Panel gene profiling of small bowel adenocarcinoma: Results from the NADEGE prospective cohort

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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...
cancerogenesis. The mismatch repair (MMR) status was assessed by immunohistochemistry. 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 ≥3 in 32.0%. Overall, at least one genomic alteration was observed in 90.4% of tumours. 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 localised tumours (72.7% vs 38.2%, \( P = .003 \)). 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, localised 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 prognosis associated with dMMR.

**KEYWORDS**

cohort study, Crohn’s disease, genomic profiling, Lynch syndrome, MMR status, small intestine adenocarcinoma

1 | 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 United States.\(^3\) 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 cohorte Nationale d’ADEnocarcinome de l’intestin GrêLE (NADEGE) cohort has enrolled prospectively consecutive patients with all stages of SBA during a 4-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 analyse BIOlogique de la cohorte Nationale d’ADEnocarcinome de l’intestin GrêLE (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.

**What’s new?**

Because small bowel adenocarcinomas (SBAs) are quite rare, genomic analyses and prognostic biomarkers are lacking. In this study, the authors found at least one genomic alteration in 90.4% of SBAs. The most frequent alterations occurred at KRAS, TP53, PIK3CA, APC, SMAD4 and ERBB2. Additional alterations were specific to SBAs from patients with Lynch syndrome, while IDH1 mutations were associated with Crohn disease. No association was found between SBA prognosis and specific genomic alterations, except for a trend toward better prognosis with tumours deficient in mismatch repair (dMMR).
2 | PATIENTS AND METHODS

2.1 | 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.

2.2 | Immunohistochemistry

Tissue microarrays (TMA) were constructed from 0.6-mm diameter tissue cores obtained from formalin-fixed paraffin embedded tumour specimens. Haematoxylin and eosin (H&E) staining was performed on each TMA slide to confirm the presence of tumour tissue. The expression of MLH1, MSH2, MSH6 and PMS2 was assessed as previously described. Briefly, 4 μm 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 minutes. 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) and 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 tumours with a negative staining of one of the MMR protein were considered as dMMR.

2.3 | 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 macrodissected with reference to the H&E stained section.

FIGURE 1  Flow chart
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 and mutation calling were performed as described previously.10

2.4 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 subgroup of patients was assessed with the $\chi^2$ 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 localised tumours, and second, as cases with metastases.

Overall survival (OS) was defined as the time from diagnosis of a primary tumour (localised 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

| 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, n = 346    |                                      |                                  |                                  |
| No                               | 278 (80.3)                           | 159 (81.1)                       | 100 (80)                         |
| 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, N = 343     |                                      |                                  |                                  |
| Duodenum                         | 208 (60.6)                           | 128 (65.6)                       | 75 (60)                          |
| Jejunum                          | 71 (20.7)                            | 35 (18.0)                        | 28 (22.4)                        |
| Ileum                            | 64 (18.7)                            | 32 (16.4)                        | 22 (17.6)                        |
| Stage at diagnosis               | N = 343                              | N = 194                          | N = 124                          |
| Localised and resected           |                                      |                                  |                                  |
| Stage 0 (T in situ)              | 202 (58.9)                           | 135 (69.6)                       | 102 (82.3)                       |
| Stage I                          | 5 (2.5)                              | 4 (3.0)                          | 1 (1.0)                          |
| Stage II                         | 17 (8.4)                             | 13 (9.6)                         | 11 (10.8)                        |
| Stage III                        | 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)                          |
to determine baseline characteristics associated with OS for patients with mutational status available. All variables with \( P \) values of <.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 the SAS 9.3 software (SAS institute Inc., Cary, NC). As the analyses were exploratory, \( P \) values were not adjusted for multiple testing.

### RESULTS

#### 3.1 Patient and tumour characteristics

Among the 347 patients included in the analysis of clinical NADEGE data set, 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\textsuperscript{13} and the BIONADEGE cohorts except for metastatic stage at diagnosis under-represented in the BIONADEGE cohort (36\% in NADEGE vs 18\% in BIONADEGE, $P < .0001$).

