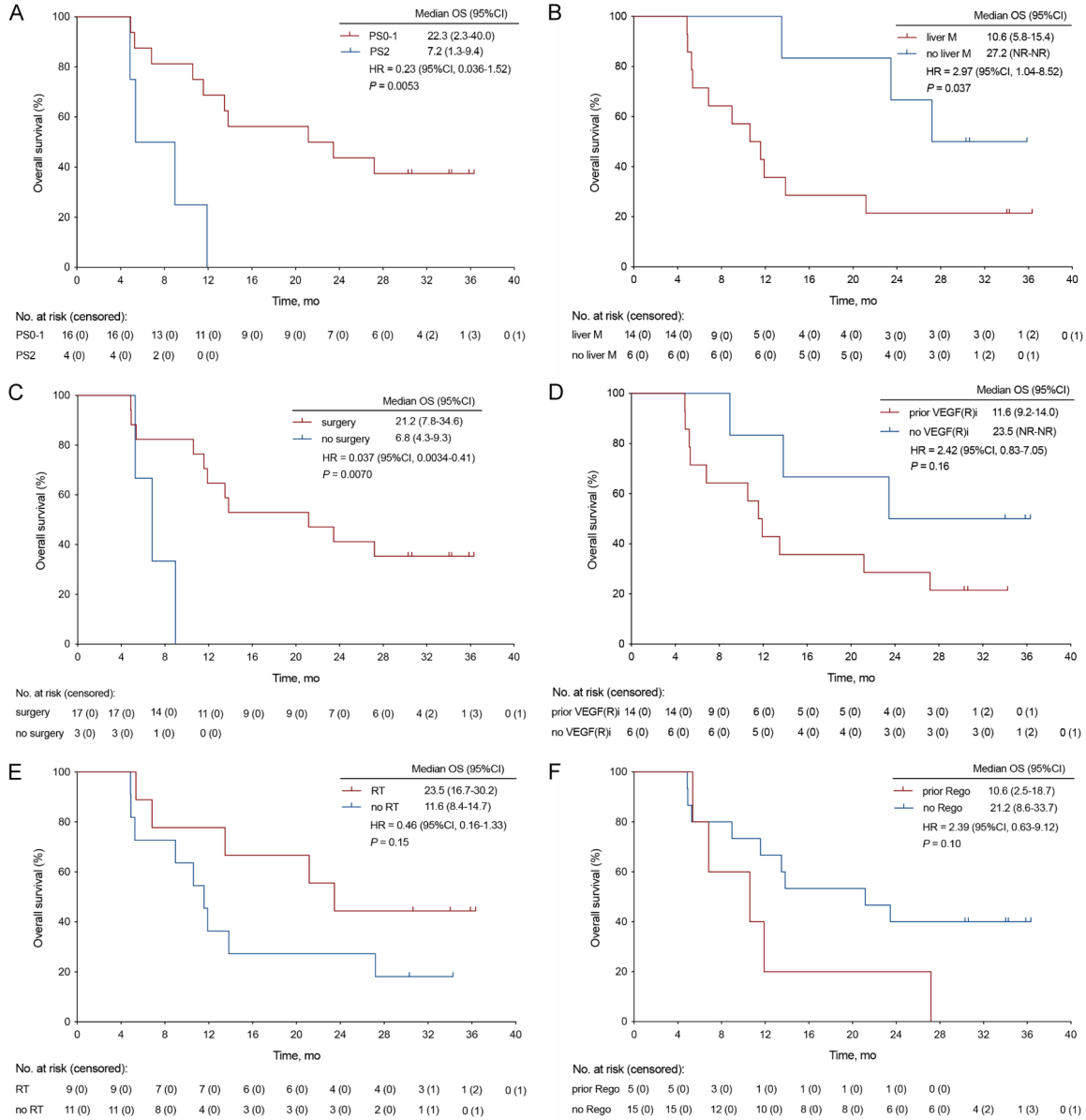
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**Table S2.** Multivariate survival analysis after variable selection for OS (n = 20)

