Tumour necrosis is significantly associated with reduced recurrence-free survival after curative resection of gastrointestinal stromal tumours

Mr. Robert Tyler MBChB MRCS[a], Miss Emma Davies MBBS MRCS[c], Dr. Dominic Tan MBChB[b], Mr. James Hodson BSc[a], Dr. Phillipe Taniere MD PhD[a], Dr. Khin Thway FRCPath BSc[c], Dr. Mariam Jafri MRCP PhD[a], Mr. Max Almond FRCS MD[a], Mr. Samuel Ford FRCS PhD[a], Mr. Dirk Strauss FRCS MMed[c], Mr. Andrew Hayes FRCS PhD[c], Mr. Myles Smith FRCSI PhD[c], Mr. Anant Desai FRCS MD[a].

a) Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Edgbaston, Birmingham, B15 2TH, United Kingdom.
b) University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom
c) Royal Marsden Hospital, 203 Fulham Road, Chelsea, London, SW3 6JJ, United Kingdom

Corresponding author: Mr. Robert Tyler

Postal Address: Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Edgbaston, Birmingham, B15 2TH, United Kingdom.

Email Address: Robert.tyler@nhs.net

Phone Number: 07825031336

Shortened Running Title: Tumour necrosis as a risk factor in GIST

Synopsis:

We found tumour necrosis to be an independent predictor of recurrence-free survival in operable GIST. Tumour necrosis identifies high-risk patients at risk of early relapse, appears independent of genotype and should be routinely reported.

Background + Objectives:

The impact of tumour necrosis as a prognostic factor in gastrointestinal stromal tumour (GISTs) is still debated. The objective was to determine whether tumour necrosis is an independent risk factor for survival in patients with GISTs.

Methods:

Patients undergoing surgery for primary GIST from March 2003 – October 2018 at two sarcoma referral centres were retrospectively identified. Patients who received neoadjuvant imatinib were excluded. Multivariable Cox regression models were produced, to assess whether tumour necrosis was an independent predictor of either overall or recurrence-free survival.

Results**:**

41/195 (21.0%) patients had tumour necrosis. Tumour necrosis was associated with a significantly higher modified NIH risk score, with 29/41 (70.7%) patients with necrosis classified as high risk, compared to 52/153 (34.0%) without ($p<0.001$). Tumour necrosis was found to be independently predictive of recurrence-free survival (hazard ratio [HR]: 5.26, 95% CI: 2.62 – 10.56, $p<0.001$) on multivariable analysis. At five years, 44.3% of patients with necrosis had either died or developed recurrence, compared to 9.9% of those without.

Conclusion:

Tumour necrosis is an independent predictor of recurrence-free survival in patients with operable GISTs. It should be routinely reported by pathologists, and used by clinicians when counselling patients and deciding on adjuvant therapy.

Key words: Tumour necrosis, GIST, survival, predictive factor

Introduction

Gastrointestinal stromal tumour (GIST) is the most common sarcoma of the gastrointestinal tract[1]. GISTs occur from the distal half of the oesophagus to the anorectum, with an overall incidence of between 10 and 20 per million[2]. The majority of GISTs are driven by activating mutations in *KIT* or *PDGFRA*, encoding for mutated tyrosine kinase receptors, which can be targeted by small molecule inhibitors, such as imatinib and sunitinib[3]. Three years of adjuvant imatinib can improve overall survival (OS) in patients with sensitive mutations, but administration depends on the estimated risk of recurrence[4].

Estimating the risk of recurrence is central to the management of GIST and, as a result, several risk classification systems exist. The most widely used comprise the Armed Forces Institute of Pathology (AFIP) classification proposed by Miettinnen, and Joensuu's 2008 modified Institute of Health (NIH) criteria[5]. The modified NIH risk score adopted by UK guidelines comprises tumour size, mitotic index and primary tumour site, and differs from previous risk scores with the addition of tumour rupture. It categorises patients into very low, low, intermediate, and high risk. As with all risk scores, this has its limitations[6]. Significant prognostic heterogeneity can exist in tumours with identical risk scores. Half of patients in the intermediate to high risk groups will not develop metachronous disease progression, resulting in a number of patients receiving inappropriate treatment[5]. Independent prognostic

markers are therefore useful, especially in GIST, where adjuvant therapy can be so beneficial.

Tumour necrosis as a prognostic factor has garnered attention over recent years, and has been found to be an independent prognostic factor in renal, lung, and bladder cancer, as well as being associated with a poorer survival in many others[7-9]. Authors of these studies conclude that tumour necrosis has high interobserver reproducibility, hence should be routinely reported and, in some cases, incorporated into scoring systems.

In GIST, there are conflicting reports on the prognostic relevance of tumour necrosis, with no such study having been performed in the UK to date. Miettinen et al in a study of 1765 gastric GISTs found tumour necrosis to be a significantly unfavourable factor, but only tumour size was independently predictive of recurrence-free survival (RFS)[1].

