Relationship of tumor grade and recurrence in the context of mismatch repair (MMR) status, tumor location, grading schema, mucinous histology, and the 12-gene Recurrence Score in 504 stage II colon cancer patients treated with surgery alone at the Cleveland Clinic

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Background

- High tumor grade is included in practice guidelines as a marker of higher recurrence risk in stage II colon cancer[1]
- Published studies of the prognostic power of tumor grade in colon cancer have reported variable findings[2-6]
  - Recent large studies, including QUASAR (n=711), PETACC-3 (n=420), and studies from NSABP and Cleveland Clinic (n=1007), have consistently found that high tumor grade is not associated with higher recurrence risk in stage II colon cancer
  - Known association of grade with pathological markers such as MMR, tumor histology and tumor location underscores the need to examine grade in the context of these markers
- An added challenge is the existence of multiple systems for colon tumor grading, without a standardized approach[7]
  - Standardized, reproducible assays are needed for decision-making in clinical practice.
  - The 12-gene colon cancer Recurrence Score, as an example, is a standardized, clinically validated assay which has been analytically validated for reproducibility and precision[8]
- There is little data regarding inter-reader reproducibility of colon cancer tumor grade[9-11]

Table 1. High Tumor Grade is Not a Marker of Higher Recurrence Risk in Stage II Colon Cancer

Source	Stage / Treatment	N (patients)	HR (High Vs. Low Grade) p-value
O'Connell, et al[2], NSABP, CCF ASCO 2010	Stage II Surgery alone	634	0.58 for RFI p=0.033
Quah, et al[3], MSKCC DCR 2008	Stage II Surgery alone	448	HR for DSS not reported p=ns
QUASAR[4], ASCO 2009	Stage II Surgery alone	711	0.62 for RFI p=0.026
PETACC-3[5], ASCO 2009	Stage II 5FU ± Irinotecan	420	0.60 for RFS p=0.55
CALGB 9581[6], ASCO 2011	Stage II Surgery alone	690	0.74 for RFI p=0.11

RFI - recurrence free interval; DSS - disease specific survival; RFS - recurrence free survival

Objectives

- Examine association of tumor grade with recurrence in the context of:
  - Clinical and pathological covariates such as mismatch-repair (MMR), mucinous histology and tumor location
  - 12-gene colon cancer Recurrence Score (RS) previously validated in stage II colon cancer patients from QUASAR[4]
- Characterize agreement of two methods for tumor grading

Methods

- Tumors from 504 stage II colon cancer patients treated with surgery alone at the Cleveland Clinic were graded independently by two academic GI pathologists (P1, P2) using the grading methods from their colon cancer clinical practice

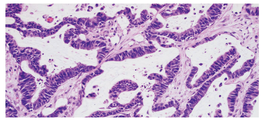
Table 2. Tumor Grading Methods

% tumor with gland-like structures	Pathologist 1	Pathologist 2*
>95%	Well	Low
50-95%	Moderate	
<50%	Poor	High

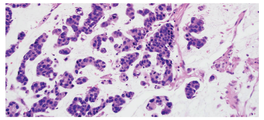
* Considered all mucinous tumors as high grade

- MMR was assessed by immunohistochemistry (IHC) for hMLH1 and hMSH2 using two 5 µm sections on glass slides
  - The IHC testing was conducted by the Cleveland Clinic Department of Pathology using antibody clones MSH2 (FE-11) and MLH-1 (G168-15) from Biocare Medical (2940 Camino Diablo, Suite 300 Walnut Creek, CA 94597).
- Gene expression was quantitated by RT-PCR from 30µm manually microdissected, fixed paraffin-embedded primary colon cancer tissue to obtain the 12-gene RS

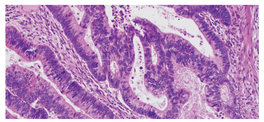
Figure 1. Colon Carcinomas



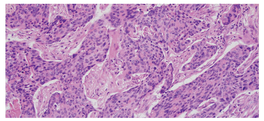
Low Grade, Mucinous Histology



High Grade, Mucinous Histology



Low Grade, Non-Mucinous Histology



High Grade, Non-Mucinous Histology

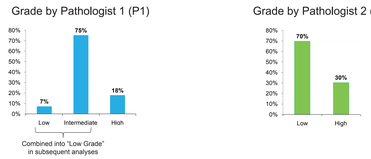
Note: Mucinous histology as assigned by pathologist P2

