

Panel gene profiling of small bowel adenocarcinoma, results from the NADEGE prospective cohort

Thomas Aparicio, Magali Svrcek, Julie Henriques, Pauline Afchain, Astrid Lièvre, David Tougeron, Johan Gagniere, Eric Terrebonne, Guillaume Piessen, Jean-Louis Legoux, et al.

▶ **To cite this version:**

Thomas Aparicio, Magali Svrcek, Julie Henriques, Pauline Afchain, Astrid Lièvre, et al.. Panel gene profiling of small bowel adenocarcinoma, results from the NADEGE prospective cohort. International Journal of Cancer, 2021, 148 (7), pp.1731-1742. 10.1002/ijc.33392 . hal-03038573

HAL Id: hal-03038573
https://hal.science/hal-03038573

Submitted on 15 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Accepted Article

Panel gene profiling of small bowel adenocarcinoma, results from the NADEGE prospective cohort

Running title: Genomic profiling of small bowel adenocarcinoma

Thomas Aparicio (1), Magali Svrcek (2), Julie Henriques (3, 4), Pauline Afchain (5), Astrid Lièvre (6), David Tougeron (7), Johan Gagniere (8), Eric Terrebonne (9) Guillaume Piessen (10), Jean-Louis Legoux (11), Cédric Lecaille (12), Marc Pocard (13), Jean-Marc Gornet (1), Aziz Zaanan (14), Sandrine Lavau-Denes (15), Thierry Lecomte (16), David Deutsch (17), Dewi Vernerey (3, 4), Pierre Laurent Puig (18, 19).

(1) Department of Gastroenterology and Digestive Oncology, Saint Louis Hospital, APHP, Université de Paris, Paris, France
(2) Sorbonne Université, Department of Pathology, Saint Antoine Hospital, APHP, Paris, France
(3) Methodology and Quality of Life Unit in Oncology, EA 3181, University Hospital, Besançon, France
(4) Bourgogne Franche-Comté University, INSERM, Etablissement Français du Sang Bourgogne Franche-Comté, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France
(5) Department of Oncology, Saint Antoine Hospital, APHP, Paris, France
(6) Department of Gastroenterology, Pontchaillou Hospital, Rennes 1 University; INSERM U1242, Rennes, France
(7) Department of Hepato-Gastroenterology, CHU de Poitiers, France
(8) Department of Digestive and Hepatobiliary Surgery, University Hospital of Clermont-Ferrand, U1071 INSERM, Clermont-Auvergne University, Clermont-Ferrand, France
(9) Department of Gastroenterology, CHU Haut-Lévêque, Pessac, France
(10) Department of Digestive and Oncological Surgery, Claude Huriez University Hospital, University Lille, Lille, France
(11) Department of Hepato-Gastroenterology and Digestive Oncology, CHR La Source, Orléans, France
(12) Department of Gastroenterology, Polyclinic Bordeaux Nord, Bordeaux, France

(13) Department of Digestive Surgery, Lariboisière Hospital, APHP, Paris, France

(14) Department of Gastroenterology and Digestive Oncology, Georges Pompidou Hospital, APHP, Université de Paris, Paris, France

(15) Department of Oncology, CHU Dupuytren, Limoges, France

(16) Department of Hepato-Gastroenterology and Digestive Oncology, Trousseau Hospital, CHU Tours, Tours, France

(17) Department of Gastroenterology, Avicenne Hospital, APHP, Bobigny, France

(18) Centre de Recherche des Cordeliers, Sorbonne Université, Inserm, Université de Paris, Paris, France

(19) Department of Biology, Georges Pompidou Hospital, APHP, Université de Paris, Paris, France.

Correspondence: Prof Thomas Aparicio

Gastroenterology and Digestive Oncology, Saint Louis Hospital, Université de Paris, 1 avenue Claude Vellefaux, 75010, Paris, France

E-mail: thomas.aparicio@aphp.fr, Tel: +33 1 42 49 95 97, Fax: +33 1 42 49 91 68

Key words: small intestine adenocarcinoma, genomic profiling, cohort study, Crohn's disease, Lynch syndrome, MMR status.

Novelty and impact:

- A genomic alteration is observed in 90.3% of the small bowel adenocarcinoma.

- The most frequent gene alterations are in *KRAS*, *TP53*, *PIK3CA*, *APC*, *SMAD4* and *ERBB2*.

- Tumours associated with Crohn disease and Lynch syndrome have specific alterations.

- Genomic alteration had no prognostic effect except dMMR status.

List of abbreviations

Accepted Article

AGEO: Association des Gastro-Entérologues-Oncologues

AJCC: American Joint Committee on Cancer

BIONADEGE: analyse BIOlogique de la cohorte Nationale d'ADEnocarcinome de l'intestin GrèlE

CT: Computed tomography

CI: confidence interval

dMMR: deficient mismatch repair

DNA: desoxyribonucleic acid

GERCOR: Groupe Coopérateur Multidisciplinaire en Oncologie

HR : hazard ratio

IHC: immunohistochemical

MSI: microsatellite instability

MMR: mismatch repair

NADEGE: cohorte Nationale d'ADEnocarcinome de l'intestin GrêlE

NGS: Next generation sequencing

OS: Overall survival

pMMR: proficient mismatch repair

SBA: Small bowel adenocarcinoma

TMA: Tissue microarrays

UICC: Union for International Cancer Control.

Accepted Article

Accepted Article

Abstract

Small bowel adenocarcinoma (SBA) is a rare tumour. Large genomic analyses with prognostic assessments are lacking. The NADEGE cohort has enrolled 347 patients with all stage SBA from 2009 to 2012. Next generation sequencing investigates the presence of 740 hotspot somatic mutations in a panel of 46 genes involved in carcinogenesis. The mismatch repair (MMR) status was assessed by immunochemistry. We have collected 196 tumour samples and 125 had conclusive results for mutation analysis. The number of mutations was 0 in 9.6% of tumours, only 1 in 32.0%, 2 in 26.4% and $\geq$3 in 32.0%. Altogether, at least one genomic alteration was observed in 90.4% of tumour. The most frequent genomic alteration was in *KRAS* (44.0%), *TP53* (38.4%), *PIK3CA* (20.0%), *APC* (18.4%), *SMAD4* (14.4%) and *ERBB2* (7.2%) genes. *KRAS* mutations were more frequent in synchronous metastatic tumours than in localized tumours (72.7% vs 38.2%, p=0.003). There was no significant difference in the mutation rates according to primary location for the most frequently altered gene. *ATM*, *FGFR3* and *FGFR1* gene alterations were associated with Lynch syndrome and *IDH1* mutations with Crohn disease. dMMR tumours were associated with younger age, localized tumours, less *KRAS* but more *SMARCB1* mutations. No genomic alteration was associated with overall survival. There is a trend for better survival in patient with dMMR tumours. In conclusion, there is a different genomic alteration profile in SBA according to predisposing diseases. No association between genomic alterations and prognoses was observed except for a trend of better prognoses associated with dMMR.

