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Original research

Molecular determinants of survival in metastatic uveal melanoma: The impact of *SF3B1* mutations

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ABSTRACT

Purpose: To determine whether key molecular alterations in primary uveal melanoma (UM), including mutations and somatic copy number alterations (SCNAs), serve as prognostic markers in metastatic UM (MUM).
Experimental design: Retrospective analysis of a prospective cohort study of clinical and molecular data from UM and MUM patients.
Results: A total of 220 patients with primary UM treated at Hospital de Bellvitge, including 79 (36 %) who developed metastases, primarily in the liver. Genetic analyses of primary tumors included hotspot mutation testing for *GNAQ, GNA11,* and *SF3B1,* along with SCNA assessment (chromosomes 3, 8, 1, and 6) via Multiplex Ligation-dependent Probe Amplification (MLPA). Kaplan-Meier and Cox proportional hazards models assessed the impact of genetic alterations on relapse-free survival (RFS) and overall survival (OS).
Results: Monosomy 3 (M3) and chromosome 8q amplification (8A) were associated with shorter RFS ($p < 0.0001$) in primary UM but did not impact OS in MUM ($p = 0.33$). *SF3B1* mutations (SF3B1m) conferred significantly longer OS in MUM (31.7 vs. 11.8 months, $p = 0.001$), independently confirmed in multivariate analysis (HR=0.26, $p = 0.01$), irrespective of tebentafusp treatment.
Conclusions: Traditional chromosomal markers stratify primary UM but fail to predict OS in MUM. *SF3B1m* emerges as a novel prognostic factor, indicating a distinct biological phenotype with potential therapeutic implications. Further studies are warranted to validate its prognostic and therapeutic relevance in MUM.

1. Introduction

Uveal melanoma (UM) is a rare yet the most common intraocular cancer in adults. Despite its rarity, UM has a substantial clinical impact due to its aggressive nature. Treatment options, including enucleation and brachytherapy, can provide effective local control of the primary tumor. However, these treatments often do not prevent disease spread, and approximately 50 % of patients develop metastatic UM (MUM), with the liver being the most frequent site of metastasis [1].

UM is driven by distinct molecular alterations. Initiating mutations occur in the G protein-coupled receptor signaling pathway, with common mutually exclusive mutations in *GNAQ* or *GNA11*, and occasional

Abbreviations: UM, Uveal melanoma; SCNAs, somatic copy number alterations; MUM, metastatic UM; MLPA, Multiplex Ligation-dependent Probe Amplification; K-M, Kaplan-Meier; RFS, relapse-free survival; OS, overall survival; M3, monosomy 3; 8A, 8q amplification; SF3B1m, SF3B1 mutations; TCGA, The Cancer Genome Atlas Program; CI, confidence intervals; HR, hazard ratios; NR, not reached; LDH, lactate dehydrogenase; AP, alkaline phosphatase; Yo, years old.

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mutations in *CYSLTR2* or *PLCB4*. While these mutations characterize the disease, they alone do not drive malignant progression or correlate with prognosis in primary disease [2,3]. As UM progresses, additional mutations appear, particularly in genes *BAP1*, *SF3B1*, and *EIF1AX*, which occur in a largely mutually exclusive manner [2].

In primary UM, *EIF1AX* mutations are associated with a low metastatic risk, *SF3B1* with later-onset metastasis, and *BAP1* loss with early metastasis onset [4]. Somatic copy number alterations (SCNAs), with the most significant being monosomy 3 (M3) and chromosome 8q gain (8A), link genetic alterations to varying relapse probabilities in primary disease [5]. This chromosomal-based classification groups patients by M3 and 8A, distinguishing among four prognostic groups with variable metastasis rates [5]. The four-cluster model proposed by The Cancer Genome Atlas Program (TCGA) working group in primary tumors provides the most comprehensive view of genetic alterations and their association with prognosis [6]. This classification, based on the analysis of SCNAs alongside other molecular data, delineates primary UM into four clusters (C1, C2, C3, and C4) with distinct relapse free survival (RFS) profiles and underlying biology. C1 shows disomy 3 (D3) and minimal SCNAs and has the best prognosis. C2 includes D3 tumors with added SCNAs, frequently involving *SF3B1* mutations and later-onset metastasis. C3, linked to higher metastatic risk, is associated with M3 and 8q gains. Finally, C4 includes M3 tumors with multiple 8q gains, marking aggressive biology and early metastasis. Both, C3 and C4, are associated with frequent *BAP1* mutations.

Although molecular classification has proven to be useful to stratify relapse risk for primary UM, there no molecular or chromosomal markers have been shown to predict survival in the metastatic setting and only clinical variables have been associated with survival prognosis in metastatic UM (MUM) [7]. With the advent of the approval of tebentafusp for MUM [8], and other potential novel therapies getting close in the horizon, there is a need to identify new molecular stratification factors to better balance the risk of progression and death, and probably use them as stratification factors in clinical trials [9]. This study investigates whether key molecular alterations, including mutations and SCNAs, identified in primary UM, can serve as prognostic markers in the metastatic setting.

2. Patients and methods

2.1. Study design and patient population

We retrospectively identified 312 prospective primary UM patients treated at a single institution, the Hospital de Bellvitge, and with available molecular study performed at the pathology unit. Patients were treated between June 2000 and March 2023. Study entry required complete information of hot spot mutation analysis in *GNAQ*, *GNA11*, and *SF3B1*, as well as SCNAs in chromosomes 3, 8, 1, and 6. A total of 220 patients were included in the study. The reason for not including 92 patients is because of no tissue available (41 patients), poor sample quality (28 patients), and unreliable/inconclusive results (23 patients). A CONSORT diagram is shown in Figure 1. The demographics and clinical characteristics of the cohort are summarized in Table 1. The mean age was 60 years (range: 13.4–93.9 years), with 159 male patients. Primary tumor was treated through enucleation in 99 cases (45 %), brachytherapy in 65 cases (30 %), and eye sparing surgery in 56 cases (25 %). The Hospital de Bellvitge/Catalan Cancer Institute Institutional Review Boards and Ethics Committee at IDIBELL approved this study for clinical investigation. All the methods were conducted in conformity with the Declaration of Helsinki and relevant guidelines.

