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Loss of SMARCB1 expression in colon carcinoma

S. Melloul^a, J.-F. Mosnier^b, J. Masliah-Planchon^{c,d,e}, C. Lepage^{f,g}, K. Le Malicot^{g,h}, J.-M. Gornetⁱ, J. Edeline^j, D. Dansette^b, P. Texereau^k, O. Delattre^{c,d,e}, P. Laurent Puig^{l,m}, J. Taieb^{l,m} and J.-F. Emile^{a,b,c,d,e,f,g,h,i,j,k,l,m,n,*}

^aDepartment of Pathology, Ambroise Paré Hospital, APHP, Boulogne, France

^bDepartment of Pathology, Hotel Dieu, Nantes, France

^cSomatic Genetic Unit, Institut Curie, Paris, France

^dParis-Sciences-Lettres, Institut Curie Research Center, INSERMU830, Paris, France

^eSIREDO, Institut Curie, Paris, France

^fFrançois Mitterrand University Hospital, Dijon, France

^gEPICAD INSERM LNC-UMR 1231, University of Burgundy and Franche-Comté, Besançon, France

^hFrancophone Society of Digestive Cancer, Dijon, France

ⁱSt Louis Hospital, APHP, Paris, France

^jEugène Marquis Center, Rennes, France

^kLayne Hospital Center, Mont-de-Marsan, France

^lG. Pompidou European Hospital, APHP, Paris, France

^mINSERM UMR-S1147, Paris, France

ⁿEA4340-BCOH, Versailles SQY University, Paris-Saclay University, Boulogne, France

Abstract. *SMARCB1* is a tumor suppressor gene, which is part of SWI/SNF complex involved in transcriptional regulation. Recently, loss of *SMARCB1* expression has been reported in gastrointestinal carcinomas. Our purpose was to evaluate the incidence and prognostic value of *SMARCB1* loss in colon carcinoma (CC).

Patients with stage III CC ($n = 1695$), and a second cohort of 23 patients with poorly differentiated CC were analyzed. Immunohistochemistry for *SMARCB1* was performed on tissue microarrays, and cases with loss of expression were controlled on whole sections. Loss of *SMARCB1* was compared with the clinico-pathological and molecular characteristics, and the prognostic value was evaluated.

Loss of *SMARCB1* was identified in 12 of 1695 (0.7%) patients with stage III CC. Whole section controls showed a complete loss in only one of these cases, corresponding to a medullary carcinoma. *SMARCB1* loss was not associated with histological grade, tumor size nor survival. In the cohort of poorly differentiated CC, we detected 2/23 (8.7%) cases with loss of *SMARCB1*; one was rhabdoid while the other had medullary and mucinous histology. These 2 cases were deficient for Mismatched Repair (dMMR) and mutated for *BRAF*.

SMARCB1 loss is rare in stage III CC, but appears more frequent in poorly differentiated CC.

Keywords: *SMARCB1*, colon carcinoma, *BRAF* V600E, mismatch repair deficiency

Abbreviations

CC colon carcinoma
CRC colorectal carcinoma

dMMR deficient for Mismatched Repair
IHC Immunohistochemistry
FFPE frozen tissue and formalin-fixed paraffin-embedded tissue
MSI Microsatellite instability
PETACC8 The Pan-European Trials in Alimentary traCt Cancer
PFS Progression-free survival

*Corresponding author: J.-F. Emile, Service de Pathologie, Hôpital Ambroise Paré, 9 Av Ch. De Gaulle, 92104 Boulogne, France.
E-mail: jean-francois.emile@uvsq.fr

SMARCB1 SWI/SNF-related matrix-associated
actin-dependent regulator of
chromatin subfamily B member 1
TMA Tissue microarray

1. Background

SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) is a chromatin-remodeling gene and a tumor suppressor gene located on chromosome 22q11 [6, 8, 18]. *SMARCB1* encodes for the protein SMARCB1 which is part of the multimolecular SWI/SNF complex involved in transcriptional regulation. The biallelic inactivation of *SMARCB1* was first described in pediatric rhabdoid tumors, and is the main oncogenic mechanism. Then, loss of SMARCB1 protein expression has been reported in a range of malignant neoplasms including pediatric rhabdoid tumors (atypical teratoid/rhabdoid tumors of the nervous system and malignant rhabdoid tumours) [5], epithelioid sarcomas [8, 9] and renal medullary carcinoma [7]. *SMARCB1* mutation/inactivation has been reported to be correlated with the loss of nuclear expression of SMARCB1 determined by immunohistochemistry (IHC) [8], and IHC for SMARCB1 is already used by pathologists to confirm the diagnosis of rhabdoid tumor.