Accepted Article

BACKGROUND

Small bowel adenocarcinoma (SBA) is a rare tumour of poor prognosis (1). Nevertheless, it is the first aetiology of small bowel cancer in France (2) and second aetiology in the USA (3). Concordant findings report an increasing incidence of SBA (2,4,5).

Few studies have investigated the molecular phenotype of SBA. A previous study reports that the genomic profile of SBA is closer to colon adenocarcinoma rather than gastric adenocarcinoma (6). Recently, a large genomic analysis mainly on stage IV tumours has reported a distinct profile of SBA compared to gastric or colon adenocarcinoma (7). Indeed, if *RAS* mutation prevalence is similar to colon cancer, *APC* mutations are much less frequent, *BRAF* rarely involved V600E point mutations and *ERBB2* mutations or microsatellite instabilities (MSI) are more frequent than in colon cancer (7–10). A prognostic value had been inconsistently associated with *ERBB2* mutations (11), MSI (9) or *TP53* mutations (12). Some differences of genetic profile were reported according to the small bowel segment. Indeed, several studies found that *ERBB2* mutations were more frequent in duodenum (7,8,10), but conflicting results are reported for other genetic alterations according to localisation across the studies. The limits of most studies are the small number of patients and the lack of clinical data or prognosis evaluation.

The NADEGE cohort has enrolled prospectively consecutive patients with all stages of SBA during a four-year period in France. Clinical tumour characteristics differ according to sporadic SBA or secondary to a predisposing disease. Crohn disease was significantly associated with younger age, poor differentiation, and ileum location, whereas Lynch syndrome was associated with younger age, poor differentiation, an early stage, and duodenum location. Tumour grade and stage were the main prognostic factors (13). The BIONADEGE study is an

ancillary study of the NADEGE cohort aimed to assess the genomic profile according to a predisposing disease for SBA, to SBA localisation or stage and assess the prognostic value of these genomic alterations.

Accepted Article

PATIENTS AND METHODS

Study population

The NADEGE cohort has recruited 347 patients in 74 participating French institutions from January 2009 to December 2012. All consecutive stage I-IV patients with histologically proven, newly diagnosed or with recurrent SBA (local or distant) were enrolled into the NADEGE cohort. Ampullary and non-adenocarcinoma tumours were excluded. TNM staging was done according to the criteria of AJCC and UICC (7th UICC TNM Staging System) performed at diagnosis by computed tomography (CT) scan and/or magnetic resonance imaging. The following clinical data were prospectively collected: demographics, cancer treatment history, tumour stage, lymph node invasion, tumour differentiation, initial treatment, and survival. The predisposing disease or genetic syndrome was assessed by investigator declaration. The tumour blocks of either tumour biopsy from primary or metastasis or tumour surgical resection were collected.

Immunohistochemistry

Tissue microarrays (TMA) were constructed from 0.6-mm diameter tissue cores obtained from formalin fixed paraffin embedded tumor specimens. Hematoxylin and Eosin staining was performed on each TMA slide to confirm the presence of tumor tissue. The expression of MLH1, MSH2, MSH6, and PMS2 was assessed as previously described (9). Briefly, 4 µm

Accepted Article

sections were cut onto silane-treated Super Frost slides (CML, Nemours, France) and left to dry at 37°C overnight. The slides were deparaffinised in xylene and rehydrated in pure ethanol. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol for 30 min. Before immunostaining, antigen retrieval was performed by immersing sections in citrate buffer (pH 6.0). Sections were then incubated for 15 minutes at room temperature with antibodies to MLH1 (dilution 1/70, clone G168-728, Pharmingen, San Diego, CA), MSH2 (dilution 1/100, clone FE11, Calbiochem, Oncogene Research Products, Cambridge, MA), MSH6 (dilution 1/100, clone 44, Becton Dickinson, Lexington, NC), PMS2 (clone A16-4, 1:150 dilution, BD PharMingen, Le Pont de Claix, France). The Bond Polymer Refine Detection kit (Leica) was used as the detection system. Immunostaining of MLH1, MSH2, MSH6 and PMS2 in tumour cells was evaluated as positive or negative as assessed in TMA. Tumours were considered negative when there was a complete absence of nuclear staining of neoplastic cells in the presence of an internal positive control assessed in a whole slide. All the tumour with a negative staining of one of the MMR protein were considered as dMMR.

Molecular analysis

The same paraffin blocks were used for DNA extractions and for IHC analyses. DNA was extracted from formalin-fixed, paraffin-embedded neoplastic tissue that had been macro-dissected with reference to the Hematoxylin and Eosin stained section.

Next generation sequencing (NGS) investigates the presence of 739 hotspot somatic mutations in 46 genes involved in carcinogenesis using cancer hotspot panel from Thermofisher (Table S1). DNA extraction, NGS sequencing and mutation calling were performed as described previously (10).

Accepted Article

Statistical analysis

Descriptive analysis of the initial tumour stage (reference) and variables measured at baseline was performed. Categorical variables were summarised as frequencies and percentages and continuous variables as medians and ranges. The comparison of gene alteration frequencies according the sub-group of patients was assessed with the χ^2 test or Fisher's exact test, as appropriate, for categorical variables.

Patients with metastatic disease were defined as those who had metastasis at the time of the inclusion and those who developed additional metastatic recurrence tumours during follow-up. Therefore, some patients in this trial were analysed twice: first, as cases with localized tumours, and second, as cases with metastases.

Overall survival (OS) was defined as the time from diagnosis of a primary tumour (localized tumour) or of metastasis (synchronous or metachronous) until death due to any cause. Patients who were still alive at the last follow-up were censored. Patients with synchronous resected metastasis were excluded from the analysis of metastatic patient subgroup in order to assess OS of patients with unresectable metastases.

The survival curves for OS were estimated by the Kaplan-Meier and were compared using the log-rank test. The follow-up time was assessed by the reverse Kaplan-Meier method. The medians and 95% confidence intervals (95% CIs) were calculated and 3-year rates with 95%CI were also provided.

The hazard ratios (HRs) and their 95% CIs were estimated with the Cox proportional hazard model. Univariate analysis was performed to determine baseline characteristics associated with OS for patients with mutational status available. All variables with p values of <0.1 were

included in multivariate analysis. The correlations between variables were assessed and proportional hazard assumptions were examined graphically by log-minus-log plots of survival.

All statistical analyses were conducted with a two-sided alpha significance level of 5% using SAS 9.3 software (SAS institute Inc., Cary, NC, USA). As the analyses were exploratory, p values were not adjusted for multiple testing.

Accepted Article

RESULTS

Patient and tumour characteristics

Among the 347 patients included in the analysis of clinical NADEGE dataset, 196 tumour blocks were collected for immunohistochemistry and molecular analysis. The quantity or quality of extracted DNA could not allow molecular analysis in 71 tumours. Finally, the mutation status was obtained for 125 patients (Figure 1). Patient characteristics are presented in table 1. The clinical and tumour characteristics were comparable in the patients from the whole NADEGE (13) and the BIONADEGE cohorts except for metastatic stage at diagnosis underrepresented in the BIONADEGE cohort (36% in NADEGE vs 18% in BIONADEGE, $p<0.0001$).

