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Intra-tumoural stromal morphometry predicts disease recurrence but not response to 5-fluorouracil – results from the QUASAR trial of colorectal cancer

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Running title - Stromal morphometry in stage II colorectal cancer

Abstract

Introduction: The biological importance of tumour-associated stroma is increasingly apparent, yet clinical utility remains ill-defined. In stage-II / Dukes B colorectal cancer (CRC), clinical biomarkers are urgently required to direct therapeutic options. We report here prognostic/predictive analyses, and molecular associations, of stromal morphometric quantification in the Quick and Simple and Reliable (QUASAR) trial of CRC. Materials and methods: Relative proportions of tumour epithelium (PoT) or stroma (PoS) were morphometrically quantified using digitised haematoxylin and eosin sections derived from 1,800 patients enrolled in QUASAR which randomised 3,239 (91% stage II) CRC patients between adjuvant fluorouracil/folinic acid (FUFA) chemotherapy and observation. The prognostic/predictive value of PoT/PoS measures were determined by stratified log-rank analyses. Results: High tumour stroma ($\geq 50\%$) was associated with increased recurrence risk: 31.3% (143/457) recurrence for $\geq 50\%$ versus 21.9% (294/1,343) if $< 50\%$ [Rate ratio (RR)=1.62; 95%CI 1.30-2.02, $p < 0.0001$]. For stromal proportions of $\geq 65\%$, 40% (46/115) of patients had recurrent disease within 10 years. The adverse prognostic effect of high stroma was independent of established prognostic variables, and maintained in stage II / Dukes B patients (RR=1.62; 95%CI=1.26-2.08; $p=0.0002$). *KRAS* mutation in the presence of high stroma augmented recurrence risk (RR=2.93; 95%CI=1.87-4.59; $p=0.0005$). Stromal morphometry did not predict response to FUFA chemotherapy. Discussion: Simple digital morphometry applied to a single representative H&E section identifies CRC patients with over 50% higher risk of disease recurrence. This technique can reliably partition patients into sub-populations with differential risks of tumour recurrence in a simple and cost-effective manner. Further prospective validation is warranted.

Keywords

Colon

Colorectal

Rectal

Cancer

Stroma

INTRODUCTION

The use of adjuvant chemotherapy in colorectal cancer (CRC) following presumptive curative resection is directed by high-quality pathological assessment.¹⁻³ Detection of tumour within lymph nodes (stage III disease) is generally regarded as an absolute indication for adjuvant therapy^{1,4,5} because of significant clinical benefit (~10% absolute improvement in overall survival [OS]).^{6,7} The value of adjuvant chemotherapy in locally advanced, node-negative (stage II) disease is less clear because the more modest benefits (~4% improvement in OS),⁸ may not outweigh the toxicity and patient inconvenience.^{1,9,10}

Thus, adjuvant chemotherapy is generally restricted to a minority of stage II patients with 'high-risk' pathological features including extra-mural vascular and/or peritoneal invasion.^{1,11,12} Such features impart a recurrence risk of similar magnitude to that associated with lymph node metastasis² and so serve to identify stage II patients who may derive worthwhile benefit from adjuvant regimens.^{1,4,5} A limitation of this approach is that identification of such features is subjective with variability in reporting quality and reproducibility.^{3,13} Also, only a minority of recurrences among these 'high-risk'

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patients will be prevented by 'appropriate' chemotherapy,^{8,10,14} and it is not currently possible to identify who is more or less likely to benefit.¹⁵ Consequently, identification and clinical validation of effective prognostic and, in particular, predictive biological indicators would help facilitate therapeutic decisions.

Mismatch repair (MMR) / microsatellite instability (MSI) testing can identify CRC patients at a reduced risk of recurrence for whom adjuvant therapy is usually not indicated.^{14,16-18} However, only a minority of CRC patients, 12-15% at most, demonstrate MSI and/or attenuated expression of one or more MMR proteins.^{16,17} The Oncotype DX[®] recurrence score has been prospectively validated for assessment of recurrence risk in post-operative stage II colon cancer patients but is of only modest prognostic value and is not predictive of chemotherapy benefit.¹⁹ Widely applicable biomarkers are thus required, particularly ones that negate the need for expensive molecular testing,²⁰⁻²³ for cost-effective application in a diverse, non-specialist setting.

Associations between disease recurrence and cancer-associated stromal gene²³⁻²⁷ or protein²⁸⁻³⁵ expression has been reported in a variety of malignant conditions with some evidence of differential chemotherapeutic response defined by stromal gene expression profiles.³⁶⁻³⁸ In CRC, the potential prognostic value of stromal gene expressional analysis is highlighted by inclusion in the Oncotype DX[®] colon recurrence score.²³ Whilst the majority of studies have been performed at the transcriptomic or proteomic level, several studies indicate that, using established mathematical principles,³⁹⁻⁴² simple, inexpensive, visual evaluation of tumour composition⁴³⁻⁴⁷ or stromal phenotype^{48,49} may yield equally valuable prognostic information.

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Studies by Mesker *et al*,^{50,51}, West *et al*,⁵² and Huijbers *et al*,⁴⁷ suggest that simple visual^{47,50,51} or morphometric⁵² assessment of CRCs provides independent prognostic information, a premise recently tested in the VICTOR CRC trial.⁴⁷ To improve on the reproducibility of these visual estimation methods described by Huijbers *et al* (2013),⁴⁷ we have developed a quantitative compositional analysis technique utilising digital pathology.⁵³ This methodology, however, has so far only been tested in a small cohort of 145 all-stage CRC patients.⁵²

Given biological plausibility, existing literature, and the recent recognition of CRCs with mesenchymal/stroma rich gene signatures as biologically distinct and clinically significant tumour sub-populations⁵⁴, we hypothesise that quantitative tumour-stromal compositional analysis might represent a simple yet powerful methodology to determine risk of disease recurrence and differential response to adjuvant chemotherapy in CRC. We report here results of a test of this hypothesis utilising digitised material and clinicopathological data from the QUASAR trial, which randomised 3,239 predominantly stage II (91%) CRC patients between 6 months of fluorouracil and folinic acid (FUFA) chemotherapy and observation.⁸

MATERIALS AND METHODS

Ethics

Ethical approval was obtained from both the West Midlands Multi-Centre Research Ethics Committee (JR/MT/MREC/02/7/56a) and the Northern and Yorkshire Research Ethics Committee (08/H0903/62).

Patients

The design and details of QUASAR (ISRCTN82375386) are reported elsewhere.⁸ Briefly, 3,239 patients (post-curative resection for colon or rectal cancer, 91% stage II disease) were randomized to FUFA chemotherapy (n=1622) or observation only (n=1617) with chemotherapy considered in the event of recurrence.

Study design

Datasets were randomly partitioned into exploratory and validatory groups (figure 1). For initial analyses, an exploratory dataset (n=399), representative of the entire study cohort, was used to develop *a priori* prognostic⁵⁰⁻⁵² and predictive hypotheses using data derived from 3 specific analytical tumour regions (figure 2). Independent validation was performed using a separate validatory patient cohort dataset (n=1,800). All pathological and laboratory assessments were undertaken blind to the patients' treatment allocation and clinical outcomes.

Clinicopathological data

Pathological data (TNM 5) were abstracted by central review of anonymised histopathological reports collected from local units.

