An insight into the driver mutations and molecular mechanisms underlying mucinous adenocarcinoma of the rectum

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Abstract

Background: Mucinous adenocarcinoma of the rectum accounts for 10% of all rectal cancers and has an impaired response to neoadjuvant chemoradiotherapy and worse overall survival. To date very little genomic research has been carried out on this histological subtype.

Objective: To define the mismatch repair deficiency rate and the driver mutations underpinning mucinous adenocarcinoma of the rectum and compare it to rectal adenocarcinoma not otherwise specified.

Design: Immunohistochemistry and sequencing were performed on tumour samples from our tumour biobank.

Settings: This study was conducted across two tertiary referral centres.

Patients: Patients with mucinous adenocarcinoma and adenocarcinoma not otherwise specified who underwent rectal resection between 2008 and 2018 were included.

Main Outcome Measures: Mismatch repair status was performed by immunohistochemical staining. Mutations in the panel of oncogenes and tumour suppressor genes were determined by sequencing on the MiSeq V3 platform.

Results: The study included 33 patients with mucinous adenocarcinoma of the rectum and 100 patients with rectal adenocarcinoma not otherwise specified. Those with mucinous adenocarcinoma had a mismatch repair deficiency rate of 12.1% compared to 2.0% in the adenocarcinoma not otherwise specified cohort (p=0.04). Mucinous adenocarcinoma and adenocarcinoma not otherwise specified rectal tumours had similar mutation frequencies in

the majority of oncogenes and tumour suppressor genes. No difference was found in the *KRAS* mutation rate (50.0% vs 37.1%, p=0.29) or *BRAF* mutation rate (6.7% vs 3.1%, p=0.34) between the cohorts. No difference was found between the cohorts with regard to recurrence-free (p=0.29) or overall survival (p=0.14).

Limitations: The major limitations of this study were the use of formalin fixed paraffin embedded tissue over fresh frozen tissue and the small number of patients included, particularly in the mucinous rectal cohort.

Conclusions: Most mucinous rectal tumours develop and progress along the chromosomal instability pathway. Further research in the form of transcriptomics, proteomics and analysis of the effects of the mucin barrier may yield valuable insights into the mechanisms of resistance to chemoradiotherapy in this cohort.

Introduction

Colorectal cancer (CRC) is a frequently encountered malignancy with an estimated 135,430 cases diagnosed and 50,260 deaths in the USA in the year 2017.[1] Rectal cancer accounts for 25-32% of all CRCs.[2] There are several histological subtypes of CRC with adenocarcinoma not otherwise specified (NOS) being the most common. Mucinous adenocarcinoma is defined by extracellular mucin comprising more than 50% of the tumour[3] and accounts for approximately 10% of all rectal cancers.[4] This subtype has been shown to have a poorer response to neoadjuvant chemoradiotherapy and poorer overall survival when compared to adenocarcinoma NOS.[5]

The mismatch repair (MMR) mechanism is able to identify and repair base-pair insertions, deletions and mis-incorporations that occur within the genome during DNA replication. Deficiency or dysfunction of one of the MMR proteins results in deficient MMR (dMMR). Defects in the MMR system can result in an accumulation of mutations and CRCs with dMMR have a markedly elevated tumour mutation rate.[6] The types of DNA errors that are usually identified and repaired by the MMR system tend to occur at areas of DNA repeats, known as microsatellites. The variation that results in these microsatellites when comparing sequences from normal and tumour is termed microsatellite instability (MSI).[7] Approximately 15% of CRCs have dMMR,[8] it is however noteworthy that dMMR is more common in right sided tumours and only rarely occurs in rectal cancers.[9] Determining the MMR status is important from a clinical perspective as dMMR tumours have a better stage adjusted survival compared to proficient MMR (pMMR) tumours and may respond

differently to 5-fluorouracil (5-Fu) based chemotherapy,[10] it also allows clinicians to identify patients with Lynch Syndrome.

