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CCR New Strategies

Clinical Cancer Research

New Strategies in Pleural Mesothelioma: BAP1 and NF2 as Novel Targets for Therapeutic Development and Risk Assessment

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Abstract

Malignant pleural mesothelioma (MPM) is a highly lethal cancer with limited therapeutic options. Recent work has focused on the frequent somatic inactivation of two tumor suppressor genes in MPM—*NF2* (Neurofibromatosis type 2) and the recently identified *BAP1* (BRCA associated protein 1). In addition, germline mutations in *BAP1* have been identified that define a new familial cancer syndrome, which includes MPM, ocular melanoma, and other cancers. These recent advances may allow screening of high-risk individuals and the development of new therapies that target key pathways in MPM. *Clin Cancer Res; 18(17); 4485–90. ©2012 AACR.*

Background

Malignant pleural mesothelioma (MPM) is an almost universally fatal disease striking approximately 3,000 individuals each year in the United States (1). Currently available therapies for MPM have a limited effect on outcomes. Even with aggressive multimodality therapy, including surgery, for early-stage disease, the median overall survival is 18 months from the time of diagnosis. For patients with advanced disease, first-line chemotherapy with pemetrexed and cisplatin improves median survival by a few months, but the benefit is limited and the use of second-line chemotherapy has not been established (2).

In this context, numerous clinical trials have been conducted in MPM to evaluate a variety of targeted agents. These include angiogenesis inhibitors such as bevacizumab and thalidomide, receptor kinase inhibitors such as erlotinib and sorafenib, and histone deacetylase inhibitors such as vorinostat (3). Despite the activity of these drugs in other diseases, they have shown little activity in MPM. Ideally, treatments that more specifically target the unique biology of MPM should be more effective. Just as the identification of somatic mutations in other malignancies has led to the development of effective disease-specific therapeutics, the potential identification of similar targetable somatic mutations in MPM would be important.

This review calls attention to emerging data on the frequent inactivation of 2 genes in MPM—neurofibromatosis type II (*NF2*) and BRCA1-associated protein 1 (*BAP1*)—that could help elucidate key pathways that drive tumorigenesis and could subsequently be exploited as rational targets for drug development in this cancer.

NF2 Loss and mTOR Blockade

About 35% to 40% of MPMs carry inactivating mutations at the neurofibromatosis 2 (*NF2*) locus, which encodes for the FERM domain protein Merlin (moesin–ezrin–radixin–like protein; refs. 4, 5). The mechanisms by which Merlin suppresses tumorigenesis have remained poorly defined. Merlin is homologous to the ERM proteins ezrin, radixin, and moesin and, like these proteins, it associates with the cortical actin cytoskeleton. Because loss of Merlin causes activation of multiple mitogenic signaling pathways, such as HER1/2, mTOR, extracellular signal-regulated kinase (ERK), and focal adhesion kinase (FAK), it has been postulated that Merlin inhibits signaling by negatively regulating multiple cell surface receptors (6). However, Merlin lacks an actin-binding motif and its dephosphorylated conformer, which is the only one active as a tumor suppressor, is not enriched at the cell membrane (7). Recent studies have revealed that the dephosphorylated, active form of Merlin accumulates in the nucleus and interacts with DCAF1, the receptor component of the E3 ubiquitin ligase $CRL4^{DCAF1}$ (8). Notably, Merlin inhibits $CRL4^{DCAF1}$, which in turn promotes a broad oncogenic gene expression program, presumably by ubiquitinating transcription factors, histones, or chromatin-remodeling enzymes. This deregulation of nuclear ubiquitination events is an

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intriguing common thread with BAP1 inactivation as described below, and the possible overlap between DCAF and BAP1 targets merits further investigation. Genetic epistasis experiments and an analysis of several Merlin missense mutations from NF2 patients support the hypothesis that the dephosphorylated form of Merlin suppresses tumorigenesis by inhibiting $CRL4^{DCAF1}$ (8). These results suggest that Merlin affects multiple mitogenic signaling pathways by controlling, through $CRL4^{DCAF1}$, the expression of components of regulators of these pathways (Fig. 1; ref. 9). It has also been proposed that Merlin loss in MPM, along with inactivating mutations in *LATS2* in a small percentage of cases (10, 11), may also contribute to oncogenesis through activation of the Hippo signaling cascade (12). However, it remains unclear whether loss of Merlin inactivates the Hippo kinase in the cytosol, as genetic studies in the fly suggest, or it deregulates the oncoprotein and transcriptional coactivator YAP through activation of $CRL4^{DCAF1}$ (9).

