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PRAME as a Potential Target for Immunotherapy in Metastatic Uveal Melanoma

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Abstract

IMPORTANCE—Uveal melanoma (UM) is an intraocular primary malignant neoplasm that often gives rise to metastatic disease for which there are no effective therapies. A substantial proportion of UMs express the cancer-testis antigen PRAME (preferentially expressed antigen in melanoma), which can potentially be targeted by adoptive T-cell therapy.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Harbour reported being the inventor of intellectual property related to gene expression profiling and *BAP1* mutations in uveal melanoma and receiving royalties from its commercialization and serving as a paid consultant for Castle Biosciences, licensee of this technology. No other disclosures were reported.

OBJECTIVE—To determine whether there may be a rationale for PRAME-directed T-cell therapy for metastatic UM.

DESIGN, SETTING, AND PARTICIPANTS—An experimental study using a retrospective cohort of 64 patients with UM (median follow-up, 62 months) was conducted from January 8, 2015, to November 20, 2016, at the Leiden University Medical Center. Clinical, histopathologic, and genetic parameters were compared between 64 *PRAME*-positive and *PRAME*-negative UMs. HLA class I restricted, PRAME-specific T cells were stimulated with UM cell lines to measure their antigen-specific reactivity against these cell lines, which were analyzed for *PRAME* expression by real-time quantitative polymerase chain reaction. Uveal melanoma metastases from 16 unrelated patients were assessed for *PRAME* expression by messenger RNA fluorescence in situ hybridization and for HLA class I expression by immunofluorescence staining.

MAIN OUTCOMES AND MEASURES—Interferon γ production for antigen-specific reactivity and detection of *PRAME* and HLA class I expression in primary and metastatic UM.

RESULTS—Of the 64 patients in the study (31 women and 33 men; mean [SD] age at the time of enucleation, 60.6 [15.6] years), *PRAME* expression was negative in 35 primary UMs and positive in 29 primary UMs. Positive *PRAME* expression was associated with a high largest basal diameter (15.0 vs 12.0 mm; $P = .005$), ciliary body involvement (59% vs 26%; $P = .008$), and amplification of chromosome 8q (66% vs 23%; $P = .002$). PRAME-specific T cells reacted against 4 of 7 UM cell lines, demonstrating that T-cell reactivity correlated with *PRAME* expression. Metastatic UM samples were positive for *PRAME* messenger RNA in 11 of 16 patients and for HLA class I in 10 of 16 patients, with 8 of 16 patients demonstrating coexpression of both *PRAME* and HLA class I.

CONCLUSIONS AND RELEVANCE—*PRAME* is expressed in many primary and metastatic UMs, and about half of the metastatic UMs coexpress *PRAME* and HLA class I. The finding that PRAME-specific T cells in this study reacted against *PRAME*-positive UM cell lines suggests a potential role for PRAME-directed immunotherapy for selected patients with metastatic UM.

Uveal melanoma (UM) is a pigment cell–derived malignant neoplasm that occurs in the eye and can lead to metastases, usually affecting the liver. Several characteristics are associated with the development of metastases, such as large tumor size, the presence of epithelioid cells, the loss of 1 chromosome 3 and the presence of additional copies of chromosome 8q,[1,2] and a specific gene expression profile known as class 2.[3,4] In addition, high-risk tumors are characterized by an inflammatory phenotype, with high numbers of infiltrating $CD4^+$ and $CD8^+$ T cells, macrophages, and increased expression of HLA class I and II.[5–9]

Treatment options currently used to treat metastatic UM include liver-directed chemotherapy and systemic targeted and immune therapies.[10] However, these treatments have resulted in durable responses in very few patients.[11] The potential efficacy of immunotherapy has been limited, presumably owing to the small number of mutations leading to neoantigen expression in UM; immunotherapy with checkpoint inhibitors has shown very low response rates in metastatic UM.[12–14]

Another possible treatment option for patients with metastasized UM is targeted therapy with T cells directed against tumor-associated antigens. Recently, Field et al[15] reported that the cancer-testis antigen PRAME (preferentially expressed antigen of melanoma) is