Morphometric analysis of tumour components

One tumour block per patient was selected for analyses. When more than one block was available, blocks were chosen to optimally represent the area of maximal tumour infiltration of the bowel wall. Histological sections (5µm thick) were cut from selected tumour blocks and stained with haematoxylin and eosin (H&E) using standard methodologies.

H&E sections were digitised at x20 magnification (0.46 μm per pixel) using an automated scanning system (Aperio XT, Aperio Technologies, Vista, CA, USA). Slide viewing for quality control (QC) and all subsequent quantification processes were performed using open source slide viewing software (Aperio ImageScope v10.2.2.2352, Aperio Technologies, Vista, CA, USA). Following image QC, and methodological and statistical validation using existing datasets, a systematic random sample of 50 points was superimposed on selected areas (figure 2) using web-based virtual graticule software (RandomSpot, University of Leeds, Leeds, UK).

For exploratory analyses (figure 1), three areas from each selected H&E section were identified for random point grid application (figure 2). The luminal tumour area (LT) was defined as a 9mm^2 area of greatest tumour epithelial cell density at the luminal surface of the tumour (figure 2A). The highest tumour density (HT) area was defined as a 9mm^2 area of highest tumour cell density (if not located at the luminal surface); figure 2B. The whole tumour (WT) area included the total area of tumour infiltration extending from the luminal surface and encompassing the lateral and deep invasive fronts of tumour (figure 2C). For 9mm^2 regions, large areas of necrosis and mucin were avoided if possible.

Tumour morphometry was determined by counting the frequency of occurrence of specific phenotypic categories underlying each of the 50 points. The following categories were used; 1: tumour epithelium, 2: tumour-associated stroma, 3: necrosis, 4: vessel, 5: inflammation, 6: tumour lumen, 7: mucin, 8: muscle and 0: non-informative / unclassifiable. Tumour morphometry was performed by technical staff under direct supervision of experienced pathologists (GH / PQ).

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For final outcome analyses, relative tumour composition was expressed as a percentage of total informative points for tumour epithelium (PoT – phenotype category 1 only) or stroma (PoS – phenotype category 2 only) or other categories (phenotype categories 3-8).

Determination of morphometric stratification cut-off points

For prognostic / predictive analyses, we applied the ‘pool adjacent violators’ algorithm to the continuous variables PoT or PoS to determine points where the relationship between the measured variable (e.g. stroma) and the recurrence risk demonstrated a distinct change⁵⁵⁻⁵⁹. Analyses were performed (by KH/RG) across all tumour / stromal measurements derived from each area within the training set (n=399) indicating the most appropriate cut-off values to stratify scores into two (<50%, ≥50%) or four groups (<35%, 35-49%, 50-64%, ≥65%).

Statistics and bioinformatics

The prognostic/predictive value of PoT/PoS measures were determined by stratified log-rank analyses.⁶⁰ For analyses of the prognostic value of PoT or PoS, recurrence was used as outcome measure. Recurrence is a reliable marker of the natural history of the cancer and correlates well with the effects of adjuvant chemotherapy on survival.⁶¹ Recurrence was calculated as the time elapsed from randomisation to recurrence with censoring at last contact with patient or death without recurrence. For assessment of the prognostic value of variables, recurrence rates over the whole follow-up period were analysed. Because adjuvant FU/FA reduces the risk of recurrence only in the first two years following randomisation with no further benefit, or loss of benefit, thereafter,⁸ investigations of differential treatment efficacy within subgroups included recurrences over the first two years only. Analyses were generally performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) by biostatisticians within the Birmingham Clinical Trials Unit, University of Birmingham, UK (KH)

and The Nuffield Department of Population Health, University of Oxford, Oxford, UK (RG).

Integrity of scoring data was validated by inter-observer comparison analysis of scores generated by 2 independent observers using kappa statistics on 2,975 individual data points. Inter-observer variability analyses were discontinued after 10 cases because of extremely high inter-observer agreement (see below). Subsequent to inter-observer analyses, all generated data were visually screened by experienced pathologists (GH/PQ).

RESULTS

Tumour tissue was obtained for 75% (2,439/3,239) of QUASAR participants with material from 2,199 patients suitable for analysis (figure 1). Comparisons of morphometry scores generated by two independent observers on 2,975 informative data points confirmed high agreement of 99.1%, Cohen's kappa=0.986 (95%CI=0.985-0.990; $p<0.001$).

Exploratory dataset analyses

Analyses of the 399-patient exploratory dataset provided no indication of any significant association between tumour or stroma density and chemotherapeutic efficacy (Supporting information, figure S1); prognostic analyses of the exploratory dataset therefore included both treated and control patients. A higher percentage of stroma cells was associated with worse prognosis in the exploratory data set, particularly in the 'highest' and 'whole' stroma categorisations (Supporting information, figures S2-S7). A lower percentage of tumour cells was also associated with worse prognosis in the exploratory data set, particularly in the 'highest' and 'luminal' tumour categorisation.

Validatory dataset analyses

Analyses of the 1800-patient validatory dataset also provided little evidence for any association between tumour or stroma density and chemotherapy efficacy (see below). Therefore, prognostic analyses of the validatory data again included both chemotherapy treated and control patients.

There was a highly significant trend of increasing 10-year recurrence risk with increasing stromal proportions in the whole tumour area in the test data set (log-rank $p < 0.0001$; figure 3). By contrast, other stromal and tumour scores were only weakly associated with worse prognosis (Supporting information, figures S8-S12). This highly significant association in the whole stroma categorisation is consistent with the exploratory data analyses where the association was strongest in the whole and highest stroma categories, whereas the lack of association in the highest stroma category is not.

For stromal proportions of $\geq 65\%$ in the whole tumour region, the 10-year risk of recurrent disease was 43% compared to 25% for tumours with $< 50\%$ scores. The adverse prognostic impact of higher whole tumour PoS scores remained highly significant ($p = 0.0002$) in analyses restricted to stage II / Dukes B patients only (figure 4). Notably, there was no association between the pattern of recurrence (local versus distant) and increasing stromal proportions within the whole tumour region ($p = 0.105$).

The distribution of PoS risk categories derived from the WT region differed significantly by tumour site, stage, histological subtype and MMR phenotype (table 1). There were significantly more tumours classified as PoS $\geq 65\%$ in the rectum than colon: 11.4% (43/377) compared to 5.1% (72/1,423), $p < 0.0001$. There were more low ($< 35\%$) PoS scores in Dukes' B than Dukes' C lesions 41.1% (658/1,603) versus 26.7% (43/161), $p = 0.002$. Predictably, there was a lower frequency of PoS $\geq 65\%$ lesions in mucinous tumours when compared to adenocarcinomas 1.9% (3/162) versus 6.4%

(95/1,473) respectively, $p < 0.0001$). The weak ($p = 0.012$) association of lower scores in the dMMR phenotype is also unsurprising given the previously reported interactions between dMMR, anatomical location and mucinous and medullary histology.