Merlin mediates contact-dependent inhibition of cell proliferation in normal cells, primarily through inhibition of mTOR in an AKT-independent manner (7). Without Merlin, mTOR activity is aberrantly upregulated, leading to increased cell proliferation (13). This mTOR activation has also been observed in other Merlin-deficient tumors (14). This increased proliferation can be reduced but not completely suppressed by mTOR inhibition. In the presence of Merlin, mTOR inhibition has little effect on growth. In MPM tumors and cell lines, there is an inverse relationship between phospho-mTOR expression and NF2 expression confirming the link between NF2 and the mTOR pathway, and we have found that immunohistochemical staining for NF2 and phospho-mTOR could be useful in selecting patients with MPM for trials targeting mTOR pathway activation due to NF2 loss (15). These preclinical observations have provided the rationale for studying mTOR inhibitors in patients with MPM. Everolimus, an oral derivative of the organ transplant immunosuppression drug rapamycin, which has also been approved for the treatment of advanced renal cell carcinoma, is being investigated as second-line therapy in a phase II trial run by the Southwest Oncology Group (SWOG). In addition, due to compensatory parallel pathway activation, mTOR inhibition alone may not be sufficient to suppress tumor growth. Indeed, we have found that MET, epidermal growth factor receptor, and insulin-like growth factor-1R are variably activated after mTOR inhibition in MPM cell lines, contributing to AKT feedback activation (16).

Preclinical evidence indicates that isolated mTOR inhibition alleviates feedback inhibition on PI3K and thereby allows restoration of PI3K and downstream AKT signaling (17). To address this mechanism of mTOR resistance, numerous dual PI3K/mTOR inhibitors are in early development. Two compounds, BEZ235 and GDC0980, have shown promise in MPM. BEZ235, a dual PI3K/mTOR inhibitor, is able to inhibit the growth of peritoneal mesothelioma cells (18). In addition, in a phase I trial of GDC0980, 3 of 6 enrolled patients with MPM had tumor shrinkage ranging from 23% to 28% and 1 patient had stable disease on this compound for more than 1 year (19). However, cases of grade 2 and 3 pneumonitis have been reported with GDC0980, albeit at a dose above the maximum tolerated dose. It remains unclear whether the pulmonary toxicity will pose a clinical obstacle to the further application of these agents in the treatment of MPM.