expressed in many primary UMs and is a marker for increased risk of metastasis in Class 1 and disomy 3 UMs. PRAME was first identified as a tumor-associated antigen through analysis of the specificity of tumor-reactive T-cell clones derived from a patient with metastatic cutaneous melanoma.[16] Subsequently, it has been shown that *PRAME* (Ensembl: ENST00000398741.5) is expressed in many malignant neoplasms, including cutaneous melanoma, breast carcinoma, non–small cell lung cancer, and leukemia,[16,17] whereas normal healthy tissues express minimal or no *PRAME*, with the exception of the testis and endometrium.[16] Griffioen et al[18] have shown that PRAME-specific $CD8^+$ T cells isolated from healthy individuals and patients with advanced melanoma were able to recognize and lyse cells expressing HLA class I and high levels of *PRAME*. However, these T cells had a low avidity for *PRAME*-expressing tumor cells, and their T-cell receptors (TCRs) will therefore unlikely be useful for therapeutic applications. A previous study isolated PRAME-specific HLA-A2 restricted T-cell clones, which exhibited a high specificity and reactivity for *PRAME*, derived from a patient who underwent an HLA-A2 mismatched stem cell transplant.[19] These PRAME-specific T cells were able to lyse multiple *PRAME*-positive malignant cell lines, including cell lines derived from cutaneous melanoma, lung cancer, breast cancer, and acute myeloid leukemia. It was demonstrated by TCR gene transfer that the high-affinity, PRAME-specific TCR could potentially be used for PRAME-TCR gene therapy. A clinical trial to evaluate PRAME-TCR gene therapy is being initiated. To determine whether patients with metastatic UM may be potential candidates for PRAME-TCR gene therapy, we expanded our analysis to UM and tested whether *PRAME* is expressed in primary and metastatic UM and whether high-affinity, PRAME-specific T cells can recognize *PRAME*-expressing UM cell lines.

Methods

Study Population

Formalin-fixed, paraffin-embedded (FFPE), and fresh-frozen tissue was obtained from 64 patients with primary UM who underwent enucleation at the Leiden University Medical Center (Leiden, the Netherlands) between September 21, 1999, and October 6, 2008, with a median follow-up of 62 months (range, 5–181 months). Patient DNA and RNA were isolated from fresh-frozen tissues for chromosome and gene expression analyses. Each tumor sample was processed for histopathologic evaluation. Patient medical records were scrutinized for clinical and histopathologic features, including age at enucleation, largest basal diameter (in millimeters), thickness (in millimeters), extraocular extension, ciliary body involvement, cell type, and mitotic count. Survival data and information on cause of death were obtained from patient medical records and from the Integral Cancer Center West, and they were updated in 2015. Follow-up time is indicated as the time from enucleation until death or last follow-up. Formalin-fixed, paraffin-embedded tissue blocks containing UM metastases from 17 unrelated patients were collected from our pathological archives for HLA class I staining and messenger RNA (mRNA) fluorescence in situ hybridization (FISH) because metastatic samples were not available from the original cohort. Because positive controls demonstrated a poor quality of the FFPE tissue sample from 1 patient, 16 cases were analyzed further. Tumor material was handled according to the Dutch National Ethical Guidelines (Code for Proper Secondary Use of Human Tissue) and the tenets of the Declaration of Helsinki.[20] The

Medical Ethics Committee of the Leiden University Medical Center approved this study and waived the need for informed consent, following the regulations laid down for use of patient material according to the Federation of Medical Scientific Societies (FEDERA).

DNA and Gene Expression Analysis

From 64 fresh-frozen specimens, DNA for single-nucleotide polymorphism and copy number analyses was extracted with the QIAmp DNA Mini kit (Qiagen). Single-nucleotide polymorphism analysis was performed with the Affymetrix 250K_NSP-chip and Affymetrix Cytosc an HD chip (Affymetrix) to assess aberrations in chromosomes 3 and 6. Information on the copy number of 8q was obtained by droplet digital polymerase chain reaction. The RNA for gene expression profiling was isolated with the RNeasy mini Kit (Qiagen). *PRAME* RNA expression was measured on the Illumina HT-12v4 chip (Illumina) using probe ILMN_1700031.[21] Expression data for disomy 3 UMs were included in a previous publication.[15]

Recognition of UM Cell Lines By PRAME-Specific T Cells

Uveal melanoma cell lines were cultured in Roswell Park Memorial Institute medium 1640 supplemented with fetal calf serum, 10%, glutamine, 1%, penicillin, 2%, and streptomycin (Gibco; Thermo Fisher Scientific Inc) at 37°C and 5% carbon dioxide. We used the following primary tumor-derived cell lines: 92.1,[22] Mel202, Mel270, Mel285, and Mel290,[23] as well as cell lines OMM2.3 and OMM2.5, which were derived from metastases of the same patient from which cell line Mel270 was derived.[24] T-cell clones and UM cell lines were coincubated at a responder to stimulator ratio of 1:4. We incubated 5000 T cells with 20 000 tumor cells in a round-bottomed, 96-well plate for 18 hours. Two PRAME-specific T-cell clones (HSS1 and HSS3) recognizing the SLLQHLIGL epitope of PRAME in the context of HLA-A*02:01 were used.[19] If HLA-A2 was not present in the UM cell lines, we introduced *HLA-A2* (European Nucleotide Archive: AF055066.1) using retroviral vectors.[25] To confirm HLA-A2 expression on the UM cell lines, we used the HLA-A2 restricted T-cell clone HSS12, which recognizes peptide FTWEGLYNV from the ubiquitously expressed gene *USP11* (Ensembl: ENST00000377107.6).[26] Our negative control clone was pp65-A2, which is also HLA-A2 restricted but recognizes a peptide from the *pp65* (European Nucleotide Archive: EF531301.1) gene of cytomegalovirus that is not expressed on the UM cell lines.[27] After 18 hours of coincubation, supernatants were harvested and interferon γ (IFN-γ) secretion was measured by enzyme-linked immunosorbent assay (Sanquin Reagents).