There are three members of the *RAS* family, *KRAS*, *HRAS* and *NRAS* and these proteins function as molecular switches downstream of growth factor receptors such as the epidermal growth factor receptor (EGFR) family . These *RAS* proteins exert effects on downstream signalling cascades such as the mitogen-activated protein kinase (MAPK) and PI3K pathways.[11] The *BRAF* protein kinase is directly activated by *RAS* proteins and activates the MAPK effectors *MEK1* and *MEK2*. *KRAS* somatic mutations can be found in approximately 40% of CRCs.[12] Mutations in the gene for *BRAF* are found in 5-10% of CRCs.[13] With regards to mucinous CRC *RAS* mutations are found in 40% of cases and *BRAF* mutations are found in 28% of cases.[14] In the setting of rectal cancer Yang et al analysed 140 cases and found that 37% had a *KRAS* mutation, 4% had an *NRAS* mutation and 0.7% had a *BRAF* mutation.[15] Accurate data on the mutational status of mucinous adenocarcinoma of the rectum is currently lacking. Determining the mutational status in these patients is important as only patients with wild-type *RAS* and *BRAF* can benefit from anti-EGFR targeted therapy.[16]

There are numerous publications documenting the proportion of CRCs that are dMMR/MSI-H, *RAS* and *BRAF* mutated.[17, 18] There are also numerous publications demonstrating that mucinous CRC is more likely to be dMMR/MSI-H and *BRAF* mutated,[14, 19] however, most of these publications deal predominantly with tumours arising in the colon. The molecular associations of mucinous rectal adenocarcinoma, from here on referred to as MC, have yet to be elucidated. The aim of this study was to compare and contrast the dMMR/MSI-H rate

and the genomic landscape between an MC cohort and a rectal adenocarcinoma NOS cohort, from here on referred to as AC.

Materials & Methods

Inclusion/Exclusion Criteria

Ethical approval for this study was granted by the Beaumont Hospital Ethics Committee. Our rectal cancer database was interrogated to identify patients potentially suitable for this study. Patients diagnosed with rectal MC or AC between January 2008 and December 2018 were eligible for inclusion. Similarly, the rectal cancer database in Imperial College London was interrogated to identify cases of rectal MC where tumour samples were available. The minimum tumour cell percentage that was accepted was 30%. Patients with any disease stage were eligible for inclusion. The haematoxylin and eosin (H&E) diagnostic slides for all included cases were re-reviewed by a consultant histopathologist with a special interest in gastrointestinal pathology to ensure that the histological diagnosis assigned to each case in our database was correct. This study adhered to the STREGA recommendations.[20]

Data Extraction

The following clinicopathological data was extracted for each case where possible; age, sex, neoadjuvant chemoradiotherapy status, disease stage determined from the resection specimen, presence or absence of lymphovascular invasion (LVI), perineural invasion (PNI), extramural venous invasion (EMVI), positive resection margin, tumour regression grade (TRG) and presence of pathological complete response (pCR). Tumour regression was graded using the Mandard tumour regression grade, a good response was defined as a TRG of 1-2 and a bad response as a TRG of 3-5.[21] The MMR status was documented for all cases. Regarding the panel of oncogenes, tumour suppressor genes (TSGs) and mucin glycoprotein genes, each case has been documented as either wild-type or mutated. Only non-

synonymous mutations such as single nucleotide variations (SNVs) and insertion-deletion (InDels) mutations were deemed relevant.

Techniques of Determining Mismatch Repair Status / Microsatellite Instability Status

Mismatch repair status was determined by immunohistochemistry (IHC), in cases where the IHC result was equivocal an MSI polymerase chain reaction (PCR) test was carried out [See supplementary methods for a full description]. Any patients with loss of staining of MMR proteins on IHC underwent further investigation. Those with loss of MSH2 and MSH6 on IHC were referred to clinical genetics for further evaluation and consideration of germline testing. Those with loss of MLH1 and PMS2 were tested for a mutation in the *BRAF* gene, those who were *BRAF* wildtype were referred to clinical genetics for further evaluation and consideration of germline testing while those who were *BRAF* mutant were deemed to be sporadic unless there was strong clinical suspicion for Lynch Syndrome at which point a clinical genetics referral could be made.

Techniques of Determining mutation status of oncogenes, tumour suppresser genes and mucin glycoproteins

The tumour containing areas were macro-dissected from the slide containing formalin fixed paraffin embedded tissue (FFPE). DNA extraction was carried out using the Qiagen GeneRead™ DNA FFPE kit. Sequencing was then performed using the Sequenom or Roche Nimblegen Heat Seq kits followed by sequencing on the MiSeq V3 sequencing platform in Beaumont Hospital (see supplementary methodology for a detailed explanation). The oncogenes and TSGs included in the Heat Seq Oncology panel can be found in Supplementary Table 1.