Variable	Multivariate assay for OS HR (95% CI)	P
ECOG performance status		
0-1 vs 2	0.31 (0.061-1.58)	0.16
Liver metastasis		
Yes vs No	2.59 (0.62-10.80)	0.19
Prior surgery		
Yes vs No	0.16 (0.029-0.90)	0.037*
Prior VEGF(R) inhibitors		
Yes vs No	3.49 (0.67-18.12)	0.14
Prior radiotherapy		
Yes vs No	0.88 (0.26-2.98)	0.84
Prior regorafenib		
Yes vs No	1.19 (0.28-4.99)	0.81

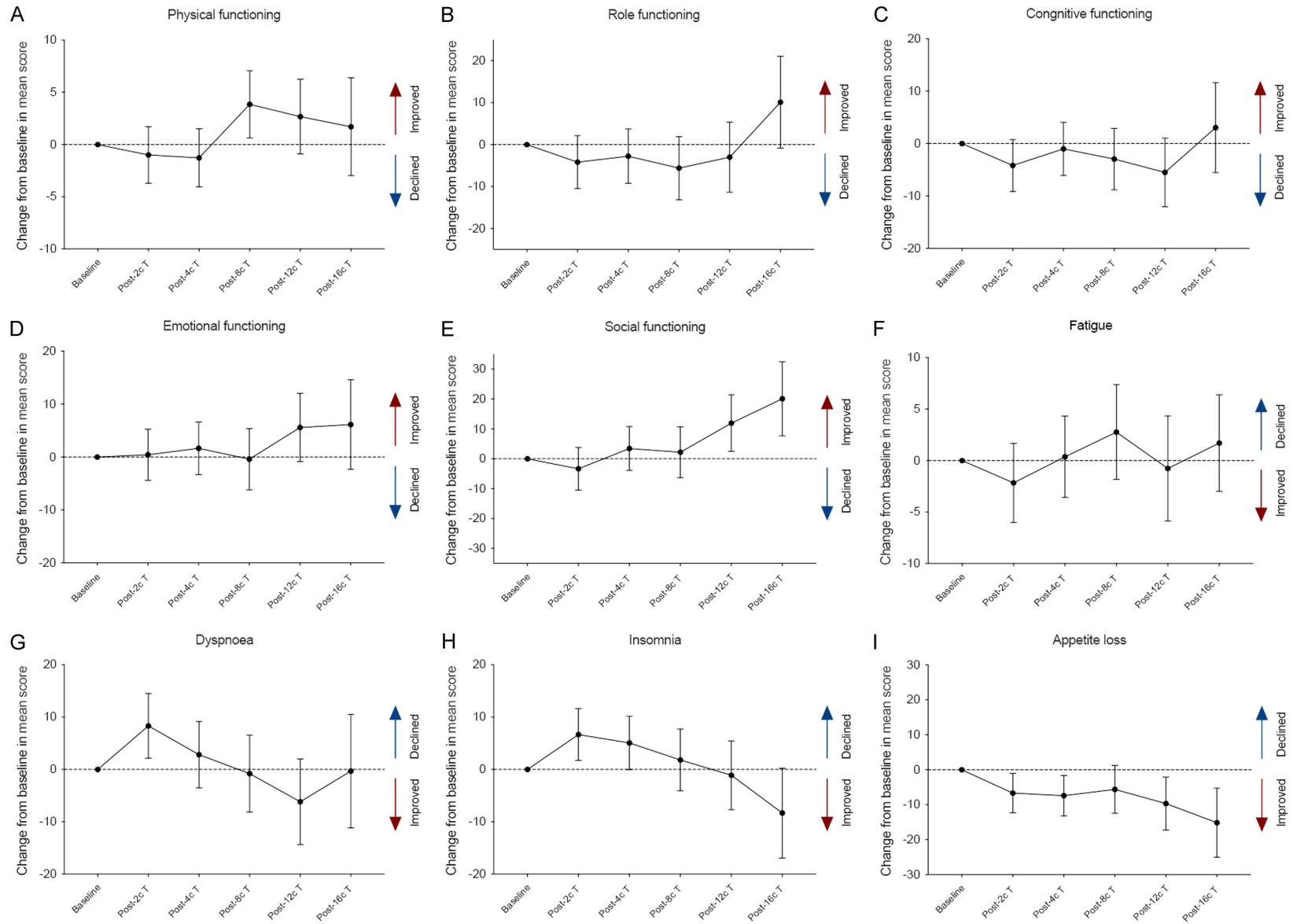
CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; OS, overall survival. \* $P < 0.05$ .

