

GCC expression in lymph nodes as a significant determinant of recurrence in stage II colon cancer patients.

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Background

Treatment decisions for patients with stage II colon cancer (CC) are determined primarily by disease stage. While staging remains an important prognostic factor, in many cases it fails to identify node-negative patients who have a high risk of recurrence and those from whom the likelihood of disease recurrence is very low, and may be safely managed without chemotherapy¹. Attempts to identify stage II patients at greater recurrence risk have focused mainly on identification of molecular markers in the primary tumor; less attention has been paid to lymph node (LN) tissues. Recent reports have suggested that the presence of Guanylyl Cyclase C (GCC or GUCY2C) gene expression in LN increased the likelihood of disease recurrence in stage II CC patients, independently of traditional high risk features^{2,3}. GCC is a colon-specific biomarker normally found in gastrointestinal epithelium whose expression is preserved in primary and metastatic colorectal cancer cells⁴. This multi-center retrospective study aims to assess the relationship between GCC mRNA LN status and time to recurrence (TTR) in stage II CC patients not treated with adjuvant chemotherapy. This planned analysis is performed on a training set of 241 patients.

Methods

GCC mRNA was quantified by RT-qPCR using formalin fixed LN tissues from untreated stage II CC patients blinded to clinical outcome. Individual LN GCC status was determined by relative quantification of GCC ($\Delta Ct = Ct \text{ GUSB} - Ct \text{ GCC}$) with a pre-specified cut-off (-5.0). Consideration of alternative cut-points (-5.9) and, specifically, consideration of the LN ratio (LNR; number of positive nodes by GCC testing divided by number of informative nodes) were prospectively included in this initial analysis. **Table 1** The prospectively defined primary outcome, TTR, was defined as the time from surgery until first event of recurrence (local or distant), or death related to first primary. Cox regression models were used to examine the relationship between GCC nodal status and the pre-specified primary endpoint of recurrence risk.

Table 1 Cut-offs and risk group classification

Risk group classifications	GCC ΔCt cut-off value	No. of Patients (%)	HR	95% CI	p
Number of GCC positive LNs					
0		110 (45.6%)			
1-3	-5.0 (pre-specified)	107 (44.4%)	1.13	0.66 - 1.96	0.65
4+		24 (10.0%)			
0		85 (35.3%)			
1-3	-5.9 (secondary)	120 (49.8%)	1.69	0.99 - 2.88	0.05
4+		36 (14.9%)			
LN ratio (LNR)					
0-0.10	-5.9	157 (65.1%)	2.38	1.15 - 4.96	0.02
>0.10	(secondary)	84 (34.9%)			

References

- O'Connell JB, et al. (2004) *J Natl Cancer Inst* 96:1420-1425
- Waldman SA, et al. (2009) *JAMA* 301:745-752
- Haince JF, et al. (2010) *J Clin Pathol* 63:530-537
- Cagir B, et al. (1999) *Ann Intern Med* 131:805-812

Population

- Inclusion Criteria:
 - Stage II CC patients diagnosed between 1999-2006
 - No rectal tumors were included
 - Patients had at least 10 LNs assessed by pathology
 - Not treated with adjuvant chemotherapy
 - Minimum of 36 months of follow-up data
- Twenty-nine patients (12%) had a disease recurrence or cancer related death, 71 died of any cause and median follow-up was 60 months.
- Median number of LNs: 15 (Range: 10-41).
- Proximal and distal margins were free of tumor for all patients.
- Two cases had a radial/circumferential margin involved by invasive carcinoma. **Table 2**
- MMR status was available for all cases (except 12)

Table 2 Baseline Characteristics (n=241)

