The mammalian switch/sucrose non-fermenting complex (mSWI/SNF) is a highly conserved ATPase-dependent chromatin remodeling unit involved in a wide range of biological functions. It is assembled from at least 15 protein subunits encoded by nearly 22 genes. These complexes comprise 1 of the 2 mutually exclusive catalytic subunits: Brahma (BRM), encoded by SMARCA2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2), or Brahma-related gene 1 (BRG1), encoded by SMARCA4 (SWI/SNF–related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 1) to large cell/poorly differentiated carcinomas (n = 1) with clear cell cytology in 2 cases. All showed loss/reduction of BRM with variable cytokeratin and SALL4 expression, and were negative for TTF-1, p40, Hep Par 1, ALK, ROS1, and EGFR mutations. CD34 and SOX2 were negative in all 4 cases. Isolated BRM loss was common (21%), distributed across all NSCLC subtypes including squamous cell carcinomas and a hepatoid adenocarcinoma.

Conclusions. —BRG1 loss occurs in a subset of TTF-1/p40–negative poorly differentiated NSCLCs. Identification and follow-up will clarify the prognosis, diagnostic criteria, and potential for therapeutic personalization.

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rhabdoid morphology occurring in adult men that were found to consistently harbor somatic SMARCA4 mutations and BRG1 loss. These SMARCA4-deficient thoracic sarcomas were transcriptionally similar to the malignant rhabdoid tumors and small cell carcinoma of ovary, hypercalcemic type, but differed from them by virtue of their increased genomic instability, frequent TP53 mutations, higher tumor mutation burdens, and lack of germline SMARCA4 alterations. They frequently expressed CD34, SALL4 (Sal-like protein 4), and SOX2 (SRY-box 2); they did not respond to therapy and were invariably fatal within months.

Other than sarcomas, SMARCA4/BRG1 loss is increasingly reported in subsets of poorly differentiated/undifferentiated carcinomas in adults arising in a wide range of anatomical sites, including the lung. Although the frequent occurrence of somatic SMARCA4 mutations and/or BRG1 protein loss in non–small cell lung carcinoma (NSCLC) cell lines and tumor samples has been well documented for the last 2 decades, it is only in recent years that SMARCA4-deficient NSCLC (SMARCA4-dNSCLC) has emerged as a distinct NSCLC subset, likely because of increased recognition of these alterations in routine next-generation sequencing platforms. A few authors have also suggested that these tumors may differ in their therapeutic responses to immunotherapy and chemotherapy. There are very limited morphologic descriptions of SMARCA4-dNSCLC. These tumors are typically negative for TTF-1 (thyroid transcription factor 1), and frequent immunopositivity for cytokeratin 7 (CK7) and Hep Par 1 has been reported in 2 series. Data on their prognosis are inconsistent and there is no clarity on their diagnostic criteria. Although a recent study has produced strong molecular evidence that SMARCA4-dTS represents the undifferentiated counterpart of SMARCA4-dNSCLCs, differentiation between the 2 may still be important because of the consistently documented poor prognosis of the former.

In this study, we describe in detail the morphologic features and immuno-profiles of 4 cases of SMARCA4-dNSCLCs identified on screening of 100 NSCLC tumor samples for BRG1 and BRM protein loss, and discuss the diagnostic, pathogenic, and possible therapeutic significance of BRG1 and BRM protein loss in thoracic tumors.

MATERIALS AND METHODS

The study was of retrospective design approved by the institute ethics committee. Informed consent was waived in view of the retrospective nature of the study. All cases of NSCLC diagnosed in our department during the last 6 years with adequate tissue in the formalin-fixed, paraffin-embedded blocks were reviewed for reconfirmation of NSCLC diagnosis, reclassified according to the latest World Health Organization classification of lung tumors, and subject to IHC using primary antibodies directed against BRG1 (1:800; E8VS5, Cell Signaling Technology) and BRM (1:2000 dilution; D9E8B clone, Cell Signaling Technology). Cases with loss of BRG1 in tumor cells using criteria previously described were further evaluated by an extended IHC panel comprising primary antibodies against TTF-1 (1:300; 8G7G3/1 clone, Bio-SB); p40 (1:100; ZR8, Bio-SB); Hep Par 1 (1:100; OCHIE5, Bio-SB); CK7 (1:200; OVTL12/30, ScyTec); cytokeratin 20 (CK20) (2D40; Ks20.8, Bio-SB); stem cell markers CD34 (1:200; QBEND, ScyTec); SALL4 (1:200; D4D6, Cell Signaling Technology). DNA extracted from formalin-fixed, paraffin-embedded tumor tissue was analyzed for the presence of hotspot EGRF (epidermal growth factor receptor) driver mutations by real-time polymerase chain reaction as previously described. Clinical details including follow-up were retrieved from clinical records of the department of surgical oncology and pulmonary medicine. We also included a case of SMARCA4-dTS from our archives for comparison of morphology and IHC profile. This particular case had already been published with limited IHC data.