Detection and Scoring of *PRAME* mRNA in UM Cell Lines and FFPE Tissue Sections

Detailed information about detection of *PRAME* expression by real-time quantitative polymerase chain reaction on UM cell lines and by mRNA FISH in primary as well as metastasized FFPE tissue sections is described in the eAppendix in the Supplement.

HLA Class I Staining and Scoring

Immunofluorescence staining for expression of human HLA-A, HLA-B/C, and β2-microglobulin (β2M) was performed on paraffin-embedded samples of metastases as

previously described,[28] and we used the scoring system of Ruiter et al[29] and other studies[30,31] (eAppendix in the Supplement).

Statistical Analysis

Statistical analysis was performed with SPSS, version 20.0.0 (IBM Corp). *PRAME* gene expression was dichotomized as negative and positive. Clinical, histopathologic, and genetic parameters were compared between both groups using the Pearson χ^2 test for categorical prognostic parameters and the Mann-Whitney *U* test for continuous prognostic parameters. The Kaplan-Meier curve and log-rank test were used to perform disease-specific survival analysis for patients with primary UM with negative and positive *PRAME* expression. Death due to metastasis was considered an event. Patients who died owing to another cause or an unknown cause were censored.

Results

Distribution of *PRAME* Expression in Primary UM

PRAME gene expression was analyzed in 64 primary UMs using an RNA expression microarray (Figure 1A). The mean (SD) age of the patients at enucleation was 60.6 (15.6) years, and 33 of the 64 patients (52%) were men. When expression values were plotted from lowest to highest, an inflection point in the slope was noted at the sample with expression of 7.23 Illumina units, so we took this point as the threshold for positive *PRAME* expression. Tumors with expression less than 7.23 Illumina units were categorized as *PRAME*-negative UM (n = 35), and those with expression of 7.23 Illumina units or more were categorized as *PRAME*-positive UM (n = 29).

We compared the clinical, histopathologic, and chromosome data between the 2 groups of patients with tumors (Table 1). Both groups did not differ in age or sex. *PRAME* expression was associated with prognostically poor tumor characteristics; *PRAME*-positive tumors had the largest median basal diameter (15.0 vs 12.0 mm; $P = .005$) and a more frequent involvement of the ciliary body (59% vs 26%; $P = .008$) compared with *PRAME*-negative tumors. Of the 29 *PRAME*-positive tumors, 21 (72%) showed monosomy of chromosome 3, whereas this was the case in 20 (57%) of the 35 *PRAME*-negative tumors ($P = .21$). *PRAME* expression correlated with amplification of chromosome 8q (66% vs 23%; $P = .002$). Tumors with chromosome 8q copies were categorized as follows: a copy number between 1.9 and 2.1 was categorized as normal, between 2.2 and 3.1 as gain, and more than 3.1 as amplification of chromosome 8q. When comparing the Kaplan-Meier survival curves, we found that patients with *PRAME*-positive tumors had a shorter disease-specific survival than patients with *PRAME*-negative tumors (median survival, 47 vs 88 months; $P = .02$) (Figure 1C).

Recognizing UM Cell Lines by PRAME-Specific T-Cell Clones

Two previously identified PRAME-specific T-cell clones (HSS1 and HSS3) were used to determine whether UM cell lines can potentially be recognized by PRAME-specific T cells.[19] First, *PRAME*-dependent recognition of the PRAME-specific T-cell clones was demonstrated by introducing full-length *PRAME* into HLA-A2–positive cell line SW480.