To investigate whether confounding with other pathological prognostic variables might at least partly explain the association of high stroma counts with recurrence, we undertook analyses stratified by these other variables using a binary dichotomisation (<50% low, $\geq 50\%$ high) of PoS scores. The risk of recurrence was over 50% higher in the 25% (457/1,800) of patients with high ($\geq 50\%$) PoS scores than in patients with low (<50%) PoS scores: [Rate ratio (RR)=1.61; 95%CI 1.30-2.00, $p < 0.0001$]; figure 5. Analyses stratified by chemotherapy allocation, tumour site and stage did not indicate any significant variability in the prognostic importance of stroma. Nor did any other of the variables tested except for the analyses stratified by *KRAS* mutation status where significant ($p = 0.001$) heterogeneity was seen with a stronger prognostic association seen in *KRAS* mutant tumours (RR=2.93; 95%CI=1.85-2.55) than in *KRAS* wild-type tumours (RR=1.14; 95%CI=0.60-1.62), (figure 5).

We investigated any differential chemotherapeutic response by PoS measurements using 2 year disease recurrence as outcome (figure 6). There were one third fewer recurrences with chemotherapy than control within the 2 year post-randomisation period [unstratified analyses; RR=0.64; 95%CI=0.49-0.84; $p = 0.001$]. Analyses sub-stratified by increasing WT PoS measures, failed to demonstrate a significant interaction between chemotherapy benefit and PoS measurements ($p_{trend} = 0.22$; figure 6).

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Unexpectedly, the beneficial effect of chemotherapy was as strong in analyses of all recurrences as in analyses of 2-year recurrence (Supporting information, figures S13-S16): 20.8% (183/880) of adjuvant treated patients recurred at 10 years compared to 27.6% (254/920) of untreated patients [RR=0.71; 95%CI=0.59-0.85; p=0.0003]. There was a suggestion ($p_{trend}=0.039$) that the reduction in 10-year recurrence with chemotherapy increased with increasing stromal proportions (PoS) in the WT region (Supporting information, figures S13-S16).

Analysis of other prognostic variables were performed to compare the prognostic strength of these variables with that of stroma count, and to determine if their prognostic value was independent of stromal count. (Supporting information, figures S17-S23) A borderline significant interaction between stroma and Dukes stage ($p_{trend}=0.032$) was seen with no apparent prognostic effect of nodal status in tumours with $\geq 65\%$ PoS values. No interactions between TNM T-category (TNM 5) and increasing PoS values were identified with the favourable prognostic effect of T3 versus T4 disease appearing similar across PoS subgroups (Supporting information, figure S18). Small subgroup numbers (2 recurrences in 5 patients with dMMR tumours with $\geq 65\%$ PoS measurements) precluded any meaningful investigation of potential interactions (Supporting information, figure S20). *KRAS* mutation was associated with a higher disease recurrence rate when compared to *KRAS* wild-type tumours [115/401; 28.7% versus 170/780; 21.8% recurred (RR=1.39; 95%CI=1.09-1.78, p=0.009); (Supporting information, figure S21) with the adverse prognostic effect of tumour PoS appearing greater in *KRAS*-mutant than *KRAS* wild-type tumours (Supporting information, figure S21). These observations parallel the analysis of dichotomised stroma stratified by *KRAS* mutation status (figure 5). *BRAF* status was non-prognostic (Supporting information, figure S22).

DISCUSSION

By applying simple digital morphometry to tumour blocks from 2,199 QUASAR CRC trial patients,⁸ we have demonstrated the powerful prognostic value of tumour-stromal counts; our division of tumours into four prognostic groups based on cut-offs in the validity cohort was confirmed in the test data with high intra-tumoural stroma (>65%) being associated with a disease recurrence risk approximately twice that of patients whose tumours contained low intra-tumoural stroma (<50%).

Our primary analysis with 2-year disease recurrence as outcome failed to substantiate any predictive chemotherapeutic effect of stroma count (figure 6). However, benefits of chemotherapy appeared to increase with increasing stroma count and this trend reached statistical significance in analyses of all recurrence stratified by intra-tumoural stromal proportion, suggesting that the benefits of chemotherapy are at least as good in the higher risk stroma groups. This contrasts with previous reports suggesting that tumour associated stroma may attenuate the efficacy of chemotherapeutic agents.⁶²⁻⁷⁰ Although some propose pharmacological targeting of tumour associated stroma as a mechanism of countering chemoresistance (and thus enhancing chemotherapeutic efficacy) in cancer⁷⁰⁻⁷³ the present study provides no support for this approach, at least in CRC.

The mechanism by which varying proportions of intra-tumoural stroma influence clinical outcome (also reported in breast,^{43,46} lung,^{44,74} ovarian,⁷⁵ cutaneous,⁴⁵ and prostatic⁷⁶ neoplasia) is unknown. Postulated theories commonly relate to stroma-associated cellular phenotypes and include enhanced pro-invasive signalling by intra-stromal myofibroblasts⁷⁷ or growth factor/cytokine production by cancer associated fibroblasts (CAF) inducing angiogenesis, increased tumour growth and invasion.^{71,78-81} Other possible explanations may involve mechanisms relating to tumour hypoxia⁸² and/or tumour associated inflammation.^{71,83} The possibility that differential proportions of

tumour and stroma may simply indirectly reflect the stage of disease has been previously suggested.^{51,52} We found no evidence for this potential explanation of the prognostic effect of stroma with similar findings in stratified and unstratified analyses and no interaction between stromal proportions, Dukes or TNM T categories.

Stromal TGF β ,^{84,85} a critical regulator of epithelial-mesenchymal transition,⁸⁶ is required to support metastatic dissemination when mutational inactivation of TGF β has occurred, as is common in CRC.⁸⁷ In view of the possible role of stromal-derived TGF β in the promotion of metastases in CRC,⁸⁷ the observation that *KRAS* mutant, high stromal tumours had a significantly more elevated recurrence risk than *KRAS* wild-type high stromal lesions is noteworthy. There is abundant evidence indicating that RAS signalling operates in unison with TGF β to enhance tumour cell invasiveness, either directly^{88,89} or by suppressing TGF β mediated growth inhibitory signals.^{90,91} A note of caution, however, is that the association between stroma and *KRAS* mutation was unanticipated, so our findings need confirming, but should encourage further investigation.

In summary, our analyses of the largest reported validation dataset confirm the prognostic value of simple morphometric analyses and reciprocate similar findings of morphological / morphometric prognostic studies in CRC reported by Mesker *et al*^{50,51}, Huijbers *et al*⁹² and West *et al*.⁵² Collectively, our data and that of others^{50-52,92} adds substance to the concept that simple morphometric appraisal of the quantitative relationship of tumour and / or tumour stroma can reliably identify patient sub-populations with differential risks of tumour recurrence in a simple and cost-effective manner. Our group (led by AW/DT) are now in the process of using the described dataset to develop an automated computational analytical platform for CRC prognostication.

