

Supplemental Data

Article Title: Prognostic value of whole lymph node molecular analysis in colorectal cancer: a prospective multicentre study

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Supplementary Table 1. Manual Validation of the Optimal TTL Cut-off Determined by X-tile Software

TTL subgroups, copies/ μ L	No. (%)	3-Year RFS (95% CI), %	HR (95% CI) ^a	Log-rank test (Mantel-Cox) ^b	
				χ^2 Value	P value
[0, 250)	106 (75.2)	96.9 (93.5, 100)	ref	ref	/
[250, 2000)	11 (7.8)	90.9 (75.4, 100)	3.44 (0.36, 33.13)	1.367	0.242
[2000, 15000]	9 (6.4)	85.7 (63.3, 100)	3.67 (0.38, 35.27)	<0.001	0.987
(15000, ~)	15 (10.6)	60.0 (39.7, 90.7)	17.53 (4.38, 70.18)	2.333	0.127

Abbreviations: TTL, total tumor load (cytokeratin 19 mRNA copies/ μ L); RFS, recurrence-free survival; HR, hazard ratio; CI, confidence interval; ref, reference.

^a All HRs and 95% CIs were generated by the Cox proportional hazards analyses, with TTL <250 copies/ μ L as reference. All subgroups were ranked by HRs from the smallest (TTL <250 copies/ μ L) to the greatest (TTL >15000 copies/ μ L).

^b Pairwise Log-rank tests were performed sequentially between two adjacent subgroups. The most appropriate TTL cut-off was identified by the peak χ^2 value, suggesting the greatest group disparity in survival probability.

Supplementary Table 2. Clinicopathological Risk Factors Associated with TTL >15000 copies/μL in Colorectal Cancer Patients

Clinicopathological variables	No. (%)	Univariate analysis		Multivariate analysis	
		OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value
Sex					
Male	90 (57.0)	ref			
Female	68 (43.0)	1.00 (0.39, 2.48)	0.995		
Age (years), continuous	158 (100)	1.00 (0.97, 1.05)	0.836		
Age (years), categorical					
< 75	73 (46.2)	ref			
≥ 75	85 (53.8)	0.93 (0.38, 2.33)	0.881		
Tumor location					
Colon	131 (82.9)	ref			
Rectum	27 (17.1)	1.25 (0.36, 3.62)	0.700		
Tumor size (cm), continuous	158 (100)	0.99 (0.78, 1.22)	0.907		
Tumor size (cm), categorical					
< 4.0	76 (48.1)	ref			
≥ 4.0	82 (51.9)	0.82 (0.33, 2.04)	0.675		
Grade					
Low (G1, G2)	135 (85.4)	ref			
High (G3, G4)	23 (14.6)	1.56 (0.45, 4.60)	0.457		
Tumor budding					
Bd1	109 (69.0)	ref			
Bd2	35 (22.1)	1.72 (0.58, 4.72)	0.315		
Bd3	14 (8.9)	2.37 (0.55, 8.46)	0.228		
Perineural invasion					
Absent	145 (91.8)	ref			
Present	13 (8.2)	3.48 (0.93, 11.48)	0.062	0.81 (0.18, 3.33)	0.771
Vascular invasion					
Absent	126 (79.7)	ref			
Present	32 (20.3)	5.93 (2.29, 15.70)	<0.001	5.97 (1.94, 18.48)	0.002
pT stage					
T1	15 (9.5)	ref			
T2	27 (17.1)	3.04 (0.23, 430.05)	0.437	4.02 (0.28, 588.06)	0.337
T3	84 (53.2)	6.38 (0.77, 830.72)	0.097	6.27 (0.69, 838.24)	0.118
T4	32 (20.2)	6.20 (0.63, 834.42)	0.134	4.96 (0.45, 686.26)	0.220

