Clinical and Molecular Validation of BAP1, MTAP, P53, and Merlin Immunohistochemistry in Diagnosis of Pleural Mesothelioma

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Abstract

BAP1 and MTAP immunostains play an important role in diagnosis of mesothelioma, but additional markers are needed to increase sensitivity. We analyzed 84 pleural mesotheliomas (51 epithelioid, 27 biphasic, 6 sarcomatoid) by a hybrid-capture next-generation sequencing (NGS) panel including complete coverage of coding and splicing regions for BAP1, MTAP, NF2, and TP53 and correlated molecular findings with diagnostic immunostains for BAP1, MTAP, Merlin, and p53, respectively. Fifty-seven reactive mesothelial proliferations served as benign comparators. Loss of BAP1, MTAP, and Merlin protein expression were, respectively, 54%, 46%, and 52% sensitive and 100% specific for mesothelioma. Two-marker immunopanels of BAP1 + MTAP, BAP1 + Merlin, and MTAP + Merlin were 79%, 85%, and 71% sensitive for mesothelioma, while a three-marker immunopanel of BAP1 + MTAP + Merlin was 90% sensitive. Diffuse (mutant-pattern) p53 immunostaining was seen in only 6 (7%) tumors but represented the only immunohistochemical abnormality in 2 cases. Null-pattern p53 was not specific for malignancy. An immunopanel of BAP1 + MTAP + Merlin + p53 was 93% sensitive for mesothelioma, and panel NGS detected a pathogenic alteration in BAPI, MTAP, NF2, and/or TP53 in 95%. Together, 83 (99%) of 84 tumors showed a diagnostic alteration by either immunohistochemistry or panel NGS. Adding Merlin to the standard BAP1 + MTAP immunopanel increases sensitivity for mesothelioma without sacrificing specificity. p53 immunohistochemistry and panel NGS with complete coverage of BAP1, CDKN2A/MTAP, TP53, and NF2 may be useful in diagnostically challenging cases.
1. Introduction

Malignant mesothelioma is a rare tumor, with approximately 3000 new cases annually in the United States\(^1\),\(^2\). 85–90% arise in the pleura, with most of the remainder affecting the peritoneum\(^3\)–\(^5\). Pleural mesothelioma carries a poor prognosis, and accurate distinction from benign mesothelial proliferations is paramount. However, this distinction may be challenging, particularly in small biopsy specimens.

Accurate classification of mesothelial proliferations has been significantly aided by recognition of molecular correlates of malignancy, including alterations in BRCA1-associated protein 1 (BAP1)\(^6\)–\(^7\), cyclin-dependent kinase 2A (CDKN2A)\(^8\)–\(^11\), and neurofibromatosis 2 (NF2)\(^12\),\(^13\). Next-generation sequencing (NGS)-based studies have identified BAP1 alterations in 36–57%\(^6\),\(^14\)–\(^19\), CDKN2A alterations in 41–68%\(^6\),\(^14\),\(^15\),\(^17\),\(^18\),\(^20\), and NF2 alterations in 50–75%\(^6\),\(^14\)–\(^18\),\(^21\),\(^22\) of cases. Inactivation of BAP1 and NF2 occurs through a broad spectrum of alterations, including missense, truncating, and splice site mutations (often with concurrent loss of heterozygosity\(^23\)); small insertions and deletions; large intragenic deletions; whole-gene deletion; and structural variants. In contrast, CDKN2A inactivation occurs largely through deletion (with co-deletion of neighboring gene methylthioadenosine phosphorylase (MTAP) in 50–75%\(^17\),\(^20\)), with loss-of-function rearrangements reported in a small subset\(^15\). Additionally, NGS-based studies have identified TP53 inactivation in 5–10% of pleural mesothelioma, via whole-gene deletion and missense, truncating, and splice site mutations with loss of heterozygosity\(^14\),\(^15\),\(^17\)–\(^19\). A subset of mesotheliomas show genomic “near-haploidization,” which may result from chromosomal instability consequent to p53 dysfunction\(^17\).

BAP1 and MTAP immunohistochemical stains are routinely deployed as surrogates for molecular testing. Loss of nuclear BAP1 immunostaining is highly correlated with a broad spectrum of pathogenic BAP1 alterations\(^7\),\(^24\),\(^25\) and is highly specific for mesothelioma\(^26\)–\(^28\). Owing to frequent co-deletion of MTAP with CDKN2A\(^17\),\(^20\),\(^29\),\(^30\), loss of cytoplasmic MTAP immunostaining is 59–84% sensitive for CDKN2A deletion\(^8\),\(^20\),\(^31\)–\(^33\) and highly specific for malignancy in mesothelial lesions\(^8\),\(^34\). An immunopanel of BAP1 and MTAP (i.e., loss of either marker) is 74–90% sensitive for mesotheliomas\(^8\),\(^26\),\(^33\)–\(^35\).

To date, there has been limited study of immunohistochemistry for Merlin (the protein encoded by NF2) and p53 in diagnosis of mesothelioma. Diagnostic fluorescence in situ hybridization (FISH) for hemizygous NF2 deletion is reportedly ~50% sensitive for mesothelioma and 100% specific in the differential with reactive mesothelial hyperplasia\(^12\),\(^36\). Two studies of Merlin immunohistochemistry yielded inauspicious results\(^19\),\(^37\); however, newer commercially available anti-Merlin antibodies warrant further exploration.
Using arbitrary non-biological thresholds (usually 10%) to distinguish “low” versus “high” p53 expression, numerous older studies found no use for p53 immunohistochemistry to distinguish benign and malignant mesothelial proliferations\(^{38}\). However, using empirical thresholds derived from high-grade serous carcinoma\(^{39,40}\), one recent study found that p53 immunohistochemistry corresponds to \(TP53\) mutational status in mesothelial proliferations and has a role in mesothelioma diagnosis\(^{41}\). Those findings warrant confirmation together with robust molecular correlation.

As the number and reliability of diagnostic immunostains have grown, so too has routine application of multigene NGS panels to mesothelioma diagnosis and management, offering the opportunity for orthogonal validation of immunostains. We studied 84 pleural mesotheliomas to 1) correlate immunohistochemical and molecular results for \(BAP1\), \(MTAP\), \(TP53\), and \(NF2\); 2) specifically explore the diagnostic characteristics of Merlin and p53 immunohistochemistry; and 3) re-evaluate current ancillary testing algorithms.