RESULTS

Case Selection

A total of 100 cases of NSCLC were subject to IHC for BRG1 and BRM. The mean patient age at diagnosis was 58 years (SD = 10.3 years) with a male to female ratio of 5.5:1. The specimens included predominantly lung resections (n = 96), excision biopsy of cervical lymph nodes (n = 2), and True-Cut biopsies (n = 2). The histomorphologic diagnoses included squamous cell carcinoma (n = 48), adenocarcinoma (ADCA) (n = 38), adenosquamous carcinoma (n = 5), large cell carcinoma (n = 5), and non–small cell carcinoma not otherwise specified (n = 4).

Clinicopathologic Features of SMARCA4-dNSCLCs

Loss of BRG1 protein was noted in only 4 cases of all NSCLCs tested (4 of 100; 4%), all of which showed complete and diffuse loss of BRG1. The clinicopathologic features of these 4 SMARCA4-dNSCLCs (cases 1–4) are summarized in Table 1. All patients were males, with history of smoking available in 2. All except for case 1 presented with unresectable/metastatic disease.

On histopathology, cases 1 and 2 were composed of sheets and nests of relatively uniform large polygonal tumor cells with clear cytoplasm, whereas cases 3 and 4 were composed of more pleomorphic tumor cells with prominent nucleoli. Frequent mitoses and necrosis were seen in all cases. Inflammation was prominent in cases 1 and 3 (Figure 1, A through I). All tumors were relatively monotonous, lacking significant morphologic heterogeneity or foci of better differentiation. On IHC, all 4 cases showed either reduction or loss of BRM. Expression was completely lost in cases 3 and 4 and significantly reduced in intensity in tumor cells in comparison with normal cells (endothelial cells, inflammatory cells) in case 1, whereas hybrid loss (subpopulation of tumor cells with absent staining in a background of tumor cells with intact expression) was seen in case 2. All 4 SMARCA4-dNSCLCs were negative for TTF-1 (staining in isolated tumor cells in case 4), p40 (staining in isolated tumor cells in case 3), chromogranin, synaptophysin, CK20, cyclin D1, ALK, and ROS1, and showed retained INI-1 staining. Cytokeratin 7 was variably expressed, ranging from strong and diffuse in cases 1 and 2 to focal in cases 3 and 4. Hep Par 1 was expressed in isolated cells in case 1 and focally in case 3. Stem cell markers were consistently negative except for SALL4, which was expressed in a diffuse, weak fashion in cases 3 and 4. P53 immunostaining was diffuse and strong in cases 3 and 4, negative (null phenotype) in case 1, and wild type in case 2. Hot spot EGRF mutations were not detected in any of the 4 cases.

One patient with stage IIb SMARCA4-dNSCLC who underwent curative resection and received no adjuvant therapy was alive without evidence of disease at 12 months.
post-surgery (case 1), whereas 1 patient (case 4) died within 2 weeks after biopsy, likely because of tumor-related complications; however, the patient’s relatives did not consent to an autopsy. Outcome data were not available in the remaining patients.

### Clinicopathologic Features of the SMARCA4-dTS

The SMARCA4-dTS patient presented with a chest wall mass without a primary lung mass. The tumor was composed of monotonous large tumor cells with focal rhabdoid phenotype. Although frequent mitoses and necrosis were present, inflammation was insignificant (Table 1; Figure 2, A through D). BRG1 and BRM loss was complete and diffuse in tumor cells. The protein expression profile was largely similar to that of SMARCA4-dNSCLC except for the stem cell markers, SOX2, CD34, and SALL4, which were diffusely and strongly expressed in SMARCA4-dTS (Table 1). The SMARCA4-dTS patient was discharged after the biopsy procedure in stable condition; however, he collapsed at home a few days later and died immediately.21

### Isolated BRM Protein Reduction/Loss in NSCLCs

Interestingly, isolated BRM reduction/loss, that is, BRM loss with retained BRG1 staining, was observed in 21 NSCLCs. Complete loss was seen in 15 cases, including 8 ADCAs, 5 squamous cell carcinomas, 1 adenosquamous carcinoma, and 1 large cell carcinoma. Reduction in staining intensity was seen in 6 cases, including 3 ADCAs, 2 adenosquamous carcinomas, and 1 squamous cell carcinoma. The majority (8 of 11) of BRM-deficient ADCAs showed acinar- and papillary-predominant patterns, whereas only 3 were solid-pattern predominant. One of these 3 had been previously diagnosed as hepatoid ADCA based on the presence of large tumor cells with abundant granular eosinophilic cytoplasm with prominent nucleoli arranged in trabeculae; diffuse positivity for Hep Par 1, arginase, CK7, and p53; and cytoplasmic staining for TTF-1 (Figure 3, A through F). Further immunotyping in this case revealed negative staining for CD34, SOX2, and SALL4, with retained BRG1 and INI-1 expression.

