VOLUME 36 · NUMBER 35 · DECEMBER 10, 2018

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

A Subset of Mesotheliomas With Improved Survival Occurring in Carriers of *BAP1* and Other Germline Mutations

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Published at jco.org on October 30, 2018.

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0732-183X/18/3635w-3485w/$20.00

ABSTRACT

Purpose

We hypothesized that four criteria could help identify malignant mesotheliomas (MMs) most likely linked to germline mutations of *BAP1* or of other genes: family history of MM, BAP1-associated cancers, or multiple malignancies; or age younger than 50 years.

Patients and Methods

Over the course of 7 years, 79 patients with MM met the four criteria; 22 of the 79 (28%) reported possible asbestos exposure. They were screened for germline *BAP1* mutations by Sanger sequencing and by targeted next-generation sequencing (tNGS) for germline mutations in 55 additional cancer-linked genes. Deleterious mutations detected by tNGS were validated by Sanger sequencing.

Results

Of the 79 patients, 43 (16 probands and 27 relatives) had deleterious germline *BAP1* mutations. The median age at diagnosis was 54 years and median survival was 5 years. Among the remaining 36 patients with no *BAP1* mutation, median age at diagnosis was 45 years, median survival was 9 years, and 12 had deleterious mutations of additional genes linked to cancer. When compared with patients with MMs in the SEER cohort, median age at diagnosis (72 years), median survival for all MM stages (8 months), and stage I (11 months) were significantly different from the 79 patients with MM in the current study ($P < .0001$).

Conclusion

We provide criteria that help identify a subset of patients with MM who had significantly improved survival. Most of these patients were not aware of asbestos exposure and carried either pathogenic germline mutations of *BAP1* or of additional genes linked to cancer, some of which may have targeted-therapy options. These patients and their relatives are susceptible to development of additional cancers; therefore, genetic counseling and cancer screening should be considered.

J Clin Oncol 36:3485-3494. © 2018 by American Society of Clinical Oncology

INTRODUCTION

Malignant mesothelioma (MM) is an aggressive cancer with a median survival of approximately 12 months from diagnosis. Conventional wisdom dictates that MM occurs in professions exposed to high levels of asbestos for many years. MM is commonly diagnosed when individuals are 70 to 80 years old and approximately 30 to 60 years from initial exposure. Since the 1980s, asbestos use has been entirely banned in western Europe and restricted in the United States. Cohorts of professionals exposed to high levels of asbestos are reduced disappearing because of old age. Presently, MM is increasingly being diagnosed in young individuals, and in women, with no known history of exposure.[1,2]

Through a 14-year study of an epidemic of MM in Cappadocia, Turkey, where more than 50% of the population exposed to erionite fibers died of MM,[3,4] we hypothesized, and then proved, that susceptibility to MM was transmitted in a Mendelian fashion. We formulated the hypothesis that the cause of the epidemic was gene–environment interaction.[3-5] While investigating this hypothesis, we discovered that heterozygous germline *BAP1*-inactivating mutations (*BAP1*$^{+/-}$)

ASSOCIATED CONTENT

Appendix
DOI: https://doi.org/10.1200/JCO.2018.79.0352

DOI: https://doi.org/10.1200/JCO.2018.79.0352

caused a very high incidence of MM in some US families apparently not exposed to asbestos.[6] We found that *BAP1* mutations and susceptibility to MM were transmitted through the course of multiple generations.[7] We reported that MM developed in *BAP1*$^{+/-}$ mice (homozygous *BAP1*$^{-/-}$ knockout mice mutations are embryonically lethal) after exposure to low doses of asbestos, which rarely cause MM in wild-type mice.[8] We and others found that human germline *BAP1* mutations were also associated with uveal melanoma (UVM), cutaneous melanoma (CM), clear-cell renal cell carcinoma (ccRCC), and breast and other cancers.[9-19] We named this condition the BAP1 cancer syndrome.[9,10] In parallel studies, we and others discovered that acquired somatic *BAP1* mutations and deletions were present in more than 60% of patients with MM,[20-24] 90% of metastatic UVM,[25] 15% of ccRCC,[26] and in other cancers.[27] BAP1 regulates DNA repair by homologous recombination,[28,29] Ca^{2+}-dependent cell death,[29] and cellular metabolism.[30,31] To our knowledge, it is unknown if, in addition to *BAP1*, germline mutations in other genes predispose to MM.

Here, we studied patients with MM who had a family history of MM and/or other cancers and/or early-onset MM. We found that inherited germline mutations are more frequent in this subgroup of patients, and that their presence influenced survival and helped identify relatives at risk for MM. We discuss opportunities for prevention and early detection in carriers of germline mutations that predispose to MM, and possible therapeutic implications.

METHODS

Study Oversight and Study Population

After we determined that carriers of *BAP1*$^{+/-}$ mutations developed MM,[6] some patients with MM contacted us directly, through their physicians, or through the Mesothelioma Applied Research Foundation to have germline testing for *BAP1*$^{+/-}$. We offered free testing to patients with pleural and peritoneal MM who met one or more of the following criteria that, based on our experience,[6,7] would make them and/or their relatives more likely to carry *BAP1*$^{+/-}$ in the germline: (1) first- or second-degree relatives with MM; (2) proband or one first- or second-degree relative diagnosed with UVM, CM, and ccRCC—malignancies frequent in carriers of *BAP1*$^{+/-}$; (3) history of multiple cancers (any cancer) in the majority of first- and second-degree relatives; and (4) early MM onset (age $<$ 50 years; the incidence of MM before age 50 years is very rare and suggestive of genetic predisposition or environmental exposure since childhood).[1,32]

Written informed consent was received from all patients. Collection and use of patient information and samples were in accordance with the Declaration of Helsinki (1995) and the World Medical Association (2013 revision), approved by University of Hawaii (institutional review board [IRB] no.CHS14406), New York University (IRB no. i8896), and Hyogo College of Medicine (IRB no. RINHI244).

Over 7 years, 79 patients with MM who met the inclusion criteria were tested for *BAP1*$^{+/-}$ and were screened for mutations in 55 additional genes (including tumor suppressor genes, oncogenes, DNA repair genes, and genes somatically mutated in MM) by targeted next-generation sequencing (tNGS).

Germline DNA was extracted from saliva or peripheral blood.[20] Clinical information was collected through the medical records and patient interviews. Personal and family histories of cancers and asbestos exposure were self-reported and obtained using a standardized questionnaire approved by the IRBs and complemented, when possible, with patient interviews (Appendix Fig A1, online only). Patients were observed up to 20 years.

BAP1 *Sequencing and Immunohistochemistry*

BAP1 was amplified by polymerase chain reaction and sequenced in its entirety.[20] BAP1 staining was performed as described.[20]

tNGS and Data Analysis

tNGS was performed using an Illumina Truseq Custom Amplicon (Illumina, San Diego, CA), and the Agilent Haloplex Custom kit (Agilent Technologies, Santa Clara, CA) as described previously.[21] tNGS sequencing data were submitted to the DDBJ Japanese genotype-phenotype archive for genetic and phenotypic human data (http://www.ncbi.nlm.nih.gov/pubmed/25477381) under accession number JGAS00000000108. Pathogenic variants were extracted using Combined Annotation Dependent Depletion (CADD) score (version 1.3; http://cadd.gs.washington.edu/), which is among the recommended strategies for selection of deleterious mutations according to the guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[33] CADD can quantitatively prioritize functional, deleterious, and disease-causing variants across a wide range of functional categories, effect sizes, and genetic architectures, and is used to prioritize causal variation in research and clinical settings.