As demonstrated in Figure 2A, the PRAME-specific T-cell clones do not recognize the HLA-A2–negative, *PRAME*-negative SW480 cells. However, retroviral introduction of *PRAME* into SW480 resulted in efficient recognition, comparable to HLA-A2–positive, *PRAME*-positive LCL-JY and K562+A2. Before the UM cell lines were tested for recognition by the PRAME-specific T-cell clones, the HLA-A2 expression of the UM cell lines was measured by fluorescence-activated cell sorting analysis using specific antibodies directed against HLA class I (W632) and HLA-A2 (BB7.2). We confirmed that only UM cell line Mel290 was HLA-A2 positive. The remaining UM cell lines were HLA-A2 negative and were therefore retrovirally transduced with HLA-A*02:01. After retroviral transduction, all 7 UM cell lines were recognized by the USP11-specific T-cell clone HSS12, confirming that HLA-A*0201 was now expressed on all UM cell lines. The results shown in Figure 2B demonstrate that 4 of 7 UM cell lines were efficiently recognized by the PRAME-specific T-cell clones HSS1 and HSS3, as coincubation led to production of IFN-γ by both of the PRAME-specific T-cell clones. Using real-time quantitative polymerase chain reaction, we confirmed that the 4 UM cell lines recognized by the PRAME-specific T-cell clones expressed *PRAME* (Figure 2C). These data demonstrate that *PRAME*-positive UM cell lines can be recognized by PRAME-specific T cells and that the recognition potential of the T cells correlated with *PRAME* expression in these cell lines.

PRAME Expression in UM Metastases

Next, we analyzed *PRAME* expression in 16 UM metastatic samples using an mRNA FISH assay to stain *PRAME* mRNA in FFPE tissue sections. We validated this technique on primary UM with known *PRAME* expression levels. Two consecutive FFPE tissue sections from 14 primary tumors were hybridized with both *GAPDH* (Ensembl: ENST00000229239.9) and *PRAME* in a dye-mirrored way. One section of each tumor was stained using the *GAPDH* probe set labeled with the dye Quasar 570 together with the *PRAME* probe set labeled with Quasar 670, while the other section was stained with the same probe sets but labeled the other way around. The presence of *PRAME* was determined as described in the eAppendix in the Supplement by 2 independent observers (M.H.M.H. and S.J.L.), with a κ score of 0.857 (Figure 1B). All tumors that were *PRAME* negative according to the Illumina data (<7.23) were also scored as *PRAME* negative by the mRNA FISH technique. All tumors with a *PRAME* expression level greater than 10 according to the Illumina data were scored positive by the mRNA FISH technique. We subsequently tested *PRAME* by mRNA FISH in metastases from 16 patients with UM. *GAPDH* but not *PRAME* expression was seen in tumors from 5 patients, whereas *GAPDH* as well as *PRAME* expression was present in metastases from 11 patients. An example of negative and positive *PRAME* expression by mRNA FISH is shown in Figure 3. In 2 patients, multiple metastases were available (2 in 1 patient and 4 in the second patient), and *PRAME* expression was identical between the different metastases of each patient. The results of all patients together demonstrate expression of *PRAME* in metastatic UM in 11 of 16 patients.

HLA Class I Expression in UM Metastases

For TCR-mediated immunotherapeutic approaches, target antigens are recognized in the context of HLA class I molecules. It is therefore essential that not only *PRAME* but also HLA class I is expressed on UM metastases to induce a potent immune reactivity of TCR-

modified, PRAME-specific T cells. We therefore analyzed HLA class I expression by triple immunofluorescence HLA class I staining on the FFPE tissue sections of the 16 patients with UM metastases. When multiple metastases from a single patient were available, HLA class I expression patterns were similar between different metastases. For 10 of the 16 patients with UM metastases, expression of HLA-A and HLA-B/C, as well as β2M expression, was high and homogeneous. In 2 patients, the staining for HLA-A and HLA-B/C, as well as β2M, was weak but positive, whereas in 3 patients, expression of HLA-A and HLA-B/C, as well as of β2M, was negative. Overall, UM metastases from 10 of 16 patients (63%) demonstrated a high HLA class I expression.

As mentioned previously, *PRAME* expression was observed in 11 of 16 patients (69%). In 8 of the 16 patients, concomitant expression of *PRAME* and HLA class I was observed in the metastases, suggesting that half of metastatic UMs could be positive for both *PRAME* and HLA class I (Table 2) and, therefore, can potentially be candidates for treatment with PRAME-TCR gene therapy.

Discussion

In the present study, we found that *PRAME* was expressed at the RNA level in 45% of primary UMs (29 of 64), and *PRAME* expression was associated with ciliary body involvement, largest basal diameter, and amplification of chromosome 8q. *PRAME* expression occurred in disomy 3 as well as in monosomy 3 tumors, which confirms the findings reported by Field et al.[32] Among all primary UMs, *PRAME* expression was associated with a poorer disease-specific survival.

We have demonstrated that the high-affinity, PRAME-specific T cells are able to efficiently recognize *PRAME*-positive UM cell lines. Furthermore, we demonstrated that 50% of the 16 patients with UM have metastases that express both *PRAME* and HLA class I. In 2 patients from whom multiple metastases were available, we were able to demonstrate that *PRAME* expression was identical between the different metastases of each patient.