### DISCUSSION

First reported in 2000,22 SMARCA4 homozygous mutations/deletions have been consistently identified at a high frequency in NSCLC cell lines (~33%)9,23–25 and in a smaller proportion of NSCLC tumor samples (~3%–6%).26,27 Non–small cell lung carcinomas harboring SMARCA4 mutations, hereafter referred to as SMARCA4-mutated NSCLCs, are

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**Table 1. Clinicopathologic Features of the SMARCA4-Deficient Tumors Described in the Current Study**

| Parameters | SMARCA4-dNSCLC | SMARCA4-dTS21 |
|------------|----------------|---------------|
| **Age, y/Sex** | 67/M | 58/M | 81/M | 41/M | 60/M |
| Smoking status | Beedi smoker (SI 800) | Former smoker (SI 300) | NA | NA | Beedi smoker (SI 40) |
| Biopsy type/site | Left upper lobectomy, left upper lobe mass | Debuxing, right endobronchial mass | Excision biopsy, right cervical lymph node | Core biopsy, cervical lymph node | Excision biopsy, chest wall mass |
| TNM | pT2N0cM0 | cT2NxMx | T4NxM4 | cT2NpM1 | NA |
| Treatment | Left upper lobectomy, no adjuvant therapy | Endobronchial mass debuxing | NA | NA | NA |
| Outcome (duration) | Alive without disease (12 mo) | NA | Died immediately after biopsy | Died soon after biopsy | Died soon after biopsy |
| Histopathology | ADCA solid subtype (solid sheets, clear tumor cells with prominent cell borders and focal mucin, mixed dense inflammation, necrosis) | LCC (solid irregular nests with intervening fibrovascular septa, clear tumor cells with prominent cell borders, no mucin, no necrosis) | NSCLC, NOS (PDCA) (solid irregular nests with thin fibrovascular septa, pleomorphic tumor cells with vesicular chromatin, moderate septal lymphoplasmacytic infiltrate, necrosis) | NSCLC, NOS (PDCA) (solid sheets, pleomorphic tumor cells with vesicular chromatin) | PDMT (solid sheets, pleomorphic tumor cells with vesicular chromatin and focal rhabdoid appearance, necrosis, no significant inflammation) |
| BRM | Significantly reduced | Hybrid | Loss | Loss | Loss |
| SOX2, CD34 | Neg, Neg | Neg, Neg | Neg, Neg | Neg, Neg | Pos (d), Pos (d) |
| TTF-1, CK7, CK20 | Neg, Pos (d), Neg | Neg, Pos (d), Neg | Neg, Pos (i), Neg | Neg, Pos (i), Neg | Neg, Neg, Neg |
| Hepar1, SALL4 | Pos (i), Neg | Neg, Neg | Pos (d), Pos (d, w) | Pos (d), Pos (d, w) | Neg, Pos (d, s) |
| CG, SYN | Neg, Neg | Pos (i), Neg | Neg | Neg | Neg |
| P40 | Neg | Neg | Pos (i) | Pos (i) | Neg |
| INI-1 | Retained | Retained | Retained | Retained | Retained |
| P53 pattern | Wild type | Null type | Wild type | Wild type | Wild type |
| EGFR/ALK/ROS1 | Wild type | Wild type | Wild type | Wild type | Wild type |