A CADD score of 15 is the recommended cutoff to identify deleterious mutations. A CADD score of 20 indicates that a variant is among the top 1% of deleterious variants in the human genome.[34] Final selection of potentially pathogenic variants was verified by the assessment of quality scores and visual inspection of the data using StrandNGS (Agilent Technologies, Santa Clara, CA). The Database for Annotation, Visualization and Integrated Discovery, which provides a comprehensive set of functional gene annotation tools, was used for functional and biologic annotation analysis (version 6.8; https://david.ncifcrf.gov/).

RESULTS

BAP1 *Testing*

A total of 52 unrelated patients with MM met the recruitment criteria and their DNA was sequenced for the presence of *BAP1* mutations. Deleterious mutations were detected, in the *BAP1* gene, in 16 patients (30.7%), and 36 carried germline wild-type *BAP1* (*BAP1*^{WT}). A total of 153 first- and second-degree relatives of 12 of the 16 *BAP1*$^{+/-}$ patients volunteered for *BAP1* testing (n = 90 women [58.8%]; n = 63 men [41.2%]). Among them, 66 (43.1%) carried *BAP1*$^{+/-}$: 23 men (34.8%) and 43 women (65.2%). As expected, their *BAP1* mutations were identical to those found in their related probands. Among the 153 relatives, MM developed in 27 of 66 carriers of germline *BAP1*$^{+/-}$, therefore, these relatives were included in our study, resulting in a total of 79 MM patients examined (Tables 1-3). There were no significant survival or other differences among the MMs in relatives and probands. The remaining 39 family members with *BAP1*$^{+/-}$ were too young for MM to have developed and were alive at the end of the study. MM did not develop in any of the 87 relatives with *BAP1*^{WT}.

Among *BAP1*$^{+/-}$ carriers, the oldest patient in whom MM developed was age 75 years; the youngest was age 29 years (Table 1). Pathology reports documenting the tumor histologic subtype or tumor biopsy–specimen slides for review were available for 37 of 79 patients. All *BAP1*$^{+/-}$ MMs and almost all *BAP1*^{WT} MMs were of the epithelial type (Table 2). Fifty-seven of 79 patients (72%) did not report any asbestos exposure. Unstained MM tissue sections available for seven patients carrying *BAP1*$^{+/-}$ were analyzed by immunohistochemistry, and no nuclear BAP1 staining was

Table 1. Patient Characteristics

ID	Sex	Asbestos Exposure*	Cancer Type (age at diagnosis,† years)	MM Site	MM Histology‡	*BAP1* Status	Other Mutations (tNGS)	FDR/SDR With Cancer
FM-01	F	No	Uterine leiomyosarcoma (32); UVM (48); MM (55); giant bone cell tumor (71)	Pleural and peritoneal	Epithelioid	MUT	RAD50	2
FM-02	M	Yes	MM (62)	Pleural	Unknown	MUT	N/A	5
FM-03	M	Yes	MM, CM	Pleural	Epithelioid	WT	None	4
FM-04	F	Yes	MM (53); hairy cell leukemia (56); lung ca (67); RCC (68)	Peritoneal	Biphasic	WT	None	5
FM-05	F	No	Breast ca (33); MM (34); leiomyosarcoma (63)	Peritoneal	Unknown	WT	TP53	2
FM-06	M	No	MM (61)	Peritoneal	Unknown	WT	None	6
FM-07	F	No	BCC (42); MM (54)	Pleural and peritoneal	Epithelioid	WT	None	5
FM-08	M	No	UVM (50); MM (52)	Peritoneal	Epithelioid	MUT	None	6
FM-09	F	No	Breast ca (42); MM (69); bronchoalveolar ca (69)	Pleural	Epithelioid	WT	None	3
FM-10	M	No	MM (51); bladder ca (57)	Peritoneal	Epithelioid	MUT	SMARCA2	5
FM-11	F	Yes	MM (55); CM (55); bladder ca (55); breast ca (58)	Pleural	Unknown	MUT	N/A	5
FM-12	M	Yes	MM (33)	Pleural	Epithelioid	WT	None	4
FM-13	F	No	MM (40)	Pleural	Unknown	WT	KDR	5
FM-14	M	No	MM (46)	Pleural	Unknown	WT	NCOR1	4
FM-15	F	No	MM (54)	Peritoneal	Epithelioid	MUT	None	6
FM-16	F	No	MM (31)	Pleural	Unknown	WT	RBM6	1
FM-17	M	No	MM (30)	Pleural	Unknown	WT	SETD2	5
FM-18	F	Yes	MM (37)	Peritoneal	Unknown	WT	MLH1	3
FM-19	F	Yes	MM (35)	Peritoneal	Unknown	WT	None	3
FM-20	F	No	MM (36)	Pleural	Unknown	WT	None	2
FM-21	F	No	MM (20)	Peritoneal	Epithelioid	WT	None	None
FM-22	F	No	MM, lymphosarcoma	Pleural	Unknown	WT	ARID1A	3
FM-23	F	Yes	MM (41)	Pleural	Unknown	WT	SMO	Unknown
FM-24	F	Yes	MM (47)	Pleural	Epithelioid	WT	None	4
FM-25	F	No	MM (30)	Peritoneal	Unknown	WT	None	Unknown
FM-26	F	No	MM (44)	Peritoneal	Epithelioid	WT	None	Unknown
FM-27	M	No	UVM, MM (55)	Peritoneal	Epithelioid	MUT	None	2
FM-28	M	No	BCC (64); MM (67); RCC (69); UVM (70)	Peritoneal	Epithelioid	MUT	SMARCE1	4
FM-29	F	Yes	MM (49); liver ca (49)	Pleural and peritoneal	Unknown	MUT	None	6
FM-30	F	Yes	BCC (47); MM (52)	Pleural and peritoneal	Unknown	MUT	N/A	6
FM-31	F	No	BCC (35); MM (48)	Pleural	Unknown	MUT	N/A	6
FM-32	F	No	MM (43)	Peritoneal	Epithelioid	MUT	MLH1	6
FM-33	F	No	Breast Ca (46); MM (47)	Peritoneal	Epithelioid	MUT	N/A	6
FM-34	M	No	RCC (53); MM (56)	Pleural	Epithelioid	MUT	N/A	3
FM-35	M	No	MM (60); UVM (60)	Pleural	Unknown	MUT	ARID2	5
FM-36	M	Yes	BCC (63); MM (64)	Pleural	Unknown	MUT	N/A	3
FM-37	M	No	MM (68)	Pleural	Unknown	WT	None	6
FM-38	F	No	MM (78)	Peritoneal	Biphasic	WT	CREBBP	6
FM-39	M	Yes	MM (63);UVM (69)	Pleural and peritoneal	Unknown	WT	None	3
FM-40	F	No	UVM (44); MM (48); breast ca (53)	Pleural and peritoneal	Unknown	MUT	None	13
FM-41	F	Yes	CM (32); MM (33)	Peritoneal	Unknown	WT	None	2
FM-42	M	No	MM (45)	Peritoneal	Epithelioid	MUT	N/A	7
FM-43	M	No	CM (60); MM (67)	Pleural	Epithelioid	WT	N/A	4
FM-44	M	No	MM (48); UVM (48); RCC (48); CM (49)	Peritoneal	Epithelioid	MUT	N/A	3
FM-45	M	Yes	MM (69)	Pleural	Epithelioid	WT	N/A	2
FM-46	F	No	MM (60); BCC (60)	Peritoneal	Epithelioid	MUT	N/A	8
FM-47	F	No	MM (65)	Peritoneal	Epithelioid	MUT	N/A	15
FM-48	M	No	MM (54)	Pleural	Unknown	MUT	N/A	15
FM-49	M	No	MM (37)	Pleural and peritoneal	Unknown	MUT	N/A	10
FM-50	F	No	MM (59)	Pleural	Epithelioid	MUT	N/A	10
FM-51	F	No	MM (63)	Pleural and peritoneal	Unknown	MUT	N/A	10
FM-52	M	No	MM (50)	Pleural	Epithelioid	MUT	N/A	8
FM-53	M	Yes	MM (59)	Pleural	Biphasic	WT	None	1
FM-54	M	Yes	MM (49)	Pleural	Epithelioid	WT	SMARCA4	1
FM-55	F	No	MM (41)	Pleural	Biphasic	WT	None	1
FM-56	F	No	MM (61)	Pleural	Epithelioid	WT	None	1