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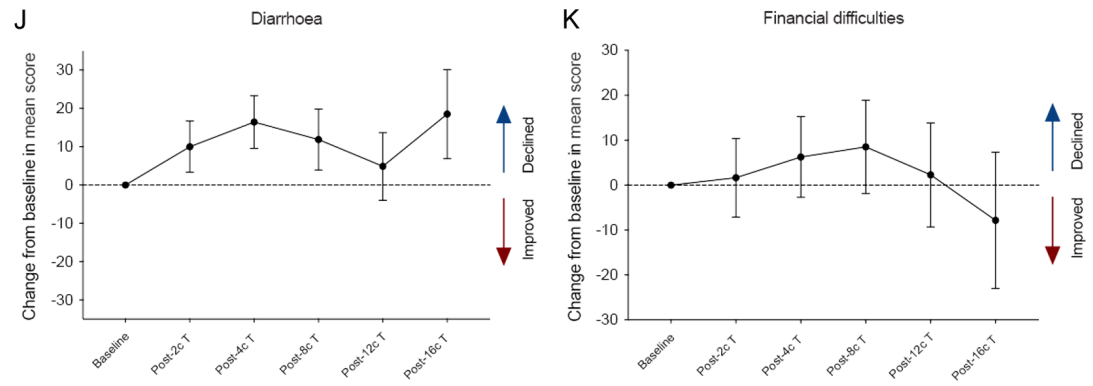


**Figure S1.** Subgroup analysis of Kaplan-Meier curves for overall survival. CI, confidence interval; HR, hazard ratio; OS, overall survival; RT, radiotherapy; Rego, regorafenib; VEGF(R)i, vascular endothelial growth factor (receptor) inhibitor; liver M, liver metastasis; PS, (ECOG) performance status.

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**Figure S2.** LSM change of other functional and symptom scale in EORTC QLQ-C30. LSM, least-squares mean; European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30.

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**Table S3.** Mean change of EORTC QLQ-C30 GHS/QoL, functional and symptom scale scores