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Statement of author contributions

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Financial support: RG, PQ

Administrative support: GH, RG, PQ

Provision of study materials or patients: LM, MS, DK, DT, AW, RG, PQ

Collection and assembly of data: GH, KS, KH, LM, ETH, RG, PQ

Data analysis and interpretation: GH, KH, MS, DK, RG, PQ

Manuscript writing: GH, DT, AW, KH, LM, ETH, KS, MS, DK, RG, PQ

Final approval of manuscript: GH, DT, AW, KH, LM, ETH, KS, MS, DK, RG, PQ

Disclaimer

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References

1. Benson AB, 3rd, Schrag D, Somerfield MR, et al: American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol* 22:3408-19, 2004
2. Morris EJ, Maughan NJ, Forman D, et al: Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology. *Gut* 56:1419-25, 2007
3. Quirke P, Morris E: Reporting colorectal cancer. *Histopathology* 50:103-12, 2007
4. NIH consensus conference. Adjuvant therapy for patients with colon and rectal cancer. *JAMA* 264:1444-50, 1990
5. Marshall JL, Haller DG, de Gramont A, et al: Adjuvant Therapy for Stage II and III Colon Cancer: Consensus Report of the International Society of Gastrointestinal Oncology. *Gastrointest Cancer Res* 1:146-54, 2007
6. IMPACT Trial Investigators: Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. *Lancet* 345:939-44, 1995
7. Moertel CG, Fleming TR, Macdonald JS, et al: Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *N Engl J Med* 322:352-8, 1990
8. Quasar Collaborative Group, Gray R, Barnwell J, et al: Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet* 370:2020-9, 2007
9. Arbuck SG: Overview of clinical trials using 5-fluorouracil and leucovorin for the treatment of colorectal cancer. *Cancer* 63:1036-44, 1989
10. Segal NH, Saltz LB: Is adjuvant therapy for stage II colon cancer worthwhile, and for

whom? *Nat Clin Pract Gastroenterol Hepatol* 5:422-3, 2008

11. Merkel S, Wein A, Gunther K, et al: High-risk groups of patients with Stage II colon carcinoma. *Cancer* 92:1435-43, 2001
12. Petersen VC, Baxter KJ, Love SB, et al: Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. *Gut* 51:65-9, 2002
13. Shepherd NA, Quirke P: Colorectal cancer reporting: are we failing the patient? *J Clin Pathol* 50:266-7, 1997
14. Tejpar S, Bertagnolli M, Bosman F, et al: Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist* 15:390-404, 2010
15. Locker GY, Hamilton S, Harris J, et al: ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 24:5313-27, 2006
16. Sinicrope FA, Sargent DJ: Clinical implications of microsatellite instability in sporadic colon cancers. *Curr Opin Oncol* 21:369-73, 2009
17. Hutchins G, Southward K, Handley K, et al: Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 29:1261-70, 2011
18. Ward RL, Turner J, Williams R, et al: Routine testing for mismatch repair deficiency in sporadic colorectal cancer is justified. *J Pathol* 207:377-84, 2005
19. Gray RG, Quirke P, Handley K, et al: Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. *J Clin Oncol* 29:4611-9, 2011
20. Webber EM, Lin JS, Evelyn PW: Oncotype DX tumor gene expression profiling in stage II colon cancer. Application: prognostic, risk prediction. *PLoS currents* 2, 2010
21. Kelley RK, Venook AP: Prognostic and Predictive Markers in Stage II Colon Cancer: Is There a Role for Gene Expression Profiling? *Clin Colorectal Cancer* 10:73-80, 2011
22. Jiang Y, Casey G, Lavery IC, et al: Development of a clinically feasible molecular assay to predict recurrence of stage II colon cancer. *J Mol Diagn* 10:346-54, 2008
23. O'Connell MJ, Lavery I, Yothers G, et al: Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. *J Clin Oncol* 28:3937-44, 2010
24. Planche A, Bacac M, Provero P, et al: Identification of prognostic molecular features in the reactive stroma of human breast and prostate cancer. *PLoS One* 6:e18640, 2011
25. Finak G, Bertos N, Pepin F, et al: Stromal gene expression predicts clinical outcome

in breast cancer. *Nat Med* 14:518-27, 2008

26. Lenz G, Wright G, Dave SS, et al: Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med* 359:2313-23, 2008
27. Saadi A, Shannon NB, Lao-Sirieix P, et al: Stromal genes discriminate preinvasive from invasive disease, predict outcome, and highlight inflammatory pathways in digestive cancers. *Proc Natl Acad Sci U S A* 107:2177-82, 2010
28. Sung CO, Lee KW, Han S, et al: Twist1 Is Up-Regulated in Gastric Cancer-Associated Fibroblasts with Poor Clinical Outcomes. *Am J Pathol* 179:1827-1838, 2011
29. Gupta V, Bassi DE, Simons JD, et al: Elevated expression of stromal palladin predicts poor clinical outcome in renal cell carcinoma. *PLoS One* 6:e21494, 2011
30. Koo JS, Park S, Kim SI, et al: The impact of caveolin protein expression in tumor stroma on prognosis of breast cancer. *Tumour Biol* 32:787-99, 2011
31. Wang D, Zhu T, Zhang FB, et al: Expression of ADAMTS12 in Colorectal Cancer-Associated Stroma Prevents Cancer Development and Is a Good Prognostic Indicator of Colorectal Cancer. *Dig Dis Sci*, 2011
32. Henry LR, Lee HO, Lee JS, et al: Clinical implications of fibroblast activation protein in patients with colon cancer. *Clin Cancer Res* 13:1736-41, 2007
33. Cohen SJ, Alpaugh RK, Palazzo I, et al: Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. *Pancreas* 37:154-8, 2008
34. Ngan CY, Yamamoto H, Seshimo I, et al: Quantitative evaluation of vimentin expression in tumour stroma of colorectal cancer. *Br J Cancer* 96:986-92, 2007
35. Tsujino T, Seshimo I, Yamamoto H, et al: Stromal myofibroblasts predict disease recurrence for colorectal cancer. *Clin Cancer Res* 13:2082-90, 2007
36. Farmer P, Bonnefoi H, Anderle P, et al: A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. *Nat Med* 15:68-74, 2009
37. Garrido-Laguna I, Uson M, Rajeshkumar NV, et al: Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. *Clin Cancer Res* 17:5793-800, 2011
38. Petty RD, Samuel LM, Murray GI, et al: APRIL is a novel clinical chemo-resistance biomarker in colorectal adenocarcinoma identified by gene expression profiling. *BMC Cancer* 9:-, 2009
39. Weibel ER: Stereological principles for morphometry in electron microscopic cytology. *Int Rev Cytol* 26:235-302, 1969
40. Dunhill MS: Quantitative methods in the study of pulmonary pathology. *Thorax* 17:320-28, 1962