(continued on following page)

Table 1. Patient Characteristics (continued)

ID	Sex	Asbestos Exposure*	Cancer Type (age at diagnosis,† years)	MM Site	MM Histology‡	*BAP1* Status	Other Mutations (tNGS)	FDR/SDR With Cancer
FM-57	M	Yes	MM (41)	Pleural	Epithelioid	WT	None	None
FM-58	M	Yes	MM (76); BCC	Pleural	Epithelioid	WT	None	2
FM-59	M	Yes	MM (64)	Pleural	Sarcomatoid	WT	None	1
FM-60	F	No	MM (33)	Pleural	Epithelioid	WT	MLH1	None
FM-61	F	No	MM (20)	Peritoneal	Epithelioid	WT	None	None
FM-62	M	No	MM (60); CM	Pleural	Epithelioid	WT	SMARCA2	1
FM-63	M	Yes	MM (49); lung ca (49)	Pleural and peritoneal	Unknown	MUT	None	9
FM-64	F	No	MM (50); RCC (50)	Pleural and peritoneal	Unknown	MUT	N/A	9
FM-65	F	No	MM (58)	Pleural and peritoneal	Unknown	MUT	N/A	9
FM-66	F	Yes	MM (43); lung ca (43) breast ca (49)	Pleural	Epithelioid	MUT	N/A	6
FM-67	F	No	MM (61); CM (61); breast ca (61)	Peritoneal	Unknown	MUT	N/A	9
FM-68	M	No	MM (44)	Pleural	Unknown	MUT	N/A	6
FM-69	F	No	MM (58)	Peritoneal	Unknown	MUT	N/A	6
FM-70	F	No	Breast ca (50); MM (70)	Peritoneal	Unknown	MUT	N/A	10
FM-71	F	No	MM (71); BCC	Pleural	Unknown	MUT	N/A	10
FM-72	M	No	MM (52)	Unknown	Unknown	MUT	N/A	6
FM-73	F	No	MM (29)	Peritoneal	Unknown	MUT	N/A	6
FM-74	M	No	MM (46)	Peritoneal	Unknown	MUT	N/A	3
FM-75	F	No	MM (62)	Pleural	Unknown	MUT	N/A	9
FM-76	M	No	MM (50)	Unknown	Unknown	MUT	N/A	12
FM-77	F	No	MM (71)	Unknown	Unknown	MUT	N/A	14
FM-78	F	No	MM (75)	Unknown	Unknown	MUT	N/A	15
FM-79	F	No	MM (70)	Unknown	Unknown	MUT	N/A	14

NOTE. Bold type indicates probands.
Abbreviations: BCC, basal cell carcinoma; Ca, cancer; CM, cutaneous melanoma; FDR, first-degree relative; MM, malignant mesothelioma; MUT, mutant; N/A, samples not tested by tNGS; RCC, renal cell carcinoma; SDR, second-degree relative; tNGS, targeted next-generation sequencing; UVM, uveal melanoma; WT, wild type.
*Yes: patients reported asbestos exposure; no: patients ruled out or stated that they were not aware of asbestos exposure.
†When available.
‡Unknown: Pathology report did not specify subtype; slides not available for review.

observed (Appendix Fig A2, online only), providing evidence of biallelic *BAP1* inactivation.[1,20,35]

Among 33 patients with MM who were younger than 50 years, 13 carried the $BAP1^{+/-}$ mutation, nine had germline mutations in other genes (discussed later in this section), and 11 had no germline mutations among the genes tested. Among the 13 patients with $BAP1^{+/-}$ who were younger than 50 years, 12 had an extensive family history of cancer types associated with the *BAP1* cancer syndrome (ie, MM, UVM, ccRCC); one had a strong family history of cancers not associated with the *BAP1* cancer syndrome. In contrast, only one of 20 patients with $BAP1^{WT}$ who were younger than 50 years had a first-degree relative with MM ($P < .01$).

Survival

Survival data were available for 77 of the 79 patients with MM: 43 of 43 patients with germline $BAP1^{+/-}$ mutations and 34 of 36 with $BAP1^{WT}$ (Fig 1). Median survival was 5 years among the 43 patients with $BAP1^{+/-}$, and 15% of these 43 patients were alive 10 years after diagnosis of MM, compared with a median survival of 9 years among the 34 patients with $BAP1^{WT}$, of whom 41% survived ≥ 10 years after diagnosis. The survival curve in each of the two MM subgroups of our cohort, $BAP1^{+/-}$ and $BAP1^{WT}$, was compared with that of the SEER cohort[36] (Fig 1A). Median survival in the SEER cohort (all stages) was 8 months from diagnosis. When patients in SEER with stage I MM were selected, median survival was 11 months. Therefore, the MM cohort we studied, including patients with $BAP1^{+/-}$ or $BAP1^{WT}$ mutations had a significantly better survival compared with the MM SEER dataset ($P < .001$; this applies to all comparisons except $BAP1^{+/-}$ *v* $BAP1^{WT}$, where $P < .16$; Fig 1A).

Irrespective of *BAP1* status, 33 patients who developed MM before they reached age 50 years had a median survival of 10 years, compared with a median survival of 4 years among the 44 patients who developed MM at a later age ($P < .0006$; Fig 1B). There was no significant survival difference based on MM site, single versus multiple malignancies or patient's sex (Appendix Fig A3, online only).