Recently, SMARCB1 loss has been described in series of gastrointestinal adenocarcinomas with rhabdoid morphology, including colon adenocarcinomas (CC) [1, 11, 12, 19]. Wang showed that the loss of SMARCB1 in CC was rare (0.46%), and associated with higher histological grade, larger tumor size, lower survival, MSI and *BRAF* V600E status ($p < 0.001$). However, the frequency and prognostic value of SMARCB1 loss according to the stages of CC remains unclear. We therefore wanted to evaluate the incidence as well as the clinical pathological and molecular associations of SMARCB1 loss in a large, homogenous and well characterized cohort of patients with stage III CC, included in a prospective clinical trial and all receiving FOLFOX adjuvant.

2. Methods

2.1. Patients

The stage III CC series corresponds to 2043 patients included in the PETACC8 study [13], who signed

a specific consent for translational research and for whom FFPE samples were available. PETACC8 is an open, randomized, controlled, multi-center, multi-national, phase 3 study in 18–75 years old patients, who underwent complete resection of stage III colon carcinoma (clinical trials # NCT00265811). Patients were randomized to receive adjuvant therapy: 6 months of FOLFOX 4 or FOLFOX and Cetuximab. The study was done in accordance with the Declaration of Helsinki (amended 2000) and the International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) Note for Guidance on Good Clinical Practice and approved by the appropriate Ethics Committees.

The second series consists in 23 patients with poorly differentiated CC treated in Ambroise Paré hospital (Boulogne, France) or Laennec hospital (Nantes, France). In particular, the serie of Nantes consisted in 17 medullary carcinomas.

2.2. *SMARCB1* immunohistochemistry

Immunohistochemistry for SMARCB1 was performed using a mouse monoclonal antibody (clone 25/BAF47, BD Bioscience). SMARCB1 staining was interpreted by two observers (JFE, SM) who were blinded to clinical, pathological and molecular data at the time of analysis. The loss of SMARCB1 expression was established when the nuclei of tumor cells were not stained and the adjacent stromal cells, inflammatory and/or endothelial component as well as normal colonic cells (positive control) were positive. Normal liver tissue was used as a positive control for each TMA. We used an evaluation score of the loss of SMARCB1 in 3 categories according to the percent of positive tumor cells [19], and 2 subcategories: focal or diffuse loss. We only considered the percentage of labeled cells with SMARCB1 antibody, and not the intensity of the marking (Fig. 1 and Supplementary file 1). The score was noted “not interpretable” when neoplastic cells and also internal controls were negative for SMARCB1.

For the first series of patients, IHC of all cases was performed on tissue micro array (TMA) sections, and those who had focal or diffuse total loss of SMARCB1 underwent staining on whole sections. Immunohistochemistry on whole sections was interpreted as diffusely negative when there was negative staining in all neoplastic cells and a positive control in non-neoplastic cells (Fig. 2).

We then analyzed the expression of SMARCB1 in whole sections in the small independent cohort of