The gene mutation frequency according to tumour stage and primary are presented in Table 2. The details of raw NGS data are presented in Table S2. 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 localised 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 duodenum, jejunum or ileum, respectively.

\begin{table}[h]
\centering
\caption{Gene mutation according to predisposing disease}\\
\begin{tabular}{|c|c|c|c|c|}
\hline
 & No predisposing & Lynch & & \hline
disease & disease & syndrome & $P$ value & Crohn's & $P$ value \\
 & (n = 100) & (n = 14) & Lynch vs no predisposing & disease & Crohn vs no predisposing \\
 & & & disease & (n = 7) & disease \\
\hline
KRAS & 48.0 & 21.4 & .0611 & 42.9 & 1 \\
TP53 & 39.0 & 21.4 & .2018 & 71.4 & .1211 \\
PIK3CA & 18.0 & 28.6 & .4671 & 42.9 & .1348 \\
APC & 20.0 & 14.3 & 1 & 0.0 & .3435 \\
SMAD4 & 16.0 & 7.1 & .6899 & 14.3 & 1 \\
ERBB2 & 7.0 & 14.3 & .3046 & 0.0 & 1 \\
ATM & 4.0 & 21.4 & .0389 & 0.0 & 1 \\
PTEN & 5.0 & 7.1 & .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 & .2918 \\
CDK2A1 & 3.0 & 7.1 & .4124 & 0.0 & 1 \\
FBXW7 & 2.0 & 14.3 & .0731 & 0.0 & 1 \\
ABL1 & 2.0 & 0.0 & 1 & 14.3 & .1853 \\
FGFR3 & 1.0 & 14.3 & .0394 & 0.0 & 1 \\
GNAS & 2.0 & 7.1 & .3276 & 0.0 & 1 \\
IDH1 & 1.0 & 0.0 & 1 & 28.6 & .0108 \\
SMARC1 & 2.0 & 7.1 & .3276 & 0.0 & 1 \\
EGFR & 1.0 & 7.1 & .2315 & 0.0 & 1 \\
ERBB4 & 1.0 & 0.0 & 1 & 14.3 & .1271 \\
FGFR1 & 0.0 & 14.3 & .0141 & 0.0 & – \\
MET & 1.0 & 7.1 & .2315 & 0.0 & 1 \\
SMO & 2.0 & 0.0 & 1 & 0.0 & 1 \\
AKT1 & 0.0 & 7.1 & .1228 & 0.0 & – \\
CDH1 & 0.0 & 7.1 & .1228 & 0.0 & – \\
FGFR2 & 0.0 & 7.1 & .1228 & 0.0 & – \\
FLT3 & 1.0 & 0.0 & 1 & 0.0 & 1 \\
IDH2 & 0.0 & 7.1 & .1228 & 0.0 & – \\
JAK3 & 1.0 & 0.0 & 1 & 0.0 & 1 \\
KDR & 0.0 & 0.0 & – & 14.3 & .0654 \\
PDGFRA & 1.0 & 0.0 & 1 & 0.0 & 1 \\
PTPN11 & 0.0 & 7.1 & .1228 & 0.0 & – \\
SRC & 1.0 & 0.0 & 1 & 0.0 & 1 \\
RB1 & 0.0 & 0.0 & – & 0.0 & – \\
\hline
\end{tabular}
\end{table}
**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%)                  | .5912   |
| Women                                                | 63 (48.5%)                  | 22 (44.0%)                  |         |
| **Age (median)**                                     | 64                          | 58                          | .1760   |
| **Primary**                                          |                             |                             |         |
| Duodenum                                             | 83 (64.3%)                  | 33 (66.0%)                  | .4889   |
| Jejunum                                              | 22 (17.0%)                  | 11 (22.0%)                  |         |
| Ileum                                                | 24 (18.6%)                  | 6 (12.0%)                   |         |
| **Stage at diagnosis**                               |                             |                             | <.0001  |
| Localised 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%)                    |         |
| **Grade**                                            |                             |                             |         |
| Well/moderately differentiated                       | 106 (83.5%)                 | 40 (80.0%)                  | .5851   |
| Poorly differentiated                                | 21 (16.5%)                  | 10 (20.0%)                  |         |
| Lynch syndrome                                       | 0 (0.0%)                    | 17 (34.0%)                  | <.0001  |
| Crohn disease                                        | 9 (6.9%)                    | 2 (4.0%)                    | .7300   |
| **Subgroup of patients with molecular phenotyping**  |                             |                             |         |
| n = 81 (66.9%)                                       | n = 40 (33.1%)              |                             |         |
| **KRAS**                                             | 44 (54.3%)                  | 9 (22.5%)                   | .0009   |
| **TP53**                                             | 35 (43.2%)                  | 11 (27.5%)                  | .0940   |
| **PIK3CA**                                           | 17 (21.0%)                  | 8 (20.0%)                   | .8996   |
| **APC**                                              | 17 (21.0%)                  | 5 (12.5%)                   | .25481  |
| **SMAD4**                                            | 14 (17.3%)                  | 3 (7.5%)                    | .1451   |
| **ERBB2**                                            | 3 (3.7%)                    | 6 (15.0%)                   | .0580   |
| **ATM**                                              | 3 (3.7%)                    | 4 (10.0%)                   | .2175   |
| **PTEN**                                             | 3 (3.7%)                    | 4 (10.0%)                   | .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%)                    | .5983   |
| **FBXW7**                                            | 2 (2.5%)                    | 2 (5.0%)                    | .5983   |
| **ABL1**                                             | 2 (2.5%)                    | 1 (2.5%)                    | 1       |
| **FGFR2**                                            | 1 (1.2%)                    | 2 (5.0%)                    | .2537   |
| **GNAS**                                             | 1 (1.2%)                    | 2 (5.0%)                    | .2537   |
| **IDH1**                                             | 3 (3.7%)                    | 0 (0.0%)                    | .5500   |
| **SMARCB1**                                          | 0 (0%)                      | 3 (7.5%)                    | .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       |

