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Improved Uveal Melanoma Copy Number Subtypes Including an Ultra–High-Risk Group

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Purpose: To evaluate the clinical relevance of low-frequency copy number aberrations (CNAs) in uveal melanoma (UM) and to discern residual genomic and clinical heterogeneity within established molecular subtypes based on genome-wide CNA profiling of 921 primary tumors.

Design: Retrospective single-center case series.

Participants: Patients with primary UM referred for genetic testing between 2008 and 2016 (n = 921). The Cancer Genome Atlas cohort with clinical outcome data available (n = 70) was used to validate findings.

Methods: Genome-wide CNAs were generated for primary tumors from 921 patients and for 19 metastatic UM (mUM) in the liver. Of the 921 patients, metastatic outcome was known for 678 patients with a median time to metastasis of 4.5 years. The primary tumors were processed on the Affymetrix arrays SNP-5.0 (n = 140), SNP-6.0 (n = 359), or CytoScanHD (n = 422), and the metastatic tumors on the CytoScanHD array (n = 19). Recurrent CNAs were identified, and the prognostic effect of individual CNAs and multiple CNA clustering strategies, including more specific molecular subgroups with rare CNAs, were evaluated.

Main Outcome Measures: CNA recurrence, and effect of CNAs and derived molecular subtypes on metastatic-free survival.

Results: Genomic profiling revealed CNAs associated with risk of metastasis and demonstrated a strong association between chromosomal instability and patient prognosis. Using standard prognostic CNAs, 6 clusters were detected, and inclusion of chromosome 16q deletion revealed an additional cluster. Of these 7 genomic clusters, 5 patient groups showed distinct rates of metastasis, indicating that different genomic patterns can have similar patient outcomes. A small group of patients with a significantly higher rate of metastasis was characterized by monosomy 3, 8q amplification, and deletion of 1p or 16q. Although this ultra–high-risk group accounts for only 7% of this cohort, 88% demonstrated metastasis within 4 years, compared with 45% in the second-highest risk group.

Conclusions: These results suggest that 1p and 16q deletion should be incorporated in clinical assays to assess prognosis at diagnosis and to guide enrollment in clinical trials for adjuvant therapies. *Ophthalmology Science 2022;2:100121* © *2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material available at www.ophthalmologyscience.org/.

Uveal melanoma (UM) is relatively rare, yet is the most common intraocular malignancy, occurring in up to 1 in approximately 142 000 individuals.[1] Despite low risk of local recurrence, metastatic disease, primarily to the liver, develops in 25% to 30% of patients within 5 years and in approximately 50% of patients within 10 years.[1–3] No adjuvant treatments have been identified to delay metastasis, and the median survival time after diagnosis of metastatic UM is 6 to 9 months.[1,4]

Recurrent DNA copy number alterations (CNAs) in primary UM can help to assess risk of metastasis. Specifically, monosomy 3, gain of 8q, and deletions of 1p and 8p are all associated with poor prognosis, whereas gain of 6p is associated with favorable prognosis.[5–8] These CNAs stratify patients into molecular subgroups with distinct rates of metastasis. Clinical assays to measure these CNAs are available, as well as genetic assays to assess for DNA sequence variants and gene expression profiles, to identify newly diagnosed patients at high risk of metastasis developing.[9–12] Additionally, multivariate prognostic algorithms have been developed using epidemiologic variables, tumor characteristics, gene expression profiling, recurrent CNAs, or a combination thereof.[13–17] The Cancer Genome Atlas (TCGA) Study, using a cohort of 80 patients with UM, showed that 4 patient subtypes with distinct rates of metastasis could be defined by recurrent CNAs and single nucleotide variants and displayed distinct RNA and DNA methylation profiles.[18]

Despite the established importance of CNAs for prognosis in UM, a large, genome-wide CNA analysis has not

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been conducted to determine whether additional prognostic CNAs exist beyond the highly recurrent and large chromosome arm-level CNAs that have been well studied: monosomy 3, gain of 8q, and deletions of 1p and 8p. Other rare but recurrent CNAs, such as deletion of 16q and gain of 18q, have been associated with poor prognosis in some studies.[6,19–24] In particular, deletion of 16q has repeatedly been identified as a lower frequency, but recurrent, finding in UM[19,20,24]; however, only 1 study found it to be an important predictor of patient metastasis.[19] Indeed, 16q and other rare CNAs are not evaluated consistently in studies, nor are they included in clinical assays. In this retrospective study, we aimed to conclusively validate whether rare CNAs can improve patient prognostication and to evaluate any residual genomic and clinical heterogeneity in established molecular subtypes using genome-wide copy number profiles of 921 primary UM and 19 metastatic UM liver tumors.

Methods

Patients

Retrospective analysis of patients referred for prognostic testing of UM identified 921 patients with newly diagnosed primary UM with high-resolution CNA data available (Table 1; Supplemental Table 1). Patients were managed by the Ocular Oncology Service at Wills Eye Hospital, Philadelphia, Pennsylvania, between 2008 and 2016, as previously described.[25] Metastasis status, time to metastasis, and additional clinical information was collected based on retrospective chart review, when available. Tumors were classified as stage T1 to T4 according to American Joint Committee on Cancer 2010 guidelines.[26] Additionally, 19 metastatic liver tumor core biopsy specimens were collected from patients with hepatic metastases by ultrasound-guided needle biopsy.[27] The samples were snap-frozen in liquid nitrogen and stored at −70 °C. The institutional review boards of the University of Pennsylvania, Thomas Jefferson University, and Wills Eye Hospital approved this research. Written informed consent for use of tissues and data for research was obtained from all patients. The study was conducted following the tenets of the Declaration of Helsinki.

DNA Extraction and Array Analysis

Chromosome analysis of UM samples was carried out by the Genetic Diagnostic Laboratory at the University of Pennsylvania. Genomic DNA was extracted from fine-needle aspiration biopsy (FNAB) samples and from solid open biopsy samples of enucleated tumors for a minority of cases (n = 89). Genomic DNA was isolated as previously described.[7] DNA was processed on 1 of 3 Affymetrix copy number genotyping arrays based on date of service: SNP-5.0 (n = 140), SNP-6.0 (n = 359), or CytoScanHD (n = 422) platforms, following manufacturers' recommendations (Supplemental Table 2). All 19 metastatic tumor samples were processed on the CytoScanHD platform.