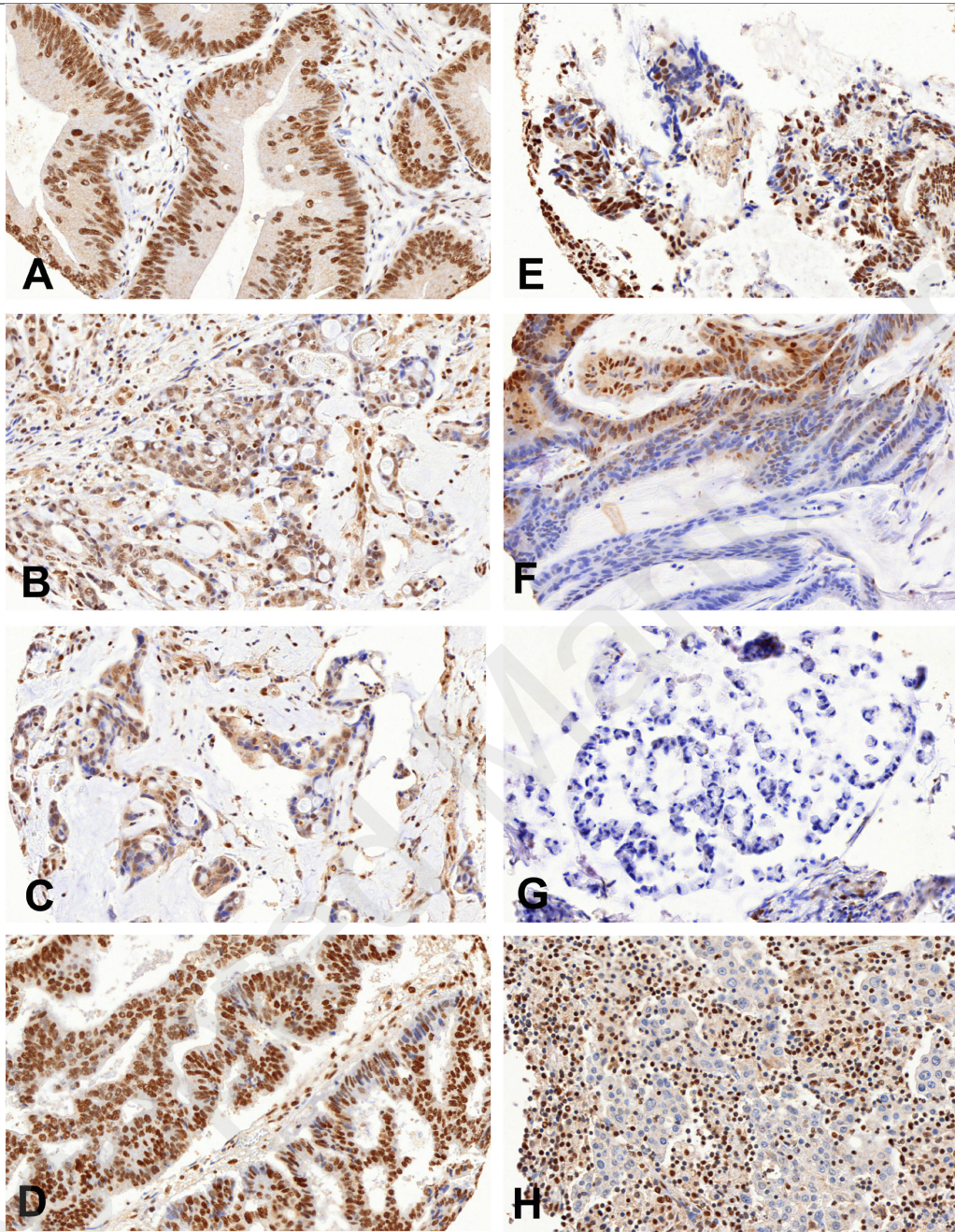


Fig. 1. SMARCB1 immunohistochemistry on TMA of colon carcinoma. A: More than 90% of tumor cells were positive (score "3d"). *Of note, neoplastic and non-neoplastic cells had nuclear expression of SMARCB1, because the expression of SMARCB1 is ubiquitous in non-tumor cells.* B: 51 to 90% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "2d"). C: 5 to 50% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "1d"). D: 51 to 90% of tumor cells were positive with a focal or multifocal distribution of loss of SMARCB1 (score "2f"). *Note that a loss is said to be "focal" if there is a loss of SMARCB1 expression on at least 25% of the TMA spot and if possible on more than one TMA spot out of 4.* E: 5 to 50% of tumor cells were positive with a focal distribution of loss of SMARCB1 (score "1f"). F: Less than 5% tumor cells were positive with a focal distribution of loss of SMARCB1 (score "0f"). G: Less than 5% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "0d"). H: In this case of medullary carcinoma, more than 95% of neoplastic cells are negative as opposed to lymphoid stroma. The non-neoplastic cells retain nuclear expression of SMARCB1 and act as an internal positive control (score "0d").