- Accepted Article
41. Chalkley HW: Methods for quantitative morphological analysis of tissue. *Journal of the National Cancer Institute* 4:47-53, 1943
 42. Aherne WA, Dunhill MS: Point counting and the estimation of volume fraction, *Morphometry* (ed 1st). London, Edward Arnold, 1982
 43. Baak JP, Van Dop H, Kurver PH, et al: The value of morphometry to classic prognosticators in breast cancer. *Cancer* 56:374-82, 1985
 44. Maeshima AM, Niki T, Maeshima A, et al: Modified scar grade: a prognostic indicator in small peripheral lung adenocarcinoma. *Cancer* 95:2546-54, 2002
 45. Breuninger H, Schaumburg-Lever G, Holzschuh J, et al: Desmoplastic squamous cell carcinoma of skin and vermilion surface: a highly malignant subtype of skin cancer. *Cancer* 79:915-9, 1997
 46. de Kruijf EM, van Nes JG, van de Velde CJ, et al: Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple-negative carcinoma patients. *Breast Cancer Res Treat* 125:687-96, 2011
 47. Huijbers A, Tollenaar RA, v Pelt GW, et al: The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. *Ann Oncol* 24:179-85, 2013
 48. Ueno H, Jones AM, Wilkinson KH, et al: Histological categorisation of fibrotic cancer stroma in advanced rectal cancer. *Gut* 53:581-6, 2004
 49. Ueno H, Jones A, Jass JR, et al: Clinicopathological significance of the 'keloid-like' collagen and myxoid stroma in advanced rectal cancer. *Histopathology* 40:327-34, 2002
 50. Mesker WE, Liefers GJ, Junggeburst JM, et al: Presence of a high amount of stroma and downregulation of SMAD4 predict for worse survival for stage I-II colon cancer patients. *Cell Oncol* 31:169-78, 2009
 51. Mesker WE, Junggeburst JM, Szuhai K, et al: The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. *Cell Oncol* 29:387-98, 2007
 52. West NP, Dattani M, McShane P, et al: The proportion of tumour cells is an independent predictor for survival in colorectal cancer patients. *Br J Cancer* 102:1519-23, 2010
 53. Treanor D, Dattani M, Quirke P, et al: Systematic random sampling with virtual slides: A new software tool for tissue research. Abstract P175. *J Pathol* 216:76, 2008
 54. Guinney J, Dienstmann R, Wang X, et al: The consensus molecular subtypes of colorectal cancer. *Nat Med* 21:1350-6, 2015
 55. Ancukiewicz M, Finkelstein DM, Schoenfeld DA: Modelling the relationship between continuous covariates and clinical events using isotonic regression. *Stat Med* 22:3151-9, 2003

56. Salanti G, Kurt U: A nonparametric changepoint model for stratifying continuous variables under order restrictions and binary outcome. *Stat Methods Med Res* 12:351-67, 2003
57. Salanti G, Ulm K: A non-parametric framework for estimating threshold limit values. *BMC Med Res Methodol* 5:36, 2005
58. Pace NL, Stylianou MP: Advances in and limitations of up-and-down methodology: a precis of clinical use, study design, and dose estimation in anesthesia research. *Anesthesiology* 107:144-52, 2007
59. Fawcett T, Niculescu-Mizil A: PAV and the ROC convex hull. *Mach Learn* 68:97-106, 2007
60. Peto R, Pike MC, Armitage P, et al: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. *Br J Cancer* 35:1-39, 1977
61. Sargent DJ, Wieand HS, Haller DG, et al: Disease-free survival versus overall survival as a primary end point for adjuvant colon cancer studies: individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol* 23:8664-70, 2005
62. Kouniavsky G, Khaikin M, Zvibel I, et al: Stromal extracellular matrix reduces chemotherapy-induced apoptosis in colon cancer cell lines. *Clin Exp Metastasis* 19:55-60, 2002
63. Pietras K, Rubin K, Sjoblom T, et al: Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res* 62:5476-84, 2002
64. Loeffler M, Kruger JA, Niethammer AG, et al: Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J Clin Invest* 116:1955-62, 2006
65. Miyamoto H, Murakami T, Tsuchida K, et al: Tumor-stroma interaction of human pancreatic cancer: acquired resistance to anticancer drugs and proliferation regulation is dependent on extracellular matrix proteins. *Pancreas* 28:38-44, 2004
66. Heldin CH, Rubin K, Pietras K, et al: High interstitial fluid pressure - an obstacle in cancer therapy. *Nat Rev Cancer* 4:806-13, 2004
67. Damiano JS, Hazlehurst LA, Dalton WS: Cell adhesion-mediated drug resistance (CAM-DR) protects the K562 chronic myelogenous leukemia cell line from apoptosis induced by BCR/ABL inhibition, cytotoxic drugs, and gamma-irradiation. *Leukemia* 15:1232-9, 2001
68. Friedland JC, Lakins JN, Kazanietz MG, et al: alpha6beta4 integrin activates Rac-dependent p21-activated kinase 1 to drive NF-kappaB-dependent resistance to apoptosis in 3D mammary acini. *J Cell Sci* 120:3700-12, 2007
69. Weaver VM, Lelievre S, Lakins JN, et al: beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell* 2:205-16, 2002
70. Sebens S, Schafer H: The Tumor Stroma as Mediator of Drug Resistance - A Potential

Target to Improve Cancer Therapy? *Curr Pharm Biotechnol*, 2011

71. Allen M, Louise Jones J: Jekyll and Hyde: the role of the microenvironment on the progression of cancer. *J Pathol* 223:162-76, 2011
72. Micke P, Ostman A: Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy? *Lung cancer* 45 Suppl 2:S163-75, 2004
73. Micke P, Ostman A: Exploring the tumour environment: cancer-associated fibroblasts as targets in cancer therapy. *Expert opinion on therapeutic targets* 9:1217-33, 2005
74. Nakajima I: [Immunohistochemical study of the extracellular matrix in non-small cell lung cancer: relation to lymph node metastasis and prognosis]. *Hokkaido Igaku Zasshi* 66:356-68, 1991
75. Schipper NW, Smeulders AW, Baak JP: Evaluation of automated estimation of epithelial volume and its prognostic value in ovarian tumors. *Lab Invest* 61:228-34, 1989
76. Yanagisawa N, Li R, Rowley D, et al: Stromogenic prostatic carcinoma pattern (carcinomas with reactive stromal grade 3) in needle biopsies predicts biochemical recurrence-free survival in patients after radical prostatectomy. *Hum Pathol* 38:1611-20, 2007
77. De Wever O, Mareel M: Role of tissue stroma in cancer cell invasion. *J Pathol* 200:429-47, 2003
78. Bhowmick NA, Neilson EG, Moses HL: Stromal fibroblasts in cancer initiation and progression. *Nature* 432:332-7, 2004
79. Kunz-Schughart LA, Knuechel R: Tumor-associated fibroblasts (part II): Functional impact on tumor tissue. *Histology and histopathology* 17:623-37, 2002
80. Kunz-Schughart LA, Knuechel R: Tumor-associated fibroblasts (part I): Active stromal participants in tumor development and progression? *Histol Histopathol* 17:599-621, 2002
81. Karnoub AE, Dash AB, Vo AP, et al: Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449:557-63, 2007
82. Sivridis E, Giatromanolaki A, Koukourakis MI: Proliferating fibroblasts at the invading tumour edge of colorectal adenocarcinomas are associated with endogenous markers of hypoxia, acidity, and oxidative stress. *J Clin Pathol* 58:1033-8, 2005
83. Eltzschig HK, Carmeliet P: Hypoxia and inflammation. *N Engl J Med* 364:656-65, 2011
84. Rooke HM, Crosier KE: The smad proteins and TGFbeta signalling: uncovering a pathway critical in cancer. *Pathology* 33:73-84, 2001
85. Wong SF, Lai LC: The role of TGFbeta in human cancers. *Pathology* 33:85-92, 2001
86. Bates RC, Mercurio AM: The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. *Cancer Biol Ther* 4:365-70, 2005

87. Calon A, Espinet E, Palomo-Ponce S, et al: Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer Cell* 22:571-84, 2012
88. Gotzmann J, Huber H, Thallinger C, et al: Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-beta1 and Ha-Ras: steps towards invasiveness. *J Cell Sci* 115:1189-202, 2002
89. Fujimoto K, Sheng H, Shao J, et al: Transforming growth factor-beta1 promotes invasiveness after cellular transformation with activated Ras in intestinal epithelial cells. *Exp Cell Res* 266:239-49, 2001
90. Bulus NM, Sheng HM, Sizemore N, et al: Ras-mediated suppression of TGFbetaRII expression in intestinal epithelial cells involves Raf-independent signaling. *Neoplasia* 2:357-64, 2000
91. Jiang B, Zhang JS, Du J, et al: Growth inhibitory signalling by TGFbeta is blocked in Ras-transformed intestinal epithelial cells at a post-receptor locus. *Cell Signal* 15:699-708, 2003
92. Huijbers A, Tollenaar RA, v Pelt GW, et al: The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 24:179-85, 2013

Figure legends

Figure 1 - Study schema.