Abbreviations: ADCA, adenocarcinoma; d, diffuse staining; f, focal staining; i, isolated cells positive; LCC, large cell carcinoma; NA, not available; Neg, negative staining; NOS, not otherwise specified; NSCLC, non–small cell lung carcinoma; PDCA, poorly differentiated carcinoma; PDMT, poorly differentiated malignant tumor; Pos, positive staining; s, strong intensity staining; SI, smoking index; SMARCA4-dTS, SMARCA4-deficient thoracic sarcoma; TNM, tumor, node, metastasis; w, weak-intensity staining.
predominantly ADCAs, typically lack EGFR mutations and ALK fusions,9,27 and frequently harbor coexisting mutations in TP53,24–26 KRAS (Ki-ras2 Kirsten rat sarcoma viral oncogene homolog),9,24,25,27 CDKN2A (cyclin-dependent kinase inhibitor 2A),24,26 and STK11 (serine/threonine kinase 11).24,25 Single cases with concurrent SMARCA2 mutation,25 KEAP (kelch-like ECH-associated protein 1) mutation,27 ROS1 fusion,27 and RET (rearranged during transfection) fusion27 are also described. The SMARCA4 mutations/deletions, best investigated in NSCLC cell lines, predominantly involve the bromodomain, resulting in a truncated BRG1 protein lacking an ATPase domain, and rarely the ATPase domain itself, leading to functional inactivation of an intact protein.24 Accordingly, with the exception of few missense mutations,28 the majority of SMARCA4 mutations/deletions in NSCLC cell lines9,23 and tumors4,10,28,29 lead to complete loss of BRG1 protein by IHC. Interestingly, variable loss/reduction of the closely related BRM protein is also common in these tumor cells despite the rarity of SMARCA2 mutations.9,23,30

In the last couple of years, there have been anecdotal observations that NSCLCs with BRG1 loss, that is, SMARCA4-dNSCLCs, respond well to immune checkpoint blockade,12 mitotic spindle inhibitors,14 and platinum-based chemotherapy regimens.15 Considering that SMARCA4-dNSCLCs usually lack targetable alterations in EGFR/ALK/ROS1, there is increasing clinical interest in identifying this subset of NSCLC for therapeutic tailoring. Including our 4 cases, we reviewed a total of 68 well-annotated cases of SMARCA4-dNSCLC from literature4,7–11 diagnosed solely based on the loss of BRG1 expression in tumor cells (Table 2; Supplemental Table 1 of 2 tables; see supplemental digital content at https://meridian.allenpress.com/aplm in the January 2021 table of contents). The SMARCA4-dNSCLCs present in the fourth or fifth decade of life, predominate in males, and show strong association with smoking.12 They are primary lung parenchymal masses with nonspecific radiologic features, and have presented in all clinical stages including stage I disease. On histomorphology, reported cases range from well-differentiated ADCAs to poorly

Figure 1. Morphologic and immunohistochemical features of SMARCA4-deficient non–small cell carcinomas. Tumor cells arranged in sheets and lobules with dense lymphoplasmacytic infiltrate in the septa in case 1 (A); cases 1 (B) and 2 (C) show presence of large polygonal tumor cells with clear cytoplasm and prominent cell borders; case 3 (D) shows sheets of large pleomorphic tumor cells with prominent eosinophilic nucleoli; loss of BRG1 protein (E) and BRM (F) in tumor cells with endothelial cells and inflammatory cells serving as internal positive controls; diffuse expression of cytokeratin 7 (G) seen in case 1; focal expression of Hep Par 1 (H) in case 3; diffuse and weak nuclear expression of SALL4 (I) in case 3 (hematoxylin-eosin, original magnifications ×200 [A and B] and ×400 [C and D]; original magnifications ×400 [E, F, and H] and ×200 [G and I]).
differentiated malignant tumors, although the majority have been diagnosed as solid ADCAs or, less commonly, as large cell carcinomas. Clear cell cytology, noted as a frequent feature by Agaimy et al, was prominent in 2 of our cases as well. Occasional cases with rhabdoid morphology, spindling, and signet cell cytology and rare cases of acinar or papillary pattern–predominant ADCA, invasive mucinous ADCA, and even squamous cell carcinoma showing BRG1 loss are on record, although in our own cohort we did not observe BRG1 loss in any squamous cell carcinomas or mucinous ADCAs. Notably, BRG1 loss has not been described in lepidic or micropapillary pattern–predominant ADCAs. Yoshida et al have described rare SMARCA4-deficient ADCAs showing BRG1 loss only in the dedifferentiated component (partial loss), reminiscent of dedifferentiated SMARCA4-deficient endometrial carcinomas. Inflammation, necrosis, and frequent mitoses are also commonly seen in these tumors, as was seen in our cases as well (Table 2).

