Association of Uveal Melanoma Metastatic Rate With Stochastic Mutation Rate and Type of Mutation

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Approximately 40% of patients with uveal melanoma develop metastases during the course of the disease. In 1978, Zimmerman et al\(^1\) reported on mortality rates after enucleation for uveal melanoma, and they found a peak incidence in mortality 2 years after diagnosis/treatment of uveal melanoma (defined as the Zimmerman-McLean-Foster effect). They hypothesized that this was owing to spreading of tumor cells by enucleation (Zimmerman-McLean-Foster hypothesis). Twenty years later, the Collaborative Ocular Melanoma Study (COMS) group published the mortality rates after iodine-125 plaque brachytherapy and found that those rates did not differ from those after enucleation.\(^2\) However, the COMS group also observed a peak in incidence of death approximately 2 years after brachytherapy. Based on these observations, the Zimmerman-McLean-Foster effect was confirmed and the Zimmerman-McLean-Foster hypothesis was disproved.\(^3\)

In this study, we aim to provide a rational explanation for the Zimmerman-McLean-Foster effect. Because it is also seen after enucleation, the existence of clinically undetectable, dormant micrometastases in the liver at the time of diagnosis of uveal melanoma is assumed.\(^4\) The current understanding is that these micrometastases may show progression and enlarge in the liver as they become clinically detectable.\(^4\) This
phenomenon can be seen approximately 2 years after the diagnosis/treatment of the primary tumor, which corresponds with the findings of Zimmerman et al and the COMS. In the 2012 Zimmerman lecture, Grossniklaus hypothesized that the Zimmerman-McLean-Foster effect might be the result of stochastic properties of uveal melanoma and host response (loss of end-organ tumor suppression). Interestingly, the COMS group observed 3 peaks in the death rate graphs for patients treated both with enucleation and plaque brachytherapy. In 2016, the Rotterdam Ocular Melanoma Study Group reported an association of EIF1AX-mutated uveal melanomas with longer disease-free survival and low metastatic risk, an association of SF3BI-mutated tumors with late metastasis, and an association of BAPI-mutated uveal melanomas with early metastasis and a decreased survival rate.

The purpose of this study was to examine the stochastic properties of primary uveal melanoma relative to metastatic rates and type of mutations. We performed a pooled data analysis using data sets from previous studies to elucidate the mutation rates and metastatic rates in uveal melanoma.

Methods

To calculate the metastatic rate and mutation rate for different tumors, we first needed to account for the different tumor sizes. Assuming each tumor cell within the tumor has the same mutation rate, we needed to calculate the number of cells within the tumor. To calculate the metastatic rate for different tumor sizes, we used 2 previously published large data sets. Shields et al calculated the 5-year metastatic rate of uveal melanoma with different tumor thickness values millimeter by millimeter. The COMS Group Report No. 267 reported an estimated proportion of patients with uveal melanoma with metastasis at 5 years. Based on these studies, we categorize uveal melanomas into small (largest basal diameter [LBD], 10 mm), medium (LBD = 12 mm), and large (LBD = 16 mm) size groups, where LBD is the largest basal diameter; the values for LBD were reported. Approximating the nodular tumor shape as a sphere cap, the volume of the tumor is:

$$V_T = \frac{\pi}{3} T^2 \left(\frac{3}{2} LBD - T\right),$$

where $T$ is the thickness of the tumor that has also been measured previously. The number of cancer cells in a tumor is then $N_c = V_T/V_c$, where $V_c = 2(\pi r^3)$ is a typical tumor cell volume. The effective mutation rate per cell is then defined as the metastatic rate divided by the total number of cells $N_c$.

The Rotterdam Ocular Melanoma Study Group provided a large data set of patients with uveal melanoma with mutation analysis for BAPI, SF3BI, and EIF1AX. In the second part of our study, we calculated the yearly metastatic rates of tumors with different mutational statuses based on these data. Patients who were diagnosed with metastasis before treatment or with unknown disease-free survival were excluded from the analysis. The peak incidence of metastasis after treatment was calculated for the BAPI, SF3BI, and EIF1AX mutation group as a proportion of all metastases after treatment. Patients who harbored more than 1 mutation were excluded from the subgroup analysis. The yearly metastatic rate was plotted against the time after treatment for total population and the metastatic rate of patients with each mutation was plotted against time after treatment. A Kaplan-Meier curve was also created to assess the disease-free survival probability for the mutant patient groups.