Statistical and Bioinformatic Analysis

Statistical analysis was carried out using GraphPad Prism version 8.3.0. Fisher's exact test was used to assess for an association between categorical variables and an unpaired *t*-test was used to compare means between the two cohorts. Recurrence-free and overall survival were assessed using Kaplan Meier methods and the Log-rank test. Time to last follow up or recurrence was measured from the date of surgery in the recurrence-free survival analysis. Time to last follow up or death was measured from the date of diagnosis in the overall survival analysis. A p-value of <0.05 was deemed to be statistically significant. Detailed explanation of the bioinformatic analysis can be found in the supplementary methodology.

Results

There were a total of 620 patients diagnosed with rectal cancer who underwent surgical intervention in our institution between January 2008 and December 2018. MC accounted for 4.8% (n=30) of these 620 cases. A further 3 cases of MC were retrieved from the Imperial College London biobank with all 3 patients being treated in St Mary's Hospital in London. These 33 patients were matched with 100 AC patients for age, sex and disease stage. No differences were found between the two cohorts with regards to use of neoadjuvant chemoradiotherapy (p=0.26), LVI (p=0.46), PNI (p=0.44), EMVI (p=0.31), positive resection margin (p=0.69), tumour differentiation (p=0.07) and pCR following neoadjuvant chemoradiotherapy (p=0.99). The AC cohort were more likely to demonstrate tumour regression following neoadjuvant chemoradiotherapy with 37.66% having a TRG of 1-2 compared to only 13.64% of MC cases demonstrating the same response (p=0.04) [See Table 1]. In the MC group the MMR status and genomic data were determined from the rectal resection specimens in all patients. In the AC group MMR status and genomic data were determined from the rectal resection specimens in 89 (89.00%) patients, the pre-treatment biopsy specimens in 9 (9.0%) patients and from metastatic lesions (1 liver & 1 lung) in 2 (2.0%) patients. Figure 1 demonstrates the number of patients included in each of the individual analyses. The number of patients included in the sequencing component was limited by the number of samples with adequate quality and quantity of tumour available. Successful extraction of at least 250ng of DNA at a minimum concentration of 25ng/µL was possible from 27 of the mucinous cases and 69 of the non-mucinous cases and allowed these cases to be included in the extended sequencing component of the study. *KRAS* and

BRAF results for 3 of the remaining 6 MC samples and 28 of the remaining 31 AC samples were available from sequencing carried out previously.

Mismatch Repair / Microsatellite Instability

MMR/MSI analysis was possible on all of the MC cases and 98 of the 100 AC cases. The remaining 2 cases had insufficient residual tumour in their biopsy or resection specimens for analysis. In the AC group 2 out of the 98 cases underwent MSI testing by PCR because the IHC results were equivocal. Both of these cases were subsequently deemed to be MSS. In the MC group 12.1% (n=4) of cases were MMR deficient, this was in comparison to 2.0% (n=2) of cases in the AC group, the difference was statistically significant (p=0.04) [See Figure 2]. After discussion at the colorectal cancer multidisciplinary team meeting three patients in our cohort were referred to clinical genetics based on the pattern of immunohistochemical staining for MMR proteins and clinical suspicion for Lynch syndrome. None of these three patients were found to have germline mutations in genes coding for mismatch repair proteins.

RAS, BRAF, Oncogenes and Tumour Suppressor Gene Analysis

The mean number of reads obtained per case in the MC cohort was 169,305 while the mean number of reads obtained per case in the AC cohort was 130,387 (p=0.22). The overall *RAS* mutation rate was 50.0% and 37.1% in the MC and AC cohorts respectively with no statistical difference identified between the two (p=0.29). There was no difference in the rate of *BRAF* mutations found between the MC and AC cohorts (6.7% vs 3.1%, p=0.34). *PIK3CA* mutations were found in 70.4% of MC tumours and 66.7% of AC tumours, again with

no difference identified between the two cohorts (p=0.81) [See Figure 2]. *TP53* mutations were found in 70.4% and 72.5% of MC tumours and AC tumours respectively with no statistical difference between the two cohorts (p=0.99). Heat maps have been used to demonstrate the mutations in the panel of oncogenes and TSGs between the cohorts [See Figure 3a & 3b]. Both rectal cancer cohorts were similar from a genomic point of view when mutations in the panel of oncogenes and TSGs were compared, only *PDGFRA* (3.7% vs 33.3%, p<0.01) and *TERT* (14.8% vs 36.2%, p<0.05) were mutated at lower frequencies in the MC cohort.