The gene mutation frequency according to tumour stage and primary are presented in table 2. The detail of raw NGS data are presented in the table S1. Overall, at least one genomic alteration was observed in 90.4% of tumours. There is no difference into the frequency of at least one genomic alteration according to the tumour stage: 89.2% and 95.4% for localized or metastatic tumour at diagnosis, respectively. There was no difference into the frequency of at least one genomic alteration according to primary tumour site: 92.0%, 82.1% and 95.4% for

Accepted Article

duodenum, jejunum or ileum, respectively. Overall, the number of mutations observed per tumour was 0 in 9.6%, 1 in 32.0%, 2 in 26.4% and >3 in 32.0% of the patients. The proportion of tumours with >3 mutations were also similar according to stage: 30.4% and 40.9% for localized and metastatic tumours at diagnosis, respectively and according to primary: 33.3%, 21.4% and 40.9% for duodenum, jejunum or ileum, respectively. The most frequent genomic alteration observed were: *KRAS* (44.0%), *TP53* (38.4%), *PIK3CA* (20.0%), *APC* (18.4%), *SMAD4* (14.4%) and *ERBB2* (7.2%). A *KRAS* mutation was more frequent in metastatic tumours at diagnosis than in localized tumours (72.7% vs 38.2%, p=0.003). A *BRAF* mutation was observed in 5 (4%) cases and among them there is only one V600E mutation. There was no significant difference of mutation rate according to primary location for the most frequently altered genes.

The comparison of gene mutation frequency between patients with Lynch syndrome and those with no predisposing disease revealed different profiles (Table 3). There is a trend for less frequent *KRAS* mutations in Lynch syndrome and more frequent *TP53* and *PIK3CA* mutations in Crohn's disease compare to no predisposing disease. No *APC* mutation was observed in any Crohn's disease. There is a trend of more frequent *ERBB2* mutations in Lynch syndrome compare to no predisposing disease. Moreover, no *ERBB2* mutation was observed in Crohn's disease. Several rare mutations are more frequent in tumour with Lynch syndrome than in no predisposing syndrome such as *ATM*, *FGFR3* and *FGFR1* gene mutations. *IDH1* mutations are more frequent in tumours with Crohn's disease than in no predisposing disease.

Results according to MMR status

MMR status was determined with immunohistochemical (IHC) analysis of MMR proteins in 180 patients. A deficient MMR (dMMR) tumour was observed in 50 (28%) patients. A negative

staining was observed for both MLH1 and PMS2 in 21 (42%), MSH2 and MSH6 in 18 (36%), PMS2 with MLH1 inconclusive test in 4 (8%), MSH6 with inconclusive MSH2 in 2 (4%), PMS2 alone in 2 (4%), MSH6 alone in 2 (4%) and MSH2 with inconclusive MSH6 in one (2%).

The comparisons of patient and tumour characteristics according to MMR status are given in table 4. The dMMR tumours were associated with a younger age, a less metastatic stage at diagnosis, less *KRAS* mutations but more *SMARCB1* mutations. There is also a trend for less *TP53* mutations and more *ERBB2* mutations.

Survival analysis

The median follow-up was 56 months (95% confidence interval (CI) [47-63]). The 3-years OS of the 196 patients with block available was 64.4% (95%CI 56.6% – 71.1%) and 71.7% (95%CI 61.9% - 79.4%) for the 125 patients with mutation status available.

Survival analysis in the 125 patients with mutational status available

Univariate analysis was performed in the sub-group of patients with mutation statuses available to assess the prognostic factors for OS including clinical parameters and the gene mutation with a frequency over 10% (Table 5). No genomic alteration was associated with OS (Table 5). In the multivariate analysis including stage, Lynch syndrome and tumour differentiation, only poor tumour differentiation remained associated with higher risk of death (HR=2.48; 95%CI [1.19-5.21]; p=0.0159). There is trend for a better prognosis associated with early stage (p=0.0774) and Lynch syndrome (p=0.0648).

The results of univariate analysis according to localized or metastatic tumour are given in table S2. In the sub-group of 102 patients with localized and resected tumour, no genomic alteration

Accepted Article

Accepted Article

was associated with OS. There is a trend for a worst 3 years OS in patients with tumour *KRAS* mutation (63.3% [95%CI 43.0 - 78.1] *versus* 82.0% [95%CI 69.1 - 89.9], p=0.3551). In the sub-group of 31 patients with metastatic disease (unresectable synchronous metastasis and metachronous metastasis) the median OS was 22.6 months (95%CI 12.7-59.7). No genomic alteration was associated with OS. Median OS was 32.3 months (95%CI 12.5 – 59.7) and 21.0 months (95%CI 8.1 – 36) in patients with mutated and wild type tumour *KRAS*, respectively (p=0.5235). The median OS was 27.3 (95%CI 9.1 – 59.7) and 16.2 (95%CI 3.9 – not assessable) in patients with mutated and wild type tumour *TP53*, respectively (p=0.9123).

Survival analysis in the 180 patients with MMR status available

The 3-years OS rate was 79.9% (95%CI 64.7 – 89.1) for patients with dMMR tumours and 58.5 months (95%CI 48.5 – 67.1) for patients with pMMR tumours. There is a trend for better survival in patients with dMMR tumours (HR=0.59; 95%CI [0.32-1.06], p=0.0765) (Figure S1). In the subgroup of patients with localized tumours the 3-years OS were 82.9% (95%CI 67.2 – 91.5) and 72.5% (95%CI 60.2 – 81.6) for patients with dMMR and pMMR respectively (logrank p=0.3957; HR=0.74 [0.36-1.50], p=0.3976). Due to the small number of patients with a dMMR metastatic tumour (n=2) the comparison of median OS according to MMR status was not reported.

DISCUSSION

Our study on a large number of patients with SBA revealed different tumour mutation profiles according to predisposing diseases (Crohn's disease or Lynch syndrome compared to tumour without predisposing disease) or an MMR status.

Accepted Article

Our results are concordant with the genomic alteration profile reported in three previous studies that reported a mutation rate for *KRAS* from 43.4% to 53.6%, *TP53* from 41% to 58.4%, *PIK3CA* from 9% to 18.4%, *APC* from 13.2% to 26.8%, *SMAD4* from 9.6% to 17.4% and *ERBB2* from 8.4% to 14% (7,8,10). Moreover, we found that *KRAS* mutations as pMMR status were associated with metastasis. This is the first report showing that a genomic alteration is associated with advanced stage in SBA to our best knowledge.

We found no significant association with one of the mutations observed in more than 5% of the tumour and primary tumour site. Two previous studies have reported an association with *ERBB2* mutation and duodenal location (7,10). In our study as in the Härinnen *et al* study (8) the *ERBB2* mutation rate was higher in tumour of the proximal small bowel without reaching significance. Some rare mutations seem to have a different distribution according to the small bowel segment. *IDH1* mutations were only reported in the ileum tumour which may be explained by the association with Crohn's disease that was mainly associated with ileum tumours in the NADEGE cohort (13). *FBXW7* mutation was predominantly observed in the jejunum tumour. This result is concordant with the Schrock *et al* results that have reported a trend of more *FBXW7* mutation in non-duodenal SBA (7).