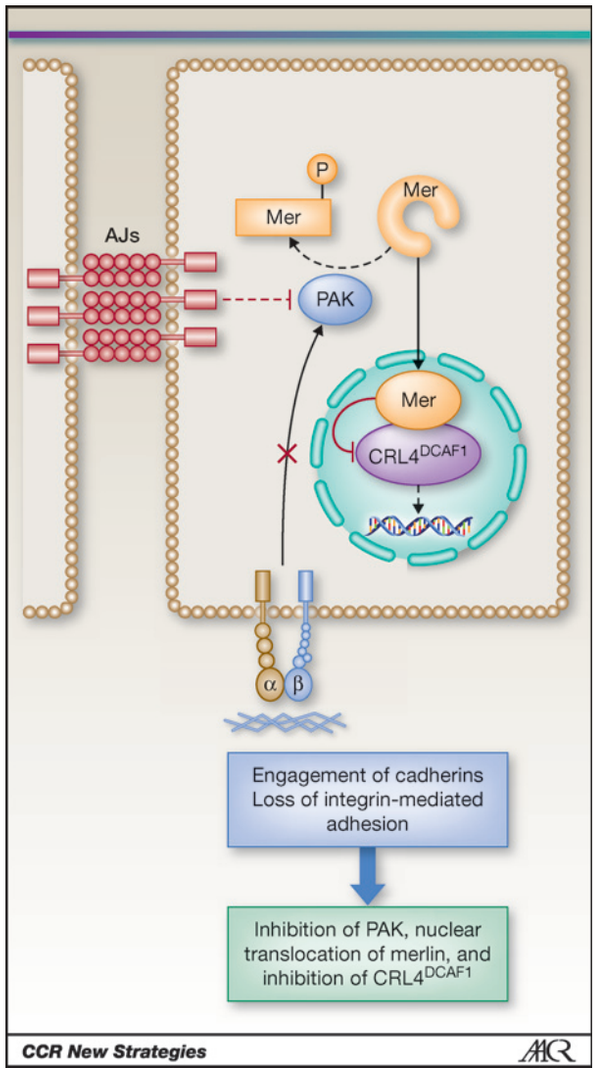

Figure 1. Mechanisms of NF2(Merlin)-mediated inhibition of proliferation. Merlin exists in an open, inactive form and a closed, active form. Matrix adhesion and the consequent activation of joint integrin receptor tyrosine kinase signaling activate the serine-threonine kinase p21-activated kinase (PAK). PAK in turn phosphorylates the C-terminus of Merlin, disrupting the intramolecular association that maintains the protein in a closed conformation. The resulting inactivation of Merlin removes a block to cell-cycle progression in normal cells. Conversely, engagement of E-cadherin and the ensuing assembly of adherens junctions (AJ) inactivate PAK, leading to an accumulation of the closed, active form of Merlin, which migrates into the nucleus to inhibit $CRL4^{DCAF1}$. When it is not inhibited by Merlin, $CRL4^{DCAF1}$ positively regulates a broad oncogenic program of gene expression, which includes mitogenic signaling components, antiapoptotic proteins, and Hippo pathway target genes (8). CRL4, cullin-ring E3 ligase. 4; DCAF1, DDB1- and CUL4-associated factor. 1 (Figure adapted and modified from ref. 9).

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BAP1 Somatic Mutations

The frequent occurrence of *NF2* gene loss in MPM, as well as the very high prevalence of p16/*CDKN2A* deletions (20–22) have been known since the mid-1990s. However, a recent integrated genomics analysis has revealed another frequently inactivated tumor suppressor gene in this cancer. By profiling genomic copy number alterations in 53 MPM tumor samples, Bott and colleagues identified candidate regions for sequencing (10). The 3 most common deletions noted were at 22q (which includes *NF2*), 9p21 (which includes *CDKN2A*), and 3p21. Within these and other candidate regions, integration with gene expression data was used to prioritize genes for sequencing. The gene implicated by deletions in 3p21 was subsequently elucidated from sequencing of this region. Of the genes selected for sequencing, the highest rate of nonsynonymous somatic mutations was in the *BAP1* gene at 3p21.1, occurring in 23% of the samples. A high rate of somatic mutations was subsequently confirmed in 6 of 25 (24%) MPM cell lines and 12 of 68 (18%) additional MPM tumor samples. In all, *BAP1* loss, mutation, or both were identified in 42% of cases. Thirty-two different *BAP1* mutations were identified: 6 nonsense, 5 missense, 13 frameshifting, and 8 at or near splice sites. The majority of truncating mutations resulted in loss of the nuclear localization signal and/or the C-terminal protein-binding domain (Fig. 2). Several missense mutations were shown to affect the ubiquitin hydrolase activity of BAP1. Splice site mutations caused exon skipping leading to aberrant, out of frame transcripts. Immunohistochemistry for BAP1 confirmed the association between *BAP1* mutation and an absence of BAP1 protein expression. Interestingly, 25% of tumors without identified *BAP1* mutations did not display any immunohistochemical staining for BAP1, suggesting the possibility of another subset of tumors with functional BAP1 loss arising by other mechanisms. A recent study has suggested that BAP1 inactivation may be more characteristic of epithelioid MPM (23), although this was not apparent in the original study (10).

BAP1 is a 729 amino acid nuclear ubiquitin hydrolase that has been implicated in numerous cellular processes such as cell proliferation and DNA repair (Fig. 2) as well as chromatin level control of gene expression (Fig. 2; ref. 24). For example, BAP1 has been shown to enhance progression through the G_1–S checkpoint and subsequently induce cell death by a process with similarities to both apoptosis and necrosis (24). In addition, BAP1 regulates cell proliferation by deubiquitinating host cell factor-1 (HCF1; ref. 25), a chromatin-associated protein believed to activate and repress transcription by linking appropriate histone-modifying enzymes to a subset of transcription factors, in particular of the E2F family (26). BAP1 knockdown using siRNA in MPM cell lines inhibited cell growth and resulted in inactivation of HCF1 and downregulation of downstream E2F-responsive genes (10). It has also been shown that BAP1 and HCF1 can form a ternary complex with the YY1 transcription factor to regulate gene expression (27).