2.2. Genetic analyses

Genomic DNA was isolated from fresh tumor tissues and reference samples using the QIAamp DNA Mini Tissue Kit (Qiagen, Hilden, Germany). For Multiplex Ligation-dependent Probe Amplification (MLPA), a MLPA was carried out with 100 ng of DNA from tumor DNA and reference samples using the SALSA MLPA Probemix P027 UM kit (MRC Holland, Amsterdam, the Netherlands). MLPA was performed using a 3130xl Genetic Analyzer (Applied Biosystems), and raw data were analyzed using Coffalyser.Net software (MRC Holland) to detect deletions and amplifications in chromosomes 3, 8, 1, and 6.

Pyrosequencing assay was performed to detect mutations in codon 209 (exon 5) of the *GNAQ* and *GNA11* genes and in codon 625 (exon 14) of the *SF3B1* gene. DNA was amplified by polymerase chain reaction as previously described [10].

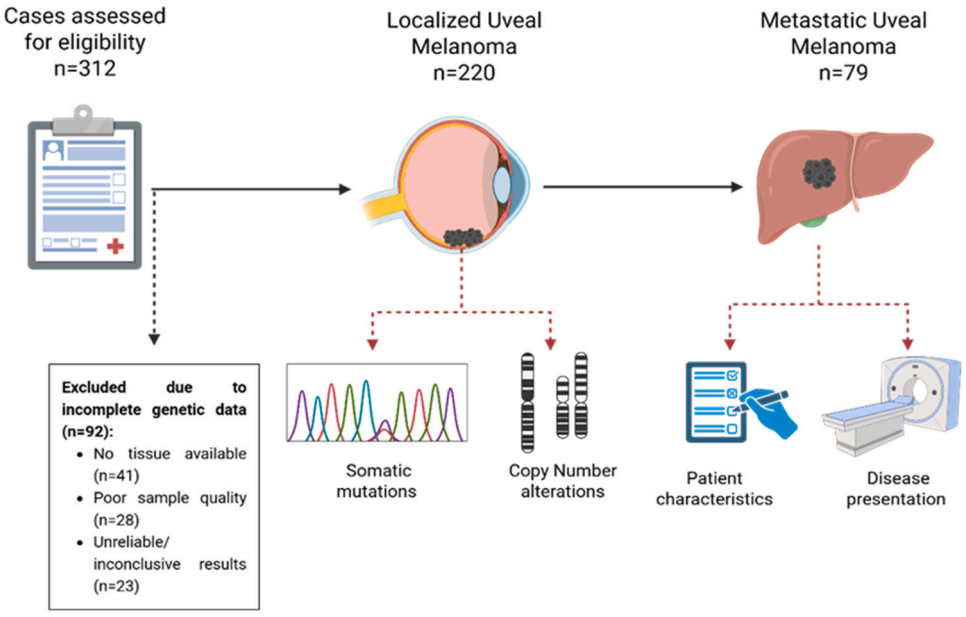


Fig. 1. CONSORT diagram. The UM cohort was composed of 220 consecutive cases with clinical and molecular information. Seventy-nine patients from this localized cohort relapsed and are part of the MUM cohort.

Table 1
Characteristics of all patients (data from n = 220). D3, chromosome 3 disomy; M3, chromosome 3 monosomy; 8 N, chromosome 8q disomy; 8A, chromosome 8q amplified; C, clusters.

Variable	Categories	Number (%)
Sex	Female	61 (27.7 %)
	Male	159 (72.2 %)
Age at Diagnosis	Mean (range)	60 (13.4–93.9)
Primary tumour treatment	Enucleation	99 (45)
	Brachytherapy	65 (30)
	Eye sparing surgery	56 (25)
GNAQ/11 Mutation	*GNAQ*	119 (54)
	GNA11	78 (36)
	Wild-type	23 (10)
Q209 Mutation	Q209P	77 (35)
	Q209L	119 (54)
	Q209R	1 (0.5)
	Wild-type	23 (10.5)
SF3B1 Mutation	R625H	19 (8.4)
	R625C	18 (8.1)
	R625G	1 (0.5)
	R625L	1 (0.5)
	R625S	1 (0.5)
	Wild-type	180 (82)
Chromosome 3	Disomy (D3)	101 (46)
	Monosomy (M3)	119 (54)
Chromosome 8q	Normal (8 N)	120 (54.5)
	Amplified (8A)	100 (45.5)
Chromosome 8p	Normal	168 (76)
	Deleted	21 (10)
	Amplified	31 (14)
Chromosome 1p	Normal	169 (77)
	Deleted	51 (23)
Chromosome 6q	Normal	182 (83)
	Deleted	38 (17)
Chromosome 6p	Normal	163 (74)
	Amplified	57 (26)
Chromosome 3 and 8q	D3 / 8 N	69 (31)
	D3 / 8A	32 (15)
	M3 / 8 N	51 (23)
	M3 / 8Q	68 (31)
TCGA Clusters	C1	69 (31)
	C2	32 (15)
	C3	94 (43)
	C4	25 (11)

2.3. Statistical analyses

Statistical analyses were performed using R version 4.1.0. Continuous variables were summarized using mean, median, and ranges, while categorical variables were summarized using frequencies and percentages. Differences in categorical variables were assessed using the Chi-square or Fisher's exact test, as appropriate. Continuous variables were compared using the Mann-Whitney *U* test or Kruskal-Wallis test where applicable.