Characteristics	Overall N = 241	LNR 0-0.10* n = 157	LNR > 0.10* n = 84
Age, years			
Median	74.0	74.0	74.0
Range	34-96	43-93	34-96
Race, n (%)			
Caucasian	186 (77.2%)	129 (82.2%)	57 (67.9%)
African American	25 (10.4%)	11 (7.0%)	14 (16.7%)
Hispanic	1 (0.4%)	-	1 (1.2%)
Asian	1 (0.4%)	-	1 (1.2%)
Not specified/ Unknown	28 (11.6%)	17 (10.8%)	11 (13.1%)
Gender, n (%)			
Male	102 (42.3%)	68 (43.3%)	34 (40.5%)
Female	139 (57.7%)	89 (56.7%)	50 (59.5%)
Date of surgery, n (%)			
1999 - 2003	127 (52.7%)	86 (54.8%)	41 (48.8%)
2004 - 2006	114 (47.3%)	71 (45.2%)	43 (51.2%)
Tumor Grade, n (%)			
Low (G1-G2)	196 (81.3%)	127 (80.9%)	69 (82.1%)
High (G3)	43 (17.8%)	28 (17.8%)	15 (17.9%)
Unknown	2 (0.8%)	2 (1.3%)	-
T stage, n (%)			
T3	229 (95.0%)	150 (95.5%)	79 (94.1%)
T4	12 (5.0%)	7 (4.5%)	5 (6.0%)
Tumor Location[#], n (%)			
Right Colon	151 (62.7%)	103 (65.6%)	48 (57.1%)
Transverse	35 (14.5%)	21 (13.4%)	14 (16.7%)
Left Colon	58 (24.0%)	33 (21.1%)	25 (29.8%)
Lymphovascular Invasion (LVI), n (%)			
Yes	22 (9.1%)	11 (7.0%)	11 (13.1%)
No	204 (84.7%)	133 (84.7%)	71 (84.5%)
Unknown	15 (6.2%)	13 (8.3%)	2 (2.4%)
Surgical Margins, n (%)			
Negative	237 (98.3%)	157 (100%)	82 (97.6%)
Positive	2 (0.8%)	-	2 (2.4%)
LN assessed, binary, n (%)			
< 12	51 (21.2%)	35 (22.3%)	16 (19.1%)
≥ 12	190 (78.8%)	122 (77.7%)	68 (81.0%)
MMR status^{**}, n (%)			
proficient (pMMR)	153 (66.8%)	91 (61.1%)	62 (77.5%)
deficient (dMMR)	76 (33.2%)	58 (38.9%)	18 (23.7%)