PRAME-TCR–specific T-cell therapy is directed against all patients with metastatic UM with *PRAME*-positive metastases, irrespective of the size of the primary tumor. The PRAME-TCR–engineered T cells used in the upcoming gene therapy study to treat acute myeloid leukemia and metastatic sarcoma recognize and lyse tumor cells when *PRAME* is processed and presented on the tumor cell surface in the context of HLA-A*02:01.[19] Therefore, this gene therapy study will be available for all HLA-A*02:01–positive patients, which comprise 50% of the Western European and North American population. In patients with primary UM, the frequency of HLA-A*02:01 was also around 50%.[33] We are currently also searching for other high-affinity, PRAME-specific TCRs restricted to other HLA class I molecules to broaden the PRAME-TCR gene therapy options. If the clinical studies using this specific TCR demonstrate promising results, we will initiate clinical studies with these newly identified PRAME-TCRs. For patients with metastatic UM, we will perform a biopsy of the metastases and analyze whether *PRAME* is present to determine whether to treat them with PRAME-TCR gene therapy. Any effect of this treatment on the primary tumor is

unlikely because most of these patients have already undergone enucleation or radiation, and therefore less or no primary tissue should remain.

The advantage of PRAME-TCR gene therapy is that a broad group of patients with different malignant neoplasms can be treated with this TCR therapy as long as the patient is HLA-A2 positive and the tumor expresses *PRAME*. The PRAME-specific T cells, however, may exhibit some reactivity against mature dendritic cells and kidney epithelial cells, which express *PRAME* at a lower level.[19] This reactivity could lead to nephrotoxicity and long-term depletion of mature dendritic cells when the PRAME-TCR therapy is applied in a clinical setting. Therefore, the PRAME-TCR–engineered T cells will be equipped with the suicide switch iCasp9 to eliminate reactive T cells in case of adverse effects. Successful elimination of iCasp9-transduced T cells has been reported in patients with graft-vs-host disease after a haploidentical stem cell transplant, leading to resolution of the graft-vs-host disease by elimination of alloreactive T cells in peripheral blood and the central nervous system.[34]

Limitations

This study has several limitations. First, we tested *PRAME* expression in a small cohort of patients with UM metastases because no other adequate FFPE tissues were available. However, we were still able to detect *PRAME* in 11 of 16 samples and in 8 of 16 patients together with HLA class I, thereby showing that concomitant expression of *PRAME* and HLA class I is present in UM metastases. Second, 2 of the 4 tumors with intermediate *PRAME* values (7.23–8.13) were positive with mRNA FISH, and the other 2 were scored negative (Figure 1B), thereby showing that the sensitivity of our PRAME probes might not be high enough to demonstrate *PRAME* gene expression at low levels with 100% sensitivity. The detection of *PRAME* might be degraded in these tissues because we used FFPE samples. Most important, however, no tumors with a negative expression in the microarray were scored by the mRNA FISH as *PRAME* positive, thereby providing high specificity.

Conclusions

We found that 45% of the tested primary UMs express *PRAME* and that *PRAME* expression in these patients is associated with known risk factors for metastasis and with poorer overall survival. We found that 50% of the analyzed UM metastases expressed both *PRAME* and HLA class I and that high-affinity, PRAME-specific T cells were efficiently recognizing UM cell lines expressing *PRAME*. These findings provide supportive evidence for including patients with metastatic UM in clinical trials using PRAME-TCR gene therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

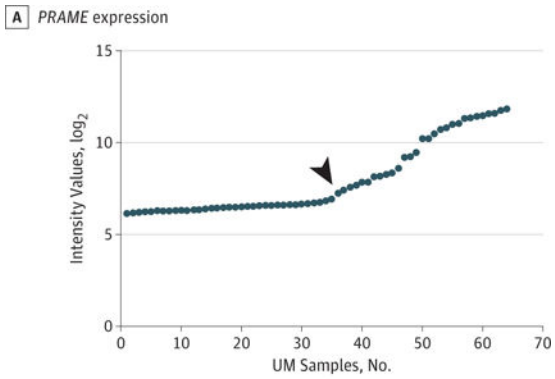
Is there a rationale for preferentially expressed antigen in melanoma (PRAME)–specific T-cell therapy for patients with metastatic uveal melanoma?

Findings

This experimental cohort study found that *PRAME* is expressed in primary uveal melanoma and its metastases, as is HLA class I. Also, PRAME-specific T cells showed reactivity against *PRAME*-positive uveal melanoma cell lines.

Meaning

For patients with metastatic uveal melanoma, the use of PRAME-specific T-cell therapy may be helpful.