A recent meta-analysis of 2320 patients across 18 studies described a decreased RFS and OS in GIST patients with tumour necrosis[10]. This was limited by different definitions of tumour necrosis, excessively high tumour necrosis rates, and the fact that only three out of eighteen studies openly excluded pre-treated patients. As it is not possible to differentiate treatment-induced necrosis and tumour-related necrosis, the question remains unanswered.

The present study investigated whether tumour necrosis was an independent risk factor for RFS and OS in primary GISTs, by combining data from two sarcoma referral centres.

Materials and Methods

Patients undergoing surgery with curative intent for primary GIST from March 2003 – October 2018 were identified from the combined clinical databases of two soft tissue sarcoma referral centres – The Queen Elizabeth Hospital (QEH), Birmingham and The Royal Marsden Hospital (RMH), London. Patients who received neoadjuvant imatinib were excluded, to prevent the inclusion of imatinib-induced tumour necrosis. Patients with metastatic disease were also excluded. After combining datasets, any inconsistencies were dealt with by personal correspondence.

Data collected were: age, sex, surgical approach (open vs. laparoscopic surgery), genetic and exonic mutation, tumour location, histological subtype, tumour necrosis, modified NIH risk score, adjuvant imatinib therapy, and survival data. RFS was defined as the time from surgery to the first event of recurrence or death, with patients censored at the time of data collection. Patients were followed up as per ESMO guidelines[11]. High risk patients underwent a CT scan every three to six months for three years during adjuvant therapy, followed by scans every three months for two years after the conclusion of treatment. Low risk patients underwent a CT scan every six to twelve months for five years.

Histopathological assessment

All histological specimens were evaluated by specialist sarcoma pathologists (PT/KT), according to international guidelines[12]. Pathological assessment was based on one formalin-fixed, paraffin-embedded tissue block per centimetre of tumour.

In accordance with previous studies, tumour necrosis was recorded as present or absent. “Tumour necrosis present” was defined as microscopic necrosis that could be recognised by the histopathologist, based on the histological evaluation of all available tumour blocks, and histologically comprised microscopic coagulative necrosis, characterized by sheets of degenerate and dead tumour cells associated with nuclear and cellular debris[13]. This was distinct from histologic changes such as hyalinisation, fibrosis, infarct and cystic changes, which were not considered to represent those of necrosis. Tumour necrosis on gross macroscopic examination was not classified or recorded as being present.

Genetic mutational analysis was performed in 194/195 (99.5%) cases, whilst exonic mutational analysis was available in 149/195 (76.4%) cases.

Statistical Analysis

Comparisons of clinicopathological variables by tumour necrosis were performed using Fisher’s exact test for nominal factors, and Mann-Whitney U tests for ordinal or continuous factors. Survival outcomes were then compared using Kaplan-Meier

curves, with hazard ratios (HRs), 95% confidence intervals (CIs) and p-values calculated using univariable Cox regression models.

Multivariable analyses were then performed, in order to assess whether tumour necrosis was an independent predictor of patient outcomes. Cox regression models were produced, which used a forward stepwise approach to variable selection. Continuous variables were divided into categories based on percentiles prior to the analysis, in order to improve model fit. Where tumour necrosis was not selected by the stepwise procedure, a new model was produced which included this alongside the factors from the stepwise model.

All analyses were performed using IBM SPSS 22 (IBM Corp. Armonk, NY). All p-values were from two-tailed tests, and $p<0.05$ was deemed to be indicative of statistical significance throughout.

Results

Tumour Necrosis

The study population consisted of a combined 261 patients, of whom 66 (25.3%) received neoadjuvant imatinib. Patients treated with neoadjuvant imatinib tended to be younger, with higher Modified NIH risk scores, a higher rate of C-KIT mutations, and were more likely to have non-gastric tumours. Further details of these comparisons are reported in *Supplementary Table 1*.

After excluding those patients treated with neoadjuvant imatinib, 195 patients (QEH: 121, RMH: 74) remained in the final study cohort, with a median age at surgery of 65 years (interquartile range [IQR]: 54-73), and of whom 116 (59.5%) were male. The majority of tumours were gastric (N=147; 75.4%), with the remainder located in the small bowel (N=41; 21.0%), extraintestinally (N=5; 2.6%) or in the colon/rectum (N=2; 1.0%). A total of 41 (21.0%) patients had tumour necrosis, and comparisons between these patients and the remainder of the cohort are reported in *Table 1*. Tumour necrosis was associated with a significantly higher modified NIH risk score, with 29 (70.7%) patients with tumour necrosis classified as high risk, compared to 52 (34.0%) in the remainder of the cohort ($p<0.001$). Patients with tumour necrosis were also significantly more likely to receive adjuvant therapy (19/41 (46.3%) vs. 22/154 (14.3%), $p<0.001$).

