

Supplementary

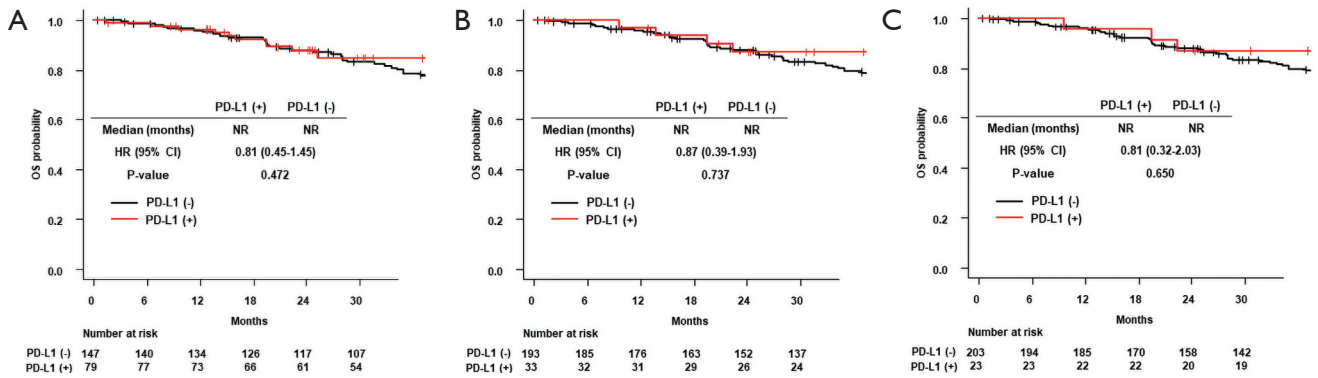


Figure S1 Kaplan-Meier plots of overall survival according to PD-L1 expression in the E1L3 assay. (A) PD-L1 CPS ≥ 1 versus < 1 ; (B) PD-L1 CPS ≥ 5 versus < 5 ; (C) PD-L1 CPS ≥ 10 versus < 10 . CPS, combined positive score; PD-L1, programmed death ligand-1; NR, not reached.

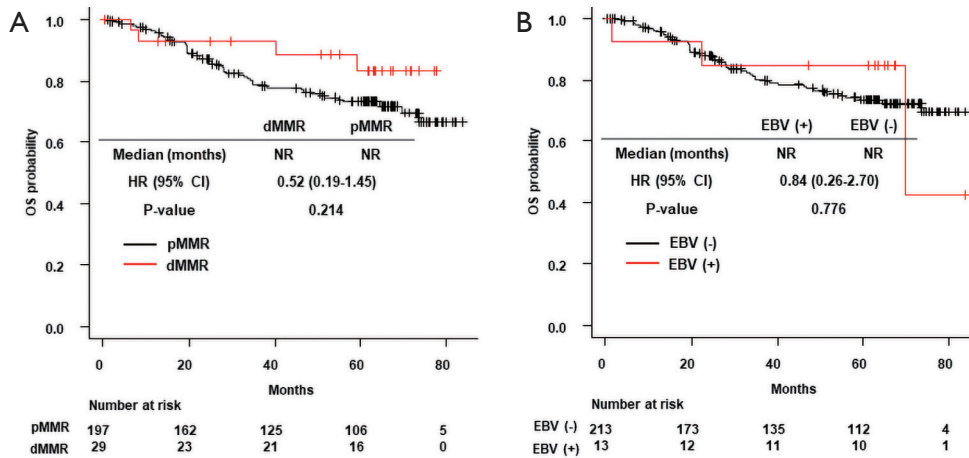


Figure S2 Kaplan-Meier plots of overall survival according to MSI status (A) and EBV status (B). OS, overall survival; HR, hazard ratio; CI, confidence interval; NR, not reached; pMMR, mismatch repair proficient; dMMR, defective mismatch repair; MSI, microsatellite instability; EBV, Epstein-Barr virus.