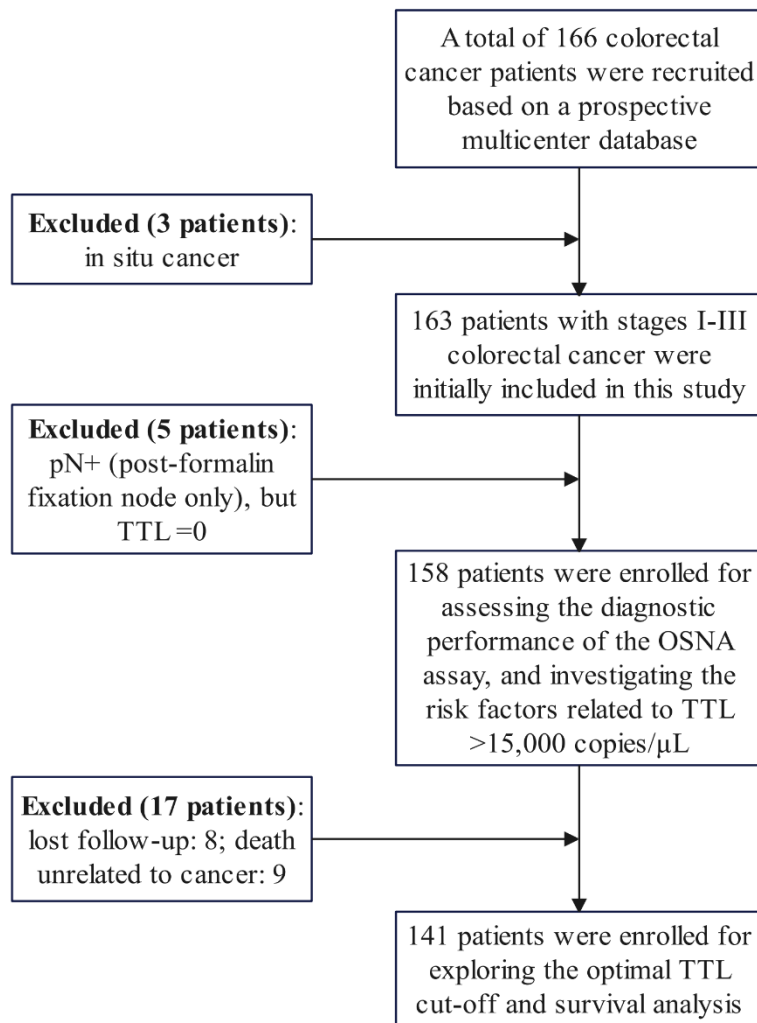
Abbreviations: OSNA, one-step nucleic amplification assay; OR, odds ratio; CI, confidence interval; TTL, total tumor load (cytokeratin 19 mRNA copies/μL); ref, reference.

Supplementary Table 3. Univariate Cox Regression Analyses According to OSNA and CS Results

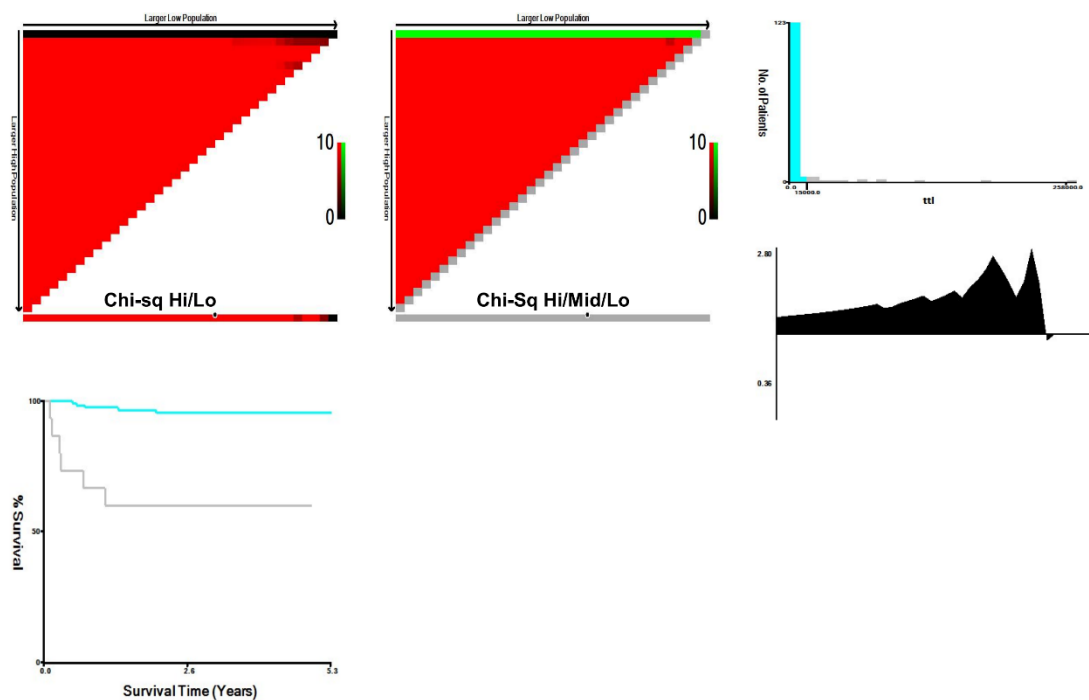
OSNA and CS results	No. (%)	Cox regression analysis for CSS		Cox regression analysis for RFS	
		Unadjusted model		Unadjusted model	
		HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
TTL (logarithmic scale)	141 (100)	1.29 (1.05, 1.58)	0.004	1.29 (1.13, 1.48)	<0.001
OSNA positive ^a					
No	106 (75.2)	ref		ref	
Yes	35 (24.8)	9.34 (1.23, 70.68)	0.009	8.04 (2.19, 29.52)	<0.001
TTL ≥6000 copies/μL					
No	121 (85.8)	ref		ref	
Yes	20 (14.2)	8.33 (1.41, 49.33)	0.013	11.40 (3.36, 38.67)	<0.001
TTL >15000 copies/μL					
No	126 (89.4)	ref		ref	
Yes	15 (10.6)	12.93 (2.18, 76.56)	0.003	12.30 (3.75, 40.34)	<0.001
TTL subgroups, copies/μL					
(~, 250)	106 (75.2)	ref		ref	
[250, 15000]	20 (14.2)	5.20 (0.39, 68.71)	0.175	3.81 (0.67, 21.48)	0.131
(15000, ~)	15 (10.6)	18.07 (2.04, 160.12)	0.002	16.28 (4.07, 65.13)	<0.001
TTL subgroups, copies/μL					
[0, 250)	106 (75.2)	ref		ref	
[250, 6000)	15 (10.6)	7.61 (0.58, 100.60)	0.102	3.15 (0.41, 24.24)	0.283
[6000, ~)	20 (14.2)	12.27 (1.39, 108.68)	0.007	12.90 (3.34, 49.93)	<0.001
CS results					
CS-	111 (78.7)	ref		ref	
CS+	30 (21.3)	12.78 (1.68, 97.05)	0.003	10.42 (2.83, 38.27)	<0.001
Combine (CS results and the optimal TTL cut-off)					
CS-	111 (78.7)	ref		ref	
CS+ (TTL ≤15000)	15 (10.6)	8.74 (0.66, 115.72)	0.084	5.32 (0.89, 32.22)	0.067
CS+ (TTL >15000)	15 (10.6)	19.36 (2.18, 171.61)	0.002	17.19 (4.30, 68.78)	<0.001