Relapse-free survival (RFS) from primary tumor diagnosis and overall survival (OS) from metastasis diagnosis were analyzed using Kaplan-Meier estimates. Survival curves were compared using the log-rank test. Median survival times and 95 % confidence intervals (CI) were reported. Statistical significance for survival comparisons among these groups was determined using the log-rank test, with a threshold of $p < 0.05$ considered statistically significant. All analyses were two-sided. Hazard ratios (HR) and 95 % CIs for univariate and multivariate survival analyses were calculated using the Cox proportional hazards regression model. Supplementary tables and figures provide detailed summaries of the statistical results.

3. Results

3.1. Patient population

The study analyzed a total of 220 primary UM with available mutational analyses for *GNAQ*, *GNA11*, and *SF3B1*; as well as SCNAs in chromosomes 3, 8, 1, and 6. Mutations in *GNAQ* were identified in 119 cases (54.1 %), *GNA11* in 78 cases (35.5 %), and *SF3B1* in 40 cases (18.2 %). Genetic imbalances such as monosomy of chromosome 3 (M3) was identified in 119 cases (54.1 %), chromosome 8q amplification (8A) in 100 cases (45.4 %), and chromosome 8p deletion in 21 cases (9.5 %), while 8p amplification occurred in 31 cases (14.1 %). Chromosome 1p deletion was observed in 51 cases (23.2 %), while chromosome 6q deletion was found in 38 cases (17.3 %), whereas 6p amplification was detected in 57 cases (25.9 %). Detailed clinical and molecular characteristics are provided in Table 1 and Fig. 2A.

Patients were grouped following 2 different approaches according to current literature (Fig. 2A). First, patients were divided according to chromosome 3 (D3 vs M3) and chromosome 8q (normal (8 N) vs amplified (8A)) statuses to form 4 groups: D3/8N (31 %), D3/8A (15 %), M3/8N (23 %), and M3/8A (31 %). Additionally we performed a subdivision according to the TCGA classification in 4 clusters. Cluster 1 (C1) included patients with no significant chromosomal copy SCNAs apart from partial or total 6p gain. Cluster 2 (C2) included D3 patients with 8A gains with or without 6p amplification. M3 patients were included in Cluster 3 (C3) and Cluster 4 (C4). C3 included M3 patients without 8A, or with 8A but without 8p or 6q loss. Finally C4 included M3 and 8A with additional 8p or 6q loss showing higher genetic instability. The resulting patients per cluster were 69 (31 %) for C1, 32 (15 %) for C2, 94 (43 %) for C3 and 25 (11 %) for C4.

3.2. Molecular determinants of survival in primary UM

We used this primary UM dataset to validate the association of SCNAs and recurrent hot-spot mutations with RFS, in an attempt to demonstrate our patient cohort is representative and comparable to other similar cohorts available in the literature. With 48.3 months (95 % CI 38.7–63) follow up the median RFS was 145 months (95 % CI: 85.8–NR). As expected, classic prognostic SCNAs, including M3, 8A, and 8p deletion, were associated with RFS, while other SCNAs and mutations, such as *GNAQ*, *GNA11*, and *SF3B1*, showed no association (Supplementary Table 1 and Supplementary Figure 1).

We applied the classification based on chromosome 3 and 8 statuses. Patients with D3/8N had the longest RFS, which was not reached (95 % CI: 145–NR), while those with M3 and 8A had the shortest RFS at 34 months (95 % CI: 27.7–46.7)($p < 0.0001$)(Fig. 2B). The remaining groups showed intermediate prognosis with 167 months (95 % CI: 72.5-NR) and NR (95 % CI: 110.6-NR) for D3/8A and M3/8N respectively. The 5-y RFS rates for D8/8N, D8/8A, M3/8N and M3/8A were 91 %, 74 %, 69 % and 0 %, respectively. Then, according to the surrogate cluster classification of TCGA (C1, C2, C3, and C4). The median RFS was not reached for C1 (95 % CI: 145-NR), 167 months for C2 (95 % CI: 72.5-NR), 48.7 months for C3 (95 % CI: 40.4-NR), and 27.7 months for C4 (95 % CI: 20.5-NR)($p < 0.0001$). Additionally, the 5y RFS rates for C1, C2, C3 and C4 were 89 %, 73 %, 46 %, and 0 % respectively (Supplementary Figure 2).

It has been considered *SF3B1*m to be exclusive to D3 patients based on TCGA analysis. We observed *SF3B1*m to be distributed equally among tumors harboring either D3 and M3 (20 patients each). Interestingly, *SF3B1*m retained better prognosis independently of D3/M3 status in primary UM patients with median RFS of 110.6 (95 % CI: 42.8 - NR) for *SF3B1*m and M3 compared to 41.8 (95 % CI:33.9–55.2) for M3 without *SF3B1* mutation ($p < 0.0001$)(Supplementary Figure 3).

3.3. Molecular determinants of survival in MUM

With 48.3 (95 % CI 38.7–63) months follow up, 79 patients (36 %) developed metastasis, mainly in the liver. We decided to study if the same SCNAs and recurrent hot-spot mutations identified in primary tumors carry prognostic implications once metastases are diagnosed. The clinical and molecular characteristics of the 79 MUM are