Post-operative outcomes

Over a median of 70 months (IQR: 46-103) of follow up, there were a total of 20 deaths, giving Kaplan-Meier estimated OS rates of 99.0%, 94.1%, and 88.1% at 1, 5, and 10 years, respectively. A total of 23 patients developed disease recurrence, of whom seven subsequently died, giving RFS rates of 96.6%, 83.0%, and 78.8% at 1, 5, and 10 years, respectively. Tumour necrosis was not found to be significantly associated with OS on univariable analysis (HR: 0.94, 95% CI: 0.31 – 2.84, $p=0.907$, *Figure 1a)*. However, a significant association between tumour necrosis and RFS was detected (HR: 4.20, 95% CI: 2.16 – 8.14, $p<0.001$, *Figure 1b*). After five years of follow-up, the Kaplan-Meier curves estimated that 44.3% of patients with tumour

necrosis had either died or developed recurrence, compared to 9.9% of those without tumour necrosis.

Multivariable analyses were then performed, to assess whether tumour necrosis was independently associated with survival outcomes, after adjustment for the clinicopathological variables from *Table 1*. The resulting models (*Table 2*) found increasing patient age to be independently associated with both significantly shorter OS (p=0.032) and RFS (p=0.007), with male gender also being associated with significantly shorter RFS (p=0.008). After accounting for these factors, tumour necrosis was not found to be a significant independent predictor of OS (HR: 1.10, 95% CI: 0.35 – 3.44, p=0.876), but was found to be a significant independent predictor of shorter RFS (HR: 5.26, 95% CI: 2.62 – 10.56, p<0.001).

Tumour necrosis and the modified NIH risk score

The modified NIH risk score was not found to be a significant independent predictor of RFS on multivariable analysis. This was partly as a result of correlation with the presence of tumour necrosis, the rates of which increased progressively across the risk categories (6.0%, 17.4%, and 35.8% for low, intermediate, and high risk, respectively, p<0.001). As such, a subgroup analysis was performed to assess the role of tumour necrosis within the intermediate and high-risk groups.

Within the intermediate risk subgroup, tumour necrosis was not found to be significantly associated with RFS (HR: 1.14, 95% CI: 0.13 – 10.20, p=0.907), although this analysis was limited by the small sample size (N=5 events). However, a significant effect of tumour necrosis was observed in the high-risk group, with a hazard ratio of 5.18 (95% CI: 2.08 – 12.91, p<0.001). Patients without tumour necrosis in the high-risk subgroup had Kaplan-Meier estimated five-year RFS rates of 87%, which was similar to that of the intermediate risk patients with (83%) or without (87%) necrosis. However, the combination of high risk on the modified NIH risk score and tumour necrosis yielded a considerably lower five-year RFS rate of 45% (*Figure 2*).

Discussion

The present study found patients with tumour necrosis to have significantly shorter RFS on univariable analysis, with 44.3% of patients having either died or developed recurrence within five years, compared to 9.9% of those without tumour necrosis. This difference persisted on multivariable analysis, with tumour necrosis being a significant independent predictor of poorer RFS in GISTs (HR: 5.26, 95% CI: 2.62 – 10.56, p<0.001). On subgroup analysis, the association between tumour necrosis and RFS remained significant within those classified as high risk by the modified NIH score (HR: 5.18, 95% CI: 2.08-12.91, p<0.001). Therefore, there is a potential role for tumour necrosis to be routinely considered in multidisciplinary discussions surrounding patients at high risk of early recurrence.

Tumour necrosis has been shown to be an adverse prognostic factor in several malignancies. The deleterious effects of tumour necrosis on prognosis are likely to be multifactorial. Some argue more aggressive tumours have genetic limitations in apoptosis, leading to unscheduled cell death and the release of necrotic mediators[14]. Others believe necrosis is driven by pro-inflammatory cytokines and angiogenesis, resulting in a heterogenous tumour vasculature. This ultimately leaves some areas necrotic, and potentially drives cancer progression and metastasis[15].

Regardless of mechanism, in our data, tumour necrosis appears to be associated with poor prognosis, particularly in high-risk GIST. In the high-risk category of the modified NIH risk score, those without tumour necrosis had a five-year RFS of 87%, compared to 45% in patients with tumour necrosis. Importantly, other groups have identified the adverse prognostic effect of tumour necrosis in the high-risk setting. Liu et al found the prognostic significance of tumour necrosis on RFS was maintained in high risk patients, but not in low-risk patients[16]. Furthermore, Zheng et al. in 246 high-risk gastric GISTs found poorer OS with tumour necrosis and greater than 20 mitoses per 50 HPFs[17] and termed this group ‘very high’ risk GISTs.

A recent comparison of the five most commonly used risk scoring systems found five-year RFS rates ranging from 56.1-74.6%[5]. It is this prognostic heterogeneity within the classification systems that has led to progressive modifications since their inception. In an attempt to reduce this prognostic heterogeneity, this data, combined with others, supports the idea that the modified NIH risk score could be re-visited as very low, low, intermediate, high and ‘very high’.

Whether this new ‘very high’ group would benefit from a longer course of adjuvant therapy is debatable. Liu et al argued for prolonging adjuvant therapy in high risk patients with tumour necrosis[16]. Extending adjuvant therapy was explored in the PERSIST-5 trial, where imatinib was given to intermediate or high-risk patients for five years. Not a single patient relapsed whilst on the drug, although 49% had ended treatment early[18].