On IHC, two-thirds of tested SMARCA4-dNSCLCs are positive for CK7 and nearly all for claudin 4. Although Hep Par 1 was found to be frequently positive in a diffuse fashion in the majority of SMARCA4-dNSCLC reported by Agaimy et al, our 4 cases Hep Par 1 was expressed very focally in one and in only isolated cells in another. The SMARCA4-dNSCLCs are usually negative for p40/p63, TTF-1, napsin, neuroendocrine markers, and CK5/6. BRM immunostaining is retained in more than half of reported cases, with variable degrees of BRM loss (complete/partial/only reduced intensity) in the remaining. Among the 17 SMARCA4-dNSCLC patients with follow-up data (Supplemental Table 1), 11 who presented with stage IV disease survived for a median duration of 4.4 months, significantly shorter than those who presented with stage III or stage II disease, and the independent prognostic association of BRG1 loss could not be ascertained. In previous studies, BRG1 protein loss has been associated with shorter survival irrespective of tumor stage in a cohort of NSCLC patients treated by resection without adjuvant therapy. Other authors have also reported poorer prognosis among NSCLCs with BRG1 protein loss and low SMARCA4 expression levels. Larger studies are necessary to confirm the prognostic impact of BRG1 loss in NSCLCs.

It may be noted here that BRG1 loss is more common in NSCLC than underlying SMARCA4 mutations and the terms SMARCA4-mutated NSCLC and SMARCA4-

Figure 2. Morphologic and immunohistochemical features of SMARCA4-deficient thoracic sarcoma. Large monotonous tumor cells arranged in sheets with frequent mitoses (A); diffuse and strong expression of SALL4 (B), SOX2 (C), and CD34 (D) in tumor cells (hematoxylin-eosin, original magnification ×400 [A]; original magnifications ×400 [B and D] and ×200 [C]).
dNSCLC are not necessarily interchangeable. Underlying SMARCA4 mutations have been identified in only 81% of tested SMARCA4-dNSCLC with loss of heterozygosity of SMARCA4 locus in 80% of mutant samples (Table 2). MicroRNA (miR-155) mediated posttranscriptional inhibition and mutations in other mSWI/SNF family proteins, in particular the BAF subunit ARID1A, are implicated as alternate mechanisms for BRG1 loss in the absence of SMARCA4 mutations.

BRG1 loss appears to result in failure of ATPase-dependent eviction of polycomb recessive complexes, leading to widespread chromatin reorganization and changes in the expression levels of many genes implicated in the etiology of NSCLC.34–36 In mouse models, heterozygous SMARCA4-knockout mice develop lung adenomas, suggesting that loss of even one SMARCA4 allele is sufficient for tumor initiation.30 Biallelic SMARCA4 knockout, however, causes apoptosis of normal lung cells and loss of cell viability. Nevertheless, in transformed adenoma cells, biallelic SMARCA4 inactivation paradoxically potentiates tumor progression, likely because of presence of other background genetic alterations such as TP53, KRAS, and STK11 mutations that inhibit apoptosis.30

The functional role of BRM in BRG1-deficient tumors is not well explored. BRM and BRG1 share 75% homology at the amino acid level,37 and there is evidence that retained BRM may compensate for BRG1 loss in certain functions, such as in mediating RB- and p16-induced growth inhibitory signals.39 In NSCLC cell lines with BRG1 loss and retained BRM, inhibition of BRM can lead to tumor cell death due to synthetic lethal effect.10,38 However, in up to 50% of BRG1-deficient NSCLC cell lines and tumors, concurrent BRM loss is also seen. In these cases, BRM loss occurs because of epigenetic silencing of SMARCA2 locus,30,39 and the resultant BRM loss has been demonstrated to paradoxically potentiate epithelial to mesenchymal transition and sarcomatoid transformation.9,36 Interestingly, Rekhtman et al38 recently described 2 BRG1-deficient tumors with sharply delineated sarcomatoid areas wherein only the sarcomatoid areas showed loss of BRM, supporting this hypothesis. Epigenetic repression of SMARCA2 appears to be reversible with EZH2 inhibitors, and SMARCA4-mutant NSCLC cell lines with decreased SMARCA2 mRNA and BRM loss show a fall in proliferative activity when treated with EZH2 inhibitors.39

Isolated BRM protein loss/reduction is also common in NSCLC tumor samples, described at a frequency of 15% irrespective of histology,6,30; however, this does not lead to similar changes in gene expression profiles as with BRG1 loss35 and appears to be neither necessary nor sufficient for lung cancer development in the presence of functional BRG1.40 In our study, we found BRM loss/reduction in a slightly higher number (21%) of tested NSCLCs, including most histologic subtypes.