Items	Completed questionnaire	Mean ± SD	LSM change ± SD from baseline (95% CI) <sup>a</sup>	P
<b>GHS/QoL</b>				
Baseline	20	57.51±3.44	-	-
Post-2 cycles	20	59.26±3.44	1.75±4.87 (-7.96~11.47)	0.720
Post-4 cycles	18	69.64±3.68	12.13±5.04 (2.09~22.18)	0.019*
Post-8 cycles	11	67.67±4.64	10.16±5.77 (-1.36~21.68)	0.083
Post-12 cycles	8	63.24±5.50	5.73±6.48 (-7.21~18.67)	0.380
Post-16 cycles	4	73.83±8.70	16.32±9.36 (-2.36~34.99)	0.086
<b>Physical functioning</b>				
Baseline	20	82.38±1.91	-	-
Post-2 cycles	20	81.38±1.91	-1.00±2.70 (-6.38~4.38)	0.712
Post-4 cycles	18	81.10±2.01	-1.28±2.78 (-6.81~4.25)	0.646
Post-8 cycles	11	86.23±2.58	3.86±3.21 (-2.53~10.25)	0.233
Post-12 cycles	8	85.05±3.02	2.67±3.58 (-4.45~9.80)	0.457
Post-16 cycles	4	84.08±4.27	1.71±4.68 (-7.62~11.03)	0.717
<b>Role functioning</b>				
Baseline	20	86.77±4.47	-	-
Post-2 cycles	20	82.60±4.47	-4.17±6.31 (-16.75~8.41)	0.511
Post-4 cycles	18	84.00±4.71	-2.77±6.49 (-15.70~10.16)	0.671
Post-8 cycles	11	81.16±6.03	-5.61±7.51 (-20.56~9.35)	0.458
Post-12 cycles	8	83.78±7.06	-2.99±8.35 (-19.63~13.66)	0.722
Post-16 cycles	4	96.86±9.99	10.09±10.95 (-11.72~31.90)	0.360
<b>Cognitive functioning</b>				
Baseline	20	81.68±3.49	-	-
Post-2 cycles	20	77.51±3.49	-4.17±4.94 (-14.01~5.67)	0.402
Post-4 cycles	18	80.67±3.68	-1.01±5.07 (-11.12~9.10)	0.843
Post-8 cycles	11	78.72±4.71	-2.96±5.86 (-14.64~8.72)	0.615
Post-12 cycles	8	76.18±5.52	-5.50±6.54 (-18.52~7.52)	0.403
Post-16 cycles	4	84.72±7.81	3.04±8.56 (-14.01~20.09)	0.723
<b>Emotional functioning</b>				
Baseline	20	84.05±3.44	-	-
Post-2 cycles	20	84.47±3.44	0.42±4.85 (-9.25~10.08)	0.932
Post-4 cycles	18	85.71±3.62	1.66±4.98 (-8.27~11.59)	0.740
Post-8 cycles	11	83.64±4.64	-0.41±5.79 (-11.94~11.12)	0.943
Post-12 cycles	8	89.64±5.43	5.59±6.43 (-7.23~18.40)	0.388
Post-16 cycles	4	90.20±7.72	6.16±8.47 (-10.73~23.04)	0.470
<b>Social functioning</b>				
Baseline	20	64.25±5.05	-	-
Post-2 cycles	20	60.92±5.05	-3.33±7.13 (-17.55~10.88)	0.642
Post-4 cycles	18	67.71±5.32	3.45±7.33 (-11.16~18.06)	0.639
Post-8 cycles	11	66.46±6.81	2.21±8.48 (-14.69~19.10)	0.796
Post-12 cycles	8	76.19±7.98	11.93±9.44 (-6.88~30.75)	0.210
Post-16 cycles	4	84.33±11.29	20.08±12.38 (-4.58~44.74)	0.109