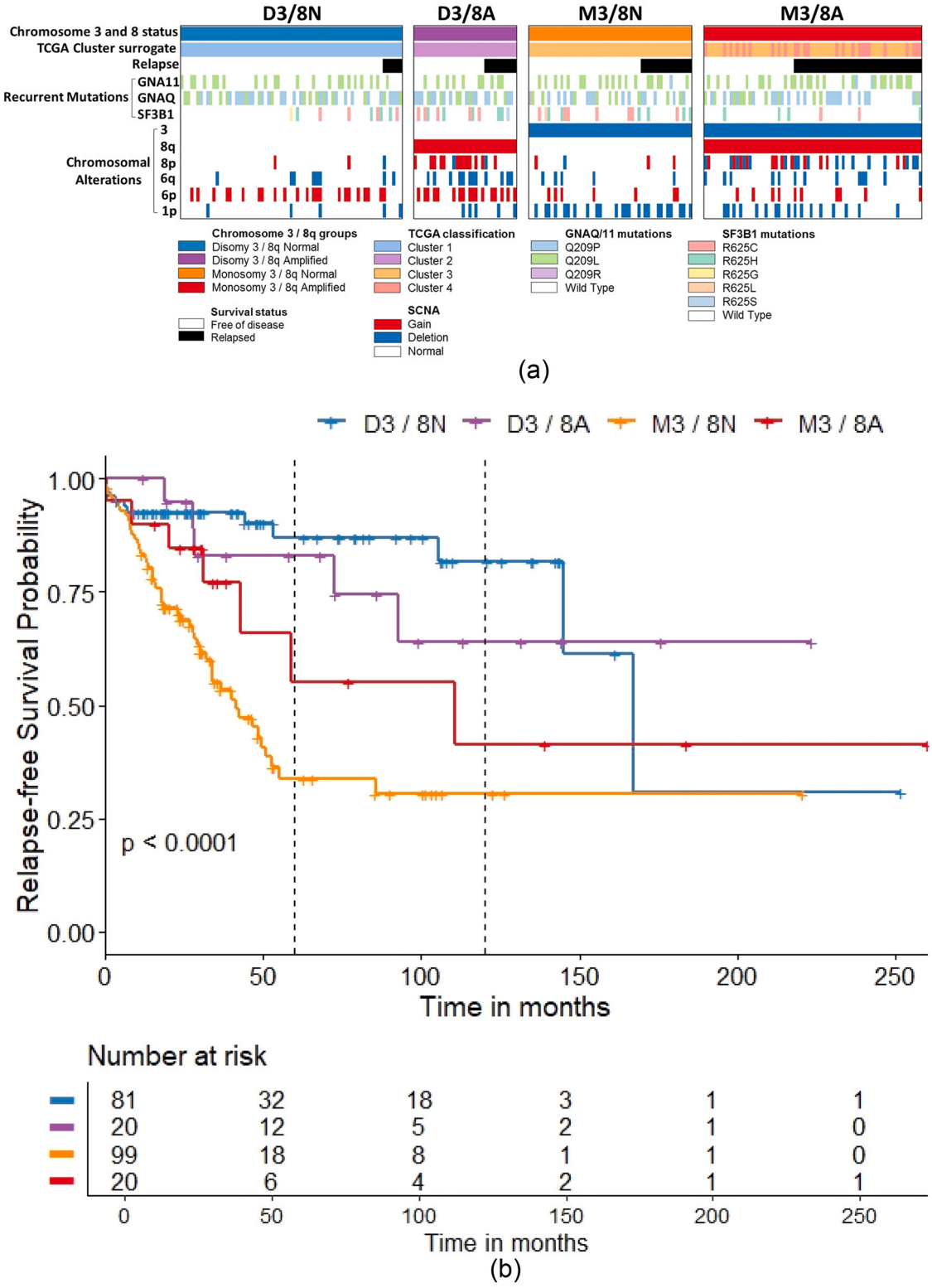


Fig. 2. (A) Heat map showing the cohort of patients (n = 220) diagnosed with primary UM patients distributed according to chromosome 3 and 8q status, recurrent mutations and chromosomal alterations studied. (B) Kaplan-Meier survival curves for relapse free survival for four different groups of patients classified according to chromosome 3 monosomy and/or chromosome 8Q amplification. Dotted line marks 60-months (5-years) and 120-months (10-years). TCGA, the cancer genome atlas; D3, chromosome 3 disomy; M3, chromosome 3 monosomy; 8 N, chromosome 8q disomy; 8A, chromosome 8q amplified; SCNA, Somatic copy number alterations; C, cluster.

summarized in Table 2 and in Fig. 3A. The median OS from MUM diagnosis was 13.2 months (95 % CI: 10.7–18.24). Key clinical variables analyzed included sex, age, performance status, levels of lactate dehydrogenase (LDH), alkaline phosphatase (AP), size of largest liver metastasis (M1a (≤30 mm), M1b (>30–≤80 mm), and M1c (>80 mm)) at time of MUM diagnosis, and presence of extra-liver metastasis and tebentafusp treatment. Clinical variables associated with survival included ECOG performance status, LDH, AP, size of largest liver metastasis, and tebentafusp treatment (Supplementary Table 2 and Supplementary Figure 4). These findings align with existing literature, supporting the representativeness and validity of our dataset.

Next, we evaluated the impact of individual SCNAs on chromosomes 1, 3, 6, and 8 on median OS in MUM patients but found no significant individual associations (Supplementary Figure 5). Neither after applying the classification based on chromosome 3 and 8 statuses (Fig. 3B). Up to 61 patients (77 %) carry M3 and patients 54 (68 %) carry 8A. When combined we identified 7 D3/8N patients (9 %), 11 D3/8A patients (14 %), 18 M3/8N patients (23 %), and 43 M3/8A patients (54 %). The median OS were 10.7 months (95 % CI: 2.6-NR), 19.4 months (95 % CI: 3.12-NR), 18.2 months (95 % CI: 14.5-NR), and 10.8 months (95 % CI: 15.51-NR) (p = 0.33) for D3/8N, D3/8A, M3/8N, and M3/8A respectively. The 1-y mOS rates for D8/8N, D8/8A, M3/8N and M3/8A were 42 %, 54 %, 77 % and 45 %, respectively. Then, we applied the classification according to the surrogate cluster classification of TCGA (C1, C2, C3, and C4). The median OS was 10.7 months for C1 (95 % CI: 2.6-NR), 19.4 months for C2 (95 % CI:3.12 - NR), 13.7 months for C3 (95 % CI:11.32–18.4), and 6.8 months for C4 (95 % CI: 4.28–38.5)(p = 0.9). Additionally, the 1-year median OS rates for C1, C2, C3 and C4 were 42 %, 54 %, 57 %, and 47 % respectively (Supplementary Figure 6).