Table S1 Antibodies used for immunohistochemical studies and in situ hybridization probes

Antibody	Clone	Manufacturer	Platform	Detection	Dilution
PD-L1	22C3	Dako	Dako Autostainer Link48	Dako pharmDx kit	RTU
PD-L1	28-8	Dako	Dako Autostainer Link48	Dako pharmDx kit	RTU
PD-L1	E1L3N	Cell signaling	Dako Autostainer Link48	Envision Flex	1:200
MLH1	G168-15	Dako	Dako Autostainer Link48	Envision Flex	RTU
MSH2	FE11	Dako	Dako Autostainer Link48	Envision Flex	RTU
MSH6	Sp93	Roche	Ventana Benchmark XT	OptiView	RTU
PMS2	A16-4	Roche	Ventana Benchmark XT	OptiView	RTU
HER2	neu	Dako	Dako Autostainer Link48	HercepTest kit	RTU

RTU, ready-to-use; NA, not applicable.

Table S2 Patient characteristics according to PD-L1 expression in the E1L3 assay

Characteristics	Categories	PD-L1, N [%]		
		E1L3		P
		CPS \geq 5 (N=33)	CPS <5 (N=193)	
Age, years	<65	14 [42]	99 [51]	0.45
	\geq 65	19 [58]	94 [49]	
Sex	Male	24 [73]	138 [72]	1.00
	Female	9 [27]	55 [28]	
Tumor location	GEJ	1 [3]	11 [6]	0.24
	U	11 [33]	36 [19]	
	M	9 [27]	76 [39]	
	L	12 [36]	70 [36]	
Depth of invasion	T1	10 [30]	84 [44]	0.23
	T2	2 [6]	23 [12]	
	T3	7 [21]	25 [13]	
	T4	14 [42]	61 [32]	
Lymph node metastasis	Absent	14 [42]	97 [50]	0.52
	Present	19 [58]	96 [50]	
pTNM stage	I	11 [33]	89 [46]	0.25
	II	6 [18]	33 [17]	
	III	13 [39]	45 [23]	
	IV	3 [9]	26 [13]	
Tumor histology	Intestinal	15 [45]	72 [37]	0.44
	Diffuse	18 [55]	121 [63]	
EBV	Positive	9 [27]	4 [2]	<0.01
	Negative	24 [63]	189 [98]	
MMR	dMMR	8 [24]	21 [11]	0.05
	pMMR	25 [66]	172 [89]	
HER2	Positive	4 [12]	20 [10]	0.76
	Negative	29 [88]	173 [90]	

CPS, combined positive score; dMMR, defective mismatch repair; EBV, Epstein-Barr virus; GEJ, esophagogastric junction; HER2, human epidermal growth factor 2; L, lower third; M, middle third; MMR, mismatch repair; PD-L1, programmed death ligand-1; pMMR, mismatch repair proficient; U, upper third.

Table S3 Pairwise comparison of PD-L1 CPS in 22C3 pharmDx and E1L3 assays

E1L3	22C3 pharmDx			
	<1	≥1, <5	≥5, <10	≥10
<1	142	5	0	0
≥1, <5	20	25	1	0
≥5, <10	1	7	2	0
≥10	0	1	5	17

Concordant, 0.82; 22C3 higher, 0.03; E1L3 higher, 0.15. CPS, combined positive score; PD-L1, programmed death ligand-1.

Table S4 Pairwise comparison of PD-L1 CPS in 28-8 pharmDx and E1L3 assays

E1L3	28-8 pharmDx			
	<1	≥1, <5	≥5, <10	≥10
<1	145	2	0	0
≥1, <5	33	13	0	0
≥5, <10	3	5	1	1
≥10	0	3	5	15

Concordant, 0.77; 28-8 higher, 0.01; E1L3 higher, 0.22. CPS, combined positive score; PD-L1, programmed death ligand-1.