Inactivating somatic mutations of *BAP1* have been identified in 47% of uveal melanomas, primarily in the metastasizing subset, where the *BAP1* mutation rate is close to 80% (28). The possible involvement of BAP1 in modulating histone modifications prompted a study of histone deacetylase inhibitors in uveal melanoma cell lines, which found that these agents seemed to counteract the expression profile of BAP1-deficient uveal melanomas, but the growth inhibitory effects on the cell lines were independent of BAP1 status (29). The relevance of these results to *BAP1*-mutated MPM is unclear. We have not seen a significant effect of BAP1 knockdown on sensitivity of BAP1 wild-type MPM lines to the histone deacetylase inhibitor suberoylanilide hydroxamic acid [SAHA (vorinostat); R. McMillan and M. Ladanyi; unpublished data]. Furthermore, a recently completed phase III trial of vorinostat in patients with advanced MPM observed few significant responses, well below what would be expected if *BAP1*-mutated cases were preferentially sensitive (30). The relationship of BAP1-related histone modifications to other previously reported epigenetic changes in MPM such as hypermethylation at gene promoters (e.g., *WIF1*, *SFRP4*, and others; refs. 31–33) remains to be defined.

BAP1 Germline Mutations

Coming on the heels of the discovery of somatic *BAP1* mutations is the recent discovery of germline *BAP1* mutations in rare families predisposed to MPM. Although the majority of MPM cases are linked to asbestos exposure with a 20- to 40-year latency, only a minority of those exposed to asbestos eventually develop malignant disease (34). For example, in the United States, it is estimated that over 30 million workers were exposed to asbestos in the second half of the 20th century, but the incidence of MPM has remained relatively stable and low in the United States over the past 15 years at 1 to 15 per 100,000. Furthermore, numerous familial clusterings of MPM both with and without exposure to asbestos or erionite have been well described with up to 50% incidence of MPM within such families (35, 36). Taken together, these observations suggest that some individuals are more susceptible to the carcinogenic effects of asbestos and erionite and some are more susceptible to the development of MPM even in the absence of asbestos or erionite exposure.

Therefore, much work has focused on identifying genetic alterations that predispose individuals to MPM. Testa and colleagues carried out array-comparative genomic hybridization on 2 MPM tumors coming from 2 different familial clusters of MPM (37). Neither family had exposure to asbestos or erionite. Alterations were noted at or near the *BAP1* locus at 3p21.1. In addition, linkage studies were conducted on germline DNA from each family, yielding a maximum lod score of 2.1 at 3p21-22. Given these observations and reports of frequent gene loss at 3p21.1 in sporadic MPM (10), germline *BAP1* sequencing was conducted in both families and revealed concordance between mutation status and linkage analysis. Furthermore, those with

