Genetic prognostication in uveal melanoma

Dogrusoz, M.

Chapter 1

General Introduction and Thesis Outline

INTRODUCTION TO UVEAL MELANOMA

Epidemiologic characteristics

Uveal melanoma (UM) comprises approximately 3-5% of all types of melanoma, most of which occur in the skin.[1-4] UM is an ocular tumor and develops from melanocytes residing in the iris, ciliary body, or choroid. The choroid is the most common (≈90%) site of origin, followed by the ciliary body (5-8%), and iris (3-5%).[5] Both eyes are affected in equal numbers, and a bilateral occurrence of UM is very rare.[6] UM has a mean annual age-adjusted incidence of 5.1 per million in Western countries and is the most frequently occurring primary intraocular malignancy in adults.[4] Up to 80% of patients are over 50 years of age, with a mean age at diagnosis of 61.[4] Albeit several studies reported no gender difference in incidence,[7, 8] a recent study involving over 7,500 UM cases reported a slight but significantly different male-to-female ratio of 1.1:1.[4] There is a racial and ethnic variation in its incidence. The incidence is 6 per million per year in whites, 1.67 in Hispanics, 0.38 in Asians, and 0.31 in blacks.[9] Northern Europe has a higher incidence of UM than Southern Europe,[10] with an annual incidence of more than 8 per million in Denmark and Norway and less than 2 per million in Spain and Southern Italy.[11] The incidence of UM in the United States has remained stable over the last decades,[12] while in Sweden, an annual relative decrease of 1% in males and 0.7% in females occurred between 1960 and 1998.[13]

Risk factors

Many host and environmental parameters have been evaluated as possible predisposing factors for UM development. Caucasians with a light iris color and a fair skin that burns easily after sun exposure have been shown to have a higher risk of developing UM than persons with a dark skin and eye color.[14] This association corresponds with the aforementioned race-dependent disparity in UM incidence, and the south-to-north increasing incidence in Europe: Northern Europeans have on average lighter eye pigmentation than Southern Europeans.[15] Besides skin and eye color, other factors have been identified as host susceptibility factors for developing UM. Oculo(dermal) melanocytosis (nevus of Ota) is a hyperpigmentation of the uvea, sclera, and episclera as well as of the periocular skin.[16] This condition affects 0.04% of the white population, while the prevalence in UM patients is 1.2-3%, which makes this condition 30 to 75 times more prevalent in UM patients than in the general white population.[16-18] Individuals with oculo(dermal) melanocytosis have a 1 in 400 lifetime risk of

developing UM, while the lifetime risk for UM in the general population is 1 in 13,000.[19] Another risk factor is the presence of a choroidal nevus. Choroidal nevi are quite frequent in Caucasians, with a prevalence ranging from 5% to 8%.[20] The risk of malignant transformation of a choroidal nevus has been estimated to be 1 in 4,300 to 1 in 8,845 per year.[20-22] The risk may depend on the size of the nevus. The rate of transformation of giant choroidal nevi (≥ 10mm diameter) into melanoma has been reported to be 18% at 10-year follow-up.[23]

Besides choroidal nevi, the presence of common/atypical cutaneous nevi, familial atypical multiple melanoma mole (FAMMM) syndrome, and cutaneous freckles is associated with a higher risk of developing UM.[24-26]

Recently, germline mutations in the *BAP1* (BRCA1-associated protein-1) gene were found to confer a higher risk of UM as well as other malignancies, such as cutaneous melanoma, mesothelioma, meningioma, renal cell carcinoma, and lung adenocarcinoma.[27, 28] We do not yet know how often Dutch UM patients carry this germline mutation. A study in Finland showed that 2% of their UM patients were affected.[29]

In contrast to cutaneous melanoma, there is no conclusive evidence for an association between ultraviolet light exposure and the risk to develop UM. While a meta-analysis regarding the relation between ultraviolet radiation and the risk to develop UM yielded contradictive results, it identified welding as a possible risk factor.[30] This may be due to occupational exposure to artificial ultraviolet light in welders; however, welding arcs also emit blue light, which has recently been associated with the risk of developing UM.[31] Dietary habits, smoking and alcohol consumption do not seem to affect the incidence of UM.[26]