Copy Number Variant Detection

Clinical calls for chromosomes 1p, 3, 6p, 6q, 8p, and 8q were based on manual review of data in Affymetrix software with default parameters. Patients with partial deletions of chromosome 3, which included *BAP1*, were grouped with the patients with monosomy 3, based on recent findings.[28] Amplification of 8q was defined as 4 or more copies of this locus. Automated CNAs for the rest of the genome were generated using the same tools for all 3 platforms. Affymetrix power tools version 1.18.2 with default parameters was used to process CEL files, and R package rCGH version 1.4.0 was used to normalize and segment the data. To generate gene by sample CNA calls, continuous copy number values were mapped to discrete values using custom thresholds depending on the platform. For SNP-5.0, thresholds equivalent to a theoretical 25% change in copy number were used, compared with 33% for SNP-6.0 arrays. For the CytoScan cohort, sample-specific thresholds based on a kernel density function were used. To identify recurrent CNAs, GISTIC version 2.0 was applied to the entire cohort with default values, except with a *P* value stringency of 0.1 and using CNA thresholds equivalent to a theoretical 33% change in copy number. Recurrent CNAs were manually reviewed to remove spurious calls. Percent of genome altered was calculated by summing the length of the CNA segments divided by the size of the genome, excluding sex chromosomes.

Statistical Analyses

R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to analyze CNA and clinical data. Survival analysis was performed with packages survival (version 2.41.3) and survminer (version 0.4.2), and data visualization was performed with packages ggplot2 (version 2.2.1) and BPG (version 5.9.2).[29,30] Patient clustering was initially performed with established UM-associated CNAs (deletion 1p, monosomy 3, gain 6p, deletion 6q, deletion 8p, gain or amplification 8q) and subsequently repeated to include 16q deletion. Final patient grouping was based on evaluation of all molecular subtypes to pair patients with similar prognostic outcomes. In this analysis, clusters are determined by presence of chromosomal features (i.e., related to genomic heterogeneity), whereas patient risk group*s* are defined as clusters with similar prognostic outcomes based on Kaplan-Meir analysis (i.e., related to clinical heterogeneity).

The Cancer Genome Atlas Cohort

The CNA data (.seg files) were downloaded from the National Institutes of Health Genomic Data Commons data portal and processed with GISTIC using the same settings as the Genetic Diagnostic Laboratory cohort. The CNA status for chromosome 1p, 3, 6p, 6q, and 8q was available from the supplementary files of the original publication,[18] and the CNA status for the remaining relevant chromosome arms (4p, 8p, and 16q) was determined by the GISTIC broad CNA results. Time to metastasis and other clinical features were extracted from the supplementary material of the original publication. Of the 80 TCGA patients, 70 had metastasis-free survival information available that was used for validation, consistent with the metastasis-free survival analyses in the original TCGA study.

Results

Patient Characteristics

Retrospective analysis of patients referred for prognostic testing at diagnosis of primary UM identified 921 patients with high-resolution CNA data available (SNP-5.0, n = 140; SNP-6.0, n = 359; and CytoScanHD, n = 422; Table 1). Most patients had a diagnosis of choroidal tumor (n = 722) and the most common stage at diagnosis was T1 disease (n = 298). The median age at diagnosis was 59.6 years. Follow-up information was available for 678

Table 1. Clinical Characteristics of the Retrospective Primary Uveal Melanoma Cohort

Characteristic	Full Cohort		678 Patients with Information on Time to Metastasis			
			With metastasis		*Without Metastasis*	
	No.	%	*No.*	%	*No.*	%
No.	921		161		517	
Median time to metastasis (yrs)	4.50		NA		NA	
Sex						
Male	440	47.8	86	53.4	239	46.2
Female	481	52.2	75	46.6	278	53.8
Median age (yrs)	59.6		60.9		59.0	
Metastasis						
No	517	56.1				
Yes	161	17.5				
Unknown	243	26.4				
Stage						
T1	298	32.4	14	8.70	213	41.2
T2	234	25.4	41	25.5	137	26.5
T3	230	25.0	70	43.5	121	23.4
T4	82	8.90	34	21.1	35	6.77
Unknown	77	8.36	2	1.24	11	2.13
Tumor location						
Choroid	720	78.2	109	67.7	411	79.5
Ciliary body	4	0.434	2	1.24	2	0.387
Iris	34	3.69	3	1.86	26	5.03
Mixed	146	15.9	44	27.3	76	14.7
Unknown	17	1.85	3	1.86	2	0.387

NA = not available.

patients, with 161 patients having had documentation of metastatic disease, of which 33 and 80 developed metastases within 1 and 2 years, respectively. Overall, the median time to metastasis was 4.5 years (range, 1–127 months). Patients who went on to demonstrate metastasis were more likely to have a diagnosis of stage T3 or T4 disease and have multiple parts of the uveal tract involved (Supplemental Fig 1). Although not statistically significant, a slight increase in men and older patients demonstrating metastatic disease was also found, as has been noted elsewhere.[31,32]

Copy Number Profiles

Genome-wide copy number profiling revealed known arm-level regions on chromosomes 1p, 3, 6p, 6q, and 8q to be the most frequent events in UM primary tumors (Fig 1A, B). Monosomy 3 was observed in 44.7% patients, and partial deletion of chromosome 3 was observed in 6.8% of patients, 64.6% cases of which involved the *BAP1* gene (or 4.4% of total cohort). In addition, 27.1% patients showed gain of 6p, and 41.8% showed gain of 8q, 47.5% of whom harbored 4 copies or more (i.e., amplifications; 20.0% of total cohort). Monosomy 3 and gain of 6p were largely mutually exclusive of one another (389 patients with monosomy 3, 250 patients with gain of 6p, and 66 patients with both). The frequency of these changes did not differ significantly between FNAB samples and enucleated tumors, further supporting the role of CNA analysis based on FNAB samples (Supplemental Table 3) Overall, the percentage of genome altered by CNAs varied widely per patient (range, 0%–53.0%; median, 3.45%) and was associated with patient prognosis (Fig 1C, D). This measure of generalized genomic instability or copy number burden is known to be prognostic in many cancers, generally with a higher percentage of aberrations associated with worse outcome.[33–35] So-called C-class tumors have multiple CNAs, but relatively few sequencing variants,[36] consistent with published mutational profiles of UM.[9,11,12]

Recurrence analysis using GISTIC analysis identified 23 high-quality, recurrent focal CNAs (3 gains or amplifications and 20 deletions) and 16 recurrent whole chromosome or chromosome arm-level CNAs (Supplemental Fig 2, Supplemental Table 4). Compared with previous studies evaluating more rare CNAs,[19,22] deletion of 16q was a recurrent finding, but gain of 18q was not. Of the 23 focal CNAs, 9 were identified within recurrent chromosome arm-level CNAs. Comparing the frequency and prognostic effect of these 9 CNA pairs revealed that the broad change was both more frequent and showed similar or stronger prognostic effects (Supplemental Fig 3).