Results

Based on the 5-year metastasis rate data published by Shields et al, mutation rates ranging from $1.09 \times 10^{-8}$ to $7.68 \times 10^{-7}$ per cell divisions were observed when using our calculation algorithm as shown in the Table. A substantially higher mutation rate was found for tumors with smaller thickness values (Table). Using the 5-year metastasis rate published by the COMS group, we observed a mutation rate of $6.02 \times 10^{-8}$ for small, $2.13 \times 10^{-8}$ for medium, and $1.54 \times 10^{-8}$ for large uveal melanomas based on their LBD values.

The Rotterdam Ocular Melanoma Study Group investigated the mutation status for BAPI, SF3BI, and EIF1AX in uveal melanoma and compared this with survival. In that study, there were 255 patients, of which 162 had BAPI mutations, 43 had SF3BI mutations, 21 had EIF1AX mutations, and 29 had no mutation. For the total study population, a peak incidence of metastasis occurred 3 years after treatment of the primary uveal melanoma, and 2 smaller peaks occurred at 7 and 11 years (Figure 1A). The subgroup analysis included patients who were diagnosed with uveal melanoma metastasis after treatment. A total of 114 patients with metastatic uveal melanoma were included in the final analysis. Ninety-one patients harbored BAPI mutations, 31 had GNAQ mutations, 31 had GNA11 mutations, 2 had EIF1AX mutations, and 12 had SF3BI mutations. Two patients with metastatic uveal melanoma and mutated EIF1AX were excluded because they harbored a BAPI mutation. GNAQ and GNA11 mutations were not mutually exclusive with other mutations. Twenty-five patients with mutated GNAQ harbored BAPI mutations, and 5 with GNAQ mutations had SF3BI mutations. Twenty-four patients with GNA11 mutations harbored BAPI mutations, and 4 of them had SF3BI mutations. After plotting yearly metastatic rate against the time...
after treatment for the different mutations, we observed a small peak in metastases at 1 year after treatment and a large peak at 3.5 years for BAP1 mutations, with an early peak between 2 and 3 years and a late peak at 7 years for SF3B1 mutations (Figure 1B). There was a lack of metastases in patients with tumors that harbored EIF1AX mutations.

**Discussion**

For uveal melanoma, a peak incidence in mortality occurs at approximately 2 years after treatment/diagnosis. We hypothesized that this effect is, at least in part, because of stochastic properties of uveal melanoma.4,8 In other words, metastasis and metastasis-related death from uveal melanoma can be determined in part by random variables. In this study, we investigated the stochastic properties of primary uveal melanoma, focusing on the mutation rate and type of mutation.

Investigations in 2016 and 2017 confirmed that greater tumor thickness, larger basal diameter, and more advanced primary tumor, regional lymph nodes, and distant metastasis stage significantly increased the odds of developing metastatic disease in patients with uveal melanoma.5,7,9,10 Importantly, these studies showed that tumor thickness added prognostic information to molecular data, including gene expression profile and chromosome 3 status.9,11 Several mathematical models have been proposed to calculate the tumor volume.12 To our knowledge, there is no validated algorithm to estimate the volume of uveal melanoma, and a wide variation has been observed in calculated tumor volume when using mathematical modeling.13