Within our high-risk group, 18/29 (62%) with tumour necrosis received adjuvant imatinib therapy compared to 20/52 (38%) without necrosis. Despite this, the group with tumour necrosis still had a poorer RFS. Clinicians should therefore remain vigilant to the high chance of relapse in this group by ensuring appropriately timed surveillance imaging. This facilitates an early switch to second line agents where appropriate, if recurrence is then confirmed.

Subgroup analysis within modified NIH risk score categories did not find tumour necrosis to be significantly associated with RFS for the intermediate risk subgroup, which would have been useful in what is clinically a grey area. This lack of significance could be explained in our data by the lower mortality and low incidence of tumour necrosis within this population, which lead to insufficient statistical power to detect a clinically relevant effect in this subgroup.

Whether the adverse effect of tumour necrosis is due to associated genomic aberrations or independent from them is an important consideration. Yin et al. found C-KIT exon 9 mutations to be independently associated with radiological evidence of

tumour necrosis[19]. Whilst our data did not find the distribution of genomic mutations to differ significantly between those with and without tumour necrosis, there was a tendency for a higher rate of tumour necrosis in C-KIT exon 9 (38%) compared to the other groups (0-26%).

Our results do not suggest that the presence of tumour necrosis is independently predictive of OS. A recent analysis of the major risk classification systems found the majority unable to detect an OS difference between risk groups[5]. The differences between RFS and OS in this study could be explained by the fact that recurrent GIST is a treatable disease. Although patients develop recurrence, adjuvant imatinib therapy overcomes the risk-loading that tumour necrosis is identifying. Furthermore, there was a relatively long median follow-up of 70 months.

Tumour necrosis has repeatedly been shown to be an important prognostic factor in soft-tissue sarcoma with respect to survival and is a key component of the FNCLCC grading system[20, 21]. However, in GIST, tumour necrosis is not routinely reported, and reporting varies between centres, both in stating its presence or absence, or in how it is defined. In this study, we defined true tumour necrosis as simply microscopically present or absent, to increase its external validity and limit interobserver variability. We found a 21% tumour necrosis rate, which is consistent with previous studies[16, 22]. Importantly, the rates were comparable between hospitals, with 26/121 (21.5%) at the QEH and 15/74 (20.3%) present at the RMH. In light of these results, and the fact that reporting tumour necrosis in other sarcomas is well established, we feel that in GIST pathology reports should always state the presence or absence of tumour necrosis.

The decision to exclude patients pre-treated with neoadjuvant imatinib was based on evidence that imatinib therapy can induce significant histological changes, including focal to widespread tumour necrosis[23, 24]. For example, Agaram et al found 23/28 (82%) patients pre-treated with imatinib developed some degree of necrosis. It is therefore not possible to confidently differentiate between true tumour necrosis and necrosis induced by imatinib therapy. As a result of excluding these patients, the results of this study will only be generalizable to patients that do not receive neoadjuvant imatinib, who in our data are a lower risk subset. Nevertheless, the decision to give neoadjuvant therapy is primarily a surgical one, with guidelines stating its use to enhance the chances of an R0 resection or to facilitate function-sparing surgery in the future[11]. The decision is not entirely based on tumour biology. As our data shows, patients from all risk categories can be commenced on imatinib prior to surgery.

Patients in this study were identified from two reference sarcoma centres, which may potentially take more complex referrals and operate on higher-risk patients. Regarding risk, 81/194 patients (41.8%) included in the main analysis of this study were classed as high-risk. In the original proposal of the modified NIH risk score, Joensuu quoted a 44% incidence of high-risk GIST, which has subsequently been validated in other large population based studies. As such, the risk level of patients in the current study appear to be representative of the GIST population at large[25].

Although advances continue to be made in next generation sequencing, for the foreseeable future it will complement rather than replace or surpass the current

histological assessment. Tumour necrosis is a simple and reproducible independent predictor of RFS in patients with operable GIST, appears independent of genotype and location and appears most clinically relevant in those with high-risk GIST. It should be routinely reported by pathologists and used by clinicians when counselling patients and deciding on adjuvant therapy. This study also supports a growing body of evidence to add a 'very high' risk category to the modified NIH risk score.

1. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. The American journal of surgical pathology. 2005;29(1):52–68.
2. Miettinen M, Lasota J, editors. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Seminars in diagnostic pathology; 2006: Elsevier.
3. Hirota S, Isozaki K. Pathology of gastrointestinal stromal tumors. Pathology international. 2006;56(1):1–9.
4. Joensuu H, Eriksson M, Hall KS, Hartmann JT, Pink D, Schütte J, et al. One vs three years of adjuvant imatinib for operable gastrointestinal stromal tumor: a randomized trial. Jama. 2012;307(12):1265–72.
5. Schmieder M, Henne-Bruns D, Mayer B, Knippschild U, Rolke C, Schwab M, et al. Comparison of different risk classification systems in 558 patients with gastrointestinal stromal tumors after R0-resection. Frontiers in pharmacology. 2016;7:504.
6. Judson I, Bulusu R, Seddon B, Dangoor A, Wong N, Mudan S. UK clinical practice guidelines for the management of gastrointestinal stromal tumours (GIST). Clinical sarcoma research. 2017;7(1):6.
7. Sengupta S, Lohse CM, Leibovich BC, Frank I, Thompson RH, Webster WS, et al. Histologic coagulative tumor necrosis as a prognostic indicator of renal cell carcinoma aggressiveness. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2005;104(3):511–20.
8. Hodgson A, Xu B, Satkunasivam R, Downes MR. Tumour front inflammation and necrosis are independent prognostic predictors in high-grade urothelial carcinoma of the bladder. Journal of clinical pathology. 2018;71(2):154–60.
9. Gkogkou C, Frangia K, Saif MW, Trigidou R, Syrigos K. Necrosis and apoptotic index as prognostic factors in non-small cell lung carcinoma: a review. Springerplus. 2014;3(1):120.
10. Yi M, Xia L, Zhou Y, Wu X, Zhuang W, Chen Y, et al. Prognostic value of tumor necrosis in gastrointestinal stromal tumor: A meta-analysis. Medicine. 2019;98(17).
11. Casali P, Abecassis N, Bauer S, Biagini R, Bielack S, Bonvalot S, et al. Gastrointestinal stromal tumours: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2018;29(Supplement_4):iv68–iv78.
12. Jo VY, Fletcher CD. WHO classification of soft tissue tumours: an update based on the 2013 (4th) edition. Pathology. 2014;46(2):95-104.

13. Zhang X-K, Zhang Z-L, Yang P, Cai M-Y, Hu W-M, Yun J-P, et al. Tumor necrosis predicts poor clinical outcomes in patients with node-negative upper urinary tract urothelial carcinoma. Japanese journal of clinical oncology. 2015;45(11):1069–75.
14. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nature Reviews Immunology. 2004;4(8):641–8.
15. Vaupel P, editor Tumor microenvironmental physiology and its implications for radiation oncology. Seminars in radiation oncology; 2004: Elsevier.
16. Liu X, Qiu H, Zhang P, Feng X, Chen T, Li Y, et al. Prognostic role of tumor necrosis in patients undergoing curative resection for gastric gastrointestinal stromal tumor: a multicenter analysis of 740 cases in China. Cancer medicine. 2017;6(12):2796–803.
17. Zheng J, Li R, Qiu H, Chen T, Zhou Y, Huang C, et al. Tumor necrosis and> 20 mitoses per 50 high-power fields can distinguish 'very high-risk'and 'highest-risk'within 'high-risk'gastric gastrointestinal stromal tumor. Future Oncology. 2018;14(7):621–9.
18. Raut CP, Posner M, Desai J, Morgan JA, George S, Zahrieh D, et al. Surgical management of advanced gastrointestinal stromal tumors after treatment with targeted systemic therapy using kinase inhibitors. J Clin Oncol. 2006;24(15):2325-31.
19. Yin Y-q, Liu C-j, Zhang B, Wen Y, Yin Y. Association between CT imaging features and KIT mutations in small intestinal gastrointestinal stromal tumors. Scientific reports. 2019;9(1):1-7.
20. Pisters PW, Leung D, Woodruff J, Shi W, Brennan MF. Analysis of prognostic factors in 1,041 patients with localized soft tissue sarcomas of the extremities. Journal of clinical oncology. 1996;14(5):1679-89.
21. Coindre J-M. Grading of soft tissue sarcomas: review and update. Archives of pathology & laboratory medicine. 2006;130(10):1448-53.
22. Novitsky YW, Kercher KW, Sing RF, Heniford BT. Long-term outcomes of laparoscopic resection of gastric gastrointestinal stromal tumors. Annals of surgery. 2006;243(6):738.
23. Agaram NP, Besmer P, Wong GC, Guo T, Socci ND, Maki RG, et al. Pathologic and molecular heterogeneity in imatinib-stable or imatinib-responsive gastrointestinal stromal tumors. Clinical Cancer Research. 2007;13(1):170–81.
24. Bümming P, Andersson J, Meis-Kindblom J, Klingenstierna H, Engström K, Stierner U, et al. Neoadjuvant, adjuvant and palliative treatment of gastrointestinal stromal tumours (GIST) with imatinib: a centre-based study of 17 patients. British journal of cancer. 2003;89(3):460–4.
25. Joensuu H, Vehtari A, Riihimäki J, Nishida T, Steigen SE, Brabec P, et al. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. The lancet oncology. 2012;13(3):265–74.