Mucin Glycoprotein Gene Analysis

MUC16 mutations were marginally more common in the AC cohort when compared to the MC cohort (97.7% vs 85.2%, p<0.05). Aside from this difference, the frequency of non-synonymous mutations in *MUC1*, *MUC2*, *MUC3A*, *MUC4*, *MUC5AC*, *MUC5B* and *MUC6* was quite high in both the MC and AC cohorts with no discernible differences identified between the two [See Figure 4].

Recurrence and Survival Analysis

No difference was found in recurrence-free survival between the MC and AC cohorts (p=0.29) [See Figure 5a]. The median overall survival was 96.3 months in the AC cohort. The median overall survival in the MC cohort was undefined because survival still exceeded 50% at the longest time point. No difference was found in overall survival between MC tumours and AC tumours (p=0.14) [See Figure 5b].

Discussion

To date most studies describing the molecular mechanisms of mucinous CRC have focused predominantly on colon cancer. Furthermore, the quality of mucinous tumours is such that extraction of adequate quantities of DNA for sequencing is difficult due to the vast quantities of mucin and relative acellularity of these specimens when compared to adenocarcinoma NOS. In the case of rectal cancers DNA extraction can be more difficult following chemoradiotherapy. We felt it was possible that these tumours would have high rates of dMMR and share similar mutation frequencies, particularly in *BRAF* and *RAS*, to that of mucinous colon cancer. While the dMMR rate was higher in the MC cohort (12.1%) when compared to AC cohort (2.0%) it was far less than then what is typically found in mucinous colon tumours where the dMMR rate is often greater than 30%.[22] A proportion of MC rectal tumours share similar genetic features to those seen in mucinous colon cancers, however, the majority appeared to arise from similar genetic origins to rectal AC. The two rectal cancer cohorts were well matched with regards to clinicopathological variables aside from a higher rate of improved tumour regression in the AC cohort. In keeping with the genomic analysis results recurrence-free and overall survival were also similar in the two rectal cancer cohorts.

A previous study by Liddell et al demonstrated that 33% of mucinous CRCs met the criteria for MSI-H compared to only 4% in the adenocarcinoma NOS group, however, most of the mucinous cases in this study originated in the right colon.[14] While the majority of mucinous tumours do not exhibit the features of MMR deficiency it is apparent that this histological subtype is associated with an increased risk of being dMMR or MSI-H when compared to

adenocarcinoma NOS and we have again demonstrated this point in a cohort of patients with rectal cancer. We believe it is worth dividing mucinous tumours into two broad subtypes, one that is associated with the MSI pathway and one that is associated with the chromosomal instability (CIN) pathway.[23] MSI tumours can arise through a number of mechanisms. Firstly they may arise due to acquired or germline mutations in one of the MMR genes (*MLH1*, *MSH2*, *MSH6* & *PMS2*). These mutations result in impaired DNA MMR and genetic hypermutability and this is what occurs in Lynch Syndrome associated CRC.[24] MSI due to germline mutations in one of the MMR genes is less frequent than MSI due to sporadic or acquired causes and this study did not include any patients with a germline mutation in an MMR gene although it is noteworthy that 22%-40% of cases of Lynch Syndrome associated CRC meet the criteria for mucinous histology.[25] Secondly, MSI can occur in sporadic cases in patients with the CpG Island Methylator phenotype (CIMP). In these cases hypermethylation of the promoter CpG islands results in silencing of tumour suppressor genes that normally suppress oncogenesis such as *MLH1*.[26] Previous studies have shown that MSI-H mucinous tumours appear to have better outcomes with regard to recurrence and survival when compared to MSS mucinous tumours,[27] however, when MSI tumours do recur they tend to have a poor prognosis, particularly if they are *BRAF* mutated.[28] We believe that the finding of mucinous differentiation in a rectal cancer should prompt the clinician to test for MMR deficiency if it is not performed automatically in their institution given the increased risk of dMMR in this histological subtype.