The spontaneous mutation rate for a gene is assumed to be $2 \times 10^{-7}$ per gene per division. Neoplastic cells generally contain more mutations compared with nonneoplastic cells.14 In our study, calculated mutation rates ranged from $1.09 \times 10^{-8}$ to $7.86 \times 10^{-7}$ per cell division for uveal melanomas when we used our calculation method. Williams et al15 investigated neutral tumor evolution and mass growth. The highest mutation rates were observed in lung adenocarcinoma (median of $6.79 \times 10^{-7}$), lung squamous cell carcinoma ($5.61 \times 10^{-7}$), and prostate cancer ($1.04 \times 10^{-7}$).15 We observed substantially lower mutation rates for uveal melanoma in our study.
Gass\textsuperscript{46} reported that tumor growth rate was associated with mitotic index in uveal melanomas. Tumor growth rate can be characterized by the tumor volume doubling time. The median doubling times for primary uveal melanoma have been reported to range from 154 to 511 days.\textsuperscript{16-19} Other tumors of neuroectodermal origin were also found to have longer doubling times (mean doubling time, 144 days) for cutaneous melanoma\textsuperscript{20} and 86.3 days for lung small cell carcinoma\textsuperscript{21} than those for malignant lymphomas (29 days), mesenchymal sarcomas (41 days), squamous cell carcinomas (58 days), and adenocarcinomas (83 days).\textsuperscript{22} The mitotic rate in uveal melanoma is low compared with lung squamous cell carcinoma and prostate cancer. Uveal melanocytes of neuroectodermal origin likely cycle at a much lower rate than epithelium. Based on previously reported growth rates, primary uveal melanoma appears to be initiated approximately 10 years before it is clinically diagnosed, and dormant liver micrometastases appear to have been seeded approximately 5 years before they are clinically detected.\textsuperscript{3,16-19,23} Alternatively, Tomlinson et al\textsuperscript{24} demonstrated in a model that increased mutation rates might not be required for evolution and progression of a sporadic cancer. Over several generations, a cell can acquire many mutations and that also depends on cell turnover. Previous authors emphasized the importance of the type of mutation and the selection of a mutator phenotype in tumorigenesis rather than the mutation rate.\textsuperscript{24}

Our results support the hypothesis that for uveal melanoma, tumor size correlating with metastatic rate can largely be explained by the number of neutral mutations in the tumors, consistent with the notion that tumor heterogeneity arises from subclonal accumulation of mutations.\textsuperscript{15} However, we observed a higher mutation rate for smaller tumors. It has been suggested that the mutation rates of different cells might be affected by different cellular growth rates.\textsuperscript{25} The numbers of cell divisions occurring during the expansion phases of uveal melanoma are likely higher for small tumors, ie, when the tumor initially starts growing (eFigure in the Supplement), because doubling time of tumor cells lengthens as the tumor grows.\textsuperscript{26} Before a tumor becomes detectable at 10\textsuperscript{6} cells (ie, 1 g) it must undergo approximately 30 population doublings. However, the lethal tumor mass size (1 kg) requires only 10 more doublings.\textsuperscript{26} A 2018 study\textsuperscript{27} identified punctuated tumor evolution in the early stage of uveal melanoma followed by neutral evolution during its progression. Williams et al\textsuperscript{15} highlighted that mutation rate and mutational timeline are the most important characteristics of tumors possessing neutral growth, whereas selection and the microenvironment may play a key role for nonneural cancer types.

Luzzi et al\textsuperscript{28} investigated the multistep nature of metastatic inefficiency and found that the main contributors for this phenomenon were the failure of extravasated cells in the target (end) organ to initiate growth and the failure of micrometastases to grow into macroscopic tumors. There is experimental evidence that the host response related to this can be both immunologic and biological. In the 2012 Zimmerman lecture, Grossniklaus suggested that the liver controls tumor progression by changes in innate immunity (natural killer cells) and macrophage aging (immunologic suppression).\textsuperscript{4} Production of pigment epithelium–derived factor in the liver inhibits micrometastatic melanoma progression to metastases owing to its dual antitumor/antiangiogenic activities (biological suppression).\textsuperscript{29}

Chromosome status and gene mutations are associated with metastatic risk and prognosis in uveal melanoma.\textsuperscript{30} Monosomy 3 is associated with an increased risk of metastasis and a poor prognosis.\textsuperscript{31,32} A 2017 study\textsuperscript{33} estimated clonal and subclonal somatic copy number alterations in uveal melanoma specimens and identified distinct subtypes with different times to metastasis. That study highlighted the role of coding and noncoding genes that were differentially expressed between somatic copy number alterations subtypes of monosomy 3 uveal melanomas that were associated with metastasis.\textsuperscript{33} Five genes have been reported to be frequently mutated in uveal melanoma.\textsuperscript{34} Mutations in GNAQ and GNA11 occur early in tumor formation, while BAPI, SF3B1, and EIF1AX mutations likely occur later in tumor progression.\textsuperscript{34} Mutation in EIF1AX is an indicator of good prognosis, whereas mutations in SF3B1 and BAPI are associated with intermediate and poor prognosis. GNAQ and GNA11 have shown to be mutated in choroidal nevi,\textsuperscript{35} and they have not been proved to be associated with metastasis and survival in uveal melanoma.\textsuperscript{34} However, as previously indicated, not only the mutation rate and type of mutation in the primary tumor may explain the metastatic rates; the host response (leukocyte and macrophage infiltrate) is also important.\textsuperscript{33,36,37} In contrast with intuition, the presence of an inflammatory phenotype in uveal melanoma is associated with poor prognosis.\textsuperscript{38}