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<b>Fatigue</b>				
Baseline	20	30.26±2.73	-	-
Post-2 cycles	20	28.10±2.73	-2.17±3.84 (-9.83~5.49)	0.576
Post-4 cycles	18	30.64±2.87	0.37±3.95 (-7.51~8.25)	0.925
Post-8 cycles	11	33.03±3.68	2.77±4.59 (-6.39~11.92)	0.549
Post-12 cycles	8	29.50±4.31	-0.76±5.11 (-10.94~9.42)	0.882
Post-16 cycles	4	28.66±6.13	-1.61±6.73 (-15.02~11.81)	0.812
<b>Nausea and vomiting</b>				
Baseline	20	13.68±3.24	-	-
Post-2 cycles	20	8.34±3.24	-5.33±4.59 (-14.47~3.81)	0.249
Post-4 cycles	18	7.34±3.42	-6.34±4.71 (-15.73~3.05)	0.183
Post-8 cycles	11	6.28±4.37	-7.39±5.45 (-18.25~3.46)	0.179
Post-12 cycles	8	0.09±5.13	-13.59±6.07 (-25.68~-1.50)	0.028*
Post-16 cycles	4	4.44±7.29	-9.23±7.99 (-25.16~6.69)	0.252
<b>Pain</b>				
Baseline	20	26.84±3.81	-	-
Post-2 cycles	20	23.51±3.81	-3.33±5.39 (-14.07~7.41)	0.538
Post-4 cycles	18	15.08±4.02	-11.76±5.54 (-22.80~-0.72)	0.037*
Post-8 cycles	11	4.94±5.14	-21.9±6.40 (-34.66~9.14)	0.001**
Post-12 cycles	8	14.05±6.04	-12.80±7.14 (-27.03~1.44)	0.077
Post-16 cycles	4	5.36±8.53	-21.48±9.34 (-40.09~-2.88)	0.024*
<b>Dyspnoea</b>				
Baseline	20	16.57±4.37	-	-
Post-2 cycles	20	24.90±4.37	8.33±6.19 (-3.99~20.66)	0.182
Post-4 cycles	18	19.38±4.61	2.81±6.36 (-9.86~15.48)	0.659
Post-8 cycles	11	15.78±5.90	-0.79±7.34 (-15.42~13.85)	0.915
Post-12 cycles	8	10.40±6.93	-6.17±8.19 (-22.50~10.15)	0.454
Post-16 cycles	4	16.26±9.90	-0.31±10.83 (-21.89~21.27)	0.977
<b>Insomnia</b>				
Baseline	20	15.61±3.51	-	-
Post-2 cycles	20	22.28±3.51	6.67±4.96 (-3.21~16.54)	0.183
Post-4 cycles	18	20.67±3.70	5.06±5.10 (-5.09~15.21)	0.324
Post-8 cycles	11	17.43±4.73	1.82±5.89 (-9.93~13.56)	0.759
Post-12 cycles	8	14.50±5.55	-1.11±6.56 (-14.19~11.97)	0.866
Post-16 cycles	4	7.28±7.84	-8.33±8.59 (-25.44~8.77)	0.335
<b>Appetite loss</b>				
Baseline	20	25.51±4.01	-	-
Post-2 cycles	20	18.85±4.01	-6.67±5.64 (-17.90~4.57)	0.241
Post-4 cycles	18	18.10±4.20	-7.42±5.81 (-18.99~4.15)	0.205
Post-8 cycles	11	19.89±5.43	-5.62±6.80 (-19.17~7.93)	0.411
Post-12 cycles	8	15.85±6.35	-9.67±7.55 (-24.71~5.38)	0.204
Post-16 cycles	4	10.37±8.97	-15.14±9.87 (-34.82~4.53)	0.129
<b>Constipation</b>				
Baseline	20	23.46±3.93	-	-
Post-2 cycles	20	11.79±3.93	-11.67±5.54 (-22.71~-0.62)	0.039*
Post-4 cycles	18	16.67±4.13	-6.79±5.70 (-18.15~4.57)	0.237
Post-8 cycles	11	19.08±5.33	-4.38±6.66 (-17.64~8.88)	0.512
Post-12 cycles	8	19.75±6.22	-3.71±7.38 (-18.41~11.00)	0.617
Post-16 cycles	4	15.12±8.77	-8.33±9.60 (-27.46~10.80)	0.388