The analysis of oncogene and TSG mutations has given some insight into the molecular mechanisms underpinning rectal MC beyond MSI. The *RAS* mutation rate of 50.0% in the MC cohort was not significantly different to the 37.1% found in the AC cohort (p=0.29).

Similarly, no difference was found in the rate of *BRAF* mutations between the MC and AC cohorts where 6.7% and 3.1% of tumours were mutated respectively. Previous studies have demonstrated a higher rate of *RAS* and *BRAF* mutations in mucinous tumours, however, these studies predominantly included patients with proximally located mucinous tumours which appear to be genomically different to rectal tumours.[29] *PIK3CA* mutations were found in more than two thirds of both rectal cancer cohorts. A previous study looking at *PIK3CA* mutations in mucinous histology found the mutation rate to be 30%, again, this study included predominantly mucinous colon cancers and there is currently very little evidence about the frequency of PIK3CA mutations in mucinous rectal cancer.[30] As expected the *TP53* mutation rate was greater than 70% in both rectal cancer cohorts, this finding is not surprising given that 88% and 98% of MC and AC tumours respectively are MMR proficient. The natural assumption is that these tumours are more likely to be associated with development and progression along the CIN pathway as opposed to the MSI pathway. Rectal AC tumours did display a higher mutation rate in *PDGFRA* and *TERT.* The exact significance of the different mutation frequencies is uncertain. The tyrosine kinase inhibitor imatinib mesylate is used to treat chronic myeloid leukaemia and some gastrointestinal stromal tumours with *PDGFRA* mutations,[31] interestingly 33% of the AC tumours were found to harbour this mutation and the clinical significance of this finding requires further investigation. The *TERT* gene is responsible for producing telomerase which functions to maintain telomeres at the end of chromosomes. Mutations in *TERT* have been associated with melanoma, breast cancer and cholangiocarcinoma but there does not appear to be a strong association with CRC.[32]

While we have shown a high frequency of mutations across the panel of mucin glycoproteins sequenced in this study, it is our current understanding that the mucinous phenotype occurs due to overexpression of MUC2 as opposed to specific mutations in any of the genes encoding for mucin glycoproteins.[33] Increased or decreased expression of mucin gene products appears to be relatively common in several other types of cancer.[34] Exactly what causes this overexpression has not yet been fully elucidated.[35] The role of mucin glycoproteins and mutations in the genes encoding these proteins in other cancers has been studied, however, the results are often conflicting.[36, 37] Mucin encoding genes appear to accumulate a lot of mutations in cancers and this most likely relates to the size of these genes, however, they do occasionally acquire pathogenic mutations.[38]

The question still remains as to why MC rectal tumours appear to have a worse response to neoadjuvant chemoradiotherapy when compared to rectal AC tumours, particularly given that this study has shown the frequency of mutations in oncogenes and tumour suppressor genes is similar between the cohorts. With such small numbers it is difficult to determine if the higher rate of dMMR/MSI-H may account in part for this and relate to impaired response to 5-FU as a radio-sensitising agent. Another likely but unproven possibility is that the mucin is creating a physical barrier to the effect of radiotherapy and also creating a hypoxic environment which has previously been associated with resistance to radiotherapy.[39] We have shown that while MC tumours are different from a morphological point of view they appear to be genetically similar. We now to need to investigate this histological subtype further to better understand why it tends to have an impaired response to up-front chemoradiotherapy. With the widespread availability of rapid sequencing services, the falling price and reducing DNA input requirements, it is easier than ever before

to get a better understanding about the molecular mechanisms underpinning these tumours. The difficulty that remains is in translating these findings into data that can be used in the clinical setting to inform treatment decisions. Genomic research has yielded valuable information about the use of EGFR inhibitors in *KRAS* wildtype CRC and immunotherapy in metastatic MSI-H CRC and will hopefully continue to provide clinicians with information that can assist them in decision making and help to personalise treatment for patients with CRC. As the quantity of genomic, transcriptomic and proteomic data increases we may eventually be able to accurately predict which rectal tumours are likely to benefit from upfront chemoradiotherapy based on molecular profiling.