2. Materials and Methods

a. Cohort

This study was approved by the institutional review board at Brigham and Women’s Hospital. The electronic pathology database was searched for mesotheliomas meeting the following inclusion criteria: 1) pleural primary site, 2) resection (decortication or extrapleural pneumonectomy) specimen, 3) analyzed by the OncoPanel next-generation sequencing assay as part of a consented protocol sponsored jointly by Brigham and Women’s Hospital and Dana Farber Cancer Institute (PROFILE), 4) hematoxylin and eosin (H&E)-stained slides available, and 5) tissue blocks available in institutional archive. Localized mesothelioma and tumors reviewed only for pathological diagnostic consultation were excluded. A separate database search was carried out for reactive mesothelial proliferations with tissue blocks in institutional archives and at least one year of clinical follow-up.

b. Clinicopathologic parameters

Patient sex and age at diagnosis were extracted from the electronic medical record. Clinical and radiology reports were reviewed to confirm pleural primary site. Three representative H&E-stained slides (including, where possible, a slide from the sequenced block) were reviewed from each case to determine histotype (epithelioid, biphasic, sarcomatoid). For epithelioid tumors, predominant architecture\(^{42}\) was determined. Biphasic tumors were subclassified as epithelioid- or sarcomatoid-predominant.

c. Immunohistochemistry

All tumors and reactive mesothelial proliferations were immunostained for BAP1 (Santa Cruz, clone C-4, 1:80), MTAP (Santa Cruz, clone 42-T, 1:75), p53 (Dako, clone DO-7, 1:500), and Merlin (Cell Signaling Technology, clone D1D8, 1:250). Pressure cooker antigen retrieval in Target Retrieval Solution (pH 6.1 citrate buffer; Dako) was used for all antibodies. EnVision+ detection system (Dako) was used for BAP1, MTAP, and p53;
Novolink (Leica) was used for Merlin. Positive controls included epithelioid mesothelioma (BAP1, MTAP, and Merlin) and serous carcinoma (p53).

Immunostains were performed on freshly cut 5-micron sections of formalin-fixed paraffin-embedded tissue. When possible, immunostains were performed on the sequenced tumor block (n=45); when necessary, another tumor block from the sequenced specimen was used (n=31, principally cases with sequencing on fresh frozen tissue), or tumor from a different surgical specimen (n=8, principally cancer center transfer cases). Where possible (n=25), both the epithelioid and sarcomatoid components of biphasic tumors were immunostained, with staining profile documented for each component.

BAP1 was scored as retained (positive tumor nuclear staining) or lost (negative tumor nuclear staining with a positive internal control). Staining patterns in tumors with BAP1 loss were subclassified as “negative” (no nuclear or cytoplasmic staining) or “cytoplasmic-only.”

MTAP was scored as retained (positive tumor cytoplasmic staining) or lost (negative tumor cytoplasmic staining with a positive internal control). Percent tumor cells with cytoplasmic staining was documented to identify tumors with “heterogeneous” MTAP expression. Any spatially discrete MTAP-negative tumor cell population was regarded as evidence for clonal MTAP deletion and classified as MTAP loss.

For p53, percent tumor cells with nuclear staining and staining intensity (1+, weak; 2+, moderate or heterogeneous; 3+, strong) were documented. The following prospectively set thresholds were used: “diffuse” = ≥80% tumor nuclear positivity with 2+ or 3+ intensity; “wildtype” = >0 % but <80% nuclear staining; “null” = no identifiable nuclear staining alongside an intact positive internal control.

Merlin was scored as retained (positive tumor cell staining, irrespective of distribution or intensity) or lost (negative tumor cell staining alongside a positive internal control). To further assess molecular correlates of specific staining patterns, we also noted 1) staining intensity (classified as “weak” if evident only under 20x or 40x objective), 2) staining distribution (membranous versus cytoplasmic), and, when applicable, 3) pattern of membranous staining (linear versus granular/discontinuous).

d. Sequencing

All tumors were analyzed by tumor-only hybrid-capture NGS on the 298-gene (n=22) or 447-gene (n=62) OncoPanel platform. Briefly, samples were required to contain at least 20% tumor by pathologist’s visual estimate. DNA was extracted and subjected to library preparation as previously described. Sequencing was performed on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA). Mutect and GATK (Broad Institute, Cambridge, MA) were used for detection of single nucleotide and insertion-deletion variants. Variants were filtered to remove technical artifacts, synonymous variants, and variants at >0.1% frequency in the Genome Aggregation Database (https://gnomad.broadinstitute.org/). Copy number alterations were determined using an internally developed tool (RobustCNV). Structural variants were detected using BreakMer. Sequencing data from this study is publicly available through the AACR Genie database.
Pass-filter variants were subclassified as missense, truncating (nonsense, frameshift), or splice site and assessed for likely pathogenicity by a molecular pathologist (LMS). Genomic deletions were classified as homozygous (log2 ratio of target copy coverage:diploid normal approximating −2); shallow deletions were not further specified in light of challenges in inferring degree of copy loss in samples with tumor content <30% or genomic heterogeneity. For loss of heterozygosity analysis, population variants detected on the panel were plotted according to their genomic coordinates and variant allele fraction. Regions of the genome showing deviation of heterozygous variant allele fractions away from 0.5 in the absence of concomitant numeric copy change were considered to have copy neutral loss of heterozygosity. Tumors were deemed to show “near-genome-wide loss of heterozygosity” when loss of heterozygosity was detected in at least 17 of 22 chromosomes (X chromosome excluded). Due to limitations in our bioinformatic analysis, we did not distinguish near-genome-wide loss of heterozygosity with versus without subsequent endoreduplication, but this distinction is of uncertain biologic significance, and prior work has grouped both cases as a singular molecular class. Further, the term “near-genome-wide loss of heterozygosity” is used in this manuscript in lieu of “near-haploidization” (as previously termed by others) to acknowledge that, due to methodological differences, the tumors in our cohort with near-genome-wide loss of heterozygosity may, individually or in aggregate, differ subtly from previously described mesotheliomas with near-haploidization, as perhaps reflected by their relatively high prevalence in our study.

**e. Statistical analyses**

Summary statistics were tabulated in Excel (Microsoft, Redmond, WA). Contingency table (sensitivity, specificity) analyses were performed manually. Between-group differences in categorical and continuously distributed variables were assessed by Pearson’s chi-squared and Mann-Whitney U tests, respectively (SAS 9.4, SAS Institute, Cary, NC, USA). Tests were two-tailed with $\alpha=0.05$ for statistical significance. $P$ values for multiple comparisons were corrected by Holm’s method.

As addressed in the Discussion, neither immunohistochemical nor panel NGS can be definitively claimed as a diagnostic gold standard when comparing results for BAP1, MTAP, TP53, and NF2; accordingly, Cohen’s kappa is presented for these comparisons to quantify interassay agreement. Using the standards of Landis and Koch, 0.0–0.2 is classified as slight, 0.21–0.4 as fair, 0.41–0.6 as moderate, 0.61–0.8 as substantial, and 0.81–1.0 as near-perfect agreement. Because MTAP immunohistochemistry is regarded as a surrogate for CDKN2A deletion, sensitivity and specificity are reported for this comparison.

**3. Results**

The study cohort comprised 84 pleural mesotheliomas (51 epithelioid, 27 biphasic, 6 sarcomatoid) diagnosed between December 2012 and August 2020. Fifty-seven pleural reactive mesothelial proliferations (51 predominantly epithelioid morphology, 6 predominantly spindled morphology) diagnosed between 2013 and 2019 (median follow-up, 60 months) served as benign comparators. Clinical and morphologic characteristics are summarized in Supplemental Table 1.
a. Molecular Findings

Molecular findings are summarized in Figure 1. Of 84 tumors, 4 (5%) harbored pathogenic alterations in all 4 genes of interest, 27 (32%) in 3 genes, 39 (46%) in 2 genes, and 10 (12%) in 1 gene. Four tumors had no pathogenic alteration detected, including 2 with a revised estimate of <10% and 2 with 10–20% tumor cellularity following sequencing.