Prognostic Copy Number Aberrations

The CNAs associated with the worst outcomes on a univariate basis were deletion of 16q (hazard ratio [HR], 5.98), followed by gain of 4p (HR, 5.78), gain of 8q (for any copy number gain: HR, 5.63; for amplification of 8q: HR, 5.54), monosomy 3 (HR, 5.16), and deletion of 8p (HR, 4.27) (Fig 2). Consistent with recent results,[28] we

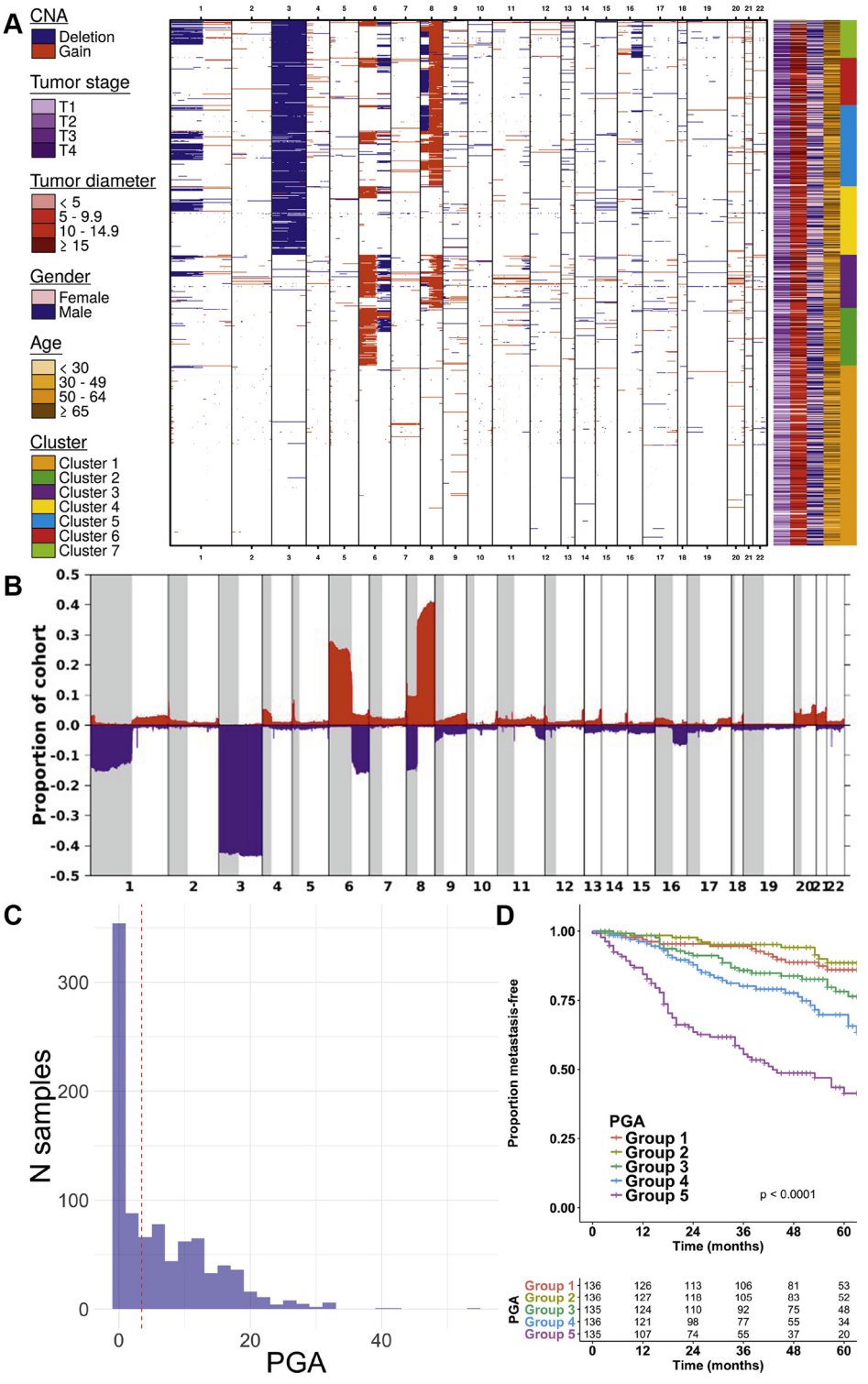

Figure 1. Genome-wide copy number alteration profile of 921 primary uveal melanoma tumors. **A**, Copy number profile in each primary tumor. Each column represents a gene sorted according to chromosomal location (x-axis), and each row represents a patient. Regions in blue and red indicate copy number deletions and gains, respectively, in the corresponding gene and patient combination. Pertinent clinical variables are shown on the right. Patients are clustered according to schema shown in Figure 3B, based on known uveal melanoma-associated copy number aberrations (CNAs; deletion 1p, monosomy 3, gain of 6p, loss of 6q, loss of 8p, gain of 8q) and 16q. **B**, Graph showing the frequency of genome-wide CNAs across the cohort. **C**, Bar graph showing the percent of genome altered (PGA) distribution in the cohort. The median (3.45%) is depicted with the red line. **D**, Prognostic impact of PGA. Patients are grouped into 5 equally sized bins with PGA thresholds of 0% to 0.0047% (group 1), 0.0047% to 1.98% (group 2), 1.98% to 6.92% (group 3), 6.92% to 12.7% (group 4), and 12.7% to 40.9% (group 5). The log-rank *P* value is shown.