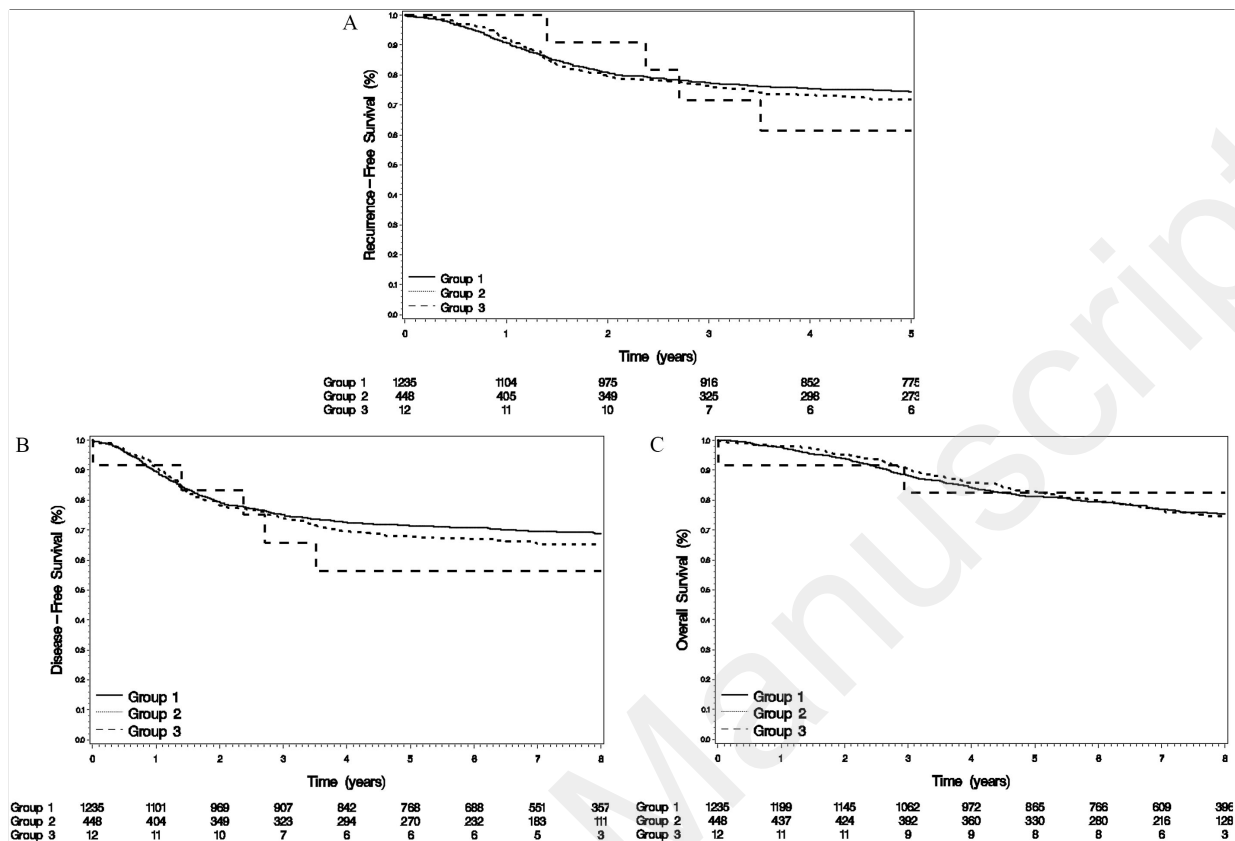


Fig. 2. Kaplan-Meier survival curves comparing SMARCB1 negative (group 3) and SMARCB1 positive CC (groups 1 and 2). A: Recurrence-Free survival according to SMARCB1 status. B: DFS according to SMARCB1 status. C: Overall survival according to SMARCB1 status.

23 patients treated in Boulogne or Nantes for poorly differentiated CC.