BAP1 pathogenic alterations were identified in 47 (56%) of 84 tumors. CDKN2A and MTAP alterations were identified in 58 (69%) and 48 (57%), respectively; 10 (17%) of 58 tumors with CDKN2A deletion had no MTAP deletion, but no tumors harbored MTAP deletion without CDKN2A deletion. Putative TP53 alterations were identified in 23 (27%) tumors, of which 12 had mutations or subgenic deletions (including 5 with probable biallelic inactivation) and 11 showed only shallow deletion of all (n=8) or part (n=3) of the short (p) arm of chromosome 17. NF2 alterations were detected in 57 (68%) tumors, of which 13 showed probable biallelic inactivation. CDKN2A and MTAP alterations were significantly more common in non-epithelioid (biphasic and sarcomatoid) than in epithelioid tumors (P=0.0002 & 0.02, respectively) (Supplemental Table 2). Prevalence of BAP1, TP53, and NF2 alterations did not differ significantly between histotypes.

Twelve (14%) of 84 tumors showed near-genome-wide loss of heterozygosity (see Figure 1; Supplemental Figure 1). Tumors with near-genome-wide loss of heterozygosity were significantly enriched in wildtype BAP1 (P=0.003), CDKN2A deletion (P=0.01), and TP53 point mutations (P=0.007) but showed no association with NF2 alteration (P=0.22).

b. Sensitivity, Specificity, and Staining Characteristics of Diagnostic Immunomarkers

Immunohistochemical results are summarized in Figure 1. Of 84 tumors, 1 showed abnormal expression of all 4 markers, 7 (8%) showed abnormal expression of 3 markers, 39 (46%) abnormal expression of 2 markers, 31 (37%) abnormal expression of 1 marker, and 6 (7%) had no abnormal immunomarkers. MTAP and Merlin were lost significantly more often in non-epithelioid compared to epithelioid tumors (P=0.01 for both comparisons). There was no difference in BAP1 or p53 staining by histologic subtype (Supplemental Table 2).

1) BAP1 and MTAP—Aberrant (negative or cytoplasmic-only) BAP1 immunostaining was observed in 45 (54%) tumors (Figure 2A–2D). BAP1 staining was retained in 56 (98%) of 57 reactive mesothelial proliferations. The sole apparently reactive proliferation with BAP1 loss showed mild cytologic atypia but no invasion (Supplemental Figure 2). (This case meets criteria for mesothelioma in situ[6]; however, the patient died of esophageal adenocarcinoma after 9 months follow-up, precluding further characterization.)

MTAP was lost in 39 (46%) (Figure 2E–2H), of which 5 showed heterogeneous staining, including three biphasic cases with predominant loss in the sarcomatoid component (Supplemental Table 3). All 57 reactive mesothelial proliferations showed retained MTAP.

2) p53—p53 immunostaining was diffuse in 6 (7%), wildtype in 66, and null in 12 (14%) tumors (Figure 3). All 57 reactive mesothelial proliferations showed wildtype (n=43) or null (n=14) p53 immunostaining. The prevalence of null-pattern p53 immunostaining did not differ between mesothelioma and reactive mesothelial proliferations (P=0.18). On the basis
of this finding, null-pattern p53 staining in mesothelioma was classified as a non-aberrant pattern, alongside wildtype staining.

3) **Merlin**—All 57 reactive mesothelial proliferations showed retained immunohistochemical expression of Merlin, including 35 with strong linear membranous staining, 19 with weak granular membranous staining, and 3 with cytoplasmic-only staining. In contrast, Merlin was lost in 44 (52%) mesotheliomas (Figure 4). Among 40 malignant tumors with retained Merlin, 15 showed strong linear membranous staining, 7 showed weak linear membranous staining, 12 showed granular membranous staining, and 6 showed cytoplasmic-only staining (Supplemental Figure 3). The intensity, distribution, and pattern of Merlin immunostaining did not differ between mesothelioma and reactive mesothelial proliferations. All sarcomatoid tumor populations (i.e., sarcomatoid tumors and sarcomatoid components of biphasic tumors) with retained Merlin (n=9) showed cytoplasmic-only staining. Among 28 epithelioid tumors with membranous Merlin staining, distribution was apico-lateral in 16 and circumferential in 12. Apico-lateral staining was associated with tubulopapillary architecture, whereas circumferential staining was associated with solid and trabecular architecture (Supplemental Table 4). Two tumors showed heterogeneous Merlin immunostaining (see below).

c. **Immunostaining Patterns in Biphasic Tumors**

Both the epithelioid and sarcomatoid components were present for immunohistochemical analysis in 25 of 27 biphasic tumors (Supplemental Table 5). BAP1 immunostaining was concordant in all cases reviewed. MTAP immunostaining was discordant (retained in epithelioid, lost in sarcomatoid component) in 4 (see Figure 2G, 2H). On Merlin immunostaining, 17 tumors showed concordant loss in both components, 5 showed concordant retention, and 2 showed loss in the sarcomatoid component only.

d. **Molecular-Immunohistochemical Correlates**

None of the studied markers showed a significant difference in molecular-immunohistochemical concordance by histotype (see Supplemental Table 2).

i. **BAP1**—BAP1 concordance statistics and specific molecular-immunohistochemical correlates are listed in Supplemental Table 6. Overall, BAP1 molecular and immunohistochemical results were concordant in 72 (86%) of 84 tumors (κ=0.71). Of 9 tumors with shallow *BAP1* deletion only, only 3 lost BAP1 protein expression. In contrast, loss of BAP1 expression was observed in 37 (97%) of 38 tumors with two-copy deletion, structural variant, and/or point mutation. (The single case (#6) with intact protein expression despite presence of a *BAP1* mutation showed a C-terminus frameshift mutation predicted to read through into the 3’ untranslated region.) Conversely, in 5 (11%) of 45 tumors with protein loss, no definite deleterious alteration was detected by molecular profiling. No significant correlation was observed between type or location of *BAP1* alteration and presence of cytoplasmic staining (see Supplemental Table 6).

ii. **CDKN2A and MTAP**—Concordance, sensitivity, and specificity statistics for MTAP immunohistochemistry and *CDKN2A & MTAP* deletion are in Supplemental Table 7.
Overall, MTAP immunohistochemistry was concordant with CDKN2A deletion status in 65 (77%) and with MTAP in 73 (87%; $\kappa=0.74$). All 39 tumors with MTAP protein loss harbored CDKN2A deletion, whereas MTAP protein expression was retained in 19 (33%) of 58 CDKN2A-deleted tumors. Of 28 tumors with shallow MTAP deletion, only 18 (64%) showed loss of MTAP protein expression, whereas protein expression was lost in all 20 (100%) tumors with two-copy MTAP deletion. One (3%) of 39 tumors with MTAP protein loss showed no MTAP deletion.