In addition to Kaplan-Meier log-rank tests, we ran a multivariate Cox proportional hazards regression analysis. The variables, entered simultaneously, were *BAP1* status ($BAP1^{+/-}$ *v* $BAP1^{WT}$; $P = .16$), age (< 50 years v ≥ 50 years; $P = .004$), site (pleural, peritoneal, or both; $P = .19$), and sex (male *v* female, $P = .87$). A notable change from the Kaplan-Meier analysis was that the difference in survival between patients with MM and other cancers and patients with MM only reached significance ($P = .02$). Additional studies in a larger cohort are needed to verify this finding.

tNGS

Among 36 patients with MM with $BAP1^{WT}$, 34 were further screened by tNGS for germline mutations in 56 genes (including *BAP1* and other tumor suppressor genes, oncogenes, DNA repair genes, and genes previously found somatically mutated in MM; Appendix Table A1, online only). In parallel, we tested 11 germline DNA samples from the MM subcohort with $BAP1^{+/-}$ mutations. To

Table 2. Clinical Characteristics of Familial and Early-Onset Mesotheliomas

Patient Characteristic	Samples (n = 79)	*BAP1* Mutation	
		Mutation (n = 43)	Wild Type (n = 36)
Sex			
Male	33 (42)	18 (42)	15 (42)
Female	46 (58)	25 (58)	21 (58)
Ethnicity			
White (United States)	75 (95)	41 (95)	34 (94)
Other (Latino, Middle Eastern)	4 (5)	2 (5)	2 (6)
Cancer types			
MM	79 (100)	43 (100)	36 (100)
MM and RCC	5 (6)	4 (9)	1 (3)
MM and UVM	8 (10)	7 (16)	1 (3)
MM and CM	7 (9)	3 (7)	4 (11)
MM and breast cancer	8 (10)	6 (14)	2 (6)
MM and one other cancer*	11 (14)	8 (19)	3 (8)
MM and ≥ 2 cancers	10 (13)	7 (16)	3 (8)
Age at diagnosis of MM, years			
Mean (*t* test)*	51.7	54.8	47.7
SD	13.3	9.7	16.1
Median	52	54	45
Range	20-78	29-75	20-78
MM site			
Pleural	36 (46)	13 (30)	23 (64)
Peritoneal	27 (34)	16 (37)	11 (31)
Pleural and peritoneal	16 (20)	14 (33)	2 (5)
Histology (available only for 37 of 79 patients)			
Epithelioid	32 (86)	16 (100)	16 (76)
Sarcomatoid	1 (3)	0	1 (5)
Biphasic	4 (11)	0	4 (19)
Asbestos exposure†‡			
Yes	22 (28)	7 (16)	15 (42)
No	57 (72)	36 (84)	21 (58)

NOTE. Data given as No. (%) unless otherwise indicated.
Abbreviations: CM, cutaneous melanoma; MM, malignant mesothelioma; RCC, renal cell carcinoma; SD, standard deviation; UVM, uveal melanoma.
*Other cancer includes all cancers other than RCC, UVM CM, and breast cancer (Table 1).
†Mean age at diagnosis and asbestos exposure in $BAP1^{WT}$ *v* $BAP1^{+/-}$: $P = .02$. Fisher's exact tests were run for all categorical data.
‡Yes, patients reported asbestos exposure; no, patients ruled out or stated that they were not aware of asbestos exposure.

exclude polymorphisms, we focused on mutations with an allele frequency less than 0.005 in the Exome Aggregation Consortium (ExAC) database, which comprises 60,706 unrelated individual sequences (exac.broadinstitute.org). Next, we used the CADD score to identify pathogenic mutations among those identified as rare with ExAC. First, we evaluated all the *BAP1* mutations detected, which included truncating, frameshift, splice site, and nonsynonymous mutations. All *BAP1* mutations found by tNGS (which were consistent with previous Sanger sequencing data) had a CADD score greater than 20 (Table 3), supporting previous work showing that they are pathogenic[1,6,7,9,10].

Therefore, we applied the same approach and cutoff values (ExAC < 0.005; CADD > 20) to identify pathogenic mutations detected using this **56**-gene panel. We found that 12 of 34 patients with MM with $BAP1^{WT}$ (35%) contained one germline mutation in 11 of the 55 additional genes tested (two patients had two different deleterious *MLH1* variants). Also, five of 11 DNA samples from patients with MM with $BAP1^{+/-}$ mutations contained one additional germline mutation, each in five different genes; all variants had a CADD score greater than 20 (Tables 1 and 4; Fig 2). All mutations were validated by polymerase chain reaction and Sanger sequencing.

DISCUSSION

Based on our experience studying families carrying germline $BAP1^{+/-}$,[6,7,9] we used a strict set of criteria to select those MMs that we hypothesized were likely caused by germline mutations of *BAP1* or of other genes. We studied 79 patients with MM (52 probands and 27 relatives; Table 1), 43 carried germline $BAP1^{+/-}$ (16 probands and 27 relatives); 36 of 52 probands were $BAP1^{WT}$.

Most patients with MM who carried $BAP1^{+/-}$ mutations had first- or second-degree relatives with MM, UVM, and ccRCC (Table 1). None of the 36 patients with MM among probands with $BAP1^{WT}$ had a family history of UVM or ccRCC; however, 12 of 36 had deleterious germline mutations of additional genes that, when mutated, cause cancer syndromes, such as *MLH1* (Lynch syndrome),[37] *TP53* (Li-Fraumeni syndrome),[38] and/or mutations in genes that regulate DNA repair,[39] or were mutations of genes previously found somatically mutated in MM[21,40](Tables 1 and 4; Fig 2).

In our cohort, selected for the clinical red flags suggesting heritability, survival was significantly better than in those with "classic" sporadic MM, even when compared with patients with stage I disease in SEER[36] (Fig 1). Within our cohort, patients with *BAP1* mutations had a worse survival than those with no *BAP1* mutations. Sex and pleural or peritoneal location did not influence survival (Appendix Fig A3). Early age at cancer onset is often related to a genetic mutation[41-43]; in our cohort, the subset of patients with early age at MM onset had the best survival rate (Fig 1B). Moreover, among $BAP1^{+/-}$carriers in whom multiple tumors developed, the improved prognosis seemed to apply to all tumor types.

The high percentage (72%) of patients with MM who reported no asbestos exposure in our cohort is not entirely surprising, given their relatively young age, in most cases, and because of the presence of pathogenic germline mutations. One could speculate that MM developing in the absence of asbestos exposure may have a different biology and improved prognosis.

Improved survival has also been observed in carriers of other germline mutations that predispose to various cancer syndromes. For example, patients with colorectal cancer who are affected by familial adenomatous polyposis or by Lynch syndrome have a better prognosis than do patients with sporadic colorectal cancer.[44] Similarly, patients with gastric cancer who carry *CDH1* mutations have a better survival rate compared with sporadic gastric cancers.[45] Our current hypothesis is that the improved prognosis is caused by changes in the tumor microenvironment, in turn caused by the presence of heterozygous germline mutations in all cells. We are investigating this hypothesis.