2.3. Statistic analyses

Prognostic and molecular status (mismatch repair (MMR) and *BRAF* V600E mutation) and detailed methods used to determine this status have been previously reported in the literature [4,14,15]. The association between CC clinicopathological and molecular variables and SMARCB1 expression were individually examined and binary logistic regression modeling was used. Kaplan Meier analysis and Cox regression modeling were employed to examine the impact of SMARCB1 expression with overall survival. A $p < 0.05$ was taken as significant. For statistical analyzes we used 3 groups as follows:

- Group 1: 3d (conserved expression of SMARCB1 on more than 90% of tumor cells)
- Group 2: 1 and 2 (labeling of SMARCB1 on 5 to 90% of tumor cells)
- Group 3: 0 (total loss of SMARCB1)

2.4. SMARCB1 molecular analysis

Tumor DNA was extracted from FFPE samples after histology control and selection of areas containing more than 80% of tumor cells. The whole coding sequence as well as the previously described hotspot in the first intron of *SMARCB1* were analyzed using next generation sequencing (NGS). Libraries obtained with Agilent SureSelect XT-HS preparation kit were sequenced on a NovaSeq 6000 system. The bioinformatics analysis included a variant calling using VarScan2 (v2.4.3) and TransIndel for intermediate insertion/deletion and a copy number profile using Facets (v0.5.1) with a sex-match control as reference [10,16].

3. Results

SMARCB1 immunostaining on TMA was interpretable in 1695 out of 2043 (82.9%) patients with stage III CC of the PETACC8 trial. In the 1695 (18.6%) analyzed cases, 315 were poorly or undifferentiated

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Table 1
Correlation of loss of SMARCB1 to clinicopathological and molecular data. Clinical and pathological characteristics of patients with loss of SMARCB1 (group 3) compared with those of patients without loss of SMARCB1 (groups 1 or 2)