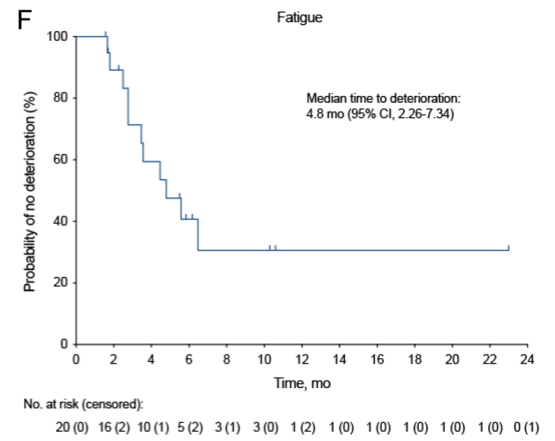
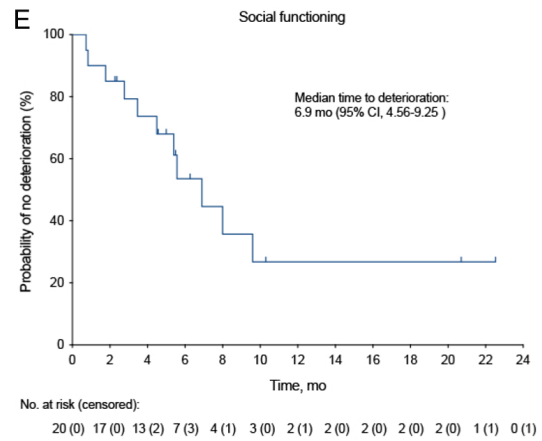
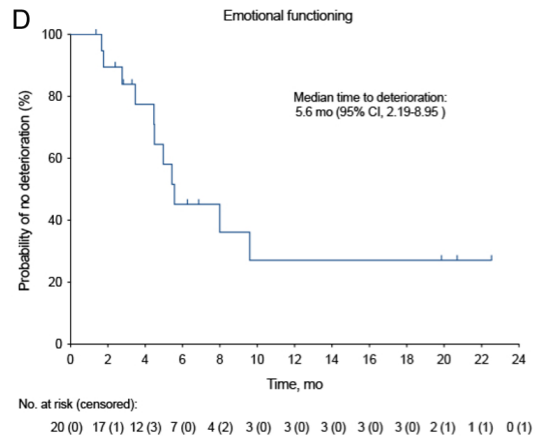
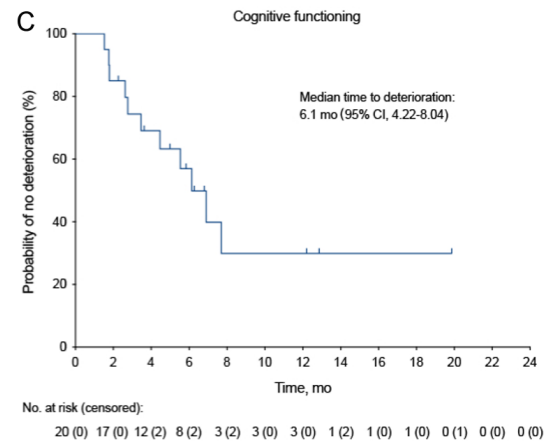
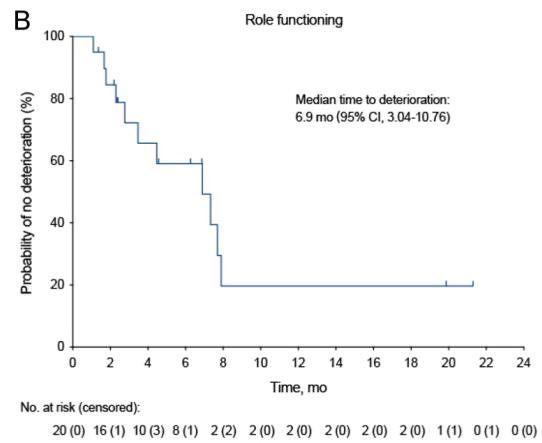
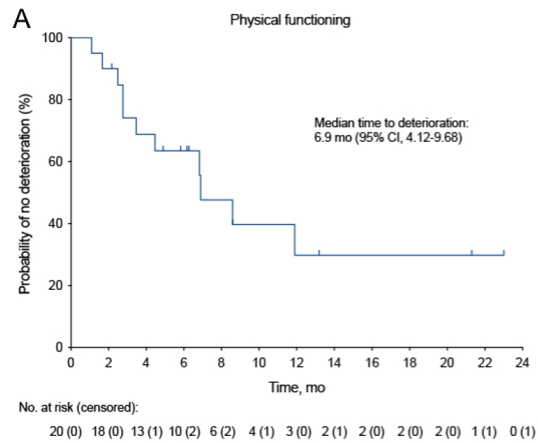
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<b>Diarrhoea</b>				
Baseline	20	10.21±4.73	-	-
Post-2 cycles	20	20.21±4.73	10.00±6.69 (-3.33~23.33)	0.139
Post-4 cycles	18	26.67±4.99	16.46±6.87 (2.77~30.15)	0.019*
Post-8 cycles	11	22.08±6.38	11.87±7.94 (-3.95~27.69)	0.139
Post-12 cycles	8	15.07±7.49	4.87±8.86 (-12.78~22.51)	0.584
Post-16 cycles	4	28.74±10.62	18.53±11.63 (-4.64~41.70)	0.115
<b>Financial difficulties</b>				
Baseline	20	53.17±6.19	-	-
Post-2 cycles	20	54.83±6.19	1.67±8.76 (-15.78~19.11)	0.850
Post-4 cycles	18	59.44±6.53	6.28±9.00 (-11.66~24.21)	0.488
Post-8 cycles	11	61.67±8.35	8.50±10.40 (-12.21~29.21)	0.416
Post-12 cycles	8	55.45±9.79	2.28±11.59 (-20.80~25.36)	0.844
Post-16 cycles	4	45.36±13.86	-7.81±15.18 (-38.06~22.45)	0.609