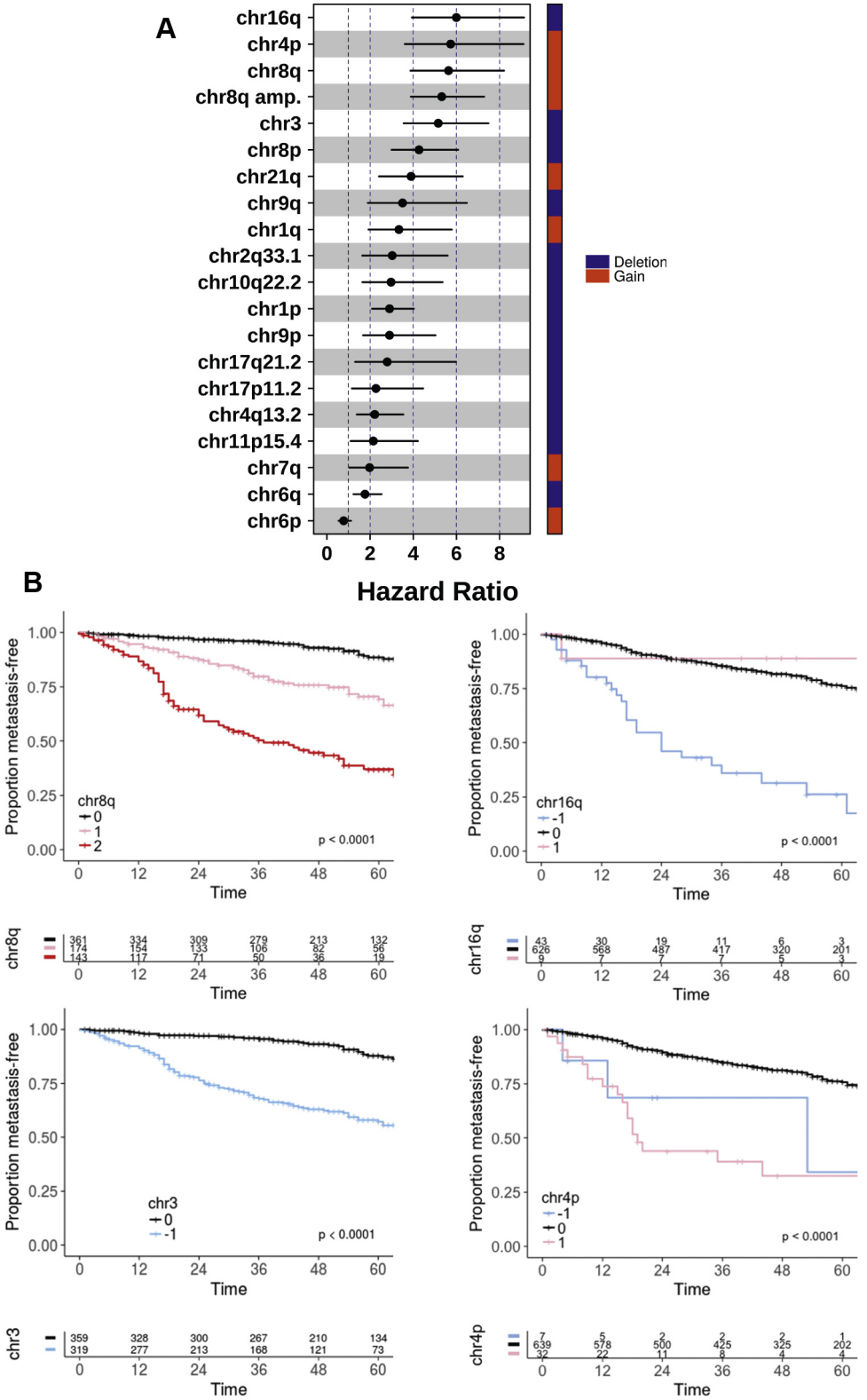

Figure 2. Prognosis of individual copy number aberrations (CNAs). **A**, Forest plot demonstrating the significant CNAs detected in the cohort. The CNAs with Benjamini-Hochberg false-discovery rate-corrected *P* values of < 0.05 are shown. Chromosome 6p is also shown, although it was not found to be significant in this cohort. **B**, Kaplan-Meier curves for metastasis-free survival based on the 4 most prognostic CNAs, deletion 16q, gain of chr4p, gain or amplification of chr8q, and monosomy 3. The patient groups refer to copy number status (0, disomy; −1, loss; 1, gain; and 2, amplification). The log-rank *P* value is shown.