Most importantly, SMARCA4 mutations and/or BRG1 protein loss are not specific to SMARCA4-dNSCLC among thoracic malignancies. SMARCA4-dTS5,8,18,21,41,42 in particular, shows overlapping demographic, histomorphologic, protein expression, and molecular genetic profiles with SMARCA4-dNSCLC (Table 2) but differs from the latter in the following aspects: (1) SMARCA4-dTSs are predominantly localized in the mediastinum or chest wall, although primary lung parenchymal localization is reported in up to one-fifth of cases; (2) they more commonly present with bulky and metastatic stage IV disease; (3) they show a higher incidence of peritoneal metastases;35 (4) SMARCA4-dTSs consistently show a poorly differentiated histology, with epithelioid, small cell, or rhabdoid phenotype cells; (5) BRM...
Table 2. Comparative Analysis of Clinicopathologic, Immunohistochemical, and Genetic Features of Published Cases of SMARCA4-dNSCLC and SMARCA4-dTS Including the Current Cases

| Parameters                        | SMARCA4-dNSCLC<sup>a</sup> | SMARCA4-dTS<sup>b</sup> |
|-----------------------------------|-----------------------------|-------------------------|
| **Clinical features**             |                             |                         |
| Age at diagnosis, median (range), y | 55–58 (34–81)              | 39–59 (27–90)           |
| M:F ratio                         | 4.7:1                       | 4.5:1                   |
| History of smoking present, %     | 92 (34/37)                  | 86 (57/66)              |
| Primary tumor location            | Lung (68/68; 100%)          | Mediastinum (31/78; 40%), mediastinum and lung (16/78; 20.5%), pleura and/or chest wall (16/78; 20.5%), lung (15/78; 19%) |
| Stage at presentation             | I–IV (nearly equally distributed) | M1 in 83% patients at presentation |
| Histology                         | ADCA (52/68; 76%)           | PDCA/LCC (5/68; 7%)     |
|                                  | Solid ADCA 63% (33/52) with clear cell cytology in one-fifth of cases | Undifferentiated rhabdoid (3/68; 4%) |
|                                  | Acinar/papillary pattern 15% (8/52) | SQCC<sup>c</sup> (8/68; 12%) |
|                                  | Invasive mucinous, signet ring cytology rare |                  |
|                                  | PDMT with monotonous sheets of tumor cells with epithelioid, rhabdoid, or small cell morphology; no squamous or glandular differentiation; no clear cell morphology described; some show myxoid stroma | |
| IHC<sup>e</sup>                   |                             |                         |
| BRG1                              | Loss (68/68; 100%)          | Loss (69/78; 88%)       |
|                                  | Partial loss exclusively in dedifferentiated component in 4 cases<sup>f</sup> | Reduced intensity (9/78; 12%) |
| BRM                               | Loss (21/66; 32%)           | Loss (69/76; 91%)       |
|                                  | Partial loss (2/66; 3%)     | Partial loss (2/76; 3%) |
|                                  | Reduced intensity (9/66; 14%) |                             |
| CK7 positive                      | 59% (23/39)                 | 9% (2/24)               |
| CK5/6 positive                    | 0% (0/10)                   | 0% (0/19)              |
| p40 positive                      | 0% (0/19)                   | 2% (1/51)              |
| TTF-1 positive                    | 2% (1/52)                   | 5% (4/75)              |
| Claudin-4 positive                | 100% (12/12)                | 0% (0/49)              |
| SOX2 positive                     | 5% (1/19)                   | 93% (42/45)            |
| CD34 positive                     | 0% (0/17)                   | 53% (36/68)            |
| SALL-4 positive                   | 3% (1/36)                   | 32% (18/56)            |
| Hep Par 1 positive                | 58% (14/24)                 | 0% (0/1)               |
| p53                               | Positive (2/4; 50%); null (1/4; 25%) | Positive (8/12; 66%); null (1/12; 8%) |
| **Genetic profile**               |                             |                         |
| Underlying SMARCA4 gene alterations | Positive (81%)—truncating mutations, deletions, splice alterations, frameshift mutations | Positive (95%)—truncating mutations, frameshift mutations, splice alterations, nonsense mutations |
| EGFR mutations                    | SMARCA4 LOH in 66% of SMARCA4-mutant tumors | SMARCA4 LOH in 100% of cases tested |
| ALK/ROS1 fusions                  | Absent (36/36; 100%)        | Absent (36/36; 100%)    |
| TP53 mutations                    | Absent (17/17; 100%)        | Absent (36/36; 100%)    |
| KRAS mutations                    | Present (15/17; 88%)        | Present (28/34; 82%)    |
| STK11 alterations                | Present (4/28; 14%)         | Present (6/36; 16%)     |
| Other gene alterations detected<sup>g</sup> | Present (3/17; 17%) | Present (6/21; 29%) |

Abbreviations: ADCA, adenocarcinoma; IHC, immunohistochemistry; LCC, large cell carcinoma; LOH, loss of heterozygosity; NSCLC, non–small cell lung carcinoma; PDCA, poorly differentiated carcinoma; PDMT, poorly differentiated malignant tumor; SMARCA4-dNSCLC, SMARCA4-deficient NSCLC; SMARCA4-dTS, SMARCA4-deficient thoracic sarcoma; SQCC, squamous cell carcinoma.