iii. P53—TP53 molecular and p53 immunohistochemical concordance is reported in Table 1 (see also Supplemental Figure 4). Overall, p53 immunohistochemistry was concordant with TP53 mutational status in 67 (80%) of 84 tumors. Of 11 tumors with shallow arm-level or sub-arm-level chromosome 17p deletion, 4 showed null-pattern and 7 showed wildtype p53 immunostaining. Of 12 tumors with point mutations or subgenic TP53 deletion, 6 showed diffuse, 5 wildtype, and 1 null-pattern p53 immunostaining. All 6 tumors with diffuse p53 expression harbored a TP53 point mutation, whereas only 5 (42%) of 12 tumors with null-pattern p53 showed any TP53 molecular alteration. Tumors with null-pattern versus wildtype p53 immunostaining did not differ in the rate of TP53 truncating mutations, deletions, or molecular alterations overall. On this basis of this immunohistochemical–molecular correlation and comparison with p53 immunostaining in reactive control samples (detailed above), null-pattern immunostaining was classified as a non-aberrant pattern alongside wildtype staining.

iv. NF2 / Merlin—NF2/Merlin concordance statistics and specific molecular-immunohistochemical correlates are listed in Table 2 (see also Supplemental Figure 5). Overall, NF2 molecular results were concordant with Merlin immunohistochemistry in 65 (77%) of 84 tumors ($\kappa=0.54$). Of 31 tumors with shallow deletion as the sole NF2 alteration, 15 (48%) retained Merlin expression, whereas Merlin expression was lost in 25 (96%) of 26 tumors with a two-copy deletion, structural variant, and/or point mutation. Three tumors showed loss of Merlin expression but no NF2 mutation. Tumors with weak, granular, or cytoplasmic (“aberrant”) Merlin immunostaining had a significantly higher rate of NF2 molecular alterations (predominantly shallow deletions), compared with tumors showing strong linear membranous staining ($P=0.005$; Supplemental Table 8). Two tumors showed heterogeneous Merlin immunostaining. One (#24) had a morphologically distinct component (<5% of sampled tumor) with Merlin loss, whereas the remainder showed aberrant weak Merlin staining. Panel NGS detected a shallow NF2 deletion. The second (#8) showed Merlin loss in morphologically distinct solid tumor nests (~10% of sampled tumor), whereas the predominant adenomatoid-pattern tumor showed non-aberrant Merlin (Supplemental Figure 6). Panel NGS detected no NF2 alteration.

e. Effect of tumor cellularity and tissue availability on molecular-immunohistochemical concordance

Based on variant allele fraction and step copy number alterations, median sequenced tumor cellularity in 8 cases showing at least 1 abnormal immunostaining result with no corresponding molecular abnormality was 17.5%, compared to 35% for all other tumors.
(P=0.03), with 7 of 8 tumors having sequenced tumor cellularity <20%. The eighth tumor (#54) showed near-genome-wide loss of heterozygosity.

Rates of immunohistochemical-molecular discordance did not differ between tumors with testing performed on the same block, different block from the same surgical specimen, or different surgical specimen (Supplemental Table 9).

f. Comparison of diagnostic algorithms

Sensitivities and comparative P values for immunomarkers and immunopanels are in Table 3. In summary, BAP1 is significantly more sensitive than MTAP for diagnosis of epithelioid (P=0.02) but not biphasic (P=0.58) mesothelioma. Merlin did not differ significantly from BAP1 or MTAP for diagnosis of epithelioid or biphasic mesothelioma. P53 is the least sensitive immunomarker for diagnosis of epithelioid and biphasic mesothelioma, though p53 was the only aberrant immunostain in 2 (4%) of 51 epithelioid mesotheliomas (see Figure 2E–2H). Among two-marker panels, there was no significant difference in sensitivity between BAP1 + MTAP, BAP1 + Merlin, and MTAP + Merlin for diagnosis of epithelioid or biphasic mesothelioma. Two-marker panels including p53 were of consistently inferior sensitivity. Adding Merlin to a panel of BAP1 + MTAP increased sensitivity from 75% to 88% for diagnosis of epithelioid mesothelioma and from 85% to 96% for biphasic mesothelioma, equating to an increase in sensitivity from 79% to 90% for all mesotheliomas (P=0.03). Seventy-eight (93%) of 84 tumors showed abnormal staining for at least 1 of the 4 immunomarkers under study.

Across all histotypes, panel NGS alone detected a pathogenic alteration in BAP1, CDKN2A, TP53, and/or NF2 in 80 (95%) of 84 tumors, including 48 (94%) of 51 epithelioid and 26 (96%) of 27 biphasic tumors. Of 4 tumors lacking one of these molecular alterations, none harbored other diagnostically significant alterations. Panel NGS sensitivity was 100% (64/64) for tumors with ≥20% estimated sequenced tumor cellularity, compared to 80% (16/20) for tumors with <20% estimated sequenced tumor cellularity. Sensitivity of panel NGS (95%) was significantly greater than immunopanels comprising BAP1 + MTAP (79%; P=0.002) and BAP1 + Merlin (83%; P=0.01) but did not differ significantly from a panel of BAP1 + MTAP + Merlin (90%; P=0.23). In combination, panel NGS and the four-marker immunopanel identified diagnostic alterations in all but one tumor (#12), a grade II epithelioid mesothelioma with tubulopapillary architecture, diagnosed in a 30-year-old woman, with sequenced tumor cellularity estimated at <10%.

4. Discussion

This study represents the first large correlation of diagnostic immunohistochemistry with clinical panel NGS in pleural mesothelioma, with four principal conclusions:

1. Immunohistochemical loss of BAP1, MTAP, or Merlin is highly specific for malignancy in mesothelial lesions, including in cases where panel NGS does not reveal a corresponding molecular alteration.
2. Adding Merlin significantly improves the performance of the standard BAP1 + MTAP immunopanel, and routine application of this three-marker panel may be recommended following independent validation.

3. Diffuse p53 immunostaining is specific for TP53 mutation and for malignancy, but we did not identify a molecularly specific “null-mutant” p53 immunophenotype among mesothelial lesions. Diffuse p53 represents the only immunohistochemical abnormality in a small subset of mesotheliomas.

4. Immunohistochemistry and panel NGS are both highly sensitive for mesothelioma and show extensive but incomplete overlap in diagnostic detection. Although immunohistochemistry remains the first-line ancillary assay for mesothelioma diagnosis, molecular testing (including panel NGS) may be required in select cases.

Despite high concordance overall, immunohistochemistry and panel NGS each detect diagnostic alterations that the other does not. In this study, immunohistochemical loss of BAP1, MTAP, or Merlin was noted in 3 of 4 tumors with no molecular alteration. Such instances are principally due to low sequenced tumor cellularity, wherein significant alterations fall below the detection threshold of panel NGS. Alternately, this discordance may be secondary to miRNA regulation or to molecular alterations (including loss-of-function rearrangements, copy-neutral gene fusions, and deep-intronic splicing alterations) that abrogate protein expression but evade detection by clinical panel NGS.

Conversely, panel NGS identified pathogenic alterations in 5 of 6 mesotheliomas with no abnormal immunostains. Most such discordances stem from poor correlation between immunohistochemistry and shallow gene deletion, noted across all genes under study. The cause of this poor correlation is unclear but could include haplosufficiency on the one hand or, on the other, undetected genetic or epigenetic alterations on the non-deleted allele or undetected genetic or epigenetic alterations on the non-deleted allele or underappreciated two-copy deletions in tumors with low sequenced cellularity. Alternately, some such discordances may result from pathogenic mutations sparing the immunohistochemical epitope.

a. BAP1

In keeping with published data, we identified a broad spectrum of mutational mechanisms for BAP1 inactivation. Interestingly, 1 distal truncating event (p.N690Gfs*36) was undetected by immunohistochemistry. This specific frameshift extends the reading frame into the 3' untranslated region, which may result in escape from nonsense-mediated decay. We observed a non-significant trend toward cytoplasmic-only BAP1 staining in tumors with truncating BAP1 mutations, which eliminate both nuclear localization signals (located at amino acids 656–661 and 717–722, or 729 total) and shallow deletion, consistent with prior reports, though two other cases with catalytic domain missense mutations had negative staining.