Figure 2 - Tumour regions for morphometric analysis. (A) Selected tumor block representing maximal lateral and deep tumor infiltration; (B) "Luminal" 9mm² area; (C) "Highest" 9mm² area of maximal tumor epithelial density (if away from luminal surface; (D) "Whole" tumor area encompassing lateral and deep invasive fronts. Scale bars=5mm

Figure 3 - Recurrence risk stratified by proportion of stroma (PoS) in the whole tumour (WT) region – validatory dataset, stage II / III patients (n=1,800).

Figure 4 - Recurrence risk stratified by proportion of stroma (PoS) in the whole tumour (WT) region – validatory dataset, stage II patients only (n=1,603)

Figure 5 - Recurrence risk by dichotomised proportion of stroma (PoS) stratified by clinicopathological variables in the whole tumour (WT) region. Validatory dataset (n=1,800); (O-E)=observed minus expected; Var=variance

Figure 6 - Recurrence risk by chemotherapy (2 years) sub-stratified by tumour morphometric subgroups. Validatory dataset (n=1,800); (O-E)=observed minus expected; Var=variance.

List of online supporting information

Figure S1 - Recurrence risk (2 years) by chemotherapy, stratified by sub-stratified by tumour morphometric subgroups

Figure S2 - Recurrence risk by proportion of stroma (PoS) in highest tumour (HT) area

Figure S3 - Recurrence risk by proportion of stroma (PoS) in the luminal tumour (LT) area

Figure S4 - Recurrence risk by proportion of stroma (PoS) in whole tumour (WT) area

Figure S5 – Recurrence risk by proportion of tumour (PoT) in the highest tumour (HT) area

Figure S6 – Recurrence risk by proportion of tumour (PoT) in the luminal tumour (LT) area

Figure S7 - Recurrence risk by proportion of tumour (PoT) in whole tumour (WT) area

Figure S8 - Recurrence risk by proportion of stroma (PoS) in highest tumour (HT) area

Figure S9 - Recurrence risk by proportion of stroma (PoS) in luminal tumour (LT) area

Figure S10 - Recurrence by proportion of tumour (PoT) in highest tumour (HT) area

Figure S11 - Recurrence by proportion of tumour (PoT) in luminal tumour (LT) area

Figure S12 - Recurrence by proportion of tumour (PoT) in whole tumour (WT) area

Figure S13 - Recurrence risk by chemotherapy stratified by proportion of stroma (PoS) in the whole tumour (WT) region across clinicopathological sub-groups

Figure S14 - Recurrence risk by chemotherapy stratified by proportion of stroma (PoS) in the whole tumour (WT) region across clinicopathological sub-groups

Figure S15 - Recurrence risk by chemotherapy stratified by proportion of stroma (PoS) in the whole tumour (WT) region across clinicopathological sub-groups

Figure S16 - Recurrence risk by chemotherapy stratified by proportion of stroma (PoS) in the whole tumour (WT) region across molecular sub-groups

Figure S17 – Recurrence risk by AJCC stage stratified by proportion of stroma (PoS) in the whole tumour (WT) region

Figure S18 – Recurrence risk by T-stage (TNM 5) stratified by proportion of stroma (PoS) in the whole tumour (WT) region

Figure S19 – Recurrence risk by lymph node yield stratified by proportion of stroma (PoS) in the whole tumour (WT) region

Figure S20 – Recurrence risk by extra-mural vascular invasion stratified by proportion of stroma (PoS) in the whole tumour (WT) region

Figure S21 – Recurrence risk by mismatch repair status stratified by proportion of stroma (PoS) in the whole tumour (WT) region

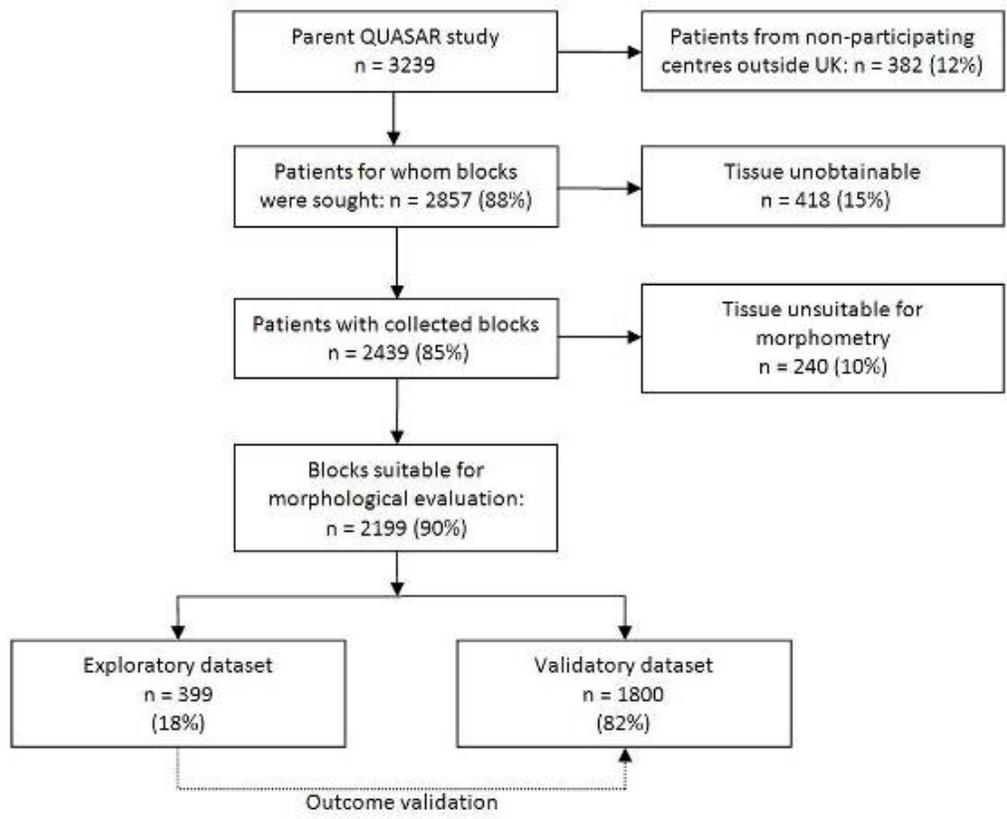
Figure S22 – Recurrence risk by KRAS mutation status stratified by whole tumour PoS