<sup>a</sup> Data compiled from 68 published cases including the 4 SMARCA4-dNSCLC described in the current study (Supplemental Table 1).

<sup>b</sup> Data compiled from 78 published cases of SMARCA4-dTS (Supplemental Table 2).

<sup>c</sup> All 8 SQCCs with BRG1 loss were described in a single study.<sup>10</sup>

<sup>d</sup> Combined NSCLC described in 5 cases in a single study.<sup>18</sup>

<sup>e</sup> Only diffuse staining in tumor cells was considered positive; focal and isolated tumor cell staining for TTF-1, p40, and SALL4 was observed in some cases (Supplemental Table 1).

<sup>f</sup> Partial loss described in dedifferentiated component in a single study.<sup>8</sup>

<sup>g</sup> Gene alterations listed here are not comprehensive, and additional gene alterations have been described in SMARCA4-mutant lung cancer cell lines and tumor samples whose BRG1 protein expression status is unknown.<sup>25,27</sup>
loss is more frequent and complete; (6) stem cell markers CD34, SOX2, and/or SALL4 are consistently and strongly expressed, whereas claudin 4 is usually negative; (7) nearly 100% of cases show the presence of SMARCA4 mutations, as well as loss of heterozygosity of SMARCA4 locus; (8) they are associated with an extremely poor survival (Table 2; Supplemental Table 2). The criteria for SMARCA4-dTS proposed by Perret et al. —rhabdoid or poorly differentiated morphology, complete BRG1 and BRM loss, and focal/diffuse expression of at least 2 of CD34, SOX2, and SALL4—effectively exclude most cases of SMARCA4-dNSCLC, although this still needs validation in larger series.

BRG1 loss has also been described in up to 30% of epithelioid mesotheliomas, the latter also harboring SMARCA4 mutations. Nevertheless, concurrent BRM loss is unusual, and the expression of other mesothelial markers (WT1, calretinin, CK5/6), which are uncommon in both SMARCA4-dNSCLC and SMARCA4-dTS, will aid in differentiation in a pleural-based mass. Other tumors that may enter the histomorphologic differential diagnosis, such as epithelioid sarcomas, NUT carcinomas, and other high-grade round cell/rhabdoid sarcomas, have demonstrated retained BRG1 in the limited numbers tested.

Although we did not find significant expression of Hep Par 1 in our 4 SMARCA4-dNSCLCs, Hep Par 1 expression has been described as a common feature of a subset of SMARCA4-dNSCLCs. The relationship of these Hep Par 1–expressing NSCLCs to hepatoid ADCAs that rarely occur has been described as a common feature of a subset of SMARCA4-dNSCLCs. Nevertheless, SMARCA4-dNSCLCs and SMARCA4-dTS, will aid in differentiation in a pleural-based mass. Other tumors that may enter the histomorphologic differential diagnosis, such as epithelioid sarcomas, NUT carcinomas, and other high-grade round cell/rhabdoid sarcomas, have demonstrated retained BRG1 in the limited numbers tested.

Although we did not find significant expression of Hep Par 1 in our 4 SMARCA4-dNSCLCs, Hep Par 1 expression has been described as a common feature of a subset of SMARCA4-dNSCLCs. The relationship of these Hep Par 1–expressing NSCLCs to hepatoid ADCAs that rarely occur is not clear. Agaيمي et al. found that Hep Par 1–expressing SMARCA4-dNSCLCs do not express other hepatocyte markers (α-fetoprotein, arginase, glypican) and are negative for TTF-1, unlike hepatoid ADCAs, which commonly express 2 or more hepatocyte markers and show characteristic cytoplasmic staining for TTF-1. The single hepatoid ADCA included in our cohort showed loss of BRM and BRG1 in human tumor cell lines: differential effects on RB-mediated cell cycle arrest. In: Travis WD, Noguchi M, Yatabe Y. Adenocarcinoma. In: Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, eds. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. 4th ed. Lyon, France: IARC; 2015:26–37.

Singh V, Guleria P, Malik PS, et al. Epidermal growth factor receptor (EGFR), KRAS, and BRAF mutations in lung adenocarcinomas: a study from India. Curr Probl Pathol. 2019;43(5):391–401.

Nambiarjnan A, Parshad R, Goyal A, Mithun NK, Jain D. Immucologic clinical profiling of a SMARCA4-deficient thoracic sarcoma arising in a patient with chronic myeloperoxidase. Pathobiology. 2015;79(5):463–474.