Presentation and Diagnosis

In a retrospective review of 2384 UM patients in an ocular oncology center, the most common symptom patients presented with was blurred vision (38%), followed by photopsia (9%), floaters (7%), visual field loss (6%), a visible tumor (3%), pain (2%), and metamorphopsia (2%). Approximately one-third of patients were asymptomatic on referral.[32]

The symptoms caused by UM depend on the location and size of the tumor. Patients with an iris melanoma are usually asymptomatic, and differentiation between a nevus and a melanoma is often difficult. The tumor may be noticed as a dark spot on the iris or have caused a distortion of the pupil. Most iris melanomas (45%) are located in the inferior quadrant. Approximately 80% of

cases are pigmented.[33] In a series of 200 patients with suspect iris lesions, 24% cases were confirmed to be UM, while most other patients were diagnosed with iris cysts (38%) and iris nevi (31%).[34] The criteria for the clinical diagnosis of melanoma were a diameter larger than 3 mm and thickness over 1 mm, replacement of the stroma of the iris, and the presence of at least 3 of the following features: growth, secondary glaucoma/cataract, prominent vascularity, or ectopion irides.[34]

In contrast to iris melanoma, a UM located in the ciliary body is not easily visible on ophthalmic examination and may be missed in the absence of symptoms. Especially small ciliary body melanomas are hidden behind the iris and may not cause symptoms. Signs that may raise the suspicion of a ciliary body melanoma are dilated episcleral vessels (sentinel vessels), extrascleral extension, cataract when the tumor touches the lens, and raised intraocular pressure if the tumor grows circumferentially. The majority of ciliary body melanomas, however, grows in a dome-shaped configuration and may be visible on dilated fundus examination.[35] Choroidal melanomas may be easily detectable, especially if they are located centrally, and may cause symptoms of e.g. vision loss and metamorphopsia when located close to the macula. Peripheral choroidal melanomas may present with a visual field defect or with photopsia. Most choroidal melanomas (77%) grow in a dome-shaped configuration and are completely or partially pigmented (85%).[36, 37] Although large and medium-sized tumors can often be accurately diagnosed by fundoscopy, the diagnosis of small melanomas can be more challenging due to their resemblance to nevi. The mnemonic “To Find Small Ocular Melanoma Using Helpful Hints Daily” (TFSOM-UHHD) which sums up risk factors for the transformation of a nevus into melanoma has been proposed as a useful tool to identify small melanomas or to determine the follow-up schedule of melanocytic lesions.[38] A tumor thickness of >2 mm, the presence of subretinal fluid, visual symptoms, or orange pigment, a margin within 3 mm of the optic disc, ultrasonographic hollowness, absence of a halo (a circular band of unpigmented area surrounding a pigmented nevus) and lack of drusen have been identified as the relevant risk factors that predict growth of choroidal nevi into melanoma.[38] As evident from the inclusion of ultrasonography (USG) in this mnemonic, the clinical diagnosis of UM can be supported or confirmed using various ancillary examinations. USG, especially B-mode ultrasound, is the most often used auxiliary method in the diagnosis of UM. On B-mode USG, the tumor may appear as a dome- or mushroom-shaped hyper-