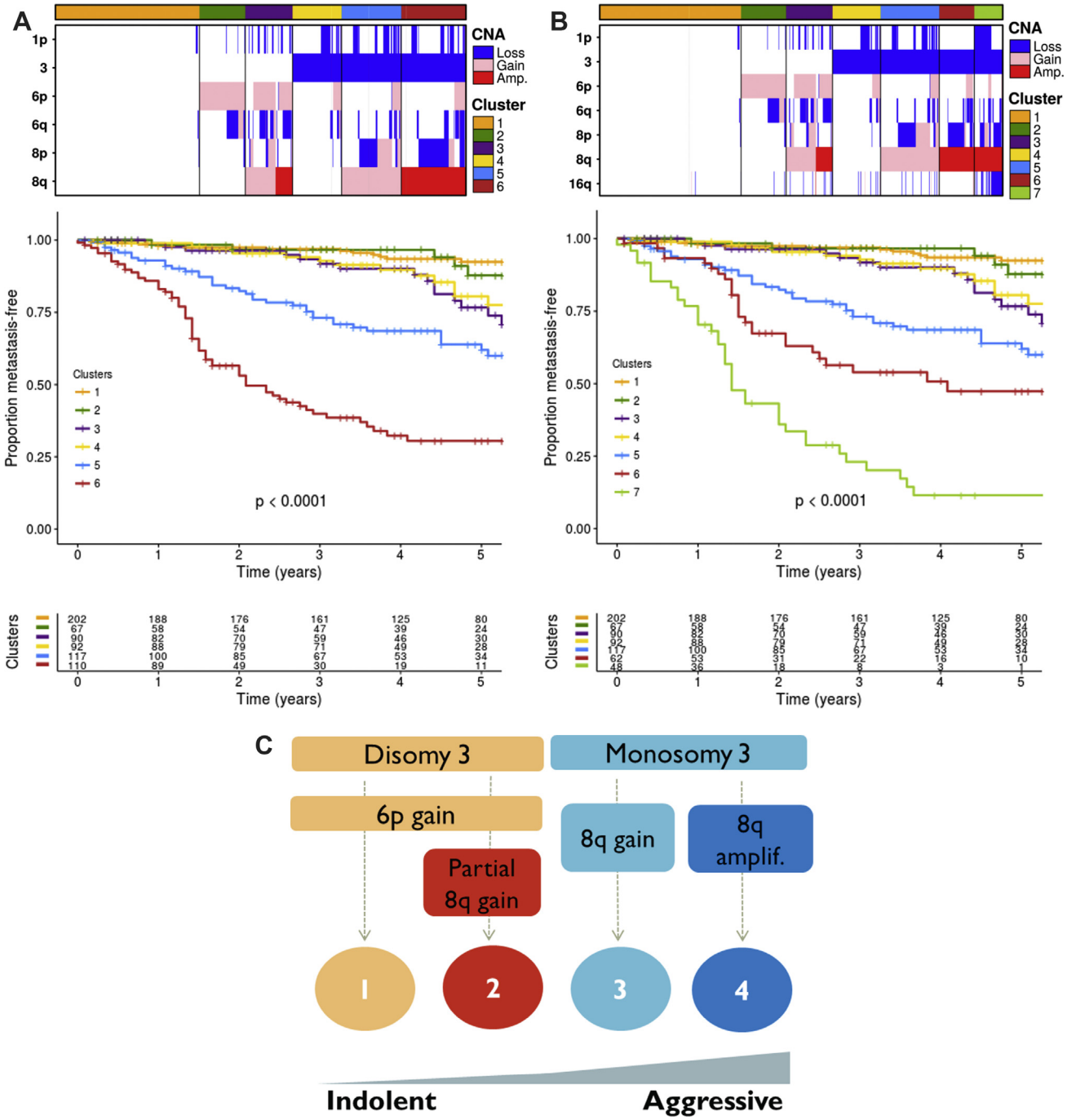

Figure 3. Recurrent copy number aberrations (CNAs) defining molecular subtypes. **A**, Patient grouping and prognosis based on known uveal melanoma (UM)-associated CNAs, namely deletion 1p, monosomy 3, gain of 6p, loss of 6q, loss of 8p, and gain of 8q. **B**, Patient grouping and prognosis based on known UM-associated CNAs (see (**A**)) and deletion of 16q. **C**, Previously published The Cancer Genome Atlas CNA-based classification. Figure adapted based on manuscript by Robertson et al.[18] **D**, Proposed Genetic Diagnostic Laboratory CNA-based risk group classification scheme for prognostication of UM. **E**, Kaplan-Meier curve for metastasis-free survival based on the Genetic Diagnostic Laboratory risk group classification scheme shown in (**D**). The log-rank *P* value is shown. amplif. = amplification.

observed that patients with partial deletions of chromosome 3 that involved *BAP1* experienced a worse prognosis compared with those with partial deletions excluding *BAP1* (Supplemental Fig 4). As expected, patients with amplification of 8q experienced a significantly worse prognosis than those with a single copy gain. Gain of 6p did not reach statistical significance, but trended toward better prognosis, as

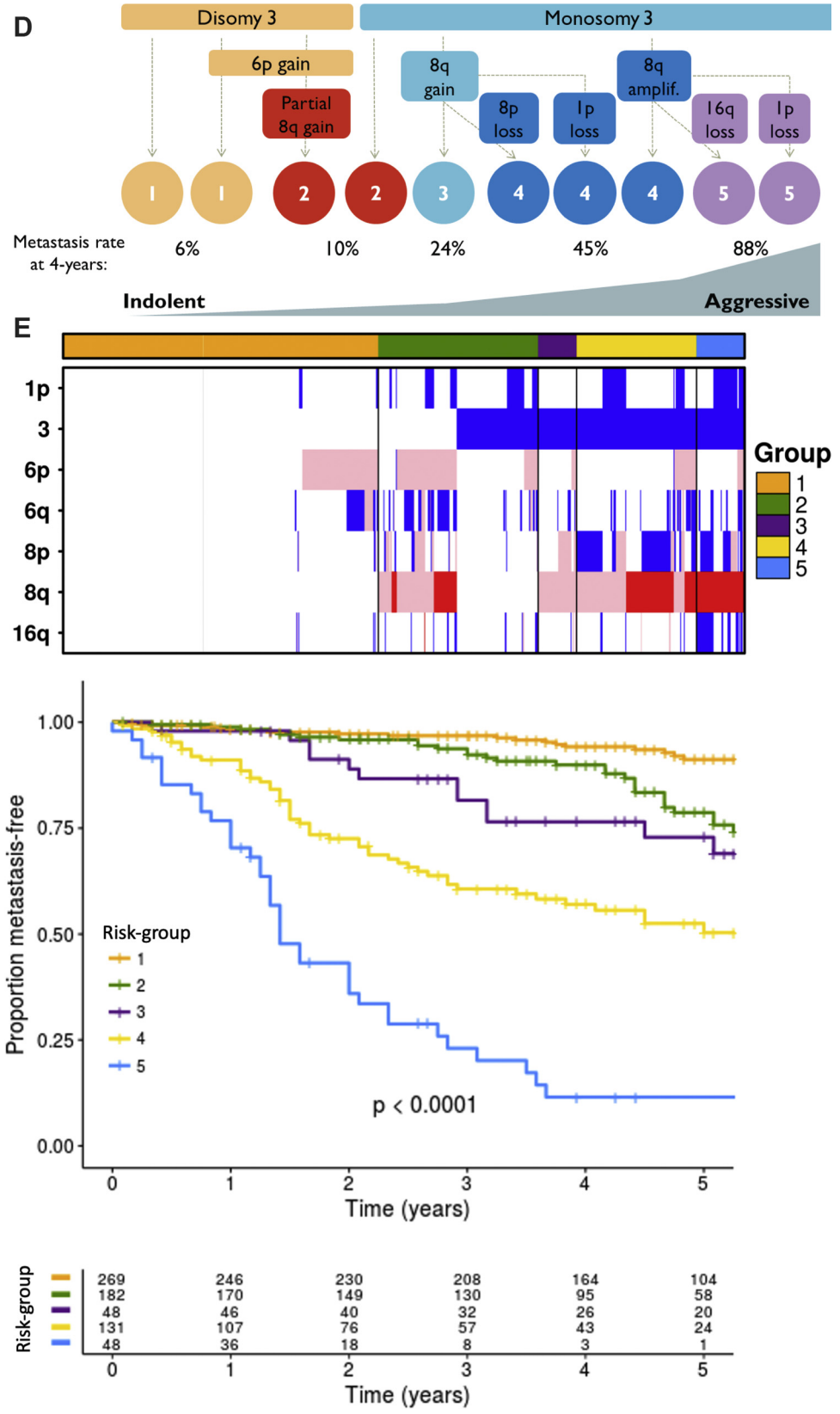

Figure 3. *(Continued).*

Table 2. Cox Proportional Hazard Models to Assess Risk of Metastasis Based on the 5 Patient Risk Groups Classification Scheme

Model	Hazard Ratio	95% Confidence Interval	P Value (Wald Test)
Univariate models			
Group 2 vs. 1	3.24	1.83–5.72	5.22×10^{-5}
Group 3 vs. 1	5.20	2.85–9.48	7.81×10^{-8}
Group 4 vs. 1	10.3	5.95–18.0	1.1×10^{-16}
Group 5 vs. 1	37.9	21.2–67.6	$<2.2 \times 10^{-16}$
Multivariate model			
Group 2 vs. 1	2.46	1.38–4.40	0.00235
Group 3 vs. 1	3.46	1.72–6.94	0.00048
Group 4 vs. 1	7.33	4.27–12.6	5.13×10^{-13}
Group 5 vs. 1	15.8	8.36–29.9	$<2.2 \times 10^{-16}$
T2 vs. T1	2.86	1.55–5.29	7.92×10^{-4}
T3 vs. T1	4.93	2.72–8.94	1.46×10^{-7}
T4 vs. T1	6.78	3.48–13.2	2.01×10^{-8}
Other vs. choroidal	1.06	0.736–1.52	0.760
Age (continuous)	1.02	1.00–1.03	0.00934
Male vs. female	1.63	1.18–2.25	0.00318

previously described (HR, 0.776; $P = 0.217$). The remaining CNAs were either not associated with prognosis or were significantly less so.