(Continues)
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 was also similar according to stage: 30.4% and 40.9% for localised 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 \textit{KRAS} (44.0%), \textit{TP53} (38.4%), \textit{PIK3CA} (20.0%), \textit{APC} (18.4%), \textit{SMAD4} (14.4%) and \textit{ERBB2} (7.2%). A \textit{KRAS} mutation was more frequent in metastatic tumours at diagnosis than in localised tumours (72.7% vs 38.2%, \(P = .003\)). A \textit{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 \textit{KRAS} mutations in Lynch syndrome and more frequent \textit{TP53} and \textit{PIK3CA} mutations in Crohn’s disease compared to no predisposing disease. No \textit{APC} mutation was observed in any Crohn’s disease. There was a trend of more frequent \textit{ERBB2} mutations in Lynch syndrome compared to no predisposing disease. Moreover, no \textit{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 \textit{ATM}, \textit{FGFR3} and \textit{FGFR1} gene mutations. \textit{IDH1} mutations are more frequent in tumours with Crohn’s disease than in no predisposing disease.

### 3.2 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 \textit{KRAS} mutations but more \textit{SMARCB1} mutations. There is also a trend for less \textit{TP53} mutations and more \textit{ERBB2} mutations.

### 3.3 Survival analysis

The median follow-up was 56 months (95% CI [47-63]). The 3-year 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.

### 3.4 Survival analysis in the 125 patients with mutational status available

Univariate analysis was performed in the subgroup 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]; \(P = .0159\)). There is a trend for a better prognosis associated with early stage (\(P = .0774\)) and Lynch syndrome (\(P = .0648\)).

The results of univariate analysis according to localised or metastatic tumour are given in Table S2. In the subgroup of 102 patients with localised and resected tumour, no genomic alteration was associated with OS. There is a trend for a worst 3 years OS in patients with tumour \textit{KRAS} mutation (63.3% [95% CI 43.0-78.1] vs 82.0% [95% CI 69.1-89.9]).
In the subgroup 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 = .5235). The median OS was 27.3 months (95% CI 9.1-59.7) and 16.2 months (95% CI 3.9—not assessable) in patients with mutated and wild-type tumour TP53, respectively (P = .9123).

## 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    |             | .5927   |
| Female                           | 59 (22)    | 1.18 | 0.64-2.19   |         |
| Age                              |            |      |             |         |
| <70                              | 87 (28)    | 1    |             | .1687   |
| ≥70                              | 38 (13)    | 1.6  | 0.82-3.15   |         |
| Primary                          |            |      |             |         |
| Duodenum                         | 75 (25)    | 1    |             | .2929   |
| Jejunum                          | 28 (7)     | 0.6  | 0.26-1.39   | .2331   |
| Ileum                            | 22 (9)     | 1.31 | 0.61-2.82   | .4864   |
| Differentiation                  |            |      |             |         |
| Well/moderately differentiated    | 102 (30)   | 1    |             | .0549   |
| Poorly differentiated             | 23 (11)    | 1.97 | 0.99-3.95   |         |
| Predisposing disease             |            |      |             |         |
| No                               | 100 (36)   | 1    |             | .1026   |
| Yes                              | 25 (5)     | 0.46 | 0.18-1.17   |         |
| Stage at diagnostics             |            |      |             |         |
| 0 or I                           | 12 (2)     | 1    |             | .0612   |
| II                               | 36 (8)     | 1.63 | 0.35-7.66   | .5393   |
| III                              | 51 (19)    | 2.69 | 0.62-11.55  | .1844   |
| IV                               | 22 (11)    | 4.75 | 1.04-21.61  | .0438   |
| pN                               |            |      |             |         |
| N0                               | 52 (10)    | 1    |             | .0206   |
| N1                               | 64 (26)    | 2.37 | 1.14-4.92   |         |
| pT                               |            |      |             |         |
| 1 or 2                           | 16 (2)     | 1    |             | .0065   |
| 3                                | 61 (14)    | 2.33 | 0.53-10.27  | .2630   |
| 4                                | 41 (20)    | 5.73 | 1.33-24.6   | .0190   |
| M                                |            |      |             |         |
| M0                               | 103 (30)   | 1    |             | .0214   |
| M1                               | 22 (11)    | 2.28 | 1.13-4.6    |         |
| MMR                              |            |      |             |         |
| pMMR                             | 81 (27)    | 1    |             | .2634   |
| dMMR                             | 40 (11)    | 0.67 | 0.33-1.35   |         |
| Lynch syndrome                   |            |      |             |         |
| No                               | 111 (39)   | 1    |             | .0895   |
| Yes                              | 14 (2)     | 0.29 | 0.07-1.21   |         |
| KRAS                             |            |      |             |         |
| Wild-type                        | 70 (23)    | 1    |             | .5760   |
| Mutated                          | 55 (18)    | 1.19 | 0.64-2.22   | .4944   |
| TP53                             |            |      |             |         |
| Wild-type                        | 77 (22)    | 1    |             | .2220   |
| Mutated                          | 48 (19)    | 1.24 | 0.67-2.29   |         |
| APC                              |            |      |             |         |
| Wild-type                        | 102 (36)   | 1    |             | .4877   |
| Mutated                          | 23 (5)     | 0.56 | 0.22-1.42   |         |
| PIK3CA                           |            |      |             |         |
| Wild-type                        | 100 (33)   | 1    |             | .5853   |
| Mutated                          | 25 (8)     | 1.32 | 0.61-2.85   |         |
| SMAD4                            |            |      |             |         |
| Wild-type                        | 107 (35)   | 1    |             |         |
| Mutated                          | 18 (6)     | 1.29 | 0.54-3.08   |         |