Table 2
Characteristics of all relapsed patients (data from n = 79). Clinical information is related to time of relapse. Molecular information was obtained from biopsy at time of primary tumor treatment. LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyl-transpeptidase; M1, metastasis; D3, chromosome 3 disomy; M3, chromosome 3 monosomy; 8 N, chromosome 8q disomy; 8A, chromosome 8q amplified; C, clusters.

Variable	Categories	Number (%)
Sex	Female	29 (36.7 %)
	Male	50 (63.3 %)
Age at Diagnosis	Mean (range)	62.3 (32.3–89.9)
Elevated LDH		34 (43 %)
Elevated ALP		18 (23 %)
Elevated GGT		35 (44.3 %)
Extra Liver M1		31 (39.2 %)
Tebentafusp use		17 (21.5 %)
ECOG	0	48 (61 %)
	1 or 2	31 (39 %)
DFS from Primary	> 2-years	43 (65.4 %)
	< 2-years	36 (45.6 %)
M1 staging	M1A	37 (47 %)
	M1B	25 (31.6 %)
	M1C	13 (16.5 %)
	No liver M1	4 (5 %)
GNAQ/11 Mutation	GNAQ	36 (45.6 %)
	GNA11	33 (41.8 %)
	Wild-type	10 (12.7 %)
Q209 Mutation	Q209P	30 (38 %)
	Q209L	39 (49.4 %)
	Wild-type	10 (12.7 %)
SF3B1 Mutation	R625H	7 (8.9 %)
	R625C	5 (6.3 %)
	R625S	1 (1.3 %)
	Wild-type	66 (83.5 %)
Chromosome 3	Disomy (D3)	18 (23 %)
	Monosomy (M3)	61 (77 %)
Chromosome 8q	Normal (8 N)	25 (31.6 %)
	Amplified (8A)	54 (68.4 %)
Chromosome 8p	Normal	55 (69.7 %)
	Deleted	16 (20.3 %)
	Amplified	8 (10.1 %)
Chromosome 1p	Normal	56 (71 %)
	Deleted	23 (29 %)
Chromosome 6q	Normal	64 (81 %)
	Deleted	15 (19 %)
Chromosome 6p	Normal	63 (79.7 %)
	Amplified	16 (20.3 %)
Chromosom 3 and 8q	D3 / 8 N	7 (8.8 %)
	D3 / 8A	11 (13.9 %)
	M3 / 8 N	18 (22.7 %)
	M3 / 8A	43 (54.4 %)
TCGA Clusters	C1	7 (8.8 %)
	C2	11 (13.9 %)
	C3	44 (55,6 %)
	C4	17 (21,5 %)

Similarly, *GNAQ* and *GNA11* mutations had no effect on median OS (see Supplementary Table 2 and Supplementary Figure 5). However, patients with the Q209L mutation showed shorter median OS (10.6 months, 95 % CI: 4.18–18.2) compared to other mutations and wild-type (14.3 months, 95 % CI: 11.8–24.2; p = 0.04), while the Q209P mutation had no impact on median OS. Lastly, we examined the impact of *SF3B1* mutations on median OS in MUM. Unlike in primary UM, *SF3B1* mutated (*SF3B1*m) MUM patients had significantly better median OS than wild-type patients (Fig. 4A). The median OS for *SF3B1*m patients was 31.7 months (95 % CI: 16.4–NR), compared to 11.8 months (95 % CI: 8.85–14.8) for wild-type patients (p = 0.001). Survival rates at 12 and 24 months were also higher for *SF3B1*m patients, at 76 % and 68 %, compared to 48 % and 15 % for wild-type patients, respectively. Given that *SF3B1*m was associated with median OS, we further explored its incidence in relation to other molecular and clinical variables. The *SF3B1*m was associated with a younger age at MUM diagnosis (54.3-yo for *SF3B1*m vs. 63.8-yo for wild-type patients (p = 0.003)) and tebentafusp use (53.8 % for *SF3B1*m vs 15.2 % for wild-type patients (p = 0.006)), and no correlation was found to any other clinical variable, SCNA, or *GNAQ/11* mutation. To better understand the interaction of tebentafusp use with *SF3B1*m, we stratified patients based on *SF3B1* status and tebentafusp use (Supplementary Figure 7). Among patients who did not receive tebentafusp, *SF3B1*m patients had a longer median OS (20.0 months, 95 % CI: 13.7–NR) compared to *SF3B1* wild-type patients (10.8 months, 95 % CI: 7.5–14.3). In the same way, *SF3B1*m patients receiving tebentafusp demonstrated the longest median OS (96 months, 95 % CI: NR–NR), compared to wild-type patients (20.3 months, 95 % CI: 4.0–NA)(p = 0.0002). Thus, *SF3B1*m patients had better prognosis independent of tebentafusp use. These findings should be interpreted with caution due to the small sample size in the subgroup analysis.

We then conducted a multivariate Cox proportional hazards analysis, incorporating the two molecular variables found to be significant in the univariate analysis (Q209L and *SF3B1* mutations) along with all key clinical variables including tebentafusp use. The forest plot summarizing these results is presented in Fig. 4B. In this multivariate model, *SF3B1*m was associated with an independent significantly lower risk of mortality, with an adjusted hazard ratio (HR) of 0.26 (95 % CI: 0.09–0.76) (p = 0.01). Elevated LDH, elevated AP and high liver metastasis burden (M1c) were linked to an increased mortality risk. Tebentafusp treatment showed a strong association with reduced mortality risk (HR = 0.15 (95 % CI: 0.06–0.37)(p < 0.0001). Additionally, another Cox model including the interaction term between Tebentafusp use and *SF3B1*m was employed. The interaction term was not statistically significant (p = 0.44), suggesting no evidence for an interaction between *SF3B1*m and tebentafusp use. Therefore the survival benefits observed for *SF3B1*m and tebentafusp are independent of each other.