Our data indicate strong correlation between BAP1 immunostaining in the epithelioid and sarcomatoid components of biphasic tumors. Previous data on this topic are

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conflicting\textsuperscript{24,52–54}, though rigorous molecular\textsuperscript{27,52} and survival\textsuperscript{52} data indicate that supposed biphasic mesotheliomas with BAP1 loss confined to the epithelioid component most likely represent epithelioid mesothelioma with a reactive spindle component.

b. CDKN2A and MTAP

Our data confirm growing evidence that MTAP immunohistochemistry is a reliable surrogate for CDKN2A deletion in diagnosis of mesothelioma\textsuperscript{8,31–33} with 100\% specificity in this NGS-based cohort in line with the 96–100\% specificity reported in FISH-based studies\textsuperscript{8,31,33}. Our data further support evidence that MTAP immunohistochemical loss is highly specific for malignancy in mesothelial lesions, including in lesions with no corresponding MTAP deletion detected\textsuperscript{34}. Previous studies have generally set a threshold of at least 50\% tumor cell MTAP loss for a diagnosis of malignancy\textsuperscript{8,12,26}. However, our experience in this work and prior studies\textsuperscript{20,31} indicates that this 50\% cutoff is overly conservative, and we regard any discrete population of MTAP-deficient tumor cells as a clonal molecular alteration supporting diagnosis of malignancy.

To our knowledge, this is the first study to compare MTAP immunostaining in the epithelioid and sarcomatoid components of biphasic mesothelioma. Although the two components were concordant in 21/25, 4 tumors showed MTAP loss confined to the sarcomatoid component, including 3 with focal (heterogeneous) MTAP loss in the epithelioid component. These observations suggest that CDKN2A deletion may be associated with transition to sarcomatoid morphology in a subset of biphasic tumors. This pattern has not been observed in FISH-based studies\textsuperscript{52,54}, possibly due to enumeration of few cells relative to immunohistochemistry. Diagnosis of biphasic mesothelioma should be regarded with skepticism if MTAP is lost in the epithelioid but retained in the sarcomatoid population\textsuperscript{52,54}.

c. TP53

Using criteria refined in high-grade serous carcinoma\textsuperscript{39,40}, we found diffuse “mutant-pattern” p53 in 7\% of mesotheliomas. Available evidence does not suggest a histotype-specific predilection for diffuse p53\textsuperscript{15,16,41}. Prior work suggests an increased prevalence of TP53 mutations among mesothelioma with genomic near-haploidization\textsuperscript{17}. We similarly found an enrichment of TP53 mutations in tumors with near-genome-wide loss of heterozygosity. Although our sequencing strategy cannot definitively distinguish true genomic near-haploidization (i.e., tumors with 24–30 chromosomes) from genomic near-haploidization followed by endoreduplication of the remaining chromosomes, Hmeljak, et al.,\textsuperscript{17} grouped both of these molecular profiles under the umbrella of “genomic near-haploidization,” which they regarded as a distinct molecular subtype of mesothelioma. The 14\% prevalence of near-genome-wide loss of heterozygosity seen in our cohort substantially exceeds the 3\% rate of near-haploidization reported by Hmeljak, et al. The clinical significance of such extensive loss of heterozygosity in mesothelioma remains unclear.

Diffuse p53 immunostaining was 100\% specific for underlying TP53 mutation and for malignancy, supporting the recent finding that diffuse p53 immunostaining may aid diagnosis in rare challenging mesothelial proliferations\textsuperscript{41}. No wildtype tumor in our cohort
showed more than 50% tumor cell staining, whereas no diffuse-pattern tumor showed <90% tumor cell staining – a quantum difference that, together with broad familiarity among practicing surgical pathologists, suggests that p53 immunostaining can be easily implemented in practice.

We did not identify a reproducible “null-mutant” p53 immunoprofile in mesothelial lesions. We observed null-pattern p53 immunostaining among reactive mesothelial proliferations and mesotheliomas alike, and we identified no difference in the rate of all mutations, truncating mutations, or deletions between mesotheliomas with wildtype versus null-pattern staining, suggesting that null p53 immunostaining is a non-specific finding in this context. This finding contrasts with a recent study which employed similar immunohistochemical methods but examined a smaller cohort with more limited molecular analysis. Further immunohistochemical-molecular correlation is necessary to resolve this discrepancy on null-pattern p53 staining in mesothelial proliferations. Until additional data are available, we advocate that null-pattern p53 staining not be regarded as evidence of underlying TP53 mutation in this context.

d. NF2

Our data indicate that Merlin immunohistochemistry could be a useful adjunct to BAP1 and MTAP immunohistochemistry in routine mesothelioma diagnosis. Merlin expression was lost in 52% of all tumors and 70% of non-epithelioid tumors; adding Merlin to an immunopanel of BAP1 + MTAP increased sensitivity across histotypes from 79% to 90%. Earlier reports found Merlin loss in just 4–8% of tumors, but potentially non-specific cytoplasmic staining in one study and absence of detailed immunophenotypic descriptions or figures in the second limit confidence in those results. Those earlier studies also employed different antibodies than the present study.

Merlin loss was seen only in malignant but not in reactive mesothelial lesions. Given poor immunohistochemical correlation with shallow NF2 deletion, we sought to define a subgroup with retained but aberrant (i.e., weak, granular, and/or cytoplasmic-only) Merlin immunostaining. However, the prevalence of aberrant Merlin immunostaining did not differ significantly between mesothelioma and reactive mesothelial proliferations (which consistently lack pathogenic NF2 alterations), and published literature indicates that granular membranous and cytoplasmic Merlin staining may be the norm in meningioma. These findings indicate that diagnostic emphasis should be placed on complete loss of Merlin immunoexpression. Validation of Merlin immunohistochemistry in independent studies is necessary for adoption in routine mesothelioma diagnosis.

Because NF2 alterations appear specific for mesothelioma in the differential with other common malignancies, Merlin immunohistochemistry (like BAP1) could be used to distinguish mesothelioma from both benign mesothelial proliferations and other malignancies, particularly lung and ovarian cancers. This exciting prospect warrants further validation.
e. Comparative sensitivities of immunomarkers and immunopanels

Under current guidelines, the diagnostic workup of a mesothelial proliferation begins with BAP1 and MTAP immunohistochemistry, with reported 67–89% combined sensitivity.\cite{8,12,26,34,35,65–67} In our study, BAP1 + Merlin was more sensitive than BAP1 + MTAP for both epithelioid and biphasic mesotheliomas, but these differences were not statistically significant. However, adding Merlin to a panel of BAP1 + MTAP significantly increased sensitivity among all histotypes (79% vs 90%), in keeping with prior studies with \textit{NF2} FISH.\cite{12,36} This may support routine application of this three-marker immunopanel, pending independent validation of Merlin immunohistochemistry for this use.