The genomic alteration profile was different according to predisposing diseases or the MMR status. Crohn's disease was associated with tumour genomic alterations of *IDH1*. Moreover, a trend for more frequent *KDR* mutations but no *APC* mutation was observed in Crohn's disease compare to no predisposing disease. *IDH1* mutations and also high mutation rate of *TP53* were already reported associated with Crohn's disease in colorectal cancer (14). A recent publication reported an association of *IDH1* and *SMAD4* mutations with Crohn's disease in SBA (15). We did not find any association of *SMAD4* mutation and Crohn's disease in our study. Tumour *KDR* gene alteration, coding for VEGFR2, has not been previously reported to

be associated to Crohn's disease. The lack of *APC* mutation in SBA associated with inflammatory bowel disease was already reported by Schrock et al (7). A lower frequency of *APC* mutation in colorectal cancer associated with inflammatory bowel disease as compared to sporadic colorectal cancer was also reported (14). No *ERBB2* mutation was observed in tumour associated with Crohn's disease in our study as it was previously observed in the Schrock study (7). Altogether ours and previous results support the hypothesis that the SBA associated with Crohn's disease has a different carcinogenesis from sporadic cancer as it is observed in colorectal cancer (16).

In SBA associated with Lynch syndrome, there is a trend of less *KRAS* mutations and more *ERBB2* mutations compared to tumours without predisposing diseases. Other rare mutations such as *ATM*, *FGFR3* and *FGFR1* are associated with Lynch syndrome in our study. The risk of developing a cancer for patient with Lynch syndrome if they had an *ATM* mutant allele is a matter of debate (17). We could not determine in our study if the *ATM* mutation was inherited or acquired. *FGFR3* R248C hotspot mutation has already been associated to the Lynch syndrome in upper tract urothelial carcinoma (18) but not with SBA until our report.

We found some specificity in the subgroup of dMMR tumours compared to pMMR tumours. Patients with dMMR tumours are younger than patients with pMMR tumours. This is the inverse result that it is observed in colorectal cancer (19). That may be explained by the fact that in our study the proportion of Lynch syndrome among dMMR tumour reach 34%. Nevertheless, as it is observed in colorectal cancer, the dMMR tumours are rarely metastatic at diagnosis. In our study, *KRAS* mutations are less frequent in dMMR tumours compared to pMMR tumours. This has not been previously reported in SBA and deserves a confirmatory study. There is also a trend for less *TP53* alterations but more *ERBB2* alterations in dMMR tumours than in pMMR tumours. The association of *ERBB2* mutations and dMMR has previously been reported (10).

Accepted Article

Accepted Article

We report a higher frequency of *SMARCB1* mutations in dMMR tumours, *SMARCB1* has already been described in dMMR colorectal tumours (20).

We did not find any association between genomic alteration and prognosis. One previous study reports a poor prognosis associated with a genomic alteration of the ERBB signalling cascade (11) but *ERBB2* mutations solely had no prognostic value. The dMMR phenotype was already reported as good prognostic factor for disease free-survival in one study (8). In our study as in a previous one (9) there is a trend for better prognosis in patients with a dMMR tumour. The prognostic effect of dMMR phenotypes seems restricted to patients with localized and resected tumours. In patients with metastatic tumours the MMR status seems to have no effect. It must be pointed out that no patient with a dMMR tumour received immunotherapy. *TP53* mutations were reported associated with poor survival in a previous study (12) but had no significant prognostic value in our study either in localized tumour or metastatic tumour like in another previous study (9). *KRAS* mutations were reported as a poor prognostic predictor in the subgroup of patients with a pT1-T3 tumour (21) but also associated with a better survival in patients with metastatic tumour (9). In our study there was no significant effect of *KRAS* mutation but a trend of a worst prognosis in localized tumours and a better prognosis in metastatic tumours. It has been previously reported that *KRAS* mutations were associated with a poor OS in colorectal stage III pMMR tumours (22). The prognostic value of *KRAS* mutations deserves further evaluation in SBA. A *BRAF* mutation was only observed in 4% of the tumours in our study. In previous studies the frequency of *BRAF* mutations range from 1% to 11% (7,8,10,21). As in the previous studies the majority of *BRAF* mutations reported in our study were not the V600E mutation. No prognostic value of *BRAF* mutations was reported in SBA. It must be pointed out that in metastatic colorectal cancer non-V600E *BRAF* mutations are not associated to a poor prognosis in contrast to the V600E *BRAF* mutations (23).

Accepted Article

Several genomic alterations reported in our study may be targeted. It has recently been reported that a treatment with immune checkpoint inhibitor gives a prolonged survival in patients with metastatic dMMR SBA (24). Preclinical data suggest that ERBB2 inhibitors reduce tumour growth of *ERBB2* mutated tumours (11). Thus, ERBB2 inhibition deserve clinical evaluation in patients with *ERBB2* mutated SBA. Other gene alterations of *PTEN*, *PI3KCA* or *PTEN* may be considered for targeted treatment (25). Some rare mutations deserve also further evaluation. Signal of efficacy have been reported with PARP inhibitors in patient with ATM deficiency (26). IDH1 inhibitions have shown efficacy in cholangiocarcinoma (27). *IDH1* mutations should be screened in patient with SBA associated with Crohn's disease and IDH1 inhibitors need evaluation in those patients.

Our study had some limitations: first, even if this study is one of the largest genomic profiling of SBA, the sample size does not allow an accurate evaluation of rare mutation impact. Secondly, the gene panel used is limited but contains the most frequently altered genes in SBA. Thirdly, the constitutional gene mutations were not assessed in case of Lynch syndrome in our study. Fourth, we did not performed MSI testing nevertheless a previous study has reported no discordance between MMR IHC and MSI testing (9). Finally, we assume that our results are exploratory and should be taken with caution for the rare mutations as we did not perform a Bonferroni correction in our analysis. Moreover, the clinical characteristics were comparable in the NADEGE (13) and BIONADEGE cohorts except for metastatic stage at diagnostic underrepresented in the BIONADEGE cohort due to missing tumour samples suitable for genomic analysis. Thus, our results in metastatic tumours are limited. Additional studies pooling several databases are needed to specify the association of genomic profile, clinical data and prognosis.

Accepted Article

In conclusion, our study shows that there are different genomic alteration profiles in SBA that depends on the existence, or lack thereof, of a predisposing disease. This advocates to analyse separately sporadic SBA and those related to predisposing disease in future studies. With caution due to sample size, genomic alteration had no prognostic impact except a trend for a favourable prognosis associated with dMMR phenotypes in localized tumour. Nevertheless, some genomic alterations may be targeted. A compilation of worldwide experiences for off label targeted therapy is urgently needed for this orphan disease.