echoic mass with a hollow appearance, due to a lower reflectivity than the surrounding choroidal tissue.[39] USG may be especially helpful in the diagnosis of UM in the presence of dense cataract or vitreous hemorrhage. Moreover, it can be used to measure tumor elevation which aids in treatment planning and the subsequent evaluation of the effect of eye-preserving treatments.[35, 40]
Fluorescein angiography (FA) is valuable in confirming the presence of orange pigment and subretinal fluid, which are helpful in identifying small melanomas.[41] FA may also reveal the presence of e.g. hyperautofluorescent drusen, which are indicative of a nevus.[42]
The accuracy of diagnosing a UM has been estimated to be over 99% when the diagnosis was based on fundoscopy, USG, and FA.[43] However, a more recent study in 2,384 patients diagnosed with UM in an ocular oncology center reported that 23% of UMs had initially been missed.[32]
Computer tomography (CT) and magnetic resonance imaging (MRI) are less commonly utilized in the diagnosis of UM and may be of use when ultrasonography is unable to visualize the lesion in patients with media opacities such as dense cataract and vitreous hemorrhages.[41] MRI may be especially helpful in differentiating UM from simulating lesions and in the detection of optic nerve involvement or orbital extension.[44] Biopsies are especially helpful in the diagnosis of suspected intraocular tumors in which it is challenging to obtain a reliable diagnosis after careful fundoscopic examination and adjunctive diagnostic techniques.[45]

Treatment

Eye-conserving therapies and enucleation are the main treatment modalities for primary UM. Enucleation is the traditional treatment option and is nowadays indicated for the treatment of UM in patients with vision loss, for large tumors, tumors in close proximity to the optic disk or which have invaded the optic disk, and cases with extraocular growth.[46, 47] Small- and medium-sized tumors are mainly treated by plaque brachytherapy using different types of radioactive isotopes, most commonly ^{125}Iodium and ^{106}Ruthenium, which are administered to the tumor by a plaque sutured to the episclera. Brachytherapy and enucleation do not provide a different metastasis rate. The Collaborative Ocular Melanoma Study (COMS) group compared enucleation versus ^{125}Iodium brachytherapy for medium-sized tumors and did not find any significant difference in mortality rates at 12-years follow-up:[48, 49] melanoma-related mortality was 21% in the brachytherapy

group and 17% in the enucleated patients.[48] Brachytherapy provides excellent local tumor control, but long-term visual loss is common: the COMS reported substantial impairment of visual acuity within 3 years following ^{125}Iodium brachytherapy in 43% to 49% of patients.[50] Part of the vision loss after brachytherapy is due to the complications of the therapy. Common vision-affecting complications include radiation-induced retinopathy, neovascular glaucoma, and macular edema.[51] Loss of visual acuity occurred mainly in diabetic patients and in patients with thick tumors and retinal detachment. Local tumor recurrence or complications such as neovascular glaucoma may lead to secondary enucleations. A secondary enucleation rate between 12-17% has been reported at 3-5 years follow-up.[50, 52]

In the past, small UMs were commonly observed for growth and only treated when tumor enlargement was documented.[53, 54] However, there is a tendency towards earlier treatment since the COMS group reported that 21% of small UMs which were managed by observation showed growth by 2 years and 31% at 5-years follow-up.[55]

Another type of radiotherapy utilized for the treatment of primary UM is proton beam irradiation. Proton beam irradiation could in theory be used for the treatment of all UMs, but is mainly reserved for large tumors in eyes with useful or salvageable vision, as it is a globe-preserving therapy for very large UMs that are not suitable for brachytherapy.[56] Desjardins et al. treated 2,413 patients by proton beam therapy, and found a 10-year metastasis rate of 27%, compared to 25% and 30% in other studies.[57]

Although recent advances in the treatment of primary UM have resulted in excellent local control and preservation of the eye, survival rates have not improved significantly.[12, 58] Damato et al. have shown that timely treatment of the primary tumor may be useful in preventing metastases in small tumors.[59] Studies on doubling times of UM metastases have indicated that micrometastases probably occur before diagnosis and treatment of the primary UM in a large portion of patients.[60] Moreover, many patients develop metastases soon after treatment of the primary UM, indicating that subclinical disseminated disease was probably already present at the time of the treatment.[61] In support of this theory, circulating tumor cells have been detected in patients who had no clinical metastases at diagnosis.[62]