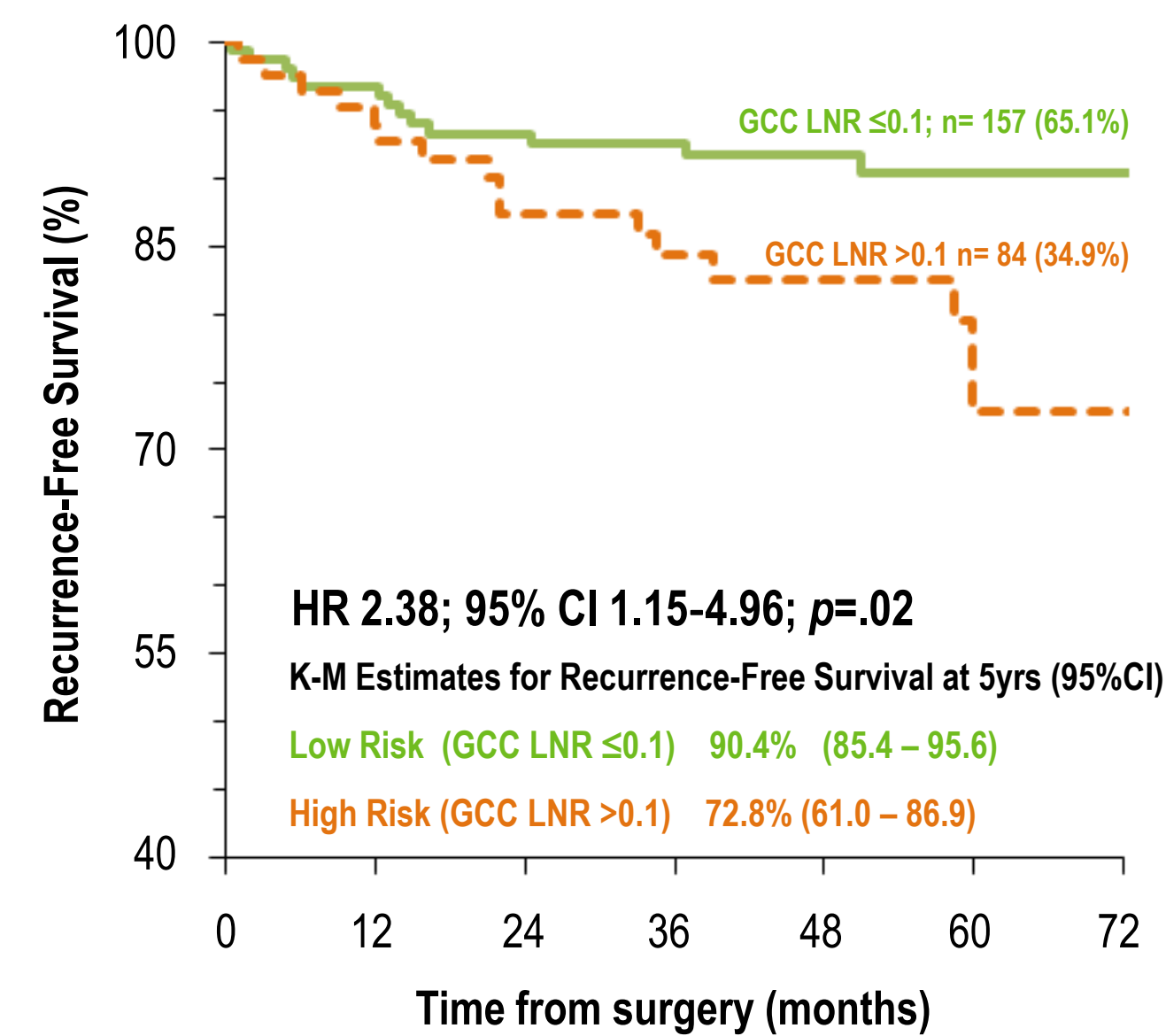
*GCC ΔCt cut-off value = -5.9

** MMR status for 12 Patients were not available

[#] Patient may have more than one value

Figure 1 Recurrence Risk based on GCC LNR

Recurrence-free survival in patients with stage II CC disease not treated with adjuvant chemotherapy. (n=241)



Patients identified as high risk based on the GCC LNR had a risk of recurrence and survival rate comparable to that of stage III CC patients

Table 3 Unadjusted Association between Pathological Factors, GCC LNR and Outcomes

Covariate [†]	No. of Patients	No. of Events	Time to Recurrence (TTR)			Overall Survival (OS)			
			Hazard Ratio	95% CI	P	No. of Deaths	Hazard Ratio	95% CI	P
LVI									
No	204	25	1.0			52	1.0		
Yes	22	4	1.55	(0.54 - 4.47)	0.44	11	2.22	(1.15 - 4.26)	0.03
Tumor Grade									
G1 to G2	196	23	1.0			61	1.0		
G3	43	6	1.15	(0.47 - 2.82)	0.77	9	0.71	(0.35 - 1.43)	0.32
T Stage									
T3	229	24	1.0			64	1.0		
T4	12	5	5.95	(2.26 - 15.70)	0.003	7	4.09	(1.85 - 9.02)	0.003
No. of LNs assessed, binary									
< 12	51	9	1.0			21	1.0		
≥ 12	190	20	0.58	(0.27 - 1.28)	0.20	50	0.57	(0.34 - 0.95)	0.04
Bowel Perforation or Obstruction									
No	216	25	1.0			63	1.0		
Yes	20	4	1.95	(0.68 - 5.63)	0.25	7	1.83	(0.83 - 4.03)	0.16
MMR									
Proficient	153	21	1.0			41	1.0		
Deficient	76	5	0.50	(0.19 - 1.32)	0.13	23	1.18	(0.71 - 1.96)	0.53
GCC LNR									
0 - 0.1	157	13	1.0			39	1.0		
> 0.1	84	16	2.38	(1.15 - 4.96)	0.02	32	1.77	(1.11 - 2.84)	0.02

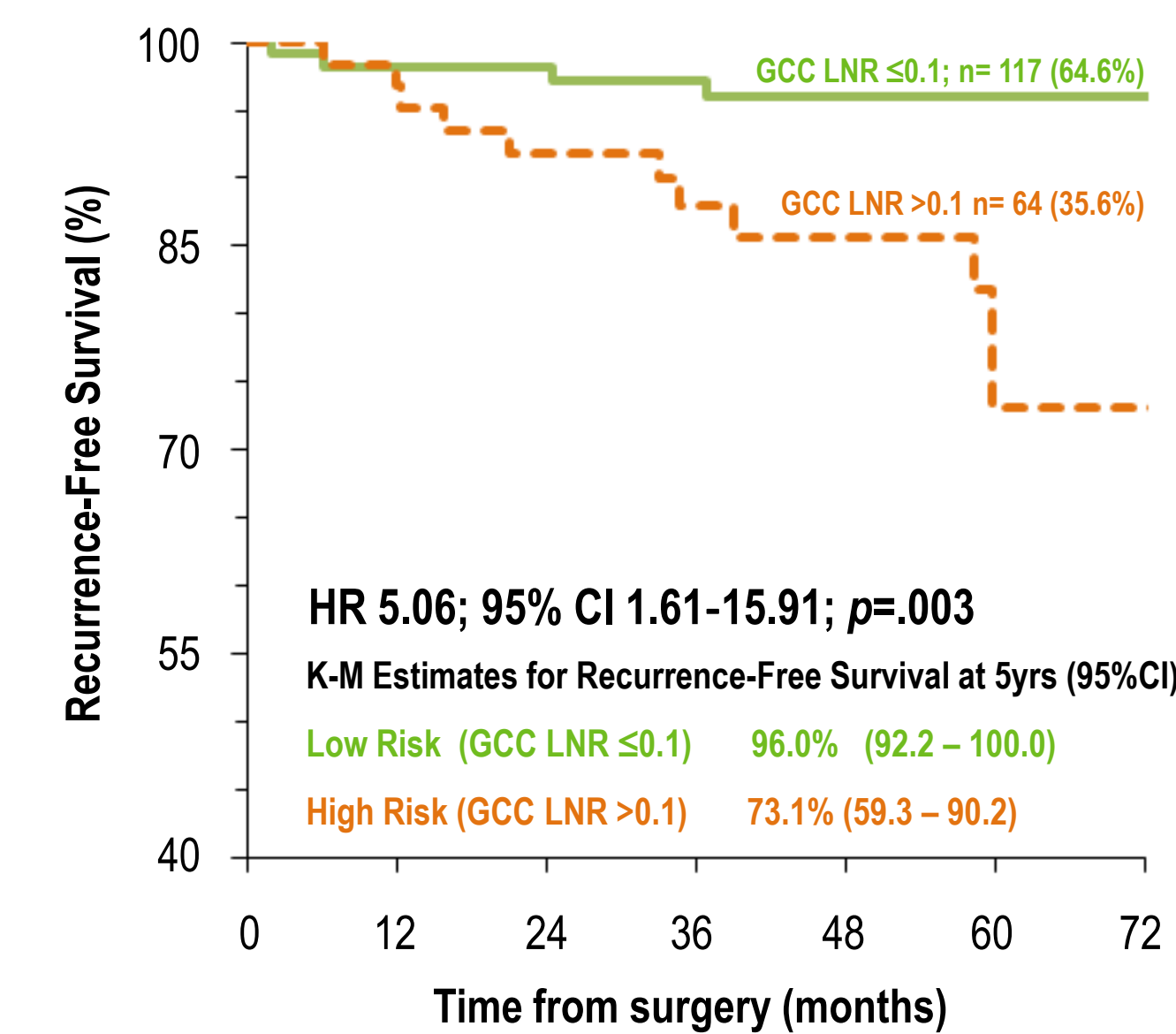
Abbreviations: CI, Confidence Interval; GCC, Guanylyl Cyclase C; LN, Lymph Node; Pts, Patients