$BAP1^{+/-}$ segregated in family members who were affected by cancer in this study and in previous studies.[6,7,9,11-19] Among the 153 relatives tested, MM developed in 27 of 66 carriers of germline $BAP1^{+/-}$ (those in whom tumors did not develop were still quite young), whereas MM did not develop in any of the 87 relatives with $BAP1^{WT}$. Moreover, in tumor cells of $BAP1^{+/-}$ carriers, BAP1 nuclear staining was absent (Appendix Fig A2), indicating loss of

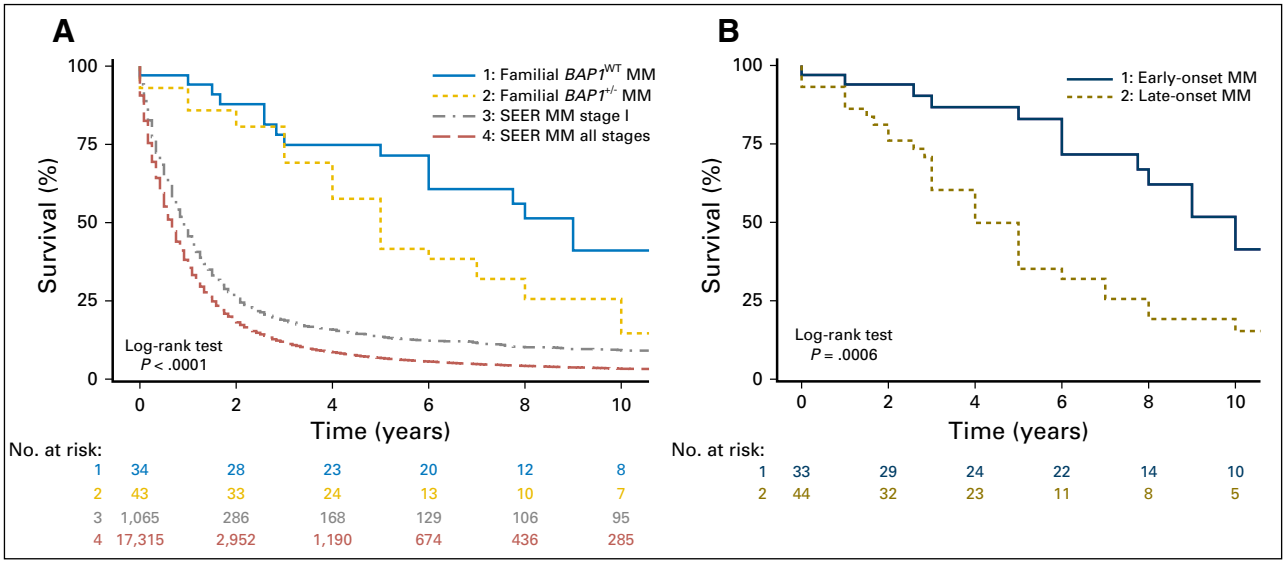

Fig 1. Kaplan-Meier MM survival probability versus years with number at risk. (A) 1. Familial and early-onset *BAP1*^{WT} MM (median survival, 9 years; 10-year survival, 41%); 2. Familial and early-onset *BAP1*$^{+/-}$ MM (median survival, 5 years; 10-year survival, 15%); 3. SEER, stage I (median survival, 11 months; 10-year survival, 9.2%); 4. SEER, all stages (median survival, 8 months; 10-year survival, 3.3%). (B) Familial MM cohort by onset: 1. age < 50 years (median survival, 10 years; 10-year survival, 41%); 2. age ≥ 50 years (median survival, 4 years; 10-year survival, 15%). Rows below figure indicate the number of patients alive in each cohort per year. All patients were treated in the United States. Because patients in our cohort and in the SEER cohort were treated at different institutions, we do not have information on the exact treatment each of them received. *BAP1*$^{+/-}$, heterozygous *BAP1*-inactivating mutations; *BAP1*^{WT}, wild-type *BAP1*; MM, malignant mesothelioma.

heterozygosity.[20] Also, MM can develop in *BAP1*$^{+/-}$ mice even when not exposed to asbestos or to other carcinogens.[46] These findings support causation. Some patients with *BAP1*$^{+/-}$ mutations (five of 11 tested) carried germline mutations of additional genes. It is possible that these mutations, together with *BAP1*$^{+/-}$, contribute to and influence the tumor phenotype; in some families, there is

Table 3. Deleterious Germline Variants in Cancer Susceptibility Genes Identified in Patients With Familial and Early-Onset Malignant Mesothelioma

Gene Name	Chr	Pos (hg19)	Ref	Alt	CADD	ExAC Freq	AA Change
ARID1A	1	27056314	G	A	25.9	ND	NM_006015:c.G1310A:p.Arg437Gln
ARID2	12	46242722	C	G	20.4	ND	NM_152641:c.C1684G:p.Leu562Val
BAP1	3	52436840	A	T	37	ND	NM_004656:c.T1938A:p.Tyr646*
BAP1	3	52437158	GGTGA	G	35	ND	NM_004656:c.1882_1885del:p.Ser628Pro*8
BAP1	3	52441334	T	C	24.4	ND	NM_004656:c.A438-2G:p.Pro147fsx48
BAP1	3	52437443	AG	A	26.1	ND	NM_004656:c.1717delC:p.Leu573Trp*3
BAP1	3	52440844	C	G	27.4	ND	NM_004656:c.G659+1C
BAP1	3	52440900	A	G	26.4	ND	NM_004656:c.T604C:p.Trp202Arg
BAP1	3	52438566	G	A	38	ND	NM_004656:c.C1153T:p.Arg385*
BAP1	3	52437432	C	A	40	ND	NM_004656:c.G1729T:p.Glu577*
BAP1	3	52436624	G	A	42	ND	NM_004656:c.C2050T:p.Gln684X
BAP1	3	52443569	C	G	34	ND	NM_004656:c.G122+1A
BAP1	3	52438516	A	AA	31	ND	NM_004656: c.1203_1204insT:p.Glu402X
BAP1	3	52442612	C	T	32	ND	NM_004656: c.G133A:p.Gly45Arg
CREBBP	16	3828047	G	T	25.6	ND	NM_001079846:c.C1964A:p.Pro655His
KDR	4	55971098	C	T	22.6	8.25E-06	NM_002253:c.G1699A:p.Val567Met
MLH1	3	37045955	T	C	28.1	8.24E-06	NM_000249:c.T370C:p.Cys124Arg
MLH1	3	37089080	A	G	28.7	ND	NM_000249:c.A1802G:p.Asp601Gly
MLH1	3	37067240	T	A	33	0.002779	NM_000249:cT1151A:p.Val384Asp
NCOR1	17	15968216	G	T	28.3	ND	NM_001190440:c.C5117A:p.Pro1706His
RAD50	5	131925354	A	G	26.1	0.0001535	NM_005732:c.A1277G:p.Gln426Arg
RBM6	3	50097113	A	G	20.9	0.002804	NM_001167582:c.A596G:p.Asn199Ser
SETD2	3	47163279	C	A	22.8	ND	NM_014159:c.G2847T:p.Arg949Ser
SMARCA2	9	2191370	G	C	25.6	0.001079	NM_003070:c.G4699C:p.Val1567Leu
SMARCA2	9	2191388	G	A	28.1	0.0007743	NM_003070:c.G4717A:p.Asp1573Asn
SMARCA4	19	11113781	G	A	32	8.24E-06	NM_001128849:c.G1889A:p.Gly630Asp
SMARCE1	17	38785194	C	T	25.1	0.001433	NM_003079:c.G1079A:p.Gly360Asp
SMO	7	128851957	A	G	24.3	9.81E-05	NM_005631:c.A2029G:p.Lys677Glu
TP53	17	7577120	C	T	27.3	0.00002628	NM_001126115:c.G422A:p.Arg141His

Abbreviations: AA, amino acid; Alt, altered nucleotide; CADD, Combined Annotation Dependent Depletion Damaging Score; Chr, chromosome number; ExAc Freq, frequency in the Exome Aggregation Consortium browser [exac.broadinstitute.org]); ND, not detected; Pos, chromosomal variant location; Ref, reference nucleotide.