4. Discussion

This study investigates the prognostic significance of molecular alterations, including SCNAs and recurrent mutations, in MUM. Our results confirm that while molecular classifications such as TCGA stratify primary UM patients, these same classifications fail to predict OS in the

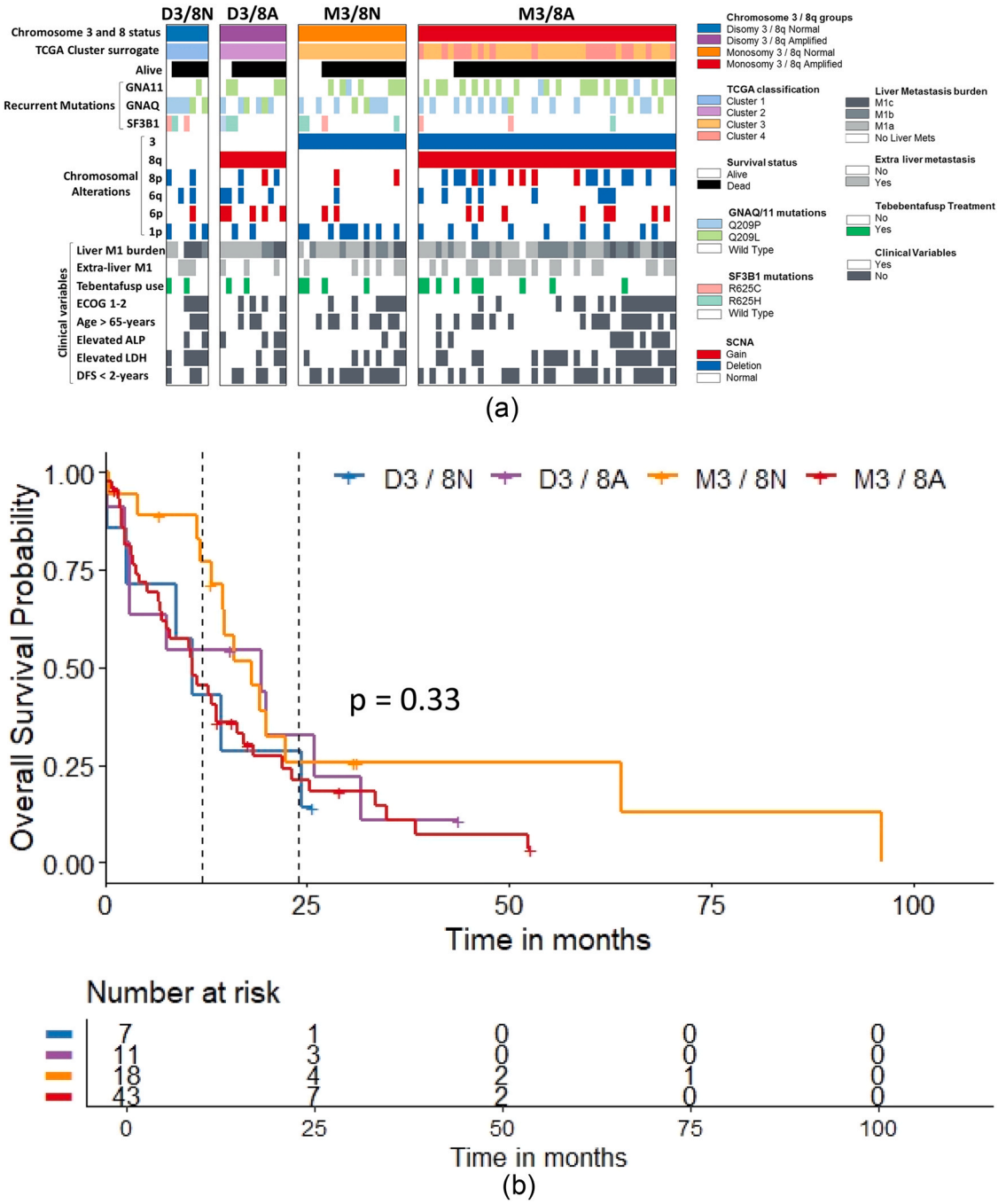
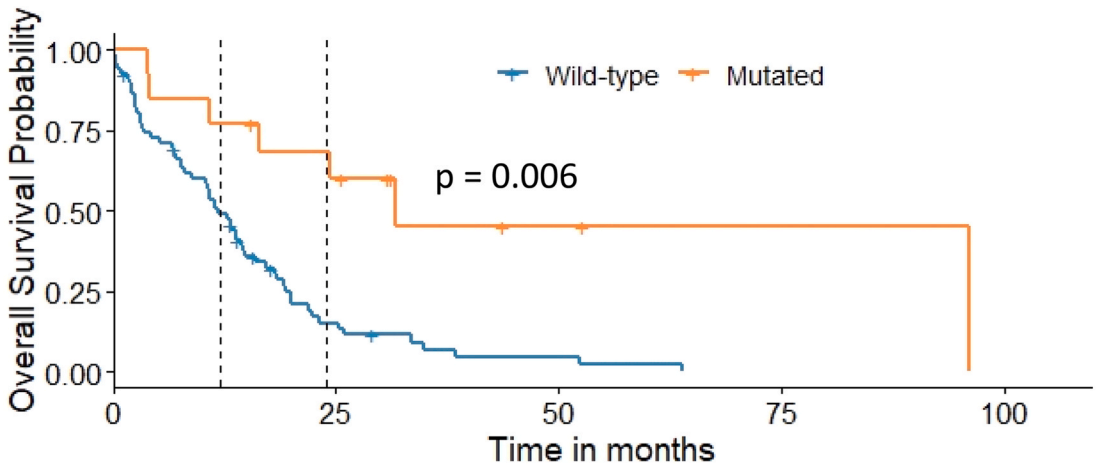
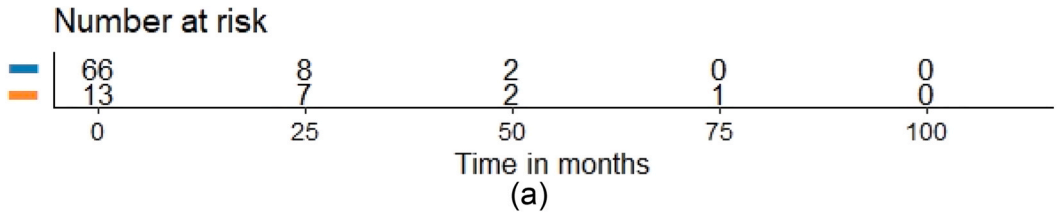