Although the prognostic effects of chromosome 16q deletion and gain of 4p are large, these are relatively rare CNAs in UM (9.3% and 4.5%, respectively). Gain of 4p has not been previously described in UM, although it was observed in 7 of 80 TCGA study patients (8.8%) and reached borderline significance (Supplemental Fig 5A). Gain of 4p is a rare event in cancers[37] and, in the present cohort, was associated with higher percentage of genome altered (median, 16.3% compared with 5.93% for tumors without gain of 4p). Further, we did not find that it influenced unsupervised classification of the CNAs in this cohort (data not shown). Thus, gain of 4p may represent a passenger change in the context of high genomic instability. Deletion of 16q was previously associated with risk of metastasis in 1 study[19] (Supplemental Table 5), and we also found it to be strongly prognostic in TCGA study patients (Supplemental Fig 5B). However, it is not considered a hallmark of UM and is not included in clinical assays used to assess patient prognosis, likely because of its low frequency in UM, and therefore its inconsistent effects on prognosis in smaller studies.[7,8,19,20] Although individual CNAs can have large prognostic effects, caution should be used when interpreting these on a univariate basis, because genomic subtypes or combinations of CNAs provide a more complete and powerful interpretation of a patient's prognosis.

Genomic Subtypes

The major recurrent CNAs observed in UM (1p, 3, 6p, 6q, 8p, and 8q) clustered patients into 6 molecular groups, or subtypes, which stratified patients into 3 major prognostic groups (Fig 3A). Patients lacking all recurrent CNAs (cluster 1) and patients with gain of 6p and disomy of 8q showed the best outcomes (cluster 2) and achieved statistically better outcomes than patients with gain of 8q in the absence of monosomy 3 (cluster 3) and patients with isolated monosomy 3 (cluster 4), although the latter 2 groups still achieved relatively good prognosis (Supplemental Table 6). Patients with monosomy 3 who also showed amplification of 8q experienced the worst prognosis, whereas those with a single copy gain of 8q showed an intermediate prognosis. Based on the strong univariate prognostic significance of 16q deletion, it was incorporated in the original 6-group clustering schema. To identify clinically significant residual genomic heterogeneity, the patients were split into 14 highly specific subtypes, which revealed that patients with monosomy 3, amplification of 8q, and deletion of either 16q or 1p experienced significantly worse outcomes compared with all other patients (clusters 13 and 14, respectively; Supplemental Fig 6). The remaining groups demonstrated a gradient in prognosis.

Although this 14-group clustering schema is overly specific, the exploratory analysis confirmed the prognostic relevance chromosome 1p and 16q deletions, even when accounting for other known prognostic CNAs, and indicated that significant clinical and genomic heterogeneity remained in the sixth cluster of the 6-group schema. Indeed, splitting this cluster based on chromosomes 16q and 1p revealed an ultra–high-risk group representing 7% of the total cohort defined by monosomy 3; amplification of 8q; and deletion of 16q, 1p, or both (Fig 3B; Supplemental Table 6). Of the patients moved from the sixth to the seventh cluster, an equal number of patients were moved because of deletions of 1p and 16q; approximately 15% harbored both deletions. Although overall rare, these patients have a significantly worse prognosis even compared with individuals in the next highest-risk cluster with monosomy 3 and amplification of 8q (HR, 2.07; $P = 0.0037$, cluster 7 vs. 6; Fig 3B) and have an approximately 40-times higher risk of metastasis than those in the lowest-risk