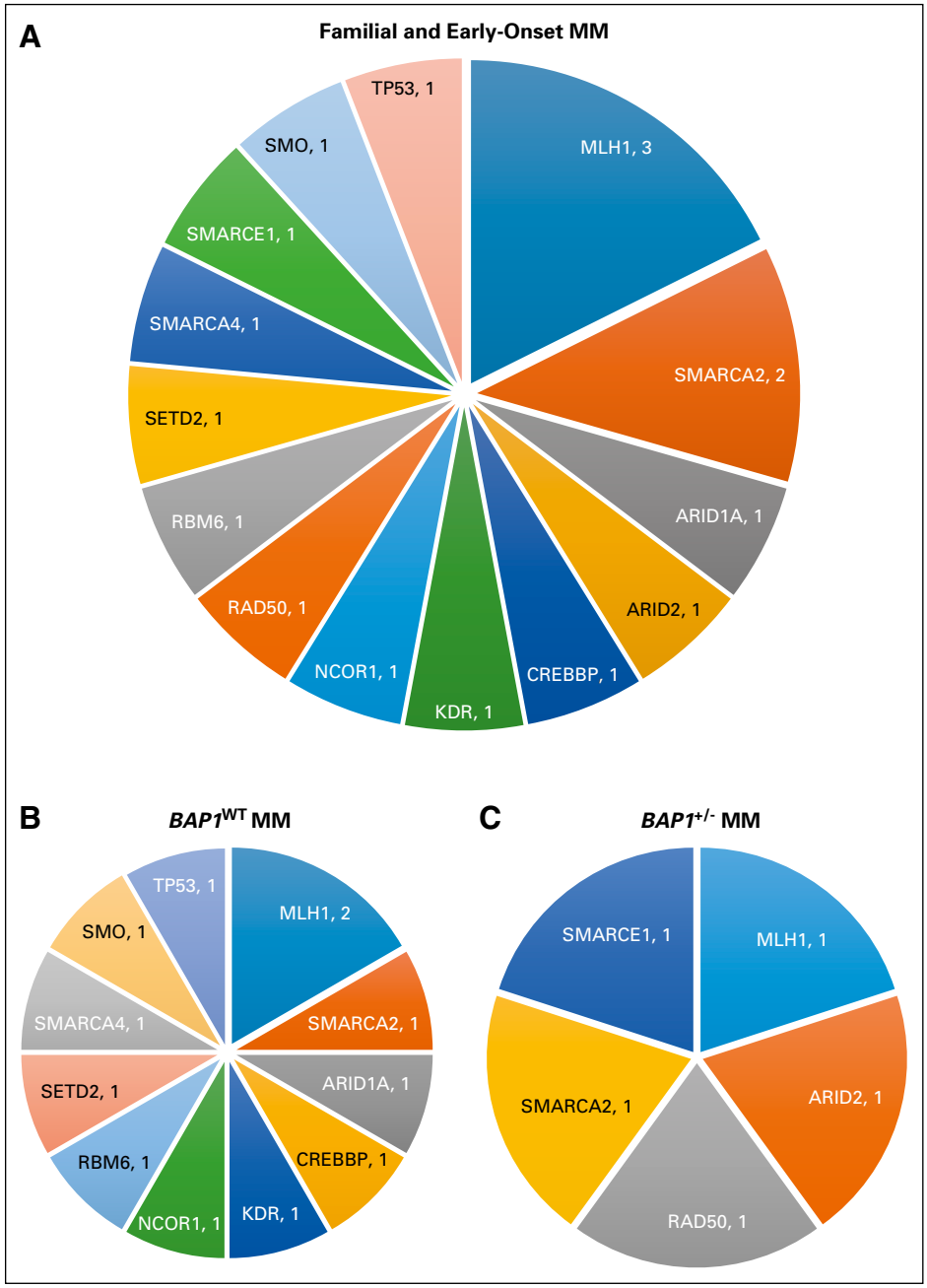

Fig 2. Deleterious germline variants identified in patients with familial malignant mesothelioma (MM). Pie charts show the number of gene variants identified by targeted next-generation sequencing in (A) the familial MM cohort and, separately, in the (B) *BAP1*^{WT} and (C) *BAP1*$^{+/-}$ family cohorts. Selected variants with a frequency < 0.005 in the Exome Aggregation Consortium database have a Combined Annotation Dependent Depletion score > 20. *BAP*$^{+/-}$, *BAP1*-inactivating mutations; *BAP1*^{WT}, wild-type *BAP1*.

a prevalence of MM; in others, a prevalence of UVM; and in others, a prevalence of ccRCC.[1]

There are some limitations to our study. Only 22 of 79 patients reported asbestos exposure, limiting the statistical power to assess any hypothesis in relation to asbestos exposure and presence of germline mutations predisposing to MM. Moreover, because the exposure was self-reported, it was subject to recall biases. It is possible that some patients carried additional germline mutations in genes not included in our 56-gene panel. Although using larger panels to identify genetic mutations increases the yield of positive findings, the results are more difficult to interpret and may cause unnecessary anxiety in patients and their relatives.[47] Therefore, we designed and used a targeted gene panel including well-known tumor suppressors, genes somatically mutated in MM, and genes associated with cancer syndromes, several of which are DNA repair genes (Table 3). Among the mutations detected, we considered only those with an allele frequency less than 0.005 in the ExAC database—thus too rare to be considered polymorphisms. To further increase specificity, we used a stricter criterion (CADD score > 20) than the recommended cutoff of a CADD score greater than 15 to identify deleterious mutations.

Although we used strict criteria, it remains possible that not all these mutations contributed to tumor development; segregation of these mutations in family members with cancer, and/or loss of heterozygosity in tumor tissue, as observed for *BAP1*, will be required to definitively prove causality. The use of strict criteria and a tNGS strategy limited to genes that, when mutated, are anticipated to be deleterious, reduced the risk of false-positive results.

Table 4. Cancer Susceptibility Genes Identified as Mutated in Patients With Familial and Early-Onset Malignant Mesothelioma

Gene	Chromosome Location	Gene Category*
ARID1A	1	Tumor suppressor, chromatin regulation
ARID2	12	Tumor suppressor, chromatin regulation
BAP1	3	Tumor suppressor, DNA repair, chromatin regulation
CREBBP	16	Tumor suppressor, transcription regulation
KDR	4	Tyrosine kinase receptor
MLH1	3	Tumor suppressor, DNA repair
NCOR1	17	Chromatin regulation
RAD50	5	DNA repair
RBM6	3	Tumor suppressor, RNA processing
SETD2	3	Tumor suppressor, DNA repair, chromatin regulation
SMARCA2	9	Tumor suppressor, chromatin regulation
SMARCA4	19	Tumor suppressor, chromatin regulation
SMARCE1	17	Chromatin regulation
SMO	7	Oncogene, G-protein couple receptor
TP53	17	Tumor suppressor, DNA repair

*Gene category was listed on the basis of gene annotations provided by the National Center for Biotechnology Information's Online Mendelian Inheritance in Man (https://www.ncbi.nlm.nih.gov/omim) and The Human Gene Database, Weizmann Institute of Science (https://www.genecards.org).