Prognosis and Prognostication

The findings that 1) survival of UM patients has not improved despite advances in

the local control of the primary tumor; 2) treatment of the primary tumor may only improve survival in small tumors; 3) micrometastases probably occur before diagnosis of the primary tumor, imply that enhancement of the survival of UM patients can mainly be achieved by inventing effective therapeutic modalities for UM metastases. Up to 50% of UM patients eventually develop metastases, usually in the liver, and die because of a lack of effective systemic treatments for disseminated UM.[63] The reported median survival time after detection of metastases is 4 to 15 months.[64]

Various therapeutic options for the treatment of UM metastases are being investigated in clinical trials. Kinase inhibition targeting the MAPK and/or PI3K pathway has been evaluated and proposed as a potential adjuvant therapy to prevent metastatic outgrowth in patients at high risk of developing disseminated disease.[65] Another focus is immunotherapy which has shown promising results in the treatment of cutaneous melanoma.[66, 67]

In view of the increasing number of studies evaluating new therapies, determining which patients are at high-risk of developing metastases by reliable prognostication is relevant for their inclusion in these clinical trials. Identification of high-risk cases is also important for planning of follow-up measures to identify metastases in an early phase and for implementation of adjuvant therapies which may prevent disseminated disease. Furthermore, prognostication allows life-planning in high-risk patients and can be used to reassure those at low-risk of developing metastases.

A variety of clinical, anatomic, histological, and genetic prognostic indicators have been identified in UM and are being utilized in clinical practice. Prognostication by genetic markers has been proven to reliably predict survival in UM and is currently a heavily investigated topic. Genetic prognostication in UM is discussed extensively in Chapter 2.

THESIS OUTLINE

This thesis is an overview of research performed to better understand the role of genetic and non-genetic factors for prognostication in UM and to identify the function of such factors.

In this introduction, I have provided an overview of the clinical aspects of UM, covering essential topics such as epidemiology, clinical presentation, diagnosis, treatment, and prognostication. **Chapter 2** provides a detailed overview of the current status of genetic prognostication in UM, evaluates various types of

genetic markers, compares genetic tests, and addresses relevant topics related to the application of genetic prognostication in daily clinical practice. In **Chapter 3**, demographic, anatomic, histological, and genetic prognostic markers that influence survival in long-term surviving patients are addressed, while results demonstrating refinement of prognostication in UM by combining genetic markers and anatomic staging are described in **Chapter 4**. Since most primary UMs are treated by radiotherapy and chromosome markers are commonly utilized for prognostication, I evaluated the effect of radiation treatment on chromosome testing in UM in **Chapter 5**.

In **Chapter 6**, the results of a study evaluating differences in the expression of DNA repair molecules between prognostically-favorable and prognostically-unfavorable UM are presented. Aberrant DNA repair is a hallmark of cancer that plays a role in the development and progression of malignancies and may be used as a target for therapy. I set out to analyze the expression of DNA repair genes in UM, since the role of DNA repair mechanisms in UM has been underexposed. Similarly, there is lack of knowledge about the role of epigenetic regulators in the pathogenesis of UM. Epigenetic modifications have been shown to contribute to cancer development and progression. I have analyzed the expression levels of a number of epigenetic modifiers in UM. **Chapter 7** reports on differences in the expression level of epigenetic markers between UMs with a favorable prognosis and UMs with an adverse prognosis. **Chapter 8** provides a summary and general discussion of the findings described in this thesis, and concludes by putting forward future perspectives on genetic prognostication in UM.