Table 1 – Demographics by tumour necrosis

Table 2 – Multivariable analysis of survival outcomes

Supplementary Table 1 – Patient demographics by neoadjuvant imatinib usage

Figure 1– *Kaplan-Meier curves of overall and recurrence-free survival by tumour necrosis*

Figure 2 - *Recurrence-free survival by modified NIH risk score and tumour necrosis*

Table 1 – Demographics by tumour necrosis

	N	Tumour Necrosis		p-Value
		No	***Yes***	
Hospital	195			1.000
QEH		95 (61.7%)	26 (63.4%)	
RMH		59 (38.3%)	15 (36.6%)	
Age at Surgery (Years)	195	66 (54 – 73)	60 (52 – 69)	0.062
Sex (% Male)	195	90 (58.4%)	26 (63.4%)	0.596
Tumour Location (% Gastric)	195	121 (78.6%)	26 (63.4%)	0.065
Gene Mutation	194			0.167
C-KIT		110 (71.9%)	33 (80.5%)	
PDGFRA		29 (19.0%)	3 (7.3%)	
Wild-Type		14 (9.2%)	5 (12.2%)	
Exonic Mutation	149			0.184
C-KIT Exon 9		8 (7.0%)	5 (14.7%)	
C-KIT Exon 11		62 (53.9%)	21 (61.8%)	
PDGFRA Exon 18		26 (22.6%)	3 (8.8%)	
Mutation: Other**		5 (4.3%)	0 (0.0%)	
Wild-type		14 (12.2%)	5 (14.7%)	
Histological Subtype	192			0.425
Spindle Cell		97 (64.2%)	26 (63.4%)	
Mixed		33 (21.9%)	12 (29.3%)	
Epithelioid		21 (13.9%)	3 (7.3%)	
Modified NIH Risk Score	194			**<0.001***
Low (+Very Low)		63 (41.2%)	4 (9.8%)	
Intermediate		38 (24.8%)	8 (19.5%)	
High		52 (34.0%)	29 (70.7%)	
Year of Surgery	195			0.337*
2003-2010		52 (33.8%)	10 (24.4%)	
2011-2013		49 (31.8%)	15 (36.6%)	
2014-2018		53 (34.4%)	16 (39.0%)	
Surgical Approach (% Open)	195	71 (46.1%)	24 (58.5%)	0.165
Adjuvant Imatinib Therapy	195	22 (14.3%)	19 (46.3%)	**<0.001**
Duration of Adjuvant Therapy (Months)***	37	36 (24 – 36)	36 (28 – 36)	0.634

*Data are reported as N (%), with p-values from Fisher's exact tests, or as median (interquartile range), with p-values from Mann-Whitney U tests, unless stated otherwise. Bold p-values are significant at p<0.05. *p-Value from Mann-Whitney U test, as the factor is ordinal. **This group comprised patients with C-KIT Exon 12 (N=3), 13 (N=1) or 14 (N=1) mutations, due to small numbers. ***For the subgroup of patients treated with adjuvant therapy*

Table 2 – Multivariable analysis of survival outcomes

	Overall Survival		Recurrence-Free Survival	
	HR (95% CI)	***p-Value***	***HR (95% CI)***	***p-Value***
Tumour Necrosis	1.10 (0.35 - 3.44)	0.876	5.26 (2.62 - 10.56)	**<0.001**
Age at Surgery (Years)		**0.032**		**0.007**
<55	-	-	-	-
55-69	0.93 (0.21 - 4.20)	0.925	1.01 (0.37 - 2.79)	0.979
70+	3.34 (0.95 - 11.82)	0.061	3.06 (1.25 - 7.46)	**0.014**
Sex (Male)	NS*	NS*	2.99 (1.33 - 6.73)	**0.008**

*Results are from multivariable Cox regression models. All factors from Table 1, except for adjuvant therapy and exonic mutation, were considered for inclusion in the models alongside tumour necrosis, with variable selection by a forwards stepwise approach. Tumour necrosis was not selected for inclusion in the model of overall survival, and so was added into a new model, alongside the factors selected by the stepwise procedure. Bold p-values are significant at p<0.05. *NS=Not selected for inclusion by the stepwise procedure. HR=Hazard Ratio, CI=Confidence Interval*

Supplementary Table 1 – Patient demographics by neoadjuvant imatinib usage

		Neoadjuvant Imatinib		
	N	***No***	***Yes***	**p-Value**
Hospital	261			**<0.001**
QEH		121 (62.1%)	18 (27.3%)	
RMH		74 (37.9%)	48 (72.7%)	
Age at Surgery (Years)	261	65 (54 - 73)	62 (49 - 67)	**0.021**
Sex (% Male)	261	116 (59.5%)	38 (57.6%)	0.885
Tumour Location (% Gastric)	261	147 (75.4%)	34 (51.5%)	**<0.001**
Tumour Necrosis	261	41 (21.0%)	18 (27.3%)	0.310
Gene Mutation	260			**<0.001**
C-KIT		143 (73.7%)	62 (93.9%)	
PDGFRA		32 (16.5%)	0 (0.0%)	
Wild-Type		19 (9.8%)	4 (6.1%)	
Exonic Mutation	193			**<0.001**
C-KIT Exon 9		13 (8.7%)	3 (5.8%)	
C-KIT Exon 11		83 (55.7%)	42 (80.8%)	
PDGFRA Exon 18		29 (19.5%)	0 (0.0%)	
Mutation: Other**		5 (3.4%)	3 (5.8%)	
Wild-type		19 (12.8%)	4 (7.7%)	
Histological Subtype	255			0.136
Spindle Cell		123 (64.1%)	48 (76.2%)	
Mixed		45 (23.4%)	12 (19.0%)	
Epithelioid		24 (12.5%)	3 (4.8%)	
Modified NIH Risk Score	257			**<0.001***
Low (+Very Low)		67 (34.5%)	3 (4.8%)	
Intermediate		46 (23.7%)	13 (20.6%)	
High		81 (41.8%)	47 (74.6%)	