However, this same strict approach increased the risk of false-negative results: deleterious mutations would not have been identified if their CADD score was less than 20 or if genes containing pathogenic mutations were not included in our gene panel. Thus, we may have underestimated the fraction of MMs linked to genetic mutations in our cohort.

Two abstracts that are relevant to our study have been presented after our initial submission. Hassan et al[48] reported that 12% of 239 patients with MM, studied at the US National Cancer Institute, carried a pathogenic germline mutation—*BAP1* was the most commonly affected gene (7%)—and that women, especially, had a second cancer diagnosis or had relatives with MM, melanoma, or breast cancer. They observed a significantly improved survival rate among patients with pleural MMs who were carriers of germline mutations. Panou et al[49] reported that 12% of 198 patients with MM studied at the University of Chicago carried pathogenic germline mutations—*BAP1* was the most common (3%)—especially those with peritoneal MM, minimal asbestos exposure, young age, and a second cancer diagnosis.

Together, these studies provide compelling evidence that there is a subset of MMs that developed in carriers of pathogenic germline mutations. Therefore, we recommend that patients with the clinical red flags denoting heritability for MM (ie, familial history of MM or other cancers and young age) should undergo genetic testing by tNGS using a gene panel similar to the one we used or a larger gene panel covering DNA repair genes and tumor suppressor genes, because these were the genes we and our colleagues[39,48,49] found most commonly mutated in the germline of patients with MM. Ideally, when economically feasible, all patients with MM should be tested. A proportion of these germline mutations may be actionable, and patients can be enrolled in targeted clinical trials. Moreover, patients with MM who carry germline mutations have a significantly improved prognosis. This knowledge is relevant to the patients, their relatives, and the physicians who have to plan their care. These patients are susceptible to development of multiple cancers, and thus must be screened, at least by a thorough history and physical examination for early detection of additional malignancies, especially UVM, CM, ccRCC, and breast cancers. Detection of these malignancies could lead to treatment with curative radical excision at an earlier stage. Furthermore, carriers of germline *BAP1* and *TP53* mutations, and of other DNA repair genes, are much more susceptible to secondary malignancies after radiation therapy; thus, whenever possible, ultrasound and whole-body magnetic resonance imaging should be used in place of computed tomography scans.[50] Finally, family members found to have inherited the same deleterious mutations will benefit from cancer screening that can be life saving.[1]

Genetic testing must be carried with proper support from genetic counselors, as thoroughly discussed in the context of pancreatic cancer, a malignancy with a similarly dismal prognosis as MM, and thus a malignancy that stands out for possible therapeutic benefits when actionable mutations are detected.[51]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Support

This work was supported by Department of Defense Grant No. CA150220 to H.Y. and M.C.; National Cancer Institute (NCI) Grant No. R01 CA198138 (M.C.); the University of Hawaii Foundation (M.C.), which received unrestricted donations to support cancer and mesothelioma research from the Melohn family endowment; from Honeywell International (M.C.); The Riviera United 4-a Cure grant (M.C. and H.Y.); the Early Detection Research Network NCI Grant No. 5U01CA111295-08 (H.I.P., H.Y.); donations from Belluck and Fox (H.I.P.); Grant-in-Aid for Scientific Research Grants KAKENHI 26460689 (Y.Y.), 25460710 (M.E.), 24590715 and 15K08658 (T.T.); and a Grant-in-Aid for Researchers from Hyogo College of Medicine, 2017 (Y.Y.).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

A Subset of Mesotheliomas With Improved Survival Occurring in Carriers of *BAP1* and Other Germline Mutations

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Acknowledgment

We thank all patients with malignant mesothelioma who kindly agreed to participate in the study.

Appendix

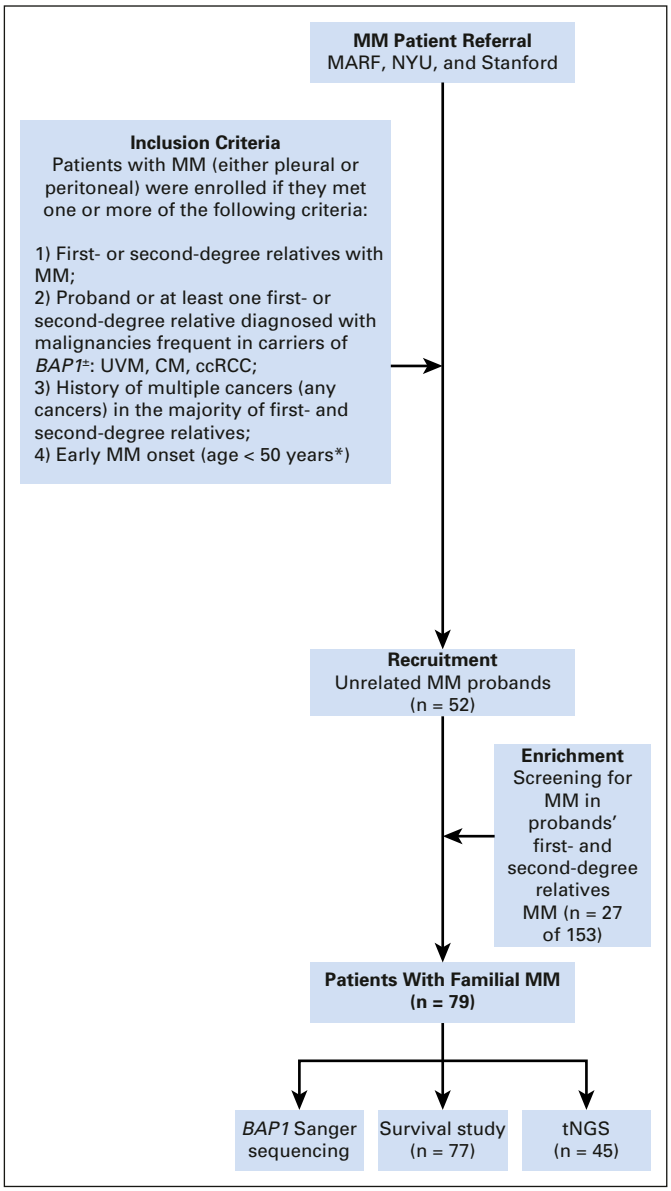

Fig A1. Study flow chart. (*) This age was chosen because the incidence of malignant mesothelioma (MM) before age 50 years is rare and suggestive of genetic predisposition or environmental exposure since childhood. ccRCC, clear-cell renal cell carcinoma; CM, cutaneous melanoma; MARF, Mesothelioma Applied Research Foundation; NYU, New York University; tNGS, targeted next-generation sequencing; UVM, uveal melanoma.