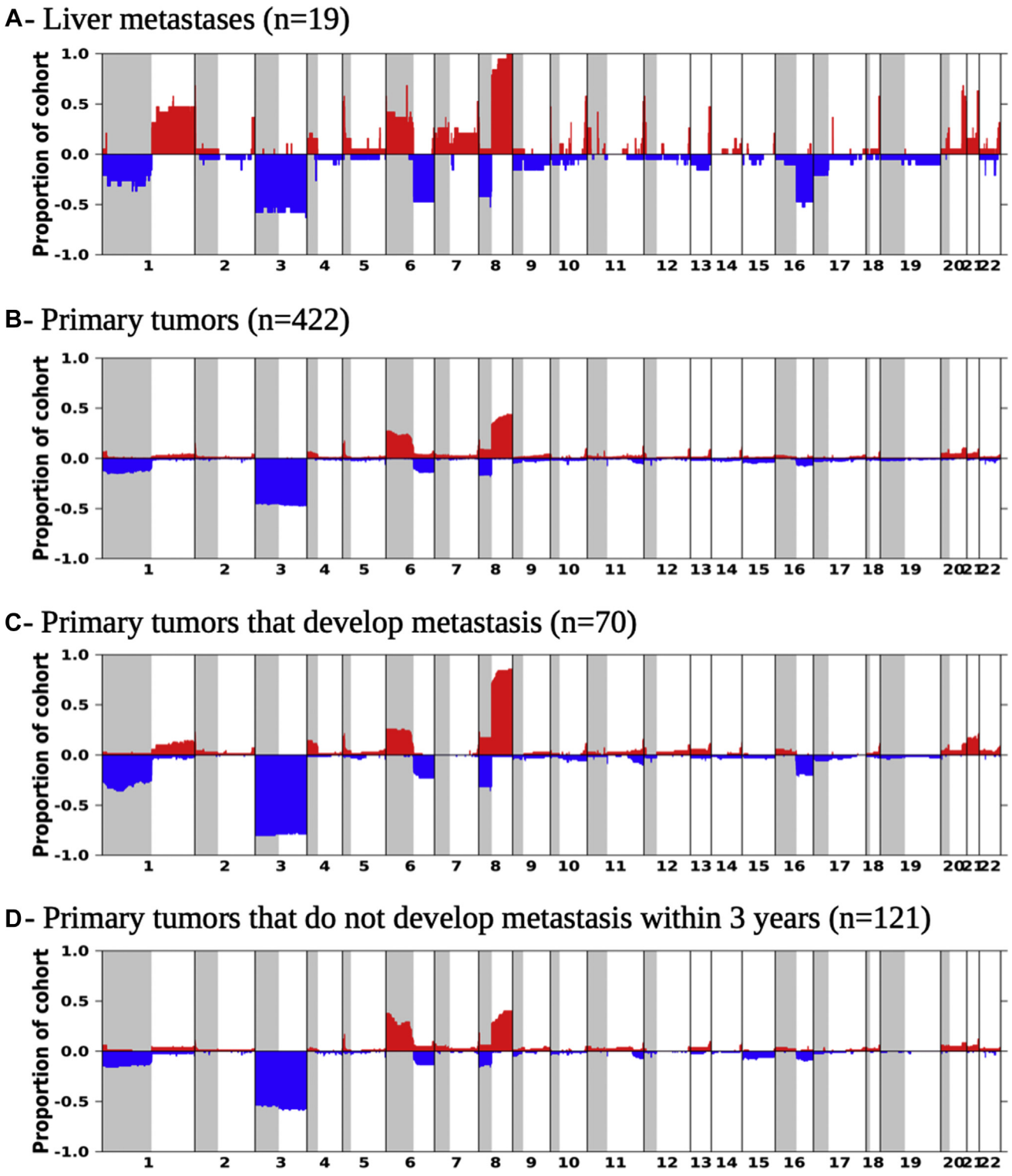

Figure 4. Copy number profile of metastatic versus primary uveal melanoma tumors. All samples were processed on the Affymetrix CytoScan HD platform. **A**, Frequency of genome-wide copy number aberrations (CNAs) in 19 liver metastases. **B**, Frequency of genome-wide CNAs in 422 primary tumors. **C**, Frequency of genome-wide CNAs in 70 primary tumors of patients who later demonstrated metastasis. **D**, Frequency of genome-wide CNAs in 121 primary tumors of patients who did not demonstrate metastasis 3 years after diagnosis.

cluster (HR, 40.5; $P < 1 \times 10^{-13}$, cluster 7 vs. 1; Fig 3B). The high-risk nature of this subgroup was also observed in TCGA patients by repeating the 6- and 7-cluster schemas and also by showing that deletions of 16q or 1p can delineate the high-risk TCGA group further (Supplemental Figs 7 and 8).

Given the gradient of prognosis observed in the highly specific 14-cluster schema (Supplemental Fig 6) and the similar prognostic rates of clusters 1 through 4 in the 7-cluster schema (Fig 3B), clusters were grouped together into 5 patient risk groups with similar risks of metastasis (Fig 3C–E, Table 2). These risk groups are largely based on the standard classification using chromosome 1, 3, 6, and 8, but allow more precision in that the presence or absence of particular CNAs, including deletion of 16q, can significantly alter an individual's predicted risk of metastasis (Fig 3D). The 5 patient risk groups remained clinically relevant when adjusted for standard clinical variables (Table 2, multivariate model) and when applied to individuals within each American Joint Committee on Cancer 2010 tumor stage, particular in patients with T2 and T3 tumors (Supplemental Fig 9). Altogether, these results support the finding that individuals with a higher proportion of genomic instability have worst outcomes and suggest that including 16q deletion in stratification of patients with UM can further refine patient prognosis.

One limitation of this study is the use of 3 different array platforms, which was the result of the nature of the clinical cohort ascertained over time. Although the prevalence of CNAs involving chromosomes 3, 6, and 8 was not dramatically biased across platforms (Supplemental Table 7), deletion of 16q was less frequently called in the SNP-5.0 cohort. However, repeating cluster prognosis analysis without the 140 patients profiled on the SNP-5.0 platform, of which 118 had known metastasis status, reproduced similar results compared with using all 3 platforms together (Supplemental Fig 10).

Comparison with Metastatic Uveal Melanoma in the Liver

Nineteen samples of mUM in the liver were also collected and analyzed by genome-wide array. All 19 metastatic tumors harbored 8q gain, 17 of 19 showed monosomy 3 or copy number neutral loss of heterozygosity, and overall, greater genomic instability was found in metastatic tumors compared with the 422 primary tumors processed on the same platform (Fig 4). The extremely high prevalence of 8q amplifications in mUM in the liver has been previously observed, and 1 study showed that an increase in 8q copy number was correlated with increased Myc protein expression by immunohistochemistry.[38] Deletions on chromosomes 6q and 16q and gains on chromosomes 1q, 7, and 8q were enriched in metastatic tumors compared with primary tumors. Primary tumors that went on to develop metastasis are more likely to have monosomy 3, gain of 8q, and deletion of 16q, but gain of 1q and 7 are rare in primary tumors and may reflect CNAs that provide growth advantages in a new locale. These findings corroborate the recent finding that gain of 1q is a common terminal branching event in metastatic tumor evolution, with a significant enrichment in metastatic tumors compared with their matched primary tumor.[39,40]

Discussion

The prognostic impact of CNAs in UM has been well studied, and clinically validated prognostic assays are available to predict risk of metastasis at the time of diagnosis based on detection of specific CNAs, among other prognostic assays.[5,7,8,41,42] However, a large-scale, systematic analysis of CNAs across the genome has not been undertaken. This may be because of the reliance on cytogenetic and targeted molecular assays for detection of chromosomal abnormalities in the clinic, research studies of small cohort sizes (fewer than 150 patients), or both.[6,16,19,41] Nonetheless, initial molecular grouping studies based on CNAs deduced important predictors of patient prognosis, most consistently chromosomes 1p, 3, 6, and 8.[14,18,20,39] Using the current large cohort of 921 primary tumors, of which two-thirds harbor information regarding metastatic-free survival, we confirm the prognostic relevance of 16q deletions, both in univariate and multivariate models accounting for additional CNAs. Although multiple studies have identified 16q as a low-frequency, recurrent abnormality in UM, reports of its prognostic significance have been conflicting, likely because of small cohorts in previous studies (Supplemental Table 5). As a result, 16q is not systematically evaluated in all UM CNA studies (for example, it was not mentioned in TCGA study) and is not included in any current clinical assay. Our study should encourage clinical laboratories to include deletion of 16q in prognostic assays based on CNAs.