| | | Group 1 (N = 1235) | Group 2 (N = 448) | Group 3 (N = 12) | Total (N = 1695) | p-value |
|-----------------------------------|--|-----------------------|----------------------|---------------------|---------------------|------------------------------|
| Gender | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.0759 |
| | Male | 688 (55.7%) | 274 (61.2%) | 5 (41.7%) | 967 (57.1%) | |
| | Female | 547 (44.3%) | 174 (38.8%) | 7 (58.3%) | 728 (42.9%) | |
| Age (Class) | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.5896 |
| | ≤ 70 ans | 1108 (89.7%) | 407 (90.8%) | 10 (83.3%) | 1525 (90.0%) | |
| | > 70 ans | 127 (10.3%) | 41 (9.2%) | 2 (16.7%) | 170 (10.0%) | |
| Age | <i>n</i> | 1235 | 448 | 12 | 1695 | KW: 0.1565 |
| | Moy (SD) | 59.06 (9.54) | 59.91 (9.23) | 54.92 (14.08) | 59.26 (9.50) | |
| | Médiane | 60.00 | 62.00 | 59.50 | 60.00 | |
| | Q1; Q3 | 53.00; 67.00 | 54.00; 67.00 | 46.00; 64.50 | 54.00; 67.00 | |
| | Min; Max | 23.00; 75.00 | 19.00; 75.00 | 28.00; 72.00 | 19.00; 75.00 | |
| WHO performance status | <i>n</i> | 1190 | 436 | 11 | 1637 | X²: 0.8160 |
| | 0 | 966 (81.2%) | 354 (81.2%) | 10 (90.9%) | 1330 (81.2%) | |
| | 1 | 218 (18.3%) | 82 (18.8%) | 1 (9.1%) | 301 (18.4%) | |
| | 2 | 5 (0.4%) | 0 (0.0) | 0 (0.0) | 5 (0.3%) | |
| | 3 | 1 (0.1%) | 0 (0.0) | 0 (0.0) | 1 (0.1%) | |
| Localisation | <i>n</i> | 1231 | 444 | 12 | 1687 | X²: 0.0624 |
| | Left localization | 711 (57.8%) | 291 (65.5%) | 7 (58.3%) | 1009 (59.8%) | |
| | Right localization | 506 (41.1%) | 147 (33.1%) | 5 (41.7%) | 658 (39.0%) | |
| | Both sides | 14 (1.1%) | 6 (1.4%) | 0 (0.0) | 20 (1.2%) | |
| Bowel obstruction and perforation | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.5507 |
| | Bowel obstruction and/or perforation | 234 (18.9%) | 90 (20.1%) | 1 (8.3%) | 325 (19.2%) | |
| | No bowel obstruction and no perforation | 1001 (81.1%) | 358 (79.9%) | 11 (91.7%) | 1370 (80.8%) | |
| Histopathology grading | <i>n</i> | 1234 | 448 | 12 | 1694 | X²: 0.7747 |
| | Well differentiated | 246 (19.9%) | 102 (22.8%) | 0 (0.0) | 348 (20.5%) | |
| | Moderately differentiated | 743 (60.2%) | 261 (58.3%) | 8 (66.7%) | 1012 (59.7%) | |
| | Poorly differentiated | 227 (18.4%) | 77 (17.2%) | 4 (33.3%) | 308 (18.2%) | |
| | Undifferentiated | 5 (0.4%) | 2 (0.4%) | 0 (0.0) | 7 (0.4%) | |
| PN classification | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.0143 |
| | pN1 | 783 (63.4%) | 260 (58.0%) | 11 (91.7%) | 1054 (62.2%) | |
| | pN2 | 452 (36.6%) | 188 (42.0%) | 1 (8.3%) | 641 (37.8%) | |
| PT classification | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.6254 |
| | pT1 | 30 (2.4%) | 13 (2.9%) | 0 (0.0) | 43 (2.5%) | |
| | pT2 | 81 (6.6%) | 30 (6.7%) | 2 (16.7%) | 113 (6.7%) | |
| | pT3 | 870 (70.4%) | 301 (67.2%) | 9 (75.0%) | 1180 (69.6%) | |
| | pT4 | 253 (20.5%) | 103 (23.0%) | 1 (8.3%) | 357 (21.1%) | |
| | pTis | 0 (0.0) | 1 (0.2%) | 0 (0.0) | 1 (0.1%) | |
| | pTx | 1 (0.1%) | 0 (0.0) | 0 (0.0) | 1 (0.1%) | |
| VELI | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.2864 |
| | Vascular invasion or lymphatic infiltration | 685 (55.5%) | 265 (59.2%) | 6 (50.0%) | 956 (56.4%) | |
| | No vascular invasion and no lymphatic infiltration | 350 (28.3%) | 128 (28.6%) | 3 (25.0%) | 481 (28.4%) | |
| | Missing | 200 (16.2%) | 55 (12.3%) | 3 (25.0%) | 258 (15.2%) | |
| Combined RAS/BRAF | <i>n</i> | 1151 | 407 | 9 | 1567 | X²: 0.1416 |
| | Double WT | 448 (38.9%) | 180 (44.2%) | 2 (22.2%) | 630 (40.2%) | |
| | RAS mutant | 569 (49.4%) | 183 (45.0%) | 7 (77.8%) | 759 (48.4%) | |
| | BRAF mutant | 134 (11.6%) | 44 (10.8%) | 0 (0.0) | 178 (11.4%) | |
| RAS | <i>n</i> | 1148 | 405 | 9 | 1562 | X²: 0.0651 |
| | Wild-Type | 578 (50.3%) | 222 (54.8%) | 2 (22.2%) | 802 (51.3%) | |
| | Mutated | 570 (49.7%) | 183 (45.2%) | 7 (77.8%) | 760 (48.7%) | |
| BRAF | <i>n</i> | 1182 | 426 | 10 | 1618 | X²: 0.0006 |
| | Wild-Type | 1045 (88.4%) | 378 (88.7%) | 8 (80.0%) | 1431 (88.4%) | |
| | Mutated | 117 (9.9%) | 41 (9.6%) | 0 (0.0) | 158 (9.8%) | |
| | Test failure | 20 (1.7%) | 7 (1.6%) | 2 (20.0%) | 29 (1.8%) | |
| MMR status | <i>n</i> | 1235 | 448 | 12 | 1695 | X²: 0.0698 |
| | pMMR | 1103 (89.3%) | 416 (92.9%) | 10 (83.3%) | 1529 (90.2%) | |
| | dMMR | 132 (10.7%) | 32 (7.1%) | 2 (16.7%) | 166 (9.8%) | |

133 CC. There was no significant difference in outcome ac- 182
134 cording of histological grade (Table 1). 183

135 Tumors from 12 of these patients (group 3 = 0.7%) 184
136 were had either focal ($n = 9$) or diffuse ($n = 3$) to- 185
137 tal loss of SMARCB1 on TMA and underwent IHC 186
138 on whole sections. These tumors were all moderately 187
139 (8/12) or poorly (3/12) differentiated. Whole section 188
140 controls disclosed a complete loss in only one case, 189
141 corresponding to a medullary carcinoma. 190