Table S5 Comparison of PD-L1 CPS with the clinical cutoff according to 22C3 pharmDx and E1L3 assays

E1L3	22C3 pharmDx				Kappa value		
	PD-L1 CPS <1	PD-L1 CPS ≥1	PD-L1 CPS <5	PD-L1 CPS ≥5	PD-L1 CPS <10	PD-L1 CPS ≥10	
PD-L1 CPS <1	142	5					0.735
PD-L1 CPS ≥1	21	58					
PD-L1 CPS <5			192	1			0.803
PD-L1 CPS ≥5			9	24			
PD-L1 CPS <10					203	0	0.836
PD-L1 CPS ≥10					6	17	

CPS, combined positive score; PD-L1, programmed death ligand-1.

Table S6 Comparison of PD-L1 CPS with the clinical cutoff according to 22C3 pharmDx and E1L3 assays

E1L3	28-8 pharmDx				Kappa value		
	PD-L1 CPS <1	PD-L1 CPS ≥1	PD-L1 CPS <5	PD-L1 CPS ≥5	PD-L1 CPS <10	PD-L1 CPS ≥10	
PD-L1 CPS <1	145	2					0.589
PD-L1 CPS ≥1	36	43					
PD-L1 CPS <5			196	8			0.827
PD-L1 CPS ≥5			0	22			
PD-L1 CPS <10					202	1	0.748
PD-L1 CPS ≥10					8	15	

CPS, combined positive score; PD-L1, programmed death ligand-1.

Table S7 Patient characteristics according to PD-L1 expression by TPS

Characteristics	Categories	PD-L1, N [%]								
		22C3			28-8			E1L3		
		Positive (N=11)	Negative (N=215)	P	Positive (N=7)	Negative (N=219)	P	Positive (N=12)	Negative (N=214)	P
Age, years	<65	6 [55]	107 [50]	1.00	3 [43]	110 [50]	1.00	5 [42]	108 [50]	0.77
	≥65	5 [45]	108 [50]		4 [57]	109 [50]		7 [58]	106 [50]	
Sex	Male	10 [91]	152 [71]	0.19	7 [100]	155 [71]	0.20	10 [83]	152 [71]	0.52
	Female	1 [9]	63 [29]		0 [0]	64 [29]		2 [17]	62 [29]	
Tumor location	GEJ	0 [0]	12 [6]	0.92	0 [0]	12 [5]	0.74	0 [0]	12 [6]	0.93
	U	4 [36]	78 [36]		4 [57]	78 [36]		5 [42]	77 [36]	
	M	4 [36]	81 [38]		2 [29]	83 [38]		4 [33]	81 [38]	
	L	3 [27]	44 [20]		1 [14]	46 [21]		3 [25]	44 [21]	
Depth of invasion	T1	3 [27]	91 [42]	0.21	1 [14]	93 [42]	0.19	1 [8]	93 [43]	0.03
	T2	0 [0]	25 [12]		0 [0]	25 [11]		1 [8]	24 [11]	
	T3	1 [9]	31 [14]		2 [29]	30 [14]		4 [33]	28 [12]	
	T4	7 [64]	68 [32]		4 [57]	71 [32]		6 [50]	69 [32]	
Lymph node met.	Absent	3 [27]	108 [50]	0.22	2 [29]	109 [50]	0.45	3 [25]	108 [50]	0.14
	Present	8 [73]	107 [50]		5 [71]	110 [50]		9 [75]	106 [50]	
pTNM stage	I	3 [27]	97 [45]	0.21	1 [14]	99 [45]	0.10	1 [8]	99 [46]	0.03
	II	1 [9]	38 [18]		2 [29]	37 [17]		3 [25]	36 [17]	
	III	6 [55]	52 [24]		4 [57]	54 [25]		5 [42]	53 [25]	
	IV	1 [9]	28 [13]		0 [0]	29 [13]		3 [25]	26 [12]	
Tumor histology	Intestinal	5 [45]	82 [38]	0.75	2 [29]	85 [39]	0.71	3 [25]	84 [39]	0.38
	Diffuse	6 [55]	133 [62]		5 [71]	134 [61]		9 [75]	130 [61]	
EBV	Positive	3 [27]	10 [5]	0.02	3 [43]	10 [5]	<0.01	3 [25]	10 [5]	0.02
	Negative	8 [73]	205 [95]		4 [57]	209 [95]		9 [75]	204 [95]	
MMR	dMMR	3 [27]	26 [12]	0.15	3 [43]	26 [12]	0.04	4 [33]	25 [12]	0.06
	pMMR	8 [73]	189 [88]		4 [57]	193 [88]		8 [67]	189 [88]	
HER2	Positive	2 [18]	22 [10]	0.33	0 [0]	24 [11]	1.00	0 [0]	24 [11]	0.62
	Negative	9 [82]	193 [90]		7 [100]	195 [89]		12 [100]	190 [89]	