B Illumina expression data and mRNA FISH scoring

Tumor	*PRAME* Expression Value With Illumina, $\log_2$	mRNA FISH *PRAME* Expression
1	6.4	–
2	6.5	–
3	6.6	–
4	6.6	–
5	6.7	–
6	7.2	+
7	7.7	–
8	7.9	+
9	8.1	–
10	10.2	+
11	11.3	+
12	11.3	+
13	11.4	+
14	11.8	+

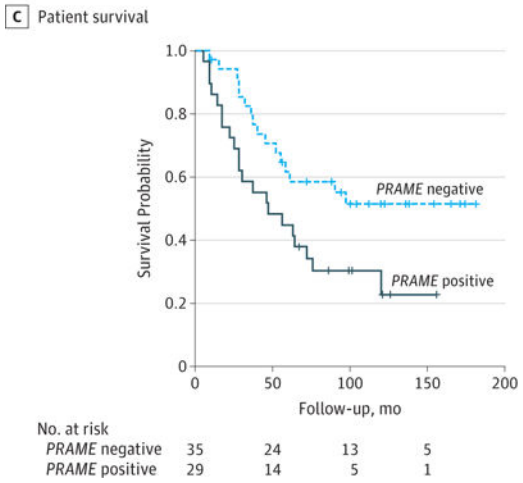
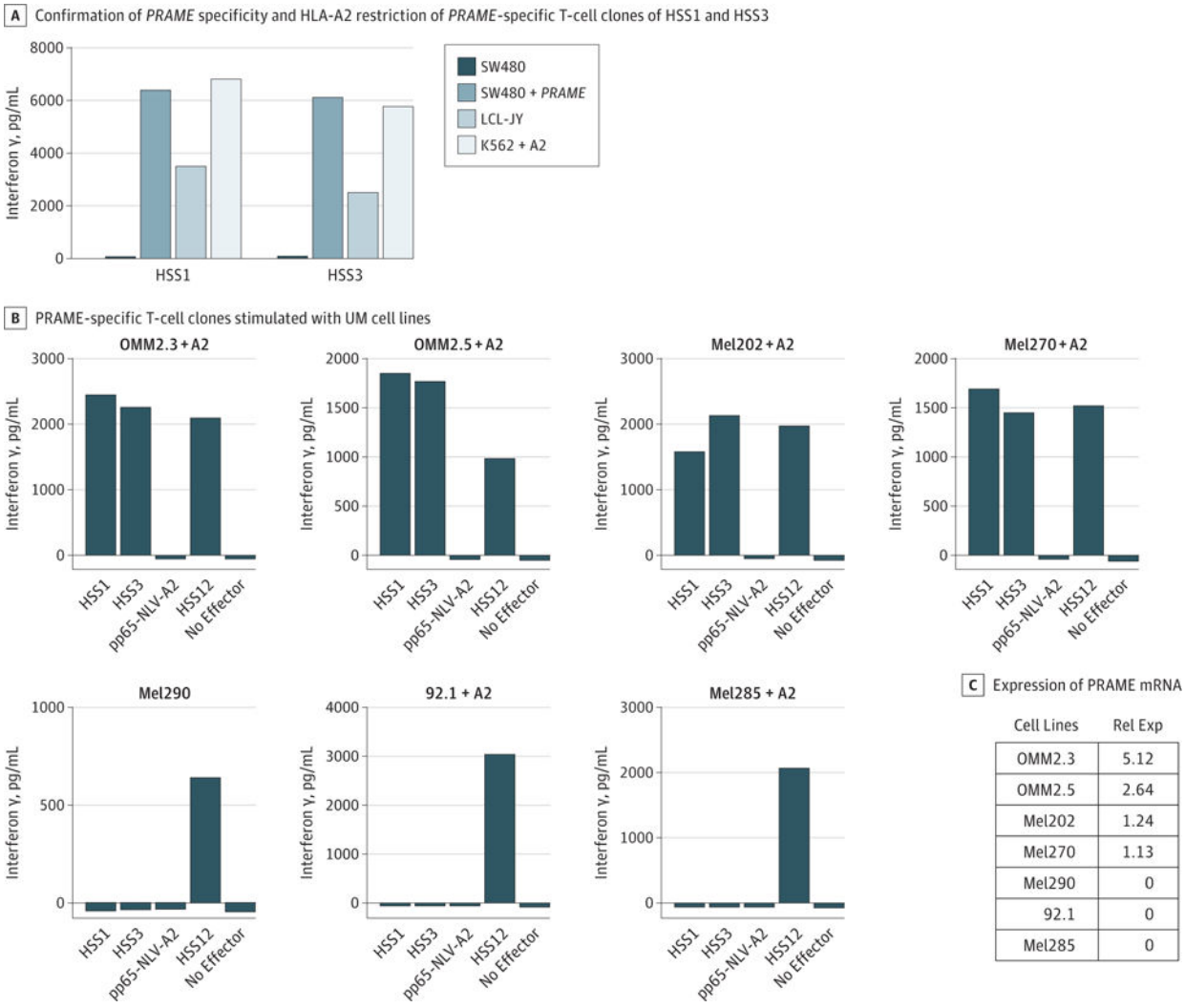