142 The clinicopathological and molecular features of 191
143 the CC with and without SMARCB1 loss are pre- 192
144 sented in Table 1. Diffuse or focal SMARCB1 loss was 193
145 not associated with clinical characteristics, histologi- 194
146 cal grade, tumor size, mismatch repair deficiency nor 195
147 *BRAF* mutation. 196

148 Loss of SMARCB1 was not associated with poorer 197
149 survival (Supplementary file 2, Fig. 2). 198

150 Surprisingly, group 1 (with a conservation of 199
151 SMARCB1 expression) had a worse node status. But 200
152 this is irrelevant clinically and is probably due to a bias 201
153 related to the low number of cases in group 3. 202

154 Among the 23 poorly or undifferentiated CC of the 203
155 second cohort, we detected 2 (8.7%) cases with total 204
156 focal loss of SMARCB1; one was rhabdoid and the 205
157 other poorly differentiated (medullary and mucinous). 206
158 These 2 cases were deficient for MMR and mutated for 207
159 *BRAF* (Supplementary files 3 and 4). 208

160 Molecular mechanisms responsible for the loss of 209
161 SMARCB1 expression were investigated in the two 210
162 cases with total and focal loss. NGS allowed a mean 211
163 coverage of 699X and 488X over the whole design in 212
164 those two cases and a minimal coverage of 100X for 213
165 *SMARCB1*, but did not reveal any pathogenic variant 214
166 in the coding sequence, nor at the hotspot within intron 215
167 1 of *SMARCB1*. Copy number profile also assessed by 216
168 NGS revealed neither large deletion nor duplication in 217
169 *SMARCB1*. Interestingly, we detected a large region of 218
170 copy-neutral loss of heterozygosity (LOH) encompass- 219
171 ing the whole *SMARCB1* locus in one of the cases. 220

172 4. Discussion 221

173 We detected a loss of SMARCB1 in 12 out 1695 224
174 (0.7%) cases with stage III CC included in the prospec- 225
175 tive international clinical trial PETACC8 and did not 226
176 found any correlation with clinical, histologic or 227
177 molecular characteristics, nor with survival. The fre- 228
178 quency of loss of SMARCB1 was higher in our second 229
179 cohort of 23 patients with poorly differentiated CC. 230

180 The incidence of loss of SMARCB1 in CC was 231
181 initially reported to be 11% (15/134) [11]. However, 232

182 in a very large series of 3041 unselected CC, Wang 183
184 et al. detected only 14 (0.46%) cases with loss of 185
186 SMARCB1 [19], which was significantly different 186
187 from the first report ($p < 0.001$, Xhi2 test). We use the 187
188 same evaluation score as Wang et al. and in our main 188
189 series of patients (cohort 1), which is limited to patients 189
190 with stage III CC, the frequency of loss of SMARCB1 190
191 was not different from Wang et al. ($p = 0.2$, Xhi2 test), 191
192 suggesting that the stage of CC has no or only limit cor- 192
193 relation with this phenotype. Altogether in these three 193
194 largest published series of CC, the frequency of loss of 194
195 SMARCB1 was 0.84% in 4880 patients, which likely 195
196 reflects a real incidence below 1% in CC of any stage. 196

197 The frequency of loss of SMARCB1 seems to be 197
198 higher in poorly differentiated CC. In fact, in the Italian 198
199 series, 8 cases with loss out of 25 poorly differentiated 199
200 CRC (32%) were observed. Among the 511 high-grade 200
201 cases of Wang, 12 were SMARCB1 negative (2.35%). 201
202 Briefly in Wang series, 12 of 14 (85.7%) of the CRCs 202
203 with SMARCB1 loss were high grade, compared to 203
204 19.6% of cases with preserved SMARCB1 staining 204
205 ($p < 0.001$). In 3 of the 7 CRCs with focal SMARCB1 205
206 loss, SMARCB1 stained in well-differentiated areas, 206
207 and lost in areas of poor differentiation. In our large 207
208 series of stage III CC, the only case with complete loss 208
209 was a poorly differentiated CC: medullary type. Further- 209
210 more in our small independent series of poorly differ- 210
211 entiated CC: 2/23 (8.7%) were SMARCB1 negative. 211