REFERENCES

1. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998;83:1664-1678.
2. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973-1997. *Ophthalmology* 2003;110:956-961.
3. McLaughlin CC, Wu XC, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. *Cancer* 2005;103:1000-1007.
4. Mahendraraj K, Lau CS, Lee I, Chamberlain RS. Trends in incidence, survival, and management of uveal melanoma: a population-based study of 7,516 patients from the Surveillance, Epidemiology, and End Results database (1973-2012). *Clin Ophthalmol* 2016;10:2113-2119.
5. Damato B. Progress in the management of patients with uveal melanoma. The 2012 Ashton Lecture. *Eye (Lond)* 2012;26:1157-1172.
6. Singh AD, Shields CL, Shields JA, De Potter P. Bilateral primary uveal melanoma. Bad luck or bad genes? *Ophthalmology* 1996;103:256-262.
7. Damato BE, Coupland SE. Differences in uveal melanomas between men and women from the British Isles. *Eye* 2012;26:292-299.
8. Zloto O, Pe'er J, Frenkel S. Gender differences in clinical presentation and prognosis of uveal melanoma. *Invest Ophthalmol Vis Sci* 2013;54:652-656.
9. Hu DN, Yu GP, McCormick SA, Schneider S, Finger PT. Population-based incidence of uveal melanoma in various races and ethnic groups. *Am J Ophthalmol* 2005;140:612-617.
10. Seddon JM, Gragoudas ES, Glynn RJ, Egan KM, Albert DM, Blitzer PH. Host factors, UV radiation, and risk of uveal melanoma. A case-control study. *Arch Ophthalmol* 1990;108:1274-1280.
11. Virgili G, Gatta G, Ciccolallo L, et al. Incidence of uveal melanoma in Europe. *Ophthalmology* 2007;114:2309-2315.
12. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology* 2011;118:1881-1885.
13. Bergman L, Seregard S, Nilsson B, Ringborg U, Lundell G, Ragnarsson-Olding B. Incidence of uveal melanoma in Sweden from 1960 to 1998. *Invest Ophthalmol Vis Sci* 2002;43:2579-2583.
14. Weis E, Shah CP, Lajous M, Shields JA, Shields CL. The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch Ophthalmol* 2006;124:54-60.
15. Candille SI, Absher DM, Beleza S, et al. Genome-wide association studies of quantitatively measured skin, hair, and eye pigmentation in four European populations. *PLoS One* 2012;7:e48294.

16. Gonder JR, Ezell PC, Shields JA, Augsburger JJ. Ocular melanocytosis. A study to determine the prevalence rate of ocular melanocytosis. *Ophthalmology* 1982;89:950-952.
17. Velazquez N, Jones IS. Ocular and oculodermal melanocytosis associated with uveal melanoma. *Ophthalmology* 1983;90:1472-1476.
18. Shields CL, Kaliki S, Livesey M, et al. Association of ocular and oculodermal melanocytosis with the rate of uveal melanoma metastasis: analysis of 7872 consecutive eyes. *JAMA Ophthalmol* 2013;131:993-1003.
19. Singh AD, De Potter P, Fijal BA, Shields CL, Shields JA, Elston RC. Lifetime prevalence of uveal melanoma in white patients with oculo(dermal) melanocytosis. *Ophthalmology* 1998;105:195-198.
20. Singh AD, Kalyani P, Topham A. Estimating the risk of malignant transformation of a choroidal nevus. *Ophthalmology* 2005;112:1784-1789.
21. Ganley JP, Comstock GW. Benign nevi and malignant melanomas of the choroid. *Am J Ophthalmol* 1973;76:19-25.
22. Sumich P, Mitchell P, Wang JJ. Choroidal nevi in a white population: the Blue Mountains Eye Study. *Arch Ophthalmol* 1998;116:645-650.
23. Li HK, Shields CL, Mashayekhi A, et al. Giant choroidal nevus clinical features and natural course in 322 cases. *Ophthalmology* 2010;117:324-333.
24. van Hees CL, de Boer A, Jager MJ, et al. Are atypical nevi a risk factor for uveal melanoma? A case-control study. *J Invest Dermatol* 1994;103:202-205.
25. Weis E, Shah CP, Lajous M, Shields JA, Shields CL. The association of cutaneous and iris nevi with uveal melanoma: a meta-analysis. *Ophthalmology* 2009;116:536-543.e532.
26. Seddon JM, Young TA. Epidemiology of uveal melanoma. In: Ryan SJ (ed), *The Retina*: Elsevier-Mosby; 2006:691-698.
27. Abdel-Rahman MH, Pilarski R, Cebulla CM, et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J Med Genet* 2011;48:856-859.
28. Pilarski R, Cebulla CM, Massengill JB, et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. *Genes Chromosomes Cancer* 2014;53:177-182.
29. Turunen JA, Markkinen S, Wilska R, et al. BAP1 Germline Mutations in Finnish Patients with Uveal Melanoma. *Ophthalmology* 2016;123:1112-1117.
30. Shah CP, Weis E, Lajous M, Shields JA, Shields CL. Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. *Ophthalmology* 2005;112:1599-1607.
31. Logan P, Bernabeu M, Ferreira A, Burnier MN, Jr. Evidence for the Role of Blue Light in the Development of Uveal Melanoma. *J Ophthalmol* 2015;2015:386986.