Wong AK, Shanahan F, Chen Y, et al. BRG1, a component of the SWI-SNF complex, is mutated in multiple human tumor cell lines. Cancer Res. 2002;63(18):5924–5931.

Araujo LH, Timmers C, Bell EH, et al. Genomic characterization of non-small cell lung cancer with massively parallel sequencing. J Clin Oncol. 2015;33(15):1690–1698.

Bell EH, Chakraborty AR, Mo F, et al. SMARCA4/BRG1 is a novel prognostic biomarker predictive of cisplatin-based chemotherapy outcomes in resected non-small cell lung cancer. Clin Cancer Res. 2016;22(10):2396–2404.

Gown A, Ussalki C. Pulmonary adenocarcinomas with hepatoid and rhabdoid features: relationship of TTF-1 and SMARCA4/BRG-1 protein. Am J Surg Pathol. 2019;43(10):suppl 1:25. Abstract 1832.

Imielinski M, Berger AH, Hamerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell. 2012;150(6):1107–1120.

Reidkman N, Montecalvo J, Chang JC, et al. SMARCA4-deficient thoracic sarcomatoid tumors represent primarily smoking-related undifferentiated carcinomas rather than primary thoracic sarcomas (published online November 18, 2019). J Thorac Oncol. doi:10.1016/j.jtho.2019.10.023.

Singh V, Guleria P, Malik PS, et al. Epidermal growth factor receptor (EGFR), KRAS, and BRAF mutations in lung adenocarcinomas: a study from India. Curr Probl Pathol. 2019;43(5):391–401.

Nambiarjnan A, Parshad R, Goyal A, Mithun NK, Jain D. Immucologic clinical profiling of a SMARCA4-deficient thoracic sarcoma arising in a patient with chronic myeloperoxidase. Pathobiology. 2015;79(5):463–474.
33. Coira IF, Rufino-Palomares EE, Romero OA, et al. Expression inactivation of SMARCA4 by microRNAs in lung tumors. *Hum Mol Genet*. 2015;24(5):1400–1409.

34. Stanton BZ, Hodges C, Calarco JP, et al. Smarca4 ATPase mutations disrupt direct eviction of PRC1 from chromatin. *Nat Genet*. 2017;49(2):282–288.

35. Orvis T, Hepperla A, Walter V, et al. BRG1/SMARCA4 inactivation promotes non-small cell lung cancer aggressiveness by altering chromatin organization. *Cancer Res*. 2014;74(22):6486–6498.

36. Marquez-Vilendrer SB, Rai SK, Gramling SJ, Lu L, Reisman DN. Loss of the SWI/SNF ATPase subunits BRM and BRG1 drives lung cancer development. *Oncoscience*. 2016;3(11–12):322–336.

37. Strobeck MW, Reisman DN, Gunawardena RW, et al. Compensation of BRG-1 function by Brm: insight into the role of the core SWI-SNF subunits in retinoblastoma tumor suppressor signaling. *J Biol Chem*. 2002;277(7):4782–4789.

38. Hoffman GR, Rahal R, Buston F, et al. Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *Proc Natl Acad Sci U S A*. 2014;111(8):3128–3133.

39. Januario T, Ye X, Bainer R, et al. PRC2-mediated repression of SMARCA2 predicts EZH2 inhibitor activity in SWI/SNF mutant tumors. *Proc Natl Acad Sci U S A*. 2017;114(46):12249–12254.

40. Bultman S, Gebuhr T, Yee D, et al. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol Cell*. 2000;6(6):1287–1293.

41. Sauter JL, Graham RP, Larsen BT, Jenkins SM, Roden AC, Boland JM. SMARCA4-deficient thoracic sarcoma: a distinctive clinicopathological entity with undifferentiated rhabdoid morphology and aggressive behavior. *Mod Pathol*. 2017;30(10):1422–1432.

42. Kuwamoto S, Matushita M, Takeda K, et al. SMARCA4-deficient thoracic sarcoma: report of a case and insights into how to reach the diagnosis using limited samples and resources. *Hum Pathol*. 2017;70:92–97.

43. Yoshikawa Y, Sato A, Tsujimura T, et al. Biallelic germline and somatic mutations in malignant mesothelioma: multiple mutations in transcription regulators including mSWI/SNF genes. *Int J Cancer*. 2015;136(3):560–571.

44. Haninger DM, Kloeker GH, Bousamra M II, Nowacki MR, Slone SP. Hepatoid adenocarcinoma of the lung: report of five cases and review of the literature. *Mod Pathol*. 2014;27(4):535–542.