Analysis Methods

- Associations between either grade and MMR status, tumor location and mucinous histology were assessed using chi-square tests
- Endpoint: time from surgery to first colon cancer recurrence or death due to recurrence of colon cancer
- Association between either grade and risk of recurrence was assessed using Cox proportional hazards regression[12]
  - Univariate
  - Multivariable Cox regression including RS, MMR, tumor location and mucinous histology
- Using the two-tier scheme, agreement between grade assessments by two pathologists was assessed using Cohen's kappa statistic[13]

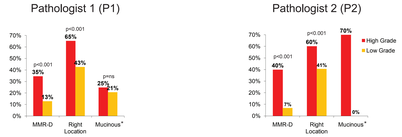
Results

Figure 2. Distribution of Grade



Combined into "Low Grade" in subsequent analyses

Figure 3. Distribution of Pathologic Markers High vs. Low Grade



Y-axis: percentage of patients with high (or low) grade, as indicated, with the tumor characteristics shown on the x-axis.

* Mucinous histology was assessed by P2 only.

- High grade tumors were more likely to be MMR Deficient and right-sided compared to low grade tumors for both P1 and P2
- Proportion of mucinous tumors was similar for high and low grade by P1 (P2 assigned all mucinous tumors to high grade)

Table 3. Association of Grade with Recurrence Risk (Univariate Analyses)

Grade	HR	HR 95% CI	P value
P1 Grade: High vs Low	0.78	(0.40,1.53)	0.46
P2 Grade: High vs Low	0.63	(0.36,1.12)	0.099

- In univariate analyses, P1 grade was not associated with risk of recurrence while P2 high grade trended to lower recurrence
  - Difference in HR's appeared to be relatively small and confidence limits overlapped substantially

Table 4. Association of Grade with Recurrence Risk in the Context of Pathological Markers

P1 Grade

MMR status	N	HR	HR 95% CI	P value
Deficient	79	0.78	(0.15,4.01)	0.76
Proficient	392	0.88	(0.42,1.86)	0.74

Interaction of P1 grade and MMR: p=0.89

P2 Grade

MMR status	N	HR	HR 95% CI	P value
Deficient	79	0.59	(0.13,2.65)	0.50
Proficient	393	0.73	(0.37,1.45)	0.35

Interaction of P2 grade and MMR: p=0.80

Tumor location	N	HR	HR 95% CI	P value
Right	234	0.31	(0.09,1.01)	0.022
Other	268	1.81	(0.80,4.11)	0.18

Interaction of P1 grade and tumor location: p=0.011

Tumor location	N	HR	HR 95% CI	P value
Right	234	0.34	(0.14,0.84)	0.009
Other	270	1.11	(0.53,2.35)	0.78

Interaction of P2 grade and tumor location: p=0.046

Histology	N	HR	HR 95% CI	P value
Mucinous	107	0.82	(0.18,3.82)	0.80
Non-mucinous	395	0.79	(0.37,1.66)	0.52

Interaction of P1 grade and mucinous histology: p=0.96

Histology	N	HR	HR 95% CI	P value
Non-mucinous	397	0.58	(0.21,1.61)	0.26

- Neither grade was significantly associated with higher risk of recurrence in subsets of patients defined by MMR, tumor location or mucinous histology
  - No evidence of interaction was observed between grade and MMR and between P1 grade and mucinous histology (all p>0.80)
  - The relationship of grade with recurrence appears to vary depending on tumor location (both p<0.05 for test of interaction)
    - High grade was significantly associated with lower risk of recurrence in right-sided tumors (p=0.022 for P1 and p=0.009 for P2)
    - Association of grade with recurrence risk was not significant in other (not right-sided) tumors though P1 high grade trended towards higher risk of recurrence (p=0.18)

Table 5. Association of Grade with Recurrence Risk in Conjunction with RS and Pathological Markers