Previous presentation: This work has been presented at the 2019 ASCO meeting

Acknowledgments

BIONADEGE was granted by INCa and sponsor by Assistance Publique Hôpitaux de Paris (Délégation à la Recherche Clinique). ARCAD-NADEGE cohort was granted by A.R.C.A.D. and sponsored by GERCOR.

The authors thank to all investigators: Pauline Afchain (Paris), Thomas Aparicio (Paris), Jean-Baptiste Bachet (Paris), Nathalie Bonichon-Lamichhane (Bordeaux), Laurent Cany (Périgueux), François Caroli-Bosc (Angers), Christos Christidis (Paris), Philippe Collin (Reims), Romain Coriat (Paris), David Deutsch (Bobigny), Joëlle Egreteau (Lorient), Pierre-Luc Etienne (Saint Brieuc), Francine Fein (Besançon), Johan Gagnière (Clermont-Ferrand), Claire Garnier-Tixidre (Grenoble), Vincenzo Giardina (Bezier), Jean-Marc Gornet (Paris), Victoire Granger (Grenoble), Rosine Guimbaud (Toulouse), Kamran Imani (Tarbes), Sandrine Lavau-Denes (Limoges), Cédric Lecaille (Bordeaux), Thierry Lecomte (Tours), Jean-Louis Legoux (Orléans), Astrid Lièvre (Rennes), Catherine Ligeza-Poisson (Saint Nazaire), Christophe Locher (Meaux), Catherine Lombard-Bohas (Lyon), May Mabro (Suresnes), Sylvain Manfredi (Dijon), André Mathieu (Narbonne), Jérôme Meunier (Orléans), Drifa Moussata (Lyon), Suzanne Nguyen

(Beauvais), Arnaud Patenotte (Semur en Auxois), Hervé Perrier (Marseille), Guillaume Piessen (Lille), Marc Pocard (Lariboisière), Fabienne Portales (Montpellier), Corinne Sarda (Castre), Eric Terrebone (Pessac), David Tougeron (Poitiers), Aziz Zaanan (Paris).

The authors also thank Sabine Helfen and Zahia ben Abdesselam from the Clinical Research Unit and Clinical Research Centre, Avicenne Hospital, Bobigny as study manager, Mourad Benallaoua from Gastroenterology department for operational assistance, Alexandra Patry for technical assistance for tumour micro-array and Elsa Benamouzig for English editing.

Authors' contributions:

Study design: TA, MS, JH, DV and PLP.

Data acquisition: TA, MA, PA, AL, DT, JG, ET, GP, JLL, CL, MP, JMG, AZ, SLD, TL, DD and PLP.

Statistical analysis: JH and DV.

Manuscript preparation: TA, MS, JH, DV and PLP.

Manuscript review: all authors

Funding

This work was supported by a grant from INCa (Programme Hospitalier de Recherche Translationelle Cancer, PRTK14 N°091) and a grant n° NA 2009 from the A.R.CA.D. foundation.

Conflict of interest statement

The authors declare no conflict of interest for this publication.

Accepted Article

Accepted Article

Data availability statement: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Statement: All patients had to give written informed consent before inclusion into the NADEGE cohort study. This study was performed in accordance with the Declaration of Helsinki and was authorised by the ethics committee “Ile de France II” No. ID-RCB: 2008-A01058-47 and had the clinical trial number: NCT02976090.

References

1. Aparicio T, Zaanan A, Svrcek M, Laurent-Puig P, Carrere N, Manfredi S, et al. Small bowel adenocarcinoma: epidemiology, risk factors, diagnosis and treatment. Dig Liver Dis. 2014;46(2):97‑104.

2. Bouvier A-M, Robaszkiewicz M, Jooste V, Cariou M, Drouillard A, Bouvier V, et al. Trends in incidence of small bowel cancer according to histology: a population-based study. J Gastroenterol. 2020;55(2):181‑8.

3. Pan SY, Morrison H. Epidemiology of cancer of the small intestine. World J Gastrointest Oncol. 2011;3(3):33‑42.

4. Legué LM, Bernards N, Gerritse SL, van Oudheusden TR, de Hingh IHJT, Creemers G-JM, et al. Trends in incidence, treatment and survival of small bowel adenocarcinomas between 1999 and 2013: a population-based study in The Netherlands. Acta Oncol. 2016;55(9‑10):1183‑9.

5. Faivre J, Trama A, De Angelis R, Elferink M, Siesling S, Audisio R, et al. Incidence, prevalence and survival of patients with rare epithelial digestive cancers diagnosed in Europe in 1995-2002. Eur J Cancer. 2012;48(10):1417‑24.

6. Haan JC, Buffart TE, Eijk PP, van de Wiel MA, van Wieringen WN, Howdle PD, et al. Small bowel adenocarcinoma copy number profiles are more closely related to colorectal than to gastric cancers. Ann Oncol. 2012;23(2):367‑74.

Accepted Article

7. Schrock AB, Devoe CE, McWilliams R, Sun J, Aparicio T, Stephens PJ, et al. Genomic Profiling of Small-Bowel Adenocarcinoma. JAMA Oncol. 2017;3(11):1546‑53.

8. Hänninen UA, Katainen R, Tanskanen T, Plaketti R-M, Laine R, Hamberg J, et al. Exome-wide somatic mutation characterization of small bowel adenocarcinoma. PLoS Genet. 2018;14(3):e1007200.

9. Aparicio T, Svrcek M, Zaanan A, Beohou E, Laforest A, Afchain P, et al. Small bowel adenocarcinoma phenotyping, a clinicobiological prognostic study. Br J Cancer. 2013;109(12):3057‑66.

10. Laforest A, Aparicio T, Zaanan A, Silva FP, Didelot A, Desbeaux A, et al. ERBB2 gene as a potential therapeutic target in small bowel adenocarcinoma. Eur J Cancer. 2014;50(10):1740‑6.

11. Adam L, San Lucas FA, Fowler R, Yu Y, Wu W, Liu Y, et al. DNA Sequencing of Small Bowel Adenocarcinomas Identifies Targetable Recurrent Mutations in the ERBB2 Signaling Pathway. Clin Cancer Res. 2019;25(2):641‑51.

12. Alvi MA, McArt DG, Kelly P, Fuchs M-A, Alderdice M, McCabe CM, et al. Comprehensive molecular pathology analysis of small bowel adenocarcinoma reveals novel targets with potential for clinical utility. Oncotarget. 2015;6(25):20863‑74.

13. Aparicio T, Henriques J, Manfredi S, Tougeron D, Bouché O, Pezet D, et al. Small bowel adenocarcinoma: Results from a nationwide prospective ARCAD-NADEGE cohort study of 347 patients. Int J Cancer. 2020;147(4):967-977.

14. Yaeger R, Shah MA, Miller VA, Kelsen JR, Wang K, Heins ZJ, et al. Genomic Alterations Observed in Colitis-Associated Cancers Are Distinct From Those Found in Sporadic Colorectal Cancers and Vary by Type of Inflammatory Bowel Disease. Gastroenterology. 2016;151(2):278-287.e6.