Figure 1. *PRAME* Expression in Primary Uveal Melanoma (UM) and Correlation With Survival

A, *PRAME* expression determined using 2 different probes in 64 cases of UM using an Illumina HT-12v4 microarray. Using probe ILMN_1700031, tumors are dichotomized into negative and positive. The samples on the right of the arrow are categorized as positive *PRAME* expression and the samples on the left of the arrow are categorized as negative *PRAME* expression. B, Illumina expression data and messenger RNA (mRNA) fluorescence in situ hybridization (FISH) scoring for 14 primary UMs demonstrating specificity and sensitivity of the *PRAME* probe sets. The κ value for mRNA FISH between both observers was 0.857. Plus sign indicates positive expression; minus sign, negative expression. C, Survival curve of patients with negative and positive *PRAME*-expressing primary UM.



Cell Lines	Rel Exp
OMM2.3	5.12
OMM2.5	2.64
Mel202	1.24
Mel270	1.13
Mel290	0
92.1	0
Mel285	0

Figure 2. Recognition of *PRAME*-Positive Uveal Melanoma (UM) Cell Lines by PRAME-Specific T Cells

A, PRAME-specific T-cell clones HSS1 and HSS3 were stimulated with the *PRAME*-negative HLA-A2–positive cell line SW480, SW480 retrovirally transduced with *PRAME* (SW480+PRAME), HLA-A2–positive LCL-JY cell line expressing low levels of *PRAME*, and HLA-A2 transduced K562 cell line (K562+A2). B, PRAME-specific T-cell clones HSS1 and HSS3 were stimulated with different UM cell lines. Uveal melanoma cell lines negative for HLA-A2 were retrovirally transduced with HLA-A2 (+ A2). Clone pp65-A2 recognizes a peptide of pp65 in the context of HLA-A2 and serves as a negative control. Clone HSS12 is reactive against the household gene *USP11*, and was used to confirm that the UM cell lines are HLA-A2 positive. Interferon γ production was measured after 18 hours of co-culture by standard enzyme-linked immunosorbent assay. Experiments were carried out in duplicate. C, The expression of *PRAME* messenger RNA (mRNA) in different cells was measured by real-time quantitative polymerase chain reaction and is shown as fold expression of the *PRAME*-positive cutaneous melanoma cell line Mel1.14, which was set to 1. Rel Exp indicates relative expression of *PRAME*.

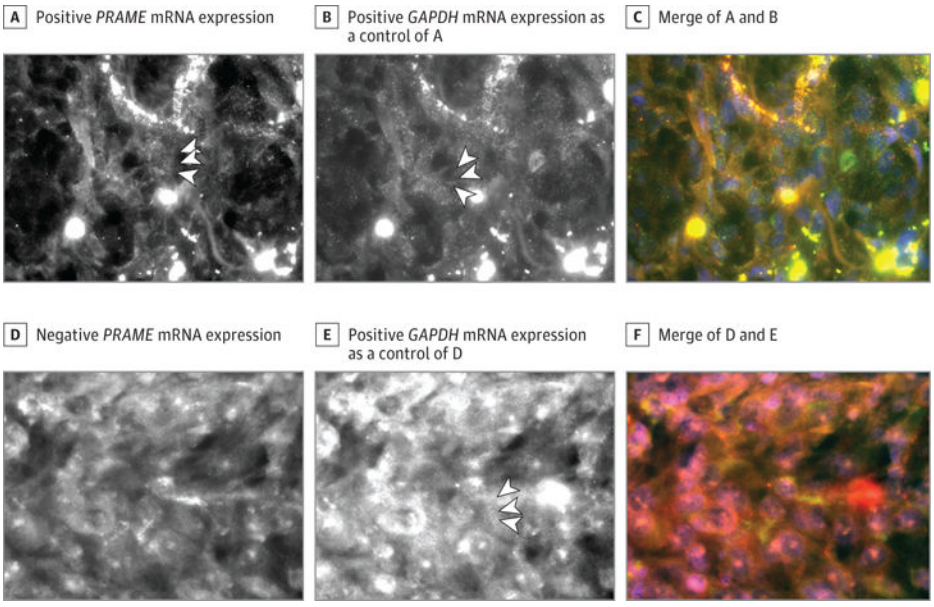


Figure 3. *PRAME* Expression by Messenger RNA (mRNA) Fluorescence In Situ Hybridization (FISH) in Uveal Melanoma (UM) Metastases

A, *PRAME* mRNA expression of *PRAME*-positive UM metastasis; *PRAME* mRNA is shown as small white speckles (arrowheads). B, *GAPDH* mRNA expression of UM metastasis shown in A; *GAPDH* mRNA is shown as small white speckles (arrowheads). C, Merged images with 4′,6-diamidino-2-phenylindole dilactate (DAPI) (blue) of A and B; *PRAME* is shown in small green dots and *GAPDH* in small red dots. D, *PRAME*-negative UM metastasis. E, *GAPDH* mRNA expression of UM metastasis shown in D; *GAPDH* mRNA is shown as small white speckles (arrowheads). F, Merged images with DAPI (blue) of D and E. *GAPDH* is shown in small red dots.