The numbers in our study were limited, particularly in the MC cohort, however it should be acknowledged that this histological subtype is diagnosed and treated infrequently making it difficult for any single institution to accumulate a large volume of these tumours in their biobank. The majority of studies to date that are focused on the genomics of mucinous CRC include mainly colonic samples and our cohort of 33 rectal MC samples is one of the largest studied to date. Another limitation of our study was in the use of samples derived from FFPE tissue as opposed to fresh frozen tissue, unfortunately fresh frozen tissue was not available for the majority of samples. It is well known that formalin fixation can increase deamination, however, FFPE samples have been shown to generate similar read counts and read quality to fresh frozen samples as long as the tissue is preserved using an adequate technique.[40] The majority of samples included in our study had a tumor cell percentage of more than 50%, there was a however a small number of mucinous samples with tumor cell percentages below 50%. While it is preferable to only sequence samples with at least 50% tumor there is evidence to suggest that good sequencing data can be generated from

samples with less than 20% tumor.[41] The data generated from the samples in our cohort with lower tumor percentages was deemed to be of acceptable quality for inclusion in the study. It should be acknowledged that sequencing in this study was carried out on both pre-treatment and post-treatment samples and some of the genomic changes described may have been induced by chemoradiotherapy. While genomic changes do occur following chemoradiotherapy it has been shown that the number of mutations induced in oncogenes and tumor suppressor genes is low.[42] There is a paucity of other sequencing studies focused on mucinous rectal cancer with which we can validate our findings and there are currently only 5 cases of mucinous rectal cancer with sequencing data available from TCGA which precludes a meaningful comparison. When compared to the rectal adenocarcinoma NOS cohort from TCGA our AC cohort had similar rates of MMR deficiency (2.9% vs 2.0%, p=0.99), *RAS* mutations (42.0% vs 37.1%, p=0.63) and *BRAF* mutations (4.3% vs 3.1%, p=0.69). Clearly more molecular research needs to be carried out on mucinous rectal tumours. Going forward, wider sequencing of the genome, which can be achieved using whole genome sequencing may yield useful insights into this histological subtype, as might transcriptomic, proteomic and methylation analysis.

Conclusion

Rectal MC has a higher rate of dMMR compared to rectal AC. The significance of this finding in relation to the observed impaired response to chemoradiotherapy is uncertain. The majority of rectal MC tumours are pMMR and appear to arise and progress through similar genetic mechanisms to rectal AC based on the similarities of their somatic mutation profile. Further efforts will need to focus on the transcriptomic and proteomic profile of MC rectal tumours to allow clinicians and scientists to gain insight into the mechanisms of resistance to chemoradiotherapy. It would also be important to determine how much of the resistance can be contributed to mucin creating a physical barrier and a hypoxic environment that might be mitigating the effects of chemoradiotherapy.

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Figure Legends

Figure 1 | Flow diagram demonstrating the number of patients from each cohort included in the individual analyses. MC = mucinous adenocarcinoma of the rectum, AC = adenocarcinoma not otherwise specified of the rectum.

Figure 2 | Bar chart showing the proportion of tumours in each cohort that are dMMR/MSI-H, *RAS* mutated, *BRAF* mutated and *PIK3CA* mutated. dMMR = Mismatch repair deficient, MSI-H = Microsatellite instability high, MC = mucinous adenocarcinoma of the rectum, AC = adenocarcinoma not otherwise specified of the rectum.

Figure 3a | Heat map demonstrating mutated genes (red) in the MC cohort. MC = mucinous adenocarcinoma of the rectum.

Figure 3b | Heat map demonstrating mutated genes (red) in the AC cohort. AC = adenocarcinoma not otherwise specified of the rectum.

Figure 4 | Bar chart showing the proportion of tumours in each rectal cohort that have mutations in MUC1, MUC2, MUC3A, MUC4, MUC5AC, MUC5B, MUC6 and MUC16. MC = mucinous adenocarcinoma of the rectum, AC = adenocarcinoma not otherwise specified of the rectum.

Figure 5a | Kaplan Meier curve demonstrating recurrence-free survival in MC and AC cohorts. No difference was found in recurrence-free survival between MC and AC cohorts (p=0.29). Number at risk is shown in the table below the x-axis. MC = mucinous adenocarcinoma of the rectum, AC = adenocarcinoma not otherwise specified of the rectum.

Figure 5b | Kaplan Meier curve demonstrating overall survival in MC and AC cohorts. No difference was found in overall survival between MC and AC cohorts (p=0.14). Number at risk is shown in the table below the x-axis. MC = mucinous adenocarcinoma of the rectum, AC = adenocarcinoma not otherwise specified of the rectum.