32. Damato EM, Damato BE. Detection and time to treatment of uveal melanoma in the United Kingdom: an evaluation of 2,384 patients. *Ophthalmology* 2012;119:1582-1589.
33. Shields CL, Furuta M, Thangappan A, et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. *Arch Ophthalmol* 2009;127:989-998.
34. Shields JA, Sanborn GE, Augsburger JJ. The differential diagnosis of malignant melanoma of the iris. A clinical study of 200 patients. *Ophthalmology* 1983;90:716-720.
35. Shields JA, Shields CL. Posterior uveal melanoma: clinical and pathologic features. In: Shields JA, Shields CL (eds), *Intraocular Tumours-A Text and Atlas* Philadelphia Pa, USA: W.B. Saunders 1992:117-136.
36. Diener-West M, Earle JD, Fine SL, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, II: characteristics of patients enrolled and not enrolled. COMS Report No. 17. *Arch Ophthalmol* 2001;119:951-965.
37. Shields CL, Kaliki S, Furuta M, Mashayekhi A, Shields JA. Clinical spectrum and prognosis of uveal melanoma based on age at presentation in 8,033 cases. *Retina* 2012;32:1363-1372.
38. Shields CL, Furuta M, Berman EL, et al. Choroidal nevus transformation into melanoma: analysis of 2514 consecutive cases. *Arch Ophthalmol* 2009;127:981-987.
39. Boldt HC, Byrne SF, Gilson MM, et al. Baseline echographic characteristics of tumors in eyes of patients enrolled in the Collaborative Ocular Melanoma Study: COMS report no. 29. *Ophthalmology* 2008;115:1390-1397, 1397.e1391-1392.
40. Laver NV, McLaughlin ME, Duker JS. Ocular melanoma. *Arch Pathol Lab Med* 2010;134:1778-1784.
41. Kivela T. Diagnosis of uveal melanoma. *Dev Ophthalmol* 2012;49:1-15.
42. Almeida A, Kaliki S, Shields CL. Autofluorescence of intraocular tumours. *Curr Opin Ophthalmol* 2013;24:222-232.
43. Accuracy of diagnosis of choroidal melanomas in the Collaborative Ocular Melanoma Study. COMS report no. 1. *Arch Ophthalmol* 1990;108:1268-1273.
44. De Potter P, Flanders AE, Shields JA, Shields CL, Gonzales CF, Rao VM. The role of fat-suppression technique and gadopentetate dimeglumine in magnetic resonance imaging evaluation of intraocular tumors and simulating lesions. *Arch Ophthalmol* 1994;112:340-348.
45. Midena E, Parrozzani R. Biopsies in uveal melanoma. *Dev Ophthalmol* 2012;49:81-95.
46. Damato B, Lecuona K. Conservation of eyes with choroidal melanoma by a multimodality approach to treatment: an audit of 1632 patients. *Ophthalmology* 2004;111:977-983.