P = .3551. In the subgroup 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 = .5235). The median OS was 27.3 months (95% CI 9.1-59.7) and 16.2 months (95% CI 3.9—not assessable) in patients with mutated and wild-type tumour TP53, respectively (P = .9123).

### 3.5 Survival analysis in the 180 patients with MMR status available

The 3-year OS rate was 79.9% (95% CI 64.7-89.1) for patients with dMMR tumours and 58.5% (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 = .0765) (Figure S1). In the subgroup of patients with localised tumours, the 3-year OS were 82.9% (95% CI 67.2-91.5) and 72.5% (95% CI
observed in the Schrock study. Overall, ours and previous results associated with Crohn's disease in colorectal cancer. A recent publication and also high mutation rate of Crohn's disease compared to no predisposing disease. KDR

IDH1 small bowel without reaching significance. Some rare mutations seem associated with ileum tumours in the NADEGE cohort. be explained by the association with Crohn's disease that was mainly reported.

The genomic alteration profile was different according to rank 60.2-81.6) for patients with dMMR and pMMR, respectively (log-rank \( P = .3957; \) HR = 0.74 [0.36-1.50], \( P = .3976 \)). 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.

4 | 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.

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%. 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. In our study as in the Härinnen et al study, 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. 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 nonduodenal SBA.

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 compared to no predisposing disease. IDH1 mutations and also high mutation rate of TP53 were already reported associated with Crohn's disease in colorectal cancer. A recent publication reported an association of IDH1 and SMAD4 mutations with Crohn's disease in SBA. 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. A lower frequency of APC mutation in colorectal cancer associated with inflammatory bowel disease as compared to sporadic colorectal cancer was also reported. No ERBB2 mutation was observed in tumour associated with Crohn's disease in our study as it was previously observed in the Schrock study. Overall, 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.

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. We could not determine in our study if the ATM mutation was inherited or acquired. FGFR3 R248C hotspot mutation has already been associated with the Lynch syndrome in upper tract urothelial carcinoma 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. 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. We report a higher frequency of SMARCB1 mutations in dMMR tumours, SMARCB1 has already been described in dMMR colorectal cancers.

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, 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. In our study as in a previous one, there is a trend for better prognosis in patients with a dMMR tumour. The prognostic effect of dMMR phenotypes seems restricted to patients with localised 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, but had no significant prognostic value in our study either in localised tumour or metastatic tumour like in another previous study. KRAS mutations were reported as a poor prognostic predictor in the subgroup of patients with a pT1-T3 tumour but also associated with a better survival in patients with metastatic tumour. In our study, there was no significant effect of KRAS mutation but a trend of a worst prognosis in localised 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. 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 ranges from 1% to 11%. As in the previous studies, the majority of BRAF mutations reported in our study were not the V600E mutation.
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 with a poor prognosis in contrast to the V600E BRAF mutations.23

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 deserves 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 has 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. Second, the gene panel used is limited but contains the most frequently altered genes in SBA. Third, the constitutional gene mutations were not assessed in case of Lynch syndrome in our study. Fourth, we did not perform 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 NADEGE13 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.

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 localised 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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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