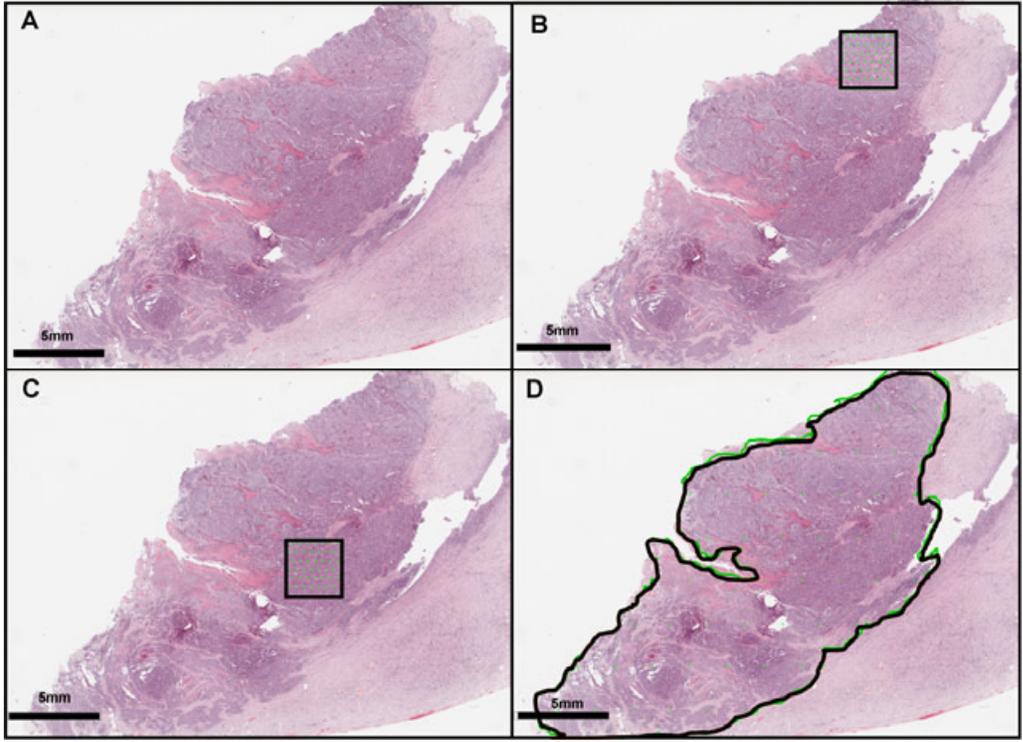
Figure S23 – Recurrence risk by BRAF mutation status stratified by whole tumour PoS

Table 1 – Patient characteristics by proportion of stroma (whole tumour area)

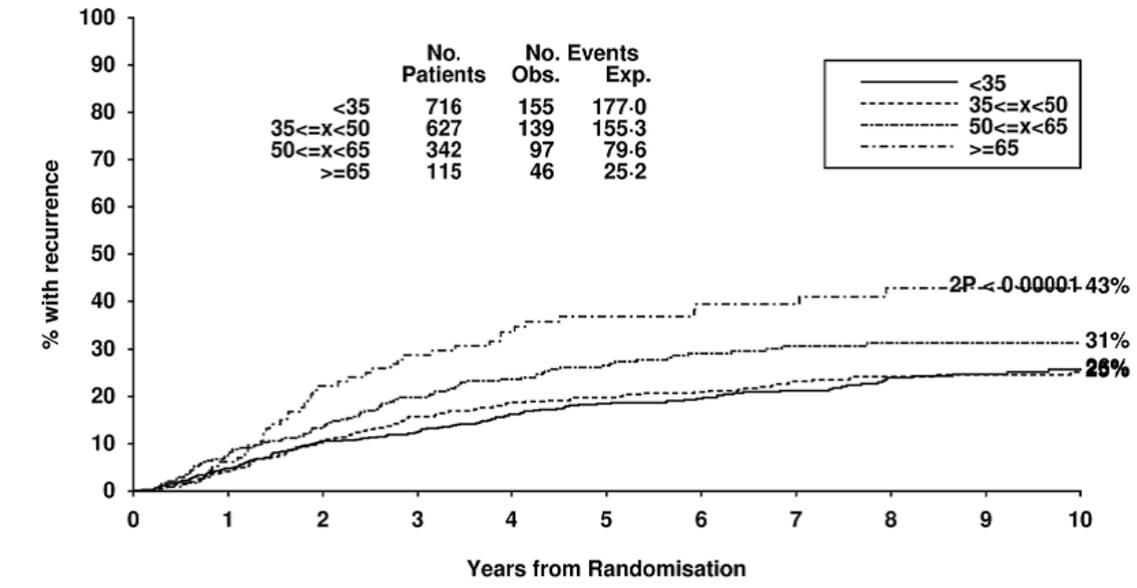
% PoS categories	<35%	35%-49%	50%<65%	≥65%	p-value
	n, (%)	n, (%)	[n, (%)]	[n, (%)]	
All patients (n=1,800)	716 (39.78)	627 (34.83)	342 (19.0)	115 (6.39)	n/a
Colon (n=1,423)	597 (41.95)	497 (34.93)	257 (18.06)	72 (5.06)	<0.0001
Rectum (n=377)	119 (31.56)	130 (34.48)	85 (22.55)	43 (11.41)	
Dukes B / Stage II (n=1,603)	658 (41.05)	553 (34.50)	295 (18.40)	97 (6.05)	0.0019
Dukes C/Stage III (n=161)	43 (26.71)	62 (38.51)	40 (24.84)	16 (9.94)	
T3 (n=1,362)	545 (40.01)	481 (35.32)	259 (19.02)	77 (5.65)	0.865
T4 (n=217)	82 (37.79)	76 (35.02)	45 (20.74)	14 (6.45)	
EMVI (n=161)	51 (31.68)	58 (36.02)	41 (25.47)	11 (6.83)	0.078
No EMVI (n=1468)	594 (40.76)	517 (35.22)	271 (18.46)	86 (5.86)	
Well diff. (n=151)	70 (46.36)	51 (33.77)	26 (17.22)	4 (2.65)	0.159
Moderately diff. (n=1,343)	521 (38.79)	485 (36.11)	256 (19.06)	81 (6.03)	
Poor diff. (n=139)	57 (41.01)	40 (28.78)	30 (21.58)	12 (8.63)	

Mucinous (n=162)	100 (61.73)	47 (29.01)	12 (7.41)	3 (1.85)	<0.0001
AdenoCa, NOS (n=1,473)	548 (37.20)	530 (35.98)	300 (20.37)	95 (6.45)	
<12 lymph nodes (n=999)	398 (39.84)	346 (34.63)	193 (19.32)	62 (6.21)	0.699
12+ lymph nodes (n=544)	217 (39.89)	199 (36.58)	101 (18.57)	27 (4.96)	
Female (n=705)	289 (40.99)	248 (35.18)	130 (18.44)	38 (5.39)	0.481
Male (n=1,113)	427 (39.0)	379 (34.61)	212 (19.36)	77 (7.03)	
Chemotherapy (n=880)	353 (40.11)	290 (32.95)	178 (20.23)	59 (6.70)	0.330
Observation (n=920)	363 (39.46)	337 (36.63)	164 (17.83)	56 (6.09)	
MMR deficient (n=162)	85 (52.47)	48 (29.63)	24 (14.81)	5 (3.09)	0.012
MMR proficient (n=1,290)	508 (39.38)	468 (36.28)	241 (18.68)	73 (5.66)	
<i>BRAF</i> -mutant (n=92)	41 (44.57)	29 (31.52)	17 (18.48)	5 (5.42)	0.821
<i>BRAF</i> wild-type (n=1,091)	434 (39.78)	389 (35.66)	205 (18.79)	63 (5.77)	
KRAS mutant (n=401)	167 (41.65)	134 (33.42)	76 (18.95)	24 (5.99)	0.816
KRAS wild-type (n=780)	307 (39.36)	282 (36.15)	146 (18.72)	45 (5.77)	