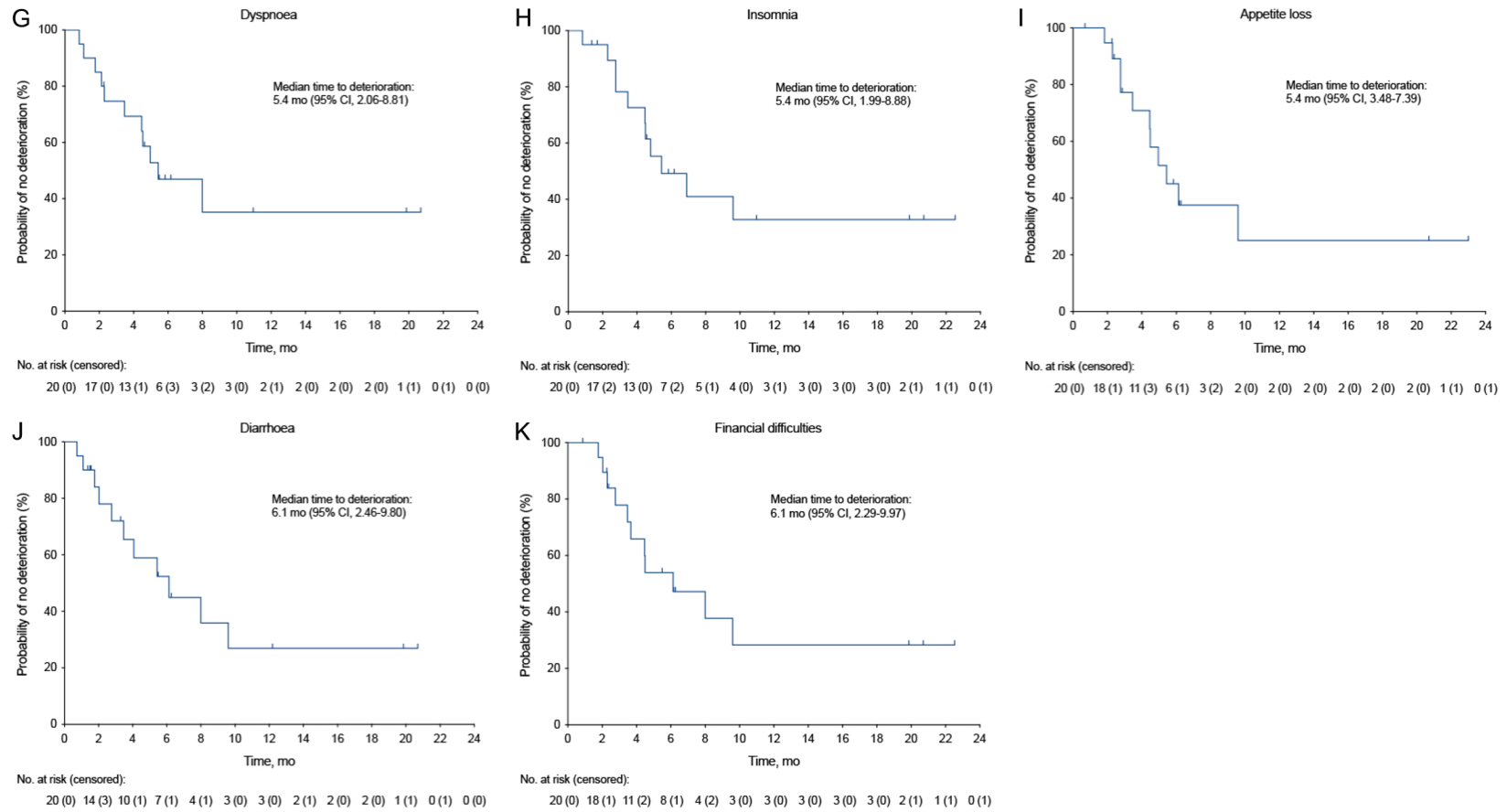
Data are n, mean ± SD, or LSM ± SD (95% CI). Error bars indicate 95% CIs around the mean. *P* values are two-sided and nominal and \**P* < 0.05, \*\**P* < 0.01. Higher scores in functional scale (physical, social, role, cognitive, and emotional functioning) represent better health status and functioning, and higher scores in symptom scale (fatigue, dyspnoea, appetite loss, insomnia, diarrhoea, and financial difficulties) indicate increased symptom severity. CI, confidence interval; EORTC QLQ-C30, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30; GHS, global health status; HRQoL, health-related quality of life; LSM, least-squares mean; SD, standard deviation. <sup>a</sup>Based on a univariate analysis model with HRQoL item scores as the dependent variable, and treatment by study visit interaction as covariates. Analysis with constrained longitudinal data analysis model involved patients with at least one baseline or post-baseline assessment for the HRQoL score being analyzed.