Table 1

Comparison of Clinical, Histopathologic, and Genetic Features Between *PRAME*-Negative and *PRAME*-Positive Primary Uveal Melanoma

Clinical and Histopathologic Feature	Patients, No. (%)[a]		P Value
	PRAME-Negative Tumors (n = 35)	*PRAME*-Positive Tumors (n = 29)	
Sex			
Male	19 (54)	14 (48)	.63[b]
Female	16 (46)	15 (52)	
Age at enucleation, median (range), y	58.9 (12.8–84.8)	62.7 (33.4–88.4)	.77[c]
Largest basal diameter, median (range), mm	12.0 (8–20)	15.0 (9–30)	.005[c]
Thickness, median (range), mm	7.0 (2–12)	9.0 (2–12)	.30[c]
Mitotic count, median (range)	5.0 (1–33)	5.5 (0–20)	.66[c]
Ciliary body involvement			
No	26 (74)	12 (41)	.008[b]
Yes	9 (26)	17 (59)	
Cell type			
Spindle	14 (40)	8 (28)	.30[b]
Mixed or epithelioid	21 (60)	21 (72)	
Extraocular extension			
None or superficial	23 (66)	17 (59)	.56[b]
Deep, total, or episcleral	12 (34)	12 (41)	
TNM staging			
I–IIB	26 (74)	15 (52)	.06[b]
IIIA–IIIC	9 (26)	14 (48)	
Metastases			
No	19 (54)	8 (28)	.03[b]
Yes	16 (46)	21 (72)	
Chromosome			
3 status			
Disomy	15 (43)	8 (28)	.21[b]
Monosomy	20 (57)	21 (72)	
8q status			
Normal	11 (31)	3 (10)	
Gain of 8q	16 (46)	7 (24)	.002[b]
Amplification of 8q	8 (23)	19 (66)	
6p status			
Normal	23 (66)	20 (69)	.78[b]
Gain of 6p	12 (34)	9 (31)	

[a] Percentages are rounded and may not total 100.

[b] Pearson χ^2 test.

[c] Mann-Whitney *U* test.

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Table 2

PRAME mRNA FISH Expression and HLA Class I Expression in UM Metastases

			Staining Intensity/% Positive Tumor Cells[a]			
Patient No.	**Location of Metastases**	***PRAME* mRNA FISH Expression**	**β2M**	**HLA-A**	**HLA-B/C**	**HLA Class I Expression**
1	Liver	Positive	Unknown	Unknown	Unknown	Unknown
2	Subcutaneous in breast	Positive	3/5	3/5	3/5	Positive
3	Liver	Positive	3/5	3/5	3/5	Positive
4	Cutaneous	Negative	3/5	3/5	3/5	Positive
	Cutaneous	Negative	3/5	3/5	3/5	Positive
	Cutaneous	Negative	3/5	3/5	3/5	Positive
	Cutaneous	Negative	3/5	3/5	3/5	Positive
	Liver	Negative	3/5	3/5	3/5	Positive
5	Liver	Positive	3/5	3/5	3/5	Positive
6	Liver	Positive	3/5	3/5	3/5	Positive
7	Liver	Positive	3/5	3/5	3/5	Positive
8	Liver	Negative	3/5	3/5	3/4	Positive
9	Bone	Positive	3/5	3/5	3/5	Positive
	Bone	Positive	3/5	3/5	3/5	Positive
10	Liver	Negative	0	Unknown	0	Negative
11	Liver	Negative	1/4	1/4	1/4	Weak
12	Mesentery of transverse colon	Negative	0	0	0	Negative
13	Liver	Positive	0	0	0	Negative
14	Liver	Positive	3/4	3/4	3/4	Positive
15	Lung	Positive	2/1	2/1	2/1	Weak
16	Liver	Positive	3/5	3/5	3/5	Positive

Abbreviations: β2M, β2-microglobulin; FISH, fluorescence in situ hybridization; mRNA, messenger RNA; UM, uveal melanoma.

[a]The staining intensity scoring system is described in the eAppendix in the Supplement.