The Rotterdam Ocular Melanoma Study Group\textsuperscript{5} investigated the association of EIF1AX, SF3B1, and BAPI mutation with disease-free survival and metastatic risk of patients with uveal melanoma. In their 2016 article, they published a Kaplan-Meier survival probability curve regarding these mutations. Three slopes on survival curves of patients treated for uveal melanoma could be identified; the first slope being at 3 years on the BAPI mutation curve, the second slope being at 7 to 8 years on the SF3B1 mutation curve, and the third slope being at 8 years on the EIF1AX mutation curve. Three peaks can be observed on the COMS graph for death rates at 3, 5, and 8 years after treatment. Therefore, the mutational analysis of these genes provided by the Rotterdam Ocular Melanoma Study Group and our pooled data analysis on their updated data may serve as an explanation for the COMS trial results (Figure 2).\textsuperscript{5} The first and second peaks are strongly associated with the poor BAPI–mutation related survival rate. The third peak may coincide with the SF3B1 mutation effects on the metastasis and survival probability. The COMS graph is a cumulative curve but may be interpreted to be representative for the mean uveal melanoma population, similar to the Rotterdam Ocular Melanoma Study Group. In the original article, 24% of patients harbored an SF3B1 mutation, and 21% of patients had an EIF1AX mutation.\textsuperscript{5} BAPI expression as determined with immunohistochemistry was lost in 38% of patients.\textsuperscript{5} This is in accordance with previous publications.\textsuperscript{34,39,40} Decatur et al\textsuperscript{34} reported a prevalence of BAPI mutations in 45%, SF3B1 mutations in 24%, and EIF1AX mutations in 17% of uveal melanomas. They found that BAPI, SF3B1, and EIF1AX mutations...
were usually mutually exclusive from each other.34 After performing a pooled data analysis of the Rotterdam data, we observed a peak of metastasis at 3.5 years for BAP1 mutations and a peak between 2 and 3 years and a late peak at 7 years for SF3B1 mutation after treatment for the primary uveal melanoma. SF3B1 mutations have been found to be associated with preferentially expressed antigen in melanoma expression.41,42 Because preferentially expressed antigen in melanoma is an independent risk factor for the development of metastasis in disomy 3 tumors,42 preferentially expressed antigen in melanoma expression might have an influence on the SF3B1 mutation-related survival curve.

Limitations
There are a few limitations of this study. It is a rough approximation that the primary uveal melanoma grows as a spherical cap. Because the first part of our study was a pooled data analysis, only 2 dimensions of tumor mass were available to calculate tumor volume based on the published data. However, computation of tumor volume is more reliable if the mass is measured in 3 dimensions. Ideally, we could use ultrasonography for a more accurate estimation. Disease-free survival of patients with uveal melanoma with BAPI and SF3B1 mutation may be influenced by the follow-up because survival is more favorable for patients with SF3B1 mutation in the earlier stages. The relevance of our study is that we developed a simplified computational algorithm to estimate the intrinsic mutation rate for different tumor sizes and to yield an explanation for the previously reported mortality/metastasis rate for primary uveal melanoma.

Conclusions
Our results suggest that for uveal melanoma, tumor size associated with metastatic rate can largely be explained by a constant (intrinsic) mutation rate for each tumor cell. However, we observed a higher mutation rate for smaller tumors, which may be interpreted to be owing to greater numbers of cell divisions occurring during the expansion phases of smaller uveal melanomas. Regarding time to clinically detected metastasis, the first 2 peaks appear to coincide with BAPI-mutated tumors, and the late peak appears to coincide with the SF3B1-mutated tumors. This provides a stochastic mutation rate and specific type of mutation explanation for the Zimmerman-McLean-Foster effect.

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