15. Liao X, Li G, McBride R, Houldsworth J, Harpaz N, Polydorides AD. Clinicopathological and Molecular Characterisation of Crohn's Disease-associated Small Bowel Adenocarcinomas. J Crohns Colitis. 2020;14(3):287‑94.

16. Ullman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011;140(6):1807‑16.

17. Jones JS, Gu X, Lynch PM, Rodriguez-Bigas M, Amos CI, Frazier ML. ATM polymorphism and hereditary nonpolyposis colorectal cancer (HNPCC) age of onset (United States). Cancer Causes Control. 2005;16(6):749‑53.

18. Donahu TF, Bagrodia A, Audenet F, Donoghue MTA, Cha EK, Sfakianos JP, et al. Genomic Characterization of Upper-Tract Urothelial Carcinoma in Patients With Lynch Syndrome. JCO Precis Oncol. 2018;2018:PO.17.00143.

Accepted Article

19. Aparicio T, Schischmanoff O, Poupardin C, Mary F, Soufir N, Barrat C, et al. High prevalence of deficient mismatch repair phenotype and the V600E BRAF mutation in elderly patients with colorectal cancer. J Geriatr Oncol. 2014;5(4):384‑8.

20. Kondelin J, Salokas K, Saarinen L, Ovaska K, Rauanheimo H, Plaketti R-M, et al. Comprehensive evaluation of coding region point mutations in microsatellite-unstable colorectal cancer. EMBO Mol Med. 2018;10(9).

21. Jun S-Y, Kim M, Jin Gu M, Kyung Bae Y, Chang H-K, Sun Jung E, et al. Clinicopathologic and prognostic associations of KRAS and BRAF mutations in small intestinal adenocarcinoma. Mod Pathol. 2016;29(4):402‑15.

22. Taieb J, Le Malicot K, Shi Q, Penault-Llorca F, Bouché O, Tabernero J, et al. Prognostic Value of BRAF and KRAS Mutations in MSI and MSS Stage III Colon Cancer. J Natl Cancer Inst. 2017;109(5).

23. Jones JC, Renfro LA, Al-Shamsi HO, Schrock AB, Rankin A, Zhang BY, et al. Non-V600 BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. J Clin Oncol. 2017;35(23):2624‑30.

24. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord J-P, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol. 2020;38(1):1‑10.

25. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol. 2018;29(9):1895‑902.

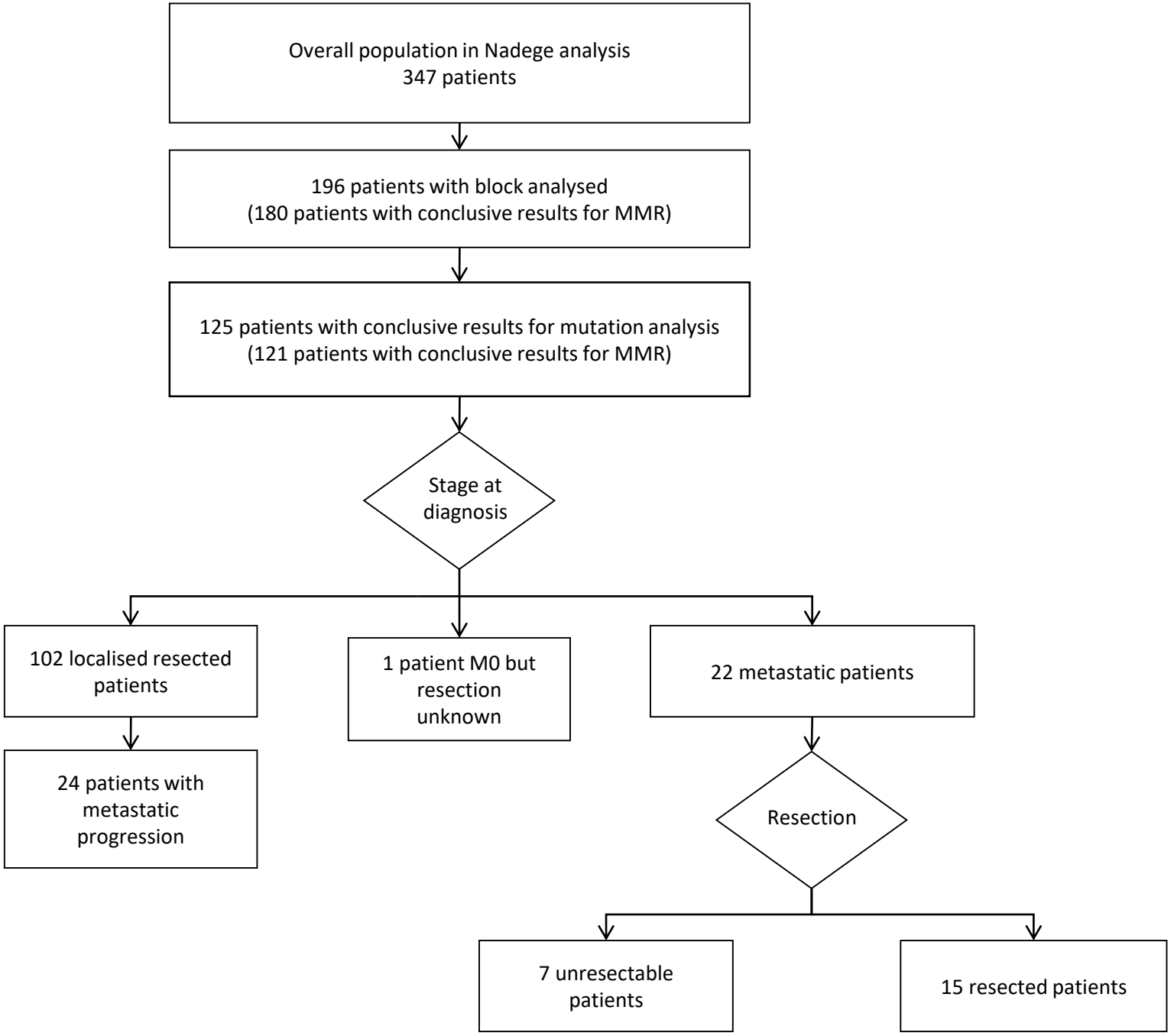
26. Jette NR, Kumar M, Radhamani S, Arthur G, Goutam S, Yip S, et al. ATM-Deficient Cancers Provide New Opportunities for Precision Oncology. Cancers. 2020;12(3).

27. Lowery MA, Burris HA, Janku F, Shroff RT, Cleary JM, Azad NS, et al. Safety and activity of ivosidenib in patients with IDH1-mutant advanced cholangiocarcinoma: a phase 1 study. Lancet Gastroenterol Hepatol. 2019;4(9):711‑20.