Year of Surgery	261			0.742*
2003-2010		62 (31.8%)	18 (27.3%)	
2011-2013		64 (32.8%)	25 (37.9%)	
2014-2018		69 (35.4%)	23 (34.8%)	
Surgical Approach (% Open)	261	95 (48.7%)	56 (84.8%)	**<0.001**
Adjuvant Therapy	261	41 (21.0%)	48 (72.7%)	**<0.001**
Duration of Adjuvant Therapy (Months)***	81	36 (28 - 36)	36 (27 - 60)	0.287

*Data are reported as N (%), with p-values from Fisher's exact tests, or as median (interquartile range), with p-values from Mann-Whitney U tests, unless stated otherwise. Bold p-values are significant at p<0.05. *p-Value from Mann-Whitney U test, as the factor is ordinal. **This group comprised patients with C-KIT Exon 12 (N=3), 13 (N=1) or 14 (N=1) mutations, due to small numbers. ***For the subgroup of patients treated with adjuvant therapy.*

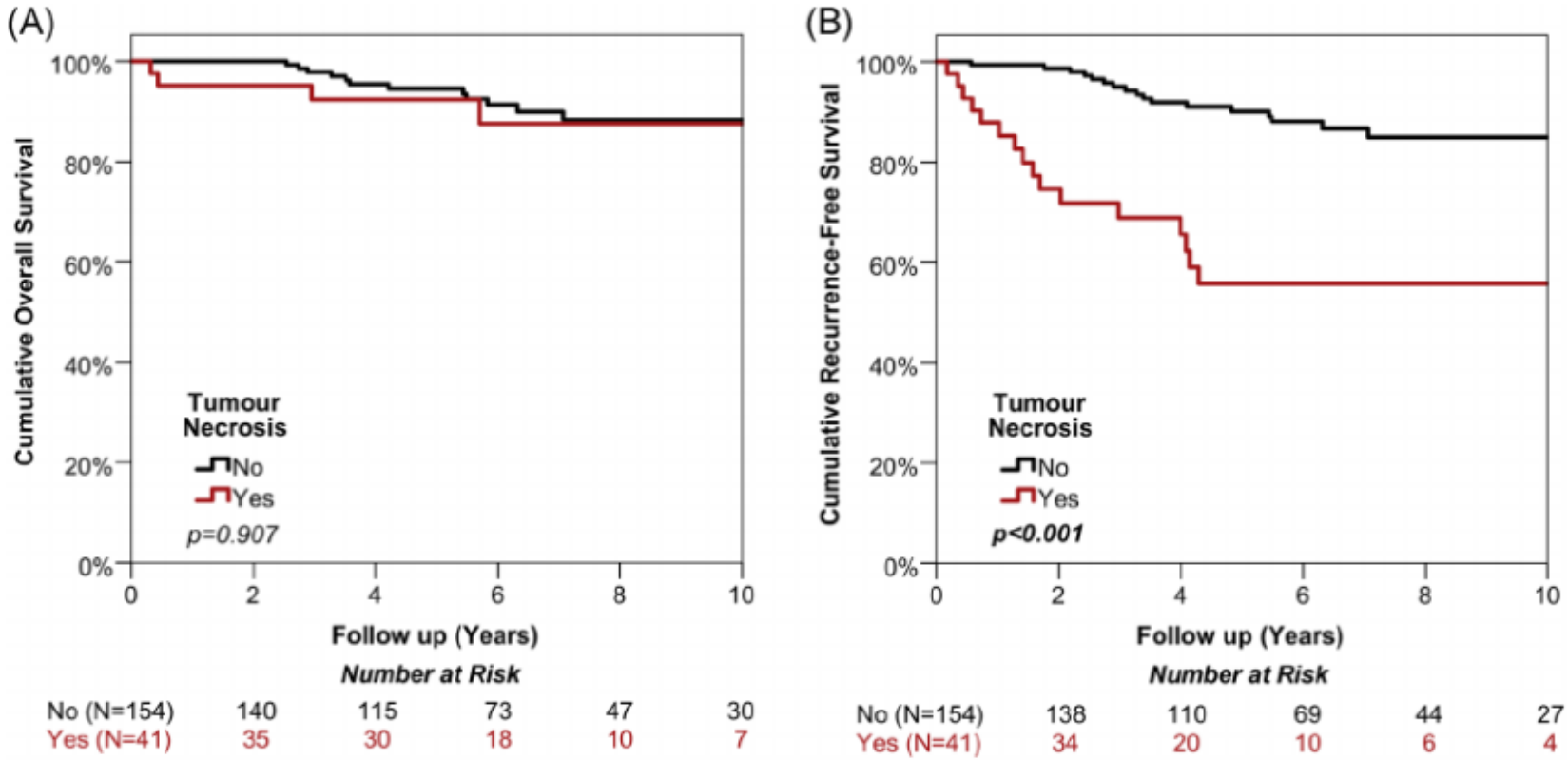
(A)
Cumulative Overall Survival
100%
80%
60%
40%
20%
0%
Tumour Necrosis
No
Yes
p=0.907
0
2
4
6
8
10
Follow up (Years)
Number at Risk
No (N=154) 140 115 73 47 30
Yes (N=41) 35 30 18 10 7
(B)
Cumulative Recurrence-Free Survival
100%
80%
60%
40%
20%
0%
Tumour Necrosis
No
Yes
p<0.001
0
2
4
6
8
10
Follow up (Years)
Number at Risk
No (N=154) 138 110 69 44 27
Yes (N=41) 34 20 10 6 4