P1 Grade

Variable	HR	HR 95% CI	P value
P1 Grade: High vs Low in Right Tumors	0.31	(0.09,1.03)	0.03
P1 Grade: High vs Low in Other Tumors	1.55	(0.64,3.73)	0.35
MMR-D vs MMR-P	0.75	(0.33,1.71)	0.48
Mucinous Tumor	0.41	(0.20,0.85)	0.01
Tumor Location (Right vs Other)	1.53	(0.89,2.63)	0.13
RS per 25 units	2.65	(1.66,4.22)	<0.001

Interaction of P1 grade and tumor location: p=0.03

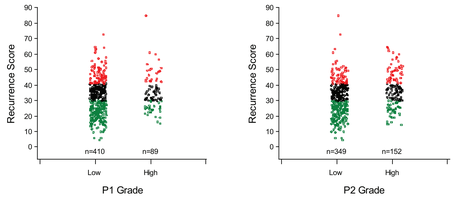
P2 Grade

Variable	HR	HR 95% CI	P value
P2 Grade: High vs Low in Right Tumors	0.47	(0.14,1.56)	0.19
P2 Grade: High vs Low in Other Tumors	1.01	(0.29,3.53)	0.98
MMR-D vs MMR-P	0.76	(0.32,1.76)	0.50
Mucinous Tumor	0.60	(0.18,2.00)	0.42
Tumor Location (Right vs Other)	1.41	(0.81,2.45)	0.23
RS per 25 units	2.81	(1.77,4.46)	<0.001

Interaction of P2 grade and tumor location: p=0.22

- After controlling for MMR status, mucinous histology, and Recurrence Score, high tumor grade was not associated with higher risk of recurrence
  - For right-sided tumors, P1 tumor grade was associated with lower recurrence risk
  - For other tumors (not right-sided), neither tumor grade was significantly associated with recurrence

Figure 4. Distribution of RS Values by Tumor Grade



- A wide range of RS values is observed for high and low grade by either pathologist, including a substantial proportion of patients with high Recurrence Score disease (RS ≥ 41) with either high or low grade
- RS values cannot be predicted from tumor grade

Table 6. Agreement Between Two Pathologists

All Patients

P1 Grade

P2 Grade	Low	High	Total
Low	315	34	349
High	98	55	153
Total	413	89	502

Kappa = 0.30, 95% CI (0.21,0.39)

Non-mucinous tumors*

P1 Grade

P2 Grade	Low	High	Total
Low	315	34	349
High	13	33	46
Total	328	67	395

* 107 (21%) of 502 patients were excluded due to mucinous histology

Kappa = 0.52, 95% CI (0.40,0.64)

- Tumor Grade: Using the two-tier scheme, agreement between the two pathologists was low in all patients and moderate if mucinous tumors were excluded

Strengths and Limitations

Strengths

- A large dataset of stage II colon cancer patients treated with surgery alone
- Central grade assessments by two academic pathologists with specialization in GI cancer
- 12-gene Recurrence Score assessment using established, reproducible RT-PCR platform
- IHC testing for MMR was performed centrally by a single laboratory (Cleveland Clinic Department of Pathology)

Limitations

- Exploratory analysis in studies designed for development of the RS
- Method of grading is confounded with pathologist effect
  - Different grading of mucinous tumors by P1 and P2

Summary

- High tumor grade was not found to be a marker of higher recurrence risk in stage II colon cancer by either of two pathologists using their methods used for clinical practice
  - Contrary to conventional expectations, but consistent with other reported studies, high grade was associated in some circumstances with lower risk of recurrence in stage II colon cancer
  - Importantly, accounting for MMR status did not affect this conclusion – the known association of MMR-D with high grade does not explain the lower recurrence risk observed with high grade in stage II colon cancer
- Inter-pathologist agreement on colon tumor grade was modest overall in this study, and moderate after excluding mucinous cases, even with central expert review

Conclusions

- These results highlight the need for standardized assays and critical evaluation of the underlying data for markers used to make treatment decisions in the clinic
- Based on data from four large studies (NSABP/CCF, QUASAR, PETACC-3, and CALGB 9581), the continued inclusion of tumor grade as a marker of high recurrence risk in clinical practice guidelines for stage II colon cancer should be questioned
- For the stage II colon cancer patient, recurrence risk should be assessed using T stage, MMR, and RS, the three key predictors of recurrence risk in stage II colon cancer, as reported in the QUASAR validation study

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