Figure legends

Figure 1: Flow chart

Accepted Article



Accepted Article

Table 1: Patient's characteristics

Characteristics		Whole NADEGE population N=347 (%)	Tumour block available N=196 (%)	Molecular genotyping N=125 (%)
Sex	Male	204 (59.0)	105 (53.6)	66 (52.8)
	Female	142 (41.1)	91 (46.4)	59 (47.2)
Age	Median (range)	62 (22-90)	63 (24 – 90)	61.7 (24-88)
Predisposing disease	no	278 (80.3)	159 (81.1)	100 (80)
n=346	Yes	68 (19.6)	37 (18.9)	25 (20)
	Lynch syndrome	24 (6.9)	17 (8.7)	14 (11.2)
	Crohn's disease	30 (8.5)	12 (6.1)	7 (5.6)
	Familial polyposis syndrome	6 (1.7)	5 (2.6)	2 (1.6)
	Coeliac disease	6 (1.7)	2 (1.0)	1 (0.8)
	Peutz-Jeghers syndrome	2 (0.6)	1 (0.5)	1 (0.8)
Primary tumour site	Duodenum	208 (60.6)	128 (65.6)	75 (60)
N=343	Jejunum	71 (20.7)	35 (18.0)	28 (22.4)
	Ileum	64 (18.7)	32 (16.4)	22 (17.6)

Accepted Article

Stage at diagnosis		N=343	N=194	N=124
Localized and resected		202 (58.9)	135 (69.6)	102 (82.3)
	Stage 0 (T *in situ*)	5 (2.5)	4 (3.0)	1 (1.0)
	Stage I	17 (8.4)	13 (9.6)	11 (10.8)
	Stage II	67 (33.2)	42 (31.1)	36 (35.3)
	Stage III	99 (49.0)	68 (50.4)	50 (49.0)
	Unknown	14 (6.9)	8 (5.9)	4 (3.9)
Locally advanced and not resected		19 (5.5)	8 (4.1)	0 (0.0)
Metastatic		122 (35.6)	51 (26.3)	22 (17.7)
Histological grade	Well/moderately differentiated	254 (73.2)	156 (79.6)	102 (81.6)
	Poorly differentiated	67 (19.3)	36 (18.4)	23 (18.4)
	Unknown	26 (7.5)	4 (2.0)	0 (0.0)

Accepted Article

Table 2: Gene mutation according to tumour stage and primary

Gene mutation	Overall population (n=125)	Localized and resected (n=102)	Metastatic at diagnosis (n=22)		Duodenum (n=75)	Jejunum (n=28)	Ileum (n=22)	
	%	%	%	P value	%	%	%	P value
KRAS	44.0	38.2	72.7	0.0031	48.0	32.1	45.4	0.3493
TP53	38.4	37.3	45.5	0.4739	33.3	43.9	50.0	0.3165
PIK3CA	20.0	19.6	22.7	0.7718	18.7	14.3	31.8	0.2759
APC	18.4	14.7	31.8	0.0690	18.7	17.9	18.2	0.9951
SMAD4	14.4	11.8	27.3	0.0899	16.0	7.1	18.2	0.4909
ERBB2	7.2	7.8	0.0	0.3484	8.0	7.1	4.5	1
ATM	5.6	6.8	0.0	0.3509	6.7	3.6	4.5	1
PTEN	5.6	4.9	9.1	0.6065	6.7	0.0	9.1	0.2983
NRAS	4.8	4.9	4.5	1	1.3	14	4.5	0.0202
BRAF	4.0	4.9	0.0	0.5848	4.0	3.6	4.5	1
CTNNB1	4.0	3.9	4.5	1	2.7	11.0	0.0	0.1397
STK11	4.0	3.9	4.5	1	4.0	0.0	9.1	0.2062
CDKN2A	3.2	3.9	0.0	1	2.7	7.1	0.0	0.3498
FBXW7	3.2	3.9	0.0	1	1.3	11.0	0.0	0.0610
ABL1	2.4	1.9	4.5	0.4464	2.7	0.0	4.5	0.5430
FGFR3	2.4	2.9	0.0	1	4.0	0.0	0.0	0.7555
GNAS	2.4	2.9	0.0	1	4.0	0.0	0.0	0.7555
IDH1	2.4	2.9	0.0	1	0.0	3.6	9.1	0.0355
SMARCB1	2.4	2.9	0.0	1	4.0	0.0	0.0	0.7555

Accepted Article

EGFR	1.6	0.9	4.5	0.3245	0.0	3.6	4.5	0.1581
ERBB4	1.6	0.9	4.5	0.3245	0.0	3.6	4.5	0.1581
FGFR1	1.6	1.9	0.0	1	2.7	0.0	0.0	1
MET	1.6	0.9	4.5	0.3245	2.7	0.0	0.0	1
SMO	1.6	1.9	0.0	1	1.3	0.0	4.5	0.3710
AKT1	0.8	0.9	0.0	1	0.0	3.6	0.0	0.4000
CDH1	0.8	0.9	0.0	1	0.0	3.6	0.0	0.4000
FGFR2	0.8	0.9	0.0	1	1.3	0.0	0.0	1
FLT3	0.8	0.9	0.0	1	0.0	3.6	0.0	0.4000
IDH2	0.8	0.9	0.0	1	1.3	0.0	0.0	1
JAK3	0.8	0.9	0.0	1	1.3	0.0	0.0	1
KDR	0.8	0.9	0.0	1	0.0	0.0	4.5	0.1760
PDGFRA	0.8	0.9	0.0	1	1.3	0.0	0.0	1
PTPN11	0.8	0.9	0.0	1	1.3	0.0	0.0	1
SRC	0.8	0.9	0.0	1	1.3	0.0	0.0	1
RB1	0.0	0.0	0.0	-	0.0	0.0	0.0	-

Accepted Article

Table 3: Gene mutation according to predisposing disease

	No predisposing disease	Lynch Syndrome	P value	Crohn's disease	P value
	(n=100)	(n=14)	Lynch vs no predisposing disease	(n=7)	Crohn vs no predisposing disease
	%	**%**		**%**	
KRAS	48.0	21.4	0.0611	42.9	1
TP53	39.0	21.4	0.2018	71.4	0.1211
PIK3CA	18.0	28.6	0.4671	42.9	0.1348
APC	20.0	14.3	1	0.0	0.3435
SMAD4	16.0	7.1	0.6899	14.3	1
ERBB2	7.0	14.3	0.3046	0.0	1
ATM	4.0	21.4	0.0389	0.0	1
PTEN	5.0	7.1	0.553	0.0	1
NRAS	6.0	0.0	1	0.0	1
BRAF	5.0	0.0	1	0.0	1
CTNNB1	5.0	0.0	1	0.0	1
STK11	4.0	0.0	1	14.3	0.2918
CDKN2A	3.0	7.1	0.4124	0.0	1
FBXW7	2.0	14.3	0.0731	0.0	1
ABL1	2.0	0	1	14.3	0.1853
FGFR3	1.0	14.3	0.0394	0.0	1
GNAS	2.0	7.1	0.3276	0.0	1
IDH1	1.0	0.0	1	28.6	0.0108