Routine upfront use of p53 immunohistochemistry may be low-yield, given the low (~5–10%) rate of diffuse mutant-pattern staining in mesothelioma. However, our cohort included two tumors in which diffuse p53 was the only immunohistochemical abnormality; p53 immunohistochemistry may therefore be a useful diagnostic tool for select mesothelial lesions suspicious for malignancy but with retained BAP1 and MTAP (and, where in use, Merlin).\cite{41}

In this cohort, panel NGS identified a pathogenic variant in \textit{BAP1, CDKN2A/MTAP, NF2,} and/or \textit{TP53} in 80 (95%) of 84 of cases. This is impressive sensitivity for a single assay; however, 1) this is not significantly greater than the 90% sensitivity achieved by a panel of BAP1, MTAP, and Merlin immunostains; 2) this figure includes shallow deletions, which should be interpreted with caution, particularly in specimens with low sequenced tumor cellularity; and 3) sequencing in this cohort was performed on resections, and it is unclear whether panel NGS would perform as well in biopsy samples, where the ability to profile potentially scant cell populations remains a strength of immunohistochemistry. Indeed, all 4 tumors with no pathogenic molecular alteration had <20% estimated sequenced tumor cellularity -- 3 of these showed a diagnostic immunohistochemical abnormality (indicating a probable false-negative molecular result), and none of these harbored a detectable molecular alteration in other mesothelioma-associated genes (e.g., \textit{PTCH1, SETD2}). \textit{(LATS2}, another gene of potential diagnostic interest, is not included in our panel, but published literature suggests \textit{LATS2} is mutated in ~10% of pleural mesotheliomas, though seldom independent of the genes examined in this study\cite{16,17,68,69}).

This study has limitations. First, we studied only pleural mesotheliomas, and extrapolation to the peritoneum or other sites should be approached with great caution. Second, our cohort included only 6 sarcomatoid mesotheliomas, limiting our ability to draw conclusions about this rare histotype. Third, our study did not include FISH or other cytogenetic data, which plays a practical role in mesothelioma diagnosis. Our recommendations should not be interpreted as removing FISH assays from the mesothelioma diagnostic workup, when appropriate. Fourth, immunostains and molecular studies were performed on different surgical specimens in 8 cases and different tumor blocks from the same surgical specimen in 31 cases. Spatial and temporal mutational heterogeneity may therefore account for a subset of discrepant results, though this effect was not statistically significant, and available data suggest that significant heterogeneity is rare in the genes under study\cite{16,55}, with an occasional exception for \textit{NF2} point mutations.\cite{55} Finally, given certain discrepancies between published literature and our findings on p53 and Merlin immunohistochemistry for
mesothelioma diagnosis, additional independent studies of these immunomarkers would be prudent prior to adoption into routine diagnostic practice.

In summary, our data emphasize the complementarity of immunohistochemical and molecular assays in classification of mesothelial lesions. We support the published literature on the molecular-immunohistochemical correlation for BAP1 and CDKN2A/MTAP and have validated p53 and Merlin immunostains as novel markers of mesothelial malignancy. Our data suggest that adding Merlin to the standard BAP1 + MTAP immunopanel could significantly increase diagnostic sensitivity. P53 immunohistochemistry, cytogenetic studies, and molecular sequencing are appropriate for worrisome mesothelial lesions with retained BAP1 and MTAP (and, when/where available, Merlin) immunostaining. These considerations are reflected in a proposed updated diagnostic algorithm (Figure 5). Additional studies are warranted to 1) validate our findings on Merlin immunohistochemistry, 2) explore the diagnostic utility of p53 and Merlin immunohistochemistry in cytology specimens (where NF2 FISH has proved fruitful), 3) assess the specificity of Merlin loss for mesothelioma in the differential with other malignancies, and 4) explore a potential role of BAP1, MTAP, and Merlin immunostains as predictive biomarkers for targeted therapies.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Conflicts of Interest:**

Dr. Chapel’s work is supported by the Ovarian Cancer Research Alliance [Ann Schreiber Mentored Investigator Award; grant number 650320]. Dr. Sholl reports consulting income to her institution from Genentech and Lilly, and research funding from Genentech. Dr. Hornick reports consulting income from Aadi Bioscience and TRACON Pharmaceuticals. Dr. Bueno reports no direct conflicts, but reports current support for other research activities, including grants from the National Institute of Biomedical Imaging and Bioengineering (grant number EB025964-02) and the National Heart, Lung, and Blood Institute, as well as participation in industry grants to Brigham and Women’s Hospital from Merck, Roche, Genentech, Varastem, Gristone, Siemens, Bicycle Therapeutics, Epizime, and Bayer and philanthropic funding to Brigham and Women’s Hospital from the International Mesothelioma Program. Dr. Bueno also reports equity interest in Navigation Sciences and holds patents through the Brigham and Women’s Hospital license to Navigation Sciences. J. Barlow reports no conflicts.

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**Data Availability Statement:**