Figure1

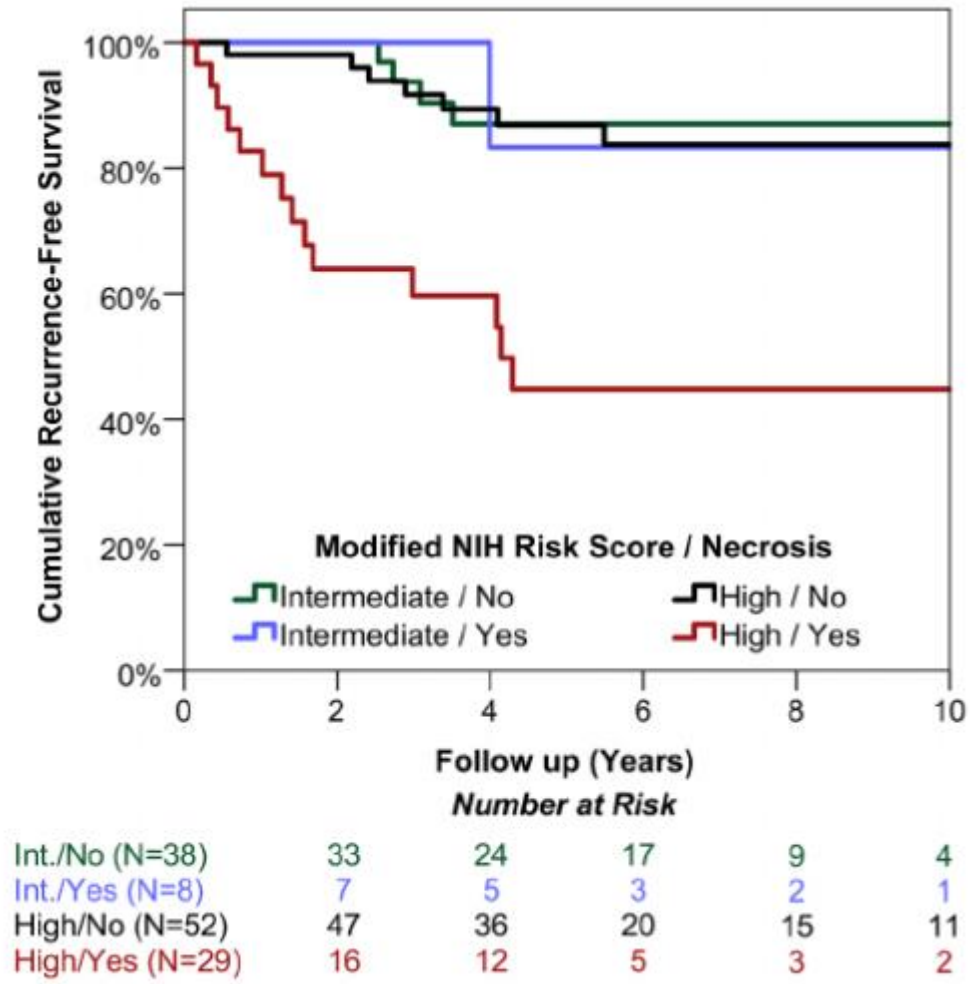
Cumulative Recurrence-Free Survival
100%
80%
60%
40%
20%
0%
Modified NIH Risk Score / Necrosis
Intermediate / No
High / No
Intermediate / Yes
High / Yes
0
2
4
6
8
10
Follow up (Years)
Number at Risk
Int./No (N=38) 33 24 17 9 4
Int./Yes (N=8) 7 5 3 2 1
High/No (N=52) 47 36 20 15 11
High/Yes (N=29) 16 12 5 3 2

Figure 2.