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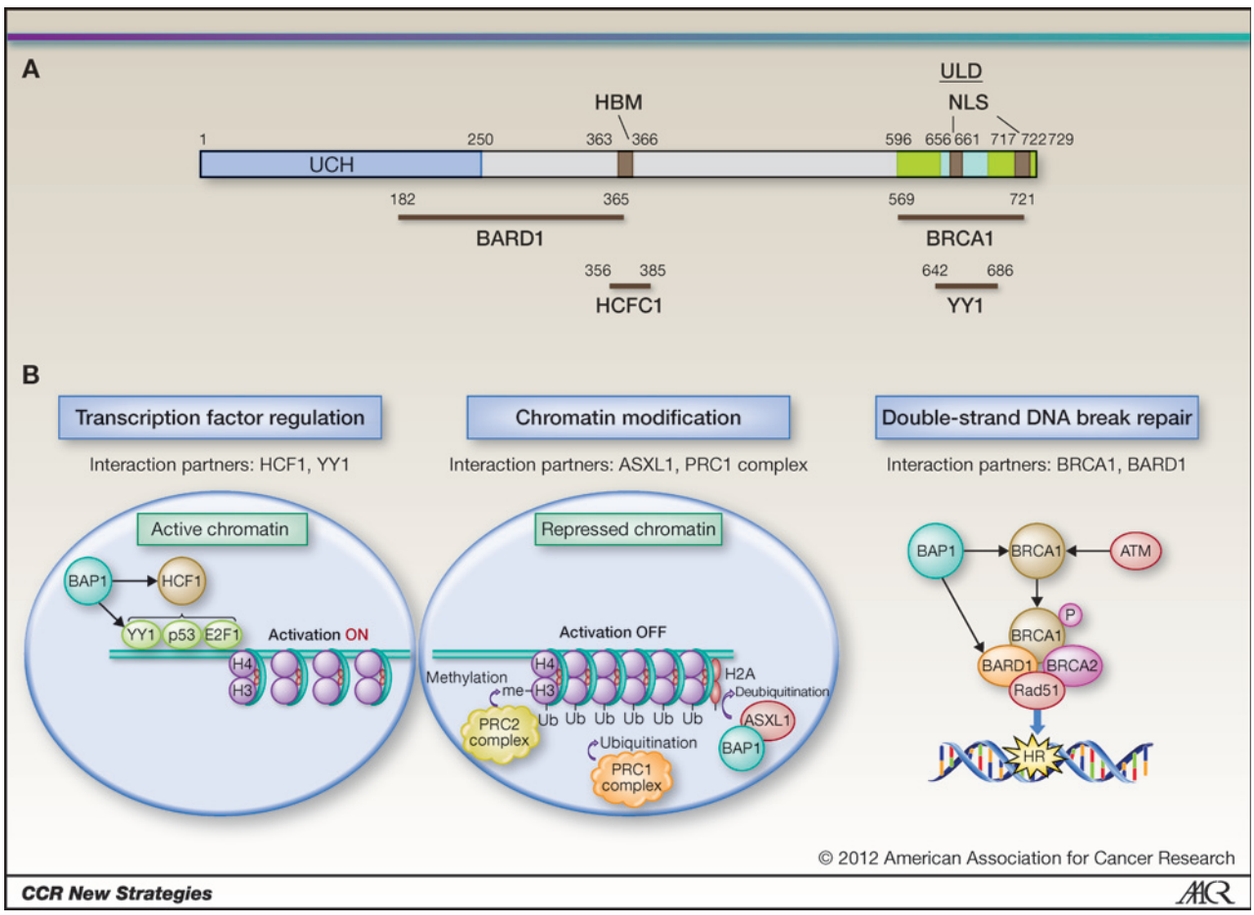

Figure 2. A, BAP1 functional domains and mapped interacting regions. BAP1 is a nuclear deubiquitinating enzyme (DUB). DUBs catalyze the removal of single ubiquitin moieties from ubiquitin chains or cleavage of the isopeptide bond between ubiquitin and the substrate protein (45). BAP1 is composed of an N-terminal UCH domain (blue; amino acid 1–250), an HCF1-binding domain (HBM)-like motif (amino acid 363–366), a motif that shares conservation with UCH37 (ULD: UCH37-like domain; green, amino acid 634–693), and a bipartite nuclear localization signal (NLS, amino acid 656–661, amino acid 717–722). BAP1 has been reported to interact with BARD1 (amino acid 182–365), HCF1 (amino acid 365–385), BRCA1 (amino acid 596–721), and YY1 (amino acid 642–686; ref. 26). B, BAP1 functions. Role of BAP1 in transcription factor regulation (left). HCF1 is detected as a major binding partner of BAP1 by mass spectrum analysis. HCF1 interacts with specific transcription factors, including OCT1, E2F1, Kox20, Sp1, and GA-binding protein. HCF1 also associates with several chromatin methyltransferases (Set1, MLL1, MLL5), chromatin acetyltransferases (tMOF), and deacetylases (HDAC1, HDAC2). HCF1 is known to recruit LSD1 to demethylate histone H4 lysine 9 and to promote the trimethylation of histone H3 lysine 4 (27, 46). YY1 is a transcriptional factor that binds to BAP1 and HCF1. BAP1 and HCF1 are recruited by YY1 to various promoters to upregulate gene expression (27). Role of BAP1 in chromatin modifications (middle). The trimethylation of lysine 27 of histone H3 (H3K27me3) is mediated by the histone methyltransferase EZH1/2, a component of the polycomb repressive complex 2 (PRC2) complex. This triggers recruitment of the PRC1 multiprotein complex through recognition of the H3K27me3 mark by the CBX subunit of the PRC1 complex. Another subunit of the PRC1 complex, the RING1 E3 ligase, then ubiquitylates lysine 119 of histone H2A (H2AK119ub1), which fixes chromatin in a repressed state, silencing gene expression. The H2AK119ub1 mark may be in a dynamic, continuously regulated state, as another part of the PRC1 complex, containing BAP1 and ASXL1 (47), and displays an opposing, deubiquitylating activity (PR-DUB). A balance between ubiquitination and deubiquitination may be needed for appropriate PRC target gene repression. Possible role of BAP1 in DNA repair (right). Other binding partners of BAP1 include BRCA1 and its partner BARD1, which have important roles in the double strand DNA repair process (48). Currently, the possible function of BAP1 in this process is not clear. The Rad51-dependent DNA repair pathway is highly regulated and includes many proteins, some of which may be potential substrates for BAP1-mediated ubiquitin hydrolysis.