Sequencing data from this study is publicly available through the AACR Genie database. Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
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Figure 1.
Summary of immunohistochemical and molecular findings. BMM, biphasic malignant mesothelioma; C, cytoplasmic-only immunostaining; EMM, epithelioid malignant mesothelioma; Hist, histotype; Indel, intragenic insertion or deletion; Mis, missense mutation; mut, strong diffuse mutant p53 pattern; N, negative BAP1 immunostaining; LOH, near-genome-wide loss of heterozygosity; n.r., BAP1 reported lost in diagnostic report, slide not re-reviewed; SMM, sarcomatoid malignant mesothelioma; Splice, splice site mutation; Trunc, truncating mutation.
Figure 2. BAP1 and MTAP. Epithelioid malignant mesothelioma (A, 400x) with deciduoid features, showing patchy but strong granular cytoplasmic BAP1 immunostaining (B, 400x). This tumor (#20) harbored a truncating BAP1 p.R666Efs*26 mutation. Epithelioid malignant mesothelioma (C, 400x), showing negative nuclear but prominent granular cytoplasmic BAP1 immunostaining (D, 400x). This tumor (#26) harbored single-copy BAP1 deletion and BAP1-NSUN3 translocation involving BAP1 exon 3. Sarcomatoid component of a biphasic malignant mesothelioma (E, 400x) with lymphohistiocytoid features. Despite strong staining of background inflammatory cells, the spindled tumor cells are negative for both cytoplasmic and nuclear MTAP immunostaining (F, 400x), imparting a “tiger-stripe” staining pattern overall. This tumor (#70) harbored no MTAP or CDKN2A alterations on panel NGS, attributed to low (<20%) tumor cellularity due to dense inflammatory infiltrate. Biphasic malignant mesothelioma (G, 200x) with distinct sarcomatoid (lower left) and
epithelioid (right) components, both of which are negative for cytoplasmic and nuclear MTAP immunostaining (H, 200x). This tumor (#77) harbored two-copy deletion of both MTAP and CDKN2A.
Figure 3.
p53. Epithelioid malignant mesothelioma (A, 100x) with micropapillary architecture and strong diffuse mutant-pattern p53 immunostaining (B, 100x). This tumor (#4) harbored a \textit{TP53} single-copy deletion and a small indel (p.N239_S241delinsT). Epithelioid malignant mesothelioma (C, 100x) with tubulopapillary architecture and strong diffuse mutant-pattern p53 immunostaining (D, 100x). This tumor (#24) harbored a \textit{TP53} single-copy deletion and a missense mutation (p.V173M). Epithelioid malignant mesothelioma (E, 100x) with tubulopapillary architecture, strong diffuse mutant-pattern p53 (F, 100x), retained nuclear BAP1 (G, 100x), retained cytoplasmic + nuclear MTAP (H, 100x), and retained apico-lateral membranous NF2 immunostaining (I, 400x). This tumor (#51) harbored a \textit{TP53} single-copy deletion and splice site mutation (c.376-1G>A). \textit{BAP1}, \textit{MTAP}, and \textit{CDKN2A} were unaltered, and \textit{NF2} harbored a single-copy deletion.
NF2. Epithelioid mesothelioma (A, 400x) with tubulopapillary architecture, showing strong apico-lateral NF2 immunostaining (B, 400x). This case (#47) showed no NF2 alteration. Epithelioid mesothelioma (C, 400x) with high-grade nuclei and rhabdoid features, showing variably weak to strong circumferential membranous and faint cytoplasmic NF2 immunostaining (D, 400x). This case (#23) showed a probable single-copy NF2 deletion. Epithelioid mesothelioma (E, 400x), showing negative (lost) NF2 immunostaining (F, 400x). This case (#19) harbored a truncating NF2 p.E460Kfs*25 mutation. Epithelioid mesothelioma (G, 400x) with high-grade nuclei, showing negative (lost) NF2 immunostaining (H, 400x). This case (#21) showed a probable single-copy NF2 deletion. Sarcomatoid mesothelioma (I, 200x), showing negative (lost) NF2 immunostaining (J, 200x). This tumor (#82) showed a probable single-copy NF2 deletion. Sarcomatoid mesothelioma (K, 40x), showing negative (lost) NF2 immunostaining in tumor (L, 200x),
with retained staining in interspersed inflammatory cells. This case (#81) showed a truncating NF2 E166* mutation. (In F, H, J, and L, note retained positive NF2 staining in interspersed inflammatory and endothelial cells.) Reactive mesothelial hyperplasia (M, N, 400x). Solid reactive nests (M, lower left; N, upper left) show granular membranous and cytoplasmic staining, whereas the single-cell lining of papillary or acinar structures showed more accentuated linear membranous staining.
Figure 5.
Proposed diagnostic algorithm. Dashed lines indicate that Merlin immunohistochemistry is a provisional diagnostic marker of malignant mesothelioma, pending independent validation in subsequent studies. FISH, fluorescence in situ hybridization; NGS, next-generation sequencing. Figure adapted from Chapel DB, et al. Mod Pathol. 2020;33:245–254.
Table 1.

Correlation between *TP53* molecular alterations and p53 immunohistochemistry. Null-pattern p53 immunostaining was grouped with wildtype staining under non-aberrant patterns based on comparison with reactive control samples and molecular correlations. Tumors with no detected *TP53* molecular alteration are not included.

| Panel NGS | Immunohistochemistry                      | kappa | 95% CI     |
|-----------|-------------------------------------------|-------|------------|
|           | **p53 diffuse (mutant pattern)**          |       |            |
| TP53 alteration |                           | 6     | 0.06–0.62  |
| No TP53 alteration |                                 | 17    |            |

| Case number | Histotype | IHC Result | Molecular Alteration                                      |
|-------------|-----------|------------|-----------------------------------------------------------|
| 4           | Epithelioid| Diffuse    | N239_S241delinsT + shallow deletion                       |
| 7           | Epithelioid| Diffuse    | A161V                                                     |
| 24          | Epithelioid| Diffuse    | V173M + shallow deletion                                  |
| 29          | Epithelioid| Diffuse    | Y126C + shallow deletion                                  |
| 51          | Epithelioid| Diffuse    | 376-1G>A + shallow deletion                               |
| 85          | Epithelioid| Diffuse    | D281E                                                     |
| 2           | Epithelioid| Wildtype   | exon 1–8 deletion                                         |
| 9           | Epithelioid| Wildtype   | shallow chr 17p arm-level deletion                        |
| 13          | Epithelioid| Null       | shallow chr 17p arm-level deletion                        |
| 14          | Epithelioid| Wildtype   | R273H                                                     |
| 21          | Epithelioid| Wildtype   | E298*                                                     |
| 22          | Epithelioid| Wildtype   | D228* + shallow deletion                                  |
| 33          | Epithelioid| Null       | shallow chr 17p arm-level deletion                        |
| 38          | Epithelioid| Wildtype   | shallow chr 17p arm-level deletion                        |
| 39          | Epithelioid| Wildtype   | shallow chr 17p arm-level deletion                        |
| 44          | Epithelioid| Wildtype   | shallow chr 17p arm-level deletion                        |
| 47          | Epithelioid| Null       | shallow chr 17p sub-arm-level deletion (del chr 17p13.1-p12) |
| 57          | Biphasic  | Null       | L93Cfs*30                                                 |
| 63          | Biphasic  | Wildtype   | shallow chr 17p sub-arm-level deletion (del chr 17p13.3-p13.1) |
| 67          | Biphasic  | Wildtype   | shallow chr 17p sub-arm-level deletion (del chr 17p13.1-p11.2) |
| 69          | Biphasic  | Wildtype   | shallow chr 17p arm-level deletion                        |
| 81          | Sarcomatoid| Null      | shallow chr 17p arm-level deletion                        |
| 83          | Sarcomatoid| Wildtype  | W146*                                                     |
Table 2.
Correlation between NF2 molecular alterations and Merlin immunohistochemistry. Tumors with no detected NF2 molecular alteration are not included.

| Immunohistochemistry | Merlin lost | Merlin retained |
|----------------------|------------|-----------------|
| Panel NGS            | NGS alteration | 41 | 16 |
|                      | No NF2 alteration | 3 | 24 |
| kappa                | 0.54        | 95% CI          | 0.36–0.72 |