212 Although originally described in malignant rhab- 212
213 doid pediatric tumors, SMARCB1 loss has now been 213
214 reported in tumors from several localization, includ- 214
215 ing the vulva [8], pancreas [2] and sinonasal tract [3]. 215
216 In these sites, as well as in the gastrointestinal tract, 216
217 SMARCB1 loss was reported to be associated with 217
218 poor prognosis [1,11,17,19]. For instance, among the 218
219 134 cases of Pancione et al. the loss was associ- 219
220 ated with poorly differentiated tumors, most often 220
221 metastatic, and with a lower survival, regardless of the 221
222 MMR status. Wang et al. also observed that loss of 222
223 SMARCB1 was associated with lower survival. Con- 223
224 trasting with these previous publications, we did not 224
225 find any prognostic value of the loss of SMARCB1. 225

226 The previous studies retrospectively included pa- 226
227 tients with different UICC stages, and with heteroge- 227
228 neous treatment and follow up. By contrast, the 1695 228
229 that we analyzed were all at stage III, prospectively 229
230 included in an international clinical trial. They all re- 230
231 ceived FOLFOX adjuvant treatment and underwent the 231
232 same follow up. For these reasons our results have a 232
233 higher level of evidence, than the previously published 233
234 series. However, the power of our series is limited by 234

the low incidence of the SMARCB1 negative phenotype.

Only few cases of CC with rhabdoid histology and loss of SMARCB1 have been reported as yet. But they had common features: mean age 70 years old, localization of the proximal colon, metastases, poor prognosis, *BRAF* mutated and dMMR status [1,12]. Wang's group showed that the loss of SMARCB1 in CC was associated with MSI-H status, and *BRAF* V600E mutation ($p < 0.001$). Molecular characteristics of the tumors of patients included in the PETACC8 series have already been published [4,14,15]. In these patients, loss of SMARCB1 was not associated with mismatch repair deficiency nor with *BRAF* V600E mutation. However, in our small series of poorly differentiated CC, one of the two cases with loss was rhabdoid, the other mixed (medullary and mucinous) and these 2 cases were *BRAF* mutated and deficient for MMR. We can therefore speculate that SMARCB1 loss could be secondary to genetic instability. However no pathogenic somatic variant were detected within the coding sequences. The previously described hotspot mutation within intron 1 may also be responsible for the loss of SMARCB1 [16], but were also absent in the two cases. By contrast we detected a copy neutral LOH in one of the two cases. Further studies are required to determine the mechanism of SMARCB1 loss in poorly differentiated gastrointestinal carcinoma.

Genes of the SWI/SNF chromatin-remodeling complex are frequently altered in human cancers [20] and may be targeted by specific therapies in the future.

To date, there is no available targeted molecular therapy against pediatric rhabdoid neoplasms, but currently a recently developed EZH2 inhibitor, is undergoing clinical trial in children with rhabdoid tumor and loss of SMARCB1 (NCT02601937). EZH2 is a catalytic subunit of the histone methyltransferase PCR2 which is blocked by the intact SWI/SNF complex [17]. When SMARCB1 is mutated, the accumulation of EZH2 promotes an undifferentiated state with maintenance of a "stem cell" program. Hedgehog-Gli pathway, Cyclin D1, Epidermal growth factor and Fibroblastic growth factor receptors [21] are other potential targets, which have been found to be up regulated in association with a disrupted SWI/SNF complex.

In view of the results obtained in the literature and in the independent cohort of undifferentiated CC studied in parallel, it seems advisable to evaluate the prognostic impact of the loss of expression of SMARCB1 by restricting the studied population to the poorly differentiated CC. The IHC could allow a pre-screening

of mutated patients, who may one day benefit from targeted therapies.

In conclusion, loss of SMARCB1 expression is rare (< 1%) in stage III CC, but more frequent (> 5%) in poorly differentiated CC. Our study did not confirm the association of loss of SMARCB1 with MMR nor *BRAF* status, neither with poorer prognostic.

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Supplementary data

The supplementary files are available to download from <http://dx.doi.org/10.3233/CBM-190287>.

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