dMMR, defective mismatch repair; EBV, Epstein-Barr virus; GEJ, esophagogastric junction; HER2, human epidermal growth factor 2; L, lower third; M, middle third; MMR, mismatch repair; PD-L1, programmed death ligand-1; pMMR, mismatch repair proficient; TPS, tumor proportion score; U, upper third.

Table S8 Pairwise comparison of PD-L1 TPS between 22C3 pharmDx and 28-8 pharmDx assays

28-8 pharmDx	22C3 pharmDx			
	0	1+	2+	3+
0	213	6	0	0
1+	0	1	0	0
2+	0	0	0	0
3+	2	0	2	2

Concordant, 0.95; 22C3 higher, 0.02; 28-8 higher, 0.03. PD-L1, programmed death ligand-1; TPS, tumor proportion score.

Table S9 Pairwise comparison of PD-L1 TPS between 22C3 pharmDx and E1L3 assays

E1L3	22C3 pharmDx			
	0	1+	2+	3+
0	209	5	0	0
1+	1	1	0	0
2+	1	0	1	0
3+	4	1	1	2

Concordant, 0.94; 22C3 higher, 0.03; E1L3 higher, 0.02. PD-L1, programmed death ligand-1; TPS, tumor proportion score.

Table S10 Pairwise comparison of PD-L1 TPS between 28-8 pharmDx and E1L3 assays

E1L3	28-8 pharmDx			
	0	1+	2+	3+
0	214	2	1	2
1+	0	0	0	1
2+	0	0	0	0
3+	0	0	1	5

Concordant, 0.97; 28-8 higher, 0.00; E1L3 higher, 0.03. PD-L1, programmed death ligand-1; TPS, tumor proportion score.

Table S11 Overall survival according to PD-L1 expression in the cases of stage III or IV

Assays	Categories	Overall survival			
		Median OS (months)	HR	95% CI	P
22C3 pharmDx	CPS ≥ 1 (vs. <1)	NR vs. 48.6	0.51	0.23–1.11	0.091
	CPS ≥ 5 (vs. <5)	NR vs. 59.1	0.75	0.29–1.91	0.54
	CPS ≥ 10 (vs. <10)	NR vs. 55.9	0.38	0.09–1.57	0.18
28-8 pharmDx	CPS ≥ 1 (vs. <1)	NR vs. 48.6	0.49	0.21–1.10	0.08
	CPS ≥ 5 (vs. <5)	NR vs. 59.1	0.75	0.29–1.92	0.55
	CPS ≥ 10 (vs. <10)	NR vs. 55.9	0.39	0.09–1.61	0.19
E1L3	CPS ≥ 1 (vs. <1)	NR vs. 48.6	0.61	0.31–1.21	0.16
	CPS ≥ 5 (vs. <5)	NR vs. 55.9	0.54	0.21–1.40	0.21
	CPS ≥ 10 (vs. <10)	NR vs. 59.1	0.59	0.21–1.67	0.32

CI, confidence interval; CPS, combined positive score; HR, hazard ratio; NR, not reached; OS, overall survival.