Fig. 3. (A) Heat map showing the cohort of patients (n = 79) that developed metastasis distributed according to chromosome 3 and 8q status, clinical variables, recurrent mutations and chromosomal alterations studied. (B) Kaplan-Meier survival curves for overall survival for four different groups of patients classified according to chromosome 3 monosomy and/or chromosome 8Q amplification. Dotted line marks 12-months (1-year) and 24-months (2-years). TCGA, the cancer genome atlas; D3, chromosome 3 disomy; M3, chromosome 3 monosomy; 8 N, chromosome 8q disomy; 8A, chromosome 8q amplified; SCNA, Somatic copy number alterations; M1, metastasis; ECOG, Eastern Cooperative Oncology Group-Performance Status; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; C, cluster.

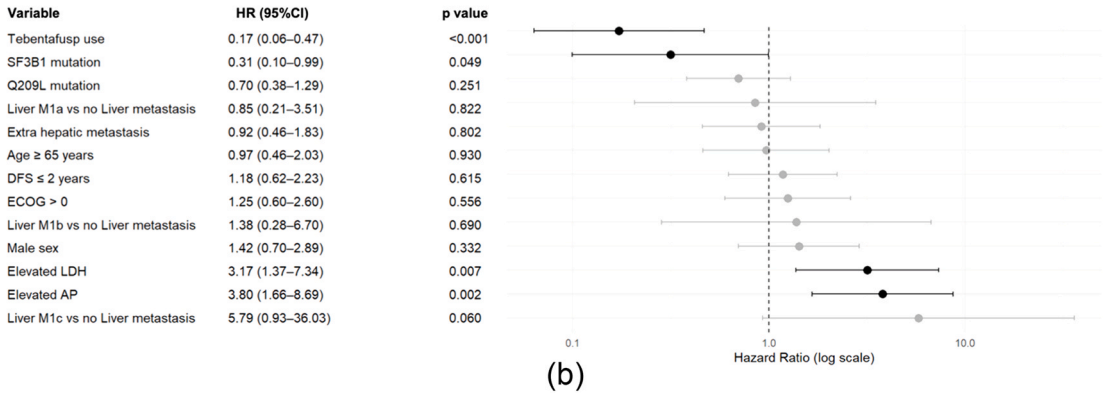
metastatic setting. However, we identify a novel association between *SF3B1* mutations and improved OS in MUM patients, suggesting a potential molecular marker for prognosis in advanced disease. *SF3B1* is a key component of the spliceosome, responsible for removing introns from pre-mRNA and joining exons to produce mature mRNA, a critical process for RNA processing, gene expression, and regulation [11]. *SF3B1* mutations are found in 22–24 % of primary UMs and are primarily localized to codon R625 [6]. *SF3B1*m particularly in patients with D3 status, have been associated with a later onset of metastasis in studies with sufficient long-term follow-up. These mutations can alter RNA interactions, leading to aberrant splicing and potentially resulting in faulty protein production. Interestingly, other spliceosome-related mutations, such as those in *SRSF2*, *RBM10*, and *SF3A1*, have also been documented and may have similar functional impacts [12].

The most striking result from our analysis is the significant survival benefit observed in *SF3B1*m MUM patients. Previous retrospective analyses suggested that patients with *SF3B1m* may have a longer median overall OS compared to historical controls [13], this study is the first to confirm these findings in a large cohort including metastatic patients. This advantage remained significant even after adjusting for key clinical





(a)



(b)

Fig. 4. (A) Kaplan-Meier curves for overall survival showing differences between the cohort of patients (n = 79) that developed metastasis distributed according to SF3B1 mutation. Dotted line marks 12-months (1-year) and 24-months (2-years). (B) Forest plot displaying hazard ratios (HR), 95 % confidence intervals (CI), and p-values for variables included in a multivariate Cox proportional hazards model. The HR is represented by points, with error bars indicating the 95 % CI. A dashed vertical line at HR = 1 denotes the null effect. Variables with a significant protective effect (HR < 1) are highlighted in green, while those associated with a worse prognosis (HR > 1) are shown in red. Covariates with CIs crossing HR = 1 are considered non-significant. This figure illustrates the relative impact of each variable on survival outcomes.

variables in multivariate analysis, supporting *SF3B1*m as an independent favorable prognostic factor in MUM. Furthermore, *SF3B1*m patients were more likely to receive tebentafusp, but survival benefits were independent of treatment, as evidenced by the lack of interaction in the Cox regression model. One of the reasons for the increased use of tebentafusp in our series is because the series includes patients treated with the drug also in second/third lines, thus probably enriching the cohort with patients with better OS characteristics such as *SF3B1*m. This phenomenon has been observed in the few studies testing novel treatments in pretreated MUM patients that have checked for *SF3B1* mutation status, as these patients tend to deteriorate more gradually and may be in better physical condition to enroll. For example, in a study by Kammula on adoptive cell therapy for MUM nearly half of the patients (43 %) were *SF3B1*m [14]. In the same line, a more recent work observed higher than expected frequency of *SF3B1* mutations in patients with MUM that were selected for liver directed therapies [15], reinforcing the idea of *SF3B1*m patient selection in trials or studies centered in successive lines of treatment or more indolent patient evolution.