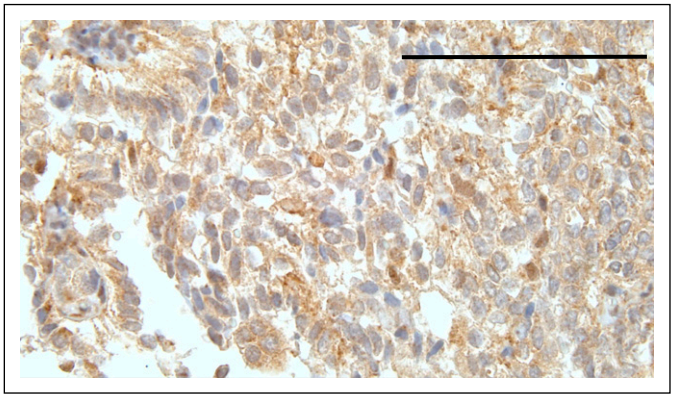

Fig A2. Representative immunostain. Note absence of nuclear staining in tumor cells, which is evidence of biallelic *BAP1* inactivation, whereas tumor-infiltrating mononuclear phagocytes show nuclear staining because they retain one *BAP1* wild-type allele.

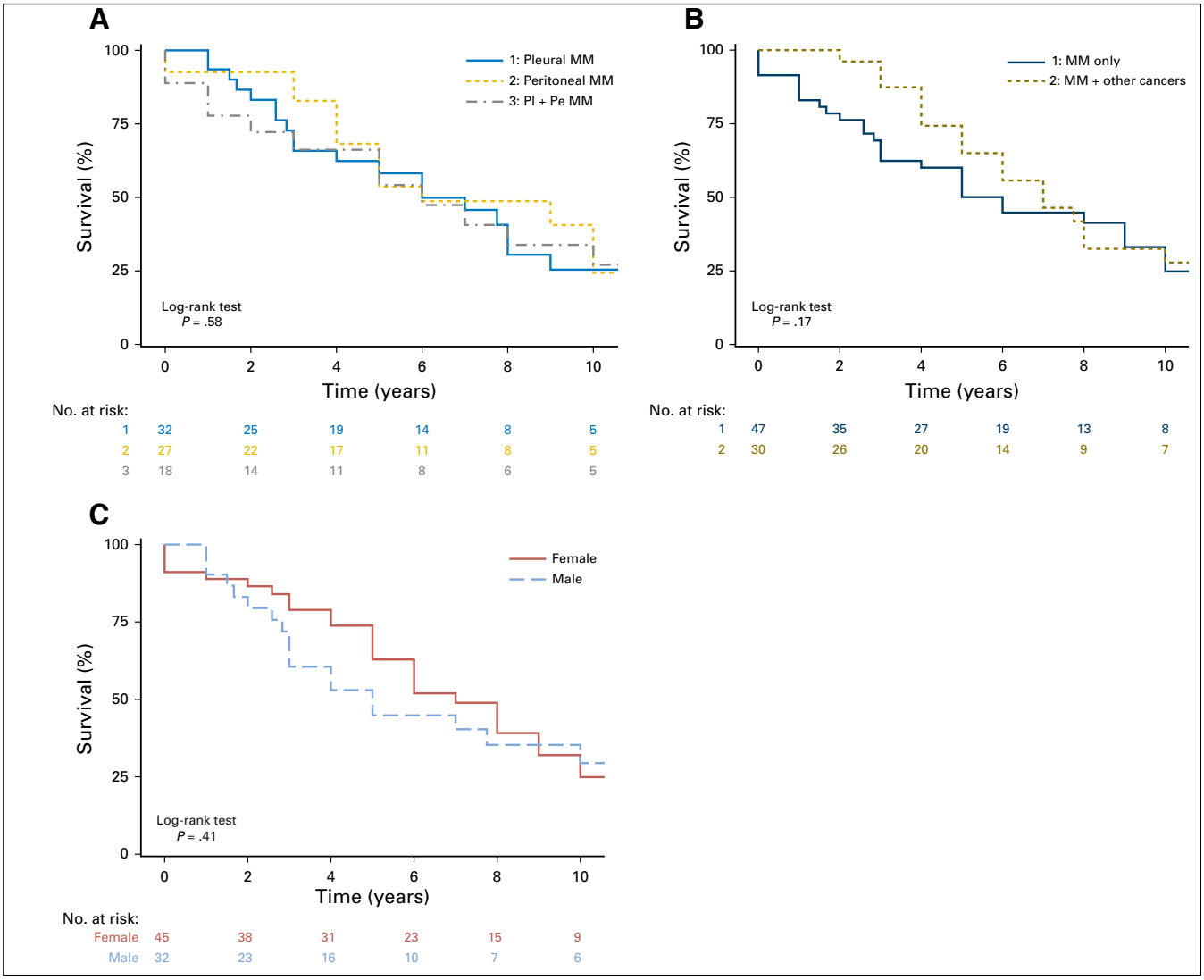

Fig A3. Kaplan-Meier familial mesothelioma survival probability versus years, with number at risk. (A) MM site: 1. pleura (median survival, 6 years; 10-year survival, 25%); 2. peritoneum (median survival, 6 years; 10-year survival, 24%); 3. both (median survival, 6 years; 10-year survival, 27%). (B) Primary relatives: 1. MM only (median survival, 6 years; 10-year survival, 25%); 2. MM plus other cancers (median survival, 7 years; 10-year survival, 28%). (C) Sex: female (median survival, 7 years; 10-year survival, 25%); male (median survival, 5 years; 10-year survival, 29%). MM, malignant mesothelioma; Pe, peritoneal; Pl, pleural.

Table A1. Targeted Next-Generation Sequencing 56 Target-Gene Panel

Target Genes	Chr	Target Genes	Chr
ARID1A	chr1	NOTCH3	chr19
ARID1B	chr6	PAX5	chr9
ARID2	chr12	PAX7	chr1
BAP1	chr3	PBRM1	chr3
BRD1	chr22	PHF10	chr6
BRD9	chr5	PRMT6	chr1
CBX2	chr17	PTCH1	chr9
CDKN2A	chr9	RAD50	chr5
CDKN2B	chr9	RBM5	chr3
CREBBP	chr16	RBM6	chr3
CTNNB1	chr3	SAV1	chr14
DISP1	chr1	SEMA3B	chr3
E2F1	chr20	SETBP1	chr18
E2F2	chr1	SETD2	chr3
E2F7	chr12	SMARCA2	chr9
EP300	chr22	SMARCA4	chr19
GNL3	chr3	SMARCB1	chr22
GTF2B	chr1	SMARCC1	chr3
GTF2H5	chr6	SMARCC2	chr12
KDM5C	chrX	SMARCD1	chr12
KDM6A	chrX	SMARCD2	chr17
KDR	chr4	SMARCD3	chr7
KIT	chr4	SMARCE1	chr17
MLH1	chr3	SMO	chr7
NCOR1	chr17	SS18	chr18
NF2	chr22	TBP	chr6
NOTCH1	chr9	TP53	chr17
NOTCH2	chr1	TUSC2	chr3

Abbreviation: Chr, chromosome.