| Case number | Histotype | IHC Result | Molecular Alteration |
|-------------|-----------|------------|----------------------|
| 5           | Epithelioid | Lost       | shallow deletion     |
| 6           | Epithelioid | Lost       | L46_T53del + shallow deletion |
| 14          | Epithelioid | Lost       | W258* + shallow deletion |
| 15          | Epithelioid | Lost       | E38* + shallow deletion |
| 18          | Epithelioid | Lost       | shallow deletion     |
| 19          | Epithelioid | Lost       | E460Kfs*25           |
| 21          | Epithelioid | Lost       | shallow deletion     |
| 25          | Epithelioid | Lost       | shallow deletion     |
| 26          | Epithelioid | Lost       | Q333* + shallow deletion |
| 28          | Epithelioid | Lost       | L535Cfs*15 + shallow deletion |
| 29          | Epithelioid | Lost       | shallow deletion     |
| 34          | Epithelioid | Lost       | 448-1G>C             |
| 35          | Epithelioid | Lost       | shallow deletion     |
| 37          | Epithelioid | Lost       | R418* + Indel - NF2 exon 12 (chr22:30069386) \(=\) NF2 exon 12 (chr22:30069386) |
| 38          | Epithelioid | Lost       | shallow deletion     |
| 42          | Epithelioid | Lost       | E465* + shallow deletion |
| 44          | Epithelioid | Lost       | shallow deletion     |
| 48          | Epithelioid | Lost       | P257L + Q178*        |
| 49          | Epithelioid | Lost       | shallow deletion     |
| 50          | Epithelioid | Lost       | R196*                |
| 54          | Biphasic   | Lost       | Q337*                |
| 56          | Biphasic   | Lost       | I280Lfs*16 + shallow deletion |
| 58          | Biphasic   | Lost       | L299Hfs*10           |
| 59          | Biphasic   | Lost       | Q538*                |
| 60          | Biphasic   | Lost       | E386*                |
| 65          | Biphasic   | Lost       | Q121Rfs*2 + shallow deletion |
| 66          | Biphasic   | Lost       | shallow deletion     |
| 67          | Biphasic   | Lost       | shallow deletion     |
| 69          | Biphasic   | Lost       | shallow deletion     |
| 70          | Biphasic   | Lost       | shallow deletion     |
| 71          | Biphasic   | Lost       | E317* + shallow deletion |
| 73          | Biphasic   | Lost       | Y144* + shallow deletion |
|   | Cell Type | Result | Description               |
|---|-----------|--------|---------------------------|
| 74| Biphasic  | Lost   | Q400*                     |
| 75| Biphasic  | Lost   | two-copy deletion         |
| 76| Biphasic  | Lost   | 363+2T>A                  |
| 78| Biphasic  | Lost   | shallow deletion          |
| 79| Biphasic  | Lost   | shallow deletion          |
| 80| Sarcomatoid | Lost | E103* + shallow deletion  |
| 81| Sarcomatoid | Lost | E166*                     |
| 82| Sarcomatoid | Lost | shallow deletion          |
| 85| Sarcomatoid | Lost | N36Ifs*4                  |
|  2| Epithelioid | Retained | shallow deletion        |
|  4| Epithelioid | Retained | shallow deletion        |
|  9| Epithelioid | Retained | shallow deletion        |
| 10| Epithelioid | Retained | shallow deletion        |
| 13| Epithelioid | Retained | shallow deletion        |
| 20| Epithelioid | Retained | shallow deletion        |
| 22| Epithelioid | Retained | shallow deletion        |
| 23| Epithelioid | Retained | shallow deletion        |
| 24| Epithelioid | Retained | shallow deletion        |
| 41| Epithelioid | Retained | shallow deletion        |
| 45| Epithelioid | Retained | shallow deletion        |
| 51| Epithelioid | Retained | shallow deletion        |
| 53| Biphasic  | Retained | shallow deletion        |
| 72| Biphasic  | Retained | shallow deletion        |
| 77| Biphasic  | Retained | shallow deletion        |
| 83| Sarcomatoid | Retained | W191*                   |
Table 3.
Comparative sensitivities of immunomarkers, immunopanels, and panel NGS for diagnosis of malignant mesothelioma. Parenthetical values are 95% confidence intervals. P values were corrected for multiple comparisons by Holm’s method. Significant P values are bolded.

| Marker / Panel          | Sensitivity for Diagnosis of Malignant Mesothelioma (%) | All MM | EMM | BMM | SMM |
|-------------------------|--------------------------------------------------------|--------|-----|-----|-----|
| BAP1                    | 54 (44–65)                                             | 59 (45–72) | 52 (33–71) | 17 (0–46) |
| MTAP                    | 46 (36–57)                                             | 35 (22–48) | 59 (41–78) | 83 (54–100) |
| Merlin                  | 52 (42–63)                                             | 41 (28–55) | 70 (53–88) | 67 (29–100) |
| P53                     | 7 (2–13)%                                              | 10 (2–18) | 0%  | 17 (0–46) |
| BAP1 + MTAP             | 79 (70–87)                                             | 75 (63–86) | 85 (72–99) | 83 (54–100) |
| BAP1 + Merlin           | 85 (75–93)                                             | 80 (69–92) | 93 (83–100) | 83 (54–100) |
| MTAP + Merlin           | 71 (62–81)                                             | 63 (49–76) | 85 (72–99) | 67 (29–100) |
| BAP1 + p53              | 60 (50–71)                                             | 67 (54–79) | 52 (33–71) | 33 (0–71) |
| MTAP + p53              | 49 (38–59)                                             | 39 (26–53) | 59 (41–78) | 83 (54–100) |
| Merlin + p53            | 62 (52–72)                                             | 49 (35–63) | 70 (53–88) | 67 (29–100) |
| BAP1 + MTAP + Merlin    | 90 (84–97)                                             | 88 (79–97) | 96 (89–100) | 83 (54–100) |
| BAP1 + MTAP + p53       | 81 (73–89)                                             | 78 (67–90) | 85 (72–99) | 83 (54–100) |
| BAP1 + Merlin + p53     | 89 (82–96)                                             | 88 (79–98) | 93 (83–100) | 83 (54–100) |
| MTAP + Merlin + p53     | 74 (64–83)                                             | 67 (54–80) | 85 (72–99) | 67 (29–100) |
| BAP1 + MTAP + Merlin + p53 | 93 (87–98)                          | 92 (85–100) | 96 (89–100) | 83 (54–100) |
| OncoPanel               | 95 (91–100)                                            | 94 (88–100) | 96 (89–100) | 100% |
| OncoPanel + BAP1 + MTAP + Merlin + p53 | 99 (96–100)                          | 98 (94–100) | 100% | 100% |

Two-marker immunopanels
All mesotheliomas (n=84)

| Marker / Panel | Sensitivity (All MM) | Sensitivity (EMM) | Sensitivity (BMM) | Sensitivity (SMM) |
|----------------|----------------------|-------------------|------------------|------------------|
| BAP1 + MTAP   | 0.02                 | <0.0001           | 0.01             | 0.29             | 0.43             |
| BAP1 + Merlin | 0.008                | <0.0001           | 0.001            | 0.07             |
| MTAP + Merlin | 0.19                 | 0.009             | 0.08             |
| BAP1 + p53    | 0.64                 | 0.22              |
| MTAP + p53    | 0.09                 |
| Merlin + p53  |                      |

Three-marker immunopanel vs two-marker immunopanels
All mesotheliomas (n=84)

| Marker / Panel | Sensitivity (All MM) | Sensitivity (EMM) | Sensitivity (BMM) | Sensitivity (SMM) |
|----------------|----------------------|-------------------|------------------|------------------|
| BAP1 + MTAP + Merlin |                      |                   |                  |                  |
| BAP1 + MTAP   | 0.03                 |                   |                  |                  |
| BAP1 + Merlin | 0.17                 |                   |                  |                  |
| MTAP + Merlin | 0.004                |                   |                  |

Molecular testing vs select immunopanels
All mesotheliomas (n=84)

| Marker / Panel | Sensitivity (Panel NGS) |
|----------------|--------------------------|
| BAP1 + MTAP   | 0.002                    |
| Combination                  | p-value |
|-----------------------------|---------|
| BAP1 + Merlin               | 0.01    |
| BAP1 + MTAP + Merlin         | 0.23    |