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**Figure S3.** Kaplan-Meier curves of time to deterioration in other EORTC QLQ-C30 item scores. European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30.

## Updated analysis of tislelizumab plus fruquintinib and FMT in MSS mCRC

**Table S4.** Mean change of EQ-5D-3L VAS and health utility scores

Items	Completed questionnaire	Mean $\pm$ SD	LSM change $\pm$ SD from baseline (95% CI)	<i>P</i>
EQ-5D-3L VAS score				
Baseline	20	55.65 $\pm$ 13.25	-	-
Post-2 cycles	20	59.55 $\pm$ 18.27	3.90 $\pm$ 3.71 (-3.50~11.30)	0.297
Post-4 cycles	18	63.06 $\pm$ 19.07	5.26 $\pm$ 3.82 (-2.36~12.88)	0.173
Post-8 cycles	11	70.91 $\pm$ 11.61	10.09 $\pm$ 4.45 (1.22~18.96)	0.026*
Post-12 cycles	8	66.75 $\pm$ 16.84	7.39 $\pm$ 4.93 (-2.44~17.22)	0.138
Post-16 cycles	4	68.75 $\pm$ 6.29	7.64 $\pm$ 6.47 (-5.25~20.52)	0.241
EQ-5D-3L health utility score				
Baseline	20	0.58 $\pm$ 0.22	-	-
Post-2 cycles	20	0.61 $\pm$ 0.22	0.03 $\pm$ 0.04 (-0.04~0.10)	0.375
Post-4 cycles	18	0.62 $\pm$ 0.27	0.02 $\pm$ 0.04 (-0.05~0.10)	0.550
Post-8 cycles	11	0.74 $\pm$ 0.12	0.10 $\pm$ 0.04 (0.02~0.19)	0.017*
Post-12 cycles	8	0.69 $\pm$ 0.24	0.10 $\pm$ 0.05 (0.00~0.19)	0.047*
Post-16 cycles	4	0.75 $\pm$ 0.04	0.11 $\pm$ 0.06 (-0.01~0.23)	0.082

Data are n, mean  $\pm$  SD, or LSM  $\pm$  SD (95% CI). *P* values are two-sided and nominal and \**P* < 0.05. CI, confidence interval; EQ-5D-3L, EuroQoL 5 Dimensions 3 Levels; LSM, least-squares mean; SD, standard deviation; VAS, visual analogue scale.