Mechanistically, two unique features of the *SF3B1* mutation may contribute to its association with a more indolent tumor behavior. First, *SF3B1* hot spot mutations in the R625 codon have been shown to generate aberrant epitopes that can be presented on cancer cells via major histocompatibility complex type I, potentially eliciting a CD8 + T-cell immune response detectable in both peripheral blood and the tumor microenvironment [16]. Second, recent findings indicate that UM cell lines with *SF3B1* R625 mutations display downregulated CINP protein, impairing their response to replication stress. This disruption leads to excessive replication origin firing, unresolved DNA intermediates, and activation of ATM signaling, causing cell cycle arrest at the G2/M checkpoint [17]. Interestingly, this vulnerability may be therapeutically exploitable with PARP inhibitor therapy. Given these findings, *SF3B1* mutation status could be proposed as a stratification factor for future clinical trials in MUM, especially in clinical trials including pre-treated patients. Additionally, this unique molecular alteration and its downstream effects may pave the way for therapies that exploit the neoepitope/T-cell axis or utilize PARP inhibitors such as talazoparib

[16,17].

Interestingly, our results challenge the assumption that *SF3B1* mutations occur exclusively in D3 tumors, as we found *SF3B1*m present in both D3 and M3 tumors. Despite M3 being a strong predictor of poor prognosis in primary UM, *SF3B1*m conferred a survival advantage in M3 primary UM. These findings suggest that *SF3B1*m tumors may exhibit distinct biological properties that influence metastatic behavior and treatment response. Our findings reaffirm the well-established role of M3 and 8A as poor prognostic markers in primary UM. Consistent with previous studies, we observed that M3/8A tumors exhibited the shortest RFS. However, when analyzing survival in MUM, neither M3 nor 8A alone, nor their combination, demonstrated a statistically significant impact on OS. This highlights the known limitation of chromosomal-based prognostication once metastasis occurs, reinforcing the need for novel biomarkers to refine risk stratification in this setting. Notably, while mutations in *GNAQ* and *GNA11* were not associated with OS in MUM, we observed that the Q209L mutation in either *GNAQ* or *GNA11* correlated with a shorter OS compared to other *GNAQ/GNA11* mutations. This finding suggests potential functional differences between Q209L and Q209P variants, warranting further mechanistic studies to elucidate their role in metastatic progression. For example, we have recently described differences in prediction to be presented on HLA-I, and immunoreactivity between Q209L and Q209P, and HLA genotypes with low Q209L affinity show higher frequency in UM patients than in the general population [18].

A limitation of our study was incomplete data regarding *BAP1* status and *EIF1AX* mutations, which prevented their inclusion in the primary analysis. In addition, our analysis of 8A by MLPA is qualitative and does not give the exact number of copy number alterations. Future studies with comprehensive molecular profiling, including these markers, will be essential to further validate and refine prognostic classifications in MUM. Recently, we have implemented a next-generation sequencing custom panel that included all recurrent genetic mutations identified in UM, and copy-number alterations to better understand the importance of these associations identified in the present work.

This study provides new insights into the prognostic landscape of MUM, demonstrating that traditional chromosomal classifications fail to predict OS once metastases develop. Instead, *SF3B1*m emerges as a novel favorable prognostic marker in the metastatic setting, independent of known clinical and molecular risk factors. Given the increasing availability of molecularly targeted therapies, further research is warranted to explore the potential implications of *SF3B1*m for treatment selection and therapeutic stratification in MUM. Future studies should aim to validate these findings in larger, multi-institutional cohorts and investigate the underlying mechanisms driving the improved prognosis observed in *SF3B1*m patients.

CRediT authorship contribution statement

Luis P del Carpio: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Funding acquisition, Formal analysis, Data curation. **Mikel Portu:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Mar Varela:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Elvira Purqueras:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Sergi Villatoro:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Daniel Lorenzo:** Writing – review & editing, Methodology, Investigation, Data curation. **Llado Laura:** Writing – review & editing, Methodology, Investigation, Data curation. **Goma Montserrat:** Writing – review & editing, Methodology, Investigation, Data curation. **Cristina Gutierrez:** Writing – review & editing, Methodology, Investigation, Data curation. **Josep M Piulats:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Emilio Ramos:** Writing – review & editing, Methodology, Investigation. **Caminal josep M:** Writing – review & editing, Methodology, Investigation.

Ethics approval and consent to participate & consent for publication

The Hospital de Bellvitge/Catalan Cancer Institute Institutional Review Boards and Ethics Committee at IDIBELL approved this study for clinical investigation. All the methods were conducted in conformity with the Declaration of Helsinki and relevant guidelines. The study was exempted from written informed consent and required ethics approval from the Institutional Review Board of Hospital de Bellvitge/Catalan Cancer Institute at IDIBELL because it was a retrospective study The manuscript does not contain any individual person's data in any form (including any individual details, images or videos), so consent for publication does not need to be obtained.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Josep M Piulats certifies that all conflict of interest, relationships, and affiliations relevant to the subject matter or materials discussed in the manuscript are the following: Josep M Piulats has acted in a consulting or advisory role for IMMUNOCORE and Bristol-Myers Squibb (BMS); and has received grants from BMS and BeiGene. Any other authors have conflict of interest relevant to the subject matter or materials discussed in the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.115591.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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