[†] Data was unknown in 15 pts for LVI, 2 pts for tumor grade, 5 pts for bowel perforation/obstruction and in 12 pts for MMR status

* Likelihood Ratio test of Cox proportional hazard model

Figure 2 Recurrence in Low Risk Stage II

Subset analysis in patients with T3 tumor, ≥ 12 LNs examined and negative margin (n=181)



With a recurrence-free survival difference of 22.9% between low- and high-risk group, the GCC LNR binary classification improved the prognostic accuracy in a subset of patients in which the likelihood of disease recurrence is very low.

Results

- In this initial analysis, the ratio of the number of GCC+ LNs over the total number of informative LNs (LNR) significantly predicted higher recurrence risk for 84 patients classified as high risk. The estimated 5-yr recurrence risk were 9.6% and 27.2% for the low and high risk group. **Figure 1**
- In a subset of 181 pts with traditionally favorable prognostic factors (negative margins, T3 tumor only and ≥ 12 LNs examined), the GCC LNR had a HR for recurrence of 5.06 (95% CI 1.61-15.91, p=0.003), translating into 5-yr recurrence rates of 4% among low risk patients and 27% for the high risk group. **Figure 2**
- In the unadjusted Cox proportional model, GCC LNR status was identified as a significant prognostic factor for both TTR and OS (TTR: HR, 2.38; 95% CI, 1.15-4.96; p=0.02; OS: HR, 1.77; 95% CI, 1.11-2.84; p=0.02). **Table 3**
- In a multivariate analysis adjusted for age, T stage, number of LNs assessed, and MMR status, the GCC LNR significantly predicted higher recurrence risk (HR, 2.61; 95% CI: 1.17-5.83, p=0.02). **Table 4**

Table 4 Multivariate Analysis for TTR

Covariate	No. of Patients*	Time to Recurrence		
		Hazard Ratio	95% CI	P
Age, continuous	229	0.98	0.96 - 1.02	0.50
T Stage				
T3	218	1.0		
T4	11	7.70	2.76 - 21.43	<0.0001
No. of LNs assessed, binary				
< 12	43	1.0		
≥ 12	186	0.53	0.22 - 1.29	0.16
MMR				
Proficient	153	1.0		
Deficient	76	0.58	0.21 - 1.60	0.29
GCC LNR				
0 - 0.1	149	1.0		
> 0.1	80	2.61	1.17 - 5.83	0.02

* 12 patients excluded from multivariate analysis due to missing MMR status

** Likelihood Ratio test of Cox proportional hazard model

Conclusions

- Our results suggest that detection of GCC mRNA in LNs is associated with risk of disease recurrence in stage II CC patients not treated with adjuvant chemotherapy.
- The GCC LNR appears to have the greatest clinical utility for T3 patients with ≥ 12 LNs assessed, which constitute the majority of stage II CC patients.
- The validation component of the study is ongoing and will explore the potential prognostic value of the GCC LNR status with an accrual goal of 500 additional patients.