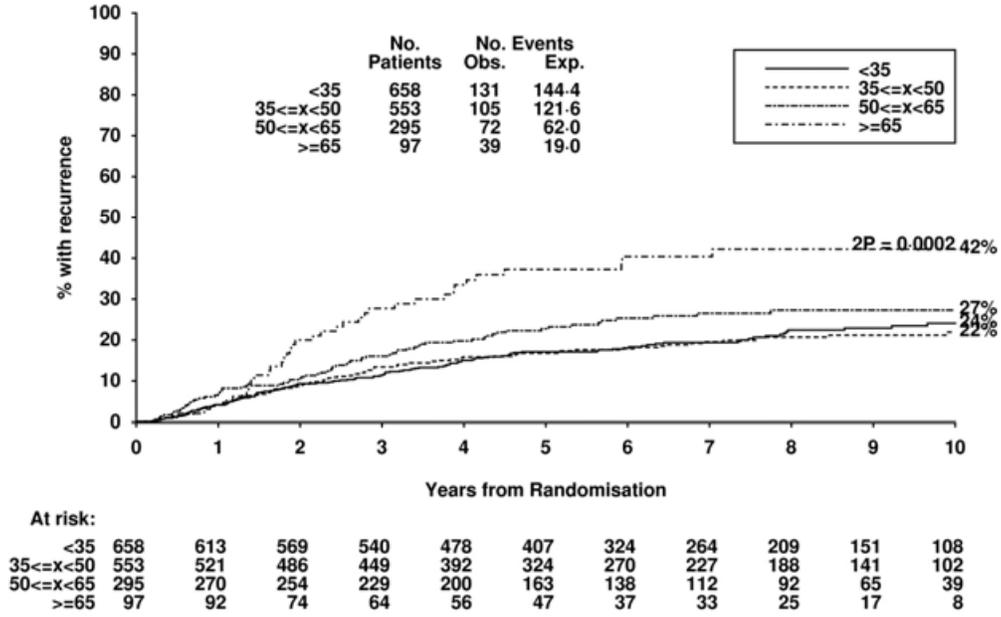
Recurrence – by whole stroma category



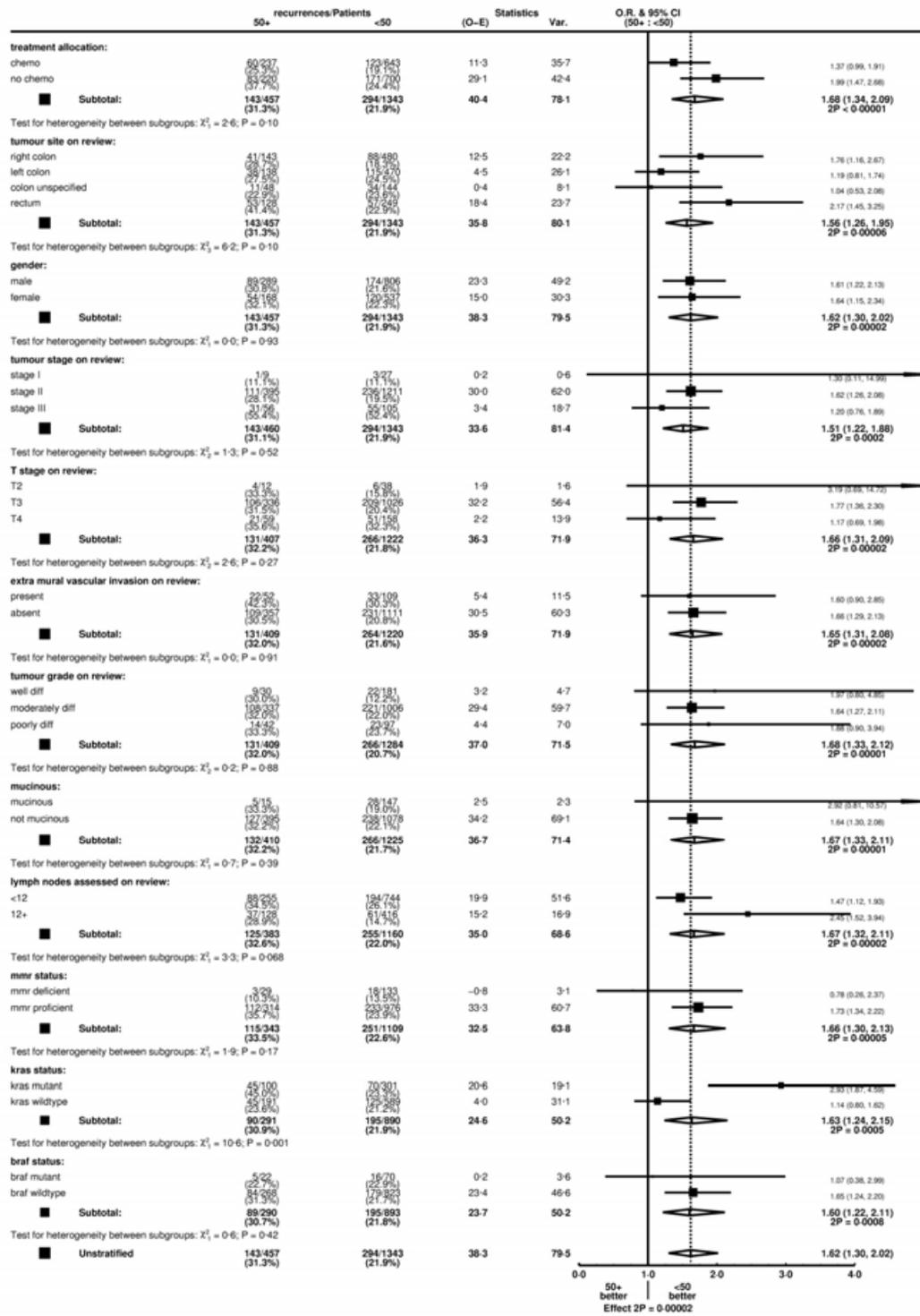
At risk:

	0	1	2	3	4	5	6	7	8	9	10
<35	716	664	612	583	515	440	350	287	231	169	121
35<=x<50	627	592	543	498	436	364	304	257	214	159	117
50<=x<65	342	311	286	255	223	182	152	124	101	74	41
>=65	115	106	85	75	66	56	45	40	30	21	10

Recurrence – by whole stroma category (stage 2)



Recurrences in QUASAR by whole stroma percentage



2 Year Recurrences in QUASAR by treatment