Abbreviations: CSS, cancer-specific survival; RFS, recurrence-free survival; HR, hazards ratio; CI, confidence interval; OSNA, one-step nucleic acid amplification; TTL, total tumor load (cytokeratin 19 mRNA copies/μL); CS, cytology smears; ref, reference.

^a A TTL < 250 copies/μL is the OSNA assay positive detection value.



Supplementary Figure 1. Flow diagram of the study design. TTL, total tumour load (cytokeratin 19 mRNA copies/μL).



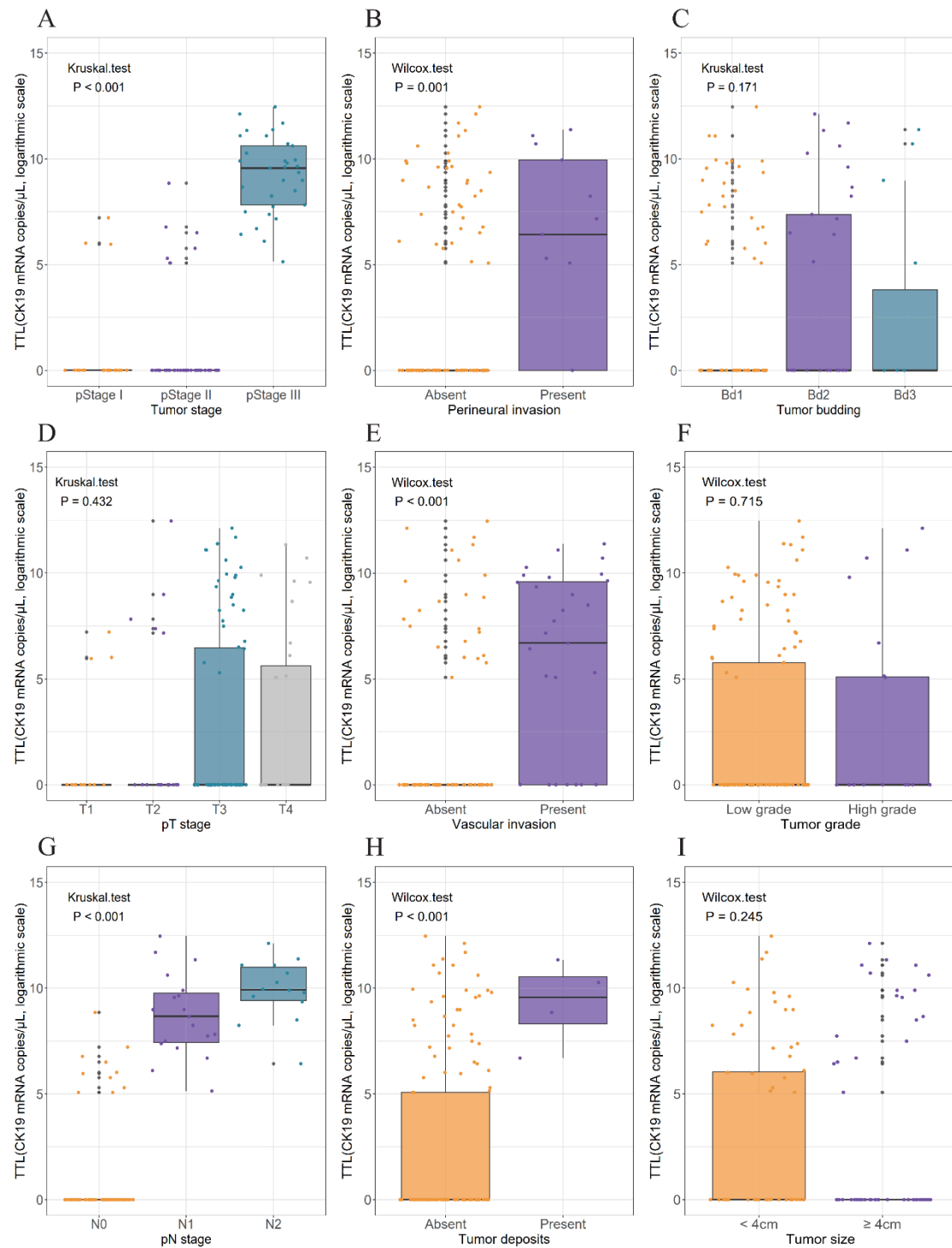
Subpopulation Cutpoints:

Pt No	% Total	Events	Rate	Rank	Range
126	89.36	5	3.97	0 to 22	0.00 thru 15000.00
15	10.64	6	40.00	23 to 36	15400.00 thru 258000.00
141	100.00	11	7.80	0 to 36	0.00 thru 258000.00

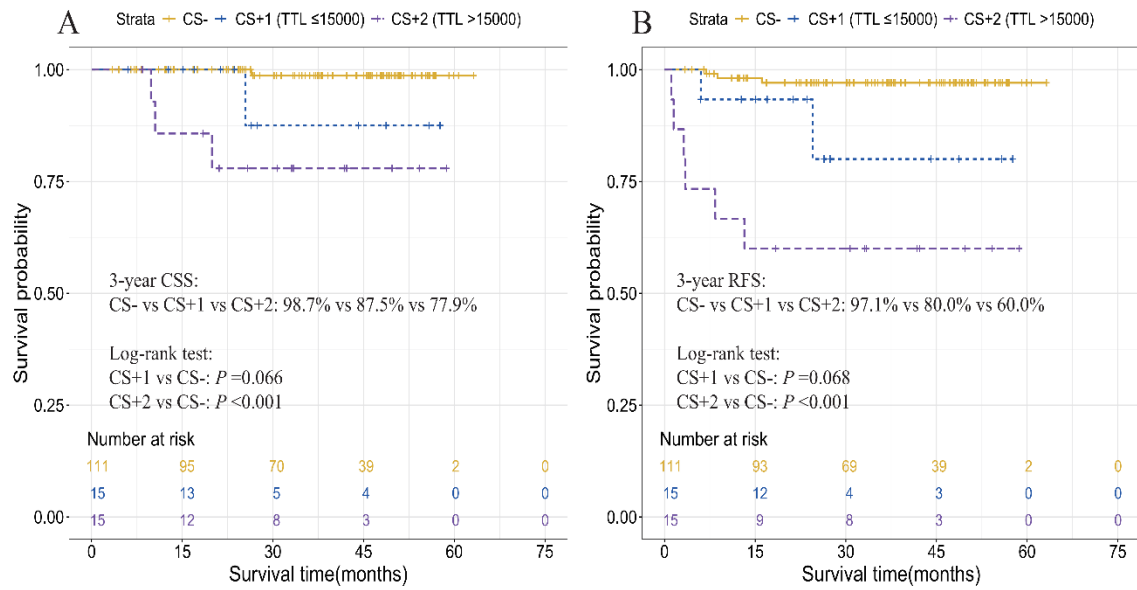
Statistics:

Variable	Value	
Miller-Seigmund P	<0.0001	Max: <0.0001
Chi-sq Hi/Lo	28.6562	Max: 28.6562
Relative Risk 1 vs 2	1.00 / 10.08	

Supplementary Figure 2. Exploration of the optimal TTL cut-off by X-tile software



Supplementary Figure 3. The distribution of TTL values assessed by OSNA across pathological risk factors in colorectal cancer. OSNA, one-step nucleic amplification assay; TTL, total tumor load (cytokeratin 19 mRNA copies/ μ L).



Supplementary Figure 4. CSS (A) and RFS (B) curves according to three subgroups determined by combining CS results with a TTL threshold of 15,000 copies/ μ L in colorectal cancer patients. TTL, total tumor load (cytokeratin 19 mRNA copies/ μ L); CSS, cancer-specific survival; RFS, recurrence-free survival.