germline mutations manifested other malignancies in addition to MPM, including uveal melanoma, breast cancer, renal cancer, and skin cancer. At the same time, Wiesner and colleagues reported an association between germline mutations in *BAP1* and familial melanocytic tumors ranging from epithelioid nevi to atypical melanocytic proliferations with features of cutaneous melanoma (38). Tumors were examined from 2 families and revealed biallelic somatic inactivation of *BAP1*. Each family had 1 person with uveal melanoma, and 1 family had multiple members with cutaneous melanoma. Currently, with only a few published pedigrees, the exact spectrum of this newly described hereditary cancer predisposition syndrome remains unclear (39). In fact, it is possible that 2 distinct cancer syndromes exist, both associated with uveal melanoma but 1 dominated by melanocytic tumors and another by MPM (40). Njauw and colleagues (41) have recently published additional melanocytic tumor-rich pedigrees of families with

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germline *BAP1* mutations. They have proposed for this phenotypic complex the acronym COMMON syndrome, which we would suggest to broaden as follows: cutaneous/ocular melanoma and atypical melanocytic proliferations, mesothelioma, and other internal neoplasms. Ultimately, the phenotype associated with germline *BAP1* mutations will likely be complex and depend on a variety of factors such as the tissue in which the second *BAP1* allele is inactivated, the mechanism of inactivation for the second *BAP1* allele, the functional consequences of a particular *BAP1* mutation, coexistent mutations, and environmental exposures.

On the Horizon

The recently published data reviewed above suggest that, together, a majority of MPMs carry either *NF2* or *BAP1* mutations. Whereas *BAP1* encodes for a ubiquitin C-terminal hydrolase [deubiquitinase (DUB)] and is therefore able to reverse the ubiquitin linkages formed by E3 ubiquitin ligases, *NF2* encodes for a negative regulator of the E3 ubiquitin ligase $CRL4^{DCAF1}$. DUBs are thought to function by binding to the substrate receptor components of specific, partner E3 ligases and by reversing the linkage that the latter form. Interestingly, total loss-of-function mutations in *NF2* and *BAP1*, as assessed by immunoblotting, occur in largely nonoverlapping subsets of patients with MPM [see immunoblotting of cell lines for Merlin (13) and for BAP1 (10)]. This raises the hypothesis that $CRL4^{DCAF1}$ and BAP1 regulate in an opposing fashion a common subset of substrates, which in turn causes a protumorigenic deregulation of gene expression in MPM. Alternatively, NF2 and BAP1 may suppress tumorigenesis by independent mechanisms. The therapeutic implications of distinguishing between these models will be significant.

If studies define a therapeutically accessible synthetic lethal target in the setting of *BAP1* loss, this could eventually benefit the approximately 40% of patients with MPM whose tumors have *BAP1* loss or mutation. More speculatively, the same synthetic lethal target could be studied as chemoprevention drug targets in individuals (or initially, mouse models) with germline *BAP1* mutations that predispose to MPM development. More fundamentally, the biologic insights into MPM pathogenesis emerging from further work on BAP1 could lead to novel, biologically rational treatment strategies for MPM. Finally, the impact of the work may also extend to other cancers with *BAP1* mutations, such as melanoma and, as recently reported, clear cell renal cell carcinoma (42–44).

Disclosure of Potential Conflicts of Interest

Lee M. Krug has commercial research support from Merck, Novartis, CanBas, and Lilly and is a consultant/advisory board member of Genentech, Morphotek, and MedImmune. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Bott

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Ladanyi, M. G. Zauderer, L.M. Krug, M. Bott

Writing, review, and/or revision of the manuscript: M. Ladanyi, M. G. Zauderer, L.M. Krug, T. Ito, F. G. Giancotti

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Ito, R. McMillan, M. Bott

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