47. Bechrakis NE, Bornfeld N, Zoller I, Foerster MH. Iodine 125 plaque brachytherapy versus transscleral tumor resection in the treatment of large uveal melanomas. *Ophthalmology* 2002;109:1855-1861.
48. Diener-West M, Earle JD, Fine SL, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, III: initial mortality findings. COMS Report No. 18. *Arch Ophthalmol* 2001;119:969-982.
49. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma: V. Twelve-year mortality rates and prognostic factors: COMS report No. 28. *Arch Ophthalmol* 2006;124:1684-1693.
50. Melia BM, Abramson DH, Albert DM, et al. Collaborative ocular melanoma study (COMS) randomized trial of I-125 brachytherapy for medium choroidal melanoma. I. Visual acuity after 3 years COMS report no. 16. *Ophthalmology* 2001;108:348-366.
51. Caminal JM, Padron-Perez N, Arias L, et al. Transscleral resection without hypotensive anaesthesia vs iodine-125 plaque brachytherapy in the treatment of choroidal melanoma. *Eye (Lond)* 2016;30:833-842.
52. Bergman L, Nilsson B, Lundell G, Lundell M, Seregard S. Ruthenium brachytherapy for uveal melanoma, 1979-2003: survival and functional outcomes in the Swedish population. *Ophthalmology* 2005;112:834-840.
53. Augsburger JJ. Is observation really appropriate for small choroidal melanomas. *Trans Am Ophthalmol Soc* 1993;91:147-168; discussion 169-175.
54. Robertson DM. Changing concepts in the management of choroidal melanoma. *Am J Ophthalmol* 2003;136:161-170.
55. Factors predictive of growth and treatment of small choroidal melanoma: COMS Report No. 5. The Collaborative Ocular Melanoma Study Group. *Arch Ophthalmol* 1997;115:1537-1544.
56. Conway RM, Poothullil AM, Daftari IK, Weinberg V, Chung JE, O'Brien JM. Estimates of ocular and visual retention following treatment of extra-large uveal melanomas by proton beam radiotherapy. *Arch Ophthalmol* 2006;124:838-843.
57. Desjardins L, Lumbroso-Le Rouic L, Levy-Gabriel C, et al. Treatment of uveal melanoma by accelerated proton beam. *Dev Ophthalmol* 2012;49:41-57.
58. Virgili G, Gatta G, Ciccolallo L, et al. Survival in patients with uveal melanoma in Europe. *Arch Ophthalmol* 2008;126:1413-1418.
59. Damato BE, Heimann H, Kalirai H, Coupland SE. Age, survival predictors, and metastatic death in patients with choroidal melanoma: tentative evidence of a therapeutic effect on survival. *JAMA Ophthalmol* 2014;132:605-613.
60. Eskelin S, Pyrhonen S, Summanen P, Hahka-Kemppinen M, Kivela T. Tumor doubling times in metastatic malignant melanoma of the uvea: tumor progression before and after treatment. *Ophthalmology* 2000;107:1443-1449.
61. Damato B. Legacy of the collaborative ocular melanoma study. *Arch Ophthalmol* 2007;125:966-968.

62. Torres V, Triozzi P, Eng C, et al. Circulating tumor cells in uveal melanoma. *Future Oncol* 2011;7:101-109.
63. Carvajal RD, Schwartz GK, Tezel T, Marr B, Francis JH, Nathan PD. Metastatic disease from uveal melanoma: treatment options and future prospects. *Br J Ophthalmol* 2017;101:38-44.
64. Augsburger JJ, Correa ZM, Shaikh AH. Effectiveness of treatments for metastatic uveal melanoma. *Am J Ophthalmol* 2009;148:119-127.
65. Surriga O, Rajasekhar VK, Ambrosini G, Dogan Y, Huang R, Schwartz GK. Crizotinib, a c-Met inhibitor, prevents metastasis in a metastatic uveal melanoma model. *Mol Cancer Ther* 2013;12:2817-2826.
66. Guan X, Wang H, Ma F, Qian H, Yi Z, Xu B. The Efficacy and Safety of Programmed Cell Death 1 and Programmed Cell Death 1 Ligand Inhibitors for Advanced Melanoma: A Meta-Analysis of Clinical Trials Following the PRISMA Guidelines. *Medicine (Baltimore)* 2016;95:e3134.
67. Marquez-Rodas I, Cerezuela P, Soria A, et al. Immune checkpoint inhibitors: therapeutic advances in melanoma. *Ann Transl Med* 2015;3:267.