Further, the current large cohort enabled evaluation of more precise molecular classification, revealing clinical and genomic heterogeneity within established molecular subtypes. The Cancer Genome Atlas CNA classification scheme is the gold standard for molecular subgrouping and is consistent with previous studies that largely categorized patients by presence or absence of 6p gain, monosomy 3, and gain or amplification of chromosome 8q: (1) patients with 6p gain show the best prognosis, (2) patients with 6p gain and partial 8q gain show relatively good prognosis, (3) patients with monosomy 3 with 8q gain show intermediate prognosis, and (4) patients with monosomy 3 and high-level 8q amplification show poor prognosis (Fig 3C).[18–21,39] In this study, we identified additional genomic drivers of molecular subtypes and clinical response. Our final 5-group classification scheme recapitulates and expands on these previous studies (Fig 3C–E); by using a larger cohort size, and therefore allowing for more specific molecular clusters and groups (Supplemental Fig 6) and including CNAs with lower frequency (e.g., 16q deletion; Fig 3B), more subtle differences can be appreciated in patient clustering. For instance, first, we showed that deletions of 1p and 16q are important in distinguishing patients with the highest risk of metastasis. We note that

deletion of 16q is present in a small proportion of TCGA patients in groups 2 and 4, but was too rare to influence their classification scheme.[18] Second, we found that presence or absence of particular CNAs can place a patient into a different risk group compared with the more traditional CNA classification schemes (Fig 3D). For example, patients in this cohort with monosomy 3 and a single copy gain of 8q fared relatively well, unless they also harbored deletion of 8p, in which case, their prognosis was more similar to those with monosomy 3 and amplification of 8q.

Overall, our classification scheme combines major findings from several previous studies. In particular, a 2009 study by Trolet et al[19] identified 5 molecular subgroups using unsupervised clustering, which largely maps to clusters 2, 3, 4, 5 or 6, and 7 of our 7-group schema, including the presence of 16q deletion in the highest risk group. However, the molecular subtypes did not distinguish between chromosome 8q gain and amplification and did not identify patients with disomy 3 and disomy 6. The Cancer Genome Atlas and earlier studies[20] clearly demonstrated the importance of identifying an 8q gain versus amplification, consistent with our proposed splitting of clusters 5 and 6. Although patients in TCGA did not harbor monosomy 3 without gains or amplifications on 8q, our cohort corroborates previous studies demonstrating a relatively good prognosis for patients with monosomy 3 in the absence of 8q changes,[19,43,44] which is an important finding because monosomy 3 is generally thought to be associated with aggressive disease. Our study showed that this analysis is confounded by the presence of additional CNAs in the genome and that it is the constellation of CNAs that drives prognostication in UM, rather than individual CNAs. Finally, the good prognosis of patients with disomy 3 and 6 (cluster 1) has been reported elsewhere.[21,43] Therefore, our molecular subtypes replicate, and more importantly unify, previously identified molecular subgroups reported across several studies based on small cohorts, targeted CNA analyses, or both.

The clinical usefulness of identifying a rare (approximately 7% of Genetic Diagnostic Laboratory cohort) but ultra–high-risk group (monosomy 3, 8q amplification, and loss of 1p, 16q, or both) needs to be further evaluated. Although patients in this group have a 4-year metastasis-free rate of only 12%, compared with 55% for the next highest-risk group (Fig 3D, E, groups 5 and 4, respectively), patients in both groups should be considered at high risk of metastasis at the time of diagnosis and should be offered clinical trials for adjuvant treatment and increased surveillance. However, given the very poor prognosis for patients in group 5, these individuals could be considered for more aggressive regimens, and the genomic heterogeneity between these groups may result in different response to treatments. For instance, deletion of tumor suppressors on 16q or 1p may modulate response to a particular treatment, or even potentially expose new targetable vulnerabilities. Chromosome 16q has one of the highest densities of tumor suppressors in the genome,[45] including CDH1 and CTCF. Measurement of 16q copy number along with the standard 1p, 3, 6, and 8 CNAs in clinical trials for adjuvant therapies will be important to decipher this question.

In this study, 50% of mUM in the liver samples harbored a 16q deletion, supporting the classification of this CNA as a high-risk feature related to the metastatic potential of UM.[19,39] Deletion of 16q has been involved in estrogen receptor (ER)-positive breast cancer and prostate cancer. The mUM in the liver harbored additional CNAs not observed in the primary tumors, such as gain of 1q and trisomy 7. Indeed, gain of 1q has been noted to be enriched in metastatic tumors even when compared with matched primary tumors.[19,39,40] Chromosome 7 has a high density of oncogenes and is commonly gained in cancer,[40] including in metastatic colorectal cancers with high genomic instability and in a subset of localized prostate cancers.[35,45,46] Given that mUM in the liver preferentially spreads to the liver, these CNAs may provide growth advantages in the liver microenvironment. Additional studies including RNA expression are needed to clarify whether and how these CNAs contribute to metastatic spread and whether distinct molecular subtypes of mUM in the liver are clinically relevant. Given the lack of drug targets for mUM in the liver, these studies could have profound clinical impact for patients going on to demonstrate mUM in the liver.

One limitation of the current study is the exclusive use of CNAs to assess patient prognosis. Because most patients underwent an FNAB rather than enucleation, the specimen was limited for molecular profiling. Although RNA expression and DNA mutations in genes such as *BAP1*, *EIF1AX*, and *SF3BP1* are known to impact patient prognosis,[9,11,12] the specimens yielded sufficient DNA for only chromosomal microarray in the vast majority of patients. Next-generation sequencing has emerged as a single platform that can simultaneously assess CNAs and DNA mutations; however, the patients included in this study were tested between 2008 and 2016, when clinical next-generation sequencing assays were not available or were just emerging. As sufficient follow-up information accumulates for large cohorts tested on now readily available next-generation sequencing assays, future studies are poised to expand on this study and TCGA study findings to elucidate how multimodal biomarkers relate to the proposed molecular subtypes defined here.

Prognostic testing at the time of UM diagnosis has become increasingly prevalent.[41,42] However, UM is a relatively rare disease, and large cohort studies including patient outcomes are lacking. The cohort used in this study is significantly larger than previous studies investigating genome wide CNAs in UM, enabling identification of possible improvements in UM prognostication. The 5 patient risk groups presented herein build on decades of work in molecular subtyping and prognostication in UM and present a unified view of genomic and clinical heterogeneity in UM at the CNA level. Future studies with large cohorts evaluating how deletion of 16q can be incorporated into existing clinical risk assessment tools are warranted.

Footnotes and Disclosures

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No animal subjects were included in this study.

Author Contributions:

Conception and design: Lalonde, Shields, Ganguly

Analysis and interpretation: Lalonde, Ewens, Ganguly

Data collection: Lalonde, Ewens, Richards-Yutz, Ebrahimzedeh, Terai, Gonsalves, Sato, Shields

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Overall responsibility: Lalonde, Ewens, Ganguly

Abbreviations and Acronyms:
CNA = copy number aberration; **FNAB** = fine-needle aspiration biopsy; **HR** = hazard ratio; **mUM** = metastatic uveal melanoma; **TCGA** = The Cancer Genome Atlas; **UM** = uveal melanoma.

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