Accepted Article

SMARCB1	2.0	7.1	0.3276	0.0	1
EGFR	1.0	7.1	0.2315	0.0	1
ERBB4	1.0	0.0	1	14.3	0.1271
FGFR1	0.0	14.3	0.0141	0.0	-
MET	1.0	7.1	0.2315	0.0	1
SMO	2.0	0.0	1	0.0	1
AKT1	0.0	7.1	0.1228	0.0	-
CDH1	0.0	7.1	0.1228	0.0	-
FGFR2	0.0	7.1	0.1228	0.0	-
FLT3	1.0	0.0	1	0.0	1
IDH2	0.0	7.1	0.1228	0.0	-
JAK3	1.0	0.0	1	0.0	1
KDR	0.0	0.0	-	14.3	0.0654
PDGFRA	1.0	0.0	1	0.0	1
PTPN11	0.0	7.1	0.1228	0.0	-
SRC	1.0	0.0	1	0.0	1
RB1	0.0	0.0	-	0.0	-

Accepted Article

Table 4: Patients and tumour characteristics according to MMR status

Characteristics	pMMR tumours n=130 (72.2%)	dMMR tumours n=50 (27.8%)	P value
Sex: Men	67 (51.5%)	28 (56.0%)	
Women	63 (48.5%)	22 (44.0%)	0.5912
Age (median)	64	58	0.1760
Primary: Duodenum	83 (64.3%)	33 (66.0%)	
Jejunum	22 (17.0%)	11 (22.0%)	
Ileum	24 (18.6%)	6 (12.0%)	0.4889
Stage at diagnosis	n=129	n=49	
Localized and resected:	81 (62.8%)	47 (95.9%)	
Stage 0 (in situ)	3 (3.7%)	0 (0%)	
Stage I	7 (8.6%)	5 (10.6%)	
Stage II	25 (30.9%)	17 (36.2%)	
Stage III	40 (49.4%)	23 (48.9%)	
Unknown	6 (7.4%)	2 (4.3%)	
Locally advanced and not resected	7 (5.4%)	0 (0%)	
Metastatic	41 (31.8%)	2 (4.1%)	<0.0001
Grade: Well/moderately differentiated	106 (83.5%)	40 (80.0%)	
Poorly differentiated	21 (16.5%)	10 (20.0%)	0.5851
Lynch syndrome	0 (0.0%)	17 (34.0%)	<0.0001
Crohn disease	9 (6.9%)	2 (4.0%)	0.7300

Accepted Article

Sub group of patients with molecular phenotyping	n=81 (66.9%)	n=40 (33.1%)	P value
KRAS	44 (54.3%)	9 (22,5%)	0.0009
TP53	35 (43.2%)	11 (27.5%)	0.0940
PIK3CA	17 (21.0%)	8 (20.0%)	0.8996
APC	17 (21.0%)	5 (12.5%)	0.25481
SMAD4	14 (17.3%)	3 (7.5%)	0.1451
ERBB2	3 (3.7%)	6 (15.0%)	0.0580
ATM	3 (3.7%)	4 (10.0%)	0.2175
PTEN	3 (3.7%)	4 (10.0%)	0.2175
NRAS	4 (4.94%)	1 (2.5%)	1
BRAF	3 (3.7%)	2 (5.0%)	1
CTNNB1	3 (3.7%)	1 (2.5%)	1
STK11	3 (3.7%)	2 (5.0%)	1
CDKN2A	2 (2.5%)	2 (5.0%)	0.5983
FBXW7	2 (2.5%)	2 (5.0%)	0.5983
ABL1	2 (2.5%)	1 (2.5%)	1
FGFR3	1 (1.2%)	2 (5.0%)	0.2537
GNAS	1 (1.2%)	2 (5.0%)	0.2537
IDH1	3 (3.7%)	0 (0.0%)	0.5500
SMARCB1	0 (0%)	3 (7.5%)	0.0343
EGFR	1 (1.2%)	1 (2.5%)	1
ERBB4	1 (1.2%)	1 (2.5%)	1
FGFR1	1 (1.2%)	1 (2,5%)	1
MET	0 (0%)	1 (2.5%)	0.3306
SMO	1 (1.2%)	1 (2.5%)	1
AKT1	0 (0%)	1 (2.5%)	0.3306
CDH1	0 (0%)	1 (2.5%)	0.3306
FGFR2	0 (0%)	1 (2.5%)	0.3306
FLT3	1 (1.2%)	0 (0%)	1
IDH2	0 (0%)	1 (2.5%)	0.3306
JAK3	1 (1.2%)	0 (0%)	1
KDR	1 (1.2%)	0 (0%)	1
PDGFRA	0 (0%)	1 (2.5%)	0.3306

PTPN11	0 (0%)	1 (2.5%)	0.3306
SRC	1 (1.2%)	0 (0%)	1
RB1	0 (0%)	0 (0%)	-

Accepted Article

Accepted Article

Table 5: Hazard ratio of death according to clinical and tumour characteristics in univariate analysis

Characteristics		**n (events)**	**HR**	**95%CI**	**P value**
Gender	male	66 (19)	1		0.5927
	female	59 (22)	1.18	0.64 - 2.19	
Age	<70	87 (28)	1		0.1687
	≥70	38 (13)	1.6	0.82 - 3.15	
Primary	Duodenum	75 (25)	1		0.2929
	Jejunum	28 (7)	0.6	0.26 - 1.39	0.2331
	Ileum	22 (9)	1.31	0.61 - 2.82	0.4864
Differentiation	Well/moderately differentiated	102 (30)	1		0.0549
	Poorly differentiated	23 (11)	1.97	0.99 - 3.95	
Predisposing disease	No	100 (36)	1		0.1026
	Yes	25 (5)	0.46	0.18 - 1.17	
Stage at diagnostic	0 or I	12 (2)	1		0.0612
	II	36 (8)	1.63	0.35 - 7.66	0.5393
	III	51 (19)	2.69	0.62 - 11.55	0.1844
	IV	22 (11)	4.75	1.04 - 21.61	0.0438
pN	N0	52 (10)	1		0.0206
	N1	64 (26)	2.37	1.14 - 4.92	
pT	1 or 2	16 (2)	1		0.0065
	3	61 (14)	2.33	0.53 - 10.27	0.2630
	4	41 (20)	5.73	1.33 - 24.6	0.0190
M	M0	103 (30)	1		0.0214
	M1	22 (11)	2.28	1.13 - 4.6	
MMR	pMMR	81 (27)	1		0.2634
	dMMR	40 (11)	0.67	0.33 - 1.35	
Lynch syndrome	No	111 (39)	1		0.0895
	Yes	14 (2)	0.29	0.07 - 1.21	
KRAS	wild-type	70 (23)	1		0.5760
	mutated	55 (18)	1.19	0.64 - 2.22	
TP53	wild-type	77 (22)	1		0.4944
	mutated	48 (19)	1.24	0.67 - 2.29	
APC	wild-type	102 (36)	1		0.2220
	mutated	23 (5)	0.56	0.22 - 1.42	
PIK3CA	wild-type	100 (33)	1		0.4877
	mutated	25 (8)	1.32	0.61 - 2.85	
SMAD4	wild-type	107 (35)	1		0.5653
	mutated	18 (6)	1.29	0.54 - 3.08	